4-2012

In vitro Transdermal Permeation of Fenoterol Hydrobromide

Ahmed H. Elshafeey
University of Rhode Island

Yassin E. Hamza

Soad Y. Amin

Hossein Zia
University of Rhode Island, hozia@uri.edu

Citation/Publisher Attribution

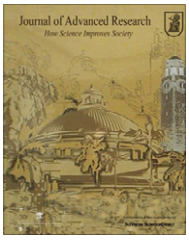
Elshafeey, A. H., Hamza, Y. E., Amin, S. Y., & Zia, H. (2012). *In vitro* transdermal permeation of fenoterol hydrobromide. *Journal of Advanced Research, 3*(2), 125-132. doi: 10.1016/j.jare.2011.05.009
Available at: http://dx.doi.org/10.1016/j.jare.2011.05.009

In vitro Transdermal Permeation of Fenoterol Hydrobromide

Journal of Advanced Research (2012) 3, 125–132

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

In vitro transdermal permeation of fenoterol hydrobromide

Ahmed H. Elshafeey [a,b,*], Yassin E. Hamza [b], Soad Y. Amin [b], Hossein Zia [a]

[a] *Applied Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA*
[b] *Pharmaceutics Department, College of Pharmacy, Cairo University, Egypt*

Received 28 March 2011; revised 25 May 2011; accepted 25 May 2011
Available online 12 July 2011

KEYWORDS

Fenoterol;
Transdermal;
Patches;
Enhancers;
Duro-Tak

Abstract The aim of this study was to determine if transdermal penetration of fenoterol, a β-agonist drug, could be enhanced and controlled by formulation modification and formulation of transdermal patches. Pre-formulation studies were performed to determine the feasibility of a transdermal dosage form of fenoterol. Penetration of fenoterol was determined using the hairless guinea pig skin with unjacketed Franz diffusion cell. Transdermal patches were formulated using drug in-adhesive technique. Several enhancers were investigated for fenoterol skin penetration. Transcutol–oleic acid co-solvent gives the highest drug flux among all tested liquid formulations. Pretreatment of the skin with oleic acid 2 h before patch application significantly increases drug diffusion. *Cis*-oleic acid gives best results compared to oleic acid. Azone derivative (1-dodecyl-2-pyrrolidinone) gives the highest drug diffusion amongst all tested enhancers. Results of this study show the feasibility of using fenoterol formulated in transdermal delivery system in the treatment of chronic asthma to improve patient compliance, bioavailability and reduce the inter-subject variability.

Introduction

The transdermal route of administration has been recognized as one of the highly potential routes. It has the advantages of obviating drug metabolism or chemical degradation in the gastrointestinal tract as well as the hepatic first-pass effect [1,2]. Transdermal delivery has the potential for highly prolonged and controlled drug delivery. In many cases, delivery can be interrupted when it is desired [3].

On the other hand, the main limitation of transdermal delivery of drugs is that the skin layers provide a great resistance to the penetrant molecules. To overcome these limitations, different strategies have been developed to minimize the skin's barrier function, such as employing appropriate components, skin enhancer [4] or applying electricity or ultrasound [5] to facilitate the penetration of drugs.

Fenoterol is a selective β_2-adrenoreceptor agonist with effect on smooth and skeletal muscles, which include bronchodilation, relaxation of the uterus. It is structurally related to orciprenaline and terbutaline but is more selective

* Corresponding author. Tel.: +20 105840261; fax: +20 2 24524223.
E-mail address: Ah_elshafeey@hotmail.com (A.H. Elshafeey).

Peer review under responsibility of Cairo University.
doi:10.1016/j.jare.2011.05.009

Production and hosting by Elsevier

than the parent drug for β_2-receptors. It has a longer duration of action than isoprenaline and has a less side effect on the heart rate [6]. A transdermal dosage form (TDD) of fenoterol may be quite useful in the treatment of chronic asthma and the reversible element of airway obstruction commonly found in chronic obstruction airways disease (COAD).

The development of a transdermal dosage form for fenoterol can be based on its pharmacokinetic properties, i.e. the short half-life and incomplete absorption from the gastrointestinal tract due to its first pass metabolism. Only 14% and 9% of fenoterol were absorbed after nasal and pulmonary administration, respectively [7].

A 2.5 mg oral dose provides bronchodilatation for about 6 h which may not be sufficient to prevent nocturnal wheezing. A sustained release fenoterol microsphere was formulated by Lin et al. [8].

In the light above, the objective of this paper was to develop a transdermal formulation for the delivery of fenoterol.

Experimental

Materials

Fenoterol, *cis*-oleic acid and dimethyl sulphoxide (DMSO) were purchased from Sigma (St. Louis, Mo, USA). Transcutol® (Gattefoss, St. Priest, France). Propylene glycol was obtained from Fisher scientific (Fair Lawn, NJ, USA). Polyethylene glycol 300 NF was provided by Union Carbide Chemicals (Danbury, CT, USA). (R)-(+)-limonene, (R)-(−)-carvone, cineole, α-pinene and 1-dodecyl-2-pyrrolidinone were purchased from Aldrich chemical company (Milwaukee, WI, USA). Duro-Tak® 87-2074 was purchased from National Starch and Chemical Company (Bridgewater, NJ, USA). Scotchpak® polyester multilam film 1006 with 2.84 mm thickness and Scotchpak® 1022 release liner with 3 mm thickness from 3 M (St. Paul, MN, USA).

