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DEVELOPMENT AND CHARACTERIZATION OF PLURONIC BASED THERMOSENSITIVE *IN SITU* GEL CONTAINING POSACONAZOLE FOR VAGINAL APPLICATION

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Abstract
The aim of the present research was to formulate and evaluate thermoreversible *in situ* gel of Posaconazole for the treatment of vaginal candidiasis. Increased residence at the site of infection for a prolonged period of time makes the i*n situ* gelling system more efficient with advantages of both gels and solution; like accuracy in dosing and ease of administration. The formulations of Posaconazole were prepared using Pluronic F-127 and Pluronic F-68 as thermo-sensitive agent and Carbopol 971P as mucoadhesive polymer. The formulations were evaluated for drug content, Gel strength, mucoadhesion, *in-vitro* diffusion and anti-fungal performance. The obtained results indicate that prepared formulations were found to release drug over the period of 8 Hrs. On basis of the results, it can be concluded that developed thermoreversible *in situ* vaginal gel of Posaconazole will be a better alternative to conventional dosage forms of Posaconazole improving patient compliance.

1. Introduction:

The currently available treatments for vaginal candidiasis are intravaginal and oral dosage forms. Intravaginal administration includes the application of cream and vaginal pessaries. Despite the fact that cream is one of the most favourable topical formulations, owing to its good Spreadability and ease of use, the cream is not appropriate for vaginal use due to its greasiness, resulting in discomfort for the patients[1]. Additionally, the use of vaginal pessaries leads to several complications, such as inconvenience and allergic issues in patients. In the case of oral administration also patient has to face several systemic adverse effects, like, cholestatic and hepatocellular damage.[2]

As an alternative for topical administration, the gel-based formulation is preferred compared to cream as it provides non-sticky properties and has a simple manufacturing process. Unfortunately, for application, the residence time of water-based topical formulation in vaginal cavity is limited. Many different vaginal formulations including gels, creams, pessaries, suppositories, rings, films, and tablets have been developed to deliver drugs for different applications such as contraception and cancer treatment. However, patient compliance, adverse effects, practicality, and biocompatibility are still the main concerns in the design of vaginal drug delivery systems.[3]

To overcome this problem, *in situ* gel can be developed using thermo-gelling components, which provide the sol-gel transformation at body temperature.

The mucoadhesive formulations emerge to be superior in their pharmacotherapeutic properties in comparison to the traditional dosage forms owing to the fact that these bioadhesive formulations ensure longer retention times and better spreadability. Similarly, intravaginal mucoadhesive dosage forms exhibit improved pharmacokinetic and pharmacodynamic outcomes on account of high perfusion, abundant surface area for absorption, and circumvention of hepatic first-pass effect, thereby ensuring a suitable safety and efficacy profile.[4]

Posaconazole[5], a second-generation triazole derivative (Fig. 1), possesses increased activity against resistance and emerging pathogens. Posaconazole is available in variety of forms, including injections, oral suspensions, and delayed release tablets.[5] Posaconazole has greater potency, and possesses increased activity against resistance and emerging pathogens. Posaconazole has also been investigated in phase III studies and approved by the regulatory agencies for the treatment and

prophylaxis of invasive fungal infections; therefore, posaconazole was selected as an active agent considering its broad-spectrum activity.[5]

When taken orally, these antifungal agents can cause, poorer stability, constipation, and stomach pain leading to patient incompliance. Hence, preparing Posaconazole loaded *in situ* gel for vaginal delivery to avoid side effects of drugs and to improve the bioavailability as well as patient compliance.[5]

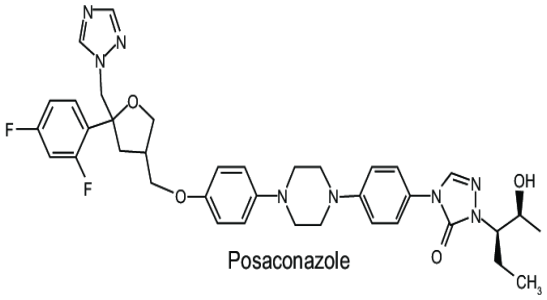


Figure 1: Structure of Posaconazole

Hence in the current work, attempts were made to prepare an *in situ* gelling system for the vaginal delivery of Posaconazole with the aim to deliver the drug with ease of administration and enhanced residence time at site of action for better antifungal effect and patient compliance.

