FORMULATION DEVELOPMENT AND EVALUATION OF IN SITU GEL FOR VAGINAL DRUG DELIVERY OF ANTI FUNGAL DRUG
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ABSTRACT
The aim of the present study was to formulate and evaluate nystatin containing thermosensitive mucoadhesive gel for vaginal drug delivery to achieve prolonging the residence time, sustained drug release, enhancing efficacy, decreasing recurrence, and increasing patient compliance in the treatment for vaginal candidiasis. Here nystatin (1%) as a model drug. A $3^2$ full factorial design was employed with two polymers: Pluronic F 68 and HPMC K4M as independent variables used in combination with Pluronic F 127. Vaginal gel was prepared by “cold method.” Vaginal gel using thermosensitive polymer, pluronic F127(15%w/v) and pluronic F68 along with mucoadhesive polymers such as HPMC K4M. The drug polymer compatibility was studied using FTIR and DSC. The prepared formulations were evaluated for parameters such as gelation temperature, viscosity, mucoadhesive strength, gel strength drug release and antifungal activity. Pluronic F 68 loading with Pluronic F 127 was found to have a significant effect on gelation temperature of the formulation and to be of importance for gel formation at temperatures 33–36 °C. HPMC K4M loading showed a positive effect on mucoadhesion force and gel strength and was also found helpful in sustain the release rate of the drug. The developed formulations had optimum viscosity, good bioadhesive strength and hence will have high retention property which is required for convenience at the site of application. Among the prepared formulations, one with the combination of pluronic F127(15%w/v), pluronic F68(15%w/v) and HPMC K4M(1%w/v) showed optimum gelation temperature, viscosity, bioadhesive strength, mucoadhesion force, antifungal activity with sustained drug release for 8 hrs. The optimized formulation (F5) showed insignificant change in physical property and drug content when stability testing was carried out at 40°C/75%RH for 1 months. The quadratic mathematical model developed is applicable to predicting formulations with desired gelation temperature and, mucoadhesion force. All the performed experiments confirm the applicability of thermosensitive and mucoadhesive In-situ gels as a novel delivery system for local therapy of vaginal candidiasis.

KEYWORDS: Nystatin, vaginal candidiasis, Pluronic F 68, Mucoadhesive, Pluronic F 127, Mucoadhesion force.

INTRODUCTION
In situ-forming systems are liquid aqueous solutions before administration, but gel under physiological conditions. There are several possible mechanisms that lead to in situ gel formation solvent exchange, UV irradiation, ionic crosslinkage, pH change, and temperature modulation. These approaches, which do not require organic solvents, copolymerization agents, or an
externally applied trigger for gelation, have gained increasing attention, such as a
thermosensitive approach for in situ gel formation.\textsuperscript{1} The development of in situ gel systems has
received considerable attention over the past few years. This interest has been sparked by the
advantage shown by these delivery systems such as ease of administration, reduced frequency of
administration improved patient compliance and comfort.\textsuperscript{2} In situ gel formulations are more
likely to be accepted by patients because of the ease of administration. Several in situ gel
formulations have been developed for the delivery of therapeutic agents [buprenorphine,
Acyclovir, Fluconazole, Insulin etc].

Nystatin (Nys) is a polyene antifungal antibiotic, one of the oldest antifungal drug,
produced by Streptomyces noursei strains commonly used for prophylaxis and treatment of
candidosis by interfering with the fungal cell membrane of the antibiotic-sensitive organism by
binding to sterols, chiefly ergosterol, and the formation of barrel-like membrane-spanning
channels playing an essential role in antifungal chemotherapy. Nys is an active substance for
treatment of susceptible cutaneous and mucocutaneous fungal infections caused by the Candida
species, which have been reported to cause infection, and many of these are classified as
emergent. But Nys also exhibits a broad spectrum of activity against fungi, including
Aspergillus, Coccidioides, Cryptococcus, and Histoplasma ssp. and it has been shown that the
antifungal activity of Nys is broader in comparison to amphotericin B other polyene antibiotic\textsuperscript{3}.

