FORMULATION ASPECT AND DEVELOPMENT OF A HERBAL TRANSDERMAL PATCH FOR ANTICANCER THERAPY AND ITS PHARMACEUTICAL EVALUATION

Dr. Babita Agarwal*, Dr. Prasad Kadam, Irshad Jamadar, Apurva Deegaonkar

Marathwada Mitra Mandal’s College of Pharmacy, Thergaon, Pune Maharashtra Pune – 33.

ABSTRACT

The Transdermal Drug Delivery System (TDDS) is the new routes for the systemic delivery of drugs through intact skin. A transdermal patch is a medicated patch placed on the skin to deliver medication through the skin into the bloodstream. The present investigation aimed to evaluate the possibility of using herbal extracts to develop transdermal delivery of ascorbic acid as an anti-cancerous drug. The investigation is also aimed at determining herbal drugs' potential as they are mostly non-toxic and have a beneficial effect on the body. The transdermal patches were formulated by the solvent casting method. Ascorbic Acid acts as a pro-oxidant at pharmacological levels, delivering H₂O₂ to tumorous tissue upon oxidation and leading cell death. The prepared formulations were assessed for various parameters like thickness, folding endurance, % elongation, % moisture content, % moisture uptake, % drug content. The herbal extract of ascorbic acid was assayed using the Brine Shrimp Lethality Assay method, which showed about 80-90% mortality. The study shows a new perspective on working with herbal drugs in pharmaceutics.

KEYWORDS: Transdermal, anticancer, Ascorbic Acid, Matrix films.

INTRODUCTION

Transdermal drug delivery systems (TDDS), are also known as "patches," dosage forms developed to deliver a therapeutically efficacious amount of drug across a patient's skin. Conventional systems of medication that require multi-dose therapy have numerous problems and complications. The design of a traditional dosage form, whether a tablet, an injection or a patch, to deliver the right amount of medicine at the right site of the target becomes
complicated if each medication were delivered in an optimal and preferred manner to the individual patient. The encouragement for the development of novel drug delivery systems apart from therapeutic efficacy is cost. Redesigning the modules and means to transport medicine into the body is a less demanding and more lucrative task. A controlled release drug delivery system, a novel drug delivery approach, evolves to address these problems, facilitating the drug release into systemic circulation at a pre-determined rate. Controlled drug release can be attained by transdermal drug delivery systems (TDDS), which carries medicines through the skin portal to systemic circulation at a pre-determined rate over a prolonged period of time.[1,4] TDDS are extended-release dosage forms that can offer a stable systemic drug concentration and avoid the first-pass metabolism. They can even avoid gastrointestinal problems associated with drugs and low absorption. These therapeutic advantages reflect the higher marketing potential of TDDS. Most of the drug molecules penetrate through the skin through an intercellular micro route. Therefore, the role of permeation or penetration enhancers in TDDS is vital as they reversibly reduce the stratum corneum's barrier resistance without damaging viable cells.

Lemon (Citrus × limon) belongs to the family Rutaceae, a hybrid of the plant genus Citrus and the common name of this small tree's popular edible fruit. The lemon plant's main characteristic is thorny branches and white flowers with purple edges; the acidic, juicy fruit is oval (shaped like an egg). It has an aromatic rind that is yellow when ripe (green as immature and under certain environmental conditions). They can contain up to 7% citric acid and are rich in vitamin C.[5]

Ascorbic acid can function as a pro-oxidant at therapeutic levels, carrying H₂O₂ to tumorous tissue upon oxidation and initiating cell necrosis. At pharmacological concentrations (i.v.), AA has shown remarkable anticancer effects in vitro, in vivo, and in small-scale human reports at concentrations non-toxic to normal cells, thus greatly increasing potential adjuvant to the standard of care. Initially, because H₂O₂ is a metabolite commonly produced by cells and is generally overproduced under malignancy conditions, besides, ascorbic acid could be substituted in parenteral administration by H₂O₂ directly. Thus, more studies are needed to illuminate the relationship between AA's carcinogenic and anti-carcinogenic activity, depending on the concentration and cellular location. This can also be employed to prepare transdermal patches for controlled and continuous drug release.[6,7,8,9]
MATERIAL AND METHOD
All the ingredients of the formulation were collected from the open market. The foreign matter and impurities were inspected with naked eyes and removed.

