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Fabrication and evaluation of lidocaine-loaded thermoresponsive organogels for enhanced pain management in dry socket wounds

Nuttawut Supachawaroj [a], Nuntachai Hanpramukkun [b], Kunchorn Kerdmanee [c], Sucharat Limsitthichaikoon [b,*]

[a] Department of Oral Surgery, College of Dental Medicine, Rangsit University, Pathum Thani, 12000, Thailand
[b] Department of Pharmaceutical Technology, College of Pharmacy, Rangsit University, Pathum Thani, 12000, Thailand
[c] Department of Periodontics, College of Dental Medicine, Rangsit University, Pathum Thani, 12000, Thailand

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ABSTRACT

The study focuses on developing mucoadhesive thermoresponsive organogels for sustained lidocaine (LD) release, aiming to improve pain management in dry socket wounds. Using a three-level, two-factor full factorial design with a center point, lidocaine-loaded thermoresponsive organogels (LTG) formulations were created with varying concentrations of polyethylene glycol 400 (PEG400) and tween80 (T80), alongside key components like poloxamer407 (P407), PEG4000, and Sacha Inchi oil. Ten formulations were prepared and evaluated for particle size, polydispersity index, swelling index, viscosity, gelation temperature, and gelation time. Results revealed that PEG400 and T80 had significant quadratic or interaction effects on the LTG properties, with P407 and PEG4000 driving the thermoresponsive behavior. PEG400 and T80 notably improved mucoadhesion and increased viscosity. Higher concentrations of T80 enhanced heat tolerance, reduced swelling, and increased formulation stability, allowing autoclave sterilization, which ensures safe use on open wounds. The optimized LTP formulations provided sustained LD release for 72 h, following the Higuchi model. Biodegradability, measured by weight remaining, inversely correlated with viscosity and drug release. *In vivo* tests using mouse tail flick and hot plate models confirmed that LTG with high PEG400 and T80 concentrations exhibited superior anesthetic effects compared to LD hydrogel and LD solutions. These results suggest that the optimized LTG formulations offer significant potential for managing pain in dry socket wounds, combining ease of application, sustained release, and effective local anesthesia.

1. Introduction

Effective pain management in dental practice is crucial, especially for conditions such as dry socket [1–3] and post-operative pain [4,5]. Conventional analgesics often require frequent administration, reducing patient compliance and increasing side effects [5,6]. Therefore, innovative drug delivery systems offering prolonged analgesic effects with minimal systemic exposure are highly sought after.

Lidocaine (LD), a widely used local anesthetic, is highly effective due to its rapid onset and potent analgesic properties. It works by blocking sodium channels in neuronal membranes, inhibiting nerve impulse initiation and propagation, effectively numbing the application area [5–7]. This makes it ideal for various medical and dental procedures, including minor surgeries, dental work, and acute pain management [5]. However, its short duration of action—typically lasting only 1-2 h—necessitates frequent reapplication, which can be inconvenient and uncomfortable for patients, especially in cases requiring prolonged pain management, such as dry socket [2,8], postoperative care [9] or chronic pain conditions [10]. To address these limitations, considerable research has focused on developing advanced formulations that can provide sustained release of LD. These innovative delivery systems aim to extend the drug's duration of action, reducing the need for frequent reapplications and enhancing patient comfort and compliance. Strategies include the use of various polymers [11], polyelectrolyte complex [12], liposomes [13], hydrogel [12,14], and organogels [15], designed to slowly release LD over an extended period. One promising approach involves thermoresponsive organogels, which transition from a liquid to a gel state at body temperature, ensuring localized and sustained drug

* Corresponding author. Department of Pharmaceutical Technology, College of Pharmacy, Rangsit University, Pathum Thani, 1200, Thailand.
E-mail addresses: nuttawut.s@rsu.ac.th (N. Supachawaroj), nuntachai.h@rsu.ac.th (N. Hanpramukkun), kunchorn.k@rsu.ac.th (K. Kerdmanee), Sucharat.l@rsu.ac.th (S. Limsitthichaikoon).

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release [16,17]. These organogels adhere to mucosal surfaces, such as in the oral cavity, providing prolonged therapeutic effects where needed [18]. Incorporating biodegradable materials like poloxamer407 (P407) enhances their biocompatibility and reduced systemic exposure [19].

Thermoresponsive organogels have appeared as a promising platform for novel drug delivery systems. These gels are liquid at lower temperatures for easy application but transition to a gel state at body temperature, ensuring sustained and localized drug release [20,21]. Their distinctive properties make them particularly suitable for mucosal applications, such as in the oral cavity, where they adhere to mucous membranes and provide prolonged therapeutic effects [18]. This extended contact with mucous membranes enhances therapeutic efficacy. Thermoresponsive organogels have been specially developed to provide precise and prolonged drug delivery, hence minimizing the need for frequent reapplications, and ensuring consistent therapeutic effects [18,20]. The gel matrix regulates the dispersion of medication, enabling a consistent and prolonged release [16]. Their liquid state at lower temperatures allows for easy and precise application to the target site, particularly advantageous in dental applications, enhancing patient comfort and compliance [22,23]. The formulation of thermoresponsive organogels can be tailored to include various active pharmaceutical ingredients (APIs) and excipients, allowing customization of properties such as viscosity, gelation temperature, and drug release profile to meet specific therapeutic needs [24]. Depending on the choice of gelator molecules and organic solvents, thermoresponsive organogels can be designed to be biocompatible and biodegradable, making them suitable for biomedical applications like topical drug delivery and tissue engineering [25,26]. Thermoresponsive organogels offer enhanced thermal, environmental, and microbial stability compared to hydrogels due to their unique composition and gelation mechanism. They effectively encapsulate hydrophobic drugs, undergo reversible sol-gel transitions, and have tunable gelation temperatures, contributing to their improved stability [27,28].

In dental applications, thermoresponsive organogels provide targeted pain relief by being applied directly to the site of pain or wound. This is particularly beneficial for conditions like dry socket, postoperative pain, and other dental procedures requiring localized treatment. The sustained drug release reduces the need for frequent reapplications, improving patient compliance and comfort, and ensuring prolonged relief from a single application. The gel matrix protects the drug from degradation, maintaining its effectiveness throughout the treatment period, which is crucial for sensitive drugs that may degrade rapidly under physiological conditions.

This study investigates the fabrication and evaluation of lidocaine-loaded thermoresponsive organogels (LTG) for enhanced pain management in dental practice. Using a full factorial design with two factors at three levels, key parameters such as particle size, polydispersity index (PdI), swelling index, viscosity, gelation temperature, and gelation time were optimized. Mechanical properties, mucoadhesion, drug release, biodegradation, sterilization by autoclave, and stability of the formulations were also assessed, along with their analgesic effects in mouse models to evaluate efficacy. The goal was to develop a formulation that provides sustained LD release with desirable physicochemical properties for ease of application and stability. This research aims to enhance dental pain management by offering a more effective and patient-friendly solution for prolonged analgesia.

2. Materials and methods

2.1. Materials

Poloxamer407 (P407), Nile red (9-diethylamino-5H-benzo[alpha] phenoxazine-5-one) and type II mucin from the porcine stomach (lot no. SLCC7713) were purchased from Sigma-Aldrich® (Missouri, U.S.A.). Lidocaine BP (LD, Batch no. 1541), polyethylene glycol 4000 (PEG4000), polyethylene glycol 400 (PEG400) and tween80 (T80) were purchased from Aketong Chemipun (Bangkok, Thailand). Sacha Inchi oil (SO) by cold press method was purchased from the organic farming group, (Chiang Mai, Thailand) and used as received.

2.2. Experiment designs

A three-level, two-factor full factorial design with a center point was applied to statistically optimize the concentrations of PEG400 (X1) and T80 (X2) for developing lidocaine-loaded thermoresponsive organogels (LTG) intended for dental applications. These organogels consist of both aqueous and oil phases, where Sacha Inchi oil (SO) serves as the oil phase, and PEG4000 acts as the organogelator. Phosphate buffer saline (PBS) at pH 7.4 was employed as the solvent, PEG400 was the solubilizer, T80 functioned as the surfactant, and poloxamer407 (P407) was used as the thermoresponsive polymer. The thermoresponsive organogels comprised both aqueous and oil phases, with SO as the oil phase and PEG4000 as the organogelator. Phosphate buffer saline (PBS) at pH 7.4 was used as the solvent, PEG400 as the solubilizer, T80 as the surfactant, and P407 as the thermoresponsive polymer. Using these components, 10 distinct LTG formulations were generated with the aid of Design-Expert® v.11.0.3 software (Stat-Ease, Inc., Minneapolis, MN, USA). The concentrations of PEG400 (X1) and T80 (X2) were designated as the independent variables (Table 1).

This experimental design effectively combines the principles of a factorial design with center points, incorporating elements to account for curvature in response surfaces, making it ideal for response surface methodology (RSM) applications [29,30]. By including a center point with replicates, the design allows for accurate estimation of experimental error while minimizing the number of experimental runs, ultimately reducing both time and cost. Importantly, the addition of center points does not interfere with the estimation of the main effects but enables the detection of curvature in the response surface, providing valuable insights into factor behavior at intermediate levels [31]. In this study, LTG10 was chosen as the center point, representing mid-levels of both factors and closely resembling the composition of LTG5. This center point is crucial for estimating experimental error, assessing model fit, and identifying any curvature effects, which are essential for precise response surface modeling [31]. Moreover, this approach requires fewer runs than a full factorial design while still producing sufficient data to fit a second-order polynomial model, ensuring both efficiency and

Table 1
The optimization of lidocaine-loaded thermoresponsive organogels (LTG), by a three-level, two-factor full factorial design with a center point. The parameters, components and levels composed of lidocaine BP (LD), poloxamer407 (P407), polyethylene glycol 4000 (PEG4000), Sacha Inchi oil (SO), polyethylene glycol 400 (PEG400) and tween80 (T80).

parameters	components	Applied levels		
		Low Level (−1)	Center Level (0)	High Level (+1)
X1	PEG400	5	10	15
X2	T80	1	2	3

Run	components					
	PEG400	T80	LD	P407	PEG4000	SO
LTG1	5	1	2	18	0.2	3
LTG2	5	2	2	18	0.2	3
LTG3	5	3	2	18	0.2	3
LTG4	10	1	2	18	0.2	3
LTG5	10	2	2	18	0.2	3
LTG6	10	3	2	18	0.2	3
LTG7	15	1	2	18	0.2	3
LTG8	15	2	2	18	0.2	3
LTG9	15	3	2	18	0.2	3
LTG10[a]	10	2	2	18	0.2	3

[a] LTG10 served as the center point, representing medium levels for both factors, similar in composition to LTG5.

statistical robustness [30].

