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Research Article

Design, Development and Characterization of Microemulsion based Hydrogel of Clotrimazole for Topical Delivery System

Dhruti P. Mehta*, Hemendrasinh J. Rathod, Dhiren P. Shah

Vidyabharti Trust College of Pharmacy, Umrakh - 394345, Gujarat, India

Correspondence: dhrutipmehta@yahoo.com (Tel.+918866309917)

Abstract

The purpose of this research work was to design, development and characterization of microemulsion based hydrogel of clotrimazole for topical delivery system. The solubility of clotrimazole was performed in different excipients. Drug-excipient compatibility study was performed using FT-IR spectroscopy. Pseudoternary phase diagrams were constructed to determine the region of existence of microemulsions. Clotrimazole loaded oil in water (o/w) microemulsion was prepared by phase titration method. A 3^2 full factorial design was applied to study the effect of independent variables, i.e., ratio of surfactant: co-surfactant (X_1) and the ratio of oil: water (X_2) on dependent variables, i.e., %Transmittance (Y_1), viscosity (Y_2) and %cumulative drug release at 12 h (Y_3) for the optimization of microemulsion formulation. Clotrimazole microemulsion formulation F9 was optimized on the basis of %Transmittance, viscosity and %cumulative drug release. The optimized formulation F9 shown in vitro drug release up to 12 h. Clotrimazole microemulsion based hydrogel was optimized based on the physical appearance, pH, viscosity, spreadability and *in vitro* & *ex vivo* permeability. The results of the skin irritation study showed that there was no irritation of skin. Optimized formulation had good antifungal activity compared to the marketed product. Results of stability study revealed that the optimized formulation was satisfactorily found to be stable for three months.

Keywords: Topical drug delivery, pseudoternary phase diagrams, microemulsion based hydrogel, clotrimazole, 3^2 factorial design, antifungal activity

Introduction

Microemulsions, that are thermodynamically stable and optically isotropic systems of water, oil, surfactant and/or co-surfactant, have been studied as drug delivery systems because of their ability to solubilize poorly water soluble drugs and also their improvement of systemic and topical availability. It helps to solubilize lipophilic drug moiety and shows rapid and effective penetration to skin. So it's beneficial for topical delivery of drug. For topical delivery, microemulsion is incorporated in polymer hydrogel base to extend the local contact to skin. Generally used topical agents such as creams, ointments, lotions have many disadvantages like causing uneasiness to the patient when applied, sticky nature, applied by rubbing and they also exhibit the stability problem. Microemulsion is having stability problem due to having low viscosity but can be overcome by incorporating into the topical drug delivery system causes improved viscosity and hydrating stratum corneum which will increase drug dermal permeation and the skin flux. Because of all these factors inside the main group of semisolid, use of transparent hydrogels has expanded both in pharmaceutical preparations and in cosmetics. In spite of the many advantages of hydrogels, there is restriction in the delivery of lipophilic drugs. Hence, to overcome this restriction microemulsion based approach is used, hence even a hydrophobic therapeutic moiety may be effectively incorporated and delivered by hydrogels. Hydrophobic drugs can be incorporated into microemulsion based hydrogel using drug/oil/water emulsions. Microemulsion based hydrogel helps in the incorporation of hydrophobic drugs in oil phase and then oily globules are distributed in an aqueous phase resulting in oil/water emulsion [1-5].

Clotrimazole is a BCS (Biopharmaceutics Classification System) class-II and imidazole derivative which have a broad spectrum of antimycotic activity [6]. It prevents the biosynthesis of sterol, ergostol and thus results in antifungal activity. Half-life of clotrimazole is 2 h. It has an oral bioavailability of 15-20%, which increases the dosing frequency of the drug. The increased dosing frequency leads to side effects like erythema, edema and skin irritation. Conventional topical hydrogel formulation of clotrimazole is proposed to exert on the outer layers of the skin which may rapidly absorb. Thus, the goal of the present investigation was to design microemulsion based hydrogel for topically controlled delivery of clotrimazole. Clotrimazole loaded microemulsion based hydrogel can be used for prolonged drug release and retention of dosage form on the skin, thus reducing fluctuation in concentration of drug, reducing drug toxicity and improving patient compliance by prolonging dosage application intervals [7].

Materials and Methods

Materials

Clotrimazole was kindly gifted by Amoli Organics Pvt. Ltd., Mumbai. Mentha oil, Propylene glycol and Triethanolamine were purchased from Sulab (Pioneer sales), Baroda. Tween 80 was purchased from Mehta sales chemical, Baroda. Carbopol 934 was purchased from Balaji Drug, Surat. All other reagents used were of analytical grade.

Screening of oils, surfactants and co-surfactants

Solubility of clotrimazole was determined in different oils, surfactant and co-surfactant. Clotrimazole was added in excess to different oils, surfactant and co-surfactant and stirred for 24 h on a magnetic stirrer. After stirring, samples were centrifuged at 1500 RPM for 10 min and drug in the supernatant was analyzed at λmax 261 nm [8].

Construction of pseudoternary phase diagram

The pseudoternary phase diagrams were constructed with screened oil, surfactant/co-surfactant and water using the water titration method at room temperature by ProSim ternary diagram software. The procedure consist of preparing solutions containing tween 80 to PG (surfactant to co-surfactant) in the ratios 1:1, 1:3, 1:5, 1:7, 1:9, 2:1, 2:3, 2:5, 2:7, 2:9,3:1,3:5, 3:7, 4:1, 4:3, 4:5, 4:7, 4:9, 5:1, 5:3, 5:7, 5:9 and 6:1, each of these solutions is then used for preparing a mixture containing mentha oil and tween 80 to PG (oil and combined surfactant to co-surfactant) solution in ratios of 1:1, 1:2 and 1:3. Add

water to each mixture and vortexed for 5 min and then placed in a water bath at 37 °C for 24 h with gentle shaking. The mixtures were observed against a dark background after illuminating the samples with white light. To such isotropic solutions a further 5% water was added and vortexed for 5 min, followed by calibration at 37 °C for 24 h with gentle shaking. Above procedure was continued until turbidity observed in the sample. The resulting phase diagram permits identifying the coarse emulsion and microemulsion regions [9].

