ORIGINAL ARTICLE

Development and Optimization of Intravaginal Mucoadhesive Microspheres of Econazole Nitrate

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Abstract

Introduction: The most common disease in the female vaginal tract is vaginal candidiasis caused by the microbe Candida Albicans. The conventional formulation for the treatment of this disease shows a lack of retention of the dosage form in the vaginal cavity due to the natural draining of vaginal fluid that leads to leakiness and Messiness, which tends to escape during normal routine work. The mucoadhesive delivery of antifungal drugs in the treatment of vaginal candidiasis is an alternative to delivering drugs for a longer time. In this research work, econazole nitrate-loaded mucoadhesive microspheres were developed by spray drying method and compressed into a vaginal tablet which disintegrates rapidly into microspheres in the vaginal cavity and adheres to vaginal mucosa for a longer time. **Methods and Materials:** Econazole nitrate-loaded microspheres were prepared and characterized in terms of particle size, drug loading, *ex vivo* mucoadhesion study, and *in vitro* drug release study. A three-factor and three-level Box–Behnken design was employed to optimize the formulation, and the effect of independent variables HPMC K 100M, Eudragit RSPO, and Eudragit RLPO on formulation was analyzed. **Results and Discussion:** The particle size of the optimized formulation was found to be 25µ. The drug loading and *in vitro* release study at 6 h were 23% and 93%, respectively. The data from the *in vitro* drug release study show that the drug is released in a sustained manner due to release retarding polymer Eudragit RSPO and Eudragit RLPO and the % mucoadhesion of the optimized microsphere was found to be 70 % after 8 h in contact with vaginal fluid due to mucoadhesive property of HPMC K100M, tablet disintegrate rapidly into microsphere which adheres to the vaginal wall and consistently release drug up to 6 h. **Conclusion:** The mucoadhesive microsphere prepared by the spray dryer provides a mucoadhesion phenomenon that allows the adherence of the microsphere to the vaginal mucosa so that drug retention time is increased. The prepared vaginal tablet overcomes the drawback of conventional formulations and disintegrates rapidly into a mucoadhesive microsphere and adheres to the vaginal lumen so that the retention time of the drug is increased.

Key words: Box–Behnken design, econazole nitrate, mucoadhesive microsphere, spray dryer, vaginal candidiasis

INTRODUCTION

Vaginal candidiasis is one of the most common vaginal infections among fertile women, accounting for 20–30% of all cases. According to epidemiological studies, approximately 75% of all women will experience at least one occurrence of vulvovaginal candidiasis during their lifetime, with 40–50% experiencing one or more subsequent episodes. It is also worth noting that the presence of Candida spp. in the vulvovaginal region does not always imply infection.[1] Vaginal candidiasis is the most common yeast infection which is caused by the over-colonization of candida albicans. The most common clinical symptoms are vaginal soreness, irritation, vulvar pruritus, and burning resulting in dysuria dyspareunia, edema, vulvar vaginal erythema, and fissures are also frequently observed.[2,3] An intravaginal topical dosage form is an efficient treatment for yeast vaginitis. Vaginal candidiasis can be treated by use of an antifungal drug, that is, econazole nitrate which is an imidazole category and has a minimum inhibitory concentration of 100 µg/ml

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against candida species.[4,5] It acts by impairing the synthesis of ergosterol by inhibiting the enzyme cytochrome P450 and leads to damage of permeable membranes resulting in leakage of intracellular phosphorous compounds and potassium ions, which inhibits macromolecular synthesis.[6,7]

The treatment requires controlling the overgrowth of candida albicans and it takes 3–7 days to get rid of this disease. The dosage forms other than those given orally are administered at night to prevent leakage or removal of dosage form from the vaginal cavity. Oral formulations, on the other hand, may not provide symptomatic relief in the case of a serious illness. In that instance, topical formulations of low-potency steroids or antifungal drugs should be utilized.[8]

Creams, gels, ointment, vaginal tablet, suppositories, and pessaries are dosage form that are available in market for treatment of vaginal candidiasis but they have drawback like leakiness, messiness, tendency to escape while performing normal routine work.[3,9] A comfortable dosage form is which can spread onto the vaginal mucosa surface and achieve intimate and prolonged contact at the site of application and can be useful to avoid the drawback of conventional therapies. As a result, localized mucoadhesive dosage forms may be an appropriate formulation design to improve drug availability, long-term therapeutic drug concentration at a site, and patient compliance.[5,10] Formulations that possess mucoadhesive properties maximize retention of dosage form and avoid leakage of it which leads to improvement in the availability of the drug and release drug for a prolonged duration of action. The rationale behind imparting mucoadhesive properties to dosage form so that it can be held on the biological surface for a longer duration of time and act as a localized drug delivery to overcome the drawback of conventional dosage form can be avoided.[11,12]

To achieve, localized drug delivery and prevent wash out of dosage form due to natural vaginal discharge use of mucoadhesive polymers can be beneficial for retention of the dosage form at the vaginal lumen and enhanced therapeutic efficacy.[13] A mucoadhesive-promoting agent or polymer is added to the formulation to aid adherence of the active pharmaceutical ingredient to the vaginal mucosa. When in contact with vagina mucosa, the agent may exhibit extra features such as swelling, which promotes the breakdown of the dosage form.[14]

The current research study aimed to develop an econazole nitrate-loaded mucoadhesive microspheres by spray dryer and further compressing into a vaginal tablet by direct compression method so that it rapidly disintegrates into microspheres and adheres to the wall of vaginal mucosa so that it releases drug for a prolonged period.[15-17] For the development of an optimized formulation of the microsphere, DoE is utilized to evaluate the multivariable system. The DOE technique used in this research work is the Box–Behnken design (BBD) to study the responses observed while changing the concentration of independent variables. The influence of independent variables on response variables (particle size, drug loading, and *in vitro* drug release) was evaluated and further surface morphology and physicochemical studies were performed by SEM, XRD, and DSC.[18]

EXPERIMENTAL

Material

Econazole nitrate was procured as a gift sample from M/s Mahrshee Laboratories Pvt. Ltd., Gujrat, India. Other excipients such as Methocel K 100M, Eudragit RSPO, and Eudragit RLPO were provided by Colorcon Asia Pvt. Ltd. and Rhom Pharma, Weiterstadt, Germany, respectively. Dichloromethane, ethanol, and triacetin were purchased from Rankem Pvt. Ltd., India.

Method of preparation of mucoadhesive microsphere of econazole nitrate

Mucoadhesive microspheres containing econazole nitrate were prepared by a spray drying process. This technique is selected because it is simple, easy to scale up, and less dependent on the solubility characteristics of drugs and polymers.[19] Drug and polymers were weighed accurately and dissolved in a solvent system containing methylene dichloride and ethanol in a ratio of 1:1. Glyceryl triacetate was added as a plasticizer. Throughout the process, the concentration of the drug (300 mg) and plasticizer (1%) with respect to polymer mass was kept constant and 1% plasticizer is optimum to provide the smooth and spherical shape of microspheres by coalescence of polymer in drug particles.[19] The solution was stirred on a magnetic stirrer for 30 min to get a clear solution. The solution was then sprayed using a spray dryer (Spray Mate, JISL, Mumbai, India) with a two-fluid nozzle (1 mm), and atomization pressure was kept at 0.2 mPa, inlet temperature and outlet temperature was set at 120°C and 60°C, respectively, feed pump was set at 10 rpm and aspirator pressure was maintained at −100 mm WC. When a drug–polymer mixture in the solvent was sprayed through a nozzle, it forms a fine mist or droplet from which the solvent rapidly get evaporated leading to the formation of microspheres which were later separated from the cyclone separator and the product was collected from the collector.

