Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 260 (2003) 93–103
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Investigation into the potential of iontophoresis facilitated delivery of ketorolac

Sandip B. Tiwari*, N. Udupa

Department of Pharmaceutics, College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal 576119, Karnataka, India

Received 9 October 2002; received in revised form 24 March 2003; accepted 13 April 2003

Abstract

The potential for iontophoresis facilitated transdermal transport of ketorolac was investigated using rat skin. Studies of electrical, physicochemical and device-related factors acting on the permeation kinetics of in vitro iontophoresis were performed. Iontophoresis increased the transdermal permeation flux of ketorolac as compared to the diffusion. Increase in applied current density or decrease in ionic strength of the donor solution enhanced the flux of the drug. Use of either platinum or silver/silver chloride electrodes resulted in similar enhancement of drug flux. Continuous current was more potent than pulsed current in promoting ketorolac transdermal permeation. Increasing the frequency or on:off ratio of pulse current induced an enhancement of the flux through the skin. An increase in donor drug loading dose or increasing the duration of current application resulted in enhancement of the drug flux. Pretreatment of the skin with D-limonene in ethanol or D-limonene in ethanol + ultrasound significantly enhanced the iontophoretic flux of the drug in comparison to passive flux with or without pretreatment. Trimodality treatment comprising of pretreatment with D-limonene in ethanol + ultrasound in combination followed by iontophoresis was found to be most potent for enhancing the rate of permeation of ketorolac.

Keywords: Ketorolac; Transdermal drug delivery; Iontophoresis; Penetration enhancer; Limonene; Ethanol; Ultrasound

1. Introduction

Ketorolac tromethamine is a non-steroidal agent with potent analgesic and moderate anti-inflammatory activities. The structure of ketorolac is shown in Fig. 1. Unlike narcotic analgesics, ketorolac does not pose potential addiction problems and respiratory depression, therefore it is a relatively more favorable therapeutic agent for the management of moderate to severe pain. Ketorolac (as the tromethamine salt) is commercially available as a racemic mixture. It is currently administered orally, intramuscularly or intravenously for short-term management of moderate to severe pain, including postoperative and postpartum pain and visceral pain associated with cancer (Buckley and Brogden, 1990; Litvak and McEvoy, 1990). The drug is administered via the oral route as a conventional tablet (10 mg q.i.d.) for the management of mild to moderate pain. Oral bioavailability of ketorolac tromethamine is reported to be 90% with a very low first pass metabolism. However, the drug is reported

* Corresponding author. Present address: Department of Novel Drug Delivery Systems, Zydus Research Centre, Sarkhej Bavala National Highway No. 8A, Moraiya, Ahmedabad 382213, Gujarat, India. Tel.: +91-79-3750-802/803/804/805x308; fax: +91-79-3750-606.
E-mail address: sandiptiwari@zyduscadila.com (S.B. Tiwari).

0378-5173/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00249-7

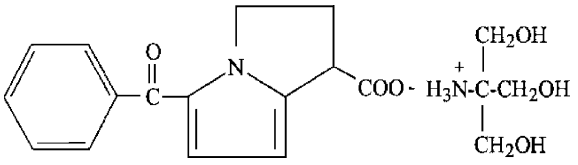

Fig. 1. Structure of ketorolac tromethamine (molecular weight = 364, $pK_a = 3.54$).

to cause severe gastrointestinal side effects (gastrointestinal bleeding, perforation and peptic ulceration) (Litvak and McEvoy, 1990). Therefore, parenteral administration of ketorolac is the preferred route of administration for moderate to severe pain management.

Regardless of the route of administration, the biological half-life of the ketorolac ranges from 4 to 6 h. Because of such a short half-life, frequent dosing is required to alleviate pain. To avoid an invasive drug delivery (i.e. intramuscular injection) and to eliminate frequent dosing regimens, there is a need for an alternative non-invasive mode of delivery of ketorolac tromethamine. Transdermal delivery of ketorolac appears to be an attractive route of administration as a non-invasive mode of delivery and to maintain the drug blood levels for an extended period of time. Transdermal delivery of ketorolac has been attempted using chemical enhancers, selection of proper vehicle and pro-drug approach by a group of researchers at Syntex laboratories, Palo Alto, USA (Roy and Manoukian, 1995; Roy et al., 1995). The peak plasma level of ketorolac following application of liquid reservoir transdermal system was comparable to that of a 10 mg oral dose administered every 6 h, while the desired plasma level of 500 ng/ml was maintained for about 16 h following a single patch application (Roy and Manoukian, 1995). The system allows significant plasma levels to be attained but the concerns regarding the lag time and the lack of opportunity to modulate the rate of the release are important disadvantages of this system.

Iontophoresis is a process by which the transport of ions into or through skin is increased by the application of an external electrical field across the skin (Riviere and Heit, 1997; Phipps et al., 2002). It could be used to improve the transdermal administration of ketorolac since the drug is weak acid ($pK_a = 3.54$) (Litvak and McEvoy, 1990), the major fraction of which is anionic at physiological pH.

The aim of the present study was to investigate the influence of electrical factors (current density, current profile and current application duration), physicochemical factors (donor drug concentration, ionic strength of the donor solution and pretreatment with chemical/physical enhancers) and device-related factor (electrode material) on the iontophoretic transport of ketorolac through rat skin.

