Effect of alcohols and enhancers on permeation enhancement of ketorolac

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Areservoir-type transdermal patch for the delivery of ketorolac was studied. The low permeability of the skin is the rate-limiting step for delivery of most of the drugs. Studies were carried out to investigate the effect of pH, alcohols, and chemical permeation enhancers on the *in vitro* permeation of ketorolac. The reservoir core of the transdermal patch was filled with the hydrogel of a nonionic polymer, methocel $K_{15}M$ (hydroxyl propyl methylcellulose, HPMC) formulated at an optimized pH of 5.4. Enhanced *in vitro* permeation was achieved after the incorporation of the alcohols. Higher enhancement was produced by short-chain alcohols like ethanol and isopropyl alcohol (IPA). Propylene glycol (PG) along with other alcohols, viz. n-propanol, n-butanol, and n-pentanol, lagged behind. An exponential rise in permeation was observed in flux with an increase in the concentration of IPA. At 25% w/w IPA concentration, the observed ketorolac flux was 18.04 μg/cm^2/h. Terpene containing eucalyptus oil was studied to determine its permeation enhancement capability. The increase in the concentration of eucalyptus oil enhanced the drug permeation and a maximum flux of 66.38 and 90.56 μg/cm^2/h was achieved at 10 and 15% w/w concentrations. The anti-inflammatory potential of the transdermal formulation was evaluated on a carrageenan-induced paw edema model, with 41.67% inhibition at 6 h. The skin irritation potential was evaluated by the Drazie test and the formulations prepared were found to be safe. The reservoir-type transdermal patch for the delivery of ketorolac appeared to be feasible for delivering ketorolac across the skin.

Key words: *Enhancer, eucalyptus oil, isopropyl alcohol, ketorolac, permeation, transdermal*

INTRODUCTION

Ketorolac (administered as tromethamine salt), a prostaglandin synthetase inhibitor is a nonsteroidal anti-inflammatory drug with potent analgesic and moderate anti-inflammatory activities.[1] Ketorolac has been studied clinically and its efficacy and safety as an analgesic in postoperative pain and cancer have been established. Administered as oral and injectable formulations, it has shown a high analgesic potency almost equivalent to that of morphine.[2] Unlike narcotic analgesics, ketorolac does not alter the gastric motility or the hemodynamic variables or adversely affect the respiration, nor is it associated with abuse or addiction potential as in the case of narcotic analgesics; therefore, ketorolac is a relatively more favorable therapeutic agent for the management of moderate to severe pain.[3]

Ketorolac thus has substantial clinical potential and

developing an alternative dosage form that is easy to administer, is painless, noninvasive, easy to comply, and avoids first-pass metabolism is worthwhile. The transdermal route encompasses all the above advantages.

Despite these advantages, only a limited number of drugs can be administered percutaneously due to low skin permeability of most drugs through the skin. The penetration through the stratum corneum is the rate-limiting step for the delivery of most of the drugs. To overcome this problem, vehicles,[4] penetration enhancers,[5] ultrasound,[6] and electrotransport[7] facilitated transdermal systems have been attempted in the development of a transdermal delivery system (TDS) of ketorolac. The prodrug approach has also been investigated for enhanced dermal delivery.[8,9] However, the most widely used technique involves use of chemical penetration enhancers or solvents that modify the thermodynamic activity.

In the present study, we investigated the effects of pH, alcohols, and chemical penetration enhancers on the *in vitro* permeation of ketorolac from hydrogel gel formulation across rat abdominal skin to examine the

feasibility of developing a transdermal system.

MATERIALS AND METHODS

Ketorolac (tromethamine salt) was obtained as a gift sample from Ranbaxy Laboratories, Devas, India. HPMC (Methocel® K$_{15}$M) was gifted by Colorcon Asia Pvt. Ltd., Goa, India, n-propanol, isopropyl alcohol, n-butanol, n-pentanol, and PG and eucalyptus oil were purchased from Central Drug House, New Delhi, India. Ethanol was procured from E. Merck (India) Ltd., Mumbai, India. Other chemicals and reagents used were of analytical grade. The experimental protocol was approved by the institutional animal ethical committee.