The dependent variables measured in the study were particle size (Y1, in micrometers), polydispersity index (PdI, Y2), swelling index percentage (Y3), viscosity (Y4, in centipoise), gelation temperature (Y5, in °C), and gelation time (Y6, in seconds). In the response surface design, the independent variables were carefully selected for their significant impact on the physicochemical properties and overall characterization of the thermoresponsive organogels. These critical properties, including particle size, PdI, swelling index, viscosity, gelation temperature, and gelation time, are key factors that influence the stability and performance of the organogels. By selecting variables that directly affect these characteristics, the design ensures an optimized formulation with reliable thermoresponsive behavior.

2.3. Formulation and characterization of LTG

LTG were fabricated using a 3^2 factorial combined with a center point to optimize the formulation. The detailed compositions of each formulation are presented in Table 1. The preparation was divided into two phases: water and oil, each requiring precise temperature control to ensure uniformity and performance. The water phase, containing PEG400 and PEG4000, was heated to 75 °C for proper solubilization and mixing, which is crucial for the gel's texture and thermoresponsive properties. Simultaneously, the oil phase was prepared by combining 2 % lidocaine (LD), Sacha Inchi oil (SO), and polysorbate 80 (T80), and heating the mixture to 70 °C. This temperature ensures complete dissolution of lidocaine in the oil and uniform mixing of T80, which is vital for the stability and effectiveness of the organogels. Once the oil phase reached 70 °C, it was slowly added to the water phase and homogenized at 7000 rpm for 5 min using a high-pressure homogenizer (Daihan® Homogenizer, model HG-15D). This high-speed homogenization step is critical for creating a stable oil-in-water emulsion, which ensures consistent drug delivery and gel behavior. After homogenization, the mixture was cooled to room temperature while being stirred with a magnetic bar. At this stage, P407 (a thermoresponsive polymer) was added. The P407 stock solution, prepared using the cold method to prevent clumping, was incorporated into the emulsion to achieve an 18 % concentration in all formulations. Continuous stirring during this process ensured even distribution of P407, essential for the desired thermoresponsive properties of the final LTG gels.

Lidocaine hydrogel (LDG) was prepared by incorporating 2 % LD into P407 solution, followed by stirring with a magnetic bar until homogenous, and labeled as LDG for use in animal studies.

Lidocaine solution (LS) was prepared by incorporating 2 % LD into PBS solution, followed by stirring with a magnetic bar until completely dissolved, and labeled as LS for use in animal studies.

Blank thermoresponsive organogels (BTG) was prepared using the same method as LTG, without the addition of LD, and labeled as BTG.

2.3.1. Appearances

The appearance of LTGs was assessed visually, and their pH was measured using a PEAK-P-310 Pocket pH meter (U.S.A.). Each LTG sample was dyed with Nile red and observed under a confocal laser scanning microscope (DMi8, Leica, Wetzlar, Germany). Nile red, dissolved in ethanol to create a 2 % stock solution, was used to stain the LTG formulations. After staining, the LTG samples were incubated at room temperature for 15 min to ensure uniform dye penetration. A thin layer of the dyed formulation was then placed on a microscope slide, covered with a cover slip to prevent evaporation and flatten the sample. The confocal microscope was set to Nile red's excitation and emission wavelengths (522 nm and 636 nm, respectively). Laser intensity and detector sensitivity were adjusted for optimal visibility of the stained vesicles. Images were captured at different focal planes to obtain the vesicle structure. The associated software was used to process and analyze the images, providing quantitative data on the distribution, size, and morphology of the vesicles within the LTG formulations.

2.3.2. Surface morphology

LTG samples were analyzed for surface morphology using a FEI Quanta 450 Field Emission SEM (Austria). The samples were diluted six times with DI water, sonicated for 5 min, then mounted on a stub, gold-coated, and visualized at 20,000 × magnification.

2.4. Evaluation of dependent variables

2.4.1. Particle size, and poly dispersion index (PDI)

Particle size, PDI, and zeta potential were determined using dynamic light scattering (DLS) with a NanoPlus® nanoparticle size and zeta potential analyzer (Model NanoPlus-3 Serial no. 409314, U.S.A.). For measurement, each LTG formulation was diluted sixfold in distilled water.

2.4.2. Swelling index

The swelling index measures the ability of the polymer components in each LTG formulation to absorb water and expand, reflecting the gel's swelling behavior. In this experiment, the formulation was first placed in a 15 mL centrifuge tube, and its initial volume of 3 mL in its dry state (V_d) was recorded. Distilled water was then added to the tube, bringing the total volume to 10 mL. The sample was allowed to sit undisturbed for 24 h, providing sufficient time for the polymer to fully interact with the water and reach its maximum swollen state. After the one-day period, the volume of the swollen gel was measured and recorded as the volume after swelling (V_s). The difference between the dry and swollen volumes gives insight into how much the polymer expands upon water absorption. The swelling index was then calculated as a percentage using the following equation (1):

$$\textit{percentage of swelling index} = \frac{V_s - V_d}{V_d} \times 100 \quad (1)$$

This measurement is critical for understanding the water-absorbing capacity of the LTG formulations, which plays a key role in their performance, stability, and gelation behavior in physiological conditions. Swelling behavior can also influence drug release rates, making it a crucial parameter for evaluating the overall effectiveness of the thermoresponsive organogels.

2.4.3. Viscosity

The viscosity of the LTG formulations was measured using a Brookfield viscometer (Brookfield Model DV-II + viscometer, Brookfield Engineering Laboratories, U.S.A.). Each gel sample was placed in a sample holder connected to a temperature-controlled jacket. Using viscometer probe no.16 at a fixed rotational speed of 10 rpm, measurements were taken at 4 °C and 37 °C to determine the viscosity at solution and gel states (n = 3).

2.4.4. Gelation temperature and gelation time

The phase transition temperature of the thermoresponsive organogels was determined using a Brookfield viscometer (Brookfield Model DV-II + viscometer, Brookfield Engineering Laboratories, U.S.A.). Each sample was placed in a container with a temperature-controlled jacket, and the viscometer probe no.16 was set at 10 rpm. The temperature was gradually increased from 4 °C to 40 °C while monitoring the viscosity. A significant rise in viscosity at a specific temperature was noted as the gelation temperature for each formulation (n = 3).

The gelation time was measured as the duration required for the organogels to transition from a solution to a gel state, suing the Brookfield viscometer. Samples were placed in a temperature-controlled container, with the viscometer probe no.16 set at 10 rpm. As the temperature increased from 4 °C to 40 °C, the viscosity was monitored. The time change at which a significant increase in viscosity occurred was recorded as the gelation time of each formulation (n = 3).

2.5. Stability assessments

2.5.1. Drug content

To ensure complete extraction of the LD from the LTG formulations, a systematic method was followed. One gram of each formulation was weight into a 10 mL volumetric flask, and methanol was added as the extraction solvent to dissolve the drug fully. The mixture was then sonicated for 15 min to help break down the gel matrix and promote the release of the LD into the solvent. After sonication, the samples were allowed to stand for 24 h, providing ample time for any remaining drug to diffuse into the methanol. The extracted samples from each formulation were then analyzed using a UV spectrophotometer (Shimadzu UV1700, Japan) at 270 nm [32,33]. This multi-step approach—sonication, prolonged extraction, and spectrophotometric analysis—ensures that the drug was thoroughly extracted from the formulation, minimizing the risk of incomplete extraction. The use of methanol as the solvent, along with the extended standing time, further enhanced the reliability of this method for total drug content determination.

2.5.2. Physical property by Heating–Cooling cycles

The heating-cooling cycle test assessed the stability of the LTG formulation by subjecting it to alternating temperature conditions. The formulation was stored at 4 °C for 12 h and then placed in a hot air oven at 45 °C for another 12 h, completing one cycle. This process was repeated for a total of 7 cycles. After these cycles, the formulation's stability was evaluated by observing any changes.

2.5.3. Thermo-sterility tolerance by autoclave

Given that thermoresponsive organogels used in dental applications may be susceptible to infection or require sterile conditions, it was essential to sterilize the formulation. Sterilization was carried out using an autoclave at 121 °C and 15 psi for 15 min. Post-sterilization, the gel integrity was evaluated by examining phase separation, pH, and drug content to ensure the formulation remained homogenous and stable.

2.5.4. Stability testing

The formulations were stored under three different conditions: refrigerated at 5 ± 3 °C, at room temperature (30 ± 2 °C), and in a hot air oven at 40 ± 2 °C [34,35]. Stability was monitored by analyzing the LD content over time using a UV spectrophotometer at 270 nm. The percentage of LD was compared to the labeled amount (%LA) at 0, 1, 3, and 6 months. These data were used to create a stability graph, visually representing the formulation's stability under varying conditions followed the Arrhenius equation [34,35]. The Arrhenius equation (2) defines the relationship between the rate of a reaction and temperature.

$$k = Ae^{Ea/RT} \tag{2}$$

where k = rate constant (s^{-1})
A = pre-exponential factor.
R = ideal gas constant (8.314 J/mol·K)
T = absolute temperature (K)

2.6. Mechanical properties

2.6.1. Texture profile analysis

The texture profile qualities of the LTG formulations were assessed in their gel state using a texture analyzer (TA.XT PlusC, Stable Micro Systems, Surrey, U.K.). Each formulation was placed onto a 55 mm culture plate and induced to solidify into a gel. The double compression technique was used [36,37]. The texture analyzer's probe descended at a velocity of 2 mm/s until it made contact with the gel surface. It then penetrated the gel matrix to a depth equal to half of its height, before being retracted back to its original surface contact position. After holding this position for 15 s, the second compression was executed in the same manner. Hardness, springiness, and resilience values were derived from the force–time graphs obtained by the texture analyzer program (n = 3).

