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Formulation and Evaluation of Transdermal Films of Lovastatin

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ABSTRACT

The monolithic matrix type transdermal films of Lovastatin (LS) were prepared by film casting technique on mercury substrate. Nine formulations were developed using Eudragit RL 100 (ERL) with Eudragit RS 100 (ERS) in ratio of 4:1 with oleic acid (OA) and menthol (MN) as penetration enhancers, alone or combination and were coded as G1, G2 G3, G4, G5, G6, G7, G8 and G9. All the formulations carried 10% w/w of Lovastatin and 30% w/w of Dibutyl Phthalate (DBP) in Chloroform as solvent system. The films were evaluated for physicochemical and *in vitro* diffusion studies using Keshary-Chien diffusion cell. All the films were found to be suitable for formulating in terms of physicochemical characteristics. The corresponding values for cumulative drug permeation for said formulation were 284.55 (G1), 283.88 (G2), 290.85 (G3), 297.12 (G4), 311.89 (G5), 353.95 (G6), 443.60 (G7), 312.98 (G8) and 356.68 (G9) mcg/cm^2. On the basis of *in vitro* permeation studies formulation G7 was having maximum rate of permeation and it was selected as optimized formulation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.95 to 0.99 indicating that diffusion mechanism of drug release. Drug-excipient interaction studies were carried out using TLC and FTIR technique; films indicated no chemical interaction between drug and excipients. Primary skin irritation study shows the films are non irritant.

Keywords: Lovastatin, Transdermal films, Eudragit RL 100, Eudragit RS 100, Oleic acid, Menthol, *in vitro* permeation study.

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INTRODUCTION

Lovastatin is a 3-hydroxy-3-methylglutaryl derivative used in the treatment of hypercholesterolaemia [1]. It acts by inhibiting HMG-CoA reductase, the enzyme that catalyzes conversion of HMG-CoA to mevalonate which is a precursor for cholesterol biosynthesis [2], after oral administration 30% of drug is absorbed and the systemic bioavailability is less than 5% owing to its presystemic metabolism by cytochrome P_{450} enzyme system. The plasma half life is about 3 hr which make frequent dosing necessary to maintain therapeutic blood levels for a long treatment [3-5]. Therefore LS is ideal candidate for transdermal delivery.

Transdermal drug delivery system has many advantages over conventional mode of drug administration; it avoids hepatic first pass metabolism, maintenance of constant and prolonged drug level, reduced frequency of dosing, minimization of inter and intra patient variability, self administration and easy termination of medication leading to patient compliance [6, 7]. Nevertheless, the primary barrier to transdermal drug delivery is the outermost layer of skin, the Stratum Corneum. The stratum corneum has been represented as a 'brick and mortar' model in which the keratinized cells are embedded in a mortar of lipid bilayers thus preventing the infiltration of exogenous agents into the body [8]. Since Lovastatin is lipophilic molecule, we decided to explore lipid based enhancers to improve drug flux.

MATERIAL AND METHOD

Material

Lovastatin (LS) - gift sample from Panacea Biotech, Chandigadh, India. Sodium laurel sulfate (SLS), sodium dihydrogen phosphate, menthol (MN) (Loba Chemie), Eudragit RL 100 (ERL), Eudragit RS 100 (ERS) (Rohm pharma, Germany), Dibutyl phthalate (DBP) (Qualingenes fine Chemie), oleic acid (OA) (Pure Chemie).

Preparation of films

Matrix type transdermal films containing LS were prepared using ERL and ERS using different ratios MN and OA by mercury substrate method (Table I). The polymers and penetration enhancers were weighted in requisite ratio and dissolved in chloroform. DBP 30% w/w of polymer composition was used as plasticizer. LS was added 10% w/w of the total weight of polymers, homogeneous dispersion was formed by slow stirring with a mechanical stirrer. The uniform dispersion was then poured into a glass ring of 6 cm diameter placed on the surface of mercury kept in a petri dish. The solvent was allowed to evaporate under ambient conditions (Temperature: 32^0, RH: 45%) for 24 hr. Aluminum foil was used as backing membrane and wax paper as release liner. The films were cut with a circular metallic die of 2 cm internal diameter to give an effective surface area of 3.14 cm^2 and stored in desiccator until used.

