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Research Article

# Statistical Optimization of Miconazole Nitrate Microemulgel by using $2^3$ Full Factorial Design

**Nishant A. Oza[1*], Ami Makwana[1], Tushar A. Gohil[2], Prashakha Shukla[3]**

[1]*C. U. Shah College of Pharmacy & Research, C. U Shah University, Gujarat, India*
[2]*Smt. C.V. Gajera Pharmacy Mahila College, Amreli, Gujarat, India*
[3]*C. U. Shah Institute of Life Science, C. U Shah University, Gujarat, India*

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ABSTRACT

Miconazole nitrate is a broad-spectrum antifungal agent, poorly water-soluble drug, and is used to treat superficial fungal infections. Microemulgel could increase drug deposition over to the skin. Therefore, the aim of this study was to prepare 2% miconazole nitrate transparent micro emulgel that contains 99% w/w liquid. Preliminary screening of oils and polymers was carried out. Oil phase of the microemulgel selected based on maximum solubility of miconazole nitrate in oil. A $2^3$ full factorial design was used to check the effect of cinnamon oil ($X_1$), HPMC K4M ($X_2$) and tween 20 ($X_3$) on viscosity ($Y_1$) and % of cumulative drug release at 6 hours ($Y_2$). Multiple linear regression analysis, ANOVA and graphical representation of the influence factor by 3D response surface plots were performed using Design Expert 7.1.5. A checkpoint batch was prepared to validate the evolved model. Optimized batch $F_5$ was found to be stable and it showed globule size 26 ± 3 µm. In *Ex-vivo* drug permeation study batch, $F_5$ was shown 87.05 ± 2.42% drug released after 6 hours and drug deposition on the upper layer of skin was found 52.5%. Accelerated stability study showed no significant change in the micro emulgel parameters within three months, and similarity factor f2 was 91.05 ± 1.67 %. Therefore, 2% miconazole nitrate micro emulgel was providing an effective treatment against topical infections.

## Introduction

Topical preparations like cream and ointment have numerous boundaries such as less spreading coefficient, less penetration through stratum corneum, less patient compliance due to stickiness, or need to apply with rubbing. Gels contain a number of favorable properties such as, a thixotropic, greaseless, spread without difficulty, easily removable, emollient, non-staining, long shelf life, bio-friendly, transparent and pleasing appearance. Despite many advantages of gels, a major limitation is in the delivery of hydrophobic drugs. Therefore, to overcome this limitation, microemulgel are prepared and used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels.[1,2]

Miconazole nitrate is a broad-spectrum antifungal agent and it is used for the treatment of fungal infections. It has poor water solubility and BCS class II drugs; hence, such drugs pose problems in a gel formulation. Therefore, miconazole nitrate was soluble in the oil phase and developed O/W microemulgel. It could also increase drug deposition over to the skin. In the development of microemulgel, an important issue is to design an optimized formulation with an appropriate viscosity and drug release.[3,4] The present study was aimed to prepare 2% miconazole nitrate stable microemulgel by decreasing interfacial tension and at the same time increasing the viscosity of the aqueous phase. A $2^3$ full factorial design was to study the effect of cinnamon oil ($X_1$), HPMC K4M

***Corresponding Author:** Dr. Nishant A. Oza

**Address:** C. U. Shah College of Pharmacy & Research, Wadhwan City-363030, Gujarat, India

**Email** ✉**:** ozanishant@gmail.com

**Tel.:** +91-9427050570

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($X_2$) and tween 20 ($X_3$) on viscosity ($Y_1$) and % cumulative drug release at 6 hours ($Y_2$).

## Materials and Methods

### Materials

Mepro Pharmaceutical Ltd., Wadhwan, India, kindly supplied Miconazole nitrate as gift samples. Cholesterol and triethanolamine were supplied as gift samples from Finar Chemicals, Ahmadabad, India. Tween 20 was purchased from Astron Chemicals, Ahmedabad, India. HPMC K4M was purchased from Colorcon Asia Pvt. Ltd. Goa, India. All other chemicals used in this study were of analytical grade and were used without further purification.

### Drug-Excipients Compatibility Study

Drug-excipient interaction plays a vital role in achieving stability of drugs in dosage form. Fourier transform infrared spectroscopy (FTIR) was used to study the physical and chemical interactions between drugs and excipients. FTIR spectra of miconazole nitrate, HPMC K4M, and physical mixture of miconazole nitrate: HPMC K4M was recorded using KBr mixing method on FTIR instrument (FTIR 1700, Shimadzu, Kyoto, Japan). Differential scanning calorimetry (DSC) was also performed for qualitative and quantitative information about the drug's physicochemical state in formulations. DSC analysis of the pure drug and the drug-polymer mixture were performed (DSC TA-60) at a rate of 10°C/min from 50 °C to 350 °C under a nitrogen flow of 20 mL/min.[5,6]

### Preliminary Screening of Oils and Polymer

Miconazole nitrate is a BCS class II drug, which has high lipid solubility and is almost insoluble in water. Therefore, it was dissolved in the oil phase of an emulsion. It has a different solubility in different oils for that purpose screening of various oils is essential for the selection of the proper oil phase. For the oil phase screening, castor oil, peaceol, almond oil, sesame oil, cinnamon oil, and liquid paraffin were used. In this study, 2 mL of oil was taken in the test tube and an excess amount of drug was added into the test tube, and it was kept in a shaker (Remi, Mumbai, India) at 25 ± 0.5 ° C for 24 hours. After 24 hours solution was centrifuged at 5000 rpm for 10 minutes in micro-centrifuge. The supernatant (0.5 mL) was diluted suitably and the amount of miconazole nitrate present in the supernatant was analyzed by UV-spectrophotometer at 272.5 nm. [7,8]. The polymer of gel phase plays an important role in release of drug from gel matrix. The concentration and class of polymer affect the viscosity and release of the drug. For screening of the polymer HPMC K4M, hydroxypropyl cellulose, sodium carboxymethylcellulose, xanthan gum, and carbopol 934 were used. Briefly, in batch T1 to T3 was prepared by dissolving 2% to 3% HPMC K4M in purified water as shown in Table 1. Batch T4 to T6 contained 1% to 3% hydroxypropyl cellulose and batch T7 to T10 formulated with 3% to 6% of sodium carboxymethyl cellulose. Batch T11 and T12 contained 1% and 2% of xanthan gum, respectively and batched T13 and T14 was formulated with 0.5% and 1% of carbopol 934.[9,10]

