Original Article

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# Formulation, optimization, and evaluation of a transdermal patch of heparin sodium

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**Summary** **The purpose of this research was to develop a matrix-type transdermal therapeutic system containing drug heparin sodium with different ratios of hydrophilic polymeric systems by the solvent evaporation technique by using 30% (w/w) of PEG 400 LR to the dry polymer weight, incorporated as plasticizer. Different concentrations of oleic acid and isopropyl myristate were used to enhance the transdermal permeation of heparin sodium. The physicochemical compatibility of the drug and the polymers studied by differential scanning calorimetry and infrared spectroscopy suggested absence of any incompatibility. Formulated transdermal films were physically evaluated with regard to thickness, weight variation, drug content, flatness, tensile strength, folding endurance, percentage of moisture content and water vapour transmission rate. All prepared formulations indicated good physical stability. *In-vitro* permeation studies of formulations were performed by using diffusion cell apparatus. Formulation prepared with hydrophilic polymer containing permeation enhancer showed best *in-vitro* skin permeation through Wistar albino rat skin as compared to all other formulations. Formulation F9 showed highest flux among all the formulations and 1.369-fold enhancements in drug permeation. These results indicate that the formulation containing 10% of oleic acid with 10% isopropyl myristate give better penetration of heparin sodium through rat skin.**

***Keywords:*** Heparin sodium, transdermal patch, permeation enhancer, *in-vitro* permeation study

## 1. Introduction

Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin (*1*). Transdermal drug delivery has many advantages over the oral route of administration such as improving patient compliance in long term therapy, bypassing first-pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizing inter- and intra patient variability, and making it possible to interrupt or terminate treatment when necessary (*2,3*).

Heparin sodium (HS) inhibits reactions that lead to the clotting of blood and the formation of fibrin clots both *in-vitro* and *in-vivo*. HS acts at multiple sites in the normal coagulation system. Small amounts of heparin in combination with antithrombin III (heparin cofactor) can inhibit thrombosis by inactivating activated factor X and inhibiting the conversion of prothrombin to thrombin. Once active thrombosis has developed, larger amounts of HS can inhibit further coagulation by inactivating thrombin and preventing conversion of fibrinogen to fibrin. HS also prevents the formation of a stable fibrin clot by inhibiting the activation of the fibrin stabilizing factor (*4*).

Intravenous or topical administration of HS has many side effects including pain, swelling, inflammation, irritation at the site of administration and even frequent doses of drug can cause hemotosis which can be avoided by preparing a transdermal patch. The bioavailability of HS transdermal patch is l-2% that

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of intravenous HS, with an elimination half life of 8 h compared to 1.5 h for *i.v.* HS hence a transdermal patch gives a sustained release profile which avoids severe patient pain and frequent application of drug in the case of topical gel.

There are reports describing the use of hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), hydroxypropyl methylcellulose (HPMC) in transdermal patches and ophthalmic preparations (*5-7*) and eudragit RSPO transdermal delivery systems as well as other dosage forms for controlled release of drugs (*8-10*). HPC, HEC, and HPMC are freely water soluble, whereas eudragit RSPO is hydrophobic. Transdermal delivery systems were prepared using all of these methods to study the effect of the hydrophilic and hydrophobic nature of polymer on release of HS. A large number of fatty acids and their esters have been used as permeation enhancers. Oleic acid has been shown to be effective as a permeation enhancer for many drugs, for example increasing the flux of salicylic acid 28-fold and 5-fluorouracil flux 56-fold, through human skin membranes *in-vitro* (*11,12*). It has also been used for ketoprofen (*13*), flurbiprofen (*14*), 5-FU, estradiol (*12*), zalcitabine, didanosine, zidovudine (*15*), *etc*.

The aims of the present study were to (*i*) prepare transdermal patches of HS using hydrophilic and hydrophobic polymers; (*ii*) optimize transdermal patch formulation using $3^2$ full factorial design; (*iii*) study the *in-vitro* diffusion behavior of prepared transdermal patch formulations in the presence and absence of penetration enhancer, and (*iv*) study skin irritation of HS on Albino Wistar rats. The purpose was to provide delivery of the drug at a controlled rate across intact skin.

## 2. Materials and Methods

### 2.1. *Materials*

HS was received as a gift sample from Intas Pharmaceutical Ltd., Ahmedabad, Gujarat, India. HPC and HEC were a generous gift from Famycare Pvt. Ltd., Ahmedabad, Gujarat, India and HPMC was a gift from Acme Pharma Ltd., Ganpat Vidhyanagar, Gujarat, Inida. Eudragit RSPO was purchased from Vikram Thermo Ltd., Mehsana, Gujarat, India. Oleic acid (OA), polyethylene glycol (PEG) 400 LR and Di-n-butyl-phthalate (DBP) were procured from Sigma Chemicals Ltd., Ahmedabad, Gujarat, India. Other materials used in the study (chloroform, methanol, dichloromethane, glycerol, potassium dihydrogen phosphate, *etc*.) were of analytical grade. Milli-Q water was used throughout the study.

