ORIGINAL ARTICLE

Transdermal delivery of Diltiazem HCl from matrix film: Effect of penetration enhancers and study of antihypertensive activity in rabbit model

Rabinarayan Parhi a,*, Padilam Suresh b

a Institute of Pharmacy, GITAM University, Gandhi Nagar Campus, Rushikonda, Visakhapatnam 530045, Andhra Pradesh, India
b Institute of Pharmacy and Technology, Salipur 754202, Cuttack, Odisha, India

ABSTRACT

The present investigation focused on the development of Diltiazem HCl (DTH) matrix film and its characterization by in-vitro, ex-vivo and in-vivo methods. Films were prepared by solvent casting method by taking different ratios of hydroxypropyl methylcellulose K4M (HPMC K4M) and Eudragit RS100. Various parameters of the films were analyzed such as mechanical property using tensile tester, interaction study by Fourier transform infrared spectroscopy (FTIR) and Thermogravimetric analysis (TGA), in-vitro drug release through cellulose acetate membrane, ex-vivo permeation study using abdominal skin of rat employing Franz diffusion cell, and in-vivo antihypertensive activity using rabbit model. The FTIR studies confirmed the absence of interaction between DTH and selected polymers. Thermal analysis showed the shifting of endothermic peak of DTH in film, indicating the dispersion of DTH in molecular form throughout the film. Incorporation of 1,8-cineole showed highest flux (89.7 μg/cm²/h) of DTH compared to other penetration enhancers such as capsaicin, dimethyl sulfoxide (DMSO), and N-methyl pyrrolidone (NMP). Photomicrographs of histology study on optimized formulation (DF9) illustrated disruption of stratum corneum (SC) supporting the ex-vivo results. The in-vivo antihypertensive activity results demonstrated that formulation DF9 was effective in reducing arterial blood pressure in normotensive rabbits. SEM analysis of films kept for stability study (40 ± 2°C/75% ± 5%RH for 3 months) revealed the formation of drug crystals which may be due to higher temperature. The findings of the study provide a better alternative dosage form of DTH for the effective treatment of hypertension with enhanced patient compliance.

Introduction

L-type of calcium channel mediated Ca²⁺ influx plays a major role in the regulation of blood pressure and manifestation of hypertension [1]. Accordingly, specific L-type of calcium channel blockers is used to prevent the entry of Ca²⁺ into the cell which results in vascular smooth muscle relaxation and subsequently vasodilatation. Out of three classes (phenylalkylamines,
dihydropyridines and benzothiazepines) of Ca \(^+\) channel blockers available for clinical use, DTH, (2S,3S)-5-[2-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1, 5-benzothiazepin-3-yl acetate hydrochloride belongs to benzothiazepines class [2]. Apart from its antihypertension application, DTH is also a drug of choice in the management of classical and vasospastic angina pectoris, supraventricular tachyarrhythmias and anal fissure [3]. Oral administration of DTH subjected to extensive and complex biotransformation (N-demethylation, O-demethylation and deacetylation) which limits the biological half-life to 3–5 h and bioavailability up to 40% [4]. The drug dosage recommended for DTH is 30 mg or above, usually three to four times a day [5]. The above conditions provide a strong rationale for the development of a transdermal formulation for DTH.

Compared to other routes, transdermal route is proved to be advantageous due to the following reasons: (i) better patient compliance as the administration is convenient and non-invasive in nature, (ii) reduction in dosing, (iii) avoidance of first pass metabolism and (iv) prevention of gastrointestinal irritation due to the contact of certain active ingredients to gastric mucosa [6]. Among transdermal systems, self-adhesive films are considered as an innovative drug delivery system to achieve systemic effect through skin application [7]. Out of various transdermal drug delivery systems such as matrix, reservoir, microreservoir, membrane matrix hybrid and adhesive, matrix diffusion type is widely accepted because of its ease of manufacturing. Many researchers have successfully developed films of DTH using combination of different polymers including ethyl cellulose–PVP, Eudragit–PVP–PEG 4000, HPMC–EC [8], taro corn mucilage–HPMC [9] and PVA–xanthan gum [5].

Due to impervious SC layer of the skin very few drugs have the ability to penetrate the skin in sufficient quantity in order to produce the required therapeutic effect. Various physical techniques and chemical penetration enhancers have been tried to breach this barrier nature of skin. Penetration enhancers are more popular than physical methods due to lack of pain at the application site [10]. Penetration enhancers increase the drug penetration across the skin by interacting with skin’s bilipid layer component, thereby causing increased fluidity in the intercellular lipid lamellae, swelling in the SC and/or leaching out some of the structural components. In addition, penetration enhancers may react with protein structure of the SC and thereby pronouncing the penetration of active ingredients. Among all, terpenes are considered as Generally Recognized As Safe (GRAS) category of penetration enhancers which could be used between 1% and 5% without any side effects to enhance either hydrophilic or lipophilic drugs penetration across the skin [11]. So, we selected 1,8-cineole as terpene along with NMP, capsaicin and DMSO as penetration enhancers to improve the flux of DTH through abdominal skin of rat.

Our objective was to develop and evaluate matrix diffusion controlled system of DTH using the combination of hydrophobic (Eudragit RS100) and hydrophilic (HPMC K4M) polymers. Different proportion of polymers was taken into consideration to select the best combination. In addition, the effect of plasticizers such as glycerol, DBT and propylene glycol (PG) on the film characteristics was also evaluated. The films were tested for physicochemical properties, mechanical characteristics, FTIR studies, thermal analysis, in-vitro release study, ex-vivo permeation using abdominal skin of rat, in-vivo study utilizing rabbit model and stability studies.

