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Factors influencing physicochemical property of *T. divaricata* extract loaded SLN

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INTRODUCTION AND OBJECTIVE

Tabernaemontana divaricata, a local plant widely distributed in Thailand, has been used for century as Thai folk medicine for treatment of fever. The major types of phytochemicals are alkaloids (van Bark 1984). Previous reports indicated that the plant extract had antipyretic and anti-inflammatory actions (Taesotikul 1989; Pratchayasakul 2008). Our preliminary in vitro study found that the anti-inflammation of the crude ethanolic extract of *T. divaricata* was through the inhibition of cyclooxygenase (data not shown). However, the physical characteristic of the extract such as solubility, texture and odor was not suitable for clinical use. The encapsulation of the extract in solid lipid nanopaticles (SLN) is an alterative to improve its properties. The aim of this study was to encapsulate *T. divaricata* ethanolic extract in the SLN. The SLN was prepared by high pressure homogenizer. The factors influencing physicochemical characteristics of the extract loaded SLN, i.e. number of homogenization cycle, amount of lipid and emulsifiers in the formula were investigated.

MATERIALS AND METHODS

Preparation of the extracts *T. divaricata* was collected in Chiang Mai, Thailand. The specimen of plant was deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai University. The stem of the plant sample was used in this study. The dried plant sample powder was prepared and macerated in ethanol. The solvent of the extracted filtrate was removed by using a rotary evaporator. The obtained extract was kept in refrigerator for further investigation.

Production of SLNs
The SLNs was prepared by using hot high pressure homogenizer technique (Muller 2000) under different processing parameters. The lipid phase, comprising 2.5% to 7.5% w/w cetyl palmitate as solid lipid ingredient, and 0.25% w/w *T.divaricata* extract as an active constituent, was heat at 70 °C. At same temperature, the water phase, comprising 10% to 20% w/w Tween 80 and Span 80 mixture as an emulsifier system, was heat and dispersed into lipid phase. A coarse pre-emulsion was formed under high-speed stirring using a Polytron® PT 3000 homogenizer at 8000 rpm for 5 min. This hot pre-emulsion was then rapidly passed through the high pressure homogenizer (Avestin C3, Canada) at pressure 1000 bars for 3 and 6 cycles, respectively. After homogenization, the produced o/w nanoemulsion was cooled down to 4 °C until the internal oil phase was solidified.

Measurement of particle diameter and zeta potential of nanoparticles The measurement of particle size diameter, polydispersity index and zeta potential of nanoparticles was performed by using photon correlation spectrophorometer (PCS) (Zetasizer ZS, Malvern Instruments, UK). All formulations were diluted with milli Q water to appropriate concentration. The polydispersity index measured the size distribution of the nanoparticle population. Each value reported was the average of at least three measurements.

RESULTS AND DISCUSSION

Effect of emulsifier concentration and homogenization cycle The influence of surfactant as a principle emulsifier of the SLN on SLN particle size and zeta potential was studied by using various amount of surfactant, i.e. 10%, 15%, and 20% w/w of the mixture of Tween80 and Span80 while maintaining amount of cetyl palmiate at 5% w/w, and *T. divaricata* extract at 0.25%w/w. The result showed that the particle size and the polydispersity index increased when the amount of surfactant increased from 10% to 20% w/w as shown in Figure 1 and Table 1. The zeta potential of the particles was in negative and the value was increased with the crease of lipid concentration as shown in Table 2. The results were in agreement with both 3- and 6- homogenization cycles. When the surfactant was at 10%w/w, the SLN particle size obtained was found to be the smallest and not different between the two homogenization cycles. Therefore, the surfactant at this concentration was considered as an optimum amount of SLN formulation. This concentration with the 3-homogenization cycles at 1000 bars was employed for further study.

Effect of solid lipid concentration The influence of cetyl palmitate as a solid lipid component in the formula on particle size and zeta potential was studied by using various amount of cetyl plamitate, i.e. 2.5%, 5%, and 7.5% w/w while maintaining amount of surfactant mixture at 10% w/w, and *T. divaricata* extract at 0.25%w/w. The result showed that the mean particle size at 3 and 6 homogenization cycles was increased then decreased when the amount of cetyl plamitate increased from 2.5% to 7.5% w/w as shown in Figure 2. Polydispersity index of these particles was in the range of 0.15 to 0.26 as shown in Table 3. The negative zeta potential of the SLN obtained from the 3- and 6- homogenization cycles was in

the range of -26 to -31 mV and -22 to -27 mV, respectively as shown in Table 4.

