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Research Article

DESIGN, DEVELOPMENT, AND EVALUATION OF CHITOSAN MATRIX-BASED SUSTAINABLE TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

The object behind the work is to formulate and characterize a transdermal patch of diclofenac sodium. Diclofenac sodium (DS) associated with the low bioavailability, short half-life and dose-related the adverse effect. To overcome these problems transdermal patch of diclofenac sodium was investigated. The matrix type transdermal patch of diclofenac sodium was design by the solvent evaporation method using chitosan as a polymer with propylene glycol as permeation enhancer and glycerol as a plasticizer. The prepared system was characterized for physicochemical properties, *in-vitro* release studies, *ex-vivo* permeation study, stability, irritation study and anti-inflammatory activity. In optimized patch FP3 (Chitosan 400 mg), drug content was found to be 94.51±0.73% and 83.36±0.84 % drug was released in 48 h. The *exvivo* study on excised rat abdominal skin showed the sustained drug permeation (54.3609%) in 24 h. Optimized formulation was safe, effective and devoid of dermal sensatization. The results of the anti-inflammatory activity, patches applied before 2 h of carrageenan injection showed 76.13±0.16% inhibition of inflammation whereas 100±0.01% inhibition of inflammation within 24 h of carrageenan insult when patches applied before 12 h of carrageenan insult. Transdermal patch has a greater potential to sustain its release characteristics.

Keywords: Transdermal patch, Controlled release, Diclofenac sodium, Chitosan, I*n-vitro* release study, Epithelial permeability.

1. INTRODUCTION

DS aryl acetic acid derivate has most potent nonsteroidal anti-inflammatory acivity. It is most widely prescribed for inflammation, arthritis, dysmenorrhea, musculoskeletal disorders, etc., for management or symptomatic relief of pain and inflammation [1]. Diclofenac inhibits the enzyme cyclo-oxygenase (COX) which is the key mediator of prostaglandins synthesis [2]. Diclofenac sodium is reportedly used in transdermal formulation. The problem associated with oral administration of the drug aprox 50-60 % of the administered amount reaches in blood, because of drug enter to hepatic circulation and subjected for hepatic first pass metabolism (HFPM).[3]. Thus, increased frequency of drug administration (150 mg tris in day) may lead to undesired response like ulcer, inestinal bleeding, stomach pain, nausea, and vomiting, etc. [4]. Diclofenac sodium also possesses a short biological half-life (1.2-2 h) [3, 4]. To omit the hurdle associated with with oral administrationnacessarly to formulate topical delivery system, which can evade the HFPM leading to decreased frequency of drug administration and its associated side effects. A topical delivery is a promising route for the administration of DS. The marix assisted drug entrapped polymeric system applied topicaly in the form of patch, release the specific dose of drug in to blood circulation over the extended period of time at the controlled and predetermined rate through the intact skin [5-7]. ALZA Corp has developed the first transdermal film, Transderm-Scop (Scopolamine) for management of motion sickness and approved by FDA in 1981and afterward Transderm-Nitro (Nitroglycerin patch) was formulated for the management of angina pectroris [6]. Transdermal delivery is more prominent then peroral drug delivery like maintain a constant and prolonged drug level, minimize intra and interpatient variability [1, 6], easy application and termination of patches leading to improve patient compliance [7], sustaining the release of and avoid HFPM [8], Improved bioavailability prompt to reduction in the dose frequency

[9], transdermal application also possesses advantages over injections, because they are painless and can be self-administered [10]. The goal of the design of matrix patch was to improve the penetration of drug across the membrane in a programmable manner from the patches to achieve a therapeutic drug concenration for the prolonged duration. The purpose of the proposed study to formulate polymeric patches of diclofenac sodium with different proportion of chitosan (Polymer), propylene glycol (Permeation Enhancer) and glycerol (Plasticizer); and was evaluated for its physicochemical properties, *in vitro* release, *ex vivo* and *in vivo* therapeutic response.

2. MATERIAL AND METHODS

2.1. Material and animals

Diclofenac sodium procured from Bennett Pharmaceutical Ltd, Baddi, India. Chitosan and carrageenan were purchased from Himedia Pvt Ltd, Mumbai, India. Propylene glycol and lactic acid were bought from S.D. Fine-Chem Ltd, Mumbai, India. Glycerol and polyvinyl alcoholwere obtained from FINAR Ltd, India and CDH Ltd, India, respectively. All other ingredient procure in propsed work were analytical grade.

The animal studies were carried out according to approval of institutional animal ethical committee (Protocol No: PH/IAEC/VNS/2K18/01). Wistar rats (Young rats, weight 200-250g) were housed in cages, given free access of laboratory diet and water. All rats were housed in laboratory having facilities of air conditioner and a cycle of 12 h dark and 12 h light.

