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IMPROVED ANTIFUNGAL ACTIVITY OF CLOTRIMAZOLE EMULGEL AGAINST *CANDIDA ALBICANS*

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ABSTRACT

Aim and objective of the study is to prepare and evaluate emulgels of Clotrimazole. Formulations were prepared by using Carbomer, Liquid Paraffin, Span 20 Tween 80, Propylene Glycol and Water. Developed formulations of Clotrimazole were evaluated for the physiochemical properties such as Spreadability 14.89 gcm/sec, Viscosity 3985 cP, pH 7.02, for optimized formulation, texture was found smooth and white cream. Melting point of emulgel was 147-149℃. Mean particle size of nanoemulsion was found 101.1 nm and SEM shows 200 nm droplet size, Cumulative percentage drug release was 98.98 in 6 hours from optimized formulation. Antifungal activity of emulgel against Candida albicans was more than marketed gel of same drug.

KEYWORDS: emulgel, clotrimazole, *candida albicans,* antifungle.

INTRODUCTION

Clotrimazole (CTM) is a broad spectrum antimycotic agent effective against pathogenic dermatophytes, yeasts, and several species of Candida, Tricophyton, Microsporum, Epidermophyton, and Malassezia1. It inhibits biosynthesis of the sterol ergostol, an important component of fungal cell membranes.[1] Its action leads to increased membrane permeability and apparent disruption of enzyme systems bound to the membrane. Many formulation approaches have been attempted to improve the solubility of Clotrimazole(c- capital) such as mucoadhesive thermo sensitive gels, nanospheres, solid lipid nanoparticles, liposomal/niosomal delivery systems, bioadhesive liposomal gels, and inclusion complexes with β-cyclodextrin. Emulgels have a higher aqueous component which permits greater dissolution of drugs, and also permit easy classical emulsion into an emulgel.[2] Emulgels are used for dermatological purpose have several favorable properties such as thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, watersoluble, and longer shelf life, bio-friendly, transparent & pleasing appearance.[3-4] Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections.[5-6] The main classes of topical antifungals are polyenes, azoles, and allylamine/benzylamines. Cicloprox is an antifungal agent also used topically.

Emulgel are also called as gellified emulsions. Emulsion in gel have emerged as one of the most interesting topical drug delivery system as it has dual release system i.e. emulsion and gel. Emulgel are either oil-in-water or water-in-oil type, which are gelled by mixing with a gelling agent. Numbers of medicated products are applied to the skin or mucous membrane that either enhances or restores a fundamental function of skin or pharmacologically alters an action in the underlined tissues Such products are referred as topical or dermatological products.[7-8] Many widely used topical agents like ointments, creams lotions have many disadvantages. They are sticky in nature causing uneasiness to the patient when applied, have lesser spreading coefficient so applied by rubbing and they also exhibit the problem of stability. In spite of many advantages of gels a major limitation is in the delivery of hydrophobic drugs so to overcome this limitation an emulsion based approach is being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels.[9-11]

MATERIAL AND METHODS

Clotrimazole received as gift sample from SIFC, SIRTP, Carbomer 941, Span 20, Tween 80, Propylene Glycol was purchased from Sigma Aldrich, USA.

Analytical methods

FTIR

The drug sample was scanned on IR spectrophotometer between 400-4000 cm^{-1} using KBr disc.

Solubility (at room temperature)

Approximately 10 mg of drug was weighed accurately and transferred to 5 different 10 ml volumetric flasks. Water, 0.1 N HCl, Ethanol, Methanol and Chloroform were taken as solvent.

Preparation of carbopol gel

Fifty grams of the carbopol gel was prepared by dispersing one gram of carbopol powder in 50 ml purified water with aid of moderate speed stirrer (50 rpm), and then the pH was adjusted to 6-6.5 using 0.5 N of sodium hydroxide.

