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Development and Optimization of Mucoadhesive Microspheres of Miconazole Nitrate

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Abstract

Vulvovaginal candidiasis (vaginal thrush) is common fungal infection caused by candida albicans in vaginal canal. The physiology mechanism of vaginal cavity offers problem of draining out of formulations with vaginal irrigation. The currently available formulation also shows problem of shorter resident time in the vaginal lumen and have feeling of uncomfort and uneasiness due to size and shape of dosage forms. Miconazole nitrate is choice of drug for the treatment of vulvovaginal candidiasis. The objective of present research work was to formulate controlled release mucoadhesive microspheres of miconazole nitrate by spray drying technique and compressing it to tablet dosage form which should disintegrate into microspheres at the site and adhere to the vaginal lumen, hence releasing the drug for longer duration of time. Formulation variables were optimized using three factor, three level Box-Behnken design composed of HPMC K100M (X1), Eudragit RSPO (X2), Ethyl cellulose 100CP (X3) as independent variables. The response surface methodology was employed and was optimized for the response variables, viz., entrapment efficiency and cumulative % drug release at different time intervals. The % mucoadhesion of optimized microspheres formulation was found to be 90 % after 8 hours of microspheres application. The tablet will disperse after contacted with vaginal fluid in the form of mucoadhesive microspheres and adhere with mucosal surface and consistently release the drug upto 12 hr.

Keyword: Miconazole Nitrate, Mucoadhesive Microspheres, Spray drying

Introduction

Vaginal lumen is susceptible site for various pathologic conditions such as bacterial, fungal and viral infections.[1] Vulvovaginal candidiasis is a relatively common form of yeast infection. It is caused by overgrowth of candidal species in the lumen. The foremost appearing symptoms are pruritus, errythma, oedema, white discharge, fissuring, satellite lesions, and vulval soreness.[2] An antifungal medication is used to treat fungal infections such as mycoses, candidiasis (thrush) either by fungicidal or fungistatic action..[3],[4],

Miconazole nitrate is drug of choice for the treatment of fungal infection[5],[6]. It prohibits the formation of lanosterol from ergosterol by blocking 14-α-demethylase enzyme, where the enzyme belongs to the cytochrome P-450 family[7],[8]. Ergosterol is the fundamental component of the yeast cell wall. Interference in the synthesis of ergosterol leads to increased permeability of the cellular membrane leading to oozing out of the cellular material.[9]

Currently available vaginal formulations, vaginal suppositories, pessaries, gels, creams have drawback of leakage, messiness, and tendency to escape from body during normal activity of their routine life.[1]

Therefore it would be beneficial to develop mucoadhesion based formulation which provides an intimate contact of the drug delivery system with vaginal mucosal surface which will contribute to improve and better therapeutic performance of the drug. [10]

Mucoadhesive based vaginal formulations have potential of delivering active substances for a prolonged duration at a predicable rate have been studied recently.[11] This type of composition offers various advantages such as localization of the drug at target site, reduction in frequency of drug dose, prolonged retention time and improved patient compliance.[12], [13].

Conventional delivery systems suffers retention and leakage problem due to self irrigation physiologic mechanism.[14]

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Utilization of mucoadhesive polymers in the pharmaceutical delivery systems help in improving retention at mucous membrane and thereby enhancing therapeutic efficacy. various mucoadhesive polymers such as synthetic cellulose derivatives (Hydroxy propyl methyl cellulose, hydroxy ethyl cellulose, hydroxyl methyl cellulose), hyaluronic acid and its derivatives, chitosan, sodium alginate, gelatine, pectin, tragacanth, carbopol, poly acrylates and its derivatives[15,16]

The objective of this study was to develop sustained release mucoadhesive microspheres of miconazole nitrate using spray drying process and compressing it to tablet dosage form which should disintegrate into microspheres at the site and adhere to the vaginal lumen, hence releasing the drug for longer duration of time.[17,18,19]The formulation of microspheres was optimized using design of experiment (DOE). The statistical methodology was incorporated to check the independent and response variable using response surface methodology. The response variables such as drug entrapment efficiency, in-vitro drug release were evaluated for optimization of formulation[20].

Material and methods

Miconazole nitrate was a gift sample from Encube Ethical Laboratories, Mumbai, India, Methocel K100M and Ethocel 100CP were received from colorcon Asia Pvt. Ltd, India, Eudragit RSPO from Rohm Pharma polymers, Dichloromethan, methanol, Ethanol, Triethyl citrate, Sodium lauryl sulphate were purchased from Loba Chemie Pvt Ltd.

Preparation of mucoadhesive microspheres of miconazole nitrate

Miconazole nitrate loaded microspheres were prepared using spray drying technique. Dichloromethane and ethanol was combined together in the ratio of 1:1. Then, accurately weighed polymers (Methocel K100M, Ethocel 100cp, Eudragit RSPO) and drug were dissolved in solvent system with continuous stirring. Spray drying was performed using SprayMate (Jay Instruments & Systems Pvt. Ltd., Mumbai, India) with a standard 0.7mm two fluid nozzle. Inlet temperature was maintained at 70° C, feed pump rate was maintained at 20 RPM. Atomizing pressure was maintained at 0.3 MPa. Solvent evaporation by flow of heated air aspirated by a pump induced the formation of discrete free flowing microspheres. The obtained microspheres were separated inside the cyclone separator and settled down in the collector. The final solution was further stirred for ten minutes using mechanical stirrer. The quantity of drug (400 mg) and Triethyl citrate as plasticizer (1 %) remains constant through out of the experimental runs. This solution was then sprayed using spray dryer and the product was collected.

Experimental design

The statistical technique response surface methodology was utilized in optimizing the formulation variables. The Box- Behnken design was choose to systemically investigate the effect of the independent and dependent variables.[21] Three-factor, three-level Box-Behnken design was used for the optimization of microsphere formulation. Box-Behnken design was used to evaluate the effects of selected independent variables were concentration of HPMC K100M (X1), Eudragit RSPO (X2), and Ethyl cellulose 100 cp (X3) on the response variables, i.e., particle size, drug entrapment, and percent cumulative drug release at different time intervals. The pre-screening of some process variables were determined from the studies conducted earlier such as solubility of drug, polymer ratio with solvent system (DCM: Ethanol), % polymer concentration, and speed of peristaltic pump. The quantity of drug (400 mg) and Triethyl citrate as plasticizer (1 %) remains constant through out of the experimental runs. Table 1 showing concentration of independent variables and dependent variables used for formulation optimization.

Table 1: Variables and their levels in Box-Behnken Design

Independent Variables	Unit	Levels		
		Low	Medium	High
X1= HPMC K100M	%	0.1	0.55	1
X2=Eudragit RSPO	%	1	1.5	2
X3=Ethyl cellulose 100 CP	%	1	1.5	2

Response Variables	Unit
R1 = Entrapment efficiency (%)	Maximum
R2 = Cumulative % drug release at 30 min	Minimum
R3 = Cumulative % drug release at 60 min	Minimum
R4 = Cumulative % drug release at 120min	Minimum
R5 = Cumulative % drug release at 240min	Minimum
R6 = Cumulative % drug release at 480min	Minimum
R7 = Cumulative % drug release at 720min	Minimum

