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Thermosensitive and mucoadhesive polymer variables affecting development of miconazole nitrate vaginal in situ gelling system

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ABSTRACT: Miconazole nitrate (MNN) is an efficient local antifungal agent with fungistatic and fungicidal activity used for vaginal candidiasis treatment. This study was aimed to formulate and evaluate MNN vaginal *in situ* thermosensitive mucoadhesive gel to enhance the residence time and potentiate its activity at the infection site. *In situ* gel formulas of MNN were formulated by employing the cold method using different concentrations of poloxamer P 407 and P188 alone or in combination as thermosensitive polymers and hydroxypropyl methylcellulose K4M (HPMCK4M), HPMCK15M and gellan gum (GG) as mucoadhesive polymers. The developed formulas were evaluated for different *in vitro* parameters such as gelation time and temperature, clarity, syrangability, pH, content uniformity, viscosity, bioadhesive force, and drug release profile. The results indicated that there is a direct association between the concentrations of poloxamer 188 with gelation temperature while further incorporation of mucoadhesive polymers caused a reduction in gelation temperature. An inverse relationship was observed between polymer molecular weight and concentration with the drug released and a direct relationship with viscosity and mucoadhesive strength. Formula 2 with 18 % P407, 2 % P188 and 0.6% HPMC K4M was selected as the optimal formula with gelation temperature of (34 ±0.033°C), gelation time (4.90 ± 0.012 min), pH value (6.13±0.05), gel spreadability (4.55± 0.02 cm), drug content (99.1 ±0.13 w/v%), mucoadhesion force (0.3136 N) and drug release of (79.5%) over 12 hours. In conclusions and according to obtained results formula 2 could be considered as a doable substitute to ordinary vaginally administered drug delivery systems.

KEYWORDS: *In situ* gel; mucoadhesion; Miconazole Nitrate**;** Poloxamers; thermoresponsive.

1. INTRODUCTION

In situ gel-developing systems are remarkable polymeric systems that endure a phase transition from a water solution to a viscous gel in a physiological site as a result of external stimuli. Different stimuli could trigger gel formation such as solvent exchange, pH alteration, ultraviolet radiation, ionic cross-linkage, and temperature [1]. *In situ* thermo-responsive gel is favored over other types of *in situ* gelling systems mainly because their gelation does not need copolymerization agents, organic solvents, or externally implemented stimuli. Over the past recent years, significant attention has been implied on the improvement of *in situ* gelling systems mainly due to the superiority provided by these systems for instance ease of administration, lessening in administration frequency, in addition to enhanced patient compliance which made patients more expected to consent comparable dosage forms [2]. Poloxamers the basic backbone of thermoresponsive *in situ* gelling systems are a group of thermosensitive amphiphilic triblock copolymers that entail a dominant block of hydrophobic propylene oxide and dual terminal blocks of hydrophilic polyethylene oxide. Included in different forms of poloxamers and due to their thermosensitive performance as well as biocompatibility, poloxamer 407 (P407) and poloxamer 188 (P188) have attracted inordinate attention for possible implementation in drug delivery systems [3]. Altering polymeric platforms could be accomplished by combining two or else more polymers, and their characteristics on persistence could be modulated by compositional modifications [4]. Most researchers have recommended the combined effect of thermo-responsive and /or mucoadhesive characteristics of polymers to attain the necessary biological influence. As stated by numerous published literature the polymer concentration can modify the characteristics of the gels, for instance, mucoadhesion, spreadability, and viscosity, as well as extend the formulation residence time, modify drug release, and enhance the bioavailability [5]. Consequently,

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mucoadhesive preparations have become of great importance for vaginal infection treatment. They provide an attachment between the mucoadhesive polymer and mucus membrane, increasing the residence time at the site of application [6].

Vaginal candidiasis is a mutual illness and up to 75 % of women suffer at least one incidence of genital frequent fungal infection throughout their life. *Candida albicans* is the most vital pathogen responsible for more than 80 % vaginal fungal infections [7]. Miconazole is an antifungal imidazole agent which is employed either as a miconazole base or else miconazole nitrate (MNN) used for vaginal candidiasis treatment. MNN has both fungistatic as well as fungicidal activity which is due to its direct interface with *Candida albicans* cell membrane instead of prevention of biochemical reactions [8]. Even though traditional vaginal dosage forms are observed most secure, many problems are associated with these formulations because of the vagina's self-cleaning behavior, mainly leakage, dripping, and discomfort. These drawbacks resulted in deprived patient compliance and consequently low medication adherence. Prolonged residence time at the targeted site is the most important characteristic of an efficient vaginal drug delivery system [9, 10]. The present study intended to develop a sustained release miconazole nitrate *in situ* thermosensitive mucoadhesive vaginal gel to upsurge the residence time of the drug delivery system allowing prolonged contact time among the drug and the site of infection by mucoadhesion.