2. Materials and methods

Ketorolac tromethamine was obtained as gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad, India. Citric acid, di-sodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride and potassium chloride were purchased from S.D. Fine-Chem Ltd., Mumbai, India. D-Limonene was purchased from Merck, Schuchrdt, Germany. All solutions were made using distilled water which had been passed through a Milli-Q® water purification system (Millipore, USA). The resulting water had a pH of 5–6 and a resistance of 18 MΩ/cm^2. Iontophoresis instrument was custom designed.

2.1. Skin and preparation of skin

Full thickness skin excised from abdominal portion of male Wistar rats (200–250 g) was used in all the experiments. Before excision, the abdominal part of the skin was shaved with electrical shaver and a uniform circle was made on the abdomen making the precise section to be positioned between the half-cells. This was done to avoid the differences of stretching the skin during removal (Durrheim et al., 1980). Prior to the actual experiments, stabilization of the skin samples was done by the method as described earlier (Varghese and Khar, 1996) using isotonic Sorensen phosphate buffer (pH 7.4) as a receptor solution.

2.2. Diffusion cell

All diffusion studies were conducted using a two-compartment horizontal glass diffusion cell. The excised skin was mounted between the two half-cells with stratum corneum facing the donor compartment. The volume of each compartment was 12 ml and the surface area of the membrane exposed to the two

solutions was 1.32 cm^2. The distance between each electrode and the membrane was 2.5 cm. The contents of donor and receptor compartments were stirred by using specially designed mini magnetic stirrers (Tiwari and Udupa, 1999).

In one experiment (effect of pretreatment with chemical/physical enhancers), two-compartment vertical Franz-type diffusion cell was used. The volume of donor and receptor compartment was 12 and 25 ml, respectively. The surface area of the membrane exposed to the two solutions was 1.77 cm^2.

2.3. Electrodes

All transport studies were carried out using a pair of platinum wires (99.99% purity, 0.5 mm diameter, Sigma Chemical Company, St. Louis, MO, USA) as working electrodes with negatively charged electrode (cathode) in the donor compartment and positively charged electrode (anode) in the receptor compartment.

In one experiment, effect of electrode material on drug permeation kinetics was studied by using silver/silver chloride (Ag/AgCl) electrodes in addition to platinum electrodes. Ag/AgCl electrodes were prepared by the method described earlier (Burnette and Bagniefski, 1988) using Ag wire (99.99% purity, 0.5 mm diameter, Sigma). Ag/AgCl electrode with Ag in receptor compartment and AgCl in donor compartment was used.

2.4. Donor and receptor solution composition

Unless stated otherwise, all ketorolac transport studies were carried out by loading 24 mg (2 mg/ml, total 12 ml) of ketorolac in donor compartment using McIlvaine buffer (citrate-phosphate buffer, ionic strength = 0.06 M) at pH 7.4 as donor solution. This buffer consisted of 0.023 M Na_2HPO_4 and 0.0012 M citric acid. The isotonic Sorensen phosphate buffer of 0.053 M Na_2HPO_4, 0.015 M KH_2PO_4 and 0.075 M NaCl at pH 7.4 was used as the receptor solution.

Effect of drug loading dose on drug permeation kinetics was studied in one experiment by loading 12, 24, 60 and 84 mg in donor compartment using McIlvaine buffer (0.06 M) at pH 7.4 as donor solution.

2.5. Experiments

All experiments were carried out at room temperature (30 ± 2 °C). Diffusion studies were carried out for the period of 6 h and the number of experiments (n) in each group were 3–6. Samples (1 ml) were withdrawn from the receptor compartment at regular intervals and were replaced with an equal volume of drug-free buffer. The dilution corrections were made in calculating the concentrations in the receptor compartment. The samples were assayed by reverse phase HPLC method (Shimadzu LC-2010C system). The method consists of mobile phase (0.1 M ammonium acetate buffer, pH 4.5 and acetonitrile in the ratio 75:25), reverse phase C18 column (Phenomenex®; 250 mm × 4.6 mm, internal diameter 5 μm), detector wavelength 323 nm and flow rate of 1.0 ml/min. The retention time for ketorolac was found to be 5.72 min. The pH of the donor and receptor compartment was measured at the start and at the end of each study using a Mettler Digital pH meter (MP 220; Mettler Toledo, Switzerland).

2.5.1. Effect of current density

The effect of current density on ketorolac skin permeation was studied using continuous direct current in the range 0–0.50 mA/cm^2.

2.5.2. Effect of donor ionic strength

The effect of ionic strength on the skin permeation of ketorolac was studied using McIlvaine buffer at pH 7.4 with ionic strength of the donor solution varying from 0.06 to 1 M. McIlvaine buffer (pH 7.4) with a ionic strength of 0.5 M was prepared by using 0.182 M Na_2HPO_4 and 0.0098 M citric acid (Elving et al., 1956). Buffers with different ionic strength, viz. 0.06, 0.12, 0.25 M were prepared by suitable dilutions with distilled water. McIlvaine buffer (pH 7.4) with 1 M ionic strength was prepared by adding 0.51 M of KCl to 0.5 M ionic strength McIlvaine buffer (pH 7.4). Constant direct current with intensity of 0.5 mA/cm^2 was used.

2.5.3. Effect of type of current

In this set of experiment, the effect of constant direct current and pulsed direct current on ketorolac permeation was studied. Constant direct current (constant dc) of density of 0.5 mA/cm^2 was used. Pulse

direct current (pulse dc) in the form of pulse square wave with the same current magnitude of 0.5 mA/cm^2 at 2.5 kHz frequency with on:off ratio of 50% was used.

