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DESIGN, DEVELOPMENT AND EVALUATION OF NOVEL NANOEMULGEL FOR TOPICAL DRUG DELIVERY SYSTEM

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Abstract

Objective: The present study is aimed to design, development of nanoemulgel and evaluate for topical drug delivery system. Nanoemulgel prove to be promising carrier for intense amount of poorly penetrable drugs to produce their effect topically. **Methods:** FTIR-study was performed to determine compatibility of drug with excipients. Various oil-in-water nanoemulsions are prepared by the homogenization method. Prepared nanoemulsions were characterized for its particle size, polydispersity index, SEM, TEM, pH, drug content, and In-Vitro drug diffusion study, etc. Based on its In-Vitro drug diffusion study optimized formulation showed higher release compared to other formulations. Incorporating the optimized nanoemulsion with gel base to produce nanoemulgel and characterize for homogeneity, pH, viscosity, spreadability, In-Vitro drug diffusion study, stability study, and antifungal study. **Results:** The FTIR-study confirmed that there was no interaction between drug and excipients. The optimized nanoemulsion (NF3) exhibited particle size 208.6nm with PDI index 0.648, drug content 90.12% and in-vitro release of 87.95% at 12 hr. The prepared nanoemulgel was homogenous, transparent yellow with good spreadability 68.5, viscosity 52500, pH 6.5. The drug release at 8 hr was 72.01%. **Conclusion:** Hence, it can be concluded that nanoemulgel of luliconazole can provide better antifungal activity and improve patient compliance.

Keywords: Luliconazole, Nanoemulgel, Gel Base, In Vitro Drug Diffusion, Stability Study

1. INTRODUCTION

Recently, nanoemulgel have emerged as the more attractive topical drug delivery system (TDDS) and it has dual release control system, one is the nanoemulsion and second is Hydrogel. Nanoemulsions being nano-sized droplets (10-100 nm), it rapidly penetrates and deliver active substances deeply and quickly. Nanoemulgel is the addition of the nanoemulsion system incorporated into gel matrix which enhances the better skin permeation. Nanoemulgel when comes in contact with skin, it discharges the oil beads from the nanoemulgel and these oil beads enter into the Stratum Corneum of the skin and discharge the medication at proposed site. Nanoemulgel has good encapsulation with high solubilizing of the API in the oil phase; nanoemulgel bring out more focused tendency towards the skin that further improves permeation of drug via skin barrier.

Luliconazole is in a class of antifungal medications called azoles. It works by slowing the growth of fungi that cause infection. Luliconazole is used to treat tinea pedis (athlete's foot; fungal infection of the skin on the feet and between the toes), tinea cruris (jock itch; fungal infection of the skin in the groin or buttocks), and tinea corporis (ringworm; fungal skin infection that causes a red scaly rash on different parts of the body). However, luliconazole suffers from drawbacks

such as lesser skin retention, low aqueous solubility and poor skin penetration because it comes under BCS Class II. Hence the study is aimed to develop luliconazole containing nanoemulgel and evaluate for topical drug delivery system.

2. MATERIALS

Luliconazole was obtained as gift sample from Akums Drugs and Pharmaceuticals Ltd Delhi, India. Labrafac were procured from Gattfose. All the other chemicals used were of analytical grade.

3. METHODS

Preformulation study:

Estimation of Luliconazole by UV-Spectroscopy:

Luliconazole 1000mcg/ml solution was prepared using PBS 7.4. From this standard dilution 1 ml was withdrawn and diluted upto 100 ml to make 10 mcg/ml of strength. Using this stock solution working dilutions of strength 0.2, 0.4, 0.6, 0.8, 1mcg/ml solutions were prepared and analysed on UV. Calibration curves were then constructed to plot linearity.

Drug – excipients compatibility study:

Drug alone and physical mixture of excipients with drug were introduced to near infrared region of 4000 cm^{-1} to 400 cm^{-1} using Perkin elmer FTIR spectroscopy. FTIR Spectrum obtained for drug and drug-excipient's physical mixture taken in 1:1 ratio was analyzed for production of any unwanted interactions.

X-Ray diffraction analysis

XRD was performed to analyse drug structural arrangement. It is used to identify amorphous or crystalline nature of API.

Preparation of nanoemulsion by spontaneous homogenization method:

Depending on the phase diagrams, Luliconazole loaded nanoemulsion were formulated at the different constituent's ratio by the spontaneous homogenization method. Appropriate quantities of oil Labrafac, tween 80 surfactant and PEG 400 co-surfactant were weighed and mixed well. Luliconazole was precisely weighed to prepare 1% w/w of the total weight of the nanoemulsion formulation, and then added to the previous mixture and stirred with a homogenizer (5000 rpm), at room temperature (25°C+0.5) until the drug is entirely dissolved. The weighed amount of water then added drop wise to the oil phase with continuous mixing stirring for 30 min.

