

## Delivery of Metoprolol by Esterification: A Comparison of Passive Permeation and Iontophoresis

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### Abstract

The present work is an attempt to assess the permeability potential of metoprolol by combining the prodrug approach and iontophoresis. Two ester prodrugs of metoprolol were synthesized (metoprolol acetate and propionate), characterized and studied for their physicochemical properties and stability. *In vitro* permeation studies were carried out by anodal iontophoresis (0.5 mA/cm<sup>2</sup>) through porcine skin using a Franz diffusion cell at different donor concentrations (5, 10, and 20mM). Evaluation of the physicochemical parameters showed significant increase in lipophilicity and a slight reduction in pKa values for prodrugs. The esters were found to be more stable in acetate buffer (pH 4) than phosphate buffer (pH 6). Passive permeation of prodrugs and iontophoresis of pure drug showed higher permeation at all concentrations. In nut shell, both prodrugs approach and iontophoresis enhanced the flux values independently but the combination contributed minimally.

**Keywords:** Transdermal, prodrugs, metoprolol, in vitro, stability, partition coefficient

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## Introduction

The topical delivery of drugs via the skin has been in existence for a long time. But at present the skin pathways have been studied extensively for the systemic delivery of drug (Banga *et al.*, 1999; Barry, 2001; Cross and Roberts, 2004). Transdermal delivery offers significant potential for the non-invasive administration of therapeutic agents (Kalia *et al.*, 2004). The route is thought to be particularly beneficial for drugs that undergo hepatic first pass metabolism as well as liable to chemical degradation in the gastrointestinal tract. Unfortunately very few drugs can compromise the formidable barrier of stratum corneum, which possess a multilamellar lipidic structure punctuated by proteinaceous corneocytes (Potts and Francoeur, 1991). Consequently, several enhancement techniques utilizing alternative forms of energy to facilitate permeation of drugs have been attempted and evaluated.

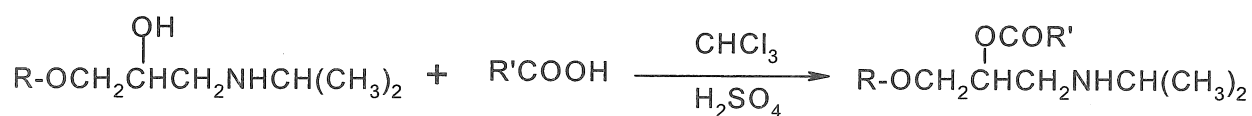
Constant current iontophoresis that uses a small electrical potential to actively propel the drug with in the skin is considered to be a promising option (Wang *et al.*, 2005). The iontophoresis delivery is proportional to the intensity and duration of electric current and is programmable in nature (Guy, 1998). Similarly, esterification represents an alternative and promising method of enhancing skin permeability of drugs by increasing lipophilicity (Buur *et al.*, 1988; Ahmed *et al.*, 1995; Doh *et al.*, 2003; Anroop *et al.*, 2005). Dermal delivery of anti-hypertensive agents has become an important research interest in recent past. Clonidine has shown promising results and a number of other drugs have been actively investigated (Groning and Kuhland, 1999; Gordon and Peterson, 2003). Work is also going on metoprolol, which is a versatile drug used in the treatment of angina pectoris, myocardial infarction and congestive heart failure as well as hypertension (Thysman *et al.*, 1992; Ghosh *et al.* 1995; Ganga *et al.*, 1996). In the present study, the potential of the combination of prodrugs and iontophoresis was evaluated for the transdermal delivery of metoprolol.

## Materials and Methods

*Materials:* Metoprolol tartrate was received as gratis sample from Astra Zeneca Pharma India Ltd., (Bangalore, India) and was converted to free base. Acetic acid (Rankem, New Delhi, India), propionic acid (Acros Organics, New Jersey, USA) and octanol (Merck-Schuchardt, Germany) were procured commercially. Acetonitrile, HPLC grade was procured from Merck, Mumbai, India. All other chemicals and reagents used were of analytical grade.

