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Research Article

Design, Development and Evaluation of Transdermal Patches of Ramipril

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ABSTRACT

The purpose of this research work was to develop and evaluate matrix-type transdermal patches containing Ramipril with HPMC E15 and Eudragit RL100 in different ratios prepared by the solvent evaporation technique. The physicochemical compatibility of the drug and the polymers were studied by Fourier Transform Infrared (FTIR) Spectroscopy. The results suggested no physicochemical incompatibility between the drug and the polymers. The prepared transdermal patches were evaluated for weight variation, thickness, folding endurance, moisture loss, moisture absorption, *in vitro* drug release, drug release kinetics and *ex vivo* permeation studies. The diffusion studies were performed by using modified Franz diffusion cells. The best formulation, F6 shows weight variation 105.9±2.71mg, thickness 0.39±0.007 mm, folding endurance 96.2 ±4.75, moisture loss 3.51 ±0.65%, moisture absorption 8.57± 2.75% and exhibited highest 94.2±1.76% of drug release in 24 hours. The formulations F6 exhibited the highest Cumulative amount of drug permeated 4760.15±29.13 µg/cm^{2}in 24hr with flux of 51.52 µg/cm^2/hr and permeation coefficient 8.79 (cm/hr)10^{-3}.Release kinetic studies revealed that the drug release from formulation F6 followed zero order release kinetics with Non-fickian diffusion mechanism.

KEY WORDS: Solvent evaporation technique, Folding endurance, *in-vitro* drug release, *ex-vivo* permeation.

INTRODUCTION

Transdermal drug delivery systems (TDDS) are defined as self contained, discrete dosage forms which, when applied to intact skin, deliver the drug(s), through the skin, at a controlled rate to systemic circulation[1]. Transdermal delivery has many advantages over conventional modes of drug administration, as because it avoids hepatic first-pass metabolism, potentially decreases side effects and improves patient compliance [2].At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration. Transdermal drug delivery system has gained popularity over the past few decades. Thus conventional drugs in the form of tablets, capsules, injectables and ointments are introduced in the body as pulses that usually produce large fluctuations of drug concentration in the blood stream and tissues and consequently unfavorable patterns of safety and efficacy[3,4].Transdermal delivery provides an improved approach to the administration of drugs by maintaining a therapeutic constant concentration of drug in the blood for desired period of time, usually between one and seven days[5].Transdermal drug delivery enables avoidance of gastrointestinal absorption, which is associated with pitfalls of enzymatic and pH associated deactivation. This method also allows for reduced pharmacological dosing due to the shortened metabolic pathway of the

transdermal route versus the gastrointestinal pathway [6,7].The transdermal drug delivery system permits constant dosing rather than the peaks and valleys in medication level associated with orally administered medication. Patients often forget to take their medicine, and even the most faithfully compliant get tired of swallowing pills, especially if they must take several each day [8,9]

Drug like Ramipril has been selected as model drug because the drug shows promising pharmacokinetics and physicochemical properties required for novel control release dosages. Ramipril is a prodrug and is converted to the active metabolite Ramiprilat by liver esterase enzymes, is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure.Ramipril has low Molecular weight of 416.5, low bioavailability of 28-30% and half life of 13-17 hours [10]. Thus, it was considered as a potential drug for transdermal drug delivery. The objective of the present research work is to formulate and evaluate transdermal patch containing Ramipril as a drug using different ratio's of polymers to avoid hepatic first pass metabolism and to increase bioavailability and to minimize the frequent dosing of the drug.

MATERIALS AND METHODS

Materials:

Ramipril was a gift sample from Dr Reddys Laboratories, Hyderabad. HPMC E15 and Eudragit RL 100 were purchased from S.D. Fine chem. Ltd, Mumbai. Polyethylene glycol-400 was purchased from Yarrow chemicals ltd, Mumbai. Dimethyl sulphoxide was a gift sample from Central Drug House, Mumbai. All other reagents used were of analytical grade.

Pre-formulation studies:

Determination of solubility of Ramipril [11]

Solubility studies were performed according to Higuchi- Connor's method. An excess amount of ramipril was weighed into conical flasks which contain 10 ml methanol, distilled water. The samples were sonicated for 24 hrs at room temperature, there after; the samples were placed on a shaker, agitated at room temperature for 48 hrs. Subsequently, the suspensions were filtered through a whatmann filter paper. The filtrate was suitably diluted and analyzed spectrophotometrically at a wavelength of 376 nm using a spectrophotometer**.**

Drug –Excipient Compatibility study[12]

This was carried out by FTIR analysis of the pure drug (Ramipril) and polymer mixtures used in formulations to study the possible interaction between drug and polymers. The pure drug, and a mixture of drug with the polymers were mixed separately with IR grade KBr in the ratio of 100:1. The base line correction was done using dried KBr. Infrared spectra of the mixture were taken over a wave number range of 4000-400 cm^{-1}. Also the infrared spectra of the drug and polymers were run individually. Then it was investigated for any possible interaction between polymer and drug.