Table 2: Box-Behnken experimental design with measured responses

S. No.	Independent variable			Response variable						
	X1(HPMC K100M)	X2: (Eudragit RSPO)	X3: (Ethyl Cellulose 100cp)	R1: Entrapment Efficiency (%)	R2: Cumulative % drug release at 0.5 hr	R3: Cumulative % drug release at 1 hr	R4: Cumulative % drug release at 2	R5: Cumulative % drug release at 4 hr	R6: Cumulative % drug release at 8 hr	R7: Cumulative % drug release at 12 hr
1	100	1000	1500	68	51.8	66.8	69.53	75.38	90.8	99.6
2	1000	1000	1500	78	31.5	45.8	68.1	86.3	92.5	98.7
3	100	2000	1500	77	34.6	44.7	67.53	88.1	95.7	99.2
4	1000	2000	1500	87	18.3	34.3	47.8	66.7	87.4	97.3
5	100	1500	1000	66	40.2	59.7	66.5	75.2	88.9	96.5
6	1000	1500	1000	74	39.2	50.6	62.8	78.9	86.8	95.2
7	100	1500	2000	72	28.3	45.7	58.7	71.5	87.6	96.7
8	1000	1500	2000	89	13.7	23.2	35.8	62.3	81.2	94.6
9	550	1000	1000	55	40.9	65.8	79.5	89.2	93.5	97.9
1	550	2000	1000	71	29.6	49.2	53.3	80.1	89.9	93.8
1	550	1000	2000	73	30.2	46.5	52.6	79.5	88.2	98.0
1	550	2000	2000	92	12.7	27.1	43.8	63.8	86.5	93.4
1	550	1500	1500	70	24.8	33.5	47.6	69.4	87.8	97.7
1	550	1500	1500	73	25.9	37.9	52.3	72.5	89.2	98.4
1	550	1500	1500	75	22.6	35.7	46.8	67.9	90.2	95.6

Characterization of miconazole nitrate microspheres

Determination of particle size of miconazole nitrate formulation

The particle size analysis of miconazole nitrate microspheres were performed by dispersing the microspheres in small amount of water and analyzing them under optical microscope (Leica microsystems) at the magnification of 100X. The particle size of 100 microspheres were observed and analyzed of each batch. The average particle size was determined under using calibrated micrometer scale on optical microscope.

Scanning electron microscopy of miconazole nitrate microspheres

The morphological characteristics of optimized microspheres were studied by scanning electron microscopy. A small amount of microspheres were spread on metal stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber (JSM 5600, JOEL, Japan). Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, at 3000 X magnification .

Determination of entrapment efficiency

Accurately weighed 50 mg of microspheres were transferred in to 50 ml volumetric flask containing adequate amount of methanol and volume was made up to 50 ml with methanol and sonicated for 10 minute. The sample was suitably diluted and analysed in UV spectrophotometrically (Shimadzu UV-1700) at 272nm. Drug content was determined and percentage entrapment efficiency of microspheres was calculated by following formula.

$$\textbf{(\%) Entrapment Efficiency} = \frac{\text{Amount of drug present in microsphere}}{\text{Initial amount of drug taken}} \times 100$$

Differential scanning calorimetric analysis

DSC study is done in order to check the presence of crystalline peaks of drug in formulation. DSC study of samples (Drug- miconazole nitrate, Polymers- HPMC K100M, Eudragit RSPO and Ethyl cellulose 100 CP). Physical mixture consisting of Miconazole nitrate : EudragitRSPO : HPMCK100M : Ethyl cellulose in the ratio of 1:1:1 and optimized Microsphere formulation were performed on DSC-6000 (PerkinElmer Thermal Analysis). Accurately weighed sample (3.1 mg) was placed and sealed in a aluminum pan, which was then heated from 50°C to 250°C melting point of individual sample at scanning rate of 10°C/min under nitrogen flow (20ml/min). An empty aluminum pan was used as reference.

In-vitro drug release study

An in-vitro drug release study was performed in order to evaluate the drug release characteristics of designed formulations. A series of 50 ml screw cap tubes were taken for every sampling time points, each containing 10 mg miconazole nitrate loaded mucoadhesive microspheres dispersed in 20 ml of 0.45 % sodium lauryl sulphate solution. The screw cap tubes were closed & sealed well with paraffin film and placed in bottle rotating apparatus (Electrolab E40W, India). At predetermined time intervals samples are withdrawn, centrifuged and filtered through 0.45µ milipore membrane filters and analyzed at 272 nm. The experiments were carried out in triplicate and average values were recorded.

In-vitro mucoadhesion study

In order to determine the mucoadhesive strength of the microspheres, ex-vivo mucoadhesion test was conducted. A strip of isolated goat vaginal mucosa (2cm long and 2cm wide) was moistened with simulated vaginal fluid (SVF) and attached on a glass plate, and plate was fixed at an angle of 45°. After this, accurately weighed microspheres (50 mg) were spread uniformly on the surface of vaginal mucosal membrane and were allowed to hydrate microspheres for 20 minutes. The mucosal surface was rinsed with simulated vaginal fluid using syringe pump (Top company, Model 5300) at a flow rate of 5 ml/hr. Washings were collected, centrifuged (Eppendorf company, minispin) for 12000 RPM for 15 minutes and dried.

$$(\%)\ \text{Mucoadhesion} = \frac{Wa - WL}{Wa} \times 100$$

Where,

Wa = weight of microspheres applied, WL = weight of microspheres leached out

Preparation of intra-vaginal tablet formulation

For development of vaginal tablet formulation, microsphere equivalent to 100 mg of miconazole nitrate were mixed geometrically with directly compressible grade excipients were selected. In present study, a combination of lactose monohydrate and microcrystalline cellulose were used as a diluent and cushioning agent. The multifunctional excipient, partially pregelatinized maize starch was used for its compressing, binding and disintegrating property.

Cross carmellose sodium was as a superdisintegrant, talc and magnesium stearate was used as glidant and lubricant. Powder blend were compressed by direct compression method using 20 station compression machine and evaluated for following parameters: weight variation, hardness, friability, drug content, disintegration, dissolution study.

Characterization of intra-vaginal tablet formulation

Weight variation test

Twenty tablets were individually weighed and average weight was calculated.

Friability test

For this test, 7 tablets having total weight of 7.04 g were taken and transferred in to the friability apparatus (Electrolab EF-2), which was rotated with a speed of 25 rpm for 4 minute. After rotation, tablets were removed and reweighted and % friability were calculated by using following formula:

$$\% \text{ Friability} = \frac{(W_i - W_f)}{W_i} \times 100$$

Where,

W_i = Initial weight , W_f = Weight obtained after conducting test

Hardness

The hardness of the tablet shows how physically stable the formulation will be in transit time from industry to the recipient. Hardness of vaginal tablet formulation was mesured using Monsanto hardness tester.

Drug content determination

Three individual tablets were crushed in pestle mortar and powder equivalent to 100 mg of miconazole nitrate was weighted and transferred in 100 ml volumetric flask containing adequate amount of methanol and sonicated for 15 minutes in a bath sonicator to disperse the powder. After this, the volume was made up to 100 ml with methanol. The above solution was filtered and filtrate was suitably diluted and analysed in UV/ Visible spectrophotometer (Shimadzu® 1700) at 272 nm and drug content was determined.

Disintegration test

The vaginal table here requires fast disintegration from the tablet in discrete mucoahesive microparticles. The disintegration test was conducted using disintegration test apparatus (Electrolab USP ED 2L) at 37°C. Six tablets were placed in disintegration beaker containing demineralised water under cylindrical basket-rack assembly without disk.

In-vitro dissolution test

Dissolution test was performed employing Electrolab Dissolution Apparatus 1 (Paddle) in which dissolution beaker containing 900 ml of 0.45 % SLS solution as a dissolution medium. The temperature of the medium was maintained at 37±0.5°C and the rotation of paddle was fixed at 50 rpm. The tablets were placed in beaker assembly and start the test. 10-10 ml aliquots of dissolution fluid were withdrawn from each vessel at suitable time interval and replaced with same volume of fresh dissolution medium. Collected sample were filtered through syring filter and suitably diluted with dissolution medium and analysed in UV spectrophotometer (Shimadzu® UV 1700) at 272nm.

