CHAPTER 5: RESULTS AND DISCUSSION

CHAPTER 5:
RESULTS AND DISCUSSION

5.1. PREFORMULATION STUDIES

Preformulation studies are essential for assessing and confirming the physicochemical qualities, as well as the identification and purity of the raw material to be utilised in formulation development, particularly the active pharmaceutical ingredient. Clobetasol propionate underwent preformulation tests to ensure its authenticity and purity, as well as to determine the most appropriate analytical approach for detecting its concentration in various formulations. The research aided in the selection of optimal formulation ingredients, solvent system process variables, and other factors in the development of a novel, patient-friendly, high-quality, and safe formulation.

5.1.1. Melting Point

The capillary method was employed for the determination of the melting point of clobetasol propionate. Which was found to be 195-196^{0}c which comes almost similar to 195.5-197^{0}c standard shown in Table 5.1.

Table 5.1 Preformulation study of clobetasol propionate.

Preformulation study	Standard sample	Test sample
Melting point	195.5-197^{0}c	195-196^{0}c
Partition coefficient (logp)	3.49	3.34
Appearance	White in colour	White in colour

5.1.2 Solubility studies

Preformulation investigations must include the determination of drug solubility Table 5.2 shows the solubility of clobetasol propionate in various solvents. It is critical in determining the best medium for many research, such as hydration, entrapment, permeability, and stability. As a result, solubility tests were carried out in a variety of solvent systems to choose the best medium for various studies throughout the development of drug-loaded carrier systems.

The saturation solubility of clobetasol propionate in various media is shown in Table 5.2. Clobetasol propionate's solubility in water was found to be negligible.

Table 5.2 Solubility profile of clobetasol propionate.

Solvent	Standard sample	Test sample
Water	4.13 ppm	(insoluble)
Ethanol	20000 ppm	18mg/ml(sparingly soluble)
Methanol	48000 ppm	47mg/ml (soluble)
Chloroform	48000 ppm	46mg/ml (soluble)
Dmso	93000 ppm	90mg/ml (soluble)
Acetone	48000 ppm	47mg/ml (soluble)

5.1.3. FT-IR Spectroscopy

FTIR spectroscopy studies were done using the FTIR spectrophotometer. The obtained spectrum was compared with the reference spectrum. Corresponding peaks for the functional groups were observed confirming the identity of the gift sample of Clobetasol propionate obtained. Spectrum showing corresponding functional groups (USP 2016) are representedin Figure 5.1 and Table 5.

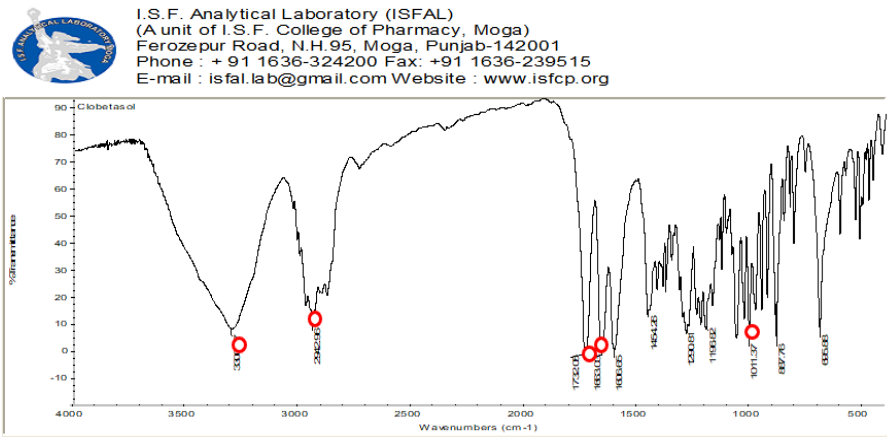


Figure 5.1 FT-IR spectra of clobetasol propionate

Table 5.3 Observed Peaks of clobetasol propionate

Interpretation	**Observed peaks Cm^{-1}**	**Standard $peak^{-1}$**
Carbonyl group of aliphatic ester and ketone	1732	1750- 1720
Stretching vibration of O-H group	3300	3570 – 3200
C=O bond stretching	1663	1750-1680
Aromatic C-H stretching	2942	3100-3000
C- F bond	1011	1150 – 1000

5.1.4. Partition Coefficient

The extent of lipophilicity of Resveratrol was evaluated in this study. The experiment was conducted thrice and the observed value (Herbig and Evers, 2013). The values of Log P are mentioned in Table 5.4. These results suggest that the drug was highly lipophilic in nature.

Table 5.4 Partition coefficient of clobetasol propionate

Preformulation Study	**Standard Sample**	**Observed**
Partition coefficient (logP)	3.49	3.34

5.1.5. Standard Curve of Clobetasol Propionate in 10 % Methanolic PBS at 239nm at pH 5.5

Table 5.5 and Figure 5.2 represent standard curve of clobetasol propionate in 10 % methanolic PBS at 239 nm. Estimation of clobetasol propionate was done using UV spectrophotometric method. Linear response was obtained in the range of 4-14 µg/ml at λ_{max} 239 nm with correlation coefficient $r^2 = 0.9982$. Results inferred that Beer's law was observed in these concentration ranges.

5.1.6. Standard Curve of Clobetasol Propionate in Octanol at 239 nm

Table 5.6 and Figure 5.3 represent standard curve of clobetasol propionate in octanol. Estimation of clobetasol propionate was done using UV spectrophotometric method. Linear response was obtained in the range of 4-14 µg/ml at λmax 239 nm with correlation coefficient r^2 = 0.9987. Results inferred that Beer's law was obeyed in these concentration ranges.

Table 5.5 Regressed data of clobetasol propionate in 10 % methanolic PBS at 239nm.

concentration (µg/ml)	Absorbance
4	**0.216**
6	**0.328**
8	**0.442**
10	**0.549**
12	**0.672**
14	**0.755**

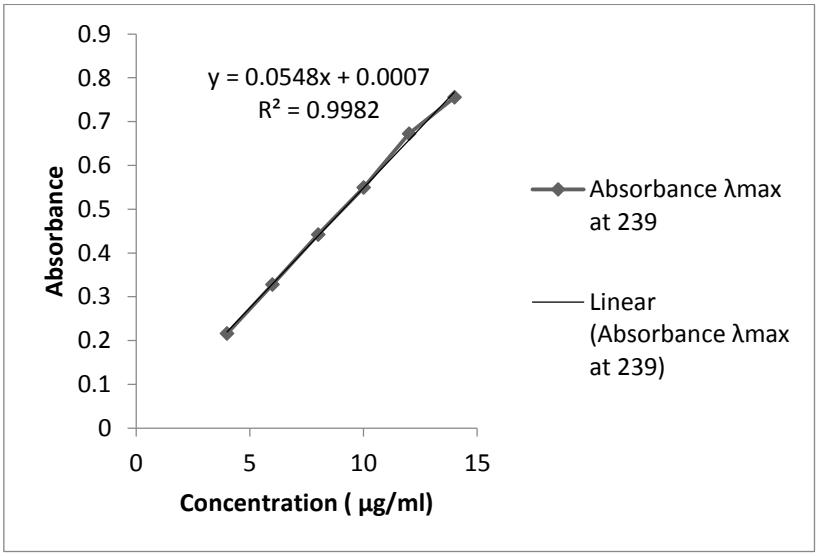


Figure 5.2 Standard curve of clobetasol propionate in 10% methanolic PBS at 239nm

Table 5.6 Regressed data of clobetasol propionate in octanol at 239 nm

Concentration (µg/ml)	Absorbance
4	**0.26**
6	**0.35**
8	**0.44**
10	**0.55**
12	**0.64**
14	**0.75**

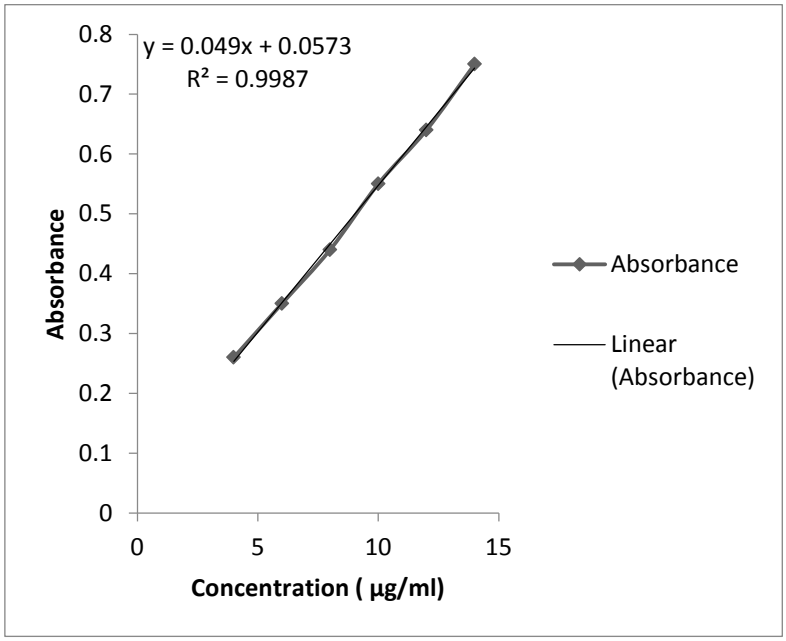


Figure 5.3 Standard curve of clobetasol propionate in octanol at 239 nm

5.2 PREPARATION OPTIMIZATION AND CHARACTERIZATION OF CLOBETASOL PROPIONATE LOADED NANOCARRIERS.

5.2.1. Preparation & Characterization of Clobetasol Propionate Loaded Nanoemulgel.

5.2.1.1. Optimization of the Blank and Drug-Loaded Nanoemulsion.

Nanoemulsion was optimized by using different surfactant concentration, lipid ratio, homogenization speed, homogenization time and sonication time for both blank and drug-loaded (Table 5.7, 5.8, 5.9, 5.10 and 5.11). This study has demonstrated that the optimized blank nanoemulsion formulation has a particle size of 212.1± 11.1nm and PDI 0.162±0.03nm. The optimized drug (clobetasol propionate) is a potent drug (dose 0.5mg through transdermal route) and we have selected its concentration on the bases of studies reported(Feldman, 2005; Gordon, 1998). The loaded formulation have a particle size and PDI of 240.5±9.2 and 0.282±0.03nm, respectively.

Table 5.7 Optimization of surfactant concentration (homogenization time 20 minutes at 12000 rpm and 20 minutes sonication)

Sr.No.	Surfactant conc. (PF68)	Lipid ratio (Squelene : SPC)	Particle size (nm)	PDI
F1	1%	4:1	-	-
F2	1.5%	4:1	-	-
F3	2%	4:1	1002±52.2	0.330±0.04
F4	2.5%	4:1	659.5±27.4	0.282±0
F5	3%	4:1	324.0±19.2	0.300±0.04

F6	**3.5%**	**4:1**	**219.8±12.5**	**0.162±0.01**
F7	4%	4:1	229.5±19.7	0.140±0.07
F8	4.5%	4:1	232.1±20.3	0.169±0.03

Table 5.8 Optimization of lipid ratio (Homogenization for 20 minutes at 12000 rpm and Sonication time 20 minutes)

Sr.No.	Surfactant conc. (PF68)	Lipid ratio (Squalene :SPC)	Particle size (nm)	PDI
F9	3.5%	1:1	292.8± 11.4	0.242±0.02
F10	3.5%	2:1	240.5± 19.8	0.287±0.0 1
F11	3.5%	3:1	219.6±9.2	0.330±0.04
F12	**3.5%**	**4:1**	**217.8±11.1**	**0.140±0.07**
F13	3.5%	5:1	248.5±8.4	0.249±0.07
F14	3.5%	4:2	283.6±21.5	0.140±0.03
F15	3.5%	4:3	321.4±11.4	0.300±0.03
F16	3.5%	4:5	430.9±38.7	0.322±0.06

Table 5.9. Optimization of Homogenization speed

Sr. No.	Surfactant conc.	Lipid ratio (Squelene : SPC)	Speed (rpm)	Sonication time and hominization time	Size (nm)	PDI
F17.	3.5%	4:1	10000	20 minutes	231.1±12.3	0.161±0.02
F18.	**3.5%**	**4:1**	**12000**	**20 minutes**	**212.1±11.1**	**0.162±0.03**
F19.	3.5%	4:1	15000	20 minutes	227.5±19.4	0.322±0.01
F20.	3.5%	4:1	18000	20 minutes	242.6±14.6	0.261±0.04

Table 5.10 Optimization of Homogenization time

Sr. No.	Surfactant conc.	Lipid ratio	Time (min)	Speed (rpm)	Sonication time	Size (nm)	PDI
F21.	3.5%	4:1	10	12000	20 minutes	292.3±10.1	0.249±0.04
F22.	**3.5%**	**4:1**	**20**	**12000**	**20minutes**	**212.1±11.1**	**0.162±0.03**
F23.	3.5%	4:1	30	12000	20 minutes	227.5±16.5	0.378±0.06
F24.	3.5%	4:1	40	12000	20 minutes	314.3±9.7	0.323±0.05

Table 5.11 Optimization of Sonication time after homogenization for 20 minutes at 12000 rpm

Sr.no.	Sonication time	Size (nm)	PDI
F25.	10 minutes	292.5± 14.5	0.249±0.02
F26.	15 minutes	232.2 ±21.3	0.169±0.01
F27.	**20 minutes**	**212.1± 11.1**	**0.162±0.03**
F28.	25 minutes	218.1± 14.9	0.140±0.03
F29.	30 minutes	313.1± 9.6	0.276±0.05

The Characterisation of drug (clobetasol propionate) loaded nanoemulsion by particle size, polydispersity index (PDI), zeta potential, drug loading and percentage encapsulation efficiency shown in Figure 5.4, 5.5 and 5.6.

Table 5.12 Characterisation of drug (clobetasol propionate) loaded nanoemulsion.

