ABSTRACT
Bisoprolol is a cardio selective β-blocker. It is given as the fumarate in the management of hypertension and angina pectoris. On oral administration, the drug under goes extensive first pass metabolism. Delivery of bisoprolol via transdermal route would minimize some of the deficiencies associated with the oral delivery and increase the bioavailability of the drug. In the present study, matrix type transdermal patches containing bisoprolol were prepared using different ratios of Ethylcellulose (EC) and Eudragit RL-100 by mercury substrate method. In vitro diffusion studies were performed for all formulations and in vitro skin permeation studies were performed for selected formulations on Pig skin by using Franz type diffusion cell. Diffused drug was quantified by UV-Spectrophotometer at 223 nm in phosphate buffer. The possible drug and polymer interaction was studied by IR Spectroscopy. All the formulations were subjected to physicochemical studies such as weight variation, thickness, drug content, moisture uptake, moisture loss and folding endurance.

KEYWORDS: Transdermal patch, Bisoprolol fumarate, in-vitro diffusion.

INTRODUCTION
Drugs administered in the conventional dosage forms usually produce large range fluctuations in plasma drug concentrations leading to undesirable toxicity or poor effectiveness. This factor as well as other factors such as repetitive dosing and unpredictable absorption, led to the concept of the controlled drug delivery system or therapeutic system.

A dosage form that releases one or more drugs continuously in a predetermined pattern for a fixed period of time, either systemically or to a specified target organ is a controlled drug delivery system. The primary objective of controlled drug delivery is to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing.  

Transdermal therapeutic systems are defined as self-contained discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at controlled rate to the systemic circulation.

METHOD AND MATERIALS
Bisoprolol fumarate was received as gift sample from Unichem Pharmaceuticals Pvt. Ltd Baddi (Himachal Pradesh). Eudragit RL-100 and ethyl cellulose were received as gift sample from Helios pharmaceutical pvt ltd Baddi, dibutyl phthalate purchased from thermo electron Ilsindia Pvt Ltd Mumbai, dichloromethane purchased from central drug house Pvt Ltd New Delhi, disodium hydrogen phosphate and potassium Dihydrogen phosphate purchased from S.D fine chem. Limited Mumbai, mercury from nice chemical Pvt. Ltd kerala, calcium chloride from Qualchem fine chemicals Pvt. Ltd New Delhi and ammonium chloride purchased from RFCL limited A-3 Okhla new Delhi, all the chemicals purchased were of high purity.

Preparation of transdermal patch
Matrix type transdermal patch containing Bisoprolol fumarate was prepared by using mercury substrate method. The polymers (total weight = 900 mg) were weighed in requisite ratio and dissolved in 10 ml of dichloromethane (solvent). Then drug was added slowly in the polymeric solution and stirred on the magnetic stirrer to obtain a uniform solution. In this mixture 5 drops (0.3 ml) of Dibutyl phthalate (Plasticizer) was added and mixed uniformly. Then the solution was poured on mercury placed in a glass Petri dish of area 47.75 cm². The petri dish was covered with an inverted funnel for uniform evaporation. The Petri dish was kept

FORMULATION AND EVALUATION OF MATRIX TYPE TRANSDERMAL PATCH OF BISOPROLOL FUMARATE BY MERCURY SUBSTRATE METHOD

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aside for drying at room temperature for 24 hrs. After drying the films were removed by using sharp blade by inserting along the edges of the film. The dried films were cut into 2x2 cm² patches, wrapped in butter paper and stored in desiccators until used.

Table 1: Formulation Ingredients of Bisoprolol Transdermal Patches.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisoprolol fumarate (mg)</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Eudragit RL 100 (mg)</td>
<td>900</td>
<td>_</td>
<td>450</td>
<td>675</td>
<td>225</td>
</tr>
<tr>
<td>Ethyl cellulose (mg)</td>
<td>_</td>
<td>900</td>
<td>450</td>
<td>225</td>
<td>675</td>
</tr>
<tr>
<td>Dichloromethane (ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Dibutyl phthalate (ml)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Determination of solubility**
The solubility of Bisoprolol fumarate was determined in different solvent medium i.e distilled water, methanol and dichloromethane. Excess of drug was added to known volume of the solvent and agitated for 24 hrs on a mechanical shaker, equilibrated at 37 ± 0.5ºC. The content was filtered. The 1 ml filtrate solution was suitably diluted with respective solvents and analyzed by UV-Visible spectrophotometer.

