

Formulation and Evaluation of Microemulsion Based Luliconazole Gel for Topical Delivery

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ABSTRACT

Introduction: Drug delivery through topical route is mostly preferred for local dermatological action. Topical action of preparations has certain limitations in terms of drug solubility, residence time, lipophilicity and permeability. Conventional dosage forms such as creams, ointments etc exhibit drawbacks like problem in stability, stickiness, poor absorption as well as permeation mainly in case of large molecule. To overcome this, the origination of emulgel came into existence which basically focus on the delivery of hydrophobic drugs.¹ The present research is proposed to prepare and evaluate microemulsion based luliconazole gel for its enhanced solubility and permeability for better antifungal activity. **Materials and Methods:** The present study was aimed to prepare and evaluate microemulgel of luliconazole for topical application by using poly unsaturated fatty acids such as linseed oil. Sodium alginate was used as a gelling agent. Luliconazole microemulsion was prepared by varying the concentration of surfactants while the oil concentration was kept fixed. It was characterized for globule size and physical stability. Microemulgel was prepared and characterized for drug content, viscosity, spreadability, *in vitro* permeation study, skin irritation test, *in vitro* antifungal study and stability study. Skin irritation test was carried out after taking approval of Institutional Animal Ethics Committee. **Results and Discussion:** Two microemulsion were selected based on the globule area, PDI and physical stability and incorporated into the gel base of sodium alginate. The optimized microemulgel (F1) showed acceptable physical properties with lowest viscosity value (2325 cps) and showed pseudoplastic behaviour, highest spreadability value (3.7 gm.cm/sec), Highest drug permeation (63.50%) after 5 hr. Skin irritation test showed no signs of irritation in any rats. Optimized formulation F1 represents greater zone of inhibition (38 mm) as compared to pure gel (34 mm) and showed acceptable stability profile. **Conclusion:** It can be concluded that luliconazole microemulgel prepared with linseed oil and sodium alginate can be a choice for topical antifungal treatment.

Key words: Luliconazole, Linseed oil, Sodium alginate, Microemulsion, Topical, Antifungal, *in vitro* permeation.

INTRODUCTION

Microemulgel is considered as one of the promising technologies among novel drug delivery system due to its dual mechanism via emulsion and gel. Moreover, it was observed that by combining emulsion with gel, its stability increased according to Pottalawathi *et al.* The reason behind choosing microemulsion system was its excellent capacity to solubilize and also its capability to permeate into the skin.^{2,3} Linseed oil belongs to the category of polyunsaturated fatty

acids that provides dual action i.e, increases solubility as well as permeability. Moreover, it is non-toxic and limits the evaporation of water from the surface. Sodium alginate is an anionic, naturally biodegradable polymer with good biocompatibility. It has comparatively low cost and provides good mechanical strength.⁴

Microemulgel preparation requires screening of oils, surfactant and co-surfactant. Based on the solubility profile of

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API, selection of these are carried out.⁵ Presence of oil leads to more penetration of API in the skin. Microemulsions helps in keeping the drug in solubilized form and the formation of small sized droplets provides large interfacial area for drug absorption.⁶

The present research is proposed to prepare and evaluate microemulsion based luliconazole gel for its enhanced solubility and permeability for better antifungal activity by using polyunsaturated fatty acids such as linseed oil and sodium alginate as gel base.

MATERIALS AND METHODS

Materials

Luliconazole was purchased from Aksharam chemicals, Ahmedabad. Linseed oil, Neem oil and olive oil were procured from Loba Chemie Private Ltd, Mumbai. Sodium alginate, Tween 20, Tween 80, Span 20 and Span 80 was procured from M/s Hi-media Pvt limited Mumbai. Propylene glycol was procured from Nice chemicals Kerala and PEG 400 was procured from Merck Specialities Private Ltd, Mumbai. All the chemicals used were of analytical grade.

