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# Investigating the influence of effective parameters on molecular characteristics of bovine serum albumin nanoparticles

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ABSTRACT

The protein nanoparticles formulation is a challenging task as they are prone to undergo conformational transitions while processing which may affect bioavailability for bioactive compounds. Herein, a modified desolvation method is employed to prepare Bovine Serum Albumin nanoparticles, with controllable particle size ranging from 100 to 300 nm and low polydispersity index. The factors influencing the size and structure of BSA NPs viz. protein concentration, pH and the conditions for purification are well investigated. The structure of BSA NPs is altered due to processing, and may affect the effective binding ability with drugs and bioactive compounds. With that aims, investigations of molecular characteristics of BSA NPs are carried out in detail by using spectroscopic techniques. UV–visible absorption and Fourier Transform Infrared demonstrate the alteration in protein structure of BSA NPs whereas the FT-Raman spectroscopy investigates changes in the secondary and tertiary structures of the protein. The conformational changes of BSA NPs are observed by change in fluorescence intensity and emission maximum wavelength of tryptophan residue by fluorescence spectroscopy. The field emission scanning electron and atomic force microscopy micrographs confirm the size and semi-spherical morphology of the BSA NPs. The effect of concentration and pH on particle size distribution is studied by particle size analyzer.


## 1. Introduction

Proteins have been extensively studied because of their relevance not only to protein engineering but also to several biological processes. The proteins adopt well defined structures in aqueous solutions, are in constant motion between open and close states and display conformational heterogeneity [1]. Noteworthy, protein domains in dynamic state with movements including the hinge bending and the shear motions within a molecular structure, play a significant role in the physiological function of molecule [2]. Protein nanoparticles (NPs) particularly Bovine Serum Albumin (BSA) nanoparticles have attracted substantial interest in the various fields of biomedical application specifically in targeted drug delivery systems [3,4]. In that concern, investigation of the structure of protein in detail at nanoscale synthesis is a challenging task. The protein conformations determined by the spectroscopic techniques are increasingly applied to investigations of conformational transitions in proteins and provide information about the three-dimensional structure of a macromolecule. It has been reported that the alcohol-induced changes observed at the secondary structural level reduces the retinol binding capacity of β-lactoglobulin [5]. Consequently, it is significant to investigate the conformational changes of BSA NPs.

The desolvation process is a promising method for the synthesis of BSA NPs [6,7] as it is a room temperature reaction and is beneficial for heat-sensitive bioactive compounds [8]. It does not require organic solvents to remove the oily residues and surfactants. This process also offers the advantage of producing NPs directly in aqueous suspension [9]. The coacervating agent used for the preparation of BSA NPs progressively changes the tertiary structure of protein in order to form submicronic aggregates of desolvated albumin [10].

BSA is a water-soluble globular protein that has the tendency to aggregate in macromolecular assemblies [11]. The three-dimensional structure is composed of three domains; each one is formed with six helices and its secondary structure is essentially α-helix. BSA NPs garnered interest recently as carriers to encapsulate hydrophobic drugs and have been extensively studied as nanoscale medicine delivery systems. The NPs prepared with BSA are biodegradable, nontoxic, metabolized in vivo to produce innocuous degradation products [12,13], non-immunogenic, easy to purify and soluble in water [14,15].

In the present work, physical characterization methods viz. Dynamic Light Scattering (DLS) and zeta potential have been

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employed to measure the particle size and to determine the surface charge respectively with regard to change in protein concentration and pH. Spectroscopic methods such as UV-visible, Fourier Transform Infrared (FT-IR), Fourier Transform Raman (FT-Raman) and fluorescence provide an orthogonal comparison with physical characterization approach. The change in optical signals corresponding specifically to BSA in comparison with BSA NPs provides the valuable information concerning protein conformational changes. The structural morphology of BSA NPs was observed by field emission scanning electron (FE-SEM) and atomic force microscopy (AFM). Earlier reports on BSA NPs are focused on fabrication of NPs for drug entrapment, size distribution and morphological studies [3,4,6,7,14]. However, to the best of our knowledge the approach by employment of physical and spectroscopic techniques to investigate the molecular characteristics of BSA NPs has not been reported yet. The BSA NPs have a strong bearing that change in conformational transitions may affect effective binding of drugs and bioactive compounds to be used as an efficient rational drug carrier.

## 2. Experimental

### 2.1. Reagent and chemicals

BSA (65,000 kDa), ethanol (95%) and glutaraldehyde (35%) were procured from HI-MEDIA, India and used without further purification. All other chemicals were of analytical grade and used as received. All aqueous solutions were prepared in double distilled deionized water.

### 2.2. Preparation of BSA NPs

BSA NPs were prepared by the well-known desolvation technique with some modifications as described previously [6,7]. Briefly, definite amounts of 25, 50 and 100 mg BSA powder were added in 1.0 mL of 10 mM NaCl solution, adjusted to pH 7.0 with 0.1 N NaOH. For the synthesis of BSA NPs continuous drop wise addition of 5.0 mL ethanol as a desolvating agent under stirring (500 rpm) at room temperature was carried out until the solution becomes ivory white (turbid). To stabilize NPs the turbid solution was stirred continuously for 30 min. After the desolvation process, 8% glutaraldehyde (11.75, 23.5 and 47 μL/mL of 10 mM NaCl solution for 25, 50 and 100 mg BSA powder respectively) was added to generate particle cross-linking for 24 h.