**Material and methods**

**Materials**

DTH (≥99%) and HPMC K4M were generous gifts of Ranbaxy Laboratories Pvt. Ltd., Gurgaon, Haryana, India. Eudragit RS100 was gifted by Evonik Degussa India Pvt. Ltd., Mumbai, India. Capsaicin and cellulose acetate membrane (molecular weight cutoff between 12,000 and 14,000 Da) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Methanol and 1,8-cineole were obtained from Merck Specialties Pvt. Ltd., Mumbai, India. NMP and DMSO were obtained from CDH, New Delhi. DBT purchased from Loba Chemie, Mumbai. All other chemicals used during experiment were of analytical grade.

**Preparation of films**

Diltiazem HCl (30% w/w of dry weight of total polymer) loaded transdermal films containing different ratios of HPMC K4M and Eudragit RS100 were prepared by solvent casting method. The requisite ratios of polymers were weighed and were allowed to swell for 6 h in methanol–dichloromethane (1:1) solvent mixture. Different plasticizers such as glycerol, DBT and PG were incorporated at 20% w/w of polymer dry weight. Calculated amount of DTH was mixed with homogeneous polymer solution and poured into a petri dish containing mercury [12,13]. A funnel was placed over the petri dish in inverted position to control the rate of evaporation. The casting solvent mixture was allowed to evaporate overnight at room temperature. The dried films were cut into required size (1.6 × 1.6 cm\(^2\)) and wrapped in aluminum foil. Then, these films were kept in desiccator containing saturated solution of CaCl\(_2\) as desiccant (29% of relative humidity) at room temperature (32 °C) until use. DMSO, 1,8-cineole, capsaicin and NMP were incorporated at 5% w/w of dry weight of polymer in optimized film.

**Evaluation of the physicochemical properties of the films**

**Thickness and weight variation**

Thickness of the prepared films was measured at six different places using digital vernier caliper (Mitutoyo, Japan) and the mean was calculated [14]. Six films (2.56 cm\(^2\)) from each batch were randomly selected for weight variation. Films were weighed individually and then the average weight was measured. The difference between individual and average weight indicated the weight variation.

**Folding endurance**

A strip of film of specific surface area (3 cm × 2 cm) was cut and folded repeatedly at one place till it broke. The number of times the film was folded before breaking at the same place represented folding endurance [12].

**Drug content analysis**

For drug content analysis, films of known area were taken in 10 ml of volumetric flask and casting solvent mixture was added to it. The flasks were shaken in a water bath at 37 °C for 24 h. Then, the solution was filtered through Whatman
filter paper No. 1 and suitably diluted prior to drug content measurement using UV–Vis spectrophotometer (UV-1800, SHIMADZU, Japan) at 236 nm.

Moisture content
The films of DTH were weighed individually and kept in a desiccator containing saturated solution of CaCl₂ (29% of relative humidity) at room temperature (32°C) for 24 h. Subsequently, the films were weighed repeatedly until a constant weight was achieved. The percentage of moisture uptake was calculated based on the difference between final and initial weight divided by initial weight [13].

Moisture uptake
The weighed films kept in a desiccator for 24 h at room temperature (32°C) were taken out and exposed to 75% relative humidity (saturated solution of NaCl) until a constant weight was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight divided by initial weight [8,13].

Water vapor permeability
For water vapor permeability (WVP) measurement, glass test tubes of 25 mL capacity were taken and filled with 20 mL of distilled water. The weight of each filled test tube was measured one hour prior to closing the openings by films. The area available for vapor permeation was 2.544 cm² and all the containers were maintained at constant room temperature (32°C) for 24 h. The final weight was calculated after one hour of test completion and the WVP was calculated using the following equation:

\[
\text{WVP} = \frac{W}{A} \times 24 \text{ h}
\]

where \( W \) is the mean loss in weight (g) of the containers and \( A \) (m²) is the area of the exposed surface [15].

Mechanical properties
The mechanical properties such as ultimate tensile strength (UTS), Young’s modulus and elongation at break (EB) were measured using universal tensile testing machine (INSTRON 3366, USA Inc). The clamps of tester were covered with silicon gum to prevent slippage of the films during the test. Then, the films were driven downward using 10 N load sensor with a fixed speed of 10 mm/min until breaking of the film. The UTS, EB (%) [15] and Young’s modulus were calculated as per the following equations:

\[
\text{UTS} = \frac{\text{Breaking force}}{\text{Cross-section area of film}}
\]

\[
\text{EB} \% = \frac{\text{Length at breaking point of film} - \text{Original length of film}}{\text{Original length of film}} \times 100
\]

\[
\text{Young’s modulus} = \frac{\text{Tensile Stress}}{\text{Tensile Strain}}
\]

Fourier transform infrared spectroscopy (FTIR) study
FTIR spectra of pure DTH and individual polymers (HPMCK4M and Eudragit RS100) were recorded along with their physical mixtures in a FTIR spectrophotometer (Alpha-FT-IR, Bruker Optics, Germany) using KBr pellets technique over the range of 4000–500 cm⁻¹.

Thermal analysis
Thermal analysis of DTH, HPMC K4M and Eudragit RS100 and the optimized film (DF9) was performed using differential thermal analyzer (DTG-60, simultaneous TGA/DTA analyzer, Shimadzu, Japan). Samples were heated from 0°C to 600°C at a rate of 10°C/min in a nitrogen purge of 50 ml/min.

