PARTICULATE DELIVERY SYSTEM OF CHITOSAN - DITERPENE LACTONE FRACTION OF SAMBILOTO (*ANDROGRAPHIS PANICULATA* NEES): PREPARATION, CHARACTERIZATION AND *IN VITRO* DRUG RELEASE

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ABSTRACT

Objective: This paper was aimed to study the effect of chitosan, sodium tripolyphosphate (TPP) as crosslinker, and diterpene lactone fraction of sambiloto (FDTL) from *Andrographis paniculata* Nees which contained 75.9% andrographolide on the characteristics of the FDTL-chitosan particulate system. Those characteristics were the physicochemical interaction, physical state, morphology, and drug entrapment efficiency. The *in vitro* drug release of the selected FDTL-chitosan particulate system was also evaluated.

Methods: The particulate system of FDTL-chitosan was prepared by ionic gelation–spray drying method with the various amounts of TPP, chitosan, and FDTL. The physical characteristics were evaluated using Fourier transform infrared (FTIR), differential thermal analyzer (DTA), and X-ray diffraction, scanning electron microscope. *In vitro* drug release was performed in 0.1% sodium lauryl sulfate media at 37°C.

Results: The results of FTIR and DTA analysis were in accordance with the results of morphology evaluation which indicated that chitosan-TPP ratio 10:8 could produce chitosan particles with a spherical and smooth surface. FDTL has been trapped in chitosan particulate systems, and the crystallinity of FDTL changed. Particulate systems with FDTL-chitosan-TPP ratio - 4:10:8 showed better characteristics compared to others with entrapment efficiency of 33.82%. The dissolution efficiency at 360 minutes (ED_{360}) of particulate systems FDTL-chitosan was higher up to 1.5 times compared to FDTL.

Conclusion: The difference in the ratio of chitosan and TPP affected the morphology of chitosan particles since the amount of drug loaded, and the amount of chitosan affected the drug entrapment. The ED_{360} of FDTL of FDTL-chitosan-TPP increased up to 1.5 times compared to the FDTL.

Keywords: Chitosan, Diterpene lactone fraction, Particulate systems, Tripolyphosphate, Physical characteristics, Drug release.

INTRODUCTION

Drug carrier system has been developed in the past decade because it can deliver hydrophilic and hydrophobic drug molecules. Drug carrier system can be used for various purposes such as improving bioavailability, improve stability, lower toxicity, reduced side effects, and prevent drug interactions. Carrier used will affect the interaction of the drug with a carrier where the drug can be trapped in a carrier or form covalent bonds with the carrier. Type of drug carrier systems is a cellular carrier, the lipid carrier, carrier, and other particulate matter [1,2].

Chitosan particulate system is a common drug carrier that can be used to enhance the dissolution of the poorly soluble drug. As a result, the drug bioavailability will also increase hence results in better drug efficacy. Chitosan is a polycationic polysaccharide which is copolymers of glucosamine and N-acetylglucosamine. This material is biodegradable, biocompatible, and nontoxic. Chitosan has primary amine group ($-NH_2$) on the main backbone structure that causes chitosan to have positively charged surface and enables preparation of the particulate system (microparticles or nanoparticles) using ionic gelation method. The ionic gelation method is based on the crosslinking reaction between chitosan and a polyanionic substance such as tripolyphosphate (TPP) as the crosslinker. The ratio of polymer and crosslinker is related with NH^{3+} protonated from chitosan that could interact with $P_3O_{10}^{5-}$ of TPP. The electrostatic interaction between NH^{3+} and $-P_3O_{10}^{5-}$ will affect the physicochemical characteristics including the physical and chemical interactions, matrix density, morphology, particle size, and drug entrapment, which could further affect the drug release, bioavailability, and effectiveness of the drug [3-8].

