Asgar Ali, Nitin Kumar, Abdul Ahad, Mohd. Aqil* and Yasmin Sultana

Enhanced delivery of diclofenac diethylamine loaded Eudragit RL 100® transdermal system against inflammation

Abstract: A transdermal therapeutic system (TTS) of diclofenac diethylamine (DDE) was developed to obtain a prolonged controlled drug delivery by the solvent evaporation technique. The matrix diffusion controlled systems used various combinations of hydrophilic (polyvinylpyrrolidone K30) and lipophilic (Eudragit RL 100® and Eudragit RS 100®) polymers containing dimethyl sulfoxide (DMSO) (0, 5 and 10% w/w) as a penetration enhancer. *In vitro* drug release was improved with an increased fraction of hydrophilic polymer. Formulation F8 containing Eudragit RL 100® and polyvinylpyrrolidone K30 in the ratio 40:60 presented the highest drug release (92.45%) and permeation rate (0.0988±0.010 mg/cm^2/h) with sustained release action for 48 h. *In vivo* pharmacodynamic study of DDE-loaded Eudragit RL 100® transdermal system (formulation F8) showed significant higher percent inhibition of rat paw edema compared with the marketed formulation of the drug. Our results suggest that a developed formulation is an efficient system for transdermal diclofenac delivery against inflammation. The optimized formulation was found to be stable and did not show physicochemical interaction. The system is envisaged to be stable for a sufficiently long period (2.52 years) at room temperature.

Keywords: antiinflammatory activity; dimethyl sulfoxide; Eudragit RL 100®; penetration enhancer; transdermal.

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1 Introduction

Transdermal delivery of drugs promises many advantages over oral or intravenous administration, although human skin provides an effective barrier to the permeation of most drugs in the form of the stratum corneum. The success of the transdermal route depends on the ability of drugs to breach this barrier and permeate the skin at a rate sufficient to attain effective plasma concentration. There are many approaches which have been employed to enhance the skin permeation rate of active moieties. However, the most convenient and widely implemented approach is the use of chemical penetration enhancers [1–4].

Dimethyl sulfoxide (DMSO) is a molecule with a long history in pharmaceutics and is now well established as an absorption promoter in topical pharmaceutical formulations.

It is one of the earliest and most widely studied compounds to be used as a penetration enhancer in pharmaceuticals. It is currently used for this purpose in diclofenac sodium topical solution (Pennsaid; Mallinckrodt, Inc., Hazelwood, MO, USA), a topical preparation approved in the United States for treatment of signs and symptoms of osteoarthritis of the knee. It is also used as a penetration enhancer in 5% idoxuridine topical solution (Herpid; Astellas Pharma, Staines, UK), a preparation approved in Europe for the treatment of herpes zoster. The clinical use of pharmaceutical-grade DMSO as an enhancer is supported by the robust data that have accumulated over the past three decades, demonstrating the favorable safety and tolerability profile. DMSO is a safe and effective mechanism for facilitating the transdermal delivery of both hydrophilic and lipophilic medications to provide localized drug delivery [4–6].

Diclofenac diethylamine (DDE) is a diethylammonium salt of 2-[(2,6-dicholorphenyl) amino] benzene acetic acid. It is a potent nonsteroidal antiinflammatory drug (NSAID) of the aryl acetic acid derivative, a nonselective cyclooxygenase-1 (COX-1) and COX-2 inhibitor. It is used widely in cases of rheumatoid arthritis and osteoarthritis, bursitis ankylosing spondylitis, dysmenorrhea, posttraumatic

and post inflammatory conditions [7]. It is extensively metabolized in the liver and because of its short half-life, the drug has to be given frequently. Therefore, developing a transdermal therapeutic system (TTS) is useful for bypassing hepatic metabolism, maintenance of constant and prolonged drug level, reduced frequency of dosing and in consequence, improved patient compliance.

The most critical parameter for transdermal absorption is the partition coefficient of a drug (log p-value); the log p-value of DDE is 0.853. A drug with a log p-value of ≤2 is considered to be a potential candidate for transdermal delivery [8]. Moreover, other physical parameters like low molecular weight (369.3) and pharmacological parameters such as poor bioavailability (40–60%), short biological half-life (2–3 h), and low dose (25–50 mg) are favorable for transdermal delivery [9]. Various transdermal delivery systems containing diclofenac have been reported, including patches [8, 10], gels [11, 12], mixed micelle formulations [13], microemulsion systems [11, 14–17], nanoemulsions [15] and vesicular systems [18–20]. Transdermal delivery of NSAIDs proved to be a convenient route of administration for a variety of clinical indications [21]. Transdermal patches offer added advantages, such as maintenance of constant and prolonged drug level, reduced frequency of dosing, minimization of inter and intra patient variability, self-administration and easy termination of medication, leading to patient compliance [22].

