Artikel Penelitian

Preparation and Characterization of Ba-Alginate Microspheres Containing Ovalbumin

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ABSTRACT : *The use of microspheres nowadays is important in the pharmaceuticals formulation. The aim of the present research was to evaluate physical characteristics of ovalbumin-loaded alginate microspheres using aerosolization technique at various concentrations of alginate and $BaCl_2$. Alginate microspheres were characterized in terms of encapsulation efficiency, yield, particle size, surface morphology and protein integrity. The comparative study between concentrations of $BaCl_2$ and alginate polymer was investigated. Increasing concentration of alginate polymer from 2.5 to 3.5% reduced their particle size and formed smoother and spherical microspheres. Increasing Ba^{2+} concentration, simultaneous increased the encapsulation efficiency and yield. Scanning electron microscope (SEM) photomicrographs revealed that with the increase in the electrolyte concentration the density of the cross-link is also increased. Smoother surface was demonstrated when the electrolyte concentration is increased from 0.5M to 0.75M. Formula prepared using 0.75M BaCl2 and 3.5% alginate polymer resulted in the highest encapsulation efficiency (92%), the highest microspheres yield (73%) and the smallest microspheres size (3.73µm). Most of the formulations maintain the integrity of ovalbumin after 2 hours incubation in pH 1.2 followed by 8 hours incubation in pH 7.4. The different formulations produced different physical characteristics of ovalbumin-loaded alginate microspheres.*

Keywords: *alginate, $BaCl_2$, microspheres, integrity, protein*

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ABSTRAK : Penggunaan mikrosfer telah banyak digunakan saat ini dalam bidang formulasi dan farmasi. Penelitian ini dilakukan untuk mempelajari karakteristik fisik dari mikrosfer ovalbumin-alginat yang diproduksi dengan teknik aerosolisasi pada beberapa perbedaan konsentrasi alginat dan sambung silang $BaCl_2$. Mikrosfer ovalbumin-alginat yang terbentuk dikarakterisasi efisiensi enkapsulasi, *yield*, ukuran, morfologi dan integritas proteinnya. Hasil yang diperoleh menunjukkan bahwa peningkatan konsentrasi alginat dari 2,5 menjadi 3,5% menurunkan ukuran partikelnya dan membentuk mikrosfer yang lebih halus dan sferis. Peningkatan konsentrasi Ba^{2+}, meningkatkan efisiensi enkapsulasi dan *yield* secara simultan. Hasil SEM memperlihatkan peningkatan konsentrasi elektrolit mengakibatkan densitas dari sambung-silang juga meningkat, yang ditunjukkan dari permukaan yang semakin halus saat konsentrasi meningkat dari 0,5M ke 0,75M. Formula menggunakan $BaCl_2$ 0,75M dan alginat 3,5% menghasilkan efisiensi enkapsulasi tertinggi yaitu 92%, *yield* tertinggi 73% dan ukuran mikrosfer terkecil (3,73µm). Dari studi integritas protein diketahui bahwa hampir seluruh formula dapat mempertahankan integritasnya setelah 2 jam inkubasi pada pH 1,2 dan 8 jam inkubasi pada pH 7,4. Mikrosfer ovalbumin-alginat yang terbentuk dengan konsentrasi polimer dan sambung silang yang berbeda menghasilkan karakteristik fisik yang berbeda.

Kata kunci: *alginat, $BaCl_2$, mikrosfer, integritas, protein*

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INTRODUCTION

Alginate microspheres have been investigated to protect antigen from acidic and enzymatic degradation in gastrointestinal tract. The aim of this research was to investigate physical characteristics of ovalbumin-loaded alginate microspheres. Ovalbumin is egg white glycoprotein that comprises 385 amino acids (molecular weight 43 kDa) that easily denatured at high temperature and acid pH (1). Ovalbumin as a model antigen, could stimulate the formation of antibodies and improve immunity. Administering oral antigen is the most effective way to induce immunological tolerance to protein antigens (2).

