

Thermoresponsive Microgel Loaded with a Clotrimazole Microemulsion Based on Bulgarian Rose Oil for the Treatment of Vaginal Candidiasis

Halah M. Anwar* ¹, Iman S. Jaafar¹

¹Department of Pharmaceutics, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

E-mail address:

halamohammed93@uomustansiriyah.edu.iq (Halah M. Anwar) <https://orcid.org/0009-0008-8533-8954>

Abstract

Background: Vulvovaginal candidiasis (VVC) is one of the most common fungal infections affecting women, typically caused by *Candida albicans*. Conventional clotrimazole (CLT) formulations provide limited relief because of their short residence time and poor drug dissolution at the vaginal site. In this study, Bulgarian rose oil (BRO) was selected as a functional, bioactive oil phase to develop a microemulsion (ME) incorporated into a thermosensitive, mucoadhesive *in situ* gel. The system was designed to simultaneously overcome CLT solubility issues and improve local antifungal retention.

Methods: Clotrimazole-loaded microemulsions were prepared using Bulgarian rose oil, Tween 60, and Transcutol P and characterized for physicochemical properties, stability, and *in vitro* drug release. Thermosensitive mucoadhesive *in situ* gels were optimized using a D-optimal design, and the optimized microemulsion was incorporated into the selected gel base. The resulting microgel was evaluated for gelation properties, drug release, antifungal activity, morphology, and vaginal irritation.

Results: The selected formulation (ME3) consisted of uniform nanosized droplets (~27 nm). It exhibited a clear appearance and a faster drug release rate (76% in 6 h) than the CLT suspension. Incorporating ME3 into a thermosensitive gel containing Poloxamer 407, Poloxamer 188, and hydroxypropyl methylcellulose (HPMC) K4M produced a transparent microgel (MG) system that gelled near body temperature (35.4 °C) and exhibited suitable viscosity and pH. The MG produced a larger inhibition zone (43 mm) against *Candida albicans* than both the plain gel and a marketed

product (32–33 mm). No signs of irritation were observed in rabbits, and microscopy confirmed the uniform distribution of ME droplets within the gel matrix.

Conclusion: Overall, this MG formulation combined effective antifungal activity with good tissue compatibility and prolonged drug release, suggesting its potential as an improved local therapy.

Keywords: Candidiasis, Clotrimazole, Volatile Oils, Hydrogels, Drug Delivery Systems

Introduction

Vulvovaginal candidiasis (VVC), predominantly caused by *Candida albicans*, is a highly prevalent gynecological infection that affects nearly three-quarters of women during their reproductive years.¹ However, treatment is challenged by vaginal self-cleansing, mucus turnover, and gravity, which rapidly remove conventional topical formulations, resulting in reduced local drug levels and frequent recurrence.^{2,3}

The efficacy of clotrimazole (CLT) is limited by its extremely poor aqueous solubility (~0.49 µg/mL), which restricts its dissolution and local bioavailability. Conventional creams and suppositories also exhibit poor mucosal retention, leakage, and uneven drug distribution, reducing therapeutic effectiveness and patient adherence.⁴ These limitations are further complicated by increasing antifungal resistance in *Candida albicans*, driven by altered membrane composition, drug-efflux pumps, and protective biofilm formation.⁵ Therefore, advanced vaginal delivery systems are needed to improve drug solubility, prolong mucosal retention, and enhance antifungal activity against resistant infections.⁶

In parallel, essential oils (EOs) exhibit intrinsic antifungal activity against *Candida albicans* and may enhance the activity of azole antifungals.⁷ Bulgarian rose oil (BRO) has demonstrated notable anti-*Candida* activity, mainly due to its geraniol and citronellol content.⁸ Geraniol disrupts fungal membrane integrity by interfering with ergosterol biosynthesis and inhibiting plasma membrane ATPases.⁹ These monoterpenes also suppress fungal virulence factors and modulate inflammatory responses.¹⁰

Microemulsions (MEs) are thermodynamically stable, transparent dispersions that improve the solubilization and dissolution of poorly water-soluble drugs and are simple to prepare.^{11,12}

For vaginal delivery, the dosage form must remain at the application site, as conventional creams and gels are rapidly cleared, limiting contact time.¹³ Thermosensitive mucoadhesive *in situ* gels (IGs) address this by remaining fluid at room temperature and gelling at body temperature,

improving placement and retention.^{13,14} Poloxamers 407 and 188 enable this sol–gel transition while improving the solubilization of hydrophobic drugs.¹⁵ Mucoadhesive polymers are commonly added to enhance contact with the vaginal mucosa and counteract the natural clearance process.¹⁶ Hydroxypropyl methylcellulose (HPMC) is particularly useful because it interacts with mucin, increasing viscosity and residence time in vaginal formulations.¹⁷ Poloxamer-based thermosensitive gels containing azole-loaded microemulsions have shown promising antifungal activity against *Candida albicans*.¹⁸

Despite these advances, most vaginal microemulsions rely on inert oils that act only as solubilizing vehicles, whereas using a therapeutically active essential oil as the oil phase can enhance the bioactivity of the microemulsion system.¹⁹ Combining azole antifungals with essential oils in a microemulsion has been reported to improve antifungal performance and mucosal penetration.²⁰ Therefore, Bulgarian rose oil was selected as the oil phase to improve clotrimazole solubilization while providing intrinsic antifungal activity.

This work aimed to develop a vaginal thermosensitive, mucoadhesive *in situ* gel incorporating a CLT-loaded ME based on Bulgarian rose oil to improve CLT solubility, local retention, and antifungal activity against *Candida albicans*.

Methods

Materials

CLT was kindly supplied by Al-Safa Pharmaceutical Industries, Iraq. BRO, derived from the flowers of *Rosa damascena* Mill., was obtained from Ji'an Xiangyuan Natural Flavors Co., China. BRO is produced by steam distillation of the flowers and is characterized mainly by the monoterpene alcohols citronellol, geraniol, and nerol. Pine needle oil (PO), tangerine oil (TO), and lemon oil (LO) were purchased from Caesar & Loretz GmbH, Germany. Tween 60 (T60) was supplied by Alpha Chemika, India, and Tween 20 (T20) by Merck Schuchardt, Germany. Labrasol® (LB) was obtained from Hubei KDG Web Science & Technology Co., China. Propylene glycol and polyethylene glycol 400 were purchased from AXO Industry, Belgium, while Transcutol® P (TP) was obtained from Macklin Biochemical Co., China. Poloxamer 407 and Poloxamer 188 were purchased from BASF, Germany. HPMC K4M, HPMC K15M, sodium alginate, and carboxymethyl cellulose (CMC) were obtained from Sigma-Aldrich, Germany. All chemicals were of analytical grade.

Preliminary antifungal activity screening

The antifungal activity of the selected essential oils and surfactants against *Candida albicans* was evaluated using the agar well diffusion method. The tested essential oils were BRO, PO, TO, and LO, while the tested surfactants were T60, T20, and LB. Inhibition zones were measured after 24 h of incubation at 37 °C. Results were expressed as mean $\pm$ standard deviation (n = 3), and statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test. Dimethyl sulfoxide (DMSO) (0.5%) served as the negative control.²¹

Determination of the saturated solubility of CLT

Excess CLT was added to the selected oils and surfactants until equilibrium was reached. After centrifugation and filtration, the concentration of the dissolved drug was quantified by ultraviolet–visible (UV–Vis) spectrophotometry.²²

Construction of pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed by the aqueous titration method using BRO as the oil phase, and T60/TP as the surfactant/cosurfactant mixture (Smix) at ratios of 1:1, 1:2, and 2:1. Clear, homogeneous microemulsion regions were identified and plotted using OriginPro.²³

Microemulsion formulation

CLT-MEs were prepared by dissolving CLT in the selected Smix, followed by the gradual addition of BRO and deionized water until clear, homogeneous formulations were obtained. Nine formulations with different Smix ratios and concentrations were prepared as shown in Table 1.²⁴

Table 1. Composition of CLT-loaded ME formulations.

Formula Code	Smix Ratio	CLT (% w/w)	BRO (% w/w)	Smix (% w/w)	Deionized water (% w/w)
ME1	1:1	0.2	4	30	q.s. to 100%
ME2	1:1	0.2	4	35	q.s. to 100%
ME3	1:1	0.2	4	40	q.s. to 100%
ME4	1:2	0.2	4	30	q.s. to 100%
ME5	1:2	0.2	4	35	q.s. to 100%
ME6	1:2	0.2	4	40	q.s. to 100%
ME7	2:1	0.2	4	30	q.s. to 100%
ME8	2:1	0.2	4	35	q.s. to 100%

ME9	2:1	0.2	4	40	q.s. to 100%
------------	-----	-----	---	----	--------------

q.s., quantity sufficient.