Formulation of clotrimazole microemulsion

Clotrimazole loaded o/w microemulsion was prepared by phase titration method. Surfactant and co-surfactant were mixed in fixed ratio and added into the water drop wise. The drug was dissolved in oil phase and added drop wise in the above solution with continuous stirring. Allowed the solution to form clear and transparent liquid, which was microemulsion.

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3^2 Full Factorial Design

It is essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y) is measured for each trial [10-11].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

Where,

β_0 = Intercept = Constant

β_1 and β_2 = Co-efficient of X_1 and X_2 variable

β_{12} = Co-efficient of interaction

β_{11}, β_{22} = Co-efficient of quadratic terms = Non linearity

X_1 and X_2 = Variables

A 3^2 full factorial design was employed to study the effect of independent variables, i.e., ratio of surfactant: co-surfactant (X_1) and the ratio of oil: water (X_2) on dependent variables, i.e., %Transmittance (Y_1), viscosity (Y_2) and %cumulative drug release at 12 h (Y_3) (Table 1). Refer (Table 2) for the composition of factorial batches F1 to F9.

Table 1: Coded value of factor in different batches of microemulsion formulations

Batch No.	X1	X2
F1	-1	-1
F2	-1	0
F3	-1	1
F4	0	-1
F5	0	0
F6	0	1
F7	1	-1
F8	1	0
F9	1	1
FV1*	0	0
FV2*	1	1

Factor and levels for 3^2 factorial design

Variables level	Low (-1)	Medium (0)	High (+1)
Ratio of surfactant: co-surfactant (X1)	2:1	4:3	5:3
Ratio of oil: water (X2)	1:1	1:2	1:3

* Extra check point batch

Table 2: Composition of factorial batches F1 to F9

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Clotrimazole (mg)	100	100	100	100	100	100	100	100	100
Surfactant and Co-surfactant ratio	2:1	2:1	2:1	4:3	4:3	4:3	5:3	5:3	5:3
Oil and water ratio	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3

Characterization of clotrimazole microemulsion:

Determination of %Transmittance of clotrimazole microemulsions

The %Transmittance was checked against distilled water using UV-visible spectrophotometer at λmax 630 nm [12].

%T = Antilog (2 - Absorbance)

Measurement of viscosity of clotrimazole microemulsions

The viscosities of microemulsions were measured using a Brookfield Viscometer LVDV-E with spindle 63 at 10, 20, 30, 50 and 100 rpm at room temperature [12-13].

In vitro permeability studies of clotrimazole microemulsions

In vitro study was carried out using cellophane membrane. The cellophane membrane was activated in glycerin for 24 h. The activated cellophane membrane was mounted on the Franz diffusion cell. The cellophane membrane was placed on receiver chamber and donor chamber was clamped in place. The receiver chamber was filled with 30 ml of phosphate buffer pH 7.4 as diffusion medium. The whole assembly was put on a magnetic stirrer. 1 gm of microemulsion was put on cellophane membrane and started stirring. Samples were withdrawn from the receiver solution at predetermined time intervals, and the cell was replenished to their marked volumes with fresh buffer solution. The addition of the solution to receiver compartment was done with great care to escape air trapping. The samples were filtered and %drug release was calculated by taking absorbance at λmax 261 nm [12-15].

Contour plot and surface plot of design

The optimization of formulation was carried out by plotting contour plots (3-D) and surface plots (2-D) for all observed dependent variables. These plots are useful in the study of the effects of two factors on response at a time.

Formulation of clotrimazole microemulsion based hydrogel

Prepared the solution of polymer (Carbopol 934) and kept for 4 hours to swell it. Add microemulsion drop wise into the gelling solution with continuous stirring on a magnetic stirrer. At last adjust the pH of the formulation with the addition of Triethanolamine as a neutralizing agent.

Characterization of clotrimazole microemulsion based hydrogel

Determination of physical parameter of clotrimazole microemulsion based hydrogel

The gel formulations were inspected for visual color, homogeneity, consistency, texture and feel upon application such as grittiness, greasiness, stickiness and smoothness characteristics [15].

Determination of spreadability of clotrimazole microemulsion based hydrogel

It indicates the degree of area to which gel freely spreads on application to the skin. The therapeutic potency of a formulation also relays on its spreading value. Spreadability is stated in terms of time

in sec occupied by two slides to slip off from gel which is placed in between the slides under the direction of certain load. Lesser the time taken for the separation of two slides, better the spreadability [15]. It is calculated by using the following formula,

$$\text{Spreadability (S)} = \text{ML/T g} \times \text{c/s}$$

Where,

M = Weight tide to upper slide, g

L = Length moved on a glass slide, c

T = Time taken to separate the slide completely from each other, s

Determination of drug content of clotrimazole microemulsion based hydrogel

For drug content determination, about 1 g of microemulsion based hydrogel was weighed in a 10 ml volumetric flask and dissolved it in methanol and diluted properly. Methanol was taken as blank and analyzed spectrophotometrically at λmax 261 nm.

