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Effect of Span 60, Labrasol, and Cholesterol on *Labisia pumila* Loaded Niosomes Quality

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Abstract*—Labisia pumila* (LP) plant extract has the potential to be applied in cosmeceutical products due to its anti-photoaging properties. The main purpose of this study was to improve transdermal delivery of LP by encapsulating LP in niosomes. Niosomes loaded LPs were prepared by coacervation phase separation method using non-ionic surfactant (Span 60), labrasol, and cholesterol. The optimum formula obtained were Span 60, labrasol and cholesterol at the mole ratio of 6:1:4. At the optimum formulation, the niosome obtained significantly improved the quality of transdermal penetration of LP compared to free LP.

Keywords*—Labisia pumila*,** niosomes, transdermal, quality.

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I. Introduction

NATURAL herbs are widely used in cosmetic. However, some of water soluble phytoconstituents (such as tannins, flavonoids, glycosidic aglycones, etc.) possess poor bioavailability due to their incompatible molecular size and poor lipid solubility [1]. Some of phytoconstituents that incorporated in the product formulation can be destroyed due to their interaction with formulation ingredients. This situation may reduce their bioavailability. Hence, a good delivery system that is capable of encapsulating plant extracts is required. Several examples of novel drug delivery system like vesicular systems (niosomes, liposomes, etc.), nanocapsules, and polymeric nanoparticles have been widely investigated for this purpose. The bioavailability of drug can be enhanced in niosomal formulation by providing the sustained and prolonged delivery [2].

LP water extract has been shown to protect fibroblast and keratinocytes from being damaged by UVB irradiation and to boost collagen synthesis [3]. However, most of LP's water soluble phytoconstituents (such as flavonoids, tannins, etc.) are poorly absorbed due to their poor lipid solubility, which limit their ability to cross the lipidic biomembrane [1]. The extract could be encapsulated in niosomes as this can reduce the barrier properties thus enhance its penetration into the skin.

The main objective of this research was to obtain the optimum formulation for LP loaded niosome. Design of experiment (DOE) was carried out to deal with the parameters that affect niosomes production. The niosomes were characterized based on encapsulation efficiency, particle size, zeta potential, and skin penetration.

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II. Materials and Methods

A. Chemicals and Materials

Span 60, Sephadex G-50 (medium), cholesterol, monobasic sodium phosphate, and dibasic sodium phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA). Absolute ethanol (99.9 %), and 1-propanol was purchased from Qrec (Chonburi, Thailand). Labrasol was purchased from Gattefosse Corporation (Saint-Priest, France).

Dried leaves of LP were obtained from Forest Research Institute Malaysia (FRIM). The dried leaves were dried further for 2 days at 45 °C before being ground to a powder, which was extracted with boiling water for 1 h, after which the infusion was filtered and the filtrate was spray-dried to form a powder [4]. 1 g of obtained powdered LP extract was dissolved in 50 ml of water and centrifuged at 10000 g-force for 5 min. The supernatant was then filtered with nylon membrane filter (Agilent techologies) with 0.2 µm pore size, and the resultant filtrate was used in this research work.

B. Preparation of Niosomes

Niosomes with LP extract were prepared by coacervation phase separation method followed by hydration as described by Vora et al. [5]. To study the effect of cholesterol, Labrasol, and surfactant on niosomes formation, first of all, Span 60 was mixed with an appropriate amount of lipid (Labrasol and cholesterol) in a 10-ml glass vial. 1 ml of solvent (absolute ethanol) was then added to the mixtures, and the vial was tightly sealed before putting in a water bath at 60°C while shaking until complete dissolution of the mixture. The specified amount of LP extract was then added to the mixture in a water bath for 3-5 min till clear or translucent solution was obtained. Niosomal suspension was then obtained by hydration of 200 ml of distilled water for 10 min in the water bath. The obtained niosomal suspensions were finally downsized with a high-pressure homogenizer (Microfluidizer M-110P, Newton, MA, USA) with constants parameter (750 bar and four cycles).

C. Characterization of Niosomes

Niosomes were characterized based on their entrapment efficiency (EE), particle size (PS), zeta potential (ZP), and penetration study.

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D.Entrapment Efficiency (EE) Determination

The EE was determined using minicolumn centrifugation method [6]. The column (EconoSpin size exclusion columns, Epoch life science, USA) were prepacked with hydrated sephadex G-50 which come with inert support. These columns were then placed in the microcentrifuge tubes and the whole assembly was centrifuged to remove excess water. Niosomes suspension was then applied to the dried bed and the assembly was centrifuged at 1700 rpm for 2 minutes. The niosomes were expelled from the column. On the other hand, the excess unentrapped extract was trapped in the void volume. The expelled niosomes collected in the micro centrifuge tubes were disrupted with 50% n-propanol with the ratio of 1:1 in an ultrasonic bath for 20 minutes. The resultant mixture was filtered with 0.2 µm syringe nylon filter, and the filtrate was subjected to total phenolic content assay. The EE was calculated as (1):

$$EE\% = \frac{\text{total phenolic content inside the vesicles x 100}}{\text{total phenolic content added}} \quad (1)$$

E. Measurement of Particle Size

Vesicular size and distribution were determined using Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). The analyses were performed by dynamic light scattering (DLS) principle at constant temperature of 25 °C, material refractive index of 1.45 and absorption index of 0.001. Each sample was measured three times, and results were expressed as mean± standard deviation.

