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Effect of cyclodextrins on the complexation and transdermal delivery of bupranolol through rat skin

R.J. Babu [a,*], J.K. Pandit [b]

[a] College of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee, FL 32307, USA
[b] Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, India

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Abstract

Bupranolol (BPL) is a potent beta-blocking agent, the extensive first-pass metabolism (>90%) and rapid elimination half-life (1.5–2.0 h) of this drug make it well suited to be developed as a transdermal delivery system (TDS). Hydroxypropyl βCD (HPβCD) and partially methylated βCD (PMβCD) were used as penetration enhancers for BPL. The formation of inclusion complex of BPL with these cyclodextrins (CDs) was characterized in solution and solid states by phase solubility, X-ray diffractometry and differential scanning calorimetry (DSC) analyses. The effect of CDs on the permeation enhancement of BPL through rat skin was studied using side-by-side diffusion cells and pH 7.4 phosphate-buffered saline (PBS). CDs were employed at different concentrations with 0.4% (w/v) BPL as well as with excess quantity of BPL (1.0%, w/v) that CDs could not complex all the BPL and the drug was in the form of an aqueous suspension. The permeation of BPL from its aqueous suspension (0.4%, w/v) significantly increased when CDs were used at low concentrations (up to 2 and 5%, w/v concentration for HPβCD and PMβCD, respectively) ($P < 0.01$). At higher CD concentrations, the permeation of BPL decreased; and both CDs at 10% (w/w), showed similar flux values to that of control (no enhancer, $P > 0.05$). The permeation of BPL from its 1.0% (w/v) aqueous suspension increased with increase in concentration of CD up to 10% (w/v) for HPβCD and PMβCD. At 10% (w/v) concentration of HPβCD and PMβCD, the flux of BPL from its 1.0% aqueous suspension increased 3.8- and 4.6-fold ($P < 0.01$ and $P < 0.001$, respectively). The permeation data of skin pretreatment with CDs indicate that HPβCD had no effect on the skin, whereas PMβCD significantly reduced the skin barrier for BPL, as shown by 1.7-fold increase in the flux by PMβCD pretreatment ($P < 0.001$). Overall, both HPβCD and PMβCD were found to be suitable for improving the solubility and penetration enhancement of BPL.

Keywords: Bupranolol; Hydroxypropyl-beta-cyclodextrin; Methylated-beta-cyclodextrin; Transdermal; Permeation; Penetration enhancer

1. Introduction

Bupranolol (BPL) is a potent beta-blocking agent, without intrinsic sympathomimetic activity. Upon oral administration in humans BPL shows rapid absorption with a peak plasma concentration of 1.5–4 ng/ml within 1.2 h with a rapid elimination half-life of 1.5–2.0 h (Wellstein et al., 1986). It is also shown that BPL is subjected to extensive first-pass metabolism (>90%) after oral administration in both humans and animals (Waller et al., 1982; Wellstein et al., 1986). The physicochemical,

* Corresponding author. Tel.: +1-850-412-7006; fax: +1-850-599-3347.
E-mail address: jaychandrababu@yahoo.com (R.J. Babu).

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pharmacokinetic and pharmacological properties of this drug make it well suited for transdermal delivery system (TDS) development (Green et al., 1989). BPL could easily penetrate through rabbit skin in the presence of some enhancers to achieve effective plasma concentrations (Ogiso et al., 2001). Cyclodextrins (CDs) form inclusion complexes with many drugs by trapping the molecule or part of it into the hydrophobic cavity and have been extensively researched for their topical use as formulation additives and transdermal absorption promoters (Loftsson and Brewster, 1996; Stella and Rajewski, 1997; Loftsson and Masson, 2001). β-Cyclodextrin (βCD) is useful for complexation of average size molecules, such as most drugs. The promising advantages of βCD as drug carrier is limited by its low aqueous solubility (Uekama and Irie, 1990), therefore chemical modification of βCD was done, resulted with products of very high water solubility (>50 g/100 ml) and minimal toxicity. Furthermore, the inclusion ability of βCD is amply magnified due to chemical modification and presently about 50 CD derivatives are now commercially available (Uekama and Irie, 1990; Szente and Szejtli, 1999; Loftsson and Bodor, 1995). Modified CDs can act as penetration enhancers by solubilizing lipophilic drugs and constantly supplying the dissolved drug molecules to the skin surface where they partition into the skin barrier (Felton et al., 2002; Masson et al., 1999). CDs have been reported to increase in drug stability along with a potential for CD associated increase in transdermal permeability (Lopez et al., 2000; Uekama et al., 1992). Also, the drug induced primary irritation of skin is alleviated by CD complexation (Uekama et al., 1982). Optimum permeation enhancement is obtained when just enough CD is used to solubilize almost all the drug in the vehicle (Loftsson et al., 1994). In the present study, we investigated the formation of inclusion complex of BPL with two modified CDs [hydroxypropyl βCD (HPβCD) and partially methylated βCD (PMβCD)] in solution and solid states. Furthermore, we studied the permeation enhancement of BPL by modified CDs. CDs were employed at different concentrations with 0.4% (w/v) BPL as well as with excess quantity of BPL (1.0%, w/v) that CDs could not complex all the BPL and the drug was in the form of an aqueous suspension.

