Available online at www.ijddt.com
International Journal of Drug Delivery Technology 2010; 2(1) 18-23

Research Article

Evaluation of Passive and Iontophoretic Transport of Lisinopril from Hydrogel Matrix Across Model Membrane *In Vitro*

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ABSTRACT

Clinical studies of lisinopril delivery through iontophoresis are highly desired for better controls over transdermal drug flux. Therefore, investigations were carried out to ascertain the relative importance of the various factor for iontophoretic transport using an ionizable drug lisinopril, which has four pK_a values 2.4, 4.0 (for amino group) and 6.7, 7.0 (for carboxylic group). Ionization of lisinopril varies with pH, hence rate and extent of transport across the skin can be enhanced, controlled and manipulated by the application of factors like anodal and cathodal current at varied pH of donor solution and current densities. To determine these parameters, experiments were performed and data were collected at 3.0, 4.0 and 7.4 pH using 4 mg/ml drug concentration and 0.1 mA/cm^2 current density for 10 hours. After establishing the pH for optimum transport of drug, effect of current density (0.1, 0.2, 0.3 and 0.4 mA/cm^2) on the transport of drug (keeping drug concentration constant) were investigated. Passive diffusion of lisinopril was maximal at pH 3.0, when unionized form of drug was 45%. Anodal iontophoresis was most effective (significant result, $p < 0.05$) in transport of drug across skin as compared to cathodal iontophoresis at pH 3.0. While at pH 4.0, cathodal iontophoretic transport of lisinopril across rat skin was highly effective (Student't' test, $p < 0.05$) compared to anodal iontophoresis. The effect of current density on steady state flux of lisinopril during cathodal iontophoresis at 7.4 pH was 1.33 ± 1.12 and 24.8 ± 3.1 µg/cm^2/h at 0.0 under passive diffusion and 4 mA/cm^2, respectively. Thus, flux was enhanced nearly 18.6 times during anodal iontophoresis as compared to passive diffusion. For cathodal flux at pH 3.0 on similar iontophoretic treatment showed enhancement nearly 4 times.

Key words: *Iontophoresis, Transport, Steady state flux, Current density, Lisinopril.*

INTRODUCTION

The skin has been identified as a route of drug administration for decades. Several drug delivery systems has been developed for utilizing this route and the ultimate goal is to ensure that compounds are delivered preferably at a specific rate to the systemic circulation. Topical drug delivery system has some limitations, arising mainly from excellent barrier properties of *stratum corneum*. Iontophoresis has potential to overcome many barriers associated with transdermal delivery of drugs and it also broadens the spectrum of drugs that can be delivered via skin, increases systemic treatment efficacy therefore, this method of transport is in high demand for increasing permeation.[1-3] Iontophoresis uses a small electrical current to enhance the transport of both ionic and nonionic molecules across the skin in controlled and programmable manner.[4,5] The enhancement of drug due to this method results from a number of possible mechanisms including the ion-electric field interaction (electrorepulsion),[5] convective flow (electro-osmosis)[6] and current-induced [7] increase in skin permeability.

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In the present investigation lisinopril was selected as a model drug which can be extensively administered transdermally as it is devoid of any pungent skin sensation and burning pain. Furthermore, for clinical implications transdermal formulation of lisinopril delivery is highly desired as it helps in avoiding the hepatic first-pass effect and shall lead to patient convenience as well as improved compliance.[8] It was therefore, decided to work on optimization of parameter affecting i.e., pH and current density in the transport of lisinopril across bio-system iontophoretically from gel formulation.[9,10]

MATERIAL AND METHODS

Materials

The constant current source (0-4 mA) was designed and fabricated by University Instrumentation Science Centre (USIC), Guru Nanak Dev University, Amritsar, India, which can be operated at a resistive load of 10KΩ and was assembled by M/S B. S. Electronics, Amritsar, India. Generous gift sample of lisinopril was obtained from Ranbaxy Research Laboratory, Gurgaon, India and analytical grade chemicals such as disodium hydrogen phosphate, sodium acid phosphate, sodium hydroxide, potassium hydrogen orthophosphate were procured from Qualigens fine chemicals, Mumbai. India.

