Research Article

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THERMOSENSITIVE MUCOADHESIVE PLURONIC-BASED *IN-SITU* VAGINAL GEL OF SERTACONAZOLE

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Abstract

The purpose of this study is to develop thermosensitive mucoadhesive pluronics based *in-situ* gel of sertaconazole for treatment of vulvovaginal candidiasis, with the aim to prolong residence time in vagina, control drug release, and enhance efficacy as well as improvement in patient compliance. *In-situ gel* formulations of sertaconazole were formulated using different concentration of pluronic-127 alone and in combination with pluronic-68 as thermosensitive polymer and HPMC K4M also added as mucoadhesive polymer. The prepared formulations were evaluated for their gelation temperature, gelling capacity, rheological properties, mucoadhesive properties, microbiological studies and *in-vitro* release studies. It was found that the viscosity and mucoadhesive force increased as the concentration of pluronic-127 increased and *in vitro* drug release decreased. In conclusion, HPMC K4M was able to improve the sustain release property of pluronics indicating that the combination of HPMC K4M and pluronics may find use in development of vaginal *in-situ* gel drug delivery system with prolong local residence time and thus better clinical outcome.

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INTRODUCTION

Opportunistic fungal infections, predominantly those caused by *Candida albicans*, are an important factor of mortality and morbidity in infants, children, and patients with compromised immune system [1,2]. Azoles are currently the most used drugs to manage fungal infections. Sertaconazole is an effectual fungicidal as well as fungistatic agent and also has a broad-spectrum activity against opportunistic filamentous fungi, dermatophytes, in addition to Gram-positive bacteria [3,4]. When used for the treatment of dermatologic and gynecological infections, it presents a superior safety report, elevated cutaneous permanence in addition low systemic absorption [5]. Despite these valuable features, the extremely low aqueous solubility of sertaconazole (< 0.01 % w/v) strongly limits its practical use and search for an adequate delivery systemis still challenging issue [6].

There are two challenges in vaginal drug delivery. First the limited contact time caused by physiological conditions imposed by protective mechanism of vagina(resulting in frequent dosing

regimen), and patient compliance is the second challenge. The conventional vaginal dosage forms such as ointment and inserts cause discomfort to the patients. Although it is known that patients tolerate gels better than insert or ointments, direct application of gels in to the vagina might be difficult as well as inconvenient [7]. Recently *in-situ* gelling liquids have been investigated as a more convenient dosage form for topical applications. These *in-situ* forming hydrogels are stimulus sensitive hydrogel that exits as aqueous solution before administration and immediately changed into standing gel after administration upon contact with the mucosa.

The most commonly used thermo-reversible gels are pluronics [8]. The pluronics *in-situ* gelling dosage form is supposed to be easily applied and give good spreading and coating of the vagina making the treatment more effective and probably result in prolong effect [7, 9].

Sertaconazole was considered as drug of choice for treatment of vulvovaginal candidiasis. Prolong treatment was found to be only effective solution to prevent high recurrence rate; however, such approaches raise concern about potential toxicity.

The objective of this study was to design new vaginal thermosensitive *in-situ* vaginal gelling formulation with mucoadhesive properties.

MATERIALS AND METHODS

Sertaconazole was kindly supplied as gift sample by Cipla Ltd. (Mumbai, India), Pluronic-127 (PF-127) and Pluronic-68 (PF-68) were kindlysupplied as gift sample by BASF, Mumbai, India. Hydroxypropyl methylcellulose (HPMC K4M) was received from Zydus-Cadila Healthcare Ltd. (Ahmedabad, India). All other chemicals and reagents were of analytical reagent grade and were purchased from S.D. Fine Chemicals Ltd., Mumbai.

PREPARATION OF PLURONIC BASED GEL FORMULATIONS

Thermosensitive and mucoadhesive pluronic *in-situ* forming gels were prepared by the cold technique first described by Schmolka with slight modification[10, 11]. Briefly, HPMC K4M was slowly added to a phosphate buffer pH 4.0 under continuous stirring in an ice bath at 4°C. PF-127 and PF-68 were then added gradually under continuous stirring. Partially dissolved pluronics solutions were stored in refrigerator at 4°C overnight and stirred periodically until clear homogenous solutions were obtained. Sertaconazole (2%) was initially dissolved in the mixture of methanol and polyethylene glycol 400 (3:5), and added to the cold PF-127 /PF-68 solution containing various content of HPMC K4M with gentle mixing. It's prerequisite for an *in-situ* gel system that it allows easy instillation into the cavity as liquid drops which undergoes sol-to-gel transition, triggered by temperature of vaginal fluid.

