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Research Article

Design of transdermal patch of ketoprofen by full factorial design for treatment of rheumatoid arthritis

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ABSTRACT

Oral therapy of NSAIDs for treatment of rheumatoid arthritis causes gastric irritation and ulceration. In the present study transdermal patch of ketoprofen was developed using hydroxyl propyl methyl cellulose E5 and Eudragit S100. Patches were prepared by solvent evaporation method. Optimization was carried out by 3^2 factorial design with polymer concentration (HPMC E5) and plasticizer concentration (propylene glycol) as independent variables. Patches were evaluated for folding endurance, surface pH, drug content, percent moisture content, water uptake and swelling studies. *Ex vivo* permeation studies of optimized patch was performed using Franz diffusion cell while bioadhesion force and tensile strength were measured by using texture analyzer. Hydrophilic nature, swelling ability and wettability of polymer and plasticizer were responsible for increase in flux and bioadhesion with increase in their concentrations in the factorial batches. Swelling index of all formulations was in the range of 17.3 ± 1.2 to 65.29 ± 4.78 up to 3h. Flux obtained from all batches was in the range of 3.37 ± 0.23 to $5.43 \pm 0.13 \mu\text{g}/\text{h}/\text{cm}^2$. Anti-inflammatory studies using carrageenan-induced rat paw edema showed greater paw swelling reduction in case of ketoprofen patch. Cumulative percent drug permeation of optimized patch through nylon 66, Wistar rat skin and cadaver skin was found to be $92.3\% > 86.28\% > 63.42\%$ in 8h, while flux order was $6.073 > 5.442 > 2.219 \mu\text{g}/\text{h}/\text{cm}^2$ respectively. The study concludes that transdermal patch of ketoprofen will be more efficacious with absence of gastric irritation observed in oral formulations.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disease of the joints characterized by synovial proliferation and inflammatory and immunological processes. These mechanisms lead to irreversible degradative and erosive changes in the articular cartilage and juxta articular bone. RA affects multiple joints, most commonly small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved^{1,2}.

Non-Steroidal Anti-Inflammatory Drug (NSAID) and steroids are used to reduce inflammation thereby decreasing pain and improving function. Disease-Modifying Antirheumatic Drugs (DMARDs) are required to inhibit the underlying immune process and prevent long-term damage^{1,3}. NSAIDs lead to gastrointestinal side effects such as dyspepsia to gastric bleeding⁴. The acidic character of NSAIDs may lead to local irritation, and lesions on the gastrointestinal mucosa are known as NSAIDs gastropathy⁵.

Ketoprofen (KTF) is a propionic acid derivative which causes inhibition of Cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway. This results in decreased levels of prostaglandin that mediate pain, fever and inflammation. KTF is a non-specific cyclooxygenase inhibitor and inhibition of COX-1 is responsible for its side effects, such as GI upset and ulceration⁶. Currently available marketed dosage form of KTF are enteric coated and extended release tablets, topical gel, liquid spray, suppositories, extended release capsules and formulation based on transfer some technology for direct application on the skin has been developed⁷.

Transdermal therapeutic systems are defined as, discrete dosage forms which, applied to the intact skin, deliver the drugs at a controlled rate to the systemic circulation via skin and this delivery system offers an advantageous alternative to conventional delivery system such as injections or oral delivery^{8,9}. Transdermal drug delivery system (TDDS) offers many advantages such as reduced side effects, less frequent administration to produce the desired constant plasma concentration associated with improved patient compliance,

elimination of the first-pass effect and sustained drug delivery. NSAID are mostly used for the preparation of transdermal patches for treatment of pain or arthritis¹⁰.

The objective of the present study was to design suitable transdermal matrix patch of (KTF) for the treatment of rheumatoid arthritis. The transdermal drug delivery system was optimized using 3² factorial designs. The patches were characterized for physicochemical parameters and also evaluated for swelling studies, ex-vivo effects of optimized batch on cadaver skin and rat skin and pharmacodynamics studies.

MATERIALS AND METHODS

Ketoprofen was obtained from BEC Chemicals Pvt. Ltd. Roha-Raighad, Mumbai, India. Hydroxy Propyl Methyl Cellulose E5, Eudragit S100 was procured from Evonik industries, Mumbai. Tween 80, Propylene Glycol, Glycerin, Ethyl Cellulose, Dibutyl phthalate was procured from Research Lab Fine Chem. Industries, Mumbai. All raw materials and chemical reagents used were of analytical grade and procured locally.