Characterization of CLT-ME

Macroscopic and stability evaluation

Formulations were visually inspected for clarity and thermodynamic stability by centrifugation and heating–cooling cycles. Formulations showing no phase separation were considered stable.²⁵

Droplet size, polydispersity index (PDI), and transmittance

Particle size, PDI, and transmittance were measured by dynamic light scattering using a Litesizer DLS 500 (Anton Paar GmbH, Graz, Austria) at 25 °C.²⁶ All tests were performed in triplicate.

Physicochemical characterization

The pH, drug content, and viscosity of the CLT-ME were determined using standard analytical methods. The pH was measured using a digital pH meter (HANNA® Instruments, HI 2210/EC214, USA). Drug content was quantified spectrophotometrically at 260 nm using the calibration curve of CLT in methanol using a UV–Visible spectrophotometer (Shimadzu, Japan), and viscosity was measured using a Brookfield DV-II Pro viscometer (Brookfield, USA) at 25 °C.²⁷

In vitro drug release test

The *in vitro* release of CLT from the microemulsions was evaluated using a dialysis bag attached to a modified USP type II dissolution apparatus (Dissolution 8000, Copley Scientific, UK) in acetate buffer (pH 4.1) containing 0.5% sodium lauryl sulfate (SLS) at 36 °C. Samples were collected over 6 h and analyzed spectrophotometrically at 264 nm using the calibration curve of CLT prepared in the acetate-buffer release medium.²⁸ An aqueous CLT drug suspension (DS) served as the control.²⁹ All tests were performed in triplicate.

Design of experiments

A D-optimal design generated using Design-Expert® v13 (Stat-Ease, Minneapolis, MN, USA) was used to prepare 12 thermosensitive IG formulations by varying the polymer type (HPMC K4M, HPMC K15M, CMC, or sodium alginate) and concentration (0.3%, 0.6%, and 0.9% w/v) (**Table 2**). The experimental design was used to evaluate the influence of the formulation variables

on the measured responses and to identify the optimized formulation with the desired characteristics.

Table 2. Composition of thermosensitive *in situ* gel formulations

Formula Name	Poloxamer 407 (%)	Poloxamer 188 (%)	Mucoadhesive Polymer	Concentration (%)
IG1	18	0.5	HPMC K4M	0.3
IG2	18	0.5	HPMC K4M	0.6
IG3	18	0.5	HPMC K4M	0.9
IG4	18	0.5	HPMC K15M	0.3
IG5	18	0.5	HPMC K15M	0.6
IG6	18	0.5	HPMC K15M	0.9
IG7	18	0.5	Sodium Alginate	0.3
IG8	18	0.5	Sodium Alginate	0.6
IG9	18	0.5	Sodium Alginate	0.9
IG10	18	0.5	CMC	0.3
IG11	18	0.5	CMC	0.6
IG12	18	0.5	CMC	0.9

Preparation of thermosensitive *in situ* gel formulations (gel base)

The thermosensitive *in situ* gels were prepared by the cold method. Poloxamers and the selected mucoadhesive polymers were dispersed in deionized water, mixed to obtain homogeneous formulations, and stored at 4 °C overnight before evaluation.¹⁸

Characterization of thermosensitive *in situ* gel formulations

Gelation temperature and gelation time measurement

Gelation temperature and gelation time were determined using a modified tube-inversion method. Approximately 5 g of each formulation was heated to determine the gelation temperature, while the gelation time was recorded at the predetermined gelation temperature as the time required for complete gel formation. All measurements were performed in triplicate.³⁰

Viscosity measurements

The viscosity of the *in situ* gels was measured at 25 °C and 37 °C using a Brookfield DV-II Pro viscometer (Brookfield, USA) fitted with spindle No. 63 at 50 rpm.³¹

Mucoadhesion strength measurement

Vaginal mucosal tissue from healthy female sheep was used to determine the detachment force between the formulation and the vaginal tissue using a modified balance method. The measured detachment force was normalized to the vaginal tissue contact area and expressed as mucoadhesive strength in dyne/cm².³² All tests were performed in triplicate.

Spreadability measurements

Spreadability was determined by placing 1 g of gel between two glass plates under a 1 kg weight for 30 min, after which the spread diameter was measured.³³

Preparation of ME-based *in situ* gel (CLT-MG) and CLT *in situ* gel (CLT-IG)

The optimized ME was incorporated into the pre-equilibrated thermosensitive gel base under gentle stirring to prepare the CLT-MG. A plain CLT-IG was prepared similarly by dispersing CLT directly into the gel base.³⁴

***In vitro* drug release study**

The *in vitro* release profiles of CLT from CLT-MG, CLT-IG, and the marketed product (MP; Cipla vaginal cream, 1% w/w CLT) were evaluated using the dialysis bag diffusion method for 12 h. Equivalent amounts of CLT were used for CLT-MG, CLT-IG, and the marketed product to ensure dose-matched comparison. Statistical differences were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test ($p < 0.05$), considering formulation type and time as the two factors.³⁵

Release kinetic modeling

The *in vitro* release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models using DDSolver (Microsoft Excel). The best-fitting model was selected based on the highest correlation coefficient (R^2), while the release exponent (n) from the Korsmeyer–Peppas model was used to determine the drug-release mechanism.³⁶

Morphological examination by FESEM

The surface morphology of the CLT-ME and CLT-MG was examined by field-emission scanning electron microscopy (FESEM; Inspect F50, FEI, Netherlands) operated at 30 kV to evaluate particle shape and surface characteristics.³⁷

***In vitro* antifungal activity**

The *in vitro* antifungal activity of CLT-MG, CLT-IG, and MP was evaluated using the agar well diffusion method. Equivalent amounts of CLT were used for all formulations to ensure a dose-matched comparison. Each formulation was tested independently in triplicate ($n = 3$), and the plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters and expressed as mean $\pm$ SD. DMSO (0.5%) served as the negative control. Statistical differences among the formulations were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.²¹ A p -value < 0.05 was considered statistically significant.

Evaluation of *in vivo* vaginal irritation potential

The vaginal irritation potential of the optimized CLT-IG and CLT-MG was evaluated in rabbits following approval by the Animal Ethics Committee of Mustansiriyah University (Approval No. 49; Ref. No. 225; 17 July 2025) and in accordance with the American Veterinary Medical Association (AVMA) 2020 guidelines.³⁸ Rabbits were divided into three groups ($n = 3$): a CLT-IG-treated group, a CLT-MG-treated group, and a negative control group. The formulations were administered intravaginally, and the vaginal tissues were examined at 1, 24, and 48 h for signs of erythema and edema using a standard 0–4 irritation scoring system, where 0 = no erythema, 1 = slight erythema (barely perceptible light pink), 2 = moderate erythema (dark pink), 3 = moderate to severe erythema (light red), and 4 = severe erythema (extreme redness).

Results and discussion

Preliminary antifungal activity screening

BRO exhibited the strongest activity against *Candida albicans* (25 mm), followed by PO (21 mm) and LO (20 mm), whereas TO showed no inhibitory activity (Figure 1). These findings are consistent with previous reports attributing the antifungal activity of BRO to its major constituents—citronellol, geraniol, and nerol—which disrupt fungal viability.³⁹ None of the surfactants (T60, T20, or LB) produced inhibition zones. Based on these findings, BRO was selected for the optimized ME and the subsequent *in situ* gel.



Figure 1. Antimicrobial screening results showing the inhibition zones (mm) of the selected essential oils and surfactants against *Candida albicans* after 24 h of incubation. Data are presented as mean $\pm$ SD (n = 3).

