UNIVERSITI PUTRA MALAYSIA

DESIGN AND DEVELOPMENT OF NEW PERFLUORODECALIN-IN-PALM OIL-IN WATER DELIVERY SYSTEM FOR OXYGEN-ENRICHED COSMECEUTICAL SKIN CARE

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By

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Chairperson: Professor Mahiran Basri, PhD

Faculty: Science

Cosmeceuticals, cosmetic-pharmaceutical hybrids, have gained much popularity in recent years due to the benefits it could provide to enhance health and beauty of the skin. In this research, a new colloidal delivery system was designed and developed to deliver oxygen into the skin while providing essential cosmeceutical actives. Oxygen, one of the most fundamental elements needed for sustaining life, is believed capable of maintaining epidermal health by facilitating the formation of collagen. Studies on the formation of simple emulsions were carried out to understand the fundamental characteristics of emulsions before the new colloidal delivery system was developed.

Simple emulsions; perfluorodecalin-in-water (PFD/W) and palm oil esters-in-water (POE/W) were formulated to facilitate the studies. Due to larger difference in the interfacial tension between perfluorocarbons (PFCs) and water as compared to hydrocarbons (HCs) and water, PFD/W emulsions generally have bigger droplet size as compared to POE/W emulsions. Non-fluorinated surfactant, such as Lipoid S75 was employed to formulate PFD/W emulsions, and their droplet size ranged between 1 to 20 µm. Meanwhile, POE was emulsified using surfactant mixture of Tween 80 and Pluronic F-68 into water. They were characterised as POE/W nanoemulsions, owning droplet size ranged from 90 to 200 nm and possessed minimum rate of Ostwald ripening. Apart from that, DL-alpha-tocopherol acetate (vitamin E) and Pluoronic F-68 have proven to enhance the stability of POE/W nanoemulsions.

Ternary emulsion, a novel complex emulsion was then developed from the simple emulsions formulated earlier. Ternary emulsion which consisted of three (3) immiscible phases was designed to deliver oxygen, lipophilic and hydrophilic cosmeceutical actives. The ternary emulsion was successfully formulated via a modified two-steps process, where PFD/W emulsion was

added gradually into POE/W emulsion. The architecture of this ternary emulsions, determined using CytoViva® Hyperspectral Imaging System revealed that perfluorodecalin-in-palm oil esters-in-water (PFD/POE/W) ternary emulsion were formed. The PFD/POE/W ternary emulsions had an enhanced physical stability as compared to its parental emulsions. The *in vitro* studies showed that PFD emulsions were not toxic and had considerably facilitated the proliferation of human fibroblast cells. When tested on human subject, it showed potential increase in collagen content in the dermis layer upon completing two weeks of treatments.

The best ternary emulsion to serve as a cosmeceutical skin care delivery system was found to be Formulation V15 consisting of 15% (w/w) PFD, 8% (w/w) POE, 19.8% (w/w) surfactant mixtures, 1% (w/w) Vitamin E, 1% (w/w) Kojic Acid, 1% (w/w) BHT, 1% (w/w) Phenonip and 53.2% (w/w) deionised water. Formulation V15 showed no physical separation during the accelerated as well as the long-term storage stability test. It not only had a high E_a value of 120.8 kJ K^{-1} mol^{-1} which indicated a strong resistance towards temperature stresses, but was also tested to have excellent shear-thinning property (n = 0.1462) for the ease of spreading the formulation onto the skin. This formulation is capable of delivering multiple cosmeceutical actives such as antioxidants (Vitamin E), whitening agents (Kojic Acid) and oxygen, and can be considered as the most efficient formulation for use in effective cosmeceutical skin care products.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

REKA BENTUK DAN PEMBANGUNAN SISTEM PENYAMPAIAN PERFLUORODECALIN-DALAM-ESTER MINYAK SAWIT-DALAM-AIR BAHARU UNTUK KOSMESEUTIKAL PENJAGAAN KULIT YANG DIPERKAYAKAN OKSIGEN

