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Formulation And Evaluation of Transdermal Patches of Metformin Hydrochloride Using HPMC K100 As Release Retarding Agent

Gopu Vijaya Sindhuri[1], Keerthi Annavarapu[2]

[1]Associate Professor, Department of Pharmaceutics, St. Mary's college of Pharmacy, Secunderabad
[2]Assistant Professor, Department of Pharmaceutics, St Mary's College of Pharmacy, Secunderabad

Abstract:
The present study was designed to develop a suitable matrix type transdermal drug delivery systems of Metformin HCL using two different polymeric combinations, E RS100 and HPMC E 15; E RL 100 with HPMC E 15. E RL100 and E RS 100 are acrylic acid matrices which have been used to make drug-polymer matrix films for transdermal delivery systems which are reported as compatible with many drugs. Penetration enhancers that alter the partitioning can be useful to enhance the drug permeation (6). In this study various penetration enhancers D-Limonene (4), Oleic acid (5) and were used in different concentrations to determine their effect on permeation of drug.

Keywords: Transdermal Patches, Permeation Enhancer, Metformin HCL, Therapeutic Activity.

I. Introduction:

Controlled drug delivery systems have been developed which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of drug to a tissue (1). Controlled drug delivery or modified drug delivery systems are conveniently divided into four categories.

1. Delayed release
2. Sustained release
3. Site-specific targeting
4. Receptor targeting

More precisely, Controlled delivery can be defined as (2): -

1. Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.
2. Localized drug action by spatial placement of a controlled release system adjacent to or in the diseased tissue.
3. Targeted drug action by using carriers or chemical derivatives to deliver drug to a particular target cell type.
4. Provide a physiologically/therapeutically based drug release system. In other words, the amount and the rate of drug release are determined by the physiological/ therapeutic needs of the body.

A controlled drug delivery system is usually designed to deliver the drug at particular rate. Safe and effective blood levels are maintained for a period as long as the system continues to deliver the drug. Controlled drug delivery usually results in substantially constant blood levels of the active ingredient as

compared to the uncontrolled fluctuations observed when multiple doses of quick releasing conventional dosage forms are administered to a patient. At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to first pass metabolism and the tendency to produce rapid blood level spikes (both high and low), leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient.

To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site specific), spatial and temporal placement within the body thereby reducing both the size and number of doses. New drug delivery system is also essential for the delivery of novel, genetically engineered pharmaceuticals (i.e. peptides, proteins) to their site of action, without incurring significant immunogenicity or biological inactivation. Apart from these advantages the pharmaceutical companies recognize the possibility of re-patenting successful drugs by applying the concepts and techniques of controlled drug delivery system coupled with the increased expense in bringing new drug moiety to the market. One of the methods most often utilized has been transdermal delivery i.e. transport of therapeutic substances through the skin for systemic effect (3).

II. Materials & Methods

Metformin HCL	:	Chandra Labs, Hyderabad.
HPMC E15	:	S.S. Pharma, Warangal.
Eudragit RS 100	:	Degussa, Germany
Eudragit RL 100	:	Degussa, Germany
D-limonene	:	Himedia Laboratories Pvt. Ltd., Mumbai
Ethanol	:	Nizam deccan sugars Ltd., Nizamabad
Dichloromethane AR	:	Merck Ltd., Mumbai
Methanol AR	:	Merck Ltd., Mumbai
Propylene glycol	:	Qualigens Fine Chemicals., Mumbai
Calcium chloride	:	Finar chemicals limited., Ahmedabad
Aluminium chloride	:	Finar chemicals limited., Ahmedabad
Dialysis Membrane	:	Himedia Laboratories Pvt. Ltd., Mumbai
Potassium dihydrogen ortho phosphate	:	Finar chemicals limited., Ahmedabad
Sodium hydroxide	:	Finar chemicals limited., Ahmedabad

Preparation of Phosphate buffer pH 7.4

To 6.8gm of potassium dihydrogen ortho phosphate and 1.564gm of sodium hydroxide, sufficient water is added to get 1000 ml of water and the pH was adjusted to 7.4 with ortho phosphoric acid or sodium hydroxide if necessary.

3.1. Construction of standard graph of Metformin HCL

3.1.2. Construction of standard graph of Metformin HCL in phosphate buffer pH 7.4.

The calibration curve is obtained by dissolving 100 mg of Metformin HCL in 100 ml of pH 7.4 phosphate buffer. From this stock-I solution 10ml solution was taken and made up to 100 ml with pH 7.4 phosphate buffer and this was stock- II. From stock-II 0.2, 0.4, 0.6, 0.8, 1.0 ml was taken made up to 10ml with pH

7.4 phosphate buffer this gave concentration 2, 4, 6, 8, 10 µg/ml. Absorbance was measured spectrophotometrically at 233 nm against pH 7.4 phosphate buffer as blank.

3.2. Preparation of Metformin HCL Transdermal Patches

Matrix type transdermal patches containing Metformin HCL were prepared by solvent evaporation technique, using different ratios of HPMC E 15, ERL100 (F1 to F5) and HPMC E 15, ERS100 (F6 to F10). The polymers were weighed in requisite ratios by keeping the total polymer weight 10.50g and allowed for swelling for about 6 hrs in solvent mixture (1:1 ratio of di- chloromethane, methanol). 15%v/w propylene glycol was incorporated as plasticizer. Then the drug solution was added to the polymeric solution, casted on to anumbra Petri plate of surface area about 69.42sq.cm, allowed for air drying overnight followed by vacuum drying for 8-10 hr. The entire sheet was cut into small patches with an area of 10cm2 i.e. with 2X5cm. About 7 patches were obtained from each sheet. Six formulations (C1 to C3 and D1 to D3) composed of HPMC E15 and ERL 100 in 5:1 ratio with two penetration enhancers d-limonene, oleic acid in three different concentrations 4%, 8% and 12% v/w were prepared. All formulations carried 15% v/w polyethylene glycol as plasticizer.

Table 1. Composition of Metformin HCL transdermal patches

Formulation code	Drug (g)	HPMC E15 (g)	ERL 100 (g)	ERS 100 (g)
F1	1.75	1.75	8.75	-
F2	1.75	3.50	7.00	-
F3	1.75	5.25	5.25	-
F4	1.75	7.00	3.50	-
F5	1.75	8.75	1.75	-
F6	1.75	3.50	-	7.00
F7	1.75	7.00	-	3.50
F8	1.75	5.25	-	5.25
F9	1.75	8.75	-	1.75
F10	1.75	1.75	-	8.75

15% v/w propylene glycol was used as plasticizer
Each patch (10 cm^2) contains 250mg of Metformin HCL

Table 2. Composition of transdermal patches with penetration enhancers

Formulation code	D-limonene (%)	Oleic acid (%)
C1	4	-
C2	8	-
C3	12	-
D1	-	4
D2	-	8
D3	-	12

3.3. Characterization of Metformin HCL Transdermal Patches [24]

3.3.1. Physicochemical properties

The Patches prepared by general procedure were evaluated for the following properties

Thickness

The thickness of the film was measured at ten different points on one film using screw gauge. For each formulation three selected Patches were used and average thickness was recorded.