2.6.2. Injectability test

The LTG formulations were filled into 1 mL syringes equipped with a 22-gauge stainless steel needle, which had a projected diameter of 0.7 mm, for use in standard clinical settings. The syringe was secured in a vertical holder that was positioned at the base platform of the texture analyzer (TA.XT PlusC, Stable Micro Systems, Surrey, U.K.). A cylindrical probe (Model P/0.5, 12.7 mm diameter) was used to exert downward pressure on the plunger rod of the syringe. The probe was moved at a constant of 10 mm/s. The peak force required to propel the formulation through the needle tip was measured. The measurements were performed at room temperature (25–30 °C), simulating typical clinical conditions.

2.7. Mucoadhesive property

The mucoadhesive properties are essential for drug delivery systems, particularly for applications in mucosal tissues such as the oral cavity. The mucoadhesive properties of LTGs were assessed using 2 methods as mucin disc test by using texture profile analyzer and examining the interactions between mucin and the organogels. Mucoadhesion ensured the formulation remains in contact with the mucosal surface for extended periods, enhancing local pain relief and anesthetic action. Adherence to the mucosa allows for localized LD release, reducing systemic absorption and side effects, which is crucial for targeted dental pain management. Strong mucoadhesion prevents the formulation from being washed away by saliva or dislodged by mouth movements, ensuring consistent pain relief without frequent reapplication. The test used crude mucin powder to mimic the natural mucous layer, providing realistic data on the formulation's adhesive performance.

2.7.1. Mucin disc test

The mucin disc test experiment evaluates the mucoadhesive properties of LTG formulation, which is essential for their effectiveness in dental applications. A texture analyzer measures the mucoadhesive force, enabling precise comparison and optimization of organogels compositions. The test also assesses how the formulation transitions from a liquid to a gel at body temperature, ensuring it adheres effectively in the mouth. Using a standardized method ensures reliable, reproducible results that validate the formulation's mucoadhesive properties across different studies.

The mucoadhesive properties of the LTG samples were assessed with slight modifications to the mucin disc model as referenced [37,38]. The mucoadhesive force was measured using a texture analyzer (TA.XT PlusC, Stable Micro Systems, Surrey, U.K.). Mucin discs were created by compressing 250 mg of crude mucin powder in a 13-mm-diameter die under a vacuum ring compression at 10 tons for 30 s. One mucin disc was secured at the center of a 60 mm Petri dish, which was fixed to the texture analyzer's base platform. Another mucin disc was attached to the cylindrical probe tip of the texture analyzer. 0.1 mL of LTG formulation was applied between the two mucin discs. The probe was lowered to leave a 1 mm gap between the discs, allowing the formulation to gel. The probe then compressed the discs together and held for 30 s before moving upward at 10 mm/s. The maximum force required to separate the discs was recorded as the mucoadhesion force of each formulation (n = 3).

2.7.2. Interaction between mucin and thermoresponsive organogels

The interaction between mucin and the thermoresponsive organogels was investigated to understand the mucoadhesive properties of the formulations. By analyzing the adhesive forces generated, we can gain insight into how effectively the organogels adhere to mucosal surfaces. This interaction is crucial for ensuring prolonged retention and

effectiveness of the drug delivery system in the oral cavity, where mucosal adhesion can significantly influence the therapeutic outcomes.

Mucoadhesion was assessed by evaluating the interactions of LTGs and mucin type II and quantifying the adhesive strength between the thermoresponsive organogels and the glycoproteins in the mucus [12,39]. During this assessment, 5 mL of each LTG sample was combined with 5 mL of mucin type II in test tubes. The tubes were gently inverted five times. The viscosity of each mucin and formulation was tested at rotational speeds of 10, 30, 60, and 100 rpm immediately, without any further shaking. The proportion of mucoadhesion was determined by analyzing the following equation (3), which measures the percentage of mucoadhesiveness.

$$\%\,\text{Mucoadhesiveness} = \frac{\text{average } \eta \text{ of mixture} - \text{average } \eta \text{ of formulation}}{\text{average } \eta \text{ of formulation}} \times 100\% \quad (3)$$

2.8. In vitro release

Experiments were conducted to evaluate LD release in an artificial environment using Franz diffusion cells. A dialysis membrane with a 12 kDa molecular weight cutoff (Sigma-Aldrich, St. Louis, MO, U.S.A.) was used, providing a diffusive interface surface area of 176.625 mm^2. Approximately 5 g of LTGs sample was loaded in a donor compartment. The receptor compartment, with an 11 mL volume, was filled with artificial saliva solution (pH = 7.4) and stirred magnetically at 300 rpm. The system was maintained at 37 ± 2 °C. At predetermined intervals, 1 mL samples of the medium containing the release drug were withdrawn and replaced with an equivalent volume of fresh medium to maintain sink conditions. The amount of release LD in each sample were analyzed using UV–Visible spectrophotometry, as previous described. The LD release kinetics were assessed by combining the dissolution data with several mathematical models, such as zero order, first order, Higuchi's, Hixson-Crowell, and Korsmeyer-Peppas models. The release mechanism was identified using DD-Solver software tool, an add-in for Microsoft Excel (Redmond, WA, U.S.A.), licensed by the Information Technology Service Center (ITSC), Rangsit University. This tool is developed in Visual Basic Applications (VBA).

2.9. Biodegradation

An important feature of thermoresponsive organogels formulations is their capacity to completely breakdown over a period of time. Throughout the therapy process, the organogels should slowly decompose, enabling the growth of epithelium tissues to replenish and occupy the wound region, successfully alleviating pain in situations such as tooth extraction, dry socket, or post-operative pain discomfort [40,41]. Assessing the biodegradation of the organogels in a controlled laboratory setting allows for estimation of its durability and longevity after application. This ensures that the organogels breaks down at a suitable pace to provide efficient drug release while avoiding any negative consequences.

The *in vitro* biodegradation of the LTG formulations was observed in a controlled laboratory setting to monitor the decomposition over time through natural biological processes. This experiment simulated various wound pathologies by using silicone molds imprinted with a tooth shape, ensuring the organogels samples remained intact for a specific period before degrading. Each wound model was injected with 1.0 mL of the LTG formulation and submerged in 20 mL of artificial saliva (pH 7.4) before being placed in an incubator shaker (model: ES-60E, MUIlab, Zhejiang, China) at 37 °C. The weight of each formulation model was measured at four different time points: 6, 12, 24, and 48 h (n = 3). The *in vitro* biodegradation was calculated using equation (4).

$$\text{Weight remaining } (\%) = \frac{W_t}{W_0} \times 100\% \quad (4)$$

Where W_t is the weight remaining at that time.

W_0 is the initial weight.

2.10. Pain tolerance and anesthetic efficacy studies

In vivo tests using mouse models, such as the tail-flick and hot plate tests, were conducted to evaluate the analgesic efficacy of thermoresponsive organogels formulations by measuring responses to thermal stimuli. These tests provided insights into the duration and effectiveness of the local anesthetic effect and helped identify the most suitable formulation for subsequent clinical trials.

The Institutional Animal Ethics Committee authorized procedures (RSU-AEC 001–2564) and monitored the study according to ethical standards [42]. Male ICR mice (*Mus musculus*, MLAC:ICR), aged 4–6 weeks with an average initial weight of 20 ± 5 g, were bred by the National Laboratory Animal Center in Nakornprathom, Thailand. They were housed in a Heating Ventilating and Air Conditioning system at the College of Pharmacy, Rangsit University, Pratum Thani, Thailand. The mice were provided with food and filtered water *ad libitum* and acclimatized in standard Plexiglas cages. Sample size determination and power analysis using the G*Power program, with each group consisting of six mice (n = 6) (effect size = 0.55, alpha error = 0.05, and power of test = 0.8) [43].

All mice were confined in cages for about one week under controlled conditions of a 12-h light-dark cycle (lights on at 06:00 h), at 25 ± 3 °C and humidity of 60 ± 5 %. They were randomly allocated to each study.

2.10.1. Evaluating of local anesthetic efficacy via tail flick test

The tail flick experiment is a commonly used technique in preclinical research to assess the pain-relieving effectiveness of anesthetics in laboratory mice [44]. This test provides data on the onset of action, duration, and intensity of anesthesia. The objective of this research was to examine the effectiveness of LTG formulation in inducing analgesia and numbness in mice [13].

The tail flick was conducted using the water bath approach [44,45]. The procedure involved measuring the response times after applying 0.1 g of blank gel (BTG), LTG formulation, and lidocaine gel (LDG) to the mouse's tail. The mouse was placed in a restraint device, leaving only its tail exposed. After the mouse adapted to the setup, its tail was immersed in a water bath maintained at 54 ± 1 °C, and the duration was recorded. The timer was stopped, and the exact moment was noted when the mouse's tail either twisted or was withdrawn from the water. The response times were recorded at intervals of 0 (baseline), 15, 30, 60, and 90 min after the application of the samples. The results were expressed as a percentage of tolerance (%T) and calculated using equation (5) provided,

$$\text{Percentage of tolerance time } (\%T_{\text{tail flick}}) = \frac{\text{Time of effective to tail flick test} - \text{time of control}}{\text{time of control}} \times 100 \quad (5)$$

2.10.2. Assessment of thermal pain response using a modified hot plate model

The technique, based on research by Hunskaar et al. (1986) [46] and modified by Chitcharoenthum and Khunkitti (1997) [47], used a water bath heated to 54 ± 1 °C with a 5-L beaker secured inside. The mice were administered BTG, LTG formulations, and LDG on their paw at a dose of 0.1 g. Measurements were taken at specific time intervals (0, 15, 30, 60, and 90 min) post-administration. When the mice exhibited abrupt movements or engaged in paw licking, the timer was stopped, and the duration was recorded. The results were expressed as a percentage of tolerance (%T) and calculated using equation (6).

$$\text{Percentage of tolerance time } (\%T_{\text{Hot plate}}) = \frac{\text{Time of effective to Hot plate test} - \text{time of control}}{\text{time of control}} \times 100 \quad (6)$$

2.11. Statistical analysis

The statistical significance of the data was evaluated using one-way analysis of variance (ANOVA) followed by the Tukey test. The 3^2 full factorial experiment was fabricated with Design-Expert® v.11.0.3 (Stat-Ease, Inc., Minneapolis, U.S.A.). The categorical variables were reported as percentage based on sample size of 3 and 6 (n = 3 and 6). The continuous variables were summarized using averages and standard deviations (SD), and the normality of the data was evaluated. Statistical significance ($p < 0.05$) was determined using either a student's t-test or ANOVA to compare the average values.

