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RESEARCH ARTICLE

Design and Optimization of Curcumin–HPβCD Bioadhesive Vaginal Tablets by 2^3 Factorial Design: In Vitro and In Vivo Evaluation

Umme Hani · H. G. Shivakumar · Atul Srivastava · Nawaz Mahammed · Shailesh Thirumaleshwar · Naga Sravan kumar Varma · Rudra Vaghela · Chetan G. Shinde · M. P. Gowrav

Abstract
Purpose Candida albicans is the major cause of candidiasis. Better patient compliance and therapeutic efficacy can be achieved by utilizing herbal antifungal agent curcumin which is 2.5-fold more potent than fluconazole at inhibiting the adhesion of *C. albicans*.
Methods Curcumin–hydroxypropyl-β-cyclodextrin (HPβCD) was first developed to increase the solubility of curcumin. The formation of the curcumin–HPβCD complex was characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR) and evaluated for its solubility. Curcumin–HPβCD complex was formulated in a bioadhesive tablets using hydroxypropyl methyl cellulose (HPMC), hydroxyethyl cellulose (HEC), and Carbopol 934P. A 2^3 factorial design was applied to investigate effect of polymers on hardness, %swelling, and drug release from tablets. Tablets were characterized by studies of friability, hardness, tensile strength, %swelling, mucoadhesion, in vitro drug release, antifungal activity, and X-ray studies in rabbit.
Results DSC and FTIR data of curcumin–HPβCD indicate that there was complex formation between the drug and HPβCD. Formulations showed 100 % drug release from 5 to 12 h with best results in terms of %swelling and mucoadhesion. In vivo X-ray studies showed that the tablet adheres to vaginal mucosa up to 8 h.
Conclusion The developed curcumin–HPβCD bioadhesive vaginal tablet using 2^3 factorial design could be a promising safe herbal medication and can ensure longer residence in the vagina and provide an efficient therapy for vaginal candidiasis.

Keyword Curcumin–HPβCD bioadhesive vaginal tablet · Vaginal candidiasis · 2^3 factorial design · 3D response curve · X-ray image

U. Hani (✉) · H. G. Shivakumar · A. Srivastava · N. Mahammed · S. Thirumaleshwar · N. S. kumar Varma · R. Vaghela · C. G. Shinde · M. P. Gowrav
Department of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore 570 015, Karnataka, India
e-mail: ummehaniahmed@gmail.com

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Introduction

Vaginal mucosa is often affected by infections or inflammations that change the physiological environment of this area. Vaginal candidiasis is a common problem among women throughout the world during their sexually active years, 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 [1].

Some of the agents used to date for treating vaginal candidiasis are metronidazole, ketoconazole, clotrimazole, fluconazole, itraconazole, secnidazole, nystatin, progesterone, acriflavine, etc., but they have side effects [2–8].

Traditional medicines provide an interesting and still largely unexplored source for the creation and development of potentially new antimicrobial agents [9, 10]. The primary and indispensable step toward this goal is screening of plants in popular medicine to provide rational means for many diseases that are obstinate and incurable with other systems of medicine. These are gaining popularity because of several merits such as fewer side effects, better patient tolerance, and

relatively less expensive and better acceptance due to long history of use. Medicinal effects of plants tend to normalize the physiological function and correct the underlying cause of the disorder [11], and also, they are less prone to the emergence of drug resistance [12].

The polyphenol curcumin (CUR) is the active ingredient in several herbal and traditional medicines of China and India [13]. It has been shown that curcumin is more efficient than fluconazole in inhibiting adhesion of *Candida* species to buccal epithelial cells [14]. Curcumin inhibits several eukaryotic P-type ATPases: Na^+/K^+-ATPase [15] and Ca^+-ATPase [16].

Curcumin has also been reported to possess anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antiarthritic, antibacterial, antifungal, antiprotozoal, antiviral, anti-Alzheimer, antipsoriatic, and neuroprotective activities [17].

Work has been carried out on estimation of antifungal activity of curcumin against *Candida*, and it was found that curcumin was 2.5-fold more potent than fluconazole at inhibiting the adhesion of *C. albicans* and has synergistic antifungal affects with azoles and polyenes [14]. However, the low solubility and instability of curcumin largely influenced its clinical application. Therefore, an effective method should be introduced to enhance the solubility and stability of the drug. Inclusion technique, a new pharmaceutical technology, is used to increase solubility and stability of the drugs. Cyclodextrins (CDs) are cyclic oligomers of glucose presenting a cage-like supramolecular structure and can encapsulate guest hydrophobic molecules inside their cavities because of their special molecular structure, which is composed of a hydrophobic internal cavity and hydrophilic external surface [18].

CDs are recognized as useful pharmaceutical excipients which form inclusion complexes to increase solubility, dissolution rate, and stability of poorly soluble compounds, to mask unwanted characteristics, or to reduce side effects [19].

Commonly used CDs are βCD, γCD, and their derivatives such as hydroxypropyl-β-cyclodextrin (HPβCD) and methyl β-CD (MβCD). Vivek et al. developed a novel formulation approach for treating experimental colitis in rat model by complexing curcumin with βCD and its derivatives HPβCD, MβCD, and γCD and evaluated them for increasing solubility of curcumin. The ability to increase the solubility of curcumin by CD increased in the order HPβCD> MβCD>βCD>γCD. Curcumin molecules with bulky side groups on the phenyl moiety seemed to fit better into the HPβCD cavity than into the cavities of MβCD. Curcumin seems to be better included in HPβCD with very significant increase in solubility when compared to pure drug. MβCD and HPβCD complexes prepared by kneading method showed an increase in solubility by 190- and 202-fold, respectively [20]. Taking this into consideration in the present study, HPβCD has been utilized for the complexation of curcumin.

Considering curcumin as promising lead compound for the design of new antifungal agent capable of inhibiting adhesion of *C. albicans*, bioadhesive formulations are developed for the treatment of candidiasis. In the present study, we have developed and evaluated a novel herbal bioadhesive vaginal tablet formulation for treating vaginal candidiasis. Curcumin was complexed with HPβCD and evaluated for increasing solubility of curcumin. The developed curcumin HPβCD bioadhesive vaginal tablet can be a promising herbal treatment for vaginal infection with no side effects.

Materials and Methods

Curcumin was purchased from Loba Chemie (purity 98 %), (2-hydroxypropyl)-β-cyclodextrin were purchased from Alfa Aesar, England. Hydroxypropylmethylcellulose (E 15 LV), hydroxyethyl cellulose high viscosity (270), Carbopol 934P, lactose monohydrate, and magnesium stearate were purchased from Loba Chemie (Mumbai, India). All other chemicals used were of analytical grade.

Curcumin HPβCD Complex

Phase Solubility Studies

The phase solubility studies were carried out in water at 25± 0.5 °C as per the method reported by Higuchi and Connors [21]. Different concentrations of aqueous HPβCD solutions such as 5, 10, 15, 20, and 25 mM were prepared and filled in screw-capped bottles. Excess amount of curcumin was added to the above solutions to attain saturation. Suspensions were capped and shaken for 72 h at 25±0.5 °C to ensure equilibrium. The bottles were also protected from light to prevent degradation. After complete mixing, the sample was filtered through 0.45-μm nylon disk filter diluted and assayed for the total dissolved curcumin content by UV analysis at 424 nm [Shimadzu 1,800 UV Visible spectrophotometer]. Experiments were performed in triplicate. The stability constant (K), which suggests inclusion effects, is an important parameter in the complexation process [22, 23]. The range of *K* between CDs or their derivatives and the majority of drugs is 100–20,000 M^{-1} [24]. A higher *K* value indicates better inclusion effects [25]. *K* value can be calculated for a 1:1 complex by using the following equation [26]:

$$K = \text{solpe}/S_0(1 - \text{slop}) \quad (1)$$

where S_0 is the solubility of curcumin in the absence of HPβCD, which can be obtained from a straight line in the phase solubility diagram.

Preparation of Curcumin–HPβCD Complex

The inclusion complex of curcumin with HPβCD in 1:2 M ratios was prepared by using the kneading method. Curcumin and HPβCD in the proportion of 1:1 and 1:2 M concentrations were mixed in a mortar for 1 h with small quantity of alcohol followed by addition of distilled water intermittently to get slurry-like consistency. The paste was dried in a hot air oven at a temperature of 45 °C for 24 h. Dried complex was pulverized into fine powder and sifted with sieve #80 [20].

