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RESEARCH ARTICLE

FORMULATION DEVELOPMENT AND EVALUATION OF MICROEMULSION GEL OF KETOCONAZOLE AS AN ANTIFUNGAL AGENT

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ABSTRACT

The aim of this investigation was to design and develop Microemulsion based gel (MBG) formulation of Ketoconazole for enhancing its solubility & permeability. Topical Permeability of Ketoconazole is very low. For this purpose, initially, solubility & permeability of Ketoconazole was determined in various vehicles. MBG was prepared with Capmul MCM NF as oil, Acrysol K150 as surfactant and Propylene Glycol as co-surfactant after preliminary screening of excipients. Carbopol 971 as gelling agent. The system was optimized by ternary phase diagram. The prepared formulations were characterized for Drug content, % Transmittance, Visual assessment, Particle size, Zeta potential and Compare In-vitro diffusion study of prepared MBG with Marketed formulation, Skin irritation study. The optimized batch contains mean particle size of 28.57nm and zeta potential (ζ) -8.25mV and viscosity study and Spreadability also in vitro diffusion/permeation study. In vitro diffusion study of optimized (MBG) formulation (F3) carried out which gives 78.95% within minute. And marketed formulation gives within 40minutes.Hence, by formulating into MBG, the solubility of Ketoconazole was found to be significantly enhanced.

Keywords: ketoconazole, antifungal, solubility; particle size, viscosity.

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INTRODUCTION

The Aim of the present work is to prepare Microemulsion based gel (MBG) may be formulated to enhance the Drug release, onset of action and to provide more topical antifungal effect of Ketoconazole. An attempt has been made to improve drug water solubility by the preparation of Micro emulsion based gel for topical drug delivery system. Rapid Drug release to on specific site of skin compare to Conventional dosage form can be achieved by this approach. [1]

Recently the concept of Microemulsion based gel formulation is become popular as novel drug delivery system because, it provide is patient with conventional mean of taking of their medication. Difficulty in oral administration is common problem of all age groups, especially elderly and paediatrics. Most reason is that, many widely used topical agents like ointment, cream, lotion have many disadvantages. They are very sticky, also they exhibit stability problem. Moreover, they also have lesser spreading coefficients and need to apply with rubbing. And they exhibit the stability problem also. [2,3.] Due to all these factors within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. Also, they have many advantages of gels but major limitations with hydrophobic drugs. So to overcome these limitations Microemulsion based gel approach is being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through Microemulsion base gels. [4-6]

Ketoconazole an imidazole containing Fungistatic compound is used as broad spectrum Antifungal agent in the treatment of superficial and systemic fungal infections. Especially against candidia albicans, it's interacts with 14-alpha demothylase a cytochrome P-450 enzyme necessary for the conversion of lanosterol to ergosterol. It works principally by inhibiting the enzyme cytochrome P450 14-alpha-demethylase (P450,14DM). It is synthetic imidazole-derived antifungal medication used primarily to treat fungal infections [7-8].

In Microemulsion based gel the reduction of drug particle size to the micron range. It's observe solubility in oil, surfactant and co-surfactant and having diameter in the range of 100-1000A (10-100 nm). Also the small size of the drug droplets in Microemulsion yields large interfacial area, from which the drug can quickly be released into external phase when it's applied through skin at particular site of action on fungal diseases, maintaining the concentration in the external phase close to initial levels for topical drug delivery system. [9-11] having logp value 4.35 i.e. drug is lipophilic in nature, so easily cross the skin barrier. Biotransformation of drug is hepatic (enzyme: cyp3A4 so, the gastric incompatibility) and patient compliance occurred so the Ketoconazole Microemulsion based gel is a newer approach for the hydrophobic drug to bypass its gastrointestinal adverse effects and directly give action in to

the specific site of action. [12] Ketoconazole given orally in single daily doses 200mg and Topically 2% apply 1-2 times daily to cover affected & surrounding area. Until at least a few days after disappearance of symptoms for the treatment of fungal diseases. Molecular weight of drug is less than (<) 600Dalton. Ionization constant value (Pka) is 6.51. (Ionised at gastric pH and unionised at intestinal pH).

