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Research paper

Optimized transdermal delivery of ketoprofen using pH and hydroxypropyl-β-cyclodextrin as co-enhancers

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Abstract

The role of pH and pK_a of ionizable drugs in transdermal delivery has been well documented by the pH partition hypothesis. Similarly the role of pH in complexation has also been addressed by many studies. Reports contrary to the well believed theory that both molecular encapsulation by hydroxypropyl-β-cyclodextrin (HP-β-CD) and transdermal delivery are considered a phenomenon of unionized drug species prompted investigation into the combined effect of pH and HP-β-CD on transdermal delivery of ketoprofen. In order to optimize the delivery of ketoprofen, solubility studies and permeation studies were conducted in vitro at pH 3.0, 4.5 and 6.0 at various concentrations of cyclodextrin. The stability constants for unionized and ionized drugs were calculated. The solubility of the ionized complex of the drug was 2.5 fold greater than the unionized complex. The flux increased linearly with increasing HP-β-CD concentration at all the pH values. However, the increase was significant at pH 6.0 where the drug is predominantly in the ionized state. The flux of the ionized drug at 10% w/v HP-β-CD concentration was enhanced to an order of approximately eight times compared to the intrinsic permeability of the unionized drug. The study shows that at higher pH, HP-β-CD can be utilized to achieve greater transdermal flux of ketoprofen. © 2002 Published by Elsevier Science B.V.

Keywords: Ketoprofen; Hydroxypropyl-β-cyclodextrin; pH; Transdermal delivery; Steady state flux

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used as analgesics and in the treatment of chronic inflammatory conditions. Limited efficacy or adverse effects like gastric irritation associated with conventional routes has prompted researchers to investigate the feasibility of alternative drug delivery systems, such as the transdermal route, for administration of these drugs [1]. Comparative studies done to evaluate NSAIDS for their intrinsic feasibility of being delivered via the transdermal route demonstrated that ketoprofen was a suitable candidate [2–4] based on its biopharmaceutical characteristics. However, studies also advocated the use of enhancers [5,6] for effective transdermal delivery of ketoprofen.

A research priority of pharmaceutical scientists is to find a safe and effective enhancer for transdermal delivery of drugs. In this regard, cyclodextrins and substituted cyclodextrins have received considerable attention. These oligosaccharides are known to solubilize lipophilic therapeutic entities through molecular encapsulation and deliver the drugs to the skin in a solubilized form from which the drug partitions into and through the skin [7]. The solubilization potential of these agents allows a greater amount of the drug to be loaded in the donor phase and at unit thermodynamic activity they are hypothesized to increase the drug transport through a kinetic barrier that exists at the skin–vehicle interface [8]. Cyclodextrins have been investigated for their affinity to ketoprofen in the dermal vehicles [9] and are known to enhance bioavailability and form stable complexes in solid states [10]. Ketoprofen, being a weak acid (pK_a 4.6), can be solubilized by adjusting the pH to a higher value as solubility increases with pH above its pK_a. Recent investigations [11] into the combined use of pH and complexation for solubilization of lipophilic drugs has resulted in greater solubilization of the drugs in their ionized state. Similarly ionic forms of NSAIDS have been shown to have better flux via improved solubility [12], contrary to the expectations of the well documented pH partition theory.

Though either pH or complexation have been used to optimize transdermal delivery, studies combining the use of both are limited. In light of these investigations, it has

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been proposed to study the effect of pH and complexation on transdermal flux and solubility of ketoprofen.

2. Materials and methods

2.1. Materials

Ketoprofen was a kind gift from Rhone-Poulenc, Mumbai, India. Hydroxypropyl-β-cyclodextrin (HP-β-CD), molar substitution 0.6, was purchased from Fluka Chemie GmbH, Buchs, Switzerland. Methanol, water, acetic acid of HPLC grade and buffer components of analytical grade were purchased from E-Merck (India) Ltd., Mumbai, India. Nylon membranes of 0.45 μm pore size were purchased from Pal-Gelman Sciences, Michigan, USA.

