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# Iontophoretic Permeation of Lisinopril at Different Current Densities and Drug Concentrations

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**ABSTRACT**

***Purpose:*** The purpose of the present work was to assess iontophoretic permeation of Lisinopril at different current densities and concentrations for development of patient-controlled active transdermal system. ***Methods:*** *In vitro* iontophoretic transdermal delivery of Lisinopril across the pigskin was investigated at three different drug concentrations and three different current densities (0.25- 0.75 mA/cm$^2$) in the donor cell of the diffusion apparatus, using cathodal iontophoresis along with the passive controls. ***Results:*** For passive permeation, the steady state flux significantly increased with the increasing of donor drug concentration. At all concentration levels, iontophoresis considerably increased the permeation rate compared to passive controls. Iontophoretic transport of Lisinopril was to be found increase with current densities. Flux enhancement was highest at the lowest drug load and lowest at the highest drug load. ***Conclusion:*** The obtained results indicate that permeation rate of Lisinopril across the pigskin can be considerably enhanced, controlled or optimized by the use of Iontophoresis technique.



## Introduction

Transdermal delivery of drugs through the skin to the systemic circulation provide a convenient route of administration to the delivery of drugs for prolonged therapy in diseases like hypertension and diabetes.[1] Cardiovascular diseases account for a large proportion of all deaths and disability worldwide. Global Burden of Disease study reported that in 1990 there were 5.2 million deaths from cardiovascular diseases in economically developed countries and 9.1 million deaths from the same causes in developing countries.[2,3] Worldwide frequency estimates for hypertension may be as much as 1 billion individuals, and approximately 7.1 million deaths per year may be attributable to hypertension.[4]

There are many oral drugs used in the treatment of hypertension. However problem may arise with oral delivery due to uneven bio-distribution throughout the body, a lack of drug targeting specificity, the necessity of a large dose to achieve high blood concentration and adverse side effects due to such high doses**.** It is also noted that difficulties in the treatment of hypertension does not necessarily lie in the inadequacies pharmacological therapeutics but it may be due to poor patient compliances during lifelong administration. Approximate 50 % of hypertensive patients do not fulfill with their prescribed treatment.[5] Reason suggested for this complication includes intolerable side effects, complex treatment and lake of reminders.[5]

Lisinopril is an angiotensin-converting enzyme inhibitor used for the treatment of hypertension. It is available only in the form of oral tablets in the market with slow and incomplete absorption after oral administration with a bioavailability of 25–30%.[6,7] Lisinopril is an ideal candidate for transdermal study because of low oral dose (2.5–20 mg), low molecular mass (405.5 g/mol), and low oral bioavailability (25%). This study investigated the iontophoretic permeability of lisinopril through excised pig skin to assess its potential for the development of a patient-controlled active transdermal system.

The first commercial patch was approved for scopolamine in 1979. Some other available transdermal patches in world drug market are: nitroglycerin, nicotin, clonidine, fentanyl, estradiol, testosterone, lidocain, and oxbutinin. Skin has been recognized as a route of drug administration for decades but limited permeability of human skin is still a basic problem, restraining its widespread therapeutic use also limiting suitability of number of drug candidates for this route. So it is the very big challenge of creating effective transdermal system because it involves adequate drug permeability through the stratum corneum.[8] Introduction of the physical enhancement techniques like iontophoresis combined with the prospect of the programmed delivery has contributed significantly in the expansion of transdermal research. Iontophoresis

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may be defined as facilitated movement of compounds, in particular charged moiety, into or across a membrane by the application of an externally applied electrical potential difference across the membrane.[9] Iontophoresis has provided a non-invasive delivery system for local or systemic delivery of drugs. Beside the common benefit of transdermal delivery iontophoresis presents a unique opportunity to provide programmed drug delivery.[10] The enhancement of permeation by the iontophoresis results from a possible mechanism including electro-osmosis, electro repulsion or current induced.[11,12] Iontophoretic products have already been launched in the US market and gained growing acceptance for the topical delivery of drugs[13,14] hence attempts have been undertaken to develop transdermal iontophoretic system for lisinopril.

