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DESIGN AND CHARACTERIZATION OF NANOEMULGEL CONTAINING GRISEOFULVIN

Shripathy D.*, Sneha V. and Shabaraya A.R.

Associate Professor, Department of Pharmaceutics, Srinivas College of Pharmacy Valachil, Farangipete Post, Mangalore, Karnataka, India-574143.

***Corresponding Author: Shripathy D.**
Associate Professor, Department of Pharmaceutics, Srinivas College of Pharmacy Valachil, Farangipete Post, Mangalore, Karnataka, India-574143.

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ABSTRACT
Nanoemulgel have emerged as one of the most interesting topical drug delivery system as it has dual release control system i.e. Nanoemulsion and gel. When gels and emulsions are used in combined form the dosage form are known as emulgel. Griseofulvin is an antifungal medication topically administerd to treat skin infections such as jock itch, athlete's foot and ring worm. The aim of the present research work was to investigate the potential of Nanoemulgel in enhacing the topical delivery of hydrophobic drug. Grseofulvin Nanoemulsions were prepared using span80, tween 80, propylene glycol and clove oil by high speed homogenization.Pseudo ternary phase diagram was generated by using water titration method. The prepared Nanoemulsions were evaluated for pH, drug content, centrifugation, globule size and zeta potential. Nanoemulgel was prepared by using carbopol 934 as gelling agent. Best formulation GF5 showed a maximum drug release of 83.42±0.03 in 8 hours, particle size of 51.9 nm and zeta potential of -24.6 mV. GF5 showed highest drug content 90%. The release kinetics of Nanoemulgel was found to obey zero order kinetics. The Nanoemulgel was found to be stable with respect to physical appearance, pH, rheological properties, spreadability and drug content at all temperature and conditions for two months. Thus, it can be concluded that Griseofulvin Nanoemulgel formulation is a promising system for the topical drug delivery and also an alternative method to deliver the hydrophobic drug in water soluble gel bases

KEYWORDS: Nanoemulsion, Nanoemulgel, Topical drug delivery.

INTRODUCTION
For topical administration skin is one of the most readily accessible parts of human body and molecules penetrate in the skin mainly by three routes: through intact stratum corneum, through sweat ducts, and through the sebaceous follicle.[1] Topical drug delivery is used for localized action on the body through ophthalmic, rectal, vaginal and skin as topical routes. The topical drug delivery system such as emulgel (gellified emulsion) generally used where the other systems of drug administration fails to directly treat cutaneous disorders such as fungal infections, acne, psoriasis etc. Absorption of drug through the skin is enhanced if the drug substance present in solution, if lipid/water partition coefficient of drug substances has favourable and if it is a nonelectrolyte.[2] For the most of the time, pharmaceutical formulation applied to the skin are intended to serve some local action and as such preparations are formulated to provide prolonged local contact with minimal systemic drug absorption. Drug such as antiseptics, antifungal agent, skin emollients are applied to the skin for their local action. Avoidance of first pass metabolism is the main advantage of topical drug delivery system, it also Avoid the risks and inconveniences of intravenous therapy and of the varied conditions of absorption like pH changes, presence of different enzymes, gastric emptying time are other advantages of topical preparations. The transparent gels use has been expanded both in cosmetics and in pharmaceutical preparations.[3] As compared with conventional ointments and creams, gel formulations generally provide faster drug release. Difficulty in delivery of hydrophobic drugs is major limitation of gels. So to overcome this limitation emulgels are prepared and with their use even a hydrophobic drug can enjoy the unique properties of gels. When gels and emulsions are used in combined form the dosage forms are referred as emulgel. In fact, the presence of a gelling agent in the water phase converts a classical emulsion into an emulgel.[4]

MATERIALS AND METHODS
Materials
Antifungal drug i.e., Griseofulvin from yarrow chemicals, Mumbai, India. Carbopol 934, Clove oil and Methanol from yarrow chemicals, Mumbai, India. Tween 80 and Span 80 from Hi- Media laboratory, Mumbai, India. Propyl paraben, Propylene glycol and

Triethanolamine from Loba cheme laboratory. Metyl paraben was purchased from SD-fine chem limited, Mumbai, India.All chemicals were of pharmaceutical grade and used without further modification.

METHODS

Preformulation Studies

1. Organoleptic characteristics[5]

The color and appearance of the drug were characterized and recorded.

2. Determination of melting point[5]

Melting point was determined by capillary method. Fine powder of Griseofulvin was filled in a glass capillary tube (sealed at one end). Capillary tube was placed in melting point apparatus attached with thermometer. Note down the temperature at which drug melts completely.

3. Preparation of phosphate buffer pH 7.4[6]

Dissolve 250 ml of potassium dihydrogen phosphate and 195.5 ml NaOH in sufficient water to produce 1000ml.

4. Screening of the absorbance maxima (λmax) of Griseofulvin[7]

The standard solution of Griseofulvin (10µg/ml) in phosphate buffer of pH 7.4 and methanol was scanned in the wavelength region of 200-400 nm to obtain λmax.

5. Preparation of stock solution of Griseofulvin using phosphate buffer pH 7.4

Stock solution A: (1000µg/ml)

Stock solution of Griseofulvin were prepared by dissolving 100mg of the drug in 10ml of methanol and the volume was made up to 100ml with phosphate buffer pH 7.4 which gives drug concentrations 1000µg/ml. (stock A).

Stock solution B:(100µg/ml)

10 ml of stock A were transferred to 100ml volumetric flask and the volume made up to 100ml with phosphate buffer pH 7.4 to get concentration of 100µg/ml (Stock B).

Standard calibration curve

0.2ml, 0.4ml, 0.6ml,0.8ml and 1ml of the stock solution B was diluted with phosphate buffer pH 7.4 to make the concentration of 2,4,6,8,10µg/ml respectively. absorbance of each solution were measured at λmax using UV spectrophotometer. Standard curve is generated for the entire range from 2 to 10µg/ml.

6. Solubility[8]

It was determined by dissolving Griseofulvin in methanol, water and phosphate buffer pH 7.4. The solubility study was conducted by taking excess quantity of the drug in 10ml of solvent. Then the sample were kept in magnetic stirrer and agitated for 24 hrs at 37±0.5˚C. The sample were filtered and diluted suitably with buffer solution. The samples were analyzed spectrophotometrically at λmax. The concentration of Griseofulvin was determined using respective standard graph.

7. Drug polymer interaction by FT-IR[9]

The samples were finely grounded and mixed with dry KBr powder. The samples were screened over a spectrum of 4000 to 400 cm^{-1}.

8. Construction of the pseudo-ternary phase diagram study[10]

Pseudo-ternary phase diagram were constructed using water titration method. Selected surfactant and co-surfactant was mixed in different volume ratios(1:1,1:2,2:1). Oil and surfactant/co-surfactant(Smix)was mixed thoroughly in different volume ratios (1:9,2:8,3:7,4:6,5:5,6:4,7:3,8:2,9:1). Titrated with water by drop wise addition under gentle agitation.The mixtures were described in terms of flowability and phase clarity. The clear emulsion with good flowability was declared as Nanoemulsion. Pseudo-ternary phase diagram was constructed using Microsoft excel.

Table.1: Construction of pseudo ternary phase diagram by water titration method for 1:1, 1:2, 2:1 ratio.

Oil (ml)	S_{mix} (ml)	Water(ml) at 1:1 ratio	Water(ml) at 1:2 ratio	Water(ml) at 2:1 ratio
1	9	6.8	23	25.7
2	8	8.2	23.2	25.9
3	7	10.3	23.9	30
4	6	12.9	24	30.8
5	5	15.2	24.5	31.2
6	4	20.1	24.9	31.5
7	3	21.9	25.2	31.9
8	2	22	25.7	32
9	1	22.5	25.9	32.3

FORMULATION OF GRISEOFULVIN NANOEMULSION[11]

Griseofulvin Nanoemulsion was prepared by high speed homogenization.

Preparation of aqueous phase 'A': Accurately weighed quantity of Griseofulvin was dissolved in methanol then addition of Accurately weighed quantity of tween 80 (S_{mix}) into it, and heated up to 80°.

Preparation of oil phase 'B': Weighed quantity of clove oil and span 80 mixed together by maintaining hot condition, simultaneously methyl paraben and propyl paraben was dissolved in propylene glycol.

Both oil phase and aqueous phase were heated upto 70-80 °separately.Incorporation of oil phase (B) in to aqueous phase (A)

After all ingredients were mixed, make up with required amount of water, then the mixture was homogenized using high speed homogenizer which was set at 1000 rpm for 60 min.

CHARACTERIZATION OF GRISEOFULVIN NANOEMULSION

1. Physical examination[12]

The prepared Nanoemulsion formulation were inspected visually for their appearance, phase separation, grittiness, homogenity and consistency.

