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ENHANCEMENT OF PERCUTANEOUS ABSORPTION OF PROPRANOLOL HYDROCHLORIDE BY IONTOPHORESIS

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ABSTRACT

Iontophoresis is defined as the migration of ions when an electric current passes through a solution containing ionized species. When applied transdermally, iontophoresis may reduce the barrier porperties of skin and may enhance the transdermal permeation of drugs. This research work was aimed at exploring the factors which influence the effectiveness of iontophoresis facilitated transport of ionized species across the skin, and comparing it with that by passive diffusion. Propranolol hydrochloride was selected as the model drug, and rat abdominal skin as the in-vitro animal model. Pulse dc was applied, at 2.16/ 3/6/9 mA/cm^2 ; 1:1 or 4:1 duty cycle and 20 KHz frequency for 15/25 minutes. Factors which modify the iontophoretic drug delivery, such as drug concentration, current density, duty-cycle of pulse dc, and duration of iontophoresis, were also evaluated. It was found that iontophoresis causes a significant increase in transdermal permeation of Propranolol hydrochloride in-vitro through rat abdominal skin, as compared to that by passive diffusion.

INTRODUCTION

It has been recognised that only a limited number of drugs can be administered transdermally at a rate which is sufficient to achieve blood levels that are therapeutically beneficial for systemic medication. To achieve and to maintain a drug concentration above the minimum therapeutic level, the barrier properties of skin must be overcome. The use of electrical current to transport ionized drugs through the skin has been shown potentially promising to accomplish the goals of reducing the barrier properties of skin and hence of enhancing the transdermal permeation of drugs[1]. Most of the earlier work on iontophoretic

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drug transport has focussed on local delivery of ionized drugs into surface tissues[2,3,4,5]. Recent developments of transdermal drug delivery systems have promoted interest in the possible use of electric current to enhance the rate of drug absorption across the skin and into the systemic circulation[6,7,8].

Iontophoresis is defined as the migration of ions when an electric current passes through a solution containing ionized species. Some advantages of iontophoresis are[1] --

a) it avoids the risks and inconveniences of parenteral therapy.
b) it prevents variation in the absorption and metabolism of oral administration.
c) it increases therapeutic efficacy by bypassing hepatic first-pass metabolism.
d) it reduces the chances of over - or under - dosing by continuous delivery of drug programmed at the required therapeutic rate.
e) it permits the use of a drug with a short biological half-life.
f) it provides a simplified therapeutic regimen, leading to a better patient compliance.
g) it permits rapid termination of the medication if needed, by simply stopping drug input from the iontophoretic delivery system.

There are several conditions necessary for iontophoretic medication[1] --

i) the drug should be charged or modified to carry a charge and the area to be medicated must be a body surface.
ii) the drug is applied under an electrode of similar polarity, i.e., negative ions are delivered by cathodal (-ve) iontophoresis, and positive ions are delivered by anodal (+ve) iontophoresis.
iii) a return electrode, opposite in charge to the drug, is placed at an indifferent site on the body.
iv) the current is allowed to flow below the level of pain threshold for an appropriate time.

This research work aims to explore the factors which influence the effectivess of iontophresis-facilitated transport of ionic species across the skin, and to compare it with that by passive diffusion only.

THEORY

Electrical Properties of Skin : The skin is known to produce a large impedance to charged molecules which are driven through skin under an applied electrical potential[1]. Electrical properties of the skin are dominated by the stratum corneum which is considered to be the least conductive layer in the skin. Stratum corneum consists of multilayers of cornified cells whose nuclei are lost. These electrically insulated horny cells are continuously replenished by the slow upward migration of cells produced from the basal cell layer of the stratum germinativum. Skin is breached by hair follicles, sweat ducts, and other glands which could provide a potential pathway for shunt-diffusion across the skin.