Curcumin Quantification

To determine the curcumin content present in the pure curcumin samples, 10 mg of the sample was diluted in 25 ml of ethanol and then filtered. The absorbance of the solution was determined at 424 nm using a UV–Vis spectrophotometer (Shimadzu, 1800, Japan).

To determine the curcumin content in the complexes, 10 mg of curcumin–HPβCD complex was dissolved in 10 ml of ethanol and filtered, and the absorbance was determined at 424 nm. For each measurement, the baseline was established using blank ethanol as a reference. C_E (%) is defined as the ratio between the amount of complexed curcumin and the total amount added initially:

C_E refers to the mass of complexed curcumin and C_T to the total mass of curcumin added initially [27].

Initial Characterization of Inclusion Complexes

Percentage Yield The efficiency of the process is determined by the yield. It was calculated using the following equation: % yield=[practical yield/theoretical yield]×100.

Solubility Studies of Cyclodextrin Complexes Excess of CD complexes was dispersed in 25 ml of distilled water in screw-capped bottles to get a supersaturated solution. These bottles were shaken continuously for 2 h at ambient temperature until equilibrium was attained. Supersaturated solution was filtered through a 0.22-μm nylon filter and diluted with methanol, and absorbance was measured at 424 nm. Solubility studies were also performed for pure drug.

FTIR Pure curcumin, HPβCD, physical mixture, and prepared curcumin–HPβCD complex were subjected to Fourier transform infrared spectroscopy (FTIR) (Shimadzu 8,400S, Japan). Samples were prepared in KBr discs and were scanned between wave number 400 and 4,000 cm^{-1}.

DSC Differential scanning calorimetry was used to assess the complexation of curcumin with HPβCD. Differential scanning calorimetry (DSC) scans of the pure curcumin, HPβCD, physical mixture, and prepared complex were performed using a Dupont 9,900 thermal analyzer with a 910 DSC module. The calorimetric measurements were made with an empty cell (high purity alpha alumina discs of Dupont Company) as the reference. The scans were taken under nitrogen atmosphere over a temperature range of 25 to 330 °C with a scan rate of 20.0 °C/min.

Morphological Analysis by SEM SEM photographs were taken using scanning electron microscope Model Joel–LV-5600, USA, at suitable magnification at room temperature. The photographs were examined for surface morphology of curcumin–HPβCD complex, physical mixture, HPβCD, and pure curcumin samples.

Experimental Design

In the present study, 2^3 full factorial design was constructed and conducted in a fully randomized order to study all possible combinations of all factors at all levels (three factors, two levels). The dependent variables measured were hardness of the tablet, %swelling, and percentage drug release. Three independent factors, concentration of Carbopol (factor A), hydroxypropyl methyl cellulose (HPMC) (factor B), and hydroxyethyl cellulose (HEC) (factor C), were set at two different levels as shown in Table 1. High and low levels of each factor were coded as +1 and −1, respectively. The range of a factor was chosen in order to adequately measure its effects on the response variables. This design was selected as it provides sufficient degrees of freedom to resolve main effects as well as the factor interactions [28, 29]. Composition of vaginal tablet formulations of curcumin–HPβCD complex is shown in Table 2. Formulation F1 is tablet without polymers F2 and F3 prepared to study the effect of Carbopol alone on tablet parameters. Similarly, F4 and F5 were prepared to study the effect of HPMC and F6 and F7 to study the effect of HEC alone on tablet parameters. And, formulations F8–F15 were obtained from 2^3 factorial design using Design of Experiments software (Design Expert 9.0.3.1.)

Table 1 Selected factor levels for the experimental design used in the formulation of curcumin–HPβCD bioadhesive vaginal tablets

Model factor	Actual values		Coded values	
	Low	High	Low	High
Factor A: amount of Carbopol 934P	5	10	−1	+1
Factor B: amount of HPMC	5	10	−1	+1
Factor C: amount of HEC	5	10	−1	+1

HPMC hydroxypropyl methyl cellulose, *HEC* hydroxyethyl cellulose

Table 2 Composition of curcumin–HPβCD bioadhesive vaginal tablets formulations

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Curcumin	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
Carbopol 934P	–	15	30	–	–	–	–	10	5	10	5	10	5	5	7.5
HPMC	–	–	–	15	30	–	–	5	10	5	5	10	5	10	7.5
HEC	–	–	–	–	–	15	30	5	5	10	10	10	5	10	7.5
Lactose	65.7	50.7	35.7	50.7	35.7	50.7	35.7	45.7	45.7	40.7	45.7	35.7	50.7	40.7	43.2
Magnesium stearate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Preparation of Bioadhesive Vaginal Tablet

A homogeneous physical blend of bioadhesive polymers Carbopol 934P, HPMC, HEC, and curcumin–HPβCD complex along with diluent lactose and lubricant magnesium stearate was gently prepared by geometrical dilution followed by blending using double cone blender (Kalweka, India) for 10 min. Tablet was prepared by direct compression method using ten-station tablet machine (minipress-1674, Rimek, India) fitted with round, flat-faced 12-mm punches to get the average weight of the 300-mg tablet.

Tablet Physical Characterization: Average Weight, Weight Variation, Thickness, Hardness, and Tensile Strength

The weight variation test of the tablets was carried out as per the Indian Pharmacopoeia guidelines. Ten tablets from each batch were weighed in a Sartorius digital balance, and the average weight and standard deviation were calculated. The thickness of tablet was determined using a digital vernier caliper. The average thickness and standard deviation were calculated. Hardness of the tablet is an indication of its strength and was tested by measuring the force required to break the tablet across the diameter. The force is measured in kilograms per centimeter. Tablet requires a certain amount of mechanical strength to withstand the shock of handling during its manufacture, packaging, shipping, and dispensing. Hardness of the tablet was determined using Erweka hardness taster. The tensile strength of tablets t was calculated using the following equation: Tensile stress $(\sigma t)=2F/\pi Dt$ (F is the crushing force, and D and t are the diameter and thickness of the tablet) [30].

Tablet Physical Characterization Friability

Friability is the measure of a tablet's ability to withstand both shock and abrasion without crumbling during manufacturing, packing, and shipping. The weight of ten tablets was taken and placed in Roche friabilator. The device subjects the tablets to the combined effect of shock and abrasion by utilizing a plastic chamber, which revolves at 25 rpm, dropping the tablets a distance of 6 in with the revolution. The preweighed tablet sample was removed after 100 revolutions, dedusted, and reweighed. Tablets that loose less than 0.5 to 1 % in weight are generally considered acceptable.

$$\text{Friability}(\%) = \frac{\text{Initial wt. of 10 tablets} - \text{final wt. of 10 tablets}}{\text{Initial weight of 10 tablets}} \times 100 \quad (1)$$

Drug Content Estimation

The Drug content of curcumin in the prepared curcumin–HPβCD bioadhesive vaginal tablets was determined by UV spectrometry. Ten tablets were finely powdered; quantity of the powder equivalent to 50 mg of curcumin was accurately weighed and transferred to a 100 ml of volumetric flask. Methanol was added and mixed thoroughly, and volume was made up with methanol and filtered. Ten milliliters of resulting solution was diluted to 100 ml with methanol, and the absorbance of the resulting solution was measured using UV Visible spectrometer (Shimadzu, 1800, Japan).

Tablet Swelling Study

Swelling behavior of prepared bioadhesive vaginal tablets was evaluated by dynamic swelling studies. Each tablet was weighed ($W1$) and immersed into a Petri plate (9-cm diameter) containing 15 ml of simulated vaginal fluid of pH 4.5. Plates were thermostated at 37 °C (±0.1) in a ventilate heater (Orbital Incubator, Sanyo Gallenkamp, Japan) for fixed times. The samples were periodically weighed after removing the excess water on the surface with a filter paper:

$$\text{Swelling}\ (\%) = [(W2 - W1)/W1] \times 100 \quad (2)$$

where $W1$ is the dry tablet weight and $W2$ is the weight after immersion in simulate vaginal fluid for predetermined time intervals (0.5, 1, 2, 4, 8, and 12 h) [31].

In Vitro Drug Release Studies

Drug release of tablet was evaluated using a Type II dissolution test apparatus (Electrolab TDT-06P, India). Five hundred milliliters of phosphate buffer of pH 4.5 with 1 % sodium lauryl sulfate (SLS) was used at 37± 0.5 °C with stirring speed of 50 rpm. Five milliliters of samples withdrawn at appropriate time intervals were filtered (Whatman filter paper), diluted with medium, and assayed at 424 nm using a spectrophotometer. This was compared against a calibration curve (r= 0.9991) and by using phosphate buffer of pH 4.5 with 1 % SLS as blank. Release experiments were run in triplicate. The release curve was described as the cumulative amount of released curcumin versus time profiles.

