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IN VITRO AND IN VIVO SKIN PERMEATION STUDIES ON TRANSDERMAL THERAPEUTIC SYSTEM OF LERCANIDIPINE HYDROCHLORIDE

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ABSTRACT

Lercanidipine hydrochloride, an effective calcium channel blocker, widely used for the treatment of chronic stable angina and hypertension seems to be potential therapeutic transdermal system candidate, mainly due to its low oral bioavailability, short half life and high first-pass metabolism. Hence an attempt was made to develop transdermal therapeutic systems for lercanidipine hydrochloride using the polymer blend of eudragit RL100 and polyvinyl pyrolidone (PVP) in the ratio 1:4 by solvent casting method. propylene glycol (PG) (15 % w/w) and tween 80 (4% w/w) were selected as plasticizer and permeation enhancer respectively based on the preliminary optimization studies. Incorporation of PVP and PG improved the flexibility, folding endurance handling properties and drug release of the films. The skin irritation studies indicated that there is no recognizable changes on skin surface after the removal of the film. The patches were also evaluated for *in vitro* skin permeation using human cadaver skin. The presence of tween 80 produced significant increase in permeability and the film exhibited the cumulative amount of drug permeation across skin of 4813.903 µg/cm^2 at the end of 24 h. *In vivo* bioavailability studies in rabbits demonstrated that the transdermal film provided steady state plasma concentrations with minimal fluctuations and nearly six fold enhancement of bioavailability of lercanidipine hydrochloride relative to oral administration. It may be concluded that the fabricated transdermal delivery system have the potential to provide controlled and extended drug release, better bioavailability and thus, they may improve the patient compliance.

KEY-WORDS: *Transdermal therapeutic system; Lercanidipine hydrochloride; Eudragit; PVP; Skin Permeation.*

INTRODUCTION

Lercanidipine hydrochloride (LD) a potent calcium channel blocker, widely used in the treatment of hypertension and angina pectoris. However, the literature survey reveals that, it undergoes variable and extensive first pass metabolism following oral administration resulting in oral bioavailability of about 10 %[1]. Hence to achieve therapeutic concentration, frequent dosing or large doses are required. But the oral administration of large dose of LD can produce nausea and other gastro-intestinal disturbances[2, 3]. Hence, to improve the bioavailability, transdermal therapeutic systems (TTS) are better suited for lercanidipine hydrochloride. Moreover, LD is an ideal candidate for the transdermal delivery because of its low molecular weight (648.2 D), low dose (10-20 mg/day) and balanced hydrophilic-lipophilic characteristics (log P = 6.4)[2, 4].

Transdermal delivery of LD avoids the firstpass effect and provides greater and more prolonged levels of unchanged LD compared to the oral regimen and overcomes the problems associated with oral administration of the drug. In addition, patient compliance, convenience of application and removal, constant and prolonged drug delivery, reduced frequencies of drug dosing, minimum inter- and intrapatient variability are some more advantages with TTS[4, 5].

In present study, matrix diffusion controlled TTS was designed and developed for extended delivery of LD using hydrophilic-lipophilic polymer combination. Even though various techniques are reported in the literature, the matrix diffusion controlled TTS was selected because of ease of fabrication (Jain, 2003).

EXPERIMENTAL

MATERIALS

Lercanidipine hydrochloride was obtained as gift sample from Cipla Ltd. Mumbai. Eudragit RL100 (ERL) was procured from Degussa India Pvt. Ltd, Mumbai. Polyvinyl pyrolidone (PVP), Propylene glycol (PG), tween 80, methanol and acetone were of analytical grade and purchased from Sree Durga Chemicals, Mangalore.

FABRICATION OF TRANSDERMAL PATCHES

Matrix type transdermal patches loaded with LD were prepared by solvent casting method. A flat square shaped, aluminium foil coated glass molds having surface area 25 cm^2 were fabricated for casting the patches. The polymeric films with ERL/PVP ratio 1:4 containing LD (30% w/w based on total polymer weight), PG (15 % w/w based on total polymer weight) as plasticizer and tween 80 (4% w/w based on total polymer weight) as permeation enhancer were selected for the *in vitro* and *in vivo* skin permeation studies based on the preliminary optimization and *in vitro* drug release studies.

a. Preparation of casting solution

The casting solutions were prepared by dissolving weighed quantities of polymers (ERL and PVP) in acetone-methanol (1:1) mixture. Required quantities of the drug (LD), plasticizer (PG) and permeation enhancer (tween 80) were then added to the polymer solution and thoroughly mixed to form a homogeneous mixture. The volume was made up to 7 ml with solvent mixture. Entrapped air bubbles were removed by applying vacuum. Composition of formulation is given in Table 1.

Table 1: Composition of formulation.

