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TRANSDERMAL AND DERMAL DELIVERY OF DEXIBUPROFEN: EFFECTS OF pH

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ABSTRACT

The objective of this work was to investigate feasibility of transdermal and dermal delivery of Dexibuprofen using porcine skin was investigated. The total flux of Dexibuprofen from the skin remarkably varied over the pH range 3.0–8.0. Transport of Dexibuprofen through and into porcine skin and effects of pH was studied *ex-vivo* using Franz diffusion cell. From aqueous donor samples, Dexibuprofen flux through the skin was 29.71±1.13 µg/cm^2/ hr with greatest permeation rate at pH 7.0. The skin permeability data were calculated. The result of this studies showed that the total flux increased with increase of pH was dependent on the lipophilicity of drug. These results suggest that the pH of formulation should be given attention to elicit the maximum permeation enhancement through the skin.

Key words: Dexibuprofen, pH, Skin permeation, Transdermal drug delivery system.

INTRODUCTION

Transdermal drug technology specialists are Non-steroidal anti inflammatory drugs (NSAIDs) are widely used for the treatment of fever and acute or chronic arthritic conditions. They act by inhibiting cyclo-oxygenase (COX) thereby reducing the release of prostaglandins (PGs), the well known inflammatory and nociceptive mediators [1]. PGs are gastro-protective in that they enhance the synthesis of mucosa, bicarbonate secretion and reduce the gastric acid secretion. However, their systemic use is often limited because of severe upper gastrointestinal ulcers and other side effects [2]. Thus, pharmaceutical dosage forms that deliver drugs to the inflammation site at a sustained, concentrated level over an extended period of time without altering pharmacodynamic activities have the advantages of avoiding the systemic side effects.

Continuing to search for new methods that can effectively and painlessly deliver larger molecules in therapeutic quantities to overcome the difficulties associated with the oral route. Transdermal Drug Delivery Device is a system in which the delivery of the active ingredients occurs across the skin. The skin is an effective medium from which absorption of the drugs takes place and facilitates its entry in the circulatory system. Gels are used to incorporate the active ingredients into the circulatory system via skin.

The term transdermal delivery includes all topically administered drug formulations intended to deliver the active ingredient into the general circulation. Transdermal therapeutic systems have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation. Moreover, such system over come various side effects like gastrointestinal disorders after oral administration of drugs and the first pass metabolism of the drug occurred by oral routes of drug delivery. Transdermal Drug Delivery System has become a great field of interest in the recent times. The main advantages of such system are that there is controlled release of the drug and the medication administration is painless [3,4].

The pH of formulation also plays an important role in the transport of drugs. The effect of pH on drug permeation across the skin was investigated.

MATERIALS AND METHODS

Hydroxy ethyl cellulose, hydroxy propyl methyl cellulose (LOBA CHEMIE PVT.LTD. Mumbai) Dexibuprofen was supplied by (Glochem Industries

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Limited, AP, India), Ethanol, Propylene glycol was obtained from (S D FINE-CHEM LIMITED, Mumbai, India).

All other reagents were of analytical grade. Stock solutions were prepared fresh when required. Swiss albino mice, 6 to 8 weeks old, were supplied by animal house.

Preparation and evaluation of transdermal dexibuprofen Formulations:

Dexibuprofen is a non-steroidal anti-inflammatory agent that has been formulated into a number of topical preparations. Its transfer across skin is well documented and Watkinson et al. have examined its solubility and diffusion properties as a function of Ph [5].

In this study, different ranges of pH were selected for *ex-vivo* diffusion study to quantitatively compare their effects. The effect of change in the pH of the donor solution on the drug transport was studied at different pH: 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 7.0 and 8.0 at 37±0.5 ^{o}C. pH of gel formulation was adjusted to below pH 6.0 using diluted phosphoric acid and above pH 6.0 by triethanolamine.

Physical evaluation of the prepared gels

Above gels were evaluated for physical parameters like homogeneity, colour, pH and consistency. Depending upon the characteristics the concentration of the gelling agent was fixed, studies carried out by Gendy E *et.al* indicates that permeation of drugs across the skin from transdermal formulations is affected by diffusion of the drug from the vehicle towards the skin surface and subsequent portioning into the stratum corneum.