3. Results and discussion

LD is the standard lidocaine described in the British Pharmacopoeia, existing in its base form [48–50]. It is less soluble in water compared to its hydrochloride counterpart (LH), making it suitable for topical and localized applications where prolonged action is beneficial [48,50]. While the side effects of LD are generally similar to those of LH due to the same active ingredient, LD poses a lower risk of systemic toxicity. This is attributed to its lower solubility and reduced likelihood of systemic absorption [51]. Thus, LD is widely used in formulations that provide effective localized anesthesia with minimal systemic impact.

3.1. Experimental design and development

This experiment study aims to develop a 2 % w/w lidocaine-loaded thermoresponsive organogels (LTG) for use in dental procedures, focusing on pain reduction. The optimization process involved varying the concentrations of PEG400 (X1) and T80 (X2), which served as key independent variables. These variables were selected for their influence on crucial formulation characteristics, including particle size (Y1), PdI (Y2), percentage of swelling (Y3), viscosity (Y4), gelation temperature (Y5) and gelation time (Y6). The organogels comprised both aqueous and oil phases, with SO as the oil phase, PEG4000 as the organogelator, phosphate buffer saline (PBS) at pH7.4 as a solvent, PEG400 as the solubilizer, and T80 as the surfactant. The Design Expert software was utilized to create 10 different LTG formulations by systematically adjusting the concentrations of PEG400 and T80 (as shown in Table 2).

Table 2 shows that the particle size ranged between 3 and 6 μm, with a PdI range of 0.05–0.7. Lower concentrations of PEG400 (X1) and T80 (X2) resulted in smaller organogels particle sizes. The percentage of swelling was less than 20 %, with higher concentrations of PEG400 leading to lower percentage of swelling, as seen in the formulation LTG7-LTG9. At refrigerator temperature, all formulations were easily injectable through a syringe. However, at room temperature, greater force was required for injection. The viscosity at 4 °C was significantly lower (1000-fold) than at 37 °C. The higher concentrations of PEG400 resulted in higher viscosity at both temperatures. The gelation temperature ranged from 20 to 28 °C, with gelation times between 50 and 180 s. The higher concentrations of PEG400 corresponded to lower gelation temperature and shorter gelation times.

Through this optimization process, an optimal balance of these independent variables was identified which resulted in a formulation exhibiting desired characteristics. The optimized formulation was designed to maintained liquid form at refrigerator (2–8 °C) and transition into a gel at room temperature (25–30 °C), allowing for *in situ* gelation. This sol-gel transformation upon exposure to body temperature ensures ease of injection and sustained drug release at the application site. In the final analysis, the independent variables PEG400 and T80 were optimized to achieve a balance between low particle size, controlled swelling, suitable viscosity, and efficient gelation properties. These optimized values ensured the gel's injectability, stability, and enhanced pain-relief performance in dental practice. Table 2 highlights the optimized experimental run with a detailed breakdown of the variables that led to the most favorable LTG formulation, ensuring an effective, patient-friendly thermoresponsive organogels system.

The prepared LTG formulations appeared as a white, viscous solution. Fig. 1A shows confocal laser scanning microscopy image of Nile-red-stained oil-in-water organogel vesicles. The image shows that the surfactant molecules (T80) self-assemble into spherical structures. In

Table 2
Outline of experimental design and results. The experimental levels (low, center, and high) are represented by the coded values of −1, 0, and +1, respectively. Dependent variables are particle size (μm), PdI, swelling (%), viscosity (cP), gelation temperature (°C), and gelation time (second).

Run	Independent Variables		Dependent Variables						
	X1	X2	Particle size (μm)	PdI	Swelling (%)	Viscosity at 4 °C (cP)	Viscosity at 37 °C (cP)	Gelation temperature (°C)	Gelation time (sec)
LTG1	−1	−1	4.26	0.26	11.11	119.97	166100	20.3	90
LTG2	−1	0	3.78	0.05	11.11	119.97	168000	24.8	120
LTG3	−1	+1	3.55	0.10	14.81	119.97	183700	27.2	180
LTG4	0	−1	5.49	0.29	22.22	279.94	173000	19.9	84
LTG5	0	0	5.80	0.42	18.52	119.97	184500	26.2	116
LTG6	0	+1	5.02	0.48	14.81	239.95	180900	22.4	96
LTG7	+1	−1	3.19	0.58	3.70	759.84	175100	17.5	54
LTG8	+1	0	4.43	0.69	3.70	1559.67	187500	26.8	66
LTG9	+1	+1	4.19	0.07	3.70	2039.56	165000	23.0	54
LTG10	0	0	5.79	0.43	14.18	240.41	181000	23.4	108

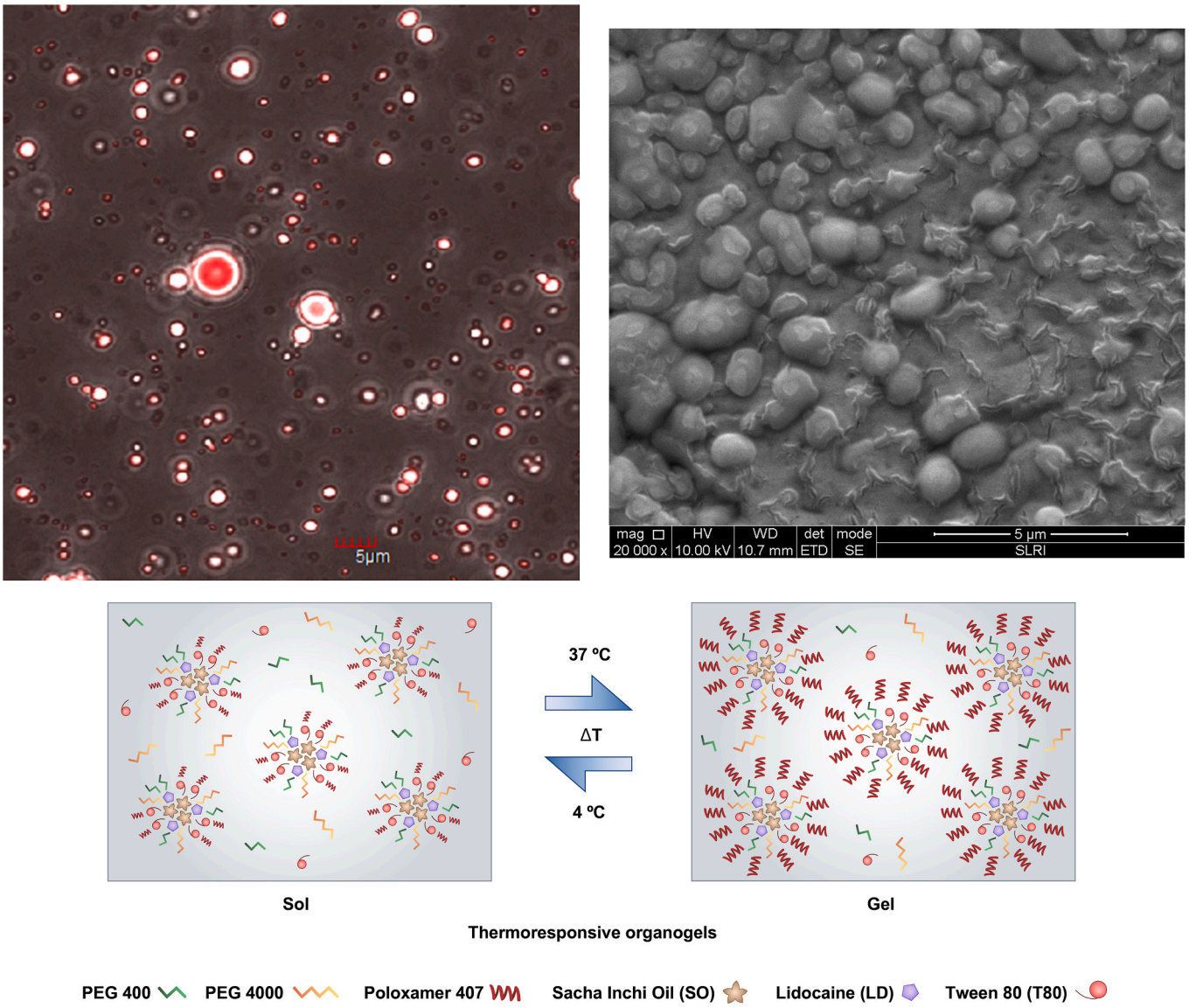

Fig. 1. Visualization of LTG stained with Nile red dye (A), showing red-stained lipid droplets under a confocal laser scanning microscope. SEM image (B) displays the surface morphology of LTG at 20,000 × magnification. Schematic illustration of lidocaine-loaded thermoresponsive organogels (LTG) (C) formulated with PEG400, PEG4000, Sacha Inchi Oil (SO), tween80 (T80), and poloxamer407 (P407). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Fig. 1B, the surface morphology of LTG is shown, revealing smooth, irregular droplet. The droplet size distribution, presented in Table 2, indicates the presence of both droplets and polymer aggregates within the gel matrix. The variations in droplet size and structure suggest differences in droplet formation, interactions among the organogels, and the coating effects of the thermoresponsive polymer network. These spherical vesicles interact to from a cohesive network that stabilizes the oil phase, resulting in a robust gel matrix. The proposed structure of these oil-in-water organogels vesicles is illustrated in Fig. 1C.