Ingredients	Quantity
Eudragit RL 100	140 mg
PVP	560 mg
Lercanidipine hydrochloride	210 mg
Propylene glycol	105 mg
Tween 80	28 mg
Methanol- Acetone (1:1) mixture	qs.7 ml

b. Preparation of transdermal patches

Casting solution (5ml) was poured into glass moulds and dried at room temperature for 24 h for solvent evaporation. An inverted funnel was placed over the glass moulds to prevent the fast evaporation of the solvent. After 24 h, the dried patches were removed by peeling and cut into square dims of 4 cm x 4 cm (16 cm^2) (Fig. 1(a)). These patches were kept in desiccator for 2 days for further drying and wrapped in aluminium foil, packed in self-sealing covers[6,7,8]

c. Lamination of Transdermal Patch

The prepared transdermal patch was cut and placed on an aluminium foil that served as the backing membrane. 5 % w/v solution of polyisobutylene was applied as adhesive along the

circumference of the aluminium foil and dried at room temperature for 10 h. The patch was covered with silicone coated release liner[9] (Fig. 1(b)).

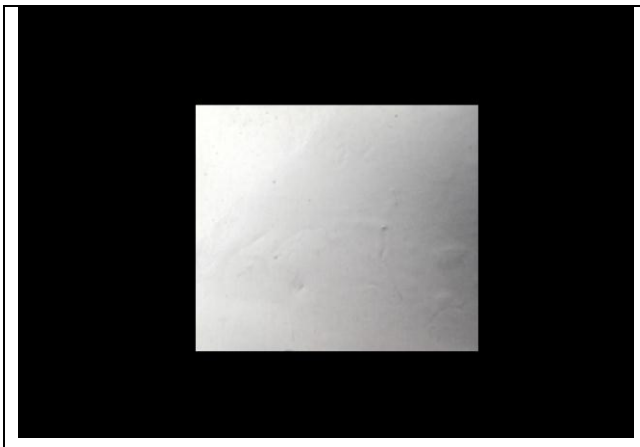

Figure 1a: Prepared transdermal film

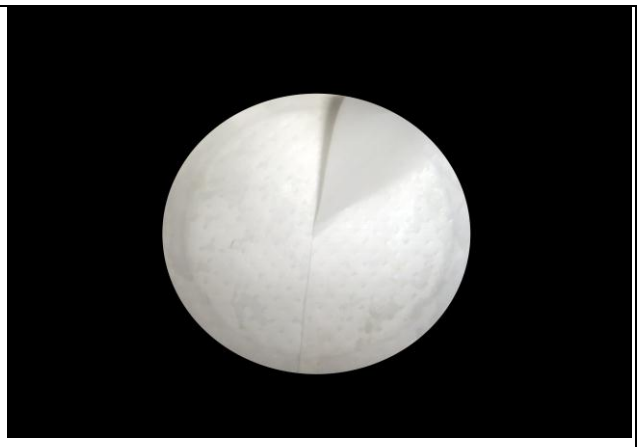

Figure 1b: Laminated transdermal patch

PHYSICO-MECHANICAL CHARACTERIZATION OF TRANSDERMAL PATCHES

The prepared film was characterized for physical appearance (colour, flexibility, homogeneity and smoothness), uniformity of thickness and weight, folding endurance and drug content. The thickness was measured at five different places of the patch using a screw gauge and the mean and standard deviation were calculated[10, 11, 12]. For weight uniformity testing, five patches having area of 1 cm^2 were dried at 60 ^oC for 4 h and then weighed on a digital balance. The average weight and the standard deviation values were calculated[10]. Folding endurance was measured manually for the prepared film. A strip of film (2 x 2 cm^2) was cut evenly and repeatedly folded until it broke. The number of times the film could be folded at the same place without breaking was observed[6, 13].

Drug content was determined by dissolving the film of 1 cm^2 area as small pieces in 20 ml of methanol. The flask was shaken continuously for 24 h in a mechanical shaker. Then the solution was filtered and residue was washed with methanol. The filtrate was made up to 100 ml with phosphate buffer pH 7.4 containing 20 % v/v PEG 400 and the absorbance was measured at 362.0 nm in a double beam UV spectrophotometer (Systronics-2203) using the blank film solution as blank and the drug content was determined[13].

PRIMARY SKIN IRRITANCY STUDY

Skin irritation studies were performed on healthy albino rabbits of either sex each weighing 2.0 to 3.0 kg on basis of the study protocol approved by the Institutional Ethical Committee

(CPCSEA Reg. No.1564/PO/a/11/CPCSEA-23-1-12) by the method described by Draize *et al.*[14]. The dorsal surface of the rabbits was cleaned and the hairs were removed by shaving. The skin was cleansed with rectified spirit. The rabbits were divided in to three groups (n =3). Group I received a 1 cm^2control patch (placebo film without any drug), group II received 1cm^2 transdermal films and group III received 0.8% v/v aqueous solution of formaldehyde as a standard irritant. The patches were applied to the shaved skin of rabbit and secured using adhesive tape. After 24, 48 and 72 h of the test article application, the test sites were examined for dermal reactions of erythema/edema and scored in accordance with the Draize scoring criteria[14] as per International Organization for Standardization (ISO) specification 1010993-10:2002/Amd 1:2006[15] (Erythema scale: 0-none; 1 very slight, barely perceptible; 2-well defined; 3-moderate; 4-beet redness and scar formation. Edema scale : 0-none; 1-very slight, barely perceptible; 2-well defined, edges of area well defined by raising; 3-moderate (raised approximately 1mm); 4-severe, raised more than 1mm and extending beyond the area of exposure). The score of Primary Dermal Irritation (PDI) was calculated as the difference between the sum of the scores for erythema and edema at particular time divided by the number of observations for the treated sites and the sum of the scores for erythema and edema at particular time divided by the number of observations for the control sites. Primary Dermal Irritation Index (PDII) was calculated by adding the PDI scores for 24, 48 and 72 h scoring intervals divided by the number of scoring intervals[16, 17, 18].

