A NOVEL EFFERVESCENT BIOADHESIVE VAGINAL TABLET OF KETOCONAZOLE: FORMULATION AND IN-VITRO EVALUATION

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Abstract: Purpose: To develop and evaluate effervescent bioadhesive vaginal tablet of ketoconazole. Methods: The vaginal tablets were prepared by direct compression. HPMC K4M, HPMC K15M, HPC, Sodium CMC, Chitosan, Sodium Alginate, Methyl Cellulose and Cabopol 941 were used as bioadhesive polymers. Effervescent was incorporated into the formulations as a disintegration agent. The amount of polymer blends and effervescent mixture was optimized using $3^2$ full factorial design. The swellings and in-vitro release were studied. The ex-vivo mucoadhesion was determined by self developed modified mucoadhesion assembly. The ex-vivo residence test was carried out by modified USP dissolution test apparatus. Anti-fungal activity of the effervescent bioadhesive tablet of ketoconazole was determined in comparison to Candida®-V3 tablet and Candida®-V gel. Results: A good sustained effect and a moderate bioadhesion were (0.088N to 0.267N) obtained with the tablet containing HPMC K4M: Chitosan (1:1) and effervescent mixture (3:1). Ex-vivo mucoadhesion time of all the formulations was in the range of 8 to 24 h. The effervescent ketoconazole tablet showed significantly higher ($p<0.05$) in-vitro antifungal activity as compare to Candida®-V3 tablet and Candida®-V gel. Conclusion: Our study may provide a potential vaginal formulation of ketoconazole against Candida albicans.

Key words: Bioadhesion, vaginal drug delivery, mucoadhesive polymers, effervescent, C. albicans

INTRODUCTION

In recent years vaginal bioadhesive preparations have been developed as a new type of controlled-release form for the treatment of both topical and systemic diseases. For drugs which are susceptible to gut or hepatic metabolism or which cause GI side effects, vaginal bioadhesive delivery may offer a number of advantages over the other routes of administration. The greatest advantage of such dosage forms is the possibility of maintaining them in the vagina for extended periods of time including daytime and nighttime, thereby enabling lower dosing frequencies. Vaginal candidiasis is a common condition and up to 75% of all women suffer at least one episode of this infection during their lifetime. Candida albicans is the most important cause of vaginal candidiasis, accounting for over 80% of the infection. Most patients with Candida vaginitis respond to topical treatment with nystatin or imidazoles. Ketoconazole (KTZ) is an imidazole derivative antifungal agent developed for treatment of human mycotic infections and plays an essential role in antifungal chemotherapy. In the literature, some additives, such as sodium bicarbonate and citric acid, were added into formulations to improve the disintegration and the dissolution of bioadhesive vaginal tablets because there is rather low moisture content in vagina under normal physiological conditions. The aim of this study was to prepare a new bioadhesive effervescent vaginal tablet formulation of
ketoconazole against *C. albicans*. Effervescent added into the formulations as disintegration agent would be expected to increase the dissolution of ketoconazole. Hence, the present work was envisaged to develop a novel and aesthetic bioadhesive vaginal delivery system with improved efficacy. The following bioadhesive polymers were screened to develop a vaginal delivery system: HPMC K4M, HPMC K15M, HPC, Sodium CMC, Chitosan, Sodium Alginate, Methyl Cellulose and Carboxpol 941. The performances of these bioadhesive polymers were evaluated by two parameters, the swelling behavior and the bioadhesive strength. For the various drug-free formulations, the effect of effervescent on polymers’ bioadhesive characteristics was investigated. On the basis of these data, suitable polymers were selected to prepare the bioadhesive effervescent vaginal tablets of ketoconazole. Swelling, bioadhesive properties and drug release of the tablets with different proportions of bioadhesive polymer and effervescent in formulations were conducted. Anti-fungal activity of the effervescent bioadhesive tablet of ketoconazole was determined in comparison to Candida®-V3 tablet and Candida®-V gel.

