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Determining optimum period of withholding irrigation for inducing maturity

Abstract

In this study, solvent worse method with further modification was used to produce biodegradable hydrophilic gelatin nanoparticles (GNPs) for delivery of proteins (BSA/HSA). The GNPs were characterized by High resolution transmission electron microscopy; scanning electron microscopy and Fourier transform infrared spectroscopy. Zeta potential showed positive value at acidic pH and negative value at basic pH. The GNPs size obtained as particles with a typical size 5-40 nm were produced. The maximum loading of BSA and HAS found as 10% and encapsulation efficiency obtained as 90% (w/w) of BSA and 80% (w/w) of HSA. The release profile for BSA/HSA have been entrapped well with the GNPs, since a controlled release of the protein is observed within 6 days in case of BSA and biphasic release was observed within 16 day for HSA.

Keywords: Bovine serum albumins, Human serum albumins, Gelatin nanoparticles, Drug delivery

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Abbreviations: GNPs, Gelatin Nanoparticles; GRAS, Generally Recognized As Safe; FDA, Food and Drug Administration; BSA, Bovine Serum Albumin; HAS, Human Serum Albumin; SEM, Scanning Electron Microscope; HRTEM, High-Resolution Transmission Electron Microscope; FTIR, Fourier Transform Infrared Spectroscopy; DLS, Dynamic Light Scattering; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; DLS, Dynamic Light Scattering

Introduction

In pharmaceutics, biodegradable and biocompatible polymeric nanoparticles have shown considerable potential as drug carriers.[1-3] Delivery of therapeutic proteins and drugs has received significant attention in recent years.[4-5] Gelatin is a natural polymer derived from animal's collagen, which is most abundant available protein. It's enormous biomedical application including plasma expander, stabilizer in a number of protein formulations, vaccines and gelatin sponge (Gelfoam®). Moreover, gelatin proved as a safety food supplement which is also documented by the classification as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA).

Gelatin nanoparticles (GNPs) are very efficient in delivery and controlled release of the drugs, proteins and peptides. GNPs based delivery system is biocompatible and biodegradable without toxic degradation products in body. They are nontoxic, biodegradable, bioactive and inexpensive. Gelatin is a poly-ampholyte consisting of both cationic and anionic groups along with a hydrophilic group. It is known that the mechanical properties such as swelling behavior and thermal properties of gelatin NPs depend significantly on the degree of cross linking between cationic and anionic groups.[6-7] Several methods have been used to synthesize GNPs, including desolvation, coacervation, and water-in-oil (w/o) emulsion, solvent worse or precipitation.[8] It has been observed that the emulsion technique require a large amount of surfactant is required to produce the small-sized NPs, which needs a complicated post-process.[9] The coacervation method is a process of phase separation followed by cross-linking step, while, the non-homogeneous cross linking occurs in this method and have unsatisfied loading efficiency. And solvent worse or precipitation method require large amount of organic solvent like acetone. Here, we have modified a solvent worse method (reverse process), to reduced synthesis time, uniform size of GNPs by using 1-methyl 3-octylimidazolium chloride (ionic liquid). We have observed that the addition of the ionic liquid reduced the extraction time of GNPs as compared to the blank solution and well dispersed with uniform size obtained. Thus, we have used these GNPs for the encapsulation of Bovine serum albumin (BSA), Human serum albumin (HSA) and their release monitored.

Materials and methods

Gelatin-B (Type B bovine skin extract, 225 blooms, pI~4.9±0.2) having molecular weight 50KDa, 1-methyl 3-octylimidazolium chloride (ionic liquid), bovine (BSA) and human serum albumins (HSA) procured from Sigma-Aldrich, USA was used as received. Others chemicals including acetone, hydrogen chloride, sodium hydroxide, silica gel (drying agent) were purchased from local supplier. All the solution prepared in deionized water without further purification.

Synthesis of gelatin nanoparticles

The gelatin nanoparticles (GNPs) have been prepared by solvent worse or precipitation method, in this method we goes from good solvent to bad solvent. A stock solution of gelatin solution (1%w/v) were prepared in distilled water by dispersing gelatin in water and kept at 40°C under continuous stirring for about 30minutes. The gelatin solution is titrated against acetone to optimize the volume (30mL) at which maximum turbidity was appeared. These GNPs were

prepared and got smaller in size and taken longer aging period for growth of the GB nanoparticles. To decrease this time, these GNPs treated with 1-methyl 3-octylimidazolium chloride (Ionic liquid), obtained uniformed settled down GNPs on the bottom of beaker. It was found that in the presence of ionic liquid, the faster growth of GNPs with optimized size of GNPs. Moreover, the addition of the ionic liquid reduced the extraction time of the GNPs as compared to the blank solution.

