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Research Article

Development of diclofenac and metronidazole thermo-responsive mucoadhesive *in situ* gel for periodontal drug delivery

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ABSTRACT

Periodontitis is a chronic inflammatory disease driven by anaerobic bacterial biofilms within periodontal pockets. Conventional oral dosage forms exhibit limitations in pocket retention and sustained drug exposure, necessitating more effective local delivery strategies. This study aimed to develop and optimize a thermo-responsive mucoadhesive *in situ* gel containing diclofenac (DIC) and metronidazole (MTZ) using a Poloxamer 407/Poloxamer 188 binary system for periodontal drug delivery. A Central Composite Design (CCD) was employed to optimize gelation temperature (Tgel), yielding a significant quadratic model ($p < 0.001$, $R^2 = 98.81\%$). The optimized formulation (P407 16.44% w/w; P188 7.93% w/w) exhibited Tgel = 33.68 ± 0.45 °C, suitable for syringe-based administration into periodontal pockets followed by *in situ* solidification at physiological temperature. Drug-loaded formulations demonstrated enhanced mucoadhesive strength compared with the blank gel. The BSA denaturation assay confirmed significant anti-inflammatory activity in DIC-containing formulations, with the dual-drug gel (G-DM) achieving 82.13% inhibition relative to the poloxamer matrix baseline. Antimicrobial testing demonstrated inhibitory activity against *Porphyromonas gingivalis*, *Streptococcus mutans*, and MRSA. *In vitro* drug release showed sustained, diffusion-controlled release of both drugs over 24 hours, markedly superior to the solution control. These findings support the potential of this binary poloxamer-based gel as a dual-action local delivery platform for periodontitis management.

Keywords:
Diclofenac; Metronidazole; Poloxamer; *In situ* gel; Periodontitis

1. INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. The disease is initiated by a dysbiotic subgingival microbiome dominated by anaerobic Gram-negative bacteria. Among over 500 bacterial species living in the oral cavity, a bacterial complex comprising *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* has been strongly linked to advanced periodontal lesions, triggering a host-mediated

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inflammatory response that leads to progressive periodontal tissue destruction and eventual tooth loss if untreated [1]. Beyond oral health consequences, periodontitis has also been associated with systemic conditions including cardiovascular disease, diabetes mellitus, and adverse pregnancy outcomes, emphasizing its broader clinical significance [2].

Conventional treatment of periodontitis primarily relies on mechanical debridement through scaling and root planing (SRP), which aims to remove bacterial biofilms and calculus from periodontal pockets. The traditional treatment modality of SRP remains the "gold standard" for the non-surgical management of chronic periodontitis; however, even clinically successful treatment carries a high probability of pocket reinfection, arising from residual biofilms, bacterial tolerance within dense mature biofilms, calculus-associated reservoirs, and bacterial colonization within the dentinal tubules of infected root surfaces [3]. Studies evaluating SRP effectiveness indicate that many teeth exhibit residual subgingival biofilm and calculus, with deeper probing depths, root concavities, grooves, restorative contours, and furcation involvements all reducing efficacy [4]. As a result, adjunctive antimicrobial therapy has been widely investigated to enhance treatment outcomes. Among antimicrobial agents, metronidazole (MTZ) has been extensively used in periodontal therapy as a nitroimidazole compound with strong activity against Gram-negative anaerobic bacteria, and its mechanism involves a series of steps culminating in bacterial cell death through DNA strand breakage and inhibition of nucleic acid synthesis [5].

In addition to microbial infection, host-mediated inflammatory responses play a crucial role in periodontal tissue destruction. The inflammatory mediator prostaglandin E2 (PGE2) is implicated in the pathogenesis of periodontitis; its synthesis via cyclooxygenase enzymes is elevated in gingival tissues and crevicular fluid of periodontitis patients compared to healthy subjects, and inhibition of PGE2 using nonsteroidal anti-inflammatory drugs (NSAIDs) has been shown to decrease periodontal disease progression and reduce alveolar bone resorption [6]. Diclofenac (DIC), a non-steroidal anti-inflammatory drug (NSAID), inhibits cyclooxygenase enzymes responsible for prostaglandin synthesis and has demonstrated potential in reducing periodontal inflammation and promoting tissue healing. Diclofenac sodium exerts its majority of anti-inflammatory effect through inhibition of prostaglandin synthesis via COX pathway inhibition, and its use as an adjunctive chemotherapeutic agent in the management of periodontal diseases has been explored in experimental models [7]. Therefore, a therapeutic strategy combining antimicrobial and anti-inflammatory agents may provide a more comprehensive approach to periodontal treatment by simultaneously targeting microbial infection and inflammatory pathways.

Despite the therapeutic potential of such combination therapy, conventional dosage forms such as oral tablets, mouth rinses, and simple gels exhibit significant limitations when applied to periodontal pockets. Scaling and root planing combined with mechanical debridement may not adequately reduce the bacterial load in deeper areas of periodontal pockets; therefore, adding local or systemic antimicrobials is advised as an adjunctive treatment to target bacteria residing in these deeper regions [8]. These formulations are rapidly cleared by saliva and gingival crevicular fluid, resulting in short residence time and insufficient drug concentration at the target site. Consequently, localized drug delivery systems have been developed to maintain therapeutic drug levels directly within periodontal pockets and improve treatment efficacy.

Thermosensitive *in situ* gelling systems represent a promising approach for localized periodontal drug delivery. Thermosensitive gels based on Poloxamer 407 (P407) have great potential for periodontal disease treatment, thanks to their ability to remain in liquid form at room temperature, facilitating easy administration, and to become viscous gels at body temperature, forming a semi-solid drug depot that enhances retention and enables sustained drug release at the target site. However, problems related to short *in situ* residence time reduce their feasible clinical use [9]. Poloxamer 407 exhibits thermos-reversible properties that are of considerable interest in optimizing drug formulations, showing a fluid state at room temperature that facilitates administration and transitioning to a gel state above the sol–gel transition temperature at body temperature, thereby promoting prolonged release of pharmacological agents [10]. Poloxamer 188 (P188), with its distinct triblock structure possessing a dissimilar PPO block length compared to P407, has been investigated as a polymer additive to modulate the temperature-dependent gelation characteristics and improve the rheological properties of poloxamer-based *in situ* gel matrices [11].

Although thermosensitive poloxamer gels have been explored for various mucosal drug delivery applications [12], relatively few studies have investigated dual-drug periodontal delivery systems that simultaneously target both bacterial infection and host inflammatory responses while optimizing gelation behavior for periodontal pocket administration. Therefore, the present study aimed to develop and optimize a thermo-responsive mucoadhesive *in situ* gel containing diclofenac and metronidazole for localized periodontal drug delivery. A central composite design was employed to investigate the influence of polymer concentrations on gelation temperature. The optimized

formulation was further characterized in terms of mucoadhesive strength, anti-inflammatory activity, antimicrobial efficacy against oral pathogens, and *in vitro* drug release behavior.

2. MATERIALS AND METHODS

2.1. Materials

Poloxamer 188 (P188; Lot No. 67DJWU, MySkinRecipes®) and Poloxamer 407 (P407; Lot No. RM39C, MySkinRecipes®) were used as thermosensitive gelling polymers. Diclofenac sodium (DIC) was used as the anti-inflammatory active pharmaceutical ingredient (API) and obtained as a gift from CHUMCHON Pharmaceutical Public Company Limited, Bangkok, Thailand. Metronidazole (MTZ) was incorporated as the antimicrobial agent and was kindly provided from T.MAN Pharma Ltd., Part, Bangkok, Thailand. Sodium chloride, potassium chloride, sodium phosphate dibasic anhydrous, and potassium dihydrogen phosphate were used as analytical grade for the preparation of phosphate-buffered saline (PBS, pH 6.8). Bovine serum albumin (BSA; Phygene®) was used as the protein substrate in the anti-inflammatory assay. Sodium ethyl paraben was used as a preservative.