Saturated solubility study of CLT in oils and surfactants

The saturated solubility of CLT in candidate oils, surfactants, and co-surfactants was evaluated to identify suitable formulation components (Figure 2). Among the tested oils, BRO exhibited the highest solubility (121 mg/mL), followed by LO, TO, and PO ($p < 0.05$). T60 showed the greatest solubilization capacity among the surfactants (62.5 mg/mL), while TP exhibited the highest solubility among the co-surfactants (48.9 mg/mL). Therefore, BRO, T60, and TP were selected for ME formulation (Figure 2).⁴⁰

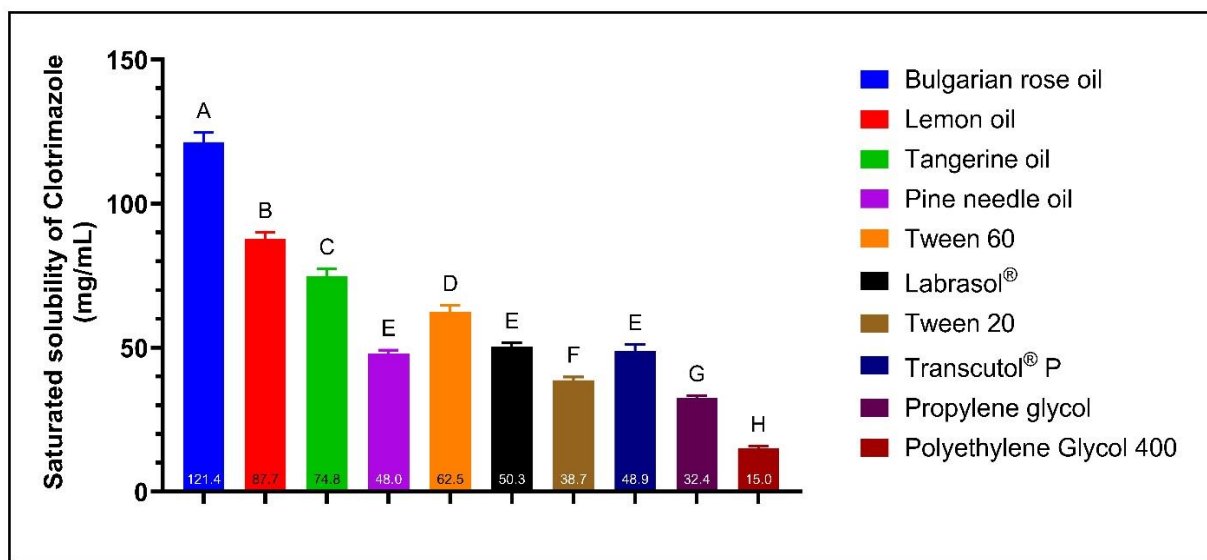


Figure 2. Saturated solubility profile of CLT across candidate ME components.

Construction of pseudo-ternary phase diagram

The pseudo-ternary phase diagrams (Figure 3) identified the ME regions of the BRO–water–Smix system, where the shaded areas represent clear, stable, single-phase MEs, and the unshaded areas indicate unstable or multiphase systems. The ME region expanded with increasing surfactant-to-cosurfactant ratio, following the order $2:1 \geq 1:1 > 1:2$, demonstrating that a higher T60 content enhanced ME formation by reducing interfacial tension and improving interfacial film flexibility. Based on these diagrams, three formulations from each Smix ratio were selected for further characterization.^{41,42}

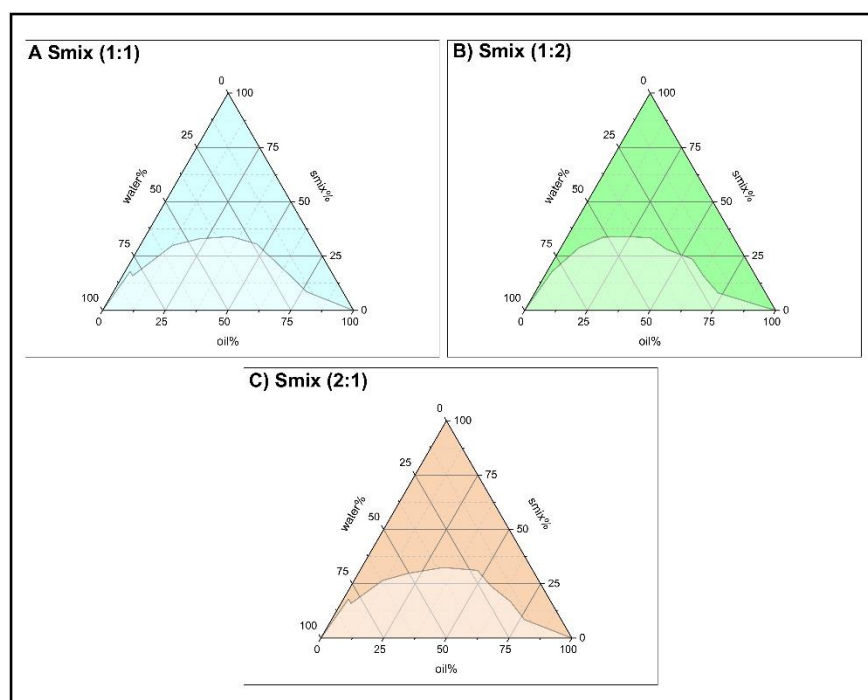


Figure 3. Pseudo-ternary phase diagrams of the BRO–water–Smix (T60/TP) system at 25 °C showing three Smix ratios: (A) 1:1, (B) 1:2, and (C) 2:1.

Characterization of CLT-ME

Macroscopic and stability evaluation

All CLT-MEs appeared translucent and free of phase separation. Following thermodynamic stability testing, ME8 and ME9 became turbid and were excluded, whereas the remaining formulations remained physically stable.^{43,44}

Droplet size, PDI, and transmittance

The selected MEs (ME1–ME7) exhibited droplet sizes ranging from 24.52 to 37.96 nm (Figure 4A). The Smix ratio significantly affected droplet size ($p < 0.05$), with the 2:1 system producing the smallest droplets (ME7; 24.52 nm) and the 1:2 system producing the largest (ME4; 37.96 nm), reflecting the effect of surfactant concentration on interfacial tension. All formulations showed a PDI below 0.3 (Figure 4B), indicating a uniform droplet distribution. Transmittance values ranged from 91.3% to 93.4%, with no significant differences among formulations (Figure 4C), confirming the formation of clear and stable MEs.^{45,46}

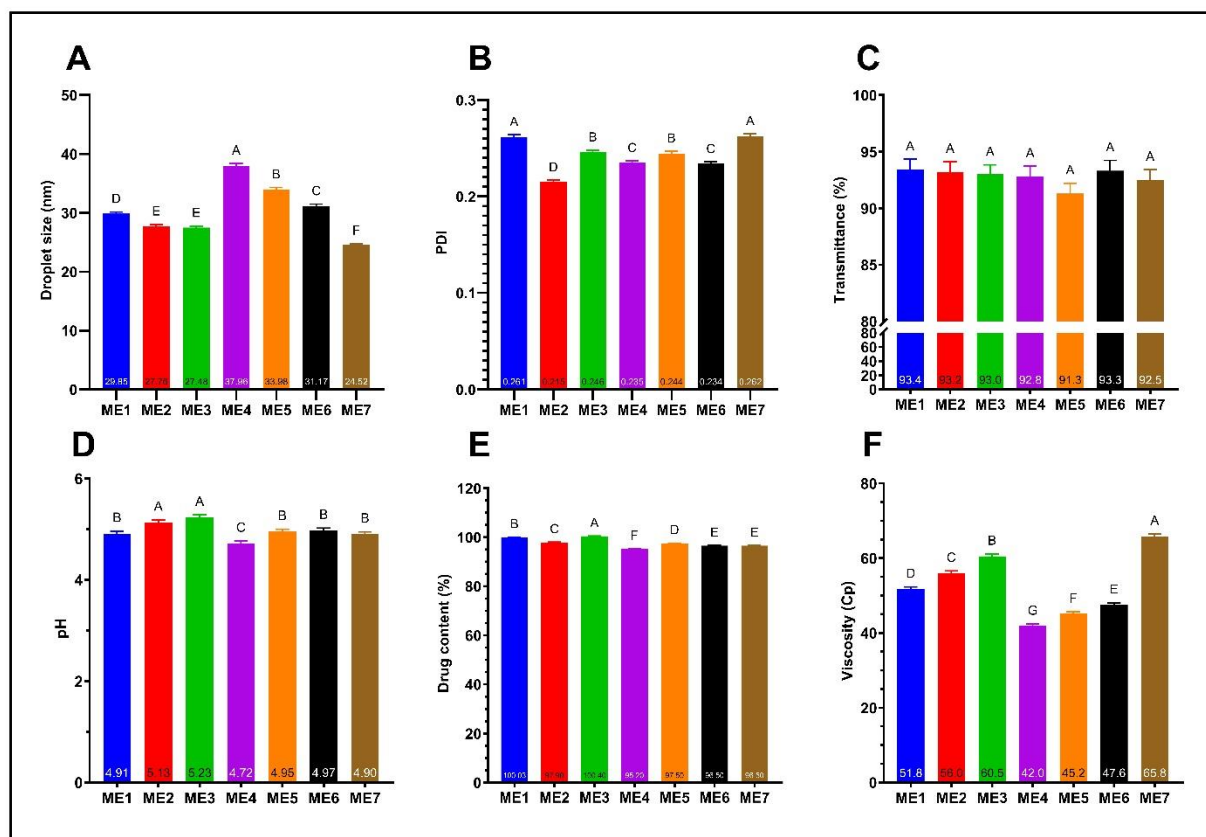


Figure 4. Physicochemical characterization of CLT-loaded MEs (ME1–ME7).

