Iranian Journal of Pharmaceutical Sciences
2018: 14 (1): 97-110
www.ijps.ir

Original Article

Influence of Chemical Permeation Enhancers on the *in vitro* Skin Permeation of Minoxidil through Excised Rat Skin: A Mechanistic Study

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Abstract

Minoxidil, a pyrimidine derivative (2, 4-diamino-6-piperidinopyrimidine-3-oxide) is the only topical medical treatment with proven efficacy for the treatment of androgenic alopecia that showed low skin penetration and bioavailability. The main aim of this research was to investigate the effect of some permeation enhancers on the *in vitro* skin permeability of minoxidil. Minoxidil permeability experiments through rat skin pretreated with some of permeation enhancers namely, Urea, Eucalyptus oil and Menthol were performed in fabricated Franz diffusion cells and compared with hydrated rat skin as control. The permeability parameters evaluated include steady-state flux (J_{ss}), permeability coefficient (Kp), and diffusion coefficient (D). The penetration enhancer's permeability enhancement mechanisms were investigated by comparing of changes in peak position and their intensities of asymmetric (Asy) and symmetric (Sym) C-H stretching, C=O stretching, C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance using Fourier transform infrared spectroscopy (FTIR), as well as by comparing mean transition temperature (Tm) and their enthalpies (ΔH) using differential scanning calorimetry (DSC). Minoxidil permeability parameters through rat skin, were evaluated with and without chemical enhancers such as Eucalyptus oil, menthol, and urea. The skin showed barrier for minoxidil permeability through whole skin and that diffusion into the skin was the rate-limiting step for drug flux. Urea, Eucalyptus oil, and Menthol were the most effective enhancers as they increased flux 1.86, 2.16, and 1.75 times and diffusion coefficient 3.25, 1.34 and 2.16 folds in comparison with hydrated skin, respectively. FTIR and DSC results showed lipid fluidization, extraction, disruption of lipid structure and irreversible denaturation of proteins in the SC layer of skin by permeation enhancers.

Key words: minoxidil, percutaneous absorption, chemical enhancers, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR)

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Cite this article as: Moghimipour E, Salimi A, Talebpour Jahromi A, Influence of Chemical Permeation Enhancers on the *in vitro* Skin Permeation of Minoxidil through Excised Rat Skin: A Mechanistic Study. Iranian Journal of Pharmaceutical Sciences, 2018, 14 (1): 97-110.

1. Introduction

Permeation of drugs through skin is the basis of transdermal delivery. Transdermal drug delivery is associated with some advantages such as controlled and continuous drug delivery which is important for drugs with short biological half-life and low therapeutic indices, first-pass intestinal and hepatic bypass, avoidance of gastrointestinal irritation which is common with oral medications, and facilitation of drug localization at target site [1].

Two main steps in skin permeation are partitioning and diffusion through the stratum corneum and viable epidermis, passage into the dermis and finally, systemic absorption or penetration into deeper tissues. The greatest barrier to drug penetration is the stratum corneum, the outermost layer of the skin [2]. The stratum corneum poses a formidable challenge to drug delivery systems. Several approaches have been used to improve entry of drugs into lower skin layer and deeper tissues. Chemical and physical permeation enhancers have been designed to facilitate delivery of high drug concentrations across the skin into systemic circulation or deeper tissues. The classes of enhancers used and the mode of action of these agents vary [3]. Increased drug diffused in the skin, stratum corneum lipid fluidization, and increase in thermodynamic activity of drug in the skin and vehicles, as well as effect on drug partition coefficient, are the most common mode of action of chemical enhancers. Minoxidil, a pyrimidine derivative (2, 4-diamino-6-piperidinopyrimidine-3-oxide) is the only topical medical treatment with proven efficacy for the treatment of Androgenic alopecia. Little is known of the effect of minoxidil on normal human hair growth and studies have been limited mainly to the response of androgenic alopecia to topical minoxidil. The drug stimulates hair growth in human by prolonging anagen through proliferative (by activating both ERK and Akt) and antiapoptotic (by increasing the ratio of Bcl-2/Bax) effects on dermal papilla cells (DPCs) of human hair follicles. Minoxidil have been reported for its poor skin penetration ability, which limits minoxidil usefulness as a potent drug in the use of hair growth treatment [4].

To develop transdermal drug delivery systems, the investigation of microstructure of intercellular or lipids in SC layer of skin is required. In the recent studies, organization of lipids and skin microstructure have been examined using various techniques including differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR)[5]. FTIR analysis of skin can be a practical tool for studying the interaction between chemical enhancers materials with SC that provides bands at different wave numbers [6].To recognize the mechanism by which the properties of

