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Formulation and Evaluation of *Econazole nitrate* Nano emulsion for Fungal Topical Use

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Abstract— *The objective of this study is to design and develop a nanoemulsion of Econazole nitrate for the effective treatment of tinea versicolor, a common fungal infection. Econazole nitrate, an imidazole-class antifungal agent, is known for its broad-spectrum activity. However, it belongs to the Biopharmaceutics Classification System (BCS) Class II, indicating that while it has high permeability, it suffers from low solubility. This limited solubility can result in incomplete absorption when administered orally, leading to variable bioavailability among different patients. In topical formulations, the drug's effectiveness may be compromised due to its poor solubility in the vehicle and low permeability through the skin. To address these challenges, nanoemulsions have been developed as a delivery system. The study involves the preparation of a topical nanoemulsion containing 1% Econazole nitrate, utilizing different oils (such as oleic acid), surfactants (Tween 20), co-surfactants (PEG 200, PEG 400), and distilled water. These oil-in-water nanoemulsions are prepared using the spontaneous emulsification method. Formulations that successfully passed thermodynamic stability tests were further characterized for their appearance, pH, FTIR spectra, viscosity, drug content, drug entrapment efficiency, and in vitro drug release profile using a Franz diffusion cell. Additionally, the stability of these formulations was assessed over time.*

Keywords— *Nano emulsion, Topical drug delivery, Econazole nitrate, Viscosity, In-vitro drug release etc.*

I. INTRODUCTION

Nano emulsions are defined as isotropic, thermodynamically stable systems consisting of oil and water stabilized by surfactants, with droplet sizes typically ranging from 5 to 200 nm. These systems offer several advantages over macroemulsions, including a significantly higher surface area and free energy, which make them more effective as transport systems. Unlike macroemulsions, nano emulsions do not experience issues like creaming, flocculation, coalescence, or sedimentation. Nano emulsions can be prepared using the spontaneous emulsification method to enhance the solubility and bioavailability of drugs with poor water solubility[1,2].

Being non-toxic and non-irritating, nano emulsions are well-suited for skin and mucous membranes application. As drug delivery systems, they can improve drug efficacy, allowing for a reduction in the total dosage and minimizing potential side effects. Nano emulsions are particularly effective as drug carriers for topical treatments, especially in skin conditions, due to their ability to incorporate hydrophobic and hydrophilic drugs. This characteristic enhances drug accumulation at the target site while reducing side effects. Moreover, nano emulsions can facilitate the sustained and controlled release of the encapsulated drug, ensuring prolonged therapeutic effects[3,4].

In this formulation, the antifungal agent Econazole nitrate is utilized to create a nanoemulsion. Although Econazole nitrate is traditionally available in topical forms such as creams and lotions, these conventional formulations can cause side effects like irritation, pain, and redness. To mitigate these issues, a nanoemulsion of Econazole nitrate has been developed. Econazole nitrate is classified under BCS Class II, indicating that while it has high permeability, it suffers from poor solubility, leading to incomplete absorption when administered orally and resulting in variable bioavailability among individuals[5].

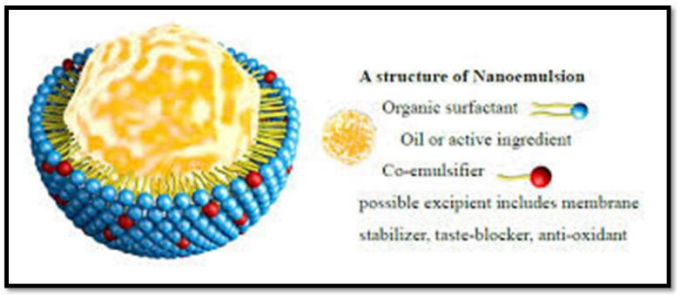

Figure 1: Structure of Nano emulsion

Econazole nitrate, an antifungal agent containing an imidazole ring, exhibits broad-spectrum activity by targeting the enzyme 14-demethylase, a cytochrome P-450 enzyme essential for converting lanosterol into ergosterol. By inhibiting ergosterol synthesis, which is crucial for maintaining fungal cell membrane integrity, Econazole nitrate increases cellular permeability, causing leakage of cellular contents and ultimately leading to fungal cell death. This drug is particularly effective when applied topically, achieving high skin

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concentrations with minimal dosing, making it useful in treating cutaneous candidiasis and tinea infections of the skin.

