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RESEARCH ARTICLE

URL of this article: <http://heanoti.com/index.php/hn/article/view/hn20905>

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## Preparation and characterization of BSA-loaded Chitosan Microspheres

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### ABSTRACT

Proteins are unstable molecules because of their complex structure and easily degraded by enzymatic systems. Biodegradable microspheres have been used in protein delivery system. Bovine serum albumin was chosen as a model protein. Chitosan/tripolyphosphate microparticles have already been used to encapsulated BSA. The aim of this work was to microencapsulate these protein-CS/TPP by ionic gelation and freeze-drying. The morphology, particle size, and encapsulation efficiency of the prepared microspheres were investigated. The results revealed that the microspheres exhibited good sphericity with ranged in size from 2.25 to 2.73  $\mu\text{m}$ . The study found that the moisture content of microspheres was 2.8%, the encapsulation efficiency of BSA in CS-TPP microspheres ranged from 88.14 to 96.25%. The results suggested that ionotropic gelation method will be an effective method for fabricating chitosan microspheres for delivery of protein..

**Keywords:** Microspheres, BSA, Ionic Gelation

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### INTRODUCTION

#### Background

Inhalation therapy was development in decades with the specificity to delivery peptides, proteins and drugs with small molecules within uptake in the lung<sup>(1)</sup>. Inhalation one of the treatment process by inhaling the drugs, that can directly enter in to the lungs as the target organ<sup>(2)</sup>.

Pharmacokinetic studies of inhaled macromolecular therapy showed very high bioavailability, with the large surface area of alveolar, low fluid volume on the lung surface, ultra thin diffusion layer, and paracellular transport ranged in pore size 1-5 nm<sup>(1)</sup>. Delivery of protein/peptide in inhalation route is difficult because the process of denaturation proteins during manufacture, aerolization and storage, inefficient deliver. To overcome this problem, biocompatible polymer-based delivery system can be used to improve protein stability and increase absorption through the lungs, improve the aerodynamic properties of particles, and have a controlled release effect and free from natural phagocytosis clearance in the lungs<sup>(3)</sup>.

Polymeric nanoparticles (NPs) have been an attractive approach for lung delivery due to their ability to enter intracellular compartments and escape macrophages phagocytosis. Furthermore, they provide the possibility of achieving high drug loading capacity, control release, increased stability and high drug absorption and appropriate therapeutic target deposition<sup>(4)</sup>. Chitosan is the second longest polymer after cellulose and the only alkaline polysaccharide in nature, with a low immunity that makes chitosan possible as a carrier for drug delivery<sup>(5)</sup>. Chitosan-based polymers in nano- microparticles are mucoadhesive and able to open tight junctions between epithelial cells. The characteristics can stimulate the absorption of proteins/antigens by epithelial cells and macrophage cells<sup>(6)</sup>.

Bovine serum albumin (BSA) has the isoelectric value of 4.7 and was used as a protein model<sup>(7)</sup>. To delivering BSA as a protein model, chitosan has a major role affecting the shape of the microsphere, 2% chitosan in the formula produces a microspheres with a very spherical shape with a homogeneous microspheres size<sup>(8)</sup>. Several methods can be used to create chitosan particulate systems, such as ionic gelation, emulsion-droplet coalescence, co-preservation/precipitation, and reverse micellar<sup>(9)</sup>. Microspheres were prepared by ionotropic gelation methods with aerolization techniques has the advantage of maintaining the integrity of proteins trapped

in microspheres<sup>(10)</sup>. Chitosan microparticles were prepared by crosslinking with tripolyphosphate (TPP) through ionic interactions between the positive charge of the amino group and the negative ion content of the TPP<sup>(11)</sup>. Enhanced crosslinker concentration in microspheres making increasing efficiency encapsulation, because the rigid and complex structure of particles are formed and can enhanced the drug content of the drug<sup>(12)</sup>.

Based on the previous consideration, we hypothesized that these TPP concentration can effect on physical the characteristics of microspheres and bovine serum albumin will be trapped in a microsphere system using ionic gelation method with freeze drying with an smaller particle size.