F. Zeta Potential Determination

Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK) was used to measure the zeta potential of niosomes with Electrophoretic Light Scattering principle. Zeta potential was determined three times for each sample, and results were automatically calculated by the analyzer using the Smoluchowski equation.

G.Skin Penetration Study

This study was performed using a transdermal Franz diffusion system (PermGear, Germany) composed of six horizontal diffusion cells with the effective area= 1.07 cm^2. The experiments were conducted using abdominal skins of 9 weeks old rats [7]. The shaved skin was cut into squares, and subcutaneous fat was carefully removed. The skin was pre-equilibrated in Phosphate Buffer Solution (PBS, pH=6.5) at 4 °C for 24 hours.

The skins were mounted on the receptor compartment with the stratum corneum side facing the donor cell. The receiver compartments (3.7 ml) were filled with PBS solution (pH 6.5), and the donor compartments are filled with 2.7 ml of the same PBS solution. The diffusion calls were equilibrated by the re-circulating water bath at 37±1 °C to provide skin surface temperature of 32±1 °C [8]. The fluid in the donor and receptor compartment was stirred continuously. 1 ml of samples was pipetted into the donor compartments after running the setup for half an hour to allow for temperature equilibrium.

Sampling was done at 0.5, 1, 1.5, 2.0, 3, 4, 5, 6, 8, 10, 14, 18, and 24 h. At each point, 1 ml of aliquots was withdrawn from the receiver compartment, and thereafter, 1 ml of PBS solution was added as replacement to the receiver compartment. The collected samples were disrupted with 50% n-propanol with the ratio of 1:1 in an ultrasonic bath for 20 minutes. The resultant mixture was filtered with 0.2 µm syringe nylon filter, and the filtrate was subjected to total phenolic content assay in order to obtain the concentration of extract.

Flux (µg/cm^2h) and the apparent permeability coefficient (× 10^{-3} cm/h) of active ingredients into the skin were calculated based on [7], [9], [10].

H.Experiment Design

Design of experiment (DOE) was done using version 6 of Design Expert® by Stat-Ease.

III. Results and Discussion

A. Formulation Optimization

Formulation optimization involved three parameters which were the amount of cholesterol (A), Span 60 (B), and labrasol (C). The lower and upper limits of parameters were determined from the previous study (unpublished data). Table I presents the DOE for LP niosomes where their responses were particle size (PS) (Y1), zeta potential (ZP) (Y2), and encapsulation efficiency (EE) (Y3).

TABLE I
DOE FOR LP NIOSOMES

Run	A (mmol)	B (mmol)	C (mmol)	Y1 (nm)	Y2 (-mV)	Y3 (%)
1	0.29	0.62	0.09	194.47	34.07	70.47
2	0.00	1.00	0.00	256.73	42.00	80.90
3	0.18	0.64	0.18	378.27	39.97	74.64
4	0.00	0.80	0.20	657.00	47.87	45.90
5	0.09	0.82	0.09	501.53	36.73	98.55
6	0.4	0.60	0.00	163.80	56.73	70.32
7	0.09	0.62	0.29	540.00	37.90	77.29
8	0.00	0.60	0.40	331.60	37.67	44.30
9	0.25	0.50	0.25	249.73	35.30	49.28
10	0.00	0.60	0.40	351.93	34.60	34.67
11	0.00	1.00	0.00	214.77	57.90	81.60
12	0.20	0.80	0.00	175.37	41.47	56.52
13	0.25	0.50	0.25	228.43	34.50	97.10
14	0.40	0.60	0.00	167.47	51.47	68.60

B. Effects of Formulation Ingredients and Their Interactions on Responses

Fig. 1 shows the three-dimensional plot representing the interactions among the three variables on PS of the LP niosomes. Based on the contour plot, particle size decreased when the amount of cholesterol (X1) was increased. Cholesterol helped to stabilize the niosomes. Higher stability will lower the tendency of niosomes to diffuse into each other, thus resulting in smaller particle size. Labrasol seemed to

increase the size of LP niosomes. The increase in size might be the result of increased fluidity of the LP niosomes. Small vesicle size can be obtained at high amount of cholesterol and Span 60, and low amount of labrasol.

Fig. 2 shows the three-dimensional plot representing the interactions of the three variables on ZP of the LP niosomes. Based on the contour plot, maximum zeta potential can be obtained at high amount of cholesterol and Span 60 and low amount of labrasol.

