Research Article

PASSIVE AND IONTOPHORETIC PERMEATION OF CAPTOPRIL GEL: AN *IN VITRO* AND *IN VIVO* STUDY

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ABSTRACT

The Objective of this work was to formulate and evaluate captopril gel to assess its suitability for transdermal delivery by passive and iontophoresis. 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. Iontophoresis was performed at a current density of 0.5 mA/cm^2 via silver /silver chloride electrodes with passive controls but for *in vivo* study current density was reduced to 0.1 mA/cm2. Blood samples were analyzed for drug content by HPLC. Results of the *in vitro* study indicated that iontophoresis considerably increased the permeation rate of captopril compared to passive controls in both the skin types (P<0.01). The plasma concentration of captopril was significantly higher (P<0.001) than that obtained in the passive controls. Results showed that the target permeation rates for captopril could be achieved with the aid of iontophoresis by increasing the area in an appreciable range.

Key words: Captopril, iontophoresis, transdermal, Rabbit, Pigskin, *in vitro*, *in vivo*.

INTRODUCTION

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. Naturally, the ultimate aim of every therapy is to restore the normalcy of life, but ironically sometimes, the requirements of treatment are such that the normal rhythm of life is disturbed. 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 is a form of transdermal delivery that use in electric field to enhances the movement of small, poorly absorbed ionic drug across the skin in controlled and program-mable manner (Nair and Panchagnula, 2003; Kalia *et al.,* 2004; Zakzewski *et al.,* 1992). 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 (Wang *et al.,* 2004; Pillai *et al.,* 2004) increase in skin permeability.

Captopril is an oral drug and a member of a class of drugs called angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors are used for treating high blood pressure, heart failure, and for preventing kidney failure due to high blood pressure and diabetes. It has a short elimination half life and its plasma half life in man ranges from 1.6-1.9 hour (Jarrott *et al.,* 1982; Raia *et al.,* 1990; Levy *et al.,* 1990). Moreover food may decrease oral absorption of captopril by up to 25-40% (Ohman *et al.,* 1985; McEvoy 1996).

The main problems associated with oral therapy include 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

(Dandagi *et al.,* 2006). Captopril being an antihypertensive agent needs prolonged administration. Results of post market surveillance performed on captopril had shown that 4.9% of the patients had to discontinue therapy because of the adverse effects (Sweetman, 2005). The drug has low stability because of the oxidation, which converts the drug into captopril disulphide. A recent study had shown that the oxidation rate of captopril in dermal homogenates is significantly lower than that in intestinal homogenates (Zohu *et al.,* 1994). This made captopril is a good candidate for transdermal delivery.

METHODOLOGY

Captopril was a gift sample from Micro Lab Bangalore. Silver plates (purity 99.99%, 5 mm diameters) were obtained from a goldsmith shop at Bangalore, India. Sodium Chloride AR, octanol , isopropyl alcohol, Silver Chloride, HPMC were obtained from SD Fine-Chem (Mumbai, India). Mixing of gel was carried in an electrical stirrer Remi model no. RQ-121/D. Cellulose membrane (9652 dialysis tubing Cut off point 12000 D) was purchased from Sigma- Aldrich, Germany. Iontophoretic DC source (digital display, current 0-10 mA, voltage 0-25 V) was purchased from C-tech Psu- 2510/lab Mumbai, India. Silver plates (purity 99.99%, 5 mm diameter) were obtained from a goldsmith shop of Bhopal. 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 (Banga, 1998). Silver wire (99.99% pure, 1.0 mm thickness) was used as connecting wire. 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.

Preparation of captopril gel

Gel formulation (100 g) were 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 (Ghosh *et al.,* 2009).

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 220 nm used. Retention times were recorded 8.4 minutes for captopril in our study. For making standard graph, working standards were prepared in phosphate buffer (5-80 mcg/mL) and injected into the column (20μL). A good linear relationship was observed between the concentrations and the peak area obtained with correlation coefficient (R^2) 0.9995. 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 (Faruk and Ishar, 2009; Wu *et al.,* 2000).

