Stability evaluation of liposomal formulation comprising of *Pueraria Mirifica* extract

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ABSTRACT

Encapsulation of active ingredients derived from natural products into the liposome vesicles is utilized for cosmetic products. Owing to their efficacy on the skin permeation enhancement, prolong stability and decrease the toxicity of active ingredients was also reported. The main aim of this study was to investigate the stability of liposome containing *Pueraria mirifica* extract. The samples of liposomes were prepared by a thin film stability parameters, such as percentage of encapsulation efficiency (%EE), color, pH, particle sizes and aggregation were investigated. The results showed that the crude extracts obtained from *Pueraria mirifica* at 1 and 2% could be loaded into the liposomal formation and exhibiting the good stability during 6 cycles of study. The particle sizes were increased with the increase in the cycles of study. The %EE 1-2% crude extract into the liposome vesicles slightly decreased after 6 cycles of heating and cooling. Therefore, these results showed the innovative technology for using in herbal cosmetic delivery systems and hence the high efficacy of cosmetic product was occurred.

Keywords: Phospholipids, Liposome, *Pueraria mirifica* extract, Encapsulation efficiency

1. INTRODUTION

Liposomes have been used for encapsulating bioactive substance, due to their efficacy on the permeation enhancement of skin, prolong stability of active ingredients and decrease the toxicity of active ingredients. Liposomes are a lipid vesicles that contains both of hydrophilic and hydrophilic structure, are widely used as cosmetic delivery systems. Liposomes are biodegradable and non-toxic, are also able to encapsulate both hydrophobic and hydrophilic materials. Varying methods for preparation of liposomes have been developed which are dependent on the vesicle diameter and aqueous volume. Liposomes could be obtained from several approaches which include the use of organic solvents, mechanical procedure and by removal of detergent from phospholipids/detergent micelle mixture. The effect on liposome formation were depend on various factors i.e., composition and concentration of phospholipids, liposomal size and surface charge [5-8].

Pueraria mirifica (PM) also known as Kwao Krua Kao (*Pueraria candollei* Graham var. *mirifica* (Airy Shaw & Suvat.) Niyomdham), is the plant found in northern and north eastern of Thailand. PM has a history of use in folk medicine. Although the name "Kwao Krua" had been applied to several species of plants having tuberous roots, it was definitively identified as PM in 1952. The PM was promoted to be champion herbal products of Thailand in 2013, is a unique herbal hormone supplement that contains various phytoestrogens including miroestrol, deoxymiroestrol, daidzein, genistin, genistein, β-sitosterol, stigmasterol, coumestrol, puerarin, campesterol, mirificoumestan, kwakhurin and mirificine. An unusual estrogenic phenol, miroestrol, was isolated eight years later from this plant. Some cosmetic products and herbal supplements claim various health benefits of the extracts of PM including increasing appetite, enlarging breasts, improving hair growth and other rejuvenating effects. [1-4].

Thus, the purpose of this research was to evaluate the stability of liposome containing PM. A thin film hydration method was used for preparation of the liposome formations. The both of 1% and 2% crude extract of PM concentrations were chosen to this study. The stability study was assessed using heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles). The stability parameter, such as percentage of encapsulation efficiency (% EE), color, pH, particle sizes and aggregation were also investigated.

2. MATERIALS AND METHODS

Materials

Phosphatidylcholine from soy bean (SPC) was purchase from Fluka, (USA) while cholesterol was obtained from sigma Aldrich. All other solvents and chemicals used are of analytical grade. The PM SARDI 190 extract were collect to Kasetsart University.