T80 is a highly versatile nonionic surfactant known for its excellent solubility in both aqueous and oil phases, making it an effective emulsifier for stabilizing oil-in-water (O/W) emulsions [52]. Although primarily used in the aqueous phase as a hydrophilic emulsifier, T80 can also be incorporated into the oil phase when needed, offering greater flexibility in different emulsion formulations [53,54]. In this specific formulation, T80 is added to the oil phase to minimize significant viscosity differences between the phases. The water phase, which contains PEG400 and PEG4000, exhibits relatively high viscosity, while the oil phase, consisting of a small amount of Sacha Inchi oil, has lower viscosity. Balancing the viscosities of both phases enhances the formulation's mixing efficiency, resulting in a more stable and homogeneous emulsion. Furthermore, T80 can be utilized in the oil phase to stabilize water-in-oil (W/O) emulsions, especially when combined with lipophilic emulsifiers like polyglycerol polyricinoleate (PGPR) [52,53]. This combination has been shown to create stable water-in-oil-in-water (W/O/W) emulsions, underscoring the versatility of T80 [54]. Additionally, incorporating T80 into the oil phase, particularly alongside solvents, improves solubility, reduces viscosity, and enhances the overall stability of the emulsion [52]. T80's ability to lower interfacial tension also makes it a valuable component in microemulsion systems, commonly used in pharmaceutical and food applications, where it helps create stable formulations [55]. Its broad functionality in both oil and water phases highlights its significant role in improving emulsion stability, texture, and performance across various industries.

The phase transformation of thermoresponsive organogels, specifically involving P407 and PEG4000, is highly dependent on temperature. P407 undergoes micellization, which increased gel-state viscosity as temperature rises [56,57]. PEG4000, acting as an organogelator,

further enhances the viscosity due to its properties [58,59]. At lower temperatures (e.g., 4 °C), the viscosity is significantly lower, as the LTG formulation remains in a liquid state. However, at body temperature (37 °C), the viscosity increases markedly, demonstrating the temperature-dependent behavior of the organogels. This intricate interplay between temperature and molecular interactions determines the organogels' phase and viscosity characteristics. Thermogelation occurs due to hydrophobic interactions between the P407 copolymer chains. As the temperature rises, these copolymer chains begin to aggregate into micellar structures. This micelle formation is triggered by the dehydration of the hydrophobic PPO units, marking the initial step in the gelation process [60].

3.2. Evaluation of dependent variables

The factorial design is an efficient experimental method for studying multiple factors with a small sample size, making it both cost-effective and time-saving. A three-level full factorial design, in particular, explores the quadratic relationships between responses and factors [29,30]. In this study, we used this design to investigate how varying concentrations of PEG400 (A) and T80 (B) affect the properties of LTG. The response surface and contour plots (Fig. 2) illustrated the impacts on particle size, PDI, swelling index, viscosity, gelation temperature, and gelation time.

The particle size response was best described by a quadratic model with an F-value = 14.70, a p-value of 0.0111, and an R^2 = 0.9484, indicating a significant and strong fit. This result suggested that both PEG400 and T80 concentrations significantly influence the particle size of the LTGs. The high R^2 value indicates that the model effectively accounts for a significant proportion of the variation in particle size. The quadratic model indicates a non-linear interaction between PEG400 and T80, as described by the following equation:

Particle size = 5.73 + 0.0367A–0.0300B + 0.4275AB – 1.56 A^2 – 0.4071 B^2

The PdI followed a linear model with an F-value of 2.52 and a p-value of 0.1512, which is not statistically significant, and an R^2 of 0.4172. This indicates that the linear model does not significant explain the variability in PdI, and other factors may also influence it. The low R^2 value suggests that the model only explained a small portion of the variability, indicating that PEG400 and T80 may not be the primary determinants of PdI in these formulations. The linear relationship for PdI is expressed by the equation below:

PDI = 0.3370 + 0.1550A–0.0800B

The swelling index was fitted to a quadratic model with an F-value of 6.70, a p-value of 0.0446 and an R^2 of 0.8934. This significant model indicates that both PEG400 and T80 concentrations significantly affect the swelling behavior of the organogels. The quadratic fit implies that the interaction between the two components is non-linear and has a substantial impact on the swelling index. The quadratic equation representing the swelling index is provided below:

Swelling index = 16.70–4.32A - 0.6183B–0.9250AB – 9.65A2 + 1.46B2

The viscosity of the organogels was best described by a quadratic model with an F-value of 17.79, a p-value of 0.0078, and an R^2 of 0.9670. This indicates a highly significant and strong relationship between the concentrations of PEG400 and T80 and the viscosity of the organogels. The high R^2 value indicates that the model effectively accounts for the majority of the variations in viscosity, highlighting the critical role of these components in determining the gel's viscosity. The quadratic equation of viscosity is described by the below:

Viscosity = 225.83 + 666.53A + 206.62B + 319.93AB +568.35A2 – 11.53B2

Gelation temperature followed a quadratic model with an F-value of

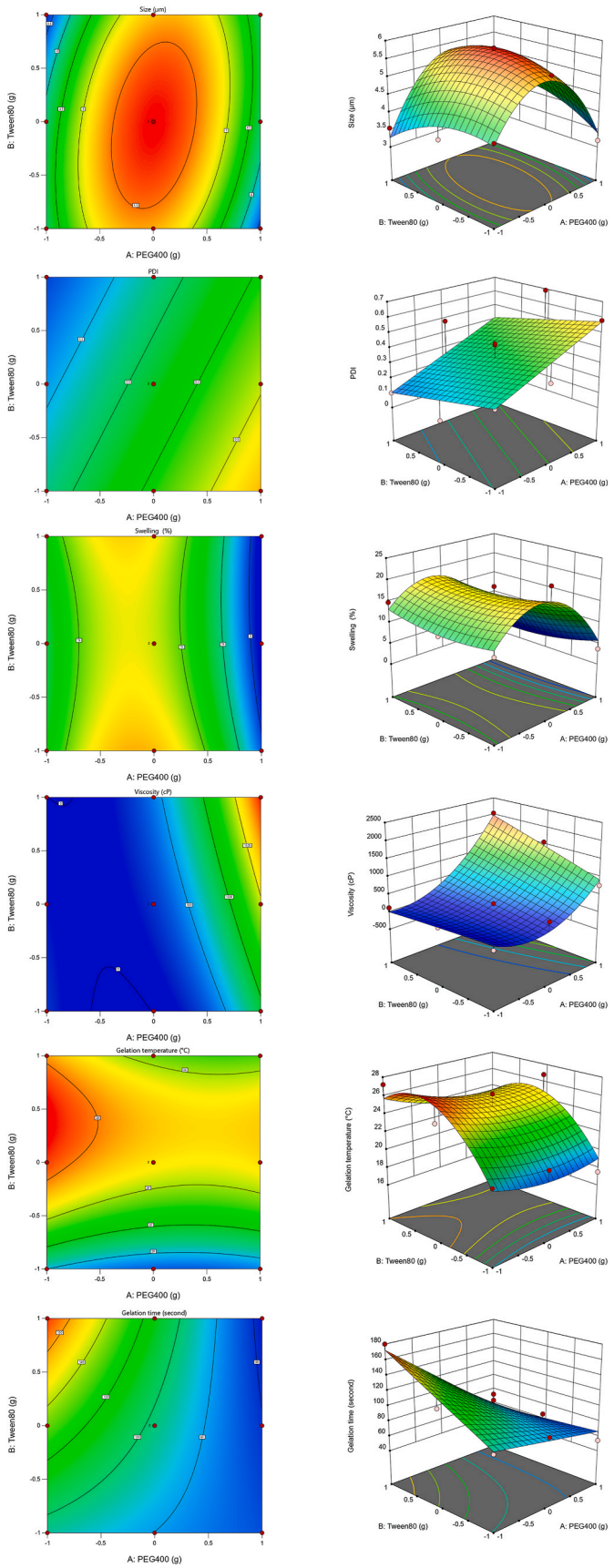

Fig. 2. Response surface and contour plots of the independent variables, PEG400 (A) and T80 (B), illustrating their impact on (a) particle size, (b) PDI, (c) swelling index, (d) viscosity, (e) gelation temperature, and (f) gelation time.

3.20, a p-value of 0.1411 (not significant), and an R^2 of 0.8002. While the model fits moderately well, the lack of statistical significance suggests that other factors might also influence the gelation temperature. The moderate R^2 value indicates that while PEG400 and T80 concentrations are important, they do not fully explain the variability in gelation temperature. The quadratic relationship for gelation temperature is represented by the equation below:

$$\text{Gelation temperature} = 24.84 - 0.8333A + 2.48B - 0.3500AB + 0.9143A2 - 3.74B2$$

Gelation time was fitted to a two-factor interaction (2FI) model with an F-value of 18.57, a p-value of 0.0019, and an $R^2 = 0.9028$. This significant model indicates that the interaction between PEG400 and T80 concentrations significantly affects the gelation time. The high R^2 value implies that the model explains most of the variability, highlighting the importance of both factors in determining gelation time. The 2FI model for gelation time is outlined in the equation below:

$$\text{Gelation time} = 96.80 - 36.00A + 17.00B - 22.50AB$$

The factorial design proved effective for examining the effects of PEG400 and T80 on various properties of LTG. Most response parameters, including particle size, swelling index, viscosity, and gelation time, showed significant quadratic or interaction effects, underscoring the complex interplay between these components. However, the PdI and gelation temperature models were not statistically significant, indicating the potential influence of other factors. These insights can guide further optimization and development of thermoresponsive organogels for enhanced performance in dental applications.

3.3. Stability assessment

3.3.1. Stability assessment through Heating–Cooling cycles

The stability of the LTG formulations was evaluated using a heating-cooling cycle test. This test subjected the formulations to 7 cycles of alternating temperature: 12 h at 4 °C followed by 12 h at 45 °C. The goal was to evaluate the physical stability of the formulations under these stress conditions [61]. Throughout the 7 cycles, formulations LTG7-9 retained their original white, opaque appearance without any visible phase separation, discoloration, or texture changes, as shown in Fig. 3. This indicates a stable emulsion system, crucial for consistent drug delivery [62,63]. The pH of the formulations had remained close to the initial value of 7.4 before and after the cycles, ensuring non-irritating and biocompatible properties for dental applications. Using UV spectrophotometry, the LD content was measured before and after the cycles, revealing no significant loss of the API. This is essential for maintain the therapeutic efficacy of the formulation over time.

