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Formulation and Characterization of Poloxamer-Based Mucoadhesive Vaginal In Situ Gelling System of Miconazole Nitrate

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Formulation and Characterization of Poloxamer-Based Mucoadhesive Vaginal *In Situ* Gelling System of Miconazole Nitrate

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Abstract

Background: Vaginal candidiasis is the most prevalent illness among women and is mostly caused by *Candida albicans*, which causes most clinic visits.

Objectives: The present study aimed to formulate and improve miconazole nitrate-containing thermosensitive bioadhesive gel for vaginal drug delivery to achieve better therapeutic effectiveness and patient compliance in the management of vaginal candidiasis.

Materials and Methods: Miconazole nitrate (MN) was prepared as a (2%) vaginal gel by using 18% poloxamer (PLX) 407, 2% PLX 188 as thermoresponsive polymers, and Carbopol (CP) 934 and CP 940 as bioadhesive polymers. The prepared formulations were assessed for parameters such as gelation temperature, viscosity, bioadhesive strength, spreadability, and in vitro drug release.

Results: The gelation temperatures for F1 were found at 35°C, and the developed formula had optimum viscosity, good bioadhesive strength, respectable spreadability, and (25%) *in vitro* drug release over 12 hours.

Conclusion: The mucoadhesive in situ gels of MN would be an alternative candidate for vaginal candidiasis treatment since they have suitable gel properties and good vaginal retention.

Keywords: Miconazole nitrate, Poloxamer, Mucoadhesion, Vaginal candidiasis

1. Introduction

One of the most common complications that arise in women is vaginal candidiasis (VC). Fungal vaginitis is a public fungal infection that nearly 80% of women may experience at least one infection during their lives. More than 10% of visits made to women's health clinics occur due to this disorder. The most common etiological cause of VC is documented as Candida albicans [1].

For a long time, vaginal delivery has been an applicable route of drug delivery to achieve local and systemic pharmacological action. The benefits of local application are escaping the gastrointestinal side effects, less drug content for dosage formulation, and a reduction in hepatic side effects of medications [2].

Poor retention, leakage, and messiness that happen with usual vaginal dosage preparations (for instance cream, gel, liquid formulations, vaginal films, tablets, and pessaries) are leading to unfavorable patient compliance and the defeat of therapeutic effectiveness [3].

In recent times, more convenient dosage formulas that have been proven to work well for vaginal applications are in situ gel formulations that have a lot of benefits, for example, ease of administration, excellent spreadability, less frequent consumption, increased patient compliance, and are more comfortable compared to ordinary dosage forms [4].

In situ is a Latin term means 'in position'. The *in situ* gelling system is converted from sol-gel due to the effect of different factors. In which, gel formation

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influenced by a number of variables, for example, pH differences, ionic basis, temperature variations, ultraviolet radiation, and the enzyme that releases the active ingredient [5].

In drug delivery systems, one of the most researched types of polymer systems are temperature-responsive hydrogel systems. The most appropriate range for the system's crucial temperatures are the physiological and ambient temperatures. At the same time, gel formation may occur without the need for an external heat source outside the patient's body [6].

Poloxamer (PLX) is a synthetic triblock copolymer consisting of polyoxyethylene and polyoxypropylene that exhibits thermosensitive manners in aqueous solutions. Formulations that are made from it are characterized by good properties such as the extended release of the active ingredient and excellent compatibility [7]. However, they have minimal mucoadhesive force. Therefore, carbapol (CP) was sponsored as a mucoadhesive polymer to improve mucoadhesive strength and mechanical properties and to confirm prolonged residence time [8].

Miconazole nitrate (MN) is a local imidazole antifungal drug that is a key player in the management of fungal infections. MN functions by inhibiting the cytochrome P450 complex, including the 14α-demethylase enzyme, which is required for ergosterol and fungal cell membrane biosynthesis. MN demonstrated a high safety profile in the treatment of VC with no serious adverse effects [9].

