Design Expert Assisted Formulation of Topical Bioadhesive Gel of Sertaconazole Nitrate

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A B S T R A C T

Purpose *:* The objective of this work was to develop a bioadhesive topical gel of sertaconazole nitrate with the help of response-surface approach.

Methods: Experiments were performed according to a 3-level factorial design to evaluate the effects of two independent variables [amount of Carbapol 934 = X1) and Sodium carboxymethylcellulose (NaCMC) = X2)] on the bioadhesive character of gel, rheological property of gel (consistency index), and *in-vitro* drug release. The best model was selected to fit the data.

Results: Mathematical equation was generated by Design Expert® software for the model which assists in determining the effect of independent variables. Response surface plots were also generated by the software for analyzing effect of the independent variables on the response. The effect of formulation variables on the product characteristics can be easily predicted and precisely interpreted by using a 3-level factorial design and generated quadratic mathematical equations.

Conclusion: On the basis of product characteristics viscosity, bioadhesiveness, permeation study, in-vitro release, in-vivo studies, TPA and spreadability it can be concluded that the best batch of topical bioadhesive gel of Sertaconazole nitrate would be with 1% Carbopol 934 and 1% NaCMC.

Introduction

Sertaconazole Nitrate is an imidazole derivative antifungal agent. It has several actions like fungistatic, fungicidal, anti-bacterial, anti-inflammatory, antitrichomonal and antipruritic.[1-3] Sertaconazole blocks the synthesis of ergosterol by inhibiting the 14α-demethylase enzyme Ergosterol is a critical component of the fungal cell membrane.[4,5] Inhibition of ergosterol synthesis prevents fungal cells from multiplying and impairs hyphae growth. Chemically, Sertaconazole contains a benzothiophene ring which makes is unique from other imidazole antifungals.[1-6]

Percutaneous drug delivery has some advantages of providing the controlled delivery of drugs. In case of their application such as ointments, creams, it is difficult to expect their effects, because wetting, movement and contacting easily remove them.[7-10] There is a need to develop the new formulations that have suitable bioadhesion. The percutaneous administration of bioadhesive gels has good accessibility and can be applied, localized and removed easily. Because of its excellent accessibility, self-placement of a dosage form is possible .[11,12]

Oleic acid use as penetration enhancer in order to promote absorption of drug, it disrupts the skin barrier, fluidized the lipid channels between corneocytes, alter the partitioning of drug into skin structure or otherwise enhances the delivery into skin.

The mechanism of bioadhesion may include wetting and swelling of polymer, Interpenetration of bioadhesive polymer chain and entanglement of polymer and mucin chain and Formation of weak chemical bond between entangled chains. Carbopols are excellent bioadhesive polymers but they have very low pH in the range of 2.5-3.0 (1% aqueous solution). If they used alone, may cause irritation following topical application due to their low pH.[13-19] Its irritant properties can be reduced by combining it with other non-irritant bioadhesive polymers. Therefore, it was proposed to develop a topical bioadhesive gel systems of Sertaconazole nitrate.

As a result, the aim of the present study was to formulate and evaluate the bioadhesive Sertaconazole nitrate gel by using the combination of Carbopol 934 and Sodium carboxymethylcellulose. In the development of pharmaceutical dosage form with appropriate characteristics, an important issue is to design an optimized pharmaceutical formulation in a short time of period with minimum trials. For that now a day's response surface methodology (RSM) gaining attention to identify and quantify the effect of different formulation variables on the important characteristics.

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Factorial design (full factorial) is orthogonal experimental design. It also addresses the interaction between two variables and determines the effect on independent variables such as amount of NaCMC and Carbopol 934 on the formulation characteristics such as bioadhesion, viscosity, permeation study and in-vitro release studies .[20,21]

Materials and Methods
Materials
Sertaconazole nitrate was gifted from Glenmark pharmaceuticals, Carbopol 934 was obtained from Vishal Chem, Mumbai and Sodium CMC was obtained from Loba Chemie, Mumbai, oleic acid was obtained from Loba Chemie, Mumbai. All chemicals were used of analytical grade.

Experimental design
A 3-level factorial design was used to study the effect of two variables on characteristics of topical gel such as bioadhesiveness, viscosity, permeation study and % of drug release in 8 h. Dependent and in dependent variables along with their levels are listed in Table 1. Experimental design of different batches of bioadhesive topical gel is summarized.[21,22]

Table 1. Factors (independent variables), factor levels and responses (dependent variables) used in 3-level full factorial experimental design.