To determine the release mechanism, the release dates were fitted to empirical or semiempirical models, namely zero-order, first-order, and Higuchi and Peppas models. These were described by the following equations:

Zero-order model:

$$\mathrm{Mt}/M = kt \quad (3)$$

First-order model:

$$\mathrm{Ln}(1 - \mathrm{Mt}/M) = -kt \quad (4)$$

Higuchi model:

$$\mathrm{Mt}/M = k_h t^{0.5} \quad (5)$$

Peppas model:

$$\mathrm{Mt}/M = kt^n \quad (6)$$

where Mt/M is the fraction of released drug at time t, k is the release rate constant, K_h is the Higuchi rate constant, and n is an indicator of release mechanism. As the k value increased, the drug was released faster. The n value of 1 corresponded to zero-order release kinetics, $0.5<n<1$ to a non-Fickian release model, and n=0.5 indicated Fickian diffusion (Higuchi model) [32].

In Vitro Antifungal studies

In vitro antifungal studies were performed against clinical isolates of *C. albicans* j1023 (obtained from JSS Medical College) in Sabouraud's agar medium by the cup-plate method. The marketed formulation (100 mg Candid V) was suspended in 2 ml of sterilized water and transferred into the well of an agar Petri dish. Optimized tablets earlier sterilized by autoclaving for 30 min at 120 °C were also placed into wells in the same manner. One hundred milligrams of curcumin suspension was also prepared. Two milliliters of distilled water was used as the control. All the samples were applied in triplicate. Covered Petri dishes were incubated at 32 °C in the BOD incubator (LHC-78-Labhospmake, India) for 40 h. The zone of inhibition was measured after incubation [33].

In Vivo X-ray Studies

The in vivo X-ray studies were approved by the Institutional Animal Ethics Committee of JSS College of Pharmacy (proposal number 144/2013 Mysore, Karnataka, India). The study was performed on a healthy female rabbit, weighing between

1.5 and 2 kg. The optimized formulation was modified by adding X-ray-grade barium sulfate. The prepared tablet was placed in the vaginal cavity of healthy rabbit. The rabbit was exposed to X-ray examinations, and photographs were taken at 0, 2, 6, and 8 h after administration of the tablet.

Result and Discussion

Phase–Solubility Studies

The effect of cyclodextrins on the aqueous solubility of curcumin was evaluated using the phase solubility method. Phase–solubility diagrams fall into two main categories according to Higuchi and Connors, i.e., A- and B-types. A-type curves are indicative for the formation of soluble inclusion complexes, while B-type behavior is suggestive of the formation of inclusion complexes of poor solubility. ABS-type response denotes complexes of limited solubility, and a BI-curve is indicative of insoluble complexes. The A-curves are subdivided into AL (linear increases of drug solubility as function of cyclodextrin concentration), AP (positively deviating isotherm), and AN (negatively deviating isotherms) subtypes [34]. Figure 1 presents the phase solubility diagram curcumin and HPβCD. The plot shows that aqueous curcumin solubility increases linearly with increasing HPβCD concentration, which indicates AL-type phase solubility diagram. *K* value was found to be 634 M^{-1}. Curcumin content determined from the abovementioned curcumin quantification method was found to be 98 %±0.12.

FTIR Studies

The IR spectra of pure curcumin, HPβCD, and curcumin–HPβCD physical mixtures and complex are depicted in Fig. 2. Pure curcumin showed that stretching vibration of C=O and –OH group appeared at wave numbers 1,627.9 and 3,404 cm^{-1}, respectively, whereas

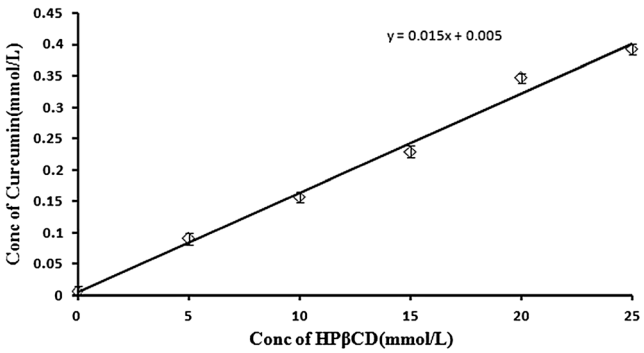

Fig. 1 Phase solubility diagram of curcumin–HPβCD complex

ether group (–C–O–) at 1,026.7 cm^{-1}, C=C aromatic stretching frequency at 1,429.3 cm^{-1}, C=C olefinic stretching frequency at 1,512.2 cm^{-1}, and a significant band were observed at 813.9 cm^{-1} being assigned to the C–H stretch. In the FTIR spectra of the physical mixtures, the broad absorption bands at 3,410 cm^{-1} arise from the stretching mode of OH groups. The bands in the range of 3,079–3,000 cm^{-1} can be attributed to aromatic C–H stretching vibration which may indicate intercalation of curcumin in CD complex as reported by Vivek et al.

In the case of curcumin CD mixture, the typical O–H stretching bands shift that might indicate the formation of hydrogen bonds between curcumin and HPβCD. These results showed appreciable shifts and variation in intensity of the characteristic curcumin bands, evidencing the chemical interactions between the curcumin and HPβCD due to inclusion of the drug in the CD cavity.

However, Tang et al. (2002) stated that curcumin has a 19 Å length and 6 Å width, which enables the possibility of curcumin rings entering the βCD cavity. The authors also affirmed that it is reasonable to consider complex formation with two molecules of βCD because, according to the molecule dimensions, curcumin appears to be too large to be entirely included in one βCD cage [35].

DSC Analysis

DSC is a very useful tool in the investigation of thermal properties of CD complexes and can provide both qualitative and quantitative information about the physicochemical state of the drug inside the CD complexes [22]. When guest molecules are embedded in CD cavities or crystal lattices, their melting, boiling, and sublimation points shifted to different temperatures [36].

DSC analysis of pure curcumin resulted in gradual enthalpy change producing a linear sharp endothermic peak at temperature 176.63 °C which is indicative of its melting temperature as shown in Fig. 3. Linearity and sharpness of the endothermic peak indicate the purity of the compound. In the case of HPβCD owing to its amorphous nature, a broad endothermic peak was observed at about 314.5 °C. The physical mixture of curcumin with HPβCD shows an endothermic peak at 177.9 and 314.5 °C, respectively, while the solid inclusion complex formed by kneading method showed a complete disappearance of the endothermic peak of both curcumin and HPβCD. A new endothermic peak at 338.3 °C appeared which may indicate the formation of a true inclusion complex between curcumin and HPβCD. The thermogram of the physical mixture was similar to the superimposition of the thermograms of individual curcumin and HPβCD [36].

