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RESEARCH PAPER

Formulation and Efficacy Studies of New Topical Anesthetic Creams

Lisheng Kang[#] and H. W. Jun[*]

Department of Pharmaceutical and Biomedical Sciences, The University of Georgia College of Pharmacy, Athens, Georgia, USA

ABSTRACT

Local anesthetics (lidocaine or tetracaine) spontaneously melted at 25°C when mixed with thymol and aqueous isopropyl alcohol solution (IPA) at proper ratios and formed novel two-phase melt systems (TMS). The TMS consisted of a homogeneous oil phase containing primarily a local anesthetic agent (lidocaine or tetracaine) and thymol, and a homogeneous aqueous phase containing primarily IPA and pH 9.2 buffer. The relationship between melting of the solid components and system composition was determined from the phase diagram obtained by a titration method. A select TMS of a local anesthetic agent (lidocaine or tetracaine) was directly emulsified to prepare an O/W cream and tested for the anesthetic efficacy on intact human skin. While both lidocaine (6%) and tetracaine (4%) creams were highly effective for dermal anesthesia with a similar onset time, the tetracaine cream exhibited a significantly longer duration of action than the lidocaine cream. An accelerated stability study indicated that lidocaine was significantly more stable than tetracaine in the creams.

Key Words: Two-phase melt system; Topical; Local anesthetic; Lidocaine; Tetracaine; Efficacy.

INTRODUCTION

A topical anesthetic preparation that can ease the pain during minor dermal procedures would be of considerable value in clinical practice. Among several anesthetic preparations currently available, a eutectic mixture of lidocaine and prilocaine called EMLA (Eutectic Mixture of Local Anesthetics by AstraZenica USA) is particularly effective on intact skin.[1–3] As concentrated eutectic oil, its active

[#]Current address: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA.
*Correspondence: H. W. Jun, Department of Pharmaceutical and Biomedical Sciences, The University of Georgia College of Pharmacy, Athens, GA 30602, USA; E-mail: wjun@rx.uga.edu.

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ingredients possess high thermodynamic activities for enhanced permeation through the stratum corneum that is the main barrier for topical anesthetics. It is known that the metabolites of prilocaine can cause methemoglobinemia, a serious blood condition more developed in children with impaired oxygen-carrying capacity.[4] A topical preparation containing lidocaine alone as the active agent would thus be of significant advantage over EMLA in pediatric clinics. However, the use of lidocaine alone in the topical anesthetic preparations has been clinically less successful due to its low permeation through the skin. Although the anesthetic efficacy of lidocaine has been achieved by higher lidocaine concentrations in the formulation[5–7] or using iontophoresis,[8] these methods are not widely adapted due to the risk of potential toxicity, skin irritation, or complexity of the technology.

A previous report from this laboratory described a new method for converting solid lidocaine into a highly concentrated oil in the presence of aqueous ethanol and thymol at ambient temperature.[9] Using the GC/MS assay, it was shown that the oil phase of the TMS system formed consisted of as much as 87% (w:w) of melted lidocaine, thus possessing a high driving force for enhanced skin permeation and high anesthetic activity of the drug. The new method offered the possibility of formulating an effective topical preparation containing lidocaine alone as the active agent.

In this study, the anesthetic efficacy of a lidocaine cream and a tetracaine cream prepared using thymol and aqueous isopropyl alcohol (IPA) as the melting point depressing agents was determined on the intact skin of 14 volunteers as compared with EMLA and placebo creams. The physical and chemical stabilities of the creams were studied under an accelerated temperature condition.

MATERIALS AND METHODS

Materials

The following chemicals and solvents were used as received from various sources: lidocaine, tetracaine, thymol (Sigma Chemical Co., St. Louis, MO), Carbopol 980, NF (BFGoodrich, Cleveland, OH), Cremophor EL, USP (BASF, Mount Olive, NJ), IPA, acetonitrile, methylene chloride, sodium carbonate anhydrous, sodium bicarbonate, sodium hydroxide (JT Baker Chemical Co., Phillipsburg, NJ). Distilled and deionized water was used.