Loading of BSA and HSA in GNPs

Aqueous solution of GNPs was made using milli Q water (20mg/mL). 2mL of the solution was dialyzed for one hour and diluted to 22mL and the solution concentration was made up to 1.8mg/mL for the loading of BSA and HSA in GNPs, an aqueous solution of BSA and HSA were made to 100 and 33.5mg/mL concentration respectively. The unloaded proteins were estimated by UV-vis absorption peak at 420nm and used to determine loading and encapsulation efficiency using the following equations: Loading efficiency (%) = weight of proteins in GNPs / weight of NPs x 100 Encapsulation efficiency (%) = weight proteins in GNPs / Weight of total NPs x 100

Release of proteins (BSA, HSA) from GNPs

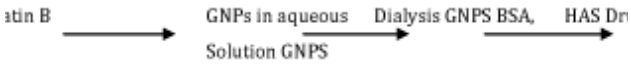
For release studies, each sample solution was loaded at its maximum in GNPs and was poured into a dialysis bag (12kD). The dialysis bag is immersed in a solution containing PBS: ethanol (2:1) solution for a period of about 3 weeks and incubated at 37oC. The drug released in PBS: ethanol was estimated every 2 4hours by measuring the absorbance at 425nm. The encapsulation efficiency of each sample was checked after first four hours and the PBS solution was replaced with new solution. The release profiles of BSA and HSA from GNP s were carried out by measuring the cumulative release over time.

Characterization

The GNPs size and morphology were determined scanning electron microscope (SEM, Zeiss, EVO, 40), High-resolution Transmission electron microscope (HRTEM) images studies were carried out using a JEOL JEM-2200 FS (Japan) instrument operating at a voltage of 200 kV. Samples for TEM analysis were prepared by spreading a drop of as-prepared products dilute dispersion on amorphous car bon-coated copper grids and then dried in air at room temperature. Fourier transform infrared spectroscopy (FT-IR) spectra of GNPs were recorded on a Varian 7000FT. The zeta-potential measurements were performed on an electrophoresis instrument (model ZC-2000, Microtec, Japan). Dynamic light scattering (DLS) measurements were performed at a scattering angle of θ=900 and laser wavelength of He/Ne laser of λ= 632.8nm on RINA Netzwerk RNA- technologies.

Results and discussion

The maximum loading of BS A and HAS found as 10% and encapsulation efficiency obtained as 90% (w/w) of BSA and 80% (w/w) of HSA, indicated that high encapsulation efficiency of the GNPs indicates good permeability. The post loaded of all these (BSA, HSA) in dialyzed GNPs. The tentative reaction takes place as follows:



GNPs: Gelatin nanoparticles
BSA: Bovine serum albumin
HSA: Human serum albumin

Figure 1 shows the zeta potential of GNPs, indicated zwitter ionic nature in which charge of the nanoparticles is varies with pH value. Zeta potential showed positive value at acidic pH and negative value at basic pH.

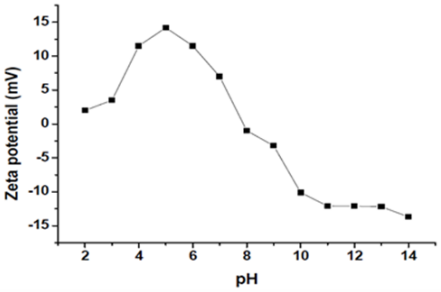


Figure 1 Zeta potential of GB nanoparticles at different PH.

Scanning Electron Microscopy (SEM)

The GNPs have spherical surface morphology of diameter 100-350nm observed from the SEM images (Figure 2a & 2b), due to agglomeration of GNPs. Most of t he nanoparticles were individually dispersed and some of them were present in the form of clusters.

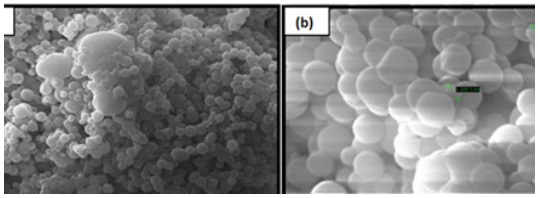


Figure 2 SEM images of the GNPs.

