

Uv

spectrophotometric
method: captopril and
lisinopril



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100 mg of Captopril was weighed accurately and transferred in to 100 ml volumetric flask. Drug dissolved in small quantity of 0.9% NaCl and sufficient quantity of NaCl solution to added to produce 100 ml of stock solution having a concentration of 1 mg/ml. Further 20 mcg/ml concentration was prepared by appropriately diluting the stock solution. The standard solution was scanned between the wavelength ranges of 200 to 300 nm in Shimadzu UV spectrophotometer to determine the wavelength of maximum absorbance. Maximum absorbance for Captopril was recorded at 227 nm.

Preparation of the working standard solution and calibration curve

100 mg of Captopril was accurately weighed and transferred into 100 ml volumetric flask. Drug dissolved in volumetric flask using small quantity of 0.9% NaCl. The volume was made up with the NaCl to 100 ml to produce a stock solution having a concentration of 1 mg/ml. An aliquot of 5 ml of the stock solution was diluted to 50 ml to get a standard solution having a concentration of 100mcg/ml using NaCl solution.

Working standard solutions ranging in concentration from 5 to 30 mcg/ml were prepared by appropriately diluting the standard solution with 0.9% NaCl. The absorbance of each working standard solution was measured at 227 nm using a Shimadzu UV spectrophotometer. Data for each and every experiment was obtained in triplicates and statistically analyzed. The Calibration curve for Captopril in 0.9% NaCl is shown in Fig: 4. 1. 1.

UV Spectrophotometric Method of Lisinopril in Phosphate Buffer pH 7.4

100 mg of Lisinopril was accurately weighed and transferred in to 100 ml volumetric flask where it dissolved in small quantity of phosphate buffer pH 7.4. Sufficient volume of phosphate buffer pH 7.4 added to produced 100
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ml of stock solution having a concentration of 1 mg/ml. A standard solution having a concentration of 20 mcg/ml was prepared by appropriately diluting the stock solution. The standard solution was scanned between the wavelength ranges of 200 to 300 nm in Shimadzu UV spectrophotometer to determine the wavelength of maximum absorbance. Maximum absorbance for Lisinopril was recorded at 217 nm.

Preparation of the working standard solution and calibration curve

100 mg of Lisinopril was accurately weighed in to 100 ml volumetric flask and dissolved in small quantity of Phosphate Buffer pH 7.4. The volume was made up with the Phosphate Buffer pH 7.4 to 100 ml to produce a stock solution having a concentration of 1 mg/ml. An aliquot of 5 ml of the stock solution was diluted to 50 ml to get a standard solution having a concentration of 100mcg/ml using Phosphate Buffer pH 7.4.

Working standard solutions were prepared in concentration range of 5 to 30 mcg/ml by appropriately diluting the stock solution with Phosphate Buffer pH 7.4. Measure the absorbance of each working standard solution at 217 nm by shimadzu UV spectrophotometer. Data for each and every experiment was obtained in triplicates and statistically analyzed. The Calibration curve for Lisinopril in Phosphate Buffer pH 7.4 is shown in Fig: 3.2.1.

Solubility Determination

For determination of solubility, excess amounts of Captopril and Lisinopril were taken into glass vials and dissolved in measured amount of different solvent to obtain saturated solutions. The same were kept at rest for 24 hours to get the equilibrium with the undissolved drug particles. The

supernatant was filtered from these solutions to separate the undissolved drug particles then diluted suitably with the same solvents and the concentrations were measured [119]. Same procedures were followed for both drugs and data for each and every experiment was obtained in triplicates and statistically analyzed.

