
Research Article

Delivery of Thermo-responsive-Tailored Mixed Micellar Nanogel of Lidocaine and Prilocaine with Improved Dermatokinetic Profile and Therapeutic Efficacy in Topical Anaesthesia

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Abstract. The topical delivery of local anaesthetics has always been a difficult task due to the limited percutaneous absorption of local anaesthetic drugs across the various barriers of the skin. In this pursuit, a thermo-responsive mixed micellar nanogel (MMNG) system of lidocaine and prilocaine has been attempted in the current piece of work. The system relies on the ability to alter its phase state (sol-to-gel) for feasibility of the topical application in response to change in temperature. The composition of MMNG entails majorly of Pluronic® F127 and Tween 80 in a fixed combination so as to provide the desired thermoreversibility for the skin application. The gels were optimized with respect to phase transition temperature ($T_{sol/gel}$), turbidity and viscosity. The optimized systems were then characterized for particle size, spreadability, syringeability, bioadhesive strength, *ex vivo* skin permeation, retention and dermatokinetic studies. The skin compatibility revealed that no histological changes were observed for optimized formulation, while the conventional system showed changes in the skin-tissues. Further, the enhanced intensity of anaesthetic effect was noted in an *in vivo* rabbit model and tail flick model in mice. The overall results suggest that the prepared MMNG system possesses the potential in providing an efficacious, safe and acceptable alternative therapeutic system for topical anaesthesia.

KEY WORDS: dermatokinetic; local anaesthetic; permeation; spreadability; thermo-responsive mixed micellar gel; topical anaesthesia.

INTRODUCTION

Anaesthesia in skin is important for relieving pain in procedures associated with skin such as venipuncture, lumbar puncture and skin biopsy. The condition of analgesia is mostly achieved through direct intradermal injection of local anaesthetic drugs which causes much discomfort (1). Injections of local anaesthetics are painful which in turn cause tissue oedema and distortion of the surgical site (2). Topical anaesthetics are of considerable importance in clinical practice primarily to relieve the pain during cutaneous procedures in the outpatient setting. The major advantage of topical anaesthesia lies

in it being as painless and does not distort tissues as infiltrated local anaesthetics can do otherwise (1).

Lidocaine and prilocaine are extensively used topical anaesthetic drugs and provide anaesthesia in the case of pain itching and burning related to cutaneous inflammatory responses with minor surgical operations. Due to the advantages of the eutectic mixture, these are combined together for local anaesthetic property. Lidocaine is an amino-amide type local anaesthetic agent, widely indicated in topical anaesthesia and has a faster onset of action (3). The combination with prilocaine is available as EMLA™ (eutectic mixture of lidocaine and prilocaine) in which liquid is emulsified with water to form an oil-water emulsion base cream (4). In 1992, the eutectic mixture of 2.5% lidocaine and 2.5%, known as EMLA, was approved by the US FDA for skin anaesthesia due to its efficacy and safety for a variety of procedures in skin conditions (5). However, the currently available formulations of topical anaesthetics have a number of disadvantages, particularly a long delayed time between application and anaesthetic effect (EMLA™ cream instructions indicate that painful procedures should only be performed after 60-min application). The stratum corneum (SC) is the main barrier to topical anaesthetic delivery as well as the rate-limiting step in the penetration process, since the free nerve endings responsible for pain sensation are present in the dermis layer of skin. Hence, while administering through

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topical route, local anaesthetics should be targeted to the dermis (6).

Lipid-based systems overcome the barrier properties of SC due to their ability to distort and loosen the highly organized intercellular arrangement of lipids, thereby improving the intradermal drug penetration and enhancing the duration of local action. Over the last decade, injectable *in situ* gels have caused much attention in the field of drug delivery, due to their meritorious advantages like ease of preparation, avoidance of organic solvents, and improved patient compliance (7–9). The *in situ* gel systems may be ion-sensitive, pH-sensitive or thermo-sensitive, according to the varied kind of materials employed (10–12). The focus of the current investigation lies on thermo-responsive systems which remain as a mobile liquid (sol) at low temperature whereas get converted to gel at physiological temperature (13). Pluronic® F127 has a thermosensitive property and can form a micelle gel. To increase the solubility of hydrophobic drugs lidocaine and prilocaine in the Pluronic® F127, Tween 80 has been incorporated to form a mixed micellar gel. Such type of systems at body temperature converts into the gel and stay at application site for the required time. After treatment, the gel can be removed by simple rinsing with cold water (14). The thermosetting mixed micellar solutions based on two block copolymers Lutrol® F127 and Lutrol® F68 of lidocaine and prilocaine have been reported for topical anaesthesia by Scherlund *et al.* (15).

The aim of the present research investigation was therefore to develop thermo-responsive mixed micellar nanogels (MMNG) of lidocaine and prilocaine based on Pluronic® F127 and Tween 80 for topical delivery. Different molar concentrations of Pluronic® F127 and Tween 80 were optimized mainly with respect to gel formation temperature and viscosity. The optimized system was characterized for micromeritics, rheology, spreadability, syringeability, bioadhesive strength and skin biocompatibility. The *ex vivo* and *in vivo* evaluation has also been performed *vis-à-vis* conventional formulations.

MATERIALS AND METHODS

Materials

Lidocaine and Prilocaine were provided by M/s IPCA Laboratories Ltd. Mumbai, India, while Pluronic® F127 (PF127) was procured *ex-gratis* from M/s BASF India Limited, Mumbai, India. Tween® 80 was purchased from Himedia Laboratories Pvt. Limited, Mumbai, India. Double distilled water was employed throughout the studies. EMLA™ and Prilox (commercial formulations of lidocaine and prilocaine) were procured from the local drug house.

Preparation of the Thermo-responsive Mixed Micellar Nanogel

The sol state of MMNG was prepared using cold method (16). In brief, a fixed concentration of PF127 (20, 18, 17%, *w/w*) and different concentration of Tween 80 (1:1 to 1:9 molar ratios of PF127 and Tween 80) were added to water in glass vials as depicted in Table I. Formulation codes J₁–Q₁ represent the formulations containing PF127 (20%, *w/w*),

J₂–Q₂ and J₃–Q₃ containing PF127 (18%, *w/w*) and (17%, *w/w*), respectively. The mixtures were then kept at 4°C and gently mixed on the magnetic stirrers until all the PF127 granules were completely dissolved. Lidocaine and prilocaine were mixed together in the ratio of 1:1 to produce a eutectic mixture (EMLP) which was then added to the mixed micelle solution at 4°C and gently mixed at intervals until a clear solution (sol) was obtained (17). The final concentration of the EMLP was 0.05 g in 1 g of sol.

Characterization of Developed Formulations

Turbidity Measurement

Per cent transmittance (%*T*) is inversely proportional to the turbidity of the formulation. The UV-visible spectrophotometer (UV 1601, M/s Shimadzu, Kyoto, Japan) at a wavelength of 650 nm was employed for the %*T* measurement. The sample readings were recorded by setting the %*T* of water as 100% (blank). MMNG are supposed to be clear transparent solutions at low temperature. Thus, %*T* determines whether the solution is clear or not (18,19).