Formulation of transdermal patch:[13]

In the present study, drug loaded transdermal patchs of ramipril were prepared by solvent evaporation method. Weigh accurate quantity of HPMC E15 and Eudragit (polymers) and Ramipril (drug) in the beaker and mix well. Dissolve the above mixture in the solution of methanol and to this solution, a measured volume of Dimethylsulpoxide (permeation enhancer) and poly ethylene glycol (plasticizer) should be added and stirred well. This solution was allowed to for 10min without disturbing until clear solution is formed. This solution is poured slowly in the center of the Petri dish which is previously lubricated with glycerin to prevent sticking. To control the evaporation of solvent the funnel should be inverted on the Petridish and allow to evaporate in the room temperature for 24hrs The films were cut into small patches containing equivalent of 5mg of the drug per patch.

Evaluation of transdermal patches

The physicochemical evaluation of transdermal patches of Ramipril was done by using the following evaluation methods.

Weight variation[14]

The patches were subjected to weight variation by individually weighing three randomly selected patches and average weight of three patches was found such determinations were carried out for each formulation.

Thickness[15]

The thickness of transdermal patches was measured at three different places using a screw gauge and the mean values were calculated.

Drug content [16]

To determine the drug content, the patch was placed in a 100 ml volumetric flask containing pH 6.8 phosphate buffer and sonicated and the sample was filtered and analysed by UV spectrophotometer at 376 nm.

Folding endurance [17]

Folding endurance was measured manually for the prepared patches. The patches were repeatedly folded at the same place till it broke. The number of times the patches could be folded at the same place without breaking gave the value of folding endurance.

Percentage moisture loss [18]

The patches were weighed accurately and placed in a desicator containing calcium chloride at room temperature for 24hr. Then the final weight was noted when there was no further change in the weight of individual patch. The percentage of moisture loss was calculated as difference between initial and final weight with respect to final weight.

% Moisture Loss =

[Initial weight- Final weight/ Final weight] ×100

Percentage moisture absorption [19]

The patches were weighed accurately and placed in the desiccators containing 100ml of saturated solution of aluminum chloride, which maintains 84 % relative humidity. After 3 days, the patches were taken out and weighed. The percentage moisture absorption was calculated using the following formula.

% Moisture Uptake =

[Final weight- Initial weight/ initial weight] ×100

***In- vitro* drug release studies** [20]

In-vitro skin permeation studies were performed by using a modified Franz diffusion cell with a receptor compartment capacity of 25 ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated patches were cut into size of 4cm^2 and placed over the drug release membrane and the receptor compartment of the diffusion cell was filled with phosphate buffer Ph 6.8.The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm the temperature was maintained at 37 ± 0.5 ^{0}C. The samples of 2 ml were withdrawn preset time points up to 24 hrs, analyzed for drug content spectrophotometrically at 376 nm against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal.

***Ex-vivo* permeation studies**[21]

Franz diffusion cell was used for *ex-vivo* permeation studies. Excised rat abdominal skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The stratum corneum side of the skin was kept in intimate contact with the transdermal patch under the test. The receiver compartment contained 25 ml of pH 6.8 phosphate buffer, stirred with a magnetic stirrer at a speed of 50 rpm. The whole assembly was kept on a magnetic stirrer and study was conducted at 37 ± 0.5 ^{0}C. The amount of the permeated drug was determined by removing 2 ml at preset time points up to 24 hrs and replacing with an equal volume of fresh medium. The absorbance was measured at 376 nm spectrophotometrically. The cumulative amount of drug permeated was calculated and plotted against time.

Determination of permeation parameters

Flux(J)

Flux is defined as the amount of material flowing through a unit cross-sectional area in unit time. The flux (µg/cm^2/hr) of Ramipril was calculated from the slope of the plot of the cumulative amount of drug permeated per cm^2 of skin at a steady state against the time using linear regression analysis.

Permeability Coefficient (P)

Permeability coefficient is the velocity of drug passage through the membrane. The permeability coefficient (Pc) was calculated by dividing the flux by the initial drug load (C)

$$Pc = Jss/C$$

Where Jss is the steady state flux and C is the concentration of drug in the patch.

