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AND RELEASE STUDY OF  
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MICROSPHERES PREPARED  
BY DIFFERENT  
CONCENTRATION OF  
ALGINATE AND BACL2 USING  
AEROSOLIZATION

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**TECHNIQUE**

*by* Tristiana Erawati



## PHYSICAL CHARACTERISTICS AND RELEASE STUDY OF OVALBUMIN FROM ALGINATE MICROSPHERES PREPARED BY DIFFERENT CONCENTRATION OF ALGINATE AND BaCl<sub>2</sub> USING AEROSOLIZATION TECHNIQUE

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### ABSTRACT

Microsphere formulations have been widely used for oral applications. The aim of this research was to study physical characteristics and release study of ovalbumin from alginate microspheres prepared by different concentration of alginate polymer and BaCl<sub>2</sub>. This research used concentrations of BaCl<sub>2</sub> of 0,5M and 0,75M and concentrations of alginate of 2,5% w/v and 3,5% w/v. Ionotropic gelation using aerosolisation technique was applied in this study. All ovalbumin – loaded alginate microspheres were characterized in terms of size, morphology, protein loading, encapsulation efficiency, yield, and release profile of ovalbumin. In vitro release study was conducted in the simulated gastric fluid (HCl pH 1.2) and simulated intestinal fluid (PBS pH 7,4) at temperature 37°C. Results showed spherical and smooth microspheres were produced. In addition, smaller particle size of less than 8 µm was produced by increasing alginate and BaCl<sub>2</sub> concentration. A factorial design ANOVA and one way ANOVA were used for statistical analysis at a 95% confidence interval. No significant effect was shown by increasing alginate and BaCl<sub>2</sub> concentration on the protein loading, encapsulation efficiency, and yield. No significant differences of ovalbumin release were found when increasing concentration of BaCl<sub>2</sub> from 0,5M to 0,75M, however ovalbumin release decreased by increasing alginate concentration and slower release in HCl

pH 1,2 during 2 hours followed by complete release in PBS pH 7,4 after 17 hours.

Keywords: Microspheres, Ovalbumin, Sodium alginate, Aerosolisation, Release.

### INTRODUCTION

Alginate microspheres have been investigated to protect antigen from acid pH 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 aminoacids (molecular weight 43 kDa) that easily denatured at high temperature and acid pH (O'neil et al., 2001). 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 (Mowat, 1985).

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 (Yeo et al., 2001). Polymer is required to coat drug or the core of active substance (Dubey et al., 2009). Sodium alginate is a biodegradable and biocompatible natural polymer, non toxic to the body, cheap and most



commonly used as polymer in the microparticles (Maria et al., 2012). Crosslinking agents are usually cations such as Pb<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, dan Sr<sup>2+</sup> (Gombotz et al., 1998). Barium ions have been extensively used as crosslinking agents because its ability to produce strong gel and high potential (Ciofani et al., 2007). In addition, Ba<sup>2+</sup> resulted high biocompatibility with alginate and able to protect human cell from xenorejection following transplantation (Lanza et al., 2007).

Several factors affect the microparticles preparation such as concentration of polymer and crosslinking agents (Jin et al., 2009). Higher polymer concentration produced bigger microspheres, but more spherical in shape (Joshi et al., 2012). Crosslinking agents also influenced particle size. Lower crosslinking agents, produced fragile and amorphous microspheres, even it could not form the microspheres (Suksamra et al., 2009). Higher concentration of crosslinker produced smaller microspheres size as a result of stronger binding between them, but often resulted rough surface (Jin et al., 2009). Therefore, this research were conducted to study the potential of ovalbumin-alginate microspheres

#### MATERIAL AND METHODS

Alginate microsphere preparation using Ionotropic Gelation – Aerosolization Method

Preparation of alginate microsphere using ionotropic gelation method by aerosolization techniques could be explained as follows: Alginate solution (concentration of 2.5 and 3.5%) containing 2.5% ovalbumin was sprayed into crosslinking agent BaCl<sub>2</sub> solution (concentration of 0.5 and 0.75M) at 40 psi 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. Alginate microspheres formulation were summarized in Table 1.

Table 1. Ovalbumin-alginate microspheres formulation

BaCl <sub>2</sub> concentration (M)	Alginate concentration (%)	
	2.5	3.5
0.5	F1	F2
0.75	F3	F4

F1: Alginate 2.5% and BaCl<sub>2</sub> 0.5 M; F2: Alginate 2.5% and BaCl<sub>2</sub> 0.75 M

F3: Alginate 3.5% and BaCl<sub>2</sub> 0.5 M ; F4: Alginate 3.5% and BaCl<sub>2</sub> 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.