### Development of 2% Miconazole Nitrate Microemulgel

All the required ingredients of the microemulgel were weighed accurately. The gel phase of the formulation was prepared by dispersing HPMC K4M in purified water with constant stirring at a moderate speed using an in-line high shear homogenization (Megatron MT300, Kinematica AG Switzerland). Cinnamon oil acted as an oil phase of emulsion while the aqueous phase was prepared by dissolving tween 20 and propylene glycol in purified water. Methyl paraben was dissolved in propylene glycol

**Table 1:** Preliminary screening of polymer for 2% miconazole nitrate microemulgel

| *Batch code* | *HPMC K4M* | *Hydroxypropyl cellulose* | *Sodium carboxymethyl cellulose* | *Xanthan gum* | *Carbopol 934* |
|---|---|---|---|---|---|
| T1 | 2 % | - | - | - | - |
| T2 | 2.5 % | - | - | - | - |
| T3 | 3 % | - | - | - | - |
| T4 | - | 1 % | - | - | - |
| T5 | - | 2 % | - | - | - |
| T6 | - | 3 % | - | - | - |
| T7 | - | - | 3 % | - | - |
| T8 | - | - | 4 % | - | - |
| T9 | - | - | 5 % | - | - |
| T10 | - | - | 6 % | - | - |
| T11 | - | - | - | 1% | - |
| T12 | - | - | - | 2 % | - |
| T13 | - | - | - | - | 0.5 % |
| T14 | - | - | - | - | 1 % |

whereas 2% miconazole nitrate was dissolved in cinnamon oil. Both the oil and aqueous phases were separately heated to 70-80 $^0$C, then the oily phase was added to the aqueous phase with continuous stirring until it cooled to room temperature. The obtained emulsion was mixed with the gel phase with gentle stirring to obtain microemulgel.[11,12]

## Evaluation of 2% Miconazole Nitrate Microemulgel

All formulations were inspected visually for their color, homogeneity, consistency, grittiness and phase separation. The pH of the Microemulgel was determined using digital pH meter and viscosity was determined using a Brookfield viscometer. Extrudability was calculated by filling an aluminum tube with 15 gm of gel and consistency of gel was observed on pressing the tube applying mild force. Globule size of micro emulgel was determined by the microscopic method.

### *Drug Content*

To evaluate the miconazole nitrate content in formulation, microemulgel equivalent to 10 mg of drug was dissolved in 100 mL of water. 1 mL of solution was withdrawn and volume was made up to 100 mL. The absorbance was measured after suitable dilution at 272.5 nm against the corresponding blank solution by using UV Spectrophotometer (UV-1700, Shimadzu).[13,14]

### *In-vitro Drug Release*

*In-vitro* drug release study of micro emulgel was carried out in a modified Franz diffusion cell using dialysis cellophane membrane. The membrane was soaked in phosphate buffer pH 7.4 for 12 h and mounted between the donor and receptor compartment. A 1 gm of microemulgel was placed on one side of the dialysis membrane with a diffusion area of 2 cm$^2$ and receptor medium containing 25 mL of phosphate buffer pH 7.4. The temperature of the receptor medium was maintained at 37 ± 0.5°C and the medium was agitated at 100 rpm speed using a magnetic stirrer. Aliquots of 2 mL sample were withdrawn periodically and replaced with equal volume to maintain the volume constant of the receptor's phase. The collected samples were analyzed for the drug containing at 272.5nm absorbance using the UV spectrophotometer.

### *Kinetic Modelling of Dissolution Data*

In order to understand the kinetics and release mechanisms of drugs, the result of *in-vitro* drug release study of micro emulgel was fitted in with various kinetics models like zero order, first order, higuchi model and korsmeyer peppas model. The linearity of the plots was obtained from the value of regression coefficient (R). The model with the highest linearity (R value approaches unity) was chosen as the best-fit kinetic model.[15,16]

### *Skin Irritation Study*

Wistar rats were divided into three groups ($n$ = 3 per group) and were treated once daily over a period upto 7 days as following groups. Group 1 Normal, Group 2 0.8% v/v aqueous Formalin solution and Group 3 2% miconazole nitrate microemulgel. On the 2$^{nd}$ day, the application sites was evaluated visually for erythematic and oedema,

### *Ex-Vivo Drug Permeation Study*

The permeation of the drug from 2% miconazole nitrate micro emulgel was determined by using franz diffusion cell. The Wistar rat skin was mounted on the receptor compartment with stratum corneum side facing upwards into the donor compartment with a diffusion area of 2 cm$^2$. The top of the diffusion cell was covered with paraffin paper. The donor compartment was filled with the microemulgel. The receptor compartment was maintained at 37 ± 0.5 °C and stirred by a magnetic bar at 600 rpm. Aliquots of 2 mL sample were withdrawn periodically and replaced with equal volume to maintain the volume constant of the receptor's phase. The collected samples were analyzed for the drug containing at 272.5nm absorbance using the UV spectrophotometer.

