FORMULATION AND EVALUATION OF ANTIFUNGALNANOEMULGEL

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Abstract: The present research study was to develop a nanoemulsiongel formulation for topical drug delivery for effective treatment of various topical fungal infections and improve the solubility of poorly water soluble drug i.e. Terbinafine hydrochloride. Theseformulation compose of Terbinafine Hcl as API and Oleic acid as a oil phase, tween 80 as surfactant, ethanol, PEG-400, Propylene glycolusing different co-surfactatnts by using Ultrasonichomoginization technique. The all nanoemulsionswere characterized for appearance, pH, % Drug content,% Drug entrapment efficiency and in-vitro drug diffusion study; based on these results optimized batch were evaluated for SEM-TEM, Zeta Potential, and stability study. These optimized nanoemulsion were converted to gel using carbapol 940 as a gelling agent; followed by characterization of gel for viscosity, spread ability, pH, in-vitro drug diffusion and accelerated stability study.

Keywords: Terbinafine HCl,in-vitro drug diffusion, SEM, TEM, Zeta potential, Stability study.

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Introduction

Topical drug delivery systems have been used for centuries for the treatment of local skin disorders and relieve the pain. One side the topical applications of the drug offer the potential advantages of delivering the drug directly to the site of action and delivering the drug for an extended period of time at the effected site that mainly acts at related regions. On the other hand, the topical delivery system increases the contact time and mean resident time of the drug.The most common side effect of some oral dosage forms is a gastrointestinal irritation. The long-term use of such drugs is associated with severe gastropathy. So, to overcome this limitation an emulsion based approach is being used, so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels. In recent years, there has been great interest in the use of novel polymers which can function as emulsifiers and thickeners because the gelling capacity of these compounds allows the formulation of stable mulsions and creams by decreasing surface and interfacial tension and at the same time increasing the viscosity of the aqueous phase. In fact, the presence of a gelling agent in the water phase converts a classical nanoemulsion into a nano-emulgel. Nano-Emulgel for dermatological use have several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining ,water-soluble, greater shelf life, bio-friendly, clear & pleasant appearance[1].

Nano-Emulsions are biphasic system in which one immiscible liquid is dispersed into other having nano size globules, due to this the system becomes unstable which are stabilized by using emulsifying agents. Nano-Emulsion can be either o/w or w/o these are used as vehicles to deliver drug. They can be easily washed off from skin, nail and have better penetrability as compare to conventional emulsion. Emulsions are of different types depending on the size of droplets ornature of distribution. Manufacturing of nano-emulgel is generally carried out through involvement of multistep process in which the first, Nano

emulsion is developed and then mixed with a suitable gel base. Therefore, here we will first discuss about the development and manufacturing of Nanoemulsion and then preparation and incorporation into gel base TerbinafineHCl[2,3].

MATERIALS AND METHODS

Materials

Terbinafine HClis purchased fromSwapnroop research Pvt. Ltd Sambhajinagar, Maharashtra, India, and oleic acid, propylene glycol, PEG 400 are purchased form S.D. Lobachem, Mumbai, India and Carbapol 940 is purchased form S.D. Lobachem, Mumbai, India, All chemicals and solvents are of analytical grade.

Methods

Preformulation

Physical appearance[4,5]

Terbinafine HCl sample which was supplied form Swapnroop API Pvt. Ltd, Sambhajinagar, Maharashtrawas closely observed for physical appearance.

Determination of Melting point[5]

The medication was distinguished by its melting point. By adding a little amount of Terbinafine Hcl to a capillary tube, transferring it to a graduation thermometer, and applying continuous heat to an assembly suspended in a Thiel's tube with paraffin bath, the melting point was determined. Melting point is the temperature at which a medication begins to melt.

Solubility study[6]

Solubility of Terbinafine HCl was determined in various oils such as Water, Ethanol, and methanol by shake flask method. An excess amount of drug was taken in 10 ml of the oil in vials, and mixed using vortex mixer. The vials were then kept at 25 ± 10C in an isothermal shaker. The samples were then centrifuged at 3,500rpm for 15min. The supernatant was filtered through whatman filter paper. The filtrate was suitably diluted. The amount of drug dissolved in the oil was determined using UV spectrophotometer at their respective wavelength.

Standard calibration curve of API[6]

Terbinafine hydrochloride, accurately weighed at 50 mg, was dissolved in 50 ml of methanol, and 1 ml of this was diluted with phosphate buffer at pH 7.4 in a 50 ml volumetric flask to create the stock solution of 1000 g/ml. 5 ml were pipetted out of the stock solution and were then further diluted to obtain 100 g/ml solutions, use up to 50 ml of buffer. A concentration range of 2–10 g/ml was obtained by taking an aliquot of 2, 4, 6, or 8 ml from a 100 g/ml solution and diluting it to 50 g/ml to generate a 10 g/ml solution. These aliquots were then removed and diluted to 10 g/ml with buffer. A UV spectrophotometer was used to test the solutions' absorbance at 283 nm. The relationship between concentration and absorbance was graphed.

Estimation of drug by Fourier transforms Infra-red Spectroscopy (FTIR)[7]

The FTIR investigation was carried out by scanning the substance in an FTIR spectrometer over the range of 4000-400 cm-1. The TerbinafineHcl FTIR spectrum revealed the presence of peaks that match the reference spectra.

