Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

The effect of combination of functional and nonfunctional acrylic polymers on transdermal patches of : in vitro permeation, in vivo evaluation using biochemical parameters, and stability studies

Niharika Lal[1*] and Navneet Verma[2]

Abstract

Background: A double-layer transdermal drug-in-adhesive patch of carvedilol was developed using functional and nonfunctional grades of acrylic adhesives, DURO-TAK® 387-2051, DURO-TAK® 387-2510, and DURO-TAK® 87-4098. The patch was designed to provide adequate permeation of the drug up to 2 days, with effective adhesion attributes. An optimized formulation was selected, the effect of the combination was studied and a 180° peel strength test was performed to evaluate adhesive properties. Further, the patch was assessed for in vivo studies on basis of biochemical parameters, skin irritation, and stability studies. The stability study was carried out on optimized fresh (S1) and 6 months old patches stored at room, and accelerated condition (40 ± 2 °C/75 ± 5% RH) using FTIR, DSC, and SEM techniques.

Result: It was studied that the steady-state flux (*Jss*) or permeation rate of the drug through excised rat skin has relied on the nature of acrylic and the combination of acrylic polymers. The TDDS containing –OH functional group DT 387-2510 with nonfunctional pressure-sensitive adhesives (PSAs) DT 87-4098, with Span 80 as penetration enhancer exhibited maximum flux (19.12 ± 0.64 μg/cm^2/h) and form homogeneous and stable blends, controlling permeation of drug at a desired steady rate for 48 h. The data obtained from in vivo studies using biochemical parameters suggested that there were no statistical differences observed in results for the control and treated group while analyzing observations for serum creatinine, glucose test, sodium test, albumin, and potassium ($p > 0.05$). Also, the optimized formulation showed no sign of localized reactions and was confirmed by a skin histological study indicating the formulation was safe and compatible with the skin. A significant shift of peaks was not observed in FTIR spectra and DSC thermograms of the patches after the stability period.

* Correspondence: niharikalal24@gmail.com
[1]Department of Pharmaceutics, Faculty of Pharmacy, IEC Group of Institutions, Greater Noida, Uttar Pradesh 201310, India
Full list of author information is available at the end of the article

Conclusion: The investigation reveals that the drug-in-adhesive patch of carvedilol, by a combination of functional and nonfunctional PSAs, provides a good and effective option for controlled delivery of carvedilol. From our findings, it has been concluded that drug in the adhesive patch has been able to provide satisfactory adhesion, drug uniformity, drug permeation, marked positive biochemical results, and good stability.

Keywords: Transdermal patch, Acrylic polymers, Carvedilol, Drug in adhesive

Background

The stratum corneum is a defensive and outer layer of skin, selectively permeable to certain drugs to reach in systemic circulation via passive diffusion. The physicochemical nature of a drug could foresee the safety and effectiveness of transdermal drug delivery. The Lipinski rule for 5 provides a framework of selecting drugs that are suitable for transdermal administration.

Owing to attributes of Lipinski rule 5, carvedilol (CV) belongs to a racemic mixture of two enantiomers of α-1 receptor blocking and β adrenoreceptor blocking activity. The drug is used in the treatment of mild to moderate congestive heart failure (CHF) and has been accepted in many nations for the treatment of long-term hypertension, left ventricular dysfunction, angina pectoris, and congestive heart failure [1]. Due to poor solubility of CV in water, gastric fluid, and intestinal fluid, considerable first-pass metabolism in the liver, and poor bioavailability (25–30%), an alternate route of administration is required for sustained therapeutic action and also for improving the compliance of patients [2].

The consideration of biological properties, favorable physicochemical properties of carvedilol (low molecular weight, 406.5; high lipophilicity, log P = 3.97), its suitability for long-term therapy, and reproducibility of dose could make carvedilol an interesting candidate for transdermal drug delivery. Of several classifications of transdermal patches, increasing interest is of adhesive matrix type transdermal drug delivery patches in which the bioactive substance is incorporated into a pressure-sensitive adhesive layer, which serves not only to carry the bioactive substance but to also attach the patch to the skin.

Additionally, due to their small size, dose adjustments, flexibility, high adhesion attribute, and patient compliance they provide good choices among various transdermal designs. The critical criteria are choosing a suitable adhesive in the fabrication of DIA patches. Many pressure-sensitive adhesives (PSAs), such as polyisobutylenes (PIBs), polysiloxanes (silicones), and polyacrylates (acrylics), have been marked as safe adhesives that could be used by the FDA. But selection of suitable PSA relies on many factors including formulation system, patch design, wear conditions, wear time, and processibility [3].

Most of the drugs exhibit relatively good solubility in acrylic pressure-sensitive adhesives than silicone pressure-sensitive adhesives, thus providing satisfactory drug loading and drug flux. Many researchers have successfully developed DIA transdermal patches using various grades of Durotak, an acrylic polymer. Most of drugs, used in fabrication of DIA systems are present in metastable state like supercooled liquids or amorphous solids. The metastable form is thermodynamically changeable and will, in general, go through phase transition, like recrystallization, all through the lifetime of transdermal products [4]. Recrystallization of the active pharmaceutical ingredient (API) can adversely affect the efficacy (slowdowns of drug release due to low solubility) of transdermal products. Transdermal patches fabricated from functional grades of Durotak shows excellent permeation of drugs but chances of recrystallization and degradation from both external and internal conditions of the pharmaceutically active ingredient, as well as certain contents of the patch, such as permeation enhancers, matrix materials, or other components, are more susceptible in them. So, patches affected to degradation cannot be reserved for an economic acceptable amount of time, hence leading practical obstacles in their distribution [5].

It would be desirable to provide a transdermal drug delivery device package that provides controlled and sustained release of an active agent for an extended period time to treat cardiovascular diseases. From many studies, it was found that a functional acrylic polymer had improved in drug permeation but the drug-excipient interaction could affect adhesiveness and stability of the formulation. The other challenging factor in transdermal patches is drug loading. High drug concentration results in recrystallization of a drug whereas low drug concentration could result in difficulties in achieving an acceptable delivery rate of medicament.

To overcome this problem, the present investigation has been directed to transdermal drug delivery matrix system for the application of drug carvedilol, which includes a blend of two acrylic-based polymer having different functionalities and therapeutically effective amount of a drug. An endeavor has been attempted to fabricate double-layer transdermal patches during which the second layer would adhere to a backing membrane that acts as a reservoir, and the first layer would be in contact with the skin that acts as a rate-controlling membrane. The blending of polymers of different functionalities would provide adequate permeation of the

drug, better stability, and use of nonfunctional acrylic as a skin contact layer will provide adequate adhesion of patch to the skin. The patches were prepared and assessed for physicochemical evaluation, drug permeation studies. An optimized transdermal formulation was chosen on basis of peel measurement studies to assess adhesion efficacy and the final formulation was assessed for in vivo evaluation using biochemical parameters, skin irritation studies by histopathological testing, and stability studies.

The acrylic grades used in this investigation were functional Durotak 387-2510 (OH group), Durotak 387-2051 (-COOH group), and nonfunctional Durotak 87-4098.