2. Centrifugation stability study[13]

Nanoemulsion were diluted with purified distilled water. Then Nanoemulsion were centrifuged at 1000 rpm for 15 minute at 30°C and observed for any change in homogenecity of Nanoemulsions.

3. Meaurment of pH[14]

1 ml of Nanoemulsion was dissolved in 10 ml of distilled water and pH was determined using digital pH meter.

4. Drug content[15]

The drug content of emulsion was measured using UV-Visible spectroscopic method. Volume equivalent to dose of 500 mg was dispersed in suitable quantity of methanol. The samples were measured at λmax. results were taken in triplicate.

$$\text{Amount of drug} = \frac{\text{concentration from the standard graph} \times \text{DF}}{1000}$$

Where DF = dilution factor

5. Gobule size determination[16]

The globule size of the Nanoemulsion was measured by Malvernzeta sizer.

6. Zetapotential[17]

Measurement of zetapotential of the Nanoemulsion was done by using Malvern zeta sizer instrument. Measurements were performed on the samples prepared for size analysis. Zetapoetntial indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

7. *In-vitro* drug diffusion study of Nanoemulsion[18]

The franz diffusion cell was used for the drug release studies. Volume equivalent to dose of 500 mg was placed over the drug release membrane. The cellophane membrane was clamped between donor and receptor chamber of diffusion cell. The receptor chamber was filled with mixture of freshly prepared phosphate buffer pH 7.4 and methanol (80:20) solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples were collected at suitable time interval and sample were analyzed for drug content by UV visible spectrophotometer at λ_{max} after appropriate dilutions.

FORMULATION OF GRISEOFULVIN NANOEMULGEL

Preparation of carbopol 934 gel[19]

The carbopol gel formulations were prepared by dispersing carbopol 934 in purified water with constant stirring at a moderate speed and the pH was adjusted to 6 to 6.5 using triethanolamine.

Nanoemulsion mixed with prepared gel, gentle stirring to obtain the Griseofulvin Nanoemulgel.

Table.2: Formulation chart of Griseofulvin Nanoemulgel

Nanoemulsion formulation code	Carbopol 934 (gm)	Triethanolamine (ml)	Water (ml)	Glutaraldehyde (ml)
Best Formulation – 25ml	1gm	0.5ml	5ml	0.5 ml

CHARACTERIZATION OF NANOEMULGEL

1. Physical examination[12]

The prepared Nanoemulgel formulaion were inspected visually for their colour, homogeneity, consistency, grittiness and phase separation.

2. Measurement of pH of the Nanoemulgel[20]

The pH of Nanoemulgel formulation was determined by using digital pH meter.1g Nanoemulgel was mixed in 10 ml distilled water. Electrodes are then immersed in the developed gel solution and readings were documented from digital pH meter in three times and mean value was estimated.

3. Viscosity determination[21]

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number 64 and rpm 100. The formulation whose viscosity was to be

determined was added to the beaker. Spindle was lowered perpendicular in to the centre of Nanoemulgel taking care that spindle does not touch bottom of the adapter and rotated at a speed of 100 rpm for 10 min.

4. Spreadability[22]

The spreadability of the gel formulation was determined by taking two glass slides of equal length. On one glass slide, 1gm gel was applied. To the other slide, weights (125g) are added and the time taken for the second glass slide to slip off from the first glass slide was determined. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula,

S=M*L/T

where, S = spreadability, M = Weight kept on upper slide, L = Length of glass slides, T = Time taken to slip off the slides completely from each other.

5. Drug content[23]

One gram of gel was dissolved in 100 ml methanol. stirred constantly for 10 minutes. From this 1 ml of solution was diluted to 10 ml with methanol. The resultant solution was filtered and was analysed by U.V spectrophotometer at λmax.

$$\text{Amount of drug} = \frac{\text{concentration from the standard graph} \times DF}{1000}$$

Where DF = dilution factor

6. *In-vitro* diffusion study of Nanoemulgel[24]

The franz diffusion cell was used for the drug release studies. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated Nanoemulgel of 1gm was placed over the drug release membrane (In the donor compartment) and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4 and methanol (80:20). The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor. And the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5°C by surrounding water in jacket. The samples were collected at suitable time interval and sample were analyzed for drug content by UV visible spectrophotometer at $\lambda_{max.}$

7. Kinetic studies[25]

The release data was fitted to Zero order, First order kinetics, Higuchi's and Korsmeyers peppa's equations to investigate the mechanism of drug release from the topical gel.

8. To compare the *in-vitro* diffusion study with marketed formulations.[26]

In-vitro diffusion study was carried out in phosphate buffer pH 7.4 and methanol (80:20) by using Franz diffusion cell and the samples were analyzed spectrophotometrically at λmax.

9. Stability studies[27]

Best formulation is subjected to stability testing at 40 ±2 ºC, 75 ±5% RH conditions for 6 months. Parameters such as appearance, drug content and *in-vitro* diffusion were examined at 0,3,6 month intervals.

RESULTS AND DISCUSSION

1. PREFORMULATION STUDIES OF PURE DRUG.

Table. 3: Preformulation studies of pure drug.

PROPERTIES	REPORTED		OBSERVED	
Description	White to pale cream-colored crystaline powder		Whitish cream amorphous powder	
Colour	white		White	
Melting point	220˚C		220˚C	
Solubility	Water	0.08mg/ml	water	0.089mg/ml
	Methanol	2.46mg/ml	Methanol	2.01mg/ml
	Phosphate buffer pH 7.4	2.11mg/ml	Phosphate buffer pH 7.4	1.87mg/ml

Organoleptic characteristics

Organoleptic characteristics like general description, colour was determined. It was found that Griseofulvin is White to pale cream-colored crystalline powder and whitish cream amorphous powder and was found to be within the reported literature limits. The results obtained were shown in table.3.

Melting point

The study was carried out and found that the drug melted at 220˚C which is within the reported range of 220˚C and indicating that the drug is pure. The results obtained were shown in table.3.

Solubility

Griseofulvin was found to be soluble in phosphate buffer pH 7.4, was found to be 1.87mg/ml, methanol was found to be 2.01mg/ml, water was found to be 0.089mg/ml. Solubility in all the solvents was within the reported literature limits. The results obtained were shown in table.3.

2. Screening of absorption maximum (λmax) of Griseofulvin

The λmax of Griseofulvin was determined in phosphate buffer of pH 7.4 and methanol which was scanned between 200-400nm in the UV spectrophotometer.

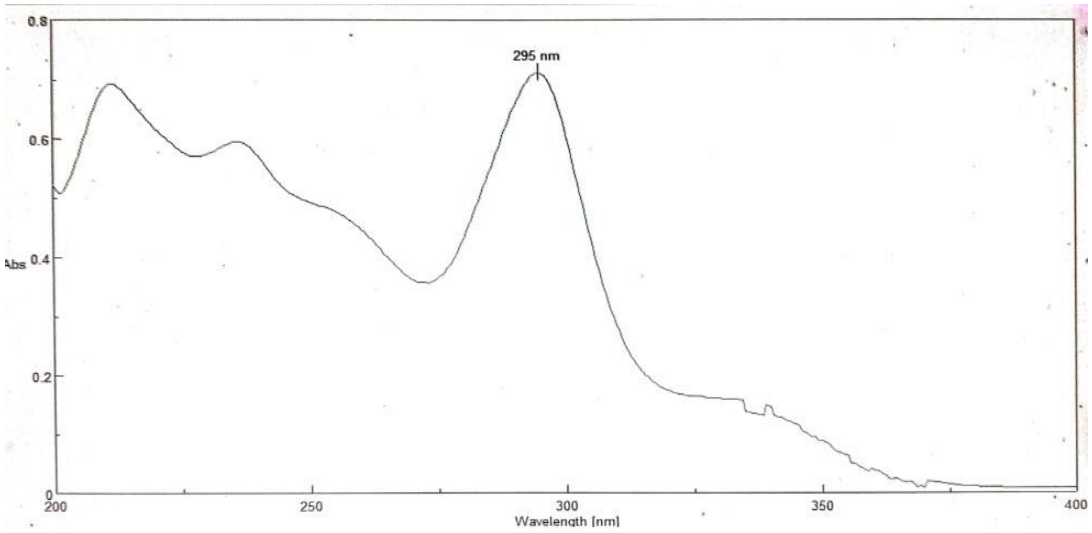

Fig.1: λmax of Griseofulvin.

The absorption spectrum of pure Griseofulvin was scanned between 200-400nm. The λmax of pure Griseofulvin was found to be 295nm by using phosphate buffer pH 7.4. The curves obtained were shown in fig.1.