IN VITRO SKIN PERMEATION STUDIES

According to latest OECD draft guideline (22 October, 2010) and European Medicines Agency (22 August 2012), the human cadaver skin is the best choice for *in vitro* permeation study**.** So the study was carried out across the human cadaver skin on basis of the study protocol approved by the Institutional Ethical Committee (Reg. No. IAD/IEC/13/14). The healthy skin from the forearm region of cadaver brought for autopsy was taken in a sealed evacuated plastic bag in a thermos containing ice. The human skin was freed from fat by the use of Irish scissors till the dermis is seen. The hair was cut and then the skin was allowed to stand at room temperature and rehydrated by immersing in distilled water for 15 min[19]. Then the cadaver skin was mounted between donor and receptor compartment of the diffusion cell with epidermis facing towards the donor compartment. 30 ml of phosphate buffer pH 7.4 containing 20 % v/v PEG 400 was used as elution medium. The film of 1 cm^2 was applied on the epidermal surface of the skin with a pressure sensitive adhesive in such a way that the drug releasing surface faced toward the skin**.** The elution medium was

magnetically stirred for uniform drug distribution at a speed of 100 rpm. The temperature of the whole assembly was maintained at 37±1°C by thermostatic arrangements. An aliquot of 3 ml was withdrawn at a suitable interval and an equivalent volume of fresh buffer was replaced. The amount of drug permeated across the membrane was determined at 362.0 nm spectrophotometrically after proper dilution with the elution medium. The cumulative amount of drug permeated was calculated and plotted against time[19, 20, 21].

The study state flux (Jss) of the drug permeated was calculated from the slope of the steady state portion of permeation profile by linear regression analysis according to the equation; $Jss = (dQ/dt)_{ss}$. 1/S, where, $(dQ/dt)_{ss}$ is the steady state slope and S is the effective surface area of the film subjected to diffusion. The permeability coefficient (Kp) was calculated using the equation; Kp = Jss/Cs, where, Cs is the Cs is the initial concentration of the drug in the film placed in donor compartment[17, 22, 23]. The lag time was determined by extrapolating the linear portion of the cumulative amount of drug permeated versus time plot to the abscissa[23]. The mathematical equation for the required drug release rate from a formulation to provide therapeutic drug concentration (Kr) can be given as; Kr = Ke.Cd.Vd.BW, where, Ke is the first order rate constant for drug elimination, Cd is the therapeutic drug concentration, Vd is the volume of distribution and BW is the standard body weight[24, 25]. For lercanidipine hydrochloride, $t_{1/2}$ = 2.8 h, Cd = 3.3 µg/l and Vd = 2.5 l[1, 2, 3] and therefore the desired drug release rate can be calculated as; $Kr_{ler} = (0.693/2.8) \times 3.3 \times 2.5 \times 70 = 142.93$ µg/h. The area of patch (A) required for desired release rate can be given as; $A = Kr/(K_{app}/S)$, where, Kr is the required drug release rate, K_{app} is the diffusion rate constant (µg/h) calculated from the slope of the linear drug diffusion profiles and S is the surface area of the film subjected to diffusion[25].

IN VIVO SKIN PERMEATION STUDIES

a. Selection of animals

The *in vivo* performance of lercanidipine hydrochloride following oral and transdermal administration was studied on basis of the study protocol approved by the Institutional Ethical Committee (CPCSEA Reg. No.1564/PO/a/11/CPCSEA-23-1-12) and was based on two-way cross over design. Six healthy rabbits (New Zealand white) of either sex having the weight of 2.2 - 2.8 kg were chosen for the study. They were divided into two groups (n=3), and both the products (oral solution and transdermal film) were administered to each group in two different study periods. Before the second study period, an appropriate wash out period

of 1 week time was allowed for the drug to be virtually eliminated. Animals were fasted 12 h prior to the administration of the drug formulation and until 12 h post dosing but had free access to water during the entire study period. One day before the experiment, the hair on the abdominal area was removed by applying depilatory for 10 min and washed with distilled water. To study the oral pharmacokinetics, 1.50 mg of LD was administered as solution in distilled water with 20 %v/v PEG 400 to rabbits (n=3) using an oral catheter. The catheter was flushed with 2 ml of distilled water with 20 %v/v PEG 400 to ensure complete dosing. The remaining rabbits (n=3) were applied 0.25 cm^2 (0.5 cm x 0.5cm) of formulated transdermal film containing 1.5 mg of LD on the hair free abdominal skin with a pressure sensitive adhesive and occluded with plaster[26, 27].

b. Estimation of lercanidipine hydrochloride in serum

About 2 ml of blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h for oral dose 0, 2 ,4, 6, 8, 12, 24, 36, 48 h after transdermal administration from the marginal ear vein of the animals into sterilized glass tubes. The serum was separated by centrifugation at 2000-3000 rpm for 10 min. The plasma samples were stored at cold temperature until analysis. Safety was evaluated by monitoring adverse events, vital signs and physical examinations[28].

