



UNIVERSITI PUTRA MALAYSIA

**FORMATION OF NANOSTRUCTURED LIPID CARRIER SYSTEMS
LOADED WITH PALM PHYTONUTRIENTS FOR
APPLICATION IN COSMETICS**

LOO CHEW HUNG

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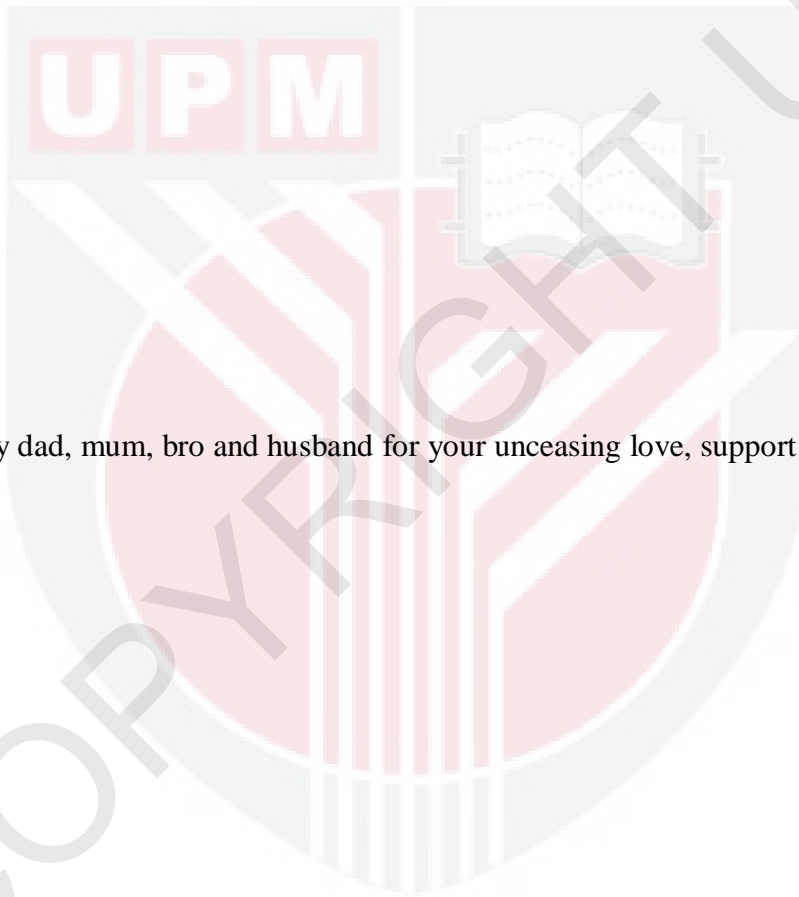
**FORMATION OF NANOSTRUCTURED LIPID CARRIER SYSTEMS LOADED
WITH PALM PHYTONUTRIENTS FOR APPLICATION IN COSMETICS**

By

LOO CHEW HUNG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

July 2013



To my dad, mum, bro and husband for your unceasing love, support and faith in me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

FORMATION OF NANOSTRUCTURED LIPID CARRIER SYSTEMS LOADED WITH PALM PHYTONUTRIENTS FOR APPLICATION IN COSMETICS

By

LOO CHEW HUNG

July 2013

Chair: Professor Mahiran Basri, PhD

Faculty: Science

Nanostructured lipid carrier has gained increasing interest in cosmetic field but the major problems are physical stability of NLC, chemical stability of bioactives in NLC and efficacy of NLC. The aim of this study was to develop nanostructured lipid carrier for delivery of antioxidant, palm phytonutrients (PP). The study was divided into four parts consisting of characterization of PP, development of preparation methods of NLC, optimization of formulation of NLC, and efficacy of NLC as delivery system for PP. Safety tests (*in-vitro* dermal and ocular irritation assay, human patch test and human repeated patch test) showed that PP was safe to be used as cosmetic bioactives. PP showed much higher fibroblasts cell viability compared to commercial phytonutrient (Tocomin 50% and Gold Tri. E) which indicated that PP was more effective in promoting proliferation of fibroblasts cells. Tocols and beta-carotene were found as the most abundant components in PP. Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) nitric oxide