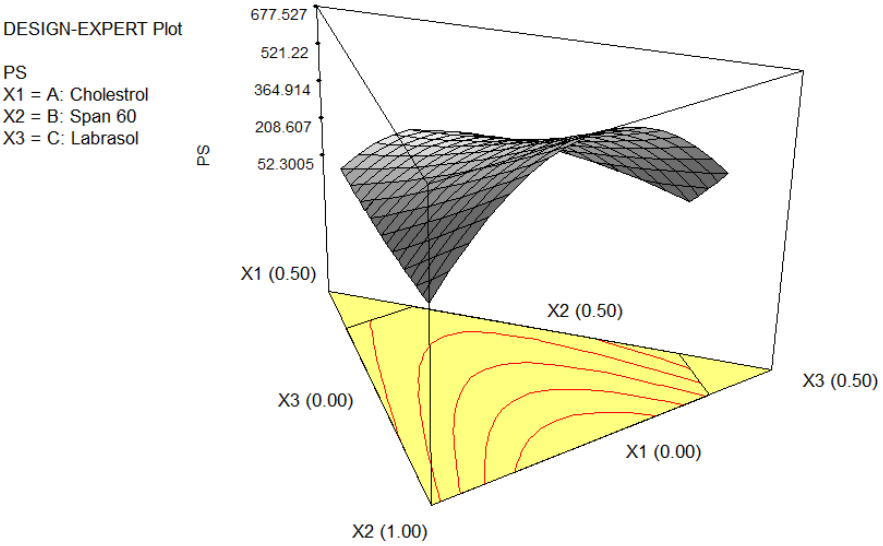

Fig. 1 Three-dimensional response surface plot representing the interactions of cholesterol, Span 60, and labrasol on particle size of LP niosomes

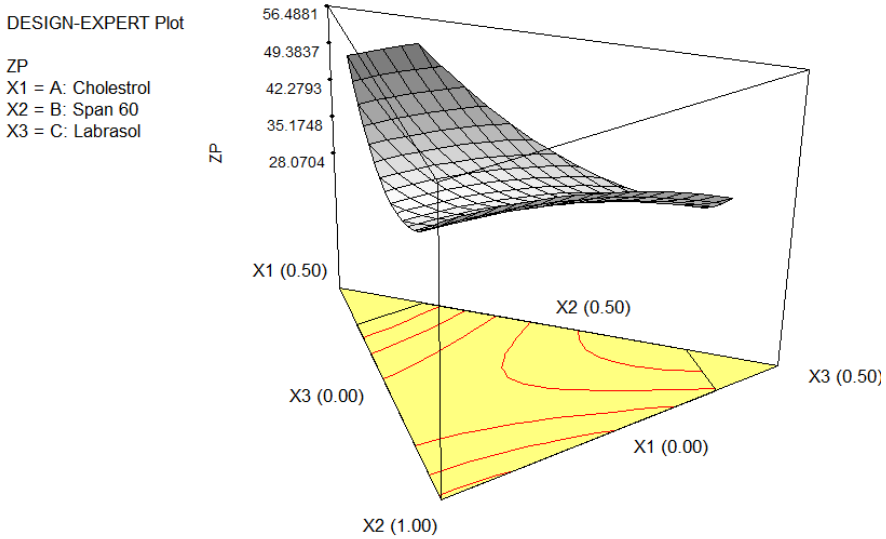

Fig. 2 Three-dimensional response surface plot representing the interactions of cholesterol, Span 60 and labrasol on zeta potential of LP niosomes

C. Penetration of LP Niosomes in Rat Skin

Penetration study was done using Franz horizontal diffusion cells with rat skin used as the barrier. The LP niosomes were obtained using the optimized formulation of 6:1:4 of Span 60: labrasol: cholesterol. The permeations of free LP extracts and LP niosomes through rat skin were compared. Fig. 3 shows the trend of cumulative permeation of free LP extracts and LP niosomes through lab skin for the period of 24 hours and permeation coefficient through rat skin, which were calculated and tabulated in Table II.

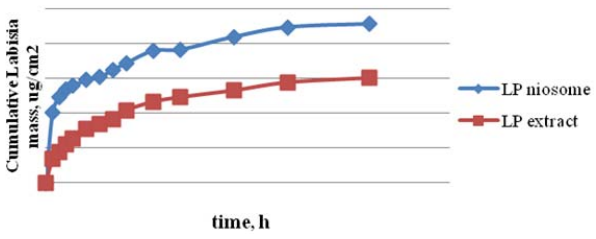

Fig. 3 Trend of cumulative permeation of free LP extracts and LP niosomes through rat skin for the period of 24 hours

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TABLE II
COMPARISON OF TRANSPORT PARAMETERS FOR THE PENETRATION OF FREE AND ENCAPSULATED LP EXTRACTS THROUGH RAT SKIN

Sample	Cumulative Permeation Q_t / S (μg/cm^2)	J_{ss} (μg/cm^{2}h)	Kp (×10^{-1}cm/h)	Amount of active ingredients in the skin, μg
LP extract without encapsulation	0.3012±0.01	0.091	1.51	0.279±0.008
LP niosomes	0.4571±0.20	0.261	4.35	0.314±0.038

IV. CONCLUSION

The optimum formula obtained for LP loaded niosomes was Span 60, labrasol, and cholesterol in the ratio of 6:1:4. The optimized niosomes showed the feasibility to be applied in cosmetics formulation.

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