3^2 FULL FACTORIAL DESIGN

A 3^2 full factorial design was adapted to optimize the variables. Here in 3^2 full factorial design 2 factors were evaluated, each one at 3 levels, along with experimental trials were performed at all 9 possible combinations. Mucoadhesive force, viscosity and Q_3 (Amount of drug released in 3h) were selected as dependent variables (response; Y). The preparation and evaluation method for *in-situ* vaginal gel and amount of sertaconazole were kept constant for all the trials. The full factorial design lay out, coded values for polymer ratio(X_1) and concentration of gelling agent (X_2), and composition of factorial batches A1 to A9 is shown in Table 1 and Table 2 respectively.

Table 01: Full factorial design layout

CODED VALUE	PLURONIC-68: PLURONIC-127	HPMC K4M
-1	0:1	0.5
0	0.5:1	1.0
1	0.75:1	1.5
Check point	0.4:1	1.3

Table 02: Composition of 3^2 full factorial design batches

INGREDIENTS (MG)	A1	A2	A3	A4	A5	A6	A7	A8	A9
Sertaconazole	2	2	2	2	2	2	2	2	2
Pluronic-68	0	0	0	9	9	9	13.5	13.5	13.5
Pluronic-127	18	18	18	18	18	18	18	18	18
HPMC K4M	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Phosphate buffer pH 4.0	q.s.								

PHYSICOCHEMICAL CHARACTERIZATION *IN-SITU* VAGINAL GELS OF SERTACONAZOLE

The clarity of the formulations after and before gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

Vaginal formulations should have pH range in between 3.5 to 4.0. The pH of gel (1gm) was determined using calibrated digital pH meter. Experiments were performed in triplicate.

Rheological studies of sample were carried out using Brookfield Digital viscometer (DV-E model). Viscosity was measured at different temperatures of 20, 25, 30 and 37°C. The viscosity of each sample was measured before and after gel formation. Each point is mean of three readings.

MEASUREMENT OF GELATION TEMPERATURE (T1) AND GEL MELTING TEMPERATURE (T2)

A 5 ml aliquot of gel was transferred to a test tube, immersed in a thermostat water bath. The temperature of water bath was increased with the increment of 1°C and left to equilibrate for 5 min at every new setting of temperature. After that the sample was examined for gelation, and it was said to have occurred when the meniscus would no longer moves upon tilting the test tube. After attaining the gelation temperature T1, further heating of gel causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 i.e. gel melting temperature [12, 13].

MUCOADHESIVE FORCE DETERMINATION

The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from vaginal mucosal tissue. The assemblies developed for in vitro measurement of mucoadhesive strength in a simulated vaginal environment are a modification of the previously reported bioadhesion test assembly [14]. A section of tissue specimen obtained from the mucosal side of sheep (procured from a slaughter house) vagina was fixed on each of two glass slides using thread. 50 mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed on flexible support in inverted position to the underside of the same pan. The gel formulation sandwiched between both the slides held in contact with each other, for 2 min to make sure intimate contact amid them. After that weight was kept increasing in second pan until slides get detached from each other. After the adhesive bond has formed, the force required to separate the bond was measured and calculated as mucoadhesive force.

IN-VITRO RELEASE OF SERTACONAZOLE FROM *IN-SITU* GELLING FORMULATIONS

The in vitro drug release was, performed by means of a Franz diffusion cell, diameter 20 mm, with water jacketed receptor chamber (15 ml) and a donor chamber thermo stated at 37°C. The receptor chamber containing phosphate buffer pH 4.0 solutions was constantly stirred by magnetic stirrer. The two chambers were separated by a semi-permeable cellophane membrane and each formulation was spread on a circular portion of the membrane. Aliquots (1mL) were withdrawn from the release medium at each sampling time point for up to 10 h. The aliquots were replaced with equal volume of freshly prepared release medium kept at the same temperature. The aliquots were diluted, and the amount of drug release was calculated by measuring the absorbance at 260 nm against blank (Shimadzu UV spectrophotometer, Japan). The results were mean of three runs. The release profile of sertaconazole was obtained by plotting cumulative % amount of drug released from each formulation against time.

KINETIC ANALYSIS OF DRUG RELEASE DATA

The drug release data also fitted to Higuchi's (cumulative percentage of drug released vs. square root of time) [15], and Korsmeyer equation (log cumulative percentage of drug release vs. time) to describe the drug release from polymeric systems.

$$M^t / M^\alpha = Kt^n$$

where M^t / M^α is fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterize the drug transport mechanism [16].

STATISTICAL ANALYSIS

The in vitro data were analyzed by statistical software and test for significant difference were performed by Student's t-test. The differences were regarded as significant at $P < 0.05$ level.

IN VITRO MUCOADHESION STUDY

The mucoadhesive potential of the sertaconazole vaginal gel was evaluated in comparison with the marketed clotrimazole gel (Candid-V® gel) by an in vitro method reported by Nakamura [17]. Candid-V® gel was used for the comparison, as there is no sertaconazole vaginal gel available in the market. Briefly, an agar plate (1%, w/w) was prepared in pH 4.0 phosphate buffer.

