Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(6): 456-464

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Enhanced Transdermal Permeability of Diclofenac Diethylamine Using Nanoemulsion Vehicle

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ABSTRACT

The transdermal delivery of Diclofenac diethylamine has attracted considerable interest in recent years due to a lot of significant properties. Transdermal delivery system exhibits very poor permeation through the skin. Thus, an attempt was made to enhance the transdermal permeability of drug using nanoemulsion as vehicle. The main objective of this study was to develop a potential of nanoemulsion formulation for transdermal delivery of Diclofenac diethylamine for inflammatory disease. Transdermal patches were prepared using solvent evaporation technique. In this investigation, the transdermal patches (control) and nanoemulsion containing transdermal patches were prepared, compared and characterized for different physicochemical parameters (moisture content, moisture uptake folding endurance, *in-vitro* drug release). The optimized batch was also subjected for in-vivo anti-inflammatory study, pharmacokinetics ($C_{max,}$ T_{max} and AUC) study and skin irritation study. The physical evaluation indicated that the prepared batches were smooth, flexible and translucent. The transdermal patch having 1:5 ratio of polyvinyl pyrrolidone and ethyl cellulose, exhibited a 1.7 fold increase in skin permeation compare to Diclofenac diethylamine patch. Nanoemulsion containing transdermal patch showed a significant increase in permeation parameters such as steady state flux (J_{ss}); 0.061 $mgcm^{-2}hr^{-1}$ and permeability coefficient (K_p); 0.025 $cmhr^{-1}$, when compared to the control. The mean C_{max} of drug for optimized transdermal patch (TD) and nanoemulsion transdermal patch (NETD) was 2.56µg/ml and 3.42µg/ml, respectively. The *in-vivo* studies revealed a constant plasma concentration of drug for long period. Thus, results indicated that the nanoemulsion containing transdermal patches can be a promising tool for enhancing the percutaneous delivery of Diclofenac diethylamine.

Keywords: Nanoemulsion, Transdermal, Diclofenac diethylamine, *In-vivo*, Pharmacokinetic.

INTRODUCTION

Transdermal drug delivery system is being extensively investigated as a viable alternative to drug delivery with improved bioavailability. It offers many advantages over conventional administration such as enhanced efficacy, increased safety, and greater convenience and improved patient compliance. Transdermal route permits the use of a relatively potent drug with minimal risk of systemic toxicity and avoids gastrointestinal degradation and hepatic first-pass metabolism. [1-3]

Nanoemulsion in transdermal drug delivery enhances therapeutic efficacy and also the bioavailability of the drugs without any adverse effect. It is also regarded as

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Received: 02 November, 2015; **Accepted:** 18 November, 2015

promising technique with many advantages including high storage stability, low preparation cost, thermodynamic stability, absence of organic solvents and good production feasibility. They have also made the plasma concentration profiles and bio-availability of drugs reproducible. These systems are being used currently to provide dermal and surface effects for deeper skin penetration.

In order to overcome the epidermal barrier as to increase transdermal transport, various transdermal carrier systems have been developed. Due to the permeability of substance through the skin is inversely related to its size under certain conditions. [4] The investigations of particulate system are promising for transdermal patches such as solid lipid nanoparticle (SLN), nano structured lipid carrier (NLC), liposomes, microemulsions, nanoemulsion and hexagonal phase nanodispersion. [5] Among these nanoemulsions appear to be attractive and competitive, when used as a vehicle for transdermal drug carrier system.

Nano-sized emulsions are a class of stable emulsions composed of surfactant and oil suspended in water with a particle diameter ranging 50-200 nm. [6] Nanoemulsions seem to be ideal liquid vehicles for drug delivery since they provide all the possible requirements of a liquid system including easy formation, low viscosity with Newtonian behaviour, high solubilization capacity for both lipophilic and hydrophilic ingredients, and very small droplet size. [7] The small droplets confer that the nanoemulsions have large surface to volume ratio, favouring to close contact with the skin providing high concentration gradient and improved substance permeation. Moreover, low surface tension ensures better adherence to the skin. Also, the dispersed phase can act as a reservoir making it possible to transport bioactive molecules in a more controlled fashion. [8]

Diclofenac is an established nonsteroidal anti-inflammatory drug (NSAID) that is widely used to symptomatically alleviate the pain and swelling associated with conditions such as arthritis, toothache, dysmenorrheal and other musculoskeletal disorders. [9] Unfortunately, oral diclofenac use is associated with extensive first pass metabolism as well as potentially gastrointestinal irritation. Intramuscular injection of this drug can cause skin lesion formation. Consequently, the transdermal delivery of diclofenac has attracted considerable interest in recent years. [10-11] Particularly, this research has tended to focus on developing effective topical formulations of Diclofenac diethylamine (DDEA), which is a diethylamine salt of diclofenac. In terms of physicochemical properties, DDEA could be a good candidate for transdermal dosage form development. [12]

In this research, the main aim is to develop the nanoemulsion containing transdermal patches with control patches (drug containing patches) so that the permeation of the drug can be improved which ultimately lead the better bioavailability. Preparation, optimization and evaluation of nanoemulsion were already discussed in previous study thus in this research work the focus has been given only on the capacity of transdermal patches for nanoemulsion and their bioavailability.