2. Materials and methods

2.1. Materials

Bupranolol hydrochloride and bupranolol base (R.S.) were provided by Schwarz Pharma AG (Manheim, Germany) as generous gift samples. Partially methylated β-cyclodextrin (PMβCD) and hydroxypropyl β-cyclodextrin (HPβCD) were gifts from Rue Ballu (Paris, France) and Roquette Corp. (Lille Cedex, France). All other chemicals were obtained from Qualigens Ltd. (Bombay, India).

2.2. Methods

2.2.1. Preparation of bupranolol base

Bupranolol HCl (4.0 g) was dissolved in 100 ml of distilled water by warming and 10 ml of dilute aqueous ammonia was added to it. The precipitated base was extracted with 3 × 50 ml fractions of chloroform and the combined chloroform solution was washed with 3 × 50 ml portions of distilled water. The chloroform phase was dehydrated with anhydrous sodium sulphate and evaporated to dryness at 37 °C. The resulted product was purified by recrystallization from cyclohexane. The purity of the base was confirmed by melting point (76 °C) and IR spectrum by comparing with the bupranolol RS (Schwartz Pharma, Germany).

2.2.2. Phase solubility studies

Phase solubility studies of bupranolol with cyclodextrin derivatives were carried out according to Higuchi and Connors (1965). Bupranolol in excess of its solubility was weighed into a series of screw-capped vials containing aqueous solutions of HPβCD or PMβCD of concentrations ranging from 0 to 0.10 M. The sealed vials were agitated on a rotary shaker for 48 h at room temperature (∼27 °C) and equilibrated for further 24 h. The clear supernatant was passed through 0.45 μm Millipore filter. The drug content of samples was determined spectrophotometrically at λ_{max} of 275 nm. The absorbance of the calibration curve was linear in the range 5–100 μg/ml with a slope of 0.0065 ($r^2 = 0.99989$).

2.2.3. Preparation and characterization of complexes

The inclusion complex of BPL with CDs was prepared by flash evaporation (Amdidouche et al., 1989;

Babu and Pandit, 1995). 0.5 g of BPL and 2.392 g of HPβCD (1.84×10^{-3} moles each of BPL and HPβCD) were dissolved in 50 ml of isopropanol to obtain a solution containing BPL and HPβCD in 1:1 molar ratio. Similarly, 0.5 g of BPL and 2.461 g of PMβCD (i.e. 1.84×10^{-3} moles each of BPL and PMβCD) were dissolved in 100 ml of isopropanol and a clear solution of BPL and PMβCD (1:1 molar ratio) was obtained. The solvent from the solution was rapidly removed on a rotary vacuum evaporator at 50 °C. The white powder obtained was dried at 40 °C for 24 h, after which the samples were stored in sealed glass containers at 25 °C for further investigations. The BPL content of the complex was determined by dissolving an accurately weighed quantity in phosphate buffered saline, pH 7.4 (PBS) followed by UV spectrophotometric assay. 5.80 mg of HPβCD complex or 5.92 mg of PMβCD complex is equivalent to 1 mg of BPL. The complex formation in the solid state was confirmed by X-ray diffractometry and differential scanning calorimetry analyses.

2.2.3.1. X-ray diffractometry. The X-ray diffraction patterns of the samples were recorded using Philips X-ray diffractometer (PW-1710) equipped with graphite monochromator, under the following operating conditions: Ni filtered Cu Kα radiation, 30 kV voltage, 20 mA current and scan speed 1° 2θ/min.

2.2.3.2. Differential scanning calorimetry. Differential scanning calorimetry of the samples was conducted on a Perkin-Elmer instrument equipped with a low temperature cell. The sample weight was 2 mg (approximately) and the heating rate was 10 °C/min.

2.2.4. Preparation of BPL–CD suspensions/solutions

The CDs and their concentrations employed in the study are given in Table 1. HPβCD, PMβCD were added to water at 2, 5 and 10% (w/v) concentrations to obtain clear aqueous solutions. To these solutions, BPL was added at 0.4 or 1.0% (w/v) concentration and the mixture was stirred for 12 h so as to obtain uniform suspensions. In case of HPβCD or PMβCD solutions of 10% (w/v) concentration, the entire drug at 0.4% (w/v) concentration was solubilized and a clear solution was obtained.

2.2.5. Preparation of skin samples for in vitro studies

Wistar rats of either sex (150–200 g, Zoological Animal Emporium, Varanasi) were used in the study. The rats were killed by an over dose of diethyl ether anesthesia. Hair on the abdominal area of the rats was removed by trimming with a clipper followed by shaving with an electrical shaver. Care was taken not to damage the stratum corneum. The hair-free abdominal skin was excised with a surgical blade and a pair of scissors and the adhering subcutaneous fat, tissue and capillaries were removed. The skin was cut into 3 cm × 3 cm samples for permeation studies.