In the present study, matrix type transdermal delivery systems for DDE were developed using varying proportions of polymers (Eudragit RL 100®/Eudragit RS 100®) and polyvinylpyrrolidone K30 (PVP K30) and enhancer (DMSO). DMSO was selected as the best penetration enhancer from a group of enhancers (such as dimethylacetamide, dimethylformamide, menthol, thymol, etc.) on the basis of preliminary skin permeation studies. The purpose was to develop a stable TTS for delivering DDE in a controlled manner to achieve a therapeutically effective drug level for a prolonged period of time.

2 Materials and methods

2.1 Chemicals and reagents

DDE was received as a gratis sample from Oscar Laboratories, New Delhi, India. Eudragit RL 100 and Eudragit RS 100 were procured from Rohm Pharma (Weiterstadt, Germany). PVP K30 was purchased from La Grande Pvt. Ltd. (New Delhi, India). Dibutyl phthalate (DBP) was obtained from Merck (Mumbai, India). All other chemicals were of analytical reagent grade.

2.2 Animals

Male Wistar rats (150–200 g) were supplied by the Central Animal House of Hamdard University and inhabited under standard laboratory conditions in 12 h light/dark cycle at 25±2°C. Animals were nourished with a pellet diet (Lipton, India) and water *ad libitum*. The animals were received after the study was duly approved by the University Animal Ethics Committee, and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA), Government of India.

2.3 Fabrication of patches

The transdermal films of DDE were prepared using varying proportions of film forming polymers (Table 1) by the solvent evaporation technique in novel film casting assembly.

The novel circular casting assembly consisted of two stainless steel plates of internal diameter 8 cm (area 50 cm^2). Aluminum foil was placed in between the plates and screwed together with nuts and bolts to prevent solvent leakage. The polymeric solution was prepared by dissolving the combination of polymers (Eudragit RL 100®, Eudragit RS 100®, PVP K30), along with DDE (57.65 mg/patch of area 9 cm^2), plasticizer (DBP 5% w/w) and penetration enhancer (DMSO 0, 5 and 10% w/w) of polymer, in a solvent system of methanol and dichloromethane (6 ml) (1:1). The solution was poured onto the aluminum foil placed in the circular pocket of the casting assembly. The solvent was allowed to evaporate by keeping the assembly in an oven at 32±0.5°C for 12–14 h. The dried film, along with the aluminum foil backing, were then cut into films with the help of a circular stainless steel die to get films of 9.0 cm^2 areas. An adhesive gateway paper was then laminated on the backing film leaving a circular adhesive rim with a little flap for easy removal. The transdermal films were stored in airtight container at ambient conditions for 7 days prior to use.

2.4 Physical evaluation of transdermal films

2.4.1 Thickness

The thickness of the patches was measured at five different places on a single patch of each formulation using a screw gauge and the mean values were calculated [23].

2.4.2 Weight

The weight of the films (one patch size) was determined using a digital electronic balance (Mettler, Japan).

Table 1: Composition of transdermal drug delivery system of diclofenac diethylamine (DDE).

	Formula (mg/patch of area 9 cm²)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
DDE	58.05	58.05	58.05	58.05	58.05	58.05	58.05	58.05	58.05	58.05
Eudragit RL 100®	112.5	112.5	90	90	–	–	75	60	–	–
Eudragit RS 100®	37.5	37.5	–	–	90	90	–	–	75	52.5
PVP K30	–	–	60	60	60	60	75	90	75	97.5
DMSO	7.5	–	7.5	–	7.5	–	15	15	15	15
DBP	30	30	30	30	30	30	30	30	30	30
Polymer ratio	75:25	75:25	60:40	60:40	60:40	60:40	50:50	40:60	50:50	35:65
Total	245.55	238.05	245.55	238.05	245.55	238.05	253.05	253.05	253.05	253.05

DBP, dibutyl phthalate; DMSO, dimethyl sulfoxide; PVP K30, polyvinylpyrrolidone K30.

2.4.3 Folding endurance

For determining folding endurance, a film of 9 cm² areas was folded end to end on the long side along the center, in between the thumb and the finger and then opened. This was called one folding. The procedure was repeated until films broke in the center. The total number of foldings was called the folding endurance value [24].

