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In vitro permeation and stability studies on developed drug-in-adhesive transdermal patch of simvastatin

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ABSTRACT

The transdermal drug-in-adhesive (DIA) patch of simvastatin (SM) was developed using acrylic adhesives such as DURO-TAK® 87-9301, DURO-TAK® 87-4287, DURO-TAK® 87-235A. The patches were evaluated for in vitro drug permeation across the pork ear skin using diffusion cell and stability studies. Among the three acrylic adhesives used, DURO-TAK® 87-9301 exhibited maximum flux (5.18 ± 0.23 μg/cm^2/h). To further enhance the drug permeation, isopropyl myristate (IPM), D-limonene and 1,8-cineol as penetration enhancers (PEs) were incorporated into the DIA patch prepared from DURO-TAK® 87-9301. Out of those, IPM containing patch exhibited a flux of 16.45 ± 1.67 μg/cm^2/h revealing that IPM is the best PE. The stability study was carried out on optimized fresh (SA4) and 6 months old patches stored at room and at accelerated condition (40 ± 2 °C/75 ± 5%RH) using FTIR, DSC and SEM techniques. Significant shift of peaks were not observed in FTIR spectra and DSC thermograms of the patches after the stability period. SEM micrographs of patches did not show any evidence of recrystallization indicating the presence of drug in the molecular form throughout the adhesive matrix. The investigation reveals that the DIA patch studied as above is stable and may serve as a potential drug delivery system for simvastatin.

1. Introduction

The formulations applied onto the skin surface are broadly categorized into two groups viz. topical and transdermal. Topical formulations deliver drug into local area of skin without systemic exposure. In contrary, transdermal formulations deliver effective concentrations of drugs into systemic circulation and thereby minimizing local effect [1]. With regard to transdermal application, a patch is the most convenient formulation with reference to productivity, cost involved in manufacturing and ease of application [2]. Furthermore, patches meant for transdermal application are usually widely accepted and represent a better replacement when oral administration is difficult such as in case of patient unable to swallow, or may results in erratic absorption (e.g. nausea, vomiting), or is in coma [3].

Transdermal patches are basically classified into three types viz. reservoir, polymer matrix monolithic or multi-laminate drug-in-adhesive (DIA) and microreservoir. A monolithic DIA patch is composed of an active ingredient, additives, pressure-sensitive-adhesive (PSA) [4,5] backing film and release liner [2]. DIA patch system has advantage over other patches due to its small size and thickness, better flexibility, uncomplicated production process, straightforward design and patient choice [6–8]. Moreover, dose adjustment, for example for patients with impaired hepatic or renal functions, can easily be attained by cutting/dividing the patch [9]. In case of DIA patch system, drug incorporated adhesive layer is in contact with the skin surface after application. Therefore, the selection of a suitable adhesive is important. Frequently used PSA polymers in transdermal DIA patch are polyisobutylenes, silicones and acrylics [10–12].

The outer layer of skin is stratum corneum (SC), which only allow lipophilic drugs with small molecular weight (< 500 Da) to penetrate via passive diffusion to reach systemic circulation in desired concentration [13]. As a result only a limited number of transdermal therapeutic systems (TTS) are available commercially. Presently, two approaches such as chemical and physical have been employed to enhance permeation of drugs across the skin in order to attain therapeutic concentration in blood. The chemical approach using penetration enhancers (PEs) has been considered most often for the development of successful DIA patches [2].

Simvastatin (SM) belongs to statin class of hypolipidemic drug whose mechanism of action is to inhibit 3-hydroxy-3-methylglutaryl

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coenzyme A (HMG-CoA) reductase and thereby decreases the biosynthesis of cholesterol [14]. SM exhibits poor aqueous solubility of 6.3 μg/ml at 25 °C, leading to poor absorption across the gastro-intestinal tract [15]. SM is considered as suitable candidate for transdermal drug delivery as it undergoes substantial pre-systemic metabolism in liver. In addition, it has molecular weight less than 500 Da (418.566), low melting point of 135–138 °C, [16] and short oral half-life of 2 h [17]. Extensive review of literature could not show any appreciable work on DIA patch of SM.

In the earlier investigation the researchers have successfully developed the matrix [17] and reservoir [18] type of transdermal drug delivery system (TDDS) of SM. The matrix patch of SM was prepared with polyvinyl alcohol (PVA) and eudragit (EG) in different ratio as polymer and dibutyl phthalate (DBT) as plasticizer using Box-Behnken design. At the optimum condition of 2% of SM; 2:1 ratio of PVA:EG; and 40% of DBT, values of tensile strength and flux (dependent variables) were found to be 11.871 MPa and 43.569 μg/cm^2/h, respectively. In the later study the effect of independent variables such as percentage of SM, poloxamer 407 (PX 407), and D-limonene on Q48 (cumulative amount of drug permeated per unit area after 48 h of permeation study) were studied employing Box-Behnken design. The highest Q48 (76.94 μg/cm^2) of SM across full thickness human cadaver skin was exhibited by the formulation composed of 1.5% w/w of SM, 25% w/w of PX 407 and 10% w/w of D-limonene. Furthermore, SM permeation was found to be increased with the increase in SM and D-limonene concentration. Recently, a monolithic DIA patch for co-delivery of SM and olanzapine was developed, wherein the drugs were encapsulated in nanostructured lipid carrier (NLC) and then incorporated into patch [19]. In the present study, both passive (using NLC and PEs) and active methods (pre-treatment of skin with dermaroller) were used and the result showed that propylene glycol as PE exhibited highest permeation rate.

