Formulation and evaluation of transdermal patch containing amphotericin B

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Abstract
Transdermal drug delivery system are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. These devices allow for pharmaceuticals to be delivered across the skin barrier. The human skin is a readily accessible surface for drug delivery. It is an alternative to oral delivery and hypodermic injection. In addition, transdermal system are non-invasive and can be self administered. Amphotericin B is effective against serious or life-threatening systemic fungal infection but not to treat less serious fungal infection of mouth, throat or vagina. It is insoluble in water and having high permeability through stomach. This results in poor bioavailability after oral administration. Therefore transdermal patch containing Amphotericin B was formulated to increase its solubility, bioavailability and patient compliance. solvent casting method was used to formulate transdermal patch. PEG was selected as the permeation enhancer and Glycerin was selected as plasticizer for the formulation and mixed with different concentration of polymeric casting solution which contains HPMC (polymer at different ratios) and Chloroform:Methanol at 1:1 ratio used as solvent. Formulated transdermal patch were then evaluated for drug content, weight variation, flatness, folding endurance, % moisture content, tensile strength, and physical appearance. The in vitro drug diffusion studies revealed that transdermal patch formulation that is F1 shows better drug release profile (97.87%) when compared with other formulations.

Keywords: Amphotericin B, Transdermal Patch.
drugs. Polyethylene Glycol (PEG) has shown to be effective as permeation enhancer for many drugs [4].

The purpose of this research work is to formulate transdermal patch of Amphotericin B using hydrophilic polymer which is intended to increase the bioavailability by penetrating poorly water soluble compounds through the surface of the skin and also making possible to avoid hepatic metabolism. Side effects caused due to over dosing can be decreased and dose can be terminated immediately at the time of irritation.

**MATERIALS AND METHODS**
Amphotericin B from SRL Pvt Ltd, Maharashtra, India; Methanol and polyethylene glycol was bought from MERCK chemicals, Mumbai, India; Glycerin and chloroform was supplied by SDFCL, S d fine-chem limited, Mumbai; Hydroxypropyl methylcellulose was from Oxford laboratory.

**COMPATIBILITY STUDY**
Fourier Transfer Infrared Spectroscopy can (FTIR) can be used to investigate and predict of any possible chemical interactions between the drug and excipients. Therefore, it can be applied to the selection of suitable chemicals for desired formulation.

The aim of the present study was to find out the possible interactions between the drug Amphotericin B and polymer (HPMC) and also to detect the compatibility between drug and other excipients (Glycerin, methanol, PEG, chloroform). 10mg of the sample and 400mg of KBr were taken in a mortar and triturated. A small amount of triturated sample was compressed to form a transparent pellet using a hydraulic press at 10 kg/cm². Pellets were scanned from 4000 to 400 cm⁻¹ in Jasco FTIR spectrophotometer. For liquid samples, a drop of liquid sample is placed on a KBr pellet and scanned. The IR spectrum of the physical was compared with those of pure drug, polymer and other excipients to detect any appearance or disappearance of peaks [2,4,5].

**Table 1. Composition of transdermal patches**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Ingredient</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F₁</td>
</tr>
<tr>
<td>1</td>
<td>Drug (mg)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>HPMC (mg)</td>
<td>150</td>
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<tr>
<td>3</td>
<td>Glycerin (ml)</td>
<td>0.017</td>
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<tr>
<td>4</td>
<td>Polyethylene glycol(ml)</td>
<td>0.011</td>
</tr>
<tr>
<td>5</td>
<td>Methanol (ml)</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform (ml)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Formulation of transdermal patch**
Transdermal patches containing Amphotericin B were prepared by solvent casting technique. The patches were prepared by incorporating glycerin (15% w/w of dry polymer) as a plasticizer and Polyethylene glycol 400 (PEG 400, 10% w/w of dry polymer) as a permeation enhancer. The polymeric casting solutions were prepared by dissolving HPMC (hydroxypropyl methylcellulose) in a Chloroform:Methanol (1:1) mixture by using a magnetic stirrer. Glycerin 15% (w/w of dry polymer) and PEG 400 is added into the above mixture. The drug 50mg was added slowly to the solution and dissolved by continuous stirring for 30min. This polymeric solution was poured in the laboratory fabricated moulds. The moulds were kept on a horizontal surface and covered by an inverted funnel to control the rate of evaporation. The polymeric solution is allowed to dry for 24hrs. After 24hrs the dried films/patches were then detached and cut to generate transdermal patches of 1cm² diameter. The formulated patches were stored in dessicator until further evaluation. A thin layer of hypoallergenic adhesive polymer is applied on the external surface of transdermal patches to provide contact between transdermal patch and skin [4,6].

**EVALUATION OF PATCHES**

**Physical Appearance**
All the prepared patches were visually inspected for colour, clarity, uniformity, flexibility and smoothness [7].
Folding Endurance
Folding endurance of the film was determined manually by repeatedly folding a small strip at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance.