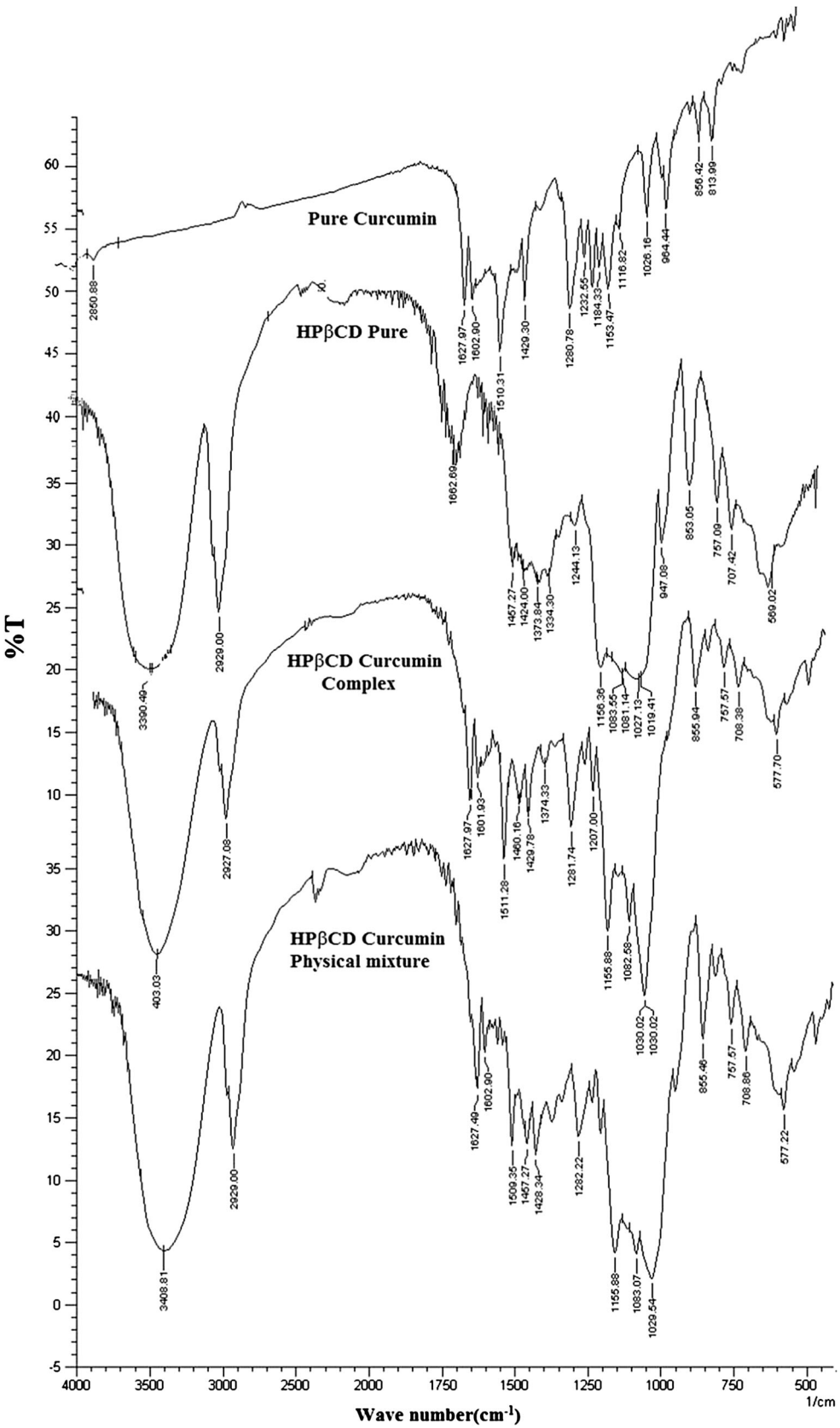

Fig. 2 FTIR spectra of curcumin, HPβCD, physical mixture, and curcumin–HPβCD complex

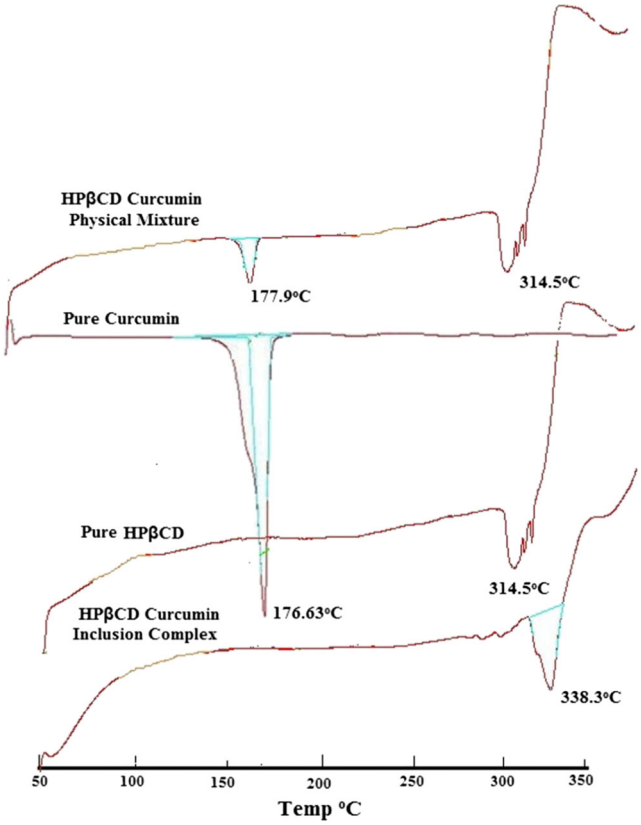

Fig. 3 DSC spectra of curcumin, HPβCD, physical mixture, and curcumin–HPβCD complex

SEM Studies

SEM pictures of pure curcumin, HPβCD, physical mixture, and HPβCD complex of curcumin are given in Fig. 4 which illustrated the arrangement of curcumin, HPβCD, and the physical mixture and the inclusion complex. Pure HPβCD and curcumin were observed to be irregular in shape. In the physical mixture, the characteristic curcumin particle, when mixed with cyclodextrin, was found to adhere onto the surface of cyclodextrin as observed by Vivek et al. Original morphology of both components HPβCD and curcumin disappeared in SEM image of HPβCD curcumin complex which shows aggregation of particles into irregularly shaped amorphous particles. When the HPβCD is kneaded with curcumin, the cavities of the HPβCD are filled by molecules of curcumin but are not clearly observed in SEM images.

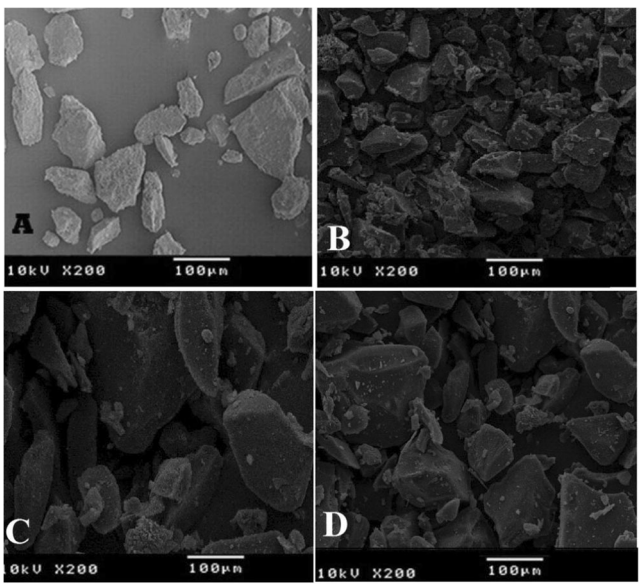

Fig. 4 SEM images of curcumin and its cyclodextrin complexes: **a** pure curcumin, **b** physical mixture, **c** HPβCD, and **d** curcumin–HPβCD complex

Tablet Characterization

In order to resist mechanical stress, the prepared tablet must show optimum compactness and hardness. Hardness of formulation F1 prepared without polymers was found to be 1.5± 0.13 kg. Formulation F2 which has 30 % *w*/*w* of Carbopol showed highest hardness, i.e., 10.3 kg, compared to all other formulations. In formulations F4 and F5 containing HPMC polymer alone, hardness was found to be 2.7±0.11 and 3.08± 0.12, respectively, and disintegrated within 5 min. In formulations F6 and F7 containing HEC polymer alone, hardness was found to be 0.86±0.22 and 0.90±0.21, respectively, and disintegrated within 3 min. Hence, formulations F1 and F4–F7 were excluded from the study as the tablet formulations showed low strength that cannot resist mechanical stress and disintegrated within a matter of 5 min. Mean hardness of the formulations F2, F3, and F8–F15 ranged from 5.1±0.2 to 10.3 ±0.23 kg as shown in Table 3, which is the optimum hardness for the vaginal tablet.

From the hardness of the various formulations prepared, it can be observed that the polymer used in the formulation is acting as a binder of the tablet which is responsible for developing strength to the tablet. When polymer is used alone, the hardness is less when compared to the combination of polymers, and hence, HPMC and HEC with the concentration of 15 and 30 % *w*/*w* cannot give optimum hard tablet for vaginal delivery, but a combination of polymers with different ratios results in an optimum hard tablet.

The physicochemical properties of tablets are summarized in Table 3. Thickness and diameter of the tablet are also important factors influencing drug release from the tablet. As the tablet has to be inserted into the vaginal cavity, thickness of the tablet was given special care. All the formulations have the optimum thickness ranging from 5.1±0.04 to 5.4±0.05 mm and diameter ranging from 10.4 to 10.6 mm suitable to be inserted into the vaginal cavity. Mean tablet weight for all the prepared formulation was in the range of 300 to 304 mg.