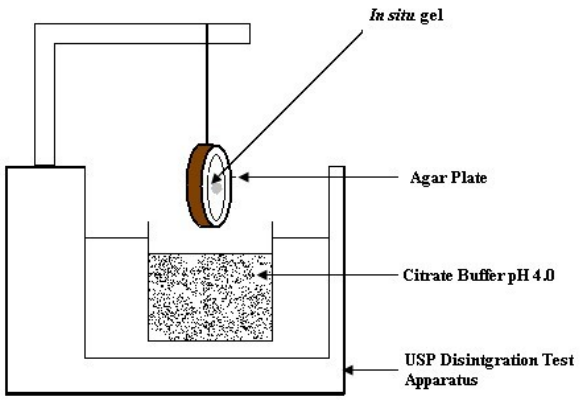

Figure 01: The apparatus used for *in vitro* mucoadhesion study

Test sample, 50 mg was placed at the center of plate. And after 5 min, the agar plate was attached to a USP disintegration test apparatus (Figure 01) and moved up and down in pH 4.0 phosphate buffer at 37±1 °C. The sample on the plate was immersed into the solution at the lowest point and was out of the solution at the maximum point. Finally, the residence time of the test samples on the plate was noted visually.

IN VIVO LOCAL RETENTION TIME MEASUREMENT

Female Wistar Kyoto rat (250±15 g, provided by Torrent Research Center, Ahmedabad) was acclimated for at least 1week before being used. Rats were allowed to free access to standard food and tap water. The dose of sertaconazole was 200µg sertaconazole /10µL (20mg sertaconazole / g gel) per rat, selected according to the dosage of commercially available sertaconazole vaginal formulations used in clinical study and practice. Three formulations were tested, including: (i) sertaconazole suspension (formulation as control group); (ii) sertaconazole-loaded 18% pluronic-127 formulation ; (iii) sertaconazole loaded 18% pluronic-127 -1.0% HPMC K4M formulation; For each formulation, rats were divided into three groups to provide amount of drug retained data for the following five time points: 0 h (immediately after administration), 3 h, 6 h, 9 h and 12 h. Intravaginal administration of the studied thermosensitive gel was performed with a micro liter syringe with a blunt needle. After insertion of the needle approximately 0.5cm into the vagina, 10µL of in-situgel or the control drug suspension pre-cooled to the liquid state was discharged rapidly and the animal was kept in an inverted position for about 10 sec. to assure the time for gel formation. To determine the local resident amount of sertaconazole, the vagina was thoroughly washed with distilled water and the washing solution was collected for analysis [18].

MICROBIOLOGICAL STUDIES

The microbiological studies were performed against Candida albicans in Sabouraud's agar medium by the cup plate method. The microbiological studies were carried out on the *in-situ* gelling formulation and 2% w/v of plain drug solution of sertaconazole. A layer of nutrient agar (20 mL) seeded with the test micro-organism (0.2 mL) was allowed to solidify in the petri-plate. Cups were made on the solidified agar layer with the help of sterile borer having 4 mm diameter. Then volume of the formulations (*in-situ* gelling formulation and plain drug solution) containing equivalent amount of drug was poured into the cups. After keeping petri-plates at room temperature for 4 h, the plates were incubated at 37°C for 24 h. The zone of inhibition was obtained. The diameter of zone of inhibition was measured [19].

RESULTS AND DISCUSSION

PHYSICOCHEMICAL CHARACTERIZATION OF *IN-SITU* GELLING FORMULATIONS

The *in-situ* gelling formulation was characterized for various physicochemical parameters, like clarity, viscosity and pH. The gel base was transparent liquid at 4°C whereas transparent semisolid gel was formed at body temperature.

Rheological behavior is key part in the formulation of pluronics formulations. Figure 2 shows the viscosity of pluronics solutions at different concentration. The same concentration of pluronics has different viscosity at different temperature (20, 25, 30 and 37 °C), where the viscosity increased with increasing temperature. This may be attributed to the fact that pluronics being nonionic PPO tri block copolymer, aggregate in to micelles at 37 °C. The dehydration of polymer blocks with temperature resulting into micellization. And also, it has been revealed that gel formation is a result of micelle enlargement and packing and that the gel is more entangled at higher PF-127 concentrations. As a result of these micelle entanglements, they cannot

distinguish simply from each other, which accounts for rigidity and high viscosity of gels containing high concentration of pluronics [20,21].Results of viscosity profiles of formulations at 37° C shows that the mucoadhesive polymer HPMC K4M had a viscosity enhancing effect.

