Original Research Article

IN-VITRO AND *IN-VIVO* TRANSDERMAL IONTOPHORETIC DELIVERY OF LISINOPRIL GEL

Ashish Jain[1]*, Satish Nayak[1], Vandana Soni[2]

[1] Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal-462021, India.

[2] Department of Pharmaceutical Sciences, Dr H.S.Gour Vishwavidyalaya, Sagar 470003, M.P. India

ABSTRACT

The feasibility of transdermal delivery of Lisinopril gel by cathodal iontophoresis using Ag/AgCl electrode was investigated. A polymer gel was prepared using hydroxypropyl methyl cellulose and *in vitro* skin permeability was assessed in full thickness skin of rabbits and pigs. For *in vivo* studies New Zealand rabbits were used. *In vitro* passive permeation was carried out in Franz diffusion cell but for iontophoresis, diffusion cell was modified according to Glikfield design. For in-vitro study 0.5 mA/cm^2 current density was used in iontophoresis with passive controls but for *in vivo* study current density was reduced to 0.1 mA/cm2. High performance liquid chromatography was used for analysis of drug content in blood samples Blood samples. Results of the *in vitro* study indicated that iontophoresis considerably increased the permeation rate of Lisinopril compared to passive controls in both the skin types ($P<0.01$). The plasma concentration of Lisinopril was significantly higher ($P<0.001$) than that obtained in the passive controls. Results showed that the target permeation rates for Lisinopril could be achieved with the aid of iontophoresis by increasing the area in an appreciable range.

Keywords: Lisinopril, iontophoresis, transdermal, Rabbit, Pigskin, in-vitro, in-vivo.

***Corresponding author: Ashish Jain,** Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal-462021, India. Tel.: +91-9981574693, Email: aashish.pharmatech@gmail.com

INTRODUCTION

Iontophoresis is a powerful technique to enhance percutaneous permeation of ionized drugs poorly absorbed by skin. Application of an external electric field provide an additional force which drives ions through the skin, modifies skin permeability and enhance solvent stream that can carry different species include neutral molecules[1-3]. Today most of drugs are taken orally and are found not to be as effective as desired. To improve such characters transdermal drug delivery system was emerged. To achieve and maintain drug concentration above the minimum therapeutic level it is the need of transdermal system to overcome the barrier properties of skin. Iontophoresis has been proved promising new technique for permeation enhancement of many drugs. The enhancement of drug due to this method results from a number of possible mechanisms including the ion-electric field interaction (electro repulsion), convective flow (electro-osmosis) and current-induced [4,5] increase in skin permeability.

Lisinopril is an angiotensin-converting enzyme inhibitor used for the treatment of hypertension. Lisinopril is available only in the form of oral tablets in the market and it has slow and incomplete absorption after oral administration with a bioavailability of 25–30% [6,7]. To rise above the problems associated with oral delivery like incomplete absorption, low oral bioavailability, and for the effective treatment of chronic hypertension, alternative transdermal route of administration may be beneficial. Lisinopril is a ideal candidates for transdermal study because of low oral dose (2.5–20 mg), low molecular mass (405.5 g/mol),and low oral bioavailability (25%).The main problems associated with oral therapy include hepatic first-pass elimination, 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 [8].

MATERIALS & METHODOLOGY

Lisinopril was obtained from Lupin laboratories Bhopal as a gift sample. Silver plates (purity 99.99%, 5 mm diameters) were purchased from a goldsmith shop at Bhopal, India. Silver Chloride, Sodium Chloride AR, Octanol, isopropyl alcohol, HPMC were purchased from SD Fine-Chem, Mumbai, India. For the mixing of gel an electrical stirrer Remi model no. RQ-121/D was used. Cellulose membrane was obtained from Sigma- Aldrich, Germany. Iontophoretic DC source having digital display, current 0-10 mA, voltage 0-25 V was purchased from C-tech Psu- 2510/lab Mumbai, India. Iontophoretic diffusion cell was made-up by P. K. Scientific Bhopal, India, as per given specifications. Silver/silver chloride electrode was arranged as per the standard procedure [9]**.** 99.99% pure, 1.0 mm thickness silver was used as connecting wire. All the reagents/chemicals used were of analytical grade. Ultra pure water (resistivity, 18.2 MW cm) used to conduct experiments which were obtained from Milli-Q Academic System.

