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Formulation, Characterization, *In Vitro* and *Ex-Vivo* Evaluation of Transdermal Patches by Inhibiting Crystallization of Lovastatin

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ABSTRACT

Lovastatin (LST) transdermal matrix patches were prepared by the solvent evaporation method using hydroxypropyl methylcellulose (HPMC E15) as polymer, dibutyl phthalate as plasticizer and citral as permeation enhancer. The crystallization of Lovastatin in the transdermal patch is a major problem that makes the patch unstable and decreases the drug release. The additives were used to inhibit the crystallization of a Lovastatin. Among the different types of additives, Tween 80 was found to be highly effective in inhibiting the crystallization of the drug. Tween 80 acts as a solubilizing agent for Lovastatin in matrix patches. The characterizations showed the homogeneous patches without the crystal form of the Lovastatin matrix patches. The prepared patches were evaluated for thickness, uniformity of weight, folding endurance, moisture content, moisture absorption. The *In-vitro* and *ex-vivo* permeation of the Lovastatin from the patches was studied by Franz diffusion cells using phosphate buffers pH 5.8 & pH 7.4 as receptor medium respectively. The optimized formulation showed flux higher than the targeted flux.

INTRODUCTION

Transdermal drug delivery offers numerous advantages over other routes of delivery including its accessibility and non-invasiveness allowing for ease and convenience of administration. Swift removal of the drug can be undertaken if required and there is the opportunity for controlled or sustained drug delivery over prolonged periods. This approach results in direct entry of bioactive molecules into the systemic circulation, thereby avoiding first-pass metabolism, efflux transporters, as well as metabolizing/digestive enzymes and unfavorable conditions associated with the oral route of administration (Panchagnula, 1997; Ozguney et al., 2006).

However, the skin has evolved to protect the body and acts as a barrier, preventing not the only entry of pathogens but also the entry of other penetrants including bioactive molecules. As a result of the low skin permeation of drugs and the advantages and enormous potential rewards afforded by the transdermal route for drug delivery, much effort has gone into investigating strategies to increase drug penetration rates. Because most drugs do not rapidly nor readily permeate the skin achieving clinically useful therapeutic concentrations, many chemical and physical approaches have been trialed to lower the stratum corneum barrier properties and enhance transdermal permeation (Kost et al., 1999; Monti et al., 2001). A wide body of literature deals with chemical enhancers, which act mainly by transiently altering the permeability characteristics of the stratum corneum.

Lovastatin is one of the widely accepted *HMG CO-A reductase* inhibitors suggested for prescription by various government healthcare agencies. This was the first identified statin drug which faces the problem of lower bioavailability (5%), due to high lipophilicity and short half-life (2-5hrs). Myopathy is one of the potential side effects of Lovastatin when administered through orally, is the matter of concern is controlled and precise delivery of statin drugs through such dosage forms. These potential problems can be easily overcome by using transdermal delivery of Lovastatin but the crystallization of many statins in polymers used in transdermal drug delivery was reported earlier(Sarvaiya*et al*,2013).

However, the crystallization effect of the drug is a serious problem for the formulation design of the matrix transdermal patches. It alters the physicochemical properties, responsible for the instability of the patches, reduces the amount of drug release from the patches, and decreases

the flux &it especially makes the patch lose its aesthetic appeal after crystallization. Thus, the crystallization inhibitors studied and used to inhibit the crystallization of the drug.

Literature review

The effects of various additives (poloxamer 407, Tween 80, polyvinylpyrrolidone (PVP K30), and PEG-8 glyceryl caprylate/caprate) were investigated for the inhibition of crystallization of Ketoprofen in polyisobutylene adhesive matrix which is reported by Kim and Choi (2002). These various additives significantly increased the permeation rate of ketoprofen from the polyisobutylene adhesive matrix. The PVP K30 is found to be the most effective as a crystallization inhibitor of the ketoprofen in a matrix & may significantly affects the efficacy and quality of the matrix transdermal patches.

Variankaval*et al*. (1999) reported needle-like crystal and aggregates around the needles of estradiol in the transdermal patches when a drug has been dissolved in the polymeric adhesive patches.

Jain and Banga (2010) studied the various additives such as poloxamer407, PVP K90, and copovidone for crystallization inhibition of captopril and levonorgestrel in the patches. The PVP K90is the most effective additive in inhibiting the crystallization of the drugs.