Preparation of the ketorolac gel system and fabrication of the reservoir-type patch

The ketorolac gel system was prepared in phosphate-buffered saline (PBS) solution by dissolving ketorolac and adding methocel K$_{15}$M (HPMC) with continuous stirring so as to uniformly disperse the polymer. Permeation enhancers were mixed with the vehicle before adding the polymer. The gel was kept overnight at an ambient temperature in a tightly closed container to allow uniform gelling (cold dispersion method).

Transdermal patches (reservoir type) of ketorolac were fabricated by filling the ketorolac gel preparation (0.25 g/cm^2) within a shallow compartment made of a hollow ring-shaped device and drug impermeable backing membrane (laminated aluminum foil). A microporous adhesive tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side [Figure 1].

In vitro skin permeation studies of ketorolac through the excised rat dorsal skin

The dorsal abdominal skin of Sprague–Dawley rats was clamped between the donor and the receptor chamber, with
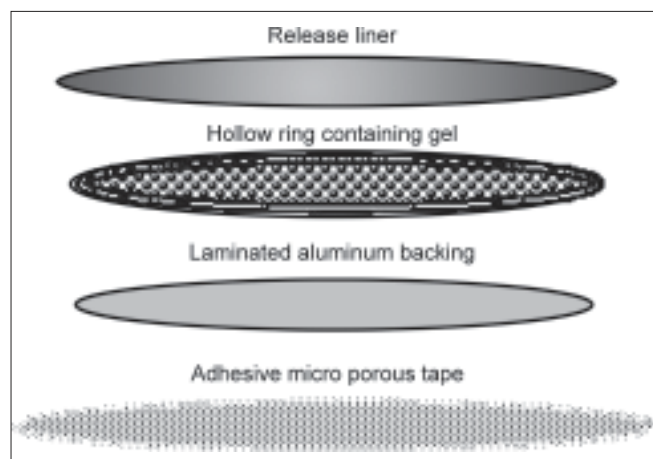
Figure 1: Design of the reservoir-type patch for transdermal administration of ketorolac

the stratum corneum surface facing the donor compartment of the modified Keshary Chien diffusion cell after removing the hair with a clipper. The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 11 ml. The receptor chamber was filled with freshly prepared PBS solution of pH 7.4. The diffusion bath was maintained at 37 ± 1°C and the solution in the receptor chamber was stirred continuously with the help of a magnetic bead. Ketorolac gel (0.5 g) was gently placed in the donor chamber and was spread evenly on the rat abdominal skin. The donor compartment was covered with Parafilm M® to prevent evaporative loss. One milliliter of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of fresh PBS solution pH 7.4. The drug concentration was determined UV spectrophotometrically.

Analysis of *in vitro* drug release study samples

Samples withdrawn were filtered through Whatmann filter paper no. 42 and diluted whenever necessary. The samples were analyzed spectrophotometrically at λ_{max} 324 nm. The concentration of the drug was determined from the regression equation generated from the calibration curve, $y = 0.0285x + 0.0012$, $R^2 = 0.999$.

Effect of pH on *in vitro* skin permeation of ketorolac through the rat skin

The ketorolac gel system was prepared, consisting of 2% w/w ketorolac and 2% w/w methocel K$_{15}$M swelled in PBS solution of defined pH by the cold dispersion method. Three different PBS solutions of pH 5.4, 6.4, and 7.4 were employed for preparing the formulation.

Effect of permeation enhancers on the *in vitro* skin permeation of ketorolac through the rat skin

The ketorolac gel system was prepared, which consisted of 2% w/w ketorolac and 2% w/w methocel K$_{15}$M in PBS solution at an optimized pH of 5.4 by the cold dispersion method. Various alcohols, viz. ethanol, n-propanol, IPA, n-pentanol, n-butanol, and PG, were evaluated at a 10% w/w concentration. The selected alcohol was further optimized at 10, 15, 20, and 25% w/w concentration. The enhancement potential of eucalyptus oil (5, 7.5, 10, and 15% w/w) was further studied at the optimized alcohol concentration in order to determine the most preferred composition for the transdermal gel system.