Vulvovaginal candidosis is estimated to be the second most common cause of vaginitis
after bacterial vaginosis and up to 75% of all women suffer at least one episode of this infection
during their lifetime, and 5–8% of adult women have recurrent vulvovaginal candidosis, defined
as four or more episodes every year, in fact it is diagnosed in up to 40% of women with vaginal
complaints in the primary care setting. Candida albicans accounts for 85–90% of cases.\textsuperscript{4}

Most patients with Candida vaginitis respond to topical treatment with nystatin or
imidazoles. However excessive use of azole antifungal drugs as fluconazole, has increased the
fungi resistances by multiple mechanisms, and non-C. albicans-related disease is less likely to
respond to azole therapy, and particularly Candida glabrata does not respond to azole treatment.
Moreover azoles have a fungistatic effect, whereas Nys has both an antifungal and fungistatic
activity. But its clinical use is currently limited almost exclusively to the topical treatment of
superficial Candida infections, since it is not effective when given orally and is severely toxic as
an intravenous application.\textsuperscript{3}

Over the years much more attention has been paid to azoles versus Nys, but Nys has been
found to possess broader spectrum of activity of the former drugs towards fungi in Candida
vaginitis. On the other hand, vaginal administration has been reported to be more suitable than oral and in contrast to azoles, there is hardly Nys formulations for vaginal administration in the market; currently in some countries do not exist. In this sense, traditional vaginal drug delivery systems include solutions, suspensions, gels, ointments, foams, tablets, tampons, suppositories, pessaries and vaginal inserts, among others. These vaginal formulations often require a frequent dosing regimen owing to the self-cleansing action of the vagina, namely the secretion of mucus and humid site of administration in addition. The chemist structure of Nys reveals formulation challenges by being both amphiphilic and amphoteric what contributes to its poor solubility in aqueous media.

The conventional dosage forms i.e preformed gel and solution have a number of lacunas, which has limited their use in vaginal drug delivery. Direct application of gels onto the infected sites of the vagina might be difficult, inconvenient as well as have frequent dosing because the conventional gels do not remain for long time at the site of application. A new and recent approach is to try to combine advantages of both gels and solution so that an accurate dose can be administered with ease of administration i.e in-situ gel system. These formulations remain to a solution state before administration but however transforms to gel after administration in to vaginal cavity. In the present study a thermosensitive bioadhesive vaginal gel was developed by incorporating the anti-fungal drug nystatin which is a most commonly employed drug in the treatment of vaginal infections.

Nowadays, in situ gel forming systems are of great importance, having the combined advantage being patient convenient with favorable residence time for enhancing vaginal bioavailability and for reducing systemic side effects. The sol-gel transition can be induced by a shift in the pH (Carbomer), temperature (poloxamer) or by the presence of deacetylated gellan gum cations (Gelrite).

Poloxamer is a triblock copolymer made of polyethylene oxide (PEO) and polypropylene oxide (PPO) units. Formation of highly ordered structures such as cubic crystalline phase and intramolecular hydrogen bonds might promote gelation. The mucomimetic property of poloxamers is proposed to be due to their hydrophobic and hydrophilic sequences simulating mucin action by adsorption of the aqueous layer of tears on the hydrophobic epithelium. This makes them suitable for use as a drug delivery system.