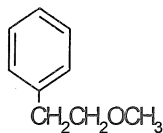
*Synthesis of Prodrugs:* The synthesis was carried out in the following way. Metoprolol (1mM) dissolved in chloroform (100 ml) was refluxed for 12 h with acetic acid or propionic acid (2 mM), and a few drops of conc. sulphuric acid under anhydrous conditions. The reaction was monitored by TLC using solvent system chloroform- methanol (95: 05) and visualized using anisaldehyde (Luch, 1983). After completion of the reaction chloroform was removed under vacuum. To the residue thus obtained was added water, then extracted with chloroform and washed with 1% sodium carbonate repeatedly. The chloroform layer was dried over anhydrous sodium sulphate and concentrated to obtain the respective prodrugs.

*Schematic representation:*

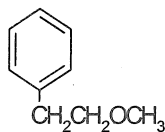


(I)

R



(a)



(b)

(II)

R'

CH<sub>3</sub>-

CH<sub>3</sub> CH<sub>2</sub>-

(III)

*IR and NMR Study:* IR spectra were recorded using Jasco FT/IR -5300 in nujol.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) spectra were recorded on Bruker (200 MHz) using TMS as internal standard.

*Metoprolol acetate (III a):* Gummy solid (71%) IR  $\nu_{\text{max}}$ : nujol: 3340(-NH), 2940 (=CH), 1735 (COOR), and  $1235\text{cm}^{-1}$  (aryl ether).  $^1\text{H-NMR}$   $\text{CDCl}_3$   $\delta$ : 1.33 (*d*,  $j = 2.2$  Hz, 6H, -NHCH ( $\text{CH}_3$ )<sub>2</sub>), 2.80 (*t*,  $j = 2.4$  Hz, 2H, Ar - $\text{CH}_2$  CH<sub>2</sub> O CH<sub>3</sub>), 3.13-3.3 (m, 3H, -NHCH ( $\text{CH}_3$ )<sub>2</sub>), 3.32 (br s, 3H, Ar -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.53 (*t*,  $j = 3.4$  Hz, 2H, Ar -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.95 (br s, 2H, Ar -OCH<sub>2</sub>CHOH CH<sub>2</sub>NHCH ( $\text{CH}_3$ )<sub>2</sub>), 4.40 (m, 1H, Ar -OCH<sub>2</sub>CH<sub>2</sub>OH), 6.77 (*d*,  $j = 8.75$  Hz, 2H, ArH), 7.07 (*d*,  $j = 8.70$  Hz, 2H, ArH), 7.25 (br s, 1H, Ar -OCH<sub>2</sub>CHOHCH<sub>2</sub>-NHCH( $\text{CH}_3$ )<sub>2</sub>) and 2.01 (s, 3H, Ar -OCH<sub>2</sub>CHOCOCH<sub>3</sub>CH<sub>2</sub>-NHCH( $\text{CH}_3$ )<sub>2</sub>).

*Metoprolol propionate (III b):* Gummy solid (67%) IR  $\nu_{\text{max}}$ : nujol: 3340(-NH), 2940 (=CH), 1735 (COOR), and  $1235\text{cm}^{-1}$  (aryl ether).  $^1\text{H-NMR}$   $\text{CDCl}_3$   $\delta$ : 1.33 (*d*,  $j = 2.2$  Hz, 6H, -NHCH ( $\text{CH}_3$ )<sub>2</sub>), 2.80 (*t*,  $j = 2.4$  Hz, 2H, Ar - $\text{CH}_2$  CH<sub>2</sub> O CH<sub>3</sub>), 3.13-3.3 (m, 3H, -NHCH ( $\text{CH}_3$ )<sub>2</sub>), 3.32 (br s, 3H, Ar -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.53 (*t*,  $j = 3.4$  Hz, 2H, Ar -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.95 (br s, 2H, Ar -OCH<sub>2</sub>CHOH CH<sub>2</sub>NHCH ( $\text{CH}_3$ )<sub>2</sub>), 4.40 (m, 1H, Ar -OCH<sub>2</sub>CH<sub>2</sub>OH), 6.77 (*d*,  $j = 8.75$  Hz, 2H, ArH), 7.07 (*d*,  $j = 8.70$  Hz, 2H, ArH), 7.25 (br s, 1H, Ar -OCH<sub>2</sub>CHOHCH<sub>2</sub>-NHCH( $\text{CH}_3$ )<sub>2</sub>), 1.46 (*t*,  $j = 2.4$  Hz, 3H, Ar-OCH<sub>2</sub>CH(OCOCH<sub>2</sub> CH<sub>3</sub>) CH<sub>2</sub>-NHCH( $\text{CH}_3$ )<sub>2</sub>) and 2.13 (s, 2H, Ar-OCH<sub>2</sub>CH(OCOCH<sub>2</sub> CH<sub>3</sub>) CH<sub>2</sub>-NHCH( $\text{CH}_3$ )<sub>2</sub>).