**MATERIALS AND METHODS**

**Materials**

Ketoconazole was kindly gifted by Ranbaxy Laboratories Limited (Mumbai, India). Carboxpol 941 was kindly gifted by Corel Pharmaceutical Ltd. (Ahmedabad, India). HPMC K4M, HPMC K15M and HPC were received as gift samples from Zydus-Cadila Healthcare Ltd. (Ahmedabad, India). Sodium CMC was kindly gifted by Mann Pharmaceuticals Pvt. Ltd. (Mehsana, India). Chitosan was obtained as a gift sample from Troikka Pharmaceuticals Ltd. (Ahmedabad, India). Sodium Alginate, Methyl Cellulose and MCC were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India). All other ingredients were of laboratory grades. Candida®-V3 tablet and Candida®-V gel were purchased from local market.

**Preliminary screening of ketoconazole effervescent vaginal tablet**

Different mucoadhesive polymers were screened for ketoconazole vaginal tablet. The formulations were prepared by direct compression technique. The required quantity of polymers, ketoconazole, effervescent (sodium bicarbonate and citric acid at the mole ratio of 3:1) and the other formulation ingredients were compressed using single punch tablet machine (Cadmack, Ahmedabad, India). The tablet mould was especially designed using stainless steel and the formulated tablets had an average weight 800 ± 1.5 mg, 20.7 ± 0.2 mm height, 8.5 ± 0.3 mm width and 5.27 ± 0.057 mm thickness.

**Swelling study**

The swelling behavior of tablet described as the water absorbing capacity. Drug-free tablets were weighed individually (W₀) and placed separately in 2% agar plates and incubated at 37 ± 1°C. At regular time intervals up to 4 hours, the tablets were removed from the petri dish and wiped with filter paper carefully. The swollen tablet was then reweighed (Wₜ) and the % swelling were calculated using the following formula:

\[ \% \text{ Swelling} = \left\{ \frac{(W_t - W_0)}{W_0} \right\} \times 100 \]  

Where, W₀ is the weight of the tablet at time t and W₀ is the initial weight of tablet. The swelling was calculated and then plotted as a function of time. The slope of the linear plots was taken as the swelling rate.

**Optimization using 3² full factorial design**

A statistical model incorporating interactive and polynomial term was used to evaluate the response:

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \]

Where, Y is the dependent variables, b₀ is the arithmetic mean response of the nine runs, and bᵢ is the estimated coefficient for the factor Xᵢ. The main effects (X₁ and X₂) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X₁X₂) show how the response changes when two factors are simultaneously changed. The polynomial terms (X₁² and X₂²) are included to investigate non-linearity.

A 3² full factorial design was adapted to optimize the variables. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. Polymer ratio (X₁) and amount of effervescent (X₂) were selected as independent variables. Mucoadhesive strength, % swelling, Qₘ and tₘ₀ were selected as dependent variables (response; Y). The preparation and evaluation method for tablets and amount of Ketoconazole were kept constant for all the trials. The full factorial design layout, coded values for polymer ratio(X₁) and amount of effervescent mixture(X₂), and composition of factorial batches A1 to A9 are shown in Table I and Table II respectively.

**Physicotechnical parameters of effervescent mucoadhesive tablet**

The uniformity of weights of all tablets was determined by using sartorious balance (Model CP-224 S). The hardness of the tablets was determined by diametral compression using a dial type hardness tester (Model no 1101, Shivani Scientific India). Tablet thickness was measured using vernier calipers.
Ex-vivo mucoadhesion study
Several types of mucosa including rat intestine, pig oral, bovine sublingual, cow vaginal mucosa \(^9,10\), have been used as model biological tissues for the evaluation of bioadhesion. A modified self developed force detachment devise was used to measure the minimum detachment force (Figure 1). A piece of rat intestine (2.0 cm x 1.0 cm) removed from newly sacrificed rat was adhered to a piece of glass, which was fixed on a plank and the plank was assembled with a little crown block. After hydrating the rat intestine with distilled water, the tablet was brought into contact with the rat intestine by applying little force for minute. After the initial contact, the tablet was encircled by a thread which fastened a light plastic beaker through the crown block. Next, water was dropped into the beaker at a speed of 3.0 ml/min using peristaltic pump until the tablet and rat intestine were pulled apart by the gravity of water. The beaker containing water was weighed and the minimum detachment force was calculated accordingly. The experiments were performed in triplicate and average values with standard deviation (SD) were reported. The study was approved by Shree S. K. Patel College of Pharmaceutical Education and Research, Institutional Animal Ethics Committee and the approval document no. was SKPCPER-IAEC/2008/10.

