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Formulation Development and Evaluation of Topical Nanoemulgel of Ciclopirox

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ABSTRACT

The aim of the present work was to formulate Ciclopirox (1% w/w) in the form of emulgel, containing Nanoemulsion of the drug. Nanoemulsion were prepared by high speed homogenization and studied for different parameters. The problems associated with emulsion stability was also overcome by formulating drug loaded Nanoemulgel by 32 full factorial design in which Almond oil (%) and speed of homogenizer (rpm) were taken as independent factors in 3 different levels. Before formulating this formulations Preformulation testing were performed for drug characterization and to analyse its purity and compatibility. Organoleptic properties, melting point, solubility testing, UV spectroscopy studies and FTIR were performed for the Ciclopirox and the drug sample procured were found to be and compatible with the excipients used in formulation. The drug loaded Nanoemulsion's were evaluated for Particle size, Polydispersibility index, Zeta potential and scanning electron microscopy analysis. Drug loaded emulgel were evaluated for physical appearance, pH, viscosity, spredability, drug content, in vitro drug release study(diffusion study), antifungal activity and Accelerated stability studies. It was concluded that the Nanoemulsion loaded emulgel of Ciclopirox can be one of the promising tool in controlling the drug release via. Percutaneous mechanism for effective and longer treatment required for fungal infections with increased stability.

Keywords: -Nanoemulsion, Ciclopirox, emulgel, Zeta potential, scanning electron microscopy analysis, In-vitro drug release.

1. INTRODUCTION

Ciclopirox is a topically used antifungal agent, having specific and significant fungicidal effect on Trichophyton, Micros Porum and Epidermophyton. It is the most potent drugs for treatment of TineaPedis, which affects approximately 10% of the population. Ciclopirox is a BCS class IV drug. Ciclopirox possesses poor water solubility and high hydrophobicity which leads to low permeability. Such drugs posses a challenge in development of topical drug delivery system. Hence, for solubilization of Ciclopirox, formulation of emulsion appeared to be a viable approach. Emulsions have gained lot of attention for delivery of hydrophobic agents for local treatment; because of its ability to increase solubility, permeability of drug. Hence, for solubilization of Ciclopirox. Formulation of Nanoemulsion appeared to be a viable approach. Nanoemulsions have gained lot of attention for delivery of hydrophobic agents for local treatment; because of its ability to increase solubility, permeability of drug.

Drug delivery through the skin to the systemic circulation is convenient for a number of clinical conditions due to which there has been a considerable interest in this area. It offers the advantage of steady state controlled drug delivery over extended periods of time, with self- administration also being possible, which may not be the case with parental route. The drug input can be eliminated at any time by the patient just by washing off the applied dosage. An extra advantage is the total absence of gastrointestinal side effects like irritation and bowel ulcers which are invariably associated with oral delivery. Topical delivery has been developed for a number of disease and disorders. The treatment of skin diseases as well as musculoskeletal disorders might be advantageous from topical administration obtaining a considerable reduction in oral side effects with improved patient compliance.

Hence the present work was done with an objective to formulate Ciclopirox (1% w/w) in the form of emulgel, containing Nanoemulsion of the drug, with a view to design a stable topical nanoemulgel of Ciclopirox that will effectively release drug for prolong period time, To reduce the frequency of dosing by preparing formulation for prolong release and thus improve patient compliance and to study the antifungal activity of formulation against the Trichophytonrubrum.

MATERIALS AND METHOD

MATERIALS

Ciclopiroxwas received as a gift sample from Aarti Drugs Ltd. Carbopol 934 from Molychem, Mumbai. Almond Oil, Tween 80 and Propylene

GlycolFrom Research -lab Fine Chem Industry, Mumbai. All other solvent and reagent are used was of analytical grade.

2. EXPERIEMENTALS

2.1 Identification of Drug

2.1.1 By UV Spectroscopy

The UV spectrum of Ciclopirox was obtained using UV-Visible Double Beam Spectrophotometer. Accurately weighed 10 mg of the drug was dissolved in sufficient quantity of methanol. Stock solutions (100 µg/ml) of Ciclopirox were prepared in methanol. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectrophotometerexhibited wavelength of absorbance maxima at 256 nm.λmax of Ciclopirox in Methanol has been shown in the following figure 1.

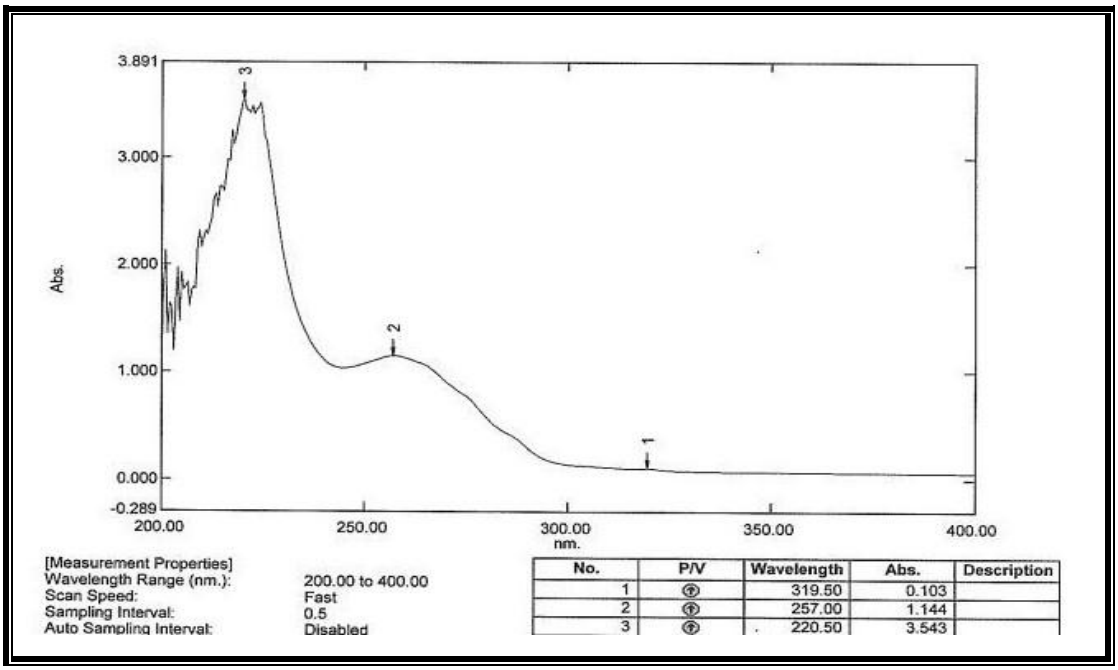


Figure 1: Ultraviolet Spectra of Ciclopirox in Methanol

2.1.2 By melting point determination

The melting point of the drug was determined by using open capillary method using the melting point apparatus. The melting point was found in the range of 110-114^0 C.

