OPTIMIZATION OF FORMULATION AND PROCESS CONDITION FOR DOWNSIZING OF *LABISIA PUMILA* AND *FICUS DELTOIDEA* LOADED NIOSOME

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ABSTRACT

Niosome, a novel drug delivery system was proposed to encapsulate *Labisia pumila* (LP) and *Ficus deltoidea* (FD) extract in order to enhance their absorption through skin. However, niosomes preparation involves several parameters such as formulation and process condition which affect the niosomes production. Thus the aim of this study is to obtain the optimum formulation and process condition for encapsulation of niosomes loaded LP and FD extract. Niosomes loaded with both extracts were prepared by coacervation phase separation method and further downsized with high pressure homogenizer, Design of experiment (DOE) was employed to ease this research. Mixture design experiment (14 batches) was applied to obtain the optimum formulation while Central Composite Design (11 batches) was applied to obtain optimum process condition. The factors involved in formulation were amount of Span 60, cholesterol and Labrasol while the parameters that affect the process condition were homogenization cycle and pressure. Their corresponding responses for both designs were the same, which were entrapment efficiency (EE), particle size (PS) and zeta potential (ZP). From the study, the optimum formulation obtained consists of 0.6 mmol of Span 60, 0.4 of mmol cholesterol and 0.1 mmol of Labrasol. Meanwhile, the optimum process condition for both LP and FD niosomes is 1100 bar of pressure and 6 cycles. It has been indicated that the mixture design and Central Composite design of experiment have succeeded in optimization of the formulation ingredients and processing parameters. The obtained optimized niosomes also has manifested a remarkable improvement in skin penetration and skin retention.

ABSTRAK

Niosom yang merupakan sistem penyampaian ubat novel telah dicadangkan untuk mengkapsulkan ekstrak Kacip fatimah (LP) dan Mas cotek (FD) dengan tujuan untuk meningkatkan kadar penyerapan ekstrak melalui kulit. Proses penyediaan niosom melibatkan beberapa parameter seperti formulasi dan pemprosesan. Oleh itu kajian ini bertujuan mendapatkan formula dan parameter pemprosesan niosom LP dan FD yang optimum untuk pengkapsulan. Niosom LP dan FD telah disediakan dengan kaedah pemisahan fasa koaservatan dan seterusnya saiznya dikecilkan dengan penghomogenan bertekanan tinggi. Reka bentuk eksperimen (DOE) telah digunakan untuk memudahkan kajian ini. Reka bentuk campuran (14 set) telah digunakan untuk mendapatkan formulasi yang optimum manakala reka bentuk komposit pemusatan (11 set) telah digunakan untuk mendapatkan parameter pemprosesan yang optimum. Faktor yang terlibat dalam formulasi ialah jumlah *Span 60*, jumlah kolesterol dan jumlah *Labrasol* manakala parameter yang terlibat sebagai parameter pemprosesan ialah kitaran penghomogenan dan tekanan penghomogen. Respon untuk kedua-dua reka bentuk adalah sama, iaitu kecekapan pemerangkapan (EE), saiz zarah (PS) dan potensi zeta (ZP). Formulasi optimum diperoleh ialah 0.6 mmol *Span 60*, 0.4 mmol kolesterol, dan 0.1 mmol *Labrasol*. Parameter pemprosesan yang optimum untuk kedua-dua LP dan FD niosom ialah tekanan 1100 bar dan 6 kitaran pemprosesan. Kedua-dua reka bentuk telah berjaya dalam mengoptimum formulasi dan parameter pemprosesan, niosom yang dioptimumkan juga menunjukkan peningkatan kadar penyerapan ekstrak dalam kulit.

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LIST OF ABBREVIATION

LP	-	*Labisia pumila*
FD	-	*Ficus deltoidea*
OFAT	-	One factor at a time
EE	-	Entrapment efficiency
ZP	-	Zeta potential
PS	-	Particle size
DOE	-	Design of experiment
PEVs	-	Penetration enhancer-containing vesicles
CCD	-	Central Composite Design
SEDDS	-	Self emulsifying drug delivery systems
SMEDDS	-	Self micro emulsifying drug delivery systems
SLMs	-	Solid Lipid Microparticles
SLNs	-	Solid lipid nanoparticles
NLC	-	Nanostructure lipid carriers
LDC	-	Lipid drug conjugates
HLB	-	Hydrophilic-lipophilic Balances
PBS	-	Phosphate buffer solution

