UNIVERSITI PUTRA MALAYSIA

DESIGN AND OPTIMIZATION OF TOCOTRIENOL RICH FRACTION NANOEMULSION SYSTEM FOR COSMECEUTICAL APPLICATION

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By

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Chairman : Professor Mahiran Basri, PhD
Faculty : Science

Highly stabilized nanoemulsion system requires high concentration of surfactant, high homogenization pressures and process cycles. However, the high surfactant concentrations may induce skin irritation while the high homogenization pressures and process cycles may incur higher operation cost. Another important problem is the absorption inefficiency of active such as tocotrienol rich fraction (TRF) into skin which affects product efficacy. This study aims to improve the stability of TRF nanoemulsion and effective absorption of TRF into skin by designing TRF nanoemulsion using Hansen Solubility Parameter (HSP) concept. The HSP concept allows alteration of the oil phase solubility which reduces Ostwald ripening and improves solubility of TRF into skin.

TRF nanoemulsion was prepared by optimizing high shear homogenization conditions with pressure of 15,000 psi and minimum of 3 process cycles which yielded nanoemulsions of average droplet size of 137 ± 3 nm with zeta potential of -24.9 ± 2.2 mV and polydispersity index of 0.22. Nanoemulsions with less than 3% surfactant resulted in less significant droplet size reduction compared to nanoemulsion with more than 5% surfactant. From Stokes Equation, the velocity of TRF nanoemulsions with droplet size between 50 and 53 nm was calculated in the region of 10^{-15} $m.s^{-1}$. Oswald Ripening, which is the main destabilization factor affecting nanoemulsion stability, was effectively reduced by increasing volume fraction of TRF to $\varphi = 0.4 – 0.5$ of the nanoemulsion disperse phase. The solubility gap based on HSP was higher at 2.46 - 3.12 indicating that the modified oil phase has lower solubility which inhibited Ostwald ripening. At these volume fractions, the system is approaching thermodynamic stability where Ostwald Ripening rate has plateau. Optimization of nanoemulsion with TRF in combination with glycerine and octocrylene was predicted and proven to have

better delivery of TRF into skin compared to other combinations of TRF. The solubility gap based on HSP was lower at 4.2 indicating the oil phase has higher solubility into skin. The penetration profiles *via* tape stripping technique showed that optimized TRF nanoemulsion recorded highest TRF at 0.493 µg.cm^{-2}. The steady-state flux proved that TRF nanoemulsion optimized with HSP concept delivered the highest average flux value (0.2556 µg/cm^2.h), followed by the TRF nanoemulsion (0.1998 µg/cm^2.h) and TRF Macroemulsion (0.1360 µg/cm^2.h). This indicated that optimized TRF Nanoemulsion allows more TRF to permeate through the skin *via* passive diffusion.

In vitro ocular and dermal irritection based on protein assays and *in vitro* ocular and dermal irritation using reconstructed human epidermis and human epithelial models showed that TRF nanoemulsions did not induce any ocular or dermal irritations. *In vitro* sun protection factor test and *in vivo* skin hydration showed that TRF nanoemulsion was having better UV protection and effective in maintaining higher level of skin hydration. This study has shown that stable TRF nanoemulsion with higher absorption of TRF into skin can be achieved by designing TRF nanoemulsion based on HSP concept.

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

REKABENTUK DAN PENGOPTIMUMAN SISTEM NANOEMULSI PECAHAN KAYA TOKOTRIENOL UNTUK KEGUNAAN KOSMESEUTIKAL

Oleh

ZAFARIZAL ALDRIN BIN AZIZUL HASAN

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Sistem nanoemulsi yang stabil memerlukan penggunaan surfaktan berkepekatan tinggi dengan proses penghomogenan bertekanan dan kitaran proses yang tinggi. Walau bagaimanapun, penggunaan kepekatan surfaktan yang tinggi boleh menyebabkan kerengsaan kulit manakala proses penghomogenan dengan tekanan dan bilangan kitaran yang tinggi menyebabkan peningkatan kos operasi. Satu lagi masalah penting ialah penyerapan bahan aktif seperti pecahan kaya tokotrienol (TRF) yang tidak cekap ke dalam kulit yang mempengaruhi keberkesanan produk. Kajian ini bertujuan untuk meningkatkan kestabilan nanoemulsi TRF dan keberkesanan penyerapan TRF ke dalam kulit dengan merekabentuk nanoemulsi TRF menggunakan konsep Parameter Kelarutan Hansen (HSP). Konsep HSP membolehkan perubahan kelarutan fasa minyak yang dapat mengurangkan pematangan Ostwald dan meningkatkan kelarutan TRF ke dalam kulit.