Animal models

Male hairless Guinea pig 8–10-week-old of 400 g weight obtained from Charles River laboratories (MA, USA). The study protocol was reviewed and approved by the Institutional Animal care and use Committee IACUC (University of Rhode Island, Kingston, RI, USA).

High performance liquid chromatography

A water liquid chromatography (Waters associates, Milford, MA, USA) was equipped with an automated gradient sampler controller, model 717 plus auto sampler, model 515 HPLC pump, model 480 LC spectrophotometer and model 746 data module integrator. The column used was supelcosil LC-18-DB (5 μm, 4.6 × 250 mm) and the mobile phase consisted of 0.01 M potassium dihydrogen phosphate adjusted to pH 6.5 and methanol (30:70). The absorbance wavelength was set to 231 nm.

Determination of fenoterol saturated solubility in different solvents or co-solvents systems

The saturated solubility of fenoterol in a variety of solvents viz. water, transcutol, polyethylene glycol 300 (PEG 300), propylene glycol (PG), dimethyl sulfoxide (DMSO), dimethyl isosorbide (DMI), ethyl acetate and oleic acid was determined in triplicate at room temperature of 25 °C. In addition, fenoterol solubility was determined in co-solvents viz. 90% propylene glycol in isotonic phosphate buffer (pH 7.4), 10% PG in isotonic phosphate buffer (pH 7.4), dimethyl isosorbide/transcutol (1:1) ratio and oleic acid/transcutol (1:1) ratio. An excess amount of fenoterol was suspended in different media in tightly closed screw cap vials equilibrated in a rotating bottle for 24 h. Then, an aliquot of the suspensions was transferred to a 1-ml microcentrifuge filter, fitted with a 0.22 μm nylon filter (Corning Incorporated, Corning, NY, USA), and centrifuged. The filtrates were appropriately diluted with methanol before assaying by HPLC.

Preparation of different fenoterol liquid systems

Different fenoterol liquid systems were prepared by dissolving or suspending 40 mg/ml of fenoterol in different solvents such as polyethylene glycol 300, propylene glycol, transcutol, a mixture of 90% v/v and 10% v/v of propylene glycol in isotonic phosphate buffer of pH 7.4 or in co-solvent systems formed from transcutol/dimethyl isosorbide (1:1) and transcutol/oleic acid of the same ratio.

Physicochemical investigation of the interaction

DSC analysis was carried out on pure substances (fenoterol and Duro-Tak® adhesive) and their physical mixtures. Thermograms were performed using a Perkin Elmer DSC7 system equipped with a computerized data station. All samples (5 mg) were weighed and heated at scanning rate of 10 °C/min between 30 and 300 °C. The measurements were performed in triplicate.

Preparation of fenoterol patches with different drug concentration

Prototype patches were prepared by using a lab hand coater. The calculated amount of fenoterol was weighed and dispersed in oleic acid. The drug/oleic acid dispersion was then mixed with Duro-Tak® containing 28% total solids as a selected adhesive in a ratio of 1:9 inside a screw-cap bottle having a hole at the center of the cap. Samples with different drug concentrations 2%, 5%, 9% and 12% (w/w) were mixed with stirrer (Cole Parmer instrument Co., Chicago, IL) equipped with an appropriate size propeller at 100 rpm for 1 h. Following drug mixing the drug/adhesive solutions were casted using a lab-hand coater adjusted at clearance of 500 μm onto a Scotchpak® 1022 release liner (3M, St. Paul, MN), left overnight and then, cured at 65 °C for half an hour, then the laminate was covered with a heat seable Scotchpak® packing polyester film 1006 (3M, St. Paul, MN) and kept in the dark at room temperature until use. In all the above prepared patches 10% (v/v) oleic acid in Duro-Tak® adhesive was used.

Cis-oleic acid in a concentration of 10% v/v was also tried instead of oleic acid and was included in the 12% (w/w) fenoterol patch with the same above procedures.

Effect of the skin pretreatment with oleic acid on fenoterol permeation

Pretreatment of the skin with 25 µl oleic acid 2 h before patches application was also tested to evaluate the drug permeation with and without pretreatment with oleic acid.

Preparation of fenoterol transdermal patches using different enhancers

Different enhancers were tested instead of oleic acid as carvone, α-pinene, cineole, limonene, dimethyl sulfoxide (DMSO), dimethyl isosorbide (DMI) and dodecyl pyrolidinone (DPY) which is one of the azone derivative. Preparations of transdermal patches proceeded as previously discussed except that the 10% (v/v) oleic acid was replaced by 10% from the different selected enhancers with Duro-Tak® adhesive. Control patch was also fabricated using 12% (w/w) drug in adhesive only without oleic acid or any other enhancer.

Permeation study of fenoterol through hairless guinea pig skin

Guinea pigs were injected by pentobarbital sodium till sacrificed and the full thickness abdominal and dorsal skin was excised. Skin was slowly thawed and was cut into small circular pieces of 2.5 cm diameter. The lower surface of the skin was allowed to hydrate for 1 h at 37 °C prior to experimentation.

The permeation of fenoterol was studied using flat flange Franz diffusion cells. The membrane was mounted carefully onto the diffusion cell with the stratum corneum side facing the donor compartment. The receiver buffer was de-aerated before use with a sonicator fitted with vacuum pump.

