



Original Article

Physical Properties of Bovine Serum Albumin Microspheres Using HPC-L and Hypromellose 606 Polymer

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The aim of this study was to investigate the preparation of microspheres as potential protein carriers. Bovine serum albumin (BSA) was chosen as a hydrophilic model protein to be encapsulated within hydroxypropyl cellulose low viscosity (HPC-L) and hydroxypropyl methyl cellulose 606 (Hypromellose 606) microspheres using spray drying method, which was called by L and M formula respectively. Both polymers have two different concentrations, low (F1L and F1M) and high concentration (F2L and F2M). The microspheres were evaluated to morphology, particle size, entrapment efficiency (EE), protein content, yield, and FTIR. The spray drying method produced rough surfaced microspheres with small particle size ranging from $1.18 \pm 0.07 \mu\text{m}$ for F1L to $1.70 \pm 0.05 \mu\text{m}$ for F2M. BSA microspheres had a high EE% ranging from $75.84 \pm 2.75\%$ for F1L to $99.29 \pm 0.56\%$ for F2L, and highest protein content was produced by microspheres with HPC-L polymer at low concentration (F1L) $2.51 \pm 0.06\%$. The FTIR spectra of protein alone and in microspheres with polymers did not show any shift in major peak, which indicated no protein-polymer interaction. The results demonstrated this spray dried microspheres can be explored for potential protein carriers.

Keywords: Microspheres, bovine serum albumin, spray drying, HPC-L, Hypromellose 606.

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1. INTRODUCTION

Bovine serum albumin (BSA) is a protein consisting of 582 amino acids and has a molecular weight of 67.000 Da. This protein is widely available in blood and protein as a protein transport for various endogenous and exogenous substances.^{1, 2} In this study, BSA used as a model protein because the structure is known and ease of quantitative assay.³

Microspheres are spherical particles with a diameter of 1-1.000 µm which can regulate the release and can protect drug from negative effects of the environment.^{4, 5} Microspheres have great potential in delivering proteins, because this delivery system can protect proteins from enzym degradation after administration and deliver the protein into the targeted site.^{6, 7} One of the methods that can be used to formulate microspheres is spray drying.⁵ This method can produce microspheres quickly and continuously. The main advantage of this method is that it can be used to dry up a variety of compounds, including those that are susceptible to high temperatures, without causing adverse effects.^{8, 9, 10}

To form a microsphere, an expandable and hydrated polymer network is required, resulting in increased contact between the formula and the mucous layer. Thus, the drug absorption can be prolonged.¹¹ Hydroxypropyl cellulose (HPC) is a water-soluble mucoadhesive polymer, and has been widely used in some formulations as an excipient which can provide a sustained release profile. Based on previous research, HPC-L, delivered a number of proteins in high numbers.^{12, 10} In addition to HPC, another mucoadhesive cellulosic derivative polymers is hydroxypropyl methyl cellulose (Hypromellose). Hypromellose was also often used for the development of formulations with controlled release profiles.^{13, 14}

The objective of the present study was to develop polymeric microspheres of bovine serum albumin using hydroxypropyl cellulose low viscosity (HPC-L) and hydroxypropyl methyl cellulose 606 (Hypromellose 606) to improve BSA effectiveness and stability, and to offer good physical characteristic microspheres for the development of drug delivery system.

2. MATERIALS AND METHODS

Materials

BSA was used as the model protein and was purchased from Sigma-Aldrich, USA. Hydroxypropyl cellulose low viscosity (HPC-L) was purchased from Wako Pure Chemical Industries Ltd, Osaka, Japan. Hypromellose 606 was purchased from Shin-Etsu Chemical Co. Ltd, Niigata, Japan. Coomassie Brilliant Blue (CBB) protein assay kit was purchased from Sigma-Aldrich, USA. All the ingredients were of pharmaceutical grade.

