

Improving Relative Bioavailability through Drug-in-Adhesive Transdermal Patch of Duloxetine:Me β CD

Rajiv Kumar

Panjab University Faculty of Pharmaceutical Sciences

Vivek Ranjan Sinha

Panjab University Faculty of Pharmaceutical Sciences

Lalita Dahiya

Kurukshetra University

Tamas Sohajda

Cyclolab Cyclodextrin Research and Development Laboratory Ltd.

Amita Sarwal (✉ sarwalamita@gmail.com)

Panjab University Faculty of Pharmaceutical Sciences

Research Article

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Abstract

Aim of the study was to develop optimised drug-in-adhesive (DIA) transdermal patch of duloxetine HCl. It is known that acrylic polymers having different functional groups play significant role in enhancing drug permeation. Among various permeation enhancers (PEs), Transcutol P exhibited most enhanced permeation ($ER \approx 1.99$) in terms of flux and Q_{24} compared to control group having no PE. Hence, a transdermal DIA patch having DURO-TAK 87-2287 as DIA polymer and TP as PE loaded with 40% DLX previously complexed with Me β CD and duly characterised (FTIR, DSC and SEM) was developed for *in vivo* study. Mean of maximum plasma concentration (C_{max}) and area under time-concentration curve (AUC_{0-72}) in Wistar rats ($n=6$) for transdermal patch (10 mg/kg) was found to be 70.31 ± 11.2 ng/ml and 2997.29 ± 387.4 ng/ml*h respectively and these values were considerably higher than oral dose of DLX (20 mg/kg and 10 mg/kg). Albeit, $T_{1/2}$ was higher in case of transdermal delivery but this was due to sustained behaviour of delivery system. These findings highlight the significance of both inclusion complexation and transdermal delivery of DLX using DIA patch for efficient drug absorption and thus reducing the dose of drug and related side effects.

Introduction

Cyclodextrins (CDs) are cyclic oligomers of 6, 7 or 8 D-glucopyranosidic units, large molecular weight (1000 to > 2000 Da) and are poorly absorbed from biological membrane (French 1957). It represents the class of excipients having lipophilic internal cavity and hydrophilic outer surface comprised of (α -1, 4)-linked α -D-glucopyranose units. Chair formation of these glucopyranose units renders CDs as cones shape having secondary and primary hydroxy groups extending from wider edge and narrower edge respectively. Such arrangement gives CD the desired shape with hydrophilic outer shape and internal hydrophilic cavity. Natural CDs are 6 membered (α CD), 7 membered (β CD), 8 membered (γ CD) glucopyranose units. Substituted CDs include hydroxypropyl, dimethyl, randomly methylated, sulfobutylether- β CD. Human pancreatic amylase is reported to hydrolyse parent β CD, although get fermented in intestine and are non-toxic at low to moderate doses. Intrinsic reactivity of these enzymes and/or affinity of CDs to these enzymes get diminished on substitution of hydroxyl groups (Marshall & Miwa 1981).

DLX is an effective antidepressant with poor solubility profile. Several attempts have been made to overcome this drawback of low oral bioavailability. Micro-emulsion loaded with DLX was prepared. Permeation of DLX from micro-emulsion was 1.5 times more compared to DLX suspension. PK/PD study outcomes were also in complete agreement of permeation study data (Sindhu et al. 2018). Avoidance of first pass metabolism along with predictable controlled transport is added advantage of TDDS. Albeit barrier property of skin is a deterrent which can be overcome by using PEs to appreciable extent and require lower initial concentration of drug for effective pharmacology (Hillery & Park 2016). For this purpose, we developed drug-in-adhesive (DIA) patches. DIA patches are comprised of active drug incorporated in pressure sensitive adhesive, a backing film and release liner. It has advantages of smaller

size, less thickness, better flexibility and drug loaded adhesive layer remains in contact with skin surface after its application. DLX-SBE β CD spray dried inclusion complexes equivalent to 22.7 mg of DLX optimized on the basis of various studies were incorporated in place of pure DLX for enhanced permeation and reduction in dose.

Pressure sensitive adhesives (PSAs) are very critical in designing TDDS. Although, their main function is to adhere the patch to skin but they function as matrix for drug constituent and for most of excipients too. PSAs also affect the flux of drug, its release and physicochemical stability. In DIA systems, drug is mixed in polymeric mixture and skin itself act as rate-controlling membrane. There are three main types of PSAs namely polyisobutylene (PIB), silicones and acrylates. Acrylates or polyacrylic esters are clear and amorphous polymers exhibiting biocompatibility, fair adhesion and need no stabilisers or plasticisers and thus can be used directly for developing TDDS. Acrylates have low glass transition temperature (T_g) of -55 °C to -15 °C and thus have good flexibility and softness (Lobo et al. 2016).

Materials And Methods

2.1 Materials

DLX was procured from Shodhana Pharmaceuticals Pvt. Ltd. (Ahmedabad, India). High purity Me β CD (98%, MW \approx 1310) was gratis sample from Cyclolab Cyclodextrin Research and Development Laboratory Ltd., (Budapest, Hungary). Duro-Tak 87-900A, 87-2287 and 87-235A pressure sensitive adhesives were procured from Henkel Ltd. India. All other chemicals and reagents used were of analytical grade and purchased from local suppliers (Loba Chemie and Himedia Lab). Transcutol P was received as gift sample from M/s Gattefosse, Mumbai, India.

2.2 Methods

Phase solubility studies

Phase solubility diagram is a widely accepted method of determining the effect of cyclodextrin complexation on the solubility of low soluble drugs and these studies were performed by method of Higuchi and Connors. In the study, increasing amounts of Me β CD (mM) were added to dilutions containing excess amount of DLX (mM). Suspensions so formed were stirred at room temperature (25 °C \pm 2°C) for successive three days to ensure equilibrium. Suspensions were then filtered (0.2 μ membrane filter), diluted, analysed spectrophotometrically (PerkinElmer UV-Vis Lambda 35 spectrophotometer, Singapore) at 289 nm and quantified for drug solubilised. Stability constant ($K_{1:1}$) was calculated using Eqn. 1 from the straight line portion of the phase solubility graph.

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept} (1 - \text{Slope})} \quad \text{Eqn. 1}$$

Preparation of Solid Binary Systems

Solid binary systems of DLX and Me β CD were prepared using equimolar amount of DLX and Me β CD in 1:1 stoichiometric ratio. 1:1 stoichiometric ratio was selected on the basis of A_p type of phase solubility graph. It is evident from our previous studies that spray dried complexes yield better solubility enhancement for DLX so we prepared the DLX-Me β CD by spray drying process and for comparison purpose we prepared physical mixture also.

Physical mixture and Spray dried Systems

PM was prepared by blending equimolar amount of DLX and Me β CD (1:1) in a ceramic mortar without trituration or applying any external force. For spray drying, required amount of DLX was dissolved in MeOH and Me β CD was dissolved in distilled water. Both solutions were mixed and stirred for 24 h in a shaking bath. The solution so obtained was spray dried using lab scale Labultima LU228 advanced spray dryer with following parameters: inlet temperature 120 °C, outlet temperature 80 °C, flow rate of 1 ml/min, and atomising air pressure of 2kg/cm².

Thermal Analysis

DSC thermograms of both materials and their binary systems were obtained by heating the sample (1-2 mg) over a range of 50-250 °C with heating rate of 10 °C/min using sealed aluminium pans purged with dry nitrogen maintained at flow rate of 50 ml/min. An empty aluminium pan duly sealed was used as reference. Samples were suitably dried before experimentations.

Fourier Transform Infrared Spectroscopy

FTIR analysis was performed using Perkin Elmer FTIR-400 spectrometer by preparing KBr pellets of the drug. Spectral acquisitions were made over the range of 4000-400 cm⁻¹.

Scanning electron Microscopy

Similarly surface morphology of different samples was studied using scanning electron microscope (Jeol, JSM-6100, Tokyo Japan) with an excitation voltage of 20 kV. For this purpose, samples were fixed on a brass stub using a double-sided tape and samples were made electrically conductive with the help of copper layer coating.