Phase Diagrams of Local Anesthetics–Thymol–Aqueous IPA Systems

To study the relationship between the melting of the solid components and system composition, the phase diagrams were obtained using a titration method as described in an earlier report.[9] At 25°C, the local anesthetic agent (lidocaine or tetracaine) and thymol were mixed at various ratios ranging from 100:0 to 0:100 (w:w), while keeping the total amount as 0.06 g. To these mixtures in glass test tubes, 1 mL of pH 9.2 carbonate buffer (0.02 M) was added and then titrated with anhydrous IPA using a 500-μL microsyringe with frequent shaking. With the continuous titration, the solid components slowly melted and completely transformed into an oil phase surrounded by aqueous IPA. The total amount of IPA added (M_1) was recorded when the solid-to-oil phase transition of the compounds was completed. At this time, a two-phase melt system (TMS) was formed with the oil phase remaining at the bottom of the test tube. Continuous addition of IPA, however, resulted in complete solubilization of the oil phase in the aqueous alcoholic phase. At this time, only a homogeneous aqueous alcoholic solution of the compounds was present in the tube without any remaining oil phase. The amount of IPA used (M_2) to completely dissolve the oil phase in the dispersion and form a homogenous solution was also recorded. To obtain compositional phase diagrams of the multicomponent systems, the values of M_1 and M_2 were plotted against the drug:thymol ratios. The entire titration was carried out at 25°C.

Preparation of Anesthetic Creams

An O/W cream of lidocaine or tetracaine used for the efficacy test was prepared using a TMS composition selected from the phase diagram representing a drug:thymol ratio of 90:10 (w:w) and an IPA content of 15% (w:w). This composition produced a TMS with high local anesthetic concentrations in the oil phase at 25°C.[9] In preparation of the cream, all materials were mixed at 25°C. Cremophor EL and Carbopol 980 were used as the surfactant and the thickening agent, respectively. Adjustment of the pH of the mixture was made to 9.2 with a NaOH solution. The mixture was then processed with a high pressure homogenizer (KU-1, Erweka-Apparateban, Germany) three times to make the O/W cream. The compositions of 6% lidocaine cream and 4% tetracaine cream prepared

Table 1. Composition of 6% lidocaine cream.

Lidocaine		6.0 g
Thymol		0.67 g
IPA		15.0 g
Carbopol NF980		1.0 g
Cremophor EL		1.0 g
Water	q.s.	100.0 g

Table 2. Composition of 4% tetracaine cream.

Tetracaine		4.0 g
Thymol		0.44 g
IPA		15.0 g
Carbopol NF980		1.0 g
Cremophor EL		1.0 g
Water	q.s.	100.0 g

for the efficacy test are shown in Tables 1 and 2, respectively.

Efficacy Test on Intact Human Skin

The dermal anesthetic activities of 6% lidocaine cream and 4% tetracaine cream were determined on intact skin of 14 volunteers (10 males and 4 females, 22–59 years of age). The study was performed based on a randomized double-blind design using EMLA cream (AstraZenica, Wilmington, DE) as a reference. A placebo cream prepared without the anesthetic agent was similarly applied.

An informed consent was signed by the volunteers, and the study was approved by the Institutional Review Board of the University of Georgia (Project # H990355). On the test day, 1 g each of the lidocaine cream, EMLA cream, and the placebo cream and 0.5 g of the tetracaine cream were applied randomly on four marked areas (1.5×1.5 cm) of the volar surface of both right and left forearms of the volunteers. All applications were covered with Saran wrap. At the end of 60 min application, the creams were completely removed using a gauze pad. At 0, 30, 60, 90, and 12 min after removal of the creams, 10 pin-pricks using a sharp toothpick were applied by a trained examiner, covering the entire area of each application site. The subjects were instructed to record the number of times (x) they felt pain for the pin-pricks. The anesthetic scores ranging between 0 and 10 at each time interval were obtained by subtracting x from 10 pin-pricks applied ($10 - x$) for each formulation tested. The duration of anesthesia, indicated as the time (min) for the recovery of sensation with a mean anesthetic score less than 5 at each pin-prick test, was also determined. The sharp tooth-picks were used instead of hypodermic needles to avoid potential damage to the skin.

