

# Enhanced Iontophoretic Permeation of Rizatriptan Benzoate from Thermo-reversible Gel Based System: Effect of Penetration Enhancers and Pulsed Current

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## ABSTRACT

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The purpose of present research work was to enhance the iontophoretic delivery of Rizatriptan Benzoate (RZB) through optimized gel based formulation by using chemical penetration enhancers and to study the effect of pulsed current on permeation through guinea pig, Wistar rat and human cadaver skin. Modified Franz diffusion cell was used to study the in vitro permeation across various types of skin by iontophoresis. The thermo-reversible gel was prepared and optimized by cold method using poloxamer 407 as a gelling agent. Thermo-reversible gel with 20% w/v poloxamer 407 has suitable gelation temperature and viscosity for iontophoretic delivery of RZB. The highest permeation obtained was with iontophoresis in combination with 10% v/v PEG-400 as penetration enhancer. The permeation of RZB was significantly higher with pulsed current (1:1 as a ON: OFF ratio) at 0.5 mA/cm<sup>2</sup> as a current density than direct continuous current. It was observed that highest permeation was obtained through the rat skin as compared to guinea pig skin, and the permeation through human epidermis was lowest. The study reveals that it is feasible to deliver RZB by iontophoresis in combination with penetration enhancer and pulsed current from Poloxamer thermo-reversible gel.

**Keywords:** Transdermal, poloxamer, Iontophoresis, Rizatriptan

## INTRODUCTION

Rizatriptan Benzoate (RBZ) is a potent and selective 5-HT<sub>1B/1D</sub> receptor agonist which has been shown to be clinically useful in the treatment of migraine headache<sup>1</sup>. It selectively constricts isolated human middle meningeal arteries, inhibits neurogenic dural and extravasation. These peripheral actions are thought to contribute its anti-migraine activity<sup>2,3,4</sup>. It has been shown to relieve migraine within 2 hr in 67 to 77 % of patients and indicated in the treatment of acute migraine attack<sup>5</sup>. It has short half life of only 2-3 hr. which requires frequent dosing. Absorption is rapid following oral administration but bioavailability is only 45% due to high first pass effect. Food has no effect on the bioavailability of RBZ. However, its administration with food will delay the time to reach peak plasma concentration by 1 hr. Moreover vomiting, inhibition of gastric motility, and delayed gastric emptying associated with migraine may affect the absorption of orally administered drug<sup>6</sup>. Transdermal iontophoresis may be one of the ways to overcome the above limitations of oral drug delivery.

US patent by Angelov on transdermal methods and systems for the delivery of rizatriptan disclosed that an effective amount of rizatriptan could be delivered in less than 45 minutes using an integrated iontophoretic patch.<sup>7</sup>

The studies by Patel et al revealed that pharmacokinetics following transdermal iontophoretic delivery of Sumatriptan Succinate, another 5-HT<sub>1B/1D</sub> receptor agonist, was comparable to those after oral, nasal or rectal administration.<sup>8</sup> Sumatriptan Iontophoretic Transdermal Patch had successfully completed Phase-I clinical trials in 2008.<sup>9</sup>

Iontophoresis is the method where the movements of ions across a membrane enhanced using an externally applied potential difference. When the membrane under consideration is skin, the method is called transdermal Iontophoresis.<sup>10</sup> Another way to enhance the transdermal drug delivery was use of penetration enhancers. The advantage of using iontophoresis in combination with penetration enhancers was to enhance the transdermal permeation by synergistic approach and to limit the area of application.

In the view of above literature and the problems associated with oral therapy of RZB, present research work was undertaken to enhance the iontophoretic permeation of RZB by coupling with penetration enhancers using optimized thermoreversible gel formulation and to study the effect of pulsed current on permeation through guinea pig skin, Wistar rat skin and human cadaver skin.

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## MATERIALS AND METHODS

RZB was obtained as a gift sample from Cipla Pharmaceuticals, Mumbai, Poloxamer-407 purchased from BASF, Mumbai, Silver Chloride was purchased from Research fine lab, Mumbai, PEG-400 and ethanol was purchased from Merck Chemicals, India. All other reagents used were of analytical grade.

