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Pharmacodynamics of a losartan transdermal system for the treatment of hypertension

M. Shahbaz Shams, M. Intakhab Alam, Asgar Ali, Yasmin Sultana and M. Aqil

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Abstract
Aims: Transdermal therapeutic systems were developed using the polymers, Eudragit E 100 and polyvinyl pyrrolidone VA 64 in a film casting assembly. The medicated films were evaluated for physical properties, in vitro drug release studies, in vitro skin permeation studies, and pharmacodynamic studies. Results: The physical parameters were found to be very satisfactory with high drug content (>99%). The in vitro drug release studies were performed using paddle-over-disc assembly specified in USP XXIII. The pharmacodynamic studies were carried out using tail cuff method in Wistar albino rats. Hypertension was induced by methyl prednisolone acetate subcutaneously for 2 weeks. The developed matrix patch was found to decrease the blood pressure (25.42% reduction in mean systolic blood pressure of rats) significantly (P < 0.001) in proximity of the normal value and it was maintained for 24 hours. Conclusion: It can be concluded that the developed transdermal matrix patch holds promise for the management of hypertension that needs to be validated by clinical trials.

Key words: Antihypertensive agent; hypertension; matrix film; permeation; TTS

Introduction

The skin is the most extensive and readily accessible organ in the body. Its chief functions are concerned with protection, temperature regulation, control of water output, and sensation. The understanding of the skin and its barrier functions has allowed the development of more reliable and more efficacious means of safely delivering therapeutic agents through the skin. The success of this approach is evidenced by the fact that there are currently more than 35 transdermal therapeutic system (TTS) products approved in the United States for treatment of wide variety of diseases. Transdermal drug delivery systems are self-contained discrete dosage forms that, when applied to the intact skin, deliver the drug through the skin at a controlled rate to the systemic circulation. Transdermal drug delivery systems have been in use for over 30 years and had generated tremendous interest amongst pharmaceutical companies on introduction in the early 1980s. The interest was owing to the advantages offered by the transdermal route, which bypassed the traditional liver metabolized route, offered higher patient compliance with the ability to control release of drugs with short half-lives, and reduced adverse effects associated with traditional drug delivery. However, the promise was short-lived as several problems emerged upon development of transdermal drugs that included dose limitation, skin irritation, delayed time-of-action, and site-dependent absorption. The waning of interest from big pharmaceutical industries resulted in limited number of transdermal delivery-based drugs entering the market and several transdermal drug delivery-focused companies merging with other drug delivery companies. The emergence of interest in other drug delivery routes like pulmonary further resulted in a dramatic decline in the pipeline for transdermal delivery-based companies. However, with thinning pipelines and fewer blockbusters, pharmaceutical companies are increasingly looking to innovative drug delivery routes to extend patent life. The success of transdermal drugs, especially nicotine-based transdermal products in the 1990s, has brought back interest amongst pharmaceutical companies.

Hypertension is a health condition in which the blood pressure (BP) is persistently elevated (high BP).
Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm and is a leading cause of chronic renal failure. Even moderate elevation of arterial BP leads to shortened life expectancy. Nearly 1 billion people worldwide have high BP (defined as >140/90 mmHg), and that number is expected to increase to 1.56 billion people by next two and half decades. The prevalence of hypertension is predicted to increase by 24% in developed countries and by 80% in developing regions. From 1999 to 2004, 78% of adults with hypertension were aware of their disease, 68% were treated for their hypertension with medications, and less than two-thirds were controlled to BPs below 140/90 mmHg with medication. WHO 1999 reports that one out of every three deaths in India is because of heart diseases. Amongst several risk factors associated with this growing menace, hypertension or high BP has been established as a key risk factor for heart diseases.

Losartan potassium (LP) is one such drug that is used frequently for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents. The drug is generally given for a longer duration of time. This causes daily dosing schedule and patient inconvenience. Its absolute bioavailability is approximately 33% after oral dosing. The low bioavailability is primarily because of incomplete absorption and partly because of presystemic metabolism. Because of its low bioavailability after oral administration, and the inconveniences related to parenteral administration, the development of transdermal drug delivery device has reasonable importance. The rationale of the study was to develop a delivery system that would provide the release of drug for a longer duration of time with improved bioavailability. The TTSs of LP would also improve the treatment by providing higher comfort (lower dosing frequency and no traumatic administration) and consequently improving patient compliance.