Preparation of crude extract loaded liposomes

The suitable ratio between phosphatidylcholine and cholesterol were weight at various molar ratios and added into round bottom flask. Phosphatidylcholines were dissolved in appropriate amount of chloroform. phosphatidylcholine were dried by a rotary evaporator with a suitable condition of pressure and temperature at 150 bars and 40°C to produce a thin lipid film for 10 min. After thin film from phosphatidylcholine and cholesterol was obtained, 5 ml of PM crude extract (1% and 2%) in phosphate buffer solution pH 7.4 was then added and the mixture was vortexes for 5 min and then sonicated with ultrasonicator for 10 min. The suspensions of liposomes were annealed with the rotary evaporator for 20 min (150 bars and 40°C). The blank liposome was prepared with the similar condition but it used phosphate buffer solution pH 7.4 without PM crude extract.

Stability evaluations

The stability studies of liposomal formulation containing PM was assessed using heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles). The stability parameter, such as percentage of encapsulation efficiency (%EE), color, pH, morphology, particle sizes and aggregation were also investigated (Figure 1).

Particle size measurement

The diameter of the liposome was detected by the method of light diffraction (Horiba L 950, Japan). All analyses were performed in triplicate.

Determination of pH:

The pH of the samples was determined by pH meter (pH 700, German). The samples were determined in triplicate.

Morphology

The morphology and surface property of liposomes were investigated by using inverts microscope (Eclipse TE2000-s, Japan).

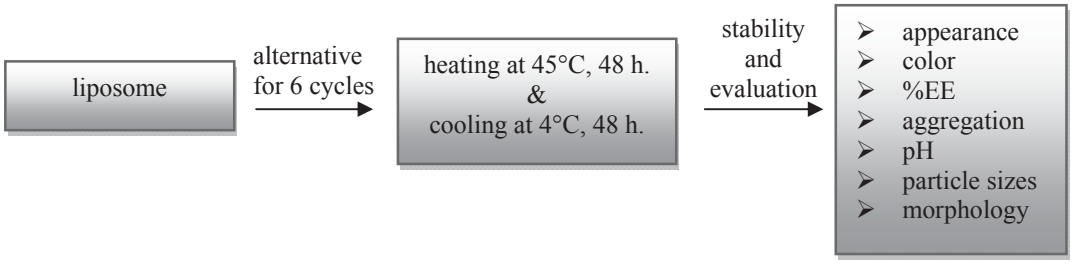

Figure 1. Diagram of stability testing by heating and cooling method

Determination of Color

The colors of liposome were investigated by color measurement (Miniscan EZ, USA). The color of the product was investigated based on three parameters including: L*, a* and b*.

Encapsulation efficiency (%EE)

The %EE of liposomes containing crude extracts at 0.25 and 0.5% were assessed. Initially, the unencapsulated was separated from the liposome dispersion by centrifugation. The liposome was centrifuged at 6,000 rpm, 4ºC for 1 h in a centrifugation in order to separating the incorporation of the active ingredient from the free form. The supernatant was analyzed by using total phenolic content to determine the amount of active ingredients for determination of percentage of encapsulation efficiency of crude extract within liposome vesicles.

3. RESULTS

Liposome containing PM extract could be developed, predicting by the morphology and %EE of liposomes. The morphology was shown in figure 1 that presented the droplet shape. However, the stability of liposome formulation was concerned a critical rule. Therefore, the stability of liposomes loaded PM extracts at 1 and 2% were also study as displayed in figure 2-3 and table 1-2.

