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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 Candid®-V3 tablet and Candid®-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 Candid®-V3 tablet and Candid®-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.[1]
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.[2] Ketoconazole (KTZ) is an imidazole derivative antifungal agent developed for treatment of human mycotic infections and plays an essential role in antifungal chemotherapy.[3] It is a weak base with limited water solubility.[4] 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.[5]
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 Carbopol 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 Candid®-V3 tablet and Candid®-V gel.

MATERIALS AND METHODS

Materials

Ketoconazole was kindly gifted by Ranbaxy Laboratories Limited (Mumbai, India). Carbopol 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. Candid®-V3 tablet and Candid®-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 (W_0) and placed separately in 2% agar gel 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:[6,7]

$$\% \text{ Swelling} = \{(Wt - W_0)/ W_0\} \times 100 \ldots..(1)$$

Where, Wt is the weight of the tablet at time t and W_0 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 3^2 full factorial design

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

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

Where, Y is the dependent variables, b_0 is the arithmetic mean response of the nine runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity.

A 3^2 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 (X_1) and amount of effervescent (X_2) were selected as independent variables. Mucoadhesive strength, % swelling, Q_8 and t_{80} 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 lay out , coded values for polymer ratio(X_1) and amount of effervescent mixture(X_2), 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.[8]

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 I). 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 *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 effervescent mucoadhesive vaginal tablet (n=3) was determined using *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 ºC. 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 eqation. Release exponent 'n' was calculated (N. A. Peppas et al., 1985 and Korsemeyer R. W. et al., 1993).[13]

$$\mathbf{M_t / M_{\square} = Kt^n}$$

Where, $M_t / M_{\square}$ 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 *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_3) 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 effervescent 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 *in-vitro* release profile and mucoadhesive strength on storage,

accelerated stability study of batch A4 was carried out at 40 ºC in a humidity chamber having 75% RH for 3 months.[15] After 3 month samples were withdrawn and evaluated for change in physical appearance, in-vitro drug release pattern and mucoadhesive strength.

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 mucoadhesive 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 (X_1) and amount of effervescent (X_2) on dependent variables mucoadhesive strength, % swelling, Q_8 and $t_{80.}$

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.[16]

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, Q_8 and t_{80} 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, t_{80}, and Q_8 (% drug release after 8 hrs.) are strongly dependent on the independent variables. The fitted equations (full and reduced) relating the responses mucoadhesive strength, % swelling, Q_8 and t_{80} 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.[17]

The high value of correlation coefficient for mucoadhesive strength, % swelling, Q_8 and t_{80} 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 b_{12} 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 b_1, b_2, b_{11} and b_{22} 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 b_{12} 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 $\alpha = 0.05$ 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 b_{12} 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 b_1 and b_2 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 b_{12} and b_{22} 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 b_1, b_2 and b_{11} 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 b_{12} and b_{22}, contribute significant information for the prediction of t_{80} or not. The results for testing the model in portions are depicted in Table IV. The critical value of *F* for $\alpha = 0.05$ 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 is 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)
$Y = 20.3 + 3.083X_1 + 1.608X_2 - 0.313X_1X_2 - 1.15X_1^2 - 0.735X_2^2$
Reduced model (% swelling)
$Y = 19.81 + 3.083X_1 + 1.608X_2 - 1.15X_1^2$

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)
$Y = 81.99 - 22.541X_1 + 4.431X_2 + 2.84X_1X_2 - 5.435X_1^2 + 0.235X_2^2$
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_3®- 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_3 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 t_{80}. 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 3^2 full factorial design revealed that the polymer ratio and amount of effervescent significantly affect the responses, mucoadhesive strength, % swelling, Q_8 and t_{80}. 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 X_1 kept optimum level and X_2 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 I : FULL FACTORIAL DESIGN LAYOUT

Batch code	Variable level in coded form	
	X_1	X_2
A1	-1	1
A2	-1	0
A3	-1	1
A4	0	-1
A5	0	0
A6	0	1
A7	1	-1
A8	1	0
A9	1	1
Check point	- 0.3	0.7
Coded value	**HPMC K4M: Chitosan X1**	**Amount of effervescent X2**
-1	1:0.5	50
0	1:1	75
1	1:1.5	100
Check point	1:0.8	92.5

