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International Journal of Pharmaceutics 206 (2000) 35–42

international journal of pharmaceutics

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Physicochemical studies of lidocaine–menthol binary systems for enhanced membrane transport

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Received 8 February 2000; received in revised form 20 June 2000; accepted 21 June 2000

Abstract

The melting properties of lidocaine and *l*-menthol binary systems were studied using differential scanning calorimetry (DSC). A eutectic mixture was obtained for the lidocaine:menthol ratio of 30:70 (w:w) with a eutectic point of 26°C. The binary melt systems formed within a range of 30:70–50:50 (w:w) remained as homogeneous oils at ambient temperature. The solubilities of pure lidocaine and lidocaine from the binary melt systems were determined with and without propylene glycol in pH 8.0 phosphate buffer. Lidocaine from the melt systems was less soluble in the buffers due to the partition of lidocaine between the oil and aqueous phases. The addition of propylene glycol to the buffer significantly increased both the solubility and heat of solubilization of lidocaine. The permeation rates of lidocaine from the binary melt systems across shed snake-skin were concentration dependent and significantly higher than those from the reference solutions.

Keywords: Lidocaine–menthol binary systems; Enhanced membrane transport; Melting properties

1. Introduction

The search for a safe and effective topical anesthetic preparation for minor dermal procedures such as venipuncture, intravenous cannulation, vaccination and circumcision has seen partial success with the introduction of a eutectic mixture of lidocaine and prilocaine (EMLA) in an emulsified topical cream (Russell and Doyle, 1997). Due to the low permeability through the stratum corneum and the necessity of high local drug concentration at the receptor site, topical anesthetic formulations are generally not effective in inducing local anesthesia through intact skin. The improved efficacy of EMLA, as compared to conventional topical formulations, can be attributed to the high drug concentration in its oil phase.

As shown by EMLA, emulsification of eutectic oils is a promising approach to improving transdermal delivery of therapeutic agents (Hallen et al., 1984). In traditional methods of preparing anesthetic creams, the active ingredients simply

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0378-5173/00/$ - see front matter
PII: S0378-5173(00)00505-6

are dissolved in inert pharmaceutical oils and then emulsified. Due to the limited solubility of drugs in these oils, such methods often result in relatively low concentrations. In comparison, the melting point depression method allows the oil phase to contain primarily the melted drug itself. Based on a prilocaine–menthol eutectic system, a topical anesthetic formulation in which 75% of the oil phase was the melted prilocaine was prepared (Nortier and Koks, 1995). With a melting point of 38°C, it was relatively easy to convert solid prilocaine into an oily phase at ambient temperature, with prilocaine as the major component. Terpenes such as menthol, thymol and cineole, depress the melting points of other compounds such as local anesthetics (Nortier and Koks, 1995; Stott et al., 1998). In this study, menthol was selected as the melting point depressing agent for lidocaine due to its safety and availability.

The objectives of the present study were to investigate, first, the physicochemical properties of a eutectic system formed by lidocaine and *l*-menthol; second, the permeation profiles of lidocaine in the melt systems across a model animal skin; and last, the method of achieving the highest possible lidocaine concentration in the oil phase at ambient temperature.

2. Experimental

2.1. Materials

The following chemicals and solvents were used as received: lidocaine and *l*-menthol (Sigma Chemical Co., St. Louis, MO), HPLC grade acetonitrile, disodium phosphate, phosphoric acid and propylene glycol (J.T. Baker Chemical Co., Phillipsburg, NJ). Distilled, deionized water was filtered using 0.45 μm membrane filter (Millipore Corporation, Bedford, MA).

2.2. Differential scanning calorimetry (DSC) of lidocaine–menthol binary systems

Lidocaine and *l*-menthol were mixed at various ratios between 5:95–90:10 (w:w) and melted at 70°C. The resulting homogeneous oils were kept at −20°C for 1 week to allow for complete solidification of the melt systems.

For the DSC studies (Perkin Elmer DSC7, Norwalk, CT), the solidified dispersions were ground into fine powders using a mortar and pestle. Approximately 15 mg of the ground sample was placed into an aluminum DSC sample pan for scanning. To avoid premature melting during sample preparation, all samples were prepared in a cold room just prior to DSC analysis (Betageri and Makarla, 1995; Doshi et al., 1997). Thermograms of the samples were obtained between 0 and 70°C against an empty reference pan at the heating rate of 1°C/min. The temperature–composition phase diagram of the binary melt systems was constructed according to a reported method (Hoelgaard and Moller, 1975).

