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Effect Of Incubation Time On Protein Loading And Encapsulation Efficiency Of Ovalbumin-Alginate Microspheres

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Abstract- The aim of this research was to investigate effect of incubation time that was important part on determination protein content method toward encapsulation efficiency and protein loading of microspheres. Determination method of encapsulation efficiency and protein loading used on this research by addition of sodium citrate solution 0.5 M pH 8.5 for 3, 6, 9, and 12 hours. Results showed that encapsulation efficiency and protein loading of ovalbumin-alginate microspheres after 3, 6, 9 and 12 hours incubation were above 99% and above 38% respectively. Based on statistical analysis, it was found that no significant differences between encapsulation efficiency and protein loading at different incubation times. Results of size before incubation was 5.87µm and after 3, 6, 9 and 12 hours incubation was 2.45µm; 2.38µm, 2.39µm, and 2.42µm respectively. Different incubation times produced similar size of particle; and same high encapsulation efficiency and protein loading as size. In addition, Biuret test demonstrated that protein was still existed after several hours' incubation.

Keywords: ovalbumin, alginate microspheres, incubation time, efficiency encapsulation, protein loading

Introduction

The development of proteins and peptides for therapeutic purposes has been widely increased. However, some drawbacks such as low stability, easily and quickly deactivated, short half-life and difficulties in oral absorbtion [1]. Microspheres for oral delivery system are an alternative to deliver the protein effectively. There was another problem in the development of microspheres related with accuracy of active content and precision in the determination of the amount of protein which is encapsulated in the microspheres. Some factors that affected microencapsulation techniques including manufacture process and microsphere formulations on the encapsulation efficiency of protein, protein loading as well as the amount of protein release.

Addition of sodium citrate pH 8.5 is one way method to break the microspheres and determine the protein content [2]. This method had advantages compare to other protein determination methods, especially in avoiding use of organic solvent, exposure from heat denaturation, overheating or overstirring and addition of chemical agents such as ammonium salt, heavy metals and alcohol. The disadvantages method was mostly resulted in irreversible denaturation [3]. According to Bilati *et al.*, 2005, several factors influenced the amount of protein released for example affinity of active agent to polymer, protein adsorption, instability during incubation period [4]. Ovalbumin-loaded alginate microspheres used Calcium Chloride as crosslinker was produced by ionotropic gelation method. Sodium alginate is used as polymer in the microspheres because of safe, cheap and biodegradable. Ca^{2+} was selected to crosslink with alginate forming hydrogel microspheres and has ability to produce strong gel and high potential[5].

The determination of protein using the current study was aiming to dissolve and break the microspheres. CBB (Coomassie Brilliant Blue) reagent to determine ovalbumin content has been used commonly resulting accurate, high sensitivity, fast and reproducible results. In previous study, same method has been conducted to determine ovalbumin content and encapsulation efficiency calculation; however 12 hours incubation time was the only time used [2]. Some researchs suggested that short incubation time was also efficient in producing as same high efficiency and loading as longer incubation time [6]. Therefore, this current study investigated effect of incubation time on the determination of protein loading and encapsulation efficiency.

Materials and Method

Alginate solution (2.5%) containing ovalbumin (2.5%) was sprayed into crosslinking agent $CaCl_2$ solution (1.5M) and was stirred continuously for 2 hours at 1000 rpm. The microspheres were collected by centrifugation at 2500 rpm for 6 minutes. 5% Lactose as lyoprotectant was added and finally microspheres were freeze dried 29 hours at -80°C. The particle size and morphology of microspheres was characterized by optical microscope. Freeze-dried ovalbumin-alginate microspheres were then incubated for 3,6,9 and 12 hours into Sodium Citrate pH 8.5 and were named F1, F2, F3 and F4 respectively (Table 1). Protein loading, encapsulation efficiency were then calculated using Protein Quantification Assay

(Coomassie Brilliant Blue/ CBB Assay). To investigate the presence of ovalbumin protein after several hours' incubation times, Biurett test was conducted.

Results and Discussion

Aerosolization technique produced spherical ovalbumin-Ca alginate microspheres with average particle size of 5.87 µm. Following incubation, particle size of microspheres were measured as well as protein loading and encapsulation efficiency. Summary of the particle size, protein loading and encapsulation efficiency of four formulas is presented in Table 1

Table 1 Particle size, protein loading and encapsulation efficiency of ovalbumin-Ca alginate microspheres

Formula	Protein Loading (%)	Encapsulation Efficiency (%)	Particle Size (µm)
F1	38.25 ± 4,61	99.10 ± 0,93	2.45
F2	38.25 ± 4,63	99.09 ± 0,96	2.38
F3	38.24 ± 4,62	99.08 ± 0,97	2.39
F4	38.24 ± 4,58	99.09 ± 0,88	2.42

Particle size of the microspheres before incubation (5.87µm) was bigger than after 3, 6, 9 and 12 hours incubation at around 2.4µm. This smaller size may be due to Ca-alginate microspheres especially the crosslinked part (Ca^{2+}) were dissolved in the alkalinized solution of sodium citrate pH 8.5 caused microspheres erosion, irregular morphology until formed clear solution. This is as mentioned by Rowe et al [5]. Replacement of Ca^{2+} with Na^{+}, reduced density of alginate gel and affected on the gel strength [7]. The complete process of microspheres from diffusion, dissolution, and erosion commonly was taken longer, however this study found that after 3, 6, 9 and 12 hours there was no significant differences of particle size of about 2.4 µm indicated that after 3 hours the microspheres were completely dissolved to release the ovalbumin protein.

For protein loading and encapsulation efficiency, results showed that no significant differences between all formulas after different time incubation. Based on particle size, shortened incubation time (3 hours) produced same size as longer time (12 hours), this caused ovalbumin protein released from alginate microspheres therefore loadings and efficiency were calcucated. Some literatures mentioned that addition of NaOH, DMSO and SDS were able to obtain protein recovery and loadings of protein from PLGA microspheres after 1 hour incubation. Higher encapsulation efficiency was also reported using SDS alkalinized after 12 hours. They discussed that the optimum method for production of microspheres was able to break the microspheres and released the active agent in any incubation time. This explanation confirmed that the method of this study using sodium citrate at pH 8.5 is the best method to break the microspheres completely; therefore different incubation time did not affect the protein loadings and efficiency.

In order to confirm whether protein was present after several hours incubation, Biurett test showed that protein was identified and existed by violet color in the solution after 3, 6, 9 and 12 hours incubation. All results in this study suggested further recommendation that it was only needed 3 hours incubation to break the Ovalbumin-Ca alginate microspheres and released the protein.

Conclusion

No significant differences were found in particle size, protein loading and entrapment efficiency following incubation of ovalbumin-Ca alginate microspheres in 3, 6, 9 and 12 hours. This tudy recommended that determination of optimum protein loading and encapsulation efficiency can be achieved during 3 hours incubation.

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