### Preparation of electrodes:

Rod shape silver wire (1mm diameter, 2cm length 99.9% pure) was used as anode. Another silver wire was dipped in melted silver chloride in porcelain dish. This silver wire was connected to negative pole of the power source at one end and immersed in 0.1 N hydrochloric acid solution at other end. A gray silver chloride layer was coated on anodal silver wire and after 24h these wires were ready for use as cathode in iontophoretic experiments.<sup>11</sup>

### Development of microcontroller based electrical circuit (Iontophoresis device):

The power supply used was designed and constructed using microcontroller Atmel 89S51. The input to the power supply was through a 12 V direct current mains adapter and the 5V direct current regulated supply to the microcontroller is derived using L 7805 voltage regulator. As the application does not demand for high speed operation, 2MHz crystal was used to provide clock pulses required for the operation of microcontroller.

The experiment was designed keeping in view the provision for applying the voltage to the cell under the following conditions.

- Continuously ON
- ON for one second and OFF for one second (Pulsed current)

This feature is implemented in program in the microcontroller and for selecting one of the above programs; a thumb wheel switch is used. The thumb wheel switch is connected to the microcontroller. When the thumb wheel is set at '0' the output of the supply is continuous, when '1' is set, it is ON for 1 second and OFF for 1 second. The microcontroller output port bit P3.5 is used to control the duration, as the microcontroller can not source the required range of current this port pin is used to drive an electromagnetic relay. The main driven by an IC ULN 2003 that works as a driver for the relay. The main function of the microcontroller is to read the value set in thumb wheel switch and according to the scheduled selected, it switches the relay making the power available at the output of relay. For indicating the ON and OFF state of the power supply a LED (Light emitting diode) is provided which glows when the supply is ON and it does not glow when it is OFF.

The timing of the circuit is calibrated and appropriate values are programmed in the microcontroller to provide the desired timing sequence. The supply to the relay is taken directly from the 12V supply and to the output current, a potentiometer (1 K $\Omega$ ) is used. The current level desired could be selected keeping the thumb wheel switch in position and adjusting the potentiometer to give the desired value of current through the cell.

### Preparation of guinea pig Skin and Wister Rat Skin:

Guinea pig which had been given free access to food and water were sacrificed by respiratory paralysis by chloroform immediately before experiment. The hairs of the guinea pig skin at dorsal side were removed with hair remover clipper 24 hr before experiment. The skin was carefully excised; adhering fat and other visceral debris were removed manually. Separated epidermis was washed with normal saline solution before starting the experiment.<sup>12</sup> Westar rat skin was prepared by similar way as of guinea pig skin. Protocol for animal study was approved by Institutional animal ethical committee (IAEC) of Y.B. Chavan College of Pharmacy, Aurangabad. (Proposal no: CPCSEA/IAEC/ PHARMACHEM.03/2009-10/13).

### Separation of human epidermis from human cadaver skin:

The human cadaver skin was obtained from Govt. Medical College and Hospital, Aurangabad. The epidermis was prepared as per method used by Bhatia, *et al.*, which involves soaking the whole skin in water at 60°C for 45 s. The skin was removed from water, blotted dry and pin with dorsal side down. The intact epidermis was teased off from the dermis with forceps, washed with water and used for the in-vitro permeation studies.<sup>13</sup>

### Preparation of thermo-reversible gel:

Gels were prepared by the cold method.<sup>14</sup> Gels containing 18%, 20%, and 22% w/v of Poloxamer 407 were prepared in distilled water to optimize the gelation temperature and viscosity (Table 1). Exactly 250 mg of drug was dissolved in 25ml distilled water and stirred using a Teflon-coated magnetic bead. Exactly 18%, 20%, 22% w/v of Poloxamer 407 was dispersed slowly into this drug solution, and the resulting mixture was then refrigerated at 4°C for 24 hr to

Table 1: Formulations of Poloxamer 407 thermo-reversible gel of RBZ.

Formulation Code	Poloxamer 407 concentration (%w/v)	Drug concentration (mg/ml)
A	18	10
B	20	10
C	22	10

obtain a completely hydrated, homogeneous, and clear sol. The sol was removed from refrigerator placed in water bath and temperature was slowly increased until it forms a completely hydrated, homogeneous, and clear gel.

#### Evaluation of thermoreversible gel:

##### Measurement of Gelation Temperature by Visual Inspection:

Each formulation was taken in 25 ml beaker containing magnetic bar and formulations were placed in a water bath. The beaker was heated and temperature was increased at a constant rate (2°C/min) with stirring. The temperature at which the magnetic bar stopped moving was recorded as a gelation temperature.<sup>15</sup>

##### Viscosity Measurement:

Viscosity of the gels were measured by using cup and bob type of viscometer i.e. by using Brookfield viscometer (Model No.CAP2000+, Spindle no.64) at different rpm and torque (Table 2).