2.4.4 Tensile strength

The tensile strength of the transdermal films was determined by the percent elongation at break value [25]. Briefly, the films of known dimensions in a rectangular shape were placed between the jaws of the tensile tester apparatus. One jaw was kept stationary and another was pulled slowly until the film was just broken. Percentage of elongation at the break was determined using the following equation:

$$\%\ \text{Elongation at break} = \frac{\text{Length of patch at break} - \text{Initial length of patch}}{\text{Initial length of patch}} \times 100$$

2.4.5 Moisture content

Individual weight of transdermal films of different composition was measured and kept in desiccator containing activated silica at room temperature for 48 h. Final weight of each film were taken in achieving constant weight. Percentage of moisture content was determined as follows:

$$\text{Moisture content} = \frac{\text{Initial wt. of patch} - \text{Final wt. of patch}}{\text{Final wt. of patch}} \times 100$$

2.4.6 Moisture uptake capacity

Transdermal films of different composition of specific dimensions were weighed and exposed to 84% relative humidity (saturated solution of potassium chloride) in a humidity chamber until constant weight for the films was achieved. The final weight of each film was taken in achieving the constant weight. The moisture uptake capacity of each film was determined by taking the difference of initial and final weights with respect to the initial weight [26].

2.4.7 Content uniformity test

The content uniformity of films was measured at different sites of the same films. Pieces of specified size of different films were cut and dissolved separately in 10 ml of methanol in different conical flasks. These were kept in a mechanical shaker for 12 h at 37±1°C. Aliquots of 1 ml of the patch solutions were filtered and centrifuged, and determined spectrophotometrically at 278 nm [27, 28]:

$$\text{Drug content} = \frac{\text{Concentration} \times \text{Dilution factor} \times \text{Volume}}{1000}$$

2.5 *In vitro* drug release studies

An *in vitro* release test was performed using a modified paddle over a disc assembly (USP Apparatus V). The TTS

was mounted on the inner stainless steel mesh of a Teflon disc assembly and was placed at the bottom of the dissolution vessel at a distance of 25±2 mm from the paddle blade. The dissolution medium of 900 ml of 30% methanol: 70% isotonic phosphate buffer (IPB), pH 7.4 was filled in a vessel and equilibrated to 37±0.5°C. The dissolution vessel was covered during the study to minimize evaporation. The apparatus was operated at 50 rpm. Aliquots (3 ml) of dissolution medium were withdrawn from the zone midway between the surface of the dissolution medium and the top of the blade, at 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 24, 32, 40 and 48 h and replaced with equal volumes of fresh dissolution medium. The samples were analyzed for drug content by the UV spectrophotometric method at 278 nm [27, 28].

2.6 *In vitro* skin permeation studies

For *in vitro* skin permeation studies, the hairs on the skin of animals were clipped and subcutaneous tissues were surgically removed, and the dermis side was wiped with isopropyl alcohol to remove residual adhering fat [29, 30]. The skin samples were mounted over the diffusion cells in such a way that the stratum corneum (SC) side faced the donor compartment, whereas the dermis faced the receiver compartment [31, 32].

A cell similar to the one designed by Keshary and Chein was used for permeation studies and it had a water jacket for maintaining the temperature at 37±0.5°C [33, 34]. The vertical double-walled diffusion cell consisted of two half cells with diffusion areas of 9.0 cm^2 and the capacity of the receiver chamber was 40 ml. The skin sample was excised from an abdominal portion of Wistar rats, pretreated and stabilized [35–37]. The release surface of TTS under test was placed in intimate contact on the stratum corneum side of the skin in the donor compartment. A Teflon coated magnetic bead was kept in the receiver compartment for agitating the contained vehicle (30% v/v methanolic IPB, pH 7.4), at 500 rpm. The samples (1 ml) were withdrawn at time intervals as in the case of the release studies for 48 h. The samples were filtered with Whatman filter paper No. 42, diluted and analyzed for drug content by the UV spectrophotometric method at 278 nm. The cumulative amount of drug permeated was plotted against time. The flux (*J*) values were calculated from the linear portion of the plots. The permeability coefficient (K_p) was calculated by the following equation:

$$K_p = \frac{J}{C_d}$$

where C_d is the drug concentration in films.

2.7 Interaction studies

Interaction studies were conducted on the selected optimized formulation (F8) in order to find any interference, interaction, or drug degradation in TTS by: (i) assay (drug content in TTS), (ii) infrared spectrophotometric study (IR study) and (iii) thin layer chromatography (TLC).

