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Effect of Iontophoresis On In-vitro Transdermal Delivery of Tramadol, A Centrally Acting Analgesic

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ABSTRACT

The aim of the present work was to characterize the in vitro transdermal absorption of tramadol hydrochloride (TH) through pig ear skin. Studies of electrical and physicochemical factors acting on the permeation kinetics of in vitro iontophoresis were performed. Iontophoresis increased the transdermal permeation flux of TH as compared to the diffusion. Increase in applied current density or enhanced the flux of the drug. Continuous current was more potent than pulsed current in promoting TH transdermal permeation. Increasing the frequency or on:off ratio of pulse current induced an enhancement of the flux through the skin. The binary system did not cause an enhancement in the permeation of TH compared to water alone. An increase in donor drug loading dose or increasing the duration of current application resulted in enhancement of the drug flux. Based on these results, and taking into account the pharmacokinetics of TH, therapeutic drug plasma levels could be achieved via transdermal iontophoresis using a reasonably sized (around 20 cm^2) patch.

Keywords: analgesic; anodal iontophoresis; tramadol hydrochloride; transdermal delivery

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INTRODUCTION

Tramadol hydrochloride (TH) is synthetic drug of opioid analgesic group which has a dual mechanism of action like binding with μ-opioid receptors and inhibiting the neuronal reuptake of norepinephrine and serotonin.[1,2] This centrally acting drug available mainly in two dosage forms (injection and oral formulations) has pharmaceutical applications in the treatment of moderately severe chronic pain.[3,4] These routes of administration have limitations with subject to patient discomfort due to multiple injections and swallowing that can lead to poor patient compliance. Other problems are high labour costs, and risks of toxicity due to sudden peaks in plasma drug concentrations after intravenous injection, and variable interpatient bioavailability after oral administration.[5] Alternative to these dosage forms, is the transdermal drug delivery, a non-invasive route, offers number of advantages over oral and parenteral routes like efficacious and maintained a constant blood drug concentration for an extended period of time with interpatient variations within acceptable range.[6–8] Also the transdermal system helps in avoiding possible addiction to this drug by decreasing peak and trough plasma concentrations and also by reducing the amount of drug intake.[9,10] Hence, a transdermal drug delivery system is a desirable alternative administration route for TH for patients with chronic pain.

The delivery of drugs through human skin, an efficient natural barrier, is big challenge for the scientists. Therapeutic agents used specific characteristic to enable them to permeate through the stratum corneum and ellicit therapeutic responses. Various methods have been used to enhance the percutaneous absorption of drugs. Iontophoresis is the most commonly used physical enhancer and is based on the application of physiologically acceptable levels of electrical current (with a current density 0.5 mA/cm^2) to drive charged or neutral drug molecules across a biological membrane. Iontophoresis, an active trandermal drug delivery technology, is popularly used for the delivery of small and large molecules and reported in literature.[11,12,13] The common mechanism involved in iontophoresis is the transport of ions across a membrane under the influence of an applied electric potential,[14,15] which has been described as electrorepulsion and electro-osmosis by different authors.[11,16–21]

High daily dose (50– 400 mg) and low lipophilicity (log P – 1.35 at pH 7) of TH, makes it unsuitable for passive diffusion across the skin. Anodal iontophoresis has been widely used for transdermal delivery of cationic compounds and positively charged macromolecules.[22,23] It is the method whereby a cationic drug is delivered across an epithelial barrier when placed under a positively charged delivery electrode (anode) from which it is repelled. A counter electrode completes the circuit by drawing physiological anions (i.e. Cl^-) from the body. The ionization

constant (pKa) of TH is 9.41, and anodal iontophoretic technology is thus applicable to transdermal delivery of TH.

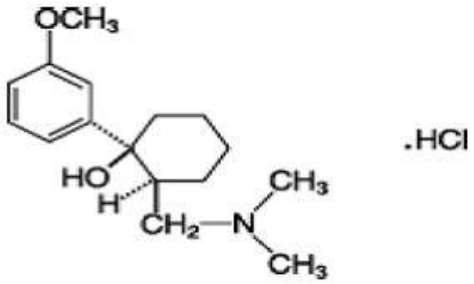
The aim of the present study was to investigate the influence of electrical factors (current density, current profile and current application duration), physicochemical factors (donor drug concentration and donor vehicle composition) on the iontophoretic transport of TH through porcine skin.