## Materials and Methods

Lisinopril was a gift sample from Lupin laboratories Bhopal. Silver plates (purity 99.99%, 5 mm diameters) were obtained from a goldsmith shop Bhopal, India. Sodium Chloride AR, octanol, isopropyl alcohol, silver chloride were obtained from SD Fine-Chem (Mumbai, India). Franz-diffusion cell was designed by Peekay scientific glass wares, Bhopal, All the reagents/chemicals used were of analytical grade. Experiments were conducted with ultra pure water (resistivity, 18.2 MW cm) obtained from Milli-Q Academic System.

### *Equipment*

Iontophoretic DC source (digital display, current 0-10 mA, voltage 0-25 V) was purchased from C-tech Psu-2510/lab (Mumbai, India) and iontophoretic diffusion cell was fabricated by Navin Scientific Glass Product (Bangalore, India) as per given specifications. Silver/silver chloride electrode was prepared as per the standard procedure.[14] Silver wire (99.99% pure, 1.0 mm thickness) was used as connecting wire. U-V Visible Spectrophotometer- Shimadzu UV-1700 PC Shimadzu Corporation, Japan was used for analysis.

### *Experimental Design*

Iontophoretic transdermal permeation study of Lisinopril was carried out at different concentrations and current densities. Different systems having different concentration (current density 0.5 mA/cm$^2$) had been designed as System A, System B, System C having concentration 25 mg/ml, 50 mg/ml and 75 mg/ml respectively. Moreover system with different current densities (concentration 25 mg/ml) designed as System A1, System A2, SystemA3 having current densities 0.25, 0.5 and 0.75 mA/cm$^2$ respectively.

### *Preparation of skin membrane*

From a local abattoir, ear was 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 tap water and stored at refrigerator in aluminum foil packing and was used within two days.[15]

### *In vitro passive permeation studies*

The *in vitro* passive permeation studies were performed using vertical type Franz diffusion cell having a receptor compartment capacity of 10 ml. The excised skin was mounted between the half-cells with the dermis in contact with receptor fluid (Phosphate buffer pH 7.4) and equilibrated for 1 h. The area available for diffusion was about 1.21cm$^2$. The donor cell was covered with an aluminum foil to prevent the evaporation of vehicle. The fluid in the receptor compartment was maintained at 37±0.5 °C. Under these conditions, the temperature at the skin surface was approximately 32 °C. Different solution of lisinopril in phosphate buffer pH 7.4 (each one ml) was placed in the donor compartment. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from the receptor compartment at regular intervals and assayed for drug content.[16]

### *Procedure of iontophoretic diffusion*

For the iontophoretic study, diffusion cell was modified according to Glikfield et al.[17] The apparatus essentially consisted of a glass molded large receiving chamber provided with two parallel ports on the topside and a sampling port on the side. Two upper chambers are made from open-ended cylindrical glass tubes, the outer diameters of which were equivalent to the inner diameter of the parallel ports. The lower 10 mm of these tubes were slightly constricted to allow a clearance of 1 to 1.5 mm on the side. This ensured easy fitting. After the skin was tied at this constricted end, the effective diameter increased and became exactly equal to inner diameter of the extended ports. Once slipped into parallel ports, they stay attached by glass joints forming two separate chambers with skin at the base. Both the skin touched the receptor solution at the same depth and each chamber housed one electrode. Once the battery was switched on, current flowed through the skin placed in anodal compartment into receiving solution below and reached the cathodal electrode through the skin tied to cathodal end. Donor solution was filled in one of the top chambers depending on the polarity of the drug and the other serve as return electrode chamber. For our study, silver/silver chloride electrode was inserted into the donor compartment whereas silver plate was inserted into anodal chamber as return electrode. Direct current (0.25-0.75 mA cm$^{-2}$) was used throughout experiment. The receptor fluid (5 ml) was withdrawn at regular intervals and replaced with fresh buffer to maintain sink condition. Samples were assayed by the U-V spectrophotometrically.