The sensitive and validated method reported by Selvaduai *et al.*, was followed for the estimation of lercanidipine hydrochloride in serum. A volume of 0.5 ml of serum was pipetted into 2.0 ml centrifuge tube and to this 0.5 ml of precipitating agent (10% perchloric acid) was added. The resulting solution was vortexed for 5 min and centrifuged at 4000 rpm for 10 min. Supernatants from the above solutions was separated and filtered using 2 micron pall syringe filter which was then used for the estimation. Analysis was performed using Agilent-1120 lc Compact HPLC which consists of binary pump, rheodyne manual injector, 20 µl sample loop and UV detector. Column used for the analysis was Waters x-bridge C18 Column (250 X 4.6 X 5 µm). HPLC grade methanol and millipore water (80:20) was used as mobile phase. The mobile phase was filtered using 45 micron pall nylon membrane. Sample volume taken is 20 µl and the flow rate was set to 1 ml/min at ambient temperature. Run time was 15 min and the sample was detected at 267 nm. Linearity was evaluated over LD concentration range 1 to 60 µg/l with minimum detection limit of 0.5 µg/l[29].

c. Pharmacokinetic analysis

From the HPLC analysis of the rabbit plasma samples, the plasma concentrations of the drug were determined from its calibration curve and the plasma drug concentration-time profile

was plotted. Pharmacokinetic parameters such as maximum serum concentration (C_{max}) and time to reach maximum concentration (t_{max}) were read directly from the individual serum concentration-time profiles. The elimination rate constant (Ke) was calculated by regression analysis from the slope of the terminal phase of concentration-time plot and biological half life ($t_{1/2}$) was determined by 0.693/Ke. Area under the curve (AUC) and area under the first moment curve (AUMC) were found by linear trapezoidal rule and MRT was calculated by dividing the AUMC by AUC[28]. The relative bioavailability of lercanidipine hydrochloride after the transdermal administration versus the oral administration was calculated[30] as follows:

$$\% Fr = \frac{AUC_{transdermal}}{AUC_{oral}} \times \frac{Dose_{oral}}{Dose_{transdermal}} \times 100$$

The statistical significances of the differences between the results of *in vivo* studies were analyzed by unpaired t-test at 5 % level of significance.

IN VITRO – IN VIVO CORRELATION

An *in vitro* - *in vivo* correlation (IVIVC) has been defined by the FDA as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an in vivo response". In the present study, Level A correlation was undertaken which is a point to point relationship between in vitro drug permeation through skin and in vivo absorption rate of a drug from the dosage form. Here *in vivo* percentage drug absorbed was plotted against *in vitro* drug permeated for the same period of time to determine the correlation coefficient[31].

RESULT AND DISCUSSION

PHYSICO-MECHANICAL CHARACTERIZATION

In the present study, efforts have been made to prepare lercanidipine hydrochloride TTS by solvent casting technique. The casting solution (5ml) was spread in to 25 cm^2 so that each cm^2 contains approximately 6.0 mg of the drug. The prepared films were slightly pale yellow coloured with homogeneous appearance and possessed uniform surface. Drug was uniformly distributed through the matrix film. There were no observable particles of drug in the matrix film. The physico-mechanical valuation data of the films is presented in Table 2.

Table 2: Physico-mechanical characters of formulated transdermal film (Mean ± S.D., n=5).

Film Parameters	Characteristic values
Physical apperance	Flexible, Smooth & Nonsticky
Colour	Pale yellow
Thickness (mm)	0.243 ± 0.005
Weight (mg)	33.614 ± 0.663
Folding Endurance	224 ± 5.025
Drug content/cm^2 (mg)	5.902 ± 0.044
Percentage drug content (%)	98.358 ± 0.738

The folding endurance was measured manually and it was found that ERL / PVP films without adjuvants were hard, brittle and fragile with low folding endurance. To prevent embrittlement, plasticizer (PG) was incorporated, which can diffuse into and softens the polymer matrix. Folding endurance values of the film were 220-240, which show that hydrophilic polymer (PVP) and the presence of plasticizer can provide higher folding endurance and good flexibility. The results suggested that the patches would not break and would maintain their integrity with general skin folding when applied[32]. The films were evaluated for the film thickness at various points. It was found that the thickness at the edges of the rectangular tray was a bit higher and uneven compared to the rest of the parts of the film. It may be due to the curvature of the viscous slurry at the edges of the foil due to surface tension. After removing these edges, films were remeasured for thickness and it was observed to have uniform thickness and low standard deviation. It indicates the uniformity of the films prepared by the solvent casting method[8]. No significant difference in the average weight among each group indicating that the patches are uniform throughout. Good uniformity of the drug content among the patches was observed for the formulations which ranged from 97.45 % to 99.21% (Table 2). Based on the initial drug loading, the entire sample films were containing above 5.847 mg, which proves that the process employed to prepare the films in this study was capable of producing films with uniform drug content and minimum batch variability.

PRIMARY SKIN IRRITANCY STUDY

Skin irritation study was carried out on rabbits revealed that the formulated transdermal film of lercanidipine hydrochloride showed no erythema and edema. The photographs taken are shown in figure 2(a) to figure 2(d). The score assigned to the skin reactions at various time points are given in table 3.

