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Full Length Research Paper

Production of biological nanoparticles from bovine serum albumin for drug delivery

Rahimnejad M., M. Jahanshahi*, G. D. Najafpour

Faculty of Chemical Engineering, Noshirvani Institute of Technology Mazandaran University, Babol, Iran

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Bovine serum albumin (BSA) was used for generation of nanoparticles in a drug delivery system. The size of the fabricated nano-particles was measure by laser light scanning. Several process parameters were examined to achieve a suitable size of nanoparticle such as pH, temperature, BSA concentration, agitation speed, glutaraldehyde concentration, organic solvent adding rate and the ratio of BSA/organic solvent. The smallest size of nanoparticles achieved, was 101 nm and the largest size was 503 nm. The most effective parameters for the fabrication of the nanoparticles were the agitation speed and the media temperature. The minimum size of nanoparticles at the desired incubator of 4°C and constant agitation rate of 300 - 400 rpm was obtained. The impact of protein concentration and additional rate of organic solvent (i.e. ethanol) upon the particle size was investigated. The protein concentration of 5-40 mg.ml^{-1} was resulted; the main effect on the particle size and minimum mean size diameter gained was 30 mg.ml^{-1} protein concentration. The nanoparticle sample was purified with 50,000 g centrifuge then followed by dialysis, micro and ultrafiltration and then analyzed by SEM, PCS as well as SDS gel electrophoresis.

Key words: Biological nanoparticles, bovine serum albumin, drug delivery, coacervation method, scaning electron microscopy.

INTRODUCTION

In the past two decades, great interests were developed for the fabrication of nanoparticles, as drug delivery vehicles (Zhang et al., 2000). The nanoparticles must be manufactured in advanced stages of purity, with material definition and sophisticated formulation to rival those demanded by the pharmaceutical macromolecules (Zhang et al., 2000; Jahanshahi et al., 2004). Nano-particle sizes are defined from 10 to 1000 nm. When the drug is encapsulated by the nanoparticles, the product are called nanocapsules, if the medicine is uniformly coated on the exterior of the nanoparticle then is called nanosphere. The carrier itself should be non-toxic and able to be degraded *in vivo* so that it does not accumulate indefinitely in tissues. BSA is a suitable carrier for drug therefore the nanoparticle generated from a protein base is easily adaptable to human body (Jahan-shahi et al., 2005).

Nanoparticles are colloids, generally composed of sophisticated biological structures. There are one or more types of proteins, lipids and/or nucleic acids (Langer et al., 2003). Colloidal drug delivery system such as microspheres, liposomes and emulsions are used as intravenously injected carriers for drug delivery to specific organs or targeted sites within human body (Davis and Illum, 1986). In colloidal system, the size of particle is very important in distribution of drug in human body. Generally, the large particle is easily removed by liver and spleen. The stability of small particle is higher than the large particle in drug delivery devices. Reducing the size of colloidal particle carriers in the range of 100 to 200 nm, enhance the stability of the carrier nanoparticles and creates the chance of escaping from the vascular system via cavities in the lining of blood vessel (Muller et al., 1996).

Liposomes are in nanoscale size range, self-assemble based on hydrophilic and hydrophobic properties and used for encapsulation of drug. Liposomes are quite pot-

*Corresponding author's E-mail: Mjahan@nit.ac.ir. Tel: +98-111-3234204. Fax: +98-111-2296583.

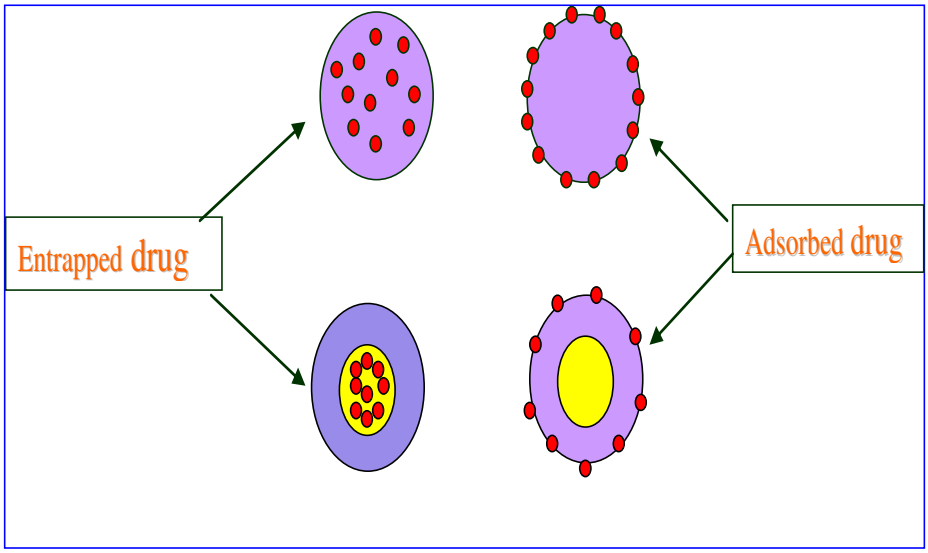

Figure 1. Schematic diagram of encapsulated and coated nanoparticle in drug delivery system.