2.2.1 Preparation of DLX transdermal DIA adhesive matrix

Different PSAs were used for preparing DLX transdermal DIA patches. After optimizing appropriate PSA, suitable penetration enhancer was also selected. We used different acrylic PSAs like DURO-TAK 87-900A (no functional group), DURO-TAK 87-2287 (-OH), DURO-TAK 87-235A (-COOH) were procured from Henkel. Initial step in formulating the DIA patch was to select the appropriate adhesive. Various PSAs were thus screened keeping all other formulation factors including drug content constant.

2.2.2 Evaluation of drug content

Drug contents of medicated patches were evaluated as described previously. Briefly, DLX in the patches was extracted with methanol to determine the DLX content in the patch. For this purpose, each patch sample (1.2 cm radius) was immersed in 50 ml of methanol and stirred for 24 h, and then resultant solution was filtered and analysed. Release liner was separated from adhesive layer before the extraction (Jung et al. 2015).

2.2.3 Fabrication and preparation of matrix DIA patch

Finally, HPMC was used as matrix forming rate-controlling polymer. Required amount of HPMC was weighed and dissolved in organic solvent methanol (15 ml) and sonicated for 20 minutes. Thereafter, DIA matrix patches were formulated. Before developing medicated DIA patches, we optimised the DLX content (on the basis of slide crystallisation studies), amount of PSA (optimised on the basis of flux provided), PE (on the basis of effect of PE on the permeability studies outcomes). For developing DIA patch, DLX-Me β CD spray dried complex equivalent to 22.7 mg of DLX, required amount of PSA, PE and HPMC were dissolved in 15 ml of solvent. 5 ml of different PEs were added in formulations DIA-2 to DIA-5 but formulation DIA-1 was treated as control formulation and contains no PE. The resulting solution was magnetically stirred for 2 h at 40 °C for complete solubilisation of various excipients in matrix followed by pouring the prepared mass on to 3M Scotchpak™ 1022, which is a transparent, occlusive fluoropolymer release coating acting as release liner. All patches so formed were dried at 40-50 °C in an oven for almost 2 h and then films were removed carefully and backing membrane (3M Scotchpak™ 9723) was placed on the top of film firmly. Finally, medicated patch was cut into circular pieces of 1.2 cm radius, wrapped in aluminium foil and stored in air tight container till further use, as describes in our previous publication (Kumar et al. 2021).

2.2.4 Characterization of fabricated patches

Patch thickness

Thickness of the suitably cut DIA patches were measured at three different places with the help of a calibrated micrometer and the results were expressed as mean \pm SD.

Drug content uniformity

For content uniformity, each patch was first separated from backing layer and release liner. The DIA film was then cut into four equal quadrants. The rest of the procedure was same as discussed above.

Surface pH, Moisture content (MC) and Moisture uptake (MU)

For determining surface pH, each film was first moistened and allowed to swell with one drop of distilled water and then electrode of pH meter was brought in contact with the even surface. For moisture content, active silica contained in desiccators was loaded with the DIA films cut with area of 4.9 cm² for 3 days. During these days, each film was weighed again and again until it shows constant weight indicating no

residual moisture present. Finally, MC was computed taking into consideration the weight difference with final weight. For moisture uptake study, fresh DIA film was placed in saturated solution of KBr for 24 h.

Ex vivo permeation study of DIA patch

For this purpose, abdominal rat skin was used. Wistar rats of average weight 225 g were sacrificed and their abdominal skin was excised and to remove the hairs and underlying fat, skin was treated with 0.3 N ammonium hydroxide solution in normal saline. After removing underlying fat, skin was washed with normal saline to wash out residual amount of ammonium hydroxide. The skin was then mounted in between the donor and receptor chambers of Franz diffusion cell with the dermis facing the receptor compartment, previously filled with 30 ml of PBS (pH 6.8).

2.2.5 Pharmacokinetics of DLX from in blood

For carrying out pharmacokinetic studies, we used three groups of Wistar rats having 6 animals each. Hairs from dorsal side of these animals were carefully removed without causing any damage to skin using commercial depilatory. Also, skin was washed with alcohol swab to remove any physical residue present on skin which could hinder the adhesion of formulation. A single medicated transdermal patch after removing the release liner was applied to clean and dry hairless skin for 72 h. DLX was administered via oral route in 20 mg/kg dose in suspension form, pellet of DLX:Me β CD spray dried complex equivalent to 10 mg/kg DLX and 10 mg/kg transdermal DIA patch applied on dorsal side of abdominal skin. After regular intervals, blood samples were withdrawn and collected in pre-heparinised Eppendorf vials upto 72 h post application of medication. Plasma samples were obtained by centrifugation (4500 rpm for 10 min) of blood samples and were stored at -20 °C until analysed using validated HPLC method.

Pharmacokinetic parameters like C_{max} , T_{max} , $T_{1/2}$ MRT and AUC were calculated using PKSolver software performing non-compartmental analysis for oral doses and one compartmental modelling for transdermal dose. After analysis, DLX pharmacokinetics of transdermal dose was compared with oral doses of DLX and the data is presented as mean \pm SD (Su et al. 2020).

2.2.6 Histopathology

Histopathological examination on organs and tissues of all animal groups were conducted. After autopsy, the organs and tissues were removed and harvested to prepare histopathological slides according to the standard protocol. Organs and tissues were fixed in 10% formalin solution. The organs observed were lungs, kidney, liver and spleen of animals of all groups.

2.2.7 Statistical analysis

Results expressed are mean \pm SD of three experiments. Difference of statistical significance among various groups was determined using GraphPad Prism 7.0 software. One-way ANOVA followed by *post-hoc* test was used and comparisons were deemed significant when *p*-value was lower than 0.05 (*p* < 0.05).

Results And Discussion

3.1 Determination of stoichiometry and apparent stability constant

Initially a calibration curve of DLX was constructed at 289 nm using methanol as solvent ($R^2=0.996\pm 0.02$). Higuchi and Connors have classified the phase solubility diagram into two types i.e. "A" and "B" on the basis of type of graph obtained. Type "A" exhibits when substrate solubility linearly increases with increase in ligand concentration and the slope was less than unity over the concentration range. Such graph can be further classified as A_L , A_P or A_N type corresponding to linear, positive and negative curvature respectively. As we can easily observe in the graph (figure 1) to exhibit A_P type and is suggestive of formation of inclusion complex in 1:1 stoichiometric ratio.

Different possible effects concerned with inclusion complexation rely upon the stability of these complexes formed as is indicated by stability constant (K_s). Lower value of K_s indicates weak interaction between the host and guest moiety and therefore more free ligand remaining while higher value of K_s indicates most of ligand to be included in the cyclodextrin inner cavity shifting the equilibrium towards formation of inclusion complex. K_s values ranging between 200-5000 M^{-1} deemed fit for application to dosage forms (Miranda et al. 2011). In table 1 various parameters calculated from linear portion of phase solubility graph at room and body temperature are shown. S_0 is the intrinsic solubility of the DLX which does not change on complexation and is indicative of innate solubility of the host in absence of any host. K_s values were determined from the slope and intercept of the linear portion of A_P type of phase solubility graph obtained. The value of apparent stability constant was higher at body temperature ($221.7\pm 1.4 M^{-1}$) than room temperature ($189.3\pm 2.6 M^{-1}$) over the same concentrations of Me β CD. It is interesting to see that in presence of maximum concentration of Me β CD, DLX solubility increased almost 13.7 times at room temperature and 18.38 at body temperature compared to its intrinsic solubility S_0 . From table 1 one can also visualise increase in efficiency of solubilisation i.e. coefficient of DLX solubility in absence and in presence of Me β CD where around 13 fold and 17 fold increase in the solubilisation of DLX was observed at room temp and body temp respectively indicating the significance of inclusion complexation. For this very reason we Me β CD was carried forward with the formulation process.

3.1.1 Thermal analysis

DSC is a very powerful analytical technique to characterise solid state interaction between drug and cyclodextrins. On inclusion and stabilisation of guest molecule in the hydrophobic cavity of cyclodextrin, peaks characterising the melting/boiling/sublimation point of guest and dehydration peak of Me β CD tend to shift to a different temperature point/disappeared. DSC thermograms of DLX, Me β CD, their corresponding physical mixture and spray dried complex are shown in figure 2. Me β CD is a water-soluble cyclic heptasaccharide consisting of a β -glucopyranose unit having ability to solubilise non-polar hydrophobic drugs and is also used as cholesterol-depleting reagent (Gotoh et al. 2014, Mundhara et al. 2019). Me β CD can also enhance cell permeability and thus increase uptake of small molecules.