MATERIALS & METHODS

Materials

Diclofenac Diethylamine B.P. (Pulverised) was obtained as free gift from the Pee-Medica, Agra. Ethyl cellulose (CDH, Mumbai), Polyvinyl pyrrolidone (Hi Media Laboratories Pvt. Ltd., Mumbai), Di-butyl phthalate (SRL Pvt. Ltd. Mumbai), Carrageenan (S.D. Fine Chemicals Ltd., Mumbai), Methanol and Chloroform (E-Merck, India) were purchased through the vendor.

Development of the Patch

Diclofenac diethylamine containing matrix-type transdermal patches were prepared in petri dish using solvent evaporation technique. Different ratios of PVP and EC were used in this study. The bottom of the Petri dish on which the backing membrane was cast by pouring 4% w/v PVA solution followed by proper drying. The two polymers were weighed in requisite ratio and then they were dissolved in chloroform. Di-n-Butyl phthalate 30% w/w of polymer composition was used as a plasticizer. The drug was added (20% w/w of the total weight of polymers) in the homogeneous dispersion with slow stirring, produced by mechanical stirrer. The uniform dispersion (2 ml each) was cast on the PVA backing membrane casted earlier and dried at 40°C for 6 hours. The backing membrane was then glued to a gummy tape keeping matrix side upward. The aluminium foils were used to give a protective covering. This was the final shape of the formulation. This was used only *in vivo* experiments. Otherwise, in every case, the drug matrix with PVA was used. They were kept in desiccators until used. [13]

Preparation of Nanoemulsion

On the basis of the solubility studies, isopropyl myristate was selected as the oil phase. Tween 80 and Transcutol P were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. The optimized ratio of surfactant and co-surfactant (1:3) was used as emulsifier in this study. The (O/W) nanoemulsion was prepared using the probe sonicator (Bendalin sonoplua, Germany). For this purpose, firstly drug (11.6 mg) was dissolved in mixture of isopropyl myristate (1.0 ml) and water (6.0 ml) and then optimized ratio of tween 80 (0.75 ml) and trancutol (2.25 ml) was added slowly and drop wise with continuous stirring until the system become transparent. The prepared transparent emulsion was undergone for sonication for 20 min, under 3 cycles. 150 pascal pressure was also used in this process.

Preparation of Nanoemulsion Transdermal Patches

The two polymers EC & PVP were weighted in 5:1 ratio and then they were dissolved in chloroform. Di-n-butyl

phthalate (30% w/w of polymer composition) was used as Plasticizer. The drug was added (20% w/w of the total weight of polymers) in the homogenous dispersion by slow stirring with a mechanical stirrer.

Evaluation of Drug Loaded Transdermal Patches

Uniformity of thickness

For measuring the thickness uniformity of the transdermal patches, micrometer (3202-25A, Mitutoyo, Japan) with least count of 0-0.01 mm was used. The thickness of the patch at five different points was measured and the average of five readings with the standard deviation was calculated. [14] The procedure was followed for all the formulation batches.

Folding endurance

The folding endurance was measured manually for the prepared patches. The patches were repeatedly folded at the same place until it broke. Number of times, the patches could be folded at the same place without breaking gave the exact value of folding endurance. [15]

Percent moisture content (% MC)

The patches were weighed individually. The moisture content was determined by keeping the drug matrix patches in a desiccators containing activated silica as desiccant at 37°C for 24 hour. The patches were weighed again and again individually until it showed a constant weight. The final weight was noted when there was no further change in the weight of individual patch. [16]

$$\%MC = \frac{(X-Y)}{Y} x100 \quad \textbf{(Eq.1)}$$

Where, X = initial weight, Y = final weight

Percent Moisture Uptake (% MU)

A weighed film kept in desiccators at normal room temperature for 24 hours then taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) in desiccators until a constant weight for the film was obtained. [16]

$$\%MU = \frac{(Y-X)}{X} x100 \quad \textbf{(Eq.2)}$$

Where, X = initial weight, Y = final weight

Percent (%) flatness study

Longitudinal strips were cut out from each transdermal patch, one from the centre and two from the either side. The length of each strip was measured. The variation in length due to non-uniformity in flatness was measured by determining the % constriction with considering the 0% constriction is equivalent to 100% flatness [16]: described by. (Eq.3)

$$\% \text{ constriction} = \frac{L_1 - L_2}{L_2} \times 100 \quad \textbf{(Eq.3)}$$

Where, L_1 = initial length of each strip. L_2 = final length of each strip

Tensile strength

The tensile strength was measured using an instrument assembled in the laboratory, and followed the method used by Sadhna *et al.* [17] The films were fixed individually to the assembly. The required weights to break the films were noted. Tensile strength was calculated by using the following formula described by (Eq.4).