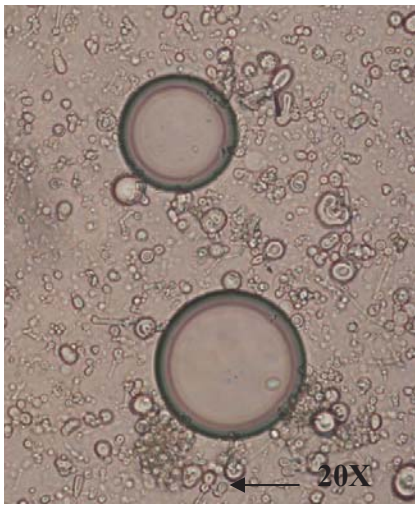

Figure 2. Morphology of liposome containing *Pueraria mirifica* extract

Stability assessment of the liposome with and without herbal extract were carried out using heating and cooling test at 45°C 24 h and 4°C 24 h for 6 cycles. The results revealed that the appearance, odor and texture of all formulation did not change. The pH, were slightly changed after 6 cycles of storage in accelerated conditions. The pH value slightly changed from 7.01, 6.99 and 7.9 to 7.06, 6.9 and 7.1 for blank liposome (Blank_LP), liposome containing 1% crude extract (LP_1% crude extract) and liposome containing 2% crude extract (LP_2% crude extract), respectively during 6 cycles of evaluation. The color of the product was investigated based on three

parameters including: L*, a*, b* which showed slight change for Blank_LP, LP_1% crude and LP_2% crude extract after 6 cycles.

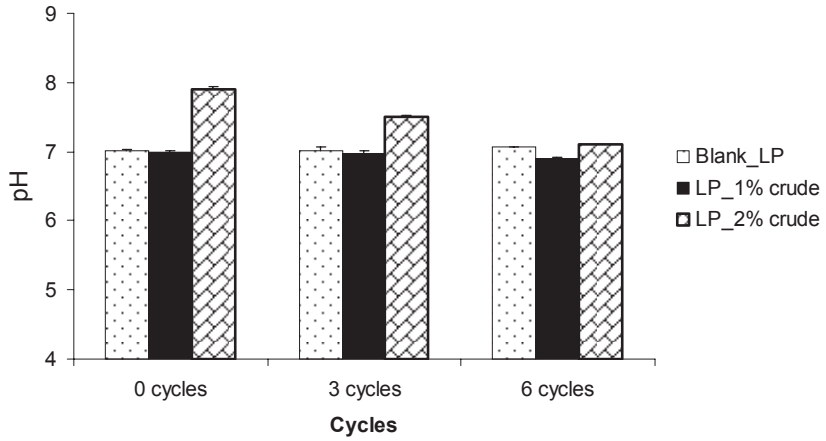

Figure 2. pH of all liposome formulations after heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles)

The result of color was in agreement with the appearance and pH of all formulations. The particle sizes were increased as increasing the cycles of heating and cooling. The particle sizes increased from 6.10, 6.70 and 6.87 µm to 7.25, 7.60, and 7.80 for blank _LP, LP_ 1% crude and LP_2% crude extract, respectively after 6 cycles of storage. This result was due to the combination between particles of liposomes. The change in sizes of liposome resulted in the change in % EE of *PM* extract loading. The %EE of LP_ 1% crude and LP_2% crude extract slightly decreased from 51.48% and 31.28% to 45.02% and 28.76% after 6 cycles of storage. Therefore, this result showed the innovative technology for using in herbal cosmetic delivery systems.

Table 1. Color of all liposome formulation after heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles)

Classification	0 cycles	3 cycles	6 cycles
Blank _LP	L* = 65.76 ± 0.22	L* = 64.12 ± 0.35	L* = 65.43± 0.10
	a* = -0.55 ± 0.03	a* = -0.56 ± 0.08	a* = -0.54 ± 0.02
	b* = 0.66 ± 0.04	b* = 0.67± 0.15	b* = 0.68 ± 0.05
LP_ 1% crude	L*=61.91 ± 0.63	L*=60.43 ± 0.45	L*=59.23 ± 0.34
extract	a*=-0.84 ± 0.03	a*=-0.87 ± 0.12	a*=-0.90 ± 0.16
	b*=9.26 ± 0.09	b*=9.78± 0.24	b*=9.98 ± 0.15
LP_2% crude	L*=61.93 ± 0.24	L*=59.67 ± 0.17	L*=58.43± 0.56
extract	a*=-0.35 ± 0.03	a*=-0.37± 0.05	a*=-0.39 ± 0.10
	b*=13.93 ± 0.15	b*=14.06 ± 0.12	b*=14.56 ± 0.07

