COMPARATIVE STUDY OF DIFFERENT CLOBETASOL PROPIONATE LOADED NANOCARRIERS TOPICAL SYSTEMS FOR THE MANAGEMENT OF PSORIASIS

A THESIS SUBMITTED TO



MAHARAJA RANJIT SINGH PUNJAB TECHNICALUNIVERSITY BATHINDA (PUNJAB)

IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

By

Ankita Dadwal Regd. No: 17201FPE04

Department of Pharmaceutical Sciences & Technology Maharaja Ranjit Singh Punjab Technical University Bathinda (Punjab), India 2022

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis, entitled "Comparative study of different clobetasol propionate loaded nanocarriers topical systems for the management of psoriasis" in fulfillment of the requirements of the award of the degree of Doctor of Philosophy in Faculty of Pharmaceutical Sciences and submitted in Maharaja Ranjit Singh Punjab Technical University, Bathinda is an authentic record of my own work carried out during a period from 2017 to 2022 under the supervision of Dr. Raj Kumar Narang. The matter embodied in this thesis has not been submitted by me for the award of any other degree of this or any other University/Institute.

(Ankita Dadwal)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Dr. Raj Kumar Narang

Professor Department of Pharmaceutics ISF College of Pharmacy, Moga

The Ph.D. Viva-Voice examination of Ankita Dadwal, has been held on

Sign. of Supervisor

Sign. of External Examiner

ACKNOWLEDGMENTS

It is usually claimed that there is an invisible force at work behind every successful trip, shaping things in the proper way and direction. I believe it is my moral obligation to bow to that holy force, "ALMIGHTY GOD," for bestowing everything upon me, and because of WHOM I was able to complete my study assignment successfully.

I'd want to express my gratitude to Prof. (Dr.) Raj Kumar Narang, my supervisor, without whom this work would not have been feasible. I'd want to use this opportunity to show my deep thanks and admiration for him. Throughout this project, he has consistently provided me with great support, encouragement, and confidence, as well as constructive criticism when appropriate, allowing me to improve my research standards.

I would want to express my heartfelt gratitude to Prof. Neeraj Kumar Mishra, who introduced me to the world of research. Thank you very much, Sir, for your unwavering support, unwavering encouragement, and unwavering assistance.

I take this opportunity to convey my heartfelt thanks to Mr. Parveen Garg, Chairman, ISFCP, Moga, for his support, well wishes, and for providing me with the greatest facilities throughout my study.

Prof. (Dr.) Rahul Deshmukh, Head, Department of Pharmaceutical Sciences Maharaja Ranjit Singh Punjab Technical University, Bathinda, has been a tremendous help to me.

I'd also like to thank all of the faculty members at Maharaja Ranjit Singh Punjab Technical University, Bathinda's Department of Pharmaceutical Sciences, especially Dr. Ashish Baldi (Head of Research and Development), Dr. Puneet Bansal, Dr. Uttam Kumar Mandal, and Dr. Amit Bhatia, for their support and encouragement. Prof. GD Gupta, ISFCP, Moga, has been a tremendous source of support, advice, and encouragement for me.

I am grateful to Mr. Abhay Pandey and the entire team of ISFAL, Moga, for providing the essential facilities for me to conduct my study.

I'd like to express my gratitude to all of the non-teaching personnel at the ISFCP, Moga, especially Ms. Rekha, Mr. Rishnu, Mrs. Krishna, Mrs. Jasveer, and Ms Gagandeep Kaur, for their prompt assistance.

Prof. Amit Goyal, Prof. Gautam Rath, Prof. Ravinder Kumar Rawal, and Prof. G S Ganti, whose blessings were always with me, deserve special mention.

Ms. Shelly Pathania, Ms. Avileen Kaur, Dr. Bhupinder Kumar, Ms. Karamjeet Kaur, Ms. Dilpreet Kaur, Mr. Amandeep Singh, Mr. Rohit Bhatia, Dr. Amit Sharma, Mr. Tanmay, Dr. Vineet Kumar Rai, and Dr. Charan Singh provided me with invaluable support, encouragement, and friendship.