Weight variation

Six Patches from each batch of an area of 10 cm^2 were weighed individually and the average weight was calculated.

Folding endurance

Folding endurance of the patch was determined manually by repeatedly folding a small strip of the medicated patch at the same place until broke. The number of times the strip could be folded at the same place without breaking gave the folding endurance number.

Estimation of drug content in polymeric Patches

The formulated polymeric patches were assayed for drug content in each case. Three polymeric patches from each formulation were assayed for content of drug.

Procedure

Patches from each formulation were taken, cut into small pieces and was allowed to dissolve in a 100 ml solution containing 50 ml of methanol and 50 ml of dichloromethane. The solution was diluted suitably and the absorbance of the solution was measured using UV-Vis spectrophotometer at a wavelength of 233 nm against methanol dichloromethane mixture (1:1) as blank.

Moisture Absorption Studies

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

$$\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Moisture Content Determination

The patches were weighed accurately and placed in a desicator containing calcium chloride at 40°C 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.

$$\% \text{ Moisture Content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Measurement of Mechanical Properties

Mechanical properties of the Patches were evaluated using a microprocessor based advanced force gauze (Ultra Test, Mecmesin, UK) equipped with a 25 kg load cell. Film strip with dimensions 60 x 10 mm and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. During measurement, the top clamp at a rate of 2mm/s pulled the strips to a distance till the film broke. The force and elongation were measured when the film broke. The mechanical properties were

calculated according to the following formulae. Measurements were run in four replicates for each formulation [32].

$$\text{Tensile strength (kg. mm}^{-2}\text{)} = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample (mm}^2\text{)}}$$

$$\text{Elongation at break (\% mm}^{-2}\text{)} = \frac{\text{Increase in length (mm)}}{\text{Original length (mm)}} \times \frac{100}{\text{Cross sectional area}}$$

$$\text{Elastic Modulus} = \frac{\text{Force at corresponding strain (kg)}}{\text{Cross-sectional area (mm2)}} \times \frac{1}{\text{Corresponding Strain}}$$

$$\text{Strain} = \frac{\text{Tensile strength}}{\text{Elastic modulus}}$$

The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength (TS) and elastic modulus (EM) and elongation at break (E/B). A soft and weak polymer is characterized by a low TS, EM and E/B; a hard and brittle polymer is defined by a moderate TS, high EM and low E/B; a soft and tough polymer is characterized by a moderate TS, low EM and high E/B; where as a hard and tough polymer is characterized by a high TS, EM and E/B. Another parameter strain has been used as an indicator of the overall mechanical quality of the film. A high strain value indicates that the film is strong and elastic. Hence, it is suggested that a suitable transdermal film should have a relatively high TS, E/B and strain but low EM.

3.3.2. *In vitro* Release Studies

The drug release studies from Metformin HCL transdermal patches were performed using Franz diffusion cell. The drug containing patches was kept between donor and receptor compartments, separated from these compartments by gelatin membrane. The receptor compartment containing diffusion medium was stirred with magnetic bead operated by magnetic stirrer, to prevent the formation of concentrated drug solution layer below the dialysis membrane. 3ml of sample was collected from the receptor compartment at appropriate time intervals and replaced with phosphate buffer pH 7.4. Analysis was carried out using UV-Visible spectrophotometer at 233nm against phosphate buffer pH 7.4 as reference.

Preparation of Rat Abdominal Skin

The male albino rats weighing 150-200gm were sacrificed using anaesthetic ether. The hair of test animals was carefully trimmed short (<2mm) with a trimmer taking extreme precaution not to damage the skin and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique, which involved soaking the entire abdominal skin in water at 60oC for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water, dried in a desiccator, wrapped in aluminium foil and stored at 4±1°C. At the time of use, the epidermis was rehydrated by immersion in water for 1hr at room temperature [33].

3.3.3. *Ex vivo* Permeation Studies

Franz diffusion cell with a surface area of 4.15cm2 was used for *ex vivo* permeation studies. The rat 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 release surface of the TDDS under test. A dialysis membrane was placed over the skin, so as to secure the patch tightly dislodged from the skin. The receiptor phase is 24ml of phosphate buffer saline (PBS) pH 7.4 stirred at 500rpm on a magnetic stirrer. The amount of drug permeated was determined by removing 3ml of sample at appropriate time intervals upto 24 hr, the volume was replenished with an equal volume of pH 7.4 buffer. The absorbance was measured at 233nm spectrophotometrically.

Cumulative amounts of drug permeated in µg/cm2 were calculated and plotted against time. Drug flux (µg/hr/cm2) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface (10cm2).

3.3.4. Drug-Excipient Compatibility study

This was carried out by FTIR analysis of pure drug (Metformin HCL), pure polymers (HPMC E 15, ERL 100 and ERS 100) and their physical mixtures as used in formulations to study the possible interaction between drug and polymers.

IV. Results and Discussion

Construction of standard graph of Metformin HCL

The standard graphs of Metformin HCL in pH 7.4 phosphate buffer constructed and shown in **Fig. 1**. The standard graphs of Metformin HCL in pH 7.4 buffer have shown good linearity over a concentration range of 2 to 10µg/ml with R^2 of 0.9992 respectively.

Development of Metformin HCL Transdermal Patches

Patches were formulated with E RS 100, E RL 100 and HPMC E15 (**Table 1**). Many experiments were performed by varying the concentrations of polymer. The experiment was initiated by taking 7g of polymer and as the polymer concentration increased the patch could accommodate more amount of Metformin HCL. Precipitation of the drug was predominant with 7g of polymer and as the polymer concentration was increased to 10.5g, the precipitation decreased. No precipitation was observed with 10.5g of the polymer and the Patches were flexible. Therefore, the polymer amount taken was 10.5g.

In addition, experiments were conducted to know optimal concentration of plasticizer to be used in all kind of Patches. Plasticizer at concentration of 5%v/w of film former was insufficient to form Patches. Plasticizer concentration at 5-10% v/w yielded hard and inflexible Patches. Further, increasing the concentration of plasticizer above 20% v/w resulted in enormous increase in drying time. Therefore, patches were prepared using 15%v/w of plasticizer and the prepared patches were soft and flexible but not brittle.

Patches were also formulated with penetration enhancers d-limonene, oleic acid in different concentrations (**Table 2**).