It is evident from the thermograms that all samples exhibited endothermic peaks as presented by heat flows. Also, as our previous publications shows (Kumar et al. 2020), DLX has sharp endothermic peak at 169 °C with $T_{\text{onset}} = 168.43$ °C with $\Delta H = 28.36$ J/g which corresponds to pure DLX compound indicating a typical crystalline state. Me β CD has shown a broad peak at 62 °C corresponding to loss/volatilisation of water molecule present/trapped in the inner cavity of Me β CD. Another peak at 171.59 °C presented the thermal decomposition of host molecule. While analysing the DSC of physical mixture, endothermic peak of Me β CD remained unchanged but an additional peak at 170.54 °C could be visible and this may be attributed to presence of DLX. It indicates no interaction/complexation between drug and Me β CD occurs in this system. So far as DSC thermogram of spray dried inclusion complex of DLX:Me β CD is concerned, dehydration signal of Me β CD was seen to be smaller as activation energy required for its dehydration gets changed after interaction with guest molecule. Also, endothermic peak of DLX gets shifted due to its partial complexation. But no volatilisation peak of DLX was observed indicating formation of inclusion complexation in solid state (Santos et al. 2017). This type of DSC curve of spray dried complex is indicative of molecular encapsulation of drug in the hydrophobic cavity of Me β CD resulting in formation of an amorphous complex.

3.1.2 FTIR

FTIR spectra of DLX, Me β CD, physical mixture and spray dried inclusion complex are shown in figure 3. IR spectra of Me β CD shows large band at 3394 cm^{-1} (O-H stretching), 2932 cm^{-1} (aliphatic stretching C-H), 1157 and bands in range of 1050 cm^{-1} i.e. 1034 cm^{-1} (C-O- stretching to bonds on ether and hydroxyl groups) and can be ascribed with stretching frequency of primary and secondary C-OH groups of Me β CD (Santos et al. 2017). In IR spectra of inclusion complex, there could be found no other peaks than those of DLX or Me β CD while the IR spectra of physical mixture exhibited peaks corresponding to original compounds.

3.1.3 ScanningElectronMicroscopy

SEM is a qualitative technique for studying the structural aspects of raw materials. Photomicrographs of different compounds are shown in figure 4. Scanning was performed at 100 and 200X magnifications for better visualisation of the compounds of interest. DLX shows a needle like smooth surface but in different shapes and sizes. While Me β CD exhibited amorphous shape of different sizes. SEM micrographs of physical mixture could show both drug particles and Me β CD particles separated from each other but in close proximity to each other suggesting no interaction between the two. Spray dried inclusion complex exhibited irregular shape Me β CD particles whose original morphology has been lost. Such changes of surface morphology of host molecule indicate the interaction with guest and formation of a new moiety (Periasamy et al. 2014).

3.1 DIA patch formulation

On the basis of phase solubility studies and characterisation of inclusion complexes formed by spray drying, we could conclude that DLX:Me β CD exhibited 1:1 stoichiometry for inclusion complexation. The

results corroborated with our previous studies (Kumar et al. 2021) and others (Siva et al. 2020).

3.2 Selection of adhesive for formulating DIA patch

Adhesives can be multifunctional in TDDS. PSAs are the adhesives forming a bond with substrate on application of minor pressure. These adhesives leave no residue on the substrate after their removal. Viscoelastic materials have pressure sensitivity characteristics. PSAs form no chemical bond to adhere to substrate but form intermolecular and can be used without using solvents without Acrylic acid based PSAs were used for preparing the DIA patches. PSAs having different functional groups were used as shown in table 2.

3.2.1 Effect of adhesive type on the permeation characteristics of DLX

Selection of PSA is critical to the transdermal delivery of DLX. We considered three types of acrylic acid based PSAs for our study having different functional groups (see table 2). Therefore, every PSA was mixed with equal amount of DLX i.e. 10% and the release behaviour of the prepared DIA patches was observed by keeping all factors constant. Thickness of the patches was also kept uniform and equal for all three PSAs. Impact of adding different PSAs on the release behaviour of DLX is shown in figure 5 and different parameters like flux and Q_{72} derived from the release profiles are tabulated in table 3 respectively.

***Therefore, on the basis of *In vitro* release study of DLX, DURO-TAK 87-2287 was selected for carrying out further studies.**

3.2.2 Effect of drug content on DLX permeation

After screening the optimum PSA for incorporating the drug to prepare DIA patch, DURO-TAK 87-2287 adhesive was loaded with different DLX content i.e. 5, 10, 20, 30 and 40% w/w of DLX. The effect of drug content on the *in vitro* permeability was evaluated as shown in figure 6 and various permeability parameters have been calculated and tabulated in table 4 to optimise the desired concentration of DLX for loading in DIA patch development.

It is evident from the figure 6 and table 4 that DLX flux from the skin increased with increase in its content in the DIA patch. Up to 40% DLX could be loaded in the DIA patches prepared with DURO-TAK 78-2287 as PSA but above this concentration, drug crystallisation starts as evident in section of drug crystallisation studies observed with the help of optical microscope. Such observation may be attributed to the maximum solubility of DLX in the PSA. Therefore, on the basis of these results, 40% DLX was selected for loading the drug into optimized PSA i.e. DURO-TAK 78-2287.

3.2.3 Selection of penetration enhancer

For selecting the optimum penetration enhancer, different PEs were mixed in fixed 40% w/w concentration of DLX (table 5) and DIA patch were prepared.

One formulation was having no PE and treated as control group. Various PEs tested include oleic acid (OA), Brij 98, Transcutol P (TP) and isopropyl myristate (IPM). Different PEs were added in the same concentration. The results of *ex vivo* permeation study are shown in figure 7 and various permeation parameters are tabulated in table 6 and indicate that TP, Brij 98 and IPM were almost showing same enhancement in the permeation of DLX across the skin. Among these PEs TP and Brij 98 were almost equally capable of enhancing the permeation demonstrated by the enhancement ratio (ER) having values of 1.986 and 1.988 respectively. Oleic acid is a fatty acid added in formulation DIA-2, can also act as crystallization inhibitor which can be the reason for increased flux of DIA-2.

*Therefore, on the basis of *ex vivo* permeation studies, TP was selected as suitable permeation enhancer (PE) for developing final DIA patch.

3.3 Characterization of DIA films

Spray dried complexes of DLX loaded DIA patches were characterised for various physicochemical properties and the results are shown in table 7. All film batches exhibited fair organoleptic properties like colour and transparency. The per cent drug loading or drug content was found to be in the range of 95.4 ± 2.8 (for DIA-3)- 98.7 ± 4.2 (for DIA-4), although other formulation groups also exhibited fair drug loading of more than 96%. The uniformity of drug content and low SD values indicate the robustness of formulation development methodology.

Low value of moisture content of all films is also indicative of its stability during storage as well as its non-brittleness as the complete dryness is not attained during storage. Low values ranging from 0.55 ± 0.003 (for DIA-4) to 1.03 ± 0.005 (for DIA-1) reflect formulated DIA films to be enough stable. Interestingly, moisture uptake for all films was also very low ranging 0.54 ± 0.01 (for DIA-5) to 1.1 ± 0.012 (for DIA-1). Such low values of moisture uptake assure that the formulations will not attract microbial contamination and will certainly deter bulkiness. Surface pH of all films ranged from 6.2 ± 0.03 (for DIA-5)- 6.6 ± 0.06 (for DIA-3) which indicate closeness to pH 7 near to skin pH and thus films are suitable for transdermal application and probably would not cause any local irritation during or after application (Su et al. 2020).

