FORMULATION AND DEVELOPMENT OF LONG-ACTING THERMOSENSITIVE AND MUCOADHESIVE VAGINAL GEL OF METRONIDAZOLE

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ABSTRACT

The development of an *in situ* metronidazole gel that is mucoadhesive and thermo sensitive was the aim of this study in the fight against bacterial vaginosis. Numerous evaluation criteria have been carried out, including mucoadhesive force, pH, viscosity, syringe capability, medication content, gelation temperature, and gelation time. All of the solutions were found to have pH values between 6-7. The viscosity was correlated with the mucoadhesive force. The drug release parameter indicates that *in situ* gels containing Carbopol 934 provide superior drug release compared to other polymers. Additionally, Carbopol 934 has 118 g more mucoadhesive strength than HEC and HPMC. Compared to non-ionic polymers like HPMC and HEC, Carbopol 934 effectively slows down drug release since it is a cationic polymer. After a 30-day stability test, the gel's properties barely changed. It follows that the developed formulation of metronidazole *in situ* gels is more reliable and efficient than earlier vaginal gels.

Keywords: Poloxamer 407, Carbopol 934, *in situ* vaginal gel, Metronidazole, TPA

INTRODUCTION

The most frequent reason for irregular vaginal secretions in women of reproductive age is bacterial vaginosis[1]. A lack of vaginal acidity, an increase in vaginal anaerobes, and a decrease in the normal Lactobacillus population characterize this unnamed disorder[2]. This acknowledged both the absence of conventional signs of inflammation as well as the presence of many facultative or anaerobic bacteria. Most often, the short residence time of the antibacterial drug in the vaginal cavity may be to blame for the inadequate clearance of these anaerobic infections[3]. Another cause might be the decomposition of antimicrobial substances in the vaginal fluid. Delivering the medicine to a local site of action is one strategy to boost its efficacy in eradicating the infection[4,5]. For improved stability and longer residence time, more antibacterial agents will permeate the vaginal mucous layer, allowing them to act for a longer period of time[1-5].

As a result, several researchers developed and published various innovative alternative formulations, such as gels, pills, and microspheres that might stay in the vaginal canal for an extended period. According to Hani et al., development of miconazole nitrate, a thermosensitive bio-adhesive vaginal gel for vaginal candidiasis is a priority. The goal of this study was to develop and test a thermosensitive bio-adhesive gel containing metronidazole nitrate for vaginal medication delivery to improve therapeutic efficacy and patient compliance in the treatment of vaginal candidiasis[6]. Sapra et al. investigated the development and optimization of *in situ* periodontal gel containing levofloxacin for the treatment of periodontal disorders," in which site-specific levofloxacin medication delivery for periodontitis was produced[7]. Bruschi et al. describe the preparation and characterization of mucoadhesive thermo responsive systems consisting of poloxamer 407 (P407), Carbopol 934P (C934P) and propolis to treat vulvovaginal candidiasis in their study, and preparation and characterization of mucoadhesive thermo responsive systems containing propolis for the treatment of vulvovaginal candidiasis (VVC)[8]. The goal of this study was to combine the advantages of both the thermo-sensitive and mucoadhesive gelling systems for symbiotic performance in the design and development of intra-vaginal *in situ* gels for prolonged metronidazole release.

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MATERIALS AND METHODS

Metronidazole was a gift sample from McCoy Drugs Pvt. Ltd. India. A gift sample of Poloxamer-407 was obtained from BASF, Mumbai. Carbopol 934 was obtained as a gift sample from Corel Pharma Chem, Ahmedabad. Hydroxyethylcellulose was purchased from Merck Private Ltd., Mumbai. HPMCK100M was purchased from Yarrow Chem., Mumbai. All other materials were of analytical grade.

Determination of absorption maxima (max) of metronidazole

In citro-phosphate buffer pH 4.5, a stock solution of metronidazole 4 µg mL^{-1} was produced. Metronidazole's UV spectrum was measured in the 200-400 nm range using a twin beam UV visible spectrophotometer (Shimadzu, UV-2450) with a 1.0 cm slit width and citro phosphate buffer pH 4.5 as the solvent[9,10].

Preparation of *in situ* gelling system

An initial dose of metronidazole was diluted in distilled water with constant stirring and then sonicated to completely dissolve. The aforesaid sample was then dissolved in the needed amount of another mucoadhesive polymer until it was soluble. Then, with constant stirring, the needed amount of preservatives (propyl paraben) was added to the solution. To obtain a clear solution, the needed quantity of poloxamer 407 was added and maintained overnight in the refrigerator at 40 ºC[10,11].

