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Fabrication of Bovine Serum Albumin Nanoparticles Self-assembled Coacervation Method for Drug Delivery Systems

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Abstract: Bovine Serum Albumin (BSA) nanoparticles have been produced in the particle size between 112 to 468 (nm). Nanoparticles were purified with 48,800 g centrifuge following by dialysis, micro and ultra filtration. Photon Correlation Spectroscopy (PCS) and Scanning Electron Microscopy (SEM) were used to characterize the nanoparticles. Photographs obtained from SEM image shown nanoparticles have semi-spherical shape. However the influence of several parameters such as the rate of addition desolvating agent,the concentration of BSA, the gluttaraldehyde concentration, agitation speed and BSA/Ethanol volumetric ratio have been investigated while the temperature kept in 14°C.The mean nanoparticle size diameter has not been relatively changed by variation of solvent adding rate and cross linker concentration and in contrast mainly depended on the volumetric ratio of BSA/ Ethanol, BSA concentration and agitation speed.

Key words: Simple Coacervation method · Nanoparticles · Bovine serum albumin · Drug delivery · SEM

INTRODUCTION

Nanoparticles were first developed around 1970 [1]. They were initially devised as carrier for vaccines and anticancer [2]. Over the past few decades, there has been considerable interest in developing biodegradable nanoparticles as effective drug delivery device [3]. Nanoparticles have become an important area of research in the field of drug delivery [4].The major advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the enhancement of the cellular uptake of a number of substances and their ability to deliver a wide range of drugs to varying areas of the body for sustained periods of time [4, 5]. Nanoparticles generally vary in size from 10 to 1000 nm [3]. The drug is dissolved, entrapped, encapsulated or attached to a Nanoparticles matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed [3, 4]. Figure 1 shows schematic diagram of nanocapsulated and nanosphere particles loaded with drug.

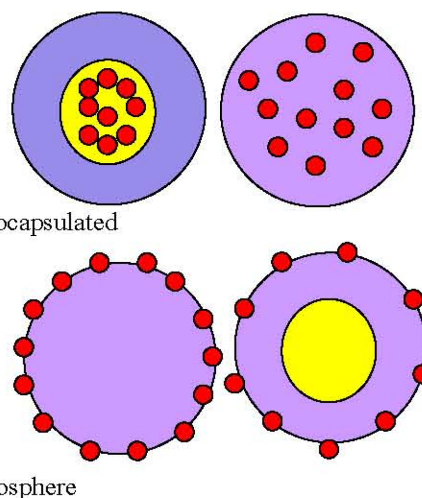

Fig. 1: Schematic diagram of encapsulated and coated nanoparticle in drug delivery system

Among these colloidal system those based on protein may be vary promising. since they are biodegradable and non-antigenic relatively easy to prepared and their size distribution can be monitored easily [6]. A wide variety of drugs can be delivered using nanoparticulate carriers via a number of routes.

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Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. [7]

Another promising class of nano-sized vehicles that have been considered in drug delivery application is liposomes [3-5].These vesicles prepared from lipids have been used as potential drugs carrier because of the protection they can offer drugs contained in their core. However liposomes have shown a low encapsulation efficiency, poor storage stability and rapid leakage of water-soluble drugs in the blood [3, 4]

The bio distribution of intravenously injected carriers is mainly influenced by their physicochemical properties, such as particle size and surface characteristics [6]. Larger particles are more rapidly removed by the liver and spleen than smaller particles. Reducing the particle size of colloidal carriers below a threshold of 100 to 200 nm introduces the possibility of escaping the vascular system via fenestrations or cavities in the lining of blood vessels [7].

Albumin nanoparticels can be obtained by different methods[8]. Albumin nanoparticles have been extensively studied as suitable for drug delivery since they are biodegradable, non-toxic and non antigenic. Because of their defined primary structure and high content of charged amino acids (i.e. lysine).The albumin-based nanoparticles could allow the electrostatic adsorption of positively or negatively charged molecules without the requirements of other compounds. In addition, protein nanoparticles can be easily prepared under soft condition, by coacervation or controlled desolvation processes [9, 10].

Among the available potential colloidal drug carrier systems covering the size range described, protein-based nanoparticles play an important role [3, 6]. Simple and complex coacervation, spry drying and emulsification represent three major techniques for fabrication protein nanoparticles [11].