Nanoemulsi TRF yang stabil dihasilkan dengan mengoptimumkan proses penghomogenan ricihan tinggi pada tekanan 15,000 psi dan 3 kali kitaran proses bagi menghasilkan nanoemulsi dengan purata saiz 137 ± 3 nm, keupayaan zeta -24.9 ± 2.2 mV dan indeks polidispersi sebanyak 0.22. Nanoemulsi yang dihasilkan dengan kepekatan surfaktan yang kurang dari 3% menyebabkan pengurangan saiz zarah tidak berkesan berbanding penggunaan surfaktan berkepekatan 5%. Melalui persamaan Stokes, kelajuan zarah nanoemulsi bersaiz antara 50 dan 53 nm adalah dianggarkan pada kelajuan 10^{-15} $m.s^{-1}$. Pematangan Ostwald iaitu faktor utama yang menyebabkan ketidakstabilan nanoemulsi telah berjaya dikurangkan dengan meningkatkan pecahan isipadu TRF dalam fasa minyak nanoemulsi kepada 0.4 - 0.5. Jarak kelarutan HSP telah meningkat kepada 2.46 – 3.12 menunjukkan fasa minyak mempunyai kelarutan lebih rendah yang merencat pematangan Ostwald. Pada pecahan isipadu ini,

sistem tersebut telah menghampiri kestabilan termodinamik di mana kadar pematangan Ostwald telah mendatar. Konsep parameter kelarutan Hansen membolehkan penyerapan TRF secara optimum di mana kombinasi TRF, gliserin dan oktokrilena telah membuktikan penyerapan TRF adalah berkesan berbanding kombinasi yang lain. Jarak kelarutan HSP adalah lebih rendah pada nilai 4.2 menunjukkan fasa minyak mempunyai kelarutan yang tinggi ke dalam kulit. Profil penembusan melalui teknik pelucutan pita menunjukkan nanoemulsi TRF yang optimum menghasilkan jumlah penembusan TRF yang lebih tinggi iaitu 0.493 $\mu g.cm^{-2}$. Fluks berkeadaan tetap membuktikan nanoemulsi TRF yang dioptimumkan melalui konsep parameter kelarutan Hansen menghasilkan purata fluks TRF yang paling tinggi (0.2556 $\mu g/cm^2.h$), diikuti nanoemulsi TRF (0.1998 $\mu g/cm^2.h$) dan makroemulsi TRF (0.1360 $\mu g/cm^2.h$). Ini menunjukkan penggunaan nanoemulsi yang optimum menyebabkan lebih banyak TRF meresap melalui kulit secara penyebaran pasif.

Ujian bagi iritasi mata dan dermis menggunakan analisa protein secara *in vitro* dan ujian bagi iritasi mata dan dermis menggunakan epidermis manusia dibina semula dan epithelial manusia dibina semula menunjukkan nanoemulsi TRF tidak merengsakan membran mata atau dermis. Ujian faktor perlindungan matahari secara *in vitro* dan ujian kelembapan kulit secara *in vivo* menunjukkan nanoemulsi TRF boleh melindungi kulit dari cahaya ultralembayung dan mengekalkan kelembapan kulit dengan berkesan. Kajian ini menunjukkan nanoemulsi TRF yang stabil dengan penyerapan TRF ke dalam kulit yang tinggi dapat dihasilkan dengan merekabentuk nanoemulsi TRF berdasarkan konsep HSP.

