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SYNTHESIS AND CHARACTERIZATION OF BOVINE SERUM ALBUMIN NANOPARTICLES AS A DRUG DELIVERY VEHICLE

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ABSTRACT

Nanoparticles prepared from Bovine serum albumin (BSA) are versatile carrier systems for drug delivery and can be prepared by an established desolvation process, under the aspect of the controllable particle size range between 100 to 300 nm with narrow size distribution. Various components were evaluated influencing the size of BSA nanoparticles (BSA NPs) viz pH, salt content and the conditions for purification. Washing the particles by subsequent cycles of differential centrifugation significantly led to decrease in the poly disparity index (PDI). FTIR was used to determine the purity of the prepared BSA NPs. Atomic Force Microscopy (AFM) and Field emission scanning electron microscopy (FE-SEM) displays the size and spherical shape of nanoparticles. Zeta potential with varying pH unveils the surface charges of BSA NPs. The size controlled BSA nanoparticles can be used as the standard material for drug loading capacity for bioactive molecules.

KEY WORDS: **Bovine serum albumin Nanoparticles, Desolvation Technique, Particle size, Drug delivery, FTIR.**

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1.INTRODUCTION

Nanotechnology refers to the research and technological developments at atomic, molecular and macromolecular scales [1]. Proteins represent good raw materials since they have the advantages of synthetic polymers together with the absorbability and low toxicity of the degradation end products [2, 3]. The protein nanotechnology holds a great promising material for the improvement in drug delivery systems, labeling agents and biosensors [4-6]. Synthetic or natural polymers viz proteins, polysaccharide, liposome and virus like particles can be used for the preparation of nanoparticles [7]. Protein nanoparticles are an attractive biomolecule which have the unique characteristic properties including biodegradability, nontoxicity, metabolized in vivo to produce innocuous degradation products, water solubility, simplicity in purification and non-immunogenic allowing ease of delivery by injection and thus an ideal candidate for nanoparticles preparation [8, 9].The classical techniques like desolvation, emulsification, thermal gelation as well as recent technique such as nanospary drying, nab-technology and self-assembly were used for the preparation of albumin nanoparticles. Among all other techniques, desolvation process for synthesizing BSA NPs has several advantages; it does not require an increase in temperature and therefore may be useful when heat-sensitive bioactive compounds are used [10]. And most importantly, unlike emulsification, it does not require organic solvents to remove the oily residues and surfactants which are required for the emulsion stabilization. This process also offers the advantage of producing nanoparticles directly in aqueous suspension [11], but the size of the nanoparticles prepared by this method may be affected by several preparation conditions, viz initial protein concentration, temperature, pH, salt content, glutaraldehyde concentration, agitation speed, organic solvent (ethanol) addition rate and the storage conditions. In addition to the transformation at the secondary structural level, the alcohol-induced changes in structure have also been shown to reduce the retinol binding capacity of β-lactoglobulin [12]. This is of particular significance for investigations on the encapsulation of bioactive material in protein NPs. So, in this context the technique Fourier transform infrared spectroscopy has been used to investigate the valuable information concerning the purification of BSA NPs. The factors that influence the protein hydrodynamic size such as the pH was determined. The suppression and expression of hydrophobic interactions provides a way to control the size of polymeric particles during desolvation [13, 14]. Hence, a balance between attractive and repulsive forces is necessary for fabricating nanoparticles of the appropriate size.The aim of the present work is to synthesize BSA NPs in the unique range which is suitable for the drug delivery systems by desolvation method. These nanoparticles were characterized by Fourier transformed infrared, Dynamic Light Scattering (DLS) and Zeta potential to investigate the physiochemical properties like size and charge at different pH for the prepared BSA NPs. The structural morphology of BSA NPs was observed by FE-SEM and AFM showing spherical smooth surface. The particle size analyzer showed 100-300 nm BSA NPs particle size with narrow PDI. Zeta potential showed different surface charges of BSA NPs with varying pH. This intended study will help to establish the controlled production of BSA NPs as a potential drug carrier.