Factors	Type of factors	Factor level used				Response
		-1	0	1		
X_1	Carbopol 934 (%W/W)	0.5	1	1.5	Y_1	Bioadhesiveness (gf)
X_2	NaCMC (%W/W)	0.5	1	1.5	Y_2	Viscosity (cp)
	-				Y_3	Permeation study (%)
	-				Y_4	In-vitro studies (%)

Preparation of Sertaconazole Gel
The required amount of gelling polymer NaCMC and CB934P was weighed. Weighed polymers were added slowly in the beaker containing distilled water (40 ml) with continuous stirring at 400-600 rpm. The mixture was stirred continuously for 1 h until it forms a clear gel. Accurately weighed Sertaconazole nitrate was dissolved in 30 ml of ethanol and the ethanolic solution of drug was added slowly with stirring (400-600 rpm) in the previously prepared polymer gel. Oleic acid was added as a penetration enhancer (0.03 ml) was added with stirring. The final quantity was made up to 100 g with distilled water. The prepared gel was kept for 24 h for complete polymer desolvation .[17-19]

Viscosity Measurements
The samples were placed in beaker and were allowed to equilibrate for 30 min before measuring the viscosity. Viscosities of formulations before and after gelation were measured by using Brookfield DV-E viscometer using Spindle number-3 at 100 rpm shear rate at room temperature. The average of the reading was used to calculate the viscosity.[15-18]

Drug Content Uniformity
All prepared gels were analyzed for the desired range of Sertaconazole nitrate content and the samples which come within the range of 100 ± 10 were taken for in-vitro release studies. A 100 mg of gel was taken in 10 mL of water and mixed for 15 min. After filtration, 0.5 mL of solution was diluted to 5 mL by means of water and the absorbance of the solution was measured at 260 nm spectrophotometrically. The experiments were done in triplicate.[19]

Bioadhesive testing
The method developed by Singh was slightly modified for studying the bioadhesive character of the prepared gels. The apparatus used for study comprised of two arm balance, one side of which contains two glass plates and other side contains a container. One of the two glass plates were attached permanently with the base of the stage and other one attached with the arm of the balance by a thick strong thread. The membrane used for bioadhesive testing was fresh rat intestinal membrane. Fresh rat intestine was glued to the upper side of the lower plate and another was glued to the lower side of the upper plate by using cyanoacrylate adhesive. The weighed gel (0.5 g) was placed on the rat intestine glued to the upper side of the lower plate. Then upper plate was placed over the lower plate and 50 g preload force (or contact pressure) was applied for 5 min (preload time). After removal of the preload force, the water kept in a bottle at some height was siphoned in the container at the rate of 10 ml per min till the plates were detached from each other. The rate of dropping of water was controlled with on-off switch same as in infusion bottle. The weight of water required for detachment of glass plates was considered as the bioadhesion force of the applied gel.[18,19]

In vitro diffusion studies
In-vitro diffusion study of formulated bioadhesive gels was carried out on Franz diffusion cell having (Diameter of 1.5 cm with a diffusional area of 1.76 cm2). Rat abdominal skin was used as diffusion membrane. Pieces of rat abdominal skin membrane were soaked in phosphate buffer (PB) pH 6.8 for 8 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.8; Rat abdominal membrane was

mounted on cell. The temperature was maintained at 37±0.5 °C. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 2.0 mg of Sertaconazole was placed in the donor chamber. At predetermined time points, 0.5 mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.8 after each sampling, for a period of 8 hrs. The samples withdrawn were filtered and used for analysis. Blank samples (without Drug) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-spectrophotometer at 260 nm.[19-21]

In vivo study

The digital plethysmometer (Ugo Basile, 7140), which was used for determination of % of inhibition edema. The rats of either sex (150-200 g) were divided into four groups containing six animals in each. The rats were fasted for 12 hours prior to induction of edema however water was available *ad libitum*. Inflammation of hind paw was induced by injecting 0.1 ml of 1% carrageenan in normal saline into the subplantar region of right hind paw. The negative control group was received gel formulation without active drug, the positive control group was received standard marketed gel formulation (standard group), third group was treated with novel formulation (test group) and fourth group was normal. The gel formulations or the reference was applied to the plantar surface of the left hind paw by gently rubbing 0.5 g of the formulation 50 times with the index finger 1 hr before the carrageenan injection. The paw volume was measured with a digital plethysmometer before and 1, 3, 6 hrs after carrageenan injection.

The % inhibition will be calculated by following formula:

$$\% \text{ inhibition of edema} = 100\,(1 - Vt/Vc)$$

Where Vt: volume of test; Vc: volume of control

Ex-vivo permeation studies

The percutaneous permeation studies were done by using modified Franz diffusion cell (Diameter of 1.5 cm with a diffusional area of 1.76 cm2) and membranes used was rat abdominal skin. Prepared skin samples (rat skin) was mounted on the receptor compartment of the permeation cell with the stratum corneum facing upward and the dermis side facing downward.

