Preparation of size-controlled bovine serum albumin (BSA) nanoparticles by a modified desolvation method

Ji Yeon Jun, Hoang Hai Nguyen, Sae-Yeol-Rim Paik, Hyang Sook Chun, Byeong-Cheol Kang, Sanghoon Ko

A R T I C L E   I N F O
Article history:
Received 9 October 2010
Received in revised form 19 December 2010
Accepted 8 February 2011
Available online 12 February 2011

Keywords:
Nanoparticle
Bovine serum albumin (BSA)
Size control
Surface-area-to-volume-ratio
Flocculation

Abstract
The size effect of nanomaterials is of major interest, since it may affect their bioavailability and toxicity. In this study, bovine serum albumin (BSA) nanoparticles were prepared using a modified desolvation method. Bare BSA nanoparticles and calcium (Ca)-loaded BSA nanoparticles were fabricated at the targeted sizes, 100, 400, and 800 nm. The mean diameters of the prepared BSA nanoparticles were 125, 393, and 713 nm; those of the Ca-BSA nanoparticles were 260, 353, and 919 nm. The surface-area-to-volume-ratios of the prepared BSA nanoparticles were 4.82, 1.53, and 1.03 nm⁻¹; those of the Ca-BSA nanoparticles were 2.34, 1.72, and 0.90 nm⁻¹. The size and the surface-area-to-volume-ratio of the BSA nanoparticles were controlled by adjusting BSA concentration, pH, and NaCl content, which affected the coagulation of the BSA molecules. The surface-area-to-volume-ratio is a more useful parameter than the mean diameter of particles for comparing effectiveness of nanoparticles.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction
Nanotechnology has been introduced into several aspects of the food science, including encapsulations and delivery systems, which protect and deliver functional food ingredients. Bioactive ingredients such as nutrients, phytochemicals, nutraceuticals, and drugs may be incorporated into nanoparticles to maximise delivery efficiency and increase desirable benefits (Rhaese, von Briesen, Rubsam-Waigmann, Kreuter, & Langer, 2003). Since nanoparticles are submicron and sub-cellular in size, they have versatile advantages such as increased surface area and reactivity, increased gastric residence time and permeability, and improved solubility in both aqueous and organic phases. Brownian motion can provide enough energy to keep exceptionally small particles agitated and hence precipitation is less likely to happen with nanoparticle suspension. Therefore, suspensions of nanoparticles are easier to stabilise because precipitation is less likely.

Reduction in size of particles up to nanoscale changes their physical, chemical, and biological properties significantly (Moghimi, Hunter, & Murray, 2001). It is presumed that the functionality and toxicity of nanoparticles could be increased more than microparticles; by considering the fact that the submicron particles are not easily removed by the liver and spleen and have increased circulation times (Muller, Leuenberger, & Kissel, 1996). To ascertain the size effect in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size. Only a few researches have studied such methods to prepare size-controlled nanoparticles until now (Desai, Labhasetwar, Amidon, & Levy, 1996). However, up to date the previous studies have kept inherent problems. In general, it is difficult to produce nano-, micro-, and macroparticles using a common methodology. Desai et al. (1996) manufactured poly[(lactic-co-glycolic acid)—poly(vinyl alcohol) particles in 100 nm, 500 nm, 1 μm, and 10 μm sizes by sonication, micro-fluidisation, high pressure homogenisation, and simple vortex mixing, respectively. However, difference in manufacturing processes affect surface chemistry of particles and correspondingly even similar sized particles may possess different bioavailability and toxicity (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001a). Unfortunately, so far no study has explained the preparation of size-controlled nanoparticles by the similar manufacturing procedure that is being used for the nanoparticles fabrication without size-control. Furthermore, no study has been established to manufacture size-controlled nanoparticles from food-grade materials. Therefore, the size-controlled particles prepared by similar manufacturing processes, having unaltered physicochemical properties would be useful for studying size effects by minimising variation from different processes.
Bovine serum albumin (BSA) was chosen as the material for the particle matrix. BSA has great potential as a nanocarrier in food and pharmaceutical applications. BSA is non-toxic and degradable in vivo, so the nanoparticles generated by using it are easily adaptable to the human body (Jahanshahi, Najafpour, & Rahimnejad, 2008). Calcium is being used as a model bioactive ingredient in which it has been incorporated into the particle matrix during formation of BSA nanoparticles. The desolvation process has been successfully used to prepare nanoparticles using food-grade polymeric materials such as BSA. Desolvation is a thermodynamically driven self-assembly process for polymeric materials. The addition of desolvating agents such as ethanol or acetone separates and coacervates the polymeric molecules in the aqueous phase (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001b). The self-assembly of the polymer molecules occurs with electrostatic interactions, since the overall free energy in the system is minimised during desolvation (Arnedo, Espuelas, & Irache, 2002). So, the polymeric molecules form particles of different shapes and sizes depending on the preparation conditions (Langer et al., 2003). Hence, a balance between attractive and repulsive forces is necessary for fabricating particles of an appropriate size. The suppression and expression of hydrophobic interactions provide a way to control the size of polymeric particles during desolvation (Krimm & Bandekar, 1986). We hypothesise that the optimisation of manufacturing conditions including BSA content, pH, and ionic strength is needed to control size of nanoparticles.