Tensile strength = (break force/a × b) × (1+L/I) (Eq.4)

Where, a, b, L and I are the width, thickness, length and elongation of the films.

Drug content

The uniformity of drug distribution was determined by taking known weight of the patches at three different places of the patch. The patch was dissolved in 2 ml of methanol and subsequently diluted with phosphate buffer pH 7.4. [18] After appropriate dilutions, solutions were analyzed spectrophotometerically (UV-1700, Shimadzu, Japan) at 275 nm for Diclofenac Diethylamine.

Surface Morphology

Morphology of the transdermal patches was analyzed using scanning electron microscopy (SEM, JSM 6100 JEOL, Tokyo, Japan).The morphological characteristics were observed at an acceleration voltage of 10 KV with suitable magnification. [19]

***In-vitro* Permeation Study**

The permeation studies were performed in a modified Franz diffusion cell- Electrolab (India) Pvt. Ltd., Mumbai (cell capacity; 15 ml, cross-sectional area; 1.13 cm^2) using rat skin. The skin was used after fulfilling all the ethical requirements. The epidermis was separated from the full thickness tissue after immersion in phosphate buffer of pH 7.4, at 60°C in distilled water for 2 min. [15] The skin was used after removing all the adhering fat. A section of skin was cut, measured, and placed on the dermal side of the skin in the donor compartment facing the drug matrix side of the patch to the skin and backing membrane upward. The holder containing the skin and formulation was then placed on the receiver compartment of the modified diffusion cell, containing phosphate buffer of pH 7.4. The donor and receiver compartment were kept in an intimate contact. The temperature of the diffusion cell was maintained at 37°C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead. The samples were withdrawn (2 ml each time) at different time intervals and an equal amount of phosphate buffer (pH 7.4) was added each time. [20] The absorbance of the samples was read by spectrophotometer at 275 nm with taking the phosphate buffer solution (pH 7.4), as blank. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. Steady-state flux (J_{ss}), permeability coefficient (K_p) and enhancement ratio (E_r) for selected formulations were also determined.

***In-vivo* anti-inflammatory study**

In vivo studies were carried out by Deshpande Laboratory Pvt. Ltd., Bhopal, India, having CPCSEA Approval No.: 1414/C/11/CPCSEA and IAEC Approval No PG/DL/2013/a. The committee's guidelines were followed throughout the studies. The

bred wistar rats were maintained at 12 h light and dark cycle, provided with sterile food water and bedding material. The anti-inflammatory activity of the optimized control formulation and nanoemulsion formulation were evaluated using carrageenan induced hind paw edema method. This method was developed by winter *et al.*, [21] in wistar rats. Paw edema was induced by injecting 0.1 ml of the 1% homogeneous solution of carrageenan in 0.9% saline solution. Young wistar rats (180-200 g weight) were randomly divided into two groups (G1 and G2) and each group contained the 6 rats. Group G1 and G2 treated with optimized control formulation and nanoemulsion formulation, respectively. All the animals were kept under standard laboratory conditions with temperature of 25°C ± 1°C and relative humidity 55% ± 5%. The animals were housed in poly propylene cages. Each cage contained the 6 animal with free access to a standard laboratory diet and water ad libitum. The dose of the formulation was calculated according to the weight of the rat (surface area ratio). The abdominal region of the rats was shaved 12 hours before the experiment started, except in control group. Transdermal and nanoemulsion transdermal patch were applied on the shaved abdominal region of all animals (except in control group) half an hour before of sub plantar injection of carrageenan in right paws. Paw edema was induced by injecting the 0.1 mL of homogeneous suspension of carrageenan. This homogenous suspension was prepared by dissolving the 0.1% w/w carrageenan in normal saline solution. After 1 h of injection, the volume of paw was measured using a digital Plathysmometer.

$$\%\text{Inibition} = \frac{\%\text{Edema (Control)} - \%\text{Edema (Drug)}}{\%\text{Edema (Control)}} \times 100$$

Primary skin irritancy studies

Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee. Albino rabbits of either sex each weighing 1.5 to 2.0 kg and 24 months of age were used in this study (n = 6 in each group). The dorsal surface of the rabbits was cleared. The hairs were also removed by shaving. The skin was cleaned with rectified spirit. The patches were applied to the shaved skin of rabbits and secured using adhesive tape USP (Leucoplast TM). On one side of the back, a control patch (without any drug, group I) and on the other side, an experimental patch (group II) were secured. A 0.8% (V/V) aqueous solution of formaldehyde was applied as a standard irritant (group III) and its effect was compared with test. The animals were observed for any sign of erythema or edema for a period of 7 days and scored as reported by Draize *et al.* [22] The application sites were examined for edema and erythema at 24 and 72 h and graded (0-4) according to a visual scoring scale. The final score represented the average of the 24 and 72 h readings. The primary irritancy index (PII) was determined for each preparation by adding the edema and erythema scores; the formulations were accordingly classified as non-irritant if PII <2, irritant if PII=2-5, and highly irritant if PII=5-8. The data was also analyzed statistically by the one-way analysis of variance (ANOVA) test followed by the least significant difference procedure. This statistical analysis was computed using the SPSS® software.