Physicochemical properties of CLT-ME

Regarding local compatibility, the pH of all preparations (Figure 4) was slightly acidic (4.72 to 5.23), safely falling within the physiological range of the vaginal mucosa.⁴⁷ Drug content exceeded 95% in all formulations, indicating efficient drug incorporation.⁴⁸ Viscosity increased significantly ($p < 0.05$) with increasing Smix concentration and higher T60 content.⁴⁵

***In vitro* drug release test**

All CLT-MEs exhibited significantly ($p < 0.05$) higher drug release (60.0–76.2%) than the raw DS ($15.5 \pm 0.6\%$) over 6 h (Figure 5), reflecting the improved dissolution provided by the nanosized droplets.⁴⁹ ME3 exhibited significantly ($p < 0.05$) higher cumulative drug release ($76.2 \pm 0.4\%$) than the other ME formulations.

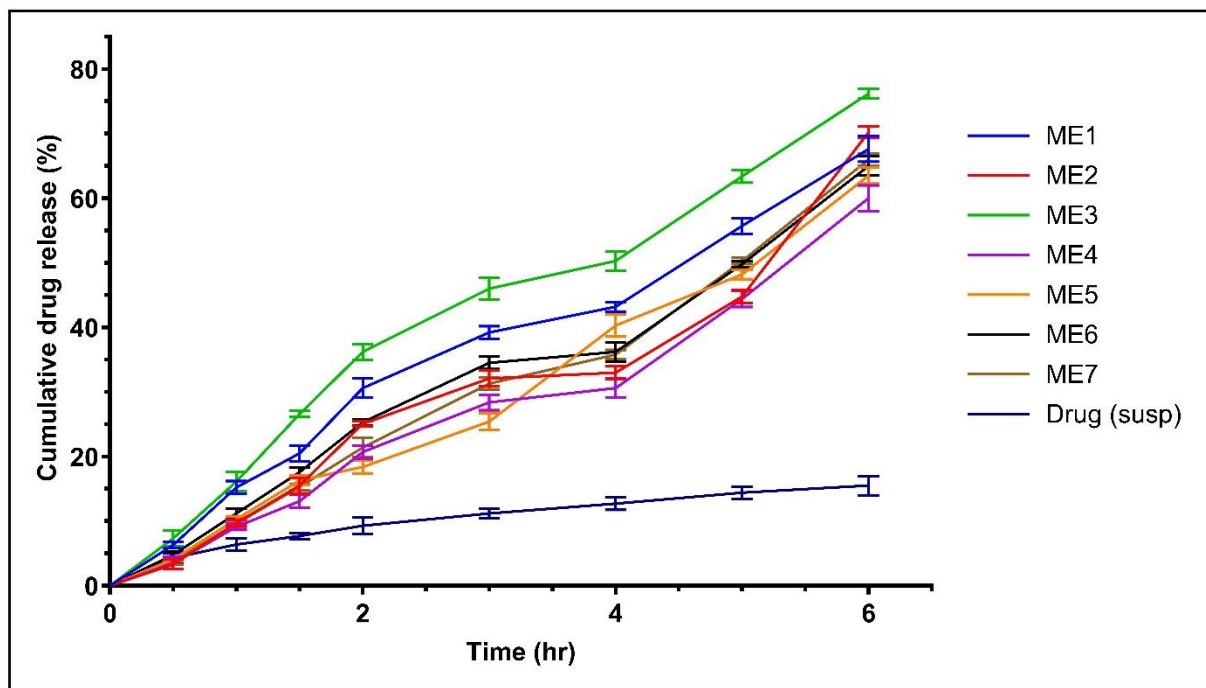


Figure 5. *In vitro* cumulative drug-release profiles of CLT from ME formulations (ME1–ME7) compared with DS. Data are presented as mean $\pm$ SD ($n = 3$).

Selection of the optimized CLT-ME formulation

ME3 was selected as the optimized formulation because it remained physically stable, exhibited a uniform droplet size (~ 27 nm; $PDI \approx 0.24$), acceptable physicochemical properties, and achieved the highest cumulative drug release ($\sim 76\%$ after 6 h). It was therefore incorporated into the thermosensitive IG.

Thermosensitive *in situ* gel

Gelation temperature and gelation time of IG

The gelation temperature and gelation time of IG1–IG12 ranged from 29.0 ± 0.22 to 38.5 ± 0.8 °C and 80.4 ± 1.5 to 210.7 ± 3.8 s, respectively (Table 3). Increasing the polymer concentration from 0.3% to 0.9% significantly reduced both gelation temperature ($p = 0.0005$) and gelation time ($p <$

0.0001), indicating enhanced polymer–poloxamer interactions and faster gel network formation. Formulations containing HPMC K4M and HPMC K15M exhibited lower gelation temperatures and shorter gelation times than those prepared with sodium alginate or CMC, reflecting the stronger hydrogen bonding and micellar interactions of HPMC-based systems.^{50,51}

Table 3. Experimental runs (IG1–IG12) generated by D-optimal design with measured responses.

Run	Polymer (A)	Concentration (B) (% w/v)	Gelation Temp. (°C)	Gelation Time (s)	Mucoadhesive Force (dyne/cm ²)	Spreadability (cm)	Viscosity at 37 °C (cP)
IG1	HPMC K4M	0.3	37.1 ± 0.11	150.3 ± 2.6	13.4 ± 0.51	5.1 ± 0.12	2100 ± 21
IG2	HPMC K4M	0.6	36.2 ± 0.52	120.5 ± 1.1	16.5 ± 0.62	4.8 ± 0.28	3000 ± 35
IG3	HPMC K4M	0.9	34.3 ± 0.62	90.2 ± 2.0	17.6 ± 0.85	4.4 ± 0.38	3500 ± 40
IG4	HPMC K15M	0.3	33.6 ± 0.51	120.6 ± 1.5	14.9 ± 0.60	4.6 ± 0.11	2500 ± 11
IG5	HPMC K15M	0.6	31.1 ± 0.42	100.1 ± 1.7	18.4 ± 0.81	4.3 ± 0.55	3500 ± 22
IG6	HPMC K15M	0.9	30.0 ± 0.11	80.4 ± 1.5	20.3 ± 0.92	4.0 ± 0.42	5200 ± 34
IG7	Sodium Alginate	0.3	38.5 ± 0.80	210.7 ± 3.8	12.0 ± 0.66	5.2 ± 0.15	2000 ± 21
IG8	Sodium Alginate	0.6	35.3 ± 0.32	180.5 ± 3.2	13.9 ± 0.71	4.6 ± 0.33	2500 ± 40
IG9	Sodium Alginate	0.9	34.1 ± 0.32	150.6 ± 1.6	15.2 ± 0.63	4.3 ± 0.61	2800 ± 51
IG10	CMC	0.3	33.4 ± 0.24	180.5 ± 2.0	13.0 ± 0.46	5.0 ± 0.58	2300 ± 42
IG11	CMC	0.6	32.1 ± 0.21	150.4 ± 2.3	14.8 ± 0.83	4.5 ± 0.29	3100 ± 21
IG12	CMC	0.9	29.0 ± 0.22	120.4 ± 2.3	16.3 ± 0.91	3.9 ± 0.51	4000 ± 73

Viscosity measurements

The viscosity data (Table 3) indicated that all IGs remained fluid at 25 °C (approximately 1000–2000 cP) and became noticeably thicker at 37 °C, with viscosity significantly increasing with polymer concentration ($p = 0.0007$), consistent with the thermogelling behavior of poloxamer systems.⁵⁰

Mucoadhesion strength measurement

Mucoadhesive strength ranged from 12.0 to 20.3 dyne/cm², with IG6 exhibiting the highest value and IG7 the lowest (Table 3). Increasing polymer concentration significantly enhanced mucoadhesion ($p = 0.0037$), particularly in HPMC-based formulations, owing to stronger hydrogen bonding and chain entanglement with mucin.⁵² The measured detachment force was normalized to the vaginal tissue contact area and expressed as mucoadhesive strength in dyne/cm².

