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OPTIMIZATION BY 3^2 FACTORIAL DESIGN AND COMPONENT SCREENING OF MICONAZOLE NITRATE NANOEMULSION.

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ABSTRACT

The objective of the present investigation was to develop and evaluate nanoemulsion for the vaginal delivery of Miconazole nitrate(MCZ). The solubility of MCZ in oils and surfactants was evaluated to construct ternary phase diagram by detecting the area for preparation of nanoemulsion and its optimization done by 3^2 factorial design using dx-8 trial version software. The formulations that passed thermodynamic stability tests were characterized for pH, refractive index, viscosity, droplet size, drug content, transmission electron microscopy, stability study and in vitro drug release. The MCZ nanoemulsion exhibited globule size of 22nm and polydispersity index of 0.98. A significant increase in drug release, and good in vitro antifungal efficacy were observed in optimized nanoemulsion formulation FM4 which consist of 1%wt/wt MCZ, 20%wt/wt Captex200EP, 40% wt/wt Twen80:CapmulMCM(2:1) and 40% wt/wt distilled water & *In vitro* antifungal efficacy of formulation FM4 showed a significant increase in percent inhibition when compared with MCZ cream.

KEYWORDS: Nanoemulsion, Pseudotrnary phase diagram, Screening, Optimization and Validation.

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INTRODUCTION

Miconazole nitrate (MCZ) is a broad-spectrum antifungal agent of the imidazole group[1]. It acts by means of a combination of two mechanisms: ergosterol biosynthesis inhibition, which causes lysis of fungal cell membranes because of the changes in both membrane integrity and fluidity, and direct membrane damage of the fungal cells. The drug is primarily used as a topical treatment for cutaneous mycoses[2]; poor dissolution and lack of absorption make it a poor candidate for oral administration.However,MCZ can be used as a systemic antifungal agent when amphotericin B or ketoconazole is either ineffective or contraindicated. MCZ's poor skin-penetration capability presents a problem in the treatment of cutaneous diseases by topical application. For effective treatment, the drug must be delivered in sufficient concentration to the site of infection[3]. Various approaches have been used to enhance the access of such poorly skin-partitioned drug molecules. For example, the use of complexation with cyclodextrins has been reported to improve oral and topical delivery of MCZ[4,5]. Other approaches have used submicron emulsions of MCZ for improved topical delivery[6,7] and chewing gum containing MCZ for buccal delivery[8,9]. To design of effective formulations for drugs has long been a major challenge, because drug efficacy can be severely limited by instability or poor solubility in the vehicle. One of the most promising technologies is the nanoemulsion drug delivery system, which is being applied to enhance the solubility and bioavailability of lipophilic drugs. The nanosized droplets leading to an enormous increase in interfacial areas associated with nanoemulsion would influence the transport properties of the drug[10,11]. Nanoemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water, and surfactants with a droplet size usually in the range of 10–100nm. Their long-term stability, ease of preparation (spontaneous emulsification), and high solubilization of drug molecules make them promising as a drug delivery tool. They have found wide applications in oral drug delivery to enhance the solubility and bioavailability of the lipophilic drugs. Recently, there has been a surge in the exploration of nanoemulsions for transdermal drug delivery. They are also being investigated ardently for potential applications in ocular, pulmonary, nasal, vaginal, and parenteral drug delivery These systems often require high surfactant concentration, and this may lead to toxicity and irritancy problems. Therefore, judicious selection of surfactants along with their optimum concentration is required & determination of the influence of the surfactant-to-cosurfactant mass ratio (Smix) on the nanoemulsion formation region also formed an important aspect of the study. Optimum selection would aid in better formulation with desirable attributes[12]. The main objective of this study was to provide an efficient screening approach for the proper selection of oils, surfactants, and cosurfactants and optimization by 3^2 factorial design for the nanoemulsion formulation.

MATERIALS AND METHODS

MATERIALS

Miconazole was a gift sample from Camlin Fine Chemicals Ltd. (Bombay, India). Propylene Glycol Dicaprylocaprate (Captex200EP) & Glycerol monocaprylocaprate (CapmulMCM) was a gift sample from USabitec (US). Propylene glycol monocaprylate (Capryol 90) and caprylocaproyl macrogol-8-glyceride (Labrasol) (Gattefosse, Gennevilliers, France) were gift samples from Colorcon Asia (Mumbai, India), and polyoxy-35-castor oil (Cremophor EL) were purchased from Merck Schuchardt (Hohenbrunn, Germany) and Sigma Aldrich (St.Louis, MO), respectively. Isopropyl myristate, castor oil, methanol, and ammonium acetate were purchased from E-Merck (Mumbai, India). Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monostearate (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), ethanol, isopropyl alcohol, PEG 400, and propylene glycol were procured from S.D Fine Chemicals (Mumbai,

India). Water was obtained from Milli Q water purification system (Millipore, MA). All other chemicals and solvents were of analytical grade.

