

Formulation, Optimization, and *In vivo* Evaluation of Clozapine Loaded Transdermal Drug Delivery System for the Treatment of Schizophrenia

Milan B. Agrawal, Mayur M. Patel

Department of Pharmaceutics, Institute of Pharmacy, Nirma University, SG Highway, Ahmedabad, Gujarat, India

Abstract

Introduction: The present research work was intended to develop and optimize transdermal matrix patch of clozapine using Box–Behnken experimental design (Box–Behnken design [BBD]) for improved bioavailability as compared to oral formulation. The 3-factor, 3-level BBD was employed to investigate the combined influence of formulation variables on flux, tensile strength (TS), and *in vitro* drug release. The generated polynomial equation was validated and desirability function was utilized for optimization. **Materials and Methods:** Optimized formulation evaluated for physicochemical characterization, Fourier transform infrared, differential scanning calorimetry, *in vitro* drug release, permeability enhancement potential by *ex vivo*, skin irritation, and *in vivo* pharmacokinetics and stability studies. **Results:** The results of the optimized formulation (F_{15}) showed TS of 6.84 ± 0.64 MPa, flux of 104.80 ± 1.39 ($\mu\text{g}/\text{h}/\text{cm}^2$), and % drug release after 20 h (Q_{20}) of $82.19 \pm 1.12\%$ which was stable up to 6 months in accelerated condition. Observed and the predicted values of the responses were found to be in good agreement. Optimized transdermal patch of clozapine found free from skin irritation as per Draize score method. The pharmacokinetic result had shown the bioavailability of clozapine improved about 2.18-fold after transdermal drug delivery when compared with oral marketed formulation. **Discussion and Conclusion:** The results of the study revealed that the developed transdermal patch of clozapine can be a promising alternative which provides effective management of schizophrenia in terms of improved patient compliance.

Key words: Atypical antipsychotic, Box–Behnken experimental design, *Ex vivo* permeation, permeation enhancers, transdermal drug delivery

INTRODUCTION

Schizophrenia is one of the most dangerous, consequential, and frightening of all mental illnesses. No other disorder arouses as much anxiety in the patient and caretakers along with doctors. Effective treatments are available, yet patients and their families often find it hard to access high standard care. Schizophrenia is a severe form of mental illness affecting about 21 million people worldwide. It is more common among males (12 million) than females (9 million).^[1,2] Schizophrenia is characterized by disintegration of thought processes and of emotional responsiveness. The prevalence is high due to its chronic nature.^[3]

A tricyclic dibenzodiazepine manifested as an atypical antipsychotic agent. It binds various types of receptors and displays a unique

pharmacological profile. Clozapine is a serotonin antagonist, with strong binding to 5-HT 2A/2C receptor subtype. It also displays strong affinity to several dopaminergic receptors, but shows only weak antagonism at the dopamine D_2 receptor, a receptor which is responsible to modulate neuroleptic activity.^[4]

Clozapine is taken twice daily, orally, in the form of tablet. However, when taken by oral route, it undergoes extensive

Address for correspondence:

Mayur M. Patel, Department of Pharmaceutics, Institute of Pharmacy, Nirma University, SG Highway, Chharodi, Ahmedabad - 382 481, Gujarat, India.
Tel.: +91 79 71652727; Fax: +91 2717 241916.
E-mail: drmayurmpatel@gmail.com

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first pass metabolism and oral bioavailability of clozapine is reported to be <27%. Clozapine is extensively metabolized by hepatic microsomal enzymes (CYP1A2 and CYP3A4) and forms N-demethyl and N-oxide metabolites.^[5] These make delivery of clozapine, a challenging task for the efficacious therapy of nervous disorders such as schizophrenia, where prolonged drug delivery is essential for the people who may need assistance to receive medication by oral or by parenteral route.

The topical drug delivery provides various benefits as compared to traditional dosage forms, namely, improved compliance of patients on long-lasting therapy, maintaining a prolonged and constant plasma level of drug (thereby diminishing the side effects associated with the oral route), bypassing biotransformation, reducing inter- and intra-patient variability, and making it possible to put an end to drug therapy whenever needed.^[6] As clozapine plays a significant role for antipsychotic treatment, it is desirable to achieve low-dose preservation therapy of clozapine by specializing the drug delivery phasing so that the desired concentration of the drug can be administered for the betterment of the ill conditions and can lead to a lower side effects than seen with the oral delivery.^[7]

Considering this proposition, the prime objective of this research is to evolve the transdermal drug delivery system of clozapine to reduce the risk of significant oral side effects along with the poor patient compliance. In addition to this, topical patch provides sustained delivery of drug, thereby reducing the dosage frequency and hence facilitates the caregivers by bringing down the hurdles as associated with the oral route.

MATERIALS AND METHODS

Materials

Clozapine was given as a gift sample by Piramal Enterprises Ltd., India. Different grades of hydroxypropylmethylcellulose (HPMC) were procured from Dow Chemicals. Isopropyl myristate (IPM) was procured from Triveni Interchem Pvt. Ltd., Vapi, India. D-limonene and oleic acid (OA) were purchased from A.B. Enterprises, Mumbai, India. 1, 8-Cineole was purchased from Sanket Enterprises, Mumbai, India. Other reagents and chemicals used in the research were procured from reliable and standard sources. The animal study was conducted as per the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying and approved by the Institutional Animal Ethics Committee (Approval no. ROFEL/IAEC/2018/2, ROFEL/IAEC/2018/3) Government of India.

Dose calculation

The amount of dose to be incorporated in a patch was calculated using the following mathematical equation.^[8,9]

$$\begin{aligned} \text{Drug input (theoretical)} &= C_{ss} \times K_e \times V_d \quad (1) \\ &= 122 \times 0.086 \times 96(\mu\text{gL}^{-1} \times \text{L} \times \text{h}^{-1}) \\ &= 1007.23 \mu\text{g/h} \end{aligned}$$

Where, C_{ss} = Steady-state concentration; K_e = Elimination rate constant; and V_d = Volume of distribution.

$$\begin{aligned} \text{Drug required for a patch} &= \text{drug input} \times \text{delivery time} \quad (2) \\ &= 1007.23 \times 24 (\mu\text{g} \cdot \text{h}^{-1} \times \text{h}) \\ &= 24.17 \text{ mg} \\ \text{So, } &\sim 25 \text{ mg.} \end{aligned}$$

Solubility studies

Solubility studies of clozapine were carried out by adding an excess amount of drug in different solvents by keeping the flasks on a mechanical stirrer (Remi Instruments Ltd., Mumbai, India) for 24 h at room temperature.^[9] After 24 h, the solutions were filtered and filtrate is used for drug estimation. The filtrate was analyzed by making use of ultraviolet (UV)/visible spectrophotometer (Shimadzu double-beam spectrophotometer 1800, Kyoto, Japan) at 259 nm. The quantity of drug dissolved was estimated using standard curve ($y = 0.0603 \times -0.013$, $r^2 = 0.9993$).

Formulation of transdermal patches

Transdermal patches of clozapine were prepared by solvent casting technique. HPMC K15M and HPMC K4M were mixed in ratio of 0.7:0.3 and dissolved along with clozapine (25 mg/4.41 cm²) in a mixture of methanol and dichloromethane (1:1) solvent system using magnetic stirrer (Remi Instruments Ltd., Mumbai, India). In addition to this, polyethylene glycol (PEG) (20% w/w of dry polymer weight) was used as plasticizer and OA (7.5% w/w of dry polymer weight) as permeation enhancer was added to the above solution. The resulting solution was cascaded on the laminated aluminum foil placed at the bottom of the cylindrical cup and the solvent was allowed to evaporate at room temperature for 24 h. The patch was cut into small patches containing amounts equivalent to 25 mg of drug.

Statistical optimization using Box–Behnken design (BBD)

A three-level and three-factor BBD^[10] was applied to assess the impact of selected variables on the tensile strength (TS) (Y_1), flux (Y_2), and Q_{20} (% drug release after 20 h) (Y_3) of

transdermal patch. This statistical model is useful in creating second-order polynomial equations and quadratic response surfaces plots.^[11] In regard of prediction variance, the authenticity of the response surface design is significantly much higher than the full factorial design.^[12] Quadratic equations and three-dimensional (3D) response surface plots were generated for each response. Significant *P* values indicated goodness of fit for all responses, revealed by statistical analysis of quadratic model.^[13] Checkpoint batches were manufactured and for each response, the percentage relative error was estimated to validate the model.^[14,15] The dependent and independent variables are listed in Table 1. The model of mathematical concept for deriving equations showing correlation between dependent and independent variables is as follows:

$$Y_i = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 \quad (3)$$

Where, Y_i is the dependent variable, B_0 is the intercept, B_1 to B_{33} are regression coefficients, and X_1 , X_2 , and X_3 are the independent variable selected from the preliminary trials. Overlay plots were generated using the Design-Expert software (version 10.0) (Stat-Ease, Inc. Minneapolis, MN, USA). For responses, the constraints were deliberated to evaluate the robustness of the established design space.

