



Transdermal self-permeation enhancement of ibuprofen

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Abstract

The objective of this study was to prepare saturated solutions of ibuprofen, of different concentrations, and to investigate their effect on permeation of ibuprofen across rat epidermis. Ibuprofen saturated solutions were prepared using 0.1, 0.2, 0.3 and 0.4 M disodium hydrogen phosphate solution (DHP). The solubility of ibuprofen in DHP increased as the molarity of DHP increased. Thus the four saturated solutions of ibuprofen (0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU) have different concentrations of the same drug, and showed same pH (pH 7.0 ± 1). The permeability study was also carried out using human epidermis and silastic membrane. Permeation rate of ibuprofen across rat epidermis and human epidermis from 0.4M-DHP-IBU was much greater than from 0.1M-DHP-IBU. The magnitudes of increase in the drug flux were 46.4-fold with rat epidermis and 9.4-fold with human epidermis. Such a great increase in drug flux was not observed with silastic membrane, only 1.4-fold. This suggests that the increased drug flux is likely due to drug–skin interaction and not the increased concentration of ibuprofen per se. Surface tension (ST) measurements of DHP versus ibuprofen concentration showed ST reduction of DHP, from 72 to 27.9 dyn/cm. This is an indication that ibuprofen acted as ionic surfactant and the observed skin permeability enhancement is attributed to disruption of stratum corneum barrier. Results of DSC study supported this assumption. DSC of untreated rat stratum corneum samples showed lipid transitions at 41.9 ± 0.0 °C (T_1), 55.1 ± 1.6 °C (T_x), 70.2 ± 0.1 °C (T_2) and 77.5 ± 0.1 °C (T_3), while those pretreated with 0.4M-DHP-IBU did not show the first three lipid transitions. Also, pretreatment of rat epidermis with 0.4M-DHP-IBU enhanced permeation of diclofenac sodium greater than 1250-fold. This corroborates that ibuprofen not only enhances its own permeation but also that of other drugs, such as diclofenac sodium.

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1. Introduction

The greatest obstacle for transdermal drug delivery is the stratum corneum that forms a primary rate-

limiting barrier to the permeation of drugs across the skin [1]. It consists of dead, flattened cells filled with keratin that are embedded in a lipid matrix [2,3]. The stratum corneum has been described as hydrophilic protein “bricks” embedded in a hydrophobic lipid “mortar” [4]. There has been a considerable interest in the potential usefulness of the topical application of

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non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin and diclofenac [5]. These weak acidic drugs are effective in the treatment of rheumatoid arthritis and osteoarthritis [6]. However, the disadvantage of the topical route for drug delivery is that a relatively high dose is required to deliver therapeutic amounts of drug across the skin. To improve the topical delivery of drugs, several strategies are available including the use of penetration enhancers and optimisation of drug release from the formulation [7]. The pH of the formulation has an impact on the penetration rate of weak acidic and weak basic drugs [5].

The flux of ibuprofen from a saturated solution at pH values ranging from 2.2 to 9.0 using human skin *in vitro* was investigated [8]. It was reported that flux of the drug increased with an increase in the pH of the solution. The reason behind this effect was unclear. However, ibuprofen was reported to show a considerable surface activity [9]. Since surfactants are well-known penetration enhancers [10], it is possible that ibuprofen has acted as surfactant and thus impaired the skin permeability barrier. Hence, it is planned to study effect of ibuprofen concentration, in saturated solutions, on its permeation across skin. It is a known concept that an increase in concentration of drug in the vehicle results in enhanced flux due to increased thermodynamic activity. However, in this study, novel vehicles were designed by keeping thermodynamic activity constant while increasing the drug concentration in the vehicle. This was done by preparing saturated solutions of ibuprofen, of different concentrations, using disodium hydrogen phosphate solutions of various molar strengths. The permeation of ibuprofen from its saturated solutions across rat epidermis and human epidermis was studied, and the results were compared with those obtained from silastic membrane.

2. Materials and methods

2.1. Materials

Ibuprofen and diclofenac sodium were obtained from Sigma, Germany. Acetonitrile (HPLC grade) and acetic acid (HPLC grade) were obtained from BDH Chemicals, Germany. Disodium hydrogen

phosphate, sodium dihydrogen phosphate and sodium acetate (AR grade) were obtained from Fluka Chemie, Germany. Water was collected from Millipore (Milli-Q) unit. Trypsin (Type III bovine) was obtained from Sigma, USA. Acetone was obtained from Fluka Chemie.

2.2. Preparation of epidermis

Neonatal rats (Albino out-bred, Sprague–Dawley) were used within 24 h of birth. The care of the animals was in accordance with the institutional guidelines. The animals were sacrificed and the full thickness skin was removed. The rat epidermis was prepared by a heat separation technique [11,12]. The entire skin was soaked in water at 60 °C for 15 s followed by careful removal of the epidermis. The rat epidermis, so prepared, was washed with water and used in the *in vitro* skin permeability studies. In a similar manner, human epidermal membranes (abdominal skin from female cadavers aging 67–69 years) were also prepared except that the skin was immersed in water at 60 °C for 60 s. The silastic membranes (polydimethyl siloxane, 0.005-in. in thickness) were obtained from Dow Corning, Midland, MI.