METHODS

SCREENING OF COMPONENTS FOR PREPARATION OF NANOEMULSION

The solubility of Miconazole nitrate in various oils (Ethyl oleate, Oleic acid, Olive oil, Captex200P, Capryol90, Isopropyl myristate), was determined by dissolving an excess amount of drug in 5ml of individual oil separately using stoppered vials (10ml capacity). The liquids were mixed using an isothermal orbital shaker at 37.0^{o} C $\pm$1.0^{o}C for 72 hours to reach equilibrium. The concentration of Miconazole nitrate in diluted or extracted sample was determined by UV spectroscopy at 272nm. Five types of surfactants were screened for nanoemulsion formulation, which included Labrasol, Cremophor EL, Tween 20 and Tween 80. In water, 2.5 mL of 15 wt.% surfactant solution was prepared, and 4 µL of oil was added with vigorous vortexing. If a one-phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy. Selected surfactant was combined with three types of solubilizers as cosurfactants, namely CampulMCM, PEG 400 and propylene glycol. At a fixed Smix ratio of 1:1, the pseudoternary phase diagrams were constructed. Nine different combinations in different weight ratios of oil and Smix, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9(w/w) were taken so that maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams.

EFFECT OF SURFACTANT & COSURFACTANT MASS RATIO ON NANOEMULSION REGION IN PSEUDOTERNARY PHASE DIAGRAM

Surfactant was blended with cosurfactant in the weight ratios of 3:1, 2:1, 1:1, 1:2, and 1:3. Aqueous titration method was used for the construction of the pseudoternary phase diagrams, which involves stepwise addition of water to each weight ratio of oil and surfactants, and then mixing the components with the help of vortex mixer at 25°C[13]. The nanoemulsion phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained based on the visual observation. Nine different combinations in different weight ratios of oil and Smix, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9(w/w), were taken.

OPTIMIZATION BY 3^2 FULL FACTORIAL DESIGN FOR SELECTION OF NANOEMULSION FORMULATIONS

From phase diagram constructed, 9 full factorial design points given in table 1 were selected from the nanoemulsion region so that the drug could be incorporated into the oil phase[14]. Exactly 1% wt/wt of Miconazole Nitrate which was kept constant in all the selected formulations, dissolved in the oil phase of the nanoemulsion formulation. Selected formulations were subjected to different thermodynamic stability tests. This design generally involves independent variable X and dependent variables Y with different levels shown in table 2 and 3 rept.

Table 1
Formulation code for preparation of various nanoemulsion compositions

Formulation Code		FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	FM9
Code Value	X_1	-1	-1	-1	0	0	0	1	1	1
	X_2	-1	0	1	-1	0	1	-1	0	1

Table 2
Independent & Dependent Variable

Independent variables	Dependent variables
X_1 = Surfactant : Co-surfactant Ratio	Y_1 = Viscosity
X_2 = Concentration of Smix..(%w/w)	Y_2 = Transmittance
	Y_3 = % drug release

Table 3
Levels of Independent Variables

Code Value		-1	0	1
Actual Value	X_1	1:1	2:1	3:1
	X_2	40	50	60

X_1 = Surfactant : Co-surfactant Ratio & X_2 = Concentration of Smix.. (%w/w).

THERMODYNAMIC STABILITY

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Selected formulations were taken for the heating and cooling cycle. Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 hours were done. The formulations, which were stable at these temperatures, centrifuged at 3500rpm for 30 minutes. The formulations that did not show any phase separations were were subjected to a freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulation between −21°C and +25°C. The formulations that survived thermodynamic stability tests were selected for further study.

CHARACTERISATION OF NANOEMULSION

pH, refractive index, % transmittance, conductance, viscosity and Drug content(USP) were evaluated.

TRANSMISSION ELECTRON MICROSCOPY

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM), with Topcon 002B operating at 200 kV (Topcon, Paramus, NJ) and capable of point-to-point resolution. To perform the TEM observations, a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying[15].

NANOEMULSION DROPLET SIZE ANALYSIS

Droplet size distribution of the nanoemulsion was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles,26 using a Zetasizer 1000 HS (Malvern Instruments, Worchestershire, UK). Light scattering was monitored at 25-C at a 90- angle[16].

