Design, Development, Physicochemical, and In Vitro and In Vivo Evaluation of Transdermal Patches Containing Diclofenac Diethylammonium Salt

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ABSTRACT: In this study, matrix-type transdermal patches containing diclofenac diethylamine were prepared using different ratios of polyvinylpyrrolidone (PVP) and ethylcellulose (EC) by solvent evaporation technique. The drug matrix film of PVP and EC was casted on a polyvinylalcohol backing membrane. All the prepared formulations were subjected to physical studies (moisture content, moisture uptake, and flatness), in vitro release studies and in vitro skin permeation studies. In vitro permeation studies were performed across cadaver skin using a modified diffusion cell. Variations in drug release profiles among the formulations studied were observed. Based on a physico-chemical and in vitro skin permeation study, formulation PA4 (PVP/EC, 1:2) and PA5 (PVP/EC, 1:5) were chosen for further in vivo experiments. The antiinflammatory effect and a sustaining action of diclofenac diethylamine from the two transdermal patches selected were studied by inducing paw edema in rats with 1% w/v carrageenan solution. When the patches were applied half an hour before the subplantar injection of carrageenan in the hind paw of male Wistar rats, it was observed that formulation PA4 produced 100% inhibition of paw edema in rats 12 h after carrageenan insult, whereas in the case of formulation PA5, 4% mean paw edema was obtained half an hour after the carrageenan injection and the value became 19.23% 12 h after the carrageenan insult. The efficacy of transdermal patches was also compared with the marketed Voveran® gel and it was found that PA4 transdermal patches produced a better result as compared with the Voveran® gel. Hence, it can be reasonably concluded that diclofenac diethylamine can be formulated into the transdermal matrix type patches to sustain its release characteristics and the polymeric composition (PVP/EC, 1:2) was found to be the best choice for manufacturing transdermal patches of diclofenac diethylamine among the formulations studied. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association

Keywords: transdermal patches; diclofenac diethylamine; in vitro skin permeation; in vivo skin permeation

INTRODUCTION

Diclofenac is a well-established nonsteroidal anti-inflammatory agent, widely used in musculoskeletal disorders, arthritis, toothache, dysmenorrhea, etc., for symptomatic relief of pain and inflammation.1 Diethylammonium salt of diclofenac (diclofenac diethylamine) is reportedly used for topical applications.2 The drug undergoes substantial hepatic first-pass metabolism and
thus only about 50% of the administered dose reaches systemic circulation.\textsuperscript{3,4} This originates the need of an alternative route of administration, which can bypass the hepatic first-pass metabolism. Transdermal route is an alternative choice of route of administration for such drugs. The drug diclofenac diethylamine also possesses the ideal characteristics such as poor bioavailability (40–60%), short biological half-life (2–3 h), smaller dose (25–50 mg), etc., to be formulated into a transdermal patch. Transdermal patches offer added advantages such as maintenance of constant and prolonged drug level, reduced frequency of dosing, minimization of inter- and intrapatient variability, self administration, and easy termination of medication, leading to patient compliance.\textsuperscript{5}

The aim of the present study was to develop different transdermal matrix patches with varied ratios of polyvinylpyrrolidone (PVP) and ethylcellulose (EC), containing the drug diclofenac diethylamine and to perform the physicochemical, \textit{in vitro}, and \textit{in vivo} evaluation of the prepared patches. The purpose was to provide the delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer period of time from transdermal patches.

### EXPERIMENTAL SECTION

#### Materials

EC (ethoxy content 47.5–49%, viscosity 14 cps in 5% w/w solution in 80:20 toluene/ethanol at 25°C) was purchased from BDH Chemicals Ltd., Poole, England. PVP (K value: 26–35) and Polyvinylalcohol (PVA) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India and S.D. Fine-Chem. Ltd. Boisar, India, respectively. di-\textit{n}-Butylphthalate was purchased from Central Drug House (P) Ltd., Mumbai, India.

A gift sample of diclofenac diethylamine was received from Kothari Laboratories, Saugor, India. All the chemicals purchased or received were of high purity.