Pharmacokinetic studies

Male adult rabbits having 3 kg weight were used in this study. Each rabbit from one group of 6 rabbits was received diclofenac diethyl amine through transdermal patches. The other group also received the drug through nanoemulsion transdermal patch. This study was also conducted by Deshpande Laboratories Pvt. Ltd., Bhopal, India (CPCSEA No. 14/10/c/11/CPCSEA, IAEC Approval no. CPCSEA/DL/PG/b).

24 hours before the experiment, the rabbits were kept fasting. During the experiment, they received water ad libitum. Each rabbit was weighed before the experiment. 24 hours before of applying the investigational pharmaceutical dosage from to the skin, the hair was removed from the dorsal area of 50 cm^2 using a manual cutting machine. For each dosage form the experiment was simultaneously conducted on 6 rabbits. 24 hours after the depilation the control samples were collected (3 ml of blood). The collection was conducted from the marginal ear vein under the conditions of asepsis using single use needles and syringes. The formulation TD (optimized transdermal patch) and NETD (nanoemulsion containing transdermal patch) were applied on the 50 cm^2 cleared area. The blood plasma sample was collected at 1, 15, 30, 60, 120, 240, 480, 720 minutes, after the application of formulation. Each plasma sample was analyzed by HPLC. For plasma separation, the blood samples were centrifuged at 4800 rpm for 10 min. For this purpose, 500µl of blood sample was collected from each rabbit. Each plasma sample (500µl of serum) was treated with 100µl of phosphate buffer and then the internal standard (10µl naproxen in methanol) was added. The probe was homogenized for 1 minute and then 500µl of chloroform was added. The mixture was horizontally homogenized for 2 minutes and then it was centrifuged for 5 minutes at 4500 rpm. [23] Finally, organic layer was drawn and then dried up at 35°C. The obtained residue was dissolved in 100µl of methanol phosphate buffer (80:20) and then samples were analyzed using HPLC. The amount of drug was determined using HPLC at λ_{max} 276 nm. The mobile phase was a mixture of methanol and phosphate buffer of pH 7.6 (80:20), pumped from the solvent reservoir to the column (Bonda pack 5µm) at flow rate of 1.0 ml/min. R_t for DDEA was 4.3 min. Each withdrawal sample was measured three times. Calibration curve was plotted with analyzing the 1, 5, 10, 20, 40µg/ml of the standard diclofenac diethylamine solution.

Table 1: Evaluation of the Transdermal Patches (Mean ± SD)

Formulation Code	Ratio of EC/ PVP	Thickness (mm)	Drug Content (%)	Flatness (%)	Folding Endurance	Moisture Content (%)	Moisture Uptake (%)	Tensile Strength ($KGCM^{-2}$)
TD-1	2:01	178 ± 4.60	98.1 ± 2.12	99.98 ± 0.002	182 ± 7.52	3.4 ± 0.01	5.4 ± 0.05	2.76 ± 0.118
TD-2	3:01	182 ± 4.88	97.6 ± 2.35	99.94 ± 0.004	190 ± 7.07	3.3 ± 0.03	5.2 ± 0.03	2.98 ± 0.104
TD-3	5:01	188 ± 4.95	97.5 ± 2.21	99.92 ± 0.003	196 ± 6.93	3.2 ± 0.04	5.1 ± 0.01	3.05 ± 0.092
TD-4	1:02	197 ± 4.83	96.9 ± 2.69	99.90 ± 0.001	176 ± 7.63	4.2 ± 0.02	7.6 ± 0.09	2.53 ± 0.104
TD-5	1:03	201 ± 4.71	95.8 ± 2.85	99.89 ± 0.002	172 ± 7.70	4.5 ± 0.05	8.4 ± 0.02	2.46 ± 0.108
TD-6	1:05	206 ± 5.29	97.9 ± 2.75	99.96 ± 0.005	162 ± 7.00	4.7 ± 0.03	8.8 ± 0.07	2.15 ± 0.113
TD-10	5:01+ Nanoemulsion	202 ± 4.14	99.1 ± 1.86	99.99 ± 0.002	228 ± 5.84	3.6 ± 0.002	5.3 ± 0.02	3.34 ± 0.101

Note: Chloroform amount for all batches; 10 ml, Total weight of EC and PVP for all the batches; 600 mg, Amount of drug for all batches; 20% w/w, Plasticizer amount for all batches; 30% w/w