Phase Transition Temperature

The sol-gel temperature ($T_{\text{sol/gel}}$) of each formulation under examination was determined through the tube inversion experiment. Samples (1 mL) were taken in sealed glass tubes of internal diameter about 10 mm and kept in the controllable water bath. The temperature of the water bath was elevated from 4 to 50°C at a heating rate of 1°C/min. The $T_{\text{sol/gel}}$ was recorded when the liquid in the tube was immobile within 30 s (20).

Rheological Studies

Viscosity of the prepared formulations was determined using a cup and bob viscometer (Rheometer; MCR 102, PaarPhysica, Germany) equipped with Z4 sensor probe (in rotation mode at a speed of 20 rpm). The formulations were subjected to different shear stress and shear rate conditions, and the measurements were carried out at two different temperatures *viz.* 25 and 5°C (21).

Micromeritics

The particle size distribution of mixed micellar aqueous (10%, *w/w*) solutions was determined at 4°C using Delsa™ Nano C (M/s Beckman Coulter, India Pvt. Ltd., Mumbai, India) at IMTECH, Chandigarh, India. The mean size was directly obtained from the instrument software (22).

Texture Profile

Spreadability and Syringeability. The spreadability and syringe ability of the optimized formulations (O₂ and P₃) was evaluated using Texture Analyzer™ (TA.XT Plus Texture Analyzer; M/s Stable Microsystems, Surrey, UK) with specific settings. For spreadability, calibration was performed for maintaining the same height (25 mm) at each measurement. The sample was placed in the female cone (lower cone) and

Table I. Per cent Turbidity, Gel Formation Temperature and Rheological Measurements of Thermoresponsive MMNG Systems

Formulation code	Molar ratio (PF127:T80)	PF127 (w/w%)	%T	$T_{sol/gel}$ (°C)	Viscosity (Pa.s)	
					25 ± 2°C	5 ± 2°C
J ₁	1:1	20	92.7	11	18.6 ± 0.56	2.4 ± 0.43
K ₁	1:2	20	94.2	11	26.7 ± 0.35	4.1 ± 0.57
L ₁	1:3	20	93.3	11	32.9 ± 0.75	5.8 ± 0.27
M ₁	1:4	20	93.6	11	35.3 ± 0.87	6.2 ± 0.95
N ₁	1:5	20	91.9	13	41.2 ± 0.37	6.9 ± 0.87
O ₁	1:6	20	19.9	16	43.1 ± 0.13	7.1 ± 0.17
P ₁	1:7	20	20.5	21	44.9 ± 0.32	7.4 ± 0.11
Q ₁	1:8	20	20.3	27	46.1 ± 0.21	7.9 ± 0.23
J ₂	1:1	18	28.2	20	18.3 ± 0.55	2.2 ± 0.14
K ₂	1:2	18	92	17	18.5 ± 0.29	2.1 ± 0.35
L ₂	1:3	18	92.9	17	25.9 ± 0.14	3.8 ± 0.24
M ₂	1:4	18	92.1	20	27.6 ± 0.45	3.9 ± 0.11
N ₂	1:5	18	90.3	25	31.1 ± 0.35	4.6 ± 0.27
O ₂	1:6	18	93.4	27	34.5 ± 0.76	5.0 ± 0.15
P ₂	1:7	18	62.2	28	36.9 ± 0.28	6.3 ± 0.39
Q ₂	1:8	18	63.5	30	37.6 ± 0.61	6.8 ± 0.53
J ₃	1:1	17	Turbid	16	17.1 ± 0.63	2.2 ± 0.44
K ₃	1:2	17	93.3	14	18.2 ± 0.36	2.7 ± 0.36
L ₃	1:3	17	91.8	14	23.5 ± 0.93	3.5 ± 0.97
M ₃	1:4	17	93.1	14	25.2 ± 0.57	4.9 ± 0.85
N ₃	1:5	17	94.5	16	30.8 ± 0.84	5.1 ± 0.56
O ₃	1:6	17	93.3	28	33.9 ± 0.24	7.0 ± 0.68
P ₃	1:7	17	91.8	36	39.9 ± 0.84	7.6 ± 0.34
Q ₃	1:8	17	93.1	Sol up to 45	41.3 ± 0.16	7.9 ± 0.13

the test was started. The upper cone probe penetrated to a depth of 2 mm from its start point at the speed of 3 mm/s (23,24). The method determines the force of penetration and detachment in grams and work of shear and adhesion in grams per second by the male cone to penetrate and detach from the test sample present in the female cone.

Syringeability was measured as the force required in expelling the test formulation from a 2-mL syringe fitted with

a needle of 18 gauge in the compression mode. Formulation was packed into the syringe, and an inert polycarbonate probe was used to expel the contents of syringe at a rate of 2.0 mm/s (25).

Measurement of Ex Vivo Bioadhesive Force. Bioadhesive strength was estimated using Texture Analyzer™. Goat buccal mucosa procured from local slaughter house was used

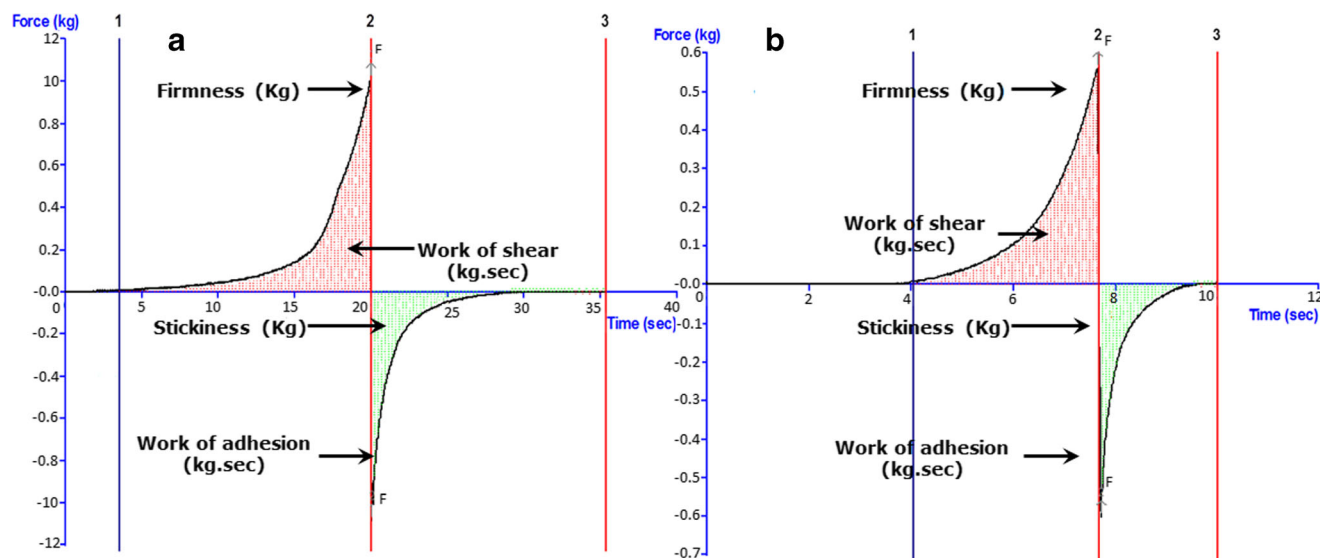


Fig. 1. Variation of force of upper probe of texture analyser while moving in/out of the gelled system: **a** O₂ and **b** P₃

Table II. Various Spreadability Parameters of the Optimized Thermo-responsive MMNG Systems

Formulation code	Firmness (kg)	Work of shear (kg/s)	Stickiness (kg)	Work of adhesion (kg/s)
O ₂	0.558	0.551	-0.605	-0.212
P ₃	1.008	2.817	-1.092	-1.428

to carry out the bioadhesion studies. Before testing, the mucosa was kept in simulated saliva (pH 5.7) for 5 h. The mucosal membrane was fixed on the lower base of device with the help of attachment, and optimized thermo-responsive MMNG gel (approx. 1 g) was applied on the membrane. The mobile arm (with attached membrane) was moved downwards at a rate of 0.5 mm/s until contact with the test formulation was made. A contact force of 10 g was maintained for a period of 500 s, and the peak detachment force was recorded for each sample in triplicates (23,26).