Poloxamer 407 gives a colorless and transparent gel but requires higher concentration of about 25 to 30 % (m/V) to exhibit sol-gel phase transition at 37 °C when used alone. Gelation temperature can be adjusted within the range of 33–36 °C by modifying cross-linking agents, by
mixing the different series of poloxamers, by changing the weight of poloxamers, or by changing
the pH and ionic strength. However, studies have been focused on modulating only gelation
temperatures of poloxamer solutions. There is lack of knowledge of the strength and bioadhesive
force of gelled poloxamers.6

In the present study, an attempt was made to solve this problem by combining two
poloxamers, i.e., Pluronic F 127 (PF 127) and Pluronic F 68 (PF 68), and developing a series of
combinations with gelation temperature ranging from 30 to 36 °C (12). They were found suitable
for formulating an in situ gelling vaginal drug delivery system of nystatin, polyene anti funagal
with enhanced activity against Candida species.6

PF 127 (15 %, m/V) was selected as the basis of formulation because below this
concentration it loses its sol-gel transition properties (6). Optimization of PF 68 and HPMC K4M
used in combination with PF 127 was done using a 3² full factorial design. The effect of
these polymers on gelation temperature, bioadhesion force, gel strength and in vitro release
pattern of the drug was also studied.7

MATERIALS AND METHOD

Materials

Nystatin was gift from Astra lifecare Pvt Ltd, Ahmadabad, India. Pluronic F127 and
pluronic F68 was purchased from Chemdyes corporation, Rajkot, India. Hydroxy Propyl Methyl
Cellulose K4M was supplied by Purvi enterprise, Ahmadabad, India. All other reagents were of
analytical grade and used without further purification.

Method

Preparation of the Simulated Vaginal Fluid8

Simulated vaginal fluid (SVF) was prepared from 3.51 g/l NaCl, 1.40 g/l KOH, and
0.222g/l Ca (OH) 2, 0.018 g/l bovine serum albumin, 2 g/l lactic acid, 1 g/l acetic acid, 0.16 g/l
glycerol, 0.4 g/l urea, 5 g/l glucose. The pH of the mixture was adjusted to 4.2 using 0.1M HCl.

Preparation of citrate phosphate buffer (0.1 M, pH 4.0)9

Take the 38.6 ml of 0.2 M Na₂HPO₄ and add the 61.4 ml of 0.1 M Citrate to produce 100
ml.

Preparation of the nystatin thermosensitive and mucoadhesive in situ gel8.

The gel formulation was prepared aseptically using the cold method. Nystatin (1%w/v)
was accurately weighed and dissolved in Cold citro phosphate buffer pH 4.5 and mixed with
mucoadhesive polymer solution, i.e. HPMC-K4M solution previously prepared in cold citro
phosphate buffer pH 4.5 and cooled to room temperature. To this solution, required amounts of PF 127/PF 68 were added. And preservative such as benzalkonium chloride (0.001 %, m/V), was added to the mixture. The volume was adjusted with Cold citro phosphate buffer pH 4.5. The solutions were adjusted to a pH in the range from 4 to 5. The solutions were mixed well and stored at 2–8 °C for 24 h.

**Table 1: Preparation of 3² factorial batches F1 to F10**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Nystatin (%w/v)</td>
<td>1</td>
</tr>
<tr>
<td>Pluronic F127 (%w/v)</td>
<td>15</td>
</tr>
<tr>
<td>Pluronic F68 (%w/v)</td>
<td>12</td>
</tr>
<tr>
<td>HPMC-K4M (%w/v)</td>
<td>0.5</td>
</tr>
<tr>
<td>Benzalkonium chloride (%w/v)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cold citro-phosphate buffer pH 4.5 (to make up 100 ml)</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

**Drug – Excipient Compatibility Study**

**Infra-Red Spectroscopy study**\(^{10}\):

An FT-IR spectrum of the Nystatin, polymers and optimised formulation was obtained on a Shimadzu Corporation, Japan. A Pellet of the drug prepared with KBr (Spectroscopic Grade) using hydraulic pellet press at a pressure of 7-10 tones. The scanning range was 400 to 4000 cm\(^{-1}\) and the resolution was 1 cm\(^{-1}\).
Different scanning Calorimetry (DSC) study\(^{10}\):

DSC analysis of Pure Drug (Nystatin), and the mixture of drug and polymer in (1:1) ratio was performed with Shimadzu DSC 60 thermal analyzer at the heating flow rates of 20 (ml/min) between 50 and 200 °C under static air using aluminum pans.

