

## ***In Vitro* Percutaneous Absorption Enhancement of Metoprolol Tartrate through Porcine Skin using Propylene Glycol and Fatty Acids**

**D.C. Bhatt<sup>1\*</sup>, A. S. Dhake<sup>2</sup>, R. K. Khar<sup>3</sup> and D. N. Mishra<sup>1</sup>**

<sup>1</sup>Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125 001, India

<sup>2</sup>L. B. Rao Institute of Pharmaceutical Education and Research, Khambhat-388 620, India

<sup>3</sup>Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India

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### **Abstract**

The effect of fatty acids and propylene glycol on the *in vitro* permeation of Metoprolol Tartrate (MT) through porcine skin was investigated. Skin permeation studies were conducted in the Franz diffusion cells. As vehicle, propylene glycol (PG) was used alone or mixed with phosphate buffered saline pH 7.4 (PBS). Compared to the PBS alone, the PG/PBS did not show any effect on the lag time, but significantly increased the transdermal permeation of MT ( $p < 0.05$ ). Based on the permeation enhancing effect, the binary solution PG-PBS (80:20, v/v) was used as a vehicle for fatty acids. The permeation enhancing effect of fatty acids (caprylic acid, capric acid, lauric acid, myristic acid, oleic acid and stearic acid) at 5% w/v concentration was studied across the excised porcine skin. The highest flux of MT was observed with oleic acid. The results of this investigation indicate that the synergistic effect of PG and oleic acid can be used to enhance the skin permeation of MT.

**Keywords:** Transdermal flux, Fatty acids, *In Vitro* release, Porcine skin, Metoprolol tartrate, Permeation enhancer, Propylene glycol

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### **Introduction**

A lot of research work has been devoted to developing techniques to overcome the barrier properties of the intact human skin and gaining a better understanding of the barrier properties of the stratum corneum (Kevin Li, 2007; Karande et al., 2005; Benson, 2005; Williams and Barry, 2004; Hadgraft and Guy, 2003; Sinha and Maninder, 2000). Penetration enhancers improve the ability of skin to absorb drugs either by reversibly altering the lipid and protein domains of the stratum corneum or by increasing the partitioning of the drug into the stratum corneum. Fatty acids along with suitable co-solvents have been shown to be effective in enhancing the permeation of drugs (Jantharaprapap and Stagni, 2007; Wang et al., 2005; Young and Gwak, 2004; Jiang and Zhou, 2003). The use of vehicles to improve transdermal permeation has been investigated by several groups. Many investigators have demonstrated enhanced transdermal permeation by using a mixture of hydrophilic vehicles such as propylene glycol/water, isopropyl alcohol/water, ethanol/water and polyethylene glycol 400/water. An improved permeation of hydrophilic drug zidovudine has been demonstrated in a system containing a mixture of hydrophilic and hydrophobic vehicles (Thomas and Panchgula, 2003;

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\* Corresponding author: bhatt\_2000@yahoo.com

Jim et al., 2000; Kim and Chien, 1996). Although there has been progress in understanding the mechanisms of permeation enhancers, screening remains the primary method to select appropriate enhancers in the development of transdermal formulations. In this study Metoprolol Tartrate (MT) was used as a model drug because this  $\beta$ -adrenergic blocking drug undergoes extensive first pass metabolism and has a short biological half life. Therefore, in order to reduce its high hepatic extraction ratio, enhance bioavailability, circumvent dose related side effects and improve patient compliance, MT has been considered to be a good candidate for being developed into a transdermal delivery system (Csoka et al., 2007; Nair et al., 2006; Aqil et al., 2004; Kommuru, 1999). Pig skin was used as an *in vitro* model skin because it closely resembles human skin in terms of similar histological and permeability characteristics. (Schroeder et al., 2007; Andega et al., 2001; Wu et al., 1997; Fang, et al., 1995; Dick, 1992; Sato et al., 1991; Wester and Maibach, 1987).

The objective of this study was to investigate the effects of binary vehicles and fatty acids on transdermal permeation of porcine skin using MT as a model drug.

## Materials and Methods

### Materials

Metoprolol tartrate was received as a gift from Astra Zeneca, Bangalore, India. The fatty acids- caprylic acid, capric acid, lauric acid, myristic acid, oleic acid and stearic acid were obtained from S D Fine chemicals, Mumbai. Propylene glycol was obtained from Hi Media Ltd, Mumbai. All other reagents were of analytical grade.

