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Matrix Type Transdermal Delivery System of Diclofenac Potassium-Skin Permeation and Pharmacokinetic Profile

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The aim of this study is to evaluate and compare matrix type patch formulations of diclofenac potassium, prepared by hydroxylpropylmethylcellulose (HPMC 4000 cps), polyvinylpyrolidine (PVP K-30) and ammonio-methacrylate copolymer type A (Eudragit RL-100). The effect of various skin permeation enhancers on permeation characteristics of the diclofenac potassium from the prepared formulations through hairless abdominal rabbit skin was studied by using modified Franz diffusion cell. The patch formulations were compared with formulation controls (without enhancers). The cumulative amounts permeated and the fluxes were higher for the prepared formulations as compared to the controls. Skin permeation studies revealed better skin permeation characteristics of diclofenac potassium using isopropyl myristate than isopropyl palmitate and Tween 80. The cumulative amount permeated at 36 h (μg/cm^2), steady-state flux Jss (μg/cm^2 h), lag time tL (h), permeability coefficient kp (cm/s) and diffusion coefficient D (cm^2/s) were determined for the prepared formulations in comparison with the controls. Skin permeation enhancers like isopropyl myristate (IPM), isopropyl palmitate (IPP) and nonionic surfactant (Tween 80) showed significant ($p < 0.05$) increment in the permeation of diclofenac potassium. The pharmacokinetic parameters of the optimized formulation (F4) were calculated from the blood levels of the drug revealed a profile typical of sustained release formulation with the ability to maintain adequate plasma levels for 24 h (*i.e.*, up to the next application). $AUC_{(0-24)}$, T_{max} and C_{max} were 28.59 ± 5.34 ng h/mL, 5 h ± 1.1 and 2.606 ± 0.21 ng mL^{-1}, respectively. The amount of drug bioavailable for targeting the site of action is higher than that of market control. Based on experimental results, preparation of 5 % diclofenac potassium matrix type patch formulation containing isopropyl myristate is promising.

Key Words: Diclofenac sodium, Permeation enhancers, Permeation studies, Pharmacokinetics.

INTRODUCTION

The success of transdermal therapeutic system has created much interest in the pharmaceutical industry and has activated research activities related to it[1]. Transdermal drug delivery offers many important advantages[2]; however, the barrier properties of intact skin limit the permeability of a wide variety of substances, including pharmaceutical active agents[3]. Therefore, considerable attention has been directed recently to overcome the low permeability of drugs through the skin[4]. One strategy overcoming this constraint is the incorporation of various chemical skin enhancers into the vehicle[5] such as azone[6], urea[7], propylene glycol[8], isopropyl alcohol (IPA)[9], isopropyl myristate (IPM)[10,11], Tween 80[12,13] and isopropyl palmitate (IPP)[14-16]. Following penetration, across the stratum corneum, drugs diffuse across the viable epidermis and dermis and transported away by the cutaneous microvasculature. The blood supply is very rich, with a flow rate of 0.05 mL min^{-1} cm^{-1} of skin, within 0.2 mm of the skin surface. This generous blood volume usually functions as a 'sink' with respect to the diffusing molecules which reach it during the process of percutaneous absorption[17]. Considering the fact that most inflammatory diseases occur locally and near the surface of the body, topical application of nonsteroidal antiinflammatory drugs (NSAIDs) to the inflamed site can offer the advantage of delivering the drug directly to the diseased site and producing high local concentrations. This bypasses gastric irritation and also reduces adverse systemic effects[9]. Dermatological drug products include a broad array of preparations which are designed to exert a local effect in diseased skin following topical application on the skin surface[18]. Chemical enhancers can be formulated with the active therapeutic as a topical cream or gel or a skin patch that can be applied anywhere on the body for prolonged systemic delivery of drug[19].

Diclofenac potassium (DP) or potassium 2-[(2,6-dichlorophenyl)amino]benzene acetate is a potent NSAID advocated for use in painful, inflammatory and certain non-rheumatic conditions. In addition, few studies have yet been recently

carried out to investigate the percutaneous transport of diclofenac potassium. However, no report has dealt with the transdermal delivery of the drug from such formulations. Therefore, the purpose of this study was to investigate the usefulness of such formulations, having different permeation enhancers, for transdermal delivery across excised hairless abdominal rabbit skin. The effect of incorporation of certain skin permeation enhancers such as IPM, Tween 80 and IPP on the *in vitro* permeation was investigated with *in vivo* characterization of the optimum formulation. In addition, accelerated stability studies for 6 months were also performed for optimal formulation.