Characterization of Thermosensitive Mucoadhesive Gel

Gelation temperature (Tgel) measurement\(^{8}\)

Ten milliliters of cold sample solution was put into a beaker (25 ml) placed in a low temperature water bath at room temperature. A thermometer was immersed in the sample solution. The solution was heated at a constant rate of 200 rpm under continuous stirring using a magnetic bar. The temperature at which the magnetic bar stopped moving due to gelation was reported as the gelation temperature (Tgel). Each sample was measured in triplicate.

pH measurement\(^{11}\)

2.5gm of gel was accurately weighed and dispersed in 25ml of purified water. The pH of dispersions was measured using pH meter.

Rheological study\(^{12}\)

The rheological studies were carried out using Brookfield digital viscometer with LV spindle number 61 at speed 0.3 to 60 rpm.

Procedure for measurement of viscosity using Brookfield viscometer

- Leveled the rheometer by using three leveling screws; adjusted so that the bubble level on top of rheometer was centered within the circle.
- The power switch was turned on and pressed to auto zero. Entered spindle number that has to be used and also speed of rotation required.
- The introduced the spindle into the sample and attach the spindle to coupling and recorded the viscosity in centipoises.

The viscosity and shear stress of the sample solutions were measured at various shear rates at 25°C and 37°C, respectively. The temperature was maintained within ±0.1°C by a recirculating bath (Wisdom) connected to the sample cup of viscometer. The samples were equilibrated on the plate for 5 min to reach the running temperature prior to each measurement.

Drug Content\(^{12}\)

Drug content was determined by dissolving 10ml in situ gels in methanol. After suitable dilution absorbance was recorded by using UV/Vis double beam Spectrophotometer at 307 nm. Drug content was determined using slope of standard curve, previously plotted.
Gel Strength

In situ gel (50 g) was put in a 100 ml-mass cylinder and gelled in a thermostat at 36.5°C. The apparatus for measuring gel strength (weight: 20 g) was then placed onto the gelled poloxamer. The gel strength was determined by the time(s) it took to move the apparatus 5 cm down through the poloxamer gel.

In vitro mucoadhesion study

The mucoadhesion of each formulation was determined by measuring the force required to detach the formulation (maximum detachment force, MDF) from Cellophane membrane treated with simulated vaginal fluid. The assemblies developed for in vitro measurement of mucoadhesive strength in a simulated vaginal environment are modified physical balance shown in figure 5.2. Cellophane membrane fixed onto each glass vial using rubber band. The vial with cellophane membrane was connected to the balance in inverted position while first vial was placed on a height adjustable pan. The in situ system (250 mg) was added onto the cellophane membrane of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising (water added) in the pan until vials get detached. After the adhesive bond has formed, the force (amount of water) required to separate the bond was measured and calculated as mucoadhesive strength. From the mucoadhesive strength, the following parameters were calculated:

\[ \text{Mucoadhesion force (N)} = \frac{\text{mucoadhesive strength} \times 9.81}{1000} \]

In vitro drug release

The in vitro diffusion of the drug through a membrane was carried out in a system composed of a glass tube in which a cellophane membrane (HIMEDIA LA 393-1MT) was stretched and securely fastened with a rubber band; 10 ml of the 1% w/w formulation (100 mg) was placed in the tube (phase I or the donor phase). This was hung vertically in a beaker containing 100 ml freshly prepared release medium (SVF). (Phase II or the acceptor phase). The diffusion system was placed in a thermostatically shaking water bath at 37 ± 1°C and 75 rpm. At predetermined time intervals, 1 ml of the solutions were removed from the acceptor phase at each sampling time for up to 6 h and 24 h in case of comparison with the marketed preparation. The aliquots were replaced with an equal volume of the freshly prepared release medium kept at the same temperature. The amount of nystatin released was calculated by measuring the absorbance at 308 nm against a blank (UV spectrophotometer). The results were the mean of
three runs. The release profile of drug was obtained by plotting the % cumulative amount of drug released from each formulation against time.