Figure 2 Melting Point of Ciclopirox

Drug	Observed Value	Reported Value
Ciclopirox	110-114^{0}c	110-113^{0}c

2.3 Preparation of standard Calibration curve of Ciclopirox

Accurately weighed 10 mg Ciclopirox and transferred to 10 ml volumetric flask. The volume was made up to 10 ml with methanol and sonicated for 5 min. to produce stock solution of 100 µg/ml. Working standard solutions of strengths 2-10 µg/ml were made from the stock solution by appropriate dilutions. The above solutions were analysed by UV spectrophotometer at λ_{max} 256 nm. The calibration curve was found to be linear in the concentration range of 100 µg/ml given in following table.

Table 3: Calibration Curve of Ciclopirox in Methanol

Sr. No.	Conc.(ppm)	Absorbance
1	2	0.276
2	4	0.458
3	6	0.680
4	8	0.888
5	10	1.144

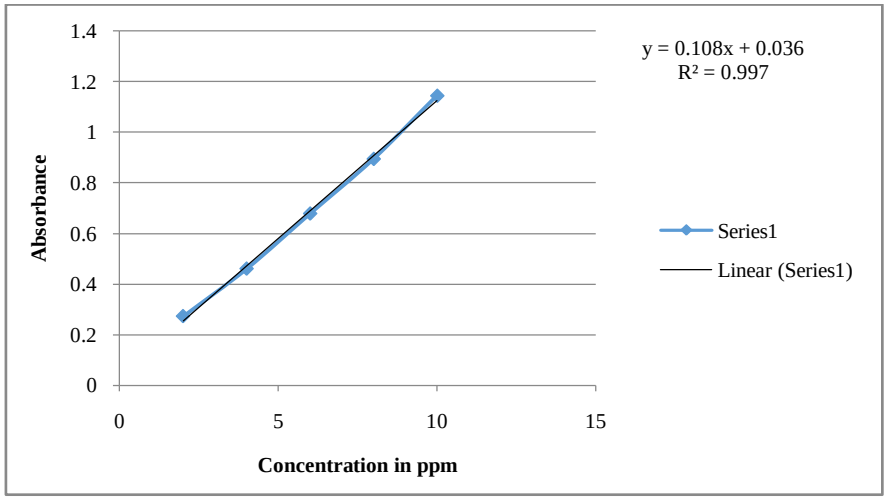


Figure 4: Calibration curve of Ciclopirox in Methanol

2.4 Solubility studies of drug

The solubility of Ciclopirox in various oils, surfactants was determined by adding an excess amount of drug to 5 ml of selected oils, surfactants, separately in 10 ml capacity stopper vials, and mixed using a vortex mixer. The mixtures were then kept on magnetic stirrer for 48 hrs at 40±0.5°C (RAJ 305-C). Further kept for 24 hours at room temperature to reach equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min followed by filtration through a 0.45-µm membrane filter. The filtrates were diluted with methanol and Ciclopirox solubility was subsequently quantified by UV.

Solubility study of drug in different oils:

Table 5 : Solubility of Ciclopirox in different oils:

Sr. No.	Oils	Solubility
1	Castor oil	10.30
2	Oleic acid	12.32
3	**Almond oil**	**31.01**
4	Liquid paraffin	9.35
5	Isopropyl myristate	20.67

Solubility determination of Ciclopirox in surfactants and cosurfactant

Table 6: Solubility of Ciclopirox in different surfactants and cosurfactant

Sr. No.	Excipients	Solubility (mg/ml)
1	Tween 20	28.04
2	Span 20	3.06
3	**Tween 80**	**37.29**
4	Span 80	30.43
5	**Propylene glycol**	**35.67**

2.5 FTIR spectroscopy:

The IR spectrum of Ciclopirox was recorded at wave number 4000 to 50cm^{-1} using Fourier transform infrared spectrophotometer (Mode- FTIR, Bruker). Method used for analysis was ATR. The methods above measure the infrared spectrum for powder samples mixed in a medium such as KBr or liquid paraffin. However, ATR method is able to measure powder samples directly. Attenuated Total Reflection (ATR) method involves pressing the sample against high refractive index prism and measuring the infrared spectrum using infrared light that is totally internally reflected in prism.

The FTIR spectrum of Ciclopirox has been shown in figure 15. The major peaks observed and corresponding functional groups are given in Table 7. The spectrum shows characteristic peaks for Ciclopirox.

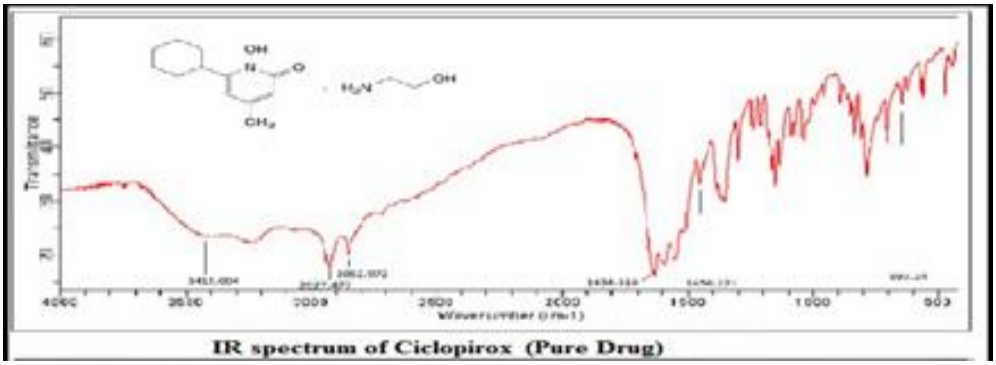


Figure 7: Representative IR spectrum of Ciclopirox

Table no.8: Interpretation of FTIR spectrum of Ciclopirox

Range(cm-1)	Values(cm-1)	Bond
2923(m)	2923	Aliphatic C-H stretching
1680-1640(m)	1616	Aromatic C=C stretching
1466 , 1236(s)	1238, 1210	C=S stretching link to nitrogen
1335-1250(s)	1210	C-N stretching
1153, 1112 (s)	1158	C-O stretching
3100-3000(s)	3059,3026	Aromatic C-H stretching

The absorption bands shown by Ciclopirox are characteristics of the groups present in its molecular structure. The presence of absorption bands corresponding to the functional groups present in the structure of Ciclopirox confirms the identification and purity of the Ciclopirox sample used in the study.