Ex-vivo mucoadhesion time
The Ex-vivo residence time was studied using a locally modified \textit{USP} paddle apparatus (Figure 1, Dissolution test apparatus type-I). The dissolution medium (500 ml citrate buffer pH 4.0) was maintained at 37 °C. A segment of rat intestine, 2.5 cm long, was glued to the surface of a glass slab, vertically attached to the paddle. The mucoadhesive tablet was hydrated from one surface using 15 µl phosphate buffer and then the hydrated surface was brought into contact with the mucosal membrane. The glass slide was vertically fixed to the paddle and allows rotating at 50 rpm. The time required for complete detachment of the tablet from the mucosal surface was recorded (mean of triplicate determinations).\(^11\)

In-vitro ketoconazole release study
The release rate of ketoconazole effervescence mucoadhesive vaginal tablet (n=3) was determined using \textit{The United States Pharmacopoeia} (USP) XXIV dissolution testing apparatus I (basket method) using 500ml of citrate buffer pH 4.0 as a dissolution medium. The tablet was placed in a settling basket to prevent the tablet from floating.\(^12\) The rate of stirring was 50 rpm and the medium temperature was maintained at 37 ± 0.5 °C. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered through a filter and diluted to a suitable concentration with citrate buffer pH 4.0. Absorbance of these solutions was measured at 221 nm using a Shimadzu UV-1601 UV/Vis double beam spectrophotometer. Cumulative percentage of drug release was calculated using the equation obtained from a standard curve.

Statistical analysis
Tests for significant differences between means were performed by Student’s \(t\)-test or one-way ANOVA by using the software Sigma State version 10. Differences were considered significant at \(P < 0.05\) level.

The surface response plot
The surface response plots were drawn using Sigma plot software version 10 (Jandel Scientific, San Rafael, CA).

Kinetic modeling of drug release
Curve fitting was performed using Microsoft Excel 2007 version. The dissolution data were fitted to following equation. Release exponent ‘\(n\)’ was calculated (N. A. Peppas et al., 1985 and Korsemeyer R. W. et al., 1993).\(^13\)

\[
\frac{M_t}{M_{\infty}} = Kt^n
\]

Where, \(M_t / M_{\infty}\) is the fraction of the drug released at time \(t\), \(k\) is the kinetic constant of the system, and \(n\) is the exponent characteristic of the mode transport. The release exponent takes various values depending upon different geometries. For the drug release from a cylindrical or a flat swellable polymer, if \(n\) approaches to 0.89, the release mechanism could be Case-II transport and if \(n\) is close to 0.45, the release mechanism can be Fickian. On the other hand if \(0.45 < n < 0.89\), non-Fickian transport could be obtained.

In-vitro antifungal study
In-vitro antifungal study was performed against \textit{Candida albicans} in Sabouraud’s agar medium by the cup plate method.\(^14\) The cups cut in the inoculated solidified media were filled with different formulations using sterilized syringes. The marketed tablet (Candid®-V gel) was crushed into a powder and dissolved in 2 mL of sterilized water was applied using sterilized syringe. The marketed gel (Candid®-V gel) was applied using the sterilized syringe. The developed ketoconazole effervescence mucoadhesive vaginal tablet was swelled in 2 mL of sterile water applied into the cups. The covered petri plates were incubated at 22°C in the BOD incubator for 48 hours. The zone of inhibition was measured at the end of 48 hours.