The donor compartment was kept on the receptor compartment and secured tightly with the help of clamps. The receptor compartment was then filled with 30 ml of pH 6.8\ phosphate buffer. The temperature of media was maintained at 37 ± 0.5 °C with the help of temperature controlled water jacket. Weighed bioadhesive gel (2 g equivalent to 50 mg of SN) was then placed in the donor compartment. The receptor compartment containing pH 6.8 phosphate buffer was stirred at 200 ± 5 rpm to maintain the hydrodynamics of the receptor fluid. Sampling (0.5 ml) was carried out at the different intervals up to 8 h. The volume of

release media was maintained by adding equal volume of the fresh media after every sampling. The concentration of SN in the sample was measured spectrophotometrically at 260 nm.[18,19-21]

Texture Profile Analysis

Texture profile analysis (TPA) was performed using a CT3 Texture Analyzer in TPA mode. Formulations (35 g) were transferred into 50-ml bottles, taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (35 mm diameter) was forced down into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). At least five replicate analyses of each sample were performed at temperatures of 25 °C and 35 °C. From the resulting force–time plots, the hardness (the force required to attain a given deformation), compressibility (the work required to deform the product during the first pass of the probe) and adhesiveness (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived.[27]

Spreadability test

Spreadability test was performed by using CT3 Texture Analyzer in Compression mode. A cone analytical probe (60°) was forced down into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). The test was performed and results were observed.[27] When a trigger force of 10 g has been achieved, the probe proceeds to penetrate the sample at a test speed of 2 mm/s to a depth of 25 mm. During this time, the force to penetrate the sample increases. When the specified penetration distance has been reached, the probe withdraws from the sample at the post-test speed of 2 mm/s. The maximum force value on the graph is a measure of the firmness of the sample at the specified depth. The area under the positive curve is a measure of the energy required to deform the sample to the defined distance (Hardness Work Done). Research has shown that the firmness and energy required deforming a sample to a defined depth grades samples in order of spreadability. A higher peak load (firmness) and hardness work done value indicate a less spreadable sample. Conversely, a lower peak load (firmness) value coupled with a lower hardness work done value indicates a more spreadable sample.

Statistical analysis of the data

Various RSM computations for the current study were performed employing 45 days Trial Version of Design-Expert software (Version 8.0.6., Stat-Ease Inc., and Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all response variables using multiple linear regression analysis. Equations were calculated to determine the effect of each variable on the formulation characteristics. Statistical validity of the model was established on the basis of Analysis of variance

(ANOVA) and the 3D response graphs were constructed using Design-Export software.[20, 21]

Results and Discussion

An ideal formulation for local delivery should exhibit ease of drug release, a good retention at application site and controlled release of drug. The application of bioadhesive gel provides a long stay, adequate drug penetration, high efficiency and acceptability. The NaCMC and carbopol 934 biocompatible and biodegradable polymer has been widely used for pharmaceutical and medical application.

Viscosity Measurements

From this study we can observe that as the concentration of polymer increases the viscosity of the formulation increases consequently. From the results at the high concentration of polymer i. e 1% carbopol and 1% NaCMC the viscosity found highest.

Bioadhesive testing

In this case we have observed that as the concentration of polymer increases the viscosity and bioadhesion property of the formulation also increases simultaneously. From the results we can conclude that at the high concentration of polymer i.e. 1% carbopol and 1% NaCMC the viscosity and bioadhesion was highest amongst all batches.

In vitro diffusion studies

The Figure 1 shows comparative in vitro performance of various batches of bioadhesive gel . *In vitro* diffusion study was performed by using dialysis membrane. The % Cumulative drug release from bioadhesive gel in 8 hrs was found to be 95.32% respectively. The result indicates that, the bioadhesive gel having higher conc. of polymer i.e. 1% carbopol and 1% NaCMC has given the highest drug release.
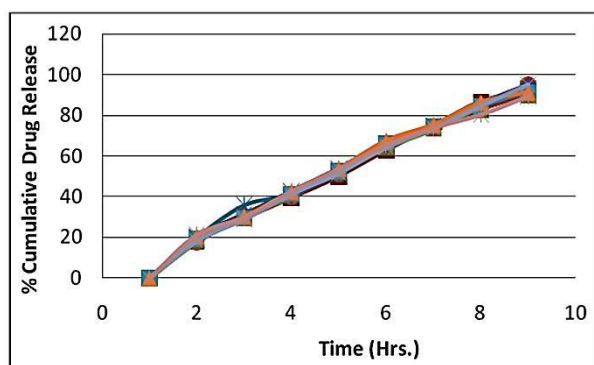

Figure 1. In vitro diffusion study

In vivo anti-inflammatory study
Carrageenan induced paw edema

The Anti-inflammatory effect of developed Bioadhesive gel formulation was compared with marketed volini gel. The results of these studies are given in Table 2. The Figure 2 shows that the mean percent edema value after 6 h application was found to be highest for bioadhesive gel as compared to marketed volini gel. This difference was extremely significant at 5% level of significance ($p < 0.05$). Initially the mean percent (%) edema value was high for bioadhesive gel formulation but after 3 h there was a marked decrease in this value which indicates the increase in inflammation. The anti-inflammatory effect of marketed volini gel was up to 6 h significantly increases the percent inhibition of rat paw edema volume then that of prepared bioadhesive gel formulation. So formulated bioadhesive gel indicates that it can be used for local as well as for transdermal drug delivery system. An increase in systemic anti-inflammatory effect of sertaconazole leads to complete inhibition of the inflammation process.

