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Research Article

DEVELOPMENT AND *IN VITRO* EVALUATION OF TRANSDERMAL PATCHES OF LOVASTATIN AS A ANTILIPIDEMIC DRUG

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ABSTRACT
In present work was designed to develop suitable transdermal matrix patches of Lovastatin (LS), using hydroxy propyl methyl cellulose (HPMC) and Eudragit RL 100 with Triethyl citrate as a plasticizer. A 3^2 full factorial design was employed, the amount of HPMC(X_1) and Eudragit RL- 100(X_2), was used as independent variables. The folding endurance, tensile strength, moisture content, moisture uptake and diffusion of drug were selected as dependent variables. The Casting solvent technique was employed for the preparation of HPMC, ERL-100 film. The dry films were evaluated for Physical appearance, thickness uniformity, moisture content, moisture uptake, tensile strength, flatness and folding endurance. In vitro diffusion studies were performed using cellulose acetate membrane (pore size 0.45 µ) in a Franz's diffusion cell. The concentration of diffused drug was measured using UV-visible spectrophotometer (Jasco V-530) at λ max 254 nm. The experimental results shows that the patch containing HPMC in higher proportion gives increase in the release of drug. It indicates that as the concentration of X2 (Eudragit RL 100) increase, the drug release from the matrix was decrease. The present study has demonstrated the potential of the fabricated matrix film for prolonged release of Lovastatin.

KEYWORDS: Lovastatin**,** Transdermal delivery, Draize test, Eudragit, Hydroxy propyl methyl cellulose.

INTRODUCTION

Delivering medicine to the general circulation through the skin is seen as a desirable alternative for administrating it by mouth. Recently it is evident that the benefits of intravenous drug infusion can be duplicated, without its hazards, by using the skin as the port of drug administration to provide continuous transdermal drug infusion in to the systemic circulation. Skin consists of membrane barriers, which are mainly composed of lipids & proteins. The penetration across epithelial borders is a slow process due to the effect of the barrier properties. The skin, in particular the stratum corneum, possesses a barrier to drug penetration due to its high density (1.4 g/cm^2 in dry state), its low hydration of 15 to 20%. The barrier function is further facilitated by the continuous replacement of stratum corneum, thereby limiting the topical & transdermal bioavailability.

Transdermal drug delivery system attracts many scientists around the world. Transdermal therapeutic systems are designed for controlled drug delivery through the skin in to systemic circulation maintaining consistent efficacy and reducing dose of the drug and it's related side effects. There are a number of routes by which a molecule can cross the stratum corneum, these are, intercellular, transcellular and appendageal but the intercellular route is considered to be the major pathway for permeation of most drugs across the stratum corneum[1]. The skin as a site of drug delivery has a numbers of significant advantages over many other routes of drug administration, including the ability to avoid problems of gastric irritation, pH, and emptying rate effects; avoid hepatic first pass metabolism thereby increasing the bioavailability of drug; reduce the risk of systemic side effects by minimizing plasma concentrations compared to oral therapy; provide a sustained release of drug at the site of application; rapid termination

of therapy by removal of the device or formulation[2]; the reduction of fluctuations in plasma levels of drugs[3] and avoids pain associated with injections. Transdermal therapeutic systems may produce sustained, constant and controlled levels of drug in the plasma, thereby improving patient compliance, since frequent intake of the drug is not necessary. Transdermal therapy also has its some disadvantages, like, higher molecular weight candidates (>500 Dalton) fail to penetrate the stratum corneum without modifying the nature of stratum corneum, drugs with very low or high partition coefficient fail to reach systemic circulation and high melting drugs, due to their low solubility both in water and fat[4]. Molecules may activate allergic responses and the drug may be metabolized by mircoflora on the surface of skin or by enzymes in the skin[5,6,7]. An ideal penetration enhancer reversibly reduces the barrier resistance of the stratum corneum without damaging the skin [8,9].

