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Formulation development and *in vitro* characterisation of ethosomes for the enhanced transdermal delivery of clotrimazole

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ABSTRACT

The target of the study was the development and *in vitro* characterisation of an effective ethosomal formulation of clotrimazole and to the performance of the formulation was investigated under different preparation conditions. The EE and from 9 formulations, the optimisation was done on the basis of its results which considering the concentration of lecithin and ethanol as the two factors and EE and in vitro release as their responses. The optimization was done on the basis of its results which considering the concentration of lecithin and ethanol as the two factors and EE and *in vitro* release as their responses. F6 formulation gained a very good release of drug and highest entrapment efficiency of 93.203%. The results of permeation flux were obtained in the order of MLVs > ESUVs > SSUVs > LUVs. This study gives an idea about the vesicles structure and the size, the technique used, the drug EE and the permeation rate. They were taking the above factors into consideration results in improving clinical effectiveness in transdermal delivery of an ethosomal formulation of clotrimazole.

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INTRODUCTION

Transdermal delivery is an alternative route for the delivery of orally acting drugs. The advantages of transdermal delivery are the following; circumvent the variables that could influence gastrointestinal absorption such as pH, food intake, and gastrointestinal motility, hepatic metabolism and can give a constant, controlled drug input, decreasing the variations in drug plasma levels, thus reducing the side effects.

Additionally, TDD has some other therapeutic benefits such as sustained drug delivery to provide a steady state plasma profile and hence reduced systemic side effects, thus generating the potential for improved patient compliance, the bypass of first-pass metabolism effect for drugs with poor oral bioavailability. Drug input can be terminated simply by removing drug application from the skin surface. Above all this, TDD represents a convenient, patient-friendly, easily allowing dose changes according to patient needs and self-regulation of dosing by the patient (Amit and Ramesh, 2003; Bain *et al.,*1993).

However, this system has its limitations and is principally associated with the barrier function of skin, which severely constrains the absolute amount of drug that is absorbed across a reasonable area of skin during a dosing period. Thus it is limited only to a potent drug that should be 10mg or less. A drug that needs high blood levels cannot be administered and can cause skin irritation. The high cost of the drug is also a major limitation for the acceptance of the product. TDD system is of a first, second and third generation. Using this novel second transdermal delivery is poised to increase the impact on medicine significantly (Mohammed and Mortada., 1999).

1.1 Ideal Properties of a Drug to Penetrate through Skin (Vyas *et al.,* 2002; Weiner and Flynn, 1985).

The ideal characters of drugs are:
Molecular weight and size: less than 500 Dalton.

Compound lipophilicity: Should be high for better absorption (through Trans cellular route), although hydrophilic small molecular compounds absorb through aqueous channels.

MATERIALS AND METHODS

Clotrimazole IP was obtained from Srikem chemicals, Mumbai and Chettana Pharmaceuticals, Kerala. Soya Lecithin, Sabouraud fluid media, Sabouraud's Dextrose agar obtained from Himedia, Mumbai.Carbapol934P and Potassium Bromide are obtained from Labachemie Pvt. Ltd., Mumbai. Potassium dihydrogen phosphate, Disodium hydrogen phosphate were obtained from Fisher Scientific. Sodium Chloride was obtained from Spectrum, Kochi. DMSO from E. Merck Ltd. Mumbai. Methanol and Formalin was obtained from Nice Chemicals, Kochi

METHODOLOGY

Pre-formulation studies: The physical and chemical properties of the drug alone or with its excipients are studied under preformulation studies. The main aim of this study is to generate structurally and functionally stable dosage form.

Identification of drug

FTIR Spectroscopy: FTIR of the standard drug spectrum is used for the comparison of FTIR of the obtained pure drug clotrimazole.

Partition coefficient of the drug

The partition coefficient of clotrimazole in octanol-water and concentration of solute was determined by UV spectroscopy at 261.6nm.

Compatibility studies of the drug with the excipients

The excipient selection assists the administration, promote the consistent release and bioavailability of the drug and protect it from degradation gives a successful, stable and effective dosage form.

Physical drug- excipients compatibility

At high temperature of 400C and 75% relative humidity conditions, the stability studies were combined with the physical compatibility studies. For 30 days, as above specified temperature and humidity conditions, these mixtures were stored in the humidity chamber. The prime state of the mixtures was evaluated for the possible occurrence of any interaction that was performed after the 15th and 30th day.