Preparation of Lisinopril Gel

100 g Gel formulation was prepared by soaking 5 g of HPMC overnight (12 h) in a part of solvent mixture (ethanol: propylene glycol: water in ratio of 50:30:20). The drug 2.5 g separately dissolve in the same vehicle was added to the polymer dispersion. Glycerol (5 % v/v) was added as humectant and volume was adjusted to 100 ml by adding the vehicle. Finally the mixture was stirred using an electrical stirrer (500 rpm, 1 h) to ensure uniform transparency [10].

Estimation of the drug (Content uniformity & in vitro diffusion study)

Analysis of the samples were performed with Waters binary gradient HPLC system, equipped with 515 HPLC pump, PDA detector, C-18 column (ODS; 25 cm X 4.6 mm; 5µm) at ambient temperature. The column was eluted with the mobile phase consisting of phosphate buffer (25 mM potassium dihydrogen ortho phosphate, pH 5.0) and acetonitrile at a ratio 88:12. A flow rate of 1 ml/min was maintained, and the detection wavelength was 215 nm used. Retention times were recorded 2.3 minutes for Lisinopril in our study. For making standard graph, working standards were prepared in phosphate buffer (5-80 mcg/ml) and injected into the column (20 µL). A good linear relationship was observed between the concentrations and the peak area obtained with correlation coefficient (R^2) 0.9997. Samples obtained from the diffusion and content analysis studies were injected into the column after suitable dilution and peak area were noted. The concentrations were determined by comparing the peak areas from the regression equation of the standard curve [11, 12].

Estimation of Drugs in Blood Samples

Blood was directly collected into vacuum tube (BD vacutainer TM) from rabbit ear vein and mixed with mixed with 0.05 ml of a solution of EDTA-3Na (0.2 M) and ascorbic acid (0.2 M) and centrifuged immediately at 5000 rpm for 7 min at 4°C. A 1ml aliquot of plasma was added to a screw-cap glass tube containing 0.1 ml of a derivative agent 4-bromophenacyl bromide (*p*-BPB, 2 mg/ml in acetonitrile) and 1 ml of phosphate buffer (pH 7.4). The tube was vortexed for 30 s and then left at room temperature for 30 min. After this, 0.2 ml 2 N HCl was added, and the resulting plasma samples were frozen at -20°C until assayed. Then 6 ml of 1:1 mixture of ethyl acetate:benzene was used as extracting solvent. The tube was vortexed for 30 s and then shaken gently for 10 min. After centrifugation, the organic layer was removed, and evaporated to dry under reduced pressure. The residue was reconstituted 7.4 pH phosphate buffer and aliquots of 0.02 ml were injected into the HPLC system [13,14].

Drug Content Analysis

Content uniformity of the gel formulation was carried out in cellulose membrane, same procedure followed as our previous study that was performed for glipizide gel [10].

Preparation of Pigskin Membrane

Ears of freshly slaughtered pigs were obtained from local slaughter house and skin was removed carefully from the outer regions of the ear and separated from the underlying cartilage with a scalpel. Fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol. Then skin was washed with tap water and stored at refrigerator in aluminum foil[15].

Preparation of Rabbit Skin

Prior permission was obtained from CPCSEA approved Institute Animal Ethics Committee (Registration No 1252/ac/09/CPCSEA). The Rabbits were sacrificed by the I.V injection of chloroform [16]. Skin samples were obtained from the back area of rabbits. The adherent fat and other visceral debris were detached. The dermal side of full thickness skin was soaked in buffer (phosphate buffer, pH 7.4) for 12 h at 4°C to equilibrate the skin and used on the same day.