2.2. Preparation of thermosensitive *in situ* gels

Four gel formulations were prepared using the cold method and are designated throughout this study as follows: blank poloxamer gel without drug (G-BLK), poloxamer gel loaded with DIC only (G-DIC), poloxamer gel loaded with MTZ only (G-MTZ), and poloxamer gel loaded with both DIC and MTZ (G-DM). Deionized water (WAT) was used as an aqueous reference control in the mucoadhesion test only.

P407 and P188 were incorporated according to the experimental design, with concentrations of 13.96–21.04% w/w for P407 and 7.93–22.07% w/w for P188. The required amounts of polymers were gradually dispersed in cold distilled water (8 ± 1 °C) under continuous magnetic stirring to prevent polymer aggregation. The dispersion was maintained at 4–8 °C for 24 h to ensure complete hydration and formation of a homogeneous solution.

For drug-loaded formulations (G-DIC, G-MTZ, and G-DM), DIC sodium and/or MTZ were subsequently incorporated at 1% w/w each under gentle stirring at refrigerated temperature to ensure uniform distribution. Sodium ethyl paraben (0.01% w/w) was added as a preservative to all formulations. The final formulation weight was adjusted to 30 g with distilled water. All formulations were stored at 4 °C prior to characterization. Visual inspection was performed under normal lighting to assess clarity, color, homogeneity, and the presence of phase separation or crystallization.

2.3. Experimental design

A two-factor Central Composite Design (CCD) with five center replicates was employed to investigate the effect of polymer concentrations on the gelation temperature (Tgel) of the thermosensitive *in situ* gel. The design consisted of 13 experimental runs, including four factorial points, four axial points ($\alpha = 1.414$), and five center points. The selected independent variables were the concentrations of P407 (X_1) and P188 (X_2), while gelation temperature (Tgel) was the response variable. The relationship between the independent variables and the response was described using a second-order polynomial model:

$$Y=\beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2$$

where Y represents the predicted gelation temperature, β_0 is the intercept, β_1 and β_2 are the linear coefficients, β_{12} is the interaction coefficient, and β_{11} and β_{22} are the quadratic coefficients. Statistical analysis and model fitting were performed using Minitab® software (Minitab Inc., State College, PA, USA). The independent and dependent variables evaluated in this study are summarized in Table 1.

2.4. Gelation temperature determination

Gelation temperature (Tgel) was determined using an oscillatory rheological method. Approximately 1–2 mL of each formulation was carefully loaded onto the lower plate of a rheometer (HAAKE™, Thermo Fisher Scientific, Germany) equipped with a 25 mm parallel plate geometry and a solvent trap system to minimize evaporation. The gap was set at 0.5 mm. Temperature ramp measurements were performed from 0 °C to 80 °C over 600 s at a constant frequency of 1 Hz under controlled oscillatory stress conditions. The storage modulus (G′) and loss modulus (G″) were continuously recorded as a function of temperature. The gelation temperature was defined as the crossover point at which G′ became equal to or greater than G″, indicating the transition from sol to gel state. All measurements were performed in triplicate and results were expressed as mean ± standard deviation.

2.5. UV–visible spectrophotometric analysis

Measurements were performed using a UV–visible spectrophotometer (Mettler Toledo, Columbus, OH, USA). DIC and MTZ were analyzed to determine drug concentration in the prepared formulations and release samples. All samples were placed in 1 cm

Table 1. Independent and dependent variables in the central composite design.

Independent variables	Low (−1)	Medium (0)	High (+1)
X_1 = P407 (% w/w)	15.00	17.50	20.00
X_2 = P188 (% w/w)	10.00	15.00	20.00
Dependent variable	**Target**		
Y_1 = Tgel (°C)	35 °C		

quartz cuvettes, and absorbance was recorded at 275 nm for DIC and 340 nm for MTZ. The instrument was auto-zeroed using deionized water as blank prior to measurement.

Standard calibration curves were constructed using DIC and MTZ solutions within the concentration ranges of 2–16 µg/mL and 5–25 µg/mL, respectively. Linear regression analysis was applied to obtain the calibration equations. Drug concentrations were calculated from the corresponding regression equations and corrected by the dilution factor. All measurements were performed in triplicate and reported as mean ± standard deviation.

2.6. Mucoadhesive force determination

Mucoadhesive strength of five test groups was evaluated: deionized water (WAT), blank poloxamer gel (G-BLK), DIC-loaded gel (G-DIC), MTZ-loaded gel (G-MTZ), and dual-drug gel (G-DM), using a modified beaker–lever method. A mucosa-simulating gel substrate was prepared by dissolving agar (0.6% w/v) in phosphate-buffered saline (PBS, pH 6.8), then casting into a flat layer with a thickness of approximately 0.5 cm. The substrate was equilibrated on a temperature-controlled hot plate at 37 °C prior to testing.

A fixed amount of each gel formulation was placed onto the agar substrate. A predefined weight (probe/weight system) was carefully positioned on top of the gel to ensure intimate contact. After a contact time of 15 min, the weight was connected to a simple lever system using a string, with the opposite end connected to an empty beaker. Water was added dropwise into the beaker until detachment occurred, defined as the point at which the weight was lifted and the gel separated from the substrate. The amount of water required to initiate detachment was recorded as the detached weight (g). Mucoadhesive force (N) was calculated as:

$$F = m \times g$$

where m is the detached mass (kg) and g is gravitational acceleration (9.81 m/s²). Each formulation was tested in triplicate (n = 3). Statistical analysis was performed using one-way ANOVA at a significance level of 0.05, Fisher's Least Significant Difference (LSD) post hoc test to identify significant differences between formulation pairs.

2.7. *In vitro* anti-inflammatory activity

The anti-inflammatory activity of four formulations; G-BLK, G-DIC, G-MTZ, and G-DM, was evaluated using a bovine serum albumin (BSA) denaturation assay, adapted from previously reported protein-denaturation methods [13]. BSA solution (0.2% w/v) was prepared in phosphate-buffered saline (PBS, pH 6.8). Each formulation was mixed with BSA solution and incubated at room temperature prior to heat-induced denaturation. The mixture was then heated at 70 °C for 5 minutes to induce protein denaturation and subsequently cooled to room temperature. Absorbance was measured spectrophotometrically at 660 nm using PBS as blank.

Since the poloxamer matrix (G-BLK) demonstrated higher absorbance than water under the assay conditions, indicating that the poloxamer itself may contribute to protein denaturation, G-BLK was used as the reference control rather than water. This approach ensures that the calculated percentage inhibition reflects the net anti-inflammatory activity attributable solely to the drug content of each formulation, corrected for any matrix-induced protein denaturation effect. The percentage inhibition of protein denaturation was therefore calculated as:

$$\% \text{ Inhibition} = \frac{(A\_G\text{-}BLK - A\_sample)}{A\_G\text{-}BLK} \times 100$$

where A_G-BLK is the absorbance of the blank poloxamer gel formulation and A_sample is the absorbance of the drug-loaded formulation being tested.

All experiments were performed in triplicate (n = 3). Results were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with statistical significance set at $p < 0.05$.

2.8. *In vitro* antimicrobial activity

The antimicrobial activity of four formulations; G-BLK, G-DIC, G-MTZ, and G-DM, was evaluated using the agar-cup diffusion method, performed according to established agar-diffusion procedures [14] against three oral pathogens: methicillin-resistant *Staphylococcus aureus* (MRSA 4330), *Porphyromonas gingivalis*, and *Streptococcus mutans*. Bacterial suspensions

were prepared in broth and calibrated to the 0.5 McFarland standard. Each suspension was swabbed uniformly onto the surface of Mueller-Hinton agar plates and allowed to dry.