EXPERIMENTAL

The materials used were: diclofenac potassium (China); HPMC 4000cps (China); IPM (UK); Tween 80 (Singapore); IPP (China), Eudragit RL-100 (China), PVP K-30 (China). All other chemicals were analytical and were purchased from Merck, Germany.

Preparation of formulation: Colloidal matrix type nine patch formulations were prepared. The formulation contents are shown in Table-1. Eudragit RL-100 and PVP K-30 were dissolved separately in 16.25 g of ethanol by continuous stirring to prepare Eudragit RL-100 phase and PVP K-30 phase, respectively. 3 g of permeation enhancer was mixed in 16.25 g of ethanol. Then the drug was incorporated in this mixture at three concentrations (1, 5 or 10 g) by continuous stirring until clear solution achieved (active phase). 16.25 g of ethanol was mixed in 22 g of WFI grade water. Then 4 g of HPMC was mixed in this solution by continuous stirring till the formation of homogeneous mixture (HPMC phase) Eudragit RL-100 phase was mixed with PVP K-30 phase with stirring. Then HPMC phase was mixed in this mixture followed by the mixing of active phase homogeneously. Formulation controls (formulations without enhancers) C1, C2 and C3 were prepared for formulations F1-F3, F4-F6 and F7-F9, respectively. The commercial product (market control) contained diclofenac diethylamine 1.16 % w/w; the active substance corresponds to 1 % w/w diclofenac sodium. The base of product is composed of an oily emulsion in an aqueous gel and contains acrylic acid polymer, cetomacrogol 1000, caprylic acid/capric acid fatty ester, IPA, propylene glycol, liquid paraffin, perfume and water.

Permeation studies across abdominal rabbit skin: Full-thickness skin was obtained after shaving hair from male rabbits 7-8 weeks old and weighing 700-1000 g. The rabbits were sacrificed with chloroform under desiccation. The abdominal skin was carefully shaved before sacrificing the animal and washed with warm water and kept in normal saline at -20 ºC[20]. The skin samples (0.81 cm^2) were mounted on Franz diffusion cells (2.0 cm i.d. PermeGear, USA) with the epidermal side upwards. 2 g equivalent to 20, 100 and 200 mg drug of each formulation was spread on this side, facing the donor compartment. The dermal side faced downward into the receptor compartment, which consisted of normal saline at 37 ± 0.5 ºC and stirred horizontally at 30 rpm in a thermostatically controlled shaker. The whole receptor phase was taken[21] as sample at 0, 2, 4, 6, 8, 16, 20, 24 and 36 h and was replaced with the fresh normal saline. All experiments were carried out on triplicate samples.

Drug assay: In all skin permeation experiments, the withdrawn samples were filtered using disposable filters (Millipore, USA) and analyzed for diclofenac potassium by using UV spectrophotometer (UV 1601, Shimadzu-Japan) at 280 nm[22]. A prefect sink condition was maintained throughout the experiment.

Calculation of permeation parameters across abdominal rabbit skin: The permeation profiles were constructed by plotting the cumulative amount (μg/cm^2) of drug permeated *versus* time. Diclofenac potassium steady-state flux, *J*, was estimated from the slope of the straight line portion of the cumulative amount of drug absorbed against time profiles and the lag time from the x-intercept[23]. The permeability coefficients and diffusion coefficients were calculated according to the method of Chow *et al.*[24].

$$D = \frac{h^2}{6tL} \quad (1)$$

$$K_p = \frac{J_{ss}}{Cs} \quad (2)$$

where tL = lag time, D = diffusion coefficient within the skin, h = thickness of the skin (0.81 cm), J_{ss} = steady-state flux, K_p is the permeability coefficient through the skin and C_s = initial drug concentration in the donor compartment.

TABLE-1
COMPOSITION OF VARIOUS TDDS PATCH FORMULATIONS

Formulation code	DP (g)	HPMC (4000 cps) (g)	Eudragit RL-100 (g)	PVP K-30 (g)	Ethanol (g)	WFI grade water (g)	IPP (g)	IPM (g)	Tween 80 (g)
F1	1	4	3	2	48.25	22	–	3	–
F2	1	4	3	2	48.25	22	3		–
F3	1	4	3	2	48.25	22	–	–	3
F4	5	4	3	2	48.25	22	3	–	–
F5	5	4	3	2	48.25	22	–	3	–
F6	5	4	3	2	48.25	22	–	–	3
F7	10	4	3	2	48.25	22	3	–	–
F8	10	4	3	2	48.25	22	–	3	–
F9	10	4	3	2	48.25	22	–	–	3
C1	1	4	3	2	48.25	22	–	–	–
C2	5	4	3	2	48.25	22	–	–	–
C3	10	4	3	2	48.25	22	–	–	–

Enhancing factor (EF): The enhancing factor (EF) was estimated by dividing the cumulative amount of diclofenac potassium of any prepared formulation by that of control formulation (without enhancer).

