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In Vitro Permeation Study of Diclofenac Hydroxyethyl Pyrrolidine

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***In vitro* diffusion of diclofenac hydroxyethyl pyrrolidine (DHEP), a novel salt of a known non-steroidal anti-inflammatory drug (NSAID) was carried out using 'Valia-Chien' glass diffusion cell assembly. Effect of penetration enhancers such as d-(+)-limonene, oleic acid, deet, isopropyl myristate (IPM) on the flux of DHEP was studied by carrying out *in vitro* permeation studies in presence of these enhancers. The enhancement brought about by d (+)-limonene was approximately 17 times and IPM enhanced flux 5 times, deet 3 times, oleic acid 2 times when compared to flux of DHEP alone. These findings helped in the selection of proper penetration enhancers to be incorporated in semi-solid formulations containing DHEP.**

DICLOFENAC is a well known and one of the most widely prescribed NSAID whose oral, parenteral and rectal dosage forms have been widely used for the treatment of inflammatory and musculoskeletal diseases[1]. However, the most frequently reported adverse effects with diclofenac are gastric pain and discomfort. In recent years there has been an increasing interest in the development of antiinflammatory drugs for topical and transdermal use. The efficacy of a particular formulation for topical administration is dependent on the ability to release the drug which then diffuses through the skin[2]. Previous studies have shown that diclofenac, when applied topically, penetrates the skin barrier in an amount sufficient to reach muscles, joints and synovial fluid and to exert the desired local therapeutic activity[3]. A topical form of diclofenac diethylammonium salt, already available in the market, has been shown to exert direct activity on the site of inflammation, thus avoiding possible systemic effects of the drug[4].

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A new water soluble salt of diclofenac, diclofenac hydroxyethyl pyrrolidine (DHEP) also known as epolamine salt has been found to be very effective in providing symptomatic relief of pain and inflammation when applied through the transdermal route, to patients suffering from musculoskeletal diseases[5]. This salt is a white low-melting powder, containing a balanced combination of hydrophilic and hydrophobic moieties[6]. Diclofenac in the form of DHEP, is the first salt with surfactant activity in the class of NSAIDs and it's affinity for a lipid medium promotes the absorption across the membrane. Due to it's surface activity, DHEP lowers surface tension of the interface of skin/ pharmaceutical dosage form and improves hydration of the skin surface. Recently DHEP has been formulated into a plaster for topical application in the therapy of articular diseases[5]. The present study was taken up to investigate the *in vitro* release pattern of DHEP across guinea pig skin in an aqueous buffer system and chemical enhancement of its flux using few well known penetration enhancers, d- (+)-limonene, oleic acid, deet and isopropyl myristate.

MATERIALS AND METHODS

Reagents and chemicals

Diclofenac hydroxyethyl pyrrolidine was a generous gift from Amoli Organics Ltd., Mumbai. D (+)-limonene and phosphate buffered saline (PBS) was purchased from Sigma Chemical Co., USA. Deet (N, N-diethyl-3-methyl benzamide), oleic acid and isopropyl myristate (IPM) were of pharmacopoeial grade and were available in-house.

Preparation of Drug solution

Saturated solution of diclofenac hydroxyethyl pyrrolidine was obtained by addition of excess amount of the drug to phosphate buffered saline (pH 7.4) and the suspension shaken for 24 h at 37°.

Preparation of guinea pig skin

Full thickness abdominal skin was obtained from freshly sacrificed guinea pigs (male, 250-300 g) and was washed thoroughly with distilled water to remove any subcutaneous matter. It was then immersed in phosphate buffered saline (pH 7.4) at 37° for 30 min for equilibration.

In vitro permeation study

'Valia-Chien' glass diffusion cell was used for this part of the study[7]. Freshly isolated and washed full thickness guinea pig abdominal skin was clamped between the two compartments of the diffusion cell. Both receptor and donor compartments were filled with phosphate buffered saline (pH 7.4). This assembly was maintained with tiny magnetic stirring bars rotating in both chambers to achieve optimum mixing. The temperature of the cell was maintained at 37±0.5° by circulating water through the outer jacket of the cell using a thermostatically controlled recirculating waterbath (Braun Co., Germany).

Mounted skin was then washed for 2 h in PBS at 37° to remove any interfering leachings from the skin. Phosphate buffered saline (pH 7.4) from both receptor and donor compartments were drained off and receptor chamber was filled with fresh buffer, while donor chamber contained saturated DHEP solution with or without appropriate amount of penetration enhancer (1% w/w). At hourly intervals, 1 ml sample was withdrawn from receptor chamber and replaced with 1 ml of fresh buffer. The total duration of this study was 8 h. These samples were analyzed on a Perkin-Elmer spectrophotometer at 276 nm to determine the amount of drug that penetrated across the skin with time.

Mean cumulative amount of the drug permeated through guinea pig skin was plotted against time (h). Each time, one set of corresponding reagent blank i.e. PBS on both sides of skin was run to eliminate absorbance due to interferences during analysis.

