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Development and characterization of ketoconazole emulgel for topical drug delivery

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ABSTRACT

The aim of the present research work was to investigate the potential of emulgel in enhancing the topical delivery of ketoconazole. Emulgel formulations of Ketoconazole were prepared using 2 types of gelling agents: Carbopol 934 and Carbopol 940. The influence of the type of the gelling agent and the concentration of both the oil phase and emulsifying agent on the drug release from the prepared emulgel was investigated using a 2^3 factorial design. The prepared emulgel were evaluated for their physical appearance, viscosity, drug release, globule size, skin irritation test, antifungal activity, transmission electron microscopy and stability. Commercially available ketoconazole cream was used for comparison. All the prepared emulgel showed acceptable physical properties concerning color, homogeneity, consistency, spreadability, and pH value. The antifungal activity and drug release were found to be higher for optimized formulation as compared to the marketed ketoconazole cream. The highest activity was observed with F1 and F3, where the percentage inhibition found to be 47.5 ±1.15 % and 46.6 ± 1.34 % respectively, as compared marketed product was found 34.43±1.06 %. The drug release from all the emulgel was found to higher 93.8±0.34 %, 76.2±0.65 % at 24 hr. by diffusion-controlled mechanism. No irritation was observed on the skin of the rabbits. Stability studies showed that the physical appearance, rheological study, in vitro drug release, and antifungal activity in all the prepared emulgel remained unchanged upon storage for 3 months. In general conclusion, it was suggested that the emulgel formulation succeed the drug release for sustained drug delivery in a controlled manner in comparison with cream.

Keyword: Emulgel, Ketoconazole, Topical drug delivery and antifungal activity

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INTRODUCTION

Emulgel are emulsions, either of the oil-in-water or water in oil type, which are gelled by mixing with a gelling agent. Emulsified gel is stable one and better vehicle for hydrophobic or poorly water soluble drugs [1]**.** They have a high patient acceptability since they possess the advantages

of both emulsions and gels. Direct (oil-in-water) systems are used to entrap lipophilic drugs, whereas hydrophilic drugs are encapsulated in the reverse (water-in-oil) systems [2]. Therefore, they have been recently used as vehicles to deliver various hydrophobic drugs to the skin. In the local market, 2 Emulgel are available: Voltaren emulgel (Novartis Pharma, Switzerland), containing diclofenac diethylamine and Miconaz-H emulgel (Medical Union Pharmaceuticals, Egypt), containing miconazole nitrate and hydrocortisone [3].

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. The emulsion gels are hydrogels containing randomly distributed oil microdroplets.[4-9] Topical drug delivery systems have been used for centuries for the treatment of local skin disorders, one side the topical applications of the drug offer the potential advantages of delivering the drug directly to the site of action and delivering the drug for extended period of time at the effected site that mainly acts at the related regions[10-14]. On the other hand, topical delivery system increases the contact time and mean resident time of drug at the applied site leading to an increase in local drug concentration while the pharmacological activity of Emulgel formulations may not change as rapidly as the solution form [15].

Several antifungal agents are available on the market in different topical preparations (e.g. creams, ointments, and powders for the purpose of local dermatological therapy)[16-18]**.** One of these antifungal agents is ketoconazole, which has both antifungal and antibacterial properties. It is applied locally in mild uncomplicated dermatophyte and other cutaneous infections [19-20]**.**

Both oil-in-water and water-in-oil emulsions are extensively used for their therapeutic properties and as vehicles to deliver various drugs to the skin. Emulsions possess a certain degree of elegance and are easily washed off whenever desired[21-24]. They also have a high ability to penetrate the skin. In addition, the formulator can control the viscosity, appearance, and degree of greasiness of cosmetic or dermatological emulsions. Oil-in-water emulsions are most useful as water washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, compatible with several excipients, and water-soluble or miscible. The rheological properties and the breakdown behaviour of gels filled with emulsions droplets can be varied by changing the interactions between oil droplets and gel matrix, the oil content and the oil droplet size [25-26]**.**

MATERIALS AND METHODS

Materials: Antifungal drug i.e. ketoconazole gifted from Gufic Bioscience Ltd Mumbai, Carbopol 934; Carbopol 940; Light liquid paraffin; Tween 20; Span 20; Propylene glycol; Methyl paraben; Propyl paraben were purchased from loba chemie , Mumbai. Ethanol; were purchased from c.y.company china. Double distilled water was used for all experiments. All chemicals were pharmaceutical grade and used without further modification.