Sambiloto (*Andrographis paniculata* Nees) is a medicinal plant that has many activities such as analgesic, antipyretic, anti-inflammatory, hepatoprotective, antiviral, antithrombotic, and anticancer [9,10]. The diterpene lactone compound of sambiloto is andrographolide, which has low oral bioavailability due to its high lipophilicity (log P=2.632), low solubility in water (3.29±0.73 mg/L), and short biological half-life [11,12]. Andrographolide is known to inhibit the growth of malaria parasite, *Plasmodium sergei* [13]. Nevertheless, the poor solubility of this plant compounds hinders its effectiveness to treat this disease effectively. Entrapment of the poorly soluble substance in the hydrophilic polymers, such as the chitosan particulate system, could improve dissolution of this poorly soluble plant compound hence increases its bioavailability and effectiveness.

This research is aimed to enhance the characteristics of diterpene lactone fraction of sambiloto (FDTL) containing 75.9% andrographolide which is poorly soluble substance by forming a particulate system with chitosan. The particulate system was prepared by ionic gelation–spray drying method in the various amounts of chitosan, FDTL, and TPP as a crosslinker. The interaction between the polymer and crosslinker, thermodynamics, solid state, morphology, drug content, and *in vitro* drug release of the formed chitosan particulate system was studied.

MATERIALS AND METHODS

Materials
FDTL containing 75.9% andrographolide was obtained from Pharmacognosy and Phytochemistry Department, Faculty of Pharmacy, University Airlangga. Low viscosity chitosan (DD 85%, pharmaceutical

grade) was purchased from Biotech Surindo, and pentasodium TPP (analytical grade) was obtained from Nacalay Tesque. Acetic acid glacial (Emsure®) and all other reagents used in this experiment were of analytical grade.

Methods

Preparation of chitosan particles

The unloaded chitosan particles were produced by ionic gelation–spray drying methods with the ratio of chitosan:TPP (as a crosslinker) vary from 10:1 to 10:10. Chitosan solution was prepared by dissolving 100 mg chitosan in 100 ml of 0.15% acetic acid solution. Various concentrations of TPP solution were prepared by dissolving TPP (10-100 mg) in 80 ml aqua dest. TPP solution was then added dropwise into chitosan solution while stirring at 500 rpm. The mixture was continuously stirred for 1 hr. Following to this, each mixture of chitosan-TPP was then spray-dried using SD-Basic Labplant spray dryer. The spray drying process was performed with using 0.5 mm nozzle diameter at inlet temperature 100°C, the flow rate of 5 ml/min and pressure at 2 mBar. The dry particles were collected and analyzed by Fourier transform infrared (FTIR), differential thermal analyzer (DTA), and scanning electron microscope (SEM). From the results obtained, one optimum composition of chitosan-TPP was then selected and used for the preparation of FDTL loaded chitosan particles.

Preparation of FDTL loaded chitosan particles

The drug-loaded particles were prepared with compositions as detailed in Table 1. Chitosan solution was added into FDTL solution in ethanol. Then, the TPP solution was added dropwise to the mixture solution of FDTL-chitosan while stirring at 500 rpm. The mixture was stirred continuously for 1 hr. Then, the process was continued as mentioned above.

Fourier transform infrared (FTIR)

FTIR spectra analysis was performed for the unloaded chitosan particles, and FDTL loaded chitosan particles. Before analysis, about 2 mg samples was mixed with 300 mg KBr powder then pressed to form a pellet. The KBr pellet was observed at 400-450/cm using Jasco FT-IR 5300, Easton MD, USA.

Particles size and morphology

The particles size and morphology were evaluated using SEM FEI, Type Inspect S-50 in various magnifications. Particles were dried and coated with gold palladium before the SEM analysis.

DTA

Thermal analysis of FDTL, chitosan and the FDTL-chitosan particles obtained were performed with DTA (FP-65 P900 Thermal, Mettler Toledo, USA). Approximately 5 mg samples were scanned from 50 to 250°C with a heating rate of 5°C/min.

X-ray diffraction (XRD)

The diffraction pattern of unloaded particles and loaded particles of chitosan were recorded using X-ray diffractometer (X'Pert analytical, The Netherlands). The analysis was conducted at room temperature at 40 kV voltages and 40 mA current. The angle of 2Ø was set between 5 and 40°.