Table 1: Formulation of nanoemulsion

Formulation	S:CoS (S mix)	Oil:S mix	% w/w of components in Nanoemulsion formulation			Drug % w/w
			Oil	S mix	Water	
NF1	1:1	1:4	5	20	75	1
NF2	1:1.5	1:5	5	25	70	1
NF3	1:2	1:6	5	30	65	1
NF4	1:2.5	1:7	5	35	60	1
NF5	1:3	1:8	5	40	55	1

Formulation of luliconazole loaded nanoemulsion gel:

Based on evaluations optimized nanoemulsion was transfer to gel form using Carbopol 940 in various concentration and analyse for different evaluation.

Nanoemulsion base gel was prepared by dispersing the weight amount of the Carbopol-940 in a sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24 hrs for complete swelling of Carbopol-940. The Carbopol dispersion was mixed with optimized formulations containing 1 gm of luliconazole. The mixture was stirred well to get homogeneous solution so that concentration of Carbopol 940 will become 0.5% w/w. The appropriate amount of triethanolamine was added to maintain the pH with continuous stirring to get homogeneous gel.

Table 2: Formulation of nanoemulgel

Formula code	Nano Emulsion (ml)	Carbopol 940 (gm)	Water (ml)	Methyl Paraben (ml)	Glycerin (ml)	Triethanol Amine (ml)
NEG1	50	1	50	0.1	5	Q. S.
NEG2	50	1.5	50	0.1	5	Q. S.
NEG3	50	2	50	0.1	5	Q. S.
NEG4	50	2.5	50	0.1	5	Q. S.

Evaluation of luliconazole loaded nanoemulsions:

Thermodynamic stability:

The developed formulations are subjected to undergo thermodynamic stability tests by 3 different stability cycles:

Freeze thaw cycle

Developed nanoemulsions were freezed by keeping at -20°C for 24 hr followed by at RT for 24 hr. The nanoemulsions regain their original form within 2-3 min were considered as stable.

Centrifugation studies

In that nanoemulsions were subjected to centrifugation at 5,000 rpm for 30 min. by Remi centrifuge. Those formulations that do not show phase separation are stable.

Heating cooling cycle

Heating at 40°C and sudden cooling to 4°C is performed on nanoemulsions for 48 hr. Formulations which were stable at this temperature, subjected to further study.

Measurement of pH:

10 ml of formulation was introduced to digital pH meter to determine pH.

% Drug content:

Nanoemulsion (1 ml) was dissolved in pH 7.4 phosphate buffer. After filtering the solution it is diluted and is analysed by UV spectroscopy. Drug content is calculated by using the equation obtained from linear regression analysis of calibration curve.

In vitro Diffusion studies:

5ml Nanoemulsion sample was taken in donar compartment and the diffusion are carried out at 37±0.5°C using 250 ml of phosphate buffer pH 7.4 as the dissolution medium. 5 ml of each sample was withdrawn periodically at 1, 2, 3, 4, 5, 6, 7 and 8 hrs and each sample is replaced with equal volume of fresh dissolution medium in order to maintain sink conditions. Samples are analysed on UV- spectrophotometer to calculate % drug diffused. The diffusion studies of the developed nanoemulsions were determined by making diffusion cell assembly with the aid of cellophane membrane.

X-Ray diffraction analysis

Optimized formulation was introduced to XRD to analyse formulation's structural arrangement. It is used to identify amorphous or crystalline nature of formulation.

SEM:

To identify the surface morphology of formulation; spanning electron microscopy was performed on optimized batch.

Zeta potential

Optimized formulation of nanoemulsion was tested for zeta potential using Malvern Zetasizer instrument. Zeta potential was used to assess potential stability of colloidal particles. The analysis was carried out at 25°C.

Particle size & Particle size distribution:

The particle size & particle size distribution of the Optimized formulation was measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer particle size analyzer.

Evaluation of nanoemulgel:

Appearance:

The appearance and clarity of nanoemulgel was observed visually.

pH:

The 5% nanoemulgel was prepared using PBS 7.4 subjected for pH determination by digital pH meter.

% Drug content:

Nanoemulgel (2 gm of sample) were taken in 100 ml flask mixed with 10ml ethanol and stirred on magnetic stirrer for 5 minutes. The solutions were filtered and analyzed on UV (Shimadzu) to calculate % drug content in nanoemulgel.

Determination of viscosity:

Nanoemulgel (20 gm of sample) was introduced to the viscosity determination by Brookfield viscometer using spindle no. 6 at RPM 50.