#### Development of the Patch

Matrix-type transdermal patches containing diclofenac diethylamine were prepared using the different ratios (Table 1) of PVP and EC by solvent evaporation technique in cylindrical both sides open glass molds. The bottom of the mold was wrapped with aluminum foil on which the backing membrane was cast by pouring 4% w/v PVA solution followed by drying at 60°C for 6 h. The two polymers were weighed in requisite ratio and they were then dissolved in chloroform. di-\textit{n}-Butylphthalate 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, by slow stirring with a mechanical stirrer. The uniform dispersion (2 mL each) was cast on the PVA backing membrane cast earlier and dried at 40°C for 6 h. The backing membrane was then glued to a gummy tape keeping matrix side upward. The wax papers were used to give a protective covering as shown in Figures 1–3. This was the final shape of the formulation. This was used only in \textit{in vivo} experiments. Otherwise, in every case, the drug matrix with PVA was used. They were kept in desiccators until used.

#### Solubility Studies

The solubility studies were performed in phosphate buffer solution, pH 7.4,\textsuperscript{6} by adding excess amounts of drug in each case and keeping the excess drug containing phosphate buffer flasks on

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<table>
<thead>
<tr>
<th>No.</th>
<th>Formulation Code</th>
<th>Ratio of PVP/EC</th>
<th>Total Weight of PVP and EC (mg)</th>
<th>Chloroform (mL)</th>
<th>di-\textit{n}-Butylphthalate</th>
<th>Drug</th>
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<td>20% w/w of polymers</td>
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<td>20% w/w of polymers</td>
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<td>20% w/w of polymers</td>
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</tbody>
</table>
a water bath shaker NSW-133 (REMI Equipment, Mumbai, India) for 24 h at 32°C. After 24 h, solutions were analyzed spectrophotometrically at 275 nm, which was the absorption maxima determined earlier and drug concentrations were calculated.

**Determination of Partition Coefficient of Drug**

The partition coefficient study was performed using n-octanol as oil phase and phosphate buffer, pH 7.4, as aqueous phase. The two phases were mixed in an equal quantity and were saturated with each other on a mechanical water bath shaker NSW-133 at 32°C for 24 h. The saturated phases were separated by centrifugation at 2000 rpm on a REMI R-23 centrifuge. Standard plots of drug were prepared from both the phosphate buffer and octanol. Equal volumes (10 mL each) of the two phases were taken in triplicate in conical flasks and, to each, 100 mg of weighed amount of drug was added. The flasks were
shaken at 32°C for 6 h to achieve a complete partitioning at 100 rpm. The two phases were separated by centrifugation at 1000 rpm for 5 min and they were then analyzed for respective drug contents. The partition coefficient of drug $K_{o/w}$ was calculated using the following formula:

$$K_{o/w} = \frac{\text{Concentration in octanol}}{\text{Concentration in phosphate buffer pH 7.4}}$$

**Drug–Excipient Interaction Study**

The drug–excipient interaction study was performed using silica gel–coated TLC (Thin Layer Chromatography) plates and a mixture of one volume of hydrochloric acid, one volume of water, six volumes of glacial acetic acid, and 11 volumes of ethylacetate as a mobile phase.\(^8\) The TLC plates were prepared using a slurry of silica-G. The

**Figure 1.** (Continued)

**Figure 2.** Percentage of moisture content from diclofenac diethylamine containing different matrix films prepared by using different ratios of PVP and EC. Data are mean ± SE ($n = 3$).

**Figure 3.** Percentage of moisture uptake from diclofenac diethylamine containing different matrices prepared by using different ratios of PVP and EC. Data are mean ± SE ($n = 3$).
prepared plates were activated at 110°C for 1.5 h. On the activated plates, 2 μL of each solution in methanol containing (a) 12 mg/mL diclofenac diethylamine and (b) 12 mg/mL diclofenac diethylamine containing different experimental ratio of excipients, that is, PVP, EC, and di-n-butylphthalate, were applied. The plates were dried in a stream of warm air for 10 min and then sprayed with ninhydrin solution. The plates were heated at 110°C for 15 min. The \( R_f \) values were calculated from the chromatogram obtained.

