ABSTRACT

Many monoamine oxidase inhibitors (MAOIs) have been used to treat major depressive disorder (MDD). However, the prescription of MAOIs has decreased considerably as a result of side effects such as tyramine-induced hypertensive crisis, known as “Cheese Effect”. The drug delivery system itself can affect the efficacy and bioavailability of certain drugs when medications are given by oral, intravesical and intravaginal routes. Therefore there is a need for advanced drug delivery techniques that can avoid toxic effects and improve the bioavailability at the same time. In this context, the transdermal patches of selegiline hydrochloride (SH) were prepared by the solvent casting method using hydroxy propyl methyl cellulose (HPMC), polyvinyl alcohol (PVA) and methyl cellulose (MC) as reservoir polymers in different ratios (1:1, 1:2 and 1:3). Rate controlling membrane was caste by using 2% ethyl cellulose (EC) membrane. The prepared patches possessed satisfactory physicochemical characteristics. The formulation exhibited flexibility, uniform weight, thickness, smoothness, drug content (93 to 97 %), little moisture loss and moisture absorption. The in-vitro permeation studies in phosphate buffer (pH 7.4) revealed formulations released drug in the range of 86.77 to 96.79% and followed diffusion mechanism. Formulations with 1:1 and 1:2 ratio released drug up to 10 to 20 h only. The optimized formulation F6 containing PVA (1:3) showed good release rate of 90.08 % for 24 h. The patches were seemingly free of potential hazardous skin irritation. FT-IR and DSC studies revealed no interaction between the drug and polymers used.

Keywords: Selegiline hydrochloride, In-vitro permeation study, Polyvinyl alcohol, Transdermal patch

INTRODUCTION

A recent approach to drug delivery is to deliver the drug into systemic circulation at predetermined rate using skin as a site of application. A transdermal drug delivery (TTDS) is a formulation or device that maintains the blood concentration of the drug within the therapeutic window ensuring that drug levels neither fall below the minimum effective concentration nor exceed the minimum toxic dose. TTDS promises many advantages over oral and/or intravenous administration, such as better control of blood levels, a reduced incidence of systemic toxicity, avoids hepatic first-pass metabolism and improves patient compliance. An ideal drug to be formulated as transdermal drug delivery should possess several physico-chemical prerequisites, such as short half-life, small molecular size and low dose. However, the highly organized structure of stratum corneum forms an effective barrier to the permeation of drugs, which must be modified if poorly penetrating drugs are to be administered. Selegiline hydrochloride, a preferential MAO-type B inhibitor, is currently used in the treatment of depression. Selegiline hydrochloride has steady state half life of 2h, oral dose of 10mg daily, oral bioavailability 4.4% and protein binding of 94%. Selegiline is readily absorbed from gastrointestinal tract from conventional preparations and crosses the blood brain barrier. It undergoes extensive first pass metabolism in the liver to produce at least 5 metabolites excreted mainly in the urine and about 15% appears in the faeces. Oral tablet produces side effects like, nausea, vomiting,
constipation, diarrhea, dry mouth, sore throat and difficulty in micturition. The amphetamine metabolites of Selegiline may cause insomnia and abnormal dreams; evening doses should be avoided. Transient increases in liver enzymes have been reported. Mouth ulcers and stomatitis may occur with the oral lyophilisate. Selegiline is interacted with tyramine in food. To improve its therapeutic efficacy, bioavailability, patient compliance and as well as to reduce the frequency of dosing and side effects, the transdermal drug delivery approach was considered to be better suitable for Selegiline hydrochloride.

The aims of the present study were to (1) design and develop transdermal patches of Selegiline hydrochloride with various ratios of drug: polymer combinations (2) perform physicochemical characterization and in-vitro permeation studies through rat skin. The purpose was to provide the delivery of the drug at a controlled rate across intact skin to improve efficacy and bioavailability of drug.

MATERIALS AND METHODS

Selegiline hydrochloride was obtained as a gift sample from Embio Limited, Mumbai. Hydroxy propyl methyl cellulose was gifted from Colorcon Ltd, Goa. Polyvinyl alcohol, Ethyl cellulose and Methyl cellulose were purchased from S.D Fine Chemicals Pvt Ltd, Mumbai. All other chemicals and reagents used were of analytical reagent grade.

