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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and evaluation of Nano emulsion based Nanoemulgel of Itraconazole

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ABSTRACT
Solubility and emulsification experiments informed the selection of O/W Tween 80 and propyl glycol as the surfactant and co-surfactant, respectively, in the aqueous titration procedure used to produce nano emulsions. Using pseudo ternary phase diagrams, we were able to determine the nano emulsion area. Gels were prepared by mixing carbopol 934 and future-optimized NE at a range of concentrations. This made it simpler to apply the therapy topically. We examined the particle size, viscosity, rheological behaviour, and thermodynamic stability of NE and NEG that had been loaded with drugs. The purpose of this research was to develop and evaluate a nanoemulsion-based Itraconazole formulation for the treatment of fungal infections. The improved formulation showed increased penetration in vitro and in vivo, suggesting that the nanoemulsion gel could serve as a vehicle for Itraconazole transdermal delivery. The medication release rate of the optimised formulation was 90.4%, while the rate of release from the standard gel was just 60.3% after 9 hours. Transdermally administered nanoemulsion greatly increased bioavailability. Itraconazole.
Keywords: *Solubility, Ternary Phase Diagram, Nanoemulsion, Nano emulgel*

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INTRODUCTION

Itraconazole, an antifungal medication belonging to the triazole class. It has been found that itraconazole significantly inhibits sterol C-14 demethylation by fungi cytochrome P-450.There is a correlation between the accumulation of 14-methyl sterols in fungi and the fungistatic effects of isoxaconazole, suggesting that the loss of natural sterols is responsible for these effects. When compared to other triazoles like fluconazole, ravuconazole, and Posaconazole 3-4, itraconazole offers greater tolerability and a broader spectrum of antifungal action. Aspergillosis, widespread non-meningeal histoplasmosis, chronic cavitary pulmonary disease, and blastomycosis are only some of the fungal infections that itraconazole is used to treat. The bioavailability of itraconazole is drastically decreased because of its low water solubility (less than 1 g/ml) and considerable first-pass metabolism in the liver [1, 2, 3]. Recent pharmaceutical technology research has focused on novel drug delivery systems (NDDS) because of its potential to enhance the efficacy of future treatments [4]

Because of its vast solubilizing qualities, nano emu gel is being used as a new dosage formulation for a variety of drugs that are either oil- or water-soluble. Since instability or reduced solubility in the medium can significantly reduce pharmacological efficacy, developing effective pharmacological formulations is a significant challenge. One of the most advanced methods for improving the bioavailability and solubility of lipophilic medicines is the nano emu gel drug delivery system. Nano-emulgel, also known as nano-sized emulsions, is used as a drug carrier to increase the body's ability to absorb the medicine. This improved technique allows for the controlled and targeted administration of physiologically active substances [5, 6]. By combining a water-immiscible oil phase with a highly strained aqueous phase, these emulsions can be manufactured in vast quantities with minimum effort. Lipids, surfactants, co-surfactants, and co-solvents are the raw materials utilised to create them [7, 8]. They cause no skin irritation, are kinetically and thermodynamically stable, and can be administered in a number of ways. They have many potential medical

applications, including as a germicide, an antibiotic, a mucosal vaccine, and a cancer treatment. 21 Scientists working on formulations of poorly water-soluble medicines still confront difficulties, in part because of the growth of new drug research initiatives. The basic properties of these formulations that have piqued the interest of scientists are their ease of manufacture and scalability, their stability, and the increased bioavailability they provide [9, 10].

MATERIAL AND METHODS

Materials:

Glenmark Pharmaceuticals Ltd. of Nashik, India, generously gave us some itraconazole to try out. Rosemary oil, Tween 80, propylene glycol, Carbopol 934, methyl paraben, propyl paraben, and triethanolamine were purchased from Research-Lab Fine Chem Industry, Mumbai. All of the experiments used double-distilled water. All other chemicals used in formulation of nanoemulgel were of Analytical grade.

Methods

Screening of excipients by solubility studies

For example, to regulate itraconazole solubility in oleic acid, rosemary oil, turmeric oil, castor oil, olive oil, and eucalyptus oil, dissolved it in 2 ml of each of those oils, surfactants, and cosurfactants in individual 5-ml limit plug vials λnm=262nm [11].

