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DESIGN, DEVELOPMENT AND EVALUATION OF SELECTED ANTIFUNGAL LOADED ETHOSOMAL GEL FOR TOPICAL DRUG DELIVERY

Dr. S. Chandra*, S. Sangeetha, Sonam Sasi, R. Suresh, B. Nandhini, S. Kavibharathi

India.

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***Corresponding Author**
Dr. S. Chandra
India.

ABSTRACT

In the present research work, an attempt was made to develop ethosomal gel of suitable antifungal drug and efficiency of ethosomes as novel lipid carriers for topical delivery of suitable drug has been evaluated. The ethosomal approach was selected to enhance the permeability of ketoconazole that increases bioavailability, reduce side effects, reduce large doses and increase the therapeutic efficacy. The ethosomal gel of ketoconazole was prepared by using cold method by using different concentration of ethanol and phospholipid. The prepared formulations were evaluated for drug entrapment efficiency, drug content, spreadability, viscosity, pH, homogeneity and grittiness, percentage yield and *in vitro* drug release studies. *In vitro* drug release studies were performed by using Franz Diffusion Cell for 8h. The results of FTIR analysis showed that there was no physical and chemical interaction between drug and other excipients. The formulation F5 was considered as best formulation. The data obtained from in vitro release study were fitted into various mathematical models like zero order, first order, Higuchi and Peppas. The results of mathematical modeling obtained indicated that it was best explained by zero order followed by Higuchimodel. The study indicated that the ethosomal gel of ketoconazole can effectively improve the bioavailability of drug by penetration enhancement and thereby reduce the frequency of administration. The ethosomal gel could be successfully prepared.

KEYWORDS: Ketoconazole, ethanol, phospholipid, ethosomes, topical drug delivery systems.

INTRODUCTION

Novel drug delivery is designed to improve the topical delivery of antifungal agents by

enhancing their dermal localization with concomitant reduction in their side effects. Various novel drug delivery strategies have the potential in optimizing and enhancing the topical delivery of antifungal agents. Human skin is an important target site for topical drug delivery. Fungal infections grow slowly and often occur in tissues that are poorly penetrated by antifungal agent, making its treatment difficult. This also necessitate prolong treatment of fungal infections. Various topically active agents are available for the treatment of fungal infections. Upon application, they release the active ingredients which form a concentrated layer of active ingredients that is rapidly absorbed. Ethosomal vesicles are used for the delivery of drugs to reach the deep skin layers and are the advanced forms of liposomes that are high in ethanol content. They can incorporate hydrophilic and hydrophobic drugs to enhance the accumulation of drug. Ethosomes are administered in semisolid form, hence producing high patient compliance.

Ethosomes are system containing soft vesicles, composed of hydro alcoholic or hydro glycolic phospholipids, water, alcohol (ethanol and isopropyl alcohol) in relatively high concentration. This high concentration of ethanol makes the ethosomal system unique. The range of ethanol in final product will be 20%-30%. The size of the ethosomes will be in the range of nanometers to microns.

The source of the phospholipids can be egg, soybean, semi-synthetics and synthetics. Some preferred phospholipids are soya phospholipids such as Lipoid S100, Phospholipon 90 (PL-90). High concentration of alcohol (20-45%) in the formulation provides soft, flexible characteristics and stability to the vesicles and it also disrupts lipid bilayers structure of the skin which results in an increase in the membrane permeability

Examples of alcohols, which can be used, include ethanol (commonly used) and isopropyl alcohol. Glycols can also be used in preparations as a penetration enhancer. Among glycols, propylene glycol and transcutol are generally used. For providing further stability to ethosome vesicles, cholesterol at concentrations ranging between about 0.1-1% can also be incorporated.

Gels are relatively newer classes of dosage form created by equipment of large amount of aqueous or hydro-alcoholic liquid in network of colloidal solid particles, which may consist of inorganic substance such as aluminum salt or organic polymers of natural or synthetic origin. Gels are going more popular now a days because they are more suitable and also can

provide controlled release than other semisolid preparation. The gel formulation can provide better absorption characteristics and hence the bioavailability of drug.