**Evaluation of Polymeric Films**

**Moisture Content**

The prepared films were marked, then weighed individually and kept in a desiccator containing activated silica\(^9\) at room temperature for 24 h. The films were weighed again and again individually until it showed a constant weight. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.

**Moisture Uptake**

A weighed film kept in a desiccator at normal room temperature for 24 h was taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) in a desiccator until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.\(^9\)

**Flatness**

Longitudinal strips were cut out from each film, one from the center and two from either side. The length of each strip was measured and the variation in the length because of nonuniformity in flatness was measured by determining percent constriction, considering 0% constriction is equivalent to 100% flatness:\(^9\)

\[
\text{% Constriction} = \frac{l_1 - l_2}{l_2} \times 100
\]

where \(l_1\) = initial length of each strip and \(l_2\) = final length of each strip.

**In Vitro Release–Dissolution Studies**

The release-rate determination is one of the most important studies to be conducted for all controlled-release delivery systems. The dissolution studies of patches are very crucial, because one needs to maintain the drug concentration on the surface of stratum corneum consistently and substantially greater than the drug concentration in the body, to achieve a constant rate of drug permeation.\(^10\)

The dissolution of patches was performed using USP Basket Type Dissolution Apparatus. The patches were placed in respective baskets with their drug matrix exposed to phosphate buffer, pH 7.4. All dissolution studies were performed at 32°C, at 50 rpm, with each dissolution jar carrying 900 mL of buffer. Samples were withdrawn at different time intervals and analyzed using a UV spectrophotometer at 275 nm against blank. Cumulative amounts of drug released were plotted against time for different formulations.

**In Vitro Permeation Studies**

The permeation studies were performed in a modified Keshary-Chien cell (cell capacity 45 mL, cross-sectional area 4.906 cm\(^2\)).

The permeation studies were performed using human cadaver skin. The skin was used after fulfilling all the ethical requirements. The skin was stored at ~80°C until usage. The epidermis was separated from the full thickness tissue after immersion in phosphate buffer, pH 7.4, at 60°C in distilled water for 2 min. Heat-stripped skin was stored at 5°C for up to 1 week before usage.\(^11\) 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 pH 7.4. The donor and receiver compartment were kept in an intimate contact by wrapping parafilm at the junction. The temperature of the diffusion cell was maintained at 32°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 (1 mL each time) at different time intervals and an equal amount of phosphate buffer, pH 7.4, was replaced each time. Absorbances of the samples were read spectrophotometrically at 275 nm taking 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. Release-rate constants for different formulations were also determined.

**Scanning Electron Microscopy**

The external morphology of the skin and the transdermal patches before and after the application were analyzed using scanning electron microscopy.

**In Vivo Study**

The antiinflammatory activity and sustaining action of the drug-loaded matrix patches were evaluated using the “carrageenan-induced hind paw edema” method developed by Winter et al. (1962) in Wistar rats. Young male rats, weighing 120–250 g, were randomly divided into four groups, each containing four rats. The rats were given free access to water and food, which was supplied by Ashirwad Industries, Ropar, India. The rats were kept under observation for 24 h. The backsides of rats were shaved 12 h before starting the experiments. Patches were applied on the shaved backs of all the animals (except the control group) half an hour before subplantar injection of carrageenan in the right paws. Paw edema was induced by injecting 0.1 mL of a 1% w/v homogeneous suspension of carrageenan in double-distilled water. The volume of injected paw was measured immediately (0 h) and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h after injection using a IMCORP plethysmometer (Ambala, India). The amount of paw swelling was determined time to time and expressed as percent edema relative to the initial (0 min) hind paw volume. The mean values of percentages were determined for each time interval. Percent inhibition of edema produced by each patch-treated group was calculated against the respective control group using the following formula

\[
\% \text{ Inhibition} = \frac{\% \text{ Edema (control)} - \% \text{ Edema (drug)}}{\% \text{ Edema (control)}} \times 100
\]

Experiments were performed with formulations PA4 and PA5 and with Voveran® gel. The same set of experiments were performed with the formulation PA4 which showed no edema in previous experiments by applying the patch 12 h before subplantar carrageenan injection in the hind paw.