### *Microbial Antifungal Test*

The microbiological activity of the optimized 2% miconazole nitrate micro emulgel was carried out using cup plate technique. *Candida albicans* was used as an indicator strain. The culture medium selected for this purpose is nutrient agar and the final pH of the medium was kept at 5.6 ± 0.2 to retard the growth of unlike organisms. The medium was sterilized using an autoclave at 121 °C for 20 min. Freshly prepared culture was used for inoculum preparation, which was prepared by suspending 1-2 colonies in tubes containing media and 10 mL of 0.9% w/v NaCl solution. The inoculum was spread over the surface of the media after appropriate solidification micro emulgel were applied with the help of borer. The experiment was replicated two times to confirm the reproducible results. Standard 2% miconazole nitrate solution was used as positive control for comparison of the antibacterial activity with 2% miconazole nitrate microemulgel. The complete experiment was carried out in a sterile area. Finally, the plate was incubated at 37 ± 2 °C for 48 h in reverse position. The zone of inhibition was calculated in millimeters of diameter.[17,18]

### *Accelerated Stress Stability Study*

Stability studies were done as per ICH guidelines. The optimized micro emulgel was kept in an amber color glass bottle then placed in an accelerated stability chamber at 40°C ± 5°C temperature and 70% ± 5% RH. After three month, gel was tested for pH, Viscosity, Entrapment efficiency, drug content and *In-vitro* release profile. The dissolution profile of products were compared using a $f_2$ which is calculated from following formula,

$$f_2 = 50 \log \left[ \{1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \}^{-0.5} x100 \right]$$

where log is logarithm to the base 10, n is the number of time points, $\sum$ is summation over all time points, $R_t$ is the mean dissolution value of the reference profile at time t and $T_t$ is the mean dissolution value of the test profile at the same time point. The USFDA draft guidance document contains more information on similarity factor ($f_2$). The value of similarity factor ($f_2$) between 50 and 100 suggests that the two dissolution profiles are similar.

*In-vitro* Skin Deposition Analysis: After the *Ex-vivo* drug permeation study the skin surface was washed with methanol. Then, skin was then cut into small pieces. The tissue was kept in methanol: distilled water (1:1) and left for 6 h at room temperature. After manual shaking for 5 minutes and centrifuging the mixture for 5 minutes at 5000 rpm, the miconazole nitrate content was analyzed at 272.5nm absorbance using the UV spectrophotometer.[19]

### Optimization of Variables using Full Factorial Design

A $2^3$ randomized full factorial design was used in the present study. In this design 3 independent factors were evaluated, each at 2 levels, and experimental trials were performed for all possible combinations. The cinnamon oil ($X_1$), the HPMC K4M ($X_2$), and the tween 20 ($X_3$) were chosen as independent variables in $2^3$ full factorial designs,while viscosity ($Y_1$) and% Cumulative drug release at 6h ($Y_2$), were taken as dependent variable. Multiple linear regression analysis and ANOVA was performed using Design Expert 7.1.5 software to generate statistical relationships between independent variables and 3D Response surface plot were also generated. The formulation layout for the factorial design batches are shown in Table 2.[20,21]

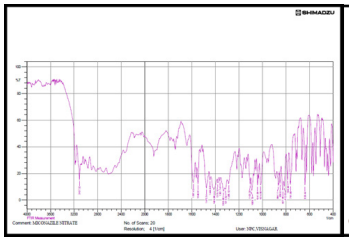

**Fig. 1A:** IR spectra of miconazole nitrate

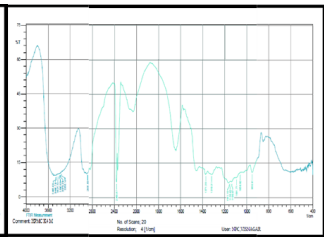

**Fig .1B:** IR spectra of HPMC K4M

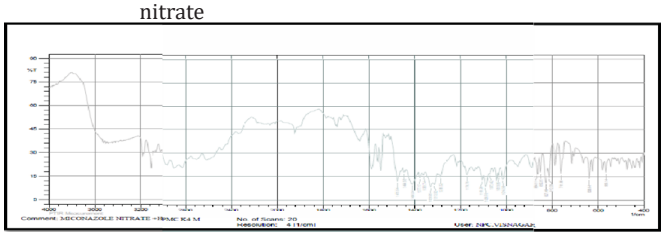

**Fig. 1C:** IR spectra of mixture of miconazole nitrate and span 60

## Result and Discussion

### Drug excipients compatibility study

Fourier transform infrared spectroscopy (FTIR) was used to study the physical and chemical interactions between drug and excipient. FTIR spectra of miconazole nitrate, HPMC K4M and mixture of miconazole nitrate and HPMC K4M (Fig. 1) were recorded using KBr mixing method on FTIR instrument. The drug exhibited peaks due to C-H, C-C, N-H, C-Cl and C=C stretching. It was observed that there were no changes in miconazole nitrate functional groups peaks in the IR spectra of the HPMC K4M and miconazole nitrate mixture. The thermograph of pure miconazole nitrate showed an endothermic melting peak at 186.93 °C. In the thermograph of the HPMC K4M and miconazole nitrate peak, mixture was observed at 184.07°C (Fig. 2). DSC study shows that there is no change in drug melting peak after the mixing of miconazole nitrate and HPMC K4M. Therefore, we can conclude that drugs and excipients are compatible with each other.[22]

### Preliminary Screening of Oils and Polymer

The calibration curve of miconazole nitrate was developed in phosphate buffer pH 7.4 containing 0.3% sodium lauryl sulphate at 272.5 nm wavelength. Miconazole nitrate

**Table 2:** Runs and measured responses of $2^3$ factorial design batches

| *Batch Code* | *%Cinnamon oil ($X_1$)* | *% HPMC K4M ($X_2$)* | *% Tween 20 ($X_3$)* | *Viscosity* | *% Cumulative drug release at 6 h ($Q_6$ %)* |
|---|---|---|---|---|---|
| $F_1$ | -1 | -1 | -1 | 1460 ± 10 | 90.84 ± 1.03 |
| $F_2$ | +1 | -1 | -1 | 1585 ± 08 | 85.55 ± 1.23 |
| $F_3$ | -1 | +1 | -1 | 1733 ± 09 | 82.20 ± 0.89 |
| $F_4$ | +1 | +1 | -1 | 1810 ± 03 | 74.69 ± 1.84 |
| $F_5$ | -1 | -1 | +1 | 1535 ± 06 | 94.04 ± 0.92 |
| $F_6$ | +1 | -1 | +1 | 1660 ± 08 | 87.80 ± 0.84 |
| $F_7$ | -1 | +1 | +1 | 1752 ± 11 | 84.97 ± 0.29 |
| $F_8$ | +1 | +1 | +1 | 1848 ± 10 | 77.25 ± 1.11 |