Chemicals and Reagent
Ascorbic Acid, Hydroxypropyl methylcellulose, Carbopol, Methanol, Glycerol, Tween 80, Span 80, PEG, Calcium chloride, Dimethyl sulfoxide and Oleic acid.

Extraction of ascorbic acid from lemon
The juice was first squeezed from lemons manually for the design and development of anticancer transdermal patches as a novel dosage form. The lemon juice was diluted with water and extracted by adding calcium hydroxide to it, and the insoluble precipitate is filtered. The filtrate is then treated with sulphuric acid to recover ascorbic acid.

Preparation of reagent
Ascorbic acid is assayed by Brine shrimp (Artemia salina, fairy shrimp, or sea monkeys) lethality assay to determine herbal drugs' in vitro cytotoxic actions of extracts. A stock solution was prepared by diffusing 10 mg of plant extract (soluble in water) in 1 mL of water. Concentrations of 1 mg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL were prepared by serial dilution from the stock solution. Five test tubes were labeled as 1-5. Then 1 mL of prepared solution was taken into the respective test tubes containing 10 nauplii and 1 mL of seawater. The number of dead nauplii was counted after 24 hours.\textsuperscript{[10]}

Assay of ascorbic Acid/Brine shrimp lethality assay
Measure 3 liters of water using a measuring cylinder and pour it into the rectangular jar. Weigh about 27 g of table salt by a balance and add it into the jar containing water. Mix the water with a spatula. Place the airline's tip from the air pump into the bottom of the jar maintaining proper aeration. Add about 15 g of brine shrimp eggs at the jar's top water level and mix with the water. Switch on a light (60-100 Watt bulb) placed a few inches away from the jar. After 20-24 hours, the nauplii will hatch. Observe the eggs and nauplii. Collect the nauplii after the next 24 hours. Hatched nauplii must be separated from the empty egg. It can be done by turn off the air and switch off the lamp. The empty egg will float while the brine shrimp will concentrate in the water column—transfer 10 nauplii to a test tube using a Pasteur pipette. Expose the nauplii to different concentrations of the plant extract.
Count the number of survivors and calculate the percentage of death after 24 hours.[11,12]

**Preparation of TDDS**
Transdermal patches were prepared by a solvent casting technique. An ethanolic solution of polymer and drug along with polyethylene glycol (plasticizer) was prepared. The homogenous mixture was cast into a plastic mold or petri dish. The solvent was allowed to evaporate at a controlled rate by placing an inverted funnel over the plastic mold. The control of evaporation is necessary for uniform drying of films. The drying was carried out at room temperature for a duration of 24 hours. After 24 hours, the dry films were removed from the plastic mold and stored in desiccators until used.

**Evaluation parameter of transdermal patches**

**Basis physical appearance, weight & thickness**
The weight, thickness, and physical consistency of the films were assessed immediately after formulation. On fulfilling the film's desired characteristics, the same was also subjected to storage for one month at normal room temperature conditions. This was done to determine the effect of storage conditions on the physical characteristic of the prepared films.[13]

**Thickness of the film**
The thickness of the drug-loaded polymeric films was measured at three different places using a Vernier caliper, and mean values were calculated.[14]

**Weight variation**
Each patch was weighed individually, and an average weight of three patches was found.

**Moisture content**
The prepared films weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24 hrs. The films are weighed again after a specified interval until they show a constant weight. The following formula calculated the percent moisture content:

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

**Moisture uptake**
Weighed films were kept in desiccators at room temperature for 24 hrs. These were then taken out and exposed to 84% relative humidity using a saturated potassium chloride solution in a desiccator until a constant weight is achieved.[16]
% moisture uptake is calculated as given below:

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

**Water vapor permeability (WVP) evaluation**

A natural air circulation oven can determine water vapor permeability. The following formula can determine the WVP. 

\[
\text{WVP} = \frac{W}{A}
\]

Where WVP is expressed in gm/m² per 24 hrs, 

A is the surface area of the samples expressed in m². 