Evaluation parameters

Determination of gelation time

Using the test tube inverting approach, the gelation time was calculated. The solution was placed in a thin-walled tube and kept in a water bath at the appropriate gelation temperature. Every 30 seconds, the test tube was removed and inverted to assess the status of the sample. With the test tube inverted, the gelation time was assessed using the flow or no-flow criterion[12].

Determination of gelation temperature

In a 15 mL borosilicate glass test tube, the gelation temperature was determined by heating the formulation (1-2°C min^{-1}). A 3 mL formulation solution was poured into each test tube and heated with gentle stirring until the formulation solution was gelled. When the test tubes were gradually immersed, the point where there was no flow was considered as gel formation[13].

Determination of pH

The pH was measured in each of the *in situ* gels (1 g) of metronidazole by using a calibrated digital pH meter. The readings were taken for an average of 3 samples[14].

Determination of viscosity

A Brookfield viscometer was used to determine the viscosity of the formulation (Brookfield viscometer, model-LVDV-II pro, USA). The samples were sheared at a rate of 20 rpm min^{-1} at room temperature using an S-62 spindle[15].

Determination of syringe ability

Each formulation's syringe ability was assessed. The viscosity of formulations increased as the polymer content increased, as did the force necessary to expel each formulation from the syringe fitted with 20 gauge needles[15,16].

Determination of drug content

1 mL of *in situ* solution was added to 100 mL of citro-phosphate buffer pH 4.5 in a volumetric flask and set aside overnight. The next day, the fluid was sonicated for 5-10 minutes in a bath sonicator. The drug concentration was evaluated using a visible spectrophotometer (UV-2450 Shimadzu, Japan) at 320 nm against an appropriate blank solution[17].

Determination of *in vitro* drug release

The dialysis sac method was used to conduct diffusion research. A dialysis sac measuring 15.9 mm in diameter was taken. 2 mL of the formulation was placed in the dialysis sac, which was hermetically sealed on one end and open on the other. To close both ends of the sac, it was folded and sealed. This "dialysis bag" was then threaded into a beaker containing 50 mL of buffer pH4.5 as the diffusion medium, which was kept at 37±0.5 ºC on a magnetic stirrer. At regular intervals, 1 mL aliquots were withdrawn and refilled with an equal volume of new buffer pH 4.5 kept at 37±0.5 ºC. The amount of drug released was measured by taking absorbance using a UV spectrophotometer at 320 nm[18].

Determination of mucoadhesive force

Using a QTS texture analyzer, the force required to separate the formulations from an acrylic 38 probe was measured to assess the mucoadhesive force of the systems. The instrument was then controlled by software using texture pro software. The following test parameters were chosen: The force necessary to remove the probe from the surface of the formulation was determined as

the maximum value in the resultant relationship between load and time, after which the probe was pushed upward at a constant speed of 30 mm min^{-1} [19].

Drug release kinetics

The release data were fitted to the equations mentioned below to study the kinetics of metronidazole release from the gel[19,20].

Zero-order equation

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation Qt = k0.t, *where Qt = percentage of drug released at time t* and *k0 = release rate constant*. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as the cumulative amount of drug released versus time.

First-order equation

This model has also been used to describe the absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug that followed first-order kinetics can be expressed by the equation: ln (100-Qt) = ln 100–k1 t, *where k1 is the first release rate constant.* The obtained data are plotted as a log cumulative percentage of drugs remaining vs. time, yielding a straight line with a slope of -K/2.303.

Higuchi's equation

In a general way, it is possible to simplify the Higuchi model as follows (generally known as the simplified Higuchi model): $Qt = kH\ t_{1/2}$ where kH denotes the Higuchi release rate constant. The obtained data were plotted as a cumulative percentage of drug release versus the square root of time.

Stability study

A stability study was carried out according to ICH Q1A (R2) guidelines at 0-8 ºC for 30 days. The pH, viscosity, gelation temperature, and drug content were measured one month later[14,19,20].

RESULTS AND DISCUSSION

Absorption maxima (λmax) of metronidazole

By scanning 4 µg mL^{-1} of metronidazole, the wavelength of maximum absorption at 320 nm was found to be sharp and satisfactory, as shown in (Fig. 1).