2.2.6. In vitro skin permeation studies of BPL solution/suspension

Horizontal, side-by-side diffusion cells were used in studies with all solution/suspension formulations. The excised skin was mounted between the donor and receptor chambers with epidermal side facing the donor fluid. A thin film of silicone grease was spread on the lapped glass surfaces of the cell to provide a watertight seal. The diffusion cell was clamped and immersed in a water bath maintained at 37 ± 0.5 °C on the magnetic

Table 1
Cyclodextrin derivatives and their concentrations used in the skin permeation studies of bupranolol

CD concentration (% w/v) in water	Drug concentration (% w/v)	CD concentration (% w/v) in water	Drug concentration (% w/v)
HPβCD (2)[a]	0.4[b]	HPβCD (2)	10
HPβCD (5)	0.4	HPβCD (5)	10
HPβCD (10)[a]	0.4[b]	HPβCD (10)	10
PMβCD (2)[a]	0.4[b]	PMβCD (2)	10
PMβCD (5)	0.4	PMβCD (5)	10
PMβCD (10)[a]	0.4[b]	PMβCD (10)	10

[a] Pretreatment studies were done CDs at this concentration.
[b] Drug was used at 0.4% (w/w) concentration in the pretreatment studies.

stirrer. The volume of donor and receptor chamber was 5 ml and the effective surface area available for permeation of drug was 3.14 cm^2. The donor chamber was filled with drug solution/suspension, while PBS containing 10% (w/v) ethanol was taken in the receptor chamber. The contents of both donor in the receptor chambers were stirred at 600 rev/min using magnetic stirring bars. Samples (3 ml) were withdrawn from the receptor chamber at predetermined time intervals. The fluid remaining in the receptor cell was drained off at every sampling interval, rinsed thrice quickly and filled with the fresh, warmed (37 °C) buffer solution. Sink condition was maintained by inclusion of ethanol (10%, v/v) in the receptor fluid and also by replacing the fluid at every sampling interval. All experiments were carried out at least three times.

2.2.7. Pretreatment of rat skin and in vitro skin permeation studies

In order to study the effect of pretreatment of rat skin on the skin permeation of BPL, the CD solution was filled in the donor chamber and PBS containing 10% (w/v) ethanol was taken in the receptor chamber. CD concentrations for the skin pretreatment are shown in Table 1. The diffusion cell was maintained at 37 °C for 3 h in a water bath. At the end of 3 h, the enhancer solution and the receptor fluid were discarded. The donor chamber was thoroughly washed with water and then filled with 0.4% (w/v) BPL suspension. The receptor chamber was filled with fresh buffer and permeation study was performed as described in the previous section. The experiments were carried out in triplicate.

Analysis of BPL in the skin permeation samples was carried out by the method reported by LeBrun et al. (1989). The samples (3 ml) were collected into 30 ml capacity screw capped vials with Teflon lined caps. The pH of each sample was adjusted to 12.5 by adding 0.2 ml of 4N NaOH. To this 5 ml of dichloromethane was added; the BPL content of samples were extracted into dichloromethane by shaking for 30 min on a rotary shaker. The samples were centrifuged; dichloromethane layer was separated and dehydrated with anhydrous sodium sulphate. The UV absorption of dichloromethane layer was determined at λ_{max} of 285 nm. The concentration of samples was calculated from the slope of calibration curve. The absorbance was linear in the range 5–100 μg/ml with a slope of 0.00826 ($r^2 = 0.99987$).

2.2.8. Data analysis

The cumulative amount of BPL permeated through the skin (mg) was plotted as a function of time (h). The slope of the linear portion of the plot was calculated as the flux (μg/cm^2/h). The flux data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test to determine level of significance between various groups. The data were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Inclusion complexation in the liquid state

The phase solubility diagram of BPL as a function of CD concentration at room temperature is shown in Fig. 1. The solubility of BPL with increase in concentration of CDs indicates an A_L type of phase solubility diagram (Higuchi and Connors, 1965). An apparent 1:1 stability constant (K_s) of the complex was calculated from the slope (R) and intercept (S_o) of the phase solubility diagram according to the equation

$$K_s = \left(\frac{R}{S_o}\right)(1 - R)$$

The K_s of BPL–HPβCD complex and BPL–PMβCD complexes were calculated to be 294.24 and 1275.00 M^{-1}, respectively. The K_s values of BPL–HPβCD and BPL–PMβCD complexes make them suitable for practical applications (Pitha et al., 1983).

3.2. Inclusion complexation in the solid state

The inclusion complexes of BPL with CDs were prepared and characterized in the solid state. The existence of BPL–CD complex in the solid state was confirmed by X-ray diffractometry and differential scanning calorimetry. The X-ray diffraction patterns of powder samples made with HPβCD and PMβCD are shown in Figs. 2 and 3, respectively. The diffraction peaks of BPL indicate the crystalline nature of the drug, whereas the CD derivatives are amorphous as evidenced from the absence of diffraction peaks in Figs. 2 and 3. The characteristic diffraction peaks of BPL are completely absent in the inclusion complexes of BPL with HPβCD and PMβCD, whereas some of these peaks are evident in the physical mixtures of BPL