In-vitro release studies
The in-vitro release of DTH from the prepared film was performed using vertical type of Franz diffusion cell (Murthy glasswares, Hyderabad, India) with an exposed surface area of 3.8 cm² and receptor compartment capacity of 22 mL. The jacketed diffusion cell with inlet and exit port for the circulation of water was used in order to maintain medium temperature at 32 ± 0.5°C. A film specimen of surface area 2.56 cm², equivalent to 15 mg of the drug was placed on 0.22-μm cellulose acetate membrane. The membrane was equilibrated by soaking in phosphate buffer pH 7.4 for 24 h prior to the experiment. The above medium was used to ensure the sink condition and stability of the drug. The membrane having film on it was immediately placed between the chambers (donor and receptor) and subsequently, secured firmly by a stainless steel clip. The receptor compartment was stirred at 200 rpm with a Teflon coated magnetic bead. Aliquots of 0.5 ml were withdrawn from the receptor medium at specified time intervals (0.5, 1, 2, 3, 4, 5, 6, and 8 h) and replaced with equal volumes of fresh buffer maintained at same temperature. The samples were analyzed using a UV-Spectrophotometer at 236 nm after suitable dilution and the concentrations of DTH were calculated using calibration curve.

Ex-vivo permeation studies
Skin preparation
The male Wister rats (200–250 g) were collected once the experiment on them was completed. The rat abdomens were shaved using electric clipper and then with hand razor from tail to head direction. The skin was removed surgically and adhering subcutaneous fat was carefully cleaned with forceps and cotton swab. The skin pieces were washed thrice with phosphate buffer pH 7.4 and wrapped in aluminum foil. Then, it was stored in deep freezer and was used on the following day [16].

Ex-vivo permeation study
The ex-vivo permeation study of DTH through abdominal skin of rat was performed using the same diffusion cell with all the conditions (receptor compartment was filled with 22 mL phosphate buffer of pH 7.4 maintained at 32 ± 0.5°C with continuous stirring) as mentioned above. A thawed piece of skin was hydrated in receptor medium for 1 h followed by mounting it on receptor compartment such that the SC end is faced toward donor compartment. A desired size (1.6 × 1.6 cm²) of optimized film specimen with and without penetration enhancers was placed over the hydrated skin and then it was securely clamped between donor and receptor compartment. At specified
time intervals, aliquots of 0.5 ml were withdrawn from the receptor medium and the concentration of DTH was analyzed by HPLC assay as mentioned below.

The HPLC system consisted of an Agilent HP 1100 series equipped with autosampler and DAD detector (G1315B). A reverse-phase C18 column (150 × 4.6 mm, Luna C18 column, 5 μm) with a guard column was used as the stationary phase at 25°C. The mobile phase used was a mixture of acetonitrile and phosphate buffer of pH 3 in 6:4 ratio. The injection volume was 30 μL and the flow rate was set at 1.2 mL/min. DTH was detected at 236 nm and the retention time was 10 min. The proposed method was validated for linearity and precision. The linearity was evaluated by determining DTH at five concentration levels: 10.0, 20.0, 30.0, 40.0, and 50.0 μg/mL. The precision and accuracy of the above method was established by analyzing pure samples of DTH. Three concentration levels (10.0, 20.0, and 30.0 μg/mL) were analyzed within one day as well as for five consecutive days. Each concentration level was analyzed three times and standard deviations (SD) of each concentration were analyzed.

**In-vivo study**

The antihypertensive activity was performed on normotensive rabbits (New Zealand white rabbit) as per the standard operating procedure of Deshpande Lab, Bhopal, India. All animal experiments were performed according to the “CPCSEA Guidelines for the Care and Use of Laboratory Animals”, India, with approval no. DL/RP/2014/a. Briefly, nine rabbits in three groups having equal number in each group were considered in this study and the grouping was made as follows:

- **Group I** was applied with film without drug (−ve control group).
- **Group II** was received per-oral drug solution (+ve control group), and
- **Group III** was applied with film containing DTH.

Rabbits were housed in individual cages with sufficient access to pellet diet and water. The temperature of ~25°C and relative humidity of 55 ± 10% were maintained in the animal room. Invasive method was employed to determine antihypertensive effect of optimized formulation (DF9) and oral drug solution in rabbits and the result was compared with −ve control group. Each rabbit of the second group has received pure drug solution (5 mg/kg) orally. In another two groups, thigh regions were shaved using a razor to prepare 3.5 × 3.5 cm area for the film sample application. Film without DTH and optimized film piece equivalent to 30 mg of DTH was applied on shaven skin area of rabbits of DTH and optimized film piece equivalent to 30 mg of DTH.

The thickness of the prepared films was varied between 270 ± 30 μm and 240 ± 0 μm as shown in Table 3. This indicates expressed in percentage decrease in arterial blood pressure in comparison with −ve control group.

**Histological studies**

Two skin samples (control and treated) were used in histology study. The control skin sample was collected without any treatment whereas the treated one was collected once the ex-vivo permeation on the optimized film (DF9) was completed. Each specimen was stored in 10% formalin solution in HPLC water prior to the experiment. The skin samples were sectioned carefully without damage, treated with absolute isopropyl alcohol for dehydration, fixed in paraffin wax, and stained with xylol. The resulted specimen was then observed under a light microscope (Microtome-1200 Weswox, Western electrical scientific work).

**Scanning electron microscope (SEM) and stability studies**

The morphological character of the optimized fresh and aged film was studied by scanning electron microscopic method. The aged film, used in this study, was generated by keeping a fresh film in accelerated stability condition (40 ± 2°C/75% ± 5%RH in a stability chamber, JRIC 11, Osworld, Mumbai, for 3 months) [19]. The sample films were mounted on a clear-glass stub, air-dried and coated with polaron E5100 sputter coater. Then, it was visualized under a SEM (Leo-435 VP; Leo, Cambridge, UK).