Preparation of nanoemulsion by High energy method[11]

TerbinafineHCl-loaded nanoemulsions were created using the High-energy methods for preparation of nanoemulsionsat various constituent ratios depending on the phase diagrams. Weighed in the proper amounts were drugs (TerbinafineHCl), oils (oleic acid), surfactants (tween80), and co-surfactants (ethanol), (Propylene glycol), (PEG 400). water with tween 80 added and heated to 40^0C while stirring.

The specific weight of Terbinafine HCl, which makes up 1% w/w of the formulation's total weight, is then dissolved in oleic acid.When the aqueous phase and oil phase were combined,Preparation of TerbinafineHClNanoemulsion.The O/W Nanoemulsion were prepared according to the following procedure: For the preparation of Nanoemulsion by using the 'HIGH ENERGY EMULSIFIATION' technique.Place the vessel in an ultrasonic homogenizer and apply ultrasonic energy to the mixture. The ultrasonic energy helps break down the oil phase into small droplets and promotes the formation of a stable nanoemulsion.Continue ultrasonication for a specific duration, typically ranging from a few minutes to several minutes, depending on the desired droplet size and stability.After ultrasonication, allow the nanoemulsion to cool down to room temperature and stabilize for a certain period to ensure the formation of a thermodynamically stable system.

Table 1: Formulation of Nanoemulsion

Ingredients	**Formulation batch**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Terbinafine HCl (%)	1%	1%	1%	1%	1%	1%	1%	1%	1%
Oleic acid(mL)	10	10	10	10	10	10	10	10	10
Tween 80(mL)	10	10	20	10	10	20	10	10	20
Ethanol(mL)	-	-	-	-	-	-	10	20	10
Propylene Glycol(mL)	10	20	10	-	-	-	-	-	-
PEG 400(mL)	-	-	-	10	20	10	-	-	-
Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Figure 1:Formulation of Nanoemulsion

Formulation of terbinafine HCl loaded nanoemulsion gel[12]

Optimized nanoemulsion tested for stability study for 1 month, stable nanoemulsion then transferred to gel form using Carbopol 940 in various concentration and analyzed for different evaluation. 98mL of distilled water was used to dissolve 2 gram of carbopol-940. The solution was then kept in the dark for 24 hours after complete dispersion to allow Carbopol-940 to fully swell. Then, a slow addition of terbinafine hydrochloride (1% w/w) nanoemulsion was made to the carbopol-940 aqueous solution. Triethanolamine (TEA) was added in the proper amount to the gel to achieve a homogenous dispersion, also add the glycerine for as humectants in proper quantity and add Benzyl alcohol as preservative in that base and continuous stirring to get homogenous gel.

Table 2: Formulation table of nanoemulsion gel

Formulation code	**Drug loaded nanoemulsion**	**Gelling agent Carbapol 940**	**water**	**Benzyl alcohol**	**Glycerine (ml)**	**Triethanolamine (ml)**

NEG 1	10	0.5	50	0.1	5	Q.S
NEG 2	10	1.0	50	0.1	5	Q.S
NEG 3	10	1.5	50	0.1	5	Q.S
NEG 4	10	2.0	50	0.1	5	Q.S

Evaluation of Terbinafine HCl Nanoemulsion

Appearance[13]

The formulation was observed for the presence of any particular matter. Clarity is one of the most important features of topical preparations. The appearance and clarity are determined by visual testing.

pH[14]

Digital pH meter at 25° ± 1°C were used to estimate the pH of the nanoemulsion.

Thermodynamic stability[15]

The selected formulation is subjected to different thermo dynamic stability tests.

1. Freeze thaw cycle: The compositions were subjected to six cycles at temperatures ranging from 4^0 C in the refrigerator to 45^0 C, with storage at each temperature lasting at least 48 hours. Centrifugation tests were conducted on those formulations that remained stable at these temperatures.
2. Freeze thaw stress cycle:For the formulations, three freeze-thaw cycles between - 21^0C and +25^0C were performed, with storage at each temperature for at least 48 hours. The formulations that passed these thermodynamic stress tests were then subjected to the dispersibility test to determine how well they would form an emulsion on their own.
3. Centrifugation: Centrifugation at 3500 rpm for 30 min was performed on formulations that remained stable after freeze-thaw cycling. The freeze-thaw stress test was conducted on those formulations that did not exhibit any phase separation.

Entrapment Efficiency (EE)[16]

The Ultracentrifugation method, which involved centrifuging the dispersion of nanoemulsion at 10,000 rpm for 90 minutes, was used to determine the effectiveness of nanoemulsion entrapment. The resulting centrifuged solution's clear supernatant was diluted with pH 7.4 phosphate buffer before being spectro photometrically tested for the presence of grise of ulvin and the percentage entrapment efficiency (EE%) was calculated using the formula below:

$$\text{Entrapment Efficiency} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

Percent drug content[16]

To get the necessary drug concentration, the nanoemulsion (1ml) was appropriately diluted with methanol, and absorbance was measured using a UV-visible spectrophotometer (UV- 1700, Pharmaspec, Shimadzu Ltd, Japan) at 283 nm.