In Group I, there was no sign of either erythema or edema after 24 h of application, but there was very slight edema observed in one rabbit after the application of 48 hrs. The primary irritation index for the test was found to be 0.553 and it indicates barely perceptible irritation. According to Draize test, the formulation producing scores of 2 or less are considered as negative (no irritation)[14, 18]. Hence the developed transdermal film is free from skin irritation and safe for application on skin.

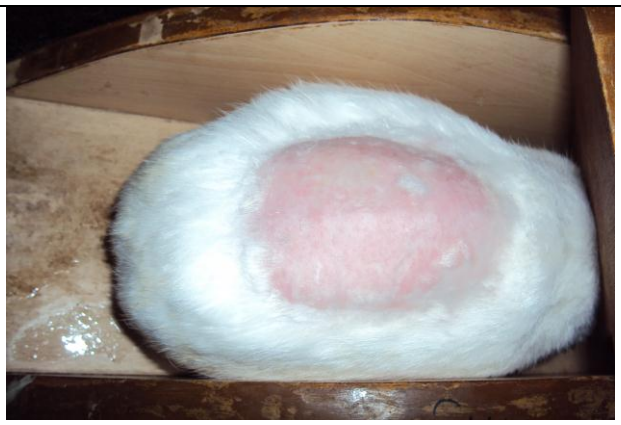

Figure 2a: Rabbit skin after the removal of control patch

Figure 2b: Rabbit treated with transdermal film

Figure 2c: Rabbit skin after the removal of transdermal film

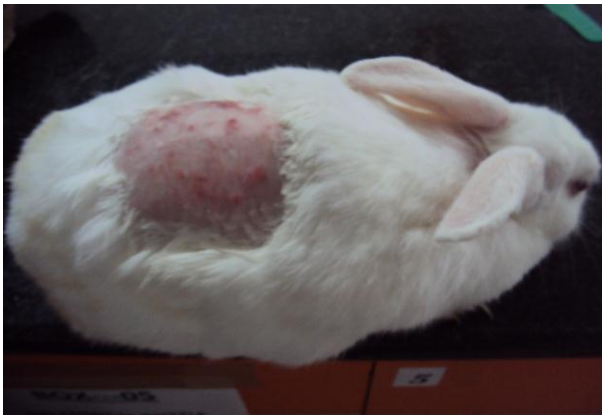

Figure 2d: Rabbit treated with standard irritant

Table 3: Skin irritation scores following transdermal patch administration.

Group	Rabbit No	24 h		48 h		72 h	
		Erythema	Edema	Erythema	Edema	Erythema	Edema
Group I (Control patch)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	1	0	1	0	0
	Mean	0	0.33	0	0.33	0	0
	PDI	0.33		0.33		0	
	PDII	(0.33 + 0.33 +0)/3 = 0.22					

Group II (Formulated transdermal film)	1	0	1	0	1	0	1
	2	0	0	0	0	0	0
	3	0	1	1	0	1	1
	Mean	0	0.66	0.33	0.33	0.33	0.66
	PDI	0.66 – 0.33 = 0.33		0.66 - 0.33 = 0.33		1 – 0 = 1.0	
	PDII	(0.33 + 0.33+ 1)/3 = 0.553					
Group III (Standard: 0.8%v/v Formailin Solution)	1	1	2	2	2	2	3
	2	1	3	1	3	2	3
	3	2	2	2	2	3	2
	Mean	1.33	2.33	1.66	2.33	2.33	2.66
	PDI	3.66 – 0.33 = 3.33		3.99 – 0.33 = 3.66		5 – 0 = 5.0	
	PDII	(3.33+3.66+5.0)/3 = 4.0					

IN VITRO SKIN PERMEATION STUDY

In vitro skin permeation studies from transdermal patches were carried out through human cadaver skin and the results are summarized in Fig. 3. The prepared film exhibited the cumulative percentage of drug release of 82.763 % and the cumulative amount of drug permeation across skin 4813.903 µg/cm^2 in 24 h. The incorporation of tween 80 can significantly improve the drug permeation characteristics as it reversibly perturbs the permeability barrier of the stratum corneum. The *in vitro* skin permeation studies revealed that the incorporation of PG into the LD-ERL-PVP films may be useful for improving the physico-mechanical and drug release properties of the films and the incorporation of tween 80 into the films may be useful for attaining required drug permeation.