2. Material and Method:

2.1 Materials:

Posaconazole was obtained as gift sample from Glenmark Pharmaceuticals, Goa. Pluronic F-127 and Pluronic F-68 were received from BASF Ltd, Mumbai. Carbopol 971P was obtained from Noveon Ltd, Mumbai. All other reagents and chemicals used were of AR grade.

2.2 Methods:

Drug-Excipient Compatibility Study by FTIR:

FTIR analysis was carried out to assess the physicochemical interaction of drug with excipients. The FTIR spectra of pure drug Posaconazole and physical mixture of Posaconazole, Pluronic F-127, Pluronic F-68 and Carbopol 971P were recorded using an FTIR Spectrophotometer (Agilent).[6] Physical mixture of, carbopol 971P, HPMC K4M, Sodium alginate, Pluronic F68, Pluronic F127 and Posaconazole were prepared individually and in combination. To study interactions between distinct components, both spectra were compared for possible changes.[7]

Formulation of *in situ* gel:[8,9]

Thermosensitive Vaginal *in situ* gel of Posaconazole was prepared by cold method using pluronic F-68(7%) and Pluronic F-127(23%) as thermosensitive polymers, and Carbopol 971P (0.2-1%), HPMC K4M, and Sodium al as mucoadhesive agent. Pluronic F 127 and Pluronic F 68 were slowly added to the distilled water previously cooled to 4˚C with continuous agitation. The dispersions were left at 4˚C until a clear solution was obtained. Posaconazole, Carbopol 971P and Benzalkomium chloride (Table 1) were added with stirring.[8,9]

Table 1: Formulation Table

Batch	Pluronic F-127 (%)	Pluronic F-68 (%)	Posaconazole (%)	Carbopol 971P (%)	HPMC K4M (%)	Sodium alginate (%)	Benzalkonium chloride (%)
H1	23	7	0.3	--	0.2	--	0.02
H2	23	7	0.3	--	0.4	--	0.02
H3	23	7	0.3	--	0.6	--	0.02
H4	23	7	0.3	--	0.8	--	0.02
H5	23	7	0.3	--	1.0	--	0.02
S1	23	7	0.3	--	--	0.2	0.02
S2	23	7	0.3	--	--	0.4	0.02
S3	23	7	0.3	--	--	0.6	0.02
S4	23	7	0.3	--	--	0.8	0.02
S5	23	7	0.3	--	--	1.0	0.02
C1	23	7	0.3	0.2	--	--	0.02
C2	23	7	0.3	0.4	--	--	0.02
C3	23	7	0.3	0.6	--	--	0.02
C4	23	7	0.3	0.8	--	--	0.02
C5	23	7	0.3	1.0	--	--	0.02

pH[9]:
pH is an important parameter in order to avoid the irritability of the formulation to the mucus membrane.[2] pH of all 15 formulations was noted using previously calibrated pH meter (Equiptronics EQ-810). The readings were taken in triplicates[9].

Viscosity[10]:
Viscosity of all formulated batches of *in situ* gel was measured by using Brookfield Viscometer. Viscosity of *in situ* gelling solutions was measured at different angular velocities at temp. 37°C. The tests were performed in triplicate.[10]

Drug Content[11]:
One ml of *in situ* gel was dissolved in phosphate buffer pH 5.8 with 1% w/v Tween 80. The solution was then filtered and suitably diluted, and the resulting solution was analyzed at 256 nm using UV-visible Spectrophotometer.[11]

Gelation Temperature: [11]
A 2 ml aliquot of the prepared solution was transferred to a test tube and put in a water bath. The temperature of the bath was slowly increased in the increment of 1 °C per minute till the occurrence of sol-gel transition. Sol-gel transition was confirmed when the meniscus would no longer move upon tilting through 90 °.[11]