2. RESULTS and DISCUSSION

2.1. Gelation Temperature (GT)

The gelation temperature is the temperature threshold at which the liquid spontaneously transforms into a gel. Vaginal gel preparation ought to be at the appropriate GT, which is between 30 and 36 ^{O}C. Preparation with GT lower than 30 ^{O}C may develop early gel formation at room temperature prior to administration. Alternatively gels with GT higher than 37 ^{O}C will fail to develop gel inside the body thus affecting drug and retention time [11]. The gelation temperature of the prepared blank formulas was detected and the results are shown in Table 1. The results indicate that the GT reduced significantly ($p < 0.05$) as the concentration of P407 increased (FP1-FP3). This outcome is mainly due to micellar structure establishment as a result of P407 chain aggregation by hydrophobic interaction and dehydration of poly propylene oxide (PPO) entities at raised temperature [12]. The reverse was perceived by increasing P188 concentration (FP4-FP6). Even though both P407 and P188 have similar hydrophobic PPO entities, the latter has longer hydrophilic poly ethylene oxide (PEO) blocks than that of P407. The longer PEO blocks of P188 could interrupt the inter-micellar packing, shifting the GT to upper temperatures. Comparable observation was noticed by Fakhari *et al* [13]. Utilizing P188 addition (FP7-FP12), as the concentration of P188 in the mixture increased the GT increased significantly. This phenomenon could be clarified by the difference in hydrophilicity of P407 and P188. P188 is more hydrophilic than P407 due to the higher fraction of PEO/PPO related with that of P407. Consequently, the addition of a slight amount of P188 can only alter the PEO fraction in the polymer solutions blend, leading to a rise in the GT [14].

Table 1. Gelation temperature of pilot *in situ* gel formulas

Formula Code	GT (^{O}C)
FP1	≥50
FP2	33 ± 0.41
FP3	28 ± 0.17
FP4	≥50
FP5	≥50
FP6	≥50
FP7	≥50
FP8	≥50
FP9	39 ± 0.31
FP10	37 ± 0.26
FP11	35 ± 0.36
FP12	≥50

Data are stated as (means, ± SD, n = 3).

2.2. Gelation Time (G Time)

Gelation time is defined as the time point at which the elasticity modulus turns greater than the viscosity modulus. In addition to GT, G time is considered another critical point in the advance of *in situ* thermosensitive mucoadhesive gel since prolonged retention of the formulated dosage form by drainage prevention from the site of the application is beneficial [15]. Table 2 demonstrates the G time for the blank formulas (FP1-FP12) and it was in the range of >10 - 2.20 ± 0.33 min. Similar observations was obtained by Karavana *et al* [16]. A significant ($p < 0.05$) decrease in G time was observed as the concentration of P407 used increased (FP1-FP3). The reverse was observed by increasing P188 concentration where the G time was increased significantly ($p < 0.05$) by increasing P188 concentration (FP4-FP6) this is related to the interference of the extended PEO blocks of P188 with the inter micelle structure packing [17]. The incorporation of a small concentration of P188 with P407 (FP7-FP12) resulted in a significant increase in the G time ($p < 0.05$) as the concentration of P188 in the mixture increased. This outcome is related to the different behavior of both P407 and P188 in aqueous solution since the latter is more hydrophilic than the former [18]. Depending on GT and G time FP10 showed a satisfactory GT (37 ± 0.26 °C) to be used within the vaginal cavity temperature as well as an acceptable G time of 6.25 ± 0.13 min. As a result, it was selected for incorporation of MNN and different types (HPMCK4m, HPMCK15M, and GG) at different concentrations (0.4%, 0.6%, and 0.8%) of mucoadhesives polymers and was used for additional studies. Formula 1- F9 were used to study the influence of the addition of mucoadhesive polymers (F1, F4, and F 7), increasing their concentrations (F1-F3), (F4-F6) and (F7-F9) as well as increasing their molecular weight (F2 and F5). The results for the effect of these variables indicated a significant reduction ($p < 0.05$) in both GT and G time upon the addition of mucoadhesive polymers as well as increasing their concentrations and molecular weight, as shown in Table 3. This could be attributed to hydrogen bond formation between the mucoadhesive polymer and the poloxamer's PEO chain. This binding will lead to the early development of micellar structures as a result of the restriction of the hydration of the PEO chain [19]. These outcomes were in agreement with that acquired by Gratieri et al [20].