The shunt pathway may be important for the skin penetration of ionic penetrants, which show an extremely poor permeation through transcellular route. Under the influence of the application of an electrical field, ionic species or charged molecules may penetrate through skin via the shunts as well as the intercellular routes in the stratum corneum as the skin is likely to be perturbed during the iontophoresis treatment, which may reorient the intercellular lipids resulting in the formation of artificial shunts.

Stratum corneum shows two important electrical features. First, it will be polarized by a direct electrical field[9]. Secondly, its impedance changes with the pulse frequency[6]. These properties may be represented by an electrical equivalent circuit shown in Figure 1[10].

In this equivalent circuit, R_{vs} denotes the pure resistance, which originates from the viable skin. This resistance (R_{vs}) to charged molecular current does not change with pulse frequency. The parallel combination of R_{sc} and C_{sc} represent the resistive and capacitive

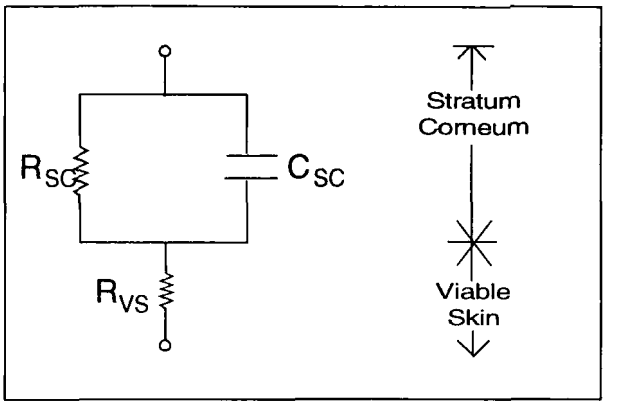

FIGURE 1

Analogous Equivalent Circuit of Skin Impedance. Rvs denotes the pure resistance, which originates from the viable skin. This resistance to charged molecular current does not change with pulse frequency. The parallel combination of Rsc and Csc represent the resistive and capacitive components for the impedance of the stratum corneum and is a function of pulse frequency.

components for the impedance of the stratum corneum and is a function of pulse frequency. It has been found that the impedance of human skin decreases with the increasing frequency.

When an electrical field with dc-mode is used to facilitate the penetration of ionic molecules, electrochemical polarization may occur in the skin[9]. This polarization operates against the applied electrical field and greatly reduces the magnitude of the input current. The polarization of the stratum corneum is analogous to the charging of the capacitor C_{sc} in Figure 1 with an input current, i. Therefore, the effective current decays across the skin when a constant dc voltage is applied. Consequently, the efficiency of current dependent penetration of ionic species through skin is expected to be reduced with application time of dc iontophoresis.

To avoid the polarization of the stratum corneum the dc should be applied in a periodic manner (Figure 2) which is called pulse dc.

The pulse dc mode is a dc voltage which periodically alternates with the "on" and "off" of the applied voltage. The number of on/off cycles is controlled by the frequency chosen. In the state of "on", charged molecules are forced into skin and the stratum corneum soon becomes polarized. On the other hand, in the state of "of", no external stimulation is present and stratum corneum becomes depolarized. It is analogous to the discharging of the current from the capacitor C[1]. The on/off ratio controls the time proportion for polarization and depolarization process in each cycle. The number of the on/off cycles in each second is called frequency.

The optimum value of on/off ratio varies with the frequency applied. When a proper on/off ratio is selected, every new cycle starts with no residue polarization left in the skin from the previous cycle, i.e., effect of polarization is eliminated.