Accelerated stability study of best batch
In order to determine the change in \textit{in-vitro} release profile and mucoadhesive strength on storage,
In-vitro the polymers could be hydrated more easily. Contacting between polymer particles and water so that tablets. The porous channels increased the area of and construct porous channels on surface and inside of effervescent, which made tablets increase in volume effervescent. The phenomenon of swelling increasing formulation, increased with increasing amount of swelling rate was developed as effervescent applied to tablets without effervescent. It was observed that the effervescent showed a higher swelling capacity than tablets without effervescent. It was observed that the swelling rate was developed as effervescent applied to formulation, increased with increasing amount of effervescent. The phenomenon of swelling increasing could be explained by the good disintegration effect of effervescent, which made tablets increase in volume and construct porous channels on surface and inside of tablets. The porous channels increased the area of contacting between polymer particles and water so that the polymers could be hydrated more easily.

RESULTS AND DISCUSSION

Preliminary screening

Swelling study

Swelling is important for the assessment of adhesion. Shortly after swelling, adhesion does occur, but with a weak bond formed. To develop maximum adhesion strength, an optimum water concentration was needed for polymer particles.

It was observed that the order of swelling rate was Carbopol 941 > Chitosan > Sod.CMC > HPMC K4M > HPMC K15M > Sod.alginate > MC in drug free formulations. According to the comparison of the corresponding swelling profiles of formulations with/without effervescent, it could be seen that the effervescent resulted in a marked increase in swelling rate. Furthermore, most tablets with 100 mg effervescent showed a higher swelling capacity than tablets without effervescent. It was observed that the swelling rate was developed as effervescent applied to formulation, increased with increasing amount of effervescent. The phenomenon of swelling increasing could be explained by the good disintegration effect of effervescent, which made tablets increase in volume and construct porous channels on surface and inside of tablets. The porous channels increased the area of contacting between polymer particles and water so that the polymers could be hydrated more easily.

In-vitro dissolution study

The dissolution of tablets were carried out in citrate buffer pH 4.0. In all the formulations, the burst release of ketoconazole was observed within 2 hrs. and gradually increasing upto 8-10 hrs. Formulations (F1 to F8) contained single mucosahesive polymer. Formulations F1 and F2 (carbopol 941 and chitosan) swelled rapidly with aqueous fluid and formed rigid structure and provided a stronger sustained release effect on the drug release. Formulations F2, F3, F4 and F6 ( HPMC K4M, HPMC K15M, HPC and Sod.alginate) were more erodible resulting in less prolong release. Formulations F5 and F7 ( Sod. CMC and MC) swelled rapidly when in contact with aqueous fluid to form gel which prevented fast disintegration of the tablet and formation of prolong release gel.

Formulations F9 and F14 containing Carbopol 941 led to gradual swelling. The drug release was in a controlled manner for more than 8 hrs. The percentage drug release after 8 hrs was 72.36% and 67.11 % respectively. Formulations F11, F13 and F16 shows rapid disintegration , more erodibility and complete drug release within 5-7 hrs. Formulations F10 and F12 shows good drug release, more than 90% of drug within 8-10 hrs.

Ex-vivo mucoadhesion study

Mucoadhesive strength of formulation F1 to F16 was determined using self developed force detachment method (Figure 1). The mucoadhesive strength was observed within the range of 0.181 to 0.393 N. Based on results obtained from dissolution study formulation F10 and F12 both show good release profile. Furthermore, formulation F10 (0.258N) show slight more adhesion property compare to formulation F12 (0.247N). So on the basis of mucoadhesive strength and dissolution release studies, formulation F10 (Chitosan and HPMC K4M) was considered a good candidate for development of a ketoconazole effervescent mucoadhesive vaginal tablet.

Optimization using 3^2 full factorial design

The number of experiments required for these studies are dependent on the number of independent variables selected. The response (Yi) is measured for each trial. In order to investigate factors systematically, a factorial design was employed in the present investigation. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables i.e. Polymer ratio (X1) and amount of effervescent (X2) on dependent variables mucoadhesive strength, % swelling, Q8 and t60.

