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Design and optimization of clotrimazole-hydroxypropylβ cyclodextrin bioadhesive vaginal tablets using *Anacardium occidentale* gum by $3^2$ factorial design

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ABSTRACT
Clotrimazole (CTZ), a BCS class II drug is widely employed in the treatment of vaginal candidiasis. However, attributable to its poor solubility repeated administrations are required to maintain a therapeutic concentration. To increase its aqueous solubility, it was complexed with Hydroxypropyl β Cyclodextrin (HPβCD) and formulated as vaginal tablets using a natural *Anacardium occidentale* gum in combination with Carbopol934P. The formation of drug-HPβCD complex was confirmed by characterization techniques viz., scanning electron microscopy (SEM), differential scanning colorimetry (DSC), X-ray diffraction (XRD), $^1$H NMR, 2D [$^1$H, $^1$H] NOESY and Fourier Transform Infrared spectroscopy (FT-IR) studies. Further, $3^2$ factorial design was employed to optimize the gum and Carbopol 934P concentration in the development of CTZ-HPβCD vaginal tablets. In vitro analysis revealed the higher susceptibility of *Candida albicans* strains and a significant mucoadhesion of the tablet formulation. Based on our findings, combined with *Anacardium occidentale* gum, CTZ-HPβCD complex could be used for treating fungal infections and offering greater advantage of higher solubility of CTZ with therapeutic importance of higher bioavailability at the site of action. Additionally, use of natural polymers could also serve to minimize the drug toxicity that would be particularly useful to treat vaginal candidiasis that is safe in pregnancy.

**Keywords:** Clotrimazole, HPβCD, Vaginal candidiasis, $3^2$ factorial design, Bioadhesion
1. Introduction

Urogenital infection such as vaginal candidiasis has become a major public health concern affecting pre-menopausal women worldwide. Specifically, *Candida albicans*, a commensal fungal pathogen and most prevalent species in the human gastrointestinal tract is associated with this pathology, affecting >70% of female population of childbearing age at least once during their lifetime.\(^1\)

Clotrimazole (CTZ), a tritylimidazole broad spectrum antifungal agent has been used for treatment of *Candida* and other common fungal infections.\(^2\) Although CTZ is generally used for treatment of oral candidiasis, studies have described their topical applicability in treating vaginal candidiasis.\(^3\) CTZ is a weak base (pKa value of approximately 6.1) with a log octanol-water partition coefficient (log $K_{ow}$) of 6.26.\(^4\) CTZ, belongs to the class II Biopharmaceutics Classification System (BCS) possessing low solubility and shorter half-lifetime that limits its applicability to effective therapeutic use. Hence, various approaches have demonstrated to achieve enhanced solubility by nanocapsule formation,\(^5\) nanostructured lipid carriers\(^6\) among others that are quite successful in promoting in vitro dissolution, stability, and adsorption.

Recent years have witnessed the rise of drug complexation with cyclodextrins (CDs) as a major topic of interest to enhance drug solubility and subsequently onset of action. CD are macrocyclic oligosaccharides with six to eight $\alpha$-D-glucopyranose units linked by ($\alpha$-1,4)-glucosidic bonds possessing unique molecular structure of lipophilic inner and hydrophilic outer surface. Due to its molecular structure, they possess ability to form inclusion complexes with various molecules through non-covalent interactions.\(^7\) Given the presence of large number of hydrogen atoms, CDs are widely employed as versatile complexing agents in the pharmaceutical formulation design including its primary use as a solubilizer for poorly water soluble drugs leading to corresponding increase in bioavailability with reduced side effects and drug-associated toxicity.\(^8\) Additionally, CD and their derivatives have also been used for development of assemblies with higher drug loading efficiency,\(^9\) and reduce gastrointestinal irritation.\(^10\) Recently, Tonglairoum and colleagues\(^11\) reported that CTZ-composited electrospun blend of hydroxypropyl-$\beta$-cyclodextrin (HP$\beta$CD) with polyvinylpyrrolidone (PVP) exhibited rapid *in vitro* antifungal activity attributed to enhanced dissolution of clotrimazol in. In addition, CTZ-PVP/HP$\beta$CD complex also reduced the cytotoxicity associated with drug exposure.
Various innovative approaches have been developed for the improvement of CTZ antifungal activity such as CTZ-loaded chitosan-tailored cubic nanoparticles, and CTZ-composite electrospun HPβCD blended nanofiber mats.\textsuperscript{12,13} An innovative CTZ-loaded nanostructured lipid carrier hydrogels observed to be 4-fold more active than Fungizone\textsuperscript{®} and CTZ-loaded microemulsion-containing nanofiber mats exhibited more rapid killing activity compared to CTZ lozenges against \textit{C.albicans}.\textsuperscript{14,15}

Vaginally applied antifungal agents require long retention time to maximize absorption with reduced number of doses needed for treatment. However, conventional vaginal formulations are limited by short residence time and prolonged courses of treatment that could interfere with plans for conception in women of childbearing age. Hence, vaginal inserts with better mucoadhesion ability provide for increased contact of the drug with the vaginal mucosa. In this regard, natural gums constitute a promising source of compounds that can be used for their binding property in tablet formulation. Hence, increasing interest has been devoted towards utilization of gums from plant origin that could also increase the residence time of the drug, minimize the side effects associated with the use of synthetic polymer and biocompatible. Cashew gum, exudate of the cashew tree (\textit{Anacardium occidentale} L.), is a complex polysaccharide constituted by galactose, arabinose, rhamnose, glucose, glucurine acid (Family: Ancardiaceae) has been previously studied as a binder\textsuperscript{16} and emulsifier.\textsuperscript{17} Further, studies have also shown the utility of bioadhesive property of \textit{A. occidentale} gum in sustained release curcumin buccal tablets providing an improved phytochemical bioavailability.\textsuperscript{18}

Multivariate formulation optimization strategy is important to understand the influence of factors on the formulation quality and also helps in the selection of factors to develop optimized formulation. This can be achieved by using well established statistical analysis tool such as factorial designs. Factorial experimental designs are considered the most effective statistical optimization technique to estimate the effects of various formulation variables.\textsuperscript{19} The optimization technique based on the factorial designs encompasses the generation of model equations for investigated responses over the experimental design to determine the settings of factor values to achieve optimum formulation.\textsuperscript{20} In the present study, a two-factor, three-level\textsuperscript{3} factorial design-based optimization was employed to investigate the effects of two independent variables (factors) such as the amount of cashew gum and Carbopol\textsuperscript{934P} on the dependent variables on the properties of CTZ-HPβCD vaginal bioadhesive tablets.
In the present study, initially we determined the possibility of formulating a novel CTZ-HPβCD complex and ascertained its solubility by phase-solubility studies through stoichiometry and equilibrium constants. Further, we confirmed the formation of drug-HPβCD complexes by characterization techniques viz., scanning electron microscopy, differential scanning colorimetry, X-ray diffraction, $^1$H NMR, 2D [$^1$H, $^1$H] NOESY and Fourier Transform Infrared spectroscopy (FT-IR) studies. Furthermore, considering the enhanced solubility of clotrimazole by complexation with HPβCD, we developed vaginal bioadhesive tablets using natural A. occidentale gum by $^2$ factorial design and evaluated its antimicrobial activity on C. albicans. Based on our findings, combined with cashew gum polymer, CTZ-HPβCD complexes could be used for treating fungal infections and offering greater advantage of higher solubility of CTZ with therapeutic importance of higher bioavailability at the site of action. Additionally, use of natural polymers could also serve to minimize the drug toxicity that would be particularly useful to treat vaginal candidiasis.