In-vitro Diffusion studies[16]

The diffusion studies of the prepared nanoemulsions are performed by using Franz diffusion cell with the aid of cellophane membrane. Nanoemulsion sample (5ml) is taken in cellophane membrane and the diffusion studies are 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 1,2,3,4,5,6,7,8, 9, 10, 11, and 12 hrs and each sample are replaced with equal volume of fresh dissolution medium in order to maintain sink conditions. Samples are analysed by UV-spectrophotometer at 283 nm for drug content.

Zeta Potential[18]

The characterization of the surfaces charges property of optimized batch of Nanoemulsion was determined bymeasuring zeta potential. The formulation of nanoemulsion was tested for zeta potential using Malvern Zetasizer instrument. The analysis was carried out at 25°C.

Tem[18]
To identify the shape and morphology of the optimized batch of nanoemulsion by which the actual diameter and shape of the droplet can be acquired, transmission electron microscopy (TEM) examination was conducted.

Sem[18]
The morphology of the improved optimized batch of Nanoemulsion formulation was examined using a scanning electron microscope.

Stability study[18]
The optimized batch of nanoemulsion formulation was examined for stability study. The formulations were taken in 20ml sealed glass vial andstored in 40 ± 2^0C, room temperature and 40^0C$\pm2^0$C,75%±5% RH for a period of one months.

Evaluation of nanoemulsion gel[18,19]
Appearance[19]
The formulation was examined to determine whether any specific materials were present. One of the most crucial characteristics of topical medicines is clarity. Visual assessment is used to determine the clarity and appearance.

Measurement of pH[19]
pH of all batches nanoemulsion gel formulations is determined by using digital pH meter. 1 gm of nanoemulsion gel is dissolved in 100 ml of distilled water and pH was measured. The measurement of formulation is done in triplicate to avoid error.

Drug content[19]
Each formulation's 2 g of nanoemulsion gel was put to a 100 ml volumetric flask along with 10 ml of ethanol, and the liquid was stirred with a magnetic stirrer for 5 minutes. The solution was filtered using Whatman filter paper. The absorbance of the solution was determined spectrophotometrically (UV 1800, Shimadzu) at 283 nm using a standard curve against a blank.

Determination of viscosity
A Brookfield viscometer was used to measure the viscosity of the nanoemulsion gel. A 25 ml beaker containing 20 g of nanoemulsion gel was filled, and spindle number S6 and RPM 10was used to calculate the viscosity.

Spreadability
On a glass plate, which was then covered by another glass plate, 0.5 g of the test formulation was placed inside a 1 cm-diameter circle that had been previously marked. Five minutes were given for a weight of five grammes to rest on the upper glass plate. It was observed that the formulation's spraedability caused the diameter to grow.

In vitro drug diffusion study
Using a cellophane membrane, a Franz diffusion cell was used to conduct diffusion tests on the generated nanoemulsion gel. A gel sample (0.5g) in a cellophane membrane was used for the diffusion investigations, which were conducted at 37°C with a dissolution media of 250 ml of phosphate buffer (pH

7.4).In order to keep the sink condition, 5ml of each sample were regularly removed at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours, and each sample was replaced with an equal volume of new dissolution medium. Samples were examined for drug content using a UV-visible spectrophotometer at 283 nm.

Stability study

In accordance with ICH Guidelines, the formulation of optimized nanoemulsion gelis placed in collapsible tubes with suitable sealing and subjected to a short-term accelerated stability study at 40°C and 75% RH. The formulation was taken off the market after a specific interval, and its physical stability was assessed for drug content and in vitro drug diffusion as well as for physical changes such phase separation and drug precipitation.

RESULT AND DISCUSSION

Preformulation results

Organoleptic properties

Organoleptic properties of Econazole nitrate are found to be as per USP NF monograph**.**

Table 3: Organoleptic properties

Sr. No.	Test	Specification (USP NF)	Observation
	Colour	White	A white crystalline powder, hygroscopic
	Odour	Odourless	Odourless
	Nature	Crystalline	Crystalline

Melting point analysis

The melting point analysis of Terbinafine HCl was observed to be 196^0C that complies with the IP standard melting point range.

Solubility

The solubility study of TerbinafineHCl in different solvent was found to be,

Table 4: Solubility of profile of drug

Sr.No.	Solvent System	Inferance
1	Water	Slightly soluble
2	Ethanol	Soluble
3	Methanol	Freely soluble

Standard calibration curve of drug in UV spectrophotometer

UV shows that Terbinafine HCl gives maximum absorption 283 nm and figured in linear standard calibration curve shown in fig no. 2. The UV absorbance of Terbinafine HCl standard solutions in the range of 0.2-1.2 µg/ml of drug in buffer pH 7.4 calculated at λ max 223 nm. The linearity was plotted for absorbance (A) against concentration (C) with R^2 value 0.992 and with the slope equation y=0.0268x + 0.002. The absorbance values and standard curve were in the figure no. 2 below.