3.Formulation and Development of Nanoemulsion:

3.1 3^2Full Factorial Design:

For the present work 3^2 full factorial designs was selected. It has been summarized in Table 14. In this design, 2 factors were evaluated each at 3 levels and experimental trials were performed at all 9 possible combinations as reflected in table no. 9. The two independent variables selected were Almond oil (x_1) and Speed of homogenizer (x_2).

Table 9: Experimental Design as per 3^2 Full Factorial Designs

Formulation code	Coded values		Coded Values	
	X_1	%	X_2	RPM
F1	+1	3	+1	25000
F2	+1	3	0	20000
F3	+1	3	-1	15000
F4	0	2	+1	25000
F5	0	2	0	20000
F6	0	2	-1	15000
F7	-1	1	+1	25000
F8	-1	1	0	20000
F9	-1	1	-1	15000

Table 10 : Composition of Nanoemulsion formulation as per 3^2 full factorial designs

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ingredients	**%**								
Ciclopirox (w/w)	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Almond Oil (v/v)	**3**	**3**	**3**	**2**	**2**	**2**	**1**	**1**	**1**
Tween 80 (v/v)	**5.25**	**5.25**	**5.25**	**5.25**	**5.25**	**5.25**	**5.25**	**5.25**	**5.25**
Propylene glycol (v/v)	**1.75**	**1.75**	**1.75**	**1.75**	**1.75**	**1.75**	**1.75**	**1.75**	**1.75**
Methyl Paraben (w/w)	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**
Propyl Paraben (w/w)	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
BHT	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Water (v/v)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

3.2 Method of preparation for Nanoemulsion:

The quantities of drug and other ingredients were weighed by calculating equivalent amounts as per table 16 and formulations were prepared in following manner.

Cleaning of glassware and container: All the glassware were washed with distilled water and then sterilized by drying at 160-165^{0}c for 1 hr. in hot air oven.

Preparation of aqueous phase 'A': Accurately weighed quantity of propylene glycol was added into distilled water (80^{0}c).

Preparation of Oil phase 'B': Weighed quantity of Almond oil and tween 80 mixed together by maintaining hot condition, simultaneously accurately weighed quantity of Ciclopirox was added into it then addition of methyl paraben, propyl paraben and BHT in it.

Incorporation of solution 'A' in dispersion 'B': Both the phases were mixed properly with the help of High pressure Homogenizer maintaining the respective rpm.

Preparation of gel:

Table 11: Composition of gel

Sr. No.	Ingredients (% w/w)	Quantity
1	Carbopol 934	1%
2	Triethanolamine	0.1%
3	Water (q.s.)	100

The weighed quantity of carbopol 934 was mixed in distilled water (40^{0}c) further addition of triethanolamine to maintain the desired pH range of the solution. The uniformity in the stirring was maintained and then the gel was kept in the refrigerator for 24 hrs.

Preparation of Emulgel:

Further incorporation of nanoemulsion containing 1% drug was incorporated to obtain emulgel.

Filling to container:

The formulation was transferred into previously cleaned and dry containers.

3.3 Evaluation of Nanoemulsion:

3.3.1 Scanning Electron Microscopy:

The morphology of nanoemulsion can be determined by scanning electron microscopy (SEM). SEM gives a three-dimensional image of the partical. 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.

sScanning electron microscopy of Nanoemulsion is shown in figure 13. The shape of Nanoemulsion was Spherical and the size of the Nanoemulsion was below micrometer range. Moreover, the micrograph also revealed the some agglomeration of nanoemulsion which might be due to the evaporation of water present in formulation during sample preparation prior to SEM analysis.

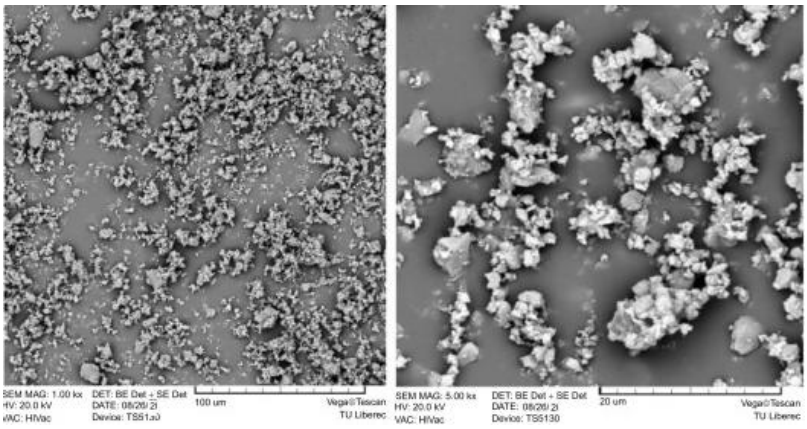


Figure 12: Scanning Electron Microscopy

3.3.2 Particle Size Analysis:

Formulated Nanoemulsion should be analysed for their hydrodynamic particle size. Generally, in case of nanoemulsion dynamic light scattering method used for the measurement of particles and further particle size distribution.

The Particle size of the Nanoemulsion of optimised batch was found to be 100 nm. It is seen with increase in concentration of Almond oil with high speed of homogenizer decrease in particle size.