*Factors and the levels in the design*

| *Independent variables* | *% Cinnamon oil ($X_1$)* | *% HPMC K4M ($X_2$)* | *% Tween 20 ($X_3$)* |
|---|---|---|---|
| Low (-1) | 1.5 | 2.0 | 0.5 |
| High (+1) | 2.0 | 2.5 | 1.0 |

(n=6)

solubility in castor oil and peaceol was found 5.015 mg/mL and 8.184 mg/mL. Miconazole nitrate solubility in almond oil, sesame oil, cinnamon oil, and liquid paraffin was 11.67 mg/mL, 10.49 mg/mL, 19.5 mg/mL, and 12.33 mg/mL respectively. It was shown higher solubility in cinnamon oil. So, cinnamon oil was selected as oil phase of an emulsion.

In the preliminary screening of polymer, gel formulations have to be translucent color and have a smooth feel on an application that remained the same on the stability testing period. In this study batch T1 to T3 was found stable for a longer time, which contained HPMC K4M. Batch T4 to T6 failed to formulate the microemulgel that was containing hydroxypropyl cellulose. Batch T7 to T10 formulation was broken down, which was containing sodium carboxymethyl cellulose. Batch T11 and T12 microemulgel was formed but not stable for a longer time, containing xanthan gum. T13 and T14 was composes the microemulgel, but it was very thick and not uniform in nature. Therefore, HPMCK4M was selected as a gel phase of miconazole nitrate micro emulgel formulation.

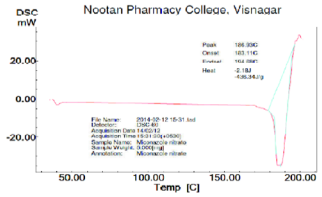


**Fig 2A:** DSC spectrum of miconazole nitrate

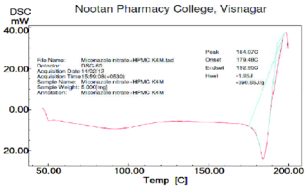


**Fig 2B:** DSC spectrum of miconazole nitrate and HPMC K4M.

## Evaluation of the Factorial Batches of 2% Miconazole Nitrate Microemulgel

A $2^3$ randomized full factorial design was used in the present study. In this design, 3 factors were evaluated, each at 2 levels, and experimental trials was performed for all 8 possible combinations. Evaluation data for factorial batches are shown in Table 3. All factorial batches of micro emulgel were white viscous creamy preparation with a smooth homogeneous texture and glossy appearance. pH of the all prepared micro emulgel was found in the range of 6.40 ± 0.10 to 6.80 ± 0.10, which is an acceptable range for the topical preparations. Viscosity of the all factorial batches was found in the range of 1460 ± 10 cps to 1848 ± 10 cps. All factorial batches of microemulgel were shown good or excellent extrudability. Drug content in the prepared miconazole nitrate micro emulgel was found in the range of 97.42 ± 0.13 % to 98.57 ± 0.13 %. *In-vitro* release data of factorial batches are shown in Table 4.

## $2^3$ Full Factorial Design Model Evaluation

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{123} X_1 X_2 X_3$$

**Table 3:** Evaluation of factorial batches F1-F8

| *Batch* | *Phase Separation* | *Grittiness* | *Homogeneity* | *Consistency* | *pH* | *Viscosity (cps)* | *Extrudability* | *DrugContent* |
|---|---|---|---|---|---|---|---|---|
| $F_1$ | Phase separation | Non gritty | ++ | ++ | 6.53 ± 0.11 | 1460 ± 10 | +++ | 97.84 ± 0.14% |
| $F_2$ | Not observed | Non gritty | ++ | ++ | 6.40 ± 0.10 | 1585 ± 08 | +++ | 97.42 ± 0.13% |
| $F_3$ | Not observed | Non gritty | +++ | +++ | 6.83 ± 0.05 | 1733 ± 09 | ++ | 98.54 ± 0.18% |
| $F_4$ | Not observed | Non gritty | +++ | +++ | 6.66 ± 0.05 | 1810 ± 03 | ++ | 97.87 ± 0.10% |
| $F_5$ | Not observed | Non gritty | +++ | +++ | 6.50 ± 0.10 | 1535 ± 06 | +++ | 98.57 ± 0.13% |
| $F_6$ | Not observed | Non gritty | +++ | +++ | 6.80 ± 0.10 | 1660 ± 08 | +++ | 97.63 ± 0.15% |
| $F_7$ | Not observed | Non gritty | +++ | +++ | 6.73 ± 0.05 | 1752 ± 11 | ++ | 97.69 ± 0.13% |
| $F_8$ | Not observed | Non gritty | +++ | +++ | 6.70 ± 0.04 | 1848 ± 10 | ++ | 98.37 ± 0.17% |