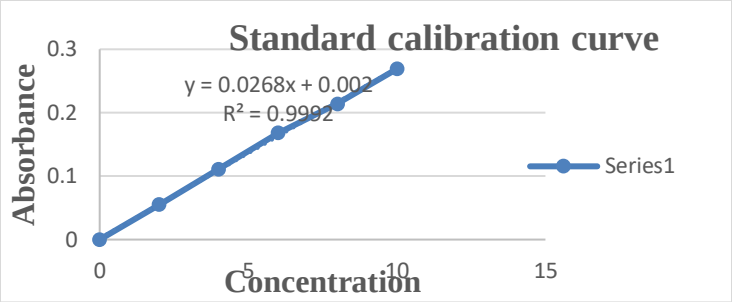

Figure 2: Standard calibration curve of Terbinafine HCl in methanol

Table 5: Standard calibration curve of Terbinafine HCl

Concentrarion (µg/mL)	**Absorbance at**
0	0
0.2	0.055
0.4	0.111
0.6	0.168
0.8	0.214
1.0	0.269

Identification of drug by FTIR spectroscopy

The IR spectrum of the pure Terbinafine HCl sample recorded by FTIR spectrophotometer is shownin fig no. 3.

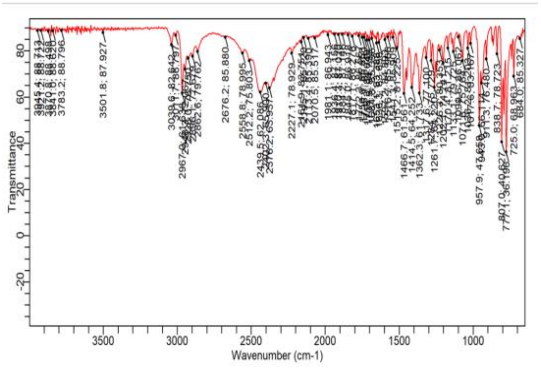

Figure 3: FTIR of Terbinafine HCl

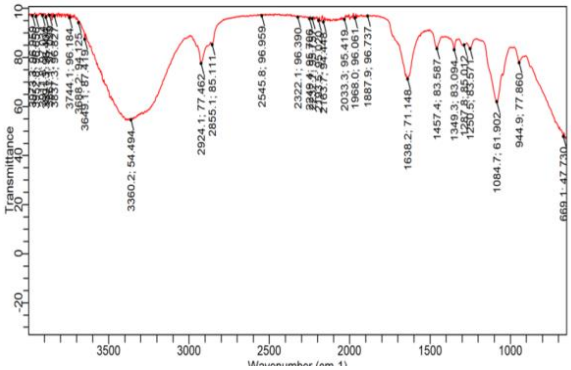

Figure 4: FTIR of Nanoemulsion Formulation

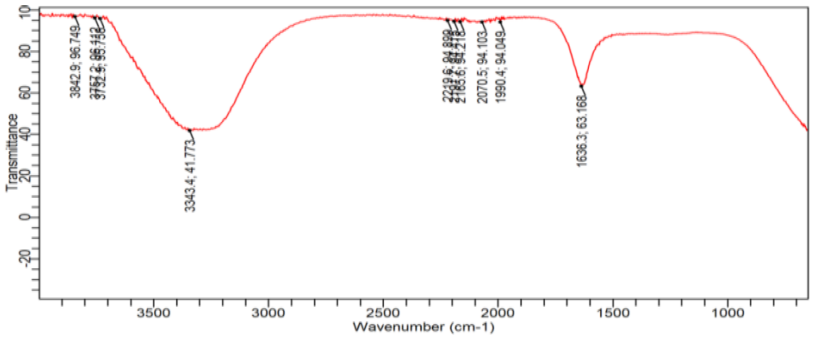

Figure 5: FTIR of Nanoemulsion gel Formulation

Evaluation result of nanoemulsion

Appearance

The nanoemulsion formulation was slightly whitish transparent in nature.

pH

pH of Nanoemulsion formulation was found to be in the range of 5.2 – 6.0 as shown in table no.7.

Thermodynamic stability study

The thermodynamic stability study of nanoemulsion formulation at different parameters Freeze thaw stress cycle, centrifugation and freeze thaw cycle were tested and result are givenin table no. 6.

Table 6: Thermodynamic stability study

Formulation	Freeze thaw stress cycle	Centrifugation	Freeze thaw cycle
F1	Stable	No phase separation	No change
F2	Stable	No phase separation	No change
F3	Stable	Foam	Unstable
F4	Stable	No phase separation	No change
F5	Stable	No phase separation	No change
F6	Stable	Foam	Unstable
F7	Stable	No phase separation	No change
F8	Unstable	No phase separation	No change
F9	Unstable	No phase separation	No change

% Entrapment efficiency

The % entrapment efficiency of all formulation was to be 50.12-70.84%. Among all formulations, F7 shows highest entrapment efficiency given in table no.7.

Drug content

The drug content was found to66.12–93.23% acceptable for all formulation. F7 shows maximum % of drug content.