FTIR studies: Compatibility of clotrimazole with the individual excipients of the formulation was established by Infrared Absorption Spectral Analysis (FTIR). Any changes in the Chemical composition after combining with the excipients were investigated with IR spectral analysis.

Formulation of Ethosomal Suspension (Gupta *et al.,* 1999; Luana and Cinzia., 2008).

Method

MLVs were obtained by TLE, and the lipid mixture was dissolved in CHCl3, at 58°C in a rotary evaporator, and a thin film of dry lipid is thus obtained on the flask wall. It was then subjected to evaporation for 2 hours and thereby obtaining a dry residue. Then the film was hydrated by addition of the hydrophilic phase, which contains dissolved drug of 1% w/v which on stirring at a 30Hz frequency with a vortex mixer until vesicle formation.

Characterisation of Ethosomal Suspension (Delgada *et al.,* 2007; Khan *et al.,* 2001; Marijia *et al.,* 2005; Suvakanta *et al.,* 2010).

Characterisation of vesicles

Microscopic evaluation: A small quantity of ethosomal suspension was hydrated with 10 ml of phosphate buffer (pH-7.4). We can individualize the vesicles clearly by shaking the dispersion of ethosome manually and occasionally. The slide was placed with a drop of dispersion and examined under the microscope. Circular vesicle bodies were observed at 100xs.

Vesicle size distribution: Dynamic light scattering technique with a computerised inspection system was used for the measurement of vesicle size and the dilution of ethosomal suspension with Millipore water.

Zeta potential determination: Using zetasizer, zeta potential was calculated.

Entrapment efficiency: Ultracentrifugation is the technique used to determine the entrapment efficiency. On centrifuging at 17,000 rpm at 40C for 45 minutes, clotrimazole loaded ethosome get separated from the unentrapped drug. Thus a supernatant was obtained, and to this, a phosphate buffer was added in order to maintain the pH. The concentration of clotrimazole was then assayed spectrophotometrically at 261.6 nm.

In vitro drug permeation studies (Somchitb *et al.,* 2003).

The human skin and porcine skin shows similar histological and biochemical properties. Moreover, the stratum corneum and epidermal thickness of ear skin of pig are comparable to human skin. It shows similar permeability to the skin of a human.

Skin preparation (Carrillo *et al.,* 2002).

The skin of pig ear was used for the reviewing of skin penetration. The subcutaneous fatty tissue was removed first, and then the skin surface was cleaned using Ringer's solution and allowed to dry for 20 min, packed in aluminium foil and stored at – 20C in polyethene.

Preparation of Franz diffusion cell

The surface area of Franz cell is 3.14 cm2 were used and the capacity of receptor compartment of approximately 15 ml. Using ringer's solution, the frozen skin was cleaned and then moved on to the Franz diffusion cells. The phosphate buffer methanol mixture was then filled to the receptor compartment. The epidermal side and dermal side of the skin were exposed and bathed in the donor compartment and the receptor solution respectively. While removing the bubbles between the beneath of the dermis and the receptor solution, care should be taken. The room temperature was maintained at 370C with external constant water circulator, and the reservoir medium was continuously stirred, to mimic the body condition.

Permeation studies (Faiyaz *et al.,* 2008)

From prepared Franz diffusion cell to the donor compartment, ethosomal suspension of 100mg was applied and from this 0.5ml of the sample was withdrawn at a predetermined rate, like 1, 2,3,4,5,6,7,8 and 24hrs. These samples were then analysed, and then calculate the concentration of the drug withdrawn and the amount of drug permeated to the receptor compartment.

Skin Deposition Studies: Using methanol, the donor compartment was washed for five times, and this was done after performing the above mentioned in vitro permeation study. Then the skin was extracted and kept for a time period of 12 hours. The amount of the drug was then determined by spectrophotometer at 261.6 nm. Ethanolic receptor solution will diffuse into the skin throughout this stage which causes the disrupting of ethosomal vesicular structure and thus results in releasing of both bounded and free clotrimazole.

In-vitro Drug Release (Krishnapriya *et al.,* 2017; Mohamed., 2004; Peter *et al.,* 2010; Piyusha *et al.,* 2010; Raphael.,2003).

For all the 9 selected formulation, release studies were carried.