**Statistical analysis**

One way analysis of variance (One-way ANOVA) with Bonferroni multiple comparison test was used to measure statistical significant differences between various data obtained from different evaluation tests at 95% confidential level (P < 0.05). It was performed employing demo version of GRAPHPAD INSTAT software (Graph-Pad Software Inc., San Diego, CA).

**Results and discussion**

All the films were prepared by solvent casting method using methylene chloride and methanol in 1:1 ratio as solvent system. Films from DF1 to DF4 were developed with plasticizer glycerol, whereas DF5 and DF6 were prepared using DBT and PG, respectively. The composition of all films is mentioned in Table 1.

The calibration graph was constructed between the standard concentrations of DTH and area (μV sec) measured at 236 nm. The correlation coefficient (R²) value was found to be 0.998, which showed a linear response in the concentration range of 10–50 μg/mL. The accuracy and precision of the developed method was performed by carrying out five independent analyses at each concentration level and the result is shown in Table 2.

**Evaluation of the physicochemical properties of the films**

**Thickness and weight variation**

The thickness of the prepared films was varied between 570 ± 30 μm and 240 ± 0 μm as shown in Table 3. This indicates
that there was no such significant difference ($P < 0.05$) in thickness among the films. Decrease in thickness of films (DF1–DF4) was observed with the decrease in the HPMC K4M percentage [20]. The variation of weight ranged from 77.93 ± 3.49 to 81.76 ± 5.01 mg. This represents different proportions of polymers have not such significant impact on weight variation.

**Folding endurance**
The main aim of the folding endurance study is to test the ability of the film to endure rupture during the application and use [9]. The folding endurance values were ranged from 240.25 ± 7.4 in case of DF1 to 210.1 ± 5.78 in case of DF5 (Table 3). It was observed that with decrease in HPMC K4M proportion in the film the folding endurance value decreases. When film formulations containing penetration enhancers (DF7–DF10) were compared, formulation DF8 demonstrated highest value of folding endurance (245.26 ± 5.79). The higher values demonstrated that films would maintain the integrity and shape with the natural folding of the skin when applied on it.

**Drug content determination**
The drug content data showed good uniformity with low standard deviation. This is an indication that the films of DTH with HPMC K4M and Eudragit RS100 can be prepared with higher degree of reproducibility.

**Moisture content and moisture absorption**
The moisture absorption is a tool to indicate how the film would behave during the initial stage of drug release [13]. The result of moisture content and moisture absorption study is shown in Fig. 1(a). The moisture content and moisture absorption values varied from 11.34% to 8.2% and from 22.62% to 7.65%, respectively. We did not observe any regular pattern of increase or decrease of moisture content among the formulations. However, there was a decreasing order of moisture absorption from polymer ratio (HPMC K4M:Eudragit RS100) of 100:0 (DF1) to 25:75 (DF4). This is obvious that with increase in hydrophilic polymer proportion there was an increase in the moisture absorption capacity of the films. The change in moisture content and moisture absorption among the film formulations containing different types of penetration enhancers (DF7–DF10) was found to be non-significant ($P < 0.05$) suggesting that the presence of penetration enhancers has negligible impact on above properties.

![Table 1 Composition of all transdermal films of DTH.](image)

<table>
<thead>
<tr>
<th>Film code</th>
<th>HPMC (mg)</th>
<th>ERS (mg)</th>
<th>Glycerol (mg)</th>
<th>DBT (mg)</th>
<th>PG (mg)</th>
<th>DMSO (mg)</th>
<th>1,8-Cineole (mg)</th>
<th>Capsaicin (mg)</th>
<th>NMP (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF1</td>
<td>1000</td>
<td>–</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF2</td>
<td>750</td>
<td>250</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF3</td>
<td>500</td>
<td>500</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF4</td>
<td>250</td>
<td>750</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF5</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF6</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>200</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF7</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>200</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF8</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>200</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF9</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>200</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DF10</td>
<td>500</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>200</td>
<td>50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

![Table 2 Test of accuracy and precision of the developed method.](image)

<table>
<thead>
<tr>
<th></th>
<th>Intra-day assay</th>
<th>Inter-day assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken (µg/mL)</td>
<td>Observed (µg/mL) (± SD, n = 5)</td>
<td>Taken (µg/mL) (± SD, n = 5)</td>
</tr>
<tr>
<td>10</td>
<td>10.01 ± 0.03</td>
<td>10.03 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>20.05 ± 0.08</td>
<td>20.05 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>30.13 ± 0.06</td>
<td>30.13 ± 0.04</td>
</tr>
</tbody>
</table>

![Table 3 Results of physicochemical parameters.](image)
Water vapor permeability

The water vapor permeability (WVP) values of the prepared film are shown in Fig. 1 (b). All the WVP values were found to be ranging between 388.85 ± 30.85 g/m²/day in case of DF4 and 766.66 ± 34.53 g/m²/day in case of DF1. It was observed that increasing the Eudragit RS100 proportion in the film decreases the WVP values which could be attributed to the water insoluble nature of the Eudragit polymer. The average transepidermal water loss by diffusion through the skin is 300–400 ml/day which corresponds to 157.894–210.526 g/m²/day (the normal body surface area was considered as 1.9 m²) [21]. According to BP 1993, the limit above which a substance is considered as permeable is 500 g/m²/day. All the WVP values were above this limit except DF4 (388.85 ± 30.85) with the highest value of 766.66 ± 34.53 in case of DF1. This indicated that the prepared films were not occlusive in nature and thereby not disturbing natural process of water loss from body surface. The decreasing order of WVP values was observed from DF1 to DF4. This result was attributed to decrease in hydrophilic polymer proportion in the film. It was also observed that the addition of different penetration enhancers does not influence the WVP significantly (P < 0.05).

