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Formulation and Evaluation of Transdermal Patch containing Anti-hypertensive drug Valsartan

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ABSTRACT

The Aim of the present investigation was to formulate and evaluate Valsartan transdermal drug delivery system. A 3^2 full factorial design was employed to explore the effects of HPMC K4M and Polyvinyl Pyrrolidine (independent variables) on Tensile strength, % cumulative drug release at 6 hours and % cumulative drug release at 24 hours(Dependent variables). Drug-polymer compatibility studies was determined by Fourier Transform Infrared Spectroscopy. The result of compatibility study revealed that there was no interaction between drug and polymers. Results showed drug release in the range 78.73± 0.38 - 96.32±0.33 and drug content in the range of 95.49±0.444 - 98.40 ±1.21. Moisture content and moisture uptake were increased for patches containing higher amount of HPMC K4M. Patch containing HPMC K4M in higher proportion gives increase in the drug release. It indicates that as PVP K30 increase drug release was decreased. On the basis of In-Vitro drug release performance F9 was selected as the optimized formulation. Ex-vivo drug release study carried out for optimized batch and it showed 96.32±0.33 % drug release after 24 hours. F9 shows ideal higuchi release kinetic. Skin irritation study for F9 revealed that it was free of irritation. Optimized batch F9 was found to the stable at 40 ± 0.5 °C and 75 ± 5% RH during the test period of 1month.

Keywords: Valsartan, HPMC K4M, Eudragit RL 100, Polyvinyl Pyrrolidone K30, Reservoir method, Transdermal Patch, *Ex-vivo* study, 3^2Factorial Design.

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INTRODUCTION

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Conventional oral dosage forms such as tablets and capsules provide specific drug concentration in systemic circulation without offering any control over drug fluctuation in plasma levels. Administration of drug normally distributes throughout the body and interacts not only with target cells but also with the normal healthy cells which often results in toxic effect.

Transdermal drug delivery is a technique that is used to deliver a drug into systemic circulation across the skin. This mechanism of drug delivery route has many advantages, including steady drug plasma concentration, improved patient compliance, elimination of hepatic first pass, and degradation in the gastrointestinal tract, controlled release over extended period besides providing a convenient non-invasive and easily terminable means for systemic as well as topical drug delivery.

Valsartan is an angiotensin II receptor antagonist (more commonly called an ARB, or angiotensin receptor blocker), with particularly high affinity for the type I (AT1) angiotensin receptor. The bioavailability of Valsartan following oral administration is 25% due to high hepatic first pass metabolism. When administered orally, frequent dosing is needed due to its short biological half-life (6 hours). So Transdermal patches are one of the better option. bioavailability can be increased by transdermal route. Transdermal permeability has linear dependency on partition coefficient. Drug with partition coefficient indicating an ability to dissolve in both lipid and water are favorably absorbed through the skin and would be ideal candidate for transdermal delivery and Valsartan has satisfactory log p value (3.68). [1-5,6]

Molecular weight is 435.519 g/mol which is suitable for transdermal route. Dose and Half-life are also suitable. So the Aim of the present investigation was to formulate and evaluate Valsartan transdermal drug delivery system.

MATERIALS AND METHOD

Valsartan Active Pharmaceutical Ingredient was Gift sample from Torrent Research Centre , Ahmedabad

Partition coefficient determination[7]

The partition coefficient of the drug was found out by taking 100 mg of drug in a separating funnel containing 10 ml each of N-octanol and phosphate buffer pH 7.4 and the funnel is kept aside for 24 hrs. The sample from each phase was withdrawn, filtered and diluted suitably to get the absorbance and converting it to concentration using standard curve. The partition coefficient of Valsartan was found out by following Equation.

$$\text{Partition coefficient} = \frac{\text{Concentration of drug in N} - \text{octanol}}{\text{Concentration of drug in Phosphate buffer pH 7.4}}$$

Preparation of standard curve of Valsartan in phosphate buffer pH 7.4

The stock solution (100µg/ml) of Valsartan was prepared by dissolving accurately about 10 mg of Valsartan in quantity sufficient methanol in 100 ml volumetric flasks and making up the volume with phosphate buffer pH 7.4. Working standard solutions of Valsartan was prepared from standard stock solution. The linearity of Valsartan was found to be in the concentration ranges of 02-10 µg/ml at their respective maxima. The coefficients of correlation were found. The absorbance of each solution was measured at λ_{max} 248 nm using phosphate buffer pH 7.4. The assay was performed triplicate and average absorbance was considered.

Method of preparation of Transdermal Patch[8]

Transdermal patches containing Valsartan were prepared by the reservoir method. The polymers in selected ratios were weighed and dissolved in specified solvent system. The plasticizers were added to the polymeric solution and mixed uniformly using magnetic stirrer. Finally the drug was incorporated with continuous agitation. The patches were prepared by casting the drug loaded polymeric solutions in a petridish. The casting solution was dried at room temperature for a period of 12 hours. The dried patches were packed in aluminum foil and stored in desiccators till further studies.

Dose calculation of drug for Transdermal patch

According to surface area of Petri dish dose calculate

Total Surface area of Petri dish (A) = πr^2

Radius of Petri dish = 4 cm

Total area of Petri dish = $3.14(4)^2$

= $50.24\ cm^2$

2cm*2cm ($4cm^2$) area of patch containing dose is 20 mg Valsartan

$50.24\ cm^2$ area of patch contains = 251.2 mg Valsartan

Total 251.2 mg Valsartan incorporated into the $50.24cm^2$ area of Petri dish with polymers.

Drug-Excipients Compatibility Study

Fourier Transform Infrared Spectroscopy

The compatibility study was carried out using Fourier Transform Infrared Spectroscopy (FTIR). The IR studies were carried out by the pressed pellet technique using a KBr press. Potassium bromide was taken and kept in a hot air oven for two hours for the removal of any moisture if present. The drug powder sample was mixed with dried KBr crystals and the mixture was pressed to form pellets using KBr press. The prepared pellet was placed in the sample holder and kept in the instrument to record the IR peaks. The same process is repeated with the physical mixture sample of drug and polymers and IR peaks were recorded.