2.5.4. Effect of varying on:off ratios and frequency of pulsed current

The effect of on:off ratios of pulse direct current (2.5 kHz frequency) on ketorolac permeation was studied by varying on:off ratios from 10 to 90%. The effect of frequency of pulse current on permeation kinetics was studied by varying frequency of pulse current from 1.2 to 10 kHz while fixing the on:off ratio to 50%. In both the experiments, applied current magnitude was 0.5 mA/cm^2.

2.5.5. Effect of duration of current application

The experiment was conducted with constant dc of 0.5 mA/cm^2 applied for a period of 0, 1, 4 and 6 h.

2.5.6. Effect of pretreatment with chemical/physical enhancers

The study followed three pretreatment protocols, viz. (1) chemical enhancer pretreatment (ethanol and 5% D-limonene in combination with ethanol), (2) physical enhancer pretreatment (ultrasound), and (3) combination of chemical and physical enhancer pretreatment (ultrasound +5% D-limonene in ethanol).

Chemical enhancer pretreatment was given as follows: the diffusion cell was assembled with the rat skin clamped between the two half-cells. The donor side was filled with the enhancer solution and the system was maintained at room temperature (32 ± 2 °C) for 2 h. The enhancer solution was then drained and the donor cell was rinsed thoroughly with the buffer. Physical enhancer pretreatment (ultrasound) was given by introducing the probe of sonicator (Model-Electroson-408, Physiomed Electronics, Chennai, India) into the donor compartment and ultrasound was applied at 1 MHz with an intensity of 1.4 W/cm^2 for 30 min. The ultrasound pretreatment was given using McIlvaine buffer (0.06 M, pH 7.4) as coupling media. In the third set, ultrasound pretreatment (1 MHz, 1.4 W/cm^2, 30 min) was combined with chemical enhancer pretreatment (5% D-limonene in ethanol, duration 2 h). In this study, enhancer solution served as a coupling media for ultrasound application. After the completion of pretreatment procedures as described above, the permeation experiments were conducted. Constant dc of 0.5 mA/cm^2 was applied.

2.6. Data analysis

The cumulative amount of ketorolac permeated per unit membrane surface area was plotted against time, and the slope of the linear plot was estimated as the steady-state flux (J), expressed as (mg/(cm^2 s)). The permeability coefficient (P, expressed as cm/s) was calculated by dividing the steady-state flux (J) with initial donor drug concentration (mg/cm^3). From the ratio between the flux at a given current density and the passive flux, a quantitative evaluation of the transport improvement, determined by current application, is achieved. This value is termed as enhancement factor (E) (Srinivasan and Higuchi, 1990). The results were expressed as means ± standard deviation. Statistical comparisons were made with ANOVA. The level of significance was taken as $P < 0.05$.

3. Results and discussion

3.1. Effect of current density

Ketorolac is anionic at physiological pH (pK_a = 3.54) with almost 100% ionization (≈99.98%) and as the pH is decreased its percent ionization and solubility also decreased (Litvak and McEvoy, 1990). Given the permselectivity of skin at physiological pH (Sage, 1995), it is true that, all other things being equal, during iontophoresis cations cross the skin better than anions. But, then the permselectivity of the skin is not only dependent on the charge but also on the valence and molecular weight (more precisely molecular volume) (Green, 1996; Li et al., 1997). Successful iontophoretic delivery of low molecular weight anionic compounds has been demonstrated (Green et al., 1991a,b; Singh and Roberts, 1993). Looking at the low molecular weight of ketorolac anion (255.27) coupled with its monovalent nature, it follows that cathodal iontophoresis is always possible. By passive diffusion alone, ketorolac permeates through skin at a very low rate. Its permeation rate was significantly enhanced by iontophoresis (Table 1). The extent of enhancement in skin permeation fluxes and enhancement factors were observed to increase

Table 1
Effect of current density on rate of permeation of ketorolac through rat skin

Current density (mA/cm^2)	Flux, J_{ss} (μg/(cm^2 h))	Permeability coefficient, P ($\times 10^{-6}$ cm/s)	Enhancement factor[a]
0	00.89 ± 0.37	0.12	–
0.11	11.87 ± 2.12	1.65	13.33
0.22	29.12 ± 3.80	4.04	32.71
0.50	57.98 ± 5.21	8.04	65.14

[a] Enhancement factor = iontophoretic flux/passive flux. In all cases, ketorolac donor concentration was 2 mg/ml in 0.06 M McIlvaine buffer pH 7.4.

with increasing current density. The density of current, expressed in mA/cm^2, represents the number of electrical charges travelling per unit time per unit surface area. An enhancement in intensity allows an increase in the driving force to induce the movement of an ionised drug. The relationship between intensity and flux has been experimentally verified by several authors (Lelawongs et al., 1989; Sanderson et al., 1989; Thysman et al., 1994). The application of current allowed the increase in permeation of the ions to be controlled. This phenomenon is one of the most advantageous aspect of iontophoresis; the change in applied current density allows one to control the quantity of the drug released from the device. The flux was found to be fairly linear over the current density range studied, with a slope (also termed iontophoretic efficiency) of 115.42 μg/(mA-h). It is clear from the result that in order to achieve a daily single systemic dose of 30 mg ketorolac at an applied current density of 0.5 mA/cm^2, a minimum transport area of approximately 40 cm^2 for 12 h application will be required from a formulation containing 24 mg of the drug.