Tinea infections are characterized by skin pigment changes caused by the colonization of the stratum corneum by Malassezia furfur, a lipophilic fungus that is part of the normal skin flora. Econazole nitrate eradicates M. furfur from the outer skin layers, effectively treating these infections. The Nano emulsion formulation is designed to be applied to the skin surface, providing either local or systemic effects[6,7,8,9,10].

- The primary aim of this research is to develop a topical nano emulsion.
- To select suitable excipients by considering the physicochemical characteristics of the drug.
- To formulate and assess the performance of the nano emulsion.
- To enhance the drug's solubility through nanonization.
- To improve the therapeutic effect at the targeted site.
- To minimize dosing frequency and associated side effects.

II. Materials and Methods[11,12,13]

Econazole nitrate was provided as a gift sample by Lee Pharmaceuticals Pvt. Ltd., Mumbai. Oleic acid, PEG 200, and Tween 20 were procured from S.D. Fine Chemicals, Mumbai, India. All chemicals and solvents used were of analytical grade.

Methods

Determination of Melting Point[14,15]: The melting point of Econazole nitrate was determined using the capillary method. The drug was packed into a capillary tube to a height of 3 mm, with one end sealed. The capillary was then placed in a digital melting point apparatus. The temperature at which the drug began to melt was recorded, continuing until the entire sample had melted.

Solubility Study[16,17]: For the solubility test, an excess amount of Econazole nitrate was added to a solvent (aqueous or non-aqueous) at room temperature and left for 24 hours with occasional shaking. The supernatant was then analyzed using a Shimadzu UV 1800 double beam spectrophotometer.

Identification of Drug by FTIR[18,19]: The pure drug was combined with an IR grade solvent in an appropriate ratio and pressed onto an IR plate. The sample was then scanned over a range of 4000-400 cm^{-1} using a Perkin Elmer FTIR spectrometer. The FTIR spectrum of Econazole nitrate was analysed to confirm the presence of characteristic peaks matching the reference spectra.

Identification of Drug by UV Spectroscopy[20,21]: To prepare the stock solution, 100 mg of Econazole nitrate was accurately weighed and dissolved in methanol in a 100 ml volumetric flask. The solution was then diluted with methanol to achieve a concentration of 100 µg/ml.

Preparation of Standard Calibration Curve of Econazole Nitrate in Methanol[22,23]: From the stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml were transferred into separate 10 ml volumetric flasks and diluted with methanol to yield concentrations of 2, 4, 6, 8, 10, and 12 µg/ml. The absorbance of each solution was measured at 271 nm using a UV spectrophotometer, with methanol as the reference. All measurements were performed in triplicate (n=3) using the same instrument.

Compatibility Study[24,25]: Compatibility studies were conducted using a Perkin Elmer FTIR spectrophotometer to detect any potential chemical interactions between Econazole nitrate and the excipients (oil, surfactant, and co-surfactant). A physical mixture of the drug, oil, surfactant, and co-surfactant was prepared and mixed with potassium bromide. Approximately 100 mg of this mixture was compressed into a transparent pellet using a hydraulic press at a pressure of 15 tons. The pellet was then exposed to IR radiation in the range of 4000-400 cm^{-1} using the Perkin Elmer FTIR spectrometer. The resulting IR spectrum was compared with those of the pure drug and individual excipients to identify any new or missing peaks that might indicate interactions.

Pseudo-Ternary Phase Diagram Study[26,27]: Constructing pseudo-ternary phase diagrams is a meticulous process aimed at precisely defining phase boundaries. Care is taken to avoid metastable systems, ensuring that the emulsions formed are thermodynamically stable. Phase behaviour and composition relationships are mapped out using phase diagrams. For this study, oleic acid (as the oil phase), Tween 20 (as the surfactant), and PEG 200 (as the co-surfactant) were chosen to examine the phase diagrams in detail. Pseudo-ternary phase diagrams were created for each surfactant mixture ratio to identify the oil-in-water (o/w) nano emulsion regions, using an aqueous titration method. Surfactant (Tween 20) and co-surfactant (PEG 200) were mixed in different volume ratios (1:1, 1:2, 2:1) to explore the effects of varying co-surfactant concentrations on phase behaviour.