Formulation Code	Drug concentration	Surfactant concentration (PF68)	Lipid ratio (Squelene : SPC)	Particle size (nm)	Zeta potential (mV)	PDI %	E.E. w/v	% Drug loading w/v
F30	0.05%	3.5%	4:1	240.5 ± 9.2	-51.21	0.282 ± 0.03	89.8 ± 7.11%	45.12%

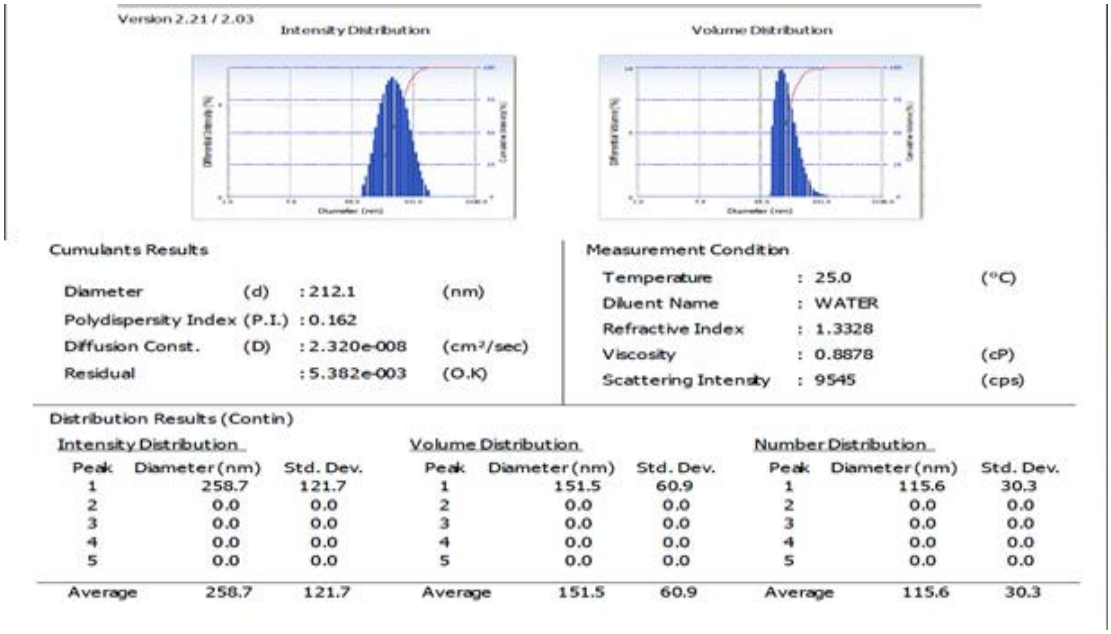


Figure 5.4 Globule size and polydispersity index of optimized formulation (F27)

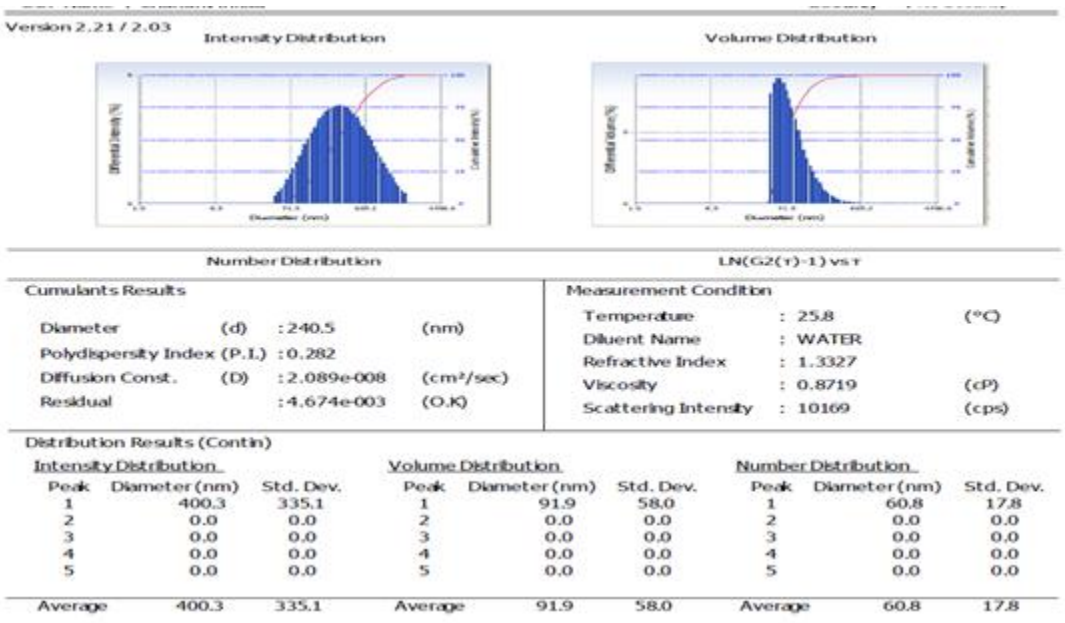


Figure 5.5 Globule Size and Polydispersity Index of drug loaded formulation (F30)

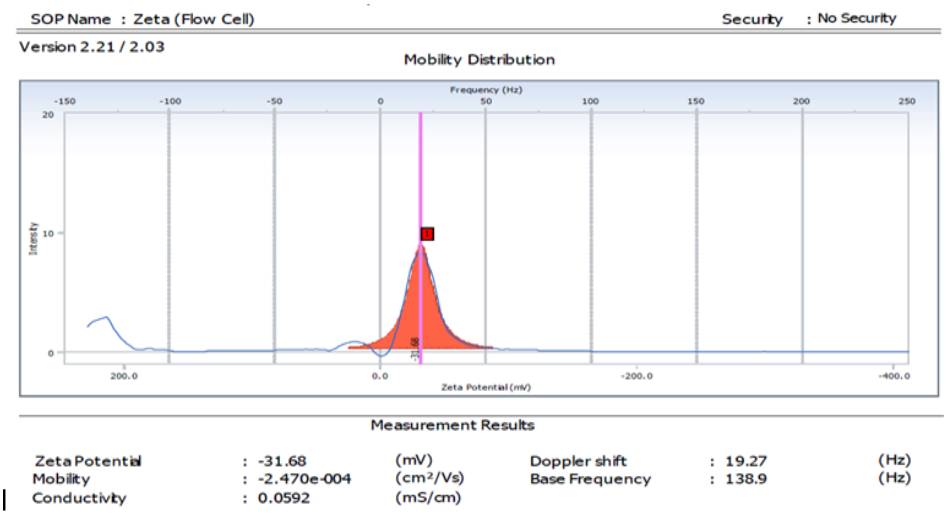


Figure 5.6 Zeta potential of drug loaded formulation (F30)

Prepared nanoemulsion was optimized on the basis of their size by different lipid ratios and surfactant concentration. The optimum ratios were found to be 3.5% PF68 surfactant and 4:1 Squelene: SPC, under Homogenization speed of 12000 rpm for 20 minutes then Sonicated for 20 minutes in formulation which had shown particle size of 212.1± 11.1 and PDI of 0.162± 0.03. The nanoemulsion size was determined by using Malvern Zetasizer. Comparative mean vesicle size of nanoemulsion prepared by different method using different surfactants and varied composition. Results of vesicle size reveal that size decreases as the concentration of the surfactant increased. Deformability was one of the characteristics for nanoemulsion to differ from other systems like liposomes, NLCs etc. When surrounding stress was enforced on nanoemulsion to penetrate the skin pores, nanoemulsion could spontaneously undergo deformation to avoid the risk of structure rupture because of its flexible nature and it is helpful in transporting drug thorough scaly keratinized psoriatic skin. Formulation prepared by using 3.5% surfactant and lipid ratio (squelene and SPC) 4:1 was found to be optimized particle size of optimized formulation was shown 212.1±11.1nm. Initially particle size was decreased by increasing homogenization speed, time and sonication time after a point of time size increased. This might be because of fussion

of nanoemulsion particle.

5.2.1.2. Morphology

The morphology of nanoemulsion can be determined by scanning electron microscopy (SEM). SEM gives a three-dimensional image of the globules(Verma *et al.*, 2016). The samples are examined at suitable accelerating voltage, usually 20 kV, at different magnifications. A good analysis of surface morphology of disperse phase in the formulation is obtained through SEM. Image analysis software, may be employed to obtain an automatic analysis result of the shape and surface morphology.(Barea *et al.*, 2010) The morphological characterization prepared nanoemulsion shows small spherical shape and uniform size distribution as observed in the SEM photograph (Figure. 5.7).

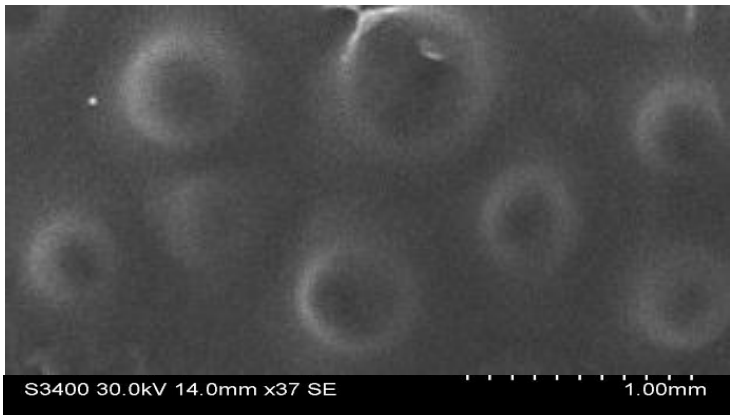
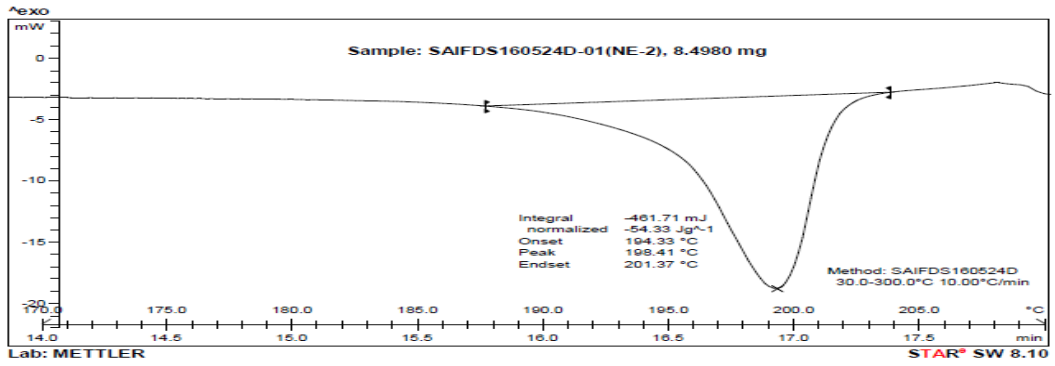


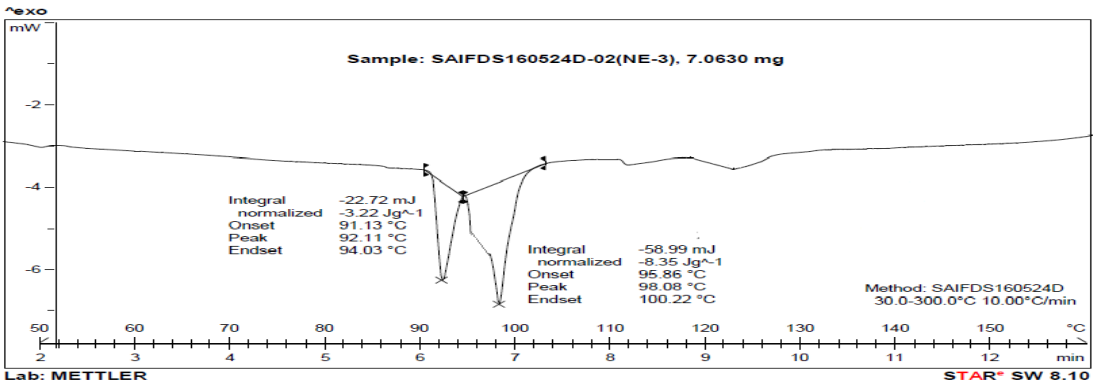
Figure. 5.7 SEM (Scanning electron microscopic) image of clobetasol propionate loaded nanoemulgel (F30).

5.2.1.3. DSC study

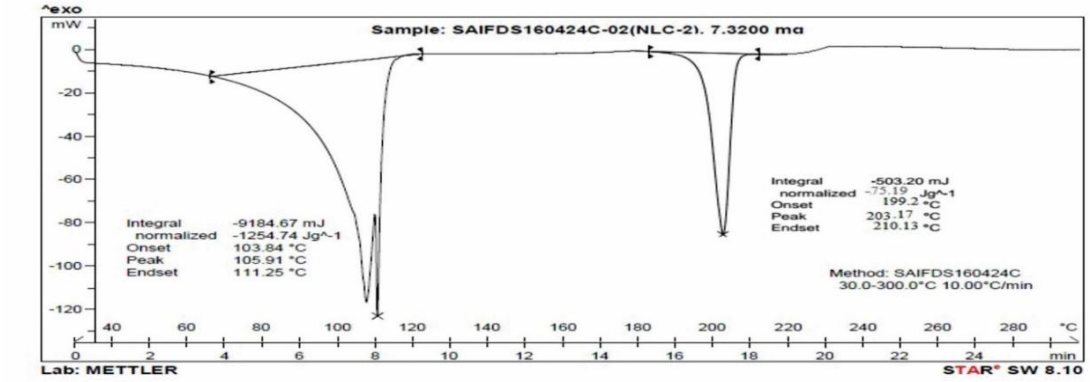
As shown in Figure. 5.8 (a), (b) and (c), DSC of the drug (clobetasol propionate) shows its melting point at 201°C, blank formulation and drug incorporated in the nanoemulsion system shows its melting point slightly higher *i.e.* 237.37°C that may protect the nature of nanoemulsion system. This might be because of chemical bond formation between drug and lipids used in the preparation of nanoemulsion.



(5.8a)



(5.8b)



(5.8c)

Figure. 5.8 (a) DSC of pure drug (Clobetasol propionate) (b) DSC of blank nanoemulgel (c) DSC of clobetasol propionate loaded nanoemulgel

Nanoemulgel were prepared in different batches by using a different concentration of carbopol 940 w/v with formulation code and optimized for the various parameters such as pH, spreadability, % of carbopol and % drug content. The optimized nanoemulgel formulation was prepared by using carbopol 940 (0.25%w/v). Carbopol gel 0.25% shows good rheological properties, swelling index, and spreadability. A prepared gel containing nanoemulsion was a transparent gel with a smooth and homogeneous appearance.