MATERIALS AND METHOD

Ketoconazole was obtained from Stallion Laboratory Pvt Ltd. Ahmadabad, India. Transcutol CG, Lubrizolwas obtained from Gattefosse, France. Capmul MCM NF, Captex 200P obtained from Abitec Corporation US. And other Tween 80, Tween 20 obtained from Finar Chemical Limited, Ahmedabad. Acrysol EL 135, Acrysol K 150, Acrysol K 140, Carbomer 974P, 971, 971P was obtained from Corel Chem, Ahmedabad, India. Methanol obtained from Finar chemical Limited, Ahmedabad, and India. Propylene glysol obtained from S.D. Fine Chemicsl Limited, Mumbai. India.

METHOD:

Solubility method of Ketoconazole in different vehicles[13-15]

Select unknown amount of vehicles was added to each vial cap containing an excess of Ketoconazole. After sealing, the mixture was heated at 400C in a water bath to facilitate the solubilisation. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 15 minutes. The Supernant layer of centrifuge sample 1 gm accurately weighed and dilute with methanol. The concentration of Ketoconazole was then quantified by U.V Spectrophotometer. Solubility study was performed at three times and standard deviation was calculated.

Construction of phase diagram[16-18]

Pseudo-ternary phase diagrams were constructed to obtain the appropriate components and their concentration ranges that can result in large exixtance area of Microemulsion. Once the appropriate Microemulsion components have been selected, ternary pseudo phase diagram was constructed to define the extent and nature of the Microemulsion regions. To produce such diagrams, a large number of samples of different composition must be prepared. The Microemulsion region is initially delineated by its isotropic nature and low viscosity. To optimize the concentration of oil phase, surfactant and co-surfactant, different batches of varied concentration were prepared and titrated with distilled water until turbidity appeared. Two dimensional ternary phase diagram can be prepared by either keeping the composition of one component fixed and varying the other three or by using a constant ratio of surfactant to co-surfactant fixed and varying the other three or by using a constant ratio of surfactant to co-surfactant.

Ternary phase diagrams study for Microemulsion

Ternary phase diagrams of Microemulsion were prepared by Sigma plot version 10.0 software to decide the Microemulsion zone in which at any point, Microemulsion can be prepared. For the ratios of surfactant and Co-surfactant were at first selected. Here three ratios of surfactant (Acrysol K 150) and Co-surfactant (Propylene Glycol) were selected (2:1, 3:1, 4:1).

For each ratio, Microemulsion were prepared by decreasing the Oil phase (Capmul MCM NF) concentration from 90% to 10% with respect to increasing the concentration of Surfactant/Co-surfactant from 10% to 90% to decide the maximum uptake of water by Microemulsion up to which they remained transparent. Optimized the concentration of oil phase, surfactant, and co-surfactant was based on maximum uptake of water by Microemulsion.

Preparation of microemulsion

(%W/W) The formulations were prepared by initially dissolving required quantity of Ketoconazole in oil. Then Surfactant and Co-surfactant mixer were added and the final mixture was mixed by vortexing until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hr, and examined for signs of turbidity or phase separation prior to particle size studies. we selected different ratio like as 2:1, 3:1, 4:1 after we were prepared Micro emulsion and will optimize one ratio from it. It was checked different evaluation parameter of Microemulsion like particle size, zeta potential, thermodynamic stability studies.

Table No.1: Preparation of microemulsion

Formulation Code	Smix (ratio)	%w/w of oil	%w/w of smix	%w/w of water	Drug (%w/w)
M1	2:1	3.5	21.5	75.0	2
M2	2:1	4.6	23.4	72.2	2
M3	3:1	5.7	28.3	66.0	2
M4	3:1	3.4	23.6	73.0	2
M5	4:1	4.3	27.7	68.0	2
M6	4:1	5.5	26.3	69.0	2

Evaluation of microemulsion[19-22]

a) Microscopic evaluation

Microscopic analysis was carried out in order to observe the homogeneity of Microemulsion formulations. Any change in colour and transparency or phase separation occurred during normal storage condition (37±2ºC) was observed in optimized Microemulsion formulation.