Measurement of viscosity of clotrimazole microemulsions based hydrogel

The viscosities of microemulsions were measured using a Brookfield Viscometer (Model LVDV-E) with spindle 63 at 10, 20, 30, 50 and 100 rpm at room temperature.

In vitro permeability study of clotrimazole microemulsion based hydrogel

In vitro study was carried out using cellophane membrane. The cellophane membrane was activated in glycerine for 24 h. This cellophane membrane was mounted on Franz diffusion cell using fevi-quick glue at the edge of the donor compartment to escape leakage of the test sample. The cellophane membrane was placed on the receiver chamber and the donor chamber was clamped in place. The receiver chamber was filled with 30 ml of phosphate buffer pH 7.4 as diffusion medium. The whole assembly was put on a magnetic stirrer. 1 gm of microemulsion based hydrogel was put on the cellophane membrane and stirring was started with note down of time. Samples were withdrawn from the receiver solution at predetermined time intervals, and the cell was replenished to their marked volumes with fresh buffer solution. The addition of the solution to receiver compartment was done with great care to escape air trapping. The samples were filtered and %drug release was calculated by taking absorbance at λmax 261 nm [15-19].

Ex vivo permeability study of microemulsion based hydrogel

Ex vivo study was carried out by using rat skin that was mounted on Franz diffusion cell using fevi-quick glue at the edge of the donor compartment to escape leakage of the test sample. The rat skin was placed on the receiver chamber and the donor chamber was clamped in place. The receiver chamber was filled with 30 ml of phosphate buffer pH 7.4 as diffusion medium. The whole assembly was put on a magnetic stirrer. 1 gm of microemulsion based hydrogel was put on the rat skin and stirring was started with note down of time. Samples were withdrawn from the receiver solution at predetermined time intervals, and the cell was replenished to their marked volumes with fresh buffer solution. The addition of the solution to receiver compartment was done with great care to escape air trapping. The samples were filtered and %drug release was calculated by taking absorbance at λmax 261 nm [15-19].

Flux and permeability co-efficient

The flux (mg cm^{-2} min^{-1}) of clotrimazole was calculated from the slope of the plot of the cumulative amount of clotrimazole permeated per cm^2 of skin at steady state against the time using linear regression analysis [20].

The steady state permeability coefficient (Kp) of the drug through rat epidermis was calculated by using the following equation:

$$Kp = \frac{J}{C}$$

Where,

J = Flux,

C = Concentration of Clotrimazole, cm/hr

Drug release kinetics

The drug release kinetic study was performed to find drug release mechanism from dissolution parameter by using various kinetic model equations. The zero-order, first-order, Hixon Crowell, Korsmeyer Peppas and Higuchi Plot models were tested [21-24].

Skin irritation study

The Male Wistar rats (150-200 gm) were divided into three groups of six rats in each group. The healthy albino rats were selected and weighed. The rats were anesthetic by diethyl ether. The dorsal surfaces of Wistar rats were cleaned and hairs were removed by shaving. Approximately 2 cm^2 area of the dorsal portion of all the rats were shaved and wiped with surgical spirit. The formulations were applied over the sites on dorsal surface of Wistar rats than after 48 h test sites were examined by using a scoring system as per coding system [2, 25-26]. Refer (Table 3) for Protocol of the study and (Table 4) for the standard skin irritancy score.

Table 3: Protocol of the study

Groups	Route of drug administration	No. of Animals
Group-I: Control (Topical hydrogel without drug)	Topical	6
Group-II: Standard Marketed conventional Clotrimazole gel	Topical	6
Group-III: Optimized Clotrimazole microemulsion based Hydrogel	Topical	6

Table 4: Standard skin irritancy score

Skin reaction	Standard score
(A) Erythema and Eschar formation	
Very slightly erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema	4
Total possible erythema score (A)	**4**
(B)Edema formation	
Very slight edema	1
Slight edema	2
Moderate edema	3
Severe edema	4
Total possible edema score (B)	**4**
Total possible score for primary skin irritation (A+B)	**8**

Antifungal activity

Antifungal activity of the formulation was checked by cup-plate method. A certain volume of Candida albicans suspension was poured into sterilized dextrose agar media (cooled at 40ºC) and mixed systematically. About 20 ml of this suspension was poured aseptically in a petri dish and kept till the solidification. The surface of agar plates was pierced by using a sterile cork borer. The prepare wells were filled with equal volume of the optimized batch of microemulsion based hydrogel and marketed hydrogel (1% clotrimazole gel) after that it was incubated at 18-24 ºC, for 72 h. Fungal growth was detected and the zone of inhibitions was measured using antibiotic zone reader [15].

Accelerated stability studies

Accelerated stability studies were carried out for optimized microemulsion formulation (F9) and optimized microemulsion based hydrogel formulation at 40 °C ± 2 °C/75 % RH ± 5 % RH. The samples were tested after 3 months. Microemulsion formulation (F9) was evaluated by %Transmittance, viscosity and % CDR at 12 h of formulation. Optimized microemulsion based hydrogel was evaluated by transparency, %drug content, viscosity, pH and stability [15].

Results and Discussion

Screening of oils, surfactants and co-surfactants

The higher solubility of the clotrimazole in the oil phase is important because clotrimazole is poorly water soluble drug. Clotrimazole solubility in the various oils such as oleic acid, lemon oil, olive oil and mentha oil were tested. Amongst the oils tested, the maximum solubility of clotrimazole was found in the mentha oil. Mentha oil itself has an analgesic and cooling sensing properties. So, mentha oil was selected as oil phase for the formulation of microemulsion of clotrimazole [25].

Clotrimazole shown maximum solubility in tween 80. Therefore, tween 80 was selected as the surfactant for clotrimazole microemulsion formulation.