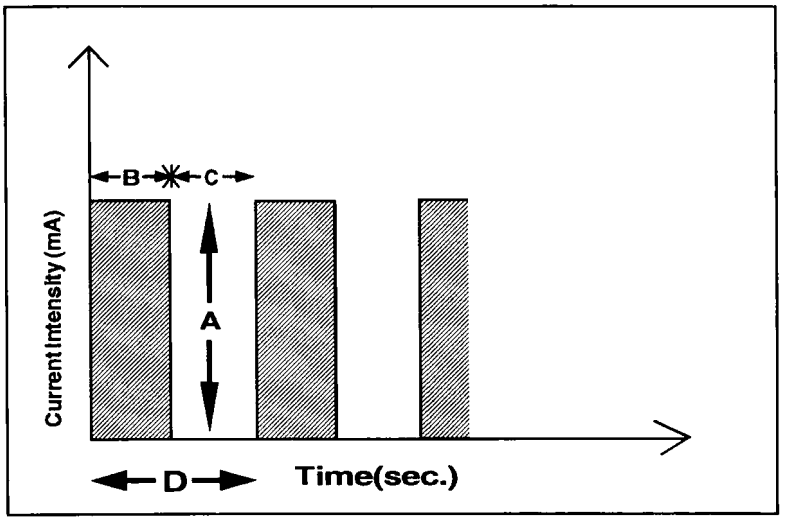

FIGURE 2

Diagram showing various components of a pulse waveform profile, where 'A' is the amplitude of a current intensity (mA), B/C is the on/off ratio, D is the duration (sec.) of a complete cycle, and 1/D is the frequency.

The energy (E) required to overcome the penetration barrier - stratum corneum - can be expressed by :

$$E = \int I.V.dt = \int I^2.R dt \quad .....(1)$$

Where E is the energy required to deliver a charged molecule with a current 'I' and a voltage 'V'. The 'R' denotes the impedance of the skin. As can be seen from equation (1) above, less energy will be required to overcome the barrier as skin impedance is reduced[11]. This may be achieved by applying the current with proper frequency and on/off ratio. Therefore, it is essential to select optimum pulse dc mode parameters to attain best facilitating effect of iontophoresis. The sensitivity and the tolerance of the skin in response to the frequency used should be considered.

MATERIALS

The following chemicals were used as obtained:

1) Propranolol hydrochloride was a gift from Ranbaxy Research Centre, India.
2) Isotonic Phosphate Buffer, pH 7.4 (USP). This was made afresh using Sodium Chloride (BDH), Sodium Phosphate Dibasic (Loba Chemie), Sodium Dihydrogen Orthophosphate (Loba Chemie) and glass double - distilled water made in the laboratory.

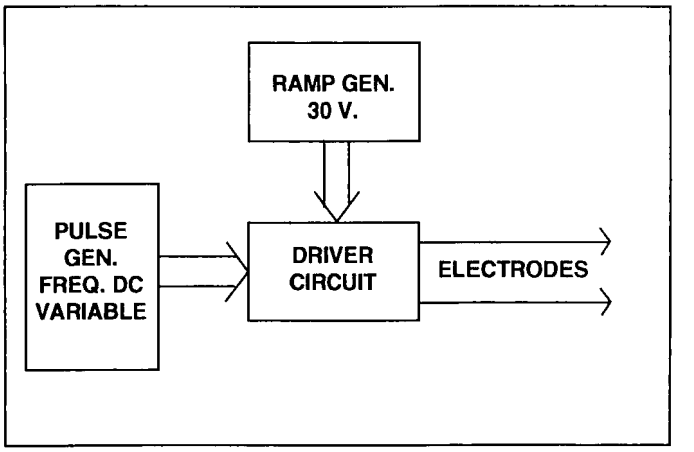

FIGURE 3

Block Diagram of the Iontophoretic Drug Delivery System.