Enhancement ratio (ER_{flux}): The enhancing ratio (ER_{flux}) was estimated by dividing the steady-state permeation rate of any formulation by that of control formulation (without enhancer).

Mathematical analysis-drug release kinetic studies: Following model dependent approaches were applied to investigate the comparison between various permeation data and interpret drug release kinetics; zero-order[25], first order[26] and Higuchi's model[27], Hixson-Crowell rate equation[28].

$$C = K_0 t \quad (3)$$

where K_0 = zero-order rate constant.

$$\log C = \log C_0 - \frac{K_t}{2.303} \quad (4)$$

where C_0 = initial concentration of drug, K_t = first order constant

$$C = (K_t)^{1/2} \quad (5)$$

where K = constant reflecting the design variables of the system

$$C_0^{1/3} - C_t^{1/3} = K_{HC} \times t \quad (6)$$

where C_t = amount of drug permeated in time t, C_0 = initial amount of the drug in the patch and K_{HC} = rate constant.

To evaluate the mechanism of drug diffusion from the formulation, data was plotted in Korsmeyer-Pappas[29].

$$\frac{M_t}{M\infty} = Kt^n \quad (7)$$

where M_t/M_∞ = fractional solute release, t = release time, K = kinetic constant characteristic of the drug/vehicle system and n is an exponent that characterizes the mechanism of release. If the exponent n = 0.45, then the drug diffusion mechanism is Fickian diffusion and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of case-II transport or typical zero-order release.

Pharmacokinetic studies: This study was approved by the Board of Advance Studies and Research, the Islamia University of Bahawalpur and was conducted according to USA guidelines for laboratory animal use and care and Halenski declaration of human use in research.

Human volunteers: Five healthy male aged from 20-35 years were selected. The average height was 175 ± 10 cm and the average weight was 74 ± 9 Kg. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, gastrointestinal, neurological, hematological and dermatological disease and without hypersensitivity or contraindication to diclofenac as determined by medical history. They did not receive any medication before or during the study and had not been involved in any other clinical trial. Each volunteer agreed a consent form. Volunteers were selected on a random basis.

Dosing procedure: A 1 cm^2 area was marked on the interior surface of left forearm near elbow. The marked area received a single topical application of 2 g patch formulation containing 100 mg of diclofenac potassium. After topical application, the dosed area was kept clear of clothing for the first 10 h, then clothing was allowed.

Blood sample collection: A 20 gauge sterilized canola (B. Braun, Germany) was placed in the ventral right forearm (the opposite one) near the elbow flexure before dosing. Blood (10 mL) was sampled at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 h using the catheter. The blood samples were immediately placed in heparin containing tubes and then centrifuged (Kubota 1710, Korea) at 4500 rpm for 10 min. Plasma was separated and stored at -20 ºC till further process.

Bio-analysis using HPLC: For extraction, 2 mL of acetonitrile per 1 mL of each plasma sample was used to precipitate the proteins followed by vortexing for 1 min and centrifugation (Kubota 1710, Korea) for 5 min at 4000 rpm. The supernatant layer was evaporated to dryness under nitrogen flux. The residue was dissolved in 80 µL of mobile phase and then injected 20 µL into the rheodyne of HPLC (Shimadzu, Japan). The HPLC method[30] involved; UV detector (SPD-10 ATVP Shimadzu, Japan) operated at 280 nm, C_{18} stainless steel analytical column (4.6 cm × 150 cm, 5 µm, Phenomenex, UK). A mixture of acetonitrile and ammonium acetate buffer 0.01 M with pH 3.4 adjusted by glacial acetic acid (60:40, v/v) was used as mobile phase at an optimum flow rate of 1.5 mL/min. Drug stock solutions (100 µg/mL) of diclofenac potassium[2,3] was prepared in acetonitrile. A calibration curve, with 250-2500 ng/mL range and 0.9895 regression coefficient (r^2), was employed for mathematical analysis of data.