**Drug-excipients interaction studies**
The FT-IR spectrum was recorded for drug and physical mixture of drug and polymer in the wavelength range 4000 – 400 cm⁻¹. The I.R spectra were compared and compatibility was studied.

**EVALUATION PARAMETERS**[2,10]
The transdermal patches prepared were evaluated for following parameters:

**Physical appearance**
All the prepared transdermal patches were visually inspected for colour, clarity and surface texture.

**Weight variation test**
The patches of size 2x2 cm² were cut from the film and weighed individually using single pan electronic balance. The average weight of patch was calculated.

**Thickness**
The thickness of patches was measured at three different places using vernier caliper. Average and standard deviation was calculated.

**Tensile strength and % elongation**
Tensile strength and percentage elongation at breaking point of film were measured using a pully based tensile strength apparatus. The apparatus consist of a base plate with a pully aligned on it. The films were cut into strips (15 x 2 cm). The film was fixed in insert holder at one end of the base plate and another end is kept with the help of forceps having triangular end to keep the film straight during stretching. A thread was tied to the triangular end and passed over the pully to which a small pan is attached to hold weights. A small pointer is attached to the thread that travels on the graph paper affixed on the base plate. The weights are gradually added to the pan till the film is broken. The weight necessary to break the film is noted as force to break the films and the simultaneously distance travelled by the pointer on the graph paper indicated the elongation at break. The tensile strength was calculated by the formula;

\[
\text{Tensile strength} = \frac{\text{Cross sectional area}}{\text{Film length}}
\]

The percent elongation was determined by noting the length just before the break point using the formula;

\[
\% \text{ Elongation} = \left( \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \right) \times 100
\]

**Folding endurance**
Folding endurance of patches was measured manually by repeatedly folding a small strip of film (2 cm x 2 cm) at the same place till it break. The number of time the film could be folded at the same place without breaking gave the value of folding endurance.

**Percentage moisture loss**
The patches were weighed individually and kept in a desiccators containing calcium chloride. The final weight was noted when there was no change in the weight of individual patch. Percentage of moisture content was calculated as weight difference between initial and final weight, with respect to initial weight. It is expressed as follows;

\[
\% \text{ Moisture loss} = \left( \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100
\]

**Percentage moisture uptake**
The patches were weighed accurately and placed in desiccators where a humidity condition of 80-90% RH was maintained by using saturated solution of ammonium chloride. The patches were kept until uniform weight is obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight, with respect to initial weight. It is expressed as follows;

\[
\% \text{ Moisture uptake} = \left( \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \right) \times 100
\]
Drug content uniformity
The patches were tested for the content uniformity. The patch of size 2 cm$^2$ was cut and each dissolved in 100 ml of phosphate buffer (pH 6.8) by using magnetic stirrer for 24 hrs. Subsequent dilutions were made with phosphate buffer. The absorbance was then measured at 223 nm. From the absorbance and the dilution factor, the drug content in the film was calculated. The experiment was repeated three times to validate the result.

