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LIPOSOME AS TRANSDERMAL CARRIER FOR LABISIA PUMILA AND FICUS DELTOIDEA WATER EXTRACTS

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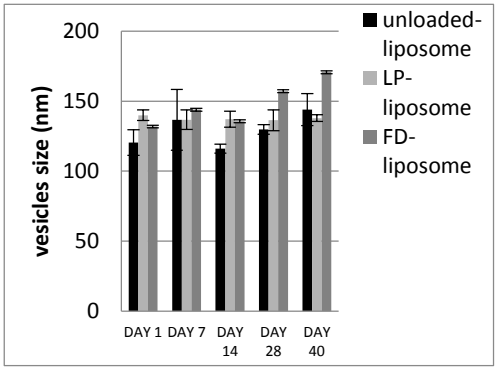
Graphical abstract



Abstract

In this study, liposome has been investigated as the potential transdermal carrier for *Labisia pumila (Lp)* and *Ficus deltoidea (Fd)*. The encapsulation efficiency, zeta potential, particle size and transdermal transport properties of the herbal extracts loaded liposomes were determined to characterize the delivery system. The entrapment efficiency for Lipo-*Lp* and Lipo-*Fd* were 35.47 ± 2.71% and 31.23 ± 7.65%, respectively. The average diameter were153.35nm and 131.9nm, respectively and the zeta potential were -47.2 and -46.8, respectively. The permeability and partition coefficient (K) of Lipo-*Lp* were 1.31cm h^{-1} and 10.05, respectively and the permeability and partition coefficient (K) of Lipo-*Fd* were 1.28cm h^{-1} and 7.62, respectively. *Labisia pumila* and *Ficus deltoidea* loaded liposomes showed better transdermal permeation compared to un-encapsulated *Labisia pumila* and *Ficus deltoidea* indicative of potential actives herbal cosmetic formulations.

Keywords: *Labisia pumila*, *Ficus deltoidea*, liposomes, transdermal delivery, cosmetic

Abstrak

Dalam penyelidikan ini, liposom dikaji sebagai pembawa transdermal untuk *Labisia pumila (Lp)* dan *Ficus deltoidea (Fd)*. Kecekapan pengkapsulan, keupayaan zeta, saiz zarah dan sifat aliran transdermal ekstrak herba yang dimasukkan liposom dikenal pasti untuk menentukan ciri sistem penghantaran. Kecekapan pemerangkapan Lipo-*Lp* dan kecekapan pemerangkapan Lipo-*Fd* masing-masing ialah 35.47 ± 2.71% dan 31.23 ± 7.65%. Purata diameter masing-masing ialah 153.35nm dan 131.9nm, dan keupayaan zeta masing-masing ialah -47.2 dan -46.8. Pekali ketelapan dan pekali sekatan Lipo-*Lp* (K) masing-masing ialah 1.31cm h^{-1} dan 10.05, manakala pekali ketelapan dan pekali sekatan Lipo-*Fd* masing-masing ialah 1.28cm h^{-1} dan 7.62. *Labisia pumila* dan *Ficus deltoidea* yang dimasukkan liposom menunjukkan penelapan transdermal yang lebih baik berbanding dengan *Labisia pumila* dan *Ficus deltoidea yang tidak berkapsul,* menunjukkan potensi formula aktif kosmetik berasaskan herba.

Kata kunci: Labisia pumila, Ficus deltoidea, liposom, penghantaran transdermal, kosmetik

1.0 INTRODUCTION

The outermost layer of the skin is called epidermis, followed by dermis and hypodermis [1]. The epidermis layer consists of stratum corneum (SC) as the outermost layer, followed by stratum lucidum, stratum granulosum (granular layer), stratum spinosum (spinous layer), and stratum germinativum (basal layer).

The transdermal delivery systems needs to overcome the natural features of the stratum corneum as the skin barrier. Stratum corneum acts as a protective layer which prevents unnecessary compounds from the external environment to penetrate the skin and also to prevent moisture loss from the inner skin [2].

Liposome is a beneficial tool for transporting desired active ingredients through the skin barrier into the inner layers of the skin. Liposome is used for transdermal delivery due to its bilayer structure, imparted from phospholipids, which is similar to skin structure [3]. Phospholipids have many advantages as a carrier of active ingredient such as strong tissue affinity, biodegradability and low toxicity [4].