Oleh

BRIAN TEO SHENG XIAN

Januari 2014

Pengerusi: Profesor Mahiran Basri, PhD

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Kosmeseutikal, satu hibrid antara kosmetik dan farmaseutikal, telah mendapat popularity sejak beberapa tahun kebelakangan ini kerana manfaat yang boleh disampaikan untuk meningkatkan kesihatan dan kecantikan kulit. Dalam kajian ini, satu sistem penyampaian koloid yang baru telah direka dan dihasilkan untuk menyampaikan oksigen ke dalam kulit dan di samping menyediakan aktif kosmeseutikal yang penting. Oksigen, salah satu daripada elemen keperluan yang paling asas untuk mengekalkan kehidupan, dipercayai mampu mengekalkan kesihatan epidermis dengan membantu pembentukan kolagen. Kajian tentang pembentukan emulsi-emulsi asas telah dijalankan untuk memahami ciri asas emulsi tersebut sebelum menghasilkan sistem penyampaian koloid yang baru.

Emulsi asas seperti perfluorodecalin-dalam-air (PFD/W) dan ester minyak sawit-dalam-air (POE/W) telah digubal untuk memudahkan kajian ini. Oleh kerana perbezaan dalam ketegangan-antara-muka antara perfluorocarbons (PFC) dan air adalah lebih ketara berbanding kepada hidrokarbon (HC) dan air, emulsi PFD/W pada umumnya mempunyai saiz titisan yang lebih besar berbanding dengan emulsi POE/W. Surfaktan yang tidak memiliki fluorin, seperti Lipoid S75 telah digunakan untuk menghasilkan emulsi PFD/W, dan saiz titisan mereka adalah antara 1 hingga 20 μm. Sementara itu, POE boleh diemulsikan di dalam air dengan menggunakan campuran surfaktan Tween 80 dan Pluronic F-68. Mereka disifatkan sebagai nanoemulsi POE/W kerana memiliki saiz titisan antara 90-200 nm dan mempunyai kadar *Ostwald ripening* yang minimum. Selain daripada itu, DL-alfa-tokoferol asetat (vitamin E) dan Pluoronic F-68 telah terbukti boleh meningkatkan kestabilan nanoemulsi POE/W.

Emulsi ternari merupakan suatu emulsi komplek yang novel telah dihasilkan daripada pengkajian emulsi asas yang digubal sebelum ini. Emulsi ternari

terdiri daripada tiga (3) fasa yang tidak boleh larut di antara satu sama lain telah direka untuk menyampaikan oksigen dan aktif kosmeseutikal yang bersifatkan lipofilik dan hidrofilik. Emulsi ternari ini telah berjaya digubal melalui kaedah yang telah diubahsuai iaitu kaedah dua-langkah, di mana emulsi PFD/W ditambahkan secara beransur-ansur ke dalam emulsi POE/W. Struktur emulsi ternari ini telah ditentukan dengan menggunakan Sistem Hiperspektra CytoViva® dan telah mempamerkan pembentukan emulsi ternari perfluorodecalin-dalam-ester minyak sawit-dalam-air (PFD/POE/W). Emulsi ternari PFD/POE/W mempunyai kestabilan fizikal yang lebih kukuh berbanding dengan emulsi asal yang digunakan untuk pembentukkannya. Kajian *in vitro* menunjukkan bahawa emulsi PFD adalah tidak toksik dan telah membantu pertumbuhan sel fibroblas manusia. Apabila diuji pada subjek manusia, ia menunjukkan peningkatan kandungan kolagen yang agak merangsangkan di lapisan dermis selepas subjek menerima rawatan selama dua minggu sahaja.