Table 1: Composition of FDTL-chitosan particles

Formula	Chitosan (mg)	TPP (mg)	FDTL (mg)
A	100	80	30
B	100	80	40
C	100	80	50
D	100	80	40
E	125	100	40
F	150	120	40

FDTL: Diterpene lactone fraction of sambiloto

Drug loading and entrapment efficiency

About 5 mg sample was dissolved in 10 ml ethanol then filtered and analyzed by high-performance liquid chromatography (HPLC) (Agilent 1100 Series), HPLC column (Merck LiChrospher® 100 RP 18, 4 mm × 250 mm, 5 µm). The mobile phase consists of methanol: Orthophosphoric acid pH 3=50:50. The flow rate was set at 0.75 µl/min. The sample was measured at wavelength 228 nm. The assays were performed in triplicate. The drug loading and entrapment efficiency were calculated using the following equations:

$$\% \text{ Drug content} = \frac{\text{Drug amount}}{\text{Particle weight}} \times 100\% \qquad (1)$$

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug amount}}{\text{Theoretically drug amount}} \times 100 \qquad (2)$$

In vitro drug release

The *in vitro* drug release test was carried out in 0.1% w/v sodium lauryl sulfate (SLS) in water. SLS was used to improve the dissolution of andrographolide [14]. FDTL-chitosan particles equivalent with 1 mg FDTL was weighed accurately and sprinkled in the medium. The test was conducted in 25 ml medium at 37±0.5°C in water bath shaker. 0.5 ml sample was taken at a predetermined time during 24 hrs and analyzed by HPLC (Agilent 1100 Series), HPLC column (Merck LiChrospher® 100 RP 18, 4 mm × 250 mm, 5 µm) using ethanol – orthophosphate pH 3=5:5. Replacement of the medium was done at each sampling time with the same volume of the withdrawn sample.

RESULTS AND DISCUSSION

In this study, unloaded chitosan particles prepared with ionic gelation–spray drying with various amount of TPP to study the effect of TPP amount on chitosan particles formation. The dry particles obtained were analyzed using FTIR spectrophotometer, SEM, and DTA.

FTIR

As showed in Fig. 1, the FTIR spectra indicated that increasing TPP amount changed the vibrational pattern of the infrared spectra. The spectra of particles with chitosan-TPP at ratio 10:3 showed a new absorption band at 1640-1650/cm. This indicates the bond between NH^{3+} ions of chitosan with $-P_3O_{10}{}^{5-}$ of TPP. The intensity of the absorption band at 1640-1650/cm was higher as TPP amount increased. This result indicated an increase in the crosslinking degree and inter- and intramolecular interactions of chitosan. As chitosan – TPP ratio >10:5, the intensity of the ionic interaction between $-NH^{3+}$ cations of chitosan and multivalent anions $-P_3O_{10}{}^{5-}$ of TPP increased along with the availability of $-P_3O_{10}{}^{5-}$. Increasing the ionic interaction caused an increase in the density of inter- and intramolecular bonds of the cross-linked chitosan. This resulted in strengthening the density of cross-linked chitosan structure that generates spherical particles with a smooth surface structure.