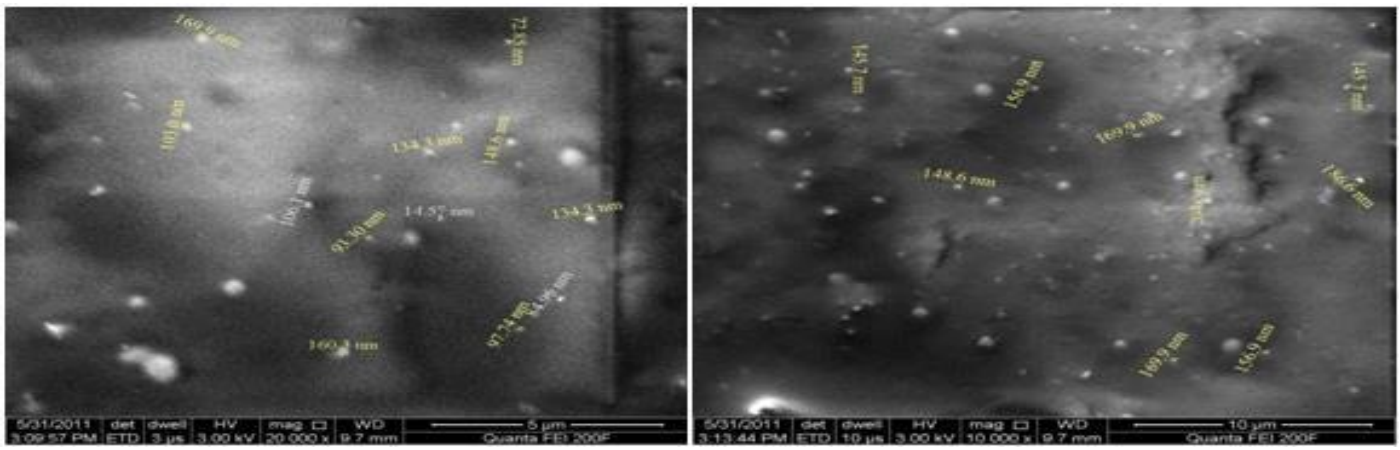

Fig. 1: SEM image of nanoemulsion containing transdermal patch (TD-10)

RESULTS AND DISCUSSION

The matrix transdermal patches of Diclofenac diethylamine were prepared using PVP and EC in different ratios. Ethyl cellulose is considered as nontoxic, non-allergic and non-irritating material and has good film forming properties that enable to form tougher films. However, the water permeability of pure EC is very low. The benefits of EC had been utilized by mixing with other hydrophilic polymer-PVP, in different ratios. In this study, Di-n-butyl phthalate was used as plasticizer to prepare the patches. All the patches were prepared using solvent evaporation technique with employing the petri dish of known diameter. All the prepared patches showed the flexibility, smoothness, and also removed easily from the petri dishes.

It is already known that the PVP and EC are popular in controlled/sustained release drug delivery system due to their compatibility with a number of drugs. Thus, EC was used to design a polymer matrix that allows one to control the release of Diclofenac Diethylamine via the most appropriate choice of polymeric blend of EC and PVP.

Characterization of Transdermal Patches

Moisture Content and Moisture Uptake

The percent moisture content of the formulated patches was varied from 3.2 to 4.7 (%w/w). Similarly, formulated patches also exhibited the percent moisture uptake which was varied from 5.1 to 8.8 (%w/w). The moisture content and moisture uptake of the formulations were gradually increased with the increasing concentration of hydrophilic polymer- PVP. The small moisture content in the formulations helps them to remain stable and from being a completely dried form a brittle film. Also, a low moisture uptake protects the material from microbial contamination and bulkiness of the patches. Thus, in this study small amount of PVP was used to prepare the patches.

Flatness and Thickness

The percent flatness of the formulated patches (TD-1 to TD-10) was varied from 99.89 to 99.99%. This result showed that the formulations were much closed to 100% flatness. The thickness of the formulated patches (TD-1 to TD-10) was also varied from 178 to 234µm. However, incorporation of any of the surfactant did not show any significant (p>0.05) change in the thickness of film. No amount of constriction in the formulated transdermal patches ensured their 100% flatness. These formulations also maintained a smooth and uniform surface when they were administered onto skin. A low standard deviation value in the patch thickness measurement confirms uniformity of the patches prepared by solvent evaporation technique.

Folding endurance

The folding endurance values of all the patches were found in between 172 to 225. The folding endurance measures the ability of patch to with stand rupture. The patches were prepared using plasticizer Di-n-butyl phthalate (DBP) (30% w/w of polymer) exhibited the optimum flexibility. The folding endurance was measured manually and results indicated that the patches cannot be break and would maintain their integrity with general skin folding when used.

Tensile strength

The Tensile strength of the patches TD-1 to TD-10 was found in between 2.15 to 3.34 (kg cm^{-2}). Those patches which were contained the hydrophilic polymer (PVP) in higher amount exhibited the lesser strength. The result of tensile strength indicates the strength of film and the risk of film cracking. However, no sign of cracking was observed in the prepared patches, which can be attributed to the addition of the plasticizer. Therefore, it may be suggested that the formulation of various blends of polymers used here are suitable for transdermal formulations in terms of their physical stability.

