ABSTRACT

Lipid nanoparticles are colloidal carrier systems that have extensively been investigated for controlled drug delivery, cosmetic and pharmaceutical applications. In this work, a series of stearic-oleic acid nanoparticles (SON) with different oleic acid compositions were successfully prepared by melt-emulsification combined with ultrasonication method. The SONs prepared have average particle size range from 200 nm to 250 nm depends on the oleic acid concentration in the mixture. TEM micrographs showed that high oleic acid compositions in SON induced the formation of elongated spherical particles. Encapsulation efficiency of SONs increased from 50% to 70% for both salicylic acid and lidocaine as increasing the oleic acid composition in the mixture. Differential scanning calorimetry analysis showed that the SONs prepared have lower crystallinity as compared to pure stearic acid. Different oleic acid composition in the mixture gave different degree of perturbation to the crystalline matrix of SONs hence result in lower degrees of crystallinity, thereby improve their encapsulation efficiencies. The optimized formulation, SON with 30 wt% was further incorporated into cream and evaluated the *in vitro* release for payload ingredients. The *in vitro* release showed a gradual release for 24 hours that strongly indicates the incorporation of payloads in solid matrix of SON and thus prolonged the release for both salicylic acid and lidocaine. Cream formulations with SONs containing salicylic acid and lidocaine showed about 3 times slower release rate as compared to their solution form, suggesting the potential use of SONs as carriers to improve therapeutic efficacy in cosmetic and pharmaceutical products.

ABSTRAK

Zarah lipid bersaiz *nano* merupakan sistem pembawa koloid yang telah dikaji secara meluas dalam aplikasi sistem penghantar terkawal, kosmetik dan farmaseutikal. Dalam kajian ini, sesiri zarah asid stearik-oleik bersaiz *nano* (SON) dengan jumlah komposisi asid oleik berlainan telah berjaya disediakan dengan kaedah pengemulsian cairan serta ultrasonkasi. SON yang disediakan mempunyai julat saiz purata zarah dari 200 nm hingga 250 nm bergantung kepada jumlah kandungan asid oleik dalam campuran. Mikrograf TEM menunjukkan bahawa jumlah kandungan asid oleik yang tinggi akan menggalakkan pembentukan zarah berbentuk sfera membujur. Kecekapan pengkapsulan SON bagi asid salisilik dan *lidocaine* meningkat dari 50% sehingga 70% dengan peningkatan komposisi asid oleik dalam campuran. Analisis kalorimeter pengimbasan perbezaan menunjukkan bahawa SON yang disediakan mempunyai darjah kehabluran yang lebih rendah berbanding dengan asid stearik tulen. Penambahan kandungan asid oleik yang berbeza dalam campuran menghasilkan darjah gangguan yang berbeza terhadap matriks hablur SON, serta menyebabkan darjah kehabluran yang lebih rendah, oleh itu dapat meningkatkan kecekapan pengkapsulan. Formulasi optimum, SON dengan 30% kandungan asid oleic telah dicampur dalam krim dan kajian pelepasan *in vitro* menunjukkan pelepasan secara beransur-ansur selama 24 jam dimana secara kukuhnya menunjukkan pemerangkapan asid salisilik atau *lidocaine* dalam matriks pepejal SON dan memanjangkan pelepasan *in vitro*. Kadar pelepasan formulasi krim tambahan SON yang mengandungi asid salisilik atau *lidocaine* adalah tiga kali lebih perlahan berbanding dalam larutannya, cadangan kegunaan potensi SON sebagai pembawa untuk meningkatkan keberkesanan terapeutik produk kosmetik dan farmaseutikal.

ACKNOWLEDGEMENTS

I would like to express my highest gratitude to Professor Dr. Misni Misran for his guidance, advises, supports and encouragements throughout the period of the research carried out. His guidance and encouragement given enable me to overcome the hardship encountered throughout the period of this project.