Spreadability:

Spreadability of nanoemulgel was determined by sandwiching 5 gm sample in between two glass slides and applying weight to the upper slide. A shorter interval indicates better spreadability, which is calculated by the formulae:

$$S=M.L/T$$

In vitro drug diffusion study:

Gel sample (0.5g) was taken in cellophane membrane and the diffusion studies were carried out at 37±1°C using 250 ml of phosphate buffer (pH 7.4) as the dissolution medium. 5ml of each sample was withdrawn periodically at 1, 2, 3, 4, 5, 6, 7, and 8 hrs and each sample was replaced with equal volume of fresh dissolution medium in order to maintain sink condition. Samples were analysed by UV-visible spectrophotometer at 425 nm for drug content. The diffusion studies of the prepared nanoemulgel were carried out in Franz diffusion cell through a cellophane membrane.

Stability study:

Optimized nanoemulgel is subjected to a short term accelerated stability study at 40±2°C, 75±5%RH as per ICH Guidelines. After stability period formulation was evaluated for any physical changes, drug content and in vitro drug diffusion.

4. RESULTS AND DISCUSSION

Estimation of Luliconazole by UV-Spectroscopy:

The UV spectrum of Luliconazole in buffer solution pH 7.4 in the range of 400-800nm. The spectrum indicates that the absorption ʎ max of luliconazole was found to be 297 nm. The linearity was found with the equation $y = 0.061x - 0.002$ and $R^2 = 0.997$. Absorbances and standard curve were mentioned below.

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Table 3: Standard calibration curve of Luliconazole

Conc (µg/ml)	Absorbance (λmax)
0.2	0.109
0.4	0.255
0.6	0.376
0.8	0.488
1	0.609

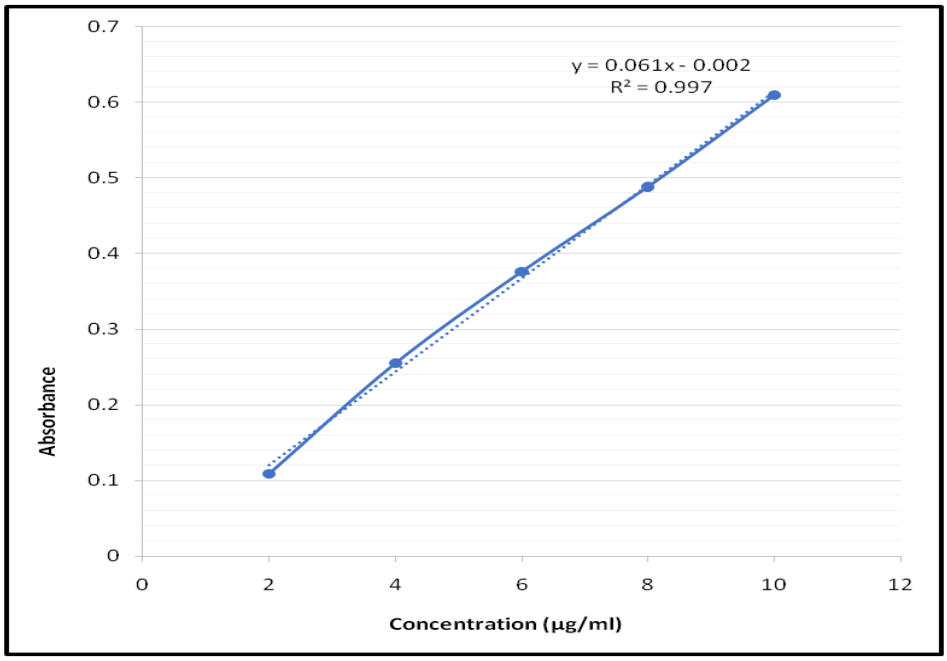

Figure 1: Standard calibration curve of Luliconazole

Identification of drug by FTIR spectroscopy:

The IR spectrum of the pure luliconazole as well as drug + excipients mixture was recorded by FTIR spectrophotometer. The frequencies of the functional group of luliconazole were found in the reported range which indicates that the obtained sample was of luliconazole and was pure. It was observed that there were no any peak deletion and creation in the drug's chemical structure and it can be concluded that drug is compatible with all other excipients.