Lovastatin lowers cholesterol levels through reversible and competitive inhibition of 3-hydroxy- 3-methylglutaryl coenzyme A reductase, an enzyme involved in the biosynthesis of cholesterol. It exhibits poor oral bioavailability (5%) because of rapid metabolism in the gut and liver. Cytochrome P450 3A4 metabolizes the lactone form of lovastatin into hydroxy acid and its metabolites.[10] To overcome hepatic first-pass metabolism and to enhance bioavailability. Hence a potential candidate for the present work on transdermal studies. In this study we observed the effect of different types of polymers (i.e. hydrophilic and lipophilic) on the release of drug from the prepared transdermal matrix patches.

MATERIALS AND METHODS

Lovastatin (LS) was a gift sample from Aurobindo Pharma Pvt. Ltd. (Hydrabad, India). Eudragit RL-100 was obtained from Corel Pharma Ahmedabad, India, HPMC obtained from Colorcon Asia Pvt. Ltd. (Goa, India). Dimethyl formamide obtained from Loba Chemie, Mumbai. 3MTM ScotchpackTM 9733 backing membrane and 3MTM ScotchpackTM 1022 release liner were obtained from 3M (USA). Cellulose acetate membrane was purchased from Sartorious Biotech GmbH (Germany). All other ingredients were used of pharmaceutical grade.

Preparation of transdermal film

The transdermal films containing HPMC:Eudragit RL 100 with 20% wt/wt of LS, 5% wt/wt of plasticizer (triethyl citrate) were prepared by film casting technique on the mercury. Hydrophilic ingredients were dissolved in water and hydrophobic ingredients were dissolve in dimethyl formamide: methanol (60:40) then mixed both solution and stir on magnetic stirrer to accomplished homogeneous mixture. The resulting solution was poured in a petri dish containing mercury. The solvent was allowed to evaporate at 45°C for 24 hours to obtain medicated transdermal film. A backing membrane (3MTM ScotchpackTM 9733) and a release liner (3MTM ScotchpackTM 1022) on either side of the film were applied to complete the transdermal therapeutic system for LP. The prepared LP patches were store in dessicator until further use.

Factorial Design

A 3^2 factorial design was used in this study and two factors were evaluated, each at three levels; experimental batches were performed at all nine possible combinations (Table-1). In Group A the amount of HPMC(X1) and Eudragit RL- 100(X2) was used as independent variables. The folding endurance, tensile strength, moisture content, moisture uptake and diffusion of drug were selected as dependent variables for both the Groups. The data were subjected to 3-D response surface methodology in PCP Disso 2.08 to determine the effect of polymers on the release of drug, dependent variable. The values of variables in a 3^2 Factorial Design are indicated in Table-2. A statistical model incorporating interactive and polynomial terms was used to calculate the responses.

$$Y = bo + b1X1 + b2X2 + b12X1X2 + b11X1\ 2 + b22X22$$

Where, Y is the dependent variable, bo is the arithmetic mean response of the 9 trials, and bi (b1, b2, b12, b11 and b22) is the estimated coefficient for the corresponding factor Xi (X1, X2, X1X2, X12 and X22), which represents the average result of changing one factor at a time from its low to high value. The interaction term (X1X2) shows how the response changes when 2 factors are simultaneously changed. The polynomial terms (X12 and X22) are included to investigate the nonlinearity.

Evaluation of transdermal films

The physical parameters such as thickness, folding endurance, tensile strength, moisture content, moisture uptake and drug content were determined.

Thickness

Patch thickness was measured using digital micrometer screw gauge (Mitutoyo, Japan) at three different places and the mean value was calculated.

Folding endurance

Folding endurance of patches was determined by repeatedly folding a small strip of film (2 cm x 2cm) at the same place till it broke. The number of time the film could be folded at the same place without breaking was the folding endurance value.

Tensile strength

The tensile strength was determined by using a modified pulley system. Weight was gradually increased so as to increase the pulling force till the patch broke. The force required to break the film was consider as a tensile strength and it was calculated as kg/cm^2.

Drug Content

A 5 cm^2 film was cut into small pieces, put into a 100 ml buffer (pH 7.4), and shaken continuously for 24 hours. Then the whole solution was ultrasonicated for 15 minutes. After filtration, the drug was estimated by spectrophotometer at wavelength of 254 nm and the drug content was determined.