Table 7: Results of Nanoemulsion

Formulation	Appearance	pH	% Entrapment efficiency	% Drug Content
NE 1	Clear	6.2±0.2	50.12±0.2	66.12±0.2
NE 2	Clear	5.8 ± 0.2	51.85±0.5	67.59±0.2
NE 3	Turbid	5.7 ± 0.2	54.03±0.2	73.67±0.2
NE 4	Clear	5.6 ±0.2	60.23±0.4	77.36±0.3
NE 5	Clear	5.4 ±0.2	63.25±0.2	77.89±0.4
NE 6	Turbid	5.6 ±0.2	66.02±0.1	89.09±0.2
NE 7	Clear	5.7 ±0.2	70.84±0.2	93.23±0.1
NE 8	Clear	5.4 ±0.2	70.11±0.2	90.86±0.2
NE 9	turbid	5.6 ±0.2	70.06±0.3	91.77±0.3

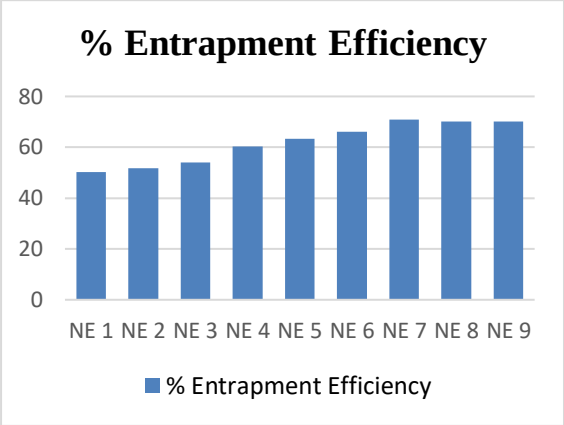

Figure 6: % Entrapment efficiency.

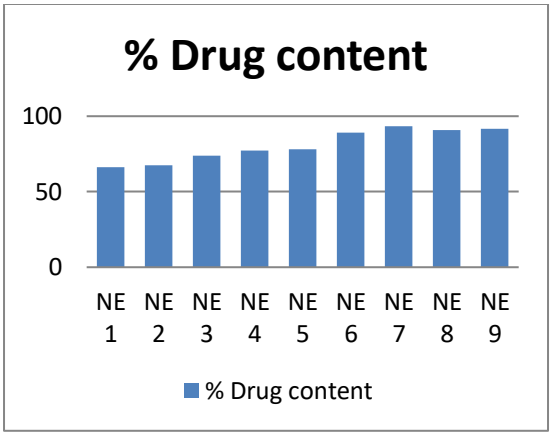

Figure 7: % Drug content

In-vitro drug diffusion

The in vitro drug diffusion study performed for all Nanoemulsion formulation batches upto 12 hrs. The Nanoemulsion formulation batch F7 shows highest percentage drug diffusion i.e., 95.56%.

Table 8: In-vitro drug diffusion of Nanoemulsion formulations

(n=3) values are expressed as ± (SD)

Time(hrs)	% Cumulative drug diffusion								
	NE 1	NE 2	NE 3	NE 4	NE 5	NE 6	NE 7	NE 8	NE 9
0	0	0	0	0	0	0	0	0	0
	6.42±0.3	6.92±0.2	6.86±0.2	5.02±0.2	7.05±0.2	8.34±0.4	9.02±0.2	8.34±0.3	8.04±0.2
	15.02±0.2	15.76±0.1	16.78±0.2	14.29±0.2	13.45±0.2	15.78±0.3	17.05±0.2	15.21±0.3	14.82±0.3
	23.01±0.4	23.78±0.1	25.80±0.2	21.87±0.2	19.67±0.3	21.02±0.2	23.09±0.4	19.89±0.3	18.74±0.2
	28.76±0.2	29.67±0.3	30.67±0.2	27.87±0.2	25.32±0.3	27.05±0.2	29.78±0.3	27.05±0.2	24.61±0.1
	34.89±0.3	35.89±0.3	34.89±0.4	33.04±0.2	34.78±0.3	37.67±0.2	39.56±0.3	35.56±0.3	33.02±0.1
	40.06±0.6	42.67±0.4	43.90±0.3	39.44±0.2	39.90±0.3	42.09±0.2	46.28±0.4	40.60±0.4	38.74±0.4
	43.12±0.2	49.87±0.4	53.09±0.3	43.08±0.3	45.54±0.2	49.15±0.4	51.06±0.5	48.73±0.5	46.23±0.4
	49.67±0.2	54.67±0.5	58.76±0.3	49.55±0.4	49.12±0.3	54.73±0.4	58.99±0.5	57.92±0.4	55.51±0.5
	52.34±0.2	58.05±0.2	62.63±0.3	52.37±0.3	52.23±0.4	60.67±0.4	67.54±0.2	67.51±0.2	63.05±0.5
	57.70±0.2	63.34±0.2	69.01±0.3	58.82±0.2	59.52±0.2	67.34±0.3	76.22±0.2	75.02±0.2	72.92±0.3
	62.90±0.3	69.74±0.3	73.32±0.3	67.29±0.2	67.78±0.2	74.56±0.3	85.34±0.3	82.55±0.3	80.78±0.2
	70.21±0.4	75.67±0.2	79.15±0.6	72.09±0.2	76.42±0.3	80.02±0.2	95.56±0.3	91.78±0.5	89.67±0.2

