List of Abbreviations

% CDR Percentage cumulative drug release

% Percentage

% RSD Percentage relative standard deviation

% EE
% w/w
% ercent entrapment efficiency
% w/w
% ercentage weight by weight
% v/v
% ercentage volume by volume
% w/v
Percentage weight by volume

< Less than

 \leq Less than equal to

> More than

More than equal toApproximately equal to

± Plus or minus= Equal to

 λ_{max} Wavelength of maximum absorbance

g Gram mg Milligram

°C Degree centigrade

 $\begin{array}{ccc} \mu m & Micrometer \\ \mu L & Microliter \\ cm & Centimeter \\ mL & Milliliter \\ nm & Nanometer \end{array}$

ng/mL Nanogram per milliliter
µg/mL Micrometer per milliliter
mg/mL Milligram per milliliter
G' Storage modulus
G'' Loss modulus

ANOVA Analysis of variance

ACN Acetonitrile

FTIR Fourier transform infrared spectrometer API Active pharmaceutical ingredient

DMSO Dimethyl sulfoxide
Cmax Maximum concentration

AUC Area under curve BSA Bovine serum albumin

CPCSEA Committee for the purpose of control and supervision of experiments on animals

Conc. Concentration

CP Clobetasol propionate

VD3 Vitamin D3 CoQ10 Coenzyme Q10

DSC Differential scanning calorimetry

FESEM Field emission scanning electron microscopy

DLS Dynamic light scattering

h Hour

EDTA Ethylene diamine tetra acetic acid GC-HS Gas chromatography - Headspace

RP-HPLC Reverse phase - High performance liquid chromatography

IPA Isopropyl alcohol

HETP Height equivalent to theoretical plates

HQC Higher quality control sample

ICH International council for harmonization IAEC Institutional animal ethical committee

IL Interleukin
IS Internal standard
LOQ Limit of quantification
LLOO Lower limit of quantification

LOD Limit of detection

MeOH Methanol

MDA Malondialdehyde LQC Lower quality control

MRI Magnetic resonance imaging

min Minute

mPEG Methoxy (polyethylene glycol)

MQC Medium quality control

mPEG-PLA Methoxy (polyethylene glycol)-(polylactide)

NMR Nuclear magnetic resonance MWCO Molecular weight cut off

p value Significance level in statistical tests

n Diffusional exponent indicating the drug release mechanism in Korsmeyer-

peppas model

LPNs Lipid-polymer hybrid nanoparticles

pH Negative log to the base 10 of hydrogen ion concentration

 $\begin{array}{lll} PLA & Poly \, (D,L\text{-lactide}) \\ PDI & Polydispersity \, index \\ R_t & Retention \, time \\ QC & Quality \, control \\ R_s & Resolution \end{array}$

R² Regression coefficient
SD Standard deviation
rpm Rotations per minute
SLNs Solid-lipid nanoparticles
RT Room temperature

sec Second

PDC Polymer drug conjugate

LPs Liposomes

NLCs Nanostructure lipid carriers

t_{1/2} Half-life

SOD Superoxide dismutase

HR-TEM High resolution – Transmission electron microscopy

United States Food and Drug Administration Tumor necrosis factor - $\boldsymbol{\alpha}$ USFDA

TNF-α

Imiquimod
Zeta potential IMQ ZP

LIST OF TABLES

Table No.	Caption	Page No.
1.1	List of lipidic nanosystems explored topically for psoriasis	18
	management	
1.2	List of polymeric nanosystems explored topically for psoriasis	24
	management	
2.1	Chromatographic conditions for the CP methods	49
2.2	Intra-day and Inter-day precision and accuracy of analytical	58
	samples of CP	
2.3	Intra-day and Inter-day precision and accuracy of CP spiked in	58
	skin extract	
2.4	Intra-day and Inter-day precision and accuracy of CP in mice	59
	plasma	
2.5	System suitability parameters for various methods	59
2.6	Robustness studies of CP	60
2.7	Chromatographic conditions for analytical methods	61
2.8	Intra-day and Inter-day precision and accuracy of analytical	66
	samples of VD3	
2.9	Intra-day and Inter-day precision and accuracy of analytical	67
	samples of CoQ10	
2.10	System suitability parameters	67
2.11	Robustness of analytical samples of VD3	68
2.12	Robustness of analytical samples of CoQ10	68
3.1	Composition of LPNs and their characterization for particle	90
	size, PDI, zeta potential and encapsulation efficiency (%). In	
	addition to the composition provided, all batches contain	
	clobetasol propionate (4 mg) and mPEG-PLA (60 mg) as	
	amphiphilic copolymer	
3.2	Systemic absorption of clobetasol propionate after topical	100
	application of Clobetamos™ gel or CP/LPNs-PL gel on the back of	
	IMQ induced psoriatic mice	