Evaluation of transdermal patches

Weight variation and thickness

The prepared transdermal patches (4.41 cm²) were evaluated for uniformity of weight by digital electronic balance (Mettler Toledo, Ohio, USA). Thickness of the patch was measured using digital Vernier caliper (Mitutoyo, Japan) at random points.^[16]

Folding endurance

Folding endurance was characterized by folding the patch repeatedly at the same point until it was broken. The

amount of time the patch can be folded at the same point without getting broken which was considered as the folding endurance.^[16]

TS

TS was characterized by weight pulley method (Tensile testing machine, SE – 2000, Medford, USA) with a 50 KN load cell. Three samples of each formulation were tested at an extension speed of 5 mm/min.^[17]

Drug content

Drug content was quantified by dissolving the patch of size 4.41 cm² in 100 mL of methanol. The complete solution was filtered and then analyzed by UV analysis using a spectrophotometer (Shimadzu double-beam spectrophotometer 1800, Kyoto, Japan) at a wavelength of 259 nm.^[18] Each measurement was obtained in triplicate and the mean and standard deviation were calculated.

Moisture content

The prepared patches were weighed individually and kept in a desiccator containing activated silica at room temperature for 24 h. The patches were weighed at regular time interval individually until it revealed a persistent weight. To calculate the percentage of moisture content, formula of the difference between initial and final weight with respect to final weight was applied.^[19]

Moisture uptake

This study was carried out at room temperature for 24 h where a weighed patch was kept in a desiccator and exposed to 84% relative humidity (saturated solution of potassium chloride) in a desiccator until a steady weight for the patch was obtained. To calculate the percentage of moisture uptake, formula of the difference between final and initial weight with respect to initial weight was applied.^[19,20]

In vitro release study

A modified paddle over disc apparatus (USP apparatus V) was used for the assessment of the release of the drug from the patches. The patch was mounted on the disc and placed at the bottom of the dissolution vessel. The dissolution medium consists of 500 ml of phosphate buffer (pH 7.4). The apparatus was equilibrated to 32 ± 0.5°C and operated at 50 rpm.^[21] At predetermined time intervals, 5 ml sample was withdrawn and exchanged with fresh medium up to 24 h. The concentration of clozapine was determined spectrophotometrically at wavelength of 259 nm (Shimadzu double-beam spectrophotometer 1800, Kyoto, Japan). All the readings were determined in triplicate.

Ex vivo permeation study

The *ex vivo* permeation studies were carried out in vertical Franz diffusion cell using Wistar rat skin (Approval no.

Table 1: Actual and coded values of the dependent and independent variables

Independent variables	Levels		
	Low	Intermediate	High
Coded value	-1	0	+1
X_1 : Drug:polymer ratio	1:2	1:3	1:4
X_2 : Concentration of PE (%)	5	7.5	10
X_3 : Concentration of plasticizer (%)	15	20	25
Dependent variables	Constraints		
Y_1 =Tensile strength (MPa)	>4 MPa		
Y_2 =Flux (µg/h/cm ²)	>100 (µg/h/cm ²)		
Y_3 = Q_{20} (%)	>80%		

ROFEL/IAEC/2018/3) after detaching the adhering fat and hair. The formulation was fixed on the skin in such a way that the drug matrix was facing the donor side. Phosphate buffer (pH 7.4) was used as receptor fluid maintained at temperature of $32 \pm 0.5^\circ\text{C}$ along with the agitation speed of 50 rpm. At different time intervals, the samples were withdrawn and exchanged with equal amounts of fresh media. Aliquots were analyzed spectrophotometrically at a wavelength of 259 nm.^[22] The drug permeated per cm of patch was calculated as per following equation and plotted against time, and the flux was calculated as drug permeated per cm^2 per hour.^[23]

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{S} \quad (4)$$

Where, C_n is the drug concentration of receptor medium after each sampling time, C_i is the drug concentration for i_{th} sample, V_0 and V_i are the volumes of the receiver solution and sample, respectively, and S is the effective diffusion area.^[24]

Fourier transform infrared (FTIR)

The optimized formulation was assessed for interaction studies by comparing with pure drug, different polymers, and mixture of drug and polymers using FTIR (Jasco 1800, Tokyo, Japan).^[25] The characterization was carried out with the frequency ranges of $4000\text{--}400\text{ cm}^{-1}$ by employing KBr pellet method.

Differential scanning calorimetry (DSC)

To carry out the calorimetric analysis, TDA trend line software connected with DSC 60A (Shimadzu, Kyoto, Japan) was used. Around 5 mg of analytes were weighed on aluminum pan by maintaining the reference aluminum pan vacant. For the analytes, thermograms were measured at a scan rate of $10^\circ\text{C}/\text{min}$ from 40°C to 300°C and then cool down to 40°C , underneath liquid nitrogen.^[26]

In vivo studies

The study was conducted as per the guidelines given by CPCSEA, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying and approved by the Institutional Animal Ethics Committee (Approval no. ROFEL/IAEC/2018/2) Government of India.

Skin irritation study

Skin irritation potential of the patches was assessed on Wistar rats by Draize score method. The hair from the dorsal area of the rats was removed with the help of clipper, 24 h before the test. The placebo, test, and positive control (0.8% v/v formalin solution) were applied to intact site of animal skin and wrapped with an occlusive dressing (non-irritant Medisurge tape). After a period of 24 h, patches were removed and the areas of the skin occupied with patch were monitored for any sign of erythema or edema. On daily basis, the process was

replicated up to 3 days and the average score was computed and reported on the basis of the degree of edema or erythema from 0 to 4 where 0 for none, 1 for slight, 2 for well defined, 3 for moderate, and 4 for scar formation. The scores, for each animal, at 24 h, 48 h, and 72 h after removal of the patch were averaged to obtain a mean irritation score, for each time point.^[27]

In vivo pharmacokinetic study

For pharmacokinetic evaluation of patches, Wistar albino rats weighing between 200 and 250 g were used. The standard laboratory environment of 12 h light/dark cycle at $25 \pm 2^\circ\text{C}$ was followed when rats were housed in polypropylene cages along with autoclaved clean rice husk as bedding material and with free access to a standard laboratory diet and water *ad libitum*. The animals were selected after visual examination of the skin surface for abnormalities. The hair from dorsal area was trimmed with a clipper and to monitor any unwanted reactions of shaving the rats which were kept under observation for 24 h; they were kept on fast for overnight. The animals were bifurcated into two groups where each group was having total six animals. Group I was given marketed formulation orally (25 mg/kg),^[8] computed based on the body surface area; for Group II, optimized formulation of patch F_{15} applied with an equivalent square centimeter piece of patch (calculated as per body weight of rats, i.e., 25 mg/kg for clozapine).^[9] Whole blood was collected in Eppendorf tubes containing disodium ethylenediaminetetraacetic acid at 0, 1, 2, 4, 8, 12, and 24 h. Plasma was separated by centrifuging at $2000 \times g$ for 15 min and then transferred to clean tubes, which were stored at -20°C until analysis

For estimation of clozapine in rat plasma, a simple and reliable high-performance liquid chromatography (HPLC) method was used for analysis of clozapine.^[28] An isocratic HPLC (Shimadzu, Kyoto, Japan) with manual injector was used. Chromatographic parameters were optimized by validation using spiked plasma samples. The analytes were concentrated from the plasma samples by extraction with isopropyl alcohol, n-hexane, and ethyl acetate, in the ratio 5:15:80, v/v/v. Clozapine was separated using a C18 reversed-phase column, $150 \times 2.1\text{ mm ID}$, and an isocratic mobile phase consisting of acetonitrile and 62.4 mM phosphate buffer (containing 0.3% triethylamine, pH 4.5) in a ratio of 40:60 (v/v). The drug was estimated with a UV detector at 220 nm. The chromatographic separation was found to be linear in the range of 50–2000 ng/ml, with a good correlation coefficient ($R^2 = 0.981$). The method was found to be specific and sensitive with detection limits of 29.4 ng/ml, for clozapine.

