Preparation of BSA Nanoparticles by desolvation method as a delivery system for nutraceuticals

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Abstract

Desolvation method was successfully used to produce BSA nanoparticles using different desolvating agents (ethanol, acetone and mixtures of them). The SEM images, shows that the nanoparticulation was different for various desolvating agents. BSA NPs produced using ethanol were completely spherical, while the NPs produced using acetone were a mixture of spherical and rod-shape particles. We conclude that ethanol and mixture of ethanol and acetone are better desolvating agents for production of the spherical BSA NPs. The Encapsulation efficiency (EE) in different molar ratios of Curcumin: BSA was between 10 to 20 %. High solubility of curcumin in the desolvating agent (Ethanol) could be the main reason for low EE. Surfactant was used to decrease separation of curcumin-BSA. By using surfactant, the Encapsulation Efficiencies increased by 2-3 times in the different molar ratios.

Keywords: desolvation, BSA, curcumin, nanoparticles

1. Introduction

Nanoparticles are very effective tools for protection and delivery of nutraceuticals, particularly when they are produced from biopolymers with high affinity for hydrophobic and hydrophilic compounds. Reduction of particle size in the nanoscale range alters significantly the physical, chemical and biological properties of particles [1]. Bovine Serum Albumin (BSA) is very strong carrier in blood and milk. BSA shows a wide range of physicological functions like binding, transport and distribution of bioactive compounds [2]. The BSA protein contains two tryptophan residues (Trp 134 and Trp 213), and in the native form, Trp 134 and Trp 213 are located on the surface of the molecule and in a hydrophobic pocket, respectively [3]. Elzoghby et al. [4] reviewed the different preparation methods for production of nanoparticles from albumin proteins such as human serum albumin (HSA), BSA and ovoalbumin. They considered the albumin-based nanoparticles as potential nano-carrier systems for controlled release of drug. The desolvation process has been successfully used to produce HSA nanoparticles (NPs) [5]. Langer et al. [6] optimized the effect of different parameters on the particle size during preparation of NPs from HSA like protein types, concentration, cross-linking method, environmental conditions and especially pH. They used ethanol as desolvating agent. The size and surface properties of protein particles can be significantly changed by the number of disulphide bonds and thiol groups, degree of unfolding, electrostatic repulsion between protein molecules, ionic strength and pH. The cross-linking of the protein molecules lead to the formation of the particles with the potential to entrap bioactive compounds [7]. Rahimnejad et al. [8] evaluated the effect of different parameters on the particles size of BSA NPs. They used different pH, temperature, BSA concentration, the flow rate of desolvating agent (ethanol) addition, agitation speed and Glutaraldehyde concentration. Curcumin is the main yellow pigment of turmeric and has been used as a spice and food coloring agent for centuries. It has also been used as a medicine for some disease such as inflammation, skin wounds, and tumors [9], as an antioxidant and anti-cancer agent [10]. There are a few publications on the application of serum albumin NPs to encapsulate bioactive compounds and minerals. Kim et al. [11] produced nanoparticles from curcumin-HSA using albumin bound nanoparticle technology; in this method they used homogenization at 20,000 psi for nine cycles. Curcumin-HSA nanoparticles improved curcumin solubility in water (300-fold) which has high effectiveness to improve biological activity of curcumin. The average particle size and the curcumin loading were 135.5±2.9 nm and 7.2±2.5%, respectively. HSA-nanoparticles can be used as potential carriers to improve solubility and antitumor activity of curcumin [11].

Our objective was to use different desolvating agents (ethanol, Acetone and mixtures of them) to produce BSA NPs. We want to study the effect of different desolvating agents on particle morphology, size and size distribution of the particle. Finally we want to evaluate the possibility of encapsulation of a hydrophobic model bioactive (curcumin) inside the BSA NPs, and improve the encapsulation

efficiency using sodium dodecyl sulfate (SDS) as the surfactant.