Long exposure under sunlight, harsh chemical skin care, ageing factors and environmental pollution can lead to skin problems such as wrinkles, acne and pigmentation.

Labisia pumilla, also known as Kacip Fatimah by locals, is the queen of the herbs in Malaysia. Traditionally, *L. pumilla* has been used to induce childbirth, as post-partum medications to contract the birth channel, as well as regain energy during post-partum [5]. *L. pumilla* extract has also been proven to have anti-ageing properties. The herbal extract has been shown to restore collagen synthesis of human fibroblast after exposure of the cells to UVB [6].

Ficus deltoidea, also known as Mas Cotek, is from the Moraceae family. *F. deltoidea* is traditionally used to contract the uterus and vaginal muscles after birth, regulation of menstrual cycle, and to treat leucorrhoea [7]. *F. deltoidea* extract has also been scientifically shown to be responsible in controlling LDL oxygenation, prevents blood clot, reduce blood sugar level, decrease blood pressure, and assist the effectiveness of vitamin C. [8]. Recently, *F. deltoidea* extract was shown to possess anti-melanogenic property. Oh *et al.*, (2011) discovered that *F. deltoidea* can directly inhibit tyrosinase enzyme activity and down-regulate the expression of genes involved in melanogenesis pathway [7]. Similar to *L. pumilla, F. deltoidea* extracts was also shown to possess anti-ageing properties [9].

Due to the promising and remarkable properties of *L. Pumilla* and *F. deltoidea* extracts, they have the potential to be employed as active ingredients in cosmetic products. Thus, appropriate tools need to be consider in delivering the herbal extracts into the skin layers to ensure efficacy. In this study, the potential of using liposome as the carrier for the transdermal delivery of both herbal extracts was investigated

2.0 METHODOLOGY

L-α- Phosphatidylcholine type II-S (from soybean), sodium phosphoric monobasic, sodium phosphoric dibasic, sodium ascorbate, Sephadex G-50, Coomassie Brilliant Blue G-250, chloroform and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *Ficus deltoidea* water extracts and Labisia pumila water extracts were provided by Phyto Biznet Sdn Bhd (Malaysia). All chemicals used as received unless otherwise noted.

2.2 Preparation of Liposome

L-α- Phosphatidylcholine (from soybean) was used as the main component in the formulation. The liposome vesicles were prepared by lipid film hydration method. 0.5 % w/v of lecithin was dissolved in 125 ml chloroform, which was then evaporated at 60°C for 24 hours under vacuum using a rotary evaporator. The resulting thin film was hydrated with 250 ml PBS (phosphate buffered saline) pH 6.5 and 0.1% w/v *Labisia pumila* or *Ficus deltoidea* under mechanical agitation for 1 hour at 60°C. The obtained vesicles were extruded through polycarbonate membrane filter equipped in Liposofast (Avestin) extrusion device under nitrogen flow. The vesicles were extruded once through 450 nm pore size filter and the procedure repeated nine times using 200 nm pore size filter. After 10 rounds of extrusion, the mixture was mixed with 50mg/ml of sodium ascorbate at 1:1 ratio.

2.3 Size Analysis of Liposome

The size of liposome was measured based on dynamic light scattering (DLS) technique using Zetasizer Nano ZS (Malvern Instrument Ltd., United Kingdom). The parameters for measurement and calculation were set as follow: 173° backscatter measurement angle, 1.33 material refraction index, 25°C, and water as dispersant. Each sample was measured 3 times using a clear disposable cell. Number and duration of run were optimized for each sample in order to obtain result meeting measurement quality criteria. The sample was dissolved in deionized water (1:9 dilution) prior to measurement.

2.4 Zeta Potential Measurement

Zeta potential was measured based on laser Doppler electrophoresis technique using Zetasizer Nano ZS (Malvern Instrument Ltd., United Kingdom). All measurement parameters and sample preparation were conducted as described in size measurement procedure above. The measurement duration was set as automatic so that the measurement was continue until result met the quality criteria.

2.5 Morphology Study

The morphology of liposomes loaded with herbal extracts were observed using Transmission Electron Microscope (TEM) (JEOL JEM 2100 LaB6, Japan). Freshly prepared liposomes were dropped onto a copper grid and dried for 3 minutes at room temperature. The samples were then dyed with uranyl acetate for 1 minute before observed under TEM.

