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**Journal of Chemical and Pharmaceutical Research, 2017, 9(3):346-350**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Iontophoretic Delivery of Nebivolol Hydrochloride: Effects of Current Density, Chemical Enhancers, pH, Polymer Concentration

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**ABSTRACT**

*The purpose of the present study was to assess the feasibility of iontophoretic transdermal delivery of Nebivolol hydrochloride, an agent used in the treatment of hypertension with iontophoresis using Ag/AgCl electrodes. The effect of process variables and formulation variables like current intensity (0.05-0.5 mA/cm$^2$), pH, concentration of polymer (Eudragit L100, HPMC E15) and permeation enhancers (D-limonene and Tween 80) on the skin permeability were examined in in vitro skin permeation studies using rat abdominal skin as the membrane. Transdermal patch was formulated and subjected for in vitro studies and cumulative amount of drug permeated and flux across the rat abdominal were calculated. The results conclude that the flux increased with the current (44.46 µg/h/cm$^2$, $R^2$ 0.8732) and the combination of chemical enhancers with iontophoresis provided a synergistic effect on skin permeation. The results suggest that iontophoresis can be used as transdermal drug delivery of Nebivolol hydrochloride using patches with acceptable levels of current intensity.*

**Keywords**: Iontophoresis; Transdermal drug delivery; Nebivolol hydrochloride; Hypertension; Chemical enhances

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## INTRODUCTION

Nebivolol Hydrochloride is a ß1-receptor selective antagonist with vasodilatory property used in the management of the hypertension. Clinically oral administration is preferable, but the bioavailability of Nebivolol is 12%, mainly due to extensive hepatic metabolism and transdermal administration of Nebivolol is a possible solution to overcome this problem, however, there are no reports on iontophoretic delivery of Nebivolol [1].Transdermal delivery technologies are divided into active methods (physical and chemical) and passive methods [2]. For a drug to be delivered passively *via* the skin, it must have a molecular weight <500 and adequate lipophilicity [3, 4], because of low molecular weight and lipophilicity Nebivolol made a suitable candidate for transdermal drug delivery. However, the stratum corneum forms an effective barrier for the permeation of drugs, especially the poorly penetrating drugs must be modified while administrating with the help of penetration enhancers and iontophoresis. Iontophoresis defined as the facilitation of active therapeutic agents through the skin by applying low-level electric current (0.05 mA/cm$^2$) [5].The aim of the present study was to assess the possibility of transdermal delivery of Nebivolol using iontophoresis by examining the effect of polymer concentration, pH, current intensity and permeation enhancer on the permeability of Nebivolol across the rat abdominal skin as the membrane [6, 7, 8].

## MATERIALS AND METHODS

Nebivolol Hydrochloride was a gift sample from Aurobindo pharmaceuticals, Hyderabad, India. HPMC E15 and Eudragit L100 from Qualikems fine chemicals Ltd, Delhi, India, sodium hydroxide, phosphate buffer from Finar Chemicals, Ahmedabad, India. All other chemicals used were pure analytical grade.

**Preparation of skin**

The male albino rats weighing 200 g were sacrificed using ether. The hair was carefully trimmed short (<2mm) taking precaution not to damage the skin and the full thickness skin was removed from the abdominal region [9]. The pieces of skin obtained were wrapped individually in aluminium film and stored in freezer until use. The required pieces of skin were defrosted at room temperature, washed with water for hydration purpose and used for permeability studies.

**Methodology**

The *in vitro* skin permeation studies of Nebivolol hydrochloride solution was performed using Franz diffusion cells with rat abdominal skin. The donor solution was adjusted to of pH 6.8, 7, 7.4 using acid or base to examine the effect of pH [10]. Other experiments were performed at pH 7.4. To study the effect of polymer concentration transdermal patches were prepared with a blend of Eudragit L100 and HPMC E15 (1:2; 1:3; 1:5) polymers respectively, and are used to study the amount of drug release, whereas, 1:5 Eudragit L100: HPMC E15 polymer ratio was chosen for other experiments. To evaluate the effect of permeation enhancer on skin permeability different concentrations of permeation enhancers (3%, 5% D-Limonene and Tween 80) were used and for further experiments 5 % D-Limonene was used as penetration enhancers. To assess the combined effects of iontophoresis and permeation enhancer on the skin permeability using different current intensity (0.05-0.5 $mA/cm^2$) with 5 % D-Limonene as A chemical enhancer [11, 12,13].

**Skin permeation study**

The *in vitro* permeation study by iontophoresis was performed using Franz diffusion cells with rat abdominal skin as the membrane. Rat skin was mounted between the donor and receptor compartment in such a way that the stratum corneum facing the donor compartment. The donor and receptor chambers were filled with Nebivolol hydrochloride solution and 20 mL phosphate buffer solution (PBS). The temperature of the solution in the receptor was maintained at 37 ± 0.5°C and silver–silver chloride electrodes (Fine Chemicals) were placed on donor and receptor compartment as anode and cathode respectively. Both electrodes were connected to the electric current and a constant current was applied. Aliquots of 1 mL were collected at 1, 2, 3, 4, and 8 h and replaced with fresh PBS. The withdrawn samples were stored until analyzed by UV-Visible spectrophotometer to measure the concentration of Nebivolol hydrochloride at 282 nm [1].