Table 3. Standard graph of Metformin HCL in pH 7.4 phosphate buffer

Concentration	Absorbance
0	0
2	0.1563
4	0.2901
6	0.4199
8	0.5801
10	0.7184

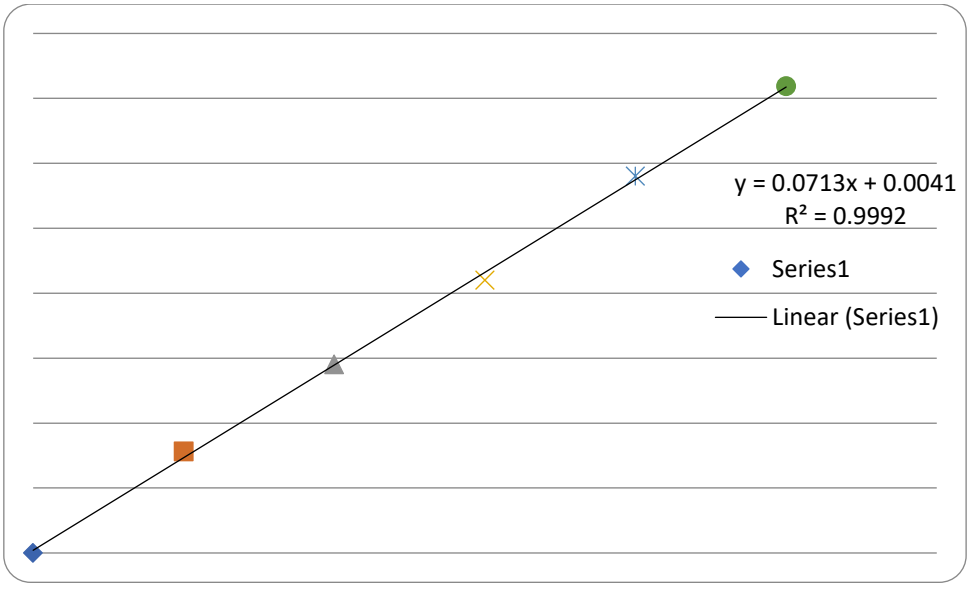


Fig 1. Standard graph of Metformin HCL in pH 7.4 phosphate buffer

Characterization of Metformin HCL Transdermal Patches

Physicochemical properties

The Patches prepared by general procedure were evaluated for the following properties:

Weight Variation Test:

The results of weight variation test for various transdermal Patches were shown in **Table 4 & 5.** Results of weight variation test indicated uniformity in weight of patches, as evidenced by SD values, which were less than 2.0 for all formulations. In formulations F1 to F10 the weight of the patches decreased with decrease in HPMC E15 concentration .The order of weight of patches is F8>F10>F4>F9>F7>F5>F3>F6>F1>F2 the weights of the patches are almost in the same range.

Thickness Variation Test:

The results of thickness variation test for various transdermal Patches were shown in **Table 4 & 5**.In thickness variation test, the thickness was found to be uniform. The thickness increased with increase in HPMC E15 concentration in A and B series formulations (order of thickness in A series (F4>F3>F5>F2>F1and B series F6>F9>F7>F10>F8). The SD values were less than 2 for all formulations, an indication of more uniform patches.

Folding endurance number:

The folding endurance numbers of formulations are presented in the **Tables 4& 5** .patches did not show any cracks even after folding for more than 80 times.ERS 100 containing patches has in the range of 40 to 90, ERL 100 containing patches has in the range of 18 to 85and for the formulations prepared with penetration enhancers has in the range of 70 to 105. The folding endurance number gives the mechanical property of the patches, high folding endurance number indicate that has high mechanical property. The folding endurance number was increased with increasing HPMC E15 content. These results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

Estimation of drug content in polymeric Patches:

The results of drug content for various transdermal Patches were shown in **Table 6 & 7**.The results of content uniformity indicated that the drug was uniformly dispersed in all transdermal patches as evidenced by low SD values. The drug content is ranged from 242 to 250.3mg per 10 cm^2 patch area .The drug

content analysis of the prepared formulations had shown that the process shown employed to prepare patches in the study was capable of giving patches with a uniform drug content and minimum batch variability.

Moisture Absorption and moisture Content study

The results of moisture content and moisture absorption studies were shown in **Table 6 & 7** and **Fig 2& 3**. The moisture content in the patches was ranged from 3.21 to 5.3% and 3.3 to 5.63% (for formulation A-series and B-series respectively). The moisture absorption in the formulations is ranged from 3.18 to 9.63% and 5.85 to 10.1% (for formulation A-series and B-series respectively). The results revealed that the moisture absorption and moisture content was found to increase with increasing the concentration of hydrophilic polymer (HPMC E15). The small moisture content in the formulations help them to remain stable and from being a completely dried and brittle film.

Table 4. Weights, thickness and folding endurance of Metformin HCL transdermal patches

Formulation	Weight (mg)	Thickness (mm)	Folding endurance
F1	1750.2±0.17	0.38±0.25	85±7.64
F2	1758.2±0.61	0.39±2.05	72.5±1.05
F3	1760.1±1.23	0.42±0.45	76.31±3.83
F4	1751.2±0.27	0.43±0.42	56.16±5.04
F5	**1753.2±0.84**	**0.42±0.29**	**58.33±2.58**
F6	1748.2±0.82	0.48±0.14	90±8.91
F7	1753.3±0.96	0.45±2.17	80.83±2.15
F8	1751.1±0.54	0.39±0.19	83.5±5.95
F9	1746.3±1.67	0.46±1.63	64.5±3.90
F10	1747.3±0.28	0.42±1.23	69.67±3.46

Table 5. Weight, thickness and folding endurance of Metformin HCL transdermal patches with penetration enhancers

Formulation code	Weight (mg)	Thickness (mm)	Folding endurance
C1	75.15±0.15	0.34±0.71	105.1±1.20
C2	76.5±1.53	0.36±0.42	88.21±0.78
C3	**75.06±0.84**	**0.33±0.41**	**75.25±2.92**
D1	75.43±0.94	0.36±0.70	85.25±0.56
D2	76.65±0.69	0.34±1.35	92.05±1.38
D3	**75.25±0.44**	**0.37±0.24**	**78.75±1.6**

Table 6. Drug content, % Moisture absorbed and % Moisture content of Metformin HCL transdermal patches, mean ± S.D (n=3)

Formulation	Drug content (mg)	% Moisture content
F1	246.35±0.64	5.3±0.24
F2	238.08±0.56	4.38±0.46

F3	246.72±0.55	4.03±0.88
F4	237.1±0.95	3.21±0.80
F5	**249.3±0.07**	**3.98±0.60**
F6	247.3±0.86	3.3±0.52
F7	248.17±0.29	4.88±0.57
F8	247.6±0.03	5.63±0.45
F9	239.01±0.06	4.9±0.66
F10	248.93±0.64	3.95±0.05