Because of the use of ethanol in the preparation of the ethosomes, the deformability of the prepared vesicles is increasing. Besides, the high alcohol content partially extract the stratum corneum lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. The ultra-deformable vesicles can forge paths in the disordered stratum corneum and finally release drug in the deeper layers of the skin. Therefore, a path through the skin can be expected to result, permitting the fusion of ethosomes with the cells from the deepest skin layers.

MATERIALS AND METHODS

Drug used for the studies and the corresponding suppliers are summaries in table. The drug was purchased from Yarrowchem Products, Mumbai, India.

Materials	Supplier
Drug(Ketoconozole)	Yarrowchem Products, Mumbai, India
Soya lecithin	Yarrowchem Products, Mumbai, India.
Propylene glycol	Yarrowchem Products, Mumbai, India.
Carbopol 934	Yarrowchem Products, Mumbai, India.
Methyl paraben	Yarrowchem Products, Mumbai, India.
Propylparaben	Yarrowchem Products, Mumbai, India.
Glycerol	Yarrowchem Products, Mumbai, India.

Preformulation Studies

Preformulation studies are the first step in the rational development of dosages forms. It can be defined as an investigation of physical and chemical property of a drug substance alone and in combined with excipients.

Preformulation studies not only helps to guide dosages form selection, but also provide knowledge that how drug products should be processed and stored to ensure their quality.[9,10]

Organoleptic Properties of Drug

The drug sample (Ketoconozole) was noted for its organoleptic properties such as colour, odour, taste and appearance.

Identification of Drug

λmax for pure ketoconazole

Preparation of stock solution

Accurately weighed quantity of 100 mg ketoconazole was taken in 100 ml volumetric flask and was dissolved by using 5 ml of methanol, finally the volume was made up with 7.4 pH phosphate buffer upto 100 ml to produce 1 mg/ml of solution.

Determination of λmax

A series of concentrations i.e. 4, 8, 12, 16, 20 µg/ml was prepared by using above stock solution and scanned between 200-400nm.

Preparation of Standard Calibration Curve of Ketoconazole

Preparation of standard solution

Accurately weighed amount of ketoconazole equivalent to 100 mg was dissolved in small volume of 0.01M HCL, in 100 ml volumetric flask and the volume was adjusted to 100 ml with pH 7.4 phosphate buffer. This resultant solution had the concentration of 1mg/ml (1000 µg/ml) which was labeled as stock solution.

Solubility determination

Solubility test of ketoconazole was performed by using various solvents such as water, ethanol, ether, DMSO and chloroform were used as solvent. The results were shown in below.

Table 2: Solubility analysis of Ketoconazole.

Sl. No	Solvent	Solubility
1	Water	Less Soluble
2	Ethanol	Soluble
3	Ether	Soluble
4	Chloroform	Soluble
5	DMSO	Soluble

Drug- Excipient Compatibilitystudies

Ftir

Integrity of the drug in the formulation was checked by taking an IR spectrum of the selected formulation along with the drug and other excipients. The spectra were taken by using Shimadzu IR Prestige-21 Spectrophotometer were compared with standard spectras. In this study, pelletisation of potassium bromide (KBr) was employed. Before forming the pellets of

potassium bromide, it was completely dried at 100 ℃ for 1 h and after drying it was thoroughly mixed with the sample in the ratio of 1 part of sample and 100 parts of KBr. The mixture was compressed to form a disc using dies. This disc was placed in the sample chamber and a spectrum was obtained through the software program which is further subjected to interpretation.

Formulationdevelopment

Composition of different ethosomal formulation of ketoconazole.