Methods

Raw materials and chemicals

The crystalline form carvedilol (99.2% w/w) was obtained as a generous gift sample from Cipla Pharmaceuticals Limited, Mumbai, India. Acrylic adhesives such as Durotak® 387-2510, 387-2051, and 87-4098 were obtained from Henkel Limited, UK. The properties of acrylic adhesives were made supplementary. The polyester film laminate (ScotchPak^{TM} 1012) backing films and fluoropolymer-coated polyester film (ScotchPak^{TM} 1022) release liners were purchased from 3M Drug Delivery system (St. Paul, MN, USA). Ethyl acetate has been used as solvent and Span 80 as permeation enhancer was purchased from Sigma Aldrich Merck, Delhi, India. Rests of the chemicals used in the study were procured from reliable sources.

Formulation of double-layer patches

The constituents of double-layer composition were of two layers; the first layer is in skin contact and the second layer will be known as the reservoir layer. The drug PSA solution was uniformly blended using a magnetic stirrer. The constituents of reservoir solution were cast on backing membrane with a thickness of 50 μm, whereas constituents of the first layer were cast on the release liner with a thickness of 40 μm. The constitution of the first layer for all formulations is identical as mentioned in Table 1. The coated film was initially placed at room temperature for 12 h and then stored in an oven at 50 °C for 30 min to remove the entire volatile solvent. Then, the first layer was layered over the second layer on the defined surface of the adhesive matrix by the help of stainless rolling ball. The entire thickness of the transdermal patch was 50 μm. Considering, the equilibrium situation laminate was fabricated into a circular shape, having a surface area of 3 cm^2 and stored in air-tight container prior use.

Animals

Wistar albino rats were obtained from the Animal House of the Institute. The investigation follows ARRIVE guidelines to improve ideals of reporting the results of animal experiments. The Wistar albino rats, weighing 180–200 g, were used for the present study. They were housed in the cleaned propylene cage and were maintained under the standard laboratory condition (25 ± 2°C with dark/light cycle 12/12 h). All experimental protocols associating animals were performed according to the basis stated in the Guide for the Care and Use of Laboratory Animals and guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Ministry of Social Justices and Empowerment, Government of India. Institutional Animal Ethics Committee approved the experimental protocol (837AE/PO/Re/S/04/CPCSEA). Male Wistar rats weighing between 180 and 200 g and approximately 8–9 weeks of age were selected for the study. Rats were maintained in an animal house with standard facilities. Animals were fed with standard feed with free access to purified drinking water. Animals were acclimatized to laboratory conditions for at least 1 week before starting the experiment.

In vitro skin permeation study

The in vitro transdermal permeation of drug from prepared patches, across hairless mouse abdominal skin, was considered by using a modified Franz diffusion cell [6]. Rats were sacrificed by using cervical dislocation.

Table 1 Formulation composition of double-layer drug-in-adhesive formulation

Double-layer device total component (%, *w/w*)						
Formulation number	**Carvedilol***	**Cetyl alcohol***	**Ethyl acetate: Span 80***	**Durotak 4098***	**Durotak 2510***	**Duro-Tak 2051***
S 1	6	2	8	42	42	..
S 2	6	2	8	42	..	42
S 3	6	2	8	84	..	..
S 4	6	2	8	..	84	..
S 5	6	2	8	..	..	84
The material composition of a skin contact layer (first) of double-layer TDDS						
Skin contact layer	3	2	8	87	..	..

*% w/w of polymer concentration

Hair of the abdominal region was carefully removed using an electric shaver (Phillips). The dermis side was cleaned with isopropyl alcohol to take off the residual fat. The skin was dipped and soaked in normal saline solution. If not used immediately, excised skins were wrapped in aluminum foil, stored in a deep freezer at −18 °C, and used within 3 days. The receptor compartment of the diffusion cell was filled with 25 ml of filtered and degassed phosphate buffer solution of pH 7.4 as a receiver medium and was maintained by 37 °C. The prepared patch (3 cm^2) was applied to the epidermal side of rat skin with modest pressure and then mounted over the receptor compartment. The entrapped air bubbles present in the receptor compartment and below the skin were delicately evacuated by considerate inclining of the diffusion cell. The receptor medium was stirred by a magnetic stirrer. The 5 ml of samples were withdrawn from a receptor compartment at a predetermined time interval up to 48 h and are restored with the same volume of the fresh phosphate buffer solution to maintain sink conditions. The concentration of carvedilol has resulted in a validated UV spectrophotometer (*Shimadzu UV-1800* spectrophotometer, Japan*)* at 239.5 nm and the cumulative amount of drug was calculated [7].

As per the concentration of each sample, the cumulative amount of the drug permeated per unit area at time point "n" (Q, g/cm^2) was calculated by the following formula:

$$Q = \frac{Cn \times V + \sum_{i=1}^{n=1} \times C_1 \times 5}{S}$$

where S is the effective area, V is the volume of the receptor cell, Cn is the drug concentration at time point "n", and Ci is the drug concentration at time point "i". The cumulative amount per unit area (Q, μg/cm^2) of drug permeated through the skin was plotted as a function of time (t, h). The slope of the linear portion of the plot was represented as the permeation flux (Js, μg/cm^2/h).

The permeation rate or skin flux (Jss) was calculated from Fick's law of diffusion as mentioned:

$$J_{SS} = (V\,dc/dt)/A$$

Whereas Jss is skin flux (μg/cm^2/h), C is the cumulative drug concentration in the receiver fluid at time t, V is the receiver volume (ml), and A is the active diffusion area (cm^2). The steady-state flux was calculated from permeation profiles. Permeation coefficient (K_p, cm/h) was calculated by dividing J_{ss} with the concentration of the drug in the donor cell (C_0) by using the following equation:

$$K_p = J_{SS}/C_O$$

All the transdermal permeation experiments were repeated three times and their mean values with standard deviations were calculated [6, 7].

Evaluation of characteristics of the optimized patch

The selection of optimized formulation was conferred with a peel strength measurement test. The formulation which possesses desired physical characteristics, good compatibility with the drug as well as an excipient, adhesive properties, and desired permeation of drug will be chosen for further studies.

Peel strength measurement at 180°

The peel test was done as per ASTMD3330 at adhesive coated tapes for 25-mm width. The patches were applied on stainless steel at room temperature as long as 20 min. By using Chemie Instrument adhesive/release tester AR-1000 (Fairfield), a peel force in 180° side was considered by a peel rate of 30.50 cm/min at room temperature. The analysis was attained three times on a particular sample [8, 9].

In vivo studies by use biochemical parameters

Animals used

The antihypertensive activity of the optimized transdermal patch was carried out on 18 male albino Wistar rats aged 6–8 weeks and weighing 180–215 g. Animals were housed in standard environmental conditions under a 12/12-h light/dark natural cycle in the laboratory animal house of the institute. All animals had free access to a standard diet and tap water ad libitum.

Experimental design

The antihypertensive activity of the optimized transdermal patch was evaluated, based on biochemical and histological results. Hypertension in Wistar rats was induced by using dietary based models, in which mixtures of ethanol and sucrose solutions were given to Wistar rats to develop hypertension. Wistar rats were randomly divided into three groups, controlled group, untreated group, and treated group. Each group contains six Wistar rats and rats of untreated and treated groups were administered daily with ethanol and sucrose solutions for 5 consecutive weeks to induce hypertension [10, 11].