Preparation of emulsion

The oil phase was prepared by dissolving Span 20 in liquid paraffin in the ratio of (1:1, 1:2 and 1:3) while the aqueous phase was prepared by dissolving Tween 20 in purified water as given in table 1. Two grams of Clotrimazole was dissolved in 5 ml of ethanol, while 0.15 g of methylparaben and 0.05 g of propylparaben were dissolved in 5 gm of propylene glycol and both were mixed with aqueous phase. Both the oily and aqueous phases were separately heated to 70-80°C. Then, the oil phase was added to the aqueous phase with continuous stirring at 500 rpm until cooled to room temperature.

Formulation of Clotrimazole emulgel

Eight formulations of Clotrimazole were prepared by dispersing the obtained emulsions with the gel in 1:1 ratio with gentle stirring until get homogenous emulgel as shown in table. 1.

Table 1: Different formulas of Clotrimazole emulgel (% w/w).

Formulation	Drug (mg)	Carbomer 941	Liquid paraffin	Span 20	Tween 20	Propylene glycol	water
F1	100	1	5	2	5	5	100
F2	100	1	5	2	7.5	5	100
F3	100	1	5	2	10	5	100
F4	100	1	10	2	5	5	100
F5	100	1	10	2	7.5	5	100
F6	100	1	10	2	10	5	100

Evaluation of nanoemulsion

Measurement of mean particle size and zeta potential

The mean size of the nanoemulsion was determined by particle size analyzer (Malvern Instruments) at a scattering angle of 90°. Sample of the emulgel was suspended in 5 ml of distilled water was used for the measurement.[12]

The zeta potential of the drug-loaded nanoemulsion was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

Evaluation of emulgel

Physical Characteristic

The Physical Characteristic was checked for gel formulations (colour, clogging, homogeneity and texture) and observations were shown in Table 2.

Determination of pH

The pH of the gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained and constant reading was noted. The measurements of pH of each formulation were replicated three times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table 2.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Spreadability

An important criterion for emulgel gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip of from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. Two glass slides of standard dimensions (6×2) were selected. The emulgel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms

along the slide. 100 grams of weight was placed up on the upper slide so that the emulgel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the emulgel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each emulgel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6 cms).
t = time taken is seconds.

Viscosity
The measurement of viscosity of the prepared gel was done using Brookfield digital viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25°C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±/1^{0}C) before the measurements.[13]

In vitro drug release studies using the prehydrated cellophane, membrane
The cellophane membrane approximately 25 cm x 2 cm was taken in study. *In vitro* diffusion of drug from the different gel preparations were studied using the classical standard cylindrical tube fabricated in the laboratory; a simple modification of the cell is a glass tube of 15 mm internal diameter and 100 mm height. The diffusion cell membrane was applied with one gram of the formulation and was tied securely to one end of the tube, the other end kept open to ambient conditions which acted as donor compartment. The cell was inverted and immersed slightly in 250 ml of beaker containing neutralizing phthalate buffer, freshly prepared (pH 5.4) as a receptor base and the system was maintained for 2 hrs at 37± 0.5^{o}C. The media was stirred using magnetic stirrer. Samples were withdrawn periodically at predetermined time interval of up to 6 hrs and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 272.0 nm using neutralizing phthalate buffer as blank.[14]
The results of *in-vitro* release profile obtained for all the formulations were plotted in kinetic models as follows
1. Cumulative of drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining to be absorbed versus time (First order model)

In vitro antifungal activity
This study was performed by well diffusion method the well diffusion method depends upon diffusion of antifungal from a solidified agar in a petridish or plate. Different concentration 10, 20 and 30 µg/ml of prepared and marketed sample was bored in already prepared agar plate and zone of inhibition was measured by Vernier calliper.

RESULTS AND DISCUSSION

Melting point was found to be 147-149^{0}C, no interaction observed by drug and polymer Fig.1 and Fig.2, Clotrimazole showed maximum absorption at a wavelength of 272 nm in ethanol. It was found that Clotrimazole was freely soluble in methanol and ethanol, slightly soluble in water, and insoluble 0.1 N HCl, 7.2 phosphate buffer and chloroform.