Fourier transmission infrared spectroscopy (FTIR) Study

Figure 3a & 3b shows the FTIR spectra of avoid GNPs, BSA, and HSA and loaded GNPs with BSA and HSA. In both cases the GNPs electrode shows all the characteristics amide I, amide II and amide III bands at about 1652, 1542 and 1241 cm^{-1}, respectively, which corresponds to C=O stretching, N–H in-plane bending and C–H stretching and C–N and N–H in-plane stretching, respectively. The absorption band at 2947 and 3081 cm^{-1} assign to –CH anti symmetric and symmetric stretching of $–CH_3$, CH_2 and =C-H, respectively. The bands near the region of 3422 cm^{-1} assign to NH_2 antisymmetric stretch of primary amide of gelatin and also corresponding to O–H stretching due to physically adsorbed water. The changes in the intensity of band and slightly shifts in the IR bands after the loading of both proteins (BSA and HSA) confirm the loading of protein in gelatin nanoparticles.

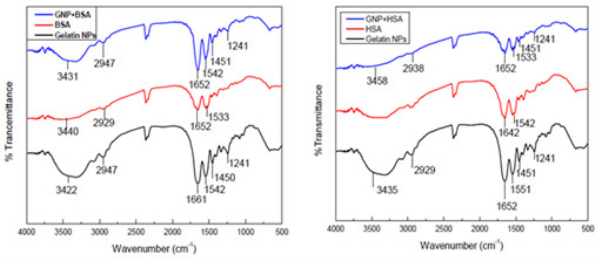


Figure 3 FTIR spectra of GNPs and loaded with proteins (BSA and HSA).

Transmission Electron Microscopy (TEM)

Transmission microscope gives the significant information about the particle size found as 5-40nm (Figure 4). The TEM images of the

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void GNPs (image a) shows uniform dispersion of nanoparticles. The changes in size in TEM after loading BSA (image b) and HSA (image c) indicates the presence of proteins in GNPs.

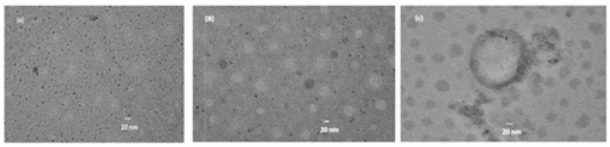

Figure 4 TEM images of

(a) Void GNPs

(b) BSA-GNPs

(c) HSA-GNPs.

Dynamic Light Scattering (DLS)

The bare GNPs have been characterized using DLS. It has been found that the hydrodynamic radius (R_H) of bare GNPs as 350 nm, and loaded with BSA and HSA as 370, 370, respectively (data not shown). These results indicate that the R_H increases indicated that proteins are encapsulated in GNPs.

Release of Proteins from GNPs

The protein release profile we can say that BSA/HSA have been entrapped well with the GNPs, since a controlled release of the protein is observed within 6 days in case of BSA and linear release was observed within 16 day for HSA. Thus we infer that proteins have been successfully encapsulated in GNPs and their released HSA been studied. It has been observed that B SA is fast released about 25% during first 48 h, and then slow released up to 29% behave as a diffusion-controlled slower release phase (Figure 5). However, in case of HSA bi-phase released is observed. Around 10% of the drug was gradually released during the initial 86 hours [phase-I], further 22% release was observed in the following 200 hours [phase-II].

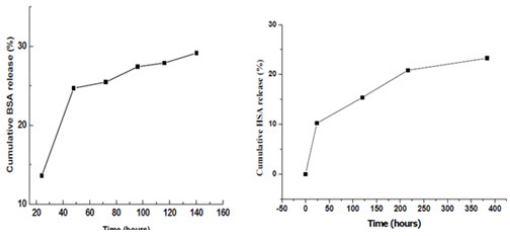

Figure 5 Release profile of BSSA (left side) and HAS (right side) from GNPs.

Conclusion

We synthesized solvent worse method with further modification was used and loaded BSA/HSA into it to evaluate their loading and encapsulation efficiency. The maximum loading of BSA and HAS found as 10% and encapsulation efficiency obtained as 90% (w/w) of BSA and 80% (w/w) of HSA. The release profile for BSA/HSA have been entrapped well with the GNPs, since a controlled release of the protein is observed within 6 days in case of BSA and biphasic release was observed within 16 day for HSA. Thus we infer that proteins have been successfully encapsulated in GNPs and their released HSA been studied.

Acknowledgments

None.

Conflicts of interest

None.

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