Table 13: Size distribution and PDI

Formulation code	Particle size (nm)	PDI
Optimized Batch (F1)	100	0.196

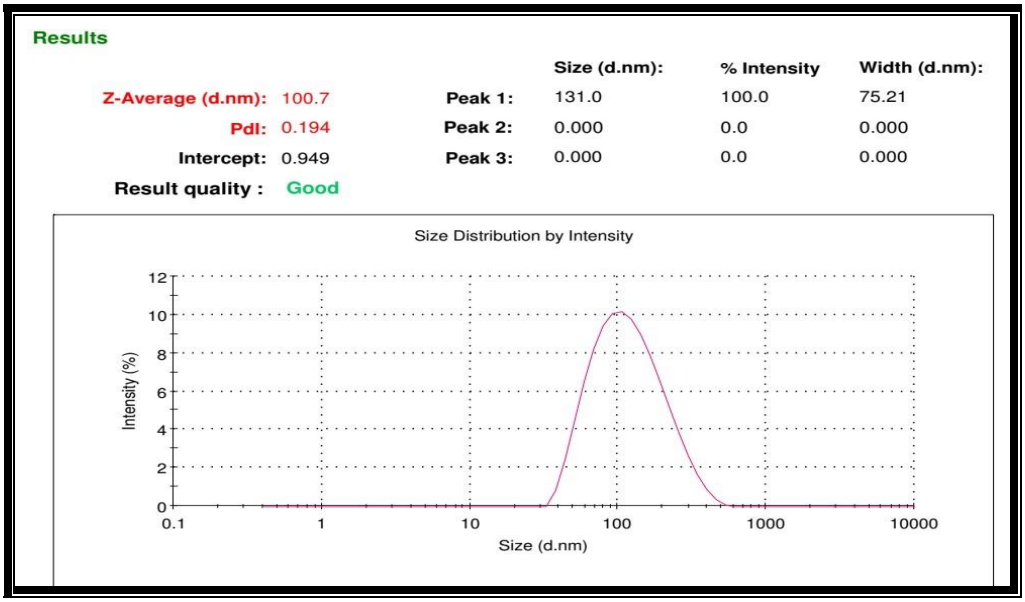


Figure 14: Graph of Particle size of Optimized formulation (F1)

3.3.3 Zeta potential measurements:

Zeta potential for nanoemulsion was determined using zetasizerhsa 3000 (Malvern instrument Ltd., UK). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment.

Zeta potential shows the stability of the (colloidal dispersion) nanoemulsion under the stress testing condition according to ICH guidelines of stability studies of various pharmaceutical formulations. Zeta potential is affected by particle size, lowest particle size in nano size i.e. 100, shows -32 mV. Zeta potential which indicate thermodynamic instability of the dispersion.

Table 15 : Zeta Potential

Formulation Code	Zeta Potential
Optimized Batch	-32.0

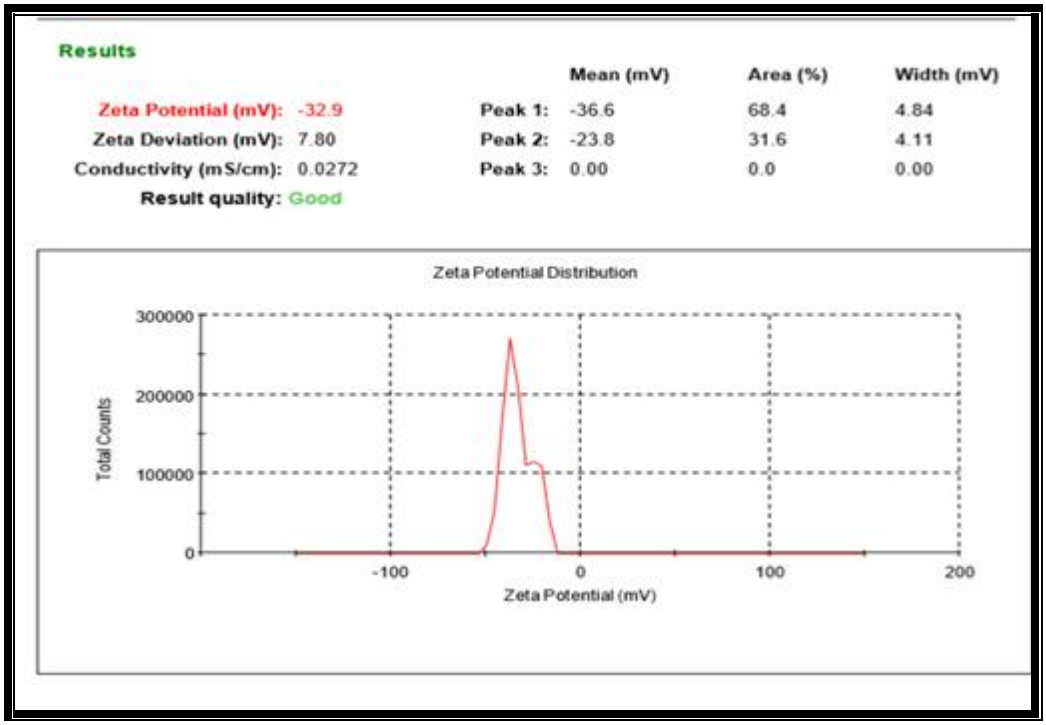


Figure 16: Graph of Zeta Potential of optimized formulation

3.3.3 Entrapment efficiency:

Entrapment efficiency is defined as the percentage amount of drug which is entrapped by the Nanoemulsion. For the determination of entrapment efficiency, the unentrappped drug was first separated by centrifugation at 15000 rpm for 30 minutes.

The resulting solution was then separated and supernatants liquid was collected. The collected supernatants was then diluted appropriately with methanol and estimated using UV visible spectrophotometer at 256 nm.

The maximum Entrapment efficiency was found to be 96.5% and the minimum Entrapment efficiency was found to be 69% in figure. It has been observed that the drug entrapment efficiency was highest for optimised batch (F1).

Table 17: Entrapment efficiency of formulation F1 to F9.

Sr. No.	Formulation code	% Entrapment Efficiency
1	**F1**	96.4
2	**F2**	94.1
3	**F3**	91.6
4	**F4**	89.1
5	**F5**	86.2
6	**F6**	83.1
7	**F7**	76.2
8	**F8**	69.1
9	**F9**	85.0

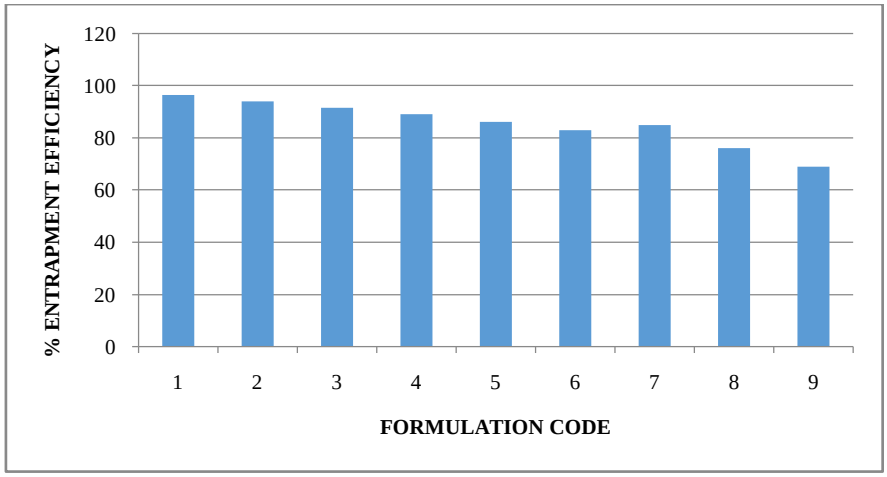


Figure 18: Entrapment efficiency of F1 to F9

3.4 Evaluation of Nanoemulsion based Gel:

3.4.1 Appearance:

The prepared nanoemulgel formulations were inspected visually for their colour, homogeneity and consistency.