Drug Release kinetics [22]

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into Zero order, First order, Higuchi, model and Korsmeyer-Peppas release model.

Zero order release rate kinetics

To study the Zero-order release kinetics the release rate date are fitted to the following equation.

$$F = Kt$$

Where ‘F’ is the fraction of drug release, ‘K’ is the release rate constant and‘t’ is the release time.

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the date obeys Zero-order release kinetics, with a slope equal to K.

First order release rate kinetics

The release rate date are fitted to the following equation

$$Log (100-F) = kt$$

A plot of log % drug release versus time is linear.

Higuchi release model

To study the Higuchi release kinetics, the release rate data was fitted to the following equation.

$$F = K.t^{½}$$

Where, 'F' is the amount of drug release, 'K' is the release rate constant, and 't' is the release time.

When the data is plotted as accumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer- Peppas release model

$$Mt / M\infty = K.t^{n}$$

Where, $Mt / M\infty$ is the fraction of drug release, 'K' is the release constant,'t' is the release time, 'n' is the diffusional exponent for the drug release that dependent on the shape of the matrix dosage form.When the data is plotted as Log of released versus Log time, yields as straight line with a slope equal to 'n' and the 'K' can be obtained from Y–intercept.

RESULTS AND DISCUSSION

Determination of solubility

The solubility of Ramipril was determined and found to be 0.418 mg/ml in 6.8 pH phosphate buffer.

Compatibility studies:

The incompatibility between the Drug and Excipients were studied by FTIR spectroscopy. The spectral data of pure drug and various drug-excipient mixtures are presented in Fig.01-03. The results indicate that there was no chemical incompatibility between drug and excipients used in formulation

Evaluation studies of Ramipril transdermal Patches.

The ramipril transdermal patches has been developed by using with HPMC E15, Eudragit RL100 and polymeric blend (HPMC E15 and Eudragit RL100). The results of the prepared patches has been illustrated briefly as follows.

Weight Variation

Weight variation for all the formulations (F1-F6) were found to be 108.5±2.96 mg, 102.6±2.93mg, 104.8±1.45mg, 105.7±1.86mg,104.7±1.96mg and 105.9±2.71mg respectively. The results are shown in Table 02.

Thickness

In thickness variation test, thickness variation values of the formulations (F1-F6) prepared with HPMC E15, Eudragit RL 100 are 0.61±0.04mm, 0.30±0.04mm, 0.47±0.00mm,0.42±0.04mm, 0.35±0.06mm and 0.39±0.03mm. The results are shown in Tables 02**.**

Folding endurance

The folding endurance numbers of formulations (F1-F6) prepared with HPMC E15, Eudragit RL100 are 148.2±4.55, 60±4.09, 80.8±4.49, 108.5±6.21, 82.5±3.65 and 96.2± 4.73.Highest folding endurance number in case of was shown of formulation F1 due to its more hydrophilic nature. The folding endurance numbers decreased with decrease in HPMC E15 concentration. This data revealed that the patches had good strength along with good flexibility. The results are shown in the Table 02.

Estimation of drug content.

The drug content of formulations (F1-F6) has in the range of 5.76±0.03mg to 5.92±0.02mg. The results of content uniformity indicated that the drug was uniformly dispersed in all transdermal patches as evidenced by low S.D values. The results of drug content for formulations (F1-F6) are shown in Table 02.

Moisture absorption and Moisture loss study

The moisture absorption and moisture loss of formulations F1 to F6 is (15.55±2.07%, 6.08±0.52%), (6.16±1.34%, 2.03±0.39%), and (8.41±2.12%, 1.99±0.54%)(9.75±1.06%, 4.51±0.43**%)**, (7.44±1.46%, 2.70±0.61%) and (8.57±2.25**%,** 3.51±0.65**%)** respectively. The results revealed that the moisture absorption and moisture loss was found to decrease due to the different concentration of hydrophilic polymer HPMC E15and Eudragit RL100.The small moisture content in the formulations help them to remain stable. The results of moisture absorption and moisture loss studies were shown in Table 02 and Fig 4.

***In- vitro* drug release studies from Ramipril transdermal Patches**

Formulation (F6) exhibited highest 94.2±1.76% of drug release in 24 hours, which were significantly different compared to the lowest values observed with the formulations (F3) 71.7±1.61% in 24hr. In the present study it was observed that the concentrations of hydrophilic polymer (HPMC E15) increased with decreased Eudragit RL100 in this formulation, the complete drug release was found to be increased 94.2±1.76% in 24 hours. The release profiles of Ramipril from transdermal patches (Formulation F1-F6) are shown in the Fig 5.