Our objectives of this study were to: (1) fabricate BSA and Ca-BSA nanoparticles of a controlled size (100, 400, and 800 nm) using a desolvation method, (2) characterise size, surface-area-to-volume-ratio, and morphology of BSA particles, and (3) investigate flocculation of BSA particles in liquid.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, BAH62-1000, purity >96%) was commercially supplied by Equitech Bio, Inc. (Kerrville, TX, USA). Analytical grade acetone, calcium citrate tetrahydrate, glutaraldehyde (25% solution), and other chemicals were supplied by Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of BSA nanoparticles

BSA nanoparticles were prepared using a desolvation method with minor modifications (Ko & Gunasekaran, 2006; Langer et al., 2003; Weber, Coester, Kreuter, & Langer, 2000). A certain amount of BSA powder was added to distilled water; subsequently, pH and NaCl concentration were adjusted as shown in Table 1 and pH was adjusted to 7 and 9 with 0.1 M NaOH under stirring at 500 rpm. The BSA aqueous solution with 1% protein content at pH 9 was the preparation condition for 100 nm-sized BSA nanoparticles. The 400 and 800 nm-sized BSA particles were prepared at pH 9 and 7, respectively, with 5% BSA content and 10 mM NaCl aqueous solution. If needed, calcium citrate tetrahydrate, a tenth of BSA content, was mixed under stirring at 500 rpm.

<table>
<thead>
<tr>
<th>Target sizes</th>
<th>Preparation conditions</th>
<th>Calcium^4 content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>BSA 1% at pH 9 and no NaCl</td>
<td>0.1</td>
</tr>
<tr>
<td>400</td>
<td>BSA 5% at pH 9 and 10 mM NaCl</td>
<td>0.5</td>
</tr>
<tr>
<td>800</td>
<td>BSA 5% at pH 7 and 10 mM NaCl</td>
<td>0.5</td>
</tr>
</tbody>
</table>

^4 Calcium is calcium citrate tetrahydrate.

The solutions were stirred overnight at 500 rpm using a magnetic stirrer for complete hydration. If needed, calcium citrate tetrahydrate was added during stirring. A desolvating agent, acetone, was added dropwise at a rate of 1 ml/min into the BSA solutions until the solutions became just turbid. Finally, 0.01 ml of a 4% glutaraldehyde–ethanol solution was mixed to induce intra-particle cross-linking. The solution was stirred continuously at 500 rpm and room temperature for 3 h.

The BSA nanoparticles formed were purified by two cycles of centrifugation and redispersion to remove unreacted chemicals and free BSA molecules. For each centrifugation step, the BSA nanoparticle solutions were centrifuged at 20,000 g (5810R, Eppendorf, Hamburg, Germany) for 30 min. After the centrifugation, the pellets were redispersed to the original volume of absolute ethanol using a stick-type ultrasonicator (VCX-750, Sonics and Materials, Inc., Newtown, CT, USA). The purified BSA nanoparticles were stored in absolute ethanol at 4 °C.