Swelling study of factorial design batches

Comparing with drug-free tablets employing the same amount of polymer and effervescent, all ketoconazole effervescent mucoadhesive vaginal tablets showed lower swelling rates, which is related with the poor solubility of ketoconazole.

Ex-vivo mucoadhesion study

In general, the swelling state of polymer contributes to its bioadhesive behavior. It was observed that the swelling rate was developed as effervescent applied to formulation, increased with increasing amount of effervescent; however, the effervescent led to a significant drop in adhesive strength. The influences of effervescent on swelling and mucoadhesion were opposite, mainly due to the tiny bubbles created by effervescent. These tiny bubbles depressed the mucosa-polymer interaction, resulting in a decrease in the mucoadhesive strength. The minimum adhesion strength (0.088N) was observed in formulation A3, which could be due to the lower ratio of HPMC K4M: Chitosan and the higher content of effervescent. On the contrary, with an increase in HPMC K4M: Chitosan ratio and decrease in effervescent, the maximum adhesion strength (0.267N) was obtained for formulation A7.
Ex-vivo mucoadhesion time
The time for the tablet to detach from the rat intestine was recorded as the mucoadhesion time. The increase in concentration of polymer blend in series from formulation A1 to A9, showed a gradual increase in mucoadhesion time. The factorial batches A1 to A3 showed mucoadhesion time 8-10 hours, batches A4 to A6 showed 12-15 hours and batches A7 to A9 showed 24 hours.

In-vitro drug release study of factorial design batches
The release rate of ketoconazole from effervescent mucoadhesive vaginal tablet was described as a function of time as shown in Figure 2. In all the formulations, the burst release of ketoconazole was observed within first 2 hrs, and then gradually increased up to 8-12 hrs. For the polymer mixture of HPMC K4M and Chitosan, more drug release could be seen as decreasing HPMC K4M and Chitosan and increasing amount of effervescent mixture

Full factorial design batches
The mucoadhesive strength, % swelling, Q8 and t80 for the nine batches showed wide variation. The results clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the nine batches (A1 to A9).
Drug content for ketoconazole was carried out by measuring the absorbance of samples at 221 nm using Shimadzu UV-1601 UV/Vis double beam spectrophotometer and comparing the content from a calibration curve prepared with standard Ketoconazole in the same medium. The total amount of Ketoconazole present in each tablet was found to be in the range of 97.5 to 99%.

Statistical analysis of factorial design batches
The statistical analysis of the factorial design batches was performed by multiple linear regression analysis carried out in Microsoft Excel 2007. The data clearly indicate that the values of mucoadhesive strength, % swelling, t80, and Q8 (% drug release after 8 hrs.), are strongly dependent on the independent variables. The fitted equations (full and reduced) relating the responses mucoadhesive strength, % swelling, Q8 and t80 to the transformed factors are shown in Table 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and mathematical sign it carries (i.e. positive or negative). Table 8 shows the results of the analysis of variance (ANOVA), which was performed to identify insignificant factors.

The high value of correlation coefficient for mucoadhesive strength, % swelling, Q8 and t80 indicates good fit i.e., good agreement between dependent and independent variables. The equations may be used to obtain estimates of the responses as small error of variance was noticed in the replicates. The significant test for regression coefficients was performed by applying student F test. A coefficient is significant if the calculated F value is greater than the critical value of F.