Pre-optimization study

During pre-optimization studies, polymers like Hydroxy Propyl Methyl Cellulose E5 (HPMC), Eudragit S100 (EGT), Xanthan gum, Sodium alginate, Polyvinyl pyrrolidone (PVP), Hydroxyl Ethyl Cellulose (HEC), Methyl Cellulose, plasticizers like Polyethylene Glycol 400 (PEG), Propylene glycol (PG), Dibutyl phthalate (DBT) and permeation enhancers like tween 80, oleic acid, Dimethyl sulfoxide (DMSO) were used for formulating transdermal matrix patches. Patches were prepared using each of these polymers in different ratios like 1:1, 1:2, 3:1:2, 2:2:1, 2:1 by solvent casting method.

Development of transdermal patch by solvent evaporation technique

The transdermal patches were prepared by solvent evaporation technique¹¹. Polymeric solutions were prepared

using polymers, HPMC E5 and EGT S100 in different ratios of 3:1, 4:1, 5:1 in mixture of chloroform: methanol (1:1 v/v). KTF was then added to this solution by calculating area of the petri dish so that final patch of 1cm would contain 50 mg of drug. Plasticizer (PG) and penetration enhancer (Tween 80) were added separately to the polymeric solution and poured in petri dish which was previously lubricated with glycerin and allowed to air dry overnight.

Preparation of backing membrane

Backing membrane solution containing 500mg of ethyl cellulose and 2% w/v DBT in 10ml acetone was poured into the glass petri dish and air dried for 1h¹². Patch with backing membrane was prepared by pouring backing membrane solution on preformed patch. Circular patches (diameter-9.2cm) were removed from petridish and placed in desiccators.

Drug polymer compatibility studies

Compatibility of drug and polymers like HPMCE5, EGT S100 was analyzed by FT-IR (Shimadzu- FTIR- 460 plus) for determination of drug polymers interactions. Drug was mixed with polymer in 1:1 ratio and samples were stored for 30 days at 40 ± 2°C/ 75 ± 5% RH. FT-IR spectra of these samples were then obtained after 30 days.

Experimental design

Optimization of transdermal patches was performed using a randomized 3² full factorial design. This design was carried out with Design Expert 9 software (State- Ease Inc, Minneapolis, USA) to study effect of two factors on response variables like flux, tensile strength and bioadhesion force. The two factors were ratio of polymers (HPMC: EGT S100) and concentration of plasticizer (PG) and three levels coded as -1, 0 and +1 respectively. The selected factor levels and composition of 3² factorial designs are given in Table 1.

Table 1: Composition for Trial Runs with Factor Level of 3² Factorial Design

Formulation code	Drug (KTF) (mg)	HPMC E5:EGT S 100	HPMC E5 (g)	EGT S100 (g)	Tween 80 (g)	PG (g)	Methanol :chloroform 1:1 (ml)
F1	50	3:1 (-1)	0.6	0.2	0.2	0.3(-1)	20
F2	50	3:1 (-1)	0.6	0.2	0.2	0.4(0)	20
F3	50	3:1 (-1)	0.6	0.2	0.2	0.5(1)	20
F4	50	4:1 (0)	0.8	0.2	0.2	0.3(-1)	20
F5	50	4:1 (0)	0.8	0.2	0.2	0.4(0)	20
F6	50	4:1 (0)	0.8	0.2	0.2	0.5(1)	20
F7	50	5:1 (1)	1	0.2	0.2	0.3(-1)	20
F8	50	5:1 (1)	1	0.2	0.2	0.4(0)	20
F9	50	5:1 (1)	1	0.2	0.2	0.5(1)	20

Factor level shown in bracket (-1) low, (0) medium (1) high while EGT-Eudragit and PG-Propylene glycol.

Physicochemical evaluation of Transdermal Patches

Thickness, flatness and weight variation

The thicknesses of the formulated patches were measured at four different points using a thickness dial gauge (Mitutoyo, Japan) and average thickness was calculated¹³. To study flatness, 3 longitudinal strips were cut out from each patch. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness¹⁴. Constriction (%) = (L1-L2) / L2 X 100 Where, L1 initial length of each strip, L2 final

length. Such determination was performed for each formulation and mean value was calculated¹⁵.