Methods

Preparation of Bovine Serum Albumin-Loaded HPC-L and Hypromellose 606 Microspheres

For preparation of BSA microspheres, spray drying methods were adopted and four distinct formulations were selected on the basis of different concentration of HPC-L and Hypromellose 606. Each formula contains 0.01% of BSA as an active compound of microspheres formulation. BSA was dissolved in demineralized water and mixed with aqueous solutions of HPC-L (1% and 1.5%, w/v) and Hypromellose 606 (1.21% and 1.875%, w/v). As shown in table 1.

Solutions were spray dried through a 1.0 µm nozzle using a Labplant Spray Dryer SD-Basic with an inlet temperature 60°C and a feed flow rate of 4-5.5 ml/min. The resulting BSA-loaded HPC-L/Hypromellose 606 microspheres were collected and stored in a desiccator until further use.^{9, 10}

Table 1: Composition of different excipients used for BSA Microspheres

F.No.	BSA (% w/v)	HPC-L (% w/v)	Hypromellose 606 (% w/v)	Viscosity (cps)
F1L	0.01	1	-	5.4 ± 0.05
F2L	0.01	1.5	-	6.6 ± 0.05
F1M	0.01	-	1.21	5.4 ± 0.05
F2M	0.01	-	1.875	6.6 ± 0.05

All values are expressed as mean ± SD; (n=3)

The formulation consists of different composition and same method was followed for all the formulations. Then the prepared microspheres formulation were evaluated for particle size, morphology, entrapment efficiency, protein content, yield, and FTIR.

Scanning electron microscopy

Surface morphology and shape of microspheres was investigated by scanning electron microscopy (Carl Zeiss MA10, USA) at 5.000x and 20.000x magnification at room temperature. The microphotographs were observed for morphological characteristics and to confirm spherical nature of the microspheres.

Entrapment Efficiency and Protein Content

The BSA loading HPC-L/Hypromellose 606 microspheres was determined by dissolve 10 mg of microspheres of each formulation in 10 ml demineralized water. Further the samples were analyzed for BSA content using coomassie brilliant blue protein assay. The entrapment efficiency and protein content was analyzed by UV-Vis spectrophotometry, at an absorbance wavelength of 486 nm and calculated by comparison with a calibration curve constructed using a series dilution of BSA in demineralized water (concentration of 1, 5, 10, 15, and 20 µg/mL). Samples were analyzed in triplicate. The entrapment efficiency and protein content was expressed as the mean entrapment efficiency and protein content in %.^{3, 13, 15}

Percentage entrapment efficiency =

$$\frac{\text{Amount of protein extracted from microspheres}}{\text{Theoretical protein content of microspheres}} \times 100\%$$

$$\text{Percentage protein content} = \frac{\text{Amount of protein extracted from microspheres}}{\text{Amount of microspheres recovered}} \times 100\%$$

Microspheres recovery yield

Recovery yield of the microspheres after spray drying was calculated for all batches formulated. Percentage recovery yield was evaluated as a function of the proportions of polymer used in the formulation. It was calculated using the following formula.¹³

$$\text{Percentage microparticle recovery yield} = \frac{\text{Weight of microspheres after spray drying}}{\text{Weight of all ingredients before spray drying}} \times 100\%$$

Fourier-transform infrared spectroscopy

FTIR spectra of pure drug, polymer (HPC-L and Hypromellose 606), and microspheres were obtained in KBr pellets at moderate scanning speed between 4000-450 cm by using Perkin Elmer FTIR Spectroscope.¹⁶

Statistical Analysis

The result are presenter as mean \pm standard deviation. The particle size, entrapment efficiency, protein content, and yield of BSA loaded microspheres were treated statistically using two-way analysis of variance (ANOVA). When there was a statistically significant difference, a post-hoc Tukey-HSD (Honestly Significant Difference) test was performed. A statistically significant difference was considered at $p < 0.05$.