#### RESULTS AND DISCUSSION

The Aerosolization technique produced homogenous, small, smooth and spherical microspheres. Small particle size of less than 8 μm was produced by increasing alginate and BaCl<sub>2</sub> concentration (Table 2).

Table 2. Particle size of formula F1,F2.F3 and F4.

Formula	Average of particle size (μm)
F1	6.54
F2	5.22
F3	4.99
F4	3.73

using different concentration of alginate polymer and BaCl<sub>2</sub> crosslinker.

This smaller ovalbumin-loaded alginate microspheres were suitable for oral administration. Mishra et al (2008) that immune response after oral administration could be achieved from microspheres with 1-30 μm in size. Manjanna et al (2010) reported that by increasing concentration of Ba<sup>2+</sup> formed



spherical and smaller microsphere's size. This report was in agreement with Joshi et al (2012) and Singh dan Kumar (2012).

Figure 1 shows that almost spherical morphological microspheres were produced by scanning electron microscope. Some rough surface was maybe caused by no cryoprotectant agent to protect microspheres during freeze drying was added to stabilize microspheres.



F2

F4

Figure 1. Scanning electron microscope of ovalbumin-loaded alginate microspheres

Encapsulation efficiency, protein loading and yield of microspheres can be seen in Table 3.

Table 3. 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

It was observed that larger amounts of BaCl<sub>2</sub> (from 0.5M to 0.75M), increased encapsulation efficiency 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<sup>2+</sup> that crosslinked with carboxylates from guluronic acid in alginate indicates more ovalbumin was entrapped within alginate microspheres (Gu-

lati, et. al., 2011). This trend was also similar to an increase of alginate concentration. The more number of alginate amounts, the more number of crosslinked alginate-BaCl<sub>2</sub>, resulted the more ovalbumin was encapsulated (Manjanna et al, 2010). Similar studies were also confirmed that encapsulation efficiency increased by increasing concentration of polymer and crosslinking agents (Joshi et al, 2012 ; Singh dan Kumar, 2012).

In terms of yield, similar results were shown. Alginate microspheres produced using both alginate concentration (2.5 and 3.5%) using highest concentration of CaCl<sub>2</sub> (0.75M) indicated the highest yield of about 72% compare to formulas produced using lower concentration of BaCl<sub>2</sub>. In the case of microspheres crosslinked using higher alginate concentration, the yield was also increased. This behaviour indicates that the more number of Ba<sup>2+</sup> contact with alginate provide a gel network that able to increase yield of microspheres (Jin et.al., 2009).

There was no significant differences of all loading's formula. This may be explained by similar strength and network between carboxylates and Ba<sup>2+</sup> ions produce similar amount of space for ovalbumin inside microspheres. In terms of release study, formula F2 and F4 were incubated in HCl medium for 2 hours followed by PBS buffer pH 7.4 for 1020 minutes.

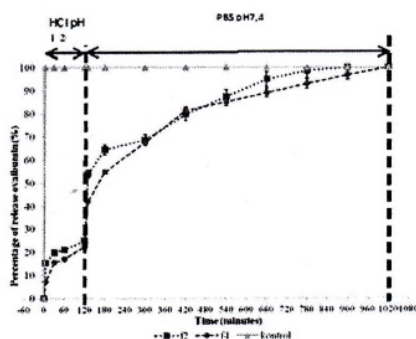




Figure 2. Profile of ovalbumin release from alginate microspheres

Results of release profile showed that ovalbumin-loaded alginate microspheres was able to protect protein from acid degradation, indicated by very small amount of ovalbumin was released during 2 hours incubation in acid pH (less than 25%) in both formulas (Figure 2). Moreover, 100% ovalbumin released from F2 microspheres in 900 minutes, whereas complete released of ovalbumin from F4 microspheres was occurred after 1020 minutes. However, the differences of ovalbumin release were not significant. It may be due to the similar swelling behaviour of alginate microspheres in pH 7.4 followed by diffusion of ovalbumin from the matrix. By increasing alginate concentration, the viscosity of alginate increased therefore avoid ovalbumin release from the matrix and slower the rate of release. From the results, this delivery system may be potential as protein or vaccine delivery system.

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