The animals of the control group received distilled water (1 ml/kg/day) by oral gavage, while rats of the untreated group were administered with a mixture of ethanol 40° (3 g/kg/day) and sucrose 10%. The treated group received a mixture of ethanol 40° (3 g/kg/day) and sucrose 10% (the same that has been given to the untreated group), beside to an optimized transdermal patch that was adhered with their abdominal skin for 48 h. A replacement patch would adhere to on the abdominal site of the treated group after every 48-h interval.

The body weights were assessed twice a week during the experimental period [10].

Biochemical and histological analysis

Rats were sacrificed and free run blood was collected and centrifuged at 3000 rpm for 15 min. The serum obtained was stored at − 20 °C for the determination of biochemical markers. Serum samples were assayed for serum creatine and albumin, using the commercial diagnostic kit Fortress. The glucose assay was performed using the kit Inmesco while sodium and potassium ions were quantified using the Atlas Medical kit.

After blood collection, the heart of each animal was dissected out for histological analysis. The examined organs were firmed in 10% formalin for 2 days. It was paraffin fixed for microscopic examination as per laboratory procedure. After that, paraffin-firmed segments of 4-μm sections were prepared and stained with alongside hematoxylin and eosin for histological investigations [11].

Histopathological investigation of skin irritation studies was carried out and compared with the micrograph of untreated skin sample, to throw light on the mechanism of drug permeation. The skin samples were sectioned with the utmost care and were preserved in formalin solution (10% v/v) before investigation. The samples were then stained with eosin, and followed by visualization of a specimen under a light microscope (Microtome-1200, Weswox, Western electric scientific work)

Stability studies

Stability studies were performed to determine the stability of the drug present in the preferred formulation. The transdermal patches were packed in heat sealable pouching films prior to stability studies. The study has been performed at room (27 ± 0.5 °C) temperature and 30 ± 5% relative humidity (RH), and accelerated conditions for the selected DIA patch. It was carried out for 6 months, as per International Conference on Harmonisation (ICH) guidelines under the following conditions: 40 ± 2 °C temperatures and 75 ± 5% RH using a stability chamber (model no. BIT-2U, BioTechnics, India).

The physicochemical stability of the DIA patch was assessed for visual inspection, drug content, permeation study, attenuated total reflectance (ATR), differential scanning calorimetry (DSC), and scanning electron micrographs (SEM) after 6 months under room and accelerated study conditions [12].

Statistical analysis

Interpretation of data obtained from different experiments was performed using t-test. The tests were assessed with Graphpad INSTAT 3 SOFTWARE (Graph-Pad Software Inc., San Diego, CA) to determine statistically significant differences. The level of significance was fixed at $p < 0.05$.

Results

As a selection of appropriate acrylic adhesives plays an important role in the fabrication of DIA patches, in initial studies a proper screening was conducted on DT 387-2051, DT 387-2510, and DT 87-4098. Double-layer formulations were evaluated for in vitro permeation studies and from which the best formulation would be considered for further studies.

The effect of adhesives on permeation of CV from double-layer patch

The combination of adhesives on CV permeation across excised rat skin was assessed and the results were represented in Fig. 1 and Table 2 by keeping factors 6% drug content in reservoir layer and 3% drug content in skin contact layer, of 50 μm thickness of dried matrix, constant. Among formulations S1–S5 investigated, formulation S1 where DT 4098 was used in combination with either DT 2510 (1:1) showed the highest cumulative CV permeation. As shown in Table 2, the Jss of CV was also higher (19.11 ± 0.03 μg/cm^2/h). In formulation S2 where DT 4098 was used in combination with DT 2051 (1:1) provides sustained drug delivery of 78.21 ± 2.3%, respectively, for 48 h. The presence of only DT 2510 in S4 and DT 2051 in S5 resulted in sustained drug permeation of 82.65 ± 2.3% and 67.45 ± 2.4%, respectively. Further, DT 4098 alone when used in reservoir and skin contact layer (in formulation S3) could not be able to sustain the release of drug till 48 h and 98.32 ± 3.6% of the drug was released at the end of 30th hour, due to no chemical interaction between polymer and drug. Significant differences were observed ($p < 0.05$) in cumulative CV permeation through rat skin were observed among formulations containing different PSAs.

The combination of nonfunctional PSA with OH functional PSA provides higher permeation in comparison to its combination with the COOH group. The diffusion of the drug from the polymer matrix depends on the nature of the polymer mixture. The high thermodynamic activity of drugs in nonfunctional, as well as OH functional PSA, provides adequate permeation rate, while permeation of drug from carboxylate group PSAs was found to be low be due to chemical interference between a carboxylate group of DT 387-2051 with α hydroxyl secondary amine of the drug resulting in the low diffusion coefficient of the drug. The chemical interaction of energy between intermolecular forces of the carboxylate group with free base form of secondary amine could result in formation of salt. It is believed that free base form of secondary amine drug is available for permeation across the skin. The amount of a free base available for

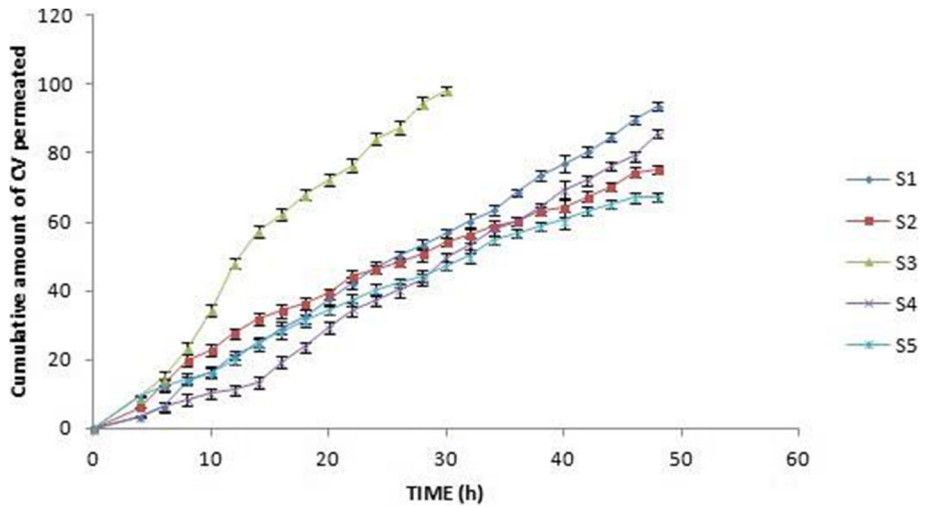

Fig. 1 Comparison of in vitro permeation profiles of CV across excised rat skin from patches with S1–S5 (mean ± SD, $n = 3$)

permeation will depend on its dissociation of salt form which will depend on the association and dissociation equilibrium between the free base and carboxylic acid salt form. The salt present in the system provides a constant source of the free base out of the system and penetrates drug out of matrix to skin, permitting sustained, controlled delivery of pharmaceutical effective amount of drugs. The release of carvedilol was not much affected in the presence of –OH functional group, as $-COO^{-\delta}$ is a substantial electron withdrawing group than $-O^{-\delta}$. Thus, the positive charge on $H^{+\delta}$ of -COOH group is more than -OH group, which results in firm hydrogen bonding between $H^{+\delta}$ and $-N^{-\delta}$. It was also noted that release of drug from its polymer depends on the solubility and diffusion coefficient of drug in a polymer. Perhaps the interaction between drug and polymer could affect drug coefficient [13, 14].