Stability study

The accelerated stability testing study of the optimized transdermal patch was performed for 6 months, according to the International Conference on Harmonization guidelines.^[29]

Adequate replicates of optimized patch were composed and stability study was carried out at temperatures of $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for 6 months. Samples were withdrawn at an interval of 1, 3, and 6 months and drug content, flux, and TS analyzed for the stability samples and results were compared with freshly prepared patches.^[30]

RESULTS AND DISCUSSION

Selection of solvent system for film casting

Solvent system for film casting was selected on the basis of highest solubility of drug and polymer. Solubility of clozapine was evaluated in different organic solvents and in binary mixture of solvents to attain the highest solubility of drug [Figure 1].

Highest solubility of drug was found in methanol (2.017 ± 0.04 mg/ml) and most of the hydrophilic polymers were miscible with the solvent system, but higher viscosity grades of HPMC such as HPMC K4M, K15M, and K100M were insoluble in methanol and the same observation was also found in case of methanol and dichloromethane (75%:25%) solvent system. In case of methanol:acetone (50%:50%) solvent system, the solubility of drug was found to be 1.957 ± 0.02 mg/ml, but most of hydrophilic polymers were insoluble with the said solvent system. Hence, the solvent system of methanol:dichloromethane (50%:50%) having drug solubility of 1.904 ± 0.02 mg/ml was evaluated for further study. Most of the polymers were soluble with the system including higher viscosity grades of HPMC. Hence, based on solubility of drug and polymer, methanol and dichloromethane mixture (50%:50%) was selected as a best suitable system for the preparation of matrix patch of clozapine.

Screening of polymer for patch preparation

To determine the film-forming property of polymer with drug, clozapine (25 mg/4.41 cm² patch area) was dissolved

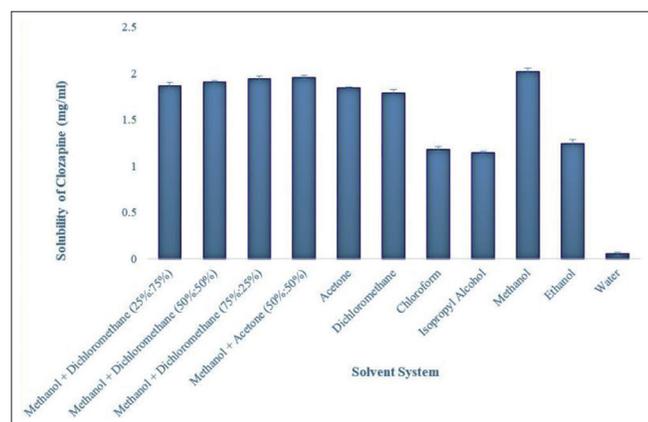


Figure 1: Solubility of clozapine in different solvents

in methanol:dichloromethane solvent system (1:1). Various batches were prepared using different polymers such as Eudragit® RL PO (batch PM1 and PM2), Eudragit® RS PO (batch PM3 and PM4), polyvinyl acetate (PVA) (batch PM5 and PM6), polyvinylpyrrolidone (PVP) K30 (batch PM7 and PM8), ethyl cellulose (batch PM9 and PM10), HPMC K4M (batch PM11 and PM14), HPMC K15M (batch PM12 and PM15), and HPMC K100M (batch PM13 and PM16). The polymer (drug:polymer; 1:5) was dissolved using methanol and dichloromethane (1:1) as solvent system. Total 10% w/w (based on dry polymer weight) of plasticizer, PEG, or dibutyl phthalate (DBP) were added to the polymeric solution. For initial screening of polymer, drug-to-polymer ratio and the concentration of plasticizers were kept constant.

Batches PM1 and PM2 were prepared using Eudragit® RL PO as a polymer and PEG and DBP as a hydrophilic and hydrophobic plasticizer, respectively. The resulting polymeric solution obtained was clear but the patch formed thereof was found to be opaque and brittle. The same results were obtained in case of Eudragit RS PO when formulated with PEG and DBP for batches PM3 and PM4, respectively. In case of PVA, resulting dispersion was found to be hazy for both the plasticizers PEG (batch PM5) and DBP (batch PM6), and hence, the said polymer was not studied further. Batches PM7 and PM8 were prepared with PVP K30 using PEG and DBP as a plasticizer, respectively, where resulting polymeric solution was found to be clear but the patch formed was brittle.

In case of ethyl cellulose, resulting dispersion was found to be clear for both the plasticizers PEG (batch PM9) and DBP (batch PM10), but the formation of patch was found to be opaque. Batches PM11 to PM13 were prepared using various grades of HPMC such as HPMC K4M, K15M, and K100M, respectively, using PEG as a plasticizer. The resulting polymeric solution was obtained clear and the patch formed was found to be smooth and clear. The same results were obtained for batches PM14 to PM16 prepared with DBP as a plasticizer for different grades of HPMC such as K4M, K15M, and K100M, respectively.

The consequence of the study disclosed that different grades of HPMC formed smooth patch with the selected solvent system. Other polymers such as polyvinyl alcohol (PVA) formed hazy dispersion while ethyl cellulose, PVP K30, and Eudragit gave clear solution with opaque brittle patch. Hence, different grades of HPMC were selected for further development of matrix patch.

Screening of plasticizer type and concentration

Plasticizer plays a critical role in determining elastic characteristic of patch. According to the American Society for Testing Materials (ASTM), the materials with TS of more than 4.0 MPa possess good elastic characteristic.^[31] Patches should be elastic in nature to withstand external forces such as

wear and tear during handling, storage, or use.^[32] To determine the elastic property of patch, clozapine (25 mg/4.41 cm² patch area) was dissolved in methanol:dichloromethane solvent system (1:1). Polymer HPMCK15M was dissolved in 1:5 drug:polymer ratio. Batches PS1 to PS12 were prepared by assorting the concentration of plasticizer from 10% w/w to 30% w/w (based on dry polymer weight) [Table 2]. Prepared patches were evaluated for thickness, TS, folding endurance, drug content, % moisture content, and % moisture uptake.

Formulations having TS less than 4 were not evaluated for characterization and dropped from the further study of *in vitro* drug release. It was depicted from the results that as the concentration of plasticizer increases, TS decreases. This effect was attributed to the fact that the plasticizer molecules may disrupt the interchain cohesive forces of polymer.^[33] Moreover, TS for hydrophilic plasticizer was more as compared to hydrophobic plasticizers. These results were observed due to the hydrophilic-hydrophobic interactions of polymer and plasticizers, respectively, where hydrophobic plasticizers disturb the miscibility of polymer chains and result in decreased TS.^[26] Moisture content and moisture uptake were higher in case of PG and PEG which was again due to the hydrophilicity of the plasticizers which provide humectant type activity to the formulation during storage. Drug content and folding endurance were found satisfactory for the patches having TS of <4. Hence, all these formulations were further evaluated for *in vitro* drug release studies [Figure 2a].

The results of *in vitro* drug release studies revealed that as the proportion of plasticizer increases, drug release increases. This could be due to increased flexibility and mobility of polymer chain molecules which further leads to the weakening of interaction between polymeric chains, thus decreasing the glass transition temperature, thereby, increasing the flexibility of polymer films (resulting in

increased drug release).^[34] As shown in Figure 2, batch PS1 prepared with the hydrophobic plasticizer exhibited slowest

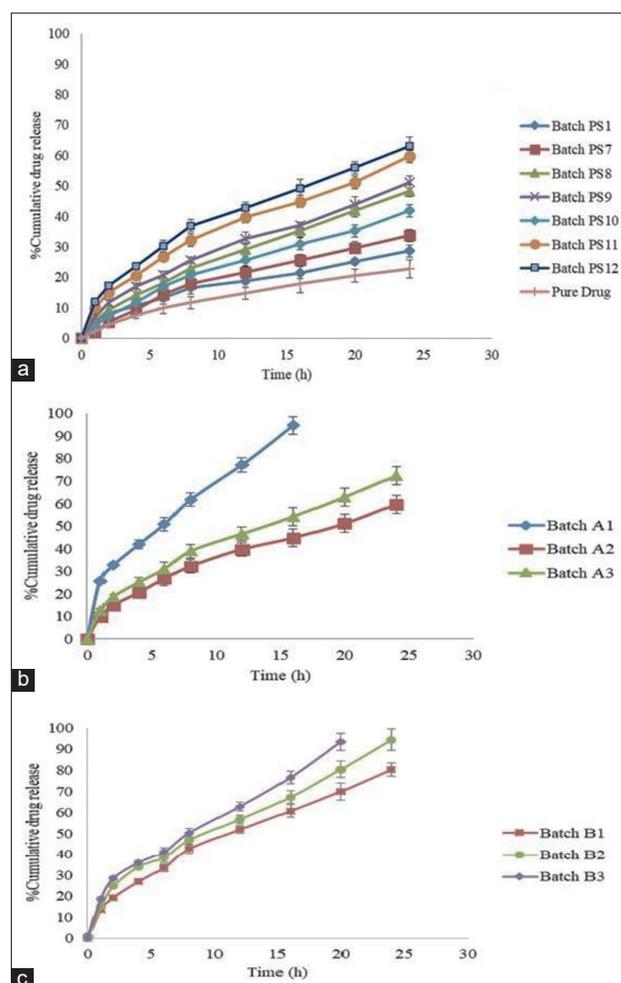


Figure 2: Impact on drug release (a) plasticizer type and concentration, (b) drug:polymer ratio, (c) polymer combinations