I would like to state my appreciation to Dr. Teo Yin Yin, Mrs. Radziah bt. Muhamad @ Mohd Annuar, Dr. Tan Hsiao Wei, Ms. Jane Gew Lai Ti, Ms. Chia Si Wai, Mr. Vicit Rizal Eh Suk, Mrs. Hairani Tahir, Ms. Yew Han Choi, Mr. Kino Chin Guo Feng, Ms. Evelyn Lim Hui Mei, Ms. Tiew Shu Xian, Ms. Ainnul Hamidah Syahadah Azizan, Mr. Mohd Nashrul Mohd Zubir, Mrs. Sumaira Naeem, Ms. Premanarayani Menon, Ms. Syahirah Sazeli, Nur Amejeida Bt. Ismail, Nursyamimi Bt. Abd. Halim and other laboratory members and friends for their sincere comments, suggestions, helps and advices.

I would also like to express my gratitude to my grandmother, mother, sister, brother and family members who always supporting and giving encouragements for my success.

Last but not least, I specially thank Ministry of Science, Technology and Innovation for funding this project under e-Science Fund 03-01-03-SF0647. I would also like to state my appreciation to University of Malaya for kindly providing financial assistance for this project under Postgraduate Research Fund PV 004/ 2011A.

TABLE OF CONTENTS **Page**

Abstract	ii
Abstrak	iii
Acknowledgements	iv
Table of Contents	v
List of Figures	viii
List of Tables	x
List of Abbreviations and Symbols	xi

CHAPTER ONE: INTRODUCTION

1.1	Introduction	1
1.2	The Structure and Function of Skin	3
1.3	Lipid Nanoparticles	6
	1.3.1 Solid Lipid Nanoparticles	7
	1.3.2 Nanostructured Lipid Carriers	8
1.4	Preparation of Lipid Nanoparticles	9
	1.4.1 High Pressure Homogenization	9
	1.4.2 Microemulsion Technique	10
	1.4.3 Melt-Emulsification Combine with Ultrasonication Method	11
1.5	Materials Used in Formulation of Lipid Nanoparticles	13
	1.5.1 Fatty Acids	13
	1.5.2 Surfactants	16
1.6	Lipid Nanoparticles as Advanced Topical Delivery System	19
1.7	Objectives of Research	22

CHAPTER TWO: MATERIALS AND METHODOLOGY

2.1	Materials	23
2.2	Preparation of SONs	24
2.3	Particle Size and Polydispersity Index of SONs Measurement	25
2.4	Zeta Potential Measurement	27
2.5	Determination of Critical Micelle Concentration (CMC) of Surfactants	28
2.6	Transmission Electron Microscopy (TEM)	29
2.7	Differential Scanning Calorimetry (DSC) Analysis	30
2.8	Preparation of Standard Concentration Curve of Salicylic Acid for UV-Visible Spectrophotometry Measurement	31
2.9	Preparation of Standard Concentration Curve of Lidocaine for UV-Visible Spectrophotometry Measurement	32
2.10	Encapsulation Efficiency of SONs	33
2.11	Sample Preparation for *In Vitro* Release Evaluation	34
2.12	Preparation of Standard Concentration Curve of Salicylic Acid for *In Vitro* Release Study	35
2.13	Preparation of Standard Concentration Curve of Lidocaine for *In Vitro* Release Study	36
2.14	*In Vitro* Release Evaluation	37