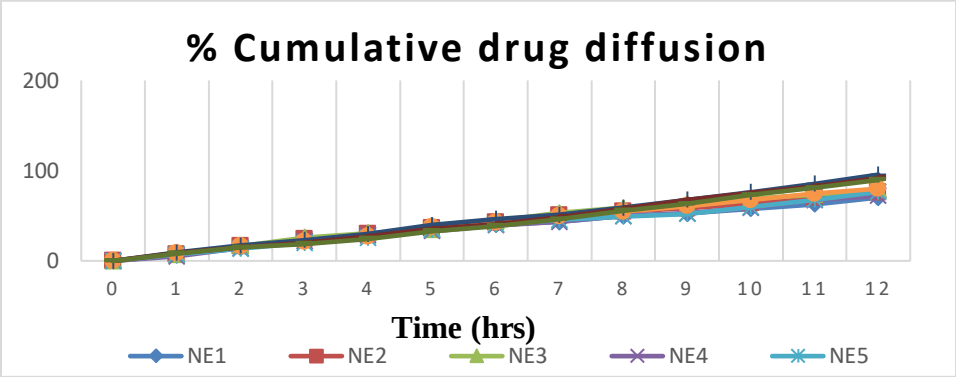

Figure 8: In-vitro drug diffusion of Nanoemulsion formulations

Zeta potential

Zeta potential of nanoemulsion was shown in fig. no. 21 Zeta potential value of optimized batch F7 was found to be – 19.mV.

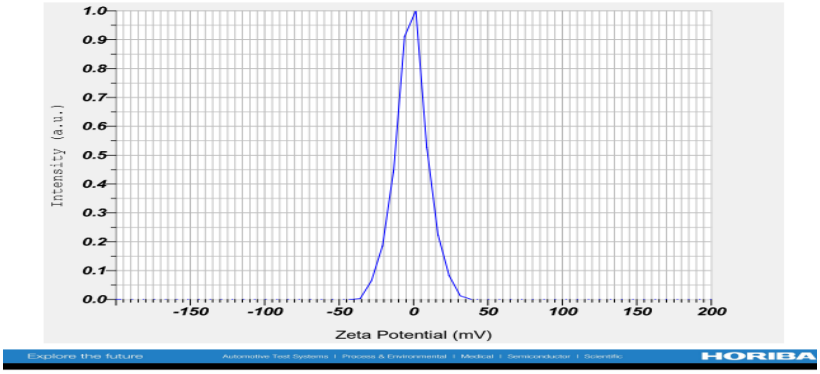

Figure 9: Zeta Potential

SEM analysis

Particle size of optimized formulation batch was done by Scanning Electron Microscope. A SEM images of microscopic evaluation of optimized NE 7 formulation batch. Nanoemulsion prepared by using tween 80 and Ethanol with ratio of 1:1, the particle size of nanoemulsion was measured and which is within the range i.e.300 nm.

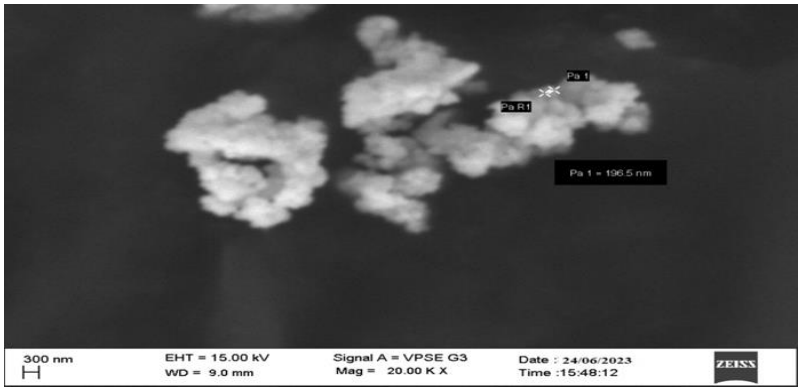

Figure 10: SEM images

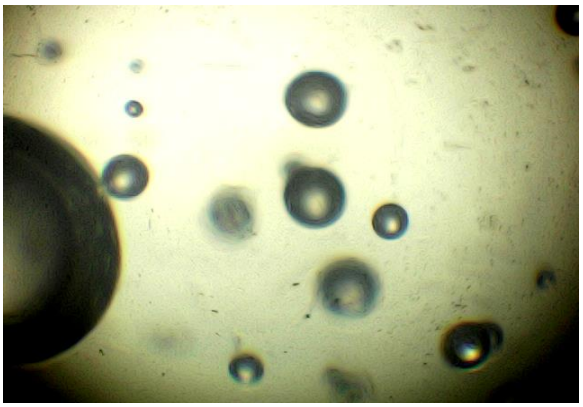

Figure 11: Dispersed phase of nanoemulsion under light microscope

TEM analysis

TEM images of microscopic evaluation of optimized formulation F7 was found to be the globule of nanoemulsion are having irregular shape within the range of 10 to 200 nm.