2.3. Solubility of pure lidocaine in buffers

The solubility of pure lidocaine in phosphate buffer (PB) and in a mixed solvent containing PB and 40% (v:v) propylene glycol (PG) was determined at 15, 25, 32 and 37°C. An excess amount of crystalline lidocaine was dispersed into 5 ml of PB in a glass vial. The vials were shaken for 24 h in a water bath before removing the aqueous phase and filtering it through a 0.45 μm membrane filter for HPLC assay of lidocaine. The solubility data were plotted according to the Arrhenius equation to calculate the heat of solubility ($\Delta H_{\text{solubility}}$).

2.4. Solubility of lidocaine from melt systems in buffers

Mixtures consisting of lidocaine and *l*-menthol at the ratios of 30:70, 40:60, 50:50 and 60:40 (w:w) were heated slowly to 70°C to obtain the homogenous oil. After cooling to 25°C, 1ml of each melt system was carefully added to 5 ml of pH 8.0, 0.05 M PB in a glass vial. The vials containing the two immiscible phases were sealed and stored without disturbance at 25°C for 3 months. After this period, 0.5 ml of the aqueous phase was removed with a syringe and analyzed by HPLC for lidocaine.

The effects of temperature and PG on the solubility of lidocaine from the lidocaine:menthol melt system (50:50, w:w) in PB were studied. It was found that this melt system possessed relatively higher thermodynamic activity of lidocaine and was both physically and chemically stable. The samples ($n = 5$), prepared in PB as described above, were stored at 25, 35, 45 and 55°C. These temperatures were different from those used for the pure lidocaine because at 15°C in PB, crystals appeared in the 50:50 (w:w) lidocaine–menthol melt system, while PG caused the crystalline lidocaine to liquefy at 45°C. However, no phase change was observed for the samples tested at the temperature range used. After storage for 3 months at these temperatures, a small volume of the aqueous phase was removed and analyzed for lidocaine content using HPLC. Another set of samples were similarly prepared and tested with the exception that 40% (v:v) PG was added to PB.

2.5. Stability of lidocaine in melt systems

The oily melt system containing lidocaine and *l*-menthol (50:50, w:w) was stored at ambient temperature (25°C) for 2 years. A small amount of the sample was periodically removed and dissolved in the mobile phase. The lidocaine amount was quantitated by HPLC to determine recovery of lidocaine. The study was performed with five replications. Any crystal formation in the melt system was monitored using an optical microscope.

2.6. HPLC analysis of lidocaine

Lidocaine was analyzed on a reversed phase ODS C_{18} column (Phenomenex, Prodigy 5u, 4.5 × 150 mm, Torrance, CA) with UV detection (Varian 2550, Monrovia, CA) at 210 nm (Kang et al., 1999). The mobile phase was acetonitrile and pH5.9 PB (0.05 M) containing 20% (v:v) acetonitrile. The flow rate was set at 1.0 ml/min. Using phenacemide as the internal standard, weekly calibrations using the peak area ratios were obtained for a range of 20–1000 ng/ml of lidocaine.

2.7. Permeation studies of lidocaine through shed snake-skin

Over the 2-year storage at 25°C, the melt systems containing lidocaine and *l*-menthol within 30:70–50:50 (w:w) remained as homogeneous oils without crystal formation. The permeation rates of lidocaine from these oily mixtures were determined using shed snake-skin. A large piece of skin was placed in distilled water for 30 min to allow complete hydration. The skin was cut to the proper size and mounted on the Franz diffusion cells with the stratum corneum side contacting the donor phase. At the start of the study (0 h), 0.5 g of the test preparations was placed onto the skin surface and covered with parafilm (American National Can, Neenah, WI) at 25°C. The receiver phase of 0.05 M PB (pH 6.0) was maintained at 32 ± 0.5°C by circulating water from a thermostatted pump (Haake, Model F4391, Berlin, Germany) and continuously stirred at 300 rpm using a star head magnetic stirring bar. The effective diffusion area of the skin was 2.0 cm^2, and the volume of the receptor compartment was 6.0 cm^3. Each test was replicated with three samples. During the test, 0.2ml of the receptor phase was periodically removed using a microsyringe and immediately replaced with fresh receptor solution. The samples were analyzed by HPLC for lidocaine within 24 h. The steady state flux, J_{ss} (μg/cm^2 h) of lidocaine was calculated using Fick's first law: $J_{ss} = \Delta M / S\Delta t$, where S is the effective diffusion area (cm^2) and $\Delta M/\Delta t$ is the amount of drug passing through the membrane per unit time at steady state (μg/h). The permeation profiles of lidocaine from saturated aqueous solutions of lidocaine in PB and in 40% (v:v) PG in PB were determined as controls. ANOVA was performed to determine significant differences in J_{ss}.