Emulgel preparation: Ketoconazole Emulgel was prepared by the method reported by Mohammad *et al* (2004) with minor modification. The Gel in formulations were prepared by dispersing Carbopol 934 in purified water with constant stirring at a moderate speed and Carbopol 940 in purified water with constant stirring at a moderate speed then the pH are adjusted to 6 to 6.5 using Tri Ethanol Amine (TEA).

The oil phase of the emulsion were prepared by dissolving Span 20 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water. Methyl and Propyl paraben was dissolved in propylene glycol whereas drug (Ketoconazole) was dissolved in ethanol and both solutions was mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70° to 80°C; then the oily phase were added to the aqueous phase with continuous stirring until cooled to room temperature. And add Glutaraldehyde in during of mixing of gel and emulsion in ratio 1:1 to obtain the emulgel.

Optimization of emulgel:

Experimental design: Eight Ketoconazole emulgel formulations (Table 3) were prepared according to a 2^3 factorial design employing the qualitative factors and levels show in table 1 and table 2.

Table 1: Factor and Level for the 2^3 Factorial Designs

Factors	Levels
(A) Gelling agent type	+10 % - 5 %
(B) Liquid paraffin concentration	+7.5% -5%
(C) Emulsifying agent concentration	+2.5% - 1.5%

Table 2: Composition of Ketoconazole Emulgel Formulation

Composition	Formulation	Composition		
		A	B	C
(1)	F1	+	-	-
A	F2	+	+	-
B	F3	+	-	+
AB	F4	+	+	+
C	F5	-	-	-
AC	F6	-	+	-
BC	F7	-	-	+
ABC	F8	-	+	+

A, Gelling Agent type, B, liquid paraffin concentration, C, emulsifying agent concentration
- Factor at low level, + factor at high level.

Characterization of Emulgel

Physical appearance: The prepared Ketoconazole emulgel formulations were inspected visually for their color, homogeneity, consistency and pH. The pH values of 1% aqueous solutions of the prepared emulgel were measured by a pH meter (Digital ph meter).

Table 3: Various composition of ketoconazole Emulgel formulation

Ingredients (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8
Ketoconazole	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 934	0.5	0.5	0.5	0.5	-	-	-	-
Carbopol 940	-	-	-	-	0.25	0.25	0.25	0.25
Light liquid paraffin	2.5	3.75	2.5	3.75	2.5	3.75	2.5	3.75
Tween 20	0.3	0.3	0.5	0.5	0.3	0.3	0.5	0.5
Span 20	0.45	0.45	0.75	0.75	0.45	0.45	0.75	0.75
Propylene glycol	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ethanol	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Methyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propyl paraben	.005	.005	.005	.005	.005	.005	.005	.005
Glutaraldehyde	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Purified water (q.s.)	50	50	50	50	50	50	50	50

Spreadability: One of the criteria for an emulgel to meet the ideal quantities is that it should possess good spreadability. It is term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula.

$$S = M. L / T$$

Where M = wt. tied to upper slide
L = length of glass slides
T = time taken to separate the slides

Rheological Study: The viscosity of different emulgel formulation was determined at 25^0C using a brook field viscometer (Brookfield DV-E viscometer). The emulgel were rotated at 10 (min.) and 100 (max.) rotation per minute with spindle 6.

Drug Content Determination: drug concentration in emulgel was measured by UV spectrophotometer. Ketoconazole content in emulgel was measured by dissolving Known quantity of emulgel in solvent (methanol) by Sonication. Absorbance was measured after suitable dilution at 226 nm in UV/VIS spectrophotometer (UV-1700 CE, Shimadzu Corporation, Japan).

In Vitro Release Study: Franz diffusion cell (with effective diffusion area 3.14 cm^2 and 15.5 ml cell volume) was used for the drug release studies. Emulgel (200 mg) was applied onto the surface of egg membrane evenly. The egg membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 5.5) solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 226 nm after appropriate dilutions. Cumulative

corrections were made to obtain the total amount of drug release at each time interval .The cumulative amount of drug released across the egg membrane was determined as a function of time (Guido *et al*., 2007).