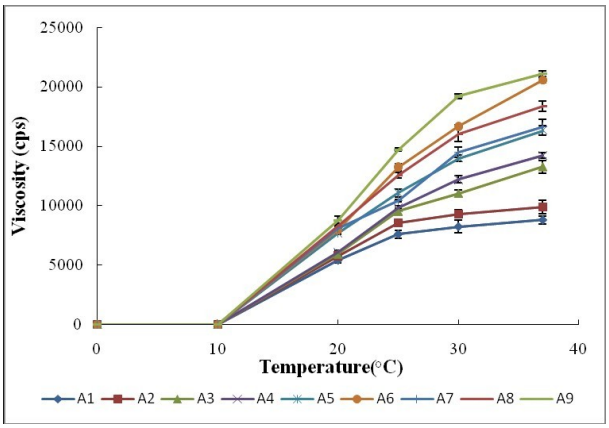

Figure 02: Rheological study of *in-situ* gelling formulation at various temperatures

GELATION TEMPERATURE (T1) AND GEL MELTING TEMPERATURE (T2)

Gelation temperature is temperature at which the liquid phase makes transition to gel. Aperfect*in-situ* gel must be a free-flowing liquid at room temperature so as to allow reproducible administration into site of application where it undergoes *in-situ* phase transition to form a strong gel [22]. The human vaginal temperature is 37.2°C [23], so gelation temperature of vaginal thermoreversible gels were considered to be suitable if they were in the range of 25-37 °C. If the gelation temperature is lesser than 25°C, a gel maybe formed at room temperature, escorting to difficulties in manufacturing, handling, as well as administering. But when the gelation temperature higher than 37°C, a liquid dosage form still exits at vaginal temperature, ensuing in drainage of the formula from the vagina at an early stage.

Table 03: Gelation temperature and gel melting temperature of *in-situ* gelling formulations

FORMULATION CODE	GELATION TEMPERATURE (T1°C)	GEL MELTING TEMPERATURE (T2°C)
A1	29	46
A2	25	50
A3	20	66
A4	30	43
A5	27	52
A6	21	62
A7	32	41
A8	28	55
A9	23	59

The results shown in Table 03 indicate that the mucoadhesive polymer, HPMC K4M has significant T1 lowering effect might be caused due to increased viscosity after dissolution of mucoadhesive polymer. The decreased gelation temperature was owing to the interaction between the hydrophobic portion of the polymer molecule, which could disrupt micellar structure and increase entanglement of micelle [24]. The gel melting temperature (T2) was also found to increase with increasing concentration of HPMC K4M.

MUCOADHESIVE FORCE DETERMINATION

The mucoadhesive force is an important and crucial physicochemical parameter for *in-situ* forming vaginal gels since it prevents the formulation from rapid drainage and hence prolong it residence time in vagina. Results of the mucoadhesive force determination of all the formulations are depicted in the Table 04.This table shows that the prepared sertaconazole *in-situ* gelling formulations with pluronics possessed satisfactory adhesive property. Increasing the mucoadhesive polymer concentration significantly increased the mucoadhesive force. Similar results were previously reported [25].

Table 04: Mucoadhesive force, pH and drug content of *in-situ* gelling formulations

Formulation Code	pH* (Mean ± SD), n=3	Drug Content* (Mean ± SD), n=3	Mucoadhesive force* (N) (Mean ± SD), n=3
A1	3.8±0.15	98.55±0.98	0.103± 0.024
A2	4.2±0.06	98.61±1.21	0.235±0.010
A3	4.4±0.10	99.70±1.03	0.267±0.029
A4	4.2±0.	97.69±2.	0.198±0.029

	25	18	
A5	4.4±0.15	98.22±1.41	0.257±0.023
A6	4.3±0.25	98.64±1.08	0.289±0.05
A7	4.4±0.15	96.29±1.04	0.212±0.013
A8	4.5±0.15	98.82±0.43	0.279±0.019
A9	4.3±0.10	97.46±1.49	0.298±0.016
Check point	4.3±0.06	98.34±1.03	0.261±0.013

*Data are expresses as Mean ± S.D. (n=3)

Addition of PF-68, which is a homologene of PF-127, enhanced the mucoadhesive force, since the pluronics with hydrophilic oxide group could bind to oligosaccharide chains. It was observed that the higher the concentration of PF-68, the greater the mucoadhesive force of pluronic gels. Furthermore, as level of HPMC K4M increases, mucoadhesive strength also increases. This was due to wetting and swelling of HPMC K4M, permits intimate contact with vaginal tissue and formation of weak chemical bond between entangled chains. Because of stronger mucoadhesive force, it can avoid the gelled solution coming out of vagina.

IN VITRO DRUG RELEASE

The cumulative amount of sertaconazole released *vs*. time profile for various *in-situ* gelling formulations presented in Figure 03.

