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DEVELOPMENT AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER LOADED EMULGEL OF ECONAZOLE NITRATE

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ABSTRACT

The primary goal of this work was to create and assess an Emulgel that contains an Econazole Nitrate nanostructured lipid carrier (NLC) for topical administration. An imidazole derivative substance called econazole nitrate (ECN) is mostly used to treat surface infections. Fungal infections are treated by topical application of econazole nitrate. Using a high-pressure homogenization process, for solid lipid stearic acid and liquid lipid oleic acid is used and it combined with surfactants (Span 80 and Tween 80) to create econazole nitrate NLC. The zeta potential, SEM, and particle size of this nanostructured lipid carrier containing econazole nitrate were assessed. Lipid nanoparticles were mixed with emulgel to be applied topically, and their antifungal, in vitro drug release, pH, drug content, viscosity and stability tests were assessed. Up to three months, the stability testing revealed no appreciable changes in the formulation. Ultimately, it can be said that one of the more promising methods for regulating the release of the medication is the Econazole Nitrate emulgel loaded with NLC topical medication administration for more thorough and successful care.

KEYWORDS: Econazole Nitrate, Nanostructured lipid carriers (NLCs), Emulgel, Topical Delivery,

INTRODUCTION

An imidazole derivative substance called econazole nitrate (ECN) is mostly used to treat surface infections. Econazole nitrate is reportedly applied topically to treat fungal infections, according to some reports.[1,2] It is necessary to include the drug into a lipid matrix in order to increase its solubility because it falls under BCS class II (low solubility, high permeability). When utilized as drug delivery vehicles, stable colloidal systems such as nanostructured lipid carriers (NLC) and solid nanoparticles (SLN) provide a number of advantages. Colloidal carrier systems like SLN and NLC offer numerous compounds regulated release characteristics.[3] A common pharmaceutical element of BCS class II, econazole nitrate (ECZN) $C_{18}H_{15}Cl_3N_2O$ is used to treat topical fungal diseases like cutaneous candidiasis, athlete's foot, and pityriasis (tinea versicolor). The purpose of the Econazole Nitrate co-crystals was to improve the water solubility of the BCS class II drug.[4]

NLC is created by combining solid lipids (oil) that have been prepared with different amounts of stearic acid and homogenizing them quickly. The development of a topical emulgel incorporating NLC dispersions loaded with econazole nitrate was the goal of this investigation. The high-pressure homogenization technique was used to create the NLC. Zeta potential, scanning electron microscopy, and particle size were used to describe the nanoparticles. Because of the impact of Nanostructured lipid carrier on in vitro drug release, NLC-loaded Econazole Nitrate emulgel has better penetration into the stratum corneum and can prolong drug release.[3]

MATERIALS AND METHODS

Materials

Mahrshee Laboratories Pvt. Ltd. provided a gift sample of econazole nitrate. Stearic acid and Oleic acid was purchased from Modern Industries Sinnar, Nashik. Tween 80 and Span 80 was obtained from Loba Chemie Pvt. Ltd. and Carbopol 934 was purchased from Mumbai's Research-Lab Fine Chemical Industry. In a lab, distilled water was made. The Mumbai-based Research-Lab Fine Chem. Industry supplied the triethanolamine and methanol.

Methods

Preparation of nlc dispersions

Using the heated high-pressure homogenization process (HPH), the NLC dispersions were made. The manufactured NLC dispersions' composition is listed in Table 1. To make NLC, to form the lipid phase, oleic acid and stearic acid were combined in the appropriate amounts. After that, the mixture was heated to 80°C, which is greater than solid lipid's melting point, and weighed volumes of econazole nitrate were added. Simultaneously, an aqueous phase was made by heating distilled water to the same temperature and adding the appropriate amounts of Tween 80 and Span 80. After that, the heated lipid phase was mixed with the hot surfactant solution for 5 min. using a magnetic stirrer. The generated pre-emulsion was homogenized with an IKA digital ultra turrax T25 homogenizer. [5]

Table 1: The batches' composition according to the 3^2 Full Factorial Design.