Ex Vivo Permeation and Retention Studies

The animal studies were approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University, Chandigarh, India (Ref. letter no. CAH/09/70; IAEC/156). The studies were conducted on excised abdominal skin of Laca mice using Franz diffusion cells (PermeGear, Inc., PA) having diffusional cross section area of 3.14 cm². Prior usage, methylene blue dye test was performed to determine the integrity of excised abdominal skin (27). The receptor compartment has a volume of 30.0 mL and was maintained at 37°C containing water: ethanol in ratio (7:3; v/v) (28). The excised abdominal LACA mice skin was clamped between the donor and receiving compartments. The test formulations (1 g), each containing 25 mg/g lidocaine and 25 mg/g prilocaine, were applied onto the mice skin in the donor compartment. An aliquot of 1 mL each were withdrawn at different time intervals, through the sampling port and

replaced with equal amount of medium to maintain constant volume throughout the receptor compartment (29). The samples were analysed using validated Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) technique at 210 nm. After the completion of the studies, the skin was removed from the Franz cells and washed with normal saline to remove any remaining formulation. The skin was cut into small fragments and kept in methanol (5 mL) for 24 h for the complete drug extraction followed by filtering the solution through a membrane (0.45 μm). The filtrate was analysed for drug content using RP-HPLC method at 210 nm. Fresh skin tissue, treated in the similar manner, was taken as blank for the above study (29,30).

Dermatokinetic Modelling

The excised skin of Wistar rats was employed for the study. The skin tissue was prepared as discussed in “Ex Vivo Permeation and Retention Studies” section. At the respective sampling intervals, the whole skin was washed to wipe off the remaining formulation and further soaked in hot water (60°C) for 30 s to separate the epidermis from the dermis (31,32). The sections were cut in small pieces and macerated in ethanol (5 mL) for 24 h. The filtrate was filtered and analysed using the RP-HPLC technique. The obtained data were fitted into one-compartment model, as per Eq. (1) (29).

$$C_{Skin} = \frac{K_p \cdot C_{max}^{Skin}}{(K_p - K_e)} (e^{-K_p t} - e^{-K_e t}) \tag{1}$$

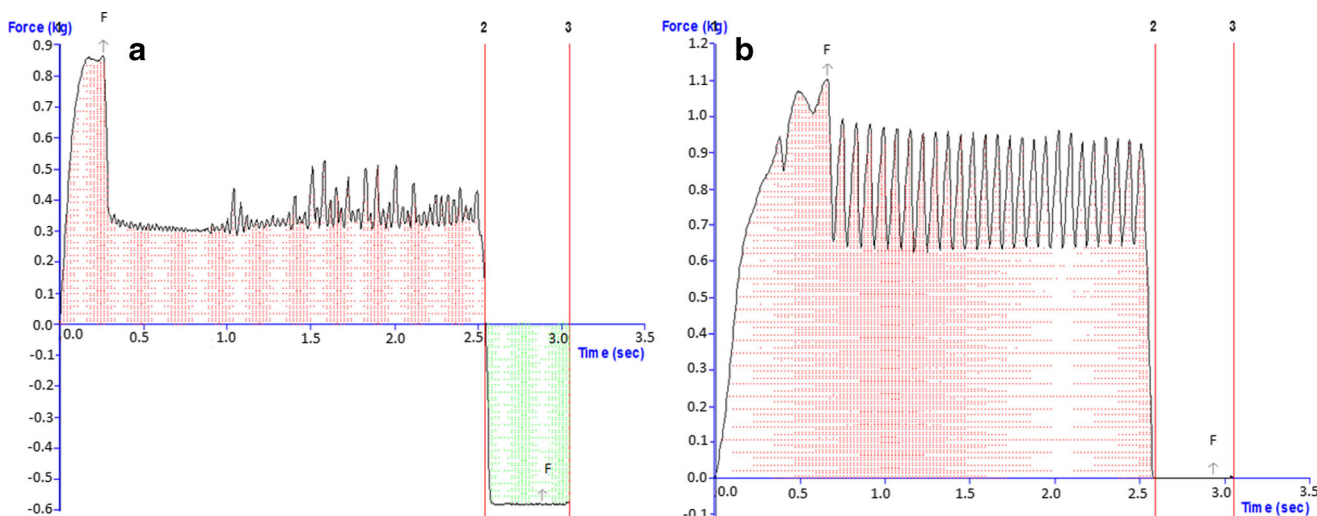


Fig. 2. Syringeability of the optimized formulations: **a** P₃ and **b** O₂

Table III. Syringeability of the Optimized Thermoresponsive MMNG Formulations

Formulation code	Force 1 (kg)	Area F-T 1:2 (kg/s)	Force 2 (kg)	Area F-T 2:3 (kg/s)
O ₂	1.105	2.016	-0.003	0
P ₃	0.866	0.967	-0.585	-0.284

where C_{skin} is the concentration of drug either in epidermis or dermis at time t , K_p is the dermal permeation constant, $C_{\text{max}}^{\text{Skin}}$ is the maximum concentration achieved either in epidermis or dermis, and K_e is the skin elimination constant. Win-Nonlin Ver 5.0 software was employed to calculate the various dermatokinetic parameters viz. K_p , $C_{\text{max}}^{\text{Skin}}$, K_e and $T_{\text{max}}^{\text{Skin}}$ (time required to achieve $C_{\text{max}}^{\text{Skin}}$ and area under the curve ($\text{AUC}_{0-3\text{h}}$) using Wagner-Nelson method (33).

Skin Compliance Studies

The hair on the dorsal side of Laca mice (20–25 g) was removed with the help of shaving razor, and the animals were divided into four groups with six animals in each group. One group was kept as control (untreated). The optimized formulations O₂ and P₃ (equivalent to 1 g, containing 25 mg/g lidocaine and 25 mg/g of prilocaine) and conventional formulations were topically applied on the skin within the area of 4 cm² once a day for 7 days. On completion of the study, animals were sacrificed by spinal dislocation and the exposed dorsal area was cut (31,34). The skin samples were fixed in 10% formalin and microtoned. The microtoned

sections were further stained with haematoxylin and eosin to observe any structural changes under a microscope.