Further, the addition of nonfunctional PSAs with DT 2510 improved produces homogeneous and stable blends, and the drug was easy to permeate through the skin because of low lattice energy, thus exhibited high thermodynamic activity in the medium or matrix, for permeation of drug.

Evaluation of optimized formulation

Changes in the thermodynamic and rheological properties of PSA after the combination of functionalities could affect the adhesion performance of patches. Therefore, determination of the adhesive properties of final patch needs to be assessed since peel strength and tack properties are strongly influenced by surface properties and backing layer mechanical properties. In the formulation S1 and S4, both were assessed for peel strength test to evaluate adhesive properties.

Peel strength measurement

The peel strength of formulation S1 shows the desired elasticity up to 2.5 N/m. The results depict that the patch fabricated were flexible which resulted in higher adhesive modulus, resulting in a linear correlation between peel strength and peel rate (Fig. 2).

However, significant deviations deformations were observed in testing the peel strength of formulation S4 (Fig. 2). Significant, stress distributions like crack and sharpness were observed when a patch was pulled apart from the substrate at a constant speed [15]. This could be due to stringiness properties of high tack DT 387-2051 which could lead to interfacial stresses and causes deformations when a patch has adhered with the substrate. It was concluded that peel strength depends on the nature of acrylic adhesives [16].

Table 2 In vitro permeation parameters of CV from single-layer drug-in-adhesive formulations (mean ± SD, $n = 3$)

Formulation code	Time of drug permeation (h)	Flux (mg/cm^2/h)	Permeability coefficient (cm/h × 10^{-2})
S1	Up to 48 h	**19.11 ± 0.03**	**32.9 ± 0.02**
S2	Up to 48 h	16.23 ± 0.05	22.7 ± 0. 03
S3	Up to 30 h	18.13 ± 0.04	27.6 ± 0.03
S4	Up to 48 h	18.05 ± 0.15	29.3 ± 0.06
S5	Up to 48 h	15.18 ± 0.02	20.1 ± 0.06

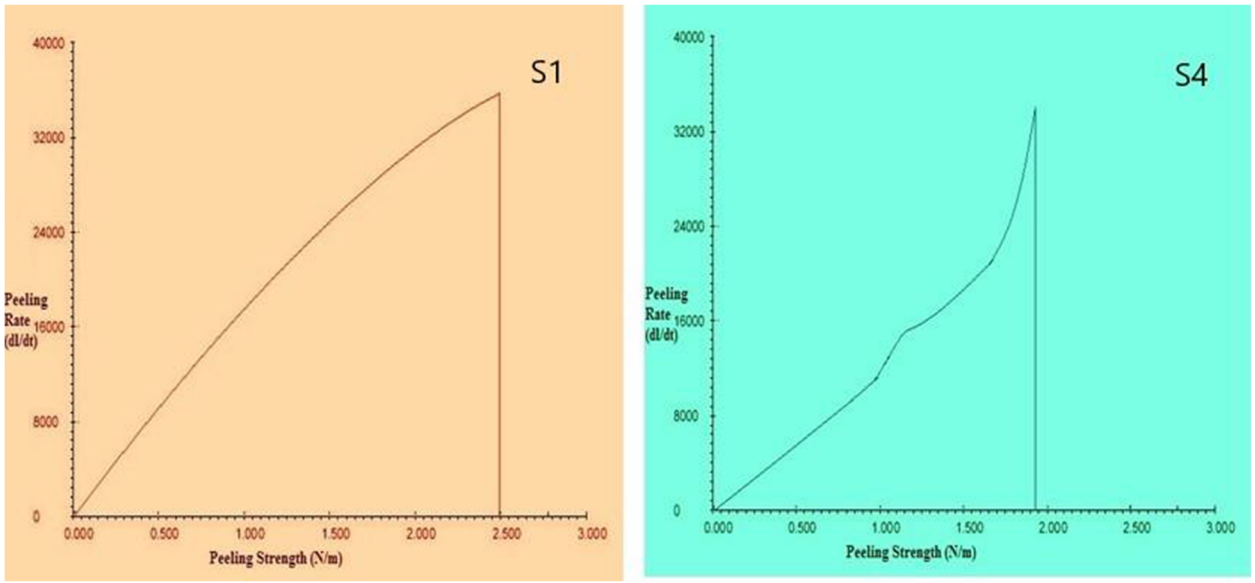

Fig. 2 Peel strength vs peel rate graph of selected formulation S1 and S4

The final formulation S1 (Duro-Tak® 387-4098 and Duro-Tak® 387-2510) was chosen as an optimized formulation as it possesses appropriate physicochemical properties, better permeation profile up to 48 h, and showed excellent adhesion without leaving any kind of residue on the substrate or the release liner. The S1 was chosen for further assessed for in vivo studies by biochemical and histological analysis, skin irritation studies by Histopathology analysis, and stability studies.

In vivo study using biochemical parameter

Experimental design

The recorded result of the body weight of control animals was mentioned in Table 3. Increased body weight was noticed in the untreated group ($p < 0.05$). A gain in body weight may well be due to consumption of calories of ethanol and sucrose solution. For the treated group, the weight gain was initially found to be increased, but then it gradually stabilizes after 2 weeks.

Biochemical studies

Serum creatinine The results of serum creatine are mentioned in Table 3. It was observed that a much significant increase in creatinine level ($p > 0.05$) was found in the untreated group comparison to control and treated groups. The increased hypertension could lead to a distorted renal function which has affected the number of functioning nephrons and loss of glomeruli function and causes a significant increase in serum creatinine level. The decreased serum creatinine level in the treated group was due to the vasodilatory alpha- and beta-blocker action of carvedilol, which exhibits additional effects on ameliorating oxidative stress and inflammation, rendering it an attractive candidate for the prevention of early diabetic nephropathy [17].

Effect on albumin, sodium, and potassium level As mentioned in Table 3, it was observed that chronic ethanol/sucrose consumption has affected the kidney's function which causes a significant increase in sodium and potassium level in the untreated and treated group, while comparing with a control group that had only strived on distilled water. Carvedilol shows the renal sparing effect and protect from a chronic renal failure of treated Wistar rats.

Due to the hypertensive effect, the concentration of albumin was found to be higher in the untreated group. The treated group prevented a rise in glucose, sodium, and potassium level induced by ethanol and sucrose.

Table 3 Assessment of bodyweight and biochemical studies for control, untreated, and treated groups (mean ± SD, $n = 3$)

Group	Body weight (g)	Albumin (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Sodium (mEq/L)	Potassium (mmol/L)
Control (group I)	219 ± 2.3	26.07 ± 0.05	0.8 ± 1.3	109.73 ± 9.65	135.71 ± 1.2	6.21 ± 0.54
Untreated (group II)	228.2 ± 3.6	35.78 ± 0.12	2.3 ± 0.43	158.50 ± 10.21	159.98 ± 1.6	11.21 ± 0.21
Treated (group III)	221.3 ± 3.6	25.98 ± 0.09	1.0 ±0.02	112.65 ± 3.45	139.87 ± 1.6	7.98 ± 0.61

The maintenance of renal blood flow due to the antihypertensive action of carvedilol resulted in a significant reduction in renal vascular resistance and it prevented the occurrence of renal failure in Wistar rats.