Skin irritation studies

The transdermal patch of ketorolac gel was applied onto the dorsal skin of the Wistar rats (220–250 g), which was shaved 24 h before the study. The site of application was occluded with gauze and covered with a nonsensitizing microporous adhesive tape. After 24 h, the gel was removed and the score of erythema was determined by the Drazie test[10] as follows: 0 - no erythema; 1 - mild erythema; 2 - moderate erythema; 3 - severe eryhtema.

Anti-inflammatory studies

The anti-inflammatory efficacy was evaluated by carrageenan-induced paw inflammation in the Wistar rat (approx. 220–250 g). The rats were assigned to treatment groups so that each group was weight balanced. Ketorolac was administered via the oral (2 mg/kg) and transdermal routes 1 h before carrageenan injection. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm^2). The rats received an intraplantar injection of 0.05 ml of 0.5% w/v carrageenan suspension into the left hind paw subcutaneously by inserting the needle into the central part of the paw. The paw volume was measured and compared with that found in animals treated with carrageenan alone. The right hind paw, which served as the control, was treated with physiological saline solution without carrageenan.[11] The inflammatory response was determined by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) at 0, 2, 4, 6, 8, and 10 h after the carrageenan injection.

The area under the curve (AUC) was determined by the trapezoidal method.

Data analysis

The *in vitro* skin flux was determined by Fick's law of diffusion, considering the transport of drugs across the skin barrier as a process of passive diffusion. J_{SS}, the skin flux (μg/cm^2/h), was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus the time plot. The lag time (T_{lag} h) was determined by extrapolating the linear portion of the curve to the abscissa. Kp, the permeability coefficient (cm h^{-1}), was determined from the equation[12]:

$$Kp = J_{SS}/C_O$$

C_O is the donor phase concentration.

The enhancement ratio (ER) was calculated from the following equation:

$$ER = Jss\ of\ test\ gel\ /\ Jss\ of\ control\ gel$$

Statistical analysis

The results were analyzed by Student's *t*-test (paired, two tailed) using Statistica for Windows (Version 5.0) from StatSoft Inc., USA. The results were evaluated at the probability level of *P* < 0.05.

RESULTS AND DISCUSSION

Effect of pH on *in vitro* skin permeation of ketorolac through the rat skin

The effect of pH on gel containing 2% w/w of ketorolac and 2% w/w methocel K$_{15}$M on the permeation rate of ketorolac was studied at pH 5.4, 6.4, and 7.4. From the results, it was concluded that higher permeation of ketorolac occurred at lower pH [Figure 2]. Flux attained for the gel system prepared in pH 5.4, 6.4, and 7.4 was 1.24. 0.99, and 0.79 μg/cm^2/h and the observed T_{lag} was 7.70, 8.99, and 9.60 h, respectively. The permeation of a molecule depends primarily on its solubility and its state (ionized/unionized). Because the drug was present at a subsaturation concentration and was completely soluble at all pH, it was probably the degree of the unionized to ionized fraction that affected the permeation rate across the skin. Thus, PBS solution of pH 5.4 was selected for further studies.

Effect of permeation enhancers on *in vitro* skin permeation of ketorolac through the rat skin