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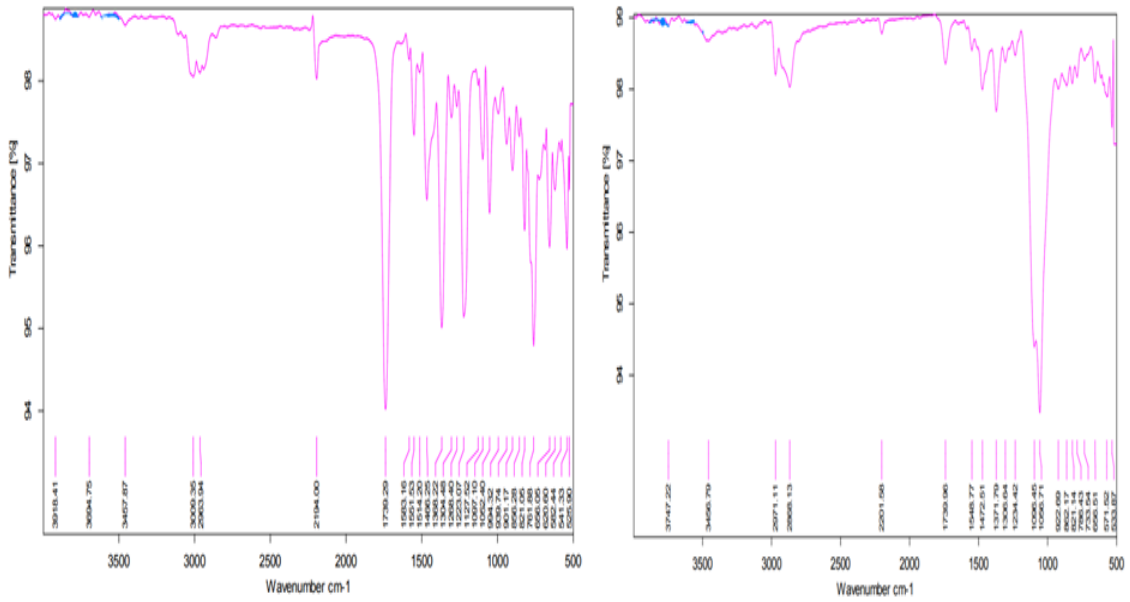

Figure 2: FTIR of Luliconazole & FTIR of physical mixture of drug + exceipients

Table 4: FTIR Intepretations

Functional group	Standard frequencies cm-1	Observed frequencies cm-1
C-H aromatic stretch	3000-3100	3033, 3015
C-H aliphatic stretch	2840-2950	2898
S-H stretch	2550-2600	2562, 2521
CN stretch	2100-2400	2195
C=C stretching	1650-2000	1738, 1815, 1867
C=N stretching	1600-1700	1690
C-Cl Stretching	600-800	763
Aromatic C=C	1450-1650	1470, 1514, 1584

X-Ray diffraction analysis

The diffraction pattern of drug showed that it is highly crystalline in nature as indicated by its numerous distinctive peaks, with major characteristic diffraction appearing a diffraction angle of 2θ at 15.69, 17.58, 20.65, 21.10, 22.65, 23.81, 25.01, 26.28, 27.24, 29.11, 31.26, 35.06.

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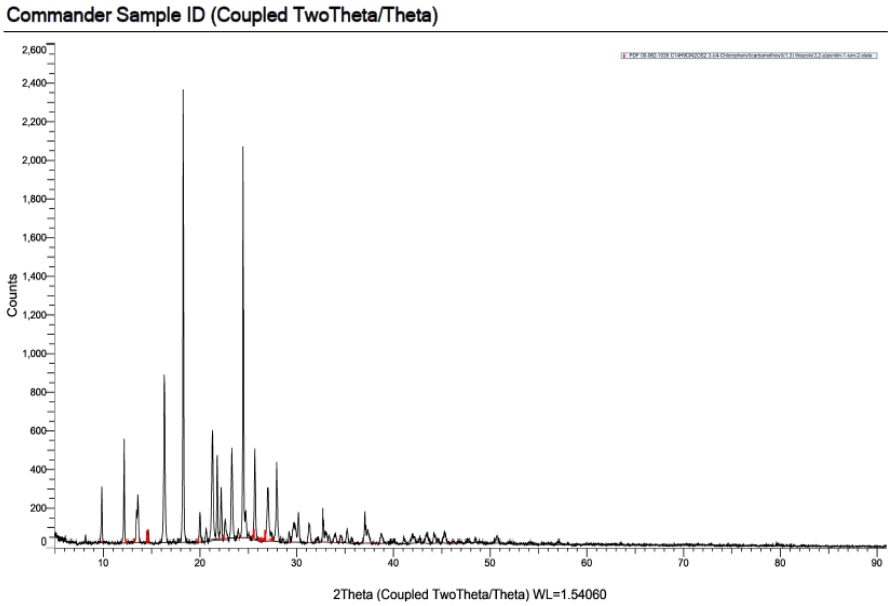

Figure 3: XRD of Luliconazole

Evaluations results of Nanoemulsion:

Thermodynamic stability:

All the batches have passed the thermodynamic stability study.

Appearance, pH, and % Drug content:

All the batches were found to be milky white dispersion in appearance. However NF3 batch showed highest drug content as 90.12%. pH of all batches was found within the range of 5.2 – 5.7. From the results of drug content batch NF3 batch was considered as optimized batch.