Throughout, the continuous antihypertensive dose of carvedilol in the systemic circulation, renal blood flow is maintained and thus it protected the integrity of the renal autoregulatory system. Additionally, the drug inhibits renal rennin secretion due to its β blocker action, which in turn blocks renin angiotensin aldosterone system, which prevents any potential for increasing sodium and potassium ions [10, 11].

Histological analysis

Hypertension accelerated the aging process of the heart like hypertrophic changes which led to structural destruction, while in the present study carvedilol has reduced and alleviated the pathological damage in the heart.

The light microscopic study of cardiac muscles of the control group shows the normal myofibrillar structure with striations, a continuity, and branched appearance with adjacent myofibrils (Figs. 1a, b and 3).

The myocytes show an acidophile sarcoplasm with central oval single nuclei. The longitudinally arranged fibers were found to be cylindrical with central oval vesicular nuclei and faint striations. The cardiac myocytes were connected to make fibers through an interrelated disk. The spaces between interfibers were narrow which contain fibroblast and blood vessels [18].

The cardiac muscles studies for group II (hypertensive) presents segregation of myofibres with expansion in interfiber spaces and erupt red blood cells. Further, a considerable area of hemorrhage could also be identified [19]. The degenerated myocytes appeared to be swollen with evident focal degeneration. The myocytes were found to be pale with vacuolated cytoplasm. Focal mononuclear cellular aggregation was detected in many areas (Figs. 2a–c and 3).

The histological pattern of the treated group was found similar to the control group (Fig. 3a, b). The cardiac muscle fibers appear to be cylindrical with central oval nuclei. The presence of wide interspaces was detected, but they were found to be low. Few areas show pale vacuolated cardiac muscle fibers [20].

The outcome of the study shows that formulation S1 showed a noticeable effect in the protection of cardiac muscles from hypertensive effect.

The dermal test for potential irritation that could be caused by acrylic polymer or penetration enhancer (Span 80) would be confirmed by histopathological studies. As observed in the histology of the rat skin Fig. 4, the control group showed the normal architectural framework of the epidermis, dermis, and subcutaneous layer, and optimized formulation showed thinning of the epidermal and distorted stratum corneum. This resulted in narrow barrier quality and increased permeation of CV. The outcome of the study shows that the optimized patch S1 did not show any kind of notable inflammation, ulceration, or edema on rat skin [21].

Stability studies

The drug content of chosen DIA patches on aging after 6 months of storage was found to be between 94.98 ± 0.43% and 96.23 ± 0.12% compared to 98.72 ± 0.21% for fresh patches. The data were statistically tested and show no significant differences were present between fresh and aged patches ($p > 0.05$). The results show that there

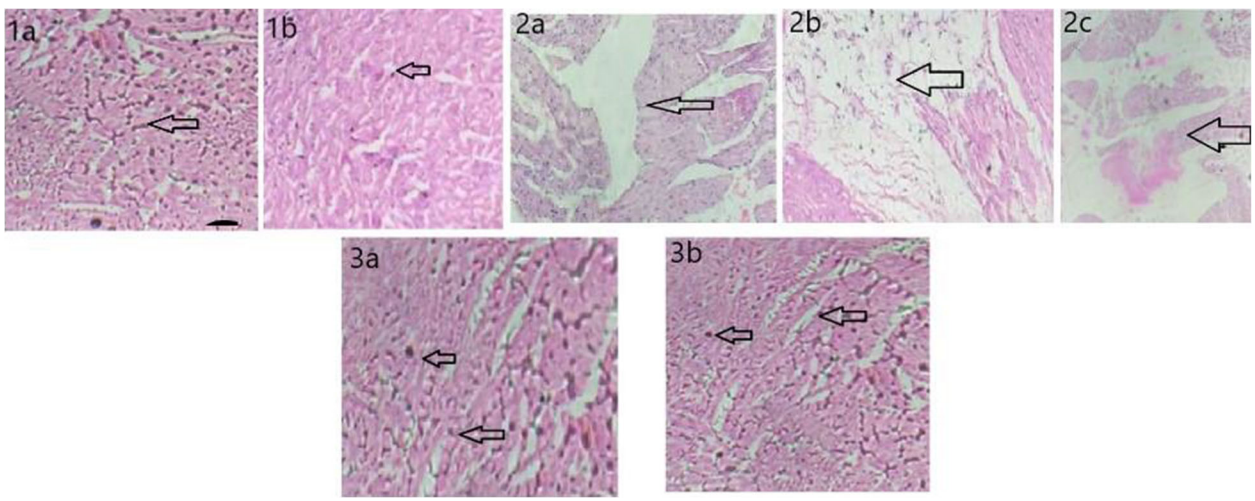

Fig. 3 (**a**) Photomicrograph of a section in cardiac muscles of control group (**1a**, **1b**), (**b**) hypertensive group (**2a**, **2b**, **2c**), and (**c**) treated group (**3a**, **3b**)

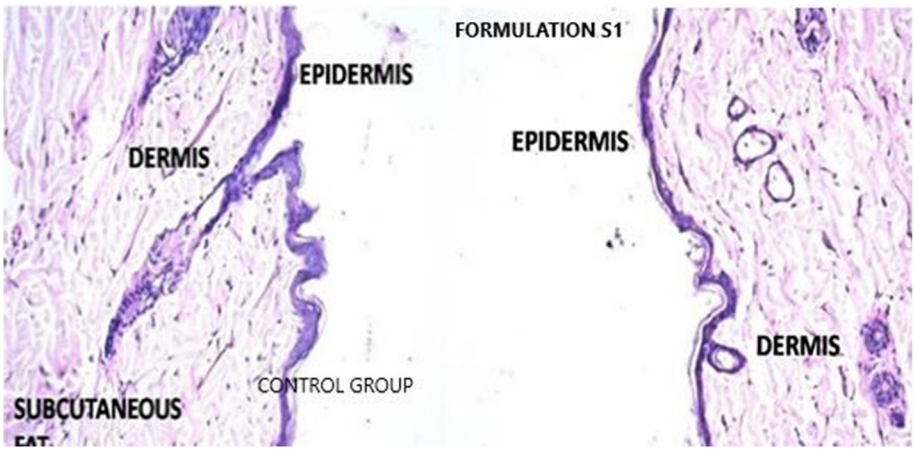

Fig. 4 Histopathology section of rat skin of control group and of treated group received formulation S1

is uniformity in drug content, with a low standard deviation value. The effect of temperature and relative humidity did not show any notable effect on drug content uniformity of transdermal patches. No visual changes were found in a characteristic of transdermal patches during the period of analysis. No variations were observed specifically in terms of color, smoothness, clarity, shape, homogeneity, stickiness, uniformity, and flexibility. Furthermore, stored DIA patches did not show any significant changes ($p > 0.05$) in Jss (i.e., 18.84 to 19.02 for stored patch vs 19.38 for a fresh patch). No burst release effect was observed in the subject's transdermal film. SEM micrograph (Fig. 5) of patches stored under stability condition along with fresh patch does not show any trace of crystallization [12].