Emulsi ternari yang mempamerkan keserasian yang tertinggi sebagai kosmeseutikal penjagaan kulit adalah Fomula V15 yang terdiri daripada 15% (w/w) PFD, 8% (w/w) POE, 19.8% (w/w) campuran surfaktan, 1% (w/w) Vitamin E, 1% (w/w) Asid Kojic, 1% (w/w) BHT, 1% (w/w) Phenonip dan 53.2% (w/w) air terdeionised. Formula V15 menunjukkan tiada pemisahan fizikal semasa menjalani ujian kestabilan penyimpanan jangka panjang. Ia bukan sahaja mempunyai nilai E_a yang tinggi iaitu 120.8 kJ K^{-1} mol^{-1} dimana ia menunjukkan ketahanan yang kuat terhadap tegasan suhu, malah ia dilaporkan mempunyai sifat *shear-thinning* yang sangat baik (n = 0.1462) bagi memudahkan penyebaran formula tersebut ke atas kulit. Formula ini mampu menyampaikan pelbagai aktif kosmeseutikal seperti antioksidan (Vitamin E), agen pemutihan (Asid Kojic) dan oksigen, dan boleh dianggap sebagai formulasi yang paling berkesan untuk digunakan dalam produk penjagaan kulit kosmeseutikal.

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Teo, B.S.X.

I certify that a Thesis Examination Committee has met on 10 January 2014 to conduct the final examination of Brian Teo Sheng Xian on his thesis entitled "Design and Development of New Perfluorodecalin-In-Palm Oil-In Water Delivery System for Oxygen-Enriched Cosmeceutical Skin Care" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

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DECLARATION

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I hereby confirm that:

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LIST OF ABBREVIATIONS

AOC	Artificial oxygen carrier
BHT	Butylated hydroxytoluene
d	Mean droplet size
D	Diffusion coefficient of the disperse phase in the continuous phase
DO	Dissolved oxygen
DMSO	Dimethyl sulphoxide solution
E_a	Energy of activation for viscous flow
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EYP	Egg yolk phospholipids
f	Frequency
F-44E	Bis(perfluobutyl)ethane
FMCP	Perfluoromethylcyclohexylpiperidine
FTPA	Perfluorotripropylamine
g	Gravitational acceleration
G	Complex modulus
G'	Storage modulus (Elastic component)
G"	Loss modulus (Viscous component)
HC	Hydrocarbon
k	Consistency coefficient
LVER	Linear viscoelastic region
MRI	Magnetic resonance imaging
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	Power law index
N/A	Not available
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
OD	Optical density
PBS	Phosphate buffered saline
PFC	Perfluorocarbon
PFC/O/W	Perfluorocarbon-in-oil-in-water
PFC/W	Perfluorocarbon-in-water
PFD	Perfluorodecalin
PFD/POE	Perfluorodecalin-in-palm oil esters
PFD/POE/W	Perfluorodecalin-in-palm oil esters-in-water
PFD/W	Perfluorodecalin-in-water
PFDB	Perfluorodecyl bromide
PFDCO	1,8-Dichloroperfluorooctane
PFOB	Perfluorooctyl bromide
PFTBA	Perfluorotributylamine
PO_2	Partial pressure of oxygen
POE/W	Palm oil esters-in-water
R	Universal gas constant (8.314 $J\,K^{-1}\,mol^{-1}$)
R^2	Linear regressions

r^3	Cubic radius of droplets
rpm	Rotation per minute
S(∞)	Bulk solubility
SC	Stratum corneum
SLN	Solid lipid nanoparticles
T	Absolute temperature
UV	Ultraviolet
UVB	Ultraviolet B
v/v	Volume over volume
V_m	Molar volume of the dispersed phase
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water
w/w	Weight over weight
γ	Shear rate
η	Apparent viscosity
σ	Shear stress

CHAPTER 1

INTRODUCTION

1.1 Background

The term cosmeceuticals is now commonly used to describe a hybrid category of products found on the spectrum between drugs and cosmetics (Choi and Berson, 2006). Skin care dominated about 63% of a total U.S. cosmeceutical product demand of $6.5 billion in 2010 (Freedonia, 2011). The demand is expected to increase at 5.8% per annum to $8.5 billion in 2015. The rising awareness for maintaining healthy skin and as well as the need to keep youthful appearance, regardless of gender and age, have contributed to the current market trend. In addition, hectic lifestyles and constant exposure to environmental aggressions such as UV radiation have raised some concerns about premature skin aging.