***Ex-vivo* diffusion study of Dexibuprofen gels**

Preparation of skin sample for diffusion study

Pig ear was used for the study[8]. Hair was removed from dorsal side of ear with razor without damaging the skin and washed with isopropyl alcohol to remove all fat. Whole thickness of skin was used for diffusion study. It was rinsed with normal saline solution. The skin was cut in proper size to fit on Franz diffusion cell having area 3.14 cm^2. Skin was stabilized on Franz diffusion cell till a zero absorbance reading was obtained. This skin sample was taken for further diffusion study.

Drug diffusion study

Franz diffusion cell with a surface area of 3.14 cm^2 was used for *ex-vivo* permeation studies [6]. Porcine skin was mounted between the compartments of the diffusion cell. The diffusion cell was maintained at 37°C using a re-circulating water bath and the solution in the receptor chamber was stirred continuously at 300 rpm. The formulation (0.5 g) was gently placed in the donor chamber. At 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hr, 2 ml of the solution in the receptor chamber was withdrawn and replaced immediately with an equal volume of fresh phosphate buffer. After suitable dilution, solution was subjected to UV-spectroscopic determination. All experiments were performed in triplicate. The cumulative amount of Dexibuprofen permeated through skin was plotted as a function of time. The permeation rate of Dexibuprofen at the steady state (J, µg cm^{-2} h^{-1}) was calculated from the slope. The data are presented as means ± SD obtained using 4–8 skin fragments from at least two animals. Statistical significance was determined using t-test or Rank Sum Test, where appropriate, for evaluation of the effect of the enhancers. ANOVA or ANOVA on Ranks with Dunn's post test was used for multiple comparisons.

Skin Accumulation Studies

Full thickness porcine skin over which transdermal gel was applied was removed. The excised skin was homogenised in 25 mL of mobile phase. The resulting solution (25 mL) is filtered through whatmann paper (0.45µm) and was injected into the HPLC system, and concentration of the drug accumulated in the skin was determined after calculations [7-15].

Calibration curve of Dexibuprofen in presence of skin

Calibration curve of dexibuprofen was developed with pig skin homogenate in mobile phase. Full thickness pig skin of 3.14 cm^2 was taken for calibration curve. Initially skin was chopped by sharp blade then it was homogenized in 25 mL of mobile phase. The resulting solution (25 mL) was filtered and volume was made up to 50 ml with mobile phase in a volumetric flask. Dexibuprofen 50 mg was added to get 1000 µg/mL stock solution. This stock solution was appropriately diluted with mobile phase to obtain different concentrations of Dexibuprofen 2, 4, 6, 8 and 10 µg/mL. The diluted standard solution was filtered through 0.2 µ nylon membrane and injected into HPLC system and the chromatogram was recorded at 223 nm. Calibration curve was plotted as peak area vs. concentration (fig 1) [15-20].

Histological studies

Transdermal gel formulations (500 mg) were applied for 24 hr on the excised skin mounted on the diffusion cell. The transdermal gel formulations were removed; the porcine skin was wiped off with tissue paper and fixed with 10% v/v formalin solution in saline for atleast 72 hr before routine processing. The skin was sectioned vertically and each section was dehydrated and embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were then examined under a microscope (10X). Skin not treated with any formulation served as a control [20-28].

Skin Irritation Study

Skin irritation is the production of reversible damage to the skin following the application of a test substance. Primary irritation is due to irritants which cause

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inflammation. These irritants involve the interaction of chemicals with the sensory receptors in the skin at the site of application. Secondary irritation causes progressive inflammation on repeated contact.

The Draize patch test using rabbit as an animal model was used to evaluate the skin irritation potential of the developed formulations to intact skin and rabbit skin.

As per the OECD guidelines, the skin irritation test was performed. Three rabbits were used for the Draize patch test. Skin irritation study was carried out according to Draize test protocol approved by the Institutional Animal Ethical committee of C.U. Shah College of Pharmacy.