### 2.2. *Investigation of physicochemical compatibility of drug and polymer*

The physicochemical compatibility between HS and polymers used in the patch was studied using differential scanning calorimetry (DSC-Shimadzu 60 with TDA trend line software, Shimadzu Co., Kyoto, Japan) and Fourier transform infrared (FTIR- 8300, Shimadzu Co., Kyoto, Japan) spectroscopy.

In DSC analysis, the samples were weighed (5 mg), hermetically sealed in flat bottom aluminum pans, and heated over a temperature range of 50 to 300°C at a constant increasing rate of 10°C/min in a nitrogen atmosphere (50 mL/min). The thermograms obtained for HS, polymers, and physical mixtures of HS with polymers were compared. The infrared (IR) spectra were recorded using an FTIR by the KBr pellet method and spectra were recorded in the wavelength region between 4,000 and 400 $cm^{-1}$. The spectra obtained for HS, polymers, and physical mixtures of ACF with polymers were compared.

### 2.3. *Preparation of transdermal films*

Transdermal patches containing HS were prepared by the solvent evaporation technique in cylindrical glass molds with both sides open (*16*). The backing membrane was cast by pouring a 2% (m/V) polyvinyl alcohol (PVA) solution followed by drying at 60°C for 6 h. The drug reservoir was prepared by dissolving polymer in Milli-Q water. PEG 400 LR 30% (w/w of dry polymer composition) was used as a plasticizer. Ten mg of the drug was added into the homogeneous dispersion with slow stirring on a magnetic stirrer. The uniform dispersion was cast on a PVA backing membrane and dried at room temperature (Table 1). The films were stored between sheets of wax paper in a desiccator.

### 2.4. *Physicochemical characterization of films*

#### 2.4.1. *Thickness*

The thickness of patches was measured at three different places using a micrometer (Mitutoyo Co., Japan) and mean values were calculated (*16*).

#### 2.4.2. *Weight variation*

Mass variation of patches was measured by individually weighing randomly selected patches. Such determinations were carried out for each formulation (*17*).

#### 2.4.3. *Drug content*

Patches of specified area (1 $cm^2$) were dissolved in 5 mL of Phosphate buffer pH 7.4 and the volume was made up to 10 mL with the same buffer. A blank was prepared using a drug-free patch treated similarly. The solutions were filtered through a 0.45 µm membrane,

**Table 1. Composition of transdermal patches**

| Formulation code | PVA (backing membrane) | HEC | HPC | HPMC K4 | HPMC K15 | HPMC K15: RS 100 | Oleic acid | PEG 4000 LR | Water |
|---|---|---|---|---|---|---|---|---|---|
| HS 1 | 2% | 3.0% | - | - | - | - | 0.2 mL | | q.s. |
| HS 2 | 2% | 4.0% | - | - | - | - | 0.2 mL | | q.s. |
| HS 3 | 2% | - | 3.0% | - | - | - | 0.2 mL | | q.s. |
| HS 4 | 2% | - | 4.0% | - | - | - | 0.2 mL | | q.s. |
| HS 5 | 2% | - | - | 2.0% | - | - | 0.2 mL | | q.s. |
| HS 6 | 2% | - | - | 3.0% | - | - | 0.2 mL | 30 % w/v | q.s. |
| HS 7 | 2% | - | - | 4.0% | - | - | 0.2 mL | of total ploymer | q.s. |
| HS 8 | 2% | - | - | - | 2.0% | - | 0.2 mL | composition | q.s. |
| HS 9 | 2% | - | - | - | 3.0% | - | 0.2 mL | | q.s. |
| HS 10 | 2% | - | - | - | 4.0% | - | 0.2 mL | | q.s. |
| HS 11 | 2% | - | - | - | - | 3:7 | 0.2 mL | | q.s. |
| HS 12 | 2% | - | - | - | - | 5:5 | 0.2 mL | | q.s. |
| HS 13 | 2% | - | - | - | - | 7:3 | 0.2 mL | | q.s. |

Note: * 30 % w/w of dibutyl phthalate to the polymer weight, incorporated as plasticizer.

diluted suitably and absorbance was read at 201 nm in a double beam UV-Visible spectrophotometer.

2.4.4. *Flatness*

Three longitudinal strips were cut out from each film: 1 from the center, 1 from the left side, and 1 from the right side. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness (*18*).

2.4.5. *Folding endurance*

Folding endurance was determined by repeatedly folding one film at the same place until it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value for folding endurance (*19*).