Formulation of factorial design batches

Table 1 Independent and Dependent variables

Independent Variable	Dependent Variable
HPMC K4M	Tensile strength
Polyvinyl Pyrrolidone	% CDR at 6 hours
	% CDR at 24 hours

3 levels were taken for each factor.

Table 2 Formulation using 3^2 factorial design

Batch Code	Primary Layer				Secondary Layer		
	HPMC K4M(mg)	Polyvinyl Pyrrolidone K30(mg)	PEG400* (%W/W)	Methanol-Water (20 ml)	EUDRAGIT RL100	PEG400* (%W/W)	Methanol-Water (10 ml)
F1	200	75	30%	10-10	100	30%	5-5
F2	300	75	30%	10-10	100	30%	5-5
F3	400	75	30%	10-10	100	30%	5-5
F4	200	100	30%	10-10	100	30%	5-5
F5	300	100	30%	10-10	100	30%	5-5
F6	400	100	30%	10-10	100	30%	5-5
F7	200	125	30%	10-10	100	30%	5-5
F8	300	125	30%	10-10	100	30%	5-5
F9	400	125	30%	10-10	100	30%	5-5

- Added 251.2 mg Valsartan in all batches.
- Where * = % of total weight of dry polymers.

Evaluation parameters of transdermal patch[9]

1. Thickness of the patch

The thickness of the drug loaded patch was measured in different points by using a digital micrometer and determines the average thickness and standard deviation for the same to ensure the thickness of the prepare patch.

2. Weight uniformity

The prepared patches were dried at 60°c for 4hrs before testing. A specified area of patch will be cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values were calculated from the individual weights.

3. Folding endurance

A strip of specific was cut evenly and repeatedly folded at the same place till it broke. The number of times the film would be folded at the same place without breaking gave the value of the folding endurance.

4. Percentage Moisture content

The prepared films were weighed individually and be kept in a desiccators containing fuse calcium chloride at room temperature for 24 hrs. After 24 hrs the films were reweighed and determine the percentage moisture content from the below mention formula,

$$\% \textbf{ Moisture content} = \frac{\textbf{Initial weight of patch– Final weight of patch}}{\textbf{Final weight of patch}} * \textbf{100}$$

5. Percentage Moisture uptake

The weighed films were kept in desiccators at room temperature for 24 hrs containing saturate solution of potassium chloride in order to maintain 84% RH. After 24 hours the films were reweighed and determine the percentage moisture uptake from the below mention formula,

$$\% \textbf{ Moisture uptake} = \frac{\textbf{Final weight of patch} - \textbf{Initial weight of patch}}{\textbf{Initial weight of patch}} * \textbf{100}$$

6. Drug content

A specify area of patch was dissolved in a suitable solvent in specific volume. Then the solution will be filtered through a filter medium and analyses the drug content with the suitable method (UV or HPLC technique). Each value represents average of three different samples.

7. Percent elongation at break (%E)

The percent elongation was determined by noting the length just before the break point and substituting the formula,

$$\% \textbf{ Elongation} = \frac{\textbf{Final length of patch} - \textbf{Initial length of patch}}{\textbf{Initial length of patch}} * \textbf{100}$$

8. Tensile Strength

Mechanical properties of the polymeric patch were conveniently determined by measuring their tensile strength. The tensile strength of the patch was determined using handmade tensile strength instrument. Average reading of three patch were taken as the tensile strength. The patch was fixed to the assembly, the weights required to break the film was noted. Tensile strength was calculated using the following formula,

$$\textbf{Tensile Stregth} = \frac{\textbf{Force at break}}{\textbf{Initial cross sectional area of patch}(\textbf{cm2})}$$

9. *In-vitro* diffusion studies[10]

An in vitro permeation study would be carried out by using modified franz diffusion cell. The temperature of the cell was maintained at 32 ± 0.5°C using a thermostatically controlled heater. The cellophane paper was mounted between the compartments of the diffusion cell. Sample volume of definite volume was removed from the receptor compartment at regular intervals, and an equal volume of fresh medium is to be replaced. Samples were to be filtered through filtering medium and would be analyzed spectrophotometrically.

10. *Ex-vivo* diffusion studies[11]

The *Ex-vivo* diffusion study was carried out with the abdominal rate skin using Franz diffusion cell. The cylinder consists of two chambers, the donor and the receptor compartment. The donor compartment opens at the top and was exposed to atmosphere. The temperature was maintained at 37 ± 0.5^{o}C and receptor compartment was provided with sampling port. The diffusion medium used will be phosphate buffer (pH 7.4). The amount of the permeated drug was determined by removing 3 ml at preset time points up to 24 hrs and replenishing with an equal volume of fresh medium. The samples were filtered and the absorbance was measured.

11. Skin Irritation studies[12-13]

Transdermal patch should be free from skin irritation and it is also an ideal property of transdermal drug delivery system. So, designed transdermal patch should not irritate the skin and hence skin irritation study was performed. The skin irritation test was performed on six healthy albino rabbits weighing between 1.8 to 2.2 kg. Aqueous solution of formalin 0.8% was used as standard irritant. Drug free polymeric patches were used as test patches. 0.8% of formalin is applied on the left dorsal surface of each rabbit, whereas the test patch was placed on identical site, on the right dorsal surface of the rabbit.

Matrices were applied to the shaved skin on the back of 4 albino rabbits and secured using adhesive tape. On one side of back, a control patch (without any drug) and on another side an experimental patch were secured. The animals were observed for any sign of erythema or edema for a period of 7 days. The patches were removed after a period of 24 hours with the help of alcohol swab. The skin was examined for erythema/edema. Erythema and edema observed using Draize scoring method.

Optimization of formulation using 3^2 full factorial designs

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions. The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Yi) is measured for each trial. Where Y is the dependent variable, b0 is the arithmetic mean response of the nine runs and

bi is the estimated coefficient for the factor Xi. The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2) show how the response changes when two factors are simultaneously changed. Coefficients with one factor represents the effect of that particular factor while the coefficients with more than one factor and those with second order terms represents the interactions between those factors and the quadric nature of the phenomena respectively. Positive sign in the front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factor.