Following application of the creams, the volunteers were asked to record any sense of irritation on the treated sites which were also examined for any signs of erythema, pallor, and edema upon removal of the creams. The anesthetic scores and the durations of anesthesia were statistically analyzed using ANOVA and Fisher's LSD tests and were compared among the test creams. The anesthetic scores of the creams after a shorter application time (45 min) were similarly determined and analyzed in seven subjects.

Stability of Lidocaine and Tetracaine in Creams

The creams after preparation were kept at ambient temperature for 24 hr prior to conducting the tests for the drug content, particle size, and viscosity. For the stability study, a portion of the creams were placed in small amber glass jars with polyvinyl-lined closures and stored at 25°C for 8 months and at 15 and 40°C for 3 months. For the tests, the samples removed after storage at 25 and 40°C were left at ambient temperature for 24 hr prior to conducting the tests. The 15°C storage condition represents a stressed condition for crystallization of the active. For tests, a portion of the creams stored at 15°C was removed and examined weekly using an optical microscope to observe any crystals formed in storage.

Quantitation of Lidocaine and Tetracaine in Creams

For the extraction of the drugs from the creams, 0.2 g of each cream was placed in 1 mL of 0.1 N NaOH in a test tube, followed by addition of 2 mL methylene chloride, vortexing for 1 min, and centrifugation for 5 min at 2000 rpm. The extraction was repeated three times for each sample, and the solvent layer was collected and combined. After evaporation of methylene chloride, the residue was reconstituted with 1 mL of the mobile phase used for HPLC assay.

Lidocaine and tetracaine in the creams were separated using a C_{18} column (Phenomenex, Prodigy 5u, 4.5×150 mm, Torrance, CA) with UV detection

(Varian 2550, Monrovia, CA) at 210 nm. The mobile phase was pH 5.9 phosphate buffer (0.05 M) containing 20% (v:v) acetonitrile and the flow rate was 1.0 mL/min.[10] The retention times of lidocaine, tetracaine, and phenacemide, the internal standard, were 6.4, 10.1, and 7.5 min, respectively.

Measurement of Particle Size, Viscosity, and Melt State

The particle size distribution of the droplets in the O/W creams was determined using an optical particle sizer (Nicomp Accusizer 780, Santa Barbara, CA). A pH 9.2 carbonate buffer (0.02 M) saturated with lidocaine or tetracaine and filtered through a 0.22-μm membrane filter (Millipore Corp., Bedford, MA) was used as the diluent. Approximately 0.2 g of the creams was first diluted with 1 mL of the diluent to obtain a concentrated emulsion. For the particle size analysis, a few drops of the emulsion were introduced into the mixing chamber of the particle sizer filled with the diluent.

The viscosity of the creams was measured at 25°C with a Brookfield digital viscometer (Model DV-II, Brookfield Engineering Laboratories, Inc., Stoughton, MA) using a No. 7 spindle. The rotation speed of the spindle was set at 10 rpm.

An optical microscope was used to monitor reappearance of solid drug crystals in the creams during storage at 15°C for 3 months. The samples were prepared weekly by removing a small amount of the creams and mixing with a drop of pH 9.2 carbonate buffer. The sample slide, cover glass, and buffer were all stored at 15°C before use to avoid any temperature effects on the microscopic examination.