In-vitro skin permeation study
A fresh pig skin was purchased from the local market and transferred to lab within one hour in 0.9% normal saline solution and washed thoroughly before use. Fatty layer completely removed from the skin by using a sharp blade or by skin grafting handle. Fresh pig skin mounted between clamped donor and receptor cells of modified version of Franz diffusion cell in such a way that its epidermal surface faced the donor compartment and dermal surface faced to receptor compartment. Cell was placed on magnetic stirrer in holding position. The receptor compartment was filled with 18 ml of freshly prepared phosphate buffer solution (pH 6.8) and stirred using Teflon coated magnetic beads. Drug solution (2 ml) was placed to the epidermal side of skin in donor cell and stirring of the receptor fluid (jacketed with water at 34±1°C) was started as shown in the “Figure 1”. At appropriate intervals, 2 ml samples were withdrawn from the receptor compartment and withdrawn sample volume was replaced with equal volume of fresh phosphate buffer solution to ensure sink conditions. Withdrawn samples were analyzed using spectrophotometer (Shimadzu, UV Visible-1800 Spectrophotometer) by measuring absorbance at $\lambda_{max}$ of 223 nm. Each experiment was continued for about 24 hours and was performed at least in triplicate.

Figure 1: In-vitro diffusion cell fitted with Pig Skin.

RESULTS AND DISCUSSION
Transdermal patches of bisoprolol fumarate were prepared by mercury substrate technique. The prepared transdermal patches were transparent, smooth, uniform and flexible. The solubility of the bisoprolol fumarate was found to be 1gm/ml. The characteristic peaks of bisoprolol fumarate were not affected in the presence of polymer and prominently observed in IR spectra. From the data, it was calculated that there was no interaction between drug and polymer. Drug content in different formulations was found to be 89.5 to 97.9%. The results of weight variation test indicated uniformity in weight of the patches, as evidenced by SD values, which were found in variation between 1.15mg to 3.05mg. Thickness of patches was varied from 20.33 ± 1.52mm to 23.66 ± 2.08mm. The thickness was found to be maximum for F1 and minimum for F2 formulation Table. This was due to the viscosity variation of the polymeric solutions used in the polymeric films making. The percentage moisture absorbed ranged from about 2.91 ± 0.051 to 4.47 ± 0.065% w/w. The moisture absorption was found to be more in formulation F5, F3 and F2. The moisture absorption was less in patches formulated with EC polymer. Low moisture uptake protects the material from microbial contamination and bulkiness of the patches. The percentage moisture loss ranged from about 2.87 ± 1.422 to 4.47 ± 0.065% w/w. It was observed that the percentage moisture loss increased with increasing hydrophilic nature of polymers Fig.1. Folding endurance was found to be satisfactory for all formulations. The % elongation was found in the range of 33.53% to 111.29%. The results are shown in table 11. The order of % elongation of patches are F5 < F2 < F3< F4 < F1. The tensile strength was found to be in the range of 1.697 to 2.841 kg. The results obtained from all the formulation and order of tensile strength of patches are F2 < F5 < F3< F4 < F1. From the results, it can be concluded that as the concentration of Eudragit RL 100 polymer is increased, the tensile strength of patch is also increased. The % elongation was found in the range of 33.53% to 111.29%. The results are shown in table 11. The order of % elongation of patches are F5 < F2 < F3< F4 < F1.
Table No. 2: Weight uniformity, thickness, tensile strength and % elongation variation test.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Weight uniformity (mg)</th>
<th>Thickness (mm)</th>
<th>Tensile strength (kg)</th>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>69.57 ± 1.52</td>
<td>23.66 ± 2.08</td>
<td>2.84 ± 0.10</td>
<td>111.2 ± 0.96</td>
</tr>
<tr>
<td>F2</td>
<td>68.66 ± 2.08</td>
<td>20.33 ± 1.52</td>
<td>1.69 ± 0.08</td>
<td>49.66 ± 1.52</td>
</tr>
<tr>
<td>F3</td>
<td>69.18 ± 2.51</td>
<td>20.66 ± 3.21</td>
<td>1.84 ± 0.09</td>
<td>80.56 ± 1.15</td>
</tr>
<tr>
<td>F4</td>
<td>69.33 ± 1.15</td>
<td>21.03 ± 1.08</td>
<td>2.22 ± 0.10</td>
<td>95.63 ± 1.20</td>
</tr>
<tr>
<td>F5</td>
<td>68.95 ± 3.05</td>
<td>20.37 ± 2.51</td>
<td>1.82 ± 0.12</td>
<td>33.53 ± 1.36</td>
</tr>
</tbody>
</table>

