FORMULASI DAN EVALUASI MIKROSFER Ca-ALGINAT DENGAN MODEL ANTIGEN OVALBUMIN DENGAN TEKNIK AEROSOLISASI

FORMULATION AND EVALUATION OF Ca-ALGINATE MICROSPHERES ENTRAPPING MODEL ANTIGEN PRODUCED BY AEROSOLIZATION TECHNIQUE

Dewi Melani Hariyadi[1*], Esti Hendradi[1], Octavia Librayanti Vierdina P[1], Lailil Muharromah[1]

[1]Faculty of Pharmacy Airlangga University, Surabaya, Indonesia

*Corresponding author:

Dewi Melani Hariyadi, PhD

Faculty of Pharmacy Airlangga University, Surabaya, Indonesia

Jl. Dharmawangsa Dalam Surabaya

Phone: +6287855989394; Fax: +62315020514

Email: dewi-m-h@ff.unair.ac.id

Abstract

Ovalbumin protein was generally used as a model of vaccine antigen for oral delivery system. This research highlighted the advantages of Ca-alginate microspheres entrapping ovalbumin which prepared by ionotropic gelation using aerosolisation technique. It involved alginate as polymer and calcium chloride as crosslinker. The objective of this study was to investigate the preparation and characterization of microspheres produced from $CaCl_2$ with different concentration and alginate in terms of size, protein loading, entrapment efficiency, and yield. The alginate concentrations used in this study were 1.5% and 2.5% w/v. While, the $CaCl_2$ concentrations used in this study were 0.1, 0.5 and 1.5 M. The results showed that low concentration of $CaCl_2$ could not produce microspheres, instead it produced gelling sheet. Microspheres were formed by increasing the $CaCl_2$ concentration. Infra red spectra were also showed that crosslinked between alginate molecule and Ca^{2+} was occurred. Smaller microspheres was produced by increasing $CaCl_2$ concentration from 0,5 M to 1,5 M. The effect of

increasing alginate concentration from 1.5% to 2.5% w/v showed an increase of protein loading, entrapment efficiency, and yield. From particle size examination, increasing alginate concentration showed a decrease of particle size. In summary, high entrapment efficiency, high protein loading and microspheres yield, and small particle size of ovalbumin-alginate microsphere were successfully produced using the ionotropic gelation-aerosolization method which can be utilized as one of oral protein or vaccine delivery systems.

Keywords : Microspheres, Ovalbumin, Alginate, characterization, Aerosolization.

Introduction

Ovalbumin is egg white glycoprotein that comprises 385 aminoacids (molecular weight 43 kDa) (1). It is easily denatured at high temperature and acid pH (1). Administering oral antigen is the most effective way to induce immunological tolerance to protein antigens (2). Thus, ovalbumin as an antigen, could stimulate the formation of antibodies and improve immunity. Polymer microspheres have been investigated to protect antigen from acid pH and enzymatic degradation in gastrointestinal tract. Therefore, it may sustain or control the release of the antigen vaccine (3).

Umer *et al* (4) defined microencapsulation as a process to entrap materials using polymer form microspheres from 1 to 1000 micron. In general, microencapsulation techniques consist of chemical and mechanical methods. 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, simple technique. Moreover, it does not involve organic solvent which can contribute to protein integrity (5).

Polymer is required to coat drug or the core of active substance (6). Sodium alginate is a biodegradable and biocompatible natural polymer, non toxic to the body, cheap and most commonly used as polymer in the microparticles (7).

Crosslinking agents are usually cations such as Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Ba^{2+}, dan Sr^{2+} (8). Calcium ions have been extensively used as crosslinking agents due to low toxicity and ability to form a good gel and provided significant stability (9).

Several factors affect the microparticles preparation such as concentration of polymer and crosslinking agents (10). Higher polymer concentration produced bigger microspheres, but more spherical in shape (11). Crosslinking agents also influenced particle size. Lower crosslinking agents, produced fragile and amorphous microspheres, even it could not form the microspheres (12). Higher concentration of crosslinker produced smaller microspheres size as a result of stronger binding between them, but often resulted rough surface (10). Therefore, this research was conducted to study the formulation and characterization of Ca-alginate microspheres entrapping ovalbumin using different concentration of alginate polymer and $CaCl_2$ crosslinker.