W is the amount of water vapor permeated through the patch is expressed in gm/24 hrs.\(^{[16,17]}\)

**Folding endurance**

A specific area of strip is taken and repeatedly folded at the same place till it broke. The frequency that the film could be folded without breaking gave the value of folding endurance.\(^{[18]}\)

**Drug content**

A specified area of patch is dissolved into a specific volume of a suitable solvent. The solution is filtered through a filtering medium and analyze the drug-containing a suitable method (UV or HPLC method). Then take the mean of three different samples.

**Percentage elongation break test**

The percentage elongation break is determined by noting the length just before the breaking point. The percentage of elongation can be determined from the below formula. Elongation percentages = \((L1-L2/L2) \times 100\).

Where L1= is the final length of the strip. L2= is the initial length of the strip.

**Drug content uniformity**

A specified area (2 cm²) of the Transdermal system was cut into small pieces and placed into a 100 ml volumetric flask with 100 ml of pH7.4 phosphate buffer was added and kept for 24 hrs with occasional shaking. The suitable dilution was then made with a phosphate buffer of pH 7.4 similarly; a blank was carried out using a drug–free patch.\(^{[19]}\)
RESULT AND DISCUSSION
The physicochemical characteristics of prepared patches are shown in Table. Thickness (mm) ranged from 0.2±0.3 to 0.29±0.18mm. Weight variation observed ranged from 1.43 to 1.58. Good uniformity in drug content was observed, and it ranged from 90.5±0.7% to 91.3±0.5% in all the formulation. The percentage of moisture uptake in the range of 3.184 to 4.312, and moisture content to be found in the range of 1.948 to 2.175. Water vapour transmission (gm/cm²/hours) observed is 0.0041 to 0.0063. Percent elongation was determined to be in the range of 15.2 to 18.5. The folding endurance was found to be satisfactory.

Table No. 1: Evaluation of TDDS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td>0.29</td>
<td>0.20</td>
<td>0.28</td>
</tr>
<tr>
<td>Weight Variation(gm)</td>
<td>1.58</td>
<td>1.55</td>
<td>1.43</td>
</tr>
<tr>
<td>% Moisture Content</td>
<td>2.175</td>
<td>1.981</td>
<td>1.948</td>
</tr>
<tr>
<td>% Moisture Uptake</td>
<td>4.312</td>
<td>4.122</td>
<td>3.814</td>
</tr>
<tr>
<td>Water Vapour Permeation(gm/cm²/hrs)</td>
<td>0.0063</td>
<td>0.0054</td>
<td>0.0041</td>
</tr>
<tr>
<td>% Elongation</td>
<td>18.5</td>
<td>16.3</td>
<td>15.2</td>
</tr>
</tbody>
</table>

The test sample's lethal concentration resulted in 80-90 % mortality of the brine shrimp larvae in the following concentrations of 10, 100, 1000 µg/ml, except in 1µg/ml. The result indicates that the test sample has potent activity towards the nauplii. This type of trend was expected as the sample acted on the receptors/ tissues, either additively or synergistically.
The mechanism of drug release in this transdermal drug delivery system is based on Matrix-diffusion-controlled TDDS. In this approach, the drug reservoir is prepared by homogenously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The resultant medicated polymer is then molded into a medicated disc with a specified surface area and controlled thickness. This drug reservoir containing polymer disc is then pasted on to an occlusive base plate in a section fabricated from a drug impermeable plastic backing. The adhesive polymer is then stretched along the skirts to form a strip of the adhesive rim around the medicated disc.\(^{[20]}\)

**CONCLUSION**

Recent advances in technology & science have led to the outstanding discovery of TDDS as a novel drug delivery system providing the release of the drug directly to the site of action with ease of application. The TDDS patch was successfully evaluated for their various physiological properties giving satisfactory results. Patient acceptability & compliance has increased tremendously, making TDDS patches gain the upper hand in long term treatment permeation enhances, and drug forms have shown superior safety over their corresponding parent forms.

**REFERENCES**

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