3. Standard graph of Griseofulvin

Table 4: calibration data of Griseofulvin in phosphate buffer pH 7.4.

S No.	Concentration (µg/ml)	*Absorbance
1	2	0.1611±0.01
2	4	0.3141±0.04
3	6	0.4712±0.02
4	8	0.6312±0.01
5	10	0.7911±0.04

*Data expressed as a mean ±SD, n=3

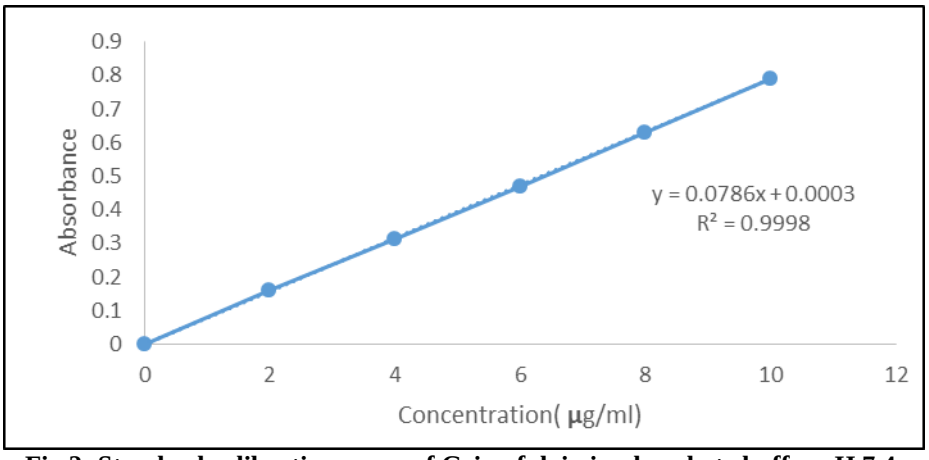

Fig.2: Standard calibration curve of Griseofulvin in phosphate buffer pH 7.4.

The calibration curve of Griseofulvin was obtained in the concentration ranging from 2- 10µg/ml in phosphate buffer pH 7.4. Data were reported in table.4. Fig 2. shows the standard calibration curve of Griseofulvin in phosphate buffer, which was found to be linear with values 0.0786 and 0.9998 as slope and regression value for phosphate buffer pH 7.4 respectively.

4. Drug-polymer interaction by FTIR

Compatibility studies of Griseofulvin with different polymers were carried out prior to the formulation of emulgel.

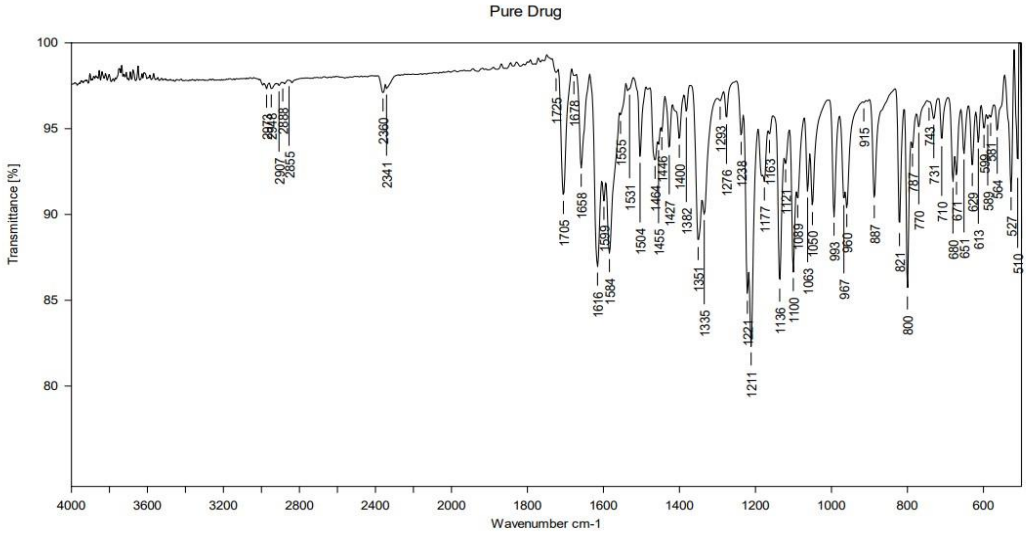

Fig. 3: FTIR-spectrum of pure drug Griseofulvin.

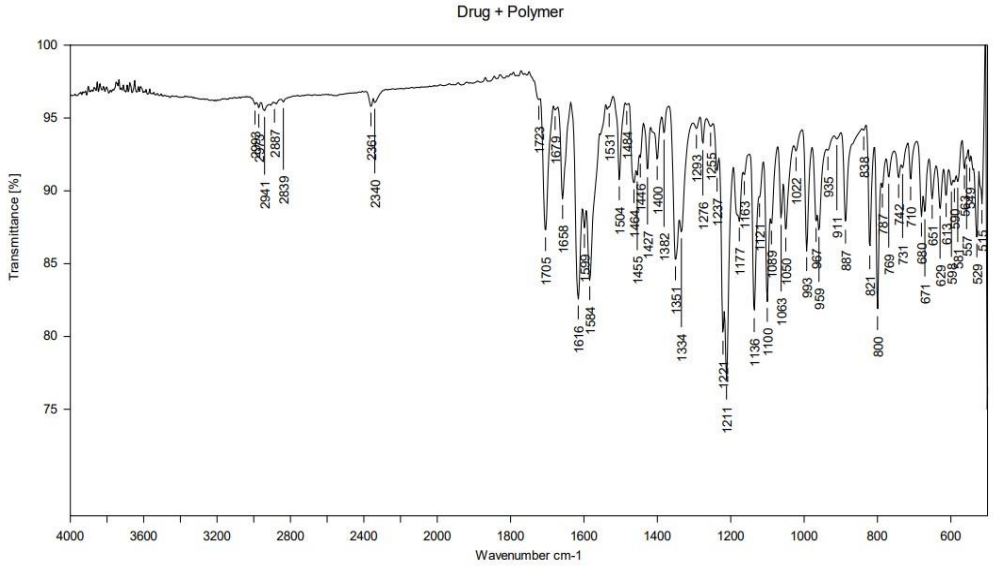

Fig. 4: FTIR-spectrum Griseofulvin + Polymer.

Table. 5: comparison of FTIR spectra of Griseofulvin and Polymer

Sl. No.	Functional groups	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})	
			Drug	Drug – Polymer
1	C=C Stretching	1678-1668	1678	1679
2	C-O Stretching	1275-1200	1238	1237
3	C=O stretching	1730-1685	1725	1723
4	C-Cl Stretching	850-550	770	769

The IR spectra of the Griseofulvin was compared with the mixture of drug and polymer and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence were noted in table.5 and the overlay of pure drug and mixture was shown in fig. 3 & 4.

The prominent peaks associated with C=C stretch was found to be 1678-1668, C-O stretch was found to be 1275-1200, C=O stretch was found to be 1730- 1685, C-Cl stretch was found to be 850-550, were analysed. The range of peak values were found to be the same indicating that there were no interaction of Griseofulvin with polymer conforming the stability of drug in the formulation.

5. Pseudo ternary phase diagram study by water titration method

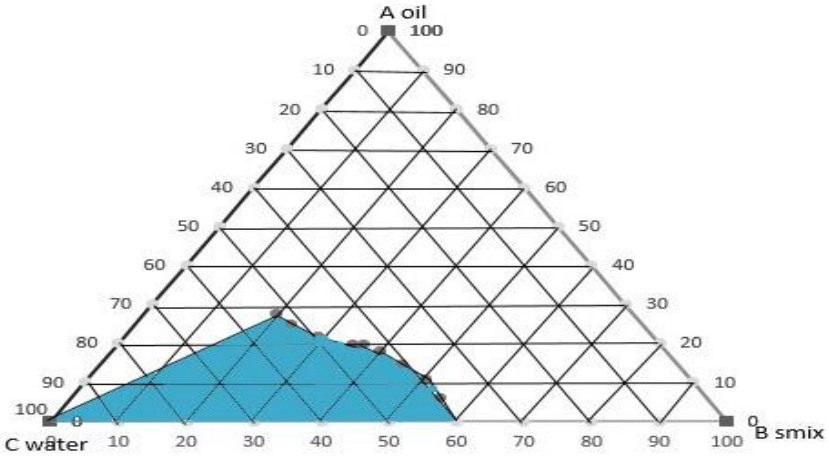

Fig.5: Ternary diagram (1:1 ratio).