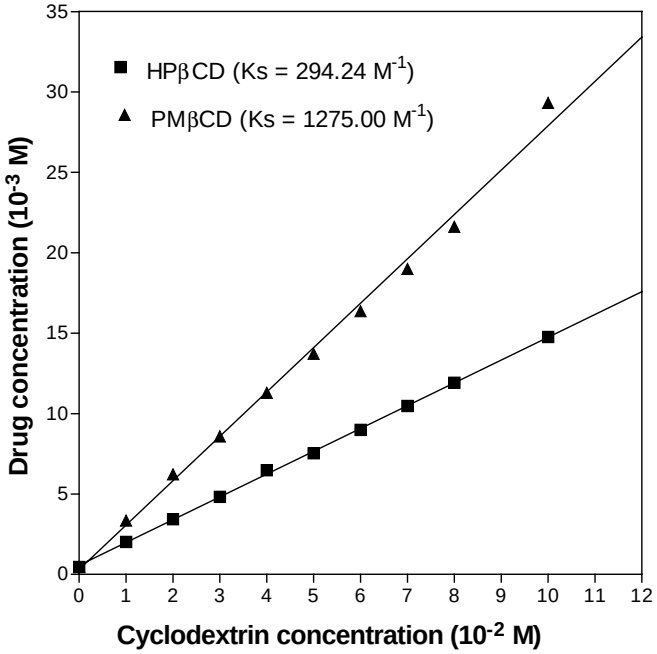

Fig. 1. Phase solubility diagram of bupranolol with HPβCD and PMβCD in water at room temperature (∼27 °C).

with respective CDs. This indicates that the complexes constitute a new solid state. There was an amorphous structure in both HPβCD and PMβCD complexes.

More direct evidence of complex formation was obtained from DSC thermograms shown in Figs. 4 and 5, respectively, for HPβCD and PMβCD complexes. BPL shows an endothermic peak corresponding to its melting point (∼77 °C). The physical mixtures of BPL with CDs also show the endothermic peak that is characteristic of BPL. This indicates that there was no interaction of BPL with HPβCD and PMβCD on simple mixing. In case of inclusion complexes the characteristic melting point peak of BPL has almost completely disappeared, showing the interaction of BPL with HPβCD and PMβCD. These results indicate that the inclusion complexes prepared by flash evaporation exist in the solid state.

3.3. Skin permeation studies

3.3.1. Studies with 0.4% BPL aqueous suspension

The effect of HPβCD and PMβCD on the permeation of bupranolol (as 0.4%, w/v aqueous suspension/solution) is shown in Figs. 6 and 7, respectively. The flux data is shown in the insets of respective figures. The skin permeation of BPL increased with HPβCD up to 5% (w/v) concentration and the permeation decreased when HPβCD increased to 10% (w/v). The flux at 5% (w/w) HPβCD concentration was significantly higher than control ($P < 0.01$), whereas the values at 2 and 10% (w/w) HPβCD concentrations were similar to control ($P > 0.05$) (Fig. 6). Similarly, the permeation of BPL increased with 2% (w/w) PMβCD and the permeation decreased with increase in PMβCD concentration up to 10% (w/w). The flux at 2% (w/w) PMβCD concentration was significantly higher than control ($P < 0.01$), whereas the values at 5 and 10% (w/w) PMβCD concentrations were similar to control ($P > 0.05$) (Fig. 7).

From the phase solubility diagram (Fig. 1) it is evident that the CDs are potent solubilizers of BPL. Addition of HPβCD to 0.4% BPL suspension dissolved a major fraction of the suspended drug and at 10% (w/v) HPβCD concentration; the entire suspended drug was solubilized. PMβCD, being a still more potent solubilizer, at 5% (w/v) concentration, completely solubilized the BPL in the suspension. At low CD concentration, when BPL was in sus-

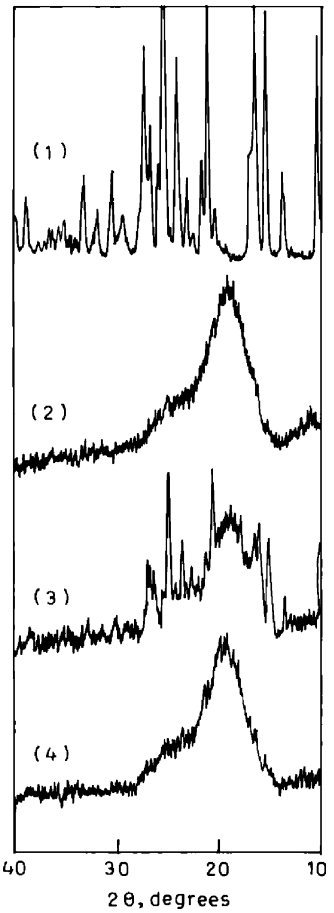

Fig. 2. X-ray diffraction pattern of bupranolol (1), HPβCD (2), physical mixture of bupranolol and HPβCD (3) and inclusion complex of bupranolol and HPβCD (4).

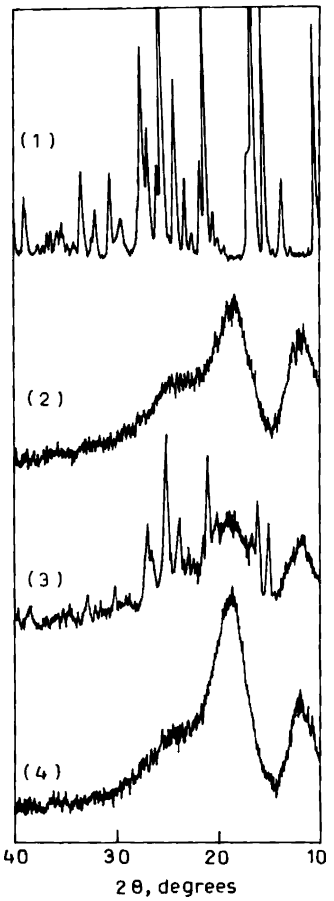

Fig. 3. X-ray diffraction pattern of bupranolol (1), PMβCD (2), physical mixture of bupranolol and PMβCD (3) and inclusion complex of bupranolol and PMβCD (4).