Table 2. Encapsulation efficiency of all liposome formulation after heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles)

Classification	Times		
	0 cycles	3 cycles	6 cycles
LP_1% crude extract	51.48 ± 3.41	45.19 ± 4.63	45.02 ± 1.09
LP_2% crude extract	31.28 ± 4.89	30.54 ± 2.54	28.76 ± 2.45

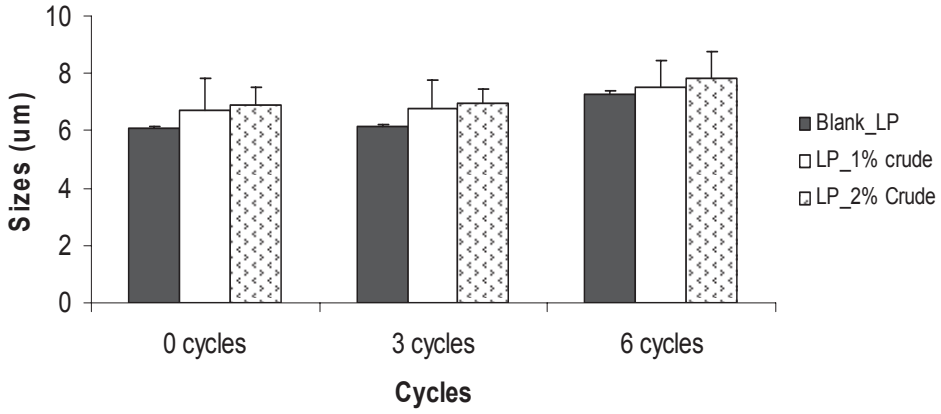

Figure 3. Particle sizes of all liposome formulations after heating and cooling method (45°C, 48 h and 4°C, 48 h for 6 cycles)

4. CONCLUSIONS

The formulation of liposome consisting of a PM extract was successfully prepared from the thin film hydration method. The formulation of liposomes containing PM of both concentrations showed good formulation and good stability for applying to cosmetic delivery and adding to cosmetic products.

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REFERENCES

1. Pope GS, Grundy HM, Jones HEH, Tait SAS. 1985. The estrogenic substance (miroestrol) from the tuberous roots of *Pueraria mirifica*. J Endocrinol. 17:15-16.
2. Chandeying V, Sangthawan M. 2007. Efficacy comparison of *Pueraria mirifica*. Against conjugated equine estrogen (CEE) with/without medroxyprogesteronr acetate (MPA) in the treatment of climacteric symptoms in perimenopausal women: phase II study. J Med Assoc Thai 90(9): 1720-1726.
3. Yusakul G, Putalun W, Udomsin O, Juengwatanatrakul T, Chaichantipyuth C. 2011. Comparative analysis of the chemical constituents of two varieties of *Pueraria candollei*. Fitoterpia 82:203-207.
4. Cherdshewasart W, Subtang S, Dahlan W. 2007. Major isoflavnoid contents of the phytoestrogen rich-herb *PM* In comparison with *Pueraria lobata.* J Pharm Biomed Anal. 43:428-434.
5. Manosroi A, Manosroi J. 2002. Liposomes in Pharmaceuticals and Cosmetics, Odian Publishing, Bangkok.
6. Nobnorb N. 2008. Development of proliposomes containing *Phyllanthus emblica* extract. Master of Pharmacy Thesis Pharmaceutical Sciences. Prince of Songkla University Thailand.
7. Weiner N, Martin F, Riaz M. 1989. Liposomes as drug delivery system. Drug Develop Ind Pharm. 15, 1523-1554.
8. Szoke F Jr, Papahadjopoulos D. 1980. Comparative properties and methods of preparation of lipid vesicles (liposomes). Annu Rev Biophys Bioeng. 9, 467-508.