Full and reduced model for mucoadhesive strength
The significant level of coefficient b12 was found to be p = 0.07 hence it was omitted from full model to generate reduced model. The results of statistical analysis are shown in Table III. The coefficients b1, b2, b11 and b22 were found to be significant at p < 0.05; hence they were retained in reduced model. The reduced model was tested in portions to determine whether the coefficient b12 contribute significant information for the prediction of mucoadhesive strength or not. The results for testing the model in portion are shown in Table IV. The critical value of F for α = 0.05 is equal to 10.13 (DF = 1, 3). Since the calculated value (F = 5.0) is less than critical value (F = 10.13), it may be concluded that the interaction term b12 do not contribute significantly to the prediction of mucoadhesive strength and therefore can be omitted from the full model. The results of multiple linear regression analysis (reduced model) revealed that, on increasing the concentration of polymer an increase in mucoadhesive strength was observed; the coefficients b1 and b2 bear positive and a negative sign respectively. When high concentration of polymer was used, higher adhesion was expected in the tables. While in case of amount of effervescent, on increasing amount a decrease in mucoadhesive strength was observed. It is obvious that the presence of low amount of effervescent adhesion is facilitated. The fitted equation for full and reduced model relating the response is given below.

Full model (mucoadhesive strength)

\[ Y = 0.221 + 0.0412X_1 - 0.054X_2 + 0.011X_1X_2 - 0.029X_1^2 - 0.027X_2^2 \]

Reduced model

\[ Y = 0.221 + 0.0411X_1 - 0.055X_2 - 0.029X_1^2 - 0.027X_2^2 \]

Full and reduced model for % swelling

The significance level of coefficients b12 and b22 was found to be p = 0.27 and p = 0.11 respectively, hence they were omitted from the full model to generate the reduced model. The coefficients b1, b2 and b11 were found to be significant at P < 0.05, hence they were retained in the reduced model. The reduced model was tested in portions to determine whether the coefficients b12 and b22, contribute significant information for the prediction of t80 or not. The results for testing the model in portions are depicted in Table IV. The critical value of F for α = 0.05 is equal to 9.55 (DF = 2, 3). Since the calculated value (F = 3.366) is less than the critical value (F = 9.55), it may be concluded that the interaction term and polynomial terms do not
contribute significantly to the prediction of % swelling. Hence, conclusions can be drawn considering the magnitude of the coefficient and the mathematical sign (positive or negative) it carries. An increase in the concentration of polymer leads to an increase in % swelling being observed; both the coefficients $b_1$ and $b_2$ bear a positive sign. The amount of effervescent is increased the swelling increases as well as concentration of polymer increases the swelling increases. But both having opposite action on drug release i.e. as the amount of effervescent is increased the drug release increases while in case of polymer concentration as the concentration increases the drug release decreases. Hence the fitted equation for full and reduced model relating the response was given below.

Full model (% swelling)

$$Y = 20.3 + 3.083X_1 + 1.608X_2 - 0.313X_1^2 - 0.735X_2^2$$

Reduced model (% swelling)

$$Y = 19.81 + 3.083X_1 + 1.608X_2 - 1.15X_2^2$$

**Full and reduced model for $Q_8$**

The significant level of coefficient $b_{12}, b_{11}$ and $b_{22}$ was found to be $p = 0.16, p = 0.08$ and $p = 0.92$ respectively, hence it was omitted from full model to generate reduced model. The coefficients $b_1$ and $b_2$ were found to be significant at $p < 0.05$; hence they were retained in reduced model. The results for testing the model in portion are shown in Table 8. The critical value of $F$ for $\alpha = 0.05$ is equal to 9.28 ($DF = 3, 3$). Since the calculated value ($F = 3.286$) is less than critical value ($F = 9.28$), it may be concluded that the interaction term and polynomial terms $b_{12}, b_{11}$ and $b_{22}$ do not contribute significantly to the prediction of $Q_8$ and therefore can be omitted from the full model. The fitted equation for full and reduced model relating the response was given below.

