



## ASSESSMENT OF LERCANIDIPINE HYDROCHLORIDE FOR TRANSDERMAL DELIVERY: PHYSICOCHEMICAL, *IN-VITRO* AND *EX-VIVO* CHARACTERIZATION OF MATRIX TYPE LERCANIDIPINE HYDROCHLORIDE TRANSDERMAL PATCHES

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### ABSTRACT

This article reports the assessment of lercanidipine hydrochloride (LH) for transdermal delivery. *In-vitro* permeation studies were carried out across the porcine ear skin in presence of various penetration enhancers. Pharmaceutical excipients and chemicals namely disodium salt of ethylenediaminetetraacetic acid (Na EDTA), tween-80, span-80 and hyaluronidase were facilitated to evaluate as effective penetration enhancer. Among all, hyaluronidase emerged as effective penetration enhancer with highest flux  $111.8 \pm 0.030 \mu\text{g}/\text{cm}^2/\text{hr}$ , Kp  $0.0172 \pm 0.040 \text{ cm}/\text{hr}$  and enhancement ratio  $5.37 \pm 0.01$  than others. Later, HL1, HL2, HL3, HL4, HL5 and HL6 LH transdermal patches were prepared by incorporating HPMC K-100 and PVP K-30 as polymers in 3:2, 2:3, 2:1, 1:2, 4:1, and 1:4 ratios respectively. All, six transdermal patches loaded with 10 mg of LH/ $3.14 \text{ cm}^2$  area of patch, optimized hyaluronidase (5% w/w) as penetration enhancer, *n*-dibutyl phthalate (30% w/w) as a plasticizer. Transdermal patches revealed satisfactory physicochemical properties. Among six formulations, HL3 exhibited highest cumulative percent of drug permeation ( $77.73 \pm 1.98\%$ ), transdermal flux ( $104.81 \pm 2.42 \mu\text{g}/\text{cm}^2/\text{hr}$ ), permeability coefficient ( $1.04 \times 10^{-2} \pm 2.08 \text{ cm}/\text{hr}$ ) and diffusion coefficient ( $7.758 \times 10^{-8} \pm 0.16 \text{ cm}/\text{hr}$ ). The curve fitment data indicated that the *in-vitro* permeation data of model formulations fitted well into zero order equation (average  $R^2 = 0.9931 \pm 0.014$  to  $0.980 \pm 0.028$ ) better than first order and Higuchi model. The stability studies revealed that no major morphological changes in patches and found insignificant variation in drug content. Further, the satisfactory results were supported by one way ANOVA.

**Keywords:** Lercanidipine hydrochloride, penetration enhancers, flux, matrix type transdermal patches, hyaluronidase.

### INTRODUCTION

The credentials of transdermal drug delivery as an alternative and attractive route for systemic medication associated with numerous advantages. For instance, it is simple and painless, it protects the API from gastrointestinal inactivation and the influence of food, it avoids the hepatic first-pass effect, avoids the variable absorption associated with the large areas of gastrointestinal tract, effective in nauseated patients, less frequent doses and improved compliance etc<sup>1</sup>.

Conceivably, the major defiance for transdermal delivery is that only a limited number of drugs are tractable to administer by this route, as the outermost layer of the skin, the cornified envelope or stratum corneum, has been evolved as the principal diffusion barrier for substances, including water<sup>2,3</sup>.

To overcome this effective barrier property of skin, second generation transdermal drug delivery systems developed with the incorporation of conventional chemical enhancers like terpenes/terpenoids,<sup>4,5</sup> an-ionic and non-ionic surfactants,<sup>6,7</sup> fatty acid and esters,<sup>8,9</sup> aprotic and its related solvents<sup>10,11</sup> etc., or by iontophoresis or noncavitational ultrasound method<sup>12,13</sup>.

In the development of a transdermal drug delivery system, the screening of APIs for percutaneous permeation is one of the primary task in the initial design and subsequently in the evaluation of dermal or

transdermal pharmaceuticals. Numerous published reports revealed that many factors can affect the transdermal permeation of a drug, including the formulation composition, nature and type of penetration enhancer, partition coefficient, source of skin etc., So it is desirable to evaluate the skin permeation characteristics of drug *in-vitro* before conducting *in-vivo* studies in human volunteers<sup>14,15</sup>. Tactication of these physicochemical properties and the co-administration of penetration enhancers may increase transdermal delivery.

Various biological membranes from mice, rats, pigs, guinea pigs, snakes, rabbits, and humans as well as synthetic membranes have been used for these drug diffusion studies<sup>16,17</sup>.

Lercanidipine hydrochloride (LH), a potent antihypertensive and antianginal drug was selected as a model drug for the study. It has molecular weight 648.19 g/mol, melting point 197-201°C, pKa 6.83 at 37°C and an octanol/water partition coefficient is 6 at 20-25°C. Rationale for selection of LH, includes complete and aberrant absorption from gastrointestinal tract after an oral dose of 10-20 mg, reduction in absolute bioavailability to approximately 10% due to extensive first pass metabolism to inactive metabolites and low half-lives of drug with 2.8 and 4.4 hours in human after single dose of 10 and 20 mg of LH respectively<sup>18-20</sup>.



Result of literature survey and patent watch revealed that no initiation of transdermal formulation of LH in view of enhancing the bioavailability. So, the purpose of present investigation was to develop the second generation transdermal delivery system (transdermal patch) of LH by employing HPMC K-100 and PVP K-30 and to study the effect of polymers and desired chemical penetration enhancers on the physicochemical properties of transdermal film and permeation of drug across porcine ear skin respectively.

Polymer matrices make good reservoirs for drugs in sustained release medications. There are many hydrophilic polymers with low and high viscosity grades used for transdermal drug delivery systems like polyvinyl alcohol, polyvinyl pyrrolidone and hydroxyl propyl methylcellulose<sup>21,22</sup>. In this study, hydrophilic polymers HPMC K-100 and polyvinyl pyrrolidone K-30 (PVP K-30) were used to control the release of LH. In addition to this, an attempt has been made to assess the percutaneous permeation of LH, in presence of various pharmaceutical excipients as penetration enhancers includes disodium salt of ethylenediaminetetraacetic acid (Na EDTA), tween-80, span-80 and hyaluronidase across the porcine ear skin.