Drug Content

The percentage drug content of all the formulations was found in between 95.8% to 99.1%. These results show that insignificant batch variability (p> 0.05).Thus, method employed to prepare the patches was capable of producing with almost uniform drug distribution. The drug content of all the formulations was found satisfactory.

Scanning Electron Microscopy (SEM)

Surface, shape and size of nanoparticles present in nanoemulsion were studied using SEM. The SEM image of transdermal patches (nanoemulsion containing) has been shown in Fig. 1. SEM revealed that all particles of diclofenac diethylamine nanoemulsion were spherical to oval in shape (Fig. 1). Mostly nanoparticles present in different batches were found with smooth surface, whereas, some nanoparticles were found with rough surface. The size of particles present in nanoemulsion was observed below 200 nm.

***In-vitro* Permeation Study**

The *in-vitro* permeation of drug from the formulated patches were carried out in Franz diffusion cell through rat skin membrane using 15 ml phosphate buffer (pH 7.4) as diffusion media for a period of 24 hours. In case of batch TD-1, TD-2 and TD-3, where ethyl cellulose (hydrophobic polymer) concentration is in gradual increasing order, showed a controlled release of the loaded drug over an extended period of 24 hours. It was also observed that the patches prepared with higher concentration of hydrophilic polymer just like in formulations TD-4, TD-5, TD-6, where concentration of PVP is in gradual increasing order, the drug release was very quick and the patches releases more than 98% of the loaded drug far before 24 hours. In this case the drug release was not in a controlled manner. A burst release was also observed for the patch formulations where concentration of hydrophilic polymer is in gradual increasing and hydrophobic polymer is in decreasing order (formulation - TD-4, TD-5, and TD-6). These patches exhibited the highest and fastest releasing pattern within 16-18 hours with no control on releasing of drug. This may be due to rapid dissolution of the surface and the rapid leaching of hydrophilic fraction of the film former which results in the formation of pores and thus leads to the decreasing of mean diffusional path length of the drug molecules to permeate into the dissolution media and hence higher permeation rate was observed.

Formulation TD-3 showed a most prolonged and controlled release over a period of 24 hours. The percentage cumulative drug permeation per cm^2 of this patch formulation at 24 hours was 55.04%. Thus, on the basis of the results obtained from *in vitro* diffusion study, and regression values obtained after fitting these data, formulation TD-3 was chosen for further skin permeation and other study.

In case of formulation TD-10, the maximum percentage cumulative drug release was 98.84%, which showed the enhanced effect due to presence of particle size in nanometer range, having lower viscosity and permeation enhancer used as surfactant and co-surfactant in nanoemulsion transdermal formulation.

The steady state flux rate, permeability coefficient, and enhancement ratio were calculated for TD-3 and TD-10 patches, out of which we found maximum for TD-10. The results showed that nanoemulsion containing transdermal patch (TD-10) enhanced the in vitro permeation which is about 1.6 fold in comparison of TD-3 formulation. All the permeability parameters (J_{ss}, K_p & E_r) for batch TD-10 were enhanced due to presence of nano size drug particles in the formulation. The permeation enhancer that was used as surfactant and co-surfactant in nanoemulsion containing transdermal formulation also enhanced the values of all the permeability parameters.

Table 2: Percentage cumulative drug release of transdermal patches

Time (h)	% Cumulative drug release/cm^2 from different transdermal patches composed of EC and PVP						
	TD-1	TD-2	TD-3	TD-4	TD-5	TD-6	TD-10
1	6.12	4.79	2.98	16.28	17.78	19.59	12.8
2	9.23	7.47	6.12	20.47	22.09	27.67	21.8
3	11.54	10.27	9.09	30.86	34.51	42.78	29.6
4	15.78	13.18	11.48	36.23	42.59	55.18	35.9
5	21.29	17.71	13.98	43.84	51.71	62.22	41.5
6	24.56	21.2	18.88	49.46	58.4	69.13	47.7
7	29.88	26.76	22.64	54.2	62.21	74.44	54.1
8	34.96	31.32	24.86	60.89	69.6	79.63	63.2
9	39.23	35.92	27.46	65.69	74.91	84.53	69.8
12	52.16	45.48	35.4	74.58	81.01	89.65	81.5
18	71.96	60.82	45.06	97.12	97.71	100.2	91
21	76.89	64.66	52.07	99.84	100.3		94.8
24	78.94	67.91	55.04				98.8

Comparison of permeation data in-between transdermal and nanoemulsion containing transdermal patches

In the case of nanoemulsion transdermal formulation (TD-10), the permeation rate was markedly enhanced in comparison of transdermal patch (TD-3), as shown in figure 2. At the end of 24 hrs, the maximum percentage cumulative drug release for patch TD-10 was 98.84.The steady state flux rate of patch TD-3 and TD-10 was 0.0363, 0.0611 (mg cm^{-2} hr^{-1}), respectively. Similarly, the permeability coefficient of patch TD-3 and TD-4 was 0.0151, 0.02548 (cm hr^{-1}), respectively. Enhancement ratio flux of patch TD-10 was 1.684. Thus, the results indicated that all the permeation parameters for

nanoemulsion transdermal patch were greater in comparison of transdermal patches.

