

Full Length Research Paper

Formulation, physical, *in vitro* and *ex vivo* evaluation of diclofenac diethylamine matrix patches containing turpentine oil as penetration enhancer

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In this study, turpentine oil was used for enhancing the transdermal penetration of diclofenac diethylamine (DDEA) from matrix patches prepared using carboxypolymethylene (CPM) and ethyl cellulose (EC) by solvent evaporation technique. The drug matrix film was subjected to different physical testes like moisture content, moisture uptake and flatness, *in vitro* release and permeation studies and *ex vivo* permeation studies. *In vitro* permeation studies were performed across artificial skin, while *ex vivo* across rabbit abdominal skin. The effect of increasing concentration of turpentine oil on transdermal permeation of diclofenac diethylamine was studied on Franz cell apparatus. The results obtained were encouraging showing an increase in transdermal permeation with an increase in turpentine oil.

Key words: Diclofenac diethylamine (DDEA), carboxypolymethylene (CPM), turpentine oil (T. oil), ethyl cellulose (EC).

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 (John, 1979). Diethylammonium salt of diclofenac (diclofenac diethylamine) is reportedly used for topical applications (Chien, 1987). The drug undergoes substantial hepatic first-pass metabolism and thus only about 50% of the administered dose reaches systemic circulation (John, 1979; Keith, 1983). 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

to 60%), short biological half-life (2 to 3 h), smaller dose (25 to 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 intra patient variability, self administration and easy termination of medication, leading to patient compliance (Chien, 1987).

Terpenes are essential oils which are used as fragrance, flavoring agents and as medicines (Naseem et al., 2008). They have been found be effective penetration enhancers for a number of hydrophilic and lipophilic drugs (Arellano et al., 1996; Okabe et al., 1992). Terpens are highly lipophilic due to their isoprene (C₅H₈) units (Williams and Barry, 1991). They are generally recognized as safe by FDA (Added to Food in the United States: A Food Additive Database, FDA, 1998) (Naseem et al., 2008). They increase the drug diffusivity in the stratum corneum for hydrophilic drugs and they enhance partitioning of drug into the stratum corneum for lipophilic

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Table 1. Composition of prepared patches.

S/N	Formulation code	Ratio of CPM/EC	Total weight of CPM/EC (mg)	Chloroform/ Acetone	PEG-400	T. oil (%)	Drug
1	P1	1:1	600	5 and 5 ml	30%w/w of polymer	-	20% w/w of polymers
2	P2	2:2	600	5 and 5 ml	30%w/w of polymer	0.5	20% w/w of polymers
3	P3	3:3	600	5 and 5 ml	30%w/w of polymer	1	20% w/w of polymers
4	P4	1:3	600	5 and 5 ml	30%w/w of polymer	1.5	20% w/w of polymers
5	P5	2:2	600	5 and 5 ml	30%w/w of polymer	2	20% w/w of polymers
6	P6	3:1	600	5 and 5 ml	30%w/w of polymer	2.5	20% w/w of polymers

drugs, besides causing increased diffusivity (Williams and Barry, 1991; Cornwell et al., 1996).

The aim of the present study was to develop different transdermal matrix patches with varied ratios of carboxypolymethylene (CPM) and ethylcellulose (EC), containing the drug diclofenac diethylamine and to perform physical, *in vitro* and *ex 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. An attempt was made to establish the best possible combination of polymeric ratio of formulated transdermal patches with maximum controlled and sustained drug release capability as well as physical stability.

MATERIALS AND METHODS

Diclofenac diethylamine, PEG 400 and carboxypolymethylene were provided by Leads Pharma (Islamabad), ethyl cellulose (Dow Chemical Co. Midland, USA), chloroform, turpentine oil, artificial skin and poly vinyl alcohol (Sigma Aldrich). All chemicals were used without further purification.

Solubility study

The solubility study for diclofenac diethylamine was performed by the method previously described by previous authors (Priyanka and Biswajit, 2002; Akhlaq et al., 2011) by adding excess amount of DDEA into phosphate buffers of different pH and keeping the flasks on a water bath shaker for 24 h at 32°C. After 24 h, solutions were analyzed spectrophotometrically at 276 nm, which was the absorption maxima determined earlier and drug concentrations determined. Each sample was analyzed three times and averaged.

Development of the patch

Matrix type transdermal patches containing diclofenac diethylamine were prepared using different ratios of ethyl cellulose and carboxypolymethylene by solvent evaporation technique. The formulations are given in Table 1.