**RESULTS**

**Solubility Study**

An attempt was made at this point to learn whether the media phosphate buffer, pH 7.4, was able to maintain sink conditions in dissolution as well as in permeation studies. \(E_{1%}^{1cm}\) was 317.512 [where mean absorbance was 0.0335 \((n = 3)\), drug concentration was 2.637 mg/mL phosphate buffer pH 7.4, volume taken was 10 mL, dilution used was 1:2500] obtained from the solubility studies. Thus, phosphate buffer was chosen as the dissolution and permeation media because sufficient amount of drug dissolved in it (4–5 times the drug incorporated in patch), which is necessary to maintain sink condition.

**Partition Coefficient Determination**

Octanol and in vitro study fluid (here phosphate buffer, pH 7.4) are considered to be the standard system to determine drug partition coefficient between skin and in vitro study fluid. To assess the partitioning of drug between skin and in vitro study fluid, the partition coefficient was determined using the formula shown in Experimental Section.

The partition studies were performed in hexaplicate. The result shows mean of all these experiments. The logarithmic value of partition coefficient (log P) value was experimentally found to be 0.853. The results obtained also indicate that the drug possesses sufficient lipophilicity, which fulfills the requirements of formulating it into a transdermal patch. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

**Drug–Excipient Interaction Studies**

TLC studies were performed to assess any interaction between the drug and the excipients. The data obtained suggested that there was no interaction between the drug and the excipients because the \(R_f\) values of both the drug and the drug–excipient solutions were nearly similar (Table 2).

**Evaluation of the Formulated Patch**

**Moisture Content**

The moisture content was determined by keeping the drug matrix patches in a desiccator containing
activated silica for 24 h. The percentage moisture content was calculated from the weight differences relative to the final weight (see Experimental Section). The results of the moisture content studies for different formulations are shown in Figure 2. The moisture content in the formulations was found to increase with the increasing concentration of hydrophilic polymer, PVP. Moisture contents in the formulations were found to be low.

**Moisture Uptake Studies**

The percentage moisture uptake was calculated from the weight difference relative to the initial weight after exposing the prepared patches to 84% relative humidity (saturated potassium chloride solution). The results of moisture uptake studies for different formulations are shown in Figure 3. The percentage moisture uptake was also found to increase with increasing concentration of hydrophilic polymer, PVP.

**Flatness Study**

An ideal patch should be formulated in such a way that it possesses a smooth surface and it should not constrict with time. Flatness studies were performed to assess the same. The results of the flatness study showed that none of the formulations had the differences in the strip lengths before and after their cuts. It indicates 100% flatness observed in the formulated patches. Thus, no amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the patches.

**Scanning Electron Microscopy**

The surface morphology of the transdermal patches before and after the in vitro drug release study was scanned using a scanning electron microscope (JSM 6100 JEOL, Tokyo, Japan) (Figs. 4–6). Figure 4 shows the uniform distribution of drug in the polymer matrix. Figures 5 and 6 depict how the polymer–matrix (PA4) behaves after the release of drug molecules. Figure 6 indicates that the formulation maintains the elastic nature of the film after the release of drug molecule without affecting the other parts of the patch. Figure 7 shows one of the skin appendages (shown by an arrow), which are to be the main diffusion pathways of molecules.

**In Vitro Dissolution Studies**

Dissolution studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. Dissolution studies for different formulations were performed in a USP basket dissolution apparatus using phosphate buffer, pH 7.4, as dissolution media at 32°C.

It was observed that as the concentration of hydrophilic polymer, PVP, increased in the formulations, the rate of dissolution increased subsequently. “Burst effect” was observed in formulations PA1 to PA3 (Fig. 8). This may be because the hydrophilic layer might need a very little “time lag” to establish a concentration profile. Maximum percentage of drug released (88%) was found for the formulations PA1 (PVP/EC, 5:1) and minimum percentage of drug released (41%) was observed for the formulation PA5 (PVP/EC, 1:5).

