THE COMPARISON STUDY OF DIFFERENT ADDITIVES ON CHARACTERISTIC AND PERFORMANCE OF *FICUS DELTOIDEA*- LOADED NIOSOMES

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To my beloved mother, Mrs Halimah @ Fatimah Binti Awang. My supported family and friends. May Allah S.W.T bless us always.

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ABSTRACT

To ensure the effectiveness of any cosmetic formulation, the active ingredients should be able to penetrate the natural barrier of the skin, which is the stratum corneum (SC). In order to ensure that active ingredients penetrate the skin barrier, a carrier must be utilized to carry the active ingredients. The purpose of this study was to develop a stable niosomal delivery system for *Ficus deltoidea* by using sorbitan monostearate (Span 60) as a surfactant. In addition, cholesterols, β-sitosterols, and PEG-8 caprylic/capric glycerides (Labrasol) were applied as additives. These vesicles were characterized based on zeta potential, vesicle size distribution, encapsulation efficiency (EE %), niosome morphology, and *in-vitro* permeation. Following application of *Ficus deltoidea* loaded niosome on reconstructed human pigmented epidermis, the efficacy of the niosome in reducing melanin level was tested based on the microscopic observation of melanin distribution and measurement of melanin content. The developed niosomes (Span 60/cholesterol/Labrasol and Span 60/β-sitosterols/Labrasol) were found to be the most stable and promising niosomes after 3 months of storage, as both niosomes preserved their stability in terms of zeta potential, vesicle size and loading capacity. Zeta potential for both niosomes was measured at approximately -30 mV. The vesicle size was found to be in the range of 140 nm to 170 nm and both niosomes had high encapsulation efficiency (87.55 ± 5.35 % and 78.38 ± 0.37 % for Span 60/cholesterol/Labrasol and Span 60/β-sitosterol/Labrasol niosomes, respectively). These results indicated that β-sitosterols could also be option as additive since it displayed similar characteristics as cholesterol and the stability of loaded niosomes was improved with the inclusion of Labrasol. This study suggested the potential use of loaded niosome as a stable carrier for delivery of anti-melanogenic effects of *Ficus deltoidea*.

ABSTRAK

Bagi memastikan keberkesanan formula kosmetik, bahan aktif harus dapat menembusi halangan semulajadi pada kulit, iaitu stratum korneum (SC). Dalam usaha untuk memastikan bahan-bahan aktif itu dapat menembusi halangan kulit dan pergi terus ke sel-sel yang disasarkan, satu agen pembawa haruslah digunakan untuk membawa bahan aktif ini. Tujuan kajian ini adalah untuk membangunkan satu sistem pembawa "niosomal" yang stabil untuk *Ficus deltoidea*, Di mana, monostearate sorbitan (Span 60) digunakan sebagai surfaktan. Di samping itu, kolesterol, β-sitosterol dan PEG-8 kaprilik / kaprik gliserida (*Labrasol*) telah digunakan sebagai bahan tambahan. Ciri-ciri vesikel ini telah dikatogerikan berdasarkan potensi zeta, taburan saiz vesikal, kecekapan pengkapsulan (EE%), morfologi niosom dan penyerapan *in-vitro*. Kemudian keberkesanan niosom diuji berdasarkan pemerhatian mikroskopik terhadap melanin kerana *Ficus deltoidea* mempunyai kesan anti-melanogenik. Span 60/cholesterol/*Labrasol* dan Span 60/β-sitosterol/*Labrasol* niosom telah ditemui sebagai niosom yang paling stabil dan tetap kekal setelah berada dalam simpanan selama 3 bulan di mana kedua-dua niosom mengekalkan kestabilan mereka dari segi potensi zeta, saiz vesikal, dan kecekapan pemerangkapan. Zeta potensi untuk kedua-dua niosom diukur pada kira-kira -30 mV. Saiz vesikal didapati dalam lingkungan 140 nm hingga 170 nm dan kedua - dua niosom mempunyai kecekapan pengkapsulan yang tinggi iaitu (87.55 ± 5.35%, 78.38 ± 0.37%) masing- masing untuk Span 60 /cholesterol/ *Labrasol* dan Span 60 / β -sitosterol / *Labrasol*. Keputusan ini menunjukkan bahawa β -sitosterol boleh menjadi pilihan sebagai sokongan tambahan kerana ia memaparkan ciri yang sama dengan kolesterol dan kestabilan niosom telah dipertingkatkan dengan penambahan *Labrasol*. Kajian ini mencadangkan potensi dan keberkesanan niosom sebagai pembawa yang stabil untuk menyampaikan kesan anti-melanogenik *Ficus deltoidea*.