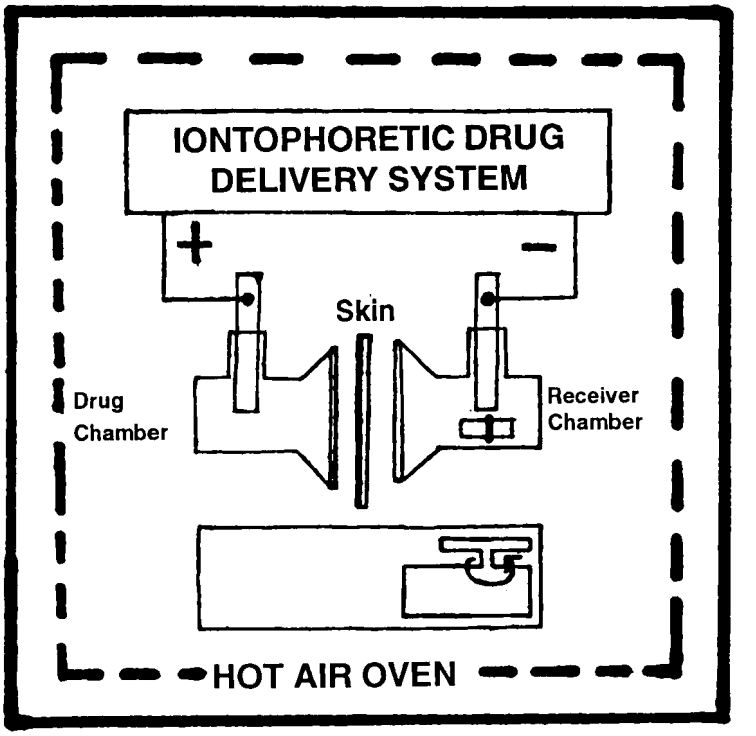

FIGURE 4

Diagrammatic illustration of the experimental set - up, using modified Valian - Chien diffusion cells, in the in-vitro transdermal iontophoretic permeation studies (drawings not to scale).

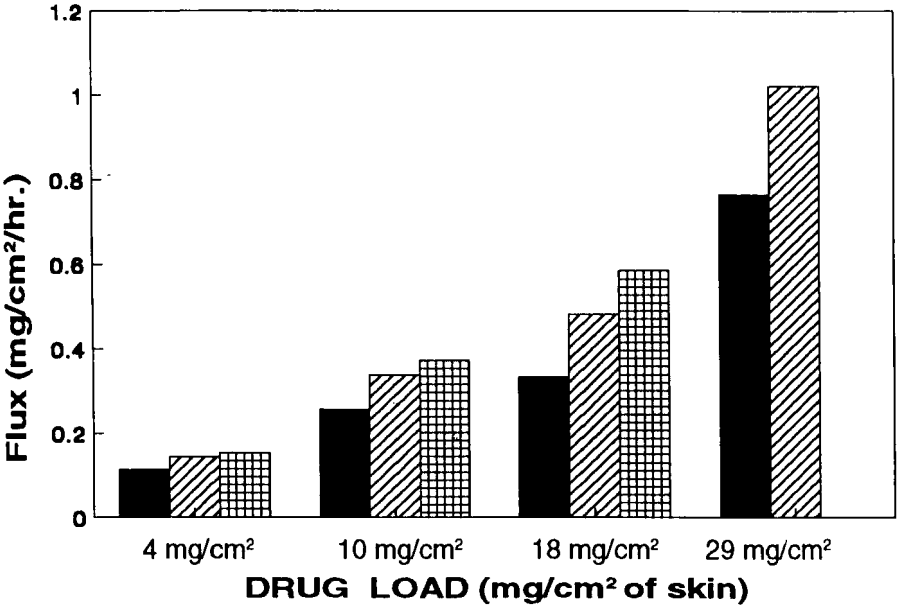

FIGURE 5

Effect of increase in drug concentration loaded on transdermal permeation of Propranolal hydrochloride through rat abdominal skin in-vitro by iontophoresis and by passive diffusion (the drug solution was loaded throughout the experiment).

Key:
- Passive Diffusion
- Iontophoresis at 2.16 mA/cm² ; 1:1 and 20 KHz for 15 minutes.
- Iontophresis at 2.16 mA/cm² ; 1:1 and 20 KHz for 25 minutes.

3) Ammonia solution, sp. gr. 25%, extrapure, (s.d. fine chem., India) was diluted in water to make a 0.32 M solution.

4) Anaesthetic Ether I.P. (Vitramika Pharm, India) was used undiluted.

5) Albino rats (Winstor strain) weighing above 300 grammes, were procured from Central Animal House, Hamdard University, India.