Data analysis: Non-compartmental pharmacokinetic parameters from plasma levels of each volunteer *versus* time data were determined by using the program, Kinetica® 4.0. C_{max} is the peak drug concentration; T_{max} is the time at which C_{max} occurred. $T_{1/2}$ is the half life of terminal rate constant. $AUC_{(0\text{-last})}$ is determined as area under the plasma concentration-time curve up to the last measured time point. One way was calculated by using SPSS 13.0 with a level of significance set at 0.05.

Stability studies: The optimal formulation (F4) was subjected to accelerated stability studies. The conditions were 40 ºC and 75 % relative humidity by following the ICH guidelines for zone four. The samples were drawn at each month and analyzed. The samples were subjected to chemical and physical assays.

RESULTS AND DISCUSSION

***In vitro* skin permeation studies:** The skin permeation profiles and permeation characteristics of diclofenac potassium from the formulations prepared with 3 g isopropyl myristate, isopropyl palmitate and Tween 80 and 1, 5 and 10 % w/w drug in comparison with the formulation controls (C1, C2, C3; without enhancers) are shown in Table-2. The cumulative amount permeated at 36 h was higher for all the prepared formulations compared to the controls (Table-2). The cumulative amounts permeated at 36 h were 1325.27 ± 96.16, 646.98 ± 36.89, 676.76 ± 57.86, 3665.74 ± 148.02, 1010.33 ± 85.64, 618.60 ± 33.99, 3085.89 ± 133.58, 1802.77 ± 107.45, 451.18 ± 34.87 µg/cm^2 for the prepared formulations F1-F9, compared to 108.92 ± 6.51, 112.24 ± 4.0, 119.17 ± 29.19 µg/cm^2 for controls C1, C2 and C3, respectively (Table-2). The formulations with isopropyl myristate have shown higher permeation which may act by penetrating into the stratum corneum and increasing

TABLE-2
PENETRATION CHARACTERISTIC OF DICLOFENAC POTASSIUM ACROSS ABDOMINAL RABBIT SKIN FROM SELECTED PATCH FORMULATIONS IN COMPARISON WITH FORMULATION CONTROL (WITHOUT ENHANCERS)

Formulation code	Cumulative amount at 36 h (μg/cm^2)	Lag time (h)	Diffusion coefficient (cm^2/s × 10^{-4})	Permeability coefficient (cm/s × 10^{-5})	Flux (*J*) (μg h^{-1} cm^{-2})	Enhancing factor*
F1	1325.27 ± 96.16	4.6 ± 0.15	3.90 ± 0.43	8.91 ± 0.51	34.07 ± 1.91	12.39
F2	646.98 ± 36.89	12 ± 3.0	1.51 ± 0.39	6.23 ± 0.81	23.84 ± 2.01	6.05
F3	676.76 ± 57.86	3 ± 0.19	6.07 ± 0.44	6.60 ± 0.31	25.23 ± 1.32	6.33
F4	3665.74 ± 148.02	8 ± 1.8	2.27 ± 0.42	6.34 ± 0.91	121.18 ± 34.37	32.66
F5	1010.33 ± 85.64	12 ± 2.12	1.51 ± 0.39	1.95 ± 0.64	37.40 ± 2.01	9.00
F6	618.60 ± 33.99	9 ± 2.19	2.02 ± 0.43	1.08 ± 0.25	20.77 ± 9.24	5.51
F7	3085.89 ± 133.58	5.5 ± 0.61	3.31 ± 0.63	2.51 ± 0.12	96.22 ± 3.67	25.89
F8	1802.77 ± 107.45	6 ± 0.46	3.03 ± 0.49	1.41 ± 0.51	54.02 ± 9.14	15.13
F9	451.18 ± 34.87	3.0 ± 0.18	6.07 ± 0.43	0.30 ± 0.18	11.62 ± 0.05	3.79
C1	108.92 ± 6.51	16 ± 0.20	1.14 ± 0.43	0.87 ± 0.81	3.34 ± 0.11	A
C2	112.24 ± 4.0	16 ± 0.42	1.14 ± 0.31	0.98 ± 0.83	3.76 ± 0.14	A
C3	119.17 ± 29.19	7.20 ± 0.39	2.53 ± 0.33	0.10 ± 0.41	3.97 ± 0.14	A