### Table 2. Determination of Drug–Excipient Interaction Using the TLC Method

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<tr>
<th>Formulation Code</th>
<th>PVP/EC</th>
<th>Drug R_f Value</th>
<th>Drug–Excipient R_f Value</th>
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<tr>
<td>PA1</td>
<td>5:1</td>
<td>0.794</td>
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<td>PA2</td>
<td>2:1</td>
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<td>PA3</td>
<td>1:1</td>
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<td>PA4</td>
<td>1:2</td>
<td>0.709</td>
<td>0.756</td>
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<tr>
<td>PA5</td>
<td>1:5</td>
<td>0.803</td>
<td>0.797</td>
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**Figure 4.** SEM photograph of the transdermal patch before application, shows a homogeneous dispersion of drug in the patch (original magnification × 500).
In Vitro Permeation Studies

In vitro permeation studies are predictive of in vivo performance of a drug. Permeation studies were performed for different formulations across cadaver abdominal skin using phosphate buffer, pH 7.4, as an in vitro study fluid in the receptor compartment of a modified diffusion cell at 32°C. Drug release profiles from the formulations PA4 and PA5 (Figs. 9 and 10) were more or less rectilinear and were indicative of the steady and slow release of drug in in vitro medium and formulation PA5 showed the slowest release-rate constant of all the formulations studied. It is interesting that despite the “burst effect” of formulations PA1, PA2, and PA3, their drug-release profiles behaved differentially during in vitro drug skin permeation study (Figs. 9 and 10). Skin permeation of drug as well as much slower exposure of PVP to phosphate buffer, pH 7.4, may be considered the reason for the same.

In Vivo Study

Variable effects of controlling paw edema induced by carrageenan were observed in the formulations PA4 and PA5 (Tables 3–7). The formulation PA4 was found to provide maximum protective effect as compared with PA5 and Voveran® gel application in the rat paw edema model.

Figure 5. SEM photograph of the transdermal patch after the release of drug molecules from a zone (original magnification × 1500).

Figure 6. SEM photograph of the transdermal patch showing how the patch (PA4) behaves after the release of drug (original magnification × 3500).

Figure 7. SEM photograph of a section of experimental skin showing one skin appendage (shown by an arrow) (original magnification × 250).

Figure 8. In vitro drug dissolution profile of diclofenac diethylamine from different PVP/EC drug matrix patches, using USP basket dissolution apparatus in phosphate buffer pH 7.4. Data are mean ± SE (n = 6).
DISCUSSION

Diclofenac diethylamine is a well-established non-steroidal antiinflammatory drug, which undergoes substantial hepatic first-pass metabolism, and thus only about 50% of the administered drug reaches the circulation.\(^1,2\) Therefore, there is a need to search for an alternative route of administration, which may bypass the hepatic first-pass metabolism. The transdermal patch delivery system may be an attractive choice of an alternative route of administration of this drug because the drug also possesses characteristics such as sparing solubility in water, short biological half-life (2–3 h), and a smaller dose range (25–50 mg).\(^{14–17}\) Moreover, the logarithmic value of the partition coefficient of the drug in octanol-phosphate buffer, pH 7.4 (in vitro study)

**Figure 9.** In vitro skin permeation profile of diclofenac diethylamine from different PVP/EC matrix patches through cadaver abdominal skin, in phosphate buffer, pH 7.4. Data are mean ± SE (n = 6).

**Figure 10.** Release-rate constants of diclofenac diethylamine from different formulations: PA1 (PVP/EC, 5:1), PA2 (PVP/EC, 2:1), PA3 (PVP/EC, 1:1), PA4 (PVP/EC, 1:2), and PA5 (PVP/EC, 1:5).