One of the major problems of these patches is the crystallization of drug in the matrix upon storage. According to Fick's law of diffusion, the amount of dissolved drug in the matrix determines the drug release. Thus, drug re-crystallization can have a grave consequence on the drug release and eventually the therapeutic efficacy suffers. Furthermore, re-crystallization of drug may have effect on the adhesive properties of the patch. It was observed from the previous studies that the adsorption of drug such as ethyl estradiol and levonorgestrel onto the insoluble carrier cross-povidone (CPVP) prevented the drug re-crystallization [10,20]. This was attributed to the formation of hydrogen bond between the drug and CPVP thereby prevented the drug molecules from forming nuclei and subsequently crystal formation.

The concept of stability in pharmaceutics is understood as the ability of a pharmaceutical product to retain its properties within the specified limits throughout its declared shelf life [21]. Investigations/tests must be carried out according to international standards to define shelf life of a drug which provide information about various aspects of stability such as chemical, physical and microbiological [22]. Therefore, there is an intense need to study the stability aspects of drug in different types of patches along with other characterization. The objectives of the current research were to develop DIA patches of SM employing three different grades of acrylic adhesives and to study the effect of PEs on in vitro permeation of SM across ear skin of pig and assess the stability potential of selected patch at room and accelerated conditions for six months so as to throw some light on the shelf life of the formulation apart from conducting mechanistic study on treated and untreated skin specimens using histopathology technique.

2. Materials and methods

2.1. Raw materials and chemicals

SM (99.1% w/w) was a generous gift of Ranbaxy Laboratories Pvt. Ltd., Gurgaon, Haryana, India. Acrylic adhesives such as DURO-TAK® 87-9301, 87-4287, 87-235A were received as gift samples from Henkel electronic material LLC, 825 Cedar springs RD, Salisbury, NC 28147, US. The properties of acrylic adhesives were made supplementary. Polyster film laminate (ScotchPak™ 1012) backing layer, fluoropolymer coated polyster film (ScotchPak™ 1022) release liner were received as gift sample from #M Pharmaceuticals (St. Paul, MN, USA). Isopropyl myristate (IPM) was purchased from Sisco Research Lab., Mumbai, India. 1, 8-Cineole was procured from Merck Specialties Pvt. Ltd., Mumbai, India and D-limonene was obtained from Loba Chemie, Mumbai, India. Remaining chemicals used in the investigation were procured from standard and reliable sources.

2.2. Pre-formulation study

2.2.1. FTIR analysis

In order to investigate possible interaction between SM and PSAs, FTIR study was performed on pure drug and physical mixtures of drug with PSAs (1:1) employing α-FTIR, Bruker Optics, Germany. The analysis was performed by KBr pellet method with frequency ranges from 4000 to 400 cm^{-1}. The samples were dried prior to the analysis. The FTIR study was also performed on selected fresh patch, and patches before and after stability study.

2.2.2. DSC study

DSC measurements on SM and its physical mixtures with PSAs, and selected DIA patch before and after stability study were performed using a Differential scanning calorimeter (DSC Q10 V9.4 Build 287 with TDA), Shimadzu, Japan. Upon removal of the moisture from the sample by heating, accurately weighed (5 mg) sample was transferred into a standard aluminium crucible. Empty pan was used as reference. Heating of samples was done at a rate of 10 °C/min over a temperature range of 20–400 °C [10].

2.3. Preparation of patch

SM incorporated transdermal patches were prepared with various acrylate adhesives viz. DURO TAK® 87-9301, 87-4287, 87-235A and PEs such as IPM, D-limonene, 1,8-cineole by solvent evaporation technique [2,7] as per the details furnished in Table 1. Weighed amounts of SM and PEs were dissolved in ethyl acetate. PSAs were added to the above solution and agitated at room temperature for 30 min and the mixed solution was cast at a thickness of 400 μm on a polyster film (ScotchPak™ 1022) release liner with a micrometer adjustable film applicator (Culture Instruments, Bangalore, India). They were kept at room temperature for 24 h for evaporation of the residual solvent. The patches were then laminated with polyster film laminate (ScotchPak™ 1012) backing layer and cut into cut into suitable sizes (figure was made supplementary), packed in aluminium foil, and stored at ambient temperature until further study. The final dry thickness of the DIA matrix was fixed at 100 μm. The PEs were incorporated to the selected patches containing DURO TAK® 87-9301.