The aim of this study was to develop a low-dose matrix-type transdermal drug delivery system of LP (ATII antagonist), which undergoes hepatic first-bypass metabolism and shows low bioavailability. It was planned to design the formulation in such a way that it provides the delivery of drug at a controlled rate across intact skin to achieve a therapeutic effective drug level for a longer period of time. The polymeric matrix systems are widely used to provide controlled delivery of drug substances because of their versatility, effectiveness, and low cost. These types of systems are also suitable for in-house development because they are usually manufactured using conventional equipment and processing. As the formulation was designed to deliver the drug by transdermal route, it will bypass hepatic first-pass metabolism and hence would provide better bioavailability compared with conventional dosage forms.

With this background the main purpose of this study was (i) to develop a stable, reproducible, and patient compliance drug delivery system in the form of transdermal therapeutic system, (ii) to reduce the side effects by the optimization of the blood concentration time profile, (iii) to provide extended duration of activity, which allows greater patient compliance owing to elimination of multiple dosing schedules, and (iv) to obviate low absorption, first-pass effect, and formation of metabolites that cause side effects.

Materials and methods

The materials used include losartan potassium (Ranbaxy Laboratory Ltd., Gurgaon, India), ethanol AR (Merck India Ltd., Mumbai, India), propanol AR (Merck India Ltd.), isopropyl alcohol (Merck India Ltd.), Eudragit E-100 (Dr. Reddy’s Laboratories, Hyderabad, India), copovidone (PVP VA 64) (Jubilant Organosys Ltd., Noida, India), acetone (S.D. Fine Chemicals, New Delhi, India), dichloromethane (Merck India Ltd.), n-octanol (Merck India Ltd.), methanol (S.D. Fine Chemicals), polyethylene glycol 400 (Central Drug House, New Delhi, India), methyl pyrrolidone (S.D. Fine Chemicals), dibutyl phthalate (Merck India Ltd.), ammonia solution [Thomas Baker (Chemicals) Ltd., Mumbai, India]; other chemicals were of analytical reagent grade.

Development of transdermal system

Preparation of films

The medicated films using the polymers, Eudragit E 100 (EE 100) and polyvinyl pyrrolidone VA 64 (PVP VA 64), were prepared in a film casting assembly with aluminum foil. The film casting assembly was fabricated for this purpose. It consisted of two stainless steel plates of same external diameter. The upper plate was made hollow such that the internal diameter was 7.90 cm. The two plates were then screwed together with nuts and bolts. Aluminum foil was placed in between the plates and screwed tightly to prevent solvent leakage. The accurately weighed amount of LP (168.12 mg) was dissolved in methanol (8 mL). The different proportions of polymers, plasticizers (dibutyl phthalate and glycerol), and permeation enhancers [methyl pyrrolidone (MP)—10%, w/w of total weight of polymers] were then dissolved in methanol using a magnetic stirrer (Table 1). The resulting solution was poured carefully into the film casting assembly upon which an inverted funnel was placed to control the evaporation rate of solvent. This minimized the chances of cracking or wrinkling of the films. The open end of the funnel was plugged with
cotton to allow uniform evaporation of the solvents. The solvent was allowed to evaporate undisturbed. The films got dried up in approximately 24 hours. The prepared medicated films were cut with a die (3.5 cm²) and one side of the film (releasing side) was covered by the wax paper in such a way as to leave a flap for easy peel off. Adhesive tape was applied on the backing side of the patch.

**Evaluation of physical properties of polymeric films**

**Thickness**

The thickness of the films was assessed at three different points using a thickness apparatus (screw gauge) and the average thickness was determined (Table 2).

**Folding endurance**

The number of times the film could be folded at the same place without breaking/cracking gives the value of folding endurance. This was determined by repeatedly folding the film at the same place until it broke (Table 2).

**Weight variation**

Three films from each batch as whole were weighed individually and average weight was calculated (Table 2).