IN-VITRO DRUG RELEASE

The drug release kinetics from prepared nanoemulsion formulations was studied using a modified method. A glass cup with a cross-sectional area of 1.5 cm^2 was filled with 0.2ml of the nanoemulsion, covered with a cellophane membrane, sealed with a rubber band and adhesive tape, and inverted under the surface of 30 ml of simulated Vaginal fluid[17] of pH 4.2 at 37°C ± 0.5°C in USP XXIII Type I Dissolution Test Apparatus with a speed of 30 rpm[18]. 1ml of aliquots were withdrawn at specified time intervals and immediately replaced with fresh dissolution medium. The drug content in the withdrawn samples was determined spectrophotometrically at 272.5nm, keeping simulated vaginal solution as a blank. The drug content in sample was determined by software PCP disso version 3.08.

DATA ANALYSIS

Various computations for the current optimization study using Response Surface Methodology (RSM) were carried out, employing the Design Expert Software (Version 8.0.1, Stat-Ease Inc., Minneapolis, MN). Statistical second-order model including interaction and polynomial terms were generated for all the response variables using multiple linear regression analysis (MLRA). The general form of the model is represented as in equation (1),

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2+\beta_6X_1X_2^2+\beta_7X_1^2X_2+\beta_8X_1^2X_2^2 \quad \ldots\ldots\ldots(1)$$

Where β_0, is the intercept & the arithmetic average of all quantities outcomes of 9 runs, β_1 to β_8 are the coefficient computed from the observed experimental values of Y, X_1 and X_2 are the coded levels of the independent variable(s). The terms X_1X_2 and X_i^2 (i = 1, 2) are the interaction and polynomial terms, respectively. The statistical validity of the

polynomials was established on the basis of Yates's ANOVA provision in the Design Expert Software. Subsequently, feasibility as well as grid search was performed to locate the composition of optimum formulations. Also, three-dimensional response surface graphs were drawn in MS-Excel using the output files generated by the Design Expert Software[19].

VALIDATION OF OPTIMIZATION MODEL

Three optimum formulations were selected by intensive search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations. The criterion for selection of optimum was primarily based on the values of viscosity, transmittance, conductance & %drug release. The resultant experimental data of response properties were subsequently quantitative compared with predicted values. Linear regression plots were obtained using MS-Excel[20].

RESULTS AND DISCUSSION

SCREENING OF OIL

Lipophilic drugs are preferably solubilized in o/w nanoemulsions, whereas w/o systems seem to be a better choice for hydrophilic drugs. Novel semisynthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties, are being preferred. Fig. 1 indicates the order of solubility of Miconazole nitrate is Captex200EP>Oleic acid>Capryol90>Ethyl oleate. This may be attributed to the non-polar nature of the poorly water soluble drugs that favors their solubilization in oils with small/medium molecular volume (containing medium chain triglycerides or mono- or diglycerides[21,22]. Among the various oils tested, Captex200EP made the most suitable oil phase viz. a synthetic ester (Propylene Glycol Dicaprylocaprate) with good stability, ability to dissolve large amounts of lipophilic drugs hence low volume required for solubilisation of drug and emulsification can be achieved with smaller quantities of surfactants with no risk of toxicity due to large concentration.

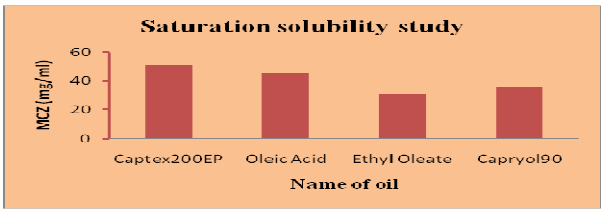

Figure 1
Saturation solubility of Miconazole nitrate in different oils

SCREENING OF SURFACTANT

The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water. After selection of captex200M as the oil phase, the goal was to identify the surfactant that has the highest solubiliza-tion capacity for the oil. In the present study, five nonionic surfactants, namely, Labrasol, Cremophor EL, Tween 20, and Tween 80, were chosen for screening. Nonionic surfactants were selected since they are known to be less affected by pH and changes in ionic strength, are generally regarded as safe, and are biocompatible. Ionic surfactants were excluded from the study due to toxicological concerns. As Tween 80 solubilized the maximum amount of Captrex200EP, i.e., 2.84 wt.%, it was chosen as the surfactant for the nanoemulsion development. Surfactant–oil miscibility can thus give an initial indication on the possibility of nanoemulsion formation with this system.