**(++)** Good, **(+++)** Excellent (n=6)

**Table 4:** Dissolution profile of factorial batches F1-F8

| | *% Cumulative drug release* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Time (min)* | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 21.55 ± 0.32 | 18.44 ± 0.43 | 15.87 ± 1.24 | 15.25 ± 1.64 | 27.66 ± 0.65 | 20.71 ± 0.53 | 16.52 ± 1.46 | 20.45 ± 0.84 |
| 60 | 39.11 ± 1.24 | 42.96 ± 0.53 | 37.60 ± 1.48 | 30.12 ± 1.13 | 45.02 ± 1.35 | 38.98 ± 1.67 | 40.76 ± 0.95 | 33.56 ± 1.75 |
| 120 | 48.64 ± 1.45 | 49.30 ± 0.57 | 49.28 ± 1.23 | 43.70 ± 1.54 | 54.12 ± 0.75 | 50.36 ± 0.87 | 50.33 ± 0.48 | 47.61 ± 0.94 |
| 180 | 61.45 ± 0.93 | 62.99 ± 1.45 | 58.88 ± 1.03 | 50.05 ± 1.25 | 66.58 ± 0.83 | 59.33 ± 1.05 | 60.78 ± 1.94 | 52.13 ± 1.74 |
| 240 | 66.28 ± 1.02 | 69.65 ± 0.89 | 64.19 ± 1.92 | 58.62 ± 0.64 | 73.36 ± 1.42 | 66.41 ± 0.84 | 67.23 ± 1.92 | 61.97 ± 1.02 |
| 300 | 78.34 ± 0.89 | 79.80 ± 0.93 | 75.89 ± 0.53 | 63.28 ± 1.53 | 84.05 ± 1.86 | 75.22 ± 1.75 | 78.83 ± 1.64 | 74.54 ± 0.82 |
| 360 | 90.84 ± 1.03 | 85.55 ± 1.23 | 82.20 ± 0.89 | 74.69 ± 1.84 | 94.04 ± 0.92 | 87.80 ± 0.84 | 84.97 ± 0.29 | 77.25 ± 1.11 |

(n=6)

Where Y is the dependent variable, $b_0$ is the arithmetic mean response of the 8 runs and any $b_i$ is the estimated coefficients for the related factor $X_i$. The $b_{12}$, $b_{13}$, $b_{23}$ and $b_{123}$ are the coefficient of interaction terms. The main effect ($X_1$, $X_2$, and $X_3$) represents the average result of changing the factor at a time from its low to high values. The interaction term ($X_1X_2$, $X_1X_3$, $X_2X_3$ and $X_1X_2X_3$) shows how the response changes when two factors are simultaneously changed. The fitted equations relating the response, that is, viscosity and % cumulative drug release at 6 h ($Q_6$%) were to be transformed factor are shown in Table 5. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e., positive or negative). ANOVA results suggested that calculated *F* values for viscosity and % cumulative drug release at 6 h ($Q_6$%) are 485.75 and 723.68, respectively (Table 6). Calculated F values were found greater than tabulated values for all the dependent variables. Therefore, one can conclude that all selected factors showed a significant effect. $R^2$ value of viscosity, % cumulative drug release at 6 hwas 0.999 and 0.998 respectively, indicating good correlation between dependent and independent variables. The reduced models were developed for response variables by omitting the insignificant terms with P>0.05. The terms with P < 0.05 were considered statistically significant and retained in the reduced model. The coefficients for full and reduced models for response variables were shown in Table 5. From the results of multiple regression analysis, it was found that both factors had statistically significant influence on all dependent variables as P < 0.05.[23]

## Full and Reduced Model for Viscosity

$$\text{Viscosity} = 1672.88 + (52.88 * X_1) + (112.88 * X_2) + (28.87 * X_3) - (9.63 * X_1X_2) + (2.8 * X_1X_3) - (11.63 * X_2X_3)$$

From the 3D response surface plot (Fig. 3) and the regression coefficient values of factors, it was concluded

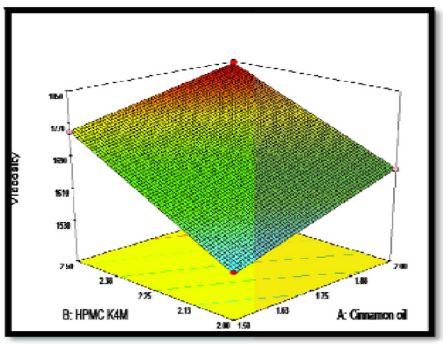


**Fig 3A:** 3D surface plot showing the effect of cinnamon oil ($X_1$) and HPMC K4M ($X_2$) on viscosity

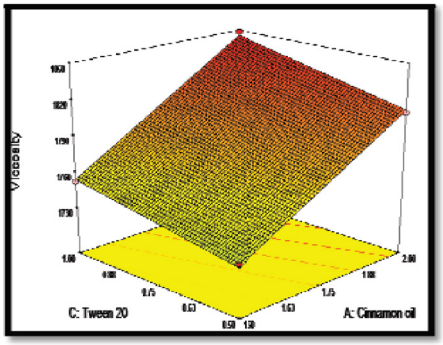


**Fig 3B:** 3D surface plot showing the effect of cinnamon oil ($X_1$) and tween 20($X_3$) on viscosity

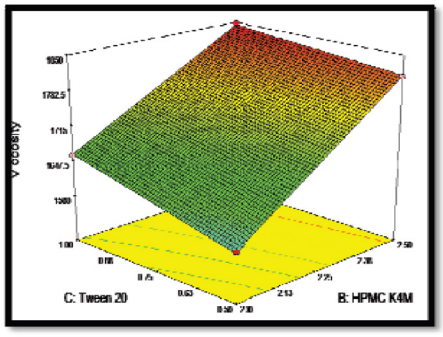


**Fig 3C:** 3D surface plot showing the effect of HPMC K4M ($X_2$) and tween 20 ($X_3$) on viscosity

**Table 5:** Summary of regression output of factors for measured responses

| | | *Coefficient of regression parameters* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Responses* | *Model* | $b_0$ | $b_1$ | $b_2$ | $b_3$ | $b_{12}$ | $b_{13}$ | $b_{23}$ | $R^2$ |
| Viscosity | Full | 1672.88 | 0052.88 | 0112.88 | 0028.87 | -0009.63 | 0002.38 | -0011.63 | 0.999 |
| | Reduced | 1672.88 | 0052.88* | 0112.88* | - | - | - | - | |
| % Cumulative drug release at 6 h ($Q_6$%) | Full | 0084.67 | -0003.35 | -0004.89 | 0001.35 | -0000.46 | -0000.14 | -0000.01 | 0.999 |
| | Reduced | 0084.67 | -0003.35* | -0004.89* | 0001.35* | - | - | - | |