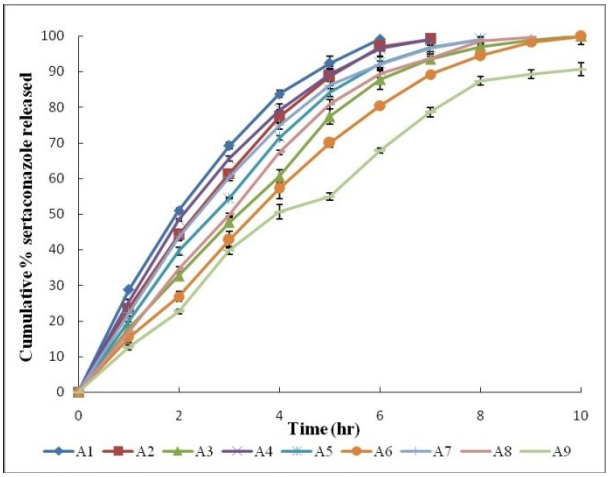

Figure 03: Release profile of sertaconazole from *in-situ* gelling formulations

The release of drug from these gels was characterized by initial phase of high release (burst effect) and as the gelation ensued, the left-over drug was released at a slower rate followed by second phase of moderate release. This biphasic pattern of release is characteristic feature of matrix diffusion kinetics. In addition, the initial burst effect was considerably reduced with increase in polymer concentration.

To study the effect of PF-68 concentration on drug release of sertaconazole from different PF-68 concentration was investigated. Drug concentration (2% w/v) and temperature 37 ± 0.2 °C were kept constant, while the concentration of PF-68 was varied (0, 9, and 13.5 %). The results show that as the concentration of PF-68 increased from 9 to 13.5%, the amount of drug released decreased. This indicates that the structure of gel functioned as an increasingly resistant barrier to drug release as the concentration of PF-68 increased. Such enhanced resistant may be due to reduction in number and dimension of water channels and to the increase in the number and size of micelles within the gel structure. Shorter the intermicellar distance leads to a larger number of cross-links sandwiched between neighboring micelles, leading to elevated viscosityas well as lower rate drug release [26]. This assumption may be potential by the rheology study. From rheological study, it was observed that there is an inversely proportional relationship between sertaconazole viscosity and release rate. The release profile of sertaconazole from all the formulations reveals that as the level of HPMC K4M is increasing, the drug release is decreasing due to higher viscosity of the formulation.

As shown in Figure 03, the addition of 9% PF-68 resulted in slight decrease in release rate as compared to 0% PF-68. In case of 18 % PF-127 the addition of 13.5 % PF-68 showed a higher reduction of release rate compared to 9% PF-68. This decrease in release rate may be attributed to the increase in viscosity.

RELEASE KINETICS

The value of 'n' in the range of 0.571 to 0.886, which indicates drug release, follows non-fickian diffusion release mechanism. The amount of sertaconazole released from all the formulation showed a linear relationship with the square root of time and log time, therefore a release rate of sertaconazole were expressed by Higuchi and Korsmeyer model.

STATISTICAL ANALYSIS OF FACTORIAL DESIGN BATCHES

The statistical analysis of the 3^2 full factorial design batches was performed by multiple linear regression

analysis carried out in Microsoft Excel 2007. The results are shown in Table 05.

Table 05: Summary of results of regression analysis

MODEL	COEFFICIENTS FOR MUCOADHESIVE STRENGTH						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	0.267	0.031	0.057	0.0195	-0.016	-0.0292	0.9711
RM	0.0238	0.031	0.057	-	-	-	0.8456
	Coefficients for viscosity at 37º C						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	16426.66	4013.33	2548.33	10	-2360	905	0.9916
RM	17030	4013.33	2548.33	-	-2360	-	0.9806
	Coefficients for Q_3						
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
FM	54.87	-4.68	-10.82	0.26	0.43	-0.93	0.9945
RM	54.55	-4.68	-10.82	-	-	-	0.9917

The data clearly indicate that the values of mucoadhesive strength, viscosity at 37° C and Q_3 are strongly dependent on the independent variables. The fitted equations (full and reduced) relating the responses mucoadhesive strength, viscosity at 37° C and Q_3 to the transformed factors are shown in Table 05.

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and mathematical sign it carries (i.e. positive or negative). Table 6 shows the result of the analysis of variance (ANOVA), which was performed to identify insignificant factors [27].