2.6 Encapsulation Efficiency

The Bradford reagent stock solution was freshly prepared by dissolving 100mg Coomassie Brilliant Blue G-250 in 50ml of 95% ethanol. Then, 100ml of 85% phosphoric acid (reagent grade) was added. The water was added to the mixture up to 1 liter after the dye completely dissolved. The mixture was then filtered through Whatman #1 paper. The filtration was repeated to get rid of the blue colour until the mixture was completely turned to light brown. BSA was used to calibrate the Bradford assay. The BSA standard was prepared in phosphate buffer 6.5 pH in a range of 20µg - 150µg for the calibration curve.

The concentration of protein was evaluated to determine the encapsulation efficiency of the extract. The liposome suspension was separated using gel filtration. Sephadex G-50 was diluted in deionized water (1g in 9ml- dry beads diameter 20-80µm) and stirred. The mixture poured into a column (20mm x 130mm) and then deionized water was allowed to flow through the column. Flow was set at the rate of 2ml/min, which was measured manually by maintaining the level of water at the top of the column. 5ml of the samples (Lipo-LP or Lipo-FD) were loaded into the column and the level of water was maintained at the top of the column throughout the sampling process. Collected samples were treated with methanol to break the liposomes wall (1ml samples: 3ml methanol). Prior to analysis, the mixture of samples-methanol was sonicated using sonic dismembrator equipment for 20 minutes. The samples were then analysed using Bradford protein assay (0.1ml samples were used to react with Bradford reagent). The absorbance was taken at 595nm. The amount of entrapped drug was calculated using the following equation.

$$\%EE = \frac{Extract\ in\ liposomes}{(Extract\ \ in\ liposomes + free\ extract)}\ x\ 100\% \quad (1)$$

2.7 Permeation Experiment Using Rat Skin

Rat skin was excised from Sprague-Dawley female rats (200 – 400 gram of weight) after euthanasia procedure. The euthanasia method was approved by the Ethics Committee from Universiti Kebangsaan Malaysia (UKM). The abdominal hair was removed using a razor and the subcutaneous layer was also detached. The excised skin was stored at -10°C and can be used within 6 months. Prior to analysis, the skin was soaked in 0.1mM sodium phosphate buffer (PBS) overnight before the experiment. The excised skin was mounted on a Franz's diffusion cells for 24 hours. The temperature was set at 32°C ± 0.5 [10] and the diffusion chambers were subjected to 300rpm magnetic stirring throughout the experiment. The volume of the donor and receptor chambers were 3ml. 1ml of sample and 2ml PBS was pipetted into the donor compartment, whereas PBS was used as the receptor medium. The initial concentration of the donor compartment was measured. The surface area exposed for diffusion was 1.27cm^2. Aliquots of 0.5ml sample from receptor chamber was withdrawn every 30 minutes for 6 hours. Fresh buffer was used to replace the withdrawn volumes. The experiment was continued up to 24 hours. The samples were analysed using Bradford assay.

2.7.1 Data Analysis

The data from permeation experiment was calculated according to Fick's first law to obtain the flux and permeability coefficient of *L. pumila* and *F. deltoidea* loaded liposomes. The cumulative amount penetrated through rat skin was calculated through a unit of surface of the skin (Q = µg/cm^2) [11]. The steady state condition was obtained by the following equation;

$$Q = J_{SS}\ (t - t_L) \quad (2)$$

Where Q is the cumulative amount of samples over time, t_L is the lag time represented as the intercept of the linear portion of the graph when y=0 and the flux (J_{SS}) is the slope of the linear curve. The lag time is the time engaged by the liposomes to initiate its diffusion through the skin. The diffusion coefficient (D) was calculated according to the following equation;

$$D = \frac{h^2}{6.t_L} \quad (3)$$

Where h is skin thickness measured as 0.077cm. The permeability coefficient (P) was calculated according to *Eq.4*, where C_d is the concentration of donor at t = 0.

$$P = \frac{Jss}{C_d} \quad (4)$$

The liposomes partition coefficient (K) was calculated as follows;

$$K = \frac{P.h}{D} \quad (5)$$

All data presented are the mean of triplicate experiment.