Methods

Preparation of Luliconazole microemulsion

For formulating microemulsion, Span 20 concentration used in (E1= 1%, E2= 0.75%, E3= 0.5%, E4= 0.85%) along with drug (5%) was dissolved in linseed oil (5%) which gives the oil phase. For preparation of aqueous phase, Tween 20 in concentration (E1= 0.25%, E2 = 0.5%, 0.75%, E4= 0.4%) was dissolved in purified water. The concentration of oil and surfactants were selected based on HLB value. Both the phases were kept on water bath and separately heated at 70-80°C. Both the phases were combined with continuous stirring for 15 min at 5 rpm and cooled to room temperature.^{7,8}

Globule size determination

For determining the globule size of emulsions, Dynamic light scattering particle size analyser (Nanotracs A-150) was used. It detects the particle size from the range of 0.08 nm to 6.8 µm. Dilution was made by taking 1 ml emulsion and diluted to 20 ml with millipore water and readings were taken.⁹ Readings were taken in triplicate.

Physical stability of microemulsion

Microemulsion stability was carried out by processing it for centrifugation for 15 min at 3000 rpm. Any changes like phase separation and clarity was observed visually. Stability against freeze through cycle or heating

and cooling cycle was not conducted as centrifugation process showed the desired results.¹⁰

Preparation of microemulgel

For preparation of gel base, sodium alginate in concentration of (2%, 4%, 6%) was dispersed in sufficient amount of water and it was kept overnight. Formation of luliconazole microemulgel was done by incorporation of emulsion containing (1g luliconazole) into the gel base by continuous stirring at room temperature using magnetic stirrer at 3000 rpm to remove any formation of lumps and to provide proper consistency to formulation.

Evaluation of microemulgel

Drug content

Drug content was calculated by dissolving 1g of emulgel into 100 ml of acetate buffer of pH 5.5. From this 1 ml was withdrawn and diluted upto 10 ml with acetate buffer. Since the luliconazole was insoluble in water, acetate buffer was used as it showed highest solubility in acetate buffer. This solution was subjected to sonication for 30 min and filtered. No precipitation of drug was observed as it dissolved completely after sonication. Measured the absorbance spectrophotometrically at 272 nm.¹¹

pH

Digital pH meter was used for determination of pH. For measurement of pH, firstly the pH electrode was calibrated using acetate buffer pH 5.5 and 1% aqueous solution of formulation was taken and electrode was immersed into the microemulgel formulation till the fluctuation in readings stopped and a constant reading was obtained.¹¹

Viscosity

Brookfield viscometer cap 2000+ was employed for measurement of viscosity. Approximately 1g of emulgel was taken on the plate of Brookfield viscometer and it was allowed to settle for 5-10 min and then spindle was placed on the plate containing gel and rotated at a speed of 5 rpm with the help of spindle. The temperature was maintained at 25°C and corresponding dial reading was noted down. Reading indicates the viscosity of the formulations in cps.¹²

Spreadability

Spreadability was performed by using two glass slides of length 7.5 cm. 350 mg of microemulgel was weighed accurately and it was taken on one glass slide. Another glass slide was placed above it from a height of 5 cm. A weight of 5 gm was kept on the upper slide and after 1

min, diameter of circle that was spread was noted in cm. The observed diameter indicates the type of gel.¹³

In vitro permeation study

The study was performed by modified Franz diffusion cell using dialysis membrane. Before carrying out the study, membrane was kept in acetate buffer pH 5.5 for 24 hr and it was mounted carefully between the donor and receptor chamber. 200 mg of emulgel was weighed and homogeneity spread on the dialysis membrane. 12 ml of acetate buffer (pH 5.5) was placed in receptor medium as dissolution media. Both donor and receptor compartment were kept in contact with each other and whole assembly was maintained at constant temperature of $32 \pm 0.5^\circ\text{C}$. Magnetic bead was used to stirred the solution of receptor chamber. 1ml of sample was withdrawn after specific time intervals and equal amount was replaced with fresh dissolution media. Sample absorbance was calculated spectrophotometrically at 272 nm and % cumulative drug permeation was calculated.¹⁴

In order to predict the drug release mechanism, the obtained values was fitted into various model like Zero order, First order, Higuchi matrix and Korsmeyer-Peppas model. Best fit model was selected according to highest R^2 values obtained.¹³

Skin irritation test

The test was carried out according to OECD guidelines for *in vitro* skin irritation test No. 439. Wistar rats (12) of either sex was used to carry out skin irritation test. Selected rats were segregated into two groups i.e., control and test. Each group comprised of 6 animals. Back skin area of 5 cm² of rats was shaved one day before the starting of the study. After 24 hr, control group was applied with gel base (without drug) and test group was applied with the optimized emulgel (F1) and rats were examined for any signs of irritation. Scores were given according to observed signs of irritation.