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2}$$

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The factors were selected based on preliminary study. The concentration of Eudragit RL 100 (X1) and concentration of HPMC K15M (X2) were selected as independent variables for transdermal patch. Folding endurance, drug content and Drug release in pH 7.4 phosphate buffer were selected as dependent Variables.

Kinetic modeling of drug release

The release data obtained from various batches were studied with respect to effect of polymer concentrations. To analyze the mechanism of drug release from the formulation, the diffusion profile of all the batches were fitted to zero order, first-order, Higuchi and Korsemeyer-Peppas models to ascertain the kinetic modeling of drug release.

To analyze the mechanism of drug release from the patches, the release data were fitted to the following equations:

Zero Order

In many of the modified release dosage form particularly controlled or sustained release dosage form (those dosage forms that release the drug in planned, predictable and slower than normal manner) is zero order kinetics.

$$Q = K_o t$$

Where, Q is the amount of drug release at time, t and K_o is the release rate constant.

First Order

Most conventional dosage form exhibits this dissolution mechanism some modified release preparations, particularly prolonged release formulation adhere to this type of dissolution pattern.

$$\text{Log } Q = K_1 t$$

Where, Q is the percent of drug release at time, t and K_1 is the release rate constant.

Higuchi Equation

A Large number of modified release dosage form contain some sort of matrix system was such instances the drug dissolves from this matrix. The dissolution pattern of the drug was dictated by water penetration rate (diffusion control) and thus the following relationship applies.

$$Q = K_2t/2$$

Where, Q is the percentage of drug release at time t andK_2 is the diffusion rate constant.

Peppas Equation

$$Q = Ktn$$

Where, Q is the percent of drug release at time, t and K is the diffusion rate constant and n is diffusional exponent. If n is equal to one the release is zero order. If n is equal to 0.5 the release was best explained by fickian diffusion and if $0.5 < n < 1$ then the release was through anomalous diffusion or case II diffusion. In this model a plot of drug released versus log time was linear.

Stability Study

Stability of a drug has been defined as the stability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic an toxicological specifications throughout its shelf life.

Long Term testing: 25ºC ± 2ºC / 60% RH ± 5% for 12 months

Accelerated Testing: 40ºC ± 2ºC / 75% RH ± 5% for 6 months

Stability studies were carried out at 40ºC / 75% RH for the selected formulation for 1 month.

Method

The selected formulation was wrapped in aluminium foil. It was then stored at 40ºC / 75% RH for 1 month in humidity chamber and evaluated for their Physical Appearance and Flexibility, Thickness, Weight Variation, %drug content and *in-vitro* diffusion study.

RESULTS AND DISCUSSION

In the present study different formulations with variable concentration of polymers were prepared and evaluated for physicochemical parameters, *In-vitro* diffusion studies, Ex-*vivo* diffusion studies, Skin irritation studies and Stability studies.

Partition Coefficient Determination

The partition coefficient of the drug in Octanol/Phosphate buffer pH 7.4 systems was found to be 3.68. The result indicates that drug posses sufficient lipophilicity which meets the requirement of formulating it into a transdermal patch.

Standard Calibration Curve of Valsartan in Phosphate buffer pH 7.4

The linear regression analysis was done on Absorbance data points. The result for standard curve in phosphate buffer pH 7.4 is given.

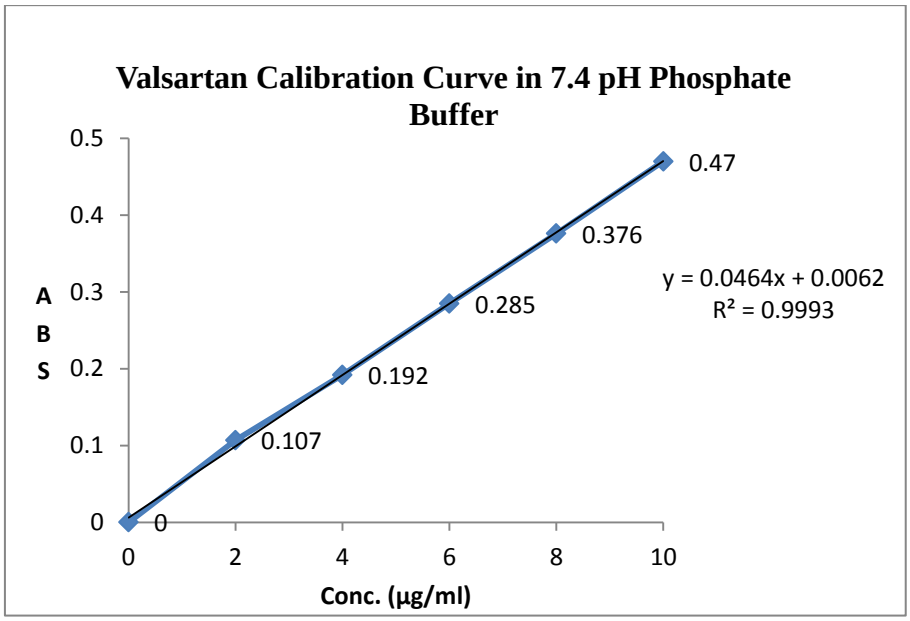

Figure 1 Standard Calibration Curve of Valsartan in phosphate buffer pH 7.4

Drug-Excipients Compatibility study by FTIR

The FTIR spectra of drug and drug+excipients were recorded and analyzed. For investigation of any chemical interaction between added excipients and Valsartan in the formulated products, the FTIR of pure Valsartan and Valsartan + HPMC K4M +PVP K30+ Eudragit RL 100 were recorded. It was observed however, that all the characteristic peaks observed for both Valsartan and Valsartan + HPMC K4M +PVP K30+ Eudragit RL 100 remained unchanged. This observation ruled out the possibility of chemical interaction between the drug and added excipient during solvent evaporation technique to form the patch.