The objective of this study was to develop a suitable mucoadhesive in situ gel formulation of MN by utilizing PLX 407, PLX 188, CP 934, and CP 940 to achieve an *in-situ* gel with respectable mucoadhesive properties. In vitro characterization of the prepared formulations was conducted.

2. Materials and methods

2.1. Materials

Miconazole nitrate was kindly provided by Al-Safa Pharmaceuticals Industries (Baghdad, Iraq). PLX 407, PLX 188, CP 934, CP 940, and acetic acid were supplied by Sigma-Aldrich, USA. Sodium lauryl sulfate (SLS) and sodium citrate were obtained from Alpha Chemika (India). All other materials and chemicals were of analytical grade, and no further purification was needed.

2.2. Methods

2.2.1. Preparation of MN vaginal formulations

In a previous study, a cold method was used to prepare in situ gel primary formulas of different PLX 407 and PLX 188 concentrations and proportions to achieve an optimum formula that has an applicable gelation temperature for vaginal delivery. Mucoadhesive in situ gel preparations of MN were formulated by the modified cold method using a mixture of PLX 407/188 and different grades of CP as mucoadhesive polymers. Accurately weighed amounts of PLX 188 and 407 were slowly dispersed in 60 mL of distilled water (DW) at 4°C with continuous stirring using a magnetic stirrer (APOPS, MS300HS) [10]. The obtained solution was then kept at 4°C to ensure complete dispersion, which was confirmed by obtaining a clear gel. Then, an accurately weighed amount of CP was gradually added to 30 mL of distilled water at 60°C with the addition of a few drops of triethanolamine for pH adjustment with constant stirring at 300 rpm, and then it was left at 25°C for 24 h. Finally, both solutions were incorporated, and 2% MN was added with stirring. The final formulation volume was calibrated up to 100 mL [11]. Table 1 demonstrates the composition of prepared formulas.

Table 1. Composition of MN mucoadhesive in situ gel formulas.*

Formula code	CP 934 w/v (%)	CP 940 w/v (%)
F1	0.4	–
F2	0.6	–
F3	0.8	–
F4	–	0.4
F5	–	0.6
F6	–	0.8

*All in situ gel formulas contain (PLX 407 18%, PLX 188 2% and MN 0.2%).

2.2.2. Characterization of thermoresponsive mucoadhesive gel

2.2.2.1. Screening of gelation temperature (T gel). The prepared formulas' gelation temperature was evaluated as follows: A 20-mL clear test tube with a magnetic bar inside was filled with 5 mL of the solution. After that, the test tube was placed in a water bath set at 25°C. The solution was then heated to a constant temperature of 2°C per minute while being stirred constantly at 100 rpm. The gelation temperature was confirmed to be the temperature at which the magnetic bar remained immobile. Every measurement was made in triplicate (n = 3) [12].

2.2.2.2. Appearance and clarity determination. The clarity of the prepared formulas was assessed by visual inspection in light by using white and black frames. The classification of the clarity degree of formulas was done as follows: unclear gel (+), clear gel (++), and extremely clear gel (+ + +) [13].

Fig. 1. Modified balance.

2.2.2.3. pH of in situ gel formulations. A digital glass electrode pH meter (Hanna Instruments, Italy) was utilized in the pH measurement of established formulas. The test was repeated in triplicate (n = 3), and the data was documented [14].

2.2.2.4. Syringeability measurement. A 5 mL syringe (20-gauge needle) was filled with prepared formulas. For syringeability, the formulas were permitted to pass freely through the syringe needle if they passed easily, categorized as "pass," or if they passed with difficulty, categorized as "failed" [15].

2.2.2.5. Spreadability studies. For spreadability measurement, one gram from each formula was positioned at the central point of the glass license plate measuring 23 cm by 10 cm. With care, the glass plate was then shielded by an additional glass plate (same dimensions), and 1000 mg of weight was placed on the top of both plates (without sliding). After 30 minutes, the cover plate was removed, and the spread gel diameter (cm) was documented. This test was measured in triplicate (n = 3) [16].