## METHODS

### Chemicals

Chitosan with a dacencylation degree of 87.7% was purchased from Biotech Surindo (Cirebon, Indonesia), Bovine Serum Albumin (BSA) was purchased from Sigma-Aldrich, Castle Hill, NSW, Australia. TPP was obtained from Sigma-Aldrich. Maltodextrin (Dextrose equivalence 12-15) was obtained from Qingdao Shengda Commercial & Trade Co.,Ltd, All other reagents were of analytical grade and were used without further purification.

### Preparation of chitosan microspheres

Ionic gelation with freeze drying was used as a method in this study. Amount of BSA was dissolved in aqua dem. Acetic acid solutions was used to make the swelling properties of chitosan by stirred at 500 rpm. BSA solution was added into chitosan solution. TPP solution were prepared by dissolving TPP in aqua dem. Dispersion solution of BSA-chitosan was sprayed into an aqueous TPP cross-linking solution via peristaltic pump using speed controller. Microspheres were purified were centrifugated for 15 minutes at 2500 rpm to wash the crosslinker and acetic acid in solution. The sediment then was suspended in water. Freeze-dried BSA-loaded CS microspheres were prepared by adding maltodextrin as a lyoprotectant. Samples were initially frozen by storing at -80 °C overnight and freeze-dried.

### Size and Surface Morphological

Microsphere particles were analyzed to provide data on particle size and size range using low angle laser light scattering (Mastersizer2000, Malvern Instruments, United Kingdom). The particle size was directly determined and was estimated as mean of 300 particle measurements (n=300) using optical microscopy.

### Fourier transform infrared (FTIR)

FTIR spectra analysis was performed for the microsphere particles. Before analysis, 2 mg samples was mixed with 300 mg KBr powder to for a pellet. The KBr pellet was observe at 4000-450/cm using Jasco FT-IR 5300, Easton MD, USA.

### Determination of protein loading capacity of microspheres

Particles microspheres ( $\pm 100$  mg) was dissolved 30 ml aquadem and stirred at 1000 rpm for 4 hours and was centrifuged at 15,000 rpm for 20 minutes to precipitate particles. BSA concentration was measured using Bradford protein assay, measuring the absorbances by spectrophotometer UV/VIS V-630 (Jasco, jepang) at 586 nm. Each sample was assayed in triplicate (n=3). The encapsulation efficiency (E.E.) and loading capacity (L.C) of microspheres was determined as follows:

$$\text{E.E. (\%)} = \frac{\text{theoretical BSA amount} - \text{BSA in supernatant}}{\text{Theoretical BSA amount}} \times 100$$

$$\text{L.C. (\%)} = \frac{\text{theoretical BSA amount} - \text{BSA in supernatant}}{\text{Microspheres weight}} \times 100$$

### Statistical analysis

Statistical analysis was performed by two-way analysis of variance (ANOVA) for repeated measurements. The post hoc (Bonferroni and Scheffe) test was used to perform multiple comparison analysis (SPSS program, USA, version 15) and differences were considered significant at a level of  $p < 0.05$ .

**RESULTS**

Table 1. Composition of BSA-loaded CS microspheres and the effect of concentration TPP on physical characteristic BSA-loaded CS microspheres

Formula	Ratio chitosan:TPP			Σ Particle Size ± SD (μm)	Σ Entrapment Efficiency ± SD (%)
	BSA	Chitosan	TPP		
1	0.01	1	2	2.40 ± 0.20	96.25 ± 3.91
2	0.01	1	2.6	2.41 ± 0.31	92.51 ± 0.14

**Size and Surface Morphological**

The chitosan and TPP composition of the microspheres has no influence on their morphology, were illustrated in figured 1 and figured 2.