2.7.1 Assay

The TTS was powdered in a mortar and pestle and then slowly extracted using 30% methanolic IPB, pH 7.4. The extract was filtered through Whatman filter paper No. 42. The UV absorbance of the filtered extract was taken at 278 nm. The content of DDE in TTS was calculated from the following formula:

$$\text{Drug Content} = \frac{\text{Concentration } (\mu g/ml) \times DF}{1000}$$

where DF is dilution factor.

2.7.2 IR study

TTS (F8) was grounded along with KCl and a pellet was formed (test sample). An IR spectrum was taken using a Fourier transform infrared spectrometer (Perkin Elmar, Germany). An IR spectrum of pure drug was also taken by making KCl pellet (reference sample) and compared with the test sample spectrum.

2.7.3 TLC study

A TLC study of pure drug and the drug in TTS (F8) was performed using silica gel GF 254 plate and water:hydrochloric acid:glacial acetic acid:ethyl acetate (1:1:6:11 v/v) as the mobile phase [38]. The plates were activated at 110°C for 1.5 h. The activated plates were applied with 20 µl of each sample of 5% w/v pure drug in methanol (reference sample) and TTS, extracted in methanol and filtered (test sample). The mobile phase was allowed to travel 3/4 of the plate. After that, it was allowed to dry in a current of warm air for approximately 30 min and cooled. It was observed under UV light at 254 nm in a UV chamber. Spots were marked and R_f values were calculated and compared.

2.8 Stability studies

The stability studies for optimized formulation (F8) were carried out according to WHO guidelines by storing the

TTS samples at 40±0.5°C, 50±0.5°C and 60±0.5°C for 60 days. The samples were withdrawn and analyzed at intervals of 20, 40 and 60 days for drug content by HPLC [39]. A Shimadzu LC-10 VP series HPLC system (Kyoto, Japan) with 2 LC-10AT pumps, variable wavelength programmable UV/VIS detector SPD-10AVP system controller and RPC-18 column (Supelco, Sigma, Germany, 250 mm×4.6 mm, particle size 5 µm) was used. The system was equipped with class VP series version 6.12 software. The mobile phase consisted of methanol:acetonitrile (60:40). The mobile phase was filtered through a 0.4 µm membrane filter; the flow rate (1.2 ml/min) was monitored at 278 nm. The total run time of the method was set at 10 min, and the retention time was 3 min. A standard stock solution of DDE (100 µg/ml) was prepared and calibration curve standard solutions (2–50 µg/ml) were prepared by diluting the stock solution with the mobile phase.

The logarithm of percentage drug remaining in TTS (F8) was plotted against time (in days). Slope of the straight lines for each temperature were obtained and the degradation rate constant (K) was calculated by the formula given below:

$$\text{Slope}=\frac{-K}{2.303}$$

The shelf life ($t_{0.9}$) was calculated by the following equation:

$$t_{0.9}=\frac{0.1054}{K_{25}}$$

K_{25} (value of K at 25°C) was determined by extrapolation from the plot between log K vs 1/T (absolute temperature).

2.9 *In vivo* antiinflammatory activity

Approval to carry out *in vivo* studies was obtained from the Institutional Animal Ethics Committee Jamia Hamdard, New Delhi, India and their guidelines were followed for the studies. Carrageenan-induced rat paw edema volume model [31, 40] was used to assess the antiinflammatory activity of developed DDE optimized formulations. Left hind paws of each rat were marked just beyond the tibiotarsal junction, so that every time the paw is dipped up to the fixed mark to ensure constant paw volume.

Wistar rats, weighing 150–200 g, were randomly divided into four groups: control, toxic control (carrageenan only), F8 and marketed gel-treated groups, each containing six rats. The dose for the rats was calculated based on the mass of the rats according to the surface area ratio [41]. The abdominal region of the rats was shaved 12 h before starting the experiments except in the control group. Paw edema was induced by subplantar injection of 0.1 ml of 1% w/w homogeneous suspension of carrageenan in distilled water in the hind paw. The Patch F8, and marketed gel formulations were applied on the shaved abdominal region of all animals (except in the control group) with the entire release surface in intimate contact with the rat skin. An adhesive tape (Johnson & Johnson, New Brunswick, NJ, USA) was rolled over the formulation to keep it secured at the site of application. The volume of the paw was measured at 0.5, 1, 2, 3, 6, 12, 24, 36 and 48 h after injection using a digital plethysmometer. The amount of paw swelling was determined at time interval and expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group. Percentage inhibition of edema produced by each treated group was calculated against the respective control group using the following formula:

$$\%\ \text{Inhibition}=\frac{\%\ \text{Edema (Control)}-\%\ \text{Edema (treated group)}}{\%\ \text{Edema (Control)}}\times 100$$

Mean edema=mean of final paw volume-mean of initial paw volume.