**Kinetic modeling of drug release profile**

To analyze the mechanism for the drug release and release rate kinetics of the dosage foam, the data obtained from in-vitro drug release studies was fitted in to zero order (cumulative % drug releases vs. time), first order (Log cumulative of % drug remaining vs. time), Higuchi (Cumulative % drug releases vs. square root of time), and korsmeyer model (Log Cumulative % drug releases vs. log time) equation $$M_t / M_\infty = K t^n$$ Where $M_t$ is the amount of drug release at time $t$, $M_\infty$ is the amount of drug released after infinite time, $K$ is a kinetic constant incorporating structural and geometric characteristic and $n$ is the release exponent indicative of the drug release mechanism. If the values of ‘n’ are less than 0.45 then it is considered as fickian release mechanism, 0.45 to less than 0.85 it is considered as Non-fickian, 0.89 for case II and more than 0.85 it is understood as super case II release.

**In vitro antifungal activity**

Name of the analysis method: Agar diffusion method

Fungi analyzed: Candida albicans

**Methodology**

Media Used: Potato Dextrose Agar (PDA). 250 g of peeled potato were boiled for 20 min and squeezed and filtered. To this filtrate 20 g of dextrose was added and the volume was made up to 1000 ml by distilled water. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 μl 10^-4 CFU) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control plates with standard antibiotics were also prepared. All the plates were incubated at 27°C ±1 for 48 hrs and the diameter of inhibition zone were noted in cm.

**Accelerated stability study of optimized formulation batch**

Stability study of selected formulation was carried out at 40 ±2°C temperature and75±5% RH for one month as per modified ICH guidelines. The formulation was finally evaluated for pH, gelation temperature, and drug content and drug release.

**Formulation design**

A $3^2$ full factorial design was used in the study, in which two factors were evaluated and experimental trials were performed with all 9 possible combinations. The concentrations
of PF68 as $X_1$ (12, 15 and 18 %, $m/V$) and HPMC K4 as $X_2$ (0.5, 1 and 1.5 %, $m/V$) were selected as independent variables. Gelation temperature (GT in °C), mucoadhesion force (BF in N), were selected as dependent variables. The experimental design is outlined in Table III. DESIGN EXPERT 7.0.11 (STAT-EASE) demo version software was used for the formulation design. In this design, there are 2 independent variables and 3 levels (low, medium, and high) of each variable:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_{11} + b_{22}X_{22} + b_{12}X_1X_2$$

where $Y$ is the dependent variable, $b_0$ is the mean response of the 9 runs, and $b_i$ is the estimated coefficient for factor $X_i$. The main effects ($X_1$ and $X_2$) represent the average result of changing a factor at a time from its low to high value. The interaction term ($X_{12}$) shows how the response changes when the factors are simultaneously changed. Polynomial terms ($X_{11}$ and $X_{22}$) are included to investigate nonlinearity.

Statistical analysis

Statistical analysis of the factorial design batches was performed by multiple regression analysis using Microsoft Excel®. To evaluate the contribution of each factor with different levels to the response, the two-way analysis of variance (ANOVA) was performed. To graphically demonstrate the influence of each factor on the response, the response surface plots were generated with the help of design expert software.

RESULT AND DISCUSSION

Drug – Excipient Compatibility Study

Infra-Red Spectroscopy study

Fig1: FT-IR Spectra of Nystatin Pure drug

Fig2: FT-IR Spectra of Optimized formulation
FT-IR spectrum of Optimized formulation was given in Figure 2 that the peaks of different functional groups of nystatin in formulation were not much deviated from the peak of pure drug.