For each phase diagram, oleic acid (selected as the oil phase based on solubility studies) was mixed with the surfactant mixture in various volume ratios ranging from 1:9 to 9:1. A total of thirteen combinations of oil and surfactant mixtures (e.g., 1:1, 1:2) were prepared to precisely define phase boundaries. The aqueous phase was gradually titrated into each combination of oil and surfactant mixture under continuous magnetic stirring, with visual observations made regarding phase clarity and flowability. These observations were then used to construct a pseudo-ternary phase diagram, with one axis representing the aqueous phase, another representing the oil phase, and the third representing the surfactant/co-surfactant mixture[28,29].

Method of Nanoemulsion preparation[30,31]:

A homogenous mixture of oil, surfactant & drug econazole nitrate was mixed thoroughly the drug was accurately weighed to represent 1%W/W of the total formulation of nanoemulsion and added to the homogenous mixture of the previous preparation. the oil phase is then added drop by dropwise to the aqueous phase medium with continuous stirring by using the magnetic power for 30 mins, and O/W nanoemulsion is formed.

Evaluation of Nanoemulsion[32,33]:

Thermodynamic Stability: The selected formulations were subjected to a series of thermodynamic stability tests.

Heating-Cooling Cycle[34]: The formulations were subjected to a heating-cooling cycle, with temperatures alternating between

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4°C and 45°C for six cycles, with each temperature maintained for at least 48 hours. Formulations that remained stable under these conditions were then subjected to centrifugation.

Centrifugation[35]: The formulations that passed the heating-cooling cycle were centrifuged at 5000 rpm for 30 minutes using a centrifuge. Only those formulations that did not exhibit any phase separation were selected for further testing.

TABLE 1: Formulation of nanoemulsion

Sr. No.	Formulation	Surfactants mixture (ratio)	Oil/surfactants mixture (ratio)	%w/w of components in Nano emulsion Formulation			Drug % w/w
				Oil	S. mix	Water	
1	F1	1:1	1:9	5.00	45.00	50	1
2	F2	1:1	1:8	5.00	40.00	55	1
3	F3	1:1	1:7	5.00	35.00	60	1
4	F4	1:2	1:9	5.00	45.00	50	1
5	F5	1:2	1:8	5.00	40.00	55	1
6	F6	1:2	1:7	5.00	35.00	60	1

pH Measurement[36]: The pH of different nanoemulsion formulations was determined using a digital pH meter. One gram of nanoemulsion was dissolved in 100 ml of distilled water and the pH was measured. Each formulation was tested in triplicate to ensure accuracy.

Percentage Drug Content[37]: One milliliter of nanoemulsion was mixed with 10 ml of an appropriate solvent. After preparing aliquots of different concentrations and filtering the stock solution, the absorbance was measured using UV spectroscopy. The drug content was then calculated using the equation obtained from the linear regression analysis of the calibration curve.

Determination of % Transparency and Drug Precipitation[38]: Formulations with varying ratios were selected based on the ternary phase diagram. The transparency study was conducted to determine the maximum percentage of transparency and to check for any drug precipitation in the system, which includes oil, surfactant mixtures (surfactant and co-surfactant), and water containing 1% drug. A nanoemulsion system is expected to be clear and transparent when diluted with distilled water.

Viscosity Determination[39]: The viscosity of the nanoemulsions was measured using a Brookfield viscometer. Twenty milliliters of the nanoemulsion were placed in a 25 ml beaker, and the viscosity was measured using spindle number 6 at a speed of 10 rpm.