Table 2. Effect of Sertaconazole bioadhesive gel on carrageenan induced paw edema

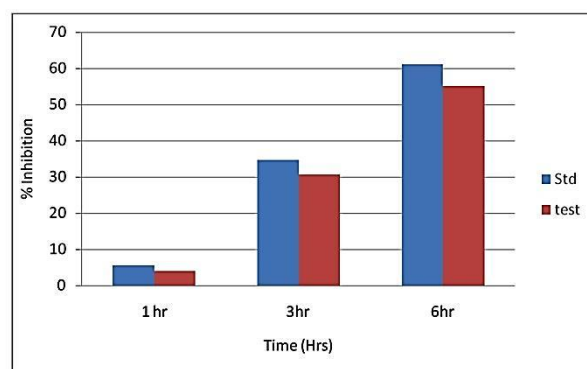| Group	Formulation	N	Mean wt.(g)	Time (h)	Mean %Edema	%inhibition
1	Control	6	230	1	29.2	-
				3	73.9	-
				6	57.8	-
2	Bioadhesive gel	6	245	1	28.1	3.91%
				3	51.2	30.71 %
				6	25.9	55.19 %
3	Marketed gel	6	235	1	27.6	5.47 %
				3	48.2	34.77 %
				6	22.5	61.07 %
N: Number of rats in each group						

Figure 2. Percent inhibition of rat paw edema volume

Ex-vivo permeation studies

The *Ex-vivo* study was carried out by using rat abdominal skin. The % Cumulative drug release from bioadhesive gel in 8 hrs was found to be 97.23% respectively. The graphical presentation of Ex-vivo permeation study is shown in Figure 3. The result indicates that, the bioadhesive gel having higher conc. of polymer i.e. 1% carbopol and 1% NaCMC found the highest drug release.
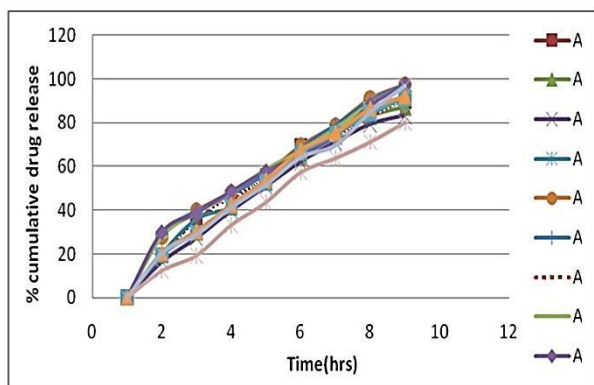
Figure 3. Ex vivo permeation study by using rat abdominal skin

Experiments of 3-level factorial design

Response data for all the 13 experimental runs of 3-level factorial design, performed in accordance with Table 2, are presented in Table 3.

Mathematical modeling

Mathematical relationship was generated between the factors (independent variables) and responses (dependent variables) using the statistical package Design-Expert. First step in mathematical modeling was fitting the experimental data to appropriate model. A suitable model was selected by software on the basis of different parameter obtained from regression analysis such as p-value, adjusted R2, predicted R2 and Predicted Residual Sum of Square (PRESS) value (Tables 4 and 5). Table 3 lists the values of various response parameters of the prepared batches. ANOVA

was applied for estimating the significance of model, at 5% significance level. If more than one model was significant (p < 0.05) for the response, the adjusted R2 and PRESS value of the model were compared to select the best mathematical model for that response. Focus on maximizing the value of adjusted R2 and predicted R2. Low PRESS value indicated adequate fitting of model . General quadratic equation for two independent variables is as follow:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2$$

Where: $\beta 0$ is the intercept representing the arithmetic averages of all the quantitative outcomes of 13 runs. $\beta 1$ to $\beta 5$ are all coefficients calculated from the observed experimental values of Y. X_1 and X_2 are the coded levels of factors. The terms $X_1 X_2$ and $X_i 2$ (i ϵ {1, 2}) represent the interaction and quadratic terms, respectively. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Synergistic effect and antagonistic effect of factor were indicated by positive sign and negative sign in front of that factor term, respectively.

Drug Content Uniformity

The drug content of the formulated batches was ranging from 92.2% to 99.12%.