SCREENING OF COSURFACTANT

A single surfactant may not give a stable nanoemulsion when used at low concentration. Hence, it is necessary to add a cosurfactant to nanoemulsion. The presence

of cosurfactant decreases the bending stress of interface & imparts sufficient flexibility to the interfacial film to take up different curvatures required to form nanoemulsion over a wide range of composition. Thus the cosurfactants viz. PG, PEG400 & CapmulMCM were selected. The shaded portion in phase diagram indicate transparent nanoemulsion region. Based on the area of this zone CapmulMCM was selected as cosurfactant(Fig.2).

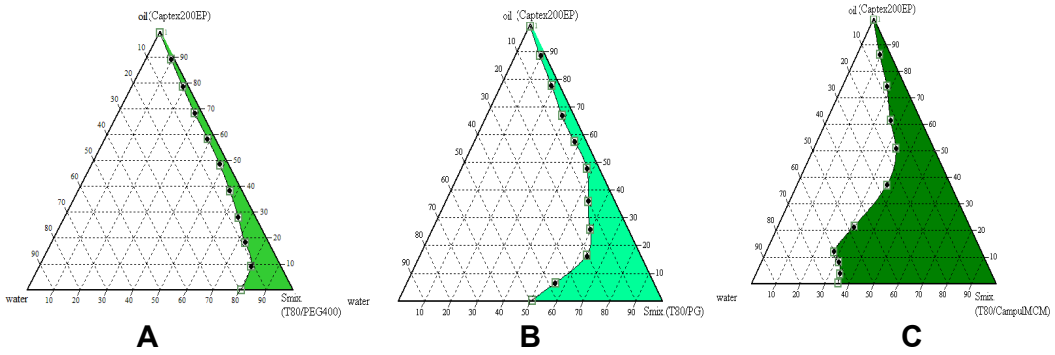

Figure 2
Pseudoternary phase diagram of with different S/CoS (1:1) viz. PEG400(A), PG(B) & CapmulMCM(C)

EFFECT OF SURFACTANT & COSURFACTANT MASS RATIO ON NANOEMULSION REGION IN PSEUDOTERNARY PHASE DIAGRAM

Phase diagrams were constructed using Captex200P as the oil phase and Tween 80 and CapmulMCM as the surfactant and cosurfactant, respectively(Fig. 3). No distinct conversion from w/o to o/w nanoemulsions was observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions[23].

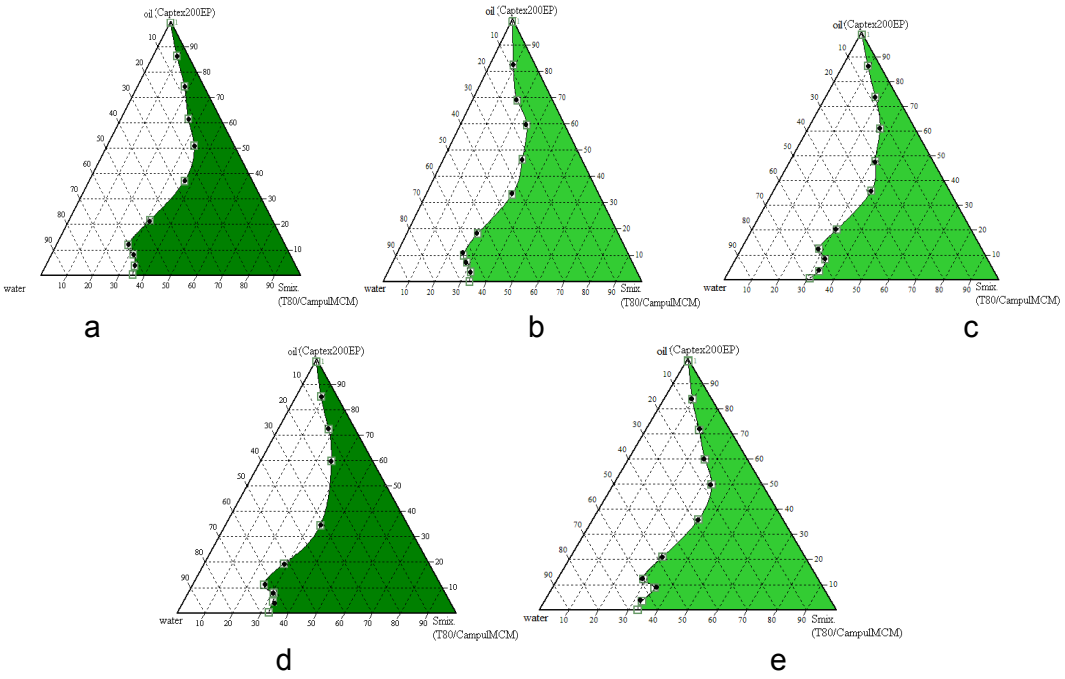

Figure: 3
Pseudoternary phase diagrams using Captex200EP as the oil phase and Tween 80/ CapmulMCM as the S/CoS rept 1:1(a), 2:1(b), 3:1(c), 1:2(d) & 1:3(e)