Table 2: Evaluation parameters of Poloxamer 407 thermo-reversible gel

Formulation Code	Gelation Temperature (°C)	pH	Viscosity (cp)*
A (18% w/v)	32	5.3 ± 0.1	1632 ± 19
B (20% w/v)	28	5.4 ± 0.1	9156 ± 61
C (22% w/v)	27	5.6 ± 0.1	10130 ± 46

#### In vitro permeation studies

##### In vitro iontophoretic permeation (continuous current):

For the iontophoretic permeation with various current densities through thermoreversible poloxamer 407 gel, a Modified Franz diffusion cell was used with 20 ml of phosphate buffer (pH 7.4) as receptor medium. The whole assembly was maintained at 37±1°C. It was kept on a magnetic stirrer (100rpm) and a current was applied as per study using silver-silver chloride electrode. Samples (1ml) were withdrawn from the receptor compartment at hourly interval for a period of 8 hr and assayed for drug content by U.V. spectrophotometer (JASCO V-630) at 225 nm. For optimization of current density, permeation studies were carried out with continuous direct current (continuous DC) of current density 0.1 mA/cm<sup>2</sup>, 0.3 mA/cm<sup>2</sup> and 0.5 mA/cm<sup>2</sup>.

##### Effect of Pulsed current (ON: OFF ratio):

For optimization of type of current, permeation studies were carried out with pulsed direct current (pulsed DC) of 0.5

mA/cm<sup>2</sup> current density with ON: OFF ratio of 1:1, 1:2 and 1:4 seconds.

##### Synergistic effect of penetration enhancer and iontophoresis:

Further to enhance the iontophoretic permeation of RZB, it was used in combination with various penetration enhancers namely, ethanol, PEG-400, Tween-80, dimethyl sulphoxide (DMSO), and urea. Penetration enhancer (5%w/v) was incorporated in the gel, and the effect on iontophoretic permeation was studied.

##### Comparison of in vitro permeation through rat skin, guinea pig skin and human epidermis:

The optimized formulation (20% w/v poloxamer 407, 5% v/v PEG-400) showed very encouraging results with in vitro iontophoretic studies on guinea pig skin with pulsed DC 1:1 at 0.5 mA/cm<sup>2</sup>. The Iontophoretic permeation studies RZB through rat skin, guinea pig skin and human epidermis were carried out.

The human epidermis and animal's skin were prepared as per method described above and used for in-vitro permeation studies.

#### Data analysis

##### Cumulative amount of drug permeated (Q<sub>s</sub>):

It was calculated by using following equation. CADP at the end of 8<sup>th</sup> hr. was given as Q<sub>s</sub>

CADP = Total amount of drug permeated / Area of permeation

##### Steady state flux (J<sub>ss</sub>):

The cumulative amount of drug permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as steady-state flux (µg/cm<sup>2</sup>/hr).

##### Permeability coefficient (K<sub>p</sub>):

It can be calculated by following equation.

$$K_p = J_{ss} / Cd$$

Where,

K<sub>p</sub> = permeability coefficient.

J<sub>ss</sub> = steady state flux.

Cd = initial concentration of drug in donor compartment.

##### Enhancement Ratio (ER):

Enhancement ratio is calculated as per following equation.

ER = Q<sub>s</sub> with iontophoresis and penetration enhancer / Q<sub>s</sub> with iontophoresis only

**Statistical analysis:**

Statistical analysis was done by using one way ANOVA followed by Dunnet test using Graph Pad InStat 3 Demo software.

**Skin retention study (DR):** After the permeation experiments, the skin was cut into the number of pieces and shaken in 3 ml distilled water for 12 h, after which 1 ml sample was analyzed for RZB content.<sup>16</sup>

**RESULT AND DISCUSSION**

**Preparation and evaluation of thermoreversible gel:** The reversible sol-gel property allows the cool solution to flow onto the skin and spread across it during its transformation to a non-occlusive gel at body temperature. Furthermore, because of the poloxamer 407 solution's ability to form a hydrogel, it can show good electrical conductivity.<sup>17</sup> This property can be exploited for refillable unit dose iontophoretic drug delivery systems. Poloxamer 407 was selected because it forms a thermoreversible gel at the optimized iontophoretic conditions with acceptable viscosity and release characteristics. The viscosity of the polymeric solutions containing 18%, 20%, and 22% w/v of poloxamer 407 in distilled water was determined to assess their gelling characteristics. It was found that an increase in the concentration of poloxamer 407 increases the gelling property of the gel. Poloxamer 407, at 20% w/v forms the gel with a good viscosity to hold the formulation in the electrode cavity for application to the skin and showed optimum gelation temperature (Table 2).