Mucoadhesive strength:[12]
Mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from membrane. It was measured by modified balance. Intestinal mucosa of goat was obtained from slaughterhouse. Gel equivalent was placed on membrane surface. Empty beaker was attached to another side of the balance. Membrane surface with gel formulation and upper membrane surface were held in contact with each other for 2 min to ensure intimate contact. Water was added to the beaker until detachment takes place. Weight of water in beaker was noted. The readings were recorded in triplicate.[12]

Gel strength:[13]
6 ml of the gel was kept in water bath and complete gelation was allowed to take place. The Formed gel was transferred to a syringe. 50 gm constant weight was put on a plunger and it was allowed to penetrate the gel in syringe. The time required for the plunger to travel down the syringe was noted in seconds.[13]

***In vitro* drug release study: [14,15]**
In vitro drug release of *in situ* gel was executed using Franz diffusion cells. The activated cellophane membrane was placed between the compartments of diffusion cell. An accurately weighed amount of samples were placed on the donor compartment. The receptor medium contains phosphate buffer (pH 5.8) with 1% w/v Tween 80. The medium of receptor compartment were maintained at 37°C ±1°C with continuous stirring at 50 rpm. At fixed time interval aliquots of 2 ml were withdrawn and recovered with equal volume of fresh phosphate buffer (pH 5.8) with 1%w/v Tween 80. The withdrawn samples were evaluated by using UV spectrophotometer at a wavelength of 256 nm.[14,15]

Antifungal Activity:[16-18]
The diffusion method was employed to assess the antimicrobial activity of batch C2 against *Candida albicans*. After preparation and sterilization of Sabouraud dextrose agar medium at room temperature was inoculated with *Candida* and then the medium was poured into the Petri dish and allowed to cool for some time at room temperature until it solidifies and then three cups were bored in the Petri dish with the help of sterile bore of 6mm diameter and calculated concentration of the marketed Posaconazole formulation, *in situ* gel formulation batch C2, and negative control were placed in the bores and the petri plate was incubated. The zone of inhibition was observed and the radius of the zone of inhibition was calculated.[16-18]

Stability Study: [19]
Stability studies was carried out on optimized formulation for the interval of 1, 2 and 3 months. The physicochemical parameters of formulated Posaconazole loaded *in situ* gel were studied after stability interval.[19]

3. Result and Discussion:

Drug-Excipient Compatibility Study by FTIR:
From Figure 2, it was observed that there was no significant change in the peak values of drug when compared with the standard values. This indicated absence of any chemical reaction between drug and polymer.