PREPARATION OF DRUG RESERVOIR

The polymeric solution was prepared by dissolving the required quantity of polymer in distilled water (2.5 ml) and glycerin (30% w/w of polymer) was added as plasticizer to this solution with stirring. The weighed amount of Selegiline hydrochloride was added to the above solution. After proper mixing the casting solution was poured in a clean glass bangle (an area of 9.61 cm²) which was placed on the mercury surface. The films were dried at room temperature for 24 h. The dried patches thus obtained were cut by cork borer into circular discs of definite size of 20 mm diameter (an area of 1.539 cm²) containing 10 mg of drug (Table 1).

PREPARATION OF RATE CONTROLLING MEMBRANE

The rate controlling membrane was prepared separately by dissolving required quantity of ethyl cellulose in chloroform. Dibutyl phthalate (30% w/w of polymer) was added as plasticizer. The polymeric solution was poured on a clean glass petridish and dried at room temperature for 12 h. Circular discs of 20 mm diameter were cut using cork borer (Table 1).

FABRICATION OF TRANSDERMAL PATCHES

The reservoir films containing the drug were sandwiched in between the rate controlling membranes. They were fixed by applying chloroform on the edges of the rate controlling membrane.

EVALUATION OF TRANSDERMAL PATCHES

Physicochemical properties such as thickness, weight variation, moisture content, moisture absorption, folding endurance, content uniformity and tensile strength were observed for the prepared Selegiline hydrochloride transdermal patches.

Table 1: Composition of Selegiline hydrochloride transdermal patches

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>HPMC (mg)</th>
<th>PVA (mg)</th>
<th>MC (mg)</th>
<th>Water (ml)</th>
<th>Glycerine (ml)</th>
<th>Rate limiting membrane ethyl cellulose (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>63</td>
<td>63</td>
<td>–</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>63</td>
<td>120</td>
<td>–</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F3</td>
<td>63</td>
<td>180</td>
<td>–</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F4</td>
<td>63</td>
<td>–</td>
<td>63</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F5</td>
<td>63</td>
<td>–</td>
<td>120</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F6</td>
<td>63</td>
<td>–</td>
<td>180</td>
<td>–</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F7</td>
<td>63</td>
<td>–</td>
<td>–</td>
<td>63</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F8</td>
<td>63</td>
<td>–</td>
<td>–</td>
<td>120</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>F9</td>
<td>63</td>
<td>–</td>
<td>–</td>
<td>180</td>
<td>2.5</td>
<td>0.015</td>
<td>2</td>
</tr>
<tr>
<td>Casting Solvent</td>
<td>Water (2.5 ml)</td>
<td>Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPMC-hydroxy propyl methyl cellulose, PVA-polyvinyl alcohol, MC-methyl cellulose.
Physical appearance
All the prepared transdermal patches were visually inspected for color, clarity, flexibility and smoothness.

Thickness
Patch thickness was measured using micrometer at three different places and the mean value plus standard deviation (S.D.) was calculated.7

Uniformity of weight
The film was cut into 5 patches of 1 cm² each and their average weight was calculated. Percentage deviation from average weight for each patch was also determined.8

Percentage moisture loss
The films were weighed accurately and kept in a desiccators containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture loss was calculated using the formula:

\[
\text{Percentage moisture loss} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100
\]

Percentage moisture absorption
The films were weighed accurately and placed in the desiccators containing 100 ml of saturated solution of potassium chloride, which maintains 80–90% RH. After 3 days, the films were taken out and weighed. The study was performed at room temperature.9 The percentage moisture absorption was calculated using the formula:

\[
\text{Percentage moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100
\]

Folding endurance
It was determined by repeatedly folding a small strip of film at the same place till it breaks. The number of times, the film could be folded at the same place without breaking gave the folding endurance value.10

Drug content
The patches (1 cm²) were cut and added to a beaker containing 100 ml of phosphate buffered saline of pH 7.4. The medium was stirred with magnetic bead for 4 h. The contents were filtered using whatmann filter paper and the filtrate was examined for the drug content against the reference solution consisting of placebo films (contains no drug) at 258 nm spectrophotometrically.11

Tensile Strength
Tensile strength of the film was determined with Universal Strength Testing Machine (Hounsfield, Slinfoeld, Horsham, U.K). The sensitivity of the machine was 1 g. It consisted of two load cell grips. The lower one was fixed and upper one was movable. The test film of size (4/1 cm²) was fixed between these cell grips and force was gradually applied till the film broke. The tensile strength of the patch was taken directly from the dial reading in kg/cm². Tensile strength is expressed as follows12:

\[
\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross section area}}
\]