LIST OF SYMBOLS

Kp	-	The apparent permeability coefficient
$Q_t/S.$	-	The cumulative drug permeation per unit of skin surface area

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In recent years, natural herbs are widely used in cosmeceutical area due to their fewer side effects as compared to the synthetic or chemical based active ingredient products. Consumer awareness towards hazardous side effects of those chemical derived cosmetic products highly increase the demand of herbs based cosmetic. However, there are several common problems to have herbs applied in cosmeceutical field, as several phytoconstituents (such as flavonoids, terpenoids, tannins etc.) have poor absorption through skin. This is due to their poor lipid/water solubility and/or incompatible molecular size (Manach *et al.*, 2004).

Multiple approaches have been carried out to ameliorate the absorption. Due to amphiphilic (lipohilic and hydrophilic) nature of phytochemical, some may isolate or purify the constituents in order to make the process of absorption enhancement easier. However, this may lead to a partial or total loss of specific bioactivity for the isolated constituent, i.e. the natural constituent synergy becomes lost. Furthermore, some constituents from plant extract may be destroyed during formulation due to the interaction between extract and other ingredients. Therefore, a good delivery system

which able to encapsulate the plant extract without purification or isolation, and able to formulate for topical is needed.

Novel drug delivery system such as polymeric nanoparticles, nanocapsules, vesicular systems (liposomes, niosomes, etc.), solid lipid nanoparticles, phytosomes, nanoemulsions and microsphere, have been significantly investigated since they are able to fulfill the requirements mentioned above. There are some drawbacks from the suggested delivery systems, for example, polymer-based nanoparticles, the toxicity of polymers and of solvent residues used in their production, high cost and difficulty of the large-scale production of biodegradable polymers, possibility of polymer erosion leads to drug diffusion through the matrix or desorption from the surface and potentially toxic or allergic final products of biodegradable polymers (Muller and Keck, 2004).

In order to overcome the problems of nano capsules and polymeric nanoparticles, considerable attention has been focused on the development of lipid-based drug delivery systems (LBDDSs). This is an accepted method for delivery systems because of their remarkable inherited properties, their biocompatibility and biodegradability of physiologically tolerated lipids, physiochemical diversity, and lower toxicity (Chen *et al.*, 2010; Wissing *et al.*, 2004).

Vesicular systems as a LBDDS have potential applications in the delivery of both hydrophilic and hydrophobic drugs which are encapsulated in interior hydrophilic compartment and outer lipid layer respectively (Gangwar *et al.*, 2012). Among all vesicular systems, liposomes have been widely used. However, liposomes undergo some problems like oxidation of phospholipids which cause chemical instability. Hence, another vesicular system, which is niosome is proposed, the phospholipid of the vesicular systems was replaced with non-ionic surfactant which have longer shelf life, besides, it is non toxic and less production cost.

Labisia pumila (LP) and *Ficus deltoidea* (FD) are chosen as encapsulated materials since they both have their own unique benefits to be applied in cosmeceutical field. *In-vitro* study showed that LP was able to protect fibroblast and keratinocytes from being damaged by UVB irradiation and boost the collagen synthesis, both of the properties are very ideal to be applied as anti-photoaging in cosmeceutical field (Choi *et al.*, 2010). Findings suggested that FD shows strong anti-melanogenic activity by prohibiting the process of tyrosinase oxidation, prove that FD extract has potential to be used as a novel depigmenting agent for cosmetics (Oh *et al.*, 2010; Hasham *et al.*, 2013). However, LP and FD extract are both hydrophilic in nature, most of the water soluble phytoconstituents (such as flavonoids, tannins, etc) are poorly absorbed due to their poor lipid solubility , thus limiting their ability to cross the lipidic biomembrane (Manach *et al.*, 2004). These poor solubility extracts could be embedded in niosomes as they can reduce the barrier properties thus enhance the penetration of the skin.