Flatness

Three longitudinal strips were cut out from each film: one from the center, one from the left side, and one from the right side. The length of each strip was measured and the variation in length because of nonuniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness[11, 12].

Percentage of Moisture Content

The films were weighed individually and kept in a desiccator containing activated silica at room temperature for 24 hours. Individual films were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight[13].

Percentage of Moisture Uptake

A weighed film kept in a desiccator at room temperature for 24 hours was taken out and exposed to 84% relative humidity (a saturated solution of aluminum chloride) in a desiccator until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight[14].

In vitro Drug Release study

In vitro drug release studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 22 ml. The cellulose acetate membrane (pore size 0.45µ) was mounted between the donor and receptor compartment of the diffusion cell. The prepared transdermal film was placed on the cellulose acetate membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. [15,16,17] The whole assembly was fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads and the temperature was maintained at 32 ± 0.5°C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically.[18,19] The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.

Skin irritation study

Skin irritation studies were performed on healthy rabbits (average weight 1.5 to 2.25 kg). The dorsal surface of the rabbit was cleaned, and hairs were removed by shaving. The skin was cleaned with rectified spirit. Representative patches were placed over the skin with the use of adhesive tape. Treated skin areas were evaluated according to a modified Draize scoring method and the irritation index was evaluated. [20,21] The rabbits were divided in to two groups (n = 6). Group I received transdermal patch and Group II received 0.8% v/v aqueous solution of formalin as a standard irritant. At 24 and 72 hours after test article application, the sites were examined for dermal reactions in accordance with Draize scoring criteria.(Table 7)

Stability studies

Stability studies were carried out for selected formulation at 40 ± 0.5ºC and 75 ± 5% relative humidity for 3 months using programmable environmental test chamber.[22, 23] The samples were evaluated for physicochemical parameters and drug diffusion.

RESULTS AND DISCUSSION

The results of the thickness and flatness of both the groups shown in Table-3. The flatness of films was found much closed to 100%. The folding endurance and tensile strength were lies in between 76 & 203 and 0.395 & 0.734 kg/cm^2; the difference depended on the composition of polymer and excipients used. (Table-4) The low value of moisture content and moisture uptake are recommended for good stability of patch, moisture content and moisture uptake was found in the range of 2.63% to 4.87 % and 4.12% to 6.84 %.(Table-5) The drug content and release found shown in (Table-6). The Response Surface plot for drug release shown in Figure-1. The drug release profiles of drug shown in Figure-2. The response surface plot for the drug release is clearly observed that the drug release was increased with increasing the concentration of HPMC and as the amount of Eudragit increased the drug release was sustained. The trial LP7 shows 81.55 % release in 12 hours, which is the maximum concentration of drug release. The diffusion studies reveled that as the concentration of HPMC increased, the rate of drug released also increase. The dermal observation of skin irritation study is shown in (Table 7). In Group I, there is a no sign of either erythema or edema after 24 hours of application, but there is very slight erythema or edema observed in some rabbit after the application of 72 hours. The primary irritation index for test was found to be 0.083 and it indicates barely perceptible irritation. According to Draize test formulation producing scores of 2 or less are considered negative (no irritation). Hence the developed transdermal formulations are free from skin irritation. The final polynomial equation of both the groups shows the effect of dependent variables on the response. Final Equation: $Y = 68.11 + 4.01\ X1 - 6.93\ X2$

The bo is the arithmetic mean of all nine trials. The value of bo was found to be 68.11. This might be because of the Eudragit RL100 has a higher alkali value, so; the ERL is less hydrophilic than HPMC. Due to the low hydrophilicity of ERL, formulations shows the higher sustaining of the drug release. The positive X1 coefficient of the both groups indicates that as the concentration of X1 (HPMC) increases; there is increase in the release of drug. The negative X2 coefficient indicates that as the concentration of X2 (Eudragit RL 100) increase, the drug release from the matrix was decrease.

