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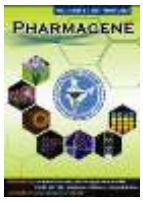

Preparation and Evaluation of Effervescent Bioadhesive Vaginal Tablet of Itraconazole for Vaginal Candidiasis

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ABSTRACT: The objective of the present work is to develop and evaluate effervescent bioadhesive vaginal tablet of Itraconazole. The vaginal tablets were prepared by direct compression method. HPMC K4M, Sodium CMC, Cabopol 934P was used as bioadhesive polymers. Effervescent materials, sodium bicarbonate and citric acid was incorporated into the formulation because Itraconazole is a weak base with limited water solubility and it also improves the dissolution of bioadhesive vaginal tablets because there is rather low moisture content in vagina under normal physiological conditions. The amount of polymer blends and effervescent mixture was optimized using 3^2 full factorial design. The swelling and in-vitro release were studied. The *ex-vivo* bioadhesion was determined by using a physical modified balance. The *ex-vivo* mucoadhesion was determined by self developed modified mucoadhesion assembly. Anti-fungal activity of the effervescent bioadhesive tablet of Itraconazole was determined in comparison to drug solution by cup plate method. The effervescent Itraconazole tablet showed significant results ($p<0.05$) in all the four dependent factors. *In-vitro* antifungal activity was compared with pure drug solution and shown better antifungal activity due to sustained release of the drug. Thus our study may provide a potential vaginal formulation of Itraconazole against Vaginal candidiasis.

KEY WORDS: Vaginal drug delivery, Bioadhesion, Candidiasis

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INTRODUCTION

The human vagina remains to be a relatively unexplored route of drug delivery despite its potential as a non-invasive route of drug administration. The presence of dense network of blood vessels has made the vagina an excellent route of drug delivery for both systemic and local effect. The main advantages of vaginal drug delivery over conventional drug delivery are the ability to by-pass first pass metabolism, ease of administration and high permeability for low molecular weight drugs. Moreover, the administration of drugs for systemic effects via the vagina is also feasible.[1] Traditionally, solutions, suppositories, gels, foams and tablets have been used as vaginal formulations. More recently, vaginal ring has been introduced for hormone replacement and contraceptive therapy. Vaginal formulations may be designed to produce local effect such as spermicidal or antibacterial effect or to produce a systemic effect by continuous release of drugs such as contraceptives.[2] Conventional vaginal formulations are associated with disadvantages of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. To circumvent these problems, bioadhesive drug delivery systems are being propagated. Bioadhesive polymers that have been used for vaginal formulation include polycarbophil, hydroxypropylcellulose and polyacrylic acid. Various peptide and protein drugs have also been attempted to administer via bioadhesive microparticulate vaginal delivery system.[3] 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. Itraconazole is an antibiotic used to treat superficial yeast and fungus infections. Itraconazole is used for the treatment of serious fungal infections. It may also be used against some yeast and dermatophyte (ringworm) infections. This medication is normally taken on a once or twice per day basis depending on the condition.

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MATERIALS AND METHODS:

Itraconazole was a gift sample from Alembic Research Center, Vadodara. Carbopole 934P, Citric acid and MCC were purchased from Loba Chemicals Pvt Ltd., Mumbai. HPMC K4M was purchased from Chemdyes Corporation, Vadodara. Sodium CMC was purchased from S.D. Fine Chemicals Ltd and Sodium bicarbonate from Sisco Research Lab Pvt Ltd, Mumbai. Magnesium stearate was purchased from Qualikems, Vadodara. Talc from Sulab Chemicals, Vadodara

Method of preparation of Bioadhesive effervescent vaginal tablet:

Bio adhesive effervescent vaginal tablets, each containing 200 mg Itraconazole were prepared by direct compression method. Composition of various formulations containing Carbopol 934P, HPMC K4M in different combinations and effervescent material 1:3 (citric acid: sodium bicarbonate) were used. All the ingredients of tablets were blended in geometrical ratio in mortar with a pestle for 15 min to obtain uniform mixture. The blended powder was sieved and then compressed into approximate 500 mg tablets (at 5 kg/cm^2) in 10 station rotary tablet machine with 10 mm round shape punch.