Table 19 : Physical appearance of formulations

Sr. No.	Parameters	Inference
1	Colour	Translucent gel
2	Homogeneity	Homogeneous
3	Consistency	Consistent

The physical appearance of the emulgel formulation was found to be Translucent, homogeneous and consistent.

3.4.2Measurement of viscosity :

The viscosity of the formulated batches was determined using a Brookfield Viscometer (RVDV-I Prime, Brookfield Engineering Laboratories, USA) with spindle 63. The formulation whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 min at the assay temperature (25±1ºC) before the measurement was taken. Spindle was lowered perpendicular in to the centre of emulgel taking care that spindle does not touch bottom of the jar and rotated at a speed of 50 rpm for 10 min. The viscosity reading was noted.

The viscosity values of formulations are shown in the following table 20:

Table 20: Viscosity of formulations

Rpm	Viscosity (cP) at Room Temperature								
	Formulation Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
10	14960	13450	14500	13750	12500	13500	14500	13500	12000
20	14200	12390	14000	13400	12250	12440	14250	12500	11709
30	13050	12050	13445	12350	11200	12203	13900	12000	10500
40	13000	11500	12230	12010	11000	11253	12750	11500	98500
50	12350	10420	11520	11250	10950	10504	12520	11200	92300

Viscosity is resistance to flow, which is important physicochemical property for topical preparations because it influences Spreadability and drug release as well as jellification. Rheological behavior of the emulgel indicates that the system was shear thinning in nature showing decrease in viscosity at increasing shear rate. The values of
viscosity measurement of all formulation are listed in table 32. This viscosity result resembles that the increase in proportion of Almond oil and increase in speed of homogenizer results in decrease in viscosity.

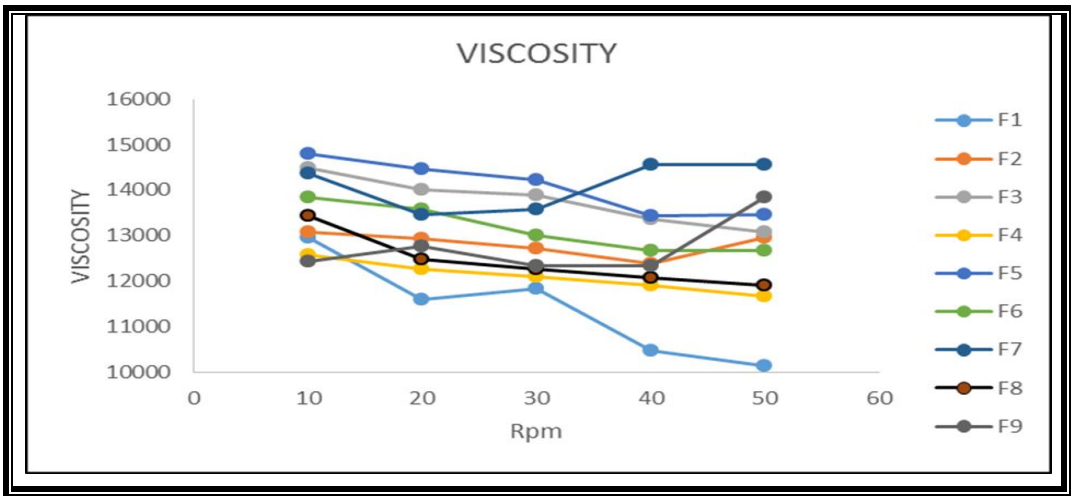


Figure 21: Viscosity of formulation

3.4.3Spreadability:

To determine spreadability of the gel formulations, two glass slides of standard dimensions were selected. Formulation whose spreadability was to be determined was placed over one slide and the other slide was placed over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower slide is held firm by the opposite fangs of the clamp allowing the upper slide to slip off freely by the force of weight tied to it. 20 gm weight was tied to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the following formula.

S = M. L/T

Where, M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides

gel having optimum viscosity provides proper spreadability to the formulations. Formulation F1, Having optimum viscosity and spreadability of this formulation is 17.77 gm.cm/sec.

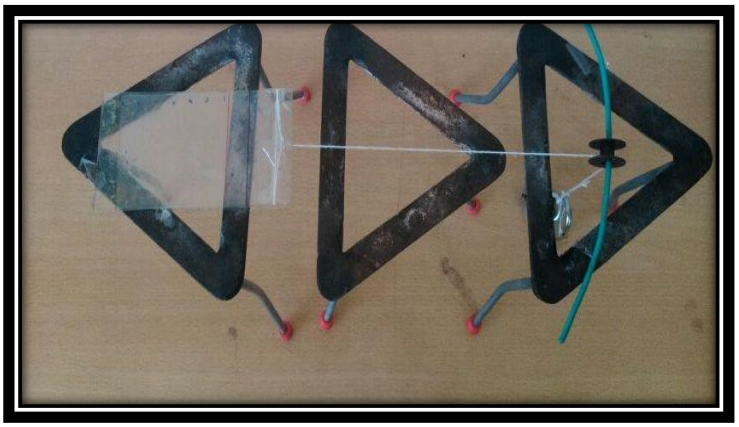


Figure 22: Spreadability Apparatus

Table 23: Spreadability values of formulation

Sr. No.	Formulation code	Spreadability (g.cm/sec)± S.D.
1	F1	17.74 ± 0.025
2	F2	16. 0±0.035
3	F3	15.34 ±0.028
4	F4	15.67 ±0.018
5	F5	15.10 ±0.032
6	F6	14.82 ± 0.012
7	F7	15.54 ± 0.012
8	F8	15.21 ± 0.011
9	F9	15.86 ±0.018

3.4.4Drug content study :

Drug content study was done to determine the amount of the drug present in the certain quantity of the formulation. Took 1 g of the formulation into 10 ml volumetric flask added methanol in it and shake well and make up the volume with methanol. The Volumetric flask was kept for 2 hr and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered the mixer then measured absorbance by using spectrophotometer at 256 nm.