Table 2: Screening of plasticizers

Batch	Type of plasticizer	Concentration of plasticizer (%)	Thickness (mm)	Tensile strength (MPa)	Folding endurance	Weight (mg)	% drug content	% moisture content	% moisture uptake
PS1	Dibutyl	10	0.07±0.01	5.41±1.14	>300	167.6±0.51	99.04±1.38	3.18±0.68	3.93±0.52
PS2	phthalate (DBP)	20	0.10±0.02	3.17±0.23	>300	171.7±1.23	----	----	----
PS3		30	0.14±0.02	1.85±0.46	~100	183.9±1.05	----	----	----
PS4	Dibutyl	10	0.08±0.01	3.42±0.17	>300	165.4±0.89	----	----	----
PS5	sebacate (DBS)	20	0.10±0.02	2.26±0.62	~240	170.9±1.41	----	----	----
PS6		30	0.10±0.01	1.19±0.18	~100	184.1±1.74	----	----	----
PS7	Propylene glycol (PG)	10	0.08±0.01	11.94±1.43	>300	168.1±1.61	98.74±1.43	5.06±0.49	6.42±0.61
PS8		20	0.13±0.02	8.05±1.26	>300	179.8±1.14	97.78±1.26	6.24±0.76	7.87±0.58
PS9		30	0.16±0.02	4.57±0.68	>300	189.2±1.26	96.31±1.59	7.16±0.52	9.31±0.73
PS10	Polyethylene glycol (PEG)	10	0.06±0.01	16.81±1.89	>300	165.2±1.38	99.26±0.62	4.39±0.35	5.21±0.49
PS11		20	0.12±0.02	12.51±1.67	>300	178.2±1.59	98.46±1.49	5.19±0.43	6.43±0.57
PS12		30	0.15±0.02	9.79±1.52	>300	188.4±1.64	97.23±1.03	6.42±0.71	8.12±0.78

drug release as compared to the batch PS10 prepared with the hydrophilic plasticizer at the same concentration of 10% w/w (based on dry polymer weight). This could be due to the fact of hydrophilic-hydrophobic interactions of polymer and plasticizers which interrupt the miscibility of polymer chains and manifest in reduced drug release. As compared to batch PS10, batch PS11 showed higher drug release due to increased concentration of hydrophilic plasticizer. As reported in earlier studies, it has been observed that increasing plasticizer concentration increases the diffusion rate of the active substance.^[35] Based on TS and *in vitro* drug release studies, PEG was found to be the best suitable plasticizer. Batches PS11 and PS12 containing PEG 20% and 30%, respectively, showed comparable drug release profile. Hence, based on TS batch, PS11 was selected for further screening and optimization studies.

Selection of drug-to-polymer ratio

To determine the optimum drug-to-polymer ratio (that provides the highest TS and highest drug release after 24 h), different ratios of drug to polymer were evaluated for the formulation of transdermal patch. Initially, batches with HPMC K4M (batch A1) and K15M (batch A2) with drug:polymer ratio of 1:5 were prepared and evaluated for thickness, TS, folding endurance, drug content, % moisture content, and % moisture uptake [Table 3]. The results of the study revealed that the TS for batches A1 and A2 was found to be 6.19 ± 0.71 and 12.51 ± 1.33 MPa, respectively. These values were <4 and hence drug release studies were carried out for both the batches. *In vitro* drug release studies of batches A1 and A2 depicted complete release of drug from batch A1 at the end of 20 h. In case of batch A2, it was observed that the drug release was sustained for more than 24 h with $59.69 \pm 2.13\%$ drug release at the end of 24 h which suggests that formulation with HPMC K4M alone was not able to withstand up to 24 h. On the contrary, formulation with HPMC K15M with ratio of 1:5 was giving very slow drug release.^[36] Hence, to achieve complete drug release in 24 h, formulations A3 and A4 were prepared with HPMC K15M by evaluating drug-to-polymer ratio of 1:3 and 1:1, respectively [Table 3]. The results of the study revealed formulation of smooth and transparent films; however, the TS for the formulation A4 was found to be 3.29 ± 0.47 MPa which was less than the ASTM standard of 4 while for A3, it was found 9.23 ± 0.86 MPa. Hence, batch A4 was not evaluated for further characterization and dropped from the further study of *in vitro* drug release. The *in vitro* drug release profile of batch A3 depicted $72.37 \pm 2.84\%$ drug release at the end of 24 h [Figure 2b]. As desired drug release profile was not achieved by reducing the drug-to-polymer ratio, a combination of high and low viscosity grade of HPMC was further explored to achieve the desired drug release profile. The results of the study revealed that there was no significant impact of polymer quantity on thickness as well as on moisture content of patch.^[37] As from the above discussion, it could be inferred that the drug:polymer ratio

Table 3: Composition and physicochemical characteristics of clozapine formulations (mean \pm SD, n=3).

Batch	Ratio of drug:polymer (HPMC K15M)	Ratio of HP MCK15M:HP MCK4M	Thickness (mm)	Tensile strength (MPa)	Folding endurance	Weight (mg)	% drug content	% moisture content	% moisture uptake
A1	1:5	----	0.09 \pm 0.02	6.19 \pm 0.71	>300	176.8 \pm 1.36	98.14 \pm 1.66	4.23 \pm 0.21	5.34 \pm 0.39
A2	1:5	----	0.11 \pm 0.03	12.51 \pm 1.33	>300	178.2 \pm 1.92	98.46 \pm 2.41	5.19 \pm 0.56	6.43 \pm 0.45
A3	1:3	----	0.10 \pm 0.02	9.23 \pm 0.86	>300	116.7 \pm 0.84	99.19 \pm 1.28	4.12 \pm 0.32	5.03 \pm 0.64
A4	1:1	----	0.05 \pm 0.01	3.29 \pm 0.47	~130	63.9 \pm 0.73	----	----	----
B1*	----	0.9:0.1	0.11 \pm 0.03	8.59 \pm 1.09	>300	115.3 \pm 1.02	97.12 \pm 0.76	4.79 \pm 0.34	5.91 \pm 0.47
B2*	----	0.7:0.3	0.10 \pm 0.02	7.12 \pm 0.68	>300	115.6 \pm 0.94	99.67 \pm 1.15	4.38 \pm 0.14	5.68 \pm 0.23
B3*	----	0.5:0.5	0.10 \pm 0.03	5.04 \pm 0.52	>300	114.8 \pm 1.16	98.14 \pm 0.84	4.23 \pm 0.29	5.34 \pm 0.18

*Drug:polymer ratio was kept 1:5

of 1:3 for HPMC K15M resulted in incomplete drug release of $72.37 \pm 2.84\%$. Hence, we have not studied the impact of HPMC K100M because of its high viscosity (100,000 mPa s) as compared to the HPMC K15M (15000 mPa s).

Batches B1, B2, and B3 were prepared by employing combination of HPMC K15M:HPMC K4M at different ratio of 0.9:0.1, 0.7:0.3, and 0.5:0.5, respectively [Table 3]. The TS for all the batches B1 to B3 was found to be >4 MPa. It was seen from the results that as the quantity of high viscosity grade of HPMC decreases TS decreases, but this change was statistically insignificant ($P > 0.05$). Folding endurance for all prepared formulations was >300 which represents the in-line correlation with TS. Drug content was found in accordance with USP limit.^[38] Objective of this polymer combination was to achieve the complete drug release at the end of 24 h. Hence, as the prepared batches were in compliance of physicochemical properties, formulations B1, B2, and B3 were evaluated for the *in vitro* drug release. For batches B1 and B2, the drug release was found to be 80.31 ± 3.27 and $94.42 \pm 2.82\%$, respectively, after 24 h while batch B3 was able to withstand up to 20 h only [Figure 2c]. As highest drug release after 24 h was achieved with B2 formulation, ratio of 0.7:0.3 of HPMC K15M and HPMC K4M was selected for further studies. This outcome of study suggests that optimum combination of higher and lower viscosity grades of HPMC provides complete drug release after 24 h along with comfortable mechanical properties.^[39] Hence, this formulation was evaluated for *ex vivo* permeation studies.