Various alcohols, viz. ethanol, n-propanol, IPA, n-pentanol, n-butanol, and PG, at 10% w/w were evaluated for their ability to enhance permeation of ketorolac. Ketorolac gels were prepared in a PBS solution of pH 5.4. ER was calculated by comparing the steady state flux of ketorolac from the alcohol-containing gel system and that of the gel system at an optimized pH. The release profile is presented in Figure 3. From the study, it was perceived that smaller chain alcohols were more efficient in enhancing the dermal permeation. The shift of the hydroxyl group away from the edge side to the center considerably increased the permeation. Highest permeation coefficients were observed for IPA and ethanol. The steady state flux achieved was 4.67 and 3.91 μg/cm^2/h, respectively, but a reduced lag time of 3.26 h was noted for ethanol in comparison with 3.55 h for IPA. Other alcohols, viz. n-propanol, n-butanol, and n-pentanol, showed a flux of 3.53, 3.18, and 2.71 μg/cm^2/h, respectively. Despite the fact that ethanol demonstrated an enhanced diffusivity, higher Q_{24} (amount of drug permeated across the skin at the end of 24 h) was achieved for IPA. Alcohol as a solvent may extract some of the lipid fraction from within the stratum corneum, thereby enhancing permeation. Various findings also reveal that longer chain alcohols possess a higher enhancement potential toward lipophilic drugs[13] and poor enhancement toward hydrophilic drugs.[14] Reports concerning the efficacy of PG as a permeation enhancer are mixed. Evidence suggests at best only a very mild enhancement effect for molecules such as estradiol and 5-fluorouracil.[15] In case of ketorolac, a lag time of 5.68 h is observed with PG as the alcohol component of the gel system and the flux attained was 5.68 μg/cm^2/h.

Increasing the concentration of IPA further enhanced the permeation of ketorolac [Figure 4]. An ER of 14.55 was attained at 25% w/w of IPA concentration. There was an exponential ($1.9167e^{0.0895x}$, $R^2 = 0.9996$) rise in the ketorolac flux.

The effect of eucalyptus oil on the permeation of ketorolac from the transdermal gel system across the rat abdominal skin was investigated. Gels were prepared using ketorolac (2% w/w), methocel K$_{15}$M (2% w/w), IPA (25% w/w), and eucalyptus oil at varying concentrations (5, 7.5, 10, and 15% w/w) in PBS solution of pH 5.4. The effectiveness of the
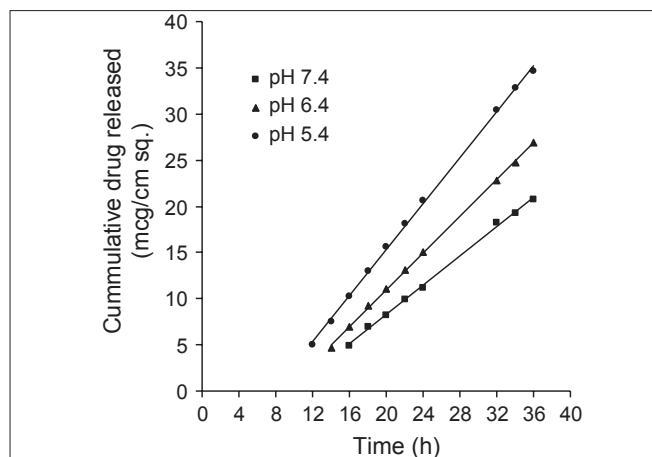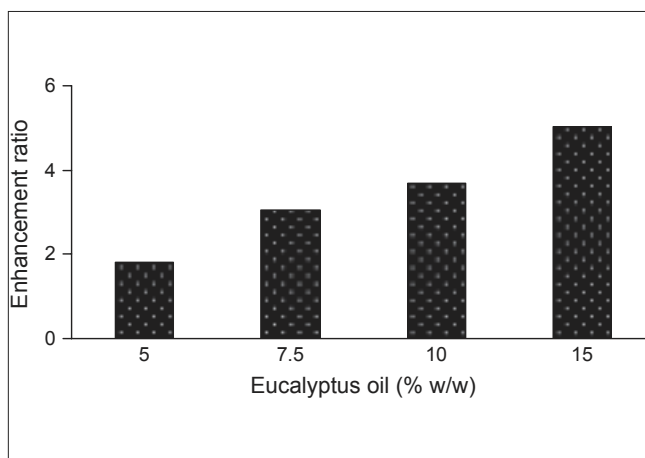
Figure 2: *In vitro* permeation profile of ketorolac through the rat abdominal skin from the gel system containing ketorolac (2% w/w) and methocel $K_{15}M$ (2% w/w) and formulated using phosphate-buffered saline solutions of different pH
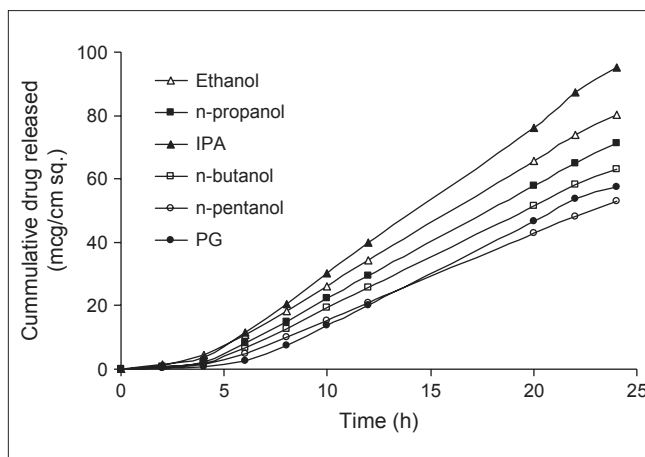
Figure 3: *In vitro* permeation profile of ketorolac through the rat abdominal skin from the gel system containing ketorolac (2% w/w) and methocel $K_{15}M$ (2% w/w) and different alcohols at a 5% w/w concentration formulated using phosphate-buffered saline solution of pH 5.4
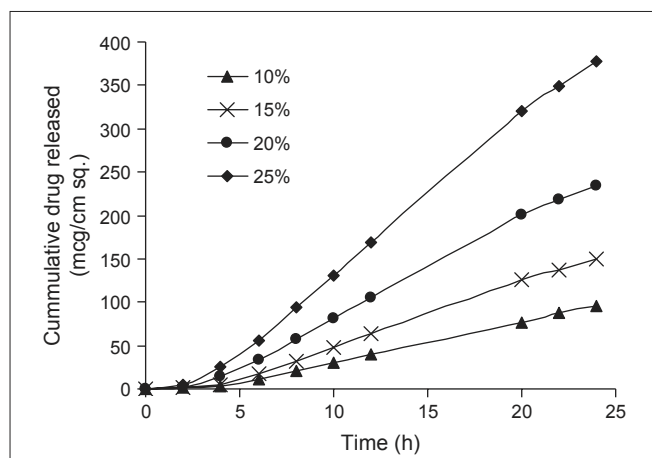
Figure 4: *In vitro* permeation profile of ketorolac across the rat abdominal skin from the gel system containing ketorolac (2% w/w), methocel $K_{15}M$ (2% w/w), and isopropyl alcohol at varying concentrations formulated in phosphate-buffered saline solution of pH 5.4