*Enhancing factor was calculated by dividing the cumulative amount of diclofenac potassium permeated at 36 h by any formulation by that of control formulation (without enhancer) C1, C2 and C3 are formulation controls (without enhancers) for 1, 5 and 10 % prepared patch formulations. Data are given as mean ± SD (n = 3).

the lipid fluidity by disruption of lipid layer packing[31]. The drug contents permeated from formulation of diclofenac with Eudragit were 0.32 ± 0.01 and 0.34 ± 0.01 mg/cm^2. The steady-state flux was highest (121.18 ± 34.37 μg/cm^2 h) for formulation F4 containing 5 % w/w drug with isopropyl myristate, followed by F7 (10 % drug with isopropyl myristate) with flux of 96.22 ± 3.67 μg/cm^2 h and was lowest for formulation F9 (11.62 ± 0.05 μg/cm^2 h). There was a significant difference in the fluxes among all formulations studied. ($p < 0.05$) diclofenac sodium in aqueous medium was reported[32,33] to have flux value of 0.07 μg/cm^2 h. Flux of 9.28 ± 0.53 μg/cm^2 h was reported from gel containing isopropyl myristate with carbopol 900 base[23]. The value of flux of diclofenac potassium[34] through the regenerated cellulose membrane from the microemulsions was 117.94 μg cm^{-2} h^{-1}. Microemulsion with 10 % w/w /propylene glycol resulted flux value[35] of 3.7 × 10^{-2} ± 0.006 mg cm^{-2} h^{-1}. Formulation with DMSO was reported[20] the flux value of 1.4 μg h^{-1} cm^{-2}. Flux of 6.215 ± 4.032 μg/cm^2 h was reported with 5 % limonene in horses[36]. Diclofenac formulation with isopropyl palmitate was reported[37] to have flux of 0.2 × 10^{-2} ± 0.1 μg h^{-1} cm^{-2} and permeability coefficient of 2.0 ± 1.0 × 10^{-3} when compared to formulation with lecithin flux of 0.7 × 10^{-2} ± 0.1 μg h^{-1} cm^{-2}. The permeability coefficient was highest (8.91 ± 0.51 × 10^{-5} cm/s) for formulation F1 containing isopropyl myristate with 1 % drug and lowest for formulation F9 (0.30 ± 0.18 cm/s × 10^{-5}). The permeability coefficient value of 6.02 × 10^{-3} cm/h was reported for unionized diclofenac in aqueous formulation[33].

Effect of added permeation enhancers: Figs. 1-4 and Table-2 show the influence of penetration enhancers like isopropyl myristate, isopropyl palmitate and Tween 80 on permeation of diclofenac potassium from patch formulations prepared in comparison with formulation control (without enhancers). The cumulative amounts permeated at 36 h for 3 g isopropyl myristate, isopropyl palmitate and Tween 80 with 1 % drug formulations, were 1325.27 ± 96.16, 646.98 ± 36.89, 676.76 ± 57.86 μg/cm^2, respectively (F1-F3, Fig. 2). For the 5 % drug formulation, the cumulative amounts of drug permeated were 3665.74 ± 148.02, 1010.33 ± 85.64, 618.60 ± 33.99 μg/cm^2 (F4-F6, Fig. 3).

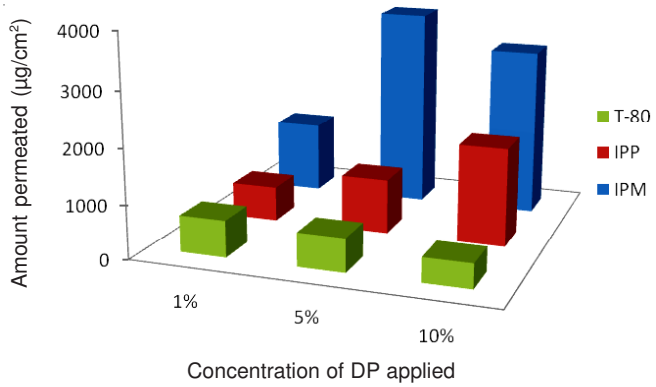

Fig. 1. Effect of enhancers on cumulative amounts permeated (at 36 h) of diclofenac potassium from matrix type patch formulations through excised hairless abdominal rabbit skin