CONCLUSION

The study was designed to formulate a transdermal system of LV using a polymeric matrix film. This allows one to control the overall release of the drug via an appropriate choice of polymers and their blends studied, utilizing the several diffusion pathways created due to the blend of the polymers to generate overall desired steady and sustained drug release from the patches. Thus, the molecular diffusion through polymer matrix is an effective, simple and reliable means to achieve sustained/controlled release of a variety of active agents from the transdermal therapeutic system. The results of the study give a rational guideline for formulating a sustained release transdermal therapeutic system of Lovastatin for effective therapy lowers cholesterol levels and prophylaxis of hypertension.

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Table 1: 3^2 Full Factorial Experimental Design Layout

Trials	Variable level in coded form	
	X_1	X_2
1	-1	-1
2	-1	0
3	-1	+1
4	0	-1
5	0	0
6	0	+1
7	+1	-1
8	+1	0
9	+1	+1

Table 2: Values Amount of Variables in 3^2 Factorial Design

Coded Values	Actual Values	
	X_1 = HPMC (mg)	X_2 = Eudragit RL 100 (mg)
-1	350	350
0	450	450
+1	550	550

* For X1 is HPMC and X2 is Eudragit RL 100

Table 3: Results of Thickness and Flatness

Trial	Thickness (mm)	Flatness (%)
LP1	0.14 ± 0.02	100.02 ± 0.02
LP2	0.17 ± 0.07	99.94 ± 0.06
LP3	0.19 ± 0.03	99.23 ± 0.03
LP4	0.16 ± 0.02	99.15 ± 0.05
LP5	0.20 ± 0.01	100.05 ± 0.02
LP6	0.21 ± 0.02	100.01 ± 0.73
LP7	0.19 ± 0.03	99.17 ± 1.27
LP8	0.21 ± 0.04	100.10 ± 0.29
LP9	0.23 ± 0.03	98.51 ± 0.12

*Results are the mean of triplicate observation ± SD

Table 4: Results of Folding endurance and Tensile Strength

Trials	Folding Endurance	Tensile Strength (kg/cm2)
LP1	76 ± 0.56	0.395 ± 0.024
LP2	94 ± 0.95	0.459 ± 0.031
LP3	112 ± 0.03	0.632 ± 0.011
LP4	119 ± 0.43	0.443 ± 0.083
LP5	137 ± 0.44	0.562 ± 0.014
LP6	185 ± 0.03	0.694 ± 0.094
LP7	173 ± 0.13	0.402 ± 0.055
LP8	194 ± 0.65	0.621 ± 0.046
LP9	203 ± 1.04	0.734 ± 0.081

* Results are the mean of triplicate observations ± SD

Table 5: Results of Moisture Content and Moisture Uptake

Trials	Moisture Content (%)	Moisture Uptake (%)
LP1	3.15 ± 0.11	5.32 ± 0.05
LP2	2.76 ± 0.06	4.93 ± 0.02
LP3	2.63 ± 0.01	4.12 ± 0.03
LP4	3.68 ± 0.04	6.03 ± 0.08
LP5	3.53 ± 0.10	5.58 ± 0.02
LP6	3.31 ± 0.03	4.74± 0.05
LP7	4.87 ± 0.05	6.84 ± 0.02
LP8	4.56 ± 0.02	6.01 ± 0.02
LP9	4.43 ± 0.01	5.32 ± 0.03

*Results are the mean of triplicate observations ± SD

Table 6: Results of Drug Content and Drug Release

Trials	Drug Content (%)	Drug Release (%)
LP1	99.32 ± 0.03	69.35 ± 0.21
LP2	98.29 ± 0.11	62.75 ± 0.07
LP3	100.03 ± .02	59.94 ± 0.32
LP4	97.56 ± 0.05	75.72 ± 0.82
LP5	99.03 ± 0.09	65.33 ± 1.23
LP6	99.74 ± 0.23	63.81 ± 0.91
LP7	98.32 ± 0.15	81.55 ± 0.73
LP8	99.42 ±0.05	73.28 ± 1.06
LP9	97.90 ± 0.01	61.27 ± 0.87