Partition Coefficient

The octanol/phosphate buffer partition coefficient of both the drugs was determined by shaking equal volume of octanol and phosphate buffer in a separating funnel for 10 min and allowing to stand for 24 hours. Aqueous phase was assayed before and after partitioning to get the partition coefficients [119]. Skin/vehicle partition coefficients were determined by dipping the skin in known concentrations of drug in water for 24 hours and estimating the concentration after partitioning. Data for each and every experiment was obtained in triplicates and statistically analyzed.

pKa Determination

The determination was made using a Digisun DI -707 pH meter with a glass electrode combined with a silver-silver chloride reference system. Solutions were made at different concentration levels and pH was noted the pKa was determined by the equation [120]

$$H^+ = (K_a \cdot C)^{1/2}$$

Preparation of Pigskin Membrane

Superficial skin of pig ear was used. Ear of freshly slaughtered pigs were procured from a local abattoir. From the outer regions of the ear, skin was detached carefully and separated from the underlying cartilage with a scalpel. Adhered fat removed from dermis side the help of a scalpel and <https://assignbuster.com/uv-spectrophotometric-method-captopril-and-lisinopril/>

isopropyl alcohol. At last, the skin was washed with tap water and stored at refrigerator in aluminum foil packing and was used within two days [121].

Preparation of Rabbit Skin

Permission was grabbed from CPCSEA approved Institute Animal Ethics Committee (Reg. No. 1252/ac/09/CPCSEA). The rabbits were sacrificed by the I. V injection of chloroform [122]. Skin samples were taken from the back area of rabbits. From the skin, adherent fat and other visceral debris were detached. To equilibrate the skin, dermal side of full thickness skin was soaked in buffer (phosphate buffer, pH 7.4) for 12 hours at 4°C and used on the same day.

Experimental Design

For the study of effect of concentration and current densities on iontophoretic delivery, experiment was carried out with different donor drug concentrations and current densities. Different systems with diverse concentrations (Current density 0.5 mA/cm²) were designed as System A, System B, System C having concentration 25mg/ml, 50mg/ml and 75 mg/ml respectively. Moreover systems with diverse current densities (Concentration 25 mg/ml) designed as System A1, System A2, System A3 having current densities 0.25 mA/cm², 0.5 mA/cm² and 0.75 mA/cm² respectively.

Moreover to assess the effect of chemical enhancers and iontophoresis on delivery of Captopril and Lisinopril by transdermal route, permeation of drugs was studied using permeation enhancers and iontophoresis. Sodium lauryl sulphate, Dimethyl sulfoxide, Menthol, Oleic acid, Peppermint oil, and Poly ethylene glycol were used as permeation enhancers. Donor compartment contained 1 ml solution having 25 mg/ml of drug and 1 % w/w of enhancer.

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Cathodal iontophoresis was performed at current density 0.5 mA/cm².

Combine effect enhancers and iontophoresis on permeation of drugs studied also.

In-Vitro Passive Permeation Studies

Franz diffusion cell having a receptor compartment capacity of 10 ml were used for in-vitro permeation study. The excised pig/rabbit skin was mounted between the half-cells with the dermis in contact with receptor fluid (Phosphate buffer pH 7.4 / 0.9% NaCl) and was equilibrated for 1 hour. The area available for permeation was noted about 1.21 cm². Temperature was maintained at 37 ± 0.5 °C in the receptor compartment. Under these situations, the temperature at the skin surface was approximately 32 °C. Donor cell was enclosed with an aluminum foil to avoid the evaporation of vehicle. Different solution of Lisinopril in phosphate buffer pH 7.4 and Captopril in 0.9% NaCl solution (each one ml) were placed in the donor cell. The complete assembly was placed on a magnetic stirrer and with the help of magnetic bead, the receiver fluid was stirred constantly. The sample solution was withdrawn from the receptor compartment at regular intervals and assayed for drug content [123].