2. Materials and methods

2.1 Chemicals and reagents
Clotrimazole (MW: 344 g/mol) was obtained as a gift sample from Glenmark Pharmaceuticals, India. Hydroxypropyl-β-cyclodextrin (MW: ~1480) was procured from Alfa Aesar, England. Carbopol 934P, lactose monohydrate, and magnesium stearate were purchased from Lobachemie, Mumbai, India. All other chemicals and reagents used were of analytical grade.

2.2 Isolation and extraction of the water-soluble fraction of A. occidentale (cashew) gum
A. occidentale gum was collected from various places of Andhra Pradesh, India. The water-soluble fraction from cashew gum was extracted as described earlier. Briefly, grounded crude gum (100g) was dissolved in water (300mL), and the solution was filtered. The filtrate was purified by precipitating the gum out with about 350 mL of alcohol (90%, v/v) and washed with diethyl ether and dried in the hot air oven at 50°C for 8 hr. The dried purified gum was milled and sieved through sieve number 80. A yield of 750 mg was obtained. It is calculated using the following equation:

\[
\% \text{ yield} = \left[ \frac{\text{practical yield}}{\text{theoretical yield}} \right] \times 100
\]
2.3 Preparation of CTZ-HPβCD complex

Physical mixture of CTZ-HPβCD complexes was prepared using Kneading method based on the previous report by Hani et al with minor modifications. Briefly, CTZ(0.344.8g) and HPβCD (1.54g) in the proportion of 1:1 molar ratio were grinded in a mortar for 1 h with a small quantity of alcohol. Thick slurry of the mixture was prepared by adding intermittently distilled water. The product was dried for 24 h at 45 °C. Finally, the dried complex was pulverized into a fine powder and sieved through mesh #80.

2.4 Phase solubility studies

Phase solubility studies were performed according to the method reported earlier by Higuchi and Connors method. In brief, excess amounts of CTZ were added to increasing concentrations (5, 10, 15, 20, and 25 mM) of HPβCD in screw capped bottles to attain saturation. Further, suspensions were shaken for 72 h at 25 ± 0.5 °C and filtered through 0.45µm nylon disk filter. CTZ concentration in the filtrate was spectrometrically analyzed at 264 nm. Each experiment was performed in triplicate. The stability constant ($K_s$), according to 1:1 stoichiometric ratio hypothesis of drug-HPCβD complex was calculated from phase solubility diagram using the equation:

$$K_s = \frac{S}{S_0(1-S)}$$

2.5 CTZ-HPβCD Complex Characterization

2.5.1 Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of CTZ, HPβCD and CTZ-HPβCD complex were obtained using an FT-IR-8400S Shimadzu (Tokyo, Japan) by KBr disk method (3mg sample in 300 mg KBr) in the scanning range of 400-4,000 cm$^{-1}$.

2.5.2 Nuclear magnetic resonance (NMR) analysis

1H NMR spectra of CTZ, HPβCD and CTZ-HPβCD complex, were acquired using BrukerAvans spectrometer (Fallanden, Switzerland) operating at 1H frequency of 500 MHz. The spectra of samples in CDCL$_3$ were acquired at 298 K.
2.5.3 Differential Scanning Calorimetry (DSC)
DSC was performed using a Shimadzu Q2000 scanning calorimeter using 3mg samples in crimped aluminium pans. Aluminium pan as a reference standard and nitrogen as a purge gas was used. Each sample was scanned at a rate of 20 °C/min from 25 to 330 °C under N₂ atmosphere (flow rate 30 mL/min).

2.5.4 X-Ray Diffraction (XRD)
XRD patterns of CTZ, HPβCD and CTZ-HPβCD complex, were recorded at an ambient temperature using diffractometer (Rigaku, Japan). Diffraction patterns were recorded using Ni-filtered CuKα radiation (λ=1.5418Å), 40 kV voltage, 20mA current and step of 0.02° for 2s with scan speed 0.01° on the interval 2θ=10°-60°.

2.5.5 Scanning electron microscopy (SEM)
To analyze morphology, SEM micrographs of CTZ, HPβCD and CTZ-HPβCD complex were obtained using a scanning electron microscope (Zeiss, EVO LS 15, Smart SEM 5.05, Germany) at an acceleration voltage of 15 kV at suitable magnification at room temperature. Briefly, 0.5 mg samples were mounted onto 5 mm silicon wafer and sputter-coated with gold under argon atmosphere and the surface morphology was visualized.

2.6 Experimental design for optimization
A 3² randomized factorial design was employed for formulation optimization. Two independent variables (factors), *Anacardium occidentale* gum (A) and Carbopol 934P(B) were selected and evaluated at three different levels: low (-1), medium (0) and high (+1). The hardness, % swelling, mucoadhesive strength and percentage drug release of tablets were used as dependent variables (responses). Data were evaluated using a DESIGN EXPERT® (version 8.0.7.1) software available from the Stat-Ease Inc. Table 1 provides the details of the composition and their levels for the CTZ-HPβCD bioadhesive tablet preparation. The study designs including the investigated responses are shown in Table 2. The range of a factor was chosen in order adequately to measure its effects on the response variables. For optimization, the effects of independent variables on measured responses were modeled using the following quadratic model equation

\[ Y = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2; \]  

where, \( Y \) is the response, \( b_0 \) is the arithmetic mean response of the 9 runs, and \( X_i \) is the estimated
coefficient for the factor A. The main effects (A and B) represent the average result of changing one factor at a time from its low to high value. The interactions (AB) showed the response changes when two factors are simultaneously changed. The polynomial terms ($A^2$ and $B^2$) are included to investigate nonlinearity. The response surface plots and counter-plots were analyzed to determine the effect of independent variables on the measured responses.

2.7 Preparation and evaluation of CTZ-HPβCD vaginal bioadhesive tablet formulation
A homogeneous powder blend of CTZ-HPβCD complex along with bioadhesive polymers *A. occidentale* gum, Carbopol934P with diluent lactose and lubricant magnesium stearate (1%, w/w) were gently prepared by geometrical dilution followed by blending using double cone blender (Kalweka, India) for 10 min. Tablets were prepared by direct compression using 10-station tablet machine (Minipress-1674, Rimek, India) fitted with round, flat-faced 12 mm punches to get the average weight of 300 mg tablet.