In vitro antifungal study

In vitro antifungal study was carried out using agar well diffusion technique (Cup-plate method). Mueller Hinton HIVE agar was used as media for growth of fungal strain (*Candida albicans*) using Spread plate technique. 50 μl of sample (F1 containing 1g of luliconazole) and pure gel (containing only API with gel base) were placed in the wells of the petriplates and it was kept at 37°C for 72 hr in incubators. Zone of inhibition was measured (in mm) in triplicates.¹⁵

Stability study

Short-term stability study was carried out (due to shortage of time) for optimized formulation. Formulation was

exposed to different conditions of temperature as well as relative humidity $25^\circ\text{C}/ 60\% \text{RH}$ and $40^\circ\text{C}/ 65\% \text{RH}$ for a period of 1 month. After 1 month, formulation was examined for any changes in pH, rheological properties and drug permeation profiles.¹³

RESULTS AND DISCUSSION

In the present research work, an attempt has been made to prepare and evaluate microemulsion based luliconazole gel for its enhanced solubility and permeability for antifungal activity by using poly unsaturated fatty acids such as linseed oil. Semi-synthetic polymer i.e. sodium alginate was used as gelling agent.

Formulation of Luliconazole microemulsion

Four microemulsions E1, E2, E3 and E4 were formulated by varying the concentration of Tween 20 and Span 20 based on the general required HLB value for vegetable oils i.e., (4:2:1). [Table 1] Based on the solubility (27.85 mg/ml), 1g of luliconazole was added to the formulation. Linseed oil was used as oil phase, Tween 20 (with solubility of 23.84 mg/ml) as hydrophilic surfactant and Span 20 (with solubility of 18.98 mg/ml) as lipophilic surfactant. HLB value for E1 and E4 was found to be 12.77 and 13.89 respectively.

Globule size determination

By using Nanotrak, the globule size of microemulsion was determined. Based on the observed MA i.e., surface area distribution of globules and polydispersity index E1 and E4 were selected as optimized microemulsion with 273.7 nm and 189.6 nm and with PDI of 0.632 and 0.197. [Table 3, Figure 5,6] The reason behind lowest globular area can be the HLB value of surfactants as described by Hosseini *et al.* in his work that lowest particle observed in the HLB value ranging from 11-13. The HLB value for emulsion E1 and E4 was 12.77 and 13.89 respectively. Thus, it can be concluded that particle size can be influenced by HLB value of surfactants. Another reason for this can be the incorporation of linseed oil in the formulation. Low PDI value indicates that uniformity in the globule size of formulation was more. The structure

Table 1: Formulation composition of luliconazole microemulsion.

Ingredients	E1	E2	E3	E4
Luliconazole (g)	1 (5%)	1 (5%)	1 (5%)	1 (5%)
Tween 20 (%)	0.25	0.5	0.75	0.4
Span 20 (%)	1	0.75	0.5	0.85
Linseed oil (%)	5	5	5	5
Water (ml) q.s.	20 ml	20 ml	20 ml	20 ml

Table 2: Formulation table for Luliconazole microemulgel.

Ingredients (%w/w)	F1	F2	F3	F4	F5	F6
Luliconazole emulsion equivalent to Luliconazole	1g	1g	1g	1g	1g	1g
Sodium alginate	2%	4%	6%	2%	4%	6%
Linseed oil	5	5	5	5	5	5
Tween 20	0.25	0.25	0.25	0.4	0.4	0.4
Span 20	1	1	1	0.85	0.85	0.85
Propylene glycol	5	5	5	5	5	5
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water q.s.	100	100	100	100	100	100

Table 3: Globule size and PDI of luliconazole microemulsions.