3.3	Various parameters indicating skin damage score after topical	104
	application of Clobetamos TM gel or CP/LPNs-PL gel on the	
	right back of IMQ induced psoriatic mice	
3.4	Various parameters indicating liver and spleen damage score	105
	after topical application of Clobetamos™ gel or CP/LPNs-PL	
	gel on the right back of IMQ induced psoriatic mice	
5.1	Improved total skin damage score of the right ear (ES) and back	184
	skin (BS) after topical application of CoQ10/LPNs gel	
	compared to CoQ10 gel using IMQ-induced plaque psoriatic	
	mice model	
5.2	Improved total liver and spleen damage score after topical	185
	application of CoQ10/LPNs gel compared to CoQ10 gel using	
	IMQ-induced plaque psoriatic mice model	

LIST OF FIGURES

Figure No.	Captions	Page No.
1.1	Pathophysiology of psoriasis	5
1.2	Advantages of nano-carrier based drug delivery systems over	10
	conventional formulations	
2.1	Representative chromatogram of (A) mobile phase spiked with	55
	formulation excipients and (B) analytical sample containing CP	
	dissolved in ACN ($45\mu g/mL$)	
2.2	Representative chromatogram of (A) blank skin extract, (B) zero i.e.	56
	IS (DTX) (Rt:7.77 min) spiked in the skin extract, (C) CP (Rt: 14.03	
	min) and DTX (Rt: 7.78 min) spiked in the skin extract at the	
	concentration of 500 ng/mL and 250 ng/mL respectively, (D) blank	
	plasma sample, (E) zero i.e. plasma sample spiked with IS and (F)	
	plasma sample spiked with IS (Rt: 10.539 min) and CP (Rt: 20.445	
	min) at concentration of 300 ng/mL and 75 ng/mL respectively	
2.3	Representative calibration curve of (A) CP in analytical samples, (B)	57
	skin extract samples spiked with CP and (C) plasma samples spiked	
	with CP	
2.4	Representative chromatogram of (A) mobile phase of VD3 spiked	65
	with formulation excipients, (B) analytical sample containing vitamin	
	D3 dissolved in methanol (45 μ g/mL), (C) mobile phase of CoQ10	
	spiked with formulation excipients and (D) analytical sample	
	containing CoQ10 dissolved in methanol ($45\mu g/mL$)	

2.5	Calibration curve for (A) VD3 and (B) CoQ10 in methanol	66
3.1	Characterization CP/LPNs and CP/LPNs gel. (A) photographic images	91
	of undiluted and 100 X diluted CP/LPNs-PL, (B) particle size	
	distribution of CP/LPNs-PL determined by Malvern zetasizer, (C and	
	D) transmission electron microscopic images of CP/LPNs-PL and	
	CP/LPNs-PL gel, (E, F, and G) scanning electron microscopic images	
	of clobetasol propionate loaded gel (CP gel), CP/LPNs-PL and	
	CP/LPNs-PL gel, and (H and I) shear stress versus shear rate and	
	viscosity versus shear rate curves of CP/LPNs-PL gel	
3.2	(A) FTIR spectra of pure CP, CP/LPNs-PL, CP/LPNs-PL gel, blank	92
	LPNs-PL and blank LPNs-PL gel and (B) DSC thermograms of pure	
	CP, blank LPNs-PL, blank LPNs-PL gel, CP/LPNs-PL and CP/LPNs-	
	PL gel	
3.3	In vitro release of clobetasol propionate from (A) freshly prepared	93
	CP/LPNs gels and (B) CP/LPNs-PL gels stored for 3 months and 6	
	months at 2-8°C and room temperature (RT)	
3.4	(A) Fluorescence microscopy and (B and C) flow cytometry analysis	95
	of cellular uptake following incubation with free Coumarin-6 (C6), C6	
	loaded LPNs-PL (C6/LPNs-PL) and C6/LPNs-PL with various	
	endocytosis inhibitors for 4 h in HaCaT cells (Magnification 40X;	
	Scale bar 200 µm). A visible shift (in B) in the peak (towards right) in	
	C6/LPNs-PL indicating higher internalization while a visible shift (in	
	C) in the peak (towards left) in C6/LPNs-PL plus M β CD or MST	
	indicating lipid raft or caveolin mediated endocytosis, (D) Percent	
	fluorescence intensity of HaCaT cells determined by flow cytometry	