6) Iontophoretic Drug Delivery System (Pulse dc generator) :
This pulse dc generator was designed and fabricated at Electronic Regional Test Laboratory (North), India. Figure 3 gives a block diagram of the circuit involved. This instrument is capable of delivering simple/pulse dc, with the following features:

Current Amplitude	:	1 to 10 mA (manual control)
Pulse dc waveform	:	Square waveform
Pulse dc frequency	:	10 to 50 KHz (manual control)
Pulse dc duty-cycle	:	20% to 80% (manual control)
Time taken to charge	:	1 minute
Time taken to discharge	:	3 minutes

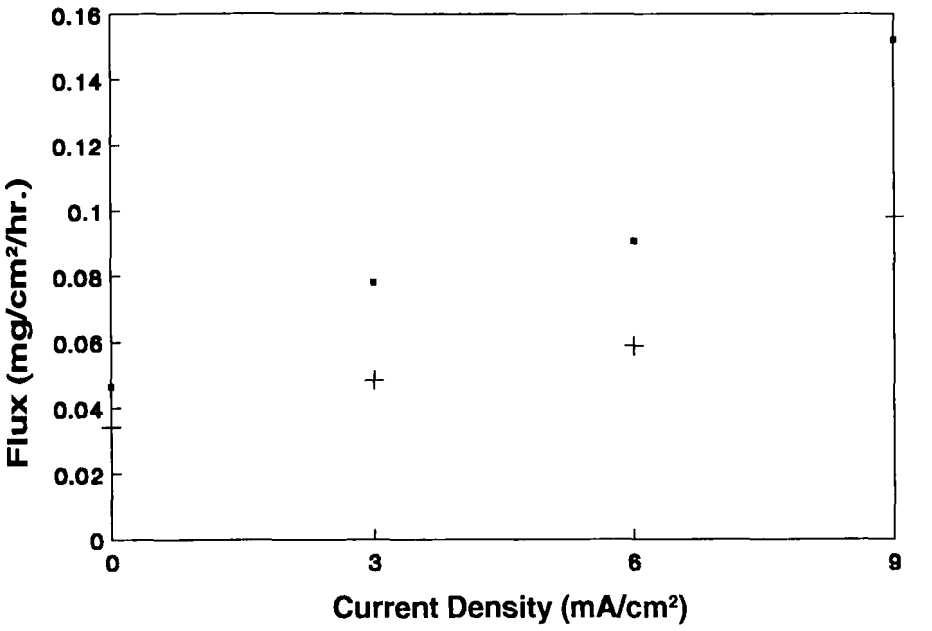

FIGURE 6

Effect of increase in current density on transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro by iontophoresis, at different durations of iontophoretic treatment.

Key : + Drug solution, 18 mg/cm² of skin, loaded for 20 minutes.
▪ Drug solution, 18 mg/cm² of skin, loaded for 30 minutes.

An external circuit LED emits signal when the external circuit is completed, and an audio-visual signal is generated at currents exceeding 7.5 mA. A notable feature of this device is that it takes one minute to charge, and three minutes to discharge, so that the patient does not get any shock (due to sudden on/off of current). It is powered by three, 9-volts dry cell battteries.

7. Permeation Cells : Modified Valian - Chien Diffusion Cells, as shown in Figure 4, were used.

EXPERIMENTAL METHODS

Skin Preparation : Adult rats were killed by exposing them in a closed chamber to Anaesthetic Ether, I.P.. The abdominal skin was removed using surgical scissors immediately thereafter, and the skin was treated with 0.32 M NH_4OH[12]. The hair and underlying fat layers were thus separated, and the skin was washed with water and examined for cuts/holes, if any. Immediately thereafter, the skin was stabilized by clamping suitably cut pieces between the two half-cells of the two-chambered, modified Valian - Chien diffusion cells (Figure 4). The skin was stabilized for 6 hours using the Isotonic Phosphate Buffer, pH 7.4..