2.4.6. *Tensile strength*

In order to determine the elongation as tensile strength, the polymeric patch was pulled by means of a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation *i.e.* the distance traveled by the pointer before break of the patch was noted with the help of a magnifying glass on the graph paper, and tensile strength was calculated as $kg \cdot cm^{-2}$.

2.4.7. *Percentage of moisture content*

The films were weighed individually and kept in desiccators containing activated silica at room temperature for 24 h. Individual films were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight (*20*).

2.4.8. *Water vapor transmission rate (WVTR)*

WVTR is defined as the quantity of moisture transmitted through a unit area of film in unit time (*21*). Glass cells were filled with 2 g of anhydrous calcium chloride and a film of specified area was affixed onto the cell rim. The assembly was accurately weighed and placed in a humidity chamber (80 ± 5% RH) at 27 ± 2°C for 24 h.

2.5. *In-vitro skin permeation studies*

*In-vitro* skin permeation studies were performed by using a Diffusion Cell Apparatus (EDC-07) with a receptor compartment capacity of 12 mL. Excised rat abdominal skin (Wistar albino) was mounted between the donor and receptor compartment of the diffusion cell. The formulated patches were placed over the skin and covered with paraffin film/or having backing layer. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5°C. Samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer pH 7.4 at each sample withdrawal. The cumulative percentages of drug permeated per square centimeter of patches were plotted against time.

2.6. *Full factorial design*

A $3^2$ randomized full factorial design was used in the present study. In this design two factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The amount of isopropyl myristate ($X_1$) and the amount of oleic acid ($X_2$) were selected as independent variables. Drug release at 10 h was selected as dependent variable. The design layout is depicted in Table 4.

2.7. *Permeation data analysis*

The flux ($\mu g \cdot cm^{-2} h^{-1}$) of HS was calculated from the slope of the plot of the cumulative amount of HS permeated per $cm^2$ of skin at steady state against time using linear regression analysis (*22,23*).

The steady state permeability coefficient ($K_p$) of the drug through rat epidermis was calculated by using the following equation (*24*): $Kp = J/C$ (**1**), where $J$ is the flux and $C$ is the concentration of ACF in the patch. The enhancing effect of penetration enhancer was calculated in terms of enhancement ratio (ER), and was calculated by using the following equation (*25*): *ER* = (*Kp with penetration enhancer*)/(*Kp without penetration enhancer*) (**2**).

2.8. *Kinetic modeling of drug release*

To analyze the mechanism of drug release from the patches, the release data were fitted to the following equations. Zero-order equation, $Q = K_0 t$ (**3**), where $Q$ is the amount of drug released at time $t$, and $k_0$ is the release rate. First-order equation, $\ln(100 - Q) = \ln 100 - k_1 t$ (**4**), where $Q$ is the percent of drug release at time $t$, and $k_1$ is the release rate constant. Higuchi's equation, $Q = k_2 t^{1/2}$ (**5**), where $Q$ is the percent of drug release at time $t$, and $k_2$ is the diffusion rate constant.

2.9. *Skin irritation study*

The skin irritation test was performed on six rats, which were divided into 3 groups ($n = 2$), to evaluate the irritant properties of the drug HS. For this purpose, rats were shaved and an aqueous solution of formalin 0.8% was used as standard irritant. Drug reservoir patches of 2 $cm^2$ were used as test patches. The patches were removed after a period of 24 h with the help of an alcohol swab. The skin response was examined for development of erythema and edema for each rat at the end of 24 h, with respect to appearance of redness, flare, wheals, and rashes. The area was observed for 3 days. Finally, the application sites were graded according to a visual scoring scale from 0 to 4 (*26*).

2.10. *Stability study of optimized formulation*

A stability study was carried out for optimized patch formulation at 40°C temperature in a humidity chamber having 75% RH for 3 months. After 3 months samples were withdrawn and evaluated for physicochemical properties and *in-vitro* diffusion studies.

## 3. Results and Discussion

3.1. *Investigation of physicochemical compatibility of drug and polymer*

Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (*i.e.*, endothermic or exothermic phase transformations). The thermograms of **(A)** HS **(B)** HS with HPMC K15, and **(C)** HS with eudragit RSPO/ammonio methaacrylate copolymer type B are presented in Figure 1. The HS showed a melting peak at 26.83°C. Peak of HS at 271.03°C was present at the same position *i.e.* near to 269°C in the physical mixture of drug with both HPMC and eudragit RSPO patch formulation excipients. This confirmed the physicochemical stability of drug with the formulation excipient used in the study.