At 1:1 ratio of S:CoS, a nanoemulsion region was observed, perhaps because of the further reduction of the interfacial tension and increased fluidity of the interface. At this ratio 30%w/w of Smix was required for solubilization of 20%w/w oil and when this ratio increased upto 2:1 (Fig.2 b), a broader nanoemulsion region was observed (Fig.2 b). The maximum 20%w/w Smix. required for solubilization 28%w/w oil. When the S:CoS ratio was increased to 3:1 (Fig 2 c), a smaller nanoemulsion region was observed (Fig 2 c). Hence, it is observed that with increase in proportion of surfactant in Smix upto 2:1 there was an increase in the nanoemulsion region which further, on increase in the proportion of surfactant, could not enhance the zone. Hence, the areas of one phase nanoemulsion zones are dependent on amount of surfactant in Smix.[24,25].

CHARACTERISATION OF NANOEMULSION BY 3^2 FULL FACTORIAL DESIGN

Parameter	Range
PH	5.3 to 5.5
refractive index	1.461±0.018 to 1.478±0.025
conductance	13.8 to 22.7mhos
%drug content	98.6% to 99.6%.
droplet size	12nm to 78nm
drug content	NLT 98% & NMT 102%(USP2004)

For the measured responses Y_1, Y_2 & Y_3, simple linear , interactive or quadratic model was fitted by carrying out multiple regression analysis. Mathematical relationships generated using MLRA for the studied response variables using Design Expert Software (Version 8.0.1, Stat-Ease Inc., Minneapolis, MN) are expressed in equation 1 to 3.

Viscosity (Y_1) = $145.00 + 3.50 X_1 + 3.67 X_2 + 0.50 X_1X_2 – 2.50 X_1^2 - 2.00 X_2^2$ ……..1

Transmittance(Y_2)=$113.31 + 1.20 X_1 + 5.05 X_2$ …….2

%Drug Release after 300min.(Q_{300}) (Y_3) = $93.42 – 1.09 X_1 – 5.23X_2$ 3

Table 4
Values of dependent variables

Sr. No	Formulation Code	Code Value*		Viscosity at 100rpm(cps) (Y1)	Transmittance (Y2)	%Drug release after300min(Q_{300}) (Y3)
		X_1	X_2			
1	FM1	-1	-1	134	106.5	99.363
2	FM2	-1	0	139	113.5	94.400
3	FM3	-1	1	140	116.9	90.319
4	FM4	0	-1	139	107.4	98.337
5	FM5	0	0	145	113.2	93.488
6	FM6	0	1	147	118.2	87.392
7	FM7	1	-1	140	110.3	97.894
8	FM8	1	0	146	114.4	93.133
9	FM9	1	1	148	119.4	86.490

The significance test for regression coefficients was carried out by applying Students t- test. A coefficient is significant if the calculated‘t’ value is greater than the critical value of‘t’.

Table 5
Significance values (Probe value) of response coefficients

Coefficients	Significance values (probe value)		
	Viscosity(Y_1)	Transmittance(Y_2)	Q_{300}(Y_3)
b_0	0.0003	0.0001	0.0001
b_1	0.0001	0.0104	0.0098
b_2	0.0001	0.0001	0.0001
b_{12}	0.0577	-	-
b_{11}	0.0018	-	-
b_{22}	0.0034	-	-

From table 5 it was clear that the significance value (Probe value) of coefficient b_0, b_1, b_2, b_{12}, b_{11}, b_{22} are significant i.e.<0.1 and hence, they are retained as such. The results of multiple regression analysis and analysis of variance test (ANOVA) are summarized in Table 7.10.

Table 6
Regression analysis data for measured responses

Coefficients	Viscosity (Y_1)	Transmittance (Y_2)	Q_{300} (Y_3)
b_0	175.67	161.66	171.44
b_1	73.50	8.64	7.18
b_2	80.67	153.01	164.25
b_{12}	1.00	-	-
b_{11}	12.50	-	-
b_{22}	8.00	-	-
R^2	0.9981	0.9768	0.9822
Significance	0.0003	0.0001	0.0001
F- value	316.20	126.49	165.83

For the viscosity, transmittance & Q_{300} of nanoemulsions calculated F-values are 316.20, 126.49 & 165.83 respectively given in table 6. Hence it can be concluded that the selected variables contribute significantly in the regression of measured responses Y_1, Y_2 and Y_3. The effects of these variables can be explained by response plots and contour plots generated using equations 1, 2 and 3 (Fig.No.7.10, Fig.No.7.11, Fig.No.7.12 respectively).