Result and Discussion

Optimization of miconazole nitrate microspheres by response surface methodology

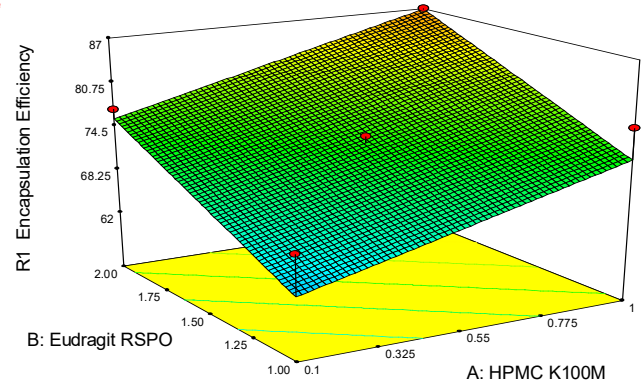
The result obtained from the optimization formulations were statistically analysed for response variables by using Design Expert 7.1.6 (trial version) software (Stat-Ease Inc., Minneapolis, USA). A total of 15 experiments were proposed by software according to Box-Behnken design. Models were selected on the basis of sequential comparison and lack of fit test. Significance of the models was further confirmed by statistical analysis. The design was evaluated using statistical analysis by sum of square and R-squared, and p value. On the above mentioned tool it was inferred that In-vitro release followed quadratic and mean model and drug content followed linear model. The following polynomial equations in terms of actual factors were generated to demonstrate the relationship between the formulation variables.

Table 3 : Statistical summary of response variables (a) Sum of squares and (b) R- squared

	R_1			R_2			R_3			R_4		
	Sum of squares	**F value**	**p-value**	**Sum of squares**	**F value**	**p-value**	**Sum of squares**	**F value**	**p-value**	**Sum of squares**	**F value**	**p-value**
a) Sum of squares												
Mean vs total	83626.67	-	-	13166.09	-	-	29624.5	-	-	48477.7	-	-
Linear vs mean	1054.25	22.07853	< 0.0001	1308.476	15.90263	0.0003	1959.00	18.28	0.0001	1329.4	7.504	0.0052
2 FI vs linear	22.5	0.393228	0.7614	59.7881	0.659076	0.5998	74.78	0.627	0.6175	250.87	1.678	0.2480
Quadratic vs 2 FI	60.16667	1.085062	0.4354	168.4464	3.821696	0.0915	307.59	49.22	0.0004	357.05	14.29	0.0069
Cubic vs quadratic	79.75	4.197368	0.1984	67.8139	8.006364	0.1131	0.733	0.050	0.9813	23.96	0.904	0.5632
Residual	12.66667	-	-	5.646667	-	-	9.68	-	-	17.66	-	-
Total	84856	-	-	14776.26	-	-	31976.4	-	-	50456.76	-	-
b) R- squared												
	Adjusted R squared	**Predicted R squared**	**PRESS**	**Adjusted R squared**	**Predicted R squared**	**PRESS**	**Adjusted R squared**	**Predicted R squared**	**PRESS**	**Adjusted R squared**	**Predicted R squared**	**PRESS**
Linear	0.818	0.714	350.44	0.761	0.652	560.09	0.787	0.743	603.85	0.582	0.401	1185.16
2 FI	0.782	0.422	709.52	0.737	0.424	926.66	0.763	0.704	695.65	0.647	0.364	1257.42
Quadratic	0.789	-0.061	1304.5	0.872	0.318	1097.72	0.987	0.985	33.52	0.941	0.786	423.09
Cubic	0.927	-	+	0.975	-	+	0.971	-	+	0.937	-	+

Table 4: Statistical summary of response variables (a) Sum of squares and (b) R- squared

	R_5			R_6			R_7		
	Sum of squares	**F value**	**p-value**	**Sum of squares**	**F value**	**p-value**	**Sum of squares**	**F value**	**p-value**
a) Sum of squares									
Mean vs total	84654.23	-	-	119028.7	-	-	140747.3	-	-
Linear vs mean	425.94	2.633	0.1021	62.702	2.330	0.1306	18.912	2.089	0.1597
2 FI vs linear	314.15	3.004	0.0948	30.525	1.1946	0.3717	0.562	0.045	0.9860
Quadratic vs 2 FI	220.49	6.303	0.0376	61.122	14.523	0.0067	22.749	3.841	0.0907
Cubic vs quadratic	47.28	2.864	0.2694	4.107	0.9420	0.5519	5.622	0.882	0.5700
Residual	11.0	-	-	2.906	-	-	4.246	-	-
Total	85673.11	-	-	119190.1	-	-	140799.4	-	-
b) R- squared									
	Adjusted R squared	**Predicted R squared**	**PRESS**	**Adjusted R squared**	**Predicted R squared**	**PRESS**	**Adjusted R squared**	**Predicted R squared**	**PRESS**
Linear	0.259	-0.156	1178.58	0.221827	-0.29151	208.4031	0.189337	-0.27539	66.43914
2 FI	0.521	-0.080	1101.01	0.261057	-1.17342	350.7123	-0.09577	-1.96164	154.2818
Quadratic	0.839	0.233	781.39	0.87829	0.552193	72.26	0.469535	-0.91032	99.515
Cubic	0.924	-	+	0.873908	-	+	0.429358	-	+



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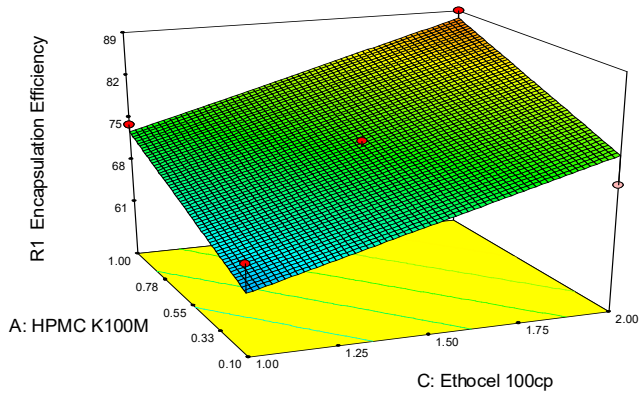

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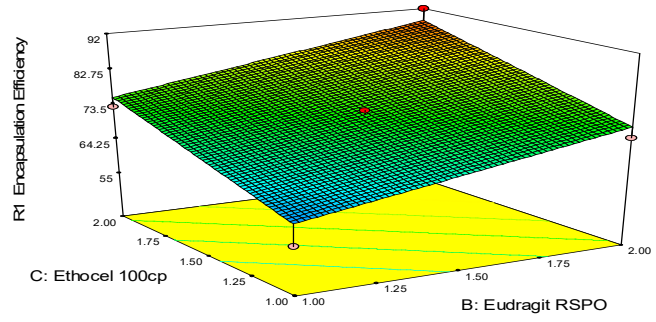
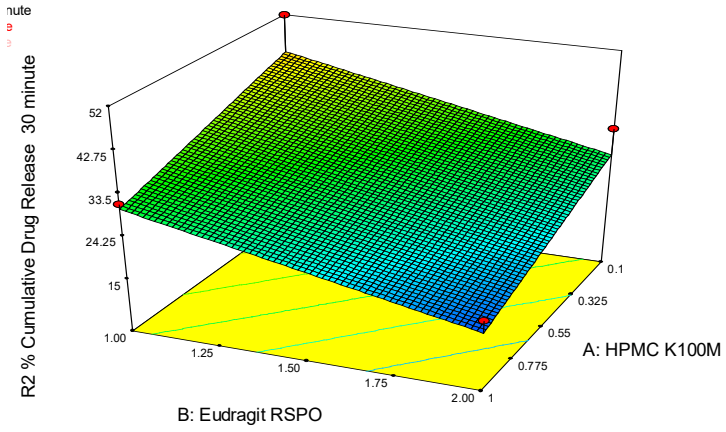


(C)

Fig 1: Three dimensional response surface plot showing (A) the effect of Eudragit RSPO and HPMC K100M on encapsulation efficiency (B) the effect of Ethocel 100cp and HPMC K100M on encapsulation efficiency (C) the effect of Eudragit RSPO and Ethocel 100CP on encapsulation efficiency