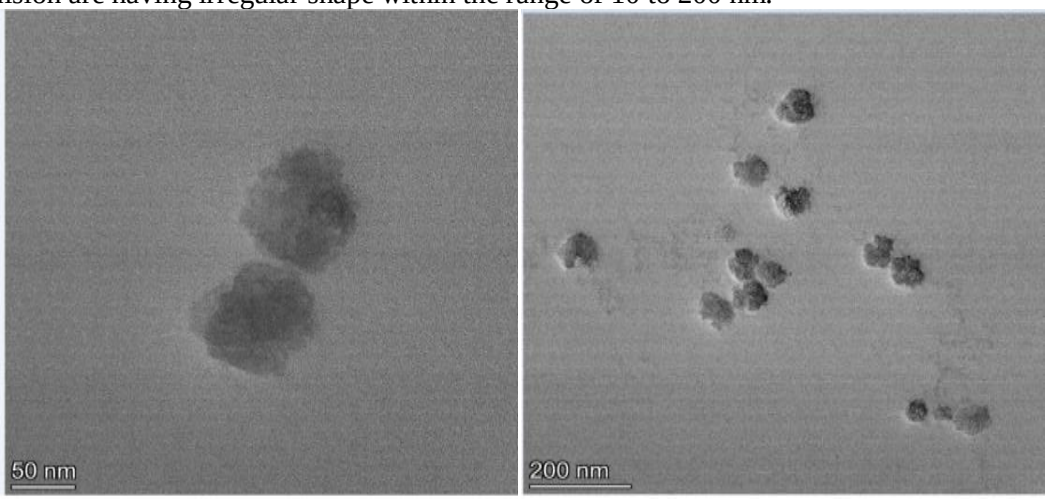

Figure 12: TEM images of optimized nanoemulsion formulation

Stability study

The batch F7 formulation show the highest drug content therefore subjected to acceleratedstability study result was given below.

Table 9:Accelarated Stability study of nanoemulsion

Parameters	**Initial**	**1 month**
Appearance	White	White
pH	5.7	5.7
Drug content (%)	93.23±0.1	92.70±0.2
Entrapment efficiency (%)	70.84±0.6	69.71±0.6

Evaluation result of nanoemulgel

Appearance, pH, Viscosity, Drug content, Homogeneity, Spreadability

The appearance of Nanoemulsion gel was Transparent white is visually analysed.pH of NEG was found to be in between 6.2 to 6.6 shown in table No. 10. The Nanoemulsion gel shown viscosity form 2567 to 12400cps and the spreadibility of NEG show 25.68 to 31.80. All developed gels were tested for homogeneity by visual inspection.

Table 10: Appearance, pH, Viscosity, Drug content, Homogeneity, Spreadability

Sr. no	Formulation code	Appearance & Colour	Viscosity (cps)	pH	Homogeneity	% Drug content	Spreadability
1.	NEG1	Transperent white	2567	6.5	Homogeneous	75.78±0.4	31.80
2.	NEG2	Transperent white	5251	6.5	Homogeneous	79.67±0.4	31.77
3.	NEG3	Transperent white	7562	6.6	Homogeneous	87.23±0.15	28.40
4.	NEG4	Transperent white	12400	6.2	Homogeneous	81.16±0.9	25.68

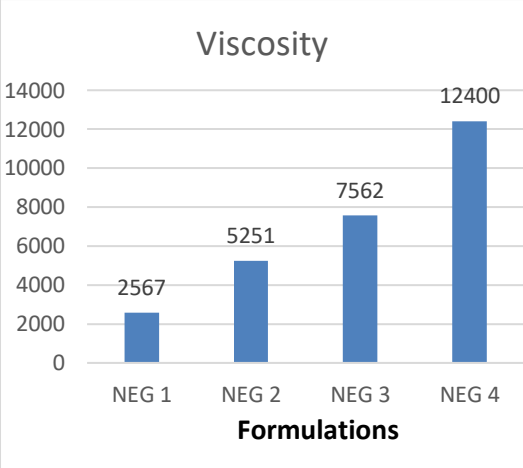

Figure 13: Viscosity of NEG

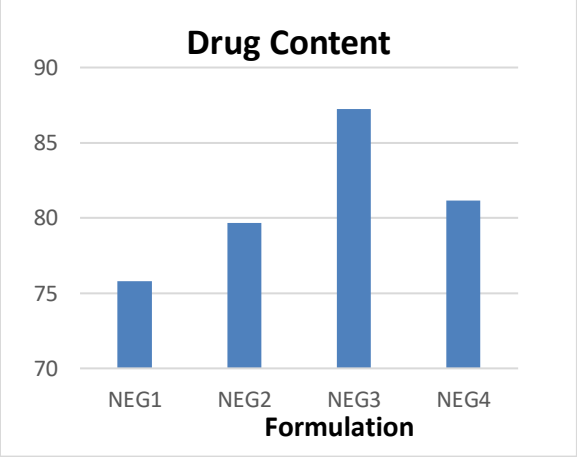

Figure 14: % Drug content of NEG

In vitro diffusion studies

The amount of TerbinafineHcl that diffused in vitro from the nanoemulsion gel varied depending on the formulation's polymer content. The amount of% drug diffuse is in the ascending sequence of Conventional gel: NEG4<NEG2<NEG1<NEG3. 72.25<80.95%< 83.02%<85.35%<90.07%. The study found that within 12 hours, nanoemulsion gel of TerbinafineHcl had better diffusion than Conventional cream.