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 0.1mL of a derivative agent 4-bromophenacyl bromide (*p*- BPB, 2 mg/mL in acetonitrile) and 1mL of phosphate buffer (pH 7.4). The tube was vortexed for 30 s and then left at room temperature for 30 min. After this, 0.2mL 2 N HCl was added, and the resulting plasma samples were frozen at -20°C until assayed. Then 6mL 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 (Wu *et al.,* 2000; Popovici *et al.,* 2011)

Drug content analysis

Content uniformity of the gel formulation was determined in cellulose membrane. irrelevant material was removed from the membrane, by washing the tubing in running water for 3-4 h and sulfur contaminations was removed by treating the tube with a 0.3 % (w/v) solution of sodium sulfide at 80° C for 1 min. The tube was washed with hot water (60° C) for 2 min followed by acidification with 0.04N sulfuric acid and rinsed with hot water to remove the acid. Gel formulation (2g) was taken in the treated membrane, kept in 50 mL beaker containing 50 mL phosphate buffer of pH 7.4 and stirred with magnetic stirrer. At the end of 24 h, sample was removed and estimated for drug content by HPLC (Ghosh *et al.,* 2009).

Preparation of pigskin 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 -20°C in aluminum foil packing and was used within two days (Suwanpidokkul *et al.,* 2004).

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 (Taro *et al.,* 2001). Skin samples were obtained from the back area of rabbits. The adherent fat and other visceral debris were removed. 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

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 was equilibrated for 1 h. The area available for diffusion was about 1.21cm². 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. One mL of captopril gel 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 (Mutalik *et al.,* 2006).

Procedure of iontophoretic diffusion

For iontophoresis diffusion cell was modified as suggested by Glikfield *et al.,* (1988). 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 (0.5 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 spectro-photometrically.

In vivo pharmacokinetic study

Rabbits maintained in the CPCSEA approved Institute animal house and maintained in accordance with NIH guidelines were used. For pharmacokinetic studies the method of Anigbogu *et al.* was utilized (Anigbogu *et al.,* 2000). Healthy New Zealand rabbits of both sexes (body weight approximately 2 kg) were used. On the day of the experiment, hair was removed from two spots and skin areas were wiped with alcohol swabs. The animals were allowed to acclimatize 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 under the silver plate which served as return electrode (anode). The circuit was completed by connecting both the electrodes to the respective negative and positive poles of the constant power supply. Studies were carried out at 0.1 mA/cm^2. The area for application of the electrode was 4 cm^2. Time was noted for the application of gels and blood samples (1 mL) were collected every hour from the ear veins using vacuette tubes. The concentration was determined from the standard curve.

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 (Sung *et al.,* 2000):

$$KP = JSS / Cd \ldots\ldots\ldots (1)$$
$$D = KP \ h / K \ldots\ldots\ldots (2)$$

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. (Bolton 1999) At 95% confidence intervals, p values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

According to previous research of Wu *et al* (Wu *et al.,* 1997) permeability of captopril had been evaluated by in a series of animal skins. Intrinsic skin permeability was found to be extremely low and it could not be detected in the rat plasma in an *in-vivo* experiment carried out by Hao *et al.,* (Hao *et al.,* 2000); This preliminary results indicate that skin permeation must be assisted with enhancement technique so attempt had been taken up in this study.

