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Research Article

Preparation process by desolvation method for enhanced loading of acyclovir nanoparticles

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Abstract

The aim of this investigation was to qualitatively study on preparation process of enhanced loading acyclovir (ACV) in ACV-loaded bovine serum albumin (BSA) prepared by desolvation method with submerged jet of desolvating agent. The prepared ACV-loaded BSA nanoparticles in sterile water for injection (SWI) and isotonic trehalose solution were shown to be monodisperse with sizes of around 120 to 200 nm and zeta potentials of around -7 to -50 mV. However, those in phosphate buffer saline (PBS) were found to exhibit much larger sizes with polydispersity, which might be attributed to the effect of ionic strength. The loading efficiency was found to be around 60%. An increase in the amount of ACV added to the system could significantly improve the loading capacity by almost the same ratio, which may be due to molecular mixing behavior of submerged jet of desolvating agent.

Keywords

Acyclovir, bovine serum albumin, drug loading, nanoparticles, submerge mixing

Introduction

Acyclovir (ACV), an antiviral drug with a highly specific activity against herpes viruses, is widely used in the treatment of various ocular viral diseases. In particular, herpes simplex keratitis (in the most severe cases) is characterized by the spread of the virus into the deeper corneal layers, leading to damage of the stromal cells (Schwartz and Holland 2000; Bartlett 2013). Therefore, the treatment requires a suitable permeation of the antiviral drug through the epithelium in order to reduce the virus load. Many attempts have been made to improve the ocular bioavailability and therapeutic efficacy of acyclovir by incorporating the drug into colloidal systems such as liposomes, microspheres (Genta et al. 1997; Chetoni et al. 2004) and nanoparticles (Giannavola et al. 2003).

Development of nanoparticles by using protein as a drug carrier is promising for delivery of many types of drug due to biocompatibility and biodegradability. Besides, they can be prepared by using simple methods with mild conditions. According to their well-defined primary structure and conformations, albumin-based nanoparticles such as bovine serum albumin (BSA) could also be surface-modified by various approaches including covalent attachment of drug or targeting ligand (Elzoghby et al. 2012; Lohcharoenkal et al. 2014; Suwannoi et al. 2017; Suwannoi et al. 2019). With numerous advantages of the albumin-based nanoparticles, one among the crucial points

to be considered is drug loading. Among various preparation methods of nanoparticles, the desolvation method is quite interesting due to its simplicity and potential to be scaled up to industrial batch size (Noomwong et al. 2011). However, certain amount of drug added into the system might be lost during denaturing step of BSA so that improvement of the drug entrapment is of prime challenge. The aim of this investigation was thus to qualitatively study on preparation process with introducing submerged jet of desolvating agent into bulk solution in order to prepare ACV-loaded BSA nanoparticles with enhanced percentage loading without using glutaraldehyde in the formulation.

Materials and methods

Materials

BSA (fraction V) was purchased from Merck, Darmstadt, Germany. ACV micronized was obtained from Zhejiang Wuyi Pharmaceutical Factory, Jinhua, China. Glacial acetic acid, ethanol, and acetonitrile were obtained from Labscan, Gliwice, Poland. Sodium hydroxide was from Carlo Erba, Val-de-Reuil, France. Trehalose was from Sigma-Aldrich, St. Louis, MO, USA. All chemicals were of analytical or reagent grade.

Preparation of ACV-loaded BSA nanoparticles

ACV-loaded BSA nanoparticles were prepared by desolvation method as previously described (Suwannoi et al. 2017). BSA for 100 mg and 0, 6.5, or 12.5 mg of ACV were dissolved together in 5 mL of sterile water for injection (SWI) and allowed for 20 min incubation. Subsequently, 30 mL of ethanol was submergely pumped at the rate of 15 mL/min (Model 505S, Watson Marlow, Wilmington, MA, USA) through a 0.5-mm spinal needle into the ACV-BSA bulk solution with continuous stirring at 500 rpm. The mixture was then stirred for 10 min and subject to hardening process at 60 °C for 2 h with continuous stirring. Finally, the obtained nanoparticles were purified by ultrafiltration technique using centrifugal filter unit (Vivaspin, 100,000 MWCO, GE Healthcare, Little Chalfont, Buckinghamshire, UK), and then resuspended in various vehicle media, i.e. SWI, phosphate buffer saline (PBS) pH 7.4, or isotonic trehalose solution. The purified formulations were kept at 4 °C until use.

