



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY

“Development and evaluation of lipid nano-carriers for the treatment of dermatitis”

A thesis submitted

By

Layla Hamodi Hashem

Bachelor of Pharmacy (2006), Al Mustansiriyah university

Teaching assistant, at Department of Pharmaceutics-Faculty of pharmacy, Al Muthanna university in Iraq

*For partial fulfillment of the requirements for the Master Degree in
Pharmaceutical Sciences (Drug Technology)*

Under supervision of

Prof. Dr. Omaima Ahmed Sammour

***Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy, Ain Shams University***

Dr. Heba Abdel Moniem Gad

***Associate professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy, Ain Shams University***

**Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy, Ain Shams University**

2021

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

سَبِّحْ تَبَّحْ لَا تَعْزُبْ عَنْكَ
إِلَٰهًا مَا عَلِمْتُنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, praise is to ALLAH the Cherisher and Sustainer of the worlds, without His Guidance and Help this work would not have been accomplished.

I would like to express my sincere appreciation to ***Prof. Dr. Omaima Ahmed Sammour***, Professor of pharmaceutics and industrial pharmacy, faculty of pharmacy, Ain shams university, for giving me the opportunity to pursue this degree under her guidance and support. Her timely appreciation of my work was a great source of encouragement. Her expertise in pharmaceutical science improved my research skills and prepared me for future challenges. Then, I would like to express my sincere appreciation and my deep gratitude to ***Dr. Heba Abdel Moniem Gad***, Associate professor of pharmaceutics and industrial pharmacy, faculty of pharmacy. Ain shams university, for her suggestions and comments during all this research work with her help, I gained much knowledge and experience.

Great thanks to ***Prof. Dr. Rania Hathout***, Professor of pharmaceutics and industrial pharmacy, faculty of pharmacy. Ain shams university for her help in *ex-vivo* study.

Finally, I am profoundly grateful to all the members of my family, my beloved father (God bless his soul), my beloved mother, my sisters, my brothers, who supported and helped me through the work period. Extended gratitude to my dear husband, for the great help and effort he devoted for the fulfillment and completion of this thesis.

List of Contents

List of Abbreviations.....	I
List of Tables.....	II
List of Figures.....	III
Abstract	V
General Introduction	1
Skin structure.....	1
Factor affecting skin absorption.....	2
-Dermatitis.....	3
1-Atopic dermatitis.....	3
2-Irritant contact dermatitis.....	4
3-Dyshidrotic type of dermatitis.....	5
4-Seborrhic dermatitis.....	5
Management of atopic dermatitis.....	6
1-Non-pharmacological approaches.....	6
2-Natural remedies (emollients).....	6
3-Topical treatments.....	7
A-Corticosteroids	7
B-Immunosuppressive drugs (Topical Calcineurin Inhibitors).....	8
C-Topical doxepin.....	8
4-Other medications	8
A- Antiviral therapy	8
B- Antibiotic therapy.....	9
C- Oral antihistamine	9
D- Ultraviolet light	9
E- Vitamin D	9
F-Biologics... ..	10
I-Anti-CD 20.....	10
II-Anti-IL-5.....	10
III-Anti-IgE.....	10
IV-Anti-TNF-a	10
Corticosteroids as the first line therapy in dermatitis	11
Systemic side effects of corticosteroids.....	11

A- Cutaneous	11
B- Hypokalemia and myopathy	11
C- Glucose intolerance	12
D- Hypertension	12
E- Cardiac effect.....	12
F- Bone effect	12
G- Digestive system.....	12
Betamethasone 17-valerate.....	12
Nanocarriers.....	15
1. Lipid-based carrier systems	15
1.1. Nanoemulsions.....	15
1.2. Liposomes	16
1.3. Niosomes	18
1.4. Ethosomes	18
1.5. Solid lipid nanoparticles (SLNs)	19
1.6. Nanostructured lipid carriers (NLCs)	20
Scope of work	24
Chapter One: Preparation and characterization of BMV-almond oil loaded SLNs	
1. Introduction.....	25
1.1. Solid lipid Nanoparticles.....	25
1.2. Preparation of SLNs	26
1.2.1. High pressure homogenization	26
1.2.1.1. Hot homogenization technique.....	26
1.2.1.2. Cold homogenization technique.....	26
1.2.2. Microemulsion procedure.....	26
1.2.3. Ultrasonication /high-speed homogenization method.....	27
1.2.4. Solvent evaporation method.....	27
1.2.5. Solvent emulsification /diffusion method.....	27
1.2.6. Supercritical fluid method.....	27
1.2.7. Film ultrasound dispersion method.....	28
1.2.8. Phase inversion temperature (PIT) method.....	28
1.2.9. Solvent injection technique.....	28
1.3. Almond oil.....	28
1.4. Skin permeation model.....	29