Drug-excipient interactions play a vital role with respect to release of drug from the formulation among others. FTIR techniques have been used here to study the physical and chemical interaction between drug and excipients used. Infrared (IR) spectra of HS **(A)**, physical mixture of HS + HPMC K15 **(B)**, and physical mixture of HS + HPMC K15 + eudragit RSPO/ ammonio methacrylate copolymer type B **(C)** are shown in Figure 2. Infrared absorption spectroscopy of HS showed a band at 1,230 and 1,040 $cm^{-1}$ due to symmetrical and asymmetrical stretching of S-O in $SO_3$ heparin groups, respectively. From the figure it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers, and that excipients are compatible with HS within the formulation.

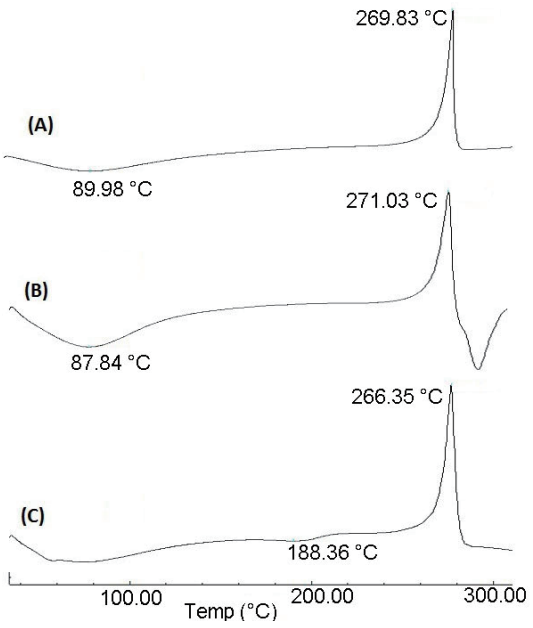


**Figure 1. DSC thermogram of heparin sodium and its physical mixture with different excipients. (A)** Heparin sodium. **(B)** Heparin sodium with HPMC K15. **(C)** Heparin sodium drug with eudragit RSPO/Ammonio methaacrylate copolymer type B.

3.2. *Physicochemical characterization of films*

The results of the physicochemical characterization of the patches are shown in Table 2. The thickness ranged between 0.063 and 0.146 mm, which indicate that they were uniform in thickness. The weights ranged between 5.70 mg and 9.93 mg, which indicates that different batches patch weights, were relatively similar.