*Results are the mean of triplicate observations ± SD

Table 7: Dermal Observation of Skin Irritation Test

Rabbit No.	Reaction	Standard		Test	
		24 hours	72 hours	24 hours	72 hours
1	Erythema	2	3	0	0
	Edema	1	2	0	0
2	Erythema	1	2	0	0
	Edema	2	3	0	1
3	Erythema	1	2	0	0
	Edema	2	2	0	0
4	Erythema	2	3	0	1
	Edema	1	3	0	0
5	Erythema	2	3	0	0
	Edema	1	2	0	0
6	Erythema	1	3	0	0
	Edema	1	2	0	0

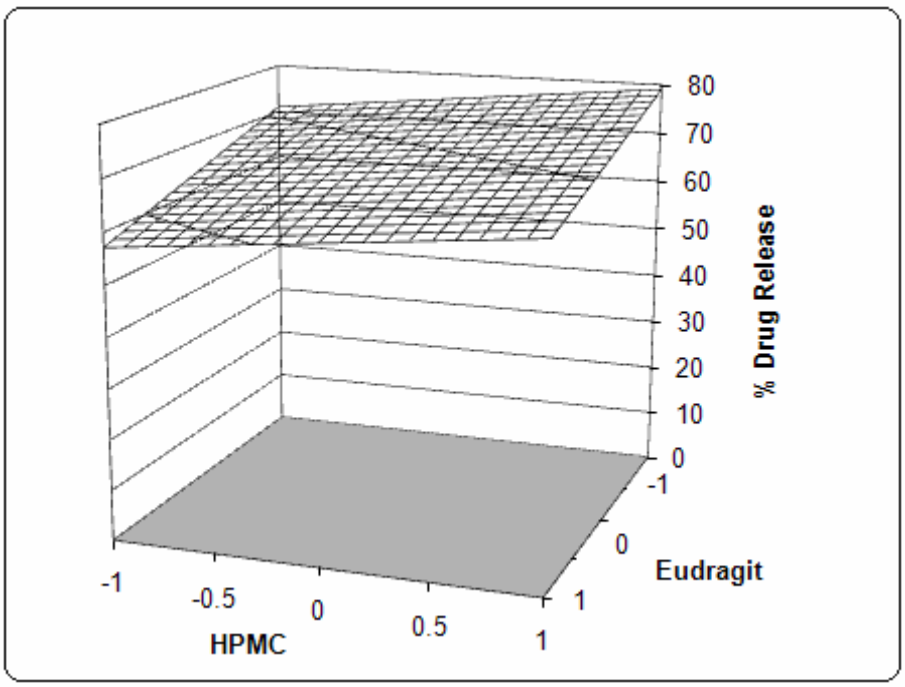

Figure 1: Surface response plot for drug release

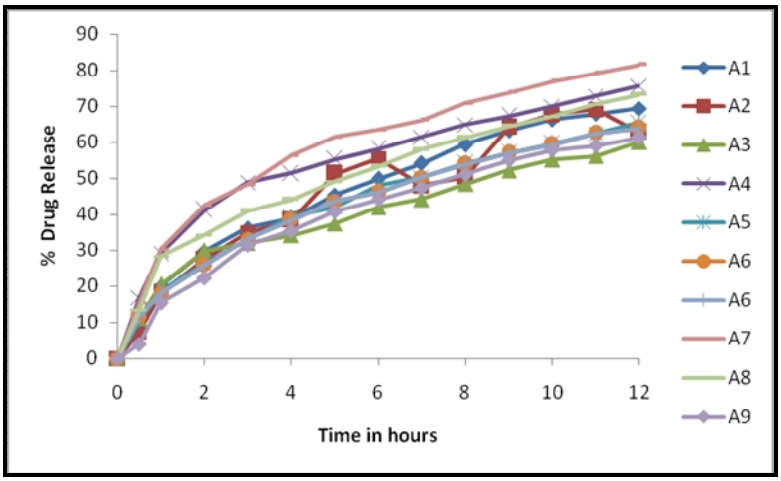

Figure 2: Drug Release Profile lovastatin patches (% of Drug Release Vs Time)

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