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I certify that a Thesis Examination Committee has met on 25 January 2018 to conduct the final examination of Zafarizal Aldrin Azizul Hasan on his thesis entitled "Design and Optimization of Tocotrienol Rich Fraction Nanoemulsion System for Cosmeceutical Application" 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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TABLE OF CONTENTS

				Page
ABSTRACT				i
ABSTRAK				iii
ACKNOWLEDGEMENTS				v
APPROVAL				vi
DECLARATION				viii
LIST OF TABLES				xiii
LIST OF FIGURES				xv
LIST OF ABBREVIATIONS				xviii
CHAPTER				
1	**INTRODUCTION**			1
	1.1	Background of Study		1
	1.2	Problem statement		2
	1.3	Significance of Study		3
	1.4	Scope of Study		3
	1.5	Objectives		4
2	**LITERATURE REVIEW**			5
	2.1	The Skin Structure and Functions		5
		2.1.1	Skin Antioxidants Network	6
		2.1.2	Vitamin E – Palm TRF	7
	2.2	Delivery Systems		8
		2.2.1	Nanoemulsion	8
		2.2.2	Formation of emulsions	11
		2.2.3	Preparation of Nanoemulsion	16
		2.2.4	High Energy Homogenization	18
		2.2.5	Control of Nanoemulsion Droplet Size	20
	2.3	Stability of Nanoemulsions		21
		2.3.1	Destabilization Mechanisms and Ostwald Ripening	21
		2.3.2	Applications of Nanoemulsion	26
	2.4	Characterization of Nanoemulsions		29
		2.4.1	Photon Correlation Spectroscopy	29
		2.4.2	Zeta Potential	30
	2.5	Optimization of Nanoemulsion with HSP		32
		2.5.1	JSME Molecular Editor	32
		2.5.2	Simplified Molecular-Input Line-Entry System	33
		2.5.3	Hansen Solubility Parameter	35
	2.6	Formulating with Hansen Solubility Parameters		45
		2.6.1	Active Formulation Gap	46
		2.6.2	Skin Formulation Gap	46
		2.6.3	Optimising the Skin Delivery of Active	48
		2.6.4	Determination of Skin Delivery Gap (SDG)	48
		2.6.5	Tape Stripping	49

	2.7	Safety Assessment	50
		2.7.1 *In Vitro* Cytotoxicity	51
		2.7.2 *In Vitro* Dermal and Ocular Irritation Assay	52
		2.7.3 *In Vitro* Reconstructed Human Skin Models	53
	2.8	Efficacy Assessment of Nanoemulsion	55
		2.8.1 Skin Hydration	55
		2.8.2 *In Vitro* Sun Protection Factor	56
	2.9	Summary of Research Gap	58
3	**MATERIALS AND METHODS**		60
	3.1	Materials	60
	3.2	Methods	60
		3.2.1 Determination of TRF composition using High Performance Liquid Chromatography	60
		3.2.2 Preparation of Pre-Mix Emulsion	61
		3.2.3 Preparation of TRF Nanoemulsions using High Pressure Homogenization.	62
		3.2.4 Particle Size Measurements	62
		3.2.5 Zeta Potential	62
		3.2.6 Density, conductivity and viscosity measurements	63
	3.3	Generation of SMILES using JSME Molecular Editor	63
	3.4	Safety Assessment	63
		3.4.1 *In Vitro* Dermal and Ocular Irritection Assay	63
		3.4.2 *In Vitro* Reconstructed Human Epidermis	64
		3.4.3 *In Vitro* Reconstructed Corneal Epithelial	65
	3.5	Efficacy Assessment	66
		3.5.1 Tape Stripping	66
		3.5.2 *In Vitro* Permeation Studies	66
		3.5.3 Skin Hydration Measurement	67
		3.5.4 *In Vitro* Sun Protection Factor	68
4	**RESULTS AND DISCUSSION**		69
	4.1	Characterization of Palm Tocotrienol Rich Fractions	69
		4.1.1 Determination of TRF composition using HPLC	69
		4.1.2 Calculation of Hansen Solubility Parameters for TRF	70
	4.2	Development of TRF Nanoemulsion	72
		4.2.1 Preparation of TRF Emulsion	72
		4.2.2 Prediction of emulsion type based on HSP	74
	4.3	Preparation of Nanoemulsion using High Pressure Homogenizer	77
		4.3.1 The Effects of Pressure and Process Cycles	77
		4.3.2 Effect of Surfactant Concentrations.	78
	4.4	Stability of Nanoemulsions	79
		4.4.1 Flocculation and creaming	80
		4.4.2 Effect of Mixed Oil Phase on Formation of Nanoemulsion	80
		4.4.3 Effects of mixed disperse phase on Ostwald Ripening	82