pension, the flux of BPL increased with increase in CD concentration, but when all BPL was in solution, the flux decreased with increase in CD concentration. When BPL is in suspension, the CDs provide high free drug concentration by rapidly liberating the drug molecules from the CD-complex, leading to larger flux. On the other hand when all the BPL is in solution, increasing the amount of CD results in decrease in the free BPL molecules in solution due to high affinity of BPL to CD molecules in solution. As a result, the complexed BPL permeates at a much slower rate than the free BPL molecules in solution. In this connection it is pertinent to mention that the flux of hydrocortisone–HPβCD complex through a synthetic membrane was found to be 10–15 times slower than free hydrocortisone molecules (Loftsson et al., 1991). CDs keep the drug molecules in solution and deliver them to the surface of the barrier where they partition into and through the barrier (Loftsson et al., 1994). Excess CD in solution reduces the flux by suppressing the BPL–CD complex from dissociation into free BPL and CD by shifting the equilibrium towards association rather than dissociation of the complex.

3.3.2. Studies with 1.0% BPL aqueous suspension

In these studies, the BPL content of suspensions was increased from 0.4% (w/v) to 1.0% (w/v) so that there was sufficient excess drug in the suspension to maintain maximum thermodynamic activity.

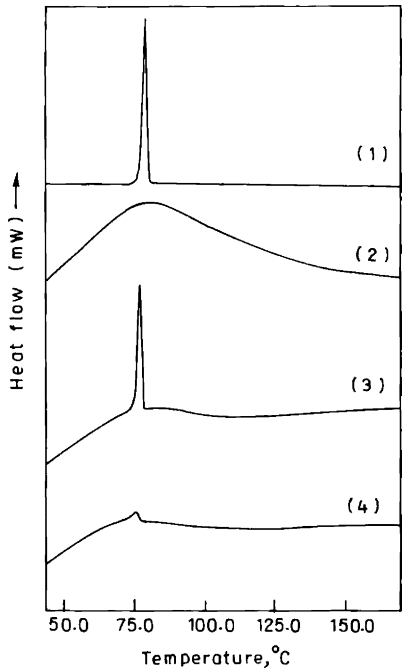

Fig. 4. Differential scanning calorimetric thermograms of bupranolol (1), HPβCD (2), physical mixture of bupranolol and HPβCD (3) and inclusion complex of bupranolol and HPβCD (4).

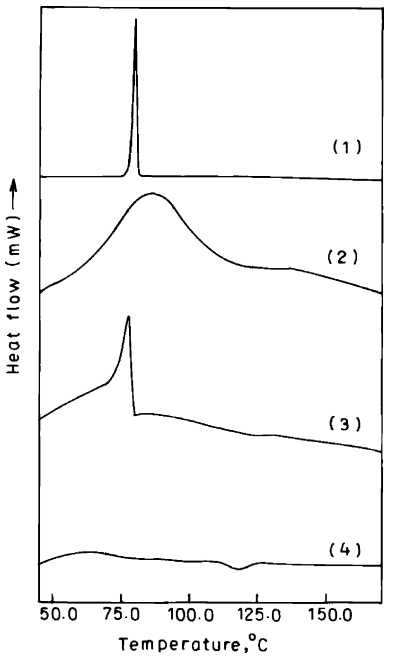

Fig. 5. Differential scanning calorimetric thermograms of bupranolol (1), PMβCD (2), physical mixture of bupranolol and PMβCD (3) and inclusion complex of bupranolol and PMβCD (4).

The effect of HPβCD and PMβCD on the permeation of bupranolol (as 1.0%, w/v aqueous suspension) is shown in Figs. 8 and 9, respectively. The flux data is shown in the insets of respective figures. The skin permeation of BPL increased with HPβCD and PMβCD concentration up to 10% (w/v). While the flux values of BPL did not rise to statistically significant levels at 2% (w/v) HPβCD concentration, the values increased significantly at 5 and 10% (w/v) HPβCD concentration ($P < 0.05$ and $P < 0.01$, respectively). The flux of BPL with PMβCD showed significant differences at 2% ($P < 0.01$), 5 and 10% PMβCD ($P < 0.001$) as compared with control (Fig. 9). Overall HPβCD and PMβCD increased the permeation of BPL by 3.8- and 4.6-fold, respectively.

HPβCD and methylated βCDs are known to interact with stratum corneum components of rat skin and improve drug absorption (Larrucea et al., 2002; Felton et al., 2002; Lopez et al., 2000; Bentley et al., 1997). Contrary to these reports, Shaker et al., 2003 showed that HPβCD did not alter the barrier properties of hairless mouse stratum corneum to any signifi-

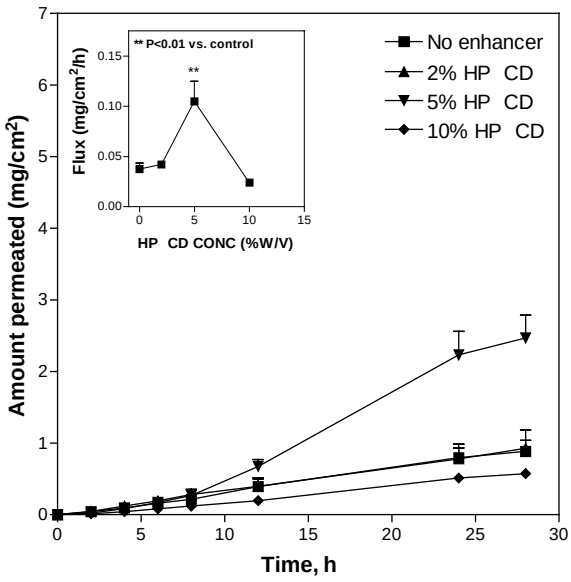

Fig. 6. Effect of HPβCD on the permeation of bupranolol (as 0.4%, w/v aqueous suspension/solution) through excised rat skin.