Data were analyzed using the GraphPad Instat software (Graph-Pad Software Inc., CA, USA). Data are expressed as mean±standard deviation (SD) and were assessed by the Dunnett test throughout the comparisons of edema size. A p-value <0.05 was considered to show a significant difference for all comparisons made.

3 Results

3.1 Physicochemical evaluation of transdermal patch

A transdermal patch of DDE was prepared using different combinations of acrylate and PVP K30 (Table 1). Physical properties of the optimized films are summarized in Table 2.

The films formed were slightly opaque and had uniform thicknesses. Amongst all prepared films, formulation F8 showed the highest folding endurance (165±13.46) and thickness (0.273±0.035 mm) (Table 2).

Further, tensile strength of the film suggested that film F8 containing polymer Eudragit RL 100® and PVP K30 in the ratio 2:3 presented the highest tensile strength and thus a high folding endurance (Table 2). The decreasing order of moisture content and moisture uptake capacity of these films was F10>F8>F9>F7 (Table 2). Drug content of the films varied from 98.5±1.1% to 99.5±1.5%.

3.2 *In vitro* drug release and skin permeation studies

Drug release and permeation studies for different formulations were performed in a USP basket dissolution apparatus and Keshary-Chein diffusion cell using 30% methanolic IPB, pH 7.4, as a release medium at 37±0.5°C. *In vitro* drug release study of formulation F1–F6 suggests that formulation F3 showed significantly ($p<0.05$) higher drug release as compared to the others (Figure 1). The decreasing order of drug release from different formulations was F3>F5>F1>F4>F6>F2 (Figure 1).

A similar trend was observed in permeation studies [F3>F5>F1>F6>F4>F2 (Figure 2)] with F3 (having polymer Eudragit RL 100®: PVP K30, 60:40) showing significantly high flux (0.0959±0.023 mg/cm²/h) and permeability coefficient (1.663±0.032 cm/h) followed by F5 (carrying polymer Eudragit RS 100®: PVP K30, 60:40) (Figure 2 and Table 3) ($p<0.05$).

Results also showed significant ($p<0.05$) improvement of the transdermal flux of DDE on the addition of DMSO (Table 3). Formulations F1 and F2 were discarded due to lesser drug release (55%) and slow permeation rate (0.0529±0.021 mg/cm²/h). Hence, further patches were prepared having a combination of acrylate (hydrophobic) and PVP K30 (hydrophilic) polymers and 15% DMSO as enhancer, by modifying formulations of F3 and F5 (formulations F7–F10). The decreasing order of drug release and permeation rate was found to be F8>F7>F10>F9 and F8>F10>F7>F9, respectively (Figures 3 and 4).

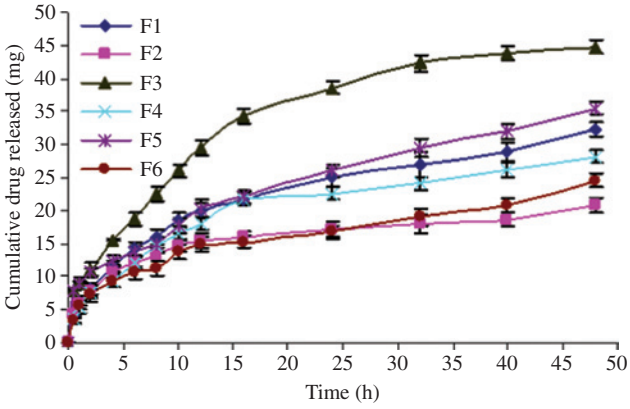

Figure 1: *In vitro* release of diclofenac diethylamine (DDE) from transdermal therapeutic system (TTS) (F1–F6) with (F1, F3 and F5) and without (F2, F4 and F6) 7.5% dimethyl sulfoxide (DMSO) as enhancer (mean±SD; n=3).

The highest drug release (92.45%) and permeation rate (0.0988±0.010 mg/cm²/h, $p<0.05$) (Table 3) from F8 (Eudragit RL 100: PVP K30; 40:60) is attributed to an optimized combination of acrylate (Eudragit RL 100®, permeable polymer) and PVP K30, hydrophilic soluble polymer (Table 1). The release rate curve in the case of F8 was smooth with almost constant sustained release of the drug (Figure 3). This result substantiated that the higher the drug release across skin from the TTS, the higher was the rate and extent of drug permeation. Hence, F8 TTS, which showed the highest drug release and skin permeation amongst all the patches, was selected as the optimized formulation and was investigated for drug excipients interaction, stability and antiinflammatory activity.