Microbiological assay: Ditch plate technique was used. It is a technique used for evaluation of bacteriostatic or fungistatic activity of a compound. It is mainly applied for semisolid formulations. Previously prepared Sabouraud's agar dried plates were used. Three grams of the emulgel are placed in a ditch cut in the plate. Freshly prepared culture loops are streaked across the agar at a right angle from the ditch to the edge of the plate. After incubation for 18 to 24 hours at 25°C, the fungal growth was observed and the percentage inhibition was measured as follows (Mohamad *et al.*, 2004):

$$\% \text{ inhibition} = L2 / L1 \times 100$$

Where L1 = total length of the streaked culture, and L2 =length of inhibition.

Skin irritation test: A 0.5 gm sample of the test article was then applied to each site (two sites per rabbit) by introduction under a double gauze layer to an area of skin approximately 1" x 1" (2,54 x 2,54 cm) square. The emulgel re applied on the skin of rabbit. Animals were returned to their cages.
After a 24 hour exposure, the emulgel are removed. The test sites were wiped with tap water to remove any remaining test article residue.

***Globule size and its distribution in emulgel*:** Globule size and distribution was determined by Malvern zetasizer. A 1.0 gm sample was dissolved in purified water and agitated to get homogeneous dispersion. Sample was injected to photocell of zetasizer. Mean globule diameter and distribution was obtained (Gondaliya *et al.*, 2003, Weiss *et al.*, 2005).

Transmission electron microscopy: The emulgel formulation after hydration was confirmed by Transmission electron microscopy (TEM). Samples were prepared by adding phosphate buffer (pH 5.5) to emulgel and shaking the mixture manually for 1 minute. A drop of the sample was placed on a carbon-coated copper grid after 15 minutes and negatively stained with 1% aqueous solution of phosphotungstic acid. The grid was allowed to air dry thoroughly and samples were viewed on a TEM (Philips, TEM, New Brunswick, Canada) (Jain *et al.*, 2006).

Stability studies: The prepared Ketoconazole emulgel formulations were stored away from light in collapsible tube at 25±2°C, 40±2°C and 4±2°C for 3 months. After storage, the samples are tested for their physical appearance, pH, rheological behavior, drug release, skin irritation test and microbiological assay.

RESULTS

***Physical examination*:** The prepared ketoconazole emulgel formulations were white viscous creamy preparation with a smooth and homogeneous appearance. The pH values of all prepared formulation ranged from 5.4 to 5.8, which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

***Spreadability*:** The values of spreadability indicate that the emulgel is easily spreadable by small amount of shear. Spreadability of marketed product was 13.42±0.93 g.cm/sec while F1 and F3 was 18.31±0.84 g.cm/sec and 17.78±0.75 g.cm/sec, indicating spreadability of emulgel containing ketoconazole was good as compared to the marketed gel. This may be due to low level of liquid paraffin concentration in F1 and F3.

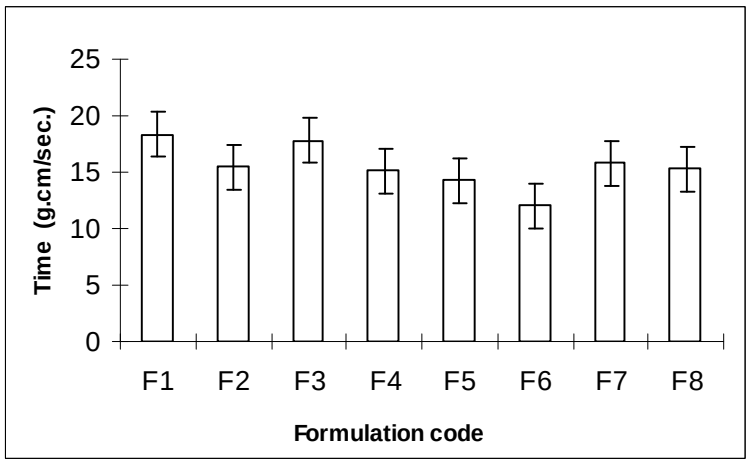


Fig.1: Spreadability of the various emulgel formulations

***Rheological studies*:** The measurement of viscosity of the prepared emulgel was done with Brookfield viscometer (Brookfield DV-E viscometer). The highest viscosity was found in Emulgel F1 it may be due to low level of the liquid paraffin concentration and emulsifying agent concentration. The lowest viscosity was found in formulation F6 due to the low level of emulsifying agent concentration. (fig.2)