Preparation of the rat skin
The experiment was conducted according to the protocol approved by the institutional animal ethics committee (IAEC). The experiment was conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animal). The male albino rats were sacrificed by decapitation. The fresh abdominal skin was excised from male albino rat weighing 170–190 g. The abdominal skin of excised hairless rat skin was separated along the epidermal junction. The hair of skin was removed using depilatories. The process of the removal of hair did not alter the skin properties and delivery of the drug. It was kept at water bath maintained 60°C for exactly 50 s. The heat treated skin was cleared of its subcutaneous fatty substance and immediately kept in refrigerator at 10°C. This step maintained integrity and viability of the skin.13

In-vitro permeation studies
In-vitro skin permeation studies were performed by using a Keshery-Chein diffusion cell with a receptor compartment capacity of 75 ml. The excised rat abdominal skin was mounted between the donor and receptor compartment of the diffusion cell. The transdermal patches were placed over the skin. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5°C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal. The cumulative amounts of drug permeated per square centimeter of patches were plotted against time.14

Skin irritation studies
The skin irritation test was performed on healthy albino rabbit weighing 3.5 kg. Aqueous solution of formalin 0.8% was used as standard irritant. Drug free polymeric patch of 20 cm² was used as test patch. 0.8% of formalin
was applied on the left dorsal surface of rabbit, where as the test patch was placed on identical site, on the right dorsal surface of the rabbit. The patches were removed after a period of 24 h with the help of alcohol swab. The skin was examined for development of erythema /edema.15

**Kinetic study**

To know the mechanism of drug release from the transdermal patches, the data were treated according to zero-order (percentage of drug released vs time), first-order (log percentage of drug to be released vs time) and Higuchi’s (percentage of drug released vs square root of time) release pattern.16

**Fourier-transform infrared spectroscopy (FT-IR) studies**

The compatibility between drug and polymer was detected by FT-IR spectra (Shimadzu 8400, Japan). The pellets were prepared on KBr-press (spectra lab, India). The spectra were recorded over the wave number range of 4000 to 500 cm\(^{-1}\).

**Differential scanning calorimeter (DSC) studies**

Thermograms were obtained by using a differential scanning calorimeter (SD600, TA instrument, USA) at a heating rate of 10°C/min over a temperature range of 35–250°C. The sample was hermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 10 ml/m for maintaining inert atmosphere.

**Stability studies**

The stability studies of formulated transdermal patches were carried out at 40±2°C/75±5% RH over a period of 60 days. The selected transdermal patches were wrapped in aluminium foil and stored in stability chamber.

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**RESULT AND DISCUSSION**

**Physicochemical characteristics of patches**

All the patches prepared with different drug: polymer concentration (1:1, 1:2 and 1:3) were found to be flexible, smooth, opaque, non-sticky and homogeneous in nature. This may be due to the presence of plasticizer. Marginal difference in thickness was observed among each group indicating that thickness of the transdermal patches was found to be directly proportional to the polymeric concentration. The calculated standard deviation values were very low suggesting that the prepared transdermal patches were uniform in weight. All the nine patches showed good folding endurance which proved that the patches have good flexibility (Table 2).

Moisture absorption studies revealed that as the concentration of polymers increased from 1:1 ratio to 1:3, the moisture absorption also increased. Among all the patches, F6 (PVA) patch showed high moisture content. This may be due to the hydrophilic nature of the PVA. The least percentage of moisture absorption was observed for F9 patch (MC) because of less hydrophilic nature of methyl cellulose than PVA and HPMC. In contrast to moisture absorption studies the opposite was observed in percent moisture loss study. The percent moisture loss decreased as the polymer concentration in the patch increased and the same was observed with all the polymers used.

As the ratio of concentration of drug: polymer is increased tensile strength also increases. PVA patch showed better tensile strength due to the nature of polymer. There was no significant difference in the drug content among all the patches, indicating content uniformity (Table 2).