Ingredients (w/v)	Quantity								
	F 1	**F 2**	**F 3**	**F 4**	**F 5**	**F 6**	**F 7**	**F 8**	**F 9**
Econazole Nitrate	1	1	1	1	1	1	1	1	1
Stearic acid	35	35	35	20	20	20	5	5	5
Oleic acid	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Tween 80	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Span 80	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (q.s)	100	100	100	100	100	100	100	100	100
Speed (RPM)	15000	20000	25000	15000	20000	25000	15000	20000	25000

Gel preparation

Table 2: Composition of gel

Sr No	Ingredients	Quantity
1	Triethanol-amine	0.1 %
2	Carbopol 934	1 %
3	Distilled Water (q.s)	100ml

Triethanolamine was added to the distilled water (40°C) mixture along with the weighed amount of Carbopol 934 to ensure the solution stayed within the intended pH range. After ensuring consistency in the stirring, the gel was refrigerated for 24 hours.[6]

Preparation of emulgel:

Further incorporation of NLC dispersion containing Emulgel were created by incorporating 1% drug.

EVALUATION

Zeta potential, polydispersity index and particle size determination

Based on Brownian motion, the (NanoPlus) equipment at 25°C determined the optimal batch's particle size analysis. Samples were diluted to a scattering intensity of around 150–300 keps in particle-free filtered water. The polydispersity index and mean z-average diameter were determined using cumulative analysis utilizing NanoPlus software.[8,9] The zeta potential is a crucial indicator of the stability of the formulation. The zeta potential's magnitude represents the strength of the electronic repulsion between the adjusters and the dispersed charge particle. Folded capillary cells were used to measure the optimized batch's zeta potential using a zetasizer. From the produced nanosuspension, a 1 milliliter sample was taken out and combined with 10 milliliters of double-distilled water. To determine primary particle size, the samples were ultrasonically treated for five minutes before being assessed for size. After that, the sample was removed out of a disposable cuvette and put inside the device to evaluate its size and zeta potential.[7,8,9]

SEM

Utilizing scanning electron microscopy, examine the optimized batch of NLC's morphology, or its forms and surface properties (SEM). In order to achieve a homogeneous coating on the sample and permit high-quality SEM photos, the sputtering process took about five minutes. The SEM was run with a load current of roughly 80MA at a low accelerating voltage of roughly 25KV.[2,11]

CHARACTERIZATION OF NLC LOADED EMULGEL FORMULATIONS

Physical appearance

Prepared formulations of Econazole Nitrate emulgel were visually evaluated for consistency, homogeneity, and color.

pH

A digital pH meter that had been previously calibrated using pH 5 and pH 7 was used to measure each composition's pH. After preparation, the pH values were taken immediately away. The formulation batches' pH values were measured three times, and the pH ranges were calculated using the average mean. [12]

Viscosity

At room temperature, using a Brookfield viscometer, the viscosity of numerous emulgel compositions was determined. type T-bar. Fixed to the viscometer at 3 rpm, spindle number 96F was submerged in the beaker holding the NLC emulgel. It was established what the formulation batches' viscosity was. Plotting the viscosity values versus speed allowed for the determination of the formulations' rheological behavior.[12]

Spreadability

Good spreadability is one of the requirements for an emulgel to meet the appropriate amounts. This term describes the region of the skin or other affected area where gel spreads readily. The spreading value of a formulation also affects how effective it is as a medicine. It's done with a modified spreading apparatus and the "slip and drag method." It made up of 7 x 2 cm glass slide that is mounted on a tripod stand. Over the fixed slide, which has another slide with a weight affixed by thread, is inserted an excess of 2 grams of emulgel. For five minutes, a 100g weight is

placed over each slide to release air. Subsequently, the two slides have the weight removed. A 50-gram weight is attached, and the amount of time it takes for the slide to move 6.5 cm, the designated distance, was recorded. The amount of time needed to transition between two slides decreases with increasing spreadability. The formula is used to calculate it..[12]

$$S = ML / T$$

Drug Content

In a volumetric flask, 100 mg of the drug-containing emulgel was taken. The formulation was fully dissolved in 100 mL of methanol after sufficient methanol had been added to reach a concentration of 1000µg/ml. 100 ml were created by diluting 1 ml of this solution. Using a UV visible spectrophotometer, the produced solution's absorbance was measured at 220 max, and the drug content percentage was calculated.[3]