	4.5	Optimizing the Delivery of TRF using HSP Concept	87
	4.6	Tape Stripping	91
	4.7	*In Vitro* Permeation	94
	4.8	Safety Assessment of TRF Nanoemulsion	97
		4.8.1 *In Vitro* Ocular and Dermal Irritection Assay	97
		4.8.2 *In Vitro* Irritation assay with Human Reconstructed Epidermis and Human Corneal Epithelium	99
	4.9	Efficacy Assessment of TRF Nanoemulsion	100
		4.9.1 Effects of nanoemulsions on Skin Hydration	100
		4.9.2 Effects of nanoemulsions on Sun Protection	101
5		**SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH**	103
REFERENCES			106
BIODATA OF STUDENT			129
LIST OF PUBLICATIONS			130

LIST OF TABLES

Table		Page
2.1	Comparison of macroemulsions, nanoemulsions and microemulsions with respect to size, shape, stability, method of preparation, and polydispersity	10
2.2	HSP for solvents	40
2.3	Polymer solubility in benzene and methanol	41
2.4	Calculation of Linalool HSP using the Van Krevelen method	42
2.5	Calculation of Linalool HSP using the Hoy method	43
2.6	Calculation of Linalool HSP using the Beerbower method	43
2.7	Summary of the calculated Linalool HSP using the different group contribution methods	44
2.8	Hansen Solubility Parameters of ingredients used in cosmetics	45
2.9	Predicted Ocular Irritancy Classification and IDE Scores	53
2.10	Predicted Dermal Irritancy Classification and HIE Scores	53
2.11	Mean UVA/UVB ratio, Star Rating and its category	58
3.1	HPLC analysis method specifications for determination of tocotrienols	61
4.1	Composition of Tocotrienol Rich Fractions (70% concentrate) based on HPLC analysis	70
4.2	Molecular structure and SMILES string for Tocopherol and Tocotrienols	71
4.3	Hansen Solubility Parameters for tocopherol and tocotrienols based on their respective SMILES Strings	72
4.4	Hansen Solubility Parameters for Tocotrienol Rich Fractions of 70% Concentrate	72
4.5	Preparation of Tocotrienol emulsion with different HLB values	73

4.6 Volume-weighted mean particle size (µm) of Tocotrienol emulsions with different HLB values (mean ± s.d., n = 3) and storage time (days) 74

4.7 Calculation of the macroemulsion oil phase solubility parameters 75

4.8 Winsor R0 values and the predicted emulsion type 75

4.9 Density, viscosity and calculated droplet velocity of nanoemulsions 80

4.10 Viscosities, droplet size and polydispersity index of nanoemulsions prepared using different oil compositions 82

4.11 Effects of increasing amount of insoluble oil (TRF) on the rate of Ostwald ripening of 15% medium chain triglyceride nanoemulsion stabilized by the 5% Tween 20 surfactant 84

4.12 Calculation of HSP distance between skin and ingredients 88

4.13 HSP characteristics of TRF and ingredients 90

4.14 HSP characteristics of TRF in different formulation 91

4.15 Average flux (µg/cm2.h) and the average cumulative concentration (µg/cm2) values of TRF that diffused through skin after 10 h 96

4.16 Calculation of Skin Delivery Gap using FFE™ program 96

4.17 Average Human Irritancy Equivalent (HIE) scores (Mean ± Standard Deviation) of Tocotrienol Emulsion, Tocotrienol Nanoemulsion and Sodium Lauryl Sulphate solution (0.5%, w/v) 97

4.18 Average Irritation Draize Equivalent (IDE) scores (Mean ± Standard Deviation) of Tocotrienol Emulsion, Tocotrienol Nanoemulsion and Sodium Lauryl Sulphate solution (0.5%, w/v) 98