3.2. Effect of electrode material

Platinum electrodes have been reported to cause electrolysis of water and alter the pH of the donor and receptor compartments (Phipps et al., 2002). The use of Ag/AgCl electrodes has been suggested to circumvent this problem (Phipps et al., 2002). These electrodes do not cause electrolysis of water, instead they take part in electrochemistry (or are consumed by electrochemistry) resulting in net flow of current. In the present study however, no significant differences either in the ketorolac flux or the change in pH of the donor compartment were observed with Ag/AgCl electrodes when compared with platinum electrodes (Fig. 2). The electrochemistry occurring at respective electrodes might be responsible for the observed insignificant difference in the flux. In case of Ag/AgCl electrodes, oxidation of silver wire at the anode releases Ag^+ which associates with Cl^- to precipitate as AgCl on the electrode. Reduction of AgCl at the cathode yields Ag and Cl^- (Riviere and Heit, 1997). The generated Cl^- ions at cathode are much smaller than ketorolac ions and they may supercede the ketorolac ions in carrying the current, thereby decreasing the flux of drug. One potential limitation of Ag/AgCl electrode design is the necessary inclusion of Cl^-, usually as NaCl, in sufficient amounts to allow the formation of AgCl to continue. If there is no adequate supply of Cl^- ions, the electrode may breakdown in an attempt to mobilize Cl^- and decreasing the pH of solution by evolution of oxygen. In such a case, the released Ag^+ may cause irritation or discoloration of the skin. It is clear from the electrochemical reactions that the use Ag/AgCl electrodes suits better for cations (especially hydrohalide salts of cationic

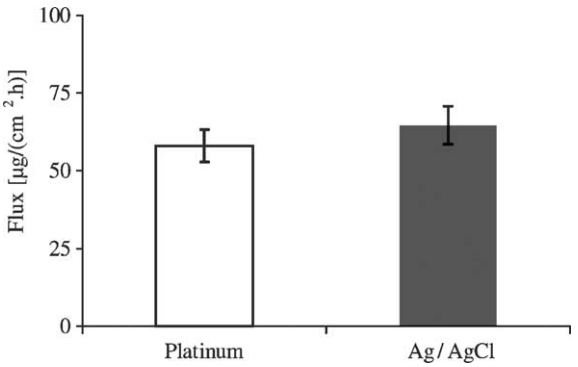

Fig. 2. Effect of electrode material on ketorolac permeation through rat skin. Ketorolac at 2 mg/ml and current density of 0.5 mA/cm^2 was used.

drugs) for efficient delivery. Their use for other drug salts or for anions may lead to unpredictable results. Moreover, use of Ag/AgCl electrode requires frequent oxidation–reduction pretreatment coupled with their ability to cause precipitation of ions (Lelawongs et al., 1989).

After analyzing the above points, we used platinum electrodes for all the diffusion experiments.

3.3. Effect of donor ionic strength

Buffers are used during iontophoresis to maintain the pH of the solution in the event of electrolysis of water (Banga and Chien, 1988; Riviere and Heit, 1997). Without buffers, the generated H^+ and OH^- ions can shift the formulation pH, affecting both delivery efficiency (due to a shift in the skin permselectivity, formation of competitive ions, or alteration of the charge state of the drug) and skin tolerability (Phipps et al., 2002). The ionic strength of the buffer should be high enough to resist the change in pH (sufficient buffering capacity), however it should not be too high that the buffering species itself becomes the major competitor for drug ions to carry the current. To analyze the effect of ionic strength on ketorolac transport, study was conducted by varying the concentration of buffering species in the donor solution. The ionized ketorolac makes only a small contribution to the total ionic strength of the donor solution, so its effect on ionic strength can be ignored.

The results in the Table 2 show that the iontophoresis facilitated permeation profile of ketorolac decreases as the ionic strength of the donor solution increases. The membrane permeation flux and enhancement factors are both declined as the ionic strength of donor solution increases.

Table 2
Effect of ionic strength of donor solution on the iontophoretic transport of ketorolac across rat skin

Ionic strength (M)	Flux (μg/(cm^2 h))	Enhancement factor[a]
0.06	57.98 ± 5.21	65.14
0.12	32.32 ± 5.80	36.31
0.25	14.40 ± 4.50	16.16
0.50	8.12 ± 2.80	9.12
1.00	6.29 ± 1.90	7.06

[a] Enhancement factor = iontophoretic flux/passive flux. In all cases, ketorolac donor concentration was 2 mg/ml in 0.06 M McIlvaine buffer pH 7.4.

The explanation for the observed phenomenon is the competition of drug ions and buffer ions for the current applied. Since most of the current would be carried by buffer ions with relatively high mobilities, the actual fraction of the applied current carried by ketorolac ions would be proportionally reduced as the concentration of buffering species in the donor solution increases, resulting in lower membrane permeation rate. In addition to the competition between ketorolac and buffer ions for the current, the activity coefficient of the drug can also be changed with the variation of ionic strength in the donor solution (Lelawongs et al., 1989; Martin et al., 1994). Simply speaking, the activity coefficient is nothing but the "effective concentration" of ions with unhindered movement in solution. Ionic strength of solution affects this effective concentration because of presence of phenomenon such as electrostatic forces of attraction between the oppositely charged ions and formation of 'ion-pairs'. The activity coefficient of ketorolac in the aqueous solution can be estimated using an extended Debye–Huckel equation (Martin et al., 1994):

$$\log \gamma_{\pm} = -\frac{Az^+z^-\sqrt{\mu}}{1+\sqrt{\mu}} \quad (1)$$

where $\gamma_{\pm}$ is the activity coefficient and A is a constant, the value of which is 0.51 in aqueous medium, z^+z^- is the net charge (ignoring algebraic signs) on the ketorolac and equals 1 and μ is the ionic strength of the solution.