3. Results and discussion

3.1. DSC studies of lidocaine–menthol binary systems

The DSC thermograms of lidocaine and *l*-menthol are shown in Fig. 1. The major endotherms

at 68 and 41°C represent the melting points of lidocaine and *l*-menthol, respectively. The thermograms of the various mixtures clearly indicated that these compounds formed a binary eutectic system. The mixture containing lidocaine and *l*-menthol at a ratio of 30:70 (w:w) showed a single sharp peak at 26°C, representing the eutectic melting point of this composition. The mixtures on either side of the eutectic composition typically produced double endothermic peaks. The first peak consistently appeared near 26°C, where the initial melting of the mixtures occurred. This point is also known as the temperature of solidus on a phase diagram. The second peak (temperature of liquidus) was generally wider, indicating that complete melting took place over a temperature range. The complete phase diagram is shown in Fig. 2.

3.2. Stability of lidocaine in melt systems

The melting point of the eutectic mixture was 26°C, however, microscopic examination showed that the melt system containing 30–50% (w:w) of lidocaine remained a homogeneous oil at 25°C for at least 2 years. After the melt system containing lidocaine and *l*-menthol (50:50, w:w) was stored at 25°C for 2 years, lidocaine was completely

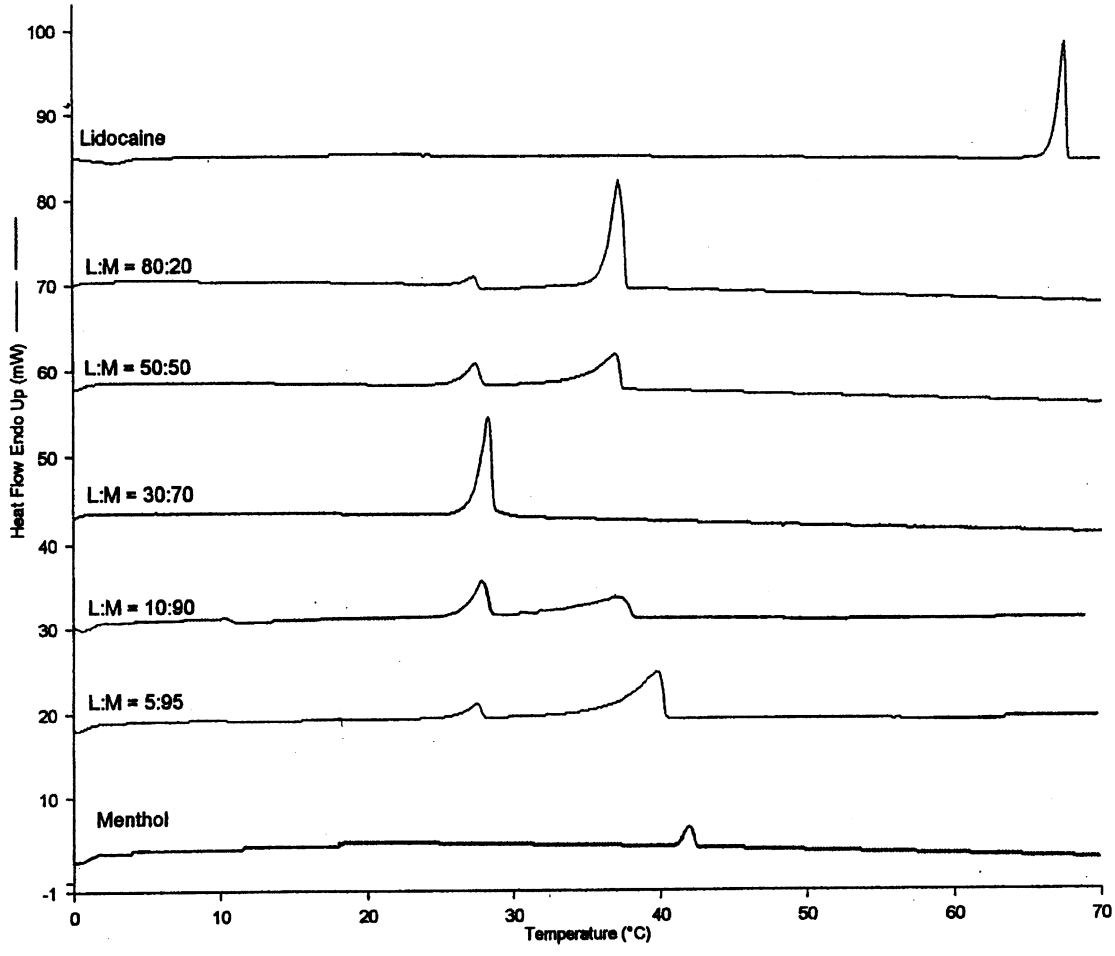


Fig. 1. DSC thermograms of lidocaine, *l*-menthol, and their binary mixtures. Ratios by weight.

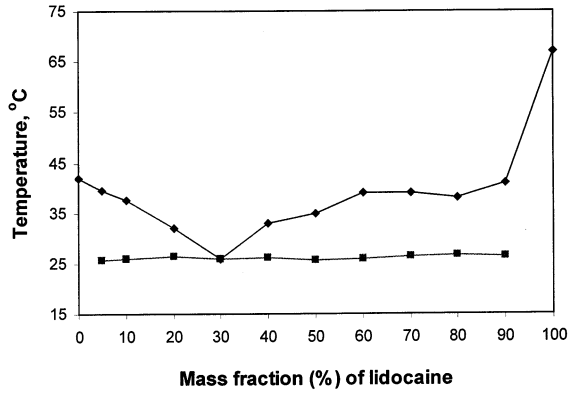


Fig. 2. The temperature/composition phase diagram of lidocaine–menthol binary system determined by DSC.

recovered ($101.2 \pm 2.4\%$, $n = 5$), thus indicating that lidocaine was stable both physically and chemically for at least 2 years. Such a system would be suitable as the oil phase for the preparation of topical lidocaine creams.