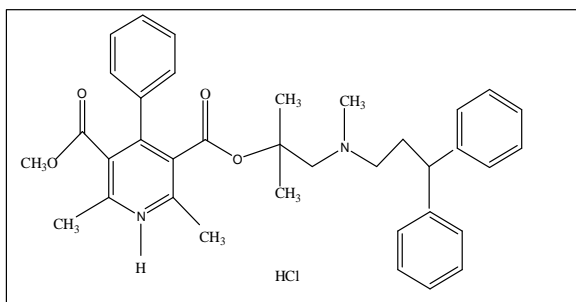


Figure 1: Structure of Lercanidipine hydrochloride

## MATERIALS AND METHODS

### Materials

Lercanidipine hydrochloride and hyaluronidase were procured from Leo Chem (Bangalore, India), Hydroxyl propyl methylcellulose K-100, polyvinyl pyrrolidone K-30, sodium ethylene diamine tetracetate (Na EDTA), tween-80, span-80, *n* - Dibutyl phthalate were supplied by S.D. Fine Chemicals Ltd., (Mumbai, India). All remaining chemicals and solvents were reagent grade.

### Methods

#### Investigation on physicochemical compatibility of drug and polymers

#### FTIR (Fourier transformed infrared spectroscopy) analysis

FT-IR spectroscopy (Perkin-Elmer FTIR spectrophotometer, Model 1600, Japan) was utilized as analytical tool to ascertain any interaction between LH and polymers.

An FTIR spectrum of LH was obtained using KBr disc method as per the following working condition: 5 mg of

sample was triturated well with 15 mg KBr in a mortar. The triturated sample was kept in a holder, scanned in the wave length region between 400 and 4000  $\text{cm}^{-1}$  and recorded<sup>23</sup>.

An FTIR spectrum of transdermal patch loaded with LH and polymers was obtained using NaCl plate's method as per the following operational condition: solution of patch (triturated powder-5 mg) in a co-solvent system methanol and distilled water (5:5 ml) was prepared and casted onto NaCl plates. The spectrum was recorded in the wave length region between 400 and 4000  $\text{cm}^{-1}$ <sup>24</sup>.

#### DSC (Differential Scanning Calorimetry) aspects

LH and transdermal film loaded with LH and polymers (HPMC and PVP K-30) were subjected to DSC (Mettler-Toledo Star System, IISc, Bangalore, India) analysis. The analysis was conducted as per the following working condition: 5 mg of drug sample was hermetically sealed in flat-bottomed aluminum pan and subjected for heating between 50-250°C. The heating speed during the test was 10°C/minute and liquid nitrogen was used as cooling agent. The thermograms obtained for LH and transdermal film were recorded and compared<sup>23</sup>.

#### Effect of pH on drug solubility

The solubility of LH was determined in distilled water and phosphate buffer solutions (PB) of various pHs 4.0, 5.0, 6.2, 7.4, 8.0, and 9.0. In this method, excess amounts of LH were weighed into conical flasks containing 10 ml of PB solution until saturation was noticed by the presence of undissolved material. Next, all samples were kept for agitation on a mechanical shaker for 24 hours at 37±0.2°C. After the specified time, the suspensions were filtered using 0.22  $\mu$  nylon membrane filter (Millipore, India). Next, 2 ml of filtered solution was diluted to 10 ml using PB solution. The concentrations of dissolved LH were assayed spectrophotometrically (Shimadzu UV-1800, Japan) measured at 236 nm<sup>25</sup>.

#### Partition coefficient of drug in *n* - octanol/aqueous solution system

Determination of partition coefficient of drug was carried out using equal volumes of *n*-octanol and *in-vitro* aqueous solution (in this case, PB 7.4 pH) in a separating funnel. In this study, a drug solution of 25  $\mu\text{g/ml}$  was prepared in *n*-octanol<sup>26</sup>. 25 ml of this solution was taken in separating funnel and shaken with equal volume of PB 7.4 pH solution on mechanical shaker for 24 hours. The mixture was then centrifuged at 2000 rpm for 10 min and concentration of drug extracted in PB phase was assayed spectrophotometrically (Shimadzu UV-1800, Japan) by measuring absorbance at 236 nm. The partition coefficient (Kp) was calculated from the below equation.

$$K_p = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}} \quad (1)$$



## **Ex-vivo permeation kinetic studies of lercanidipine hydrochloride with penetration enhancers**

### **Skin preparation**

In this study, porcine ear skin was used because of its close relationship in terms of histologically and biochemically to human skin<sup>27</sup> and was found to have a closer permeability character to human skin<sup>28,29</sup>. The fresh full thickness (75-80  $\mu\text{m}$ ) porcine ear skin was procured from local slaughter house. Superficial skin was excised from the back of porcine ear and hair was removed by shaving process. The cleared area was washed with PB pH 7.4. The skin was dipped in water, thermostated at 58°C for a period of 2 min to separate the epidermis and dermal joint. The isolated epidermis (25 $\pm$ 5  $\mu\text{m}$ ) was rapidly rinsed with hexane to devoid the lipid content. Next, the skin was immediately rinsed with water and then either used or stored at frozen conditions (for not more than 78 hours) in an aluminum foil for further use<sup>25,30</sup>.