CHAPTER THREE: RESULTS AND DISCUSSIONS

3.1 Particle Size Analysis 39

3.1.1 Effect of Homogenization Time on Mean Particle Size of SONs 39

3.1.2 Effect of Homogenization Speed on Mean Particle Size of SONs 40

3.1.3 Effect of Sonication Time on Mean Particle Size of SONs 41

3.1.4 Effect of Lipid Content on Mean Particle Size of SONs 42

3.1.5 Effect of Oleic Acid Composition on Mean Particle Size of SONs 43

3.1.6 Effect of Surfactant Chain Length on the Size of SON Particles 44

3.1.7 Effect of Surfactant Concentration on the Size of SON Particles 47

3.1.8 Effect of Active Compounds in the Composition on the Particle Size of SONs 49

3.2 Accelerated Stability Studies of SON 51

3.2.1 Effect of Surfactant Concentration 51

3.2.2 Effect of Storage Temperature 53

3.3 Effect of Oleic Acid Composition on the Morphologies of SONs 55

3.4 Differential Scanning Calorimetry (DSC) 57

3.5 Encapsulation Efficiency of SONs 61

3.6 *In Vitro* Release Evaluation 64

CHAPTER FOUR: CONCLUSION

4.1 Conclusion 68

REFERENCES

APPENDIX

LIST OF FIGURES

Figure	Title	Page
1.1	Illustration of cross-section through human skin adapted from reference [13].	5
1.2	Schematic diagram of melt-emulsification combine with ultrasonication method for preparation of lipid nanoparticles	12
1.3	Molecular structure of stearic acid (octadecanoic acid).	15
1.4	Molecular structure of oleic acid (*cis*-9-octadecenoic acid).	15
1.5	Schematic diagram of a surface active agent.	16
2.1	UV-Vis absorption spectrum and standard concentration curve (small) of salicylic acid in deionized water at 25 ℃.	31
2.2	UV-Vis absorption spectrum and standard concentration curve (small) of lidocaine in deionized water at 25 ℃.	32
2.3	Schematic diagram of centrifugal ultrafiltration by using Vivaspin.	33
2.4	UV-Vis absorption spectrum and standard concentration curve (small) of salicylic acid in 10 mM PBS solution at 25 ℃.	35
2.5	UV-Vis absorption spectrum and standard concentration curve (small) of lidocaine in 10 mM PBS solution at 25 ℃.	36
2.6	Schematic diagram of Automated Franz Diffusion Cell System employed in this experiment.	37
2.7	Photograph of Franz Diffusion Cell.	38
3.1	Plot of mean particle size (■) and polydispersity index (○) of SONs as function of lipid content at 25 ℃.	43
3.2	Plot of mean particle size (■) and polydispersity index (○) of SONs as function of surfactant chain length at 25 ℃.	45
3.3	Plot of surface tension as a function of ln[surfactants] of Tween 20 (□), Tween 40 (○) and Tween 60 (△) at 25°C.	46
3.4	Plot of mean particle size (■) and polydispersity index (○) of SONs as function of surfactant concentration (%$^{w}/_{v}$) at 25 ℃.	48
3.5	SON dispersions stabilized by (a) 0.25% $^{w}/_{v}$, (b) 0.50% $^{w}/_{v}$, (c) 1.00% $^{w}/_{v}$, (d) 1.50% $^{w}/_{v}$ and (e) 2.00% $^{w}/_{v}$ of Tween 60.	48
3.6	Accelerated stability of SON dispersions with different surfactant concentration in formulation at 45°C for 1 day (■) and 4 days (□) of storage.	52

Figure	Title	Page
3.7	Plot of mean particle size of SON dispersions as a function of storage time at 5°C (■), room temperature (about 28 °C) (●) and 45 °C (♦) and the TEM micrographs showed the changes in shape of nanoparticles, scale bar is 1 μm.	54
3.8	Photograph of SONs stored at different temperatures (a) 5 °C (left), (b) room temperature (middle) and (c) 45 °C (right) after 28 days showing denser sedimentation.	54
3.9	TEM micrographs of SONs. (a) SON without oleic acid, (b) SON with 30 wt% oleic acid composition, (c) SON with 50 wt% oleic acid composition, scale bar is 500 nm.	56
3.10	Endothermic thermogram of stearic acid at the heating rate of 5 °C min^{-1}.	57
3.11	Endothermic thermograms of salicylic acid loaded SONs with 0 – 50 wt% oleic acid ratio at the heating rate of 5 °C min^{-1}.	59
3.12	Endothermic thermograms of lidocaine loaded SONs with 0 – 50 wt% oleic acid composition at the heating rate of 5 °C min^{-1}.	60
3.13	Plot of encapsulation efficiency of salicylic acid (■) and lidocaine (○) loaded SONs as function of oleic acid composition at 25 °C.	63
3.14	*In vitro* release study of salicylic acid solution (□), salicylic acid cream (○), salicylic acid loaded SON with 30 wt% oleic acid composition (△) and salicylic acid loaded SON with 30 wt% oleic acid composition in cream (▽) over a period of 24 hours at 37 °C.	62
3.14	*In vitro* release study of lidocaine solution (◇), lidocaine cream (⬡), lidocaine loaded SON with 30 wt% oleic acid composition (☆) and lidocaine loaded SON with 30 wt% oleic acid composition in cream (⬠) over a period of 24 hours at 37 °C.	65
3.15	Schematic diagram for *in vitro* release mechanism of SON with active ingredients in Franz Diffusion Cell.	65