2.2.2.6. Drug content measurement. One mL of in situ formulations (0.2% MN) was measured and dissolved with a 30 mL acetate buffer solution of pH 4.10. The solution was then placed in the ultrasonic bath for 15 minutes to ensure thorough dissolution, after which a syringe filter (0.45 mm) was used to get rid of the undissolved material. Samples were collected from the upper, middle, and lower regions of the gel formula. MN concentration was detected by a spectrophotometer at 230 nm [17].

2.2.2.7. Formulations viscosity measurement. Brookfield viscometer (DV-II PRO) was used to determine in situ gel formula viscosity at 25°C and 37°C, in which at low temperature, 30 mL of formulas were placed in a small glass transparent container. The formula temperature was elevated to 37°C by using a water bath. The viscosity measurement was documented at both 25°C and 37°C by utilizing spindle no. 63. This test was analyzed in triplicate [18].

2.2.2.8. Mucoadhesive force measurement. An improved balance method was utilized to assess the mucoadhesive strength of the prepared in situ gel formulations, in which it was measured by determining the force required to separate the MN formulations from a vaginal sheep's tissue. This mucosal tissue was acquired from a local slaughterhouse [19].

Ten grams of the sample were fixed to a small, customized container that was located beneath the inferior surface of the right side of the balance. At which an isolated piece of sheep vaginal mucosa was secured to the mobile wooden platform as shown in Fig. 1. The exposed tissue was submerged in 1 mL of an acetate buffer solution of pH 4.1 and left for 30 seconds for hydration. The hydrated tissue came into contact with the gel surface by moving the platform downward. A 20-gram preload was positioned precisely above the right pan for three minutes to apply

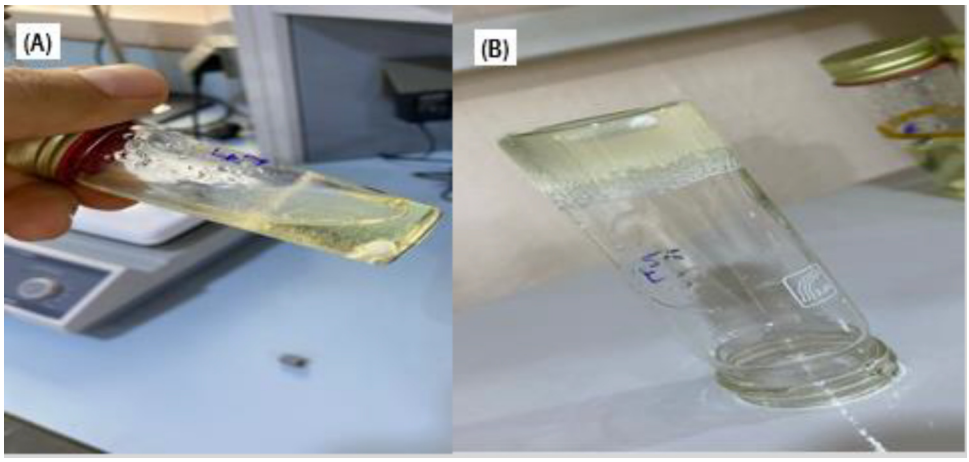

Fig. 2. In situ gel formulations (A) at 4°C in solution form (B) at 36°C in gel form.

initial pressure. The preload was moved away from the right pan, followed by the gradual addition of water drop by drop to the left pan till the gel detached from the surface of the vaginal mucosa. The total water weight needed for the separation of the gel was documented as the mucoadhesive strength.

Force of adhesion was calculated by Eq. (1)

$$F = 1/4\, G * W \quad (1)$$

Where F in (dynes/cm2) is the force of mucoadhesion. W mass in grams. G in (cm/s2) is the acceleration owing to gravity [20].