1.5. Selection of the skin type	30
1.6. Precaution during <i>ex-vivo</i> studies	31
2. Experimental.....	34
2.1. Materials.....	34
2.2. Animals	34
2.3. Equipment.....	34
2.4. Methods.....	36
2.4.1. Determination of $\lambda_{\max}$ of betamethasone valerate.....	36
2.4.2. Construction of the calibration curve of BMV in hydro- alcoholic solution...	36
2.4.3. HPLC assay for BMV.....	36
2.4.4. Validation	36
2.4.4.1 Linearity and range.....	36
2.4.4.2. Determination of limit of quantification (QL) and limit of detection (DL)....	37
2.4.4.3. Specificity.....	37
2.4.4.4. Precision (Repeatability and Reproducibility)	37
2.4.4.5. Accuracy.....	37
2.4.5. Preparation of betamethasone valerate solid lipid nanoparticles	38
2.4.5.1. Preliminary study.....	38
2.4.5.2. Effect of different preparation variables.....	38
2.4.6. Characterization of BMV loaded SLNs	38
2.4.6.1. Determination of particle size, polydispersity index and zeta potential	38
2.4.6.2. Determination of entrapment efficiency percent.....	39
2.5. <i>Ex-vivo</i> studies	39
2.5.1. Skin preparation.....	39
2.5.2. <i>Ex-vivo</i> skin permeation and deposition studies.....	39
2.5.3. Tape stripping study.....	40
2.5.4. Skin images by confocal laser scanning microscopy (CLSM).....	41
2.6. Transmission electron microscopy (TEM).....	41
2.7. Differential scanning calorimetry (DSC).....	41
2.8. Fourier transform infrared spectroscopy (FT-IR) measurements	41
2.9. Powder X-ray diffraction measurements	42
2.10. pH measurements	42
2.11. Viscosity measurements of the prepared formulae.....	42
2.12. Stability study.....	42

2.13. Statistical Analysis	43
3. Results and Discussion.....	44
3.1. Determination of $\lambda_{\max}$ of BMV.....	44
3.2. Construction of the calibration curve of BMV.....	44
3.3. HPLC assay for BMV.....	45
3.4. Method Validation	46
3.4.1. Linearity and range.....	46
3.4.2. Determination of limit of quantification (QL) and limit of detection (DL).....	47
3.4.3. Specificity	48
3.4.4. Precision.....	48
3.4.5. Accuracy.....	48
3.5. Preparation of BMV loaded SLNs.....	49
3.5.1. Preliminary screening to select lipid type.....	49
3.5.2. Effect of different preparation variables on the physicochemical properties of SLNs prepared with precirol.....	51
3.5.3. Effect of total lipid concentration.....	51
3.5.4. Effect of Tween 80 concentration	52
3.5.5. Effect of Precirol to almond oil ratio.....	52
3.6. <i>Ex-vivo</i> skin studies.....	53
3.6.1. <i>Ex-vivo</i> skin permeation and deposition studies.....	53
3.6.2. Tape stripping studies.....	54
3.6.3. Skin images by confocal laser scanning microscopy (CLSM).....	55
3.7. Transmission electron microscopy (TEM).....	58
3.8. Differential scanning calorimetry measurements.....	59
3.9. Fourier transform infrared (FT-IR) measurements	60
3.10. X-ray Diffraction measurements.....	61
3.11. pH of the prepared formulae.....	62
3.12. Viscosity of the prepared formulae.....	62
3.13. Stability study.....	63
4. Conclusions.....	66
Chapter Two: <i>In-vivo</i> evaluation of BMV-almond oil loaded SLNs	
1. Introduction.....	67
1.1. Topical Drug Delivery.....	67
1.2. Topical corticosteroids for dermatitis.....	67

2. Experimental.....	70
2.1. Materials.....	70
2.2. Animals.....	70
2.3. Equipment.....	70
2.4. Methodology.....	71
2.4.1. In-vivo animal evaluation.....	71
2.4.2. Induction of atopic dermatitis and treatment protocol.....	71
2.4.3. Histopathological examination and Morphometric analysis.....	72
3.Results and discussion.....	73
3.1. Histopathological examination results.....	73
4. Conclusions.....	78
Future perspective.....	79
Summary	80
References.....	84
الملخص باللغة العربية.....	1

List of Abbreviations

<u>Abbreviation</u>	<u>Terms</u>
°C	Degree Celsius
AD	Atopic dermatitis
ANOVA	Analysis of variance
AUC	Area under curve
BMV	Betamethasone valerate
CD	Contact dermatitis
CLSM	Confocal laser scanning microscope
CS	Corticosteroid
DNCB	Dinitrochlorobenzene
DSC	Differential scanning calorimetry
EE %	Entrapment Efficiency percent
HPLC	High performance liquid chromatography
h	Hour
ICH	International Conference on harmonization
O/W	Oil in water
PBS	Phosphate buffer saline
PCS	Photon correlation spectroscopy
PDI	Poly dispersity index
PIT	Phase inversion temperature
PS	Particle size
QL	Quantification limit
RSD	Relative standard deviation
SC	Stratum corneum
SCR	Size change rate
SD	Standard deviation
SLNs	Solid lipid nanoparticles
TEM	Transition electron microscope
UV	Ultra violet
W/O	Water in oil
W/W	Weight by weight
ZP	Zeta potential