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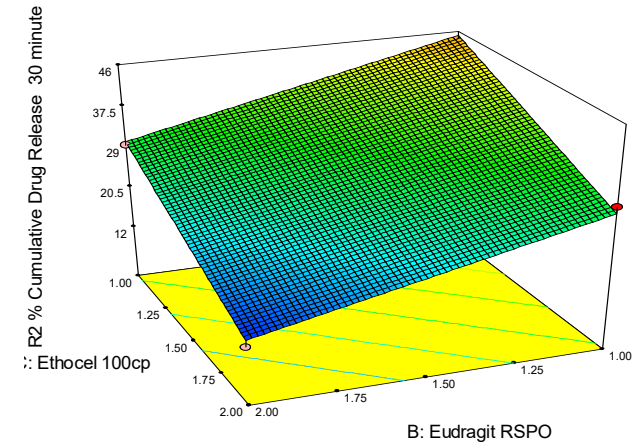

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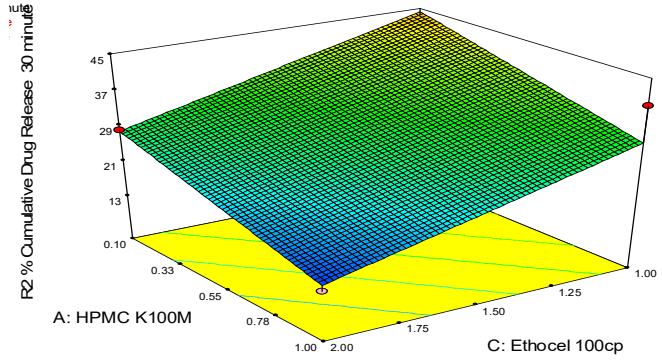
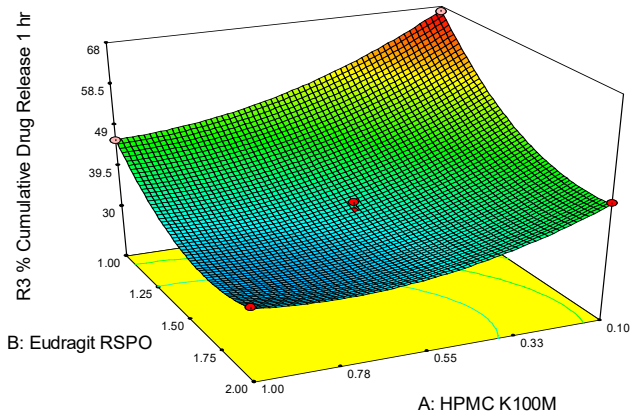


(C)

Fig 2: Three dimensional response surface plot showing (A) the effect of Eudragit RSPO and HPMC K100M on % CDR in 30 min (B) the effect of Ethocel 100cp and Eudragit RSPO on % CDR in 30 min (C) the effect of HPMC K100M and Ethocel 100cp % CDR in 30 min



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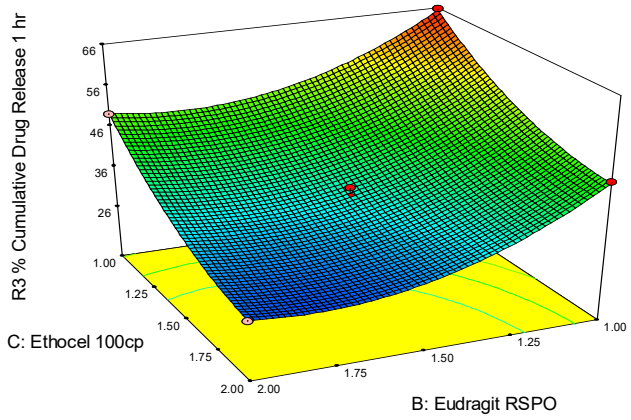

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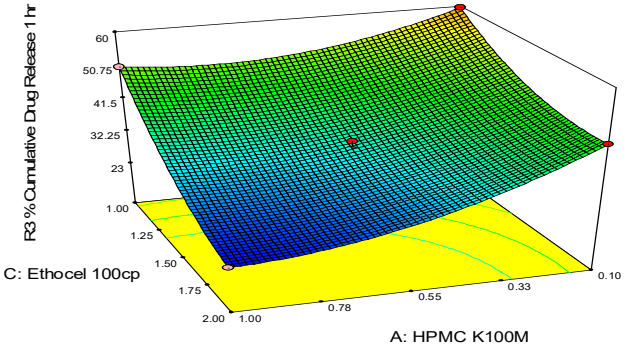
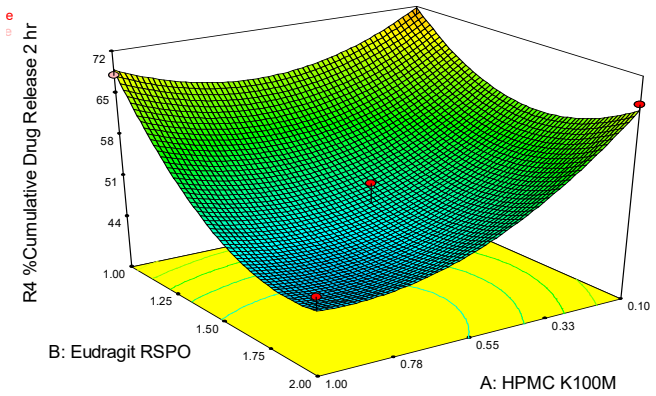


(C)

Fig 3: Three dimensional response surface plot showing (A) The effect of Eudragit RSPO and HPMC K100M on % CDR in 1 hr (B) The effect of Ethocel 100cp and Eudragit RSPO on % CDR in 1 hr (C) The effect of HPMC K100M and Ethocel 100cp % CDR in 1 hr



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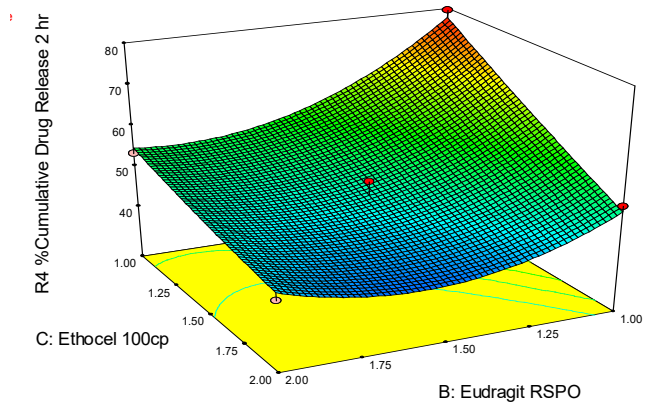

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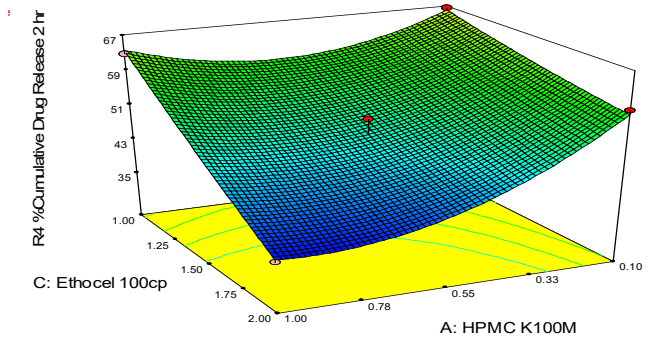

(C)

Fig 4: Three dimensional response surface plot showing (A) The effect of Eudragit RSPO and HPMC K100M on % CDR in 2 hr (B) The effect of Ethocel 100cp and Eudragit RSPO on % CDR in 2 hr (C) The effect of HPMC K100M and Ethocel 100cp % CDR in 2 hr