Volumes of 150 µl of the tested liquid systems under investigation containing 6 mg fenoterol were placed in the donor chamber and covered with a rubber plug. The active diffusion area was 0.64 cm^2.

For permeation study from the transdermal patches, the same procedure as in liquid formulation were done except the patches were cut into pieces of 2.5 cm diameter and applied to the skin instead of the liquid formulation. Pretreatment of the skin with 25 µl oleic acid 2 h before patches application was also tested to evaluate the drug permeation with and without pretreatment with oleic acid.

Finally, the receptor solution was completely withdrawn after 4, 12, 24, 36 and 48 h. The fenoterol concentration was determined using HPLC. Each experiment was performed in six replicates.

Data analysis

The cumulative amounts of fenoterol penetrating per unit skin area (Q) were plotted against time. From the slope of the linear portion of the plot a steady state flux (SSF) was determined. The permeability coefficient (Kp) was calculated as the ratio of SSF and the critical concentration of fenoterol in the formula. Lag time (t_{lag}) was estimated by extrapolation of the linear portion of the plot to the time axis. The cumulative amount of drug permeated per unit area (Q_{24}) obtained for various formulae was compared using a one-way ANOVA test followed by Fisher's least significant test. Differences between the treatments were assumed to be significant at $p < 0.05$. All data analysis and statistical calculations were performed using Stat view Statistical Software 4.57 for Windows (CA, USA).

Results and discussion

Fenoterol solubility in different solvents

It was observed from Table 1 that, the highest fenoterol solubility was given in dimethyl sulfoxide (DMSO) which was amounted to be 170.2 mg/ml. However, lower drug solubility was recorded in solvents having moderate polarity like propylene glycol (136 mg/ml), polyethylene glycol 300 (118.5 mg/ml) and transcutol (103 mg/ml). On the other hand, on increasing the buffer percent mixed with propylene glycol, a decrease in fenoterol solubility was observed as given with 90% of propylene glycol in isotonic phosphate buffer (97.96 mg/ml) and 10% of propylene glycol in the same buffer (70.28 mg/ml). Transcutol co-solvent with dimethyl isosorbide (DMI) increased drug solubility from 9.05 mg/ml in DMI alone to 39.43 mg/ml in the mixture while, transcutol mixture with oleic acid increased drug solubility from 0.028 mg/ml in oleic acid alone to 8.68 mg/ml in the mixture. However, a limited solubility of the drug was watched in ethyl acetate (0.07 mg/ml). The variability in drug solubility would depend upon the dielectric constant of the tried solvents or co-solvents.

Effect of vehicles type on the skin permeation of fenoterol

The cumulative amount of fenoterol permeated through hairless guinea pig skin from different liquid systems was compiled in Table 2 and graphically represented in Fig. 1. It was obvious that, the highest drug permeation observed after 24 h was reached with the system composed of 10% propylene glycol (PG) in phosphate buffer compared to each of PG, 90% PG in phosphate buffer and polyethylene glycol 300 (PEG 300). These vehicles could be arranged in an ascending order in relation to the cumulative amount of drug permeated as follows: PEG 300, PG, 90% PG in phosphate buffer and 10% PG in the same buffer. On the other hand, transcutol as a pure vehicle did not show a significant drug permeation except after 36 h which was very low compared with its mixtures with di-

Table 1 Solubility study of fenoterol in different solvents.

Solvent type	Solubility mg/ml ± SD
Water	61.45 ± 3.65
Transcutol	103.0 ± 8.97
PEG 300	118.5 ± 11.23
Propylene glycol	136.0 ± 13.23
Dimethyl sulfoxide (DMSO)	170.2 ± 19.68
Ethyl acetate	0.07 ± 0.015
Dimethyl isosorbide (DMI)	9.05 ± 1.24
Oleic acid	0.0284 ± 0.009
90% PG in Buffer	97.96 ± 19.4
10% PG in Buffer	70.28 ± 12.48
DMI/Transcutol (1:1)	39.43 ± 13.72
Oleic acid/Transcutol (1:1)	8.68 ± 4.49

Table 2 Permeation parameters of different fenoterol liquid systems through guinea pig skin.

Liquid formulae	SSF μg cm^{-2} h^{-1} ± SD	Q_{24} μg cm^{-2} ± SD	t_{lag} h	Permeability coefficient cm h^{-1} × 10^{-3}	r^{2*}
PEG 300 $n = 5$	0.257 ± 0.103	11.6 ± 8.9	0.44	0.042	0.9924
PG $n = 4$	0.338 ± 0.224	22.82 ± 11.11	0.989	0.056	0.948
Transcutol $n = 4$	0.13 ± 0.08	13.37 ± 8.92	0.436	0.021	0.9967
90% PG $n = 4$	14.319 ± 13.72	65.81 ± 45.43	1.824	2.386	0.9904
10% PG $n = 4$	9.191 ± 6.8	202.79 ± 46.78	0.391	1.53	0.9939
Transcutol:DMI $n = 5$	340.37 ± 88.0	262.496 ± 119.8	3.261	56.72	0.9237
Transcutol:Oleic acid $n = 6$	4645.76 ± 528.0	2004.04 ± 386.82	0.669	774.29	0.9915

All formulas between the same parentheses are not significantly different from each other in the Q_{24} data but differ significantly from those included in other ($P < 0.0001$).
(PEG 300, PG, Transcutol, 90% PG, 105PG), (Transcutol:DMI), (Transcutol:Oleic acid).
* The r^2 describes only the linear portion in the graphs.