Formulation code	Globule size (nm)	Polydispersity index
E1	273.7	0.632
E2	342.0	0.964
E3	480.0	0.763
E4	189.6	0.197

of globules was observed by using optical microscope with magnification of 40X. [Figure 1]

Physical stability of microemulsion

It was visualized after centrifugation, that there was no separation of phases in E1 and E4. Slight coalescence appeared in case of E3 and E4 microemulsion. So based on the stability, E1 and E4 were selected as optimized emulsion.

Preparation of microemulgel

Optimized emulsion E1 and E4 were used for preparation of emulgel. Luliconazole emulsion equivalent to 1g was added to gel matrix of sodium alginate using magnetic stirrer to remove any formation of lumps. Methyl and propyl paraben were mixed in propylene glycol and added to the gel base containing emulsion. Triethanolamine was used for adjusting pH. Formulation F1, F2 and F3 was prepared with E1 emulsion having sodium alginate concentration of 2%, 4% and 6% respectively while F4, F5 and F6 was prepared with E2 emulsion having sodium alginate concentration of 2%, 4% and 6% respectively. [Table 2]. Sodium alginate concentration (2%, 4%, 6%) was selected as most of the microemulgel were prepared by using these concentration only.

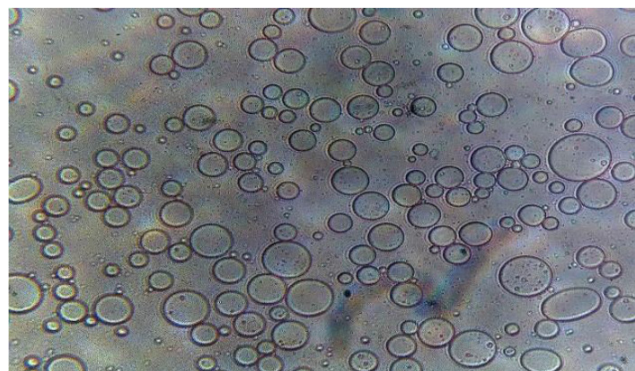
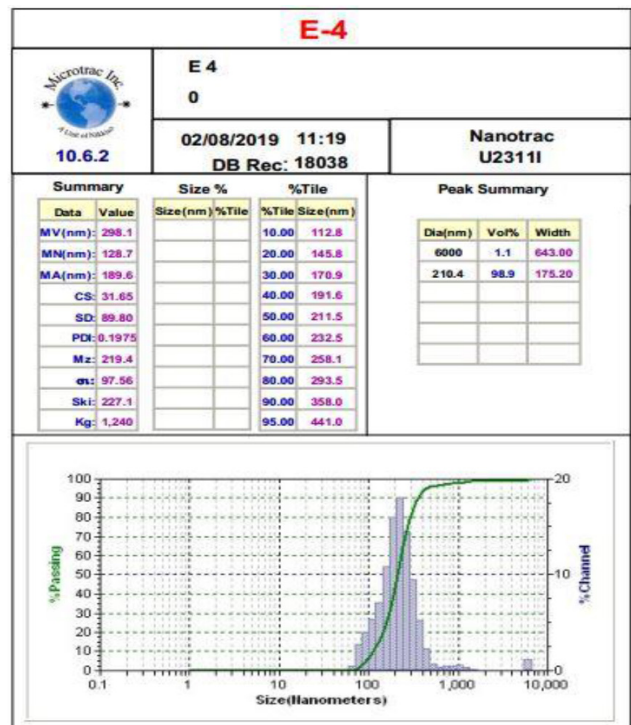


Figure 1: Photograph showing globular structure of microemulsion under 40x magnification.

Drug Content

The observed range of percentage drug content for all the formulation was from 74.77 to 95.49 %. [Table 4]. It was observed that with decrease in gelling agent concentration, drug content was found to be highest.

pH

All formulation showed pH within a range of 5.54 to 6.71 which is in accordance with pH of skin indicating skin compatibility and avoids any risk of skin irritation upon application. [Table 4]

Viscosity

The values of viscosity ranged from 2325 to 8850. Lowest viscosity was exhibited by optimized formulation F1 i.e., 2325 formulated with 2% sodium alginate

Table 4: Drug content and pH of formulations F1 to F6.