*pKa Measurement:* pKa values were determined experimentally by conductometry. Various molar concentrations of metoprolol and prodrugs were prepared in deionised water. Then the conductivity of the prepared solutions were measured by using a conductivity meter (Elico CM 180 conductivity meter) at room temperature. The pKa was calculated as  $-\log$  of acid dissociation constant (Ka) (Silcocks, 1980).

*Stability studies:* The hydrolysis of the synthesized ester prodrugs was studied in aqueous acetate (pH 4) and phosphate buffers (pH 6). The total buffer concentration was 0.01 M and a constant ionic strength ( $\mu$ ) of 0.5 was maintained for both buffers by adding a calculated amount of potassium chloride. The rates of hydrolysis were

determined by using a reverse phase HPLC procedure capable of separating the prodrugs from metoprolol. The HPLC system consisted of a solvent delivery pump (LC 10 AT, Shimadzu, Japan). The column used was Phenomenex C<sub>18</sub> Column (5 $\mu$ m, 4.6x250 mm, Luna). The mobile phase constituted of acetonitrile: 100 mM pH 7.0 phosphate buffer (20:80) at a rate of 1.0 ml/min. The column effluent was monitored at 222 nm (Kaliszan *et al.*, 1995). The ester prodrugs had greater retention times in the column than the parent drug. The retention times were 4.11, 4.79 and 5.09 min for metoprolol, metoprolol acetate and propionate respectively.

Quantitation of the ester as well as free drug formed upon hydrolysis was done by measuring the peak height in relation with those of standard chromatograph under same conditions. The hydrolysis studies were carried out for 6 h (Huang *et al.*, 2005), samples were taken in regular intervals and immediately chromatogrammed.

*Solubility measurements:* A normal equilibrium solubility determination was carried out by the method of Okumara et al. (1989). An excess amount of drug or prodrug was added and dissolved in a measured amount of 0.01M acetate buffer (pH = 4) in a glass vial to get a saturated solution. The system was stirred for 24 h at 25°C and kept at rest for 1 h to assist the attainment of equilibrium. The solution was then filtered through a membrane filter (pore size 0.22 $\mu$ m) and after dilution, the solubility of each drug and prodrugs was determined spectrophotometrically (Shimadzu, Japan) at 274 nm wavelength.

*Measurement of partition coefficient:* The partition coefficients of drug and prodrugs were determined in mutually saturated (at 25°C for 24 h) 1- octanol - pH 4 acetate buffer ( $\mu = 0.5$ ) system. For drug the standard solution was prepared in acetate buffer whereas for prodrugs it was prepared in octanol. The partitioning was done by stirring equal volumes of both phases for 24 h to ensure the attainment of equilibrium. In both cases, the aqueous solution was suitably diluted and determined spectrophotometrically for drug content. The partition coefficient was determined from the equation.

$$K_{o/w} = C_o/C_w$$

Where  $C_o$  is the concentration of drug or prodrugs in the octanol and  $C_w$  is the concentration of drug or prodrugs in pure water.

*Skin membrane preparation:* From a local abattoir, an ear was obtained from a freshly slaughtered pig. The skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. After separating the full thick skin, the fat adhering to the dermis was removed using a scalpel and isopropyl alcohol. Finally, skin was washed in tap water and stored at  $-20^{\circ}$  C in aluminum foil and was used within a week (Pillai and Panchagnula, 2003).

*Preparation of electrodes:* The silver chloride electrodes were prepared by immersing silver plate ( $1\text{cm}^2$  area) in 0.1M HCl solution and applying a current of  $0.4\text{ mA/cm}^2$  for 24 h (Banga, 1998).