Current study applies ionotropic gelation method based on polyelectrolyte capability to form hydrogel using polymer and crosslinking agent. Aerosolization technique was used because it is a cost effective, fast, and simple technique. Moreover, it does not involve organic solvent which can interfere with the protein integrity (3). Polymer is required to coat drug or the core of active substance (4). Sodium alginate is a biodegradable and biocompatible natural polymer, non toxic to the body, cheap and most commonly used as polymer in the microparticles (5). Crosslinking agents are usually cations such as Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Ba^{2+}, dan Sr^{2+} (6). Barium ions have been extensively used as crosslinking agents because its ability to produce strong gel and high potential (7). In addition, Ba^{2+} resulted high biocompatibility with alginate and is able to protect human cell from xenorejection following transplantion (8).

Several factors could affect the microparticles preparation such as concentration of polymer and crosslinking agents (9). Higher polymer concentration produced larger microspheres, but more spherical in shape (10). Crosslinking agents also influenced particle size. Lower crosslinking agents produced fragile and amorphous microspheres, or even it could not form the microspheres. Higher concentration of crosslinker produced smaller microspheres size as a result of stronger binding between them, but often resulted rough surface (9). Therefore, this research were conducted to study the potential of ovalbumin-alginate microspheres using different concentration of alginate polymer and $BaCl_2$ crosslinker.

MATERIAL AND METHODS

Material

Sodium alginate (low viscosity grade), ovalbumin and protein quantification kit were purchased from Sigma (Sigma-Aldrich Inc). $BaCl_2.2H_2O$ *pharmaceutical grade* (Solvay Chemicals Internationals; Natrium citrate *pharmaceutical grade* (Weifang Ensign Industry).

Alginate microsphere preparation

Preparation of alginate microsphere using ionotropic gelation method by aerosolization techniques could be explained briefly as follow. Alginate solution (concentration of 2.5 and 3.5%) containing 2.5% ovalbumin was sprayed into crosslinking agent $BaCl_2$ solution (concentration of 0.5 and 0.75M) at 40 psi and was stirred continously for 2 hours at 1000 rpm. The microspheres were collected by centrifugation at 2500 rpm for 6 minutes, washed twice with *aqua destilata* and finally freeze dried for 20 hours at -80°C. Alginate microspheres formulation were summarized in Table 1.

Table 1. *Ovalbumin-alginate microspheres formulation*

$BaCl_2$ concentration (M)	Alginate concentration (%)	
	2.5	3.5
0.5	F1	F2
0.75	F3	F4

F1: Alginate 2.5% and $BaCl_2$ 0.5 M; F2: Alginate 2.5% and $BaCl_2$ 0.75 M
F3: Alginate 3.5% and $BaCl_2$ 0.5 M ; F4: Alginate 3.5% and $BaCl_2$ 0.75M

Morphology analysis

The morphology of microspheres were characterized by optical microscope with camera and scanning electron microscopy (SEM).

Protein Loading

Loading of ovalbumin into microspheres was analyzed following breakdown of 400 mg of microspheres suspensions in 50 mL sodium citrate solution over 12 hours at 1000 rpm at room temperature. The drug content was determined using protein quantification assay using UV spectrophotometry.

Encapsulation efficiency and *yield*

Encapsulation efficiency and yield were determined as equations below:

$$\textit{Encapsulation efficiency (\%)} = \frac{\textit{amount of ovalbumin}}{\textit{Theoretical amount of ovalbumin}} \times 100$$

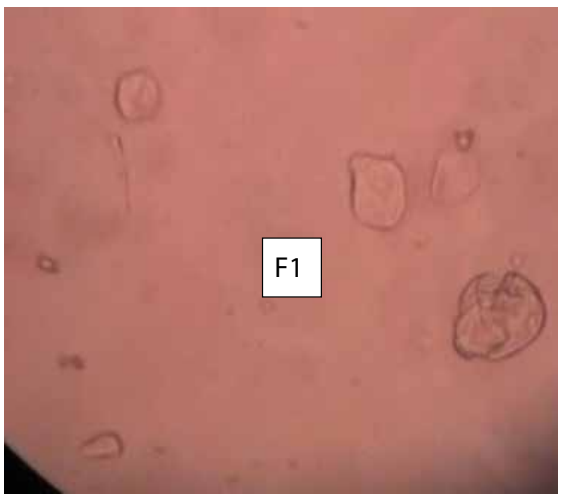
$$\textit{Yield (\%)} = \frac{\textit{weight of microspheres}}{\textit{total weight of polymer and protein}} \times 100$$