Preparation of buffer

The buffer used for receptor solution was prepared by dissolving 2.1 g of sodium acid phosphate and 4.4 g of

sodium chloride in 1.0 lit of water (deionized double distilled water purified in a Milli-Q ® water system, Millipore).

Preparation of gel formulation[11]

Gel formulations (100g) were prepared by dissolving lisinopril (3%) in a solvent mixture (ethanol:propylene glycol: water in the ratio of 50:30:20). The solution was finally gelled by adding 8 % HPC carefully with constant stirring at 500 rpm for 15 min. After stirring, the beaker containing the gel was allowed to stand in a water bath at normal temperature of 25 ^{0}C for 30 min and set aside for 24 h.

Collection and preparation of skin sample [12]

Skin diffusion experiments were carried out on full-thickness skin from Wistar male rats[13] (8-12 week old) supplied from animal house of Guru Nanak Dev University, Amritsar. The experiments using animal were carried out as per the ethical guidelines and housed at appropriate conditions 12 hour light and 12 hour dark side (CPCSEA No 226).

Rats were scarified by cervical dislocation. The abdominal hair of the rat was removed with electric clippers and the skin was excised. The whole thickness of skin was removed and divided along the sagittal plane into two pieces (left and right sides); excess adipose tissue was removed by gentle scraping. The skin pieces were soaked in the receptor buffer solution for approximately 45 min prior to being placed in the cells.[12]

***In vitro* permeation studies** [14]

The *in vitro* permeation studies were performed using horizontal glass diffusion cells. The dorsal side of the excised skin was used as the model skin barrier. The skin was mounted between the donor and receptor half-cells of each skin permeation system with the *stratum corneum* facing the donor half-cell. A thin film of petroleum gelly was spread on the lapped glass surface to the cell to provide a watertight seal. The receptor phase contained 5.0 ml of phosphate buffer (pH 7.4). For permeation studies from hydrogel vehicles, 5.0 g of hydrogel-containing drug was used as the donor vehicle. The cells were clamped and immersed in a water bath 37 $\pm$ 0.5 ºC placed on the magnetic stirrer. The maximum capacity of each of the donor and receiver compartments was 5.0 ml and the surface area of skin exposed to the solution was 2.855 cm^2. The medium of the diffusion cell was stirred at the rate of 200 rpm using small glass magnetic bead. For passive diffusion, this assembly was used as such.

Iontophoresis protocols [12]

Iontophoresis was carried out by inserting a pair of platinum wires having an effective length of 15 mm (99.99 % purity, 0.5 mm in diameter) as electrodes[15] into gel. The electrodes were each positioned 3 cm from the side of rat skin. The electrodes were connected to a constant current device. Anodal iontophoresis was carried out by inserting anode in the donor compartment and cathode in receiver compartment. Cathodal iontophoresis was done by reversing the polarity. The samples (300 µl) were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh buffer solution. The samples were then analyzed by the HPLC methods.

Optimization of iontophoretic application

Different studies were conducted and data were collected at three different pH values (3.0, 4.0 and 7.4) using 4.0 mg/ml drug concentration, and 0.1 mA/cm^2 current density for 10 hour. After obtaining the optimum transport of drug at a particular pH, effect of current density (0.1 mA/cm^2, 0.2 mA/cm^2, 0.3 mA/cm^2 and 0.4 mA/cm^2) on transport of drug was investigated at the same drug concentration for 10 h.

Sample analysis [16]

At regular interval of time 1.0 ml receptor solution were withdrawn and replace the volume with equal amount of receptor buffer. The receptor solution was then filtered through a 0.45 µm polyvinyldifluoride filter (Millipore), and then evaporated to dryness in vacuum at 50 ^{0}C. The dry residue was dissolved in 1.5 ml of HPLC mobile phase and the solution was centrifuged (Sigma 2-15, Germany) for 10 min at 3500 rpm. The supernatant was filtered and lisinopril was quantified in the filtrate by HPLC.

Chromatography[17]

Quantitative HPLC was performed on gradient High Performance Liquid Chromatograph (Perkin Elmer 200 series) with Series 200 pump, UV-VIS detector 200 Series set at 215 nm and Link 600 Series and Spheri 5-RPC-18 Column (150 mm x 4.6 mm, particle size 5 µm). Gradient elution was carried out with 0.02 M phosphate buffer solution (pH = 3.2, eluent A, 90 %) and acetonitrile:methanol: Tetrahydrofuran (4:4:2; eluent B, 10 %). The chromatograph was run for 10 min at flow rate of 0.1 ml/min of the mobile phase; the temperature of the column was held at 55 °C. The data were collected and analysed using computer software TCN version 6.2.1(Perkin Elmer).