Pharmacodynamic Evaluation

Surface Anaesthesia on the Cornea of Rabbits

The anaesthetic activity of lidocaine and prilocaine in MMNG and conventional formulations was assayed *in vivo* in New Zealand white rabbits, according to the conjunctival reflex test (35,36). Male New Zealand white rabbits (4–5.5 kg body weight) were obtained from CCS Haryana Agriculture University, Hisar, India. The animals were divided into four groups (each containing three rabbits). Group 1 received O₂, group 2 P₃, group 3 EMLA™ and group 4 Prilox cream. A sample of (30 mg) of each formulation was instilled in the right eye of the rabbit, and a blank formulation (without drug) serving as control was instilled in the left eye. The external sides of rabbit eyes were stimulated at suitable intervals with a bristle (human straight hair) to induce the conjunctival reflex. The efficacy of local anaesthetic activity is evidenced by

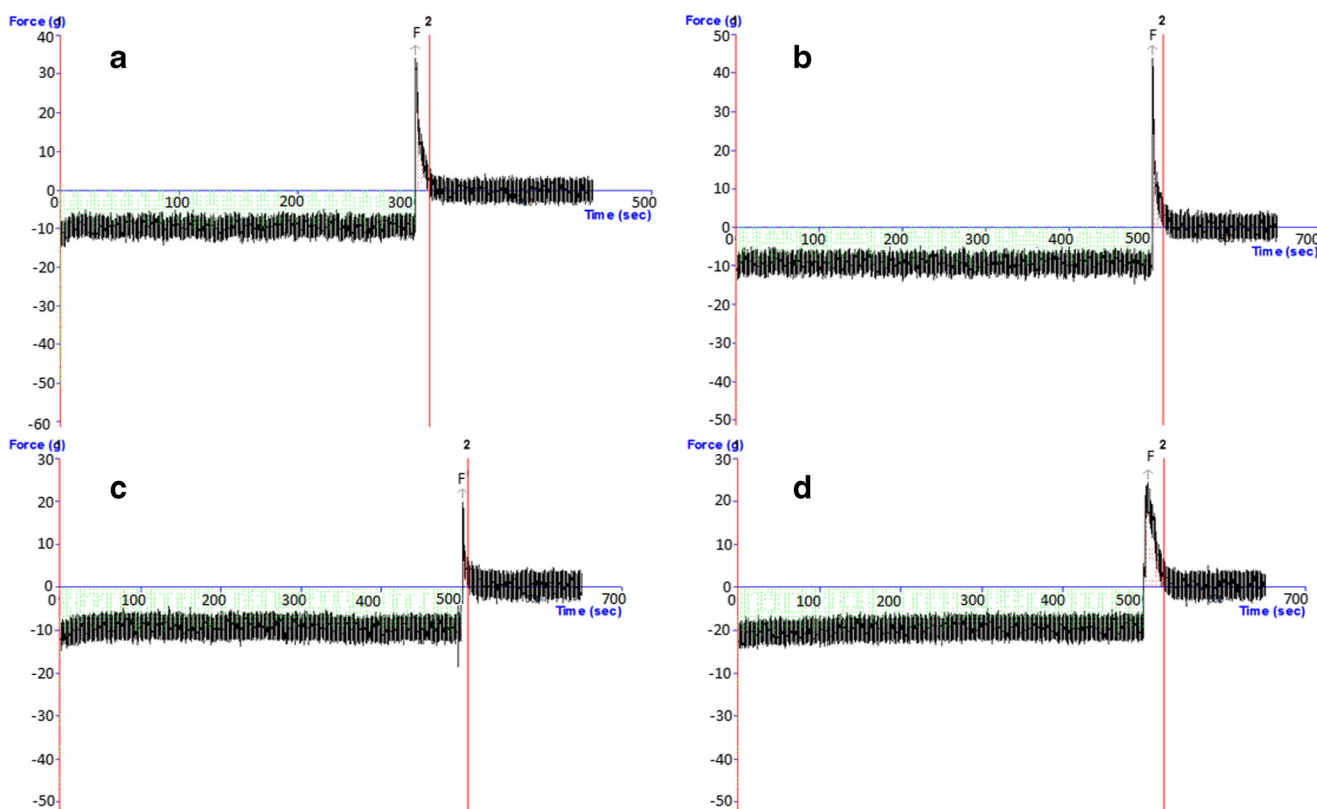


Fig. 3. Bioadhesive strength of **a** O₂, **b** P₃, and conventional formulations **c** EMLA™ and **d** Prilox

the requirement of number of stimuli to provoke the reflex (28). The observations were taken every 15 min after application of the formulation.

Radiant Heat Tail-Flick Method

Radiant heat tail-flick latency test on male Wistar rats employing analgesiometer (IMCORP, Ambala, India) was used for ascertaining the analgesic effect of the prepared formulations (29,37). The animals were divided into four groups containing five mice each (group 1 received O₂, group 2 P₃, group 3 EMLA™ and group 4 Prilox cream). About 30 mg of the formulation was applied on the root of the tail on midline. All the animals in the respective groups were individually exposed to analgesiometer maintained at 55°C. As a standard protocol, the cut-off for the basal reaction time was fixed at 10 s. The reaction time was measured at various time intervals (15, 30, 45 and 60 min) for each group (38). The per cent analgesic effect was determined using Eq. 2.

Percent analgesic effect

$$= \frac{(\text{Observed reaction time} - \text{Basal reaction time})}{(\text{Cut off time period} - \text{Basal reaction time})} \times 100 \quad (2)$$

Stability Studies

The optimized MMNG formulations were subjected to stability studies at different storage temperatures *viz.* 5 ± 2, 25 ± 2 and 45 ± 2°C for 45 days in sealed ampoules. The per cent drug content and per cent drug loss were ascertained on every seventh day. The physical stability of the MMNG formulations was also studied *via* clarity, phase separation observation and globule size determination (39).

Statistical Analysis

The data was statistically analysed by one-way ANOVA analysis followed by *post hoc* analysis using Student's *t* test. Statistical significance was considered at *p* < 0.05.

RESULTS AND DISCUSSION

Turbidity

%*T* determined at 4°C of the formulations is depicted in the Table I. While preparing the MMNG, it was observed that the turbidity of the system was greatly affected by temperature. Some formulations appeared to be turbid when kept at low temperature *i.e.* 4°C. With the temperature increasing from 4 to 30°C, the turbidity of the system decreased since turbidity of a system changes

Table IV. Bioadhesive Strength of the Optimized Thermo-responsive MMNG and Conventional Formulations

Formulation code	Force 1 (g)	Area F-T 1:2 (g/s)
O ₂	34.269	-2723.682
P ₃	43.846	-4608.682
EMLA	20.077	-4744.481
Prilox	24.692	-4652.858

as a function of temperature according to Chen *et al.* hypothesis (40). The reduced solubility and hydrogen bond formation of poloxamer at low temperature resulted in the formation of physical junctions, ultimately increasing the turbidity of the system. With increase in temperature, the weaker hydrogen bonds present in poloxamer skeleton and water molecules make poloxamer molecules free, thus leading to the decrease in turbidity.