Data were expressed as mean ± standard deviation (S.D.) and the cumulative amount of drug permeated against time was plotted, the flux was calculated from the straight line portion of the curve. Linear regression analysis was performed to observe the correlation between the steady-state flux and current intensity. The effects of pH, polymer concentration, permeation enhancer and current intensity on the cumulative amount of drug permeated were analyzed.

## RESULTS

***In vitro* permeability studies**

The effects of polymer concentration were shown in table 1. The amount of drug permeated across the skin with 1:2 and 1:3 ratios of Eudragit L100 and HPMC E15 were almost identical and the flux didn't change with different polymer ratio. Only with 1:5 ratio of polymer concentration the cumulative amount of drug permeated is maximized and considered for remaining experiments.

Table 2 shows the effect of pH solution present in the donor compartment induced with iontophoresis 0.5 $mA/cm^2$. The in vitro cumulative amount of Nebivolol permeated across the membrane increases with pH. The flux increased significantly at pH 7.4. Hence, pH 7.4 solution is used in the donor compartment for performing all relevant experiments except for the effect of pH study. Results of table 3 show the effect of chemical permeation enhancers in combination with iontophoresis (0.5 $mA/cm^2$) on the permeability of drug across the membrane. The flux was 1.52 times higher with 5 % D-Limonene compared with control. The flux didn't differ significantly between 3 % D-Limonene and control and also the flux didn't differ between 3 % Tween 80 and control. Initially the power was supplied for 1 h ahead disconnected and the drug permeation followed by passive diffusion.

**Table 1: Effect of polymer concentration in the patch on the amount of drug permeated across the membrane with constant permeation enhancer (% 5 D-Limonene) after 8 h**

| Eudragit L100 : HPMC E15 | Cumulative amount of drug permeated ($\mu g/cm^2$) | Flux $J_{ss}$ ($\mu g/h/cm^2$) |
|---|---|---|
| 01:02 | 1527.82±4.8 | 31.04±1.13 |
| 01:03 | 1676.28±9.52 | 31.6±0.56 |
| 01:05 | 1987.14±10.69 | 33.4±0.97 |

**Table 2: Effect of donor pH on the amount of drug permeated across the membrane with constant current density (0.5 mA/cm$^2$)**

| pH | Cumulative amount of drug permeated ($\mu g/cm^2$) | Flux $J_{ss}$ ($\mu g/h/cm^2$) |
|---|---|---|
| 6.8 | 779.14±7.95 | 22.4±0.31 |
| 7 | 845.2±9.5 | 26.5±0.46 |
| 7.4 | 940.62±7.5 | 29.2 ±0.57 |

**Table 3: Effect of penetration enhancers on Iontophoretic transdermal delivery of drug permeated and flux at constant current 0.5 mA/cm$^2$ and polymer concentration (1:5) after 8 h**

| Permeation enhancer | Cumulative amount of drug permeated ($\mu g/cm^2$) | Flux $J_{ss}$ ($\mu g/h/cm^2$) | ER (enhancement ratio) |
|---|---|---|---|
| Without permeation enhancer (control) | 1417.72±7.5 | 29.15±1.54 | 1 |
| 3 % D-Limonene | 1487.45±2.68 | 30.74±0.72 | 1.05 |
| 5 % D-Limonene | 2403.4±0.69 | 44.46±0.73 | 1.52 |
| 3 % Tween 80 | 1437.17±2.97 | 29.32± 0.64 | 1 |

**Table 4: Effect of current intensity mA/cm$^2$ on amount of drug permeated at different intervals of time from transdermal patch containing 5% D-Limonene as penetration enhancer**

| Time h | Cumulative amount of drug permeated ($\mu g/cm^2$) at 0.05 mA/cm$^2$ current intensity | Cumulative amount of drug permeated ($\mu g/cm^2$) at 0.25 mA/cm$^2$ current intensity | Cumulative amount of drug permeated ($\mu g/cm^2$) at 0.5 mA/cm$^2$ current intensity |
|---|---|---|---|
| 1 | 269.01±10.5 | 276.35±4.2 | 602.98±6.45 |
| 2 | 412.14±7.5 | 447.74±10.55 | 961.54±10.55 |
| 4 | 751.24±8.9 | 822.08±2.95 | 1529.28±11.12 |
| 8 | 1367.93±18.56 | 1585.4±12.56 | 2403.85±8.54 |
| Flux | 32.82±1.36 | 33.4±0.97 | 44.46±0.73 |