Formulation code	Drug content *(%)	pH*
F1	95.49±1.22	6.48±0.04
F2	85.58±2.17	6.51±0.22
F3	74.77±1.47	6.10±0.06
F4	93.69±1.41	5.83±0.08
F5	84.68±1.52	6.71±0.15
F6	75.67±2.20	5.54±0.11

*Data are expressed as Mean ±S.D. (n=3).

Table 5: Viscosity, Spreadability of formulations F1 to F6.

Formulation code	Viscosity (cps)*	Spreadability* (gm.cm/sec)
F1	2325 ±271.44	3.7 ±0.15
F2	4087 ±361.74	3.3 ±0.05
F3	8850 ±312.24	2.9 ±0.23
F4	3975 ±474.92	3.4 ±0.17
F5	4275 ±354.22	3.3 ±0.15
F6	7350 ±114.56	2.6±0.28

*Data are expressed as Mean ±S.D. (n=3).

concentration and E1 emulsion. It may be due to lower concentration of gelling agent employed in the formulation. Viscosity can directly influence the release profile of drug as increased viscosity can lead to more rigidity in the structure which can decline the rate of drug release. All luliconazole microemulgel exhibits pseudoplastic behaviour with shear thinning property as viscosity was found to decrease with increase in shear rate. [Table 5]

Spreadability

Spreadability values contributes to efficacy of formulation. The value of spreadability was found to be higher in case of optimized formulation F1 with 3.7 gm.cm/sec whereas it was found lowest in case of F6 i.e., 2.6 gm.cm/sec. [Table 5] When the concentration of gelling agent decreased, the spreadability values was found to be increased. All the gels belong to fluid gel category [Table 6]. There was no ideal value for spreadability reported in earlier studies. Based on the observed diameter, gels are classified.

In vitro permeation study

All the formulations showed percentage cumulative drug permeation within a range of 51.93% to 63.50% after 5 hr. [Figure 2] Maximum drug permeation was observed in case of F1 i.e., 63.50% with incorporation of E1 emulsion. The possible reason for this can be

Table 6: Types of gel based on Spreadability values.

Types of gel	Measurement (in cm)
Fluid gel	More than 2.4
Semi- fluid gel	1.9-2.4
Semi stiff gel	1.9-1.6
Stiff gel	1.6-1.4
Very stiff gel	Less than 1.4

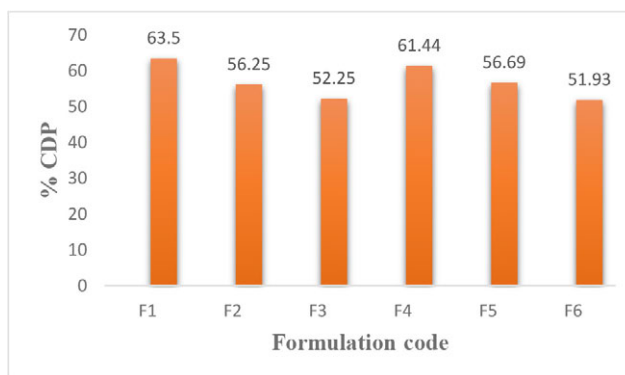


Figure 2: Comparison of in vitro percent cumulative drug permeation of all the formulations at the end of 5 hr.

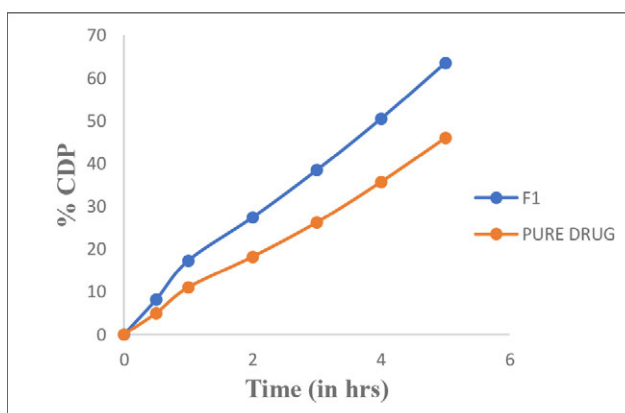


Figure 3: Comparison of in vitro permeation profile of optimized formulation F1 and pure drug.

the lower viscosity value found in F1 formulation. As discussed by Ayoub *et al.* that lower viscosity can lead to faster release of drug. Minimum drug permeation was observed in case of F6 (51.93%) with 6% sodium alginate concentration. This is because higher polymer concentration tends to develop more resistance for diffusion of drug by entrapment of drug molecules. Another reason for higher permeation of drug from F1 could be the presence of surfactant and co-surfactant which provides enhanced penetration of drug.