Spreadability measurements

Spreadability ranged from 3.9 to 5.2 cm (Table 3). Increasing polymer concentration significantly affected spreadability ($p = 0.0003$). Lower-viscosity formulations exhibited greater spreadability, whereas more viscous gels showed reduced spreading, demonstrating the expected inverse relationship between viscosity and spreadability.³¹

Design-Expert® modeling and multi-response optimization

Two-factor interaction (2FI) models successfully described all responses, with significant ANOVA results ($p < 0.05$) and non-significant lack of fit ($p > 0.05$), confirming good model predictability. All models exhibited high R^2 values (>0.93), supporting the reliability of the optimization process (Table 4, Figure 6).⁵³

Table 4. Fit statistics for two-factor interaction (2FI) models, confirming model robustness and reliability.

Response	R ²	Adjusted R ²	Predicted R ²	Adequate Precision	C.V. (%)
Mucoadhesion	0.936	0.883	0.744	13.65	5.22
Gelation Time	0.991	0.984	0.966	38.46	3.61
Gelation temperature	0.971	0.947	0.885	17.88	1.94
Spreadability	0.952	0.912	0.808	15.00	2.79
Viscosity	0.955	0.917	0.819	16.92	7.29

Numerical optimization was performed using the desirability function in Design-Expert® v13 based on a D-optimal design using two-factor interaction (2FI) models. Polymer type (HPMC K4M, HPMC K15M, CMC, and sodium alginate) and polymer concentration (0.3%, 0.6%, and 0.9% w/v) were evaluated as the independent variables, whereas gelation temperature, gelation

time, mucoadhesive strength, spreadability, and viscosity at 37 °C were selected as the responses for optimization.

Gelation temperature (32.5–37.1 °C) was constrained within the acceptable range for vaginal formulations, whereas gelation time (100–150 s) was maintained within a predefined range to ensure rapid gel formation after administration. Mucoadhesive strength was maximized, and spreadability (4.0–5.2 cm) and viscosity at 37 °C (2500–3500 cP) were maintained within predefined ranges. The optimized formulation was selected based on the highest overall desirability value and subsequently verified experimentally.

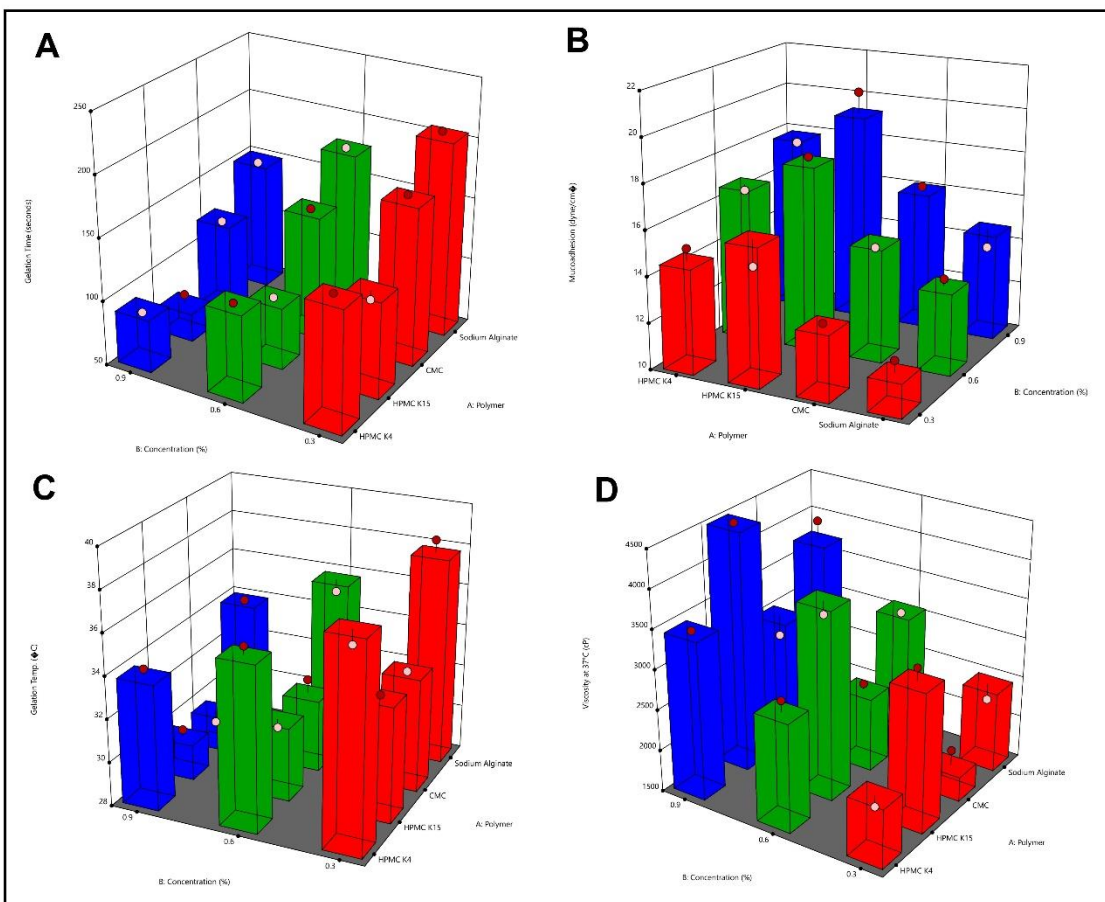


Figure 6. Three-dimensional response surface bar plots generated by Design-Expert® v13 using the D-optimal design, showing the influence of polymer type and polymer concentration on the critical quality attributes of the thermosensitive IG: (A) gelation time, (B) mucoadhesive strength, (C) gelation temperature, and (D) viscosity at 37 °C.

The numerical optimization identified the formulation containing HPMC K4M (0.6% w/v; IG2) as the optimized formulation, with an overall desirability value of 0.756 (Figure 7). Experimental verification confirmed the predictive capability of the optimization model, with prediction errors ranging from 3.58% to 6.25%, demonstrating good agreement between the predicted and observed responses (Table 5).

Factor Coding: Actual

Desirability

● Design Points

X1 = A

X2 = B

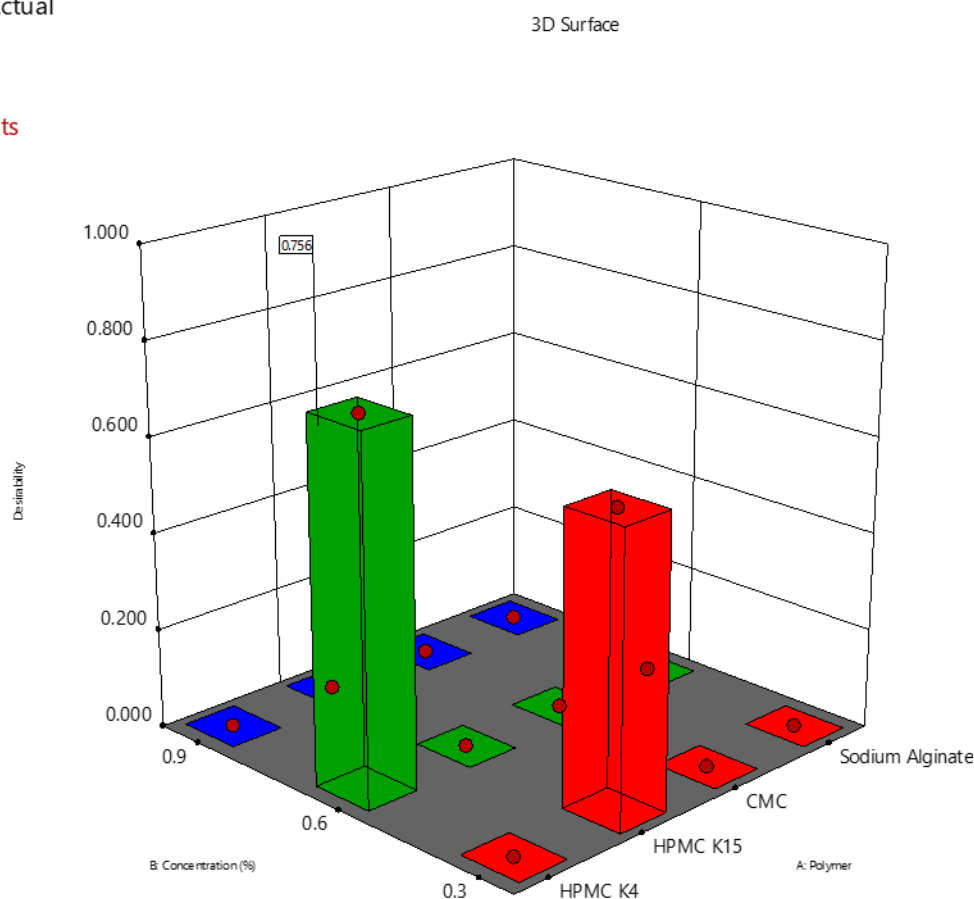


Figure 7. Overall desirability plot generated by Design-Expert® v13 for numerical optimization of the thermosensitive IG formulation (overall desirability = 0.756).