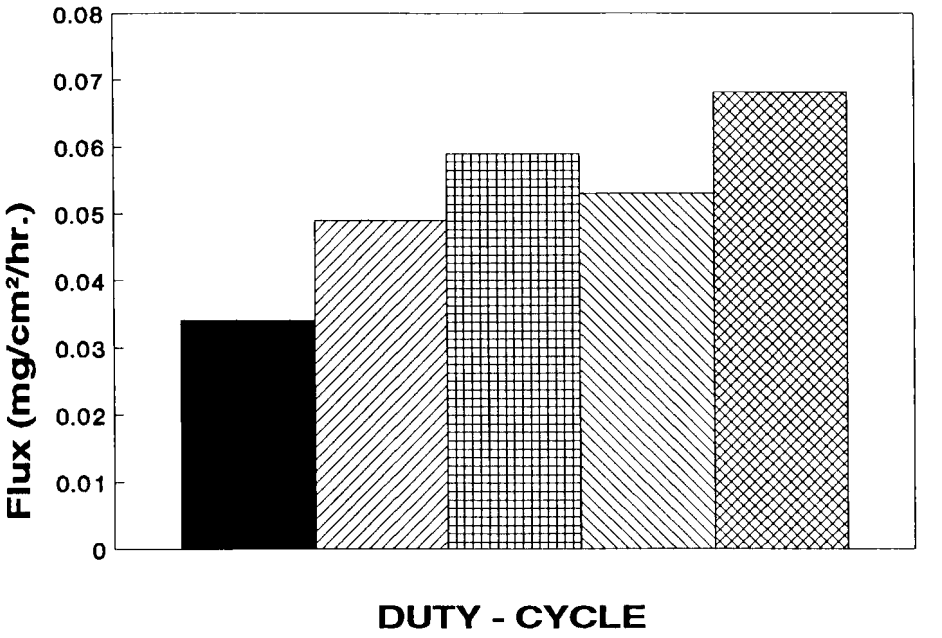

FIGURE 7

Effect of increase in duty-cycle of pulse dc on transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro by iontophoresis, when drug solution, 18 mg/cm² of skin, was loaded for 20 minutes only.

- Passive Diffusion
- Iontophoresis at 1:1 duty cycle, 20KHz frequency, and 3 mA/cm² for 15 minutes.
- Iontophoresis at 1:1 duty cycle, 20 KHz frequency, and 6 mA/cm² for 15 minutes.
- Iontophoresis at 4:1 duty cycle 20 KHz frequency and 3 mA/cm² for 15 minutes.
- Iontophoresis at 4:1 duty cycle 20 KHz frequency and 6 mA/cm² for 15 minutes.

Permeation Studies : Following the stabilization of skin, the receiver chamber was loaded with fresh Isotonic Phosphate Buffer, pH 7.4, and the donor chamber was filled with the solution of Propranolol hydrochloride in water. The drug solution was loaded for 20/30 minutes or 18 hours, in separate experiments. For iontophoresis, current was applied at different strength (2.16/3/6/9 mA/cm²) ; 20 KHz frequency and 1:1 or 4:1 duty cycle for 15/25 minutes. The anodal current was applied to the drug chamber, and cathodal current to the receiver chamber. For passive diffusion, no current was applied. Samples were withdrawn from the receiver chamber upto 18 hours, and fresh buffer was replaced. Temperature was maintained throughtout at 37°C ± 0.5 °C by keeping the assembly in a hot air oven, and the receiver solution was continuously stirred by a magnetic stirrer at a constant speed (100 rpm).