***Solubility determination***

Excess amounts of drug were taken into glass vials and dissolved in measured amount of different solvent to get saturated solutions. The solutions were kept at rest for 24 h to assist the attainment of equilibrium with the undissolved drug particles. From these solutions, the supernatant was filtered to separate the undissolved drug particles and diluted suitably and the concentrations were measured.[18]

***Partition coefficient***

The octanol / phosphate buffer partition coefficient of the lisinopril was determined by shaking equal volume of octanol and phosphate buffer in a separating funnel for 10 min and allowing to stand for 24 h. Aqueous phase was assayed before and after partitioning to get the partition coefficients.[18,19] Skin/vehicle partition coefficient was determined by dipping the skin in known concentrations of drug in phosphate buffer for 24 h, assaying aqueous phase remaining drug for estimation of concentration after partitioning.

***Data analysis***

The cumulative amount permeated was plotted against time, and the slope of the linear portion of the plot was estimated as the steady state flux. Permeability coefficient and diffusion coefficient were calculated using following formulas[20]:

$$KP = JSS / Cd \quad \text{..... (1)}$$

$$D = KP\ h / K \quad \text{..... (2)}$$

where Kp represents permeability coefficient, Jss is the steady-state flux, Cd is the concentration of drug in donor compartment, D is the diffusion coefficient, K is the skin/vehicle partition coefficient and h the thickness of the skin. Flux enhancement was calculated by dividing iontophoretic steady state flux by the corresponding passive steady state flux.

***Statistical analysis***

Statistical analysis was carried out using 2- way ANOVA. The effect of concentration on steady state flux was separately evaluated by one-way ANOVA followed by Bonferroni's test.[21] At 95% confidence intervals, p values less than 0.05 were considered to be significant.

**Results and Discussion**

Permeability of drug through skin is strongly influenced by its physicochemical parameters. Molecular weight for ideal candidate of transdermal drug delivery suggested around 200-500 Da by Doh and co-workers.[22] Lisinopril having molecular weight 405.5 fits into this category. Physicochemical parameters of lisinopril were investigated and the results were listed in Table 1. The drug showed good solubility in water (90.47 mg/ml). Phosphate buffer (pH 7.4) was used as receiver fluid in our study, by this basis saturation solubility was also determined in this media. Solubility of Lisinopril in phosphate buffer (pH 7.4) was found 90.68 mg/ml which is very close to solubility of Lisinopril in water. The octanol–water partition coefficient is a measure of the relative lipophilicity of a compound. The experimentally determined partition coefficient (octanol/phosphate buffer pH 7.4) was found to be 0.675 ± 0.0124, which indicated that the drug had less affinity towards the lipids compared to phosphate buffer. However, the lipophilicity of a drug moiety is an intrinsic character and cannot be changed.

At pH 7.4 mammalian skins are negatively charged and ionic forms have low affinity toward skin. Lisinopril, being an acidic drug with the pKa value of 3.85, was largely ionized which reduced its affinity toward the skin.[23] In iontophoresis, the ionized moieties actively propelled through natural pore pathways of skin while the unionized fraction could pass the unbroken horny layers by passive permeation resulting in enhanced permeation.[24] To simulate the physiological condition, the diffusion cell was modified where both the electrodes were place on the same side of skin. The receiving chamber with phosphate buffer reflected the body. The permeability and diffusion parameters are very important for comparison purpose.[25] It is evident that as the concentrations increases in donor the permeability coefficients decrease. Result for the permeability and diffusion coefficients of lisinopril in different systems in our study for passive diffusion and iontophoresis are provided in Table 2, which found to be obey above hypothesis.

**Table 1.** Physicochemical Parameters of Lisinopril

| Trial No. | Solubility (mg/ml) | | Partition Coefficient Octanol/Phosphate Buffer 7.4 pH | Molecular Weight (g/mol) |
|---|---|---|---|---|
| | Phosphate buffer 7.4 pH | Water | | |
| **1** | 90.12 | 93.22 | 0.671 | 405.5 |
| **2** | 92.14 | 90.87 | 0.689 | |
| **3** | 89.78 | 87.34 | 0.665 | |
| **Mean±SD** | 90.68±1.2757 | 90.47±2.9596 | 0.675±0.0124 | |