Table 1
Formulation of different DIA patches of SM.

Ingredient (% w/w)	Formulation code					
	SA1	SA2	SA3	SA4	SA5	SA6
SM	2	2	2	2	2	2
DURO TAK® 87-9301	98	–	–	93	93	93
DURO TAK® 87-4287	–	98	–	–	–	–
DURO TAK® 87-235A	–	–	98	–	–	–
IPM	–	–	–	5	–	–
D-Limonene	–	–	–	–	5	–
1, 8-Cineole	–	–	–	–	–	5

2.4. Drug content uniformity

Upon removal of the adhesive matrix, the drug in the patches was extracted by immersing patch sample (1 cm × 1 cm) in 10 ml of solvent mixture (acetonitrile:ethylacetate, 40:60) followed by stirring for 24 h. The resultant solution was filtered, suitably diluted and analyzed for SM content at 238 nm using UV spectrophotometer.

2.5. In vitro permeation study

Excised hairless pork ear skin was used for the in vitro percutaneous absorption study because; it is readily accessible in sufficient size and quality compared to human skin, and this animal skin has shown histological and biochemical properties similar to human skin. It is supported by the fact that pork skin is particularly suitable for the permeation studies and providing results comparable to human skin [23]. Thus, it is expected that the flux of SM in human skin should be similar to the results obtained with pork ear skin.

Fresh pig ears were obtained from a local slaughter house. With the use of distilled water [13] and forceps, blood stains and fatty tissues were removed from ears. Then, the skin sample was cut into desired size and kept in the deep freezer (−20 °C) until its use [24].

The in vitro permeation study of SM from the prepared DIA patch was carried out employing six-station vertical diffusion cell (Kshitij International, Ambala, India) with 3.8 cm^2 exposed surface area and 20 ml receptor compartment capacity. All the skin samples were used up within 7-days. Phosphate buffer of pH 7.4 prepared from HPLC grade water and acetonitrile (HPLC grade) in the ratio of 70:30 was used as the receptor medium [25], which aimed to accomplish following objectives; (i) to provide and maintain sink condition, (ii) to avoid alteration in thermodynamic activity of the drug in the donor compartment (iii) to shun possible contamination of skin till the end of study [26]. The temperature of the receptor compartment was maintained at 37 ± 1 °C throughout the experiment which in turn provides a skin surface temperature of approximately 32 ± 1 °C [27]. Thawed skin samples were hydrated in the receptor medium for 1 h and then mounted on receptor compartment such that the SC faced toward donor compartment. A patch specimen of 3.8 cm^2 size (equivalent to 5 mg of SM) was placed over the hydrated skin and then, securely clamped between donor and receptor compartment. Aliquots of 0.5 ml were withdrawn at predetermined time from the receptor medium through the sampling port of diffusion cell and the concentration of SM was analyzed by HPLC assay method [17].

Briefly, the HPLC system consisting of a Hitachi L2130 model, UV–Visible detector (L-2400) and reverse-phase C18 column (Develsil) with 5 µm, 15 cm × 4.6 mm internal diameter. Phosphate buffer pH 2.8 and acetonitrile in a ratio of 2:8 was used as mobile phase. The flow rate and the run time were set at 1.1 ml/min and 13 min, respectively. The retention time observed at 1.83 min and 5.67 min for hydroxy acid (active form) and SM, respectively. The detection was made at 238 nm.

The sink condition of SM in receptor medium was maintained on the following basis. The saturation solubility (Cs) of SM in above medium was found to be 7.384 ± 0.03 mg/ml at 37 °C. Sink condition was maintained for permeation studies when maximum concentration of drug in receptor medium is less than 1/10th of its Cs value in the same medium ($C < Cs \times 0.1$). Therefore, final concentration of SM i.e. after the complete release of SM in phosphate buffer of pH 7.4 and acetonitrile in the ratio of 70:30 was maintained below 738 µg/ml, which is in compliance with the sink condition.

Steady state flux (Jss, µg/cm^2/h), permeability coefficient (Kp, cm/h), and enhancement ratio were calculated by utilizing the data obtained from in vitro permeation study. Measurement of Jss was carried out from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot. The slopes were obtained for all the formulations and the linearities were found to be between 7 h and 24 h for SA4 and SA5 between 8 h and 24 h for SA1 and SA6, and between 8 h and 22 h for SA2 and SA3. The Kp and ER were calculated by using following equations [28]:

$$Kp = \frac{Jss}{C}$$

$$ER = \frac{\text{Flux of SM with PE}}{\text{Flux of SM without PE}}$$

where C is the concentration of SM incorporated in DIA.