Table 3. Results for bioadhesion, viscosity, permeation and in-vitro studies of prepared topical bioadhesive gel with 3-level factorial experimental design.

Run	Viscosity (cp)	Bioadhesiveness (gf)	Permeation (%)	In vitro drug release (%)
1	799	94	90.12	91.23
2	789	91	87.12	90.18
3	891	92	83.24	92.34
4	512	82	90.46	93.2
5	751	98	97.23	95.32
6	751	98	97.23	95.32
7	678	90	91.12	90.12
8	751	98	97.23	95.32
9	751	98	97.23	95.32
10	647	92	91.12	91.22
11	643	91	92.09	91.23
12	751	98	97	95.32
13	544	70	80.23	89.33

Table 4. Fit summary of model for the measured responses Y 1 (Viscosity in cp), Y2 (Bioadhesion in gf), Y3 (Permeation study in %) and Y4 (Cumulative percentage release at 8 h in %).

Source	Y1		Y2		Y3		Y4	
	f-value	p-value	f-value	p-value	f-value	p-value	f-value	p-value
Linear vs Mean	23.75	0.0002	2.56	0.1266	2,617E-0.003	0.9974	0.24	0.7899
Quadratic vs 2FI	8.25	0.0144	28.78	0.0004	44.06	0.00001	12.67	0.0047

Viscosity Measurements

From the p-values presented in Table 4, linear model and quadratic model was found to be significant for viscosity. Quadratic model was selected on the basis of maximum value of adj.R^2 and low PRESS value indicating adequate fitting of model mentioned in Table 5. Quadratic model was significant with model f-value of 29.73 (p-value<0.0001). The quadratic equation generated by software is as follows:

$$Y1 = 741.41 + 78.67X_1 + 109.17X_2 + 27.50X_1X_2 + 3.55X_1^2 - 66.95X_2^2$$

Equation reveals that both factors (X1 and X2) affect viscosity characteristics of gel significantly. Equations also indicated that the effect of the change in NaCMC concentration seems to be more pronounced in comparison with that of the change in Carbopol 934 concentration since the coefficient of factor X2 has a larger value than that of factor X1. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots (Figure 4A), which demonstrated that Y1 varies in a linear fashion with the amount of both the polymers. However, the steeper ascent in the response surface with NaCMC (X2) – instead of Carbopol (X1) – is clearly discernible from response surface plots, indicating that the effect of NaCMC is comparatively more pronounced than that of Carbopol. From this discussion, one can conclude that the bioadhesion may be changed by appropriate selection of the levels of X1 and X2. (Figure 4B) shows a linear relationship between the observed response values and the predicted values indicating the correctness of the model. The details of Analysis of variance (ANOVA) table for measured responses is presented in Table 6.
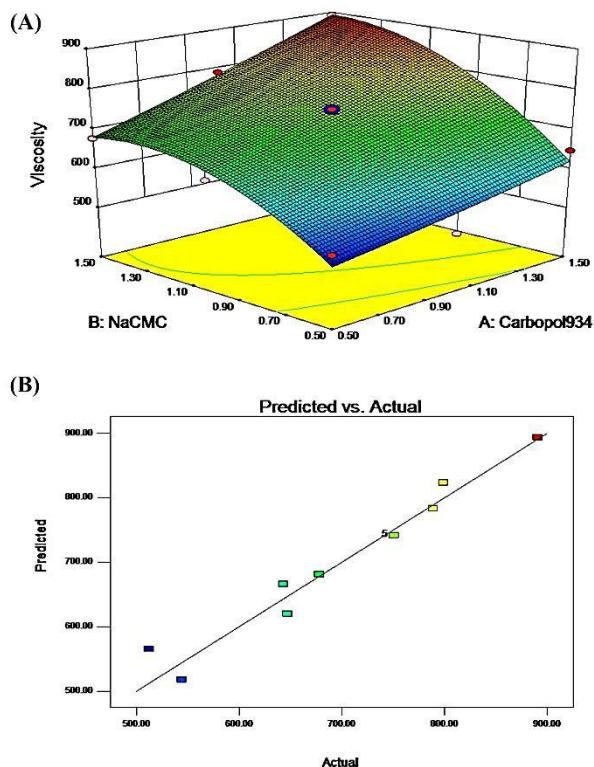
(A)

(B)

Figure 4. (A) Response surface plot showing the effect of Carbopol 934 and NaCMC on Viscosity (Y1); (B) Linear plot between observed and predicted value of Y1.

Table 5. Model statistical summary of responses for selection suitable model.

Source	Linear			Quadratic		
	Adj. R^2	Pred.R^2	PRESS	Adj. R^2	Pred.R^2	PRESS
Y1	0.7913	0.6788	42242.55	0.9229	0.6274	49000.59
Y2	0.2063	-0.3564	1048.22	0.9011	0.4878	395.81
Y3	-0.1994	-1.1123	801.30	0.9032	0.5706	185.64
Y4	-0.1447	-0.6797	111.14	0.6463	-0.7489	114.67

Table 6. Analysis of variance (ANOVA) table for measured responses.