5.2.1.4. Optimization and characterization of Gel

Nanoemulgel were prepared in different batches by using different concentration of carbopol 940 w/v with formulation codes and optimized for the various parameters such as pH, spreadibility, % of carbopol and % drug content which are shown in Table 5.13 nanoemulgel prepared by using carbopol 940 was 0.25%w/v found to be optimized.

Table 5.13 Characterization of gel loaded nanoemulsion.

Sr. No.	Carbopol 940 (w/v)	pH	Spreadability (gm.cm/sec)	% Drug content
F31	**0.25%**	**5.51±0.91**	**21.12±0.15**	**88.61±0.39**
F32	0.3%	5.10±0.28	19.91±0.12	88.12±0.31
F33	0.35%	5.30±0.91	18.41±0.31	88.27±0.42
F34	0.4%	5.51±0.71	16.34±0.71	89.19±0.21
F35	0.45%	5.78±0.07	15.24±0.88	89.24±0.11
F36	0.5%	6.41±0.21	14.29±0.23	90.02±0.23

5.2.1.5. Spreadibility of optimized blank nanoemulgel (F30)

Hardness Cycle 1: 150.0 g **Deformation at Hardness:** 12.50 mm

Load at Target: 150.0 g **Deformation at Target:** 12.50 mm

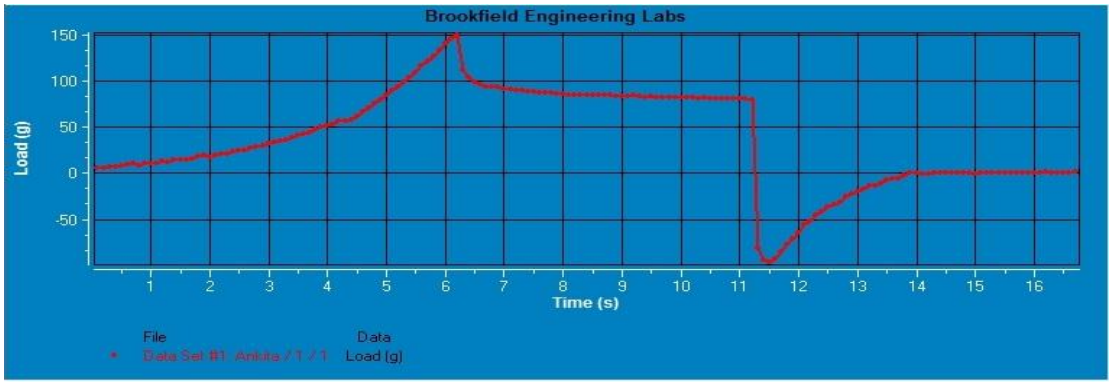


Figure 5.9 Spreadibility study of optimized blank nanoemulgel

5.2.1.6. Spreadibility of optimized nanoemulgel (F31)

Hardness Cycle 1: 147.0 g **Hardness Work Cycle 1:** 0.54 mJ

Load at Target: 147.0 **Deformation at Target:** 13.92 mm

Deformation at Hardness: 13.92 mm

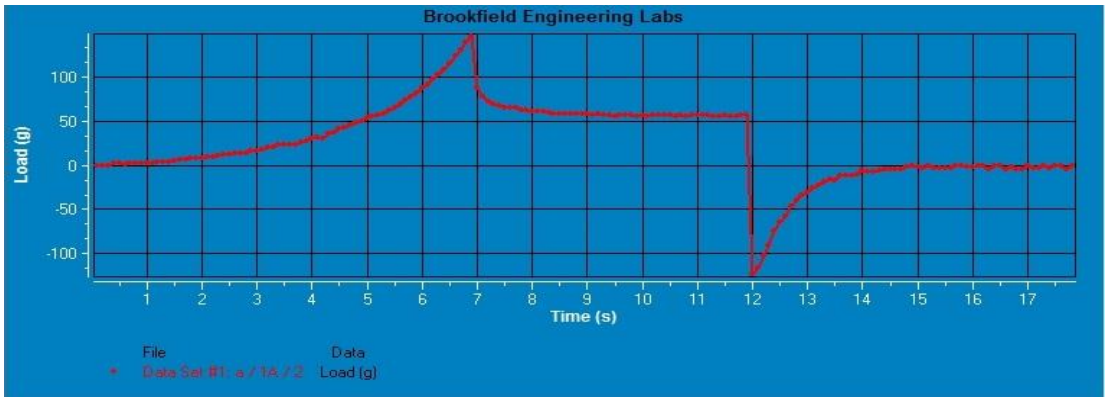
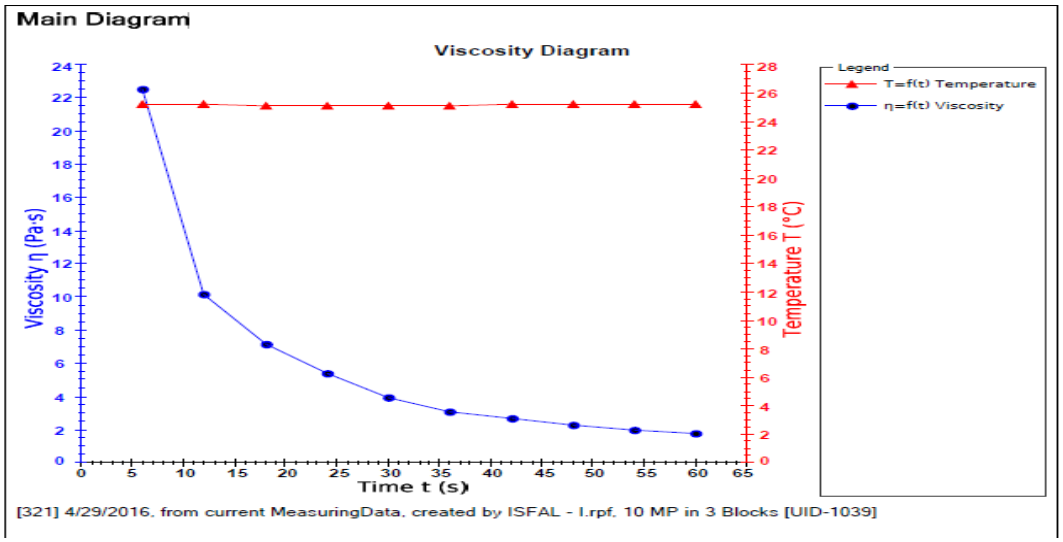


Figure 5.10 Spreadibility of optimized nanoemulgel (F31)

As the drug is added in the gel deformation at target changes from 12.50 mm to 13.92 mm which is shown in Figure 5.9 and 5.10 which shows that when drug concentration was increased the spreadibility was decreased.

5.2.1.7. Rheology

Clobetasol propionate loaded nanoemugel (F31) exhibited rheological behaviour at 37^0C as depicted in Figure 5.11. Gel follows Newtonian flow at below 37^0C while at below 37^0C gel shows pseudoplastic behaviour. Studies suggested that viscosity decreases with increase in shear rate.



Number Nr.	Time (s) t	Shear Stress (Pa) τ	Shear Rate (1/s) Ẏ	Viscosity (Pa·s) η	Temperature (°C) T
1	6	219.918	9.783	22.4796	25.2
2	12	203.194	20.076	10.1213	25.2
3	18	214.960	30.048	7.1539	25.1
4	24	215.028	39.960	5.3811	25.1
5	30	196.028	50.073	3.9148	25.1
6	36	185.181	60.057	3.0834	25.1
7	42	185.603	69.975	2.6524	25.2
8	48	180.559	80.073	2.2549	25.2
9	54	175.037	90.069	1.9434	25.2
10	60	174.525	99.975	1.7457	25.2

Figure 5.11 Rheology study of clobetasol propionate loaded nanoemugel (F31)

5.2.1.8. Percentage *in vitro* release graph of clobetasol propionate in 10% methanolic PBS (pH 5.5)

The rate of drug release across the dialysis membrane was slower for nanoemulgel than the nanoemulsion and was least for the marketed gel (topinate). The drug release from nanoemulsion in PBS (pH 5.5) was approximately 84.24±1.35% after 24hrs. The nanoemulgel formulations showed release of 66.83±2.05% while marketed gel showed release of 57.67±1.63% after 24 hrs. *In-vitro* release profile of different formulations is shown in Table 5.14 and Figure 5.12 showed the % cumulative release profile. The release profiles of clobetasol propionate from nanoemulgel showed biphasic release processes, where initial burst release of the surface-adsorbed drug was observed, followed by slow diffusion from the lipid nanoemulsion. At the initial 4 hrs, the little higher drug release of nanoemulgel was observed. Afterward, lipid nanoemulsion diffusion in gels played an important role in the release profiles and drug release rate slowed down.

Table 5.14 Percentage *in vitro* release data of clobetasol propionate in 10% methanolic PBS (pH 5.5)

Time (hrs.)	Nanoemulsion F27	Topinate gel	(Nanoemulgel) F31
0.00	0.00	0.00	0.00
0.15	18.87±2.05%	8.78±1.84%	13.12±2.02%
0.30	39.03±1.34%	14.57±1.63%	22.14±1.84%
1	46.67±1.84%	20.68±1.34%	28.68±1.63%
2	51.36±2.05%	24.79±1.43%	32.24±2.05%
4	56.48±2.05%	29.32±1.35%	36.32±1.34%
6	61.98±1.43%	34.67±2.05%	41.34±1.43%
8	68.17±2.04%	39.24±2.02%	45.87±2.05%
12	74.59±2.02%	45.67±1.43%	52.13±1.35%
18	79.97±2.05%	51.32±2.05%	59.61±1.84%
24	84.24±1.35%	57.67±1.63%	66.83±2.05%

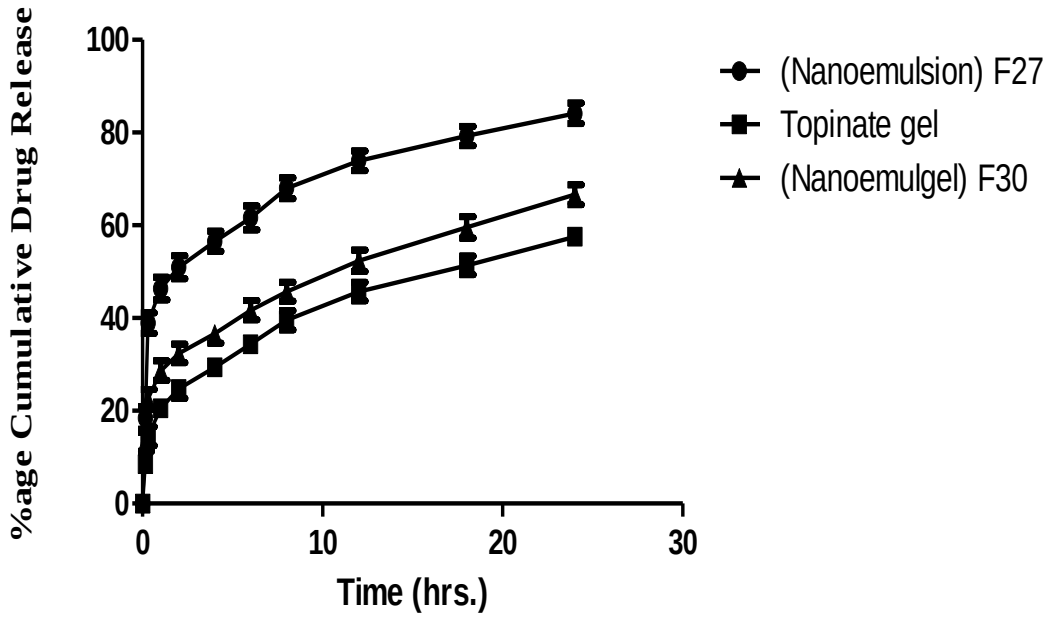


Figure 5.12 Percentage *in vitro* release of clobetasol propionate in 10% methanolic PBS (pH 5.5)

5.2.2. Preparation & Characterization of Clobetasol Propionate Loaded Nanostructured Lipid Carrier

5.2.2.1. Optimization of Blank and Drug-Loaded Nanostructured Lipid Carrier

Blank NLCs were prepared in different batches and optimized for the various parameters such as size and PDI on the basis of homogenization speed, time, sonication time, surfactant ratio which are shown in following tables (5.15, 5.16, 5.17, and 5.18). Formulation (f 25) was found to be optimized with respect to particle size (227 ± 6.21 nm), zeta potential (-40.32 ± 4.20 mV), PDI (0.262±0.04).

Table 5.15 Optimization of lipid ratio

Form code	SPC: Squalene	Homo. speed (rpm)	Homo. time (min)	Soni. Time (min)	Particle size (nm)	Zeta potential (mV)	PDI
f1	1:1	15000	20	15	267±9.32	-17.21	0.189±0.04
f2	**1:2**	**15000**	**20**	**15**	**227±6.21**	**-40.32**	**0.262±0.04**
f3	1:3	15000	20	15	238±7.65	-23.22	0.432±0.05
f4	1:4	15000	20	15	259±8.56	-39.31	0.611±0.32
f5	3:2	15000	20	15	290±9.23	-19.11	0.410±0.43
f6	4:2	15000	20	15	320±16.50	-26.11	0.226±0.02
f7	5:2	15000	20	15	374±20.2	-33.11	0.226±0.02
f8	6:2	15000	20	15	510±29.2	-31.05	0.521±0.31

Table 5.16 Optimization of surfactant ratios (GMS: PF68)

Formulation code	GMS: PF68	Particle size (nm)	Zeta potential (mV)	PDI
F9	0.5:1	290±9.23	-51.21	0.13±0.07
F10	**1:1**	**231 ±7.81**	**-46.33**	0.13±0.07
F11	1.5:0.5	-	-	-
F12	1:0.5	231 ±7.81	-10.55	0.191±0.21

Table 5.16 depicts the optimization of NLCs by using different ratios of surfactants (GMS: PF68) and formulation F10 was found to be optimized (n=3).