2.2. Methods

2.2.1. Patch Fabrication

The matrix patches of diclofenac sodium were formulated using different quantities of chitosan (Table 1) by a solvent evaporation method. A backing membrane of the transdermal patch was formulated with 4%w/v aqueous solution of PVA (polyvinyl alcohol) in petri dish followed by drying for 24 h. The polymeric solution was prepared by taking the appropriate quantity of chitosan and dissolved in 4% v/v aqueous solution of the lactic acid with agitation at 1500 rpm using Homogenizer until a clear solution obtained. The drug (Diclofenac sodium), Plasticizer (Glycerol) and permeation enhancer (Propylene glycol) was incorporated to the above homogeneous dispersion with continuous stirring. About 3 to 4 mL methanol (Work as co-solvent for the drug) was added into the dispersion for uniform distribution and dissolution of the drug. The uniform Matrix dispersion was poured on the backing membrane of PVA which cast earlier. This matrix dispersion in petri plate was covered with an inverted funnel and kept undisturbed at room temperature (26±1°C) for 48 hours in order to evaporate the solvent. After complete evaporation of the solvent, the matrix patches were peeled from the Petridish and cut in square patches. The patches were properly covered in aluminum foil and kept in desiccators for further uses [11].

Fig. 1: The prepared transdermal patch of Diclofenac sodium

Rationale behind developing transdermal system of diclofenac sodium with new combination of polymer, plasticizer and permeation enhancer. In none of reported article chitosan was used as polymer with diclofenac sodium. 100 mg of drug incorporate in the system in order to achieve optimum therapeutic conc. in blood over the period of 48 hrs. The Prepared transdermal patch of diclofenac sodium is shown in Fig. 1.

Table 1: Composition of Prepared matrix patches.

S.NO.	Formulation code	Weight of chitosan (mg)	Drug (mg)	Plasticizer Glycerol (mL)	Permeation enhancer Propylene glycol (mL)	4%w/v aqueous solution of lactic acid (mL)
1	FP1	200	100	0.15	0.25	15
2	FP2	300	100	0.15	0.25	15
3	FP3	400	100	0.15	0.25	15
4	FP4	500	100	0.15	0.25	15
5	FP5	600	100	0.15	0.25	15

2.3. Evaluations

2.3.1. Physicochemical evaluation

2.3.1.1. Physical Appearance

Physical appearance such as clarity, smoothness, color and flexibility of all transdermal patches were visually scrutinized [12].

2.3.1.2. Thickness

The thickness of the prepared patches was measured by a using digital Vernier caliper (Aerospace, range 0-150 mm) at three random sites. Mean and standard deviation of three readings was calculated [13].

2.3.1.3. Weight Variation

The three patches of each formulation were cut in 3.5×3.5 cm^2 and weigh in digital balance (Contech Analytical Balance) individually. The average weight and standard deviation value were calculated [14].

2.3.1.4. Folding Endurance

Folding endurance is one of the important parameters for determination of the efficiency of plasticizer. The number of times polymeric film folds without break and crack is known as folding endurance. Folding endurance of matrix films was determined manually by repetitively folding a patch at the same location until it broke [8].

2.3.1.5. Tensile strength and percentage elongation

The studies were performed for determination of mechanical characteristics of formulated patches, which mainly depends on formulation components such as matrix polymer and plasticizer. The tensile strength (TS) of prepared patches was determined by the tensile strength apparatus Texture analyser Stable Micro System UK using A/MTG tensile grip. The transdermal patches were fixed onto the tensile strength assembly having two jaws (Arms), upper one jaw was fixed with no possibility of further movements and the lower one was moving. The load on the lower jaw was gradually increased until patches breaks and tensile strength was noted at a point just before the patch break. Tensile strength was calculated by using the equation 1:

$$\mathrm{T\,S\,(F)} = W.t\left(1+\frac{L}{I}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

Where the latter F is Force necessary for complete film breakage, W is a width of the film (cm), t is a thickness of the film (cm), L is a length of the patch (cm) and I is an elongation of the patch just before patch breakage (cm).

% elongation describes the elasticity of patch and assessed by noticing length just before the patch breakage and can be calculated by equation 2:

$$\%\ \mathrm{Elongation} = \frac{L2-L1}{L1}\times 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.2$$

Where L_1 refers to the final length just before patch breakage and L_2 refers to initial length. [11, 15, 16, 17]

2.3.1.6. Loss on Drying or % moisture loss

Loss on drying was determined the physical stability of patches in dry condition. For the measurement of moisture loss (%), the three patches (1.5×1.5 cm^2) from all formulations were individually weighed accurate and kept in a desiccator having anhydrous $CaCl_2$at ambient temperature (26±1 °C) for 72 h. During this period, weight of the pach examine after 24, 48, and 72 hr. Percentage moisture loss(ML) was calculated based on the equation 3 [11].

$$\%\ \mathrm{ML} = \frac{Initial\ Mass - Final\ Mass}{Iniial\ Mass}\times 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.3$$

2.3.1.7. Percentage Moisture Uptake

Percentage moisture uptake was utilized to check the integrity and physical stability of patches under high humid conditions. The Moisture uptake test was carried out by three accurately weighed patches (1.5×1.5 cm^2) from every formulation and placed in desiccators consist a saturated solution of KCl at ambient temperature (26±1 °C) for 48h. During this interval, the patches were weighed again after 24 and 48 h. The individually percentage moisture uptake (MU) was calculated using the equation 4: [5, 17]

$$\%\ \mathrm{MU} = \frac{Initial\ Mass - Final\ Mass}{Iniial\ Mass}\times 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.4$$