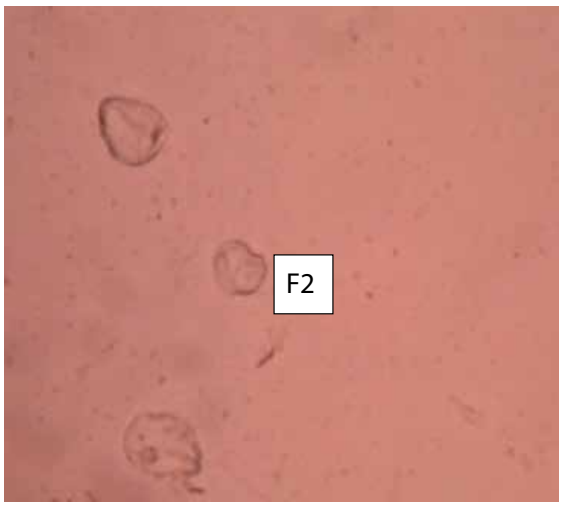
Integrity test

The Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method was applied for investigating integrity structure of ovalbumin inside all microspheres formulas during preparation and incubation.

RESULTS AND DISCUSSION

Ovalbumin-loaded microspheres were succesfully produced from different concentration of alginate and $BaCl_2$. In respect of morphology, Figure 1 shows that almost spherical morphological microspheres were produced by optical microscope. As can be seen from Figure 2, we can see that small, spherical and almost smooth microspheres were produced from all formulas. Some rough surfaces maybe caused by no cryoprotectant agent to protect microspheres during freeze drying was added to stabilize microspheres.





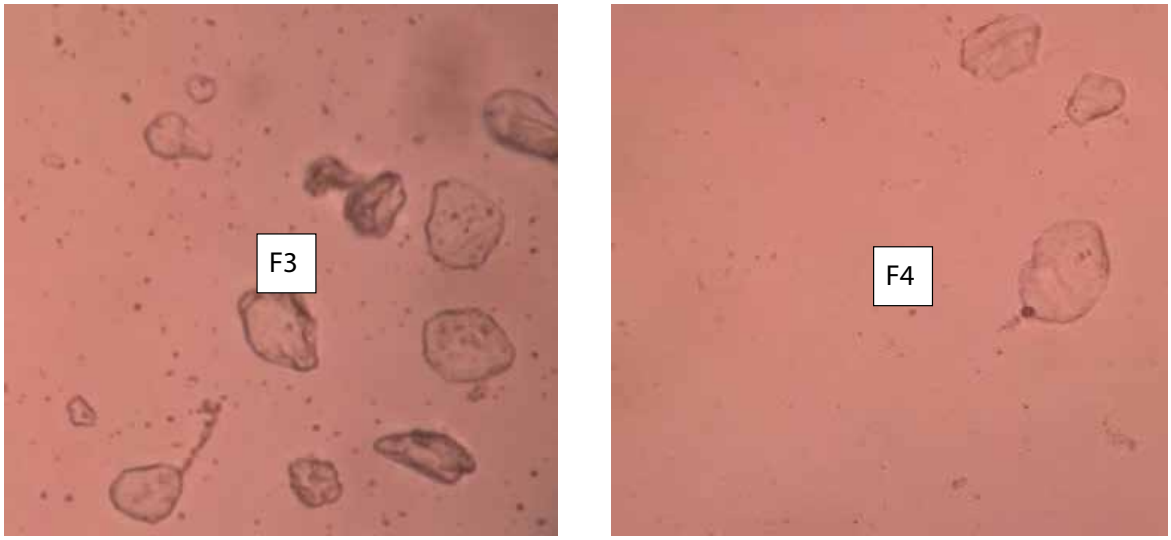


Figure 1. *Optical microscope images of ovalbumin-loaded alginate microspheres (F1 to F4)*