2.7.1 Physical characteristics
To determine weight variation, 10 tablets from each batch were weighed using a Sartorius digital balance and the mean weight and standard deviation were calculated. The thickness of tablets was determined using a digital vernier caliper. Hardness of the tablets was determined using a tablet hardness tester (TBH 125, Erweka, Germany). Tensile strength of tablets was checked by Universal Tensile Strength Testing Machine (Lloyd Instruments Ltd, UK) by determining the force at which the tablet broke. The tensile strength was calculated using the formula:

$$\text{Tensile strength (MPa)} = \frac{2F}{\pi Dt}$$

Friability of tablets was evaluated by using a Roche friabilator (FT2, Sotax, Switzerland). Briefly, tablets ($n = 10$) were placed in the friabilator and the instrument was operated at 25 rpm for 4 min. The % friability was calculated using the following equation:

$$\text{Friability (F\%)} = \frac{Wi - Wf}{Wf} \times 100$$

where $Wi$ is the initial weight, and $Wf$ is the final weight of the tablets.

Disintegration time was investigated as per Indian Pharmacopoeia.
2.7.2 Swelling study
To determine the water-uptake ability of the bioadhesive CTZ-HPβCD complex tablets, 10 tablets were weighed, and the average initial weight was calculated as \( W_1 \). The tablets were soaked in a petri plate (9 cm diameter) containing 15mL of simulated vaginal fluid (containing 3.51 g/L NaCl, 1.40 g/L KOH, and 0.222 g/L Ca(OH)\(_2\), 0.018 g/L bovine serum albumin, 2 g/L lactic acid, 1 g/L acetic acid, 0.16 g/L glycerol, 0.4 g/L urea and 5 g/L glucose; pH 4.5 maintained at 37 ± 0.5°C) and were removed periodically (at predetermined time intervals) from media and reweighed after blotting excess water carefully with a filter paper. Each experiment was performed in triplicate. The average weight \( W_2 \) was determined, and the degree of swelling was calculated by the formula:
\[
\% \text{ swelling} = \left(\frac{W_2 - W_1}{W_1}\right) \times 100
\]

2.7.3 Drug content estimation
The drug content of the prepared tablet formulation containing was determined by UV spectrometric method. Briefly, 50 mg CTZ equivalent tablet powder was transferred to a 50 mL of volumetric flask containing methanol. The reaction mixture was mixed thoroughly, and volume was made up with methanol and filtered. 5mL of resulting solution was diluted to 50mL with methanol, and the absorbance of the resulting solution was measured at 264 nm. Determination of the percentage drug content was carried out using the standard graph.

2.8 Evaluation of in vitro bioadhesive strength
Freshly excised goat vaginal mucosa was obtained from a local slaughter house (Mysore, India) and used for the study within 2 h. The surface of the mucosal membrane was initially separated; underlying fat removed to obtain the thickness of about 0.5 mm. The membrane was then washed with distilled water, blotted with filter paper and then moistened with 25 µL of simulated vaginal fluid at 37°C. The vaginal mucosa was sectioned into smaller pieces, and a piece of mucosa was used to evaluate the bioadhesive strength of formulated vaginal tablets was evaluated as previously reported with minor modifications. Three experiments were performed in triplicate and the force required to detach the tablet from the mucosal surface was taken as the measure of bioadhesive strength.
2.9 In vitro drug release studies
Type II dissolution test apparatus (Electrolab TDT-06P, India) was used to study drug release from the tablet formulations. In brief, the tablets were subjected to dissolution study in 500 mL dissolution media (simulated vaginal fluid, pH 4.5) maintained at 37±0.5 °C with paddle rotating at 50 rpm. An aliquot of samples (5 mL) were withdrawn at periodic time intervals and replaced with fresh dissolution media to maintain sink condition. The amount of drug released was determined by UV analysis measuring the absorbance spectrometrically at 264 nm against the standard curve of the drug. The release data of all formulations were fitted to various kinetic models viz., zero-order, first-order, Higuchi and Korsmeyer-Peppas to determine the drug release patterns and establish mechanism of CTZ release.

2.10 In vitro antifungal studies
In vitro antifungal studies were performed on clinical isolates of *Candida albicans* (J-1023) (obtained from JSS Medical College) in Sabouraud’s glucose agar medium (2% w/v) by the cup plate method. *C. albicans* (J-1023) suspension (100μL) and 30 mL of sterile medium were introduced into sterile dishes (60 mm × 15 mm). The plates were agitated carefully to allow homogenous mixing of the agar with test organism and allowed to solidify. In each plate, three cups (each 8mm in diameter) were bored in the medium with Cork borer. The disks of agar were removed using sterilized dissecting needle carefully without damaging the cups. 0.1mL of the sample (control, CTZ tablet and CTZ-HPβCD tablet) has been withdrawn at predetermined time intervals from the dissolution medium and placed in each cup (Table 5). The plates were incubated at 37 ± 5°C for 24 h. The entire experiment was performed under aseptic condition in triplicates and zone of inhibition was measured.

2.11 Mucoadhesive (X-ray) studies
Adult female rabbit (New Zealand strain, 2-2.5 kg) were obtained from Institute animal house facility. Specialized tablet with addition of aradiopaque agent – barium sulfate in the optimized tablet formulation (OF) was prepared. The radiopaque agent BaSO4, confer ‘contrast’ to X-ray films by their ability to absorb X-rays. The area where barium localizes will appear white on the X-ray film, creating distinctive definition and visual contrast. The prepared bioadhesive tablet was placed in the vaginal cavity of rabbit. The rabbit was exposed to X-ray examinations, and
photographs were taken at 1h and 10h after tablet administration. All experimental procedures including handling were approved by the Institutional Animal Ethics Committee (Registration number: 144/2013) complied with the guidelines set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Ministry of Environment and Forests, Government of India, India.

