Intermittent desolvation method to prepare size-controlled BSA nanoparticle

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ABSTRACT

The development of standard method for nanoparticle preparation using food-grade materials is helpful for various nanotechnology-related applications for food product. In this study, bovine serum albumin (BSA) nanoparticles were prepared using a desolvation method. The size of BSA nanoparticles was controlled by adjusting pH and NaCl content as well as controlling rate of ethanol addition, a desolvating agent. At a basic condition such as pH 9, coagulation of the BSA molecules reduced. On the other hand, pH 7, coagulation of the BSA molecules increased; as a result, larger BSA nanoparticles were achieved. The size of the BSA nanoparticles increased as NaCl content increased. In order to control the particle size and improve reproducibility, the rate of ethanol addition was controlled. To induce a controlled aggregation between BSA molecules in the aqueous system was added intermittently under stirring until an opalescent solution was obtained. Size of the BSA nanoparticles prepared was close to the target size: 100, 300 and 500 nm. In addition, BSA nanoparticles formed with narrow size distribution compared to those prepared by a conventional continuous method. The size-controlled BSA nanoparticles can be standard materials made from food ingredients to investigate benefits and risks of nanofoods.

Keywords: Nanoparticle, Size-controlled, Bovine serum albumin (BSA), Desolvation

1. INTRODUCTION

Nanotechnology has become a fashionable term in the current food science and technology. Nanotechnology is attractive because of application in many fields of industry. Nanotechnology has been used in the food and drug industry. They can be produced with different structures such as nanoparticles, dendrimers, nanocages, micelles, liposomes and nanocomposites [1]. Delivery system is a kind of the major role of nanotechnology using nanoparticles [2]. For this reason, the efficient delivery system of nanoparticles is the one of the major factor enhancing the efficacy of the therapeutic agent. For application of nanotechnology in food and pharmaceutical industry, various nano structured vehicles such as nanoemulsions, solid lipid nanoparticles [3] and surface modified nanoparticles have been developed. Nanoparticles can be prepared from proteins, polysaccharides and synthetic polymers, nanoparticles by proteins play an important role among them [4]. Commonly, serum albumin from human and bovine have been mainly studied. In delivery system, the particles should be small enough to penetrate through the capillary bed. Bioactive compounds which have poor stability or bioavailability, such as figments, nutrients, phytochemicals, minerals and drugs, can be incorporated into nanoparticle to improve delivery efficiency [5]. Generally, nanoparticles are prepared by emulsification or desolvation methods [6]. However, the emulsification method required the organic solvent for emulsion stability. Recently, desolvation process has been successful to prepare nanoparticles. Factors affecting properties of nanoparticles have been reported by many researchers [7-9], but these methods indicated unreliable reproducibility. Thus, establishing the preparation method of size-controlled nanoparticles with high reproducibility is important. In the preparation of protein nanoparticles, pH and salt affect the aggregation of protein molecules. The pH and salt are important factors in size controlling. In the preparation of protein nanoparticle using desolvation method, continuous addition of desolvating agent indicate low reproducibility because there is not enough time to react, while an intermittent addition could overcome this limitation by providing enough time to react. The objective of this study is to optimize the desolvation procedure for preparation of BSA nanoparticle with narrow particles size distribution.

2. MATERIALS AND METHODS

2.1 Materials

BSA was commercially supplied by Equitech-bio, Inc. (Kerrville, TX, USA) and other chemicals were supplied by Sigma-Aldrich (St. Louis MO, USA).
2.2 Preparation of BSA nanoparticles by intermittent method

BSA nanoparticles were prepared using a desolvation method. First of all, 2% of BSA powder was dissolved in deionized water with NaCl under stirring at 500 rpm. The pH of the solution was adjusted to 6, 7, 8 and 9 with NaOH or HCl, followed by continuous stirring. In order to fabricate the nanoparticles, ethanol, a desolvating agent, was added intermittently using a peristaltic pump (BT100-1F, Baoding Longer Precision Pump Co., Ltd., Hebei, China) until the solution turns to ivory white color. To stabilize the BSA nanoparticles the turbid solution was stirred continuously for 30 min without ethanol addition.

2.3 Preparation of BSA nanoparticles by continuous method

BSA solution was prepared under the same condition with intermittent method, and ethanol was added continuously until the solution become turbid, followed by continuous stirring for 30 min.

2.4 Particle size measurement

Size distribution of BSA nanoparticles was measurement by commercial particle size analyzer (Delsa Nano, Beckman Coulter, Inc., Fullerton, CA, USA). The size distribution was measured at 25°C with a fixed scattering angle of 165°. Particle size was measured at least 3 times.

3. RESULTS

At the same preparation condition, the mean particle size by the intermittent addition was smaller and homogeneous compared with that by the continuous addition. In the intermittent addition, the particle size was about 300 nm, 200 nm and 100 - 150 nm at pH 6, 7 and 8, respectively. A pH is an important factor coagulating BSA molecules during desolvation because the chemical characteristics and structure of the protein molecule strongly affected by pH alteration. In the results, the particle size increased with decreasing the pH. When the pH of the solution containing BSA approaches a pI value of BSA, a dominant protein-protein interaction can be caused dominantly instead of a protein-water interaction [10]. Fig 1 also indicates that the particle size of the BSA nanoparticle decreased with increasing the pH. At pH7, small-sized particle is fabricated because the aggregation between BSA molecules is hindered by electrostatic repulsion. On the other hand, large-sized particle can be fabricated at low pH due to the decreased net charge of the BSA. In addition, the hydrophobic property of protein decreased by increased hydrogen bonds at alkaline pH. However, the intermittent method strongly affects the size controlling of the BSA nanoparticle than hydrophobic interaction [11].

Fig 1. The size of BSA nanoparticles by the intermittent and continuous method

Salts such as NaCl also affect the aggregation of BSA molecules because salts offset the charges at the surface by changing the electric property of protein: Na⁺ ion couples to the surface of the BSA molecule, while Cl⁻ ion interacting with (+) charges reduces a net charge of the BSA molecule. In this study, the size of the BSA nanoparticle increased with increasing the NaCl content because NaCl decreased a repulsive force between BSA molecules during desolvation. In order to secure the reliability of the data from intermittent addition standard deviation of particle size values from repeated tests were calculated. Fig 2 indicates the mean standard deviation values, which represented low value in the intermittent addition in comparison to the continuous addition.

Fig 2. Mean standard deviation of the BSA nanoparticle by the intermittent and continuous method

From the results, it is considered that development of an intermittent addition of desolvating agent could improve reproducibility of the BSA nanoparticle preparation.

4. CONCLUSIONS

Recently, food materials have been used to nanoparticles for several food and pharmaceutical industry applications. Our study may provide the protocol method for the protein nanoparticle. The particle size was controlled with narrow size distribution. Therefore, the intermittent method can be useful for size control for food materials. In particular, our study can be expanded to the area of in vitro digestion of nanoparticles,
in vivo tracking and quantification of nanoparticle toxicity study. In addition, size-controlled nanoparticles can be used to assess the risk of nanomaterials.

REFERENCES