MATERIALS AND METHODS

Materials

TH was obtained as gift sample from Zydus Ahmedabad, India. Its physicochemical properties are shown in Table 1. Potassium dihydrogen phosphate, sodium hydroxide, ethyl alcohol absolute, isopropyl alcohol were purchased from S.D. Fine-Chem Ltd., Mumbai, India. All solutions were made using distilled water. Iontophoresis instrument was custom designed.

Table. 1. Physicochemical properties of Tramadol Hydrochloride

Generic Name	Tramadol Hydrochloride
IUPAC Name	(±)cis-2-[(dimethylamino)methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride
Structural formula	
Empirical formula	$C_{16}H_{25}NO_2$
Molecular weight	263.4 g/mol
Dosage forms	Oral; Parenteral
Oral bioavailability	68-72%
Solubility	Soluble in water
Octanol/water partition coefficients	log P = 1.35

Methods

Skin membrane preparation

From a local abattoir, ears were obtained from freshly slaughtered pigs. The skin was removed carefully from the outer regions of the ear and separated from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol. Finally the skin was washed with water and stored at −20°C and was used within a week.

Preparation of Electrodes[20]

Iontophoresis experiments were conducted using silver/silver chloride electrodes. The silver chloride electrodes were prepared as follows: silver plate (1cm^2 diameter) was immersed in 0.1N HCl solution and connected to the anode of an electric current source (12V). Silver chloride powder was melted in a basin and picked up by another silver wire, which was connected to the negative pole of the current source. A gray silver chloride layer was gradually coated on the anodal silver wires, and after 24 hours these wires were ready for use as iontophoresis cathodal electrodes.

***In vitro* permeation studies**

The *in vitro* permeation studies were carried out in a Franz diffusion cell using porcine skin. The excised skin was mounted between the half-cells with the dermis in contact with receptor fluid. The receiving chamber contained 60mL of phosphate buffered saline (PBS pH 7.2) and the area available for diffusion was 7.06 cm^2. The receiver compartment was stirred at 600 rpm with a 3-mm magnetic stir bar. The receptor fluids were thermostated at 37 ± 0.5°C. Under these conditions the temperature at the skin surface was 32 ± 0.5°C. Porcine skins were left for 1 hour to hydrate before the start of the experiment. After this hydration period, 4 ml of drug solution was applied on top of each skin in the donor chamber. Then the donor compartments of the diffusion cells were covered with an aluminum foil to prevent the evaporation of vehicle.

Anodal iontophoresis was carried out by using a single channel power supply Ag/AgCl electrodes (1 cm^2 diameter) were fixed at a distance of 2 mm from the skin surface in donor and receiver chambers. The anode was connected to the donor and the cathode to the receiver chamber. At predetermined times (1, 2, 3, 4, 5, 6, 7 and 8 hours), 5mL samples were withdrawn from the receptor compartment and were immediately replaced by the same volume of the buffer solution. These samples were accounted for in the calculation of the corrected TH concentration in the samples for the subsequent calculation of the diffusion parameters. All experiments were conducted for 8 hours. The estimation of TH was done by UV-spectroscopy at 271nm. The iontophoresis experiments were conducted according to the general procedure described above. The following factors affecting TH iontophoretic delivery were studied.

Current Density

Four current densities were used, in the range 0–0.50 mA/cm^2. Direct current was used throughout. The current effect was studied using TH water solutions at 20mg/ml. All experiments were carried out for 8 h.

TH Concentration

Four concentrations of TH were used, 5 mg, 10 mg, 15 mg, and 20 mg/ml. Constant direct current (constant dc) of density of 0.5 mA/cm^2 was used. Now onwards in all the experiments donor concentration of TH taken was 20mg/ml.

Composition of the Donor Vehicle

The donor vehicle effect was studied using ethanol-water systems at different concentrations of ethanol (0%, 25%, 50%, 75% and absolute alcohol). Constant direct current (constant dc) of density of 0.5 mA/cm^2 was used.