Table 5: pH and drug content of all formulations

Batch	pH	Drug content (%)
NF1	5.2±0.3	78.55
NF2	5.7±0.3	83.87
NF3	5.3±0.2	90.12
NF4	5.2±0.3	82.48
NF5	5.5±0.3	76.37

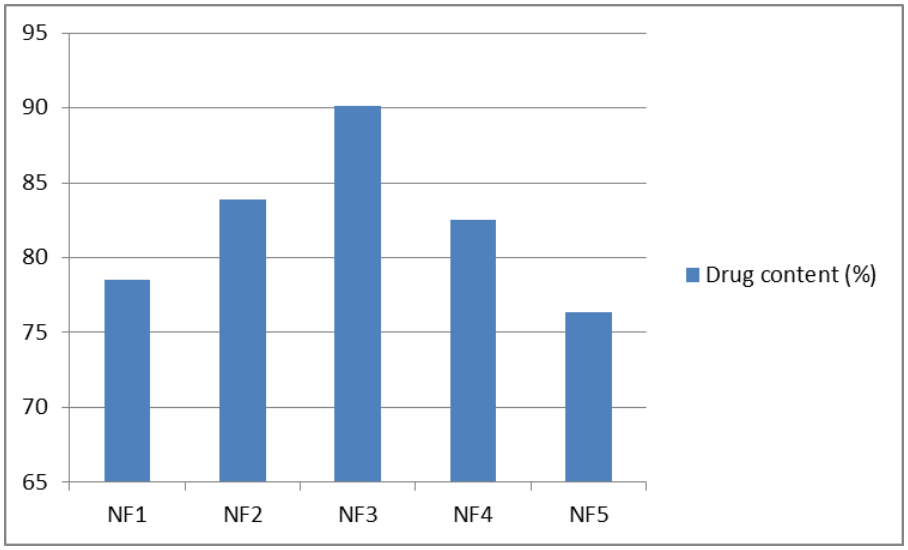

Figure 4: %Drug content of NE

% In vitro Drug Diffusion:

All the prepared batches show good drug diffusion. The drug release of the nanoemulsions shown in below table. The percentage drug diffusion at 12 hrs is maximum in F3 batch among all the batches i.e. 87.95%. From these results it is considered to be best optimized one.

Table 6: % In vitro Drug Diffused

Time (hr)	% In vitro Drug Diffused				
	NF1	NF2	NF3	NF4	NF5
0	0.0	0.0	0.0	0.0	0.0
1	10.17	12.08	9.64	13.12	11.98
2	14.40	16.05	11.07	17.49	18.58
3	21.80	21.98	16.86	22.93	25.07
4	27.40	23.75	22.09	24.98	30.98
5	30.68	36.03	29.95	39.40	37.74
6	37.20	40.73	36.08	43.73	43.85
7	40.6	49.21	43.67	49.98	51.90
8	48.37	60.68	46.10	63.68	56.39
9	55.97	68.96	54.53	69.20	63.83
10	68.58	73.68	62.12	76.70	74.61
11	71.37	76.92	76.75	79.90	80.28
12	72.60	80.90	87.95	83.20	73.04

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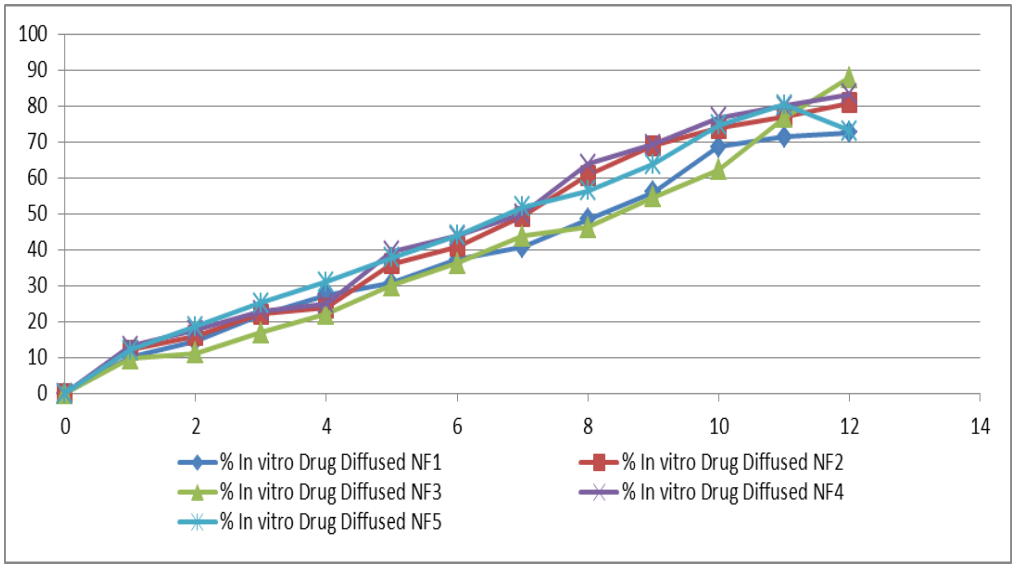

Figure 5: % In vitro Drug Diffused

SEM:

A SEM images of microscopic evaluation of optimized formulation was found to be spherical shape of droplets as shown below figure. Results shown that formulation was found to be in nano sized range of 150-200nm.