Experimental design (ED)

ED is the method of designing an experiment in such a manner that appropriate data can be collected and statistically evaluated, providing a valid and objective conclusion. This method is employed to get meaningful conclusions from experimental data.[20] BBD provides designs for a spherical domain whose most specific property is that

independent variables take only three levels. The designs in this are based on the construction of balanced incomplete block designs.[21,22] The formulation optimization of the mucoadhesive microsphere is done by BBD where, X_1, X_2, and X_3-X_8 are dependent variables corresponding to particle size, drug loading, and cumulative percentage drug release at different time points, and independent variables are HPMC K100M (Y_1), Eudragit RSPO (Y_2), and Eudragit RLPO (Y_3) concentration, respectively. The amount of drug (300 mg) and concentration of plasticizer (1% of polymer weight) were kept constant throughout optimization runs. Pre-optimization of formulation variables, such as feed viscosity and drug–polymer ratio, was also carried out. In addition, process variables such as inlet and outlet temperature, feed atomization, and peristaltic pump speed were optimized. The quantity of the independent variable was taken as per the BBD and after experimenting, their responses are noted and shown in Tables 2 and 7. A total of 15 batches of econazole nitrate-loaded microsphere were prepared according to the composition provided by BBD and a 3-D surface plot of all responses was constructed, as shown in Figures 1–16, and all batches were evaluated for the dependent variable, as shown in Table 1. With the help of ANOVA, the data of the responses of experimental batches were fitted using linear, two-factor interaction (2FI), quadratic, cubic models to examine the agreement with R-square values, sequential p-values, and lack of fit. The data of all responses followed below quadratic equation:

$$X_0 = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

Where, X_0 is the dependent variable, Y_1, Y_2, and Y_3 are the independent variable, and b_0-b_{33} are regression coefficients, as shown in Tables 3 and 4.

To determine the optimized formula for econazole nitrate-loaded microsphere, maximum particle size, maximum drug loading, and minimum cumulative % drug release during various time intervals, such as 15, 30, 60, 120, 240, and 360 min, respectively, were chosen as dependent variables. The software-predicted formulation with the highest desirability of 0.96 was chosen as the optimized formulation, and its quantities are depicted in Table 5 and Figures 17 and 18.

The optimized batch of econazole nitrate-loaded microsphere was prepared according to the formula suggested by the software and all desired responses were evaluated. The data are shown in Figure 19 and Table 5 which imply that changes in the concentration of independent variables affect particle size (X_1), drug loading (X_2), and *in vitro* drug release (X_3-X_8). Theoretical response values predicted by the software and experimentally observed response variables of the optimized

Table 1: Experimental plan for formulation optimization of econazole nitrate-loaded mucoadhesive microsphere by BBD

Independent variables	Unit	Levels		
		Low	Medium	High
Y_1 = HPMC K 100M	mg	100	300	500
Y_2 = Eudragit RSPO	mg	1000	1500	2000
Y_3 = Eudragit RLPO	mg	1000	1500	2000
Dependent variable			**Constraints**	
X_1 = Particle size			Maximum	
X_2 = Drug loading			Maximum	
X_3 = Cumulative % drug release in 15 min			Minimum	
X_4 = Cumulative % drug release in 30 min			Minimum	
X_5 = Cumulative % drug release in 60 min			Minimum	
X_6 = Cumulative % drug release in 120 min			Minimum	
X_7 = Cumulative % drug release in 240 min			Minimum	
X_8 = Cumulative % drug release in 360 min			Minimum	

Table 2: Box–Behnken experimental design with an independent variable for optimizing econazole nitrate mucoadhesive microspheres

Batch No.	Independent variable		
	Y_1 HPMC K 100M (mg)	Y_2 Eudragit RSPO (mg)	Y_3 Eudragit RLPO (mg)
Batch-1	100	1000	1500
Batch-2	500	1000	1500
Batch-3	100	2000	1500
Batch-4	500	2000	1500
Batch-5	100	1500	1000
Batch-6	500	1500	1000
Batch-7	100	1500	2000
Batch-8	500	1500	2000
Batch-9	300	1000	1000
Batch-10	300	2000	1000
Batch-11	300	1000	2000
Batch-12	300	2000	2000
Batch-13	300	1500	1500
Batch-14	300	1500	1500
Batch-15	300	1500	1500

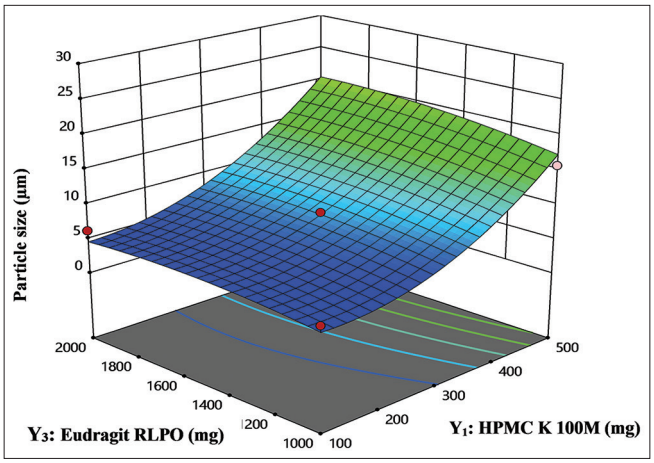

Figure 1: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on particle size

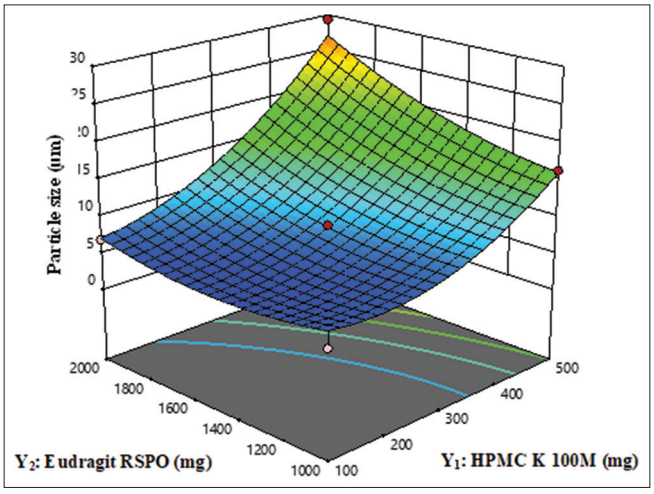

Figure 2: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on particle size

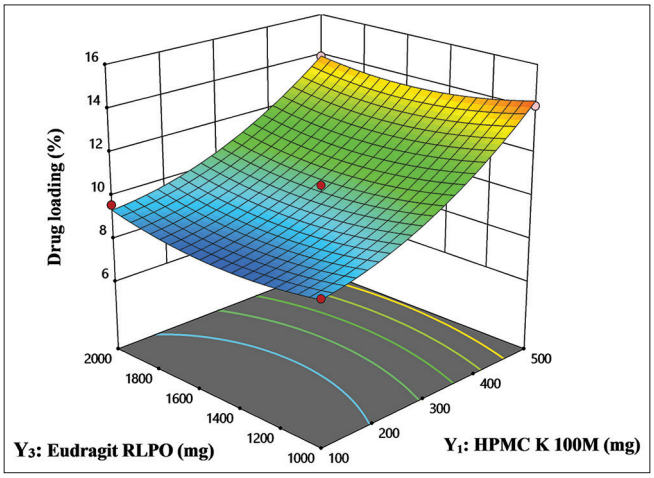

Figure 3: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on drug loading

final formulation batch were compared, and the results indicate that there is a close agreement between the predicted