**Different scanning Calorimetry (DSC) study**

![DSC spectra of nystatin](image1)

![DSC spectra of Physical mixture](image2)

The DSC thermogram of Nystatin and physical mixture shown in figure(3,4). From the DSC analysis of the drug alone elicited a endothermic peak at 165.16°C, very close to the reported value of nystatin’s melting point, which is 160-170°C. The DSC thermogram of mixture shows the peak of drug & polymer. So, there was no evidence of interactions between nystatin and the used polymer.
Gelation temperature (Tgel) measurement, Rheological study, Drug Content, Gel Strength of batch F1 to F9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Gelation temperature* (°c)</th>
<th>Viscosity at 37°C (cps)</th>
<th>Gel strength (s)</th>
<th>Drug content* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>40±1</td>
<td>3510</td>
<td>22</td>
<td>98.4±0.3</td>
</tr>
<tr>
<td>F2</td>
<td>36±3</td>
<td>4790</td>
<td>30</td>
<td>96.9±0.4</td>
</tr>
<tr>
<td>F3</td>
<td>32±0</td>
<td>5320</td>
<td>38</td>
<td>95.0±0.5</td>
</tr>
<tr>
<td>F4</td>
<td>38±2</td>
<td>3650</td>
<td>24</td>
<td>98.8±0.3</td>
</tr>
<tr>
<td>F5</td>
<td>36±3</td>
<td>5050</td>
<td>33</td>
<td>95.6±0.3</td>
</tr>
<tr>
<td>F6</td>
<td>33±0</td>
<td>5610</td>
<td>39</td>
<td>96.4±0.1</td>
</tr>
<tr>
<td>F7</td>
<td>32±1</td>
<td>4150</td>
<td>25</td>
<td>97.2±0.3</td>
</tr>
<tr>
<td>F8</td>
<td>30±2</td>
<td>5630</td>
<td>34</td>
<td>96.1±0.1</td>
</tr>
<tr>
<td>F9</td>
<td>29±0</td>
<td>5850</td>
<td>41</td>
<td>97.5±0.1</td>
</tr>
</tbody>
</table>

*(mean±SD,n=3)

Formulation batches were prepared by cold method, using the pluronic F127 & pluronic F68 as thermosensitive polymer and HPMC-K4M as mucoadhesive polymer. Results formulation batches F1 to F9 was depicted in table 2. Which show that as increase the concentration of HPMC-K4M, gelation temperature was decreased because of their ability to bind with poly ethylene oxide (PEO) chain present in pluronic molecule & promoting dehydration. Viscosity and gel strength were also increase with HPMC-K4M. The drug content of batch F1 to F9 was in range.
In vitro mucoadhesion study

<table>
<thead>
<tr>
<th>Table 3: Mucoadhesion force of formulation batches F1 to F9</th>
</tr>
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<tbody>
<tr>
<td>Batch code</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>F1</td>
</tr>
<tr>
<td>F2</td>
</tr>
<tr>
<td>F3</td>
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<tr>
<td>F4</td>
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<td>F7</td>
</tr>
<tr>
<td>F8</td>
</tr>
<tr>
<td>F9</td>
</tr>
</tbody>
</table>

The mucoadhesive potential of formulation was evaluated by in vitro method. The results of the study are shown in table 3.

The increased mucoadhesion of pluronic based Nystatin in situ gel can be attributed to presence of HPMC-K4M.

One could conclude that the elasticity of the in situ gel composed of pluronic F127/pluronic F68/HPMC-K4M (15/15/1.5% w/v) was higher than the one composed of pluronic F127/pluronic F68/HPMC-K4M (15/15/0.5% w/v), because the total amount of HPMC-K4M is higher in F6.

Also the elasticity of the in situ gel composed of pluronic F127/pluronic F68/HPMC-K4M (15/15/0.5% w/v) was higher than the one composed of pluronic F127/pluronic F68/HPMC-K4M (15/14/0.5% w/v), because the total amount of pluronic F127 is higher in F4.