Procedure of Iontophoretic Diffusion

For iontophoretic permeation study, diffusion cell was modified as suggested by Glikfield [124]. Apparatus consist of a large receiving chamber of glass having two parallel ports on the topside and one sampling port on the side. Both upper chambers are prepared from open-ended cylindrical glass tubes, the outer diameters of which were correspondent to the inner diameter of the parallel ports for ensuring easy fitting. The lower 10 mm of these tubes

were slightly constricted to allow a clearance of 1 to 1.5 mm on the side. Skin was tied at this constricted end, effective diameter increased and became just equal to inner diameter of the extended ports. Skins are tied in this manner so that it touched the receiving solution. Now as the battery was switched on, current flowed through the skin placed in donor anodal compartment into receiving solution below and the subsequently reached to 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. In the present study, silver/silver chloride electrode was inserted into the donor compartment because similar charge repels each other, 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 U-V spectrophotometer.

Permeation Enhancement Studies

Dimethyl sulfoxide, oleic acid, peppermint oil, menthol, poly ethylene glycol, and sodium lauryl sulphate were used for enhancement of permeation. The donor compartment contained drug solution with 1% w/w concentration of different enhancers. Combined effect of chemical enhancers and iontophoresis also studied.

Preparation of Gel formulation

Gel formulations were prepared by soaking 5 g of HPMC overnight (12 hours) in a part of solvent mixture (ethanol: propylene glycol: water in ratio of 50:30:20). Separately dissolved 2.5 g of drug in the similar vehicle was

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incorporated 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 with the help of an electrical stirrer (500 rpm, 1 hour) to make sure uniform transparency [125].

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

Waters binary gradient HPLC system was used for analysis of samples. HPLC system equipped with PDA detector, 515 HPLC pump, C-18 column (ODS; 25 cm X 4.6 mm; 5 μ m) at ambient temperature was used. Mobile phase consisting of phosphate buffer (25 mM potassium dihydrogen ortho phosphate, pH 5.0) and acetonitrile at a ratio 88:12 was used for elution of column. Flow rate of 1 ml/min was maintained, and the detection wavelengths were detected at 215 nm and 220 nm for Lisinopril and Captopril respectively. Retention times were noted 2.3 minutes and 8.4 minutes for Lisinopril and Captopril respectively. Working standards were prepared in phosphate buffer in the range of 5-80 mcg/ml and injected into the column (20 μ L), and standard graph were prepared. A good linear relationship was observed between the concentrations and the peak area obtained with correlation coefficient (R²) 0.9997 and 0.9995 for Lisinopril & Captopril respectively. Samples collected from the diffusion study and content analysis, were injected into the column after appropriate dilution and peak area were noted. The concentrations were determined by comparing the peak areas from the regression equation of the standard curve [126-127].

Physical Examination

The prepared gels were visually inspected for clarity, color and transparency. Gels were also evaluated for the occurrence of any particles using clarity chamber with black and white background [128]. Smears of gel formulations were made on glass slide and placed under the microscope to observed presence of any particle or grittiness. All gel formulations were found to be clear and transparent.

Determination of the Viscosity and pH

Viscosity of the gel formulations was determined using DV-E (Brookfield Viscometer). The samples were taken in a 250 ml beaker, in sufficient quantity so that the spindle of the instrument was fully dipped into the samples. Viscosity determined at 12 r. p. m using Spindle-F, torque value was kept between 50% and 95%. [128, 129]. The pH of gel was determined (using digital pH meter) after diluting and dispersing it in distilled water (10% w/v) [129]. All the measurements were made in triplicate and mean calculated.

Compatibility Studies

Compatibility studies were performed to judge any incompatibility between drug and polymer by IR spectra studies. Spectra of pure drugs and formulations were taken individually by the potassium bromide pellet method using FTIR spectrophotometer (FTIR BX-II, Perkin Elmer, USA).

Stability Studies

The short term stability studies of formulated gels were performed at different temperature and humidity according to ICH guidelines at room temperature (25 ± 2 o C, 60 % RH) and elevated temperature (45 ± 2 o C, 75
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% RH) for a period one month. Prepared gels were visually inspected daily for any change in physical appearance. Zero time samples were used as controls. Samples withdrawn at predetermined intervals (0, 15, 30 days) were analyzed for various performance parameters i. e., Physical appearance, viscosity, pH, drug releases and drug content [130].