Optimization using 3^2 full factorial design

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

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

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_{12} and X_{22}) 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. % swelling, Mucoadhesive strength, Q_8 and t_{80} were selected as dependent variables (response- Y). The preparation and evaluation method for tablets and amount of Itraconazole were kept constant for all the trials.

Table 1: Full factorial design lay out

Batch code	Variable level in coded form	
	X1 (Polymer ratio)	X2 (Amount of effervescent)
BEV 1	-1	-1
BEV 2	-1	0
BEV 3	-1	+1
BEV 4	0	-1
BEV 5	0	0
BEV 6	0	+1
BEV 7	+1	-1
BEV 8	+1	0
BEV 9	+1	+1

Evaluation parameters of vaginal bioadhesive effervescent tablet:

The vaginal bioadhesive effervescent tablets were subjected to following tests.

Thickness:

Tablet thickness is a function of the die fill and compression force. The thickness of bioadhesive tablets were determined by using Vernier Calipers.

Hardness:

Hardness is sometimes termed as the tablet crushing strength. Hardness test was conducted for three tablets from each batch using Monsanto hardness tester and average values were calculated. Hardness of the tablets from each batches were measured using the Pfizer hardness tester.

Friability:

For this test ten tablets were weighed and placed in the friabilator, which was then operated for 100 revolutions and the tablets were then dusted and reweighed to determine weight loss. Tablet that loss less than 0.5% to 1.0% of their weight is generally considered acceptable.

Weight variation test:

As per USP 20 tablets were weighed individually and average weight was calculated and maximum % deviation was found.

Content uniformity:

Two tablets were weighed and grounded in a mortar with pestle to get fine powder, powder equivalent to the mass of one tablet was dissolved in 100 ml citrate buffer pH 4.0. The drug content was analyzed spectrophotometrically at 268 nm using an UV spectrophotometer in triplicate.

Swelling index[4]:

The swelling behavior of tablet described as the water absorbing capacity. Tablets were weighed individually; initial weight was considered as W1 and placed separately in Petri-dish containing 15 ml of citrate buffer (pH 4) solution. Tablets were completely immersed in the buffer solution. At regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr to till 24 hrs), the tablets were carefully removed from the Petri-dishes and excess surface water was removed carefully using the filter paper. The swollen tablets were then reweighed W2. This experiment was performed in triplicate. The degree of swelling (water uptake) was calculated according to the following equation:

$$\text{Swelling index (SI)} = \frac{W2 - W1}{W1} \times 100$$

Where,

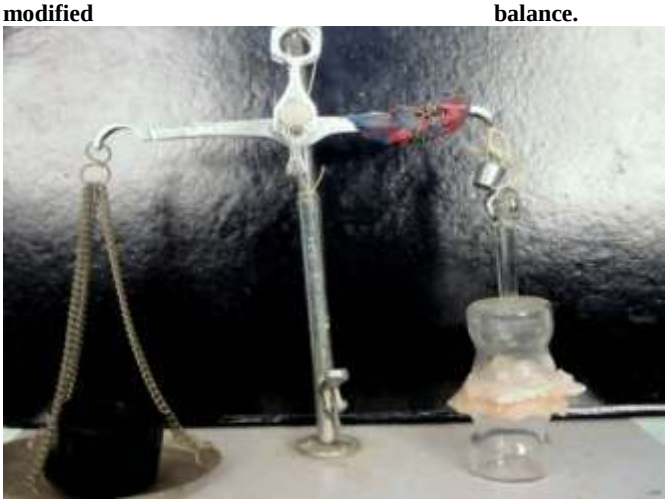
W1 = Weight dry of tablet, W2 = Weight of wet tablet

Measurement of *Ex-vivo bioadhesive* strength[4,5]:

Mucoadhesive strength of tablet was measured on modified two arm physical balance. The goat vaginal mucosa was used as vaginal membrane. The mucosa was stored in krebs buffer at 4℃ from the time of collection and used within 3 hr of procurement. The membrane was washed with distilled water and then with citrate buffer pH 4.0 at 37℃. The piece of vaginal mucosa was cut into pieces and tied to glass vial, which was filled with citrate buffer. The glass vial is tightly fitted into glass beaker (filled with citrate buffer pH 4.0) at 37℃ ± 0.5℃. So that it just touches the mucosal surface. The vaginal tablet were suck to lower side of a rubber stopper by keeping 5 gm on right hand pan,

which lower the pan tablet over the mucosa. The balance was kept in the position for 5 min contact time. Mucoadhesive strength was assessed in terms of weight required to detach the tablet from the membrane.