We conducted different studies to observe the physicochemical and skin permeability of DLX across the skin. Our findings led to the conclusion that DIA formulations have better drug loading and better drug permeation across the same skin surface area used compared to matrix type of transdermal patches. Among DIA formulations, the outcomes of *ex vivo* permeation studies conducted using skin samples of Wistar rats, it was evident that formulation DIA-4 containing TP as permeation enhancer was having significant permeation enhancement compared to control group having no PE. Therefore, formulation DIA-4 was selected for conducting further studies. Here onwards, all studies are conducted using DIA-4 as optimized formulation.

3.4 *In vivo* pharmacokinetic study

Under optimum HPLC conditions, method developed exhibited fair selectivity and specificity for DLX. Good linear regression for DLX was observed with R value of 0.997 (over the linearity range of 0.5-20µg/ml with retention time of 3.13 min (Kumar et al. 2021). We conducted pharmacodynamics study where DLX could alleviate depression progression in rat CUMS models. Median lethal dose of DLX (LD_{50}) in female rats is 279 mg/kg, so we determined DLX pharmacokinetics at dose equivalent to 10 mg/kg (DLX-Me β CD pellet) and 20 mg/kg (pure DLX in suspension form) for oral route.

As we try to circumvent hepatic first pass metabolism of DLX via transdermal route expecting 100% bioavailability of DLX through this route, the dose of the drug can be reduced to 10 mg/kg in place of 20 mg/kg. Therefore for administering DLX via transdermal route we administered dose equivalent to 10 mg/kg in laboratory animals (group 3). Plasma concentration-time profiles of DLX after oral and transdermal administration are reported in figure 8 and different parameters calculated are summarised and compared in table 8 respectively.

As shown in figure 8, after administration of oral dose at 10 mg (pellet of DLX complexed with Me β CD) and 20 mg/kg (suspension form), DLX was seen to be absorbed and excreted rapidly from the body. Mean of peak plasma conc. was 41.7 ± 5.5 and 34.7 ± 8.3 ng/ml respectively. AUC_{0-72} was 646.27 ± 66.3 and 1332.36 ± 232.7 (ng/ml)*h and it increased non-linearly for doses extending from 10 mg/kg to 20 mg/kg. As it is seen that $T_{1/2}$ and T_{max} values did not appreciably change for both doses, the change in AUC values may be attributed to non-linearity in absorption of the drug (de Velde et al. 2016). C_{max} was found to be 34.7 ± 8.3 ng/ml for 20 mg/kg oral dose of DLX administered in suspension form while it was 41.7 ± 5.5 ng/ml for 10 mg/kg dose administered in the form of DLX complexed with Me β CD. The non-significant difference ($p > 0.05$) in the C_{max} values indicated that there appears no appreciable increase in rate of absorption of drug in its complexed form. But respective $AUC_{0-\infty}$ values 889.59 ± 42.2 and 2054.65 ± 176.2 (ng/ml)*h indicated oral bioavailability to increase in case of complexed form of DLX with Me β CD.

So far as DLX in its complexed form with Me β CD is concerned, transdermal patch was applied and blood samples were analysed at different time intervals. C_{max} (70.31 ± 11.2 ng/ml) and AUC_{0-72} (2997.29 ± 387.4 ng/ml*h) was found to be improved via this route compared to oral administration of naïve as well as pellet dose of DLX. This increased AUC value clearly indicates significance of transdermal route in enhancing bioavailability of DLX. If we consider this bioavailability to be 100%, then relative bioavailability (dividing AUC of oral route by AUC of transdermal route x 100) through oral route for 20mg/kg dose of DLX would be merely 44.45%, which is near to reported bioavailability of DLX through oral route. This finding demonstrates significance of transdermal route in enhancing bioavailability of DLX and thus reducing the dose of DLX. T_{max} for reaching C_{max} was approximately 4 ± 0.7 h for transdermal administration. C_{max} obtained from transdermal patch was even higher than that obtained from oral administration of 20 mg/kg suspension which indicated the significance of this route in circumventing hepatic metabolism and significance of inclusion complexation process. But as the C_{max} is higher, so the side effects related to high plasma drug concentration may not be reduced using

transdermal delivery of DLX. Also, increased $T_{1/2}$ (35.04 ± 5.2) for transdermal delivery compared to oral delivery of naïve DLX (22.2 ± 3.7) and pellet of DLX-Me β CD spray dried inclusion complex (21.02 ± 3.2) indicated a steady DLX plasma level to a certain extent and a sustained delivery of DLX to extended period of time *in vivo*. Also, increased MRT for transdermal delivery (53.43 ± 5.1) compared to same dose of DLX (27.8 ± 3.5) is indicative of practicability of sustained behaviour through transdermal delivery.

It is interesting to see that transdermal delivery *ex vivo* exhibits around $11025.8 \pm 343 \mu\text{g}/\text{cm}^2$ (for a loading dose of 22.7 mg equivalent to 20 mg duloxetine) in 24 h while results of transdermal delivery *in vivo* indicated DLX to release for 72 h (for dose of 10 mg/kg DLX). This means that skin permeation in real animal body is lower than *ex vivo* models. This warrants some more study to establish better *in vitro-in vivo* correlations.

3.5 Histopathological examination

As shown in figure 9, different tissues of different treated groups did not show any remarkable histopathological changes compared to control group. Liver of control group (a), at 100X showed normal hepatic architecture of liver along with lymphocytes except a mild portal tract while at 400X (b) and at low power (c), thickening by lymphocytes and possibly fibrosis is seen. There is a fine particulate matter in or over liver cells that may be an artefact. Low dose group (d) at 40X, looks normal at low magnification, while (e) at 100X, mild fatty change in liver cells and (f) at 400X, further details are provided. At (g) 40X, liver of high dose group looks normal at this low magnification, but at (h) 400X, marked pathological changes characterized by an increased cellularity of portal tract and extension towards the next portal region is seen. This may suggest chronic hepatitis.

Conclusion

Spray drying, loading of complexed drug in transdermal matrix followed by enhanced penetration and permeation advocate the potential application of inclusion complexation. The developed patch was stable, non sticky, and had a fair folding endurance. *Ex vivo* studies suggested DURO-TAK 87-2287 based patches to better permeate across the skin. With these interesting outcomes, it is evident that the said technique can work for better permeation and retention through drug amorphization and its concomitant inclusion. *In vivo* pharmacokinetic study in rats indicated a steady sustained absorption for transdermal delivery of DLX with enhanced AUC and $T_{1/2}$ suggesting obvious advantages of this route over oral administration. Therefore, it is assured that DIA patch transdermal delivery can help in reducing dose and related side effects of DLX by augmenting bioavailability of the drug.

Declarations

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Credit Authorship Contribution statement

V.R.S. and A.S. designed the research study. R.K. performed majority of experimental protocols and was a major contributor in writing first draft of manuscript. L.D. and T.S. contributed to reviewing and editing. All authors read and approved the final manuscript.

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Data availability

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

Ethical approval

All protocols involving laboratory animals are approved from institutional animal ethical committee, Panjab University, Chandigarh.

Consent for publication

Not applicable.