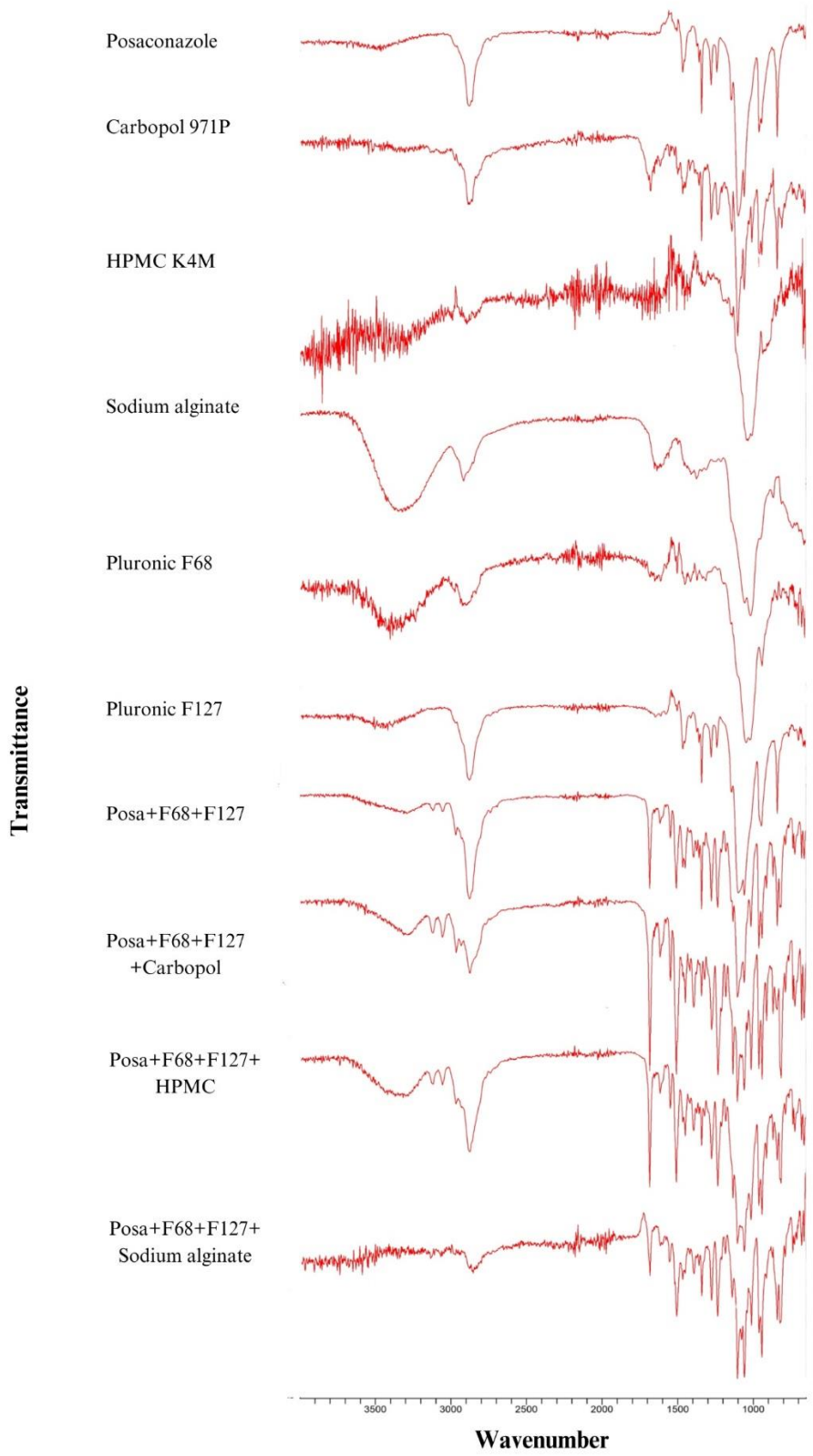


Figure 2: FTIR spectra for compatibility study

Determination of pH:
The pH of all batches was noted using previously calibrated pH meter (Equiptronics) the pH values were observed between 4.8 to 5.8 (Table 2)

Viscosity: Viscosity of formulations is closely linked to the product characteristics, such as spreadability, ease of application, drug release, and stability. The average formulation viscosity was found in the 792 to 1080 cps range. Table 2 shows the viscosities of all formulations.

Table 2: Evaluation for *in situ* vaginal gel

Batch	pH	Drug Content	Gelation temperature (°C)	Mucoadhesive strength (g)	Viscosity (Cps)
H1	4.81	100.40	36.4°C	27.617	899
H2	5.79	99.47	37.2°C	30.442	902
H3	5.82	98.13	37.4°C	34.951	928
H4	4.92	100.22	37.2°C	31.541	945
H5	4.89	92.12	36.4°C	32.066	1015
S1	5.13	97.33	36.6°C	26.549	792
S2	5.01	94.37	36.8°C	23.321	818
S3	4.98	93.41	36.6°C	30.502	887
S4	5.77	91.73	36.4°C	21.465	940
S5	5.09	90.42	37.2°C	25.174	998
C1	5.87	89.78	37.0°C	23.513	842
C2	5.04	98.56	37.2°C	32.233	914
C3	5.21	92.54	37.4°C	27.186	963
C4	5.14	91.23	36.8°C	26.288	1013
C5	4.94	93.24	36.4°C	32.012	1080

Gelation Temperature:
The temperature of vaginal cavity is 36.5 – 37.5 ˚C. At elevated temperature Poly-oxy-ethylene (POE) and Poly-oxy-propylene (POP) fragments of Poloxamer get cross-linked to yield a gel like structure. The proportion of Pluronic F68 and Pluronic F127 in the formulation batches was optimized to show the gelation temperature at about 37˚C. Gelation temperatures were noted on a water bath. It was found to be in a range 36.4-37.4ºC. The results are illustrated in Table 2 and Fig 3.