**Table 3.** Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats (Control Group)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Paw Vol. (mL)</th>
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**Table 3.** Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats (Control Group)

<table>
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<tr>
<th>Formulation</th>
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<th>2</th>
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</table>

Mean % edema ± SD (n = 4) 0.62 ± 1.08 14.02 ± 2.06 32.05 ± 4.44 46.10 ± 6.83 63.61 ± 7.85 72.26 ± 28.87 82.08 ± 39.21 91.48 ± 34.29 104.51 ± 37.66 111.94 ± 37.66 114.02 ± 38.39
### Table 4. Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats Half an Hour after the Application of the Formulation PA4 (PVP/EC, 1:2)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Paw Vol. (mL)</th>
<th>Formulation PA4 (PVP/EC, 1:2)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Edema w.r.t. initial paw volume</td>
<td>0.25</td>
</tr>
<tr>
<td>1</td>
<td>0.30</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.31</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mean % edema ± SD (n = 4)</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mean % inhibition w.r.t. control group</td>
<td></td>
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<td>100</td>
</tr>
</tbody>
</table>

### Table 5. Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats Half an Hour after the Application of the Formulation PA5 (PVP/EC, 1:5)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Paw Vol. (mL)</th>
<th>Formulation PA5 (PVP/EC, 1:5)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Edema w.r.t. initial paw volume</td>
<td>0.25</td>
</tr>
<tr>
<td>1</td>
<td>0.30</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.36</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.36</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>0.40</td>
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<tr>
<td>Mean % edema ± SD (n = 4)</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mean % inhibition w.r.t. control group</td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>
### Table 6. Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats Half an Hour after the Application of the Voveran® Gel

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Paw Vol. (mL)</th>
<th>Voveran® Gel</th>
<th>Time (h)</th>
<th>% Edema w.r.t. initial paw volume</th>
<th>Mean % edema ± SD (n=4)</th>
<th>% Inhibition w.r.t. control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>% Edema</td>
<td>0.25</td>
<td>0 0 0 0 0 3.33 10 20 23.33 30 36.46</td>
<td>0 0 0 0 3.33 ± 1.44 9.16 ± 3.63 15.83 ± 2.76 22.49 ± 4.93 33.42 ± 4.92 38.28 ± 7.27</td>
<td>100 100 100 100 96.60 88.84 82.68 78.48 70.14 66.42</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>w.r.t.</td>
<td>0.5</td>
<td>0 0 0 0 3.33 13.33 16.66 30 36.66 50</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>initial paw</td>
<td>1</td>
<td>0 0 0 0 3.33 13.33 16.66 33.33 36.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>volume</td>
<td>2</td>
<td>0 0 0 0 3.33 10 13.33 20 23.33 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % edema ± SD (n=4)</td>
<td>0 0 0 0 3.33 ± 1.44 9.16 ± 3.63 15.83 ± 2.76 22.49 ± 4.93 33.42 ± 4.92 38.28 ± 7.27</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### Table 7. Paw Edema Data Obtained on Carrageenan Challenge in Male Wister Rats 12 h after the Application of the Formulation PA4 (PVP/EC, 2:1)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Paw Vol. (mL)</th>
<th>Formulation PA4 (PVP/EC, 2:1)</th>
<th>Time (h)</th>
<th>% Edema w.r.t. initial paw volume</th>
<th>Mean % edema ± SD (n=4)</th>
<th>% Inhibition w.r.t. control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.33</td>
<td>% Edema w.r.t.</td>
<td>0.25</td>
<td>0 0 0 0 6.06 12.12 18.18 27.27 36.36 45.45</td>
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</tr>
<tr>
<td>2</td>
<td>0.36</td>
<td>initial paw</td>
<td>0.5</td>
<td>0 0 0 0 5.55 13.88 25 27.77 36.11</td>
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</tr>
<tr>
<td>3</td>
<td>0.32</td>
<td>volume</td>
<td>1</td>
<td>0 0 0 0 3.03 9.37 18.75 28.12 37.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.32</td>
<td></td>
<td>2</td>
<td>0 0 0 0 6.25 12.5 21.87 31.25 43.75 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % edema ± SD (n=4)</td>
<td>0 0 0 0 3.07 ± 1.53 8.3 ± 2.05 15.82 ± 1.33 25.56 ± 2.26 34 ± 3.29 42.26 ± 2.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Inhibition w.r.t. control group</td>
<td>100 100 100 100 95.80 89.88 82.70 75.55 69.62 62.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
fluid used by us) system, in our study showed that the value is well within the range of 0.8–3.0 required for a transdermal patch delivery system.10 Drug–excipient interactions were studied using a silica gel G-coated TLC plate with water-hydrochloric acid–glacial acetic acid-ethyl acetate system as mobile phase.8 No distinct difference in the $R_f$ values of both the drug and drug excipient solution used in our study indicates that the excipients do not alter the performance characteristics of the drug from the patches studied. It is already known that the common polymers such as PVP and EC are popular in controlled/sustained release matrix type patches because of their compatibility with a number of drugs.18