Mechanical properties

The mechanical properties such as UTS, EB% and Young’s modulus of all the films were measured and the data are shown in Table 4. A hard and tough polymeric film, generated from high values of both UTS and EB%, is always desired as it has qualities suited best as a drug delivery system for the skin application. This implies that higher UTS values prevent abrasion of the film caused for example by contact with clothing whereas higher EB% values allow the film to follow the movement of skin without breaking. In case of films (DF1–DF4), containing glycerin as plasticizers, a decreasing trend in all the above parameters from DF1 to DF4 was observed with highest value for film DF1. Decreasing trend of UTS, and EB (%) may be due to decrease in HPMC K4M proportion from 100% in case of DF1 to 25% in case of DF4. Formulation DF5 containing DBT as plasticizers demonstrated highest value of UTS (12.173 ± 2.591 MPa) and lowest percentage of EB (40.913 ± 6.134) when the films containing different plasticizers such as glycerol, DBT and PG at 20% concentration level were compared. This result was attributed to the hydrophobic nature of DBT which increased the strength but reduced elasticity [22]. Similar type of result was obtained when formulation containing penetration enhancers (DF7–DF10) was compared. Formulation DF8 incorporated with 1,8-cineole (hydrophobic) showed highest UTS value of 17.179 ± 2.513 MPa and lowest percentage of EB (40.913 ± 6.134) when the films containing different plasticizers such as glycerol, DBT and PG at 20% concentration level were compared. This result was attributed to the hydrophobic nature of DBT which increased the strength but reduced elasticity [22]. Stiff materials are having higher Young’s modulus value, thus difficult to stretch or deform. The Young’s modulus is a measure of materials stiffness or rigidity. Stiff materials are having higher Young’s modulus value, thus difficult to stretch or deform. The Young’s modulus values were found to be highest (618.457 ± 65.864 MPa) in case of DF1 indicating more force is needed to deform it compared to other film formulations.

![Diagram of Water vapor permeability](a) Percentage of moisture content and moisture absorption by different films containing DTH, and (b) percentage of water vapor permeation through the films containing DTH.

Water vapor permeability

The water vapor permeability (WVP) values of the prepared film are shown in Fig. 1(b). All the WVP values were found to be ranging between 388.85 ± 30.85 g/m²/day in case of DF4 and 766.66 ± 34.53 g/m²/day in case of DF1. It was observed that increasing the Eudragit RS100 proportion in the film decreases the WVP values which could be attributed to the water insoluble nature of the Eudragit polymer. The average transepidermal water loss by diffusion through the skin is 300–400 ml/day which corresponds to 157.894–210.526 g/m²/day (the normal body surface area was considered as 1.9 m²) [21]. According to BP 1993, the limit above which a substance is considered as permeable is 500 g/m²/day. All the WVP values were above this limit except DF4 (388.85 ± 30.85) with the highest value of 766.66 ± 34.53 in case of DF1. This indicated that the prepared films were not occlusive in nature and thereby not disturbing natural process of water loss from body surface. The decreasing order of WVP values was observed from DF1 to DF4. This result was attributed to decrease in hydrophilic polymer proportion in the film. It was also observed that the addition of different penetration enhancers does not influence the WVP significantly (P < 0.05).

Mechanical properties

The mechanical properties such as UTS, EB% and Young’s modulus of all the films were measured and the data are shown in Table 4. A hard and tough polymeric film, generated from high values of both UTS and EB%, is always desired as it has qualities suited best as a drug delivery system for the skin application. This implies that higher UTS values prevent abrasion of the film caused for example by contact with clothing whereas higher EB% values allow the film to follow the movement of skin without breaking. In case of films (DF1–DF4), containing glycerin as plasticizers, a decreasing trend in all the above parameters from DF1 to DF4 was observed with highest value for film DF1. Decreasing trend of UTS, and EB (%) may be due to decrease in HPMC K4M proportion from 100% in case of DF1 to 25% in case of DF4. Formulation DF5 containing DBT as plasticizers demonstrated highest value of UTS (12.173 ± 2.591 MPa) and lowest percentage of EB (40.913 ± 6.134) when the films containing different plasticizers such as glycerol, DBT and PG at 20% concentration level were compared. This result was attributed to the hydrophobic nature of DBT which increased the strength but reduced elasticity [22]. Similar type of result was obtained when formulation containing penetration enhancers (DF7–DF10) was compared. Formulation DF8 incorporated with 1,8-cineole (hydrophobic) showed highest UTS value of 17.179 ± 2.513 MPa and lowest percentage of EB (40.913 ± 6.134) when the films containing different plasticizers such as glycerol, DBT and PG at 20% concentration level were compared. This result was attributed to the hydrophobic nature of DBT which increased the strength but reduced elasticity [22]. Stiff materials are having higher Young’s modulus value, thus difficult to stretch or deform. The Young’s modulus is a measure of materials stiffness or rigidity. Stiff materials are having higher Young’s modulus value, thus difficult to stretch or deform. The Young’s modulus values were found to be highest (618.457 ± 65.864 MPa) in case of DF1 indicating more force is needed to deform it compared to other film formulations.