Clotrimazole shown maximum solubility in propylene glycol among other co-surfactant such as IPA and ethanol. Propylene glycol also has the good permeation ability through the skin. Therefore, propylene glycol was selected as the co-surfactant (Figure 1).

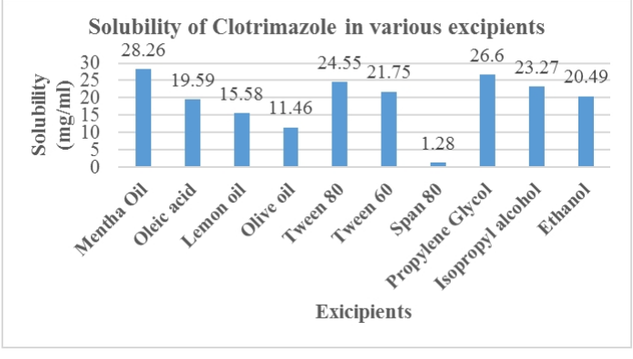


Figure 1: Solubility of clotrimazole in various oils, surfactants and co-surfactants

Construction of pseudoternary phase diagram

It was found that formulation F1, F6, F7, F8, F11, F15, F16, F17, F18, F19, F21, F22 and F23 shown clear yellow colour microemulsion formulation whereas Formulation F2, F3, F9, F10, F13 and F14 shown clear Pale yellow colour microemulsion formulation whereas Formulation F20 and F24 shown clear dark yellow colour microemulsion formulation whereas Formulation F4 and F12 shown slightly clear and separated microemulsion formulation (Figure 2 a-c).

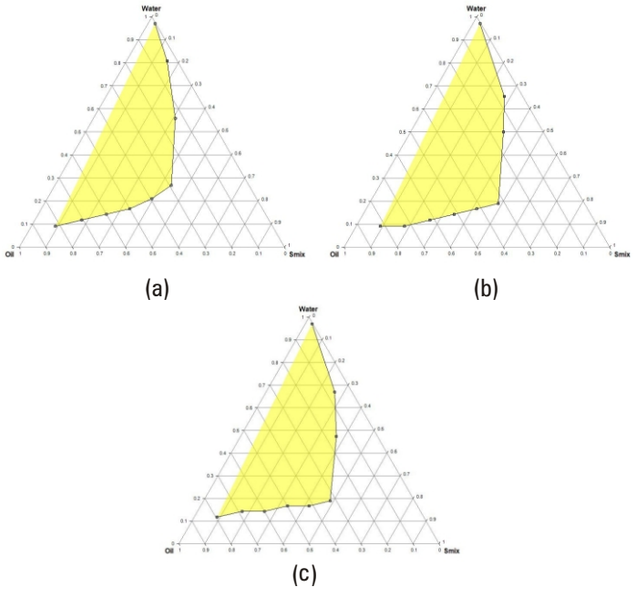


Figure 2: Pseudoternary phase diagram with (a) ratio of surfactant: co-surfactant = 2:1 and oil: water = 1:3, (b) ratio of surfactant: co-surfactant = 4:3 and oil: water = 1:1, (c) ratio of surfactant: co-surfactant = 5:3 and oil: water = 1:3

It was found that formulation F1, F7, F8, F11, F15, F17, F18, F19, F22 and F23 were contained oil: water ratio 1:1, 1:2 and 1:3 shown unstable formulation.

The results of phase diagram revealed that formulation F6, F16 and F21 have shown clear and stable microemulsion formulation. Formulation F6, F16 and F21 contain surfactant: co-surfactant ratio 2:1, 4:3 and 5:3 respectively with 1:1, 1:2 and 1:3 oil: water ratio [27-29].

Experimental design

3^2 full factorial design has been applied to optimize the formulation variables with the basic requirement of understanding the interaction of independent variables. The pseudoternary phase diagram shown that, the factors like the ratio of surfactant: co-surfactant (X_1) and the ratio of oil: water (X_2) showed significant influence on %Transmittance (Y_1), viscosity (Y_2) and % cumulative drug release at 12 h (Y_3) of clotrimazole microemulsion formulations. Therefore, they were utilized for further systematic studies. For all 9 batches, both the selected dependent variables (X_1 and X_2) showed a wide variation in %Transmittance, viscosity and % cumulative drug release at 12 h. The data clearly indicated strong influence of X_1 and X_2 on selected responses (Y_1, Y_2 and Y_3). The polynomial equations can be used to draw conclusions after considering the magnitude of co-efficient and mathematical sign it conveys either positive or negative.

Characterization of clotrimazole microemulsion formulations

Determination of %Transmittance of clotrimazole microemulsions

%Transmittance of clotrimazole microemulsions F1-F9 was found to be 92.5 ± 0.26, 93.1 ± 0.21, 93.5 ± 0.15, 93.9 ± 0.18, 94.4 ± 0.29, 95.1 ± 0.11, 95.9 ± 0.23, 96.8 ± 0.09 and 97.5 ± 0.22, respectively. The clarity of microemulsions was checked by transparency, measured in terms of transmittance (%T). Formulation F7, F8 and F9 have %Transmittance values greater than 95.5% indicate the high clarity of microemulsion formulations.

Measurement of viscosity of clotrimazole microemulsions

Results of viscosity of various batches shown that formulation F1 has

high viscosity and formulation F9 has less viscosity as compared to other formulations. As the ratio of oil:water as well as surfactant: co-surfactant increases, the viscosity of clotrimazole microemulsion formulations was decreases (Figure 3).