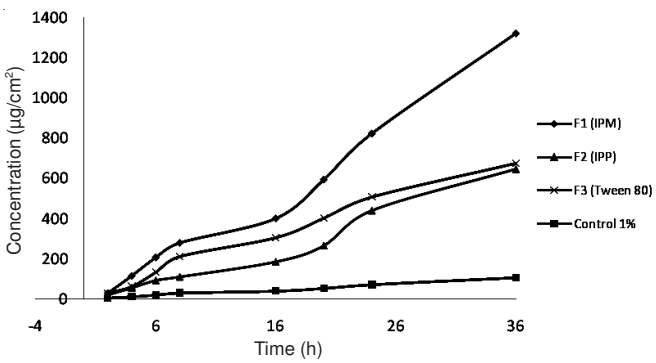

Fig. 2. Effect of enhancers on permeation of diclofenac potassium from various patch formulations (1 % drug) across abdominal rabbit skin in comparison to formulation control data

For 10 % drug formulation, the cumulative amounts permeated at 36 h were 3085.89 ± 133.58, 1802.77 ± 107.45, 451.18 ± 34.87 μg/cm^2 for formulations F7-F9, respectively (Fig. 3). It is obvious from the data that the isopropyl myristate has significantly influenced the permeation of diclofenac potassium through abdominal hairless rabbit skin. isopropyl myristate is an aliphatic ester, which is widely used as a safe penetration enhancer in dermatological formulations. Its mechanism of action is not precisely understood, but it seems that isopropyl myristate penetrated between the lipid layers of

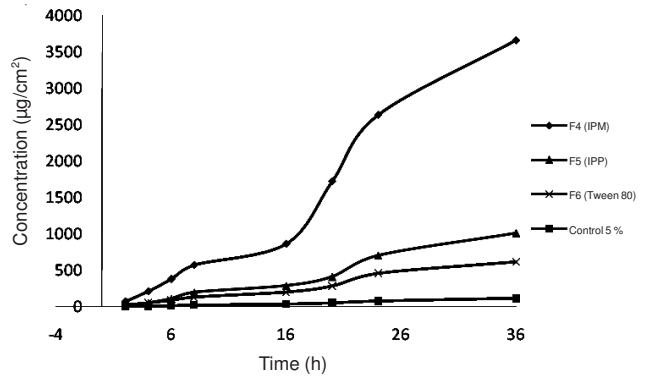

Fig. 3. Effect of enhancers on permeation of diclofenac potassium from various patch formulations (5 % drug) across abdominal rabbit skin in comparison to formulation control

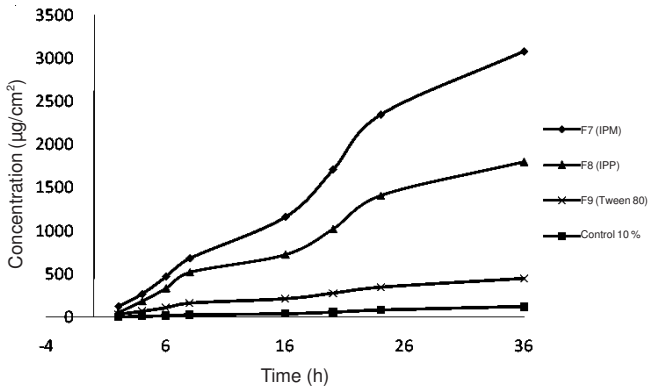

Fig. 4. Effect of enhancers on permeation of diclofenac potassium from various patch formulations (10 % drug) across abdominal rabbit skin in comparison to formulation and control

stratum corneum and due to its chain structure disrupts the order and arrangement of lipid bilayer of stratum corneum and hence improves drug permeation into this layer[38].

Consequently, based on more data, the intercellular rout is now considered to be the major pathway for permeation of most drugs across the stratum corneum[39]. The permeation rates (flux, J_{ss}) of diclofenac potassium from all the prepared formulations containing enhancers were higher than that of the formulation control (Figs. 1-4 and Table-2). The ER_{flux} was 32.66 (highest) for the formulation F4 as compared to 4.01 ± 2.604 for 5 % limonene in horses[36]. Diffusion coefficients obtained were 6.07 ± 0.44 $cm^2/s \times 10^{-4}$ for formulation F3, in comparison to 1.14 ± 0.43 $cm^2/s \times 10^{-4}$ of control without enhancer C 1, 2.27 ± 0.42 $cm^2/s \times 10^{-4}$ for formulation F4 in comparison to 1.14 ± 0.31 $cm^2/s \times 10^{-4}$ of C2, 6.07 ± 0.43 $cm^2/s \times 10^{-4}$ for formulation F9 in comparison to 2.53 ± 0.33 $cm^2/s \times 10^{-4}$ of C3. The diffusion coefficient of the drug is increased as the enhancer molecules form microcavities within the lipid bilayers, hence increasing the free volume fraction[39]. In addition, the minimum lag time (apparent diffusivity)[40] was 3.0 h ± 0.18, 17 h ± 0.39 for the formulation F3 and F9 compared to 16 ± 0.20 and 7.2 ± 0.39 for controls C1 and C3, respectively (Table-2). The reduction in the lag time of the prepared formulations can be useful for rapid onset of the therapeutic effect[9]. The data reveal the importance of permeation enhancers for improving the permeation rate of the drug. Maximal enhancement of diclofenac potassium permeation was obtained with isopropyl myristate for 5 % drug (F4) followed by F1 with isopropyl myristate and 1 % drug formulation having the enhancing factor value of 32.66 and 12.39, respectively (Table-2).