3.5 (A) Microscopic images of HaCaT cells treated with free clobetasol propionate (CP) and CP/LPNs-PL (Magnification 40X; Scale bar 200 μ m), (B, C and D) cell viability, apoptosis and cell cycle analysis of HaCaT cells treated with free CP and CP/LPNs-PL. For cell viability, each value is represented as mean \pm SD (n = 4) and statistical analysis was performed using paired t test with significant difference of #, p < 0.01 free CP versus CP/LPNs-PL

3.6

3.7

98

97

Ex vivo permeation of LPNs in psoriatic skin of *Swiss albino* mice.

(A) Bio-imaging using IVIS spectrum of *ex vivo* psoriatic skin tissue treated with free C6 and C6/LPNs –PL gel after 6 h, 12 h and 24 h (n = 3) and (B) Quantification of clobetasol propionate in the remaining skin i.e. viable epidermis and dermis after treatment with ClobetamosTM gel and CP/LPNs-PL gel. Each value is represented as mean ± SD. #, p < 0.01 versus ClobetamosTM gel (0.05% w/w) group

101

CP/LPNs-PL gel inhibited IMQ-induced psoriasis-like skin condition in *Swiss albino* mice (n = 6). (A to C) Scoring of erythema, scaling, and thickening based on the clinical Psoriasis Area and Severity Index (PASI) measured on scale from 0-4, (D) cumulative score indicating the extent of psoriatic inflammation on scale from 0-12, (E) back skin thickness measured using digital vernier caliper, (F) percent change in body weight, (G) Comparative thickness of right and left ear measured using digital micrometer, (H) Average spleen weights for different treatment groups, and comparison of spleen size of treatment groups viz. (a) negative control, (b) positive control, (c) ClobetamosTM gel (0.05% w/w), (d) CP/LPNs-PL gel (0.025% w/w), (e) CP/LPNs-PL

gel (0.05% w/w). Each value is represented as mean ± SEM. *, p < 0.05 and #, p < 0.01, versus ClobetamosTM gel (0.05% w/w) group

IMQ-induced skin inflammation phenotypically simulates psoriasis

like skin condition. Swiss albino mice were treated with Imiquad®

(5% w/w) on the shaved back skin and right ear on daily basis. (A)

Experimental protocol for IMQ-induced psoriasis like mouse model,

(B) phenotypical presentation of mouse back skin after 5 days of

3.8

different treatments, (C) histopathological (H&E staining) evaluation of right ear skin (ES), back skin (BS), liver and spleen of animals at the end of the study. For **ES** and **BS** abbreviations are as follow- **RED**

Stratum corneum: Yellow Arrow: Infiltration of inflammatory Cells;

Arrow: Epidermis; Plain green arrow: Dermis; Black arrow:

Blue Arrow: Capillary proliferation; Orange Arrow: Epidermal

hyperplasia. For liver abbreviations are as follow- RED Arrow:

Hepatocytes; Black arrow: Infiltration of mononuclear inflammatory

Cells Yellow Arrow: Hepatocytes degeneration; Blue Arrow:

Central vein. For spleen abbreviations are as follow- RED Arrow:

White pulp; Black arrow: Splenic central artery Yellow Arrow: Red

pulp; Blue Arrow: Depopulation of lymphocytes in white pulp.