It is well-known that excessive exposure to UV radiation from the sun could cause the skin to age prematurely. Harmful UV rays from sunlight, when penetrated deep into the skin, can cause damage to the collagen in the dermis (Noguchi and Djerassi, 2009). Collagen is one of the key components that provide firmness and elasticity to the structure of the skin. Supplying oxygen (O_2) to the skin could facilitate the formation of collagen (Sen, 2009) which in turn would rejuvenate the skin and maintain its youthfulness. Oxygen was also claimed by several researchers to be useful for wound healing processes. Hence, it is believed that consumers will benefit from an oxygen-enriched cosmeceutical product.
However, oxygen is extremely insoluble in almost any liquid, which makes it very difficult to be administered topically. Hence, oxygen must be entrapped or dissolved in a carrier so that it can be delivered effectively to the targeted site. Perfluorocarbon (PFC) liquids are the best option to dissolve oxygen thus far, due to the exceptionally large gas-dissolving capacity of PFCs (Riess and Krafft, 2006). This unique property of PFCs was highly-favoured in the development of artificial oxygen carrier (AOC) during the past two decades as a substitute to human blood during emergencies. However, literature associated with the development of oxygen carrier for cosmeceutical applications continues to be scarce.

A few reportedly stable PFC emulsions have been formulated in the recent years; however, these were emulsified using fluorinated surfactants. As some fluorinated surfactants are bioaccumulative and toxic to the environment, non-fluorinated surfactants are more favourable in light of environmental consciousness. There was also an effort made by Weers *et al*. in 2004, to stabilise the PFC/W emulsion by the addition of a small percentage of long-chain triglycerides. However, their efforts were thwarted when the addition of the long-chain triglyceride (soybean oil) resulted in oil de-mixing and formed two distinct populations of emulsion droplets. The implications would be great

if they were to succeed by adding the triglyceride, to result in the formation of a multifunctional colloidal delivery system.

The future of the cosmeceutical industry lies in the development of new products which can decrease the amount of time and tedium of a consumer's skin care routine and yet be able to yield appreciable results. For this reason, a multifunctional colloidal delivery system could be useful for the delivery of multiple cosmeceutical actives or substances. Colloidal systems are commonly employed to deliver cosmeceutical actives, owing to the fact that some of these colloids are capable of encapsulating actives (Yang and McClements, 2013; McClements, 2012; Mihranyan *et al*., 2012; Velikov and Pelan, 2008; Flanagan and Singh, 2006; Taylor *et al*., 2005) and delivering them effectively to the targeted cells. Hougeir and Kircik (2012) have identified and reviewed some of those delivery systems used in consumer health products, especially cosmetics.

1.2 Problem Statements

Supplying oxygen (O_2) to the skin could facilitate the formation of collagen which in turn would rejuvenate the skin and maintain its youthfulness. However, it is nearly impossible to deliver pure oxygen to the cellular level without a proper delivery system because the oxygen solubility in water is extremely low, even more so in oil. Oxygen must be entrapped or dissolved in PFC liquids for effective transportation and then released on site. In addition to these challenges, such a hypothetical idea saw another potential challenge which involves the extreme hydrophobicity of PFCs, being considerably more hydrophobic than hydrocarbons. Therefore, possible administrations are usually in the form of colloids such as PFC-in-water emulsions. That being said, stable PFC emulsions are very difficult to be formed due to the high interfacial tension between PFC and water and the tendency of PFC liquids to keep to themselves. To make the matter worse, the density of PFC liquids are approximately two-fold of that of water. The majority of PFC emulsions formed thus far have very short shelf-lives, approximately two weeks to several months and usually requiring storage at lower temperatures.

1.3 Objectives

The objective of this research is to design and develop a novel PFC-based colloidal delivery system from palm oil-based esters for oxygen-enriched cosmeceutical skin care formulations. Thus, the research embarks on the following specific objectives:

a) To develop and investigate the formation of simple PFC emulsions and palm-based esters emulsions;
b) To develop a ternary (triphasic) emulsion as a novel multifunctional colloidal delivery system for oxygen-enriched cosmeceutical applications;

c) To evaluate the physicochemical characteristics of the ternary emulsion in terms of physical stability, flow behaviour, microstructural behaviour and architecture of the ternary emulsion; and,
d) To evaluate the potential application of the ternary emulsion using *in vitro* and pre-*in vivo* approach.

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