Surface pH

The surface pH of the patches was determined by placing the probe of the pH meter in close contact with the wetted surface of the patch. The pH of three randomly selected films was measured using a pH meter (Deluxe pH meter 101, India)¹⁶.

Folding endurance

The films were repeatedly folded in the same place 300 times or until they broke, whichever was less, to determine their folding endurance¹⁷.

Drug content

The drug content was determined using a UV-visible spectrophotometer (Lab India Analytical UV 3000, India). Patches of 1cm² were cut and placed in test tubes containing 10ml methanol for 24h to extract the drug. This solution was filtered and further diluted with methanol and analyzed by UV-visible spectrophotometry at a wavelength of 254nm. Drug content was calculated using equation obtained from the standard calibration curve of KTF¹⁸.

Determination of percent moisture content and moisture uptake

The films were weighed individually and kept in a desiccators containing activated silica at room temperature for 24h¹⁹. Individual films were weighed repeatedly until they show constant weight. Percentage moisture content was calculated by using the following formula:

Percent Moisture Content =

$$\frac{\text{Initial weight} - \text{final weight}}{\text{final weight}} \times 100$$

The films were also subjected to moisture uptake studies by exposing the patches to 84% RH and ambient temperature in a desiccators containing saturated solution of potassium chloride until a constant weight was achieved²⁰. Then the films were weighed and percentage moisture uptake was calculated by using the following formula:

Percentage moisture uptake =

$$\frac{[\text{Final wt} - \text{Initial wt.}] / \text{Initial wt.}}{\times 100}$$

Swelling studies

Patches of 1cm² were weighed separately (initial weight = W1) and placed in petri plates containing 6ml distilled water. The swollen films were weighed individually at regular time intervals up to 3h (Final weight = W2). Swelling index of each system was calculated using the following formula²¹:

$$\text{Swelling index} = (W2 - W1) / W1 \times 100$$

Ex vivo permeation studies

The permeation of KTF from all transdermal patches were studied using excised rat skin. Male Wistar rats were sacrificed by cervical dislocation with prior permission from the Institutional Animal Ethics Committee (Protocol approval No. CPCSEA/IAEC/PT-03/01-2K 16). Skin was shaved and the subcutaneous fat and connective tissue was removed and the excised skin was washed with phosphate buffer pH 6.8 and skin was stored in the freezer at -20°C. Saline phosphate buffer pH 7.4 (7ml) was filled in the receiver compartment of the Franz diffusion cell. Excised rat skin was positioned between the donor and receiver compartment and the transdermal patch of area 3.14cm² was placed onto the skin. The buffer was continuously stirred with a magnetic bead and the entire assembly was maintained at 32 ± 0.5°C. Samples were withdrawn at 1h time intervals up to 8h and analyzed by UV-visible spectrophotometry at 258nm to determine the amount of drug permeated. The flux (J) was calculated from the graph slope of percent permeation Vs time in h^{22, 23}.

Tensile strength

Tensile strength of the patches was determined using a texture analyzer (CT-3/10,000, Brookfield, USA) equipped

with a 10kg load cell. Patches of 2cm² area were randomly selected and fixed between the two clamps of probe and for a hold time of 60s. The film was pulled apart at a speed of 2.0mm/s by the upper clamp²⁴. The peak load corresponding to the time the film broke was recorded. The tensile strength was calculated using formula:

Tensile Strength (M Pa) =

$$\frac{\text{Peak load at break (kg)}}{\text{Initial cross-sectional area (mm}^2\text{)}} \times 9.80665$$

Bioadhesion studies

The bioadhesion force was measured using Texture Analyzer (CEB Texture Analyzer, Make-Brookfield Engineering Labs, Inc., Model Texture Pro CT V1.4 Build 17) equipped with a 100g load cell. The measurement of bioadhesive force was done on excised rat skin as the model membrane. The rat skin was tied to the probe and the patch was placed between two circular discs which were at lower Perspex support and was wetted with 10 µl of distilled water. The probe and circular cavity were aligned to ensure that film comes into direct contact with exposed surface of rat skin membrane. The probe with the skin was lowered at a speed of 1.0mm/s and skin was kept in contact with the patch for 20s with 80g load. Data collection and calculations were performed using Texture- Pro CT V1.3 Build 14 software²⁵.