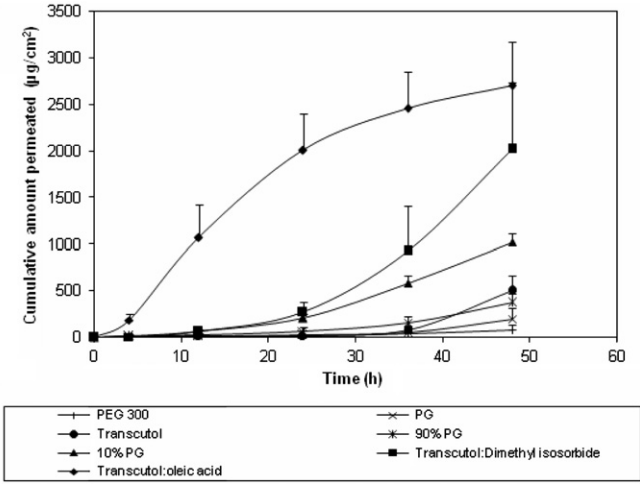


Fig. 1 Permeation of fenoterol from different liquid formulation through guinea pig skin.

methyl isosorbide in 1:1 ratio and transcutol: oleic acid with the same ratio. However, the tried transcutol or its mixtures with dimethyl isosorbide and oleic acid at 1:1 ratio exhibited a drug permeation profile that could be arranged in an ascending order as such: transcutol, transcutol: dimethyl isosorbide mixture and transcutol: oleic acid mixture.

It was remarkable that, the higher the drug solubility in each of pure propylene glycol, transcutol and polyethylene glycol 300, the lower the drug permeation through animal skin quantified as cumulative amounts of drug permeated after 24 h (Q_{24}). However, blends of 90% and 10% propylene glycol with phosphate buffer showed a noticeable increase in the cumulative amounts of the drug permeated after 24 h (Q_{24}). Furthermore a high extent of drug permeation was recorded with blends of transcutol/ dimethyl isosorbide and transcutol/ oleic acid at 1:1 ratio where the latter showed the highest value (2004.04 μg cm^{-2}) compared with others. It was obvious as well that, transcutol: oleic acid mixture was shown to be approximately ten times more effective than 10% PG in buffer with regard to the cumulative amount of fenoterol permeated after 24 h (Q_{24}).

With relation to the permeability coefficients of fenoterol in the tested liquid preparations shown in Table 2, it was obvious that, the highest reached permeability coefficient of fenoterol was shown by transcutol/oleic acid mixture in 1:1 ratio that amounted to be 774.3 cm h^{-1} × 10^{-3}. The latter finding would indicate the superiority of drug diffusivity when placed in transcutol/ oleic acid mixture of 1:1 ratio as measured by Q_{24}, SSF and permeability coefficient. This finding might be attributed to the excellent solubilizing power of transcutol for fenoterol in oleic acid which aided the diffusivity of the drug in this system. Also, the increased permeation parameters of fenoterol from transcutol–oleic acid binary mixture might be explained by the limited solubility of fenoterol in oleic acid that is amounted to be 0.028 mg/ml [9–11]. Almirall et al. [9] noticed that, increasing the drug solubility in the vehicle will lower its thermodynamic activity in this vehicle and hence decreases the drug permeation through the skin.

Furthermore, PG tried in 10% in isotonic phosphate buffer showed higher value of (Q_{24}) for fenoterol than 90% of the same solvent because it was evident from the solubility study that, the drug has a higher solubility in PG than water. Also, higher percentage of water opens up the compact structure of the horney layer [12].

Differential scanning calorimetry of fenoterol and its physical mixture with adhesive

The DSC thermograms (figure not shown) of fenoterol and Duro-Tak declared that, fenoterol exhibited an exothermic peak at 240.39 °C while Duro-Tak declared an exothermic peak at 91.95 °C. The physical mixture consisting of fenoterol: Duro-Tak with 1:1 ratio showed the aforementioned original peaks with no evidence of any interaction between the drug and the adhesive.

Effect of fenoterol concentration on its permeation through hairless guinea pig skin

The influence of different fenoterol concentrations viz., 2%, 5%, 9% and 12% on its permeation across hairless guinea pig skin was shown in Table 3 and graphically represented in Fig. 2. It was obvious that, the cumulative amount of fenoterol permeated per unit area after 24 h showed a gradual increase upon increasing drug concentration, where, 12% of fenoterol in a matrix composed of 10% oleic acid or *cis*-oleic acid with 90% Duro-tak exhibited the highest values.

It was noteworthy that, the use of 12% of fenoterol together with 10% of *cis*-oleic acid instead of oleic acid appreciably increased Q_{24}, steady state flux and permeability coefficient

Table 3 Permeation parameters of fenoterol transdermal patches using different drug concentrations through guinea pig skin.

