



UNIVERSITI PUTRA MALAYSIA

***FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF PALM
KERNEL OIL ESTERS NANOEMULSION SYSTEM CONTAINING
SODIUM DICLOFENAC***

MALAHAT REZAE

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SODIUM DICLOFENAC**

By

MALAHAT REZAE

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

November 2014

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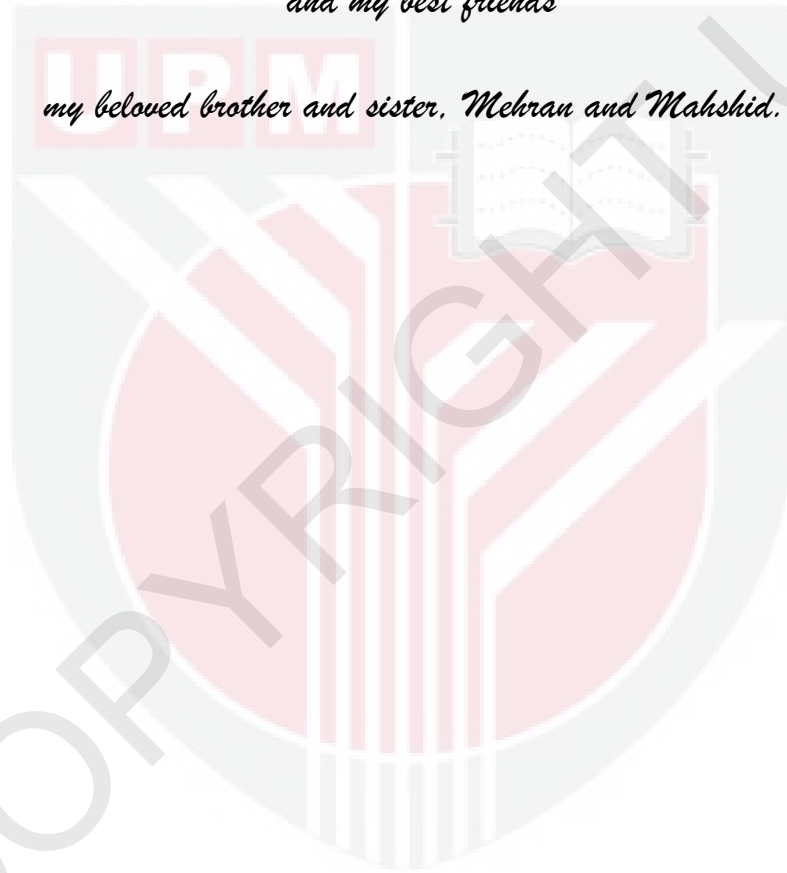
This thesis is dedicated to:

The first and best leaders of my life

my beloved father and mother, Naser and Mina with love and gratitude,

and my best friends

my beloved brother and sister, Mehran and Mahshid.



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

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SODIUM DICLOFENAC**

By

MALAHAT REZAEE

November 2014

Chairman: Professor Mahiran bt. Basri, PhD
Faculty: Institute of Bioscience

Nanoemulsions are nano-size emulsions which are used in many applications such as cosmetics and pharmaceuticals as delivery systems for parenteral, oral, ocular or transdermal administration. Transdermal administration is a suitable route for sodium diclofenac to prevent its adverse side effects which exhibited in other routes. Palm kernel oil esters (PKOEs) were selected as the oil phase for the preparation of nanoemulsion formulations containing sodium diclofenac. Special characteristic of PKOEs which has been reported is excellent wetting behavior without the oily feeling which makes it suitable for delivery of actives. Ternary phase diagrams of palm kernel oil esters with three surfactants (lecithin, Tween 20, Cremophor EL) as single and binary surfactants were constructed in three systems. The binary systems consisted of lecithin/Tween 20 and lecithin/Cremophor EL in two ratios of 80:20 and 60:40 for both systems. The increase of hydrophilic lipophilic balance (HLB) value and synergism effect between the surfactants in the binary surfactants systems gave larger homogenous and isotropic phases.

The isotropic region in the PKOEs/Lecithin:Cremophore EL (60:40)/water system was selected as nano-size emulsions composition range to be optimized and evaluated for their independent variables on responses of particle size and viscosity using Response Surface Methodology. The independent variables were water content, oil and surfactant ratio, stirring rate and stirring time. The study showed that third-order polynomial model was sufficient to describe and predict the responses. The smallest P-value and largest F-value belonged to O/S ratio for both responses. The optimized formulation (Opt.F) was formulated based on the achieved optimum conditions for preparation of sodium diclofenac nanoemulsions with the lowest values of particle size and viscosity to provide better drug penetration. As the O/S ratio was found to be the most efficient factor on the particle size and viscosity, in addition of Opt.F, five other formulations with different O/S ratio were selected for characterization.