(A) Valsartan

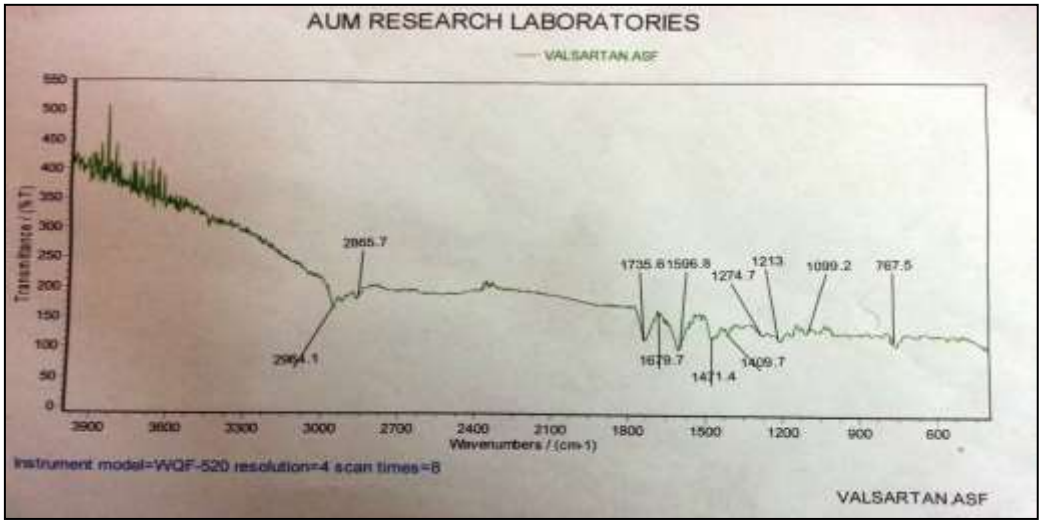

Figure 2 FTIR spectra of Valsartan

(B) Valsartan + Eudragit RL 100+ HPMC K15M

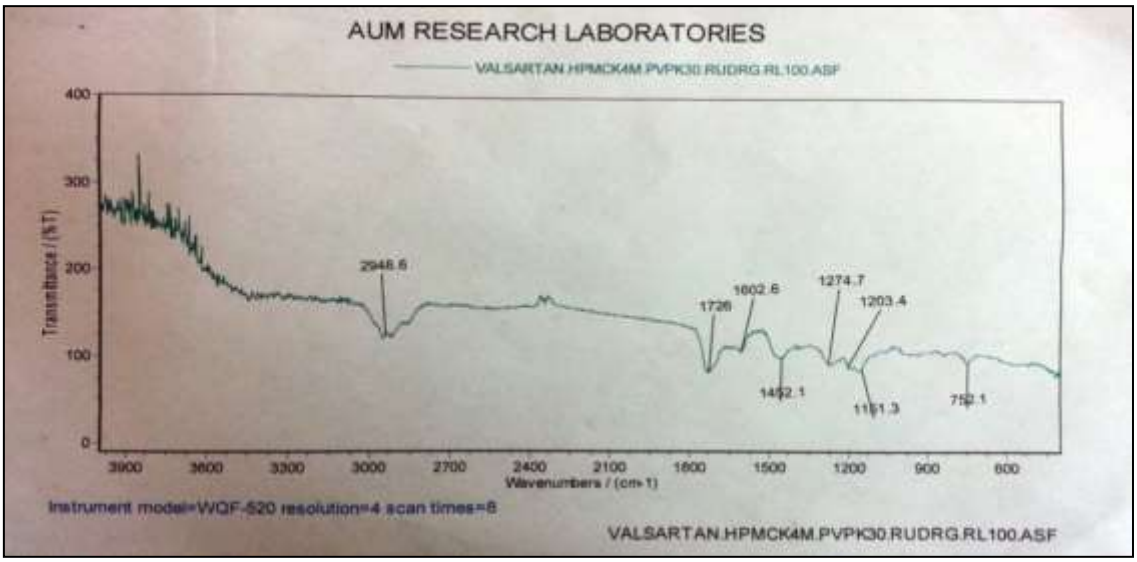

Figure 3 FTIR spectra of Valsartan + Eudragit RL 100 + HPMC K4M + PVPK30

Table 3 Different functional peaks in FTIR

Sr. No.	Functional Group	Valsartan Standard IR peak	Valsartan Peak	Valsartan+ Polymers Peak
1	-CH aromatic	2963 cm-1	2964.1 cm-1	2948.6 cm-1
2	O=C-	1600 cm-1	1596.8 cm-1	1602.6 cm-1
3	C=N	1729 cm-1	1735.6 cm-1	1726 cm-1

There was no significant difference in characteristic peak at wave numbers of the drug in presence of the excipients. So it can be concluded that there is no interaction Between Drug and polymers under study.

EVALUATION OF FACTORIAL DESIGN BATCHES

Transparency, Weight uniformity, Thickness, % moisture content and % moisture uptake

The different batches of patch were evaluated for Transparency, Weight uniformity, Thickness, % moisture content and % moisture uptake.

Table 4 Different batches of patches were evaluated for Transparency, Weight uniformity, Thickness, % moisture content and % moisture uptake

Batch code	Transparency	Wt Uniformity for 4 cm2 Patch(mg)*	Thickness (mm)*	%Moisture content*	% Moisture uptake*
F1	Transparent	0.225±0.001	0.122±0.002	2.31±0.005	4.12±0.011
F2	Transparent	0.239±0.004	0.137±0.001	2.39±0.011	4.32±0.01
F3	Transparent	0.252±0.0005	0.143±0.001	2.44±0.005	4.60±0.015
`F4	Transparent	0.234±0.002	0.135±0.001	2.37±0.005	4.59±0.01
F5	Transparent	0.248±0.001	0.143±0.001	2.41±0.005	4.79±0.011
F6	Transparent	0.261±0.001	0.149±0.001	2.60±0.015	5.46±0.015
F7	Transparent	0.239±0.001	0.138±0.001	2.58±0.01	5.02±0.001
F8	Transparent	0.260±0.001	0.150±0.001	2.65±0.011	5.89±0.021
F9	Transparent	0.273±0.001	0.154±0.0005	2.80±0.015	6.23±0.001

*Values expressed as Mean ± SD, n=3

The results suggested of weight variation and thickness suggested that the patch have sufficient weight and thickness so it would maintain its integrity when applied to the skin.