Sterilized stainless steel cylindrical cups (8 mm diameter, 10 mm height) were placed onto the inoculated agar surface. Each cup was filled with one of the four test formulations (G-BLK, G-DIC, G-MTZ, or G-DM). Plates were then incubated at 37 °C for 24–48 hours under anaerobic conditions to support optimal growth of the test organisms. After incubation, antimicrobial activity was quantified by measuring the diameter of the inhibition zone (mm) using a digital caliper. All experiments were performed in triplicate (n = 3) and results were expressed as mean inhibition zone diameter ± standard deviation.

2.9. *In vitro* drug release study

The *in vitro* release of DIC and MTZ from the gel formulations and a solution control was evaluated using a dialysis bag diffusion method. Four test groups were included: G-DIC (poloxamer gel loaded with DIC only), G-MTZ (poloxamer gel loaded with MTZ only), G-DM (dual-drug poloxamer gel loaded with both DIC and MTZ), and SOL-DM (solution control containing both DIC and MTZ dissolved in deionized water without any gel matrix), which was included to demonstrate the contribution of the poloxamer gel matrix to sustained drug release.

For gel formulations (G-DIC, G-MTZ, and G-DM), approximately 5.0 g of each formulation (containing DIC and/or MTZ at 1% w/w) was accurately weighed and transferred into a pre-wetted dialysis membrane bag (MWCO 12,000 Da). For the solution control (SOL-DM), an equivalent amount of DIC and MTZ (corresponding to 1% w/w each relative to 5.0 g) was dissolved in deionized water and transferred into the dialysis bag in the same manner.

Each bag was tightly sealed and fixed in a beaker containing 200 mL PBS (pH 6.8) maintained at 37 ± 0.5 °C under continuous magnetic stirring at 330 rpm throughout the experiment. At predetermined time points (5 min to 24 h), a 10 mL aliquot of the release medium was withdrawn and immediately replaced with an equal volume of fresh pre-warmed PBS (37 °C) to maintain constant volume and near-sink conditions. Collected samples were analyzed by UV–Vis spectrophotometry at 275 nm for DIC and 340 nm for MTZ. A matrix-matched blank corresponding to the final sample diluent was used. All measurements were performed in triplicate (n = 3). The percent cumulative drug release was calculated at each time point with correction for sampling and medium replacement, and reported as percent cumulative release (%) relative to the initial theoretical drug content in each sample.

3. RESULTS AND DISCUSSION

3.1. Experimental design

A central composite design (CCD) comprising 13 experimental runs (α = 1.41421) was employed to investigate and optimize the gelation temperature (T_{gel}) of the thermosensitive *in situ* gel as a function of poloxamer 407 (P407, X_1) and poloxamer 188 (P188, X_2) concentrations (% w/w) (Table 2). The CCD design, consisting of four factorial points, four axial points, and five center point replicates, is well recognized as an efficient and flexible approach for fitting a second-order quadratic model, enabling the simultaneous evaluation

Table 2. CCD matrix for gelation temperature optimization.

Experiment	PtType*	P407 (%w/w)	P188 (%w/w)	Tgel (°C)
1	1 (cube)	20.00	10.00	28.75
2	-1 (axial)	21.04	15.00	24.38
3	0 (center)	17.50	15.00	—**
4	1 (cube)	20.00	20.00	17.64
5	0 (center)	17.50	15.00	32.50
6	1 (cube)	15.00	20.00	40.27
7	1 (cube)	15.00	10.00	—**
8	0 (center)	17.50	15.00	33.22
9	0 (center)	17.50	15.00	31.12
10	0 (center)	17.50	15.00	30.87
11	-1 (axial)	17.50	7.93	33.74
12	-1 (axial)	17.50	22.07	25.38
13	-1 (axial)	13.96	15.00	45.31

*PtType: 1 = factorial/cube point, 0 = center point, −1 = axial point
**Tgel could not be obtained.

of linear, interaction, and quadratic effects [15]. The use of five center point replicates further allowed reliable estimation of pure experimental error and was essential for assessing the lack-of-fit significance, a criterion recognized as critical for confirming model adequacy in pharmaceutical optimization [16].

A second-order polynomial model was fitted to the T_{gel} response by regression analysis and analysis of variance (ANOVA). The overall model was highly significant ($F = 83.14$, $p < 0.001$), while the lack-of-fit test was not significant ($p = 0.422$), collectively confirming that the quadratic model adequately described the T_{gel} behavior within the experimental domain. The goodness-of-fit statistics demonstrated excellent model performance: $R^2 = 98.81\%$, adjusted $R^2 = 97.62\%$, and predicted $R^2 = 90.57\%$. These values are consistent with accepted benchmarks, wherein R^2 above 0.90, a non-significant lack-of-fit ($p > 0.05$), and are indicative of a well-fitting, predictive model. The close agreement among R^2, adjusted R^2, and predicted R^2 in the present study suggests that the model was not overfit and retained strong external predictability, which was subsequently verified through experimental confirmation of the optimized formulation [17].

The fitted regression equation in uncoded units was:

$$T_{gel} = 85.4 - 6.91(P407) + 5.17(P188) + 0.2081(P407^2) - 0.0537(P188^2) - 0.2353(P407 \times P188)$$

ANOVA confirmed that all model terms were statistically significant, including the linear terms X_1 ($p < 0.001$) and X_2 ($p = 0.002$), the interaction term X_1X_2 ($p = 0.011$), and the quadratic terms X_{12} ($p = 0.040$) and X_{22} ($p = 0.036$) (Table 3). The significance of both quadratic terms and the interaction term confirms that the T_{gel} response surface is genuinely curved and non-additive, underscoring the necessity of the CCD framework over simpler factorial designs in this formulation context.

The contour and 3D surface plots revealed a pronounced dependence of Tgel on P407 and P188 concentrations. In general, Tgel decreased substantially as P407 increased (as shown in contour plot in Figure 1(A)), consistent with poloxamer micellization and enhanced micellar packing occurring at lower temperatures when the P407 content is higher. Meanwhile, P188 exerted a significant effect and contributed to curvature in the response, as evidenced by the significant quadratic term (X_2^2) and the interaction term (X_1X_2) (as shown in 3D response surface plot in Figure 1(B)).

Notably, the significance of X_1X_2 ($p = 0.011$) indicates that the influence of P188 on Tgel is not independent but varies depending on the level of P407. This interaction is visually reflected in the tilted/curved contour bands across the design space, demonstrating that optimal Tgel is achieved through a balance between the two poloxamers rather than by adjusting a single component alone.

3.2. Effect of P407 concentration on gelation temperature

The negative coefficient of X_1 (−6.91) in the regression equation indicates that T_{gel} decreased substantially as P407 concentration increased. This inverse relationship is well-explained by the thermogelation mechanism of P407, an amphiphilic ABA-type triblock copolymer composed of a central hydrophobic polypropylene oxide (PPO) block flanked by two hydrophilic polyethylene oxide (PEO) blocks. At low temperatures, P407 chains exist as individual unimers surrounded by a hydration layer. At this stage, the hydrophilic PEO blocks are extensively hydrogen-bonded to the surrounding water molecules, forming an ordered hydration shell that keeps the more hydrophobic PPO segments solubilised; the copolymer therefore remains molecularly dispersed as free unimers, and the system stays a clear, low-viscosity sol with

Table 3. ANOVA significance of model terms for Tgel.