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Table. I: Formulation Composition

Formulation Code	ERL:ERS 600 mg	DBP % w/w of polymer	Chloroform (ML)	Lovastatin % w/w of polymer	OA % w/w of polymer	MN % w/w of polymer
G1	4:1	30	10	10	-	-
G2	4:1	30	10	10	-	2
G3	4:1	30	10	10	-	5
G4	4:1	30	10	10	-	10
G5	4:1	30	10	10	2	-
G6	4:1	30	10	10	5	-
G7	4:1	30	10	10	10	-
G8	4:1	30	10	10	5	5
G9	4:1	30	10	10	10	10

Drug - Excipient interaction study [3]

Drug – Excipient interaction study was performed using TLC and FTIR.

TLC analysis was conducted using silica plate with toluene: acetone (70:30) as mobile phase. The TLC plates were prepared using slurry of Silica Gel GF. The prepared plates were activated at 110^0 for 1.5 hr, on the activated plates 6 µL of each solution in methanol containing (a) 10 mg/ml LS (b) 10 mg/ml LS containing different excipients, i.e. ERL, ERS, DBP were applied. The plates were dried in a stream of hot air and then observed for the spots in UV cabinet. The Rf values were calculated from the chromatogram obtained.

The pure drug, LS and a mixture of it with polymers, ERL and ERL were mixed separately with IR grade KBr in the ratio of 100:1 and corresponding pellets were prepared by applying 10 metric ton of pressure in hydraulic press. The pellets were then scanned over a wave range of 4000 – 400 cm^{-1} in FTIR instrument (8400 S Shimadzu).

Evaluation of films

Thickness

The thickness of the patch was measured by micrometer (Acculab) at three different places; average of three values was calculated.

Tensile strength and percent elongation

An instrument fabricated according to ASTM standards to measure tensile strength and percent elongation. The specimen's thickness and breadth were measured at least at 3 different places with traveling microscope. The specimen was held between two jaws in such a way that the marking towards the first jaw was just inside it, whereas the marking towards the movable jaw is just visible through traveling microscope and marking towards the movable jaw

was measured. The change in the length of the specimen that occurred with increasing stress was measured. The rate of change in stress was kept constant by increasing the load on the pan at the rate of 100 gm/ 2 min, as stress – strain relationship changes with the rate of change in stress.

Flatness

Longitudinal strips were cut out from each film, one from the centre and two from either side. The length of each strip was measured and the variation in length because of non uniformity in flatness was measured by determining percent constriction, considering 0% constriction equivalent to 100% flatness.

$$\% \text{ constriction} = L1 - L2 / L2 \times 100.$$

Where, L1 = initial length, L2 = final length of each strip.

Folding endurance

The folding endurance of the films was determined by repeatedly folding a small strip measuring 2×2 cm size at same place till it breaks [9].

Moisture content

The films were weighted and kept in desiccator containing calcium chloride for at least 24 hr or more until it showed a constant weight. The percentage moisture content was the difference between the initial and final weight with respect to final weight.

Moisture uptake

A weighted film kept in a desiccator at normal room temperature for 24 hr was taken out and exposed to two different relative humidity of 75% (saturated solution of sodium chloride) and 93% (saturated solution of ammonium hydrogen phosphate) in two different desiccators, at room temperature. Then the weights were measured to constant weight. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight [10].

Drug content

Films of specified area were cut and weighted accurately. Pieces were taken into 100 ml volumetric flask and 60 ml of phosphate buffer solution (pH 7.0) with 2% SLS was added and kept in a mechanical shaker for 24 hrs. A blank was performed using drug free films. The solution was filtered and samples were analyzed spectrophotometrically at 238 nm [11].

Preparation of skin

Albino Wistar rats (150 – 175 gm) which had been given free access to food and water were sacrificed by snapping the spinal cord at the neck immediately before experiment. The skin was carefully excised, hair, adhering fat and other visceral debris was removed by treating the skin with 0.32 M ammonia solution for 35 minutes [12]. Separated epidermis was washed with normal saline solution before starting the experiment.