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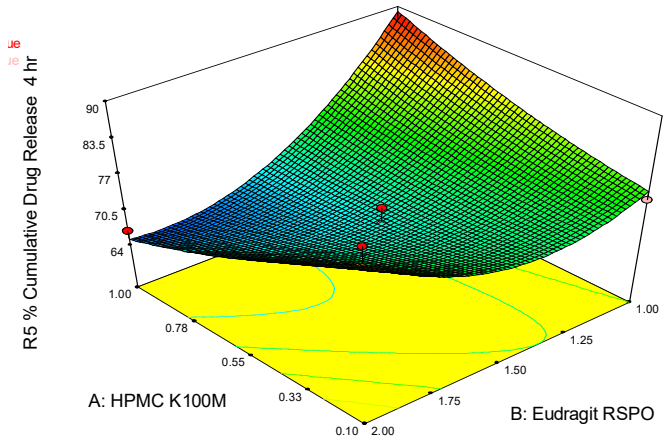
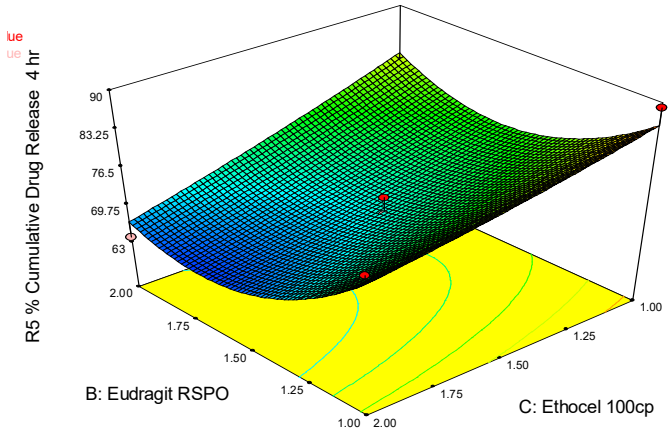


(A)



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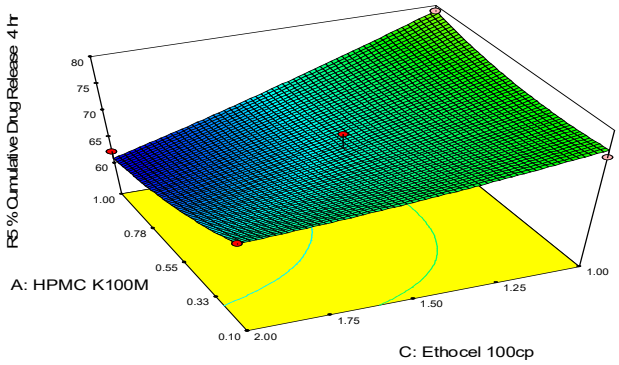
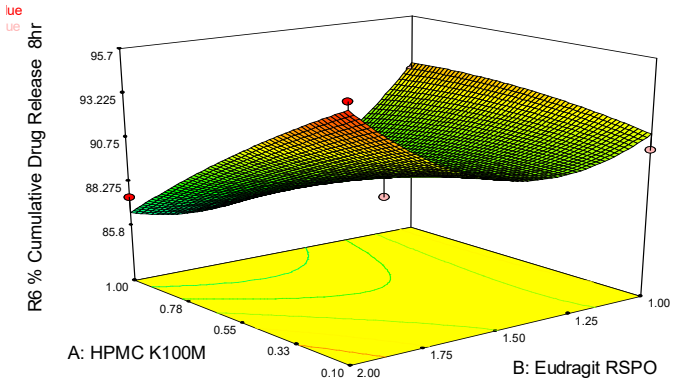
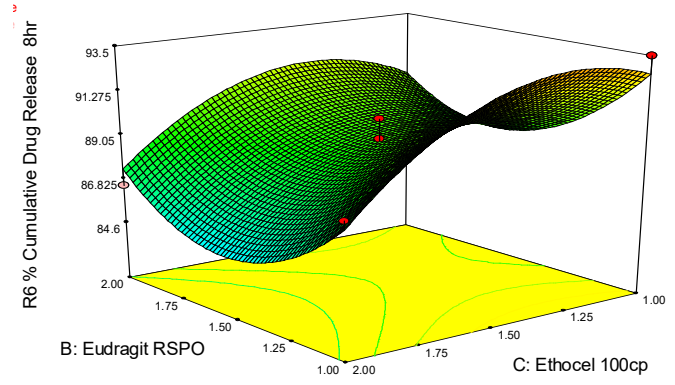


(C)

Fig 5: Three dimensional response surface plot showing (A) The effect of Eudragit RSPO and HPMC K100M on % CDR in 4 hr (B) The effect of Ethocel 100cp and Eudragit RSPO on % CDR in 4 hr (C) The effect of HPMC K100M and Ethocel 100cp % CDR in 4 hr



(A)



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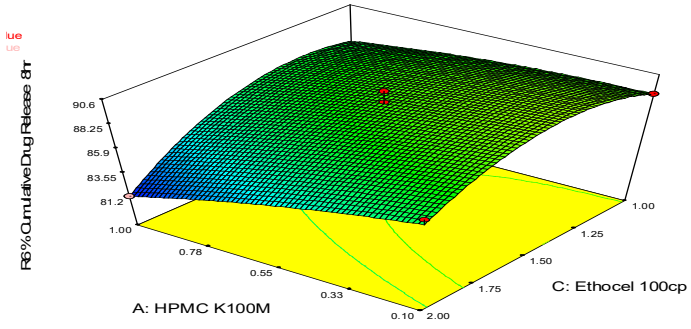
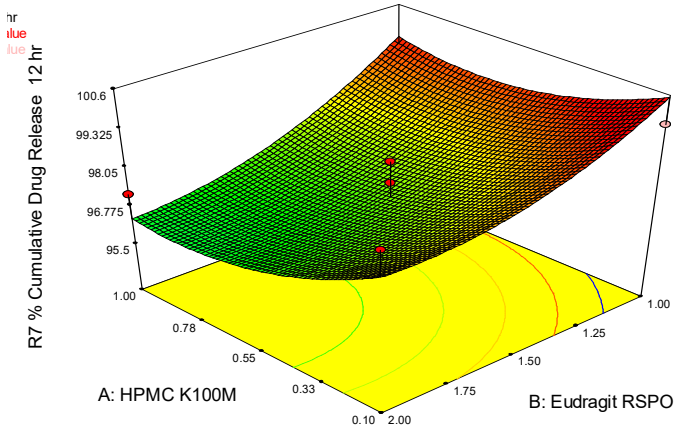
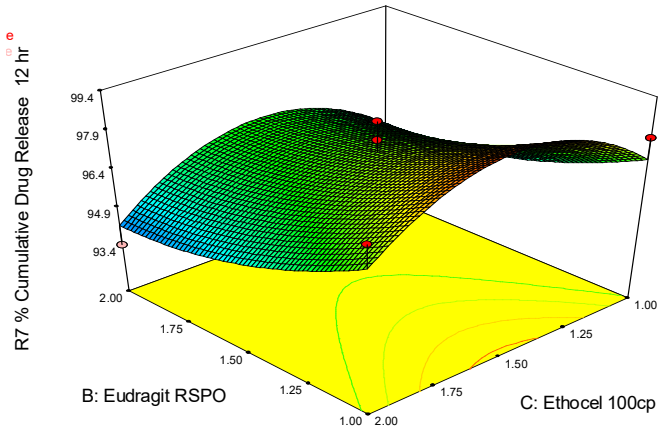


(C)

Fig 6: Three dimensional response surface plot showing (A) The effect of Eudragit RSPO and HPMC K100M on % CDR in 8 hr (B) The effect of Ethocel 100cp and Eudragit RSPO on % CDR in 8 hr (C) The effect of HPMC K100M and Ethocel 100cp % CDR in 8 hr



(A)



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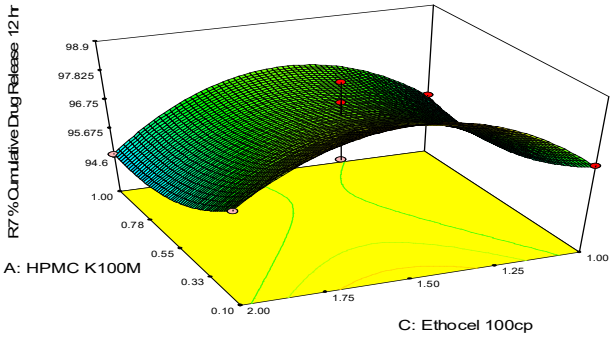