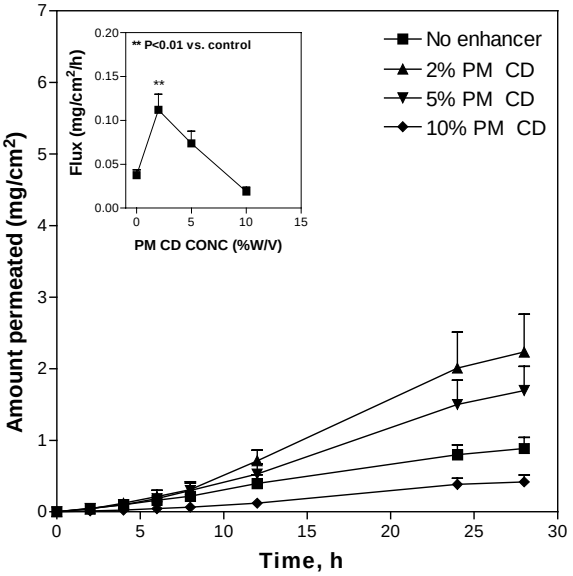

Fig. 7. Effect of PMβCD on the permeation of bupranolol (as 0.4%, w/v aqueous suspension/solution) through excised rat skin.

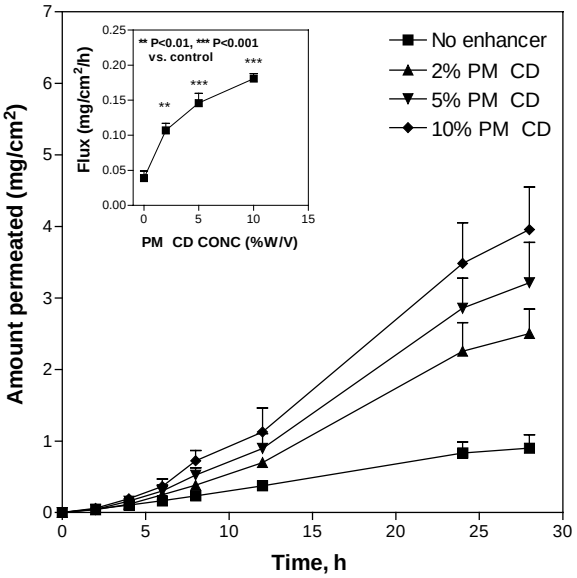

Fig. 9. Effect of PMβCD on the permeation of bupranolol (as 1.0%, w/v aqueous suspension/solution) through excised rat skin.

cant extent nor did it enhance corticosterone transport in any other manner such as by a carrier mechanism involving the aqueous boundary layer or by a carrier mechanism within the stratum corneum. βCD and HPβCD themselves were not penetration enhancers for 5-fluorouracil or estradiol in human skin as they did not enhance the flux of these compounds (Williams et al., 1998). Methylated βCDs are reported to extract all the major lipid classes and proteins and reduce barrier function of skin, whereas HPβCD had limited specificity towards stratum corneum lipid structure (Vollmer et al., 1993, 1994; Legendre et al., 1995). In the present study, PMβCD was found to be a more potent enhancer than HPβCD. This may be due to the combined effect of increased aqueous solubility of BPL and reduced barrier function of skin by PMβCD.

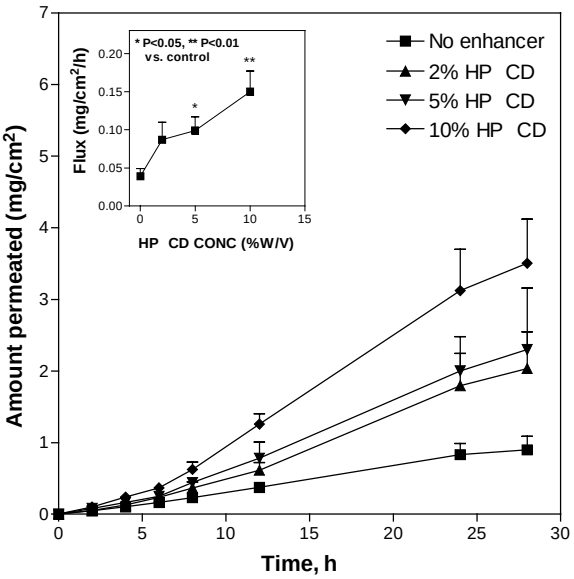

Fig. 8. Effect of HPβCD on the permeation of bupranolol (as 1.0%, w/v aqueous suspension/solution) through excised rat skin.