Antifungal study

Agar diffusion method was employed to ascertain the formulation's antifungal efficacy. The media were placed in a standard 9 cm diameter petri dish with a 0.5 cm depth. Prior to use, the lots' sterility was regulated. To prepare the inoculums, one or two colonies of candida albicans (NCIM NO.3102) were suspended for a whole day. 10 ml of sterile saline is added to a tube containing cultures in potato dextrose agar medium. Saline was used to dilute the tubes. The agar medium's surface was covered in the inoculum. Before the formulation was placed on the plates, they were dried for 15 minutes at 35° C. Prepaid boars with a 0.5 cm diameter had a formulation sample (1% w/v) added to them. Following a 24-hour incubation period at 35°C, the boars' zone of inhibition was evaluated.[10]

In-vitro drug release study

For the in-vitro drug release study, optimized batch was utilized. To ascertain the profile of drug release for emulgels, a Franz diffusion cell-like laboratory setup was put together. The setup consisted of a donor chamber and a receptor compartment, separated by a diffusion membrane that was attached to the egg membrane, comprised cell. The donor compartment was open, having an inner diameter of 24 mm. One end of the receptor compartment was exposed to the environment, allowing for sampling. The diffusion medium employed was a phosphate buffer with a pH of 6.8.

The medication that contains emulgel was placed inside the egg membrane that divides the donor compartment from the receptor compartment. Egg membrane was first immersed in phosphate buffer for a whole day. A clamp was used to hold the donor and receptor chambers together. The donor compartment was positioned in a way that minimally made contact between the diffusion media and the egg membrane. All the parts were attached to a magnetic stirrer. A thermostat-controlled magnetic stirrer is employed to transfer the receptor compartment, which holds 100 milliliters of phosphate buffer. It was continuously spun at 50 rpm and maintained at 37 ± 0.5°C. At predetermined intervals, one milliliter samples were collected, suitably diluted with methanol to produce a ten-milliliter solution, and the drug concentration was then measured at 220 max in comparison to a blank using a UV Spectrophotometer. Every time a sample was withdrawn, an equivalent volume of pH 6.8 phosphate buffer solution was added to the receptor phase.[3]

Drug release kinetics

To look into the drug release kinetics, the release data were fitted to Higuchi's square root of time kinetics models and zero order models. Using plots of CDR vs. Time (hrs.) for zero order and % CDR vs. Sq.Rt. for the Higuchi model—where %CDR is the amount of medication released occasionally—the coefficient of determination of R^2 values was computed.[3]

Optimization Study

3^2 full factorial designs were used to examine formulation optimization. Antifungal activity (Y1) was selected as the dependent variable, while the homogenizer speed (X2) and stearic acid levels (X1) were selected as the independent factors. Design Expert software was applied to process the gathered information, and an analysis of variance (ANOVA) was employed for statistical analysis. To find out how homogenizer speed and stearic acid affect the dependent variable, the data were additionally exposed to the 3-D response surface methodology. For example, antifungal action.[3,13]

Stability Study

Stability studies in accordance with ICH recommendations. The Stability Studies employed the optimized formulation. At 0 and 3 months, the formulations were assessed mostly based on their physical attributes. For an ideal batch, physical characteristics like clarity, pH, viscosity, and medication concentration were assessed.[3]

RESULT AND DISCUSSION

Evaluation of Nanostructured Lipid Carrier

PDI and particle size

Table provides the optimized batch's particle size:

Table 3: PDI and particle size

Formulation Code	Particle size (nm)	PDI
F7	380.6	0.258

It was discovered that the optimized batch's Nanostructured Lipid Carrier formulation had a particle size of 380.6 nm.