Drug Conc.	SSF μg cm^{-2} h^{-1} ± SD	Q_{24} μg cm^{-2} ± SD	t_{lag} h	Permeability coefficient cm h^{-1} × 10^{-3}	r^{2*}
2% in oleic acid $n = 6$	2.438 ± .083	8.29 ± 3.48	8.59	1.52	0.9999
5% in oleic acid $n = 6$	35.05 ± 6.47	21.74 ± 8.0	11.5	21.9	0.9990
9% in oleic acid $n = 6$	141 ± 23.68	45.64 ± 20.81	10.88	88.12	0.9920
12% in oleic acid $n = 5$	1191 ± 20.4	247.34 ± 55.9	5.74	744.37	0.9915
12% in *cis*-oleic acid $n = 6$	1435.7 ± 48.88	266.459 ± 87.78	5.86	897.31	0.9900

All formulas between the same parentheses are not significantly different from each other in the Q_{24} data but differ significantly from those included in other. ($P < 0.0001$).
(2%, 5% and 9% in oleic acid), (12% in oleic acid, 12% in *cis*-oleic acid).
* The r^2 describes only the linear portion in the graphs.

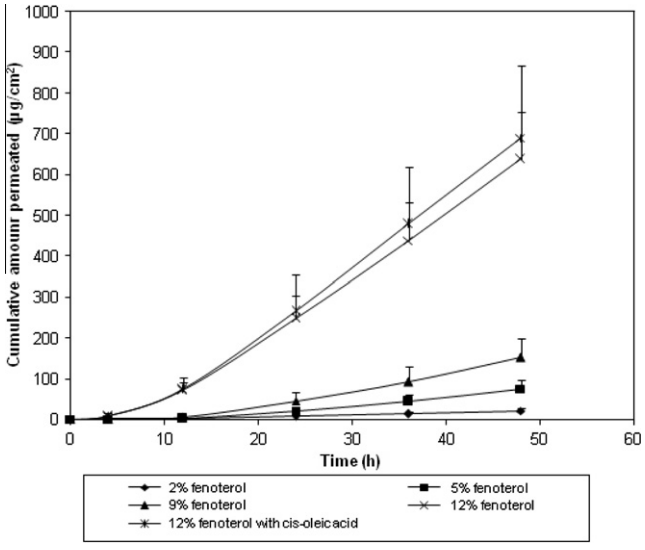


Fig. 2 Permeation of fenoterol from different concentration patches.

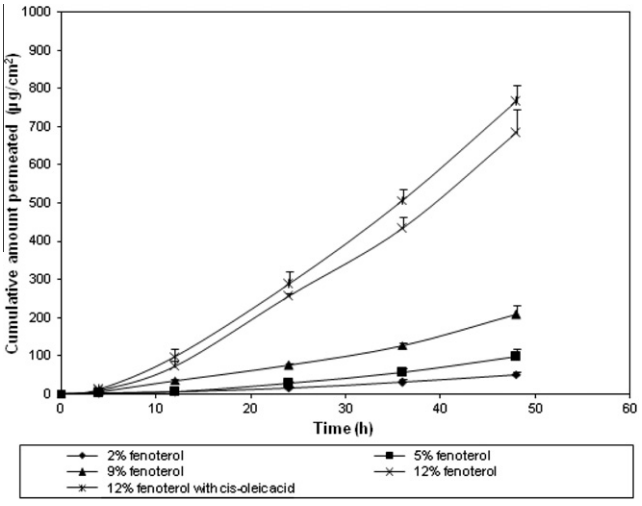


Fig. 3 Permeation of fenoterol from different concentration patches after pretreatment the skin with oleic acid.

but without variability in the lag time value. This would indicate the permeation benefit of *cis*-oleic acid over oleic acid. This finding could be attributed to the higher ability of *cis*-oleic acid to penetrate the skin in a faster way rather than normal oleic acid and this may be due to the lower hindrance effect of its chemical structure.

Effect of skin pretreatment with oleic acid on fenoterol permeation

It was obvious from Table 4 and Fig. 3 that, the pretreatment of guinea pig skin with oleic acid resulted in a remarkable increase in drug permeation in terms of Q_{24} all over the different drug concentrations in patches compared with the untreated skin shown in Table 3. The highest extent of drug permeation occurred as a result of pretreatment of guinea pig skin with oleic acid was recorded with 2% fenoterol patches which was double the quantity of Q_{24} without treatment.

One way to increase drug penetration is to pre-treat the skin with the enhancers before drug application. Another possibility is to add the enhancer to the drug formulation, but this may prolong the lag time of drug penetration depending on the partitioning delay and the subsequent effect of the enhancer in the skin [13].

Table 4 Permeation parameters of fenoterol transdermal patches after guinea pig skin treatment with oleic acid using different drug concentrations.

Drug Conc.	SSF μg cm^{-2} h^{-1} ± SD	Q_{24} μg cm^{-2} ± SD	t_{lag} h	Permeability coefficient cm h^{-1} × 10^{-3}	r^{2*}
2% in oleic acid $n = 6$	7.72 ± 0.078	16.26 ± 4.33	7.26	4.825	0.9913
5% in oleic acid $n = 6$	44.09 ± 1.22	26.92 ± 6.95	10.088	27.55	0.9923
9% in oleic acid $n = 6$	49.88 ± 0.189	75.3 ± 5.96	3.516	31.175	0.9957
12% in oleic acid $n = 6$	1627.3 ± 48.75	255.19 ± 9.73	7.17	1017.06	0.9999
12% in *cis*-oleic acid $n = 6$	1901.8 ± 64.78	285.88 ± 35.47	6.6	1188.6	0.9979

All formulas between the same parentheses are not significantly different from each other in the Q_{24} data but differ significantly from those included in other ($P < 0.0001$).
(2% and 5% in oleic acid), (9% in oleic acid), (12% in oleic acid), (12% in *cis*-oleic acid).
* The r^2 describes only the linear portion in the graphs.