***In-Vitro* Passive Permeation Studies**

Franz diffusion cell was used for *in vitro* passive permeation studies, having a receptor compartment capacity of 10 ml. The remove skin was mounted between the half-cells with the dermis in contact with receptor fluid (Phosphate buffer pH 7.4). 1.21cm^2 area was available for diffusion. Aluminum foil was used to cover the donor cell to prevent the evaporation of vehicle. The fluid of receiver compartment was maintained at 37±0.5 °C. Temperature at the skin surface was approximately 32 °C maintained. One ml of Lisinopril gel was placed in the donor compartment. The whole assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred constantly using a magnetic bead. The sample solution was reserved from the receptor compartment at regular intervals and assayed for drug content [17].

Procedure of Iontophoretic Diffusion

Diffusion cell used fort iontophoretic studies was modified as suggested by Glikfield et al [18]. Detail of apparatus deign we have already discus in our previous study (Bijya et al 2009) so that is not discussed here [10]. In present study, silver/silver chloride electrode was inserted into the

donor compartment whereas silver plate was inserted into anodal chamber as return electrode. Direct current (0.5 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.

In-vivo Pharmacokinetic Study

Rabbits maintained in the CPCSEA permitted Institute animal house and maintained in harmony with NIH guidelines were used. Pharmacokinetic studies were performed as method suggested by Anigbogu *et al.* [19]. Both sexes and healthy New Zealand rabbits (body weight approximately 2 kg) were used. Hair was removed from two spots and skin areas were wiped with alcohol swabs, on the day of experiment. The animals were allowed to become accustomed for 30 min, in restrainers. For passive study, blank and drug gels were applied parallel to one another on the right dorsum separated by a distance of approximately 3 cm. In iontophoresis, the drug was delivered from cathodal electrode (silver/silver chloride) and blank gel was applied beneath the silver plate which served as return electrode (anode). The circuit was accomplished by connecting both the electrodes to the respective negative and positive poles of the constant power supply. Studies were performed out at current density of 0.1 mA/cm^2. The area for application of the electrode was 4 cm^2. Time was noted for the application of gels. Blood samples were collected every hour (1 ml) from the ear veins using vacuette tubes. The concentration was measured from the standard curve.

Data analysis

For determination of steady state fluxes, 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. Using following formulas permeability coefficient and diffusion coefficient were calculated [20]:

$KP = JSS / Cd$ ……. (A)

$D = KP\ h / K$ …….. (B)

where Kp stands for 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 performed 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 & DISCUSSION

If we analyze the history of human suffering, we observe that diseases and compulsions imposed by diseased states are considered to be a greater enemy to mankind rather than the death. 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 life long administration. Approximate 50 % of hypertensive patient do not fulfill with there prescribed treatment. Reason suggested for this complication includes intolerable side effects, complex treatment and lake of reminders [22]. Although half-life of the drug is 12 h, thus effective as single daily dose medication,but it severely suffers from average intersubject bioavailabilty of 25%[23,24].