### **Permeation kinetic studies**

The studies were performed in triplicate. The permeation kinetic studies were conducted in Franz diffusion cell (25 ml capacity),<sup>25</sup> consisting of donor and recipient compartments. The diameter of donor compartment provided 3.14  $\text{cm}^2$  effective constant area for diffusion studies. The excised epidermal skin was sandwiched between the donor and recipient compartments using a clamp, such that the dermis side of skin attached to donor compartment was exposed to environment and stratum corneum was facing receptor compartment. Initially, the diffusion studies were done by placing 5 ml of filtered (through Nylon membrane filter-0.22  $\mu$ ) saturated solution of drug in PB pH 7.4 in donor compartment. Later, studies were continued with LH solution [6.5 mg in 5 ml PB solution of pH 7.4 and constant concentration (5% v/v) of each permeation enhancer includes Na EDTA, tween-80, span-80 and hyaluronidase individually] in the donor compartment. The receptor compartment was introduced with 25 ml of PB solution (pH 7.4) as the diffusion medium and a small teflon coated bar magnet was placed to stir the medium for uniform drug distribution. The diffusion cells were water jacketed, and controlled at a temperature of 37 $\pm$ 1°C by thermostatic arrangement. The entire assembly was placed on the ready to operate thermostatic controlled magnetic stirrer (Remi Motors Pvt Ltd, Vasai, India). The amount of drug permeated through the skin was determined by withdrawing aliquots of 5 ml at predetermined time intervals for 24 hours duration and replacing them with an equal volume of fresh PB. The drug concentration in the aliquots was estimated spectrophotometrically (Shimadzu UV-1800, Japan) at 236 nm.

### **Determination of flux, permeability coefficient, diffusion coefficient and enhancement ratio<sup>25,31</sup>**

Flux ( $J_{SS}$ ) of drug permeated in case of *in-vitro* was calculated from slope of the steady-state portion of

permeation profile by linear regression analysis. Lag time was calculated from back extrapolation. Diffusion coefficient ( $D/h^2$ ) and permeability coefficient ( $K_p$ ) was also calculated for the *in-vitro* studies using below mentioned equations respectively,

$$D/h^2 = 1/6 \times T_{Lag}, \quad (2)$$

$$J_{SS} = (dq/dt).1/A, \quad (3)$$

$$K_p = J_{SS}/C_s, \quad (4)$$

Where,  $T_{Lag}$  is the lag time,  $J_{SS}$  the flux at steady state,  $C_s$  is concentration in donor compartment,  $D$  is diffusion coefficient;  $(dq/dt)$  is the steady state slope and  $h$  is the diffusion path length.

Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by using below mentioned equation:

$$\text{Enhancement ratio} = \frac{K_p \text{ of drug with penetration enhancer}}{K_p \text{ of drug without penetration}}, \quad (5)$$

### **Fabrication of matrix type transdermal patches**

A set of initial trials were undertaken to optimize the concentration of polymer, plasticizer, volume of casting solution, drying temperature, and drying period to prepare films of uniform thickness.

Inherently developed solvent casting technique<sup>32</sup> was employed for fabrication of LH (10 mg/3.14  $\text{cm}^2$ ) matrix type transdermal patches. A mixture of methanol and distilled water in a ratio of 1:1 was used as a casting solvent to prepare the polymeric solution of different ratios of polymers i.e., HPMC K100, PVP K-30 and LH. *n*-dibutyl phthalate was added as a plasticizer at a concentration of 30% w/w of the dry weight of the polymers and hyaluronidase was added as penetration enhancer at a concentration of 5% w/w of the dry weight of the polymers. Accurately weighed polymers were dissolved in a beaker. The resultant homogeneous polymeric solution containing the drug, plasticizer and penetration enhancer was agitated on a magnetic stirrer (Remi Equipments Ltd., Mumbai, India) for 1 hour. The casting solution was poured on the surface of petriplate which was previously smeared with small amount of plasticizer. The solvent was allowed to evaporate at room temperature by placing inverted funnel on it to obtain the dried patch. The dried films were removed, wrapped in aluminum foil and kept in desiccators. Compositions of prepared patches were tabulated in table 1.

### **Evaluation of transdermal patches**

The transdermal patches were evaluated for physicochemical properties such as thickness, weight variation, drug content, percentage moisture absorption, percentage moisture loss, percentage elongation, tensile strength and *in-vitro* skin permeation studies. Transdermal patches were subjected to evaluation



parameters with the 3.14 cm<sup>2</sup> area of film except for percentage elongation and tensile strength.

### Uniformity of thickness

Thickness of the transdermal patches was recorded at three different points using a digital caliper (Baker Digital Vernier Caliper, Pune, India). The average thickness and mean value of transdermal patches of all batches were determined<sup>33,34</sup>.

**Table 1:** Compositions of the casting solutions used to prepare model matrix type transdermal patches

Batch	Polymeric Blend	Drug (mg/3.6 cm <sup>2</sup> )	Polymer Ratio	n -Dibutyl phthalate (w/w)	Hyaluronidase (w/w)	Solvent system (1:1)
HL1	HPMC K100:PVP K-30	10	3:2	30%	5%	Methanol:Water
HL2	HPMC K100:PVP K-30	10	2:3	30%	5%	Methanol:Water
HL3	HPMC K100:PVP K-30	10	2:1	30%	5%	Methanol:Water
HL4	HPMC K100:PVP K-30	10	1:2	30%	5%	Methanol:Water
HL5	HPMC K100:PVP K-30	10	4:1	30%	5%	Methanol:Water
HL6	HPMC K100:PVP K-30	10	1:4	30%	5%	Methanol:Water

56 cm<sup>2</sup> was total surface area of the petriplate. On this basis drug was incorporated for 3.14 cm<sup>2</sup> area.

### Drug content uniformity

The uniformity of drug distribution in transdermal patches was determined by transferring known area of the film in to screw capped bottle (25 ml capacity) containing 10 ml of methanol and dissolved. The solution was subjected to sonication (Servewell Instruments India Pvt Ltd, Bangalore, India) for 20 min and filtered by using 0.22 μ nylon membrane filter (Millipore, India). The filtrate was diluted appropriately and assayed in a UV-visible spectrophotometer (Shimadzu UV-1800, Japan) at 236 nm to determine the drug content. The drug content was estimated in triplicate using the calibration curve constructed in methanol and the results were expressed as average and mean of three determinations<sup>33,34</sup>.