The highest percentage of moisture content was found as 2.80% and the highest moisture uptake was found as 6.23 % because of one hydrophilic polymer which allows polymeric network to absorb maximum moisture.

Tensile strength and Drug Content

The tensile strength was found by using tensiometer and the % drug content was found by UV visible spectrophotometer.

Table 5 Different batches of patch were evaluated for Tensile strength and Drug Content parameters

Batch code	Tensile Strength	% Drug content*
F1	0.371±0.0005	95.58±0.577
F2	0.390±0.0010	96.91±0.508
F3	0.393±0.000	95.49±0.444
F4	0.397±0.0011	97.36±0.259
F5	0.425±0.0015	95.80±0.167
F6	0.453±0.0015	96.77±0.386
F7	0.428±0.0015	98.40 ±1.21
F8	0.463±0.015	98.15±0.835
F9	0.506±0.0021	98.12±0.827

* Values expressed as Mean ± SD, n=3

The maximum tensile strength was found as 0.506 kg/cm^2 which mainly depend on the concentration of the polymer. In general the % elongation and tensile strength were increases with increases in the concentration of Eudragit RL 100.

The maximum drug content was found as 98.40%.

***In-vitro* diffusion study**

The *in-vitro* diffusion study was carried out using modified franz diffusion cell. The Cellophane paper was used as semi permeable membrane. Phosphate buffer pH 7.4 was used as diffusion media.

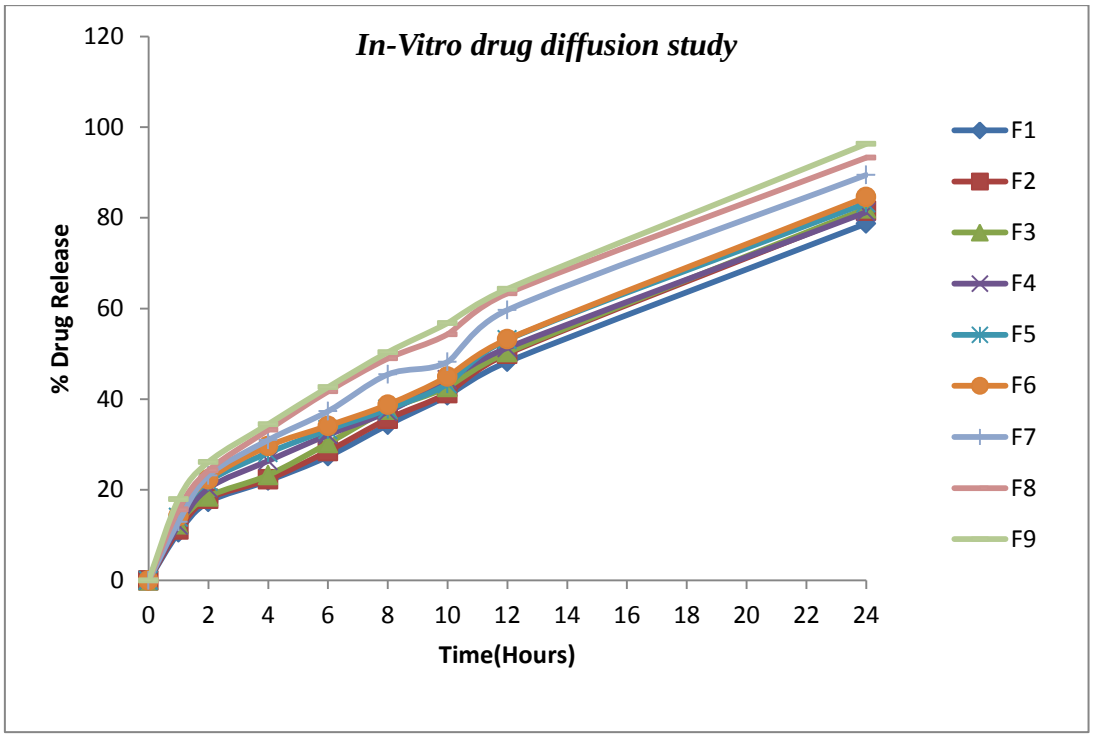

Figure 4 *In-vitro* diffusion studies for formulation F1 to F9

The purpose of this study was to investigate the *In-vitro* release studies of all formulations made of polymers such as For primary layer HPMCK4M and PVP K30 and Secondary layer Eudragit RL 100. The *in-vitro* diffusion of drug from the patches was carried out for 24 hours and showing drug release of 82.05%, 81.57%, 78.73%, 84.56%, 83.26%, 81.25%, 96.32%, 93.28%, 89.46%, for F1, F2, F3, F4, F5, F6, F7, F8, F9 respectively at the end of 24 hours. The results of *in-vitro* permeation studies of Valsartan from transdermal patches are shown in Figure 29. Batch F7 shows maximum release after 24 hours.

Response surface analysis for Optimization of final batch

Table 6 Design summary and response data

Std.	Run.	Factor 1 A: HPMC K4M (mg)	Factor 2 B: PVP K30 (mg)	Response 1 Tensile strength	Response 2 % CDR at 6 hours	Response 3 % CDR at 24 hours
9	2	300	100	0.425	33.21	93.26
8	1	300	125	0.463	41.69	93.28
7	8	300	75	0.39	28.54	81.57
6	9	400	100	0.453	34.12	84.56
5	7	200	100	0.397	32.11	81.25
4	3	400	125	0.506	42.63	96.32
3	6	200	125	0.428	37.33	89.46
2	5	400	75	0.393	30.25	82.05
1	4	200	75	0.371	27.36	78.73

Table 7 Results of ANOVA study

Response model	F value	P value	R^2 value	Adequate precision
Y1	374.21	0.0001	0.9956	55.167
Y2	73.16	0.0001	0.9606	21.166
Y3	271.48	0.0004	0.9978	44.473

Response model for Tensile Strength (Y1)

The Model F-value of 374.21 for tensile strength implied the model was significant. Values of "Prob > F" was 0.0001 which was less than 0.05 indicated model terms were significant. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 374.21 indicated an adequate signal. This model could be used to nevigate the design space.