2.3. Measurement of particle size and zeta-potential

Size distribution and zeta-potential of BSA nanoparticles were measured by a commercial zeta-potential and particle size analyzer (DelsaNano, Beckman Coulter, Inc., Fullerton, CA, USA) with an autotitrator. For the size measurement, 5 ml of the BSA nanoparticles dispersed in absolute ethanol were collected; subsequently, 0.1 ml of 11.5 M NaOH was added in order to maintain pH above 9 to prevent particles aggregation. The dispersions were stirred continuously at 500 rpm and room temperature for 30 min before particle size measurement. The particle size was measured at 25 °C with a scattering angle of 90°.

For the zeta-potential measurement, the BSA nanoparticles were dispersed in 5 ml of distilled water, and pH was adjusted to 11 with 0.1 M NaOH under stirring at 500 rpm. The dispersion was stirred continuously at 500 rpm and room temperature for 30 min. The zeta-potential was measured at 25 °C. Since the pH adjustment was fully automated, the zeta-potential data were scanned continuously at different pHs from 11 to 2. The pH was adjusted with 0.1 M HCl. Both size and zeta-potential measurements were performed in triplicate.

2.4. Scanning electron microscopy

The size-controlled BSA nanoparticles were examined in a low vacuum scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Ibaraki, Japan) under an accelerating voltage of 10 kV, 3.0 nm resolution at high voltage, and 4.0 nm resolution at low voltage. The monochrome images of the BSA nanoparticles were obtained as tagged image file format.

3. Results and discussion

3.1. BSA nanoparticle formation

The preparation conditions for BSA and Ca-BSA nanoparticles for 100, 400, and 800 nm plans are shown in Table 1. The strategy to control size was to adjust BSA concentration, pH, and ionic strength in the desolvating conditions. BSA nanoparticles formed in different sizes at the conditions since the desolvation process was influenced by the electrostatic attraction and repulsion among BSA molecules which varied on pH and ionic strength. As a result, the different desolvating conditions needed different amounts of acetone for desolvating. Consumed volumes of acetone for the BSA and Ca-BSA nanoparticle preparation are listed in Table 2. The amounts of acetone for the 100 nm targeted BSA nanoparticle preparation were 35 ml. Consumption of acetone decreased as...
targeted size increased. One or two milliliter less acetone was added for Ca-BSA nanoparticles compared to the amount of acetone for fabricating bare BSA nanoparticles. Scanning electron micrographs of the BSA nanoparticles formed are shown in Fig. 1. The BSA nanoparticles were a sphere shape since self-assembly of BSA molecules in the aqueous desolvating condition resulted in the spherical shape (Pan, Yu, Yao, & Shao, 2007; Rahimnejad, Jahanshahi, & Najafpour, 2006). Fig. 2 shows the repeatability of the desolvation process to prepare BSA nanoparticles at 100, 400, and 800 nm plans. Generally, the desolvation by acetone successfully formed BSA nanoparticles at the targeted sizes. Especially, the repeatability in preparing bare BSA and Ca-BSA nanoparticles for the 100 nm plan was stable. The desolvation processes were also stable for bare BSA at 800 nm and Ca-BSA at 400 nm plans. The preparation conditions of BSA content, pH, and ionic strength affected self-assembly of BSA molecules to form size-controlled particles. However, the desolvation processes were unsteady for bare BSA at 400 nm and Ca-BSA at 800 nm plans. The self-assembly of BSA molecules or the incorporation of calcium molecules into the BSA matrix may be more sensitive at the preparation conditions for bare BSA at 400 nm and Ca-BSA at 800 nm plans. Therefore, it is important to adjust the preparation conditions more precisely. Table 3 shows the mean diameters of the BSA and Ca-BSA nanoparticles formed. The mean diameters of BSA nanoparticles were 125, 393, and 713 nm, which were close to the plans, respectively. Statistical analysis showed that the particles prepared were different in the mean size. On the other hand, the preparation of Ca-BSA nanoparticles was also successful except for the 100 plan. The mean diameters of Ca-BSA nanoparticles were close to 400 and 800 plans, respectively. However, the mean diameters of Ca-BSA nanoparticles for the 100 plan were significantly large due to interference of calcium molecules in self-assembly of BSA molecules.