3.3 Drug excipients interaction study

The interaction studies were performed in order to establish any kind of interaction of the drug with excipients used in transdermal patch formulation. For this, the optimized formulation F8 and pure drug were subjected to

Table 2: Physicochemical characterization for optimized patch of diclofenac diethylamine (DDE).

Formulation code	Thickness (mm)	Weight (mg)	Folding endurance	Tensile strength (% elongation)	Moisture content (%)	Moisture uptake capacity (%)	Content uniformity%±SD
F7	0.261±0.041	251.11±0.49	147±9.23	56±7.58	3.05±0.54	2.31±0.59	99.2±1.2
F8	0.273±0.035	252.85±0.51	165±13.46	63±6.19	3.81±0.51	3.06±0.63	98.8±1.5
F9	0.269±0.043	250.91±0.56	143±11.84	48±6.94	3.47±0.49	2.33±0.74	98.5±1.1
F10	0.281±0.038	252.05±0.89	158±8.91	51±8.05	4.05±0.63	3.15±0.55	99.5±1.3

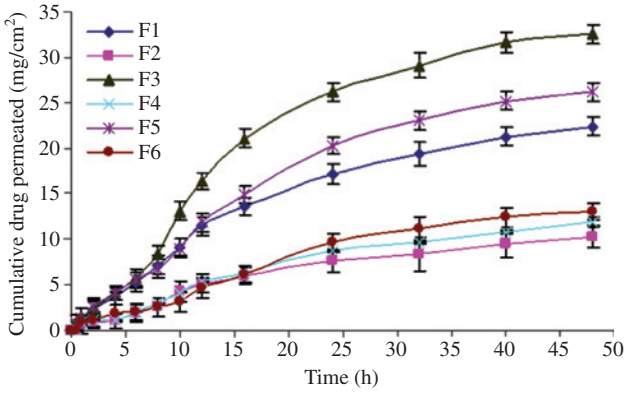

Figure 2: *In vitro* skin permeation profile of diclofenac diethylamine (DDE) from transdermal therapeutic system (TTS) (F1–F6) with (F1, F3 and F5) and without (F2, F4 and F6) 7.5% dimethyl sulfoxide (DMSO) as enhancer (mean±SD; n=3).

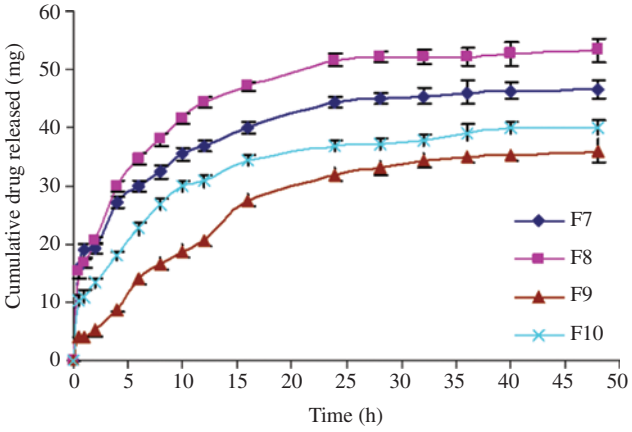

Figure 3: *In vitro* release of diclofenac diethylamine (DDE) from transdermal therapeutic system (TTS) (F7–F10) with 15% dimethyl sulfoxide (DMSO) as enhancer (mean±SD; n=3).

Table 3: *In vitro* transdermal flux and permeability constant (K_p) of diclofenac diethylamine (DDE) from various formulations.

Formulation	Flux (*J*) (mg/cm²/h)	$K_p \times 10^{-3}$ (cm/h)
F1	0.0529±0.021	0.918±0.029
F2	0.0448±0.011	0.777±0.012
F3	0.0959±0.023	1.663±0.032
F4	0.0397±0.009	0.689±0.015
F5	0.0694±0.013	1.204±0.033
F6	0.0388±0.010	0.673±0.027
F7	0.0772±0.010	1.339±0.023
F8	0.0988±0.010	1.714±0.021
F9	0.0815±0.010	1.414±0.010
F10	0.0870±0.010	1.509±0.013