The backing membrane was prepared by dissolving sufficient polyvinyl alcohol (PVA) in chloroform to make 15% of the solution. Volume of 1.5 ml of PVA solution was poured into a petri dish followed by drying under ambient conditions for 24 hrs. Matrix solution was prepared by dissolving 20% w/w of the total weight of

polymer in chloroform and acetone (1:1), followed by addition of polymer in different ratios and PEG 400 as plasticizer in concentration of 30% w/w of the polymers and turpentine oil in increasing concentration started with 0.5% of polymer concentration. The uniform matrix dispersion was cast on prepared and dried backing membrane and allowed to dry for 24 h under ambient conditions. The prepared patches were stored in desiccator till further use.

Evaluation of patches

The prepared patches were evaluated on physical basis for moisture content, moisture uptake and flatness, by the methods described previously (Barhate et al., 2009). Briefly, moisture content were determined by individually weighing each patch and kept in dessicator containing activated silica at room temperature for 24 h. The patches 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.

For moisture uptake, the weighed patches 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.

For 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 non uniformity in flatness was measured by determining percent constriction, considering 0% constriction is equivalent to 100% flatness:

$$\text{Constriction (\%)} = \frac{L_1 - L_2}{L_2} \times 100$$

where L1= initial length of each strip and L=2 final length of each strip.

In vitro permeation studies

The DDEA patches were evaluated for their permeation capability across artificial skin which was fixed in between the receptor and donor compartment of Franz cell in such a way that the epidermis was facing outward and open to air. The receptor compartment was filled with phosphate buffer pH 7.4 which was stirred with a magnetic stirrer continuously throughout the permeation experiment. The patch was mounted on the upper surface of the skin so that drug matrix will be facing epidermis and backing membrane facing outward. The temperature of the cell was maintained at 32°C by

Table 2. Solubility results of DDEA.

S/N	Solvent	Solubility (mg/ml) \pm SD
1	Phosphate Buffer pH 6.8	1.892 \pm 0.01
2	Phosphate Buffer pH 7.2	2.301 \pm 0.01
3	Phosphate Buffer pH 7.4	2.637 \pm 0.02

continuous water circulation through cell jackets. Sample volume of 2 ml was withdrawn at time intervals of 0.5, 1, 1.5, 2, 4, 8, 12, 16, 20 and 24 h. The samples were analyzed spectrophotometrically at 276 nm for extent of DDEA permeated (Priyanka and Biswajit, 2002). Percentage cumulative drug permeated was determined and plotted against time.

Ex vivo animal studies

Preparation of rabbit skin

Different animal models have been proposed by various authors for permeation studies like rat (Catz and Friend, 1990), rabbit (Hirvonen et al., 1993), hairless mouse skin (Catz and Friend, 1990), shed snake skin (Buyuktimkin et al., 1995) and human cadaver skin (Roy et al., 1994). Although, human cadaver skin is the best choice to study the transdermal formulations penetration behavior, but because of difficulty in availability, rabbit abdominal skin was used in this study.

The present study was approved by the local ethical committee for research, Gomal University, D.I.Khan, KPK, Pakistan. The rabbit was anesthetized with chloroform to facilitate easy cleaning of the belly and hairs from the dorsal region were shaved carefully with an electric hair trimmer to avoid any damage to the skin and was swabbed using distilled water. The rabbit was then sacrificed and the hairless skin was excised from the animal with a surgical scissors. Since the skin was not firmly attached to the viscera, it was lifted easily from the animal after the incision was made. The subcutaneous fats were removed by scalpel, and the epidermis removed by heat separation. This involved immersion of full thickness skin in water at 60°C for 1 min, followed by careful teasing of the epidermis from the dermis. The thickness of the epidermis samples was measured by screw gauge. The epidermis was rinsed with distilled water, and was used.

Ex vivo permeation studies

The permeation studies were performed in a Franz cell apparatus (cell capacity 5 ml, cross-sectional area 1.16 cm²). The permeation studies were performed using rabbit abdominal skin. 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 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 276 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.

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 (Sood and Panchagnula, 1999). 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 276 nm; blank cumulative amounts of drug released were plotted against time for different formulations.

RESULTS

Solubility study

An attempt was made at this point to learn whether the media phosphate buffer of pH 7.4, was able to maintain sink conditions in dissolution as well as in permeation studies. $E^{1\%}_{1\text{cm}}$ 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 to 5 times the drug incorporated in patch), which is necessary to maintain sink condition (Devi and Paranjothy, 1999). The solubility results are as shown in 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. The results of the moisture content studies for different formulations are as shown in Figure 1. The

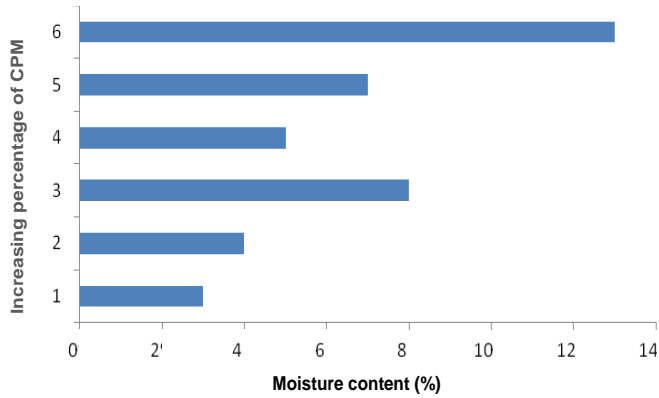


Figure 1. Percentage of moisture content from diclofenac diethylamine containing different matrix films prepared by using different ratios of CPM and EC with increasing concentration of CPM. Data are mean \pm SE (n = 3).

