FORMULATION AND EVALUATION OF NANOEMULGEL FOR TOPICAL APPLICATION CONTAINING ANTIFUNGAL DRUG

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Abstract:
Objective: *The aim of this study is to formulate and evaluate nanoemulgel for topical application. From all other topical drug delivery systems, nanoemulgel systems are attractive due to enhanced skin permeation potential and minimum toxicity. Nanoemulgels contain both gelling agents and nanoemulsion and are able to enhance skin permeation with sustained effects at the site of application.*
Methods: *Drug-excipients compatibility study was performed by FTIR. At first nanoemulsions are formulated by the homogenization method. Nanoemulsions preparations were characterized for its particle size, polydispersity index, SEM, TEM, pH, drug content, and In-Vitro drug diffusion study, etc. Based on results obtained by in-vitro drug diffusion study optimized batch was converted to nanoemulgel using gelling agents. Further nanoemulgels are evaluated for appearance, pH, spreadability, viscosity, in-vitro drug diffusion study, stability study, and antifungal study.*
Results: *FTIR study concluded that drug is compatible with all the excipients. Among all nanoemulsion NF2 exhibited; better drug content 81.12% and in-vitro release of 82.62% at 12 hr and was optimized. The prepared nanoemulgel was homogenous, transparent yellow with high % drug content 88.23, spreadability 65.50, viscosity 18829, pH 6.5. The drug release at 8 hr was 74.61%.*
Conclusion: *Research can conclude that luliconazole loaded nanoemulgel can be best alternative to conventional dosage system as it provides better antifungal activity and improved patient compliance.*

Keywords: *Luliconazole, Nanoemulgel, Gel base, in vitro drug diffusion, stability study*

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INTRODUCTION

Topical drug delivery is a better non-invasive route of administration with confined drug delivery to skin which leads to lower systemic absorption and adverse reactions. Transdermal delivery of therapeutic drugs would be considered for sustained delivery approaches and skin permeation enhancement which can raise patient compliance in dosage form consumption.
Number of nanocarriers such as lipid nanoparticles, liposomes, niosomes, nanoemulsions, hydrogels, nanoemulgels, etc. has been projected for topical delivery with variety of benefits and lacunas that should be conquered. Among all aforementioned-nanocarriers, nanoemulgels are extremely favourable and have the benefits of both nanoemulsions and gels which could enhance skin permeation and provide longer drug deposition in the target site.
Topical gels have the limited ability in proficient delivery of hydrophobic drug while nanoemulgels may overcome this limitation, for instance, highly lipophilic drugs such as luliconazole and luliconazole were encapsulated within the nanoemulsion to enhance transdermal delivery. Further, the occurance of gelling agents in nanoemulgels, with suitable pharmaceutical characteristics including good spreadability, skin softening, and moisturizing properties, not only can raise patient compliance, but also develop pleasing formulation appearance, sustained release pattern, and drug protection against irratic in vivo environment.

Luliconazole is in a class of medications called azole antifungals. It works by declining the growth of fungi that cause infection. Luliconazole is used to prevent serious fungal infections that can spread throughout the body in adults and children's 2 years of age and older with a weakened ability to fight infection. However, Luliconazole show drawbacks of poor skin permeation due to less aqueous solubility as it comes under BCS Class II. Hence the present study is focused on formulation and evaluation of Luliconazole containing nanoemulgel for topical application.

Materials

Luliconazole sample obtained from Akums Drugs and Pharmaceuticals Ltd Delhi, India. Oleic acid from Hi Media Laboratories, Tween 80 from Lobachem, Mumbai, PEG 400 from Thermo fisher scientific India pvt.ltd., Carbopol 934 from Lobachem, Mumbai, HPMC K15 from Meherchemi, Mumbai, Methyl Paraben from Ozone international, Glycerine from Vikash pharma, Triethanolamine from Molychem Thane, Mumbai.

Methods

Preformulation study

Estimation of Luliconazole by UV-Spectroscopy

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

Drug – excipients compatibility study

Drug and excipients were scanned in near infrared range of 4000 cm -1 to 400 cm -1 using Perkin elmer FTIR spectroscopy. Spectrum obtained for drug alone and in combination with excipient's mixture taken in 1:1 ratio was assessed for production of any unwanted interactions.