3.3. Solubility of pure lidocaine in buffers

The solubility of pure lidocaine in PB decreased with increasing temperature (Fig. 3), which agrees with an earlier report (Nakano, 1979). This unusual temperature effect on the solubility of lidocaine and other local anesthetics was possibly attributed to the decreased pK_a values of the compounds at higher temperatures. It has been found that the pK_a of lidocaine changed from 8.2 at 15°C to 7.7 at 35°C (Nakano, 1979). On the other hand, the presence of PG in the buffer not only significantly increased the solubility of lidocaine, but also reversed the temperature effect on solubility. $\Delta H_{solubility}$ of pure lidocaine in the absence and presence of PG in PB was -7.3 and 3.1 kJ/mol, respectively. The large change in ΔH indicated the physicochemical interaction between lidocaine and PG, although its mechanism is yet to be elucidated.

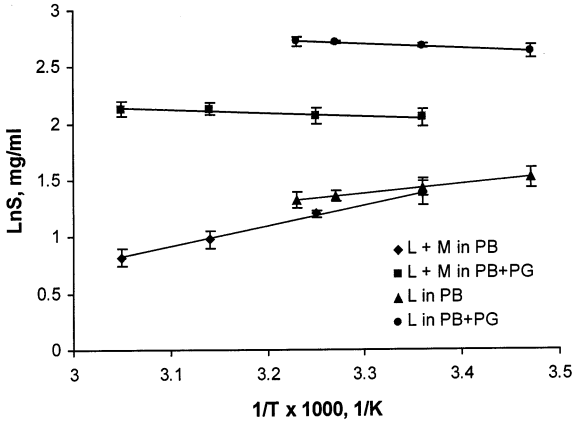


Fig. 3. Effects of temperature, propylene glycol and *l*-menthol on the solubility of lidocaine in PB at pH 8.0.

3.4. Solubility of lidocaine from lidocaine–menthol melt systems

The partition coefficient (K_m) of lidocaine was calculated from the ratio of the concentrations of lidocaine in the melt systems to those in the buffers after equilibration. At 25°C, with and without PG in the aqueous phase, the K_m values calculated from Table 1 were 62.9 ± 4.3 ($n = 12$) and 127.0 ± 6.7 ($n = 12$), respectively. The higher thermodynamic activity of lidocaine in the more concentrated melt systems may allow more lidocaine to partition into the aqueous phase. This could also increase the oil to skin partitioning of the drug, resulting in a higher permeation rate through the skin.