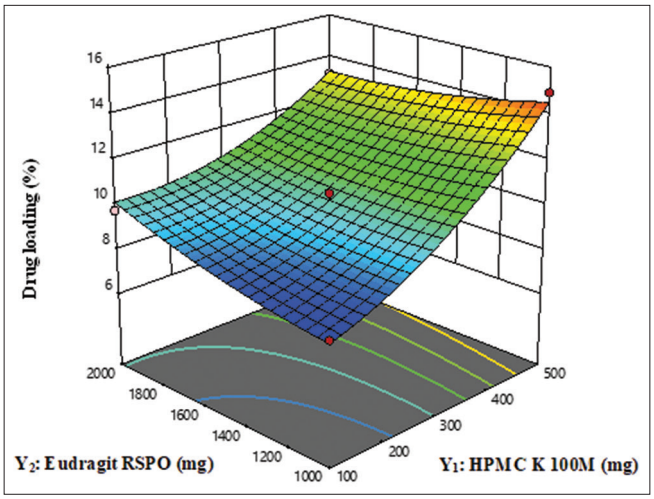

Figure 4: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on drug

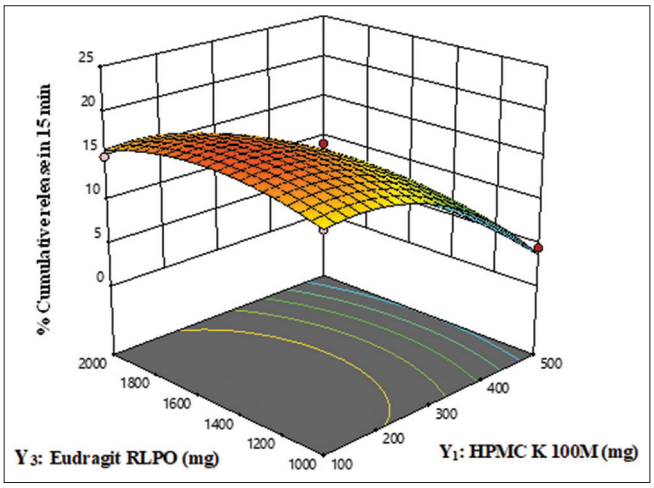

Figure 5: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 15 min

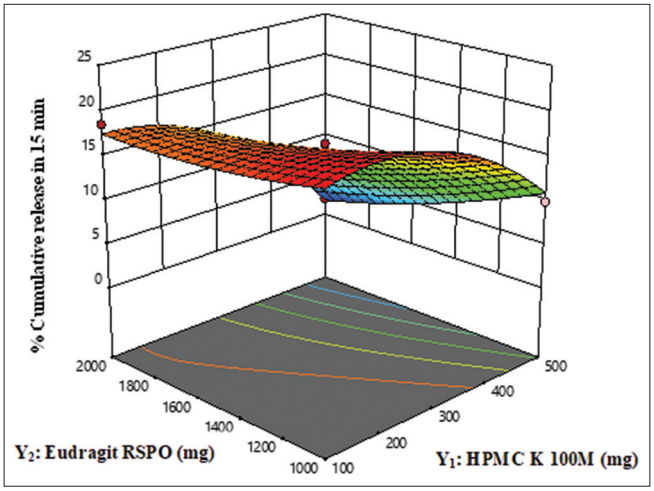

Figure 6: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 15 min

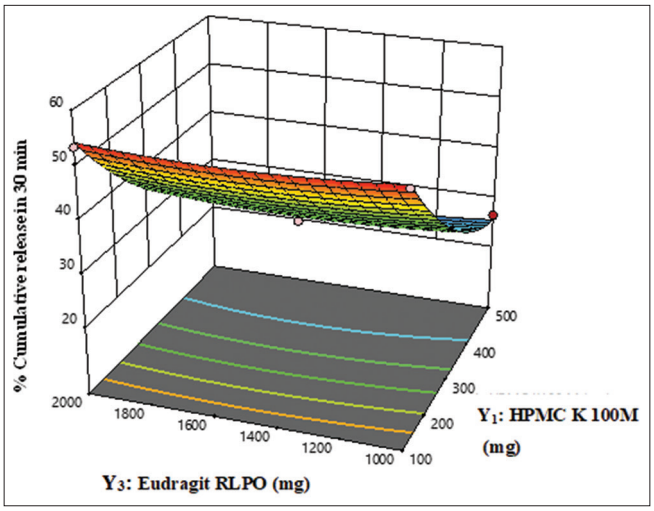

Figure 7: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 30 min

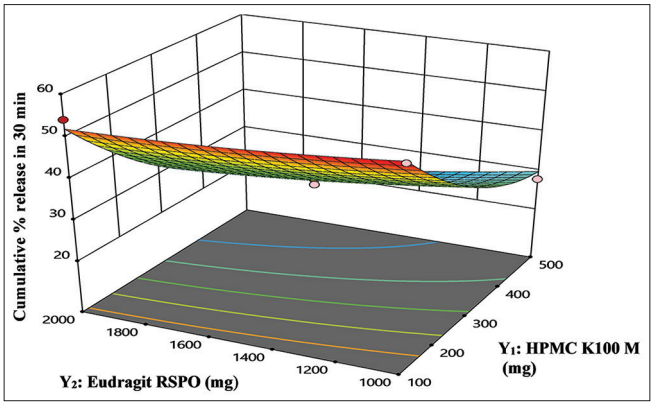

Figure 8: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 30 min

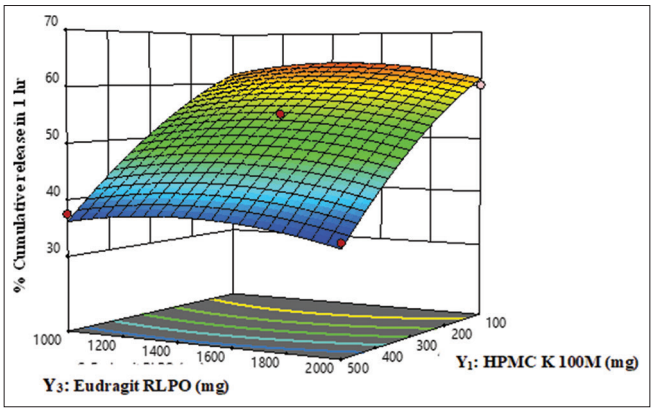

Figure 9: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 1 h

value of the software and the experimentally observed response, as shown graphically in Figure 20. With the help of a linear regression plot between software-predicted and theoretically observed values of response variables, the

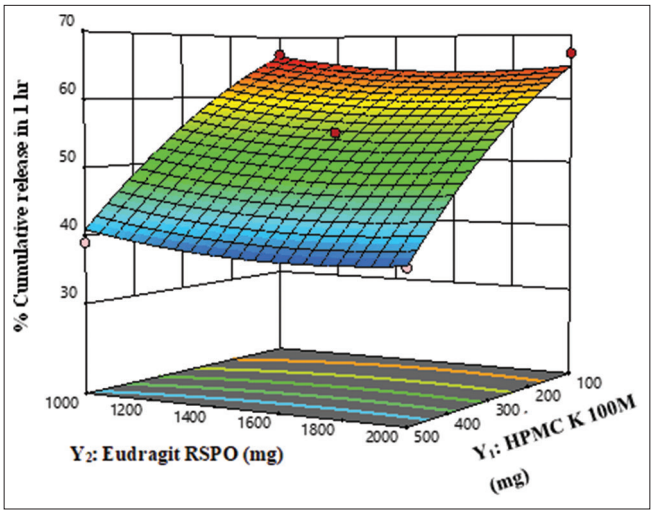

Figure 10: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 1 h

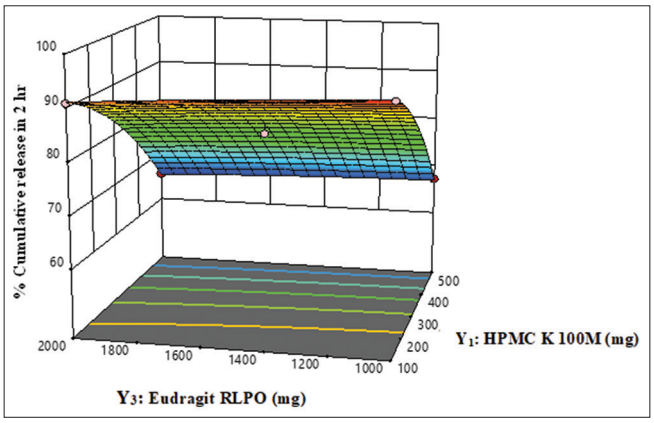

Figure 11: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 2 h

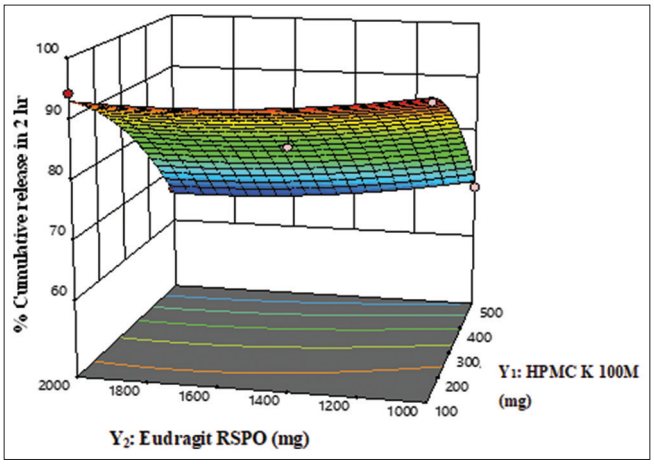

Figure 12: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 2 h

relative percentage error was calculated and a high R^2 value was obtained which shows that there is a smaller difference