Table No. 3: Folding endurance, percent moisture loss, percent moisture uptake and drug content variation test of different formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Folding endurance</th>
<th>% Moisture loss</th>
<th>% Moisture absorption</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>157 ± 7.505</td>
<td>2.94 ± 0.305</td>
<td>2.91 ± 0.051</td>
<td>97.9 ± 0.84</td>
</tr>
<tr>
<td>F2</td>
<td>87 ± 6.12</td>
<td>2.87 ± 1.422</td>
<td>3.37 ± 0.788</td>
<td>89.5 ± 0.09</td>
</tr>
<tr>
<td>F3</td>
<td>98 ± 6.327</td>
<td>3.42 ± 0.796</td>
<td>3.45 ± 0.771</td>
<td>92.9 ± 0.07</td>
</tr>
<tr>
<td>F4</td>
<td>12 ± 6.027</td>
<td>3.02 ± 0.051</td>
<td>3.01 ± 0.066</td>
<td>96.5 ± 0.06</td>
</tr>
<tr>
<td>F5</td>
<td>92 ± 4.932</td>
<td>4.47±0.065</td>
<td>4.47±0.065</td>
<td>91.7±0.20</td>
</tr>
</tbody>
</table>

**In vitro skin permeation study**

*In vitro* skin permeation study of all the formulations F1-F5 were carried out by using Franz diffusion cell. Formulations F1 to F5 contain the polymers Eudragit RL 100 and Ethyl cellulose in different ratio. These polymers were used alone and in combination. Formulation F1 containing polymer Eudragit RL100 alone showed the release (67.05%) in 24 hrs. Formulation F2 containing only Ethyl cellulose showed the minimum release (38.17%) in 24 hrs. Formulation F3 containing polymers Eudragit RL100 and Ethyl cellulose in combination with ratio (1:1) showed the maximum release (84.32%) in 24 hrs. Formulation F4 containing polymers Eudragit RL100 and Ethyl cellulose in combination with ratio (3:1) showed the release (66.30%) in 24 hrs which is comparable as of formulation F1. Formulation F5 containing polymers Eudragit RL100 and Ethyl cellulose in combination with ratio (1:3) showed the release (50.06%) in 24 hrs. The release profiles from various patches were in the following order: F3 > F1 > F4 > F5 > F2

The release profiles of these formulations are given in table 13-17. The formulation F3 showed highest release with polymers Eudragit RL 100 and Ethyl cellulose in ratio 1:1. Formulation F2 showed lowest release with polymer Ethyl cellulose used alone. Ethyl cellulose attributed to the relatively hydrophobic nature of polymer which was having less affinity for water, results decreased drug release and skin permeation. *In vitro* release profile of formulations F1-F5 are shown in “Figure 2”.

**CONCLUSION**

Bisoprolol fumarate an antihypertensive agent is selected for the preparation of transdermal delivery system as it complies with physicochemical properties required to permeate through skin. The suitable analytical method based on UV-Visible spectrophotometer was validated for bisoprolol fumarate. λ max 223 nm was identified in phosphate buffer solution (pH 6.8). The pre-formulation studies involving description, solubility, melting point, partition coefficient of the drug were found to be comparable with the standard. The FTIR and UV visible spectrophotometer shows that bisoprolol fumarate did not interfere with the polymers used. The patches were prepared by mercury substrate method using different polymers Eudragit RL 100 and Ethyl cellulose. The patches were subjected for following evaluation parameters such as physical appearance, weight variation, thickness, folding endurance, drug content, percentage moisture loss, percentage moisture uptake, tensile strength, in vitro skin permeation studies. All the parameters shows were within the limits. Based on results, formulation F3 was considered as the best.
formulation containing Eudragit RL 100: Ethyl cellulose in ratio (1:1). Formulation F3 showed better drug release 84.03% in 24 hrs. The stability study indicated no significant physical changes in the prepared formulation. The evaluation parameters and the pattern of release were also not altered.

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REFERENCE