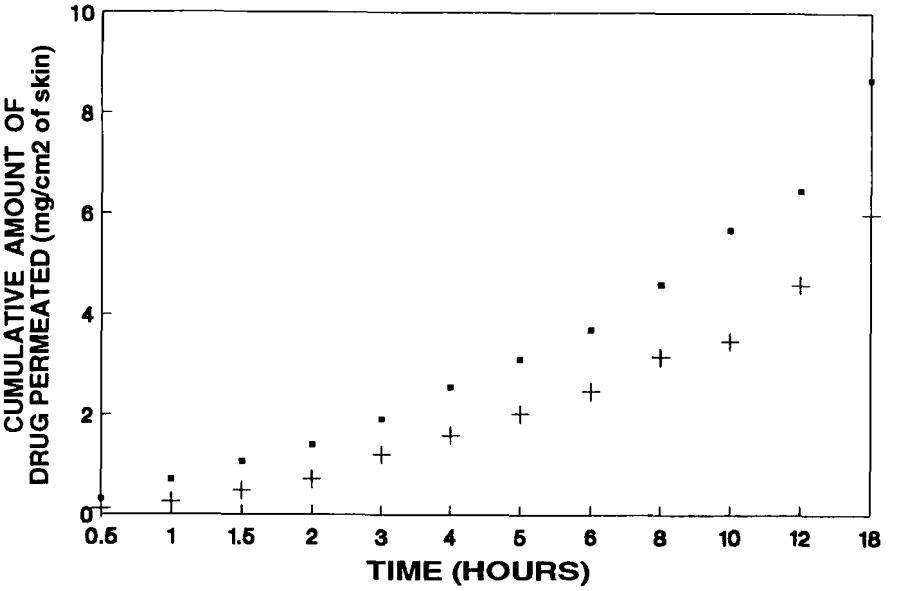

FIGURE 8

Cumulative amount of Propranolol hydrochloride permeated through rat abdominal skin in-vitro by iontophoresis and by passive diffusion, when drug solution, 18 mg/cm^2 of skin, was loaded throughout the experiment.

Key : ▪ Iontophoresis at 2.16 mA/cm^2 of skin ; 1:1 and 20 KHz for 15 minutes.
\+ Passive Diffusion

The content of Propranolol hydrochloride was analysed in the withdrawn samples by UV - absorbance spectroscopic analysis, at λ max. = 289 nm, using a double-beam Hitachi Spectrophotometer (Model 150-20).

The influence of following experimental variables was studied :

1) Loading the drug solution throughout the experiment, the effect of increasing the drug concentration, and the effect of increasing the current density on transdermal permeation was studied.
2) Loading the drug solution for 20/30 minutes only, the effect of increasing the current density on transdermal permeation was evaluated.
3) Loading the drug solution for 20 minutes only, the effect of increasing the duty cycle at different current densities on transdermal permeation was evaluated.

RESULTS AND DISCUSSION

The results of transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro by pasive diffusion, and by iontophoresis, have been illustrated in Figures 5,6,7,8 and 9.