The ATR studies were conducted for optimized formulation S1 to detect any kind of changes in the characteristic peaks. The peak shift was seen for the hydroxyl functional group from 2359.41 to 2360 to 2361 cm^{-1} of a fresh patch and for patch stored at room and accelerated conditions, respectively. The characteristic peak for alcoholic O-H stretch vibration at 3540.254 was disappeared in the ATR spectrum of the DIA patch that has been stored under accelerated conditions, while the same was found to be intact in case of patch that has been stored at room condition for 6 months. As Duro-Tak 87-4098 has no functional group in it, therefore, the chances of interaction between the drug and adhesive could not be considered for the above shifting and disappearance of peaks (Fig. 6). The change could be

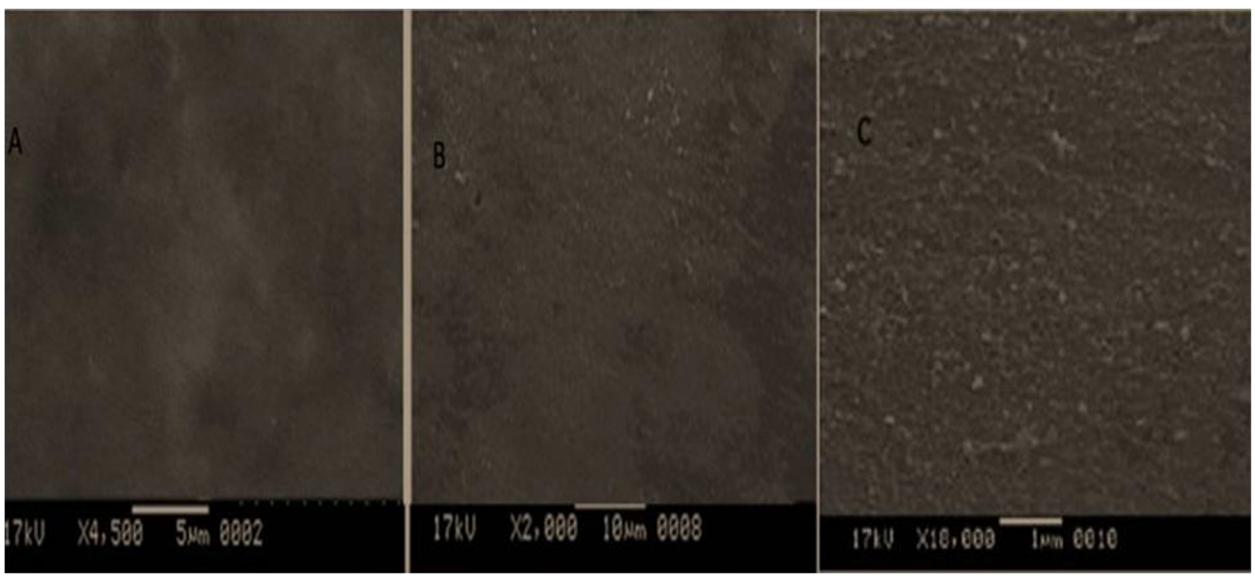

Fig. 5 SEM images of DIA patch at **A** 0 months, **B** 6 months under room temperature, and **C** 6 months under storage condition

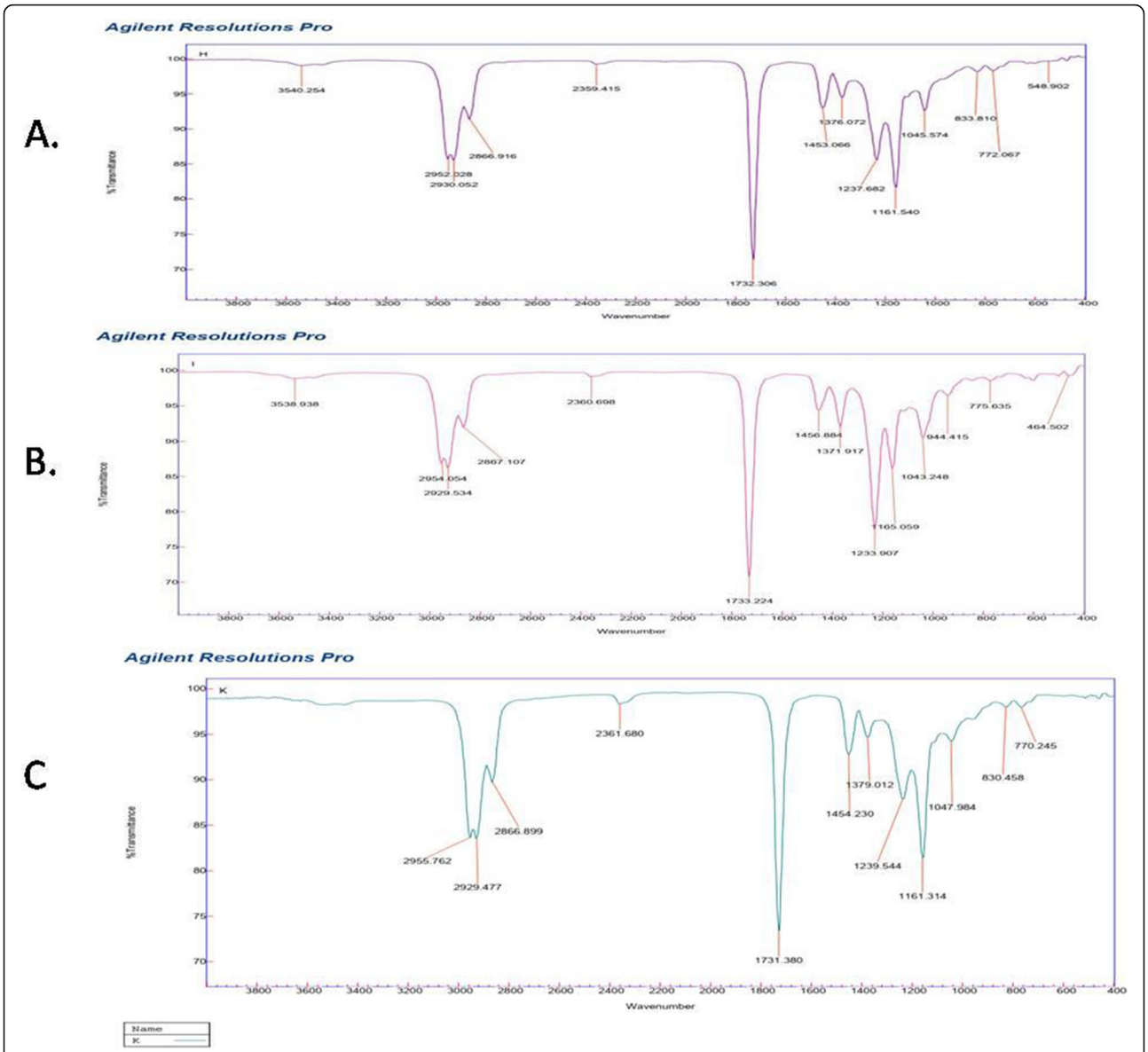

Fig. 6 ATR spectra of DIA patch at **A** 0 months, **B** 6 months under room temperature, and **C** 6 months under storage condition

assumed due to the drug loading process or complete loss of organic solvents during the storage period. The DSC thermogram of the drug-in-adhesive-based transdermal patches before and after stability study did not show any significant changes. It was observed that endothermic peak of the drug was found at 119 °C for fresh prepared patches which was later found to be disappeared in the next two thermograms (Fig. 7). This could indicate towards saturation of drug within acrylic adhesive indicating non-crystallinity of drug in the patch during storage. Hence, it could be concluded that the drug might be homogeneously dispersed in matrix. Thus, the DSC profile of optimized formulation S1 did not exhibit any significant changes indicating the stability and non-crystallinity of drug samples during storage [22]