2. MATERIALS AND METHODS

2.1. Reagent and Chemicals

BSA (65,000 kDa), ethanol (95 %) and glutaraldehyde (35 %) were procured from HI-MEDIA, India and were used without further purification. The analytical grade chemicals were 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 desolvation technique with some modifications [15]. A definite amount of 100 mg BSA powder was added in 1.0 mL of 10 mM NaCl solution

respectively, adjusted to pH 7.0 with 0.1 N NaOH. These samples were transformed into NPs by the continuous drop wise addition of 5.0 mL of ethanol as a desolvating agent under stirring (500 rpm) at room temperature, till the solution becomes ivory white (turbid). To stabilize nanoparticles the turbid solution was stirred continuously for 30 minutes without further addition of ethanol. After the desolvation process, 8 % glutaraldehyde was added to generate particle cross linking for 24 h.

2.3. Purification of BSA NPs

The resulting nanoparticles were purified by five cycles of differential centrifugation (20,000 rpm, 8 minutes) and then the pellet was redispersed to the original volume of 10 mM NaCl (pH 7.0). Each redispersion step was performed in the ultra sonication bath over 10 minutes.

2.4. Dynamic Light Scattering

The size distribution of BSA NPs was determined by a Particle size analyzer (Particle Sizing Systems, Inc. Santa Barbara, Calif., and USA) with an autotitrator. For determination of particle size, 5 mL of BSA NPs were dispersed in PBS and data was collected. The dispersions were sonicated for 10 minutes the particle size was measured at 25 ˚C with scattering angle 90˚. The measurements were performed in triplicate.

2.5. Zeta Potential

For zeta potential measurements the samples of BSA NPs were dispersed in 5 mL 10 mM NaCl and the pH was adjusted with the fully automated systems. In order to determine zeta potential, the samples were scanned continuously at different pH 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 minutes at 25 ˚C. The measurements were performed in triplicates.

2.6. FTIR spectroscopy

The purity of fabricated BSA NPs was determined by FTIR spectroscopy and was scanned at the range (500-4000 cm^{-1}) with Alpha ATR Bruker (Eco Model). The samples for FTIR analysis were prepared by grinding 98.99 % KBr with 1-2 % nanoparticles and pressing the mix to form a tablet.

2.7. Field emission scanning electron microscopy (FE-SEM)

FE-SEM was performed using Carl Zeiss scanning electron microscope .Then 10-20 µL of the BSA NPs, were freeze dried on a polished aluminum surface. After drying the samples were sputtered with gold, and then these prepared films were scanned for imaging.

2.8. Atomic Force Microscopy (AFM)

AFM was performed using Bruker AXS Analytical Instruments Pvt. Ltd. Singapore. The 10-20 µL of the BSA NPs solution was spread on a glass slide surface and were allowed to dry at 37 ˚C, and then these prepared films were scanned for imaging.

3.RESULTS AND DISCUSSION

3.1. Particle size distribution

The BSA NPs were characterized for investigating the hydrodynamic diameter. Protein is highly sensitive to pH, any alteration in pH will directly affect the size of the BSA NPs. The isoelectric point (p*I*) of BSA is about 4.9 [16]. It was noted that at this p*I* large aggregates are formed via non-specific interactions viz. electrostatic repulsions which are mainly of hydrophobic in nature. When the pH was decreased large sized particles 262 nm and 214 nm (PDI 0.130 and 0.241) were held due to the decreased net charge. At pH 7.0 the particle size was slightly increased, i.e. 242 nm is having polydispersity index 0.078. Below the basic conditions, such as pH 9.0 and pH 11.0 electrostatic interaction increases, but hydrophobic interaction decreases, thus the particle size decreases to 185 nm and 179 nm respectively. It was observed that highly monodispersed particles were obtained which is an important criteria for drug delivery application