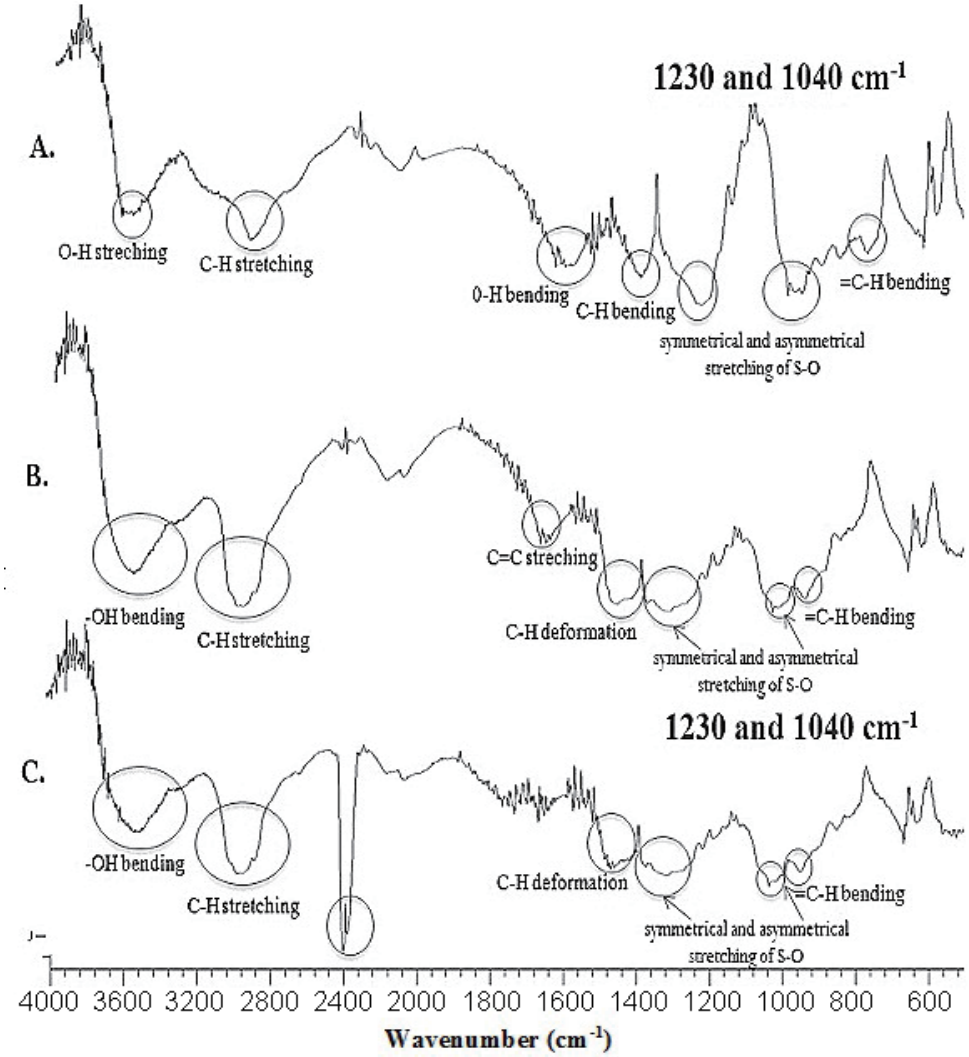


**Figure 2. FTIR spectras of heparin sodium and its physical mixture with different excipients. (A)**: Heparin sodium. **(B)**: Physical mixture of heparin sodium + eudragit RSPO. **(C)**: Physical mixture of heparin sodium + HPMC K15 + eudragit RSPO/ ammonio methacrylate copolymer type B.

**Table 2. Evaluation of transdermal patch formulations of HS**

| Formulation code | Thickness uniformity (mm) | Weight variation test (mgs) | Drug content (mcg/2 $cm^2$) | Folding endurance | Tensile strength ($kg/cm^2$) | Moisture Content (%) | Moisture uptake (%) | WVT ($mg/cm^2$ hr) |
|---|---|---|---|---|---|---|---|---|
| HS 1 | 0.063 ± 0.011 | 5.93 ± 0.94 | 9.9 ± 0.25 | 156 ± 9 | 2.0 ± 0.5 | 10.66 ± 0.2 | 1.05 ± 0.25 | 6.0 ± 0.45 |
| HS 2 | 0.076 ± 0.005 | 5.70 ± 0.45 | 9.6 ± 0.39 | 165 ± 3 | 2.5 ± 0.5 | 11.23 ± 0.5 | 1.82 ± 0.35 | 5.3 ± 0.311 |
| HS 3 | 0.065 ± 0.015 | 6.09 ± 0.56 | 9.7 ± 0.52 | 148 ± 5 | 2.5 ± 0.5 | 13.45 ± 0.4 | 0.90 ± 0.32 | 5.1 ± 0.56 |
| HS 4 | 0.086 ± 0.005 | 6.96 ± 0.66 | 9.8 ± 0.70 | 180 ± 2 | 3.0 ± 0.25 | 12.62 ± 0.6 | 1.80 ± 1.15 | 6.0 ± 0.11 |
| HS 5 | 0.113 ± 0.011 | 7.03 ± 0.73 | 9.9 ± 0.42 | 180 ± 3 | 2.0 ± 0.5 | 7.36 ± 0.78 | 1.48 ± 0.57 | 6.6 ± 0.26 |
| HS 6 | 0.112 ± 0.01 | 7.00 ± 0.75 | 9.8 ± 0.75 | 205 ± 4 | 2.5 ± 0.25 | 7.50 ± 0.01 | 3.03 ± 0.12 | 6.6 ± 0.43 |
| HS 7 | 0.116 ± 0.005 | 7.12 ± 0.45 | 9.5 ± 0.44 | 80 ± 10 | 3.0 ± 0.75 | 7.71 ± 0.78 | 1.02 ± 0.65 | 6.1 ± 0.5 |
| HS 8 | 0.139 ± 0.015 | 7.03 ± 0.95 | 10.1 ± 0.43 | 135 ± 4 | 2.0 ± 0.25 | 8.91 ± 0.20 | 1.56 ± 0.95 | 6.9 ± 0.15 |
| HS 9 | 0.143 ± 0.01 | 7.93 ± 0.95 | 9.8 ± 0.52 | 196 ± 2 | 3.5 ± 1.0 | 9.03 ± 0.45 | 2.09 ± 0.89 | 5.2 ± 0.45 |
| HS 10 | 0.125 ± 0.01 | 7.98 ± 0.70 | 9.9 ± 0.29 | 92 ± 5 | 3.5 ± 0.5 | 8.76 ± 0.26 | 2.12 ± 0.59 | 5.3 ± 0.31 |
| HS 11 | 0.146 ± 0.015 | 8.10 ± 0.54 | 9.7 ± 0.93 | 204 ± 2 | 2.0 ± 0.5 | 7.50 ± 0.40 | 3.21 ± 0.15 | 4.9 ± 0.11 |
| HS 12 | 0.145 ± 0.011 | 9.93 ± 0.41 | 9.8 ± 0.42 | 201 ± 5 | 2.5 ± 0.0 | 7.36 ± 0.80 | 2.89 ± 0.35 | 5.1 ± 0.72 |
| HS 13 | 0.144 ± 0.01 | 8.16 ± 0.32 | 9.7 ± 0.36 | 194 ± 7 | 3.5 ± 0.5 | 7.24 ± 0.50 | 2.56 ± 0.33 | 5.2 ± 0.115 |

* mean ± SD ($n$ = 3).