Preparation of nanoemulsion

Making use of high-speed homogenization and the building of a pseudo-ternary phase diagram, prepare the nanoemulsion according to the chosen formulations.

Preparation of nanoemulgel formulation

At first, nanoemulgels were prepared using 1% w/v concentrations of various polymers (carbopol 934, carbopol 940). After the nanoemulgels were made, their outward appearance was analysed.

The nanoemulsion base gels are made by fusing 1 g of Carbopol 934 speciality in enough water. This gelling operator setup is then put in a dark spot for 24 hours while the final welling framework is acquired. "Attractively stirring the thick arrangement of gelling specialist, the nanoemulsion stacked with the medication is added gradually. Concentrations of Carbopol 934 used ranged from 1% to 2%. A pH-neutralizing alternative is triethanolamine (TEA). After 24 hours, the framed nanoemulgels will have scattered uniformly throughout the gel [12].

RESULT AND DISCUSSION

Solubility Studies have evaluated a wide variety of ingredients, including: Rosemary oil is more soluble in itraconazole than other oils, hence rosemary oil used for the oil phase as seen in Table 1. Co-surfactants such propylene glycol and surfactants like Tween®80 were found to improve the drug's solubility. Solubility information was used to settle on the oil, surfactant, and co-surfactant choices (propylene glycol, tween 80, and rosemary oil), respectively. UV spectrophotometer solubility results were used to compare itraconazole concentrations in various excipients.

Table 1. Solubility studies of Itraconazole in various excipients

Sr. No.	Solvents	Solubility(mg/ml)
1	Tween 80	10.35 ±0.41
2	Tween 20	7.00 ± 0.36
3	Propylene glycol	4.12 ±0.45
4	PEG 20	1.90 ±0.32
5	Oleic acid	91.42 ± 1.22
6	Rosemary oil	84 .9± 1.39
7	Turmeric oil	20.80 ±1.20
8	Castor oil	30.01 ± 1.33
9	Oilve oil	54.00 ±1.51
10	Eucalyptus oil	14.04 ± 1.50

Different combinations of oil, surfactants and co surfactants:

Solubility trials led to the selection of five distinct combinations of oil, surfactants, and co-surfactants with different S_{mix} ratios for further study. Visually observed combinations I–III and their thermodynamic stability studies Visual inspections indicated that some formulations of the nanoemulsion in combination I, S_{mix} ratio 1:2, were more thermodynamically stable than others, with some falling into the A group and others into the C category.

Construction of Pseudoternary phase diagram:

Itraconazole Pseudo Ternary Phase Diagram in Various Combinations S_{mix} Ratio. Propylene glycol: Tween 80 (2:1).

The solubility experiments led to the conclusion that rosemary oil would be the best choice for the oil phase. Tween 80 and propylene glycol were chosen as the surfactant and co-surfactant, respectively. "An aqueous phase was created by mixing chemicals with sterile water. One, two, and three distinct mass ratios were used to form blends of surfactant and cosurfactants (S_{mix}). In order to perform a thorough examination of the phase diagrams, these ratios were chosen in increasing order of surfactant concentration relative to cosurfactants concentration. For each phase diagram, oil and S_{mix} were mixed together in equal quantities in individual glass vials. In order to establish the phases' borders exactly and to cover all possible ratios for the investigation, many different combinations of oil and S_{mix} were constructed.

