DESIGN AND DEVELOPMENT OF IONTOPHORETIC DRUG DELIVERY SYSTEM OF L-TYROSINE

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ABSTRACT: This study was aimed to design and develop iontophoretic drug delivery of L-Tyrosine in the treatment of phenylketonuria. The In-house iontophoretic drug delivery device was designed and validation was performed respect to Current intensity, Voltage, and Power Resistance. The study was also focused various parameters such as effect of different drug concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, 15mg/5ml) and effect of different current intensities (0.1mA/cm², 0.25mA/cm², 0.5mA/cm², 0.75mA/cm²). The results concluded there is a significant increase in Percentage of drug release in iontophoretic drug delivery of L-Tyrosine from 1-47 folds when compare to passive diffusion. The drug release increased up to certain concentration range after that if concentration increased means there is a decrease of drug release due to skin boundary saturation. Among the four drug concentrations 2.5mg/5ml,5mg/5ml showed good reliable release. 10mg/5ml, 15mg/5ml showed very less release due to saturation. Among the different current intensities the drug release increased with increase of current intensities.

INTRODUCTION: There has been a huge awareness in recent years of potential therapeutic importance of achieving true controlled drug delivery manner where the release rate of drug output may be modulated in a previously controlled, predictable manner. In that, the iontophoretic technique is one of the most desirable to enhance the transdermal drug delivery of high molecular weight substances like peptide and proteins using a lower current intensity with a short time period. Iontophoresis is the introduction by means of a direct electrical current of ions of soluble salts form of drug into the tissue or the body for therapeutic purposes. It is the novel technique used to increase the absorption of drugs across biological tissues such as the skin membrane. Electro migration and Electro osmosis is the major principles involved in the iontophoretic drug delivery process.

L-Tyrosine is an important amino acid. Under normal conditions the body synthesis sufficient amount of L-Tyrosine from phenylalanine. But in phenylketonuria condition, there is a severe deficiency level in the enzyme phenylalanine hydroxylase and this enzyme is responsible for the conversion of phenylalanine to tyrosine. This enzyme deficiency leads to need for the external

Keywords: L-Tyrosine, Iontophoresis, Transdermal, Current intensity, Electro osmosis

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supplementation of L-Tyrosine for the normal physiological process. 6, 7

Thus the objective of this study is to delivery L-Tyrosine through transdermal route for the therapeutic purposes at controlled, predictable manner.

MATERIALS AND METHODS:

Materials:
The drug L-Tyrosine was purchased from Loba Chemie Private Limited, Mumbai. All other chemicals and solvents were of analytical grade. All solutions were prepared in HPLC grade water Purchased from RFCL Limited, New Delhi.

Iontophoresis Set up Preparation:
The iontophoretic circuit was designed according to normal basic electric circuit method with reference to www.dadasheetcatalog.com/XTR using the basic electrical components such as electrical circuit motherboard, ammeter, anode, cathode, resistance, voltage stabilizer, and power connection box etc.. 8

Preparation of the skin:
Full thickness Youkshire Swine (albino pig, weighing around 90kg) porcine skin was obtained from local abattoir. The skin was rinsed using distilled water. The skin slices were taken from the epidermal side with the use of knife at the thickness of 750µm. then these slices were wrapped in parafilm and stored at -20°C in ultra low freezer (Remi) up to 4 weeks 9

Iontophoretic instruments and in-vitro permeation procedures:
Into Franz cell set up means the interconnection between iontophoresic devices with the Franz diffusion cell apparatus. In that, the power supply anode was connected to the donor compartment of Franz diffusion cell with anode wire such as Silver wire. The power supply cathode was connected through the receptor compartment of Franz diffusion cell with cathode wire such as Silver Chloride wire.