2.3.1.8. Water vapor permeation test

Water vapor permeation test was carried out for determining the amount of moisture transmitted through a per unit area/time of the patch. Equal volume of glass vials was used in this study. Glass vials were washed properly and dried in a hot air oven. 1 gm anhydrous $CaCl_2$ was taken in every glass vial and the medicated films were fixed over the brim of the glass vials by using adhesive tape. Then these glass vials were weighed and stored in desiccators containing a saturated solution of KCl at 84% RH. These glass vials were removed from the desiccators and weighed after 24,48,72,96 and 120 h to determine the weight gain. Water vapor permeation (WVP) was calculated by using the equation 5:

$$WVP = \frac{WT}{S} \ldots\ldots\ldots\ldots\ldots\ldots Eq.5$$

Where, W is the weight of water vapor permeated, T is the thickness and S is surface area exposed by film over the brim of vials in the square centimeter. The weight of water vapor permeated (W) was determined as follows: W= Final weight – Initial weight (After different time interval) [5].

2.3.1.9. Determination of surface pH

The surface pH was determined by placing patches in 5 ml of double distilled water for half an hour in a petri dish and was allowed to swell. A digital pH meter was brought near the surface of patches and pH reading was recorded after 1-2 min [18].

2.3.1.10. Determination of drug content

Films of 3.5×3.5 cm^2 were cut and placed in a volumetric flask containing 100ml of phosphate buffer saline, pH7.4 at room temperature (26±1 °C). The mixture was stirred at a magnetic stirrer for 24 h and the solution was filtered through Whatman filter paper (pore size 0.45µm). The absorbance of the filtrate was taken by a UV visible spectrophotometer at 277nm. From the absorbance value, the drug content was calculated [19].

2.4. Release Studies

2.4.1. In Vitro Drug Release Studies

The *in vitro* release is one of the most essential parameters, because of requires maintaining consistently the drug concentration on the surface of the stratum corneum and considerably greater than the drug concentration in the body, to attain a constant rate of drug permeation [1]. The *in vitro* release studies were conducted using modified Franz diffusion cell. The release studies were performed using an egg membrane (Biological membrane). A section of egg membrane was cut and ties on donor compartment using thread [20]. The transdermal patch was placed on a donor compartment in such manner facing the drug matrix side of the patch to the egg membrane and backing membrane upward. The receptor compartment of the modified diffusion cell was filled with the adequate volume of phosphate buffer saline (pH 7.4), whose temperature was maintained at 37±2 °C and stirred at 500 RPM. Followed by the holder (Donor compartment) containing membrane and the patch was placed on receptor compartment of the modified diffusion cell. The whole assembly was placed on a magnetic stirrer and solution of the receptor compartment was stirred continously at 500 rpm using a magnetic bead. The release study was carried out for 48 hours. The samples (1.0 ml each time) were withdrawn from the receptor compartment at different time intervals and equal volume of phosphate buffer saline, pH 7.4 was replaced to maintain sink condition. The collected samples were analyzed by using to UV spectrophotometer at 277nm against phosphate buffer saline, pH 7.4 as blank.

2.4.2. Ex-vivo permeation studies

The permeation studies were carried outon optimized transdermal patches, FP2 and FP3 showed the maximum. The dimension of applied patch for *ex vivo* skin permeation was 1.25×1.25 cm^2. Abdominal skin of Evan excised rat and modified Franz diffusion cell were used in permeation study. Subcutaneous fat and other extraneous tissues were trimmed from Wistar skin rat. Excised skin of rat were mounted in such a way on the Franz diffusion cells that the stratum corneum (SC) facing towards the donor compartment and patches under study were placed in such as intimate contact with the excised skin. 100 mL of pH 7.4 saline phosphate were filled in receptor compartment. The assembly was kept on a magnetic stirrer and content of the receptor compartment was stirred with a magnetic bead at 500 rpm at the temperature of 37±1 °C. The samples were withdrawn (2mL) at different time intervals and replaced with an equal volume of pH 7.4, phosphate buffer saline. The collected samples were subjected to UV-Visible spectrophotometer analysis at 277 nm against pH 7.4, phosphate buffer saline as blank [21]. For data analysis: *Ex vivo* permeation study data were plotted as cumulative percentage drug release versus time. Several parameters such as a steady state drug flux (J_{ss}), a permeability coefficient (K_p) through the membrane, lag time (T_{lag}) and distribution parameters (D) within the membrane were calculated. The plot was regressed to yield linear curve and the slope of the linear curve taken as a drug flux (J_{ss}) and its X-intersect took as lag time (T_{lag}). Distribution parameters (D) and permeability coefficient (K_p) were resolved by following equation [22]:

$$D = \frac{h^2}{6Tlag} \ldots\ldots\ldots\ldots\ldots Eq.6$$

$$Kp = \frac{Jss}{Cd} \ldots\ldots\ldots\ldots\ldots Eq.7$$

Where D=Distribution parameter (cm^2/h), h=Path length and C_d=Initial drug concentration.

2.5. Stability study

The sability study is carried out to determine the effect time, emperature, humidity and light on their characteristics and drug conent [23]. The accelerated stability study of the optimized transdermal patch (FP3) was performed for 3 months, according to ICH guideline under the following conditions: temperature 40±2 °C and relative humidity 75±5% to establish a shelf life and storage conditions of the best-optimized formulation [24]. The optimized films were kept in a desiccator containing a saturated solution of NaCl at 40±2 °C and 75% RH for three months [25]. Theparametersnamely weight, surface pH, and drug content were determined.