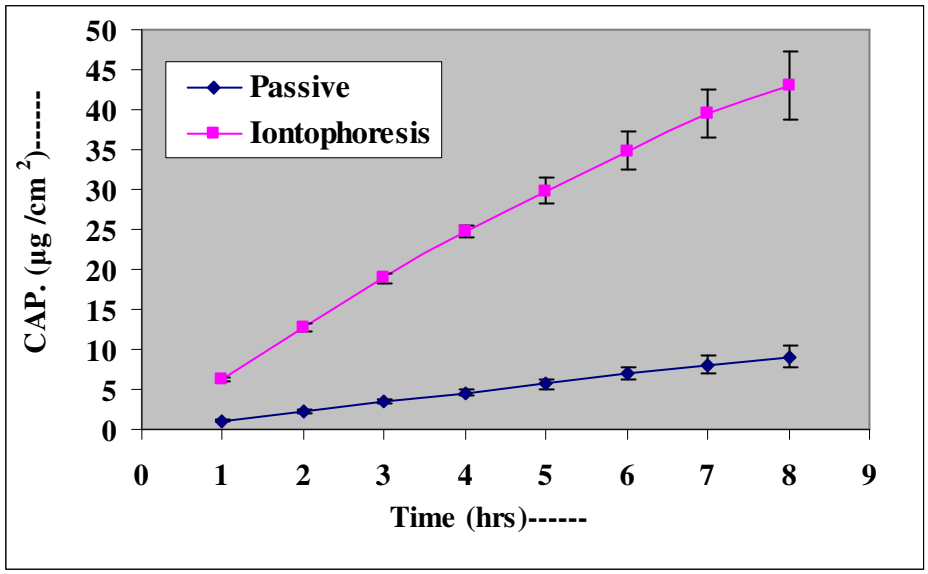

Figure 1. Comparison of passive and iontophoretic permeation of Lisinopril in excised pigskin. Data expressed as Mean ± SD (n=3). CAP= Cumulative amount permeated.

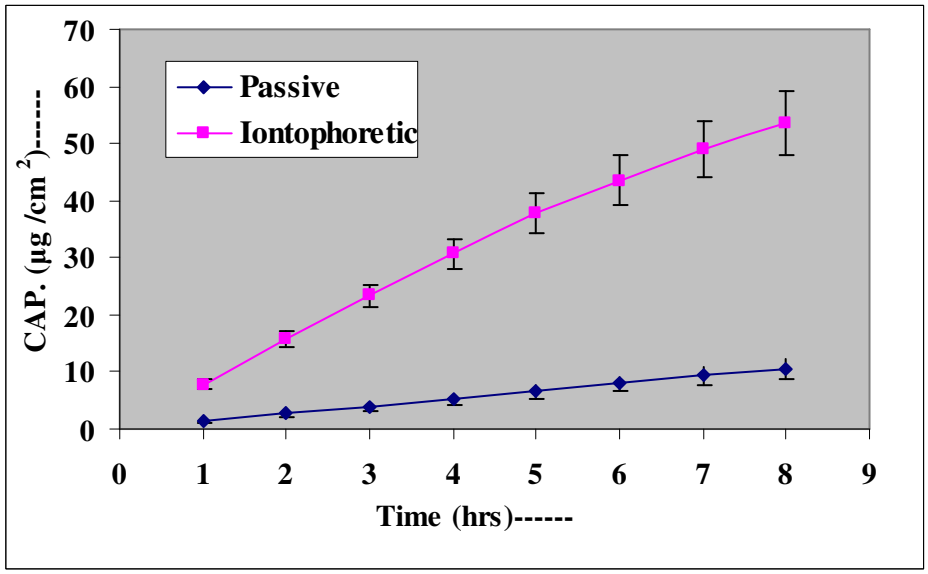

Fig 2 Comparison of passive and iontophoretic permeation of Lisinopril in excised Rabbit skin. Data expressed as Mean ± SD (n=3). CAP= Cumulative amount permeated