Table 2. Gelation time of blank pilot formulas

Formula Code	G Time (min)
FP1	≥10
FP2	4.10 ± 0.18
FP3	2.20 ± 0.33
FP4	≥10
FP5	≥10
FP6	≥10
FP7	≥10
FP8	≥10
FP9	8.10 ± 0.21
FP10	6.25 ± 0.13
FB11	5.34 ± 0.24
FB12	≥10

Data are stated as (means, ± SD, n = 3).

2.3. Appearance and Clarity

All prepared formulas were clear as well as transparent as stated in Table 3**.** These characteristics are required mainly for *in situ* gel formulations, as they enable easy handling as well as simplify the calibration of précised dose [21].

Table 3. Physical characteristics MNN vaginal *in situ* gel formulas

Formula Code	GT (°C)	G time (min)	Appearance	pH	Syringeability	Spreadability (cm)	% Drug content
F1	36 ±0.029	5.50 ± 0.031	+++	5.61 ± 0.121	Pass	5.25 ± 0.01	100.21 ±0.21
F2	34 ±0.033	4.90 ± 0.012	+++	5.47 ± 0.203	Pass	4.55 ± 0.02	99.1 ±0.13
F3	31.5 ±0.022	4.30 ±0.051	+++	5.31 ± 0.261	Pass	3.95 ± 0.02	96.43 ± 0.31
F4	35.5 ±0.031	5.20 ± 0.015	+++	5.16 ± 0.121	Pass	4.80 ± 0.13	102.1± 0.22
F5	33 ±0.061	4.70 ± 0.042	+++	5.31 ± 0.052	Pass	4.10 ± 0.17	100.5 ± 0.54
F6	31 ±0.051	3.90 ± 0.21	+++	5.48 ±0.105	Pass	3.50 ±0.085	98.3 ± 0.43
F7	34 ±0.062	5.00 ±0.053	+++	5.52 ± 0.122	Pass	4.50 ± 0.022	100.21± 0.22
F8	32 ±0.051	4.55 ±0.021	+++	5.31 ± 0.21	Pass	4.05 ± 0.01	95.21 ± 0.37
F9	30 ±0.074	3.75 ±0.032	+++	4.92 ± 0.15	Pass	3.10 ± 0.015	99.29 ± 0.81

Data are stated as (means, ± SD, n = 3)

2.4. pH Determination

The normal pH of the vagina is about 3.5–4.5 which may be altered by different causes mainly physiological or pathogenic. Consequently, and to assure vaginal compatibility and stability of formulas, it is important to formulate gels with an optimum pH to prevent irritation and enhance patient compliance. The obtained pH values for the prepared formulas as shown in Table 3 were found to be around (4.92 ± 0.15-5.61 ± 0.121) which is close to the neutral pH of the vagina and assures the stability and patient acceptance of prepared formula to be administered vaginally [22].

2.5. Syringeability

The syringeability of the formulas was resolute as per material and concentration. The syringeability of all the formuls is shown in Table 3. All the formulas were passed spontaneously through the used syringe needle. This finding is important to assure dose measurements precision, simplicity of withdrawal of the dosage form from the container and successive application to the targeted site [23].

2.6. Spreadability

The efficacy of local treatment is influenced mainly by spreading a uniform layer of the prepared formula on the site of application to ensure accurate dose delivery. For that reason, an assessment of the spreadability of the formulas is required. A significant reduction in spreadability ($p < 0.05$) was observed when the polymer concentration was increased. Correspondingly a significant reduction in formulas spreadability ($p < 0.05$) was noticed as the polymer grade was increased as shown in Table 3. Increasing both polymer concentration and grade resulted in a higher degree of cross-linking of the polymer chain and hence increased viscosity subsequently spreadability decreased [24].This could be attributed to the inverse correlation between spreadability and viscosity. The higher viscosity of the gel, the higher the surface tension and accordingly the harder for the gel to be spreadable. A related observation was reported by Lucero et al [25].

