Comparison of the Effect of Essential Oils on the Permeation of Diclofenac Diethylamine Through Various Barriers

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Abstract

Ethylcellulose transdermal patches for diclofenac diethylamine (DDA) using essential oils as penetration enhancers developed. Effect of drug loading and penetration enhancers on the in vitro permeation of drug through cellophane membrane, rat skin and human cadaver skin was investigated. Incorporation of essential oils enhanced the moisture content, moisture uptake capacity and permeation of diclofenac diethylamine across skin barriers. Among the penetration enhancers used, menthol oil found to be most effective. Comparison of steady state flux (J) values obtained with various barriers revealed that permeation was in following order: rat skin > cellophane membrane > human cadaver skin. Stability studies did not show any degradation of the drug. In conclusion, stable and effective diclofenac diethylamine transdermal patches can be prepared using essential oils as penetration enhancers.

Keywords: Transdermal patches; diclofenac diethylamine, essential oils; permeation study.

Introduction

Diclofenac is a well-established NSAID agent, widely used in musculo-skeletal disorders, arthritis, toothache, dysmenorrhea, symptomatic relief of pain and inflammation (John 1979). Diclofenac diethylamine (DDA) salt of diclofenac is reportedly used for topical application (Kriwet 1993) to overcome the problems such as, gastric irritation, hepatic metabolism and poor bioavailability (Zevin et al. 2000). Diclofenac diethylamine also possess the ideal characteristics such as short biological half-life (2-3 hr), smaller dose (25-50 mg) etc, to be formulated into transdermal patch. Patch offers added advantages such as, maintains constant and prolonged drug level, reduced frequency of dosing, minimized of inter- and intra-patient variability, self-administration, and easy termination of medication leading to patient compliance (Keith 1983). For delivering a drug via transdermal route, an extensive application area may be needed for desired therapeutic effect. One way to reduce device size/application area is to incorporate penetration enhancers to improve the permeation characteristics of the drug or barrier resistance of skin. In the present study, objective was to develop, matrix type transdermal patch containing DDA and evaluate in vitro transdermal DDA permeation through various diffusion barriers including excised rat skin, human cadaver skin, and synthetic membrane. Functionality of various essential oils as penetration enhancers across different barriers is also evaluated.

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Materials and Methods

Materials

Diclofenac diethylamine (Emcure Pharmaceuticals Ltd., India) was received as a gift sample. Ethylcellulose- 45 cps (Colorcon Asia., Gao), menthol oil, lemon oil, eucalyptus oil and clove oil (Rajesh Chemical Co. Mumbai) and dibutylphthalate (Qualigens Lab. Mumbai) were purchased. All other reagents were of analytical grade.

Preparation of Transdermal patches

Matrix type transdermal patches of diclofenac diethylamine in ethylcellulose were prepared by solvent evaporation technique in petri dish. The ethylcellulose was dissolved in acetone - chloroform (2:1) solvent system and dibutylphthalate (10% w/w of polymer) was used as a plasticizer. Drug and enhancer were added to the polymeric dispersion and the resultant homogeneous solution was poured into a petri dish. Controlled solvent evaporation was achieved by inverting a funnel over the petri dish for 24 h. The dry films were wrapped in aluminium foil and kept in desiccator until used. The composition of different patches is given in Table 1.

Table 1. Composition of transdermal patches

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Weight of polymer (mg)</th>
<th>Drug (mg/cm²)</th>
<th>Enhancer (% w/w of polymer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1000</td>
<td>4.72</td>
<td>---</td>
</tr>
<tr>
<td>F2</td>
<td>1000</td>
<td>4.72</td>
<td>Menthol oil 5% w/v</td>
</tr>
<tr>
<td>F3</td>
<td>1000</td>
<td>4.72</td>
<td>Lemon grass oil 5% w/v</td>
</tr>
<tr>
<td>F4</td>
<td>1000</td>
<td>4.72</td>
<td>Clove oil 5% w/v</td>
</tr>
<tr>
<td>F5</td>
<td>1000</td>
<td>4.72</td>
<td>Eucalyptus oil 5% w/v</td>
</tr>
<tr>
<td>F6</td>
<td>1000</td>
<td>7.86</td>
<td>---</td>
</tr>
<tr>
<td>F7</td>
<td>1000</td>
<td>7.86</td>
<td>Menthol oil 5% w/v</td>
</tr>
<tr>
<td>F8</td>
<td>1000</td>
<td>7.86</td>
<td>Lemon grass oil 5% w/v</td>
</tr>
<tr>
<td>F9</td>
<td>1000</td>
<td>7.86</td>
<td>Clove oil 5% w/v</td>
</tr>
<tr>
<td>F10</td>
<td>1000</td>
<td>7.86</td>
<td>Eucalyptus oil 5% w/v</td>
</tr>
</tbody>
</table>