Data analysis

The drug concentration was corrected for sampling effects according to the equation described by Hayton and Chien:[18]

$$C'_n = C_n (V_t / V_t - V_s) (C'_{n-1} / C_{n-1}) \ldots\ldots\ldots \quad (1)$$

Where $C'_n = C_n$ is the corrected concentration of n^{th} sample, C_n is the measured concentration of drug in the n^{th} sample, C_{n-1} the corrected concentration of the drug in the $(n-1)^{th}$ sample, V_t the total volume of the donor solution, V_s is the volume of the sample withdrawn. The corrected drug concentration was divided by the area of the skin exposed to the donor solution to calculate the cumulative amount of drug permeated per unit area.

The cumulative amount of drug permeated per unit area is plotted against time and the flux was calculated by the slope of the linear portion of cumulative amount vs $(time)^{1/2}$ plots for Higuchi model and expressed as the mass of drug passing across 1 cm^2 of skin over time or square root of time.[19]

The permeability coefficient (K_p in cm/h)[20-22] was calculated by

$$K_p = J_{ss} / C_v \ldots\ldots\ldots\ldots \quad (2)$$

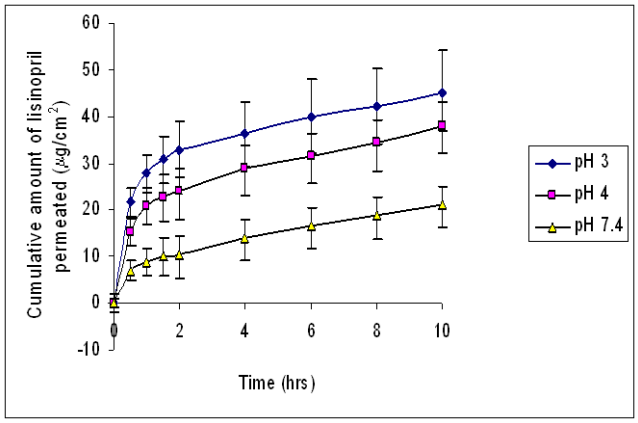

Fig. 1: Effect of pH on the transport of lisinopril across rat skin during passive diffusion

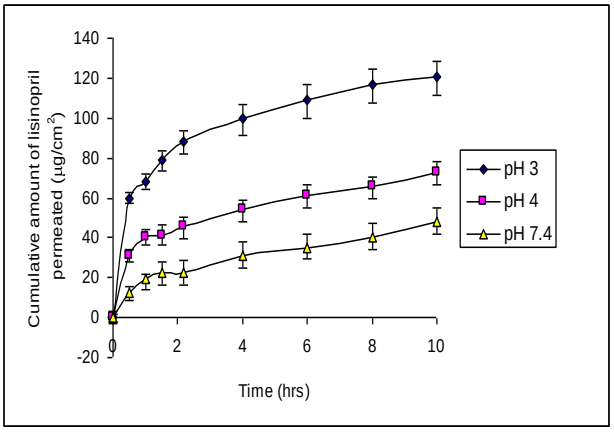

Fig. 2: Effect of pH on the transport of lisinopril through rat skin during anodal iontophoresis at current density 0.1 mA/cm^2

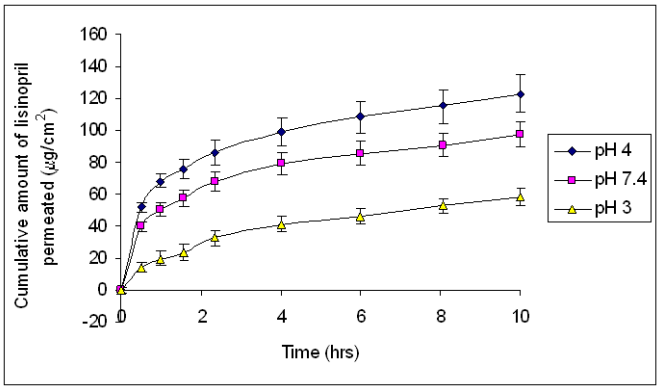

Fig. 3: Effect of pH on the transport of lisinopril during cathodal iontophoresis at current density 0.1 mA/cm^2