The heating-cooling cycle test is a standard method for simulating the stress conditions that pharmaceutical formulations might face during storage and handling [61]. The results demonstrate that the LTG formulations exhibit excellent physical stability, maintaining their appearance, pH, and drug content under stress conditions. This robustness suggests that the formulations are robust and reliable for practical use in dental pain management.

3.3.2. Stability assessment through Thermo-sterility tolerance

Sterilization is vital for drug formulations used on open wounds, particularly in the oral cavity, which is highly susceptible to bacterial exposure. Ensuring the sterility of the LTG formulations prevents infections and ensures patient safety. The LTG formulations were autoclaved at 121 °C and 15 psi for 15 min. This process effectively eliminates contaminants while maintaining the drug's integrity and efficacy. Post-sterilization analysis showed no change in drug content, remaining within 95–105 % of the labeled amount (%LA) [33]. Sterile formulations are crucial for the oral cavity due to its complex microbiome and high risk of infection. The specified autoclave conditions did not affect the drug's efficacy or quantity, preserving the lidocaine's therapeutic properties and providing consistent pain relief and anesthesia.

Sterilization ensures the safety and effectiveness of drug delivery systems, especially for dental procedures. The stability assessment results confirm that LTG formulations can withstand sterilization without loss of drug content or efficacy. The LTG 7, 8, and 9 formulations retained their white, opaque appearance with no phase separation, discoloration, or texture changes, ensuring they remain effective and safe for dental pain management.

3.3.3. Stability kinetic and accelerated stability

Table 3 shows the percentage of label amount (%LA) of LD under different storage temperatures (5 ± 3 °C, 30 ± 2 °C, and 40 ± 2 °C) over 1, 3, and 6 months. At 5 ± 3 °C (refrigerated temperature), LD concentrations remained stable within the acceptable range 95–105 % LA across all time periods. At 30 ± 2 °C (room temperature), there was slight degradation over time. LTG7 exhibited a concentration drop to less than 95 % LA (90 % LA) at 3 months storage, whereas LTG8 and LTG9 maintained concentration above 95 % LA. At 40 ± 2 °C (accelerated temperature), significant degradation occurred, particularly over 3 and 6 months. LTG7 showed a concentration below 95 % LA after 1 month, while LTG8 fell below 95 % LA after 3 months. LTG9 demonstrated the greatest stability, maintaining its concentration above 95 % LA throughout all storage concentrations and time periods.

All formulations degraded according to first-order kinetics, as shown in Table 4. This indicates that the degradation rate is proportional to the concentration of the remaining product. The formulations' degradation kinetics adhere to the Arrhenius equation, which explains how

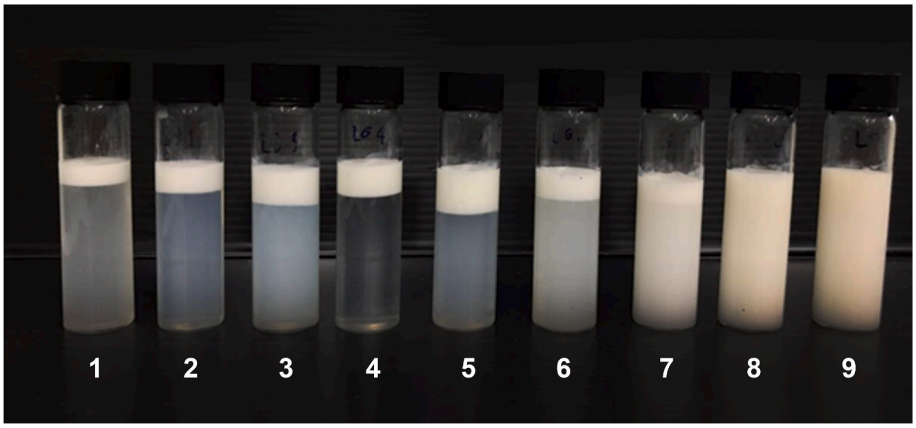

Fig. 3. The stability of LTG formulations (LTG1-LTG9) evaluated through a heating-cooling cycle test conducted over 7 cycles.

Table 3
The percentage of label amount (%LA) of lidocaine concentration was assessed under different storage temperatures (5 ± 3 °C, 30 ± 2 °C, and 40 ± 2 °C) over 1, 3, and 6 months.

Formulation	Storage Conditions								
	1 Month			3 Months			6 Months		
	5 ± 3 °C	30 ± 2 °C	40 ± 2 °C	5 ± 3 °C	30 ± 2 °C	40 ± 2 °C	5 ± 3 °C	30 ± 2 °C	40 ± 2 °C
LTG7	99.7 ± 0.7	96.2 ± 0.7	93.9 ± 0.9	98.9 ± 0.6	93.7 ± 0.5	88.2 ± 0.5	97.8 ± 0.4	90.4 ± 0.5	80.5 ± 0.2
LTG8	99.6 ± 0.8	98.9 ± 0.7	97.4 ± 1.0	99.2 ± 0.6	97.1 ± 0.6	94.5 ± 0.6	98.7 ± 0.7	96.3 ± 0.7	90.7 ± 0. 9
LTG9	99.9 ± 0.7	99.7 ± 1.0	99.1 ± 1.0	99.7 ± 0.6	99.4 ± 0.6	98.8 ± 0.8	99.4 ± 0.6	98.0 ± 0.6	95.7 ± 0.3

Table 4
Stability kinetic and accelerated stability testing.

Formulation	r^2						Accelerate stability				
	5 ± 3 °C		30 ± 2 °C		40 ± 2 °C		Absolute K			Absolute Slope	E_a (kJ/mol)
	Zero order	First order	Zero order	First order	Zero order	First order	5 ± 3 °C	30 ± 2 °C	40 ± 5 °C		
LTG7	−0.9457	−0.9446	−0.9993	−0.9996	−0.9398	−0.9265	0.3789	1.1553	2.6729	2005.19	16.67
LTG8	−0.9552	−0.9547	−0.9681	−0.9690	−0.9994	−0.9997	0.1789	0.5000	1.3342	2024.31	16.83
LTG9	−0.9586	−0.9582	−0.9707	−0.9704	−0.9464	−0.9460	0.1000	0.3500	0.7079	2052.20	17.06

temperature affects the rate of a chemical reaction. The activation energy (E_a) for each formulation indicated the energy barrier that must be overcome for the degradation reaction to occur. The values of E_a – for the LTG7, LTG8, and LTG9 formulations – are 16.67, 16.83, and 17.06 kJ/mol, respectively (as presented in Table 4). Among the formulation, LTG9 has the highest value of E_a (17.06 kJ/mol). This means that LTG9 requires the most energy for its degradation reaction to proceed, implying that it is the most stable formulation. Conversely, LTG7, with the lowest E_a value (16.67 kJ/mol), is the least stable, as it requires less energy for degradation. The LTG9 formulation, which has the highest concentration of T80, demonstrated the greatest stability. T80, a non-ionic surfactant, significantly influences the physicochemical properties of organogels [64]. It reduces surface tension, stabilizes emulsions, and maintains a consistent gel structure by preventing phase separation [65,66]. In thermoresponsive organogels, Tween 80 facilitates micelle formation, encapsulating active ingredients and protecting them from degradation while ensuring uniform distribution. It also enhances viscosity, contributing to a robust gel network, and improves thermal stability, reducing sensitivity to temperature fluctuations [66]. The stability of LTG9 highlights the crucial role of T80 in thermoresponsive organogels formulations. Its surfactant properties are essential for maintaining integrity and performance, underscoring the importance of including T80 at appropriate concentrations for optimal stability.

According to the Arrhenius equation, an increase in temperature exponentially increased the rate constant (k), resulting in faster degradation [34,35]. Therefore, formulations stored at high temperatures degraded more quickly than those stored at lower temperatures. The activation energy (E_a) could be used to predict the shelf life of each formulation under various storage conditions [67]. Formulations with a higher E_a typically have a longer shelf life because they are more resistant to temperature-induced degradation. Applying the Arrhenius equation reveals that the stability of the formulations is directly related to their activation energy values [67,68]. This analysis highlights the crucial role of activation energy in determining the stability and shelf life of pharmaceutical formulations.

3.4. Mechanical properties

The mechanical properties assessed through texture profile analysis (TPA) are essential for understanding and optimizing the performance of polymeric gels. By evaluating attributes such as hardness, springiness, resilience, cohesiveness, and adhesiveness, TPA assists design gels with the desired textural characteristics, ensuring their efficacy, stability, and user satisfaction in pharmaceutical applications [36].

3.4.1. Texture profile analysis (TPA)

TPA is commonly used in pharmaceutical technology, especially for polymeric dosage forms, to measure properties like hardness, springiness, and resilience. Many recent studies have utilized this technique to evaluate prepared dosage forms such as polymeric gel [38], in-situ forming implants [36], solvent-exchanged in-situ gel [41,69], and thermoresponsive hydrogel [37]. The texture properties of the gel-state LTG were examined to understand its behavior in the oral cavity (Table 5). It was observed that hardness, defined as the maximum force required during the first compression to penetrate the gel formulation, significantly increased with higher concentration of T80 (X2) ($p < 0.05$). This demonstrates that the gel-state hardness is directly influenced by the concentration of T80 in the formulation. The springiness and resilience properties did not show significant differences across the formulations. T80 is critical in organogels formulation, as it reduces surface tension and forms micelles that encapsulate active ingredients, thereby enhancing emulsion stability [66]. Additionally, T80 significantly influences the gel's texture and hardness, which is crucial for its, stability, and application. The effect of T80 on organogels hardness depends on the specific formulation, including the type and concentration of other components such as oils, polymers, and active ingredients [70]. A higher concentration of T80 can increase hardness by creating a denser, more cohesive network, while a lower concentration can result in a softer gel

Table 5
Mechanical properties evaluated using texture analysis.

Mechanical Properties	Texture Profile Analysis			Injectability (N)	Mucoadhesion (mN)
	Hardness (mN)	Springiness (Ratio)	Resilience (Ratio)		
Formulation					
LTG7	701.57 ± 29.04	0.36 ± 0.02	0.002 ± 0.00	1.62 ± 0.16	433.03 ± 19.00
LTG8	817.08 ± 39.14	0.38 ± 0.03	0.002 ± 0.00	1.72 ± 0.20	417.87 ± 95.25
LTG9	867.86 ± 49.09	0.39 ± 0.05	0.002 ± 0.00	1.75 ± 0.17	493.17 ± 74.13

with less structural rigidity [70]. Understanding and leveraging the role of T80 is crucial for optimizing the mechanical properties of thermoresponsive organogels, enhancing their stability, usability, and overall effectiveness.

