EFFECT OF NATURAL ENHANCERS IN TRANSDERMAL PERMEATION OF FLUOXETINE

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ABSTRACT

Matrix-type transdermal drug delivery system of fluoxetine was prepared by solvent evaporation technique. Several batches were prepared by using different proportion of ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) as a film former and dibutyl phthalate as a plasticizer. Eucalyptus oil and olive oil in three different concentrations i.e. 2, 5 and 10% were also employed as a natural enhancer to enhance the skin permeation of fluoxetine. All the prepared patches were subjected to physical characterizations (like thickness, weight variation, drug contents, moisture content, moisture uptake and flatness), in vitro drug release studies and in vitro skin permeation studies. All the formulations exhibited satisfactory physicochemical characteristics. In vitro skin permeation study showed that eucalyptus oil was a more promising enhancer than olive oil. The increases in concentration of eucalyptus oil further enhanced drug permeation with maximum flux being achieved at 10% w/w of 36.70 µg/cm²/h. The results of present study suggested that the formulation FE3 containing 10% w/w of eucalyptus oil could be feasible of delivering fluoxetine across skin.

Keywords: Natural enhancer, transdermal patches, fluoxetine, eucalyptus oil, olive oil, flux.

INTRODUCTION

The transdermal route of drug administration is recognized as one of the potential route for the local and systemic delivery of the drug. Transdermal drug delivery system can deliver medicines via the skin portal to systemic circulation at a predetermined rate and maintain clinically effective concentration over a prolonged period of time. This route of drug administration avoid the hazards and discomfort associated with parenteral therapy and improves patient compliance, as it is easy to remove also.¹ The bioavailability of the drug increased as variation in absorption when it is taken orally and its first pass metabolism by the liver is avoided.

Treatment can also be terminated rapidly by simply removing the patch when need to curtail drug delivery arises. Transdermal delivery may also eliminate side effects of that drugs cause when presented in conventional forms.² Fluoxetine is a potential selective serotonin-reuptake inhibitor, which blocks the serotonin reuptake pump of the neuronal membrane and thereby increasing the concentration of serotonin on 5HT₁A autoreceptors. Fluoxetine is used in several indications like depression, panic disorder, post-traumatic stress, bulimia nervosa and premenstrual dysphoric disorder.³ The oral dose of its hydrochloride salt is administered in ranges

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from 20 to 80 mg as 1 to 4 times a day. But fluoxetine has several drawbacks due to its frequency and dose may cause side effects related with drug and may produce patient compliance and it also has gastrointestinal side effects when orally administrated like dyspepsia, abdominal pain, nausea, vomiting, constipation and diarrhoea are more common side effects.\(^4\)

**MATERIALS AND METHODS**

**Materials**
Fluoxetine was purchased from Yarrow Chem Pvt. Ltd, Mumbai, India. Polyvinylpyrrolidone, ethylcellulose, chloroform, dibutyl phthalate and tween-80 were supplied from S.D. Fine Chem. Ltd., Mumbai, eucalyptus oil and olive oil was purchased from Central Drug House, New Delhi. All other chemicals and reagents used were of analytical grade and procured from an authorized dealer.

**PREPARATION OF TRANSDERMAL PATCHES**
Transdermal films of fluoxetine containing different ratio of EC and PVP were prepared by solvent evaporation technique, composition is given in Table-1.

The polymers were weighed in required amount and then dissolved in chloroform used as solvent. The Di-n-butyl phthalate 30% (w/w) to the total dry weight of polymers was used as a plasticizer. The drug was added in the homogeneous dispersion, by slow stirring with a mechanical stirrer for 30 minutes. On the basis of preliminary studies results, the optimized batch (F4) was mixed with the eucalyptus oil and olive oil in three different concentrations i.e. 2, 5 and 10% as a permeation enhancers. The polymers-drug dispersion was poured into a circular aluminium foil cup placed in petri dish and solvent was allowed to evaporate at room temperature for 24 hrs. The dry films were kept in a desiccator until used.\(^5\)

**PHYSICOCHEMICAL EVALUATION OF TRANSDERMAL PATCHES**

**Thickness**: Thickness of patches was measured by using digital thickness gauge (Muttato Japan) with a four different points and average thickness was taken.\(^6\)

**Weight variation**: The weight variation study was performed by individually weighing 10 randomly selected patches (4.52 cm\(^2\)) on digital weighing balance and average weight was calculated.\(^7\)

**Drug content**: To determine the drug content of patch, a known amount of patch was cut from casted film and dissolves in chloroform in 100 ml volumetric flask and placed in shaking incubator for 4 hrs. The solution was filtered and analysed at 227 nm by UV spectrophotometer (Model-1700, Shimadzu, Japan).\(^8\)