The desolvation process has been successfully used to prepare BSA nanoparticles for delivery carriers. However, the size of the nanoparticles varied but was not prepared in a controlled manner. The sizes for the bare BSA nanoparticles were 120–550 nm (Roser & Kissel, 1993) and 101–503 nm (Rahimnejad et al., 2006) whereas those for drug or nutrient carriers were 400–820 nm for an anti-tumour 5-fluorouracil carrier (Santhi, Dhanaraj, Joseph, Ponnusankar, & Suresh, 2002) and 50–400 nm for a bone morphogenetic protein-2 carrier (Wang et al., 2008). The size distributions of the particles prepared were broad. Especially, no study was conducted for size-different nanoparticles.

<table>
<thead>
<tr>
<th>Target sizes (nm)</th>
<th>Acetone consumptions (ml/20 ml of aqueous BSA solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>400</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>800</td>
<td>18 ± 1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Bare BSA particles.  
<sup>b</sup> BSA particles for Ca carrier.

Fig. 1. Scanning electron micrographs of BSA nanoparticles at the targeted sizes: (A) 100 and (B) 400 nm. Scale bar is 500 nm.
Prepared by the same manufacturing procedure in order to avoid variations in surface characteristics of particles. In this study, different sized BSA nanoparticles were prepared under the same desolvation method. As shown in Fig. 3 zeta-potentials of both BSA and Ca-BSA have similar profiles, which decreased with increasing pH. This result indicated that the different sized particles have similar surface characteristics. In the future, these types of particles are beneficial in studying size effect by avoiding surface effect. The surface of BSA and Ca-BSA was charged positively under acidic conditions and negatively at neutral and basic conditions, with the transition occurring at pH ~ 4. The size-controlled nanoparticles prepared from the same manufacturing process in this study will be useful for understanding in vivo size effect by minimizing variation from different processes.

### 3.2. Size control of BSA nanoparticles

Size control of BSA nanoparticles was conducted by modifying desolvation methods studied previously (Ko & Gunasekaran, 2006). The particle size was controlled by adjusting BSA content, pH, and NaCl content, which affect the coagulation of the BSA molecules. The pH is the most important factor in controlling the coagulation of the BSA molecules during the desolvation process. The isoelectric point (pl) of BSA is about 4.9. When pH shifts toward the pl, the enhanced protein–protein interactions increase coagulation among BSA molecules; as a result, larger BSA particles could be formed. On the other hand, the enhanced protein–solvent (or water) interactions decrease coagulation when pH is far from the pl. pH 9 provides a highly electrostatic repulsive condition for the BSA molecules (pl ~ 4.9), and concomitantly, coagulations by protein–protein interactions are limited; as a result, fine BSA particles could be formed. In this study, at pH 9, BSA molecules possessed a negative charge, which allowed the formation of small particles since coacervation of the molecules was suppressed. Under basic conditions, such as pH 9, coagulation of the BSA molecules was reduced, and the void spaces within a particle generally decreased (Schmidt, 1981). On the other hand, pH 7 medium, which alters electrostatic repulsion, increased the coagulations among BSA molecules that could result in the formation of larger BSA particles.

The content of salts such as NaCl also affected the coagulation of the BSA molecules. Salt is responsible for shielding the surface charge of protein molecules by altering their electrostatic properties (Illeda & Morris, 2002). Positive ions attach to negative charges on the surface of BSA molecules and concomitantly reduce the charge. Negative ions also interact with positive charges and reduce the net charge of BSA molecules. The change in salt concentration can increase or decrease the coagulation among BSA molecules. The size of the BSA particles increased as NaCl content increased during desolvation. Due to the shielding of the surface charges on the BSA molecules by the addition of salt, the reduced net charge on the BSA molecules enhanced the hydrophobic protein–protein interactions to form larger particles. The addition of NaCl weakened the repulsion between BSA molecules during desolvation.

High BSA concentrations increase the chances for coagulation, especially, the protein molecules have had more chances to undergo electrostatic and hydrophobic interactions. Larger hydrophobic interactions of BSA increased the coagulation of the molecules and subsequently resulted in larger particles. Thus, sufficient content of the BSA could form large particles, such as 400 or 800 nm BSA particles.