Table 5. Comparison of Design-Expert® predicted and experimentally observed values for the optimized formulation.

Response (units)	Predicted (Design-Expert)	Observed (mean ± SD)	Error (%)
Gelation temperature (°C)	35.8	34.1 ± 0.2	4.75
Gelation time (s)	120	115.7 ± 2.1	3.58
Viscosity at 37 °C (cP)	2866	2725 ± 15	4.92

Mucoadhesion (dyne/cm²)	16.8	16.1 ± 0.4	4.17
Spreadability (cm)	4.8	4.5 ± 0.1	6.25

Selection of the optimized IG formulation

Based on the numerical optimization and subsequent experimental verification, IG2 (HPMC K4M, 0.6% w/v) was selected as the gel base for incorporation of ME3 because it provided the highest overall desirability value (0.756) while satisfying the predefined criteria for gelation temperature, gelation time, mucoadhesive strength, spreadability, and viscosity.

Physicochemical evaluation of the final CLT-MG

The optimized ME3 was incorporated into IG2 to produce CLT-MG, which exhibited a gelation temperature of 35.4 ± 0.3 °C, a gelation time of 126 ± 3 s, a viscosity of 2680 ± 30 cP, a mucoadhesive strength of 16.0 ± 0.4 dyne/cm², a spreadability of 4.90 ± 0.05 cm, and a pH of 5.10 ± 0.05 . The slight increases in gelation temperature and gelation time were attributed to interactions between the dispersed droplets and the poloxamer network.⁴⁴

***In vitro* drug release test and release kinetic modeling**

The *in vitro* cumulative release profiles (Figure 8) demonstrated that the prolonged-release profile of CLT-MG was significantly different from both the rapid release of MP and the incomplete release from CLT-IG ($p < 0.05$). MP lacks a mechanism for prolonged local drug delivery. Conversely, CLT-MG demonstrated a desirable prolonged-release profile, steadily releasing $97.11 \pm 0.42\%$ of CLT over 12 h, whereas CLT-IG reached only $58.57 \pm 0.63\%$.

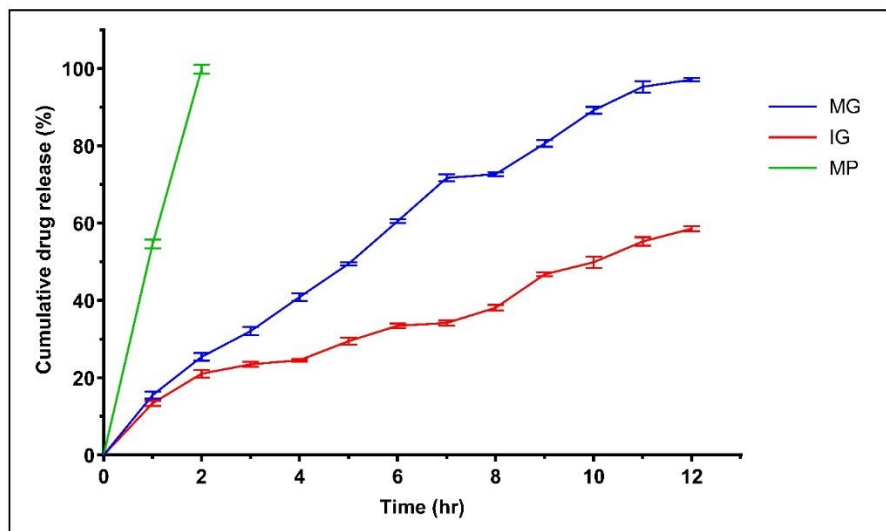


Figure 8. *In vitro* cumulative drug-release profiles of CLT from CLT-MG, CLT-IG, and MP over 12 h. Data are presented as mean $\pm$ SD ($n = 3$).

The release data were well described by the first-order model ($R^2 = 0.988$), indicating concentration-dependent release. The Korsmeyer–Peppas model showed a high correlation coefficient ($R^2 = 0.995$), with a release exponent ($n = 0.801$), indicating anomalous (non-Fickian) transport and suggesting that CLT release was governed by a combination of drug diffusion and polymer relaxation (Table 6).^{17,44}

Table 6. Kinetic model-fitting parameters for the *in vitro* release of CLT from the optimized MG formulation.

Formula	Zero-order (R^2)	First-order (R^2)	Higuchi (R^2)	Korsmeyer– Peppas (R^2)	Release exponent (n)
MG	0.977	0.988	0.939	0.995	0.801

Morphological examination by FESEM

FESEM revealed dispersed nanoscale droplets in the optimized ME. Following incorporation into the gel matrix, these droplets remained visible and well dispersed, suggesting preservation of the ME structure within CLT-MG (Figure 9).

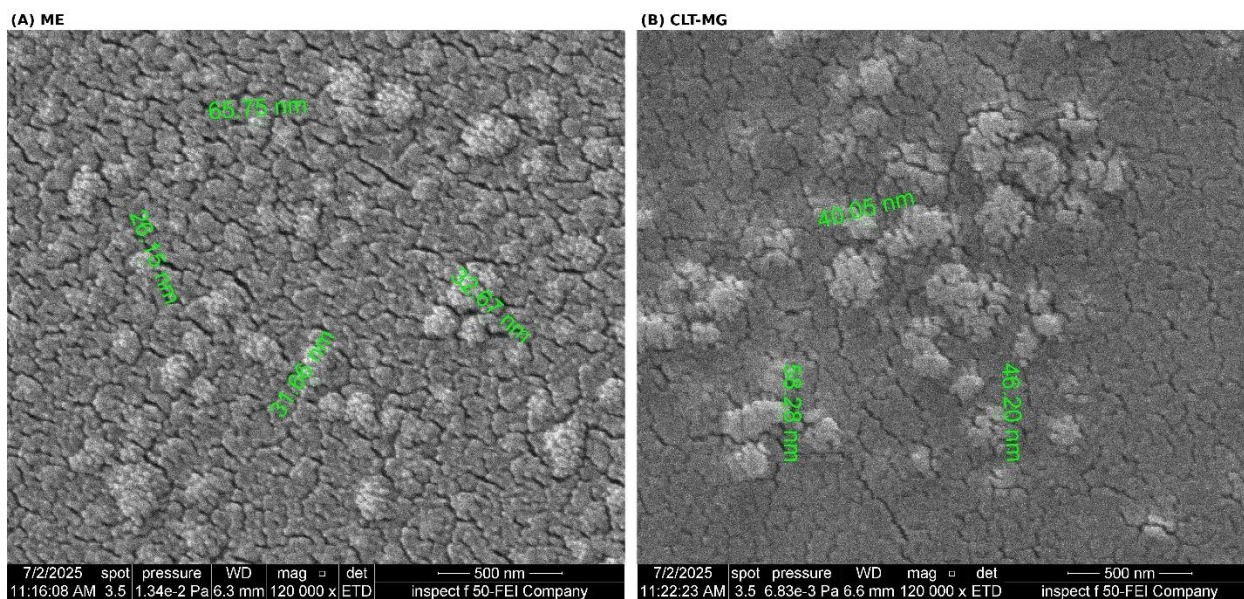


Figure 9. FESEM micrographs of (A) the optimized ME, showing dispersed nanoscale droplets, and (B) CLT-MG, showing well-dispersed droplets within the gel matrix.

Evaluation of vaginal irritation potential

No signs of erythema, edema, or other visible signs of vaginal irritation were observed in rabbits treated with CLT-MG or CLT-IG at 1, 24, and 48 h. Similar findings were observed in the negative control group. According to the 0–4 irritation scoring system, all groups received a score of 0 throughout the study, indicating excellent vaginal tolerability of both formulations (Figure 10).



Figure 10. *In vivo* vaginal irritation evaluation of the optimized formulations after 48 h.