The stability of the all formulations was evaluated by centrifugation, freeze-thaw cycle test and storage stability test. The Opt.F depicted good stability through the stability tests with no phase separation. The F-3 and F-4 formulations exhibited better stability than the other formulations. Rheology study showed that all nanoemulsion formulations exhibited non-Newtonian flow behaviour by displaying a pseudoplastic behaviour. Aerobic plate count, and yeast and mould count tests showed that there were no bacteria and fungus growth in the samples. The nanoemulsion formulations were found to be non-irritating by Irritancy Test. Permeation study was carried out by using cellulose acetate membrane and Wistar male rat skin. Opt.F and F-2 had the higher percentage of drug release. Five different terpenes based on the different chemical structure in five different concentrations were chosen as enhancer in Opt.F. All terpenes showed promoting effect on sodium diclofenac penetration. In addition, increase in terpenes concentration exhibited enhancement effect on drug permeation. Menthol had the highest permeation coefficient with the highest enhancement ratio for penetration of sodium diclofenac. Menthol and menthone in all concentrations of terpenes were shown to have significant effect on sodium diclofenac permeation. Different ratio of O/S is an effective factor on drug permeation. In conclusion, nanoemulsion formulations with good stability and suitable properties for transdermal delivery of sodium diclofenac were designed and developed.

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

**FORMULASI, PENGOPTIMUMAN DAN PENCIRIAN SISTEM NANO-
EMULSI ESTER MINYAK ISIRUNG KELAPA SAWIT YANG
MENGANDUNGI NATRIUM DIKLOROFENAK**

Oleh

MALAHAT REZAEE

November 2014

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Nano-emulsi adalah emulsi bersaiz nano yang digunakan dalam banyak aplikasi seperti kosmetik dan farmasi sebagai sistem penyampaian parenteral, lisan, okulus atau transdermal. Pemberian transdermal merupakan laluan sesuai bagi diclofenac sodium bagi mengelakkan kesan sampingan buruk seperti yang didapati pada laluan lain. Ester minyak isirung kelapa sawit (PKOEs) telah dipilih sebagai fasa minyak bagi persediaan formulasi nano emulsi yang mengandungi diclofenac sodium. Ciri-ciri khusus PKOEs yang telah dilaporkan ialah ciri pembasahan yang cemerlang tanpa rasa berminyak yang menjadikannya sesuai bagi penghantaran aktif. Gambar rajah fasa ternar (pseudo) ester minyak isirung kelapa sawit dengan tiga surfaktan (lesitin, Tween 20, Cremophor EL) sebagai surfaktan individu dan dedua telah dibina dalam tiga sistem. Sistem perduaan terdiri daripada lesitin/ Tween 20 dan lesitin/ Cremophor EL dalam nisbah 80:20 dan 60:40 bagi kedua-dua sistem. Peningkatan nilai baki hidrofilik lipofilik (HLB) dan kesan sinergisme antara surfaktan dalam sistem surfaktan dedua memberikan fasa homogen isotropik yang lebih besar.

Kawasan isotrop dalam PKOEs/Lesitin: Cremophor EL (60:40)/sistem air telah dipilih sebagai kawasan emulsi bersaiz nano bagi mengoptimum dan menilai pembolehubah bebas terhadap tindak balas saiz zarah dan kelikatan dengan menggunakan Metodologi Permukaan Gerak Balas. Pembolehubah bebas adalah kandungan air, minyak dan nisbah surfaktan, kadar adukan dan masa adukan. Kajian ini menunjukkan bahawa model polinomial tertib ketiga mencukupi bagi memperihal dan meramal tindak balas tersebut. Nilai P terkecil dan nilai F terbesar tergolong dalam nisbah O/S bagi kedua-dua tindak balas. Formulasi optimum (Opt.F) telah diformulasi berdasarkan syarat optimum tercapai bagi penyediaan nano emulsi diclofenac sodium dengan nilai terendah bagi saiz partikel dan kelikatan agar dapat memberi penembusan dadah yang lebih baik. Memandangkan nisbah O/S didapati sebagai faktor paling berkesan terhadap saiz zarah dan kelikatan, ditambah dengan Opt.F, lima formulasi lain dengan nisbah O/S yang berbeza telah dipilih untuk pencirian.