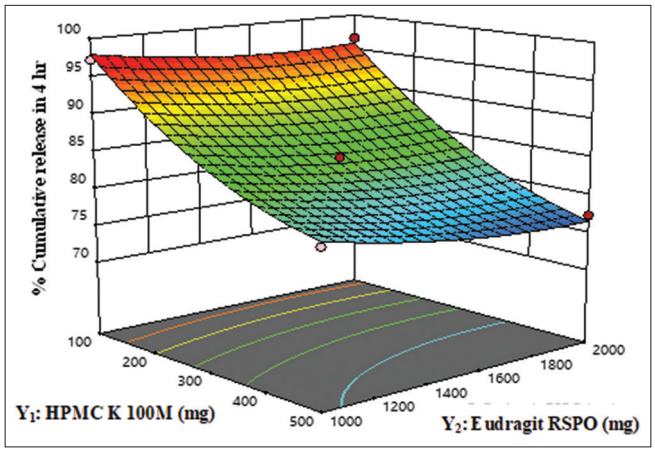

Figure 13: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 4 h

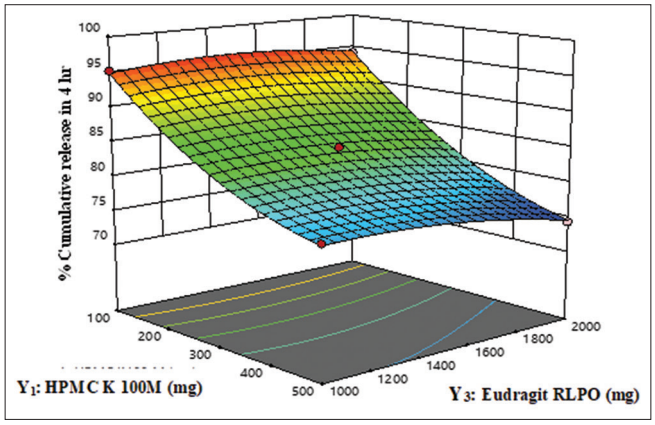

Figure 14: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 4 h

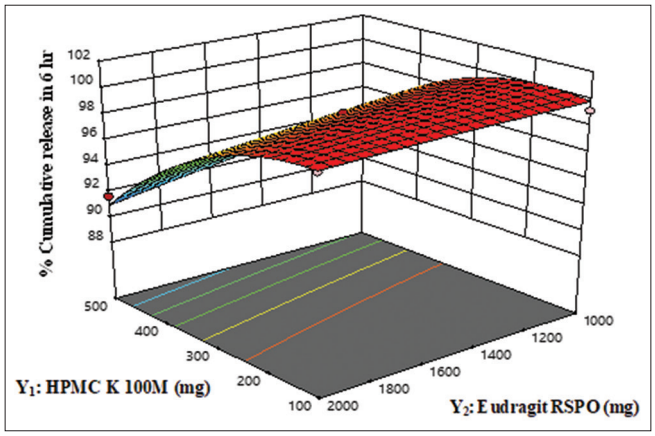

Figure 15: 3-D response surface graph showing effect of Eudragit RSPO and HPMC K100M on cumulative % drug release in 6 h

between observed data and predicted data, as shown in Figure 21.

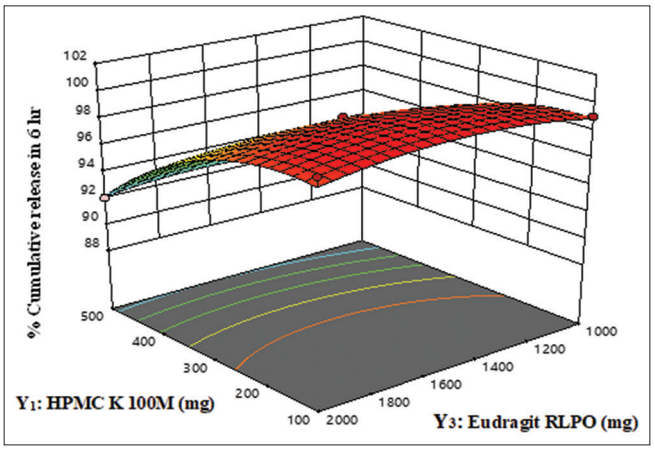

Figure 16: 3-D response surface graph showing effect of Eudragit RLPO and HPMC K100M on cumulative % drug release in 6 h

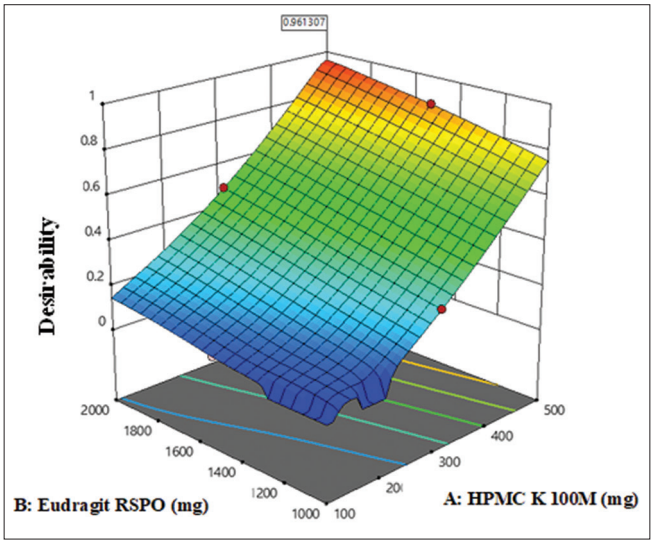

Figure 17: 3-D surface plot showing maximum desirability of a mucoadhesive microsphere formulation

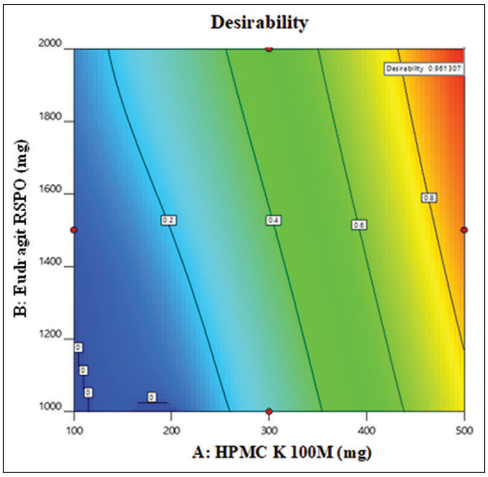

Figure 18: Contour plot showing maximum desirability of mucoadhesive formulation

Table 3: Statistical summary of responses variables

Response	Model	Sum of square	F-value	*P*-value	R^2	Adjusted R^2	Predicted R^2	SD
Particle size (X_1)	Quadratic	67.42	5.24	0.0530	0.9656	0.9037	0.4519	2.07
Drug Loading (X_2)	Quadratic	4.14	7.54	0.0265	0.9840	0.9552	0.7779	0.4227
In vitro drug release at 15 min (X_3)	Quadratic	80.59	22.19	0.0026	0.9868	0.9630	0.8102	1.10
In vitro drug release at 30 min (X_4)	Quadratic	94.46	7.79	0.0248	0.9896	0.9708	0.8479	2.01
In vitro drug release at 1 h (X_5)	Quadratic	63.59	5.93	0.0422	0.9879	0.9662	0.8251	1.89
In vitro drug release at 2 h (X_6)	Quadratic	59.34	6.25	0.0382	0.9895	0.9707	0.8986	1.78
In vitro drug release at 4 h (X_7)	Quadratic	39.90	11.55	0.0110	0.9923	0.9784	0.8923	1.07
In vitro drug release at 6 h (X_8)	Quadratic	20.10	13.60	0.0077	0.9814	0.9478	0.7054	0.7019