**Moisture uptake**

The accurately weighed films kept in a desiccator at 40 °C for 24 hours were taken out and exposed to two different relative humidities of 75% (saturated solution of sodium chloride) and 93% (saturated solution of ammonium hydrogen phosphate) in two different desiccators at room temperature until a constant weight for the films were obtained. The percentage of moisture uptake was calculated as the difference between final and initial weights with respect to initial weight (Table 2).

\[
\text{Moisture uptake} (\%) = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100
\]

**Drug content**

A film of size 9.616 cm² was dissolved in 100 mL of methanol. This was shaken in a mechanical shaker for 30 minutes to obtain a homogeneous solution and filtered. The drug was determined by UV spectrophotometry at \( \lambda_{max} \) 234.2 nm after suitable dilution (Table 2) and calculated using the following formula:

\[
\text{Drug content (mg)} = \frac{\text{concentration} \times \text{dilution factor} \times \text{volume}}{1000}
\]

**In vitro drug release studies**

The in vitro drug release studies were performed using paddle-over-disk assembly specified in USP XXIII.

The vessel of the dissolution apparatus was filled with 900 mL of isotonic phosphate buffer (IPB) of pH 7.4 and the assembly was equilibrated to 37 ± 0.5 °C. The patch of specified area was placed on a Teflon disc, covered with a muslin cloth, and fastened with cotton thread, ensuring that the release surface was facing upward. The disc was placed flat at the bottom of the vessel. A distance of 25 ± 2 mm was maintained between the paddle blade and the surface of the disc assembly. The vessel was covered during the study to minimize the evaporation. The disc assembly was

| Table 1. Formulae of medicated transdermal patches for one ring of casting assembly. |
|------------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Ingredients                          | MZ1E     | MZ1      | MZ2E     | MZ2      | MZ3E     | MZ3      | MZ4E     | MZ5E     |
| Eudragit E 100 (mg)                  | 700      | 700      | 600      | 600      | 500      | 500      | 450      | 400      |
| Polyvinyl pyrrolidone VA 64 (mg)     | 100      | 100      | 200      | 200      | 300      | 300      | 350      | 400      |
| Dibutyl phthalate (mL)               | 0.1152   | 0.1152   | 0.1152   | 0.1152   | 0.1152   | 0.1152   | 0.1152   | 0.1152   |
| Glycerol (mL)                        | 0.02     | 0.02     | 0.02     | 0.02     | 0.02     | 0.02     | 0.02     | 0.02     |
| MP (mg)                             | 80       | —        | 80       | —        | 80       | —        | 80       | —        |

MP, methyl pyrrolidone.

<p>| Table 2. Physicochemical properties of medicated transdermal films (with enhancer). |
|------------------------------------------|----------|----------|----------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Medicated films</th>
<th>Thickness (mm) ( n = 3, \pm SD )</th>
<th>Weight variation (mg) ( n = 3, \pm SD )</th>
<th>Folding endurance ( n = 3, \pm SD )</th>
<th>Moisture uptake (%) ( n = 3, \pm SD )</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ1E</td>
<td>0.237 ± 0.071</td>
<td>214.27 ± 1.83</td>
<td>41 ± 1.19</td>
<td>3.21 ± 0.924</td>
<td>99.94</td>
</tr>
<tr>
<td>MZ2E</td>
<td>0.232 ± 0.061</td>
<td>217.43 ± 1.71</td>
<td>35 ± 1.24</td>
<td>3.16 ± 0.853</td>
<td>99.94</td>
</tr>
<tr>
<td>MZ3E</td>
<td>0.228 ± 0.064</td>
<td>211.91 ± 1.97</td>
<td>37 ± 1.16</td>
<td>3.08 ± 1.119</td>
<td>99.99</td>
</tr>
<tr>
<td>MZ4E</td>
<td>0.229 ± 0.063</td>
<td>213.29 ± 1.59</td>
<td>38 ± 1.17</td>
<td>3.13 ± 0.972</td>
<td>99.93</td>
</tr>
<tr>
<td>MZ5E</td>
<td>0.233 ± 0.067</td>
<td>215.62 ± 1.93</td>
<td>40 ± 1.21</td>
<td>3.19 ± 1.014</td>
<td>99.94</td>
</tr>
</tbody>
</table>
placed horizontally to the surface of the vessel in such a way as to minimize any dead volume between the disc assembly and the bottom of the vessel. It was applied taking care that no air bubbles developed between the cloth and the release surface. The study was performed at 50 rpm. A 5 mL aliquot, which was replaced by fresh media, was collected at specified intervals for 48 hours from the zone midway between the surface of the dissolution medium and the top of the blade. The study was repeated three times with every medicated patch. Cumulative percent release of drug for each formulation was plotted against time (Figure 1). The following formulae were used for the calculation of in vitro release parameters:

\[
\text{Cumulative amount of drug released (mg)} = \frac{\text{concentration (μg/mL)} \times \text{DF} \times 900}{1000}
\]

where DF is the dilution factor.