### Percentage moisture absorption

The moisture absorption test was performed to check the physical stability or the integrity of the transdermal patches. Three transdermal patches from each batch were weighed and placed in test compartment (Economical, Thermolab equipments Pvt. Ltd., Mumbai, India) maintained at 84% RH. After three days the transdermal patches were taken out and reweighed.<sup>33,34</sup> The percentage moisture absorption was calculated using below mentioned equation:

$$\% \text{ moisture absorption} = \frac{[(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100}{(6)}$$

### Percentage moisture loss

The percentage moisture loss was carried out to check the integrity of the transdermal patches in dry conditions. Three transdermal patches from each batch were weighed and placed in desiccator containing activated silica. After 24 hours, the transdermal patches were taken out and reweighed<sup>33,34</sup>. The percentage moisture loss was calculated using below mentioned equation:

### Uniformity of weight

Three transdermal patches were randomly picked from each batch and weighed individually in a digital balance (AUX-220, Shimadzu Corporation, Japan). The average weight and mean value of transdermal patches of all batches were determined<sup>33,34</sup>.

$$\% \text{ moisture loss} = \frac{[(\text{initial weight} - \text{final weight})/\text{initial weight}] \times 100}{(7)}$$

### Percentage of elongation

Longitudinal strips of known length were cut from the prepared film from each batch. Then variation in the length due to the non-uniformity in flatness was measured. Flatness was calculated by measuring constriction of strips after the elongation and a zero percent constriction before the elongation was considered to be equal to a hundred percent flatness<sup>23</sup>.

$$\text{Constriction (\%)} = \frac{[(L1-L2)/L2] \times 100}{(8)}$$

Where L1 - initial length of strip,

L2 - final length of strip.

### Determination of tensile strength

The mechanical strength of patch can be ascertained in terms of tensile strength. It is the maximum stress given to a point at which the patch specimen breaks. Tensile strength was determined by using computerized Precisa bottom-loading balance, with necessary modifications. A patch from each batch, with dimensions of 1×1 cm was taken and subjected for studies. The weight required to break the film was noted as the break force<sup>25</sup>.

### Ex-vivo skin permeation study of transdermal patches

#### Permeation studies

The skin preparation was done as per the details mentioned in ex-vivo permeation kinetic studies of lercanidipine hydrochloride with penetration enhancers.

The *in-vitro* permeation studies of different transdermal patches were performed in a bi-chambered Franz diffusion cell (25 ml capacity), using a prepared porcine ear skin as *in - vitro* membrane. The skin used to simulate human derma, was tied to one end of an open-end





cylinder with  $3.14 \text{ cm}^2$  of diameter with stratum corneum facing it, which acted as a donor compartment. The transdermal patch was placed in donor compartment and the lower surface of skin was in intimate contact with PB solution of pH 7.4 (25 ml) placed in a receptor compartment as diffusion medium. A small teflon coated bar magnet was placed in receptor compartment to stir the medium for uniform drug distribution. The entire assembly of diffusion cell was placed on ready to operate thermostatic controlled magnetic stirrer (Remi Motors Pvt Ltd, Vasai, India). The diffusion cells were water jacketed, and maintained at a constant temperature of  $37 \pm 1^\circ\text{C}$  by thermostatic arrangement. At periodic time intervals, 5 ml of sample was withdrawn up to 24 hours duration and replaced with equal volume of PB. The aliquots were filtered using  $0.22 \mu$  nylon membrane filter (Millipore, India) and the amount of LH released was analyzed spectrophotometrically (Shimadzu UV-1800, Japan) at 236 nm against a reference standard using PB pH 7.4 as a reagent blank.

### Data analysis

To examine the drug permeation kinetics and mechanism from the different patches, the diffusion data were fitted to models representing zero-order, first-order, and Higuchi diffusion model using the below mentioned equations:

Zero order release equation:

$$Q = K_0 t, \quad (9)$$

First order release equation:

$$\log Q_t = \log Q_0 + K_t/2.303, \quad (10)$$

Higuchi's square root of time equation:

$$Q = K_H t^{1/2}, \quad (11)$$

Whereas Q is the amount of drug release at time t,  $K_0$  is zero order release rate constant,  $K_t$  is first order release rate constant, and  $K_H$  is Higuchi square root of time release rate constant<sup>35-37</sup>.

### Stability studies

The stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines<sup>38</sup>. Formulations of different batches were wrapped in an aluminum foil and placed in stability chamber (Thermolabs, India) at a temperature of  $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH for 6 months. Samples were analyzed for drug content.

### Statistical Analysis of Data

The results were subjected to analysis by one-way analysis of variance (Repeated Measures) using Graph Pad Prism software-5 version (Graph Pad software Inc., San Diego, CA, USA). Paired t-test was used to compare different formulations and p-value of  $< 0.05$  was considered as to be significant.

## RESULTS AND DISCUSSION

### Investigation on physicochemical compatibility of drug and polymer

#### IR spectroscopy analysis

Figure 2 of FTIR spectra of LH alone, exhibited principal peaks at  $3078.8 \text{ cm}^{-1}$  for C-H aromatic stretching,  $1347.03 \text{ cm}^{-1}$  for  $-\text{NO}_2$ ,  $1672.95 \text{ cm}^{-1}$  for  $>\text{C}=\text{O}$  stretching vibrations and  $1486.85 \text{ cm}^{-1}$  for  $-\text{CH}_3$  bending vibration. The obtained peaks confirm the purity and authentication of drug<sup>6</sup>. On keen observation of FTIR spectra (Figure 3) of LH transdermal patch prepared with polymers HPMC-K100 and PVP-K30 displayed the peaks at  $1347.03 \text{ cm}^{-1}$  for  $-\text{NO}_2$  stretching,  $1671.98 \text{ cm}^{-1}$  for  $>\text{C}=\text{O}$  stretching and  $1486.85 \text{ cm}^{-1}$  for  $-\text{CH}_3$  bending vibrations. However, few additional polymeric peaks were obtained in formulation.