Also, in a previous study found the crystallization of Mefenamic acid in the transdermal patches prepared by ethylcellulose and eudragit as a matrix film. PVP is interesting to be used as a crystallization inhibitor for Mefenamic acid matrix patches**(**Suksaeree*et al*, 2017).

Objective:

The objective of this study is to prepare the Lovastatin matrix patch using tween 80 as a crystallization inhibitor. The patches were made from HPMC E15 as polymer, dibutyl phthalate as a plasticizer, and citral as a permeation enhancer.

MATERIALS

Lovastatin (LST) was obtained as a gift sample from Yarrow Chem Ltd. Potassium dihydrogen orthophosphate was supplied by Czen Lab Chemicals Pvt Ltd, Hyderabad. Sodium hydroxide, dichloromethane, and dibutyl phthalate were purchased from Finar chemicals, Gujarat. Tween 80 was supplied by SDFCL, Mumbai. Citral was purchased from

Avra Synthesis Pvt Ltd, Hyderabad. HPMC E15 and Dialysis membrane were supplied by HiMedia Laboratories Pvt Ltd, Mumbai.

METHODOLOGY

Drug polymer interaction studies

To study the possible interaction between LSTand polymer used in the patches, Differential scanning calorimetry(DSC, Perkin Elmer 4000) and FTIR (KBR pellet method) were carried out on pure drug and drug-excipient physical mixture.

Preparation of transdermal patches

The preparation of transdermal patches of LST was carried out by following the solvent evaporation technique. Weighed quantity of polymer (Table I) Hydroxypropyl methylcellulose (HPMC E 15 LV), was taken in a boiling tube. About 20 ml of a solvent mixture of dichloromethane: methanol (1:1) and a sufficient volume of tween 80 was added and vortexed. Sufficient care was taken to prevent the formation of lumps. The boiling tube was set-aside for 6 hours to allow the polymer to swell. Permeation enhancer (citral) and weighed amount of drug was added to this polymeric mixture and mixed well. It was set-aside for 10-15mins to exclude any entrapped air and was then transferred into a previously cleaned Anumbra Petri plate (72.3 sq cm). Drying of these patches was carried out at room temperature overnight and then the polymeric films formed were removed carefully, placed in a vacuum oven and vacuum was applied for 8-12hrs to remove traces of solvents if any. They were stored in a desiccator until the evaluation tests were performed. The entire film was cut into patches of 4.9cm^2 (Gannu*et al, 2007*).

Table No. 1: Composition of LST transdermal patches

Formulation code	Drug (mg)	HPMC E15 (mg)	Tween 80 (mL)	Citral (%)
L0	147	600	-	-
L1	147	1800	1	-
L2	147	2000	1	-
L3	147	2200	1	-
L4	147	2400	1	-
L5	147	2200	1	6
L6	147	2200	1	8
L7	147	2200	1	10
L8	147	2200	1	12
L9	147	2200	1	14

Note:5% v/w Dibutyl phthalate to the total polymer weight

Each patch (4.9 cm^2) contains 10mg of LST. 20ml dichloromethane:methanol (1:1)

SEM Characterization: The SEM5800LV instrument (model: JSM-5800 LV, JEOL, Japan) was used to study the surface morphology of the LST matrix patches with a high vacuum and a high voltage of 15.00 kV condition and using Everhart Thornley detector.

EVALUATION PARAMETERS

Thickness

The thickness of the patch was checked using a screw gauge. Each formulation thickness of three selected patches was recorded.

Weight uniformity

For each formulation, three selected patches (4.9 cm^2) were weighed individually and the average weight was calculated.

Folding endurance

This was calculated by folding the patch at the same place until it breaks. The folding endurance number is the number of times the patch was folded without breaking.

Drug content

Patches from each formulation were taken and cut into small pieces (4.9 cm^2) and allowed to dissolve in a 100mL phosphate buffer and placed on a magnetic stirrer. After 24 hrs the solution was filtered using Whatman filter paper & diluted suitably. The absorbance was measured at 242 nm against blank using a spectrophotometer.