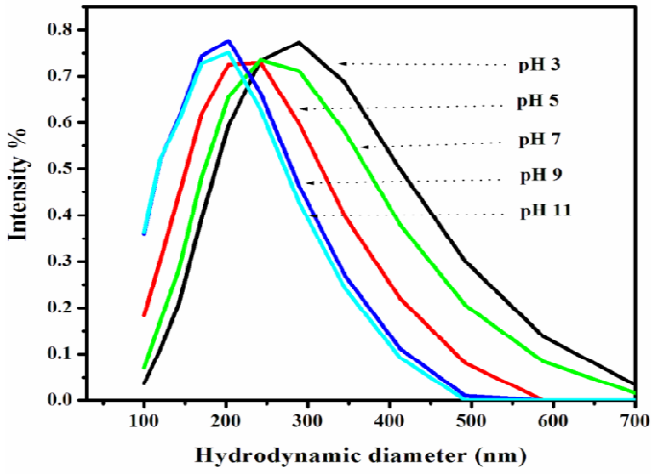

Figure 1
pH dependent hydrodynamic diameter of BSA NPS suspended in PBS

3.2. Zeta Potential of BSA NPs

The zeta potential study of BSA NPs with respect to pH i.e. from pH 2.0 to 11.0 is presented in Table No 1. Zeta potential critically influences the interaction of NP with the environment [17]. The stability and electrical behaviour of the fabricated BSA NPs were evaluated at different pH. These measurements provide the information regarding the surface charge of the colloidal system and indicate comparable surface properties of the BSA NPs at different pH. The isoelectric point (p*I*) of BSA is about 4.9. A particle aggregation was observed at higher pH and exhibited pronounced surface charge. It was noticed that BSA NPs shows 9.67 mV at acidic pH, with increasing pH value the zeta potential of the nanoparticles was reduced to -13.67 mV at pH 11.0. At higher pH, that is towards basic the value of Zeta potential changes from positive to negative [18].These results suggest that BSA NPs are having good stability when suspended in media.

Table No I
The pH-dependent zeta-potentials of BSA NPs.

pH	Zeta Potential (ζ mV)
2	9.67
3	10.08
4	-9.65
5	-14.27
6	-17.04
7	-9.87
8	-12.83
9	-9.65
10	-7.71
11	-13.67

3.3. Fourier-transform infrared (FTIR) spectroscopy.

The molecular characteristics of the resulting BSA NPs were characterized by FTIR. As shown in Figure 3. The FTIR peaks of pure BSA at 3385 cm^{-1} , 3113 cm^{-1}, 1707 cm^{-1}, 1533 cm^{-1} and 1242 cm^{-1} are assigned to the stretching vibration of OH, amide A (mainly NH stretching vibration), amide I (mainly C=O stretching vibrations), amide II (the coupling out phase of bending vibration of N-H and stretching vibration of C-N bands) and amide III (is in the phase combination of N-H in plane bending and C-N stretching) [19]. It was noticed that there is presence of all peaks in the fabricated BSANPs, and no other impurity peak was observed after purification. These results indicate that BSA NPs were purified successfully.

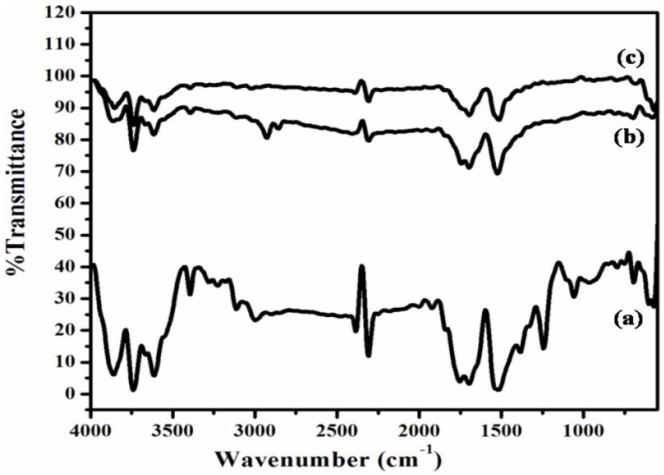

Figure 2
FTIR spectra for (a) BSA NPs without purification, (b) BSA NPs with purification, (c) Native BSA, at pH 7.0.