In vitro permeation study was also performed for pure gel (without excipients). After 5 hr, it showed permeation of 45.98% which is less than the optimized formulation F1. This is due to insolubility of luliconazole in water

Table 7: In vitro release kinetics of formulations F1 to F6.

FORMULATION CODE	ZERO ORDER (R ²)	FIRST ORDER (R ²)	HIGUCHI MATRIX (R ²)	HIXSON CROWWELL (R ²)	PEPPAS		BEST FIT MODEL
					(R ²)	(n)	
F1	0.9803	0.9779	0.8791	0.9872	0.9928	0.8311	PEPPAS
F2	0.9798	0.9815	0.8725	0.988	0.9935	0.7825	PEPPAS
F3	0.9816	0.9868	0.88	0.9909	0.9926	0.8465	PEPPAS
F4	0.9799	0.9805	0.8825	0.9885	0.9918	0.8812	PEPPAS
F5	0.981	0.9845	0.8819	0.9915	0.9924	0.8620	PEPPAS
F6	0.9792	0.9822	0.8674	0.9878	0.9967	0.7520	PEPPAS



Figure 4: Showing zone of inhibition of optimized formulation F1 and pure drug.
A-Optimized formulation F1= 38mm
B-Pure gel (without excipients) = 34mm

Skin irritation test

Skin irritation test was carried out after taking approval of Institutional Animal Ethics Committee. Wistar rats (12) of either sex was used to carry out skin irritation test. Control group was treated with gel base without drug whereas test group was treated with optimized formulation F1. It was observed that both the groups showed no signs of irritation after 24 hr and they were assigned with a score of 0 [Figure 7,8]. This indicates that the prepared formulation is safe for use.

In vitro antifungal study

In vitro antifungal study was carried out using agar well diffusion technique using *Candida albicans* strain for optimized formulation F1 and drug gel without surfactants and linseed oil. Optimized formulation F1 represents 38 mm zone of inhibition which is greater than

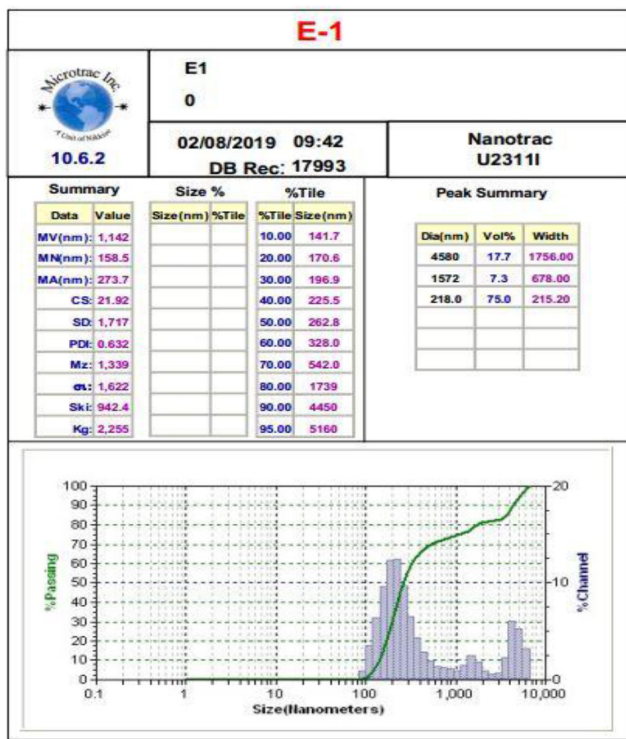


Figure 5: Globule size and PDI of optimized E1 Microemulsion

due to which drug was unable to dispersed within the gel matrix and permeation is retarded. [Figure 3] in vitro release kinetics of formulations F1 to F6. [Table 7]