Table 5.17 Optimization of homogenization speed

Formulation code	Homo. Speed (rpm)	Homo. Time (min)	Particle size (nm)	PDI
F13	9000	20	689.5±21.02	0.511±0.02
F14	10000	20	598.6±17.21	0.432±0.03
F15	11000	20	467.3±14.10	0.411±0.07
F16	12000	20	380.8±9.20	0.389±0.12
F17	13000	20	267.6±8.67	0.482±0.04
F18	14000	20	250.5±7.32	0.322±0.13
F19	**15000**	**20**	**229.8±7.76**	**0.140±0.19**
F20	16000	20	289.5±6.11	0.327±0.23

Table 5.17 depicts the optimization of NLCs on the basis of homogenization speed and formulation F19 was found to be optimized (n=3).

Table 5.18 Optimization of homogenization and sonication time

Formulation code	Homogenization time (min)	Sonication time (min)	Particle size (nm)	PDI
F21	10	15	313.6±12.3	0.140±0.12
F22	15	15	289.8±10.11	0.251±0.41
F23	20	15	256.1±9.63	0.163±0.12
F24	25	15	222.3±8.21	0.132±0.011
F25	**20**	**15**	**225.8±7.71**	**0.222±0.16**
F26	20	10	230.4±8.31	0.386±0.13

Table 5.18 depicts the optimization of NLCs at different homogenization and sonication time; finally formulation F25 was found to be optimized (n=3).

5.2.2.2. Drug loaded NLCs

Finally, formulation (f27) (Drug loaded NLCs)(As clobetasol propionate) is a potent drug (dose 0.5mg through transdermal route) we have selected its concentration on the bases of studies reported by (Gordon, 1998), (Feldman, 2005) was found to be optimized having particle size of 254 ± 10.11 nm, zeta potential -56.11 ± 6.21 mV, PDI 0.140, entrapment efficiency 89.8 ± 7.11%, drug loading 45.12%. final results are depicted in table 5.19.

Table 5.19 Optimization of drug loaded NLCs on the basis of entrapment efficiency, % drug loading

Form code	Drug Conc.	Particle size (nm)	Zeta potential (mV)	PDI	Entrapment efficiency
f27	0.05%	254±10.22	-56.11 ±6.32	0.140±0.01	89.8%±4.31

Table 5.19 depicts the optimization of drug loaded NLCs, finally formulation f27 was found to be optimized formulation.

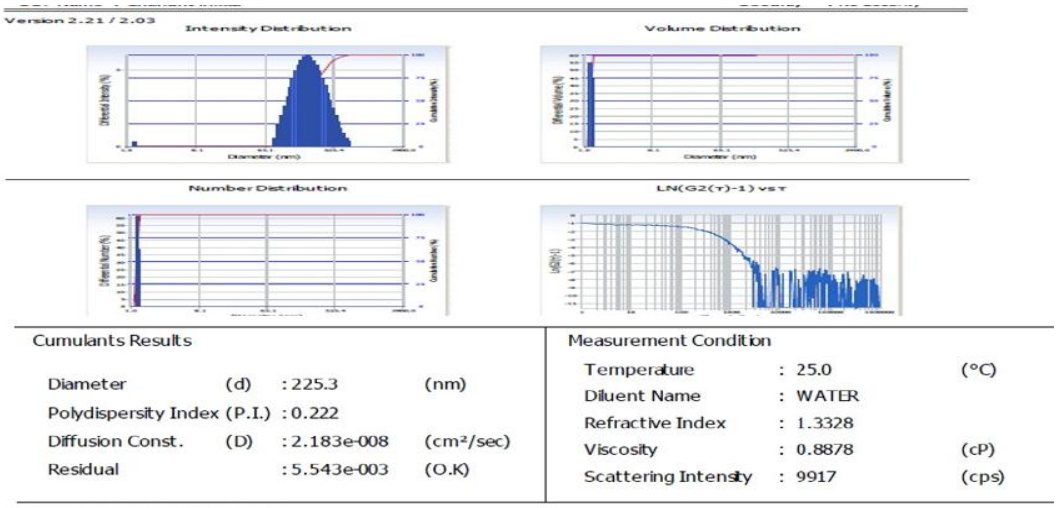


Figure 5.13 Size and polydispersity index of NLC (f25)

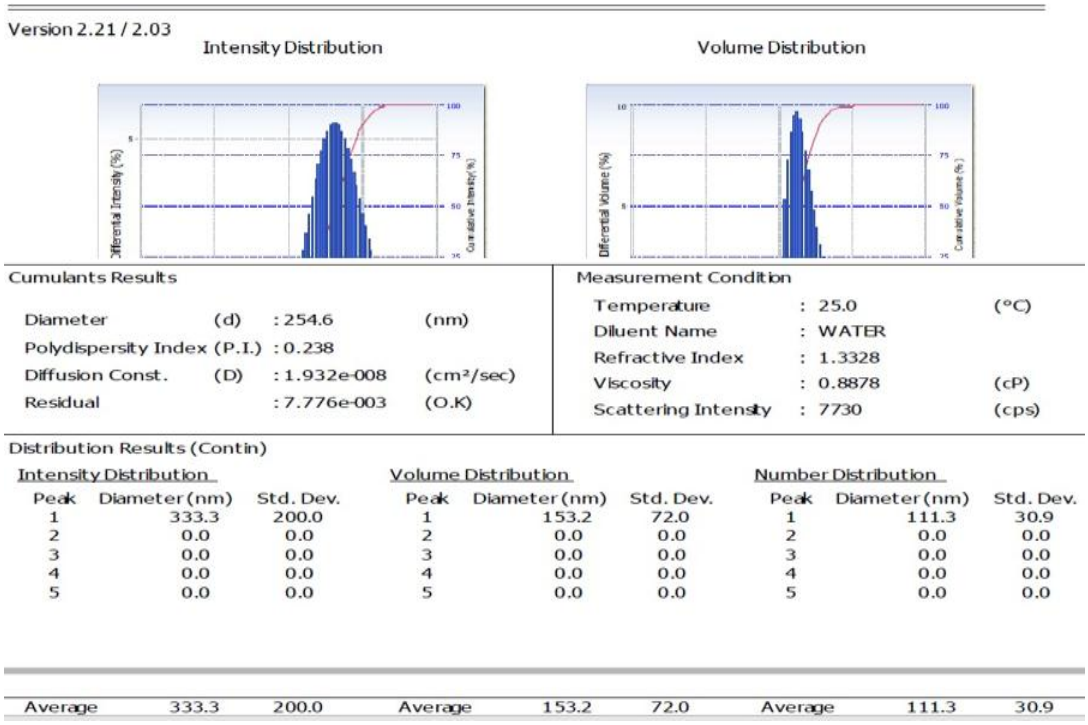


Figure 5.14 Size and polydispersity index of Drug loaded NLCs (f27)

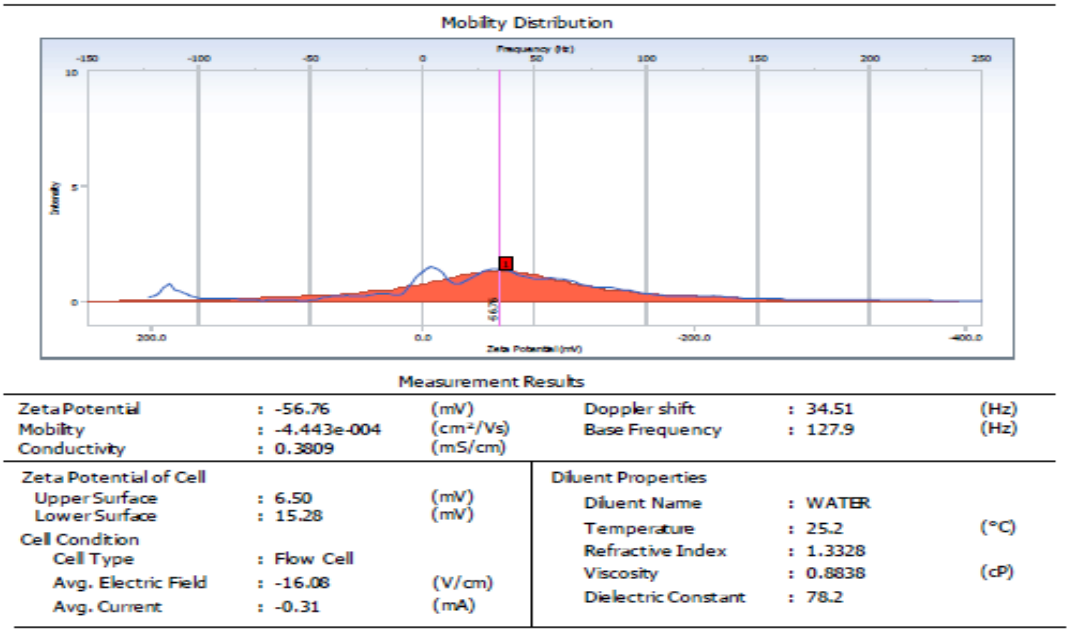


Figure 5.15 Zeta Potential of NCLs

5.2.2.3. Morphology

Shape and surface morphology of optimized formulation (F27) Motic microscopy and SEM was performed to study vesicle morphology that revealed that NLCs was spherical in shape.

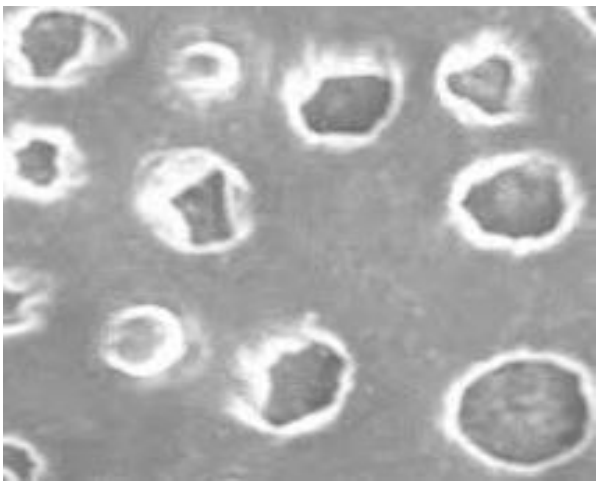
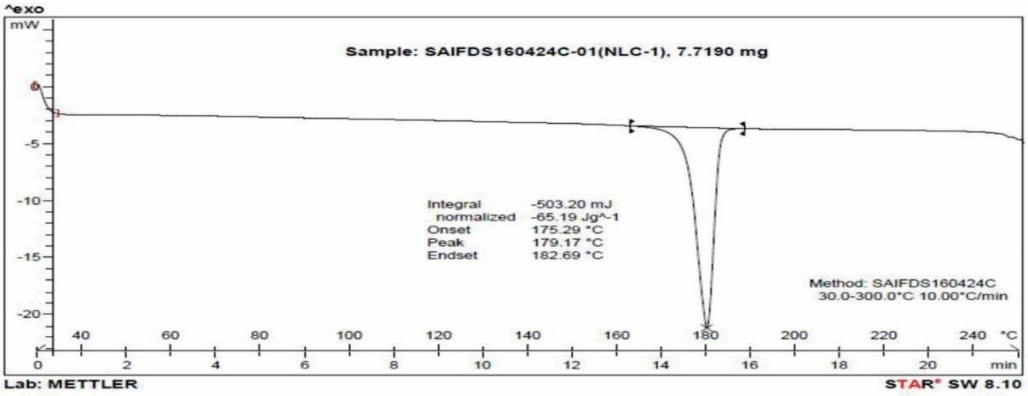


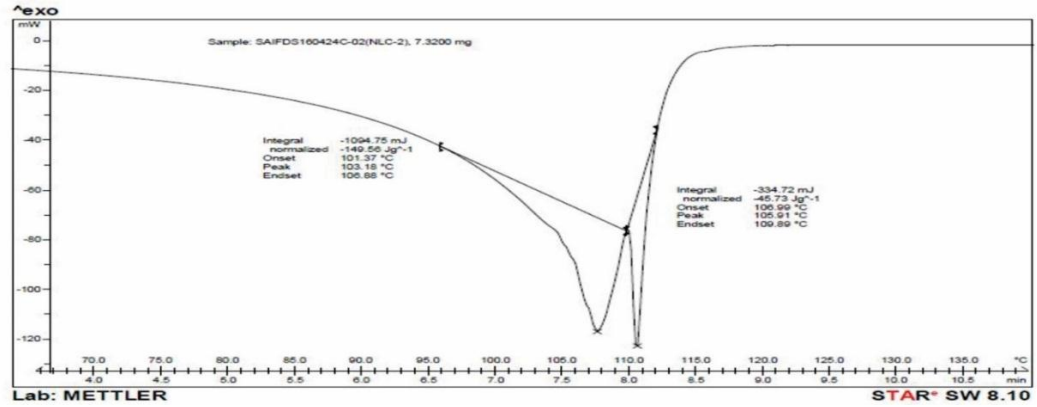
Figure 5.16 SEM image of optimized NLCs formulation

5.2.2.4. Differential Scanning Calorimetry

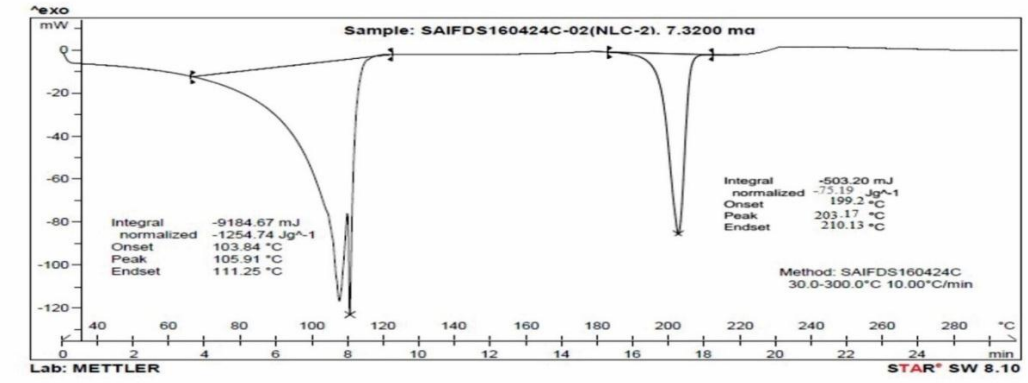
DSC was performed. It was found that the drug shows a thermo expansion peak at 179.17˚C while blank NLC shows a peak at 103.3˚C and 109.10˚C and drug-loaded NLCs show a peak at 105.91˚C and 203.17˚C, which can be seen in Figure. 5.17 (a, b,and c), respectively. The shift of drug peak might be due to the formation of chemical bond between drug and lipids used in the preparation of NLCs.