Table 11: In vitro drug diffusion study

Time in (hrs)	NEG1	NEG2	NEG3	NEG4	Conventional creams
0	0	0	0	0	0
1	7.34±0.2	8.05	9.22±0.2	8.15	5.02±0.2
2	14.39±0.3	18.99±0.2	18.56±0.3	16.01±0.2	11.28±0.4
3	21.73±0.2	25.07±0.3	27.90±0.4	23.94±0.4	16.70±0.4

4	27.02±0.3	30.32±0.2	32.78±0.3	30.78±0.2	21.26±0.2
5	33.67±0.3	36.08±0.2	38.23±0.3	37.11±0.4	29.67±0.4
6	40.99±0.4	41.52±0.2	42.53±0.4	47.25±0.2	34.12±0.4
7	47.05±0.2	47.48±0.3	50.21±0.2	54.67±0.4	41.23±0.2
8	55.71±0.2	54.76±0.3	57.01±0.4	63.07±0.2	49.05±0.4
9	60.02±0.4	60.56±0.4	67.12±0.4	70.78±0.2	55.56±0.4
10	67.27±0.2	68.01±0.2	76.56±0.2	75.99±0.4	60.07±0.4
11	75.05±0.4	75.05±0.3	84.09±0.4	80.56±0.2	67.06±0.3
12	80.95±0.3	83.02±0.4	90.07±0.3	85.35±0.4	72.25±0.3

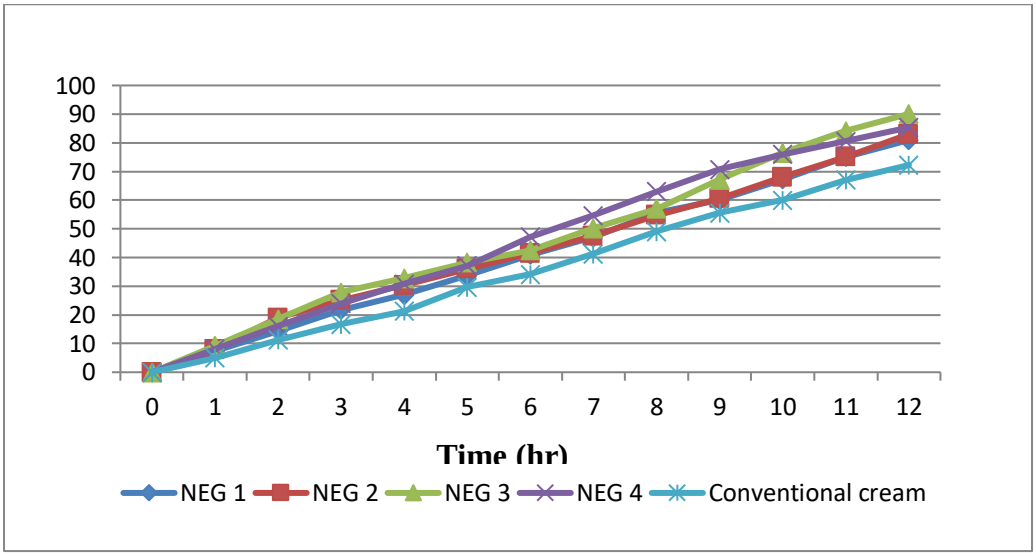

Figure 15: In vitro drug diffusion

Stability studies of NE gel

The optimized formulation NEG3 was subjected to a stability testing for the period of threemonthsas per ICH norms temperature of $40^0C\pm2^0C$ with relative humidity RH=75±5 %. The optimized formulation NEG3 were analysedfor the change in appearance, pH, Viscosity and in-vitro drug diffusion study.There was slight change observed in the pH, and In-vitro drug diffusion but the formulationwere stable at both the temperatures. The optimized formulation NEG3wasstable at roomtemperature. There was no change in the appearance and pH of the formulation but slightchange observed in In-vitro drug diffusion. The results are shown in table no.12.

Table 12: Stability studies of NE gel

Parameters	**Appearance**	**Viscosity**	**pH**	**Drug diffusion**
Initial	White	7562	6.6	90.07
After 1 Month	NC	NC	6.5	89.77
After 2Month	NC	NC	6.3	89.52
After 3Month	NC	NC	6.3	88.78

NC= No change

CONCLUSION

Terbinafine HCl loaded nanoemulsion was prepared by the 'High energy method' technique. The FTIR spectrum is revealed that drug compatable with other excipients .The formulation of nanoemulsion F7 shows good results in evaluation tests that's why we chosen that nanaemulsionfor the gel preparation. In the nanoemulsion formulation we are adding the different co-surfactants that are effects on the nanoemulsion formulation batch. In that formulation we are adding the three different co-surfactants such as Ethanol, PEG 200, propylene glycol they are effects on the nanoemulsionviscosity,in-vitro drug diffusion, and their pH. In that formulation F7 when we are adding the appropriate quantity Ethanol as co-surfactant it shows good stability, Viscosity, drug diffusion, drug contents and pH showing the good results in nanoemulsion .The optimized formulation of nanoemulsion gel i.e., NEG3 showed better drug content, In-vitro drug diffusion (as compared to conventional gel) and good anti-Fungal, anti-oxidant, anti-bacterial activity. The stability studies carried out for 90 days and the formulation was found to be stable. It can be concluded that Terbinafine HClnanoemulgel can be better alternative for conventional topical gel and effectively used for the treatment on Onycomycosis.

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