RESULT AND DISCUSSION

The pH of gels was adjusted from 3.5 to 8.0 to investigate the effect of pH on drug diffusion from gels. These gels contained only drug and polymer. No solvent and penetration enhancer were added. Gels at pH 7 and 8.0 showed good clarity and consistency as compared to gels prepared at other pH.

Effect of formulation pH on the transdermal permeability of Dexibuprofen through porcine skin was studied. The pH of the porcine skin remained relatively constant and is similar to that on human skin. Highest flux was obtained with gels formulated at pH 7 AND 8. Porcine skin treated with gel formulations at different pH was subjected to skin accumulation studies.

As the pKa value of Dexibuprofen is around 4.85, Dexibuprofen will remain in the ionized form above pH of pKa +1 i.e above pH 5. Dexibuprofen will solublise at pH above 5. Figure 3.15 depicts the effect of pH on the solubility of the drug. Below this pH Dexibuprofen being in non ionized form is less soluble in water and hence precipitates out. At pH=7.4 practically all Dexibuprofen (pKa= 4.85) is present in the salt form and hence dissolves in the solution; the acidic form fraction is determined by

Log [Dexibuprofen acid/ Dexibuprofen salt] = pKa - pH; thus % [Dexibuprofen acid] = 0.066%.

The importance of this value is that because only the non-ionized form of an acid (like Dexibuprofen) is able to pass the different membranes in the skin, the driving force for permeation is rather low (0.066%) at pH 7.4.

In our work, addition of acid to the aqueous solution of the drug resulted into the precipitation of the drug as the pH of the solution decreases below 5. This implies that the drug solution reaches to saturation level near pH 5. Such a solution is expected to impart high thermodynamic activity to the dissolved drug molecules. Hence improved drug permeability may be attributed to higher thermodynamic activity to Dexibuprofen molecule caused by pH lowering and consequent increase in saturation. Increase in the flux is observed as the pH is raised to 7. Similar findings has been reported by Hadgraft 2000. In his study they showed that although the permeability coefficient of ibuprofen at higher pH's was low, the flux of a saturated solution was greatest at the high pH's. This was due to the increased solubility of the ionized permanent. At the higher pH the flux however was higher than anticipated, possibly as a result of ion pairing, since the skin is able to buffer its outer layers to a pH around 5.0. A similar study representing the effect of pH on the flux of NSAID's has been carried out by Hadgraft, 2000. There are significant differences when *in vivo* studies are conducted where active processes may be involved in maintaining the pH balance of the skin. As the highest flux was obtained with gels adjusted to pH above 5.0, these formulations were considered for *ex vivo* diffusion studies through porcine skin.

Amount of drug accumulated in the skin layers will be available for localized management of pain. Drug amount reaching the receptor compartment will be available for systemic circulation. Thus skin accumulation studies helps in determining both, the amount of drug reaching the systemic circulation and the amount available for localized action. Accumulation of Dexibuprofen at pH 8 is about 6-7 folds higher than at pH 7.0 and the flux of the drug through the skin is greater with gel formulated at pH 7.0. This observation complies with the results obtained from *ex-vivo* studies. Hence these gels were selected for histological studies to investigate the effect of formulations on skin.

A comparative study for gels was performed on porcine skin and further compared with untreated control group. The influence of pH on the anatomical structure of the skin was elucidated with the aid of light microscope findings by pathologist (report is attached). Section of the untreated control group showed normal uniform layered stratum corneum, a two cell thick epidermis and loosely textured collagen in dermis. No significant changes in the epidermal thickness were seen in biopsies from the skin section treated with pH=7 gels. The stratum corneum treated with pH 8 gel showed mild disintegration, the epithelium showed mild desquamation and mild degenerative changes. The slide indicated a significant reduction in the thickness of stratum corneum as compared with untreated skin. Normal stratification was also lost and epidermis irregularly thickened with infiltration and inflammatory cells in the dermis of the skin for treated with pH 8 gel.

The gels at pH 7 evaluated for skin irritation potential in accordance with Draize patch test. The observations noted during study are reported in table 3. The positive control (formalin) showed erythema or edema. The negative control was placebo gel. Optimized formulation showed no irritation and edema. Thus, gel containing Dexibuprofen are non-irritant to the skin as the primary irritation index was calculated to be zero.