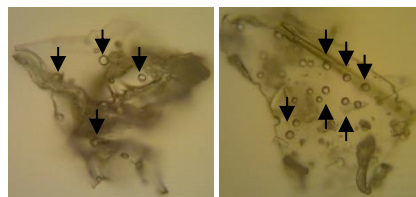


Figure 1. Optical photograph of BSA (A), chitosan (B), tripolyphosphate (C), and microspheres with chitosan-tripolyphosphate ratio: 1:2 (F1), 1:2.6 (F2) (magnification x4.000)



Figure 2. Organoleptic of chitosan microspheres with chitosan-tripolyphosphate ratio 1:2 (F1), 1:2.6 (F2)

**Fourier Transform Infrared (FT-IR)**

FTIR spectra of BSA-loaded CS microspheres are shown in Figure 3.

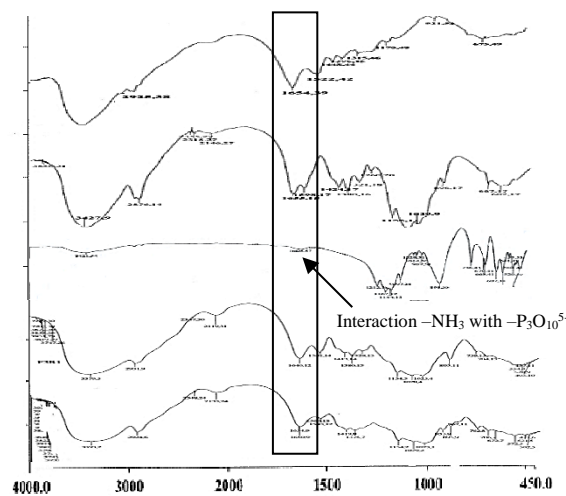


Figure 3. Fourier transform infrared spectra of BSA (A), chitosan (B), tripolyphosphate (C), and microspheres with chitosan-tripolyphosphat ratio: 1:2 (F1), 1:2.6 (F2)

## DISCUSSION

The characteristics of the BSA-loaded CS microspheres prepared with different concentration of chitosan were studied. The optical microscopy resulted BSA-loaded CS microspheres having spherical shape with mean sizes ranging from 2.38-2.41  $\mu\text{m}$  (table 1 and figure 1). Concentration range in this study, as TPP increases microscale on particles are formed. BSA-loaded CS microspheres had smaller size, indicate that using gelation ionic and stirring speed was 1000 rpm in this study resulted the smaller particles size. The ionic gelation method has several advantages such as the use of aqueous solutions, the preparation of small size particles, the control of particle size by the variation of parameters such as chitosan and TPP concentrations, and the possibility of encapsulation of a large range of molecules<sup>(13)</sup>. Stirring speed of 1000 rpm was found to be optimal, yielding microspheres of uniform size and with a narrow size range and spherical shape<sup>(14)</sup>. The structure of chitosan-microspheres was showed that the particles have a uniform spherical shape and smooth surface.

Data of FTIR spectra BSA-loaded CS microspheres resulted that the absorption band at wavenumber 1640  $\text{cm}^{-1}$  was higher, indicating that the amino groups on chitosan and TPP are involved in the formation microspheres. So we suppose that the phosphoric groups in tripolyphosphate are linkage with ammonium group of chitosan; inter- and intramolecular action are enhanced in BSA-loaded CS microspheres.

Table 1 shows the effects of concentration crosslinker on the efficiency encapsulation. To investigated the capability of BSA-loaded CS microspheres to encapsulated BSA, microspheres were prepared with various amounts of TPP. The BSA-loaded CS microspheres was resulted  $>90\%$ , indicated that the microspheres prepared with chitosan and TPP can trapped the BSA. The mechanical strength, physicochemical properties and particle absorption efficiency and extend drug release time were enhanced by crosslinking<sup>(15)</sup>.

## CONCLUSION

Although the drug delivery system based on chitosan/TPP microparticles has its own advantages, the efficiency encapsulation of macromolecules with smaller particles size. It has been found in this study that an increase in the concentration of TPP to promote the formation of a compact and sufficient cross-linking structure of chitosan/TPP particles that can enhanced the efficiency encapsulation. The particles size of BSA-loaded CS microspheres were formed in smaller size indicate the microspheres will be an effective system for delivery the protein.

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