Estimation of Drugs in Blood Samples

Blood samples were directly collected into BD vacutainer TM (vacuum tube) from rabbit ear vein and mixed with 0.05 ml of a solution of EDTA-3Na (0.2 M) and ascorbic acid (0.2 M). Centrifugation was carried out immediately at 5000 rpm for 7 minutes at 4°C. Plasma (1 ml) 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). Tube was vortexed for 30 second and then left at room temperature for 30 minutes. Subsequently, 0.2 ml 2 N HCl was mixed, and the resulting plasma samples were frozen at -20°C until assayed. After that, 1:1 mixture of ethyl acetate: benzene (6 ml) was used as extracting solvent. The tube was vortexed for 30 second and followed by gentle shake for 10 minutes. Organic layer was removed (after centrifugation) and evaporated to dry under reduced pressure. The residue was reconstituted at 7.4 pH phosphate buffer and aliquots of 0.02 ml were injected into the HPLC system [127, 131].

Drug Content Analysis

Cellulose membrane was used for determination of content uniformity of the gel preparations. Inappropriate material was removed from the membrane, by washing the tubing in running water for 3-4 hours and for removal of sulfur contaminations tube was treated with a 0.3 % (w/v) solution of

sodium sulfide at 80° C for 1 minute. Then tube was washed with hot water (60° C) for 2 minute, followed by acidification (0. 04N sulfuric acid used) and washing (hot water used) to remove the acidic contents. 2 g of gel formulation was taken in the treated membrane and placed in 50 ml beaker containing 50 ml phosphate buffer (pH 7. 4) and stirred with magnetic stirrer. At the end of 24 hours, sample was taken and estimated for drug content by HPLC [125].

In-vivo Pharmacokinetic Study

Healthy New Zealand rabbits of both sexes (body weight approximately 2 kg) were used for in-vivo studies. Animals maintained in the CPCSEA approved institute animal house and maintained in accordance with guidelines. Method suggested by Anigbogu et al. was utilized for pharmacokinetic studies [132].

Hair was clipped from two spots at the day of experiments and skin areas were wiped with alcohol swabs. In restrainers, animals were allowed to acclimatize for 30 minutes.

In the passive study, blank sample and drug gels were applied parallel to one another on the right dorsum separated by a distance of about 3 cm.

Moreover 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 anode electrode. Circuit was completed by connecting both the electrodes to the respective negative and positive poles of the constant power supply. For in-vivo study current density 0. 1 mA/cm² and area for application of the electrode 4 cm² were used. Time was noted when gels was applied and blood samples (1 ml) were collected every hour from the ear

veins using vacuette tubes. Concentrations were determined using standard curve.

Data analysis

For determination of study state flux, 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. Following formulas were used for the calculations of permeability coefficients and diffusion coefficients [133]:

$$K_P = J_{SS} / C_d \dots\dots\dots (1)$$

$$D = K_P h / K \dots\dots\dots (2)$$

where K_p stand for permeability coefficient, C_d is the concentration of drug in donor compartment, J_{ss} is the steady-state flux, D used for the diffusion coefficient, K is the skin/vehicle partition coefficient and h denoted 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 applied using One-way and two-way ANOVA. For evaluation of the effect of concentration on steady state flux, one-way ANOVA followed by Bonferroni's test used separately [134]. At 95% confidence intervals, p values less than 0.05 were considered to be significant.