*indicated the coefficient with p >0.05

**Table 6:** Results of the ANOVA for dependent variables

| *Source of variation* | *DF* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| *Viscosity (cps)* | | | | | |
| Regression | 6 | 131515.8 | 21919.29 | | |
| Residual | 1 | 0045.125 | 00045.12 | 0485.75 | 0.0347 |
| Total | 7 | 131560.9 | 18794.40 | | |
| *% Cumulative drug release at 6 h ($Q_6$%);* | | | | | |
| Regression | 6 | 0297.22 | 0049.54 | | |
| Residual | 1 | 0000.06 | 0000.06 | 0723.68 | 0.0284 |
| Total | 7 | 0297.28 | 0042.47 | | |

Viscosity was measured in cps, % Cumulative drug release at 6 h DF is degree of freedom, SS is sum of square, MS is mean square and F is Fischer's ratio.

the viscosity of microemulgel increases with an increase in the concentration of cinnamon oil and HPMC K4M. From regression, it is observed that only $X_1$ and $X_2$ were significant model terms that affect the on viscosity. Interaction and nonlinearity was not observed. The results also indicated that the HPMC K4M was given a more significant effect on viscosity as compared to cinnamon oil. The coefficients of $X_1$ and $X_2$ were found to be 0.0286 and 0.0134, respectively. Therefore, they were found to be significant and were retained in the reduced model; while coefficients $X_3$, $X_1X_2$, $X_1X_3$ and $X_2X_3$ were found to be 0.0583, 0.1540, 0.5000 and 0.1283 respectively, so they were omitted from reduced model.

Viscosity = 1672.88 + (52.88 * X1) + (112.88 * X2)

## Full and Reduced for % Cumulative Drug Release at 6 h (Q6)

$$Q6 = 84.67 - (3.35 * X_1) - (4.87 * X_2) + (1.35 * X_3) - (0.46 * X_1X_2) - (0.14 * X_1X_3) - (0.015 * X_2X_3)$$

From the 3D response surface plot (Fig. 4) and the regression coefficient values of factors, it was concluded that a correspondence decrease as the drug-releasing of micro emulgel was observed with increase in concentrations of cinnamon oil and HPMC K4M. It was also observed that drug release increases with an increase in the concentration of tween 20. From the graph and the regression coefficient values of factors, the drug release appeared to decrease more with an increase in concentration of HPMC K4M compared to cinnamon oil. Tween 20 had a positive effect on drug release. Interaction and nonlinearity was not observed. For Q6, the coefficients $X_1$, $X_2$ and $X_3$ were found to be 0.0176, 0.0120 and 0.0436, respectively. Therefore, they were found to be significant and were retained in the reduced model; while coefficients $X_1X_2$, $X_1X_3$, and $X_2X_3$ were found to be 0.1257, 0.3615 and 0.8977, respectively, so they were omitted from full model to generate reduced model.

$$Q6 = 84.67 - (3.35 * X_1) - (4.89 * X_2) + (1.35 * X_3)$$

## Formulation of Check Point Batch

To validate the evolved mathematical models (reduced models for Viscosity and Q6), one-check points were selected from the overlay plot as shown in Fig 5. The checkpoint batch (CP1) was prepared and evaluated. The observed and predicted values are shown in Table 7. Good correlation was found between observed and predicted values. Hence, it may be concluded that the evolved models may be used for theoretical prediction of responses within the factor space.

## Selection and Evaluation of the Optimized Batch in Factorial Design Study

The dissolution profile of factorial batches was fitted to kinetic models such as zero order, first order, Higuchi and Korsemeyer-peppas, to ascertain the kinetic of drug release that result are shown in Table 8. The $R^2$ value indicates that the mechanism of drug release of all batches follows Korsmeyer-peppas kinetic models. All factorial batches

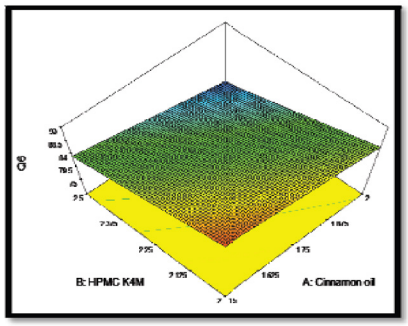


**Fig 4A:** 3D surface plot showing the effect of cinnamon oil ($X_1$) and HPMC K4M ($X_2$) on $Q_6$

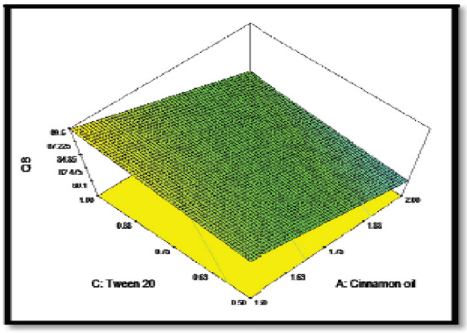


**Fig 4B:** 3D surface plot showing the effect of cinnamon oil ($X_1$) and tween 20 ($X_3$) on % $Q_6$

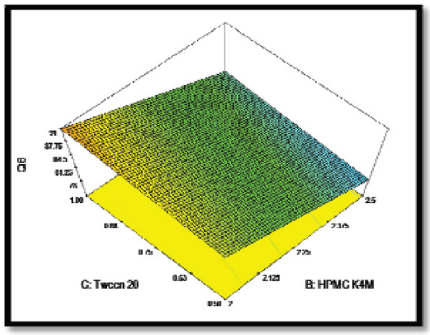


**Fig 4C:** 3D surface plot showing the effect of cinnamon oil ($X_1$) and HPMC K4M ($X_2$) on % $Q_6$

**Table 7:** Formulation and evaluation of check point batch and comparison with predicted value

| *Formulation of check point CP1* | | | |
|---|---|---|---|
| Batch Code | Variable Level | | |
| | Actual Value | | |
| | $X_1$(%) | $X_2$(%) | $X_3$ (%) |
| CP1 | 1.57 | 2.03 | 0.93 |
| *Evaluation of check point CP1* | | | |
| *Parameters* | *Predicted* | | *Actual* |
| Viscosity | 1554.55 | | 1548 |
| Q6 | 92.07 | | 93.48 |