Ex vivo permeation studies on cadaver skin

Human cadaver skin was obtained from Sassoon Hospital after taking prior permission of Head of Forensic Medicine and Toxicology Department. All subcutaneous fat from freshly excised human cadaver epidermis (from the thigh region of a male cadaver of age 41 years within 24–48h post-mortem) was removed with the scalpel. The sample was cut to appropriate size and kept in saline phosphate buffer pH 7.4 solutions overnight at room temperature and then washed several times with phosphate buffer pH 7.4 before use²⁶. The *ex-vivo* permeation of the optimized patch was studied for duration of 8h using Franz diffusion cell by previously reported procedure²⁷.

Skin irritation studies

The irritancy of the selected films was evaluated by Draize method on Wistar rats (200-210gm). The rats were anesthetized with ether and dorsal side of the rats was shaved 24h before the beginning of the experiment. The animals were divided into 3 groups each consisting of 6 rats. Group A served as control; Group B as standard and received 0.5ml of a 0.8% v/v aqueous formalin solution as a standard irritant. Group C was the test group which received medicated films for 3 days (new patch was applied daily). The application sites were examined for edema and erythema after 24h and 72h and then graded from 0-4 according to a visual scoring scale by the same investigator; the final score represented the average of the 24 and 72h readings. The primary irritancy index (PII) was determined for each preparation by adding the edema and erythema scores, the formulations was accordingly classified as non-irritant if PII < 2, irritant if (PII = 2-5) and highly irritant if PII = 5-8¹⁸.

Pharmacodynamic test (Carrageenan induced edema model)

Carrageenan induced rat hindpaw edema method was used to evaluate the anti-inflammatory activity of the formulation. Wistar rats weighing approximately 150-200g were divided into 3 groups each consisting of 6 animals. In each group the left hind paw of each rat was marked, just beyond tibiotarsal junction, so that every time the paw is dipped up to the fixed mark to ensure constant paw volume. Group A served as

control group while group B was the standard group which was given KTF suspension orally (15mg/kg; 4mg/ml KTF suspension). The transdermal patch was applied to Group C after removing the hair from the dorsal side of rats. The paw volume was measured with a plethysmometer prior to the injection. Acute inflammation was produced by injecting 0.1 ml of 1% w/v carrageenan solution in the sub plantar region of the left hind paw. The paw volume was determined after 0, 1, 2, 4, and 6h. The percentage paw swelling was calculated by the following formula²⁸.

$$\% \text{ paw swelling} = (B - A) \times 100 / A$$

Where,

A is the paw volume before induction of edema and

B is the paw volume measured hourly after induction of edema.

Stability studies

The stability studies were conducted as per ICH guidelines to investigate the influence of temperature and relative humidity on the drug content in different formulations. The optimized transdermal formulations were stuck on an adhesive backing membrane, covered with a release liner and stored in an aluminum foil. They were subjected to stability studies for 3 months using storage conditions $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$ as per ICH guidelines²⁹. Samples were taken after third month and evaluated for percent drug content, thickness, percent flatness and folding endurance.

RESULT AND DISCUSSION

Pre optimization results

The polymer combination of Xanthan gum: HPMC E5: MC produced sticky and opaque films. Some of the polymers like combination of PVP, sodium alginate and methyl cellulose formed brittle and inferior quality film. HPMC E5 and PVP in a ratio of 1:1 with PG as plasticizer and oleic acid as permeation enhancer had very good film properties. Xanthan gums in combination with other polymers produce sticky films. The polymers which produced transparent and flexible films with smooth appearance were found to be HPMC E5 and EGT S100 in a ratio of 2:1 with propylene glycol as plasticizer, tween 80 as permeation enhancer using methanol and chloroform (1:1 v/v) as solvent system. The film so produced was evaluated for pH, folding endurance, thickness, percent weight variation and percent drug content. Thickness, percent weight variation and drug content was found to be 0.27 ± 0.006 , 0.25 ± 0.1 and 99.49 ± 0.098 respectively. The pH and folding endurance were found to be 6.4 ± 0.04 and 398 ± 0.083 respectively.