Polynomial equation

Tensile Strength: +0.43+0.026 *A+ 0.041* B + 0.014 *AB

Table 8 Result of Analysis of Variance for Tensile Strength

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.43	1	1.205E-003	0.42	0.43	
A-HPMCK4M	0.026	1	1.476E-003	0.022	0.030	1.00
B-PVPK30	0.041	1	1.476E-003	0.037	0.044	1.00
AB	0.014	1	1.808E-003	9.352E-003	0.019	1.00

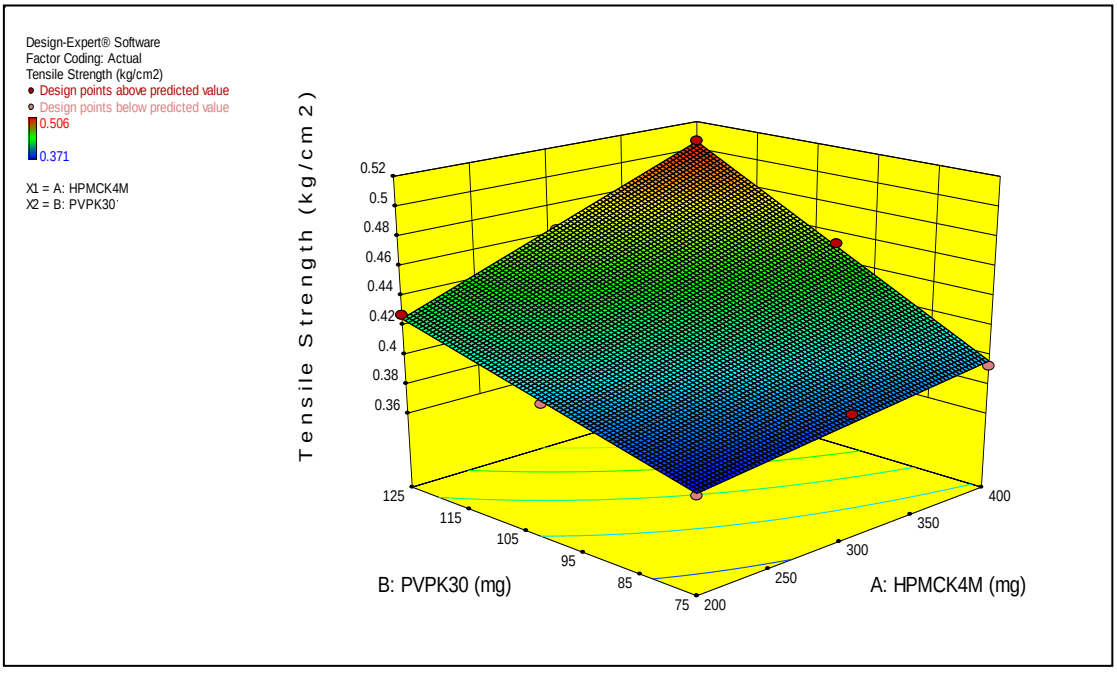

Figure 5: 3D surface plot for Tensile Strength

Discussion

In above counter plot and Response surface plot, its clearly state that both the independent factors HPMC K4M affect the dependent factor tensile strength. In counter plot different

colors shades were given to differentiate the tensile strength. As moving to the red shade the tensile strength was increases, and as moving towards blue shade tensile strength decreases.

Response model for % Drug release at 6 hours (Y2)

The Model F-value of 73.16 for % Drug release at 6 hours implied the model was significant. Values of "Prob > F" was 0.0001 which was less than 0.05 indicated model terms were significant. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 73.16 indicated an adequate signal. This model could be used to nevigate the design space.

Polynomial equation

% Drug release at 6 hours: +34.14+1.70*A+5.90*B

Table 9 Result of Analysis of Variance for % Drug release at 6 hours

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	34.14	1	0.42	33.12	35.15	
A-HPMCK4M	1.70	1	0.51	0.45	2.95	1.00
B-PVPK30	5.92	1	0.51	4.67	7.16	1.00

