



Faculty of Pharmacy

Department of Pharmaceutics and Industrial Pharmacy

Formulation and Evaluation of Vesicular Systems for Enhanced Transdermal Drug Delivery

Thesis Submitted

By

Hanaa Adel Abdel Messih Seif

(Bachelor of pharmaceutical sciences, 2010)

As a Partial Fulfillment

for Master Degree in Pharmaceutical Sciences

(Pharmaceutics)

Under the supervision

Of

Prof. Dr. Ahmed Shawky Geneidi

Prof. Dr. Samar Mansour Holayel

Professor of Pharmaceutics and
Industrial Pharmacy

Professor of Pharmaceutics and
Industrial Pharmacy

Faculty of Pharmacy
Ain Shams University

Faculty of Pharmacy
Ain Shams University

Dr. Rania Aziz Helmy Ishak

Associate Prof. of Pharmaceutics and Industrial Pharmacy

Faculty of Pharmacy
Ain Shams University

FACULTY OF PHARMACY

AIN SHAMS UNIVERSITY

2018

Acknowledgement

I would like, first and foremost of anything, to thank God that has driven me to this moment and has not left me in any step in this work and any stage of my life.

*I would like to express my sincere appreciation and gratitude to **Professor Dr. Ahmed Shawky Geneidi**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, who I am lucky and honored to work under his instructive wise supervision.*

*I am very grateful to **Professor Dr. Samar Mansour Holayel**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University and Head of Pharmaceutical Technology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo . I was so pleased and honored to work under her supervision. I had learned a lot from her in this field and I would like to thank her valuable advice, follow-up and her usual support.*

*I can not express my deep and profound gratitude to **Associate Professor Dr. Rania Aziz Helmy Ishak**, Associate Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University. I am grateful to her great help, continuous care and support, her focus in every detail and her sincere friendly supervision and guidance.*

All thanks again to my supervisors from whom I learned a lot and still learn.

I feel very thankful to all my professors, friends, colleagues and every member in my second home, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for help, encouragement and support.

Thanks for everyone who contributed in the accomplishment of this thesis in a direct or indirect way, and special thanks to Genuine Research center (GRC).

I thank all my family members and friends without whom I could not reach this step.

I extend my thanks and my pride to my husband who helped and supported me a lot and to my daughters for their patience all over the period of working and for being a motive.

List of content

Item	Page
List of Abbreviations	i
List of Tables	iv
List of Figures	vi
Abstract	x
General Introduction	1
Scope of work	27
 <i>Chapter I: Preparation and Optimization of TRO-Loaded Nano-Ethosomes</i>	 28
Introduction	28
Experimental	35
Materials	35
Animals	35
Equipment	36
Methodology	37
I. Constructing the calibration curve of TRO in deionized water using UV-Visible spectrophotometer	37
II. Constructing the calibration curve of TRO in phosphate buffer saline (PBS, pH=7.4) containing 20% ethanol using HPLC	37
III. Preparation of TRO-loaded nano-ethosomes	38

IV. Experimental design	38
1. Determination of VS, PDI, and ZP	39
2. Determination of drug EE%	39
V. <i>Ex-vivo</i> drug permeation study through rat skin	40
1. Determination of the drug permeation kinetics	42
VI. Characterization of the prepared nano-ethosomes	42
1. High-resolution transmission electron microscopy (HR-TEM) imaging	42
2. Drug–excipient interaction study	43
A. <i>Differential scanning calorimetry (DSC)</i>	43
B. <i>FT-IR spectroscopy</i>	43
VII. Physical stability study of the selected nano-ethosomes	43
VIII. Statistical analysis	43
1. Point prediction and model validation	44
2. Optimization data analysis	45
Results and discussion	46
I. Calibration curve of TRO in deionized water using UV-Vis spectrophotometer	46
II. Calibration curve of TRO in PBS (pH 7.4)/ethanol (80:20) using HPLC	48
III. Preliminary studies	50
IV. Optimization of TRO-loaded ethosomal vesicles	51
1. EE% response	53

2. VS response	57
3. PDI response	63
4. Point Prediction and model validation	65
5. Optimization analysis	67
V. <i>ex-vivo</i> drug permeation study	69
1. Mechanism of <i>ex-vivo</i> drug permeation profile	73
VI. Characterization of the prepared nano-ethosomes	76
1. HR-TEM imaging of the prepared TRO-loaded nano-ethosomes	76
2. Drug-excipient interaction study	76
A. <i>DSC study</i>	76
B. <i>FT-IR Spectroscopy</i>	79
VII. Physical stability study	81
Conclusions	84
 <i>Chapter II: Preparation and Optimization of TRO-Loaded Flexosomes</i>	86
Introduction	86
Experimental	98
Materials	98
Animals	98
Equipment	99
Methodology	99

I. Preparation of TRO-loaded Flex	99
II. Optimization Study	99
1. Visual inspection of the prepared formulations	100
2. Turbidity measurements	100
3. Determination of PS, PDI, and ZP	100
III. Characterization of the selected Flex	101
1. Determination of drug EE%	101
2. HR-TEM imaging of the selected Flex	101
3. Drug–excipient interaction study	101
A. DSC	101
B. FT-IR spectroscopy	102
IV. <i>Ex-vivo</i> drug permeation study through rat skin	102
1. Determination of the drug permeation kinetics	102
V. <i>Ex-vivo</i> assessment of the permeation enhancing mechanism of the selected ethanolic vesicles	102
1. SC isolation from rat skin	102
2. SC treatment with selected formulations	103
3. DSC study on SC samples	103
VI. Physical stability study	103
VII. Statistical analysis	103
Results and discussion	104
I. Optimization study	104