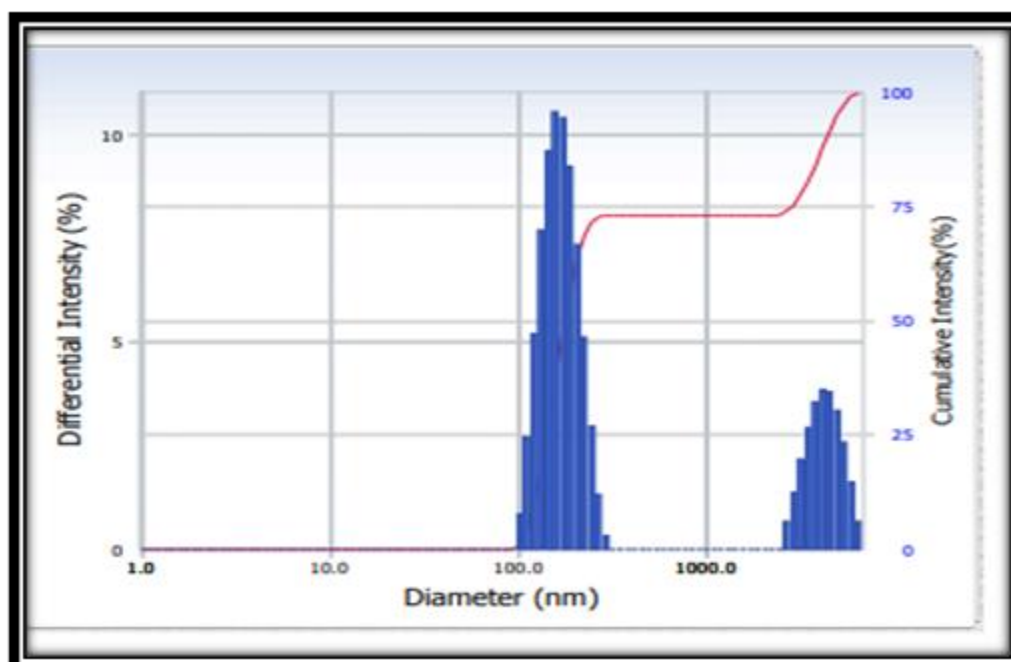

Figure 1: Graph of Particle size determination

Zeta Potential

The table displays the optimized batch's zeta potential.

Table 4: Zeta potential

Formulation	Zeta potential (mV)
(F7)	26.41

Zeta potential, as defined by ICH guidelines for stability tests of various pharmaceutical formulations, indicates the (colloidal dispersion) NLCs stability in stress testing conditions.

Particle size has an impact on zeta potential; the lowest particle size in the nanosize range, 380.6, exhibits a zeta potential of 26.41 mV.