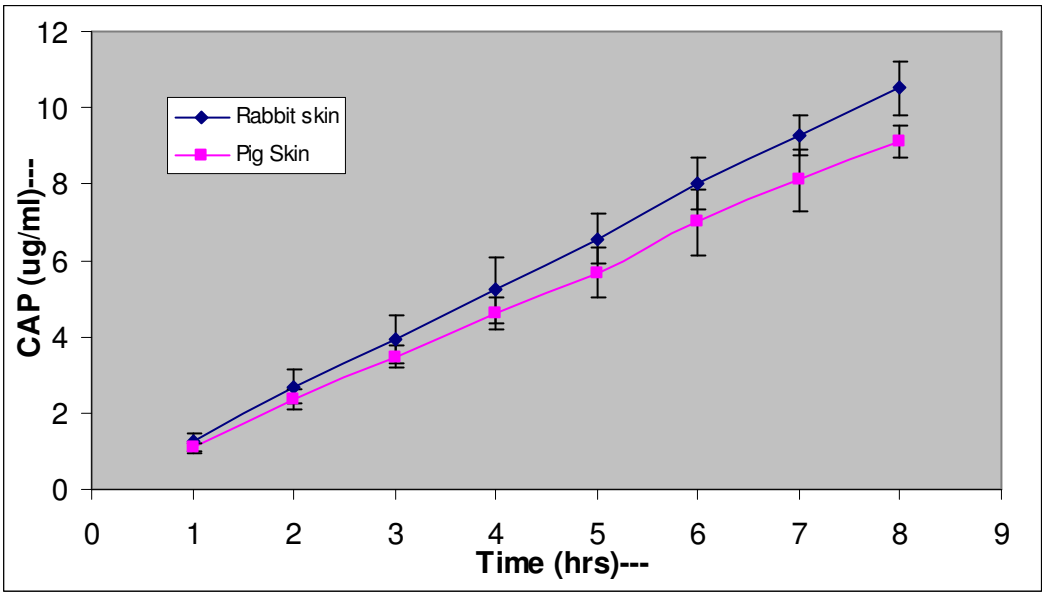

Fig 3 Comparison of Passive permeation of Lisinopril in excised pig and rabbit skin. Data expressed as Mean ± SD (n=3)

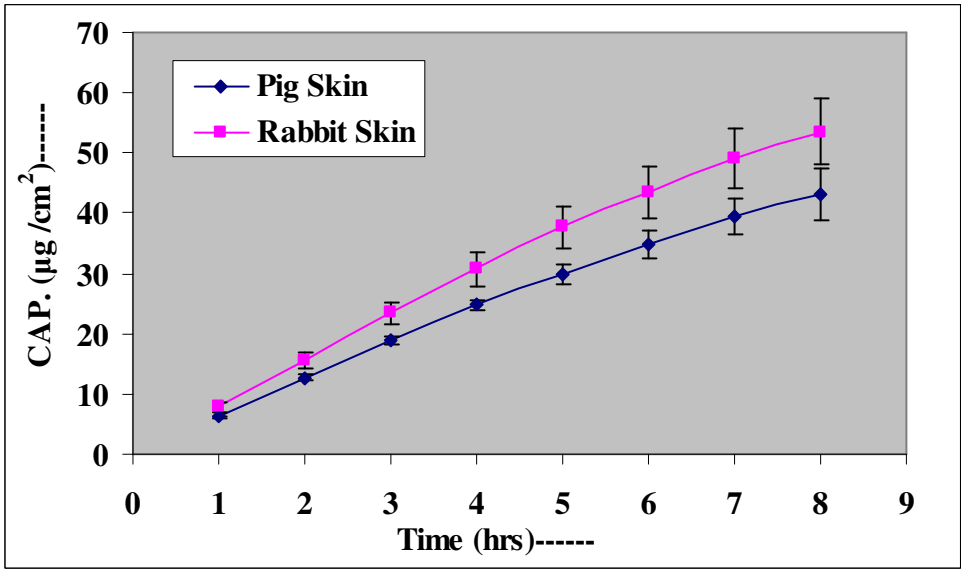

Fig 4 Comparison of Iontophoretic permeation of Lisinopril in excised pig and rabbit skin. Data expressed as Mean ± SD (n=3)

Hence, transdermal delivery could be an alternative approach that will increase the bioavailability of drug and sustained the drug release for long periods. In order to overcome the barrier properties of skin, penetration enhancers and iontophoresis were employed in this study. Skin permeability of a drug is strongly influenced by its physicochemical parameters. According to Doh and coworkers[25], drug candidates for transdermal delivery should have molecular weight around 200–500 Da. Lisinopril having molecular weight of 405.5 fits into the category. Physicochemical parameters of Lisinopril were investigated in our previous work, results

showed good solubility in water (90.47 mg/ml) and in Phosphate buffer pH 7.4 (90.68) but experimentally determined partition coefficient (0.675) indicates poor lipophillicity. Which indicates drug have less affinity towards lipid as compare to aqueous phase, but as it is the intrinsic property of molecule we can not change. To enhance the permeation of drug we utilized chemical enhancers and iontophoresis out of various available enhancement techniques.