2.7. Drug Content

The drug content was found to be around (95.21± 0.37-102.1± 0.22 %) as shown in Table 3. which is in the standard range according to USP [26]. Similarly, non-significant variances ($p > 0.05$) were observed among upper, middle as well as lower samples .These finding specifies that the formulation process implemented was able to produce gels with minimum variability and even drug distribution. [27]

2.8. Viscosity Assessment

The evaluation was carried out at the gel phase under a changed shear rate (rpm). The viscosity results for the selected formulas (F1-F9) are shown in Tables 4 and Figure 1. At room temperature (25°C), all formulas are presented in a solution form. A significant rise ($p<0.05$) in viscosity was perceived by increasing the temperature to physiological temperature. This could be justified by the existence of gel-forming thermosensitive polymers that develop gel and raise viscosity once the temperature is elevated [28].

Table 4. Viscosity of MNN vaginal *in situ* gel formulas at room temperature (24 ± 2 °C, at 10 rpm) using spindle No. 63.

Formula code	Viscosity (cp)
F1	1242 ± 5.8
F2	1253 ± 7.1
F3	1318 ± 6.7
F4	1314 ± 4.2
F5	1366 ± 3.8
F6	1385 ± 6.2
F7	1401 ± 2.9
F8	1452 ± 1.54
F9	1485 ± 3.2

Data are stated as (means ± SD, n = 3)

The obtained data also showed that viscosity decreased significantly ($p<0.05$) as the speed of rotation increased (shear rate) as seen in Figure 1 which indicates the pseudoplastic flow of prepared formulas [29]. Another observation was made related to the mucoadhesive polymer used. Formula 3 and F6 which contain HPMCK4M and HPMCK15M respectively demonstrate higher viscosity in a concentration as well as molecular weight dependent manner at both room and physiological temperature. Analogous results were observed by Kim *et al* [30] This could be correlated to the hydrogen binding capability of both HPMC and GG to a greater degree with the oxygen atom of PEO block in poloxamer [31], .Higher viscosity was observed with F9 due to a higher concentration of GG compared to other formulas. A similar outcome was observed by Tuğcu-Demiröz *et al* [32].In situ gel dissent the Arrhenius equation relates the viscosity and temperature.

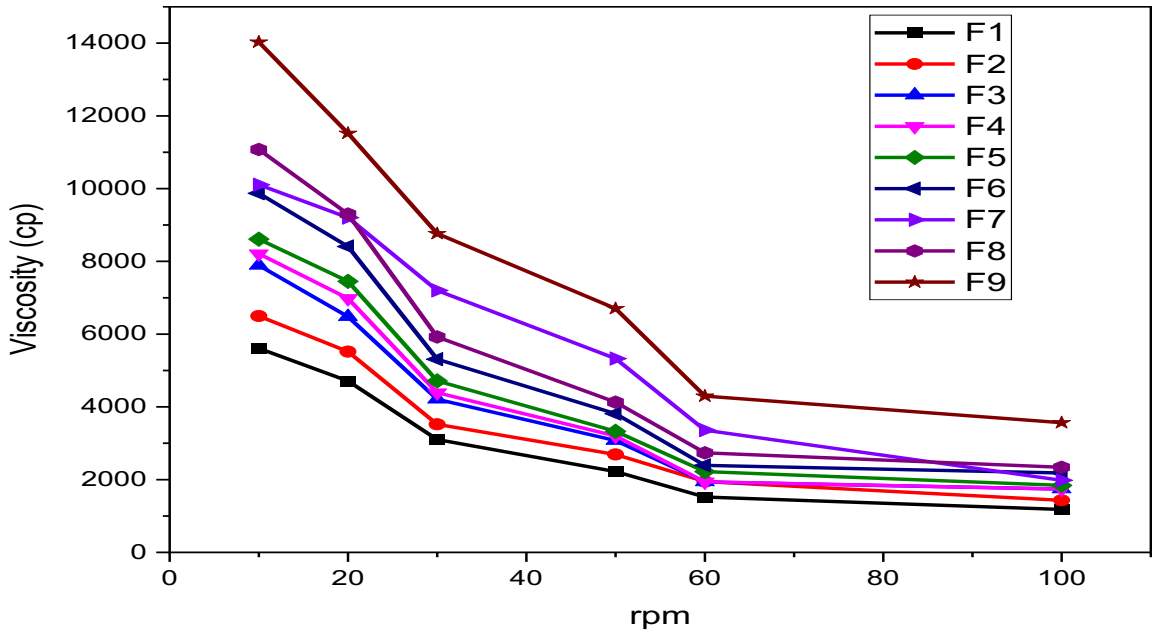

Figure 1. Rheogram profiles for MNN vaginal *in situ* gel formulas at 34 °C using spindle No. 63