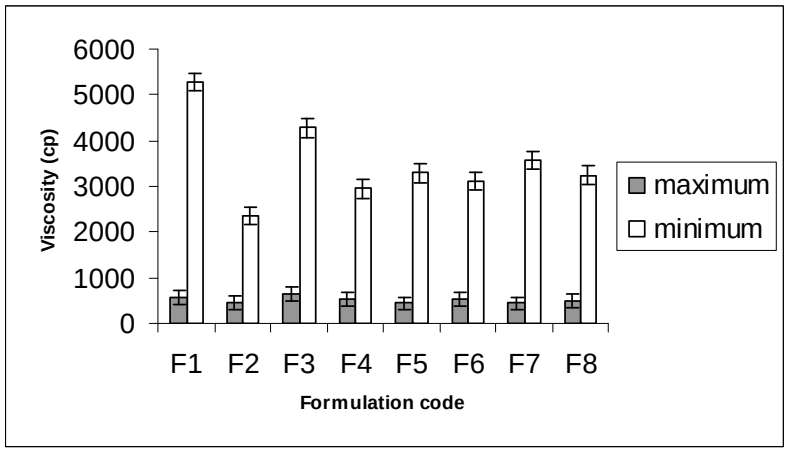


Fig.2: Viscosity of ketoconazole emulgel

***Drug content determination*:** The drug content in emulgel was found in range of 65.5 ± 1.82% to 83.87 ± 1.20%. The higher drug content found in F1 i.e. 83.87 ± 1.20% it may be due to the low concentration of liquid paraffin and emulsifying agent and the lower drug content found in F4 i.e. 65.5 ± 1.82% it may be due to the high concentration of liquid paraffin and emulsifying agent. The drug content of all emulgel formulation given below (fig.3);

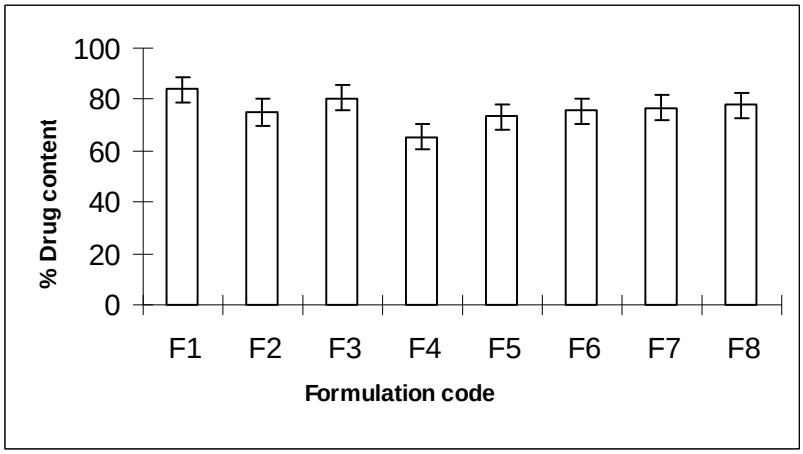


Fig.3: Comparing of drug content of various formulation of ketoconazole emulgel

In vitro Drug Release: The *in vitro* release profiles of ketoconazole from its various emulgel formulations are represented in (Figure 4). The better release of the drug from all emulgel formulation can be observed and the emulgel formulation can be ranked in the following descending order: F1 > F3 > F5 > F6 > F8 > F4 > F7 > F2, Where the amounts of the drug released after 24 hours were 93.8±0.34%, 76.2±0.65%, 67.94±0.43%, 61.14±0.46%, 60.7±0.41%, 50.06±0.35%, 41.06±0.36% and 34.19±0.41%, respectively. The higher drug release was observed with formulations F1 and F3. This finding may be due to presence of liquid paraffin in its low level and the emulsifying agent in its low level / high level respectively, which lead to an increase in the hydrophilicity of the emulgel, which, in turn, facilitates penetration of the release medium into the emulgel and diffusion of the drug from the emulgel. 0.1% of gluteraldehyde is added to retard the release rate of drug from emulgel formulation. The presence of liquid paraffin leads to retardation of ketoconazole release from its emulgel formulation. The lower drug release from F5, which is Carbopol 940-based, than the drug release from F1 and F3, which is Carbopol 934-based, this may be due to the higher viscosity of Carbopol 934 emulgel formulations as observed in table 4, this may also be due to the network structure of Carbopol 934. Opposing to F1 and F3 formulation, F7 and F2 showed the lowest drug release. In formulation F7 and F2, liquid paraffin is in its low level / high level, while the emulsifying agent is in its high level / low level respectively.