2.2. Analytical methods

The amount of ketoprofen was quantitated by a HPLC system (Shimadzu, LC10 Ai, V.P., Japan), equipped with two pumps, a photodiode array detector and a communication bus module. The analysis was performed at room temperature with a C18 Shimpack 250 × 4.5 mm, 5 μm column using a mobile phase of methanol (80%) and 0.1 M acetic acid (20%) pumped at a flow rate of 1.5 ml/min and monitored at a wavelength of 255 nm, set at AU/FS −5 to 30. The retention time of the drug was 3.2 ± 0.1 min and the calibration graph was linear in the concentration range of 0.1–10 μg/ml with $r^2 = 0.999$ and inter–intraday variation of rsd ≤5.0%.

2.3. Solubility determination

Solubility studies were conducted according to the continuous variation method proposed by Higuchi and Connors [13]. An excess drug was added to the buffer, containing increasing concentrations of HP-β-CD. The suspensions were briefly sonicated and agitated at 32 °C (to represent the surface temperature of skin) on an orbital shaker at 300 rev./min for 3 days. On equilibration and filtration through 0.45 μm nylon membranes and dilution, the samples were analyzed to determine the drug concentration. The results of the triplicate studies were averaged.

2.4. Determination of distribution coefficient

The partition coefficient of ketoprofen was determined at pH 3.0, 4.5, and 6.0 in citrate-phosphate buffers. An n-octanol/buffer mutual saturation was carried out for 24 h with gentle mechanical stirring and then each phase was separated. The ethanolic solution of ketoprofen at 500 μg/ml (1 ml) was placed in a glass tube and 1 ml of each saturated solvent was added to the tubes after completely evaporating the ethanol. The stoppered tubes were shaken for 24 h on an incubator shaker. Concentrations of the drug in each phase were determined by HPLC after appropriate dilution with methanol.

2.5. Skin permeation studies

Male Wistar rats (150 ± 10 g) were sacrificed by deep anesthesia using pentobarbitone sodium (50 mg/kg, i.v.). Abdominal skin was excised on prior clipping of the hair and removal of the subcutaneous fat by means of blunt dissection. The full thickness skin was clamped by a 'O' ring between the donor and the receptor chambers of a vertical diffusion cell (diffusion area 4.9 cm^2) with the stratum corneum side in contact with the donor phase. A 2 ml aliquot of the ketoprofen samples obtained from the solubility studies was used as reservoirs for the permeation studies. The donor phase consisted of excess drug in suspension form to maintain a unit thermodynamic activity and infinite dosing. The receptor medium consisted of 30 ml normal saline maintained at 37 °C and stirred at 100 rev./min by a bar magnet. Samples (3 ml) were withdrawn at fixed time intervals and the same volume of receptor fluid was replaced periodically up to 24 h. The drug concentrations in the samples were estimated by HPLC and the cumulative drug vs. time graphs were plotted. The steady state permeation profiles were zero order and the permeation parameters were calculated from the linear portion of the permeation profiles. The protocol was approved by the Institutional Animal Ethical Committee of the Indian Institute of Chemical Technology (IICT).

2.6. Data treatment

The stability constants for ionized and unionized drugs were calculated from the equations formulated by Li et al. [11].

K_u, the complexation constant for 1:1 complex of unionized drug, was calculated from the solubilization slope τ_u and is given as

$$\tau_u = \frac{K_u[D_u]}{1 + K_u[D_u]} \quad (1)$$

where D_u is the intrinsic solubility of the unionized drug. Similarly K_i, the constant for ionized drug, was calculated from the solubilization slope τ_i for the complexation of the ionized drug and is given as

$$\tau_i = \frac{K_i[D_u] \times 10^{(pH - pK_a)}}{1 + K_i[D_u] \times 10^{(pH - pK_a)}} \quad (2)$$

The fraction of ionized drug (f_i) was calculated from

$$f_i = 100/[1 + 10^{(pK_a - pH)}] \quad (3)$$

The expected permeability coefficient (K_p) was calculated from the predictive equation of Potts and Guy [14].

$$\text{Log } K_p \text{ (cm/h)} = -2.7 + 0.71 \log K_{oct} - 0.0061 \text{ MW} \quad (4)$$