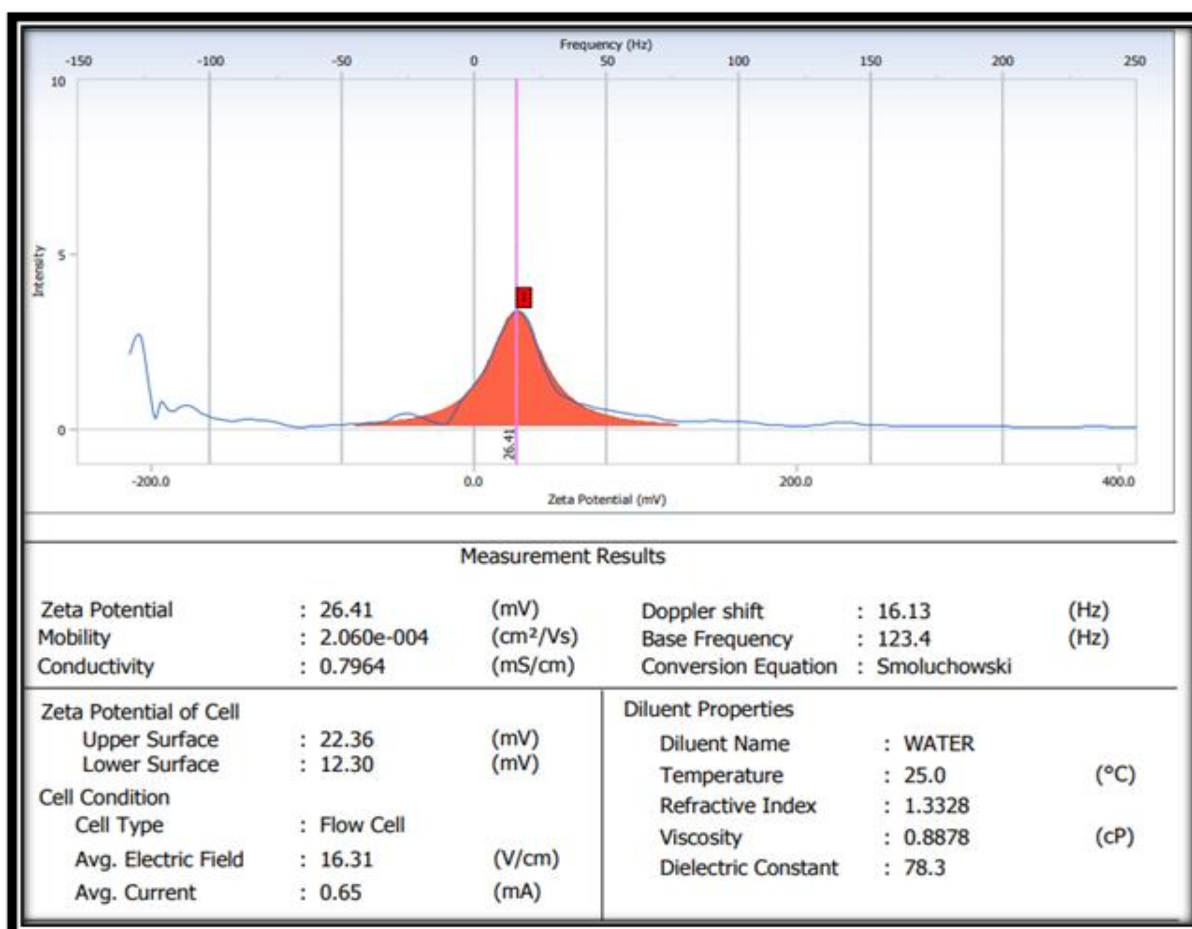

Figure 2: Graph of Zeta potential

Scanning Electron Microscopy

The emulgel formulation's consistent distribution of NLC has been revealed by the F4 batch's scanning electron microscopy. But due to homogenization of NLC, the spherical structure was found to be change and some agglomeration has been seen in the sample provided for SEM analysis.

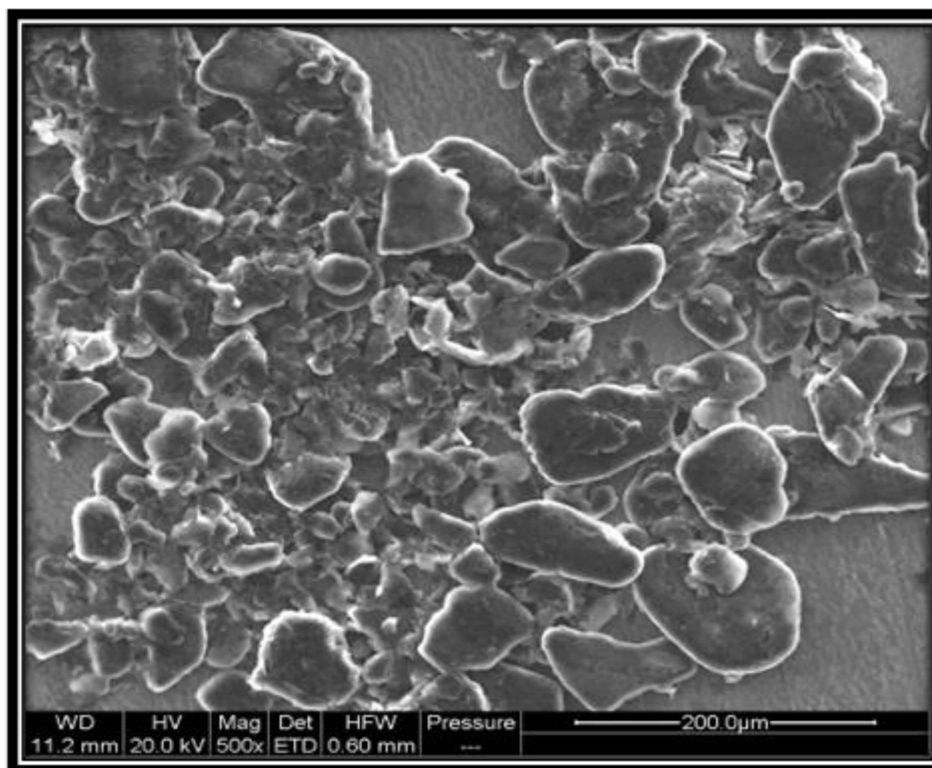

Figure 3: Scanning Electron Microscopy

CHARACTERIZATION OF NLC LOADED EMULGEL

Physical evaluation of formulations

The emulgel formulation were observed to have a uniform, homogenous, and translucent physical appearance.

pH

The range of pH values observed for the different emulgels was 5.32 to 6.08. This pH value suggests the appropriateness of the emulgel for topical administration.