***In vitro* drug permeation study**

The in vitro drug permeation studies were carried out using Keshary-Chien diffusion cell. The cell consists of two compartments, namely donor and receptor having volume capacity of 3 ml and 11 ml respectively. Phosphate buffer (pH 7.0) with 2% SLS was used as receptor fluid [13]. The polymeric films of 2 cm diameter were placed in intimate contact with the stratum corneum side of the skin. The receptor fluid was agitated using a magnetic stirrer at 100 rpm to avoid the formation of diffusant layer and the temperature of 37 ± 1^{0} was maintained, sampling port was covered with parafilm to avoid the evaporation of solvent. Aliquots of 1 ml sample was withdrawn at time interval of 1, 2, 4, 6, 8, 12 and 24 hrs and replaced with an equal volume of drug free receptor fluid to maintain the sink condition. The amount of drug permeated at each time interval was calculated spectrophotometrically at 238 nm. In vitro drug cumulative release data for various polymeric films are given in table III.

Data analysis

The cumulative amount of drug permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the steady state flux [14] (Jss), permeability coefficient (Kp) and enhancement ratio (ER) was calculated by using equation,

$$Kp = Jss / DC.$$ Where, DC- Donor concentration.

ER = drug flux with enhancer/drug flux without enhancer.

Primary skin irritation study

The films were tested for their potential to cause skin irritation/sensitization in healthy human volunteers. Each site of film application was rated with regard to presence of severity of erythema and edema [15].

RESULT AND DISCUSSION

TLC studies were performed to assess any interaction between the drug and the excipient. The data obtained suggest that there was no interaction between the drug and excipient because Rf values of both drug and drug – excipient solutions were nearly similar.

FTIR spectra were recorded to assess the interaction between drug and excipients. The figure showed no distinctive physical or chemical interactions between drug and polymers.

All the films, measured thickness with low standard deviation values ensuring the uniformity of films prepared by mercury substrate method. The tensile strength and percent elongation were found to be optimum; results are shown in table II. An ideal film should maintain smooth and uniform surface when applied to skin. The result indicated no amount of constriction in the films and ensured 100% flatness [16]. The folding endurance of all the films was found to be satisfactory. Results of thickness, tensile strength, percent elongation, flatness and folding endurance are shown in table II.

Table.II: Evaluation of transdermal films.

Formulation code	Thickness cm	Tensile strength dyne/cm^2	% Elongation	% Flatness	Folding endurance
G1	0.0396 (0.00021)	46.67×10^6	35.67	100	> 150
G2	0.0384 (0.00561)	48.58×10^6	36.81	100	> 150
G3	0.0398 (0.00754)	48.48×10^6	35.32	100	> 150
G4	0.0402 (0.00062)	50.43×10^6	39.09	98	> 150
G5	0.0416 (0.00091)	45.64×10^6	37.28	100	> 150
G6	0.0356 (0.00118)	53.01×10^6	37.93	100	> 150
G7	0.0365 (0.00268)	51.83×10^6	41.12	100	> 150
G8	0.0372 (0.00048)	47.79×10^6	38.75	98	> 100
G9	0.0408 (0.00082)	49.52×10^6	39.59	100	> 150

Values in Parenthesis are expressed as $\pm$ S.D (n =3)

The percentage moisture content was calculated from the weight differences relative to final weight. The moisture content was found to be low, low moisture content helps them to remain stable and from being completely dried and brittle (Fig.1). The results of moisture uptake study are shown in Fig.2. Low moisture uptake protects the film from microbial contamination and bulkiness. Films were subjected to test for drug content uniformity. The film does not shows significant deviation from average value (Table. III).

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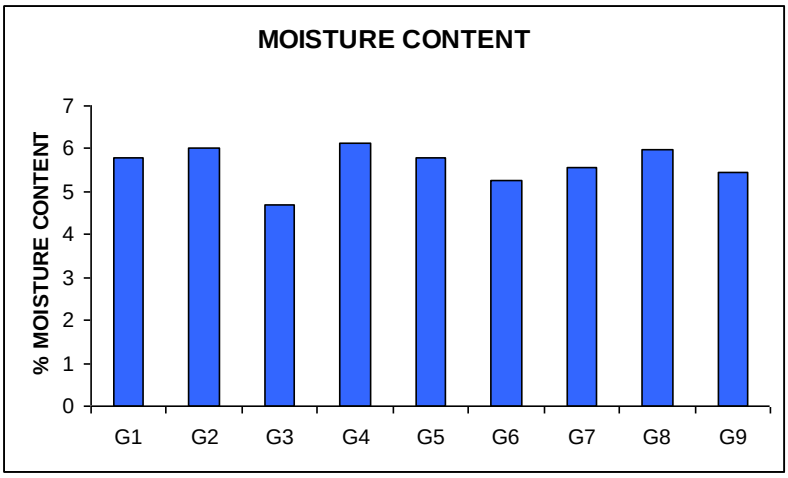

Figure 1: Moisture Content study.