Captopril, an orally effective angiotensin-I converting enzyme inhibitor, is widely used intreatment of hypertension and congestive heart failure but its oxidation in intestinal homogenates eliminates it with in 1.5 to 1.9 h (Jarrott *et al.,* 1982; Raia, 1990; Levy *et al.,* 1991) which is faster compared to its oxidation in dermal homogenates (Zhou *et al.,* 1994). Consequently delivery of captopril as transdermal may be the better dosage form. Skin permeability of a drug is strongly influenced by its physicochemical parameters. According to Doh and coworkers (Doh, *et al.,* 2003), drug candidates for transdermal delivery should have molecular weight around 200–500 Da. Captopril having molecular weight of 217.29 fits into the category.
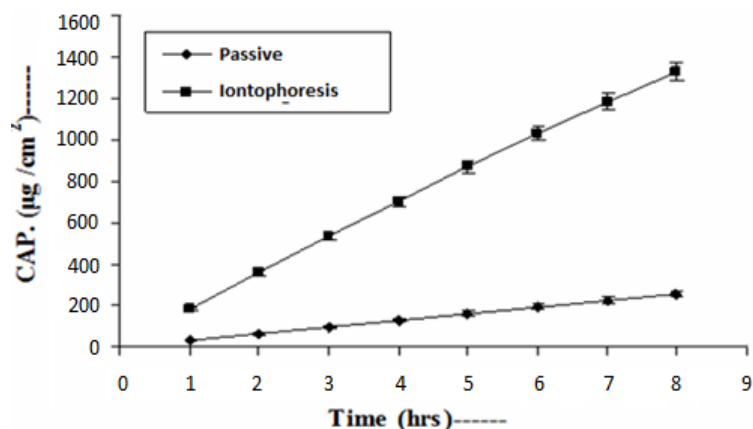
Figure 1. Comparison of passive and iontophoretic permeation of Captopril in excised pigskin Data expressed as Mean ± SD (n=3). CAP= Cumulative amount permeated
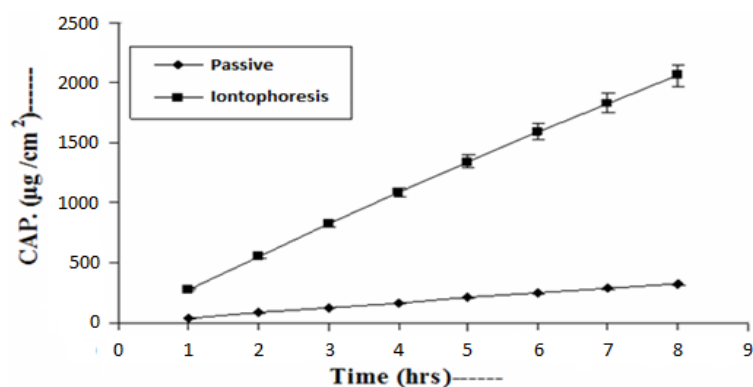

Figure 2. Comparison of passive and iontophoretic permeation of Captopril in excised Rabbit skin. Data expressed as Mean ± SD (n=3). CAP= Cumulative amount permeated

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Physicochemical parameters of aptopril were investigated in our previous work, results showed good solubility in water (156.1mg/mL) and in 0.9%NaCl (145.57mg/mL) but experimentally determined partition coefficient (0.335) indicates poor lipophillicity (Jain *et al.,* 2011). 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. Here attempt has been made to increase the permeation of captopril by the utilization of iontophoresis.

After passive and iontophoretic permeation of captopril 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 (Jain *et al.,* 2011). 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.
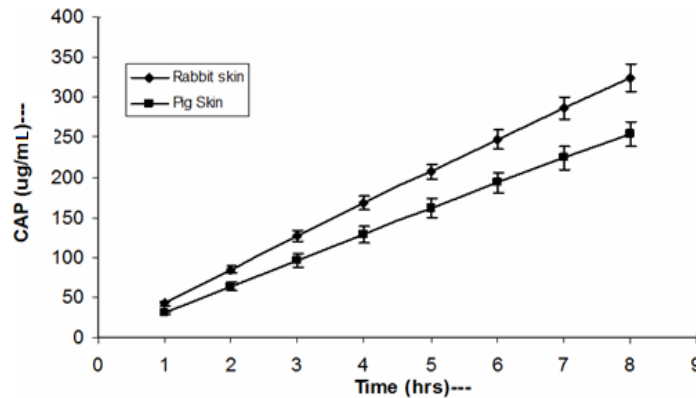
Figure 3. Comparison of Passive permeation of Captopril in excised pig and rabbit skin. Data expressed as Mean ± SD (n=3).
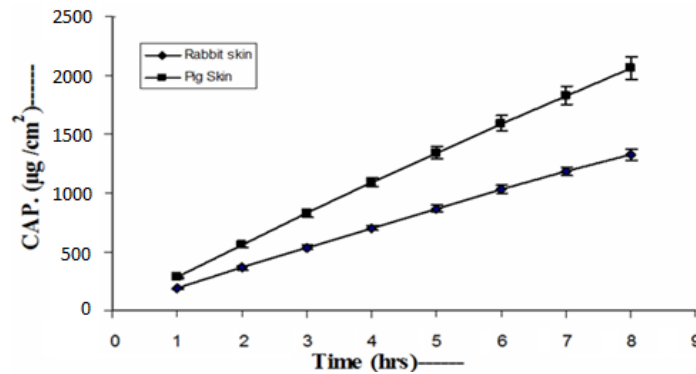