3. Results and Discussion

3.1 Phase solubility

Fig. 1 shows the phase solubility diagram of CTZ:HPβCD complex. The correlation coefficient ($r^2$) of the phase solubility diagram was 0.995. Results showed a relationship between CTZ solubility and increased in HPβCD ratio. The complex formation constant (K) obtained for the inclusion complex between CTZ and HPβCD was found to be 656 M$^{-1}$ (calculated from the straight line of the phase solubility diagram) fits an A$_1$-type profile i.e. linear increase with unchanged stoichiometry. Further, this type of curve indicates the formation of 1:1 type soluble inclusion complex$^{12,22}$ and solubility for the prepared inclusion complex increased 9.3 times at HPβCD concentration of 10mmol/L. This result corroborates with earlier findings.$^{11}$ Along these lines, it is possible that the formation of this stable complex could be due to the possible interaction between basic groups of CTZ and HPβCD which further suggests the tendency of the drug to enter the cavity of HPβCD. These findings support our previously demonstrated ability of HPβCD complexation to improve solubility of spice active principle (curcumin).$^{11}$

3.2 Characterization of the CTZ:HPβCD complexes

3.2.1 FT-IR studies

The FT-IR spectra of pure CTZ, HPβCD, and CTZ:HPβCD complex are presented in Fig. 2. The spectra of CTZ showed characteristic absorption peaks at 3057 cm$^{-1}$ (for C-H stretching vibrations), 1585 cm$^{-1}$ (for C=N stretching vibrations), 1481 and 1438 cm$^{-1}$ (for C=C stretching vibrations), 1082 cm$^{-1}$ and 1039 cm$^{-1}$ (for C-N stretching vibrations), 1202 cm$^{-1}$ and 1313 cm$^{-1}$ (for C-H bending vibrations) and 902, 823, 756 and 702 cm$^{-1}$ (for C-H bending vibrations) (Fig. 2A). The HPβCD (Fig. 2B) showed a prominent absorption band at 3394 cm$^{-1}$ (for O-H stretching vibrations), 2928 cm$^{-1}$ (for C-H stretching vibrations), 1157 and 1033 cm$^{-1}$ (for C-H-C-O stretching) as previously reported$^{27}$. In the FT-IR spectrum of CTZ-HPβCD complex peaks
corresponding to CTZ was observed (at 1481 and 1438 cm\(^{-1}\)) (Fig. 2C) which could be probably attributable to the decrease in crystallinity of the CTZ structure in the inclusion complex, as previously alluded by Garcia et al.\(^{28}\)

### 3.2.2 NMR Spectroscopy

The formation of the inclusion complex between CTZ and HPβCD was examined using 1D \(^1\)H NMR spectra of CTZ, HPβCD and CTZ-HPβCD inclusion complex (Fig. 3). Major changes were observed in the chemical shift values of HPβCD NMR spectra with large effect on the protons located inside the hydrophobic cavity i.e. H-3 (with δ value 3.5) and H-5 (with δ value 3.8) which provides information about inclusion of guest molecules. H-3 and H-5 protons of HPβCD showed upfield shift in drug-HPβCD complex spectra suggesting these protons are situated near the π-electron cloud of an aromatic nucleus (i.e. CTZ) and resulted in an upfield shift due to its magnetic anisotropy as previously suggested.\(^{29}\) Further, as explained by Garg et al\(^{30}\) an upfield shift displacement is probably due to variation in local polarity when the protons are inside the cavity of HPβCD and indicates weaker interaction with hydrogen atoms (shielding effect due to van der Waals forces between the drug and carbohydrate chains). From the NMR results, it can be concluded that CTZ was embedded in the cavity of HPβCD supporting the results of FT-IR studies.

### 3.2.3 DSC analysis

The characteristic sharp melting point of CTZ was observed at 145 °C (Fig. 4A). For the physical mixture, the endothermic peak of CTZ and the HPβCD peaks shows the absence of any interactions between the drug and HPβCD (Fig. 4B), indicating that the physical mixture system is a simple mixture of both the components. However, the smaller intensity of the CTZ peak could be due to the masking of CTZ melting endotherm or fusion between drug melting and HPβCD decomposition due to overlapping of the vicinity of the two effects Liu has reported a similar phenomenon.\(^{31}\) Concerning the complex inclusion, the endothermic peak of CTZ disappeared completely in the thermogram of the inclusion complex (Fig. 4C). This explains the amorphous solid dispersion and molecular encapsulation of CTZ into HPβCD cavity.\(^{32}\) Further, thermal curve of HPβCD alone showed a broad irregular endothermic peaks at about 305.5 °C (Fig. 4D) that could be attributable to degradation of HPβCD as previously reported.\(^{33}\)
3.2.4 X-ray diffraction

Complexation of CTZ with HPβCD was further ascertained by X-ray powder diffraction (XRD). Complexation with CD alters the crystallinity of the drug by changing its structure to an amorphous state. The XRD pattern of CTZ presented intense, sharp diffraction peaks indicating the crystalline nature of the drug molecule (Fig. 5A). CTZ has strong crystallinity peaks at 2θ of 10.3°, 12.4°, 18.6°, 19.5°, 20.7° and several minor peaks at 14.2°, 16.7°, 18.8°, 19.9°, 24.4°, 27.5° and 28.2°. The appearance of sharp peaks of the pure drug indicated the retention of the crystalline structure of CTZ in the physical mixture (Fig. 5B). However, smaller intensity of some peaks of CTZ could be due to the dilution effect of HPβCD. XRD pattern of HPβCD showed absence of diffraction peaks and presence of a broad peak in the range of 2θ 15-30° confirming its amorphous structure as previously reported by Jing Wang et al. Diffraction pattern of CTZ-HPβCD complex revealed a broad peak at 2θ 10-20° (Fig. 5C) which was similar to that of the amorphous HPβCD (Fig. 5D) and did not exhibit the characteristic peaks of CTZ, thereby demonstrating that complex has an amorphous structure. Along these lines, XRD analysis confirmed DSC results for the formation of the inclusion complex of the drug within HPβCD cavity.

3.2.5 SEM analysis

CTZ appeared in crystal-like structure (Fig. 4A) whereas HPβCD revealed amorphous spheres (Fig. 6B) as previously reported. In the CTZ and HPβCD physical mixture, the appearance of characteristic CTZ crystals mixed with CD particles was observed (Fig. 6C). However, in contrast, the CTZ-HPβCD inclusion complex appeared in the form of irregular particles in which the original morphology of both components disappeared and showed aggregation of particles into irregularly shaped amorphous deposits (Fig. 6D). Comparison of these SEM micrographsshowed that the complex was structurally distinct from the isolated components, and the physical mixture. The sizes and shapes of CTZ and HPβCD particles were different from those of the inclusion complex, which confirmed the formation of the inclusion complex. These results indirectly prove that the inclusion complex between CTZ and HPβCD was completely formed and that the improved solubility of CTZ was partially attributable to the formation of the inclusion complex.
3.3. Vaginal bioadhesive tablet formulation studies

Vaginal bioadhesive CTZ-HPβCD complex tablet formulations were prepared using *A. occidentale* gum and Carbopol 934P at different ratios. All the formulations were composed of 200 mg of CTZ-HPβCD complex as the active ingredient and magnesium stearate (0.5% w/w) as the lubricant (as shown in Table 2). The physical characterization (hardness, weight variation, thickness, diameter, friability, and drug content), swelling, mucoadhesion strength and drug release studies were performed on the tablet formulations.