Particles size and morphology

The morphology of unloaded particles was showed in Fig. 2. Morphology of chitosan particulate systems observed by SEM showed that the particles produced from chitosan-TPP with ratio 10:1-10:5 had a hollow structure. In ratio chitosan-TPP=10:1 particles had rough surface structure, and aggregate mass showed that the cross-linked bond between chitosan and TPP did not occur and the particles formed was more affected by the amount of chitosan compared with TPP. Furthermore, as the chitosan-TPP ratio was higher than 10:6, the unloaded chitosan particles had spherical and smooth surface. Increasing TPP amount strengthened the crosslinking between $-NH^{3+}$ ions of chitosan with $-P_3O_{10}{}^{5-}$ of TPP as shown by the FTIR spectra in Fig. 1. Increasing TPP amount formed more solid particles with a fewer hollow. The ratio of chitosan-TPP=10:8 could form particles with a spherical shape and smooth surface structure (Fig. 2). The addition of FDTL in the chitosan particles did not affect the morphology of the particles as shown in Fig. 3.
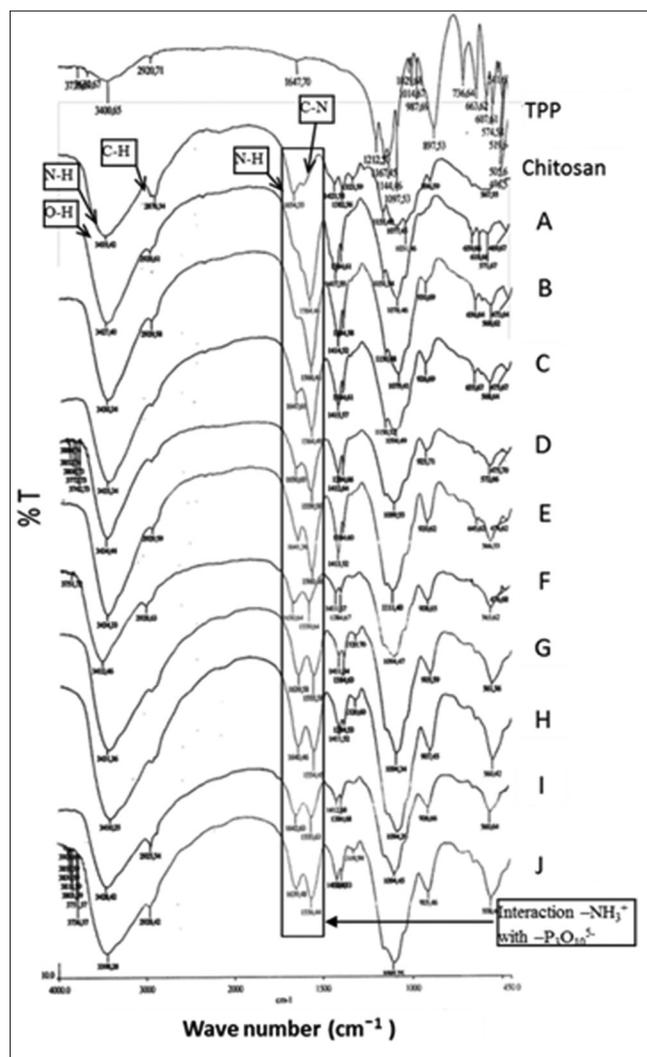
Fig. 1: Fourier transform infrared spectra of tripolyphosphate (TPP), chitosan and unloaded chitosan particles with chitosan-TPP ratio: 10:1 (A), 10:2 9 (B), 10:3 (C), 10:4 (D), 10:5 (E), 10:6 (F), 10:7(G), 10:8 (H), 10:9 (I), 10:10 (J)

DAT

The results of the DTA as in Fig. 4 showed that the unloaded particles of chitosan-TPP with the ratio of 10:1-10:3 had melting range lower than chitosan and broad endothermic peak as chitosan. Furthermore, in particles with a ratio of chitosan-TPP=10:4 and 10:5, the endothermic peak broadened. As the ratio of chitosan-TPP increased, thermogram pattern became narrower due to the interaction between the molecules of chitosan and TPP which form ionic bonds so that the molecular structure of chitosan particulate system changed. Particles with chitosan-TPP ratio 10:8 had a sharp endothermic peak, indicating an optimal chemical interaction between the $-NH_3^+$ and $-P_3O_{10}^{-5}$ resulting in more ordered structure than the other compositions. This is confirmed by the data of FTIR spectra (Fig. 1) in which the intensity of the absorption band at wavenumber 1640/cm was higher. Chitosan is a polysaccharide that is able to absorb the water and in the solid state has an irregular structure that is easily hydrated. The intensity of crosslinking in different degrees will lead to differences in the water absorption of the particulate system that affect the crystallinity of chitosan. The presence of hydrogen bonds, ionic intramolecular and intermolecular bonding in the structure of the particles will form a strong bond that requires higher energy to dehydration [15].