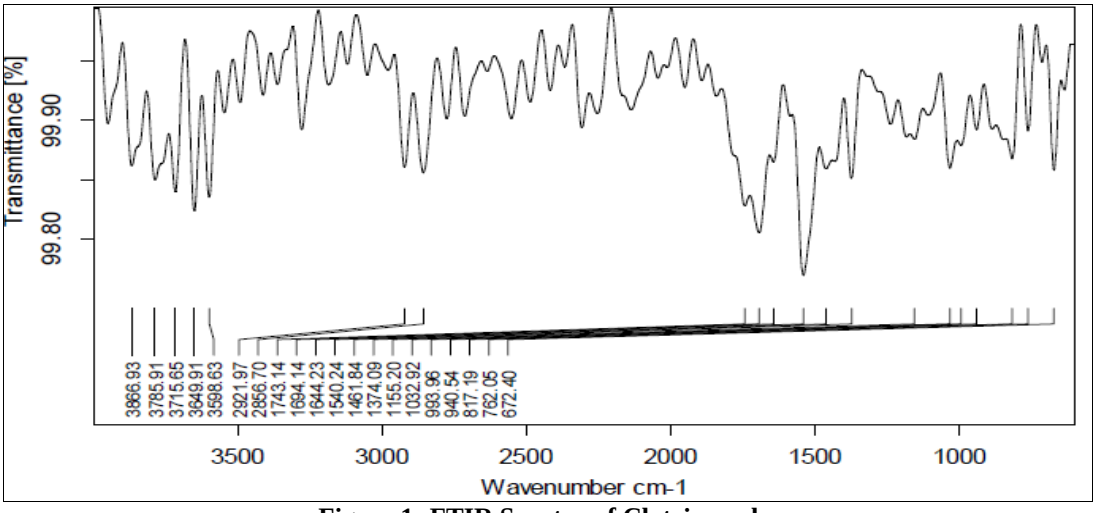

Figure 1: FTIR Spectra of Clotrimazole.

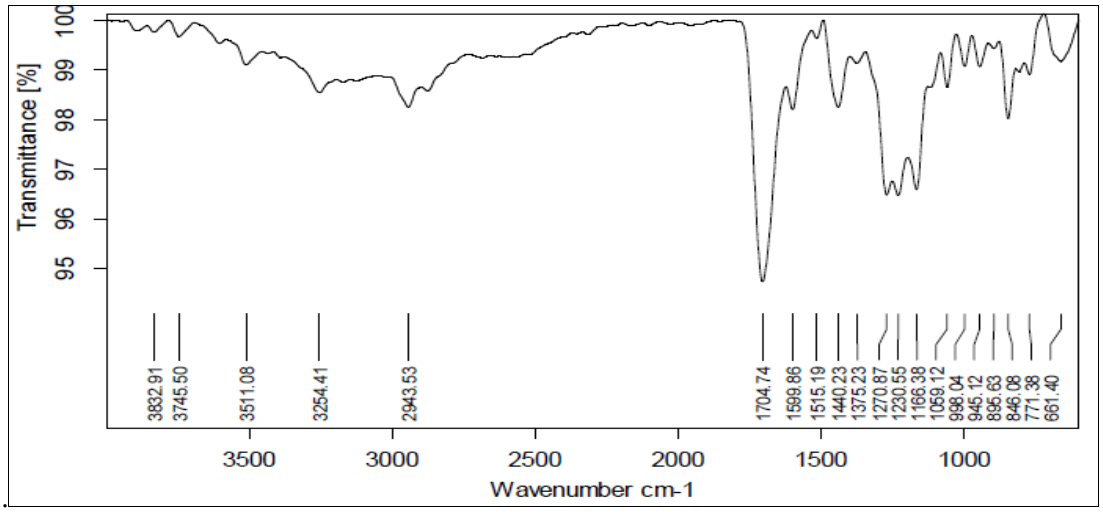

Figure 2: FTIR Spectra of drug and polymer physical mixture.