The results are further supported by pretreatment studies. Figs. 10 and 11 show the effect of pretreatment of rat skin with CDs on permeation of BPL. While pretreatment with HPβCD at 2 and 10% concentration did not increase the flux ($P > 0.05$), PMβCD significantly increased the flux at both 2 and 10% concentrations ($P < 0.001$). Thus, HPβCD pretreatment did not affect the barrier function of rat skin, whereas PMβCD pretreatment (10%, w/v concentration) reduced the barrier and the permeation of BPL increased by 1.7-fold. These results are in agreement with the findings of Vollmer et al. (1994) who observed that pretreatment of rat skin with DMβCD (20%; 4 h) produced 10-fold increase in the flux of

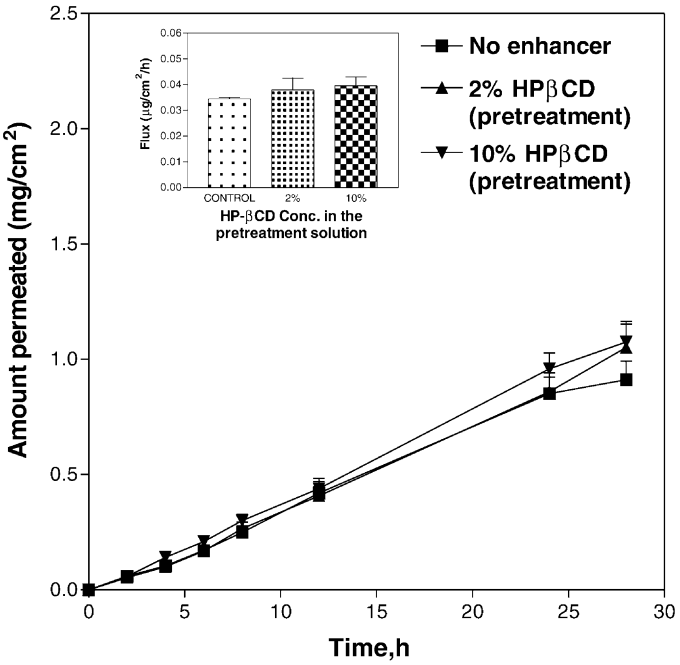

Fig. 10. Effect of pretreatment of rat skin with HPβCD on the in vitro permeation of bupranolol.

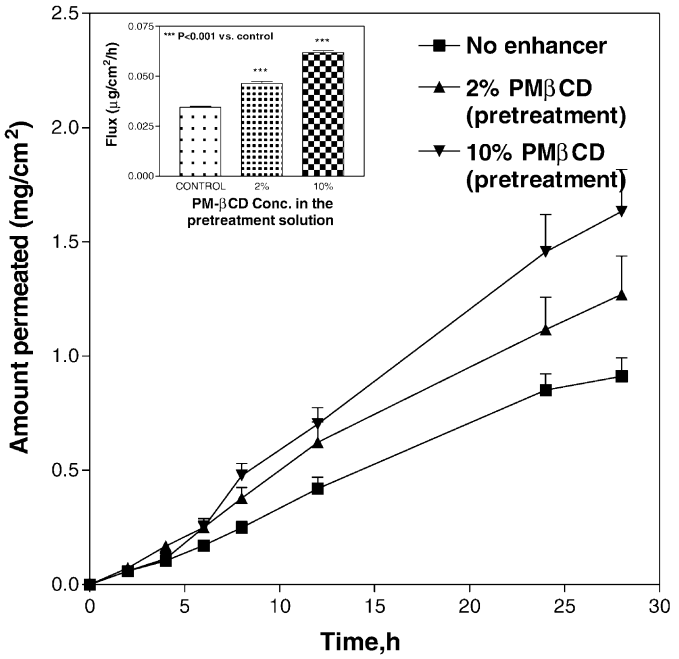

Fig. 11. Effect of pretreatment of rat skin with PMβCD on the in vitro permeation of bupranolol.

liarozole. Thus, both HPβCD and PMβCD acted as penetration enhancers by increasing the solubility of BPL and PMβCD also reduced the skin barrier function, probably by interacting with stratum corneum lipids.

3.4. Conclusion

The inclusion complex formation of BPL with HPβCD and PMβCD was characterized in solution and solid state and the stability constant (K_S) of BPL–HPβCD and BPL–PMβCD complexes was calculated to be 294.24 and 1275.00 M^{-1}, respectively. Overall, both HPβCD and PMβCD were found to be suitable for improving the solubility and penetration enhancement of BPL. The permeation data of skin pretreatment with CDs indicate that HPβCD had no effect on the skin, whereas PMβCD significantly reduced the skin barrier for BPL, as shown by 1.7-fold increase in the flux by PMβCD (10%, w/v concentration) pretreatment.

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References

Amdidouche, D., Darrouzet, H., Duchene, D., Poelman, M.C., 1989. Inclusion of retinoic acid in β-cyclodextrin. Int. J. Pharm. 54, 175–179.

Babu, R.J., Pandit, J.K., 1995. Enhancement of dissolution rate and hypoglycemic activity of glibenclamide with β-cyclodextrin. S.T.P. Pharma. Sci. 5, 196–201.

Bentley, M.V., Vianna, R.F., Wilson, S., Collett, J.H., 1997. Characterization of the influence of some cyclodextrins on the stratum corneum from the hairless mouse. J. Pharm. Pharmacol. 49, 397–402.

Felton, L.A., Wiley, C.J., Godwin, D.A., 2002. Influence of hydroxypropyl-beta-cyclodextrin on the transdermal permeation and skin accumulation of oxybenzone. Drug Dev. Ind. Pharm. 28, 1117–1124.