The weight variation of curcumin–HPβCD bioadhesive vaginal tablets for all the batches was below 5 % indicating consistency in manufacturing process. Concerning the

Table 3 Hardness and disintegration time, calculated dimensions (weight, thickness, and diameter), tensile strength, friability, drug content of curcumin–HPβCD bioadhesive vaginal tablets, and their standard deviations

Formulations	Hardness (kg)	Disintegration time (min)	Mean weight (mg±SD) (n=3)	Thickness (mm±SD) (n=3)	Diameter (mm±SD) (n=3)	Tensile strength (MPa)	Friability (%)	Drug content (% label claim±SD) (n=3)
F1	1.5±0.13	3.2	–	–	–	–	–	–
F2	6.4±0.03	DND in 1 h	303±0.2	5.3±0.02	10.4±0.01	0.750±0.01	0.332	99.1±0.24
F3	10.3±0.23	DND in 1 h	300±0.3	5.2±0.04	10.6±0.01	0.761±0.01	0.397	99.97±0.1
F4	2.7±0.11	4.5	–	–	–	–	–	–
F5	3.08±0.12	5.40	–	–	–	–	–	–
F6	0.86±0.22	2.2	–	–	–	–	–	–
F7	0.90±0.21	2.5	–	–	–	–	–	–
F8	6.5±0.03	DND in 1 h	304±0.02	5.2±0.05	10.4±0.01	0.735±0.01	0.332	99.8±0.06
F9	6.4±0.33	DND in 1 h	300±0.3	5.6±0.04	10.4±0.01	0.741±0.01	0.466	99.52±0.23
F10	6.7±0.03	DND in 1 h	300±0.2	5.3±0.04	10.6±0.02	0.746±0.01	0.331	99.62±0.08
F11	5.1±0.2	DND in 1 h	301±0.2	5.4±0.05	10.5±0.02	0.721±0.02	0.198	99.96±0.21
F12	5.7±0.2	DND in 1 h	301±0.1	5.2±0.02	10.5±0.01	0.749±0.02	0.166	99.9±0.22
F13	5.02±0.13	DND in 1 h	300±0.2	5.1±0.04	10.6±0.01	0.783±0.02	0.332	98.98±0.31
F14	6.4±0.03	DND in 1 h	301±0.2	5.3±0.03	10.4±0.02	0.761±0.02	0.397	99.62±0.12
F15	6.04±0.01	DND in 1 h	302±0.2	5.4±0.05	10.4±0.01	0.788±0.01	0.453	99.61±0.21

DND did not disintegrate

uniformity of drug content, all of the formulations were acceptable since drug content of the tablet was 98.9–99.9 % label claim indicating uniform mixing of the tablet formulation. All the prepared tablets showed good compactness with friability less than 1 %.

Swelling Studies

Bioadhesive polymers used in the curcumin tablets swell in the presence of water; hence, it is necessary to perform hydration studies in order to determine its hydration capabilities. All tablets showed high hydration percentage and found to retain their integrity even after 12 h. Initially, sharp ends of the tablet got smoothed to leave a gel layer on the tablet and gradually increased its size and changed its integrity with water penetration inside the tablet. Formulation F3 showed highest swelling compared to other formulations due to the presence of highest concentration of Carbopol in it. F15 showed lowest swelling among all the formulations. Formulations F8 and F12 having 10 % *w*/*w* of Carbopol showed very high rate of swelling, i.e., 374 and 248 %, respectively, in 30 min, whereas formulations F9 and F15 having 5 % *w*/*w* of Carbopol showed lowest swelling rate, i.e., 7 and 10 % in 30 min which is insufficient for the drug release. Formulation F13 showed optimum swelling which is significant for the drug release as shown in Fig. 5. Hence, from the swelling study, it was found that the formulations having highest concentration of Carbopol have higher swelling rate which can swell as soon as they come in contact with the aqueous medium, whereas formulations having mixture of polymers (Carbopol, HPMC, and hydroxyethyl cellulose (HEC)) reduce the swelling rate and result in optimum swelling of the tablet.

In Vitro Release Studies of Vaginal Tablet

Controlled release of the drug depends on bioadhesive polymers used in the formulation. From the literature, it was found that Carbopol 934P (CP), HPMC, and HEC are the best bioadhesive polymers for vaginal tablets. Figure 6 shows the in vitro release profiles of various curcumin tablet formulations. Formulation F2 with 15 % *w*/*w* of Carbopol and F13 with lowest concentration of all polymers showed 99.9 % drug release in 5 h. Formulation F12 and F14 with highest amount of combination of polymers showed 98.92 and 99.62 % of controlled release of the drug up to 12 h, whereas other formulations like F3 and F11 showed 98.92 and 96.7 % drug release in 8 h. F9 and F15 showed 93.32 and 99.98 % drug release in 6 h. F8 and F10 showed 99.52 and 99.2 % drug release in 10 h. It was found that as we increase the concentration of polymer in tablet formulations, the drug released in a controlled manner. Minimum inhibitory concentration of curcumin was 64 μg/ml as reported by earlier studies [14]. Based on this, F2 and F13 were selected as optimized formulation which can provide effective concentration of curcumin to achieve antifungal effect up to 5 h.

The other formulations which can provide controlled release from 8 to 12 h were not selected as optimized minimum inhibitory concentrations (MIC) cannot be achieved and hence

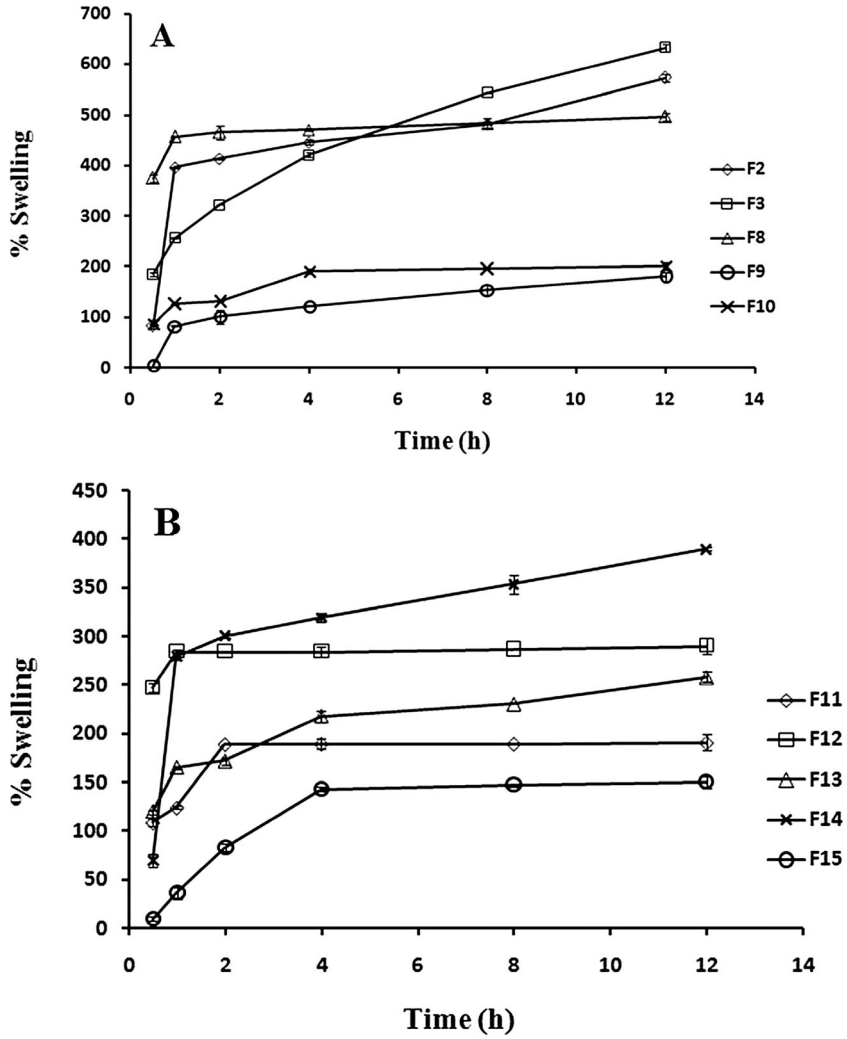

Fig. 5 %Swelling of formulation

fail to produce the required efficacy of curcumin against *C. albicans*. The results of fitting the release data to various models are shown in Table 4.

The release mechanism was better described by the Peppas model as indicated by correlation coefficients for all the tablet formulations. Results revealed *n* values between 0.543 and 0.714, indicating that the release mechanisms were non-Fickian. The release of curcumin–HPβCD bioadhesive vaginal tablet followed the coupled erosion–diffusion mechanism.