Table 4: Polynomial equation of response variables

Responses		Equation
X_1 = Particle size	=	8.63 + 7.3375 A + 2.87 B + 0.98 C + 2.625 AB + 0.6 AC + 0.42 BC + 3.78 A^2 +1.80 + B^2 - 0.86 C^2
X_2 = Drug loading	=	10.23 + 2.4 A + 0.27 B - 0.04 C - 0.86 AB - 0.175 AC -0.185 BC + 0.91 A^2 +0.31 B^2 0.55 C^2
X_3 = Cumulative % drug release in 15 min	=	15.77- 6.09 A- 2.78 B - 0.06 - 1.76 AB-0.21 AC-0.45 BC- 4.11 A^2 + 0.80 B^2- 2.15 C^2
X_4 = Cumulative % drug release in 30 min	=	33.77 - 14.63 A - 3.55 B - 0.22 C - 1.38 AB - 0.004 AC -0.73 BC + 4.96 A^2 + 0.86 B^2+1.23 C^2
X_5 = Cumulative % drug release in 60 min	=	53.79 - 13.16 A - 1.09 B - 0.59 C - 0.24 AB - 0.28 AC - 0.14 BC - 2.66 A^2 + 1.65 B^2 - 2.63 C^2
X_6 = Cumulative % drug release in 120 min	=	83.25 - 13.20 A - 1.97 B - 1.05 C - 0.59 AB + 0.245 AC - 0.45 BC - 3.57 A^2 + 1.53 B^2 - 0.30 C^2
X_7 = Cumulative % drug release in 240 min	=	83.87- 9.14 A- 1.53 B - 1.007 C - 0.52 AB - 0.74 AC + 0 .22 BC + 2.77 A^2 + 1.11 B^2 - 1.22 C^2
X_8 = Cumulative % drug release in 360 min	=	98.41 - 3.54 A- 0.87 B + 0.20 C - 0.69 AB + 0.25 AC - 0.24 BC - 2.20 A^2 - 0.10 B^2-0.91 C^2

Table 5: Software predicted versus experimental observed responses of econazole nitrate-loaded mucoadhesive microsphere

Optimized formulation composition		Response			
Component	**Quantity (mg)**	**Evaluation parameter**	**Software predicted values**	**Experimentally observed value ± SD**	**Relative error (%)**
HPMC K 100M	499	Particle size (nm)	27.45	25.03±1.70	8.8
Eudragit RSPO	1963	Drug loading (%)	21.24	23.3±1.16	9.6
Eudragit RLPO	1923	Cumulative % drug release in 15 min	8.31	9.30±1.67	11.9
		Cumulative % drug release in 30 min	20.38	22.53±2.25	10.5
		Cumulative % drug release in 60 min	35.39	38.63±1.51	9.1
		Cumulative % drug release in 120 min	64.15	66.96±1.74	4.3
		Cumulative % drug release in 240 min	74.37	75.27±2.51	1.2
		Cumulative % drug release in 360 min	90.65	93.49±2.16	3.1

Data are expressed as mean±SD, $n = 3$

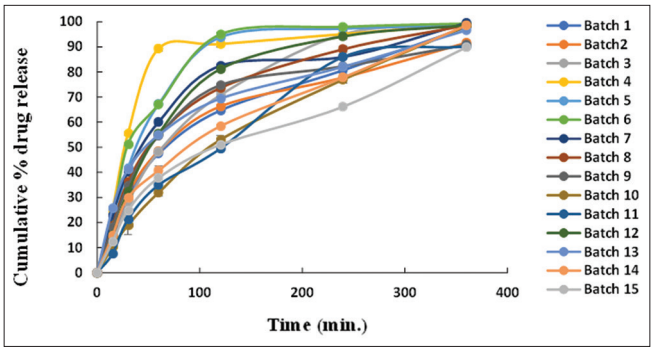

Figure 19: *In vitro* drug release profile of each optimization batch of econazole nitrate-loaded microsphere. All values shown in the graph are measured as mean ± SD, $n = 3$, error bar indicates the standard deviation of replicate

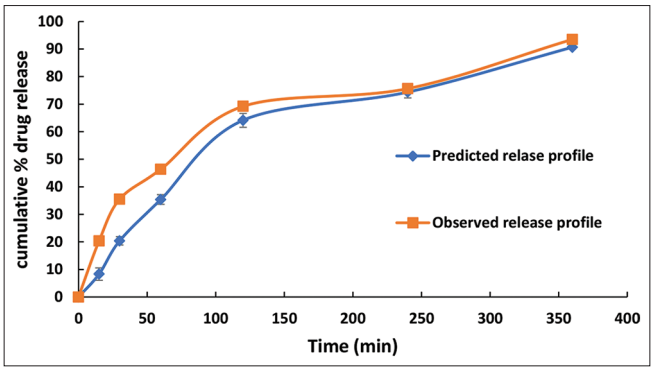

Figure 20: Comparative drug release profile of predicted drug release versus observed drug release of econazole nitrate-loaded microsphere. Data are expressed as mean ± SD, *n*=3

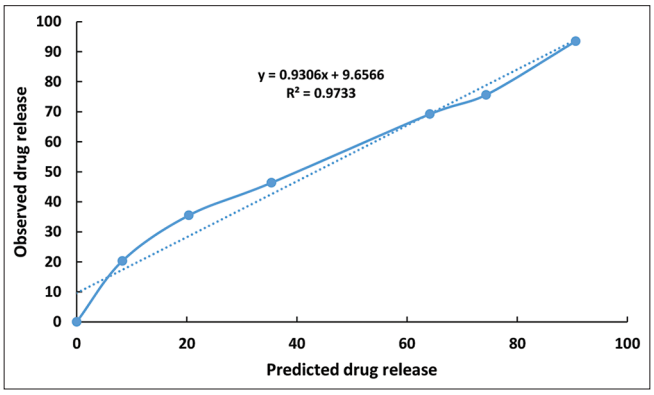

Figure 21: Observed release versus predicted release linear plot

Evaluation of econazole nitrate-loaded mucoadhesive microsphere

Particle size

The particle size of the econazole nitrate-loaded mucoadhesive microsphere was analyzed using an optical microscope (Leica microsystem) at 100× magnification. A small amount of sample was mounted on a glass slide by wetting it with water and covered with a cover slip. The particle size of 100 microspheres from each optimization batch was measured from Leica Q win software and the average particle size was calculated. The result is mentioned in Tables 5 and 7, respectively.

Drug loading

A weighed amount of 10 mg of econazole nitrate-loaded microsphere and a small quantity of ethanol was added and sonicated for 5 min, the volume was made up to 10 mL with ethanol and filtered with Whatman filter paper. The sample was then analyzed in a U.V. spectrophotometer (Shimadzu 1700) at 271 nm and absorbance was recorded. The actual drug content and drug loading were calculated using the formula mentioned below:

$$\text{Drug loading}(\%) = \frac{\text{Actual drug content}}{\text{A weighed amount of microspheres}} * 100$$

The drug loading of each optimization formulation batch and optimized final formulation is shown in Tables 5 and 7, respectively.

In vitro release study

To evaluate the drug release properties of optimized batch formulations, an *in vitro* drug release study was carried out. The drug release study of the econazole nitrate-loaded microsphere was carried out on a shaker water bath. 10 mL vials containing 10 mg of econazole nitrate-loaded microsphere were dispersed in 10 mL of acetate buffer pH 4.4. The vials were closed, sealed, placed in the shaker water bath, and allowed to shake for 6 h at 37°C. 5 ml samples were taken out at specified intervals and filtered through a 0.45 membrane filter before being analyzed at 271 nm with a U.V. spectrophotometer (Shimadzu 1700). The study was performed in a triplicate manner and average values were calculated. The *in vitro* drug release data are given in Table 7 and graphically represented in Figure 19. The software predicted versus experimental observed *in vitro* drug release data of econazole nitrate-loaded mucoadhesive microsphere is shown in Figure 20 and the regression graph is shown in Figure 21.

Scanning electron microscopy

Scanning electron microscopy was used to investigate the morphological features of optimized microspheres. A small amount of mucoadhesive microspheres were dispersed on a metal stub with double-sided adhesive tape and placed in the scanning electron microscopy chamber (Supra 55, ZEISS Gemini, Germany). A scanning electron photomicrograph was taken at appropriate magnifications with a 5 kV acceleration voltage which is shown in Figure 22.