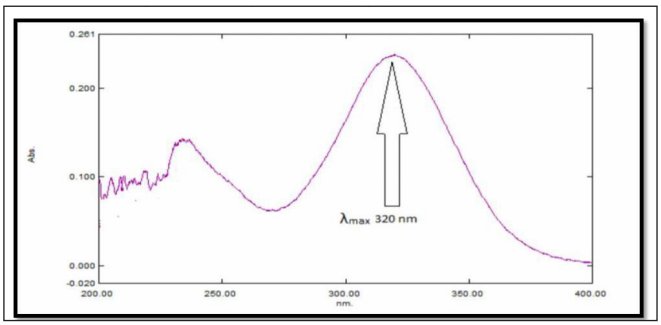

Fig. 1: Absorption maxima (λmax) of metronidazole

Formulation batches of *in situ* gelling system

The prepared formulation batches are given in Table I. In all the formulations, 0.18% methyl paraben and 0.02% propyl paraben were added as preservatives.

Table I: Formulation batches of *in situ* gelling system

Batch	% Ingredient			
	HEC	HPMC K4M	HPMC K100M	Carbopol 934
F1	0.50	-	-	-
F2	1.0	-	-	-
F3	1.5	-	-	-
F4	-	0.5	-	-
F5	-	1.0	-	-
F6	-	-	0.25	-
F7	-	-	0.50	-
F8	-	-	-	0.25
F9	-	-	-	0.50

The metronidazole and P407 were taken as 0.75 and 18% while formulating batches from F1-F9.

Table II: Gelation time of batches

Formulation batch	Gelation time (Seconds)
F1	40-42
F2	30-35
F3	25-30
F4	22-25
F5	20-25
F6	35-40
F7	25-30
F8	20-25
F9	17-20

Table III: Viscosity of batches

Formulation batch	Viscosity (cps) (n=3)
F1	81±0.65
F2	151±0.74
F3	190±0.72
F4	250±0.25
F5	376.4±0.35
F6	83.7±0.71
F7	129.3±0.75
F8	621.7±0.79
F9	935±0.86

EVALUATION PARAMETER

Gelation time

In the study, the gelation time of all batches was found to be within 40 sec. Optimized batch F9 shows a fast gelation time of 17-20 seconds, as shown in Table II.

Gelation temperature

All of the batches studied had a gelation temperature of 37±2ºC, which was found to be within the body temperature range. Tgel is the temperature at which the liquid phase changes to the gel phase. An ideal *in situ* gel is a free-flowing, room-temperature liquid that may be injected repeatedly into the application site, where it undergoes an *in situ* phase transition and forms a strong gel. The use of vaginal thermo reversible gels was deemed appropriate if it was between 25 ºC and 37 ºC, as the human vaginal temperature is 37±2 ºC. A gel may form at room temperature if the Tgel is less than 25°C, causing production, handling, and administration issues.

Batches' pH

Based on the pH of all batches analyzed, all formulation pH values were found to be in the range of 6.20-6.81. Because the vaginal pH ranges from 4.0 to 6.5, this pH range is suitable for the vaginal cavity.

Viscosity of batches

Semi-solid solutions offer the advantage of ensuring that the formulation comes out of the package in the right amount and stays in the infected area for the right amount of time. Due to their high viscosity values, oily systems such as ointments and creams are not well tolerated by patients when administered to the mouth area. As a result, preparing the formulation as a water-based

Table IV: *In vitro* drug release profile of batches

Time h	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	40.48 ± 0.33	33.84 ± 0.30	28.95 ± 0.71	37.51 ± 0.39	32.24 ± 0.36	42.06 ± 0.17	38.02 ± 0.33	35.37 ± 0.30	30.93 ± 0.43
1	46.58 ± 0.84	42.77 ± 0.23	34.90 ± 0.64	46.22 ± 0.53	42.80 ± 0.11	49.73 ± 0.29	45.20 ± 0.17	44.06 ± 0.24	39.88 ± 0.58
2	54.78 ± 1.77	54.13 ± 0.58	42.67 ± 0.20	56.41 ± 0.24	51.90 ± 0.21	58.53 ± 0.59	54.15 ± 0.46	52.18 ± 0.16	45.85 ± 0.39
3	62.10 ± 1.21	62.57 ± 0.21	49.01 ± 0.47	63.02 ± 0.16	61.28 ± 0.15	64.05 ± 0.45	62.34 ± 0.57	59.88 ± 0.54	52.36 ± 0.48
4	70.18 ± 1.52	70.25 ± 0.63	54.78 ± 0.55	70.50 ± 0.31	67.31 ± 0.16	71.53 ± 0.86	69.14 ± 0.36	69.89 ± 0.55	58.96 ± 0.20
5	79.39 ± 1.02	76.58 ± 0.27	58.54 ± 0.53	77.06 ± 0.75	71.40 ± 0.59	78.60 ± 0.73	73.73 ± 0.63	73.97 ± 0.22	65.87 ± 0.21
6	82.66 ± 1.46	79.94 ± 0.30	63.74 ± 0.50	80.27 ± 0.34	75.40 ± 0.63	81. 09 ± 0.65	77.47 ± 0.33	78.29 ± 0.39	72.31 ± 0.14
7	86.11 ± 0.61	82.44 ± 0.30	70.33 ± 0.50	82.66 ± 0.57	78.55 ± 0.54	85.08 ± 0.35	82.75 ± 1.06	80.80 ± 0.22	76.73 ± 0.63
8	89.13 ± 0.83	85.21 ± 0.26	75.33 ± 0.36	84.63 ± 0.49	81.88 ± 0.30	90.08 ± 0.30	86.14 ± 0.81	83.80 ± 0.45	80.66 ± 0.43

