

CHITOSAN BASED PARTICULATE CARRIER OF DITERPENE LACTON OF SAMBILOTO PREPARED BY IONIC GELATION-SPRAY DRYING :EFFECT OF STIRRING RATE AND NOZZLE DIAMETER

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INTRODUCTION

Chitosan, a cationic polysaccharide has many advantages as carrier for drug delivery system such as biocompatible, biodegradable and non toxic. Chitosan has amino group that could be crosslinked with polyanion such as tripolyphosphate so that the active ingredient will be entrapped (Agnihotri, 2004, Sinha, 2004). Diterpene lactone fraction of sambiloto (*Andrographis paniculata*) has antimalarial activity but it has low solubility in water. Entrapped diterpene lactone into chitosan matrix could improve the bioavailability of the active substance.

The aim of this research is to investigate the effect of process parameter of chitosan carrier preparation : stirring rate (500 rpm and 1000 rpm) during ionic gelation and nozzle diameter (0.5 mm and 1.0 mm) of spray dryer on physical characteristics of diterpene lactone fraction-chitosan particles. Evaluation of morphology, thermal analysis and drug entrapment were conducted.

EXPERIMENTAL METHODS

Material and Methods

Material

Diterpene lactone fraction of sambiloto was obtained from Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, chitosan with deacetylation degree 85% was purchased from Biotech Surindo, Natrium tripolyphosphate, pro analysis grade from Nacalay Tesque. All other reagents used in this experiment were pro analysis grade.

Preparation of chitosan particles

Independent variable	Nozzle diameter	
Stirring speed	1.0 mm	0.5 mm
500 rpm	P1	P3
1000 rpm	P2	P4

Chitosan was dissolved in 0,15% acetic acid to make 0,1% chitosan solution. Preparation of diterpen lactone - chitosan particles was done by mixing chitosan solution and diterpene lactone fraction solution and then 0,1%tripolyphosphatesolution was added while stirring with two stirring speed.. The mixture was continuously stirred with magnetic stirrer for 2 hours. Subsequently the mixture was dried with Labplant SD-Basic Spray Dryer at 100°C, flow rate 5 ml/min, pressure 2 bar with two different nozzle diameter. The ratio of drug-chitosan-TPP was 4:10:8.

Evaluation of nanoparticles morphology

The particles was evaluated by Scanning Electron Microscopy (SEM) FEI Inspect S50. Particles were dried and coated with gold palladium and then observed for its shape and surface morphology.

Thermal analysis

Thermal analysis for diterpen lactone fraction of sambiloto, chitosan and nanoparticles was performed with Differential Thermal Analyzer (DTA) Metler Toledo FP 85. Samples were scanned from 50 to 250°C at a rate of 10°C/min.

Entrapment efficiency (EE)

5 mg sample was dissolved in 10 ml of ethanol then filtered. Solution was analyzed by HPLC Agilent 1100 with mobile phase of methanol: phosphoric acid pH 3=50: 50 at wavelength of 228 nm. The assays were performed in triplicate. The entrapment efficiency (EE) of diterpenelactone in chitosan nanoparticles was calculated by this equation :

$$EE = (\text{actual drug}/\text{theoretically drug}) \times 100\%$$

RESULTS

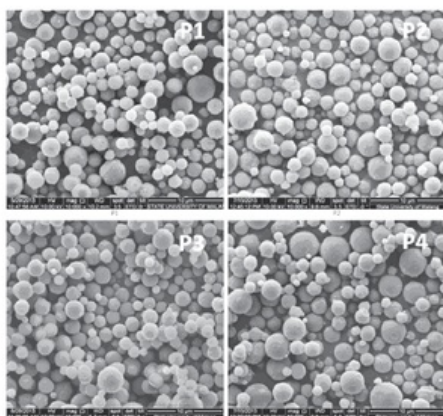


Figure 1 SEM micrographs of particles of diterpene lactone-chitosan prepared with different condition (mag 10.000x)

Sem photograph of particles diterpene lactone - chitosan (figure 1) showed that the particles have spherical shape and smooth surface with wide range particle size.

From DTA thermogram (Figure 2) it was indicated that endothermic peak of diterpene lactone appears at 222 °C and chitosan glass transition appears at 146.6 °C. Endothermic peak of diterpene lactone fraction was no longer exist in chitosan particulate system since it had been entrapped in chitosan matrix.

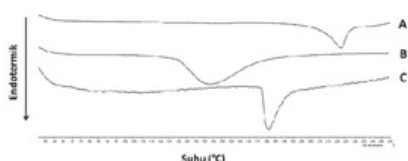


Figure 2. DTA thermogram of diterpene lactone (A), chitosan (B) and diterpene lactone-chitosan particles (C)

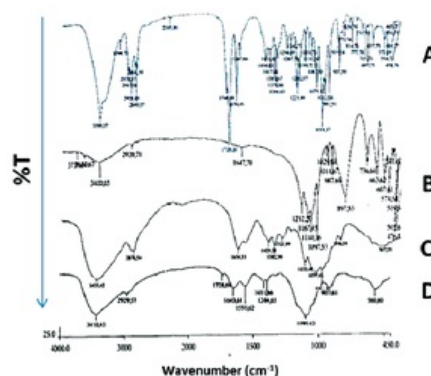


Figure 3. FTIR spectra of diterpene lactone (A), tripolyphosphate (B), chitosan (C), diterpene lactone-chitosan particles (D)

	Drug content ± SD (%)	EE ± SD (%)
P1	4.79 ± 0.04	26.36 ± 2.42
P2	3.84 ± 0.04	21.11 ± 2.04
P3	4.38 ± 0.02	24.12 ± 0.82
P4	3.82 ± 0.03	21.01 ± 1.69

Table 2. Drug content and Entrapment Efficiency (EE) of diterpene lactone- chitosan particles (n=3)

FTIR analysis was performed to confirm the crosslink interaction of chitosan and tripolyphosphate. Absorption band at 1643 cm⁻¹ attributed to amide bond of chitosan. New band at 1555 cm⁻¹ indicated hydrogen bond and 1643 cm⁻¹ band confirmed linkage between P3O5-5 of tripolyphosphate and NH₃⁺ of chitosan (Figure 3).

From drug entrapment efficiency, it was known that as stirring speed increased from 500 rpm to 1000 rpm, the entrapment of drug became lower decrease from about 24-26% to 21% (Table 2). From statistical analysis of one way Anova with α 0.05, it was known that drug entrapment efficiency of particles prepared with different stirring rate was significantly difference since nozzle diameter didn't affect the entrapment efficiency.



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CONCLUSION

The result showed that diterpen lactone – chitosan particles prepared by ionic gelation-spray drying with composition and condition in this study has spherical shape with wide range size from 400 nm to 4000 nm. Highest drug entrapment efficiency was obtained from particles prepared with 500 rpm stirring rate and 1,0 mm nozzle diameter.

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