### Preparation of drug solutions containing penetration enhancers

Propylene glycol (PG) was added to phosphate buffered saline pH 7.4 (PBS) at 20, 50, 60 and 80% v / v concentration. The penetration enhancers and their concentrations used in the study are given in table 1. The binary vehicle composed of PG and PBS (80:20, v / v) was used to make the solution formulations containing the drug and the penetration enhancers. The drug was added at 10% w/v concentration. Each of the solutions contained one of the fatty acids as penetration enhancers at 5% w/v concentration. The mixture was stirred for 1 h at 37<sup>o</sup> C. The control solution consisted of drug in the binary vehicle or the phosphate buffer mentioned above, without any penetration enhancer. The solutions were filtered through 0.45  $\mu$ m nylon filter and 0.5 ml was placed in the donor compartment of the Franz diffusion assembly. The composition of various formulations is given in Table 1.

**Table 1.** Formulations of Metoprolol Tartrate containing penetration enhancers.

Formulation	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Propylene glycol (%v/v)		20	50	60	80	100	80	80	80	80	80	80
Phosphate buffered saline pH 7.4 (%v/v)	100	80	50	40	20		20	20	20	20	20	20
Caprylic acid (%w/v)							5					
Capric acid (%w/v)								5				
Lauric acid (%w/v)									5			
Myristic acid (%w/v)										5		
Oleic acid (%w/v)											5	
Stearic acid (%w/v)												5
Metoprolol tartrate (%w/v)	10	10	10	10	10	10	10	10	10	10	10	10

### ***In vitro* permeation of Metoprolol tartrate across pig skin**

The porcine skin from pinnae was obtained from a local slaughter house (Dick and Scott, 1992; Wester and Maibach, 1987; Andega et al., 2001). The skin samples were clipped, excised and cleaned with phosphate buffered saline pH 7.4 (PBS) before being cut into 2x2 cm square pieces. Individual skin samples were wrapped with aluminium foil and put into plastic bags and stored at -20<sup>o</sup> C for a maximum of 2 weeks before use. The frozen skin samples were thawed at room temperature and mounted on the vertical Franz diffusion cell with the stratum corneum side facing the donor compartment. The diffusion area was 1.13 cm<sup>2</sup>. The receptor compartment was filled with 10.2 ml of PBS at 37<sup>o</sup> C. After equilibrating the skin for 1 h in the diffusion cell, the solution formulation (0.5 ml) was placed in the donor compartment. The donor compartment was then covered with a sheet of aluminium foil and wrapped with parafilm to prevent evaporation. At predetermined intervals, samples (0.5 ml) were taken from the receptor compartment and the cell was refilled with an equivalent amount of fresh preheated buffer solution. The samples, after suitable dilutions, were analyzed at 223 nm by a spectrophotometer (Perkin Elmer, model EZ 301). All the experiments were carried out in triplicate.

### **Sample analysis**

The amount of drug in the receptor phase samples was determined using a spectrophotometer at 223 nm. The performance of the method was assessed for linearity, accuracy and precision. The drug concentration of samples was calculated from the slope of the calibration curve. The absorbance was linear in the concentration range 2-10 µg/ml (Equation,  $y=0.0189x + 0.0004$ ). The least square regression analysis showed that the correlation coefficient of the calibration curve was > 0.999.

### **Data analysis**

The data analysis for determination of permeation parameters was performed as described by Barry (1983). The amount of drug permeated was calculated by multiplying sample concentration with the receptor volume and the dilution factor. For each skin specimen, the amount of drug permeated per unit area was calculated and plotted against time. The steady state flux was calculated from the slope obtained through regression analysis. The enhancement factor was calculated as a ratio of the drug flux in presence of a permeation enhancer to that without the enhancer. The data were expressed as mean of triplicate observations along with respective standard deviations. Student's t-test was performed to determine the level of significance between the two groups. The data were considered significant at  $p < 0.05$ .