enhancers/retardants change in a given vehicle, molecular studies of whole rat skin were carried out using DSC and FTIR [7]. Several infrared spectral bands of the skin are attributed to vibration of protein and lipid molecules in the SC [8].The lipid vibration is a good index to evaluate the microstructure of the lamellar lipids form in the intercellular area in the SC layer . Some different spectral regions of the SC was expected to have various bands, -CH2 symmetric vibration (near 2850 cm-1) and –H_2asymmetric vibration (near 2920cm^{-1}) and amide I (about 1650cm^{-1}) and amide II (near 1550cm^{-1}) stretching vibrations of SC protein have been reported. An increase in wave number and width of –CH2 stretching peaks is due fluidization of the stratum corneum lipids [9]. If the shift is to higher wave number (blue shift), it indicates SC membrane (lipid bilayer) fluidization that in change contribute to the disruption of the barrier properties that probably causes the substance permeation enhancement through the SC [10].On the other hand, lipid groups are oriented again, a phenomenon that causes a shift to lower wave number (e.g. red shift) and strengthening of subcutaneous barrier properties which finally slows down the entrance of permeant through the skin [11]. If the penetration modifier performs by affecting on lipid pathway, the phase transition of the lipids is showed by increase/decrease in the band position (wavenumber) of the signals at 2920, 2850, and near1738 cm^{-1}[12].Thermal analysis methods such as differential scanning calorimetry (DSC) have been utilized to investigate thermal transitions in mammalian stratum corneum. The stratum corneum (SC) is the outermost layer of the epidermis and is primarily responsible for the skin's barrier function [13].

The DSC technique is widely used for characterization of melting lipids and the phase transition of lipid bilayers and protein denaturation in SC layer. To obtain more detailed information about lipid components and protein conformational stability of the whole skin rat treated with pure vehicle, a DSC study was programmed [14] .

By comparing mean transition temperature (T_m) and enthalpies (H), thermotropic behavior of treated skin was assessed. Any transition in T_m to lower degrees may be due to lipid disruption in bilayer and irreversible protein denaturation in SC, while, enthalpy decrease is generally related to lipid fluidization in lipid bilayers and protein–lipid complexes [15].

2. Materialsand Methods

2.1. Materials

Minoxidil was gifted from Sepidaj pharmaceutical company (Tehran, Iran). Eucalyptus oil, containing 70% 1, 8-cineole, was obtained from Barij Essence Iranian Company in Kashan (Iran), urea supplied by Merck. Potassium phosphate monobasic and menthol were purchased from Sigma. Water was deionized and filtered in-house. All other chemicals and reagents were of analytical grade.

2.2. Animal Experiments

Male Wistar rats weighing 200-250 g were used for *in vitro* permeation study. After sacrificing under ether anesthesia, the abdominal skin hair was carefully removed with an electric clipper and razor without damaging. The skin was excised and any extraneous subcutaneous fat was removed from dermal surface. Whole skin thickness was measured using a digital micrometer [17]. The animals were treated according to the principles for the care and use of laboratory animals and approval for the studies was given by the Ethical Committee (no.GP-94138) of Ahvaz Jundishapur University of Medical Sciences. The guidelines followed were those laid down by the National Academy of Sciences and published by the National Institutes of Health (U.S. Department of Health & human services, office of laboratory animal welfare)[16].

2. 3. Minoxidil Assay

Determination of the amount of minoxidil was carried out by UV spectroscopy method at λmax= 288 nm.

2.4. In Vitro Permeation Study

Especially designed diffusion cells with an effective area of approximately 3.46 cm^2, were used for the permeation studies. The volume of the receptor compartment was 25 mL. The whole skin samples, hydrated prior to use, were mounted between the donor and receptor compartments of the cells with the epidermis facing the donor medium. Minoxidil (2 %w/v), dissolved in the distilled water, was placed in the donor compartment and the receptor chamber was filled with phosphate buffer solution (pH 7) [17]. The diffusion cell was placed and clamped in a water bath 37 ± 0.05 ^{O}C on a magnetic stirrer with a heater. The receptor medium was stirred with a small magnetic bead at 300 rpm. At predetermined time intervals (0.5, 1, 2, 4, 5, 10, 12and 24h), 2 mL of the receptor medium was withdrawn and immediately replaced by an equal volume of fresh buffer. The samples were filtered and the permeated amount of minoxidil was determined by UV spectroscopy method at 288 nm [18].

2.5. Skin Samples Pretreatment Procedure

The fully hydrated samples were pretreated with putting 2 mL of each chemical permeation enhancer on the surface of skin in the donor phase for 4 hrs. The donor and receptor compartments were then washed with water and filled with aqueous solution of minoxidil and phosphate buffer (pH 7), respectively. Eucalyptus oil (containing 70 % 1, 8-cineole), urea and menthol were used as chemical permeation enhancers. The fully hydrated samples were used as controls. To minimize experimental errors arising from biological variability, each piece of skin was used as its own control [19].

2.6. Data Analysis

The cumulative amount of minoxidil permeated through unit area of the diffusion surface into the receptor was calculated and plotted as function of time. Steady state flux ($mg/cm^2.h$) was calculated from the linear

portion of the slope of the permeation curve. Permeability coefficient (K_p, cm/h) of minoxidil through the skin was calculated using Equation 1: [20]

$$K_p = \frac{J_{ss}}{C_v} \quad \text{…….. (equation 1)}$$

Where J_{ss} and C_v are steady state flux and initial concentration of minoxidil in receptor compartment, respectively.