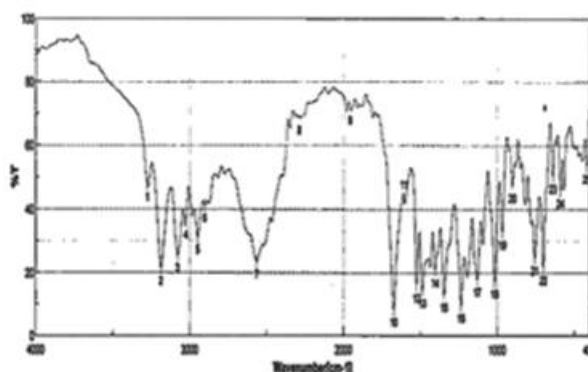


Figure 2: FTIR spectra of Lercanidipine hydrochloride

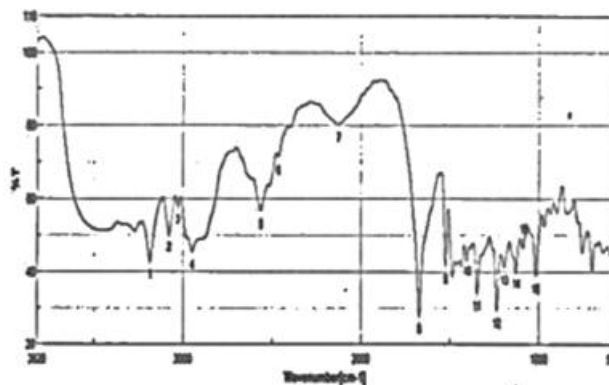
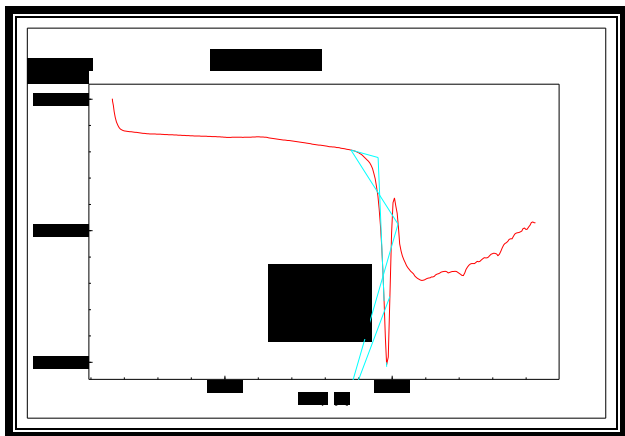


Figure 3: FTIR spectra of Lercanidipine hydrochloride transdermal patch prepared with polymers HPMC-K100 and PVP-K30

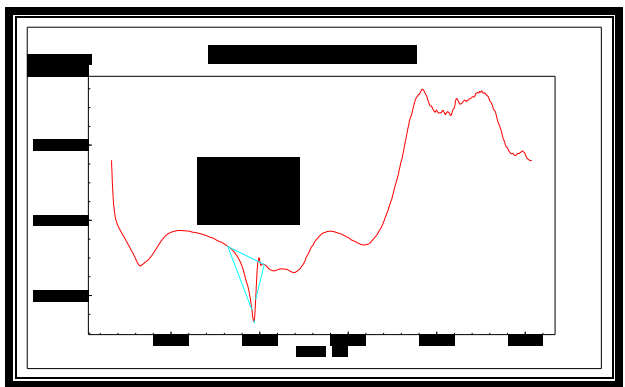
#### DSC aspects

DSC aspects were also employed as a significant analytical tool to assess any interactions between the LH and polymers, HPMC K-100 and PVP-K30 in transdermal patch in terms of their thermal behavior. DSC thermograms of LH alone and LH transdermal patch prepared with polymers, HPMC K-100 and PVP-K30 are represented in Figure 3. The DSC thermogram (Figure 4) of drug alone displayed the characteristic endothermic peak at  $196.68^\circ\text{C}$  corresponding to its melting point.

The DSC analysis (Figure 5) of LH in transdermal patch, unveiled an insignificant change in the melting point (193.74°C), due to the high molecular weight of polymers.



**Figure 4:** DSC thermogram of Lercanidipine hydrochloride



**Figure 5:** DSC thermogram of Lercanidipine hydrochloride transdermal patch prepared with polymers HPMC-K100 and PVP-K30

#### Effect of pH on drug solubility

Lipid soluble compounds cannot penetrate through the water-rich keratinocytes in skin due to repellent property. The ideal topical formulation should have both oil and water soluble properties. The skin permeation is directly related to the degree of drug solubilization, surface area as well as pH. If the drug is not properly solubilized in a suitable solvent, it will not diffuse into the skin<sup>39</sup>. Hence, in the present study the solubility of drug was carried out in PB solutions of various pHs and flux were obtained.

The solubility of drug in distilled water is not an appreciable (10.12 µgm) due to inherent property of practically insoluble in water. The values for solubility of drug were found to be 0.286, 0.547, 0.876, 1.243, 1.546, and 1.786 mg/ml in PB solutions of various pHs 4.0, 5.0, 6.2, 7.4, 8.0 and 9.0 respectively. By observing results it can clearly indicated that as the pH of the buffer increased, the solubility of drug was increased. It indicates that permeability co-efficient increased with increasing the pH value because solubility of drug was increased with increasing the pH value of PB. Figure 4 represents the effect of pH on the solubility of drug in various pHs.