Moisture absorption study

Percentage moisture uptake was studied by first checking the initial weight of patches and then patches were stored in a desiccator (saturated solution of aluminum chloride (100mL) maintained at 79.50% RH) for 3 days. After the period, the patches were taken out and the final weight was checked (Devi *et al*.,2003).

$$\% \text{ Moisture uptake} = \frac{\text{Final weight-initial weight}}{\text{Initial weight}}$$

Moisture content

The initial weight of patches was noted and then the patches were kept in a desiccator for 24 hr (calcium chloride at 40^oC). When no further change in the weight of the patch was observed then this weight was taken as final weight(Gupta *et al*., 2003).

$$\% \text{ Moisture content} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}}$$

***In-vitro* release studies**

The drug release studies from Lovastatin transdermal patches were performed using Franz diffusion cell which contains donor and receptor compartment. The transdermal patch was placed over a dialysis membrane (Himedia Mol Wt 5000) which is soaked in dissolution media overnight and sandwiched between the two compartments and fixed tightly with the help of clamps. Phosphate buffer pH 5.8 (15 mL) was used as dissolution media which was taken in the receptor compartment. The whole set up of Franz diffusion cell was placed on a magnetic stirrer. The study was conducted at a speed of 25 rpm at 37 ±0.5^oC. Samples (2 mL)

were collected at pre-determined time intervals up to 24 hrs and analyzed using a UV-Vis spectrophotometer at 242nm against phosphate buffer pH 5.8 as blank.

Preparation of rat abdominal skin(IAEC/23/UCPSC/KU/2018)

Male Wistar rats (230-250 gm) were sacrificed using ether. The hair of the animals at the abdominal region was carefully trimmed short (<2 mm) with scissors and the full-thickness skin was removed. The epidermis was prepared surgically by heat separation technique by soaking the entire abdominal skin in the water at 60^{o}C for 45 sec. The epidermis was removed carefully and cleaned with water and this was used to perform *Vivo* permeability studies.

***Ex-vivo* permeation studies**

The patch was applied over the stratum corneum side of the rat skin and sandwiched between the two compartments of the diffusion cell. The receiver phase (15 mL) contains phosphate buffer 7.4 as release media. The whole assembly was kept on a magnetic stirrer and stirred at 25 rpm by maintaining the temperature at 37 ± 0.5^{o}C. Samples (2mL) were withdrawn at pre-determined time intervals up to 24 hr and replenished with an equal volume of phosphate buffer pH 7.4 and analyzed spectrophotometrically at 242 nm. Cumulative amount of LST permeated(µg) was calculated. The graph was plotted by taking the cumulative amount permeated on the y-axis and time on the x-axis. The slope of the curve (m) obtained was divided by area of the exposed skin surface (A, 4.9 cm^2) to give flux (J, µg/cm^2/hr) at steady state (Peltola *et al*., 2003). Permeability coefficient (Kp) and enhancement ratio was calculated using the following equation (Gannu*et al*., 2007).

$$\text{Flux, } J = m/A$$

Permeability coefficient, $Kp = J/C_d$ (Initial drug load)

Enhancement ratio ER=J of formulation/J of drug solution

The following equation was used to calculate the theoretical flux of the drug.

$$J = \frac{C_{SS}Cl_{T}BW}{A}$$

C_{ss}= LST concentration at the therapeutic level (4.58µg/ml)

Cl_T = Total clearance (0.3L/min/kg)

BW = Standard human body weight of 60 kg

A = Surface area of the transdermal patch (i.e. 4.9 cm^2)

The calculated theoretical flux value for LST was 16.82µg/cm^2/hr.

Stability study for optimized formulation

The stability studies were performed by following ICH (International conference on harmonization) guidelines for the optimized formulation (L8). The patch was wrapped in aluminum foil and stored in a desiccator containing saturated sodium chloride (NaCl) solution at a temperature of 40 ± 2 °C, 75 ± 5 % RH for 3 months. The samples were withdrawn at regular intervals of 1st, 2nd, and 3rd month and analyzed for drug content and percentage cumulative drug release up to 24 hrs.

RESULTS AND DISCUSSION:

DSC study: In the DSC thermogram of pure drug, the endothermic peak was observed at 176.86ºC which corresponds to the melting point of LST at 174ºC. DSC thermogram of a physical mixture of drug and polymer showed an endothermic peak at 171.86ºC. The shift in the peak was very less which indicates the polymer (HPMC E15) used is compatible with the drug.