3.4.2. Injectability test

The injectability test of the LTG formulations were conducted at room temperature simulating clinical conditions (Table 5). All LTG formulations maintained their consistency and did not flow out of the needle before injection. The force required to inject the organogels corresponded closely with the viscosity of each LTG. Importantly, the maximum force needed to expel the formulations through the syringe needle tip was less than 2N, showing no significant differences between formulations. All tested formulations were deemed injectable. The low injection force of the gel is highly advantageous for dental applications, as it allows for easy administration into confined spaces as dry socket wounds [12]. Clinicians can inject the organogels into these narrow dental wounds with minimal pressure, ensuring a smooth and efficient application. This ease of injectability enhances precision and comfort, improving patient experience and treatment outcomes. The gel is particularly useful for the dental procedures that require precise delivery of the therapeutic agents to specific sites within the oral cavity.

3.5. Mucoadhesive properties

Mucoadhesion, or the formulation's ability to adhere to biological tissues, plays a crucial role in extending retention time and enhancing effectiveness. In the oral cavity, where the epithelium is constantly exposed to saliva, this property is vital. This study evaluated the bioadhesive properties of thermoresponsive organogels (LTG) using mucin disc and mucin interaction tests.

3.5.1. Mucin disc test

The mucin disc test is a standard method for assessing the bioadhesive properties of formulations, measuring LTG gel's ability to adhere to mucin, a key mucus component. Strong adhesion ensures prolonged retention in the oral cavity [39]. As shown in Table 5, LTG7, LTG8, and LTG9 exhibited mucoadhesive forces of 433.03 ± 19.00 mN, 417.87 ± 95.25 mN, and 493.17 ± 74.13 mN, respectively, with no significant differences. The mucoadhesion is likely due to the surfactant properties and hydrophilicity of P407, enhancing gel integration into mucoadhesive interface [71,72].

The oral cavity presents challenges for dosage-form development due to factors like saliva flow and muscle movement. The mucin disc test results demonstrate the strong and consistent mucoadhesive strength of LTG formulations, ensuring effective adherence to mucosal surfaces for sustained drug delivery and prolonged therapeutic effects. This confirms the reliability of thermoresponsive organogels for dental applications requiring extended retention and efficient drug delivery.

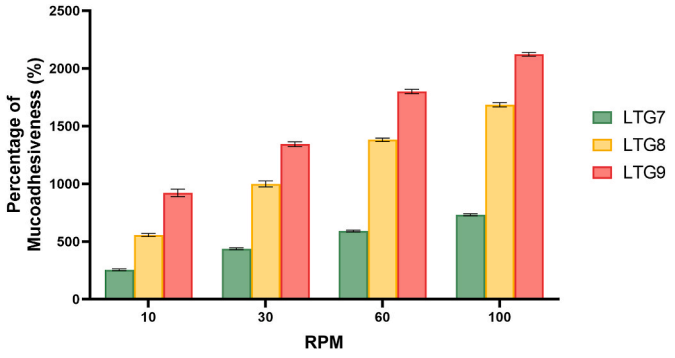

Fig. 4. The mucoadhesive index of the lidocaine-loaded thermoresponsive organogels at varying shear rate.

3.5.2. Thermoresponsive organogels and mucin interaction

Mucoadhesive testing is essential for evaluating drug delivery systems like lidocaine-loaded thermoresponsive organogels, ensuring they adhere to mucosal surfaces for localized and sustained drug release [37,38]. Analyzing the interaction between mucin and the organogels provides valuable insights into their bioadhesive properties.

This interaction is crucial for ensuring the gel remains in place within the dynamic oral environment. Mucoadhesion (%) varied significantly between formulations at the same shear rate ($p < 0.001$), as illustrated in Fig. 4, though these results differed from the mucin disc test. Increasing shear force also affected mucoadhesiveness ($p < 0.001$), primarily influenced by mucin-P407 interactions [71] and organogels properties [18]. Higher shear forces, simulating oral movements like eating, enhanced mucoadhesion by promoting deeper penetration between P407 and mucin glycoproteins, ensuring the effectiveness of thermoresponsive organogels under dynamic conditions [12,37,39].

LTG formulations exhibit excellent mucoadhesive properties, making them ideal for oral cavity applications requiring prolonged mucosal contact. The strong mucin-organogels interaction ensures localized action, crucial for effective pain relief and local anesthesia. Mucoadhesive testing also helps optimize the polymer concentration for maximum adhesion without affecting viscosity, gelation temperature, or drug release. Understanding this interaction allows for the development of more effective, patient-friendly drug delivery systems for better pain management.

3.6. In vitro release

The cumulative release plots of LD for 72 h from LTG7, LTG8, and LTG9 are showed in Fig. 5. All formulations exhibited a similar release profile, with a 15-min lag time. LTG9 showed the highest drug release rate, followed by LTG8 and LTG7. In the fourth hour, LTG9's release rate increased significantly and remained the highest for the entire 72 h. The release profiles can be divided into three phases: (1) fast release during the first 16 h; (2) sustained release from 16 to 48 h; and (3) steady-state release from 48 to 72 h [73]. All tested formulations demonstrated prompt and sustained LD release for up to 72 h, with LTG9 exhibiting the highest release rate. This release pattern is ideal for pain management, providing an initial fast release followed sustained release to achieve prolonged pain relief.

The cumulative drug release data from the formulations were analyzed using various kinetic models, including zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer–Peppas models [74]. The results of linear regression analysis for each formulation, along with their respective equation parameters, are presented in Table 6. The release rates of all formulations were best fitted to the Higuchi model, indicating that LD release was primarily controlled by diffusion through the gel matrix [75]. This suggestion that the LD molecule diffuse through the

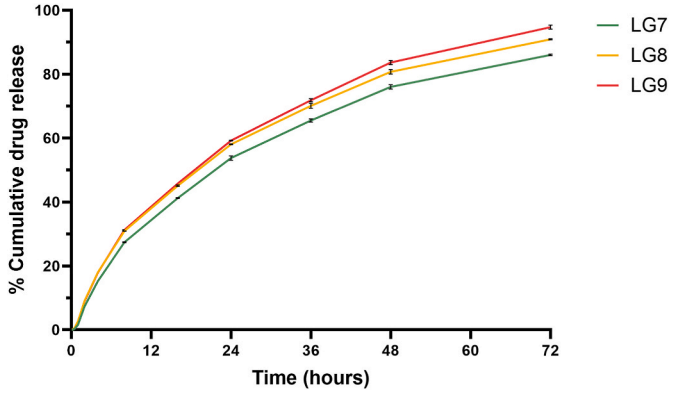

Fig. 5. Percent cumulative lidocaine release from LTG formulations (LTG7, LTG8, and LTG9). Data are mean ± SD, n = 3.

Table 6
Kinetic model fitting of AZG formulations.

Kinetic models	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer -Peppas		
	K_0	r^2	K_1	r^2	K_H	r^2	K_{HC}	r^2	K_{KP}	n	r^2
Formulation											
LTG7	2.802	0.9794	0.036	0.9915	0.047	0.9980	0.011	0.9882	4.616	0.802	0.9911
LTG8	3.110	0.9746	0.041	0.9900	10.146	0.9983	0.013	0.9858	5.779	0.754	0.9906
LTG9	3.147	0.9752	0.042	0.9906	10.231	0.9981	0.013	0.9864	5.626	0.770	0.9901

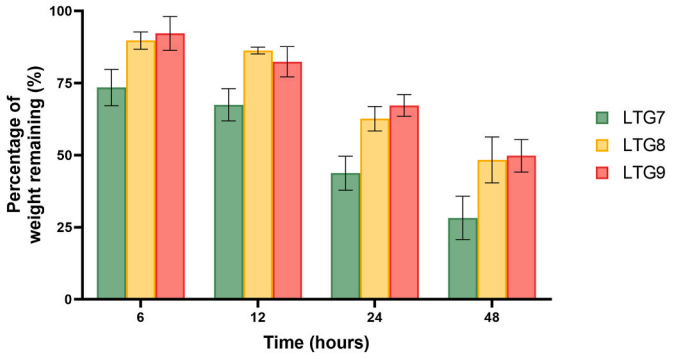

Fig. 6. Percentage of weight remaining in LTG formulations after submerged in artificial saliva at 37°C for 48 h, with data reported as the average ± standard deviation (SD) (n = 3).

polymer network of the organogels, acting as a rate-limiting barrier.

Analyzing the LD release data using the Higuchi model offers valuable insights into the release mechanism of the LTG formulations. The fitting of the Higuchi model confirms that diffusion through the gel matrix is the primary mode of drug release, ensuring controlled and sustained delivery of LD. This understanding aids in designing effective formulations for dental applications, where extended pain relief and drug retention at the site of action are critical.

3.7. Biodegradation

The *in vitro* biodegradation study, conducted in a controlled laboratory environment, observe the breakdown of thermosensitive organogels formulations over time. Fig. 6 illustrates the degradation of LTG formulations during a 48-h period, showing percentage of weight remaining (%). The LTG7 formulation exhibited significantly different biodegradation compared to LTG8 and LTG9, correlating with its lower viscosity and hardness. After 24, hours, the weight remaining percentages for LTG7, LTG8 and LTG9 were 43.74 ± 5.90 %, 62.63 ± 4.25 %, and 67.23 ± 3.80 %, respectively. LTG7 had less than 50 % of its weight remaining, while for LTG8 and LTG9 remained more than 50 %. After 48 h, the weight remaining percentages for LTG7, LTG8 and LTG9 were 28.24 ± 7.53 %, 48.35 ± 7.97 %, and 49.78 ± 5.63 %, respectively. LTG7 had less than 30 % of its weight remaining, whereas LTG8 and LTG9 remained about 50 %. All the formulations exhibited an accelerated rate of biodegradation, enhancing their biocompatibility [73]. The results of LTG8 and LTG9 indicated that the formulation with the lowest level of biodegradability might contribute to extended substance release.