The disadvantage of the emulsion method for particle preparation is the need for applying organic solvent, for the removal both of the oily residues of the Preparation process and surfactants required for emulsion stabilization [6] and it is difficult to obtain protein nanoparticle less than 500 nm by emulsification cross linking method [3]. The size and surface charge of the nanoparticles are crucial for their uptake [4].

Coacervation is the separation into two liquid phases in colloidal systems [12]. The phase more concentrated in colloidal component is the coacervate and the other phase is the equilibrium solution associative phase separation of two polymers in water occurs if there is an electro static attraction [12,13].

Concerning simple coacervation method, the protein precipitation around oil droplets is obtained by changing pH and temperature or by the “salting-out“technique [11].

As consequence, a major aspect in preparing a colloidal drug carrier system has to be establishing preparation conditions which control the resulting particle size and which lead to particles of a narrow size distribution, with special emphasis on size of 100-200 nm [6].

BSA nanoparticle can be obtained by different methods. In this study, method was based on simple coacervation and the details related to albumin nanoparticles production with the particle size between 112 and 468 (nm) were presented by affection some parameters on the size. For characterizing the particles by the size and shape, Photon Correlation Spectroscopy (PCS) and Scanning Electron Microscopy (SEM) were utilized.

MATERIALS AND METHODS

Materials: BSA (fraction V, purity 98%), Tween-20, ethanolamine and glutaraldehyde, 25% solution, were commercially supplied by Sigma Aldrich. Analytical grade and high purity of sodium azide and all other chemicals were supplied from Merck (Germany).

Fabrication of BSA Noanoparticles: Simple coacervation method was implemented for preparation of BSA nanoparticles [14]. Anhydrous ethyl alcohol was added to 150 ml BSA (5 mg/l in 10 mM Tris/HCl contained 0.02% sodium azide, pH 7.5) till the solution became turbid then 150 μl of 25% glutaraldehyde was added for cross linking. The reaction was continued at temperature 14°C. Ethanolamine was added to block the non-reacted aldehyde functional group. Also Tween-20 was added at a final concentration of 0.01% v/v to stabilize the preparation. Large aggregates were eliminated by centrifuge (4°C, 48800 g and 30 min). The supernatant was dialyzed and subsequently micro and ultrafiltrated through a 0.2 μm acetate membrane and polyvinylchloride copolymer membrane with cut off 300 kDa, respectively. The concentration of BSA was determined with Coomassic Blue reagent [13]. The size distribution and shape of BSA nanoparticles were determined by Photon Correlation Spectroscopy (PCS) and Scanning Electronic Microscope (SEM).

Determination of Nanoparticle Size and Distribution: The size distribution of the prepared BSA nanoparticle was analysed by photon correlation spectroscopy (PCS). PCS is industrially preferred method of sub-micron particle size analysis. The sample analyzed in the PCS device should consist of well dispersed particles in liquid medium. In such conditions the particles are in constant random motion, referred to as Brownian motion and PCS measures the speed of this motion by passing a laser. PCS determines the average particle size and Polydispersity Index (PI) which is a range of measurement of the particle sizes within measured samples. The accurate measurement of particle size must be blow 0.7 (70%).

Scanning Electronic Microscopic (SEM): For electronic microscopic scanning micrographs, samples were taken from nanoparticles experimentally obtained. The samples were dipped into liquid nitrogen for 10 minutes, then freeze dried for 7 hours in the Freeze Drier, EMITECH; model IK750, Cambridge, UK. The sample was fixed on the aluminium stub and coated with gold palladium by Polaron machine model SD515, EMITECH, Cambridge, UK, at 20 nm coating thickness. Finally the sample was examined under SEM using Stereoscan model S360 brand SEM-Leica Cambridge, Cambridge, UK.

Determination of Particle Purity: Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) was formed to analyse and compare the protein composition profiles of specific samples. The pore size of poly acrylamide is limited; therefore proteins higher than 800 kDa can not permeate into the gel [16]. An acrylamide concentration of 10 % has been selected to enable optimal resolution for the soluble proteins [17]. This property can be used to separate nanoparticles from monomeric proteins. Since protein monomers are separated in the gel, the nanoparticle stay on the stacking gel. The monomers are collected on top of the resolution gel (based on monomer size).

RESULTS AND DISCUSSION

Physical Characterization of Nanoparticles: A range of protein (BSA) nanoparticle having broadly similar particles size and anionic characters to other nanoparticle such as adenovirus and plasmid DNA were fabricated based on simple coacervation. Average particle size in base of size distribution was calculated by Photon Correlation spectroscopy (PCS).