Drug polymer compatibility studies

FTIR analysis of HPMC E5 and EGT S100 film formulations containing KTF did not reveal any additional peak for the drug are shown in Figure 1 which pointed to lack of interaction between drug and polymers used in the films. FTIR spectra showed compatibility of KTF with HPMC E5, EGT S100, and the functional group signals observed were -OH stretch (Carboxylic acid), C=C (Aromatic) stretch, C-O (Phenolic) stretch, C=O (Ketone) stretch, O-H (Carboxylic acid) Bending, C-H (Aromatic) in plane Bending, C=O stretching and bending at wave number range 2977.55, 1597.73-1443.46, 1284.36-1319.07, 1698.98, 1419.35, 1078.01-1133.94, 1133.94-1284.36 cm^{-1} respectively.

Figure 1: FTIR Spectra of KTF (A), KTF + HPMC E5 (B) and KTF + EGT S 100 (C) and KTF+HPMC E5+EGT S100 (D)

Optimization

A 3^2 factorial design was employed to investigate the effect of independent variables on flux, tensile strength, and bioadhesion force. This design was carried out with Design Expert 9 software (State- Ease Inc, Minneapolis, USA) to study effect of polymer combination of HPMC E5: EGT S 100 and plasticizer as PG. The two factors HPMC E5: EGT S100 ratio and PG were used at three levels -1, 0 and +1 for optimization is shown in Table 1.

Ex vivo permeation studies

Ex vivo permeation studies of KTF film formulation through rat skin showed slow and sustained permeation of the drug up to 8h are shown in Figure 2. The rank order of drug permeation from films was found to be $F_9 > F_8 > F_7 > F_6 > F_5 > F_4 > F_3 > F_2 > F_1$. The patch (F9) containing higher polymer concentration of HPMC E5 and plasticizer concentration (2.5% w/v) showed maximum flux ($5.43 \pm 0.13 \mu\text{g/h/cm}^2$). It has been widely reported that addition of hydrophilic component to an insoluble film former leads to enhanced release rate constant³⁰. Permeation study revealed that drug release increases as plasticizer and bioadhesive polymer concentration increases. F9 patch maximum release of 86.28 % in 8h among other patches.

Figure 2: Permeation profiles of KTF from all the nine formulations.

Flux is rate of mass transfer across a unit surface area of a barrier and it gives idea related to permeability³¹. The 3D graph showed (Figure 3) that the flux across the membrane increased with increase in polymer and plasticizer concentration. This could be due to the extensive swelling of the hydrophilic polymer and plasticizer³². PG was used as plasticizer which has additional property of penetration enhancer^{33, 34} and tween 80 (1%w/v) that may result in enhancement of permeation and flux. Penetration enhancement effect of tween 80 is due to interaction with intracellular protein to enhance penetration through the corneocytes, and disruption of the highly ordered structure of stratum corneum lipid with an increase in intercellular diffusivity³⁵. PG can absorb moisture into the film because of its humectants ability, thus contributing to higher flux, though it has been used as a plasticizer in the patches³⁶.

ANOVA analysis of the coefficients of the polynomial equations (eq.1) and the F-value of 32.71 implied that the quadratic model is significant.

$$\text{Flux} = 4.02 + 0.85 A + 0.26 B + 0.10 AB + 0.20 A^2 + 0.085 B^2 \dots (1)$$

Where,

A- Polymeric concentration (HPMC E5: EGT S100),

B- plasticizer concentration (PG)

Equation (1) showed that high levels of factor A gave high value of flux as evident by the higher coefficient. These results confirm the interpretation of response surface graphs.

Figure 3: 3D response surface plot showing the influence of HPMC E5: EGT S100 and PG concentration on the flux, Tensile strength and bioadhesion force.

Tensile strength

The data for all formulations for tensile strength (Table 2) indicated that films have sufficient tensile strength. It is evident from the contour and response surface plots (Figure 3) that the tensile strength increases as the concentration of plasticizer increases. Polymer concentration also has an effect on tensile strength which can be attributed to the excellent film forming properties of SHPMC E5 and EGT S100³⁷.