In this study, various transdermal matrix type patches containing diclofenac diethylamine of variable combinations of PVP/EC were prepared. It was desired 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 among the formulations studied, using the different diffusion pathways of the individual polymeric composition to produce the desired overall prolonged/sustained drug release.

The physicochemical performances and the release characteristics were different in the patches studied. The moisture content and moisture uptake of the various formulations showed that with the increase in concentration of hydrophilic polymer, PVP, both the percentage moisture content and moisture uptake increased. The small moisture content in the formulations helps them to remain stable and from being a completely dried and brittle film. Again, a low moisture uptake protects the material from microbial contamination and bulkingness of the patches. No amount of constriction in the formulated transdermal patches ensured their 100% flatness. Thus, these formulations can maintain a smooth and uniform surface when they are administered onto skin. Figures 5 and 6 show the electron microscopic representations of the patches after releasing the drug molecules from them. Figure 6 also shows that after the release of drug molecules, the distorted portion of the membrane had a tendency of maintaining elasticity in an affected small area with little effect on the other part of the membrane. Thus, this showed that very little or almost no constriction, that is, 100% flatness of the patches persists even after the patches were deprived of the drug molecules. 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.

The importance of polymer dissolution on drug release from matrices has been known for ensuring the sustained release performance and the reproducibility of rate and duration of drug release.10 Initial “burst release” was observed in patches with PVP/EC ratios 5:1 and 2:1. This may be because of the much higher percentages of PVP in those two formulations. This hydrophilic PVP layer might need very little “time lag” to establish a concentration profile in the patches resulting in a “burst effect” in dissolution studies. The PVP/EC (1:1) patches showed the moderate “burst effect” pattern, whereas, the patches with PVP/EC 1:2 and 1:5 showed comparatively controlled and sustained release. It was observed that the PVP/EC 1:5 patch released only 41% of the drug incorporated in 24 h. Homogeneous uniform distribution of the transdermal patches is one of the important characteristics that also ensures the uniform reproducible sustained release of the drug molecules from the patch.19 Electron microscopic photograph (Fig. 4) shows that diclofenac diethylamine was homogeneously dispersed in the transdermal patch formulated in our experiment. The phenomenon in this study is not a molecular dispersion; rather, drug molecules are dispersed in small (~2-μm size) aggregates. Intermolecular forces between drug molecules are stronger (ionic) as compared with those between drug and polymers (i.e., ionic drug and slightly polar polymers). Polymers known to interact physically with drug molecules through electrostatic forces20,21 might form soluble polion drug complex associate22 with an individual diameter of ca. 2 μm. Polymer molecules surrounding cores of polion complexes prevented the complexes from precipitation while they were in solution. Again, the presence of uniformly distributed about 2-μm drug aggregates indicate simultaneous precipitation of drug complexes along with polymers during solvent evaporation.

In vitro release profile is an important tool that predicts in advance how the drug will behave in vivo23. Thus, we can eliminate the risk of hazards of drugs because of direct experimentation in the living system. In vitro skin permeation experiments are known for their value for studying the rate and mechanism of percutaneous absorption of drugs.24 In our experiments, variable release profiles of diclofenac diethylamine from the different experimental patches composed of various blends of polymers, PVP and EC, were observed.
Cumulative amounts of drug permeated per square centimeter of patches, through the skin into the *in vitro* fluid when plotted against time, showed almost a rectilinear graphic of the data obtained from the formulation PA5. It may depict the zero-order drug-release kinetic of the formulation. In the case of other formulations, PA1 to PA4, the release profiles of the drug seem to follow apparent zero-order/pseudo first-order kinetics. Initially up to 24 h, the drug released in the *in vitro* study fluid followed zero-order kinetics because the dispersed drug matrix ensured constant concentration. Afterward, however, concentration-dependent release kinetic changed the system toward a first-order reaction.