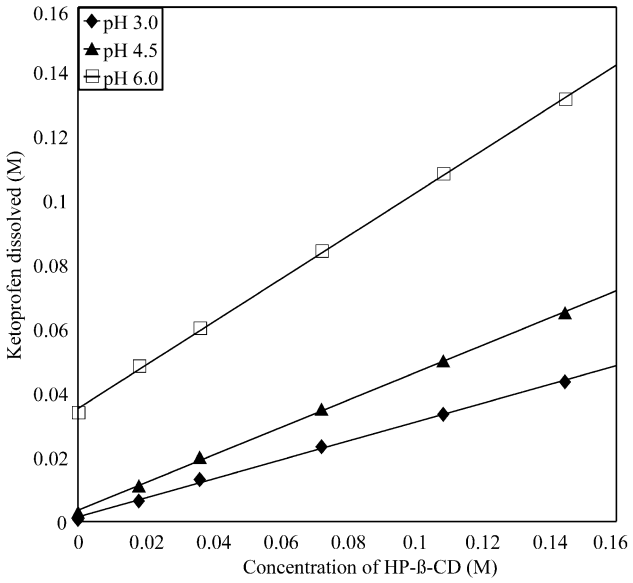

Fig. 1. Combined effect of pH and HP-β-CD on total solubility of ketoprofen, $n = 3$ (rsd ≤ 5%).

Log K_{oct} (octanol/water partition coefficient) was substituted by log D, the distribution coefficient.

The steady state flux (J_{ss}) was obtained from the linear portion of the cumulative drug permeated vs. time graph and permeability coefficient K_p was calculated from the $J_{ss} = K_p C_0$, where C_0 is the saturation solubility of the drug in the vehicle; pK_a of 4.6 for ketoprofen was taken from the Medchem database.

3. Results and discussion

3.1. Solubility studies

The solubilization profiles of ketoprofen at pH 3.0, 4.5 and 6.0 as a function of increasing concentration of HP-β-CD are linear (A_L-type) [13] at all the pH values investigated (Fig. 1) indicating the formation of a 1:1 complex irrespective of ionization of the drug. However, the solubilization slopes 0.31 (pH 3.0), 0.44 (pH 4.5) and 0.69 (pH 6.0) differ significantly. The slopes at pH 3.0 and 6.0 were substituted in Eqs. (1) and (2) proposed by Li et al. [11] (modified for an acidic drug) and the values obtained are 673 and 128 M^{-1} for unionic and ionic forms of the drug complexes.

The higher solubilization slope at pH 6.0 where the drug is 97% ionized indicates a greater solubility of the ionized complex. The ionized ketoprofen complex is 2.5 fold more soluble than the unionized drug complex though the stability constant is less than one-fifth that of the unionized drug ($K_i/K_u = 0.19$). This is a result of 50 fold greater intrinsic solubility of the ionized drug at pH 6.0 and is in agreement with the hypothesis (observation) that if

$$\frac{D_i}{D_u} > \frac{K_u}{K_i} \text{ then } [D_i L] > [D_u L]$$

where D_i and D_u are solubilities of ionized and unionized drug, respectively, [D_iL] and [D_uL] are the solubilities of the ionized and unionized complex, and K_u and K_i are stability constants of unionized and ionized complexes, respectively. The findings are in agreement with the solubility studies carried out with other drugs [11,15].

The distribution coefficient decreased as a function of pH as a result of greater affinity of the ionized drug species for the aqueous phase. The log D values were substituted in the Potts and Guy equation to calculate the predictive flux and permeability coefficient values and the results are presented in Table 1 along with the experimentally obtained values. The applicability of the above equation by substituting log D at different pH values has been validated by Hadgraft and Valenta [12].

3.2. Skin permeation studies

The experimental intrinsic flux at the three pH values agrees with the prediction that flux increases at higher pH despite ionization of the drug. The higher than predicted flux obtained in the present study was on account of the fact that rat skin was used instead of human skin, which was originally used to validate the Potts and Guy equation. The lower permeability coefficient of the ionized drug is compensated by the increasing solubility of the ionic form and thus accounts for the higher flux which is a composite term related to both solubility and permeability. The results are in agreement with studies on ibuprofen [12] where the flux of the ionized drug was higher and an ion pairing mechanism was a possible explanation.