In Vitro Diffusion Studies[40]: The diffusion studies of the prepared nanoemulsions were conducted using a Franz diffusion cell equipped with a cellophane membrane. Five milliliters of the nanoemulsion were placed in the cellophane membrane, and the diffusion studies were carried out at 37 ± 1°C using 250 ml of 25% methanolic phosphate buffer (pH 7.4) as the dissolution medium. Samples of 5 ml were withdrawn at intervals of 1, 2, 3, 4, 5, 6, 7, and 8 hours, and each sample was replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The samples were then analyzed for drug content using a UV spectrophotometer at 271 nm.

III. Results and Discussion

This study aimed to formulate a nanoemulsion of Econazole nitrate for the effective treatment of fungal infections. Six nanoemulsion formulations were prepared and evaluated based on various parameters.

Organoleptic Properties: The organoleptic properties of Econazole nitrate were consistent with those described in the USP NF monograph.

TABLE 2: Organoleptic properties

Sr. No.	Test	Specification	Observation
1.	Color	White	White
2.	Odor	Odorless	Odorless
3.	Nature	Amorphous	Amorphous

Spreadability: The spreadability of the formulation was found to be good, ensuring that it adheres well to the skin.

Melting Point Analysis: The melting point of Econazole nitrate was determined to be 160°C, which is consistent with the standard melting range of 161-165°C.

Solubility: The solubility of Econazole nitrate was tested in various solvents, with the results summarized as follows:

TABLE 3: Solubility study

Sr. No.	Solvent system	Specification as per USP	Result
1	Ethanol	Freely soluble	Freely soluble
2	Oleic acid	Soluble	Soluble
3	Tween 20	Soluble	Soluble
4	PEG 200	Soluble	Soluble
5	Methanol	Freely Soluble	Freely Soluble
6	Water	Slightly soluble	Slightly soluble
7	chloroform	Insoluble	Insoluble
8	Ether	Insoluble	Insoluble

Identification of drug through UV spectroscopy

Standard calibration curve of Econazole nitrate as a pure drug in methanol

TABLE 4: Standard calibration curve of Econazole nitrate

Concentration (µg/ml)	Absorbance
2	0.216
4	0.458
6	0.668
8	0.889
10	1.124
12	1.322

UV shows that *Econazole nitrate* gives maximum absorption at 271 nm and figured in linear standard calibration curve shown in fig no 2.

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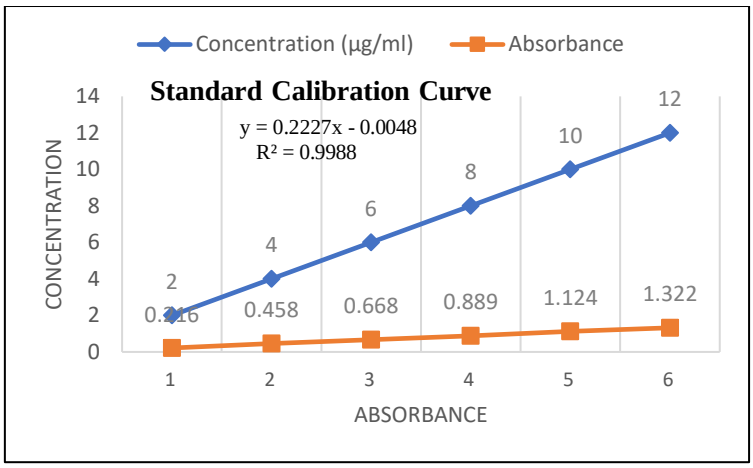