(C)

Fig 7: Three dimensional response surface plot showing (A) The effect of Eudragit RSPO and HPMC K100M on % CDR in 12 hr (B) The effect of Ethocel 100cp and Eudragit RSPO on % CDR in 12 hr (C) The effect of HPMC K100M and Ethocel 100cp % CDR in 12 hr

Table 5: Polynomial equations of response variables

% Encapsulation efficiency (R_1)	=	74.67 +5.62* A+6.62 * B +7.50 * C
% Cumulative drug release in 0.5 hr(R_2)	=	29.63-6.53 * A-7.40 * B -8.13 * C
% Cumulative drug release in 1 hr (R_3)	=	35.70 -7.88* A-8.69 * B-10.36 * C+2.64* A * B-3.35 * A * C-0.71 * B * C +4.93* A^2 +7.29 * B^2 + 4.17 * C^2
% Cumulative drug release in 2 hr (R_4)	=	48.90 -5.97 * A -7.15 * B-8.91 * C - 4.57 * A * B - 4.80 * A * C+4.33 * B*C+6.48 * A^2 +7.86 * B^2 +0.56 * C^2
% Cumulative drug release in 4 hr (R_5)	=	69.93-1.99 * A-3.96 * B-5.80 * C-8.08 * A * B-3.25 * A * C-1.65 * B * C +1.52* A^2+7.67* B^2+0.55 * C^2
% Cumulative drug release in 8 hr (R_6)	=	89.07-1.89 * A-0.69 * B-1.95* C-2.50* A * B-1.07 * A * C+0.47 * B * C-0.43 * A^2+2.97* B^2-2.51 * C^2
% Cumulative drug release in 12 hr (R_7)	=	97.23-0.75* A-1.34* B-0.11* C-0.25* A * B-0.25* A * C-0.12* B * C+0.77 * A^2+0.70* B^2-2.15* C^2

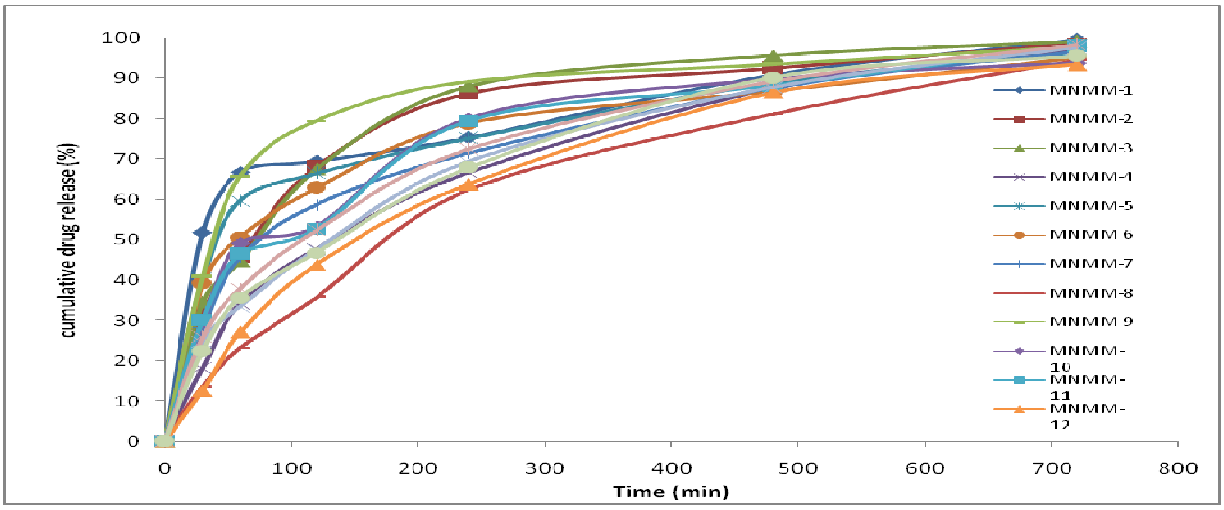

Fig 8: Cumulative % drug release v/s time plot of miconazole nitrate mucoadhesive microspheres optimization batches

Prediction of optimized miconazole nitrate mucoadhesive microspheres formulation

Statistical analysis of the data were done by design expert software keeping the constraints and criteria on the desired characteristics of the final formulation of optimization batches i.e. maximum entrapment efficiency and required sustained release drug release pattern. the software predicted formulations with desirability close to 1. The formulation with maximum desirability of 0.991 was selected as the predicted optimum formulation. The desirability contour and response surface plots predicting the formulation with maximum desirability. The cumulative % drug release of optimized batch.

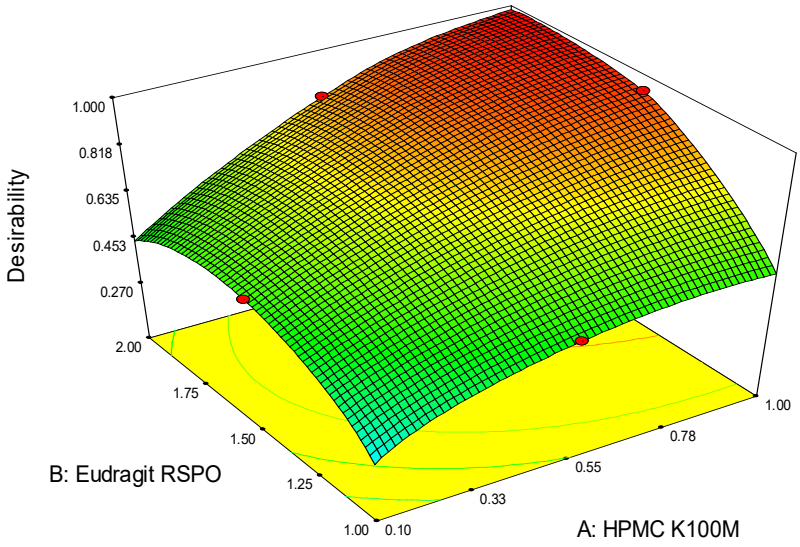

Fig 9: Three dimensional plot showing the microsphere formulation of maximum desirability

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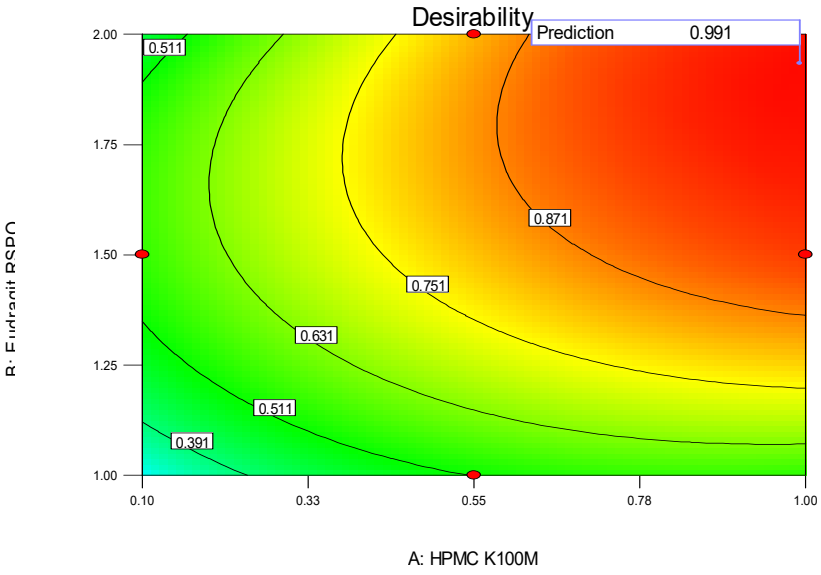

Fig 10: Contour plot showing the microsphere formulation of maximum desirability

Table 6: Predicted and optimized variables of miconazole nitrate mucoadhesive microsphere formulation