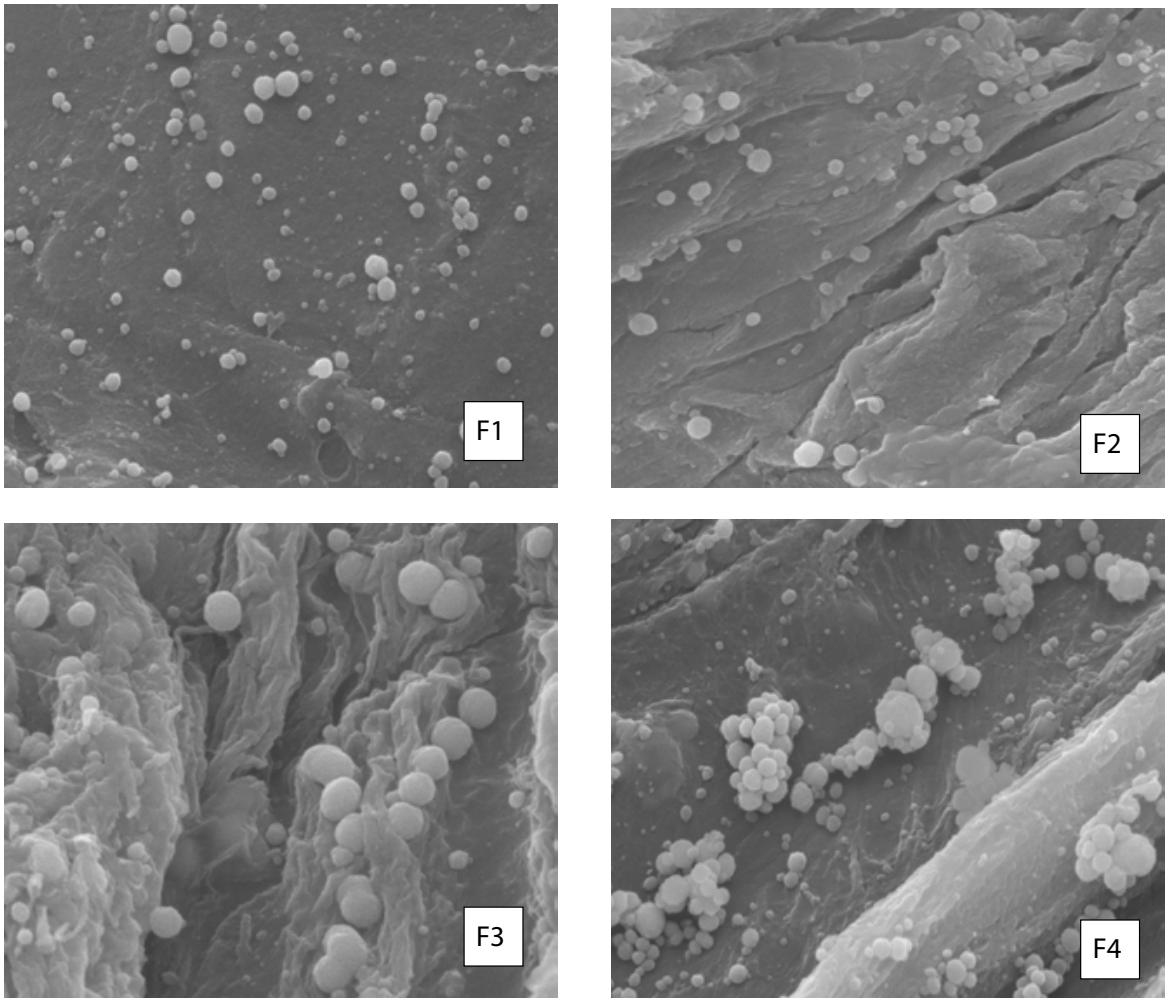


Figure 2. *Scanning electron microscope images of ovalbumin-loaded alginate microspheres (F1 to F4)*

Three hundred particles of each formula were analyzed for particle size demonstrated average particle size of all formulas were small and high percentage of particle size was about 3.31 µm (Figure 3). Smaller particle size was shown by increasing concentration of $BaCl_2$, whereas increasing of alginate concentration at same concentration of $BaCl_2$ resulted in increasing particle size. An increase of alginate concentration was due to an increase of alginate viscosity in forming bigger droplet which produced bigger microspheres'size (11). Mishra et al (12) showed that immune response after oral administration could be achieved from microspheres with 1-30 mm in size. Manjanna et al (11) reported that by increasing concentration of Ba^{2+} formed spherical and smaller microsphere's size. This report was in agreement with Joshi *et al* (10) and Singh dan Kumar (13).