radical scavenging assays analysis showed that PP had good scavenging activity. PP was found to have excellent UV-blocking activity as it absorbed light at UVA and UVB light region (290-400 nm). Particle size analysis, zeta potential analysis, rheological measurement and accelerated stability testing were used to assess the physical stability of NLC formulation. Two novel preparation methods of NLC which produced NLC with high physical stability and enhanced chemical stability of PP were successfully developed. The optimized NLC production conditions were 500-1000 bars homogenization pressure, 3 cycles of high pressure homogenization, and the cooling temperature was 25 °C after hot high pressure homogenization. The optimized NLC formulation were 6% of Span 40 and Tween 80 (50:50) as surfactants, 20% of lipid phase containing mixture of hydrogenated palm kernel and palm glycerides and isopropyl palmitate (90:10) as emollients and 0.10% of PP as bioactives, and 0.5% of MC-NP4 as preservatives. The optimized formulation exhibited good physical and microbiological stability and enhanced chemical stability of PP in the formulation. NLC loaded with PP showed high physical stability and chemical stability of PP when compared to macroemulsion loaded with PP and nanoemulsion loaded with PP. Percentage of fibroblasts cells viability of NLC was much higher at concentration of 400-2000 µg/ml when compared to macroemulsion loaded with PP and nanoemulsion loaded with PP. Increase in percentage of fibroblasts cell viability indicated that more collagen could be produced to reduce skin aging. NLC loaded with PP greatly increased skin hydration and reduced skin roughness when compared to macroemulsion loaded with PP and nanoemulsion loaded with PP. This work concluded that NLC loaded with PP which exhibited good physical stability and chemical stability of PP, and promising anti-wrinkle efficacy was successfully developed.

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

**PEMBENTUKAN SISTEM PEMBAWA BERSTRUKTUR NANO YANG
DIMUATKAN DENGAN FITONUTRIEN SAWIT UNTUK APLIKASI
KOSMETIK**

By

LOO CHEW HUNG

Julai 2013

Pengerusi: Profesor Mahiran Basri, PhD

Fakulti: Sains

Kepentingan pembawa berstruktur nano (NLC) di bidang kosmetik semakin meningkat tetapi penghalangnya adalah kestabilan fizikal NLC, kestabilan kimia bioaktif dalam NLC dan Efikasi NLC. Tujuan penyelidikan ini adalah untuk membangunkan NLC yang dimuatkan dengan antioksidan, fitonutrient sawit. Kajian ini dibahagikan kepada empat bahagian, iaitu pencirian fitonutrien sawit, pembangunan kaedah penyediaan NLC, pengoptimuman formulasi NLC, dan efikasi NLC sebagai sistem penghantaran untuk fitonutrien sawit. Kajian keselamatan (iritasi dermis dan okular *in-vitro*, kajian penambalan pada manusia dan kajian penambalan berulang pada manusia) menunjukkan fitonutrien sawit adalah selamat dipakai sebagai bioaktif kosmetik. Kebolehhidupan sel fibroblas terhadap fitonutrien sawit adalah lebih tinggi berbanding dengan fitonutrien komersial (Tocomin 50% and Gold Tri.E) menunjukkan fitonutrien sawit adalah lebih berkesan untuk mendorong pergandaan sel fibroblas. Tokol dan beta-karoten didapati sebagai komponen-komponen yang paling banyak di fitonutrien sawit. Kajian-kajian

aktiviti penyingkiran radikal bebas (Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) dan nitrik oksida) menunjukkan fitonutrien mempunyai aktiviti penyingkiran radikal yang baik. Fitonutrien sawit didapati mempunyai aktiviti penyekatan sinaran ultralembayung yang baik disebabkan ia menyerap sinaran dalam lingkungan jarak gelombang ultra ungu A dan B (290-400 nm). Analisis- analisis saiz zarah, potensi zeta, reologi dan pengujian kestabilan telah digunakan untuk mengkaji kestabilan fizikal formulasi NLC. Dua kaedah baharu penyediaan NLC yang menghasilkan NLC dengan kestabilan fizikal yang tinggi dan kestabilan kimia fitonutrien sawit yang dipertingkatkan telah dibangunkan dengan jayanya. Keadaan produksi NLC yang optimum adalah tekanan tinggi penghomogenan pada 500-1000 Pa, tiga kitaran penghomogenan tekanan tinggi dan NLC segar disejukan kepada 25 °C dengan kukusan ais selepas penghomogenan tekanan tinggi. Ramuan-ramuan NLC yang telah dioptimumkan adalah 6% Span 40 dan Tween 80 sebagai surfaktan, 20% fasa lipid mengandungi campuran hidrogenasi isirong sawit dan gliserida sawit dan isopropyl palmitat (nisbah 90:10) sebagai emolien dan 0.10% fitonutrient sawit sebagai bioaktif, dan 0.5% MC-NP4 sebagai pengawet. Formulasi ini mempunyai kestabilan fizikal dan mikrobiologi yang baik dan kestabilan kimia fitonutrien sawit dalam NLC dipertingkatkan. NLC yang dimuatkan dengan fitonutrien sawit menunjukkan kestabilan fizikal yang tinggi dan kestabilan kimia fitonutrien sawit lebih tinggi jika berbanding dengan makroemulsi dan nanoemulsi yang dimuatkan dengan fitonutrien sawit. Peratus kebolehhidupan sel fibroblas bagi sampel NLC adalah lebih tinggi pada kepekatan 400-2000 µg/ml apabila berbanding dengan makroemulsi dan nanoemulsi yang dimuatkan dengan fitonutrien sawit. Peningkatan peratus kebolehhidupan sel fibroblas menunjukkan pergandaan sel fobroblas berlaku yang