Evaluation of the FTIR and DTA results was in accordance with the results of morphology evaluation by SEM. From the details of SEM
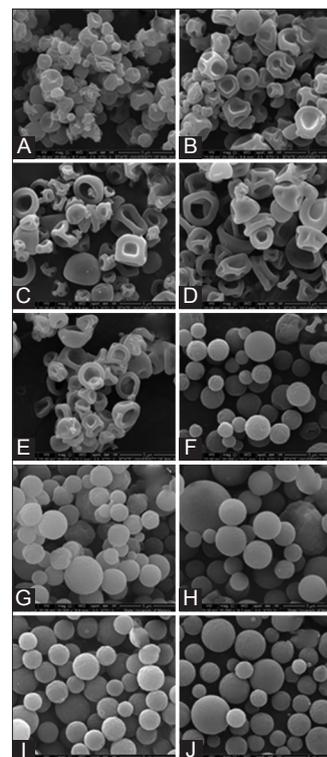
Fig. 2: Scanning electron microscope photograph of unloaded chitosan particles with chitosan-tripolyphosphate ratio: 10:1 (A), 10:2 9 (B), 10:3 (C), 10:4 (D), 10:5 (E), 10:6 (F), 10:7 (G), 10:8 (H), 10:9 (I), 10:10 (J) (magnification, ×20,000)

analysis on Fig. 2, the particle surface structure and sphericity proved that the ratio of chitosan-TPP 10:8 could form particles with good physical characteristics in terms of the interaction between chitosan and TPP, their sphericity as well as the surface structure. However, the particles sizes were in wide ranges of 500 nm to 5 μm.

Furthermore, the results of thermal analysis with DTA in Fig. 5 showed that the FDTL loaded chitosan particulate system with chitosan–FDTL-TPP with ratio of 10:3:8 and 10:5:8 had lower melting point (144°C) with broadening endothermic peaks compared to the unloaded system with chitosan-TPP ratio 10:8 (157°C). The addition of FDTL altered the arrangement of chitosan particulate system molecular structure. The thermogram of particles with FDTL-chitosan-TPP ratio=10:4:8 was different from other systems. The thermogram showed a sharper endothermic peak with lower melting range (181.6°C) compared to FDTL. This indicated that the particulate system with FDTL-chitosan-TPP ratio=10:4:8 had more stable and ordered molecular structure than the other formulas.

XRD

The XRD in Fig. 6 showed that FDTL has sharp peaks indicated a crystalline structure of FDTL while chitosan had no sharp peaks that denoted as an amorphous structure. From diffractogram of FDTL-chitosan particulate systems, it was seen that crystalline peak of FDTL disappeared, whilst the sharp peak of FDTL in the physical mixture of FDTL-chitosan still observed. This verified that FDTL had been trapped in chitosan particulate systems and the crystalline structure altered due to the recrystallization barriers of cross-linked chitosan. Entrapping FDTL in cross-linked chitosan and rapid drying during spray drying also hindered the growth of crystals so that drug particles trapped in the smaller size. Since a crystalline form of a drug has low solubility, therefore change in crystalline structure to the amorphous structure/unordered structure would be advantageous. Since the amorphous form has a higher solubility than the crystalline form, this change could increase the bioavailability of the drug [16].
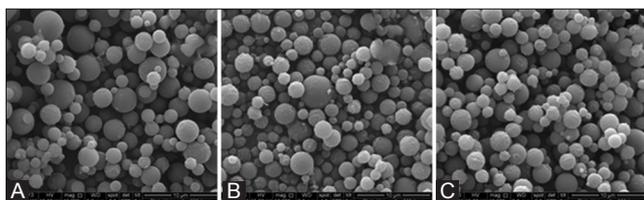
Fig. 3: Scanning electron microscope photograph of diterpene lactone fraction of sambiloto (FDTL) loaded chitosan particles with FDTL-chitosan- tripolyphosphate ratio: 3:10:8 (A), 4:10:8 (B), 5:10:8 (C) (magnification, ×10,000)
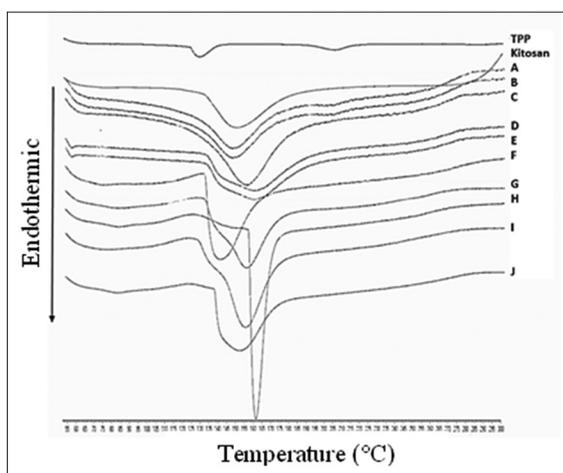