***In-vivo* anti-inflammatory study**

Variable effects of controlling paw edema induced by carrageenan were observed in the group G1 and G2, as shown in Table 4 and Fig. 3. The group G2 which was treated with TD-10 formulation was found to provide maximum protective effect as compared with G1 (treated with formulation TD-3) application in the rat paw edema model. Transdermal patches exhibited the higher ratio of un-induced to induced paw volume (1.32) and lesser % of edema inhibition, in comparison of nanoemulsion transdermal patches. This result is obtained probably due to lesser % of drug release from the transdermal patches. The reason behind this may be nano size drug present in nanoemulsion transdermal patches and low viscosity of the nanoemulsion.

Skin Irritancy study

Skin irritancy study was conducted for formulation TD-10 for seven days on healthy adult male rabbits. The final score represents the average of the 24 and 72 h readings. The erythema and edema score for formulation TD-10 was 0.91 and 0.92, respectively, as shown in Table 5. The primary irritancy index (PII) of formulation TD-10 was 1.83 ± 0.52. This value is less than 2, thus no prominent sign of skin irritation (Erythema and edema) was observed on the skin specimens, treated with transdermal patch TD-10. However, that group of rabbit which was treated with formalin solution exhibited the very high score of erythema, edema and PII (Table 5). These results indicated that developed nanoemulsion transdermal patch is safe for transdermal delivery of Diclofenac diethylamine.

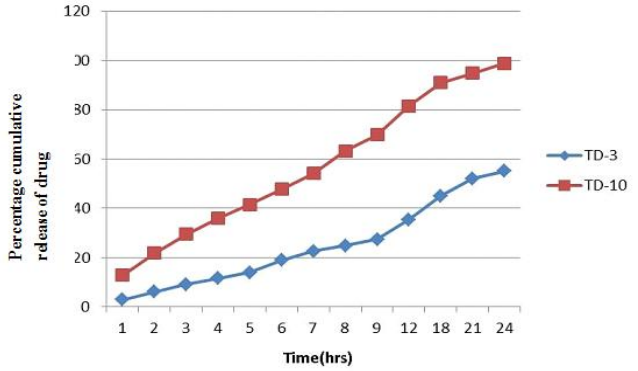

Fig. 2: Comparison of drug Release pattern in-between transdermal (TD-3) and nanoemulsion containing transdermal patches (TD-10)

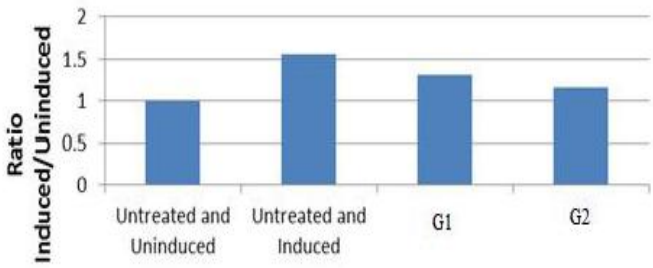

Fig. 3: Graphical representation of *In-vivo* anti-inflammatory study

Table 3: *In-vitro* skin permeation parameters for transdermal and nanoemulsion transdermal patches

Formulation Code	Flux (J_{ss}) (mg cm^{-2} hr^{-1})	Permeability coefficient (K_P) (cm hr^{-1})	Enhancement ratio (E_r)
TD-3	0.0363	0.0151	--
TD-10	0.0611	0.02548	1.684

Table 4: Tabular representation of *In-vivo* anti-inflammatory study

Animal Group		Paw volume(mm)		U/I Ratio	
Group name	Animal	Un-induced (U)	Induced (I)	Value	Mean ± SD
Untreated	1	6.1	6.1	1.00	1.00 ± 0.00
	2	6.2	6.2	1.00	
	3	6.2	6.2	1.00	
	4	6.2	6.2	1.00	
	5	6.1	6.1	1.00	
	6	6.2	6.2	1.00	
G1	1	6.2	9.5	1.53	1.57 ± 0.03
	2	6.1	9.8	1.61	
	3	6.3	9.9	1.57	
	4	6.1	9.8	1.61	
	5	6.3	9.9	1.57	
	6	6.2	9.5	1.53	
G2	1	6.1	8.2	1.34	1.32 ± 0.04
	2	6.4	8.1	1.27	
	3	6.2	8.4	1.35	
	4	6.4	8.1	1.27	
	5	6.2	8.4	1.35	
	6	6.1	8.2	1.34	
	1	6.3	7.3	1.16	1.16 ± 0.00
	2	6.1	7.1	1.16	
	3	6.2	7.2	1.16	
	4	6.3	7.3	1.16	
	5	6.2	7.2	1.16	
	6	6.1	7.1	1.16	

Pharmacokinetic study

Pharmacokinetic study was carried out on rabbits to judge the efficacy of the developed nanoemulsion transdermal formulations against the transdermal dosage form. The T_{max} of drug for both type patches (transdermal patch; TD-3 and nanoemulsion containing transdermal patch; TD-10) was 4 h.