The drug content of formulation has shown in following table:

Table 24: Drug content of formulation

Sr. No.	Formulation code	Drug content (%)± SD
1	F1	97±0.5
2	F2	91.81±0.7
3	F3	96±0.7
4	F4	93.96±0.7
5	F5	94. 81±0.7
6	F6	73±0.7
7	F7	67±1.09
8	F8	83±1.07
9	F9	63.91±1.43

The percentage drug content of all prepared emulgel formulations was found to be in the range of 64 to 96%. Therefore uniformity of content was maintained in all formulations. TheF1 Formulation drug content was found to be 96%.

3.4.5 In-vitro Drug release study:

The in vitro drug release studies of the Emulgel were carried out on Diffusion cell using egg membrane. This was clamped carefully to one end of the hollow glass tube of dialysis cell. Emulgel (1gm) was applied on to the surface of egg membrane dialysis membrane. The receptor chamber was filled with freshly prepared PBS (pH 7.4) solution. Total amount of gel filled in the tube to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1ml aliquots) were collected at suitable time interval sample were analyzed for drug content by UV visible spectrophotometer at 256 nm after appropriate dilutions. Cumulative corrections were made to obtain the total amount of drug release at each time interval. The cumulative amount of drug release across the egg membrane was determined as a function of time. The cumulative % drug release was calculated using standard calibration curve.

The in- vitro release of Ciclopirox from its various emulgel formulae are represented in the figure 27 and table 25 and 26.

Table 25: Cumulative amount of Ciclopirox diffused (%) from all the emulgel formulations through egg membrane using Modified Franz diffusion cell

Time hrs	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	9±0.70	8.17±0.76	7.14±0.22	6.25±0.071	6.13±0.75	5.34±0.82	5.01±0.70	3.41±0.85	2.31±0.53
2	17±0.70	14.08±0.73	12.96±0.51	15.22±0.24	11.13±0.75	12.96±1.06	16.74±0.97	19.15±0.75	16.24±0.79
3	25±1.07	24.21±0.74	23.01±0.16	22.11±0.17	23.12±0.74	19.66±0.39	21.30±0.81	18.12±0.74	20.11±0.74
4	34±1.06	32.65±0.38	31.09±0.12	28.23±0.79	26.61±0.84	25.66±0.64	23.49±0.88	22.44±0.31	20.66±0.94
5	41±0.70	40.42±0.85	40.97±0.52	35.49±0.88	30.99±0.50	32.67±0.95	34.69±095	39.45±0.69	39.41±0.85
6	50±0.53	48.57±0.38	47.87±0.47	45.66±0.72	45.35±0.83	40.19±0.19	38.09±0.75	35.66±0.94	30.11±0.74
7	59±1.06	57.45±0.31	55.13±0.94	52.79±0.99	48.49±0.88	44.09±0.10	41.49±0.88	38.09±0.73	37.71±0.96
8	68±1.07	65.15±0.27	62.14±0.16	60.49±0.32	57.18±0.76	54.66±1.2	51.78±0.66	48.83±1	49.89±1.03
12	78±1.41	72.30±0.28	74.25±0.32	64.49±0.33	62.16±0.75	58.19±0.19	56.99±1.07	54.97±1.04	52.31±0.81
16	85±1.04	81.89±0.50	73.41±0.64	70.89±1.05	68.12±0.74	64.69±0.45	61.44±0.86	58.10±0.73	54.14±0.54
24	96±0.66	90±0.38	87.42±0.30	78.94± 0.50	72.09±0.73	69.99±1.0	65.05±0.72	61.19±0.76	58.45±1.21

The in- vitro release of Ciclopirox from its various emulgel formula are represented in the figure 27 and table 26.

Table 26: Cumulative drug release of formulation F1 and Marketed formulation

Time (hours)	% Cumulative drug Release + S.D. (F1 formulation)	Time (hours)	% Cumulative Drug Release + S.D. (Marketed formulation)
0	0	0	0
1	9±0.70	1	8±0.77
2	17±0.70	2	15±0.707
3	25±1.07	3	24±0.70
4	34±1.06	4	34±0.70
5	41±0.07	5	42±0.72
6	50±0.77	6	55.57±0.91
7	59±0.70	7	60.45±0.83
8	68±0.71	8	69±0.707
12	78±0.70	9	76.90±705
16	85±0.77	10	82±0.73
24	96±0.71	12	92±0.76

It was observed that the release of the drug from optimized (F1) emulgel formulation was higher than the commercial cream. (Tinactin 1 % cream). The drug release of optimised formulation shows the controlled release up to 24 hrs (96 %) and marketed formulation shows (92 %) drug release upto 12 hrs. Formulation F1 showed steady state release upto 24 hours which also indicates that this formulation would show better contact with biological

membrane. The drug is entrapped in the oil phase, hence when formulation was applied on egg membrane the penetration takes place upto 24 hrs. This phenomenon of drug release also suggests that when such formulations would be applied on skin surface the drug diffusion follows mechanisms.

Drug which is adsorbed on nanoemulsion will diffuse through stratum corneum at the rapid rate and would be available quickly into the epidermal region. The formulation which has less entrapment efficiency would follow this mechanism because of the more free drug available on the surface.

The drug embedded in the Nanoemulsion may leach on the surface of the skin by diffusion process and this leached drug may further diffuse through the stratum corneum. The Nanoemulsion which has low entrapment efficiency also follows this principle in addition to the above principle A. because of which it shows rapid availability of the drug in epidermal region.

The highest drug loading Nanoemulsion follows the drug diffusion by following mechanism i.e. as the skin hydration is improved because of prevention of transepidermal water loss due to the Nanoemulsion loaded gel. The nanoemulsion moves towards the skin of higher water concentration across the stratum corneum because of longer time of hydration the Nanoemulsion are enforced through wide aqueous pores and Nanoemulsion are available in the epidermal region. From the epidermal available Nanoemulsion further the drug would be released in controlled fashion which ultimately improves the antifungal activity of the entrapped drug in the Nanoemulsion.

When marketed formulation compared with Nanoemulsion loaded gel the marketed formulation has only shown the release upto 12 hrs. The possible mechanism of drug penetration for the marketed formulation may be only by process of diffusion of the free drug across the stratum corneum.