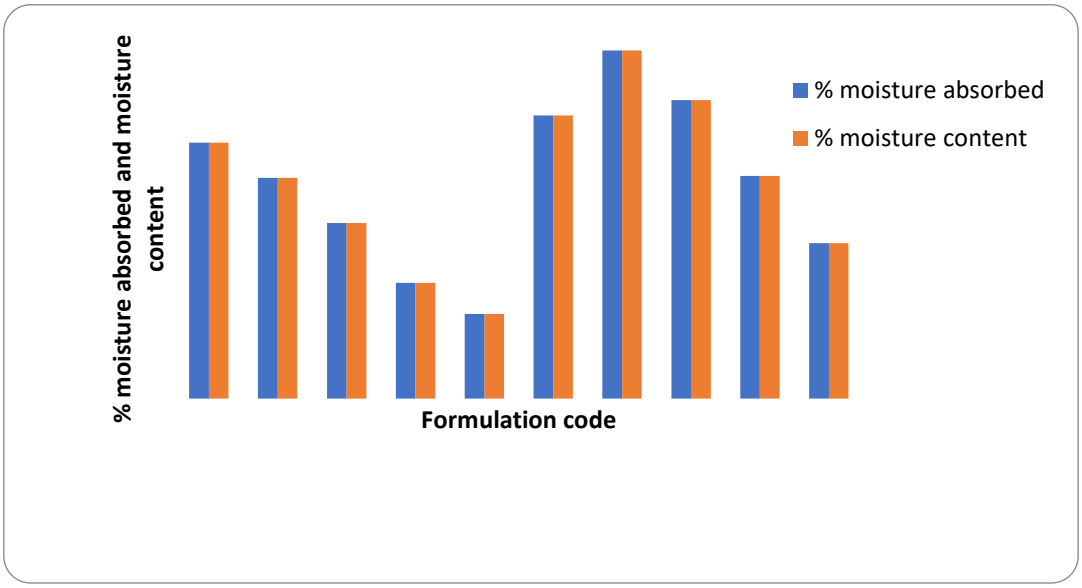


Fig 2. % Moisture absorbed and Moisture content of Metformin HCL transdermal patches, mean ± S.D (n=3)

Table 7. Drug content, % Moisture absorbed and % Moisture content of Metformin HCL transdermal patches with penetration enhancers, mean ± S.D (n=3)

Formulation code	Drug content (mg)	% moisture absorbed	% moisture content
C1	240.28±0.82	8.5±0.16	5.6±0.75
C2	239.05±1.05	13.5±1.95	7.55±0.22
C3	**249.5±0.84**	**8.25±1.47**	**4.22±1.22**
D1	236.25±0.68	6.6±2.85	5.45±1.08
D2	238.7±1.07	6.75±3.36	5.82±0.68
D3	**244.25±0.88**	**8.25±1.25**	**3.85±1.22**

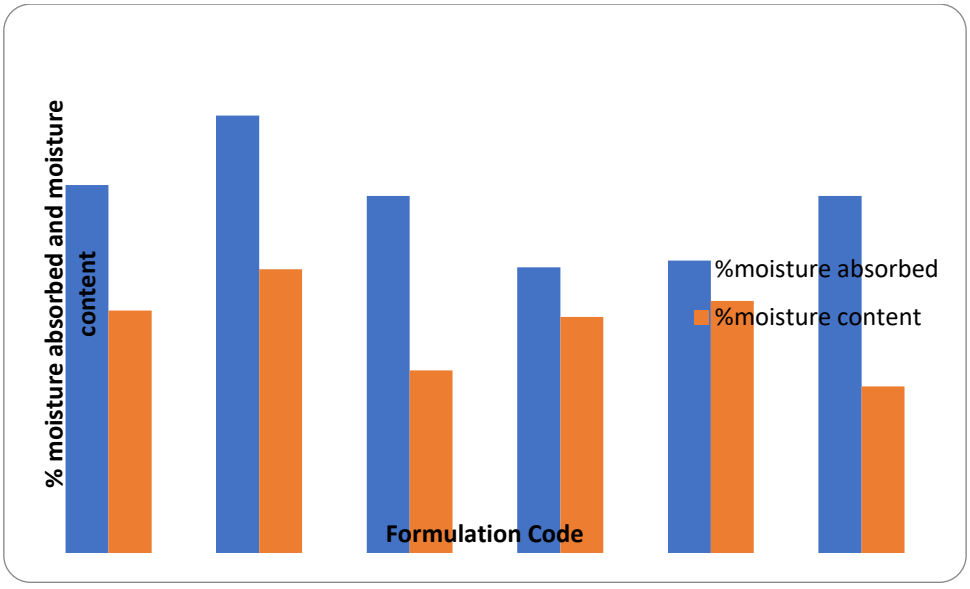


Fig 3. % Moisture absorbed and Moisture content of Metformin HCL transdermal patches with penetration enhancers, mean ± S.D (n=3)

Table 8. Mechanical properties of optimized formulations

Formulation code	Tensile strength(kg/m^2)	Elongation at break (%mm^{-2})	Elastic modulus (kg/mm^2)	Strain
F5	1.02±0.26	65.92±2.02	2.68±0.38	0.46±0.023
C3	1.09±0.31	69.7±1.06	2.09±0.41	0.52±0.018
D3	1.06±0.11	72.16±1.89	2.84±0.50	0.49±0.037

***In vitro* Drug Release Studies from Transdermal Patches**

The patch formulated with HPMC alone showed 87% of drug within 8 hrs and followed first order kinetics. This means that the patch was not suitable for the release of drug for 24 hrs to get a prolonged release of drug, copolymer that decreases the drug release rate is needed to be added. Therefore, rate controlling polymers ERL 100 and ERS100 were cast with the aim to achieve controlled release of drug.

The cumulative amount of drug released from A and B series patches are shown in the **Tables 9 and 10**.The results indicate that there was increase in the amount of drug release with an increase in HPMCE 15.There is an increase of drug release from F1 to F5 (F5> F4>F3>F2>F1) and F6 to F10 (F9>F10>F8>F6>F7).

The release profiles of Metformin HCL from transdermal patches are shown in the **Fig 4 & 5.**

Formulations F5 and F9 exhibited greatest (71.08±0.41 and 68.06±0.41 respectively) percentage of drug release values which are significantly different compared to the lowest values observed with the formulations containing ERL 100 and ERS 100 (36.07±1.98 % and 35.25±0.62 respectively). In the present study it was observed that as the concentrations of hydrophilic polymer (HPMC) increased in the formulations, the drug release rate increased substantially.

Table 9. Cumulative percent release of Metformin HCL from transdermal patches

Time (hrs)	Cumulative % of drug released, mean ± S.D (n=3)				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	6.08±0.06	4.55±2.67	6.17±1.30	5.7±1.88	6.44±0.62
2	9.92±1.09	5.95±0.02	10.75±1.03	8.13±1.09	7.29±1.08
3	10.88±1.78	9.08±0.17	13.2±1.70	12.42±0.56	9.52±0.37
4	15.5±0.43	13.83±1.19	18.02±2.50	14.06±1.09	13.71±1.21
5	19.83±0.10	17.06±1.08	22.08±1.3	19.05±1.99	16.05±1.05
6	21.16±0.80	19.12±1.60	28.05±0.62	24.38±1.80	20.12±1.96
8	24.3±0.56	23.83±0.85	33.6±1.38	28.33±1.16	34.93±1.39
10	26.33±1.08	32.16±1.31	38.4±1.05	38.5±0.30	43.53±1.38
12	29.16±0.43	36.22±1.90	41.08±3.52	44.83±1.39	56.03±0.30
24	36.07±1.98	43.65±1.07	53.16±0.80	59.15±1.03	**71.08±0.41**