Discussion

The study was undertaken for the design and characterization of acrylic pressure-sensitive adhesives based transdermal drug delivery system. The drug in the adhesive-based transdermal system was fabricated using three grades of acrylic pressure-sensitive adhesives of different functionalities and nonfunctionalities. The three grades Duro-Tak 87-4098, Duro-Tak 387-2051, and Duro-Tak 387-2510 were studied to prepare a dermal drug delivery matrix system for the application of

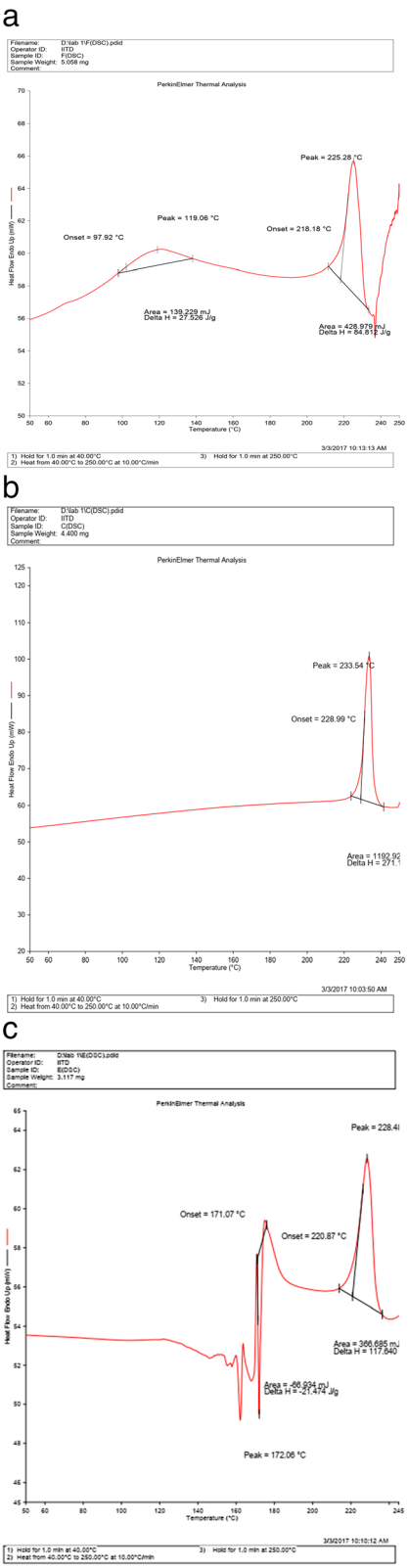

Fig. 7 DSC thermogram of DIA patch at **A** 0 months, **B** 6 months under room temperature, and **C** 6 months under storage condition

drug carvedilol, which includes a blend of two acrylic-based polymer having different functionalities and therapeutically effective amount of a drug. The transdermal patch has been fabricated with a combination of acrylic Duro-Tak of various functionalities (-OH, -COOH, and nonfunctional) comprising of carvedilol as an active pharmaceutical ingredient and was assessed for controlled drug delivery [9, 11]. The double-layer matrix transdermal system was prepared and it was observed they were able to deliver sustained drug delivery for 48 h.

The prepared transdermal films showed uniformity with less standard deviation for their various physicochemical and surface morphological characteristics. The films showed a clear and smooth surface. Percent cumulative amount of drug permeated varied with polymer composition along with the effect of functional and nonfunctionality. The utilization of nonfunctional Duro-Tak and penetration enhancers has increased the permeation of the drug which was not suitable for the proposed period. On the other hand, functional Duro-Tak containing –COOH group was responsible for low permeation of drug due to its interaction with the amine group of drugs. The result of this study indicated that using a combination of functional acrylic grade (Duro-Tak 387-2051, 387-2510) along with nonfunctional grade (87-4098) affects release rate and permeation rate can be achieved in a controlled manner for a longer period [4, 12].

The better permeation profile and adhesive properties were achieved by combing Duro-Tak 87-4098 along with a Functional –OH group Duro-Tak 387-2510. This synergistic combination of DT 87-4098 with DT 387-2051 suggests us to choose formulation S1 for further studies [13]. In vivo studies were done to assess the efficiency of transdermal patches of carvedilol in Wistar rats. Hypertension was induced by using dietary based models, in a mixture of ethanol and sucrose solutions. After completion of 5 consecutive weeks, the rats were sacrificed and free run blood was collected and serum samples were assayed for serum creatinine, albumin, glucose, sodium, and potassium ions were quantified using the suitable kit [14, 16]. The heart of each animal from the groups was dissected out for histological analysis. It was observed that the results of the treated group have a positive effect as found in the control group and no significant differences were observed in the treated and control group. The effect of hypertension in the untreated group causes significant hypertrophic changes, whereas the histological pattern of the treated group was found similar to the control group. The cardiac muscle fibers appear to be cylindrical with central oval nuclei [17]. Despite providing therapeutic drug release and good adherence properties, it is necessary to assess dermal testing for evaluation of any potential irritation that could occur due to drug, penetration enhancer, or by the

use of the acrylic polymer. The dermal test for potential irritation that could be caused by acrylic polymer, drug, or penetration enhancer was confirmed by histopathological studies [18]. The outcome of the study shows that neither penetration enhancer nor the optimized patch shows any kind of notable inflammation, ulceration, or edema on rat skin. The stability studies were also carried out for optimized formulation to assess the drug and drug product stability. The stability studies were conducted at room temperature and accelerated conditions for the selected formulation, S1. The study was carried out according to ICH guidelines under given conditions: 40 ± 2 °C temperature and 75 ± 5% relative humidity (RH) using a stability chamber. The studies were performed after six months for visual inspection, drug content, drug permeation, FTIR, and DSC, to determine physicochemical stability of choosing DIA transdermal patch of carvedilol under room and accelerated study conditions [17, 19]. There were no visual changes were found in a characteristic of DIA transdermal patches during the period of analysis and no significant differences in drug content were present between fresh and aged patches. The effect of storage conditions on cumulative percent drug permeation could not be ignored. It was noted that at room temperature (27 ± 0.5 °C), the release of carvedilol from the formulation was not influenced throughout the period [20, 21].

The transdermal films were found to be stable; the erratic drug release could be due to absorption of moisture from humid storage conditions which could lead to softening of the matrix. However, no burst release effect was observed in the subject's transdermal film. The ATR and DSC studies of optimized formulation S1 did not exhibit any significant changes indicating the stability and noncrystallinity of drug samples during storage [22]. The result of our study indicated that by using a combination of nonfunctional PSA along with functional PSA, improved permeation rate and drug permeation can be achieved in a controlled manner for a longer period.