**In vitro permeation studies:*****In vitro iontophoretic permeation (continuous current):***

Current density optimization study showed that  $Q_8$  increased with current density. In solution RZB gets ionized and acquires positive charge. The positive electrode in the donor compartment repels the positively charged RZB ions into the epidermis, so the permeation gets increased. The  $Q_8$  was found to be  $362.82 \pm 37.54 \mu\text{g}/\text{cm}^2$ ,  $1155.62 \pm 113.99 \mu\text{g}/\text{cm}^2$  and  $1256.87 \pm 148.16 \mu\text{g}/\text{cm}^2$  at 0.2 mA/cm<sup>2</sup>, 0.4 mA/cm<sup>2</sup> and 0.5 mA/cm<sup>2</sup> current density respectively. The statistical analysis of data was performed by ANOVA followed by Dunnet test which showed that there was no significant difference ( $p < 0.05$ ) in the  $Q_8$  for passive permeation and iontophoretic permeation at 0.2 mA/cm<sup>2</sup> but significant increase ( $p > 0.05$ ) in the  $Q_8$  was observed at 0.4 mA/cm<sup>2</sup> and 0.5 mA/cm<sup>2</sup>. The iontophoretic transport of RZB was linearly correlated to the applied current density the result accords with many reports and demonstrates that electrorepulsion is an important mechanism of RZB transport. When electrorepulsion plays an important role during iontophoresis, the

flux of the ion drug depends in a linear fashion on the applied current density.<sup>18,19,20</sup> So, we selected 0.5 mA/cm<sup>2</sup> as a current density for further studies.

***Effect of Pulsed current (ON: OFF ratio):***

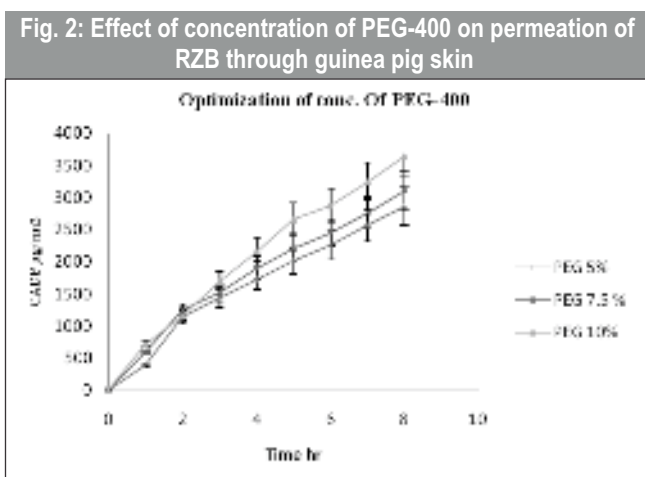
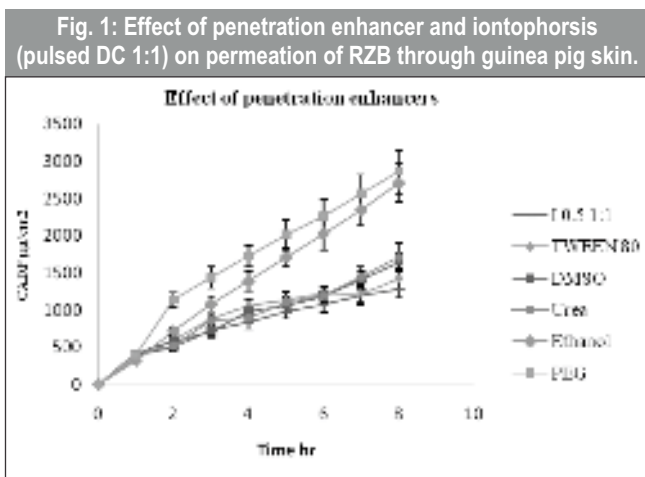
Optimization of type of current studies revealed that the pulsed DC of 0.5 mA/cm<sup>2</sup> with 1:1 ON: OFF ratio gives more permeation compared with continuous DC at 0.5 mA/cm<sup>2</sup>. Use of continuous DC for long period of time results in skin polarization, this can reduce the efficiency of iontophoretic delivery proportional to the length of continuous DC application. This can be overcome by using pulsed DC. It allows the skin to depolarize and return to its original electrical condition when current put OFF for fraction of second. There was significant increase ( $p > 0.05$ ) in the  $Q_8$  ( $1438.08 \pm 148.16 \mu\text{g}/\text{cm}^2$ ) with pulsed DC 1:1 at 0.5 mA/cm<sup>2</sup> as compared to continuous current ( $1256.87 \pm 148.16 \mu\text{g}/\text{cm}^2$ ). This might be because of continuous polarization and depolarization of skin due pulsed current. The results were significantly higher with DC 1:1 ON:OFF ratio because one second off time was sufficient for skin to regain its normal electrical properties. As the OFF time was increased more than one second then the permeation was decrease, it might be because the skin remained in a polarized condition for more time as continuous current. From the above results 0.5 mA/cm<sup>2</sup> 1:1 ON: OFF ratio pulsed DC was selected for further studies.