<table>
<thead>
<tr>
<th>Film code</th>
<th>UTS (MPa)</th>
<th>Elongation at break (%)</th>
<th>Young’s modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF1</td>
<td>42.877 ± 3.963</td>
<td>116.528 ± 15.281</td>
<td>618.457 ± 65.864</td>
</tr>
<tr>
<td>DF2</td>
<td>24.165 ± 3.678</td>
<td>58.481 ± 4.006</td>
<td>415.855 ± 33.351</td>
</tr>
<tr>
<td>DF4</td>
<td>05.328 ± 1.223</td>
<td>32.482 ± 4.782</td>
<td>145.283 ± 17.192</td>
</tr>
<tr>
<td>DF5</td>
<td>12.173 ± 2.591</td>
<td>40.913 ± 6.134</td>
<td>209.552 ± 47.569</td>
</tr>
<tr>
<td>DF6</td>
<td>09.369 ± 1.745</td>
<td>47.782 ± 7.205</td>
<td>243.457 ± 28.574</td>
</tr>
<tr>
<td>DF7</td>
<td>09.975 ± 2.423</td>
<td>48.458 ± 5.647</td>
<td>243.457 ± 28.574</td>
</tr>
<tr>
<td>DF8</td>
<td>17.179 ± 2.513</td>
<td>37.451 ± 5.246</td>
<td>197.278 ± 21.782</td>
</tr>
<tr>
<td>DF9</td>
<td>08.054 ± 1.237</td>
<td>45.316 ± 1.764</td>
<td>245.123 ± 6.127</td>
</tr>
<tr>
<td>DF10</td>
<td>09.127 ± 2.452</td>
<td>43.192 ± 4.654</td>
<td>245.123 ± 6.127</td>
</tr>
</tbody>
</table>

n = 3.
Fourier transform infrared spectroscopy (FTIR) study

FTIR study was performed to investigate the type of interaction between drug and polymers. The spectra of pure DTH, Eudragit RS100 and HPMCK4M and their physical mixtures are shown in Fig. 2(a). The principal peaks of pure DTH are 1680.21 cm\(^{-1}\) for C=O (ketone) stretching, 1511.11 cm\(^{-1}\) for C=C (aromatic ring) stretching, 1255.12 cm\(^{-1}\) for C=O (ether) stretching and 1026.86 cm\(^{-1}\) for R-O-R. The other peaks present with pure DTH spectra were at 3441.91 cm\(^{-1}\) N-H stretching, 2966.40 cm\(^{-1}\) C-H (aliphatic) stretching, 1743.53 cm\(^{-1}\) for C=O (ester) stretching, 1059.59 cm\(^{-1}\) for C=O (ester) stretching, and 781.78 cm\(^{-1}\) for C-H bending.

The FTIR spectra showed that the all the principal peaks were intact and the absence of any additional peak in all physical mixture of drug and polymers indicates that there were no interactions between the above functional groups of drug with polymers.

Thermal analysis

The thermogram of a drug sample demonstrates a single sharp endothermic peak and a broad peak indicating melting point and decomposition temperature, respectively. The DTA-thermogram of DTH in Fig. 2(b) showed a sharp endothermic peak at 205.17 °C and a broad peak at 275.15 °C. The

![Fig. 2 Interaction studies: (a) FTIR spectra of pure drug, polymers and their physical mixture, and (b) thermograms of pure drug, polymers and optimized film (DF9).](image-url)
polymers Eudragit RS100 and HPMC K4M showed sharp endothermic peaks at 365.74 °C and 339.75 °C, respectively, representing their melting point.

The thermogram of film showed a smaller and shifted endothermic peak from 205.17 °C to 198.74 °C. Shifting of peak was not definitely due to interaction as FTIR spectra of physical mixtures showed no sign of interaction. This may be due to the conversion of crystalline form of DTH to amorphous form in the film [17]. It was expected as DTH was completely dissolved in organic solvent system chosen thereby dispersed in the molecular form in hydrophilic polymer (HPMC K4M). Another shifted peak at 349.65 °C/C176 represents their melting point.

**In-vitro release studies**

In-vitro drug release profiles of transdermal film (DF1–DF6) containing different ratios of HPMC K4M and Eudragit RS100 are shown in Fig. 3(a). Among the films having different proportions of polymers (DF1–DF4), DF1 demonstrated the highest DTH release (66.51 ± 2.79) at the end of 24 h which was significantly different (P < 0.05) from formulations DF3 and DF4. It was observed that the release of DTH decreased substantially as the percentage of HPMC K4M decreased from DF1 (having matrix made from 100% of HPMC K4M) to DF4 (containing 25% of HPMC K4M). This may be explained due to the hydrophilic nature of HPMC with high water absorption property which when in contact with hydrated membrane exhibits rapid polymer dissolution [8,12]. Furthermore, the fraction of drug present on the surface of the film could also be a contributor to the higher release [23].

The drug release from the films (DF3, DF5 & DF6) was also evaluated to predict the influence of different plasticizers (Glycerin, DBT and PG) on in-vitro drug release. The highest (60.048 ± 1.51%) and lowest (56.292 ± 1.97%) drug release was found to be in case of DF3 and DF5 containing glycerin and DBT, respectively. This may be due to the hydrophilic nature of glycerin, thereby, increasing the drug solubility of DTH (a hydrophilic drug) and subsequently the drug diffusion. Similar trend of DTH release was observed among the films containing penetration enhancers (DF7–DF10). The film DF8 containing hydrophilic penetration enhancer (1,8-cineole) showed the highest cumulative percentage of DTH release (63.448 ± 2.89%). The drug release was found to be in the decreasing order of DF8 > DF10 > DF7 > DF9. Film formulations containing different plasticizers and penetration enhancers did not exhibit significant difference in drug release (P < 0.05).