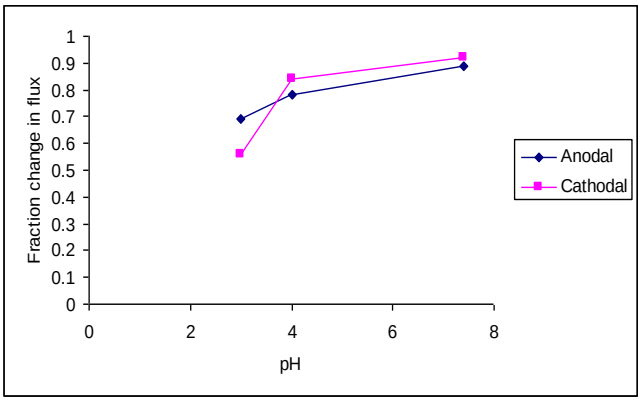

Fig. 4: Relationship between fraction change in flux and the fraction of lisinopril ionized at different pHs

Where, J_{ss} is the steady state flux and C_v is the total donor (unionized and ionized) concentration.

Enhancement factor is calculated by equation[23-25]

$$E = J_i / J_p \quad \text{.....} \quad (3)$$

Where, J_i is the flux of the drug during iontophoresis and J_p the flux of the drug during passive diffusion.

Fraction change in the flux is determined by deducting the passive flux from iontophoresis flux and then dividing it by iontophoretic flux.

$$\text{Fraction change in the flux} = J_i - J_p / J_i \quad \text{.....} \quad (4)$$

Fraction ionized (degree of ionization) is calculated on the basis of Henderson Hesselbalch equation[26,27]

$$\text{Fraction ionized} = 1 - \text{Fraction unionized} \quad \text{.....} \quad (5)$$

Statistical analysis

Statistical analysis of the data was done by student's t-test, a value of $p < 0.05$ was considered to be significant.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S\sqrt{[1/n_1 + 1/n_2]}} \quad \text{.....} \quad (6)$$

$$d.f = n1 + n2 - 2 \quad \text{.....} \quad (7)$$

$$S^2 = \frac{(n_1-1)\,S.D_1^2 + (n_2-1)\,S.D_2^2}{d.f} \quad \text{.....} \quad (8)$$

Where $\bar{X}_1$ and $\bar{X}_2$ are the means, n_1 and n_2 are number of samples, $S.D_1$ and $S.D_2$ are standard deviation of two sets of data and d.f is the degree of freedom.

RESULTS

It is reported that platinum wire electrodes caused shift in the pH of the donor compartment to the higher side during cathodal iontophoresis due to electrochemical decomposition of water.[28] Therefore, buffer of sufficient buffering capacity in the donor medium was used to avoid pH drift during experiments.

Effect of pH

The cumulative amount of lisinopril permeated under different pH conditions during passive diffusion is shown in fig. 1. The passive transport was found to decrease as the pH of the donor gel formulation was increased from 3.0 to 7.4. It is evident from the fig. 1 that the minimum cumulative amount is permeated form the donor gel of pH 7.4 during passive diffusion. Fig. 2 shows the cumulative amount of the drug transported during anodal iontophoresis. The iontophoretic transport increased as the pH of donor gel was decreased from 4.0 to 3.0 and was minimum at pH 7.4. The transport during cathodal iontophoresis is shown in fig. 3. The cumulative amount of lisinopril permeated through skin from donor gel at pH 3.0 was minimum and cathodal iontophoeretic transport was maximum at pH 4.0. The transport at pH 7.4 was less as compared to that at pH 4.0, but greater than pH 3.0. The cathodal transport was greater than anodal iontophoresis at pH 4.0 and 7.4 ($p < 0.05$, t

test).The steady state flux during iontophoresis (cathodal and anodal) was significantly higher ($p < 0.05$, t test) than the corresponding passive flux at all pH values (Table 1). The steady state flux obtained during anodal and cathodal iontophoresis showed significant difference at all the pH values investigated. The permeability coefficient (Kp) of lisinopril is given in table 2.Iontophoresis resulted in an increase in the permeability coefficient at all pH values. Permeability coefficient was highest during cathodal iontophoresis at pH 4.0. The enhancement factor for lisinopril is given in table 3. Cathodal iontophoresis shows significant enhancement in the flux as compared to anodal iontophoresis at pH 4.0 and 7.4. The relationship between faction change in flux and the fraction of lisinopril ionized is shown in table 4 and fig. 4.