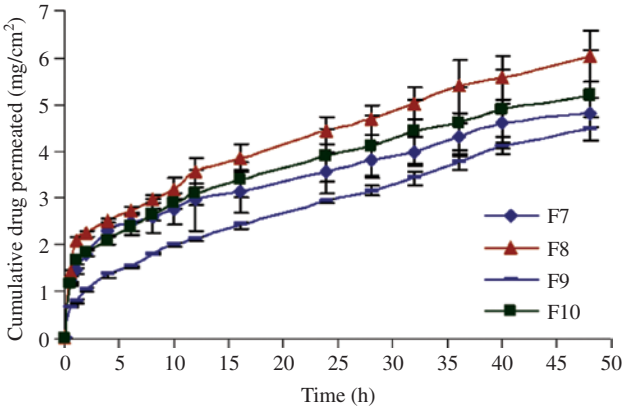

Figure 4: *In vitro* skin permeation profile of diclofenac diethylamine (DDE) from transdermal therapeutic system (TTS) (F7–F10) with 15% dimethyl sulfoxide (DMSO) as enhancer (mean±SD; n=3).

assay, IR and TLC studies. Assay of transdermal patches were performed on six units and mean drug content of patches found to be 99.8±2.05%. IR studies of pure drug and drug incorporated in patch revealed that major peaks of drug were identical, except for a slight deviation in peak height and area in the region of 2000 cm^{-1} to 1400 cm^{-1}. Any interaction between drug and excipients was further confirmed by TLC study. The R_f values of drug and drug excipients solution (F8) were found to be 0.881±0.01 and 0.875±0.006, respectively.

3.4 *In vivo* antiinflammatory activity

Carrageenan-induced rat paw edema in rats was taken as a model for studying the antiinflammatory activity. It was observed that there was a significant difference ($p<0.05$) in the percent inhibition in rat paw edema after 48 h treatment which was found to be higher (78.84%) as compared to marketed gel (45.06%) (Figure 5).

The difference was found to be significant at the 5% level of significance ($p<0.05$). The maximum percentage of inhibition was found at 18 h, and subsequently sustained action was observed up to 48 h. Previously, Baboota et al. [40] developed once-a-day transdermal gel formulation of DDE containing olesan oil and DMSO (10%) as penetration enhancers and evaluated it for antiinflammatory efficacy. They reported that the optimized formulation showed a sustained reduction in inflammation and produced a maximum 73.13% inhibition of edema. In the present study, formulation F8 (Eudragit RL 100: PVP K30; 40:60) showed a higher percentage inhibition of edema (98.24%) for 24 h time points. The formulation demonstrated a sustained inhibition of edema; however at 48 h,

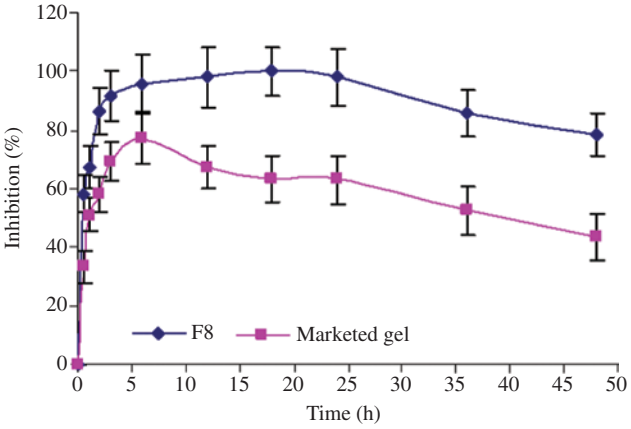

Figure 5: Antiinflammatory activity of optimized diclofenac diethylamine (DDE) formulation (F8) and marketed gel using Wistar rats (mean±SD; n=6).

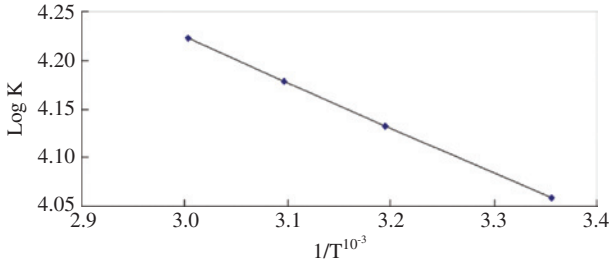

Figure 6: Arrhenius plot for the optimized diclofenac diethylamine (DDE) formulation (F8) kept at temperature 40°C, 50°C and 60°C.

percentage inhibition of edema was reduced to 78.48. The results reflect that the combination of two polymers of a different nature is pertinent for sustained antiinflammatory activity of DDE.

3.5 Stability study of optimized TTS

No significant changes in test parameters were noticed and a very low degradation rate constant ($K=0.044\times10^{-4}$ day^{-1}) was observed on performing the stability studies according to WHO guidelines and a shelf life of 2.52 years could be assigned to the developed TTS of DDE (Figure 6).