Fig. 4: Differential thermal analyzer thermogram of unloaded chitosan particles: 10:1 (A), 10:2 (B), 10:3 (C), 10:4 (D), 10:5 (E), 10:6 (F), 10:7 (G), 10:8 (H), 10:9 (I), 10:10 (J)
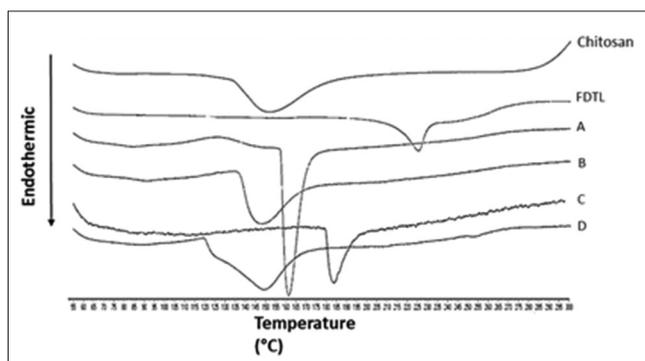

Fig. 5: Differential thermal analyzer thermogram of chitosan, diterpene lactone fraction of sambiloto (FDTL), and unloaded particles (A), FDTL loaded particles with FDTL-chitosan-tripolyphosphate ratio 3:10:8 (B), 4:10:8 (C), 5:10:8 (D)
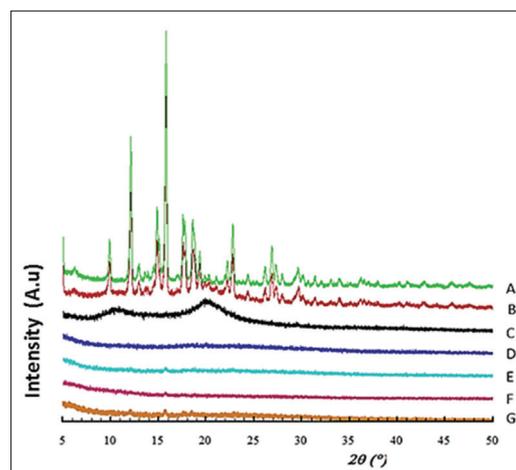

Fig. 6: X-ray diffractogram of the diterpene lactone fraction of sambiloto (FDTL) (A), physical mixture FDTL-chitosan (B), chitosan (C), unloaded particles (D), FDTL loaded particles with FDTL-chitosan-tripolyphosphate ratio 3:10:8 (E), 4:10:8 (F), 5:10:8 (G)
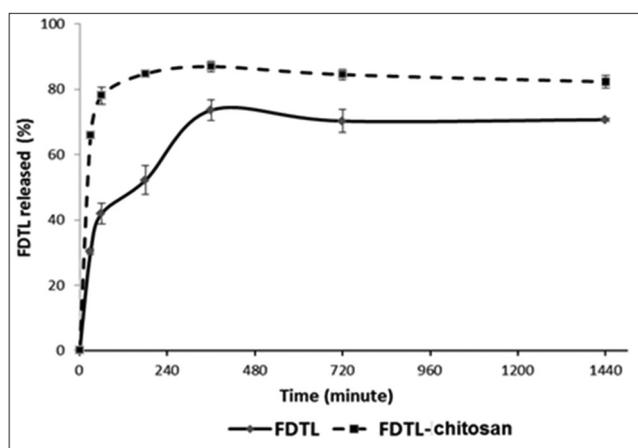

Fig. 7: The release profile of the particulate system diterpene lactone fraction of sambiloto (FDTL)- chitosan-tripolyphosphate and FDTL in 0.1% sodium lauryl sulfate in water medium at 37±0.5°C (n=3)

Drug loading and entrapment efficiency

Particulate systems of FDTL-chitosan-TPP were prepared with various amounts of chitosan and FDTL to investigate the capability of the system to entrap drug. From evaluation of the entrapment efficiency, it was found that increasing FDTL amount will lead to an increase in the entrapment efficiency. From the statistical analysis of one-way ANOVA followed by least significant difference test, it was known that formula A to B and A to C was significantly different, but there was no significant difference between B and C (Table 2).