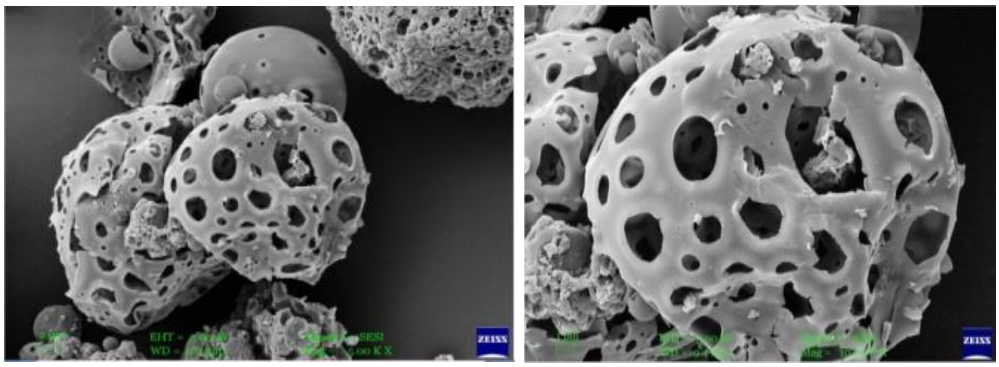

Figure 6: SEM images of optimized nanoemulsion formulation

X-Ray diffraction analysis of optimized formulation:

The diffraction pattern of optimized formulation showed that it is highly amorphous as compared to pure API's XRD. Form the XRD it was observed that luliconazole was found crysatalline in nature

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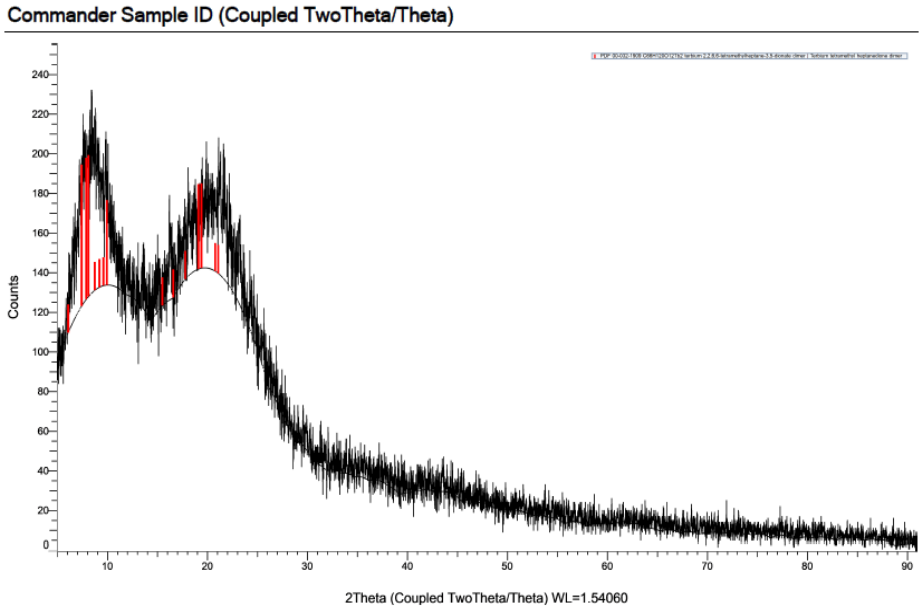

Figure 7: XRD image of optimized nanoemulsion formulation

Zeta Potential:

The surface charge on optimized batch was determined by Zeta potential. It was found to be –45mV. Values in the range of -50mv to +50mv of either charge characterize stable formulations.