Procedure: 3ml ethosomal suspension was placed in a cellophane membrane. Immerse these into dissolution medium of 30 ml, with a phosphate buffer (pH 7.4)-methanol mixture medium and at temperature 37±0.5^{0}C, in order to simulate the condition of the human cell during the experiment. From this, 5 ml samples were taken and are analysed spectrophotometrically at 261.6 nm at fixed rate 1, 2, 3, 4...,24 hours, and are substituted with an equal amount of the samples. The percentage drug released and the calculated amount of released medicament was plotted against time.

Kinetic Modelling of In-vitro Drug Release (Steptoe *et al.,* 2009).

To study the release kinetics, the data obtained from in vitro drug release studies were plotted in four different kinetic models. The plots were drawn using 6 origins, and from the value of R-value, the linearity of the plots was obtained. The best-fit kinetic model was selected if the model is with the highest regression coefficient (R-value), i.e. near to unit value.

Statistical Design of Experiment (Shammika *et al.,* 2017)

The procedure of statistical optimization was performed with the help of optimization software like Design Expert 8 and Stat Graphics Centurion 16. The interpretation of data obtained from response analysis can be performed using 3D response plot, contour plots and Pareto charts.

The statistical study was done by the method

ANOVA: The significant level of the study was done with F statistics and P value, where P value should not greater than 0.05, is considered and the F value obtained is not less than the critical F value the factor becomes significant.

Comparison Studies (Vidya Viswanad *et al.,* 2015; John *et al.,* 2013)

In-vitro comparative study of ethosomal suspension

The study was done with a hydroethanolic solution of drug and drug in buffer solution. The optimized ethosomal formulation was compared with the hydroethanolic solution of drug and drug in buffer solution. The cumulative amount of drug permeated per cm2 v/s time was plotted. The slope was then compared.

In-vitro permeation study of Optimized ethosomal gel and Gel with Marketed and Carbopol gel

The optimized 1% ethosomal gel was compared with 1% Marketed gel (Candid gel) and 1% Carbopol Gel for the in-vitro permeation studies. The cumulative drug permeated per unit area v/s the time was plotted. The slope of the plot was noted and compared.

Gel preparation: 0.5g of Carbopol 940 is suspended in distilled water (99.5 ml), Carbopol gel base of 0.5 % w/v was prepared and stirred it at

room temperature for 24 hours and kept for gelification by adding triethanolamine to a (pH-7). The gel so formed was stored in the dark at 4°C, in capped glass containers. High entrapment efficiency and optimum vesicular size are the characteristics of optimized ethosomal formulation, and in order to separate the ethosomal vesicles, this was centrifuged in temperature at 4°C and 20,000rpm for 90 minutes. The entrapped drug was collected and dispersed in the Carbopol 940 (0.5 %w/v) gel base with gentle stirring to obtain the total drug equivalent to 1%w/w of clotrimazole which is obtained from ethosomal sediment. By mixing (50/50/w/w) Carbopol gel with a 1% aqueous solution of clotrimazole, the gel loaded with the drug were prepared and thus ultimate drug concentration of 1% w/w was obtained.

In-vitro Antifungal Activity

The supplied test products are marketed formulation and optimized ethosomal formulation in a quantity of 2µl, 5µl, 10µl, 15µl respectively. Here the microbial test strain is Candida albicans are isolated into colonies by streaking and incubated at 370C for 48 hours. Agar diffusion assay is the test method, where yeast extract (2g), peptone(1g), dextrose(4g), agar(2g) and 1000ml of distilled water are the chemicals and media used respectively for the experiment, in which a PH of 6.8 and at a maintained temperature of 35°C. Some additional materials like loops, saline blanks, and slides, staining supplies, autoclave, incubators etc. are used.

Determination of MIC and zone of inhibition

The inoculums of test organisms 0.1 ml were uniformly spread over the prepared sterile YPD plate. By using a 6 mm diameter sterile borer, wells were prepared and add the samples of different concentration (2µl, 5 µl, 10 µl, 15 µl) separately to each well and thus the samples were tested at different concentration. Then at 37°C for 24 hours' plates were incubated where a period of time is sufficient for the growth. Microbial growth around the well is known as the zone of inhibition which was then measured. MIC was from the fully grown plates.