Characterization of nanoemulsion

Particle size analysis

Mean particle size of optimized nanoemulsion was found 101.1 nm respectively. Fig 3

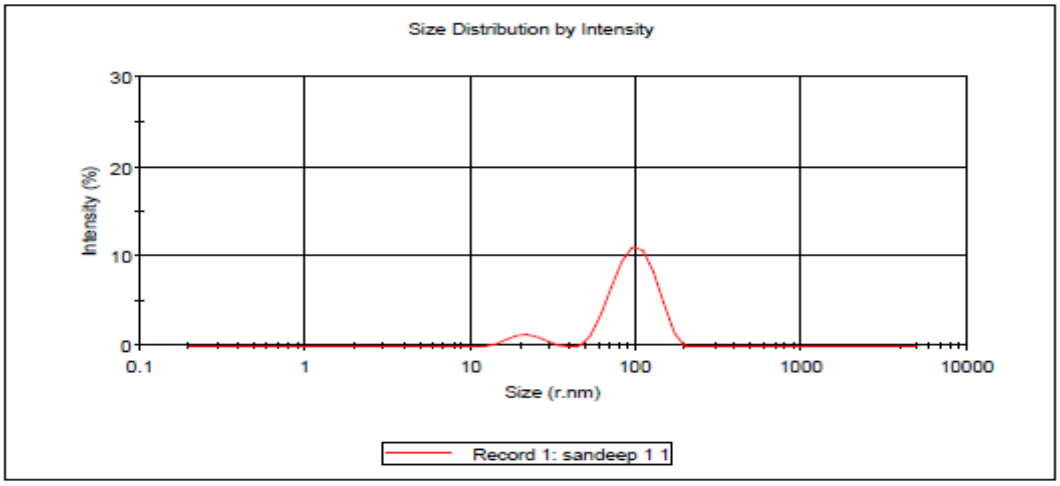

Figure 3: Particle size data of nanoemulsion.

Zeta Potential

Zeta potential of nanoemulsion was found -25.9 mV fig 4.

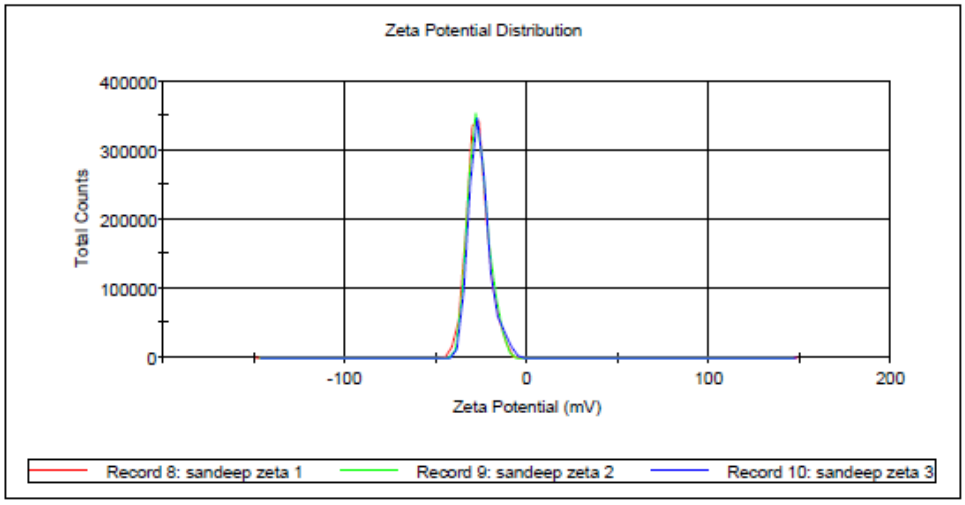

Figure 4: Zeta potential data of mucoadhesive microsphere.

Characterization of emulgel

Gels were evaluated for their clarity, pH, viscosity, spreadability, washability *in vitro* diffusion studies using standard procedure. All studies were carried out in triplicate and average values were reported shown in Table 2 Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table 2.