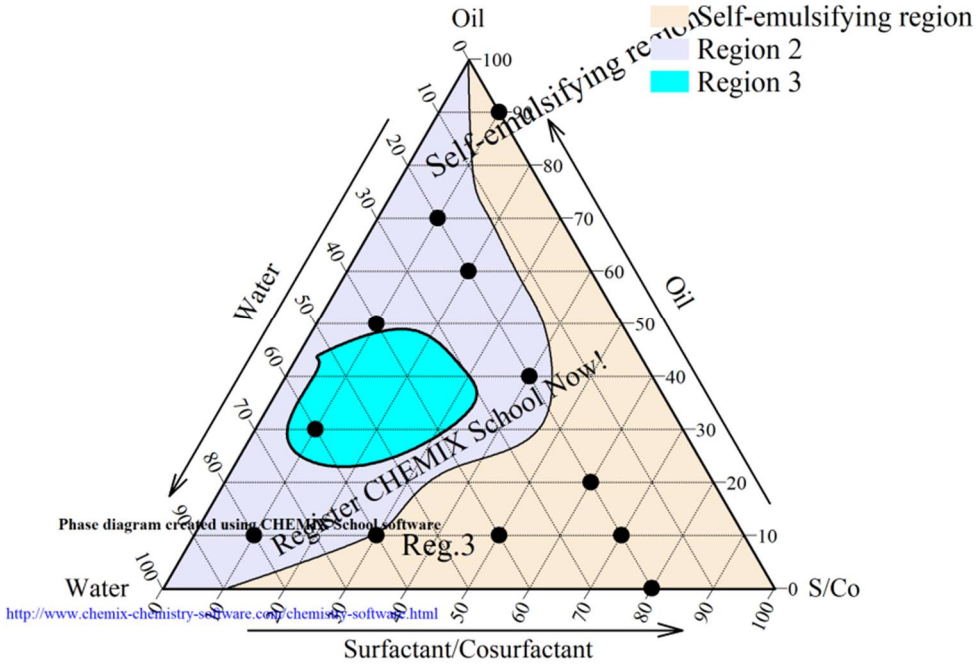

Fig1. Pseudoternary phase 1:2 (Oil: Surfactant)

In order to determine the boundaries of the phases in the phase diagrams, it was necessary to make a large number of distinct combinations of oil and S_{mix}. The aqueous titration approach was used to create pseudo ternary phase diagrams of the oil, S_{mix}, and aqueous phases. When the aqueous phase was titrated slowly into the o/w nanoemulsion at various mass ratios of oil and S_{mix}, the o/w nanoemulsion became transparent and easily flowable. By plotting the aqueous phase (represented by the x-axis), the oil phase (represented by the y-axis), and a mixture of surfactant and cosurfactants (represented by the y-axis) at a constant mass ratio, a pseudo-three-component phase diagram was constructed to represent the nanoemulsion physical state.

Characterization of Nanoemulsion:

Size of Droplets and Polydispersity Index The nanoemulsion particle size and polydispersity index were determined using photon correlation spectroscopy and a Malvern Zetasizer. The optimal Kcps values, between 50 to 202.8 were achieved by diluting samples with the aqueous phase of the formulation to a pH of 6.9 to 7.2. All measurements were collected at a constant temperature of 25 degrees Celsius and an intensity of 75%-100%. After analysing the samples.

Drug Content:

One hundred millilitres of 0.1M HCl were added to a measured quantity of nanoemulsion. The concentration of the medication in the filtrate was determined by spectrophotometric analysis at 262 nm using 0.1 M HCl as the standard. Data regression analysis yielded a calibration map that was used to determine the sample medication concentrations. The amount of itraconazole detected was compared to the calculated amount of medicine used to produce the nanoemulsion. There were three iterations of every conclusion.

Table 2. Drug content of formulation

Sr. No.	Formulation	Drug Content (%)
1	F1	94
2	F2	91.91
3	F3	95
4	F4	93.91
5	F5	96
6	F6	72
7	F7	68
8	F8	82
9	F9	64.91

pH determination:

The pH scale is used to determine Monitoring the pH value is essential for establishing the stability of the emulsions since changes in pH indicate the onset of chemical reactions that may reduce the quality of the final product. The pH of the nanoemulsion was consistently in the range of (5.22-5.52) throughout the majority of the tested formulations. Table 3 displays the results.