Ex vivo permeation study

The target skin permeation rate for clozapine was calculated using the following equation:^[40]

$$J = (Cl * C_p * W) / A \quad (5)$$

Where, J is the flux ($\mu\text{g}/\text{h}/\text{cm}^2$), A is the surface area of patch (4.41 cm^2), C_p is plasma concentration ($100 \mu\text{g}/\text{L}$), Cl is the clearance rate ($0.072 \text{ L}/\text{h}/\text{kg}$), and W is the average weight of patient (taken as 60 Kg).^[41] The theoretically required flux for clozapine as calculated from the above equation was found to be $97.95 \mu\text{g}/\text{h}/\text{cm}^2$ (which was considered as $\sim 100 \mu\text{g}/\text{h}/\text{cm}^2$). This permeation rate is a prerequisite for attaining adequate plasma concentrations of drug. Flux for formulation B2 was found to be only $59.42 \pm 0.34 \mu\text{g}/\text{h}/\text{cm}^2$. Hence, to achieve the target flux, overages of drug were evaluated. Batches O1 and O2 with 10% and 20% overages of 25 mg dose, that is, 27.5 mg and 30 mg dose were formulated, respectively. The results of the study depicted flux value of 65.95 ± 0.76 and $68.66 \pm 1.76 \mu\text{g}/\text{h}/\text{cm}^2$ for batch O1 and O2, respectively [Figure 3a].

This inference of the study suggests that there was no significant increase in the flux which was attributable due to the further addition of clozapine, leading to the supersaturation

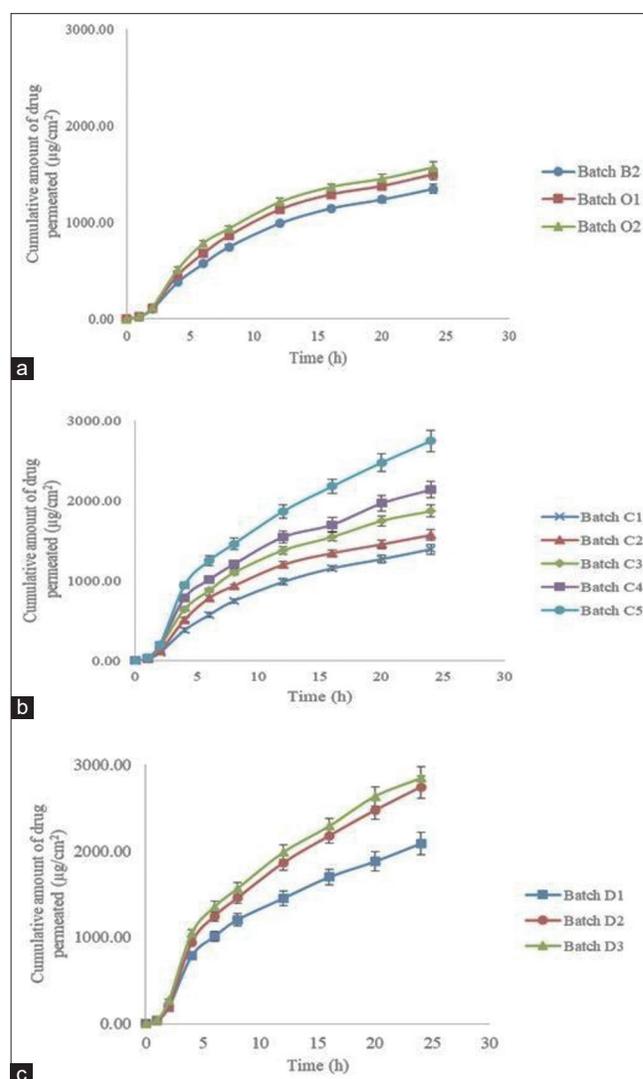


Figure 3: Effect on *ex vivo* skin permeation (a) drug concentration, (b) different permeation enhancers, (c) concentration of permeation enhancer

of drug in patches. This results are in agreement with the literature.^[6,42] For the development of transdermal patch, permeation enhancers (PEs) such as pyrrolidones, azones, and surfactants are generally used. These penetrating agents are solvents which are accountable for keratin reversible denaturation while azones are less efficacious on human skin.^[43] Surfactants enhance the drug penetration but rate of penetration relies on their physicochemical properties. Vegetable oils are natural PEs which are easily accessible and are metabolized in the body.^[44] Hence, various PEs, namely 1, 8-cineole, D-limonene, azone, IPM, and OA were evaluated to improve the permeation to achieve the target flux. Thus, different PEs manifesting to the group of fatty acids (OA), fatty acid ester (IIPM), terpenes (1,8-cineole and D-limonene), and laurocapram (azone) were explored in this study to assess their impact on the drug permeation rate through transdermal matrix patch. *Ex vivo* and *in vivo* studies were also carried out to explore the potential of matrix patch to achieve the sustained release of clozapine.

Batches C1 to C5 were prepared using 1,8-cineole, D-limonene, azone, IPM, and OA, respectively, at a concentration of 10% w/w (of dry weight polymer). The flux of clozapine was found to be 61.05 ± 0.67 , 68.39 ± 0.78 , 80.55 ± 1.04 , 90.44 ± 1.23 , and 116.46 ± 1.16 $\mu\text{g}/\text{h}/\text{cm}^2$ with the enhancement ratio of 1.03, 1.15, 1.36, 1.52, and 1.95 for batches C1 to C5, respectively [Figure 3b].

The permeation rates for prepared batches C1 to C5 were found to be in the following decreasing order: OA > IPM > Azone > D-limonene > 1,8-Cineole. The above results were ascribed to the lipophilicity of the PEs and the extent of the modifications exerted by them in the subcutaneous layer of skin. Lipophilicity of the PEs was one of the most significant factors influencing their capability to promote permeation through skin.^[45] OA is a lipophilic fatty acid^[46] having highest log $P = 7.7$ ^[47] amid the PEs evaluated. As a consequence, OA promotes the partitioning of clozapine from matrix patch to the lipophilic membrane and subsequently into the acceptor phase. Moreover, OA acts by disrupting the lipid bilayer and hence increases the drug flux, while IPM fluidizes the stratum corneum lipids and thereby enhancing flux.^[40] The log P value of IPM is 7.17^[48] while for azone log $P = 6.2$.^[49] Azone does not appear to interact with proteins; it partitioned directly into the lipid bilayer and disrupts it, making the lipids more fluid and flexible.^[50] The permeation mechanism of 1,8-cineole is depending on the highly ordered bilipid structure disruption of subcutaneous layer,^[51] whereas penetration enhancing mechanism of D-limonene is the lipid extraction from SC.^[52] It has been reported in the literature that the highly polar terpenes are more effective PEs for hydrophilic drugs while for lipophilic drugs, non-polar terpenes are more suitable PE.^[53] 1,8-cineole is considered as polar terpenes due to the presence

of oxygen atom in its structure with log $P = 2.82 \pm 0.25$. On the contrary, log p value for a non-polar (hydrocarbon) terpene, d-limonene, was found to be 4.58 ± 0.23 .^[54] The extent of alteration of subcutaneous caused by selected PEs played an important role in drug penetration through the skin. Hence, based on above findings, OA is selected as best suitable permeation enhancer for the further studies and its effect on flux was evaluated at different concentration.

Batches D1 to D3 were formulated by differing amount of OA in the range of 5%, 10%, and 15% (of dry polymer weight), respectively. Flux for batches D1, D2, and D3 was found to be 87.52 ± 0.89 , 116.46 ± 1.16 , and 120.74 ± 1.42 $\mu\text{g}/\text{h}/\text{cm}^2$ with the enhancement ratio of 1.47, 1.95, and 2.03, respectively. The permeation rate of drug increased with increasing the concentration of OA, reaching maximum at 15% [Figure 3c]. Permeation rate obtained with 10% and 15% concentration of OA was comparable and no noteworthy difference was found.^[55]

Optimization using BBD

It was inferred from the results of prelude batches that among a variety of formulation variables and critical process parameters evaluated, three most significant factors, namely, drug:polymer ratio (X_1), concentration of PE (X_2), and concentration of plasticizer (X_3) exhibited pronounced effect on the TS (Y_1), flux (Y_2), and Q_{20} (% drug release after 20 h) (Y_3). The ratio of drug:polymer and concentration of plasticizer mainly affects the TS, flux, and Q_{20} to a greater extent, while concentration of PE exerted pronounced impact on flux only. Hence, to optimize the clozapine matrix patch systematically, three levels and three factors BBD was applied [Table 4].