4 Discussion

4.1 Physicochemical evaluation of transdermal patch

Folding endurance is a measure of brittleness or fragility of the film, high folding endurance suggests more resistance to fragility. Thickness of different films (F7–F10) was found to vary with change in PVP K30 proportion. Films with a high proportion of PVP K30 had high thickness and high folding endurance. The tensile strength of the films was determined by the percentage elongation of film at break value, which could be described as the percentage change in length after elongation when the film specimens break. Determination of moisture content and moisture uptake capacity of these films suggest that films with PVP K30 as one of the polymers in a high percentage experienced higher moisture content as well as featured high uptake capacity. This might be due to the hydrophilic and hygroscopic nature of PVP K30.

4.2 *In vitro* drug release and skin permeation studies

In vitro release study is an important parameter for ensuring the sustained release performance and reproducibility of the rate and duration of drug release**.** The patch with polymer Eudragit RL 100® and PVP K30 at a high percentage, including enhancer (DMSO), showed a high drug release. High drug release and permeation rate of DDE from the patch with Eudragit RL 100® and PVP K30 is justified by the high permeability of Eudragit RL 100® and high solubility of PVP K30. Formulations F1 and F2 were discarded, due to lesser drug release and a slow permeation rate; this could be assigned to the combination of two hydrophobic polymers used in the above formulation. It was reported that the patch containing only a hydrophobic polymer, like acrylate, showed the lowest drug release [42]. Formulations containing Eudragit RL 100® and PVP polymers showed better *in vitro* drug release and *in vitro* skin permeation as compared to formulations containing Eudragit RS 100® and PVP polymers, because of Eudragit RL 100®, which had better permeability than Eudragit RS 100®. Formulation F8 showed better release than F7 because of a high concentration of PVP K30 in the former, and dissolution of this excess aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores (channels). The formation of such pores leads to a decrease in the mean diffusion path length of the drug molecules to be released into the diffusion medium, and hence, to improved release rates [43]. A similar trend was reported for matrix diffusion controlled patch of

diltiazem hydrochloride. The drug release was found to increase with increasing the concentration of hydrophilic polymer in the polymer matrix [42].

4.3 Drug excipients interaction study

Assay of transdermal patches suggests the drug remained stable in the transdermal patch, as the percentage recovery of the drug from the patches was almost complete. IR studies of pure drug and drug incorporated in a patch showed minor deviations of IR peaks, which might be due to the contribution of excipients on account of the physical interaction of drug and polymers.

No significant difference in R_f value ($p>0.05$) of both drug and drug excipients solution revealed that the excipients do not alter the integrity of the drug in the transdermal patch. It is well established that these excipients (Eudragit RL 100®, Eudragit RS 100®, PVP K30, etc.) are in sustained release matrix type transdermal patches because of their compatibility with numerous drugs [33, 44].

4.4 *In vivo* antiinflammatory activity

Formulation F8 was selected for the *in vivo* antiinflammatory activity based on higher drug release, highest skin permeation rate and stability of patch for a prolonged period of time. In the present study, maximum percentage of inhibition was found at 18 h, and afterwards, sustained action was noticed up to 48 h. Formulation F8 showed a higher percentage inhibition of edema (98.24%) at 24 h time points. Our developed formulation presented better efficacy against inflammation in comparison to a formulation developed by Baboota et al. [40]. They described that the optimized formulation demonstrated a sustained decrease in inflammation and produced maximum 73.13% inhibition of edema. Our optimized formulation demonstrated 78.48% inhibition of edema at 48 h time point. The results reflect that the combination of two polymers of a different nature is pertinent for sustained antiinflammatory activity of DDE.

4.5 Stability study of optimized TTS

The accelerated stability study indicates that the formulation is quite stable at accelerated environmental conditions. A shelf life of 920 days could be assigned to the developed TTS of DDE.

5 Conclusions

Based on these results, it was concluded that the composition of a patch and inclusion of an enhancer (DMSO) influences the drug release and permeation rate from different patches. Optimized formulation F8 (containing Eudragit RL 100®:PVP K30; 40:60) showed the highest drug release and sustained antiinflammatory action up to 48 h on single patch application. The system is envisaged to be stable for sufficiently long periods (2.52 years) at 25°C. The drug remained intact and stable in the TTS during storage, with no significant chemical interaction between the drug and the excipients. Thus, based on the above discussion, it is well justified to conclude that formulation F8 presented an ideal for a combination of polymers and enhancer, and would serve as the best choice for future fabrication of DDE patches, and for a sustained effect of DDE with better enhancement in permeation characteristics and robustness.

Conflict of interest: All authors have approved the final manuscript and the authors declare that they have no conflicts of interest to disclose.

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