Table 1 Steady state fluxex, Permeability Coefficients, and enhancement ratio of Lisinopril in Rabbit and Pigskin from gel formulation

Parameters	Process	Pig Skin	Rabbit skin
Steady State Fluxes (µg /hr. cm^2)	Passive	1.203	1.387
	Iontophoresis	5.01	6.402
Permeability Coefficients (cm/h)	Passive	0.048×10^{-3}	0.055×10^{-3}
	Iontophoresis	0.20×10^{-3}	0.256×10^{-3}
Enhancement Ratio (R)	(Iontophoresis / Passive)	4.164	4.615
Net Benefit of Iontophoresis (µg /hr. cm^2)	(Iontophoresis- Passive)	3.807	5.015

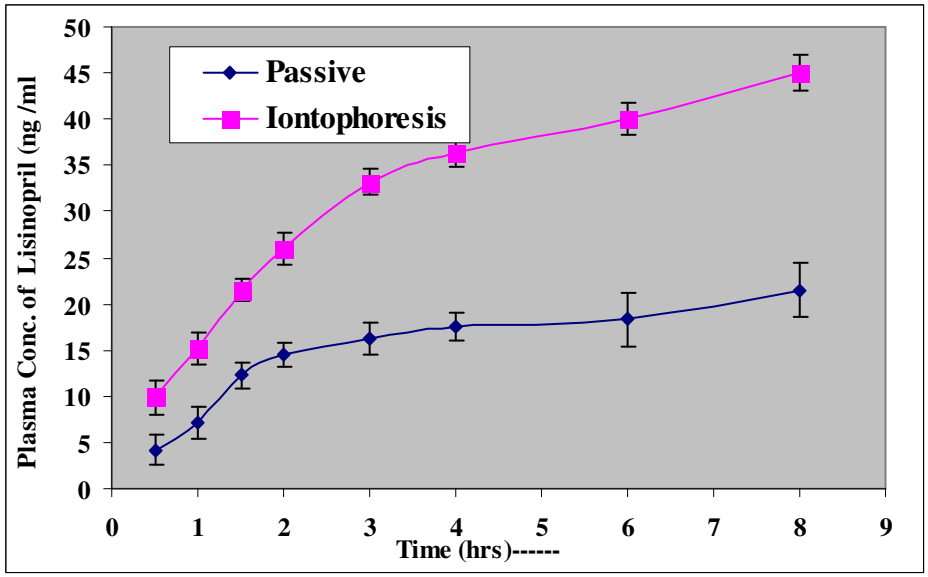

Fig 5 Plasma Concentration of Lisinopril after passive and iontophoretic administration of Lisinopril Gel in Rabbits. Data expressed as Mean ± SD (n=3)

After passive and iontophoretic permeation of Lisinopril from a solutions, we found that iontophoresis could significantly enhance the *in vitro* permeation of the drugs compared to its passive diffusion, in our previous study. However, skin permeation of a drug from dosage form is much more complex than that from a solution. In a dosage form, drug stay in intimate contact with the excipients, this can influence its release profile. The concentration gradient of the free

drug between the skin surface and plasma is the driving force of passive diffusion. Hence it is necessary to ensure that the drug is not irreversibly bound to the dosage form components and enough free drug concentration is available on the skin surface to ensure a high concentration gradient. Gel formulation was prepared using hydroxyl propyl methyl cellulose. HPMC being a neutral polymer did not show any interaction with the drug. Drug content was found uniform. The measured viscosity of the gel was about 1.3 Pa/S for Lisinopril (Brookfield viscometer, 12 rpm), suitable for transdermal application.