The enhancing effect of oleic acid on fenoterol permeation went parallel with that recorded with Cooper and Williams [14–17], who mentioned that, fatty acids like oleic acid are known to have a potent skin permeation enhancing effect when used alone or in combination with glycols. The permeation enhancing effect of oleic acid could be attributed to the disruption of the lipid bi-layer that is filling the extra-cellular spaces of stratum corneum.

Barry [18] and Kin [19] postulated that oleic acid increases the fluidity of skin liquid while Ongpipattanakul et al. [20] mentioned that, oleic acid forms a fluid phase within the stratum corneum which enhances drug permeation.

Effect of different enhancers on fenoterol permeation from transdermal patches

The permeation parameters reached by mathematical calculations are compiled in Table 5 and Fig. 4. It was obvious that, 12% of fenoterol patches in a matrix of Duro-tak containing 10% of carvone as permeation enhancer diminished the extent of fenoterol permeation quantified as Q_{24} by almost 40% in comparison with control patches. Furthermore, a pronounced decrease in steady state flux, lag time and permeability coefficient were observed which went parallel with the diminishing in drug diffusion.

On the other hand, the incorporation of 10% of α-pinene, cineole and limonene in fenoterol-Duro-tak patches improved the extent of drug permeability (Q_{24}) where, limonene was the highest (17.62 μg cm^{-2}) and α-pinene was the lowest (10.09 μg cm^{-2}) in this regard. However, there was a decrease in the steady state flux as well as the lag time and the permeability coefficient for patches containing α-pinene compared with the control patches.

Jain et al. [21] suggested that the main barrier or the rate limiting step in transdermal drug delivery of polar, water soluble drugs is the lipophilic part of subcutaneous layer in which lipids (ceramides) are arranged in a bilayer. Ceramides are tightly packed in the bilayer due to the high degree of hydrogen bonding. The amide group of ceramide is hydrogen bonded to amide group of another ceramide and a tight network of hydrogen bonding is formed at the head of ceramides. This hydrogen bonding provides strength and stability to lipid bilayer and imparts the barrier property to the subcutaneous layers.

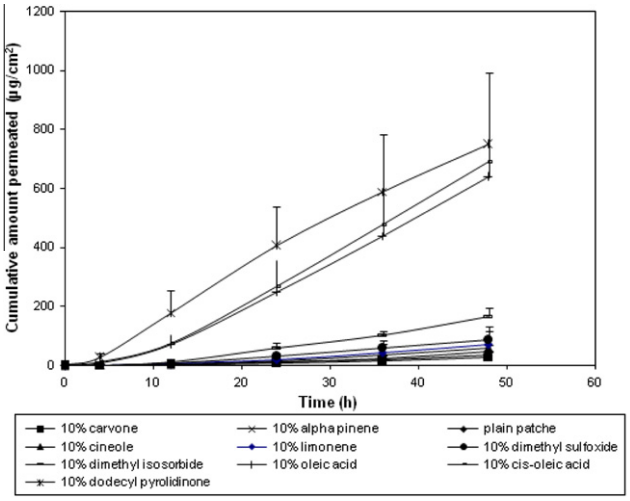


Fig. 4 Permeation of fenoterol from patches using 10% different enhancers.

Recently; it has been proved that amide 1 of ceramide 5 is laterally hydrogen bonded between head of ceramides [22].

Cineole has a greater enhancing effect than carvone because the boiling point of cineole is 173 °C which is less than carvone (230 °C) indicating that cineole has a weak cohesion or self association than carvone [23]. So, the oxygen of functional ether in cineole is in free form for interaction facilitating hydrogen bond formation. Therefore, the energy required for competitive hydrogen bonding in skin ceramides is relatively

Table 5 Permeation parameters of 12% fenoterol transdermal patches using 10% concentration of different enhancers through guinea pig skin.

Drug Conc.	SSF μg cm^{-2} h^{-1} ± SD	Q_{24} μg cm^{-2} ± SD	t_{lag} h	Permeability coefficient. cm $h^{-1} \times 10^{-3}$	r^{2***}	Enhancement Ratio (ER) Q24
(R)-(−)-Carvone $n = 6$	0.312 ± 0.02	6.12 ± 3.1	3.65	0.195	1	0.63
α-Pinene $n = 5$	3.6 ± 0.2	10.09 ± 7.8	7.85	2.25	0.9800	1.05
Plain Duro-tak® (control) $n = 3$	13.95 ± 2.6	9.6 ± 3.76	11.83	8.71	0.9764	Control
Cineole $n = 4$	48.94 ± 0.5	15.97 ± 4.5	15.64	30.58	0.9940	1.66
(R)-(+)-Limonene $n = 4$	77.5 ± 27.2	17.62 ± 13.3	16.27	48.43	0.9980	1.83
DMSO* $n = 3$	39.99 ± 17.1	31.4 ± 10.05	8.6	24.99	0.9979	3.27
DMI** $n = 4$	123.17 ± 1.7	57.06 ± 17.6	8.7	76.98	0.9999	5.94
Oleic acid $n = 5$	1191 ± 20.4	247.34 ± 55.9	5.74	744.375	0.9915	25.76
Cis-oleic acid $n = 6$	1435.7 ± 48.8	266.45 ± 87.7	5.86	897.31	0.9900	27.75
1-Dodecyl-2-pyrrolidinone $n = 6$	951.64 ± 9.2	404.69 ± 133.3	2.64	594.77	1	42.15