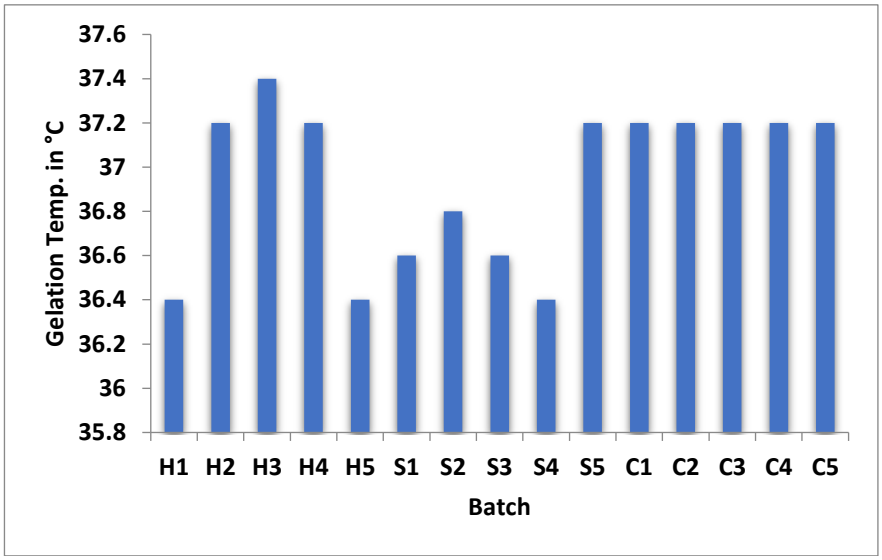


Figure 3: Gelation temperature

Drug content:
The Posaconazole content in the *in situ* gel was in a range 89.78% to 100.4%. From this, it can be concluded that Posaconazole was uniformly distributed in prepared *in situ* gel formulations. The results also exhibited that the process involved in the preparation of thermosensitive *in situ* gel did not affect the amount Posaconazole in the formulation. (Table 2)

Mucoadhesion Study:
Mucoadhesive property of gel formulations is an important parameter as it determines the residence time of formulations in vaginal cavity. A Mucoadhesive formulation interacts with the carbohydrate branches of the mucin by electrostatic interactions or hydrogen bonds.
It was observed that polymeric combination of Pluronic F127, Pluronic F68 and Carbopol 971P resulted in excellent mucoadhesion. It can be generally observed from the results that the strength of mucoadhesion is concentration dependent. But all the batches C1 to C5, H1 to H5 and S1 to S5 showed excellent mucoadhesion which would help the formulation to improve the residence time for the longer pharmacological action. (Table 2, figure 4)