Materials and Methods

Materials

Ovalbumin *pharmaceutical grade ;* Natrium alginate *pharmaceutical grade*; $CaCl_2.2H_2O$ *pharmaceutical grade;* Sodium citrate *pharmaceutical grade;* NaCl p.a; NaH_2PO_4 p.a; KH_2PO_4 p.a; HCl p.a; NaOH p.a; *protein quantification kit*, Aquadest.

Methods

Ca-Alginate microsphere preparation using Ionotropic Gelation – Aerosolization

Preparation of Ca-alginate microsphere using ionotropic gelation method by aerosolization techniques could be explained as follows: Alginate solution (concentration of 1.5 and 2.5%) containing 2.5% ovalbumin was sprayed into crosslinking agent $CaCl_2$ solution (concentration of 0.1, 0.5 and 1.5M) and was stirred continuously for 2 hours at 1000 rpm. The microspheres were collected by

centrifugation at 2500 rpm for 6 minutes, washed two times with aquadest and finally freeze dried 20 hours at -80°C.

$CaCl_2$ concentration (M)	Alginate concentration (%)	
	1.5	2.5
0.1	A1	B1
0.5	A2	B2
1.5	A3	B3

Ca-Alginate microspheres formulations were summarized in **Table 1**.

TABLE 1. Ovalbumin-alginate microspheres formulation

A1: Alginate 1.5% and $CaCl_2$ 0.1 M; A2: Alginate 1.5% and $CaCl_2$ 0.5 M
A3: Alginate 1.5% and $CaCl_2$ 1.5 M ; B1: Alginate 2.5% and $CaCl_2$ 0.1 M
B2: Alginate 2.5% and $CaCl_2$ 0.5 M ; B3: Alginate 2.5% and $CaCl_2$ 1.5 M

Protein Loading

Loading of ovalbumin into microspheres was analysed 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 at 600 nm.

Encapsulation efficiency and Yield

Encapsulation efficiency was determined as equation below:

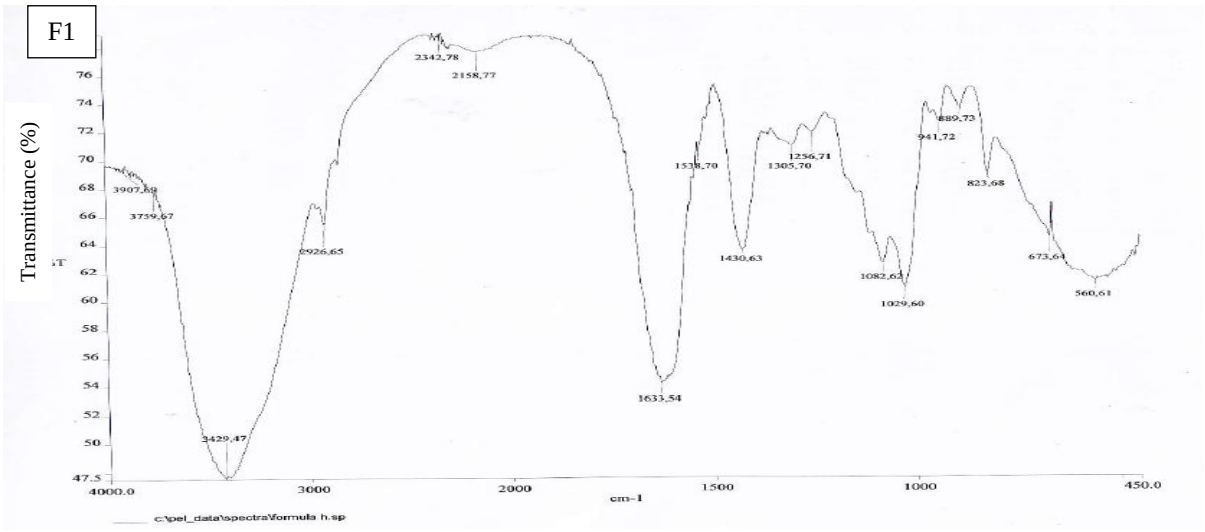
$$\text{Encapsulation efficiency (\%)} = \frac{\text{amount of ovalbumin}}{\text{Theoretical amount of ovalbumin}} \times 100$$

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

Results and Discussion

Almost spherical and some rough morphological microspheres were observed. However, microspheres with 0.1M $CaCl_2$ produced gelling sheets. Some rough surface was caused by no cryoprotectant agent to protect microspheres during freeze drying such as sucrose and lactose.