2.9. Mucoadhesive Force

Bioadhesive characteristics of vaginal drug delivery systems considered as one of the most challenging parameters due to their role in retention time enhancement and formula leakage reduction. This is mainly owing to the self-cleaning capability of the vagina and the slow flush of vaginal fluid to wash unwanted surplus [33]. Accordingly, it is beneficial to estimate formula/mucosal surface interaction when the mucoadhesive drug delivery system is been formulated. Table 5 demonstrates the *ex vivo* mucoadhesive strength, force as well as band strength of prepared formulas. Significant enhancement ($p < 0.05$) for *ex vivo* mucoadhesive strength was observed as the concentration of the used mucoadhesive polymer increased. Mucoadhesive strength is directly related to the polymer/mucosal tissue binding since hydrogen bound is mainly responsible for this binding. Consequently, increasing polymer concentration directed to an increase in the functional group number mainly hydroxyl groups available for binding, and hence increased mucoadhesive strength [34]. Results also demonstrate a significant increase in mucoadhesive force ($p < 0.05$)

as a result of increasing polymer grade (F1 and F4). This could be justified by increasing the interpenetration of the polymer chain as a result of increased polymer structure flexibility as well as increasing the hydroxyl group available for binding by increasing the polymer molecular weight [35].

Table 5. Mucoadhesive force of MNN vaginal *in situ* gel formulas.

Formula Code	Bioadhesive strength (gm)	Adhesion Force (N)	Bond Strength (Nm^{-2})
F1	22 ± 0.21	0.2156	5.39
F2	32 ± 0.165	0.3136	7.84
F3	48 ± 0.231	0.4704	11.76
F4	61 ± 0.253	0.5978	14.945
F5	83 ± 0.176	0.8134	20.335
F6	97.5 ± 0.295	0.9555	23.8875
F7	18 ± 0.176	0.1764	4.41
F8	21 ± 0.2	0.2058	5.145
F9	30 ± 0.201	0.294	7.35

Data are stated as (means ± SD, n = 3)

2.10. In vitro Drug Release

In vitro drug release evaluation test was applied for (F1-F9) to study the influence of different polymer variables on MNN release from prepared formulas.

The results as shown in Figure 2A, 2B and 2C demonstrated that as the concentration of mucoadhesive polymers increased the drug released was decreased significantly ($p < 0.05$). The hindrance of MNN release increased with rise in the mucoadhesive polymers concentration. This release impeding effect of these mucoadhesive polymers could be justified by increasing the whole gel viscosity [36] in addition to their capability to squash the extra-micellar aqueous pathways of poloxamer micelles by which the drug diffusion occurs and accordingly delaying MNN release process [37]. Alternatively a fast initial MNN release was observed with formulas containing HPMCK4M as mucoadhesive polymer (F1, F2 and F3) as shown in Figure 2A, this could be due to the presence of formula in solution form at the start and gradual conversion till complete gel formation. [38] Similar observation was reported by Dholakia *et al* [39].

Significant reduction in drug release ($p < 0.05$) was observed when the same concentration 4% of HPMCK4M (F1) was substituted by same concentration of HPMCK15M and GG respectively as shown in Figure 2D [23]. It was also found that the MMN release from F1 (HPMCK4M) formulation was significantly ($p < 0.05$) higher than the release rate from the analogous formulas comprising HPMCK15M (F4) as shown in Figure 2D. This is mainly due to increase the viscosity of the prepared formulas as the molecular weight of polymer increased and accordingly the released amount of active ingredient decreased. It was a predictable result and in accordance with the literature [40].