Good uniformity of drug content among the batches was observed with all formulations and ranged from 97.9 to 99.2%. The results indicate that the process employed to prepare patches in this study was capable of producing patches with uniform drug content and minimal patch variability. The flatness study showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness. Thus, no amount of constriction was observed; all patches had a smooth, flat surface; and that smooth surface could be maintained when the patch was applied to the skin. Folding endurance test results indicated that the patches would not break and would maintain their integrity with general skin folding when applied. Moisture content and moisture uptake studies indicated that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture content and moisture uptake of the patches. The moisture content of the prepared formulations was low, which could help the formulations remain stable and reduce brittleness during long term storage. The moisture uptake of the formulations was also low, which could protect the formulations from microbial contamination and reduce bulkiness (*27*).

### 3.3. *In-vitro skin permeation*

The *in-vitro* release profile is an important tool that predicts in advance how a drug will behave *in-vivo* (*28*). The results of *in-vitro* skin permeation studies of HS from transdermal patches are shown in Figure 3. In the present study hydrophilic (HPC, HEC, and HPMC) and hydrophobic (eudragit RSPO) polymers were used to preparepatches. Formulation HS13 exhibited the greatest, 93.25%, drug release value, while formulation HS5 exhibit the lowest, 64.72%, drug release value. The cumulative amount of drug released from formulations containing hydrophilic polymer released drug at a faster rate than hydrophobic polymer. The cumulative amount of drug released from formulations HS9, HS12 and HS13 were much higher than other formulations. In addition to nature of polymer concentration of polymer also affected drug release. The transdermal drug delivery system HS9 (HPMC K 15 M alone) showed drug release (88.16%), and lasted only for 8 h but the transdermal drug delivery system HS13 (HPMC K15:ammonio acrylate) showed the highest prolonged drug release successfully for 10 h (93.25%). HS13 achieved a high cumulative amount of drug permeation at the end of 10 h. Based on physiochemical and *in-vitro* release experiments, HS13 was chosen for further studies.

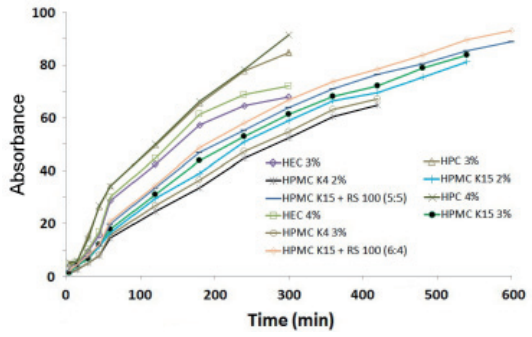


**Figure 3. Release profiles of HS from patches containing different concentrations of HPC, HEC, HPMC, and eudragit RSPO, mean ± SD (*n* = 3).**

### 3.4. *Full factorial design*

#### 3.4.1. *Physicochemical properties of factorial design batches*

The results of the physicochemical characterization of the patches are shown in Table 3.

#### 3.4.2. *In-vitro drug release study of factorial design batches*

The cumulative percentage of drug permeated through the rat epidermis from the patch containing different concentrations of penetration enhancer is shown in Figure 4.

An increase in concentration of oleic acid leads to an increase in $Q_{10hr}$ because the coefficient $b_1$ bears a positive sign. Increasing the concentration of oleic acid from 5 to 10% the $Q_{10hr}$ value increased from 81.13% to 88.14%. An increase in concentration of isopropyl myristate leads to an increase in $Q_{10hr}$ because the coefficient $b_2$ bears a positive sign. When increasing the concentration of isopropyl myristate from 5 to 10% the