Effect of type of current

In this set of experiment, the effect of constant direct current and pulsed direct current on TH 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.

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 TH 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.

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 8 h.

Data analysis

The cumulative amount of TH 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 (µg/(cm^2 h)). The permeability coefficient (*P*, expressed as cm/h) 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*).[24] Statistical analysis was performed by one-way analysis of variance (ANOVA) and a *t*-test to test the effects of various treatments. The data points provided in the graphs are an average of three trials. The error bars represents the standard deviation. A significance level of $p < 0.05$ denoted significance in all cases.

The lag time (to) was obtained from extrapolation of the linear region (steady-state portion) of the permeation profile to the intercept on the time axis. As a useful approximation, pseudo-steady-state permeation for most drugs is achieved after approximately 2.7 times the lag time.[25]

RESULTS AND DISCUSSION

The composition and architecture of the stratum corneum render it a protective barrier against transdermal administration of therapeutic agents. In spite of the many advantages offered by transdermal drug delivery by passive permeation, the route is limited to delivery of small, relatively lipophilic molecules into the systemic circulation due to the barrier function of stratum corneum.[26,27] It is difficult to exploit the transdermal route to deliver therapeutic amounts of TH by conventional passive transdermal administration, since TH is hydrophilic (log P at pH 7.0 is 1.35) and its oral daily dose is quite large (50–400 mg) with high bioavailability (~70%).[3,4] Iontophoresis is a technique used to enhance the transdermal delivery of compounds through the skin via application of a small electric current, and enables transdermal delivery of relatively large amounts of hydrophilic charged molecules compared with the conventional passive transdermal approach.[13,27-29] The pKa of tramadol hydrochloride is 9.41, and so anodal iontophoresis of tramadol hydrochloride is probably the most promising transdermal drug delivery system to attain therapeutic blood levels of tramadol.

Porcine skin has been validated as a membrane model for research and development purposes.[30-32] Porcine skin has a similar thickness of stratum corneum as human skin[33] and similar hair follicle density. *In vitro* studies with human skin have also shown to correlate well with *in vivo* studies in pigs[34]. It is readily obtainable from abattoirs for in-vitro skin permeation studies. It is also reported that porcine skin is a reasonable model for the human barrier in iontophoresis studies. The pI values of the two skins are very close and thus cationic permselectivities at pH 7.4 are confirmed. Hence the present investigation porcine skin was used.[35]

In iontophoresis, drug is propelled into the stratum corneum by the ionic repulsion. Positively charged drug gets repelled from the anodal terminal whereas negative charge gets repelled from the cathodal part of the circuit. The unidirectional propulsion of the drug ions into the skin is controlled by the electrochemistry of the cell, which may change the reaction environment as the process continues. So the choice of the electrode is of vital importance. A number of metals have been investigated as electrode in the iontophoretic permeation. Platinum electrodes have been reported to cause electrolysis of water and alter the pH of the donor and receptor compartments.[36] The use of Ag/AgCl electrodes has been suggested to circumvent this

problem.[36] 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. Hence these electrodes are the better choice and were used in the present study.

Effect of current density

By passive diffusion alone, TH permeates through skin at a very low rate. Its permeation rate was significantly enhanced by iontophoresis (Table 2). The extent of enhancement in skin permeation fluxes and enhancement factors were observed to increase 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.[37-39] 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.

Table. 2. Effect of current density on rate of permeation of TH through porcine skin

Current density, (mA/cm^2)	Flux, *J*ss (µg/(cm^2 h))	Lag time (h)	Permeability coefficient, Kp x 10^{-3} (cm/h)	Enhancement factor (E)
0	10.28 ± 5	2.25 ± 0.14	0.514	-
0.025	107.85 ± 15	1 ± 0.09	5.39	10.49
0.1	183.57 ± 20	0.83 ± 0.05	9.18	17.86
0.25	263.00 ± 25	0.66 ± 0.02	13.15	25.58
0.5	355.71 ± 25	0.42 ± 0.01	17.78	34.60

Figure. 1. shows steady-state flux dropped to107. 85 ± 15, 183.57 ± 15, 263.00 ± 25, 355.71 ± 25 µg/(cm^2 h), respectively for 0.025, 0.1, 0.25 and 0.5 mA/cm^2 corresponding to a 10-, 17-, 25-, 34-fold increase in presence of these latter iontophoresis currents in comparison to passive diffusion.