1. Effect of EA type	105
2. Effect of PC to EA molar ratio	108
3. Effect of cholesterol content	114
4. Preparation of different EA-based ethanolic vesicles using the selected cholesterol concentration.	119
II. Determination of TRO EE% in the selected Flex	121
III. <i>Ex-vivo</i> permeation study for the selected Flex	122
1. Kinetics of <i>ex-vivo</i> drug permeation profile	127
IV. <i>Ex-vivo</i> assessment of the mechanisms of permeation enhancing effect of the selected ethanolic vesicles	128
V. Characterization of the selected Flex	129
1. Particle morphology using HR-TEM imaging	129
2. Drug-excipient interaction study	130
A. DSC	130
B. FT-IR spectroscopy	134
VI. Physical stability study of the selected formulations	138
Conclusions	141
 <i>Chapter III: In Vivo Assessments of the Selected Ethanolic Vesicles</i>	143
Introduction	143
Experimental	149
Materials	149

Animals	149
Equipment	149
Methodology	150
I. Tracking the fluorescently-labelled vesicles in the rat skin layers using CLSM	150
II. Pharmacokinetic studies	151
1. Administration of the selected TRO-loaded formulations to rats	151
2. Blood sampling	152
3. Sample preparation for analysis	152
4. Quantitative determination of TRO in plasma using LC-MS/MS method	154
A. Method conditions	154
B. Method validation	154
C. Construction of TRO calibration curve using LC-MS/MS assay	155
5. Pharmacokinetic analysis	155
III. <i>in vivo</i> skin-vesicle interaction study	156
1. Transdermal administration of the selected TRO-loaded formulations to rats	156
2. Preparation of dermato-histopathology sections	156
IV. Statistical analysis	157

Results and discussion	158
I. Tracking the fluorescently-labelled vesicles in the rat skin layers using CLSM	158
II. Pharmacokinetic studies	165
1. Construction of TRO calibration curve using LC-MS/MS assay	165
2. Pharmacokinetic analysis	165
III. <i>in vivo</i> skin-vesicle interaction study	171
Conclusions	175
Summary	177
References	187

List of Abbreviations

3 D	Three dimensions
5-HT ₃ -RAs	5-HT ₃ receptor-antagonists
ANOVA	Analysis of variance
ASCO	American Society of Clinical Oncology
AUC	Area Under the concentration Vs time curve
BAV	Bioavailability
CBD	Cannabidiol
CINV	Chemotherapy-induced nausea and vomiting
CLSM	Confocal laser scanning microscope
C _{max}	Maximum plasma drug concentration
CMC	Critical micelle concentration
CPP	Critical packing parameter
CREM	Cremophor RH 40
CTAB	Cetyl trimethylammonium bromide
CTZ	Chemoreceptor trigger zone
CYP2D6	Cytochrome P2D6
d	Desirability function
D	Diffusion coefficient
DHEW	Department of Health, Education & Welfare
DLS	Dynamic light scattering
DMSO	Dimethyl sulphoxide
DOE	Design of experiments
DSC	Differential scanning calorimetry
EA	Edge activator
EAPRU	Experiments and Advanced Pharmaceutical Research Unit
EE	Entrapment efficiency
EPC	Egg phosphatidylcholine
ER	Enhancement ratio
FA	Fatty acid
Flex	Flexosomes
F _{rel}	Relative Bioavailability

FT-IR	Fourier-transform infrared
H&E	Haematoxylin and Eosin
HLB	Hydrophilic-lipophilic balance
HPLC	High performance liquid chromatography
HR-TEM	High resolution transmission electron microscope
ICH	International Conference on Harmonization
IS	Internal standard
Jss	Steady state transdermal flux
Kp	Permeability coefficient
LC	Liquid chromatography
LC-MS/MS	Liquid Chromatography/Mass Spectrometry
LDA	Laser Doppler anemometry
LOD	Limit of detection
LOQ	Limit of quantitation
MASCC	Multinational Association of Supportive Care in Cancer
MLX	Meloxicam
MS	Mass spectrometry
Mw	Molecular weight
MWCO	Molecular weight cut off
n	Diffusional release exponent
NaC	Sodium cholate
NIH	National Institute of Health
NLC	Nanostructured lipid carriers
NPs	Nanoparticles
OS	Oral solution
PBS	Phosphate buffer saline
PC	Phosphatidylcholine
PC:EA	Phosphatidylcholine to edge activator molar ratio
PDI	Polydispersity index
PE	Penetration Enhancer
PK	Pharmacokinetic
PLGA	Poly(D,L-lactide-co-glycolide)

PS	Particle size
Q ₂₄	Cumulative amount of drug permeated per unit area over 24 h
RSD	Relative standard deviation
SAA	Surface active agent
SC	Stratum corneum
SD	Standard deviation
SDC	Sodium deoxycholate
SEM	Standard error of the mean
SLN	Solid lipid nanoparticles
SPC	Soybean phosphatidylcholine
T80	Tween 80
TDDS	Transdermal drug delivery system
TEWL	Transepidermal water loss
T _{max}	Time to reach maximum drug concentration
TPGS	D- α -Tocopherol polyethylene glycol 1000 succinate
TRO	Tropisetron hydrochloride
TS	Topically applied drug solution
UV/Vis	Ultra-violet/Visible
VS	Vesicle size
ZP	Zeta potential