(Magnification: 40X). Scale bar = $50 \mu m$

(A) Immunohistochemically stained psoriatic back skin sections of mice for Ki-67, cell proliferation marker. CP/LPNs-PL gel treatment could effectively decrease the expression of Ki-67 (Magnification: 40X). Black arrow represents the overexpression of Ki-67. Scale bar = 50 μm. (B) average Ki-67 positive cells per High Power Field (HPF)

106

144

of different treatment groups. Each value is represented as mean \pm SD. #, p < 0.001 versus ClobetamosTM gel (0.05% w/w)

- 4.1 Characterization of VD3/LPNs. (A) Dynamic light scattering (DLS) measurements of size distribution of freshly prepared VD3/LPNs (red plot) and VD3/LPNs stored at room temperature for one day (green plot) in water determined by Malvern zetasizer, (B) photographic images of (a) VD3/LPNs dispersion and (b) VD3/LPNs gel (0.005% w/w), (C) field emission-scanning electron microscopic images of VD3/LPNs (scale bar = 1 μm), and (D) In vitro drug release from VD3/LPNs showing a sustained release profile compared to it free form i.e. free VD3. Error bars indicate mean ± SD
- 4.2 (A) FTIR spectra of pure VD3, VD3/LPNs, VD3/LPNs gel, blank
 LPNs and blank LPNs gel and (B) DSC thermograms of pure VD3,
 blank LPNs, blank LPNs gel, VD3/LPNs and VD3/LPNs gel
- 4.3 Rheological and oscillatory rheological measurements of VD3/LPNs gel suggested non-newtonian flow properties possessing shear-thinning behaviour with variable thixotropy as apparent from (A) shear stress (mPa) versus shear rate (1/s) and (B) viscosity (η) versus shear rate (1/s) graphs, (C) subjecting to rise in temperature did not showed sudden collapse of gel 3D matrix structure evident from viscosity (mPa.s) versus temperature (°C) graph suggesting stability of matrix, (D) formation of viscoelastic gel with G' (storage modulus) > G'' (loss modulus) within the LVE region in amplitude sweep test, and (E) long term storage stability was predicted from frequency sweep test where G' > G'' in lower frequency range

147

VD3/LPNs gel suppressed IMQ-induced inflammatory psoriatic condition in mice. Scores of various psoriatic parameters based on PASI system measured on scale from 0-4 on the days indicated that includes (A) erythema, (B) scaling, and (C) thickening, Additionally (D) cumulative score representing the degree of psoriasis that includes sum of erythema, scaling and thickening measured on scale from 0-12 on the days indicated. Each value is denoted as mean ± SEM. @, p ≤ 0.05 and \$, p ≤ 0.01, versus VD3 gel (0.005% w/w) treated group
IMQ-induced psoriatic mouse model resembles the clinical psoriasis condition. Swiss albino mice were subjected to topical treatment on daily basis with IMO on the right ear and back skin. (A) In vivo study

condition. Swiss albino mice were subjected to topical treatment on daily basis with IMQ on the right ear and back skin. (A) In vivo study protocol for IMQ-induced psoriatic mice model, (B) photographic images of back skin of mouse from various groups on day 0 and day 5, measurement of (C) back skin (BS) and (D) right ear (ES) thickness

4.6 Topical treatment with IMQ results into enlargement of spleen shown in positive control group. VD3/LPNs gel showed significant reduction

gel (0.005% w/w) treated group

using digital vernier caliper and micrometer respectively. Each value

is denoted as mean \pm SEM. @, $p \le 0.05$ and \$, $p \le 0.01$, versus VD3

148

in the spleen weights. (A) Average spleen weights from various groups, along with their comparative spleen size s viz. (a) negative control, (b) positive control, (c) VD3 gel (0.005% w/w), (d) VD3/LPNs gel (0.0025% w/w), (e) VD3/LPNs gel (0.005% w/w). During the study duration, application of IMQ leads to substantial

toxicity resulting into fluctuation in body weights with significant

151

reduction in the values from their original ones. (B) percent change in body weight indicating complete recovery in groups treated with VD3/LPNs gel. Each value is denoted as mean \pm SEM. @, $p \le 0.05$ and \$, $p \le 0.01$, versus VD3 gel (0.005% w/w) treated group