4.19 The in vitro SPF results between macroemulsion and nanoemulsions 102

LIST OF FIGURES

Figure		Page
2.1	The Human Skin (Cross section)	5
2.2	The antioxidant network in the skin - predominant role of tocopherol and tocotrienol and its recycling by co-antioxidants	7
2.3	Molecular structures of tocopherol and tocotrienol isomers	8
2.4	Illustration of increase in Laplace pressure when a spherical droplet is deformed into an ellipsoid	12
2.5	Different methods for nanoemulsion preparations. (a) High energy method such as ultrasonication and high-pressure homogenization. (b) Low energy methods such as emulsion inversion point and phase inversion temperature	17
2.6	Diagram of the high shear homogenization principle	19
2.7	Diagram of High Pressure Homogenizer	20
2.8	Diagram of various nanoemulsion destabilization mechanisms	22
2.9	Diagram of the Photon Correlation Spectroscopy	30
2.10	Diagram of the electric double layer model	31
2.11	JSME Molecular Editor interface showing molecular structure of lauric acid	33
2.12	Hansen's Volume of Interaction	39
2.13	Molecular Structure of Linalool, structural groups and its numbers	42
2.14	Hansen Space for a formulation with three major points for skin, formulation and active	47
2.15	Hansen Space for two different formulae	48
2.16	MTT assay indicating reduction of MTT to formazan derivate catalyzed by mitochondrial succinate dehydrogenases of the viable cells	52
3.1	Transdermal Diffusion Vertical Cell	67

4.1 HPLC Analysis Tocotrienol Rich Fractions: (A) Profiles for Tocopherol and Tocotrienol Standard. (B) Profiles for Tocotrienol Rich Fractions sample 69

4.2 Particle Size Distributions of Tocotrienol Emulsions with different HLB values and storage time 74

4.3 Linear correlation between Winsor Ro and HLB values 76

4.4 The effect of pressure and number of cycles towards droplet size distribution 78

4.5 Comparison of the effect of Tween 20 and Brij 721 on the droplet size of 15% MCT nanoemulsions. The error bars denote standard deviation of triplicate measurements 79

4.6 Effects of increasing amount of insoluble oil (TRF) on the rate of Ostwald ripening of 15% medium chain triglyceride nanoemulsion stabilized by the 5% Tween 20 surfactant 83

4.7 Effects of volume fraction of insoluble oil (tocotrienol rich fraction) on Ostwald ripening rate of 15% MCT nanoemulsion stabilized with 5% Tween 20 86

4.8 Potential ingredients with their respective calculated HSP for optimizing delivery of TRF to skin 87

4.9 Basic and Optimized Formulation for effective delivery of TRF 89

4.10 TEWL readings before and after multiple tape stripping 92

4.11 Calibration curve for tocotrienols at wavelength of 298 nm 93

4.12 The amount of TRF in tape strips for untreated, TRF macroemulsion, TRF nanoemulsion and Optimized TRF nanoemulsion 94

4.13 Average cumulative TRF amount per area (µg/cm2) that diffused through skin as a function of time 95

4.14 Cell viability (mean ± SD, n = 2) of reconstructed human epidermis incubated with tocotrienol emulsion and nanoemulsion. Values in the same column with different letters designate as significant differences ($p < 0.05$) 99

4.15 Cell viability (mean ± SD, n = 2) of reconstructed human corneal epithelium incubated with tocotrienol emulsion and nanoemulsion. Values in the same column with different letters designate as significant differences ($p < 0.05$) 100

4.16 Skin hydration value for untreated skin, skin treated with tocotrienol macroemulsion (TRF ME), TRF nanoemulsion (TRF NE) and Optimized TRF nanoemulsion (Opt TRF NE). N=20 101

LIST OF ABBREVIATIONS

DPPH	2,2-diphenyl-1-picrylhydrazyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
AFG	Active Ingredient Gap
c.u	Corneometric unit
ELISA	Enzyme Linked Immunosorbent Assay
HIE	Human Irritancy Equivalent
HLB	Hydrophilic-Lipophilic Balance
HPLC	High Performance Liquid Chromatography
HPH	High Pressure Homogenization
HSP	Hansen Solubility Parameters
IAG	Ingredient Active Gap
ISG	Ingredient Skin Gap
IDE	Irritation Draize Equivalent
JSME	Java Script Molecular Editor
LTC	Local Tissue Concentration
ME	Macroemulsion
MCT	Medium Chain Triglyceride
MEC	Minimum Effective Concentration
NE	Nanoemulsion
O/W	Oil-in-water
OD	Optical density
PBS	Phosphate Buffer Solution