List of Tables

Table (I): A survey on lipid based nanocarriers used for the treatment of atopic dermatitis	22
Table (1): Relationship between concentration of betamethasone valerate and absorbance at $\lambda_{\max}$ 242 nm in 20% hydro-alcoholic solution.....	45
Table (2): Relationship between concentration of betamethasone valerate and area under the curve by HPLC method.....	47
Table (3): Results for intra-day precision.....	48
Table (4): Results for inter-day precision.....	48
Table (5): Accuracy results.....	49
Table (6): Summary of validation data obtained for the developed HPLC method for BMV assay.....	49
Table (7): Effect of lipid type on the physicochemical properties of BMV loaded solid lipid nanoparticles.....	50
Table (8): Effect of different formulation variables on the physicochemical properties of BMV loaded solid lipid nanoparticles	53
Table (9): Results for <i>ex-vivo</i> skin permeation and deposition studies	55
Table (10): Effect of storage temperatures on the physicochemical properties of betamethasone valerate loaded solid lipid nanoparticles.....	64

List of Figures

Figure (I): Skin structure.....	2
Figure (II): Immunologic pathways in AD patients showing high serum eosinophils and IgE resulting from circulating Th2-type cells in blood stream.....	4
Figure (III): Palmer (A) and dorsal (B) view of left-hand showing vesicles and bullae of dyshidrotic dermatitis	5
Figure (IV): Chemical structure of betamethasone valerate.....	13
Figure (V): Conversion of betamethasone 17-ester to betamethasone 21-ester.....	13
Figure (VI): The pH-rate profile for the rate of disappearance of betamethasone 17-valerate in aqueous solutions.....	14
Figure (VII): The three types of NLCs as compared to old SLNs.....	21
Figure (VIII): Models of drug incorporation into SLN: homogenous matrix of solid solution (upper), dispersion of drug into outer shell (middle), drug-enriched core surrounded with outer lipid shell (lower).	25
Figure (IX): Structural comparison among pig, human, rat and mouse skin	31
Figure (X): Tape stripping schematic representation for determination the drug amount penetrated into the skin: a) after application the formulation, b) removing the SC by tape stripping.....	40
Figure (1): U.V. spectrum of betamethasone valerate in 20% hydro-alcoholic solution.....	44
Figure (2): Calibration curve of betamethasone valerate in 20% hydro-alcoholic solution at $\lambda_{\max}$ 242 nm.....	45
Figure (3): Chromatogram showing a sharp peak of BMV at 7.6 min.....	46
Figure (4): Calibration curve of betamethasone valerate by HPLC method.....	47
Figure (5): Surface xy images of the stratum corneum treated with F2 (A) and F4 (B).....	56

Figure (6): Z-stack images of F2 (A) and F4 (B) stained with Nile red (the skin was sectioned from zero to 200 μm with 10 μm increments).....	57
Figure (7): Concentration depth light intensity profile of F2 and F4.....	58
Figure (8): Transmmission electron microscope image for betamethasone valerate loaded solid lipid nanoparticles (F2).....	58
Figure (9): Differential scanning calorimetry thermograms of pure betamethasone valerate, Precirol lipid, their physical mixture, plain solid lipid nanoparticles and betamethasone valerate loaded solid lipid nanoparticles(F2)	60
Figure (10): FT-IR spectra of D) pure drug (betamethasone valerate), DL) drug with Precirol lipid, F) betamethasone loaded solid lipid nanoparticles (F2), L) pure Precirol lipid and P) plain solid lipid nanoparticles.....	61
Figure (11): X-ray patterns of BMV (betamethasone valerate), BMV+L (drug with Precirol lipid), F) betamethasone valerate loaded solid lipid nanoparticles (F2), L) pure Precirol lipid and P) plain solid lipid nanoparticles.....	62
Figure (12): Results for particle size measurements of F2 and F4 after six months storage.....	64
Figure (13): Results for polydispersity index measurements of F2 and F4 after six months storage.....	65
Figure (14): Results for entrapment efficiency measurements of F2 and F4 after six months storage.....	65
Figure (15): The appearance of rats ears after sensitization.....	75
Figure (16): Skin cross sections after staining with haematoxylineosin and examination under light microscope. A: negative control, B: positive control, C: after treated with F2, D: after treated with F4 and E: after treated with Betnovate® cream.....	76
Figure (17): Epidermis thickness scores as a function of treatment.....	77
Figure (18) : Dermis thickness score as a function of treatment.....	77