Table 1: Effect of pH on the steady state flux: mean ±S.D. of three determinations of lisinopril during passive diffusion and iontophoresis at current density 0.1 mA/cm^2

pH	Steady state flux, μg/cm2/h		
	Passive[a]	Anodal[a]	Cathodal[a]
3.0	5.90 ± 1.12	19.5± 2.23	13.5+1.36
4.0	3.5 ± 2.31	16.2+1.36	22.1± 1.14
7.4	1.33 ± 1.12	12.2+2.11	18.2+1.32

[a] All values are expressed as mean ± S.D.; n = 3

Table 2: Permeability coefficient of lisinopril at different pHs during passive diffusion and iontophoresis

pH	Permeability coefficient, Kp (cm/h) x10-3		
	Passive	Anodal	Cathodal
3.0	11.8	39.0	27.0
4.0	7.00	32.4	44.2
7.4	2.66	24.4	36.4

Table 3: Enhancement factors for lisinopril at different pH through rat skin

pH	Enhancement factor		
	E1	E2	E3
3.0	3.30	2.28	0.69
4.0	4.62	6.31	1.36
7.4	9.17	13.68	1.49

E1= Ja / Jp E2 = Jc / Jp E3 = Jc / Ja

Where, Ja = Steady state flux during anodal iontophoresis
Jc = Steady state flux during cathodal iontophoresis
Jp = Steady state flux during passive diffusion

Effect of current density

The effect of current density on the transport of lisinopril through rat skin at pH 3.0 and 7.4 was investigated. The cumulative amount of lisinopril at pH 3.0 during cathodal iontophoresis at different current densities is shown in fig. 5. The transport of the drug increased with the increase in current density. Similarly, anodal iontophoretic transport at pH 7.4 was found to increase with increasing current density (Fig. 6). The steady state flux of lisinopril at pH 3.0 during cathodal iontophoresis was 5.90 ± 1.12 and 23.9 ± 2.01 μg/cm^2/hr at 0.0 (i.e., passive diffusion) and 4.0 mA/cm^2 current densities, respectively. Therefore, increasing current density from 0.0 to 4.0 mA/cm^2 enhanced the flux by about 4 folds. Whereas, anodal flux at same current densities range, showed 18.6 times enhancement (Table 5). Similarly, the permeability coefficient of the drug also increased with increase in the current density. Delivery efficiency of lisinopril at pHs 3.0 and 7.4 is shown in table 6. A relationship between the steady state flux for anodal (pH 7.4) and cathodal (pH 3.0) is shown in fig. 7.

DISCUSSION

The release profile of lisinopril from gel formulation was found to be curvilinear and is shown in figure 1 to 3. Highest flux was observed during first one and half hours indicating that there is a great initial burst effect from gel bases i.e., release rate of drug ranges from 19 μg/h to 380 μg/h, which gradually decreased from 47 μg/h to around 7 μg/h at the end of 10 h. Reason for burst effect in the initial stages may be the release of drug from the surface of gel. Later on, burst release does not occur, which suggests that the bulk of the drug contained in the hydrogel matrix is released under control of iontophoresis. This biphasic pattern of drug release is characteristic of matrix diffusion kinetics.[29] Furthermore, during investigation on studying the effect of different variables on passive and iontophoretic transport, it was observed that not only final release but also the initial burst was affected increased. When the release characteristic of lisinopril from gel formulation was analysed by Higuchi equation,[30] it was found that transdermal iontophoretic flux of lisinopril from gel base was best explained (correlation coefficient, R^2 = 0.9321- 0.9643). In other words, drug release was following diffusion kinetics during 2 h of iontophoretic study and at the later-stage the flux appeared to level off. The results were in consonance with the literature reports on iontophoretic delivery of salbutamol,[31] piroxicam[32] and in vitro release of dibucaine and 5-flurouracil from hydrogel bases.[33]

Since the in vitro release studies were performed at 37 ± 0.5 ^{0}C, and at this temperature it was expected that solvent components of alcohol and water evaporate, therefore, becomes more concentrated gel of lisinopril and formation of thin film over the membrane occurs. Consequently, it is reasonable to believe that lisinopril might be released by diffusion through this film.[34] Moreover evaporation of solvents disturbs the proportions of solvent system in the gel which inturn change the thermodynamic activity of the drug; the latter is supposed to be the main driving force for drug permeation across membrane. At the later stage i.e., due to prolonged contact of the thin film over the membrane, its integrity is distorted, which leads to more complex release behavior.