**Table 3. Properties of transdermal patches containing HS**

| Formulation code | Thickness (μm) | Weight variation (mg) | Drug content | Folding endurance | Tensile strength (kg·cm$^{-2}$) |
|---|---|---|---|---|---|
| HS 1 | 160 ± 5.60 | 9.61 ± 0.57 | 97.9 ± 2.42 | 198 ± 6.93 | 2.55 ± 0.50 |
| HS 2 | 168 ± 5.88 | 10.12 ± 0.38 | 98.6 ± 2.45 | 202 ± 7.07 | 2.58 ± 0.25 |
| HS 3 | 171 ± 5.95 | 10.23 ± 0.55 | 97.5 ± 2.41 | 215 ± 7.52 | 3.00 ± 0.75 |
| HS 4 | 158 ± 5.53 | 8.20 ± 0.39 | 96.9 ± 2.39 | 218 ± 7.63 | 2.00 ± 0.50 |
| HS 5 | 166 ± 5.81 | 10.73 ± 0.37 | 98.8 ± 2.45 | 201 ± 7.70 | 2.50 ± 0.25 |
| HS 6 | 154 ± 5.39 | 9.97 ± 0.38 | 96.8 ± 3.52 | 200 ± 7.00 | 2.50 ± 0.75 |
| HS 7 | 165 ± 5.77 | 8.21 ± 0.29 | 97.8 ± 2.42 | 208 ± 7.28 | 3.00 ± 0.50 |
| HS 8 | 169 ± 5.61 | 10.87 ± 0.38 | 98.6 ± 2.45 | 196 ± 6.86 | 3.50 ± 0.25 |
| HS 9 | 152 ± 5.28 | 10.52 ± 0.40 | 99.2 ± 1.51 | 220 ± 7.42 | 3.50 ± 1.00 |

* mean ± SD (*n* = 3).

$Q_{10hr}$ value increased from 84.36% to 90.31%.

Here the coefficient of interaction terms showed a negative value. The interaction term indicated that $Q_{10hr}$ was not significantly affected by interaction of two penetration enhancers. This indicates that by changing two factors at a time there was no effect on $Q_{10hr.}$

The maximum amount ($Q_{10hr}$) of ACF that permeated during the 10 h of the study was 93.06% from formulation H8. The flux was calculated by dividing the cumulative amount of drug which permeated per $cm^2$ of the skin with time. Thus the corresponding flux of HS was 292.03 $\mu g \cdot cm^{-2} hr^{-1}$ from formulation H8. A marked effect of penetration enhancer on HS permeation was observed when they were incorporated in the patch in varying concentrations. The cumulative percentage of HS that permeated over 10 h was found to increase ranging from 67.91 to 93.06% for patches. The corresponding flux values ranged from 208.50 to 292.03 $\mu g \cdot cm^{-2} hr^{-1}$. Formulation H8 shows highest flux among all the formulations. This result indicated that the formulation containing 10% oleic acid with 10% isopropyl myristate gave better penetration of HS through rat skin (Table 4).

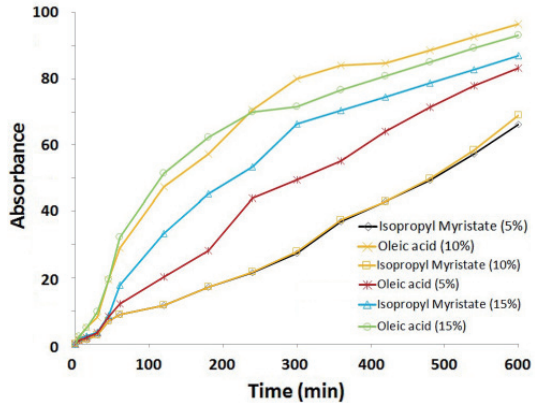


**Figure 4. Release profiles of HS having different penetration enhancers, mean ± SD (*n* = 3).**

### 3.5. *Regression analysis for $Q_{10hr}$*

The significance levels of the coefficients *b*1, *b*2, *b*11, *b*22 and *b*12 were found to be *P* = 6.570, 7.30, –0.540, –3.732 and –2.057 respectively, so they were omitted from the full model to generate a reduced model. The coefficient *b*1 was found to be significant; hence, it was retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients *b*1, *b*2, *b*11, *b*22, and *b*12 contribute significant information to the prediction of $Q_{10hr}$. The critical value of F for α = 0.05 is equal to 9.11 (df = 4.3). Since the calculated value (*F* = 2.50) is less than the critical value (*F* = 9.11), it may be concluded that the terms *b*1, *b*2, *b*11, *b*22 and *b*12 do not contribute significantly to the prediction of *Q*10 and can be omitted from the full model to generate the reduced model.

### 3.6. *Kinetic modeling of drug release*

The cumulative amount of drug which permeated per square centimeter of patches (H1 to H9) through rat skin plotted against time was fitted to zero, first and Higuchi kinetic models. The release profile of H followed mixed zero-order and first-order kinetics in different formulations. The release profile of patches H1, H2, H3, H4, H5, H6, H7, H8, and H9 as per Higuchi's equation was 0.937, 0.966, 0.974, 0.960, 0.980, 0.985, 0.969, 0.994 and 0.990 respectively. However, the release profile of the optimized formulation H8 ($r^2$ = 0.994 for Higuchi) indicated that the permeation of the drug from the patches was governed by a diffusion mechanism.