Figure 5: Enhancement ratio of ketorolac across the rat abdominal skin from the gel system containing ketorolac (2% w/w), methocel $K_{15}M$ (2% w/w), isopropyl alcohol (25% w/w), and eucalyptus oil at varying concentrations formulated in phosphate-buffered saline solution of pH 5.4

of the permeation enhancement of ketorolac, a hydrophilic drug, also support the above observations toward which oil containing oxygen-containing terpenes were effective. Eucalyptus oil at 5, 7.5, 10, and 15% w/w concentration produced a flux of 32.47, 54.79, 66.38, and 90.56 $\mu g/cm^2/h$, i.e. an enhancement of 1.80, 3.04, 3.68, and 5.02 times and T_{lag} of 1.34, 1.14, 1.08, and 1.11 h, respectively. However, the concentration of 15% w/w was not considered due to skin irritation potential. Figure 5 shows the enhancement influx of ketorolac across the rat skin attained on increasing the concentration of eucalyptus oil.

These agents probably modify the solvent nature of the stratum corneum, improving drug partitioning into the tissue. Terpenes permeate the skin well.[18] With loss of terpenes, which are generally good solvents, from a formulation, there could be an alteration of the thermodynamic activity of the

enhancer was determined by comparing the steady state flux of ketorolac from the chemical enhancer containing gel system and that of the gel system at an optimized IPA concentration.