5.17 (a)



5.17 (b)



5.17 (c)

Figure 5.17 (a) DSC of pure drug (Clobetasol propionate) (b) DSC of blank NLC gel (c) DSC of clobetasol propionate loaded NLC gel.

5.2.2.5. Preparation and characterization of gel containing nanostructured lipid carrier

For the preparation of gel containing nanostructured lipid carrier system, Carbopol 940 was used as gelling agent. Out of six different concentrations of carbopol 0.25% (F28D) was chosen for the further study on the basis of pH, spreadability, viscosity, drug content and swelling index as depicted in Table 5.20. Prepared gel was transparent with a smooth and homogeneous appearance. NLCs were finally incorporated in the gel for the increasing adherence of formulation with skin as well as it will provide stability to the formulation. Table 5.20 depicts the optimization of gel on the basis of spreadability, viscosity, % drug content and pH; formulation F28 D was found to be optimized (n=3).

Table 5.20 Optimization of Gel

Form code	**Carbopol 940 (%w/v)**	**pH**	**Spreadability (gm.cm/sec)**	**Viscosity (Pa/s)**	**%Drug content**
F28A	0.10	2.81±0.2	22.22 ± 0.17	4.11	82.23±0.41
F28B	**0.15**	3.42±0.28	21.91 ± 0.21	4.76	84.10±0.61
F28C	0.20	4.51±0.31	20.81 ± 0.32	5.31	86.74±0.32
F28D	0.25	**5.49±0.43**	**20.22±0.17**	**5.50**	**87.23±0.22**
F28E	0.30	5.91±0.51	15.72 ± 0.73	5.65	88.88±0.46
F28F	0.35	6.17±0.60	14.77 ± 0.22	5.71	89.88±0.46

Hence, F28D is finally optimized NLCs loaded gel of clobetasol propionate on the basis of parameters like particle size, zeta potential, entrapment efficiency and drug loading which is depicted in Table 5.20 (n=3).

5.2.2.6. Texture analysis of gel

Texture analysis was performed via using Brookfield Texture Analyzer. Table 5.21 depicts that drug loaded NLC gel show spreadability at load 146 gas compare to blank NLC gel (145 g); it might be due to chemical bonding between drug loaded NLC system and gel.

Figure 5.18 (a) Texture analysis of NLCs loaded gel

Figure 5.18 (b) NLCs loaded gel of clobetasol propionate

Table 5.21 Texture analysis of plain gel and drug loaded NLCs loaded gel

Sr. No.	Parameters	Gel loaded blank NLCs	Drug loaded NLCs loaded gel
1	Hardness cycle (g)	145	146
2	Hardness work cycle (mJ)	1.34	1.38
3	Load at target (g)	145	146
4	Deformation at target (nm)	11.49	9.89

5.2.2.7. Rheological behaviour of optimized formulation (F28 D)

NLCs loaded gel of clobetasol propionate (F28 D) exhibited rheological behaviour at 25^0C as depicted in Figure 5.19. Gel follows Newtonian flow at 25^0C while below 37^0C gel shows pseudoplastic behaviour. Studies suggested that viscosity decreases with increase in shear rate hence flow property of gel increased comparatively.

5.2.2.8. *In-vitro* drug release studies

The release profiles for optimized gel formulation (F28 D), drug loaded NLCs (F28) and marketed gel (Topinate gel) was determined in triplicate. In vitro release is shown in Table 5.22 & graphically shown in Figure 5.20 (n=3). This study indicates that Topinate gel showed least release in 10 % methanolic PBS buffer solution i.e. 61.12±2.31 % followed by gel loaded NLC (F28D) (72.56%) and NLC formulation (F28) (81.25± 4.81%). NLCs system showed higher release might be because of small size (nano) of the particle. NLCs loaded gel (F28D) has shown 72.56±2.31% release after 24 hour. Hence, we have chosen F28D as final formulation as it shows intermediate release in 10 % methanolic PBS buffer solution and it was selected for further studies like skin irritation test.

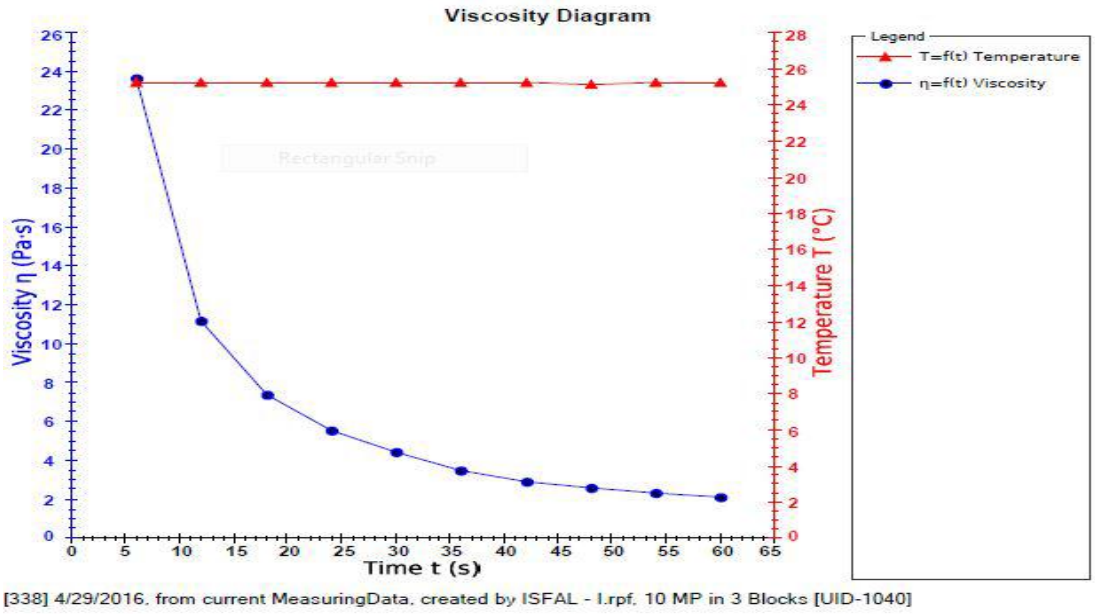


Figure 5.19 Rheological behaviour of optimized formulation (F28 D)

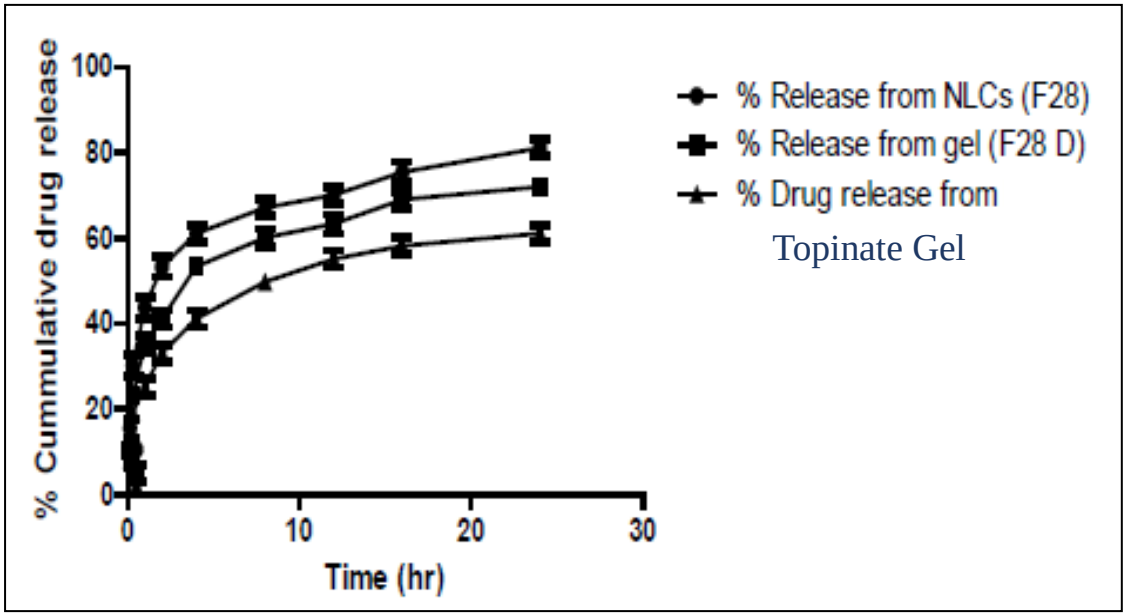


Figure 5.20 In-vitro drug release profile of Clobetasol propionate from NLCs formulation, NLCs loaded gel and Topinate gel (Marketed preparation)

Table 5.22 In vitro drug release in 10% methanolic PBS buffer at pH 5.5

Sr. No.	Time (hrs)	Drug loaded NLCs formulation (F28)	NLCs loaded gel (F28 D)	Topinate gel (Marketed)
1	0	10.23 ± 2.13%	5.21 ± 1.72%	2.11 ± 0.72%
2	15 min	15.51 ± 3.67%	10.11 ± 2.04%	8.41 ± 1.89%
3	30 min	30.67 ± 3.91 %	23.10 ± 2.55%	19.13 ± 2.32%
4	1 hr	44.11 ± 3.71%	35.20 ± 2.12%	25.17 ± 1.82%
5	2 hr	53.13 ± 4.13%	41.12± 2.14%	33.21 ± 1.96%
6	4 hr	61.63 ± 3.91%	53.64 ± 2.63%	41.16 ± 2.10%
7	8 hr	67.11 ± 4.11%	60.14 ± 3.01%	50.19 ± 2.42%
8	12 hr	70.11 ± 4.73%	63.04 ± 1.71%	55.16 ± 2.10%
9	16 hr	75.21 ± 3.79%	69.14 ± 2.32%	58.31 ± 3.71%
10	24 hr	81.25 ± 4.81%	72.56 ± 1.94%	61.12 ± 2.31%

5.2.3. Optimization of the Blank and Drug-Loaded deformable liposomes.

Table 5.23 Optimization on the basses of method of preparation:

a) Hand rotatory method:

Formulation code	Lipid ratio SPC:SC	Antioxidant	Vortex time	Size (nm)	PDI
D1	2:1	16µm	5min	--	--
D2	4:1	16µm	5min	4034.0	1.373
D3	6:1	16µm	5min	292.5	0.249
D4	8:1	16µm	5min	315.2	0.262
D5	6:2	16µm	5min	357.2	0.261
D6	6:4	16µm	5min	--	-

b) Thin film hydration method using Rota evaporator

Formulation code	Lipid ratio SPC:SC	Antioxidant	Vortex time	Size (nm)	PDI
D7	2:1	16µm	5min	--	--
D8	4:1	16µm	5min	368.8	0.261
D9	6:1	16µm	5min	219.5	0.28
D10	8:1	16µm	5min	313.0	0.30
D11	6:2	16µm	5min	630	0.32
D12	6:4	16µm	5min	--	--

Table 5.24 Optimization on the basis of vortex timing

Formulation code	Lipid ratio SPC:SC	Antioxidant	Vortex time	Size (nm)	PDI
D13	6:1	16µm	1 min	330.0	0.300
D14	6:1	16µm	5min	219.5	0.28
D15	6:1	16µm	10 min	313.5	0.276

Table 5.25 Optimization of drug loaded deformable liposomes

Formulation code	Drug conc. (%w/v)	Particle size (nm)	Zeta potential (mv)	PDI	% EE	%Drug loading
D16	0.05%	232.1 ±6.35	-30.65 ±6.21	0.169 ±0.02	72.9± 7.11%	52.2%

Finally, formulation (D16) (clobetasol propionate loaded Deformable liposomes) is a potent drug (dose 0.5mg through transdermal route) we have selected its concentration on the bases of studies reported by (Gordon, 1998), (Feldman, 2005) was found to be optimized having particle size of 232.1±6.35 nm, zeta potential -

30.65±6.21 mV, PDI 0.169±0.02, entrapment efficiency 72.9± 7.11%, drug loading 52.2%. final results are depicted in table 5.25 and figure 5.21 and 5.22.

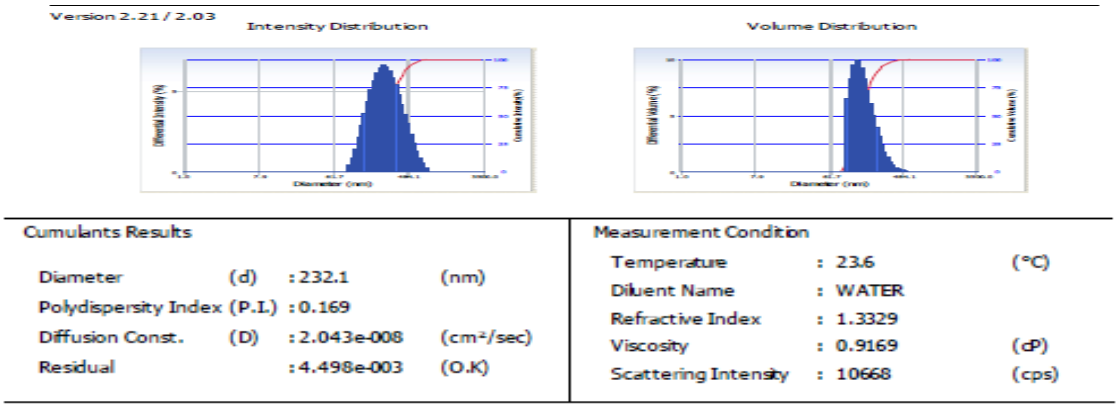


Figure 5.21 Size and polydispersity index of Drug loaded NLCs (D16)