Table 4: Composition of experimental formulations (mean \pm SD, $n=3$)

Batch	X_1 (Ratio)	X_2 (%)	X_3 (%)	Y_1 (MPa)		Y_2 ($\mu\text{g}/\text{h}/\text{cm}^2$)		Y_3 (%)	
				Observed	Predicted	Observed	Predicted	Observed	Predicted
F ₁	1:2	5	20	4.26 \pm 0.58	4.10	83.53 \pm 1.29	82.47	92.13 \pm 2.04	93.00
F ₂	1:4	5	20	8.63 \pm 0.97	8.75	76.77 \pm 1.02	75.67	71.87 \pm 1.41	71.11
F ₃	1:2	10	20	3.92 \pm 0.35	3.81	106.34 \pm 1.49	107.44	94.32 \pm 2.43	95.08
F ₄	1:4	10	20	8.34 \pm 0.68	8.50	98.52 \pm 1.32	99.58	73.64 \pm 1.59	72.77
F ₅	1:2	7.5	15	5.76 \pm 0.47	5.93	91.28 \pm 0.86	90.66	90.27 \pm 2.18	89.25
F ₆	1:4	7.5	15	10.89 \pm 1.29	10.79	85.19 \pm 1.04	84.61	64.05 \pm 2.59	64.66
F ₇	1:2	7.5	25	2.18 \pm 0.31	2.28	96.72 \pm 0.78	97.30	96.09 \pm 2.11	95.48
F ₈	1:4	7.5	25	6.93 \pm 0.53	6.76	88.07 \pm 0.92	88.69	74.86 \pm 1.89	75.88
F ₉	1:3	5	15	9.06 \pm 0.96	9.05	86.16 \pm 0.64	87.84	76.41 \pm 1.57	76.56
F ₁₀	1:3	10	15	8.89 \pm 0.87	8.83	108.41 \pm 1.65	107.93	78.23 \pm 1.14	78.49
F ₁₁	1:3	5	25	5.21 \pm 0.27	5.27	88.39 \pm 1.43	88.86	85.59 \pm 1.27	85.33
F ₁₂	1:3	10	25	4.92 \pm 0.39	4.93	119.32 \pm 1.58	117.64	87.31 \pm 1.32	87.16
F ₁₃	1:3	7.5	20	6.82 \pm 0.72	6.80	104.12 \pm 1.24	104.13	80.78 \pm 1.09	82.00
F ₁₄	1:3	7.5	20	6.74 \pm 0.79	6.80	103.46 \pm 1.13	104.13	83.02 \pm 1.40	82.00
F ₁₅	1:3	7.5	20	6.84 \pm 0.64	6.80	104.80 \pm 1.39	104.13	82.19 \pm 1.12	82.00

Influence of independent variables on TS (Y_1)

The TS of clozapine patch varied in the range of 2.18 ± 0.31 MPa– 10.89 ± 1.29 MPa as a result of the variation in the independent variables. The polynomial equation with the coefficients of the model showing the relationship between the factors and TS was as follows.

$$Y_1 = 6.80 + 2.33 X_1 - 0.14 X_2 - 1.92 X_3 - 0.013 X_1 X_2 - 0.095 X_1 X_3 - 0.030 X_2 X_3 - 0.55 X_1^2 + 0.034 X_2^2 + 0.19 X_3^2 \quad (6)$$

Here, correlation coefficient R^2 was found to be 0.9978, indicating a good fit. From Equation (6), it is noted that the quantitative effects of independent variables (X_1 , X_2 , and X_3) on the response TS was mainly affected by drug:polymer ratio (X_1) (positive correlation) and concentration of plasticizer (X_3) (negative correlation) which indicates that as polymer concentration increases, TS increases. This enhancement in TS could be due to the increase in molecular chain of the polymer which provides more rigidity and hence the mechanical stability to the patch.^[13] On the contrary, as the plasticizer concentration increases, TS decreases. This could be due to plasticizer molecules which disrupts the interchain cohesive forces of polymer and hence reduce the mechanical strength of the patch. These findings are in good concurrence with the literature.^[26,33] The interaction effects of the independent variables on the TS are shown in Figure 4 by portraying the 3D response surface graphs.

Influence of independent variables on flux (Y_2)

The flux of clozapine patch varied in the range of 76.77 ± 1.02 $\mu\text{g}/\text{h}/\text{cm}^2$ – 119.32 ± 1.58 $\mu\text{g}/\text{h}/\text{cm}^2$ as a result of the variation in the independent variables. The polynomial equation with the coefficients of the model showing the relationship between the factors and flux was as follows:

$$Y_2 = 104.13 - 3.67 X_1 + 12.22 X_2 + 2.68 X_3 - 0.26 X_1 X_2 - 0.64 X_1 X_3 + 2.17 X_2 X_3 - 11.55 X_1^2 - 1.29 X_2^2 - 2.27 X_3^2 \quad (7)$$

Here, correlation coefficient R^2 was found to be 0.9931, indicating a good fit. From Equation (7), it is evident that the quantitative effect of independent variables (X_1 , X_2 , and X_3) on the response flux was mainly affected by the concentration of PE (X_2) and concentration of plasticizer (X_3) (positive correlation) as well as drug:polymer ratio (X_1) (negative correlation) which indicates that as amount of PE increases, flux increases. This enhancement could be due to the increase in partitioning of drug in lipophilic membrane of skin which leads to enhanced lipid fluidization and thereby increases permeation.^[56] In addition to this, increase in concentration of plasticizer also enhances the permeation of the drug. This could be due to the disruption of the interchain cohesive forces of polymer. As concentration of plasticizer increases, flexibility and mobility of polymer chain also increase, thus resulting in improved release of drug from matrix patch system.^[33] On the contrary, as the polymer concentration increases, flux decreases. This could be due to the fact that increase in polymer amount increases the viscosity of polymer solution which further retards the diffusion of drug molecules from the patch to the skin surface. These outcomes are in good concurrence with the literature where it is reported that as the concentration of rate controlling polymer increases, permeation decreases.^[36,57] The interaction effects of the independent variables on the flux are shown in Figure 5 by representing the 3D response surface graphs.

Influence of independent variables on Q_{20} (Y_3)

Q_{20} of clozapine patch varied in the range of $64.05 \pm 2.59\%$ – $94.32 \pm 2.43\%$ as outcome of the variation in the independent variables. The polynomial equation with the coefficients of

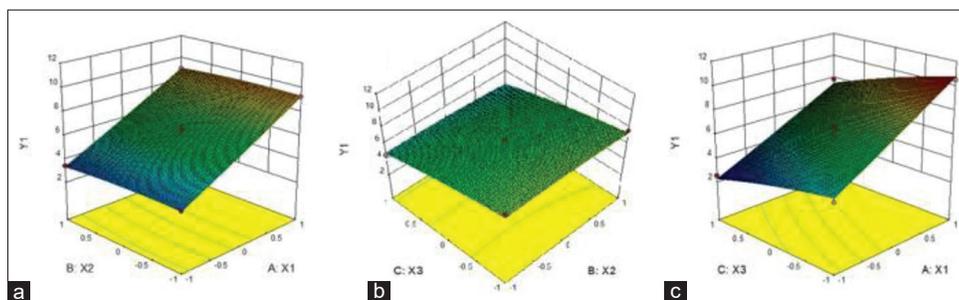


Figure 4: (a-c) Three-dimensional response surface plot for tensile strength

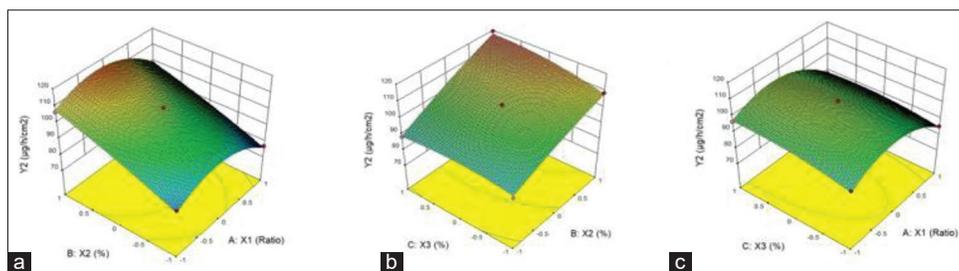


Figure 5: (a-c) Three-dimensional response surface plot for flux

the model showing the relationship between the factors and Q_{20} was as follows:

$$Y_3 = 82.00 - 11.05 X_1 + 0.94 X_2 + 4.36 X_3 - 0.10 X_1 X_2 + 1.25 X_1 X_3 - 0.025 X_2 X_3 + 0.21 X_1^2 + 0.78 X_2^2 - 0.89 X_3^2 \quad (8)$$

Here, correlation coefficient R^2 was found to be 0.9929, indicating a good fit. From Equation (8), it is evident that the quantitative effect of independent variables (X_1 , X_2 , and X_3) on the response Q_{20} was mainly affected by the drug:polymer ratio (X_1) (negative correlation) and concentration of plasticizer (X_3) (positive correlation) which indicates that as concentration of polymer increases, Q_{20} decreases. This decrement could be due to the retarding effect of polymer which ultimately formed the viscous barrier around patch and hindered the drug release in dissolution media.^[21] On the contrary, as the plasticizer concentration increases, Q_{20} increases. This could be due to disruption of the interchain cohesive forces of polymer within creased flexibility and mobility of polymer chain molecules.^[33] The interaction effects of the independent variables on the Q_{20} are shown in Figure 6 by depicting the 3D response surface graphs.