Histopathological investigation on treated skin sample was carried out and compared with the micrograph of untreated skin sample, to throw light on the mechanism of drug permeation. To prevent further change in the anatomy, skin specimens were preserved in formalin solution (10% v/v) prior to investigation. The skin specimens were sectioned with outmost care to prevent further damage and then stained with eosin [18]. This is followed by visualization of specimen under a light microscope (Microtome-1200, Weswox, Western electric scientific work).

2.6. Statistical analysis

Interpretation of data obtained from different experiments was performed using ANOVA. Bonferroni multiple comparison tests employing GRAPHPAD INSTAT 3 SOFTWARE (Graph-Pad Software Inc., San Diego, CA) was used to determine statistical significant differences. The level of significance was fixed at 95% ($p < 0.05$).

2.7. Stability studies

Stability study is usually performed to ascertain the stability potential of the drug present in the selected formulation. The stability study at room and accelerated conditions for the selected DIA patch was carried out for 6 months, according to the ICH guidelines under the following conditions: 40 ± 2 °C temperature and 75 ± 5% relative humidity (RH) using a stability chamber (Model no: BIT-2U, Bio Technics-India) [29]. Visual inspection, drug content, permeation study, FTIR, DSC, and scanning electron micrographs (SEM) were performed after 6 months to assess the physicochemical stability of the selected DIA transdermal patch of SM under room and accelerated study conditions.

JEOL scanning electron microscope (Model: JSM-6390) was used to take the surface images of the selected patch before and after the storage period. A sputter coater was used to coat sample patches with platinum and subsequently, the coated patches were visualized under microscope at an accelerating voltage of 15 kV. Magnification of 500× was used to take the micrographs.

3. Results and discussion

As the selection suitable acrylate adhesive plays an important role in the development of DIA patch, a proper screening was conducted on three acrylic PSAs by keeping all other formulation factors such as drug content (2% w/w of acrylate adhesives) and matrix thickness (100 µm) constant. Upon screening, DURO TAK® 87-9301 was selected. The effect of PEs on the skin permeation of SM was assessed after their incorporation into selected patch. Finally, the selected SM and PE content in the patches were 2% (w/w) and 5% (w/w), respectively.

3.1. Pre-formulation study

3.1.1. FTIR analysis

The FTIR spectra of pure SM (Fig. 1) demonstrated characteristic peaks at 3549.02 cm^{-1} (alcoholic O—H stretch vibration), 2962.66 cm^{-1} and 2877.89 cm^{-1} (methyl and methylene C—H asymmetric and symmetric stretching vibration), 1705.07 cm^{-1} (lactone C═O and ester C═O stretching), 1462.04 cm^{-1} and 1381.03 cm^{-1}

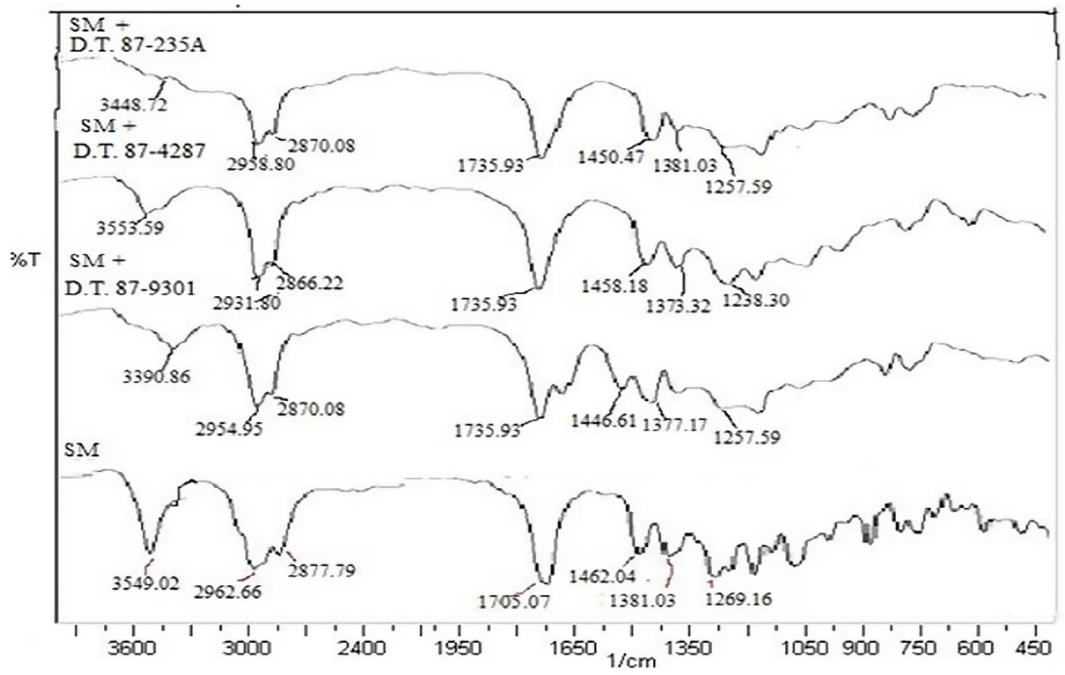

Fig. 1. FTIR spectra of pure drug and its physical mixture with different PSAs.