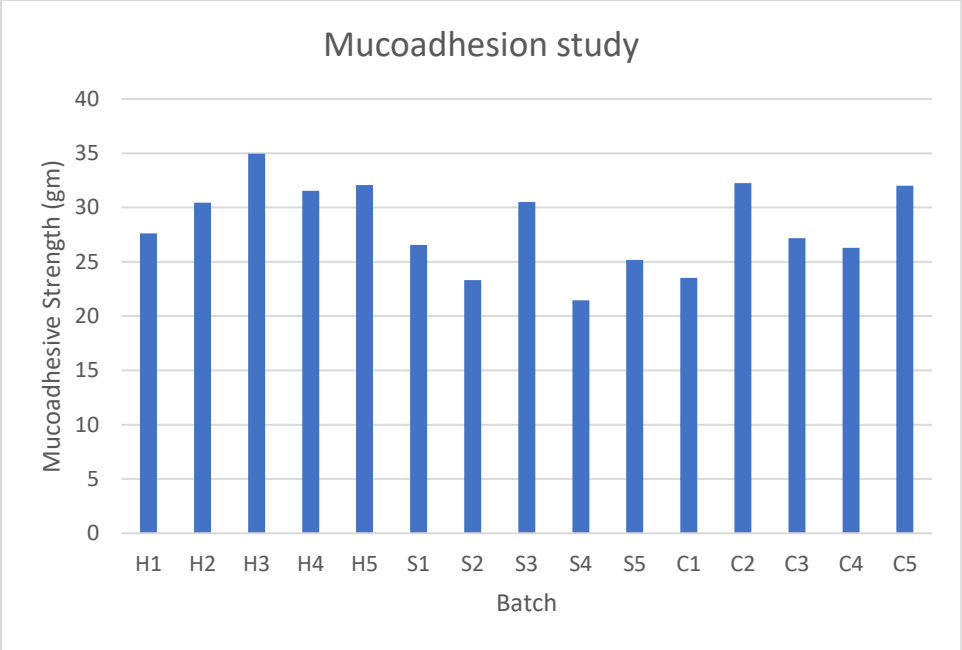


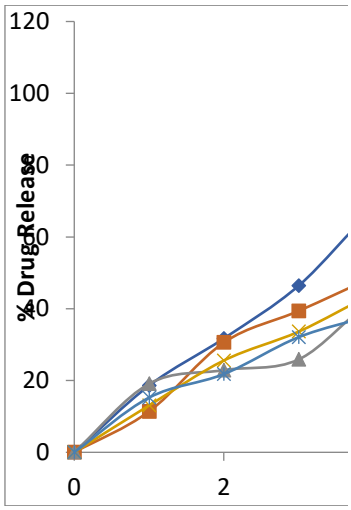
Figure 4: Mucoadhesion Study

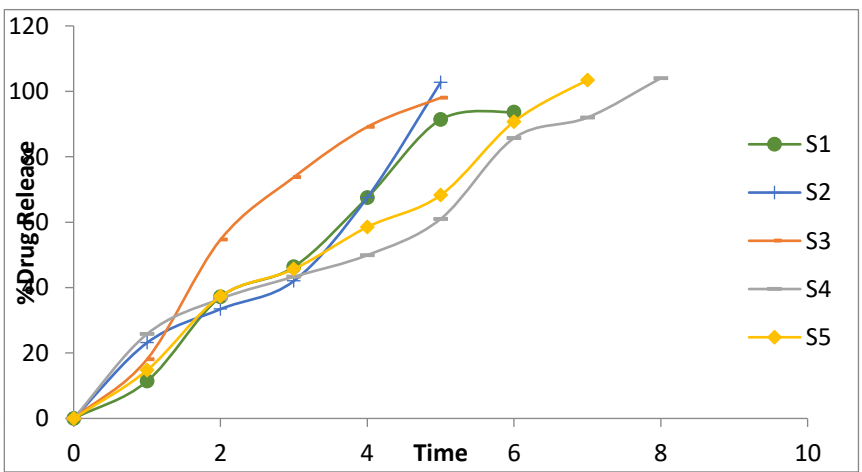
Gel strength Study:

Gel strength gives an indication about the tensile strength of the gelled mass owing to the ability of the Pluronic to undergo crosslinking after thermal exposure which has an additive effect of mucoadhesive polymers which are also known for their hydrogelling characteristics. All the batches exhibited gelation more than 20 minutes upon keeping the constant weight of 50 gm.

***In vitro* drug release study:**

To illustrate the kinetics of the drug release from the *in situ* gel, the obtained results from *in-vitro* release studies was fitted to various kinetic mathematical models such as First order, Higuchi and Krosmeyer Peppas model. The criterion for selecting the most appropriate model was based on a goodness of fit test. The dissolution profiles of all the formulation show a polymer concentration dependent behaviours for the drug release retardation. The data can be completely correlated with the results obtained in the gel strength determination. More the gel strength, greater will be the drug release retardation owing to the reduced outflux. The retarded drug release can also be attributed to the presence of swellable mucoadhesive polymer in the formulations. The drug release was retarded upto about 6 to 8 hours which show a greater residence time of the formulation in vaginal cavity. The dissolution profiles of the formulations are depicted in Fig.5