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**Table 2: Physicochemical evaluation of Selegiline hydrochloride transdermal patches**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Thickness++ (mm)</th>
<th>Weight variation++ (mg)</th>
<th>Moisture Loss* (percent)</th>
<th>Moisture Absorption* (percent)</th>
<th>Folding endurance++ (Nos)</th>
<th>Drug content* (percent)</th>
<th>Tensile strength++ (Kg/mm(^2))</th>
<th>Cumulative percentage of drug permeated*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.23±0.01</td>
<td>27.14±0.44</td>
<td>12.63±0.18</td>
<td>12.36±0.18</td>
<td>98</td>
<td>94.50±0.27</td>
<td>0.30±0.05</td>
<td>93.20</td>
</tr>
<tr>
<td>F2</td>
<td>0.29±0.01</td>
<td>36.19±0.39</td>
<td>12.60±0.13</td>
<td>12.55±0.19</td>
<td>131</td>
<td>94.05±0.22</td>
<td>0.32±0.03</td>
<td>95.37</td>
</tr>
<tr>
<td>F3</td>
<td>0.36±0.00</td>
<td>47.85±0.45</td>
<td>12.55±0.20</td>
<td>12.63±0.09</td>
<td>189</td>
<td>95.32±0.32</td>
<td>0.35±0.01</td>
<td>88.11</td>
</tr>
<tr>
<td>F4</td>
<td>0.18±0.01</td>
<td>26.80±0.49</td>
<td>12.68±0.37</td>
<td>12.49±0.18</td>
<td>105</td>
<td>95.87±0.17</td>
<td>0.47±0.06</td>
<td>95.19</td>
</tr>
<tr>
<td>F5</td>
<td>0.20±0.01</td>
<td>35.76±0.44</td>
<td>12.60±0.28</td>
<td>12.60±0.28</td>
<td>161</td>
<td>96.15±0.28</td>
<td>0.49±0.02</td>
<td>96.79</td>
</tr>
<tr>
<td>F6</td>
<td>0.22±0.01</td>
<td>45.20±0.40</td>
<td>12.49±0.11</td>
<td>12.72±0.11</td>
<td>210</td>
<td>96.70±0.10</td>
<td>0.50±0.05</td>
<td>90.08</td>
</tr>
<tr>
<td>F7</td>
<td>0.20±0.02</td>
<td>26.16±0.66</td>
<td>12.83±0.10</td>
<td>12.26±0.19</td>
<td>101</td>
<td>93.40±0.27</td>
<td>0.43±0.05</td>
<td>92.68</td>
</tr>
<tr>
<td>F8</td>
<td>0.23±0.01</td>
<td>35.14±0.36</td>
<td>12.67±0.14</td>
<td>12.39±0.14</td>
<td>151</td>
<td>93.95±0.28</td>
<td>0.45±0.03</td>
<td>95.37</td>
</tr>
<tr>
<td>F9</td>
<td>0.28±0.01</td>
<td>42.79±0.39</td>
<td>12.74±0.23</td>
<td>12.52±0.11</td>
<td>191</td>
<td>94.05±0.22</td>
<td>0.46±0.01</td>
<td>86.77</td>
</tr>
</tbody>
</table>

*All values are expressed as mean±SD, ++ n=5, * n=3.
**In-vitro permeation studies**

In-vitro permeation study revealed that formulations prepared with drug: polymer ratio 1:1, 1:2 and 1:3 exhibited release for 12h, 20h and 24h respectively (Figure 1). The transdermal patches released the drug in the range of 86.77% (F9) to 96.79% (F5). The patches prepared with 1:3 drug: polymer ratio provided prolong and controlled release of drug for a period of 24 h for all the polymers used in the study. In case of these patches, as the polymer concentration is more compared to 1:1 or 1:2 ratios, the thickness of patches is more and that contributes to the controlled and prolonged release of drug up to 24 h. The amount of drug released in patches prepared with 1:1 and 1:2 ratios was more compared to 1:3 (Table 2). To get once-a-day formulation for selegiline hydrochloride drug: polymer ratio 1:3 was used for further studies. Among the three polymers used, PVA controls and releases more amount of drug than HPMC and MC. This may be attributed to hydrophilic nature of the polymer which has more affinity for water resulting in increased thermodynamic activity of the drug in the patch. Transdermal patch F6 prepared with PVA (ratio 1:3) released 90.08% of drug compared to F3 (HPMC) – 88.11% and F9 (MC) – 86.77% (Figure 2). Patch containing HPMC and MC showed less percent of drug release compared to PVA, which may be due to their less hydrophilic nature. The release profile was correlated with the moisture absorption which further reflected the nature of polymers. From the study it was observed that approximately 85–90% of drug was released in 24 h and formulation F6 was selected as optimized formulation. Hence, the developed transdermal patches control and extend the release of drug for desired period of time.

**Skin irritation studies**

Results of the skin irritation studies revealed that optimized Selegiline hydrochloride transdermal patch (F6) did not cause any noticeable sign of erythema or edema on rabbit skin throughout the period of 24 h (Figure 3 a, b). Hence, the optimized transdermal patch F6 was found to be compatible with the skin.