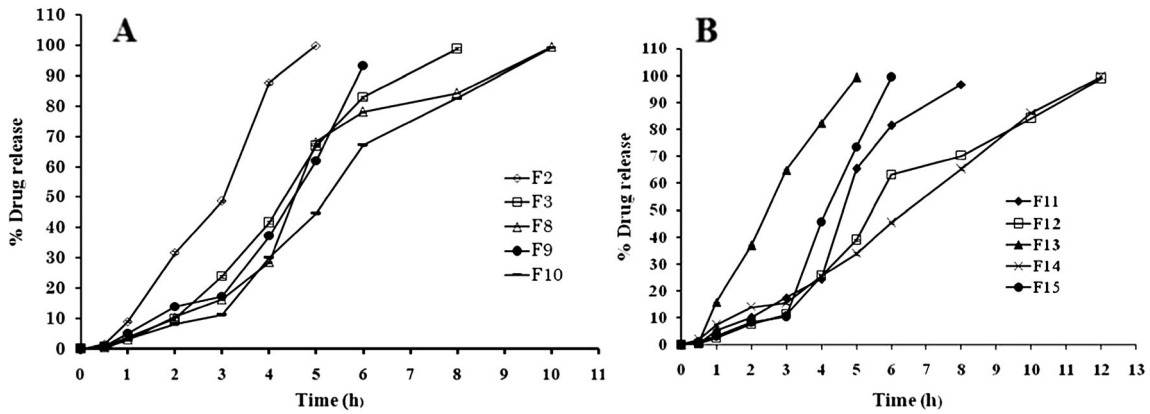

Fig. 6 In vitro release profiles of tablet formulations (n=3, mean±standard deviation)

Table 4 Calculated correlation coefficients

Formulations	Curve fitting constant (R^2)				
	Zero	First	Higuchi	Peppas	Number (for peppas)
F2	0.8982	0.8681	0.8762	0.9892	0.543
F3	0.9182	0.8782	0.8877	0.9982	0.605
F8	0.9874	0.9664	0.9879	0.9943	0.611
F9	0.8922	0.9622	0.9982	0.9841	0.647
F10	0.8935	0.9872	0.9238	0.9990	0.532
F11	0.7322	0.9176	0.9342	0.9976	0.566
F12	0.8966	0.8652	0.8977	0.9950	0.714
F13	0.9122	0.8537	0.8882	0.9891	0.702
F14	0.9021	0.8762	0.8933	0.9893	0.549
F15	0.9070	0.8888	0.8761	0.9982	0.561

Results of 2^3 Factorial Designs

In the present study, a 2^3 factorial design was employed to study the effect of independent variables, i.e., amount of polymers Carbopol 934, HPMC, and HEC on dependent variables such as hardness, X_1, and X_2, i.e., percentage swelling after 1 and 4 h, respectively, and Q_1 and Q_5 percentage drug release after 1 and 5 h, respectively, and $T_{100\ \%}$ is the time taken for 100 % drug release. The results as summarized in Table 5 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the batches. From the ANOVA test, the model was found to be significant ($P<0.05$). Concentrations of polymers used (Carbopol, HPMC, and HPC) significantly ($P<0.05$) influence the hardness of the tablet. The R^2 value for first response, i.e., hardness, was found to be 0.9988.

A negative value represents an effect that favors the optimization, while a positive value indicates an inverse relationship between the factor and the response. Eqs. (7)–(12) represent the quantitative effect of the formulation variables on the three responses.

$$\text{Hardness(kg/cm)} = 5.9844 + 0.247{*}A + 0.1474{*}B - 0.0025{*}C - 0.5225{*}A{*}B - 0.0225{*}A{*}C - 0.0725{*}B{*}C - 0.0525{*}A{*}B{*}C \quad (7)$$

$$R^2 = 0.9988$$

As in Eq. (7), Carbopol and HPMC have a significant effect on the hardness of the tablet. The variables such as concentration of Carbopol (factor A) and HPMC (factor B) have positive effect on hardness of the tablet that means that the increase in amount of A and B can increase the hardness, whereas the variable C (HEC) has negative impact on Hardness that indicates that increase in HEC concentration will reduce the hardness.

Linear correlation plots between actual and predicted values of hardness are shown in Fig. 7. The 3D response surface plots drawn using Design Expert software showing effect of independent variables on hardness are shown in Fig. 8.

$$\%\text{Swelling 1 h} = 206.4667 + 64.35{*}A + 9.375{*}B - 23.65{*}C - 8.925{*}A{*}B\ 62.6{*}A{*}C + 69.075{*}B{*}C + 9.675{*}A{*}B{*}C \quad (8)$$

$$R^2 = 0.7709$$

Table 5 Observed responses in 2^3 factorial design for tablet formulations (mean±SD, n=3)

Formulations	Dependent variables					
	Hardness	X_1	X_2	Q_1	Q_5	$T_{100\ \%}$
F2	6.4±0.03	396.6±0.2	446±0.04	1.56±0.21	99.9±0.12	5.03±0.43
F3	10.3±0.23	257±0.12	421±0.03	0.63±0.12	66.8±0.11	8.02±0.12
F8	6.5±0.03	456.6±0.32	471±0.11	0.32±0.22	68.3±0.06	10.06±0.3
F9	6.4±0.33	83.3±0.03	123±0.12	0.73±0.3	61.9±0.05	6.05±0.3
F10	6.7±0.03	126.6±0.11	192±0.22	0.34±0.3	44.6±0.08	10.04±0.12
F11	5.1±0.2	124.6±0.13	189.9±0.21	0.6±0.01	65.3±0.06	8.11±0.11
F12	5.7±0.2	285±0.05	285±0.11	0.39±0.01	38.9±0.08	12.05±0.11
F13	5.02±0.13	165.5±0.06	218±0.12	0.91±0.11	99.7±0.07	5.03±0.21
F14	6.4±0.03	280±0.04	320±0.32	1.93±0.12	33.9±0.03	10.01±0.32
F15	6.04±0.01	36.6±0.11	143.3±0.11	1.02±0.21	73.36±0.3	6.06±0.03

X_1 and X_2 are percentage swelling after 1 and 4 h, respectively, and Q_1 and Q_5 percentage drug release after 1 and 5 h, respectively, and $T_{100\ \%}$ is the time taken for 100 % drug release

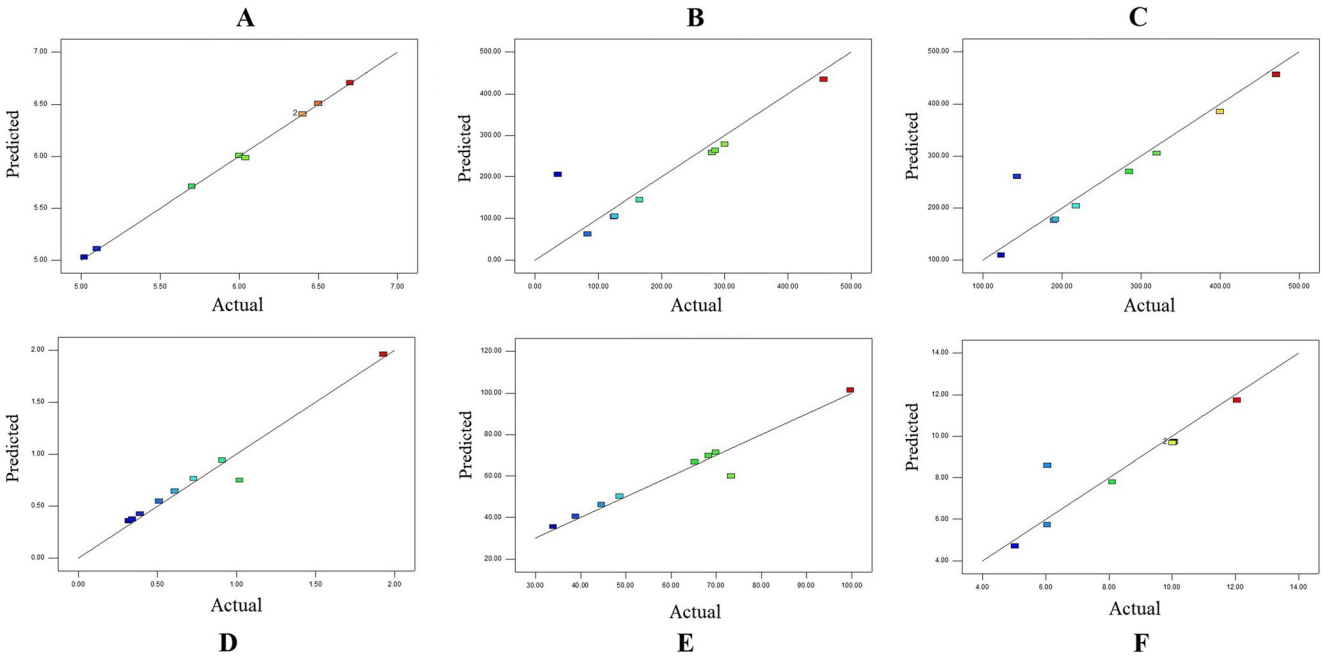

Fig. 7 Linear correlation plots between actual and predicted values of **a** hardness, **b**, **c** %swelling at 1 and 4 h, **d**, **e** %drug release at 1 and 5 h, and **f** time taken for 100 %drug release

$$\%\text{Swelling 6 h} = 260.244 + 62.137{*}A + 7.1375{*}B - 28.137{*}C - 1.637{*}A{*}B - 70.3625{*}A{*}C + 48.637{*}B{*}C - 7.6375{*}A{*}B{*}C \quad (9)$$