3. RESULT AND DISCUSSION

The microsphere formula was distinguished based on ratio of the concentration of hydroxypropyl cellulose polymer (HPC-L) and hydroxypropyl methyl cellulose (Hypromellose 606), where the main factor in producing these microspheres is viscosity. The low concentration of polymer resulted in lower viscosity of the polymer and produced smaller size of the microspheres, therefore this study used low viscosity type of HPC and Hypromellose.¹⁷ HPC-L was used as a reference polymer, because it has been previously investigated that microspheres consisting of 1% HPC-L were relatively small (1-3 μm) and their ability to encapsulate protein was high at $88.9 \pm 1.3\%$.¹⁰ Therefore, the concentration of Hypromellose 606 was obtained by equalizing its viscosity with HPC-L.

BSA Microspheres were successfully accomplished by spray drying method. HPC-L and Hypromellose 606 was selected as a polymer for the preparation of microspheres owing to its excellent biocompatibility, biodegradability, and non-toxicity. The spray drying process begins by dispersing BSA into polymer solution (HPC-L and Hypromellose 606). After the BSA dispersed homogeneously in polymer solution, the mix solution flowed to the nozzle rod through the silicon tube to be atomized through nozzle to form the droplets. Furthermore, droplets formed in the chamber are dried using air flow with high temperature and pressure. Hot air will provide energy for the process of evaporation and absorb water from the polymers. The resulting particles will be collect to the final storage container. The spray drying parameters used are inlet temperature (60°C) and feed flow rate (4 to 5.5 ml/min). The selected inlet temperature is based on BSA thermal stability (63°C).¹⁸ It is expected that BSA stability can be maintained when the inlet temperature is below the thermal stability of BSA. Moreover, encapsulated BSA using HPC-L and Hypromellose 606 can maintained BSA stability.

Based on the previous optimizations, inlet temperature below 60°C can not dry the particles completely. The low inlet temperature causes the drying process to be slower, making it less efficient in drying out the particles that are formed. Therefore, the resulting particles had higher residual moisture content and promoting particles to bind together produce aggregated particles. Moreover, high inlet temperature of the spray dryer can increasing drying, but the resulting particles had a large size with hollow surface.¹⁹ As well as a feed rate more than 6 ml/min can produce microspheres with relatively high water content. Drying also did not occur maximally if the sample flow rate is more than 5-6 ml/min, because spraying droplet solution occurs quickly and will be attached to chamber spray dryer wall which is the main component of solvent evaporation and drying process, causing droplet accumulation on chamber when the process of evaporation and drying has not happened perfectly. This results in the acquired microsphere having a relatively high water content.²⁰

Particle size is one of the most important characteristics of the microspheres. Optical micrographs of BSA – HPC-L/Hypromellose 606 microspheres showed spherical shape and the particle size of microspheres measurement resulted in increased size from 1.18 to 1.52 μm for F1L and F2L and from 1.28 to 1.70 μm for F1M and F2M with the increase of HPC-L and Hypromellose 606 concentration. The result were shown in Table 2.

Table 2: Result of physical properties parameter of BSA microspheres

F.No.	Particle Size (μm)	Polydispersity Index	Yield (%)	Entrapment Efficiency (%)	Protein Content (%)
F1L	1.18 \pm 0.07	0.003	29.79 \pm 0.71	75.84 \pm 2.75	2.51 \pm 0.06
F2L	1.52 \pm 0.15	0.003	33.74 \pm 0.84	99.29 \pm 0.56	1.94 \pm 0.05
F1M	1.28 \pm 0.05	0.003	30.78 \pm 0.58	81.67 \pm 1.4	2.17 \pm 0.01
F2M	1.70 \pm 0.05	0.003	37.80 \pm 0.67	84.33 \pm 0.8	1.18 \pm 0.01

All values are expressed as mean \pm SD; (n=3)

The results indicated that particle size increased significantly with the increase of HPC-L and Hypromellose 606 concentrations ($p < 0.05$). For instance, as the amount of polymer increased from 1% to 1.5% for HPC-L, and from 1.21% to 1.875% for HPMC 606, the particle size increased. This can be explained by the fact at higher polymer concentration, the viscosity of polymer solution increased, thereby producing bigger droplets during atomisation on spray drying process, which the droplets had thicker layers, so the particle size increase significantly. While the comparison of both polymer were not significant ($p > 0.05$), because the viscosity of both polymers was same. The polydispersity index of all formula was low (PDI = 0.003), it means the distribution was getting narrower.