Determination of particle size and zeta potential

Particle size and polydispersity index (PdI) of ACV-loaded BSA nanoparticles were determined by dynamic light scattering (DLS) technique (Zetasizer, Nanoseries, Malvern, Worcestershire, UK). The samples were measured at 25 °C with a scattering angle of 173 °. The nanoparticles were directly loaded into a cuvette of the particle electrophoresis instrument (Zetasizer, Nanoseries, Malvern, Worcestershire, UK), the zeta potential of which was determined by measuring the direction and velocity of the particle movement in the applied voltage of 150 V at 25 °C.

Determination of drug loading

The contents of ACV entrapped in BSA nanoparticles were directly determined by subtracting the initial amount of drug added into the system by the amount of extracted drug from ACV-loaded BSA nanoparticle formulations. ACV extraction was done by treating the formulation with 1.0 N NaOH at 1:1 ratio. After continuous stirring for 30 min with magnetic stirrer, the mixture was centrifuged at 2,000 rpm for 30 min (Biosan, Riga, Latvia). The supernatant was then collected and the contents of ACV were analyzed by HPLC method. The percentage loading efficiency and loading capacity were calculated, respectively, as follows:

$$\% \text{ Loading efficiency} = \frac{\text{Amount of drug recovered}}{\text{Initial amount of drug added}} \times 100 \quad (1)$$

$$\% \text{ Loading capacity} = \frac{\text{Amount of drug recovered}}{\text{Amount of carrier BSA}} \times 100 \quad (2)$$

Drug analysis

Analysis of ACV contents were done by HPLC system equipped with a high-precision pump (LC-20AD, Shimadzu), UV-vis detector (SPD-20A, Shimadzu), and system controller (LC-20AD, Shimadzu, Tokyo, Japan) as previously described (Suwannoi et al. 2017). A C_{18} reverse-phase column (Mightysil RP-18 Aqua 250–4.6 (5 µm), Kanto Kagaku Co., Tokyo, Japan) was used at ambient temperature. The mobile phase consisted of 95% of 0.1% glacial acetic acid in water and 5% of acetonitrile, which was eluted at a flow rate of 1.0 mL/min. The sample injection volume was 40 µL and detection wavelength was 254 nm. The retention time was about 2.5 min. Calibration curve with the coefficient of determination (r^2) for linear regression of at least 0.999 was acceptable. The accuracy and precision of the HPLC method were validated.

Results and discussion

Particle size and zeta potential

It was shown in Fig. 1 that all formulations of ACV-loaded BSA nanoparticles with small and monodisperse sizes around 120–200 nm could be obtained by this preparation process for those resuspended in SWI and isotonic trehalose solution. In contrast, those resuspended in PBS were found to exhibit much larger sizes with high polydispersity. This might be attributed to the effect of ionic strength of different vehicle media. The solution with high concentration of ions could neutralize the surface charge and shift the zeta potential to nearly zero, which was followed by aggregation of the particles. Adsorption of neutral molecules on

the charged surface should cause the movement of the slip plane away from the surface causing a decrease in the absolute value of zeta potential. The high absolute value of zeta potential generates a repulsive electrostatic force between particles, which is a key property of an agglomeration resistant suspension (Kaasalainen et al. 2012). With low absolute value of zeta potential, agglomeration of the particles was generated and could be determined by an increase in particles sizes. Such explanation could be evident by the results of zeta potentials shown in Fig. 2. The surfaces of BSA nanoparticles were negatively charged with zeta potentials of around -7 to -50 mV due to higher pH of vehicle medium than the pI of BSA. The highly negative charge reduced the extent of protein-protein interactions to form larger particles. SWI and trehalose solution as vehicle medium could still maintain the electrostatic stability of the colloidal system, while PBS imposed the strong ionic strength effect resulting in aggregation of particles as mentioned above. This indicated that physical stability of nanoparticles was not only affected by the intrinsic electrical properties of the nanoparticles formulations, but also influenced by microenvironments (Jun et al. 2011; Kaasalainen et al. 2012).