Consent to participate

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

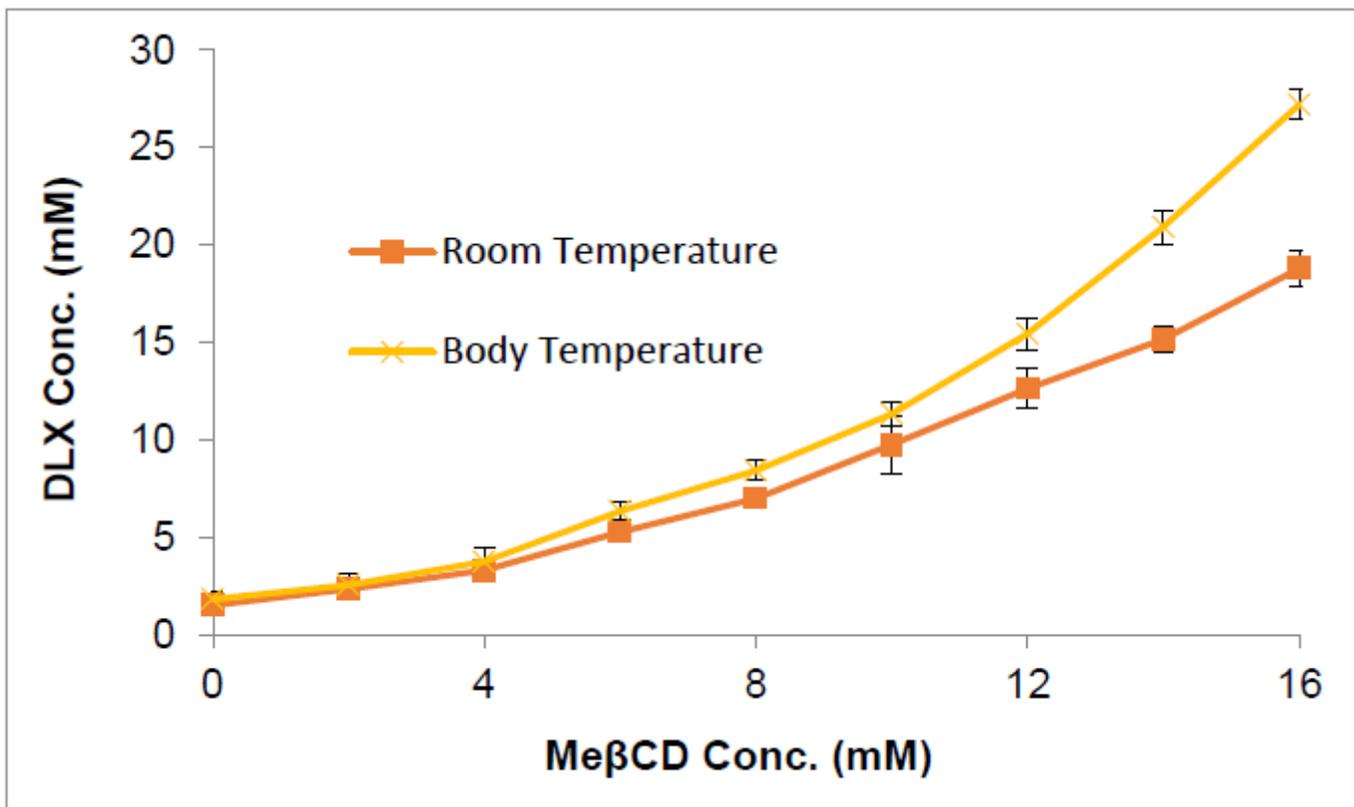


Figure 1

Phase solubility study of DLX in aqueous solution of MeβCD at 25±2 °C and 37 °C

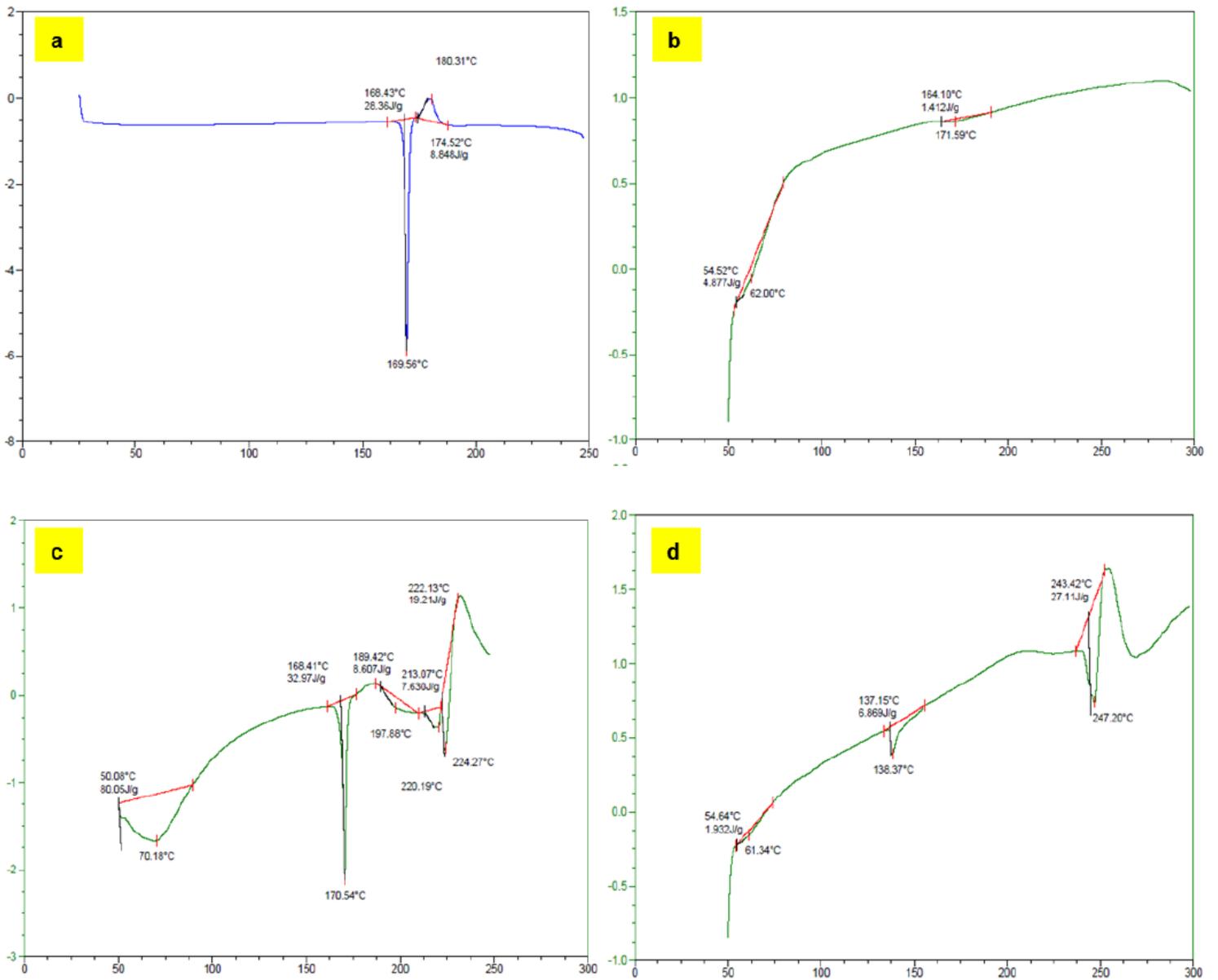


Figure 2

DSC curves of DLX (a), MeβCD (b), physical mixture (c) and spray dried inclusion complex of DLX:MeβCD respectively