Viscosity

Viscosity, or the essential physical property for topical preparations is resistance to flow. since affects the drug's release and spreadability as well as jellification. Every composition now has the necessary consistency to be applied topically. The table shows the viscosity measurement values for each formulation. This viscosity result shows that viscosity decreases with increasing homogenizer speed and decreasing stearic acid content.

Spreadability

Topical emulgel formulations, the spreadability of the emulgel is an important consideration. It has an inverse relationship with the emulgel's viscosity; higher viscosity formulas are thicker and more challenging to spread., while those with a lower viscosity appear fluid. Neither of these extremes is appropriate for any topical preparation; therefore, the formulations with a gel's optimal viscosity have proper spreadability. Additionally, there was only a slight variation in the spreadability results among the formulations; it was discovered that each formulation had good spreadability.

Drug Content

An analysis of the drug content was completed to make sure that drug concentration in every formulation was the same. It was discovered that all prepared emulgel formulations had a medication concentration that ranged from 92 to 98%. As a result, all formulations retained the same substance.

In-vitro drug release study

Enhanced emulgel formulation were shown to release the medication at a higher rate than the commercial cream. Both the marketed formulation and the improved formulation exhibit 74% and 76% drug release, respectively, over an 8-hour period. The formulation exhibited a steady state release for up to eight hours, suggesting that it would have improved biological membrane contact. Since the medication is confined in the lipid phase, it can take up to eight hours for the formulation

to penetrate the egg membrane. This drug release event also implies that drug diffusion would follow mechanisms if such formulations were put to the skin's surface.

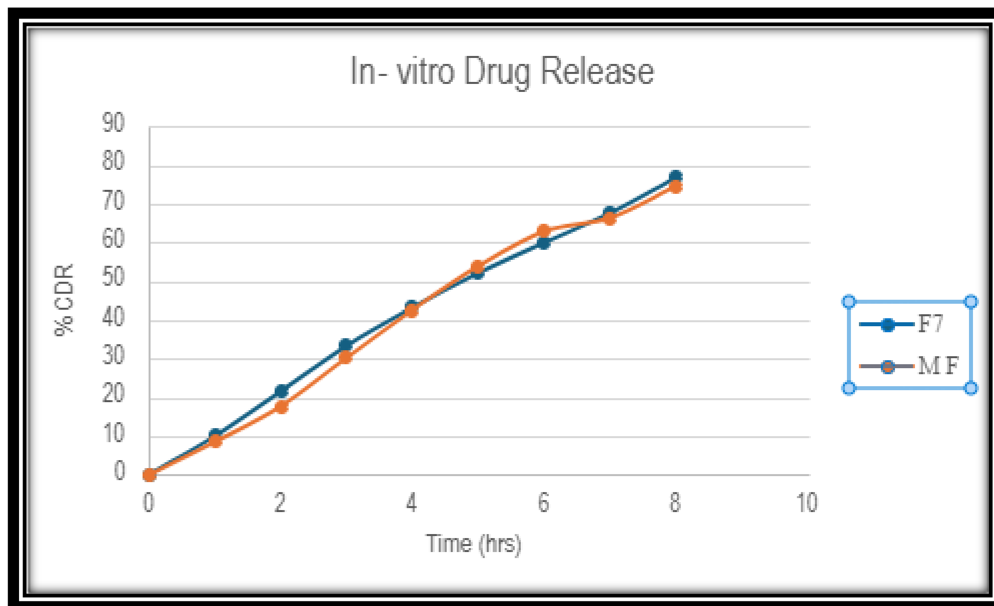

Figure 4: Drug release profile of formulations in vitro and Marketed Formulation

Drug Release Kinetics

Based on drug and NLC diffusion process from gel matrix, the zero-order mechanism is demonstrated by the optimized formulation, as per the findings of Higuchi kinetics drug release experiments and zero order drug release research. As seen in figure.