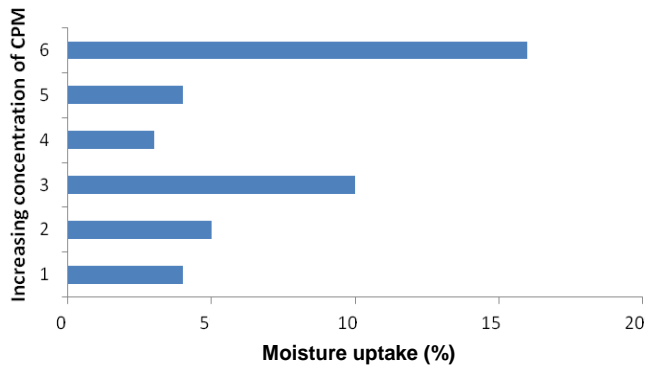


Figure 2. Percentage of moisture uptake from diclofenac diethylamine containing different matrices prepared by using ratios of CPM and EC with increasing concentration of CPM. Data are mean \pm SE (n = 3).

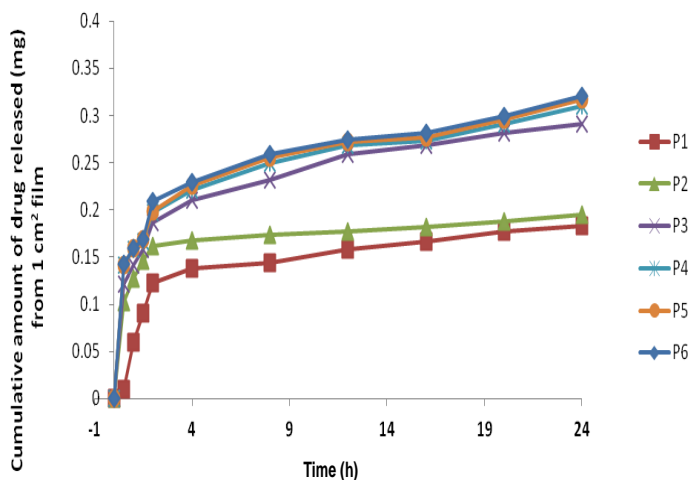


Figure 3. *In vitro* drug permeation profile of diclofenac diethylamine from different CPM/EC drug matrix containing increasing concentration of turpentine oil. Data are mean \pm SE (n = 6).

moisture content in the formulations was found to increase with the increasing concentration of hydrophilic polymer, CPM. Moisture contents in the formulations were found to be low.

Moisture uptake studies

The percentage of 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 as shown in Figure 2. The percentage of moisture uptake was also found to increase with increasing concentration of hydrophilic polymer, CPM.

Flatness studies

An ideal patch should be formulated in such a way that it possesses a smooth surface and should not constrict with time. Flatness studies can be carried out to assess them. The results of the flatness study in this research work showed that none of the formulations had 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.

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 artificial skin using phosphate buffer of pH 7.4, as an *in vitro* study fluid in the receptor compartment of Franz cell at 32°C. The permeation profile of different formulations with an increasing concentration of turpentine oil are as shown in Figure 3. It is evident from the graph that increasing concentration of turpentine oil has a direct relationship with increasing permeation of drug. Turpentine oil has different terpenes which could have a synergistic effect of drug permeation. Turpentine oil causes an increase in permeation of drugs due to increased disruption of stratum corneum layer (Naseem et al., 2008). No profound increase in drug permeation was observed between P4 and P6, which might be because no direct relationship exists between amount of diclofenac permeated and terpenes concentration (Nokhodchi et al., 2007).