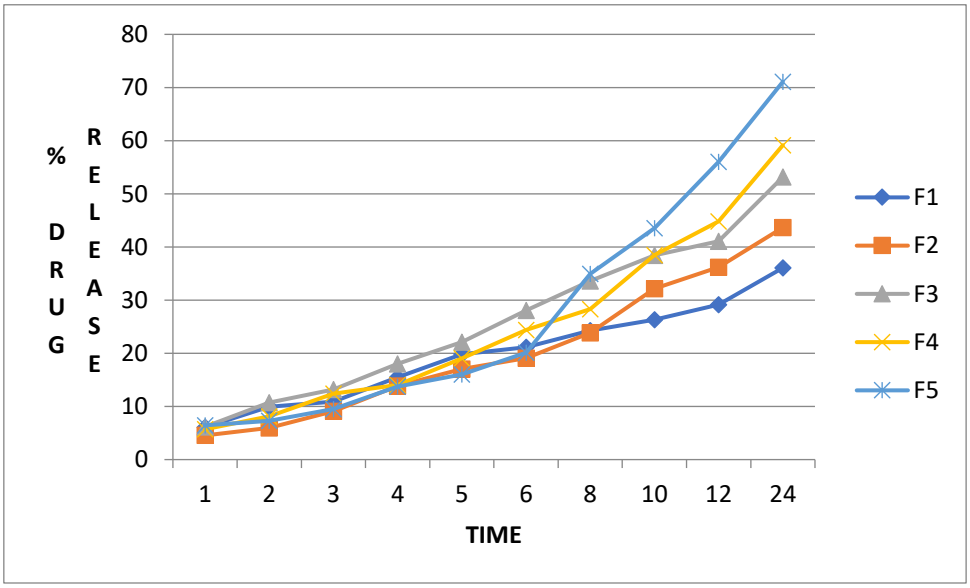


Fig 4. Cumulative percent release of Metformin HCL from transdermal patches F1-F5

Table 10. Cumulative percent release of Metformin HCL from transdermal patches F6-F10

Time (hrs)	Cumulative % of drug released, mean ± S.D (n=3)				
	F6	F7	F8	F9	F10
0	0	0	0	0	0
1	4.92±1.43	3.91±0.61	5.75±1.61	5.34±0.68	6.5±1.80
2	6.72±1.74	5.25±1.08	8.1.2±1.30	9.5±1.18	9.8±1.42
3	9.63±1.50	6.6±1.14	11.5±2.61	15.5±1.07	11.72±0.63
4	11.92±2.49	7.13±0.45	12.5±1.41	18.01±0.21	16.88±0.32
5	13.83±2.54	10.43±1.67	16.36±2.67	20.05±1.65	18.16±3.70
6	18.5±1.36	13.22±1.40	19.08±1.43	23.2±1.56	23.25±0.46
8	22.58±0.57	17.5±0.87	25.91±3.55	31.13±1.30	29.91±0.21

10	28.71±1.22	22.45±1.05	36.25±1.11	42.53±1.32	33.91±0.29
12	33.16±0.5	26.6±4.10	41.67±1.92	50.03±0.30	41.88±1.92
24	44.25±0.41	35.25±0.62	52.12±0.74	**68.06±0.41**	57.08±1.87

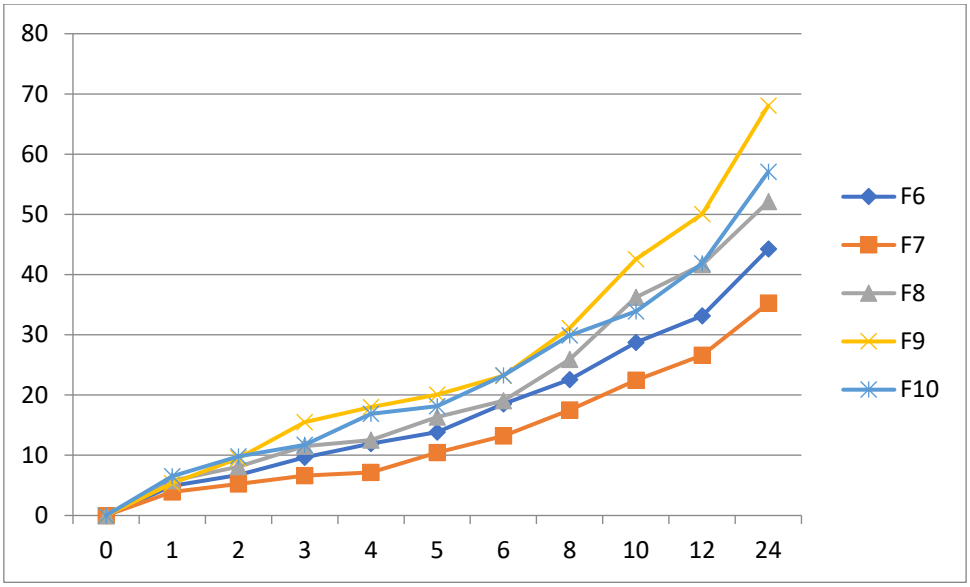


Fig 5. Cumulative percent release of Metformin HCL from transdermal patches F6-F10

***Ex vivo* permeation studies through rat abdominal skin from transdermal patches**

The results of *ex vivo* skin permeation of Metformin HCL from patches are shown in **Fig 6 & 7**. The formulations (area of 10 cm2) F5 and F9 exhibited the greatest cumulative amounts of drug permeation, which were significantly different compared to the lowest values observed with the formulations containing ERL100 (F1) and ERS100 (F7) in 24hr (**Table 11 & 12**).

As the proportion of HPMC increased in all the formulations, increased drug release and permeation in both series were observed. Initial rapid dissolution of the hydrophilic polymer occurs when the patch is in contact with the hydrated skin, resulting in the accumulation of high amounts of drug in the skin surface and thus leading to saturation of the skin with drug molecules at all times.

The flux obtained with formulation F5 was found to be maximum. But with these formulations the required flux was not obtained. Literature study gave an idea of using permeation enhancers to improve the drug permeation of formulations as they help in the permeation of drug through the skin. Oleic acid and d-limonene were used as permeation enhancers .

The results of *ex vivo* skin permeation of Metformin HCL from patches prepared with penetration enhancers were shown in **Fig 6, 7.**

The formulations C3 (containing 12% D-Limonene), D3 (containing 12% oleic acid) exhibited greatest cumulative amounts of drug permeation (**Table 13, 14**) . These formulations exhibited the required flux.

The profiles of *ex vivo* skin permeation of Metformin HCL from patches prepared with penetration enhancers were shown in **Fig 6, 7.**

The *ex vivo* permeation results of optimized formulations F5, C3 and D3 were fitted into various kinetic models (zero order, first order and higuchi model and Peppas model). The R^2 values of first order plots (0.876, 0.946 and 0.935) were greater than the R^2 values of first order plots (0.845, 0.073 and 0.889) and the R^2 values of Higuchi (0.956, 0.966 and 0.941) plots were greater than zero and first order models . The

R^2 values reveal that the permeation of Metformin HCL from the transdermal Patches followed first order and diffusion rate controlled mechanism.

According to peppas model, a value of slope (n) between 0.45 and 1 indicates an anomalous behavior (Non-Fickian). The 'n'values of formulations F5, C3 and D3 are 0.822, 0.746 and 0.731 respectively. So, it indicates that the release mechanism from the optimized formulations follows Non-Fickian diffusion (anomalous behavior).