Conclusion

The result of our study indicated that using a combination of nonfunctional PSA along with functional PSA improved permeation rate up to 48 h. The transdermal films developed by a combination of functional and nonfunctional PSAs act as a good and effective option for controlling the delivery of the antihypertensive drug. The carvedilol transdermal patches act as a good and effective option for controlling the delivery of the antihypertensive drug. The antioxidant property of the drug also prevents the heart from hypertrophic changes, although further work is required in support of carvedilol transdermal films in terms of their efficacy and bioavailability parameters by long-term pharmacodynamic and pharmacokinetic studies on viable animals and human beings.

Abbreviations

API: Active pharmaceutical ingredient; ATR: Attenuated total reflectance; ARRIVE: Animal Research: Reporting of In Vivo Experiments; CV: Carvedilol; CHF: Congestive heart failure; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; DIA: Drug-in-adhesive; DT: Durotak; DSC: Differential scanning calorimetry; ICH: International Conference on Harmonisation; PSA: Pressure-sensitive adhesive; SEM: Scanning electron microscopy

Acknowledgements

The authors acknowledge the support of Mr. Ajit Narkhede, Sales Manager of Henkel Adhesives Technologies India Pvt. Ltd., for providing gift samples of Durotak and Mr. Anjani Kumar for gift sample of carvedilol, required for completion of the study.

Authors' contributions

NI collected the data and prepared the draft of the manuscript. NV designed the study, interpreted some of the results, and made critical revisions to the entire manuscript. Both authors read and approved the final manuscript.

Funding

No external funding was received for this study. The resources used for the study were provided by the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Declarations

Ethics approval and consent to participate

This protocol was approved by the Institutional Animal Ethical Committee of Department of Pharmacy, IFTM University, Moradabad (U.P.) (reference no. 2016/837ac/08). This article does not contain any studies with human subjects performed by any of the authors; all institutional and national guidelines for the care and use of laboratory animals were followed.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Pharmaceutics, Faculty of Pharmacy, IEC Group of Institutions, Greater Noida, Uttar Pradesh 201310, India. [2]Department of Pharmaceutics, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh 244102, India.

Received: 8 March 2021 Accepted: 26 May 2021
Published online: 29 June 2021

References

1. Wokovich A, Prodduturi S, Doub W.H, Hussain A, Buhse FH.(2006) Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. Eur J Pharm Biopharm 64 1, 1–8, DOI: https://doi.org/10.1016/j.ejpb.2006.03.009.
2. Beig A, Markovic M, Dahan A. (2018) Solubility, permeability, and their interplay. Methods and Principles in Medicinal Chemistry,Wiley-VCH Verlag GmbH & Co. KGaA, 1, 171-202 .
3. Wiedersberg S, Guy R (2014) Transdermal drug delivery: 30+ years of war and still fighting! J Control Release 190:150–156. https://doi.org/10.1016/j.jconrel.2014.05.022
4. Kokubo T, Sugibayashi K, Morimoto Y (1994) Interaction between drugs and pressure-sensitive adhesives in transdermal therapeutic systems. Pharm Res 11:104–107
5. Singh A, Bali A (2016) Formulation and characterization of transdermal patches for controlled delivery of duloxetine hydrochloride. Anal Sci Technol 7:25
6. Weng W, Quan P, Liu C, Zhao H, Fang L (2016) Design of a drug-in-adhesive transdermal patch for risperidone: effect of drug-additive interactions on

the crystallization inhibition and in vitro/in vivo correlation study. J Pharm Sci 105:3153–3161
7. Taghizadeh S, Ardakani A, Mohamadnia F (2015) A statistical experimental design approach to evaluate the influence of various penetration enhancers on transdermal drug delivery of buprenorphine. J Adv Res 6:155–162 A
8. Zhao C, Quan P, Liu C, Liang F (2016) Effect of isopropyl myristate on the viscoelasticity and drug release of a drug-in-adhesive transdermal patch containing blonanserin. Acta Pharm Sin B 6(6):623–628. https://doi.org/10.1016/j.apsb.2016.05.012
9. Lal N, Yadav P, Rastogi V, Verma A, Verma N (2017) Aspects of pressure sensitive adhesives in fabricating drug-in-adhesive transdermal therapeutic systems. Drug Delivery Letter 7(1):3–15
10. Dzeufiet P, Mogueo A, Bilanda D, Aboubakar B, Tedong L, Dimo T, Kamtchouing P (2014) Antihypertensive potential of the aqueous extract which combine leaf of *Persea americana* mill. (Lauraceae), stems and leaf of *Cymbopogon citratus* (D.C) Stapf. (Poaceae), fruits of *Citrus medical* L. (Rutaceae) as well as honey in ethanol and sucrose experimental model. BMC Complement Altern Med 14:507
11. Faruk E, Elsawy N, Desoky R (2015) Histological, immunohistochemical and biochemical study on the possible cardioprotective and antihypertensive role of the flavonoid in unilateral renal artery ligation of adult albino rats. J Cytol Histol 6(3):1–8
12. Parhi R, Padilam S (2018) Invitro permeation and stability studies on developed drug-in- adhesive transdermal patch of simvastatin. Bull Fac Pharm Cairo Univ 56(1):26–33
13. Yang D, Wan X, Quan P, Liu C, Fang L (2018) The role of carboxyl group of pressure sensitive adhesive in controlled release of propranolol in transdermal patch: quantitative determination of ionic interaction and molecular mechanism characterization. Eur J Pharm Sci 115:330–338
14. Vora N, Madan L (2013) Development and in-vitro evaluation of an optimized carvedilol transdermal therapeutic system using experimental design approach. Asian J Pharm 8:28–38
15. Lal N, Yadav P, Rastogi V, Pandey L, Verma N, Verma A (2016) Development and evaluation of transdermal therapeutic system of metoprolol succinate using acrylic polymer. Asian J. Pharm. 10(3):178–187
16. Stefano FJ, Scasso AF(2005). Transdermal delivery system with two superimposed adhesive layers having different affinities to the active substance comprised. United States Patent US 20050208116 A1.
17. Malone JI, Schocken D, Morrison AD, Gilbert-Barness E (1999) Diabetic cardiomyopathy and carnitine deficiency. J Diabetes Complicat 13(2):86–90. https://doi.org/10.1016/S1056-8727(99)00039-2
18. Wang E, Casciano CN, Clement RP, Johnson WW (2001) Active transport of fluorescent pglycoprotein substrates: evaluation as markers and interaction with inhibitors. Biochem Biophys Res Commun 289(2):580–585. https://doi.org/10.1006/bbrc.2001.6000
19. Naziroglu M, Butterworth P (2005) Protective effects of moderated exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. Can J Appl Physiol 30(2):172–185. https://doi.org/10.1139/h05-113
20. Nemoto O, Kawaguchi M, Yaoita H, Miyake K, Maehara K, Maruyama Y (2006) Left ventricular dysfunction and remodeling in streptozotocin-induced diabetic rats. Circ J 70(3):327–334. https://doi.org/10.1253/circj.70.327
21. Wang J, Li Z, Sun F, Tang S, Zhang S, Lv P, Li J, Caoa X (2017) Evaluation of dermal irritation and skin sensitization due to vitacoxib. Toxicol Rep 4:287–290
22. Valenta C, Auner BG, Loibl I (2005) Skin permeation and stability studies of 5 aminolevulinic acid in a new gel and patch preparation. J Control Release 107(3):495–501. https://doi.org/10.1016/j.jconrel.2005.07.006

Publisher's Note