\[
\text{Cumulative drug released (%) = } \frac{\text{cumulative amount of drug released (mg)}}{\text{dose in one patch}} \times 100
\]

### In vitro skin permeation studies

A vertical diffusion cell fabricated by local glass fabricator similar to the Keshary Chein diffusion cell (jacketed) was used for permeation studies. Water jacket was used for maintaining the temperature at 37 ± 0.5°C. The vertical double-walled diffusion cells consisted of two half-cells. The capacity of receiver chamber of cell was 33 mL with an area of diffusion between two half-cells of 9.616 cm².

The skins were excised from the abdominal region of albino rats that were previously killed using excess environment of chloroform and were stored in the deep freezer (−20°C) until use. On the day of experiment, the skin was brought to room temperature and then treated with 0.32 M ammonia solution for 1 hour and hair and fat were removed manually. The skin was washed with water and examined for cuts or holes if any.

After pretreatment, skin was mounted between the two half-cells of the apparatus and the extra skin was cut and trimmed to prevent the lateral diffusion. The stratum corneum side of the skin was facing the donor compartment whereas the dermis faced the receiver compartment and the apparatus was assembled with springs. Both the compartments were filled with IPB of pH 7.4. The receiver fluid was stirred with a magnetic bead at a speed of 600 rpm and the assembled apparatus was placed on a magnetic stirrer. Hot water was circulated through the jackets of the cells to maintain 37 ± 0.5°C temperature. The receiver fluid was replaced every 15 minutes to stabilize the skin. It was found that the sample at 4 hours and beyond showed negligible UV absorption indicating the complete stabilization of the skin. Transdermal patch was placed above the skin and the release surface faced the stratum corneum. The samples were withdrawn by the sampling port at specified intervals up to 48 hours, filtered, and analyzed for drug content permeated. Cumulative amount of drug permeated was plotted against time for each formulation (Figure 2).

### Pharmacodynamic studies (in vivo studies)

The preclinical assessment of antihypertensive activity of the developed formulation was performed on experimental hypertensive rats. Hypertension was induced by methyl prednisolone acetate (MPA; 40 mg/kg/week) subcutaneously for 2 weeks. The studies were carried out using tail cuff method (Biopac system, Inc., Goleta, CA, USA). The instrument was based on noninvasive BP (cuff tail) measuring technique. The instrument
comprised of a scanner, a tail cuff, and a restrainer (animal holder) attached to main instrument having digital BP display panel.

Twenty-four Wistar albino rats (either sex) weighing between 150 and 180 g procured from the central animal house (Jamia Hamdard, New Delhi, India) were marked distinctly with picric acid solution for easy identification. They were put on standard feed (Lipton, Mumbai India) and drinking water ad libitum with proper monitoring on regular basis.

The restrainer was an unfamiliar environment for the rats, so they had to be trained for their stay in calm and nonaggressive manner in the restrainer during the BP measurement exercise. For this reason, a rat was inserted in the restrainer headlong until the whole body got conveniently accommodated inside. Screwing on the rear closure locked the rat holder. The front closure was previously locked. The restrainer was made of plastic and well ventilated. Initially, the rats were agitated, restless, and offered resistance to the above process as it was unfamiliar to them. Each rat was placed for few minutes in the restrainer per day and rest of the time they were kept in cages. However, they got accustomed to the proceedings with the passage of time. The animals were trained for 30 days.

The initial BPs of all the rats were recorded using the noninvasive BP instrument. The instrument was turned on. For recording the BP, the tail of the rat was first dilated by exposing it with a gentle heat emanating from lamp. Then, the rat was first made to enter in the restrainer as done in the training session. Then the protruding tail of the animal was placed in the tail cuff of the instrument and the BP recording button was pressed and systolic BP was recorded.