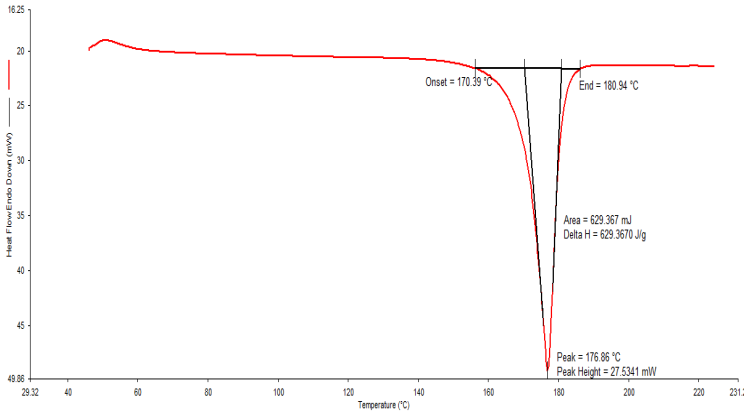

Figure No. 1: DSC thermogram of pure LST

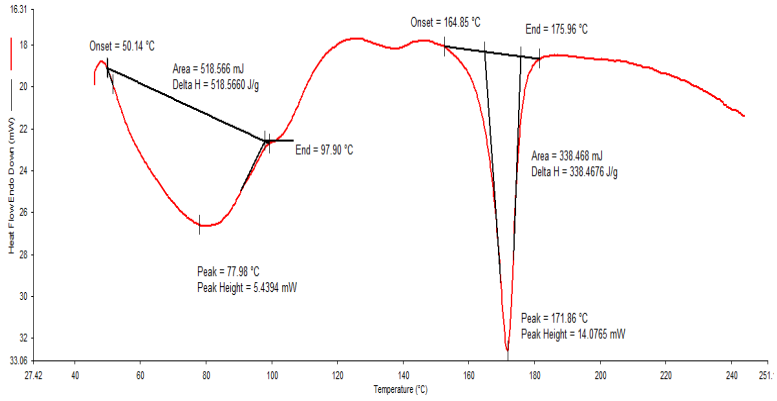

Figure No. 2: DSC thermogram of LSTand HPMC E15

FTIR spectra: The prominent peaks observed with LST were also observed in the IR spectra of a sample containing LST& HPMC E15 and compared with the literature range which indicates that the drug did not show any interaction with the polymer used and confirms the stability of the drug.

Table No. 2: FTIR data of pure drug (Lovastatin) and physical mixture of drug and polymer

Functional group	Frequency (cm^{-1})		
	Literature value	Lovastatin	A physical mixture of LST & HPMC E15
O-H	3200-3600	3288.092	3282.477
C-H	2850-2960	2920.022	2922.02
C=O	1690-1760	1678.384	1678.966
C-O-C	1080-1300	1059.672	1060.269

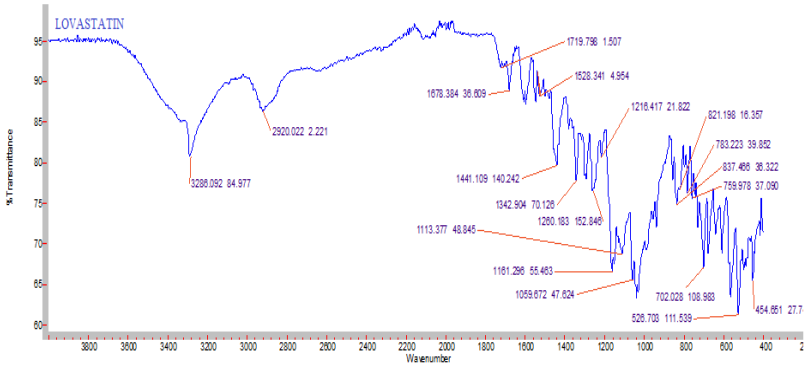

Figure No. 3: FTIR spectra of pure drug LST

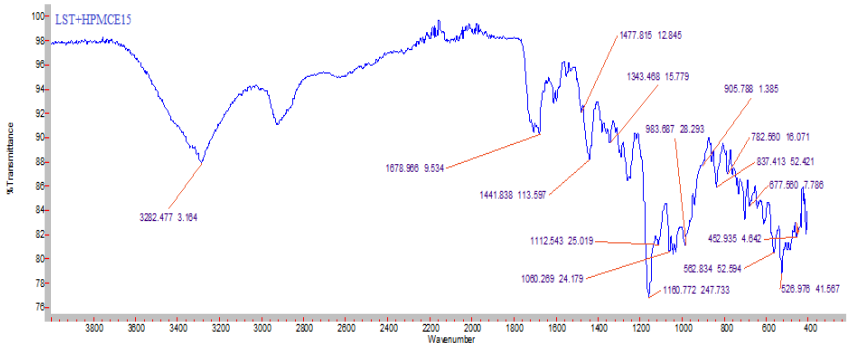

Figure No. 4: FTIR spectra of a physical mixture of pure drug LST and polymer HPMC E15