DISCUSSION

Since the first patch was commenced in 1981, for clinical benefits, industry interest and regulative precedence it predicted a strong market for transdermal therapeutic systems. This route is particularly suitable for drugs used in long-term therapy like hypertension and diabetes. Captopril and Lisinopril, an orally effective angiotensin-I converting enzyme inhibitors, routinely used to cure hypertension and congestive heart failure. Captopril has a short elimination half life and its plasma half life in man ranges from 1.6-1.9 h [6-8]. Captopril and Lisinopril being antihypertensive agents needs long time administration. Results of post market observation on Captopril and Lisinopril had shown that number of the patients had to withdraw therapy because of the unwanted effects [14-15]. In present market these drugs are available only in the form of oral tablets and have slow and incomplete absorption; moreover food may affect oral absorption of both drugs by up to 25-40% [9-12]. Major problem related to oral therapy includes irregular bio-distribution throughout the body, an absence of drug targeting specificity, the requirement of a large dose to get high blood concentration and unwanted effects due to such high doses [13]. These drugs have stability problems due to the oxidation reaction, which converts Captopril and Lisinopril into Captopril disulphide and Lisinopril disulphide respectively. A recent study had shown that the oxidation rate of Captopril and Lisinopril in dermal homogenates is considerably less than that in intestinal homogenates [15-16]. For hypertensive patients, medication become an essential part of life and noncompliance of therapy may result in chronic complication. Worldwide frequency estimate for hypertension may be as much as 1 billion people, and concerning 7.1 million deaths per year may be <https://assignbuster.com/uv-spectrophotometric-method-captopril-and-lisinopril/>

attribute to hypertension. To overcome the problems encountered with oral delivery like imperfect absorption, less oral bioavailability, and for the effective treatment of chronic hypertension, alternative transdermal route of administration suggested valuable. Being medication essential part of life, the success of the treatment depends on patient compliance too. Seeing as transdermal dosage forms can reduce the fluctuations of plasma drug concentration and increase patient compliance, the development of the transdermal therapeutic systems for these drugs has become research interest of late [17, 18].

Captopril and Lisinopril were scanned in the UV wavelength region of 200-400 nm for maximum absorption (λ_{max}). The λ_{max} were found to be at 227 nm and 217 nm for Captopril and Lisinopril respectively that were same as reported value. Linear relationships were observed between the concentration and absorbance values (Table 4. 1. 1 & Table 4. 2. 1) in the range of 5 to 30 $\mu\text{g/ml}$ (Slope = 0. 0138, $R^2 = 0. 9945$) for Captopril and same 5 to 30 $\mu\text{g/ml}$ (slope = 0. 0358, $R^2 = 0. 9966$) for Lisinopril. (Fig 4. 1. 1 & Fig 4. 2. 1)

Though it was hypothesized that skin is permeable to the lipophilic moieties of low molecular weight, in reality the extent of transdermal permeation is a composite parameter influenced by many factors. In addition to molecular weight, partition co-efficient, solubility, the pka value that determines the extent of ionization, is of prime importance. As Doh et al suggested, drug candidates for transdermal dosage form should have molecular weight around 200~500 Da [135]. Captopril and Lisinopril having molecular weights 217. 29 and 405. 5 Da respectively, well fits into this range. Various <https://assignbuster.com/uv-spectrophotometric-method-captopril-and-lisinopril/>

physicochemical parameters of Captopril and Lisinopril were investigated and the results listed in Table 4. 1. 2 & 4. 2. 2. Both drug candidates showed good aqueous solubility. The experimentally determined partition coefficients (octanol/phosphate buffer) were found to be 0.335 ± 0.0090 and 0.675 ± 0.0124 for Captopril & Lisinopril respectively, which indicated that these drugs have less affinity towards the lipid compared to phosphate buffer, which is not favorable for transdermal permeation. Although, the lipophilicity of a drug moiety is an intrinsic character therefore cannot be modified.