3.4. Surface properties

3.4.1. Field Emission Scanning Electron Microscopy.

The FE-SEM image depicts the shape and the surface characteristics of the fabricated particles. The BSA NPs were almost having a smooth surface. It was clear that most of the BSA NPs were semispheres with diameter 100 to 200 nm. The obtained nanoparticles have smaller size as compared to the other reported method [20].

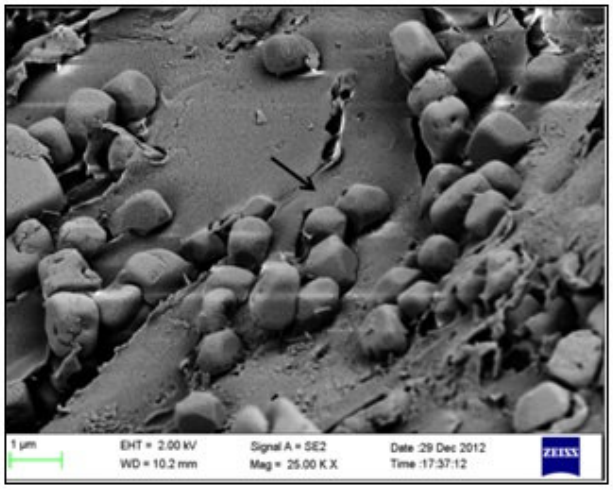

Figure 3
FE-SEM image of prepared BSA NPS. The scale bar represent 1 µm.

3.4.2. Atomic Force Microscopy.

AFM image proves the expectation that BSA NPs are spherical with a smooth surface. The average diameter of the nanoparticles is 107.9 nm. The particles obtained from AFM are much smaller than size obtained from DLS. DLS provides the data of the particle swollen in solution, while AFM shows the image of the BSA NPs spread and dried on the glass slide surface. It was observed that the height of BSA NPs was much smaller than that of diameter which suggests that the surface of nanoparticles are soft and collapsed on the glass slide. The three dimensional image of BSA NPs is shown in Figure 4. This characteristic proves that BSA NPs are good candidate for drug loading applications.

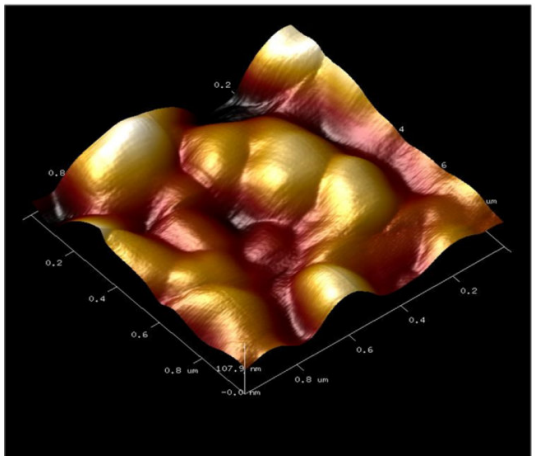

Figure 4
AFM image of BSA NPs. The smooth surface can be observed.

CONCLUSION

Nanocarriers for drug delivery can be prepared by ethanolic desolvation technique. The BSA NPs were successfully fabricated by exploiting robust process of desolvation by an adjustment of pH value in the presence of NaCl. This finding provides the basic information about the particle size after the formulation of BSA NPs. The synthezised nanoparticles were characterized quantitatively by applying orthogonal and physical approaches viz. DLS, AFM, FE-SEM and Fourier transformed infrared spectroscopy. The FE-SEM and AFM indicated that BSA NPs are semispherical with a particle size distribution from 100 nm to 200 nm. The particle size analysis monitored the particle size in the range 100 to 300 nm diameter having a low polydispersity index.The presented work serves as a template for the application of commonly used methods, such as DLS, FTIR, Zeta potential. This work provides a simple method which will be helpful in order to fabricate BSA NPs for loading and delivery of bioactive compounds like peptides, hormones as a rational colloidal carrier.

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