Ingredients	F1	F2	F3	F4	F5	F6
Ketoconazole	0.5	0.5	0.5	0.5	0.5	0.5
Soyalecithin	3	2	4	5	5	4
Ethanol	10	15	20	10	15	20
Propylene glycol	10	10	10	10	10	10
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s

Formulation of ethosomes

Ethosomal formulations were prepared by using the cold method. The ethanolic vascular system was composed of phospholipid (2-4% W/V), ethanol (20-40% V/V), propylene glycol (20% V/V), drug and distilled water. Phospholipid was dissolved along with the drug in ethanol. This mixture was heated to 40 ℃ and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 min, while maintaining the system at 40 ℃. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4 ℃ for five cycles of 3 min each with a minute rest between cycles using a probesonicator. Six formulations were prepared using different concentrations of phospholipid and ethanol, among them optimized formulation was selected for characterization and evaluation studies.

Preparation of carbopol gel base

1% carbopol gel base was prepared by dispersing 1 g of carbopol 934 in 90 ml hot distilled water which is previously added with glycerol. Accurately weighed amount of methyl paraben and propyl paraben was also added into it. The mixture was stirred until thickening occurred and then neutralised by dropwise addition of triethanolamine to achieve a transparent gel.

Incorporation of ethosomes in the gel base

The ethosomal formulation was slowly added in carbopol 934 gel base with gentle stirring. Finally, the ethosomal gel was mixed using a mechanical stirrer for 5 min.

Evaluation of Ethosomes

Drug entrapment efficiency

The total volume of the ethosomal suspension was measured. 5 ml of this formulation was diluted with distilled water up to 8 ml and centrifuged at 15,000 rpm for 45 min at 40 ℃ using a cooling centrifuge. After centrifugation, the supernatant and sediment were recovered, their volume was measured. Then sediment was lysed using n- propanol and filtered through a 0.45 µm nylon disk filter. The concentration of ketoconazole in the supernatant and sediment was analyzed by UVspectroscopic method at 223 nm. The percent drug entrapment was calculated using the following equation.

$$\%entrapmentefficiency = \frac{\text{amount of entrapped drug recovered}}{\text{Total amount of drug}} * 100$$

Drug content

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to10 mg of the drug in 100 ml volumetric flask and volume was made upto 100 ml with methanol. The content was filtered through Whatman paper no.41. 5 ml of above solution was taken into a 25 ml volumetric flask and volume was made to mark with methanol. The content of the ketoconazole was determined at 223 nm against blank using the Shimadzu UV/visible spectrophotometer. The drug content was determined from the calibration curve of ketoconazole.

Physical evaluation

The ethosomal gel formulation of ketoconazole was evaluated for organoleptic characteristics, occlusiveness and wash ability.

Measurement of pH

1 g ketoconazole ethosomal gel was mixed in 100 ml distilled water with homogenizer. Then the electrode was immersed in the prepared gel solution and readings were recorded from digital pH meter in triplicate and average value was calculated.

Franz diffusion cell

In vitro absorption studies are generally carried out in vertical Franz diffusion cell. According to Food and Drug Administration (FDA) regulations, it is an ideal tool for quality control of topical preparations. It has a receptor and a donor chamber, which is filled with phosphate buffer medium. It consists of a water jacket through which temperature controlled water is re-

circulated in order to perform the experiments at a desired temperature. The dialysis membrane is sandwiched between the two chambers and clamped in place tightly. The donor chamber is filled with a known volume of buffer and the permeation of solute through the membrane is monitored by periodic sampling of the solution from the receptor chamber. The jacketed cell embodied is stirred throughout the study at 500 rpm employing a magnetic stirrer.

Drug release kinetics

The release kinetic was studied by various kinetic models as zero order plot, first order plot, higuchi plot and korsmeyer-peppas plot. To study the release kinetics of the ethosomal gel data obtained from in-vitro drug release studies were plotted in various kinetic models: zero order as cumulative amount of dug releases v/s time, First order as log cumulative % of drug remaining v/s time, higuchi model as cumulative % of drug released v/s square root of time and korsmeyer– peppas model as log cumulative % drug release v/s. log time. The best fit model was confirmed by the value of correlation coefficient near to1.