2.2.2.9. In vitro drug release study. The *in vitro* drug release manners of the prepared in situ gel formulations was done by utilizing a semipermeable dialysis membrane (8000–14000 D) with type-II dissolution apparatus (DS8000, Lab India) as a modified release method that mimics in vivo body circumstances. Initially, the dialysis bag was immersed in dissolution media for 24 hours. before the test took place. Then, approximately 10 mL of the sample was transmitted to the dialysis bag, followed by sealing it from two sides by using a rubber band and wrapping around a paddle. By lowering the paddle, the membrane was dipped in a previously filled dissolution jar of 100 mL of acetate buffer solution with pH 4.10, 36 ± 0.5°C, and a 50-rpm paddle rotation speed.

At predetermined time intervals for 12 hours, five mL samples were taken and replaced with an equivalent volume of fresh acetate buffer to maintain the sink in good condition. Consequently, a syringe filter (0.45 μm, Millipore) was used to remove any undissolved material from the collected samples. The cumulative percentage of drug release at each time point was calculated spectrophotometrically at 230 nm using the acetate buffer. A previously assembled standard curve. The tests were conducted in triplicate [21].

2.2.3. Statistical analysis

Statistical analysis for all experimental data was conducted using IBM SPSS Statistics 25 software. All tests were repeated three times, and the data are presented as mean ± S.D. The Student's t-test, with $p < 0.05$ serving as the significance threshold, was used for statistical data analysis.

3. Results and discussion

3.1. Gelation temperature (T gel)

The temperature degree at which the liquid phase marks its conversion to a gel is considered the temperature of gelation. The T gel that is applicable for vaginal administration is around 30–36°C as showed in Fig. 2 [22]. Consequently, when the T gel of the prepared in situ formula is less than 30°C, the transition of the sol-gel might happen at 25°C and lead to troubles in the manufacturing, handling, and administration processes. If the T gel is more than 37°C, the in situ gel persists as a solution at body temperature and consequently causes rapid formula leakage, uncontrolled drug release, and low retention time in the vagina [23].

Based on the findings of a previous study, in situ gel formulations were prepared for the T gel of the primary blank. An ideal formula (with compositions of PLX 407 (18%) and PLX 188 (2%) was found to have optimistic results (T gel 37 ± 0.26°C). This indicated that the formulation was fit for further incorporation with different concentrations of CP 934 and CP 940 as mucoadhesive polymer.

Table 2. Physical characteristics of MN vaginal in situ gel formulas (Data are stated as means ± SD, n = 3).

Formula code	GT (°C)	Appearance	pH	*Syringeability*	*Spreadability* (cm)	% Drug content	Mucoadhesive force dyn/cm^2
F1	35 ± 0.029	+ + +	5.45 ± 0.121	Pass	5.15 ± 0.01	99.35 ± 0.21	13.8 ± 0.7
F2	33 ± 0.033	+ + +	5.20 ± 0.203	Pass	4.25 ± 0.02	99.1 ± 0.13	16.3 ± 1.4
F3	31.5 ± 0.02	+ + +	4.90 ± 0.261	Pass	3.75 ± 0.02	98.43 ± 0.31	17.1 ± 0.5
F4	33 ± 0.13	+ + +	5.32 ± 0.42	Pass	4.30 ± 0.101	99.21 ± 0.41	19.2 ± 0.1
F5	32 ± 0.21	+ + +	4.54 ± 0.031	Pass	3.95 ± 0.251	100.01 ± 0.28	20.5 ± 0.2
F6	30 ± 0.47	+ + +	4.65 ± 0.173	Pass	3.15 ± 0.13	98.32 ± 0.43	22.5 ± 0.3

Formulations (F1–F3) and (F3–F6) showed the influence of mucoadhesive polymer concentration, while (F1–F4) that utilize the same CP 934 and CP 940 concentrations showed the effect of mucoadhesive polymer grade. The results for these variables indicated a significant reduction ($p < 0.05$) in T gel upon increasing mucoadhesive concentrations and molecular weight, as displayed in Table 2. This could be attributed to the establishment of hydrogen bonds between the poloxamer's polyethylene oxide (PEO) chain and the mucoadhesive polymer. This binding will cause the early growth of micellar structures as a consequence of the restriction of the hydration of the PEO chain [24].