2.6. Animal studies

2.6.1. Skin irritation study

Young rats, weight 200-250g were divided into two groups, each group containing three rats. The backside of the rats was shaved 12h before conduct the experiments. An adhesive tape was used for the control group (Group I). For the test group (Groups II), transdermal patch (FP3) was applied on the dorsal surface of the rats, new patches were applied for 7 days. The patches were removed after 7 days. The rats were observed for any sign of erythema and edema for a period of 7 days and score was given by Draize scoring method (Draize et al.1944) as score 0 for no erythema, score 1 for very slight erythema (light pink), score 2 for well-defined erythema (dark pink), score 3 for moderate to severe erythema (light red), and score 4 for severe erythema (dark red) [26].

2.6.2. Therapeutic study (Carrageenan induced hind paw edema)

This method was used to assess the sustaining and anti-inflammatory action of transdermal patches. Experiment were performed on young ratshaving weight 200-250 g. approximately 12 hr before conducting experiment the dorsal surface of rats was shaved 12 h. The study was divided into two sections; section 1 and section 2. Total 28 rats were used to conduct experiments, 16 rats in section 1 and 12 rats insection 2. Section 1 has four groups and each group contain 4 rats. Section 1 was utilized for determination of immediate drug release and action. Group I (Vehicle treated+0.1 mL of 1% carrageenan subplantar) rats have received only a subplantar injection of carrageenan. Group II (Diclofenac sodium per oral+0.1 mL of 1% carrageenan subplantar) rats have received diclofenac orally. Group III (Marketed patch of diclofenac diethylamine+0.1 mL of 1% carrageenan subplantar) rats have received the marketed patch (Nu-patch 100mg) while group IV (Test patch of diclofenac sodium+0.1 mL of 1% carrageenan subplantar) rats have received test formulation. Section 2 has three groups similar to section 1 except group II. Section 2 was utilized for determination of sustained drug action. To induce paw edema in section 1 rats, after 2 h of application of formulations in all the rats were injected (subplantar) with 0.1 mL of a 1% w/v homogeneous suspension of carrageenan (in double distilled water) in the right paws. Whereas in section 2, carrageenan injection was injected in rats after 12 h of application of formulation. The hind paw volume was measured (In both of section)at 0, 1, 2, 3, 4, 5, 6 and 24 h after carrageenan injection using a plethysmometer and expressed as a percentage of edema relative to initial hind paw volume. Percentage inhibition (Inhi.) of edema was calculated using equation 8; [1, 27]

$$\%\ \text{Inhibaion} = \frac{\%\ \text{edema (control)} - \%\ \text{edema (drug)}}{\%\ \text{edema (control)}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.8$$

3. RESULTS AND DISCUSSION

3.1. Physicochemicalcharacterization of Trans-dermal Patch.

A polymeric drug incorporaed matrix patch was prepared using variable amount of chitosan. Chitosan selected as a polymer for matrix formulation by their versatile character like bidegedation, non-toxic, antimicrobial, mucoadhesion, absorption enhancer film forming and gelation abilities . Chiosan enhance cellular uptake via endocytocis and paracellular transport by transient opening of tight junction between epithelial cells [28, 29]. The formulated patches were characerized for their physicochemical properties, *in vitro* and *ex vivo* study, irritation study and for *in vivo*characterization. Transdermal patches were found to be white to slightly cream color, uniform, sufficiently flexible and smooth appearance on visual inspection. Consequently, these patches can preserve uniform and smooth surface while they adhere or administered on the skin. The thickness of the transdermal patches was resolved by digital Vernier caliper. The thickness of patches increased with increasing the concentration of chitosan in a formulation. The thickness ranged from 0.16±0.01 to 0.25±0.0057 mm, the low standard deviation value ensures that uniformity in thickness and distribution of polymer, drug and other excipients of formulations. The weight of different batches of

transdermal patch found to be in the range 525.8033±5.1829 mg to 928.3733±5.6140 mg. Surface pH of the formulation was determined as the patches will be administered on the skin, which can be cause the local irritation and toxicity on the skin. The pH of formulations (FP1 to FP5) was ranged from 5.9 to 6.4, is close to ideal pH of the skin (pH 4-6.8) and water (pH 7). Tensile strength and % elongation break of patches increasing with increase the concentration of chitosan. The tensile strength and % elongation was found to range from 2.9699 kg/cm^2 to 3.6666 kg/cm^2 and 66.67 % to 95.12% respectively. Those results suggested the patches have sufficient tensile strength and flexibility to affording the packing, transports, storage and other conditions. The drug content was determined by the spectrometer through a standard method. All formulations were shown that a near uniform drug content ranging from 83.0018±1.31% to 94.5172±0.73%. The Percentage moisture loss was found to be in the range of 4.3241±0.0992 to 9.6355±0.7405 in 48 h. All formulations were having the low level of moisture, which reflects formulation remain stable and dried. % moisture gain was found to be in the range from 5.5246±0.2960 to 13.2442±1.4977 at 48 h. The moisture gain in our study was found to be low which is necessary to protect the formulations against microbial contamination and growth [1, 30]. The moisture uptake of various formulations was found to be increased with the increase in chitosan (Polymer) concentration. The water vapor permeation test was performed to determine the permeability characteristics of patches, wheres as low water vapor permeation again emphasize the stability aspect during long-term storage. The water vapor permeation for formulated patches ranges from 0.0382±0.0001 to 0.0761±0.0031 g mm/cm^2 after 120 h. These results show that the all formulations were permeable to water vapor. The results of physicochemical properties for different transdermal patches are depicted in Table 2.