The decrease in the elasticity of the in situ gels was compensated by a gain in the viscosity modulus. It is expected that the gain in the viscosity modulus will result in an increase in the spreading of the in situ gel over the vaginal mucosa after its in vivo administration.

The higher elasticity of in situ gels will increase the possibilities of micelle interpenetration with mucus. It is well known that polymers possessing hydrophilic functional
groups such as hydroxyls are susceptible to interact more favorably with mucus gel layer.

**In vitro drug release**

![Graph showing in vitro drug release of batches F1 to F9](image)

In figure 5, the in vitro drug release of batches F1 to F9 is depicted. The graph shows that the pluronic F68 and HPMC-K4M retard the release of nystatin. The release rate of nystatin tended to decrease as the concentration of HPMC-K4M increased.

**Statistical Analysis**

Preliminary investigations of the process parameters revealed that factors X1 (%, m/V, of PF 68) and X2 (%, m/V of HPMC K4M) highly influenced the rate of in vitro drug release and hence, they were used for further systematic studies. In the present investigation, combinations of two polymers were studied using a $3^2$ full factorial design. Mathematical models developed for all the dependent variables using statistical analysis software are shown in Equation.
Regression Analysis for $Y_1$ Gelling temperature ($^\circ$C)

Table 4: Regression analysis for effect of X1 and X2 on GT.

<table>
<thead>
<tr>
<th>Regression Output</th>
<th>Full model equation</th>
<th>$Y = 35.66 - 2.83X_1 - 2.66X_2 + 1.25 X_1X_2 - 2.5 X_1^2 - 2.4 X_2^2$</th>
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</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.9962</td>
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</tr>
<tr>
<td>Coefficient(B0)</td>
<td>35.66</td>
<td></td>
</tr>
<tr>
<td>X1 coefficient</td>
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</tr>
<tr>
<td></td>
<td>X1</td>
<td>X2</td>
</tr>
<tr>
<td>P Value</td>
<td>0.0023</td>
<td>0.0028</td>
</tr>
<tr>
<td>Reduced model equation</td>
<td>$Y = 35.66 - 2.83X_1 - 2.66X_2 + 1.25 X_1X_2 - 2.5 X_1^2$</td>
<td></td>
</tr>
</tbody>
</table>

From table 4, Variable X1 has p value 0.0023 (p<0.05), and variable X2 has p value 0.0028. Variables which have p value less than 0.05, significantly affect on gelling temperature.

For further evaluation, contour plot for gelling temperature was plotted using the Design-Expert 9 Trial version.

![Fig 6: Contour plot for gelling temperature ($Y_1$)](image-url)
Regression Analysis for $Y_2$ (Mucoadhesion force (N))

<table>
<thead>
<tr>
<th>Tabl 5: Regression analysis for effect of X1 and X2 on Mucoadhesion force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression Output</td>
</tr>
<tr>
<td>Full model equation</td>
</tr>
<tr>
<td>$Y = 0.34 + 0.01X_1 + 0.03X_2 - 0.05 X_1X_2 - 0.03 X_1^2 - 0.01 X_2^2$</td>
</tr>
<tr>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>0.9856</td>
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<tr>
<td>Coefficient(B0)</td>
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<td>0.3415</td>
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<td>X1 coefficient</td>
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<td>P Value</td>
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<tr>
<td>0.03</td>
</tr>
<tr>
<td>Reduced model equation</td>
</tr>
<tr>
<td>$Y = 0.34 + 0.01X_1 + 0.03X_2 - 0.01 X_2^2$</td>
</tr>
</tbody>
</table>

From table 5, Variable X1 has p value 0.03 (p<0.05), and variable X2 has p value 0.0008. Variables which have p value less than 0.05, significantly affect on Mucoadhesion force.