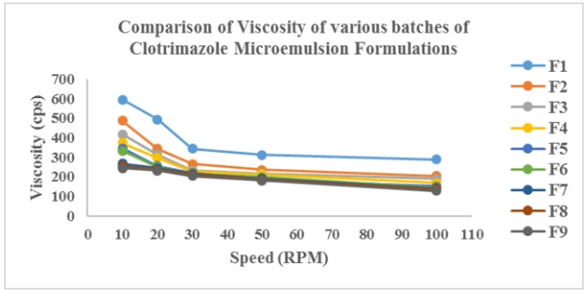


Figure 3: Comparison of viscosity of various batches of clotrimazole microemulsion formulations at room temperature

A viscosity value plays an important role in controlling the release of the drug into the receptor compartment. The comparative graph of viscosity of various batches signifies that the all prepared microemulsion formulations exhibited Newtonian flow.

In vitro permeability studies of clotrimazole microemulsions

%CDR increases with an increase in the ratio of oil: water as well as the ratio of surfactant: co-surfactant, as compared to batch F1-F9 (Figure 4). %Cumulative drug release data and graph indicate that formulation F1 has a less permeation rate where formulation F9 has high permeation rate. Formulation F9 shows the maximum permeation of clotrimazole after 12 hours with compared to other formulations [30].

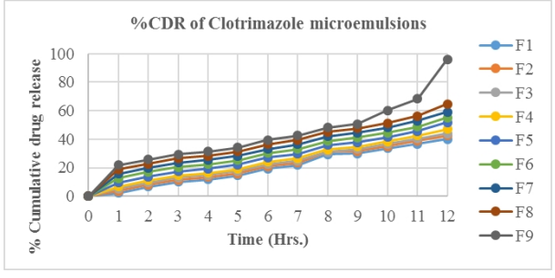
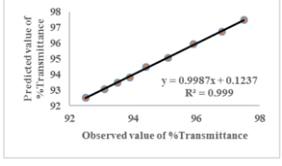


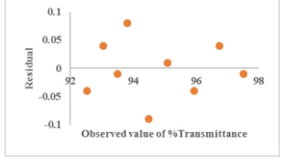
Figure 4: % Cumulative drug release of microemulsion formulation (F1-F9)

Statistical analysis

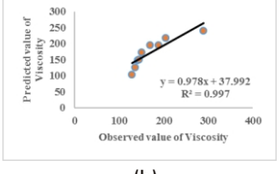
Statistical analysis was done using Microsoft Excel 2013. Linear correlation plot between observed and predicted value of viscosity, %Transmittance and %CDR at 12 h are shown in Figure 5 (a-f).



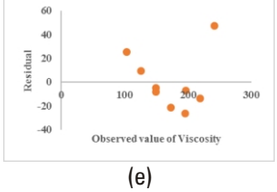
(a)



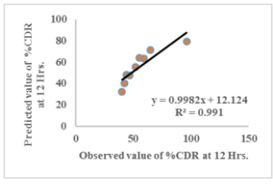
(d)



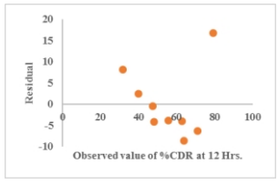
(b)



(e)



(c)



(f)

Figure 5: Linear co-relation plot of (a) %Transmittance (b) Viscosity and (c) %CDR at 12 h. & Residual plot of (d) %Transmittance (e) Viscosity and (f) %CDR at 12 h

The linear correlation plots drawn between predicted and observed responses show values of R^2 are 0.999, 0.997 and 0.991 for %Transmittance, viscosity and %CDR at 12 h. The corresponding residual plots show nearly uniform and random scatter around the mean values of response variables.

Factorial equation for %Transmittance

The %Transmittance of F1-F9 batches varied from 92.5 to 97.5 and showed correlation coefficient 0.999. Negative sign in the regression equation indicates the response value decreases as the amount of factors increases. Positive sign in the regression equation indicates the response value increases as the amount of factors increases. The P value for variable X_1 and were <0.0001 and 0.0003 respectively ($P<0.05$), it indicates that X_1 and X_2 have the prominent effect on the %Transmittance.

$$\%\text{Transmittance} = 94.49 + 1.85\,X_1 + 0.63\,X_2 + 0.15\,X1X_2 + 0.42\,X_{12} - 0.33\,X_{22}$$

In the present study, coefficients b_1 and b_2 possessed positive sign indicating the synergistic effect of variables, X_1 and X_2, on response Y_1 (%Transmittance). Both the independent variables, X_1 (Ratio of surfactant: co-surfactant) and X_2 (Ratio of oil: water) has a prominent effect ($b_1 = 1.85$ and $p = <0.0001$) ($b_2 = 0.63$ and $p = 0.0003$) over all the three independent variables. The significance, F value is less than 0.05. Higher values of the coefficient of determination indicate a good fit i.e., good agreement between the dependent and independent variables. The coefficients b_1 and b_2 were found to be significant at $p < 0.05$.

Factorial equation for viscosity

Observed and predicted values for viscosity studies (Y_2) for all the 9 batches are shown in figure 5 (b & e). R^2 value in the plot of predicted v/s observed responses was 0.997 which indicated excellent goodness of fit. The Y_2 (viscosity) values observed for different batches showed wide variation i.e., values ranged from 129 to 289.

$$\text{Viscosity} = 172.67 - 46.17X_1 - 22.67\,X_2$$

Coefficient b_1 and b_2 possessed negative sign, which indicated a negative effect of X_1 and X_2 variable on response Y_2 (viscosity). Independent variables, X_1 (Ratio of surfactant: co-surfactant) ($b_1 = -46.17$ and $p = 0.0061$) and X_2 (Ratio of oil: water) ($b_2 = -22.67$ and $p = 0.0884$) have a negative effect on viscosity. The significance, F value is reported in Table 5.