Partition Coefficient of Drug

The partition coefficient of the drug was determined using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. The two phases were mixed (1:1) and were saturated with each other on a mechanical bath shaker at 32°C for 24 h. The saturated phases were separated by centrifugation at 2000 rpm. Equal volume (10 ml each) of the two phases were taken in conical flask and to each, 100 mg of drug was added. Flask was shaken at 32°C for 6 h to achieve a complete partitioning. The two phases were separated and they were then analyzed spectrophotometrically (Shimadzu UV-1700, Japan) for respective drug content.

The partition coefficient of drug (Ko/ w) was calculated using the following expression,

\[ \frac{\text{Concentration in octanol}}{\text{Concentration in pH 7.4}} \]

Drug-excipient interaction studies

Thin Layer Chromatography (TLC)

Study was performed using silica gel coated TLC plate and mixture of hydrochloride, water, glacial acetic acid, ethylacetate (1:1:6:11) as a mobile phase (British Pharmacopeia, 2003). The TLC plate was prepared using slurry of silica G. The prepared plate was activated at 110 °C for 1.5 h. On the activated plate, 2 μl of each solution in methanol containing 10 mg/ml of drug and patches containing
drug, ethylcellulose and penetration enhancers used. 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. $R_f$ values were calculated from the chromatogram obtained. $R_f$ value of each formulation was determined in triplicate along with the drug.

**Fourier Transfer Infra Red Spectroscopy (FTIR)**

The IR spectra were recorded for DDA, drug free polymeric patch and drug loaded patch containing essential oils with penetration enhancer using FTIR (8400S Shimadzu Corporation, Japan) spectroscopy from KBr pellets. The scanning range was 400-3900/cm.¹

**Scanning electron microscopy (SEM)**

The surface morphology of transdermal patches before and after in vitro skin permeation experiments was studied using a scanning electron microscopy (JSM-6360, Japan).

**Thickness**

The thickness of patches was determined using micrometer gauge (Mitutoyo, Japan). Patch was measured at three different places and mean value was calculated.

**Moisture content**

The prepared patches were weighed individually and kept in desiccator containing activated silica at room temperature till a constant weight was attained. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight (Arora and Mukherjee 2002).

**Moisture uptake**

Weighed patches kept in a desiccator at room temperature for 24 h was taken out and exposed to 84% RH in a stability chamber (Lab-Care, Mumbai) until a constant weight of patch was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight (Arora and Mukherjee 2002).

**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. After appropriate dilutions, solutions were analyzed spectrophotometrically at 276 nm for diclofenac diethylamine.

**In vitro permeation study**

**Preparation of cadaver skin**

Sample of whole adult human skin (45 ages, provided by Navodaya Medical College, Raichur) were obtained from breast reduction operation. Subcutaneous fat was carefully trimmed and then rinsed with normal saline. Skin was sealed in aluminium foil and a plastic bag and stored at −20 °C until used (Fang et al. 1999).

**Preparation of rat abdominal skin**

The male albino rats were sacrificed by excess chloroform inhalation (Institutional Animals Ethics Committee, NETPC, Raichur approved the protocol). Hair on abdominal skin was removed with electrical clipper taking extreme precaution not to damage the skin. The shaved skin was excised from the animal subcutaneous tissue. The full thickness skin thus prepared was soaked in distilled water at 60°C for 60 s, followed by careful removal of epidermis. Skin was dried in desiccator at 25%RH and wrapped in aluminium foil and stored at 4°C (Das et al. 2006).
Preparation cellophane membrane

The standard cellophane membrane was presoaked in phosphate buffer pH 7.4 for 45 min before experiment (Fang et al. 1999).