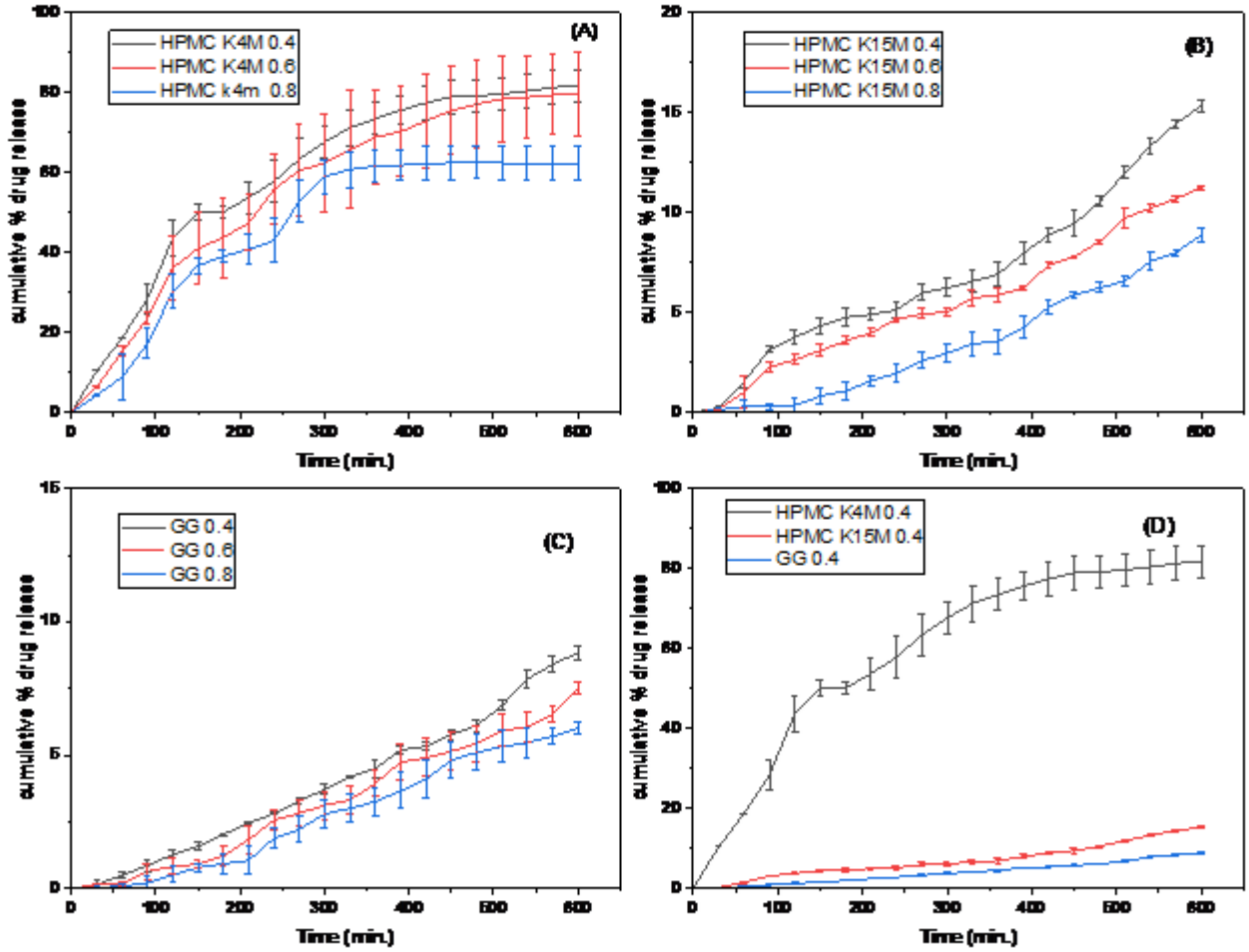

Figure 2. (A, B and C) Effect of polymer concentration on *in vitro* drug release (D) Effect of polymer type and grads on *in vitro* drug release. Data are stated as (means, ± SD, n = 3).

3.CONCLUSION

From current study, an *in situ* gel with improved mechanical and mucoadhesive properties as well as improved residence time in the vagina, was obtained by using a modified cold technique to successfully create mucoadhesive *in situ* gel formulations of MNN by utilizing a mix of the combination of poloxamer 407,poloxamer 188 and diffrent mucoadhesive polymer ratios. According to this study's in vitro characterization, it was concluded that the tempreture sensative *in situ* gel of MNN with combination of P407, P188 and HPMCK4M may be a promising and innovative therapeutic system. *In situ* gelling system containing MNN can offer some advantages in the treatment of vaginitis in terms of providing increased mucoadhesive qualities necessary for sustained drug delivery as well as regulated release. To validate the findings of this study, additional ex vivo and in vivo studies are necessary, and these investigations will be performed.