2.3. Preparation of saturated solutions of ibuprofen

The saturated solutions of ibuprofen were prepared by agitating excess amount of the drug with 0.1, 0.2, 0.3 and 0.4 M disodium hydrogen phosphate solutions in a water bath shaker for 2 h at 50 °C. After agitation, the mixtures were kept to equilibrate at 32 °C in the same place for 24 h, and the pH of each saturated solution was determined. The resultant drug solutions were coded as 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU, respectively. The concentration of drug in the saturated solution was determined using HPLC after appropriate dilution so as to determine the solubility. The saturated solutions of ibuprofen, prepared in this way, were used for *in vitro* permeation experiments.

When measured, the pH of these saturated solutions was found to be 7.0 ± 0.1 . The control vehicle to be used for comparison should have same pH value as that of the drug solution used in the study (pH 7.0), to nullify the effect of pH as a factor on transdermal permeation of drugs. For this reason, phosphate buffer

solution (PB), pH 7.0, was chosen as control vehicle instead of disodium hydrogen phosphate that has a pH value >9. The control vehicle for 0.4M-DHP-IBU was prepared from solutions of 0.4 M disodium hydrogen phosphate and 0.4 M monosodium hydrogen phosphate (0.4M-PB, pH 7.0). Similarly, the control vehicles for 0.1M-DHP-IBU, 0.2M-DHP-IBU and 0.3M-DHP-IBU were prepared from the respective molar solutions of disodium hydrogen phosphate and monosodium hydrogen phosphate.

2.4. *In vitro* permeation studies

The permeation studies were performed using an automated flow-through diffusion apparatus (Perme-Gear In-Line Cells, ILC14). The epidermal membrane of the rat or human was mounted on to the diffusion cell with the stratum corneum side facing the donor compartment and the dermal side facing the receptor compartment. The diffusion cell was equilibrated overnight at 32 °C with a continuous flow of 0.05 M phosphate buffer (pH 7.4) through the receptor compartment. Saturated solution (750 µL) of ibuprofen, prepared as described above, was placed into the donor compartment of each diffusion cell. Prior to covering, a few crystals of ibuprofen were added to donor compartment to maintain saturation. The skin permeate samples were collected at various time intervals and analyzed by HPLC. Similarly, the *in vitro* permeation studies were also carried out using silastic membrane. In all experiments, the cumulative amount of ibuprofen permeated at different time intervals were estimated by HPLC method.

2.5. *In vitro* permeation of a model drug, diclofenac sodium across rat skin

Rat skin samples were soaked in either water (overnight), 0.4M-PB (for 4 h) or 0.4M-DHP-IBU (for 4 h) at 32 °C. The skin samples treated with 0.4M-PB were rinsed and soaked overnight in water. However, those treated with 0.4M-DHP-IBU were first rinsed and soaked in 0.4M-PB for 1 h followed by rinsing and soaking overnight in water, to remove ibuprofen and phosphate salt. These pretreated skin samples were subjected to *in vitro* permeability study using diclofenac sodium as model permeant. Saturated solution of diclofenac sodium prepared in 0.05 M

phosphate buffer solution, pH 7.4, was placed in the donor compartment of the diffusion cell. The receptor solution (0.05 M phosphate buffer, pH 7.4) was continuously pumped at a flow rate of 5 mL/h. The cumulative amount of diclofenac sodium permeated at different time intervals were estimated by HPLC method.

2.6. HPLC analytical method

The amount of ibuprofen or diclofenac sodium either in skin permeates or in the vehicles was estimated by HPLC. The chromatographic system consisted of Waters 2690 automatic sample injector with a loop of 250 µL and Waters 996 Photodiode Array Detector. A reversed-phase Waters Symmetry C₁₈ column (3.9×150 mm; 5 µm) and Waters Symmetry C₁₈ guard column (3.9×20 mm) were used. The mobile phase consisted of acetonitrile and 50 mM acetate buffer (pH 5.1) in the ratio of 55:45 (v/v) delivered at a flow rate of 1.5 mL/min and the volume injected was 50 µL. The column temperature was set at 40 °C. A series of drug solutions, with varying quantity of ibuprofen or diclofenac sodium ranging from 2.5 to 20 µg/mL, were prepared and injected into the HPLC column. The eluents were monitored at 220 nm for either ibuprofen (retention time: 3 min) or diclofenac sodium (retention time: 2 min). The peak areas were obtained and subjected to regression analysis. There was a perfect linear relationship ($Y=16512X-3459.8$) between the concentration of ibuprofen and its peak area as indicated by high correlation coefficient ($R^2=0.9999$). The inter- and intra-day variation was less than 2.8%, indicating high precision of the method. The HPLC method was found to be highly accurate as shown by a mean recovery of 98.2% when ibuprofen solution (5 µg/mL) was added with known quantity (5 µg/mL) of the same drug and estimated as per the procedure described above. The HPLC analytical method for diclofenac sodium was also found to be highly accurate and precise.