Optimization and validation

Based on the polynomial models, the result of 3D response surface plots exerts the impact of significant independent factors on each observed response. To establish the correctness of the optimization procedure validation, a checkpoint analysis was carried out. Hence, two new checkpoint batches (batch F_{16} and F_{17}) were prepared. There was magnificent concurrence between the measured practical responses and predicted theoretical responses. The experimental values were in close proximity to the predicted values, with very low percentage of bias, propounding that the applied mathematical model was reliable, and hence, the proposed model could be used to navigate the design space [Table 5]. Design-Expert software

(version 10.0) was used to identify optimum conditions for clozapine matrix patch by which desirability value was found to be 0.9041. Based on the results obtained from BBD, batch F_{15} was chosen as the best formulation (chosen from the experimental batches). The best batch was selected based on the desired constraints having TS value of 6.84 ± 0.64 MPa (>4 MPa), flux of 104.80 ± 1.39 $\mu\text{g}/\text{h}/\text{cm}^2$ (>100 $\mu\text{g}/\text{h}/\text{cm}^2$), and Q_{20} of $82.19 \pm 1.12\%$ ($>80\%$). Moreover, depending on the desirability criteria, the predicted values of optimized batch suggested by software for TS were found to be 6.80 MPa, with flux value of 104.13 $\mu\text{g}/\text{h}/\text{cm}^2$ and Q_{20} value of 82.00%. As the predicted values of optimized formulation for above-mentioned responses were considered similar to the actual values, batch F_{15} was selected as best batch and evaluated for further characterization.

FTIR studies

The FTIR spectra of pure clozapine demonstrated characteristic peaks at 3296.71 cm^{-1} (N-H stretching), 2969.84 cm^{-1} (C-H stretching), 1551.45 cm^{-1} (C=N stretching), 1456.56 cm^{-1} (aromatic C=C stretching), and 822.49 cm^{-1} (C-Cl stretching) [Figure 7]. Peaks of HPMC, physical mixture of polymers, and blank patch were assigned at 2900 cm^{-1} (C-H stretching), 2550 – 2500 cm^{-1} (O-H stretching), 1650 – 1600 cm^{-1} (C-O stretching), and 1400 – 1350 cm^{-1} (C-O-C stretching). Peaks of the FTIR spectra for physical mixture of clozapine and polymers were detected at the same position as that of drug, namely, at 3296.71 cm^{-1} (N-H stretching), 2969.84 cm^{-1} (C-H stretching), 1552.42 cm^{-1} (C=N stretching), 1456.96 cm^{-1} (aromatic C=C stretching), and 822.49 cm^{-1} (C-Cl stretching).^[58] This result suggested that there was no interaction between drug and polymer. Peaks of the FTIR spectra for matrix patch were detected at 3289.24 cm^{-1} (N-H stretching), 2966.95 cm^{-1} (C-H stretching), 1553.38 cm^{-1} (C=N stretching), 1456.96 cm^{-1} (aromatic C=C stretching), and 821.52 cm^{-1} (C-Cl stretching).

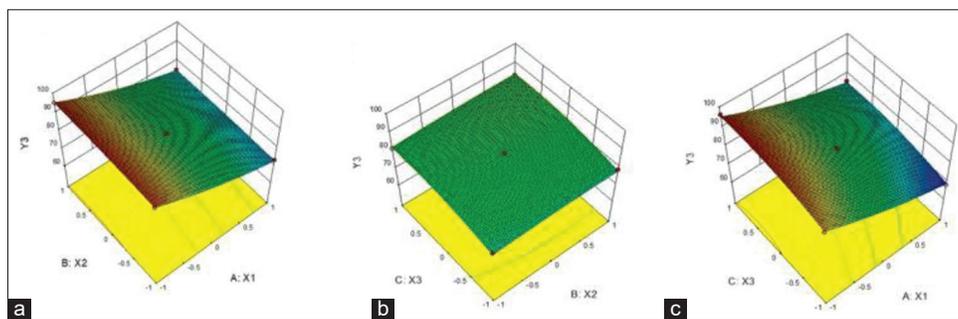


Figure 6: (a-c) Three-dimensional response surface plot for Q_{20}

Table 5: Predicted and observed responses for checkpoint batches (mean \pm SD, $n=3$)

Batch	X_1 (Ratio)	X_2 (%)	X_3 (%)	Y_1 (MPa)		Y_2 ($\mu\text{g}/\text{h}/\text{cm}^2$)		Y_3 (%)	
				Observed	Predicted	Observed	Predicted	Observed	Predicted
F_{16}	1:2.5	7.5	22.5	4.41 \pm 0.56	4.60	104.56 \pm 1.28	104.01	91.57 \pm 1.79	89.23
F_{17}	1:3.5	7.5	17.5	8.59 \pm 0.71	8.86	96.14 \pm 1.54	97.66	72.26 \pm 1.13	73.81

In IR spectrum of clozapine, the absorption at 3296.71 cm^{-1} was assigned to the stretching vibration of N-H group which was red shifted to the wavenumber of 3289.24 cm^{-1} for clozapine formulation.^[59] As reported in literature, red shift is attributable for the intermolecular hydrogen bonding,^[60] the same has been observed in case of the matrix patch of clozapine.

the melting point of clozapine [Figure 8]. The DSC analysis of the physical mixture of polymer and drug also revealed characteristic peak of drug molecule, which got disappeared from the DSC thermogram of optimized batch. It could be achieved because of the homogenous molecular dispersion of the drug in the polymeric matrix.^[61]

DSC studies

For analysis, thermogram derived for pure drug revealed an intense endothermic peak at 188.59°C , commensurable with

Skin irritation study

The main objective of the skin irritation study was to evaluate the skin irritation potential of the optimized patch

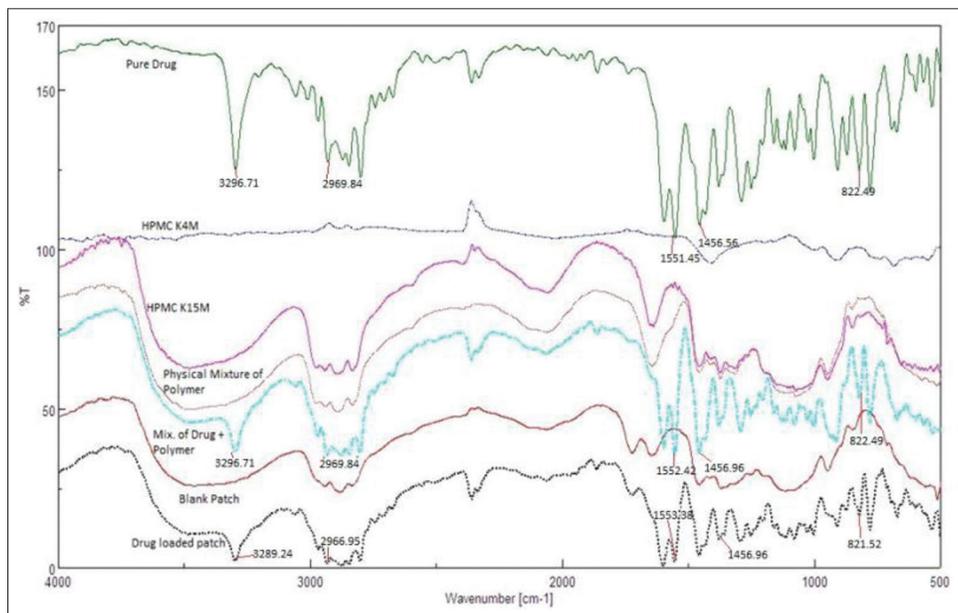


Figure 7: Fourier transform infrared spectra of pure drug, polymer, physical mixture, blank patch, and optimized transdermal patch