The addition of HP-β-CD to the vehicles increased the flux linearly at all pH values (Table 2). HP-β-CD signifi-

Table 1
The predicted and obtained flux and the permeability coefficients of ketoprofen at different pH values[a]

pH	f_i (%)	Log D	Predicted		Obtained	
			K_p (cm/h)	J_{ss} (μg/cm^2 per h)	K_p (cm/h)	J_{ss} (μg/cm^2 per h)
3.0	2.45	2.70	4.645×10^{-3}	0.801	0.0107	1.85
4.5	44.26	2.44	3.037×10^{-3}	2.197	4.47×10^{-3}	3.24
6.0	96.24	1.28	4.5×10^{-4}	3.85	5.8×10^{-4}	4.87

[a] f_i, fraction ionized; D, distribution coefficient; K_p, permeability coefficient; J_{ss}, steady state flux.

Table 2
Effect of pH and HP-β-CD concentration on the flux and permeability coefficient of ketoprofen ($n = 3$)[a]

pH	% Cyclodextrin	J_{ss} (μg/cm^2 per h)	K_p (cm/h)	EF
3.0	0.0	1.85 ± 0.11	0.0107	1.00
	2.5	4.79 ± 0.75	2.9×10^{-3}	2.57
	5.0	7.5 ± 0.7	2.24×10^{-3}	4.05
	10.0	10.0 ± 0.87	1.7×10^{-3}	5.40
4.5	0.0	3.24 ± 0.28	4.47×10^{-3}	1.00
	2.5	6.1 ± 0.66	2.17×10^{-3}	1.88
	5.0	8.58 ± 0.84	1.69×10^{-3}	2.64
	10.0	11.51 ± 0.57	1.3×10^{-3}	3.55
6.0	0.0	4.87 ± 0.22	5.8×10^{-4}	1.00
	2.5	7.59 ± 0.97	6.19×10^{-4}	1.56
	5.0	11.3 ± 1.19	7.2×10^{-4}	2.40
	10.0	14.86 ± 1.65	6.96×10^{-4}	3.08

[a] K_p, permeability coefficient; J_{ss}, steady state flux; EF, enhancement factor.

cantly increased the solubility of ketoprofen in aqueous vehicles, thus improving the diffusible form of the drug species at the skin–vehicle interface. Though the complex does not penetrate the skin, the drug in the complex is in rapid dynamic equilibrium with the drug in the aqueous phase, thus continuously supplying the drug molecules to the skin surface in a diffusible form. When in close proximity to a lipophilic membrane like skin, it is known that the lipophilic drugs in the cavity partition into the membrane for which they have a greater affinity. In other words, HP-β-CD acts as a carrier for transdermal delivery of ketoprofen.

The higher flux at pH 6.0 than at pH 3.0 on addition of HP-β-CD is a result of the increased solubility of the drug. It may be noted that both increased pH and cyclodextrin concentration decreased the permeability coefficient of ketoprofen (though the flux increased on account of higher solubility) as a result of greater affinity of the drug to the donor phase. At pH 6.0 however, HP-β-CD marginally increased the permeability coefficient. The reason for this discrepancy could not be established but it is possible that HP-β-CD affected the barrier properties of the stratum corneum by extracting the lipids [16] and easing the penetration of the anionic form of the drug into the more hydrophilic viable epidermal region, a rate limiting step for many lipophilic drugs [17]. It may be noted in this context that the enhancement factor is higher at pH 3.0 than at pH 6.0 for a similar cyclodextrin concentration. This can be attributed to an increase in the solubility of the drug by 31 times at pH 3.0 as against a factor of only two times at pH 6.0 on addition of 10% w/v HP-β-CD.

From the study, it is clear that complexation with HP-β-CD improves the transdermal flux of ketoprofen and greater flux can be achieved by optimizing the pH to achieve a better solubility of the drug.

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