#### Partition coefficient of drug in *n* - octanol/aqueous solution system

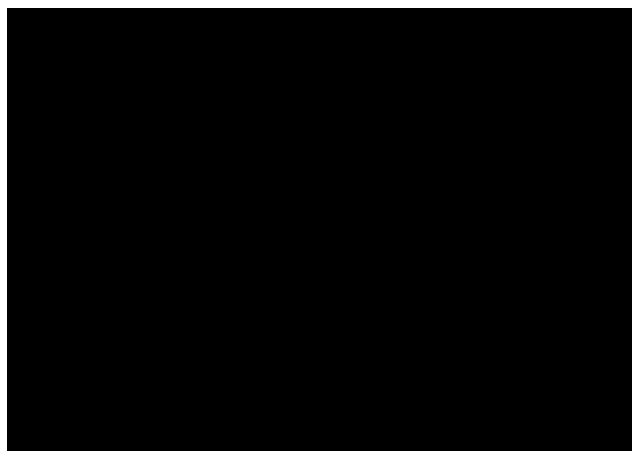
*n* - octanol and *in vitro* aqueous fluid (in this case, PB 7.4 pH) are considered as the standard co-solvent system for determining the drug partition coefficient between the skin and *in vitro* fluid. *n* - octanol was chosen as the oil phase owing to its widespread use in partitioning work and the belief that it may mimic the functions of biological membranes more accurately than other solvents<sup>40</sup>.

The value for partition coefficient of drug was found to be 6.84 with logarithmic value 0.835. The obtained satisfactory result presents the sufficient lipophilic property of drug, which meets the requirements of formulating it into a transdermal patch.

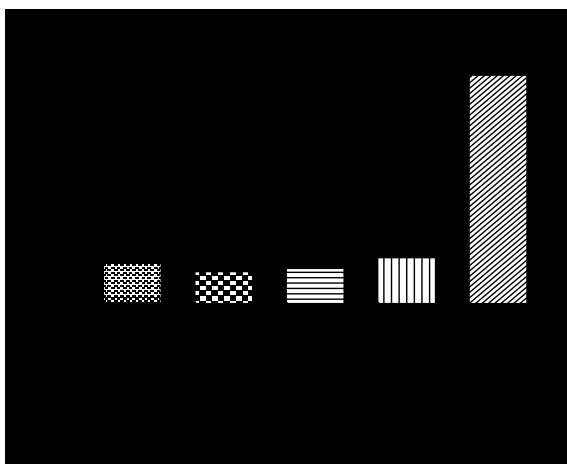
#### Ex-vivo permeation kinetic studies of lercanidipine hydrochloride with penetration enhancers

Steady state permeation kinetic studies were performed using Franz diffusion cell, employing a porcine ear skin as *in-vitro* membrane using PB solution of pH 7.4 in triplicate. The results of cumulative amount of drug and drug with various enhancers permeated are displayed in Figure 6.

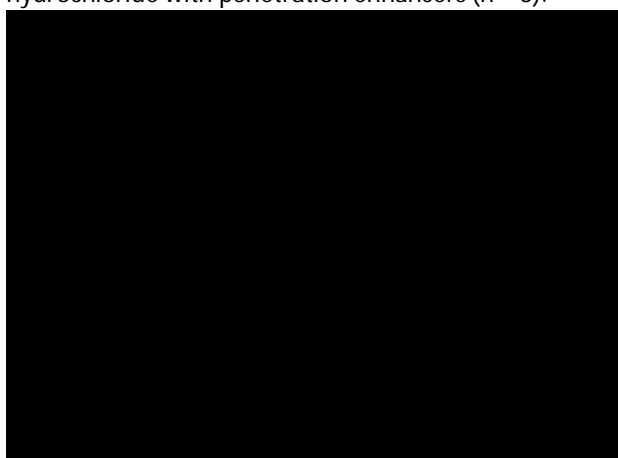
The effective barrier function of the skin is due to the intercellular lipids of stratum corneum and thus it is difficult for drugs to penetrate the skin. Permeation studies were carried out with 6.5 mg of drug, which is the measured quantity of saturation solubility of drug in 5 ml PB pH 7.4. Various penetration enhancers of constant concentration at 5% v/v were evaluated. All calculated results related to permeability coefficient, flux and enhancement ratios of drug are depicted in Table 2. The permeability coefficient, flux, and enhancement ratios of drug with different penetration enhancers found to be in increasing order as follows: Na EDTA>Span-80>Tween-80 >Hyalurodinase. Figure 7 represents the bar graph displays the enhancement ratios of the drug and penetration enhancers.



**Figure 6:** Ex-vivo permeation kinetic studies of Lercanidipine hydrochloride with penetration enhancers (n = 3).



**Figure 7:** Enhancement ratio for Lercanidipine hydrochloride with penetration enhancers (n = 3).



**Figure 8:** Effect of hyaluronidase on *in-vitro* skin permeation profile of Lercanidipine hydrochloride from transdermal patches of different ratios of Ethyl cellulose:Polyvinyl pyrrolidone K-30. Data are mean  $\pm$  SE (n = 3).

The enhancement ability of anionic surfactants such as span-80 and tween-80 exhibited highest flux  $18.95 \pm 0.092$  and  $23.90 \pm 0.042$   $\mu\text{g}/\text{cm}^2/\text{hr}$  respectively than Na EDTA. This property is attributed due interaction with the stratum corneum by increasing local water concentration with successive swelling and expansion of the thickness of the tissue<sup>41</sup>.

A major chelating agent Na EDTA, which displayed significantly lower flux value  $16.73 \pm 0.023$   $\mu\text{g}/\text{cm}^2/\text{hr}$  than others due to chelating action. This aspect is due to relative affinity of EDTA for various metals (for instance,  $\text{Ca}^{++}$ ) that could be involved<sup>42</sup>.

An enzyme hyaluronidase as a permeation enhancer impressively enhanced the flux and enhancement ratio than others. This is due to either disruption of lipid organization, making the drug permeate through micro cavities and hence increasing the volume available for drug diffusion or by increasing the permeability of connective tissues and decreasing the viscosity of body fluids by transglycosidase and hydrolytic activities, respectively<sup>25,43</sup>.

### **Evaluation of transdermal patches**

LH transdermal patches were prepared by employing hydrophilic polymers HPMC K-100 and PVP K-30, *n*-dibutyl phthalate (plasticizer) and hyaluronidase (penetration enhancer) in a co-solvent system consisting of methanol and distilled water in a ratio of 1:1. The chosen polymer combination imparts clear, smooth, uniform, substantive and flexible patches for the transdermal delivery systems of LH.