2.7. Measurement of surface tension

The surface tension of ibuprofen solutions with varying concentration in 0.1M-DHP, 0.2M-DHP,

0.3M-DHP and 0.4M-DHP was measured by Wilhelmy plate method [13] at room temperature (20 ± 2 °C) using Manual Digital Tensiometer (Sigma 703 KSV Instruments, Helsinki, Finland). The critical micelle concentration (CMC) of ibuprofen in each vehicle was determined from the plot of ibuprofen concentration versus surface tension. Also, the surface tension of saturated solutions of ibuprofen (0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU) was measured to estimate the extent of reduction in surface tension.

2.8. Preparation of rat stratum corneum for DSC

Stratum corneum sheets were obtained by digesting the rat epidermis at 37 °C for 45 min in 0.1 M phosphate buffer saline solution (pH 7.4) containing 0.1% w/v trypsin. The tissue was then smoothed out on a flat surface, and the mushy epidermis was removed by rubbing with moistened cotton wool tips. The stratum corneum sheets thus obtained were rinsed with water. The cleaned sheets were soaked overnight in water at room temperature. Finally, the membranes were rinsed with fresh deionised water and dried overnight on wire meshes under ambient conditions. The dried membranes were rinsed in acetone for 30 s at 0 °C to remove surface lipids, and were dried. Dried stratum corneum membranes were soaked in 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU at 32 °C for 24 h. After this treatment, the samples were rinsed and soaked for 2 h at 32 °C in respective control vehicles (0.1M-PB, 0.2M-PB, 0.3M-PB and 0.4M-PB) followed by overnight soaking and rinsing in water to remove ibuprofen and phosphate salt from stratum corneum. Stratum corneum samples were also treated with control vehicle alone (0.4M-PB) at 32 °C for 24 h to test the influence of phosphate salt. Then, the samples were rinsed and soaked, overnight, in water and dried. All samples were stored in desiccators over silica gel for at least 3 days before subjecting them to DSC studies. The weight of stratum corneum samples was determined before and after treatment with 0.4M-PB or 0.4M-DHP-IBU to find the percent loss in weight of stratum corneum.

2.9. Differential scanning calorimetry

Each desiccated stratum corneum sample (about 10 mg) was placed in 100 μ L aluminium pan and pushed tightly by another but slightly smaller pan to ensure better thermal contact, and hermetically sealed. The samples were heated from 0 to 140 °C at 10 °C/min using a Differential Scanning Calorimeter (DSC141, Setaram, France). After the first heating run, samples in their pans were cooled to 0 °C and then immediately reheated again to 140 °C at 10 °C/min as described before.

2.10. In vitro permeation data analysis

The flux ($\mu\text{g}/\text{cm}^2 \text{ h}$) of ibuprofen was calculated from the slope of the plot of the cumulative amount of ibuprofen permeated per cm^2 of skin/silastic membrane at steady state against the time using linear regression analysis [14,15]. The steady state permeability coefficient (k_p) of ibuprofen across rat epidermis, human epidermis/silastic membrane was calculated by using the following equation [16]: $k_p = J/C$, where J is the flux and C is the concentration of ibuprofen in donor solution. The flux of diclofenac sodium was calculated in the same way.

2.11. Statistical analysis

The in vitro transdermal permeation data involving the effect of 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU on the permeation of ibuprofen across rat epidermis/human epidermis/silastic membrane was subjected to Student's t -test to find out the statistical significance of the observed difference. The significance of the difference observed in the flux of diclofenac sodium across the rat skin before and after treatment with 0.4M-DHP-IBU was tested using Student's paired t -test. The observed difference in the permeation of ibuprofen from 0.4M-DHP-IBU across rat epidermis, human epidermis and silastic membrane was subjected to analysis of variance (ANOVA) and Duncan's multiple range test with the help of SPSS™ computer program (Release 11.5.2.1, SPSS, 1989–2002). In all the cases, a value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

When drug concentration is same in different vehicles, flux of the drug will not be the same as the vehicles provide different thermodynamic activity. However, a drug saturated in different vehicles has same thermodynamic activity and thus skin permeation should be the same provided no interaction occurs between the vehicle/drug and skin components [7,17]. In the present study, saturated solutions of ibuprofen that differ in drug concentration but have equal thermodynamic activity were prepared and subjected to *in vitro* permeability study across skin/silastic membrane. The objective of this study was to investigate the possibility of interaction between ibuprofen and skin, which might result in impairment of the skin barrier and thus permeation enhancement of the drug would be obtained. The novel vehicles, used in the present study, are hypothesized to provide enhanced permeation of ibuprofen across skin.