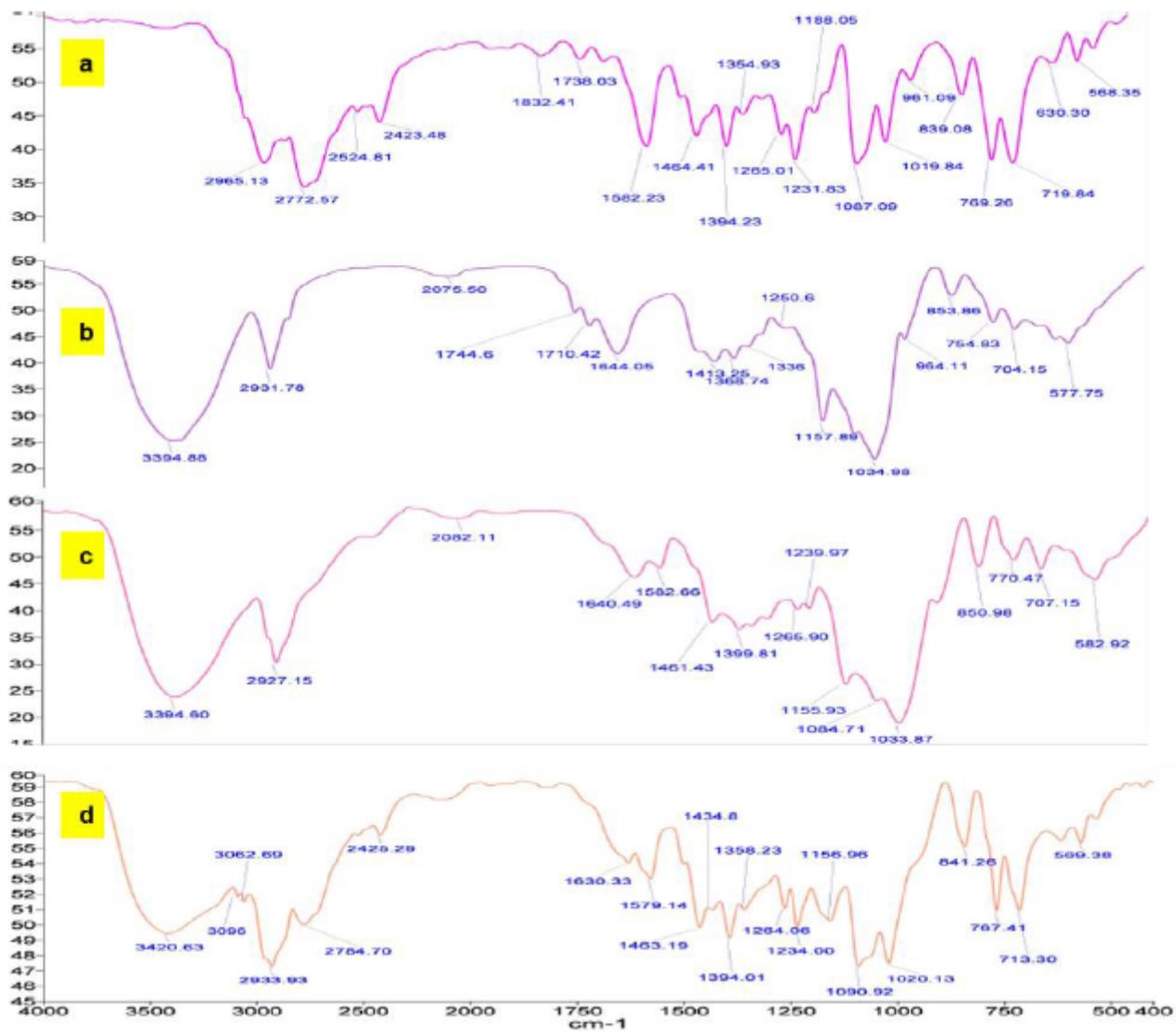


Figure 3

Comparative FTIR spectra of (a) DLX, (b) Me β CD, (c) PM and (d) spray dried inclusion complex

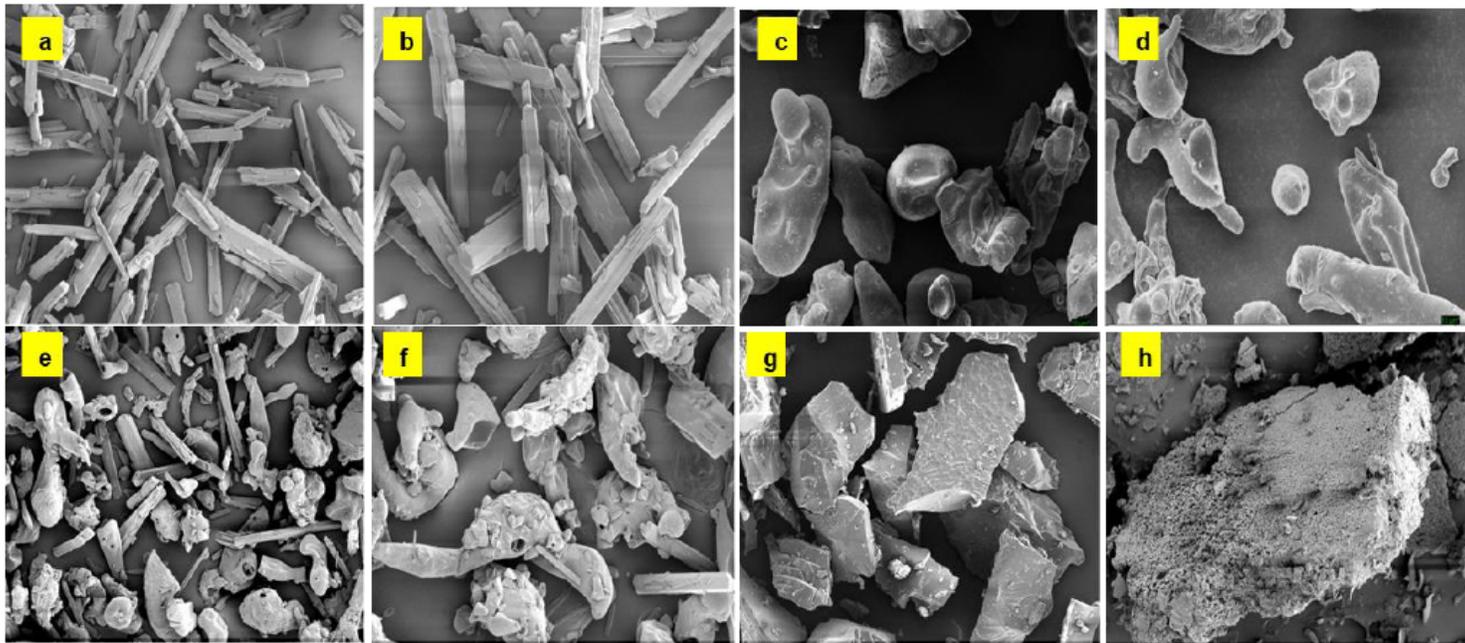


Figure 4

Photomicrographs obtained by scanning electron microscopy of DLX (a, b at 100 and 200X), MeβCD (c, d at 100 and 200X), PM of DLX:MeβCD (e, f at 100 and 200X) and DLX:MeβCD spray dried inclusion complex (g, h at 100 and 200X) respectively