Coacervation and phase separation, a method for proniosomes preparation is chosen for preparation of niosomes dispersion among other methods as this is a simple practical method for the routine and large scale production with the minimum use of organic solvent (Fang *et al.*, 2001). Main ingredient of niosomes is non-ionic surfactant, normally additives are added to enhance the stability and permeability of vesicles. Span 60 was chosen as non-ionic surfactant, and cholesterol was added to stabilize the niosomes dispersion, Labrasol, a penetration enhancer was added as well to increase the permeability of niosomes. Obtained niosomes dispersion is usually large in size and low uniformity, therefore downsizing is needed. Among the downsizing methods (such as sonication, extrusion, etc.), high pressure homogenization was chosen as it can be directly scaled up in order to obtain large scale production (Barnadas-rodrı and Sabe, 2001).

Design of experiment (DOE) was then carried out to deal with parameters that affect the niosomes production, there were two designs constructed, For instance, mixture design was carried out to deal with formulation and Central Composite design (CCD) was implemented to deal with processing parameters.

Finally niosomes were prepared based on optimum conditions obtained through the design, the permeability of the niosome were studied by Franz diffusion system.

1.2 Problem Statement

Benefits of LP and FD extract are not only on promoting health effect but also giving a beneficial effect to the skin such as anti-aging and whitening effect. However water extract of LP and FD are mainly hydrophilic in nature, which may reduce their absorption through skin as they are not able to cross the lipidic bio-membrane (Bhattacharya, 2009). Some methods were reported to overcome phytochemicals absorption problems such as purification and isolation, however this kind of method will reduce the efficacy of herbs due to the loss of synergy effect. Therefore, a good delivery vehicle is needed to deliver the extracts to the targeted site. Several herbs extract such as *Ginkgo Biloba*, *Curcuma longa linn*, and *Silybum marianum* were reported being encapsulated by niosomes, therefore niosome is assumed feasible to encapsulate LP and FD extracts as well.

Preparation of niosomes loaded with LP and FD extracts has never been reported in any literature. In this study, niosomes was aimed to have highest entrapment efficiency (EE), lowest particle size (PS) and stable zeta potential (ZP) values, parameters which have significance effect to these responses were identified. Factors such as amount of Labrasol, amount of Span 60, and amount of cholesterol were identified through niosomes formulation, while homogenization cycle and pressure were identified through downsizing process. However, the factors involved in niosomes production do not work in the same way to achieve desired responses, for example, at high pressure, lowest PS could be obtain but the EE would be low, which is not desired in this research, therefore the optimization is needed in order to tackle this problem.

Conventional optimization involves changing one independent variable at a time while keeping others at fixed levels, it is tedious because many runs are needed if number of parameters is high, and the interaction among the parameters is undefined. Hence, DOE is carried out to reduce number of runs and able to study the interactions among the factors, which resulting in saving time, resources, chemicals, and manpower.

1.3 Hypothesis

Encapsulation of LP and FD extracts with niosomes has not been reported in any literature. Thus the optimum parameters which affect the niosomes production were expected to be obtained through DOE. Percentage of cholesterol incorporated in niosomes was claimed to be optimum at 1:1 with Span 60 (Uchegbu and Florence, 1995), thus the expected to be molar ratio between cholesterol and Span 60 was in 1:1 as well. Niosomes encapsulated with Labrasol have not been reported in any literature, thus ratio of Labrasol in niosomal formulations is unpredictable. Effect of homogenization cycle and pressure on niosomes production was studied by Zidan *et al.* (2011), thus the expected optimum point were in the range of 2 to 6 cycles and 1000 bar to 1700 bar according to their research. The optimized LP and FD niosomes were expected to have higher permeability than extracts without any encapsulation.

1.4 Objectives of the Study

The main objective of this research is to obtain the optimum formulation and process condition for downsizing of niosomes loaded LP and FD extract.

1.5 Research Scope

The scopes of the research include:

1. To determine the optimum formulation for niosomes loaded LP and FD (Span 60, cholesterol, labrasol) with mixture design using Design Expert®

2. To determine the optimum process condition for niosomes loaded LP and FD (homogenization cycle and pressure) with Central Composite design using Design Expert®.

3. To characterize the physical characteristic of niosomes (particle size, zeta potential, entrapment efficiency) for obtaining the optimum formulation ingredients and process condition.

4. To compare the permeability of LP and FD niosomes with LP and FD extract through the skin.

1.6 Significance of the Study

This study is expected to provide an optimized formulation and condition for niosomes production. Thus the data can be used for up scaling, which believed that it may boost up local industrial varieties and economics. Furthermore, this study will promote the benefits of local herbs which are LP and FD because niosomes are believed can enhance the absorption and bioavailability of extract.

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