The process of drug release in most controlled-release devices including transdermal patches is governed by diffusion and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. The alteration of the crosslinking and the modification of structural arrangements of polymers by using different blends of polymer were already reported. So, different *in vitro* drug release profiles from the different blends of PVP and EC formulations could be attributable to the varied crosslinking networks of polymeric chains of the different blends of polymeric transdermal experimental formulations as tortuosity and diffusion pathway varied and they have thereby been reported to vary the release of drug and the duration of diffusion.

Moreover, the implication of skin permeation of drug on release-rate profiles of the experimental formulations should not be ignored, because the skin is known to have a substantial role in variation of release kinetic. At an early stage as well as in a steady state of skin permeation, diffusion of drug through appendages (hair follicles, sebaceous and sweat ducts) (Fig. 7) are considered to be significant and the variation of shunt pathways from one part of skin to the other may even be one of the causes of variation in the release-rate profiles of the experimental formulations.

When the release-rate constants were compared among the formulations, almost similar values of rate constants were observed in formulations PA2 to PA4, and PA5 gave the slowest release. It is also clear that the increased amount of EC in the formulations decreased the release rate of diclofenac diethylamine.

Based on physicochemical and *in vitro* release experiments, formulations PA5, PA4, PA3, and PA2 may be chosen for further *in vivo* studies. Again, when burst release as well as higher release rate were considered, formulations PA2 and PA3 may be avoided from the preparation of a physicochemically stable and sustained-release patch type formulation. Thus, it can reasonably be suggested that the formulation PA4 (PVP/EC, 1:2) and PA5 (PVP/EC, 1:5) are best suited for further animals studies.

Carrageenan-induced rat paw edema has been considered as a useful model for studying the anti-inflammatory effect of drug in rats. As described by Winter et al. (1965), paw edema was induced in rats by injecting 1% w/w carrageenan solution (in double-distilled water) in our experiments to study the antiinflammatory effect and sustaining action of diclofenac diethylamine from the two transdermal patches (PA4 and PA5 formulation) selected based on their physicochemical characteristics and *in vitro* release profiles.

Carrageenan-induced mean percent paw edema was found to increase about 114% as compared with initial paw volume, 12 h after carrageenan injection (Table 6) in the carrageenan control group of animals. Table 3 and 4 depict the comparisons of mean percentage edema as well as percentage of inhibition of edema with the duration after the application of patches, PA4 and PA5, respectively, half an hour before carrageenan injection. Formulation PA4 was very effective in terms of inhibiting carrageenan-induced edema as 100% inhibition, that is, no edema was observed even after 12 h of the carrageenan challenge. However, an application of formulation PA5 produced about 4% mean percentage edema within half an hour after the carrageenan injection and the value became 19.23% 12 h after the carrageenan insult. This may be because of the less percentage of drug release from PA5, which was not enough to control edema effectively for long hours. Approximately 83% inhibition was observed 12.5 h after the application of PA5 formulation. Application of Voveran gel half an hour before carrageenan insult showed about 3% mean percent edema value which increased eventually up to 38.28% after 12 h. As in the case of formulation PA4, there was no edema in animals after 12 h of carrageenan challenge (Table 6); further study was initiated by applying the formulation 12 h before the carrageenan insult. Table 5 shows that there was 100% inhibition of paw edema up to 3 h after carrageenan application and edema returned and mean percent edema value gradually increased with the duration. It indicates that
formulation PA4 (PVP/EC, 1:2) controlled edema effectively for about up to 19 h after its application in the in vivo rat model.

Thus, based on the above discussion, it is well justified to conclude that formulation PA4 has the best effective combination of polymers PVP and EC, among the formulations studied for further development of the transdermal matrix patch type delivery system of diclofenac diethylamine.

ACKNOWLEDGMENTS

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REFERENCES