3.3.1 Tablet physical characterization

Table 2 presents the summary of physical studies of the tablet formulation. Hardness of the tablet is directly proportional to the force applied to compress a tablet. In order to resist mechanical stress prepared tablet must show optimum compactness and hardness. The hardness of the tablet formulations (F1 to F9) varied between 64.13 to 73.64 N. The proportional increase in the tablet hardness may be attributed to increase in concentration of the gum and Carbopol 934P which are used as a binder in the formulation and the hardness increased with increasing concentration of *A. occidentale* gum and Carbopol 934P. The average tablet weight of the prepared tablet formulation varied from 300±0.12 to 304±0.22 mg and no batch varied by more than 5% from the tablet weight indicating consistency in tablet formulation. Other physical characteristics viz., thickness and diameter of the tablet are also important factors influencing drug release from the tablet. As the tablet is intended to be used in the vaginal cavity, thickness of the tablet was also measured. All the formulations showed optimum thickness and diameter between suitable to be inserted into the vaginal cavity. Concerning the uniformity of drug content, all of the formulations were acceptable since drug content of the tablet was between 98.91±0.31 and 99.86±0.24 indicating uniform mixing of the tablet formulation. Further, all the prepared tablets showed good compactness and friability less than 1% that showed that all the formulations prepared were within the pharmacopoeial limits. Mean drug content of vaginal bioadhesive CTZ-HPβCD complex tablet was found to be 98.53% thus showing drug content uniformity of the tablets (Table 2).
3.3.2 Swelling studies

Swelling capacity is of importance since it regulates the bioadhesive property of the formulation and enhances the adhesive material adsorption to onto the mucosa.\textsuperscript{35} All tablet formulations showed high percentage swelling. The dynamic swelling profile of various tablets showed that the rate of swelling was directly proportional to \textit{A. occidentale} gum and Carbopol 934P content. Visualization initially showed smoothening of the ends of the tablet to leave a gel layer and gradual change its size and integrity. Formulation F9 showed highest percentage swelling of (158.31±0.32) containing 20\% \textit{A. occidentale} gum and 10\% Carbopol 934P compared to other formulations prepared, while least % swelling was obtained with formulation F1 (108.32 ± 0.04) which contained lower concentration (5\%) of anionic polymer Carbopol 934P in the tablets (Table 2). The swelling index of the gum was found to be 11.5 ± 0.36 mL, indicating good water absorbing capacity of the polysaccharide and hence its capability to form hydrated three-dimensional networks from which drug release might follow by diffusion attributable to high porosity and lower surface tension of cashew gum.\textsuperscript{18}

3.4 Mucoadhesive strength

Mucoadhesion may be defined as the adhesion takes place between two materials, one of which is a mucosal surface. The phenomena of mucoadhesion take place in 3 stages: wetting, interpenetration, and mechanical interlocking between mucus and polymer.\textsuperscript{18} The strength of mucoadhesion depends on various factors including molecular weight and swelling rate of polymers, contact time with mucus and biological membrane used in the study. Hydration is one of the important factor involved in the mechanism of mucoadhesion. If the level of hydration exceeds, the capability of bioadhesion decreases because a large number of polymer binding sites are involved in bonds with water molecules, hence reducing the number of groups effectively available to interact with mucin chains.

In present study, goat vaginal mucosa was used as biological membrane for mucoadhesion. It was observed that as the concentration of \textit{A. occidentale} gum and Carbopol 934P increases the mucoadhesive strength was also increased. Formulation F9 with highest amount of \textit{A. occidentale} gum and Carbopol 934P (20 and 10 \%) showed maximum mucoadhesive strength (0.87±0.01N) whereas F1 containing 10\% of gum and 5\% Carbopol 934P showed minimum mucoadhesive strength of 0.321±0.02N.
The reason for higher mucoadhesion with higher *A. occidentale* gum content may be due to the formation of secondary bioadhesion bonds with mucin and its capability to undergo extensive interpenetration and physical entanglement with mucus layer.\(^\text{36}\) Carbopol 934P is the main responsible for bioadhesion with its hydrophilic groups available to interact with mucin chains, and its presence improved the mucoadhesion force. From the literature, it was found that *A. occidentale* gum also possesses the mucoadhesive property, and the mucoadhesive property of this gum was further enhanced by a combination with Carbopol934P in order to develop vaginal tablet.

### 3.5 *In vitro* release studies of vaginal tablet

The release of CTZ from different vaginal bioadhesive tablet formulations is shown in Table 3. The amount of % drug release at predetermined time intervals were recorded up to 10 h. The natural gum, *A. occidentale* may not only be used to sustain the drug release from the tablet but also reported to have sufficient mucoadhesive strength for clinical application.\(^\text{18}\) From the literature, it was found that Carbopol934P is the best bioadhesive polymers for vaginal tablets. Common swellable systems hydrate after the contact with fluids creating a gel network in which drug diffusion takes place.\(^\text{31}\) A combination of natural gum with Carbopol934P was investigated in the present work. Formulation F1 and F2 which contained lower amount of cashew gum (10 and 15% respectively) coupled with lower concentration of Carbopol934P (5%) showed highest drug release at 5 h (96.4%) and 10 h (100%) whereas F2 showed 100% drug release up to 6 h. Formulation F3 to F6 showed 98.02 and 99.64% of controlled release of the drug up to 10 h and formulation F7 to F9 controlled the drug release up to 12 h giving 99.35 to 99.76%. It can be observed from the release profile that as the concentration of polymer increased, drug release was decreased. The coefficient of correlation indicates the release mechanism. The value obtained from all the formulation is in the range between 0.523 and 0.754, indicates non-Fickian release mechanisms. The release of bioadhesive vaginal tablet of CTZ-HPβCD followed the coupled erosion–diffusion mechanism. It may be noted here that lower the swelling property of the formulation higher the release rate of the drug.

### 3.6 Data analysis and optimization of design

The results obtained from the experiment were statistically analyzed for response variables. \(^\text{32}\) full factorial design was employed to study the effect of independent variables amount of
Carbopol 934P (A) and the amount of cashew gum (B) on dependent variables hardness, mucoadhesive strength, % swelling, % drug release after 1, 5 and 10 h and T100%. The results depicted in Table 4, clearly indicated that all the dependent variables are strongly dependent on the selected independent variables as they shown wide variation among the 9 batches (F1-F9). The fitted equations (full models) relating the responses to the transformed factor are shown in the same table. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematically expressed positive or negative. The high values of the correlation coefficient for the dependent variables indicate a good fit. In the case of hardness coefficients $b_1$, $b_2$, $b_{12}$, $b_{11}$ and $b_{22}$ were found to be significant. It is observed from regression analysis that all the coefficients are positive (Fig. 7A). It signifies dependent variable, hardness increases by increasing the value of independent variables, amount of gum (A) or/and Carbopol 934P (B). In the case of % swelling coefficients $b_1$, $b_2$ and $b_{22}$ were positive, and the coefficient $b_{12}$ and $b_{11}$ were negative (Fig. 7B). This indicates that dependent variable (% swelling) is directly proportional to amount of gum (A) or Carbopol 934P (B) alone and also with polynomial terms $B^2$ but inversely proportional to interactions of A and B and polynomial terms $A^2$. Further, in the analysis of mucoadhesive strength, the coefficients $b_1$, $b_2$, $b_{11}$, and $b_{22}$ were found to be positive, and the coefficient of AB was negative (Fig. 7C). This indicates that dependent variable, i.e., mucoadhesive strength is directly proportional to amount of gum (A) or Carbopol 934P (B) alone and also with polynomial terms ($A^2$ and $B^2$) but inversely proportional with interactions of A and B. Furthermore, % drug release (after 1, 5 and 10 h) coefficients $b_1$, $b_2$, $b_{12}$, $b_{11}$ and $b_{22}$ were negative indicating that dependent variables are inversely proportional to both the dependent variables A and B (Fig. 8A-D). Additionally, the combined effects of factor A and factor B shows the effect of cashew gum and Carbopol 934P on dependent variables. These plots are useful to understand the effects of various factors on the response at a given time and to predict the responses of dependent variables at intermediate levels of independent variables.