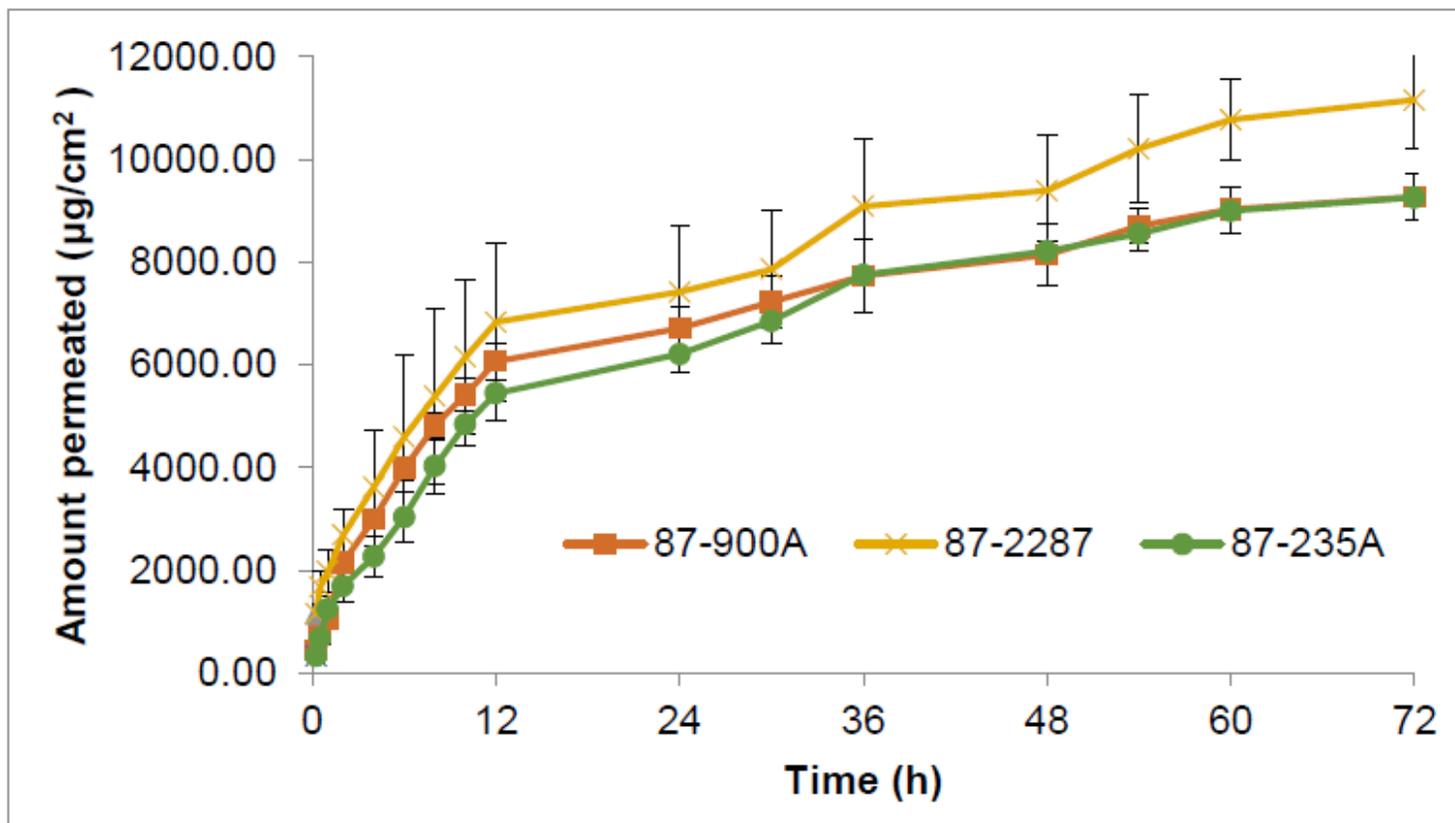


Figure 5

In vitro hairless rat skin permeation profiles of DLX from patches containing 10% (w/w) DLX in PIB 87-900A, 87-235A and 87-2287 (n=3). Each point and vertical bar represents mean and standard deviation, respectively

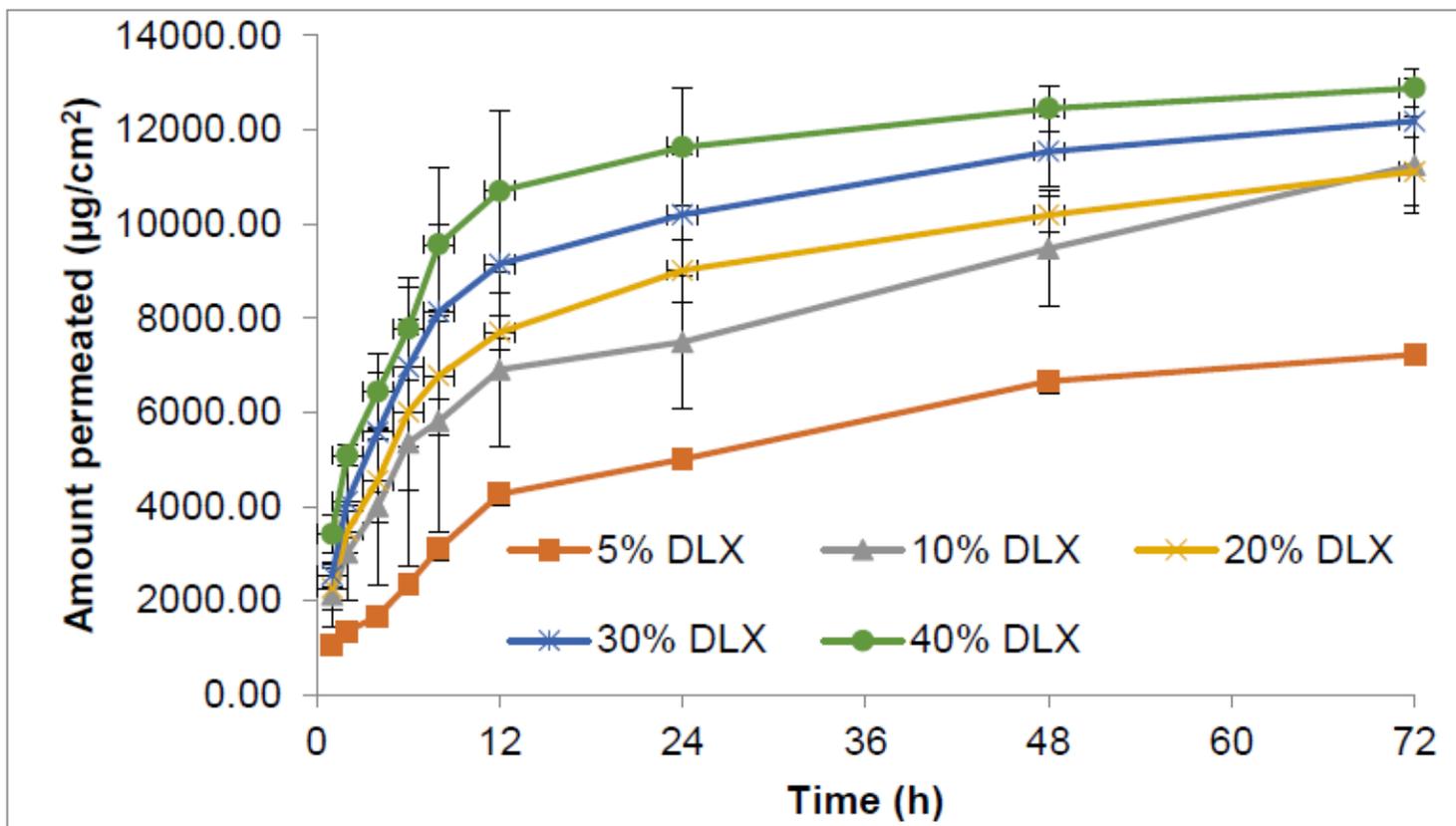


Figure 6

In vitro permeation profile of DIA patches containing different DLX content, 5 (■), 10% (▲), 20% (x), 30% (*) and 40% (●). Each data point represents mean of three experiments and each vertical bar represents standard deviation

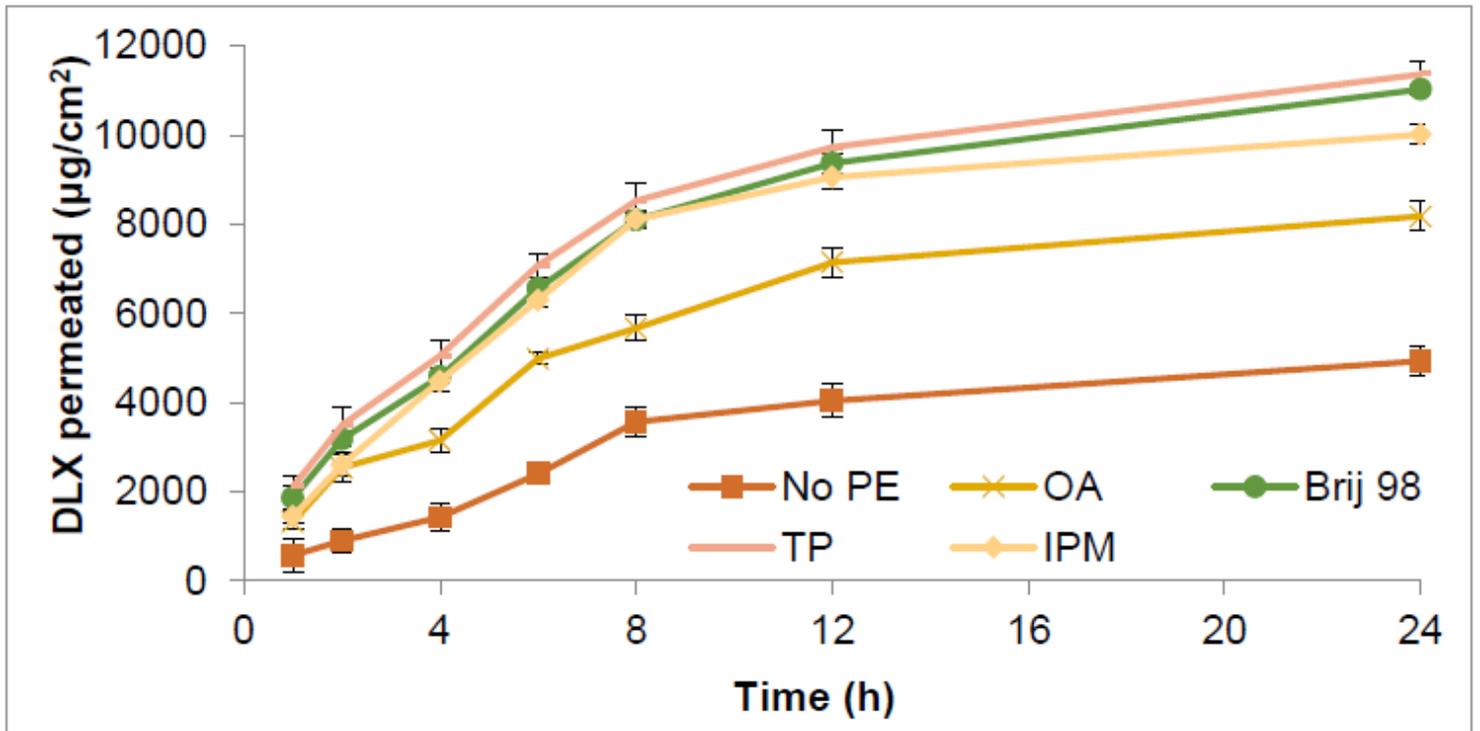


Figure 7

Ex vivo permeation results of DLX containing various PEs. One batch (NoPE) was a control batch having no PE. All values represent mean (n=3) and each vertical bar represents standard deviation