Skin Morphological Studies

Skin Preparation

The skin of pig ear was used for the reviewing of skin penetration. First, the s/c fatty tissue was removed, and then the surface of the skin was cleaned with the Ringer's solution. Then the skin was treated with various formulations like; normal saline, water, drug solution, marketed formulation, ethosomal suspension. The skin is then washed thoroughly buffered it in a formalin solution which is off concentration 10%. Specimens were chopped into a vertical section and using ethanol it is then dehydrated. This is then fixed in paraffin, and with haematoxylin and eosin, it is then stained well. These samples were then compared with a control sample after observing under a light microscope. After the treatment, changes in the skin were noted.

Stability Studies: To determine the physiochemical stability, the formulation which is off optimized were subjected for studying the stability, for 45 days at two variant temperatures. It is also subjected to drug degradation at different intervals of time.

RESULTS

Pre-formulation studies: Identification of drugs

The solubility of the drug: With reference sample, the solubility of the pure drug was then compared and tabularized in table 2. It conceals about the pure drug is freely soluble in ethanol (95%), methanol, chloroform and n, n- dimethylformamide and partially insoluble in water, 0.1N hydrochloric acid and pure phosphate buffer (7.4). So for the calibration purpose and further analytical works the solvent selected was a mixture of methanol and phosphate buffer in 8:2 ratios.

Partition coefficient of the drug: It was found that 4.09 is the partition coefficient of the Clotrimazole, which point out that it can be incorporated into an ethosomal formulation for the efficient transport through the skin, as this drug is practically insoluble in water and having high lipid solubility.

Compatibility of a drug with an excipient

Physical compatibility drug and excipient

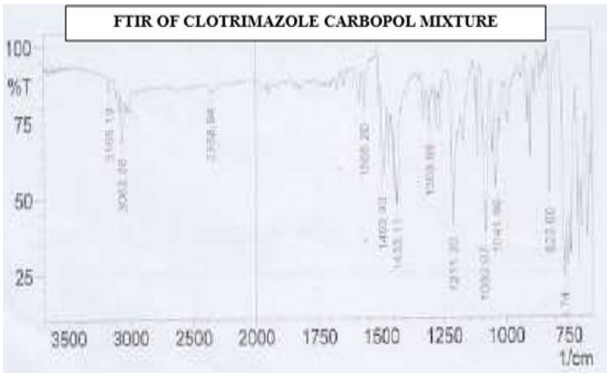

Figure 1: FTIR of Drug

FTIR Spectroscopy

Drug-excipient compatibility studies were also confirmed by FTIR and the spectra of the drug, drug lecithin mixture, drug Carbopol mixture, and the formulation is shown in figure 1 - 3 respectively.

Table 1: Composition of ethosomal vesicle prepared

Formulation code	Amount of lecithin in%	Amount of ethanol in %	Water
F1	1%	20%	Up to q.s
F2		30%	Up to q.s
F3		40%	Up to q.s
F4	2%	20%	Up to q.s
F5		30%	Up to q.s
F6		40%	Up to q.s
F7	3%	20%	Up to q.s
F8		30%	Up to q.s
F9		40%	Up to q.s

Table 2: Comparison of Solubility Profile of Pure Drug with Reference

Solvent	Solubility of	
	Reference	observation
Distilled water	-	-
Ethanol	+	+
Methanol	+	+
0.1N hydrochloric acid	-	-
Phosphate buffer(7.4)	-	-
Chloroform	+	+
Phosphate buffer methanol(8:2)	+	+

Table 3: Physical compatibility drug excipient Studies

Sl.no	Composition	Description		
		Initial	2weeks	4weeks
1	Clotrimazole	White powder	No color change	No color change
2	Clotrimazole +cholesterol	White powder	No color change	No color change
3	Clotrimazole +lecithin	Brown color powder	No color change	No color change

Table 4: Composition of different ethosomal suspension

Ethosomes formulation code	Drug (mg)	Lecithin (%w/v)	Ethanol (%v/v)	Water
Et120	20	1	20	q.s
Et130	20	1	30	q.s
Et140	20	1	40	q.s
Et220	20	2	20	q.s
Et230	20	2	30	q.s
Et240	20	2	40	q.s
Et320	20	3	20	q.s
Et330	20	3	30	q.s
Et340	20	3	40	q.s