Stability studies

The stability study was carried out for ethosomal gel formulation. The most satisfactory formulation was scaled in a glass vial to a temperature of 40ºc for month, then at 25ºc for 1 month, then At 40ºc for 1 month. After this ethosomal gel was exposed to ambient room temperature and liquid Exudates separating was notes. At the end of 3 months, the samples were analyzed for physical Characteristic study, percentage relative humidity, the drug content and diffusion study etc.

RESULT AND DISCUSSION

Organoleptic evaluation

Colour	**White**
Odour	Odourless
Taste	Bitter

Identification of the drug

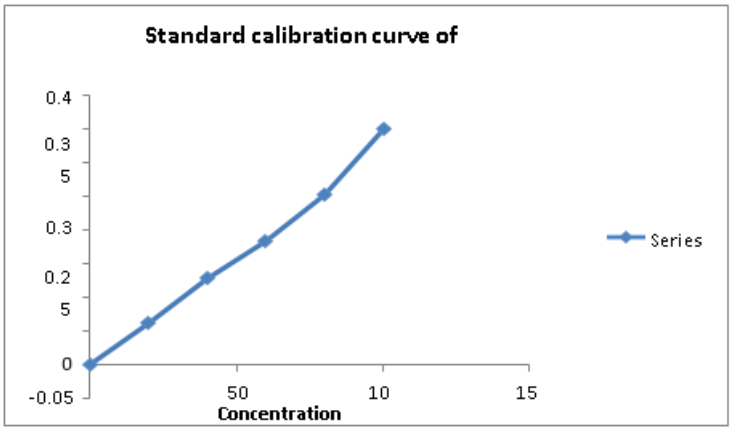
λmax for pure ketoconazole

The absorbance maximum of pure ketoconazole was determined using methodology and the absorbance was measured at 223nm.

Calibration curve for pure ketoconazole

The standard calibration curve of ketoconazole was described in the method obtained results are used to plot a graph with absorbance v/s concentration. It shows straight line that passes through origin.

Sl. No.	Concentration (µg/ml)	Absorbance (223nm)
1	0	0
2	20	0.061
3	40	0.127
4	60	0.183
5	80	0.247
6	100	0.348



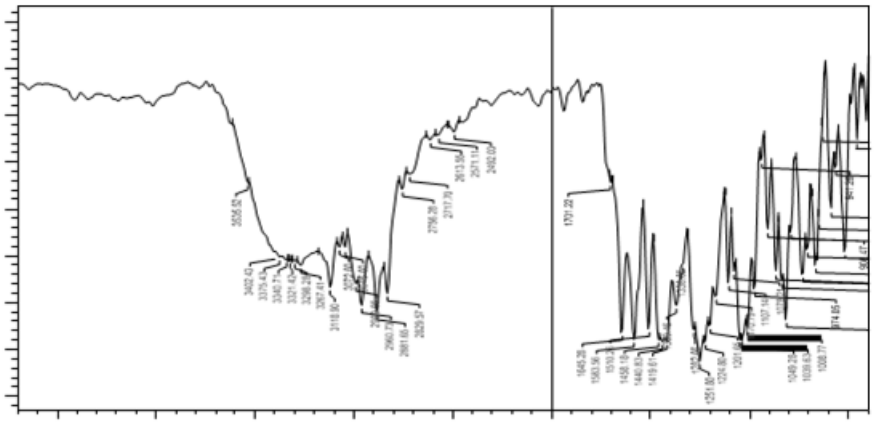
Calibration curve of ketoconazole

Solubility determination

The solubility of ketoconazole was observed that it was soluble in ethanol, ether, chloroform and DMSO.

Drug – Excipient Compatibility Studies

FTIR analysis was carried out for pure drug and drug polymer mixture and drug excipient mixtures. The FTIR studies were carried out as mentioned in method. FTIR spectrum of drug shows the prominent peaks with respect to functional groups. The FTIR spectrum of physical mixture of drug with polymer and drug with excipient concluded that there is no significant interaction between the drug, polymer and excipients. In the spectrum of drug polymer mixture, the characteristic peak of drug was not altered.