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LIST OF ABBREVIATION

β-sito	-	B-sitosterols
CH	-	Cholesterol
CPP	-	Critical packing parameter
DLC	-	Drug loading capacity
EE	-	Encapsulation efficiency
FD	-	*Ficus deltoidea*
HLB	-	Hydrophilic-lipophilic balance
LUV	-	Large uni-lamellar vesicle
LAS	-	PEG-8 Caprylic/Capric glyceride (Labrasol)
MLV	-	Multi-lamellar vesicle
mM	-	Milimolar
nm	-	Nanometer
PDI	-	Polydispersity Index
RHPE	-	Reconstructed human pigmented epidermis
SC	-	Stratum corneum
Sp 60/Span 60	-	Sorbitan monostrearate
TEM	-	Transmission Electron Microscopy
TPC	-	Total Phenolic Content
ZP		Zeta potential

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CHAPTER 1

INTRODUCTION

1.1 Background of The Study

Nowadays, health care formulations must meet high criteria of product efficacy as the masses are becoming more cognizant of the potency and side effects of a pharmaceutical product. Currently, many products have taken advantage of nanotechnology. Nanotechnology holds great prospect in all forms of industrial areas. Recently, cosmetic nanotechnology, called nanosome technology, such as liposomes, niosomes, cubosomes, solid lipid nanoparticles (SLN), polymeric micelles, nano-emulsions, and others are organized as a delivery and stabilizing system for cosmetic ingredients.

To ensure the effectiveness of any cosmetic formulation, active ingredients should be transported into the deeper layer of skin. Still, penetration of substances through the skin is determined by the skin's natural barrier, which is the stratum corneum (SC) with its "brick and mortar" architecture (Bolzinger *et al.*, 2012). To assure that the active ingredients can penetrate the skin barrier, a carrier must be employed to carry the actives. Moreover, this method can also protect the actives

from degradation and increase the stability of the formulation (Sudhamani *et al*., 2010).

Niosome is a non-ionic surfactant based vesicle obtained from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structure which can entrap both lipophilic and hydrophilic drugs (Dahiya *et al.,* 2011). The amphiphile basically consists of a hydrophilic head group and a hydrophobic tail. The hydrophobic moiety could be one or two alkyl or perfluoroalkyl group and also a single steroidal group (Ijeoma and Suresh, 1998). Its biocompatible and biodegradable nature makes them less toxic compared to other vesicular systems. Lower cost of niosome production is attributed to the greater availability of non-ionic surfactants. There is no special condition required for handling and storage of this vesicle. These properties make them versatile carriers and interesting candidates for study.

To develop any niosomal drug delivery systems, a few variables must be well controlled. These include choice of main surfactant, nature of membrane additive, and also the nature of drug (Ijeoma and Suresh, 1998). All of these factors will influence the niosome's physical properties. A good surfactant for niosome is a substance that has the ability to enhance their physicochemical and biological features, to achieve a successful therapeutic response. Advantageously, niosomes could be constructed from a variety of hydrophilic head groups. Based on that reason, different kinds of surfactants have been analysed with the purpose of obtaining innovative niosomal systems, which could upgrade the therapeutic effects of delivered active compounds.

1.2 Problems Statement

To bring out a highly stable niosome, an additive should be inserted into the formulation in order to stabilize the vesicles. According to Junyaprasert *et al.,* (2012), the stability of certain niosomes depend on the type of membrane additive. Generally, the most common additive found in niosomal systems is cholesterol.

Cholesterol is a membrane constituent that is widely found in a biological system and in all animal tissues. In cosmetic products, it is primarily used as an emollient, while in the pharmaceutical area, it is known as an emulsifying agent. The overall function of cholesterol in plasma membranes relates to the fluidity of the membrane (Liebert, 1986). It is claimed to have the ability to modulate membrane fluidity and elasticity. Nevertheless, as reported by Junyaprasert *et al.*, (2012), at certain amounts, this additive could affect membrane permeability, encapsulation, bilayer rigidity, ease of rehydration of freeze dried niosome, and toxicity. Moreover, cholesterol might cause some negative effects when used in human pharmaceuticals. Since most cholesterol is obtained from egg or wool grease, it is not suitable for human pharmaceuticals.

These days there is concern about any product derived from animal including those derived from sheep or cattle because of possible transmission of bovine spongiform encephalopathy or better known as “mad cow disease”. The prevailing opinion is that this problem was originally caused by feeding animal product. When the cholesterol is extracted from spinal cords or wool, there is always the chance of contamination with trace amount of other animal product. Although the risk of anybody contracting mad cow disease from cosmetic borders on zero, but cosmetic producers have to contend with the perception among consumer that the inclusion of any animal product is undesirable. In addition, cholesterol is readily oxidized, leading to a stability problem for lipid based drug products. Some of these oxidation by-products tend to be rather toxic in biological systems (Mahmoud *et al*., 2008).