The lag time is also one of the first limiting factor for a transdermal drug delivery system. It is obvious from the result presenting table 2 that lag time decreased when higher current density is applied. It was reduced to 2.25h ± 0.14, 1h ± 0.09, 0.83h ± 0.05, 0.66h ± 0.02, 0.42h ± 0.01 for 0, 0.025, 0.1, 0.25 and 0.5 mA/cm^2current density respectively.

Figure. 2 shows the same data in the form of a plot of TH steady state flux as a function of current density. It can be discerned that the relationship between flux and current density is linear (R^2=0.880). A direct proportionality between current density and ion flux is a general characteristic of iontophoresis, predicted by the Nernst–Planck equation[16].

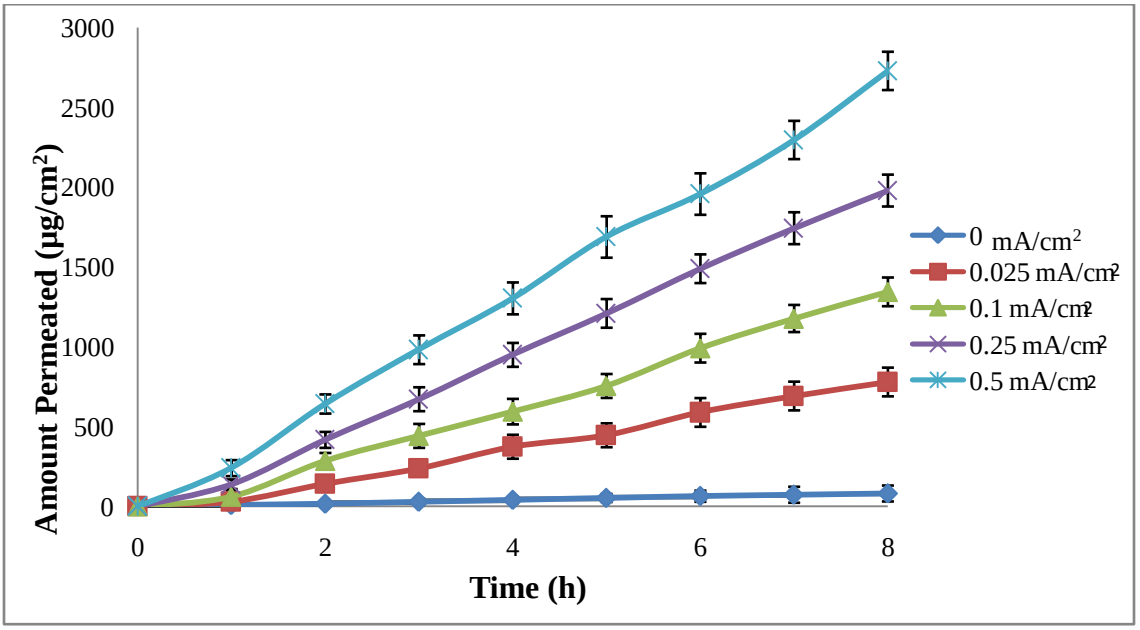


Figure 1: Effect of current density on rate of permeation of TH through porcine skin. Donor concentration of TH was 20mg/ml. (n=3)