RESULTS AND DISCUSSION

Phase Diagrams of Local Anesthetics–Thymol–Aqueous IPA Systems

The titration method was used to study the phase behavior of the local anesthetics–thymol–aqueous IPA systems. In these multicomponent systems, the external phase consisted mostly of water and, therefore, the effect of water on the melting state of lidocaine was considered essentially neutral. In addition, the amount of the local anesthetic agent and thymol together in each of the multicomponent systems was only 6% (w:w) of the total composition, and thus the lidocaine/thymol weight ratios were more important than the absolute concentrations of these two components in determining the melt states of these compounds. Therefore, the two primary variables determining the phase diagrams of these systems were the concentration of IPA and the weight ratio of lidocaine to thymol.

Figures 1 and 2 show the compositional phase diagrams of lidocaine and tetracaine, respectively, in the presence of thymol and aqueous IPA at 25°C. The plots of M_1 and M_2 values against drug:thymol ratios resulted in a lower (ABCD) and an upper (EF) curve. The curve ABCD represents the minimum amounts of IPA required to completely transform the solid compounds into the oily state, while the curve EF indicates the amounts of IPA needed to completely dissolve the oil phase formed, thus becoming a single homogeneous alcoholic solution. The curves ABCD and EF divide the phase diagrams into the four regions. Within the right and left regions below ABCD (Regions 1 and 2), crystals of the local anesthetic agent and thymol, respectively, remain in each system. Between ABCD and EF (Region 3), all the compositions spontaneously produce TMS, consisting of an oil phase and an aqueous phase. Above EF (Region 4), the oil phase no longer exists because the amount of IPA in the aqueous phase was sufficiently high to completely dissolve the oil phase.

A previous study using GC/MS showed that the oil phase of the TMS prepared with a high drug:thymol ratio (90:10, w:w) and a low alcohol content (15%, w:w) consisted primarily of the local anesthetics with a small amount of thymol and alcohol.[9] Similarly, in the present study, the concurrent use of thymol and IPA synergistically reduced the melting point of the local anesthetics, thus requiring significantly smaller amounts of the melting point depressing agents to form a TMS. On the other hand, the use of larger amounts of IPA and thymol to form a TMS could dilute the concentration of the local anesthetics in the oil phase. Therefore, TMS compositions with high drug:thymol ratios and low alcohol contents should be selected to produce systems with high thermodynamic activities of the drugs. According to phase diagrams shown in Figs. 1 and 2, for both lidocaine and tetracaine systems, compositions comprising 15% IPA, and a local anesthetic agent:thymol ratio of 90:10 (w:w) locates in Region 3 and close to boarderline CD. This indicates that such compositions are in two-phase melt state and the oil phase is close to saturated state for the drugs. Such systems were used in preparation of topical creams for in vivo efficacy study.