A pulsed waveform supposedly allows the skin to depolarize and return to its initial state before the onset of the next pulse. This is because the stratum corneum acts as a capacitor and this polarization may reduce the magnitude of a current applied as a constant current. It has also been suggested that pulsed current will be less irritating to the skin, so that patients could tolerate higher levels of current if pulsed DC at high frequency is used. It has been proposed that pulsed DC can result in lower skin resistance and higher drug delivery if the steady-state current during the 'on' phase of the pulse is very small and the frequency is low enough to allow depolarization of the skin during the 'off' phase. Therefore higher drug fluxes could be achieved with pulsed current than the equivalent DC current.<sup>21</sup>

Pulsed current was considered to be less damaging to the skin based on the passive flux of water across skin before and after iontophoresis for DC compared with pulsed current.<sup>22</sup>

***Effect of iontophoresis and penetration enhancer:***

Significant ( $p > 0.05$ ) increase in  $Q_8$  was obtained with the ethanol and PEG-400 when used in combination with iontophoresis. The  $Q_8$  obtained with ethanol and PEG-400 was  $2710.3 \pm 249.58 \mu\text{g}/\text{cm}^2$  and  $2862.52 \pm 289.91 \mu\text{g}/\text{cm}^2$  respectively (Figure-1). Combination of chemical enhancers



and iontophoresis leads to synergistic enhancement. The synergistic action may be due to skin delipidization by the penetration enhancers. Chemical enhancers have been used only to promote the drug flux across the skin. Alcohols, polyols are known to increase solubility and to improve partitioning coefficients. Ethanol, may extract lipids, making the stratum corneum more permeable. Propylene glycol have known to disrupt the horny layer intercalating into the structured lipids of the skin, which renders the structure more fluid and increases the diffusion coefficient of the permeant.<sup>23, 24</sup>

**Effect of concentration of PEG-400 and Ethanol:**

Significant ( $p > 0.05$ ) increase in  $Q_8$  and the  $J_{ss}$  obtained with the ethanol and PEG-400 as compared to other penetration enhancer (Figure-1). As concentration of PEG-400 increased there was significant ( $p > 0.05$ ) increase in  $Q_8$  (Table 3). Where as there was no significant ( $p < 0.05$ ) increase in the permeation of RZB when concentration of ethanol in the gel increases from the 5% v/v to 7.5% v/v. (Table-4)

**Comparison of *in vitro* permeation through rat skin, guinea pig skin and human epidermis:**

The optimized formulation (20% w/v poloxamer 407, 5% v/v PEG-400) showed very encouraging results with *in vitro* iontophoretic studies on guinea pig skin with pulsed DC 1:1 at 0.5 mA/cm<sup>2</sup> so, the permeation studies through various types of skin were carried out.