Table 2: Physicochemical evaluation of prepared transdermal patches.

Parameters	FP1	FP2	FP3	FP4	FP5
Thickness (mm)*	0.160±0.010	0.186±0.005	0.216±0.005	0.243±0.005	0.256±0.005
Weight Variation (mg)*	525.8±5.182	664.2±4.367	782.0±4.152	851.6±4.358	928.3±5.614
Folding Endurance	302	305	308	312	315
Surface pH	5.9	6.2	6.2	6.3	6.4
Drug Content (%)*	86..30±0.89	88.31±1.21	94.51±0.73	90.17±0.82	92.22±1.24
Tensile Strength (Kg/cm^2)	2.969	3.136	3.520	3.534	3.945
% Elongation	77.77	78.94	84.61	87.75	96.00
Moisture loss (After 48 h)*	4.324±0.099	7.081±0.094	7.9059±0.082	8.469±0.3959	9.635±0.740
Moisture Uptake (After 48 h)*	5.524±0.296	10.09±0.821	12.63±0.785	12.88±0.361	13.24±1.497
WVP (g mm/cm^2) after 5 days*	0.0382±0.0001	0.0449±0.0015	0.0721±0.0007	0.0703±0.0741	0.0761±0.0031

3.2. Release Studies.

3.2.1. In Vitro Drug Release Studies

In vitro release is a key parameter to determine drug release and to predict their *in vivo* characterstics. FormulationFP1 (chitosan 200 mg) displayed burst release. This burst release happened due to the low amount of chitosan in a formulation which may require less 'lag time' to establish a release profile in patches resulting in a 'burst effect'. Formulation FP2 (Chitosan 300 mg) and FP3 (Chitosan 400mg) shows overall high release, however FP3(83.3695±0.847 in 48 h) displays-superior drug release as comparedto FP2 (80.15 29±2.173 in 48 h). Both of these formulations were exhibited release in a controlled and sustained manner. Formulations FP4 (Chitosan 500 mg) and FP5 (Chitosan 600 mg) also displayed controlled and sustained drug release. Only 69.8764±0.6948% and 57.5849±2.7326 % of drug were found to be released from formulation FP4 and FP5 respectively with is very less as compared to FP3. The results relived that the increase in the amount of chitosan increase drug release initially than the decrease in the release of drug i.e. increasing the chitosan concentration cause sustained drug release.

The observed initial release may be helpful to reach the optimum therapeutic conc. along with a constant release rate helpful to maintain a drug conc. for a prolonged duration. The release profile data revealed that in most of the formulations, release pattern was best fitted for zero order release.In vitro release from various formulations plotted cumulative % drug released against time are mentioned in Fig. 2. From *the in vitro* release profile data, the kinetic of drug release were assessed for zero order, first order, Higuchi and Korsmeyer-peppas type release kinetics. The release profile data revealed that zero order release kinetic model shows best results for release pattern because the dispersed drug matrix ensured constant concentration as its coefficient of correlation (r^2) value predominates over first order, Higuchi-type and Korsmeyer-peppas type release kinetic. Data obtained from release studies, mechanical and physicochemical evaluation, formulations FP2 and FP3 were selected for *ex vivo* permeation study.

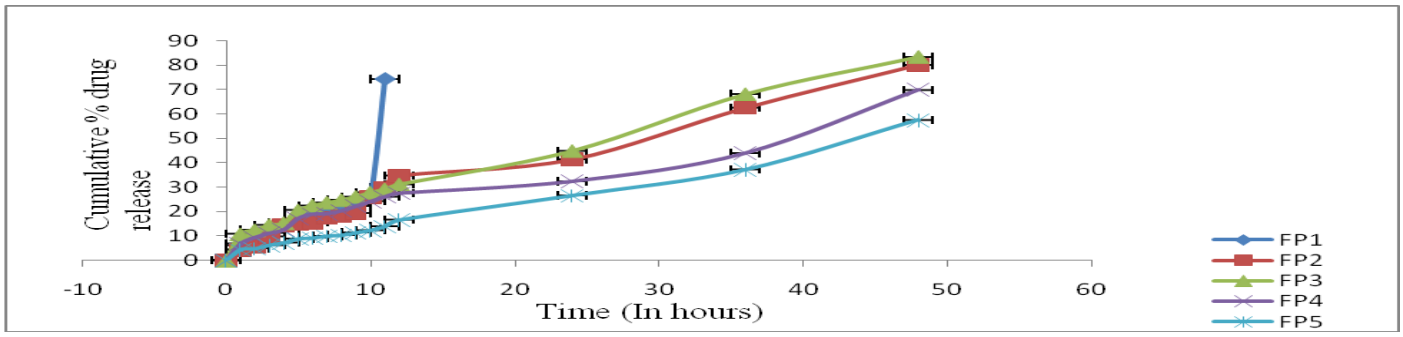

Fig. 2: In vitro drug release profile of diclofenac sodium form different formulations (FP1 to FP5) in PBS 7.4. Data are mean ± SEM (n=3).