TABLE II : COMPOSITION OF 3^2 FULL FACTORIAL DESIGN BATCHES

Ingredients	Quantity (mg)								
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**
Ketoconazole	400	400	400	400	400	400	400	400	400
HPMC K4M	50	50	50	50	50	50	50	50	50
Chitosan	25	25	25	50	50	50	75	75	75
MCC	252.5	227.5	202.5	227.5	202.5	177.5	202.5	177.5	152.5
Sod. bicarbonate	37.5	66.3	75	37.5	66.3	75	37.5	66.3	75
Citric acid	12.5	18.7	25	12.5	18.7	25	12.5	18.7	25
Mg. stearate	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Talc	15	15	15	15	15	15	15	15	15

TABLE III: SUMMARY OF RESULTS OF REGRESSION ANALYSIS

Model	Coefficients for mucoadhesive strength						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	0.221	0.0412	-0.054	-0.011	-0.029	-0.027	0.9936
RM	0.221	0.0411	-0.055	-	-0.029	-0.027	0.9781
	Coefficients for % swelling (4hrs.)						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	20.3	3.083	1.608	-0.313	-1.15	-0.735	0.9915
RM	19.81	3.083	1.608	-	-1.15	-	0.9725
	Coefficients for Q_8						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	81.99	-22.541	4.431	2.84	-5.435	0.235	0.9915
RM	78.52	-22.541	4.432	-	-	-	0.9637
	Coefficients for t_{80}						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	7.7	2.9	-0.66	-0.397	1.03	0.04	0.9994
RM	7.72	2.9	-0.66	-0.397	1.03	-	0.9993
FM indicates full model and RM indicates reduced model							

TABLE IV: CALCULATION FOR TESTING THE MODEL IN PORTION (ANOVA)						
For mucoadhesive strength						
Regression	**DF**	**SS**	**MS**	**F**	**R^2**	
FM	5	0.0311	0.0062	93.59	0.9936	*F*cal=5.0
RM	4	0.0306	0.0076	44.75	0.9781	*F*tab=10.13
Error						*DF*=(1,3)
FM	3	0.0002	0.0001	-	-	
RM	4	0.0007	0.0002	-	-	
For % swelling						
Regression	**DF**	**SS**	**MS**	**F**	**R^2**	
FM	5	76.6782	15.3356	70.19	0.9915	*F*cal=3.366
RM	3	75.2071	25.0690	58.94	0.9725	*F*tab=9.55
Error						*DF*=(2,3)
FM	3	0.6554	0.2185	-	-	
RM	5	2.1265	0.4253	-	-	
For Q_8						
Regression	**DF**	**SS**	**MS**	**F**	**R^2**	
FM	5	3258.0497	651.6099	70.25	0.9915	*F*cal=3.286
RM	2	3166.5984	1583.2992	79.65	0.9637	*F*tab=9.28
Error						*DF*=(3,3)
FM	3	27.8253	9.2751	-	-	
RM	6	119.2766	19.8794	-	-	
For t_{80}						
Regression	**DF**	**SS**	**MS**	**F**	**R^2**	
FM	5	55.8306	11.1661	968.86	0.9993	*F*cal=0.278
RM	4	55.8274	13.9569	1477.89	0.9993	*F*tab=10.13
Error						*DF*=(1,3)
FM	3	0.0346	0.0115	-	-	
RM	4	0.0378	0.0094	-	-	