Table 5: Entrapment efficiency of formulations

Sl.no	Formulation code	Entrapment efficiency in %
1	Et120	38.971 ± 0.89766
2	Et130	42.523 ± 0.67534
3	Et140	44.235 ± 1.23326
4	Et220	62.985 ± 0.96509
5	Et230	82.752 ± 0.65848
6	Et240	93.203 ± 0.55203
7	Et320	69.661 ± 0.40463
8	Et330	73.124 ± 1.98099
9	Et340	77.765 ± 1.64872

Formulation of ethosomal suspension

The preparation technique of an ethosomal formulation of clotrimazole antimitotic agent against Candida albicans was studied and considering this as worthy of interest that to study the role of the technique of preparation on vesicle properties as well as on the drug antifungal activity.

Optimization of the composition

The previous studies show that different concentration of ethanol and lecithin has marked effect

on the entrapment efficiency and other vesicle properties of ethosomes. So for fixing a constant composition, a total of 9 formulations was selected with varying concentration of ethanol and lecithin. The formulations consist of ethanol concentration in a range of 20- 40% v/v, and lecithin concentration in a range of 1- 3%w/v. To evaluate the potential of the vesicular drug delivery system, one of the important features is drug entrapment within the vesicular carrier. This is the reason that for all the formulation, the EE of clotrimazole within the ethosomal vesicles was studied and the effect of ethosome composition, i.e. Ethanol and lecithin concentration on drug loading capacity was also considered. The optimization of the formulation was done on the basis of entrapment efficiency and in vitro drug release by using a software assisted statistical method.

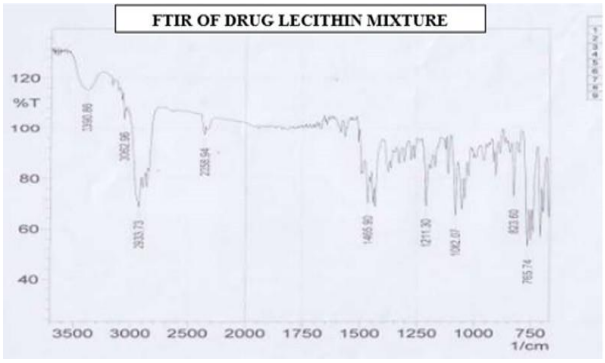

Figure 2: FTIR of Drug lecithin Mixture

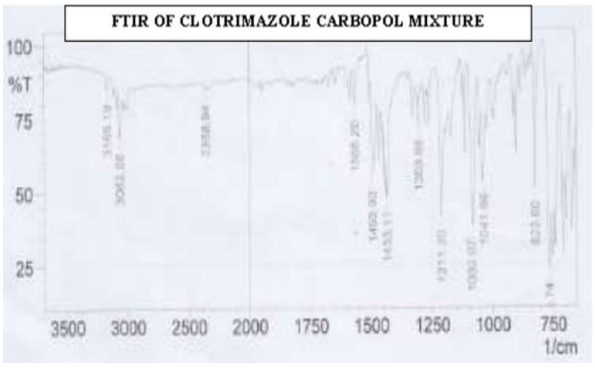

Figure 3: FTIR of Drug clotrimazole Carbopol Mixture

A composition of various ethosomal formulations

This is used to the study with a fixed concentration of drug clotrimazole 20mg are represented in table 4.

Entrapment efficiency: Solute retention is used for measuring the entrapment efficacy. If entrapment efficiency is high, less time and effort is required to remove the unentrapped drug. The important parameter which is used to study the ingredients that are used in the formulation was the vesicular entrapment efficiency. The entrapment efficiency of these formulations varied from 38.97% - 93.203% and was found statistically significant at $p< 0.05$. The entrapment efficiency of various formulations is charted in table 5. From the data in table 5, it is clear that both ethanol and lecithin concentration determines the entrapment efficiency.

Effect of ethanol concentration on entrapment efficiency

The percent total drug entrapped within the vesicles measures entrapment efficiency. The result obtained for 9 formulations were shown that the content of ethanol has a significantly positive effect on the entrapment efficiency of ethosomal carriers. Here the concentration of ethanol is directly proportional to entrapment efficiency. Increase in the ethanol concentration increases the entrapment efficiency in each formulation. Highest EE of 93.203 ± 0.55203% is for F6 which is having the highest concentration of ethanol 40%. It was observed that the vesicles would be more stable at higher concentrations of ethanol. Because of the electrostatic repulsions, stabilizing agents like dicetylphosphate, ethanol also may exert a stabilizing effect in the formulation, prevent or delay the formation of vesicular aggregation. So the concentration of ethanol is a significant factor governing stability and hence the entrapment efficiency.