(methyl and methylene C—H bending vibration) and 1269.16 cm^{-1} (lactone and ester C—O—C bending vibration). Physical mixtures of SM with all types of adhesives revealed that there is a carbonyl (—C═O) band shift from 1705.02 cm^{-1} to 1735.93 cm^{-1}. The band shift in the FTIR spectra of the physical mixtures was attributed to intermolecular hydrogen bond formation [30].

3.1.2. DSC study

The thermograms of SM and its physical mixture with DURO TAK® 87-4287, DURO TAK® 87-9301, and DURO TAK® 87-235A were presented in Fig. 2. The sharp endothermic peak at 136.5 °C was observed for SM which indicates its melting point. Similarly, physical mixture of SM and DURO TAK® 87-4287 demonstrated sharp endothermic peaks at 64.7 °C. This may be attributed to the peak of DURO TAK® 87-4287. It is clear from the thermograms of other physical mixtures that there was absence of sharp endothermic peak. The major peak of SM disappeared from DSC thermogram of all the physical mixture, which may be due to the dispersion of SM in molecular form in the adhesive and solvent mixture.

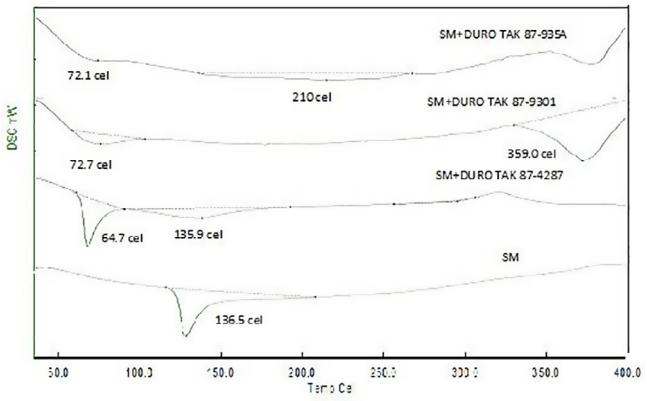

Fig. 2. DSC thermograms of pure drug and its physical mixture with different PSAs.

3.2. Drug content uniformity

Drug content of all DIA patches was measured and the results are made supplementary. The drug content values ranges from 1.26 ± 0.1 mg/cm^2 for formulation SA6 to 1.35 ± 0.08 mg/cm^2 for formulation SA2. The observed variations may be attributed to the different experimental processes such as drug-loading, cutting of specimen and analysis.

3.3. In vitro permeation study

In the present investigation, DIA patches of SM were developed and selected with regard to two formulation factors such as types of acrylate adhesives and PEs.

3.3.1. The effect of adhesives on the permeation of SM through pig ear skin

The PSAs tested in this study were DURO TAK® 87-9301, DURO TAK® 87-4287, and DURO TAK® 87-235A. The effect of adhesives on the SM permeation across the pig ear skin was assessed and the results were illustrated in Fig. 3a and Table 2 by keeping factors, such as 2% drug content and 100 μm thickness of the dried matrix, constant [31,32]. Among the three PSAs investigated, DURO TAK® 87-9301 showed highest cumulative SM permeation. As shown in Table 2, the Jss of SM was also higher (5.18 ± 0.23 μg/cm^2/h) in patches containing DURO TAK® 87-9301 than in patches containing the other adhesives. But, no significance difference ($p > 0.05$) in cumulative SM permeation through pig ear skin were observed among the formulations containing different PSAs. This may be attributed to variation in drug-loading process. We have considered DURO TAK® 87-9301 for the screening of different PEs.

3.3.2. The effect of PEs on the permeation of SM through pig ear skin

Different DIA patches (SA4-SA6) with PEs viz. IPM, D-limonene and 1,8-cineole were prepared and tested for their enhancement ability on the permeation of SM across hairless pig ear skin. SA1 was used as control as it exhibited highest drug permeation among patches without PEs. Obtained results are illustrated in Fig. 3b and the relevant data are presented in Table 2.

Formulation SA4 containing IPM demonstrated highest cumulative

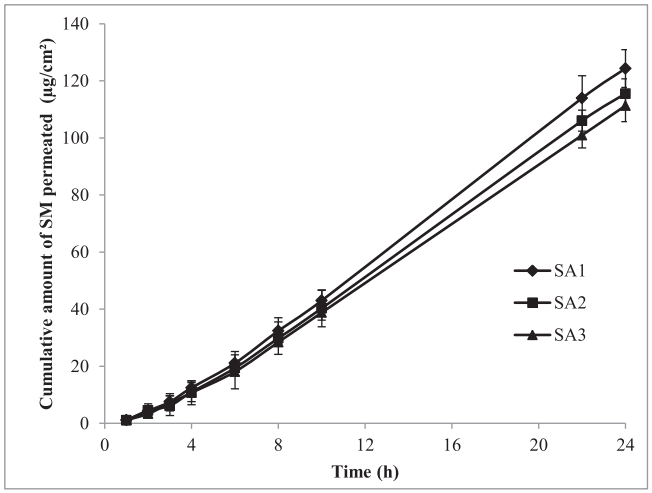

Fig. 3a. Comparison of in vitro permeation profiles of SM across pig ear skin from patches containing 2% (w/w) of SM in DURO TAK® 87-9301 (SA1), 87-4287 (SA2) and 87-235A (SA3) (Mean ± SD, n = 3).