EFFECT ON VISCOSITY AT 100RPM

In equation 1 positive coefficient of X_1 and X_2 indicate increase in response Y_1 (viscosity). This may be attributed to increase in number of droplets (dispersed phase) due to increase in concentration of Smix & S:CoS ratio leading to dense packing within the nanoemulsion[26]. With the simultaneous increase in both independent variables the total interactive effect was significant as compared to their individual effects resulting in positive sign. Fig.4 demonstrated increase in response Y_1 (viscosity) as X_1 (S/CoS ratio) and X_2 (concentration of Smix) increased from lower level to higher level. Viscosity increased drastically with increase in S/CoS Ratio & Smix concentration at the intermediate level i.e. 2:1 & 50%w/w respectively. Further increase in X_1 & X_2 resulted in slight increase in viscosity.

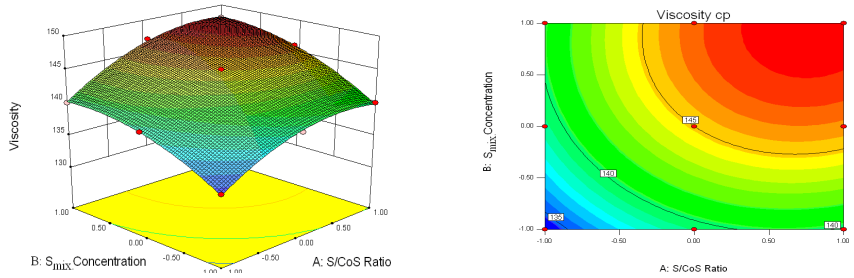

Figure 4
Surface response plot & contour plot demonstrating influence of S/CoS ratio and concentration of Smix. on viscosity of nanoemulsion

EFFECT ON TRANSMITTANCE

The positive coefficients of X_1 & X_2 in equation 2 indicate increase in transmittance (Y2) with increase in the S/CoS ratio & concentration of Smix, due to corresponding decrease in droplet size . The addition of surfactant caused the condensation of interfacial film and also imparted stability to this film which resulted into decrease in droplet size[27]. The linear equation shows, there is no significant effect of interaction of two variables on the response(Y2). Fig.5 indicate the relative effect of increasing S/CoS ratio (X1) and Smix concentration (X2) on transmittence (Y3) at different levels (-1, 0, 1) of S/CoS ratio, with the increase in concentration of Smix there is corresponding increase in transmittence. At 1:1 (0) ratio of S/CoS, when concentration of Smix increased from 40 to 60, the transmittence increased from 105 to 120. It can be observed from equation 2 that interaction between both the independent variables did not exert significant effect.

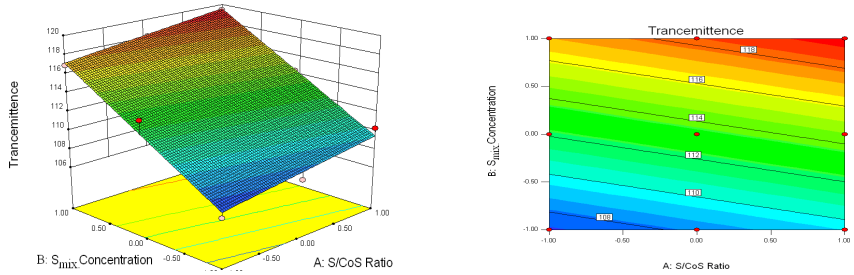

Figure 5
Surface response plot & contour plot showing influence of S/CoS ratio and Smix.concentration on transmittance of nanoemulsion

EFFECT ON Q_{300} (CUMULATIVE % DRUG RELEASE AFTER 300MIN.)

For response Y_3 (Q_{300}), i.e. %Release of MCZ after 300min, linear equation 3 is obtained suggesting insignificant interactive effect of both the independent variables. Moreover, negative coefficient of X1 & X2 indicate decreasing effect on the response Y_3. Fig.6 demonstrate a linear effect of X1 & X2 on drug release (Y3). At all three levels of S/CoS ratio (X1), the increase in the concentration of Smix caused a linear decrease in release of the drug. This is because high concentration gradient and MCZ partitioned preferentially into internal phase of nanoemulsion due to its lipophilicity . With increase in Smix concentration, this partitioning effect also increased and hence, caused slow release of the drug. Moreover, the number of droplets in internal phase increased leading to dense packing. This subsequently caused, increase in viscosity and hence, decreased the release of drug[28].