Term	p-value
Model	< 0.001
P407 (X_1)	< 0.001
P188 (X_2)	0.002
X_1X_2	0.011
X_1^2	0.040
X_2^2	0.036
Lack of fit	0.422
$R^2 = 98.81\%$;	
Adj $R^2 = 97.62\%$;	
Pred $R^2 = 90.57\%$	

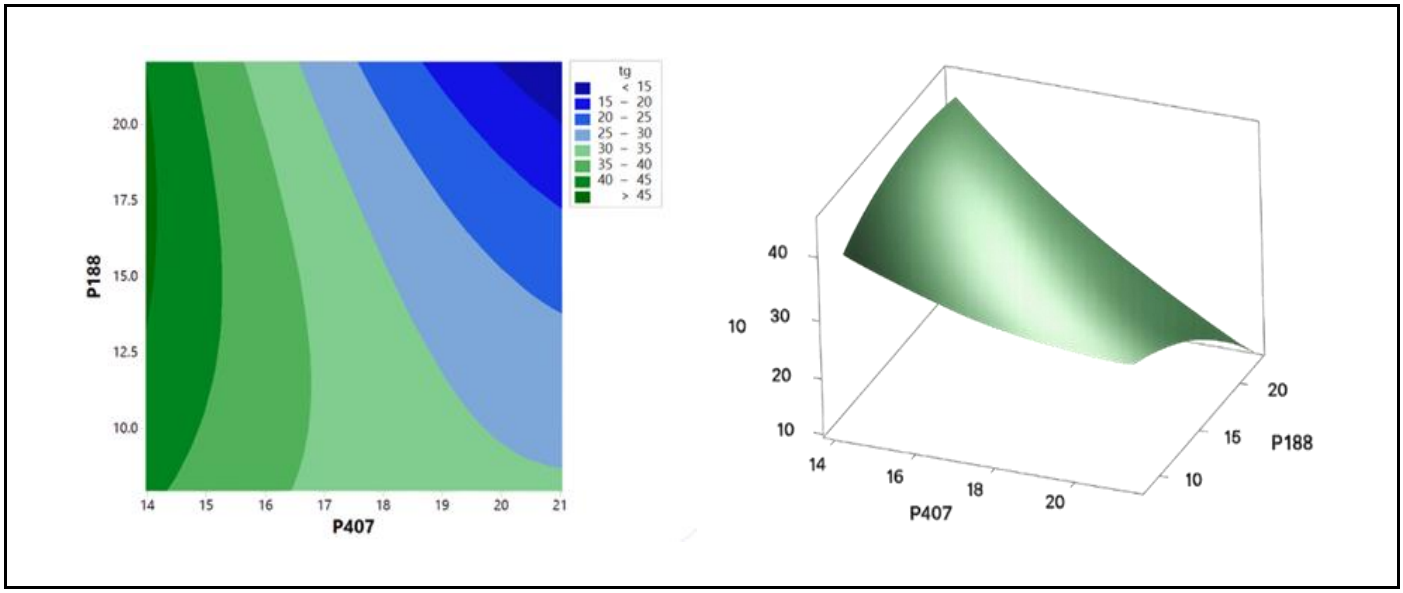

Figure 1. Contour plot of Tg as a function (A) and 3D response surface plot of Tg as a function of P407 and P188 concentrations. (B)

negligible inter-chain association. As temperature rises, hydrogen bonds between water molecules and the PEO chains are disrupted, causing dehydration of the PPO blocks and promoting hydrophobic self-assembly into spherical micelles. With further heating, these micelles pack into cubic and subsequently hexagonal phases, generating the macroscopic gel network [18]. At higher P407 concentrations, the greater number of polymer chains enhances hydrophobic inter-chain interactions and facilitates micellar aggregation at lower temperatures, thereby lowering T_{gel} [19].

The present findings are consistent with multiple reports in the literature. Hirun et al. [11] reported a clear inverse relationship between P407 concentration and T_{gel} in thermosensitive P407/P188 blends optimized via a Box–Behnken design. Menshutina et al. [20] similarly observed that T_{gel} for nasal *in situ* gels based on P407 decreased from 32.71 °C to 24.63 °C as P407 concentration increased, attributing this to enhanced hydrophobic interactions and facilitated micelle network formation at lower temperatures. Furthermore, Galara et al. [21] formulating chlorhexidine periodontal *in situ* gel with CCD noted that gelation temperature decreased considerably as P407 concentration increased, consistent with the pattern observed in the present study. Within the experimental range explored here, the data from runs 1 (P407 = 20%, T_{gel} = 28.75 °C), 6 (P407 = 15%, T_{gel} = 40.27 °C), and 13 (P407 = 13.96%, T_{gel} = 45.31 °C) visually illustrate this trend, with the effect amplified at lower P407 levels, consistent with the significant quadratic term X_{12}.

From a formulation design standpoint, the target T_{gel} of 35 °C was established to ensure the gel remains sufficiently flowable for syringe-based subgingival administration at room temperature while rapidly transitioning to a semi-solid state upon contact with the periodontal pocket environment (~37 °C) [22]. The identified inverse dependence of T_{gel} on P407 concentration therefore provided a primary lever for tuning the formulation within the therapeutically relevant range.

3.3. Effect of P188 concentration on gelation temperature and its interaction with P407

The positive coefficient of X_2 (+5.17) in the fitted model reflects an overall trend of increasing T_{gel} with rising P188 concentration, attributable to the comparatively higher hydrophilicity of P188 relative to P407. P188 is a PEO80-PPO30-PEO80 triblock copolymer with approximately 80% PEO content (HLB = 29), compared to ~70% PEO in P407 (HLB = 22) [23]. The more hydrophilic character of P188 is attributable to its higher PEO/PPO ratio, which promotes more extensive hydrogen bonding with water molecules. These interactions require greater thermal energy to disrupt, thereby elevating the critical micelle temperature and, consequently, the sol-gel transition temperature of the mixed system [11,24]. Mechanistically, the longer PEO blocks of P188 can sterically interfere with inter-micellar packing during the gelation process by intruding into the space between neighboring micelles, which shifts T_{gel} to higher values [11].

These findings are consistent with those of Hirun et al. [11], whose Box–Behnken design study of P407/P188/polycarbophil thermosensitive hydrogels demonstrated that P188, being more hydrophilic than P407 with a higher PEO/PPO ratio, caused an increasing trend in T_{gel} when added at low to intermediate quantities. The same group noted that this effect is consistent with earlier findings by Qian et al. [25] reported that in nasal thermosensitive gels, the addition of

hydrophilic P188 consistently increased T_{gel}, regardless of the baseline P407 concentration, due to the formation of additional hydrogen bonds that require extra energy to break. Similarly, Giuliano et al. [19] reported that combining P407 with P188 was necessary to achieve a T_{gel} within the clinically suitable range for periodontal gel applications, since pure P407 at concentrations sufficient for gelation often transitions below room temperature. The present observations align with the general principle established in the poloxamer literature that pure P407 alone cannot meet the requirement of forming gels at body temperature from a liquid state at room temperature, and that P188 is an effective and widely employed co-excipient to raise T_{gel} toward the physiologically desired range [25].

However, the significant negative quadratic term X_{22} ($p = 0.036$) and the significant interaction term X_1X_2 ($p = 0.011$) indicate that the relationship between P188 and T_{gel} is non-linear and that the influence of P188 is not independent of P407 concentration. The interaction term reflects that at high P407 levels, the T_{gel}-elevating effect of P188 is diminished or partially counteracted. This behavior may be explained by a mechanism recently described by Panyamao et al. [26], who demonstrated through synchrotron-based small-angle X-ray scattering (SAXS), rheology, and differential scanning calorimetry (DSC) that at high P188 concentrations relative to P407, P188 competes with P407 for water molecules. This drives accelerated dehydration of P407 chains and promotes denser micellar packing at lower temperatures, partially offsetting the T_{gel}-raising effect observed at lower P188 levels. This also aligns with findings from Qi et al. [27] who showed that in P407/P188/carbopol composite hydrogels, precise tuning of the P407/P188 ratio produced markedly different T_{gel} values, and that the interplay between the two poloxamers governed the phase transition behavior more than each component individually.

3.4. Response surface plot and formulation design space

The contour and 3D surface plots (Figure 1(A) and 1(B)) demonstrated a pronounced, curvature-containing dependence of T_{gel} across the P407–P188 design space. The tilted contour bands reflecting the significant interaction term (X_1X_2, $p = 0.011$) confirmed that the optimal T_{gel} target can be achieved through multiple combinations of P407 and P188 concentrations, rather than by modifying a single polymer in isolation. Zheng et al. [12] similarly identified significant interaction effects in their CCD-based optimization of a poloxamer/carbopol *in situ* gel for periodontal application, demonstrating that both P407 and carbopol 934P concentrations exerted significant interactive effects on T_{gel} and drug release.