Table 2
In vitro permeation parameters of SM from different DIA patches (Mean ± SD, n = 3).

Formulation code	Flux (µg/cm²/h)	Kp ($\times 10^{-4}$ cm/h)	ER
SA1 (control)	5.18 ± 0.23	10.36 ± 1.57	–
SA2	4.73 ± 0.19	9.46 ± 0.94	–
SA3	4.68 ± 0.61	9.36 ± 1.09	–
SA4	16.45 ± 1.67	32.9 ± 3.46	3.17
SA5	14.08 ± 0.94	28.16 ± 3.18	2.71
SA6	6.42 ± 0.28	12.84 ± 1.47	1.23

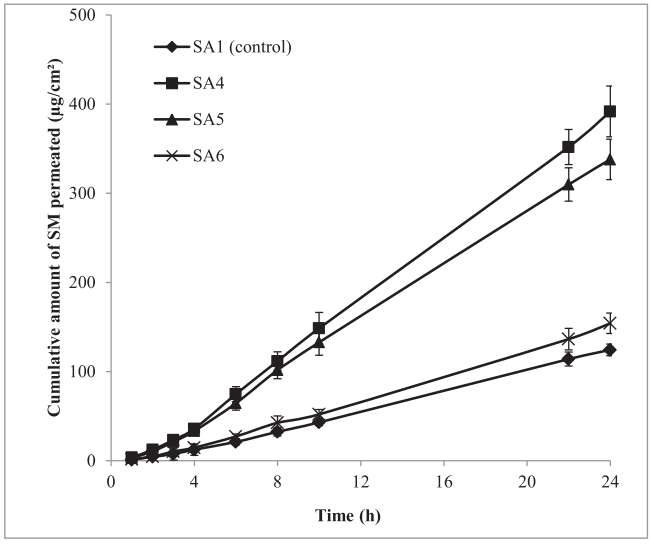

Fig. 3b. Comparison of in vitro permeation profiles of SM across pig ear skin from patches with (SA4-SA6) and without (SA1) penetration enhancers (Mean ± SD, n = 3).

SM permeation (391.8 ± 28.48 µg/cm²) than patches containing other PEs. It was observed that there were significance differences ($p < 0.05$) in cumulative amount of SM permeation between formulation pairs: SA1-SA4, SA1-SA5, SA4-SA6 and SA5-SA6. The Jss of SM from DIA formulation containing IPM was found to be highest (16.45 ± 1.67 µg/cm²/h). The permeation of SM from different DIA formulations containing PEs and control was found to be in the decreasing order of; SA4 (IPM) > SA5 (D-limonene) > SA6 (1,8-cineole) > SA1 (control).

The above result was attributed to lipophilicity of the PEs and the magnitude of alteration produced by PEs in the SC layer of skin. Lipophilicity of the PEs was one of the important factors governing their ability to enhance permeation. IPM is a lipophilic solvent [33] and having highest log P value of 7.17 among the PEs selected. As a result IPM helps in partitioning of SM from patch to lipophilic membrane and, subsequently, in to the acceptor phase. In our previous study, we have observed that IPM is the better solvent/PE than D-limonene for the diltiazem hydrochloride permeation from organogels [28]. It was reported that the terpenes with higher polarity are more potent enhancers for hydrophilic drugs whereas non-polar terpenes are better enhancers for lipophilic drugs [34]. Due to the presence of oxygen atom in 1,8-cineole structure, it is considered as polar terpene and its log P value was found to be 2.82 ± 0.25. In contrary, D-limonene is a non-polar (hydrocarbon) terpene with log P value of 4.58 ± 0.23 [35]. The magnitude of alteration of SC caused by above PEs played a major role in penetration of drug across the skin. The mechanism of IPM is considered to be related with increased lipid fluidity in the SC through the disruption of lipid packing because of higher affinity between IPM and component of SC [2,36]. The mechanism of action of 1,8-cineole is the disruption of highly ordered bilipid structure of SC [37,38], whereas extraction of lipid from SC is the penetration enhancing mechanism of D-limonene [39]. Above finding was supported by the fact that bufalin (log P = 2.78) permeation was enhanced by 22-fold with 5% (w/v) of D-limonene compared to other ten different PEs including 1,8-cineole [40].