The same is evident in the 3D response surface plots (Figure 3). Plasticizers cause a reduction in polymer-polymer chain secondary bonding and form secondary bonds with the polymer chains instead. The reasons for the use of plasticizers in transdermal drug delivery systems are the improvement of film forming properties and the appearance

of the film^{38,39}. Propylene glycol may be forming hydrogen bonds with HPMC E5 resulting in greater flexibility of films at higher plasticizer concentration. When both factors are increased simultaneously a prominent rise in the tensile strength was observed. Also it is very noticeable that high polymer concentration and low plasticizer concentration did not result in significant increase in the tensile strength. Reason behind increase in tensile strength after increase polymer and plasticizer could be cross linking structures of polymer (HPMC E5) that improves its elongation ability and reduction of fragility of polymer by plasticizer⁴⁰.

The coefficients of the polynomial equations (eq.2) and the model F-value of 27.73 supported the above findings.

$$\text{Tensile Strength} = 6.00 + 2.39A + 2.68B + 1.57AB - 0.89A^2 - 0.32B^2 \dots (2)$$

Table 2: Responses of Factorial Design Formulations

Formulation code	Flux ($\mu\text{g}/\text{h}/\text{cm}^2$) $\pm$ SD	Tensile strength (MPa X 10^{-3}) $\pm$ SD	Bioadhesion force (N X 10^{-2}) $\pm$ SD
F1	3.37 $\pm$ 0.23	1.70 $\pm$ 0.42	1.17 $\pm$ 0.23
F2	3.41 $\pm$ 0.12	2.40 $\pm$ 0.78	1.47 $\pm$ 1.86
F3	3.52 $\pm$ 0.02	3.43 $\pm$ 1.78	1.86 $\pm$ 0.07
F4	3.69 $\pm$ 0.19	2.14 $\pm$ 0.83	3.04 $\pm$ 0.42
F5	3.98 $\pm$ 0.42	6.73 $\pm$ 1.35	4.31 $\pm$ 1.12
F6	4.55 $\pm$ 0.34	8.49 $\pm$ 0.12	5.003 $\pm$ 0.39
F7	4.87 $\pm$ 0.49	3.38 $\pm$ 0.75	5.073 $\pm$ 0.16
F8	5.07 $\pm$ 0.04	7.10 $\pm$ 1.09	5.39 $\pm$ 1.55
F9	5.43 $\pm$ 0.13	11.4 $\pm$ 1.87	5.68 $\pm$ 1.74

Values are expressed as mean of triplicate reading $\pm$ standard deviation

Bioadhesion Force

Bioadhesion of the patches may be attributed to the wetting and swelling of HPMC E5 and penetration of the polymer chains into the cracks or crevices of the skin⁴¹. This can be correlated with the swelling index of the patches which is discussed in further section. Formulation F9 having a high swelling index value of 65.29 ± 4.78 % showed good bioadhesion properties. The contour and 3D response surface plots (Figure 3) show that the bioadhesion force was found to increase with increase in the concentration of HPMC E5. Similarly increase in plasticizer (PG) concentration also enhanced adhesion due to softening effect exerted by the plasticizer⁴².

The final (eq.3) of Bioadhesion force in terms of coded factors is as follows:

$$\text{Bioadhesion Force} = 4.17 + 1.94A + 0.54B - 0.021AB - 0.68A^2 - 0.086B^2 \quad \dots\dots(3)$$

The Model F-value of 24.55 implied that the quadratic model is significant.

Validation of optimum design

To obtain an optimum formula and justify the validity of the optimization, the desirability approach was used. Design expert software generated four optimum batches (O1 to O4) based on the input selection criteria which was flux in the range of 5 to $6\mu\text{g}/\text{h}/\text{cm}^2$, tensile strength $10 \times 10^{-3}\text{MPa}$ and $12 \times 10^{-3}\text{MPa}$ and bioadhesion force in the range of 5×10^{-2} to $6 \times 10^{-2}\text{N}$ (Table 3). All optimum batches were found to have desirability of approximately 1. Comparison of predicted and observed values and residual error for batch O1 were found to be in good agreement for all the three responses. Also, desirability about 0.991 and low magnitude of residual error designates a high prognostic ability of response surface methodology. Hence the optimum formulation O1 was selected for the further pharmacodynamic studies.