Preparation of nanoemulsion

Luliconazole loaded Nanoemulsion were formulated at the different constituent's ratio by the spontaneous homogenization method. Appropriate quantities of oil (oleic acid), surfactant (tween 80) and co-surfactant (PEG 400) were weighed and mixed well. Luliconazole was precisely weighed to represent 1% w/w of the total weight of the Nanoemulsions formulation, and then added to the previous mixture and stirred with a Homogenizer (3600 rpm), at room temperature (25°C) until the drug is entirely dissolved. The weighed amount of water then added drop wise to the oil phase with continuous stirring for 30 min.

Table 1: Formulation of nanoemulsion

Formulations	Sur.mix (ratio)	Oil/S.mix (ratio)	%w/w of components in Nanoemulsion formulation			Drug %w/w
			Oil	Smix	Water	
NF1	1:1	1:2	10	20	70	1
NF2	1:2	1:4	10	40	50	1
NF3	1:3	1:6	10	60	30	1
NF4	1:4	1:8	10	80	10	1

Formulation of nanoemulgel

Optimized nanoemulsion formulation was transfer to gel form using Carbopol 934in various concentration and analyse for different evaluation.

Nanoemulsion base gel was prepared by dissolving the weight amount of the Carbopol-934in appropriate quantity of water. After complete dispersal, the solution was kept in dark for 24 hrs for complete swelling of Carbopol-934. The Carbopol solution was mixed with optimized formulations containing luliconazole.

The mixture was stirred to get homogeneous solution. The appropriate amount of triethanolamine was added to maintain the pH with continuous stirring to get homogeneous gel.

Table 2: Formulation of nanoemulsion gel

Formula code	Nano Emulsion (ml)	Carbopol 934 (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

Freeze thaw cycle

Nanoemulsions were kept at -20 °C in deep freezer. After 24 hr the nanoemulsions were removed and kept at room temperature. The nanoemulsions returned to their original form within 2-3 min were considered as stable.

Centrifugation studies

After freeze thaw cycle nanoemulsions were subjected to centrifugation for 30 min. at 5,000 rpm in a Remi centrifuge. The formulations that do not show any phase separation or turbidity are stable.

Heating cooling cycle

Six heating cooling cycles are performed at temperature 4 °C and 40 °C with storage of 48 hr were performed. Formulations which were stable at these temperature, subjected to further study.

Measurement of pH

pH of all batches of nanoemulsions was determined by using digital pH meter. 10 ml of formulation was introduced to digital pH meter. The measurement of formulation is done in triplicate to avoid error.

% Drug content

1 ml of nanoemulsion was dissolved in pH 7.4 phosphate buffer. After filtering this solution is suitably 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

The diffusion studies of the developed nanoemulsions were determined by making diffusion cell assembly with the aid of cellophane membrane. 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.

Zeta potential

The formulation of nanoemulsion was tested for zeta potential using Malvern Zetasizer instrument. The analysis was carried out at 25°C.

SEM

To identify the peripheral morphology of formulation; SEM was performed on optimized batch.

Evaluation of nanoemulgel

Appearance

The nanoemulgel was observed for appearance and clarity by visual testing.

pH

pH of all batches of nanoemulgel was determined by digital pH meter. The 5% nanoemulgel was prepared using PBS 7.4 prior to be subjected for pH determination.

% Drug content

2 gm of Nanoemulgel sample were taken in 100 ml flask having 10ml ethanol and stirred by magnetic stirrer for 5 minutes. The solutions were filtered using Whatmann filter paper. The absorbance of the solution was estimated spectrophotometrically (UV 1800, Shimadzu) to calculate % drug content in nanoemulgel.

Determination of viscosity

20 gm of nanoemulgel was subjected to the viscosity determination on Brookfield viscometer using spindle no. 6 at RPM 50.

Spreadability

Spreadability of nanoemulgel was determined by sandwiching 5gm 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

The diffusion studies of the prepared nanoemulsion gel were carried out in Franz diffusion cell through a cellophane membrane. Gel sample (0.5g) was taken in cellophane membrane and the diffusion studies were carried out at 37±1°C using 250 ml of (25%) ethanolic 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.