- (A) Histopathological examination (H&E, Magnification: 40X) of right ear skin (ES) and back skin (BS) of animals from various groups. For ES and BS, arrow acronyms are as follow- Black: Epidermis; Pink: Pustule of Kogoj; Dark green: Dermis; Sky blue: Munro microabscess; Red: Infiltration of inflammatory Cells; Blue: Stratum Corneum; Yellow: Capillary Proliferation; White: Parakeratosis; Purple: Epidermal Hyperplasia; Fluorescent green: Hyperkeratosis. Scale bar = 50 μm and (B) Assessment of various psoriatic parameters representing total skin damage score for both right ear and back skin of various groups
- 4.8 (A) Histopathological examination (H&E) of liver and spleen of animals from various groups. For **liver** (Magnification: 40X), arrow acronyms are as follow- **Red:** Infiltration of mononuclear inflammatory Cells; **Green:** Hepatocytes; **Yellow:** Central vein; **Orange:** Hepatocytes degeneration. For **spleen** (Magnification: 10X), arrow acronyms are as follow- **Green:** White pulp; **Red:** Red pulp. Scale bar = 50 μm and (B) Assessment of various psoriatic parameters representing sum of total liver and spleen damage scores of various groups
- 4.9 (A) Immunohistochemical staining of Ki67, cell proliferation marker

 152
 expressed in back skin sections of psoriatic mice. Treatment with

175

176

VD3/LPNs gel efficiently downregulates Ki67 expression (Magnification: 40X, Scale bar = 50 μ m). Blue arrow exemplifies the upregulation of Ki67 expression. (B) distribution of average cells positive for Ki67 per High Power Field (HPF) among various groups indicating effectiveness of VD3/LPNs in ameliorating psoriasis. Each value is denoted as mean \pm SEM. @, $p \le 0.05$ and \$, $p \le 0.01$, versus VD3 gel (0.005% w/w) treated group

- Physicochemical characterization of monolithic CoQ10 loaded polymer-lipid hybrid nano-carrier (CoQ10/LPNs). (A) Schematic representation of CoQ10/LPNs along with the particle size distribution demonstrating unimodal size distribution with mean hydrodynamic diameter of 121 nm and polydispersity index of 0.252 determined by dynamic light scattering (Malvern Nano ZS), (B) FE-SEM image showing uniform and spherical morphology, (C) In vitro drug release profile of CoQ10 from CoQ10/LPNs exhibiting sustained drug release without any burst release (n = 3, Mean ± SD)
- 5.2 (A) FT-IR spectra of CoQ10, CoQ10/LPNs, CoQ10/LPNs gel, blank
 LPNs and blank LPNs gel and (B) DSC thermograms of CoQ10,
 CoQ10/LPNs and CoQ10/LPNs gel, blank LPNs and blank LPNs gel
- Rheological measurements and oscillatory rheological analysis of CoQ10/LPNs gel indicated non-newtonian type system with pseudoplastic properties and variable thixotropy (A) Viscosity (η) against shear rate (1/s) and (B) shear stress against shear rate (1/s) graphs, (C) stability of gel 3D matrix evident from the graph of viscosity (η) against temperature (°C) demonstrating no sudden

179

collapse of viscosity (mPa.s) with rise in temperature (°C), (D) amplitude sweep test data indicating viscoelastic gel formation with G' > G" within the linear viscoelastic (LVE) region wherein G' is storage modulus and G" is loss modulus, (E) frequency sweep test data suggesting long term storage stability as obvious from higher storage modulus than loss modulus (G' > G") in lower frequency region

- 5.4 Schematic representation of study protocol for conducting IMQ-induced in vivo anti-psoriatic efficacy studies along with representative photographic presentation of back skin of animals from various groups on day 0 and day 5
- CoQ10/LPNs gel reduces the PASI scores and ameliorated the skin lesion of IMQ-induced plaque psoriatic mouse model in Swiss albino mice (n = 6). Antipsoriatic efficacy was evaluated based on PASI scores measured on a scale from 0 to 4 for parameters (erythema, scaling, and thickening) and from 0 to 12 for cumulative score (summation of erythema + scaling + thickening) which served as an index of extent of psoriasis induced. Data is presented as mean \pm SEM. #, p \leq 0.05 and @, p \leq 0.01, versus CoQ10 gel (0.06% w/w) treated group
- 5.6 CoQ10/LPNs gel substantially inhibited the rise in skin thickness induced by topical application of IMQ. Commercial product of IMQ (Imiquad® (5% w/w)) was applied topically onto the right ear and back skin of Swiss albino mice for 5 consecutive days and resulted into thickening of the skin tissue due to underlying dermal