Table 3: Comparison of Observed and Predicted Responses for Validation Batches

Batch code	Polymer (g)	Plasticizer (g)	Tensile Strength (MPa X 10^{-3})		Flux ($\mu\text{g}/\text{h}/\text{cm}^2$)		Bioadhesion Force (N X 10^{-2})		Desirability
			P*	O*	P*	O*	P*	O*	
O1	1.200	0.495	11.26	11.24	5.48	5.44	5.857	5.961	0.992
O2	1.200	0.492	11.15	11.16	5.47	5.45	5.846	5.765	0.987
O3	1.200	0.474	10.486	10.47	5.38	5.27	5.77	5.531	0.949
O4	1.179	0.500	11.204	11.21	5.37	5.37	5.80	5.845	0.980

P*- predicted value, O*- observed value

Evaluation of factorial transdermal patches

Thickness, flatness, weight variation and drug content

The nine transdermal patches, prepared as per 3^2 factorial designs, were subjected to preliminary evaluation. All the patches were found to be very transparent, flexible and uniform in appearance. The thickness of the patches was found to be in the range of 0.173 ± 0.015 to $0.27 \pm 0.012\text{mm}$ thickness confirmed that the patches prepared were uniform in thickness, while the percent weight variation of patch ranged between 0.0927 ± 0.0351 to $0.1209 \pm 0.0027\text{g}$. The percent flatness study showed that all the formulations (F1 to F9) had the same strip length before and after their cuts, with percent flatness range between 98.39 ± 0.1 to $99.83 \pm 0.9\%$. All patches had a smooth, flat surface with no constriction^{43,44}. The drug content was found to be in range of 97.45 ± 0.19 to 99.87 ± 0.47 indicated that the drug was uniformly dispersed in the polymeric matrix patch.

Surface pH

The surface pH value of all patches (6.3 to 6.5) was close to human skin pH (6.4) which means that there will be no skin irritation and therefore the patches will have good patient compliance as the patch was intended for once-a-day application. A very high and low pH value can be harmful to the skin⁴⁵.

Folding endurance

The folding endurance of all patches were above 300 thus considered satisfactory to reveal good patch properties^{46,47} and F1 to F3 batches displayed lower folding endurance 171 ± 0.89 to 252 ± 0.42 hence, all patches indicated that the patches would retain their integrity and not break or crack during general skin folding due to bodily movements⁴⁶. Higher folding endurance supports the results of tensile strength⁴⁸.

Percent moisture content and moisture uptake

The moisture content in the patches ranged from (2.7% to 6.8% w/w). The moisture uptake in the formulations ranged from (1.34% to 3.75% w/w) in a span of 24 h. The moisture absorption and moisture content was found to increase with increasing concentration of hydrophilic polymers (HPMC E5)³⁷ as well as hydrophilic plasticizer (PG). Small moisture content in the formulations is desirable as it helps the films to remain stable and avoids formation of a completely dried and brittle film¹¹.

Swelling index

Swelling behavior of films as a function of time is illustrated in Figure 4. Formulation F9 having highest concentrations of

HPMC E5 polymers was found to have the highest swelling index of 65.29 ± 4.78 as HPMC E5 as hydrophilic polymer has high capacity of water uptake^{49,50}. The polymer will then undergo simultaneous swelling, dissolution and diffuse into the bulk medium resulting in erosion of the polymer⁵¹. However, an excessive water uptake causes a leakage in cohesiveness of dosage forms transforming the formulation into over-hydrated slippery mucilage. This will result in a drop in adhesive strength due to disentanglement at the polymer tissue interface²¹. The rate and the extent of patch hydration and swelling also affects the patch adhesion and consequently the drug release from the patch⁵². In our study the patches were found to retain their integrity as only a nominal amount of water was taken up in a span of 3h.

Figure 4: Swelling study of KTF transdermal Patch.

1 h [■] 2 h [■] 3 h [■]

Ex vivo skin permeation in cadaver skin

Permeation study of optimized batch (O1) through human cadaver skin was carried out using Franz diffusion cell. Permeation profile of optimized (O1) patch was slower and sustained release up to the 8h was obtained. The cumulative percent drug permeated at 8h was found to be 63.42 % (Figure 5) and flux was computed as $2.219 \pm 0.68 \mu\text{g}/\text{h}/\text{cm}^2$.

Figure 5: Permeation profiles of optimized batch through different membranes.

(—●—) cadaver skin, (—■—) Wistar rat skin,
(—◆—) nylon membrane.