2. Materials and Methods

2.1. Materials

Bovine serum albumin (BSA, lyophilized powder, ≥98%, essentially fatty acid free, essentially globulin free) was purchased from Sigma-Aldrich Co. Curcumin was obtained through the generosity of the ChromaDEX company as a gift sample. All other chemicals were analytical grade and used without further purification.

2.2. Preparation of BSA nanospheres

The Desolvation method was used for preparation of nanoparticles. The BSA powder was dissolved in MilliQ water at 20 $mg.ml^{-1}$; and its pH was adjusted to 9.0±0.02 with 2.0 M NaOH along with stirring system. The desolvation method was performed according to [6], [8], [12], and [13] with some modifications.

2.3. Particle size analysis, distribution and morphology

A commercial dynamic light scattering (DLS) instrument (ZetaPALS, Brookhaven Instruments Corp., Holtsville, NY) was used to analyze the size and distribution of nanoparticles. The morphology of the BSA nanoparticles and curcumin loaded nanoparticles were characterized by field emission scanning electron microscopy (FE-SEM) (S-4700, Hitachi, Japan).

2.5. The preparation of BSA NPs loaded with curcumin

The BSA solution in MilliQ water (20 $mg.ml^{-1}$, pH=9) was mixed with different curcumin concentrations (molar ratios of 0.5 to 2.5). To evaluate the effect of SDS, an aqueous solution of SDS was added to each solution and blended by using a magnetic stirrer for 2 h, and then the desolvation method was performed using ethanol as a desolvating agent.

2.6. Measuring the encapsulation efficiency

After the nanoparticulation process, the BSA NPs were separated using a centrifuge (20000×g for 20 min) and the supernatant was diluted by ethanol (1:10). To measure the curcumin concentration in the supernatant, the absorbance of the solutions was measured at 420 nm. To calculate the exact curcumin concentration, the standard curves were plotted in different conditions. Then the following formula was used to estimate the encapsulation efficiency (EE):

$$Encapsulation\ Efficiency\ (\%) = \frac{Added\ Cur.Conc. - Free\ curc.\ Conc.}{Added\ Cur.Conc.} \times 100$$

3. Results and discussion

3.1. The effect of desolvating agent ratio on the particle size and nanoparticulation efficiency

In the first step, it was necessary to find the best ratio of different desolvating agents for each desolvating agent. Fig. 1 shows the effect of this ratio on the particle size. When the desolvating ratios of different combined solvents [including Ethanol (100), Et: Ac (70:30) and Et: Ac (50:50)] increased to 2.0-3.0, the particle size started to increase and after this ratio reached to maximum, it became constant. The particulation was completed in 3X, 2X, 2X and 2X for ethanol (100), Et: Ac (70:30), Et: Ac (50:50) and acetone (100), respectively.

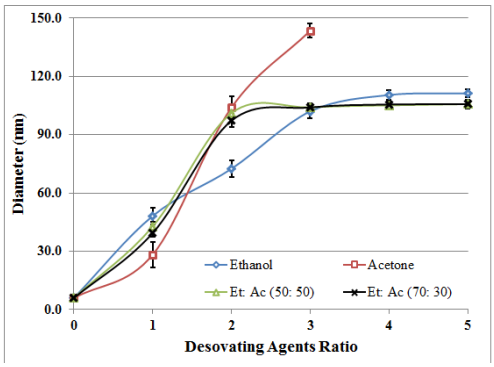

Fig. 1: The effect of different desolvating agent ratios on nanoparticle size

To select the best ratio of desolvating agents, it was necessary to calculate the nanoparticulation efficiency. As Table 1 shows the nanoparticulation efficiency by using ethanol, acetone and mixtures of them as desolvating agents. Acetone was the effective solvent to increase the nanoparticulation efficiency. According to the particle size and nanoparticulation efficiency the best conditions for nanoparticulation were Ethanol (4X), Et: Ac (70:30) (4X), Et: Ac (50:50) (4X) and Acetone (3X).