Optimization tool and the desirability approach are used as strategy for determining the effect of the levels of independent variables on the responses and to assist in obtaining the specific point that maximizes the desirability function. The optimization of multiple responses to different targets was performed using a multi-criteria decision approach (numerical optimization technique). The optimized formulation was obtained by applying constraints on dependent
variable responses and independent variables by constructing a desirability plot (Fig. 9). The constraints were: hardness - 70 N, % swelling - 0.7 N, % mucoadhesion - 148; drug release (h) at 1 - 0.91%, 5 - 63.7%, 10 - 91% and T100% - 10.9. The recommended concentrations of the independent variables were calculated by the from the plots that have the highest desirability factor near to 1.0 (0.806). The optimum values of selected variables obtained were found to be 18.51% and 8.33% of A and B respectively. The final composition of CTZ-HPβCD vaginal tablet comprised of 200 mg CTZ-HPβCD complex, 55.5 mg of cashew gum (18.51%), 24.9 mg of Carbopol 934P (8.3%), 18.1 mg of lactose as diluent and 1.5 mg of magnesium stearate as lubricant. The calculated desirability factor for optimized formulation was 0.806. The statistically optimized formulation (OF) fulfilled all the physicochemical criteria. In vitro dissolution, studies were carried out on the prepared optimized formulations were calculated to verify the theoretical prediction. The observed values: hardness - 69.2 N, % swelling - 0.62 N, mucoadhesive strength - 141%, drug release (h) at 1 - 1.2%, 5 - 65.4%, 10 - 92.2% and T100% - 10.89 h were in close agreement with the model predictions: hardness - 70 N, % swelling - 0.7 N, mucoadhesive strength - 148%, drug release (h) at 1 - 0.91%, 5 - 63.7%, 10 - 91% and T100% - 10.9. The relative errors (%) between the predicted and experimental values for each response were calculated, and the values found to be within 5%. The experimental values were in agreement with the predicted values confirming the predictability and validity of the optimization process in the present study used to prepare vaginal tablet formulation by 3² factorial design.

3.7 In Vitro Antifungal Activity

CTZ is well demonstrated to inhibit fungal cytochrome P-450 synthesis of ergosterol, the key sterol found in pathogenic fungi and enhances cellular permeability. In the present study, the biological activity of CTZ-HPβCD was evaluated against C. albicans (J) using cup-plate method. While the controls showed no zone of inhibition, pure CTZ and CTZ-HPβCD tablets showed 0.11% and 0.32% of drug release at 1 h respectively with no zone of inhibition indicating the concentration of the drug released from both tablets is less than minimum inhibitory concentration (MIC) of pure CTZ i.e. 68 µg/ml against Candida. Further, pure CTZ resulted in 24.34% of drug release at 5 h also showed no zone of inhibition. However, pure CTZ at 10 h showed 45.6% of drug release with 15.5 ± 0.22 mm of zone of inhibition. Interestingly, C. albicans showed higher susceptibility to CTZ-HPβCD (with 48.34% and 80.2% of drug release
at 5 h and 10 h respectively) with a zone of inhibition of 16.33 ± 0.18 mm and 27.4 ± 0.38 mm. The results revealed that CTZ-HPβCD vaginal tablet showed more antifungal activity than tablet containing pure CTZ. The higher antifungal activity of the prepared CTZ-HPβCD vaginal tablet could be attributed to higher solubility of CTZ in complex form in comparison with free form. As a result, greater drug release per unit time from the formulation provides higher concentration gradient and more amount of drug with improved antifungal efficacy.

3.8 Mucoadhesive (X-ray) studies

The mucoadhesion and retention property was evaluated in therabbit using x-ray photographic images (Fig. 10). Specialized tablet with addition of theradiopaque agent – barium sulfate in the optimized CTZ-HPβCD bioadhesive vaginal tablets formulation (OF) was administered into the vaginal cavity of female albino rabbit. The duration of tablet in the vaginal cavity was monitored by radiograms. It was observed that the tablet was retained in the cavity, remained intact and adhered to vaginal mucous membrane for a period of 10 h (Fig. 10). After the administration of the tablet in vaginal cavity diameter of the tablet was found to be increased slowly as a result of swelling. Further, the tablet showed swelling with change in the shape at 8h.

4. CONCLUSION

Inclusion complex of CTZ with HP•CD was successfully prepared, and vaginal bioadhesive tablet was formulated using Anacardiumoccidentale gum and Carbopol 934P as bioadhesive polymers using 3² factorial design successfully. It was found that CTZ is better included in HPβCD with asignificant increase in solubility and effect when compared to pure drug. The FT-IR, DSC, SEM, X-ray diffraction and ¹H NMR, 2D [¹H, ¹H] NOESY results produced important evidence of CTZ-HPβCD inclusion complex formation. Design of experiment (DoE) was applied successfully to develop CTZ-HPβCD bioadhesive vaginal tablet. Concentration of independent variables was found to have asignificant effect on responses like hardness, swelling, mucoadhesive strength and percentage drug release at 1, 5 and 10th h. The prepared tablet was effective against Candida albicans and showed more zone of inhibition compare to pure drug. The in vivo X-ray studies in rabbit showed that the tablet adheres to the vaginal mucosa up to 10 h. The developed CTZ-HPβCD bioadhesive vaginal tablet could be a novel vaginal tablet with increase the solubility of CTZ and reduced side effects and provide longer residence time at the
vagina because of combined bioadhesive effect of the gum and Carbopol along with therapeutic importance of higher bioavailability at the site of action leading to efficient therapy for vaginal candidiasis.

AUTHORS’ CONTRIBUTIONS
UH performed the experiments, analyzed the data and wrote the manuscript. GK helped in writing and editing the paper. HGS has supervised the experimental work.