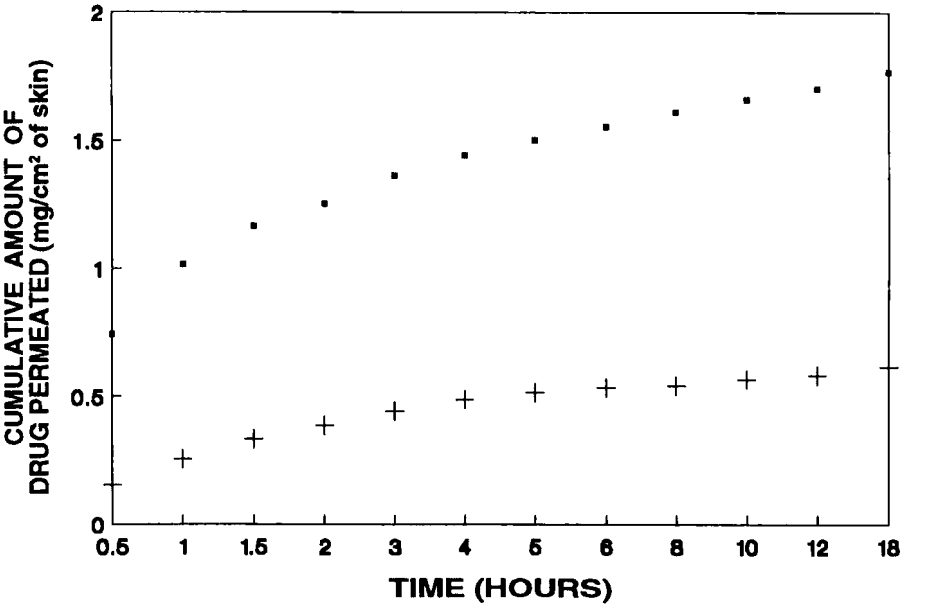

FIGURE 9

Cumulative amount of Propranolol hydrochloride permeated through rat abdominal skin in-vitro by iontophoresis and by passive diffusion, when drug solution, 18 mg/cm² of skin, was loaded for 20 minutes.

Key : ▪ Iontophoresis at 9 mA/cm² ; 20 KHz and 1:1 for 15 minutes
\+ Passive Diffusion.

It can be seen that increasing the concentration of drug solution loaded increases the transdermal permeation of Propranolol hydrochloride through rat skin in-vitro by passive diffusion and also by iontophoresis (Figure 5). This increase is appreciably more in case of iontophoresis at all drug concentrations employed, as compared to that by passive diffusion, as can be seen in Figure 8, for example. Further, increasing the time of application of current (iontophoresis) produces some increase in transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro at various drug concentrations, other factors being the same.

Further, increasing the current density of pulse dc applied (iontophoresis) increases the transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro as compared to that by passive diffusion, as can be seen in Figure 6 and Figure 9. Increasing the duration of application of current (iontophoresis) increases the transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro, other factors being the same.

Further, increasing the duty-cycle of pulse dc applied, at different current densities, increases the transdermal permeation of Proporanolol hydrochloride through rat abdominal skin in -vitro, as compared to that by passive diffusion, other factors being the same (Figure 7).

TABLE 1

Percent Increase in Efficiency of Permeation of Propranolol Hydrochloride through Rat Abdominal Skin in-vitro by Iontophoresis over that by Passive Diffusion.

Drug Concentration (loaded for 18 hrs) (mg/cm^2)	Percent increase in efficiency of permeation due to iontophoresis at 2.16 mA/cm^2 ; 1:1 and 20 KHz for	
	20 min	30 min
4	15.4	71.5
10	30.04	43.4
18	34.4	35.1
29	36.4	Not performed

TABLE 2

Percent Increase in Efficiency of Permeation of Propranolol Hydrochloride through Rat Abdominal Skin in-vitro by Iontophoresis, over that by Passive Diffusion, at Drug Concentration 18 mg/cm^2, loaded for 20/30 minutes.

Current Density (mA/cm^2)	Percent Increase in Efficiency of Permeation due to Iontophoresis at 20 KHz and at duty cycle		
	1:1 for 15 min.	1:1 for 25 min.	4:1 for 15 min
3	27.1	51	55.99
6	100.97	130.5	100
9	178.8	210.0	Not performed

A quantitative comparison of transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro with that by passive diffusion, can be made by calculating the % increase in efficiency of permeation due to iontophoresis over that by passive diffusion :

$$\% \text{ increase in efficiency of permeation} = \frac{A - B}{A} \times 100$$

where A = Cumulative amount of drug permeated in 18 hours by iontophoresis
B = Cumulative amount of drug permeated in 18 hours by passive diffusion

The results, in terms of % increase in efficiency of permeation, are summarized in Table 1 and Table 2.

CONCLUSION

It can be concluded that iontophoresis causes a significant increase in transdermal permeation of Propranolol hydrochloride in-vitro through rat abdominal skin, as compared to that by passive diffusion. Further, factors which modify the iontophoretic drug delivery, such as drug concentration, current density, duty - cycle of pulse dc, and duration of iontophoresis, can be manipulated to control the transdermal permeation of Propranolol hydrochloride through rat abdominal skin in-vitro. Hence, iontophoretic drug delivery can be accepted as an effective means of enhancing the transdermal drug delivery in-vitro of Propranolol hydrochloride.

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