**Moisture content**: To determination of moisture contents of transdermal patches were weighed individually kept in a desiccator containing calcium chloride at room temperature for 24 hrs and weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.\(^9\)

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>EC (%)</th>
<th>PVP (% w/w)</th>
<th>Permeation Enhancers (% w/w)</th>
<th>Eucalyptus Oil</th>
<th>Olive Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>95</td>
<td>05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>80</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>66.67</td>
<td>33.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>60</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F5</td>
<td>40</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FE1</td>
<td>60</td>
<td>40</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FE2</td>
<td>60</td>
<td>40</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FE3</td>
<td>60</td>
<td>40</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FO1</td>
<td>60</td>
<td>40</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>FO2</td>
<td>60</td>
<td>40</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>FO3</td>
<td>60</td>
<td>40</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Plasticizer, dibutyl phthalate (30% w/w of the polymer). The drug content in 2 cm\(^2\) of the film is 10 mg.
Moisture uptake:
Transdermal patches were kept in desiccators at room temperature for 24 hrs with silica gel and weighed and transfer to another desiccators to exposed of 75% RH using a saturated solution of sodium chloride at 25°C and patches were reweighed again and again, until a constant weight was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.\[6\]

Flatness:
Longitudinal strips from the 5 randomly selected transdermal films of each formulation were cut out. One from the centre and one from the other side. The length of each strip was measured and the variation in length because of the non-uniformity of flatness was measured. 0 %
constriction was considered to be 100 % flatness.\textsuperscript{[10]}

**Folding endurance**: Folding endurance of patches was determined by repeatedly folding a small strip of patch (approximately 2×2 cm) at the same place till it broke. The number of times patch could be folded at the same place, without breaking gave the value of folding endurance and it was recorded.\textsuperscript{[11]}

**IN VITRO RELEASE-DISSOLUTION STUDIES**

Dissolution rate test apparatus (USP-II) was used to carry out dissolution studies. The commercially available water impermeable adhesive backing membrane was applied over the transdermal film and it was further fixed on glass slide (2.3x2.3 cm) by using cyanoacrylate adhesive. Then the transdermal film was covered with dialysis membrane and placed in a dissolution vessel carrying 900 ml of 20% methanol in PBS pH 7.4 solution. The apparatus was equilibrated at a temperature 32 ± 0.5°C and operated at 50 rpm. Sample were withdrawn at appropriate time interval upto 24 hrs and analyzed at 227 nm by UV spectrophotometer. Cumulative percent released drug was calculated and plotted against time.\textsuperscript{[12-13]}

**IN VITRO SKIN PERMEATION STUDIES**

Permeation studies were carried out for different formulations across excised rat skin in Franz-type diffusion cell at 32 ± 0.5°C. The effective surface area of the diffusion cell was 2 cm\(^2\). Excised rat skin was mounted between the donor and receptor compartments of the diffusion cell with stratum corneum facing the donor compartment. The stratum corneum side of the skin was kept in intimate contact with the transdermal patch to be tested. The 20% methanol in phosphate buffer pH 7.4 solution (11 ml) was used as diffusion medium to ensure sink conditions. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously at 50 rpm using a magnetic bead and temperature was maintained at 32 ± 0.5°C. The samples were withdrawn at different time interval upto 24 hrs and analyzed spectrophotometrically at 227 nm. The flux was calculated as the amount of drug permeated per sq. cm per hour.\textsuperscript{[14]}

The effectiveness of permeation enhancers was determined by comparing drug flux in presence and absence of each permeation enhancer and obtained ratio was known as the enhancement factor (EF)\textsuperscript{[15]}

**STATISTICAL ANALYSIS**

The formulation parameters were statistically evaluated by Graph pad prism 5 using one-way analysis of variance (ANOVA), followed by Dunnett test multiple comparison tests and unpaired t-test. A difference below the probability level of 0.05 was considered statistical significant. The obtained results were expressed as the mean ± standard deviation.

**RESULTS AND DISCUSSION**

**PHYSIOCHEMICAL EVALUATION**

The formulated patches were found clear, smooth and flexible in their physical appearance. The weight of transdermal patches varied from 164.37 to 172.01 mg which indicated that the prepared transdermal films of different batches were similar in weight. The thicknesses of different batches were found in range from 0.246 to 0.273 mm. A low standard deviation value in the film thickness measurement ensures the uniformity of formulated patches. No significant (p>0.05) difference in drug content was observed for all the formulated patches which were found from 94.12 to 98.23%. The percentage of moisture content and moisture uptake were found in the range of 1.64 ± 0.31 to 6.38 ± 1.04 and 2.43 ± 0.55 to 9.4 ± 0.75 respectively. The results indicated that by increasing in concentration of hydrophilic polymer (PVP) both percentages of moisture content and moisture uptakes were increases (Figure 1). The similar results also found by other works.\textsuperscript{[16]} The 100 % flatness of all the patch formulations indicated that there was no amount of constriction in formulated transdermal patches. The folding endurance was measured manually and was found in the range from 34 - 48. The results of folding endurance indicated that the patches did not break and would maintain their integrity with general skin folding when used.\textsuperscript{[17]} The results of physiochemical characterizations are given in Table-2.
Fig. 2. In vitro dissolution profile of fluoxetine from transdermal patches containing eucalyptus oil 2% (FE1), 5% (FE2) and 10% (FE3).