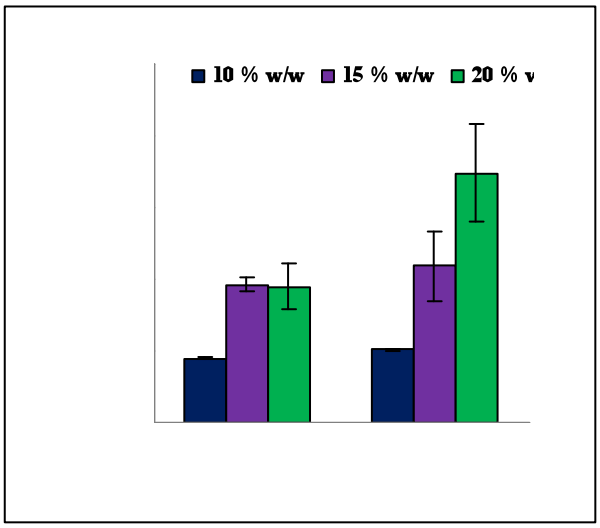

Figure 1. Effect of surfactant concentration and homogenization cycle on SLN particle size

Table 1. Effect of surfactants concentration and homogenization cycle on Polydispersity index

Concentration of surfactant (w/w)	Polydispersity index	
	3 cycles	6 cycles
10	0.199 ± 0.025	0.261 ± 0.020
15	0.531 ± 0.010	0.574 ± 0.108
20	0.513 ± 0.027	0.656 ± 0.076

Table 2. Effect of surfactants concentration and homogenization cycle on SLN zeta potential

Concentration of surfactant (w/w)	Zeta potential (mV)	
	3 cycles	6 cycles
10	-31.0 ± 2.6	-22.2 ± 1.2
15	-31.7 ± 2.4	-33.7 ± 1.5
20	-37.1 ± 1.6	-38.7 ± 1.9

CONCLUSIONS

It was concluded that the surfactant and solid lipid concentration as well as high pressure homogenization cycle played an important role on physicochemical properties of *T.divaricata* extract loaded SLN. According to the results, the optimum formula of *T.divaricata* extract loaded SLN could be performed by consisting of 2.5 w/w cetyl palmitate, 10% w/w surfactant mixture and 0.25%w/w *T.divaricata* extract. The optimum preparation condition of high pressure was of 1000 bars and 3 homogenization cycles. The optimized *T.divaricata* extract loaded SLN showed the smallest mean particle size of about 140 nm with a narrow particle size distribution, suggesting that the particles were in homogenous system. The obtained small size was suitable for transdermal skin penetration. This formula was considered for further experiment in transdermal product development of *T.divaricata* extract.

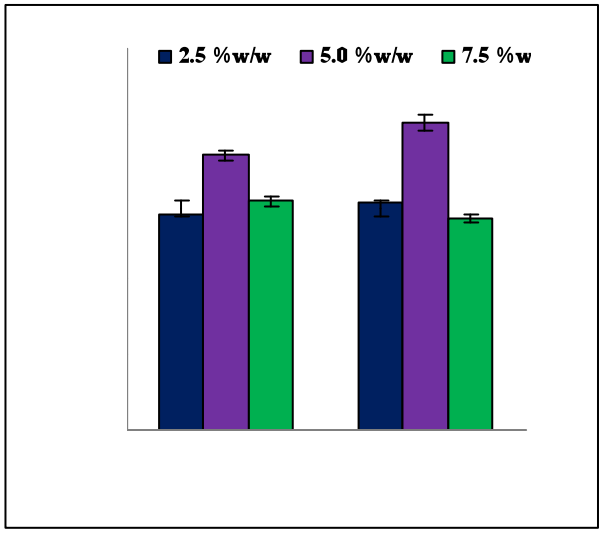

Figure 2. Effect of cetyl palmitate concentration and homogenization cycle on SLN particle size

Table 3. Effect of cetyl palmitate concentration and homogenization cycle on polydispersity index

Concentration of cetyl palmitate (w/w)	Polydispersity index	
	3 cycles	6 cycles
2.5	0.154 ± 0.020	0.197 ± 0.018
5.0	0.199 ± 0.025	0.261 ± 0.020
7.5	0.247 ± 0.021	0.149 ± 0.002

Table 4. Effect of cetyl palmitate concentration and homogenization cycle on SLN zeta potential

Concentration of cetyl palmitate (w/w)	Zeta potential (mV)	
	3 cycles	6 cycles
2.5	-28.2 ± 1.8	-27.8 ± 1.9
5.0	-31.0 ± 2.6	-22.2 ± 1.2
7.5	-26.5 ± 0.5	-27.0 ± 0.5

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