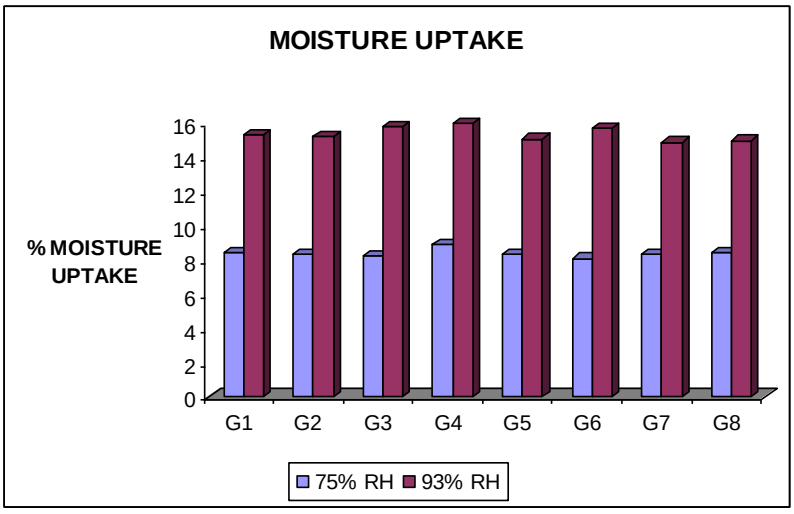

Figure 2: Moisture uptake study.

Table.III: Drug Content, Cumulative drug permeation, Permeation flux, Enhancement ratio and Permeation flux through Transdermal films.

Formulation code	% Drug content	Cumulative drug permeated mcg/cm^2	Flux mcg/cm^2	ER	Permeability Coefficient Kp× 10^3
G1	97.21	284.55	15.52	1.000	7.39
G2	97.57	283.88	15.37	1.000	7.31
G3	96.64	290.85	15.61	1.007	7.43
G4	95.78	297.12	16.28	1.050	7.75
G5	97.06	311.89	17.27	1.114	8.22
G6	99.14	353.95	19.47	1.256	9.27
G7	101.27	443.60	23.88	1.540	11.37
G8	98.32	312.98	17.53	1.130	8.34
G9	97.50	356.68	19.66	1.268	9.36

Values in Parenthesis are expressed as ± S.D (n =3)

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In vitro skin permeation studies are predictive of *in vivo* performance of drug. Permeation studies were performed for different films using phosphate buffer (pH 7.0) with 2% SLS as an *in vitro* study receptor fluid of Keshary-Chien diffusion cell at 37 ± 1^{0} C. Cumulative amount of the drug permeated per cm^2 from the different films of varied ratio of MN (G2 – G4), OA (G5 – G7) and MN: OA (G8 – G9) showed variable release pattern (Fig.3 and 4).

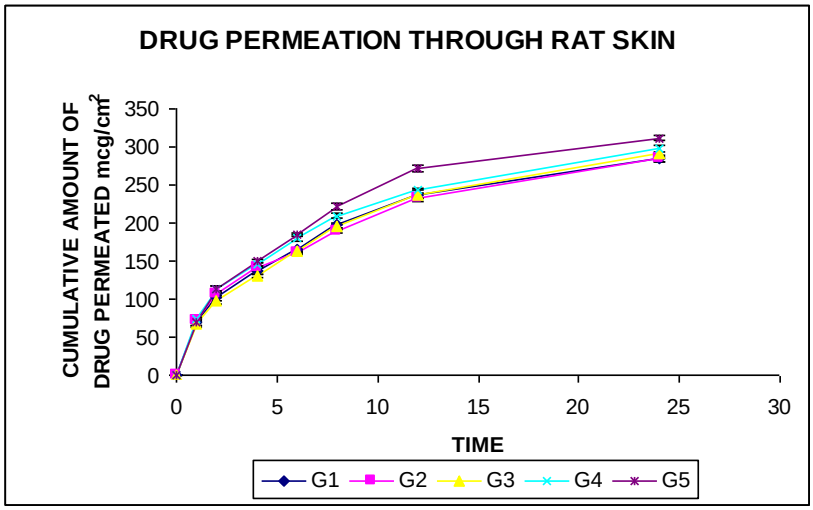

Fig.3: Plots of Cumulative Drug Permeated Verses Time (h) For G1 – G5.