The reported value of Biological half –life was also increases its residence time in the bio fluids at the site of release which improves its antifungal property.

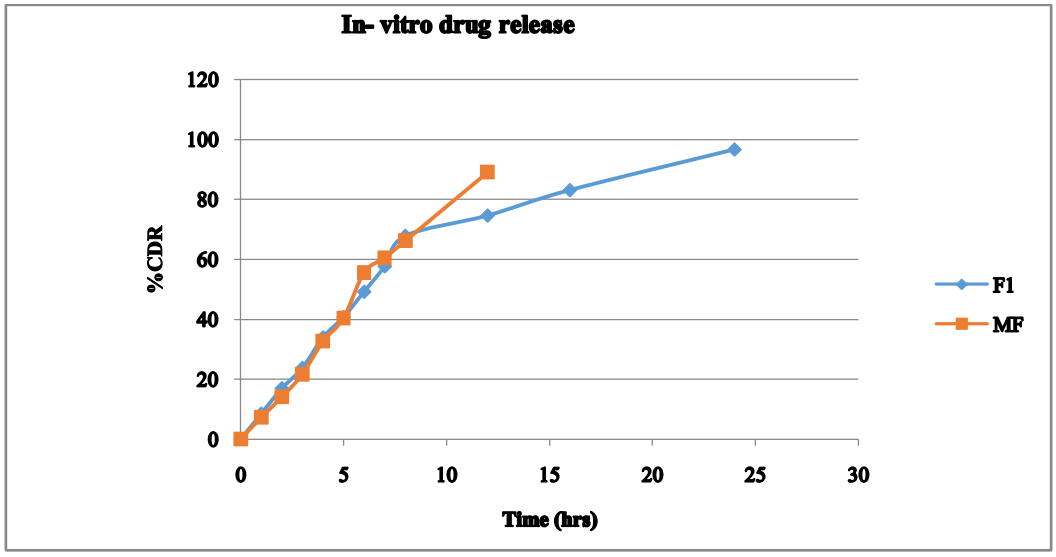


Figure 27: In-Vitro Drug release profile of optimized formulation (F1) and Marketed formulation.

3.4.6 Antifungal study :

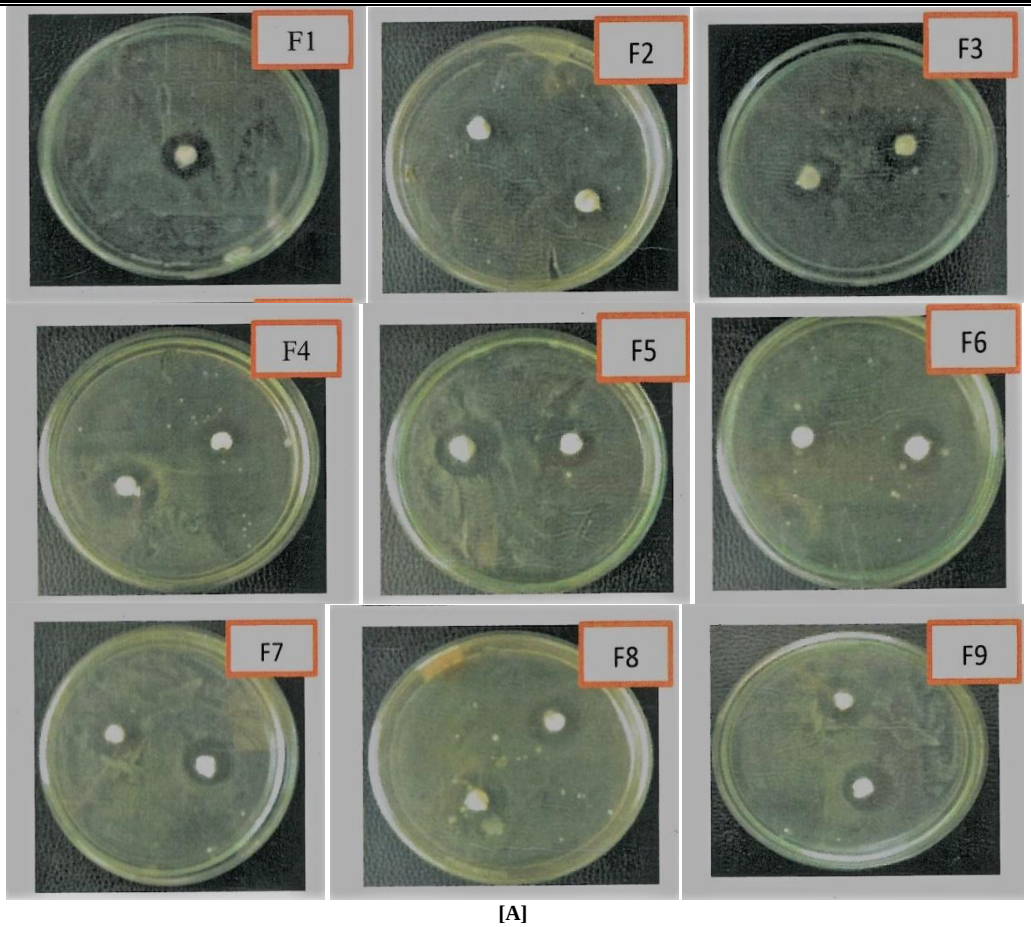
An agar diffusion method used for determination the antifungal activity of formulation. Standard petri dish 9 cm containing medium to depth of 0.5 cm were used. The sterility of the lots was controlled before used. Incubation were prepared by suspending 1-2 colonies of Trichophytonrubrum (ATCC 28188) From 24 hr. Cultures in sabouraud's medium in to tube contain 10 ml of sterile saline. The tubes were diluted with saline. The inoculum spread over the surface of agar medium. The plate was dried at 35º C for 15 min prior to placing the formulation. The boars of 0.5 cm diameter were prepaid and 20 µl sample of formulation (1 % w/v) were added in the bores. After incubation at 35ºC for 24 hr. the zone of inhibition around the boars are measure. Simultaneously the antifungal activity of the marketed formulation (Tinactin 1% cream, 1% suspension) without nanoemulsion and optimized batch was recorded.

Observed value of (% Drug suspension) for Ciclopirox against Trichophytonrubrum (ATCC 28188) for zone of inhibition is 15. The study indicates that Ciclopirox retained its antifungal activity when formulated in Nanoemulsion loaded emulgel and Ciclopirox was active against selected strain of micro-organism. F1 shows a zone of inhibition of 24 mm.