At pH 7.4 mammalian skins are negatively charged and ionic forms have low affinity to skin. Captopril and Lisinopril, being an acidic drug with the pKa value of 3.481 and 3.85 respectively, were largely ionized which decreased its affinity toward the skin [136]. However ionization make favorable for iontophoresis. In the process of iontophoresis, the ionized moieties are actively forced, using low intensity current through natural pore pathways of skin at the same time the unionized fraction could pass the unbroken horny layers by passive diffusion resulting in enhanced permeation [137]. As the permeation rate completely depends on the energy so controlling the supply of the current can control the delivery rates [138]. The diffusion cell was modified, to simulate the physiological condition, where both the electrodes were placed on the same side of skin and receiving chamber filled with phosphate buffer, reflected the body.

Proper selection of electrode is very vital in iontophoresis as in this method the electronic current gets converted to ionic current at the electrode-solution interface. The electrodes like stainless-steel, platinum or carbon graphite do not contribute in the electrochemistry thus the inert

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electrochemistry forces the water in the reservoir to become fuel for the electrochemistry. Because the oxidation take place at the anode and reduction at the cathode, hydrogen and hydroxyl ions are generated; this results to drop in pH at the anode and rise at the cathode. That change in the pH may lead to irritation or burns at the site. In addition, there may also chance for degradation of drug at the electrode. Problem does not occur with silver/silver chloride reversible electrodes, which take parts in electrochemistry and chosen for this study [138].

The main factor that affects the in-vitro transdermal permeation is the barrier properties of the skin. The present study carried out using porcine ear skin, obtained from local slaughterhouse. As the barrier properties of the skin varies with age of the skin, dermal thickness, hair follicle depth, factors controlled by experimentally. Same ear skin was used for iontophoresis as well as passive permeation studies to control the variability of results by skin. Porcine skin has similar thickness of stratum corneum as human skin. Also the hair follicle density of pig and human skin (about 11 hair follicles/cm²) is similar which is higher in other mammals. Using a number of compounds, it has been shown that the pig skin has found the closed permeability characteristics to that of human skin. In-vitro studies with human skin have been also shown to correlate fine with in-vivo studies in pigs. In one more study the skin permeability of nicorandil was find out across excised skin samples from hairless mouse, guinea pig, pig, hairless rat, dog and human. Among six species, the permeability was highest in hairless mouse while that in pig and human skin was found in good

agreement. It was observed that pig and human skin had similar barrier thickness, surface lipid and morphological features [138].

Among the various factors that affect skin permeability, the concentration of the actives in the delivery system is the most crucial. To evaluate this effect of concentration and effect of current densities the experiments were designed at three different drug concentrations and current densities (Table 4. 1. 3 & Table 4. 2. 3).

Fig 4. 1. 8 & Fig 4. 1. 9 shows passive and iontophoresis permeation profile of Captopril at different donor concentrations. The passive profiles are linear at all concentration levels indicating the permeation kinetics was more or less zero order. The rate of permeation increased with increasing donor drug load. This is expected, as increase in the donor drug concentration enhances the concentration gradient, that is the driving force of mass transport [139]. In contrast iontophoretic profiles are less linear showing the involvement of some other factors along with concentration. In iontophoresis though ionic repulsion is the dominant process, there is also a convective transport of the materials toward the direction of flow of current, moreover the permeability of skin changes. The total flux of a solute during iontophoresis is the sum of fluxes due to electro repulsion, convective flow, and passive diffusion [139]. Similar results were found to be in case of Lisinopril. (Fig 4. 2. 8 & Fig 4. 2. 9).

The total flux of a solute during iontophoresis is the sum of fluxes due to electro-repulsion, convective flow, and passive diffusion [137]. At pH 7. 4, Lisinopril (pKa 3. 85) and Captopril (pKa= 3. 48) acquired negative charges

and decided to 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 expected to contribute to the permeation. There are also possibilities to oppose the permeation from the cathodal compartment by electro-osmotic flow [140]. In our study, iontophoretic profile indicates the initial permeation was high however the permeation rate declined in the later hours. This was unexpected as the voltage gradually dropped with time and therefore the magnitude of electro-osmotic opposition was expected to be lesser in the later part of the study. The contrary of result suggested the contribution of a factor that negatively influenced the permeation as time passed. This is also possible that as the current flows, the cathodal electrode (Ag/AgCl) obtained a steady flow of electrons, which lead to the release of negatively charged chloride ions. As time passed, the concentration of this newly liberated chloride ions were probably to increase in the cathodal portion. A chloride ion, being much smaller than the drug ion, was a powerful competitor, which diminished the transport efficiency of the drugs, since the drug candidates were negatively charged and chloride ions act as competitor [141].