*In vitro permeation studies:* The *in vitro* permeation studies were performed using a Franz diffusion cell (Neutron scientific, Kolkata, India). The excised skin was mounted between the half-cells within the dermis in contact with the receptor fluid, 0.9% NaCl and was equilibrated for 1 h. The receiving chamber had a volume of 50 ml and the area available for diffusion was about  $1.74\text{ cm}^2$ . The top of the donor cell was covered with an aluminium foil to prevent the evaporation of vehicle. The drug concentration was maintained to be uniform in matching experiments by loading equivalent amounts (2ml, 5 mM, 10 mM and 20 mM) for metoprolol and its prodrugs. Metoprolol and the prodrugs were delivered from a vehicle containing 20% methanol in 0.01M acetate buffer (pH 4).

Anodal iontophoresis was carried out by using a single channel constant power supply unit (Ultra pure Scientific, Mumbai, India). A constant current of  $0.5\text{ mA/cm}^2$  was applied with a silver- silver chloride electrode by placing the anode in the donor compartment and the cathode in the receiver. The iontophoresis was performed for a period of 6 h (Marro *et al.*, 2001). Temperature was maintained at  $37\pm 0.5^{\circ}$  C. The receiver fluid (10ml) was withdrawn at regular intervals and replaced with fresh  $37^{\circ}$  C normal saline to maintain the sink condition and assayed after suitable dilution

using the standard curve at 274 nm (sensitivity 0.1mcg). All experiments were done in triplicate and the values were expressed as mean  $\pm$  S.E.

*Data analysis:* Cumulative amount of drugs permeated per unit skin surface was plotted against time and the slope of the linear portion of the plot was estimated as the steady-state flux ( $J_{ss}$ ). Permeability coefficient ( $K_p$ ) was calculated by using the eq.  $K_p = J_{ss}/C_v$ ; where  $C_v$  was the total donor concentration of the solute. Statistical analysis was performed by one-way analysis of variants (ANOVA) to test the effects of various treatments.

## Results and Discussion

*Stability Studies:* The stability of the new derivatives was evaluated at pH 4 and pH 6 (Fig.1 and 2). It is clear that both esters showed higher stability at pH 4 than at pH 6. The first order plot of the hydrolysis profile is more or less linear in both cases, but at pH 4.0 the slope is less steep after 3 h indicating a stabilizing effect. At the end of 6 h, the amounts degraded were 2.18 and 1.86% for metoprolol acetate and propionate respectively. Hence physicochemical studies were carried out at this pH. Of the two esters, metoprolol propionate was found to have better stability. However, the prodrugs were found to be broken down during the permeation process, and minimal amount was detected in the physiological saline. There is already a report that the prodrug moiety derived from the combination of alcoholic functional group of the parent drug with a carboxylic acid group readily hydrolyses to its components in vivo by esterase (Sloan, 1992).

*Physicochemical Properties:* Physicochemical properties of the substances particularly, MW, solubility, pKa and lipophilicity exert marked influences on the rate and extent of their skin permeation (Singh *et al.*, 1999) and the values obtained are listed in Table 1. Esterification reduced the solubility significantly ( $P < 0.001$ ) and also enhanced the partition coefficient ( $P < 0.001$ ) as desired. The pKa values of prodrugs decreased towards neutrality, too.

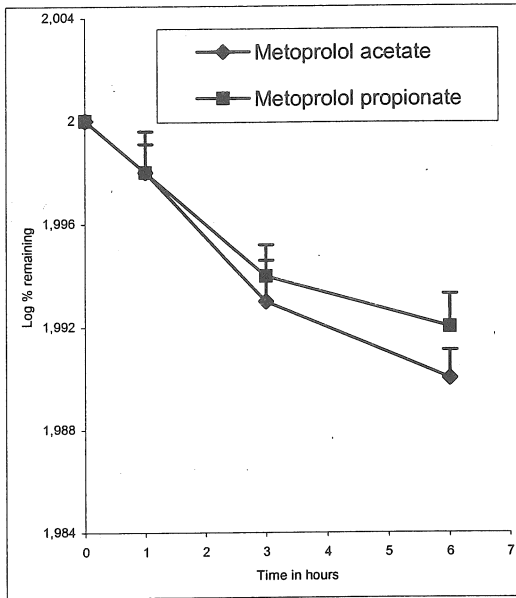


Fig. 1. Log percentage remaining versus time profiles of metoprolol prodrugs in acetate buffer solution of pH 4.0 at 25°C. Each data represented the mean  $\pm$  SE of three experiments.