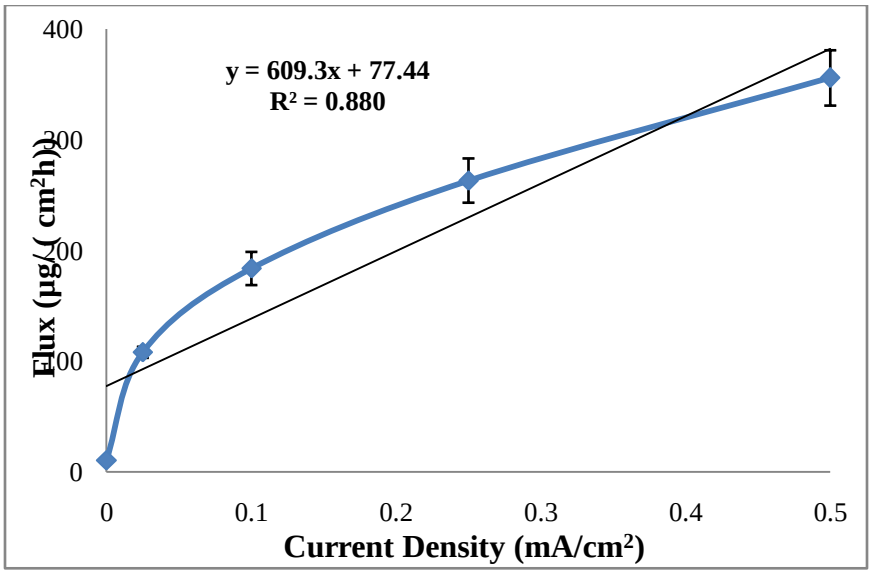


Figure 2: Iontophoretic flux of TH as a function of current density (n=3)

Controlling plasma levels of tramadol is crucial in the treatment of chronic pain to maintain therapeutic plasma concentration with high accuracy, to avoid the risk of toxicity due to sudden peaks in plasma levels and abuse/addiction potential due to overexposure. Although one of benefits of transdermal iontophoresis is controlled drug delivery based on the percutaneous permeation of drug in proportion to current, some molecules have been reported to exert a non-current dependent relationship between flux and current.[40] In the present skin permeation study, the *in-vitro* steady-state skin permeation flux of TH increased in a current-dependent manner.

These findings indicate that the percutaneous delivery of TH can be controlled directly by varying the current strength. Thus, individual dose requirements of patients for chronic pain management can be defined without the risk of side-effects by adjusting current strength.

Effect of loading dose

The effect of loading doses on iontophoretic permeation of TH was very significant, and is shown in Table 3. When iontophoresis was applied, a four-fold increase in TH concentration was associated with a three-fold increase in transdermal fluxes. 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 TH flux and loading dose (R^2=0.988) (Figure.3). This indicates that the ion conducting pathways of the skin have not reached saturation.[41]

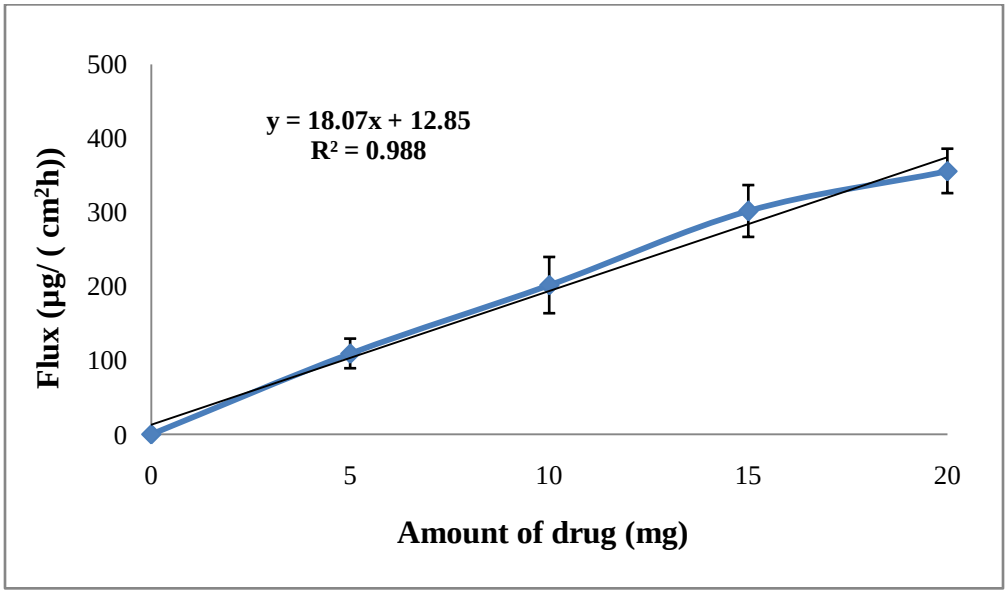


Figure 3: Iontophoretic flux of TH as a function of loading dose of drug. (n=3)