Figure 4. Comparison of Iontophoretic permeation of Captopril in excised pig and rabbit skin. Data expressed as Mean ± SD (n=3).

Gel formulation were 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 1.4 Pa/S for captopril (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 drugs with respect to the rabbit skin also. Numbers of studies have reported that the barrier function of pigskin is close to the human skin (Suwanpidokkul *et al.,* 2004) 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 Figure 1 and 2. Moreover the comparative data for pigskin and Rabbit skin also designed in figure figure 3 and 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 (Hirvonen *et al.,* 1991). 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.
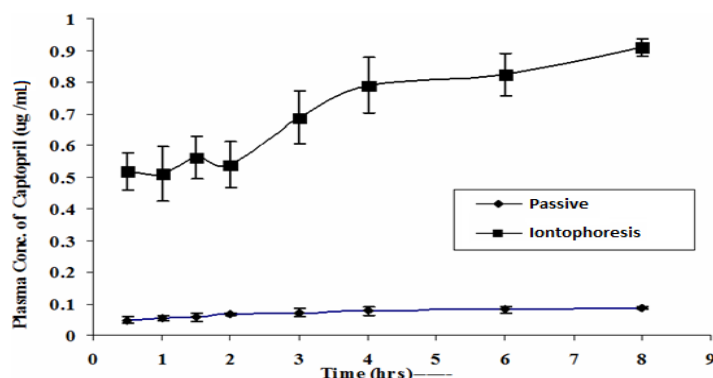
Figure 5. Plasma Concentration of Captopril after passive and iontophoretic administration of Captopril Gel in Rabbits. Data expressed as Mean ± SD (n=3)

Table I. Steady state fluxex, Permeability Coefficients, and enhancement ratio of Captopril in Rabbit and Pigskin from gel formulation.

Parameters	Process	Pig Skin	Rabbit skin
Steady State Fluxes ($\mu g/hours.\ cm^2$)	Passive	32.200	40.137
	Iontophoresis	162.185	251.471
Permeability Coefficients (cm/h)	Passive	1.28×10^{-3}	1.605×10^{-3}
	Iontophoresis	6.48×10^{-3}	10.05×10^{-3}
Enhancement Ratio (R)	(Ionto/Passive)	5.036	6.265
Net Benefit of Iontophoresis ($\mu g/hrours.\ cm^2$)	(Ionto-Passive)	129.985	211.333

Steady state fluxex, permeability coefficients, and enhancement ratio determined and provided in table I. Enhancement by iontophoresis 6.265 folds for captopril was reported in our study using rabbit skin it as slightly higher than that of pigskin. Figure 5 show the plasma concentration profile of captopril using passive diffusion and ionto-phoresis. It was seen that the captopril concentration after iontophoresis increase much rapidly than that of passive diffusion. Very less amount of captopril 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 captopril was achieved 909±28 ng/mL at the end of 8th hour. Results showed that the target permeation rates for captopril 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 1488 µg of captopril must be supplied to the systemic circulation every hour (Wu *et al., 1996*).

The *in-vitro* iontophoretic flux of captopril formulation through rabbit skin was found to be 251.471 µg/hour.cm² in our study. As the Patch in the market usually have wider area (10 cm² and above), it can be expected to achieve the target. Overall results looked quite promising for transdermal delivery of captopril.

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