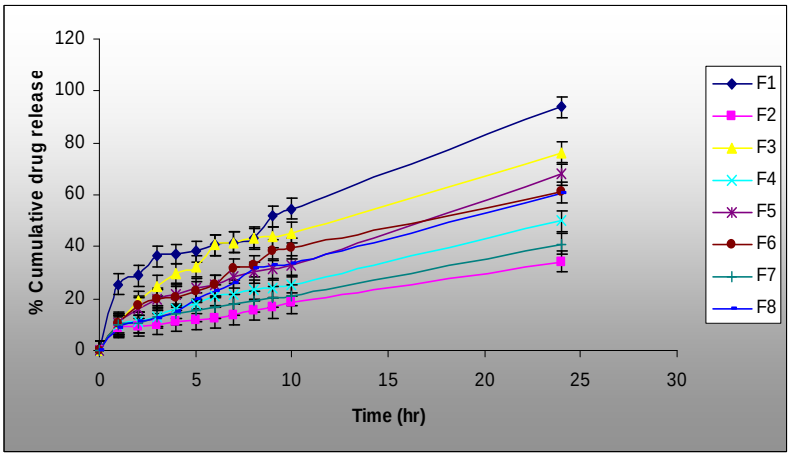


Fig.4: Release profiles of ketoconazole from its emulgel formulations at 24 hours *(Mean ±SD, n = 3)*

F3 containing emulsifying agent concentration is in its high level and liquid paraffin concentration is in its low level, exhibited greater drug release than F5, containing both emulsifying agent and liquid paraffin concentration in their level. This finding indicated that the lowering effects of liquid paraffin on the release was more pronounced than the higher effect of emulsifying agents on the drug release. The same observation was found in F1 and F4 formulation. Although F3 is Carbopol 934-based, it showed a greater drug release than F5, which is Carbopol 940-based. The same is true for F5 and F8. This finding proved that the effect of liquid paraffin in increasing the drug release from the emulgel was more predominant than the decreasing effect of Carbopol 934 on drug release.

***Microbiological assay*:** The use of control plates showed that the plain emulgel bases were microbiologically inert toward the *Candida Albicans* strain. The antifungal activity of Ketoconazole in its different emulgel formulations as well as in its commercially available Cream form is shown in figure 5 in which Percentage inhibition was taken as a measure of the drug antifungal activity. The emulgel formulations were found to have the same rank order in their antifungal activities as in the in vitro release studies. Thus, the highest activity was observed with F1 and F3, where the percentage inhibition found to be 47.5 ±1.15 % and 46.6 ± 1.34 % respectively, while the lowest activity was found with F4, where the percentage inhibition was 32.14 ±1.10 %. Where as the percentage inhibition of marketed product (Nizral cream) was found to be only 34.43±1.06% which is less than optimized formulation.

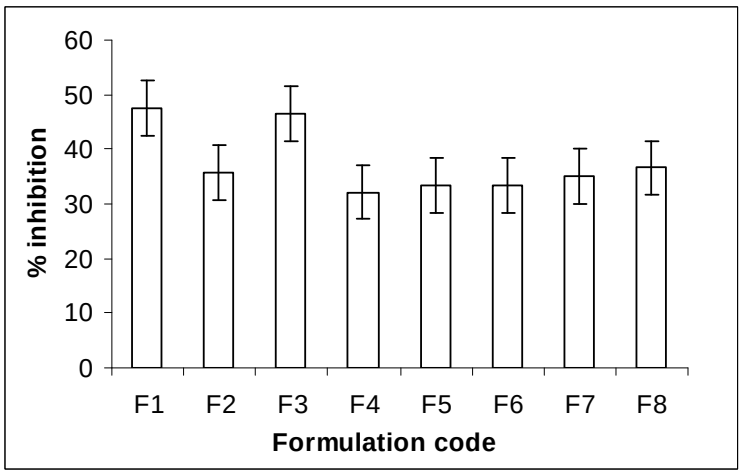


Fig.5: Percentage Inhibition as a Criterion for the Antifungal Activity of Ketoconazole in Its Different Emulgel Formulations

Skin irritation test: The Primary Irritation Index of the test article was calculated and found to be 0.00.