Table 2: Characterization of different formulation.

Formulation	Clogging	Texture	Wash ability	Observation	Viscosity (cps)	pH	Spreadability (gcm/sec)
F1	Absent	Smooth	+++	white cream	3850	6.95	13.25
F2	Absent	Smooth	+++	white cream	3950	6.85	14.26
F3	Absent	Smooth	+++	white cream	4012	7.05	14.56
F4	Absent	Smooth	+++	white cream	3985	7.02	14.89
F5	Absent	Smooth	++	white cream	4025	6.98	13.25
F6	Absent	Smooth	++	white cream	4150	6.99	14.56

Washability - Excellent: +++, Good ++, Average: +, Poor –

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked. Release of drug from Clotrimazole emulgel was significantly slower, which confirmed that slight prolonged drug release rate. Incorporation of carbomer affected the release rate of the drug. By increasing the amount of carbomer, the release rate of the drug decreased, which could be related to the increased rigidity of the formulation, followed by its decreased permeability for the drug.

In-vitro drug release and antifungal activity

In-vitro drug release studies of formulation F4 showed 98 % release in 6 hrs, order of release shown in table 3.order of release kinetics shows in fig 5

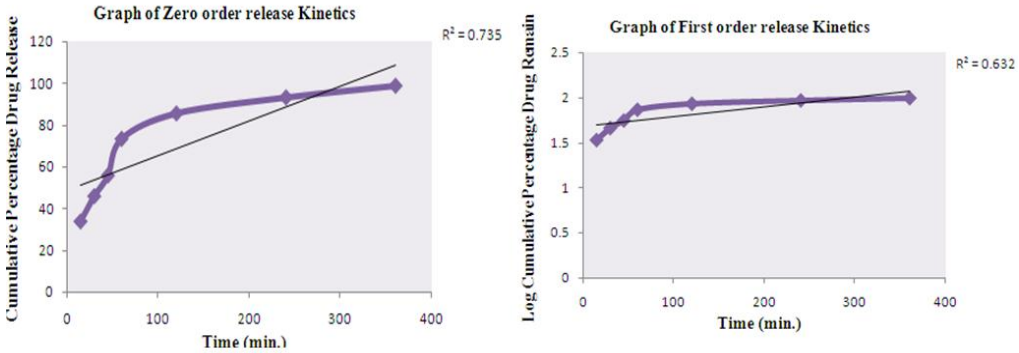

Figure 5: Zero and First order release Kinetics of optimized formulation F4.

Table 3: *In vitro* drug release data for optimized formulation F4.

S. No.	Time (min)	Square Root of Time	Log Time	Cumulative* % Drug Release ± SD	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log cumulative % Drug Remaining
1	15	3.873	1.176	33.48±1.25	1.525	66.52	1.525
2	30	5.477	1.477	45.65±0.89	1.659	54.35	1.659
3	45	6.708	1.653	55.65±2.32	1.745	44.35	1.745
4	60	7.746	1.778	73.36±2.98	1.865	26.64	1.865
5	120	10.954	2.079	85.65±3.14	1.933	14.35	1.933
6	240	15.492	2.380	93.36±4.56	1.970	6.64	1.970
7	360	18.974	2.556	98.98±4.32	1.996	1.02	1.996

* Average of three determinations

Antifungal activity of optimized formulation was more than control preparation shown in table 4.

Table 4: Antifungal Activity of formulation.

S. No.	Formulation	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Candid gel	*Candida albicans*	12±0.74	16±.57	20±0.5
2.	Optimized formulation		14±0.86	18±0.5	22±0.86

CONCLUSION

In vitro drug release from the optimized Clotrimazole emulgel formulation shows significantly improved drug release rate as compare to the marketed preparation. It can be concluded that developed formulations deliver the drug for the treatment of fungal disease and the Carbomer based preparation would providing local onset of action without need of any device for their application on skin. The preparation of emulgel has potential advantages over marketed preparation as they improved patient compliance rapid local onset of action for longer period with cost effectiveness.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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