ential drug carriers instead of conventional use of drug dosage since it may protect the drug from degradation; also reduce the toxicity of the drug and side effects (Muller et al., 1996; Soppimath et al., 2001). It was reported that liposome loaded with doxorubicin with particle size of 100 nm was commercially used as anticancer drug (Soppimath et al., 2001). However there are disadvantages in use of liposome which has low encapsulation efficiency, rapid leakage of water-soluble drug in presence of blood component and poor storage stability (Soppimath et al., 2001).

Synthetic protein nanostructure act as surrogate mimics such as viruses and plasmid for drug delivery system. The benefits of protein nanoparticles are: non-toxicity, stability for long duration, non-antigen also posse biodegradability (Muller et al., 1996; Weber et al., 2000). In fact protein is biopolymer, which is commonly used for preparation of nanostructured molecules for drug delivery (lazko et al., 2004). Figure 1 shows the schematic diagram of nanocapsulated and nanosphere particles loaded with drug. Simple and complex coacervation techniques for separation of proteins in two liquid phases in colloidal systems were used (Soppimath et al., 2001; Kruif et al., 2004; Mohanty and Bohidar, 2003). The phase with more concentrated in colloidal component is the coacervate and the other phase is the equilibrium solution. Association of two polymers in aqueous phase occurs with electrostatic attention (Arnedo et al., 2004).

The purpose of this research paper was to fabricate nanoparticles in the unique range which is suitable for the drug delivery system. The effected of manufacturing conditions such as pH, temperature, BSA concentration, agitation speed, organic solvent adding rate etc. upon the fabrication of such nanoparticle is strongly investigated.

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).

Preparation of BSA noanoparticles

Simple coacervation technique was implemented for preparation of BSA nanoparticles (lazko et al., 2004). 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 room temperature (24°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 (50,000 g, 30 min, 4°C). 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 determined with coomassie blue reagent (Bradford, 1976). The size distribution and shape of BSA nanoparticles were determined by scanning electronic microscope (SEM) (Zhang et al., 2000).

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

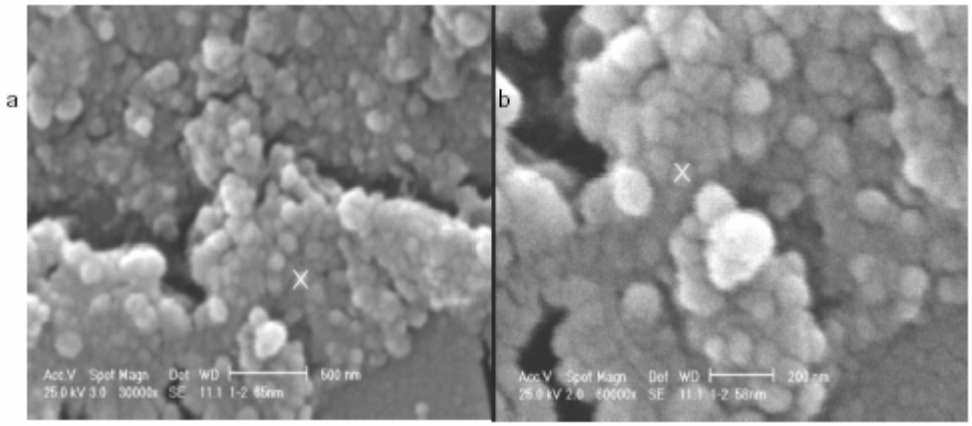

Figure 2. Scanning electronic microscopy of the outer surface of the BSA nanoparticles. **a:** Outer surface of the particle with magnification of 30000. **b:** Outer surface of the particle with magnification of 60000.

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 that were experimentally obtained. The samples were dipped into liquid nitrogen for 10 min, then freeze dried for 7 h 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 (Voet and Voet, 2004). An acrylamide concentration of 10% has been selected to enable optimal resolution for the soluble proteins (Samebrook et al., 1998). 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 particle sizes 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 PCS.