$R^2 = 0.8627$

The variables such as concentration of A and B have positive effect on swelling of the tablet that means that the increase in amount of A and B can increase the swelling, whereas the variable C has negative impact on swelling that indicates that increase in HEC concentration will reduce the swelling as in Eqs.(8) and (9).

Linear correlation plots between actual and predicted values of %swelling are shown in Fig. 7, and the 3D

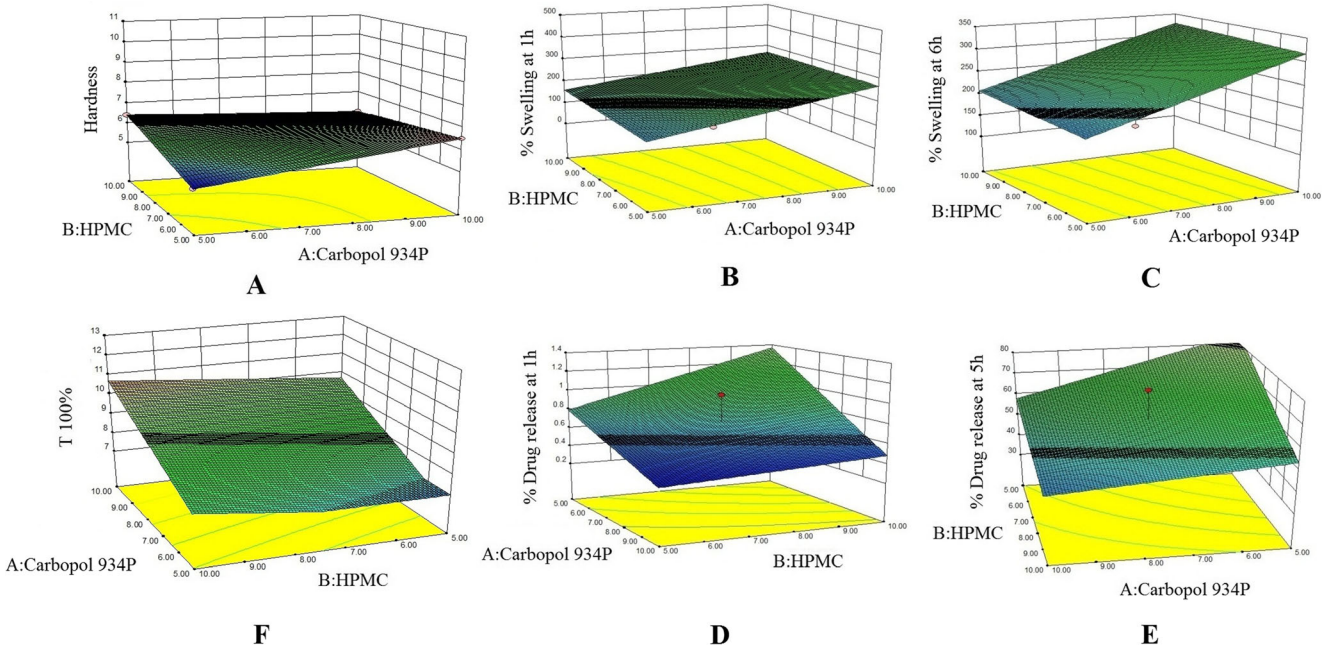

Fig. 8 Response surface plot showing effect of independent variables on **a** hardness, **b**, **c** %swelling at 1 and 4 h, **d**, **e** %drug release at 1 and 5 h, and **f** time taken for 100 % drug release

response surface plots showing effect of independent variables on %swelling are shown in Fig. 8.

$$\%\text{Drug release 1 h} = 0.7511 - 0.327*A + 0.1725*B + 0.1*C - 0.1125*A*B - 0.125*A*C + 0.17*B*C - 0.205*A*B*C \quad (10)$$

$$R^2 = 0.9603$$

$$\%\text{ Drug release 5 h} = 60.2777 - 8.55*A - 10.825*B - 12.927*C + 4.475*A*B + 4.625*A*C + 1.55*B*C + 1.95*A*B*C \quad (11)$$

$$R^2 = 0.9446$$

The variables such as concentration of A, B, and C have negative impact on percentage release of drug which indicates that increase in polymer concentration will reduce the %drug release as in Eqs. (10) and (11). Linear correlation plots between actual and predicted values of %drug release are shown in Fig. 7, and the 3D response surface plots showing effect of independent variables on %drug release are shown in Fig. 8.

$$T_{100\%} = 8.60222 + 1.62*A + 0.61*B + 1.1325*C - 0.12*A*B - 0.6275*A*C + 0.365*B*C + 0.1475*A*B*C \quad (12)$$

$$R^2 = 0.8420$$

The variables such as concentration of A, B, and C have positive effect on time taken for 100 % drug release from the tablet that means that the increase in amount of A, B, and C can increase the $T_{100\,\%}$ as in Eqs. (12). Linear correlation plots between actual and predicted values of time taken for 100 % drug release are shown in Fig. 7, and the 3D response surface plots showing effect of independent variables on $T_{100\,\%}$ are shown in Fig. 8.

In Vitro Antifungal Activity

Curcumin exhibits antifungal activity by generation of reactive oxygen species and triggers an early apoptosis in *C. albicans* cells and alteration of membrane-associated properties of ATPase activity, ergosterol biosynthesis, and protein secretion. To evaluate the antifungal activity, zone of inhibition for pure drug was determined and then compared the effectiveness of the pure drug and the drug in formulation by cup-plate method. Curcumin showed antifungal activity against *Candida* strains with minimum inhibitory concentrations (MIC) 64 μg/ml [14]. Curcumin has shown more susceptibility to *C. albicans* among the *Candida* species studied. Zone of inhibition curcumin–HPβCD bioadhesive vaginal tablet (containing 50 mg curcumin) and pure curcumin (50 mg) was found to be 18.7±0.44 and 17.3±0.6, respectively. Antifungal study with Sabouraud culture shows that the curcumin tablet was capable to control the growth of *C. albicans* for more than 24 h.

Curcumin has shown more susceptibility to *C. albicans* among the *Candida* species studied. Antifungal study with Sabouraud culture shows that the curcumin tablet was capable to control the growth of *C. albicans* for more than 24 h.

In Vivo X-Ray Studies

The mucoadhesion and retention property was evaluated in albino rabbit, and the X-ray photographic images are given in Fig. 9. Optimized formulation F13, developed by loading barium sulfate in place of curcumin in a tablet, was administered to rabbit. The duration of tablet in 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. After administration of tablet in vaginal cavity, diameter of tablet was found to have increased slowly as a result of swelling. Tablet showed swelling with change in the shape at the end of 8 h.