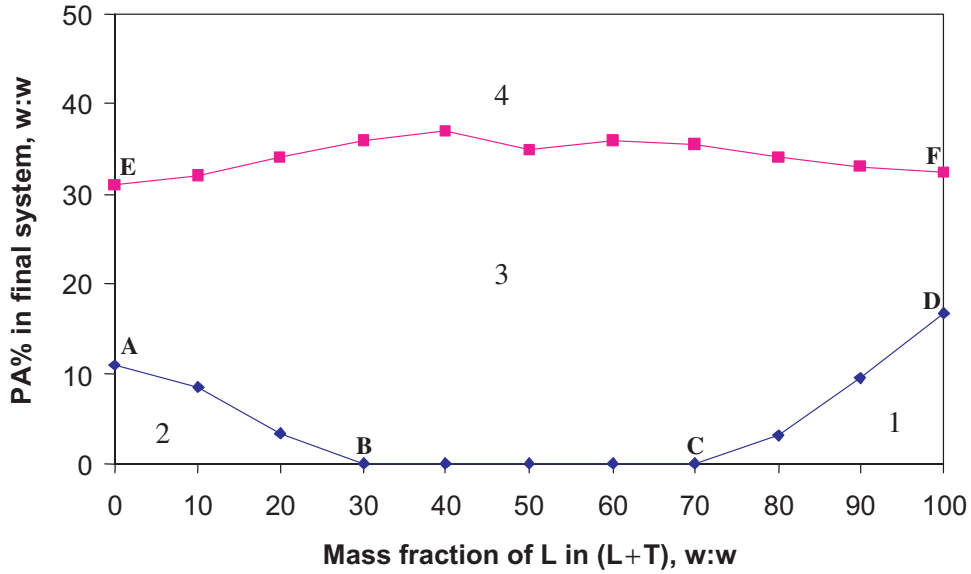

Figure 1. Phase diagram of lidocaine–thymol–aqueous IPA system. L—lidocaine; T—thymol; IPA—isopropyl alcohol; AB, CD, and EF—border lines on phase diagram; Areas 1 and 2—solid crystals remaining in each system; Area 3—two-phase melt systems; Area 4—solution systems.

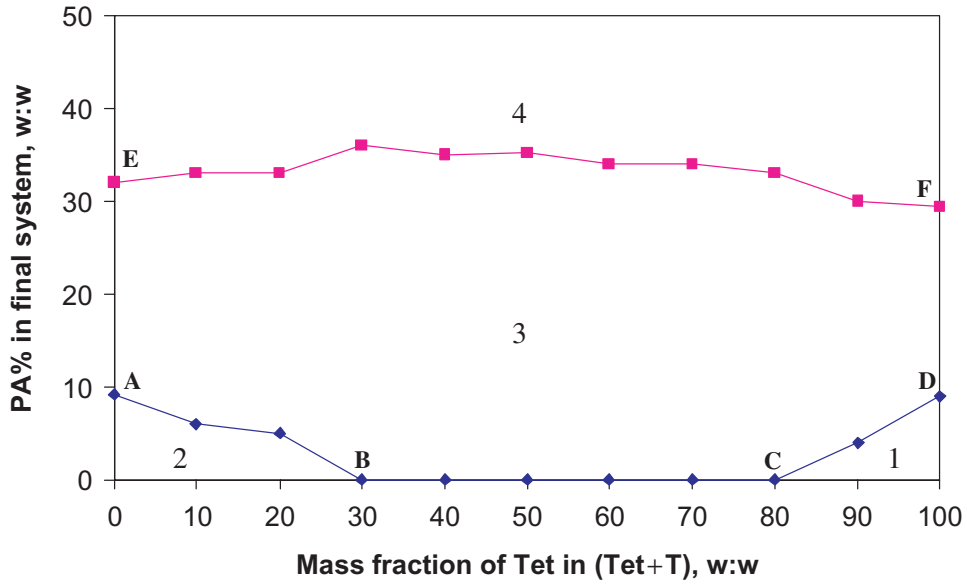

Figure 2. Phase diagram of tetracaine–thymol–aqueous IPA system. Tet—tetracaine; T—Thymon; IPA—isopropyl alcohol; AB, CD, and EF—border lines on phase diagram; Areas 1 and 2—solid crystals remaining in each system; Area 3—two-phase melt systems; Area 4—solution systems.

Efficacy Test in Human Volunteers

Figure 3 shows the statistical data of the anesthetic scores measured on the intact skin of 14 volunteer subjects after topically applying the cream formulations for 60 min. At each time point shown in Fig. 3, all of the creams tested (tetracaine, lidocaine, and EMLA) exhibited a significantly higher anesthetic activity than the placebo cream ($p < 0.001$). No significant difference, however, was found between the lidocaine and EMLA creams ($p > 0.05$). At 30, 90, and 120 min after removal of