Alternatively, loss of water and alcohol under experimental conditions may be making the gel more viscous and forming a saturated solution of drug in a gel. Previous studies have revealed that viscosity of gel formulation not only affects the passive transport, but also the transdermal iontophoretic delivery of permeant.[35,36] Thus, with gel formulation which have increased viscosity there results a decrease in the formulation conductivity and the iontophoretic flux of lisinopril. Nevertheless other factor such as change in the thickness of the film of gel by more evaporation of solvent at latter stage, change in the thermodynamic activity of the drug and nature of the rat skin may be responsible factor for influencing the diffusion of drug across rat skin. It is also possible that with the use continuous current iontophoresis,a skin polarization potential is developed that eventually

Table 4: Effect of pHs on the fraction ionized and fraction change in the flux of lisinopril

pH	Fraction ionized[a]	Fraction change in flux[b]	
		Anodal	Cathodal
3.0	0.55	0.69	0.56
4.0	0.85	0.78	0.84
7.4	0.99	0.89	0.92

[a]Fraction of lisinopril ionized has been calculated by Handerson - Hasselbalch equation

[b]Fraction change in flux = $\frac{\text{Iontophoretic flux} - \text{Passive flux}}{\text{Iontophoretic flux}}$

Table 5: Effect of current density on the steady state flux of lisinopril during iontophoresis

Current density mA/cm^2	Steady state flux, µg/cm^2/h	
	Anodal (pH 7.4)[a]	Cathodal (pH 3.0)[a]
0.0	1.33 ± 1.12	5.90 ± 1.12
0.1	12.2 ± 2.11	13.5 ± 1.63
0.2	22.1 ± 2.02	18.3 ± 2.56
0.3	24.2 ± 2.31	21.5 ± 3.26
0.4	24.8 ± 3.1	23.9 ± 2.01

[a] All values are expressed as mean ± S.D.; n = 3

Table 6: Permeability coefficient of lisinopril at different current densities

Current density mA/cm^2	Permeability coefficient, Kp (cm/h) ×10^{-3}	
	Anodal (pH 7.4)	Cathodal (pH 3.0)
0.0	2.66	11.8
0.1	24.4	27.0
0.2	44.2	36.6
0.3	48.4	43.0
0.4	49.6	47.8

decrease the magnitude of effective current.[37] Consequently, iontophoretic flux did not increased significantly after 2 hours, though it increased rapidly up to 2 hours, in all experiments.

Effect of pH

There is observed a decrease in the steady state flux of lisinopril as the pH increases from 3.0 to 7.4 during passive diffusion. Lisinopril is a weekly acidic drug with molecular weight 441.52 and has pKa value of 2.5, 4.0, 6.7 and 7.0. Therefore, the drug is in highly ionized at pH 7.4 and exits as the carboxylate ions. At pH 3.0 substantial amount of the drug will be in unionized form. The decrease in the steady state flux from 3.5 to 7.4 is due to increase in the ionized fraction of the drug. The results comply with the pH partition hypothesis according to which increased permeation of unionized species of the lisinopril occurs through lipophilic biological membrane.[38,39] Substantial transport of drug during cathodal iontophoretic transport of drug at pH 4.0 and 7.4 may be due to increased ionic mobility caused by 85% and 99% ionization of lisinopril, respectively (Table 4). Nernst-Plank equation verifies this phenomenon,[40] according to which, the steady state flux depends on the charge on the solute molecule, ionic mobility and electrical potential applied besides the concentration of solute. The steady state flux during anodal iontophoresis is maximum at pH 3.0, where fair amount of the drug exists in unionized form. This may be explained on the basis of permselective nature of the skin. The epidermis of the skin above pH 4.2 (isoelectric point) acquires negative fixed charge density in the pore at neutral and alkaline pHs, and may be positively charged at acidic pHs,[22] therefore, at pH 3.0, accumulation of positive ion concentration (counter ions) in the pores results. This leads to the movement of negatively charged or uncharged permeant, and impedes positively charged permeant in the direction of counter ions transfer (convective flow).[41,42] Therefore, combined effect of electrical and convective transport induces a net volume flow during iontophoresis at pH 3.0. The same interpretation is valid for