Procedure

Permeation studies were performed for different formulations across rat skin, cadaver skin and artificial membrane using phosphate buffer pH 7.4 as an in vitro fluid in receptor compartment of modified Keishery- Chein cell at 32°C. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. The samples were withdrawn at different time interval and replaced with equal volume of diffusion medium. Samples were analyzed in spectrophotometrically at 276 nm.

Stability studies

The stability studies were conducted according to ICH guidelines by storing the patches at 40 ± 2°C / 75% RH in stability chamber (Lab-Care, Mumbai) for six months. The samples were withdrawn after six months and analyzed for drug content (Aqil and Ali 2002).

Statistical analysis of data

The results were analyzed by one-way ANOVA with Turkey post t-test using Graph Pad Prism software-5 version (Graph Pad Software Inc., San Diego, CA, USA).

Results and Discussion

The partition study was performed in triplicate and logarithmic value was found to be 0.710 ± 0.05. These results indicate that drug possess sufficient lipopility, which fulfill the requirement of formulating in to a transdermal system and mimics the biphasic nature of skin, thus ensuring easy penetration through the skin (Arora and Mukherjee, 2002).

Drug-excipient interaction studies

The Rₜ values of pure drug and those from different patches (Table 3) were almost same. The results indicating that there was no interaction between the drug and excipient of the patch. Drug-excipient interaction was also studied by FTIR technique. Shown in fig 8 IR spectra of drug showed characteristic band at 2740-2940 1/cm (CH stretching of CH₃ group), 1720 1/cm (for C=O), 1590-1480 1/cm (for C=C of ring stretching), 3200 1/cm (for doublet NH group), 240 – 870 1/cm (for CH bending) and 690 1/cm (for CCl). Comparisons of spectra of different formulations with that of drug indicate no variation in the positions of bands for functional groups of the drug. These results clearly suggest that there was no interaction among the drug, polymer and penetration enhancers. FTIR study also implied that all the excipients were compatible with diclofenac diethylamine.

Table 3.Drug-excipient interaction study using TLC method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Rₜ value ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>0.817 ±</td>
</tr>
<tr>
<td></td>
<td>0.004*</td>
</tr>
<tr>
<td>F1</td>
<td>0.815 ± 0.008*</td>
</tr>
<tr>
<td>F2</td>
<td>0.809 ± 0.007†</td>
</tr>
<tr>
<td>F3</td>
<td>0.800 ± 0.005†</td>
</tr>
<tr>
<td>F4</td>
<td>0.805 ± 0.004†</td>
</tr>
<tr>
<td>F5</td>
<td>0.807 ± 0.007†</td>
</tr>
</tbody>
</table>

* n = 15, † n = 3

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Thickness of patches

Table 2 shows thickness of the various patches. Thickness of patches increased (from 0.29 to 0.31 mm) with increase in the drug loading from 4.72 to 7.86 mg per cm² of patch. However, incorporation of any of the essential oil (5% w/w) did not show any significant (p>0.05) change in the thickness of film.

Moisture content and Moisture uptake studies

Table 2 shows the results of moisture content and moisture uptake studies. Values of these parameters were found to be highest in patches containing menthol oil (F2 and F7) than any other essential oil (F3-F5, F8-F10). The small moisture content in the patches helps them to remain stable and never being completely dried and brittle (Mutalik and Udapa, 2004). These results indicate that presence of essential oil will enhance the quality of transdermal patches in terms of mechanical properties.

Drug content analysis

Drug content of all the formulations (table 2) was ≥ 98.02 %, with a small coefficient of variation of 8.91%. These results show that the method employed to prepare patches was capable of producing patches with almost uniform drug distribution and insignificant batch variability (p> 0.05).