***In vitro* antifungal activity**

The optimized MG exhibited the largest inhibition zone (43 mm), significantly greater than those of the MP and plain IG ($p < 0.001$), whereas DMSO showed no antifungal activity (Figure 11). The enhanced activity was attributed to improved CLT solubilization and the intrinsic antifungal activity of BRO.⁵⁴

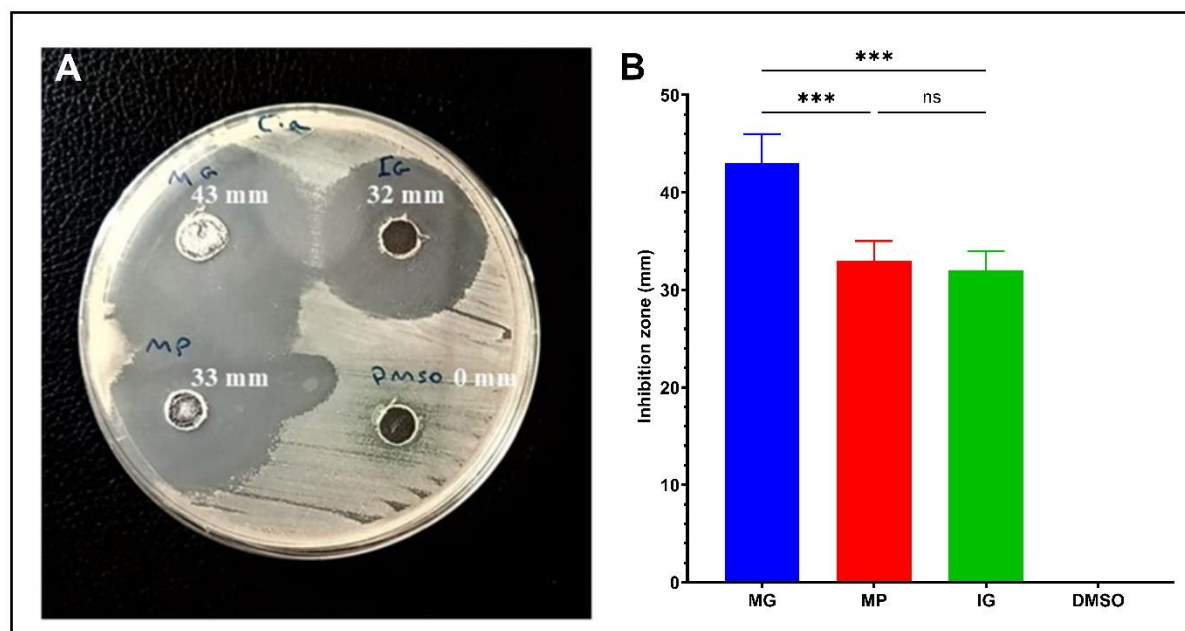


Figure 11. *In vitro* antifungal activity against *Candida albicans*. Data are presented as mean $\pm$ SD (n = 3).

Conclusion

This study successfully developed and optimized a thermosensitive, mucoadhesive microgel incorporating a clotrimazole-loaded Bulgarian rose oil microemulsion for vaginal drug delivery. The microemulsion improved clotrimazole solubilization and drug release, while Bulgarian rose oil provided intrinsic antifungal activity against *Candida albicans*. A D-optimal design was employed to optimize the *in situ* gel, resulting in suitable physicochemical and functional properties. The optimized CLT-MG provided prolonged *in vitro* drug release and significantly enhanced antifungal activity compared with the plain *in situ* gel and marketed product, with no visible signs of vaginal irritation under the tested conditions. Collectively, these findings demonstrate the potential of CLT-MG as an improved thermosensitive and mucoadhesive vaginal

delivery system combining enhanced clotrimazole delivery with the complementary antifungal activity of Bulgarian rose oil.

Ethics and policies

Ethics approval and animal welfare

"The animal study protocol was approved by the Animal Ethical Committee of the College of Pharmacy at Mustansiriyah University (approval number 49, reference number 225, dated 17th July 2025). All animal handling throughout the investigation was conducted in strict accordance with the AVMA 2020 guidelines"

Declaration of competing interests

The authors declare that they have no financial or non-financial competing interests related to this work

Funding source

"This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors."

Data availability statement

"The data that support the findings of this study are available from the corresponding author upon reasonable request."

Acknowledgment

The authors sincerely thank the College of Pharmacy, Mustansiriyah University, Baghdad, Iraq, for its support during this research.

Author's contribution statement

Halah M. Anwer: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Visualization, Writing – Original Draft, Writing – Review & Editing; Iman S. Jaafar: Conceptualization, Methodology, Validation, Supervision, Project Administration, Writing – Review & Editing.

References

1. Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: a systematic review. *The Lancet infectious diseases*. 2018;18(11):e339-e47.

2. Benedict K, Singleton AL, Jackson BR, Molinari NAM. Survey of incidence, lifetime prevalence, and treatment of self-reported vulvovaginal candidiasis, United States, 2020. *BMC Women's Health*. 2022;22(1):147.
3. Roselletti E, Warris A, De Seta F. New Insights towards Personalized Therapies for Vulvovaginal Candidiasis and Vaginal Co-infections. *Frontiers in microbiology*. 2025;16:1625952.
4. Facchinatto WM, Galante J, Mesquita L, Silva DS, Dos Santos DM, Moraes TB, et al. Clotrimazole-loaded N-(2-hydroxy)-propyl-3-trimethylammonium, O-palmitoyl chitosan nanoparticles for topical treatment of vulvovaginal candidiasis. *Acta biomaterialia*. 2021;125:312-21.
5. Ibraheem DR, Alwas NGA, Abbood SH, Nasser SM, Sulaiman GM, Jabir MS, et al. Nystatin-Based Zinc Oxide Nanoparticles Coated with Polyethylene Glycol for Enhancing the Antibacterial Activity Against Some Resistance Pathogenic Bacteria. *BioNanoScience*. 2024;14(3):2103-16.
6. Bhattacharya S, Sae-Tia S, Fries BC. Candidiasis and mechanisms of antifungal resistance. *Antibiotics*. 2020;9(6):312.
7. Tran HN, Graham L, Adukwu EC. In vitro antifungal activity of *Cinnamomum zeylanicum* bark and leaf essential oils against *Candida albicans* and *Candida auris*. *Applied Microbiology and Biotechnology*. 2020;104(20):8911-24.
8. Lee Y, Park E, Jang B, Hwang J, Lee J, Oh E-S. Antifungal Activity of Bulgarian Rose Damascena Oil against Vaginitis-Causing Opportunistic Fungi. *Evidence-Based Complementary and Alternative Medicine*. 2023;2023(1):5054865.
9. Sharma Y, Khan LA, Manzoor N. Anti-Candida activity of geraniol involves disruption of cell membrane integrity and function. *Journal de mycologie medicale*. 2016;26(3):244-54.
10. Vasconcelos PGS, Lee KM, Abuna GF, Costa E, Murata RM. Monoterpene antifungal activities: evaluating geraniol, citronellal, and linalool on *Candida* biofilm, host inflammatory responses, and structure-activity relationships. *Frontiers in pharmacology*. 2024;15:1394053.
11. Yang T-L, Hsieh C-M, Meng L-J, Tsai T, Chen C-T. Oleic acid-based self micro-emulsifying delivery system for enhancing antifungal activities of clotrimazole. *Pharmaceutics*. 2022;14(3):478.

12. Kaoud RM, Alwan MH, Amran M, Fawzi HA. Design and optimization of pantoprazole sodium mucoadhesive hydrogel microcapsules for the healing of peptic ulcers. *Pharmacia*. 2024;71:1-14.
13. Osmałek T, Froelich A, Jadach B, Tatarek A, Gadziński P, Falana A, et al. Recent advances in polymer-based vaginal drug delivery systems. *Pharmaceutics*. 2021;13(6):884.
14. Padmasri B, Nagaraju R, Prasanth D. A comprehensive review on in situ gels. *Int J Appl Pharm*. 2020;12(6):24-33.
15. Koland M, Narayanan Vadakkepushpakath A, John A, Tharamelveiyil Rajendran A, Raghunath I. Thermosensitive in situ gels for joint disorders: pharmaceutical considerations in intra-articular delivery. *Gels (Basel, Switzerland)*. 2022;8(11):723.
16. Fathalla Z, Mustafa WW, Abdelkader H, Moharram H, Sabry AM, Alany RG. Hybrid thermosensitive-mucoadhesive in situ forming gels for enhanced corneal wound healing effect of L-carnosine. *Drug delivery*. 2022;29(1):374-85.
17. Thapa R, Pandey P, Parat M-O, Gurung S, Parekh HS. Phase transforming in situ gels for sustained and controlled transmucosal drug delivery via the intravaginal route. *International Journal of Pharmaceutics*. 2024;655:124054.
18. Alwan OM, Jaafar IS. Development of synergistic antifungal in situ gel of miconazole nitrate loaded microemulsion as a novel approach to treat vaginal candidiasis. *Scientific reports*. 2024;14(1):23168.
19. Thakur D, Kaur G, Puri A, Nanda R. Therapeutic Potential of Essential Oil-based Microemulsions: Reviewing State-of-the-art. *Curr Drug Deliv*. 2021;18(9):1218-33.
20. Mamatha GT, Mahendra kMS, Pavankumar. Preparation and Evaluation of Efinaconazole Loaded Microemulsion with Tea Tree Oil. *International Journal of Pharmaceutical Research and Applications*. 2022;7(5):490-501.
21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*. 2016;6(2):71-9.
22. Habib DA, Elbedaiwy HM, Essa EA. Liquid and Solid Self-Emulsifying Drug Delivery Systems to Improve Dissolution of Carvedilol. *Journal of Advanced Medical and Pharmaceutical Research*. 2023;4(1):1-10.
23. Vlaia L, Coneac G, Muț AM, Olariu I, Vlaia V, Anghel DF, et al. Topical biocompatible fluconazole-loaded microemulsions based on essential oils and sucrose esters: Formulation design

based on pseudo-ternary phase diagrams and physicochemical characterization. *Processes*. 2021;9(1):144.