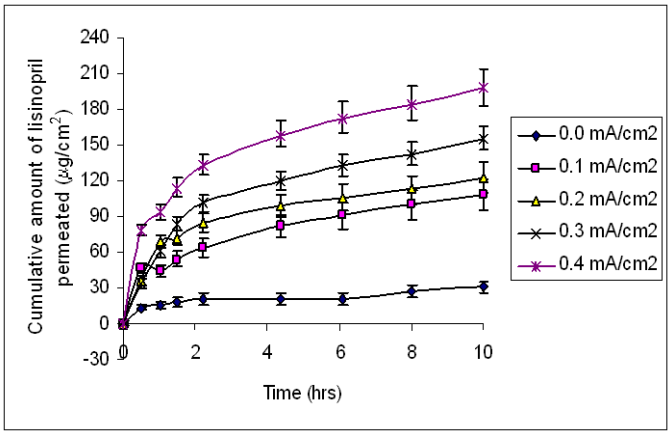

Fig. 5: Effect of current densities on the permeation of lisinopril through rat skin during cathodal iontophoresis at pH 3.0

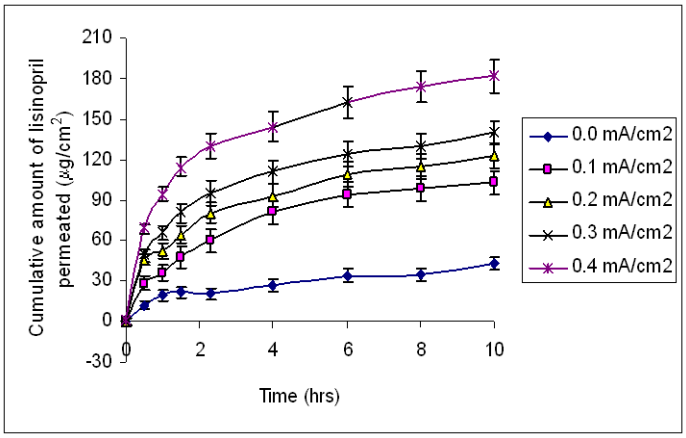

Fig.6: Effect of current densities on the permeation of lisnopril across rat skin during anodal iontophoresis at pH 7.4