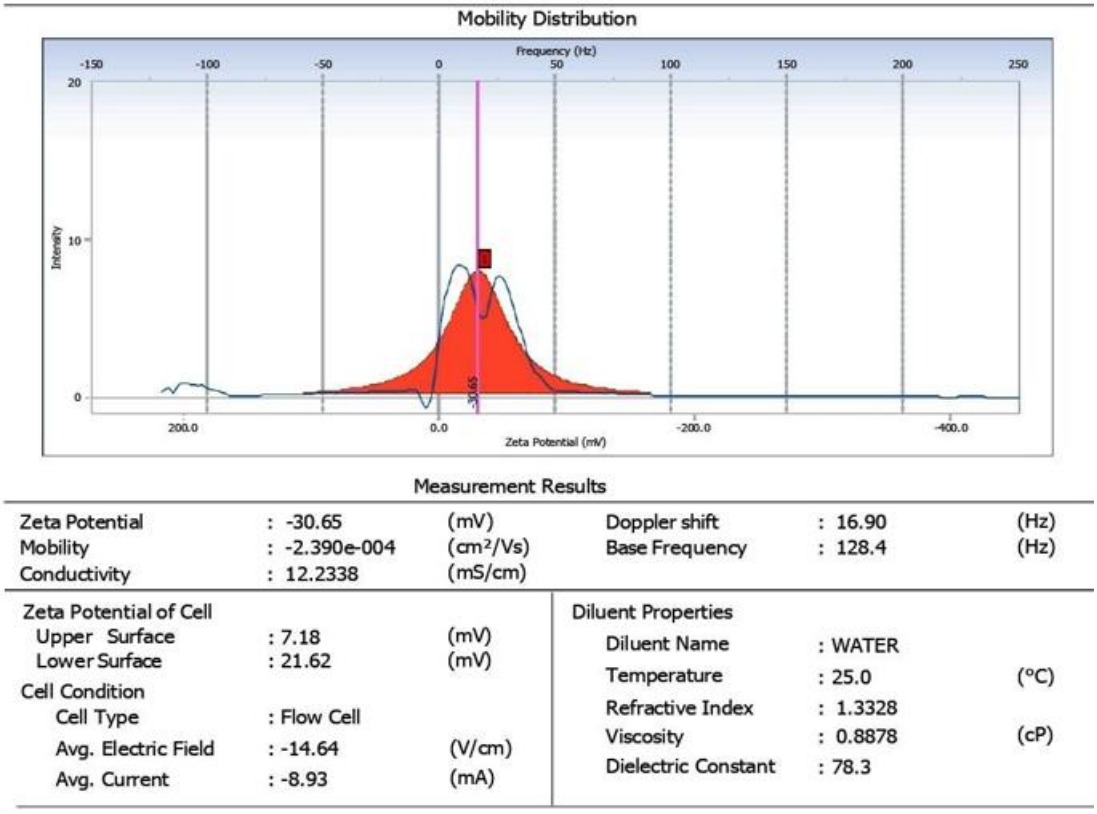


Figure 5.22 zeta potential of deformable liposomes.

5.2.3.1. Centrifugation Test

This test was performed in order to ensure the stability of the Deformable liposomes whether it is monophasic or not. In this, the optimized formulation was firstly diluted with 200ml of water and then centrifuged at 7500 rpm for 10 min. There was no sign of phase separation in the optimized formulation.

5.2.3.2. Morphology

Shape and surface morphology of optimized formulation (D16) SEM was performed to study vesicle morphology that revealed that Deformable liposome was spherical in shape as shown in figure 5.23.

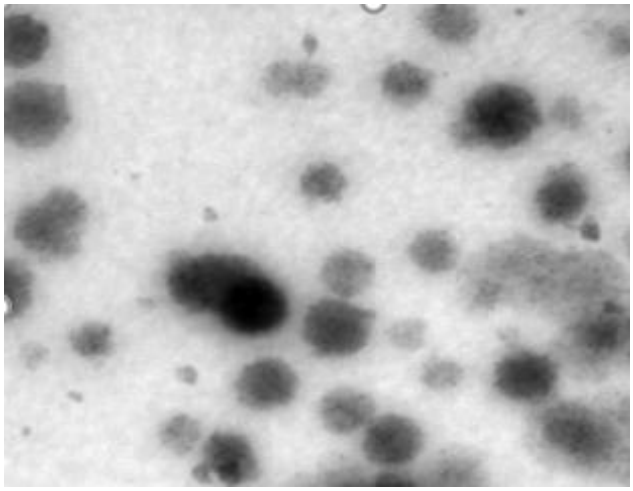


Figure 5.23 SEM image of optimized Deformable liposome formulation

5.2.3.3. Optimization and characterization of Gel

Deformable liposomes loaded gel were prepared in different batches by using different concentration of carbopol 940 w/v with formulation codes and optimized for the various parameters such as pH, spreadibility, % of carbopol and % drug content which are shown in Table 5.26 Deformable liposomes loaded gel prepared by using carbopol 940 was 0.25%w/v found to be optimized.

Table 5.26 Characterization of gel

Sr. No.	Carbopol 940 (w/v)	Ph	Spreadability (gm.cm/sec)	% Drug content
D16A	**0.25%**	**5.51±0.91**	**20.12±0.15**	**88.71±0.39**
D16B	0.3%	5.10±0.28	19.91±0.12	88.52±0.31
D16C	0.35%	5.30±0.91	19.41±0.31	88.67±0.42
D16D	0.4%	5.51±0.71	18.34±0.71	89.16±0.21
D16E	0.45%	5.78±0.07	15.24±0.88	89.14±0.11
D16F	0.5%	6.41±0.21	13.29±0.23	89.02±0.23

5.2.3.4. Rheology

Clobetasol propionate loaded Deformable liposomes gel exhibited rheological behaviour at 37^0C as depicted in Figure 5.24. Gel follows Newtonian flow at below 37^0C while at below 37^0C gel shows pseudoplastic behaviour. Studies suggested that viscosity decreases with increase in shear rate.

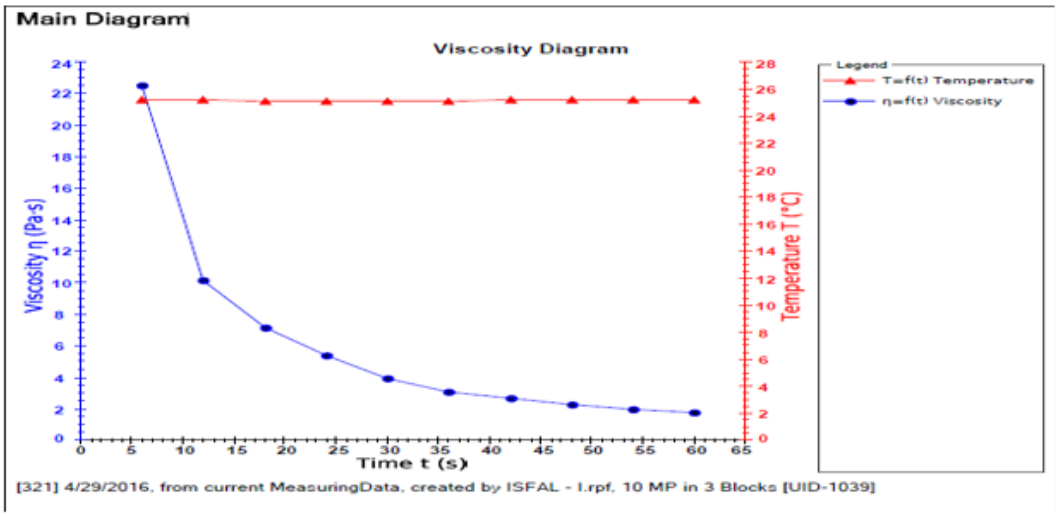


Figure 5.24 Rheology study of clobetasol propionate loaded deformable liposomes (D17)

5.2.3.5. Percentage *in vitro* release graph of clobetasol propionate in 10% methanolic PBS (pH 5.5)

The rate of drug release across the dialysis membrane was slower for Deformable liposomal gel than the Deformable liposome and was least for the marketed gel (topinate). The drug release from Deformable liposome in PBS (pH 5.5) was approximately 64.12 ±1.35% after 24hrs. The Deformable liposomal gel formulations showed release of 61.67± 2.05% while marketed gel showed release of 57.67±1.63% after 24 hrs. *In-vitro* release profile of different formulations is shown in Table 5.27 and Figure 5.25 showed the % cumulative release profile. The release profiles of clobetasol propionate from Deformable liposomal gel showed biphasic release processes, where initial burst release of the surface-adsorbed drug was observed, followed by slow diffusion from the lipid Deformable liposome. At the initial 4 hrs, the little higher drug release of Deformable liposomal gel was observed. Afterward, lipid Deformable liposome diffusion in gels played an important role in the release profiles and drug release rate slowed down.

Table 5.27 Percentage *in vitro* release data of clobetasol propionate in 10% methanolic PBS (pH 5.5)

Time (hrs.)	Deformable liposome	Topinate gel	(Deformable loaded gel)
0.00	0.00	0.00	0.00
0.15	10.87±2.05%	8.78±1.84%	9.44±2.02%
0.30	16.97±1.34%	14.57±1.63%	14.81±1.84%
2	28.12±2.05%	24.79±1.43%	26.37±2.05%
6	37.87±1.43%	34.67±2.05%	35.47±1.43%
12	49.21±2.02%	45.67±1.43%	47.52±1.35%
18	55.84±2.05%	51.32±2.05%	53.81±1.84%
24	64.12±1.35%	57.67±1.63%	61.67±2.05%

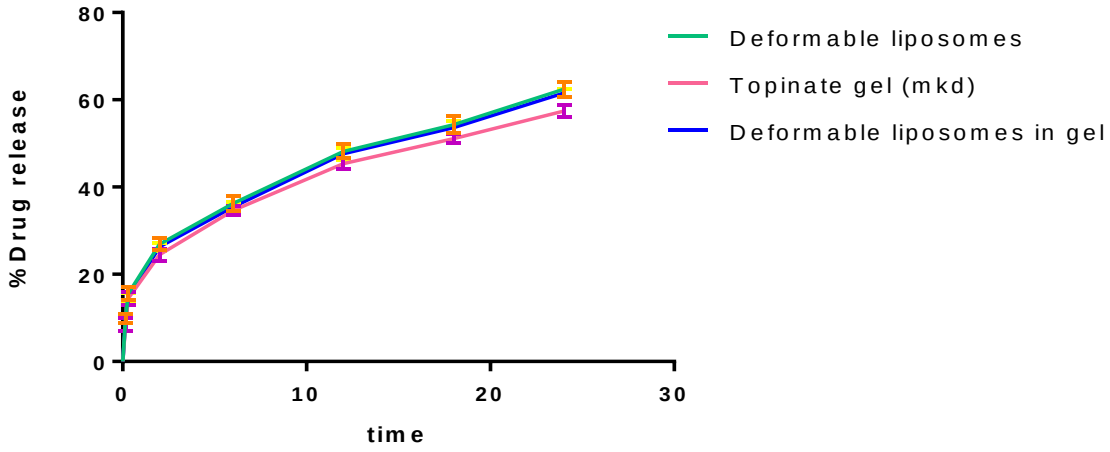


Figure 5.25 Percentage *in vitro* release of clobetasol propionate in 10% methanolic PBS (pH 5.5)

5.2.4. Ex-Vivo Studies on Rat Psoriatic Skin

5.2.4.1 Ex-Vivo Skin Permeation Studies of nanoemulgel

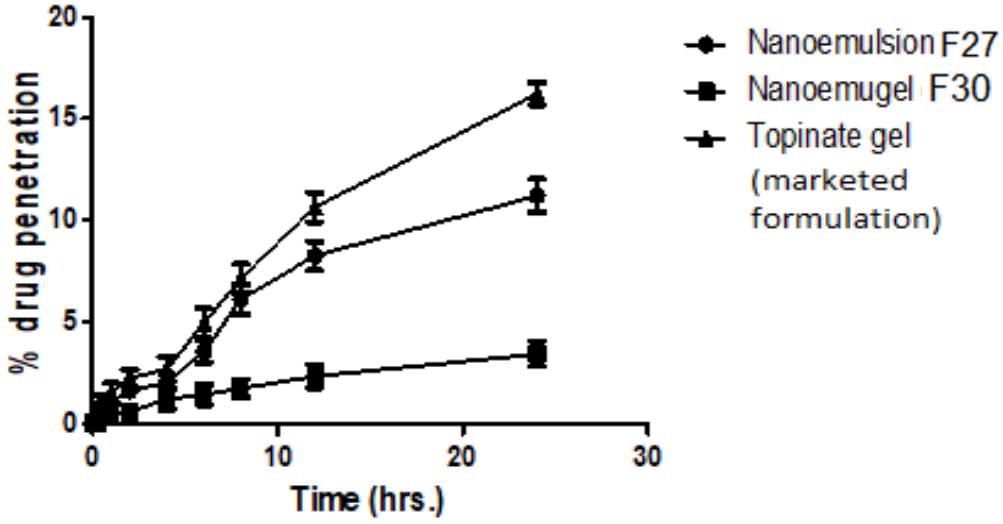


Figure. 5.26 Percentage drug penetration through psoriatic skin. Values expressed as mean ± SD (n = 3).

A Franz diffusion cell with an effective diffusion area of 1.00 cm^2 was used for the experiment. The rat abdomen skin was placed between the donor and receptor compartments of Franz diffusion cell with the stratum corneum facing donor compartment. Drug permeation profiles through rat skin, optimized gel formulation, and marketed ointment were taken as shown in Figure 5.26.

5.2.4.2 In- Vitro Skin Deposition Studies

Nanoemulgel not only acted as drug vectors but also acted as penetration enhancers. Penetration enhancement could because of surfactant contributing good deformation to nanoemulgel. Nanoemulsion owning better membrane deformability could penetrate the scaly keratinized psoriatic skin through the hydrophilic pathways and pore between the skin cells. But due to the presence of squalene the system will show an affinity towards sebaceous gland and very small part of it will reach in the blood and it shows depot effect. The cumulative amount of free drug content in the receptor compartment and the amount of drug remained on the percentage drug diffused across the rat skin was calculated at each sampling point and recorded. Amount of drug retained in the skin was calculated by subtracting the unabsorbed drug over the epidermal surface of the skin from the initial drug content of the formulation applied, The optimized gel of F30 showed better retention in the skin as compared to marketed gel. The cumulative percentage of drug retained in the skin of clobetasol propionate loaded nanoemulgel was 62 ±1.28% which was more than the marketed formulation (23.12 % ±0.54).

5.2.4.3 Drug Delivery via Sebum-Removed Skin

The difference of clobetasol propionate deposition between intact and de-sebumed skin from the marketed formulation (Topinate gel), nanoemulsion (F27) and nanoemulgel (F30) shown in (Figure 5.27). The result indicates that clobetasol propionate deposition in all formulations was lower in sebum-removed skin than in intact skin.