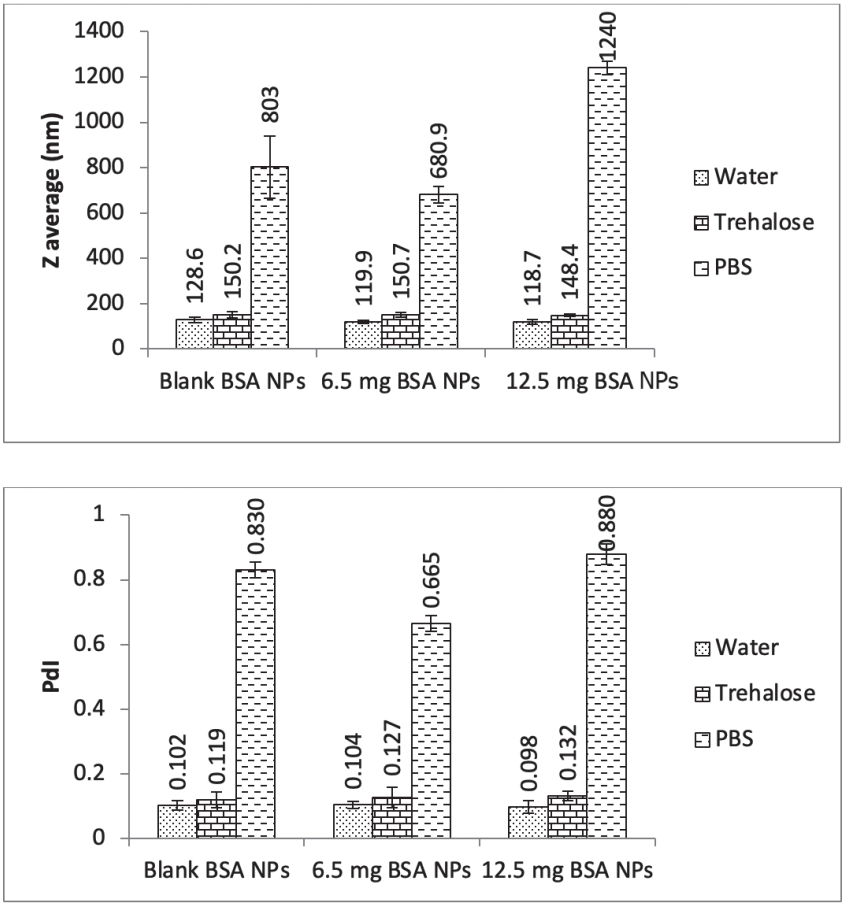

Figure 1. Particle size (upper) and PdI (lower) of BSA nanoparticles with various ACV contents in different vehicle media. (Mean ± SD, n = 3).

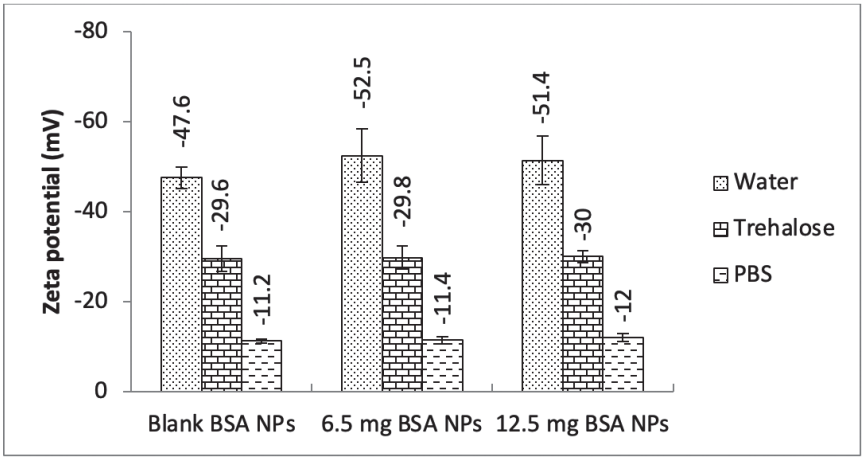

Figure 2. Zeta potential of BSA nanoparticles with various ACV contents in different vehicle media. (Mean ± SD, n = 3).

Drug loading

It can be seen in Table 1 that substantially loading efficiency of around 60% of drug could be achieved in ACV-loaded BSA nanoparticles prepared by this preparation process. Besides, the results indicated that a 2-fold increase in ACV amount added to the system could significantly improve the drug loading capacity by almost the same ratio with similar percentage loading efficiency. This may be due to molecular mixing behavior between the bulk aqueous solution containing ACV and BSA, and flow of submerged circular jet of ethanol pumped into it as illustrated in Fig. 3. The volume of liquid entrained at the nominal boundary of the jet per unit time, causing the mixing in action, is proportional to the volume of liquid leaving jet nozzle per unit time as described by the following equation (McCabe et al. 1993)

$$q_e = [(x/4.3D_j) - 1]q_o \quad (3)$$

where D_j is the diameter of submerged circular jet, q_e is the volume of liquid entrained per unit time at distance x from nozzle, and q_o is the volume of liquid leaving jet nozzle per unit time. In this study, the diameter of submerged circular jet of ethanol (D_j) is equal to that of 0.5-mm (0.05 cm) spinal needle used and leaving jet nozzle at a flow rate (q_o) of 15 mL/min. Substituting these values into Eq. (3) would yield Eq. (4)

$$q_e = 69.77x - 15 \quad (4)$$

The calculated volume flow rates (mL/min) of bulk aqueous ACV-BSA solution entrained at the nominal boundary of the ethanol jet pumped through needle at various distances from needle tip are shown in Fig. 3. It was shown that the volume flow rate of ACV-BSA solution

Table 1. Percentage loading efficiency and loading capacity of ACV-loaded BSA nanoparticles with 6.5 and 12.5 mg of ACV initially added to the system in SWI and isotonic trehalose solution (Mean ± SD, n = 3).