Differential scanning calorimetry

The DSC technique is used to evaluate the presence of crystalline drug peaks in a formulation. Samples such as econazole nitrate, physical mixture (HPMC K 100M: Eudragit RSPO Eudragit RLPO: Drug) in a ratio of (1:1:1:1),

Table 6: Evaluation of microsphere-loaded vaginal tablet

Batch no.	Optimized econazole nitrate microspheres	Thickness (mm)	Hardness (kg)	Friability (%)	Drug content (%)	Disintegration test (sec)
MMVt1	650 mg	3.47	4.2	0.3	98.2	41
MMVt2	650 mg	3.51	3.2	0.5	99.4	29
MMVt3	650 mg	3.45	3.9	0.4	98.5	35

*MMVt: Mucoadhesive microsphere-loaded-vaginal tablet

econazole nitrate microsphere, and excipients were analyzed in DSC 6000 (PerkinElmer). An aluminium pan containing an accurately weighed sample of 3 mg was sealed and heated from 50°C to 250°C at a scanning rate of 20°C/min with nitrogen flow (20 mL/min). An empty aluminium pan was used as a reference, and the thermogram is demonstrated in Figure 23.

X-ray diffraction pattern study

The crystallinities of pure econazole nitrate, blank microspheres, and drug-loaded microspheres were determined by X-ray diffractometer (D8 Advance, Bruker). The silicon drift detector (Bruker LynxEye 1D PSD detector) was connected to a sealed tube (40 kV, 40 mA) that generated monochromatized Cu-Kα X-ray radiation and analyzed on 2θ between 5° and 60°. The X-ray diffractogram is shown in Figure 24.

Ex vivo mucoadhesion study

The strength of mucoadhesion by mucoadhesive microspheres was determined using an *ex vivo* mucoadhesion study. An excised strip of goat vaginal mucous membranes (7.5 cm × 2.5 cm) was fixed to a glass slide, and 50 mg of microspheres were evenly distributed on the surface of the vaginal mucosa. The mucosa was moistened, and the slide was positioned at a 45° angle. The mucosal surface was rinsed with 4.4 pH acetate buffer using a syringe pump (Top 5300) at a flow rate of 5 mL/min, washings were collected and centrifuged for 15 minutes at 7000 rpm (Eppendorf Company, Mini spin), dried, and percent mucoadhesion was determined using the formula below:

$$\%\ Ex-vivo\ mucoadhesion = \frac{Weight\ of\ microspheres\ applied - Weight\ of\ microspheres\ washed\ out}{Weight\ of\ micropsheres\ leached\ out} * 100$$

The *ex vivo* mucoadhesion adhesion study of econazole nitrate-loaded microsphere was found to be 70.6% for 8 h.

Preparation of mucoadhesive microsphere-loaded vaginal tablet

The mucoadhesive microsphere-loaded vaginal tablets were prepared using direct compressible grade excipients such as dibasic calcium phosphate, Avicel PH 200, starch 1500, cross carmellose sodium, magnesium stearate, and talc

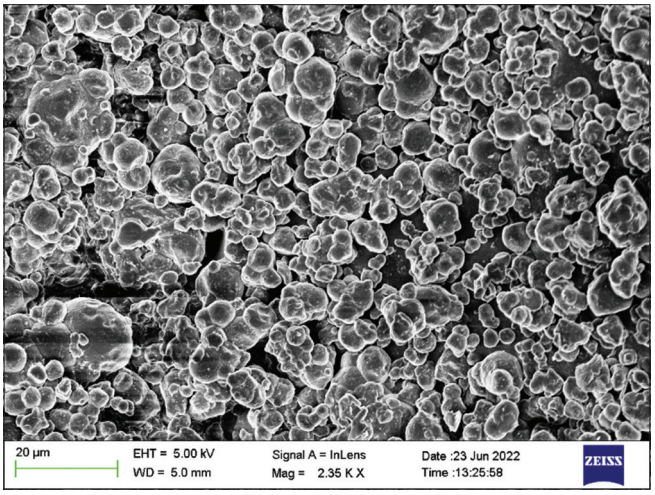

Figure 22: SEM image of econazole nitrate-loaded mucoadhesive microsphere

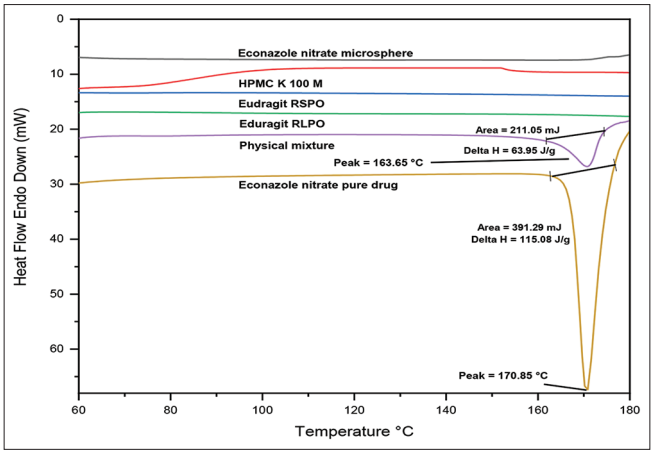

Figure 23: DSC thermogram of econazole nitrate-loaded microsphere, HPMC K 100M, Eudragit RSPO, Eudragit RLPO, physical mixture, and pure drug

were used to prepare vaginal tablets, where dibasic calcium phosphate acting as a diluent and Avicel as a cushioning agent. For binding property, starch is employed, while cross carmellose sodium, magnesium stearate, and talc, are used as disintegration agents, lubricants, and glidants. Econazole nitrate-loaded microspheres (650 mg) equivalent to 150 mg of econazole nitrate were combined with the excipients and passed through sieve #100. The tablets were compressed using the direct compression approach on a 20-station tablet compression machine.

Table 7: Measured responses of batches for optimizing formulation of econazole nitrate-loaded microsphere

Batch No.	Response variable							
	X_1: Particle size	X_2: Drug loading	X_3: Cumulative % drug release in 15 min	X_4: Cumulative % drug release in 30 min	X_5: Cumulative % drug release in 60 min	X_6: Cumulative % drug release in 120 min	X_7: Cumulative % drug release in 240 min	X_8: Cumulative % drug release in 360 min
B-1	5.4±0.96	18.5±0.5	12.0±0.11	29.86±0.23	47.49±0.24	64.63±0.35	80.72±0.46	97.85±0.58
B-2	16.0±0.6	14.81±0.44	15.2±0.16	31.3±0.33	48.4±0.50	66.24±0.67	77.76±0.84	91.66±1.01
B-3	7.2±0.30	19.41±0.96	11.4±0.30	27.8±0.60	48.2±0.90	70.70±1.20	94.92±1.31	98.54±1.51
B-4	27.2±2.35	13.5±0.36	24.9±0.22	55.51±0.55	89.31±0.77	91.10±0.99	95.09±1.21	98.94±0.99
B-5	5.5±0.81	17.6±0.62	16.2±0.14	41.7±0.29	67.20±0.45	93.81±0.60	97.26±0.75	98.47±0.90
B-6	15.8±0.80	16.55±0.56	21.00±0.16	51.14±0.33	67.07±0.50	94.95±0.59	97.96±0.68	99.28±0.77
B-7	6.8±0.85	15.7±0.56	22.8±0.20	40.25±0.40	60.07±0.60	82.43±0.46	85.99±0.41	99.54±0.40
B-8	17.4±2.79	13.57±0.33	18.56±0.22	36.24±0.44	55.02±0.66	73.59±0.88	89.06±1.10	98.57±1.32
B-9	8.4±1.06	19.25±0.30	18.07±0.13	34.67±0.26	54.57±0.27	74.70±±0.35	82.20±0.48	90.82±0.55
B-10	11.2±0.69	11.62±0.33	10.21±2.73	18.8±0.21	31.93±3.70	52.90±1.48	76.95±1.76	98.28±1.41
B-11	8.2±0.30	13.72±0.43	7.52±0.10	21.15±2.13	34.96±0.31	49.3±0.36	8597±0.48	90.05±0.48
B-12	13.5±0.6	11.68±0.58	14.93±0.23	32.2±0.47	55.45±0.71	81.12±0.83	94.1±1.06	98.52±1.19
B-13	8.51±1.19	20.53±0.70	25.6±0.07	41.18±0.15	25.88±1.13	69.35±0.26	82.0±0.40	96.62±0.40
B-14	8.2±0.3	20.56±0.50	14.77±1.16	29.82±0.25	40.67±0.19	58.3±1.79	77.81±0.40	98.56±1.32
B-15	8.9±0.50	21.13±0.91	12.32±1.13	24.78±0.27	37.80±0.41	50.96±0.55	66.17±0.70	89.87±0.56