Kestabilan kesemua formulasi telah dinilai oleh pengemparan, ujian kitaran beku-cair dan ujian kestabilan simpanan. Opt.F menggambarkan kestabilan yang baik menerusi ujian kestabilan tanpa tahap pemisahan. Formulasi F-3 dan F-4 menunjukkan kestabilan yang lebih baik berbanding yang lain. Kajian reologi menunjukkan bahawa semua formulasi nano emulsi menunjukkan perilaku aliran bukan Newtonian dengan memaparkan perilaku pseudoplastik. Setelah pelaksanaan kiraan plat aerob, ujian kiraan ragi dan acuan, didapati bahawa tiada pertumbuhan bakteria atau kulat di dalam sampel. Formulasi emulsi nano didapati tidak merengsa oleh Ujian Kerengsaan. Kajian penelapan telah dijalankan oleh membran asetat selulosa dan kulit tikus Wistar jantan. Lima terpenes berbeza pada struktur bahan kimia yang berbeza dalam lima kepekatan yang berbeza telah dipilih sebagai peningkat dalam Opt.F. Semua terpenes menunjukkan mempunyai kesan penggalak terhadap penembusan diclofenac sodium. Selain itu, peningkatan kepekatan terpenes menyebabkan kesan peningkatan terhadap penelapan dadah. Mentol mempunyai korelasi penelapan tertinggi dengan nisbah peningkatan tertinggi bagi penembusan diclofenac sodium. Mentol dan mentona dalam semua kepekatan terpenes menunjukkan kesan signifikan terhadap penelapan diclofenac sodium. Akhir sekali, ianya didapati bahawa nisbah O/S yang berbeza merupakan faktor berkesan terhadap penelapan drug. Kesimpulannya, formulasi nano emulsi dengan kestabilan yang tinggi dan sifat-sifat yang sesuai bagi penyampaian transdermal diclofenac sodium telah direka dan dibangunkan.

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I certify that a Thesis Examination Committee has met on 26 November 2014 to conduct the final examination of Malahat Rezaee on her thesis entitled "Formulation, Optimization and Characterization of Palm Kernel Oil Esters Nanoemulsion System Containing Sodium Diclofenac" 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.

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LIST OF ABBREVIATION

2P	Two phase
3P	Three phase
ANOVA	Analysis of variance
CCRD	Central composite rotatable design
COX	Cyclooxygenase
Cr EL	Cremophor El
ER	Enhancement ratios
FFA	Free fatty acids
H	Homogeneous
BA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HIE	Human irritancy equivalent
HLB	Hydrophilic-lipophilic balance
I	Isotropic
Kp	Permeability coefficients
L	Lecithin
LB	LB broth
LBA	LB agar
min	Minute
mL	Milliliter
mV	Millivolt
NA	Nutrient agar
NB	Nutrient broth
NSAIDs	Nonsteroidal anti-inflammatory drugs
O/S	Oil and surfactant
O/W	Oil-in-water
PDA	Potato dextrose agar
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2
PGs	Glycerophospholipids
PIC	Phase inversion composition

PIT	Phase inversion temperature
PRESS	Prediction error sum of squares
R ²	Coefficient of determination
RMSE	Root mean squared error
rpm	Rotation per minute
RSM	Response surface methodology
SC	Stratum corneum
T20	Tween 20
TEM	Transmission electron microscopy
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UPLC	Ultra-performance liquid chromatography
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

1.1 Background

Nonsteroidal anti-inflammatory drugs (NSAIDs) belong to a group of drugs that are most commonly used in the world for their anti-inflammatory, antipyretic and analgesic properties (Pereira-Leite et al., 2013). Oral administration of NSAIDs is a very efficient route. However, potential of NSAIDs in creating adverse effects such as irritation and ulceration of the gastrointestinal (GI) mucosa often limits its usage in clinical application. NSAIDs must be prescribed frequently in daily doses in order for the therapeutic blood levels to remain constant. Therefore, patient noncompliance is also other usual problem in the administration of the drugs in oral or parenteral routes (Vonkeman et al., 2010; Sintov and Botner, 2006).

Sodium diclofenac is one of the most commonly used drugs of this group and is especially effective in the controlling the severe conditions of inflammation and pain, musculoskeletal disorders, arthritis, and dysmenorrhea. It is often prescribed for a period of time. Formulation as nanoemulsions is one of the nanoscience approaches that has been progressively considered in pharmaceutical science for transdermal delivery of drug. These nano-delivery systems in comparison to micron-size emulsions have been shown to have higher bioavailability and increase the effect of a number of compounds such as nonsteroidal anti-inflammatory drugs (NSAIDs) (Kotyla et al., 2008).