Model	Y1		Y2		Y3		Y4	
	f-value	p-value	f-value	p-value	f-value	p-value	f-value	p-value
Model	29.73	0.0001	22.87	0.0003	23.40	0.0003	5.39	0.0238
X_1	43.95	0.0003	19.08	0.0033	0.059	0.8152	1.46	0.2602
X_2	84.64	<0.0001	22.01	0.0022	5.932E-0.03	0.9408	0.11	0.7540
$X_1 X_2$	3.58	0.1003	15.70	0.0054	28.79	0.0010	0.014	0.9089
X_1^2	0.041	0.8449	4.13	0.0816	16.57	0.0047	9.43	0.0181
X_2^2	14.65	0.0065	35.81	0.0006	39.32	0.0008	6.35	0.0398

Bioadhesive testing

From the p-values presented in Table 4, linear model and quadratic model was found to be significant for bioadhesion. Quadratic model was selected on the basis of maximum value of adj. R2 and low PRESS value indicating adequate fitting of model (Table 5). Quadratic model was significant with model f-value of 22.87 (p-value<0.0003). The quadratic equation generated by software is as follows:

$$Y1 = 97.31 + 4.50X_1 + 4.53X_2 - 5.00X_1X_2 - 3.07X_1^2 - 9.09X_2^2$$

Equation reveals that both factors (X1 and X2) affect bioadhesion characteristics of gel significantly. Equations also indicated that the effect of the change in

NaCMC concentration seems to be more pronounced in comparison with that of the change in Carbopol934 concentration since the coefficient of factor X2 has a larger value than that of factor X1. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots (Figure 5A), which demonstrated that Y2 varies in a linear fashion with the amount of both the polymers. However, the steeper ascent in the response surface with NaCMC (X2) – instead of Carbopol 934 (X1) – is clearly discernible from response surface plots, indicating that the effect of NaCMC is comparatively more pronounced than that of Carbopol. From this discussion, one can conclude that the bioadhesion may be changed by appropriate selection of the levels of X1 and X2. (Figure 5B) shows a linear relationship between the observed response values and the predicted values indicating the correctness of the model.
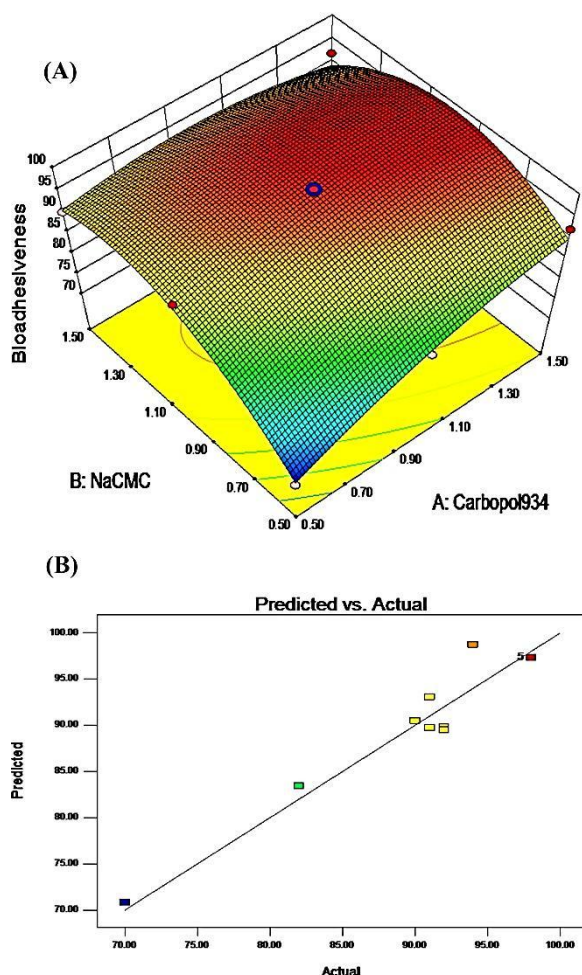

Figure 5. (A) Response surface plot showing the effect of Carbopol 934 and NaCMC on Bioadhesion (Y2); (B) Linear plot between observed and predicted value of Y1.

Permeation studies
From the p-values presented in Table 4, quadratic model was found to be significant for bioadhesion. Quadratic model was selected on the basis of maximum value of adj. R^2 and low PRESS value indicating adequate fitting of model (Table 5).