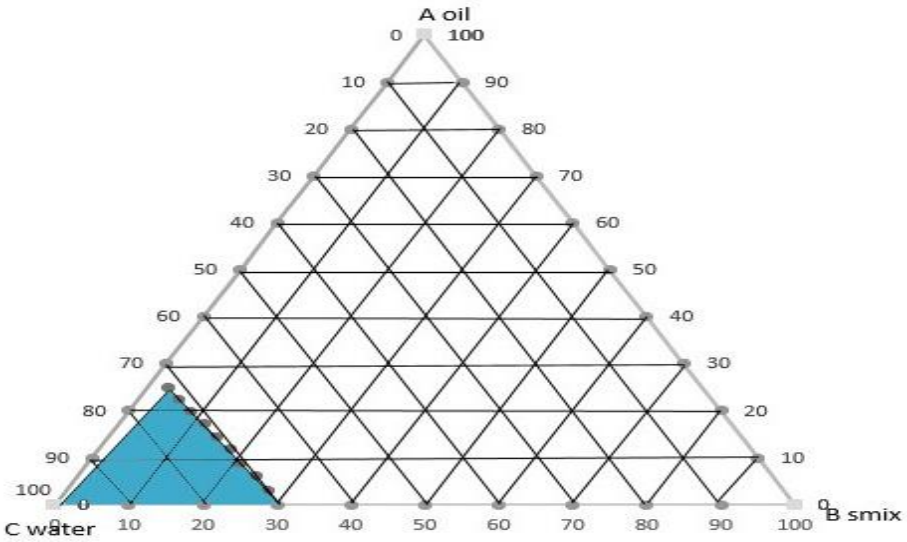

Fig.6: Ternary diagram (1:2 ratio).

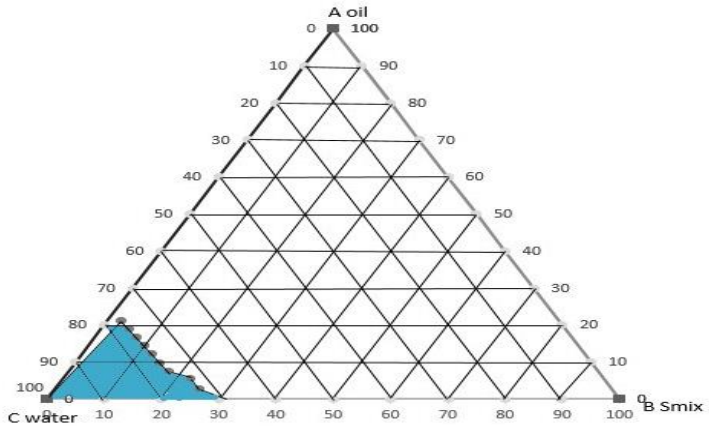

Fig.7: Ternary diagram (2:1 ratio).

The mixing of surfactant and cosurfactant was done in different ratios (1:1, 1:2 and 2:1). Corresponding pseudoternary phase diagram of each combinations were presented in Fig.5,6,7 As seen in Fig.5. ternary diagram has a wide emulsion area but the other two ternary diagrams fig.6, 7 were not stable. Based on ternary diagrams, phase regions of 1:1 showed good emulsion characteristics with stable emulsion.

Table.6: Formulation chart of Griseofulvin Nanoemulsion.

Formulation code	Clove Oil (ml)	Smix Ratio Methanol : Tween 80(1:1) (ml)	Griseofulvin (mg)	Water up to (ml)
F1	2.9	26.7	500	20.2
F2	5.4	21.9	500	22.5
F3	7.3	17.2	500	25.3
F4	8.9	13.4	500	28.1
F5	9.9	9.9	500	30.1
F6	10.4	4.9	500	32.8

EVALUATION OF GRISEROFULVIN NANOEMULSION

1. Physical examination

Table.7: Physical examination Nanoemulsion F1-F6.

Formulation	Appearance	Phase separation	Homogeneity	Consistancy
F1	Light yellow	None	Good	Good
F2	Light yellow	None	Good	Good
F3	Light yellow	None	Good	Good
F4	Light yellow	None	Good	Good
F5	Light yellow	None	Excellent	Excellent
F6	Light yellow	None	Good	Good

The prepared Griseofulvin Nanoemulsions were light yellow colour with smooth and homogeneous appearance with excellent consistency. There was no phase separation observed with the formulations. The results were depicted in the table.7. The prepared Nanoemulsion is shown in the fig.8.

Fig.8: prepared Griseofulvin Nanoemulsion.

2. Centrifugation study, pH, Drug content

Table.8: Centrifugation stability study, pH & Drug content of Nanoemulsion F1-F6.

Formulation	Centrifugation	pH	Drug content* (%)
F1	No phase Separation	6.30±0.01	77.8±0.03
F2	No phase Separation	6.26±0.01	82.4±0.02
F3	No phase Separation	6.21±0.01	86.1±0.02
F4	No phase Separation	6.12±0.01	84.7±0.01
F5	No phase Separation	6.40±0.01	93.3±0.02
F6	No phase Separation	6.32±0.01	75.4±0.01

***Data expressed as a mean ±SD, n=3

There was no phase separation which indicates that all the Nanoemulsions prepared were stable.

The pH value of the all formulation F1-F6 ranged from 6.12 to 6.40, which are considered acceptable to avoid the risk of skin irritation upon application to the skin.

The drug content of Nanoemulsions was estimated spectrophotometrically at 295 nm. The highest drug content was found with Nanoemulsion F5, 93.3±0.02 and the lowest for Nanoemulsion F1 was found to be 77.8±0.03.

The results were shown in the table.8.

3. Average particle size and size distribution

Table. 9: Average particle size of Nanoemulsion F1-F6.

Formula	Particle size (nm)
F1	69.3
F2	71.8
F3	62.3
F4	64.7
F5	51.9
F6	74.8

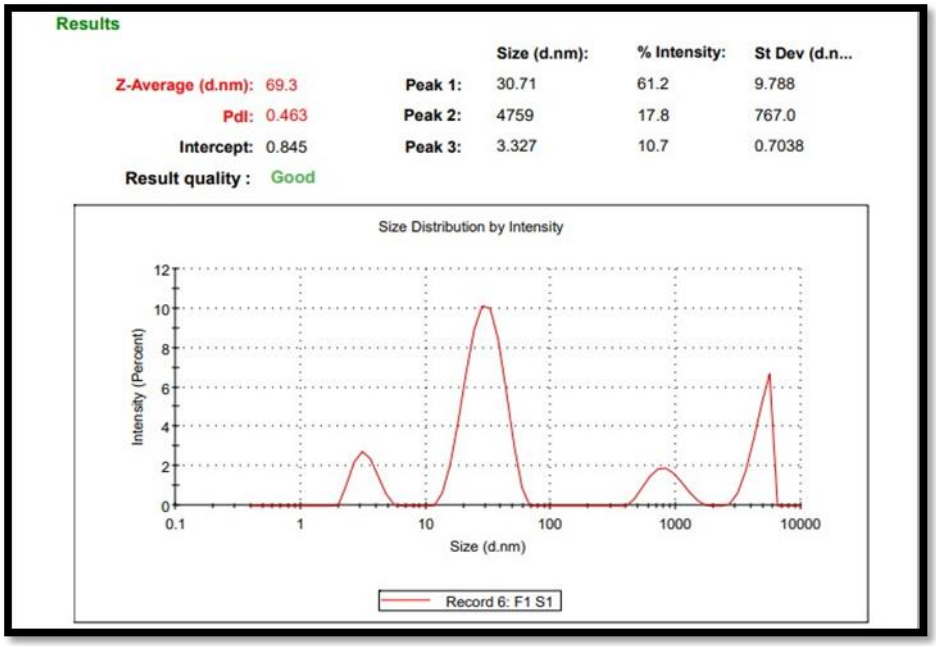

Fig.9: particle size of F1 by Malvern zeta sizer.