In addition, the morphology and size distribution of the prepared nanoparticles from BSA were examined with scanning electronic microscope. Figures 2a and b show the particle size with magnification of 30 000 and 60 000, respectively. The shape of the nanoparticles demonstrated in SEM is spherical. The sizes of fabricated nanoparticles were absolutely less than 100 nm (Figure 2).

Nanoparticle purity

The gel electrophoresis SDS-PAGE was formed to analyze and compare the purity of the prepared nanoparticles with pure BSA. Figure 3 represents the gel electrophoresis of pure BSA and the nanoparticles prepared from BSA. The experimented concentrations of pure BSA and the nanoparticles from BSA were 10, 20 and 30 µl. The protein bands are shown the nanoparticles were pure and the strips clearly demonstrate highly purity of the product.

The effect of different parameters on nanoparticle size

The impact of pH, temperature, BSA concentration, molar ratio of BSA to ethanol, agitation speed and the rate of ethanol addition on the nanoparticle size was studied. The effect of 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 204, 191, 163.6 and 145.7 nm were fabricated with BSA concentration of 5, 10, 20 and 30 mg/ml, respectively. Beyond a certain BSA concentration (30 mg/ml), the particle size was unaffected with concentration of BSA.

Figure 5 illustrates the particle size may not be influenced with the change of pH since the concentration of BSA is in the low range (5 mg/ml). The size of particles

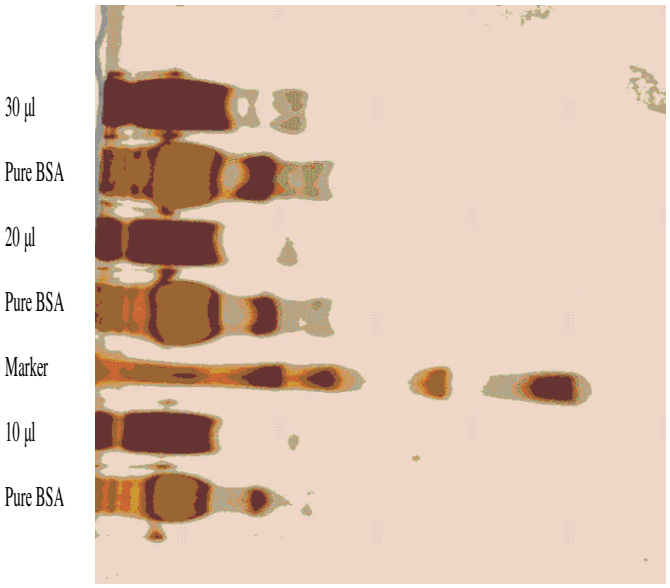

Figure 3. Gel electrophoresis of the BSA nanoparticles with 10, 20 and 30 µl. The different amount of samples (10, 20, 30 µl) were analyzed by gel electrophoresis (SDS-PAGE).All samples were prepared at general condition (pH=7.5, T=24°C, C=5 mg.ml^{-1},200 rpm)

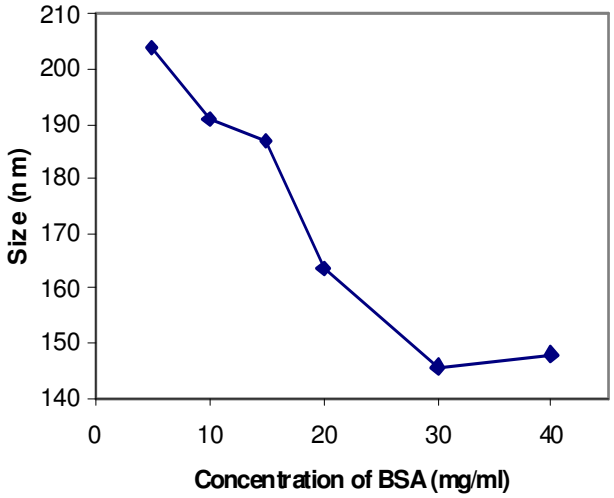

Figure 4. Effect of concentration of BSA on nanoparticle size. The influences of initial BSA concentration on BSA nanoparticle diameter have been investigated. The experiment were carried out at room temperature, by 200 rpm agitation speed, ethanol adding rate of 1.5 ml.ml^{-1} in the solution with pH=7.5. The diameters of fabricated nanoparticles subsequently were measured by PCS.