Table 5: ANOVA for dependent variables for all batches (F1-F9)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Significance F
For Y_1 = %Transmittance (F1-F9)					
Regression	23.38	5	4.68	664.52	< 0.0001
Residual	0.021	3	7.037	-	-
Total	23.40	8	-	-	-

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Significance F
For Y_2 = Viscosity (F1-F9)					
Regression	15870.83	2	7935.42	10.63	0.0107
Residual	4477.17	6	746.19	-	-
Total	20348	8	-	-	-
For Y_3 = %CDR at 12 h (F1-F9)					
Regression	1847.74	2	923.87	10.77	0.0104
Residual	514.92	6	85.82	-	-
Total	2362.67	8	-	-	-

The viscosity of F1-F9 batches varied from 129 to 289 and showed correlation coefficient 0.997. Negative sign in the regression equation indicates the response value decreases as the amount of factors increases. The P value for variable X_1 and X_2 were 0.0061 and 0.0884 respectively.

Factorial equation for %CDR at 12 h

There was not much difference between actual and predicted values for all 9 batches figure 5 (c & f). R^2 value in the plot of predicted v/s observed responses was 0.991 which indicated excellent goodness of fit. Y_3 (% CDR at 12 h) value observed for different batches showed wide variation. The response (Y_3) obtained at three levels of the two independent variables (X_1 and X_2) were subjected to multiple regression to yield a polynomial equation. Equation clearly reflects the wide range of values for coefficients (b).

$$\%CDR \text{ at } 12 \text{ h} = 55.62 + 15.53\ X_1 + 8.17\ X_2$$

The %CDR at 12 h of F1-F9 batches varied from 40.12 to 96.03 and showed correlation coefficient 0.991. Positive sign in the regression equation indicates the response value increases as the amount of factors increases. The P value for variable X_1 and X_2 were 0.0063 and 0.0740.

Results of Analysis of variance (ANOVA)

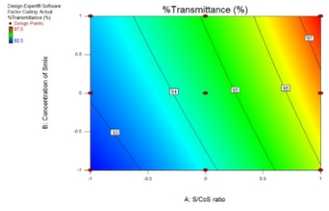
ANOVA was done using Microsoft Excel. Results of ANOVA for %Transmittance, viscosity and %CDR at 12 h are shown in Table 5.

From ANOVA results, both variables show significant F value, less than 0.05. It indicates that all model terms are significant.

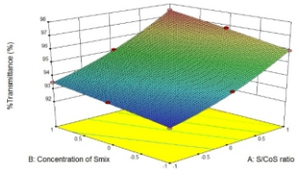
Contour plot and Response surface plot

Contour plot and Response surface plot were drawn using Design expert software version 9.0 (Stat-Ease, Inc., USA).

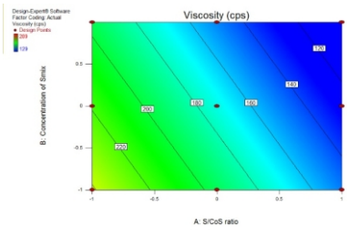
Two-dimensional contour plots and three-dimensional response surface plots are presented in Figures 6 (a-f), which are a useful tool to study interaction effects of the factors on responses. Figure 6 (a-f) shows perfect linear relationship between factor X_1 and X_2 in the form of straight lines in contour plot.



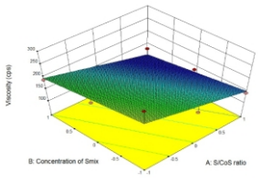
(a)



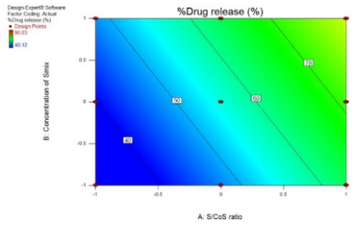
(d)



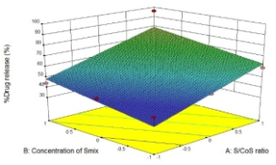
(b)



(e)



(c)



(f)

Figure 6: Contour plot of (a) %Transmittance Y_1 (b) Viscosity Y_2 and (c) %CDR at 12 h. Y_3; with X_1 and X_2 & 3D response plot of (d) % Transmittance Y_1 (e) Viscosity Y_2 and (f) %CDR at 12 h. Y_3; with X_1 and X_2

Validation of experimental design

Polynomial equations were generated using Design expert software version 9.0 (Stat-Ease, Inc., USA) for selected responses like %Transmittance, viscosity and %CDR at 12 h. The generated polynomial equations were further reduced on the basis of significant terms obtained by applying ANOVA. The 3^2 full factorial design was validated by preparing two extra check point formulation (FV1 & FV2). The predicted values for %Transmittance, viscosity and %CDR at 12 h for FV1 & FV2 were determined on the basis of respective polynomial equations while the experimental values were determined by evaluating FV1 & FV2 for the selected dependent variables (Table 6).

Table 6: Regression data for check point validation batch

Composition	Response variable	Experimental value	Predicted value
Batch FV1:	%Transmittance	94.21	94.49
X_1 = 4:3	Viscosity	154.48	172.67
X_2 = 1:2	%CDR at 12 h	53.5	55.62
Batch FV2:	%Transmittance	96.9	97.50
X_1 = 5:3	Viscosity	125.31	103.83
X_2 = 1:3	%CDR at 12 h	94.91	79.32

Selection of optimized formulation

Optimized formulation was selected on the basis of maximum %Transmittance, viscosity and %CDR at 12 h with good desirability (Figure 7). The desirability value of optimized formulation was found to be 0.910. The overlay plot of optimized formulation shown %Transmittance (95.999), viscosity (130.66) and %CDR at 12 h (70.297). The value of X_1 & X_2 of optimized formulation was 0.419 & 0.999, respectively.