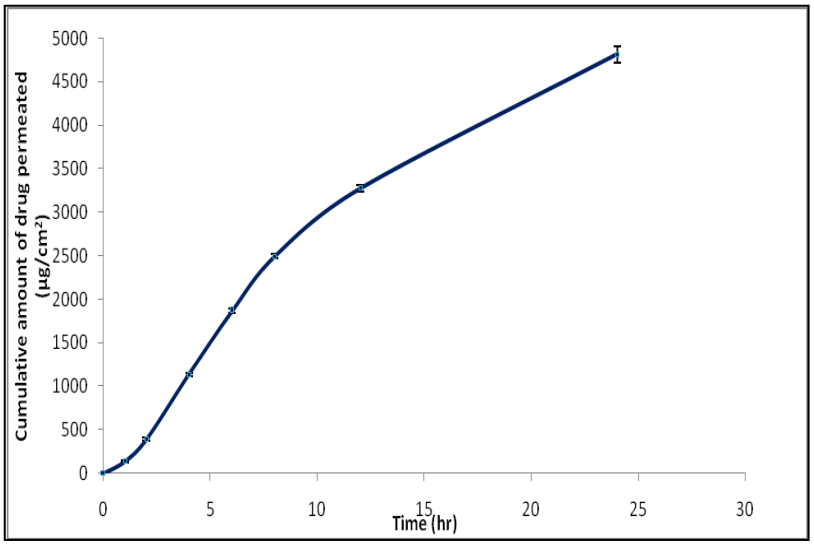

Figure 3: *In vitro* skin permeation of lercanidipine hydrochloride from transdermal film.

IN VIVO SKIN PERMEATION STUDIES

The *in vivo* performance of LD following the administration of transdermal film was studied and compared with that of oral administration. LD was not present in the samples taken prior to dosing. These samples served as negative control for the experiment. The pharmacokinetic parameters calculated from the plasma concentration - time profile of the drug after its oral and transdermal administration were given in table 4.

Table 4: Pharmacokinetic parameters after oral and transdermal administration of lercanidipine hydrochloride in rabbits (Mean ± S.D, n=6).

Pharmacokinetic Parameters	Oral Solution	Transdermal Film
C_{max} (µg/L)	49.467 ± 9.803	41.517 ± 2.330
t_{max} (h)	2.0 ± 0	8.333 ± 1.966
AUC $_{0-t}$ (µg.h/L)	159.117 ± 34.539	976.133 ± 47.903
$AUMC_{0-t}$ (µg.h^2/L)	609.396 ± 148.113	15858.33 ± 857.505
AUC $_{0-\alpha}$ (µg.h/L)	171.66 ± 41.270	1017.933 ± 53.589
$AUMC_{0-\alpha}$ (µg.h^2/L)	824.390 ± 279.858	17623.01 ± 1610.805
MRT (h)	4.730 ± 0.467	17.290 ± 0.926
K_e (h^{-1})	0.214 ± 0.034	0.174 ± 0.025
$t_{½}$ (h)	3.308 ± 0.559	4.054 ± 0.647
Relative Bioavailability		5.92 Times

The results from the oral administration of LD solution indicated that it is rapidly absorbed from the gastro-intestinal tract of rabbit having a C_{max} of 49.467 ± 9.803 µg/l and t_{max} of 2.0 h. Transdermal administration of film achieved almost steady state serum concentration of LD between 41.233 ± 2.594 and 31.817 ±1.598 µg/l up to 24 h after an initial lag time of approximately 5 h (Fig. 4).

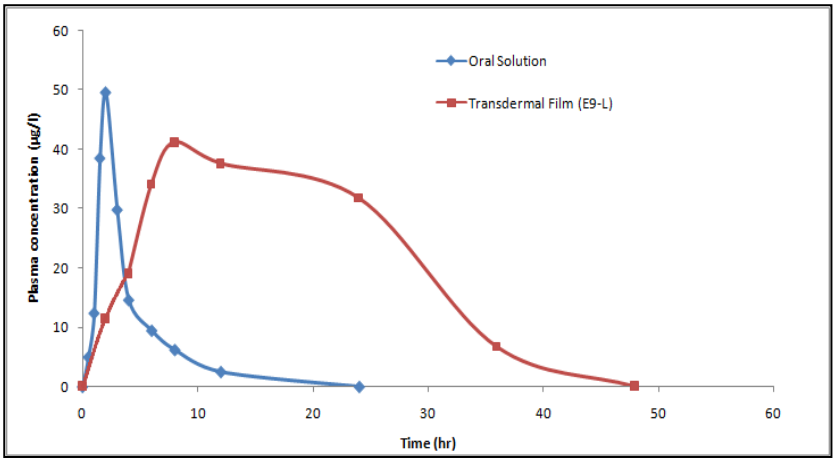

Figure 4: Plasma concentration level of lercanidipine hydrochloride after oral and transdermal administration.

The low C_{max} and prolonged t_{max} after the administration of transdermal film was due to the barrier properties of the skin and accumulation of the drug in skin tissues in the initial stages followed by continuous delivery in to systemic circulation[28]. After the removal of the transdermal film, a mild reservoir effect was observed for about 2 h, followed by normal elimination similar to that after oral administration. This reservoir effect might be due to the slow depletion of the drug accumulated in the skin tissues. The oral administration of LD solution resulted in a low and quite variable $AUC_{(0-\alpha)}$ of 171.66 ± 41.270 µg.h/l, where as the transdermal administration resulted in $AUC_{(0-\alpha)}$ of 1017.933 ± 53.589 µg.h/l. Similarly the MRT after transdermal administration was quite longer (17.290 ± 0.926 h) compared to oral administration (4.730 ± 0.467 h) which means that the drug remains in the body for a longer period of time and its action is more sustained. The reservoir effect after the removal of the transdermal film due to the slow depletion of the drug accumulated in skin tissues leads to a slightly higher terminal $t_{1/2}$ value on transdermal administration (4.054 ± 0.647 h) of LD than its oral administration (3.308 ± 0.559 h). The difference in C_{max}, t_{max}, AUC and MRT values following oral and transdermal administration was found to be statistically significant ($p<0.05$).