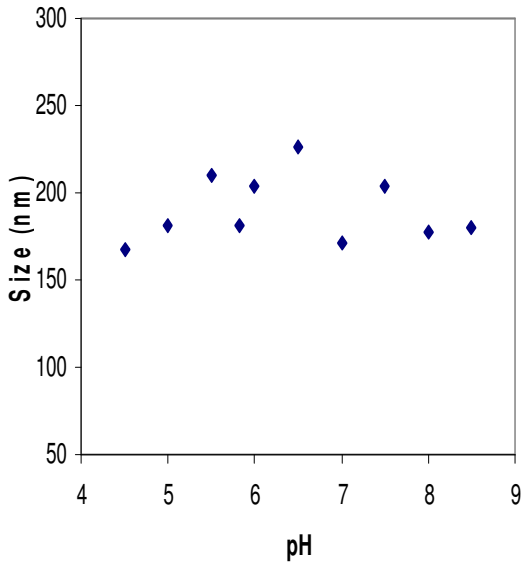

Figure 5. Effect of pH of BSA on nanoparticle size. Dependence of the pH value on the diameter of BSA nanoparticle prepared in pure water and 10 mM Tris-HCl solution, initial BSA concentration is 5 mg.ml^{-1}.

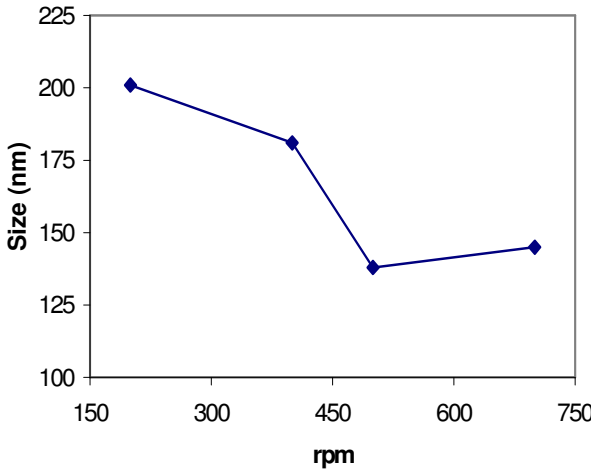

Figure 6. Effect of agitation rate on nanoparticle size. Relationship between agitation speed on nanoparticle size.

may slightly fluctuates therefore the particle size are defined in the range of 170 to 210 nm.

Figure 6 shows a minimum size of particle (138 nm) was obtained at agitation speed of 500 rpm. Generally, the size of particle is expected to reduce with increasing trend of agitation speed (Jahanshahi et al., 2003). The particle size was unaffected with agitation speed of higher than 500 rpm.

The effect of glutaraldehyde, which was used for cross linking of the nanoparticles, on size of particle was investigated. There was no influential concentration to have any impact on particle size (Figure 7). A similar result was declared on fabrication of protein nanoparticles using human serum albumin (HAS). Langer and his coworkers (2003) reported that glutaraldehyde concentrations had no influence on particle size.

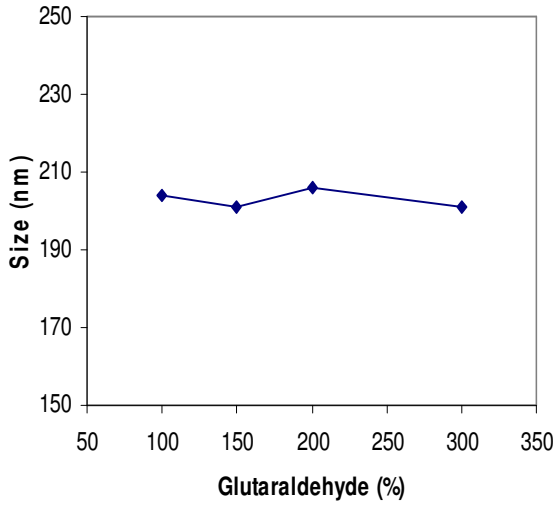

Figure 7. Effect of glutaraldehyde concentration on nanoparticle size. Influence of the cross linking process with different amount of glutaraldehyde on the diameter of BSA nanoparticle.