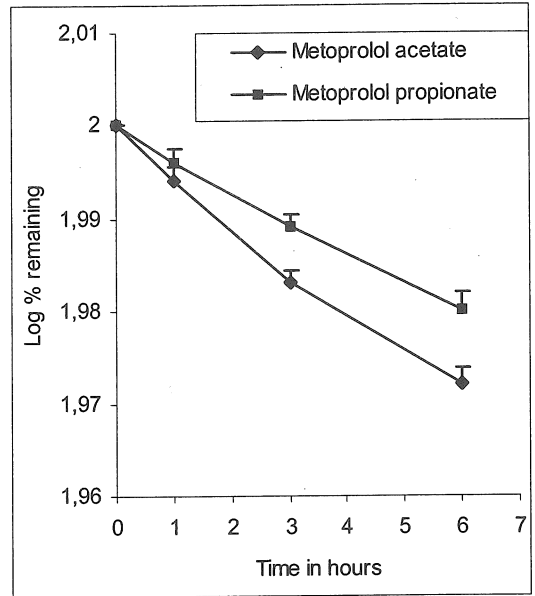


Fig. 2. Log percentage remaining versus time profiles of metoprolol esters in phosphate buffer of 6.0 at 25°C. Each data represented the mean  $\pm$  SE of three experiments.

Table 1. Physicochemical parameters\* of metoprolol and prodrugs

Drug/ prodrugs	Molecular weight	Solubility (mg/ml)		pKa	P <sup>c</sup>
		Buffer <sup>a</sup>	Receptor <sup>b</sup>		
Metoprolol	267.36	39.13 $\pm$ 0.42	23.64 $\pm$ 0.85	9.14 $\pm$ 0.03	0.76 $\pm$ 0.01
Metoprolol acetate	309.40	2.56 $\pm$ 0.05	2.25 $\pm$ 0.05	8.95 $\pm$ 0.02	2.40 $\pm$ 0.01
Metoprolol propionate	323.42	1.90 $\pm$ 0.03	1.72 $\pm$ 0.04	8.90 $\pm$ 0.01	3.85 $\pm$ 0.05

\*Each value is the mean  $\pm$  SE (n = 3) <sup>a</sup>Solubility in pH 4 acetate buffer, <sup>b</sup>Solubility in normal saline  
<sup>c</sup>Partition coefficient between octanol and acetate buffer (pH 4) at 25°C

*Permeation studies:* To compare the benefit of passive permeation and iontophoresis, experiments were carried out using equimolar concentrations of the drug and prodrug



moieties. Since prodrugs expressed much lower solubility in buffer than metoprolol, the donor vehicle contained with 20% methanol. The vehicle of this composition effectively dissolved the prodrugs (218 mg/ml for metoprolol propionate), achieving a low saturation level to ensure approximately equal activity of drug and prodrugs in the donor for the matching experiments. Fig. 3 - 5 shows the permeation profile of metoprolol and its two prodrugs at various donor concentrations. Here the beneficial effect of esterification is clearly visible for the passive permeation process. The cumulative permeation obtained with the metoprolol propionate was higher than that with metoprolol acetate and metoprolol at all concentration levels (5, 10 and 20 mM).

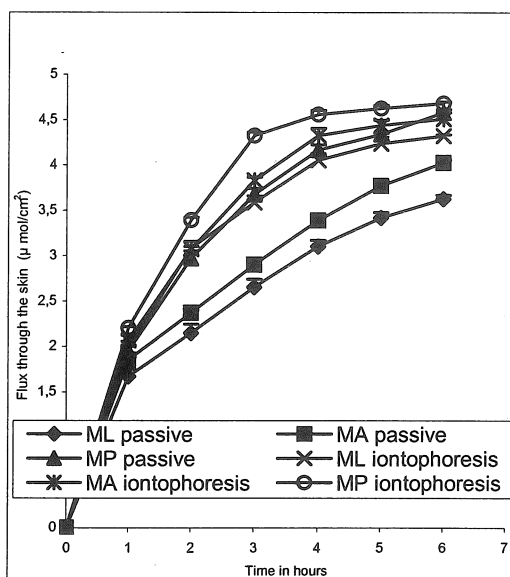


Fig. 3. Comparison of permeation profiles of metoprolol (ML), metoprolol acetate (MA) and metoprolol propionate (MP) with or without iontophoresis at 5 mM concentration. Each data represented the mean  $\pm$  SE of three experiments.