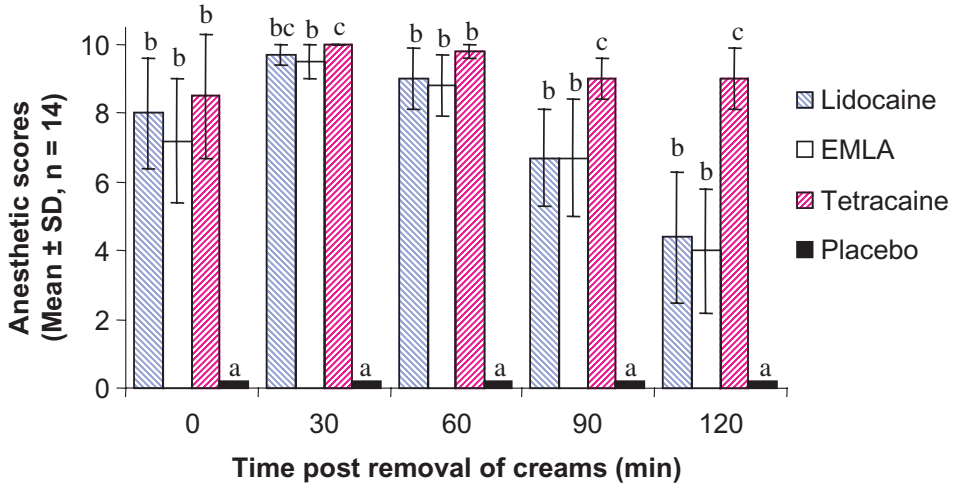

Figure 3. Anesthetic scores measured following application of the test creams for 60 min. At the same time point, $c > b > a$ ($p < 0.05$), $bc > a$ and no significant difference between bc and b or c.

the testing creams, tetracaine treatment showed significantly higher scores than both lidocaine and EMLA treatments ($p < 0.05$). The durations of efficacy exhibiting the mean anesthetic scores maintained higher than 5 after removal of the creams were compared for the three formulations tested. The lidocaine and EMLA creams, respectively, showed the durations of 1.54 ± 0.36 hr, and 1.46 ± 0.41 hr, but the difference was not significant ($p > 0.05$). The duration of the tetracaine cream was 1.96 ± 0.13 hr, which was significantly longer than both the lidocaine and EMLA creams ($p < 0.05$). The higher anesthetic scores and longer duration of the tetracaine cream (4%) despite its lower dose applied (0.5 g) could be attributed to the greater intrinsic anesthetic potency and higher lipophilicity of tetracaine as reported previously.[11]

The incidence of skin reactions observed at the end of 60 min application is listed in Table 3. Mild erythema and pallor were seen on several subjects, which was probably caused by the pharmacological action of the local anesthetics on cutaneous blood vessels.[12] Lidocaine and tetracaine are known to exihibt both vasoconstrictor and vasodilator effects depending upon their concentration in local tissues. Pallor occurred at low concentrations due to vasoconstriction, while erythema was observed at high drug concentrations due to vasodilation.[13] After application of the tetracaine cream for 60 min, two volunteers exhibited signs of edema on the site of drug application. No skin reaction was observed on the placebo-treated sites.

Table 3. Incidences of skin reactions observed following application[a] of different creams on forearms of 14 subjects.

Creams	Erythema	Pallor	Edema
Lidocaine	3	1	0
EMLA	2	4	0
Tetracaine	5	0	2
Placebo	0	0	0

[a]Application time: 60 min.

Figure 4 shows the anesthetic scores of the test formulations measured following the application time of 45 min in seven subjects. Compared with the 60 min application, the relatively higher level of deviations in the anesthetic scores found immediately after removal of the test creams were probably due to the intersubject variability in the skin permeation of the local anesthetics. The 45-min application time was not sufficient for some volunteers to achieve the onset of the anesthetic efficacy. Interestingly, however, for all the anesthetic creams applied for both 60 and 45 min, the efficacy scores measured appeared to have increased during the first 60-min interval after the removal of the creams. This finding indicated that the drugs in the stratum corneum might have continuously permeated into the dermal area where the nerve endings are present. The role of the stratum corneum as the source of prolonged efficacy of the drugs after topical application has been previously reported.[14,15]