Flatness
Flatness of patch was observed by cutting three longitudinal strips: one from centre, one from the left side and one from the right side. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness [6,7].

Constriction(%) = \( \frac{l_1 - l_2}{l_1} \times 100 \)
Where:
\( l_1 = \) initial length of each strip
\( l_2 = \) final length

Weight Variation
The patches were subjected to mass variation by individually weighing six dried patches of 1cm\(^2\) and then mean ± S.E.M (mg/cm\(^2\)) was calculated [7,8].

Tensile strength
Tensile strength can be determined by using physical balance. The polymeric patch was pulled by gradually adding weights to the pan to increase the pulling force till the patch broke. The percentage elongation (i.e. the distance travelled by the pointer before the patch broke) was calculated as Kg/cm\(^2\) [1,6].

Percentage Moisture Content
The patches of 1cm\(^2\) were cut and weighed individually and placed in a desiccator containing activated silica at room temperature for 24hrs. Each patch was weighed repeatedly after a specified interval until they showed a constant weight [4,6]. The percent moisture content is calculated using following formula:

\[ \% \text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \]

Drug content
The patches of 1cm\(^2\) were cut and transferred to 100ml flasks which contain buffer medium. The formulation were then sonicated for 8 hours, after 8 hours the content of each flask were filtered through whatman filter paper. Filtrate was diluted properly and absorbance were determined using UV Visible spectrophotometer at 416 nm. The percentage of drug content of various formulations (F\(_1\), F\(_2\), F\(_3\)) are calculated by the following formula [8,9].

\[ \text{Drug content} = \frac{\text{test absorbance} \times \text{standard dilution} \times \text{average weight}}{\text{Standard absorbance} \times \text{test dilution}} \]

\[ \% \text{Drug content} = \frac{\text{Practical yield} \times 100}{\text{Label claim}} \]

In vitro drug diffusion studies
Diffusion rate of the prepared patches was studied by using Franz diffusion cell at 50 rpm & 37\(^0\) ± 0.50 C temperature. Phosphate buffer of pH 7.4 (20 ml) was used as a dissolution medium. Samples of 5ml each were withdrawn at 15min, 30min, 1, 2, 4, 6, 8, 10 and 12 hours. The samples were suitably diluted with the dissolution fluid and assayed for Amphotericin B at 416 nm by using the corresponding dissolution medium as a blank. Each sample withdrawn vessel was replaced with a drug free dissolution medium to maintain the desired concentration [1, 6, 10].

KINETICS OF DRUG ANALYSIS
Release behaviour of the Amphotericin B from the dosage form
In order to predict and correlate the release behaviour of the Amphotericin B from the polymer matrix, also it is necessary to fit in-vitro release data into a suitable model. So the dissolution data were fitted according to the well-known exponential equation, which is used to describe the drug release behaviour from polymeric system [11].

The equation, which is used to describe drug release mechanism, is:

\[ \frac{m_t}{m_8} = k t^n \]
where;
\( m_t / m_8 = \) fraction release of the drug
\( t = \) release time of the drug
\( k = \) constant (which indicates the properties of the polymeric system) and
\( n = \) release exponent indicative of the mechanism of release.

Order of drug release
To determine the type of order of drug release graphs were plotted between cumulative % of drug release vs. Time, log cumulative % of drug release
remaining vs. Time. The plotted graphs are represented in figures [12].

**a. Zero order kinetics**

The zero order rate equation describes the systems where the drug release rate is independent of its concentration. A plot of % cumulative drug release vs. time is linear.

\[ C = K_0 t \]

Where,
- \( C \) = Zero order rate constant
- \( t \) = Time

**b. First order kinetics**

The first order equation describes the system where the drug release rate is dependent on its concentration. A plot of log % drug remaining vs time is linear.

\[ \log C = \log C_0 - \frac{Kt}{2.303} \]

Where,
- \( C_0 \) = Initial concentration of drug
- \( K \) = First order rate constant

**Korsmeyer-Peppas model**

Korsmeyer et al. (1983) derived a simple relationship which described drug release from polymeric system [25].

\[ \frac{M_t}{M_\infty} = Kt^n \]

Where,
- \( M_t / M_\infty \) = Fraction of drug released at time ‘t’,
- \( K \) = Release rate constant and
- \( n \) = Release exponent.

In this model, the value of \( n \) characterizes the release mechanism of drug. 0.45 ≤ \( n \) corresponds to a Fickian diffusion mechanism, 0.45 < \( n \) < 0.89 to Non- Fickian transport (the rates of solvent penetration and drug release are in the same range), \( n \) = 0.89 to Case II (relational) transport, and \( n \) > 0.89 to super case II transport.