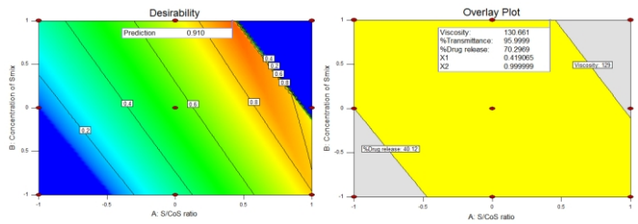


Figure 7: Desirability graph and overlay plot of optimized formulation

Characterization of clotrimazole microemulsion based hydrogel

Measurement of pH, spreadability and drug content of clotrimazole microemulsion based hydrogel

The prepared clotrimazole microemulsion based hydrogel is transparent and yellowish colour with a pleasant odour and smooth texture. It gives a cooling sensation when applied on the skin. The pH, spreadability and drug content was found to be 7.2 ± 0.10, 7.82 ± 0.10 gxc/s and 92.5%, respectively.

Measurement of viscosity of clotrimazole Microemulsion based hydrogel

Viscosity of clotrimazole microemulsion based hydrogel was increasing as the speed increases (Figure 8).

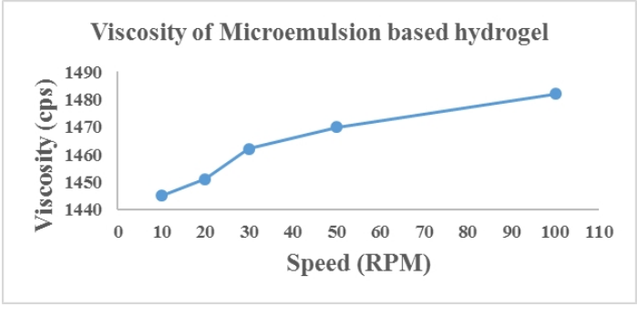


Figure 8: Viscosity graph of microemulsion based hydrogel at room temperature

Comparative *in vitro* and ex vivo **permeability of clotrimazole microemulsion based hydrogel**

Refer Figure 9 to Figure 11 for comparative *in vitro* and *ex vivo* permeability of clotrimazole microemulsion based hydrogel.

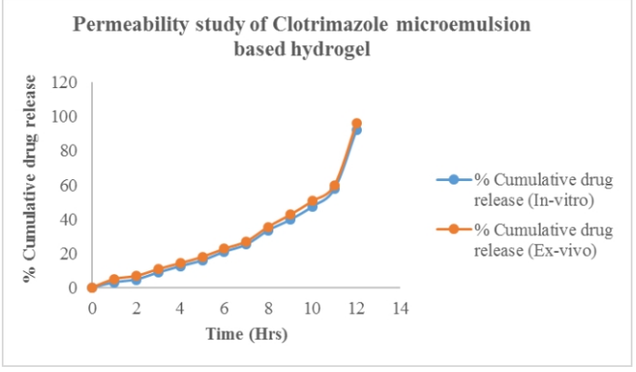


Figure 9: Comparative permeability of clotrimazole microemulsion based hydrogel

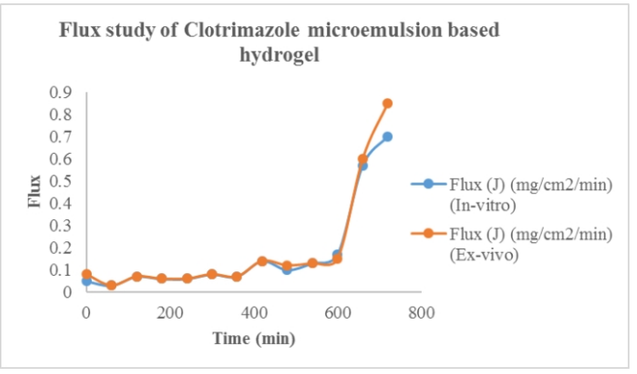


Figure 10: Flux study of clotrimazole microemulsion based hydrogel

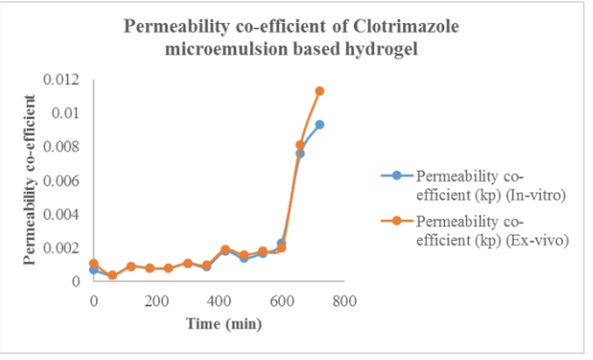


Figure 11: Permeability co-efficient of clotrimazole microemulsion based hydrogel

Drug release kinetics study

Refer Table 7 for drug release kinetics study. Microemulsion based hydrogel formulation serves as efficient promoters of the clotrimazole localization into the skin.

It was observed that the permeation rate of the drug from microemulsion based hydrogel (*in vitro*) optimized formulation (92.04%) was lower than its microemulsion based hydrogel (*ex vivo*) optimized formulation (96.12%). This might be due to the partitioning of the drug into the oil phase of microemulsion based hydrogel which decrease the steady state of drug release.