Quadratic model was significant with model f-value of 23.40 (p-value<0.0003). The quadratic equation generated by software is as follows:

$$Y1 = 34.12 + 53.39X_1 + 71.46X_2 - 18.77X_1X_2 - 17.13X_1^2 - 26.40X_2^2$$

Equation reveals that both factors (X1 and X2) affect permeation characteristics of gel significantly. Equations also indicated that the effect of the change in NaCMC concentration seems to be more pronounced in comparison with that of the change in Carbopol934 concentration since the coefficient of factor X2 has a larger value than that of factor X1. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots (Figure 6A), which demonstrated that Y3 varies in a linear fashion with the amount of both the polymers. However, the steeper ascent in the response surface with NaCMC (X2) – instead of Carbopol 934 (X1) – is clearly discernible from response surface plots, indicating that the effect of NaCMC is comparatively more pronounced than that of Carbopol. From this discussion, one can conclude that the bioadhesion may be changed by appropriate selection of the levels of X1 and X2. (Figure 6B) shows a linear relationship between the observed response values and the predicted values indicating the correctness of the model.
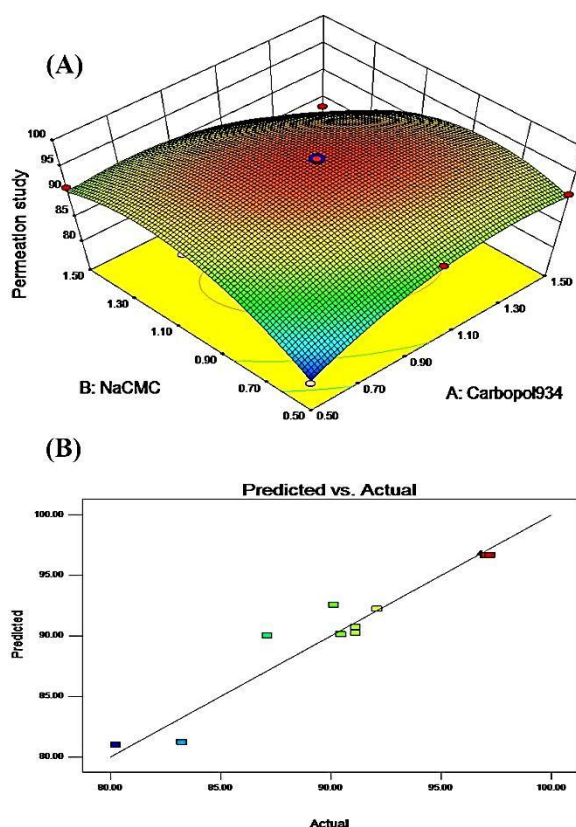

Figure 6. (A) Response surface plot showing the effect of Carbopol 934 and NaCMC on permeation (Y3); (B) Linear plot between observed and predicted value of Y1.

In vitro diffusion studies
From the p-values presented in Table 4, quadratic contribution were found to be significant since p-

value is less than 0.05 for both sources. In this case, A, B, AB, A2 and B2 are significant model terms. PRESS value for quadratic model (114.67) was found as indicating in Table 5. Therefore, quadratic model was selected to fit the data of this response. Quadratic model was significant with model f-value of 5.39 (p-value<0.0238). The quadratic equation generated by software is as follows:

$$Y1 = 94.89 + 0.68X_1 - 0.18X_2 + 0.083X_1X_2 - 2.57X_1^2 - 2.11X_2^2$$

In this case, all the model terms (X1, X2, X1X2, X12 and X22) were found to be significant (Table 6). The equation reveals that both factors have antagonistic effect on the drug release. CPR of the drug in 8 h with highest polymer content (1.5% Carbopol 934 and 1.5% NaCMC) was found to be lowest. However, in this equation, it was clearly indicating that the retarding effect of NaCMC was more prominent than Carbopol 934. Coefficient of interactions shown in above equation was also significant which confirms the formation of rigid gel structure of carbopol 934 with NaCMC. At high concentration of NaCMC and Carbopol 934, a very thick gel (highest consistency index value) was formed which provide a very slow release of drug. (Figure 7A) represented the response surface indicating the more pronounced effect of NACMC than Carbopol 934 on Y4. (Figure 7B) represented the observed response value compared with that of predicted values indicating the correctness of model.
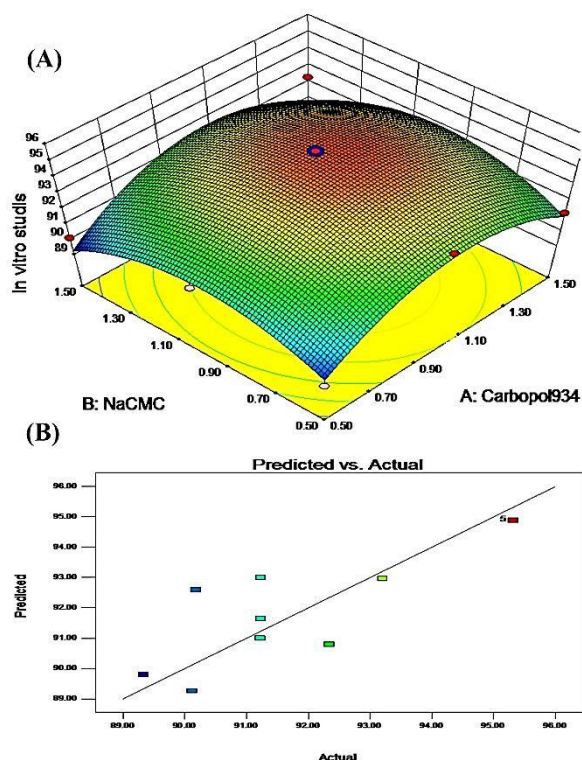