ISF Faculty members' informal assistance and encouragement have been invaluable. It is impossible to mention them all, but at this point in my life, I remember them all and thank them for their aid and support.

I want to convey my heartfelt thanks and gratitude to my parents (Mr. Gagan Singh and Mrs. Premlata) and family members (Mrs. Bhanu Dadwal, Mr. and Ashish Dadwal), Special gratitude to my parents, who have always been an inspiration to me and have always supported, encouraged, and stayed by my side during difficult times. They even helped me to become self-sufficient. Thank you so much for your neverending love, care, support, and sacrifice. I'm at a loss for words to convey my gratitude for all of their efforts and care, therefore I'd want to dedicate this thesis to them.

I'd want to take this opportunity to thank my closest friend, Mr. Rishav Thakur, for his love, devotion, and cooperation in every moment of my life.

Finally, I'd want to thank everyone whose names were left off of this list.

(Ankita Dadwal)

Figure	T:41 6 T ²	Page
No.	Title of Figure	No.
1.1	Penetration of conventional drug and nano-formulations	4
	through psoriatic skin.	
1.2	Pathophysiology of psoriasis	5
1.3	Etiology of Psoriasis	10
1.4	Problems associated with conventional therapy	19
1.5	Advantage of novel carrier over conventional system	22
1.6	Structure of clobetasol propionate	28
1.7	Mechanism of action of glucocorticoids (clobetasol	28
	propionate)	
1.8	Different carrier systems for topical and transdermal drug	30
	delivery	
1.9	Mechanism of uptake of novel carriers through topical	31
	route	
1.10	Structure of nanoemulsions	33
1.11	Types of NLCs	35
1.12	Schematic diagram of the two micro-routes of penetration	40
1.13	Diagrammatic representation showing research envisaged	46
4.1	Screening techniques for preformulation	79
4.2	Preparation of nanoemulgel	82
4.3	Preparation of nanoemulsion	83
4.4	Schematic view for preparation of NLCs	84
4.5	Preparation of deformable liposomes	85
5.1	FT-IR spectra of clobetasol propionate	98
5.2	Standard curve of clobetasol propionate in 10% methanolic PBS at 239nm	100
5.3	Standard curve of clobetasol propionate in octanol at 239 nm	101

LIST OF FIGURES

5.4	Globule size and polydispersity index of optimized formulation (F27)	105
5.5	Globule Size and Polydispersity Index of drug loaded formulation (F30)	105
5.6	Zeta potential of drug loaded formulation (F30)	106
5.7	SEM (Scanning electron microscopic) image of clobetasol propionate loaded nanoemulgel (F30).	107
5.8	(a) DSC of pure drug (Clobetasol propionate)(b) DSC of blank nanoemulgel	108
	(c) DSC of clobetasol propionate loaded nanoemulgel	
5.9	Spreadibility study of optimized blank nanoemulgel	110
5.10	Spreadibility of optimized nanoemulgel (F31)	110
5.11	Rheology study of clobetasol propionate loaded nanoemugel (F31)	111
5.12	Percentage <i>in vitro</i> release of clobetasol propionate in 10% methanolic PBS (pH 5.5)	113
5.13	Size and polydispersity index of NLC (f25)	116
5.14	Size and polydispersity index of Drug loaded NLCs (f27)	117
5.15	Zeta Potential of NCLs	117
5.16	SEM image of optimized NLCs formulation	118
5.17	(a) DSC of pure drug (Clobetasol propionate) (b) DSC ofblank NLC gel (c) DSC of clobetasol propionate loadedNLC gel.	119
5.18	(a) Texture analysis of NLCs loaded gel(b) NLCs loaded gel of clobetasol propionate	121
5.19	Rheological behaviour of optimized formulation (F28 D)	123
5.20	In-vitro drug release profile of Clobetasol propionate from NLCs formulation, NLCs loaded gel and Topinate gel (Marketed preparation)	123
	NLCs formulation, NLCs loaded gel and Topinate gel	