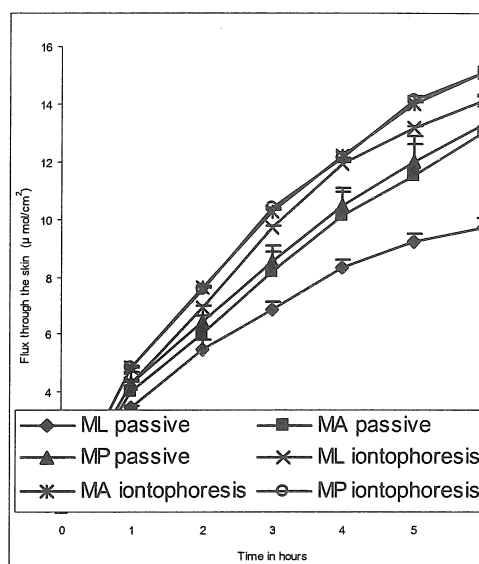


Fig. 4. Comparison of permeation profiles of metoprolol (ML), metoprolol acetate (MA) and metoprolol propionate (MP) with or without iontophoresis at 10 mM concentration. Each data represented the mean  $\pm$  SE of three experiments.

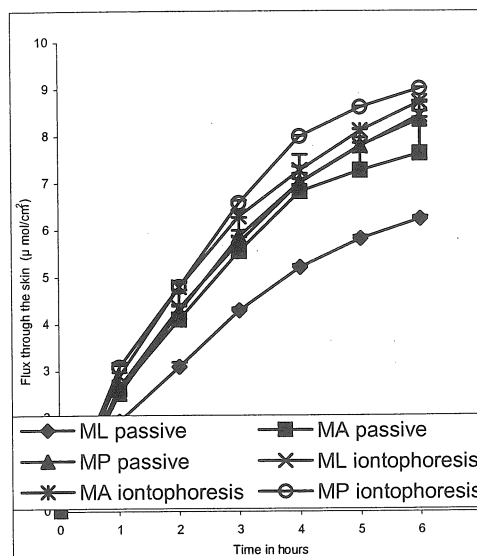


Fig. 5. Comparison of permeation profiles of metoprolol (ML), metoprolol acetate (MA) and metoprolol propionate (MP) with or without iontophoresis at 20 mM concentration. Each data represented the mean  $\pm$  SE of three experiments.

In Fig. 6, the steady state flux obtained from passive permeation showed also similar response. Hence it can be said that the enhancement of lipophilicity had made the esters more acceptable to stratum corneum and facilitated its permeation.

Iontophoresis, where drugs are actively propelled through aqueous pathways of skin usually benefits the permeation of water-soluble and ionisable drugs (Modamio *et al.*, 2000). But the pore pathways available to this process are only 0.1% of the skin and concurrent passive permeation also takes place. Hence it was thought that cumulative permeation under iontophoresis was likely to result in higher flux values. Anodal iontophoresis was carried out for the drug and prodrugs using silver – silver chloride electrode and a current of 0.5 mA / cm<sup>2</sup>. Flux through the skin for various concentrations is given in Fig. 3 – 5. It can be seen that the iontophoretic fluxes were slightly higher than the passive flux and the cumulative fluxes at the end of 6 h were higher than the passive permeation at all concentrations. The benefit of iontophoresis over that of passive permeation was mostly pronounced at 20 mM concentration. When analyzed from the viewpoint of donor drug load, it was seen that the flux

values showed a graded increase ( $P < 0.05$ ) with concentration (Fig. 6) for both metoprolol and prodrugs.