The size of BSA particles was controlled by adjusting self-assembly phenomena of the BSA molecules. The self-assembly was affected by preparation conditions including pH, protein content, and salt content, which provided different electrostatic and hydrophobic interactions during desolvation of the BSA molecules. The control of the BSA particle size can be explained by their surface charge and surface hydrophobicity. Surface hydrophobicity dictates the propensity to bind non-polar amino acid groups to a hydrophobic part of its surface. Hydrophobic interactions lead to coagulations among protein molecules, resulting in size increase (Ismond, Murray, & Arntfield, 1988). Under basic conditions, such as pH 9, electrostatic interactions increase, but hydrophobic interactions decrease. In addition, at basic pH, an increase in hydrogen bonding results in a decrease in the hydrophobicity (Krimm & Bandekar, 1986). At pH 9 there was a decrease in the hydrophobic interactions. On the other hand, the expression of the hydrophobic

### Table 3

Mean size and surface-area-to-volume-ratio of BSA and Ca-BSA nanoparticles at the targeted sizes: 100, 400, and 800 nm.

<table>
<thead>
<tr>
<th>Target sizes (nm)</th>
<th>Mean size (nm)</th>
<th>Surface-area-to-volume-ratio (nm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA⁵</td>
<td>Ca-BSA⁶</td>
</tr>
<tr>
<td></td>
<td>BSA¹</td>
<td>Ca-BSA²</td>
</tr>
<tr>
<td>100</td>
<td>125 ± 4⁴</td>
<td>260 ± 3⁸</td>
</tr>
<tr>
<td>400</td>
<td>393 ± 129⁵</td>
<td>353 ± 18⁸</td>
</tr>
<tr>
<td>800</td>
<td>713 ± 29⁶</td>
<td>919 ± 343⁷</td>
</tr>
</tbody>
</table>

The symbols (a, b and c) within the same column indicate significant differences at p < 0.05.

A Bare BSA particles.

B BSA particles for Ca carrier.

Fig. 3. The pH-dependent zeta-potentials of (A) BSA and (B) Ca-BSA nanoparticles at the targeted sizes: 100, 400, and 800 nm.
Fig. 4. Stability of BSA nanoparticles and their resuspendability after storage: (A) fresh 100 nm BSA particles, (B) 100 nm BSA particles after 1 day storage, (C) resuspended B by moderate shaking, (D) fresh 800 nm BSA particles, (E) 800 nm BSA particles after 1 day storage, (F) resuspended E by moderate shaking, (G) cake formation of 100 nm BSA particles after 3 month storage, and (H) chunky cake from 100 nm BSA particles after 3 month storage.
interactions by changing the pH to 7 increased the size of BSA particles. This result supports our hypothesis with regards to controlling hydrophobic interaction as a means for controlling the size of BSA nanoparticles.

Addition of calcium affected the size of BSA particles. The calcium was incorporated into the particle matrix during the desolvation process since its water solubility was too low to dissolve fully in water. The water solubility of the calcium citrate tetrahydrate used in this study is $9.5 \times 10^{-4}$ g/ml at 25 °C. Hydrophobic interactions between BSA molecules and calcium citrate tetrahydrate molecules led to the size increase of the BSA particles. Especially, the mean particle size for the 100 nm plan was 260 nm which was not close to the target size. The incorporation of the calcium molecules into the BSA matrix could not fully limit the coagulation among BSA molecules to form particles in a small size.

3.3. Surface-area-to-volume-ratio of BSA nanoparticles

Surface area is a material property of particles that is often a determining factor in bioavailability, dissolution rate, adsorption, catalyst activity, and toxicity (Aguzzi, Cerezo, Viseras, & Caramella, 2007). The surface area is a more useful and accurate metric than concentration or dosage, which is traditionally believed to be the most important (Oberdorster, Ferin, & Lehnert, 1994). The concept of total surface area can be used to describe the surface area in a sample.

In general, information about mean or total surface area of particles is not measured using a commercial particle size analyzer. Therefore, other relevant parameters that are based on the data from the particle size analyzer are needed to trace the surface area. One such is the surface-area-to-volume-ratio, and is more useful than the concentration or the mean diameter of particles. The surface-area-to-volume ratio is the amount of surface area per unit volume of particles. The surface-area-to-volume ratio can be calculated using size distribution data of particles under the assumption that all particles are spheres. Table 3 shows the surface-area-to-volume-ratios of BSA and Ca-BSA nanoparticles of sizes 100, 400, and 800 nm, respectively. As expected, the particles for the 100 nm plan obtained the largest surface area, whereas those for 800 nm had a relatively small surface area. In any case, the surface-area-to-volume-ratio can be used to represent a major physical property of the particles instead of diameter-related values (Phyu, Warne, & Lim, 2005). Furthermore, the surface-area-to-volume-ratio is useful for comparative studies of nano-scaled materials.