Fig. 3. In vitro dissolution profile of fluoxetine from transdermal patches containing olive oil 2% (FO1), 5% (FO2) and 10% (FO3).

Fig. 4. In-vitro permeation studies of fluoxetine from transdermal patches of FD4 (without enhancer), FE3 (10% eucalyptus oil) and FO2 (5% olive oil).
IN VITRO RELEASE-DISSOLUTION STUDIES
The importance of dissolution studies for the transdermal patches is very crucial to confirm sustained release pattern and to known the ability of duration and rate of drug release from matrix patches. It was observed that, the cumulative amount of drug release from control formulations (without enhancer) F1, F2, F3, F4 and F5 were found 40.70, 46.68, 52.38, 59.66 and 50.61 % respectively in 24 hrs. The greatest percentage of drug release (59.66%) was observed from formulation F4 (EC/PVP, 3:2) which was significantly (p>0.05) highest from lowest value observed from formulation F1. Therefore the formulation F4 was selected as optimized formulation for incorporation of eucalyptus oil and olive oil in three different concentrations i.e. 2, 5 and 10% as permeation enhancers in order to enhance permeation.

The transdermal patches containing 2% (FE1), 5% (FE2) and 10% (FE3) of eucalyptus oil were showed 68.58, 76.83 and 83.52% of drug release in 24 hrs respectively. It was observed that concentration of eucalyptus oil increased, the cumulative amount of drug release was also increased as shown in Figure-2. This may be due to presence of terpene constituents in eucalyptus oil. The eucalyptus oil containing cineol as a principal terpene which increases the drug release, this is probably due to the increase in saturation solubility of drug in matrix.

In case of formulations containing olive oil, the cumulative % of drug release was also increased with increased in concentration of olive oil from 2 to 5% (64.50 to 73.08% respectively). This may be due to presence of fatty acids in olive oil. Further increased in concentration of olive oil to 10%, the % of drug release was found to be decreased (67.93 %) as shown in Figure-3. This may be attributed to decrease in solubility of drug in presence of high concentration of oil in matrix, which cannot increase solubility of drug significantly in release media as previously reported for vegetables oils. The percentage of drug release from FE3 and FO2 were significantly (p<0.05) higher than control formulation F4.

IN VITRO PERMEATION STUDIES
On the basis of in vitro dissolution studies, the best formulations FE3 and FO2 was selected from both batches for in vitro permeation study (Figure-4). The effect of permeation enhancers on fluoxetine permeation from transdermal patches was investigated and compared with control formulation F4 (without enhancer). The several studies have been reported that, the essential oil like eucalyptus oil enhance the several time permeability of both lipophilic as well as hydrophilic drug. This is probably attributed to binding on the stratum corneum and enhancement of the lipophilic drug penetration by increasing the partition coefficient and hydrophilic drug penetration by increasing diffusion coefficient. The result of fluoxetine permeation a lipophilic drug also support above observation. The formulation FE3 containing 10% eucalyptus oil demonstrated a flux of 36.70±2.63 µg/cm²/h an enhancement of 3.64 times, as compared to control formulation F4 which demonstrated a flux of 10.06±0.86 µg/cm²/h.

The formulation FO2 containing 5 % olive oil as a permeation enhancer showed a flux of 19.85±1.31µg/cm²/h an enhancement of 1.97 times. The mechanism of penetration enhancement of olive oil is primarily believed to be due to the promotion of membrane-vehicle partitioning tendency of the drug with the oils. It is also reviewed that penetration of the vegetables oil into the intracellular lipid phase of the membrane may also increase the degree of fluidity in this phase resulting in a decreased resistance to permeation, which can in turn increase flux. The flux obtained from formulations FE3 and FO2 was significantly (p<0.05) higher than control formulation F4. Although not as good as eucalyptus oil, the olive oil also exhibited good enhancing effects.

CONCLUSION
From this study, it can be concluded that matrix type transdermal therapeutic systems of fluoxetine could be prepared with eucalyptus oil as a permeation enhancer to delivering fluoxetine across skin. The formulation FE3 was found to optimum transdermal patch of fluoxetine and it may be used for further pharmacokinetic and

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pharmacodynamic studies in suitable animal models.

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