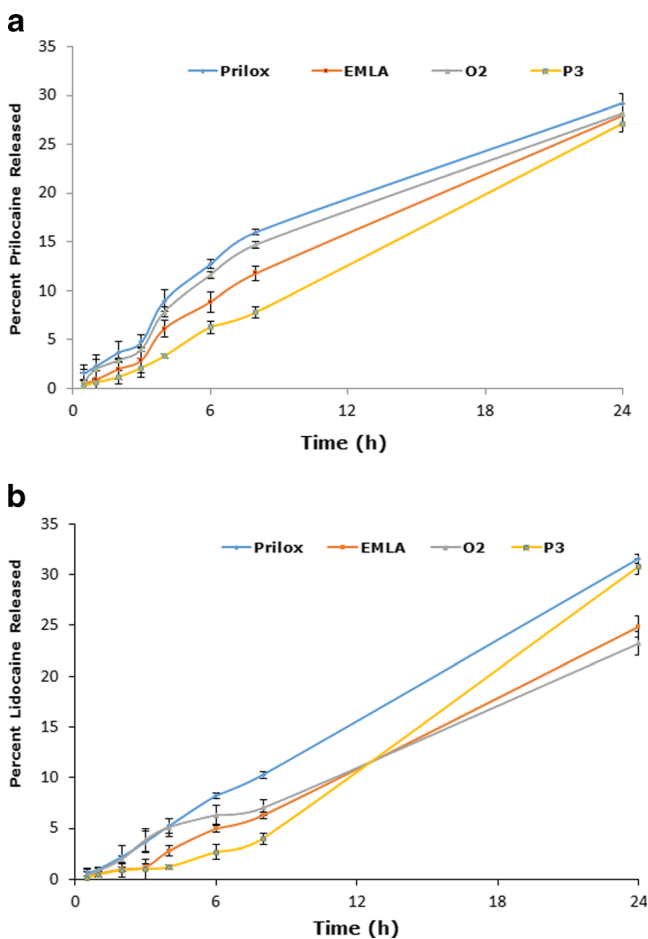


Fig. 4. Permeation profile of the optimized thermo-responsive MMNG formulations and other conventional products: **a** prilocaine and **b** lidocaine. Each cross bar indicates average value ± SD (*n* = 3)

Table V. Permeation Profile of the Optimized (O₂ and P₃) and Conventional Formulations (EMLA™ and Prilox) (*n* = 3)

Formulation code	Per cent drug permeated in 24 h (% ± SD)		Steady state flux (µg/h/cm ² ± SD)		Per cent drug retained (% ± SD)	
	Prilocaine	Lidocaine	Prilocaine	Lidocaine	Prilocaine	Lidocaine
EMLA™	27.99 ± 1.05	24.84 ± 1.09	118.73 ± 1.06	64.66 ± 0.57	0.52 ± 0.002	0.88 ± 0.003
O ₂	28.13 ± 1.05	32.25 ± 1.07	155.14 ± 1.56	98.22 ± 0.83	2.25 ± 0.006	3.09 ± 0.004
P ₃	27.09 ± 1.18	31.81 ± 2.08	149.68 ± 2.57	81.39 ± 1.52	1.94 ± 0.003	2.68 ± 0.003
Prilox	29.18 ± 1.18	32.58 ± 1.01	79.22 ± 0.99	46.3 ± 0.93	0.29 ± 0.003	0.35 ± 0.003

Phase Transition Studies

The formulations composed of PF127 (20, 18, 17%, w/w) and Tween 80 (molar ratio of PF127: Tween 80 was varied from 1:1 to 1:9) exhibited reversible sol-gel transition property. The measured $T_{sol/gel}$ values of MMNGs obtained were basically in the range of 11–45°C (Table I). The results obtained showed that as the percentage of PF127 was decreased, $T_{sol/gel}$ increased considerably (41). The unique characteristic of PF127 copolymer is reverse thermal gelation behaviour, which occurs at concentrations above 20%, w/w. Highly concentrated solutions exist in solution form below the critical micellization temperature (CMT), which forms soft gels above the CMT (42). Hence, decrease in PF127 concentration led to an increase in gelation temperature. Formulations prepared using 20% PF127 had low $T_{sol/gel}$ as compared to the formulations that were prepared using 18 and 17% PF127. At PF127 concentrations 20 or 17%, it was also observed that when the molar ratio of PF127/Tween 80 was ≤1:4, no change in the value of $T_{sol/gel}$ was observed; however, it increased considerably afterwards. On the other hand, in the case of the formulations containing 18% PF127, the higher molar ratio of Tween 80 resulted in lower $T_{sol/gel}$ values when molar ratio of PF127/Tween 80 was ≤1:2, while higher molar ratio of Tween 80 resulted in higher $T_{sol/gel}$ values when that molar ratio was ≥1:2.

Rheological Studies

Viscosity is an important rheological criterion for the utilization and *in vivo* performance of thermosetting gels (21). All the formulations at the refrigerated temperature showed low viscosity and thus indicated easy flowability (Table I). At room temperature, the formulations revealed higher values of viscosity. The developed formulations revealed that the viscosity increases with the increase in temperature. It is also noticed that as the ratio of PF127/Tween 80 increased, the viscosity of the formulation also got enhanced at both the temperatures. The formulations were liquid at refrigerated temperature and showed gelling property at room temperature (43).

Micromeritics

The mean particle size of the optimized mixed micellar solution was determined to be 32.5 nm. The log size distribution curve showed normal distribution indicating confidence in the observed particle size. It also ensured that the micelles population was uniform in size. The PDI values were found to be in acceptable range i.e. 0.281 (19,44,45).

Spreadability

Spreadability evaluates the stickiness and ease of application of topical formulations along with extrudability from the tubes. Figure 1 shows the variation of force of upper probe of texture analyser while moving in/out of the optimized MMNG systems. The results showed that the prepared formulations were non-sticky and required less force and work of shear (Table II) (23).

Syringeability Test

Syringeability test was performed in order to determine the flowability of the optimized formulations from the syringe for its easy application. The graphical repre-

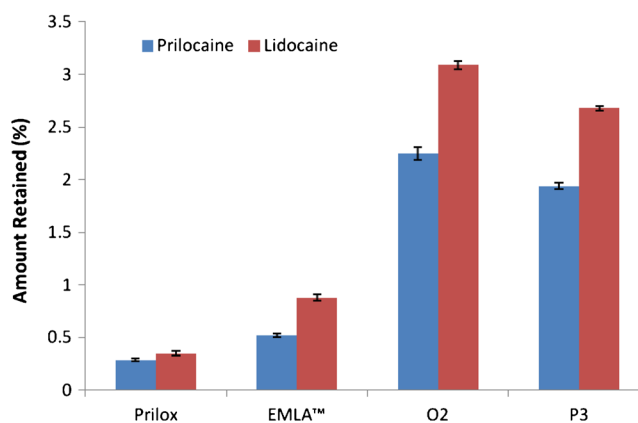


Fig. 5. Bar diagram showing the mean per cent drug retention in the skin from various formulations. Each cross bar indicates average value ± SD (*n* = 3)