3.1. Solubility studies

The saturated solutions of ibuprofen were prepared at 32 °C by dissolving the drug in disodium hydrogen phosphate solutions (DHP) of different molarities ranging from 0.1 to 0.4 M. The solubility of ibuprofen increased as the molarity of disodium phosphate increased. The saturation solubility of ibuprofen was 10.4 ± 0.9 , 15.6 ± 1.3 , 25.1 ± 1.2 and 37.5 ± 1.1 mg/mL in 0.1, 0.2, 0.3 and 0.4 M DHP, respectively. The pH values of these saturated solutions of ibuprofen were estimated and found to be 7.0 ± 0.1 . Ibuprofen being a weak acid probably formed a buffer solution, of pH 7, with disodium hydrogen phosphate. At pH 7, ibuprofen molecules would be highly ionized since its pK_a is 5.2 [18]. Since all the saturated solutions of ibuprofen, under study, have the same pH, this would eliminate “pH effect” as a factor on the skin permeation of the drug.

3.2. *In vitro* permeation of ibuprofen across rat epidermis, human epidermis and silastic membrane

The cumulative amount of ibuprofen permeated from its saturated solution across the rat epidermis was shown in Fig. 1. The amount of ibuprofen

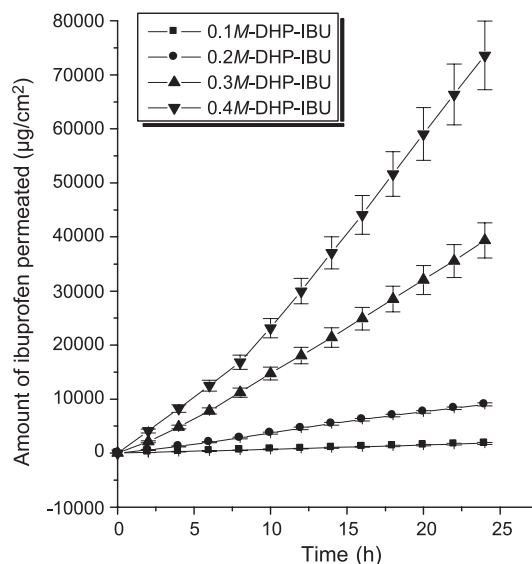


Fig. 1. Mean (\pm S.D.) amount of ibuprofen permeated ($\mu\text{g}/\text{cm}^2$) across neonatal rat epidermis ($n=6$) from 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU.

permeated across rat epidermis was found increasing with an increase in the concentration of the drug in these saturated solutions (0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU). At the end of 24 h of study, the amount of ibuprofen permeated from 0.1M-DHP-IBU was 1808.3 ± 166.3 $\mu\text{g}/\text{cm}^2$ whereas that permeated from 0.4M-DHP-IBU was 73594.7 ± 6368.2 $\mu\text{g}/\text{cm}^2$. The corresponding flux of ibuprofen from these solutions was 77.4 ± 8.3 and 3592.9 ± 724.2 $\mu\text{g}/\text{cm}^2$ h, respectively. This indicated that the permeation of ibuprofen from 0.4M-DHP-IBU was increased by 46.4 times when compared to that obtained from 0.1M-DHP-IBU. With the rat epidermis, the permeability coefficient of ibuprofen using 0.1M-DHP-IBU was $7.4 \pm 0.8 \times 10^{-3}$ cm/h whereas that obtained from 0.4M-DHP-IBU was 95.8×10^{-3} cm/h.

The flux of a drug should remain the same when its thermodynamic activity in different vehicles is maintained constant [7,17]. Since the thermodynamic activity of ibuprofen in the saturated solutions of current study is maintained constant, the value of flux should be the same and k_p should decrease with drug concentration. However, in the present study, both flux and k_p increased by about 46.4 and 12.9 times, respectively, from 0.4M-DHP-IBU as compared to that from 0.1M-DHP-IBU (Table 1). This indicates

Table 1

Permeability parameters of ibuprofen from 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU across three different membranes

