Formulation and encapsulation efficiency of crude extract derived from PM loaded liposomal formation

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ABSTRACT

Liposomes are lipid vesicles that contained both part of hydrophilic and hydrophilic structure. It is widely used as cosmetic delivery. Their efficacy on the permeation enhancement of skin, prolong stability of active ingredients and decrease the toxicity of active ingredients were also reported. Therefore, the purpose of this study was to prepare *Pueraria Mirifica* (PM) extract loaded liposome formulation. Liposomes were prepared by hydration of dried lipid films. The crude extract of both concentrations (1% and 2%) were performed to loading within the liposome vesicles. The physicochemical properties of liposomes were characterized on the morphology, sizes, color, pH and percentage of encapsulation efficiency (%EE). The %EE of liposomes containing crude extracts at 1 and 2 % were assessed using total phenolic content. The results indicated that the crude extracts obtained from PM could be loaded into the liposomal formation. The %EE of crude extract loaded liposomes was in the range of 30.0-50.0%. The particle sizes of liposomes was in the ranges of micrometer about 6.0–7.0 μm. Hence, crude extract derived from PM loaded liposome showed a good formulation, giving a high efficacy for applying to cosmetic delivery system.

Keywords: Phospholipids, Liposome, PM extract, Encapsulation efficiency

1. INTRODUTION

Pueraria mirifica (PM) is the herb as origin of Thailand. It has also been called Kwao Krua Kao and Thai Kudzu, *PM* is a plant which belongs to the family Papilionaceae (Leguminosae) same family as soy. It has a history of usage in Thai herbal medicine pharmacopia which have been used for almost 100 years and well known in benefit of PM. The tuberous of PM were found hormones from natural components; including phytoestrogens contains similar estrogen-like compounds which are more potent than those found in soy, such as miroestrol and deoxymiroestrol.

Thus PM shows a valuable role in helping to maintain a healthy hormone balance in menopausal women, when estrogen levels drop and women experience changes in mood, hot flashes, lower libido, sleep interruption and other health issues. PM has earned a reputation for its supportive effects for breast health, and has been featured in products that help support breast firmness, as well as protecting breast tissue. In addition to their hormone supporting effects, these substances have a high level of antioxidant activity, probably due to their ability to increase the cell protective substance called superoxide dismutase [1-4].

In recent years, liposomes have been widely applied as a carrier system which can improve the activity and safety of many active ingredients. Liposomes are widely used as cosmetic delivery systems and drug delivery systems. Due to their efficacy on the permeation enhancement of skin, prolong stability of active ingredients and decrease the toxicity of active ingredients. Liposomes, which 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 structure formation of liposomes was depend on many factors i.e., composition and concentration of phospholipids, liposomal size and surface charge [5-8].

Therefore, the purpose of this research was to formulate and evaluate the percentage of encapsulation efficiency of crude extract derived from PM loaded liposomes. A thin film hydration method was used for preparation of the liposome formations. The physicochemical properties were characterized on size, pH, color, morphology and encapsulation efficiency (EE %).

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.

Preparation of crude extract derived from *Pueraria mirifica*

Specific extracts of PM SARDI 190 was separately prepared by macerating the powdered plant with ethanol-water and filtering through Whatman paper No. 41. The solvent was removed under reduced pressure using a rotary evaporator (Heidolph, Hei-VAP Precision) at 45°C. An appropriate mixing ratio was used to prepare the mixed extract.

Preparation of crude extract loaded liposome

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 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 crude extract.

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.

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*.

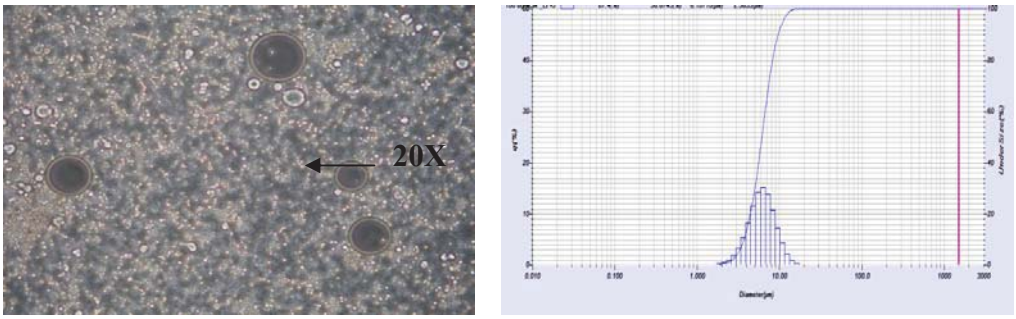
Encapsulation efficiency (%EE)