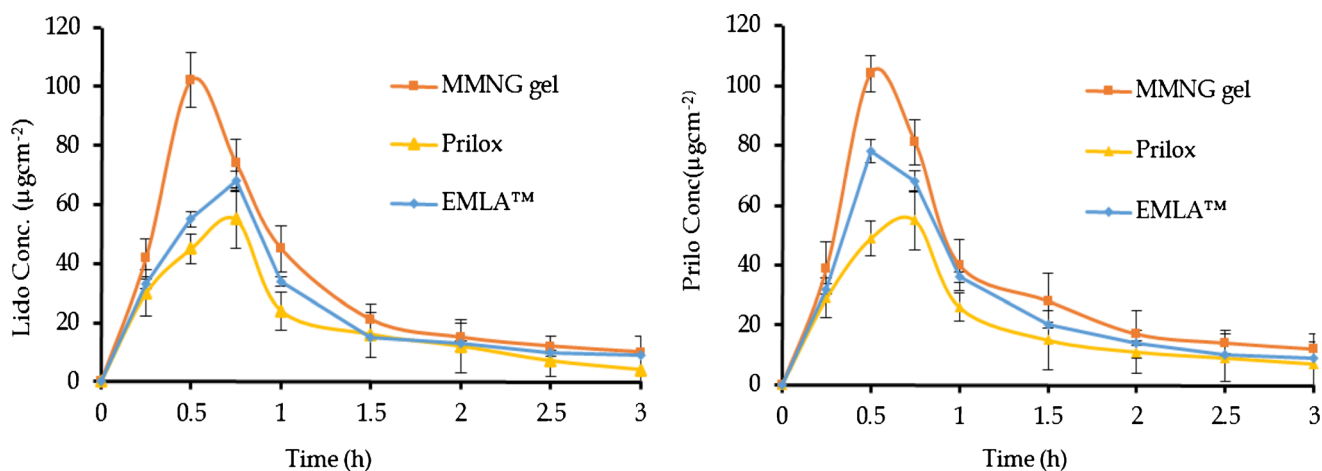


Fig. 6. Dermatokinetic profile showing the drug concentration in the epidermis of Wistar rats at various time points of lidocaine and prilocaine

sensation of the study is given in Fig. 2, and the various parameters calculated are depicted in Table III. The results of syringeability showed that the formulations were having optimum viscosity at refrigerated temperature so as to be easily applied with the help of a syringe.

Bioadhesive Strength

The bioadhesive studies were carried out in order to find out the degree of adhesiveness on the oral mucosa. This in turn will determine the retention of formulation at the site of application. The bioadhesive strength was measured in terms of force (g). The optimized formulations were compared with the conventional formulations. The graphical representation of the study is given in Fig. 3. The developed formulations, O₂ and P₃, showed higher bioadhesive strength i.e. 34.269 and 43.846 g, respectively, as compared to the conventional formulations (EMLA™ and Prilox, i.e. 20.077 and 24.692 g, respectively) as depicted in Table IV and Fig. 3. This indicates higher adherence of the formulation towards the site of

application, and therefore, there is delay in drainage of the formulation because of the biological fluids, i.e. saliva (46). Consequently, this will lead to longer duration of action and efficacy at the site of desired therapeutic site (26).

Ex vivo Skin Permeation Studies

Lidocaine and prilocaine permeation profiles from different formulations are depicted in Fig. 4, and all permeation parameters are depicted in Table V. Considering the need of a fast onset time, a high release rate is important (30). The permeation rates of lidocaine and prilocaine from the optimized MMNG formulations were higher than the conventional formulations ($p < 0.001$). The flux of lidocaine and prilocaine from O₂ and P₃ were 1.3–2.11-fold higher than EMLA™ and Prilox ($p < 0.01$). This could be ascribed to the reasons that smaller micellar aggregates are solubilizing the active components and thus the high thermodynamic activity of the drugs (47).

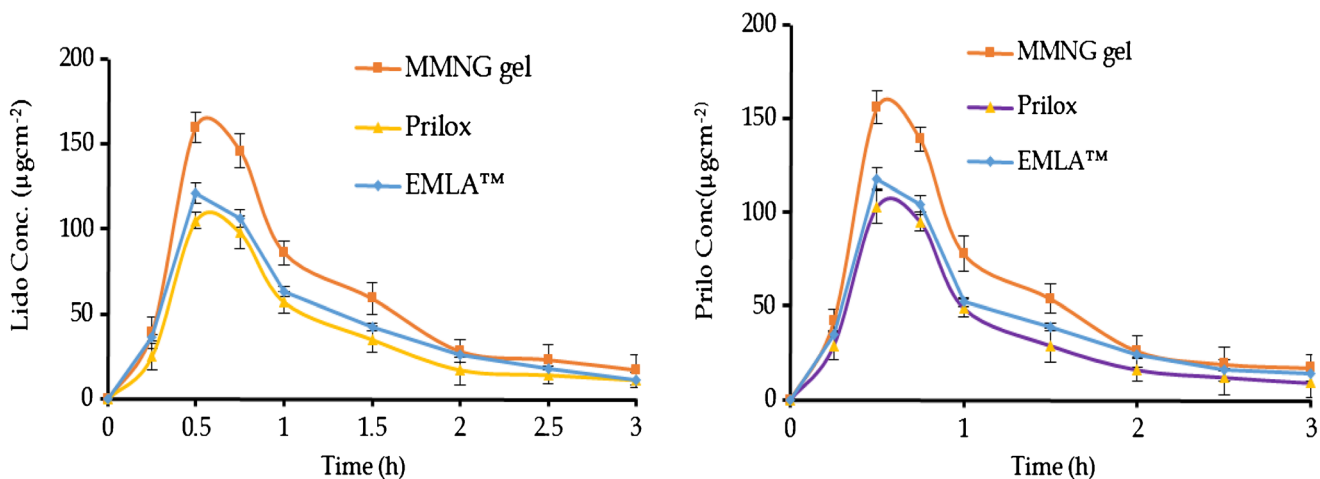


Fig. 7. Dermatokinetic profile showing the drug concentration in the dermis of Wistar rats at various time points of lidocaine and prilocaine

Table VI. Dermatokinetic Parameters (mean \pm SD) of Prilocaine- and Lidocaine-Loaded Formulations in Epidermis and Dermis of Wistar Rats ($n = 3 \pm$ SD)

Dermatokinetic parameters	Thermoresponsive MMNG gel				Conventional formulation (EMLA™)			
	Epidermis		Dermis		Epidermis		Dermis	
	Prilocaine	Lidocaine	Prilocaine	Lidocaine	Prilocaine	Lidocaine	Prilocaine	Lidocaine
AUC _{0-3h} ($\mu\text{g}/\text{cm}/\text{h}$)	105.20 \pm 13.88	115.02 \pm 12.45	187.54 \pm 29.65	189.99 \pm 19.04	57.94 \pm 9.20	107.45 \pm 22.88	128.09 \pm 28.53	88.72 \pm 19.45
C _{max} ($\mu\text{g}/\text{cm}$)	65.63 \pm 8.22	76.04 \pm 10.45	110.26 \pm 20.96	118.30 \pm 18.98	41.04 \pm 1.05	57.43 \pm 10.89	80.03 \pm 10.21	40.73 \pm 10.45
T _{max} (h)	0.48 \pm 0.35	0.43 \pm 0.09	0.53 \pm 0.10	0.52 \pm 0.13	0.28 \pm 0.08	0.59 \pm 0.19	0.48 \pm 0.25	0.59 \pm 0.13
K _a (/h)	2.01 \pm 0.10	2.89 \pm 0.10	1.83 \pm 0.34	1.62 \pm 0.98	1.86 \pm 0.04	1.04 \pm 0.17	1.23 \pm 0.22	1.11 \pm 0.18
K _e (/h)	0.99 \pm 0.16	1.05 \pm 0.25	1.20 \pm 0.18	1.15 \pm 0.96	1.56 \pm 0.10	1.34 \pm 0.18	1.04 \pm 0.16	1.31 \pm 0.16

The order of per cent skin retention of various formulation was O₂ > P₃ > EMLA™ > Prilox as depicted in Fig. 5. The optimized formulations offered significantly higher skin retention for lidocaine and prilocaine and ($p < 0.01$). High skin retention is required for topical application and longer duration of action. This, in turn, results in reduction in dosing frequency and higher patient compliance.