Encapsulation efficiency influenced by protein loading and yield of microspheres can be seen in Table 2. It was observed that larger amounts of $BaCl_2$ (from 0.5M to 0.75M) increased encapsulation efficiency of ovalbumin in alginate microspheres (from 80% to 82% in formula F1 and F2; from 90% to 92% in formula F3 to F4). An increase of encapsulation efficiency is most likely caused by larger amounts of availability of Ba^{2+} that crosslinked with carboxylates from guluronic acid in alginate, which indicates more ovalbumin was entrapped within alginate microspheres. This trend was also similar to the increasing of alginate concentration. The more number of alginate amounts, the more number of crosslinked alginate-$BaCl_2$ resulted the more ovalbumin was encapsulated (11). Similar studies were also confirmed that encapsulation efficiency increased by increasing concentration of polymer and crosslinking agents (10,13).

Figure 3. *Histogram of particle size distribution of formula F1,F2,F3 and F4.*

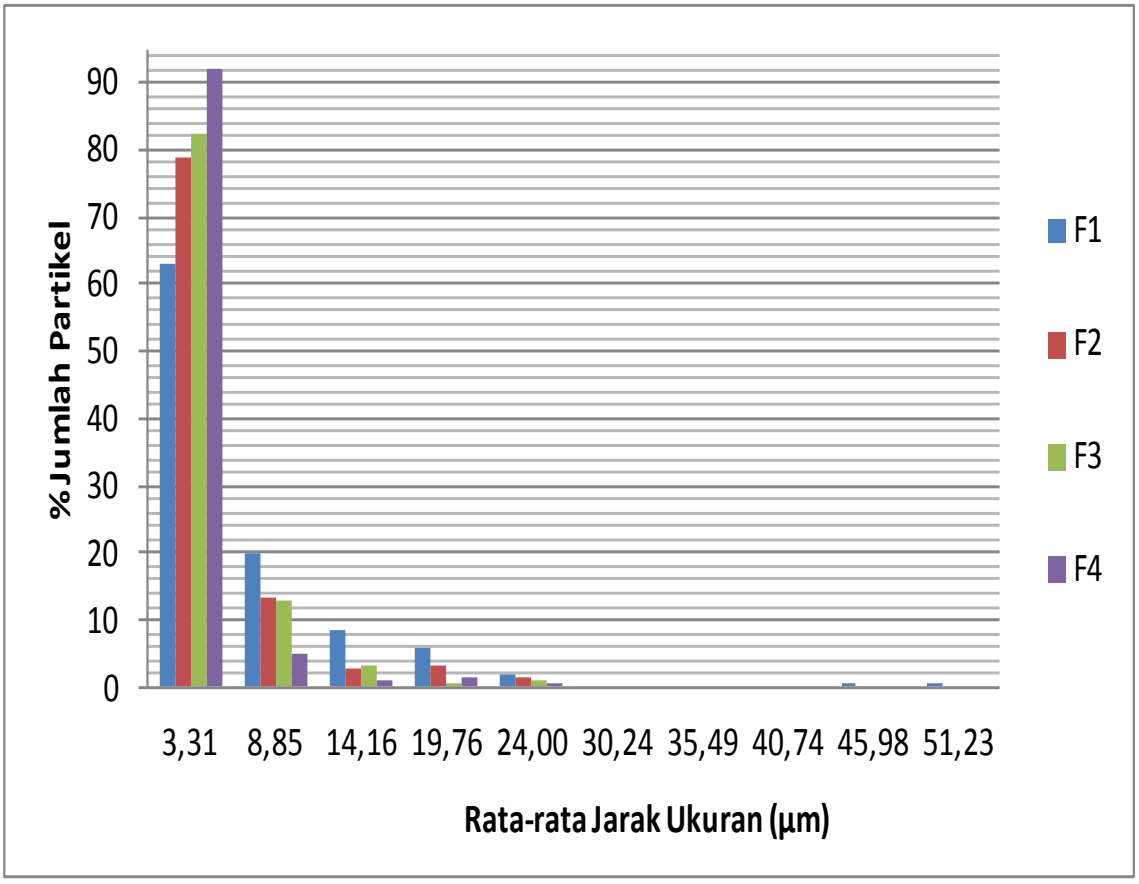


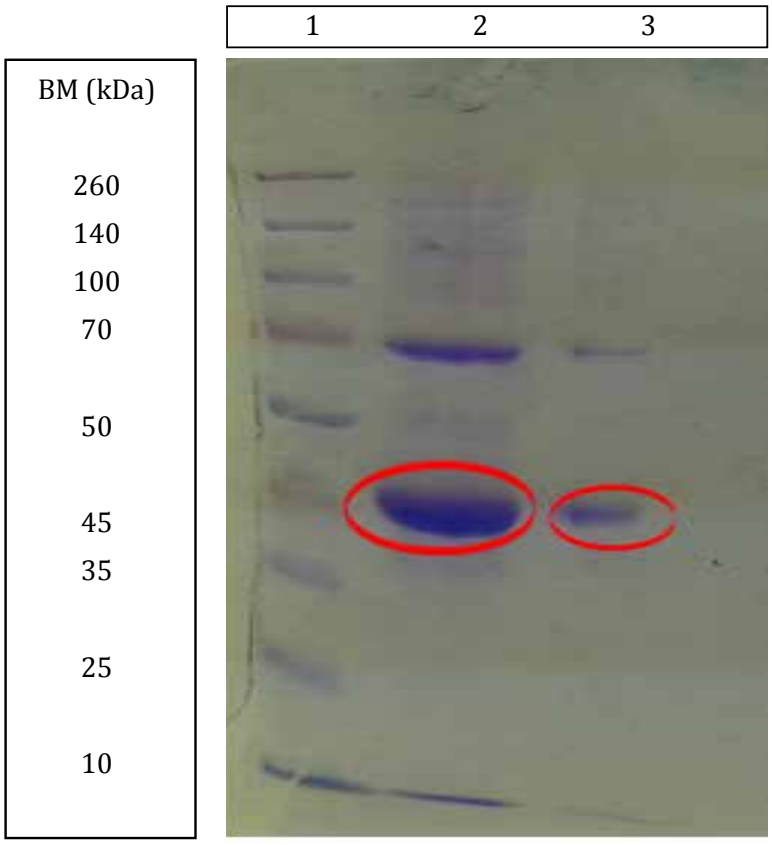
Table 2. *Encapsulation efficiency, protein loading and yield of microspheres*

Formula	Encapsulation Efficiency (EE) (%)	Protein Loading (%)	Yield (%)
F1	80,47 ± 9,52	66,86 ± 10,36	60,63 ± 3,06
F2	81,81 ± 10,77	65,17 ± 12,24	63,43 ± 4,98
F3	90,88 ± 7,37	61,34 ± 4,16	62,74 ± 5,96
F4	92,17 ± 5,57	53,59 ± 2,70	71,93 ± 6,73