Table 5: Data of the skin irritation test

Rabbit	First group (Control)		Second group (Formalin solution)		Third group (TD-10 Transdermal Patch)	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0.00	0.00	3	3	1.0	1.0
2	0.00	0.00	4	2	1.0	0.5
3	0.00	0.00	3	1	0.5	1.0
4	0.00	0.00	4	3	1	1.0
5	0.00	0.00	4	3	1	1.0
6	0.00	0.00	3	2	1	1.0
Mean	0.00	0.00	3.5	2.33	0.91	0.92
S.D.	0.00	0.00	0.5	0.75	0.33	0.18
PII (Mean ± SD)	0.00 ± 0.00		5.83 ± 1.17		1.83 ± 0.52	

The mean C_{max} of DDEA for transdermal patch (TD) and nanoemulsion transdermal patch (NETD) was 2.56µg/ml and 3.42µg/ml, respectively. Thus, C_{max} values for both patches were different (Table 6 and fig. 4). The pharmacokinetic parameters were calculated from the plasma concentrations of the drug. The mean AUC values after transdermal patch treatment was 201520 units and after nanoemulsion transdermal patch treatment was 281698. The significantly (p<0.05) high AUC value was observed with transdermal nanoemulsion patch which indicated the higher bioavailability of the drug in comparison of transdermal patch. This increased bioavailability may be due to elimination of hepatic first pass metabolism and also possible explanation of these finding could be due to very small particle size of nanoemulsion present in transdermal delivery. Thus the nanoemulsion transdermal formulation was found to provide prolonged steady state concentration of DDEA with minimal fluctuations and improved bioavailability.

Table 6: Concentration of drug for TD and NETD patches

Time (min)	Concentration (µg/ml)	
	TD	NETD
1	0.5	0.5
15	1	1
30	1.3	1.2
60	1.4	1.8
120	1.7	2.7
240	2.6	3.4
480	2.5	3.3
720	2.4	3.1

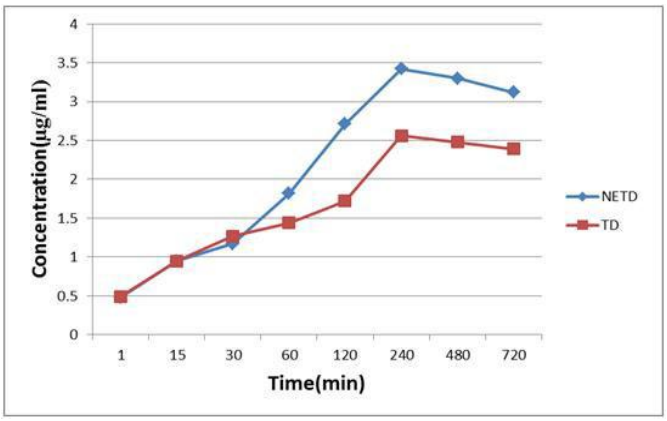

Fig. 4: Concentration of drug with respect of time for TD and NETD patches

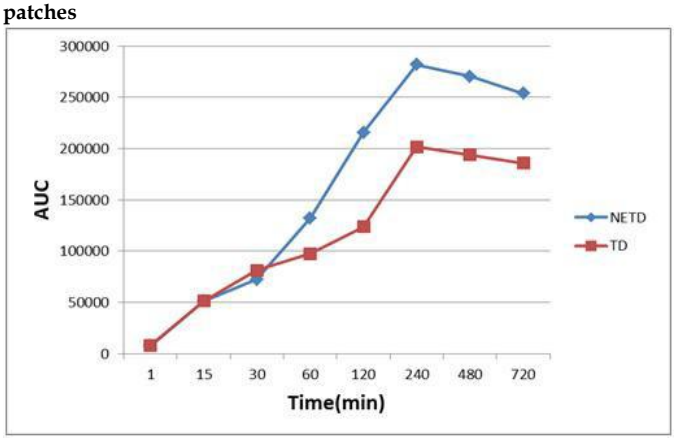

Fig. 5: AUC of drug with respect of time for TD and NETD patches

Table 7: Area under curve with respect of time for TD and NETD patches

Time (min)	AUC	
	TD	NETD
1	8149	7985
15	51359	51367
30	81458	72318
60	97568	132351
120	123671	215687
240	201520	281698
480	194061	270510
720	185671	253729

ACKNOWLEDGEMENTS

The authors wish to thanks to SMITA lab, Indian Institute of Technology (IIT), Delhi, India for SEM analysis.

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Source of Support: Nil, Conflict of Interest: None declared.