4. MATERIALS AND METHODS

Miconazole nitrate was generously gifted by Al-Safa Pharmaceuticals Industries (Baghdad, Iraq). Poloxamer 407 and Poloxamer 188 were purchased from Sigma Aldrich (USA). Hydroxy Propyl Methyl Cellulose (HPMC) K4M, HPMC K15M, Sodium acetate (SA), sodium lauryl sulfate (SLS), and Gellan gum (GG) were purchased from Alpha chemika (India). All other reagents and chemicals were of analytical grade and used deprived of additional purification.

4.1. Methods

4.1.1. Preparation of MMN Vaginal sin Situ Gel Formulas

The cold technique was employed to prepare MNN vaginal *in situ* thermosensitive gel utilizing poloxamer as a gel-forming polymer [41]. Blank pilot formulas were prepared to detect the most appropriate concentrations of the gelling polymers P 407 and P 188 and screened for their gelation temperatures. Required amount of P407 and 188 as shown in Table 6 were dispraised in cold DIW slowly with continuous stir till thorough desperation took place. To ensure complete dissolution and air bubbles elimination the prepared solutions were kept overnight at 4 °C.

Table 6. Blank pilot formulas

Formula Code	P 407 w/v (%)	P 188 w/v (%)	DIW Up to mL
FP1	10	-	100
FP2	15	-	100
FP3	20	-	100
FP4	-	10	100
FP5	-	15	100
FP6	-	20	100
FP7	15	5	100
FP8	16	4	100
FP9	17	3	100
FP10	18	2	100
FP11	19	1	100
FP12	20	5	100

Formula with an appropriate temperature of gelation (30°C -36°C) was selected for the enclosure of MNN and different types of bioadhesive polymers at different concentrations as shown in Table 7**.** MNN as well as bioadhesive polymers were dispersed totally in 30% of the total volume of DIW with continuous stir by magnetic stirrer (APOPS, MS300HS) at 60 °C and then cooled down to 25 °C. P 407 and P 188 were consciously mixed at 100 rpm with the remaining 60% total volume of DIW at 4 °C till completely dissolved. Both solutions were then mixed and the volume was measured and adjusted using cold DIW. Finally to confirm thorough solubilization the resultant formulas were kept in the refrigerator overnight at 4°C till a transparent clear solution was acquired [42].

Table 7. Composition of MNN vaginal *in situ* gel formulas

Formula Code	P407 w/v (%)	P188 w/v (%)	MNN w/v (%)	HPMC K4M w/v (%)	HPMC K15M w/v (%)	GG w/v (%)	DIW Up to (mL)
F1	18	2	0.2	0.4	-	-	100
F2	18	2	0.2	0.6	-	-	100
F3	18	2	0.2	0.8	-	-	100
F4	18	2	0.2	-	0.4	-	100
F5	18	2	0.2	-	0.6	-	100
F6	18	2	0.2	-	0.8	-	100
F7	18	2	0.2	-	-	0.4	100
F8	18	2	0.2	-	-	0.6	100
F9	18	2	0.2	-	-	0.8	100

4.1.2. Characterization of Vaginal Thermosensitive Mucoadhesive In Situ Gel Formulas

Assessment of Gelation Temperature

The gelation temperature assessment of the prepared formulas was done as follows: 5 mL of formula was poured into a 20 mL transparent test tube in which a magnetic bar was placed. The test tube was then submerged in a water bath at 25 °C. A thermometer was then submerged in the solution, which was heated at a constant rate (2 °C/min) and constant rate of stirring (100 rpm). The temperature at which the magnetic bar was completely unmoving was verified as the gelation temperature. Each measurements were done in triplicate (n = 3) [43].

Assessment of Gelation Time

The gelation time was estimated by the following procedure: 5 mL of each formula was placed in a 20 mL vial including a magnetic bar. In a thermostated bath the vial was immersed at 36 °C as well as a constant stir rate (100 rpm). The time upon which the magnetic bar was completely immobile was regarded as a time of gelation [44].

Appearance and Clarity Determination

The clearness of the prepared formulas was evaluated visually in light under substitute white and dark backgrounds. The degree of clarity for the formula was categorized as follows: turbid gel (+), clear gel (++), and very clear gel (+++) [45].

In Situ Gel pH Assessment

The pH of the developed formulas was dignified by using a digital glass electrode pH meter (Hanna Instruments, Italy). The test was performed in triplicate (n=3) and the data was recorded [46].