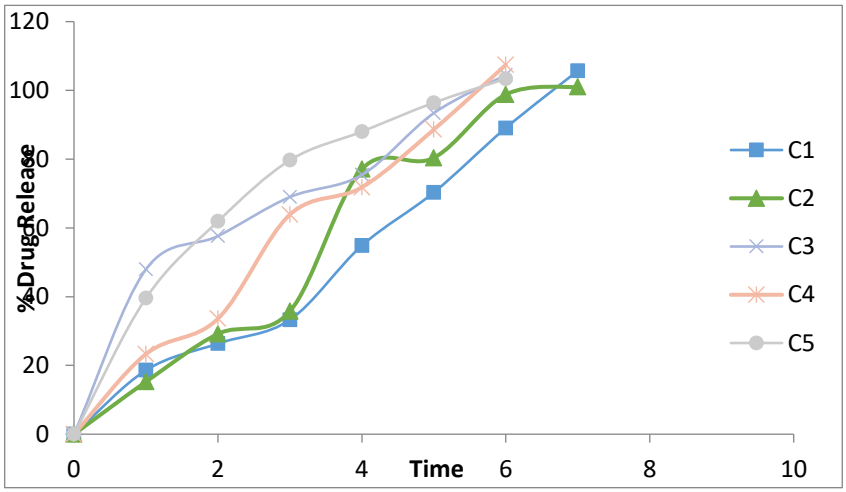


Figure 5: *In vitro* drug release study

Antifungal Study:

Antifungal assay was carried out using the optimized formulation and the activity was compared with the available marketed formulation. The agar cup diffusion method was used to find the antifungal efficacy of the prepared formulation. This was done by measuring the zone of inhibition of the Posaconazole *in situ* gel against *Candida albicans* by diffusion method The antifungal activity of batch C2 and marketed formulation against *Candida albicans* were compared. Zone of inhibitions against *Candida albicans* of Batch C2 and marketed formulation were found to be 2.958 mm and 2.176 mm respectively. Zone of inhibition of the Posaconazole *in situ* gel was greater as compared to that of the marketed posaconazole formulation. It can be concluded that the prepared Posaconazole *in situ* gel has a greater efficacy against *C. Albicans.*

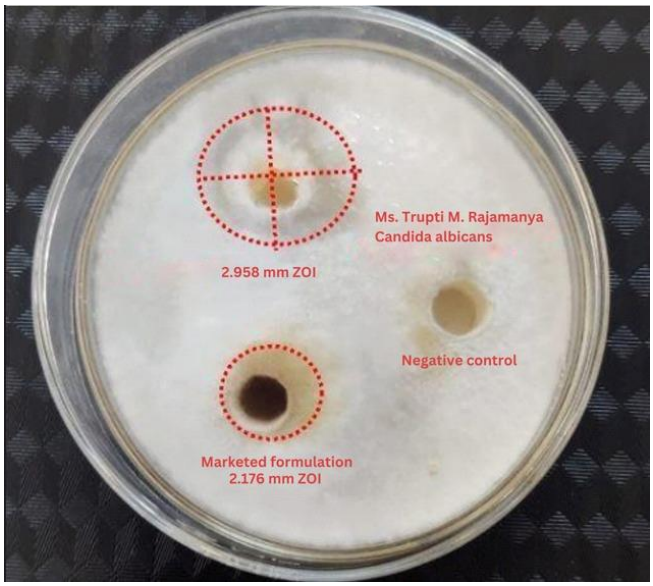


Figure 6: Antifungal Activity

Stability Study:

The physical stability of *in situ* gel formulation was found to be maintained throughout the testing period, as only negligible changes were observed. There was no significant difference in the appearance as well as pH of the formulation. Also, the drug content of the formulation was found almost similar, which suggested the absence of any drug degradation at these conditions. The formulation was opaque and white at refrigerated temperature and room temperature after 3 months. (Table 3)

Table 3: Evaluation of formulation after stability study

Interval	pH	Viscosity (Cps)	Gelation Temperature (ºC)	Drug content (%)	Mucoadhesive strength (gm)
After1 month	5.04	914	37.2	98.56	32.233
After2months	5.01	910	37.0	98.23	31.871
After3months	5.05	902	37.1	97.89	32.103

Conclusion:
The present investigation deals with the formulation of mucoadhesive Posaconazole vaginal *in-situ* gel using carbopol 971P, HPMC K4M and Sodium alginate as a mucoadhesive polymer. This *in-situ* vaginal gel was made with the intention to provide vaginal safety against *Candida albicans*. The Posaconazole vaginal *in-situ* gel was found to have excellent mucoadhesion, retention and prolonged release based on the results.

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