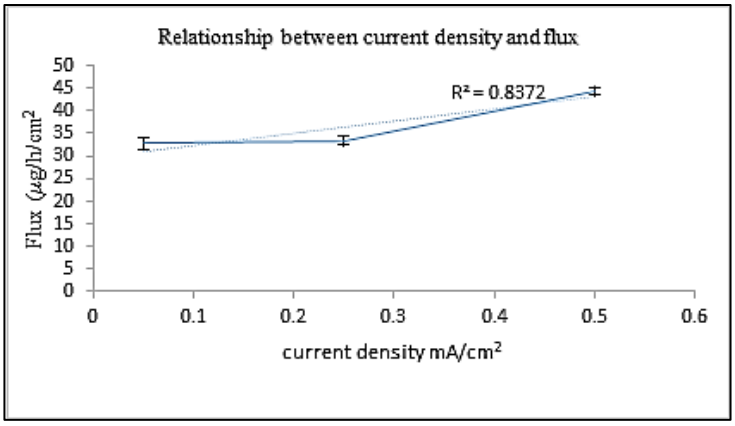


**Figure 1: Relationship between current intensity and flux**

The permeability of drug across the skin membrane from the patch using pH 7.4 donor solution, 1:5 polymer ratio and 5 % D-Limonene as a penetration enhancer at various current intensities were studied and the effects shown in Table.4. The amount of drug permeated increased with an increase in current intensity. The steady state flux produced by 0.05, 0.25 and 0.5 mA/cm$^2$ after 8 h were 32.82, 33.4 and 44.46 respectively. The permeability increased in a dependent manner with respect to current as shown in Table.4 and Fig.1. Regression analysis showed a good linear relationship between Nebivolol percutaneous flux and current intensity ($R^2$=0.8372). The results also show the effect of permeation enhancers in combination with iontophoresis on skin permeability. The steady state flux is 1.52 times higher with 5% D-Limonene.

## DISCUSSION

Iontophoresis is a method used to increase the permeability of drug by application of a small electric current. Iontophoresis is defined as the movement of ions under applied electric field. The iontophoretic delivery is possible at a pH at which relatively large proportion of drug is in an ionized form [4]. This technique allows the transdermal delivery of charged molecules compared with the passive approach [13]. In the present work, the permeation of Nebivolol from the patch through the rat abdominal skin was observed in the range of applied current intensity, from 0.05 to 0.5 mA/ $cm^2$. When no current was applied, the amount of the drug permeated was less than the one with applied current. The data indicate that the amount of electrical current applied plays a major role in the transdermal delivery of Nebivolol and suggest that iontophoresis is a promising drug delivery system through the skin for attaining therapeutic blood levels of the drug. In this work, the solubility of Nebivolol at the physiological pH was measured before the measurement of the effect of the donor pH on skin permeability. In the pH range of 6.8–7.4, the cumulative amount of drug permeated and the steady-state skin permeation flux of Nebivolol increased in a pH-dependent manner and were greatest at pH 7.4. The permeability flux at pH 7.4 was 1.32 times higher than that of pH 6.8 (Table.2). *In vitro* skin permeation flux of Nebivolol increased in a current dependent manner in the range of 0.05 to 0.5 mA/$cm^2$. The finding shows that the percutaneous delivery of Nebivolol can be controlled directly by varying the current strength. The use of chemical enhancers is one of the more widely considered techniques for increasing transdermal drug delivery. The penetration characteristics of different drugs can be modified by using various chemicals. To achieve higher drug penetration these chemicals can also be used in combination with iontophoresis. It was reported that D-limonene was the most outstanding penetration enhancer [14, 15] among other terpenes. It enhances the drug penetration by disrupting intercellular lipid and keratin and penetrates into the skin reversibly by reducing the barrier resistance. In addition Tween 80 increased the permeability of Nebivolol by changing the barrier function and thereby increasing the movement of anions through the skin. In this study, addition of D-Limonene at a concentration of 5% increased the permeability of Nebivolol. The work iontophoresis combined with permeation enhancers has often been performed by applying a high level penetration enhancer (5% D-Limonene) in the formulation of the patch is suitable for practical use and allow the drug to dissolve easily into drug donor solution across the rat abdominal skin at 0.5 mA/$cm^2$. Although transdermal iontophoresis offers potential the benefits by avoiding patient discomfort associated with oral administration with less variations based upon pre-programmed current supplied, but may cause skin irritation. However, the *in vitro* skin permeation is not relevant to evaluate skin irritation. Chemical enhancers in combination with iontophoresis increase the transdermal permeation rate synergistically.

## CONCLUSION

The present study establishes that iontophoresis can control the permeation flux of Nebivolol and deliver a therapeutic amount of Nebivolol at a pH 7.4 with a current-dependent manner. Thus, transdermal iontophoretic drug delivery is a potential alternative route for the treatment of hypertension.

## ACKNOWLEDGEMENTS

The authors are thankful to Aurobindo Pharmaceuticals for providing a gift sample, also thankful to management of college for providing facilities to carry out this work.

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