Optimization of tween 80 and polymer

Transdermal patches for Lovastatin using HPMC E15 as a matrix film were prepared. It was found that the crystals of Lovastatindispersed in the patches. The different types of additive, tween 80, PVP K30, PVP K90, and poloxamer 188were tested for the minimum concentration used to inhibit crystallization. Crystal inhibition was seen only by tween 80**.** Due to the addition of tween, an increase in polymer weight was required to maintain enough strength of the patches. Below 1800mg of polymer, patches were broken during handling, 1800-2400mg of polymer, patches were soft and flexible, and above 2400mg polymeric solution was highly viscous and leads to uniform mixing of polymer and drug.

Table No. 3: Optimization of tween 80

Sr. No.	Minimum concentration of tween 80 (ml)	Crystal inhibition
1	0.5	No
2	1	Yes
3	1.5	Yes, increased drying time and easily breakable.
4	2	No patch formed.

SEM characterization: Fig. 5 shows the surface morphology of the patches of Lovastatin without and with tween 80. Stick shaped particles of LST crystals were seen before adding tween 80and homogeneous patch was formed after tween 80 was mixed in the patch. Thus,

the inhibition of crystallization of Lovastatin in matrix transdermal patches was a successful preparation by using tween 80as crystallization inhibitor.

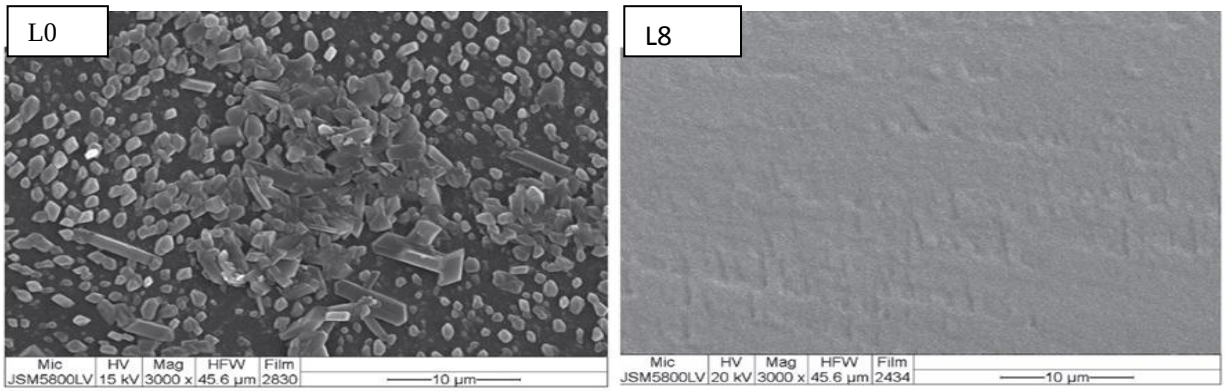

Figure No. 5: SEM photography of lovastatin patch without citral & tween 80 and a transdermal patch of lovastatin with tween 80 & citral respectively

Evaluation parameters of the Lovastatin patches

Physicochemical characteristics of patches

Weight and thickness of patches increased with an increase in polymer concentration as evidenced by the results shown below. Drug content ranges from 9.5±2.2mg to 10.3±1.34mg in all formulations. Folding endurance was found to be below 70 for all the formulations.

Moisture absorption and moisture content studies

The moisture absorption for different patches was in the range of 5.7±2.1 to 8.12±2.20%. The moisture content for different patches was in the range of 2.12 ± 2.10 to 10.32 ± 1.35%. Percentage moisture content and moisture uptake were found to increase with an increase in the concentration of polymer (HPMC E15). Microbial contamination in patches is prevented due to low moisture absorption. Patches were stable, completely dry and brittle due to low moisture content.