Drug release kinetics:

The *In-vitro* drug release results of formulations (F1-F6) were fitted into various kinetic models - Zero

order, First order, Higuchi model and Peppas model. Release kinetic studies revealed that the drug release from formulation F6 followed zero order release kinetics with Non-fickian diffusion mechanism. The results are shown in the Table 03.

***Ex-vivo* permeation studies through rat abdominal skin from Ramipril transdermal patch**

The formulations F6 exhibited the highest Cumulative amount of drug permeated (µg/cm^2) 4760.15±29.13 in 24hr.The formulation F6 exhibited greatest flux of 51.52 µg/cm^2/hr respectively with permeation coefficient 8.79 (cm/hr)10^{-3}. The results are shown in Table 04.

CONCLUSION

The prepared transdermal patches were evaluated for their physiochemical characteristics such as physical appearance, weight uniformity, thickness, folding endurance; moisture content, drug content were suitable. The polymer drug interaction study did not show any incompatibility. The formulations F6 exhibited the highest Cumulative amount of drug permeated 4760.15±29.13 µg/cm^{2}in 24hr with flux of 51.52 µg/cm^2/hr and permeation coefficient 8.79 (cm/hr)10^{-3}.So the results indicates that ramipril transdermal patches can be suitable for sustain release over a period of 24 hrs for the management of hypertension.

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Table-01
Formulation chart of Ramipril Transdermal patches (F1-F06)

Name of the ingredients	Formulation code					
	F1	F2	F3	F4	F5	F6
Ramipril (mg)	50	50	50	50	50	50
HPMC E15 (mg)	500	250				
EUDRGIT RL100(mg)	-	-	500	250	-	-
HPMC E15: ERL100(mg)	-	-	-	-	150:300	300:150
DMSO (ml)	1	1	1	1	1	1
PEG400 (ml)	18	18	18	18	18	18
Methanol (ml)	5	5	5	5	5	5

Table-02
Evaluation parameters of Ramipril Transdermal patches (F1-F06)

Formulation code	F1	F2	F3	F4	F5	F6
Weight variation(mg)	108.5±2.96	102.6±2.93	104.8±1.45	105.7±1.86	104.7±1.96	105.9±2.71
Thickness(mm)	0.61±0.04	0.30±0.04	0.47±0.06	0.42±0.04	0.35±0.03	0.39±0.07
Folding endurance	148.2±4.55	60±4.09	80.8±4.49	108.5±6.21	82.5±3.65	96.2±4.73
Drug content(mg)	5.87±0.046	5.84±0.049	5.88±0.051	5.88±0.044	5.92±0.028	5.76±0.037
Moisture absorbed (%)	15.55±2.07	6.16±1.34	8.41±2.12	9.75±1.06	7.44±1.46	8.57±2.25
Moistureloss(%)	6.08±0.52	2.03±0.39	1.99±0.54	4.51±0.43	2.70±0.61	3.51±0.65

Table 03
***In vitro* drug release kinetics of Ramipril transdermal patches (F1-F6)**

Formulation code	Zero order	Higuchi	First order	Peppas	
	R^2	R^2	R^2	R^2	n
F1	0.992	0.888	0.989	0.978	0.865
F2	0.990	0.958	0.935	0.997	0.865
F3	0.994	0.968	0.950	0.987	0.865
F4	0.993	0.934	0.965	0.970	0.733
F5	0.998	0.968	0.985	0.978	0.655
F6	0.988	0.973	0.965	0.988	0.656

Table 04
Cumulative amount of Ramipril permeated per unit area from Transdermal patch F6 through rat abdominal skin. All values are presented as mean±S.D; n=3.

Time (hrs)	Cumulative amount of drug permeated (µg/cm^2)
	F6
0	0.0
1	383.96±10.34
2	561.49±15.18
3	769.29±14.78
4	986.78±25.56
5	1233.93±19.87
6	1379.29±26.67
8	1716.53±20.35
10	2091.11±17.19
12	2512.38±24.45
24	4760.15±29.13
Flux(µg/cm^2/hr)	51.52
Permeation Coefficient (cm/hr)10^{-3}	8.79