In PB, similar temperature dependence was observed to that of pure lidocaine (Fig. 3). These results were also in agreement with the solubility of lidocaine from the eutectic mixture of lidocaine and prilocaine in 1 mM NaOH (Brodin and Nyqvist-mayer, 1984). The presence of PG significantly increased the solubility of lidocaine and reversed the temperature effect. The $\Delta H_{solubility}$ of lidocaine from the melt system in the absence and presence of PG was -15.2 and 2.4 kJ/mol, respectively. The effect of PG on the solubility of lidocaine from the melt system was similar to its effect on the solubility of pure lidocaine. The use of *l*-menthol as a melting point depressing agent and PG as a solubilizing agent significantly changed both the solubility and $\Delta H_{solubility}$ values of lidocaine in PB, suggesting a molecular interaction between lidocaine and the two additives.

Table 1
Solubility (mg/ml) of pure lidocaine and lidocaine from lidocaine:menthol (L:M) mixtures in pH 8.0 PB and PB+PG at 25°C (Mean ± S.D., $n = 3$)

	L:M ratio (w:w)					
	30:70	40:60	50:50	55:45	60:40	100:0
PB	2.27 ± 0.12	3.02 ± 0.06	3.99 ± 0.11	4.65 ± 0.15	4.70 ± 0.02[a]	5.06 ± 0.06[a]
PB+PG	4.32 ± 0.11	6.69 ± 0.41	7.77 ± 0.32	8.86 ± 0.50	10.24 ± 0.72	14.68 ± 0.21[a]

[a] Crystals observed.

3.5. Permeation studies

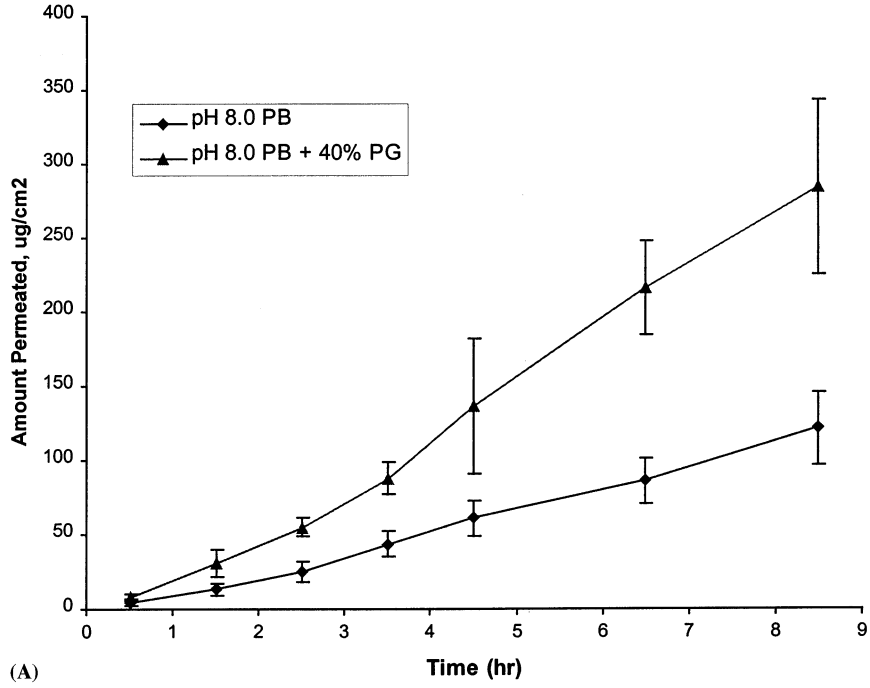
Shed-snake skin has been used as a model membrane for in vitro drug permeability studies (Higuchi and Konishi, 1987; Itoh and Rytting, 1990). Advantages of shed snake-skin include its biochemical similarity to human stratum corneum, the availability of multiple samples from the whole shed skin, easy handling, and relatively small sample variation.