**Table 8:** Factorial batches kinetic modeling of dissolution data

| *Batch* | *Zero Order* | *First Order* | *Higuchi* | *Korsmeyer – Peppas* | |
|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | *N* |
| $F_1$ | 0.926 | 0.517 | 0.989 | 0.978 | 0.753 |
| $F_2$ | 0.894 | 0.515 | 0.982 | 0.977 | 0.758 |
| $F_3$ | 0.905 | 0.536 | 0.984 | 0.983 | 0.754 |
| $F_4$ | 0.922 | 0.549 | 0.990 | 0.987 | 0.730 |
| $F_5$ | 0.898 | 0.477 | 0.992 | 0.964 | 0.758 |
| $F_6$ | 0.913 | 0.512 | 0.990 | 0.977 | 0.750 |
| $F_7$ | 0.901 | 0.529 | 0.982 | 0.980 | 0.759 |
| $F_8$ | 0.915 | 0.516 | 0.989 | 0.978 | 0.735 |

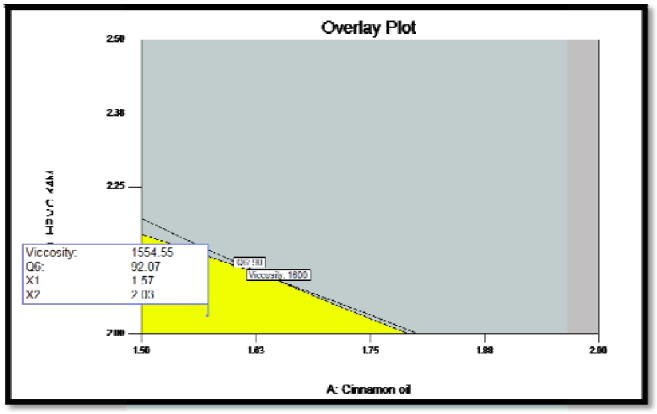


**Fig 5:** Overlay plot of applied design

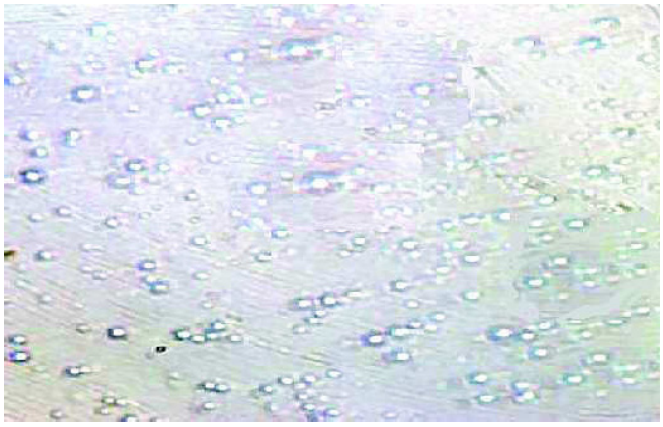

**Fig 6:** Globule size of microemulgel

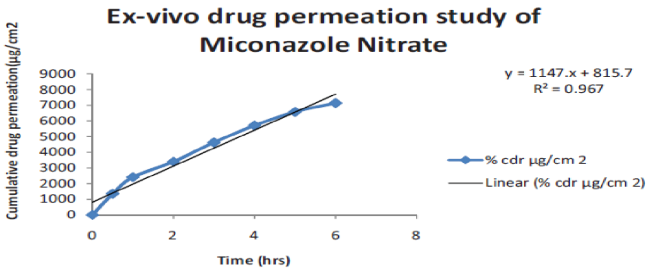


**Fig 7:** *Ex-Vivo* drug permeation study of 2% miconazole nitrate microemulgel

were shown in value in the range of 0.5 to 1.0, so drug release followed a non-fickian transport mechanism. It was observed that factorial batches $R^2$ value of Korsmeyer-peppas in the range of 0.982 to 0.992 and batch $F_5$ $R^2$ value was found 0.992. Therefore, batch $F_5$ was selected as an optimized batch. Globule size of 2% miconazole nitrate transparent micro emulgel was found 26 ± 3 µm was shown in Fig 6. The visual evaluation of the skin irritation study of batch $F_5$ showed that the no erythema and edema produced in the group-3 as compared to the group-2 treated with a standard irritant, so it can be concluded that the formulation was safe. The results of a microbial study of batch $F_5$ showed that micro emulgel formulation has the potential to inhibit fungal growth compared to 2% miconazole nitrate solution. The 2% miconazole nitrate micro emulgel Zone of inhibition was found to be 17 ± 2 mm where the 2% miconazole nitrate solution is shown as one of inhibition was 8 ± 2 mm . Therefore, it was concluded that microemulgel is a better carrier system for topical drug delivery of Miconazole Nitrate. After short-term stress stability studies, the release profile of batch $F_5$ was compared with the dissolution profile. The values of similarity factor ($f_2$) were shown 91.05 ± 1.67%. The result of *Ex-vivo* skin permeation of 2% miconazole nitrate microemulgel of batch $F_5$was shown in Fig 7.In this, study miconazole nitrate flux was found 1147 µg/$cm^2$/hr from Skin and 87.50 ± 2.42%of drug permeated in 6 hours. *Ex-Vivo* permeation study is predictive of *in-vivo* performance of a drug. *In-vitro* Skin Deposition study was shown that 52.5 % drug was retained after 6 hours on the upper layer of skin.[24]

## Conclusion

A 2% miconazole nitrate micro emulgel was prepared successfully using Cinnamon oil as an oil phase of emulsion and HPMC K4M as a gel base. Drug content in the prepared miconazole nitrate micro emulgel was found in the range of 97.42 ± 0.13% to 98.57 ± 0.13%. From the factorial batch evaluations batch $F_5$ was selected as an optimized batch. Globule size of micro emulgel was found 26 ± 3µm. In Skin irritation study, it was concluded that test micro emulgel formulation was safe. *Ex-vivo* drug permeation study was also performed and from that, it was found that 87.05 ± 1.67 % of drugs were released after 6 hours. The drug deposition study concluded that 52.5 % drug was retained on the upper layer of skin. The accelerated stability study results showed no significant change in the formulation parameters after 3 months and the Similarity factor $f_2$ was found to be 87.05 ± 1.67%. The results demonstrated that the formulations were stable and showed improved drug deposition on skin.