The observed design space also illustrates the practical consequence of formulation extremes. Formulations at the lowest P407 axial point (13.96%, Run 13) yielded T_{gel} = 45.31 °C, well above physiological oral temperature and therefore unsuitable for rapid *in situ* gelation upon administration. Conversely, high P407 with high P188 (Runs 1 and 4) produced T_{gel} values of 28.75 °C and 17.64 °C, respectively, near or below room temperature, risking premature gelation prior to injection. The operational T_{gel} window of 30–37 °C is widely regarded in the periodontal *in situ* gel literature as the acceptable range, balancing ease of syringe administration at room temperature with reliable gelation upon contact with periodontal pocket tissue [21]. Specifically, if T_{gel} falls below ~33 °C, gelation may occur at room temperature, preventing syringe delivery; if above 37 °C, the formulation may not gel within the pocket and risks leakage and subtherapeutic local concentrations [22].

3.5. Model optimization and verification

Numerical optimization was performed using the desirability function approach to identify the P407 and P188 combination yielding a target T_{gel} of 35.0 °C. The predicted optimum corresponded to P407 = 16.44% w/w and P188 = 7.93% w/w, with a predicted T_{gel} of 35.00 °C and desirability = 1.000. The choice of 35 °C as the target was deliberate, recognizing that this value lies within the critical window between room temperature and physiological body temperature, ensuring the formulation remains flowable during periodontal pocket administration and undergoes rapid *in situ* gelation upon contact with the tissue environment at ~37 °C [28]. Comparable target T_{gel} values in the 34–37 °C range have been reported for numerous poloxamer-based periodontal *in situ* gel systems [29]. Zheng et al. [12] reported an optimized poloxamer/carbopol periodontitis gel with T_{gel} = 36 °C and gelation time ≤ 213 s, consistent with the gelation performance expected from the present optimized system.

Table 4. Predicted and experimental Tgel of the optimized formulation (with 95% confidence and prediction intervals).

Response	Fit	SE Fit	95% CI	95% PI / Exp.	% Prediction Error
Tg	35.00	1.39	(31.44, 38.56)	(30.36, 39.64) / 33.68±0.45	3.77%

Experimental verification of the optimized formulation yielded T_{gel} = 33.68 ± 0.45 °C, which fell within both the 95% confidence interval (31.44–38.56 °C) and the 95% prediction interval (30.36–39.64 °C) of the model (Table 4). The prediction error relative to the target T_{gel} of 35.0 °C was approximately 3.77%, demonstrating the reliable predictive accuracy of the CCD-derived quadratic model. The agreement between predicted and experimental values confirms that the model is not merely descriptive but retains genuine predictive utility for prospective formulation work, consistent with the close proximity of adjusted R^2 (97.62%) and predicted R^2 (90.57%).

Collectively, the experimental verification demonstrates that the optimized binary poloxamer formulation (P407 = 16.44% w/w, P188 = 7.93% w/w) achieves a T_{gel} within the physiologically desired range for periodontal *in situ* gel application. This precise thermosensitive behavior is expected to translate into a clinically favorable combination of easy injection at ambient temperature and rapid, reliable gel formation within the periodontal pocket, supporting localized and sustained delivery of the incorporated active agents, diclofenac sodium and metronidazole, for the management of periodontal inflammation and infection. The use of P188 as a T_{gel}-modulating co-excipient not only fine-tuned the phase transition temperature but also contributes to the mucoadhesive capacity of the gel matrix, as previously documented for similar P407/P188-based drug delivery systems [30].

3.6. Mucoadhesive force determination

Mucoadhesive force (N) of the five test groups, deionized water (WAT), blank poloxamer gel (G-BLK), DIC-loaded gel (G-DIC), MTZ-loaded gel (G-MTZ), and dual-drug gel (G-DM), showed a statistically significant difference among groups as determined by one-way ANOVA ($F = 15.79$, $p = 0.005$; $\alpha = 0.05$). Post hoc comparison using Fisher's LSD test (95% CI) indicated the following compact letter display: G-MTZ (A), G-DIC (A), G-DM (B), G-BLK (BC), and WAT (C). Means not sharing a letter are significantly different.

This grouping indicates a clear hierarchical separation among formulations. G-DIC (0.6059 N) and G-MTZ (0.6369 N) formed the highest group (A), demonstrating that single-drug loading significantly enhanced mucoadhesion relative to all other groups. G-DM (0.5106 N) formed an independent intermediate group (B), significantly lower than single-drug formulations but significantly higher than WAT. G-BLK (0.4737 N) occupied group BC, significantly lower than drug-loaded formulations yet not significantly different from G-DM, while WAT (0.4150 N) formed the lowest group (C), significantly lower than all gel formulations (Figure 2).

Mucoadhesion is a key performance attribute for periodontal *in situ* gels because stronger adhesion directly increases residence time within the periodontal pocket, counteracting the continuous washout effect of

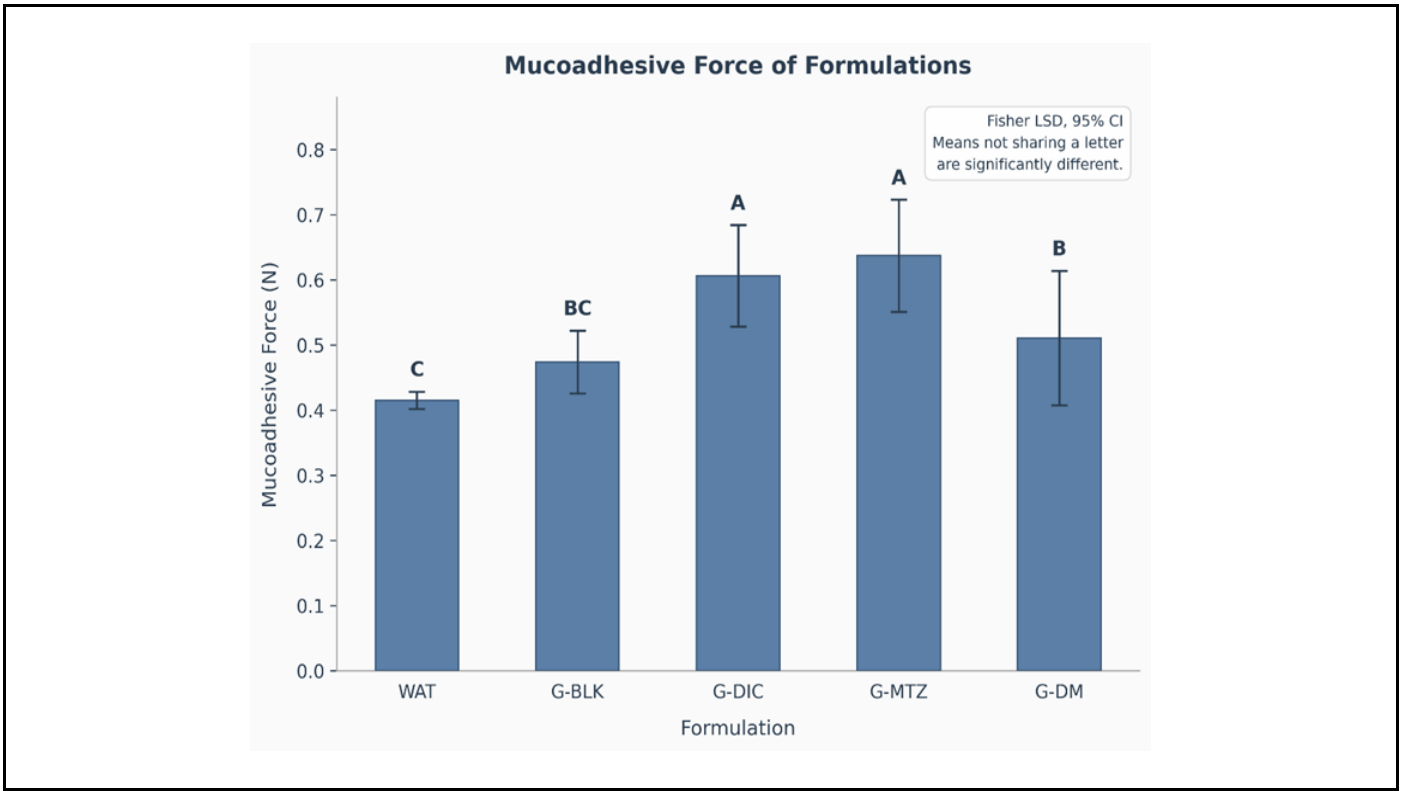

Figure 2. Mucoadhesive force of thermosensitive gel formulations (mean ± SD, n = 3).

gingival crevicular fluid (GCF) flow and thereby prolonging local drug exposure. The Fisher LSD results reveal a more refined and informative separation among formulations compared with conservative pairwise methods, allowing clearer interpretation of the drug-loading effect across all groups.