gel will be preferable. The viscosity of the formulation should be low at room temperature and high at 33°C to prevent flow and removal. To stay at the application site, the *in situ* formulation should have a low viscosity when applied and a high viscosity following administration. The viscosity of all formulation batches increases as the polymer concentration increases. The viscosity in cps is given in Table III.

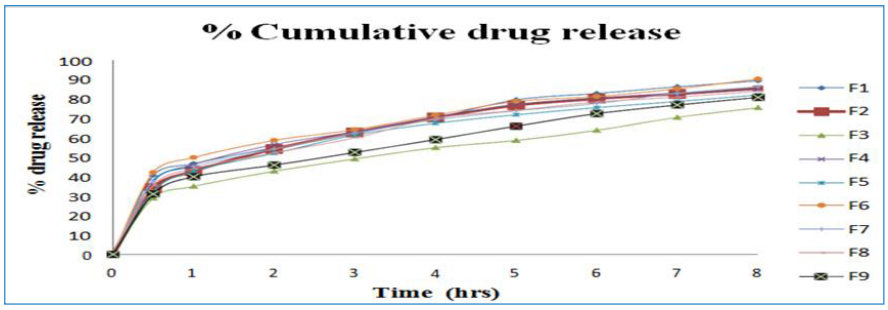

Fig. 2: *In vitro* drug release profiles of batches

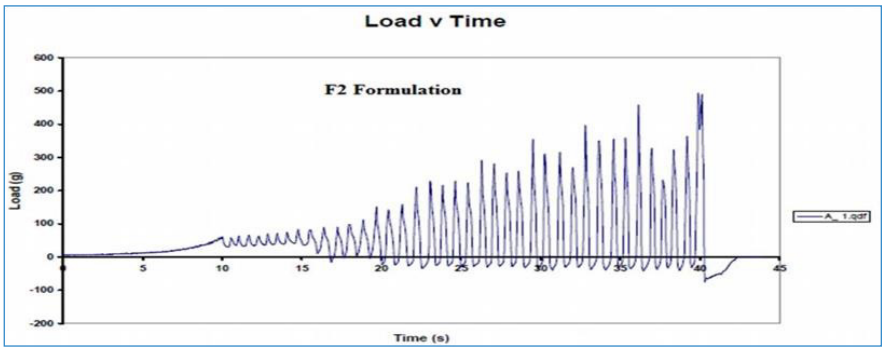

Fig. 3: TPA Graph of F2 formulation

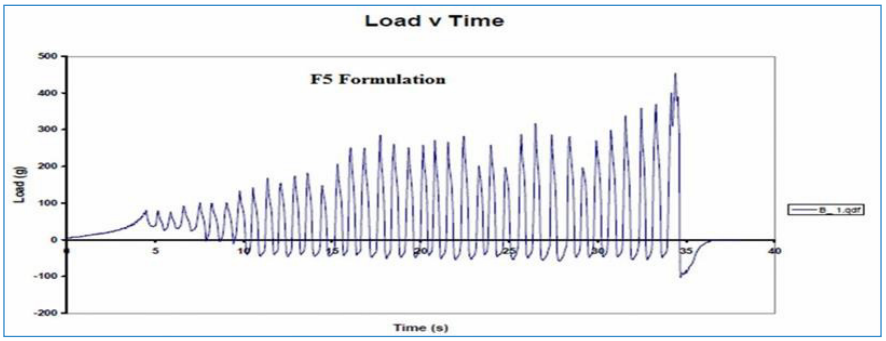

Fig. 4: TPA graph of F5 formulation

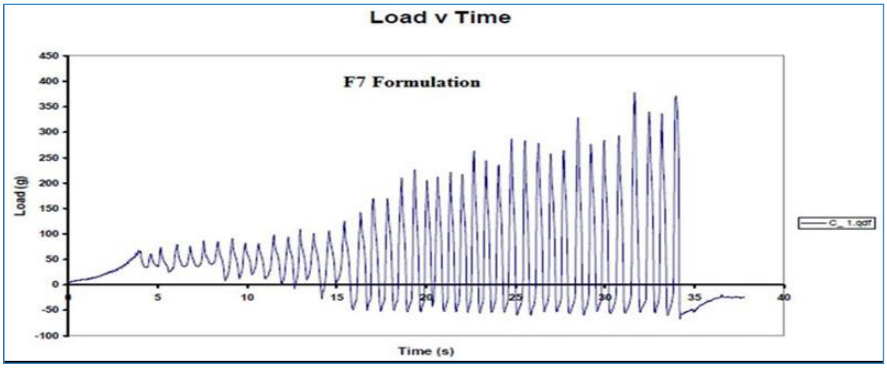

Fig. 5: TPA graph of F7 formulation

Batch syringe capacity

The viscosity of formulations increases as the concentration of HEC/ HPMC K100M increases, increasing the polymer concentration required to expel each formulation from a syringe with a 20-gauge needle. Because formulation F3 has a greater polymer concentration, it fails the syringe ability test. The syringe ability test was passed by the F9 batch.

Drug content of batches

For metronidazole analysis, the utilized spectrophotometric technique was determined to be linear (r^2= 0.9954). The drug content of the optimized batch F9 was 97.94±0.71%. All formulation batches from F1-F9 had drug content ranging from 90.94±0.71% to 97.94±0.71%.

In vitro drug release profile of batches

According to the drug release profiles of all batches investigated, batches F9 and F3 have the best release compared to other batches, with up to 80% of drug release occurring within 8 h. Fig. 2 depicts the graphical presentation. The graphical representation of the release profile revealed a burst release impact in the first half-hour due to complete polymer hydration, followed by a decline in drug release due to the polymeric matrix.