The results of drug permeation from transdermal patches of Metformin HCL through the rat abdominal skin confirmed that Metformin HCL was released from the formulation and permeated through the rat skin and hence could possibly permeate through the human skin.

Table 11. Cumulative percentage of Metformin HCL permeated from transdermal patches (A series)

Time (hrs)	Cumulative percentage of drug permeated, mean ± S.D (n=3)				
	F1	F2	F3	F4	F5
1	3.3±1.43	2.01±1.76	3.08± 1.78	3.2±1.09	4.3±1.45
2	5.3±2.22	4.88±1.92	5.01±2.37	4.1±2.07	5.7±1.90
3	6.9±2.10	5.4±0.92	6.5±2.03	7.3±1.55	9.9±2.08
4	9.8±1.20	7.01±0.89	9.8±2.18	9.4±1.87	13.1±2.78
5	11.3±0.92	9.5±1.25	11.0±1.35	13.8±0.98	17.3±1.67
6	13.5±1.25	13.9±1.84	14.9±1.11	19.9±0.99	21.2±0.56
8	14.1±1.73	15.8±1.11	16.3±1.90	21.4±1.89	26.4±1.23
10	15.5±0.83	17.09±0.92	19.1±0.98	23.1±1.45	31.02±1.09
12	16.8±0.98	19.9±0.89	22.1±1.12	25.4±2.09	36.1±2.01
24	20.1±1.25	25.4±1.56	28.3±2.07	33.2±2.34	**42.08±1.45**

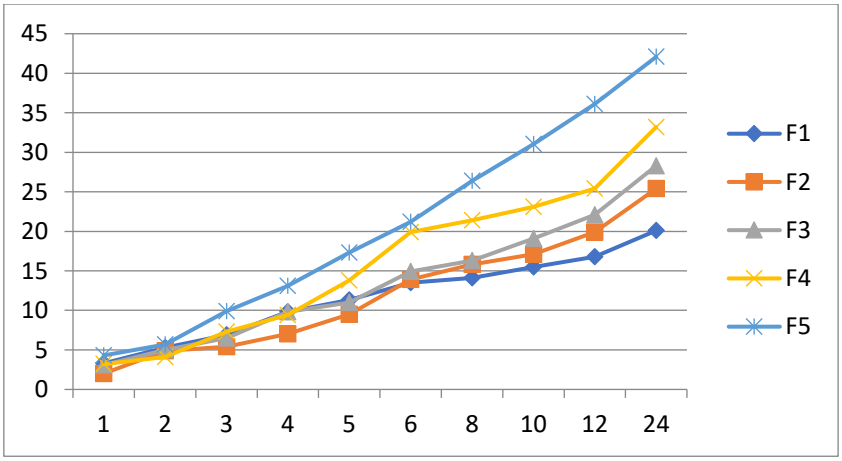


Figure 6: Cumulative percentage of Metformin HCL permeated from transdermal patches (A series)

Table 12. Cumulative percentage of Metformin HCL permeated fromtransdermal patches (B-series)

Time (hrs)	Cumulative percentage of drug permeated, mean ± S.D (n=3)				
	F6	F7	F8	F9	F10
1	2.99±1.92	2.51±1.22	3.91±0.99	3.6±2.09	3.3±1.86
2	3.5±0.78	3.9±2.66	5.5±0.78	5.02±1.79	5.9±2.35
3	4.9±0.45	4.4±2.02	7.2±1.77	7.7±1.90	8.08±2.09
4	5.6±1.23	5.9±1.23	9.8±1.44	11.9±2.45	11.08±1.58
5	8.5±1.99	6.6±1.99	12.9±1.56	15.02±2.45	17.7±1.20
6	9.9±1.67	8.1±1.45	15.3±2.09	19.9±1.89	19.9±0.85
8	13.5±0.89	11.8±2.09	17.9±2.03	22.5±1.57	23.01±1.84
10	16.3±0.99	13.9±2.66	19.5±1.22	26.1±1.01	25.2±0.47
12	19.5±1.99	17.01±1.22	21.9±1.24	29.9±0.901	27.8±1.94
24	24.5±2.08	21.01±1.90	29.3±1.89	**36.08±1.46**	32.2±1.36

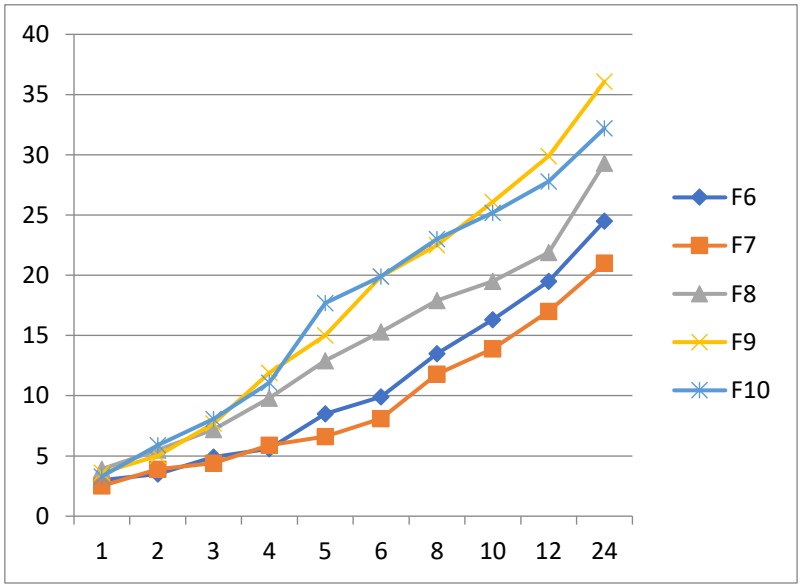


Fig 7. Cumulative percentage of Metformin HCL permeated from transdermal patches (B- series)

Table 13.Cumulative percentage of Metformin HCL permeated from transdermal patches (C1-C3)

Time (hrs)	Cumulative percentage of drug permeated mean ± S.D (n=3)		
	C1	C2	C3
1	5.3±1.89	8.8±1.34	9.1±1.17
2	7.01±2.23	12.9±0.97	12.8±1.90
3	9.9±2.12	16.1±1.34	15.6±2.01
4	12.5±1.45	20.5±1.23	20.8±1.45
5	14.9±1.67	23.4±0.67	28.1±1.11
6	17.8±1.90	26.6±0.55	33.8±1.23
8	23.09±1.99	33.3±0.97	41.9±0.35

10	31.8±2.89	42.6±1.67	52.8±0.93
12	40.3±0.98	51.8±1.23	61.09±1.23
24	53.1±1.56	62.8±1.11	**73.1±1.45**