The summary of the *in vivo* performance of lercanidipine hydrochloride after oral and transdermal administration revealed an increased bioavailability of the drug after transdermal administration compared to oral route. The formulated transdermal film was found to enhance the bioavailability of LD by 5.929 times with reference to the oral dosage form. The low oral bioavailability of LD is due to its extensive hepatic metabolism[1, 3]. The significant increase in bioavailability following transdermal administration indicated avoidance of a substantial amount of hepatic first pass metabolism associated with oral administration and it is most likely due to the fact that the vascular system of the skin predominantly drains in to the superior venacava, thereby eliminating the initial hepatic first pass metabolism[28].

IN VITRO - IN VIVO CORRELATION

To develop the Level A (point to point) correlation, the correlation coefficients were determined by plotting *in vivo* percentage drug absorbed against *in vitro* drug permeated through skin. The percentage drug absorbed was determined by Wagner Nelson method, using the equation; $Fa = [(C_t + K_e \cdot AUC_{0-t}) / K_e \cdot AUC_{0-\infty}] \times 100$, where Fa is the fraction of the drug absorbed, C_t is the plasma drug concentration at time t, K_e is the overall elimination rate constant, AUC_{0-t} and $AUC_{0-\infty}$ are areas under the curves between time zero and t time and

between time zero and infinity, respectively. The values obtained were correlated with percentage drug permeated at the same time intervals as shown in the Fig.5. Good *in vitro* – *in vivo* correlation was obtained (R^2 = 0.995).

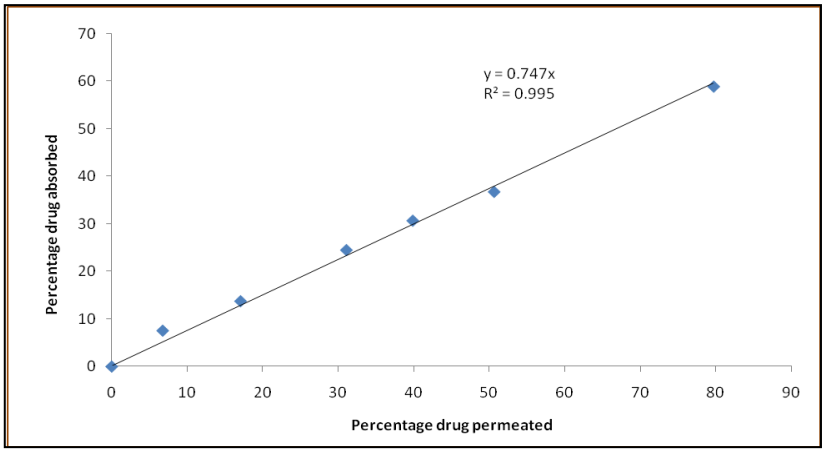

Figure 5: *In vitro* and *in vivo* correlation plot of transdermal film.

CONCLUSION

The fabricated transdermal film with ERL/PVP (1:4) with 15 % w/w PG as plasticizer and 4 % w/w tween 80 as permeation enhancer is one of the best controlled drug delivery systems having greater utility in the effective therapy and prophylaxis of hypertension, angina pectoris and cardiac arrhythmia, where the drug is made available for an extended period of time, so frequency of administration can be minimized. The *in vitro* and *in vivo* results of the study show the feasibility of designing and fabricating the rate controlled transdermal drug delivery system of lercanidipine hydrochloride in order to achieve improved bioavailability. Further, pharmacodynamic and pharmacokinetic evaluation of these systems in human volunteers is necessary to confirm these findings. However, these findings may help the industry to scale up for commercial production.

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REFERENCES

1. Brunton LL, Lazo JS, Parker KL. Goodman and Gilman's the pharmacological basis of therapeutics.11th ed. McGraw Hill Medical Publishing Division, New York. 2006: 871-896.
2. Parfitt K. Martindale: the complete drug reference. 33rd ed. Pharmaceutical Press, London. 2002: 920.
3. Tripathi KD. Essentials of medical pharmacology. 6th ed. Jaypee Brothers, New Delhi. 2007: 532.
4. Manish PP, Jayvadan KP, Nikita C, Nimisha P, Anjali P. Transdermal drug delivery system. The Indian Pharmacist. 2012; 11(3): 17-22.
5. Jamakandi VG, Ghosh B, Desai BG, Khanam J. Recent trends in transdermal cardiovascular therapy. Indian J. Pharm. Sci. 2006; 68(5): 556-561.
6. Sankar V, Johnson DB, Sivanad V, Ravichadran V, Raghuram S. Design and evaluation of nifedipine transdermal patches. Indian J. Pharm. Sci. 2003; 65(5): 510-515.
7. Mohammed A, Yasmin S, Asgar A. Matrix type transdermal drug delivery systems of metoprolol tartarate. Acta Pharm. 2003; 53: 119-125.
8. Wagh MP, Dalvi H, Bagal M. Formulation and evaluation of transdermal drug delivery system for simvastatin. Indian drugs. 2009; 46(3): 221-225.
9. Saqib Z, Aquil M, Ali A, Ahmad S. Transdermal drug delivery of labetolol hydrochloride: system development, in vitro, ex vivo and in vivo characterization. Current Drug Delivery. 2005; 2: 125-131.
10. Kusum D, Paranjothy KL. Development and evaluation of free films and transdermal patches of ketorolac trimethamine using polymers and pressure sensitive adhesives. The Eastern Pharmacist. 1998; 42(485): 97-100.
11. Kulkarni R, Doddayya H, Marihal SC, Patil CC, Habbu PV. Comparative evaluation of polymeric films for transdermal application. The Eastern Pharmacist. 2000; 3(516): 109-112.
12. Anil SJ, Kevin GC, Harinath MN. Formulation and evaluation of transdermal patches of metoprolol tartarate. The Indian Pharmacist. 2009; 8(11): 69-74.
13. Koteshwar KB, Udupa N, Vasantha K. Design and evaluation of captopril transdermal preparation. Indian Drugs. 1992; 29: 680-685.
14. Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 1944; 82: 377-379.