In vitro drug release studies

In vitro release studies give an idea about the release pattern of the drug from the vesicle. The 9 formulations selected were undergone release studies for 24hours. The release data were tabulated and represented in graphs. The first 3 formulations showed rapid release, particularly evident in the initial phase almost 40% drug released within the 4th hour. This can be increased with an increase in ethanol concentration. In these formulations, the lecithin concentration is relatively high so the lamellae formed may be of less in number. Leakage of the drug from bilayer is due to an increase in the concentration of ethanol that causes thinning of the bilayer, permeability of vesicle membrane and an increase in fluidity at high concentration of ethanol. The release rate from the formulation 4, 5 and 6 was moderate at level. And in them also the release rate increased with an increase in ethanol concentration. Formulation 7, 8 and 9 showed very slow release from the vesicle. This can be explained by relating the concentration of lecithin content. One possible reason for the result could be the rigidization effect of lecithin at higher concentrations. In all the 9 formulations the release rate decreases with increase in lecithin concentration. In last 3 formulations contains lecithin in high level thereby very slow release. The release parameters of different formulations are represented in Fig.4.

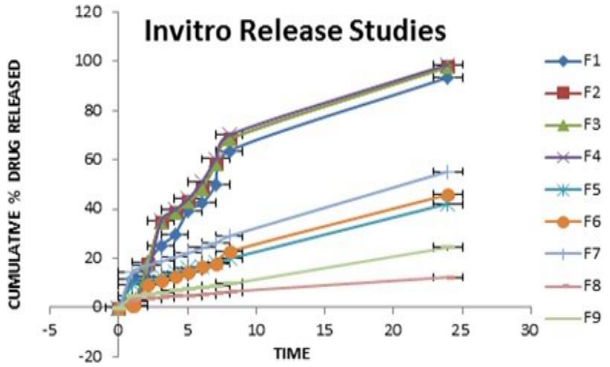

Figure 4: FTIR of Drug and Carbopol Mixture

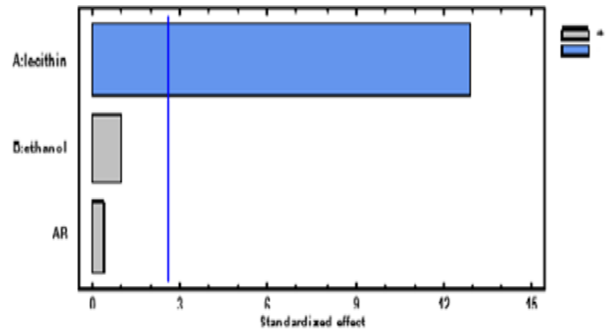

Figure 5: Percent Cumulative Release of Formulation F1 to F9

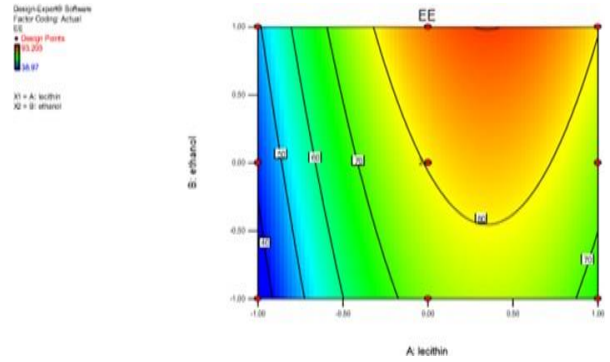

Figure 6: Pareto Plot for In vitro drug release

Software assisted optimization results: This software analyses the responses individually, and the relations between the factors and responses represent in the form of different plots like contour plots,3D surface plots, Pareto plots and main effect plots from fig.5-9. The optimization is done on the basis of desirability value which is closer to unity.