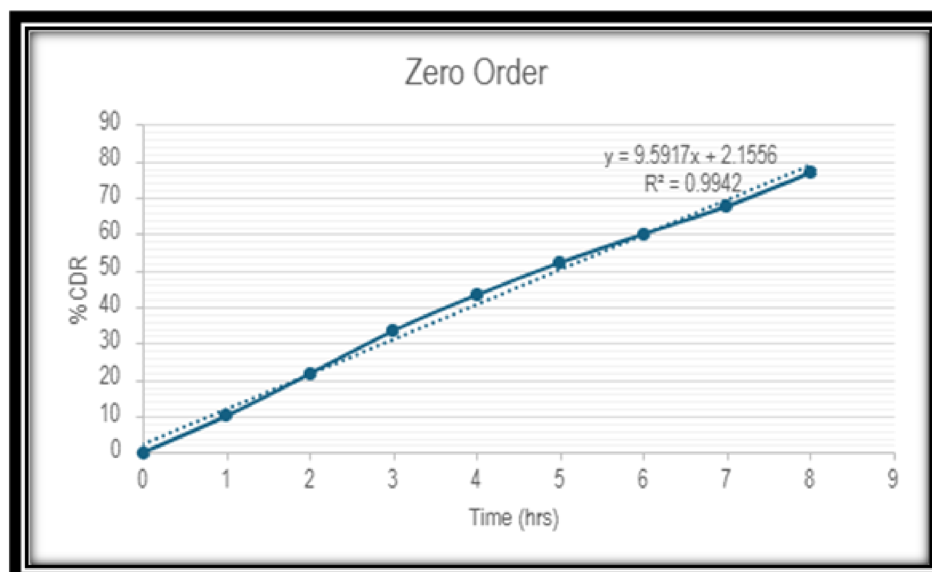

Figure 5: Model graph for Zero order release kinetics

Antifungal activity

According to the study, Econazole Nitrate was effective against specific strains of microorganisms and maintained its antifungal activity when prepared in an emulgel loaded with a nanostructured lipid carrier. A 24.2 mm zone of inhibition is displayed in F4.

Table 5: The result of antifungal activity of formulations is shown in below

Formulation Code	Zone of Inhibition (mm)
F1	23.6 ± 0.5
F2	23.8 ± 0.47
F3	22.4 ± 0.40
F4	24.2 ± 0.75
F5	22.9 ± 0.96
F6	23.4 ± 0.72
F7	18.2 ± 0.60
F8	19.9 ± 0.70
F9	21.1 ± 0.60
1% Drug suspension (10µg/ml)	16.0 ± 0.20

Marketed formulation	15.0 ± 0.25
1% Cream formulation	17.0 ± 0.32

Optimization

The table presents the experimental design in connection to the optimization investigation. The homogenizer speed (X2) and the amount of stearic acid (X1) respectively are given and the antifungal activity (Y1). It can be confirmed that both factors significantly affect the selected replies because values of X1 and X2 were determined to be significant at $p < 0.05$.

Table 6: Experimental design and Optimization study

Runs	**Factor X-1 (Stearic acid %)**	**Factor X-2 (Speed of Homogenizer RPM)**	**Response Y1 (Antifungal activity mm)**
F1	35	15000	23.6
F2	35	20000	23.8
F3	35	25000	22.4
F4	20	15000	24.2
F5	20	20000	22.9
F6	20	25000	23.4
F7	5	15000	18.2
F8	5	20000	19.9
F9	5	25000	21.1