## **Results and Discussion**

### **Preliminary experiments to determine the choice of binary vehicle**

Four binary combinations of solvents consisting of propylene glycol and phosphate buffered saline (pH 7.4) were initially investigated for their effect on *in vitro* permeation of Metoprolol tartrate (MT) across porcine skin. Table 1 shows the composition of the solution formulations prepared in this investigation. The transdermal permeation parameters of drug from formulations F0 to F5 are shown in Table 2.

Compared with the buffer alone the PG/buffer system containing 80% PG greatly increased the skin permeation of drug (Figure 1). Although PG alone showed almost similar flux compared to formulation F4, the latter was selected as a vehicle for dissolving the hydrophilic drug and the permeation enhancers.

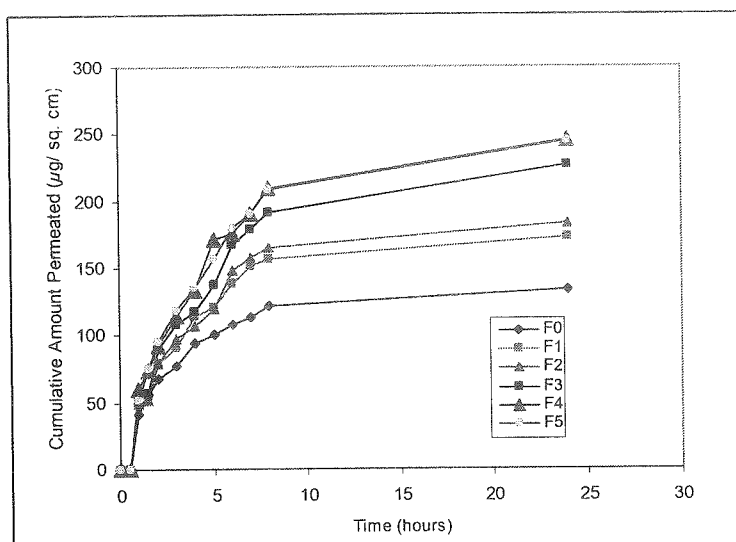
**Table 2.** Permeation parameters of Metoprolol tartrate for binary vehicles across porcine skin

Formulation	Composition (%v/v)	Flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	EF
F0	PBS, 100	4.88±0.59	1
F1	PG:PBS,20:80	6.66±0.41	1.36
F2	PG:PBS,50:50	7.18±0.24	1.47
F3	PG:PBS,60:40	8.99±0.39	1.84
F4	PG:PBS,80:20	9.66±0.34	1.98
F5	PG 100	9.61±0.11	1.96

PBS, phosphate buffered saline pH 7.4; PG, propylene glycol; EF, enhancement factor

Each formulation contained 10 % w/v of metoprolol tartrate

Data are given as mean ± SD (n=3)



**Figure 1.** Permeation profiles of MT from drug solution in binary vehicles containing propylene glycol and phosphate buffer saline (pH 7.4).

Binary combination of various solvent systems such as water, propylene glycol, ethanol, isopropyl myristate and Polyethylene glycol 400 have been reported for their effect on *in vitro* drug permeation across various model of animal membranes (Surber et al., 1991). Many researchers have investigated the transdermal permeation enhancing effect of propylene glycol and ethanol and have demonstrated that the lower concentrations affect only the intercellular lipid pathways of stratum corneum, whereas the higher concentrations affect intracellular polar pathways as well (Thomas and Panchgnula, 2003).

#### Effect of fatty acids as skin permeation enhancers

Fatty acids are known to be potent enhancers of penetration of several drugs through skin. To study the potential penetration enhancing effect of fatty acids on Metoprolol Tartrate(MT) across porcine skin, binary vehicles containing propylene glycol and phosphate buffer saline and individual fatty acids were used. The permeation parameters of MT from these vehicles containing fatty acids are shown in Table 3.