Transdermal administration of NSAIDs offer the advantage of local, enhanced drug delivery to affected tissues with a reduced incidence of systemic adverse effects. Compared to oral administration transdermal administration leads to relatively high NSAID concentration in dermis. Concentrations achieved in the

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muscle tissue below the site of application are variable, but are at least equivalent to that obtained with oral administration. NSAIDs applied transdermally reach the synovial fluid, but the extent and mechanism remains to be determined. Thus transdermally applied NSAID have a superior safety profile to oral administration. Hence attempts were made in the present study to deliver dexibuprofen through transdermal route.

Table 1. Composition of gels without solvent and penetration enhancer at different pH

Ingredients (%w/w)	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9
Dexibuprofen	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
HEC	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Purified water to q.s.	100	100	100	100	100	100	100	100	100
pH	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 8.0

Table 2. Flux data of gels at different pH without solvent and penetration enhancers (Mean±S.D.)

Formulations	pH	% Drug Content	Flux (μg/cm^2/hr)
P-1	**3.5**	99.99±0.23	23.33±1.15
P-2	4.0	99.98±0.45	11.30±0.56
P-3	4.5	99.85±0.89	17.66±0.48
P-4	5.0	99.94±0.78	14.85±0.48
P-5	5.5	99.68±0.45	15.45±0.64
P-6	6.0	99.96±0.98	13.14±0.45
P-7	6.5	100.10±0.45	19.18±0.97
P-8	7.0	99.03±0.14	**29.71±1.13**
P-9	8.0	100.3±0.65	23.42±1.03

Table 3. Dermal irritation score observation for gel formulation at pH 7after Draize patch test

Rabbit no	Reaction	24 hours (Intact)	24 hours (Abraded)	72 hours (Intact)	72 hours (Abraded)
1	Erythema	0	0	0	0
	Edema	0	0	0	0
2	Erythema	0	0	0	0
	Edema	0	0	0	0
3	Erythema	0	0	0	0
	Edema	0	0	0	0
4	Erythema	0	0	0	0
	Edema	0	0	0	0
5	Erythema	0	0	0	0
	Edema	0	0	0	0
6	Erythema	0	0	0	0
	Edema	0	0	0	0

Fig 1. Chromatogram of dexibuprofen with skin homogenate

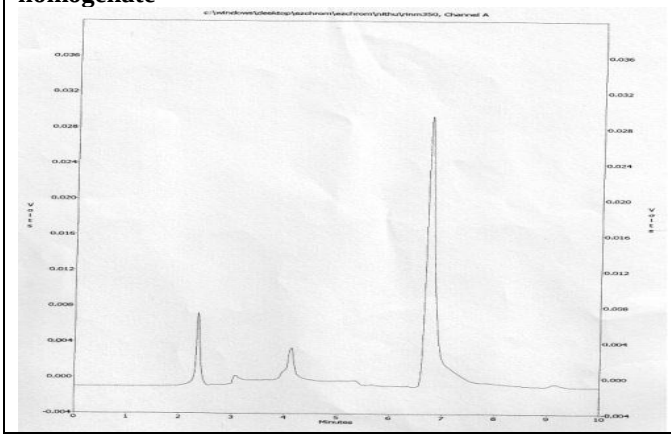

Fig 2. Effect of pH on Dexibuprofen solubility

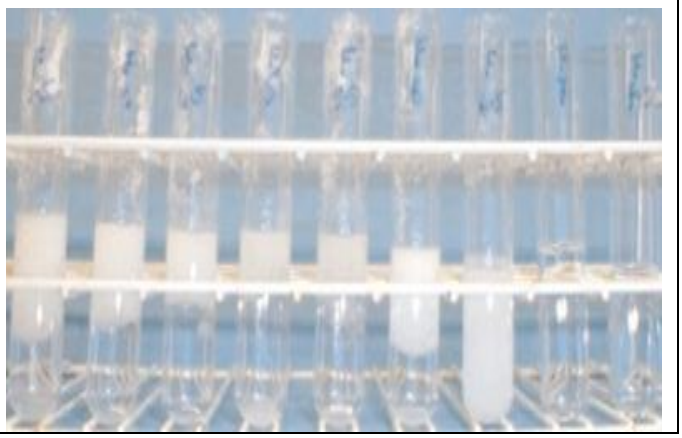

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Fig 3. *Ex-vivo* release data for gels at different pH