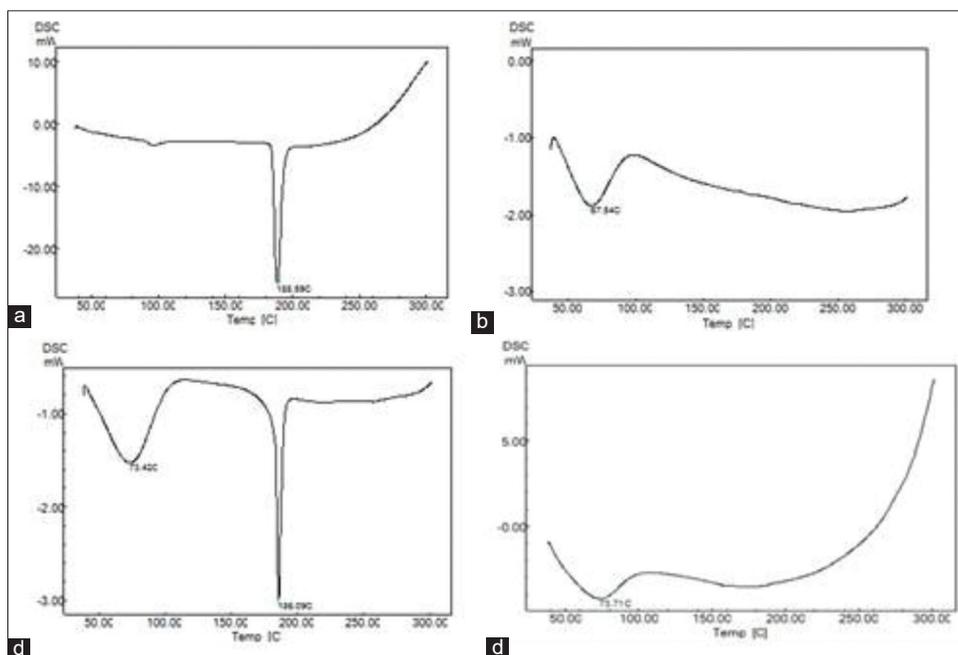


Figure 8: Differential scanning calorimetry curve for (a) pure drug, (b) physical mixture of polymers, (c) physical mixture of drug and polymers, and (d) optimized transdermal patch

of clozapine. Scores for placebo, test, and positive control are provided in Table 6. According to Draize scoring criteria, the scores of 2 or less are contemplated as negative (no skin irritation).^[57] Draize score for the present study was found up to 0.67 ± 0.52 and 0.83 ± 0.41 for erythema and edema, respectively. Hence, the transdermal patch of clozapine was found to be free from any kind of skin irritation and can be considered safe and well enduring for the transdermal drug delivery.

Stability study

Stability study is an extremely important component in the assessment of transdermal patches as it reveals the crystallization feature of drug dispersed in the patch at the time of storage. The results of the study divulged the physical appearance unchanged for the formulation after 6 months. The drug content present in transdermal patch was found to be $98.24 \pm 0.41\%$ after 6 months, while TS, Q_{20} , and flux were found to be 6.64 ± 0.15 MPa, $82.52 \pm 1.09\%$, and 102.67 ± 1.45 $\mu\text{g}/\text{h}/\text{cm}^2$, respectively. Above outcomes were insignificant ($P > 0.05$) when compared with the flux, TS, and drug content obtained from the fresh patches [Table 7]. The results of flux and drug content values of stored patches under accelerated stability conditions for 6 months confirmed the homogenous dispersion of the drug without any signs of crystallization. Hence, this proves the development of a good stable product.

In vivo pharmacokinetic studies

The pharmacokinetic characteristics of clozapine after oral administration and after application of transdermal patch were evaluated using Wistar rats. Figure 9 depicts clozapine plasma profiles for transdermal patch and oral clozapine marketed formulation LOZAPINE® tablets. The pharmacokinetic parameters of clozapine are presented in Table 8.

Results of *in vivo* studies showed C_{max} of transdermal patch as 685.39 ± 54.17 ng/mL compared to 806.34 ± 69.75 ng/mL of marketed product. The high C_{max} and short T_{max} values of clozapine were due to the rapid absorption from the gastrointestinal tract when given orally. On the contrary, the low C_{max} and prolonged T_{max} were achieved with the transdermal patch might be due to the barrier properties of the skin which lead to an early accumulation of drug in the skin followed by its sustained release into the systemic circulation.^[62] The $AUC_{(0-24)}$ value was significantly increased to $11,898 \pm 741$ ng. h/ml for optimized transdermal patch compared to 5470 ± 430 ng. h/ml for the oral marketed product. This could be attributed due to a mild reservoir effect leading to the slow exhaustion of drug accumulated in the skin tissues.^[42] This substantiates the significance of prepared sustained release topical matrix patches for better therapeutic profiles. The pharmacokinetic result showed that bioavailability of clozapine was ameliorated over 2.18-fold after transdermal drug delivery as compared to oral marketed formulation. This pronounced impact could be attributed to the avoidance of first pass hepatic metabolism which is a most common problem of oral route.^[36] Therefore, it can be concluded that the system developed improves

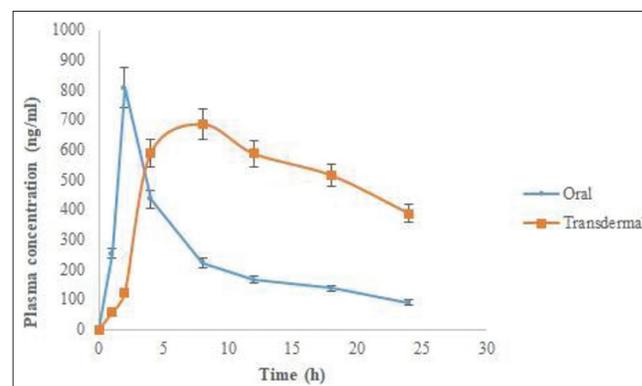


Figure 9: Plasma concentration versus time profiles of clozapine after oral administration and transdermal application

Table 6: Results of skin irritation of F15 batch

Group	Formulation	Erythema score			Edema score		
		24 h	48 h	72 h	24 h	48 h	72 h
Negative control	Placebo patch	0	0	0	0	0	0
Test batch	F ₁₅	0	0.17 ± 0.41	0.67 ± 0.52	0	0.33 ± 0.52	0.83 ± 0.41
Positive control	Formalin	2.67 ± 0.58	2.00 ± 0.58	1.33 ± 0.33	2.33 ± 0.58	1.67 ± 0.33	0.67 ± 0.33

Table 7: Stability study for optimized formulation (F15) in accelerated condition (mean \pm SD, n=3)

Time point	Drug content (%)	Tensile strength (MPa)	Flux ($\mu\text{g}/\text{h}/\text{cm}^2$)	Q 20 (%)
Initial	99.16 ± 0.73	6.84 ± 0.64	104.80 ± 1.39	82.19 ± 1.12
1 month	98.49 ± 0.59	6.69 ± 0.98	104.96 ± 1.63	83.26 ± 1.23
3 months	98.71 ± 0.67	6.61 ± 0.57	103.48 ± 1.37	81.37 ± 0.89
6 months	98.24 ± 0.41	6.64 ± 0.32	102.67 ± 1.45	82.52 ± 1.09

Table 8: Pharmacokinetic parameters of oral and transdermal clozapine formulations

Parameters	Oral	Transdermal
C _{max} (ng/ml)	806.34±69.75	685.39±54.17
T _{max} (h)	2	8
T _{1/2} (h)	7.76	20.27
AUC ₀₋₂₄ (ng. h/ml)	5470±430	11,898±741
Kel (h ⁻¹)	0.089	0.034
MRT (h)	12.12	30.58
AUCo-α (ng. h/ml)	6300±490	23,535±1280
Relative bioavailability (F)	----	2.18

pharmacokinetic profile of clozapine due to controlled and continuous release of drug into the systemic circulation over an extended period of time.

CONCLUSION

The results of preulative trials stipulated that the TS, flux, and Q₂₀ were remarkably influenced by the variables of formulations mainly ratio of drug:polymer, concentration of PE, and concentration of plasticizer. It was inferred that appropriate transdermal matrix patch for clozapine was formulated and optimized using 3-level and 3-factor BBD. *In vivo* studies on rats revealed the superiority of transdermal matrix patch over the oral formulation of clozapine in terms of enhanced bioavailability which was mainly achieved due to the avoidance of biotransformation. These studies showed encouraging results suggesting the convenient delivery of clozapine by formulating HPMC-based matrix system. The sustained release and superior bioavailability data of transdermal patch authenticate the advantage of topical drug delivery over the traditional dosage forms. Hence, the prepared formulation can be recommended for the effective low-dose maintenance therapy along with improved patient compliance.

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