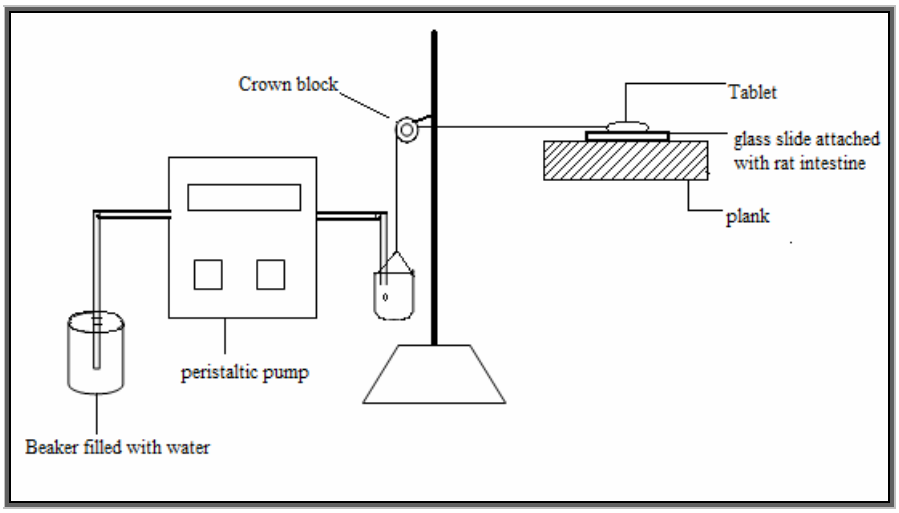

Figure-1: The scheme of the device used in the mucoadhesion studie

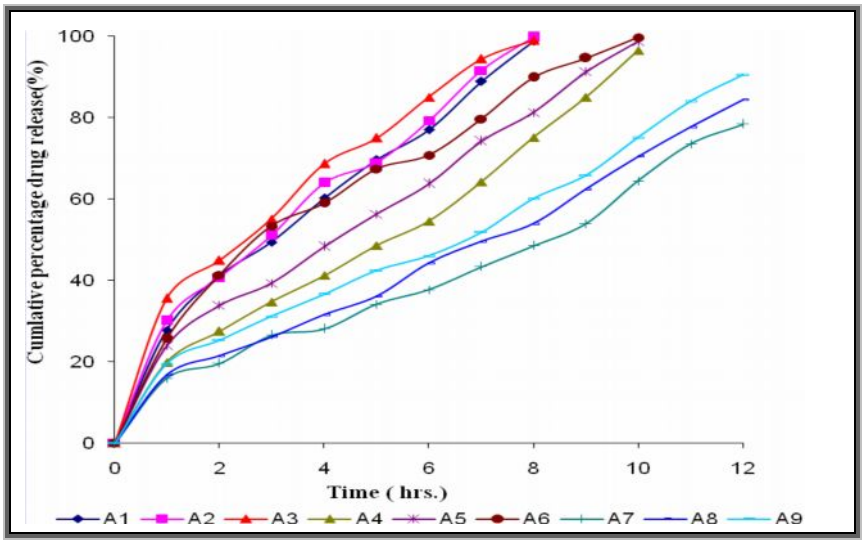

Figure-2: Release profile of ketoconazole from factorial design batches (A1 to A9)