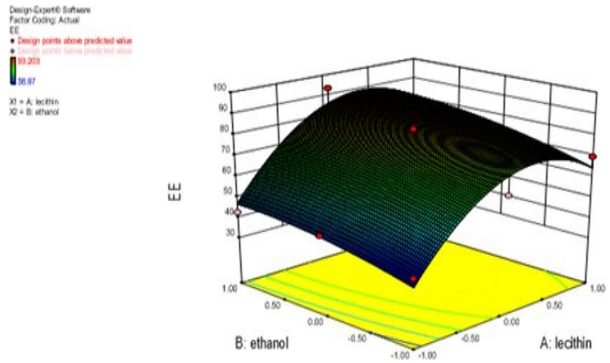

Figure 7: Counter Plot for Entrapment Efficiency

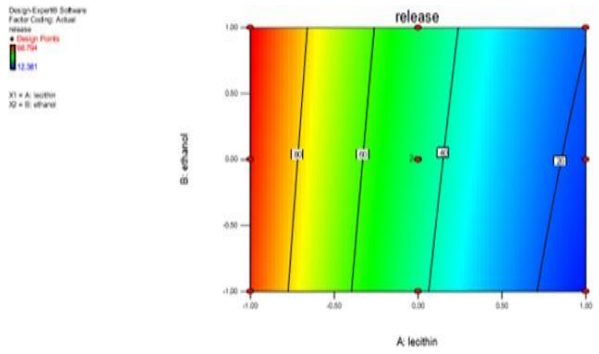

Figure 8: 3D Surface Plot for Entrapment Efficiency

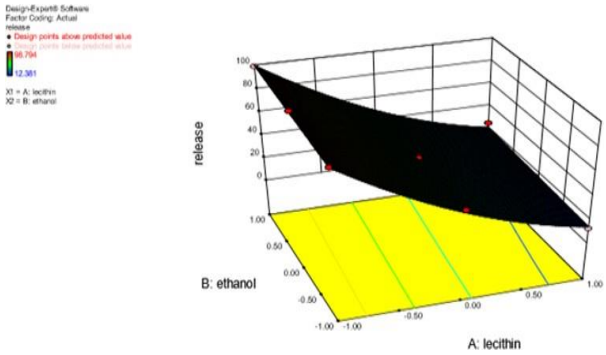

Figure 9: Counter Plot for in vitro drug release

ANOVA analysis report

As the F values are greater than the critical F values and the P values not greater than 0.05 (threshold level) that is 0.0059 and 0.0476 respectively point out that the entrapment efficiency of both lecithin and ethanol are analysed by ANOVA. So the entrapment efficiency of the clotrimazole-loaded ethosomal vesicle is influenced by the use of both the excipients. The in vitro drug release by ANOVA indicates that the F value of lecithin is greater than the critical F values and the P values not greater than 0.05 (threshold level), i.e. 0.0001. So that the permeation flux of clotrimazole loaded ethosomal vesicles is influenced by the use of lecithin.

Drug release kinetic modelling

Drug release kinetics follows a defined manner in order to supply the maintenance dose enabling the attainment of the desired drug concentration. In accordance with the data obtained by the in vitro drug release of formulations F4, F5, and F6 were used to determine the release pattern of a drug from the prepared vesicle. Selection of the best fit model release data is based on the regression coefficient 'R' value. If 'R' value is high, then that is selected as the best fit model. Kinetic modelling is compared with release profile, and it was found that the release of a drug is in accordance with Higuchi kinetics and the regression coefficient was found to be as unity for the entire 3 ethosomal formulations. The release kinetic model and plots of selected formulations are given in Fig.10-12 respectively.