Shape and surface morphological examination of microspheres were done by scanning electron microscope (SEM). Figure 1 showed the SEM photograph of the whole

microspheres. From the figures, shows that all microspheres have corrugated surface, due to low density of both polymers,¹⁰ so the polymers can't maintain the surface of microspheres due to the pressure and high temperature on spray drying process. It may be concluded that the surface of the microspheres containing higher polymer concentration (Figure 1C-1D) were smoother that of the microspheres of the lowest polymer concentration (Figure 1A-1B). This may be due to rapid diffusion of the solvents from the formulations having lowest polymer concentration. The smoothest surface was found with the HPC-L 1.5% and Hypromellose 606 1.875% (Figure 1C-1D), because of having highest viscosity it leads to slower diffusion of the solvent. The microspheres containing lowest concentration of HPC-L 1% and Hypromellose 606 1.21% showed pores on the surface due to traces of solvent evaporation.

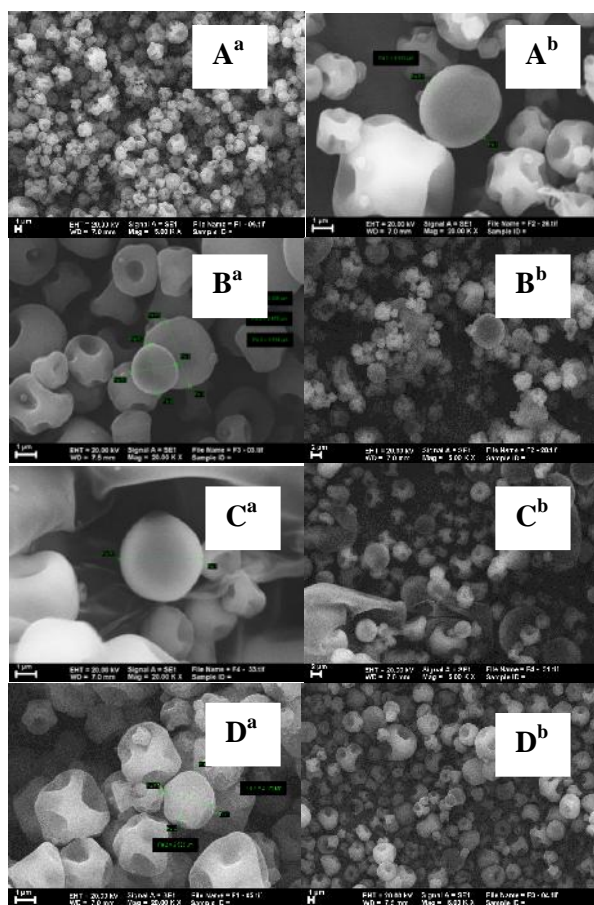


Fig 1: Scanning electron micrographs (5,000x^a) and 20,000x magnification) of (A) F1L, (B) F2L, (C) F1M, and (D) F2M.

The percentage yield of formed microspheres was shown in Table 2. The product yield for microspheres was found to be in the range of $29.79 \pm 0.71\%$ to $33.74 \pm 0.84\%$ for Formula L microspheres, and from 30.78 ± 0.58 to $37.80 \pm 0.67\%$ for Formula M microspheres. The yield of all microspheres was found to be increase with increase in the concentration of polymer.