**Table 3: Permeation of RZB from Gel with PEG-400 as permeation enhancer in combination with iontophoresis (pulsed DC 1:1)**

Sr.no.	Permeation study	$Q_8$ , ( $\mu\text{g}/\text{cm}^2$ )	$J_{ss}$ , ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )	$K_p$ , (cm/hr)	ER	DR (mg)
1	PEG 5%	2862.52±289.91	251.43±25.1 25.	1±2.51	1	0.4349
2	PEG 7.5%	3109.49±285.99 <sup>a</sup>	257.69±21.5 <sup>a</sup>	2.57±2.15	1.08	0.4193
3	PEG 10%	3645.59±298.57 <sup>a</sup>	293.4±25.69 <sup>a</sup>	29.34±2.56	1.27	0.4235

Note:  $J_{ss}$ =steady state flux,  $K_p$ =permeability coefficient,  $Q_8$ =Cumulative drug permeated at the end of 8th hr. ER=enhancement ratio. DR: drug retain in skin. All the values are given in mean ±SD, n=3. a= significant difference

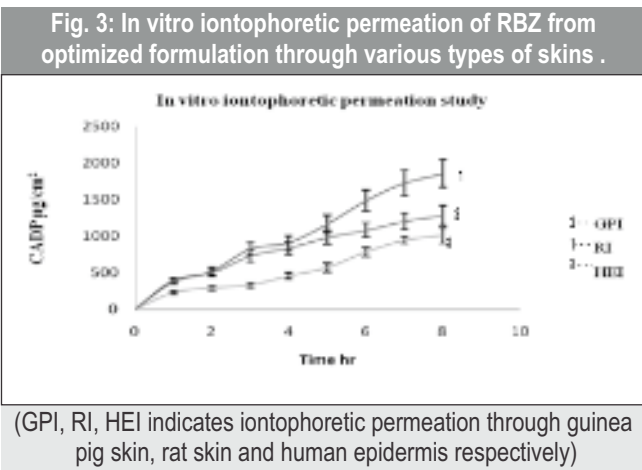
**Table No 4: Permeation of RZB from Gel with Ethanol as permeation enhancer in combination with iontophoresis (pulsed DC 1:1)**

Sr.no.	Permeation study	$Q_8$ , ( $\mu\text{g}/\text{cm}^2$ )	$J_{ss}$ , ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )	$K_p$ , (cm/hr)	ER	DR (mg)
1	Ethanol 5%	2710.3±249.58 <sup>b</sup>	236.89±21.5	23.6±2.15	2.11	0.4012
2	Ethanol 7.5%	2730.58±285.99 <sup>b</sup>	237.81±19.25	23.7±1.92	2.12	0.4193

All the values are given in mean ±SD, n=3. b= No significant difference.

Both of these studies showed that permeation through human epidermis is least among all the skin used. The  $Q_s$  through human epidermis, guinea pig skin and rat skin in passive study was  $186.82 \pm 9.5 \mu\text{g}/\text{cm}^2$ ,  $285.93 \pm 21.9 \mu\text{g}/\text{cm}^2$  and  $625.44 \pm 62.6 \mu\text{g}/\text{cm}^2$  respectively

The human epidermis showed least permeation amongst all types of skins used ( $p > 0.05$ ). This difference might be because of the difference in the number of hair follicles present on different type of skin. The rat skin has more number of hair follicles as compare to human skin and guinea pig skin (Table 5). The epidermal thickness of rat and human skin are  $18 \mu\text{m}$  and  $47 \mu\text{m}$  respectively which may reduce the permeation of drug.<sup>25</sup> N. Kanikkannan et al reported similar



**Table No 5: In vitro iontophoretic permeation of RZB from optimized gel formulation through various types of skins.**

Sr.no.	Permeation study	$Q_s$ , ( $\mu\text{g}/\text{cm}^2$ )	Jss, ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )	Kp, (cm/hr)	ER	DR (mg)
1	GPI	$1283.96 \pm 130.8$	$251.43 \pm 25.1$	$25.1 \pm 2.51$	1.00	0.4349
2	RI	$1856.24 \pm 189.6^a$	$192.58 \pm 17.7^a$	$19.2 \pm 1.77$	.40	0.5136
3	HEI	$1012.05 \pm 109.4^a$	$105.59 \pm 8.79^a$	$10.5 \pm 0.87$	0.28	0.5136

Note: GPI, RI, HEI indicates iontophoretic permeation through guinea pig skin, rat skin and human epidermis respectively.

results for iontophoretic permeation of timolol maleate.<sup>26</sup> The study by Esmail M. Niazy on penetration-enhancing effect of Azone through excised rat, guinea pig and human skins also showed similar results.<sup>27</sup>

**CONCLUSION**

The study reveals that Gel may be suitable delivery vehicles for iontophoresis of the drug. The iontophoresis in combination with penetration enhancer and pulsed current can increase the permeation of Rizatriptan Benzoate through the skin, thus minimizing the area of application.

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