When donor solution has an ionic strength of 1.0 M, the ionized ketorolac has a calculated activity coefficient of 0.55. The activity coefficient was found to increase by 43% when the ionic strength was reduced from 1.0 to 0.06 M. The increase in the activity coefficient of ketorolac in response to reduction in the concentration of competitive ions could cause an increase in the permeation of the drug. After reanalyzing the data using extended Debye–Huckel equation, a linear relationship can be established between the permeation rate of ketorolac and its activity (as a function of ionic strength) within a range of ionic strengths (Fig. 3). As expected, a deviation from the linearity is observed at a very high ionic strength. Similar results has been reported by Lelawongs et al. (1989) for arginine-vasopressin.

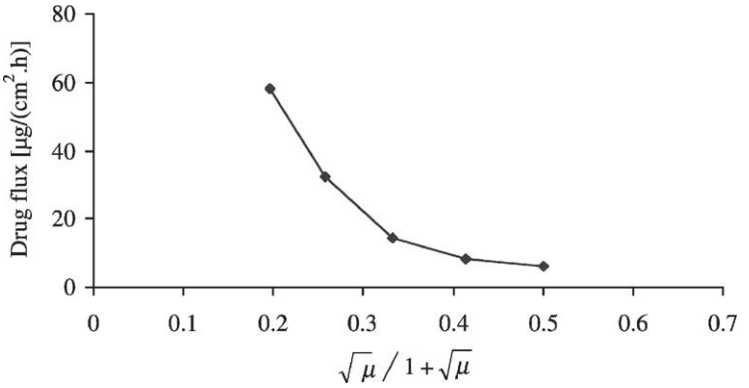

Fig. 3. Plot of flux of ketorolac through rat skin vs. activity coefficient.

Although, the enhancement of flux was higher in a buffer with less ionic strength (0.06 M), the change of pH of donor compartment after 6 h of iontophoresis was relatively less as ionic strength increased. For example, with 0.06 M strength, after 6 h of iontophoresis, the change in pH of donor compartment was +2.5 pH units while with 0.5 M strength, it was only +0.4. It means in case of iontophoresis for prolonged period, sufficiently higher ionic strength (e.g. 0.5 M) of buffer is desired.

3.4. Effect of type of current

The direct current was more efficient than pulse current to promote ketorolac permeation ($P < 0.05$). The flux of the ketorolac decreased to nearly half with pulse current (2.5 kHz, 50% on:off, flux = 30.12 μg/(cm^2 h)) when compared to constant dc of same current magnitude (flux = 57.98 μg/(cm^2 h)) (Fig. 4).

Pulse current has been widely used to allow the depolarization of skin induced by the application of direct electrical current, therefore decreasing the resistivity of the skin by reducing its capacitance (Yamamoto and Yamamoto, 1976). Moreover, the reduction of the current quantity passing through the skin diminishes the risk to skin alterations, like burns (Thysman et al., 1992). The observed two-fold efficiency of direct current over pulse current can be explained by the fact that in latter case the quantity of electric charge permeating through the skin is reduced by half as a function of the square wave current. The results obtained are in good agreement with researchers working with smaller molecules (Pikal and Shah, 1991; Thysman et al., 1992, 1994; Preat and Thysman, 1993). The observations, however, contradict results obtained by others working with macromolecules such as insulin (Banga and Chien, 1988; Thysman et al., 1992). The higher efficiency obtained from pulse current for macromolecules could be attributed to the difference in the molecular size or pathways taken by these molecules for transport (Clemessy et al., 1995). During application of constant dc and subsequent polarization, the smaller molecules (like ketorolac) may escape through the paths of low skin impedance (like skin appendages: hair follicles and sweat glands), however the bulkier molecules

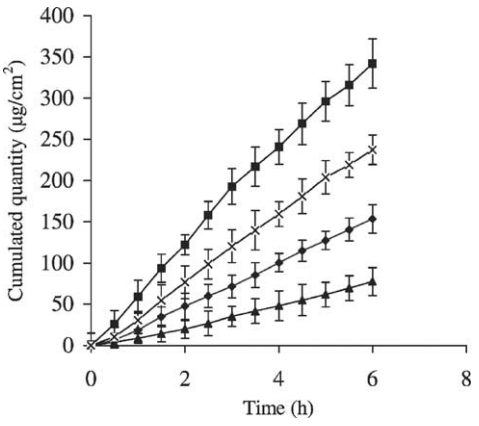

Fig. 4. Effect of on:off ratio of pulse current on permeation of ketorolac through rat skin. Current magnitude was 0.5 mA/cm^2 and on:off ratio of pulse current (2.5 kHz) was (■) 100% (constant dc); (×) 90%; (◆) 50%; and (▲) 10%.

like insulin may follow the transport through lipid layers of the stratum corneum (high skin impedance area) and polarization may be an important factor responsible for decreased flux with constant dc.

3.5. Effect of varying on:off ratios and frequency of pulsed current

Fig. 4 shows effect of varying on:off ratios of pulse current (2.5 kHz) on ketorolac permeation through rat skin. As the on:off ratio of the pulsed current decreased, its rate of permeation also decreased. For example, when the on:off ratio of pulse current decreased from 90 to 10%, ketorolac flux decreased from $41.17 \pm 4.26\,\mu g/(cm^2\,h)$ to $13.15 \pm 2.9\,\mu g/(cm^2\,h)$. This can be explained by the fact that as the on:off ratio decreased, the quantity of electric charge permeating through the skin also decreased.