Table. 3. Effect of loading dose on rate of permeation of TH through porcine skin

Loading dose, (mg/ml)	Flux, *Jss* (µg/(cm^2 h))	Enhancement factor (E)
5	109.24 ± 20	25.67
10	201.52 ± 38	27.45
15	301.61 ± 35	31.63
20	355.71 ± 30	34.60

Effect of donor vehicle

In order to determine the role of donor phase composition on the system, ethanol-water systems were used at different concentration of ethanol (0%, 25%, 50%, 75% and absolute alcohol), the flux obtained was 355.71 ± 30, 250.51 ± 43, 223.65 ± 77, 275.45 ± 56, 201.51 ± 34 µg/(cm^2 h), respectively. Current density was fixed at 0.5 mA/cm^2 throughout. The use of binary solvent in

the formulation did not affect TH in comparison to a purely aqueous vehicle. Similar result was also reported for arginine vasopressin, ethanol-water (2:1) system did not cause an enhancement in the permeation compared to control.[42]

Effect of type of current

The direct current was more efficient than pulse current to promote TH permeation ($P < 0.05$). The flux of the TH decreased to half with pulse current (2.5 kHz, 50% on:off, flux = 173.57 ± 34 µg/(cm^2 h)) when compared to constant dc of same current magnitude (flux = 355.71 ± 25 µg/(cm^2 h)) (Figure. 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.[43] Moreover, the reduction of the current quantity passing through the skin diminishes the risk to skin alterations, like burns.[44] 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.[44-46] The observations, however, contradict results obtained by others working with macromolecules such as insulin.[44,47] 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.[48] During application of constant dc and subsequent polarization, the smaller molecules (like TH) may escape through the paths of low skin impedance (like skin appendages: hair follicles and sweat glands), however the bulkier molecules 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.

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

Figure. 4 shows effect of varying on: off ratios of pulse current (2.5 kHz) on TH permeation through porcine 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%, TH flux decreased from 261.43 ± 42 µg/(cm^2 h) to 103.57 ± 22 µg/(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 TH permeation is shown in Table 4. As the frequency of the pulse current increased, the flux of TH 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.[43] This result is in agreement with the results obtained by Clemessy M et al. [48] with angiotensin.

Table. 4. Effect of frequency of pulse current (50% on:off) on rate of permeation of TH through porcine skin

Frequency , (kHz)	Flux, *Jss* (μg/(cm^2 h))	Enhancement factor (E)
1.25	150.51 ± 40	14.64
2.50	173.57 ± 34	16.88
5.00	189.72 ± 27	18.45
10.00	213.43 ± 52	20.76