### 2.3. Purification of BSA NPs

The resulting NPs were purified in order to remove the free glutaraldehyde by five cycles of differential centrifugation (20,000 rpm, 8 min) and redispersion of the pellet to the original volume 10 mM NaCl at pH 7.0. Each redispersion step was performed in ultra sonication bath over 10 min [16].

### 2.4. Characterization techniques

The UV spectra of BSA NPs and native BSA are measured in the wavelength range of 250–400 nm at room temperature with UV spectrophotometer (UV-3600 UV-Visible-NIR Spectrophotometer, SHIMADZU). The molecular structure of BSA NPs was determined by FT-IR spectroscopy and was scanned at range (500–4000 $cm^{-1}$) with Alpha ATR Bruker (Eco Model). The samples for FT-IR analysis were prepared by grinding 98.99% KBr with 1–2% nanoparticles and pressing the mix to form a tablet. The FT-Raman spectra of the films were recorded in the spectral range of 39–3600 $cm^{-1}$ using Fourier Transform Raman spectrometer (Bruker Multi-RAM, Germany Make) Nd:YAG laser source with excitation wavelength of 1064 nm and resolution 4 $cm^{-1}$ at 336 mW laser power. The fluorescence spectra were measured after excitation at 280 nm, scanned at an emission wavelength range between 300–500 nm using a fluorescence spectrophotometer (Spectrofluorimeter JASCO JAPAN Model No. FP 8300) at room temperature; using a quartz cuvette. The analysis was carried out at an excitation and emission slit wavelength of 3 and 5 nm, respectively. The FE-SEM was performed using Carl Zeiss scanning electron microscope. 10–20 μL of the BSA NPs solution was freeze dried on a polished aluminium surface. After drying the samples were sputtered with gold. AFM was performed using Bruker AXS Analytical Instruments Pvt. Ltd., Singapore. For AFM sample, 10–20 μL of the BSA NPs solution were spread on a glass slide surface and were allowed to dry at 37 °C. The size distribution of BSA NPs was determined by a particle size analyzer (Particle Sizing Systems, Inc., Santa Barbara, CA, USA) with an autotitrator. For measurement, 5 mL of BSA NPs dispersed in absolute ethanol was collected; the dispersions were sonicated for 10 min and the particle size was measured at 25 °C with scattering angle 90°. The measurements were performed in triplicate. For zeta potential measurements the samples of BSA NPs were dispersed in 5 mL 10 mM NaCl and the pH was adjusted by fully automated systems. For zeta potential data the samples were scanned continuously at different pHs from 2.0 to 11.0. The pH was adjusted with 0.1 M HCl and 0.1 M NaOH. The dispersions were sonicated for 10 min at 25 °C. The measurements were performed in triplicates.

## 3. Results and discussion

### 3.1. Optimization for preparation and purification of BSA NPs

The morphologically formed BSA NPs require to be sufficiently stabilized after dispersion in water. In order to stabilize BSA NPs, glutaraldehyde was added as a cross-linker which gives better stability and shape to the fabricated NPs. The amino moieties in lysine (Lys) residues and guanidine side chains in arginine (Arg) of albumin lead to solidification through condensation reaction with the aldehyde-group of glutaraldehyde [17]. The free groups of glutaraldehyde are involved in the condensation reaction; hence, the toxicity is diminished. The final concentration of glutaraldehyde used in this experiment was very low. Moreover, the unlinked glutaraldehyde has been removed during the repeated purification process reducing the toxicity. The use of glutaraldehyde as a cross linker is widely reported for biomedical applications [7,18–24]. The cytotoxicity study of BSA NPs synthesized with organic solvents and glutaraldehyde indicates that NPs are non-toxic [16,25].

### 3.2. Secondary structure of BSA formulated as NPs

#### 3.2.1. Absorption spectroscopy of BSA NPs

The UV–visible spectra of BSA NPs are significantly different compared to spectra of the corresponding BSA native (Fig. 1). The absorption spectrum of native BSA and BSA NPs exhibited characteristic band centred at 280 nm with no peak shifting in the wavelength. More interestingly, the peak broadening was observed in BSA NPs as compared to BSA native. The full with half maxima value for native BSA was 33.02 nm (curve *a*) and for BSA NPs the peak broadened to 52.82 nm, 65.98 nm and 66.42 nm for 25, 50 and 100 mg/mL concentration respectively (curves *b*–*d*). These data clearly indicate that there are some conformational transitions which are obtained due to NPs formation and may also be attributed due to the different conformations of BSA NPs [26,27]. It was remarkable that as the concentration of BSA increases the peak broadening also increases which may be due to the decrease in size of BSA NPs.

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