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 hrs 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

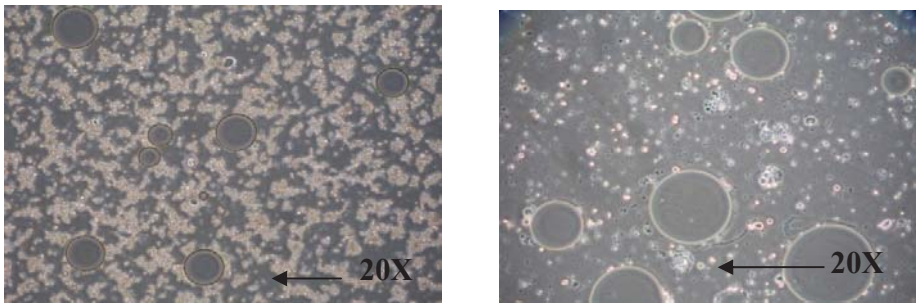
PM extract loaded liposomes were formulated through thin film hydration method varying with the composition of the phosphatidylcholine and cholesterol. The suitable ratio between phosphatidylcholine (Phospholipids) and cholesterol was 10:1, due to it was easily prepared liposomes as shown in figure 1a. The results indicated that the liposomes showed the spherical shape (1a) and white suspension of colloids. The particle sizes of blank liposomes were about 6 µm as displayed in figure 1b.

This condition was to formulate *PM* extract loaded liposomes formation. The concentrations crude extract of PM at 1% and 2% was used in this study. The results demonstrated that the crude extract of both concentrations could be added into the structure of liposomes indicating by the % EE of both concentrations. The % EE of crude extract loaded in liposomes at 1% and 2% concentrations were 51.48% and 31.28% respectively. The addition of crude extract into liposomal vesicles did not affect the change in pH of liposome while the color of liposomes was extremely changed. The higher concentrations of crude extract, the higher yellow color (b*) of liposomes were observed. The b* value increased ($p < 0.05$) from 0.66 to 13.93. The change in color was due to the influence of crude extract.



(1a) **(1b)**

Figure 1. Shows the invest micrograph (1a) and particle sizes (1b) of blank liposomes at 10:1 (lecithin and cholesterol)



(2a) **(2b)**

Figure 2. Shows the invest micrograph of 1.0% crude extract of *Pueraria mirifica* loaded liposomes (2a) and 2.0% crude extract loaded liposomes (2b)

Table1. Physicochemical properties and encapsulation efficiency of *Pueraria mirifica* extract loaded liposomes

Formulas	pH	Shape	Sizes	Color	% EE
Blank liposome	7.01 ± 0.02	Spherical	6.10 ± 0.05	L* = 65.76 ± 0.22 a* = -0.55 ± 0.03 b* = 0.66 ± 0.04	none
1% crude extract loaded liposomes	6.99 ± 0.01	Spherical	6.16 ± 0.03	L* = 61.91 ± 0.63 a* = -0.84 ± 0.03 b* = 9.26 ± 0.09	51.48 ± 3.41
2% crude extract loaded liposomes	7.90 ± 0.01	Spherical	7.25 ± 0.17	L* = 61.93 ± 0.24 a* = -0.35 ± 0.03 b* = 13.93 ± 0.15	31.28 ± 4.89

4. CONCLUSIONS

The formulation of liposomes comprising of a PM extract was successfully prepared from thin film hydration method. The formations of liposome with and without crude extract were spherical shape, indicating the suitable condition for fabrication of liposome formulation. The particle sizes of liposomes with and without crude extract were in the range of 6.0 - 7.0 µm while % encapsulations were in the range of 30.0 – 50.0% which depended on the concentrations of crude extracts. This research contributed to a new formulation of PM extract for applying to cosmetic formulation.

ACKNOWLEDGEMENT

This work was supported by the Thai Herbal Medical Plant, The Office of Kamphaengsaen Campus, Kasetsart University, Kamphaengsaen, Nakhon Pathom, 73140, Thailand.

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