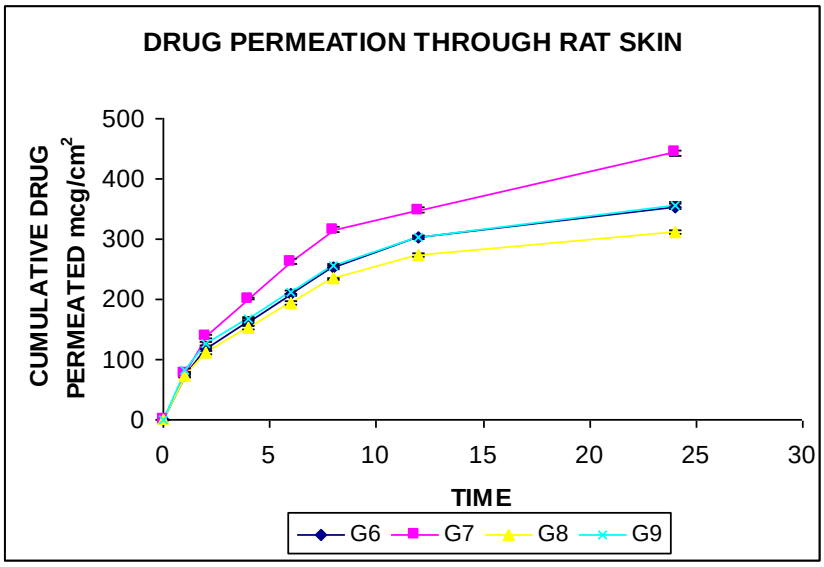

Fig.4: Plots of Cumulative Drug Permeated Verses Time (h) For G6 – G9.

The process of drug release in most of the controlled/sustained release devices including transdermal films is governed by diffusion [17], when these matrix patches comes in contact with an *in vitro* study fluid, the fluid is absorbed into the polymer matrix and this initiates polymer chain dissolution process in the matrix [18]. Polymer chain dissolution from the matrix surface involves two steps [19]. The first step involves migration of drug towards matrix surface and the second step involves transport of drug from surface into the *in vitro* receptor fluid. When the active agent is released from the matrix in such a way that the rate of

release of the drug remains constant, the release kinetic of the drug is said to follow zero order kinetics [20]. The films under study follow similar patterns of drug permeation profile i.e. initially apparent zero order and then first order permeation kinetics. Initially for first few hrs drug permeation followed zero order kinetics and with enhancement of time the permeation profile gradually changed into concentration dependant first order permeation kinetics.

Due to continuous lipid regions in the stratum corneum, it is believed that passive transdermal diffusion occurs through the lipid phase of the skin [21]. For this reason hydrophobic drugs generally have better transport through skin while water soluble ionic drugs have very limited permeability [22]. In this study permeation enhancement of LS was studied using OA and MN, alone or combination.

The cumulative amount of drug permeation was found to be 284.55 (G1), 283.88 (G2), 290.85 (G3), 297.12 (G4), 311.89 (G5), 353.95 (G6), 443.60 (G7) and 312.98 (G8) and 356.68 (G9) mcg/cm^2. Increase in drug permeation was found as the concentration of OA increased. It has been reported that the enhancing effect of OA was dependant on its concentration, thus percutaneous permeation of drugs increased with the amount of OA until maximum, after which the penetration decreases, however within the range of concentration used in present study, OA enhances the permeation of LS since maximum was not reached [23]. The mechanism of penetration enhancement of OA is by increasing the fluidity of the intercellular lipids [24].

In order to understand mechanism of drug release, *in vitro* release data were treated to kinetic models and linearity was observed with respect to Higuchi equation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.95 to 0. 99. This indicates that the mechanism of drug release was diffusion type [25]. No erythema or edema was noticed on the skin of human volunteer after the application of the films for 24hrs.

CONCLUSION

From above studies it can be concluded that the polymeric matrix-type transdermal films of LS prepared with different ratios of penetration enhancers holds potential for transdermal delivery. A slow and controlled release of drug release versus time is linear, these supporting the test products for transdermal films. Developed formulation has the best effective combination of polymer but slight modification required to achieve therapeutic plasma concentration.

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