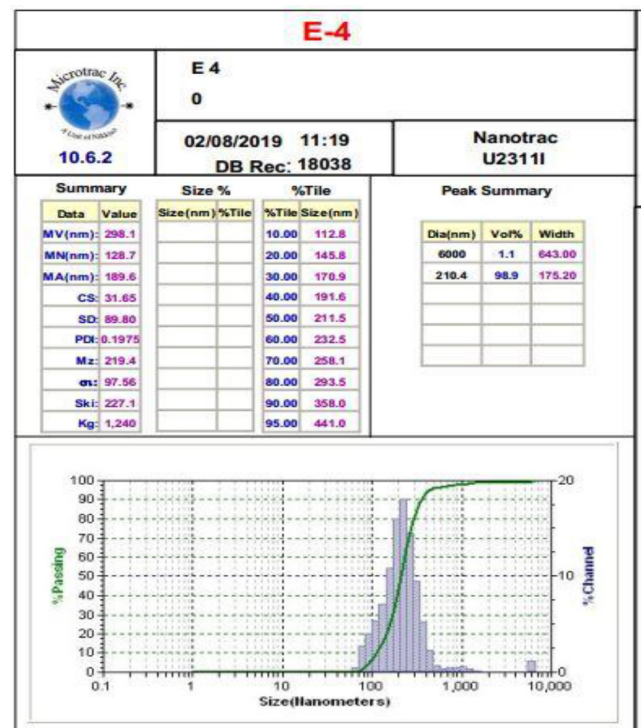


Figure 6: Globule size and PDI of optimized E4 Microemulsion.

A - Control group after shaving



B - Test group after shaving

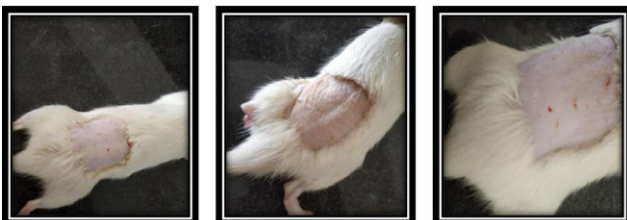
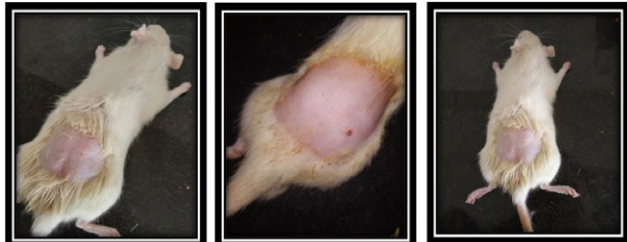


Figure 7: Skin irritation test of Control and Test group after shaving.

C- Control group after 24 hr



D- Test group after 24 hr

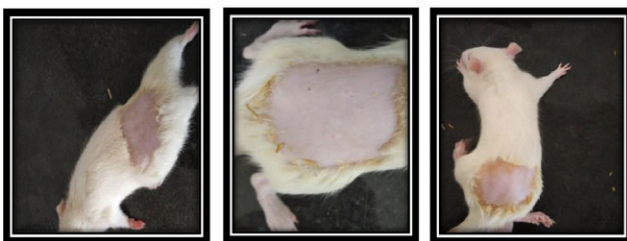


Figure 8: Skin irritation test of Control and Test group after 24 hr.

zone of inhibition observed in case of drug gel without surfactants and linseed oil (34 mm). [Figure 4]

Stability study

Optimized formulation was examined after 30 days for any changes in pH, rheological properties and drug release profiles. pH was found to be increased slightly. Viscosity was found to be increased at room temperature and then decreased at accelerated temperature. Drug release was found to be slightly decreased with temperature. Not much deviation was observed from optimized formulation F1, so it can be concluded that it showed acceptable stability on a given temperature and humidity. [Table 8]

Table 8: Stability study of optimized formulation F1.