Since cholesterol is not preferable in pharmaceutical products due to its side effects, sterols from plant membrane (phytosterol) can be used as an alternative to mammalian cholesterols. This plant membrane contains a mixture of sterols including β-sitosterol, campesterols and stigmasterols. Plant sterols attract much attention due to their ability to reduce cholesterol absorption in the intestine and hence, protect against cardiovascular diseases. Apart from functioning as a membrane modulator, β-sitosterol also has antiseptic, antineoplastic and antipyretic effects (Ovesna *et al*. 2004 ; Awad and Fink, 2000). Phytosterols may also provide protection against cancers of the colon, breast and prostate. However, the influences of β-sitosterol on the modulation of the membrane structure have yet to be studied in depth. Therefore, in this study the effect of this additive will be studied and compared with cholesterols.

PEG-8 Caprylic/Capric glyceride (Labrasol) is employed as a surfactant for two immiscible liquids, water and oil. It is used as a surfactant to form colloidal dispersions that are known as single-phase microemulsions. Drug delivery systems based on microemulsions have a special interest since they generally have low toxicity and irritancy, plus are easy to prepare (Ljiljana *et al* . 2012). Zhaopeng *et al.* 2002 and Karatas *et al*., 2005 proved that Labrasol can increase the solubility and enhance the absorption of poorly absorbable drugs. Moreover, it also has the same properties with cholesterols which means that it could act as an emulsifying agent. Nevertheless, evaluation about the potential of Labrasol as a surfactant for niosome has not been investigated. Hence, in this study, the influences of this surfactant as a niosome additive will be examined.

For this research, Mas cotek (*Ficus deltoidea*) was chosen because this herb has been recognized to possess strong antioxidant activities, and has been found to exhibit anti melanogenetic effects (Myoung J. *et.al.,* 2010 ; Hyun *et al*. 2009). The components of *Ficus deltoidea* include polyphenols, vitamin C, carotene, anthocyanins and flavonoids.

In this work, the effects of cholesterol, B-sitosterol and PEG-8 Caprylic/Capric glyceride (Labrasol) on niosome were examined. Then, the efficacy of *Ficus Deltoidea*-loaded niosome was studied.

1.3 Objective of Study

The purpose of this research is to determine the influence of various additives on the stability and characteristics of *Ficus deltoidea*-loaded niosomes.

1.4 Scope of Study

In order to achieve the objective, the study has been divided into three scopes which are:

i. Development of *Ficus deltoidea*-loaded niosomes using different additives.

ii. Characterization of FD-niosome based on zeta potential measurement vesicle size distribution by using Zetasizer Nano ZS, measurement of encapsulation efficiency by Total Phenolic Content (TPC) assay, measurement of niosomal morphology using TEM, and also *in-vitro* penetration of *Ficus deltoidea*-loaded niosome through reconstructed human epidermis.

iii. Efficacy study of *Ficus deltoidea*-loaded niosome by microscopy observation for melanin distribution using reconstructed human pigmented epidermis- SkinEthic.

87.55% for Span 60/CH/LAS niosome and 78.38% for and Span 60/β-sitosterol/LAS niosomes.

Since Span 60/CH/LAS and Span 60/β-sitosterol/LAS niosomes demonstrated good characteristic properties, these samples were chosen to undergo the *in-vitro* penetration study. From this analysis, it was found that the prepared niosomal system could enhance the permeability of *Ficus deltoidea* extract into the skin (epidermis layer). Efficacy test showed that Span 60/CH/LAS and Span 60/β-sitosterol/LAS niosomes had successfully reduced melanin distribution of reconstructed human pigmented epidermis (RHPE).

Based on the results obtained, it can be said that the overall objectives of this experiment have been achieved. By the addition of cholesterol, β-sitosterol and Labrasol ,a promising and stable niosomal system was obtained. Interestingly, β-sitosterol has been proven to have the same function as cholesterol as a membrane additive and will be more attractive to the cosmetic industry compared to cholesterol.

5.2 Recommendations

Based on the results and summary obtained from this study, the following future works are commended:

1. Different charge of membrane additives should be employed in niosome formulation. From that point, the effects of charge additives on the physicochemical properties and stability of niosomes could be examined.

2. In this study, all samples were stored at 4°C. It would be an opportunity to explore the effect of storage temperature toward the stability of niosomes.

3. Future work can also scrutinize the vesicle size effect toward the penetration rate.

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