Donor solution	Permeability parameters of ibuprofen across					
	Rat epidermis		Human epidermis		Silastic membrane	
	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	k_p ($\text{cm}/\text{h} \times 10^{-3}$)	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	k_p ($\text{cm}/\text{h} \times 10^{-3}$)	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	k_p ($\text{cm}/\text{h} \times 10^{-3}$)
0.1M-DHP-IBU	77.4 \pm 8.3	7.42 \pm 0.8	35.4 \pm 4.4	3.4 \pm 0.4	514.9 \pm 49.0	47.3 \pm 4.5
0.2M-DHP-IBU	415.1 \pm 24.4	26.7 \pm 1.6	74.8 \pm 12.8	4.8 \pm 0.8	608.5 \pm 22.0	39.41.42
0.3M-DHP-IBU	1700.4 \pm 381.8	67.7 \pm 15.2	179.8 \pm 65.1	7.2 \pm 2.5	669.6 \pm 7.2	27.6 \pm 0.3
0.4M-DHP-IBU	3592.9 \pm 724.2	95.8 \pm 19.3	332.0 \pm 26.2	8.9 \pm 0.7	698.9 \pm 28.0	20.6 \pm 0.8

that the permeation enhancement of ibuprofen from its saturated solutions is not due to the increase in concentration of the drug per se. Thus, it appears that ibuprofen in its saturated solution (0.4M-DHP-IBU) might have disrupted the skin barrier function resulting in permeation enhancement.

The dramatic increase in the permeation of ibuprofen observed across rat epidermis was also investigated using human epidermis. The amount of ibuprofen permeated across human epidermis was also found increasing with an increase in the concentration of the drug in saturated solution (Fig. 2). At end of 12 h of study, the amount of ibuprofen permeated from 0.1M-DHP-IBU was 416.5 \pm 26.6 $\mu\text{g}/\text{cm}^2$ whereas that permeated from 0.4M-DHP-

IBU was 3751.3 \pm 124.2 $\mu\text{g}/\text{cm}^2$. The corresponding flux of ibuprofen from these solutions across human epidermis was 35.4 \pm 4.4 and 332.0 \pm 26.2 $\mu\text{g}/\text{cm}^2 \text{ h}$, respectively. Thus, the increase in the flux of ibuprofen from 0.4M-DHP-IBU across human epidermis was 9.4 times when compared to that observed from 0.1M-DHP-IBU (Table 1). The permeability coefficient across human epidermis from 0.1M-DHP-IBU was 3.4 \pm 0.4 $\times 10^{-3}$ cm/h whereas that obtained from 0.4M-DHP-IBU was 8.9 \pm 0.7 $\times 10^{-3}$ cm/h. The increase in permeability coefficient of the drug across human epidermis from 0.4M-DHP-IBU was 2.6 times that obtained from 0.1M-DHP-IBU. The results of the study showed that ibuprofen affected both rat epidermis and human epidermis in a similar way. However, the observed low permeation enhancing effect of ibuprofen using human epidermis was likely due to the high resistance of human skin to chemical penetration enhancers compared to that of animals [10]. As indicated previously, the observed permeation enhancement of ibuprofen across rat epidermis and human epidermis was not due to the increased concentration of ibuprofen in the donor solution per se, but possibly due to an interaction of the drug with the stratum corneum components. This assumption was tested using permeability studies across silastic membrane. The permeability study across silastic membrane was used as a control to find whether the permeation enhancement of ibuprofen was merely due to the increased drug concentration or other factors.

The extent of increase in the flux of ibuprofen obtained across rat and human epidermal membranes was not observed across silastic membrane. At the end of 12 h of study, the total amount of ibuprofen permeated from 0.1M-DHP-IBU across silastic membrane was 13 609.3 \pm 590.6 $\mu\text{g}/\text{cm}^2$ whereas that permeated from 0.4M-DHP-IBU was 17 993.7 \pm

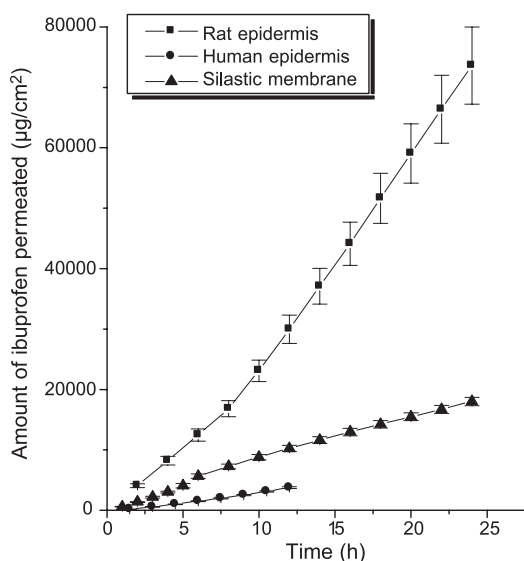


Fig. 2. Mean (\pm S.D.) amount of ibuprofen permeated ($\mu\text{g}/\text{cm}^2$) from 0.4M-DHP-IBU across neonatal rat epidermis ($n=6$), human epidermis ($n=3$) and silastic membrane ($n=3$).