Independent Variable			Dependent Variables			
HPMC K100M (%)	Eudragit RSPO (%)	Ethyl cellulose 100 CP (%)	**Responses**	**Predicted**	**Observed**	**Relative Error (%)**
0.99	1.93	2.0	Entrapment efficiency (%)	93.44	89	4.8
			Cumulative % drug release at 0.5 hr	8.65	17.8	105.8
			Cumulative % drug release at 1hr	22.81	24.24	6.3
			Cumulative % drug release at 2hr	35.79	33.6	6.1
			Cumulative % drug release at 4hr	55.02	56.06	1.9
			Cumulative % drug release at 8hr	81.19	83.22	2.5
			Cumulative % drug release at 12hr	93.76	96.2	2.6

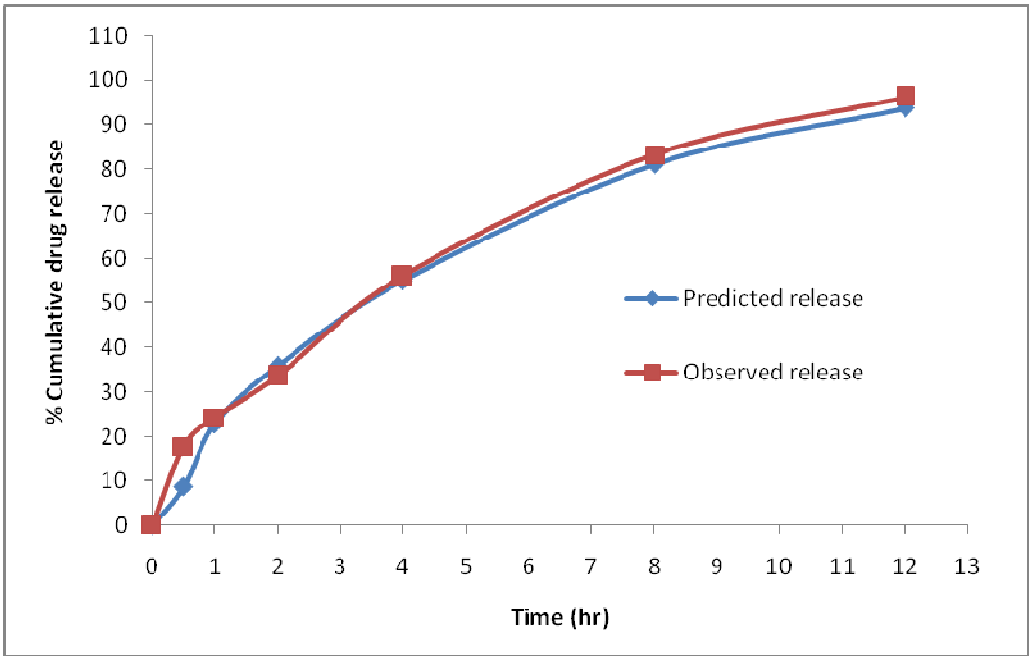

Fig 11: Release profile of predicted and observed formulation of miconazole nitrate mucoadhesive microspheres

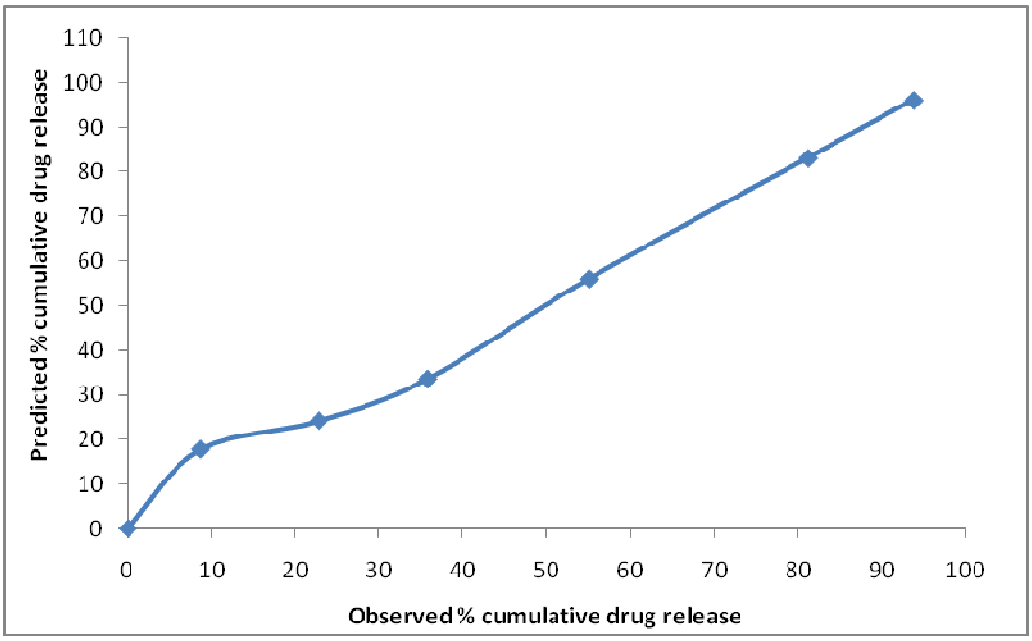

Fig 12: Linear plots between observed and predicted values of % cumulative drug release

In-vitro characterization

It was observed during the process of optimization of microspheres that the entrapment efficiency does not change much more in the spray drying process. The highest entrapment efficiency of optimization was found to be 89 %. The mean particle size of spray dried microspheres obtained by optical microscopy were in the range of 7.1-11.3µm. The scanning electron micrograph of miconazole nitrate mucoadhesive microspheres are shown in fig 13. Microspheres observed were of uniform size distribution with smooth surface. The photographs of scanning electron microscope reveal that microspheres are spherical, porous with smooth surface. The size of microspheres was found to be approximately 10 µm.

The differential scanning colorimetric patterns of the microspheres are shown in Fig .14The DSC thermograph shows the endothermic peak of miconazole nitrate at 180°C. The excipients such as HPMC K100M, Eudragit RSPO and Ethyl cellulose 100 CP. The absence of any specific at 180 °C peak in microsphere formulation confirmed that the conversion of physical form of miconazole nitrate from crystalline peak into amorphous form.

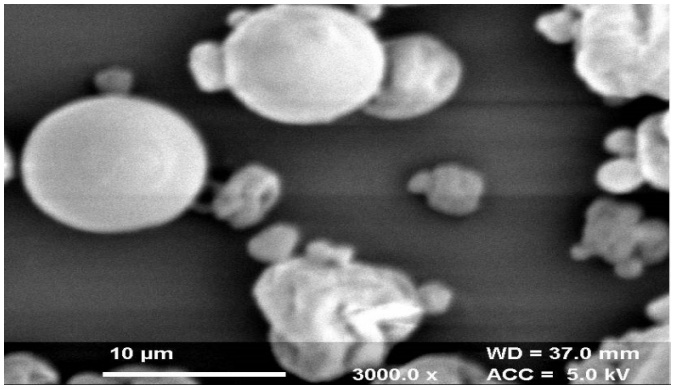

Fig 13: Photograph of scanning electron microscopy of optimized microsphere formulation