Nanoemulsions are a type of emulsion with particle sizes ranging from 20 nm to 200 nm, which are only kinetically stable (Solans et al., 2005). They may appear transparent or translucent mixtures of the oil phase, aqueous phase, surfactant(s), and co-surfactant(s) that create oil-in-water (O/W) or water-in-oil (W/O) emulsion (Solans and Sole, 2012). An emulsion is formed by the dispersion of one liquid, usually the oil phase in another immiscible liquid, water phase that is stabilized using surfactant. Palm kernel oil esters (PKOEs), in comparison to other oils, contain higher amounts of shorter chain esters, which suitable to be applied in micro and nanoemulsion systems as a carrier for actives. Furthermore, the higher iodine and saponification values, as well as lower slip melting point and the lower acidity values of PKOEs compared to other esters, provide some favorable properties for micro and nanoemulsions in many applications (Keng et al., 2009; Salim et al., 2012).

Nanoemulsions as non-equilibrium systems cannot be formed spontaneously and for their formation, extrinsic energy is necessary. There are two main methods for preparation of nanoemulsions: condensation or low-energy methods, and dispersion or high-energy methods. High-energy emulsification methods result in emulsions having the most homogeneous flow and adjustable control of droplet size and thus are suitable for industrial utilizations (Solans et al., 2005). The prepared nanoemulsions properties in addition of formation preparation mechanisms, are influenced by formulation compositions. Evaluation of nanoemulsion formulations and optimization according to the best formulation composition and preparation conditions can be carried out through an experimental design.

The experimental design allows the study of the effect of variables alone, as well as their interactions. Response Surface Methodology (RSM) which is used for experimental design is an efficient statistical and mathematical tool used for the modeling and analysis of processes that exist between the independent factors and their responses, and for optimization of the processes or products (Yuan et al., 2008). When the independent variables have complex interactions, RSM is also beneficial in simultaneously analyse their process (Hao et al., 2011). The first aim of RSM is to detect the optimum response, and the second is to understand how is the changes of response against the adjusting the variables.

1.2 Problem Statements

Although sodium diclofenac is one of the best-tolerated classical NSAIDs, like other drugs in this category, it can cause gastrointestinal adverse effects such as gastrointestinal bleeding, ulceration, nausea and vomiting by oral route, and cutaneous lesions by intramuscular injection (Suwalsky, et al., 2009; Zhai et al., 2013). It is a challenge to overcome the drug's adverse effects, high hepatic first-pass metabolism, and the short biological half-life. Therefore, development of a transdermal nanodelivery system like nanoemulsions could be beneficial.

The selection of suitable type and concentration of surfactant, and applying a proper input energy are the main challenges for preparation a nanoemulsion. Therefore, it is necessary to develop a model that in addition of formulation compositions, study and optimize the processing conditions, so as to avoid spending time and energy to save cost, particularly in industrial applications. Since particle size and viscosity are important factors for an efficient drug release (Cross et al., 2001; Tsai et al., 2011), these parameters were selected as responses in the study. In spite of importance of viscosity in stability and drug permeation, there are few reports on the effect of various factors on nanoemulsion viscosity, or its optimization especially in pharmaceutical research. Since nanoemulsions are thermodynamically unstable, they have tendency to coalescence and Ostwald ripening processes which result in phase separation. Therefore, stability of nanoemulsions is a critical factor to be analysed, especially when the achievement of formulations with long time stability (three years shelf life) was favorable.

In addition, sodium diclofenac is solved in aqueous solutions as ionized salt and has low logP value, thus it is not absorbed easily by transdermal application (Nokhodchi et al., 2007). One challenge to overcome this problem and the skin's strong barrier function is to design a functional transdermal drug delivery system by using enhancers.

1.3 Objectives

The main objective of the research was to develop an efficient palm kernel oil esters-based nanodelivery system for the transdermal delivery of sodium diclofenac. To achieve this aim the following objectives was carried out:

1. To determine the phase behaviour of palm kernel oil esters (PKOEs) system by constructing the ternary phase diagrams.
2. To optimize and evaluate the condition for preparation of nanoemulsions with the smallest values of particle size and viscosity using Response Surface Methodology (RSM).
3. To characterize the physicochemical properties of the nanoemulsion systems, such as stability, rheology, and safety evaluation.
4. To determine the delivery potential of the nanoemulsion systems containing sodium diclofenac in *in-vitro* tests through cellulose membrane and Wistar rat skin, and improve the drug permeation using enhancers.



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