The effect of frequency of pulse current on ketorolac permeation is shown in Table 3. As the frequency of the pulse current increased, the flux of ketorolac also increased. The increase in flux was insignificant up to 5 kHz frequency, however it became significant at 10 kHz. This result could be explained by a decrease in skin impedance with an increase in current frequency as shown by the skin equivalent circuit model of Yamamoto and Yamamoto (1976). This result is in agreement with the results obtained by Clemessy et al. (1995) with angiotensin.

3.6. Effect of loading dose

The effect of loading doses on iontophoretic permeation of ketorolac was very significant, and is shown in Table 4. When iontophoresis was applied, a seven-fold increase in ketorolac concentration was associated with a four-fold increase in transdermal fluxes. This increase results from a seven-fold increase in passive diffusion as predicted by Fick's diffusion law and an increased iontophoretic flux. The Nernst–Plank equation predicts that the ion flux through an inert membrane is directly proportional to the concentration. In the present study also, a fairly linear relationship was found between the ketorolac flux and loading dose ($R^2 = 0.97$). This indicates that the ion conducting pathways of the skin have not reached saturation (Brand et al., 1997). The iontophoretic efficiency was calculated for each loading dose and is presented in Table 4. It follows from the Table 4 that, in order to achieve a daily single systemic dose of 30 mg ketorolac at an applied current density of $0.5\,mA/cm^2$, a minimum transport area of approximately $20\,cm^2$ for 12 h application will be required from a formulation containing 84 mg of drug.

Table 3
Effect of frequency of pulse current (50% on:off) on ketorolac permeation through rat skin

Frequency (kHz)	Flux ($\mu g/(cm^2\,h)$)
1.25	23.65 ± 3.12
2.50	26.33 ± 4.10
5.00	31.12 ± 4.36
10.00	44.52 ± 5.01[a]

[a] Significantly different from 1.25 and 2.5 kHz ($P < 0.05$).

Table 4
Effect of loading dose on iontophoretic transport of ketorolac through rat skin

Loading dose (mg/12 ml)	Flux, J_{ss} ($\mu g/(cm^2\,h)$)	Enhancement factor[a]	Efficiency of iontophoresis ($\mu g/(mA\text{-}h)$)[b]
12	30.13 ± 4.20	33.85	58.32
24	57.98 ± 5.12	65.14	115.42
60	91.35 ± 8.40	102.64	179.79
84	121.49 ± 10.12	136.50	240.11

[a] Enhancement factor = iontophoretic flux/passive flux.
[b] Efficiency of iontophoresis was calculated from slope of flux vs. current density plot for respective loading doses. Constant dc of $0.5\,mA/cm^2$ was applied in all cases.

3.7. Effect of duration of current application

Fig. 5 shows the effect of duration of application of the electrical field on the cumulative amount of the drug permeated. The results indicate that the permeation profile of drug increases with increase in the duration of application. Without the application of current, the rate of drug permeation was low. It is clear from Fig. 5 that a lag time is evident for passive diffusion. With iontophoresis decrease in lag time was significant. The shorter lag time of ketorolac with iontophoresis indicates the possible transport of ionized species through shunts (hair follicles and sweat ducts) (Siddiqui et al., 1985). Flux was greater when iontophoresis was applied for 6 instead of 1 h. Termination of current did not cause the flux to return immediately to the passive control level in both 1

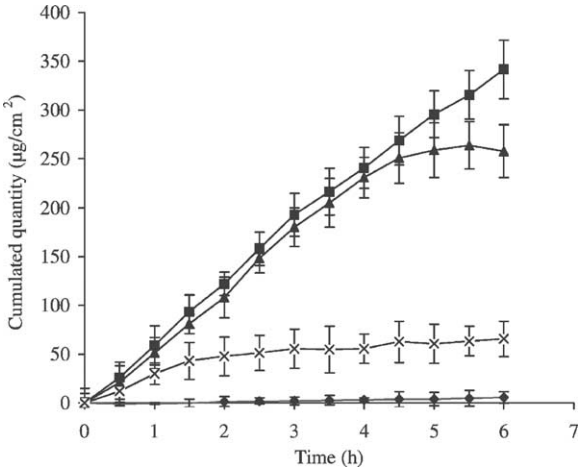

Fig. 5. Effect of duration of current application on permeation of ketorolac through rat skin. Ketorolac concentration used was 2 mg/ml and duration of current application (0.5 mA/cm^2) was (■) 6 h; (▲) 4 h; (×) 1 h; and (◆) 0 h.

and 4 h treatment. When the current was applied for 1 h (or 4 h) and terminated, the cumulated quantity of ketorolac detected in the receptor compartment increased linearly with time at a rate much higher than diffusion. Enhanced passive levels have often been attributed to an alteration of the barrier as a result of current application. However, other indirect effects might also be important: increased water content, induced by iontophoresis, may lead to increased flux of ketorolac through the skin. Another possibility is the release of the drug from a reservoir formed in the skin during iontophoresis, however this aspect was not investigated in the present study.