3.2. Appearance and clarity

As seen in Table 2, all the constructed formulas were transparent and clear. These qualities are primarily necessary for in situ gel formulations since they facilitate simple handling and make precise dosage calibration easier [25].

3.3. pH results

The natural pH of the vaginal cavity ranges between 3.5 and 5.5, which may be affected by different causes, mainly pathogenic or physiologic. To ensure the stability of formulas and vaginal compatibility, it is essential to prepare gels with an ideal pH to enhance patient compliance and prevent irritation. The results of pH values for the prepared formulations, as demonstrated in Table 2, were found to be around 4.54 ± 0.031–5.45 ± 0.121, which is near the neutral pH of the vaginal cavity, as well as promising the stability and patient acceptance of the developed formula for vaginal administration [26].

3.4. Syringeability

Syringeability is a key parameter to assure the precision of dose measurements, the simplicity of drawing the formula from the dose container, and comfortable use at the targeted site. The results of all in situ gel formulations at 4°C were passed freely through the used syringe with low hand pressure [27].

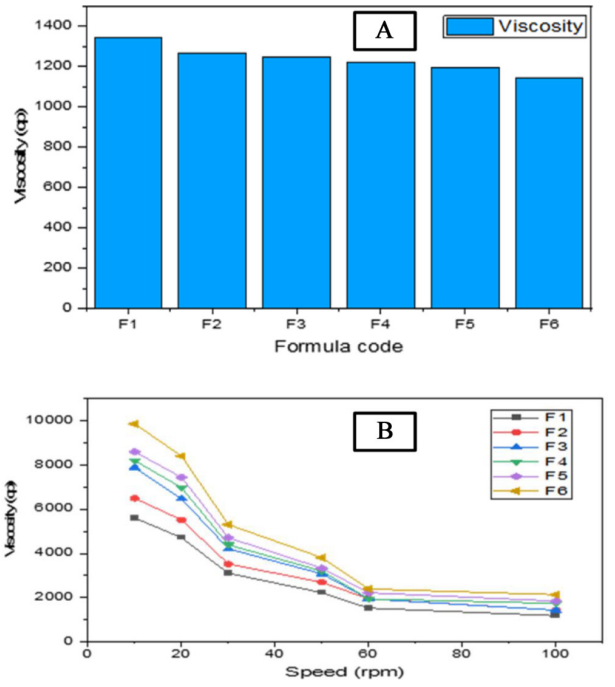

Fig. 3. A: Rheogram profiles for MN vaginal in situ gel formularies at 24°C using spindle No. 63; B: Rheogram profiles for MN vaginal in situ gel formularies at 34°C using spindle No. 63.

3.5. Spreadability

Assessment of the spreadability of the semi-solid formula is needed due to the importance of this parameter in achieving high local treatment efficacy, which is mainly achieved by spreading a uniform layer of the prepared formula on the site of application to maintain precise dose delivery [28].