Green, P.G., Hadgraft, J., Wolff, M., 1989. Physicochemical aspects of the transdermal delivery of bupranolol. Int. J. Pharm. 55, 265–269.

Higuchi, T., Connors, K.A., 1965. Phase solubility techniques. In: Advances in Analytical Chemistry and Instrumentation. Interscience, New York, pp. 117–212.

Larrucea, E., Arellano, A., Santoyo, S., Ygartua, P., 2002. Study of the complexation behavior of tenoxicam with cyclodextrins in solution: improved solubility and percutaneous permeability. Drug Dev. Ind. Pharm. 28, 245–252.

LeBrun, P.P.H., Fox, P.L.A., de Vries, M.E., Bodde, H.E., 1989. In vitro penetration of some beta adrenoreceptor blocking drugs through porcine buccal mucosa. Int. J. Pharm. 49, 141–145.

Legendre, J.Y., Rault, I., Petit, A., Luijten, W., Demuynck, I., Horvath, S., Ginot, Y.M., Cuine, A., 1995. Effects of β-cyclodextrins on skin: implications for the transdermal delivery of piribedel and a novel cognition enhancing drug S-9977. Eur. J. Pharm. Sci. 3, 311–322.

Loftsson, T., Bodor, N., 1995. Effect of cyclodextrins on percutaneous transport of drugs. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, Florida, pp. 335–342.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Frioriksdottir, H., Ingvarsdottir, G., Jonsdottir, B., Siguroardottir, A.M., 1994. The influence of 2-hydroxypropyl-β-cyclodextrin on diffusion rates and transdermal delivery of hydrocortisone. Drug Dev. Ind. Pharm. 20, 1699–1708.

Loftsson, T., Masson, M., 2001. Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. 225, 15–30.

Loftsson, T., Olafsdottir, B.J., Bodor, N., 1991. The effect of cyclodextrins on transdermal delivery of drugs. Eur. J. Pharm. Biopharm. 37, 30–33.

Lopez, R.F., Collett, J.H., Bentley, M.V., 2000. Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone. Int. J. Pharm. 200, 127–132.

Masson, M., Loftsson, T., Masson, G., Stefansson, E., 1999. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. J. Controlled Release 59, 107–108.

Ogiso, T., Hata, T., Iwaki, M., Tanino, T., 2001. Transdermal absorption of bupranolol in rabbit skin in vitro and in vivo. Biol. Pharm. Bull. 24, 588–591.

Pitha, J., Szente, I. Szejtli, J., 1983. Molecular encapsulation of drugs by cyclodextrins and congeeners. In: Bruck, S.D. (Ed.), Controlled Drug Delivery. CRC Press, Boca Raton, FL, pp. 126–148.

Shaker, D.S., Ghanem, A.H., Li, S.K., Warner, K.S., Hashem, F.M., Higuchi, W.I., 2003. Mechanistic studies of the effect of hydroxypropyl-beta-cyclodextrin on in vitro transdermal permeation of corticosterone through hairless mouse skin. Int. J. Pharm. 253, 1–11.

Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.

Szente, L., Szejtli, J., 1999. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv. Drug Deliv. Rev. 36, 17–28.

Uekama, K., Adachi, H., Irie, T., Yano, T., Saita, M., Noda, K., 1992. Improved transdermal delivery of prostaglandin E1 through hairless mouse skin: combined use of carboxymethyl-ethyl-beta-cyclodextrin and penetration enhancers. J. Pharm. Pharmacol. 44, 119–121.

Uekama, K., Irie, T., 1990. New perspectives in cyclodextrin pharmaceutical applications: cyclodextrin derivatives as new drug carriers. Drug. Invest. 2, 22–28.

Uekama, K., Irie, T., Sunada, M., Otagiri, M., Arimato, Y., Nomura, S., 1982. Alleviation of prochlorperazine induced primary irritation of skin by cyclodextrin complexation. Chem. Pharm. Bull. 30, 3860–3862.

Vollmer, U., Muller, B.W., Mesens, J., Wilffert, B., Peters, T., 1993. In vivo skin permeation kinetics of liarozole: percutaneous absorption studies with different formulations of cyclodextrin derivatives in rats. Int. J. Pharm. 99, 51–58.

Vollmer, U., Muller, B.W., Peeters, J., Mesens, J., Willfert, B., Peters, T., 1994. A study of the percutaneous absorption enhancing effects of cyclodextrin derivatives in rats. J. Pharm. Pharmacol. 46, 19–22.

Waller, A.R., Chasseaud, L.F., Bonn, R., Taylor, T., Darragh, A., Girkin, R., Down, W.H., Doyle, E., 1982. Metabolic fate of the beta-blocker 14C-bupranolol in humans, dogs, and rhesus monkeys. Drug Metab. Dispos. 10, 51–54.

Wellstein, A., Kuppers, H., Pitschner, H.F., Palm, D., 1986. Transdermal delivery of bupranolol: pharmacodynamics and beta-adrenoceptor occupancy. Eur. J. Clin. Pharmacol. 31, 419–422.

Williams, A.C., Shatri, S.R., Barry, B.W., 1998. Transdermal permeation modulation by cyclodextrins: a mechanistic study. Pharm. Dev. Technol. 3, 283–296.