Figure 1 : *Ex vivo* bio adhesion using a physical modified balance.



Determination *of Ex-vivo* residence time[6]:

Ex-vivo residence (ER) time was determined using a locally modified USP disintegration apparatus, which was applied by Nakamura et al. The disintegration medium was composed of 800 ml pH 4.0 citrate buffer maintained at 37°C. The vaginal tissue was tied to the surface of a glass slab, vertically attached to the apparatus. The vaginal bioadhesive effervescent tablet was hydrated from one surface using 0.5 ml of pH 4.0 citrate buffer and then the hydrated surface was brought in contact with the mucosal membrane. The glass slide was vertically fixed to the apparatus and allowed to run in such way that the tablet completely immersed in the buffer solution at the lowest point and was out at the highest point. The time taken for complete erosion or dislodgment of the tablet from the mucosal surface was noted. The experiments were performed in triplicate (n=3) and mean of triplicate was determined.

Figure 2: Schematic diagram of apparatus for determination of residence time

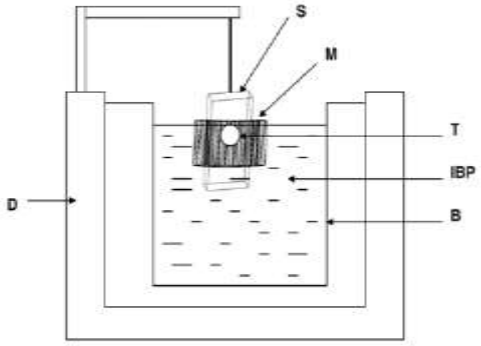
S = glass slab
D = disintegration apparatus
B = glass beaker
M = mucosal membrane
T = bioadhesive tablet
IBP = isotonic buffer

***In-vitro* drug release of vaginal bio adhesive tablet[6]:**

The release rate of Itraconazole effervescent mucoadhesive vaginal tablet was determined using USP dissolution testing apparatus I (basket method) using 900 ml of citrate buffer pH 4.0 as dissolution medium. The tablet was placed in a settling basket to prevent the tablet from floating. The rate of stirring was 50 rpm and the medium temperature was maintained at 37 ±0.5 ºC. A sample 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 268 nm using a Shimadzu UV-1800 UV/Vis double beam spectrophotometer. Cumulative percentage of drug release was calculated using the equation obtained from a standard curve.

***In vitro* Anti Fungal Activity[7]:**

In vitro antifungal activity of the optimized formulation was studied against *Candida albicans* ATCC 4215 (species of *C. albicans*) by using cup plate method. The media for inoculating *Candida albicans* was S.C.D.M. (Soybean-Casein Digest medium) The culture was inoculated one day before the actual antifungal activity is to be done. The sterilized media S.C.D.M. and freshly grown culture of *C. albicans* were mixed aseptically under the LAF and poured in a sterile petridishes. After solidifying cups were made with the help of sterile borer and lifter. Different concentrations of Itraconazole 25% w/v, 50% w/v, 75% w/v were prepared along with the intact tablet for antifungal evaluation. All three respective solutions were poured inside the cups using sterile micropipette tips aseptically. The tablet of optimized formulation also put in to the cup. The plates were kept in refrigerator for one hour and then kept for incubation at 28º C in BOD (Biochemical oxygen demand)incubation for 18 hrs. The zone of inhibition was measured and reported.