15. Hanan EN, Fakhry G, Hanaa EG, Sabry, Shereen. Effect of various penetration enhancers concentrations on diclofenac sodium release from cellulose acetate phthalate polymeric film. Asian J Pharm Sci. 2011; 12: 33-40.
16. Zvezdana K, Dobrica S, Svetlana P, Milan J, Djordje J. The irritative property of α-tricalcium phosphate to the rabbit skin. Gen. Physiol. Biophys. 2009; 28: 168–173.
17. Pattnaik S, Kalpana S, Choudhury P, Pradeepta KA, Subrata M. Alfuzosin hydrochloride transdermal films: evaluation of physicochemical, *in vitro* human cadaver permeation and thermodynamic parameters. Int. Braz. J. Urol. 2009; 35(6): 716-729.
18. Shinde AJ, Manoj PB, Harinath MN. Development and *in vitro* evaluation of transdermal patches of lovastatin as a antilipidemic drug. Int. Res. J. Pharmacy. 2010; 1(1): 113-121.
19. Narasimha Murthy S, Mini S, Hamsa V. Drug release from terbutalin sulphate transdermal films across human cadaver skin. Indian J. Pharm. Sci. 1997; 59(2): 75-76.
20. Mourya VK, Saini TR. Studies on skin permeation of piroxicam. Indian Drugs. 1997; 34(11): 656-657.
21. Jose J, Narayanacharyulu R, Mathew M (2013). Transdermal delivery of felodipine from eudragit film. Am. J. PharmTech. Res. 2013; 3(2): 517-531.
22. Mamatha T, Venkateshwara Rao J, Mukkanti K, Ramesh G. Formulation and *in vitro* evaluation of transdermal patches of lercanidipine hydrochloride. Acta Pharmaceutica Sciencia, 2010; 52: 295-304.
23. Subhash PG, Dinesh BM, Ravikumar M. Assessment of lercanidipine hydrochloride for transdermal delivery: physicochemical, *in vitro* and *ex vivo* characterization of matrix type lercanidipine hydrochloride transdermal patches. Int. J. Pharma. Bio. Sci. 2012; 3(1): 349-365.
24. Robinson JR, Eriksen SP. Theoretical formulation of sustained release dosage forms. J. Pharm. Sci. 1966; 55: 1254-1258.
25. Murty TEGK, Kishore VS. Effect of casting solvent and polymer on permeability of propanalol hydrochloride through membrane controlled transdermal delivery system. Indian J. Pharm. Sci. 2007; 69(5): 646-50.
26. Gupta SP, Jain SK. Effective and controlled transdermal delivery of metoprolol tartarate. Indian J. Pharm. Sci. 2005; 67(3): 346-350.
27. Charde S, Mudgal M, Kumar L, Saha R. Development and evaluation of buccoadhessive controlled release tablets of lercanidipine. AAPS Pharm. Sci. Tech. 2008; 9(1): 182-190.

28. Rao RR, Diwan PV. Comparative *in vivo* evaluation of diltiazem hydrochloride following oral and transdermal administration in rabbits. Indian J. Pharmacol. 1999; 31: 294-298.
29. Selvaddurai M, Kumar JR, Dhanaraj A. Simple and accurate validation of lercanidipine in human plasma by RP-HPLC. Am. J. PharmTech Res. 2012; 2(5): 605-612.
30. Ubaiullah U, Reddy MVS, Ruckmani K, Ahmad FJ, Khar RK. Transdermal therapeutic system of carvedilol: effect of hydrophilic and hydrophobic matrix on *in vitro* and *in vivo* characteristics. AAPS Pharm. Sci. Tech. 2007; 8(1): E1-E8.
31. Jayaprakash S, Mohammed H, Mohammed PF, Yasmin, Nagarajan M. Preparation and evaluation of celecoxib transdermal patches. Pak. J. Pharm. Sci. 2010; 23(3): 279-283.
32. Kulkarni RV, Mutalik S, Hiremath D. Effect of plasticizers on the permeability and mechanical properties of eudragit films for transdermal application. Indian J. Pharm. Sci. 2002; 64(1): 28-31.