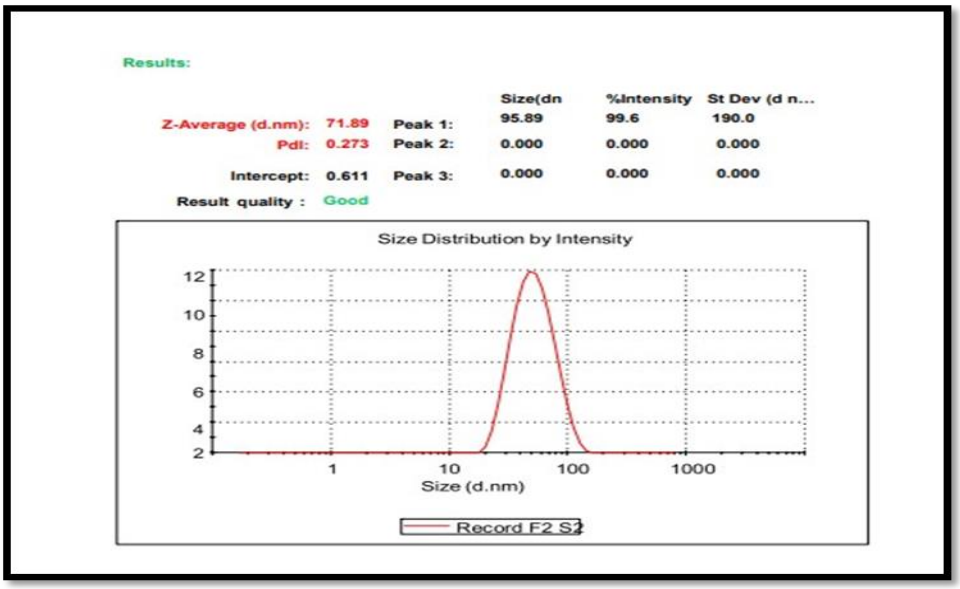

Fig.10: particle size of F2 by Malvern zeta sizer.

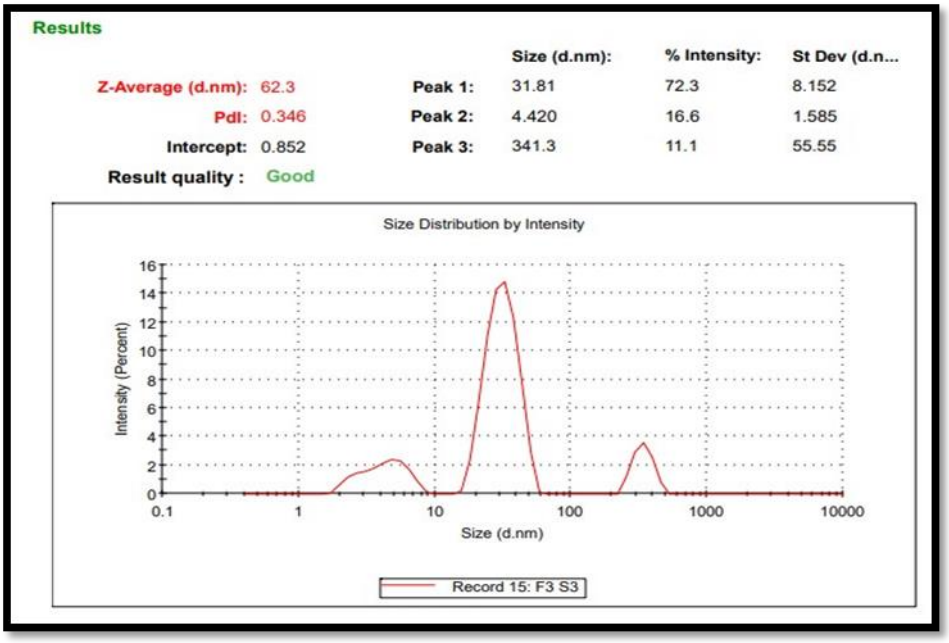

Fig.11: particle size of F3 by Malvern zeta sizer.

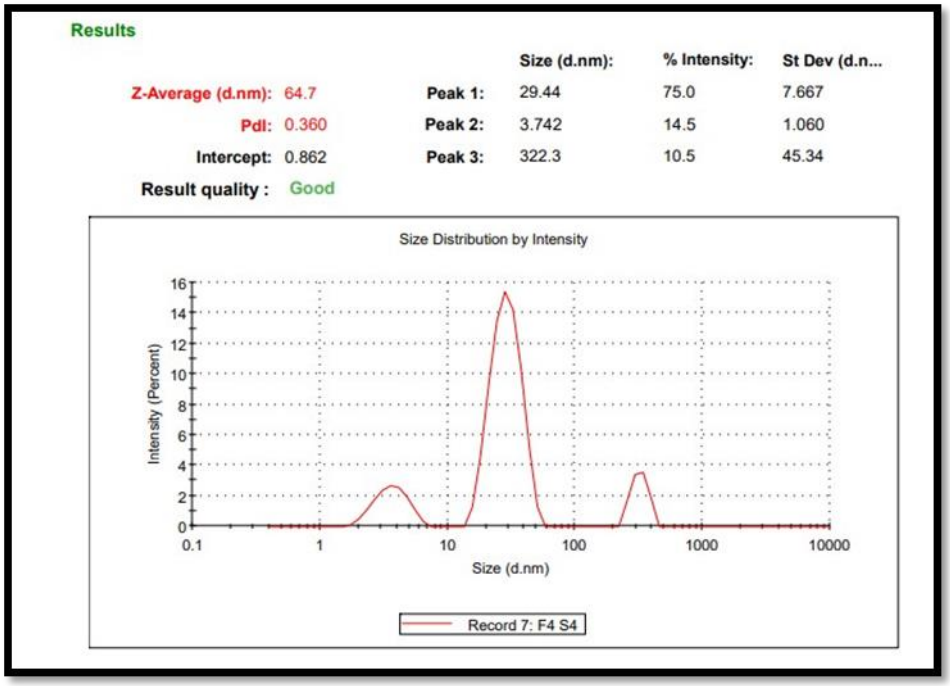

Fig.12: particle size of F4 by Malvern zeta sizer.

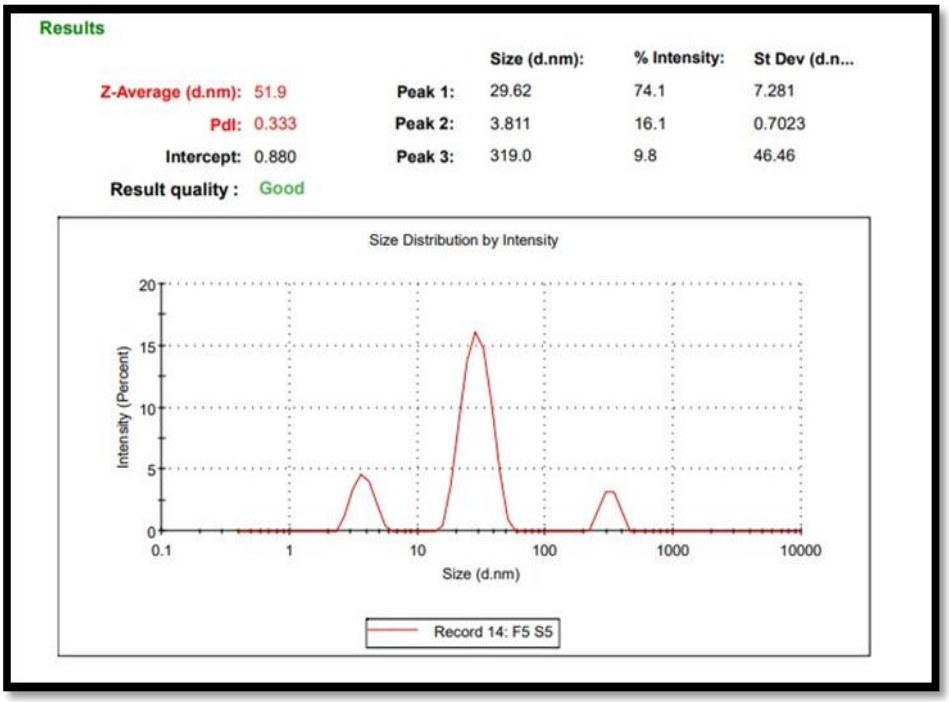

Fig.13: particle size of F5 by Malvern zeta sizer.