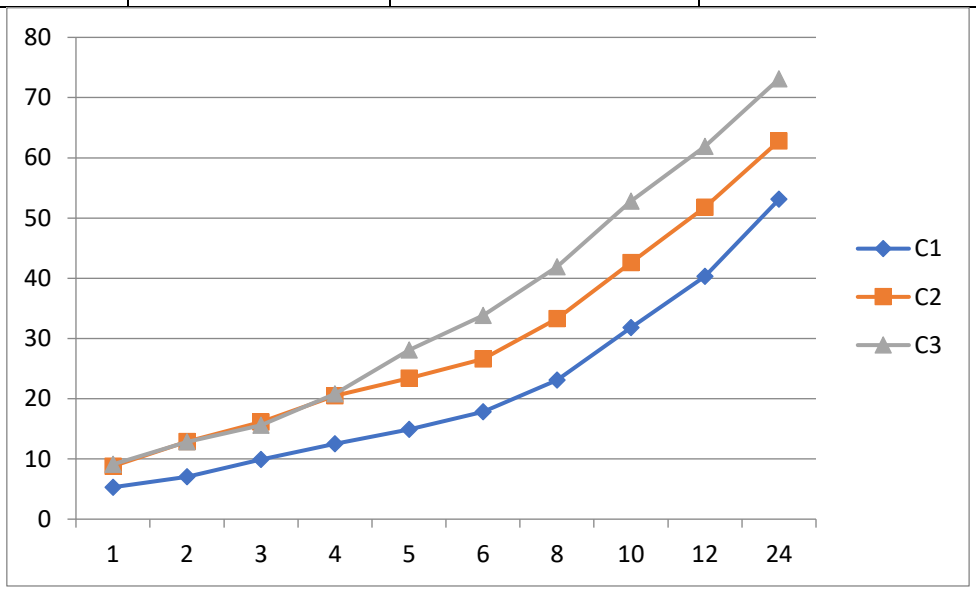


Fig 8. Cumulative percentage of Metformin HCL permeated from transdermal patches (C1-C3)

Time (hrs)	**Cumulative percentage of drug permeated, mean ± S.D (n=3)**		
	D1	D2	D3
1	6.6±1.01	8.8±0.78	9.8±1.11
2	9.9±0.97	11.9±1.20	11.6±1.23
3	11.8±1.22	15.1±1.01	14.1±0.97
4	13.3±2.01	18.8±0.99	17.9±1.98
5	16.6±1.62	22.2±0.96	21.8±2.02
6	20.01±0.92	27.6±0.89	27.7±2.34
8	26.8±1.11	35.5±1.54	38.3±1.89
10	33.1±1.99	42.1±1.76	49.9±0.99
12	42.8±1.34	50.9±1.09	58.6±1.35
24	51.1±0.87	60.5±1.97	**69.9±1.67**

Table 14. Cumulative percentage of Metformin HCL permeated from transdermal patches (D1-D3).

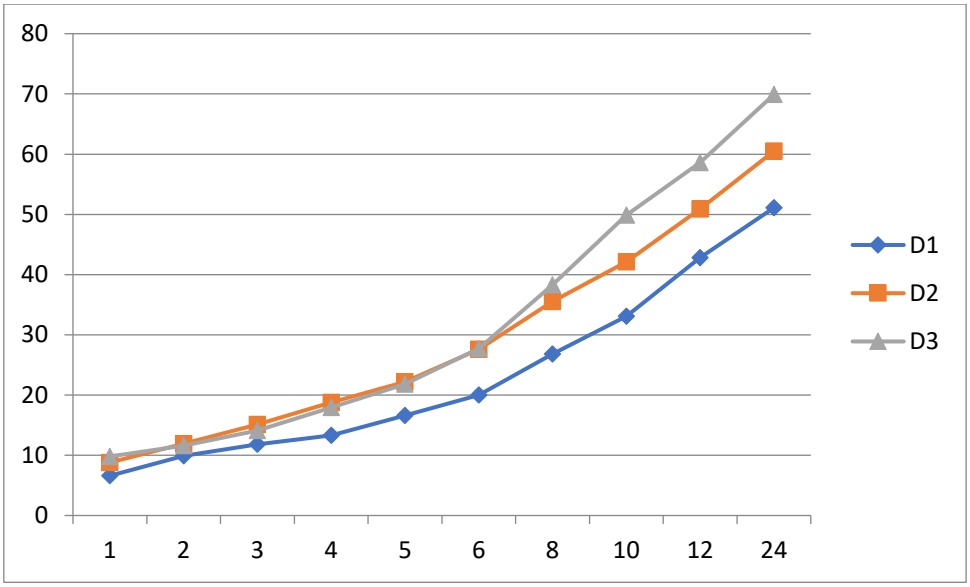


Fig 9. Cumulative percentage of Metformin HCL permeated from transdermal patches (D1-D3)Kinetic models for optimized formulations

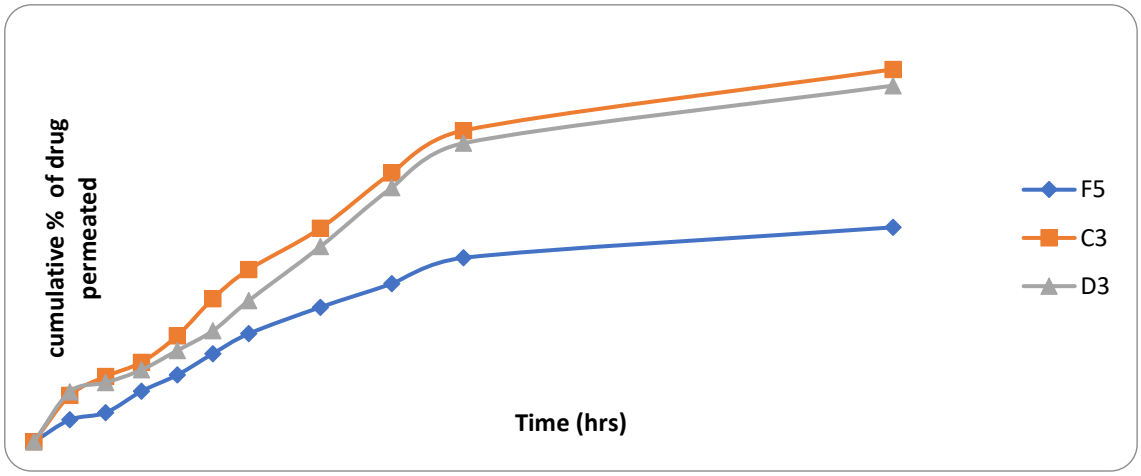


Figure 10. Zero order model (Cumulative percent of drug permeated vs time)