Table 3. pH determination

Sr. No.	Formulation	pH
1.	F1	5.03
2.	F2	5.04
3.	F3	5.06
4.	F4	5.52
5.	F5	5.16
6.	F6	5.10
7.	F7	5.11
8.	F8	5.14
9.	F9	5.07

Scanning electron microscope:

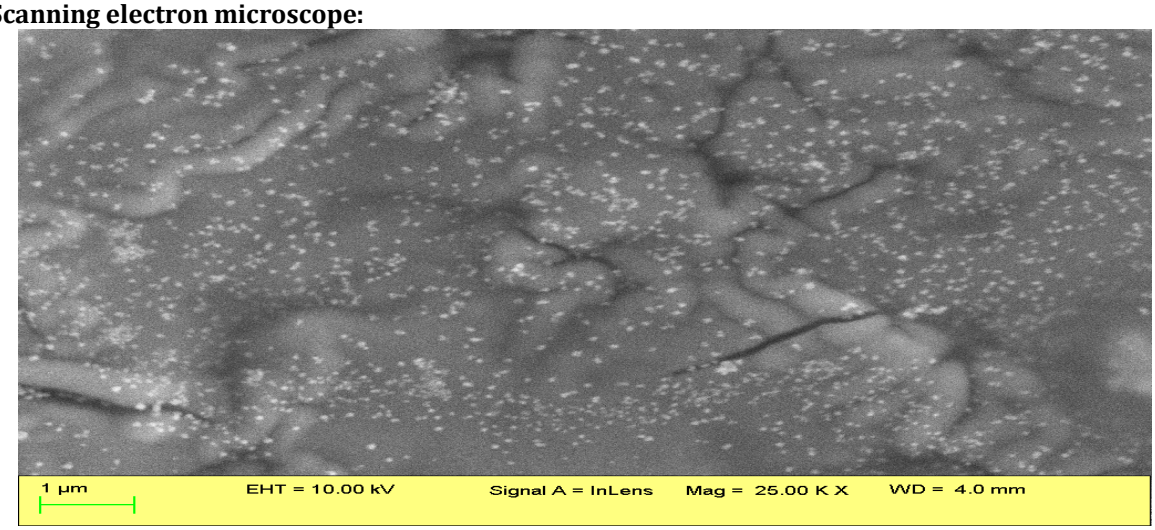

Fig2. Scanning Electron Microscopy of selected formulation

To create an image, a scanning electron microscope (SEM) moves a focused beam of electrons across the surface of a sample. Signals produced by electron interactions with atoms in the sample disclose the surface topography and chemical composition of the sample. During a raster scan, an image is created by connecting the position of an electron beam to the detected signal strength at each pixel. In the most popular type of SEM, secondary electrons produced by excited atoms are detected using an Everhart-Thornley detector. The signal strength is a function of the number of secondary electrons that can be detected, which in turn is affected by the specimen's topography.

***In-vitro* Drug Release:**

For in vitro drug release testing, the Franz diffusion cell was utilised. A cellophane membrane served as the filter. The membrane was ready to be attached to the cell after soaking in phosphate buffer for 12 hours. The itraconazole solution was placed in the donor's compartment, while the phosphate buffer at pH 7.4 was placed in the recipient's compartment. A magnetic stirrer was used to mix the contents of the cell at 37 degrees Celsius. 0.5, 1, 2, 3, 4, and 6 hour intervals were used to collect a total of six sets of serial samples.

By constantly replenishing the phosphate buffer, the volume of the receptor portion was kept constant. Spectrophotometry at a wavelength of 262 nm was used to analyse the samples.

Formulations of nanoemulgel from selected nanoemulsion:

The typically extremely low viscosity of nanoemulsion renders it unfit for topical use. Because of this, the nanoemulsion-based gel was formulated with the appropriate gelling agent.

Viscosity measurement:

Using the Brookfield Viscometer at 37±0.5°C and spindle #61, the resulting nanoemulgel formulations' viscosities were determined (0.5%w/v, 1%w/v, and 1.5%). At 37±0.5 °C and 60 rpm spindle speed, a single run was finished. There was a 2-minute pause in the process. Each sample was tested three times to determine the standard deviation.

Table 4. Viscosity (cP) of various formulations

RPM	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	9850
50	12350	10420	11520	11250	10950	10504	12520	11200	9230

Spreadability studies

A test formulation of 0.5 g was placed inside a circle marked on a glass plate with a diameter of 1 cm, and the plate was then filled with another glass plate. A 50 g weight was left on the top glass plate for five minutes. The formulation's dispersibility was seen to increase the diameter.