709.0 $\mu\text{g}/\text{cm}^2$ (Fig. 2). The corresponding flux of ibuprofen from these solutions across silastic membrane was 514.9 ± 49.0 and 698.9 ± 28.0 $\mu\text{g}/\text{cm}^2$ h, respectively. The increase in flux of ibuprofen from 0.4M-DHP-IBU was only 1.4-fold when compared to that obtained from 0.1M-DHP-IBU (Table 1). The k_p of ibuprofen using silastic membrane decreased from 47.3 ± 4.5 to 20.6 ± 0.8 $\text{cm}/\text{h} \times 10^{-3}$. Though the concentration of ibuprofen in 0.4M-DHP-IBU was three times more than that in 0.1M-DHP-IBU, the permeation enhancement was only 1.4-fold using silastic membrane wherein it was 9.4- and 46.4-fold with human and rat epidermal membranes, respectively. Also, the k_p was increasing with human epidermis and rat epidermis, but was decreasing with silastic membrane. Again, this suggested that permeation enhancement of ibuprofen is not due to increased concentration of drug, but due to drug–skin interaction. A detailed study was undertaken to investigate the possible mechanism involved in ibuprofen self-permeation enhancement across the skin. It was reported that ibuprofen showed surface activity [9]. Hence, the surface tension of the saturated solutions of ibuprofen was measured. Also, the effect of various concentrations of ibuprofen on surface tension of disodium hydrogen phosphate solution was measured.

3.3. Surface tension measurements

Ibuprofen solution with varying concentration was prepared in 0.1M-DHP, 0.2M-DHP, 0.3M-DHP and 0.4M-DHP, and the surface tension was measured by Wilhelmy plate method [13] at room temperature (20 ± 2 °C) using Manual Digital Tensiometer. There was a decrease in the surface tension of DHP as the concentration of ibuprofen in these vehicles increased. The decrease in surface tension of ibuprofen solutions suggested that ibuprofen behaved as a surface-active agent. The critical micelle concentration (CMC) of ibuprofen in each vehicle was determined from the plot of ibuprofen concentration versus surface tension. The surface tension of ibuprofen in 0.1M-DHP decreased from 72 to 36.2 dyn/cm with a CMC of 0.8% w/v whereas that in 0.2M-DHP decreased from 72 to 30.6 dyn/cm with a CMC of 1.1% w/v. However, the decrease in the surface tension of ibuprofen in 0.3M-DHP and 0.4M-DHP was higher

when compared to that in 0.1M-DHP and 0.2M-DHP. The surface tension of ibuprofen in 0.3M-DHP decreased from 72 to 30.1 dyn/cm with a CMC of 1.5% w/v whereas that in 0.4M-DHP decreased from 72 to 29.1 dyn/cm with a CMC of 1.8% w/v. This indicates that ibuprofen has surface activity, and CMC value is depending on the solubility of ibuprofen in these vehicles. The surface tension of saturated solution of ibuprofen (0.1M-DHP-IBU) decreased from 72 to 32.7 dyn/cm whereas that of 0.4M-DHP-IBU decreased from 72 to 27.9 dyn/cm. In the donor compartment, ibuprofen solutions of lower surface tension suggest lower drug/skin interfacial tension, which in turn could have improved the contact between the drug and skin leading to permeation enhancement of ibuprofen. In this work, the surface activity of ibuprofen was attributed to its ionized form (anionic surfactant), which also might have impaired the permeability barrier of the skin resulting in enhanced drug permeation across the epidermis.

Surfactants are known to act as chemical penetration enhancers for transdermal delivery of drugs [19]. Surfactant solutions were reported to greatly enhance penetration of several drugs and their actions were attributed to their interaction with stratum corneum components [20]. The dramatic and large increase in the penetration rate of ibuprofen seen with 0.4M-DHP-IBU suggested that its major effect on skin permeability was possibly due to disruption of skin permeability barrier. The interaction of ibuprofen with the barrier components of stratum corneum might have resulted in self-permeation enhancement of the drug. To elucidate the possible mechanisms of ibuprofen–skin interaction, differential scanning calorimetry (DSC) was carried out after treating the rat stratum corneum with 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU.

3.4. DSC study

The stratum corneum acts as the principal barrier to diffusion of most substances. It essentially consists of flattened keratinocytes embedded in a matrix of multilamellar lipid bilayers [21]. Further, it was suggested that cholesterol and lipids with long saturated acyl chains (e.g., free fatty acids and ceramides) predominate in the barrier layer [22]. Differential scanning calorimetry (DSC) has been used to study thermal

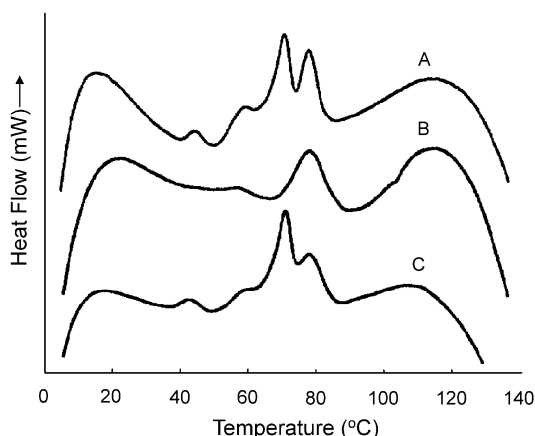


Fig. 3. DSC thermograms of neonatal rat stratum corneum: (A) untreated, (B) pretreated with 0.4M-DHP-IBU and (C) pretreated with 0.1M-DHP-IBU.

transitions in mammalian stratum corneum [23]. Typically, thermal transitions occur at 35–42, 60–77, 70–90 and 95–120 °C and are referred to as T_1 , T_2 , T_3 and T_4 , respectively. The transition temperatures T_1 , T_2 and T_3 are attributed to phase changes in the intercellular lipid bilayers and T_4 is associated with protein denaturation [10,24].