Dermatokinetic Modelling

The local anaesthetic products require a lag time of 45–60 min after the application of the topical product for effects to be observed (48). Hence, distribution of drugs in the skin layers was determined for over a short time interval only. Figures 6 and 7 depict the distribution of drugs in epidermis and dermis of the Wistar rat skin,

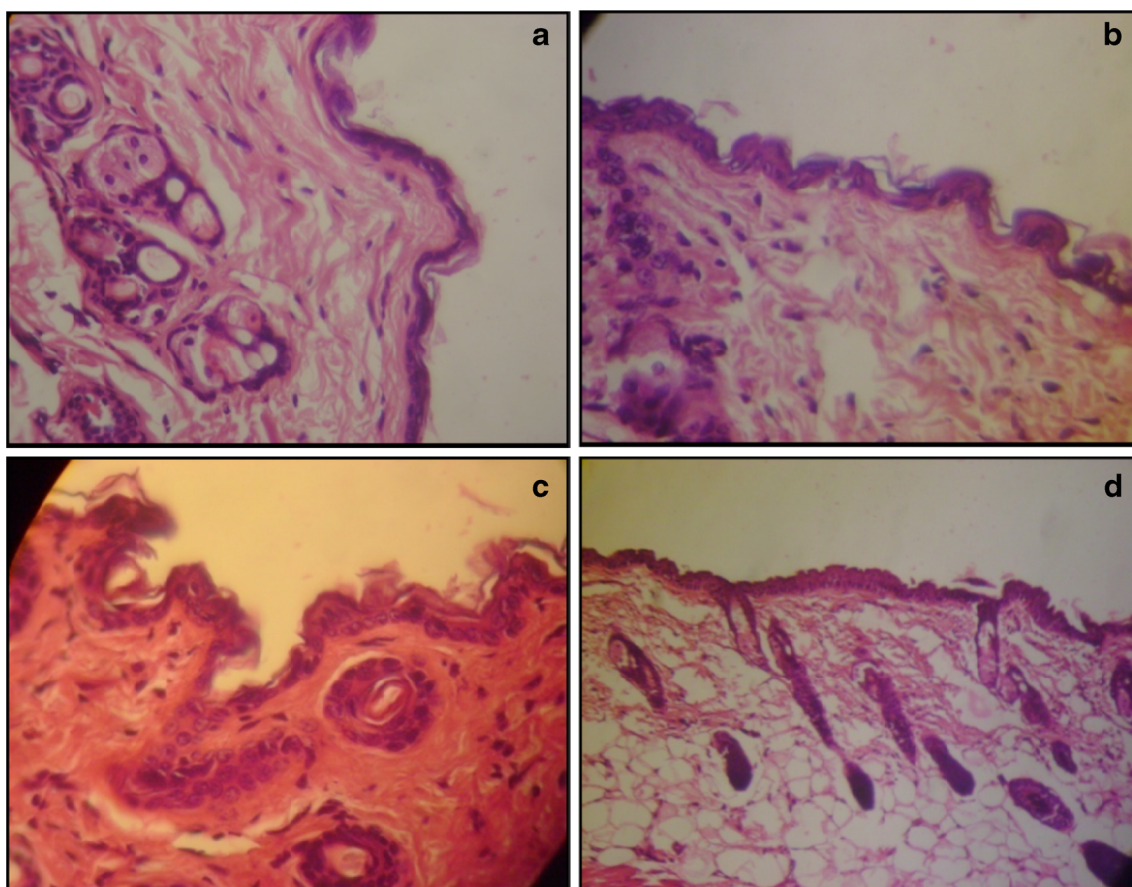


Fig. 8. The photographs of skin treated with formulations: **a** O₂ and **b** P₃, and conventional formulations **c** EMLA™ and **d** Prilox

Table VII. Anaesthetic Effect Induced by Optimized MMNG Systems and Conventional Formulations on the Rabbit Conjunctival Reflex Test ($n = 3$)

Formulations	Eye	Number of stimuli to induce conjunctival reflex time after treatment			
		10 min	20 min	40 min	60 min
O ₂	Left	1.2 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	Right	12.2 ± 2.1	28.4 ± 2.5	21.13 ± 1.0	14.3 ± 1.2
P ₃	Left	1.0 ± 0.3	1.3 ± 0.2	1.1 ± 0.1	1.2 ± 0.1
	Right	16.2 ± 1.2	22.13 ± 1.0	17.13 ± 3.0	11.3 ± 1.3
EMLA™	Left	1.2 ± 0.2	1.0 ± 0.2	1.4 ± 0.1	1.1 ± 0.2
	Right	8.2 ± 3.4	15.4 ± 2.8	13.13 ± 2.1	6.3 ± 2.0
Prilox	Left	1.3 ± 0.2	1.0 ± 0.1	1.2 ± 0.3	1.4 ± 0.2
	Right	12.6 ± 1.9	16.5 ± 2.4	12.8 ± 2.7	7.3 ± 1.8

respectively. The delivery of Lido and Prilo by thermo-responsive MMNG gel was found to be significantly greater ($p < 0.05$) as compared to the conventional formulations. Table VI details the numeric values of AUC_{0-3h} , C_{max}^{Skin} , skin penetration rate constant (K_p), T_{max}^{Skin} and skin elimination rate constant (K_e). The results suggest that the MMNG gel delivered local anaesthetic drugs to the dermis layer efficiently, hence providing the drug supply adequately to the pain-sensitive nerve endings.

Skin Compliance Studies

The photographs of optimized thermo-responsive MMNG systems (O₂ and P₃) and conventional formulations (EMLA™ and Prilox)-treated skin is depicted in Fig. 8. Microscopic examination showed no marked changes in the skin structure of optimized formulations and EMLA™. The skin showed normal epidermis and cell maturation. The dermis was normal and no inflammatory changes were seen in the skin. The hair follicles were evenly spread and normal. The photograph indicated that the formulations did not disrupt the integrity of normal skin. Little changes in the skin structure were observed in the Prilox-treated skin as can be seen from the photograph. The skin histopathology examination indicated the biocompatible nature of the optimized

MMNG formulations, and hence, the formulations indicated to be well tolerated by skin (34,49,50).