ACKNOWLEDGMENTS
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REFERENCES
Figure Legends

Fig. 1.
Phase solubility diagram of clotrimazole (CTZ) as a function of increasing HPβCD concentration at 25 °C. Values are presented as mean ± SD (error bars smaller than symbols) from three experiments performed in triplicate/concentration. CTZ, Clotrimazole; HPβCD, hydroxyl propyl β cyclodextrin.

Fig. 2.
FT-IR spectra of Pure Clotrimazole, Pure HPβCD and CTZ-HPβCD complex.

Fig. 3.
1D 1H NMR spectrum of (A) CTZ (pure drug), (B) Expanded aromatic region of 1H NMR spectrum of CTZ, (C) hydroxypropyl-β-cyclodextrin, (D) CTZ-HPβCD inclusion complex and (E) Expanded aromatic region of 1H NMR spectrum of CTZ-HPβCD inclusion complex (F) Expanded 1H NMR spectrum of CTZ-HPβCD inclusion complex showing H3 and H5 proton shift.

Fig. 4.
DSC thermograms. (A) CTZ (pure drug), (B) CTZ-HPβCD physical mixture, (C) CTZ-HPβCD inclusion complex and (D) HPβCD.

Fig. 5.
Powder X-ray diffractograms of A: CTZ (pure drug), B: CTZ-HPβCD obtained by physical mixture, C: HPβCD and D: CTZ-HPβCD inclusion complex. Test condition: Cu Kα radiation (λ=1.5418 Å), voltage: 40 kV; 20 mA current; step size: 0.02°, time per step: 2 s; scan speed: 0.01°.

Fig. 6.
Scanning electron micrographs of (A) CTZ (pure drug), (B) HPβCD, (C) CTZ-HPβCD physical mixture and (D) CTZ-HPβCD inclusion complex at 500X magnification.

Fig. 7.
3D response surface plot showing the effect of independent variables i.e, Factor A (Anacardium occidentale gum) and Factor B (Carbopol 934P) on dependent variables like a) hardness, b) % swelling, c) % mucoadhesive strength.
Fig. 8.
3D response surface plot showing the effect of independent variables i.e, Factor A (*Anacardium occidentale* gum) and Factor B (Carbopol 934P) on dependent variables like a, b, c ) % Drug release at 1, 5 and 10 h and d) Time taken for 100% drug release.

Fig. 9.
Optimization of vaginal bioadhesive CTZ-HPβCD tablets represented by desirability plot.

Fig. 10.
X-ray radiographic images of the rabbit vaginal cavity at 1 h and 10 h after administration of optimized BaSO₄-loaded CTZ-HPβCD bioadhesive vaginal tablet.
Fig. 1

![Graph showing the relationship between Concentration of Clotrimazole (mmol/L) and Concentration of HPβCD (mmol/L). The equation is $y = 0.0062x + 0.0119$ and the $R^2 = 0.9956$.](image)
Fig. 2

CTZ-HPβCyD Complex

Pure HPβCyD

Pure CTZ

wavenumber cm⁻¹

% T

A

B

C
Fig. 3
Fig. 5
Fig. 6
Fig. 7

A

Carbopol 934P vs. Anacardium occidentale gum

B

Carbopol 934P vs. Anacardium occidentale gum

C

Carbopol 934P vs. Anacardium occidentale gum
Fig. 8

A

Release at 1h (%)

Carbopol 934P

Anacardium occidentale gum

B

Release at 5h (%)

Carbopol 934P

Anacardium occidentale gum

C

Release at 10h (%)

Carbopol 934P

Anacardium occidentale gum

D

T 100 % (h)

Carbopol 934P

Anacardium occidentale gum
Fig. 9

Desirability

A: Cashew gum
B: Carbopol 934P

Prediction 0.802
Table 1
Composition of independent variables and their levels for the preparation for CTZ-HPβCD bioadhesive vaginal tablets.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Actual values (%)</th>
<th>Coded values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td><em>Anacardium occidentale</em> gum (A)</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Carbopol 934P (B)</td>
<td>5</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Table 2: Results of hardness and disintegration time, calculated dimensions (weight, thickness and diameter), tensile strength, friability, drug content of tablet formulations containing CTZ-HPβCD complex (200 mg) prepared using Carbopol and cashew gum at different concentrations using lactose as diluent and 0.5% magnesium stearate as a lubricant.

<table>
<thead>
<tr>
<th>Form</th>
<th>Cashew gum (%)</th>
<th>Carbopol 934P (%)</th>
<th>Hardness (N)</th>
<th>Disintegration time (min)</th>
<th>Mean weight (^a) (mg)</th>
<th>Thickness (^a) (mm)</th>
<th>Diameter (^a) (mm)</th>
<th>Tensile strength (MPa)</th>
<th>Friability (%)</th>
<th>Drug content (^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>10</td>
<td>5</td>
<td>66.97 ± 0.13</td>
<td>DND in 1hr</td>
<td>303 ± 0.12</td>
<td>5.3 ± 0.01</td>
<td>10.5 ± 0.03</td>
<td>0.735 ± 0.01</td>
<td>0.363</td>
<td>99.80 ± 0.22</td>
</tr>
<tr>
<td>F2</td>
<td>15</td>
<td>5</td>
<td>64.92 ± 0.23</td>
<td>DND in 1hr</td>
<td>304 ± 0.22</td>
<td>5.4 ± 0.06</td>
<td>10.5 ± 0.03</td>
<td>0.741 ± 0.01</td>
<td>0.422</td>
<td>99.62 ± 0.12</td>
</tr>
<tr>
<td>F3</td>
<td>20</td>
<td>5</td>
<td>67.07 ± 0.11</td>
<td>DND in 1hr</td>
<td>300 ± 0.12</td>
<td>5.6 ± 0.03</td>
<td>10.4 ± 0.02</td>
<td>0.746 ± 0.01</td>
<td>0.314</td>
<td>99.12 ± 0.18</td>
</tr>
<tr>
<td>F4</td>
<td>10</td>
<td>7.5</td>
<td>64.13 ± 0.21</td>
<td>DND in 1hr</td>
<td>303 ± 0.12</td>
<td>5.5 ± 0.04</td>
<td>10.5 ± 0.03</td>
<td>0.721 ± 0.02</td>
<td>0.189</td>
<td>99.86 ± 0.24</td>
</tr>
<tr>
<td>F5</td>
<td>15</td>
<td>7.5</td>
<td>65.80 ± 0.09</td>
<td>DND in 1hr</td>
<td>301 ± 0.13</td>
<td>5.3 ± 0.01</td>
<td>10.2 ± 0.03</td>
<td>0.749 ± 0.02</td>
<td>0.154</td>
<td>99.40 ± 0.06</td>
</tr>
<tr>
<td>F6</td>
<td>20</td>
<td>7.5</td>
<td>68.45 ± 0.11</td>
<td>DND in 1hr</td>
<td>304 ± 0.22</td>
<td>5.6 ± 0.07</td>
<td>10.5 ± 0.01</td>
<td>0.783 ± 0.02</td>
<td>0.364</td>
<td>98.91 ± 0.31</td>
</tr>
<tr>
<td>F7</td>
<td>10</td>
<td>10</td>
<td>69.52 ± 0.03</td>
<td>DND in 1hr</td>
<td>301 ± 0.12</td>
<td>5.1 ± 0.03</td>
<td>10.6 ± 0.03</td>
<td>0.749 ± 0.02</td>
<td>0.411</td>
<td>99.11 ± 0.38</td>
</tr>
<tr>
<td>F8</td>
<td>15</td>
<td>10</td>
<td>70.80 ± 0.11</td>
<td>DND in 1hr</td>
<td>301 ± 0.12</td>
<td>5.3 ± 0.03</td>
<td>10.4 ± 0.02</td>
<td>0.783 ± 0.02</td>
<td>0.462</td>
<td>99.64 ± 0.13</td>
</tr>
<tr>
<td>F9</td>
<td>20</td>
<td>10</td>
<td>73.64 ± 0.21</td>
<td>DND in 1hr</td>
<td>301 ± 0.12</td>
<td>5.4 ± 0.06</td>
<td>10.3 ± 0.03</td>
<td>0.761 ± 0.02</td>
<td>0.325</td>
<td>99.54 ± 0.18</td>
</tr>
</tbody>
</table>