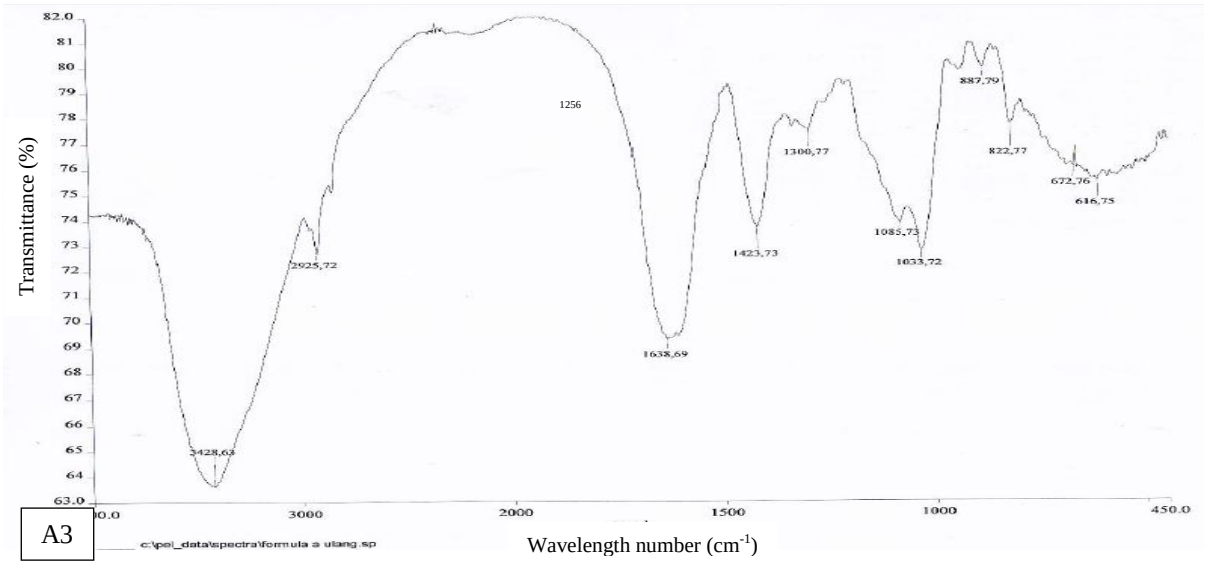
From evaluation of infra red spectra we can see that wavelength number of Calcium ion and alginate molecule showed that crosslinked was occurred of all Ca-alginate microspheres (Figure 1-4).



A2

Wavelength number (cm^{-1})

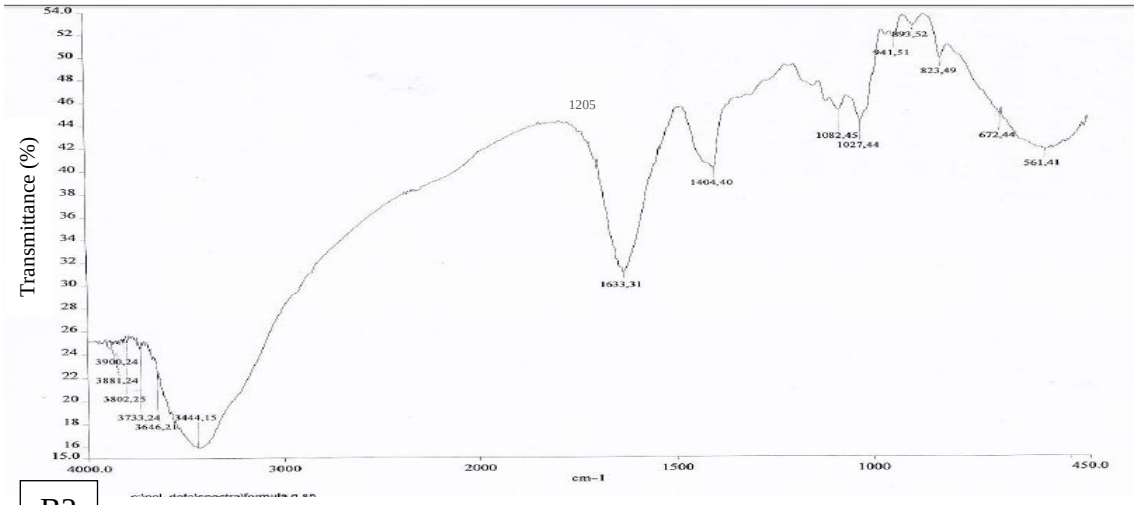
Figure 1. Infra Red spectra of formula A2