In addition, the morphology of the prepared Nanoparticles from BSA were examined with scanning electronic microscope. One of the samples (condition: T=14°C, pH=7.5, C = 5 mg/ml of BSA and 400 rpm agitation) was analyzed by Scanning Electron Microscopy (SEM) is presented in Figure 2.Figure 2 show the particle size with magnification of 30000. The shape of the nanoparticles demonstrated in SEM is spherical. The diameter of fabricated nanoparticle was determined by SEM is 65 nm (shown in Figure 2).

The Effect of D ifferent Parameters on Nanoparticle Size: The impact of pH, BSA concentration, molar ratio of BSA to ethanol, agitation speed and the rate of ethanol addition on the nanoparticle size was studied.

Figure 3 illustrated that the rate of addition ethanol during the simple coacervation procedure was not an important parameter. The result of PCS showed the average diameter was around 191 nm. Similar result with HAS nanoparticles have been reported before [5].

The effect of protein (BSA) concentration on nanoparticle size was conducted and shown in Figure 4. As the concentration of BSA increased the particle size was decreased. The particle size of 194, 176, 136,112.6,114,116 and 115 nm were fabricated with BSA concentration of 5, 10, 15, 20, 25, 30 and 40 mg/ml, respectively. Beyond a certain BSA concentration (20mg/ml), the particle size was unaffected with concentration of BSA.

The different of glutaraldehyde concentration used for cross-linking appeared to have no significant effect on particle size (Figure 5). Also, Langer and his team work [5] reported that concentration of glutraldehyde was not important parameter for fabricating of Human Serum Albumin (HSA) nanoparticles either.

Figure (6) showed BSA nanoparticles not regular changed with the change of pH since concentration of BSA is in the low (5 mg/ml). but the largest particle in this condition obtained around isoelectric pH (PI), around of PI, which the nanoparticles became unstable[5]and its diameter increased.

Figure (8) presented that agitation speed was one of the important parameter in particle size. The results illustrated that the size of particles would increase if the agitation increased. The particle size was unaffected with agitation speed of higher than 500 rpm. Minimum size obtained in 500 rpm was 121 nm.

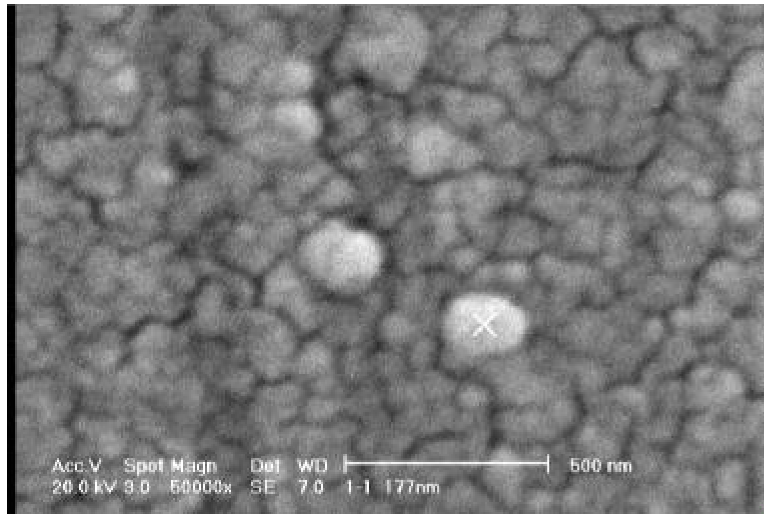

Fig. 2: Scanning electronic microscopy from BSA nanoparticles with magnification of 50000