As CP concentration and its molecular weight within the formula were increased, a significant reduction in spreadability ($p < 0.05$) was detected, as shown in Table 2. This increase in mucoadheisve polymer concentration and molecular weight leads to a higher degree of cross-linking of the polymer chain and, therefore, increased viscosity as well as

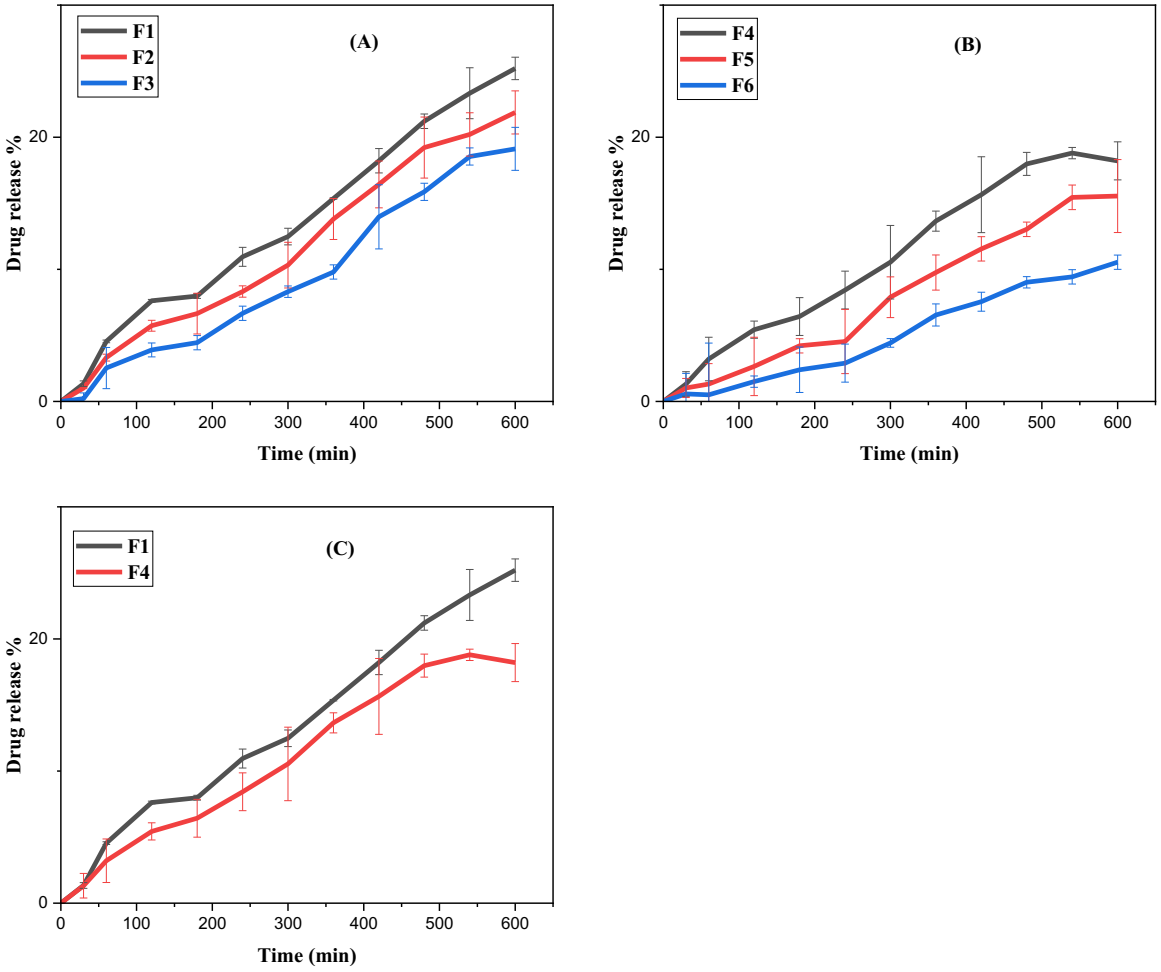

Fig. 4. Drug release profile for MN in situ formulations in acetate buffer (pH4.10) A- release of F1, F2 and F3 B-release of F4, F5 and F6 C-release of F1 and F4.

decreased spreadability. Such observations were described by Limpongsa *et al*. [29].