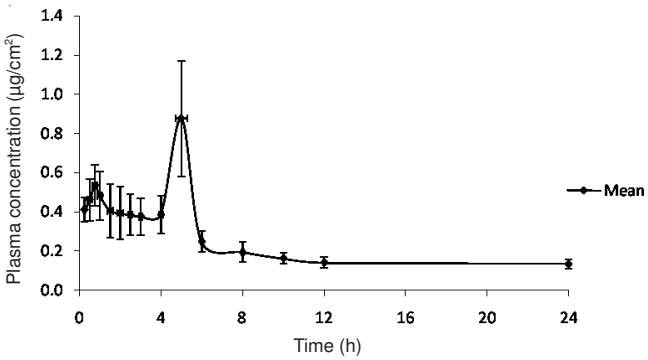

Fig. 5. Plasma concentration-time profile of diclofenic potassium after topical application of trandermal formulations

Data analysis according to kinetic models: The kinetic parameters of drug permeation for different formulations are presented in Table-3. The zero order plots of all the formulations were found to be fairly linear as indicated by their high regression values. The n value from Korsmeyer-Peppas equation gives an indication of the release mechanism.

Optimized formulation resulting from *in vitro* studies: Formulation F4 was selected as the optimized formulation by virtue of maximum skin permeation as compared to other formulations and the control (without enhancers). Initially rapid permeation was observed, gradually approaching to constant

TABLE-3
RELEASE KINETICS OF DICLOFENAC POTASSIUM DIFFUSION FROM MATRIX TYPE TRANSDERMAL DRUG DELIVERY SYSTEM

Formulation code	Kinetics of drug release	Determination coefficient	Zero order rate K_0 (h^{-1})	Mechanism of release	n value	Best fit equation for permeation plot
F1	Zero order	0.9760	0.5603	Super case-II transport	0.9783	Q = 36.28t - 53.245
F2	Zero order	0.9648	0.2780	Super case-II transport	1.5429	Q = 17.076t - 26.239
F3	Zero order	0.9870	0.3031	Non-Fickian	0.8384	Q = 19.307t - 10.787
F4	Zero order	0.9583	1.6452	Super case-II transport	1.2011	Q = 108.14 - 299.26
F5	Zero order	0.9642	0.4440	Super case-II transport	0.9832	Q = 29.103t - 73.307
F6	Zero order	0.9705	0.2730	Non-Fickian	0.7996	Q = 17.649t - 22.472
F7	Zero order	0.9848	1.4014	Super case-II transport	1.1089	Q = 90.095t - 74.463
F8	Zero order	0.9783	0.8157	Super case-II transport	1.0569	Q = 51.944t - 0.9716
F9	Zero order	0.9789	0.1969	Non-Fickian	0.6882	Q = 12.189t - 31.502

Data are given as mean ± SD (n = 3).

values for the rest of time as in agreement with the literature[41]. Linear curves were obtained on plotting the graphs for cumulative per cent drug permeated *versus* time suggesting zero order diffusion mechanism of drug release (r^2 = 0.9870, Table-3). Higher flux value (121.18 ± 34.37 μg h/cm^2) was obtained which presents the better permeation rates from the formulation as compared to control (Table-2). An increase in diffusion coefficient (2.27 ± 0.42 cm^2/h × 10^{-4}) as compared to formulation control (without enhancer) causes an increase in permeability as evident in literature[39].