All of the formulations demonstrated burst release when the release trial began. This could be because the formulations take so long to transition from sol to gel. The drug release from the gels decreased as the concentration of P-407 increased. After 0.5 h of dissolving, formulation F3 (18% w/V of P-407) released 28.93±0.71% of the drug, whereas

Table V: Effect of mucoadhesive force

Batch	Mucoadhesive force (g)
F2	75
F5	105
F7	61
F9	118

formulation F9 (18%w/V of P-407) released 30.93±0.43%, as shown in Table IV. As the polymer concentration grows, drug burst release appears to diminish. At higher P-407 concentrations, the sol-to-gel transition occurred faster, resulting in a reduction in burst release.

After 8 h of dissolving, formulation F3 (18%w/V of P-407) released 75.33 ±0.36% of the medication, while formulation F9 (18%w/V of P-407) released 80.66 ±0.43%. A decrease in the number and size of the channels in the gel structure may be the likely cause of such a delay in a release as the concentration of Carbopol

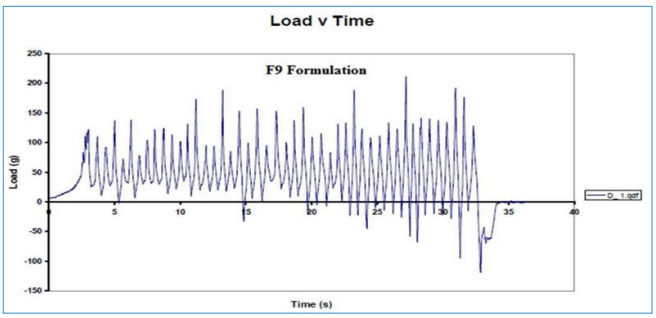

Fig. 6: TPA graph of F9 formulation

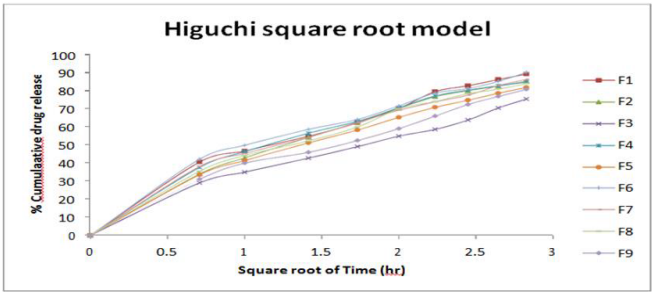

Fig. 7: Higuchi square root model

940 rises. When the inter-micelle distance inside the gel is reduced, bridging between neighboring micelles and an increase in gel viscosity results in a decrease in drug release. The presence of a mucoadhesive polymer in these channels would lower the amount of free water while also causing hydrogen bonding with the water. This would cause a rise in viscosity and thus a decrease in drug release.