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-45.0 mV	-0.000348 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -45.0 mV
Electrophoretic Mobility Mean : -0.000348 cm^2/Vs

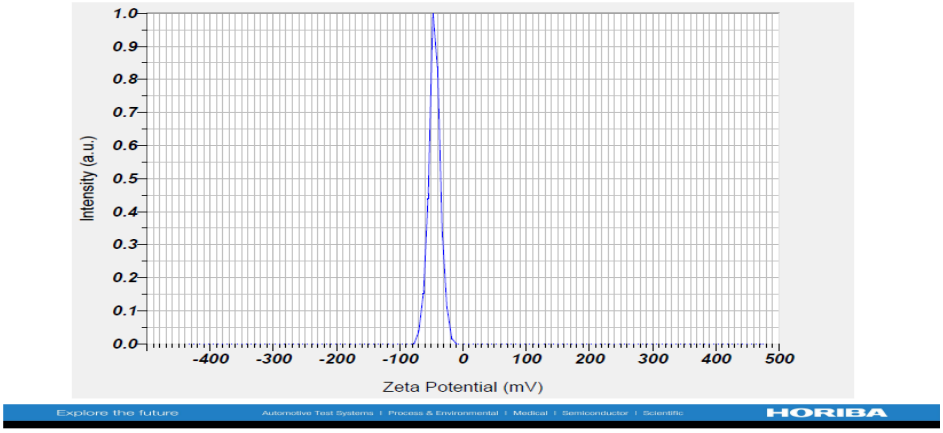

Figure 8: Zeta Potential

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Particle size & Particle size distribution:

The particle size & particle size distribution of the Optimized formulation was measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer particle size analyzer. Results obtained can conclude that, average particle size of formulation is 208.6nm with PDI index 0.648.

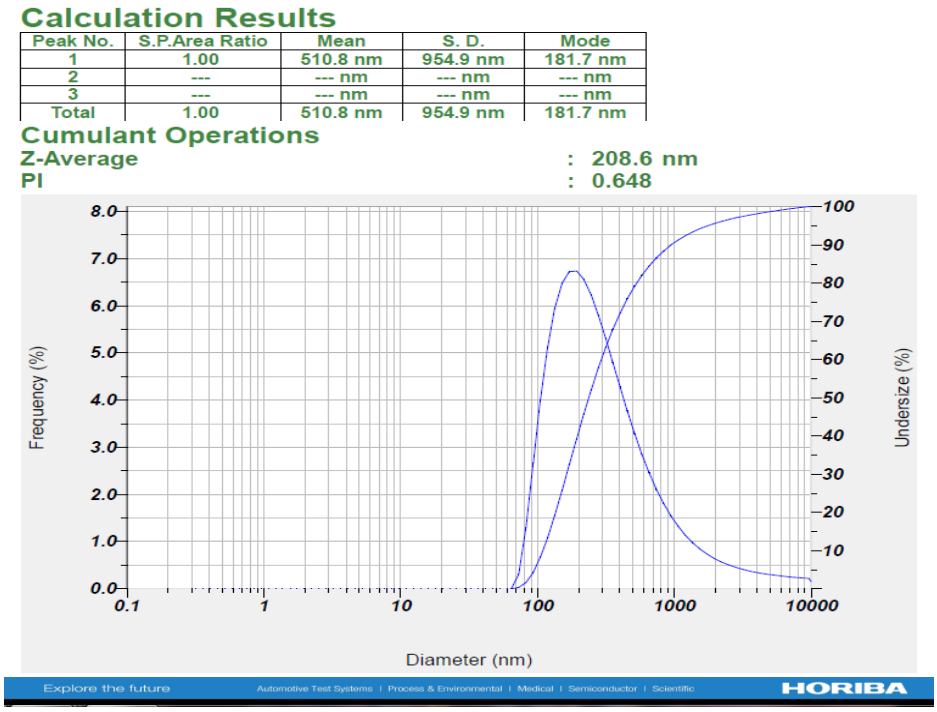

Figure 9: Particle size & Particle size distribution

Evaluation of nanoemulgel:

All the batches of nanoemulgel shows transperant yellow appearance with homogeneous consistancy. Having pH in the range of 6.3-6.5. Spreadability was found to be in 40.84 – 68.50 & viscosity 43400 – 65300. % Drug content was found to be 78.88 – 89.23. NEG 3 batch was optimized with highest drug conent.

Table 7: Evaluation parameter of nanoemulgel

Formulation Code	Viscosity (cps)	pH	% Drug content	Spreadability (gms.cm/sec)
NEG1	43400	6.5	78.88	57.4
NEG2	46567	6.4	87.90	40.84
NEG3	52500	6.5	89.23	68.5
NEG4	65300	6.3	80.45	59.68