3.8. Effect of pretreatment with chemical/physical enhancers

Table 5 shows the effect of pretreatment with chemical and physical enhancers either alone or in combination on passive and iontophoresis assisted transport of ketorolac. We have selected ethanol as chemical enhancer because of reports of synergism between iontophoresis and ethanol as enhancer on transport of peptides through human epidermis (Srinivasan and Higuchi, 1990; Srinivasan et al., 1989; Bhatia et al., 1997). Ethanol pretreatment alone could not significantly alter the iontophoretic transport of ketorolac. It has also been reported that the percutaneous enhancement effect of ethanol markedly increased in the presence of D-limonene, a constituent of orange or lemon oils (Bhatia and Singh, 1999). Hence, the effect of combination of D-limonene and ethanol pretreatment was studied. Pretreatment with D-limonene/ethanol significantly enhanced the passive flux of drug in comparison to control (no pretreatment) ($P < 0.05$). The iontophoretic flux of ketorolac was also significantly enhanced with D-limonene/ethanol pretreatment when compared with passive flux with or without pretreatment ($P < 0.001$) and iontophoresis alone or in combination with ethanol ($P < 0.05$). This result indicates the disruption of stratum corneum barrier by limonene/ethanol. Bhatia and Singh (1999) reported that pretreatment of human skin with limonene/ethanol resulted in the expansion of normal stratum corneum cytoplasm and creation of more porous intracellular structure. Further, this pretreatment in combination with iontophoresis resulted in swelling of stratum corneum cells and creation of a looser network of

Table 5
Effect of pretreatment with chemical/physical enhancers on iontophoretic permeation of ketorolac through rat skin

Pretreatment modality	Flux (μg/(cm^2 h))
Control (no pretreatment)	0.99 ± 0.39
Iontophoresis alone	62.80 ± 6.78
Ethanol	
Passive	1.19 ± 0.54
Iontophoresis	64.21 ± 5.93
Liomonene + ethanol	
Passive	7.26 ± 1.50[a]
Iontophoresis	114.87 ± 9.05[b]
Ultrasound (1.4 W/cm^2, 1 MHz, 30 min)	
Passive	1.28 ± 0.67
Iontophoresis	64.25 ± 5.84
Limonene + ethanol + ultrasound (1.4 W/cm^2, 1 MHz, 30 min)	
Passive	10.12 ± 1.18[c]
Iontophoresis	136.11 ± 9.10[d]

[a] Significantly different from control (no pretreatment) ($P < 0.05$).
[b] Significantly different from iontophoresis alone or with ethanol or ultrasound pretreatment ($P < 0.05$).
[c] Significantly different from control (no pretreatment) ($P < 0.01$).
[d] Significantly different from iontophoresis alone or with ethanol or with ultrasound pretreatment ($P < 0.01$) or with D-limonene/ethanol pretreatment ($P < 0.05$). In all cases, ketorolac at 2 mg/ml and current density of 0.5 mA/cm^2 was used.

filaments leading to an increased free volume for distribution and decreased diffusional resistance within stratum corneum. Ultrasound is another physical modality presently being explored for non-invasive delivery of drugs and it has been reported to disrupt the lipid barrier of the stratum corneum (Simonin, 1995). When ultrasound pretreatment is combined with D-limonene/ethanol pretreatment, diffusional resistance of stratum corneum barrier is further expected to drop because ultrasound may enhance the penetration of limonene/ethanol inside the stratum corneum and further loosening of stratum corneum cells. In addition, enhanced temperature during ultrasound pretreatment coupled with cavitational effects may facilitate the disruption of stratum corneum barrier either independently or in combination with chemical enhancers. Trimodality treatment comprising of pretreatment with D-limonene/ethanol + ultrasound in combination followed by iontophoresis was found to be most potent for enhancing the rate of permeation of ketorolac. The enhancement in flux was significant ($P < 0.01$) in comparison to iontophoresis alone or in combination with ethanol pretreatment or ultrasound pretreatment or D-limonene/ethanol pretreatment ($P < 0.05$). It is clear from the result that in order to achieve a daily single systemic dose of 30 mg ketorolac at an applied current density of 0.5 mA/cm^2, a minimum transport area of approximately 18 cm^2 for 12 h application will be required from a formulation containing 24 mg of the drug when D-limonene/ethanol + ultrasound pretreatment was applied. In brief, it can be concluded that application of D-limonene/ethanol + ultrasound pretreatment resulted in 45% reduction in patch area when compared with iontophoresis without pretreatment. Such an approach of combination of iontophoresis and chemical/physical enhancers is interesting for realization of transdermal systems capable of providing sufficient drug levels with lower quantity of enhancer and current.

4. Conclusion

From the results, it can be concluded that the transdermal penetration of ketorolac tromethamine can be significantly enhanced by iontophoresis. The enhancement in flux was dependent on drug loading dose, ionic strength of donor solution, the type and nature of current applied, duration of current application and pretreatment with chemical or physical enhancers. Pretreatment of skin with combination of D-limonene/ethanol and ultrasound lead to maximal enhancement in iontophoretic flux of the drug. The synergistic effect of iontophoresis and combination of chemical + physical enhancer may permit the use of lower quantity of chemical enhancer and current within the delivery system for achieving therapeutically effective systemic drug concentrations.

Acknowledgements

The authors thank the University Grants Commission, New Delhi for financial support to this research work.

References

Banga, A.K., Chien, Y.W., 1988. Iontophoretic delivery of drugs: fundamental development and biomedical application. J. Cont. Rel. 7, 1–24.

Bhatia, K.S., Singh, J., 1999. Effect of linoleic acid/ethanol or limonene/ethanol and iontophoresis on the in vitro percutaneous absorption of LHRH and ultrastructure of human epidermis. Int. J. Pharm. 180, 235–250.

Bhatia, K.S., Gao, S., Freeman, T.P., Singh, J., 1997. Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin. J. Pharm. Sci. 86, 1011–1015.

Brand, R.M., Duensing, G., Hamel, F.G., 1997. Iontophoretic delivery of an insulin-mimetic peroxovanadium compound. Int. J. Pharm. 146, 115–122.