Table 06: Calculation for testing the model in portion (ANOVA)

FOR MUCOADHESIVE STRENGTH						
Regression	DF	SS	MS	F	R^2	
FM	5	0.0287	0.0057	20.14	0.9711	Fcal =4.11
RM	2	0.0250	0.0125	16.	0.8	Ftab
				43	456	=9.28
Error						DF= (3,3)
FM	3	0.0009	0.0003	-	-	
RM	6	0.0045	0.0008	-	-	
For Viscosity at 37ºC						
Regression	DF	SS	MS	F	R^2	
FM	5	148382733.00	29676546.00	70.77	0.9916	Fcal =1.95
RM	3	146744283.33	48914761.11	84.44	0.9806	Ftab =9.55
Error						DF= (2,3)
FM	3	1257866.66	419288.88	-	-	
RM	5	2896316.66	579263.33	-	-	
For Q_3						
Regression	DF	SS	MS	F	R^2	
FM	5	835.65	167.13	108.48	0.9945	Fcal =0.51
RM	2	833.29	416.65	357.53	0.9917	Ftab =9.28
Error						DF= (3,3)
FM	3	4.62	1.54	-	-	
RM	6	6.99	1.16	-	-	

The high value of correlation coefficient for mucoadhesive strength, viscosity at 37° C and Q_3 indicates good fit i.e., good agreement between dependent and independent variables. And also, the equations might be employed to get estimates of the responses as small error of variance was noticed in the replicates. In addition, the significant test for regression coefficients was performed by applying student *F* test. If the calculated *F* value is greater than the critical value of *F* a coefficient is considered to be significant. The significance levels of coefficients b_{12}, b_{11} and b_{22} for mucoadhesive strength were omitted from the full model to

generate the reduced model. And the critical value of *F* for α = 0.05 is equal to 9.28 (df= 3, 3). Since the calculated value for mucoadhesive strength (*F* = 4.11) is less than the critical value, so it can be concluded that the interaction term b_{12} and polynomial terms b_{11} and b_{22} do not contribute significantly to the prediction of mucoadhesive strength.

The critical value of *F* for α = 0.05 is equal to 9.55 (df = 2, 3). Since the calculated value for viscosity at 37° C (*F* = 1.95) is less than the critical value, so it can be concluded that the interaction term b_{12} and polynomial terms b_{22} do not contribute significantly to the prediction of viscosity at 37° C.

The critical value of *F* for α = 0.05 is equal to 9.28 (df = 3, 3). Since the calculated value for Q_3 (*F* = 0.51) is less than the critical value, it may be concluded that the interaction term b_{12} and polynomial terms b_{11} and b_{22} do not contribute significantly to the prediction of Q_3.

The data demonstrate that both X_1 and X_2 affect the drug release. It may also be concluded that the high level of X_1 (P 188: PF-127) and the medium level of X_2 (concentration of HPMC K4M) favor the preparation of *in-situ* vaginal gel and that the drug release pattern may be changed by appropriate selection of X_1 and X_2 levels. It was arbitrarily decided to select a batch of *in-situ* gel that showing 90% drug release profile up to 8 h and gives moderate mucoadhesive strength (A8). The final selection is done after considering some aspects such as drug release profile, *ex-vivo* retention time and viscosity.