The thermoresponsive organogels formulation 9 (LTG9), containing of 2 % LD, was composed of 15 % PEG400, 5 % T80, 0.2 % PEG4000, 3 % SO, and 18 % P407. LTG9 demonstrated excellent physicochemical properties, such as high viscosity, strong mucoadhesion, appropriate sustained release, optimal biodegradation, and good stability. Due to these superior characteristics, LTG9 was selected for further investigation in mouse models to evaluate its efficacy in pain tolerance.

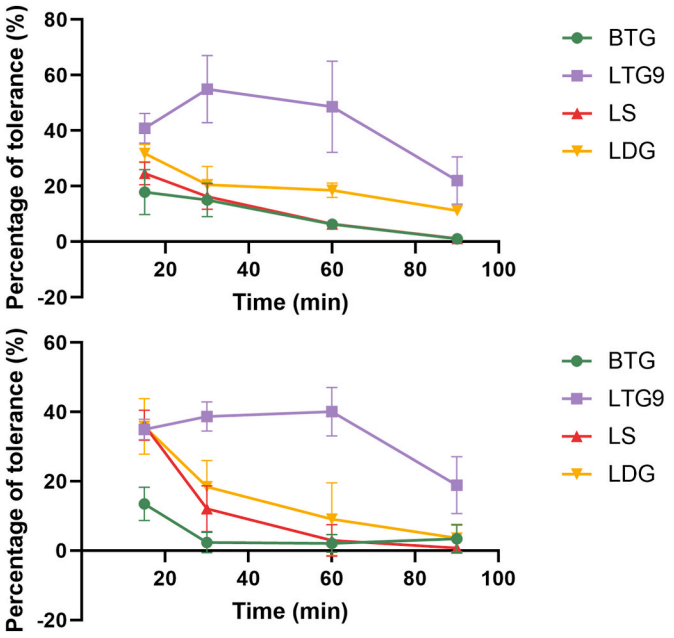

Fig. 7. The percentage of tolerance time in models measured using the tail-flick test (a) and the thermal-induced pain sensation test with a modified hot plate (b) following the administration of blank thermoresponsive organogels (BTG), lidocaine-loaded thermoresponsive organogels formulation 9 (LTG9), lidocaine hydrogel (LDG), and lidocaine solution (LS) over a period of 0–90 min. Data are presented as the average ± standard deviation (SD) (n = 6).

3.8. Pain tolerance and anesthetic efficacy studies

Before proceeding to animal and clinical research, the LTG9 formulations, blank thermoresponsive organogels (BTG), lidocaine hydrogel (LDG), and lidocaine solution (LS) were tested for microbial contamination. The results showed that the total plate count was below 10 CFU/mL, and the total yeast and mold were below 10 CFU/g. Additionally, no *Escherichia coli* or *Stapphylococcus aureus* was detected. These microbiological tests confirmed that all formulations met acceptable standards, making them suitable for animal tests.

3.8.1. Local anesthetic efficacy via tail-flick test

This study employed the tail flick test to assess baseline nociceptive levels, the analgesic effects of a topical treatment formulation, and the onset of tolerance [13,44,45]. The LTG9 formulations were compared with a thermoresponsive organogels without LD (BTG) and with LD in other dosage forms, including hydrogel (LDG) and solution (LS). All groups developed heat tolerance within 15 min of treatment, evidenced by increased tail flick time, as shown in Fig. 7a. LTG9 demonstrated a longer tolerance time compared to other dosage forms, likely due to the thermoresponsive organogels' prolonged release characteristics [58]. While the BTG, LDG, and LS groups showed a decrease in tolerance time (%) after 30 min, LTG9 exhibited an increase in tolerance time (%). LDG also provided a prolong anesthetic effect, although it was significantly less effective than LTG9 ($p < 0.01$), possibly due to the influence of P407

hydrogels [76]. LTG9 had the most potent anesthetic effect, attributed to its extended duration and high heat tolerance (%), demonstrating strong sustained release capacities [24,77]. This indicates that LTG9 formulation effectively delivered LD through the mouse's tail, prolonging the anesthesia effect. Additionally, BTG exhibited anesthetic effects due to the characteristics of P407 and organogels [78]. On the other hand, LS did not have a penetration enhancer, resulting in a rapid onset of anesthetic effect within the first 15 min but no prolong numbing effect due to the short duration of action of LD [12,13].

3.8.2. Thermal pain response using a modified hot plate model

The anesthetic efficacy of heat-induced pain blocking in a mouse paw was assessed using the hot plate method [10,79]. The study measured the percentage of tolerance time to evaluate the effectiveness of different formulations. LTG9 was compared with a blank thermoresponsive organogels (BTG) and LD in other dosage forms, including hydrogel (LDG) and solution (LS). All groups exhibited heat tolerance within 15 min of treatment, as shown by the extended tolerance duration in Fig. 7b. Similar to the tail flick test, the hot plate model demonstrated comparable results, with LTG9 showing the longest tolerance time among the formulations, likely due to the sustained release properties of the thermoresponsive organogels [58,78]. While the BTG, LDG, and LS groups showed a decrease in tolerance time after 30 min, LTG9 continued to exhibit increased tolerance time, indicating the most potent anesthetic effect due to its extended duration and high heat tolerance, demonstrating strong sustained release capacities [77]. This suggests that the LTG9 formulation effectively delivered LD through the mouse's paw, prolonging the anesthesia effect. BTG also showed anesthetic effects due to the properties of P407 and organogels [78]. In contrast, LS lacking a penetration enhancer, provided a rapid onset of anesthetic effect within the first 15 min but no prolong numbing effect due to the short duration of action of LD [12,13].

Based on the findings, the study used a full factorial design with two factors, three levels to determine the influence of PEG400 and T80 ratios on the characteristic of LTG. This experimental design revealed that the ratio PEG400 to T80 significantly affects the physicochemical properties of LTG formulation. Specifically, these ratios influence key properties such as particle size, PdI, percentage of swelling, viscosity, gelation temperature, gelation time, gel's hardness, and mucoadhesion. T80 also impacts stability, drug release and biodegradation. LD was release from the matrix via diffusion over 72 h, following Higuchi's model. The select LTG (LTG9) demonstrated prolong release effects when evaluating the effectiveness of local anesthetics in mouse's models testing. Among the formulations, LTG9 provided the most desirable characteristics for topical anesthetic medication based on its overall performance.

Thermoresponsive organogels, as developed in this study, show strong potential for treating dry socket wounds due to their unique temperature-sensitive behavior and mucoadhesive properties. Dry socket, or alveolar osteitis, occurs when the blood clot protecting the socket after tooth extraction is lost, exposing bone and nerves and causing severe pain and delayed healing. An ideal treatment must adhere to the wound site, protect it from environmental factors such as saliva and bacteria, and deliver sustained pain relief through localized anesthetic release. These organogels leverage the natural temperature of the body to transition from a liquid (sol) at cooler temperatures to a solid gel at the temperature of the oral cavity. This transformation allows for easy application in liquid form and a stable, adhesive gel upon warming to 37 °C. Once gelled, the organogels forms a protective barrier over the exposed bone, ensuring effective pain management and accelerated healing. The formulation's strong mucoadhesive properties ensure it remains in place despite the dynamic conditions of the oral cavity, including saliva flow and tongue movement. Additionally, the organogels' ability to transition between sol and gel states, combined with controlled drug release, reduces the need for frequent re-application. This minimizes patient discomfort while providing continuous pain relief and protection for the wound. Notably, these organogels also demonstrate heat tolerance, allowing for autoclave sterilization and ensuring safe application on open wounds.

The thermoresponsive organogels developed in this research offer a novel and effective solution for dry socket treatment by combining temperature-triggered gelation, enhanced mucoadhesion, sustained anesthetic release, and wound-healing properties. These innovative features address the key physiological challenges of dry socket, providing long-lasting pain relief and wound protection. Future studies will focus on clinical trials to further explore the organogels' benefits in dental pain management, particularly in terms of allergy responses and overall pain relief efficacy.

4. Conclusion

Thermoresponsive organogels provide targeted pain relief by being directly applied to the site of pain or wound, making them especially beneficial for conditions like dry socket wounds or other dental procedures requiring localized anesthetic. The sustained drug release reduces the need for frequent reapplications, enhancing patient comfort and compliance by providing prolonged relief from a single application. The gel matrix also protects the drug from degradation, ensuring its effectiveness throughout the treatment period, which is particularly important for drugs that degrade rapidly under physiological conditions. This study successfully developed 2 % lidocaine-loaded thermoresponsive organogels (LTG) using a full factorial design with two factors at three levels. The research focused on how PEG400 and tween80 affected LTG characteristics, revealing important properties such as particle size, PdI, percentage of swelling, viscosity, gelation temperature, gelation time, gel's hardness, and mucoadhesion. Tween80 influenced the bonding of the organogels, leading to reduced swelling, improved heat tolerance, and increased stability. This enhanced heat tolerance ensures the safe use of LTG on open wounds. Lidocaine was release through diffusion over 72 h, following Higuchi's model. The LTG9 formulation, in particular, demonstrated prolonged release effects in mouse models, highlighting its potential to improve local anesthesia across medical disciplines. These thermoresponsive organogels are easy to apply to specific areas, making them highly valuable for dental treatments. The ease of administration and handling enhances their clinical applicability, potentially improving patient outcomes and comfort. However, further clinical studies are necessary to validate the effectiveness and safety of these thermoresponsive organogels across different patient demographics and dental procedures, given their promising attributes and efficacy.

CRediT authorship contribution statement

Nuttawut Supachawaroj: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nuntachai Hanpramukkun:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Kunchorn Kerdmanee:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Sucharat Limsitthichaikoon:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Statement of human and animal rights

This study involved animal research conducted in accordance with the NIH (National Research Council) Guide for the Care and Use of Laboratory Animals. No human subjects were involved in any of the studies performed by the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

In preparing this work, the author(s) used ChatGPT to improve the English language. After using this tool, the author(s) carefully reviewed and edited the contents as needed, assuming full responsibility for the publication's content.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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