3.2.2. Ex-vivo permeation studies

The *ex-vivo* study of optimized formulations was performed through an abdominal skin of Wister rat. The result of ex vivo studies of formulations (FP2 and FP3) paralleled the results obtained from *in vitro* studies. The Formulation FP3 (54.3609 % in 24hr) exhibited the highest release comparison to formulation FP2 (52.5772 % in 24h). Moreover, FP3 has shown highest flux 160.7857 µg/cm^2/h and permeability coefficient 0.03108 (cm/h) $\times 10^{-3}$. Since FP3 transdermal patch was selected for further evaluations. The *ex-vivo* release is given in Fig. 3 and permeation parameter is shown in Table 3.

Table 3: Shows Slope, Drug flux (Jss), Lag time (T_{lag}), a Permeability coefficient (Kp) and Distribution parameter (D) from 1cm^2 patch.

S. NO.	Formulation code	Slope	Drug Flux (µg/cm^2/h)	Lag time (min)	Permeability coefficient (cm/h)$\times 10^{-3}$	Distribution Coefficient (cm^2/h)$\times 10^{-3}$
1	FP2	217.6	155.4285	29.58	0.0108	0.3380
2	FP3	225.1	160.7857	31.8	0.0112	0.3144

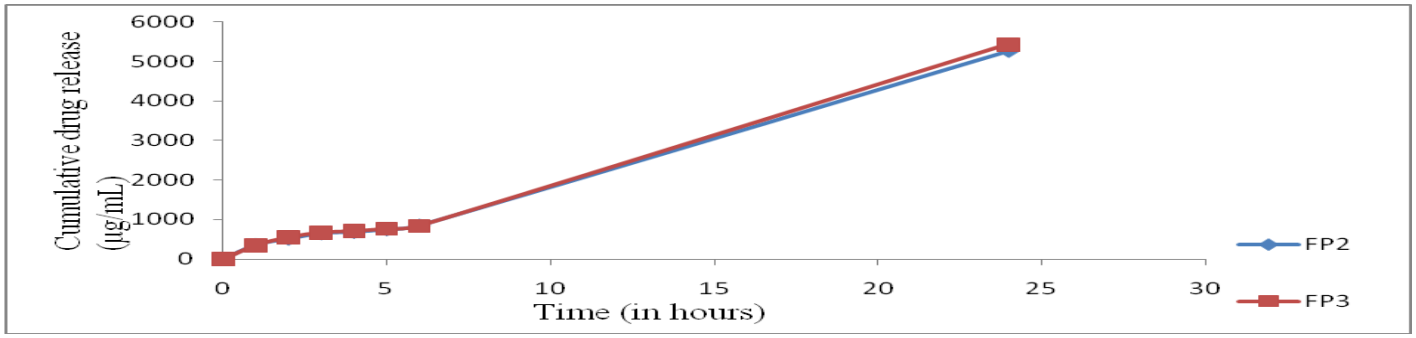

Fig.3: Permeation of Diclofenac Sodium from FP2 and FP3 formulation.

3.3. Stability study

Stability study was performed according to ICH guidelines for 90 days, the best-optimized matrix patch (FP3) of diclofenac sodium subjected to accelerated stability study. The optimized patch was found to be stable with respect to drug content uniformity, weight variation and surface pH. The drug contents were found to be uniform with respect to initial drug content. The surface pH of a transdermal patch was found in the ranging 6.3000±0.1000 to 6.3666±0.2081. Results distinctly specified that the pH of the patch was stable throughout the stability study period, which indicates the absence of skin irritation. Weight of transdermal patches was initial decreases then constant at 3rd month of study. The results of the stability study clearly indicated that the temperature and humidity do not causes many changes in the properties of the transdermal patch. The data of the stability study are mentioned in Table 4.

Table 4: Accelerated stability study of transdermal patch (FP3). (n=3)

Parameters / Days	Weight (mg)	Surface pH	Drug content (%)
0	199.45±1.6263	6.3000±0.1000	94.5172±0.7314
30	196.11±0.8485	6.3333±0.0577	93.5399±1.7029
60	194.55±1.9091	6.3661±0.1000	91.1328±1.2761
90	193.09±1.1243	6.3666±0.2081	90.9823±1.4887

3.4. Animal studies

3.4.1. Irritation study

The seven-days skin irritation study on Wistar rat revealed that the test patch demonstrated a skin irritation score 0 which means no erythema. Studies were divided into two groups, adhesive tape was applied to control group and patch was applied to test group up to seven days.