Buckley, M.M.-T., Brogden, R.N., 1990. Ketorolac: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. Drugs 39, 86–109.

Burnette, R.R., Bagniefski, T.M., 1988. Influence of constant current iontophoresis on the impedance and passive Na^+ permeability of excised nude mouse skin. J. Pharm. Sci. 77, 492–497.

Clemessy, M., Couarraze, G., Bevan, B., Puisieux, F., 1995. Mechanisms involved in iontophoretic transport of angiotensin. Pharm. Res. 12, 998–1002.

Durrheim, H., Flynn, G.L., Higuchi, W.I., Bhel, C.R., 1980. Permeation of hairless mouse skin. I. Experimental methods and comparison with human epidermal permeation by alkanols. J. Pharm. Sci. 69, 781–786.

Elving, P.J., Markowitz, J.M., Rosenthal, I., 1956. Preparation of buffer systems for constant ionic strength. Anal. Chem. 28, 1179–1180.

Green, P.G., 1996. Iontophoretic delivery of peptide drugs. J. Cont. Rel. 41, 33–48.

Green, P.G., Hinz, R.S., Cullander, C., Yamane, G., Guy, R.H., 1991a. Iontophoreitc delivery of amino acids and amino acid derivatives across the skin in vitro. Pharm. Res. 8, 1113–1120.

Green, P.G., Hinz, R.S., Kim, A., Frnacis, C., Guy Jr., R.H., 1991b. Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. 8, 1121–1127.

Lelawongs, P., Liu, J.-C., Siddiqui, O., Chien, Y.W., 1989. Transdermal iontophoretic delivery of arginine-vasopressin (I): physicochemical considerations. Int. J. Pharm. 56, 13–22.

Li, S.K., Ghanem, A.-H., Peck, K.D., Higuchi, W.I., 1997. Iontophoretic transport across a synthetic membrane and human epidermal membrane: a study of the effects of permanent charge. J. Pharm. Sci. 86, 680–689.

Litvak, K.M., McEvoy, G.K., 1990. Ketorolac, an injectable non-narcotic analgesic. Clin. Pharm. 9, 921–935.

Martin, A., Bustamante, P., Chun, A.H.C., 1994. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences. B.I. Waverly, New Delhi, p. 135.

Phipps, J.B., Scott, E.R., Gyory, J.R., Padmanabhan, R.V., 2002. Iontophoresis. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, 2nd ed. Marcel Dekker, New York, pp. 1573–1587.

Pikal, M.J., Shah, S., 1991. Study of the mechanism of flux enhancement through hairless mouse skin by pulsed dc iontophoresis. Pharm. Res. 8, 365–369.

Preat, V., Thysman, S., 1993. Transdermal iontophoretic delivery of sufentanil. Int. J. Pharm. 96, 189–196.

Riviere, J.E., Heit, M.C., 1997. Electrically-assisted transdermal drug delivery. Pharm. Res. 14, 687–697.

Roy, S.D., Manoukian, E., 1995. Transdermal delivery of ketorolac tromethamine: permeation enhancement, device design, and pharmacokinetics in healthy humans. J. Pharm. Sci. 84, 1190–1196.

Roy, S.D., Manoukian, E., Combs, D., 1995. Absorption of transdermal delivered ketorolac acid in humans. J. Pharm. Sci. 84, 49–52.

Sage, B.H., 1995. Iontophoresis. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL, pp. 351–368.

Sanderson, J.E., Riel, S.D., Dixon, R., 1989. Iontophoretic delivery of non-peptide drugs: formulation optimization for maximum skin permeability. J. Pharm. Sci. 78, 361–364.

Siddiqui, O., Roberts, M.S., Polack, A.E., 1985. The effect of iontophoresis and vehicle pH on the in vitro permeation of lignocaine through human stratum corneum. J. Pharm. Pharmacol. 37, 732–735.

Simonin, J.-P., 1995. On the mechanism of in vitro and in vivo phonophoresis. J. Cont. Rel. 33, 125–141.

Singh, P., Roberts, M.S., 1993. Iontophoretic transdermal delivery of salicylic acid and lidocaine to local subcutaneous structures. J. Pharm. Sci. 82, 127–131.

Srinivasan, V., Higuchi, W.I., 1990. A model for iontophoresis incorporating the effect of convective solvent flow. Int. J. Pharm. 60, 133–138.

Srinivasan, V., Higuchi, W.I., Sims, S.M., Ghanem, A.H., Behl, C.R., 1989. Transdermal iontophoretic drug delivery: mechanistic analysis and application to polypeptide delivery. J. Pharm. Sci. 78, 370–375.

Thysman, S., Preat, V., Roland, M., 1992. Factors affecting iontophoretic mobility of metoprolol. J. Pharm. Sci. 81, 670–675.

Thysman, S., Tasset, C., Preat, V., 1994. Transdermal iontophoresis of fentanyl: delivery and mechanistic analysis. Int. J. Pharm. 101, 105–113.

Tiwari, S.B., Udupa, N., 1999. Low cost mini magnetic stirrers for application in pharmacy. Ind. J. Pharm. Educ. 33, 223–224.

Varghese, E., Khar, R.K., 1996. Enhanced skin permeation of diclofenac by iontophoresis: in vitro and in vivo studies. J. Cont. Rel. 38, 21–27.

Yamamoto, T., Yamamoto, Y., 1976. Electrical properties of the epidermal stratum corneum. Med. Biol. Eng. 14, 151–156.