Histopathological study on treated and untreated skin samples were performed to ascertain the mechanism of SM permeation from the selected patch containing IPM as PE. It is evident from the Fig. 4a that SC is intact which is indicated by thick dark line. In contrary, micrographs (Fig. 4b) of treated skin sample demonstrated a distorted SC. This resulted in a reduced barrier quality and increased permeation of SM [41]. This result is supported by our previous work where pluronic lecithin organogel containing IPM as organic phase showed disrupted SC [28].

3.4. Stability studies

Drug content of the selected DIA patches on aging after 6 months of storage was found to be in between 1.23 ± 0.09 and 1.27 ± 0.13 mg/cm² compared to 1.28 ± 0.07 mg/cm² for fresh patches. Statistical test showed that there was no significant difference ($P > 0.05$) in drug content existed between fresh and stored patches. This indicated that there was uniformity among drug contents with comparatively low standard deviation values. No alteration in the visual characters such as shape, clarity, smoothness, homogeneity, and uniformity of the selected transdermal DIA patch was observed during the period of analysis. Furthermore, stored DIA patches did not show any significant change ($p > 0.05$) in Jss (i.e., 15.83 ± 0.87 to 16.04 ± 1.09 µg/cm²/h for stored patch vs. 16.45 ± 1.67 µg/cm²/h for fresh patch). The FTIR spectra (Fig. 5), DSC thermogram (Fig. 6) and SEM micrographs (submitted as supplementary material) of patches stored under stability condition along with fresh patch are illustrated below.

It is observed from the Fig. 5 that there was one small shift and one disappearance in the characteristic peaks during FTIR analysis. The peak shift was seen for carbonyl (−C═O) functional group from1735.93 cm^{-1} to 1666.51 cm^{-1} and 1667.38 cm^{-1} for DIA patches stored at room and accelerated condition, respectively. Characteristic peak for alcoholic O−H stretch vibration at 3390.86 cm^{-1} was disappeared in FTIR spectrum of DIA patch stored under accelerated condition while the same was intact in case of patch stored at room condition for 6 months. DURO TAK® 87-9301 has no functional group, thus the chance of interaction between drug and adhesive could not be assumed for the above shift and disappearance of peaks. These variations may be due to drug-loading process and complete loss of organic

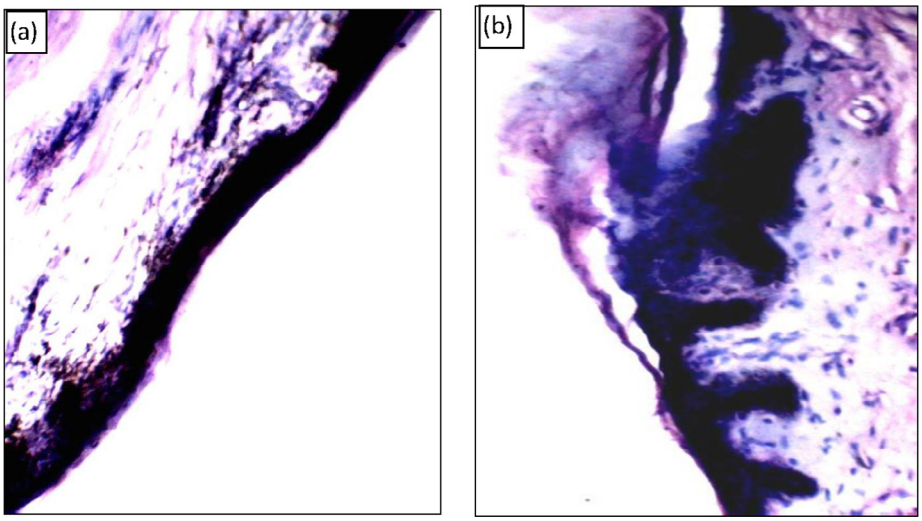

Fig. 4. Micrographs of histopathological study: (a) untreated skin and (b) treated skin.

solvents during storage. Same type of result was obtained when DIA-type patch of eserine and pralidoxime chloride undergoes accelerated stability test [8].

In case of DSC thermograms (Fig. 6), DIA patches before and after stability study did not show any significant changes i.e. peak for SM disappears in all the three thermograms. This indicates non-crystallinity of the drug in the patch during storage which may be due to lower concentration of drug in patch. Hence, it could be concluded that the drug might have been homogeneously dispersed in the patch.