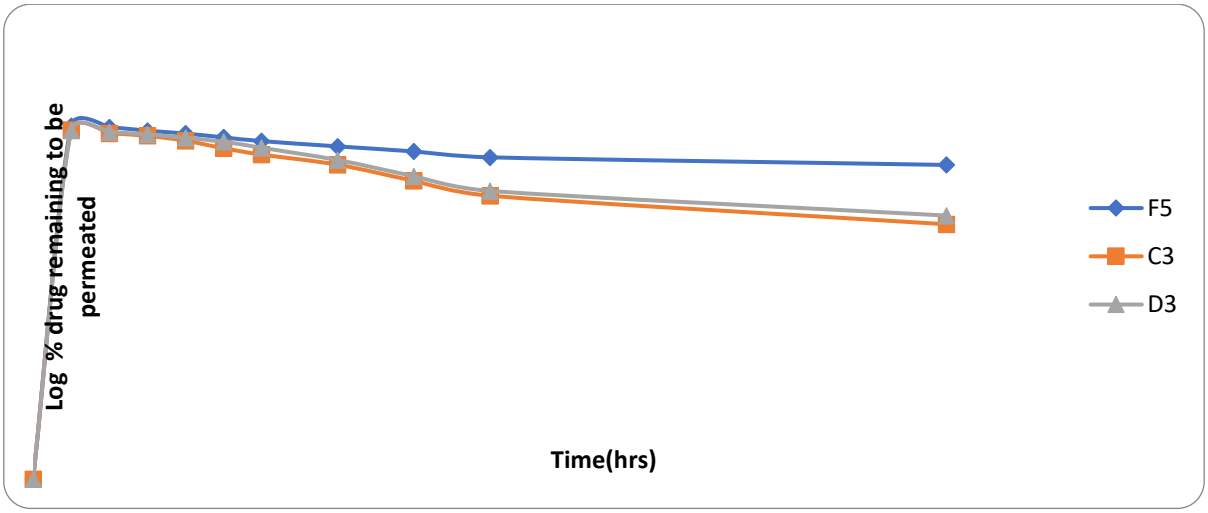


Fig. 11. First order model (- log percentage drug remaining to be permeated vs time)

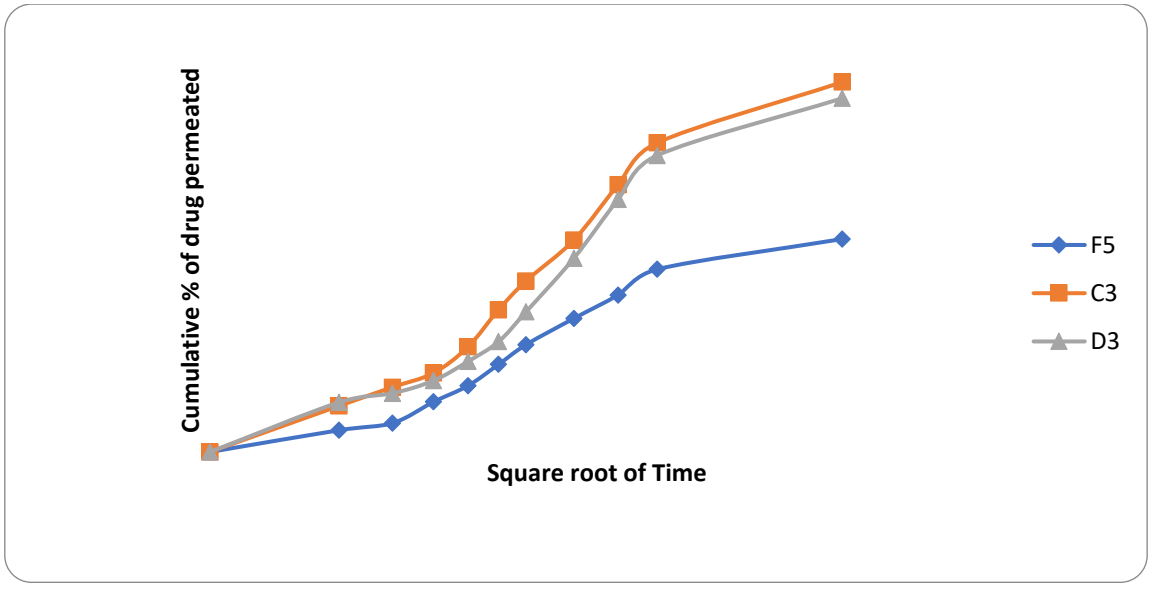


Fig. 12. Higuchi model (Cumulative percent of drug permeated vs square root of time)

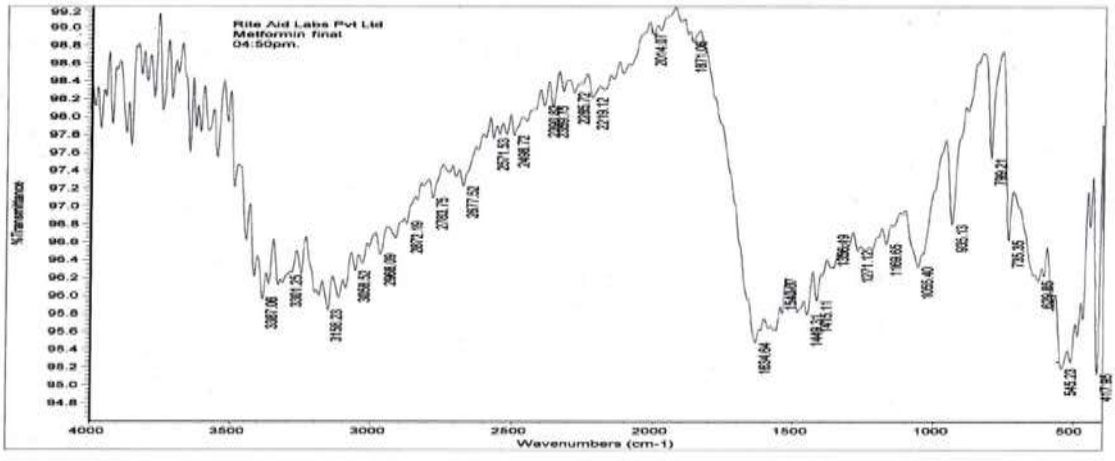
Table 15. Correlation coefficients of kinetic models of optimized formulations

Formulation code	Zero order	First order	Higuchi model	Peppas model	'n' value (Peppas model)
F5	0.845	0.876	0.939	0.956	0.822
C3	0.873	0.946	0.945	0.966	0.746
D3	0.884	0.935	0.926	0.941	0.731

Drug - Excipient Compatibility Study

The IR spectral analysis of Metformin HCL showed that the principal peaks and for the mixture of Metformin HCL, ERS 100 and HPMC E15 additional to the principal peaks, some additional peaks were observed with physical mixtures, which could be due to the presence of polymers. The presence of all the characteristic bands due to functional groups in polymer mixtures suggest that there is no interaction between the drug and polymers used in the present study. The FTIR profiles were shown in the below.

FTIR OF FINAL OPTIMIZED FORMULATION



CONCLUSION

Different polymeric Patches containing Metformin HCL were prepared and evaluated for physicochemical, in vitro drug release and permeation characteristics. Transdermal patches with ERL 100 and HPMC E15 showed better release than patches with ERS 100 and HPMC E15. The release rate was increased with an increase in HPMC E15 content. Metformin HCL transdermal Patches with penetration enhancers d-limonene, oleic acid in 4%, 8% and 12% v/w concentrations were prepared and evaluated for physicochemical and permeation characteristics. The formulations containing d-limonene (12%), 0leic acid (12%) were found to meet the required flux. The release kinetics of the optimized formulations followed zero order and release mechanism was non-fickian diffusion rate-controlled mechanism. FTIR studies showed no drug and polymer interactions. The transdermal patches of Metformin HCL with required flux could be prepared with suitable mechanical properties, further studies are recommended to find their therapeutic utility in humans by pharmacokinetic and pharmacodynamic studies.

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