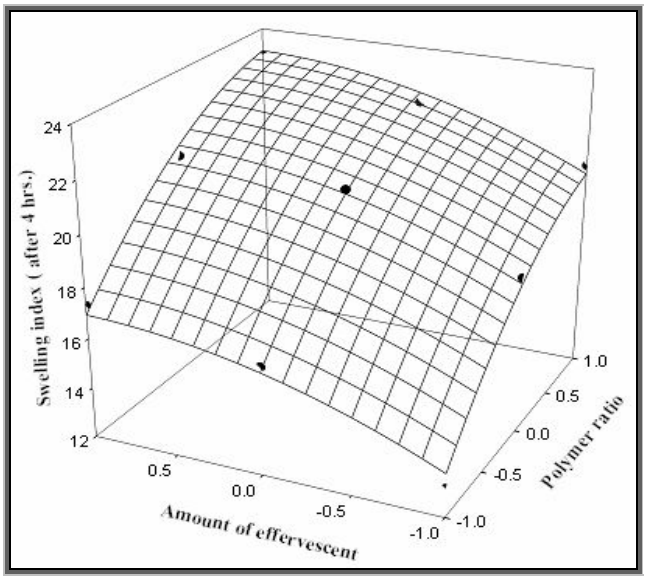

Figure-3: Response surface plot for % swelling

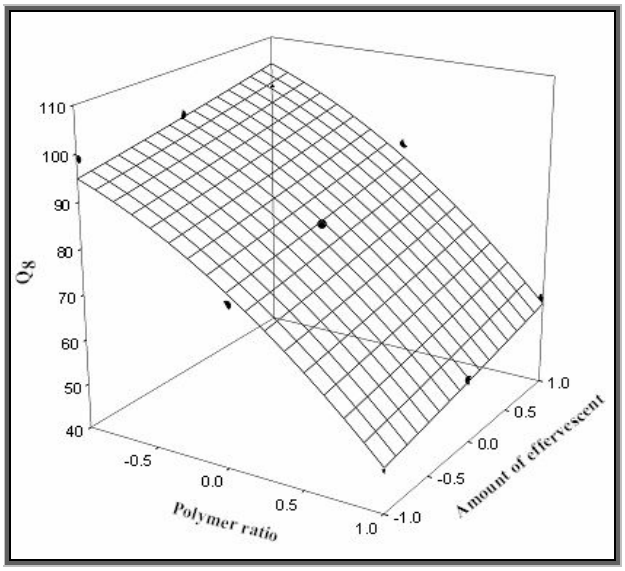

Figure-4: Response surface plot for Q_8

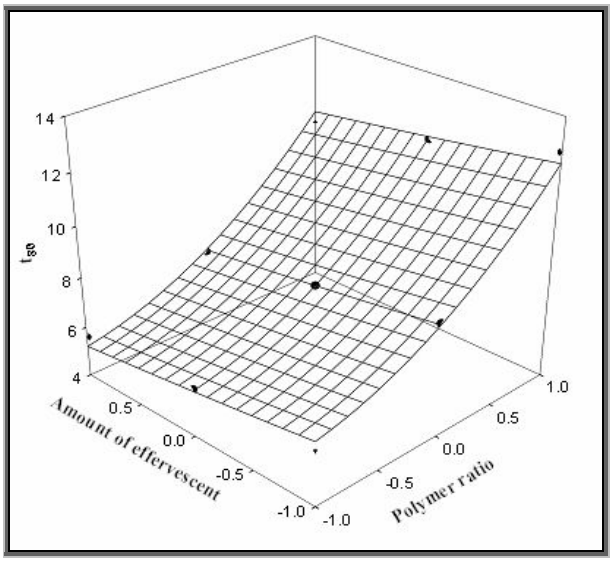

Figure-5: Response surface plot for Q_8

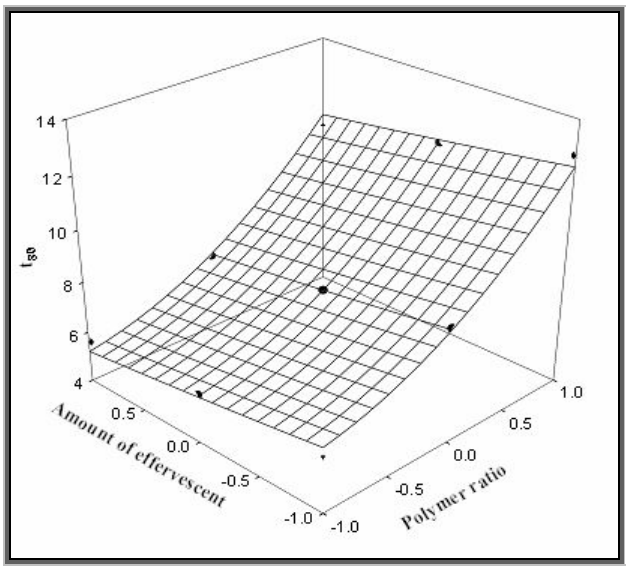

Figure-6: Response surface plot for t_{80}

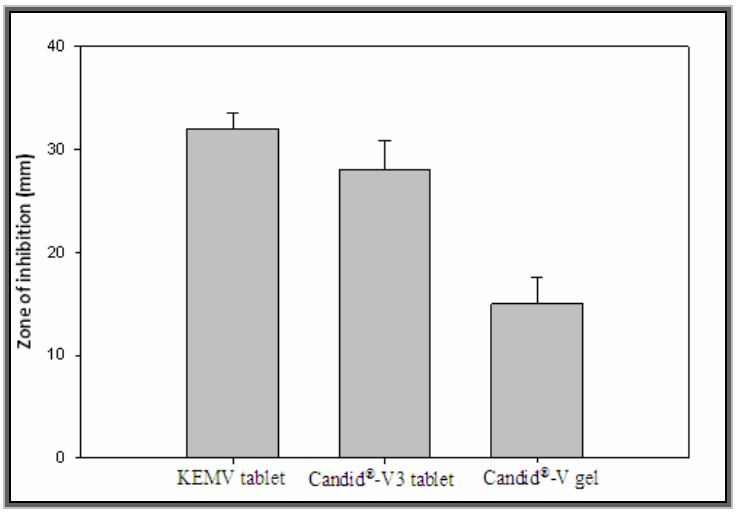

Figure-7: Zone of inhibition (mm) for KEMV vaginal tablet, marketed vaginal Candid®-V3 tablet and Candid®-V gel. Error bar represent SD (n=3), KEMV tablet- ketoconazole effervescent mucoadhesive vaginal tablet

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