Ketoconozole+methyl parabean

Formulation development

Ethosomal suspension of ketoconazole Ethosomal gel of ketoconazole

Drug Entrapment Efficiency

Sl. No	Formulation code	Entrapment efficiency (%)
1	F1	57±0.17
2	F2	63±0.12
3	F3	56±0.23
4	F4	65±0.14
5	F5	91±0.26
6	F6	72±.10

Evaluation of ethosomalgel

Physicalevaluation

Sl. No	Formulation code	Color	Odour	Appearance
1	F1	Light orange	Characteristic	Translucent
2	F2	Pale yellow	Characteristic	Translucent
3	F3	Light red	Characteristic	Translucent
4	F4	Pale yellow	Characteristic	Translucent
5	F5	Pale yellow	Characteristic	Translucent
6	F6	Pale yellow	Characteristic	Translucent

pH Determination

Sl. No	Formulation code	pH
1	F1	6.98±0.18
2	F2	7.43±0.12
3	F3	7.56±0.25
4	F4	7.81±0.16
5	F5	7.14±0.16
6	F6	7.25±0.22

Drug content

Sl. No	Formulation code	Drug content (%)
1	F1	79.4±0.21
2	F2	90.1±0.14
3	F3	87.6±0.13
4	F4	87.2±0.21
5	F5	91.8±0.28
6	F6	83.5±0.12

Drug content of ethosomal gel

The formulations were analyzed for drug content spectrophotometrically at 223 nm. All the formulations exhibited fairly uniform drug content. The drug content of all developed formulations were in the range of 79.4-91.8%.

***In vitro* diffusion studies**

Sl. No	Time (hr)	Percentage of drug released (%)					
		F1	F2	F3	F4	F5	F6
		0	0	0	0	0	0
1	1	3.01	.58	24	92	.32	.54
2	2	10.13	17.96	21.04	12.08	29.87	12.32
3	3	19.41	22.14	30.21	20.89	37.26	24.56
4	4	25.68	35.09	39.41	32.12	49.57	31.81
5	5	31.16	41.83	50.51	47.49	78.41	60.17
6	6	49.78	53.87	58.76	66.38	85.25	68.23
7	7	56.64	56.45	61.17	71.01	91.40	76.22
8	8	59.27	62.13	65.14	76.27	93.2	80.56

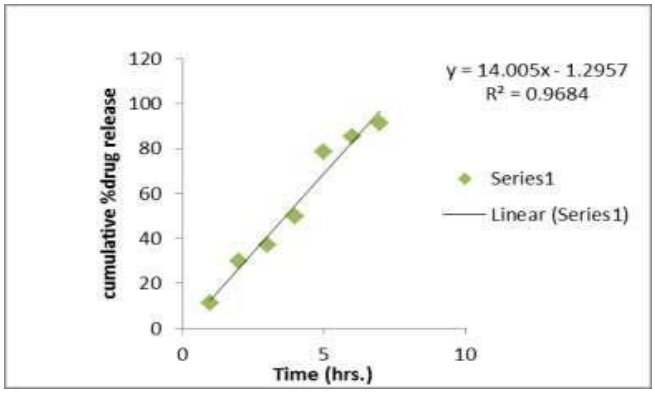
Kinetic modeling of F5

Time (hr)	Cumulative %drug release	%drug remaining	Square root time	Log cumulative % drug remaining	Log time	Log cumulative %drug released	% dru g released	Cube root of % drug remaining (w)
1	11.32	88.68	1.000	1.948	0.000	0.000	100	4.459
2	29.87	70.13	1.414	1.846	0.301	1.475	18.55	4.124
3	37.26	62.74	1.732	1.798	0.477	1.571	7.39	3.974
4	49.57	50.43	2.000	1.703	0.602	1.695	12.31	3.695
5	78.41	21.59	2.236	1.334	0.699	1.894	28.84	2.785
6	85.25	14.75	2.449	1.169	0.778	1.931	6.84	2.452
7	91.4	8.6	2.646	0.934	0.845	1.961	6.15	2.049
8	93.26	6.74	2.828	0.829	0.903	1.970	1.86	1.889

Kinetic analysis data of F5

Zero order Plot

Graph was plotted between % cumulative drug release v/s time.



