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Nanoparticle formulation having ability to control the release of protein for drug delivery application

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ABSTRACT

Controlled release of therapeutic protein is desirable for protein delivery applications. This study discuss about a unique nanomaterial which is capable to provide the protein release in controlled manner. The nanomaterial has been synthesized from folic acid molecules and bovine serum albumin (BSA[1]) is loaded in these nanoparticles as a model protein. The size distribution of the synthesized folic acid nanoparticles was observed between 200 and 300 nm. The release study using high performance liquid chromatography suggests that more than 90% of BSA can be encapsulated in the nanoparticles having BSA loaded up to 19.29 mg (57% of folic acid loaded). Release study also reveals that more than 95% of the total folic acid and BSA were released in phosphate buffer saline solution within 48 h. Investigation of folic acid release along with BSA release reveals that the particles are formed through folic acid-protein complex. Salt concentration in the release medium and crosslinked cations in the nanoparticles are found to be the key parameters to control the release rate. Thus, folic acid nanoparticles are efficient carrier for protein encapsulation and release in the controlled manner with minimum drug loss.

1. Introduction

Injection is a more effective route of protein administration as proteins are digestible in the body [1]. However, a patient requires multiple injections because these molecules have short life span [2]. Frequent injection doses cause side effects and increase the cost of the therapy. Therefore, a cost effective and more efficient therapeutics with the help of a controlled drug delivery system is desirable [3–5]. Sustained release of drug through nanocarriers has already been introduced in the previous studies [6–9]. Nanoparticles as a carrier increase the bioavailability of drugs at the targeted site [9–14]. Nanoparticles can potentially circulate for a long time in the body and provide release for multiple hours or days depending on their properties [15–19]. Hence, protein delivery through nanoparticles is an effective way to control the drug release as well as to design an efficient protein delivery system.

Folic acid (FA[2]) is a water soluble vitamin which shows liquid crystalline properties. The molecules of the self-assembled FA interact with various drugs and dyes [20]. Studies prove the associative interactions between proteins (for example, BSA) and FA using fluorescence emission spectroscopy, X-ray diffraction, FTIR spectroscopy and molecular simulations [21–24]. Studies have also analysed the impact of FA molecules on the structure of proteins. Interaction studies between FA and casein suggests that the integrity of protein structure retain even after addition of FA molecules [23]. Similarly, presence of FA molecules does not impact the microenvironment around protein like β-lactoglobulin [22]. FA has also been used in several insulin delivery strategies and these strategies have shown increase in the bioavailability of insulin [25–27]. On the other hand, a study indicates that FA molecules affect the secondary structure of BSA [21]. Therefore, it can be concluded that the impact of FA addition on the structure of protein varies with the type of protein while associative interactions with FA is applicable for casein, β-lactoglobulin, BSA and many other proteins. Hence, FA as a carrier will show similar behaviour with different proteins.

FA has already been used in a variety of drug delivery applications [28–31]. FA decorated protein delivery systems demonstrate an increase in the bioavailability of protein after administration in the body [26,27,32]. Hence, FA is a safe molecule for protein delivery. Past studies have discussed the use of FA nanoparticles in the delivery of small molecular weight drugs (molecular weight 200 to 800 g/mol) [33–36]. Previous work on FA nanoparticles shows that the variation in the type of crosslinking salts like calcium chloride, aluminium chloride and iron chloride have different effects on the release of drug [33–36]. In addition, control over the size of particles by variation in the loading of FA and other constituents during formulation synthesis also affect the protein release as shown in the previous studies by our group [33–36]. However, the behaviour of FA nanoparticles for protein delivery can differ due to larger molecular weight of proteins. Therefore, additional work is required for the application of FA nanoparticles in the protein delivery.

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[1] BSA: Bovine serum albumin
[2] FA: Folic acid

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In the present work, the effectiveness of BSA loaded FA nanoparticles has been examined to design a controlled protein delivery system. BSA is serum albumin which is derived from cows. The structure of BSA has 17 disulphide bonds and can be divided in three homogeneous domains. Two tryptophan (Trp[3]) residues in BSA structure cause fluorescence emission on excitation at 280 nm [21,24]. BSA also support to transport various biologically important compounds in the body [37]. A detailed release study was performed with nanoparticles having BSA loading up to 19.29 mg. The concentration of carrier, polymer and cross-linking salt were according to the method given in the literature for nanoparticle synthesis in the range of 200 to 300 nm [28,36]. FA nanoparticles were characterized after synthesis using various techniques. Transmission electron microscopy (TEM[4]) and dynamic light scattering (DLS[5]) techniques were used to predict the particle size distribution of these nanoparticles. Post-release concentrations of both FA and BSA in the release medium were analysed separately using high performance liquid chromatography (HPLC[6]) [38]. The release study of carrier FA was performed along with protein to demystify the mechanism of nanoparticle formation. In addition, this study was fruitful to clarify the effect of cross-linking agent concentration on the protein release behaviour. An analysis of the total FA and BSA released was executed to ensure the conservation of masses during the entire encapsulation and release process. Small loss of components during the mass balance study suggests that analytical approach for quantification was robust.