Pharmacokinetic studies: Plasma drug concentration profile for optimized formulation F4 is presented in Fig. 5 while pharmacokinetics parameters are summarized in Table-4 for the optimized formulation F4 containing 100 mg of drug per application. Detectable diclofenac in plasma appeared at 0-0.25 h after topical dosing and the drug persisted in the blood up to 24 h. This indicated that the release was sustained for a period of 24 h which is in agreement with literature[42]. The mean value of time to achieve peak level in plasma, T_{max}, is 4.50 h ± 1.73 which means that diclofenac in present study produced a prolonged delivery compared to oral dosing[43]. The C_{max} is 0.98 ± 0.59 ng mL^{-1} for all volunteers. A study reported C_{max} of 0.081 ± 0.043 μg/mL after single dose application of 300 mg dose of solution gel and 0 039 ± 0.017 μg/mL after single application of 300 mg dose of emulsion gel[44].

TABLE-4
PHARMACOKINETIC PARAMETERS OF DICLOFENAC AFTER TOPICAL APPLICATION OF PATCH FORMULATION (F4) TO HUMAN SKIN *in vivo* (n = 5)

S. No.	Parameters	Mean
		F4
1	C_{max} (ng mL^{-1})	0.98 ± 0.59
2	t_{max} (h)	4.50 ± 1.73
3	K_e (h^{-1})	0.098 ± 0.079
4	$t_{1/2}$ (h)	14.67 ± 13.05
5	K_a (h^{-1})	0.98 ± 0.079
6	$AUC_{(0-24)}$ (ng h/mL)	3.53 ± 1.04
7	$AUMC_{(0-24)}$ (ng h^2/mL)	21.04 ± 11.01
8	MRT (h)	22.38 ± 20.75

The area under the curve, AUC_{0-24} value (3.53 ± 1.04 ng h/mL) indicated an extent of drug availability of drug. The calculated parameters also indicate that biological half life of drug is prolonged from 2 h (conventional tablets) and 2-5 h (for sustained release) to 103.427 h ± 4.5, hence, the drug administered in the transdermal dosage form will remain in the body for a longer period and thus will exert a sustained action.

The elimination rate constant K_e calculated from the linear terminal phase was found to be 0.81 h^{-1} ± 0.98 with MRT of 22.38 h ± 20.75. The significantly less elimination rate constant and high mean residual time values of diclofenac obtained with formulation F4 further support the sustained action of drug from the patch formulation[45]. DPSGC based liquid formulation of diclofenac potassium for doses of 25 and 50 mg, given results of C_{max}; 1025 and 1882 ng/mL, respectively; $AUC_{0-\infty}$ of 582 and 1197 ng h/mL, respectively; and T_{max} of 0.45 and 0.48 h, respectively[46]. 60 g topical dose of diclofenac potassium resulted C_{max} of 0.034 μg/mL, T_{max} of 6 h, $t_{1/2}$ of 9.966 ± 0.834 h, $AUC_{(0-24)}$ of 0.442 ± 0.053 μg h/mL and MRT of 15.388 ± 0.426 h when administered as ethyl hexyl acrylate and vinyl acetate pressure sensitive adhesive system[47]. An IV administration of 22.5 mg and single oral doses of 12.5 and 25 mg diclofenac potassium resulted in C_{max} (μg L^{-1}) of 1892 ± 439, 334 ± 162, 588 ± 315, T_{max} of 0.28 ± 0, 0.48 ± 0.28, 0.93 ± 0.96 h and $t_{1/2}$ of 0.9 ± 0.3, 0.8 ± 0.3, 0.8 ± 0.2 h, respectively[48].

Stability studies: The optimized formulation (F4) was found stable as shown by 6 months accelerated stability studies. On performing assay, a high percentage of drug was recovered from the patches. The results indicated that the drug remained intact in the formulation and there was no chemical interaction between the drug and excipients therein.

Conclusion

Among the formulations, the more pronounced enhancing effect was obtained with isopropyl myristate, regarding permeation flux, diffusion coefficient and permeability coefficient, as well as the decreased lag time of permeation of 5 % diclofenac potassium from patch formulations (F4) as compared to formulation control (without enhancer). The faster permeation of the drug as compared to the controls could be attributed to the incorporation of skin penetration enhancer isopropyl myristate. The pharmacokinetic studies shown that there was continuous delivery of drug up to 24 h from the patch formulation (F4) into and through the skin. Therefore it is concluded that the incorporation of 3 g of isopropyl myristate as skin penetration enhancer is promising in developing matrix type patch formulation containing 5 % diclofenac potassium. Different concentrations of isopropyl myristate could be studied with the same formulation for having better optimized transdermal drug delivery system.

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