Initially, the current density was fixed in the circuit device with the help of adjustment pins present in the circuit device. Then the voltage was verified by using ammeter. The donor compartment was filled with the drug solution through the injection needle. The buffer solution was filled in the receptor compartment and the skin was mounted between the donor and receptor compartment. Further the anode and cathode wire dipped carefully in the donor solution compartment and receptor solution compartment respectively. Then power supply was switched on to activate the iontophoretic circuit device. The voltage and current density was verified each sampling time interval to maintain the constant level throughout the experimental conditions.

The in-vitro permeation procedure of iontophoresis was determined using Franz diffusion cells. The abdomen skin of excised male (8 weeks old) porcine skin was used as the model membrane. The receptor phase containing 16ml of pH 6.8 phosphate buffers was used. 10 The donor compartmental was filled with 5ml of drug solution with pH 4.7. It was selected to neutralize skin’s negative charge so as to avoid the interruption of skin changes during iontophoresis. The available diffusion surface area was 1.53cm².

The cells were agitated by magnetic stirrers at 500rpm. A pair of Ag/Agcl wires, having an effective length of 7.5cm used as electrodes were immersed in the cell with the anode in the donor and the cathode in the receptor. The electrodes were connected to current power supplier. Current density of 0.5mA/cm² was applied to stimulate the permeation of L-Tyrosine. 2 ml samples were withdrawn from the receptor at regular time intervals and immediately replaced by an equal volume of fresh receptor solution. The samples were assayed by spectrophotometrically at 274.7nm.

Validation of Designed Experimental Ionto-Franz Diffusion Setup:
The designed iontophoretic device circuit was validated with related to current density, voltage, and power resistance due to the safety precautions during experimentation and usage time. For this different length of anode and cathode was fixed and the current voltage was checked. The circuit current density and voltage was measured by using the ammeter at various time intervals. The difference
in voltage was minimized by using the stabilizer in the power supply connection. The validation of designed Experimental results showed in Table 1, 2, 3 with respect to length of anode & cathode wires, Total current density, coating amount of silver chloride on silver wire etc.

**TABLE 1: VALIDATION OF VOLTAGE OF CURRENT WITH RELATED TO LENGTH OF ANODE AND CATHODE WIRE**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Anode length in donor solution (cm)</th>
<th>Cathode length in receptor solution (cm)</th>
<th>Voltage of current</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>15.51</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.0</td>
<td>15.02</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1.5</td>
<td>11.28</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>2.0</td>
<td>9.07</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>7.51</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>3.0</td>
<td>6.88</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>3.5</td>
<td>5.38</td>
</tr>
</tbody>
</table>

**TABLE 2: VALIDATION RESULTS OF TOTAL CURRENT INTENSITY TOTAL CURRENT INTENSITY = CURRENT INTENSITY X SKIN TRANSPORT SURFACE AREA**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Current intensity mA/cm²</th>
<th>Skin Transport Diameter (cm)</th>
<th>Skin Transport surface area(cm²)</th>
<th>Total current intensity (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>1.4</td>
<td>1.5386</td>
<td>0.15386</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>1.4</td>
<td>1.5386</td>
<td>0.38465</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>1.4</td>
<td>1.5386</td>
<td>0.76930</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>1.4</td>
<td>1.5386</td>
<td>1.15395</td>
</tr>
</tbody>
</table>

**TABLE 3: VALIDATION RESULTS OF COATING OF SILVER CHLORIDE ON TO THE SILVER WIRE**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Total current intensity (mA)</th>
<th>Total In-vitro time (min)</th>
<th>Coating amount of Silver Chloride(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15386</td>
<td>240</td>
<td>3.29</td>
</tr>
<tr>
<td>2</td>
<td>0.38465</td>
<td>240</td>
<td>8.225</td>
</tr>
<tr>
<td>3</td>
<td>0.76930</td>
<td>240</td>
<td>16.45</td>
</tr>
<tr>
<td>4</td>
<td>1.15395</td>
<td>240</td>
<td>24.67</td>
</tr>
</tbody>
</table>

Preparation of L-Tyrosine drug solution for Iontophoretic drug development:

Different concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, and 15mg/5ml) of drug solutions were prepared using HPLC grade water for the investigate the effect of various concentrations on the iontophoretic drug delivery of L-Tyrosine.