Effect of mucoadhesive force

Only four gel formulations with the maximum viscosity were tested using texture profile analysis TPA: F2, F5, F7, and F9. The difficulty in measuring the TPA of mucoadhesive gels with low viscosity was the reason for this. Because the procedure cannot be validated for low-viscosity gels, TPA cannot produce accurate findings for these types of gels. The following is the mucoadhesive force obtained in Table V and Figs. 3-6.

Kinetic study

It was carried out on three kinetic models, with the following results: Diffusion data fits the Higuchi square

Table VI: Kinetic study

Batch No.	R^2 Value			Release kinetics	
	Zero-order model	First-order model	Higuchi square root model	Release exponent (n)	Kinetic constant (k)
F1	0.808	0.9753	0.958	0.4928	37.77
F2	0.810	0.9674	0.967	0.5028	31.24
F3	0.862	0.9598	0.977	0.5290	27.75
F4	0.778	0.9537	0.950	0.5357	25.18
F5	0.817	0.9637	0.968	0.5820	20.02
F6	0.774	0.9633	0.941	0.6177	20.44
F7	0.797	0.966	0.957	0.5421	25.45
F8	0.804	0.9617	0.961	0.5688	23.14
F9	0.858	0.9716	0.977	0.5722	23.24

Table VII: Stability study of optimized batch (F9)

Stability	Gelation temp	pH	Viscosity	Drug content
Before	36 ^{0}C	6.75	947	97.94%
After	36 ^{0}C	6.79	951	96.78%

root model (Fig. 7), according to the kinetic analysis. Drug release from a gel matrix is a complex process that involves drug diffusion through a hydrated component of the gel matrix and erosion of the matrix's outer fully hydrated polymer. Due to the entry of surplus water into the gel matrix's core, the hydration of the gel matrix increases, posing a diffusion barrier to drug release. The polymer chains relax to the point that they can no longer maintain the gel's integrity, resulting in disentanglement and polymer degradation from the gel matrix's surface.

P 407 and Carbopol 940 formulations F3 and F9 have n values very close to 0.5, confirming Fickian diffusion (Table VI). As a result, drug diffusion across the gel matrix largely regulated drug release from the gel formulation. As the P 407 concentration grew, the rate of drug release and the first burst release were both reduced. This was shown by the n number ranging from 0.52 to 0.58. The n value increased from 0.54 to 0.61 when mucoadhesive polymers (Carbopol 940) were introduced to P 407, demonstrating non-Fickian/anomalous behavior. As a result, drug release in these formulations was determined by both drug diffusion through the gel matrix and disentanglement or erosion of polymer chains.

Stability study of optimized batch

A stability study was conducted at 0-8 ^{0}C for 30 days under ICH Q1A (R2) criteria. A one-month stability study was conducted on batch F9. After 30 days, the stability analysis found no significant changes in pH, viscosity, gelation temperature, or medication content. As a result (Table VII), the *in situ* gel was deemed stable at 0-8 ^{o}C.

CONCLUSION

Using the thermosensitive polymer poloxamer 407 and mucoadhesive polymers HEC, HPMC, and Carbopol 934, a cold technique was used to make a vaginal *in situ* gel containing metronidazole. Because no signs of incompatibility were seen in the FTIR and DSC studies (not reported in the article), it was concluded that these polymers are compatible with the drug. In terms of gelation time and temperature, all the formulations performed well. All the formulations had pH values between 6-7. The obtained viscosity was found to be mucoadhesive dependent. A polymer concentration-dependent release profile was discovered in an *in vitro* drug release investigation.

In comparison to HEC and HPMC polymers, Carbopol-934 provides adequate release and mucoadhesion. This is because Carbopol 934 is a cationic polymer, which means it releases less than non-ionic polymers. The Higuchi square root model of diffusion-controlled drug release was used to calculate the release date. *In situ* gel properties did not change significantly during the stability investigation. As a result, the *in situ* formulation was proven to be superior to previous vaginal gels.

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