3.0 RESULTS AND DISCUSSION

Freshly prepared liposomes were stored in dark vials and kept at 4°C. The liposomes were mixed with

sodium ascorbate to minimise lipid oxidation [12] and prolong the shelf life. Size distribution of the liposomes was analysed for 40 days. In addition, the zeta potential, morphology and entrapment efficiency of the herbal extract-loaded liposomes were also studied. Permeation study of the loaded liposomes using rat skin was also carried out to investigate the capability of the liposome as the transdermal carrier for both plant extracts.

3.1 Particle Size and Zeta Potential Measurements

The size of the liposomes was measured immediately after preparation. Vesicle size for the unloaded liposome, liposome loaded with *L. pumila* water extract and liposome loaded with *F. deltoidea water extract* at day 1 were 120 ± 9 nm, 140 ± 3 nm and 132 ± 1 nm, respectively (Table 1). As expected, the unloaded liposome was smaller compared to the loaded liposomes.

Zeta potential readings derived from the potential difference between the surrounding dispersant and the slipping points of the vesicles [13]. The herbal extract loaded liposomes were found to give slightly higher zeta potential readings compared to the unloaded liposome (Table 1) suggesting that both of the loaded liposomes were more stable compared to the unloaded liposome.

Size measurement were repeated (day 7, 14, 28 and 40) to study the stability of the liposomes. Even though unloaded liposomes were expected to be less stable than the loaded liposomes, the size distribution study showed that size variations among the three types of liposomes were not significant as shown in Figure 1. One contributing factor might be the addition of sodium ascorbate to the liposomal suspensions. Sodium ascorbate is an antioxidant that can be used to prevent or minimise the oxidation of the liposome's lipid bilayer membrane [12, 14].

3.2 Morphological Investigation

The spherical structure of the liposome was confirmed using TEM (Figure 2). The size of liposomes obtained from the micrographs correlated well with the measurements obtained from the particle sizer. The micrographs indicate that both the loaded and unloaded liposome were almost spherical with smooth surfaces.

Table 1 Particle size and zeta potential values of the unloaded and loaded liposomes

Compound	Size (nm)	PDI	Zeta Potential (mV)
Unloaded liposome	120 ± 9	0.15	-27.9 ± 5.1
Lp-liposome	140 ± 3	0.22	-47.2 ± 10.5
Fd-liposome	132 ± 1	0.23	-46.8 ± 8.1

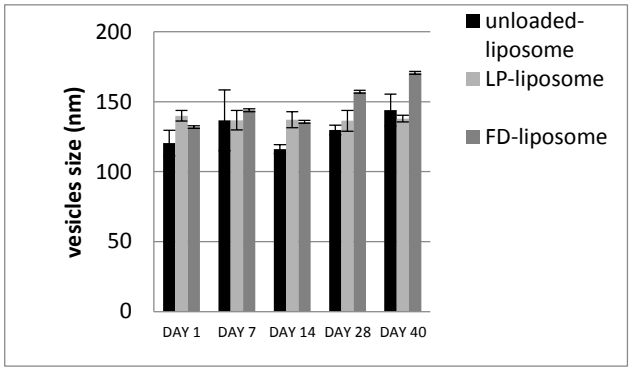


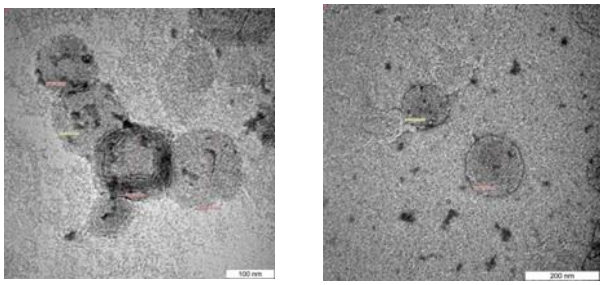
Figure 1 Variation in the size of liposomes over a period of 40 days

3.3 Entrapment Efficiency

Efficient loading of the active agents in liposomes can be achieved through passive or active entrapment procedures depending on the characteristic of the payload [15]. In passive loading, the payload can be added during liposome formation but in active loading, the payload is added to preformed liposomes.