The results on Table 2 indicated that microspheres yield increased significantly with the increase of HPC-L and Hypromellose 606 concentrations ($p < 0.05$). While the comparison of both polymer (F1L and F1M) were not significant ($p > 0.05$), but the comparison of F2L and F2M were significantly different ($p < 0.05$). The yield of spray drying method tends to be low ($< 50\%$), because the spray dried particles are attached and dry on the chamber and cyclone, therefore caused difficulty to collect.²¹

It has been observed that the entrapment efficiency (EE) of the microspheres containing HPC-L polymers has been increased significantly with an increase in the concentration of the HPC-L in the formulation ($p < 0.05$). As shown in Table 2. This may be due to an increase in the entrapment of drug in the swollen or gel structure of HPC-L, higher amount of polymer was available for cross linking which prevented drug diffusion from the microspheres. The entrapment efficiency of the Hypromellose 606 was found to be unaffected by increase in the concentration of the Hypromellose 606 ($p > 0.05$) as it was unable to form gel like structure. It was observed that the percent protein content was found to decrease significantly with the increase in the polymer ratio ($p < 0.05$). Further increasing in the polymer content was not effective in enhancing the drug loading, because of the amount of BSA in all formula was same, while the amount of polymer in formula F2L and F2M are higher than F1L and F1M. High viscosity of the polymer caused its adhesion to the chamber of spray dryer and it had been wasted without effective loading of the drug.^{15, 22, 23}

Any remarkable change in FTIR spectra would be interpreted as an interaction between protein and other components in microspheres, hence this study was done to investigate these changes. FTIR spectra of BSA, HPC-L, Hypromellose 606, and microspheres were demonstrated in Figure 2. The BSA illustrated characteristic peaks was due to stretching vibration of O–H (3463.32 cm^{-1}), amide I (1654.39 cm^{-1}), and amide II (1522.42 cm^{-1}). HPC-L demonstrated specific peaks in 3469.20 cm^{-1} due to O–H stretching vibration, 2934.28 cm^{-1} C–H asymmetric stretching vibration, 1650.35 cm^{-1} aromatic C – C stretching, 1128.36 cm^{-1} C–O stretching, and 670.42 cm^{-1} aromatic C–H bending. Hypromellose 606 demonstrated specific peaks in 1651 cm^{-1} carbonyl (C= O), 3477.16 cm^{-1} stretching vibration OH, 2932.20 cm^{-1} C– H stretch, and 2838.24 cm^{-1} methyl groups. The peaks of respective polymers and protein were also appeared in their respective spectra. There was no significant shift in major peaks observed from the spectra of protein and polymer physical mixtures, which indicated there were no major interaction between protein and selected polymers. This results suggested BSA stability in HPC-L/Hypromellose 606 microspheres and confirmed their compatibility.

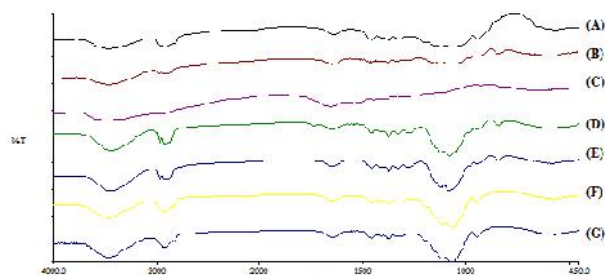


Fig 1: Protein and Polymers compatibility studies through FTIR analysis. (A) Hypromellose 606, (B) HPC-L, (C) BSA, (D) FIL, (E) F2L, (F) F1M, and (G) F2M.

4. CONCLUSION

Polymeric microspheres of BSA were prepared successfully by spray drying methods using HPC-L and Hypromellose 606. In this study, it has been demonstrated that HPC-L and Hypromellose 606 microspheres containing BSA produced high entrapment efficiency and protein contents with smaller particle size. This can be an excellent candidates for consideration in drug delivery system. As the results suggested, formula microspheres using high concentration of HPC-L polymer, can be considered as the optimum formula for further characterization and stability study.

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