**Table 2.** Comparison of Permeation & Diffusion coefficients of Lisinopril in different Systems by Passive and Iontophoresis

| Donor System | Permeability Coefficient (cm/hr) | | Diffusion coefficient ($cm^2/s$) | |
|---|---|---|---|---|
| | Passive | Iontophoretic | Passive | Iontophoretic |
| System- A | 0.0252 | 0.0797 | $0.1147* 10^{-5}$ | $0.3629* 10^{-5}$ |
| System-B | 0.0250 | 0.0789 | $0.1138* 10^{-5}$ | $0.359* 10^{-5}$ |
| System-C | 0.0235 | 0.0543 | $0.1070* 10^{-5}$ | $0.247* 10^{-5}$ |

Among the various factors that affect passive permeability, the concentration of the actives in the donor system is most crucial. To evaluate this effect, the experiment was design at three different drug concentrations. Results show that overall iontophoretic permeability at all concentration level significantly higher that of passive values. The passive and iontophoretic permeation profiles of lisinopril at different donor concentrations are shown in Figures 1 and 2. The passive profiles are linear at all concentration levels indicating the permeation kinetics was more or less zero order. In the passive process, both the rate and extent of permeation increased with increasing donor drug concentrations. This was expected as increase in the donor drug concentration enhanced the concentration gradient, which was the driving force of mass transport.[26] In contrasts iontophoretic profiles were less linear indicating the involvement of multitude of factors. When the concentration of the drug was raised from low (System A) to medium (System B), the permeation rate increased but there no significant increase was found at the highest concentration (System C) over that of next lower concentration. This was in agreement with the hypothesis that in the drug concentration increases iontophoretic delivery upto a certain point, but at still higher concentrations, the flux may become independent of concentration.[27] In iontophoresis though ionic repulsion is the dominant force, convective flow of solutes toward the direction of current, influences the permeation rate. Permeability of skin also changes under influences of current.[28]

The total flux of a solute during iontophoresis is the sum of fluxes due to electro-repulsion, convective flow, and passive diffusion.[24] Lisinopril (pKa 3.85) at pH 7.4 acquires a negative charge and was delivered from cathodal chamber**.** Since the isoelectric point of the skin varies between 3 and 4, at physiological pH, the volume flow was directed toward the cathode. Hence at pH 7.4, only passive and electro-repulsive fluxes were likely to contribute to the overall permeation. Electro-osmotic flow may even oppose the permeation from the cathodal compartment.[29] The iontophoretic profile showed the initial permeation was high but the permeation rate declined in the later hours. This was unexpected as the voltage gradually dropped with time and hence the magnitude of electro-osmotic opposition was expected to be lesser in the later part of the study. The opposite result suggested the involvement of a factor that negatively influenced the permeation as time progressed. It is possible that during the passage of current, the cathodal electrode (Ag/AgCl) received a steady flow of electron, which resulted in the liberation of negatively charged chloride ions. As time progressed, the concentration of this newly released chloride ions were likely to increase in the cathodal compartment. Since the drug was negatively charged, chloride ions served as competitor. A chloride ion, being much smaller than the drug ion, was a powerful competitor, which reduced the transport efficiency of lisinopril.[30]