After recording the initial BP of all rats, the rats were divided into four groups of six animals each (Table 3). Group A was taken as control (nonhypertensive). Hypertension was induced in the remaining groups (groups B–D) by subcutaneous injection of MPA for 2 weeks as per method by Krakoff et al.9 After MPA treatment groups C and D were subjected to application of placebo and medicated matrix patch, respectively. Group B served as toxic control and received no further treatment. The patch was applied to the previously shaven abdominal area of rat skin. The rat was then placed in the restrainer and the BP from the tail was recorded at predetermined time intervals up to 48 hours.

### Stability study

Stability studies were carried out as per WHO and ICH guidelines to determine any changes in the formulation. The optimized formulation MZ3E that gave satisfactory in vitro and in vivo results was considered for accelerated stability studies10. Sufficient replicates of the formulation were prepared, packed in aluminum foil, and stored in Petri dishes. The samples of the optimized formulation were stored at various temperature conditions (i.e., 40 ± 0.5°C, 50 ± 0.5°C, and 60 ± 0.5°C) and at 40 ± 2°C, RH 75 ± 5%, the samples were evaluated for their physicochemical characteristics, namely thickness, weight, folding endurance, and drug content after 90 days. The values obtained were compared with those of the fresh samples.

### High-performance liquid chromatographic method of analysis

The high-performance liquid chromatographic (HPLC) method of Ozkan11 was modified as per the need in this research work. HPLC system of Shimadzu class VP series was used. Separation was achieved using RP C-18 column (25 × 4.6 mm internal diameter). A shimadzu SPD-10 AVP UV detector was used to detect LP at 234.0 nm. The mobile phase used was a mixture of 0.02 M KH₂PO₄ (pH 3.2): acetonitrile (55:45). The pH was adjusted to 3.2 with phosphoric acid. The flow rate was set to 0.9 mL/min. The retention time was found to be 6.325 minutes, which is within the reported value.

### Statistical analysis

Statistical data were analyzed using one-way analysis of variance of pharmacodynamic studies. A Dunnette multiple comparison test and paired t-test were used to compare different formulations, and a P-value of >0.05 was considered to be significant.

### Results and discussion

The drug LP was found to be very soluble in methanol. The selected polymers, Eudragit E100 and Copovidone (Polyvinyl pyrrolidone VA-64), exhibited good solubility in methanol. Eudragits are basically insoluble in water.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of rats</th>
<th>Time intervals (hours) for the measurement of BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>6</td>
<td>0,1,2,4,6,8,10,12,24,30,36,48</td>
</tr>
<tr>
<td>B</td>
<td>MPA (toxic control)</td>
<td>6</td>
<td>0,1,2,4,6,8,10,12,24,30,36,48</td>
</tr>
<tr>
<td>C</td>
<td>MPA + placebo film</td>
<td>6</td>
<td>0,1,2,4,6,8,10,12,24,30,36,48</td>
</tr>
<tr>
<td>D</td>
<td>MPA + medicated patch</td>
<td>6</td>
<td>0,1,2,4,6,8,10,12,24,30,36,48</td>
</tr>
</tbody>
</table>
and soluble in most organic solvents. The various medicated formulations were fabricated, that is, MZ1, MZ1E, MZ2, MZ2E, MZ3, MZ3E, MZ4E, and MZ5E, and evaluated for weight variation, folding endurance, thickness, moisture uptake and drug contents (Table 2), and in vitro release and skin permeation studies (Figures 1 and 2).

All the films were thin (<0.3 mm) with uniform thickness and weight variation. The uniform thickness and weight of the films suggested the uniform distribution of drug and polymer over the surface selected for film-forming. The moisture uptake capacity was very less. The low moisture uptake defends the material from microbial contamination and bulkiness of the patches. The folding endurance measures the breaking ability of the films during use. Sufficient folding endurance was found for the films of each batch, suggesting the use of the system for a period of 24 hours or more without breaking or cracking. The drug content in each of the film was found to be high (>90%), suggesting high retaining capacity of LP that will be available to be permeated through transdermal route.