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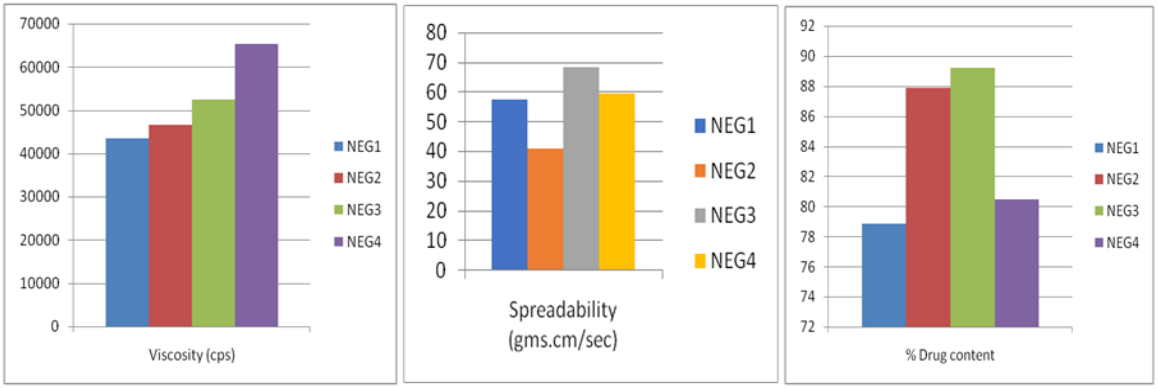

Figure 10: Viscosity , Spreadability &% Drug content

7.10 *In vitro* diffusion studies:

All the prepared batches show good drug diffusion. The drug release of the nanoemulgel batches shown in below table. The percentage drug release at 8 hrs is maximum of NEG3 among all the batches i.e. 91.93%. From these it is considered to be best optimized one.

Table 8: % In vitro drug diffusion study

Time in (hrs)	NEG1	NEG2	NEG3	NEG4
0	0	0	0	0
1	6.54±0.3	10.70±0.3	11.32±0.2	9.79±0.4
2	15.68±0.2	14.50±0.1	16.54±0.5	14.77±0.3
3	20.89±0.3	19.86±0.2	23.89±0.4	21.66±0.2
4	39.75±0.5	40.69±0.3	42.62±0.2	38.43±0.4
5	50.51±0.2	53.39±0.5	57.53±0.3	54.12±0.2
6	61.72±0.1	60.55±0.3	65.23±0.4	60.05±0.4
7	79.25±03	71.15±0.2	86.57±0.1	75.17±0.3
8	81.37±0.2	83.93±0.1	91.93±0.3	85.63±0.1

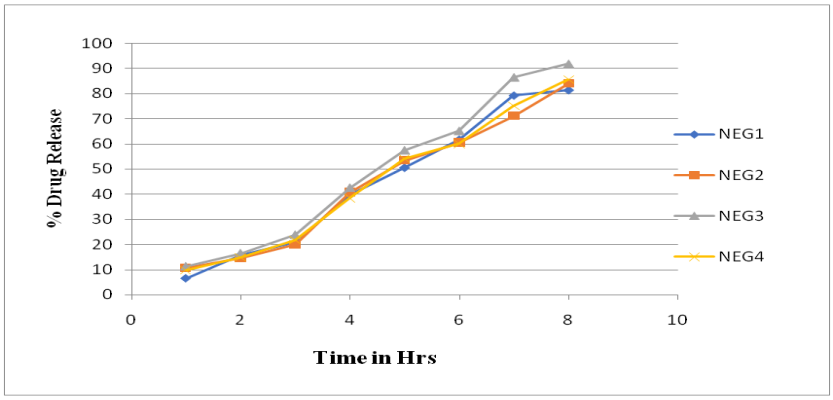

Figure 11: *In Vitro* Drug Diffusion

7.11 Stability studies:

The accelerated stability study of nanoemulgel batch 3 was carried out; there is no significant change in pH, drug content and % drug diffusion in NEG3 formulation at 40°C temperature and 75% RH. The result is shown in table below.

Table 9: Stability Study of NE gel

Parameters	Initial	1 Month	2 Month	3 Month
Appearance	Transparent yellow	NC	NC	NC
Viscosity	52500	NC	NC	NC
pH	6.5	NC	NC	NC
Drug content	89.23	88.55	87.02	86.32
***In vitro* drug diffusion**	92.93±0.2	92.33±0.2	90.57±0.3	89.87±0.1

NC= No Change

5. CONCLUSION

Luliconazole loaded nanoemulgel was prepared by the homogenization technique. All the formulations were evaluated for pH, drug content, viscosity, spreadability, drug diffusion study and accelerated stability study and results were within the limits. Among all optimized formulation of nanoemulsion gel i.e., NEG3 showed better drug content, In-vitro drug diffusion (as compared to conventional gel) and good anti-inflammatory, anti-oxidant, anti-bacterial activity. The stability studies carried out for three months and the formulation was found to be stable. It can be concluded that Luliconazole nanoemulgel can be better alternative for conventional topical gel and effectively used for the treatment of fungal infections.

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