Data are expressed as mean±SD (n = 3)

Evaluation of microsphere-loaded vaginal tablet

The vaginal tablet was prepared according to composition and evaluated as per the parameters shown in Table 6 and the result obtained is noted down.[23]

Drug content

The tablet is crushed in a pestle mortar and the powder is transferred in a 100 mL volumetric flask containing ethanol and sonicated for 20 min to dissolve the content in solvent then the volume was made up to 100 mL with ethanol. The solution was filtered on Whatman filter paper which was diluted and analyzed in a U.V. spectrophotometer (Shimadzu 1700) at 271 nm and drug content was calculated.

In vitro drug dissolution study of the vaginal tablet

To determine, the *in vitro* dissolution of the tablet USP apparatus type II (Paddle) was used. Tablets were placed in a dissolution apparatus having acetate buffer pH 4.4 at 37°C ± 0.5°C and the dissolution study was carried out. The 5 mL of sample was withdrawn at the time interval of 15, 30, 60, 120, 240, and 360 min with the help of a syringe filter of 0.45 µ. The sample was replaced by an equal amount of acetate buffer pH 4.4 kept at the same temperature. The withdrawal sample was analyzed at 271 nm on a double-beam UV-visible spectrophotometer (Shimadzu 1700). The cumulative % drug release recorded is recorded in Table 8 and the graph is shown in Figure 25.

RESULTS AND DISCUSSION

Optimization of econazole nitrate-loaded microsphere

All the desired characteristics of optimized formulation of econazole nitrate-loaded microsphere, that is, maximum particle size, maximum drug loading, and minimum drug release were set as criteria in Design Expert software. Quantities of HPMC K100 M, Eudragit RSPO, and Eudragit RLPO were set accordingly to attain the above criteria. The range of parameters was set as for HPMC K100 M (100–500 mg), Eudragit RSPO (1000–2000 mg), and Eudragit RLPO (1000–2000 mg), respectively. The particle size (X_1), drug loading (X_2), and cumulative % drug release at different time points (X_3-X_8) of prepared mucoadhesive microsphere were set as response variables, and the effect of the amount of HPMC K100 M, Eudragit RSPO, and Eudragit RLPO was studied as shown in Table 2. The data of responses are shown in Table 7. It was found that the particle size of microspheres was directly proportional to the concentration of independent variables. Figures 1 and 2 show that an increase in the concentration of HPMC K100M, Eudragit RSPO, and Eudragit RLPO is responsible for an increase in the particle size of microspheres. This is because an increase in polymer

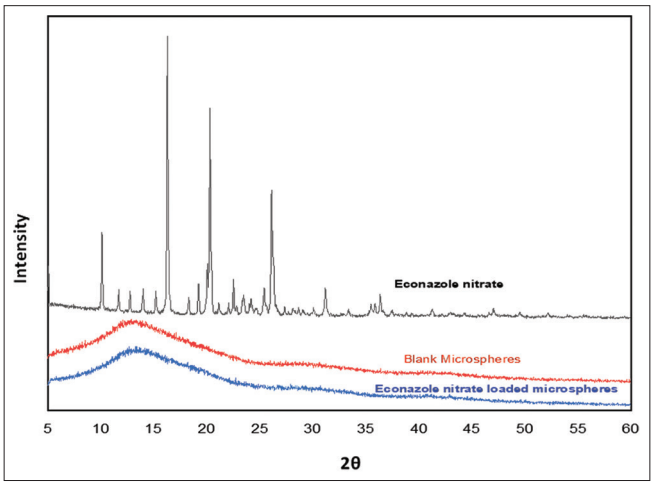

Figure 24: X-ray diffractogram of econazole nitrate, blank microspheres and econazole nitrate-loaded microsphere

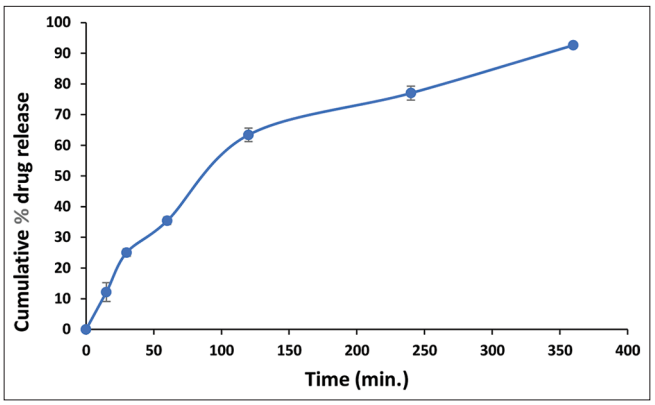

Figure 25: *In vitro* drug release graph of econazole nitrate microsphere-loaded vaginal tablet. Data are expressed as mean ± SD, $n = 3$

Table 8: *In vitro* drug release kinetic data and model fitting of optimized formulation of econazole nitrate-loaded mucoadhesive microsphere

S. No.	Drug release kinetic model	Equation	K	R^2
1.	Zero-order	C_0-$C_1 = k_0 t$	2.15×10^{-1}	0.8261
2.	First-order	Log C = log C_0 – kt/2.303	-2.60×10^{-2}	0.9549
3.	Korsmeyer-Peppas	$C_0 - C_1 = k_0 t^{1/2}$	2.15×10^{-1}	0.8261
4.	Higuchi	Log (C_0 - C_1) = log k-n log t	4.71×10^{0}	0.9685
5.	Hixon-Crowell	$C_0^{1/3} - C_1^{1/3} = kt$	6.40×10^{-3}	0.9389

concentration causes an increase in the viscosity of the polymeric solution, which is responsible for the formation of larger droplets, which were cooled and hardened by ethanol and dichloromethane evaporation. The effect of the independent variable in the response (X_2) has been shown as a 3-D surface plot in Figures 3 and 4. The graph shows that an increase in the concentration of HPMC K100M, Eudragit RSPO, and Eudragit RLPO leads to an increase in the loading of drug in microspheres. This is because a high concentration of polymer possesses a high binding capacity so more drug is loaded in a polymeric matrix.[24] It is also found that the concentration of HPMC K100M, Eudragit RSPO, and Eudragit RLPO affects dependent responses (X_3–X_8), that is, cumulative % drug release at 15 min, 30 min, 60 min, 120 min, 240 min, and 360 min, respectively. The 3-D surface plot in Figures 5–16 shows that an increase in the amount of polymers leads to a decrease in drug release from microspheres. This is due to the hydrophilic polymer HPMC K 100M that forms a gel layer after swelling, thus increasing the diffusional path length for the drug.[18] Eudragit RSPO and Eudragit RLPO follow the same mechanism, but they possess hydrophilic quaternary ammonium moiety that allows swelling after contact with the medium. Higher concentration polymer controls the release of drug from polymer because the cumulative % release of a drug directly corresponds to the quantity of polymers taken.

Prediction of optimized econazole nitrate-loaded mucoadhesive microsphere

With the help of a design expert, a statistical analysis was performed. The software provided a final optimized formula with a desirability close to 1 by keeping all the constraints and requirements on the desirable properties of the final batch of the mucoadhesive microsphere, including maximum particle size, maximum drug loading, and sustained release of a drug. The software suggested an optimum formulation with a desirability close to 1 based on the results of the evaluation of optimization batches. The formulation that had the highest predicted desirability, 0.96, was selected as the optimized formulation.