**Table 3.** Effect of fatty acids on the permeation of Metoprolol Tartrate through porcine skin

Formulations	Fatty acids <sup>a</sup>	Flux ( $\mu\text{g}/\text{cm}^2$ )/ h	EF <sup>c</sup>
F4	Control <sup>b</sup>	$9.66 \pm 0.34$	1
F6	Caprylic acid	$29.64 \pm 0.19$	3.06
F7	Capric acid	$31.51 \pm 0.37$	3.26
F8	Lauric acid	$32.73 \pm 0.52$	3.39
F9	Myristic acid	$31.26 \pm 0.18$	3.24
F10	Oleic acid	$50.56 \pm 0.24$	5.23
F11	Stearic acid	$29.88 \pm 0.10$	3.09

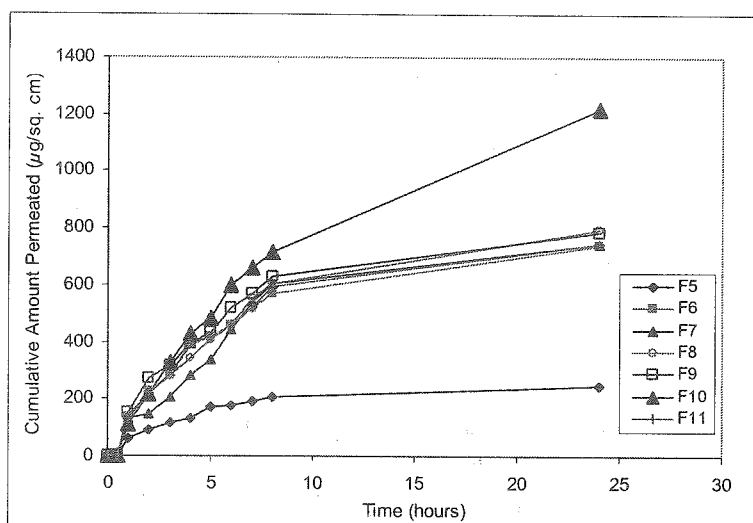
a- Each formulation contained 5 % w/v of the fatty acid in the control vehicle

b- Control was PG/buffer (80:20, v/v) binary vehicle

c- EF, Enhancement Factor.

Drug content of each formulation was 10% w/v

The fatty acids have been widely studied as permeation enhancers due to their lipophilic properties. The permeation enhancing effects of fatty acids are reported to be higher when used along with alcoholic vehicles such as ethanol and propylene glycol (Cooper et al., 1985; Aungst et al., 1986; Yamada et al., 1987). We selected some generally regarded as safe (GRAS) category of fatty acids, in order to investigate their permeation enhancing effects on the transdermal permeation of MT across excised porcine skin. The six fatty acids studied were caprylic-C8, capric-C10, lauric-C12, myristic-C14, oleic-C18 and stearic-C18. Except oleic acid, all are saturated fatty acids. It has been reported that in the permeation enhancement studies with naloxone (Aungst et al., 1986) the most effective fatty acids were found to be those with a carbon chain length in the range of C10-C12. However, the results of our study indicated (Table 3), that the increase in the carbon chain length of saturated fatty acids did not have a significant effect on the permeation of MT across the porcine skin (Figure 2).



**Figure 2.** Permeation profiles of Metoprolol tartrate across porcine skin from donor solutions containing the drug and individual fatty acids as penetration enhancers. The composition of various formulations is given in Table 3.

The enhancement in the transdermal flux due to all other fatty acids studied was found to be significantly lower than that due to oleic acid. The permeation enhancing effects of oleic acid observed in our studies are quite consistent with an earlier report (Santoyo et al., 1995) wherein the oleic acid was found to be the most effective enhancer for *in vitro* permeation of piroxicam through rat skin. The mechanism of permeation enhancement by oleic acid probably involves increased partitioning of the drug into skin due to the fluidization of the intercellular lipid domain. The surface layers of stratum corneum rapidly equilibrate with the adjacent phase of the substances applied onto it. The Differential Scanning Calorimetric analysis of human stratum corneum has established this mechanism very well (Kim et al., 1993). The oleic acid has also been reported to disorganize the lipid chains buried within the bilayer structure of stratum corneum, together with some other non-polar materials. The *cis* double bond at C-9 on oleic acid causes a bend in the alkyl chains, disrupting the ordered arrangement of predominantly saturated straight chain skin lipids, leading to their fluidization (Devi et al., 2003; Barry, 1987; Golden et al., 1987).

## Conclusion

The present study may form an important step towards the successful fabrication of a drug-in-adhesive type transdermal drug delivery system for Metoprolol Tartrate due to the synergistic permeation enhancement effects of oleic acid and alcoholic binary vehicles. In conclusion, for effective transdermal delivery, combination of fatty acids like oleic acid along with propylene glycol could be used to enhance the skin penetration of Metoprolol Tartrate.

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