Table 1: The nanoparticulation efficiency of different desolvating agents and ratios

Desolvating agent (VR[1])	NP[2] (%)	Desolvating agent (VR[1])	NP[2] (%)
Ethanol (4X)	**95.2±0.5**	Et: Ac 50:50 (3X)	**96.0±0.5**
Et: Ac 70:30 (3X)	**92.5±0.5**	Et: Ac 50:50 (4X)	**97.7±0.4**
Et: Ac 70:30 (4X)	**96.8±0.4**	Acetone (3X)	**99.2±0.2**

[1] **Volume Ratio** [2] **Nanoparticulation efficiency**

3.2. Effect of desolvating agents on the particles distribution and morphology

The drop wise addition of desolvating agents into the BSA aqueous solution is the main driving force to prepare nanoparticles. The organic solvents such as acetone and ethanol have ability to force the BSA molecules to aggregate and change them to nanoparticles. It was clear the desolvating agent could effect on the particle size and distribution of BSA nanoparticles. Although some researchers were able to use ethanol or acetone (as a

desolvating agent) to produce nanoparticles from BSA or HSA [6], [8], [12], and [13], they did not work on the phenomenon which happens during desolvation, and the role of solvents on the particle size and distribution. In this case four desolvating agents with four fabricated ratios of 4X, 4X, 4X and 3X to BSA respectively for Ethanol (100) 4X, Acetone 3X, Et: Ac (70:30) 4X, and Et: Ac (50:50) 4X were selected to produce BSA NPs. The particle distribution made with different ratios of desolvating agent and SEM Image of BSA molecules (shown in Fig. 2) are the results of this study. While before nanoparticulation most part of the BSA molecules were in the monomeric forms with the effective diameter of 5.9±0.15 nm, and there were some aggregations without any effects on the particle size formation (Fig. 2A), when Ethanol (100) used as a desolvating agent, very uniform particles were produced. The DLS result (Fig. 2B) shows that only one nanoparticles nucleation or one group (with even size) of the spherical particles were produced by ethanol. Rahimnejad et al. [8] produced semi-spherical NPs when they used ethanol in desolvation method. The SEM images confirmed the DLS results, but the diameter of the particles with DLS was a little bigger than results of SEM images. The polydispersity of the particles was 0.045±0.007. Although the best particulation was performed by Ethanol (100), but the particulation efficiency was lower for ethanol in comparison with other solvents formulation (Table 1). By using mixture of the ethanol and acetone, two groups of nanoparticles were observed (Data not shown), the diameter of the bigger one was around 100-150 nm similar to the one produced by ethanol, but the latter particle size was around 40-50 nm. By increasing acetone portion the smaller particles increased. Although the SEM images of nanoparticles produced by Et:Ac (70:30) and Et:Ac (50:50) did not show significant differences, the proportion of smaller particles in Et: Ac (50:50) were a little higher than Et:Ac (70:30). Furthermore, by using the mixture of ethanol and acetone (as desolvating agents), two groups of particles as an outcome of two separated nucleation were produced. Even though the particulation for Acetone produced two groups of particles, and the first (or major) group were around 100-150 nm. However, the DLS result showed another (or minor) group with diameter around 300-400 nm. Fig. 2C shows the particle distribution which produced by 100% acetone. The effective diameter of this group of particles increased to 143.5±3.65 nm. The SEM images confirmed and showed that the bigger nanoparticles were produced by acetone, and these bigger particles were the results of the smaller particles aggregation and they were not completely spherical. Langer et al. [6] used only ethanol as the desolvating agent and produced HSA. The optimum pH range, flow rate of ethanol addition, and stirring speed were between 8-9, 1-2 $ml.min^{-1}$, and 500 rpm in all experiments, respectively. In our study, the particle size of BSA NPs were smaller than Langer et al. [6] and Rahimnejad et al. [8] particles, most probably because of using the higher stirring speed (1200 rpm) in our case. The polydisperse of BSA NPs in this study were 0.045±0.007, 0.065±0.013, 0.091±0.012, and 0.120±0.016 for Ethanol (100) 4X, Et: Ac (70:30) 4X, Et: Ac (50:50) 4X, and Acetone (100) 3X, respectively. Gülseren et al. [14] used desolvation along with volume frequency methods and prepared NPs from whey protein isolate (WPI) with the particle size of less than 100 nm.