Full model ($Q_8$)

$$Y = 81.99 - 22.541X_1 + 4.431X_5 + 2.84X_1X_2 - 5.435X_1^2 + 0.235X_2^2$$

Reduced model ($Q_8$)

$$Y = 78.52 - 22.541X_1 + 4.432X_2$$

**Full and reduced model for $t_{80}$**

The significant level of coefficient $b_{22}$ was found to be $p = 0.63$ hence it was omitted from full model to generate reduced model. The coefficients $b_1, b_2, b_{12}$ and $b_{11}$ were found to be significant at $p < 0.05$; hence they were retained in reduced model. The critical value of $F$ for $\alpha = 0.05$ is equal to 10.13 ($DF = 1, 3$). Since the calculated value ($F = 0.278$) is less than critical value ($F = 10.13$), it may be concluded that the polynomial term $b_{22}$ do not contribute significantly to the prediction of $t_{80}$ and therefore can be omitted from the full model. The fitted equation for full and reduced model relating the response was given below. Full model ($t_{80}$)

$$Y = 7.7 + 2.9X_1 - 0.66X_2 - 0.397X_1X_2 + 1.03X_1^2 + 0.04X_2^2$$

Reduced model ($t_{80}$)

$$Y = 7.7 + 2.9X_1 - 0.66X_2 - 0.397X_1X_2 + 1.03X_1^2$$

**The response surface plot**

For drawing the conclusions, response surface plot was used. Figure 3 to Figure 6 shows the plot of polymer ratio ($X_1$) and amount of effervescent ($X_2$) versus mucoadhesive strength, % swelling, $Q_8$ and $t_{80}$ respectively. The plots were drawn using Sigma plot software (Jandel Scientific, San Rafael, CA). The plots demonstrate that $X_1$ and $X_2$ affect the mucoadhesive strength, % swelling, $Q_8$ and $t_{80}$. Batch A4 was selected as best batch. It was arbitrarily decided to select a batch of tablets that gives moderate mucoadhesive strength and drug release in a control manner. The final selection is done after considering some aspects such as drug release profile, ex-vivo retention time and $t_{80}$.

A checkpoint batch A10 was prepared at $X_1 = 0.3$ level and $X_2 = 0.7$. From the reduced model, it is expected that the value of mucoadhesive strength of the checkpoint batch should be 0.154 N; the value of % swelling should be 19.91, the value of $Q_8$ of the checkpoint batch should be 97.40 % and the value of $t_{80}$ of the checkpoint batch should be 6.96 hrs. The data indicates that the results are as expected. Thus, we can conclude that the statistical model is mathematically valid.

**Kinetic analysis of dissolution data**

The in-vitro release data obtained were fitted to korsmeyer peppas kinetic model. In the entire batches exponent ‘n’ was in between 0.45 and 0.89, so predominant drug release mechanism is non-Fickian (anomalous) transport.

**Ex-vivo mucoadhesion time**

The increase in concentration of polymer ratio in series from formulation A1 to A9, showed a gradual rise in mucoadhesion time. The factorial batches A1 to A3 showed mucoadhesion time 10 hours, batches A4 to A6 showed 15 hours and batches A7 to A9 showed 24 hours.

**Anti fungal study**

The ketoconazole effervescent mucoadhesive vaginal tablet had better antimicrobial activity as compared with the marketed formulations (Candid V®-tablet and Candid V®-gel). Mucoadhesive polymers of the tablet had prolonged drug release and provided better contact with the wells cut in the plate, while the Candid V® suspension dried up as water was not available in the wells for prolonged time to allow diffusion of drug molecule(s). The zone of inhibition was measured at the end of 48 hours. The effervescent ketoconazole tablet showed significantly higher
(p<0.05) in-vitro antifungal activity as compare to Candid®-V3 tablet and Candid®-V gel. The results of antifungal studies are reported in Figure 7.

**Selection of best batch**

It was arbitrarily decided to select a batch of tablets that gives good mucoadhesive strength. The final selection is done after considering some aspects such as drug release profile, ex-vivo retention time and t₈₀. On the basis of mucoadhesive strength and dissolution release studies A4 comprising HPMC K4M: chitosan (1:1 ratio) and amount of effervescent (50mg) was considered a good candidate. The aim of study was, tablet should release more than 90% drug within 8-10 hrs and tablet should have satisfactory adhesive strength. Batch A4 shows good release profile which exactly fit in our objective and also shows good adhesive strength (0.252 N) which was sufficient to retain the tablet in vagina for more than 8 hrs.