180

inflammatory cascade accompanied by hyperplasia of keratinocytes. Bringing back the thickness to normal or suppressing the rise in the thickness marked the efficiency of treatment. Measurement of the (A) average right ear thickness and (B) average back skin thickness for various groups using digital micrometer and vernier caliper on the indicated days. Symbols represented average thickness \pm SEM (n = 6). Data is presented as mean \pm SEM. #, p \leq 0.05 and @, p \leq 0.01, versus CoQ10 gel (0.06% w/w) treated group

5.7

181

Topical application of IMQ exhibited toxicity by inducing splenomegaly and body weight fluctuation (reduced body weights). CoQ10/LPNs gel showed improved recovery w.r.t. both spleen weights and body weights. (A) Average spleen weights of various treatment groups, along with their comparative spleen sizes viz. (a) negative control, (b) positive control, (c) CoQ10 gel (0.06% w/w), (d) CoQ10/LPNs gel (0.03% w/w), (e) CoQ10/LPNs gel (0.06% w/w) and (B) percent change in body weight indicating improved recovery in groups treated with CoQ10/LPNs gel on Day 5. Data is presented as mean \pm SEM. #, p \leq 0.05 and @, p \leq 0.01, versus CoQ10 gel (0.06% w/w) treated group

183

5.8 Comparative histological alterations in various tissues such as right ear skin (ES), back skin (BS), liver and spleen suggesting effectiveness of CoQ10/LPNs gel in reduction of inflammatory infiltration leading to suppression of keratinocyte hyperplasia in IMQ-induced plaque psoriatic mouse model that simulates the clinical psoriasis. Commercial product of IMQ (Imiquad® (5% w/w)) was

topically applied onto the ES and BS of Swiss albino mice for 5 consecutive days that resulted into development of psoriasis-like inflammatory skin condition characterized by dermal damage (rete ridges, acanthosis, hyperplasia, parakeratosis, inflammatory infiltrate etc.), liver damage (fibrosis) and spleen damage (undifferentiated white pulp and red pulp). For ES and BS (Scale bar = 50 μm at 40X magnification) arrow abbr. are as follow- Red: Dermis; Blue: Epidermis; Black: Inflammatory infiltrates; Dark green: Stratum Corneum; Pink: Capillary Proliferation; White: Hyperkeratosis; Fluorescent green: Parakeratosis; Yellow: Epidermal Hyperaplasia. For liver (Scale bar = 50 μm at 40X magnification) arrow abbr. are as follow- Orange: Inflammatory infiltrates; Black: Hepatocytes; Green: Central Vein; Red: Hepatocytes Degeneration. For spleen (Scale bar = 50 μm at 40X magnification) arrow abbr. are as follow- White: Red Pulp; Black: White Pulp

5.9

Ki67 (cell proliferating nucleoprotein) is found to be over-expressed in psoriatic lesions. (A) Immunohistochemical staining of Ki67 expressed in back skin of negative control or IMQ-induced psoriatic mice without or with treatment of CoQ10 containing formulations. CoQ10/LPNs gel resulted into significant reduction in expression of Ki67 which is over-expressed in psoriatic lesions. (Scale bar = 50 μm at 40X magnification). Red arrow signifies the higher expression of Ki67, (B) Graphs of average keratinocytes positive for Ki67 per High Power Field (HPF) of various groups indicated treatment with CoQ10/LPNs gel significantly reduced expression of cell proliferation

		marker and results were in alignment with the PASI scores. Data is		
	presented as mean \pm SD. #, p \leq 0.05 and @, p \leq 0.01, versus CoQ10			
	gel (0.06% w/w) treated group			
	5.10	Comparative assessment of various groups w.r.t. (A) MDA and (B)	187	
	GSH levels suggesting marked improvement in the antioxidant effect			
	of CoQ10 when delivered as LPNs gel			