In vitro permeation studies

A number of modes of action of penetration enhancers, depending on the nature of penetration enhancer, have been reported (William and Barry, 2004). Terpenes have been extensively investigated as skin permeation enhancers (Gao and Singh, 1998). In the present study, in vitro permeation of drug due to essential oils was evaluated across standard cellophane membrane, rat abdominal skin and human cadaver skin barriers, to know the influence of barrier functionality on the drug permeation. The cumulative amount of drug permeated / cm² was plotted against time and the steady- state permeation flux (J) was calculated from the slope of linear portion of the curve. Table 2 shows the steady-state flux values of various formulations. The different type of essential oils as penetration enhancers and drug loading were used as formulation variables. When the permeation studies were conducted using cellophane membrane as the permeation barrier, except menthol oil, which showed a flux of 185.38 mcg/cm²/hr, other essential oils did not improve (p>0.05) flux the values. While, increase in drug loading increased (p< 0.001) flux values. When rat abdominal skin was used as permeation barrier, comparison of flux value of F1 with flux
values of formulations with essential oils (F2 to F5) indicated a significant (p< 0.001) increase in the permeation of drug except with F5. The results of present study indicated that menthol oil (F2, \( J = 246.20 \text{ mcg/cm}^2/\text{hr} \)) produces higher drug flux than any other essential oils (F3-F5). The flux of the drug also significantly increased (p< 0.001) with increasing initial drug loading (F6 to F10) of the patches. The human cadaver skin was also used as a permeation barrier and permeation profiles are shown in figures 5 - 6. Comparison of flux values of F1 with those of formulations with essential oils (F2 - F5) indicated a significant (p<0.001) increase in the permeation of drug except with F5 formulation. Similarly, flux values were also significantly increased (p<0.001) with increasing initial drug loading. These results indicate that both formulation variables, i.e. essential oils and drug loading, can effectively increase the permeation of drug across both type of skins used. The permeation effect of essential oils observed only with skins justified that action of terpene containing essential oils is primarily due to altered skin diffusivity mainly by disrupting lipid network. However, absence of such skin lipid network in artificial membrane was the reason to show the insignificant effect on the permeation of diclofenac diethylamine through artificial membrane. The results also indicate that flux values are always more from rat skin than human cadaver skin with any formulations. It is well known that rodent skin is generally more permeable than human skin (Catz and Friend, 1990). Among the essential oils, menthol oil found to be more effective showing higher and significant flux values across artificial and skin barriers. Effectiveness of menthol oil may be attributed to the property of menthol oil forming eutectic mixture with the drug (Yong et al., 2003) (Greenhalgh et al., 1999) and to the direct interaction with skin lipids. These results indicate that essential oils posses the permeation enhancing capacity only due to their direct effect on the skin lipid attributes. While, menthol oil acts by interacting with skin lipids and by also changing physicochemical state of the drug.

**Analysis of release mechanism**

For matrix controlled release system, the Higuchi equation is described as per (Mehidizadeh et al., 2004).

\[
Q = (2ADCs t)^{1/2}
\]  

Where Q is the cumulative amount of drug released per unit area of the matrix, A is the total drug concentration in the matrix dissolved and undissolved, D is the diffusion coefficient of the drug in the matrix, Cs is the solubility or saturation concentration of the drug in the matrix, and t is time. When cumulative amount of a diclofenac diethylamine released were plotted as a function of square root of time, linear correlation \( (R^2 \geq 0.9614) \) were observed. The high correlation \( (R^2) \) values indicate that release of diclofenac diethylamine from the patches was in compliance with Higuchi diffusion model.