b) Transmittance Test

Stability of optimized Microemulsion formulation with respect to dilution was checked by measuring Transmittance through U.V. Spectrophotometer (UV-1800 SHIMADZU).Transmittance of samples was measured at 650nm and for each sample three replicate assays were performed.

c) Particle size analysis & Polydispersibility Index

Each Formulations of 1 ml were diluted with 100 ml of water in a volumetric flask. The volumetric flask was inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulation the droplet size of resultant Microemulsion was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a Zetasizer Nano Series (Malvern Instruments, DTS Ver.4.10, Serial No. MAL 500999). Light scattering was monitored at 25°C at 90° angle. Value of Particle size and polydespersity index are tabulated in table 5.12 .

d) Thermodynamic Stability

Heating cooling cycle

Six cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature of not less than 48h was studied. Those formulations,

which were stable at these temperatures, were subjected to centrifugation test.

Temperature Stability

Shelf life as a function of time and storage temperature was evaluated by visual inspection of the Microemulsion system at different time period. Microemulsion was diluted with purified distilled water and to check the temperature stability of samples, they were kept at three different temperature range (28°C (refrigerator), Room temperature) and observed for any evidences of phase separation, flocculation or precipitation.

Centrifugation

In order to estimate metastable systems, the optimized Microemulsion formulation was diluted with purified distilled water. Then Microemulsion was centrifuged (Remi Laboratories, Mumbai, India) at 1000 rpm for 15 minute at 0°C and observed for any change in homogeneity of Microemulsions.

e) Zeta potential

Test Zeta potential for microemulsion was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd., U.K.). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment.

f) **IR studies**[23]

2An FTIR-8400S spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients (Capmul MCM NF, Acrysol k-150, Propylene Glycol) were carried out using diffuse reflectance spectroscopy (DRS)- FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each the spectrum, 8 scans were obtained at resolution of 4 cm-1 from a frequency range of 4000-400 cm -1

Preparation of microemulsion based gel (MBG). (%W/W)

Preparation of Microemulsions based Gel (MBG)

Formulation Microemulsion based Gel were prepared by using gelling agent and penetration enhancer. The method only differed in the process of making gel in different formulations. The gel bases were prepared by dispersing Carbopol 971 in distilled water separately with constant stirring at a moderate speed using mechanical shaker. In formulation gelling agent dispersing in distilled water. And the dispersion was left overnight. The pH of all formulations was adjusted to neutral using triethanolamine (TEA). Ketoconazole is hydrophobic drug. And, it was dissolved in oil phase. Oleic acid was also mixed in oil phase. And dissolve drug completely after add it in surfactant and co-surfactant mixture (Smix). After, added aqueous solutionin oil phase with continuous stirring. After keep it room temperature for 1day. The obtained Microemulsion was mixed with gel bases with gentle stirring to obtain the Microemulsion based gel under room temperature. Applied heat whenever if necessary.

Table No. 2: Formulation of Microemulsion based Gel (100gm)

Ketoconazole Gel	Ingredients (for 100g. of gel)
Ketoconazole	2
Capmul MCM NF	0.9
Acrysol K 150	3.1
Propylene Glycol	1.56
S/cos Ratio	2:1
Carbopol 971	1.2
Oleic acid	1.0
Triethanolamine	0.1
Water	q.s.

RESULT AND DISCUSSION

Compatibility study: From the ir spectral analysis, it was found that IR spectrum of Ketoconazole, Ketoconazole Microemulsion formulations, and Polymer showed the characteristic peaks as follows.

Table No.3: Interpretation of IR of drug with ME Formulation& Carbopol 971

Functional group	Ketoconazole cm-1	Ketoconazole microemulsions formulation (cm-1)	Carbopol
-cl stre	731, 812	814	807.27
-C-H- stre	1367	1371	1303.89
-c=o- stre	1642	1644	----------
$-CH_3$	2996	2997	2948.33
-c=c- stre	1585	1589	----------