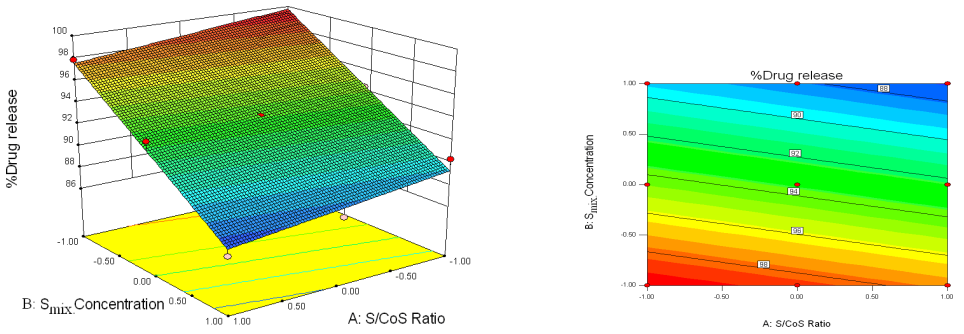

Figure 6
Surface response plot & contour plot showing influence of S/CoS ratio and concentration of Smix. on % drug release (Q_{300}) from nanoemulsion

All the nanoemulsion formulations showed zero order release with maximum drug released at 300min(Fig.7). In nanoemulsion formulations (FM1, FM4 & FM7) when S:CoS ratio was increased from 1:1 to 2:1, %drug release was slightly decreased. With further increase of S:CoS ratio to 3:1, drug release was not much changed showing 2:1 as the optimum ratio for formulation of stable nanoemulsion.

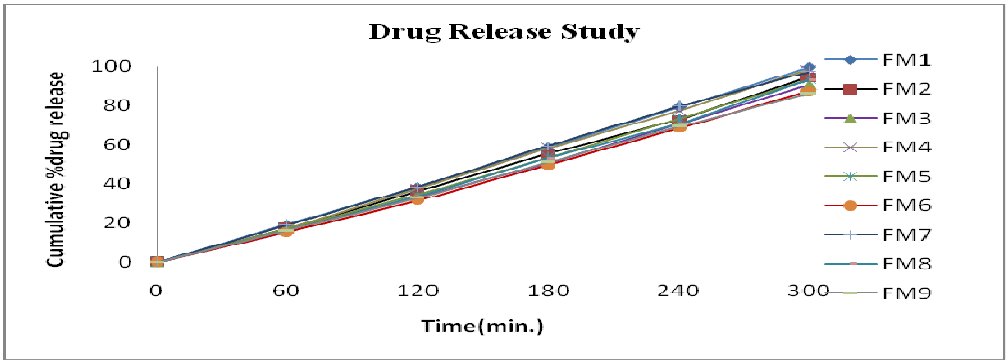

Figure 7
Cumulative % drug release of Nanoemulsions

THERMODYNAMIC STABILITY

No phase separation, turbidity, creaming/cracking was observed. All the nine nanoemulsion formulations were found to be stable after heating cooling cycles & centrifugation tests. Thermodynamic stability confers long shelf life to the nanoemulsion as compared to ordinary emulsions.

TRANSMISSION ELECTRON MICROSCOPY

The droplet size increased with increase in the concentration of the oil in the formulations. However, the droplet size of all the formulations was in the nano range. The low polydispersibility values observed for all the formulations indicated uniformity of droplet size within each formulation.The droplets in the nanoemulsion appear dark, and the surroundings are bright; a “positive” image was seen using TEM (Fig.8). Some droplet sizes were measured using TEM, as it is capable of point-to-point resolution.

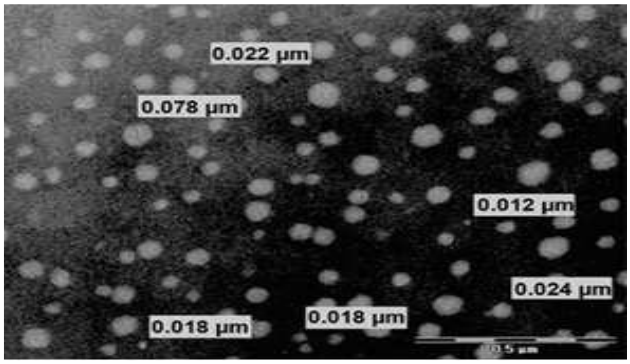

Figure 8
Transmission electron micrograph of nanoemulsion FM4 showing the size of some oil droplets

VALIDATION OF OPTIMUM NANOEMULSION FORMULATIONS

The linear correlation plots drawn (Fig. 9) between the predicted and observed responses (Table 8) demonstrated higher values of R^2 (ranging between and), indicating excellent fitting of model ($P<0.001$).