**Kinetic study**

To know the mechanism of drug release from the formulations, the data were treated according to zero order, first-order and Higuchi’s equation. When the cumulative amount of drug permeated was plotted against time, the permeation profiles of the drug followed first-order kinetics with higher regression values ($r^2 = 0.848–0.903$). The in vitro release profiles of the formulations did not fit into zero-order kinetics.

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**Figure 1:** In-vitro drug permeation of Selegiline hydrochloride transdermal patches.
Figure 2: In-vitro drug permeation of Selegiline hydrochloride from selected formulations.


Figure 3: (a) Photograph showing rabbit with applied transdermal patch.

Figure 3: (b) Photograph of rabbit showing no signs of redness or erythema after removal of the patch.

\( r^2 = 0.840–0.894 \) as the \( r^2 \) values were less. However, when the release profiles of the formulated patches were subjected to Higuchi’s equation high \( r^2 \) values were obtained \( (r^2 = 0.991–0.995) \), which indicates that the permeation of the drug from the patches was governed by a diffusion mechanism. In this context, the results obtained from the \( r^2 \) values support the results of Higuchi’s equation and the theory that the patches release the drug by a diffusion-dominated mechanism indicating that as the time increases; the diffusion path length also increases (Table 3).

Fourier-transform infrared spectroscopy (FT-IR) studies

FT-IR spectrophotometer was used to study interaction between the drug and excipients in the formulations. FT-IR study of pure drug and optimized transdermal patch F6 was carried out to assure the compatibility between drug and excipients. The pellets were prepared on KBr press. The spectra were recorded over the wave range of 4000 to 500 cm\(^{-1}\). FT-IR spectra of the pure drug showed significant bands at 3230 cm\(^{-1}\) for C-H stretching of C=C-H, 2943 and 2812 cm\(^{-1}\) C-H
stretches of CH₂ and CH₃ groups, 2123 cm⁻¹ C=C stretching, 1450 and 1371 cm⁻¹ C-H bonding of CH₃ and CH₂ groups, 1371 cm⁻¹ C-N stretching and 765 cm⁻¹ for monosubstituted phenyl ring. The spectrum of transdermal patch F6 indicates match between functional groups of pure drug with formulation and confirms the purity of the drug in the patch (Figure 4 a, b).

**Differential scanning calorimeter (DSC) studies**

Drug interaction can be studied by thermal analysis using the DSC thermograms. DSC thermogram of pure selegiline hydrochloride exhibits a sharp endothermic peak at 143°C, which corresponds to its melting point. DSC thermogram of optimized transdermal patch F6 exhibits a sharp endothermic peak at 145°C. The difference in the melting point is negligible and the
Table 3: $r^2$ values of F3, F6 and F9 Selegiline hydrochloride transdermal patches

<table>
<thead>
<tr>
<th>Code</th>
<th>F3</th>
<th>F6</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.892</td>
<td>0.894</td>
<td>0.840</td>
</tr>
<tr>
<td>First order</td>
<td>0.900</td>
<td>0.903</td>
<td>0.848</td>
</tr>
<tr>
<td>Higuchi model</td>
<td>0.991</td>
<td>0.992</td>
<td>0.995</td>
</tr>
</tbody>
</table>

small change may be due to the polymer and excipients present in the formulation. Hence DSC study showed that there was no any drug polymer interaction (Figure 5 a, b).

Stability studies

Stability studies of selected transdermal patches F3, F6 and F9 showed that, there was no significant change in physical characteristics and drug content after 60 days of study (Table 4). Based on these results it was concluded that the formulated transdermal patches were found to be physically and chemically stable during the study period.

CONCLUSION

Thin, flexible, smooth and transparent films were obtained by solvent casting method with HPMC,
PVA and MC polymers using glycerin as plasticizer. The physicochemical properties like thickness, weight and drug content of all patches were uniform with low SD values. The formulations with 1:3 ratio provided 24 h release with all polymers. Among all the prepared patches F6 (PVA-1:3 ratio) showed good skin permeation profile over a period of 24 h. Skin irritation studies revealed the compatibility of patches with rabbit skin. All the selected formulations followed Higuchi’s equation indicating diffusion-dominated mechanism. The transdermal patches were found stable during stability period. Based on the encouraging results, the Selegiline hydrochloride transdermal patches can be used as a controlled drug delivery system and frequency of administration can be minimized to reduce toxicity and increase efficacy. Though the efforts were made for the development of Selegiline hydrochloride transdermal patches, long term pharmacokinetic and pharmacodynamic studies in higher animals and controlled clinical studies on human beings can be carried out in future to establish the usefulness of these patches.

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