Pharmacodynamic Evaluation

Surface Anaesthesia on the Cornea of Rabbits

The results of the study are reported in Table VII. It is evident that MMNG formulations were successful in significantly improving both intensity and duration of drug anaesthetic effect with respect to the conventional formulations at the same concentration. The best results, in terms of improved intensity of anaesthetic effect after the gel application, were achieved in 20 min by O₂ formulation followed by P₃. The order of effectiveness was O₂ > P₃ > Prilox > EMLA™. The mixed micellar systems at the application site readily convert into the gel and maintain a close contact. This in turn facilitates a preferential uptake of drug molecules through the epithelium. The latter is accentuated by the enhanced duration of stay of the local anaesthetic at the site of action (28,35).

Radiant Heat Tail-flick Method

A significant increase ($p < 0.05$) in the basal reaction time in the groups receiving optimized thermo-responsive MMNG gel (O₂ and P₃) vis-à-vis the conventional

Table VIII. Comparison Between Analgesic Effect of O₂, P₃, EMLA™ and Prilox

Formulation	Mean basal reading (s)	Mean reaction time ± SD (s)										AUEC ₀₋₄₅	EF	
		0	5	10	15	20	25	30	35	40	45		EMLA™	Prilox
O ₂	2.25	0	2.75	3.2	3.5	4.75	6.25	5.20	4.55	3.65	2.55	234.38	1.21	1.25
P ₃	2.75	0	2.60	3.40	4.0	5.00	6.00	5.30	4.20	3.45	2.40	241.88	1.25	1.29
EMLA™	2.25	0	2.40	2.55	3.00	4.25	4.75	4.15	3.50	3.30	2.35	193.13	1.0	
Prilox	2.25	0	2.45	2.50	3.00	4.00	5.5	4.55	4.10	3.55	2.25	187.5	1.0	

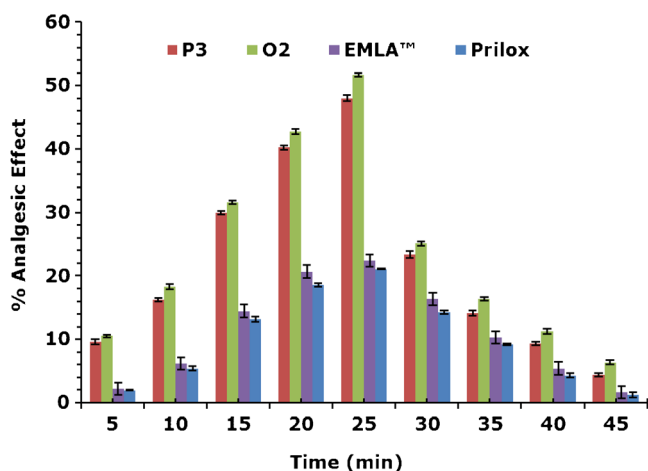


Fig. 9. Bar diagram depicting per cent analgesic effect of the various formulations. Each cross bar indicates average value \pm SD ($n=3$)

formulation groups was observed, as shown in Table VIII. The value of $AUEC_{0-45}$ of the optimized formulations O_2 and P_3 was found to be 234.38 and 241.88 min^2 , respectively, whereas 193.13 and 187.5 min^2 for EMLA™ and Prilox, respectively. Thus, the efficacy of the optimized MMNG systems was observed to be about 1.21–1.29-folds greater than the conventional formulations. Simultaneously, the per cent analgesia produced by MMNG gel (O_2 and P_3) formulation was significantly greater ($p < 0.05$) than of the conventional gel as depicted in Fig. 9. The highest analgesic effect of both the optimized formulation (i.e. 51.67% for O_2 ; 48.03% for P_3) and conventional gel (i.e. 22.41% for EMLA™; 21.09% for Prilox) was shown at 25 min. The enhanced

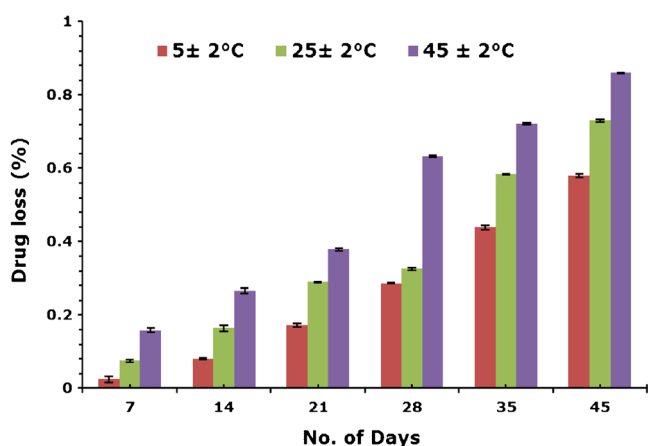


Fig. 10. Bar diagram depicting the % loss of prilocaine from thermoresponsive MMNG systems at different temperatures. Each cross bar indicates average value \pm SD ($n=3$)

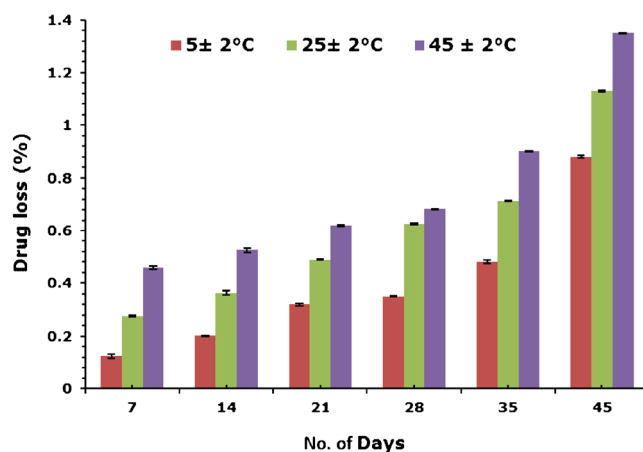


Fig. 11. Bar diagram depicting the % loss of lidocaine from thermoresponsive MMNG systems at different temperatures. Each cross bar indicates average value \pm SD ($n=3$)

pharmacodynamic effect offered by thermoresponsive MMNG system can be ascribed to better penetration, localization and extended drug action.

Stability Studies

For the stability testing, the optimized formulations were studied at 5 \pm 2, 25 \pm 2 and 40 \pm 2°C. The per cent drug loss of Lido was found to be greater than Prilo at all the storage temperatures as depicted in Figs. 10 and 11. However, the best storage temperature for the formulations was found to be at 5 \pm 2°C, followed by 25 \pm 2°C. The physical stability of the formulation was maintained throughout the study as only negligible changes were observed in the globule size or phase separation of the formulations at all the storage temperatures (51).

CONCLUSION

The study with the optimized thermoresponsive MMNG system of lidocaine (2.5%, w/w) and prilocaine (2.5%) is an indication of its potential in improving the pain relief therapy by way of improved delivery to the site of action. The formulation-specific properties like $T_{\text{sol/gel}}$ of 34.5°C, particle size 32.5 nm and bioadhesive strength of 34.00 g have been found as supportive. PF127 and Tween 80 have been the key components in a fixed proportion, in attaining the desired characteristics. The MMNG system was made possible for the thermosensitive release of the drug molecules and enhanced the stay of local anaesthetic molecules at the site of action to increase the drug-target interactions. Thus, given the overall consideration, the system based on the thermoresponsive character and novelty in the composition may provide a better and value-added alternative for topical anaesthesia.

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COMPLIANCE WITH ETHICAL STANDARDS

Declaration of Interest The authors report no declarations of interest.

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