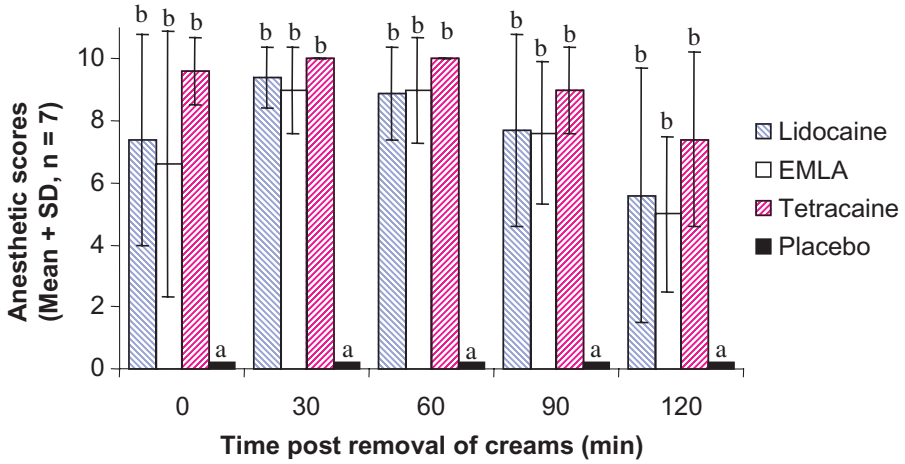

Figure 4. Anesthetic scores measured following application of the test creams for 45 min. At the same time point, $b > a$ ($p < 0.05$).

Table 4. Stability of 6% lidocaine cream.

Temp (°C)	Storage time (days)	Drug content (%)	Particle size (μm)	Viscosity (10^3 cps)
40	0	5.86 ± 0.07	4.74 ± 2.36	42.8 ± 1.6
	90	5.79 ± 0.13	5.04 ± 2.49	40.8 ± 1.8
25	0	5.86 ± 0.07	4.74 ± 2.36	42.8 ± 1.6
	240	5.74 ± 0.08	4.85 ± 2.27	44.9 ± 2.1

Table 5. Stability of 4% tetracaine cream.

Temp (°C)	Storage time (days)	Drug content (%)	Particle size (μm)	Viscosity (kcps)
40	0	4.05 ± 0.07	3.96 ± 1.79	36.3 ± 1.4
	90	2.10 ± 0.30	—[a]	—[a]

[a]Test discontinued due to chemical instability.

Stability of Lidocaine and Tetracaine in Creams

Table 4 shows the stability data of lidocaine in the creams. After storing at 40°C for 3 months, 98.8 ± 4.1% (CV) lidocaine was recovered as compared to the initial drug content analyzed by HPLC. According to a previous report,[16] lidocaine was highly resistant to heat, acid, and alkali despite its amide structure. The high stability was attributed to the sterical hindrance exhibited by the two methyl substitutes on the amide group. In comparison, only 51.9 ± 8.4% (CV) tetracaine was recovered from the cream under the same storage conditions (Table 5). Tetracaine has been reported to be quickly hydrolized in a liposomal formulation.[17]

The particle size distribution and bulk viscosity of the lidocaine cream did not change significantly after storing at both 25 and 40°C for 3 months as shown in Table 4. No crystals were formed in the creams stored at 15°C for the same period.

CONCLUSIONS

Lidocaine and tetracaine were spontaneously transformed into an oily state in the presence of thymol and aqueous IPA and formed the two-phase

melt system at ambient temperature. The solid-to-liquid phase transition was caused by the melting point depression of the compounds by the additives. The O/W creams prepared using the select two-phase melt systems possessed the high thermodynamic activity of the local anesthetics for the enhanced drug penetration through the stratum corneum. The anesthetic efficacy tests on the intact skin of volunteers showed that both 6% lidocaine cream and 4% tetracaine cream were highly effective in obtaining dermal anesthesia following topical application for 60 min. The degradation of lidocaine in the new cream was not significant as compared to the initial drug content when stored at 40°C for 90 days.

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