2. Materials and methods

2.1. Materials

FA and BSA (molecular weight: 66.5 kDa) were purchased from SRL Pvt. Ltd., India. Sodium hydroxide (NaOH) was purchased from Fisher Scientific, India. Hydroxypropyl methylcellulose (HPMC[7]) and zinc chloride ($ZnCl_2$) were supplied by Central Drug House (CDH) India. All the solutions were prepared in weight/weight basis in deionized (DI[8]) water at 25 °C during this study. Purified DI water was obtained from Milli-Q water purification system (Merck Millipore, Germany). First, FA aqueous solution of 10% concentration was prepared by mixing 10 g of FA in 100 ml of DI water. The solubility of FA in water was 0.6 mg/ml as reported in the literature [39]. Hence, 1 N NaOH was added in 10% FA stock solution with continuous stirring to dissolve such a high concentration of FA in this study [20]. FA stock solution was kept at 6.5 pH after maintaining 1:0.8 ratio between FA and 1 N NaOH. pH was measured using pH meter (make: Eutech instruments model: pH 2700).

2.2. BSA encapsulation in FA solution

Encapsulation of BSA in the liquid crystalline solution of FA was achieved by mixing 5 ml FA stock solution with 5 ml aqueous solution of BSA at different concentrations for 15 min. Both the solutions were mixed at 800 rpm with the help of magnetic stirrer (make: Tarsons Products PVT LTD model: Spinot Digital).

2.3. Synthesis of nanoparticles

A simple emulsification process was used to create BSA encapsulated FA nano-domains in the polymer solution [28,35]. The resultant FA-BSA mixture prepared from the previous step and aqueous solution of HPMC polymer at 5% concentration were mixed at 1:5 ratio (protein loaded FA solution: HPMC solution). Mixing was performed for 6 h at 800 rpm using mechanical stirrer (make: REMI Instruments). Multi-valent salts like $ZnCl_2$ or $CaCl_2$ can be used to stabilize these nano-domains according to the literature [28,36]. In this study, $ZnCl_2$ at 0.1% and 1% concentration was mixed as a cross-linking agent to maintain a ratio of 1:5 (HPMC solution having FA nano-domains: $ZnCl_2$). Mixing was performed for 8 h at 800 rpm using mechanical stirrer for crosslinking. 1 ml volume of each nanoparticle solution obtained was separated after crosslinking with $ZnCl_2$ for size distribution study and rest of the sample was processed for encapsulation study. All the synthesis process was carried out at room temperature.

2.4. Characterization of nanoparticles and release study

2.4.1. Dynamic light scattering study

The size distribution study was performed using DLS technique (Instrument: Malvern Zetasizer Nano ZS 90). Samples were prepared from 1 ml nanoparticle solution. 1 ml of each nanoparticle sample was first centrifuged at 1×10^4 rpm for 10 min. After centrifugation, the supernatant was discarded and the pellet was resuspended in water for washing purpose. The suspended sample of 400 μl was mixed in 2 ml water using vortex mixer (Make: REMI Model: CM101 PLUS). The mixed samples were sonicated for 10 min. All the studies were performed at 90° scattering angle with refractive index 1.33 and the temperature was maintained at 25 °C.

2.4.2. Drug loss study

The remaining nanoparticle solution after size distribution study were centrifuged using REMI R-24 centrifuge at 1×10^4 rpm for 10 min to remove excess salt and HPMC. Centrifugation process was applied to separate the solution into supernatant and pellet. This supernatant was stored at 2 to 8 °C and analysed by DLS and HPLC to evaluate the drug loss. Analysis of particle count from DLS results shows a small loss of less than 5% of the particle in the supernatant compare to the total particles in the solution. The amount of protein in the supernatant was considered as lost and evaluated using HPLC. The quantification of the amount of both FA and BSA was studied for each formulation. Drug loss was calculated on the basis of the amount of FA and BSA lost in the supernatant according to Eq. (1) shown below.

$$\text{Drug loss}\ (\%) = \frac{\text{Amount of compound lost in supernatant}}{\text{Amount of compound taken initially}} \times 100 \quad (1)$$

2.4.3. Release study

The pellet obtained during drug loss study was freeze-dried at −49 °C and 0.002 mbar vacuum pressure through lyophilisation using Christ Alpha 1–4 LD Plus. The powder obtained from the pellet after lyophilisation process was then used for all the release studies. Release study was executed in three different mediums 0.08% NaCl, 0.8% NaCl and phosphate buffer saline (PBS[9]) respectively. Aqueous 0.8% and 0.08% NaCl solutions were prepared by dissolving an appropriate amount of NaCl in DI water. PBS buffer was prepared by mixing 0.8% NaCl, 0.02% KCl, 0.14% Na_2HPO_4 and 0.024% KH_2PO_4 in water. The buffer was kept at 7.4 pH to maintain the similarity between blood and buffer. The concentration of the cations were kept at 0.08% NaCl (in water), 0.8% NaCl (in water) and 0.8% NaCl (in PBS buffer) respectively. Hence, the release study in these mediums will be productive to analyse the impact of salt concentration in the release medium on protein release.

The lyophilized powder was mixed for 10 min with 15 ml of release medium using a vortex mixer such that all the particles were well dispersed. These samples were stored at static conditions in the absence

[3] Trp: Tryptophan
[4] TEM: Transmission electron microscopy
[5] DLS: Dynamic light scattering
[6] HPLC: High performance liquid chromatography
[7] HPMC: Hydroxypropyl methylcellulose
[8] DI: Deionized
[9] PBS: phosphate buffer saline