3.4. Stability of BSA nanoparticles

Fig. 4 shows stability of BSA nanoparticles and their resuspendability by moderate shaking after storage. Particle suspension often becomes less stable so that gravity forces cause precipitation of the particles. Precipitation is less likely to occur in the nanoparticle suspension since Brownian motion provides enough energy to keep nanoparticles agitated and electrostatic repulsive forces among particles helps particles disperse well in the suspension. However, flocculation among particles accelerates the precipitation. In general, nanoparticles tend to come together strongly since the large surface area of particles results in a high surface energy (Peltonen & Hirvonen, 2008). The flocculation among BSA nanoparticles is more likely than that in microparticles. The BSA nanoparticles in suspensions carry electrostatic charges and these charges are dependent on the surrounding solvent phase. The rate of flocculation of the BSA nanoparticles depended on their surface characteristics which differed with the preparation conditions such as pH and ionic strength.

Just after fabricating BSA nanoparticles, all particles existed in the colloidal form which resulted in a stable suspension (Fig. 4A and C). For the preparation of the target size of 100 nm BSA particles, 1% BSA aqueous solution at pH 9 without NaCl was formulated. The 100 nm BSA particles maintained a turbid suspension and hardly flocculated during storage. This could be due to the high net repulsive force among the negatively charged surface of BSA particles (Fig. 4B). Earlier reports indicate that the fabricated BSA nanoparticles are negatively charged and stabilised by electrostatic repulsion (Schmidt, 1981). The particles surface properties at pH 9 prevented the BSA nanoparticles from flocculation to become heavy enough to gravity settle. Strong interactions between particles and solvents prevent the particles from coming too closely into contact with one another (Hoffmann & van Mil, 1999). In this condition, particle flocculation hardly occurs. However, despite stabilisation by electrostatic repulsion, the suspension became unstable slowly during long storage time. As time went on, destabilisation of BSA nanoparticles led to flocculation. The flocculation observed was slow among 100 nm BSA particles. The 100 nm particles packed closely minimising the voids between the particles. The settling resulted in formation of solid hard cakes that was difficult to resuspend (Fig. 4G and F).

BSA particles of the 400 and 800 nm target sizes were prepared at pH 9 and 10 mM NaCl and at pH 7 and 10 mM NaCl, respectively. The 400 and 800 nm BSA particles settled rapidly so that their supernatant of the suspensions was clear (Fig. 4E). The suspensions at pH 9 and 10 mM NaCl and at pH 7 and 10 mM NaCl, respectively. The 400 and 800 nm BSA particles settled rapidly so that their supernatant of the suspensions was clear (Fig. 4E). The suspensions loosely packed with 800 nm BSA particles settled faster than the 100 nm BSA particles because of their larger sizes. Stronger interactions among particles than those between particles and solvent molecules result in flocculation (Langton & Hermansson, 1996). Under this condition, the suspension form loose networks of flocs that settle rapidly. The sediment of the particles did not form solid cake but was easy to redisperse by putting in a small amount of energy in the form of moderate stirring or shaking (Fig. 4F). These conditions could be due to lowered electrostatic repulsions between particles since net charge of BSA particles decreased by lowering pH and/or charge shielding effect of salt (Ikeda & Morris, 2002). As a result, the attractive forces supersed the repulsive forces and the rate of flocculation was increased.

In conclusion, herein, BSA and Ca-BSA nanoparticles were prepared using a desolvation method by pH adjustment and salt addition which helped to control the particle size. Size-controlled nanoparticles are useful for understanding the cellular fate, uptake, metabolism, and clearance of nanoparticles in the body. In particular, our study may provide basic data to establish a procedure for evaluating the safety of nanomaterials manufactured from food-grade ingredients. In the near future, the results of these studies will help investigate the in vitro and in vivo size effect of nanomaterials. In addition, size-controlled nanoparticles can be used to assess the risk of nanomaterials.

Acknowledgement

This research was supported by a Grant (08082KFDA549) from Korea Food and Drug Administration in 2008.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.02.040.

References