On visual inspection, all the dried films appeared transparent which indicates that the added drug was completely solubilized. The transdermal patches were evaluated for physicochemical properties such as thickness, weight variation, drug content, percentage moisture absorption, percentage moisture loss, percentage elongation, tensile strength and *in-vitro* skin permeation studies. Transdermal patches were subjected to evaluation parameters with the  $3.14 \text{ cm}^2$  area of film except for percentage elongation and tensile strength.

### **Uniformity of thickness and weight**

The thickness and weight of the model transdermal formulations were found to be fairly even and consistent (Table 3). The thickness of patches was found from  $0.19 \pm 0.02$  to  $0.22 \pm 0.03$  mm whereas the weight varied between  $19 \pm 0.36$  and  $20 \pm 0.56$  mg. The low values of standard deviation indicated the reproducibility ( $p < 0.05$ ) of the solvent casting technique employed to produce transdermal patches with consistent thickness, weight and drug content.

### **Drug content uniformity**

Since the composition of matrix film was maintained same for all batches, the assay values for the drug content (Table 3) of the different batches varied from  $9.56 \pm 0.02$  to  $9.84 \pm 0.01$  mg of theoretical values. The values of drug content for all batches indicated that the drug was uniformly distributed throughout the patch with negligible batch to batch variability ( $p < 0.05$ ).

### **Percentage moisture absorption**

Transdermal patches were found to displayed good stability and physical integrity in humid conditions as shown by their physical appearance. The moisture absorbed ( $p < 0.05$ ) in the humid condition was found to vary from  $5.14 \pm 0.08$  to  $9.08 \pm 0.01\%$  (Table 3). The result unveiled that the moisture absorption was found to augment with the amount of hydrophilic polymers. The low moisture absorption also preserves the patch from microbial attack and avoids bulkiness of the patches<sup>25</sup>.

### **Percentage moisture loss**

The transdermal patches were exhibited sufficient stability and physical integrity in dry conditions as depicted by their physical appearance. The moisture lost ( $p < 0.05$ ) in the dry condition was found to vary from  $2.14 \pm 0.04$  to  $4.88 \pm 0.06\%$  (table 3). The variation in the percentage moisture loss depends on varied

concentration of hydrophilic polymers in formulations. This minute moisture content helps the patches to stay stable and prevents them from becoming a completely dry and brittle<sup>25</sup>.

#### Percentage of elongation

Percentage of elongation was found to be 100% flatness (table 3) which indicates no amount of constriction in formulated transdermal strips.

#### Determination of tensile strength

Transdermal films have relatively good tensile strength values ranged from 13.48±0.14 to 14.20±0.16 gm/cm<sup>2</sup> (table 3). These values guarantee the films found to be elastic on handling.

**Table 2:** Data of permeability coefficient, flux and enhancement ratio of lercanidipine hydrochloride and lercanidipine hydrochloride with permeation enhancers

Name	Transdermal flux (µg/cm <sup>2</sup> /hr)	Permeability coefficient (cm/hr)	Enhancement Ratio
LH	20.90±0.010	0.0032±0.052	1.00±0.03
LH+Na EDTA	16.73±0.023	0.0025±0.030	0.80±0.06
LH+Span-80	18.95±0.092	0.0029±0.062	0.90±0.04
LH+Tween-80	23.90±0.042	0.0036±0.052	1.12±0.03
LH+Hyaluronidase	111.8±0.030	0.0172±0.040	5.37±0.01

Average of triplicate results. LH - lercanidipine hydrochloride, Na EDTA - disodium salt of ethylenediaminetetraacetic acid

**Table 3:** Characterization of lercanidipine hydrochloride transdermal patches

Batch	Thickness (mm) ±SD	Weight (mg) ±SD	Drug content (mg) ±SD	% moisture loss ±SD	% moisture absorption ±SD	% of elongation ±SD	Tensile strength (gm/cm <sup>2</sup> ) ±SD
HL1	0.22±0.01	19±0.36	9.79±0.04	3.14±0.06	6.29±0.04	100%	14.01±0.18
HL2	0.20±0.02	20±0.56	9.84±0.01	3.89±0.02	7.69±0.02	100%	13.86±0.16
HL3	0.19±0.02	19±0.60	9.70±0.03	4.21±0.04	8.61±0.06	100%	13.48±0.14
HL4	0.22±0.03	19±0.82	9.56±0.02	2.86±0.08	5.84±0.06	100%	13.98±0.18
HL5	0.21±0.04	19±0.42	9.64±0.06	2.14±0.04	5.14±0.08	100%	14.08±0.10
HL6	0.20±0.01	20±0.46	9.58±0.04	4.88±0.06	9.08±0.01	100%	14.20±0.16

Average of triplicate results; Mean ±SD Standard deviation.

**Table 4:** Results of cumulative percent of drug that permeated, transdermal flux, permeability coefficient and diffusion coefficient of lercanidipine hydrochloride transdermal patches.

Batch	Q <sub>24</sub> (%) ±SD	J <sub>ss</sub> (µg/cm <sup>2</sup> /hr) ±SD	Kp (cm/hr) ±SD	Diffusion coefficient (cm/h×10 <sup>-8</sup> ) ±SD
HL1	65.87±2.86	89.32±3.14	8.93×10 <sup>-2</sup> ±2.26	6.579±0.06
HL2	61.94±2.06	82.34±2.88	8.23×10 <sup>-3</sup> ±2.24	5.551±0.09
HL3	77.73±1.98	104.81±2.42	1.04×10 <sup>-2</sup> ±2.08	7.758±0.16
HL4	63.90±2.11	86.02±1.64	8.60×10 <sup>-3</sup> ±2.11	5.575±0.18
HL5	72.17±2.64	97.59±2.98	9.75×10 <sup>-2</sup> ±2.42	7.365±0.20
HL6	60.50±2.12	80.74±1.64	8.07×10 <sup>-3</sup> ±2.28	5.308±0.22