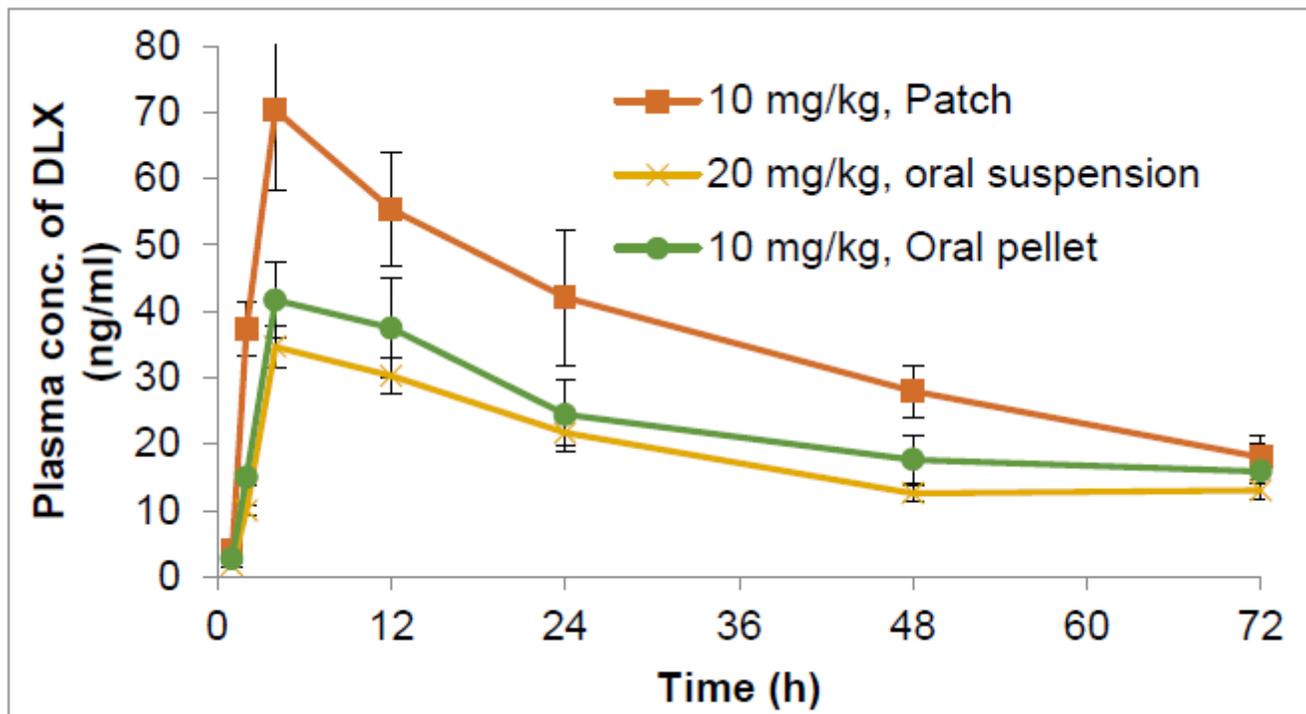


Figure 8

Mean plasma concentration-time profile of DLX after oral (10 mg/kg and 20 mg/kg) and transdermal (10 mg/kg) administration in a single dose study (mean±SD, n=6)

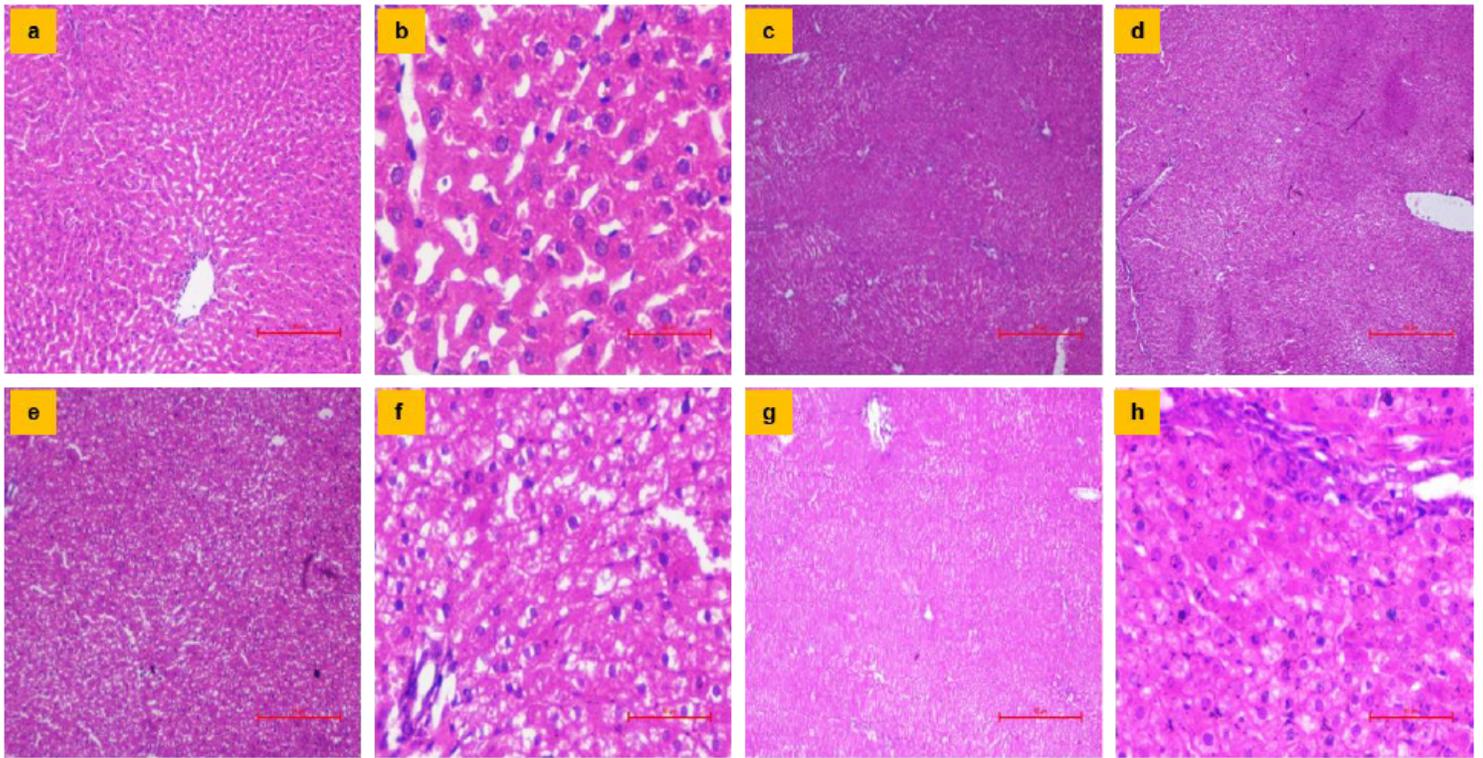


Figure 9

Histological sections of liver are shown. Micrographs of liver sections from different treatment groups are shown at different magnifications

Supplementary Files

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