SOLUBILITY STUDY AND SELECTION OF EXCIPIENTS

Before selecting suitable excipients for Ketoconazole MBG, UV Spectrophotometric analysis of drug in each individual excipients was done by scanning the methanol drug excipients mixture in the range of 200400 nm. It is expected that in the absence of any interference between the drug and the excipients, the absorption maxima of the drug remain intact even in its dissolved state in the said excipients. In this study, excipients were explored for solubility of Ketoconazole. For each excipients, max of the drug in 7.4 pH PBS i.e., 225 nm was found to be retained. This information indicates that each of these excipients is well compatible with the drug at room temperature. The important criterion for selection of the excipients is thatall the components are pharmaceutically acceptable for topical administration and fall under GRAS (Generally regarded as safe) category. It has been demonstrated that only very specific pharmaceutical excipients combination lead to efficient Microemulsions formulation. The higher solubility of the drug in the oil phase is important for the co-surfactant is contributing to drug Solubilization, there could be a risk of precipitation, as dilution of emulsions on topically will lead to lowering of solvent capacity of surfactant or co-surfactant. Safety is major determining factor in choosing surfactant, as large amount of surfactants may cause skin irritation. Nonionic surfactants are less toxic than ionic surfactants. Nonionic surfactantstypically have higher CMCs than their ionic counterparts. The hydrophilic Lipophilic balance (HLB) value has been proven to be very useful in choosing the best type of surfactant is necessary for the immediate formation of O/W droplets and/or rapid spreading of the formulation in the aqueous environment, providing a good transparent. An important criterion for selection of the surfactant is that the required HLB value to form O/W

Nanoemulsion is greater than 10. A proper surfactant HLB value was a key factor for the formation of emulsion with small droplets.

Solubility of Ketoconazole:

The maximum solubility of Ketoconazole was found in Capmum MCM NF (184.6±6.32 mg/gm) as compared to the other oils and combinations of oils (Table 2). High drug solubility was found in Tween 80 (166.1±6.7) and Propylene glycol was found (178.1±7.2).

Table No. 4: Solubility of Ketoconazole in various oil, surfactant, co-surfactant.

Components	Solubility(MG/GM) ±S.D
Oleic acid	123.9±4.3
Castor oil	28.67±2.93
Capmul MCM NF	187.6±6.39
Captex 200P	3.67±0.89
Peanut oil	9.83±0.88
Sesame oil	189.33±6.88
Acrysol K-140	25.80±1.9
Labrasol	98.0±3.19
Tween 80	169.1±6.78
Transcutol CG	129.30±3.9
Propylene Glycol	174.1±7.0
PEN 400	5.11±0.60

PSEUDO-TERNARY PHASE DIAGRAM

Microemulsion systems form fine oil-water emulsions with only gentle agitation, upon their introduction into aqueous media. Surfactant and co surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the Microemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the Microemulsion (Kang et al 2004). After performing solubility studies, components in which drug showed more solubility put forwarded for phase behavior study. In present study, combinations of surfactants (Smix) with high and low HLB values were used. Capmul MCM NF has low HLB value (5-6) and Acrysol K150 having higher (14-16). Combination of low and high HLB surfactants leads to more rapid dispersion and finer emulsion droplet size on addition to aqueous phase (Liu et al 2008). Capmul MCM NF and Acrysol K150 in the ratio of 2:1 showed wider Microemulsion existence area compared with 3:1 and 4:1 Smix.

Evaluation of microemulsion

Transmission test

% Transmittance was measured by directly taking the absorbance of the diluted Microemulsion. No significant difference was observed among the percentage transmittance of formulations F1 to F6 and formulation F2 was found to have the highest percentage transmittance value of percentage transmittance closer to 100% signified that all of the formulations were clear and transparent. Besides signifying clarity of the formulation, a percentage transmittance closer to 100% also indicates that the size of the globules in the formulation is in the nanometer range. This in turn indicates that the drug in the formulation has a large surface area for release.