Stability study

Formulation of nanoemulsion gel is placed in collapsible tubes with proper sealing and for a short term accelerated stability study at 40±2°C, 75±5%RH as per ICH Guidelines. The formulation was withdrawn after particular period of interval; the physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation) and evaluated for drug content and in vitro drug diffusion.

RESULTS AND DISCUSSION

Identification of the drug by UV spectroscopy

The UV spectrum of Luliconazole in buffer solution pH 7.4 in the range of 200-600nm. The spectrum indicates that the absorption ʎ max of Luliconazole was 297 nm which is match with ʎ max given in Indian Pharmacopoeia.

Table 3: Standard calibration curve of Luliconazole

Conc.(µg/ml)	Absorbance (ʎmax)
2	0.205
4	0.461

6	0.695
8	0.985
10	1.191

UV shows that Luliconazole gives maximum absorption at 297 nm and figured in linear standard calibration curve shown in fig no.4

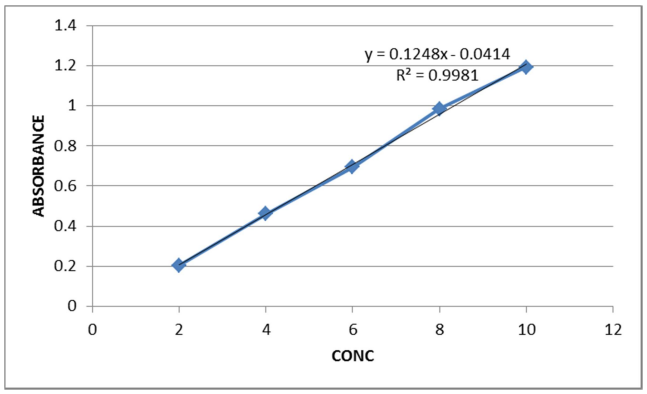

Figure 1: Standard calibration curve of Luliconazole

Identification of drug by FTIR spectroscopy

The IR spectrum of the pure Luliconazole sample recorded by FTIR spectrophotometer is shown in fig no. 5. The frequencies of the functional group of Luliconazole in the reported range which indicates that the obtained sample was of Luliconazole and was pure.

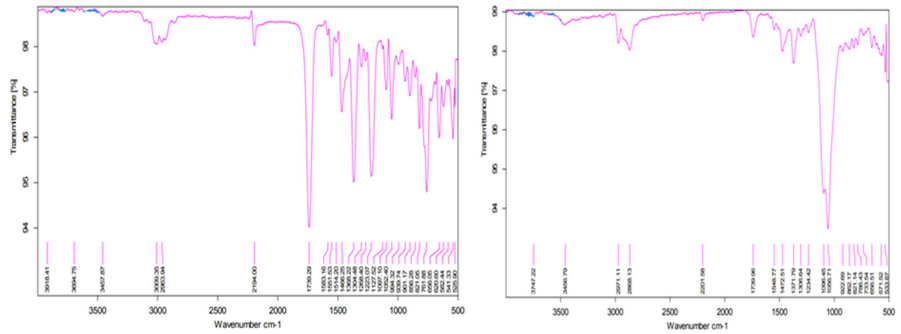

Figure 2: a) FTIR of Luliconazole b) FTIR of drug + excipients

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

Form the FTIR study drug compatible with all the excipients.

Nanoemulsion Characterization

All the batches have approved the thermodynamic stability study.

Table 5: Thermodynamic stability study

Formulation	Freeze thaw	Heating cooling	Centrifugation
F1	Stable	Stable	No phase separation
F2	Stable	Stable	No phase separation
F3	Stable	Stable	No phase separation
F4	Stable	Stable	No phase separation

pH, and % Drug content

All the batches were found to be milky white dispersion in appearance. F2 batch showed highest drug content as 81.12%. pH of all batches was found within the range of 5 – 5.5. From the results of drug content batch F2 batch was considered as optimized batch.