All formulas between the same parentheses are not significantly different from each other in the Q_{24} data but differ significantly from those included in other. ($P < 0.0001$).
(Carvone, pinene, cineole, limonene, DMSO, DMI), (Oleic acid, *cis*-Oleic acid), (1-dodecyl-2-pyrrolidinone).
* Dimethyl sulfoxide.
** Dimethyl isosorbide.
*** The r^2 describes only the linear portion in the graphs.

less for cineole than carvone. On the other hand, in case of carvone, additional energy is required to free the respective functional group from strong self-association, as reflected by higher boiling point. It has been reported that α-pinene and limonene caused a shift in transition temperature of the skin [16,24]. In other words, limonene is not able to break the hydrogen bond network between ceramides due to absence of a hydrogen bond accepting or donating group.

Pertaining to the influence of non-terpene type enhancers in 10% concentration viz., dimethyl sulfoxide (DMSO), dimethyl isosorbide (DMI), oleic acid, *cis*-oleic acid and 1-dodecyl-2-pyrolidinone (DPY), it was obvious from Table 5 that, all of them improved the drug permeation through hairless guinea pig skin compared to the plain patch.

Table 5 shows the extent of drug permeation in terms of the cumulative amount of fenoterol permeated in 24 h (Q_{24}) where, the greatest enhancement of drug permeation was achieved by the DPY (404.69 μg. cm^{-2}) while the lowest value was given by DMSO (31.4 μg cm^{-2}).

The permeation enhancement offered by 1-dodecyl-2-pyrolidinone (DPY) was shown to be 40 times as effective as the control patches without enhancer and 12 times as effective as Duro-tak patches containing 10% DMSO as an enhancer.

On the other hand, it was evident from Table 5 that, the steady state flux and the permeability coefficients were increased to an appreciable extent upon the inclusion of 10% of each of DMSO, DMI, oleic acid and *cis*-oleic acid. However, 1-dodecyl-2-pyrolidinone (DPY) showed a lower extent of steady state flux than oleic and *cis*-oleic acid only. Furthermore, the implication of the aforementioned enhancers in the drug patches in 10% concentrations resulted in shortening the lag time of the drug permeation where, the lowest value (2.64 h) was given by 1-dodecyl-2-pyrolidinone. This would indicate a greater rapidity in drug permeation in presence of all the tried enhancers where, DPY superseded the others in this regard.

In conclusion, 1-dodecyl-2-pyrolidinone (DPY) in 10% concentration showed the highest enhancement ratio compared with the other tried enhancers while, DMSO showed the lowest value compared with others in 10% concentration.

It is worthnoting that, 1-dodecyl-2-pyrolidinone (DPY) in 10% concentration showed the highest Q_{24} and the shortest lag time among the all tested terpenes or non-terpenes enhancers in 10% concentration.

Menczel et al. [25] suggested that, dimethyl sulfoxide (DMSO) and dimethyl isosorbide (DMI) disrupt stratum corneum lipid organization, making it permeable. The essential action increases the drug diffusion coefficient. The accelerant molecules jump into the bilayer, rotating, vibrating and translocating forming micro cavities and increasing the free volume available for drug diffusion.

As discussed before, fatty acids like oleic acid are known to have a potent skin permeation enhancing effect when used alone or in combination with glycols [15,26].

These effects appear to involve the disruption of lipid bilayer that is filling the extra cellular spaces of stratum corneum. It has been postulated to act by increasing the fluidity of skin liquid [18,19] and by forming a fluid phase within the stratum corneum [20].

As concluded from the previous results, it is obvious that *cis*-oleic acid has more enhancing effect than oleic acid and this can be explained to the higher ability of *cis*-oleic acid to penetrate the skin rather than normal oleic acid and this may be due to less hindrance effect of the chemical structure of *cis*-oleic acid.

From the previous results, DPY was shown to be a superior enhancer over all tested enhancers showed the shortest lag time that amounted to be 2.64 h and the highest enhancement ratios.

The superiority of DPY as permeation enhancer might be attributed to its nature as being azone derivative. The ring size of the lactam derivative increased the enhancer activity. A direct trend between ring size and permeability coefficient was identified. DPY which is five membered lactam moieties showed greater transdermal enhancement activity compared to the 6- and 7-membered (azone) lactam moieties [27].

Several authors have investigated Azone analogues and have shown that pyrolidinone and piperidinone analogues increase percutaneous absorption of various drugs [28,29].

Conclusion

In conclusion, 1-dodecyl-2-pyrrolidnon (DPY) was shown to be exclusively the best enhancer for fenoterol when tried in 10% concentration. Also, carvone was shown to be the worst enhancer for drug permeation since it diminished the drug permeation when tried in 10% concentration compared with the control Duro-tak® patches.