**Study of kinetics and mechanism of drug release**

To understand the drug release kinetics and mechanism of drug release, all the in-vitro release data were fitted to various kinetic models such as zero order, first order, and Higuchi model. The in-vitro release data followed neither zero order ($R^2 = 0.897–0.970$) nor first order kinetics ($R^2 = 0.621–0.811$), whereas it followed Higuchi model with highest $R^2$ value of 0.994 for DF10 and followed by 0.993 for DF6 as shown in Table 5. The linearity for Higuchi model indicated diffusion is the drug release mechanism. In order to know the type of diffusion all data were fitted to Korsmeyer–Peppas equation. The diffusion release exponent values ($n = 0.507–0.682$) demonstrated anomalous diffusion (non-Fickian model) i.e., the release mechanism followed the combination of diffusion and swelling. This is attributed to the presence of swelling polymer HPMC K4M in the matrix [13]. Based on the $R^2$ values along with the results obtained from physical, mechanical and release studies, formulation DF6 ($R^2 = 0.993$) was selected for further study.

**Ex-vivo permeation studies**

The ex-vivo permeation study was performed using hairless abdominal skin of rat and the cumulative amount of DTH permeated ($\mu$g/cm²) was plotted against time as shown in Fig. 3 (b). Among all the formulations, DF9 containing 1,8-cineole showed highest permeation (2691 µg/cm²) of DTH at 36 h which was significantly different (P < 0.05) from film formulations DF6, DF7, and DF8. The same formulation also showed highest flux (89.7 µg/cm²/h) and permeability coefficient (Kp) (0.0059) as shown in Table 6. The flux enhancement was found to be in the following order: DF9 > DF10 > DF8 > DF7 > DF6. The film DF9 showed highest enhancement ratio (ER) of 6.469.
The principal monoterpene present in eucalyptus oil is 1,8-cineole. It was reported that hydrophilic terpenes (alcohol, ketone and oxide terpenes) were better penetration enhancers for hydrophilic drugs compared to hydrocarbon containing terpenes. Again, among oxide terpenes cyclic ethers such as 1,8-cineole are more potent than terpenes containing epoxide ring [24]. 1,8-cineole is cyclic ether and the mechanism of action is the disruption of regular arrangements of intercellular bilayer lipid present in SC [25]. Furthermore, in the molecular level oxygen-containing monoterpenes such as cineole preferentially make hydrogen bond with ceramide head groups, thereby, breaking the lateral/transverse hydrogen bond network of lipid bilayer [25]. It was evident (hydrophilic terpenes are better penetration enhancer for hydrophilic drug) from a study where there was a 100 times increase in Kp of 5-fluorouracil (hydrophilic drug) across human epidermis pretreated with 1,8-cineole [26]. 1,8-cineole was found to be more efficient penetration enhancers for another hydrophilic model drug propranolol hydrochloride at 5% (flux of 49.3 ± 5.5 μg/cm²/h) and 10% (93.81 μg/cm²/h) as compared to menthol and PG [27]. In the present investigation, we witnessed a flux of 89.7 μg/cm²/h at 5% level of cineole suggesting that these films can provide a long-lasting effect. It was described elsewhere in the literature that PG was used as penetration enhancer for drugs such as 5-fluourouracil, progesterone and estradiol [28]. Improvement of flux of bupranolol by 1.8-fold using PG compared to control was also reported. Furthermore, PG, present in the film as plasticizer, can absorb moisture from environment because of its humectant activity, and thereby, enhances the permeation of DTH [28].

Capsaicin is a resin obtained from the plant of capsicum family. It exerts therapeutic effects on cardiovascular, respiratory and sensory nervous system apart from its penetration enhancement activity. Capsaicin at 3% level has been used as a penetration enhancer for the topical application of naproxen through pretreated full thickness human abdominal skin and rabbit ear skin and observed that the ER was 2.8-fold and 3.6-fold for human and rabbit skin, respectively. The result was attributed to (i) similarity of structure (the longest axis distance of both compounds is very similar ≈ 17 Å) of capsaicin to azone (as azone is well established penetration enhancer), (ii) vasodilatation effect of capsaicin, and (iii) the predicted log P value for capsaicin is 3.31 that helped capsaicin molecules to insert itself into the lipid bilayers leading to disruption of its packed structure [29]. In our study, we found that the ER of DTH through rat skin was 3.916 which was more than the previous study. This is attributed to the use of higher concentration (5% compared to 3% in previous case) of capsaicin and the rat skin (instead of human skin used in the previous study) as model membrane. Previous study reported that the rat skin is considered as more permeable than human skin [30].

It was reported that pyrrolidones without carbon chain enter and disrupt the lipophilic domain of SC, whereas, alkyl-substituted pyrrolidone (NMP) interacts with polar domain of the SC [31]. So, enhancement effect of NMP was due to its interaction with lipid present in SC. The solvent DMSO interacts with the polar head of the lipid and keratin structure of the corneocytes resulting in loosening of these structures [32]. Both NMP and DMSO enhance the permeation of polar drugs. We observed that the NMP present in film DF8 showed better enhancement (2.827) than DMSO (2.197) containing film DF7. This result was in accordance with cyclosporine A retention in the SC of rat using DMSO and NMP along with other penetration enhancers [33].