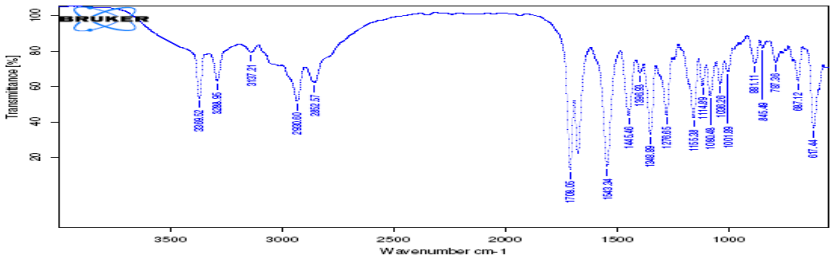

Fig 01: FTIR Spectra of HPMC E15

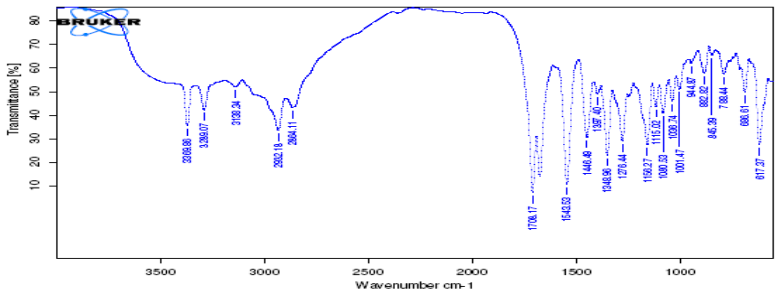

Fig 02: FTIR Spectra of Eudrgit RL100

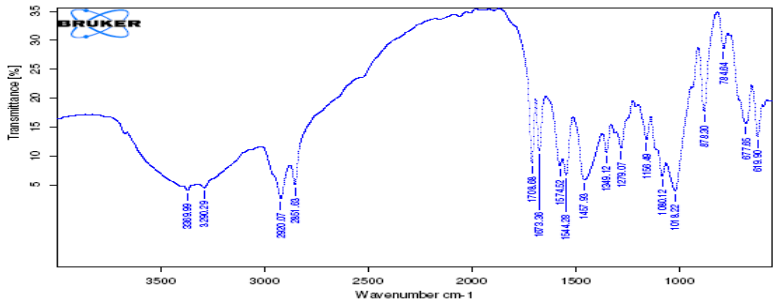

Fig 03: FTIR Spectra of Ramipril + HPMC E15 + Eudragit RL100

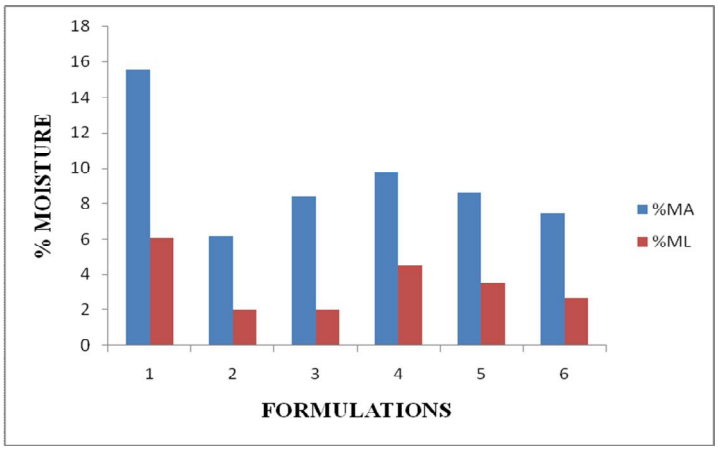

Fig.04. Moisture absorbed and Moisture loss of Ramipril transdermal patches (F1-F6). All values are presented as mean±S.D; n=3.

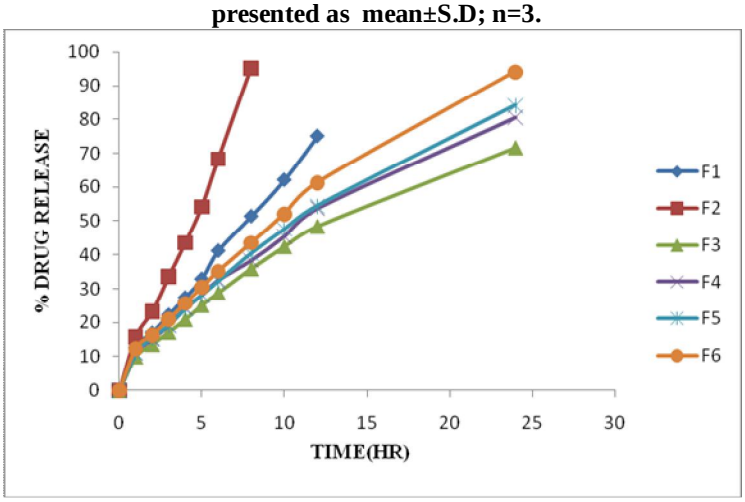

Fig 05: *In vitro* drug release profile of formulations F1 to F6

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