PCS	Photon Correlation Spectroscopy
PDI	Polydispersity Index
PEG	Polyethylene glycols
Brij 721	Polyethylene (21) Stearyl Ether
PMMA	Polymethyl Methacrylate
Brij 72	Polyoxyethylene (2) Stearyl Ether
Tween 20	Polyoxyethylene (20) Sorbitan Monolaurate
SDG	Skin Delivery Gap
SFG	Skin Formulation Gap
SMILES	Simplified Molecular Input Line Entry
SLS	Sodium Lauryl sulphate
SPF	Sun Protection Factor
T	Tocopherol
T3	Tocotrienol
TEWL	Transepidermal Water Loss
TRF	Tocotrienol Rich Fraction
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet Radiation
W/O	Water-in-Oil

LIST OF SYMBOLS

δ_P	Energy from dipolar intermolecular force between molecules
δ_D	Energy from dispersion bonds between molecules
δ_H	Energy from hydrogen bonds between molecules.
ΔH	Heat of Vaporization
V_m	Molar volume
ω	Ostwald Ripening Rate
R_o	Ratio of the intermolecular attraction of lipophilic portion of surfactant and oil molecules
δ	Solubility Parameter
δT	Total Solubility Parameter

CHAPTER 1

INTRODUCTION

1.1 Background of Study

New development in nanotechnologies has resulted in many applications in consumer products and medical field. The innovation in nanotechnologies are useful mostly as nanomaterials in cosmetic and personal care products such as sunscreens, shampoos, eye shadow, deodorants, emollients and anti-aging products. The use of the nanomaterials provides enhanced the functionality and the performance of the products particularly as novel vehicle for topical delivery. The nanomaterials may include active ingredients; vitamins, essential oil, sunscreen agents and anti-aging actives (Montenegro *et al*., 2016; Gulotta *et al*., 2014).

Cosmeceutical is defined as cosmetic products that produce pharmaceutical therapeutic benefit to skin. Their effects as cosmeceutical agents are through several routes of mechanisms on various cells such as fibroblasts, melanocytes and keratinocytes. The efficacies of cosmeceuticals in treatment of many skin disorders have created a high demand for such products. The global cosmeceuticals market was valued at USD 42.24 billion in 2016 and is expected to reach a value of USD 68.72 billion by 2022, with a Cumulative Average Growth Rate (CAGR) of 8.52% for the forecast period of 2017-2022 (Mordor Intel, 2017). Currently, the fastest growth area in skin-care market is cosmeceuticals which holds more than 57% of the overall market share. There are many cosmeceutical agents or active ingredients which provide the potential therapeutic effects such as antioxidants, proteins and biochemicals.

As a lipophilic antioxidant, tocopherols are widely used in cosmetics. However, their isomeric counterpart, tocotrienols which are more potent is currently being marketed and gaining interest as active ingredient in cosmetics. Vitamin E is a general term describing both tocotrienols and tocopherols derivatives. Tocopherols are derived from dietary intake of leafy vegetables and plant oils. The source of tocotrienols are mainly from palm oil, rice bran and barley oils (Ahsan *et al.,* 2015). Palm oil is the largest edible oil in the world and new developments in the extraction of tocotrienol rich fractions (TRF) have made it commercially viable.

In 2010, Muller and co-workers have reported that tocotrienols and tocopherols are widely used due to their excellent antioxidative properties (Muller *et al.*, 2010). However, tocotrienols were reported to provide many other biological activities compared to tocopherols. Besides its anti-inflammatory effects, tocotrienols have also been reported as having anti-cancer properties, lowering lipids and protection of neuron (Ju-Yen *et al*., 2014). Through its action as a free radical scavenger, tocotrienols serves as the most potent inhibitor of lipid peroxidation. In the skin, it forms an antioxidant network and its activity is replenished with the help of vitamin C and thiols. Vitamin E in the stratum corneum plays a vital role in protection against ultraviolet (UV) light. Dietary tocotrienols protect the skin more strongly than α-tocopherol against damage induced by UVB (Yamada *et al*., 2008). Vitamin E in human and rodent stratum corneum has also been reported to be depleted by 50% and 85% respectively upon exposure to a single dose of solar simulated UV (Thiele and Ekanayake-Mudiyanselage, 2007). Thus, effective delivery of tocotrienols directly into skin may enhance the bioavailability of antioxidants and hence increase in free radical scavenging activities which protect the skin against the sun's damaging ultraviolet irradiation and improving vital skin properties.