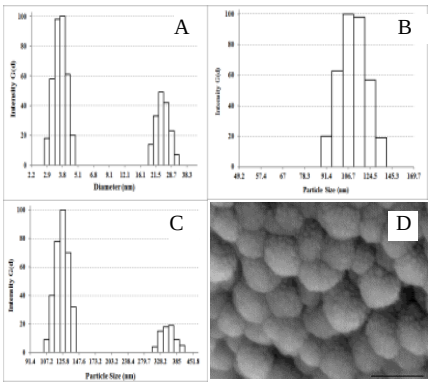

Fig. 2: The size distribution of BSA (A), BSA NPs for Ethanol (100) 4X (B), Acetone (100) 3X (C), and SEM image of BSA NPs produced by Ethanol (100) 4X (D).

3.4. Encapsulation efficiency of BSA Nanoparticles with curcumin

The encapsulation efficiency (EE) of BSA NPs with curcumin was very low, and it was around 10-20 % when molar ratio of curcumin: BSA was in the range of 0.5 to 2.5 (0.5X to 2.5X). The amounts of EE were 19.4±2.2 and 19.8±1.6 % for 1.0X and 1.5X, respectively. The main reason for low encapsulation efficiency was using ethanol. When ethanol (as desolvating agent) was added to interact with Curcumin-BSA, and produce NPs, most of the attached curcumin molecules to BSA molecules were separated, and encapsulation efficiency became very low. To improve this efficiency, after mixing the curcumin solutions with BSA solution in the two separate reactors, SDS solution was added into each reactor at specific concentration and the two separate mixtures were stirred for 2 h to complete their nanoparticulation processes. The SDS improved encapsulation efficiency, especially in lower curcumin concentrations. Two chemical reactions could be happening for each mixture, first one was changing the BSA configuration which led to entrapment of curcumin into the BSA molecules, and the second one was interaction of SDS molecules with hydrophobic pockets of BSA and consequently inhibition of curcumin molecules separation from BSA molecules during desolvation.

When the curcumin concentration increased in the mixture, the particle size of nanoparticles increased slowly, and by adding SDS to BSA solution at a molar ratio of 2, the particle size decreased. Once, the molar ratio of SDS to BSA reached 4 the particle size increased slightly. The SEM images of BSA NPs loaded with different curcumin

concentrations were similar to each other (data not shown). Nevertheless, by increasing the concentration of curcumin, sensitivity of NPs to electron flow increased, and in high concentration of curcumin imaging of BSA NPs became impossible.

4. Conclusion

In this paper, nanoparticulation was conducted with different desolvating agents in the best conditions (found from the literature). Acetone was the strongest desolvating agent, nanoparticulation efficiency for acetone was 99.2±0.18, but the particles were not completely spherical and for 3X ratio, SEM images showed some aggregations which were led to highest particle size. By using mixture of ethanol and acetone, the particles were spherical but there were two nucleation points which led to two groups of nanoparticles. Ethanol prepared uniform particle at 4X ratio, the particles were spherical with lower polydispersing, but nanoparticulation efficiency was lower than other desolvating agents. Interaction of curcumin with BSA molecules was suitable to prepare curcumin-loaded-BSA NPs; the nanoencapsulation efficiency was low (10 to 20%) when ethanol was used as desolvating agent. When SDS used in nanoparticulation process, it decreased the detaching of curcumin molecules from BSA molecules during desolvation, and improved encapsulation efficiency 2-3 times.

Acknowledgements

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