Apart from FTIR and DSC studies, surface structure analysis by SEM is also very essential components for stability as it reveals crystallization aspect of dispersed drug molecules in DIA patch and also demonstrates initial drug release pattern. In our previous study we observed that SM crystals were formed in the Eudragit and PVA based transdermal film stored at accelerated condition (40 ± 2 °C/75 ± 5% RH) for duration of 3 months [17]. In contrary, neither micrographs of fresh DIA patch nor patch stored at room and accelerated conditions (figures were made supplementary) showed any evidence of porosity or crystals of drug, which supported the findings of the DSC study that the presence of SM in molecular form throughout the storage period in the adhesive matrix [42]. Furthermore, it was reported that drugs such as ethinyl estradiol and levonorgestrel in the acrylate adhesives did not recrystallizes even after 3 months storage at 25 °C/60 RH and 40 °C/75 RH [9]. This might be due to the rigidity of the adhesive matrix system that compactly entrapped the SM molecule into its matrix and preventing it to move freely at higher storage temperature. As a result of that formation of nuclei did not happen and thus the absence of crystals in the DIA patch.

4. Conclusions

Transdermal DIA patches of SM were successfully developed using different types of acrylic adhesives (PSA). DURO TAK® 87-9301 and IPM as acrylic adhesive and PE exhibited highest flux for SM. The selected DIA patch composed of 2% (w/w) of SM, 93% (w/w) of DURU TAK® 87-9301, and 5% (w/w) of IPM was found to be stable for six months at room and accelerated condition. Permeation studies on human skin and in vivo studies on animals are essential to establish the

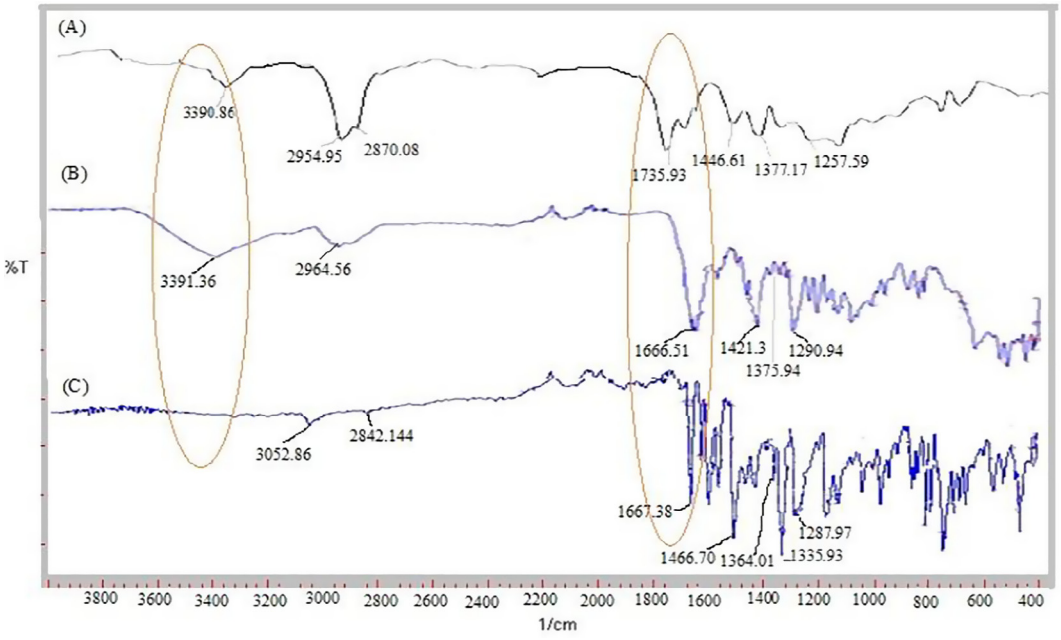

Fig. 5. FTIR spectra of DIA patch at (A) 0 months, (B) 6 months under room temperature, and (C) 6 months under accelerated condition.

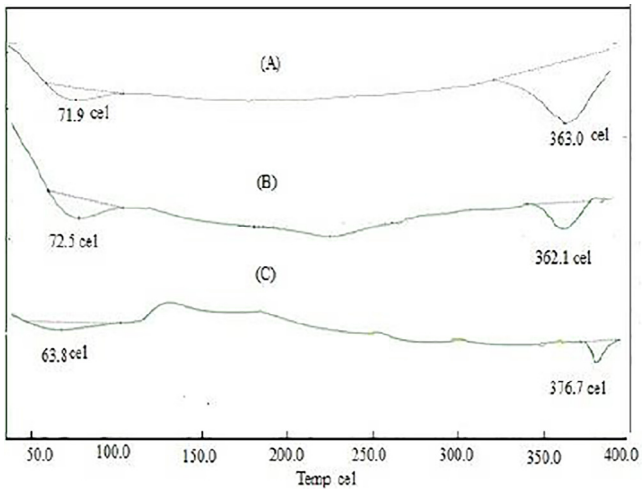

Fig. 6. DSC thermogram of DIA patch at (A) 0 months, (B) 6 months under room temperature, and (C) 6 months under accelerated condition.

potential of DIA patches of SM in the treatment of hyperlipidemia. Furthermore, selected DIA patch is expected to exhibit similar results when it is tested by in vivo methods as well as in clinical stage.

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Conflict of interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bfopcu.2018.04.001.

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