Table 7: Drug release kinetics study

Kinetic models		Zero Order	First Order	Hixon Crowell	Corse Mayer Pappas	Higuchi Plot
In vitro study	R^2 Value	0.928	0.965	0.986	-0.229	0.831
	Slope	0.104	0.002	0.011	1.338	0.247
	Intercept	-9.813	0.370	0.626	-4.522	10.568
Ex vivo study	R^2 Value	0.929	0.948	0.981	-0.269	0.836
	Slope	0.108	0.002	0.011	1.158	0.241
	Intercept	-8.798	0.482	0.918	-4.008	10.207

The bioavailability of drugs penetrating the skin can be enhanced by using microemulsion systems because the small droplet size ensures

close contact with the stratum corneum. Small droplets have better chances to adhere to the skin and transport the drugs in a more controlled Fashion.

Clotrimazole can be released from the oil phase to water phase than water phase to the skin, relative activity may monitor the skin permeation Flux.

From the results of *in vitro* and *ex vivo* permeation study, it was found that the microemulsion based hydrogel formulation of clotrimazole had shown maximum drug retention into skin as compared to a microemulsion formulation of clotrimazole.

The addition of carbopol 934 in the microemulsion formulation increased the viscosity of the formulation and transform from microemulsion to lamellar structure or a highly ordered microstructure and delay drug release.

After 12 hours (72 min) Flux value and permeability co-efficient of in vitro study was 0.70 mg/cm^2/min and 0.0093 and flux value and permeability co-efficient of ex vivo study was 0.85 mg/cm^2/min and 0.0113.

Result of kinetic models indicates that the drug release follows Hixon Crowell kinetic mechanism.

Skin irritation study of clotrimazole microemulsion based hydrogel

Refer Figure 12 (a) for skin irritation study on rat at 24 hours & Figure 12 (b) for skin irritation study on rat at 48 hours.

Refer table 8 for response score of skin irritation.

Table 8: Response score of skin irritation

Groups		Score for Erythema		
		After 4 hr	After 24 hr	After 48 hr
Control (Topical hydrogel without drug)	1	0	0	0
	2	0	0	0
	3	0	0	0
Standard marketed conventional Clotrimazole Gel	1	0	0	0
	2	0	0	0
	3	0	0	0
Optimized Clotrimazole microemulsion based hydrogel	1	0	0	0
	2	0	0	0
	3	0	0	0
		Score for Edema		
Control (Topical hydrogel without drug)	1	0	0	0
	2	0	0	0
	3	0	0	0
Standard marketed conventional Clotrimazole Gel	1	0	0	0
	2	0	0	0
	3	0	0	0
Optimized Clotrimazole microemulsion based hydrogel	1	0	0	0
	2	0	0	0
	3	0	0	0

Scoring system: For no erythema or edema 0, very slight erythema or edema 1, slightly erythema or edema 2, moderate erythema or edema 3 and severe erythema or edema 4.

Skin irritation study results show that there was no irritation on skin (Figure 12 a-b).

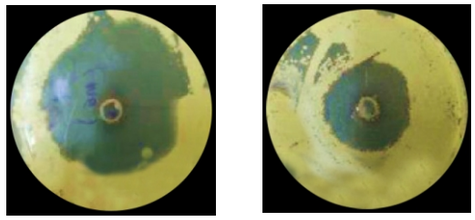
(a) (b)

Figure 12: Skin irritation study on rat at (a) 24 hours & (b) 48 hours

Microemulsion based hydrogel formulation increased the viscosity of microemulsion and formed network structure and decreased the contact chances between skin and microemulsion. Thus, very less chances of irritation by microemulsion based hydrogel applied on surface of the skin as a topical formulation [24, 26].

Antifungal activity

The values of mean zone of inhibition (in vitro antifungal activity) of optimum microemulsion based hydrogel batch and marketed formulation (Figure 13 a-b).



(a) (b)

Figure 13: Zone of inhibition of (a) optimized batch & (b) marketed product

Hence the optimized formulation had highest zone of inhibition (4.4 cm) as compared to marketed product (2.3 cm). So, it is clearly indicated that the optimized formulation had good antifungal activity compared to the marketed product.

Stability study of the optimized clotrimazole microemulsion formulation and optimized clotrimazole microemulsion based hydrogel before and after stability (3 months)

%Transmittance of clotrimazole microemulsion before and after stability was found to be 99.1 ± 0.26 & 99.5 ± 0.28, respectively. The viscosity was found to be 129 ± 4.29 cps & 129 ± 4.20 cps, respectively. %CDR was found to be 96.03% & 96.10% at 12 h, respectively.

Clotrimazole microemulsion based hydrogel was transparent and clear before and after stability. % Drug content was found to be 92.5% & 92.7%, respectively. pH was found to be7.2 ± 0.10 & 7.2 ± 0.25, respectively. The viscosity was found to be 1482 ± 7.18 cps & 1483 ± 7.37 cps, respectively.

Results of stability study revealed that clotrimazole microemulsion F9 and optimized clotrimazole microemulsion based hydrogel formulation were satisfactorily found to be stable for three months.

Conclusion

Enhancement of solubility of clotrimazole was done by microemulsification approach. The results of a 3^2 full factorial design shown that the ratio of surfactant: co-surfactant and ratio of oil: water significantly affected on the dependent variables like %Transmittance (Y_1), viscosity (Y_2) and %CDR at 12 h (Y_3). The optimized formulation F9 is shown in vitro drug release up to 12 h. Optimized microemulsion based hydrogel formulation had good antifungal activity compared to the marketed product. Stability study of optimized batch shown that

there was a negligible change in pH, viscosity, transparency and drug content after 3 months.

Conflicts of interest

The authors declare no conflict of interest.

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