Essential oils like eucalyptus oil are reported to be effective penetration enhancers for 5-flouorouracil traversing in to the human skin *in vivo* with a maximum ER of 34-fold.[16] The principal terpene element within eucalyptus oil is 1, 8-cineole, a cyclic ether and proven enhancer, but its efficacy as an enhancer is mixed. Eucalyptus oil could not enhance the permeation of a lipophilic drug like estradiol. Similar results were also reported for the permeation of indomethacin, a lipophilic molecule. The study revealed that oxygen-containing terpenes (carvone, 1, 8-cineole) were ineffective[17] whereas hydrocarbon terpenes, especially limonene, were effective toward lipophilic drugs. The results
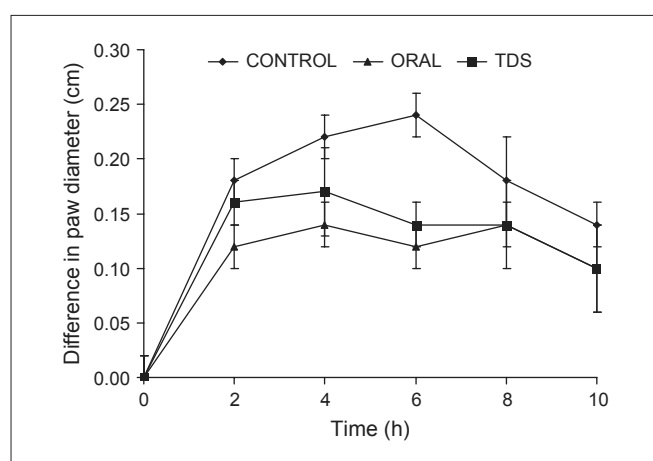
permeant. Terpenes may also modify drug diffusivity through the membrane and bring about a reduction of the lag time for permeation, indicating an increase in the diffusivity of the drug through the membrane following terpene treatment. X-ray diffraction studies have also indicated that 1, 8-cineole disrupts the stratum corneum bilayer lipids.[19] The ketorolac gel system consisting of ketorolac (2% w/w), methocel $K_{15}M$, (2% w/w), IPA (25% w/w), and eucalyptus oil (10% w/w) in PBS solution of pH 5.4 was selected as the optimized component composition of the gel system for transdermal delivery of ketorolac.

Skin irritation studies
The results of the skin irritation studies based on the visual observation score suggest that the formulations were safe to be applied on the skin. The scores for transdermal formulations containing eucalyptus was between 0 and 2. Eucalyptus oil at 15% w/w showed a slight increase in erythema.

Anti-inflammatory studies
The formulation showed a prominent increase in activity in the carrageenan-induced paw inflammation model. Figure 6 represents the anti-inflammatory activity after oral administration and after application of the transdermal formulation. Ketorolac transdermal patch formulation demonstrated a significant anti-inflammatory potential as compared with the control ($P < 0.05$). The anti-inflammatory potential was measured in terms of the AUC of the graph plotted between the difference in paw diameter and the time. Compared with the % AUC for the untreated paw, which was taken as 100%, oral administration showed swelling of 64.04% while TDS formulation demonstrated 74.16% swelling. Maximum percentage inhibition was observed at

6 h for oral and transdermal application of 50 and 41.67%. The probable reason may be that transdermal delivery is primarily through the paracellular pathway, i.e. around the cells, than through the elastin glue. The glue-like compound, elastin is composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e. the epidermis, including, e.g. stratum corneum, lucidum, granulosum, spinosus) must be dissolved and/or disrupted in order for the drug, dissolved in a solvent, to be able to transverse through the viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached. This is achieved only when the permeation enhancer is able to disrupt the upper stratum corneum layer of the skin thus enhancing the permeation rate. The reservoir-type transdermal patch consisting of ketorolac gel thus appears promising in delivering the drug across the skin.

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Figure 6: Anti-inflammatory activity of ketorolac on paw edema induced with carrageenan injection (0.05 ml of 0.5% w/w) in rats (control) and after oral administration of ketorolac solution (2 mg/kg) (oral) and on application of the transdermal patch of ketorolac (TDS). The reservoir transdermal patch contained ketorolac (2% w/w), methocel $K_{15}M$ (2% w/w), isopropyl alcohol (25% w/w), and eucalyptus oil (10% w/w) formulated using phosphate-buffered saline solution of pH 5.4

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