In terms of yield, similar results were shown. Alginate microspheres produced using both alginate concentration (2.5 and 3.5%) using highest concentration of $BaCl_2$ (0.75M) indicated the highest yield of about 72% compare to formulas produced using lower concentration of $BaCl_2$. In the case of microspheres crosslinked using higher alginate concentration, the yield was also increased. This behaviour indicates that the more number of Ba^{2+} contact with alginate provide a gel network that able to increase yield of microspheres (9). There was no significant differences of all loading's formula. This may be explained by similar strength and network between carboxylates and Ba^{2+} ions produce similar amount of space for ovalbumin inside microspheres.

Integrity structure of ovalbumin was conducted by SDS-PAGE and results of molecular weight ovalbumin as spot profiles were shown in Figure 4.

Figure 4. *Profile of SDS-PAGE*



Note:

1. Protein marker
2. Ovalbumin standard in PBS pH 7.4 (500 ppm)
3. Ovalbumin sample release from alginate microspheres in PBS pH 7.4 after incubation in HCl pH 1.2

Ovalbumin sample in pH 7.4 after incubation in pH 1.2 maintained its molecular weight of 45 kDa as same as ovalbumin standard reference. This indicated that integrity structure of ovalbumin was maintained and confirmed. Therefore this delivery system may be potential as protein or vaccine delivery system.

CONCLUSION

Ionotropic gelation using aerosolization technique was potential to produce ovalbumin-loaded alginate microspheres with high entrapment efficiency, high protein loading, high yield and small particle size. Moreover, this delivery system can be utilized as one of oral protein or vaccine delivery systems.

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