Table 6: pH and drug content of nanoemulsion formulation

Formula code	pH	Drug content (%)
F1	5.5±0.3	76.37
F2	5.4±0.2	81.12
F3	5.3±0.2	80.06
F4	5.0±0.3	79.56

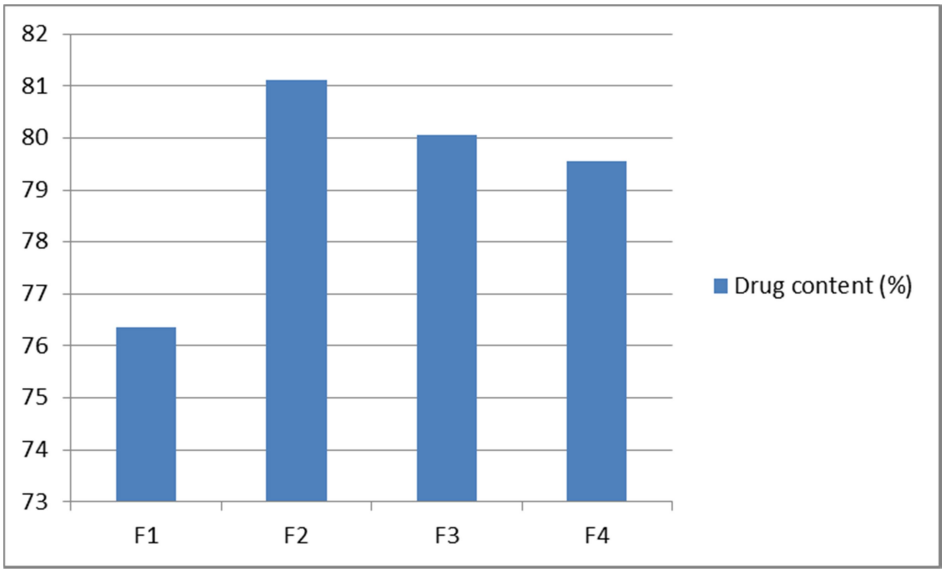

Figure 3: %Drug content of NE

% 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 F2 batch among all the batches i.e. 82.62%. From these results it is considered to be best optimized one.

Table 7: % In vitro Drug Diffused

Time (hr)	% In vitro Drug Diffused			
	NF1	NF2	NF3	NF4
0	0	0	0	0
1	9.64	14.68	9.59	10.58
2	16.2	18.34	13.78	11.98
3	23.87	29.69	22.16	16.07
4	28.09	38.35	26.4	22.98
5	31.95	42.87	31.38	26.74
6	37.08	46.78	38.59	32.85
7	47.67	51.23	40.98	37.92
8	54.1	57.18	49.96	45.39
9	58.93	60.74	54.9	51.53
10	67.12	67.85	69.82	57.61
11	75.85	74.86	70.47	64.28
12	79.07	82.62	74.19	71.95

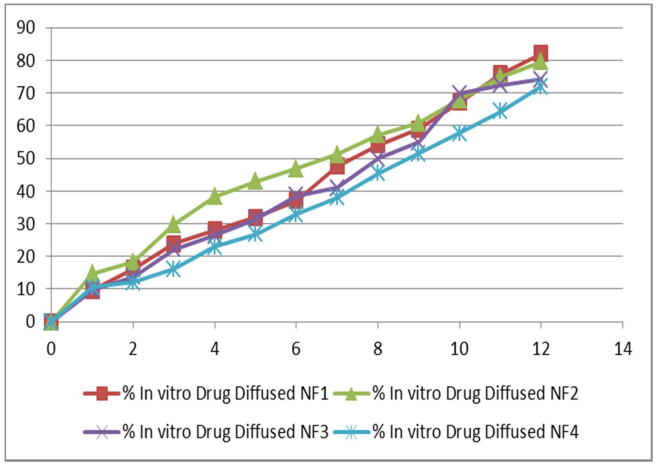

Figure 4: % In vitro Drug Diffused

SEM

A SEM images of microscopic evaluation of optimized formulation F2 was found to be spherical shape of droplets as shown below figure. Nanoemulsion prepared by using tween 80 and PEG 400 with the ratio of 1:2, the size of nanoemulsion was found to be 20-200nm shown in results.