Since one of the objectives of our study was to evaluate plasma concentration profile of drug in rabbits, it was necessary to have some basic idea about the permeability of drug with respect to the rabbit skin also. Numbers of studies have reported that the barrier function of pigskin is close to the human skin [26] whereas the permeability of rabbit skin is much higher. Comparison of passive and iontophoretic permeation of both drugs from rabbit and pig skin were shown in figures Fig 1 & 2. Moreover the comparative data for pigskin and Rabbit skin also designed in figure Fig 3 & 4, results show that permeability of rabbit skin is much higher than compare to pigskin. This was expected that rabbit skin is considered to be one of the most permeable among the laboratories animals [27]. As we assumed that rabbit skin with its higher follicular density and water content would favor iontophoresis better, it was right assumption after the study have performed. Permeation enhancement using iontophoresis was found to be higher in case of rabbit skin than pig skin.

Steady state fluxex, Permeability Coefficients, and enhancement ratio determined and provided in Table 1. Enhancement by iontophoresis 4.615 folds for Lisinopril was reported in our study using rabbit skin it as slightly higher than that of pigskin. Figure 5 show the plasma concentration profile of Lisinopril using passive diffusion and iontophoresis. It was seen that the Lisinopril concentration after iontophoresis increase much rapidly than that of passive diffusion. Very less amount of Lisinopril determined in plasma after passive diffusion but as expected it was considerably higher when iontophoresis was used in the study.

In our study the maximum plasma concentration of Lisinopril was achieved 45.076 ± 1.924 ng/ml at the end of 8^{th} hour. Results showed that the target permeation rates for Lisinopril could be achieved with the aid iontophoresis by increasing the area of application in an appreciable range.

CONCLUSION

The non compartmental analysis of the pharmacokinetic data indicate that to meet the demand of maintenance therapy for 60 kg individual 26.7 µg of Lisinopril must be supplied to the systemic circulation every hour [28]. The *in-vitro* iontophoretic flux of Lisinopril formulation through rabbit skin was found to be 6.402 µg /hr. cm^2 in our study. As the Patch in the market usually have wider area (10 cm^2 and above), it can be expected to achieve the target. Overall results looked quite promising for transdermal delivery of Lisinopril.

REFERENCES

1. Sage B. H. and Riviere J.E. Model system iontophoresis- transport- efficacity. Adv. Drug del. Rev. 1992;9:265-287.

2. Sage B.H. Iontophoresis,in encyclopedia of pharmaceutical Technology. Swarbrick j Boyalan J.C. (Eds) 1993; 8: 247-217.
3. Sims S.M., Higuchi W.I. and Srinivasan V. Skin alteration and convection solvent flow effects during iontophoresis: I Neutral solute transport across human skin. Int. J. Pharm. 1991;69:109-121.
4. Wang Y, Thakur R, Fan Q, Michniak B. Transdermal iontophoresis: combination strategies to improve transdermal iontophoretic drug delivery. Eur J Pharm Biopharm. 2005; 60(2): 179-191.
5. Pillai O, Nair V, Panchagnula R. Transdermal iontophoresis of insulin: IV. Influence of chemical enhancers. Int J Pharm. 2004; 269(1): 109-120.
6. Lancaster SG, Todd PA. Lisinopril: A preliminary review of its pharmacodynamic and pharmacokinetic properties and thera- peutic use in hypertension and congestive heart failure. *Drugs* 1988;35:646–69.
7. Marc H. Angiotensin converting enzyme inhibitors, angiotensin and calcium channel blockers. In: Williams AD, Thamas LL, editors. Foye's principles of medicinal chemistry. 5th ed. Baltimore, MD: Lippincott Williams and Wilkins; 2002. p. 533– 61.
8. Barry BW. Mode of action of penetration enhancers in human skin. *J Control Release.* 1987;68:85–97.
9. Banga, A.K. *Electrically Assisted Transdermal and Topical Drug Delivery*. 1st ed.; Taylor & Francis Ltd: UK, **1998**; pp. 1-32.
10. Ghosh B, Jain A, Ashok P, Patel B and Tarafdar K. Passive and Iontophoretic Permeation of Glipizide Gel: An *In Vitro* and *In Vivo* Study. *Current Drug Delivery,* 2009, *6,* 444-450.
11. Faruk A, Ishar MPS. *In- Vivo* Passive and Iontophoresis Delivery of Lisinopril Using Wistar Rat Model. *International journal of Pharmaceutical and clinical Research* 2009; 1(3): 131-135.
12. Wu PC, Huang YB, Chang JJF, Chang JS, Tsai YH. Evaluation of pharmacokinetics and pharmacodynamics of captopril from transdermal hydrophilic gels in normotensive rabbits and spontaneously hypertensive rats. *International Journal of Pharmaceutics* 2000; 209:87–94.
13. Wu PC, Huang YB, Chang JJF, Chang JS, Tsai YH. Evaluation of pharmacokinetics and pharmacodynamics of captopril from transdermal hydrophilic gels in normotensive rabbits and spontaneously hypertensive rats. *International Journal of Pharmaceutics* 2000; 209:87–94.
14. Popovici RF, Alexa IF, Novac O, Vrinceanu N, Popovici E, Lupusoru CE, Voicu VA. Pharmacokinetics Study on Mesoporous Silica-Captopril Controlled Release Systems. *Digest Journal of Nanomaterials and Biostructures* 2011; 6(3):1619-1630.
15. Suwanpidokkul N, Thongnopnua P, Umprayn K. Transdermal delivery of zidovudine: The effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin. AAPS Pharm Sci Tech 2004; 5(3): 48.