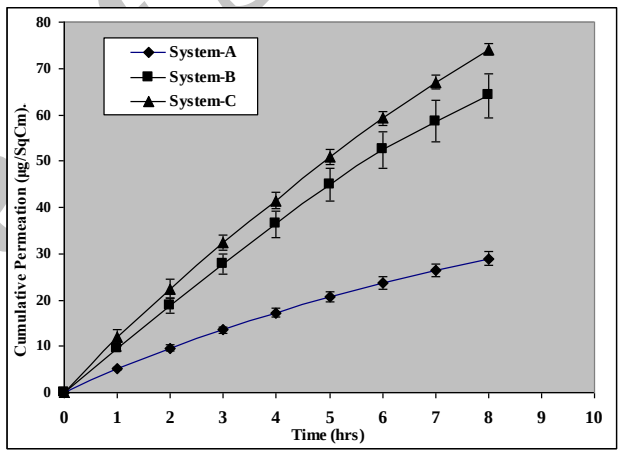


**Figure 1.** Permeation profile of Lisinopril (Iontophoresis) at different donor concentrations (n=3).

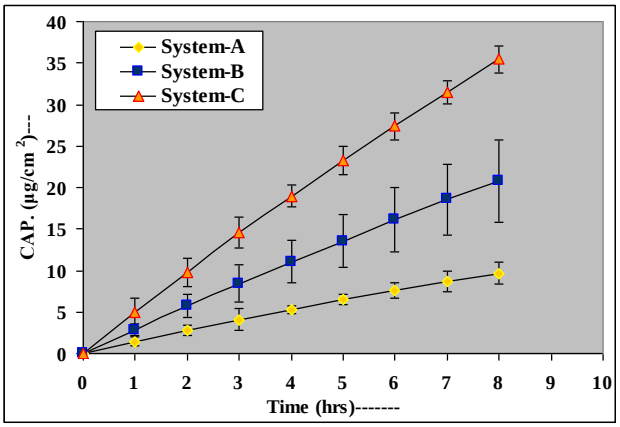


**Figure 2.** Permeation profile of Lisinopril (passive) at different donor concentrations (n=3).

Table 3 depicts the enhancement in iontophoretic flux compared to the passive flux of same donor concentration. Enhanced permeation was highest at the lowest drug load and lowest at the highest drug load. The iontophoretic contribution was found to be slightly more at higher donor concentrations.

**Table 3.** Enhancement Ratio and Benefit by iontophoresis

| Donor Systems | Steady State flux (µg/ hr.cm2) | | Benefit by Iontophoresis (µg/hr.cm2) | Enhancement Ratio (R) |
|---|---|---|---|---|
| | Passive | Iontophoretic | | |
| **System-A** | 1.429 | 4.517 | 3.088 | 3.160 |
| **System-B** | 2.841 | 8.636 | 5.795 | 3.0397 |
| **System-C** | 3.998 | 9.227 | 5.229 | 2.3079 |

The effect of current densities on the transport of lisinopril through pig ear skin, experiment was carried out at three different current densities and result was found that iontophoretic drug transport of lisinopril increased with the increasing current densities.(Figure 3).

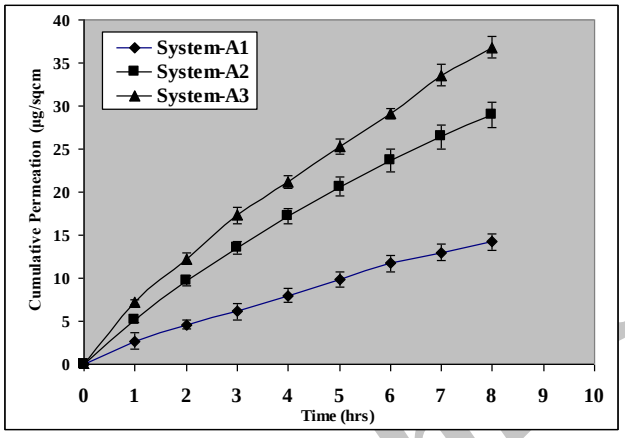


**Figure 3.** Permeation Profile of Lisinopril (Iontophoretic) at different Current Densities (n=3).

The relationship between current density and flux of lisinopril may be described by Faraday's law which is represent by following equation[31]

$$Ji = ti\ It / Zi\ F$$

where Ji, Zi are the flux and charge of lisinopril at particular pH, It is the applied current density, F is the Faraday's constant, ti is a proportionality constant.

Since in the experimental conditions ti and Zi i.e., charge of the drug was kept constant, then by above equation flux is directly proportional to the current density.

Literature survey suggested that disordering of intracellular lipid of stratum corneum by increasing of current density may cause increase in drug transport.[32] Some researchers suggested that possibility may be also that the electro-osmotic volume flow increase with an increase an current densities[33] which leads to increase in the flux of the drug.

Experimental data indicate that by proper selection of donor solution current density and some other related factors, extent of lisinopril transport across skin can be manipulated.

## Conclusion

The present work shows that the iontophoretic approach was feasible to enhance and control the rate of transdermal drug delivery of lisinopril. Study show that iontophoretic drug delivery increase with the current densities and concentration, but in case of concentration permeation increase up to certain points, afterwards flux may become independent of concentration. It can be said that lisinopril is a promising candidate for transdermal delivery and its delivery can be manipulates, controlled and optimized by iontophoresis.

## Conflict of interest

The authors report no conflicts of interest.

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