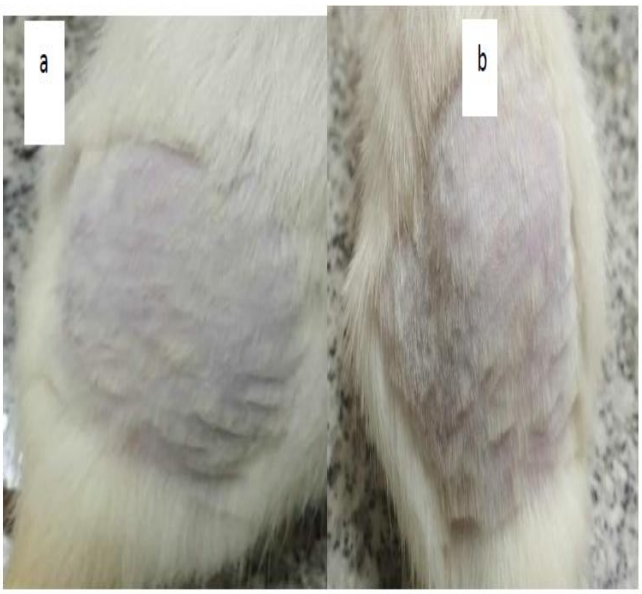

Fig. 4: Test group rat: (a) represents before appli-cation of transdermal patches (b) represent after the 7th day of application of the transdermal patches.

The score of irritation study was done according to the Draize method of scoring. According to the Draize method, substance or drugs producing the score of 2 or less than 2 are considered as non-irritant. From the Draize method of scoring, test formulation did not cause erythema and edema at the end of the study period (7 days) against a control group. Hence, from the study, it can be concluded that formulation is non-irritant and non-allergenic for human skin and harmless for therapeutic use [30]. The irritation study of test group rats were mentioned in Fig. 4 where (a) represents before application of transdermal patches and (b) represent after the 7th day of application of the transdermal patches.

3.4.2. In vivo anti-inflammatory study

The anti-inflammatory response of self-made patch and other formulations were evaluated by measuring the change in paw volume of carrageenan-induced rats. In group I rats which received carrageenan alone, a rapid and continuous increase in paw volume was observed and maximum inflammation after the 3rd hour of carrageenan insult. In group II inhibition of paw edema occurs, but not sustained inhibition of paw edema, it's due to orally short half-life (2-3 h) of diclofenac sodium. The efficacy of the prepared transdermal patch was also compared with the marketed transdermal patch of diclofenac diethylamine (Nu-patch 100mg). The inflammation due to carrageenan injection was noticeably inhibited by the marketed (Group III) and test (Group IV) patches. A higher anti-inflammatory

activity was found with the test patches (FP3 100mg) as compare to marketed patch (Nu-patch 100mg). Carrageenan injection was injected in rats after 12 hrs of application of patch (In section 2 study) showed 100% inhibition at 24hrs suggest that the released drug retained at site for a prolonged period. That effect may be due to the action of chitosan to open the tight junction of epithelium and stratum corneum membrane. The test formulation FP3 was more prominent in term of inhibiting carrageenan-induced paw edema. These results indicate the prepared transdermal patch capable of immediate release along with the sustained action. All data were analyzed by one way ANOVA followed by Tukey's multi comparison test by GraphPad In-Stat. The results of *in vivo* study are given in Table 5 and Fig. 5 describe different group rats after the 3rd hour of Carrageenan insult, Where (a) group I rat (No treatment), (b) group II rat (Oral diclofenac), (c) group III rat (Marketed patch) and (d) Group IV rat (Test patch).