References

[1] Davis SS, Illum L, Tamlinson E. Enzymatic barriers to peptide and protein absorption and the use of penetration enhancers to modify its absorption. In: Lee VHL, editor. Delivery Systems for Peptide Drugs. New York, NY: Plenum Press, Dekker; 1986. p. 389–416.

[2] Wohlrab J, Kreft B, Tamke B. Skin tolerability of transdermal patches. Expert Opin Drug Deliv 2011:21, Epub ahead of print.

[3] Burkoth T, Bellhouse B, Hewson G. Transdermal and transmucosal powdered drug delivery. Crit Rev Ther Drug Carrier Syst 1999;16(4):331–84.

[4] Squillante E, Nanda A, Needham TE, Zia H. Transdermal delivery of calcium channel blockers such as nifedipine. US Patent 2000;6(106):856.

[5] Mitragorti S, Kost J. Low frequency sonophoresis. A non-invasive method of drug delivery and diagnostics. Biotechnol Prog 2000;16:448–92.

[6] Sears MR. The evolution of beta2-agonists. Respir Med 2001;95:S2–6.

[7] Hochhaus G, Schmidt EW, Rominger KL, Mollmann H. Pharmacokinetic/dynamic correlation of pulmonary and cardiac effect of fenoterol in asthmatic patients after different routes of administration. Pharm Res 1992;9:291–7.

[8] Lin W J, WU TL. Modification of the initial release of a highly water-soluble drug from ethyl cellulose microspheres. J Microencapsul 1999;16(5):639–46.

[9] Almirall M, Montana J, Escribano E, Obach R, Berrozpe J. Effect of d-limonene, alpha-pinene and cineole on *in vitro* transdermal human skin penetration of chloropromazine and haloperidol. Arzneim-Forsch Drug Res 1996;46(7):678–80.

[10] Obata Y, Takayania K, Maitani Y, Machida Y, Nagai T. Effect of pretreatment of skin with cyclic monoterpenes on penetration of diclofenac in hairless rat. Biol Pharm Bull 1993;3:312.

[11] Minghetti P, Cilurzo F, Casiraghi A, Montanari L, Fini A. Ex vivo study of transdermal permeation of four diclofenac salts from different vehicles. J Pharm Sci 2007;96(4):814–23.

[12] Menon GK. New insights into skin structure: scratching the surface. Adv Drug Deliv Rev 2002;54(Suppl. 1):S3–S17.

[13] Koskela RV, Hirvonen J, Monkkonen J, Kiesvorra J. Transdermal delivery of Levosimendan. Eur J Pharm Sci 2000;11:343–50.
[14] Cooper ER. Increased skin permeability for lipophillic molecules. J Pharm Sci 1984;73:1153–6.
[15] Williams AC, Barry BW. Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. Pharm Res 1991;8(1):17–24.
[16] Yamane MA, Williams AC, Barry BW. Terpene penetration enhancers in propylene glycol/water co-solvent systems effectiveness and mechanism of action. J Pharm Pharmacol 1995;47(12A):978–89.
[17] Pierre MB, Ricci Jr E, Tedesco AC, Bentley MV. Oleic acid as optimizer of the skin delivery of 5-aminolevulinic acid in photodynamic therapy. Pharm Res 2006;23(2):360–6.
[18] Barry BW. Mode of action of penetration enhancers in human skin. J Control Release 1987;6:85–97.
[19] Kin CK, Kin JJ, Chi SC, Skim CK. Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. Int J Pharm 1993;99:109–18.
[20] Ongpipattanakul B, Burnette RR, Russell OP, Francoeur ML. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res 1991;8:350–4.
[21] Jain AK, Thomas NS, Panchagnula R. Transdermal drug delivery of imipramine hydrochloride. J Control Release 2001;79(1–3):93–101.
[22] Moore DJ, Rerek ME. Insights into the molecular organization of lipids in the skin barrier from infrared spectroscopy studies of stratum corneum lipid models. Acta Derm Venereol Suppl 2000;20:16–22.
[23] Martin A, Bustamante P. State of matter. In: Physical pharmacy, B.I., Waverly, New Delhi, 1996. p. 22-52.
[24] Cornwell PA, Barry BW, Bauwstra JA, Gooris GS. Modes of action of terpene penetration enhancers in human skin, differential scanning calorimetry, small-angle x-ray diffraction and enhancer uptake studies. Int J Pahrm 1996;127:9–26.
[25] Menczel EM. Delipidization of the cutaneous permeability barrier and percutaneous penetration. In: Smith, EW, Percutaneous Penetration Enhancers. Boca Raton, FL: CRS Press; 1995. p. 383–392.
[26] Williams A, Barry B. Skin absorption enhancers. Crit Rev Ther Drug 1992;9:305–25.
[27] Fuhrman Jr LC, Michniak BB, Behl CR, Malick AW. Effect of novel penetration enhancers on the transdermal delivery oh hydrocortisone: an *in-vitro* species comparison. J Control Release 1997;45:199–206.
[28] Michniak BB, Player MR, Chapman JM, Sowel JW. Azone analogs as permeation enhancers: effect of different vehicles on hydrocortisone acetate skin permeation and retention. J Control Release 1997;32:147–54.
[29] Banweer J, Pandey S, Pathak A. Formulation, optimization and evaluation of matrix type transdermal system of lisinopril dihydrate using permeation enhancers. J Pharm Res 2008;1(1):16–22.