In vitro drug release kinetics

The *in vitro* drug release profile of the optimized econazole nitrate-loaded microsphere is shown in Figure 26 which concludes that the drug is released in a sustained manner which is due to HPMC K 100M, Eudragit RSPO, and Eudragit RLPO acting as a release retarding polymer. Econazole nitrate-loaded microsphere releases drug up to 93.49% at 6 h. *In vitro* drug release data of the final optimized batch were studied for drug release kinetics by fitting into the zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixon-Crowell model equations, as shown in Table 8 and Figure 27. The optimized formulation *in vitro* drug release kinetics matched the Higuchi model shows that the regression coefficient (R^2) value was highest at 0.9685 in this model.

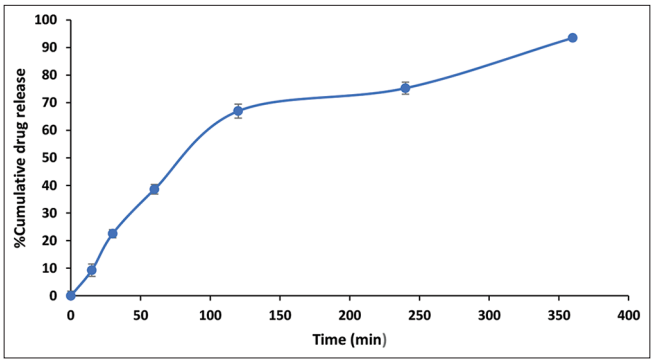

Figure 26: *In vitro* drug release graph of optimized econazole nitrate-loaded mucoadhesive microsphere. Data are expressed as mean ± SD, $n = 3$

Table 9: *In vitro* dissolution study of econazole nitrate microspheres loaded vaginal tablet, mean±SD, $n = 3$

S. No.	Time interval (min.)	% Cumulative drug release of a microsphere-loaded vaginal tablet
1.	0	0
2.	15	12.17±0.95
3.	30	25.05±3.05
4.	60	35.42±1.21
5.	120	63.4±1.20
6.	240	77.01±2.19
7.	360	92.6±2.27

SEM analysis and particle size estimation

The particle size of the econazole nitrate-loaded microsphere was determined by an optical microscope (Leica microsystems). An average of 100 microspheres was observed for optimization batches and the final formulation and microsphere size were found to be in the range of 4.1–29.5 µm. The SEM image of the econazole nitrate-loaded mucoadhesive microsphere is shown in Figure 22. The SEM image shows that the microspheres are of uniform size and smooth surface. The size of the econazole nitrate-loaded microsphere was found to be approximately 12 µm.

Differential scanning calorimeter analysis

DSC thermogram as shown in Figure 23 shows that pure econazole nitrate has a sharp endothermic peak at 170.085°C, which corresponds to the econazole nitrate melting point. The thermogram of a physical mixture shows an endotherm at 171.25°C, indicating that the crystalline drug is melting. The thermogram of the econazole nitrate-loaded microsphere revealed no peak corresponding to the melting point of crystalline econazole nitrate, indicating changes in the physical property of econazole nitrate, that is, conversion from crystalline form to amorphous form. The absence of a

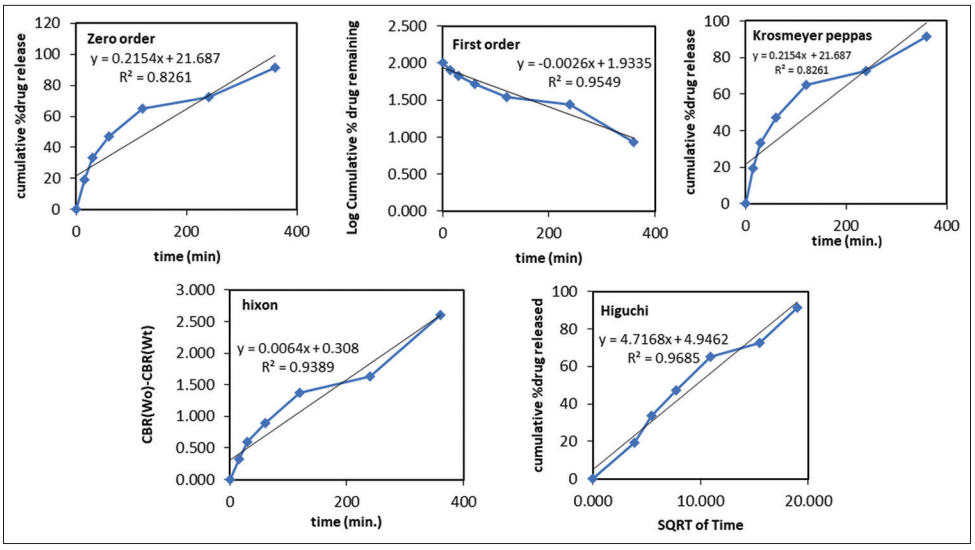

Figure 27: *In vitro* drug release kinetic graph of econazole nitrate-loaded microsphere

peak in the econazole nitrate-loaded microsphere confirms that there is no unentrapped drug on the surface of the microparticle and that it is molecularly dispersed throughout the microsphere.

X-ray diffraction pattern analysis

The XRD pattern of econazole nitrate-loaded microspheres, blank microspheres, and econazole nitrate is shown in Figure 24. The pattern shows the sharp distinctive peak at 10.11°, 16.7°, 20.28°, and 26.09° on the 2θ scale which depicts the crystalline nature of econazole nitrate. The absence of a crystalline peak in the graph of the econazole nitrate-loaded microsphere illustrates the conversion of a crystalline drug into an amorphous form and the drug was molecularly dispersed in a polymer matrix and broad peak in blank microspheres representing an amorphous form of polymer.[25,26] The result of this study is in close agreement with DSC studies.

Evaluation of econazole nitrate microsphere-loaded vaginal tablet

The econazole nitrate-loaded microsphere (650 mg) equivalent to econazole nitrate 150 mg was compressed into a tablet by direct compressible method with excipients such as dibasic calcium phosphate (7.3%), Avicel PH 200 (5.9%), starch 1500 (1.4%), magnesium stearate (2%), talc (1%), and cross carmellose sodium (1%). The hardness of econazole nitrate-loaded microsphere-loaded vaginal tablet and the % friability of tablets was found to be 3–4 kg/cm^3 and 0.39%, respectively. All prepared vaginal tablets passed for weight variation test and the average weight was found to be 816.3 and the variation limit was 5% (776.3–856.3 mg). The drug content of the tablet was found to be 98.2%. The disintegration time of the tablet was found to be 30 s. The *in vitro* dissolution test was performed to study the release pattern and % cumulative drug release versus time was plotted which is shown in Figure 25 and data is shown in Table 9.

CONCLUSION

The most common disease in the female genital tract is vaginal candidiasis which is caused by candida albicans. The marketed formulation available for the treatment of vaginal candidiasis is creams, gels, pessaries, ointments, and suppositories, but they have major drawbacks such as leakiness, Messines, and a tendency to escape from the vaginal cavity while performing normal routine work, this drawback leads to the shorter residence of vaginal formulations. The current research study focuses on the development of mucoadhesive microspheres by spray drying technique and compressing them into vaginal tablets. These vaginal tablets quickly disintegrate into individual microparticles and adhere to the mucosa of the vagina; therefore, release time is prolonged. The optimization study was performed with the help of a design expert software using Box–Behnken design. The particle size of the optimized mucoadhesive microsphere is around 25 µm and the SEM image shows the smooth and spherical surface of microspheres. The drug loading of the optimized mucoadhesive microsphere was found to be 23.1 %. The *ex vivo* mucoadhesion study showed 70% adherence of the microsphere for up to 8 h, while the *in vitro* drug release study of the optimized microspheres demonstrates a sustained release of drug from microspheres for up to 6 h. The mucoadhesive microsphere is compressed into vaginal tablets and evaluated for disintegration time, and release profile and compared with the release profile of the optimized microsphere. There is a similarity between the release profile of the vaginal tablet and the optimized microsphere and the tablet disintegrates within 28 s. This inferred that econazole nitrate-loaded mucoadhesive microsphere developed by

a spray dryer and further compressing to a vaginal tablet disintegrates rapidly into microspheres that adhere to vaginal mucosa for a longer duration of time and are effective in the treatment of vaginal candidiasis.

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