Trial release study:

The trial release study was carried out by passive (without current) and iontophoresis at 0.5mA/cm² current density for the verification of possibilities of percutaneous diffusion of L-tyrosine through the transdermal iontophoretic drug delivery system.

**Investigations of Various Assessments on Iontophoretic Drug Delivery of L-Tyrosine:**

Current density, drug concentration on iontophoretic drug delivery of L-Tyrosine was also performed to assess the enhancement of percentage of drug diffusion.

**Effect of different Current Density on the same Concentration:**

Various current densities (0.1mA/cm², 0.25mA/cm², 0.5mA/cm², and 0.75mA/cm²) with constant drug concentrations of iontophoretic drug delivery of L-Tyrosine were performed to assess the improvement of percentage drug diffusion rate.

**Effect of different Concentration on the same Current density:**

Various drug concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, and 15mg/5ml) with same constant current density were performed to assess the improvement of % drug diffusion.

**UV spectrophotometry:**

Samples were analyzed at 274.7 nm using a shimadzu UV 1700 (Pharmaspec). The reference cell was filled with 2ml of phosphate buffer solution pH 6.8 and the absorbance set at zero. Each 2ml sample was diluted up to 5ml with PBS and the absorbance read. A set of standard solutions of L-Tyrosine in PBS (50, 40, 30, 20, 10µg ml⁻¹) was also prepared and determined and used to determine the amount and percentage L-Tyrosine released and the data plotted as a function of time.

**RESULTS AND DISCUSSION:**

**Trial Relese Study:**

The trial release study was performed between passive and iontophoresis using 0.5mA/cm² current density. In the Table 4 the passive showed 0.55±0.02 % of drug release and the iontophoresis showed 25.86±0.91 % of drug release at the end of 240 minutes. This release profiles showed (Figure 1) there is a significant increase of % drug release in the transdermal iontophoretic drug delivery of L-Tyrosine when compare to passive diffusion.
TABLE 4: COMPARISON OF PASSIVE (WITHOUT CURRENT) AND IONTOPHORETIC (WITH 0.5MA CURRENT DENSITY) IN-VITRO DIFFUSION PROFILE OF L-TYROSINE (TRIAL STUDY)

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Cumulative % of Drug Diffused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Passive (Without Current)</td>
</tr>
<tr>
<td>0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>180</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>210</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>240</td>
<td>0.55±0.22</td>
</tr>
</tbody>
</table>

FIGURE 1: COMPARISON OF PASSIVE (WITH OUT CURRENT) AND IONTOPHORETIC (WITH 0.5MA CURRENT DENSITY) IN-VITRO DIFFUSION PROFILE OF L-TYROSINE (TRIAL STUDY)

Effect of different Current Density on the same Concentrations:

Effect of different Current Densities on 2.5mg/5ml drug Concentration:

Effect of different Current Densities (0.1 mA/cm², 0.25 mA/cm², 0.5mA/cm², 0.75 mA/cm²) on the same constant drug concentration of 2.5 mg / 5 ml was showed 19.28±0.25, 24.84±0.65, 28.44±1.23, and 29.5±1.2 % of drug release at the end of 240 minutes. This confirmed (Figure 2) there is an increase of % drug release with the increase of current density on the transdermal iontophoretic drug delivery of 2.5mg/5ml drug concentration of L-Tyrosine.