***In vitro* release Studies**

The *in vitro* release studies were conducted by using Phosphate buffer pH 5.8 as a release medium. Fig. 6 shows the release profiles of Lovastatin from patches containing different concentrations of HPMC E15.

Formulation L3 exhibited the highest (4.7±0.52) percentage of drug release without citral. As the concentrations of polymer (HPMC) increased in the formulations, the drug release rate increased, however with a very small decrease in formulation L4 which may be due to an increase in the diffusional path length.

Citralin different concentration (6% to 14%) was added to L3 formulation which tends to enhance the release rates (Fig 7). Good permeation enhancement was observed with 12% (L8, 94.5%) of citral and beyond this, the drug release was found to decrease which might be due to reaching of saturation point.

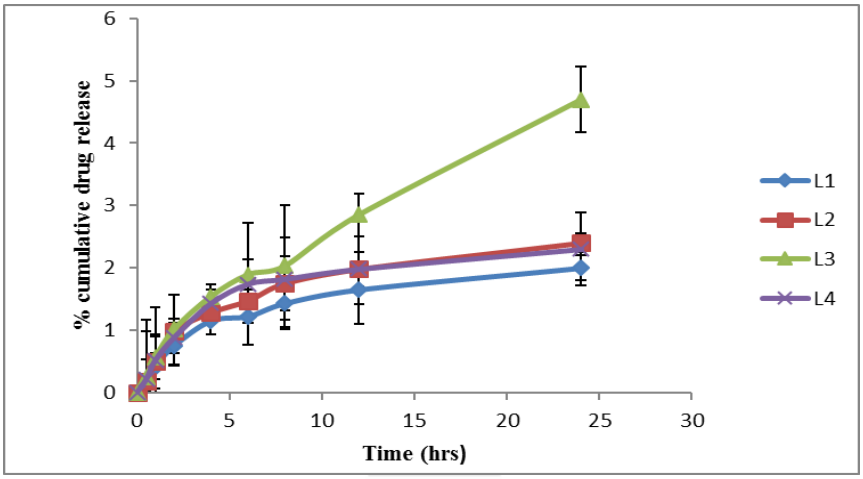

Figure No. 6: Comparison of %cumulative drug release of LST from formulations without citral

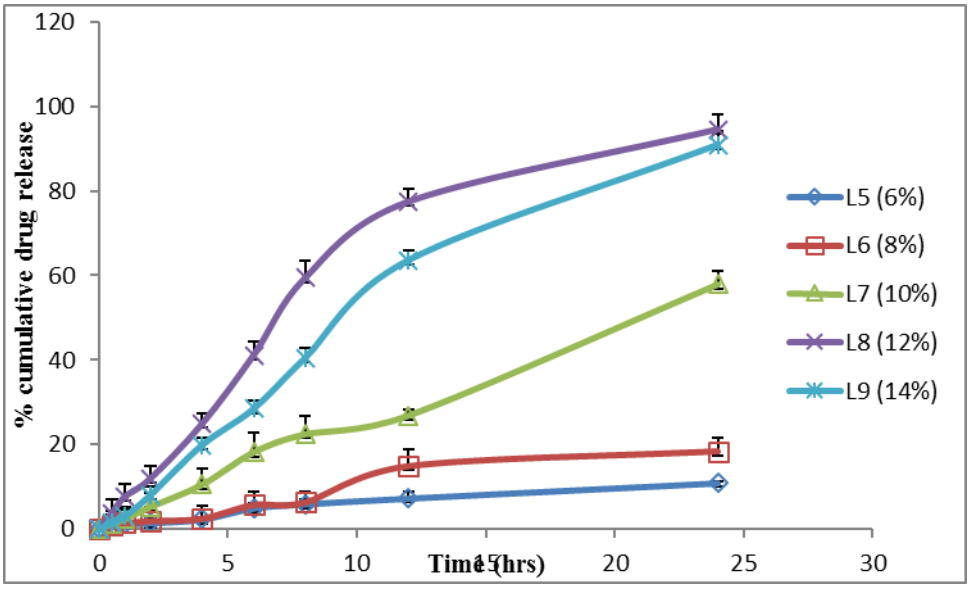

Figure No. 7: Comparison of %cumulative drug release of LST from formulations with citral

Release kinetics

Data of *in vitro* release were fitted to various kinetic models to determine the release mechanism of LST from transdermal patches. For optimized formulation, the best fit with the highest correlation value was shown by zero-order (R^2=0.9927) and n value (n>0.5) indicated non-fiction diffusion controlled drug release which is obtained from the slope of Peppas equation.