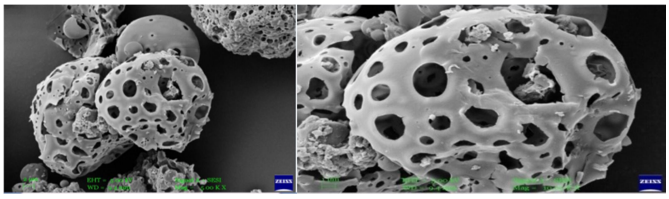

Figure 5: SEM images

Zeta Potential

Zeta potential of nanoemulsion of F2 was found to be -45 mv. The surface charge on nanoparticles was determined by Zeta potential. Values in the range of -50mv to +50mv of either charge characterize stable formulations.

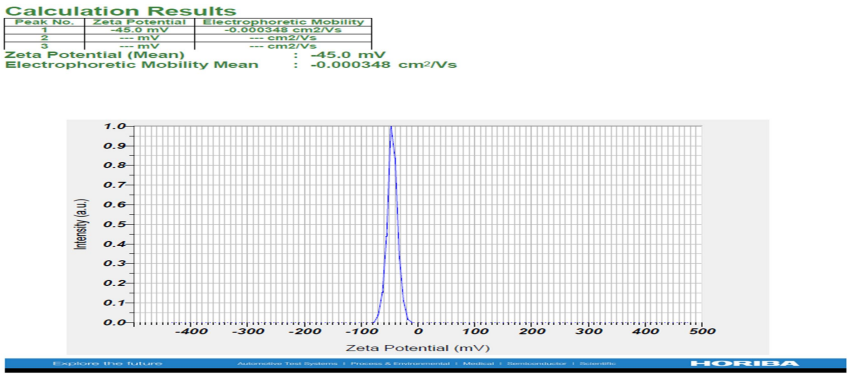

Figure 6: Zeta Potential

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 42.84 – 65.50 & viscosity 14256 – 18829. % Drug content was found to be 71.24 – 88.23. NEG 4 batch was optimized with highest drug content.

Table 8: Evaluation parameter of nanoemulgel

Formulation Code	Viscosity (cps)	pH	% Drug content	Spreadability (gms.cm/sec)
NEG1	14256	6.5	71.24	47.45
NEG2	15521	6.3	78.46	55.68
NEG3	16320	6.4	85.19	42.84
NEG4	18829	6.5	88.23	65.50