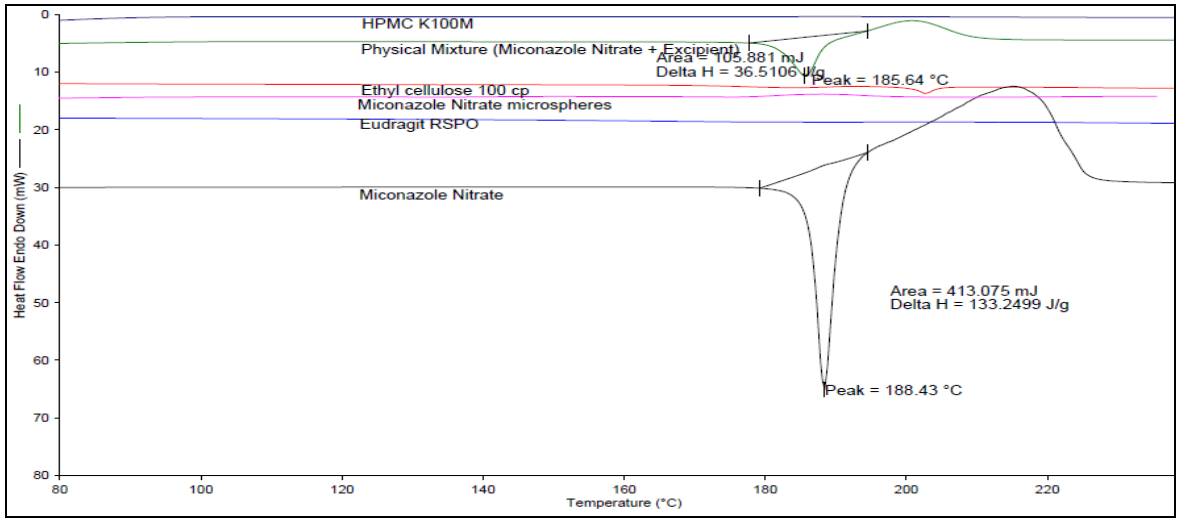

Fig 14: DSC overlay of HPMC K100M, Physical Mixture, Eudragit RSPO, Miconazole Nitrate microspheres and Miconazole Nitrate drug

Evaluation of mucoadhesion of microspheres

The in-vitro mucoadhesive properties of the optimized batch of microspheres were found to be 90% after 8 hrs of microsphere application. The percentage of mucoadhesion was notably increased with incorporation of HPMCK100M polymer in the microspheres, which indicated that HPMC K100M has a strong ability to interact with mucus. Higher retention effect was observed in the formulation having higher HPMC K100M.

Evaluation of vaginal tablet

The mucoadhesive microspheres of miconazole nitrate (76 %) were further compressed with lactose monohydrate (7%), avicel PH 102 (5%), starch 1500 (5%), primellose (5%), magnesium stearate (1%)and talc (1%). The tablets were prepared by direct compression method. All tablets were passed weight variation test and hardness of vaginal tablet formulation was found to be 8-9 kg/cm^2. The % friability was found to be 0.42%. The disintegration test was passed and disintegration time was found to be 3 minute 24 second. The *in-vitro* dissolution test was conducted successfully and % cumulative drug release vs. time plot showed consistent release of miconazole nitrate upto 12 hr.

Evaluation of tablets properties of powder blend

Angle of repose is maximum angle possible between the surface of a pile of microspheres and horizontal plan. Bulk density and tapped density was determined by using mechanical tapper apparatus (ETD-1020, Electrolab), India. The powder blend was evaluated for its micromeritic properties:

Parameters	Calculated Values
Bulk density (g/cm^3)	0.12
Tapped density (g/cm^3)	0.14
Compressibility index	11.2
Hausner's ratio	1.12
Angle of repose	29.2°

Hardness and friability

The hardness of vaginal tablet formulation was found to be 8-9 kg/cm^2 and friability test was passed by tablets and the percentage value of tablet was less than 1 %. The values are shown in table 7.

Table 7: Friability test of vaginal tablet formulation

S. No.	Parameter	Observed value
1.	Initial weight (g)	7.04
2.	Weight after conducting test (g)	7.01
3.	% Friability	0.42

Weight variation test

All 20 tablets were passed the weight variation test the average weight a tablet was 1.006g. The variation limit was 5% (0.956-1.056).

Drug content determination

The drug content was found to be 98.7 %.

Disintegration test

The disintegration time was found to be 24 second.

***In-vitro* release studies**

The *in-vitro* release study (dissolution test) was performed using Electrolab Dissolution Apparatus 1 (Paddle) in which dissolution beaker containing 900 ml of 0.45 % SLS solution as a dissolution medium. The temperature of the medium was maintained at 37±0.5°C and the rotation of paddle was fixed at 50 rpm. The tablets were placed in beaker assembly and start the test. 10-10 ml aliquots of dissolution fluid were withdrawn from each vessel at suitable time interval and replaced with same volume of fresh dissolution medium. Collected sample were filtered through syring filter and suitably diluted with dissolution medium and analysed in UV spectrophotometer (Shimadzu® UV 1700). The % cumulative drug release was calculated and recorded in table 8 and graphically presented in fig. 15.

Table 8: *In-vitro* release profile of vaginal tablet formulation

S. No.	Time Interval (hour)	Cumulative Drug Release (%)
1.	0	0
2.	0.5	19.5
3.	1	23.6
4.	2	31.7
5.	4	55.3
6.	8	86.1
7.	12	98.4

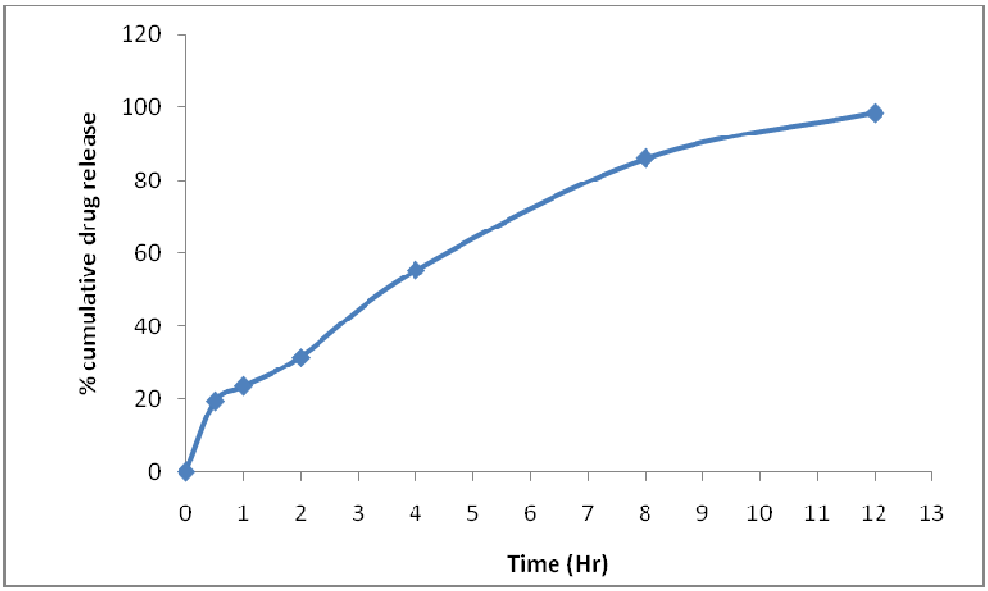

Fig 15: *In-vitro* drug release profile of vaginal tablet formulation

Conclusion

Vulvovaginal candidiasis is common type of pathologic condition in females caused by candida albican species. The conventional marketed vaginal formulations, vaginal suppositories, pessaries, gels, creams have drawback of leakage, messiness, and tendency to escape from body during normal activity of their routine life. The problem of shorter resident time in the vaginal lumen is always associated with vaginal formulations. In present research work the controlled release mucoadhesive microspheres of miconazole nitrate was prepared by spray drying technique and compressing it to tablet dosage form which should disintegrate into microspheres at the site and adhere to the vaginal lumen, hence releasing the drug for longer duration of time.

The optimization studies was performed and the results obtained from the experiments were statistically analyzed for response variables. The In-vitro drug release study of the optimized batch showed a consistent drug release of drug upto 12 h with mucoadhesion of 90 % upto 8 h. The encapusulation efficiency of optimized microsphere formulation was found to be 89 %. The result of SEM analysis showed that optimized microsphere formulation was spherical with smooth surface and the particle size was approximately 10 µm. The vaginal tablets were prepared and evaluated for the release profile of optimized microsphere formulation and vaginal tablet formulation. It was compared and concluded that there was no significant change in the release profile of compressed tablet. The prepared tablet formulation shows rapid disintegration into mucoadhesive microspheres in 24 sec and releases the drug consistently for a period of 12 hrs. Thus, we can say that the formulation has overcome the drawbacks of conventional vaginal formulations such as leakage, messiness, tendency to escape and discomfort and shows effective treatment of the disease condition.

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