**RESULTS AND DISCUSSION**

**Compatibility studies**

An I.R study was carried out to check the compatibility between the drug and selected excipients. The spectra obtained for I.R study at wavelength from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\) are shown in the figure. After interaction through the above spectra it was confirmed that there are no major shifting, loss or appearance of functional peaks between the spectra of drug, methanol, chloroform, HPMC, physical mixture of drug + HPMC. From the I.R studies it was concluded that, the selected polymer and other excipients are compatible with the selected drug Amphotericin B.
Evaluation of transdermal patches

The results of the physiochemical characterization i.e. % flatness, weight variation, moisture content, folding endurance, drug content, tensile strength values of the formulation are shown in Table 2.

Table 2. Physiochemical characterization of patches

<table>
<thead>
<tr>
<th>Code</th>
<th>Folding endurance (no. of folds)</th>
<th>Weight variation (mg cm(^{-2}))</th>
<th>Flatness (%)</th>
<th>Tensile Strength (kg cm(^{-2}))</th>
<th>Moisture Content (%)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F(_1)</td>
<td>215±2.12</td>
<td>10.16±0.47</td>
<td>100</td>
<td>3.12±0.08</td>
<td>2.01±0.36</td>
<td>97.61±0.53</td>
</tr>
<tr>
<td>F(_2)</td>
<td>220±3.08</td>
<td>11.51±0.37</td>
<td>99.97</td>
<td>3.22±0.10</td>
<td>2.79±0.56</td>
<td>90.90±0.33</td>
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<tr>
<td>F(_3)</td>
<td>216±2.04</td>
<td>12.95±0.42</td>
<td>99.87</td>
<td>3.35±0.11</td>
<td>3.30±0.89</td>
<td>86.78±0.50</td>
</tr>
</tbody>
</table>

All the formulated patches were subjected to physiochemical evaluatory parameters i.e. folding endurance, % flatness, weight variation, tensile strength, percentage moisture contant, drug content. Folding endurance value of matrix films were found within 215 to 220 no.of folds, indicates good strength and elasticity. The weights of Prepared patches were ranged between 10.16 to 12.95 mg cm\(^{-2}\), indicated that patches were uniform in weight. The flatness study showed that F\(_1\) formulation had the same strip length before and after their cuts, indicating 100% flatness. Tensile strength of the prepared patches ranges between 3.12 to 3.35, indicates good strength and elasticity. The % moisture content studies indicates that the increase in the concentration of the hydrophillic polymer increases the moisture content of the patches. Among all the three formulations F\(_1\) showed less moisture content since it has less % of polymer than the other two formulations. The drug content of the prepared patches ranged from 86.78 % to 97.61%, the polymer, plasticizer and permeation enhancer used to prepare the patch is capable of giving uniform drug content.

**In vitro drug diffusion study**

The results of *in-vitro* drug diffusion study of Amphotericin B transdermal patches (F\(_1\), F\(_2\), F\(_3\)) are shown in Figure 5. The % cumulative amount of drug released in 12 hrs from formulations (1 cm\(^2\)) F\(_1\), F\(_2\), F\(_3\) was 97.87, 91.23 and 86.81 % .

**Fig 5. In-vitro drug diffusion study of Amphotericin B**
Kinetic modelling of drug release

The kinetic profile of the drug permeated per 1cm² of F1 patches through the membrane was shown in Figures 6(a, b, c).

Fig 6. Graphical representation of drug release kinetics (Zero order, First order and korsmeyer peppas equation) for transdermal patch of F1

Table 3. Kinetic modeling of drug release

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Kinetic Models</th>
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<tbody>
<tr>
<td></td>
<td>Zero order R²</td>
<td>First order R²</td>
<td>Korsmeyer-peppas R²</td>
<td>Korsmeyer-peppas N</td>
</tr>
<tr>
<td>F1</td>
<td>0.899</td>
<td>0.911</td>
<td>0.93</td>
<td>0.519</td>
</tr>
<tr>
<td>F2</td>
<td>0.917</td>
<td>0.977</td>
<td>0.944</td>
<td>0.580</td>
</tr>
<tr>
<td>F3</td>
<td>0.929</td>
<td>0.988</td>
<td>0.877</td>
<td>0.737</td>
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</tbody>
</table>

The cumulative amount of drug permeated per 1cm² of patches through the membrane was plotted against time was not optimally fit to Zero-order kinetics (R² = 0.899). However, the release profile of the formulated patches followed First-order kinetics (R² = 0.911) and Karsmeyer peppas equation (R² = 0.93), which indicates that the permeation of drug from the F1 formulation was governed by a diffusion mechanism.

CONCLUSION

This thesis delas with the investigations carried out on the formulation and evaluation of transdermal patch containing Amphotericin B with minimum permeation enhancer concentration that could improve its skin permeability. Based on the lower polymer concentration, higher drug release and higher bioavailability the F1 formulation of Amphotericin b is concluded as the best.
formulation containing PEG as permeation enhancer, Glycerin as plasticizer, HPMC as polymer and methanol, Chloroform as solvents respectively.

REFERENCES