menghasilkan lebih kolagen untuk melambatkan penuaan kulit. NLC dimuatkan dengan fitonutrien sawit meningkatkan penghidrasian kulit dan mengurangi kekasaran kulit secara drastik apabila dibandingkan dengan makroemulsi dan nanoemulsi dimuatkan dengan fitonutrien sawit. Penyelidikan ini menyimpulkan bahawa NLC yang dimuatkan dengan fitonutrien sawit menunjukkan kestabilan fizikal dan kestabilan kimia fitonutrien sawit yang bagus dan efikasi anti-kedutan yang dijanjikan telah dibangunkan dengan jayanya.



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I certify that a Thesis Examination Committee has met on **23 July 2013** to conduct the final examination of **Loo Chew Hung** on her thesis entitled “**Formation Of Nanostructured Lipid Carrier Systems Loaded With Palm Phytonutrients For Application In Cosmetics**” in accordance with the Universities and University Colleges 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.

Members of the Thesis Examination Committee were as follows:

Zulkarnain Zainal, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
(Chairman)

Gwendoline Ee Cheng Lian, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mansor Hj Ahmad @ Ayob, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Varaporn Junyaprasert, PhD
Associate Professor
Faculty of Pharmacy
Mahidol University
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013



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

Mahiran Basri, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Bimo Ario Tejo, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Kanthimathi A/P M S Subramaniam, PhD

Associate Professor
Faculty of Medicine
University of Malaya
(Member)

Harrison Lau Lik Nang, PhD

Group Leader of Biodiesel Technology Group
Engineering and Processing Division
Malaysian Palm Oil Board
(Member)

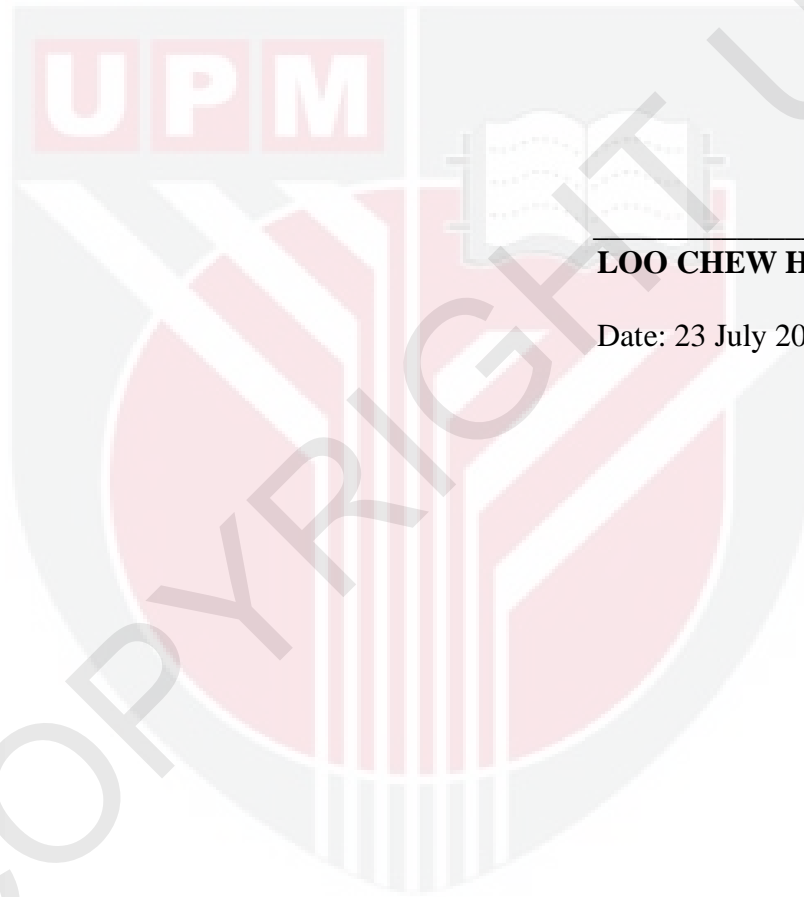
BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



LOO CHEW HUNG

Date: 23 July 2013

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