Table 5. Spreadability (gm.cm/sec) of various formulations

Sr. No.	Formulation	Spreadability (g.cm/sec)
1	F1	17.77±0.025
2	F2	16±0.035
3	F3	15.38±0.028
4	F4	15.09±0.018
5	F5	15.68±0.032
6	F6	14.81±0.012
7	F7	15.53±0.011
8	F8	15.23±0.011
9	F9	15.84±0.018

Antifungal Activity:

The activity of itraconazole 1.5% nanoemulgel formula using the agar diffusion method compared with 1.5% Itraconazole cream product against *Candida albicans* and *Microsporum gypseum* can be seen in table 6 and **fig 3**.

Table 6. Antifungal Activity of various formulations

Batches	Anti-fungal activity (mm)
F1	21
F2	23
F3	26
F4	29
F5	30
F6	32
F7	37
F8	39

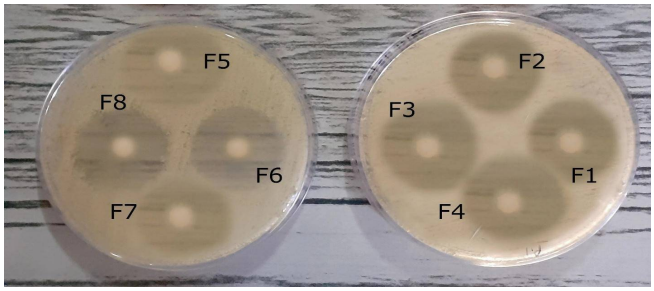

Fig3. Antifungal Activity of various formulations

***In Vitro* Drug Release Studies:**

A nano emulgel formulation was evaluated for diffusion using a Franz diffusion cell. The dialysis membrane was placed between the donor and receptor halves before they were secured. The local hydrodynamics were maintained with a blend of appealing dab, and the receptor compartment was dosed with a 7.4-pH phosphate buffer. At regular intervals, 1 cc of the tests were drained and refilled with cushion. The samples were analysed by a UV-Visible spectrophotometer at a wavelength of 262 nm to get the concentration. There were three separate runs of the experiment. The same method was used on all of the gels.

Table 7. Percent drug release from various formulations

Time (Hrs.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	6.13	8.17	7.14	6.25	9	5.34	5.01	3.41	2.31
2	11.13	14.08	12.96	15.22	17	12.96	16.72	19.15	16.24
3	23.12	24.21	23.01	22.11	25	19.6	21.30	18.12	20.11
4	26.61	32.65	31.09	28.23	34	25.66	23.49	22.44	20.66
5	30.99	40.42	40.97	35.49	41	32.67	34.69	39.45	39.41
6	45.35	48.57	47.87	45.66	50	40.19	38.09	35.66	30.11
7	48.49	57.45	55.13	52.79	59	54.66	41.49	38.09	37.71
8	57.18	65.15	62.14	60.49	68	58.19	51.78	48.99	49.89
9	62.16	72.30	74.25	64.49	78	55	56.99	54.10	52.31

Drug release comparison of nanoemulsion gel with conventional gel:

The in-vitro drug release of carbopol-940(1%)-containing conventional itraconazole and carbopol-934(1%)-containing nanoemulsion gel was compared. Table 8 displays the findings.

Table 8. Percent Drug release comparison of nanoemulsion gel with conventional gel

Time (Hrs.)	Nanoemulgel	Conventional gel
1	10.1±1.56	6.1±0.21
2	15.5±1.22	11.9±1.22
3	22.1±2.33	17.9±2.30
4	32.0±0.25	24.3±0.40
5	90.4±0.41	60.3±2.36
6	62.2±0.52	36.8±1.42
7	76.7±1.24	41.2±0.54
8	84.6±0.36	53.1±0.21
9	43.8±0.14	29.1±1.01

Stability studies at different temperature conditions:

The stress caused by temperature changes was taken into account by storing the details at different temperatures. A cooler (4°C), room temperature (25°C), and accelerated temperature (40°C) were used to store the details for a total of 90 days. Physical changes (such as clarity, stage partition, medicine precipitation, and shading change), sedative substance, and pH were assessed 1, 30, 60, and 90 days after the plans were created. Nanoemulgels remained transparent after three months of storage at 25.2°C, 40.0.1°C, and 4.0.2°C, as shown in a table containing the results of a stability study. Transparency, pH, drug concentration, and phase separations were all found to be consistent across all formulations during stability testing.