5.21	Size and polydispersity index of Drug loaded NLCs (D16)	126
5.22	Zeta potential of deformable liposomes.	126
5.23	SEM image of optimized Deformable liposome formulation	127
5.24	Rheology study of clobetasol propionate loaded deformable liposomes (D17)	128
5.25	Percentage <i>in vitro</i> release of clobetasol propionate in 10% methanolic PBS (pH 5.5)	130
5.26	Percentage drug penetration through psoriatic skin. Values expressed as mean \pm SD (n = 3)	130
5.27	Comparison of the skin deposition of Clobetasol propionate in intact skin and sebum-removal skin after in	132
	vitro application of marketed and optimized formulations *p<0.05 compared to intact skin. All data represent the	
	mean \pm SD (n=3)	
5.28	Percentage drug penetration of NLCs formulations through psoriatic skin. Values expressed as mean \pm SD (n = 3)	133
5.29	Drug retention profiles through rat skin, of nanostructured lipid carrier. Values expressed as mean \pm SD (n = 3)	134
5.30	Comparison of the skin deposition of Clobetasol propionate in intact skin and sebum-removal skin after in	135
	vitro application of marketed and optimized NLCs formulations $p<0.05$ compared to intact skin. All data represent the mean \pm SD (n=3)	
5.31	Drug retention profiles through rat skin, of Deformable liposomes. Values expressed as mean \pm SD (n = 3)	135
5.32	a) Overlay of clobetasol propionateb) Overlay of clobetasol propionate Skin irritation studies	137
5.33	Skin irritation studies	140
5.34	Histopathology studies	142-143

Table	Title of Table	Page
No.	The of Table	No.
1.1	Difference between normal skin cells and psoriatic skin	2
	cells	
1.2	Classification of types of psoriasis and their features	10
1.3	List of topical novel drug delivery systems for psoriasis	14
1.4	Correlation of transdermal approaches to conventional	20
	treatment on the basis of merits and demerits.	
1.5	List of topical novel drug delivery systems for psoriasis.	23
3.1	Physical properties of Clobetasol Propionate	69
3.2	Physical properties of Pluronic F-68	70
3.3	Physical properties of soya phosphatidylcholines	71
3.4	Physical properties of Squalene	72
3.5	Physical properties of Sodium cholate	73
4.1	List of chemicals and excipients	75
4.2	List of different instruments	76
4.3	List of various computer software used in the present	78
4.3	research	78
4.4	Experimental Grouping of the animals.	92
5.1	Preformulation study of clobetasol propionate.	97
5.2	Solubility profile of clobetasol propionate.	98
5.3	Observed Peaks of clobetasol propionate	99
5.4	Partition coefficient of clobetasol propionate	99
5.5	Regressed data of clobetasol propionate in 10 % methanolic	100
2.0	PBS at 239nm.	100
5.6	Regressed data of clobetasol propionate in octanol at 239	101
210	nm	
5.7	Optimization of surfactant concentration (homogenization	102
	time 20 minutes at 12000 rpm and 20 minutes sonication)	102

LIST OF TABLES

5.8	Optimization of lipid ratio (Homogenization for 20 minutes at 12000 rpm and Sonication time 20 minutes)	103
5.9	Optimization of Homogenization speed	103
5.10	Optimization of Homogenization time	104
5.11	Optimization of Sonication time after homogenization for 20 minutes at 12000 rpm	104
5.12	Characterisation of drug (clobetasol propionate) loaded nanoemulsion.	104
5.13	Characterization of gel loaded nanoemulsion.	109
5.14	Percentage <i>in vitro</i> release data of clobetasol propionate in 10% methanolic PBS (pH 5.5)	112
5.15	Optimization of lipid ratio	114
5.16	Optimization of surfactant ratios (GMS: PF68)	114
5.17	Optimization of homogenization speed	115
5.18	Optimization of homogenization and sonication time	115
5.19	Optimization of drug loaded NLCs on the basis of entrapment efficiency, % drug loading	116
5.20	Optimization of Gel	120
5.21	Texture analysis of plain gel and drug loaded NLCs loaded gel	122
5.22	In vitro drug release in 10% methanolic PBS buffer at pH 5.5	124
5.23	Optimization on the basses of method of preparation: a) Hand rotatory method b) Thin film hydration method using Rota evaporator	124
5.24	Optimization on the basis of vortex timing	125
5.25	Optimization of drug loaded deformable liposomes	125