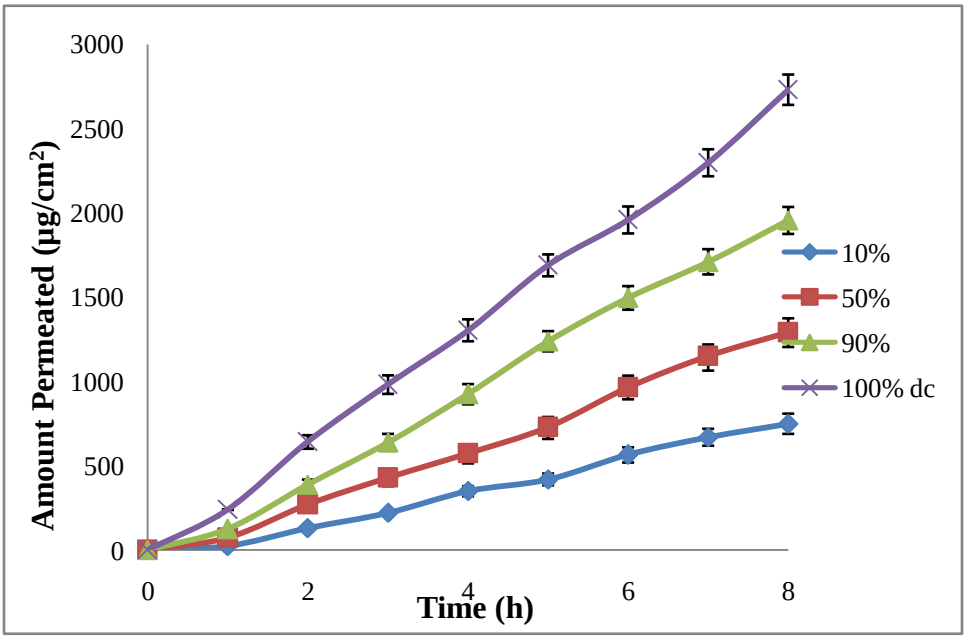


Figure 4: Effect of on:off ratio of pulse current on permeation of TH through porcine skin. Current magnitude was 0.5 mA/cm^2 and on:off ratio of pulse current (2.5 kHz) (n=3)

Effect of duration of current application

Figure. 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 lag time is evident for passive diffusion. With iontophoresis decrese in lag time was significant. The shorter lag time of TH with iontophoresis indicates the possible transport of ionized species through shunts (hair follicles and sweat ducts)[49] Flux was greater when iontophoresis was applied for 8 instead of 1 h. Termination of current did not cause the flux to return immediately to the passive control level in both 1 and 4 h treatment. When the current was applied for 1 h (or 4 h) and terminated, the cumulated quantity

of TH 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 TH 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.

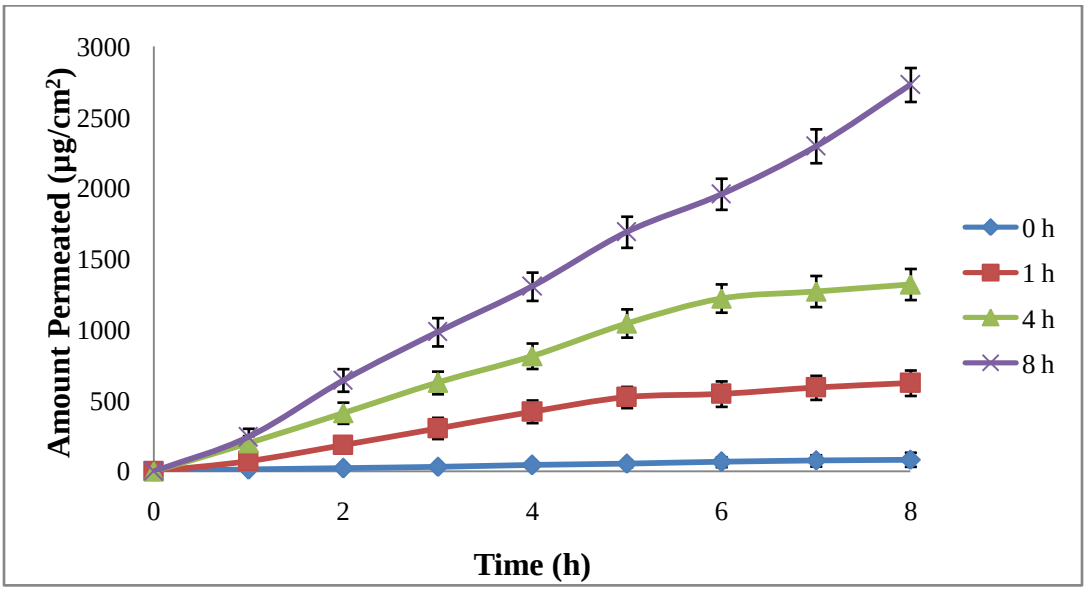


Figure 5: Effect of duration of current application on permeation of TH through porcine skin. Donor concentration of TH was 20mg/ml and current applied was 0.5 mA/cm^2. (n=3)

CONCLUSION

In summary, our work demonstrates that TH penetrates the skin and that iontophoresis is a very efficient technique for enhancing its transdermal delivery. Furthermore, as in most iontophoretic systems, there was a linear relationship between current density and drug flux. At the maximal investigated current density of 0.5 mA/cm^2, the observed flux was 355.71 ± 25 µg/(cm^2 h). The pharmacokinetic data of TH indicate that for the maintenance of the therapeutic level, a transdermal flux of 7.096 mg/h is required for a 60 kg individual. If *in vivo* follows the same profile, the requirement can be met up with a system of ~ 20 cm^2 of skin contact area.

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