### Table 5

<table>
<thead>
<tr>
<th>Film code</th>
<th>Correlation coefficient ($R^2$) (mean ± SD, n = 3)</th>
<th>Diffusion release exponent ($n$) (mean ± SD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order kinetics</td>
<td>First order kinetics</td>
</tr>
<tr>
<td>DF1</td>
<td>0.897 ± 0.005</td>
<td>0.640 ± 0.023</td>
</tr>
<tr>
<td>DF2</td>
<td>0.923 ± 0.018</td>
<td>0.621 ± 0.034</td>
</tr>
<tr>
<td>DF3</td>
<td>0.960 ± 0.008</td>
<td>0.811 ± 0.007</td>
</tr>
<tr>
<td>DF4</td>
<td>0.959 ± 0.021</td>
<td>0.793 ± 0.003</td>
</tr>
<tr>
<td>DF5</td>
<td>0.953 ± 0.005</td>
<td>0.716 ± 0.020</td>
</tr>
<tr>
<td>DF6</td>
<td>0.966 ± 0.013</td>
<td>0.787 ± 0.034</td>
</tr>
<tr>
<td>DF7</td>
<td>0.968 ± 0.008</td>
<td>0.788 ± 0.021</td>
</tr>
<tr>
<td>DF8</td>
<td>0.961 ± 0.004</td>
<td>0.783 ± 0.007</td>
</tr>
<tr>
<td>DF9</td>
<td>0.970 ± 0.011</td>
<td>0.791 ± 0.013</td>
</tr>
<tr>
<td>DF10</td>
<td>0.963 ± 0.02</td>
<td>0.772 ± 0.002</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Film code</th>
<th>Flux (μg/cm²/h)</th>
<th>Permeability co-efficient</th>
<th>Enhancement ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF6</td>
<td>13.866</td>
<td>0.0009</td>
<td>–</td>
</tr>
<tr>
<td>DF7</td>
<td>30.466</td>
<td>0.002</td>
<td>2.197</td>
</tr>
<tr>
<td>DF8</td>
<td>39.2</td>
<td>0.0026</td>
<td>2.827</td>
</tr>
<tr>
<td>DF9</td>
<td>89.7</td>
<td>0.0059</td>
<td>6.469</td>
</tr>
<tr>
<td>DF10</td>
<td>54.3</td>
<td>0.0036</td>
<td>3.916</td>
</tr>
</tbody>
</table>

**In-vivo study**

Antihypertensive activity of optimized film (DF9) was carried out by invasive method using rabbit model and the result in...
percentage decrease in blood pressure compared to +ve control group is shown in Fig. 4. The arterial blood pressure was measured up to 24 h. The result showed a decrease in arterial blood pressure in case of rabbit treated with oral solution and optimized film.

It was reported that invasive method of blood pressure measurement, where the arterial pressure was measured with the help of a fluid (mostly heparinized saline) in contact with blood, gives more accurate result as compared to non-invasive method [34]. We carried out blood pressure measurement in normotensive rabbits and observed that the antihypertensive activity of rabbit group applied with drug incorporated film was statistically significant ($P < 0.05$) compared to +ve control group throughout the study. It was also observed that there was a steep decrease in percentage of arterial blood pressure drop in +ve control group as shown in Fig. 4. Further, at 6 h of study the percentage decrease in arterial blood pressure was 15.79%. This indicated a rapid decrease in drug level in blood which may be due to short half-life of the drug. Rabbit group treated with drug loaded film showed a constant percentage decrease in blood pressure up to 10 h of study and then it gradually decreases till 24 h. This suggested a sustained antihypertensive activity of DTH when a matrix type of transdermal film was used as formulation.

**Histopathological studies**

The penetration enhancing capacity of the optimized film (DF9) containing 1,8-cineole was performed and further compared with untreated skin. The photomicrograph of untreated skin shows a well defined SC with distinct coenocytes intercalated in bilipid layers as shown in Fig. 5(a). Upon treatment with the DF9, a significant increase in SC disruption was observed (Fig. 5(b)). This was evident from the highly enlarged intercellular space within SC which confirmed the disruption lipid bilayer arrangement leading to increase in penetration of DTH.

**Scanning electron microscopic (SEM) and stability studies**

The SEM micrographs of fresh film and aged film are shown in Fig. 6(a) and (b). The SEM photographs of fresh film showed a clear and smooth surface indicating uniform distribution of drug in the polymer matrix (Fig. 6a). A clear and smooth film surface in case of fresh film indicates uniform distribution of drug in the polymer matrix having equal amount of hydrophilic and hydrophobic polymers [9]. This may be due to the good solubility of drug in hydrophilic polymer (in this case HPMC K4M). In addition, entrapment of drug molecules in the polymeric chain leads to sustained release of the drug from the films [9]. Numbers of white spots were observed in case of aged film (Fig. 6b) suggesting that the crystals of DTH were formed...
during the course of 3 months storage at 40 ± 2°C/75% ± 5%RH. The higher storage temperature may have resulted in movement/migration of drug particle and deposited on the nuclei to form the crystals. Similar phenomenon was also observed when miconazole nitrate was crystallized out in chitosan-based buccoadhesive film at same storage condition [35].

Conclusions

Diltiazem HCl in film DF9 containing equal proportion of polymers HPMC K4M and Eudragit RS100, and 1,8-cineole (5%) as penetration enhancer showed highest flux in ex-vivo study. This result was further supported by the histology study which demonstrated the mechanism (disorganization of bilipid layer of SC) of 1,8-cineole. The in-vivo study showed a consistent lowering of arterial blood pressure for the optimized film (DF9) until 10 h. Therefore, it can be concluded that the above film has the potential for transdermal drug delivery of DTH with improved permeation profile for longer period of time and thereby increasing the patient compliance.

Conflict of interest

The authors have declared no conflict of interest.

References