### 3.7. *Skin irritation study*

The skin irritation test of the transdermal formulation of optimized batch HS13 and marketed formulation on the rat gave a score less than the 1 scale for erythma as well as 0 level scale for edema as compared to the standard

**Table 4. $3^2$ full factorial design layouts for HS transdermal patches**

| Batch No. | $X_1$ | $X_2$ | $Q_{10hr}$ release (%) | Flux (J) ($\mu g \cdot cm^{-2} \cdot hr^{-1}$) | Permeability co efficient ($K_p$) ($cm \cdot hr^{-1}$) | Enhancement ratio (ER) |
|---|---|---|---|---|---|---|
| HS 1 | -1 | -1 | 68.95 | 208.50 | 4.17 | 1.04 |
| HS 2 | 1 | 1 | 87.01 | 263.12 | 5.26 | 1.32 |
| HS 3 | 0 | 1 | 88.87 | 268.74 | 5.37 | 1.34 |
| HS 4 | 1 | -1 | 69.84 | 211.20 | 4.22 | 1.06 |
| HS 5 | 1 | 0 | 83.2 | 251.60 | 5.03 | 1.26 |
| HS 6 | 0 | 0 | 82.76 | 250.27 | 5.01 | 1.25 |
| HS 7 | 0 | -1 | 69.51 | 208.50 | 4.17 | 1.04 |
| HS 8 | -1 | 0 | 96.57 | 292.03 | 5.84 | 1.46 |
| HS 9 | -1 | 1 | 93.13 | 281.63 | 5.63 | 1.41 |

Translation of coded levels in actual units

| Variables level | Low (-1) | Medium (0) | High (+1) |
|---|---|---|---|
| Amount of isopropyl myristate (% W/W of drug) $X_1$ | 0 | 5 | 10 |
| Amount of oleic acid (% W/W of drug) $X_2$ | 0 | 10 | 15 |

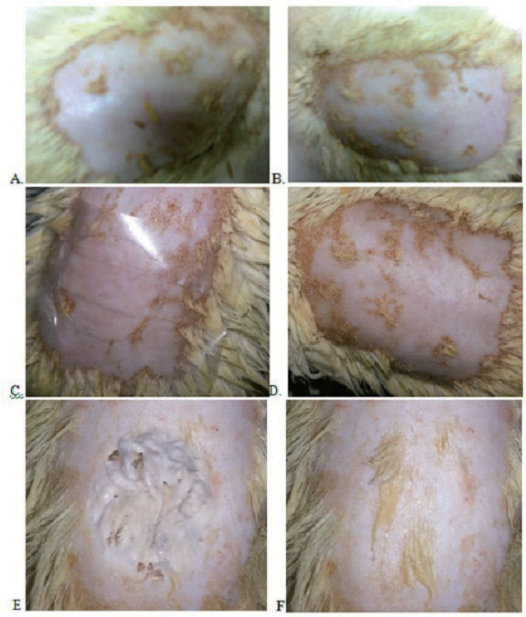

**Figure 5. Skin irritation studies. (A)**: Control group at 0 h, **(B)**: Control group at 24 h, **(C)**: Optimized batch HS13 at 0 h, **(D)**: Optimized batch HS13 at 24 h, **(E)**: Marketed Formulation at 0 h, **(F)**: Marketed Formulation at 24 h.

**Table 5. Stability studies**

| Stability condition | Sampling time | Folding endurance | Visual appearance | Drug content |
|---|---|---|---|---|
| Room tempeartue (30°C and 65% RH) | Initial ( 0 day) | > 200 | Clear film | 99.5 |
| | After 30 days | > 200 | Clear film | 99.4 |
| Accelerated condition (40°C and 75% RH) | Initial ( 0 day) | > 200 | Clear film | 99.8 |
| | After 30 days | 35 | Hazy | 99.1 |

formalin solution for 24 and 48 h after application (Figure 5). According to Draize *et al*. (*29*), compounds producing scores of 2 or less are considered negative (no skin irritation). Hence, the developed transdermal formulations are free of skin irritation.

3.8. *Stability study*

In order to determine the change in physicochemical parameters and *in-vitro* release profile on storage, a stability study was carried out. The physicochemical parameters of the optimized formulation were not significantly changed on storage. The *in-vitro* release profile before and after storage is shown in Table 5. The result indicates that the formulation was stable under the required storage conditions.

## 4. Conclusion

The method of preparation of transdermal patches of HS presented in this research work is simple. All formulations also showed good physicochemical properties like thickness, weight variation, drug content, flatness, folding endurance, moisture content and moisture uptake. The *in-vitro* release data showed that drug release from the patch formulation were affected by types of polymer and concentration of polymer. Effect of penetration enhancer like oleic acid and isopropyl myristate have been checked on *in-vitro* permeation of drug. These studies indicated that as the concentration of penetration enhancer increased drug permeation was increased. The finding of this result revealed that the problems of HS on i.v. administration like pain, swelling, inflammation, and short half life can be overcome by applying HS topically in the form of a transdermal patch.

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