***Ex vivo* permeation study of optimized formulation (L8) through rat skin**

The result of *ex vivo* skin permeation of LST from the L8 patch was shown in Fig (8). Citral acts by modifying the solvent nature of stratum corneum and increases the drug partition coefficient into the tissue. The flux obtained by formulation L8 (12% citral 17.2µg/cm^2/hr) was higher than the theoretical flux (16.82 µg/cm^2/hr) and the flux obtained by drug solution (7.38µg/cm^2/hr) was less than theoretical flux. Enhancement ratio was found to be 2.33 for L8 formulation with permeability coefficient 0.35.10^{-3}cm/hr higher than drug solution (0.15.10^{-3}cm/hr). The results of the permeation study of Lovastatin from the transdermal patch through the rat abdominal skin confirmed that drugs could permeate through the human skin.

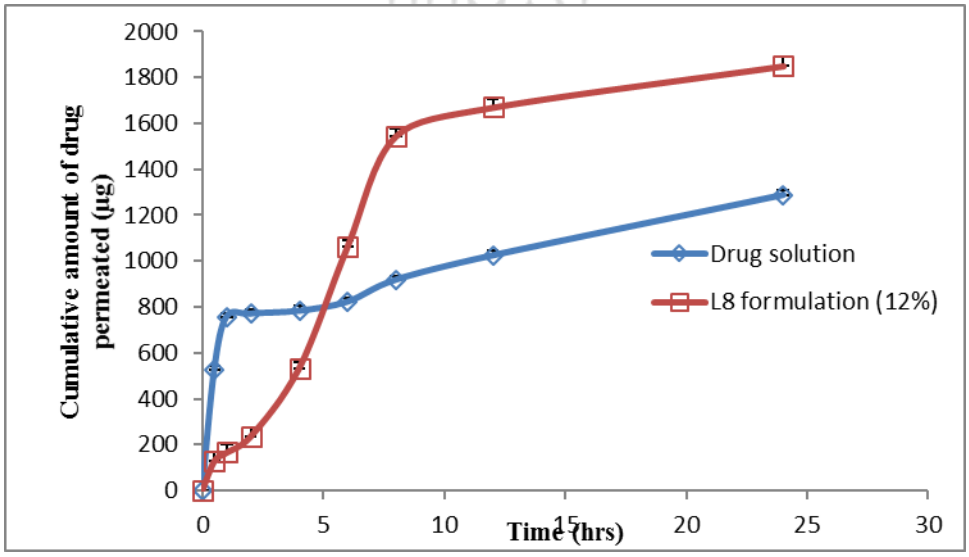

Figure No. 8: *Ex vivo* permeation study of an optimized formulation containing 12%citral through rat skin

Release kinetics data of *ex vivo* study

The *ex vivo* permeation profiles of drugs from L8 seem to follow zero-order release kinetics as it is evidenced by correlation coefficient (0.9038) and n value (0.7046) indicated that mechanism of drug release was by non Fickian diffusion.

Stability study for optimized formulation (L8)

3 months of study was performed. At different time points, samples were withdrawn and analyzed for drug content and *in vitro* drug release. Significant changes in drug content and *in vitro* drug release were not observed.

Table No. 4: Stability study for optimized formulation (L8)

Parameters	**1st month**	**2nd month**	**3rd month**
Drug content (mg)	9.9±0.32	9.8±0.37	9.85±0.66
% cumulative drug release	94.2±3.67	92.5±2.98	93.5±2.32

Values represent mean ± SD (n=3)

CONCLUSION

The transdermal patches of Lovastatin were prepared and evaluated successfully. HPMC E15 was used as a polymer. LST was found to be compatible with HPMC E15 from the results obtained from DSC and FTIR study. *In vitro* studies illustrate that LST being hydrophobic cannot be easily permeated through stratum corneumas optimum HLB value has to be maintained to cross corneum and hence permeation enhancer was required. A maximum drug release was seen with 12% citral. Citral reacts with amides of ceramide located in corneum and disrupts the barrier as a result drug diffusion occurs. *Ex vivo* permeation of optimized formulation L8 (12% citral) reveals that drug release occurred in zero-order manner and mechanism of release is by non-fiction diffusion.

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