In vitro drug release studies were carried out for all the formulations using paddle-over-disk method. It was carried out on MZ1, MZ1E, MZ2, MZ2E, MZ3, MZ3E, MZ4E, and MZ5E. It revealed that the addition of permeation enhancer (10% MP) increased the cumulative amount of drug released from the transdermal therapeutic systems, as compared with formulations without penetration enhancer. Results showed that 55.31%, 57.27%, and 58.79% of drug was released from the formulations without permeation enhancer (i.e., MZ1, MZ2, and MZ3), respectively. While the cumulative percent release was more for the formulations containing enhancer (10% MP) and it was found to be 70.64%, 75.45%, 78.18%, 83.79%, and 89.85% for the formulations MZ1E, MZ2E, MZ3E, MZ4E, and MZ5E, respectively. But MZ4E and MZ5E showed early release, that is, before 48 hours and showed almost negligible release up to the desired period of time. These formulations possessed more amount of water-soluble polymers (PVP VA 64) and hence faster release of drug because of faster leaching. Formulations MZ2E and MZ3E possessed slightly less amount of water-soluble polymer (PVP VA 64) and hence the release rates of the drug were up to more duration of time, that is, reaching toward 48 hours. These two formulations MZ2E and MZ3E released the drug 75.45% and 78.18%, respectively, up to 48 hours and after the drug was not released significantly. It was observed from the study that when water-soluble polymer is increased the release of the drug from the patch also increased and this might be attributed to providing permeability effect in the matrix by such polymers. The mechanism of drug release was found to follow Higuchi matrix mechanism when the cumulative percent of drug released was plotted against the square root of time. Linearity was seen in the plot between log amount of drug released versus log time for the formulations MZ2E and MZ3E, indicating first-order release mechanism of drug from the monolithic matrix TTS.

In vitro skin permeation studies were carried out using diffusion cells. Permeability coefficients were calculated and found to be 0.0104 × 10⁻³, 0.0115 × 10⁻³, 0.0126 × 10⁻³, 0.0147 × 10⁻³, and 0.0163 × 10⁻³ cm/h for formulations MZ1E, MZ2E, MZ3E, MZ4E, and MZ5E (with permeation enhancer), respectively, whereas 0.009 × 10⁻³, 0.0095 × 10⁻³, and 0.010 × 10⁻³ cm/h for formulations MZ1, MZ2, and MZ3 (without permeation enhancer), respectively. Permeability coefficient is the expression of the amount or degree of permeation possessed by a substance associated with simple diffusion through a membrane that is proportional to the partition coefficient and the diffusion coefficient and inversely proportional to membrane thickness. For molecules of equal size, the one with greater solubility in lipids will pass more quickly into the cell. For molecules of equal solubility, smaller ones penetrate faster. The increased permeability coefficient upon addition of the enhancer may be explained by the increased fluidity of the matrix membrane that may increase the availability of LP for the permeation through the stratum corneum.

The cumulative amount of drug permeated was also calculated for each patch through rat skin at various time intervals up to 48 hours, that is, the desired time period for which transdermal patches were planned. The formulations without permeation enhancer (MZ1, MZ2, and MZ3) did not permeate the satisfactory amount of drug through the rat skin. However, the formulations with permeation enhancer (MZ1E, MZ2E, MZ3E, MZ4E, and MZ5E) exhibited higher permeation. Among these five formulations, only two formulations MZ2E and MZ3E gave satisfactory permeation up to 48 hours, that is, 65.96% and 70.77%, respectively. This might be due to the satisfactory flux of these two formulations. The permeation enhancer to be included in the formulation was selected amongst the enhancers studied including dimethyl formamide (5%, v/v), MP (5%, v/v), isopropyl myristate (5%, v/v), orange oil (5%, v/v), rose oil (5%, v/v), and eucalyptus oil (5%, v/v). An optimum 10% MP was selected for the formulation of transdermal therapeutic system based on the increase in flux. A corresponding increase of permeability coefficient was also seen with MP. The effectiveness of these permeation enhancers was also ascertained by determination of the enhancement factor (E2) of each permeation enhancer. Methyl pyrrolidone might increase the epidermal permeability through a mechanism involving the perturbation of stratum corneum lipid bilayers and lacunae formation to enhance transdermal drug delivery.
Losartan transdermal system

The results of these two formulations were again found to be in correlation with their release of drug in paddle-over-disk method. From the above observations, it was seen that formulations MZ2E and MZ3E showed better in vitro drug release and permeation.