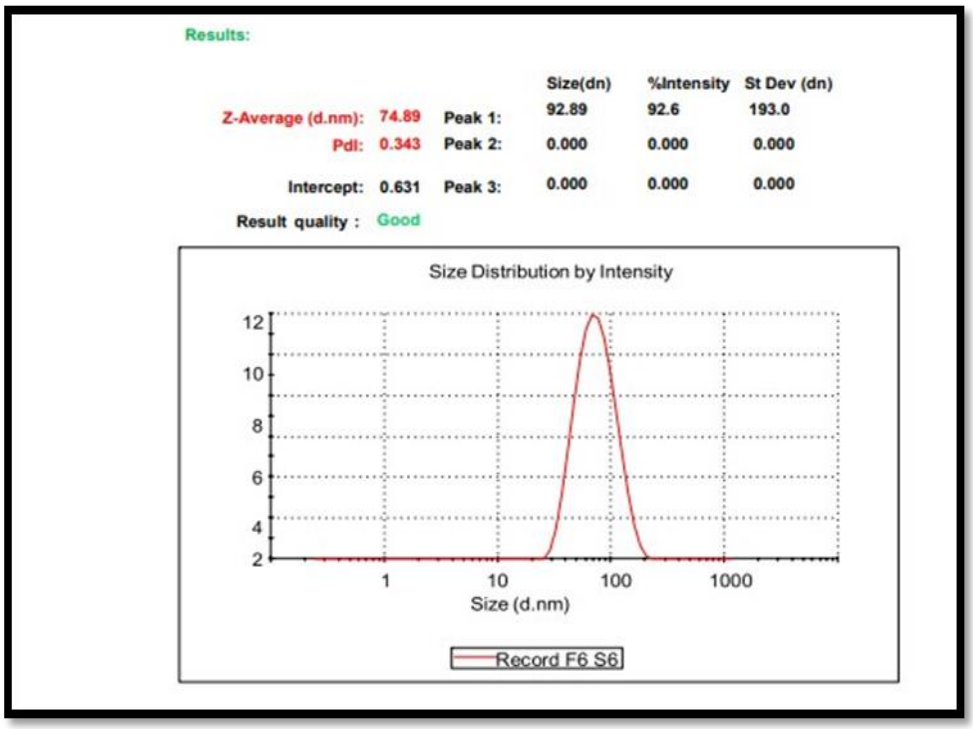

Fig.14: particle size of F6 by Malvern zeta sizer.

4. Zetapotential

Table. 10: Zeta potential of Nanoemulsion F5

Formulation code	Zeta potential
F5	-24.6mV

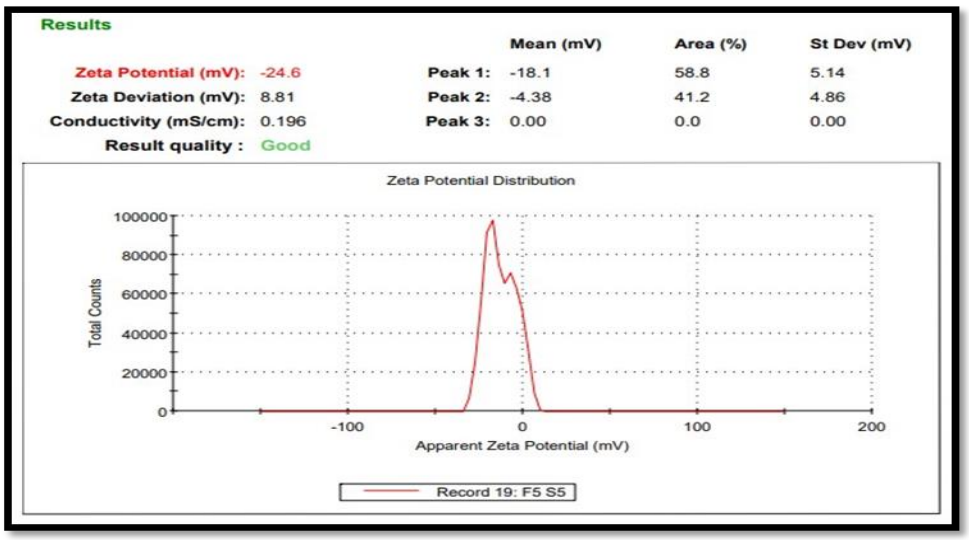

Fig. 15: zeta potential of Nanoemulsion F5.

Mean globule size of Nanoemulsion F5 was found to be 51.9 nm within the literature limits which proves the homogeneity of Nanoemulsion.

Zeta potential of Nanoemulsion F5 was found to be -24.6 mV. The negative zeta potential indicates that the droplets of nanoemulsion have no charge and there is no flocculation in the system which indicates that the system is stable.

Results were depicted in table.9,10 & fig.9,10,11,12,13,14,15.

5. *In-vitro* drug diffusion from Nanoemulsion

Table. 11: *In- vitro* drug diffusion data of Nanoemulsion formulations (F1-F6).

Time (hours)	% cumulative drug release*					
	F1	F2	F3	F4	F5	F6
0.25	19.29± 0.03	17.20±0.01	20.66±0.08	21.85±0.06	22.02±0.07	17.67±0.09
0.5	15.41±0.07	24.58±0.09	22.78±0.03	24.51±0.01	24.4±0.02	19.00±0.07
1	25.73±0.06	28.63±0.06	28.47±0.04	27.69±0.02	28.8±0.03	22.14±0.01
2	33.89±0.05	38.95±0.07	36±0.04	34.59±0.02	34.9±0.08	30.33±0.02
3	44.91± 0.03	45.68±0.03	44.58±0.01	41.62±0.06	44.19±0.04	42.00±0.08
4	52.96±0.08	59.78±0.04	58.70±0.04	51.57±0.02	59.±0.05	50.33±0.07
5	64.72± 0.09	65.79±0.05	69.65±0.09	68.78±0.08	70.11±0.08	65.38±0.09
6	72.29±0.07	75.46±0.04	75.94±0.06	75.55±0.01	76.00±0.01	72.18±0.01
7	78.01±0.07	77.99±0.07	79.3±0.09	78.11±0.04	80.33.±0.07	77.33.±0.04

***Data expressed as a mean ±SD, n=3

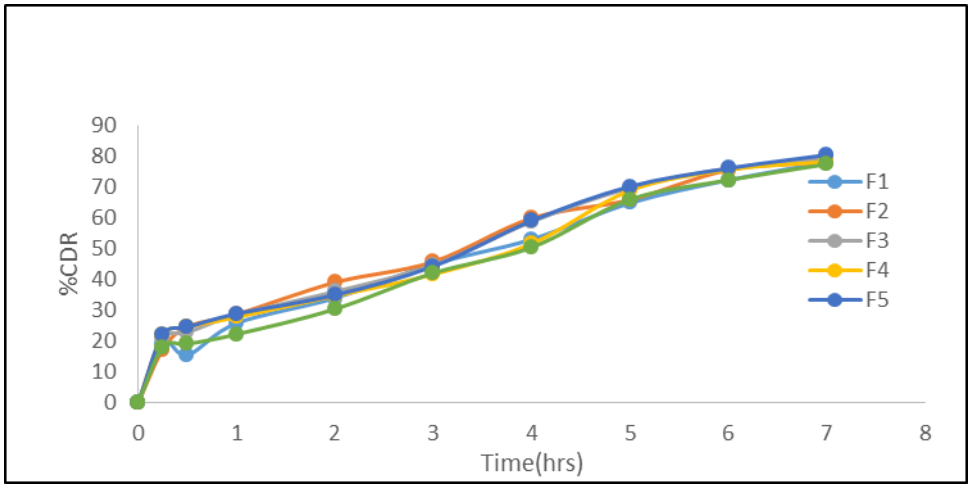

Fig.16: *In-vitro* drug diffusion profile of Nanoemulsion (F1-F6).

The results obtained for F1-F6 were shown in table.11 and fig.16. Nanoemulsion F6 showed least percentage cumulative drug release value 77±0.07 at 7 hrs and Nanoemulsion F5 showed highest percentage of drug release value 80.33±0.07 at 7 hrs.

EVALUATION OF GRISEOFULVIN NANOEMULGEL

1. Physical characteristics of Griseofulvin Nanoemulgel

Table.12: Physical characteristics of Griseofulvin Nanoemulgel.

Formulation code	Appearance	pH	Spreadability (gms.cm/sec)	Drug content (%)	Viscosity (cps)
GF5	Light yellow colour	7.2±0.05	18.03±0.1	90.14±0.02	25680

Fig.17: prepared Griseofulvin Nanoemulgel GF5.

Physical examination

The prepared Nanoemulgel GF5 were light yellow in colour. Physical examinations were shown in the table. 12.

Measurement of pH

pH of the formulations is shown in the table.12. The pH values of the nanoemulgel GF5 was 7.2±0.05.

Viscosity Study

Viscosities of the formulations were evaluated by using Brookfield viscometer at 27^{o}C using spindle no.64 at 100rpm. The viscosity of the nanoemulgel GF5 was found to be 25680 cps. The viscosity of the formulation were shown in the table.12.

Spreadability

The value of spreadability were shown in table.12. The Nanoemulgel GF5 showed 18.03±0.1 g cm/ sec. The results indicate that the polymers used gave gels spread by small amount of shear and the spreadability of the emulgel decreases with the time.

Drug content determination

The drug content of Nanoemulgel GF5 was estimated spectrophotometrically at 295 nm and drug content were found in the range of 90.14 ±0.02. The result were shown in table.12.

2. *In-vitro* diffusion study

Table.13: *In-vitro* diffusion study of Nanoemulgel GF5.