The DSC thermograms of untreated rat stratum corneum (control) and those pretreated with ibuprofen saturated solutions were shown in Fig. 3. The DSC of control samples showed four endothermic transitions at about 42 °C (T_1), 55 °C (T_x), 70 °C (T_2) and 78 °C (T_3), and were attributed to lipid melting. Similar transition values were also reported and attributed to lipid melting [10,24]. The results of the DSC study on stratum corneum samples pretreated with 0.1M-DHP-IBU, 0.2M-DHP-IBU and 0.3M-DHP-IBU showed lipid transitions at more or less the same temperatures

as that of controls (Table 2). This was not surprising since ibuprofen was removed from the treated samples by sequential soaking in the respective control vehicles (0.1M-PB, 0.2M-PB and 0.3M-PB, respectively) and water prior to desiccation and thermal analysis. This was essential; otherwise, huge endothermic transition due to ibuprofen (around 70 °C) would overlap T_x , T_2 and T_3 of stratum corneum lipids. However, the DSC thermograms of the pretreated samples indicated that the enthalpies corresponding to the lipid transition at 70 °C (T_2) were decreasing from 2.9 to 0.9 J/g as the concentration of ibuprofen in these vehicles increased. The decreasing enthalpy might be due to increasing extraction of stratum corneum lipids with an increase in the concentration of ibuprofen in the vehicles. In the case of stratum corneum samples pretreated with 0.4M-DHP-IBU, the lipid transition at 70 °C (T_2) disappeared from the thermogram, suggesting greater effect of ibuprofen on stratum corneum lipids responsible for T_2 . However, stratum corneum samples pretreated with 0.4M-PB alone showed all the lipid transitions as that of untreated samples (water-treated). The enthalpy corresponding to lipid transition at 70 °C (T_2) was 3.3 J/g, which was similar to that obtained with the untreated samples (3.2 J/g). These results indicated that ibuprofen saturated solution (0.4M-DHP-IBU) interacted with stratum corneum and extracted stratum corneum components responsible for lipid transition at T_2 .

Surfactants usually consist of a lipophilic alkyl or aryl group with a hydrophilic head group. Ibuprofen chemical structure resembles those of surfactants. Rao et al [9] also reported the surface activity of ibuprofen. As reported in the literature, surfactants can interact with keratin, swell stratum corneum and extract

Table 2

Thermal analysis (DSC) of rat stratum corneum samples ($n=3$) before and after treatment with 0.1M-DHP-IBU, 0.2M-DHP-IBU, 0.3M-DHP-IBU and 0.4M-DHP-IBU

Solution	Endothermic peak* (°C) on first heating				Endothermic peak* (°C) on second heating	
	T_1	T_x	T_2	T_3	T_1	T_2
Water (Control)	41.9±0.0	55.1±1.6	70.2±0.1	77.5±0.1	38.8±0.2	69.5±0.3
0.1M-DHP-IBU	42.2±0.9	–	71.4±0.3	76.3±1.5	41.6±0.4	68.4±0.3
0.2M-DHP-IBU	39.5±1.0	51.7±0.1	66.7±0.3	74.1±0.2	39.2±0.3	64.4±0.2
0.3M-DHP-IBU	44.3±5.1	–	69.5±1.6	77.8±0.8	39.1±0.6	67.1±0.2
0.4M-DHP-IBU	–	–	–	75.9±0.1	–	68.0±0.0

* Values in the table shown as mean±S.D.

various components from the intercellular lipid matrix of the stratum corneum besides their ability to extract lipids, thereby resulting in disruption of a pathway through the stratum corneum [20]. In the present work, most of ibuprofen molecules should be available as ionized form because the pH value of ibuprofen solution was 7.0 and the pK_a of the drug is 5.2. In the ionized form, ibuprofen might have acted as anionic surfactant and showed similar effects on stratum corneum as surfactants. In the present study, these effects were observed when stratum corneum was treated with 0.4M-DHP-IBU. During DSC study, rat stratum corneum samples treated with 0.4M-DHP-IBU showed swelling. The weight of stratum corneum samples was determined before and after treatment with 0.4M-PB or 0.4M-DHP-IBU. Samples treated with 0.4M-PB alone indicated a weight loss of 17%; however, those treated with 0.4M-DHP-IBU presented a 35% decrease in the weight. These observations possibly indicate that ibuprofen in 0.4M-DHP-IBU acted as a surfactant, interacted with stratum corneum keratin, caused hydration and partially extracted stratum corneum lipids and other components.