Though steady state flux is considered to be the most therapeutically relevant parameter, permeability and diffusion parameters are also very important for comparison purpose [142]. It is evident that as the concentration increase in donor the permeability coefficients decrease. Result for the permeability and diffusion coefficients of Captopril & Lisinopril

in different systems in our study for passive diffusion and iontophoresis are provided in Table 4. 1. 12 & 4. 2. 12, which found to obey above hypothesis.

Table 4. 1. 13 & Table 4. 2. 13 depict the enhancement in iontophoretic flux compared to the passive flux of same donor concentration. Enhancements were highest at the lowest drug load and lowest at the highest drug load for both the drugs. To analyze the net benefit of electrical energy, the active fluxes of drug at various donor drug loads was compared with the corresponding passive value. (Table 4. 1. 13 & Table 4. 2. 13) The iontophoretic contribution was found to be slightly more at higher donor concentrations.

Pikal et al suggested that permeability of skin also changes under influences of current [143]. To observe the effect of current densities on the transport of Captopril and Lisinopril through pig ear skin, experiment was carried out at three different current densities and result was found that iontophoretic drug transport increased with the increasing current densities (Fig 4. 1. 10 & 4. 2. 10).

The relationship between current density and flux of drugs may be described by Faraday's law which is represent by following equation [144]

$$J_i = t_i I_t / Z_i F$$

where J_i , Z_i are the flux and charge of drugs 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 and Z_i 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 [145]. Some researchers suggested that possibility may be also that the electro-osmotic volume flow increases with an increase in current densities [146] which leads to increase in the flux of the drug.

To assess the combined effect of chemical enhancers and iontophoresis on transdermal delivery of selected drugs, permeation was carried using permeation enhancers with iontophoresis. Dimethyl sulfoxide (DMSO), menthol, oleic acid, peppermint oil, poly ethylene glycol, and sodium lauryl sulphate were used as permeation enhancers. These chemical substances temporarily disrupting the barrier properties of skin and known as accelerants or sorption promoters can enhance drug permeation. DMSO is one of the earliest and most commonly used as penetration enhancers, it changes the intercellular keratin conformation, from α helical to β sheet [147-148] and enhances the permeation. The possible mechanism of action of oleic acid suggested as it increases the fluidity of the lipid structure by penetrating into it, that is again supported by its polar end and bent structure and peppermint oil probably operates due to opening of tight junctions [149-150]. Mechanisms of action for the permeation enhancement by propylene glycol is suggested by keratin solvation within stratum corneum by competition for the hydrogen bond binding sites with water and the intercalation in the polar head groups of the lipid bilayers [151]. Anionic surfactant like sodium lauryl sulphate open up the protein controlled polar pathway by uncoiling and extending the alpha-keratin helices and swelling the stratum corneum [152]. Menthol reported for penetration enhancing

effect by changing of dense barrier structure of the stratum cornea [153]. Cumulative amount permeation of drugs with and without enhancers provided in Fig 4. 1. 24-25 & Fig 4. 2. 24-25. Diffusion and permeability coefficients increase in the order of Pure drug < PEG < SLS < Oleic acid < Menthol < Peppermint oil < DMSO. The diffusion and permeability coefficients of both drugs with DMSO were found highest in our study, moreover with the iontophoresis synergistic effect was found (Table 4. 1. 29 and Table 4. 2. 29). Res