TREATMENT OF DISSOLUTION DATA WITH DIFFERENT MODEL:

Curve fitting was performed using Microsoft excel 2007 version. The dissolution data were fitted to following eqation. Release exponent 'n' was calculated. $M_t / M_0 = kt^n$ where M_t/M_0 is fraction of the drug released at time t, k is the kinetic constant of the system, and n is exponent characteristic of the mode transport. The release exponent takes various values depending upon different geometries.

RESULTS AND DISCUSSION

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}.

Evaluation parameters:

Effervescent bioadhesive vaginal tablet of Itraconazole were prepared by direct compression method using HPMC K4M and Carbopol 934P as a bioadhesive polymers. A total nine formulations were designed.

Thickness of vaginal tablet was ranged from 4.6 ± 0.20 mm to 4.9 ± 0.1 mm. Average Hardness of vaginal tablet 4.7Kg/cm^2. This ranges were suitable for vaginal tablet. As none of formulation deviate from range of 475 mg to 525 mg (I.P. limit = ± 5 %), the prepared formulation comply with the weight variation test. Percentage friability in entire batches is less 1%. The average content uniformity from all formulation was found to be 97.63 ± 0.48. Among all the formulations, the formulation BEV 5 was found to be good. *In-vitro* drug release studies of all formulation were carried out in pH 4.0 citrate buffer, sample were withdrawn at an interval of time 1 hour. Formulation BEV 5 has shown complete drug release with 12 h and emerged as the overall best formulation.

Swelling index of tablet containing **Carbopol 934P: HPMC K4M** in 1:1 with a **effervescent material ratio 1:3** having a good swelling. **Nature of polymer** affect on swelling index.

Table 2: Composition of 3^2 Full Factorial design batches:

Ingredients	BEV1	BEV2	BEV3	BEV4	BEV5	BEV6	BEV7	BEV8	BEV9
Itraconazole	200	200	200	200	200	200	200	200	200
HPMC K4M	50	50	50	50	50	50	50	50	50
Carbopol 943P	25	25	25	50	50	50	75	75	75
Citric acid	12.5	18.7	25	12.5	18.7	25	12.5	18.7	25
Sodium bicarbonate	37.5	66.3	75	37.5	66.3	75	37.5	66.3	75
MCC	159	124	109	134	115	84	109	74	59
Talc	10.75	10.75	10.75	10.75	10.75	10.75	10.75	10.75	10.75
Magnesium stearate	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25

Bioadhesion strength and Bioadhesion time:

Bioadhesive strength of all vaginal tablets was found to be a function of concentration of polymer and amount of effervescent material. Maximum bioadhesive strength was found to be 0.4728±0.04732 N. As the amount of effervescent material increasing the bio adhesive strength was decrease. Bioadhesive time of all vaginal tablets was found to be a function of concentration of polymer and amount of effervescent material. Maximum bioadhesive time was found to be 827± 13.50 and for a minimum bio adhesive strength found to be 470± 20.09.

Table 3 : Bioadhesion strength and Bioadhesion time

Batch	Bio adhesion strength (N)	Bio adhesion time (min)
BEV 1	0.397±0.039	549 ±10.40
BEV 2	0.3211±0.022	524±12.63
BEV 3	0.2178±0.036	470± 20.09
BEV 4	0.4728±0.047	663± 11.21
BEV 5	0.3579±0.034	589± 11.04
BEV 6	0.279±0.075	530± 12.58
BEV 7	0.4745±0.046	827± 13.50
BEV 8	0.436±0.016	763± 14.04
BEV 9	0.3652±0.014	739± 17.61

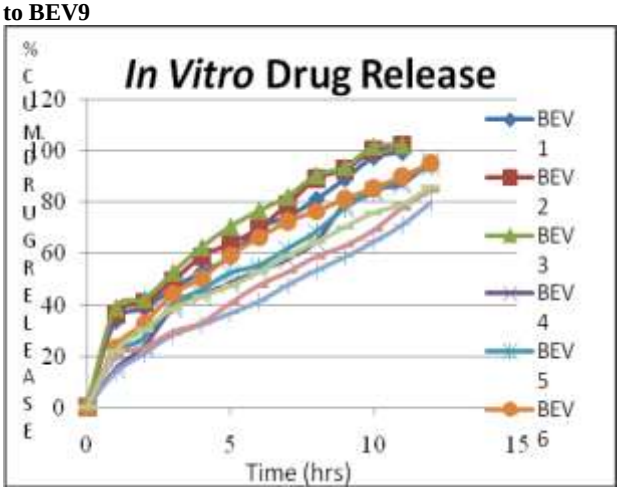
In-vitro drug release study:

The release rate of Itraconazole from effervescent mucoadhesive vaginal tablet was described as a function of time as shown in Figure. In all the formulations, the burst release of Itraconazole was observed within first 2 hrs, and then gradually increased up to 8-12 hrs. For the polymer mixture of HPMC K4M : Carbopol 934P, more drug release could be seen as decreasing HPMC K4M : Carbopol 934P ratio and increasing amount of effervescent mixture. So, here we observed that in BEV 1-3 a release all the drug within 12 hrs. Where, in BEV 7-9 release least drug compare to other formulation ranging from 71.13 to 79.04% within 12 hrs.

***In vitro* anti fungal activity**

The Microbial assay of Itraconazole for *C. albicans* with 20µg/ml, free Itraconazole having an activity on yeast *C. albicans*, it exerts an inhibition effect on a fungi. That shows in different concentrations of Itraconazole an inhibition effect. It is having a long a effect and a more zone of inhibition.

***Figure3: In-vitro* drug release study of batch no. BEV 1 to BEV9**



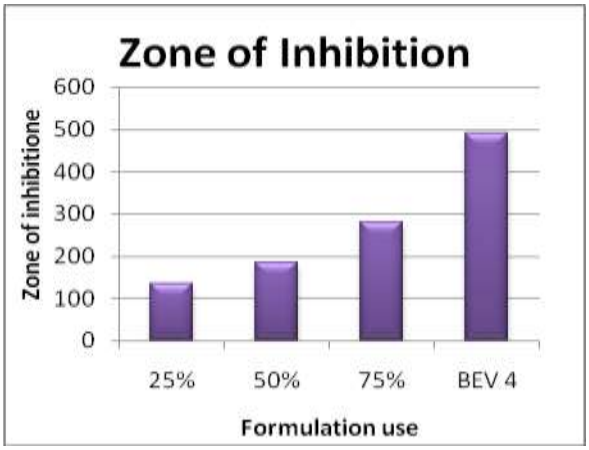


Figure 4: Zone of inhibition

TREATMENT OF DISSOLUTION DATA WITH DIFFERENT MODEL:

The *in vitro* release data obtained were fitted to krosmeyer peppas kinetic model. In the entire batches exponent 'n' was in between 0.45 to 0.89, so predominant drug release mechanism is non Fickian transport.

STABILITY STUDY:

Stability study of optimized batch BEV 5

Table 5 : Stability study of optimized batch BEV 5

Temperature condition	Drug content (%)	In-vitro drug release (%)
Room temperature	**97.10**	**88.03**
40°C ±2°C/ 75% ± 5% RH	**97.04**	**87.93**

After the 1 month stability study of optimized Formulation BEV 5, values of all parameter like % drug content and in vitro drug release were almost similar to the initial values. The drug dissolution profile was same as the initial profile. There was no significant change in any value so formulation is stable.

CONCLUSION

The bioadhesive vaginal effervescent tablet of Itraconazole was prepared by direct compression method which showed acceptable pre compression properties, post compression properties and satisfactory sustained release for minimum 8 hours. The tablets were prepared with smooth surface, no deformation without any drug - excipient interactions and found satisfactory sustained drug release. It can be used as a substitute for other marketed formulations for better bioavailability, sustained release of the drug and more effective treatment of candidiasis. The result of invitro drug release, bioadhesive strength and ex vivo bioadhesion time indicates formulating the bioadhesive vaginal effervescent tablet to increase the residence time of the drug to the vaginal mucosa and provide sustained release of Itraconazole thus prolonging the action of drug for local treatment of vaginal Candidiasis. The result of stability study for 1 month indicated that tablet is relatively stable upon storage. Thus it can be concluded that formulated bioadhesive vaginal effervescent tablet can be used for effective management of vaginal candidiasis.

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