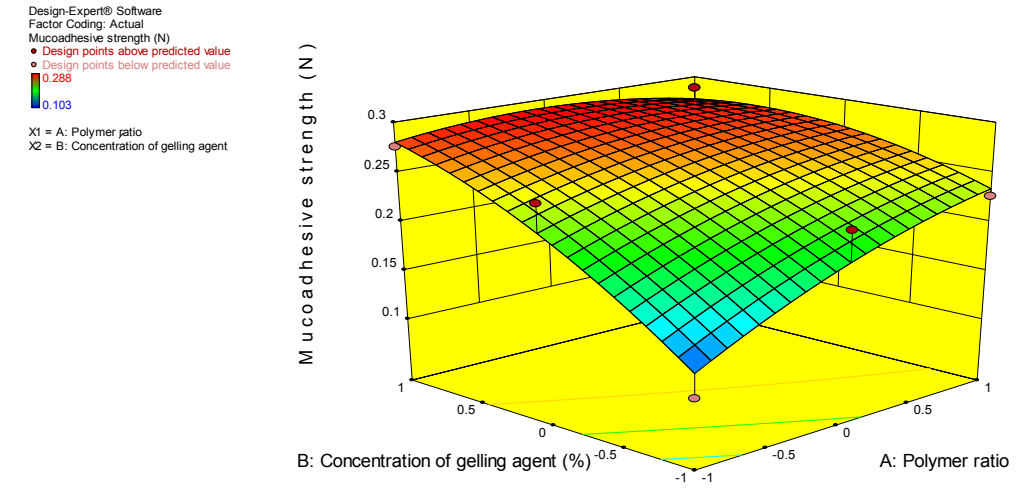

Figure 04: Response surface plot for mucoadhesive strength

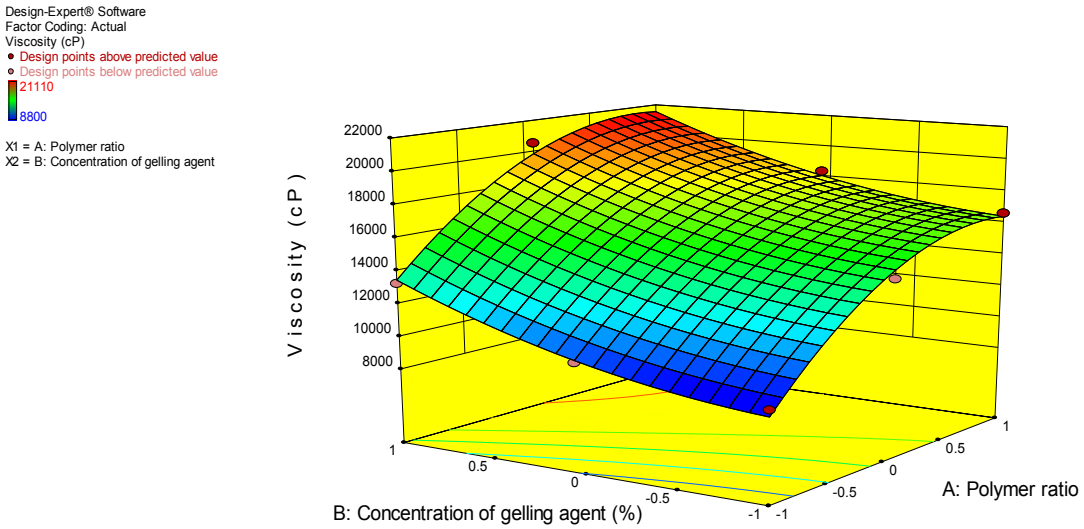

Figure 05: Response surface plot for voscosity at 37^{0} C

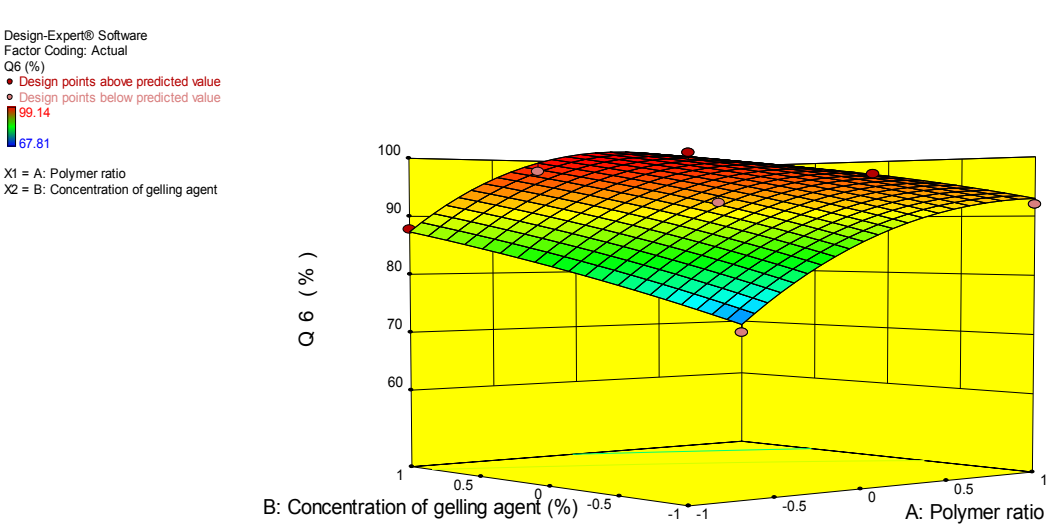

Figure 06: Response surface plot for Q_3

Figure 04 to Figure 06 shows the surface plot of X1 and X2 versus mucoadhesive strength, viscosity at 37°C and Q_3 respectively. The plots were drawn using design Expert Software Version 9.0. The plot demonstrates that both X1 and X2 affect the mucoadhesive strength, viscosity at 37°C and Q_3.

A check point batch was prepared at X1 = – 0.4 and X2 = 0.6. It was expected from the reduced model that mucoadhesive strength, viscosity at 37°C and Q_3 values of the check point batch should be 0.258N, 18295.33 and 34.53 % respectively. The mucoadhesive strength, viscosity at 37°C and Q_3 values of the check point batches were 0.261N, 18185.66 cP and 33.05% respectively which are as expected. As a result, we can conclude that the statistical model was mathematically valid.

IN VITRO BIOADHESION STUDY

The bioadhesive potential of sertaconazole *in-situ* vaginal gel and commercial formulation (Candid-V® gel) was evaluated by *in vitro* method. The residence time of the test samples on the plate showed by sertaconazole *in-situ* vaginal gel and Candid-V® gel were 60 ± 3.0min and 26 ± 1.5min, respectively (n = 3). The residence time shown by sertaconazole *in-situ* vaginal gel was significantly higher as compared to Candid- V® gel. This clearly indicates that the sertaconazole *in-situ* vaginal gel may have higher residence time in vagina as compared to Candid-V® gel.