The poloxamer base gel (G-BLK) produced significantly higher mucoadhesive force than WAT (group BC vs C), confirming that the poloxamer matrix itself contributes meaningfully to mucosal adhesion even in the absence of drug. This is consistent with the known ability of poloxamer micellar networks to interact with mucin glycoproteins through hydrophobic and hydrogen-bonding interactions, providing a baseline adhesive character to the gel platform [11].

Incorporation of a single drug further and significantly enhanced mucoadhesion above G-BLK. Both G-DIC (0.606 N) and G-MTZ (0.637 N) were classified in group A, significantly superior to G-BLK, G-DM, and WAT. The increase in mucoadhesive force upon drug loading may be attributed to formulation-dependent changes in the gel microstructure and interfacial interactions with the mucosal surface. The presence of drug molecules can modify micellar packing density and the viscoelastic character of the poloxamer network, enhancing wetting and interpenetration at the mucosal interface. Additionally, drug–polymer and drug–mucus interactions such as hydrogen bonding and weak intermolecular forces may contribute to higher detachment forces in single-drug formulations compared with G-BLK. Notably, although DIC sodium has been previously reported to reduce mucoadhesive force in poloxamer systems [30], our results demonstrate the opposite trend, suggesting that formulation-specific factors, including drug concentration, polymer ratio, and gel microstructure, may modulate this effect differently under the conditions employed in this study. This should be further investigated using molecular dynamics or functional theory approaches to confirm the molecular mechanisms underlying the gelling system.

For the dual-drug formulation G-DM (0.511 N), Fisher LSD assigned an independent group B, placing it significantly below single-drug formulations (A) but significantly above WAT (C), with no significant difference from G-BLK (BC). This result indicates that co-loading both DIC and MTZ partially attenuates the mucoadhesion-enhancing effect observed with individual drug loading. This attenuation may arise from competitive effects of the two drugs on polymer network organization, altered micellar packing, or changes in the gel–mucus interfacial properties when both molecules are simultaneously present within the matrix. Despite this partial reduction, G-DM retained a meaningfully higher mucoadhesive force than the aqueous control, confirming that the gel matrix provides adequate adhesion for periodontal pocket retention and supporting its clinical suitability as a dual-action local delivery platform.

3.7. *In vitro* anti-inflammatory activity

The anti-inflammatory activity of G-DIC, G-MTZ, and G-DM was evaluated using the BSA denaturation assay and expressed as percentage inhibition relative to G-BLK, which served as the matrix reference control, and shown in Figure 3. This approach was adopted because G-BLK exhibited higher absorbance

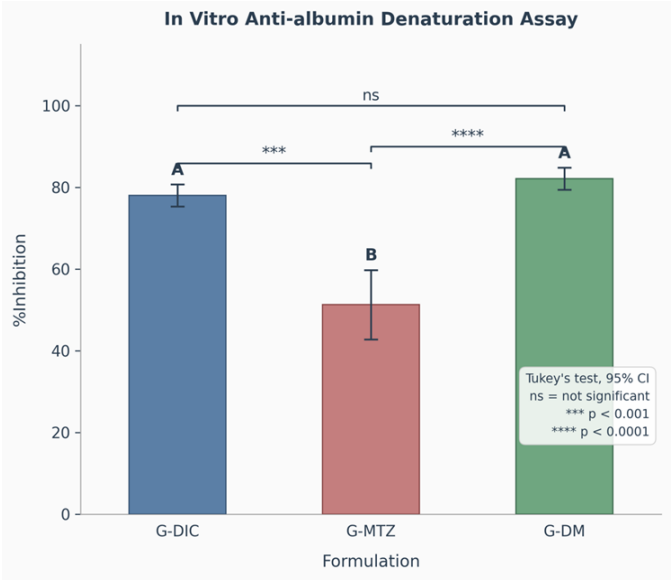

Figure 3. %Inhibition of thermosensitive gel formulations (mean ± SD, n = 3).

than water under the assay conditions, indicating that the poloxamer matrix itself promotes a degree of protein denaturation. Using G-BLK as the reference therefore isolates the drug-attributable anti-inflammatory contribution and prevents overestimation of inhibition values that would otherwise include a matrix-correction artifact. By definition, G-BLK represents 0% inhibition, the baseline against which the therapeutic effect of each drug-loaded formulation is measured.

The DIC-containing formulations (G-DIC and G-DM) demonstrated significantly higher inhibition of BSA denaturation compared with G-MTZ, confirming that DIC was the primary contributor to anti-inflammatory activity, consistent with its well-established mechanism of COX-2 inhibition and subsequent reduction of prostaglandin synthesis. G-DIC achieved 78.04% inhibition, while G-DM achieved 82.13% inhibition relative to the poloxamer matrix baseline.

Interestingly, G-DM showed numerically higher inhibition than G-DIC, although this difference was not statistically significant. This suggests that the incorporation of MTZ into the dual-drug formulation did not compromise the anti-inflammatory effect of DIC. Because the percentage inhibition values of G-DM and G-DIC were not statistically significantly different, the two drug-loaded formulations can be regarded as providing comparable anti-inflammatory activity; the numerically higher value for G-DM should not be interpreted as evidence of enhanced protein stabilisation or of a synergistic effect. G-MTZ exhibited moderate inhibition (51.31%), significantly lower than DIC-containing formulations, which is consistent with MTZ's primary antimicrobial rather than anti-inflammatory mechanism of action. The moderate but non-zero inhibition observed for G-MTZ may reflect a minor non-specific stabilizing effect of MTZ on BSA under heat stress conditions [31].

Collectively, these results confirm that the dual-drug formulation G-DM preserved the full anti-inflammatory efficacy of DIC while simultaneously incorporating antimicrobial activity through MTZ, supporting the therapeutic rationale of the combined periodontal delivery platform.

3.8. *In vitro* antimicrobial activity

The antimicrobial activity of the formulations was evaluated against three clinically relevant oral pathogens: *Porphyromonas gingivalis*, *Streptococcus mutans*, and methicillin-resistant *Staphylococcus aureus* (MRSA 4330), representing anaerobic periodontal pathogens, cariogenic oral bacteria, and resistant gram-positive cocci, respectively (as depicted in Figure 4). The zone of inhibition was shown in Table 5.

Formulations containing MTZ (G-MTZ and G-DM) demonstrated potent inhibitory activity against *P. gingivalis*, producing mean inhibition zones of 20.0 mm and 19.7 mm, respectively. This strong activity is consistent with MTZ's established mechanism as a selective agent against obligate anaerobic bacteria, making it particularly relevant for targeting the anaerobic subgingival microbiome characteristic of periodontitis. Similarly, robust inhibition was observed against *S. mutans* (19.0 mm for both G-MTZ and G-DM), further supporting the formulation's broad utility against oral pathogens.