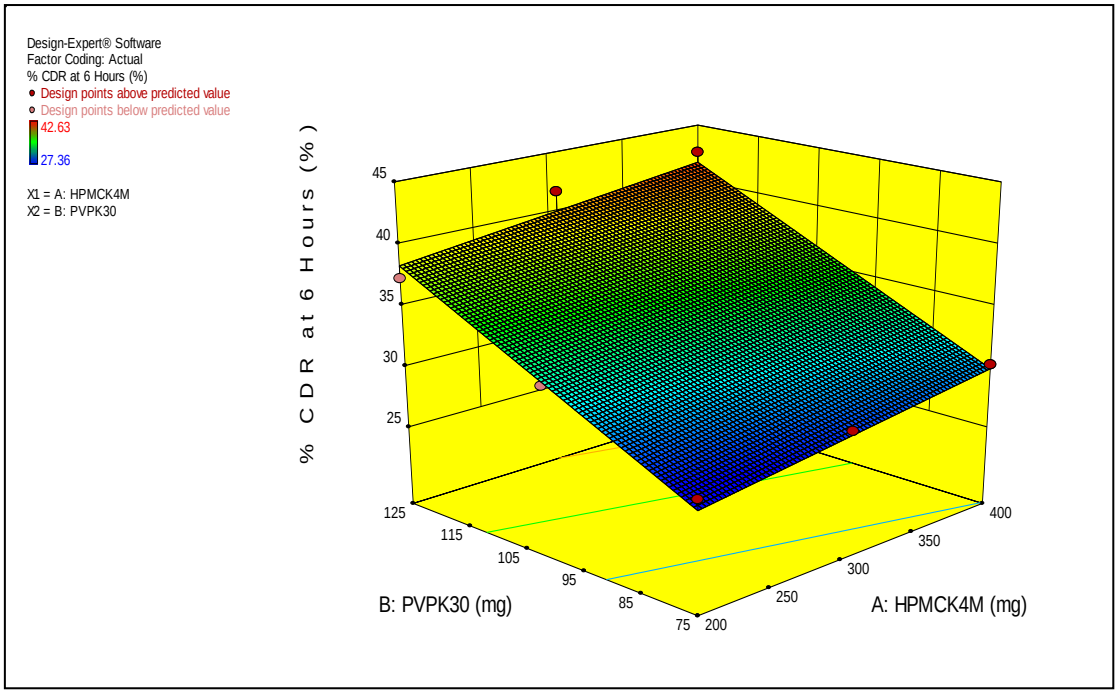

Figure 6: 3D surface plot for % Drug release at 6 hours

Discussion

In above counter plot and Response surface plot, its clearly state that both the independent factors HPMC K4M affect the dependent factor tensile % Drug release at 6 hours**.** In counter plot different colors shades were given to differentiate the % Drug release at 6 hours**.**. As moving to the red shade the % Drug release at 6 hours was increases, and as moving towards blue shade % Drug release at 6 hours decreases.

Response model for % Drug release at 24 hours (Y3)

The Model F-value of 271.48 for % Drug release at 24 hours implied the model was significant. Values of "Prob > F" was 0.0004 which was less than 0.05 indicated model terms were significant. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 271.48 indicated an adequate signal. This model could be used to nevigate the design space.

Polynomial equation: +86.72+2.25*A+6.12*B

% Drug release at 24 hours:

Table 10 Result of Analysis of Variance for % Drug release at 24 hours

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	86.72	1	1.20	83.79	89.65	
A-HPMCK4M	2.25	1	1.47	-1.34	5.83	1.00
B-PVPK30	6.12	1	1.47	2.53	9.70	1.00

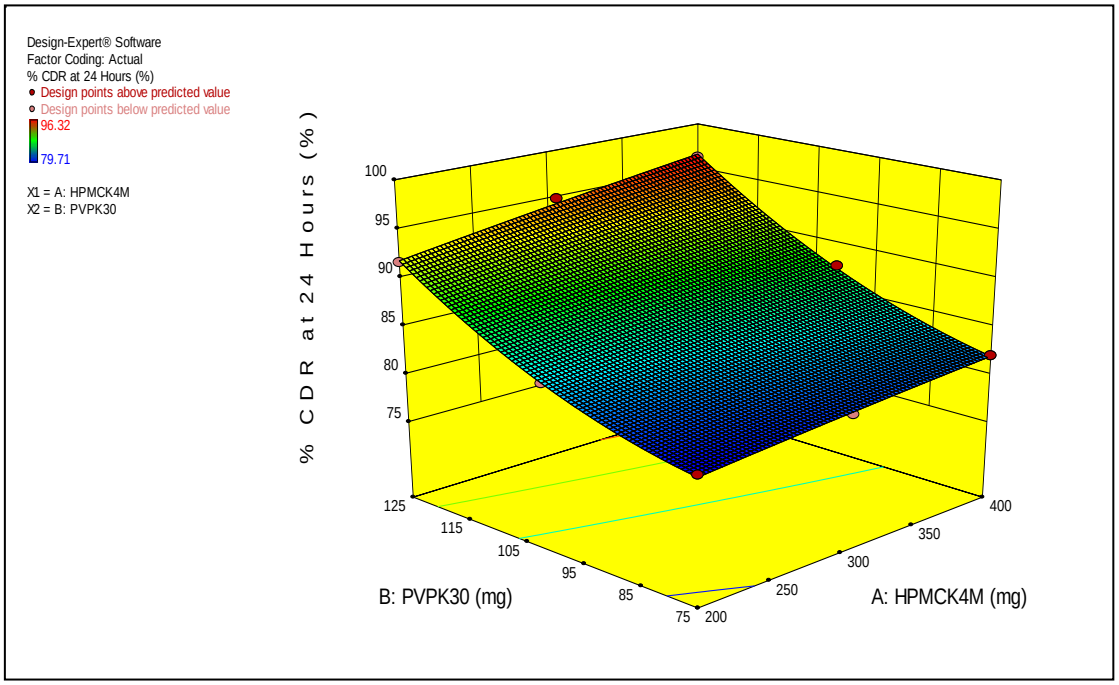

Figure 7 3D surface plot for % Drug release at 24 hours

In above counter plot and Response surface plot, it's clearly state that both the independent factors HPMC K4M affect the dependent factor tensile % Drug release at 24 hours**.** In counter plot different colors shades were given to differentiate the % Drug release at 6 hours**..** As moving to the red shade the % Drug release at 24 hours was increases, and as moving towards blue shade % Drug release at 24 hours decreases.

Optimization of Formula

After generating the polynomial equations relating the dependent and independent variables, Valsartan loaded transdermal patches were optimized for the responses Tensile Strength

(Y1), % Drug release at 6 hours (Y2) and % Drug release at 24 hours (Y3). The desirable ranges of these responses were restricted to $0.371 \leq Y1 \leq 0.506$ kg/cm^2, $27.36\% \leq Y2 \leq 47.63$ % and $78.73\ \% \leq Y2 \leq 96.32$ %. The optimum values of the variables were obtained by graphical and numerical analyses using the Design Expert software which are on criterion of desirability. The optimized formula was achieved with 150 mg of Eudragit RL 100 and 250 mg of HPMC K15M concentration. Therefore, to verify the evolved models, the optimum formulation was prepared according the above values of the factors and evaluated for Tensile Strength (Y1), % Drug release at 6 hours (Y2) and % Drug release at 24 hours (Y3). As shown in table, it was demonstrating that the observed value of a new batch was quite closer to predicted value.

Formulation of Check pint batch (F10) of Transdermal Patch

Table 11 Formulation of Check point batch (F10) of Transdermal Patch

Layers	Ingredients	Quantity
Primary Layer	Valsartan	251.2 mg
	HPMC K4M	400 mg
	PVPK30	125 mg
	Polyethylene Glycol 400	30% w/w
	Methanol:Water	10:10 ml
Secondary Layer	Eudragit RL 100	100 mg
	Polyethylene Glycol 400	30%
	Methanol:Water9	5:5 ml

Comparison of Experimental and predicted values of Check point batch

After formulating a check point batch F10, the batch was evaluated for Tensile Strength (Y1), % Drug release at 6 hours (Y2) and % Drug release at 24 hours (Y3).