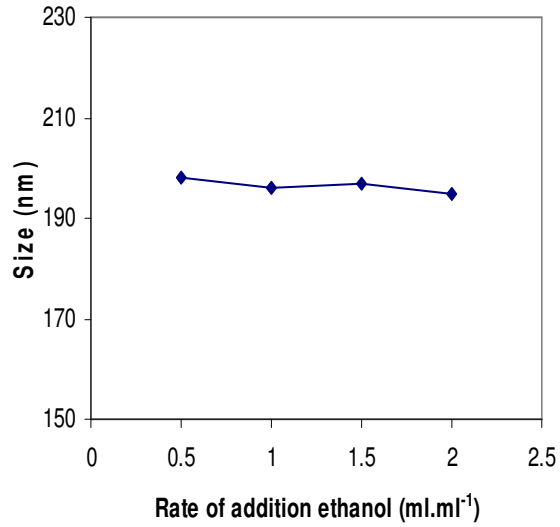

Figure 8. Effect of ethanol addition on nanoparticle size. Diameter of BSA nanoparticle fabricated in pure water at initial BSA concentration of 5 mg ml^{-1}as a function of the rate of addition ethanol.

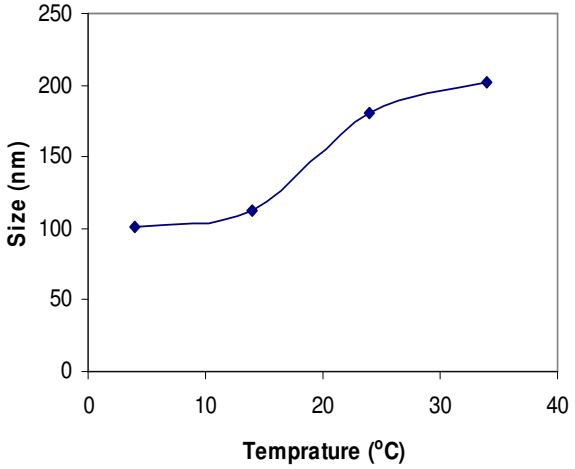

Figure 9. Effect of temperature on nanoparticle size determined by PCS.

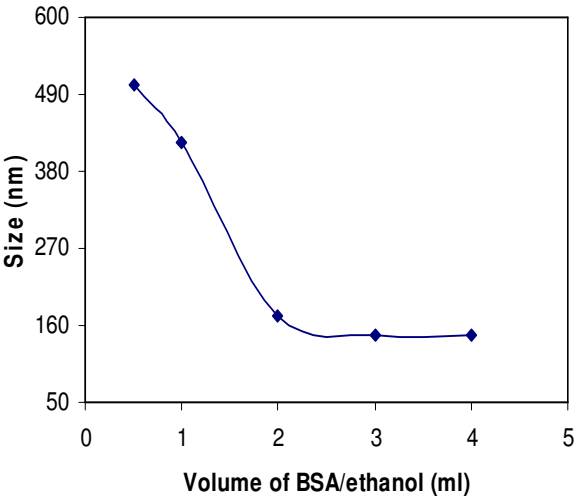

Figure 10. Effect of volumetric ratio of BSA/ethanol on nanoparticle size during the fabrication procedure based on simple coacervation at initial BSA concentration of 5 mg.ml-1.

However, the rate of addition of ethanol on particle size was also experimented. There was no influence of ethanol concentration reflected on particle size (Figure 8). Similar results with HAS nanoparticle have been reported before (Langer et al., 2003). It should be noted that application of ethanol accelerated phase transition of organic phase.

Figure 9 shows the effect of temperature on nanopar ticle size. The most influential parameter on particle size is temperature. Decreasing temperature will also decrease on the particle size. The minimum size of 101.4 nm was obtained at 4°C and the largest size of the particle obtained was 202 nm at highest temperature 34°C.

The effect of the ratio of BSA/ethanol was also investigated in this research (Figure 10), as the ratio increased the particle size was decreased. Weber et al. (2000) reported that the volumetric ratio of BSA/ethanol was very influential parameter for the fabrication of nanoparticles due to the fact that ethanol was able to dissolve BSA with high solvency power. In fact the obtained data employed 5 mg/ml BSA.

Conclusion

The biological nanoparticle (BSA) 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. Low BSA concentration of 5 $mg.ml^{-1}$ was used in a simple coacervation method for preparation of nanoparticles. The nanoparticles size fabricated from BSA was influenced by several process variables including agitation speed, temperature and BSA concentration. In predetermined conditions (protein concentration of 5 $mg.ml^{-1}$) the particle was prepared at 4°C, pH 7.5 and agitation rate of 500 rpm. The coacervation procedure appears promising in studies of BSA nanoparticle manufacture where temperature has been shown to be highly influential upon the mean size of fabricated nanoparticle. The minimum size of particle fabricated was 101.4 nm at the lowest temperature 4°C. Optimization of this fabrication method for protein nanoparticles as drug delivery systems will be the subject of next publication.

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