Figure 2: Standard calibration curve of Econazole nitrate in methanol

Determination of drug-excipients compatibility studies

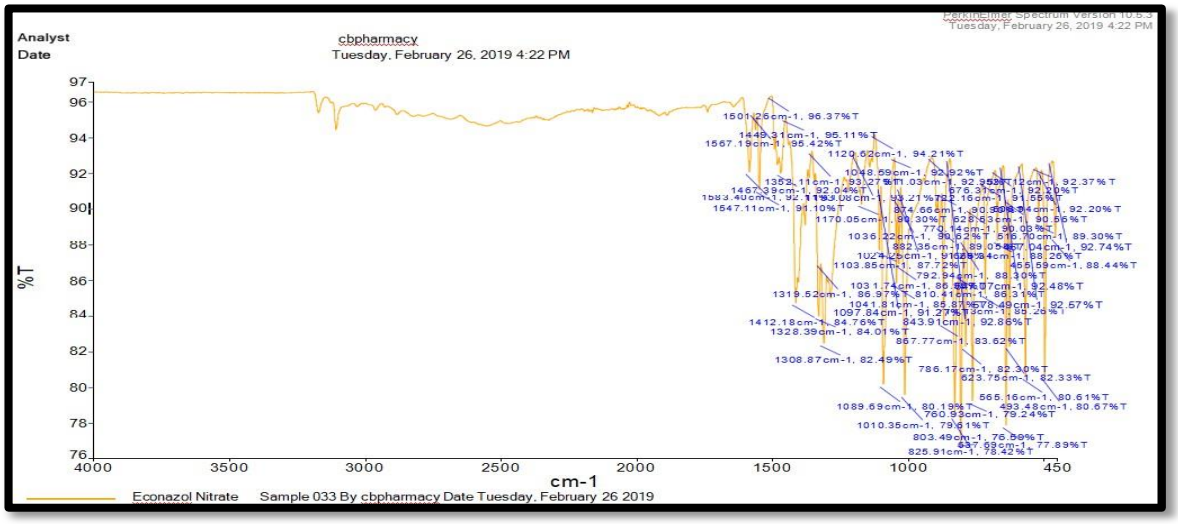

Figure 3: FTIR of Econazole nitrate

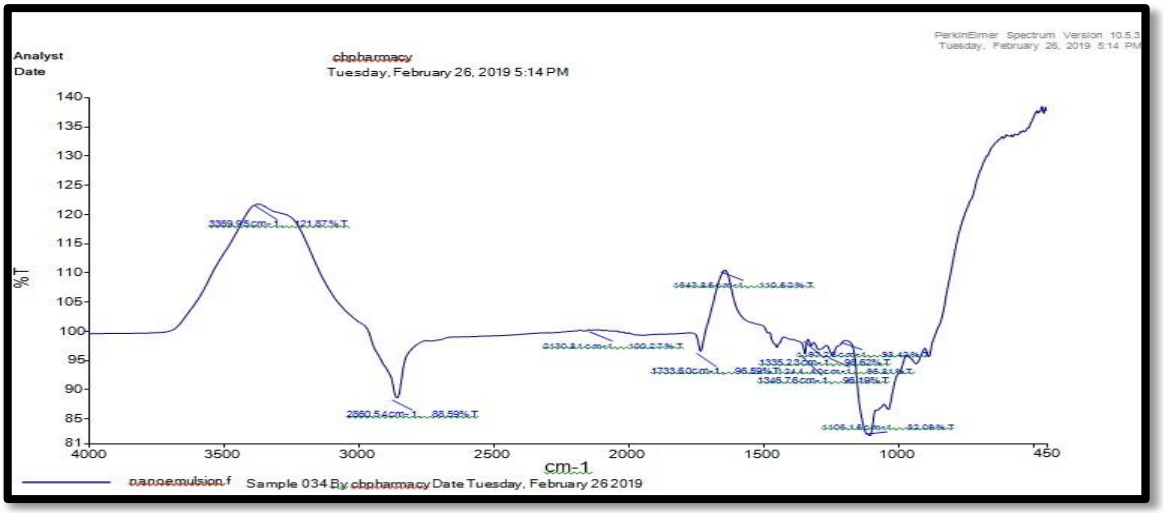

Figure 4: FTIR of Nanoemulsion formulation

From FTIR it can be said that there was no incompatibility between the drug and excipient as shown in fig. no. 3 & 4.