Table 9. Stability studies at different temperature conditions

Sr. No.	Observation		Before Accelerated Stability Testing	After Accelerated Stability Testing		
				30 days	60 days	90 days
1	Visual appearance (opaque)		Opaque	Opaque	Opaque	Opaque
2	EE(%)(±SD)		96.1	93.5	95.1	95.9
3	pH(±SD)		5.07	5.33	5.09	5.14
4	Viscosity	10	4641	4890	4999	5409
		20	5322	6669	5598	4089
		30	6578	6703	6009	6004
		40	5365	5093	6421	4092
		50	4898	4095	4132	4011

The problems associated with Itraconazole poor bioavailability were attempted to be mitigated by developing a stable nanoemulsion. The highest bioavailability products on the market right now are capsules and solutions with bioavailability between 30 and 55 percent. As a result, lipid-based nanoemulsion was developed to enhance the solubility of drugs and boost their oral bioavailability. This research confirms previous findings that Carbopol 934 can be utilised to produce a nanoemulsion of itraconazole. Based on these observations, it was concluded that lipid-based formulations may be generated at the nanoscale. The studies demonstrated a fair diameter dispersion and a mean droplet size in the nanometer range. Particle size (318.43 nm) was used to determine that Formulation 5 (F5) was the most effective. Polydispersibility index (0.436) and percent cumulative release (95.92%) with r2 values of 0.986 allow for the development of itraconazole nanoemulsion using Carbopol 934 as a lipid.

DISCUSSION

Transdermal administration of itraconazole was proposed, and nanoemulgel was proposed as a vehicle because of its low solubility and high permeability. In any case, the topical strategy may be able to sidestep the problem. The nanoemulgel drug delivery system is composed of four distinct phases: oil, surfactant cosurfactants, water, and a gel matrix. Finding the optimal concentration range for the nanoemulsion individual components. Morphology, droplet size, viscosity, and conductivity were modified for each of the eight nanoemulgels (F1-F9) that were made. The TEM image revealed a spherical structure for all of the formulations despite their nanoscale sizes (10-100 mm). A low PDI score indicates that there is consistency in droplet size among formulations. The addition of 1%, 1.5%, and 2% Carbopol 934 improved the efficacy of the successful drug-loaded formulation F5. These formulations were characterised in a variety of ways, including by their appearance, drug content, content homogeneity, viscosity, pH, Spreadability, and in vitro skin permeation. Without the usage of irritating chemical boosters, the developed nanoemulgel technology was proved to have effective penetration. The oil, surfactant, and, most notably, the cosurfactants in the nanoemulgel all contributed to improved penetration, making this system one of a kind. Studies of the stability of the drug both at room temperature and in the fridge showed no appreciable changes in concentration or ph. Because it enhances Itraconazole bioavailability and penetration during transdermal distribution, the nanoemulgel formulation shows promise for treating fungal infections.

CONCLUSION

Stable nanoemulgels were developed in an attempt to address the problems caused by itraconazole poor bioavailability. The highest bioavailability products on the market right now are capsules and solutions with bioavailability between 30 and 55 percent. As a result, adherence is reduced when using a lipid-based nanoemulgel. Carbopol 934 was successfully employed to create an itraconazole nanoemulgel in the present study. Based on these findings, it was concluded that a nanoscale lipid-based formulation was feasible. The tests revealed that, on average, the droplets were nanometres in diameter, with very little variation. The best formulation, designated as F5, was found to have a particle size of 156.36 nm, a polydispersibility index of 0.653, and an entrapment efficiency of 96.1.

DECLARTION OF COMPITING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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