1.2 Problem statement

Effective delivery of non-soluble cosmeceutical active ingredient *e.g.* TRF into skin is important since formulations with expensive active ingredient needs to be optimized to reduce production cost while maintaining its effectiveness in skin. Nanoemulsion is widely used as delivery system for lipophilic active such as TRF in topical or oral applications. However, the major problem affecting the quality of TRF nanoemulsion is destabilization due to Ostwald ripening which is due to the solubility of lipophilic ingredient through the continuous phase. Another important problem of interest to the cosmetics industry is the efficiency of delivering the active into skin. Advancement in polymer chemistry introduces a new concept in solubility, the Hansen Solubility Parameters (HSP) concept which relates to van der Waal forces, polarity and hydrogen bonding of molecules. The HSP value of oil phase in the TRF nanoemulsion can be modified by combining at least two lipophilic ingredients which affect the solubility of the oil phase. Thus, selection of different lipophilic ingredient using the HSP concept can affect the solubility of the oil phase and thus, reduction of Ostwald ripening in the TRF nanoemulsion. The HSP concept can also be used to enhance the delivery of TRF into skin. The skin penetration potential of TRF can be further enhanced by increasing the solubility of TRF to skin. This can be carried out by optimizing the HSP value of the nanoemulsion oil phase and TRF to match the HSP value of the skin. The smaller the HSP gap between the HSP value of TRF and skin enhances the solubility of TRF into skin. Based on literature review there is no specific report been published for the use of HSP concept to reduce Ostwald ripening or enhancement of TRF delivery to skin.

1.3 Significance of Study

This study will explore the use of HSP concept in reducing Ostwald ripening, which is the main destabilization factor for any nanoemulsion system. Furthermore, the HSP concept can be used to enhance the delivery of TRF into skin. Thus, this study will provide evidence that HSP concept can be used for optimization of TRF nanoemulsion stability and delivery of TRF into skin. The results will be beneficial for development of cosmeceuticals for the cosmetic industry.

1.4 Scope of Study

The Tocotrienol rich fractions were analyzed for its compositions using HPLC. The TRF compositions were then used to calculate the total HSP value. The pre-mix of TRF macroemulsions were formulated using lipophilic and hydrophilic materials with mixed surfactants system. Based on HLB concept, the most stable emulsion was chosen for preparation of TRF nanoemulsions using high pressure homogenization method. The effects of homogenization pressure to a maximum of 20,000 psi, number of homogenization cycles (maximum 5 cycles) and surfactant concentrations (1 – 10%) were studied and the optimized conditions were chosen to prepare the TRF nanoemulsion. Average particle size was monitored throughout the 28 days of storage. The stability of TRF nanoemulsions were assessed for flocculation, creaming and Ostwald ripening destabilization factors. Modification of the TRF nanoemulsion oil phase was made by selecting lipophilic material based on HSP values. The effects of different oil phase with varying HSP values on Ostwald ripening and stability of TRF nanoemulsion were determined. TRF nanoemulsions with three different oil phase systems and HSP values were developed to study the effects of different lipophilic materials on delivery of TRF into skin.

In order to introduce TRF nanoemulsion in topical applications, safety assessment against skin and eye irritation needs to be investigated. Therefore, cytotoxicity studies on *in vitro* dermal and ocular membrane were conducted and further confirm with validated *in vitro* reconstructed human epidermis and corneal epithelium studies. The potential use of TRF nanoemulsion in UV protection of skin was evaluated using in vitro sun protection factor assay. The effects of TRF nanoemulsion of skin hydration was also conducted to determine whether TRF nanoemulsion can moisturize the skin effectively.

1.5 Objectives

The development of optimized TRF nanoemulsion system, its safety and efficacy assessment for topical applications are focused on four objectives below;

i. To formulate TRF nanoemulsion delivery systems *via* high energy homogenization method;
ii. To improve TRF nanoemulsions stability by reducing Ostwald ripening using HSP concept;
iii. To optimize the TRF nanoemulsions using HSP concept for effective delivery of TRF;
iv. To assess the safety and efficacy of the formulations through ocular and dermal assays, reconstructed corneal epithelium and skin models, *in vitro* sun protection factor and *in vivo* skin hydration.

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