Table 1 : Standard curve data at 276 nm for DHEP in PBS

DHEP (µg/ml)	Mean absorbance nm
1	0.024
2	0.052
3	0.067
4	0.107
5	0.134
6	0.161
7	0.185
8	0.215
9	0.239
10	0.270
30	0.810

Serial dilutions of DHEP (1-10 µg) were made in PBS and absorbance was measured at 276 nm. The linear regression of Y on X for this data was calculated which yielded an equation Y=0.027X-0.005.

Preparation of standard calibration curve of DHEP in PBS

Series of dilutions of DHEP in PBS ranging from 1 µg to 10 µg were made and absorbance was measured at 276 nm on a Perkin-Elmer spectrophotometer (n=12). Graph of absorbance Vs amount of DHEP was plotted (mean of n=2) and linear regression of Y on X was calculated using a computerized BASICA programme.

Table 2 : *In vitro* diffusion of DHEP using Valia-Chien diffusion cell

Time in (h)	DHEP	DHEP+1% D-Limonene	DHEP+1% Deet	DHEP+1% IPM	DHEP+1% Oleic acid
1	22.91	42.18	25.30	NIL	NIL
2	29.28	131.1	29.10	NIL	NIL
3	39.87	272.5	35.00	NIL	NIL
4	51.97	498.0	57.00	110	63.97
5	61.41	939.4	114.0	214	84.97
6	67.91	1204	181.0	320	150.0
7	80.58	1300	296.0	406	161.0
8	171.0	1450	390.0	510	185.0
Flux ($\mu g/cm^2/h$)	16.51	282	52.17	79.33	30.60

Donor chamber of diffusion cell contained saturated DHEP solution with or without penetration enhancer and receptor compartment was filled with fresh phosphate buffered saline. At hourly intervals, 1 ml sample was withdrawn from receptor chamber and analysed for DHEP diffused across 1 cm^2 of isolated guinea pig skin. Average cumulative amount of DHEP ($\mu g/cm^2/h$) is tabulated above.

RESULTS AND DISCUSSION

Purity of locally procured DHEP was checked using Mass and NMR facilities of the department of Analytical Chemistry, HMR Research Centre in comparison with pure drug sample obtained from IBSA Institute of Biochimique SA, Switzerland. Standard calibration curve of DHEP in PBS yielded the following equation for regression of Y on X calculated using computerized BASICA programme (Table 1).

For 1.10 µg range -> Y= **0.027X-0.005** n=10, r=0.999

Mean cumulative amount ($\mu g/cm^2$) of DHEP alone and in presence of penetration enhancers, permeated through isolated guinea pig skin with time is given in table 2. The enhancement brought about by d (+)-limonene was approximately 17 times and IPM enhanced the flux 5 times, deet 3 times, oleic acid 2 times when compared to flux of DHEP alone.

Transdermal administration of many drugs is often difficult due to stratum corneum barrier, an impermeable outermost layer of the skin[8]. Therefore, a chemical enhancer is used in formulation to increase drug permeability and achieve higher concentration of active principle at the site of action. However it is essential to study the effect of enhancer in a suitable buffer system on rate of drug permeation before formulating it with the drug and other additives in a final dosage form. Ideally a penetration enhancer is a substance which reversibly reduces the barrier resistance of the horny layer without damaging any viable cells. It would be pharmacologically inert, nonirritating and nontoxic[9]. A penetration enhancer also must be cosmetically acceptable and pharmacologically inert. Naturally occurring terpenes such as d (+)-limonene have shown potential as penetration enhancers through human skin[8]. Transdermal administration or diclofenac, a widely prescribed NSAID, is recommended in several antiinflammatory therapies and use of enhancers to increase its concentration at the site of action is known for long time[10]. Diclofenac epolamine with it's surfactant property is considered to be more suitable for transdermal application, would yield better permeation of drug across the skin in presence of a suitable penetration enhancer.

In our *in vitro* permeation study of DHEP all the enhancers d(+)-limonene, oleic acid, deet and isopropyl myristate belong to different chemical classes and therefore would give us a broad idea about an optimum effectiveness as well as compatibility of drug-enhancer combination to be used in formulation.

Naturally occurring terpenes such as d (+)-limonene have shown potential as penetration enhancers in human skin[8]. Terpenes act by disrupting the lipid structure of the stratum corneum, thereby increasing the diffusion coefficient of polar drug in the membrane. Due to their fragrance, cosmetic acceptability is also very high. In our study, the enhancement of diffusion of DHEP brought about by d (+)-limonene was approximately 17 times when compared with flux of drug alone.

Unsaturated fatty acids such as oleic acid are well known for their penetration enhancing effect on NSAIDs. These compounds exert their effect by increasing partition coefficient of the drug. However, our results indicate that oleic acid enhanced the flux of DHEP only 2 times. Alkyl esters such as isopropyl myristate (IPM) are also known for drug penetration enhancement effect and IPM is more commonly used as a co-solvent[11]. In our *in vitro* experiments, IPM enhanced DHEP flux nearly 5 times.

We have studied the effect of deet on permeation of DHEP and it exerted a mild enhancing activity (3 times) but did not increase lag time in contrast to IPM and oleic acid. These studies have given us the insight into the right permeation enhancer which can be incorporated in a transdermal dosage form of diclofenac epolamine.

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