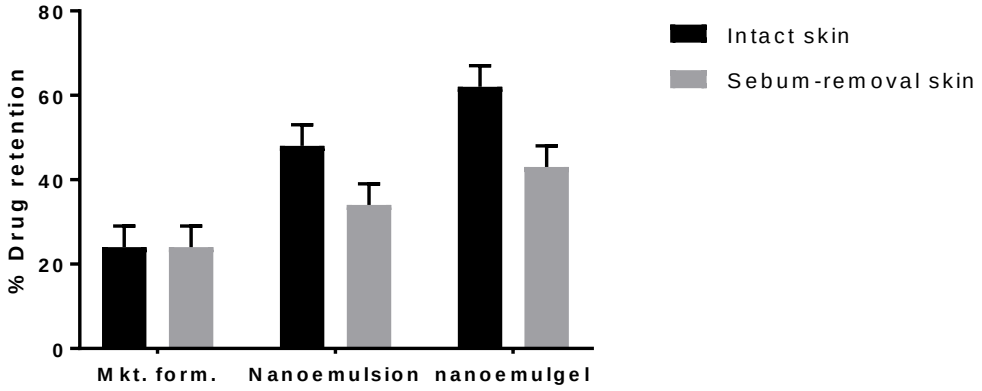


Figure 5.27 Comparison of the skin deposition of Clobetasol propionate in intact skin and sebum-removal skin after in vitro application of marketed and optimized formulations *p<0.05 compared to intact skin. All data represent the mean ± SD (n=3)

Nanoemulsion significantly enhanced the accumulation of drug into the skin layers. High drug deposition in case of nanoemulgel may be due to the formation of a drug reservoir in pilosebaceous gland due to deposition of nanoemulsion there, hence it is proved that the squelene use in the formulation has improved the depot action of the drug.

5.2.4.4. Ex-Vivo Skin Permeation Studies of NLCs (10% Methanolic PBS pH 5.5)

A percentage drug penetration study was performed in Franz diffusion cell with an effective diffusion area of 1.00 cm^2 was used for the experiment. Drug permeation profiles of nanostructured lipid carrier drug dispersion (F28), optimized gel formulation (F28D), and marketed gel (Topinate) was tested through rat psoriatic skin as shown in Figure 5.28 and it was found that loaded NLCs loaded gel of Clobetasol propionate (F28D) shows minimum drug penetration as compared to (F28) and Topinate gel. This study clearly reveals that NLC loaded gel (F28D) has shown the least penetration into keratinized psoriatic skin which indicates the depot effect of

formulation.

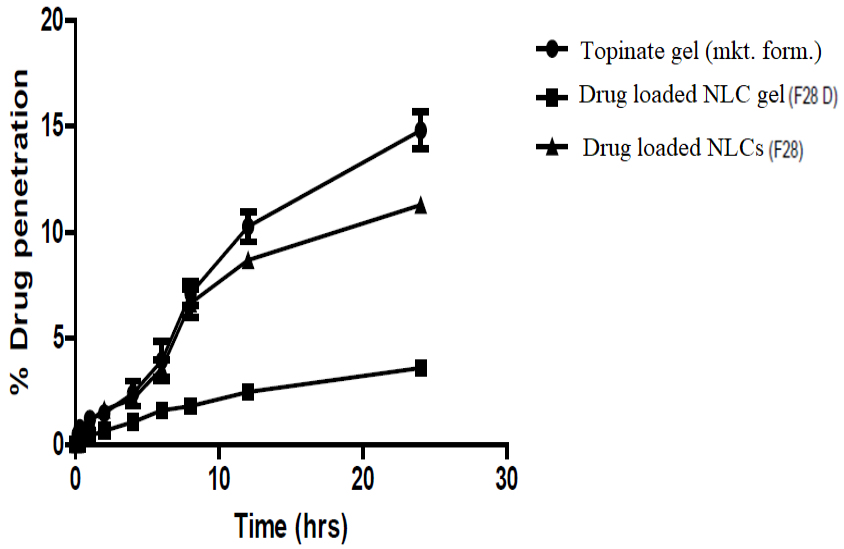


Figure 5.28 Percentage drug penetration of NLCs formulations through psoriatic skin. Values expressed as mean ± SD (n = 3).

5.2.4.5. Percentage Drug Retention Studies in 10% Methanolic PBS at pH 5.5

This study was performed by Franz diffusion cell used to compare the percentage of drug retention from different formulations. Drug retention profiles through rat skin, of nanostructured lipid carrier drug dispersion (F28), optimized gel formulation (F28D), and marketed gel (Topinate gel) were compared as shown in Figure 5.29 (n=3). It was observed (F28D) shows maximum drug retention i.e 61% as compared to (F28) and followed by Topinate gel (Marketed). The maximum drug retention might be due to presence of squalene and soy phosphatidyl choline in the nano lipid carrier system which provides depot effect at pilosebaceous gland as well as squalene, the main component of skin surface polyunsaturated lipids, shows some advantages for the skin as an emollient (Huang et al 2009).

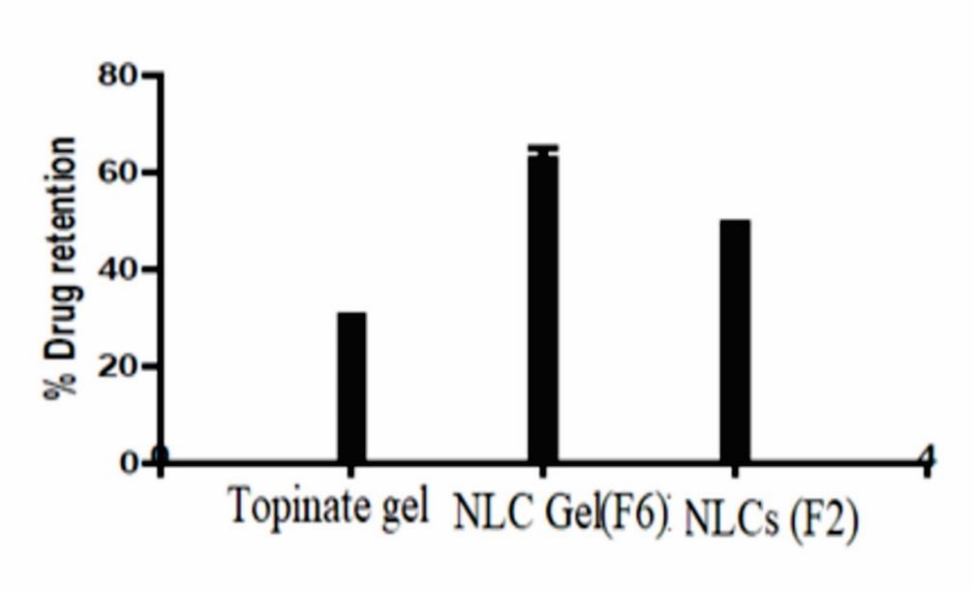


Figure 5.29 Drug retention profiles through rat skin, of nanostructured lipid carrier. Values expressed as mean ± SD (n = 3).

5.2.4.6. Drug Delivery via Sebum-Removed Skin

The difference of clobetasol propionate deposition between intact and desebumed skin from the marketed formulation (Topinate gel), nanostructured lipid carrier (NLCs) (F28) and NLCs loaded gel (F28D) shown in Figure 5.30. The result indicates that clobetasol propionate deposition in all formulations was lower in sebum-removed skin than in intact skin.

NLCs significantly enhanced the accumulation of drugs into the skin layers. High drug deposition in the case of NLCs loaded gel (F28D) may be due to the formation of a drug reservoir in the pilosebaceous gland due to the deposition of NLCs there, hence it is proved that the squalene use in the formulation has improved the depot action of the drug.

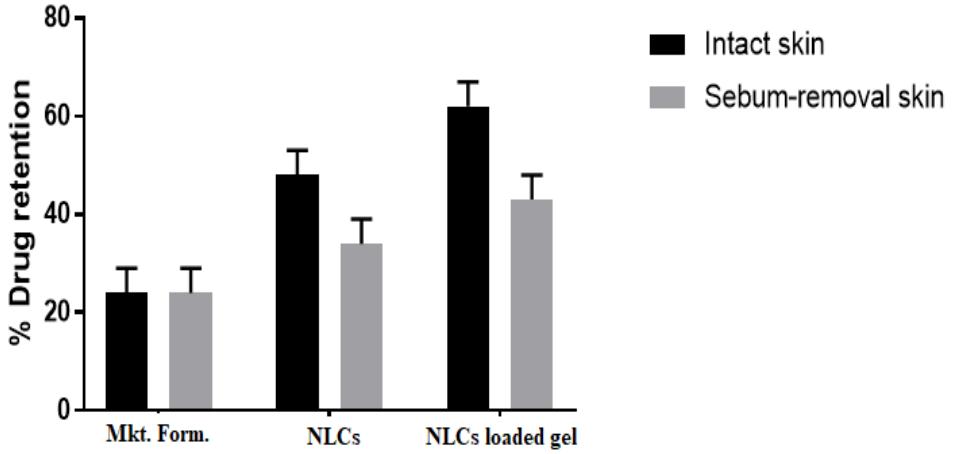


Figure 5.30 Comparison of the skin deposition of Clobetasol propionate in intact skin and sebum-removal skin after in vitro application of marketed and optimized NLCs formulations *p<0.05 compared to intact skin. All data represent the mean ± SD (n=3)

5.2.4.7. Percentage Drug Retention Studies of Deformable liposomes in 10% Methanolic PBS at pH 5.5

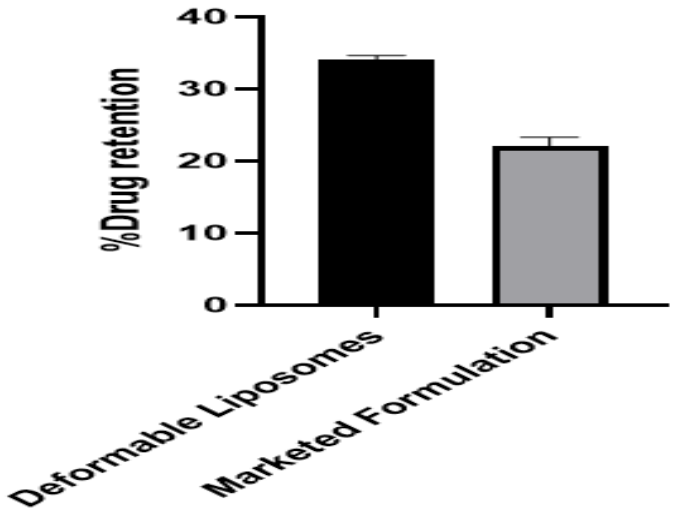


Figure 5.31 Drug retention profiles through rat skin, of Deformable liposomes. Values expressed as mean ± SD (n = 3).

This study was performed by Franz diffusion cell used to compare the percentage of drug retention from different formulations. Drug retention profiles through rat skin, of Deformable liposomes drug dispersion loaded gel (D16A), and marketed gel (Topinate gel) were compared as shown in Figure 5.31 (n=3). It was observed that (D16A) shows maximum drug retention as compared to Topinate gel (Marketed). Because of its flexible nature it will easily penetrate through psoriatic skin and then again regain its shape, so it shows better retention than marketed.

5.2.5. Physical stability studies

Formulations were found to be stable in terms of aggregation and fusion. Grittiness was not found and there is no change in the spreadibility of the formulation.

There was slight change in the size of the formulations as shown in Table 5.28. Results of the stability studies suggest that formulations in Carbopol gel under refrigerated conditions minimizes the stability problems of formulations.

Table 5.28 Physical stability studies of optimized formulations.

Storage condition	**Particle size (nm)**		**PDI**	
Temperature	Initial	After 3 months	Initial	After 3 months
$4^0C \pm 2^0C$ (Nanoemulsions)	170.5± 9.2	219.8 ±8.2	0.282 ±0.03	0.140± 0.02
$25^0C \pm 2^0C$ (Nanoemulsions)	170.5 ±9.2	232.1 ±9.1	0.282 ±0.03	0.169± 0.04
$4^0C \pm 2^0C$ (NLCs)	243 ±4.12	262 ±3.87	0.251 ±0.132	0.321 ±0.034
$25^0C \pm 2^0C$	243 ±4.12	278 ±3.11	0.251 ±0.132	0.289 ±0.037

(NLCs)				
4˚C±2°C (Deformable liposomes)	232.1±0.35	232.1±0.35	0.169±0.03	0.169±0.04
25˚C±2°C (Deformable liposomes)	232.1±0.35	413.0±0.35	0.169±0.03	0.276±0.04

5.2.6. Pharmacokinetic study

HPLC method was developed in rat plasma for estimation of clobetasol propionate. A linear response was obtained in the range of 1-10µg/mL with correlation coefficient r^2 = 0.9992 which proves that there is the least penetration of the drug in the blood circulation and our system has shown deposition in pilosebecious glands (Figure. 5.32 (a),(b) and Table 5.29, 5.30).