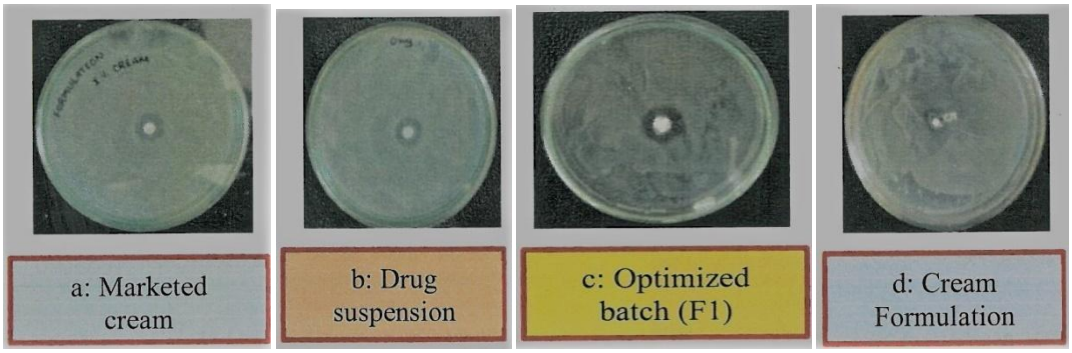
The results of antifungal activity of formulation have been shown in following table 40; Standard value of (Drug suspension) for Ciclopirox against Trichophytonrubrum for zone of inhibition is 24 mm.

Table 40: Antifungal Activity of Formulation F1 to F9

Sr. No.	Formulation code	Trichophytonrubrum
		Zone of inhibition (mm)
1.	**F1**	24
2.	**F2**	23
3.	**F3**	22
4.	**F4**	21
5.	**F5**	20
6.	**F6**	19
7.	**F7**	18.40
8.	**F8**	18
9.	**F9**	17
10.	**1% Drug suspension**	15
11.	**Marketed formulation (Tinactin cream 1%)**	16.40
12.	**1% Formulated cream**	15



[A]



[B]

Figure 28: Zone of inhibition for all formulations

A: F1 to F9 Batches

B: Comparative study of Antifungal activity

3.4.7 Accelerated stability studies of Emulgel:

Stability studies are performed by guidelines. The organized emulgels were full in aluminum collapsible tubes (5 g) and subjected to strength learns at 5°C, 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH and 60 ± 2° for a period of 3 months. Tests were pulled back at 15-day time between times and surveyed for physical appearance, pH, rheological properties and pharmaceutical substance.

The optimized formulation was evaluated after storage accelerated condition and Room Temperature. The results of stability studies show that the formulation was stable at Accelerated temperature conditions (40^0 C± 2^0 C, 75 % RH ± 5% RH). Results have been given in table 29.
Stability study of Optimized batch F1 was done at Room Temperature.

Table 29: Stability Study data for F1 formulation at Accelerated condition (40^0 C± 2^0 C, 75 % RH±5% RH)

Sr. No	Observations		Before Stability Testing	During study
				3rd month
1	**Clearity**		Translucent	Translucent
2	**pH**		6.84±0.006	6.80±0.008
3	**% Drug content**		96±0.5	95.97± 0.5
4	**Viscosity**	**10**	12961cp	10233 cp
		20	11590cp	10123cp
		30	11821cp	9876cp
		40	10478cp	10122cp

CONCLUSION

Antifungal therapy is one of most effective mechanism to eradicate the fungal infection for improving quality of life. Systemic treatment is usually reserved for infections of the nails, extensive cutaneous infections or those which have not responded to topical therapy. Conventional topical formulations are unable to retain and controlled the delivery of the drug over the skin for a prolonged period and hence necessitate longer treatment duration or have to be supplemented by oral therapy. Fungal infections require frequent application of conventional formulations for longer duration. An emulgel would facilitate prolonged contact of the drug with skin, also has capability of altering the skin properties thereby improving the topical treatment of fungal skin infections.

The strategy is to formulate drug loaded Nanoemulgel that would control the release of the drug over the dermal surface for 24 hrs.

To fulfill all this parameters, drug loaded Nanoemulgel formulations were prepared. Nanoemulsion were prepared by high speed homogenization and studied for different parameters. The problems associated with emulsion stability was also overcome by formulating drug loaded Nanoemulgel by 3^2

full factorial design in which Almond oil (%) and speed of homogenizer (rpm) were taken as independent factors in 3 different levels. Before formulating this formulations Preformulation testing were performed for drug characterization and to analyse its purity and compatibility. Organoleptic properties, melting point, solubility testing, UV spectroscopy studies and FTIR were performed for the Ciclopirox and the drug sample procured were found to be and compatible with the excipients used in formulation. For optimization design expert software (version 11) was used. The drug loaded Nanoemulsion's were evaluated for Particle size, Polydispersibility index, Zeta potential and scanning electron microscopy analysis. Drug loaded emulgel were evaluated for physical appearance, pH, viscosity, spredability, drug content, *in vitro* drug release study(diffusion study), antifungal activity and Accelerated stability studies. The dependent variables Antifungal activity and Diffusion study were statistically evaluated using ANOVA and the 3-D response surfaces were plotted for interpretation.

From all this studies, data analysis following conclusions can be drawn:

A- Amongst all the formulations, Nanoemulsion loaded emulgel prepared with the tween 80, Almond oil was found to be better with the drug diffusion.

B- The particle size of optimized formulation (Nanoemulsion) was found to be 100.0 nm which suggest the possible increased penetration of drug through biological membrane.

C- Zeta potential of optimized formulation (Nanoemulsion) -32.0 mV which indicates the thermodynamic instability of the dispersion.

D- Scanning electron microscopy shows spherical shape and size below micrometer range.

E- Decrease in viscosity value leads to increase in spredability value of all the viscosity results suggest, the suitability of formulation for external use.

F- The percentage of drug content was found to be in 64-96% hence, uniformity of content was maintained.

G- When the optimized formulation was compared with marketed formulation for in- vitro drug release, it was found to be controlled release for optimized batch (F1) in 24 hrs. And marketed formulation in 12 hrs.

H- The formulation followed the Higuchi Kinetic model of drug release.

I- Accelerated stability study showed no significant change in the formulation upto 3 months.

Finally it can be summarized and concluded that the Nanoemulsion loaded emulgel of Ciclopirox can be one of the promising tool in controlling the drug release via. Percutaneous mechanism for effective and longer treatment required for fungal infections with increased stability.

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