Increasing the amount of polymer will increase the viscosity of the polymer result in a better ability of drug entrapment of particulate systems [3]. However, the results showed that increase in chitosan concentration did not improve the entrapment ability of chitosan particulate systems (Table 3). This can be explained that the particles with chitosan-TPP ratio 10:8 have cross-linked structure that could entrap drug/substance in a limited amount. From the results of particles characterization SEM and the entrapment efficiency, it was known that the particulate systems with FDTL-chitosan-TPP ratio=4:10:8 had optimal characteristics. Chitosan particles of this composition were further evaluated for it's *in vitro* drug release.

In vitro drug release

From *in vitro* release study, it was known that the FDTL dissolved from the chitosan particles was higher than FDTL itself. The entrapment of FDTL in the chitosan particulate system changed the crystallinity of FDTL as revealed Fig. 7. This change from the ordered structure into unordered molecular structure caused lower melting point hence will enhance dissolution rate. Dissolution efficiency at 360 minutes (ED_{360}) of the particulate systems FDTL-chitosan-TPP and FDTL was 78.82% and 51.41%, respectively. From the paired t-test analysis, it was known that ED_{360} of FDTL-chitosan particulate systems and FDTL was significantly different (Table 4).

Table 2: One-way ANOVA test (p=0.05) to determine the effect of FDTL amount on the entrapment efficiency of FDTL-chitosan particles

Formula	N	Entrapment efficiency±SD	ANOVA	
			Result	Conclusion
A	3	29.43±0.25[a]	F=34.702 p=0.001	Significantly different
B	3	33.82±0.38[b]		
C	3	33.47±1.16[b]		

The ratio of the FDTL-chitosan-TPP=3:10:8 (A), 4:10:8 (B), 5:10:8 (C).
[a,b,c]Signs mean that there is no difference between the groups. N=Sample number, TPP: Tripolyphosphate, FDTL: Diterpene lactone fraction of sambiloto

Table 3: One-way ANOVA (p=0.05) to determine the effect of chitosan amount on the entrapment efficiency of FDTL-chitosan particles

Formula	N	Entrapment efficiency±SD	ANOVA	
			Result	Conclusion
D	3	33.82±0.38[a]	F=78.895 p=0.000	Significantly different
E	3	26.58±0.49[b]		
F	3	25.65±1.38[b]		

The ratio of FDTL: chitosan: TPP ratios are 4:10:8 (D), 4:12.5:10 (E) and 4:15:12 (F). [a, b, c]Signs refer to no difference between the groups. N=Sample number, FDTL: Diterpene lactone fraction of sambiloto

Table 4: Paired t-test (p=0.05) for ED_{360} of FDTL-chitosan particulate system and FDTL

Formula	N	Mean ED_{360}±SD	Paired t-test	
			Result	Conclusion
FDTL-chitosan	3	78.82±0.38[a]	t=8.567 p=0.025	Significantly different
FDTL	3	51.41±0.49[b]		

[a, b]Signs refer to no difference between the groups. N=sample number
ED_{360}: Efficiency dissolution at 360 minutes, FDTL: Diterpene lactone fraction of sambiloto

CONCLUSIONS

1. The difference in the ratio of chitosan and TPP had a high correlation of the morphology of chitosan particles such as the shape and surface structure
2. The amount of drug loaded and the amount of chitosan affected the drug entrapment ability of chitosan particles
3. The particulate system of FDTL-chitosan-TPP was able to improve the ED_{360} of FDTL up to 1.5 times compared to the FDTL. To sum up, the results obtained in this study can be used as a guidance to develop a particulate system of chitosan and also for developing other polymers of the particulate system.

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