Average of triplicate results; Mean ±SD Standard deviation; Q<sub>24</sub> Cumulative percent of drug that permeated; J<sub>ss</sub> Transdermal flux Kp Permeability coefficient

**Table 5:** Results of curve fitting data of the *in-vitro* permeation data for lercanidipine hydrochloride transdermal patches

Batch	Zero Order	First Order	Higuchi
	R <sup>2</sup> ± SD	R <sup>2</sup> ± SD	R <sup>2</sup> ± SD
HL1	0.9942±0.022	0.9510±0.029	0.8611±0.012
HL2	0.9963±0.012	0.9842±0.024	0.8798±0.031
HL3	0.9938±0.021	0.9338±0.016	0.8489±0.030
HL4	0.9954±0.026	0.9812±0.021	0.8550±0.018
HL5	0.9931±0.014	0.9581±0.028	0.8768±0.024
HL6	0.9980±0.028	0.9808±0.020	0.8659±0.026

Mean ±SD Standard deviation; Average of triplicate results

#### Ex-vivo skin permeation study of transdermal patches

Release pattern of the drug from the transdermal patches is majorly governed by the chemical properties of the drug and delivery dosage form, as well as physiological and physicochemical properties of biological membrane

used as a barrier<sup>44</sup>. An *in-vitro* skin permeation study is significant and essential in terms of prediction of *in-vivo* performance of patches and to know the rate and mechanisms of percutaneous absorption of drugs<sup>34</sup>.





Permeation kinetic studies of patches were performed using a Franz diffusion cell across porcine ear skin.

Table 4 and Figure 8 illustrate the cumulative percentage of drug permeated, transdermal flux, permeability coefficient and diffusion coefficient. From the results it can clearly indicated that batch HL3 displayed the highest cumulative percent ( $77.73 \pm 1.98\%$ ) of drug permeated after 24 hours, with the lowest value observed with the batch HL6 for cumulative percent ( $60.50 \pm 2.12\%$ ) of drug permeated. When the transdermal flux, permeability coefficients, and diffusion coefficient of different batches were compared, batch HL3 was found to be highest value  $104.81 \pm 2.42 \mu\text{g}/\text{cm}^2/\text{hr}$ ,  $1.04 \times 10^{-2} \pm 2.08 \text{ cm}/\text{hr}$  and  $7.758 \pm 0.16 \times 10^{-8} \text{ cm}/\text{h}$  respectively.

In the present the study, it was observed that formulations (HL1, HL3 and HL5) containing higher amount of HPMC K-100, drug permeation found to be increased. The controlled release of drug was attributed due to higher viscosity and higher concentration of this polymer in the patch. The polymeric molecules are arranged in more crowded fashion. Therefore, polymer defies the penetration of drug molecules through the polymeric chain meshwork.

Formulations (HL2, HL4 and HL6), exhibited decreased drug permeation rate. This outcome can be attributed to the leaching of the aqueous polymer (PVP K-30) molecules, which leads to the formation of outlets when contact with dissolution medium and thus a decrease in the mean diffusion path length of drug molecules to release into the dissolution medium.

The curve fitment data (table 5) indicated that the *in-vitro* permeation data of most of the model formulations fitted well into zero order equation (average  $R^2 = 0.9931 \pm 0.014$  to  $0.9980 \pm 0.028$ ) better than first order (average  $R^2 = 0.9338 \pm 0.016$  to  $0.9842 \pm 0.024$ ) and Higuchi model (average  $R^2 = 0.8611 \pm 0.012$  to  $0.8798 \pm 0.031$ ). The best-fit model was found to be zero order model as the R value of most of these formulations was found to approach unity. The results revealed that as the quantity of hydrophilic polymer with high viscosity in high concentration in all batch formulations, permeation of drug was increased.

### Stability studies

The values for drug content of all batches of patches prior and post stability study are depicted in Table 6. The stability studies were investigated as per ICH guidelines at a temperature  $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH for 6 months. After specified duration, visual examination of transdermal patches did not show any changes in morphology but slightly soft nature was observed. This property is attributed due to the fabrication of patch with hydrophilic polymers. Though, the drug content of the patches was determined at different time intervals, the result ( $9.50 \pm 1.88$  to  $9.76 \pm 0.84 \text{ mg}$ ) was presented at 180<sup>th</sup> day (end of 6<sup>th</sup> month) revealed no significant variations. These result confirms that drug remain stable after stability studies.

**Table 6:** Stability study data of model transdermal patches

Batch	Drug Content (mg) $\pm$ SD	
	0 <sup>th</sup> Day	180 <sup>th</sup> Day
HL1	$9.79 \pm 1.22$	$9.71 \pm 0.98$
HL2	$9.84 \pm 2.14$	$9.76 \pm 0.84$
HL3	$9.70 \pm 0.98$	$9.64 \pm 1.12$
HL4	$9.56 \pm 1.64$	$9.47 \pm 0.88$
HL5	$9.64 \pm 1.80$	$9.54 \pm 0.92$
HL6	$9.58 \pm 2.10$	$9.50 \pm 1.88$

Average of three determinations;  $\pm$  SD - Standard deviation (n = 3)

### CONCLUSION

In this study, though few results are reported earlier, an attempt has been made to prove the feasibility of LH for transdermal in presence of different penetration enhancers and can successfully fabricate the LH transdermal patches employing hydroxypropyl methylcellulose K-100 and polyvinyl pyrrolidone K-30 polymeric blend. To enhance the penetration of LH, different penetration enhancers such as disodium salt of ethylenediaminetetraacetic acid (Na EDTA), tween-80, span-80 and hyaluronidase were incorporated. Among all, hyaluronidase emerged as best with highest permeation rates. On the basis of obtained results it can be concluded that chosen polymers were compatible for the fabrication of TDDS of lercanidipine hydrochloride. Future prospect is to know the further efficacy of formulation HL3 can be subjected for *in-vivo* studies either in animals or humans.

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