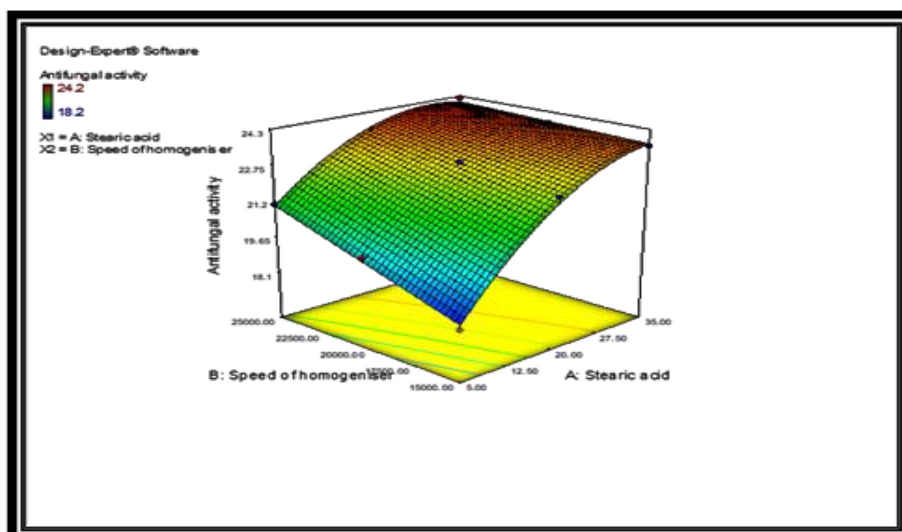

Figure 6: Surface Response Plot illustrating the Impact of Homogenizer Speed and Stearic Acid on Antifungal Activity

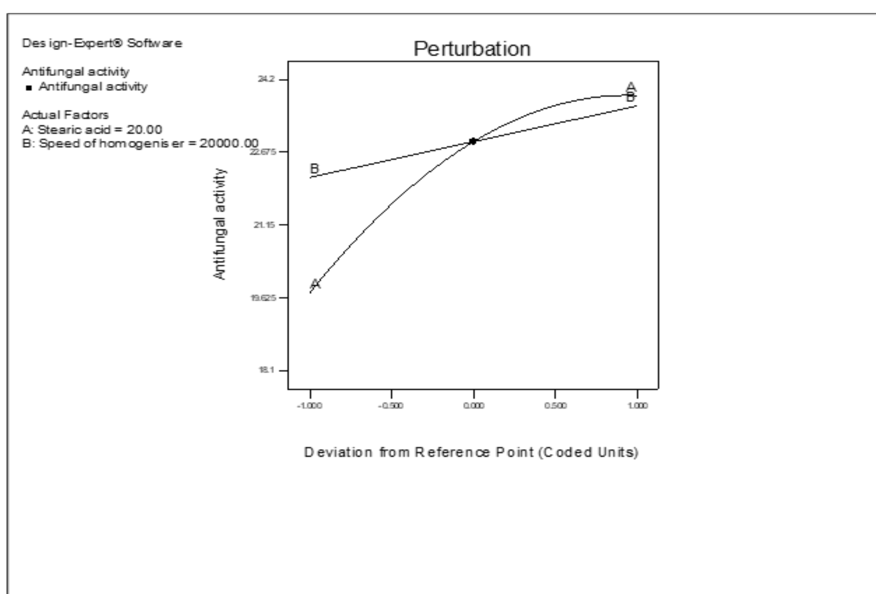

Figure 7: Plot of perturbation illustrating the impact of stearic acid and homogenizer speed on antifungal activity

Conclusion: The antifungal zone of inhibition is impacted by stearic acid and homogenization speed. The antifungal zone of inhibition grows with increasing stearic acid levels, and it also increases with increasing homogenization speed.

Stability studies

Once the adjusted formulation had been stored at room temperature, it was assessed. Both pH and viscosity showed a small increase.

Table 7: Data from stability studies for the F7 formulation at 40°C ± 2°C and 75% ± 5% relative humidity

Sr no.	Observations	Before Stability testing	After Stability testing
1.	Clarity	Creamy, white	Creamy, white
2.	pH	6.08 ± 0.025	6.10 ±0.01
3.	Viscosity (cp)	12220	14540
4.	% Drug Content	98.7	98.2

CONCLUSION

In the current investigation, the lipids used were solid stearic acid, liquid oleic acid, and Span 80 and Tween 80 as surfactants utilizing the HPH process to successfully manufacture Econazole Nitrate loaded NLCs. Using NLCs to apply Econazole Nitrate topically on the skin may reduce both its systemic absorption and adverse effects. Moreover, the fungal burden could be decreased by Econazole Nitrate loaded NLCs. Consequently, in contrast to commercially available products, econazole nitrate-NLC proved to be a viable formulation strategy for treating fungal infection.

DECLARTION OF COMPITING INTEREST

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

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