Against MRSA 4330, only MTZ-containing formulations (G-MTZ and G-DM) produced measurable inhibition zones of 15.0 mm and 14.3 mm, respectively, while G-BLK and G-DIC showed no inhibitory activity, as expected given their mechanisms. The slight reduction in inhibition zone for G-DM compared to G-MTZ against MRSA may be attributed to minor changes in drug diffusion rate within the gel matrix upon co-loading of DIC.

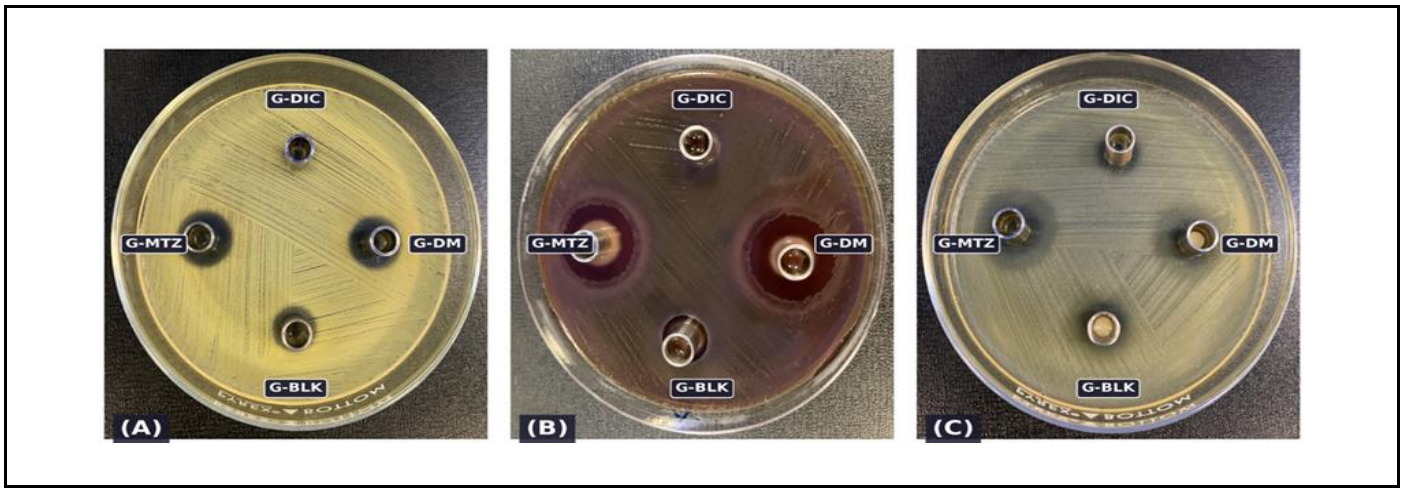

Figure 4. Inhibition zones of thermosensitive *in situ* gel formulations against oral pathogens evaluated by the agar-cup diffusion method. (A) Methicillin-resistant *Staphylococcus aureus* (MRSA 4330); (B) *Porphyromonas gingivalis*; (C) *Streptococcus mutans*.

Table 5. Inhibition zone (mm) of formulations (n = 3).

Formulation	MRSA 4330				*P. gingivalis*				*S. mutans*			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
G-BLK	—	—	—	0.0	10.0	10.0	11.0	10.3	12.0	11.0	9.0	10.7
G-DIC	—	—	—	0.0	11.0	11.0	12.0	11.3	12.0	12.0	11.0	11.7
G-MTZ	15.0	15.0	15.0	15.0	20.0	20.0	20.0	20.0	21.0	18.0	18.0	19.0
G-DM	14.0	14.0	15.0	14.3	20.0	19.0	20.0	19.7	20.0	20.0	17.0	19.0

Notably, both G-BLK and G-DIC exhibited baseline inhibitory activity against *P. gingivalis* and *S. mutans* (approximately 10.3–11.7 mm), which may reflect the inherent, albeit limited, antimicrobial properties of poloxamers reported in the literature [32]. G-DM maintained antimicrobial efficacy comparable to G-MTZ across all tested organisms, indicating no significant antagonistic interaction between DIC and MTZ within the thermo-responsive matrix. This confirms that G-DM retains full antimicrobial coverage while simultaneously delivering anti-inflammatory activity via DIC, supporting its dual-action therapeutic potential for periodontal pocket delivery.

3.9. *In vitro* drug release study

In vitro release of DIC and MTZ from the optimized thermo-responsive *in situ* gel formulations was evaluated using a dialysis bag diffusion method. The optimized binary poloxamer formulation (P407 16.44% w/w and P188 7.93% w/w) was tested as three gel formulations (G-DIC, G-MTZ, and G-DM) alongside a drug solution control (SOL-DM), in which both DIC and MTZ were dissolved in deionized water without a gel matrix, to isolate the contribution of the poloxamer network to release behavior.

The release profiles demonstrated a clear and pronounced difference between the gel formulations and SOL-DM (Figure 5). SOL-DM exhibited rapid drug release, with both DIC and MTZ being largely released within the early time points, confirming that the dialysis membrane itself does not impose significant diffusion resistance and that the gel matrix is the primary release-controlling barrier. Consistent with this, in both panels of Figure 5 the SOL-DM control shows the highest cumulative release at the early time points (0–2 h), whereas the three gel formulations release more slowly. In contrast, all three gel formulations (G-DIC, G-MTZ, and G-DM) demonstrated sustained release of both drugs over 24 h with no pronounced burst effect at early sampling points, confirming effective drug entrapment within the thermogelled poloxamer matrix and validating the role of the gel structure in modulating drug release.

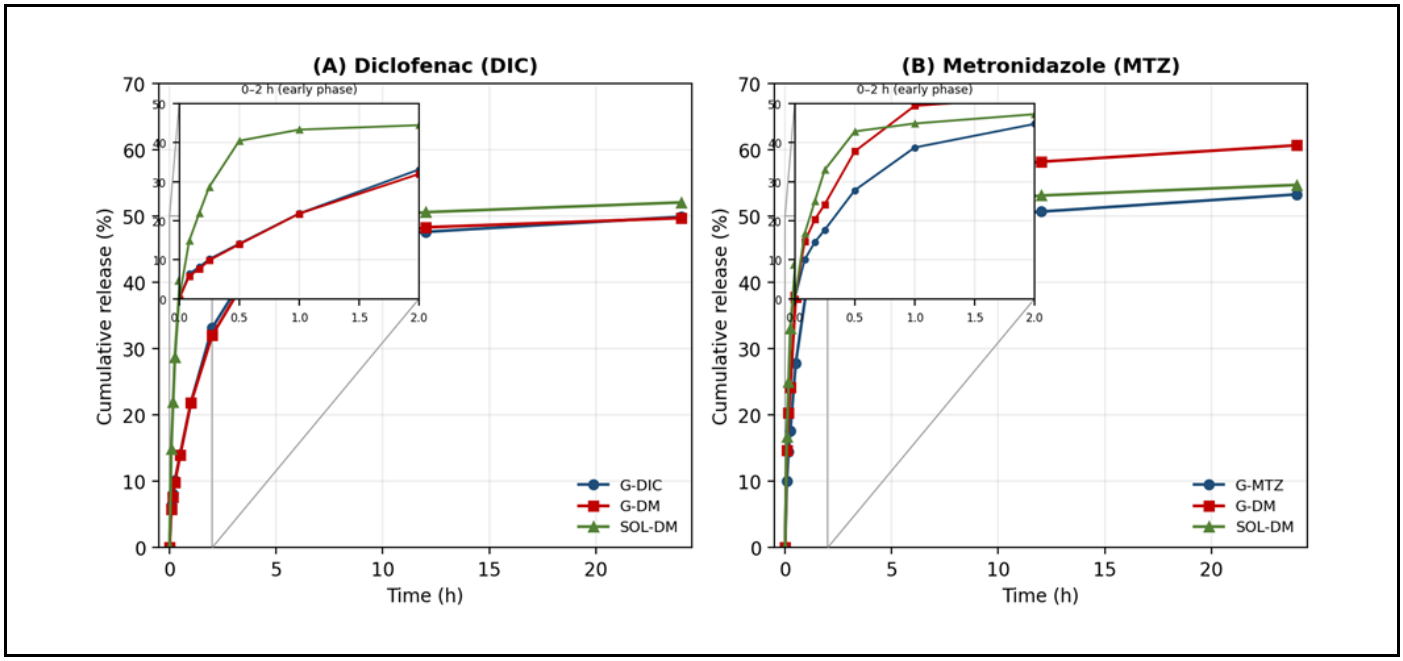

Figure 5. *In vitro* cumulative percentage release profiles of (A) diclofenac (DIC) and (B) metronidazole (MTZ) from thermosensitive *in situ* gel formulations over 24 hours. G-DIC = poloxamer gel loaded with diclofenac only; G-MTZ = poloxamer gel loaded with metronidazole only; G-DM = poloxamer gel loaded with both diclofenac and metronidazole; SOL-DM = drug solution control containing both diclofenac and metronidazole dissolved in deionized water without gel matrix.