For hydrophilic compounds, passive loading of the payload is achieved during the hydration of the dry lipid films and is based on the entrapment of the compound in the liposome's core. The efficiency of the loading of the payload into the liposome's core depends on the ability of the payload to pass through the lipid bilayer. For a hydrophilic compound, passive loading usually results in low entrapment efficiency [16]. Entrapping high molecular weight payloads in small sized liposomes will also reduce the entrapment efficiency [17].

In this study, passive loading of the hydrophilic herbal extract was performed during the hydration of the dry lipid films. The efficiency of the liposomes in entrapping *L. pumila* extracts and *F. deltoidea* were 35.47 ± 2.71% and 31.23 ± 7.65%, respectively (Table 2). These values are rather low due to the rather large molecular size of the extracts and the hydrophilic nature of the extracts.



(a) **(b)**

Figure 2 TEM micrograph of a) herbal extract loaded liposome (magnification of 200k) b) unloaded liposome (magnification of 150k)

Table 2 Entrapment efficiency of the liposomes

Compounds	% EE ± SD
Labisia pumila	35.47 ± 2.71
Ficus *deltoidea*	31.23 ± 7.65

3.4 Permeation Study Using Rat Skin

Permeability study of liposomes loaded *L. pumila* or *F.deltoidea* was carried out using Franz diffusion cells. Rat skin was used for the permeation study. Non-encapsulated *F. deltoidea* extract, non-encapsulated *L. pumila* extract, liposome loaded with *F. deltoidea* extract and liposome loaded with *L. pumila* extract were allowed to permeate through rat skin for the period of 24 hours.

The diffused amount of liposomes loaded with *L. pumila* through rat skin over 24 hours was almost twice the amount of non-encapsulated *L. pumila (*38.82 ± 0.5 % vs 21.16 ± 1.25%). The same trend was exhibited by liposomes loaded *F. deltoidea when compared to non-encapsulated F. deltoidea (*39.99 ± 0.61% vs 16.92 ± 0.86%) (Table 3). This shows that encapsulation in liposome improves the penetration of the herbal extracts through the skin.

Table 4 shows the transport properties of encapsulated and non-encapsulated herbal extracts. Encapsulation of the herbal extracts in liposome improved lag time. Lag time was the period taken by the herbal extracts to permeate through the membrane and diffuse to the receptor side before reaching steady state. The transport of the herbal extracts was purely passive as a linear relationship between flux and concentration gradient was observed (data not shown).

Particle charge and lipid solubility can influence the permeability of the liposomes. Higher partition coefficient was observed for the penetration of encapsulated extracts which might be due to the negative surface charge of the liposomes that resulted in a deeper penetration through the skin [18].

Table 3 The amount of encapsulated and non-encapsulated herbal extracts that permeated through rat skin after 24 hours

Samples	Donor t=0 (µg/ml)	Amount retained at skin (µg/ml)	Permeated amount (µg/ml)	% permeated amount
Liposomes –*L.pumila*	51.6 ± 0.57	31.57 ± 0.09	20.03 ± 0.47	38.82 ± 0.5
L.pumila extract	99.58 ± 0.37	78.5 ± 1.43	21.07 ± 1.2	21.16 ± 1.25
Liposomes-*F.deltoidea*	50.51 ± 0.49	30.31 ± 0.03	20.20 ± 0.49	39.99 ± 0.61
F.deltoidea extract	98.73 ± 0.76	82.02 ± 0.14	16.71 ± 0.69	16.92 ± 0.86

Table 4 Transport properties of the encapsulated and non-encapsulated extracts through rat skin

Samples	Lag time (h)	Flux (Jss) µg/cm²h⁻¹	Difussion coefficient (D)- cm²/h	Permeability (P)- cm/h	Partition coefficient (K)
Liposomes-*L.pumila*	0.11	67.43	9.23×10^{-3}	1.31	10.85
L. pumila extracts	0.22	15.97	$4.4x\ 10^{-3}$	0.16	2.78
Liposomes *F.deltoidea*	0.08	64.48	1.2×10^{-2}	1.28	7.67
F.deltoidea extracts	0.17	24.02	5.9×10^{-3}	0.45	5.86

4.0 CONCLUSION

In this work, the potential of liposome as a suitable transdermal carrier for herbal extracts have been investigated. The results of the study showed that liposomes loaded with *Labisia pumila* and *Ficus deltoidea* can be utilized as active ingredients in cosmetic formulation.

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