Thermodynamic stability

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TABLE 5: Thermodynamic stability study

Formulation code	Heating Cooling Cycle	Centrifugation
F1	Stable	No phase separation
F2	Stable	No phase separation
F3	Stable	No phase separation
F4	Stable	No phase separation
F5	Stable	No phase separation
F6	Stable	No phase separation

pH, Viscosity, and % Drug content:

TABLE 6: Characterization of Nano emulsion

Formula code	pH	Viscosity (cp)	Drug content (%)
F1	5.5	5229	95.92
F2	5.8	4332	96.32
F3	5.2	4320	95.45
F4	5.3	4850	94.66
F5	6.5	5920	98.78
F6	5.2	4530	94.65

The pH of the prepared nano emulsion formulations was determined to be between 5.5 and 6.5, as presented in Table 6. The drug content in the nano emulsion formulations ranged from 94% to 98%, with details also provided in Table 6. The average viscosity of the formulations was measured to be between 4000 and 6000 cp, with the F5 batch exhibiting the highest viscosity, all of which is summarized in Table 6.

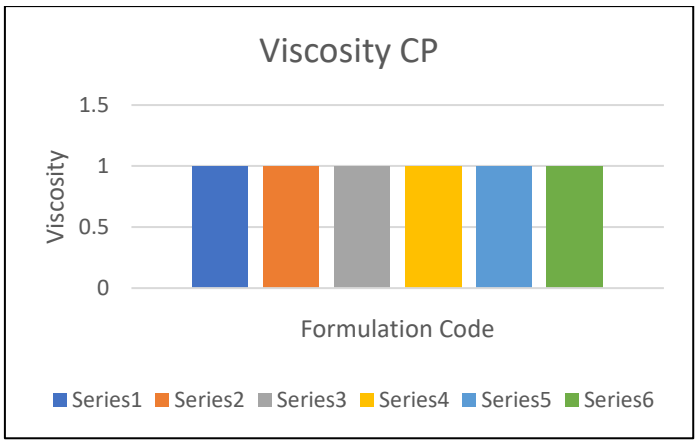

Fig. 5.

In-vitro drug release:

TABLE 7: In-vitro drug release (in %)

Time in hrs	F1	F2	F3	F4	F5	F6
0.5	6.86	5.95	4.5	3.68	8.22	5.79
1	12.77	11.86	10.95	9.59	14.13	7.97
1.5	22.75	15.95	17.29	20.45	25.49	16.09
2	35.04	33.22	30.04	28.68	38.22	27.16
2.5	46.4	44.13	43.22	40.04	52.76	40.36
3	57.31	55.04	53.22	51.58	59.58	50.6
3.5	68.3	65.53	60.12	67.20	69.10	64.23
4	71.2	70.2	71.5	71.21	75.25	71.15
4.5	76.2	75.25	76.35	76.35	85.25	76.45
5	81.45	81.78	81.23	82.10	90.24	81.65
5.5	90.23	90.52	90.23	90.78	94.70	90
6	93.25	92.23	90.7	96.12	98.12	95.25

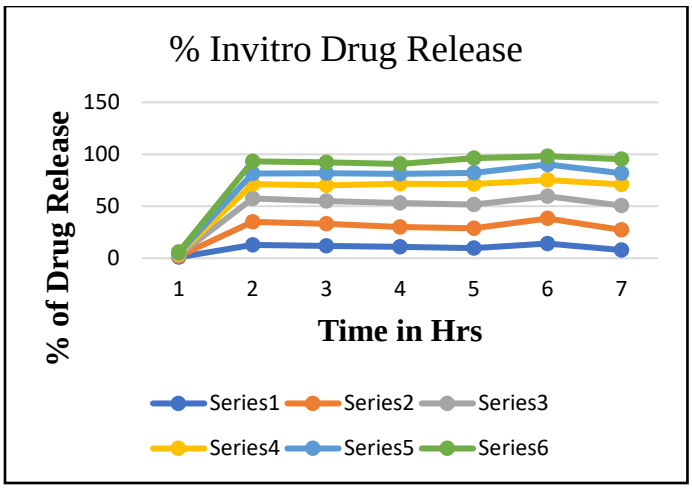

Figure 6: % In vitro drug release

Result of *in-vitro* drug release from different formulations are tabulated in table 7 and graphically shown in fig.no.6. profile as compared to other preparation F1, F2, F3, F4 and F6.

IV. Conclusion

The prepared formulation batch F5 shows the better release better release profile than others. From above result concluded that nano emulsion drug delivery system can be effective for topical application in the treatment of fungal diseases and further study on an animal being need to perform before its commercial use.

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