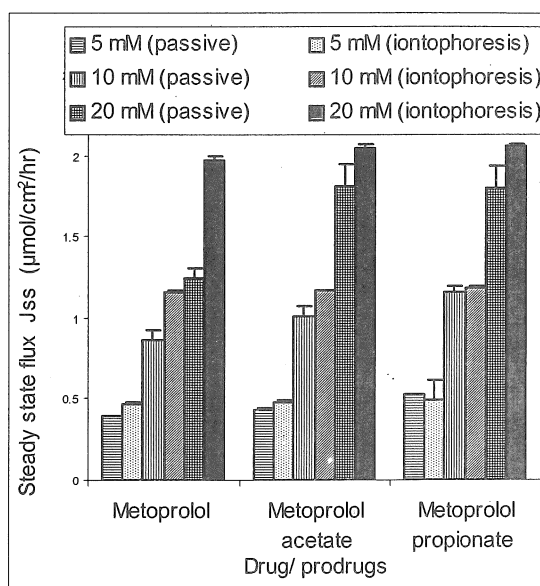


Fig. 6. Comparison of steady state flux of metoprolol and two prodrugs with or without iontophoresis at various donor concentrations. Each data represented the mean  $\pm$  SE of three experiments.

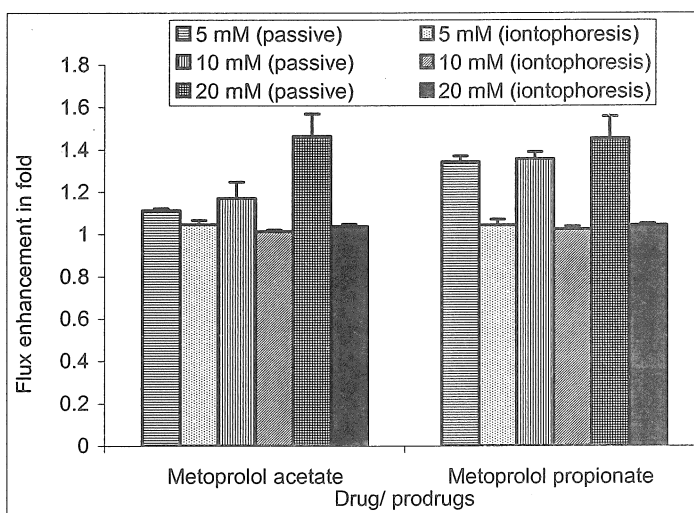


Fig. 7. Flux enhancement in folds of two prodrugs with or with out iontophoresis compared to the corresponding concentration of parent drug. Each data represented the mean  $\pm$  SE of three experiments.

The highest flux was obtained at 20 mM concentration in all cases. Metoprolol, as expected was the most responsive to the iontophoresis and at each concentration level, active flux recorded was higher than that of passive value. In contrast, prodrugs showed a nominal response to the active process, their iontophoretic permeation was minimally higher than that of passive permeation.

In Fig., we could also see the steady state flux obtained from the iontophoretic permeation of metoprolol propionate at 20 mM level which was slightly higher than that of metoprolol obtained with the same drug load, indicating that the comparable flux values can result from also the iontophoresis of unaltered drug moiety. On the other hand, the passive permeation of metoprolol acetate and propionate had shown high flux values indicating prodrug formation was an effective way and could be utilized to enhance the permeation rate without iontophoresis. Since metoprolol propionate at 20 mM level had not shown any significant enhancement over metoprolol acetate in terms of drug flux, metoprolol acetate at 20 mM concentration could be said to reach the optimum value. For both passive and iontophoresis, the permeability coefficients were found to be the highest with metoprolol propionate (10.413 and 10.670 X 10<sup>-2</sup> cm/h for passive and iontophoresis respectively). Fig. 7 shows the flux enhancement of prodrugs in folds with respect to the corresponding concentration of metoprolol carried out by a similar permeation process. For passive permeation, both metoprolol acetate and propionate have recorded enhancement (1.45 folds) at 20 mM level. In contrast, flux enhancement of the prodrugs was minimal (4 - 9%) in the iontophoretic process. Under the influence of electrical force, a number of permeation process underwent simultaneously. The charged moieties got propelled through the pathways and the permeation rate was affected by the fraction of electricity carried by the molecules (Merino *et al.*, 1999). As the rate of the active process is directly proportional to the energy supply, at a given current density the contribution of this route to the total drug flux is likely to approach a limiting value at higher drug concentrations (Kalia *et al.*, 2004). In contrast, passive process is relatively free from such reservations. Permeation usually increases with increase in

lipophilicity to a certain level. Hence for the prodrugs, the contribution of iontophoretic process was minimal to the total flux.

In nutshell it can be said that both prodrug approach and iontophoresis enhanced the flux values independently but the benefit in terms of flux enhancement was minimal when applied together. Hence it would be much practical to adopt a single process rather than combination.

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