24. Ryu K-A, Park PJ, Kim S-B, Bin B-H, Jang D-J, Kim ST. Topical Delivery of Coenzyme Q10-Loaded Microemulsion for Skin Regeneration. *Pharmaceutics*. 2020;12(4):332.

25. Wen MM, Ismail NIK, Nasra MM, El-Kamel AH. Repurposing ibuprofen-loaded microemulsion for the management of Alzheimer's disease: Evidence of potential intranasal brain targeting. *Drug Delivery*. 2021;28(1):1188-203.

26. Moghimipour E, Farsimadan N, Salimi A. Ocular delivery of quercetin using microemulsion system: design, characterization, and ex-vivo transcorneal permeation. *Iranian journal of pharmaceutical research: IJPR*. 2022;21(1):e127486.

27. Shewaiter MA, Hammady TM, El-Gindy A, Hammadi SH, Gad S. Formulation and characterization of leflunomide/diclofenac sodium microemulsion base-gel for the transdermal treatment of inflammatory joint diseases. *Journal of drug delivery science and technology*. 2021;61:102110.

28. Wolska E, Szymańska M. Comparison of the In Vitro Drug Release Methods for the Selection of Test Conditions to Characterize Solid Lipid Microparticles. *Pharmaceutics*. 2023;15(2):511.

29. Gómez-Lázaro L, Martín-Sabroso C, Aparicio-Blanco J, Torres-Suárez AI. Assessment of In Vitro Release Testing Methods for Colloidal Drug Carriers: The Lack of Standardized Protocols. *Pharmaceutics*. 2024;16(1):103.

30. Qian S, Wong YC, Zuo Z. Development, characterization and application of in situ gel systems for intranasal delivery of tacrine. *International journal of pharmaceutics*. 2014;468(1-2):272-82.

31. Salman Z, Alhamdany A, Yousif N. An Innovative Mucoadhesive Thermosensitive In situ Gelling Liquid Suppository of Metoclopramide Hydrochloride for Treatment of Nausea and Vomiting Associated with Diseases. *Indian Journal Of Pharmaceutical Sciences*. 2020;82(4):650-64.

32. Alwan OM, Jafar IS. Thermosensitive and mucoadhesive polymer variables affecting development of miconazole nitrate vaginal in situ gelling system. *Journal of Research in Pharmacy*. 2024;28(5):1472-84.

33. Chaudhary B, Verma S. Preparation and evaluation of novel in situ gels containing acyclovir for the treatment of oral herpes simplex virus infections. *The scientific world journal*. 2014;2014(1):280928.
34. Abdulla NA, Balata GF, El-Ghamry HA, Gomaa E. Intranasal delivery of Clozapine using nanoemulsion-based in-situ gels: An approach for bioavailability enhancement. *Saudi Pharmaceutical Journal*. 2021;29(12):1466-85.
35. Mirani A, Kundaikar H, Velhal S, Patel V, Bandivdekar A, Degani M, et al. Tetrahydrocurcumin-loaded vaginal nanomicrobicide for prophylaxis of HIV/AIDS: In silico study, formulation development, and in vitro evaluation. *Drug Delivery and Translational Research*. 2019;9(4):828-47.
36. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, et al. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. *The AAPS journal*. 2010;12(3):263-71.
37. Muhammed SA, Al-Kinani KK. Formulation and in vitro evaluation of meloxicam as a self-microemulsifying drug delivery system. *F1000Research*. 2023;12:315.
38. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved on March. 2020;2013(30):2020-1.
39. Mileva M, Krumova E, Miteva-Staleva J, Kostadinova N, Dobрева A, Galabov AS. Chemical compounds, in vitro antioxidant and antifungal activities of some plant essential oils belonging to Rosaceae family. *Compt Rend Acad Bulg Sci*. 2014;67(10):1363-8.
40. Borhade V, Pathak S, Sharma S, Patravale V. Clotrimazole nanoemulsion for malaria chemotherapy. Part I: Preformulation studies, formulation design and physicochemical evaluation. *International journal of pharmaceutics*. 2012;431(1-2):138-48.
41. Vlaia L, Coneac G, Olariu I, MUȚ A, Anghel DF, Maxim ME, et al. LORATADINE-LOADED MICROEMULSIONS FOR TOPICAL APPLICATION. FORMULATION, PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO DRUG RELEASE EVALUATION. *Farmacia*. 2017;65(6):851-61.
42. Hashemzadeh N, Jouyban A. Review of Pharmaceutical Applications of Diethylene Glycol-Monoethyl Ether. *J Pharm Pharm Sci*. 2022;25:349-53.

43. Mitra D. Microemulsion and its application: An inside story. *Materials Today: Proceedings*. 2023;83:75-82.
44. Hamed R, Abu Kwiak AD, Al-Adhami Y, Hammad AM, Obaidat R, Abusara OH, et al. Microemulsions as lipid nanosystems loaded into thermoresponsive in situ microgels for local ocular delivery of prednisolone. *Pharmaceutics*. 2022;14(9):1975.
45. Razali RN, Ismail NI. Effects of surfactants on extra virgin coconut oil-in-water microemulsion formation and properties for topical application. *Materials Today: Proceedings*. 2024;110:142-6.
46. Kiromah NZW, Sugihartini N, Nurani LH. Development and characterization of clove oil microemulsion. *Pharmacia*. 2023;70:233-41.
47. Lin Y-P, Chen W-C, Cheng C-M, Shen C-J. Vaginal pH value for clinical diagnosis and treatment of common vaginitis. *Diagnostics*. 2021;11(11):1996.
48. Coneac G, Vlaia V, Olariu I, Muş AM, Anghel DF, Ilie C, et al. Development and evaluation of new microemulsion-based hydrogel formulations for topical delivery of fluconazole. *Aaps Pharmscitech*. 2015;16(4):889-904.
49. Shahid M, Hussain A, Khan AA, Alanazi AM, Alaofi AL, Alam M, et al. Antifungal cationic nanoemulsion ferrying miconazole nitrate with synergism to control fungal infections: In vitro, ex vivo, and in vivo evaluations. *ACS omega*. 2022;7(15):13343-53.
50. Brambilla E, Locarno S, Gallo S, Orsini F, Pini C, Farronato M, et al. Poloxamer-based hydrogel as drug delivery system: How polymeric excipients influence the chemical-physical properties. *Polymers*. 2022;14(17):3624.
51. Chen L-C, Lin S-Y, Cheng W-J, Sheu M-T, Chung C-Y, Hsu C-H, et al. Poloxamer sols endowed with in-situ gelability and mucoadhesion by adding hypromellose and hyaluronan for prolonging corneal retention and drug delivery. *Drug Delivery*. 2023;30(1):2158964.
52. Rençber S, Karavana SY, Şenyiğit ZA, Eraç B, Limoncu MH, Baloğlu E. Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation. *Pharmaceutical development and Technology*. 2017;22(4):551-61.
53. Maded ZK, Sfar S, Taqa GAA, Lassoued MA, Ben Hadj Aayed O, Fawzi HA. Development and Optimization of Dipyridamole- and Roflumilast-Loaded Nanoemulsion and Nanoemulgel for Enhanced Skin Permeation: Formulation, Characterization, and In Vitro Assessment. *Pharmaceutics*. 2024;17(6):803.

54. Tiwari N, Sivakumar A, Mukherjee A, Chandrasekaran N. Enhanced antifungal activity of Ketoconazole using rose oil based novel microemulsion formulation. *Journal of Drug Delivery Science and Technology*. 2018;47:434-44.