LIST OF TABLES

Table	Title	Page
1.1	Examples of different types of surfactants that commonly used in cosmetic and pharmaceutical industries.	18
1.2	Examples of active ingredients and drugs incorporated into lipid nanoparticles for topical application.	19
1.3	Examples of marketed lipid nanoparticles cosmetic products.	21
2.1	Weight composition of SON preparation.	24
3.1	Effect of homogenization time on mean particle size and polydispersity index of SONs at 25 ℃.	40
3.2	Effect of homogenization speed on mean particle size and polydispersity index of SONs at 25 ℃.	41
3.3	Effect of sonication time on mean particle size and polydispersity index of SONs at 25 ℃.	42
3.4	Mean particle size and polydispersity index of freshly prepared SONs with different oleic acid compositions at 25 ℃.	44
3.5	Mean particle size and polydispersity index of freshly prepared salicylic acid or lidocaine loaded SON with 30 wt% oleic acid at 25 ℃.	50
3.6	Zeta potential value of freshly prepared SONs with different surfactant concentrations at 25 ℃.	52
3.7	Onset temperatures (T_{onset}), melting points, melting enthalpies (ΔH) and crystallinity degree of SONs with and without salicylic acid.	59
3.8	Onset temperatures (T_{onset}), melting points, melting enthalpies (ΔH) and degree of crystallinity of SONs with and without lidocaine.	60
3.9	Different kinetic model evaluations of payloads (salicylic acid or lidocaine) release for eight different samples at 37C.	66

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ATP	Adenosine-5'-triphosphate
CD	Degree of crystallinity
CMC	Critical micelle concentration
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
GC	Gas chromatography
HPLC	High pressure liquid chromatography
NLC	Nanostructured lipid carriers
PBS	Phosphate buffer saline
rpm	Rotation per minute
RO	Reverse osmosis
R^2	Regression
SLN	Solid lipid nanoparticles
SON	Steric-oleic acid nanoparticles
TEM	Transmission electron microscopy
UV	Ultraviolet
wt%	Weight percentage
$^{w}/_{v}$%	Weight to volume percentage

Symbols

A_c	Baseline of the correlation function
B	Intercept of the correlation function
$C(t')$	Correlation function of the scattered intensity
D	Stoke-Einstein diffusion coefficient
d	Diameter of the particles
EE	Encapsulation efficiency of steric-oleic acid nanoparticles
ε	Dielectric constant
$f(ka)$	Henry’s function
ΔH	Melting enthalpy
H_{SA}	Enthalpy of stearic acid
H_{SON}	Enthalpy of steric-oleic acid nanoparticles
k	Boltzmann’s constant
k_n	Release rate
η	Viscosity
n'	Refractive index of medium
q	Scattering vector
Γ	Relaxation time
T	Temperature
T_{onset}	Onset temperatures
t'	Time delay between two intensities measurements
U_E	Electrophoretic mobility
W_F	Weight of free active ingredients
W_T	Weight of active ingredients added during preparation
λ_o	Wavelength
ζ	Zeta potential
θ_{7°	Backscattering angle