**Stability study**

Drug content of the patches after stability studies was between 98 – 99.5% and did not show any significant variations. The results indicates that drug in patches remain stable after stability studies.
Table 2. Physicochemical properties of patches and in vitro flux of diclofenac diethylamine across various membrane barriers.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug content (%) ±SD, n=4</th>
<th>Thickness (mm) ±SD, n=4</th>
<th>Moisture content (%) ±SD, n=4</th>
<th>Moisture uptake capacity (%) ±SD, n=4</th>
<th>Cellophane membrane (µg/cm²/h) ±SD, n=4</th>
<th>Rat skin (µg/cm²/h) ±SD, n=4</th>
<th>Cadaver skin (µg/cm²/h) ±SD, n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>98.3 ± 0.8</td>
<td>0.292 ± 0.005</td>
<td>1.5 ± 0.3</td>
<td>2.1 ± 0.1</td>
<td>94.416±5.94</td>
<td>140.42±2.19</td>
<td>36.68±3.94</td>
</tr>
<tr>
<td>F2</td>
<td>98.4 ±0.61</td>
<td>0.294 ±0.0040</td>
<td>4.6±0.4</td>
<td>7±0.5</td>
<td>185.38±9.85*†</td>
<td>264.2±14.8‡</td>
<td>94.5±3.93*</td>
</tr>
<tr>
<td>F3</td>
<td>99.1 ±1.5</td>
<td>0.297±0.0058</td>
<td>2.7±0.2</td>
<td>5±0.2</td>
<td>122.65±2.09*″</td>
<td>192.69±2.03*</td>
<td>77.786±2.8‡</td>
</tr>
<tr>
<td>F4</td>
<td>99.2 ±1.2</td>
<td>0.290±0.0056</td>
<td>2.6±0.2</td>
<td>3.4±1.1</td>
<td>114.63±2.30*</td>
<td>178.81±2.02*</td>
<td>66.26±6.11*</td>
</tr>
<tr>
<td>F5</td>
<td>98.4 ±0.6</td>
<td>0.298 ±0.016</td>
<td>2.2±0.2</td>
<td>3.3±1.0</td>
<td>98.37±0.78*</td>
<td>153.70±5.33*</td>
<td>49.06±1.63*</td>
</tr>
<tr>
<td>F6</td>
<td>98.9 ±0.69</td>
<td>0.313 ±0.002</td>
<td>2.1±0.1</td>
<td>2.4±0.2</td>
<td>174.27±5.89</td>
<td>287.43±9.50</td>
<td>72.85±2.53†</td>
</tr>
<tr>
<td>F7</td>
<td>98.0 ±0.18</td>
<td>0.312 ±0.001</td>
<td>5.9±0.2</td>
<td>9.2±0.2</td>
<td>419.46±21†</td>
<td>499.8±40.33†</td>
<td>141.6±7.28†</td>
</tr>
<tr>
<td>F8</td>
<td>98.3 ±0.96</td>
<td>0.313 ±0.001</td>
<td>3.9±0.2</td>
<td>6.6±1.1</td>
<td>390.46±21†</td>
<td>476.3±21.35†</td>
<td>128.1±10.0†</td>
</tr>
<tr>
<td>F9</td>
<td>98.1 ±0.83</td>
<td>0.313 ±0.005</td>
<td>3.1±0.2</td>
<td>6.0±1.6</td>
<td>388.22±34.4†</td>
<td>422.6±21.71†</td>
<td>99.59±5.89†</td>
</tr>
<tr>
<td>F10</td>
<td>99.7 ±1.55</td>
<td>0.312 ±0.001</td>
<td>3.8±0.3</td>
<td>3.4±0.7</td>
<td>375.89±7.05†</td>
<td>329.89±7.52†</td>
<td>88.0±8.045*†</td>
</tr>
</tbody>
</table>

*(p<0.001) when compared with F1; **(p>0.005) when compared with F1; †(p<0.001) when compared with F6; ‡(p>0.005) when compared with F6.
Figure 1. Effect of essential oils on the permeation of diclofenac diethylamin (4.72 mg/cm²) through celophane membrane. Key: •-F1; ■-F2; ▲-F3;x-F4; ●-F5

Figure 2. Effect of essential oils on the permeation of diclofenac diethylamine through celophane membrane: o-F6; ■-F7; ▲-F8;x-F9; ●-F10

Figure 3. Effect of essential oils on the permeation of diclofenac diethyl amine (4.72 mg/cm²) through rat skin.

Figure 4. Effect of essential oils on the permeation of diclofenac diethylamine (7.86 mg/cm²) through rat skin.

Figure 5. Effect of essential oils on the permeation of diclofenac diethyl amine (4.72 mg/cm²) through human cadaver skin.

Figure 6. Effect of essential oils on the permeation of diclofenac diethyl amine (7.86 mg/cm²) through human cadaver skin.
SEM studies

Figure 7 shows SEM photographs of the patches. Figure 7(a) shows that drug was uniformly distributed throughout the patch. However, pores developed in the patches after the release study (fig 7(b) may be due to the release of drug molecule. In case of patches containing eucalyptus oil, surface appears to be peeled-off without many pores (figure 7(c)).

![Figure 7: SEM of F2 before in vitro permeation (a), after in vitro permeation (b).](image)

(c) SEM of F5 with eucalyptus oil

Conclusion

In conclusion, essential oils can be incorporated in transdermal patches of ethylcellulose for improving the permeation of diclofenac diethylamine. Among the essential oils used, menthol oil was more effective in enhancing the in vitro permeation of diclofenac diethylamine. Menthol oil shows higher flux than any other formulation through various barriers although permeation data were not uniform across different barriers.

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