IN VIVO LOCAL RESIDENCE TIME

The residence time of sertaconazole in the vagina of rat after intravaginal administration of different thermosensitive vaginal gel formulations loaded with 20mg sertaconazole/gm gel (10µg/rat) (Figure 07).

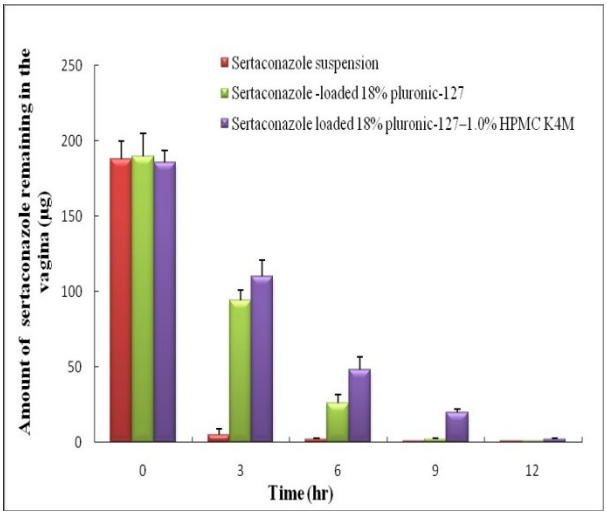

Figure 07: Residence of sertaconazole in the vagina of rat after intravaginal administration of different thermosensitive gel formulations

Aqueous suspension of the sertaconazole content is used as control. Each point is mean ± SD from three rats. As shown in Figure7, more than 85% of drug administered could be recovered in the washing fluid obtained immediately after administration (0 h), confirming that this method could constantly recover most of the drug in the vagina with good repeatability.

The aqueous suspension group (control group) did not result in significant residence of drug even after only 3 h. The drug residence for the 18% PF-127 group achieved obvious drug residence after 6 h, but fell to nearly zero after 9 h. In case of 18% PF-127 – 1.0 % HPMC K4M group, indicating that HPMC K4M improve the residence of drug up to 9 h.

The significant prolongation of drug residence caused by HPMC K4M might be attributed to both the slowdown in gel erosion and the bioadhesive property of HPMC K4M. 18% PF-127 and 1.0% HPMC K4M group achieved the longest local residence of drug (9 h), indicating significant synergistic mucoadhesive effect between PF-127 and HPMC K4M.

MICROBIOLOGICAL STUDIES

The optimized *in-situ* gelling formulation showed antimicrobial activity when tested microbiologically by cup plate technique. Clear zone of inhibition was obtained. The diameter of zone of inhibition is shown in Figure 08.

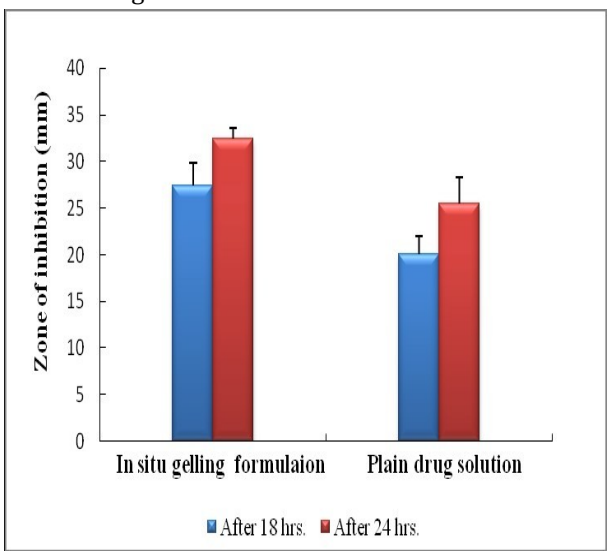

Figure 08: Zone of inhibition (mm) for *in-situ* gelling formulation of sertaconazole and plain drug solution

The results of 3^2 full factorial design revealed that the polymer ratio and concentration of gelling agent significantly affect the dependent variables mucoadhesive strength, viscosity at 37°C and Q_3. It is thus concluded that by adopting a systematic formulation approach, an most favorable point may be reached in the shortest time with minimum efforts.

CONCLUSION

Gel formulation of sertaconazole with mucoadhesive properties is promising for prolonging vaginal residence time of formulations and thereby better therapeutic effects. The ability of these systems, to adhere to the vaginal mucosa has great appeal for the treatment of localized infection. The *in-situ* gelling liquids are considered a more convenient formulation for topical application into vagina. Hence, the use of this formulation is expected to make the treatment more effective as it gives good spreading and coating properties of the vagina.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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