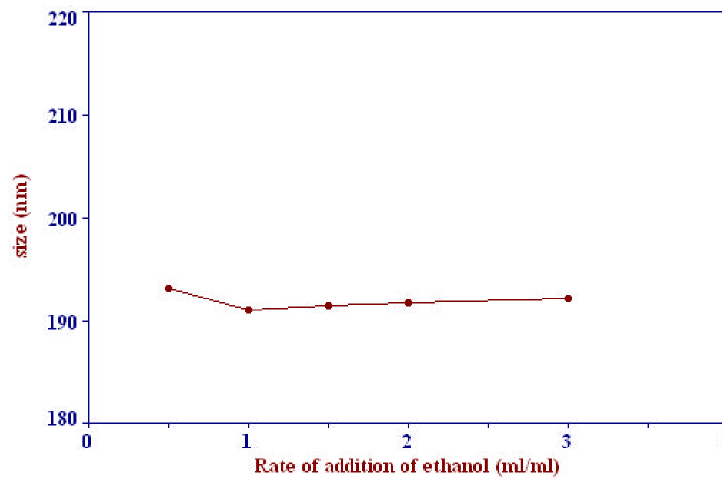

Fig. 3: Effect of ethanol addition rate on nanoparticle size

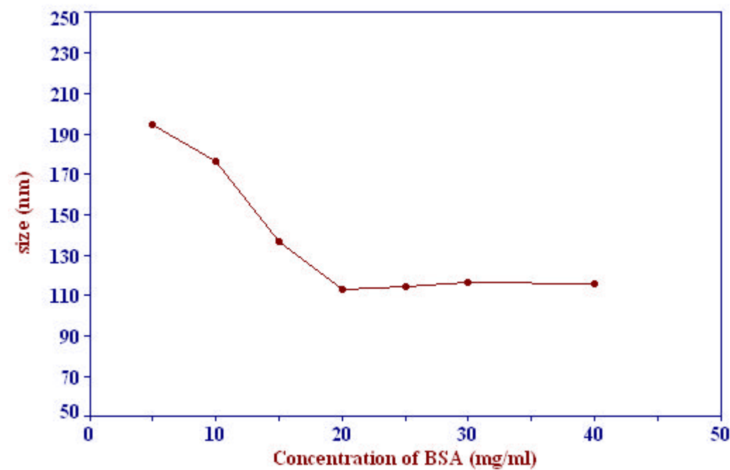

Fig. 4: Effect of initial BSA concentration on nanoparticle size

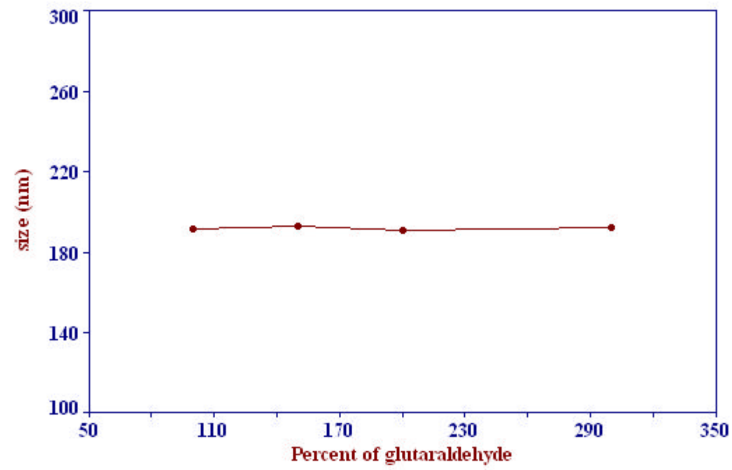

Fig. 5: Effect of glutaraldehyde concentration on nanoparticle size

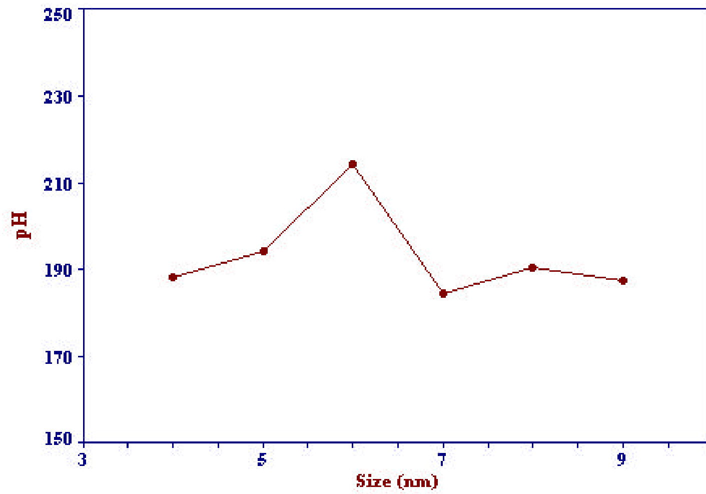

Fig. 6: Effect of pH of BSA on nanoparticle size

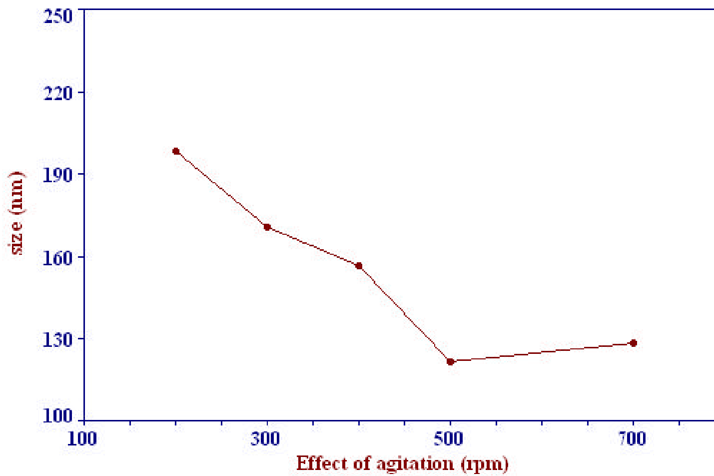

Fig. 7: Effect of agitation speed on nanoparticle size

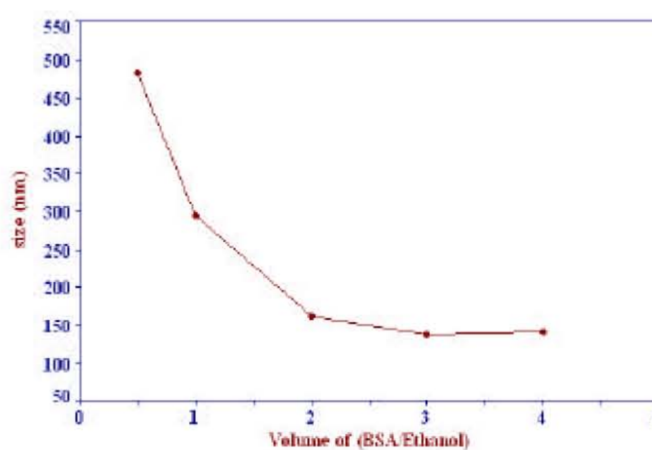

Fig. 8: Effect of volumetric ratio of BSA/ethanol on nanoparticle size

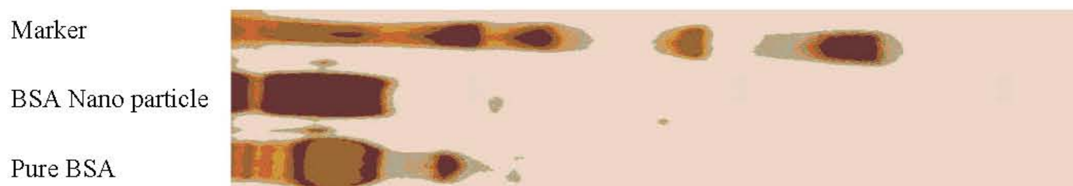

Fig. 9: Gel electrophoresis of the BSA nanoparticles

The effect of the ratio of BSA/ Ethanol was also investigated in this research (Figure 9), as the ratio increased the particle size was decreased. Weber and his coworker [4] reported that the volumetric ratio of HSA/Ethanol was very influential parameter for the fabrication of nanoparticles due to the fact that ethanol was able to dissolve HAS with high solvency power. In fact the obtained data was designated to 5 mg/ml of BSA, for any other BSA concentration, the volumetric ratio was depends on new BSA concentration.

Nanoparticle Purity: The gel electrophoresis SDS-PAGE was formed to analyze and compare the purity of the prepared nanoparticles with pure BSA. Figure 9 represents the gel electrophoresis of pure BSA and the nanoparticles prepared from BSA.. The protein bands are shown the nanoparticles were pure and the strips clearly demonstrate highly purity of the product.

CONCLUSION

The biological nanoparticle (i.e BSA nanoparticle herein) as it has been assembled here, not only mimics the size and surface chemistry of nanoparticles such as viruses and plasmid, but also can be used as drug delivery vehicles in its own right.The present study shows that BSA nanoparticles can be prepare with predictable and reproducible size, in ize between 112.6 and 480.3 nm, by simple coacervation method. In the 14°C minimum size of particle prepared was 112.6 nm at the 20 mg/ml concentration of BSA.SEM analyse indicated nanoparticles have spherical shape. Optimization of this fabrication method for protein nanoparticles as drug delivery systems will be the subject of next publication.

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