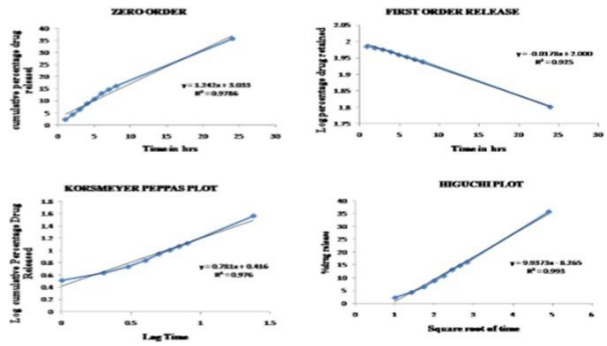

Figure 10: 3D Surface Plot of in vitro drug release

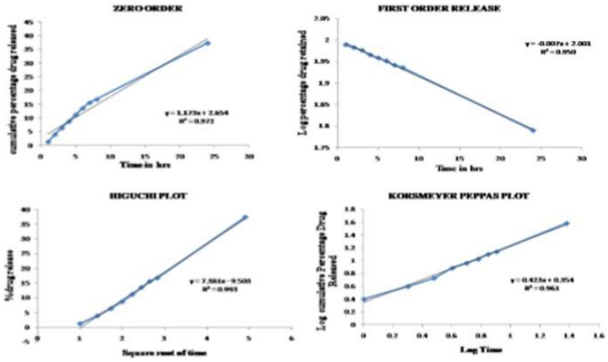

Figure 11: Drug release kinetic Plots of F4

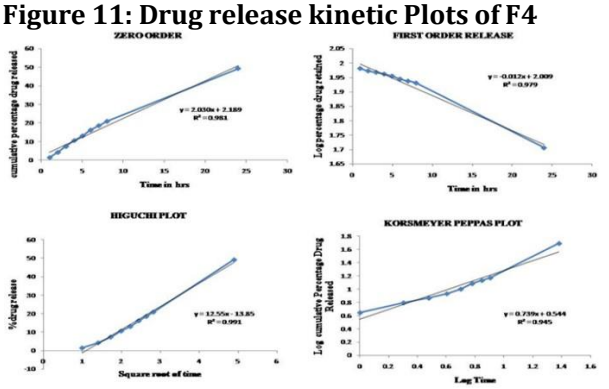

Figure 12: Drug release kinetic Plots of F5

Evaluation of Ethosomal Suspension

Entrapment efficiency

Using the ultracentrifugation technique, the EE of clotrimazole loaded various ethosomal vesicles were assessed. The entrapment efficiency of different ethosomal formulations was calculated as percent total drug entrapped and the effect of vesicle nature on the entrapment efficiency was observed. It is evident, that the highest EE% values showed by MLV and LUV vesicles, followed by SUV vesicles by sonication. These results were not only depending on vesicle characteristics, rather the method adopted for their preparation. In the presence of both the lecithin and ethanol which confirms the vesicles flexibility and deformability.

Stability studies

The stability was studied at 30 ± 2°C and 4 ± 2°C respectively for 45 days and it was confirmed that the formulations were more stable at 4 ± 2°C in Figure 13.

CONCLUSION

The present study focused on developing an ethosomal formulation of Clotrimazole and its in vitro characterisation to enhance permeation effect. Then the study focused on developing a suitable composition of ingredients which will provide an ethosomal vesicle with excellent integrity and efficiency. Based on the in vitro drug release data and entrapment efficiency obtained from the selected 9 formulations, one composition was optimized (F6) for further studies. As confirmation step software assisted, a statistical analysis method was also used for the optimization. The analysis showed the entrapment efficiency directly depends on the concentration of ethanol, but the entrapment efficiency found to be high when the lecithin concentration is optimum. The formulation with high ethanol showed a fast release rate, due to the thinning of the vesicle membrane and thereby leakage of the drug, in them, the amount of lecithin was also less. The formulation with high lecithin content showed slow release rate when compared to others. So selected a formulation to contain optimum amount of lecithin with a high amount of ethanol as optimized one, i.e. F6. ANOVA, desirability, surface plot and counterplot were also showed that F6 is the optimized formulation. By fixing that composition of ethosomal vesicles of 4 different types were prepared by 4 different preparation techniques. This study influences the structure and size of ethosomal vesicles from the preparation of the percentage of vesicles EE as well as on penetration rate. To determine the highest EE% and good permeation, one of the best methods is the thin layer evaporation method which is obtained from MLVs. The further progress based on the characterisation, permeation through porcine skin and comparison studies also revealed that ethosomes containing MLVs are a good option for transdermal delivery of clotrimazole.

From these result, the properties and effectiveness of ethosomal formulations are related to the vesicle preparation. The in vitro antifungal activity studies also showed increased activity of ethosomal formulation when compared to the market. The lipid perturbation and elasticity of ethosomes are the main contributors for improved skin permeation. Stability study at temperatures 30 ± 2°C and 4 ± 2°C respectively for 45 days were done, and it was then confirmed that the formulations were more stable at 4 ± 2°C. From the result, it was found that the ethosomes as a vesicular carrier have a vast potential of enhancing permeation of clotrimazole. Finally, it can be inferred that it has a rapid

onset and good release for attaining the effective therapeutic efficacy of clotrimazole.

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