Hypertension was successfully induced in the normotensive rats by MPA administration for a period of 2 weeks and they remained hypertensive for 72 hours after stopping the MPA injection as high significant difference (\(t\)-test, \(P < 0.001\)) was found in the pre- and post-treatment values (Table 4). This was authenticated by Dunnet test, which showed significant difference (\(P < 0.001\)) in BP values of control A and toxic control B groups, corroborating the reports that excessive production or administration of glucocorticoid is associated with systemic hypertension. The developed matrix patch was found to decrease the BP significantly (paired \(t\)-test, \(P < 0.001\)) in proximity of the normal value and it was maintained for 24 hours. This indicated that drug was permeated into systemic circulation in rats in a constant manner up to 48 hours through the optimized formulation (MZ3E). However, post-treatment BP values in control (A) and treatment group (D) were comparable (\(P < 0.01\)). On comparing the effects of all the systems, the percentage reduction in mean systolic BP of rats by LP matrix patch and placebo was 25.42% (38.61 mmHg) and 0.13%, respectively. Formulation MZ3E was successful in reverting the rat BP to normal values. The above results suggested that the developed transdermal matrix patch holds promise for the management of hypertension that needs to be validated by clinical trials. The BP reducing efficacy of the optimized formulation (MZ3E) has been depicted in Figure 3.

The shelf life of transdermal formulation MZ3E of LP was determined by accelerated stability studies on the basis of first-order degradation kinetics and \(\tau_{0.9}\) (the time required to degrade 10% of drug at 25°C). The shelf life was found to be 2.15 years for formulation MZ3E.

The formulation MZ3E was found to be fairly stable as revealed by the stability studies conducted as per ICH guidelines and degradation is >5% (3.30%) over a period of 3 months. The influence of temperature and humidity on the physicochemical properties of the formulations that were stored for 90 days were also determined and again it was found that the properties were very slightly altered under elevated temperature and humidity conditions.

The influence of temperatures and humidity on physicochemical characteristics of formulation MZ3E was also studied out. The results indicated that the values for physical characteristics (thickness, weight, and folding endurance) and drug content very slightly declined with the rise in temperature. This might be due to increased degradation of drug and excipients with the elevation in storage temperature. Slightly higher values of thickness and weight were obtained for samples stored at higher humidity (75 ± 5% RH). This could be due to increased moisture uptake by the films at elevated humidity conditions.

**Conclusion**

Based on the above observation, interpretation, and results, it was concluded that an optimized transdermal monolithic patch-type dosage form (MZ3E) of losartan potassium was obtained successfully, which can be applied on the skin of the patient for 48 hours for the

**Table 4. Influence of transdermal patch of LP on mean systolic BP in MPA-induced hypertensive rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Pre-treatment</th>
<th>Post-MPA treatment</th>
<th>Post-MZ3E treatment(^a)</th>
<th>Reduction in BP (%)</th>
<th>Post-MZ3E treatment(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>126.21 ± 11.43</td>
<td>—</td>
<td>126.21 ± 8.21</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>MPA (toxic control)</td>
<td>127.42 ± 23.53</td>
<td>162.53 ± 9.52</td>
<td>162.53 ± 5.29</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>MPA + placebo film</td>
<td>126.09 ± 16.26</td>
<td>161.18 ± 3.20</td>
<td>160.96 ± 13.25</td>
<td>0.13 ± 21.46</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>MPA + medicated Patch (MZ3E)</td>
<td>128.15 ± 29.46</td>
<td>164.05 ± 14.52</td>
<td>122.35 ± 19.33</td>
<td>25.42 ± 17.37</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\)Reflects reading at 48 hours post-MZ3E (patch) treatment. \(^b\)In case of control and toxic control no MZ3E (patch) treatment was given.

**Figure 3.** BP reducing efficacy at each time interval of the optimized formulation (MZ3E).
treatment of hypertension. The preparation was free from chemical interactions and was a stable preparation when tested after storage. It gave permeation of a drug in living animal (rat) in a steady manner over a desired period of time. It can be further evaluated for clinical trials on human volunteers.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References