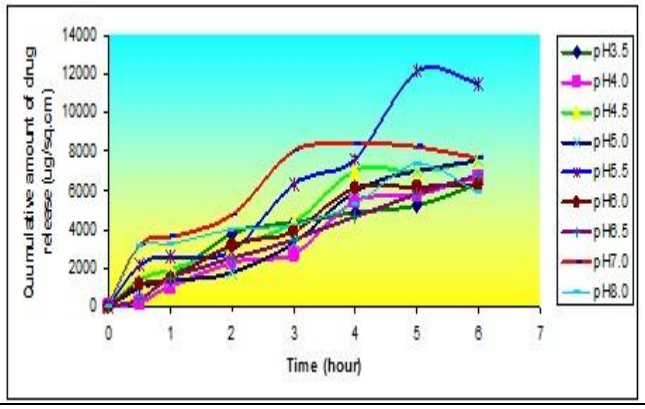


Fig 4. Comparison of flux of gels at different pH

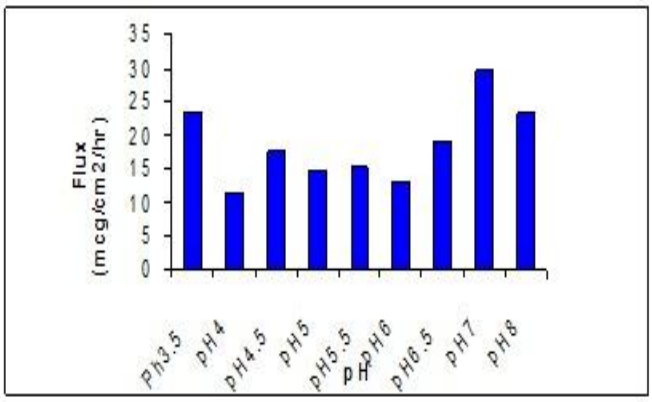


Fig 5. Accumulation of drug from various gel preparations

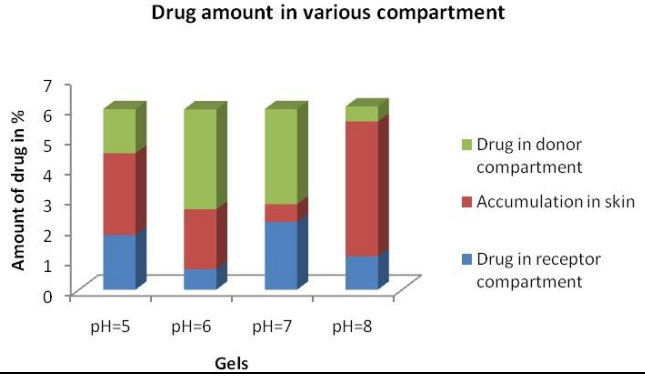
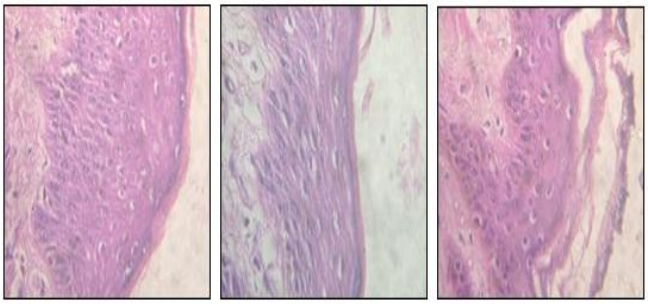


Fig 6. Histological studies for gels formulated at pH 7and pH 8



Control skin sample Skin sample treated with gels at pH 7 and pH 8

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CONFLICT OF INTEREST

No interest

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