3.6. Drug content

The results were found to be around (98.32 ± 0.43%–100.01 ± 0.28%), as originated in Table 2 which is in the typical range according to USP [30]. At the same time, there were no significant differences ($p > 0.05$) among the upper, middle, and lower gel samples. To prepare gels with low variability and even drug distribution, the formulation process should be characterized by these wonderful finding [31].

3.7. Viscosity assessment

The viscosity of the prepared formulas (F1–F6) at the gel phase with different shear rates (rpm) is shown in Figs. 3A and B. At room temperature, there are no significant differences ($p > 0.05$) between all formulations at solution phase with low viscosity, while at gelation temperature, a significant ($p < 0.05$) rise in viscosity was observed. In in situ gel flow, the Arrhenius equation connects viscosity and temperature due to the presence of the thermoresponsive PLX that converts to gel and increases viscosity as temperature increases [32].

The rheogram profiles of different polymer concentrations utilized in this study were displayed by means of the rotation speed increase, which led to a significant decrease in the pseudoplastic (shear-thinning liquid) flow of the formulations [16].

Another observation was made regarding the mucoadhesive polymer employed. Formulas 3 and 6, which contain CP 934 and CP 940, respectively, demonstrate higher viscosity in a concentration as well as molecular weight-dependent manner at both room temperature and physiological temperature. The same results were observed by Kim *et al*. [33]. This could be correlated to the hydrogen binding capability of CP to a greater degree with the oxygen atom of the PEO block in PLX as a consequence of

more physical entanglement once concentration and molecular weight rise [34].

3.8. Mucoadhesive strength

For *in situ* developing vaginal gels, the force of mucoadhesion is a fundamental and vital physicochemical parameter as it prevents the preparation from draining too quickly in addition to extending its residence time within the vaginal cavity [35].

Significant enhancement of F1–F3 ($p < 0.05$) for ex vivo mucoadhesive strength was detected as the concentration of mucoadhesive polymer (CP 934) increased, as shown in Table 2. Mucoadhesive strength is strongly correlated with polymer/mucosal tissue binding since the binding is mostly mediated by hydrogen bonds. Therefore, increased CP 934 polymer concentration increased the number of functional groups that were available for binding, mostly hydroxyl groups, and this increased mucoadhesive strength [36].

Additionally, results show that increasing polymer grades (F1 and F4) significantly increases mucoadhesive force ($p < 0.05$). This might be explained by raising the polymer molecular weight, which would increase the amount of hydroxyl group accessible for binding, and increasing the interpenetration of the polymer chain due to the greater flexibility of the polymer structure [37].

3.9. In vitro drug release

An *in vitro* drug release assessment test for F1–F6 was done to investigate the impact of various polymer concentrations and molecular weights on MN release from the generated formulations.

The findings, as illustrated in Figs. 4A and B, demonstrated that as the concentration of mucoadhesive polymers increased, the drug released significantly decreased ($p < 0.05$). The impediment to MN release increased with an increase in the concentration of the mucoadhesive polymer. This release-impeding effect of these mucoadhesive polymers could be clarified by increasing the gel's overall viscosity [38]. In addition to their capability to compress the extramicellar aqueous pathways of poloxamer micelles by which drug diffusion occurs and accordingly slow down the MN release process [39, 40].

It was also found that the MN release from F1, F2, and F3 (CP 934) formulations was significantly ($p < 0.05$) higher than the release rate from the analogous formulas comprising CP 940 (F4, F5, and F6), as shown in Fig. 4C. This is mainly due to the increased viscosity of the prepared formulas as the molecular weight of the polymer increased and, accordingly, the released amount of the active ingredient decreased. It was a predictable result and in accordance with the literature [41].

4. Conclusion

The current study indicates that MN vaginal in situ gel can be effectively developed by using a combination of P407, P188, and CP 934 with proper characteristic features, making it a suitable formulation for vaginal delivery systems with prolonged residence time. To enhance MN release from the in situ vaginal gelling system, the authors plan to investigate the use of other mucoadhesive polymers or MN microemulsions in the future study.

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