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## References

1. Bhalke RD, Kirloskar GV, Bhutada RR, Jagtap NK, Giri MA. Design, development and evaluation of topical anti-inflammatory herbal gel. Int J Pharm Sci & Res 2020; 11(3): 1467-1472.
2. Rao M, Kamble P. Formulation and evaluation of antifungal proniosomal gel for oral candidiasis. J Drug Deliv Ther 2018; 8: 291-301
3. Mahajan V, Basarkar GD. Formulation design, development and characterization of dexibuprofen Microemulgel for topical delivery: *In-vitro* and *In-vivo* evaluation. J Drug Delivery Ther 2019; 9(2S): 330-342.

4. Shah SNH, Javed H, Nisar M, Nawaz A. Design and in-vivo evaluation of topically applied Chlorpheneramine maleate gels. Int J Pharm Sci & Res 2019; 10(2): 775-782.
5. Kodre KV, Attarde SR, Yedhe PP, Patil RY, Borge VU. Differential scanning calorimetry: A review. Res Rev J Pharm Anal Differ Scanning Colorimetry 2014; 3: 11-22.
6. Singh V, Chaubey N. Design and evaluation of topical hydrogel formulation of aceclofenac for improved therapy. J Drug Deliv Ther 2019; 9(5): 118-122.
7. Joshi H. Microemulgel: An advance approach for transdermal drug delivery. Mintage J Pharm Med Sci 2019; 8(3): 4-8.
8. Yadav SK, MK Mishra, A Tiwari, A Shukla. Microemulgel: A new approach for enhanced topical drug delivery. Int J Curr Pharm Res 2016; 9(1): 15-9.
9. Vishwanath AB, Avinash BG, Anil BD. Formulation and evaluation of antibacterial topical gel of doxycycline hyclate, neem oil and tea tree oil. Indian J Pharm Educ Res 2020; 54(1) :206-212.
10. Sharadha M, Gowda DV, Vishal GN, Akhila AR. An overview on topical drug delivery system- Updated review. Int J Res Pharm Sci 2020; 11(1): 368-385.
11. Patil P, Datir S, Saudagar R. A Review on topical gels as drug delivery system. J Drug Delivery Ther 2019; 9(3s): 989-994.
12. Gadkari PN, Patil PB, Saudagar RB. Formulation, development and evaluation of topical nano Micro Emulgel of tolnaftate. J Drug Delivery Ther 2020; 9(2s): 208-213.
13. Verma S, Singh AK, Mukharjee A. Formulation and evaluation of ketoconazole nano Microemulgel. World J Pharm Pharm Sci 2016; 5 (2):899-911.
14. Shrestha S, Pokhrel S, Sharma S, Manandhar M, Alam I. Formulation and evaluation of topical micro-micro emulgel loaded with terbinafine HCL microemulsion. Int J Pharm Sci Res 2017; 8(11): 4716-4723.
15. Thatai P, Sapra B. Transungual Gel of Terbinafine Hydrochloride for the Management of Onychomycosis. Formulation, Optimization, and Evaluation. AAPS Pharm Sci Tech 2017; 18(6): 2316-2328.
16. Moustafa MA, El-Refaie WM, Elnaggar YS, Abdallah OY: Gel in core carbosomes as novel ophthalmic vehicles with enhanced corneal permeation and residence. Int J Pharm 2018; 546: 166-175.
17. Patel TB, Patel TR, Suhagia BN. Preparation, characterization and optimization of microemulsion for topical delivery of itraconazole. J Drug Deliv Ther 2018; 8: 136-45.
18. Fahmy AM, El-Setouhy DA, Ibrahim AB. Penetration enhancer-containing spanlastics (PECSs) for transdermal delivery of haloperidol: *in-vitro* characterization, *ex-vivo* permeation and *in-vivo* biodistribution studies. Drug Deliv 2018; 25: 12-22.
19. Hegde AR. Peptide dendrimer-conjugates of ketoprofen: synthesis and *ex-vivo* and *in vivo* evaluations of passive diffusion, sonophoresis and iontophoresis for skin delivery. Eur J Pharm Sci 2017; 102: 237-249.
20. SN Politis, P Colombo, G Colombo, DM Rekkas. Design of experiments (DoE) in pharmaceutical development. Drug Dev Ind Pharm 2017; 43: 889-901.
21. Madgulkar AR. Formulation and optimization of sustained release tablets of venlafaxine resinates using response surface methodology. Indian J Pharm Sci 2009; 71: 387-394.
22. R Mazzeo, S Prati, M Quaranta, E Joseph, E Kendix, M. Galeotti. Attenuated total reflection micro FTIR characterization of pigment–binder interaction in reconstructed paint films. Anal Bioanal Chem 2008; 392(1): 65-76.
23. Bolton S, Bon C. Pharmaceutical statistics: Practical and clinical Applications, 5th ed. Informa Healthcare. 2005: 472-493.
24. N Oza, S Sahoo, S Sagar. A 32 full factorial design for topical controlled release tazarotene microsponge using HPMC gel. Int J Appl Pharm. 2019, 11(5): 12-18.

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