Using dermatomed human skin, the permeation rate of the unionized form of lidocaine ($pK_a = 7.8$) was 50-fold higher than the ionized form (Kushla and Zatz, 1991). Therefore, the pH of the two reference preparations was adjusted to 8.0, where the majority of lidociane was unionized. PG was an effective enhancer for transdermal permeation of various therapeutic agents (Sarpotdar and Zatz, 1987; Megrab and Barry, 1995) and had the most significant enhancing effect for lidocaine at a concentration of 40% in water (Sarpotdar and Zatz, 1986). In the present study the addition of 40% (v:v) PG to PB significantly ($P < 0.01$) enhanced the permeation of lidocaine across shed snake skin as compared to PB. The J_{ss} of lidocaine in PB and in PB + PG was 15.4 ± 3.5 and 35.7 ± 10.7 μg/cm^2 h, respectively. The thermodynamic activity of lidocaine, which represents the concentration over the solubility in the vehicle, was the same in both saturated solutions used. The difference in J_{ss} thus indicated the enhancing effect of PG which may be due to its inhibitory action on the hydrogen bonding of drugs to alphakeratin, reducing barrier resistance to drug permeation (Potts and Guy, 1995).

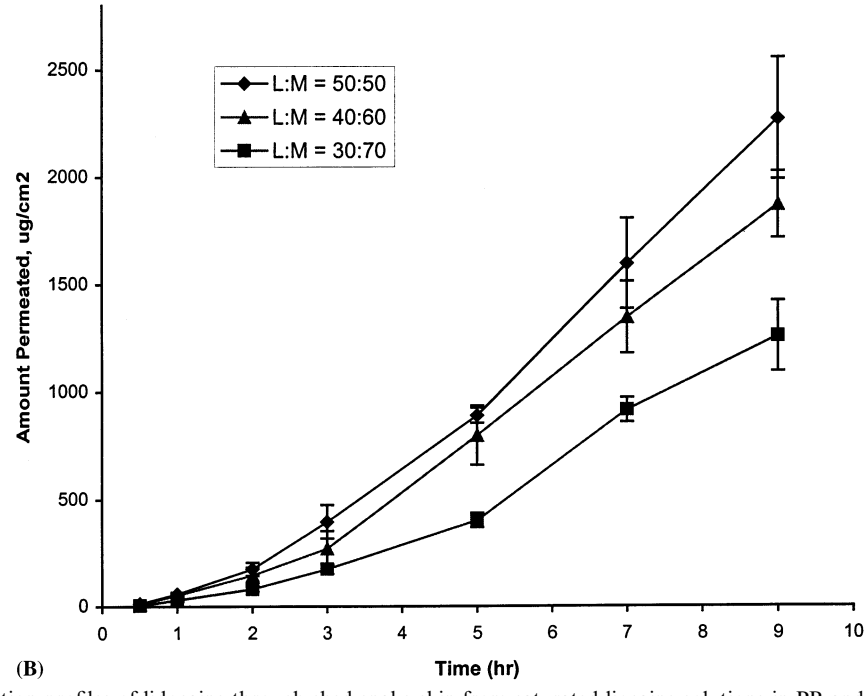
Significantly higher permeation rates of lidocaine from the melt systems were observed as compared to the saturated solutions of lidocaine both in the presence and absence of PG. The J_{ss} values were 187.8 ± 21.8, 268.6 ± 35.5 and 316.3 ± 50.1 μg/cm^2 h for the melt systems containing lidocaine and *l*-menthol at the ratios of 30:70, 40:60 and 50:50 (w:w), respectively (Fig. 4B), clearly showing a concentration dependency. It is known that the higher the drug concentration in the melt systems used, the higher the thermodynamic activity, and therefore, the higher the driving force for drug penetration. Assuming the same vehicle effects on the skin, the saturated melt systems of lidocaine which possess the maximum thermodynamic activity are expected to produce the highest permeation rate; however, crystallization of lidocaine from the saturated systems during storage may occur.

4. Summary

The physicochemical properties of lidocaine in the presence of l-menthol and propylene glycol were investigated. Menthol significantly depressed the melting point of lidocaine forming eutectic melt systems for a wide range of compositions. A melt system containing as much as 50% (w:w) lidocaine was obtained, and this concentration is much higher than the solubility of the drug in most pharmaceutical oils. The permeation rates of lidocaine from these melt systems through shed snake-skin were significantly higher than those from the saturated lidocaine solutions in the pH 8.0 PB. The addition of PG (40%, v:v) to the buffer significantly increased the solubility of lidocaine and enhanced the permeation rate of lidocaine through the skin. Emulsification of the



(A)



(B)

Fig. 4. (A) Permeation profiles of lidocaine through shed snake-skin from saturated liocaine solutions in PB and PB + 40% PG. (B) Permeation profiles of lidocaine through shed snake-skin from lidocaine–menthol binary systems.

eutectic melt systems could produce high thermodynamic activity of the drug in the oil phase and the high driving force for transdermal absorption of lidocaine.

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