Evaluation Parameters	Initial day	Room temperature (25°C/ 60% RH)	Accelerated temperature (40°C/ 65% RH)
pH	6.48	6.51	6.77
Viscosity (cps)	2325	2344	2287
Drug permeation (%)	63.50	62.20	59.08

CONCLUSION

Luliconazole was successfully formulated in the form of microemulgel by using poly unsaturated fatty acid i.e., linseed oil with sodium alginate as gelling agent. Microemulsions were prepared by varying Tween 20 and Span 20 concentration in four emulsions. E1 and E4 were selected as optimized emulsion based on surface area distribution of globules (MA), PDI and physical stability. E1 showed globule area of 273.7 nm with 0.632 PDI and E4 showed globule area of 189.6 nm with PDI of 0.197. E1 and E4 showed good physical stability. Microemulgel was prepared by incorporation of E1 and E4 emulsion in formulation F1, F2, F3 and F4, F5, F6 respectively. Concentration of sodium alginate was varied in 2%, 4% and 6%. The prepared microemulgel was pleasant in appearance and showed drug content ranged from 74.77% to 95.49%. pH of all the formulations was within a range of 5.54 to 6.71 which is in accordance with skin pH indicating compatibility with skin. The viscosity of all formulations ranged from 2325 to 8850 cps. All formulations showed pseudoplastic behaviour. Spreadability values ranged from 2.6 to 3.7 gm.cm/sec. All the formulations belong to fluid gel category. The percentage cumulative drug permeation was found to be in the range of 51.93% to 63.50% after 5 hr. F1 formulation was selected as optimized formulation based on drug content, viscosity and percentage cumulative drug permeation. Skin irritation test showed that formulation is safe for use since it showed no signs of irritation. Optimized formulation F1 showed 38 mm zone of inhibition as compared to pure gel with 34 mm indicating its good antifungal activity. Acceptable stability profile was observed after subjecting the formulation to various conditions of temperature and humidity. Hence, it can be concluded that luliconazole microemulgel prepared with linseed oil and sodium alginate can be a choice for topical antifungal treatment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

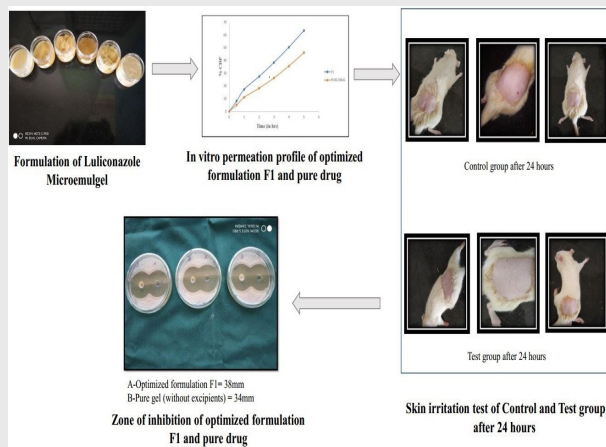
ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **CLA:** Cumulative Loss Added; **CDP:** Cumulative Drug Permeation; **Min:** Minutes; **Hrs:** Hours; **Sec:** Seconds; **mg:** Milligram; **ml:** Milliliter; **µg:** Micro gram; **gm:** Grams; **nm:** Nanometer; **m:** Meter; **cm:** Centimeters; **cps:** Centipoise; **i.e.:** That is; **ME:** Microemulsion; **RH:** Relative Humidity; **rpm:** Revolutions per minute; **UV:** Ultra-violet; **w/w:** weight by weight; **PEG:** Polyethylene glycol; **R²:** Regression coefficient; **ZOI:** Zone of inhibition.

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PICTORIAL ABSTRACT



SUMMARY

Luliconazole was successfully formulated in the form of microemulgel by using poly unsaturated fatty acid i.e., linseed oil with sodium alginate as gelling agent. F1 formulation was selected as optimized formulation based on drug content, viscosity and percentage cumulative drug permeation. Acceptable stability profile was observed after subjecting the formulation to various conditions of temperature and humidity. Hence, it can be concluded that luliconazole microemulgel prepared with linseed oil and sodium alginate can be a choice for topical antifungal treatment.

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