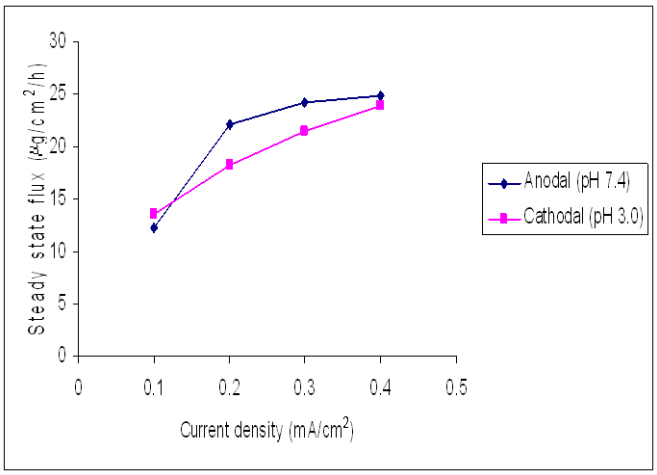

Fig. 7: Effect of current densities on iontophoretic delivery of drugs

higher flux obtained at pH 4.0 during cathodal iontophoresis. The results obtained comply with the previously established physicochemical factors such as net charge density of the membrane, ionic fraction of drug, ionic strength and the magnitude of applied electric field, that governs the iontophoretic transport of drug.[43,44] In addition, presence of two negative centers in the lisinopril at pH 7.4 also contributes in high conductivity during cathodal iontophoresis, thus enhancing the cathodal flux of the drug. While at pH 4.0 substantial amount of the drug exists in ionized form, transport of the drug was mainly due to electrostatic repulsion by the respective electrode and lesser by convective solvent flow process, which is mainly observed for unionized moiety. Therefore, during cathodal iontophoresis, maximum flux at pH 4.0 may be attributed to the contributions from both passive diffusion term (mainly due to unionized fraction of the drug) and electrical term (due to electrostatic repulsion of the ionized fraction of the drug). It is thus clear that both the form of drug species i.e., unionized and ionized can permeate through rat epidermis may be through intracellular[44] and intercellular routes.[46-48]
The maximum iontophoretic flux enhancement and fraction change in flux at pH 3.0 and 4.0 are better explained in terms of model proposed by Sims and Higuchi,[51] wherein transport of ionized and non ionized drug moiety occurs. It is thus clear that both the form of drug species i.e., unionized and ionized can permeate through rat epidermis may be through intracellular[44] and intercellular routes.[46-48]
The maximum iontophoretic flux enhancement and fraction change in flux at pH 3.0 and 4.0 are better explained in terms of model proposed by Sims and Higuchi,[51] wherein transport of ionized and non ionized drug moiety occurs from aqueous pore pathways and through the parallel lipoidal phase of the *stratum corneum* by the process of diffusion, and partition, respectively. Therefore, in general term it can be stated that when pore pathways dominate (due to iontophoretic treatment), the difference between passive flux and iontophoretic flux becomes larger.[24] The result comply with the literature reports.[49,50]
Moreover, long term application of current leads to the dissipation of heat, which in turn increase lipid fluidity and thus changes in the integrity of the skin structure,[51] consequently permeability of the skin is altered.[52,53] This may be the reason for increased permeability coefficient of drug during iontophoresis (anodal and cathodal) at all pHs compared to the corresponding values for passive diffusion.

Effect of current density

The results indicate that the rate of increase in the flux with increase in current is more during anodal iontophoresis at pH 7.4 than cathodal iontophoresis at pH 3.0 (Fig. 7), suggesting a direct relationship between flux and current density. The relationship between current density and flux of the lisinopril may be described by Faraday's law which is represented by following equation[54]

$$J_i = \frac{t_i I_T}{z_i F} \quad \text{.....} \quad (9)$$

where J_i, Z_i are the flux and charge of lisinopril at particular pH, I_T is the applied current density, F is the Faraday's constant, t_i is a proportionality constant. Since in the experimental conditions t_i (constant, transport no.) and Z_i i.e., charge of the drug (percent of the drug ionized) was kept constant, then above equation 9 is transformed into $J_i \propto I_T$. This equation justify the obtained result i.e., flux is directly proportional to the current density. The linear dependence of the lisinopril flux indicate that the resistance decreases with increasing current density.[55-57] The decrease in electrical resistance of the skin may be due to the change in electric properties of the skin. Literature reports[58] confirmed that application of iontophoretic current causes disordering of the intercellular lipids of stratum corneum which become more marked on increasing current density. Another possibility may be also that the electroosmotic volume flow increases with an increase in current density,[59] which leads to increase in the flux of the drug. Solvent flow occurs across a membrane carrying fixed charges when an external electric potential gradient is applied. Given that the skin is negatively charged, this fluid flow will occur in the direction of cation flux. Such flow will either enhance the transport of cations or retard the transport of anions.
The experimental data indicated that by proper selection of donor solution, pH and type of iontophoresis (cathodal or anodal), magnitude of lisinopril transport across skin can be manipulated.

CONCLUSION

In summary, the delivery of ionizable drug, lisinopril using iontophoresis can be manipulated, controlled and optimized, by taking into account of following features:

1. Iontophoresis (cathodal and anodal) substantially enhanced the transport of lisinopril through the rat skin as compared to passive diffusion.
2. Passive diffusion of lisinopril was maximum at pH 3.0, when 45 % of lisinopril was present as unionized species.
3. Cathodal iontophoresis was more effective as compared to anodal iontophorersis at pH 4.0.
4. Anodal iontophoresis was more effective as compared to cathodal iontophorersis at pH 3.0.

ACKNOWLEDGEMENT

Financial support was provided by All India Council for Technical Education, New Delhi under R & D scheme in the project on iontophoresis.

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