5.26	Characterization of gel	128
5.27	Percentage <i>in vitro</i> release data of clobetasol propionate in 10% methanolic PBS (pH 5.5)	129
5.28	Physical stability studies of optimized formulations	136
5.29	Regressed data of clobetasol propionate through HPLC method	138
5.30	Biopharmacokinetic parameters by using HPLC method	138
5.31	Skin irritation evaluation score	139

LIST OF ABBREVIATIONS USED

ABC	accelerated blood clearance
ALA	5-aminolevulinic corrosive
APCs	Antigen presenting cells
ATP	Adenosine triphosphate
AUC	Area under curve
CD2	cluster of differentiation
СР	Clobetasol propionate
CPCSEA	Committee for prevention, Control and
	supervision of Experiments on Animals
DSC	Differential scanning calorimetry
EE	Entrapment efficiency
EPC	egg-yolk phosphatidylcholine
FA	Fluocinolone acetonide
PS-SCS	psoriasis stratum corneum substitute
GI	Gastrointestinal
H&E	hematoxylin and eosin
HBD-2	Human b defensin-2
НРН	High pressure homogenization
HPMA	poly[N-(2-hydroxypropyl) methacrylamide]
IAEC	Institutional Animal Ethical Committee
ICAM	intercellular adhesion molecule
ICH	International Conference of Harmonization
IFN	Interferon
IL	Interleukin
IL12B	interleukin-12 subunit beta
IMQ	Imiquimod
Lab	Labrasol

LFA	leukocyte function associated
LFA-3	lymphocyte function-associated antigen-3
LNPs	large-scale synthesis
mALA	methyl 5-aminolevulinic corrosive
MHC	major histocompatibility complex
MTX	Methotrexate
MW	molecular weights
NDDS	Novel drug delivery systems
NE	Nanoemulsion
NIPAM	N-isopropylacrylamide
NK	natural killer
NLCs	Nanostructured lipid carriers
NMFs	Normal moisturizing factors
NTPDase	Nucleoside triphosphate
	diphosphohydrolases
Or NS10	Oramix® NS10
PAcM	poly(N-acryloyl morpholine)
PASI	psoriasis area and severity index
PDI	polydispersity index
PDMA	poly(N,N-dimethyl acrylamide)
PEG	polyethylene glycol
PEVs	penetration enhancer-containing vesicles
PF68	Pluronic 68
PG	propylene glycol
PMOX	poly(2-methyl-2- oxazoline)
ppm	Parts per million
PS	Particle size
PSORS	Psoriasis susceptibility
PVP	poly(vinylpyrrolidone)

QbD	Quality by design
R&D	Research and development
rpm	Rotation per minute
SAR	structure-activity relationships
SC	Stratum corneum
SCS	stratum corneum substitute
SD	Skin deposition
SEM	Scanning electron microscopy
SFEE	supercritical fluid extraction of emulsions
SLN	Solid lipid nanoparticles
SPC	Soya phosphatidyl choline
TCR	T-cell receptor
TEM	Transmission electron microscope
Th1	T helper 1
Th17	T helper 17 cells
Th2	T-helper 2
TNF	tumor necrosis factor
ΤΝΓ-α	Tumor necrosis factor-alpha
Trc	Transcutol
UV	Ultraviolet
VD	Volume distribution
VEGF	vascular endothelial growth factor
XRD	X-ray diffraction