3.5. Effect of ibuprofen on the *in vitro* permeation of a model drug, diclofenac sodium

The results of *in vitro* transdermal permeability studies, surface tension measurements and DSC study on 0.4M-DHP-IBU-treated rat stratum corneum indicated that ibuprofen resulted in self-permeation enhancement. In view of this interesting finding, additional experiments were carried out to confirm the penetration enhancing effect of ibuprofen solution (0.4M-DHP-IBU) using diclofenac sodium as a model drug. The cumulative amount of diclofenac sodium permeated across rat skin pretreated with 0.4M-DHP-IBU, 0.4M-PB or water was shown in Fig. 4. The amount of diclofenac sodium permeated at the end of 24 h of study across rat skin pretreated with 0.4M-DHP-IBU was $199\,281 \pm 27\,225.4 \mu\text{g}/\text{cm}^2$ whereas that permeated across rat skin pretreated with 0.4M-PB was only $90.7 \pm 5.8 \mu\text{g}/\text{cm}^2$. However, the amount of diclofenac sodium pretreated with water (control) was $256.2 \pm 99.3 \mu\text{g}/\text{cm}^2$. The flux of diclofenac sodium across rat skin pretreated with water was $6.7 \pm 1.3 \mu\text{g}/\text{cm}^2 \text{ h}$ whereas that obtained across rat skin pretreated with 0.4M-PB was $4.3 \pm 0.5 \mu\text{g}/\text{cm}^2 \text{ h}$. However, the

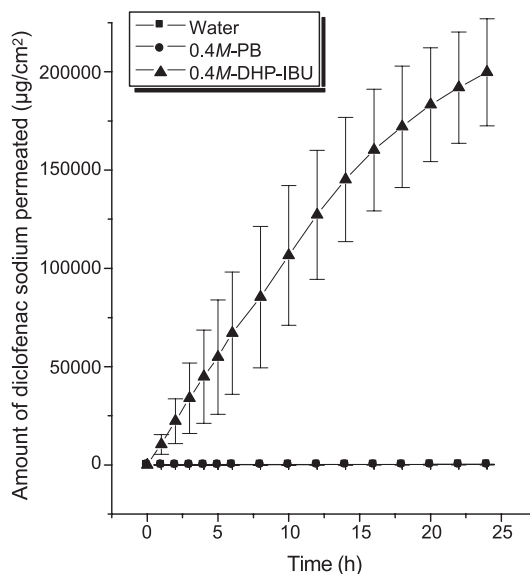


Fig. 4. Mean (\pm S.D.) amount of diclofenac sodium permeated ($\mu\text{g}/\text{cm}^2$) across rat skin ($n=6$) pretreated with water (control), 0.4M-PB or 0.4M-DHP-IBU.

flux of diclofenac sodium across rat skin pretreated with 0.4M-DHP-IBU was $8380.4 \pm 1391.7 \mu\text{g}/\text{cm}^2 \text{ h}$, which is more than 1250 times of those pretreated with the other two vehicles (water or 0.4M-PB). This suggested that the enhanced permeation of diclofenac sodium across rat skin was due to the effect of ibuprofen present in 0.4M-DHP-IBU, but not the phosphate salt.

Based on the results of *in vitro* transdermal permeability studies, surface tension measurements and DSC study, there is a reasonable evidence to suggest that ibuprofen, not the phosphate salt, is directly involved in the impairment of skin permeability barrier, resulting in enhanced permeation of the drugs (ibuprofen or diclofenac sodium) used in the study. This novel vehicle (disodium hydrogen phosphate solution saturated with ibuprofen) may lead to the development of novel membrane-moderated transdermal therapeutic systems for increasing the permeability of drugs across skin.

4. Conclusions

The saturated solution of ibuprofen containing disodium hydrogen phosphate was investigated for

its use in transdermal drug delivery. The ibuprofen saturated solution (0.4M-DHP-IBU) provided a 46.4-fold increase in transdermal permeation of the drug across rat epidermis when compared to that obtained from 0.1M-DHP-IBU, indicating self-permeation enhancement of the drug. The surface tension measurements showed that ibuprofen exhibited surface activity. Thus, the self-permeation enhancement of ibuprofen is attributed to disruption of stratum corneum barrier. This hypothesis was supported by the results of DSC study, which in turn was confirmed by conducting in vitro permeation studies across rat skin pretreated with 0.4M-DHP-IBU using diclofenac sodium as a model drug. It appears that ionized ibuprofen in disodium hydrogen phosphate solution acts as anionic surfactant, thereby resulting in self-permeation enhancement of the drug.

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