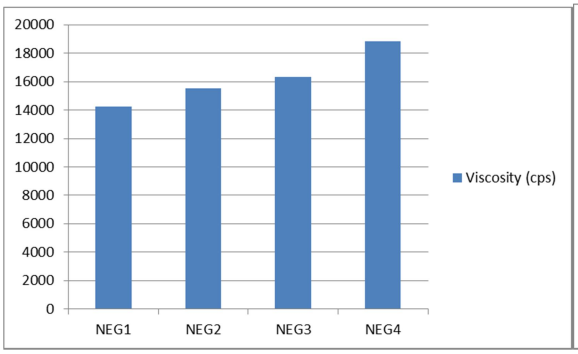

Figure 7: Viscosity of NEG

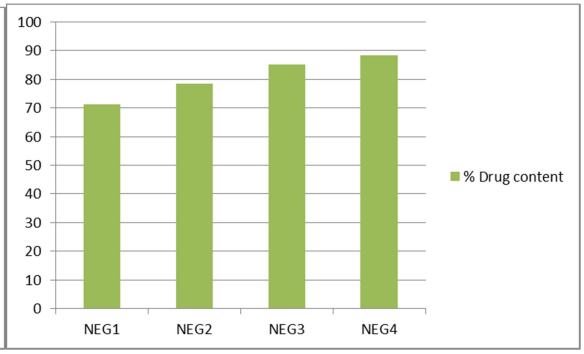

Figure 8: % Drug content of NEG

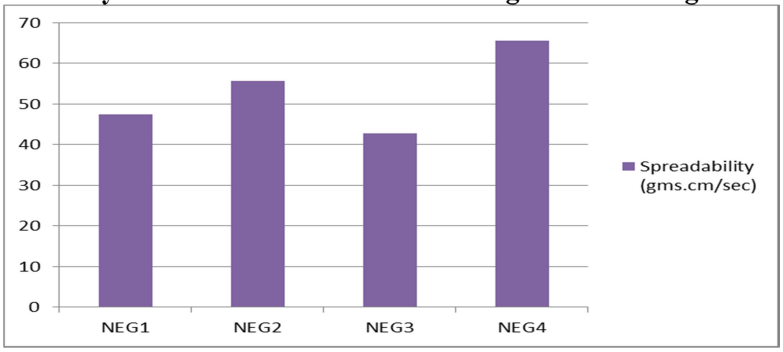

Figure 9: Spreadability of NEG

In vitro diffusion studies

All the prepared batches show good drug diffusion. The percentage drug release at 8 hrs is maximum of NEG4 among all the batches i.e. 74.61%. From these it is considered to be best optimized one.

Table 9: In vitro drug diffusion study

Time in (hrs)	NEG1	NEG2	NEG3	NEG4
0	0	0	0	0
1	5.85	10.2	9.64	11.98
2	10.86	14.4	11.07	18.58
3	15.56	21.8	16.86	25.07
4	22.37	27.4	22.09	30.98
5	35.34	36	35.95	43.83
6	42.21	42.6	47.68	55.35
7	49.2	61.4	57.2	68.28
8	54.23	68.6	65.6	74.61

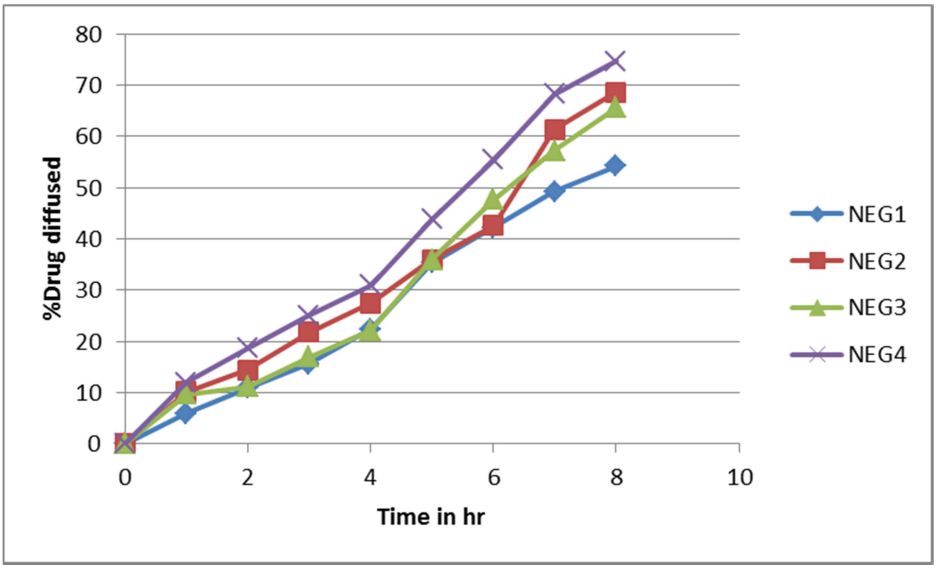

Figure 10: In Vitro Drug Diffusion

Stability studies

The accelerated stability study of nanoemulgel batch 4 was carried out; there is no significant change in pH, drug content and % drug diffusion in NEG4 formulation at 40°C temperature and 75% RH. The result are shown in table no.10

Table 10: Stability Study of NE gel

Parameters	Initial	1 Month	2 Month	3 Month
Appearance	Transparent yellow	NC	NC	NC
Viscosity	18829	NC	NC	NC
pH	6.5	NC	NC	NC
Drug content	82.23	88.55	87.02	86.32

NC= No Change

CONCLUSION

Luliconazole nanoemulgel was prepared by the homogenization technique and was evaluated for pH, drug content, viscosity, spreadability, drug diffusion study and accelerated stability study and results were within the limits. Among all the formulations of nanoemulsion F2 was the best formulation, and it was further converted to gel form. The optimized formulation of nanoemulgel i.e., NEG4 showed better drug content, In-vitro drug diffusion (as compared to conventional gel). 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 infections of skin.

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