A3

Wavelength number (cm^{-1})

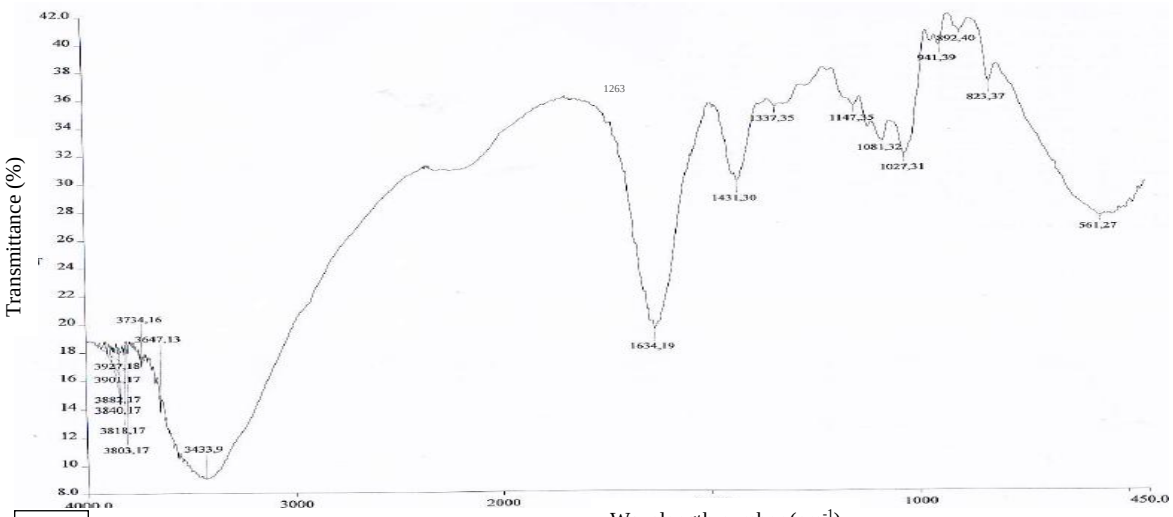
Figure 2. Infra Red spectra of formula A3



B2

Wavelength number (cm^{-1})

Figure 3. Infra Red spectra of formula B2



F4

Wavelength number (cm^{-1})

Figure 4. Infra Red spectra of formula B3

Three hundred particles of each formula were analyzed for particle size demonstrated average particle size of A2, A3, B1, B2 and B3 were 18,06 µm; 12,54 µm; 23,10 µm; 21,14 µm and 17,13 µm respectively (**Figure 5**).