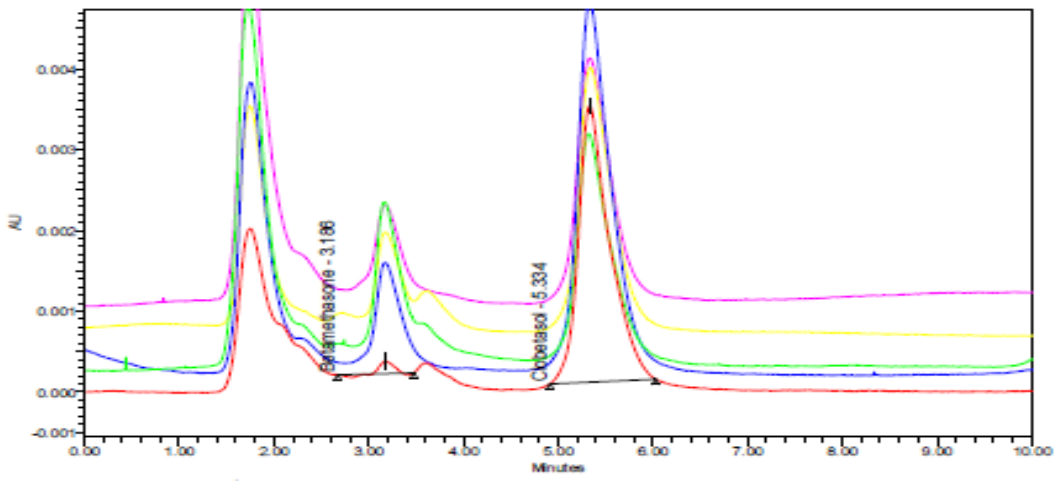
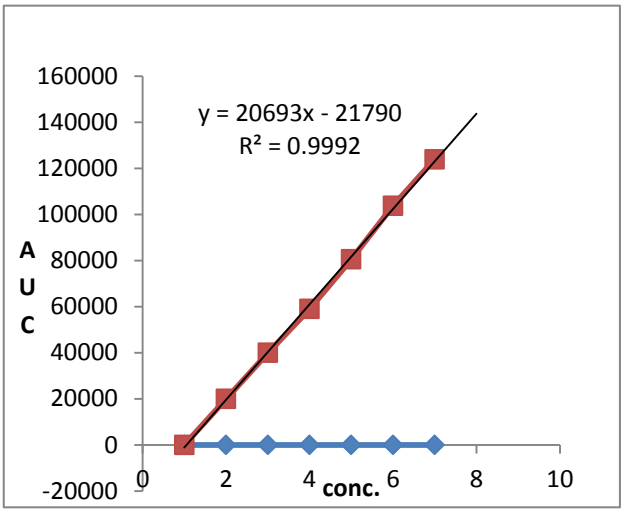


Figure 5.32 a) Overlay of clobetasol propionate

Table 5.29 Regressed data of clobetasol propionate through HPLC method

Conc. 1(µgm/ml)	AUC
1	20001
2	39935
4	58914
6	80535
8	103677
12	126819



b) Overlay of clobetasol propionate Skin irritation studies

Table 5.30 Biopharmacokinetic parameters by using HPLC method

Parameters	Test formulation (nanoemulgel)	Test formulation (NLC loaded gel)	Marketed formulation
T1/2(hours)	6.25 hrs	5.76 hrs	2.77 hrs.
Clearance	0.027 l/hr	0.067 l/hr	0.009 l/hr.
AUC (mcg hr/ml)	12.33 mcg hr/ml	11.56 mcg hr/ml	28.67 mcg-hr/ml
VD	244.029 ml	244.029 ml	36.59 ml
Tmax(hours)	12hr 25 min	13hr	12 hr
Cmax (mcg/ml)	1.932 mcg/ml	2.063 mcg/ml	9.925 mcg/ml

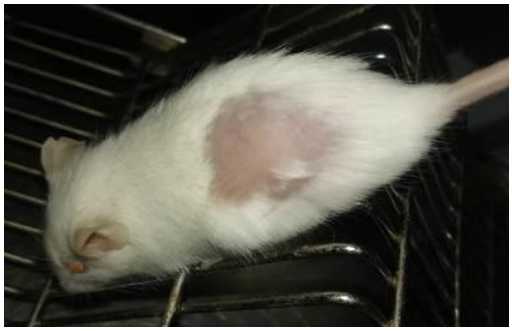
5.2.7. *In-vivo* studies

5.2.7.1 Skin irritation studies

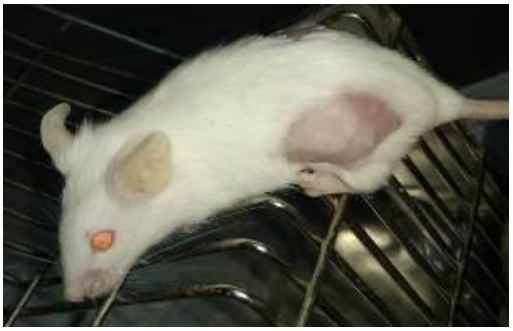
Nanoformulations were applied on dorsal rat abdominal skin and was occluded with gauze and covered with a nonsensitizing microporous tapes. The formulations were removed after 24 h and a score of erythema was recorded and compared with the standard. A score of erythema is recorded (Table 5.31). The total score obtained was zero indicating the absence of any sign of redness, irritation, erythema and scar formation after 24h of treatment with nanoemulgel, NLCs and deformable liposomes, all the animals survived. They appeared active and healthy. We also have taken care of pH of the formulation which is 5.51±0.91 near to pH of the skin. As formulation does not show any sign of redness, irritation, erythema, and scar so formulation is safe to use topically as shown in figure 5.33.

Table 5.31 Skin irritation evaluation score

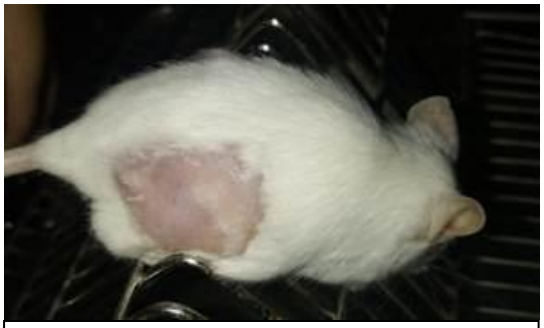
SKIN RESPONSES	**TIME (HRS)**	**SCORE**		
Erythema and Scar Formation		Nanoemulgel	NLCs loaded gel	DLLoaded gel
	1	0	0	0
	24	0	0	0
Edema formation	1	0	0	0
	24	0	0	0



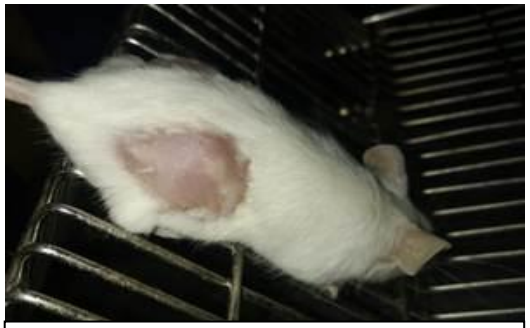
a) Before application of nanoemulgel



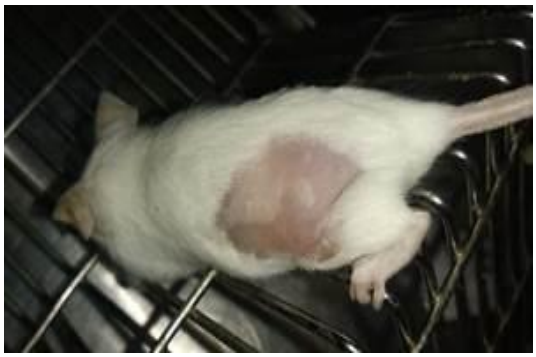
a) After application of nanoemulgel



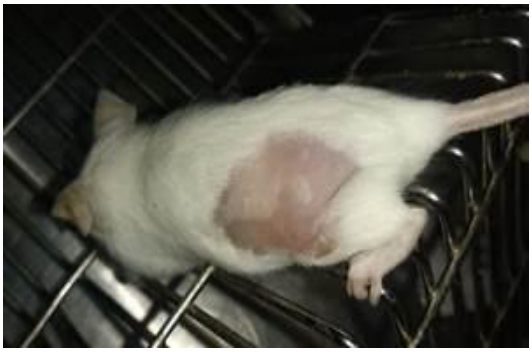
b) Before application of deformable liposomal loaded gel



b) After application of deformable liposomal loaded gel



c) Before application of NLC loaded gel



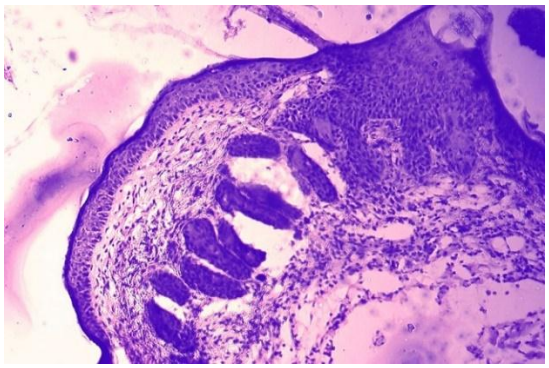
c) After application of NLC loaded gel

Figure 5.33 Skin irritation studies

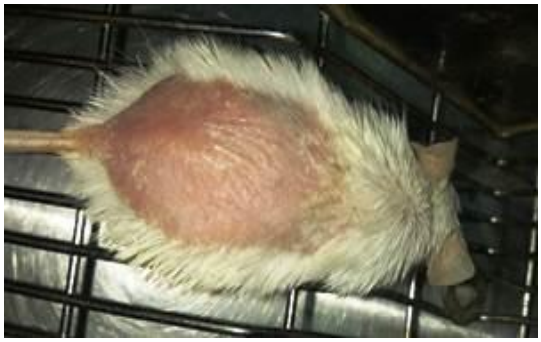
5.2.7.2. Histopathology

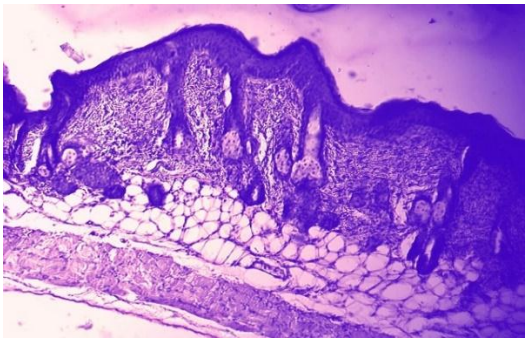
As per experiment conducted within control, diseased standard (marketed), and test formulations histopathological studies had shown control group had shown keratinized stratified squamous epithelium with normal subepidermal tissue, Diseased group had shown parakeratosis and mild acanthosis in subepidermal tissue moderate inflammatory infiltrate composed of lymphocytes, few neutrophils and histiocytes seen and no atypia noted, standard group had shown keratinized thin stratified squamous epithelium with moderate inflammatory infiltrate composed of lymphocytes and histiocytes and no parakeratosis/ acanthosis seen, clobetasol treated test groups had shown keratinized stratified squamous epithelium with mild inflammatory infiltrate composed of lymphocytes and histiocytes and no parakeratosis/ acanthosis seen. Changes in epidermal layer in various groups had shown that test formulation nanoemulgel is better as compared to other test formulation were as NCL loaded gel has also shown better effect from deformable liposomes, all the test formulations have shown better result than marketed formulation (Figure 5.34).

CONTROLED GROUP:

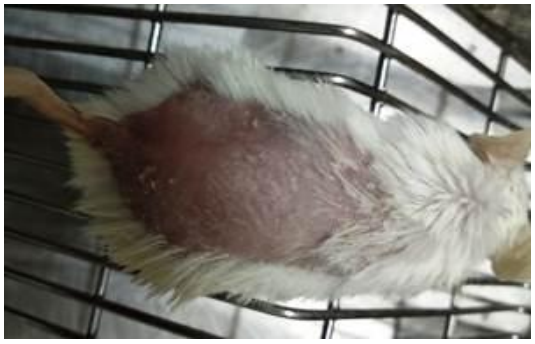


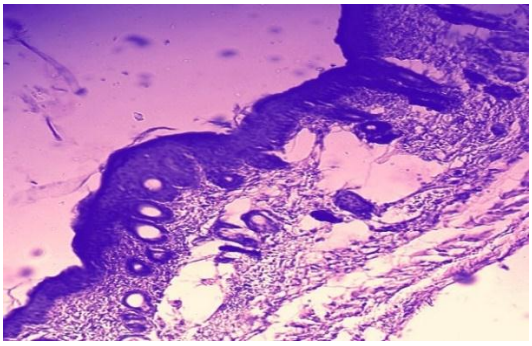
DISEASED CONTROL:



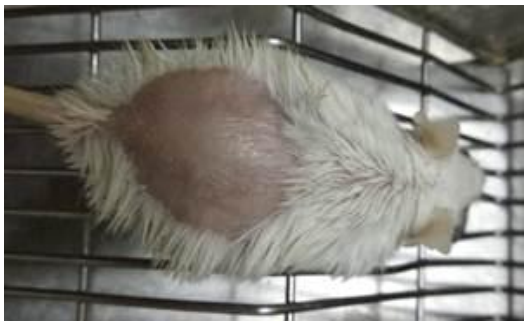
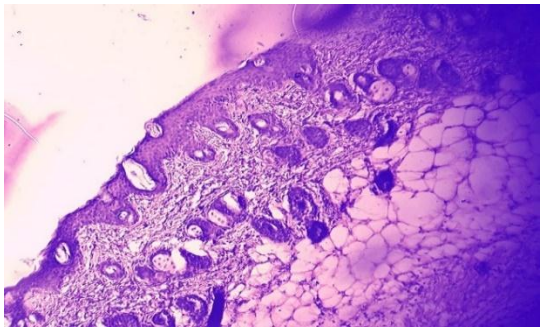


MARKETED GROUP:

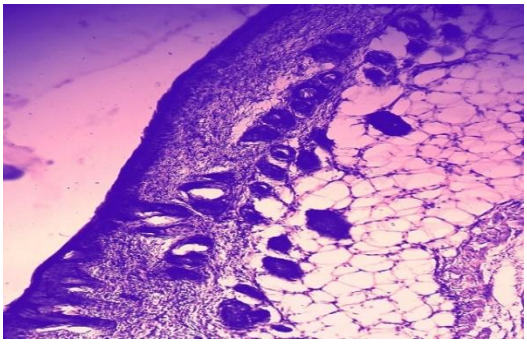




TEST FORMULAION (CLOBETASOL PROPIONATE LOADED NANOEMULGEL)

TEST FORMULAION (CLOBETASOL PROPIONATE LOADED NLCs GEL)

TEST FORMULAION (CLOBETASOL PROPIONATE LOADED DEFORMABLE LIPOSOME GEL)

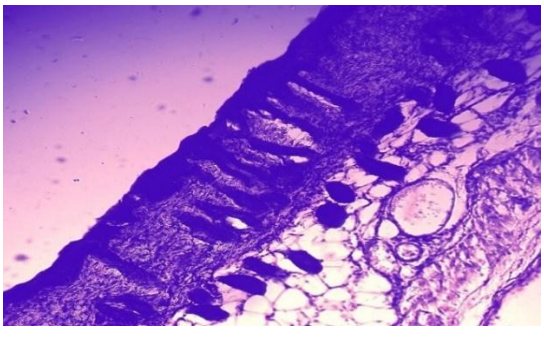



Figure 5.34 Histopathology studies