Assessment of Syringeability

One mL of prepared formula was transferred to a 5 mL disposable syringe with a 20-gauge needle. The formulas that were passed simply from the syringe were labeled as pass, while the ones that passed with difficulty were labeled as fail [47].

Assessment of Spreadability

For spreadability determination, a specified amount of formula (1 g) was located at the midpoint of the glass plate with dimensions of (23 cm × 10 cm). The glass plate was then shielded by another glass plate and 1 kg weight was placed with care on the top of the two plates (without sliding). The gel was spread between the two plates. The plate was distant after 30 min and the diameter of the spread area (cm) was measured in triplicate (n=3) [48].

Drug Content Determination

One mL of the formulation which is equivalent to 20 mg/mL MMN *in situ* gel was precisely measured and diluted by acetate buffer (pH 4.10). The filtrate which was obtained by filtration via a 0.45 µm syringe filter was then used to determine the concentration of MNN by measuring the absorbance spectrophotometrically at 230 nm. Samples were collected from three different points of the prepared gel: the upper, middle, and bottom of each formula to confirm the even distribution of MNN in the formula [49].

Assessment of In Situ Gel Viscosity

The viscosity of the formulated gels was determined by means of Brookfield viscometer DV-II PRO. At low temperatures about 30 mL of the formula was placed in a small sample adapter. The temperature of the formula was elevated to 37 °C utilizing a water bath then viscosity was measured and documented at 25 °C and 37 °C by spindle no. 64. Each data point is a mean of triplicate analysis [50].

Mucoadhesive Force Determination

The mucoadhesive strength of the prepared formulas was resolute by employing a modified balance method as shown in Figure 3 by assessing the force necessary to separate the formulas from a sheep's vaginal tissue. The mucosal vaginal tissue was gotten from a local slaughterhouse [51].

Ten grams of gel was fixed to a platform provided by a small glass container placed lower the right pan of the balance. To the right arm of the balance, a section of sheep vaginal tissue was glued to the movable wooden platform. Before testing 1 mL of acetate buffer pH 4.1 was used to submerge the exposed gel and left for half a minute for preliminary hydration. The hydrated gel comes in contact with the mucosal surface by moving the platform upwards. A 20-gram preload was located above the right pan for 3 min to provide the initial pressure required. At that point, the preload was removed from the right pan followed by gradual addition of weight to the left pan till the gel detachment from the vaginal mucosal surface. The whole weight necessary for the complete separation of the gel was recorded.

Both of force of adhesion and bond strength were calculated by Equations 1 and 2 [52].

$$1.\ldots\ldots \boldsymbol{Force\ of\ adhesion\ (N)} = \frac{\boldsymbol{Bioadhesivestrength}\times 9.8}{1000}$$

$$2\ldots\ldots Bond\ strength\ (\frac{N}{M^2}) = \frac{Force\ of\ adhesion(N)}{Surface\ area\ (M^2)}$$

Figure 3. Demonstrated modified balance method.

In vitro Drug Release Study

To mimic the *in vivo* release of MNN *in situ* gel formulas in the laboratory, a modified method including a type II dissolution apparatus was used. The semipermeable dialysis membrane (MWCO 8000-14000 D) was submersed in the dissolution medium for 24 hours and opened on both ends [53]. Then one side was firmly sealed with a rubber band. The membrane was full of 10 grams of *in situ* gel formula, then the other side of the membrane was sealed by an additional rubber band and the membrane was held around the paddle. By lowering the paddle, the membrane is then immersed in a previously filled dissolution jar with 100 mL of acetate buffer (pH 4.10) upheld at 36± 0.5°C and 50 rpm. At scheduled time intervals 5 mL samples were gotten after and were substituted by an equal volume of fresh dissolution media to preserve sink condition. After that, calm samples were filtered by using a filter paper (0.45 µm, Millipore filter syringe) and investigated spectrophotometrically at 230 nm of MNN in acetate buffer. A formerly assembled calibration curve was used to estimate the amount of MNN released. The experiments were conducted in triplicate [54].

4.1.3. Data Statistical Analysis

Statistical analysis for all experimental data was implemented using IBM SPSS statistic 25 software. Data were expressed as mean values with their standard deviation (SD). ANOVA test was empoloyed to approve the significance as statistical differences considered significant when ($p<0.05$) [55].

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