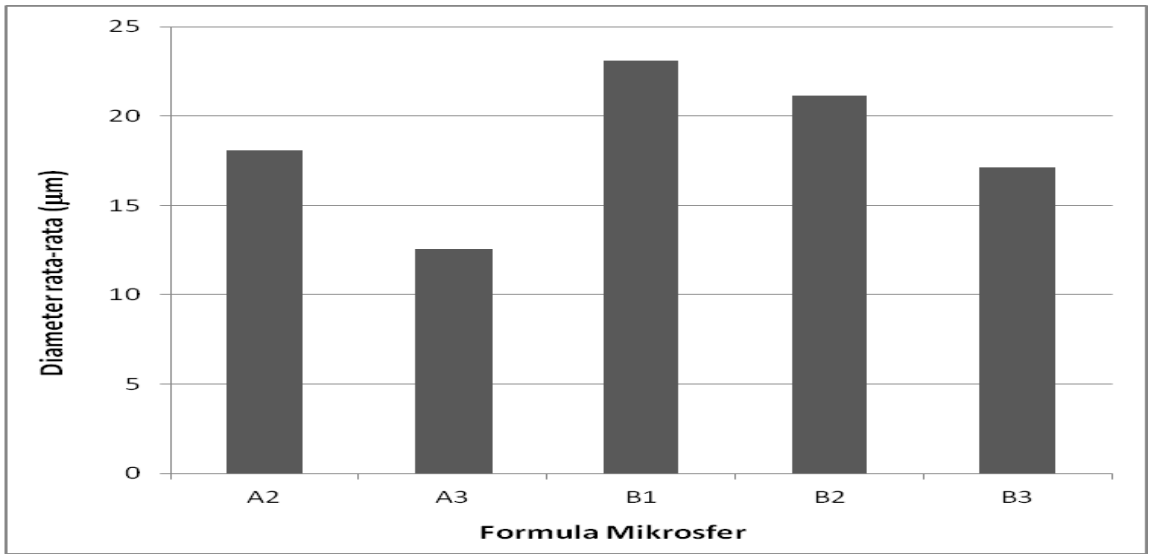


Figure 5. Particle size of ovalbumin – alginate microspheres from all formulas

Smaller particle size was shown by increasing concentration of $CaCl_2$, whereas by increasing alginate concentration at same concentration of $CaCl_2$, increased particle size. An increase of alginate concentration was due to an increase of alginate viscosity in forming bigger droplet which produced bigger microspheres'size (13). Additionally, current particle size was around 10 to 30 µm that also been demonstrated by Mishra et al (14) that immune response after oral administration could be achieved from microspheres with 1-30 µm in size. Manjanna et al (13) reported that by increasing concentration of Ca^{2+} formed spherical and smaller microsphere's size. This report was in agreement with Joshi *et al* (11) and Singh dan Kumar (15).

Formula	Appearance	Protein Loading (%)	Yield (%)	Encapsulation Efficiency (EE) (%)
A1	**Gelling sheet**	**-**	**-**	**-**
A2	**Microspheres**	**20.60 ± 5.78**	**33.56 ± 2.62**	**38.31 ± 10.38**
A3	**Microspheres**	**64.22± 18.82**	**62.30 ± 13.90**	**63.775 ± 4.33**
B1	**Microspheres**	**44.07 ± 13.17**	**50.43 ± 15.79**	**59.96 ± 28.92**
B2	**Microspheres**	**64,10 ± 9,93**	**51,84 ± 6,29**	**67,18 ± 18,03**
B3	**Microspheres**	**74.50 ± 1.70**	**69.53 ± 5.01**	**88.80 ± 0.52**

Encapsulation efficiency, protein loading and yield of microspheres can be seen in **Table 2**.

TABLE 2. Encapsulation efficiency, protein loading and yield of microspheres

It was observed that larger amounts of $CaCl_2$ (from 0.1m to 1.5M), increased encapsulation efficiency ovalbumin in alginate microspheres (from 38% to 64% in formula A2 and A3; from 60% to 89% in formula B1 to B3). Significantly differences were found within formulas by statistic analysis. An increase of encapsulation efficiency is most likely caused by larger amounts of availability of Ca^{2+} that crosslinked with carboxylates from guluronic acid in alginate indicates more ovalbumin was entrapped within alginate microspheres (16). This trend was also similar to an increase of alginate concentration. The more alginate amounts, the more number of crosslinked alginate- $CaCl_2$, resulted the more ovalbumin was encapsulated (13). Similar studies were also confirmed that encapsulation efficiency increased by increasing concentration of polymer and crosslinking agents (11 ; 15).

The increase of protein loading from 20% to 64% in formula A2 and A3 as well as from 44% to 75% in formula B1 to B3 were most probably caused by an increase of concentration of $CaCl_2$, this may be again explained by strong network between carboxylates and Ca^{2+} ions in providing more space for ovalbumin inside microspheres (16).

Alginate microspheres produced using both alginate concentration (1.5 and 2.5%) using highest concentration of $CaCl_2$ (1.5M) indicated the highest yield of about 60% compare to formulas produced using lower concentration of $CaCl_2$. In the case of microspheres crosslinked using higher alginate concentration, the yield was also increased. This behaviour indicates that the more number of Ca^{2+} contact with alginate provide a gel network that able to increase yield of microspheres (10).

Conclusion

Ca-alginate microspheres entrapping model antigen Ovalbumin high entrapment efficiency, high protein loading, high yield and small particle size were successfully produced using this ionotropic gelation-aerosolisation method which can be utilized as one of oral protein or vaccine delivery systems.

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Declaration

The Authors declares that there is no conflict of interest.

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