Initial amount of ACV added (mg)	In SWI		In isotonic trehalose solution	
	% Loading efficiency	% Loading capacity	% Loading efficiency	% Loading capacity
6.5	59.70 ± 11.94	4.02 ± 0.48	63.58 ± 3.15	4.65 ± 0.49
12.5	53.52 ± 8.44	7.79 ± 0.61	59.56 ± 0.47	7.52 ± 0.71

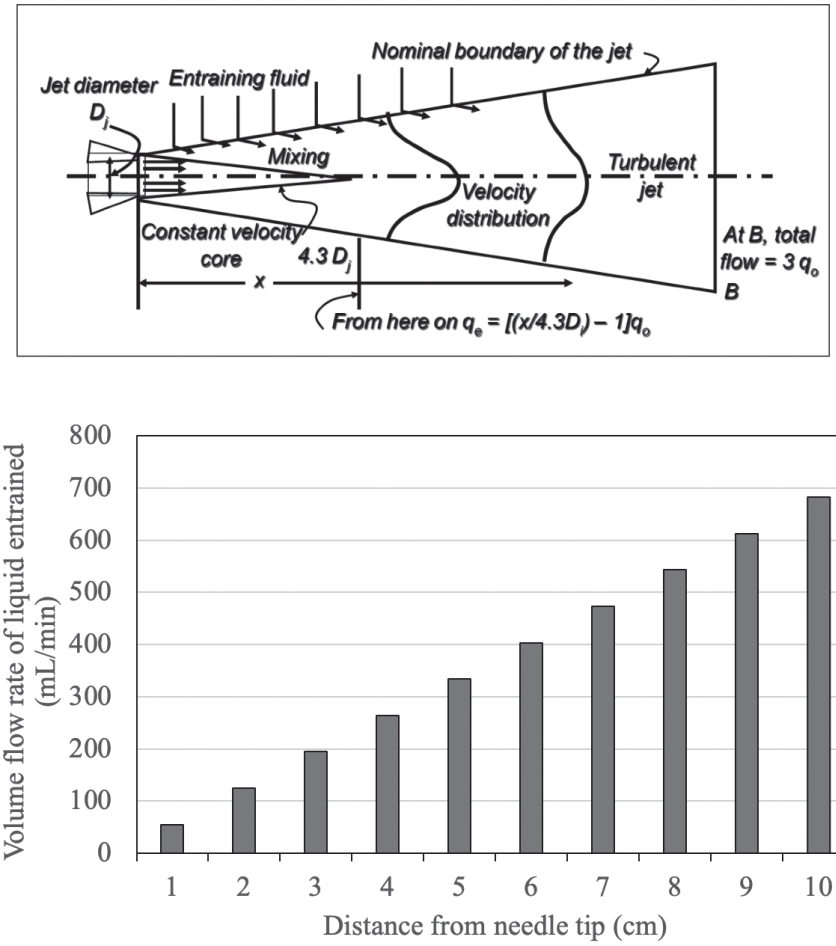

Figure 3. Molecular mixing behavior between the bulk aqueous solution containing ACV and BSA, and flow of submerged circular jet of ethanol (upper), and calculated flow (mL/min) of bulk aqueous liquid containing ACV and BSA entrained at the nominal boundary of the ethanol jet submersibly pumped at 15 mL/min through 0.5-mm spinal needle as a function of distance from needle tip (lower).

entrained increases drastically as a function of distance compared to that of ethanol jet. Therefore, ACV in solution could be efficiently adsorbed and embedded at a higher extent onto the simultaneously precipitating BSA nanoparticles. In addition, from Eq. (4) the mass flow rate (mg/min) of ACV in entrained solution that mixed with ethanol at distance x from nozzle is

$$Q_e = (69.77x - 15)\, C_{ACV} \quad (5)$$

where C_{ACV} is the ACV concentration in solution. Eq. (5) implies that increase in ACV concentration would result in an increase in mass mixing rate, and hence drug loading capacity, by almost the same increasing ratio of ACV concentration.

Conclusion

The nano-size as well as enhanced ACV loading of BSA nanoparticles could be obtained by merely increasing the amount of ACV added into the system in this preparation process employing submersion introduction of ethanol jet stream. Moreover, vehicle medium selected for the systems is of importance in the development of nanoparticles.

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