Time(hrs)	% Cumulative drug release*
1	14.43±0.01
2	20.23±0.04
3	26.21±0.02
4	39.51±0.05
5	51.46±0.02
6	62.27±0.01
8	83.42±0.03

***Data expressed as a mean ±SD, n=3

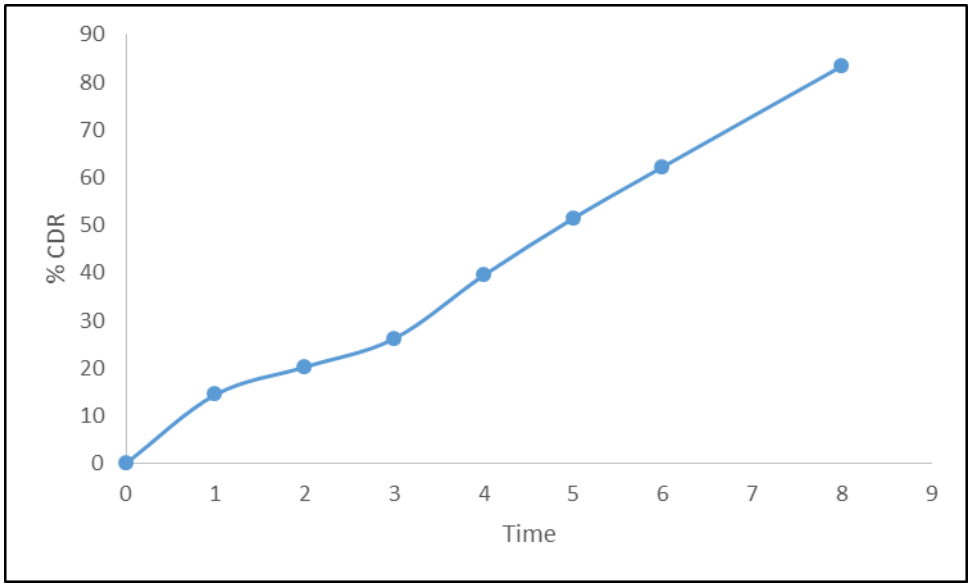

Fig.18: *In-vitro* diffusion profile of Nanoemulgel GF5.

In-vitro diffusion study was carried out in phosphate buffer pH 7.4 for 8 hrs by using franz diffusion cell and the samples were analyzed spectrophotometrically at 295 nm. GF5 showed drug release value of 83.42 ±0.03. The results were shown in table.13 and fig.18.

3. Kinetics of drug release of Nanoemulgel GF5.

Table. 14: Kinetic release study of Nanoemulgel GF5.

Time	Log time	Square root of time	% CDR of F5	Log % CDR OF F5	% cumulative remaining	Log % cumulative remaining
0	0	0	0	0	100	2
1	0.000	1.000	14.43	1.159	85.57	1.932
2	0.301	1.414	20.23	1.305	79.77	1.901
3	0.477	1.732	26.21	1.418	73.79	1.867
4	0.602	2.000	39.51	1.596	60.49	1.782
5	0.698	2.236	51.46	1.711	48.54	1.686
6	0.778	2.449	62.27	1.794	37.73	1.576
8	0.903	2.828	83.42	1.921	16.58	1.219

Zero order kinetics release

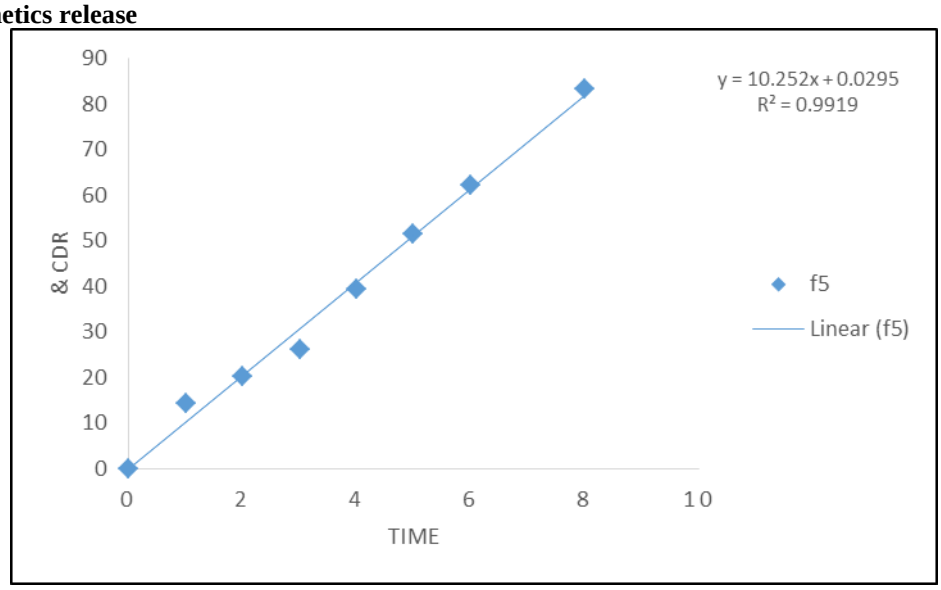

Fig.19: Plot of Percentage CDR v/s Time (Zero order kinetics).

First order kinetics release

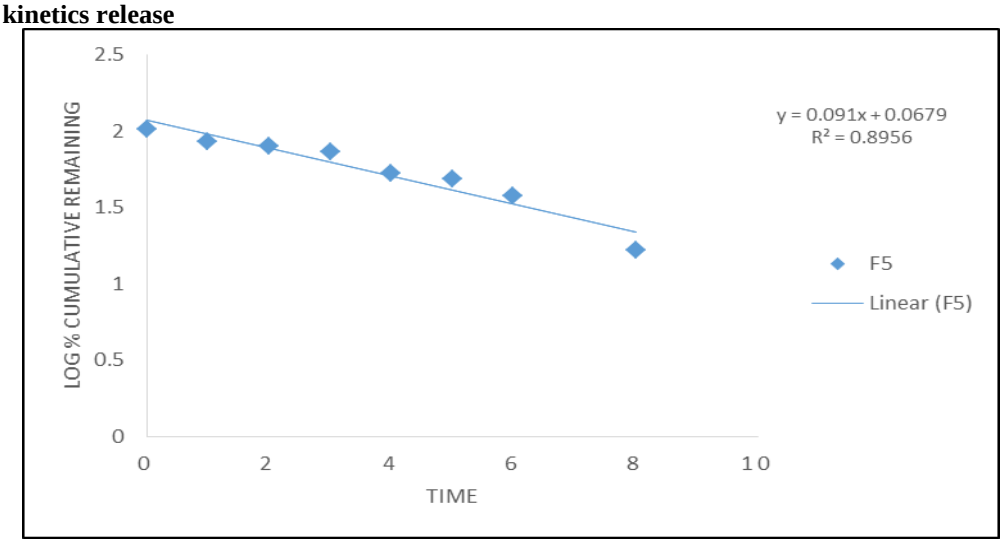

Fig.20: Plot of Log Percentage CDR v/s Time (First order kinetics).

Higuchi Model

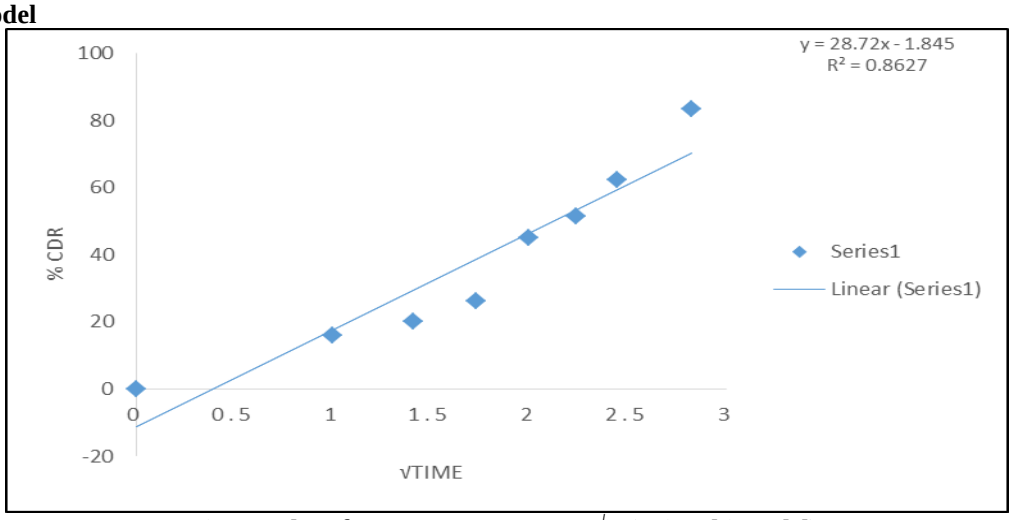

Fig.21: Plot of Percentage CDR v/s √ t (Higuchi model).