Effect of different Current Densities on 5mg/5ml drug Concentration:

The Effect of different Current Densities (0.1 mA/cm², 0.25 mA/cm², 0.5mA/cm², 0.75 mA/cm²) on the same constant drug concentration of 5 mg / 5 ml was showed 17.4±0.7, 23.18±0.57, 25.86±0.91, and 28.31±2.16 % of drug release at the end of 240 minutes. This confirmed (Figure 3) there is an increase of % drug release with the increase of current density on the transdermal iontophoretic drug delivery of 5mg/5ml drug concentration of L-Tyrosine.

Effect of different Current Densities on 10mg/5ml drug Concentration:

The Effect of different Current Densities (0.1 mA/cm², 0.25 mA/cm², 0.5mA/cm², 0.75 mA/cm²) on the same constant drug concentration of 10 mg / 5 ml was showed 12.4±0.99, 14.45±0.86, 15.15±0.74, and 19.2±2.19 % of drug release at the end of 240 minutes. This confirmed (Figure 4) there is an increase of % drug release with the increase of current density on the transdermal
iontophoretic drug delivery of 10mg/5ml drug concentration of L-Tyrosine.

**Effect of different Current Densities on 10mg/5ml Drug Concentration:**
The effect of different Current Densities (0.1 mA/cm², 0.25 mA/cm², 0.5mA/cm², 0.75 mA/cm²) on the same constant drug concentration of 10 mg/5 ml was showed 3.2±0.31, 3.38±0.26, 4.08±0.45, and 5.15±0.9 % of drug release at the end of 240 minutes. This confirmed (Figure 5) there is an increase of % drug release with the increase of current density on the transdermal iontophoretic drug delivery of 10mg/5ml drug concentration of L-Tyrosine.

**Effect of different drug concentrations on the same constant current density:**

**Effect of different drug concentrations on 0.1mA/cm² Constant current density:**
The effect of different Drug Concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, 15mg/5ml) on the same constant Current Density of 0.1mA/cm² was showed 19.28±0.25, 17.4±0.7, 12.4±0.99, and 3.2±0.31 % of drug release at the end of 240 minutes. This confirmed (Figure 6) there is no increase of % drug release with increase of drug concentration on the same constant 0.1mA/cm² current density of L-Tyrosine.
The effect of different Drug Concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, 15mg/5ml) on the same constant Current Density of 0.5mA/cm² was showed 28.44±1.23, 25.86±0.91, 15.15±0.74, and 4.08±0.45 % of drug release at the end of 240 minutes. This confirmed (Figure 8) there is no increase of % drug release with increase of drug concentration on the same constant 0.5mA/cm² current density of L-Tyrosine.

Effect of different drug concentrations on 0.75mA/cm² constant current density:
The effect of different Drug Concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, 15mg/5ml) on the same constant Current Density of 0.75mA/cm² was showed 29.5±1.2, 28.31±2.16, 19.2±2.19, and 5.15±0.9 % of drug release at the end of 240 minutes. This confirmed (Figure 9) there is no increase of % drug release with increase of drug concentration on the same constant 0.75mA/cm² current density of L-Tyrosine.

CONCLUSION: This research work mainly aimed to investigate the effect of current on permeation of L-Tyrosine through porcine skin having low passive diffusion. The results revealed that the usage of iontophoretic current have led to significant increase in % drug release (Percutaneous absorption), when compare to passive diffusion. The comparison of trial study of both passive and iontophoresis using 0.5mA/cm² current density showed there is a significant increase in % of drug release in the transdermal iontophoretic drug delivery of L-Tyrosine from 1-47 folds. The effect of different current intensities (0.1mA/cm², 0.25mA/cm², 0.5mA/cm², 0.75mA/cm²) confirmed that there is an increase of % drug release with the increase of current density on the transdermal iontophoretic drug delivery of L-Tyrosine.

The effect of different drug concentrations (2.5mg/5ml, 5mg/5ml, 10mg/5ml, and 15mg/5ml) confirmed that there is no increase of % drug release with increase of drug concentration on the same constant current intensity of L-Tyrosine. This may be due to system saturation.

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