Figure 7. (A) Response surface plot showing the effect of Carbopol 934 and NaCMC on In-vitro studies (Y4); (B) Linear plot between observed and predicted value of Y1.

Texture profile analysis (TPA) and Spreadability testing

TPA is a method to determine mechanical properties of gel in which an analytical probe is twice depression into the sample at a defined rate to a desired depth, allowing a predefined necessary period between the end of the first and the beginning of second compression shows in Figure 8 (A).

The peak / maximum force is taken as a measurement of firmness; higher the value the thicker is the consistency of the sample. The negative region of the graph produced on probe return is an a result of the weight of sample which is lifted primarily on the upper surface of the disc on return i.e. due to the back extrusion and hence gives again an indication of consistency or resistance to flow off the disc. The maximum negative force is taken as an indication of the stickiness / cohesiveness of the sample. The more negative the value the more stuff the sample.

The observations for spreadability of all formulations are shown in Figure 8 (B). The spreadability of the formulations is a characteristic derived from its more basic property i.e. viscosity. The greater the viscosity the longer will be the time taken for spreading. The gels are expected to spread easily on the skin areas when applied. The spreadability also depends on the polymer in formulation, possessing typical physicochemical properties which create surface tension between slide and product.
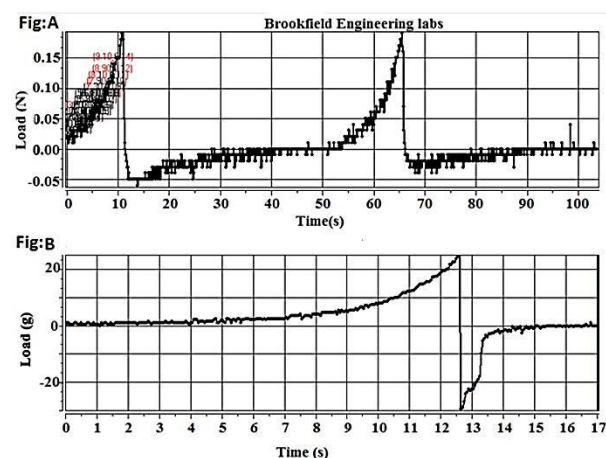

Figure 8. A] Texture profile analysis (TPA) spectra of bioadhesive gel. B] Spreadability pattern of bioadhesive gel.

Conclusion

The present study concludes that topical bioadhesive gel of Sertaconazole nitrate can be formulated by using combination of Carbopol 934 and NaCMC employing the response surface approach. The effect of formulation variables on the product characteristics can be easily predicted and precisely interpreted by using a 3-level factorial design and generated quadratic mathematical equations. On the basis of product characteristics viscosity, bioadhesiveness, permeation study, in-vitro release, TPA and spreadability it can be concluded that the best batch of topical bioadhesive gel

of Sertaconazole nitrate would be with 1% Carbopol 934 and 1% NaCMC.

In case of TPA of the bioadhesive gel, as the probe of texture analyzer returns to its starting position, the initial lifting of the weight of the sample on the upper surface of the disc produces the negative part of the graph. This gives an indication of the cohesiveness and resistance of the sample to separate (flow off) from the disc. The maximum negative force on the graph indicates sample adhesive force; the more negative the value the more "sticky" the sample. The area under the negative part of the graph is known as the adhesiveness (the energy required to break probe sample contact) and can give an indication of the cohesive forces of the molecules within the sample. The sertaconazole bioadhesive gel is more adhesive or "sticky" and therefore it is more cohesive than other batches.

Research has shown that the firmness and energy required deforming a sample to a defined depth grades samples in order of spreadability. A higher peak load (firmness) and hardness work done value indicate a less spreadable sample. Conversely, a lower peak load (firmness) value coupled with a lower hardness work done value indicates a more spreadable sample. From Figure 8, Sample of bioadhesive gel is significantly much firmer and has a higher hardness work done (area under the positive curve). This indicates that sertaconazole bioadhesive gel is more spreadable than the other batches prepared.

Conflict of Interest

The authors report no conflicts of interest.

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