For further evaluation, contour plot for mucoadhesion was plotted using the Design-Expert 9 Trial version.
Fig 8: Contour plot for mucoadhesion force ($Y_2$)

Fig 9: 3D Response surface plot for mucoadhesion force($Y_2$)
Validation of design model

![Design Expert Software](image)

**Fig 10: Overlay plot of response variables**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Predicted response</th>
<th>Actual response</th>
<th>%Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>F10</td>
<td>Gelling temperature ((^0C))</td>
<td>36.84(^0C)</td>
<td>36(^0C)</td>
</tr>
<tr>
<td></td>
<td>Mucoadhesion force (N)</td>
<td>0.3436</td>
<td>0.3337</td>
</tr>
</tbody>
</table>

The result show that the predicted values of check point batch F10 were good agreement with the actual values.

Factorial data suggest that moderate concentration of pluronic F68 and HPMC K4M 15 %w/v and 1%w/v respectively has good effect on gelling temperature and mucoadhesion force. So F5 batch is optimised batch for further study. Here check point batch was near to F5 batch so validate.

Analysis of drug release kinetic of optimized batch

<table>
<thead>
<tr>
<th>Table 7: Release kinetic data of batch F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Regression Values ((R^2))</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
</tbody>
</table>
The release kinetic data indicates that the release of drug from gels follows zero order drug release because the correlation coefficient values are higher in case of zero order equation, which describe that the release rate is independent of the concentration of the drug. The diffusion exponent n is the indicative of mechanism of drug release from the formulation. The n value of 0.45 is indicative of Fickian diffusion controlled drug release, n value between 0.45-0.85 signifies anomalous non-Fickian transport, n value of 0.82 indicates non-Fickian transport.

**In vitro antifungal activity**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zone inhibition diameter (cm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nystatin thermosensitive mucoadhesive in situ gel (1%)</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>Nystatin standard solution (1%)</td>
<td>2.3±0.1</td>
</tr>
</tbody>
</table>

*(mean±SD, n=3)*

Fig 11: Growth of candida albicans

Fig 12: Antifungal activity of Nystatin *in situ* gel
The values of zone of inhibition produced by Nystatin thermosensitive mucoadhesive in situ gel and nystatin standard solution, were 4.2±0.3, 2.3±0.1cm respectively (n = 3), which is shown in table 8. It is evident that nystatin in situ gel showed higher antifungal activity as compared to the nystatin standard solution. The enhanced in vitro antifungal activity of the gel may be attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity.

**Accelerated stability study of optimized formulation batch**

Stability study was performed only on optimized batch at 40±2°C temperature and 75±5% RH conditions. The results obtained after one month time period are shown in table 9.

<table>
<thead>
<tr>
<th>Time period for sampling</th>
<th>pH</th>
<th>Gelation temperature* (°C)</th>
<th>Drug content* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>4.5</td>
<td>34±3</td>
<td>98.23±0.3</td>
</tr>
<tr>
<td>After 1 month</td>
<td>4.9</td>
<td>33±3</td>
<td>96.9±0.1</td>
</tr>
</tbody>
</table>

*(mean±SD, n=3)*
As shown in table 10, there is no significant change in the pH, Gelation temperature and drug content of the formulation after one month.

The stability study conducted for cumulative percentage drug release of batch F5 after one month and observes the value of similarity factor $f_2$ was 83.735, which is presented between the standard values of $f_2$ 50-100. So, the optimized batch F5 show good stability for one month at 40 ±2°C temperature and 75±5% RH.

**CONCLUSIONS**

When poloxamers(PF 127 and PF 68) were used in combination for developing thermosensitive and mucoadhesive in situ gel, low to moderate amounts of HPMC K4M and PF 68 were to be used to achieve the desired gelation temperature, gel strength, mucoadhesion, drug release profile and viscosity required for a sustained vaginal drug delivery system of nystatin. It was concluded that the amounts of HPMC K4M had a significant effect on bioadhesion force and gel strength of the formulated thermosensitive and mucoadhesive in situ gel. The quadratic mathematical model developed is applicable to predicting V in situ gel vaginal formulations with desired characteristics.

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REFERENCES


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