Ex vivo permeation studies

Ex vivo permeation studies were performed across rabbit

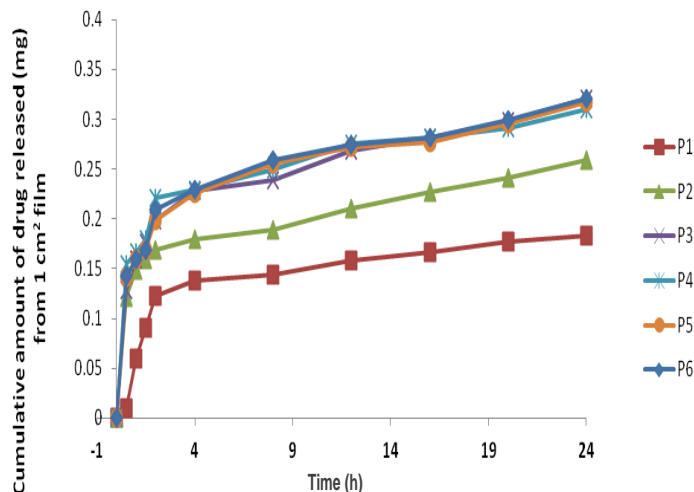


Figure 4. *Ex vivo* drug permeation profile of diclofenac diethylamine from different CPM/EC drug matrix containing increasing concentration of turpentine oil. Data are mean \pm SE (n = 6).

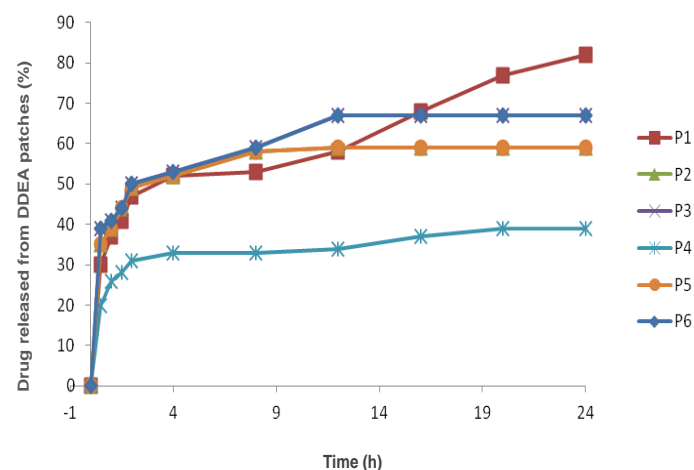


Figure 5. *In vitro* drug dissolution profile of diclofenac diethylamine from different CPM/EC drug matrix patches, using USP basket dissolution apparatus in phosphate buffer pH 7.4. Data are mean \pm SE (n = 6).

skin on Franz cell using phosphate buffer of pH 7.4 as receiving medium. The *ex vivo* permeation profile of DDEA is shown in Figure 4. No significant difference was observed in the diclofenac permeation across artificial and rabbit skin.

***In vitro* release 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 (Keith, 1983).

It was observed that as the concentration of hydrophilic polymer, CPM, increased in the formulations, the rate of dissolution increased subsequently. "Burst effect" was observed in formulations P1 to P3 (Figure 5). This may be because the hydrophilic layer might need a very little "time lag" to establish a concentration profile. Maximum percentage of drug released (82%) was found for formulations P3 and P6 (CPM/EC, 3:3 and 3:1) and minimum percentage of drug released (39%) was observed for formulation P4 (CPM/EC, 1:3).

DISCUSSION

Diclofenac diethylamine is a well established non-steroidal anti-inflammatory drug, which undergoes substantial first pass hepatic metabolism and thus only 50% of the total administered drug enter the blood stream. Therefore, an alternative route of administration is desirable, which may bypass the hepatic first pass metabolism. The transdermal patches are an attractive choice for the formulation of such type of drugs.

In this study, various transdermal matrix type patches containing diclofenac diethylamine of variable combinations of CPM/EC were prepared and turpentine oil was incorporated into the matrix patches in order to access its permeation enhancement effect on diclofenac diethylamine. The increasing concentration of turpentine oil showed an increase in total drug permeation, but the effect was not so pronounced after adding 2% concentration of turpentine oil, which might be because of the absence of direct relationship between amount of diclofenac permeated and terpenes concentration (Nokhodchi et al., 2007). Similarly, no significant difference was observed between the drug permeation across artificial as well as rabbit skin.

The two polymers (CPM/EC) were incorporated in different ratios into the patches in order to sustain the drug release from matrix. By increasing the concentration of CPM, the drug release increases due to its hydrophilic properties. Maximum percentage of drug released (82%) was found for formulations P3 and P6 (CPM/EC, 3:3 and 3:1) and minimum percentage of drug released (39%) was observed for formulation P4 (CPM/EC, 1:3).

When the release rate constants were compared among formulations, almost similar values of rate constants were observed in formulations P1, P2, P4 and P5, while P4 and P6 gave the slowest release. It is also clear that the increased amount of EC in the formulations decreased the release rate of diclofenac diethylamine.

Thus, based on aforementioned results and discussion, it is well justified to conclude that turpentine oil holds great potential to be used as natural penetration enhancer for transdermal formulations, and CPM/EC blended in specific ratio might have an advantage of

controlled release of drug from the matrix.

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