For DIC, cumulative release from gel formulations increased gradually throughout the study period, reaching approximately 49–52% at 24 h. In the early phase (0–2 h), G-DM exhibited slightly higher DIC release compared with G-DIC, but the release curves converged after approximately 4 h and remained comparable thereafter. This behavior suggests that co-loading MTZ may modestly influence the initial diffusional environment without altering the long-term release-limiting mechanism, which appears governed by diffusion through the established gel network. This is consistent with previous studies reporting sustained DIC release from poloxamer-based gel systems [33].

MTZ showed a similar sustained profile across gel formulations but with a slightly faster initial release than DIC, reaching approximately 52–60% at 24 h. The relatively higher early MTZ release is consistent with its higher aqueous solubility and weaker affinity for the hydrophobic poloxamer microdomains compared to DIC, facilitating more rapid partitioning into the aqueous release medium. Despite these inherent drug-dependent differences, co-loading of DIC and MTZ in G-DM did not compromise the release of either compound; both drugs maintained controlled release kinetics and comparable terminal release extents within 24 h.

Mechanistically, the observed profiles are consistent with diffusion-controlled release from a micellar gel matrix. At physiological temperature, P407 undergoes micellization and micellar packing, forming a three-dimensional gel network that increases viscosity and creates a tortuous diffusion pathway. The absence of an early burst effect in all gel formulations supports homogeneous drug distribution and effective incorporation within the poloxamer network, in sharp contrast to the rapid release observed from SOL-DM. This is consistent with previous studies indicating that drug release from poloxamer-based *in situ* gels involves a combination of diffusion and erosion of the gel structure [34]. Furthermore, the incomplete release within 24 h suggests that a fraction of drug remains associated with the micellar or gel domains, an outcome advantageous for periodontal pocket delivery where extended residence time and sustained drug exposure at the target site are essential for therapeutic efficacy.

The use of a large dissolution volume (200 mL) and medium replacement after each sampling was intended to preserve sink-like conditions and avoid saturation effects, strengthening the interpretation that formulation structure, rather than solubility limitation, governed the release behavior. Overall, the optimized binary poloxamer gel achieved sustained, controlled release of both DIC and MTZ over 24 h, markedly superior to SOL-DM, confirming its suitability as a platform for prolonged local anti-inflammatory and antimicrobial periodontal therapy.

To elucidate the release mechanism, the cumulative release data of all formulations were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas and Hixson–Crowell models (Table 6). For every formulation the Higuchi model gave the highest coefficient of determination (R^2 = 0.81–0.99), indicating that drug release was predominantly governed by diffusion through the poloxamer gel matrix. This was supported by the Korsmeyer–Peppas release exponent (n), which ranged from 0.18 to 0.42 for all formulations ($n \leq 0.45$), confirming a diffusion-controlled (Fickian) transport mechanism rather than one governed by polymer relaxation or matrix erosion [35]. The gel formulations (G-DIC, G-DM and G-MTZ) fitted the Higuchi model markedly better (R^2 = 0.93–0.99) than the corresponding solution control (SOL-DM, $R^2 \approx$ 0.81–0.83), reflecting the additional diffusional resistance imposed by the micellar gel network and reinforcing the sustained-release behaviour described above.

4. CONCLUSIONS

A thermo-responsive and mucoadhesive *in situ* gelling system for local periodontal drug delivery incorporating diclofenac (DIC) and metronidazole (MTZ) was successfully developed and systematically characterized. Four formulations were prepared and evaluated throughout this study: blank poloxamer gel (G-BLK), DIC-loaded gel (G-DIC), MTZ-loaded gel (G-MTZ), and dual-drug gel (G-DM), alongside a drug solution control (SOL-DM) for drug release comparison. All gel formulations underwent sol-to-gel transition

Table 6. Release kinetic model fitting (R^2) and Korsmeyer–Peppas release exponent (n) for DIC and MTZ release from the gel formulations and the solution control (SOL-DM).

Formulation	Zero-order R^2	First-order R^2	Higuchi R^2	Korsmeyer–Peppas R^2	n	Hixson–Crowell R^2
G-DIC	0.9496	0.9721	0.9927*	0.944	0.407	0.9652
G-DM (DIC)	0.9452	0.9681	0.9947*	0.945	0.420	0.9609
SOL-DM (DIC)	0.5730	0.6252	0.8327*	0.775	0.193	0.6082
G-MTZ	0.8220	0.8745	0.9730*	0.866	0.295	0.8579
G-DM (MTZ)	0.7268	0.7904	0.9293*	0.868	0.279	0.7705
SOL-DM (MTZ)	0.5470	0.6102	0.8142*	0.769	0.178	0.5894

via micellization and micellar packing upon contact with physiological oral temperature, forming a polymeric depot suitable for sustained drug delivery within the periodontal pocket. The optimized formulation, consisting of 16.44% w/w P407 and 7.93% w/w P188, exhibited a measured Tgel = 33.68 ± 0.45 °C, suitable for syringe-based administration into the periodontal pocket followed by rapid *in situ* gelation at physiological temperature. Drug incorporation in G-DIC and G-MTZ not only provided therapeutic benefits but also enhanced mucoadhesive strength compared to G-BLK, thereby increasing residence time at the mucosal surface and supporting prolonged local drug exposure against GCF washout. G-DM demonstrated intermediate mucoadhesive behavior, remaining within an acceptable range for periodontal application. Anti-inflammatory activity assays confirmed that G-DIC and G-DM effectively inhibited protein denaturation, with DIC serving as the primary contributor to this effect. *In vitro* antimicrobial testing demonstrated that G-MTZ and G-DM produced significant inhibitory zones against key periodontal and oral pathogens including *Porphyromonas gingivalis*, *Streptococcus mutans*, and MRSA. *In vitro* drug release studies demonstrated that all gel formulations (G-DIC, G-MTZ, and G-DM) achieved sustained, diffusion-controlled release of their respective drugs over 24 hours without a pronounced burst effect, markedly superior to the rapid release observed from SOL-DM. This clearly confirms that the poloxamer gel matrix governs the release behavior, validating the formulation's potential for prolonged therapeutic drug exposure within the periodontal pocket. Future studies should evaluate the formulation in periodontal pocket models, assess safety in oral mucosal tissues, and conduct *in vivo* efficacy studies in animal models of periodontitis to further validate its therapeutic potential. Overall, these findings suggest that the binary poloxamer-based *in situ* gel represents a versatile and clinically promising platform for the sustained local delivery of antimicrobial and anti-inflammatory agents for periodontitis management.

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Author contribution

Y.S. performed experiments and data analysis; J.A., N.S., S.H., S.N., N.D., and N.W. performed experiments; E.D. and T.P. provided research facilities; K.J. supervised the study, conceptualized the research, and wrote the manuscript. All authors reviewed and approved the final manuscript.

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