16. Taro O, Tomona H, Masahiro Iwaki, Tadatoshi T. Transdermal absorption of bupranolol in rabbit skin *in vitro* and *in vivo*. *Biol Pharm Bull* 2001, *24*, 588- 591.

17. Mutalik S, Udapa N. Pharmacological evaluation of membrane-moderated transdermal system of glibenclamide. *Clin Exp Pharmacol Physiol* 2006;33:17-26.

18. Glikfield P, Cullander C, Hinz R, Guy R. A new system for in vitro studies of iontophoresis. *Pharm Res* 1988; 5: 443-446.

19. Anigbogu A, Patil S, Singh P, Liu P, Dinh S, Maibach H. An *in vivo* investigation of the rabbit skin responses to transdermal iontophoresis. *Int. J. Pharm* 2000; *200*:195-206.

20. Sung KC, Fang JY , Hu OYP. Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis. *J Cont Rel* 2000; 67:1–8.

21. Bolton S. Pharmaceutical analysis practical and clinical application, ed 3, *Marcel Dekker*, New York, 1999; 250- 329p.

22. Foster SB, Kousch D. Adherence to therapy in hypertensive patients. *Nurs Clin North Am* 1981;16(2):331-341.

23. Itoh Y, Imamura S, Yamamoto K, Ono Y, Nagata M, Kobayashi T, Kato T, Tomita M, A, Itoh M and Nagasaka A. Changes of endothelin in streptozotocin induced diabetic rats: effects of an angiotensin converting enzyme inhibitor enalapril maleate. J Endocrinol , 2002, 175: 233-9.

24. Patchett AA. In: Hypertension and the angiotensin system: therapeutic approaches; Raven Press, New York, 1984; pp. 2-13.

25. H.J. Doh, W.J. Cho, C.S. Yong, H.G. Choi, J.S. Kim, C.H. Lee, D.D. Kim, Synthesis and evaluation of ketorolac ester prodrugs for transdermal delivery, J. Pharm. Sci. 92 (2003) 1008–1017.

26. Suwanpidokkul N, Thongnopnua P, Umprayn K. Transdermal delivery of zidovudine: The effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin. *AAPS Pharm Sci Tech* 2004; 5(3): 48.

27. Hirvonen J, Rytting JH, Paronen P, Urtti A, Dodecyl N. Ndimethylamino acetate and azone enhance drug penetration across human, snake, and rabbit skin. *Pharm. Res* 1991; 8*:* 933-937.

28. Wu PC, Huang YB, Lin HH, Tsai YH. Percutaneous absorption of captorpil from hydrophilic cellulose gel® through excised rabbit skin and human skin. *Int J Pharmaceutics 1996*;145(2): 215-220.