Peppas Model

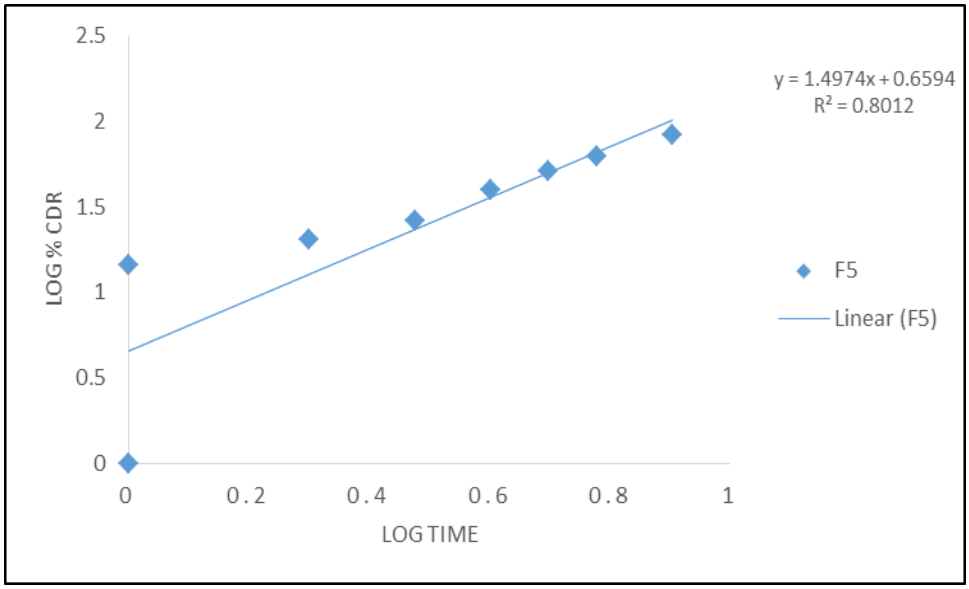

Fig.22: Plot of Log % CDR v/s Log Time (Peppas exponential equation).

In order to study the release mechanism of Nanoemulgel. various dissolution models were applied to the *in-vitro* release profiles of formulations. The kinetic models included zero order, first order, higuchi and peppas equation.

The regression values (r) for zero order, first order, Higuchi and Peppas were found to be 0.9919, 0.8956, 0.8627 and 0.8012. Based on the highest regression values (r), the best fit model for the Nanoemulgel GF5 follows zero order.

The results of kinetics analysis of the *in-vitro* drug release data for the formulation were given in Table.14. And Fig.19,20,21,22.

4.Comparison of *In-vitro* diffusion study of Nanoemulgel GF5 with marketed product

Table. 15: Comparison of *in-vitro* diffusion study of Nanoemulgel GF5 and marketed product (Grisowell (1%w/w)).

Time(hrs)	% Cumulative drug release*	
	GF5 (1%w/w)	Marketed product(Grisowell cream(1%w/w)
1	14.43±0.01	17.21±0.02
2	20.23±0.04	20.12±0.02
3	26.21±0.02	25.34±0.01
4	39.51±0.05	38.12±0.03
5	51.46±0.02	49.68±0.01
6	62.27±0.01	60.06±0.02
8	83.42±0.03	81.18±0.01

***Data expressed as a mean ±SD, n=3

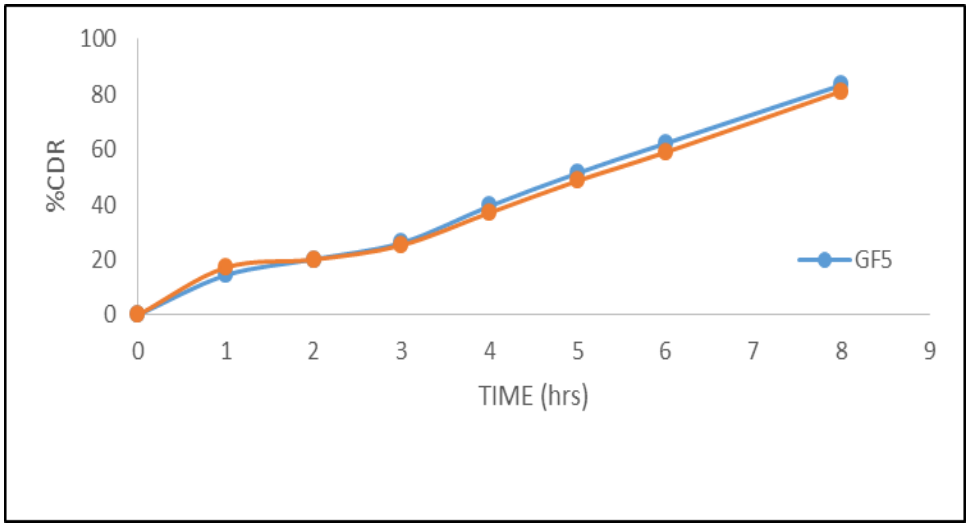

Fig.23: Comparison of *in-vitro* diffusion study of Nanoemulgel GF5 and marketed product.

In-vitro diffusion study was carried out in phosphate buffer pH 7.4 and methanol for 8 hrs by using Franz diffusion cell and the samples were analyzed spectrophotometrically at 295 nm. The nanoemulgel GF5 shows drug release of 83.42±0.03 whereas the marketed product shows 81.18±0.01 in 8 hours. The results were shown in table 15. and fig. 23.

5. Stability Studies

Table.16: Evaluation of Nanoemulgel GF5 for stability study.

Evaluation Parameters	Time (days) Accelerated condition 40±2°C, 75±5%RH			
	0 day	30 days	60 days	90 days
Colour	Light yellow colour	Light yellow colour	Light yellow colour	Light yellow colour
Drug content(%)	90.14%	89.55%	86.56%	83.1%
In-vitro diffusion in 8 hr	83.42%	81.13%	79.43%	76.1%

Stability studies were carried out on selected formulation GF5. The study was carried to evaluate physical appearance, drug content and *in-vitro* diffusion studies at accelerated condition 40±2^{0}c ,75±5%RH. Drug content of GF5 were found as 90.1, 89.55, 86.56, 83.1% and 83.42, 81.13,79.43, 76.1% of drug release in 0, 30, 60 and 90 days.

The results of stability study of the formulation were depicted in table. 16.

CONCLUSION

- Preformulation of Griseofulvin was carried out. The results of preformulation study was found to be whitish cream amorphous powder, odourless, melting point 220^{0}C and solubility of 0.089mg/ml, 2.01mg/ml, 1.87mg/ml in water, methanol, Phosphate buffer pH 7.4.
- Drug and polymers were subjected for the compatibility study using FTIR spectroscopy, which suggested that there is no interaction between the drug and polymer.
- Maximum wavelength (λ_{max}) was determined by using UV spectrophotometer by using phosphate buffer pH 7.4 as medium. Maximum absorbance was found at 295nm.
- Standard calibration curve was constructed in the concentration range of 2-10 µg/ml using phosphate buffer pH 7.4 as a medium and obtained slope of 0.0786 and R^2 value of 0.9998.
- Nanoemulsion region was selected by plotting the ternary phase diagrams by water titration method.
- The Nanoemulsions prepared by high-speed homogenization method.
- Prepared Nanoemulsions subjected to physical examinations and were found to be light yellow colour with smooth and homogeneous appearance with excellent consistency and was concluded that Nanoemulsions prepared were stable.
- The pH, drug content and centrifugation stability studies were performed and it was in the acceptable range. The drug content of all the formulations were carried out and results of the F1, F2, F3, F4, F5& F6 were found to be 77.8, 82.4, 86.1,84.7 93.3 & 75.4%.
- The Globule size distribution of Nanoemulsion F5 was found to be 51.9 nm respectively.
- From evaluation parameters, Nanoemulsion F5 was found to be best.
- From nanoemulsion F5, nanoemulgel GF5 were prepared.
- The drug content of the formulations was carried out and it was concluded that the GF5 showed 90%.
- The *in-vitro* diffusion studies of all formulations were carried by using Franz diffusion cell for 8 hrs. GF5 showed highest release of 83 % where as marketed product showed the release of 81%.
- Kinetic drug release studies GF5 were carried out based on the highest regression value of 0.9919 the best fit model for the Nanoemulgel GF5 follows zero order.
- Stability studies were carried out for 0, 30, 60 and 90 days for the best formulations GF5 and the results of appearance, *in-vitro* release and drug content were within the literature limits.
- The *in-vitro* drug diffusion studies showed no significant changes hence concluded that all the formulations were stable during the storage conditions.
- The formulated Nanoemulgels were found to be stable and showed good release of Griseofulvin.

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