**Accelerated stability study of optimized batch**

Ketoconazole effervescent mucoadhesive vaginal tablets were subjected to accelerated stability studies in aluminum pack as aluminum strip is considered the best protecting packaging material but in the present study simulation was made using aluminum foil pouch. As the dosage form is formulated for mucoadhesive vaginal drug delivery, no change should occur in its mucoadhesive strength and drug dissolution profile. The effect of aging was studied for optimized formulation. The storage condition was optimized at 40 ± 0.5°C and 75 ± 5 % RH for 3 months.

The data reveals no mark change in hardness, drug content and in vitro release. A slight reduction in residence time was noticed but no much obvious alteration. The nearly constant ‘n’ value reveals that the release behavior from dual component mucoadhesive system is not affected by the storage condition.

**CONCLUSION**

The results of the study reveal that with the developed formulations, the drug release and mucoadhesion properties of mucoadhesive vaginal tablets can be controlled by changing the polymer type, polymer concentration and effervescent content. Ex-vivo retention studies justified the prolong retention of the tablet inside the vaginal tract. Consequently, the mucoadhesive form of the drug would increase the time of contact with the vaginal mucosa and thus its therapeutic effect.

The results of 3² full factorial design revealed that the polymer ratio and amount of effervescent significantly affect the responses, mucoadhesive strength, % swelling, Q₈ and t₈₀. Full and reduced models were derived for the prediction of the response variable, Y. Based on result of multiple linear regression analysis, it was concluded that satisfactory mucoadhesion and good drug release profile of tablet could be obtained when X₁ kept optimum level and X₂ kept at low level. The effervescent ketoconazole tablet showed significantly higher (p<0.05) in-vitro antifungal activity as compare to Candid®-V3 tablet and Candid®-V gel.
**TABLE II: COMPOSITION OF 3² FULL FACTORIAL DESIGN BATCHES**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A1</th>
<th>A2</th>
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<th>A4</th>
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**TABLE III: SUMMARY OF RESULTS OF REGRESSION ANALYSIS**

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficients for mucoadhesive strength</th>
<th>Coefficients for % swelling (4hrs.)</th>
<th>Coefficients for Qₘ₈</th>
<th>Coefficients for tₘ₀</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>b₀</td>
<td>b₁</td>
<td>b₂</td>
<td>b₁₂</td>
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<tr>
<td>FM</td>
<td>0.221</td>
<td>0.0412</td>
<td>-0.054</td>
<td>-0.011</td>
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<tr>
<td>RM</td>
<td>0.221</td>
<td>0.0411</td>
<td>-0.055</td>
<td>-0.029</td>
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FM indicates full model and RM indicates reduced model
TABLE IV: CALCULATION FOR TESTING THE MODEL IN PORTION (ANOVA)

For mucoadhesive strength

<table>
<thead>
<tr>
<th>Regression</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
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<td>0.0311</td>
<td>0.0062</td>
<td>93.59</td>
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<td>Error</td>
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For % swelling

<table>
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<th>R²</th>
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<td>70.19</td>
<td>0.9915</td>
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<td>RM</td>
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<td>25.0690</td>
<td>58.94</td>
<td>0.9725</td>
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<td>FM</td>
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For Q₈

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<th>R²</th>
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For t₈₀

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<th>SS</th>
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</thead>
<tbody>
<tr>
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<td>11.1661</td>
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<td>RM</td>
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Figure-1: The scheme of the device used in the mucoadhesion study
Figure-2: Release profile of ketoconazole from factorial design batches (A1 to A9)

Figure-3: Response surface plot for % swelling

Figure-4: Response surface plot for $Q_s$
Figure-5: Response surface plot for $Q_8$

Figure-6: Response surface plot for $t_{90}$

Figure-7: Zone of inhibition (mm) for KEMV vaginal tablet, marketed vaginal Candid®-V3 tablet and Candid®-V gel. Error bar represent SD (n=3), KEMV tablet- ketoconazole effervescent mucoadhesive vaginal tablet
REFERENCES


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