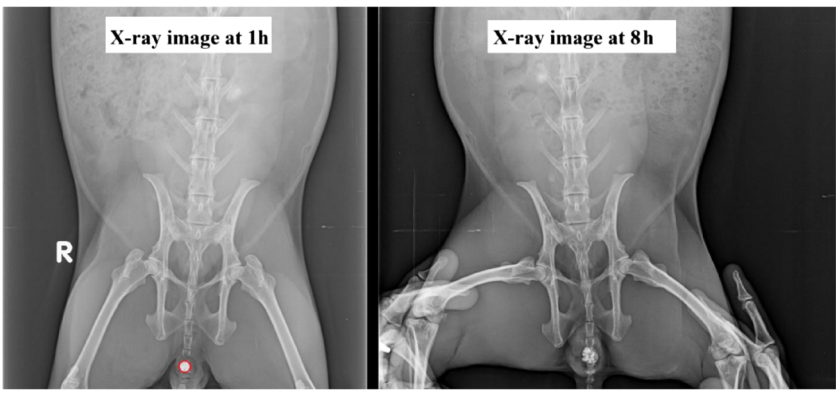

Fig. 9 X-ray radiographic images of curcumin–HPβCD bioadhesive vaginal tablets at 1 and 8 h after ingestion of BaSO4-loaded optimized F13 tablet in rabbits

Conclusion

A herbal approach of using curcumin as an antifungal agent to treat vaginal candidiasis by formulating curcumin–HPβCD complex in a bioadhesive vaginal tablet was successful. HPβCD inclusion complex of curcumin significantly increased curcumin solubility compared to pure drug. The FTIR, DSC, and SEM results produced important evidence of curcumin–HPβCD inclusion complex formation. Design of experiment (DoE) was applied successfully to develop curcumin–HPβCD bioadhesive vaginal tablet. Concentration of independent variables A, B, and C was found to have significant effect on responses like hardness, swelling, and percentage drug release. The prepared curcumin–HPβCD bioadhesive vaginal tablet was effective against *C. albicans*.

Among all formulations, F13 was suitable to administer into the vaginal cavity as it was found to provide effective concentration of curcumin to achieve antifungal effect up to 5 h and also good mucoadhesion and retention examined from X-ray studies and showed optimum thickness suitable to be inserted into the vaginal cavity. The combination of all results showed that curcumin–HPβCD complex bioadhesive vaginal tablet prepared using single polymer results in tablet with lower hardness. Tablet with combination of polymers like Carbopol, HPMC, and HEC exhibited required hardness, interesting swelling, and release properties with a very good antifungal activity making them promising formulations for the vaginal administration of herbal-based curcumin for treating vaginal candidiasis. The antifungal activity of curcumin can be further studied on other suitable systems.

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References

1. Hani U, Bhat RS, Shivakumar HG. Formulation design and evaluation of metronidazole microspheres in a bioadhesive gel for local therapy of vaginal candidiasis. Lat Am J Pharm. 2012;30(1):161–7.
2. Hani U, Bhat RS, Younus MP. Formulation design and evaluation of bioadhesive vaginal films of metronidazole for vaginal candidiasis. Lat Am J Pharm. 2012;31(1):84–90.
3. Hani U, Shivakumar HG, Gowrav MP. Formulation design and evaluation of hydrogel-based metronidazole bioadhesive tablet for vaginal candidiasis. Iranian J Pharma Sci. 2013;9(1):114–70.
4. Hani U, Shivakumar HG. Formulation design and evaluation of a novel vaginal delivery system of clotrimazole. IJPSR. 2014;5(1):220–7.
5. Hani U, Shivakumar HG. Formulation and evaluation of thermosensitive bioadhesive vaginal gel of miconazole nitrate for vaginal candidiasis. American J Adv Drug Del. 2013;1:66–78.
6. Hombach J, Palmberger TF, Bernkop-Schnürch A. Development and *in vitro* evaluation of a mucoadhesive vaginal delivery system for nystatin. J Pharm Sci. 2009;98:555–64.
7. Valenta C, Kast CE, Harich I, Bernkop-Schnürch A. Development and *in vitro* evaluation of a mucoadhesive delivery system for progesterone. J Control Release. 2001;77:323–32.
8. Gavini E, Sanna V, Juliano C, Bonferoni MC, Giunchedi P. Mucoadhesive vaginal tablets as veterinary delivery system for the controlled release of an antimicrobial drug Acriflavine. AAPS Pharm Sci. 2002;3:E20.
9. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. 3rd ed. Council of Scientific and Industrial Research: New Delhi; 1992. p. 7–246.
10. Bruneton J. Pharmacognosy. Phytochemistry: Medicinal Plants, France, Lavoisiler Publishing Co.; 1995. p. 265–380.
11. Murray MT, Pizzorno JE. Textbook of natural medicine. China: Churchill Living; 1999.
12. Vermani K, Garg S. Herbal medicines for sexually transmitted diseases and AIDS. J Ethnopharmacol. 2002;80:49–66.
13. Ammon H. WahlMA Pharmacology of *Curcuma longa*. Planta Med. 1991;57:1–7.
14. Martins CVB, da Silva DL, Neres, Magalhaes ATM, Watanabe TFFG, Modolo A, et al. Curcumin as a promising antifungal of clinical interest. J Antimicrob Chemother. 2008;63:337–9.
15. Yasser MA. Curcumin modulation of Na, K-ATPase: phosphoenzyme accumulation, decreased K+ occlusion, and inhibition of hydrolytic activity. Br J Pharmacol. 2005;145:236–45.
16. Smith MJL, Lockyer PJ, East JM, Lee AG. Curcumin, amolecule that inhibits the Ca2+ ATPase of sarcoplasmic reticulum but increases the rate of accumulation of Ca2+. J Biol Chem. 2001;276:46905–11.
17. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007;5951:75.
18. Aguerosa M, Aresesb P. Miguel Angel Campaneroa, Hesham Salmana, Gemma Quincocesb, Ivan Pe˜nuelasb, Juan Manuel Irachea. Bioadhesive properties and biodistribution of cyclodextrin–poly(anhydride) nanoparticles. Eur J Pharm Sci. 2009;37:231–40.
19. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci. 1996;85:1017–25.
20. Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. 2009;10:752–62.
21. Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instrum. 1965;4:117–212.
22. Wang LL, Zheng WS. Inclusion effects of β-CD and HPβCD on momobenzone. Chin Pharm J. 2006;41:529–31.
23. Zarzycki PK, Lamparczyk H. The equilibrium constant of beta-cyclodextrin-phenolphtalein complex; influence of temperature and tetrahydrofuran addition. J Pharm Biomed Anal. 1998;18:165–70.
24. Stella VJ, Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res. 1997;14:556–67.
25. Banerjee R, Chakraborty H, Sarkar M. Host–guest complexation of oxicam NSAIDs with β-cyclodextrin. Biopolymers. 2004;75:355–65.
26. Masson M, Sigurdardottir BV, Matthıasson K, Loftsson T. Investigation of drug-cyclodextrin complexes by a phase-distribution method: some theoretical and practical considerations. Chem Pharm Bull. 2005;53:958–64.
27. Mangolim CS, Moriwaki C, Nogueira AC, Sato F, Baesso ML, Neto AM, et al. Curcumin–b-cyclodextrin inclusion complex: stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application. Food Chemistry. 2014;153:361–70.
28. Nitin B, Dobaria A, Badhan C, Mashru RC. A novel itraconazole bioadhesive film for vaginal delivery: design, optimization, and physicodynamic characterization. AAPS PharmSciTech. 2009;10:951–9.

29. Derringer G, Suich R. Simultaneous optimization of several responses variables. J Qual Technol. 1980;12:214–9.
30. Kendal GP, Matthew GH. Determination of the tensile strength of elongated tablets. Powder Technol. 2013;238:169–75.
31. Geeta P, Anita P. A novel effervescent bioadhesive vaginal tablet of ketoconazole: formulation and in vitro evaluation. Int J Pharm Tech Res. 2010;2:656–67.
32. Zhou Q, Zhong L, Wei X, Do W, Choua G, Wang Z. Baicalein and hydroxypropyl β cyclodextrin complex in poloxamer thermal sensitive hydrogel for vaginal administration. Int J Pharm. 2013;454: 125–34.
33. Mirzaa MA, Talegaonkara S, Farhan JA, Iqbal Z. A novel and multifunctional excipient for vaginal drug delivery. J Excipients and Food Chem. 2011;2:98–112.
34. Tang B, Ma L, Wang H, Zhang G. Study on the supramolecular interaction of curcumin and b-cyclodextrin by spectrophotometry and its analytical application. J Agric Food Chem. 2002;50:1355–61.
35. Martin Del Valle EM. Cyclodextrins and their uses: a review. Process Biochem. 2003;39:1033–46.
36. Marque HM, Hadgraft J, Kellaway IW. Studies of cyclodextrin inclusion complexes. I. The salbutamol–cyclodextrin complex as studied by phase solubility and DSC. Int J Pharm. 1990;63:259–66.