Table 12 Comparative levels of predicted and observed responses for optimized Valsartan loaded transdermal patch

Responses	Response(Predicted)	Response(Observed)	Predicted error a(%)
Y1	0.505	0.471	-6.73
Y2	41.75	42.39	1.53
Y3	96.65	97.09	0.45

Where, a Predicted error (%) = (observed value-predicted value)/predicted value×100 %.

Kinetic analysis

The release kinetics was determined by using mathematical models of first order, zero order kinetic, Higuchi's square root model, Hixon-crowel model and Korsemeyer Peppas model.

Table 13 Kinetics Treatment of Permeation Profile of Transdermal Patches Containing Valsartan

Optimized Batch	Zero order	First order	Highchi model	Korsemeyer-Peppas		Hixson-Crowell	Best fit model
	R^2	R^2	R^2	R^2	n	R^2	
F7	0.933	0.623	0.993	0.634	0.663	0.729	Higuchi Model

***Ex-vivo* diffusion study**

The purpose of this study was to investigate the *Ex-vivo* release studies of optimized batch F9. After 24 hours the 91.25% drug permeated through rat abdominal skin.

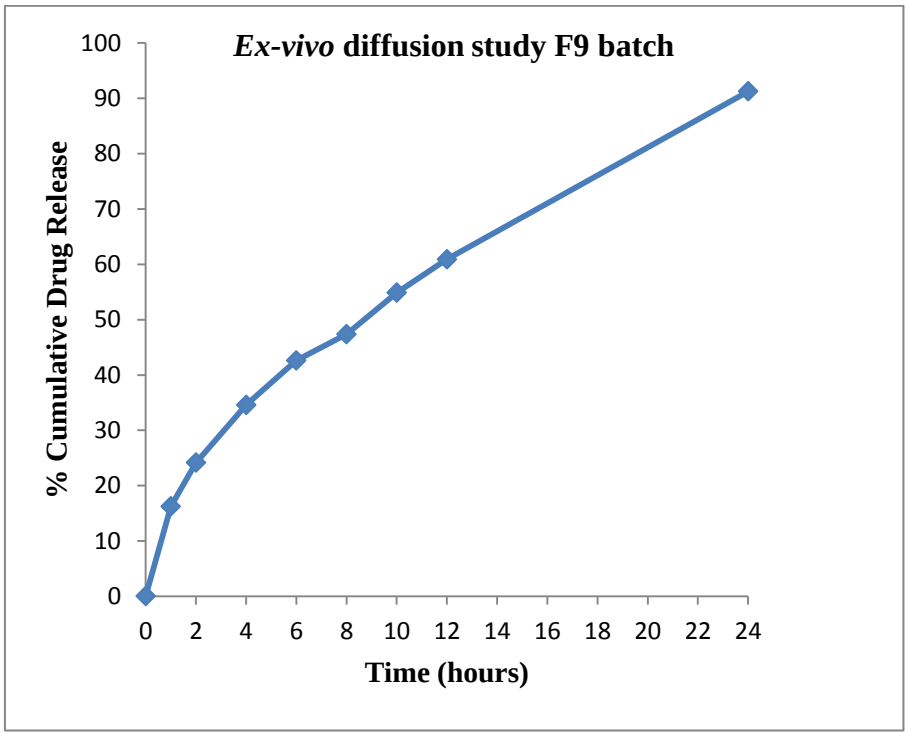

Figure 8 *Ex-vivo* diffusion study for Optimized batch of patch (F9)

Skin Irritation Study

Skin irritation test of the transdermal formulation F9 showed a skin irritation score (erythema and edema) of less than 2. According to Draize et al, compound producing score of 2 or less are considered negative (no skin irritation). Hence the developed transdermal formulation is free of skin irritation.

Table 14 Skin Irritation study

Rabbit No.	Control		Formulation (F1)	
	Erythema	Edema	Erythema	Edema
1	0	0	0	0
2	0	0	0	1
3	0	0	0	0
4	0	0	1	0
5	0	0	0	0
6	0	0	0	0

Stability Study

Formulations showing optimum %CDR (F9) was selected for stability studies. Selected formulation (F9) was stored at 40ºC ± 2 º C temperatures and 75 ± 5 % relative humidity (RH) for a period of 1 month. Formulations were evaluated at periodical intervals of 15 days for physical appearance, drug content and % CDR.

Table 15 Stability Study

Batch Code	Parameter	Storage (40ºC ± 2ºC temp. and 75 ± 5 % RH) 0 days	15 days	30 days
F9	Physical Appearance and Flexibility	Transparent and flexible	Transparent and flexible	Transparent and flexible
	Thickness*	0.154±0.0005	0.153±0.01	0.152±0.01
	Weight Variation*	0.273±0.001	0.272±0.02	0.272±0.02
	%Drug Content*	98.12±0.827	98.01±0.38	97.90±0.32
	% CDR at 24 Hours*	96.32±0.33	96.30±0.25	96.28±0.26

*All values are the mean of three readings

CONCLUSION

The present study was aimed at preparation of transdermal patch of Valsartan to show its prolonged release. The highest drug release was found 96.33±0.33% in 24 hours in formulation F9 which contains 400 mg HPMC K4M, 125 mg Polyvinyl Pyrrolidone K30 for primary layer and 100 mg Eudragit RL 100 for secondary layer. The drug permeation profile was also found to follow Higuchi model kinetics. Hence this combination of polymers can be successfully manipulated to attain desired efficacy of Valsartan with minimum fluctuations in plasma levels. The formulation F9 was optimized and Ex-*vivo* drug release study carried out for optimized batch F9 using rat abdominal skin. It showed satisfactory drug release 94.98±0.47 after 24 hours. Skin irritation study was also carried out on rabbit, it revealed that it was free of irritation.

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