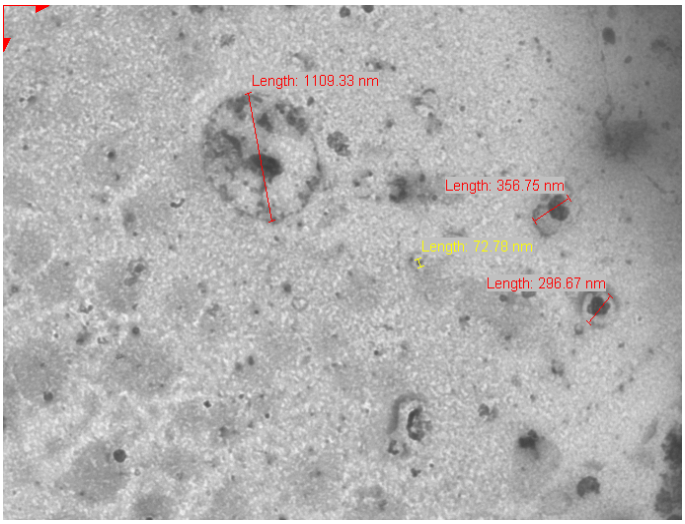
Table 4: Skin irritation test of Ketoconazole emulgel on rabbit skin.

No. of Rabbit	Erythma			Edema		
	4 hr	24hr	72hr	4hr.	24hr.	72hr
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0

0 indicate no irritation.

Globule size and its distribution in emulgel: Mean globule size in emulgel was found to be 666.0 d.nm. The poly dispersity index (PDI) of emulgel was found to be 0.962 which prove the homogeneity of emulgel.

Transmission electron microscopy: The globule of Emulgel formation was confirmed by Transmission electron microscopy. TEM image of the emulgel was observed which confirm that emulgel with approximate diameter within the range of 1 µm was formed from the emulgel, following contact with PBS (pH 5.5).



Stability studies**:** Stability studies of optimized formulation were performed as per ICH guideline (International Conference on Harmonization). It can be observed that the emulgel formulation showed no major alteration in relation to the pH, microbiological study, consistency, skin irritation test and in vitro release study. The formulation shows stability for the period of 3 months. No significant changes in the pH of formulations were observed for 3 months in all storage conditions.

DISCUSSION

The drug ketoconazole is antifungal drug with wide spectrum of antifungal activity. The physical appearance and melting point of drug were found to be concordant with that mentioned in USP (2002), which shows purity of sample. Solubility of Ketoconazole was determined in various aqueous and non aqueous solvents. The drug was found to be freely soluble in methylene chloride, soluble in methanol, sparingly soluble in ethanol, and slightly soluble in PBS (pH 5.5), and insoluble in water. The λmax for drug in PBS (pH 5.5) was 226 nm. The spreadability of formulations ranges from 12.02±0.95g.cm/sec to 18.31±1.17 g.cm/sec. The higher spreadability of emulgel formulation (F1) is 18.31±0.84 g.cm/sec. It may be due to the low concentration of emulsifying agent added in this formulation. The highest viscosity was found in Emulgel F1 it may be due to low level of the liquid paraffin concentration and emulsifying agent concentration.

The drug content in emulgel is 65.5 ± 1.82 % to 83.87 ± 1.20 %. The higher drug content determination in emulgel F1 and F3 are 83.87 ± 1.20 % and 80.4 ± 1.50 % respectively.

The higher drug release observed with formulations F1 and F3. This finding may be due to presence of liquid paraffin in its low level and the emulsifying agent in its low level/high level respectively, which lead to an increase in the hydrophilicity of the emulgel, which, in turn, facilitates penetration of the release medium into the emulgel and diffusion of the drug from the emulgel. The percentage inhibition was taken as a measure of antifungal activity of the drug. The emulgel formulations were found to have the same rank order in their antifungal activities as in the *in vitro* release studies. Thus, the highest activity was observed with formula F1 and F3, where the percentage inhibition found to be 47.5 ±1.15 % and 46.6 ± 1.34 % respectively.

The Primary Irritation of the emulgel formulation was calculated and found to be nil. The formulations found to be stable for period of 3 months; it can be observed that the emulgel formulation showed no major alteration in relation to the pH, microbiological study, consistency, skin irritation test and in vitro release study. Mean globule size in emulgel was found to be 666.0 d.nm. The poly dispersity index (PDI) of emulgel was found to be 0.962 which shows the homogeneity of emulgel is good. Emulgel formation was confirmed by Transmission electron microscopy. The size of emulgel particle found within the range of 1µm.

The prepared formulation (emulgel) show better release profile than marketed preparation. Emulgel will act as depot of drug which releases drug in sustained manner. Hence the optimized formulation may be used to treat the topical fungal diseases.

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