The permeability studies of optimized (O1) formulation were carried out across rat and cadaver skin and nylon 66 model membranes. Comparative studies showed slower drug permeation in human cadaver skin as compared to rat skin. Human skin is a multilayered organ which causes an efficient permeation obstacle for exogenous molecules⁵³. The rank order of drug permeation from optimized (O1) patch was found to be Nylon membrane > rat skin > cadaver skin (Figure 5). Cumulative percent drug permeation order of three different model membranes was found to be $92.3\% > 86.28\% > 63.42\%$ and flux value was to be $6.073 > 5.442 > 2.219 \mu\text{g}/\text{h}/\text{cm}^2$ respectively.

Skin irritation study

Skin irritation studies of optimized KTF transdermal patch average skin irritation score was about (erythema) 0.66 ± 0.0173 and standard irritant formalin score (erythema) 2.50 ± 0.0473 . According to Draize test²⁷, compounds producing scores of 2 or less are considered as safe for use on skin (no skin irritation). Thus we may infer that none of the components of the patch will result in skin irritation and consequent discomfort.

Pharmacodynamic studies (Carrageenan induced hind paw edema model)

Development of paw edema in rats induced by carrageenan is commonly correlated with the early exudative stage of inflammation, one of the important processes of inflammatory pathology⁵⁴. Carrageenan is a sulphated polysaccharide obtained from a sea weed (*Rhodophyceae*)⁵⁵. In this test the efficacy of the optimized KTF transdermal patch was compared with orally administered KTF. The

percent paw swelling for each group is given in comparative plot of percent paw swelling (Figure 6). The average percent paw swelling reduction values of all groups were compared statistically using one-way ANOVA. The percent paw swelling for standard and test group was significantly less than the control group ($p < 0.01$). However, there wasn't any significant difference in the efficacy of the oral KTF and transdermal patches ($p > 0.05$). The average percent paw swelling of KTF patch and KTF oral suspension was found to be 58.60 ± 6.34 and 64.39 ± 3.86 respectively. Hence, it can be concluded that the KTF transdermal patches are as effective as the orally administered drug. However chronic oral administration of KTF or other NSAIDs for symptomatic treatment of arthritis results in severe gastric irritation and ulceration which is absent in transdermal delivery. Thus we may conclude that a transdermal NSAID patch is more effective without the concomitant gastric damage.

Figure 6: Comparative plot of percent paw swelling.

(—◆—) control (—■—) oral suspension
(—▲—) KTF transdermal patch

Stability studies

In order to determine the change in physicochemical parameter on storage, stability studies were carried out. The physicochemical parameter of the optimized O1 formulation did not significantly change on storage. The thickness, percent drug content, flatness and folding endurance of the optimized patch was found to be 0.25 ± 0.20 mm, $97.32 \pm 0.14\%$, $98.87 \pm 0.19\%$ and 429 ± 0.43 respectively. The patch retained its original properties even after exposure to accelerated conditions of temperature and humidity.

CONCLUSION

In the present study transdermal patches of KTF were developed using a hydrophilic and hydrophobic polymeric matrix of HPMC E5 and EGT S100. Formulation containing higher concentration polymer HPMC E5 and plasticizer PG showed maximum values of flux, tensile strength, bioadhesion and physicochemical parameters because polymer and plasticizer having its hydrophilic nature. *Ex vivo* permeation studies concluded that permeation of drug from patch in different skin membrane up to 8h. It also concluded that the KTF transdermal patches are as effective as the orally administered drug. However chronic oral administration of KTF or other NSAIDs for symptomatic treatment of arthritis results in severe gastric irritation and ulceration which is absent in transdermal delivery. Thus we may conclude that a transdermal NSAID patch is more effective without the concomitant gastric damage. Stability studies concluded that the patch retained its original properties even after exposure to accelerated conditions of temperature and humidity.

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CONFLICTS OF INTEREST

The authors report no conflict of interest, financial or otherwise associated with this project.

ABBREVIATIONS

RA: Rheumatoid arthritis; **KTF:** Ketoprofen; **NSAID:** non steroidal anti inflammatory drug; **HPMC:** Hydroxyl propyl methyl cellulose; **EGT:** Eudragit S100; **PG:** Propylene glycol; **TDDS:** Transdermal drug delivery system; **PVP:** Polyvinyl pyrrolidone; **HEC:** Hydroxyl Ethyl Cellulose; **MC:** Methyl Cellulose, **DBT:** Dibutyl phthalate; **DMSO:** Dimethyl sulfoxide

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