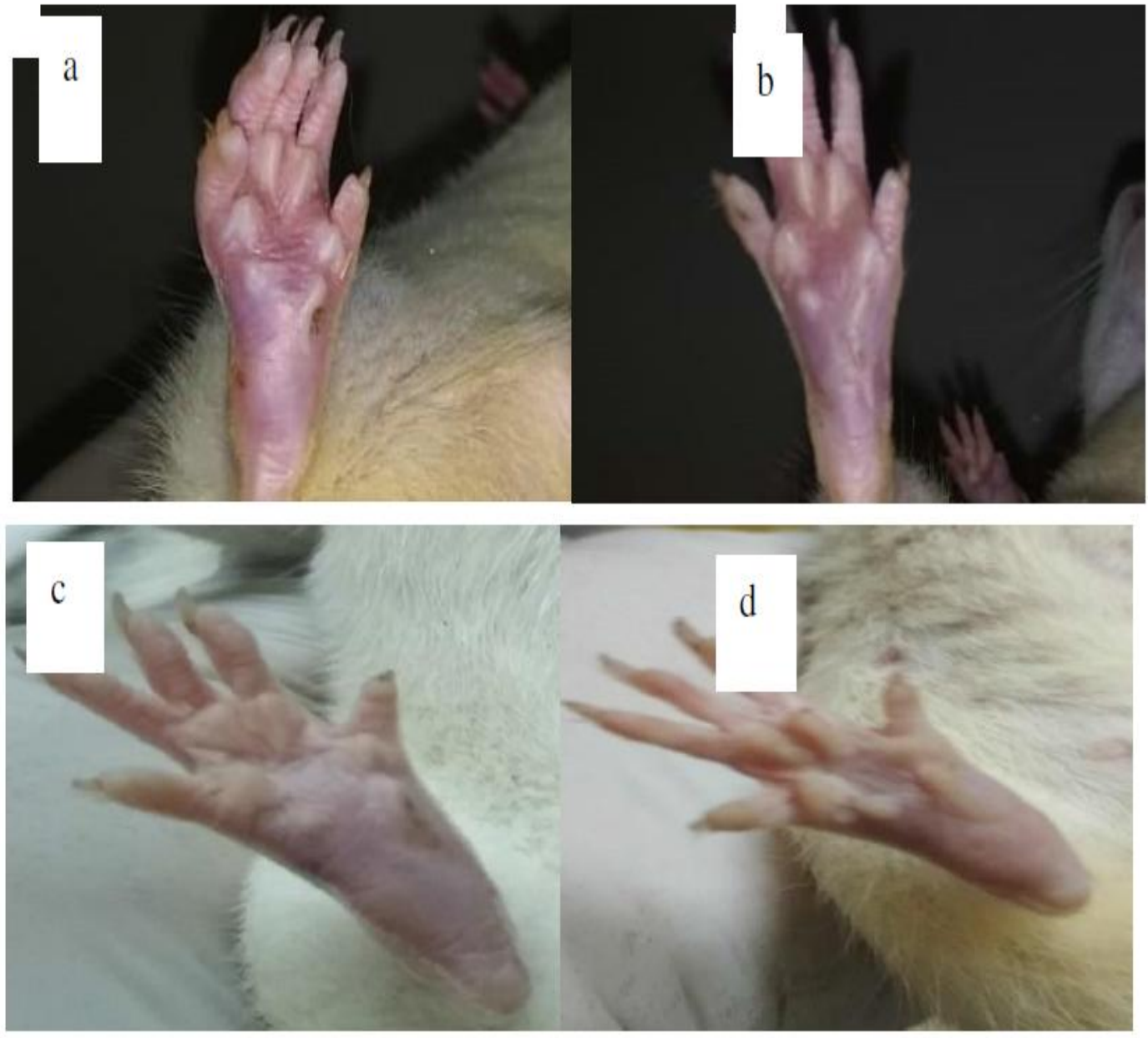

Fig. 5: Different group rats after the 3rd hour of Carrageenan insult, Where (a) group I rat (No treatment), (b) group II rat (Oral diclofenac), (c) group III rat (Marketed patch) and (d) Group IV rat (Test patch).

Table 5: % Inhibition in paw volume of rats (Carrageenan induced paw edema).

Group / Time (h)	I (Vehicle treated + 1 % Carrageenan 0.1ml S.P.)	II (Diclofenac 10.41 mg/kg P.O. +1 % Carrageenan 0.1ml S.P.)	III (Diclofenac Marketed patch + 1 % Carrageenan 0.1ml S.P.	IV (Diclofenac Test formulation +1 % Carrageenan 0.1ml S.P.
Section 1: Effect of drug with 2 h pretreatment of Carrageenan Insult				
1	-	48.86±0.05 [a, c]	52.52±0.29 [a, b]	60.33±0.23 [a, b, c]
2	-	71.16±0.21 [a, c]	55.52±0.44 [a, b]	66.64±0.13 [a, b, c]
3	-	63.12±0.11 [a, c]	62.33±0.28 [a, b]	66.74±0.0 [a, b, c]
4	-	56.25±0.27 [a, c]	63.65±0.17 [a, b]	69.39±0.08 [a, b, c]
5	-	54.84±0.01 [a, c]	67.33±0.28 [a, b]	73.83±0.10 [a, b, c]
6	-	52.57±0.08 [a, c]	73.70±0.15 [a, b]	74.00±0.06 [a, b, c]
24	-	30.10±0.16 [a, c]	78.46±0.16 [a, b]	76.13±0.16 [a, b, c]
Section 2: Effect of drug with 12 h pretreatment of Carrageenan Insult				
1	-	Not used	30.73±0.11 [a]	64.36±0.01 [a,c]
2	-	Not used	41.31±0.07 [a]	66.04±0.05 [a, c]
3	-	Not used	51.62±0.04 [a]	75.04±0.03 [a, c]
4	-	Not used	52.48±0.07 [a]	82.17±0.03 [a, c]
5	-	Not used	55.05±0.10 [a]	95.74±0.03 [a, c]
6	-	Not used	63.72±0.05 [a]	97.18±0.01 [a, c]
24	-	Not used	83.67±0.03 [a]	100.0±0.01 [a, c]

4. CONCLUSION

From above results, it could be concluded that sustained-release transdermal patch of Diclofenac sodium can be efficaciously prepared with chitosan (polymer) which offers better compliance than conventional marketed formulation. Transdermal delivery of Diclofenac sodium overcome the problem associated with an oral administration like low bioavailability, high hepatic first-pass metabolism, frequent dosing and its associated adverse effects. As compare to conventional delivery system it is temporal or prolonged drug release system which helps to minimize dose frequency and side effect and sustain the therapeutic effect in the body. Data of release study and animal study supports the maintenance of effective concentration of drug for a prolonged period. On the basis of in vitro drug release study it was found that 83% of drug release from the system during 48 hr which is the characteristics sign of temporal control drug delivery system, *exvivo* permeation study give the idea about improvement in the bioavailability of drug, drug release Kinetic of transdermal system indicate steady infusion of drug over prolonged period reduce dose in patch avoiding the adverse effect and intermittent dosing. The developed system will be useful for 2 days on once application. The optimized transdermal patch could be a better choice for symptomatic relief of pain and inflammation in chronic diseases such as arthritis, gout, etc. Furthermore, transdermal patches have been duly screened may be used for pharmacokinetic studies in animals and human for their best therapeutic uses.

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Conflict of interest

The author declares manuscript has no conflict of interest.

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