Formulation	Kinetic modeling			
F5	Zero order	First order	Higuchi	Korsmeyer Peppas
R2	0.9684	0.9372	0.9596	0.9116

Kinetics of drug release of F5 formulation

The permeation profile of most satisfactory formulation F5 was fitted to zero order, first order, Higuchi's model and korsmeyer-peppas model. It was found that the *in vitro* drug release of ketoconazole was best explained by zero order model as it showed the highest linearity (R^2 = 0.9684), followed by Higuchi model (R^2 = 0.959).

RESULT OF STABILITY STUDIES

Parameter	Initial value	After 3 Months
Physical Evaluation • Colour • Odour • Taste	 White Odourless Bitter	 No Changes No changes No Changes
Relative Humidity	25ºC±2ºC/60% RH	40ºC±2ºC/75% RH (Accelerated stability study)
Uniformity Drug Content	91.8±0.28	No Changes
Diffusion study	93.20 (% Drug release in 8hr)	93.22%

Stability studies performed at 40°C±2°C for 3 months showed good storage stability. It was observed that optimized gel kept for 3 months under 40ºC ± 2º c /75% RH conditions showed no change in their physical appearance. Optimized ethosomal gel kept for 3 months under 40ºC±2ºC temperature with relative Humidity 75% RH conditions was studied for uniformity of content. The results showed no significant change in content uniformity. In diffusion study carried out at 40ºC±2ºC/75% RH for 8 hr shows slight changes are seen after 3 months.

DISCUSSION

The present study was to design the ethosomal gel containing ketoconazole using different concentration of ethanol and phospholipid. It is an attempt to utilize the immense potential of ethosomes as a carrier to increase the permeability. The ethosomal gel of ketoconazole was prepared by using cold method and is then evaluated for drug entrapment efficiency, drug content, spreadability, viscosity and percentage yield. The result of these studies were within the prescribed limits of Pharmacopoeial specifications.

FTIR studies showed that there was no marked incompatibility between drug and excipients. In vitro drug release from the gel showed significantly improved drug permeation. Among all the formulation, in vitro release of F5 formulation showed sustained action upto 8 h with maximum drug release of 93.26%. Based on R^2 value, the optimized formulation F5 follows zero order followed by Higuchi model for the mechanism of drug release. The ethosomal gel of ketoconazole could be successfully prepared in a cost effective manner and had better drug release than other conventional dosageforms.

Stability studies performed at 40°C±2°C for 3 months showed good storage stability. It was observed that optimized gel kept for 3 months under 40ºC ± 2º c /75% RH conditions showed no change in their physical appearance. Optimized ethosomal gel kept for 3 months under

40ºC±2ºC temperature with relative humidity75% RH conditions was studied for uniformity of content. The results showed no significant change in content uniformity (91.8±0.28). In diffusion study carried out at 40ºC±2ºC/75% RH for 8 hr shows slight changes are seen after 3 months (93.2).

CONCLUSION

Among all the formulation, in vitro release of F5 formulation showed sustained action upto 8 h with maximum drug release of 93.26%. Based on R^2 value, the optimized formulation F5 follows zero order followed by Higuchi model for the mechanism of drug release. The ethosomal gel of ketoconazole could be successfully prepared in a cost effective manner and had better drug release than other conventional dosage forms. FTIR studies showed that there was no marked incompatibility between drug and excipients. In vitro drug release from the gel showed significantly improved drug permeation. In diffusion study carried out at 40ºC±2ºC/75% RH for 8 hr shows slight changes are seen after 3 months (93.2).

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