Table 8
Comparison of experimental results with predicted Responses of Nanoemulsion formulations.

Code	Composition		Response	Predicted Value	Experimental Value	Percentage Error
	X_1	X_2				
V1	2:12	40.12				
			pH	-	5.4	-
			Conductance(mhos)	-	18.8	-
			%Drug content	-	99.24	-
			Droplet size(nm)	-	56	-
			Transmittance(%)	107.435	107.4	0.4
			% Release(Q_{300})	98.2936	98.30	0.4
V2	2:14	40.14	Viscosity at100rpm(cps)	139.1418	139	0.9
			pH	-	5.4	-
			Conductance(mhos)	-	19.0	-
			%Drug content	-	99.24	-
			Droplet size(nm)	-	57	-
			Transmittance(%)	108.81	108.8	0.5
			% Release(Q_{300})	98..8144	98..80	0.4
V3	2:16	40.16	Viscosity at100rpm	140.048	140	0.7
			pH	-	5.5	-
			Conductance(mhos)	-	19.6	-
			%Drug content	-	99.60	-
			Droplet size(nm)	-	57	-
			Transmittance(%)	110.185	110.1	0.4
			% Release(Q_{300})	99..5352	99.50	0.4
			Viscosity at100rpm	142.096	142	0.8
Mean (± S.E.M.) of Percentage Error						

Upon comparison of the observed responses with that of the anticipated responses, the percentage error varied between 0.4 and 0.9 . Thus, the low magnitudes of error as well as the significant values of R^2 in the current study indicated a high prognostic ability of nanoemulsion formulations of Miconazole Nitrate using RSM optimization.

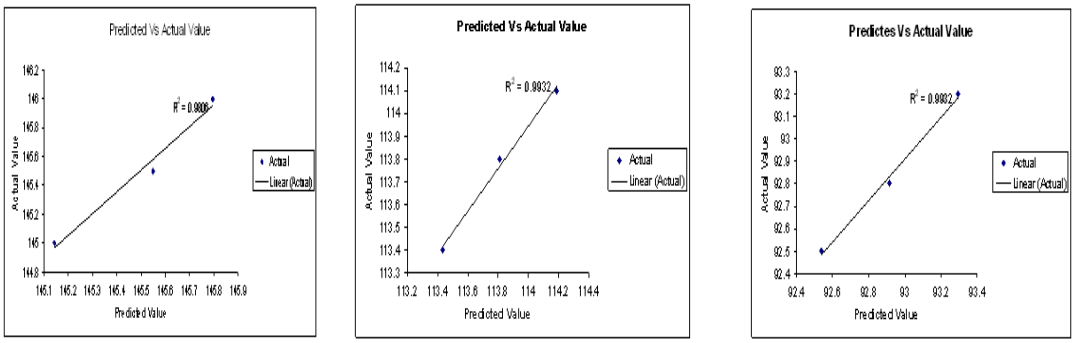

Figure 9
Linear plots between observed and predicted values of (a) Viscosity (b) Transmittance (c) %drug release after 300min

CONCLUSION

Proper selection of components is critical to an efficient nanoemulsion formulation. Low-molar-volume oils viz. Captex200M for Miconazole nitrate are preferable instead of high-molar-volume oils, as they usually show better solubilization of the drug. As of late, novel semisynthetic medium chain derivatives, which can be defined as amphiphilic compounds viz tween80 and capmulMCM with surfactant properties, are being preferred. Attention should be paid with regard to the tolerability of the constituting excipients. Recent efforts have, therefore, been focused on how to decrease or eliminate the toxicity or irritation of the nanoemulsion formulations. The study clearly illustrated the impact of the surfactant/cosurfactant weight ratio in the formulation of nanoemulsion systems. It is possible to achieve desirable properties by appropriately varying the level of oil, surfactants, and secondary surfactants. Optimization by factorial design showed a significant effect on the emulsification rates as well as the physical properties of the resultant nanoemulsion. The quantitative effect of these factors at different levels was predicted by using polynomial equations. Factorial design expert was then used to selection & validation of optimum formulation. A new formulation was prepared according to these levels. Observed responses were in close agreement with the predicted values of the optimized formulation, thereby demonstrating the feasibility of the optimization procedure in developing nanoemulsion formulations.

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