Table No. 5: % Transmission test of Microemulsion formulation in water

Formulation code	Transmittance (%)
M1	99.3
M2	99.8
M3	98.8
M4	98.6
M5	99.4
M6	99.3

Drug Content

Irrespective of difference in composition, the drug content of formulations M1 to M6 was found in range of 95.71±0.81 – 99.39±0.25 %

Table No.6: % Drug content of Microemulsion formulation

Formulation code	% drug content (Values are expressed as mean ± S.D, n=3)
M1	98.46±0.24
M2	99.56±0.25
M3	95.79±0.81
M4	94.44±0.09
M5	96.65±0.45
M6	99.39±0.08

Visual Assessment

Ketoconazole Microemulsion concentration (approximately 0.2 ml) was diluted with purified water (100 ml) and gently stirred with magnetic stirrer. Temperature should be 370C.

Particle Size Determination

The droplet size of the emulsion is a crucial factor in MBG performance because it determines the rate and extent of drug release as well as absorption. Average droplet size was found in water, which range from 28.57 – 82.10 nm indicating all the particles were in the nanometer range. The result shows that the higher Smix proportion led to a increase in mean droplet size. The smallest particles were observed for formulation F2 (28.57) and largest droplets were obtained for formulation F3.

Table No.7: Visual assessments various formulation

Formulation code	Colour	Grade
M1	Transparent	Grade-Il
M2	Bluish transparent	Grade-I
M3	Bluish transparent	Grade-I
M4	Transparent	Grade-I
M5	Transparent	Grade-Il
M6	Slightly transparent	Grade-Il

Polydispersibility Index (PDI)

Polydispersibility which determines size range of particle in the system. It is expressed in terms of polydispersibility index (PDI). Polydispersibility index (PDI) (0.3) indicates a homogenous distribution, while a PDI (>0.3) indicates a higher heterogeneous dispersion. The data are as shown in table 2.14 The results show that formulations F1, F3 and F4, F5, F6 does not pass the test as they have PDI more than 0.3 whereas remaining all formulations pass the test as they have PDI less than 0.3. PDI value of formulation F2 is less compare to other Formulations. Report of particle size analysis are represents in figure .

Table No. 8: Particle size and PDI of Microemulsion in water

Formulation code	Mean Particle size ± S.D (nm)	Polydispersibility Index
M1	43.06	0.321
M2	29.14	0.307
M3	82.89	0.879
M4	56.45	0.802
M5	75.49	0.868
M6	59.31	0.858

Thermodynamic stability Microemulsion are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking.

Table No. 9: Result of Thermodynamic stability of M1 to M6 formulation

Formulation code	Phase separation	Flocculation	Precipitation
M1	Not Seen	Not Seen	Not Seen
M2	Not Seen	Not Seen	Not Seen
M3	Not Seen	Not Seen	Not Seen
M4	Not Seen	Not Seen	Seen
M5	Not Seen	Not Seen	Not Seen
M6	Not Seen	Not Seen	Seen

Table No. 10: Centrifugation stability study of M1 to M6 Formulation

Formulation code	Phase Separation
M1	Not Seen
M2	Not Seen
M3	Not Seen
M4	Not Seen
M5	Not Seen
M6	Not Seen

Zeta potential

Determination Zeta potential can be defined as the difference in potential between surface of the tightly bound layer (shear plane) and the electro neutral region of an emulsion. The ideal zeta potential for ME is less than-30, formulation M1 and M3 were not pass the zeta potential test. Formulation M2 has lowest zeta potential (-8.25) on this basis M2 is good.

Table 11: Zeta potential of M1 to M6 Formulation

Formulation code	Zeta potential (mV)
M1	-34.9
M2	-8.35
M3	-36.9
M4	-7.42
M5	-12.9
M6	-23.9

CONCLUSION

Formulation of Ketoconazole microemulsion with antifungal properties is promising in short time gave effect of antifungal action on local area. The microemulsion based gel formulation of Ketoconazole containing 4% oil phase (Capmul MCM NF), 8% surfactant phase (Tween 80), 16% cosurfactantphase(Propylene Glycol). 68% distilled water has been optimized. In addition they provide intimate contact

between dosage form and fungal skin which may result in hiwh drug concentration in local area. The Ketoconazole Microemulaion based gel could be successfully formulated for the topical treatment of fungal candidiasis. The developed ketoconazole microemulsion based gel showed good in vitro antifungal activity against rhizomes fungi. It can be concluded that the developed Microemulsion based gel have great potential for topical drug delivery.

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