All values are mean ± SD, \(n = 10\).  
DND: Did not disintegrate
Table 3
3² full factorial design and the observed responses for tablet formulations containing CTZ-HPβCD complex.

<table>
<thead>
<tr>
<th>Form</th>
<th>Hardnessᵃ</th>
<th>% swellingᵃ</th>
<th>Mucoadhesive strengthᵃ</th>
<th>Drug releaseᵃ</th>
<th>T₁₀₀%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 h</td>
<td>5 h</td>
</tr>
<tr>
<td>F1</td>
<td>66.97 ± 0.13</td>
<td>108.32 ± 0.04</td>
<td>0.321 ± 0.02</td>
<td>1.45 ± 0.11</td>
<td>96.40 ± 0.22</td>
</tr>
<tr>
<td>F2</td>
<td>64.92 ± 0.23</td>
<td>111.71 ± 0.03</td>
<td>0.355 ± 0.02</td>
<td>1.73 ± 0.23</td>
<td>85.50 ± 0.21</td>
</tr>
<tr>
<td>F3</td>
<td>67.07 ± 0.11</td>
<td>169.34 ± 0.11</td>
<td>0.381 ± 0.02</td>
<td>1.43 ± 0.32</td>
<td>74.90 ± 0.16</td>
</tr>
<tr>
<td>F4</td>
<td>64.13 ± 0.21</td>
<td>129.32 ± 0.12</td>
<td>0.563 ± 0.03</td>
<td>1.83 ± 0.12</td>
<td>79.41 ± 0.32</td>
</tr>
<tr>
<td>F5</td>
<td>65.80 ± 0.09</td>
<td>131.66 ± 0.22</td>
<td>0.600 ± 0.01</td>
<td>1.64 ± 0.11</td>
<td>78.48 ± 0.22</td>
</tr>
<tr>
<td>F6</td>
<td>68.45 ± 0.11</td>
<td>137.82 ± 0.21</td>
<td>0.626 ± 0.03</td>
<td>1.21 ± 0.03</td>
<td>72.44 ± 0.13</td>
</tr>
<tr>
<td>F7</td>
<td>69.52 ± 0.03</td>
<td>149.51 ± 0.11</td>
<td>0.811 ± 0.03</td>
<td>0.91 ± 0.11</td>
<td>70.24 ± 0.23</td>
</tr>
<tr>
<td>F8</td>
<td>70.80 ± 0.11</td>
<td>152.62 ± 0.12</td>
<td>0.845 ± 0.01</td>
<td>0.64 ± 0.31</td>
<td>60.48 ± 0.31</td>
</tr>
<tr>
<td>F9</td>
<td>73.64 ± 0.21</td>
<td>158.31 ± 0.32</td>
<td>0.870 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>48.34 ± 0.03</td>
</tr>
</tbody>
</table>

ᵃ mean ± SD, n = 3
T₁₀₀%, time required for 100% of drug release
Table 4
Regression coefficients of formulations for dependent variable responses of tablet formulations containing CTZ-HPβCD complex prepared as per $3^2$ factorial designs.

<table>
<thead>
<tr>
<th>Response</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$b_{12}$</th>
<th>$b_{11}$</th>
<th>$b_{22}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>65.37</td>
<td>1.423</td>
<td>2.5006</td>
<td>1.005</td>
<td>1.122</td>
<td>2.694</td>
<td>0.9643</td>
</tr>
<tr>
<td>Mucoadhesive strength</td>
<td>126.195</td>
<td>13.0533</td>
<td>11.845</td>
<td>-13.055</td>
<td>10.1066</td>
<td>8.7016</td>
<td>0.9627</td>
</tr>
<tr>
<td>% swelling</td>
<td>0.599</td>
<td>0.0303</td>
<td>0.2448</td>
<td>-0.00025</td>
<td>-0.00467</td>
<td>0.000833</td>
<td>0.9765</td>
</tr>
<tr>
<td>1</td>
<td>1.6568</td>
<td>-0.2056</td>
<td>-0.4568</td>
<td>-0.1425</td>
<td>-0.1453</td>
<td>-0.47883</td>
<td>0.9734</td>
</tr>
<tr>
<td>Drug release (h)</td>
<td>82.018</td>
<td>1.1933</td>
<td>-4.925</td>
<td>-15.005</td>
<td>-10.7533</td>
<td>-1.19833</td>
<td>0.9446</td>
</tr>
<tr>
<td>5</td>
<td>100.901</td>
<td>-2.59667</td>
<td>-1.835</td>
<td>-6.875</td>
<td>-5.9166</td>
<td>-2.3816</td>
<td>0.9753</td>
</tr>
<tr>
<td>$T_{100%}$</td>
<td>9.7033</td>
<td>-0.7216</td>
<td>0.2616</td>
<td>1.8175</td>
<td>1.235</td>
<td>-1.155</td>
<td>0.8420</td>
</tr>
</tbody>
</table>

$T_{100\%}$, time required for 100% of drug release
Table 5
Diameter of zone of inhibition for *in-vitro* release samples of control, tablet containing plain CTZ and optimized vaginal bioadhesive tablet containing CTZ-HPβCD.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Drug release</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 1 h</td>
<td>At 5 h</td>
</tr>
<tr>
<td>Control (Tablet without drug)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tablet containing Plain CTZ</td>
<td>0.11±0.04</td>
<td>24.34±0.08</td>
</tr>
<tr>
<td>OF (Tablet containing CTZ-HPβCD complex)</td>
<td>0.32±0.02</td>
<td>48.34±0.03</td>
</tr>
</tbody>
</table>