Enhancement ratios (ER) were calculated from equation 2: [21]

$$ER = \frac{\text{permeability parameter after treatment}}{\text{permeability parameter before treatment}} \quad \text{…….. (equation 2)}$$

Statistical comparison was made using one-way ANOVA and $p<0.05$ was considered statistically significant. Correlation analysis was performed by least square linear regression method and correlation coefficient examined for significance by Student's t-test. All statistical analyses were conducted using MINITAB software version 17.0.

2.7. Differential Scanning Calorimeter (DSC)

The changes in structure of whole skin induced by permeation enhancers were examined using a DSC (Mettler-Toledo DSC1 System) equipped . The fully hydrated skin samples were first immersed in a chemical permeation enhancer for 4 h and the excess of the enhancer was blotted out before they were hermetically sealed to avoid evaporation of water [22]. Approximately, 6–10 mg of pretreated skin samples were placed in hermetically sealed aluminum pans. Simultaneously, an empty pan was used as a reference. The skin samples were repeatedly exposed to heat ranging from 20 to 200 ˚C scan rate: 5 ^{0}C/min. All experiments were repeated at least in triplicate. In order to ensure accuracy and repeatability of data, DSC analyzer was calibrated and checked with indium standard [12].

2.8. FT-IR Experiments

The excised rat skin samples were treated by urea, menthol, Eucalyptus oil for 24 hrs, vacuum dried (650 mm Hg, 25±1°C) for 30 minutes and stored in desiccators to remove traces of permeation enhancer [19] The skin samples scanned in the range of 4000 to 500 cm^{-1} using an FT-IR facility (Uker, Vertex70, Germany)[12].

3. Results and Discussion

3.1. Effect of Chemical Enhancers on Minoxidil Permeability

Permeability parameters after skin pretreatment with chemical enhancers compared with control and amount of minoxidil permeated from various enhancers across the rat abdominal skin are presented in table 1 and figure 1, respectively. Table 1 shows the effect of chemical enhancers on minoxidil permeability compared with control as ER_{flux} (ratio of drug flux after and before skin pretreatment with enhancer) and ER_D (drug diffusion coefficient after and before skin pretreatment with enhancer)[12]. Hydrated skin with no enhancer pretreatment

Table 1. Permeability parameters after pretreatment with permeation enhancers compared with control (Mean ± SD, n = 3).

parameter	J_{SS} (mg.cm-2.h-1)	D ($cm^2.h^{-1}$)	P($cm.h^{-1}$)	Tlag (h)	ER_{flux}	ER_D	ER_P
Control (Water)	0.0095±0.0001	0.059± 0.002	0.0001±0	0.91± 0.037	------	-----	-----
Menthol	0.0166± 0.0002	0.129± 0.041	0.0002±3.54	0.441± 0.141	1.75± 0.03	2.16± 0.6	1.747±0.029
Eucalyptus oil	0.017±0.001	0.194± 0.04	0.0002±0	0.285±0.059	2.16± 0.04	1.34± 0.23	1.863±0.02
Urea	0.02±0.0003	0.079±0.01	0.0002±4.419	0.685±0.091	1.86±0.03	3.25± 0.55	2.163±0.037

Table2. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (Mean ± SD, n = 3).

Permeation Enhancer	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester	
	Peak height	%D	Peak height	%D	Peak height	%D
Water	0.360±0.01	-	0.558±0.002	-	1.722±0.005	-
Urea	0.657 ±0.01	*N.D	0.428 ±0.015	23.2	2.011±0.05	N.D
Menthol	0.508±0.005	N.D	0.527±0.008	5.6	2±0.004	N.D
Eucalyptus oil	0	100	0	100	0	100

%Decrease in peak height(%D) =(peak height from untreated whole skin - peak height from solvent treated whole skin)/ peak height from untreated whole skin x 100

*N.D = Not Decrease in peak height

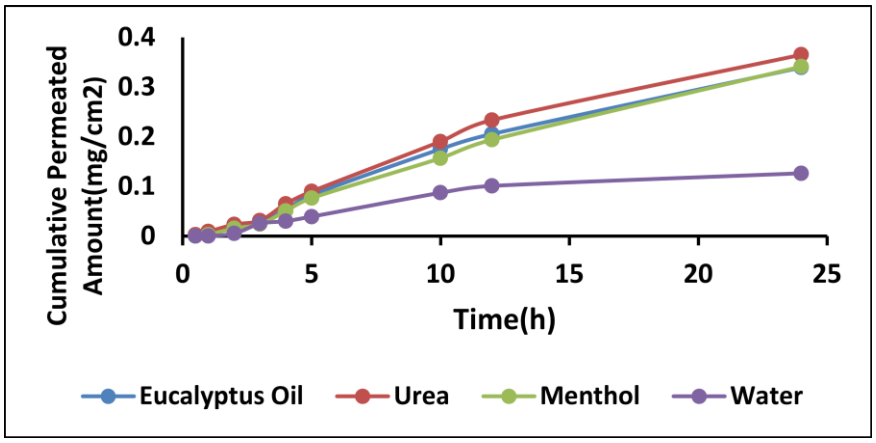
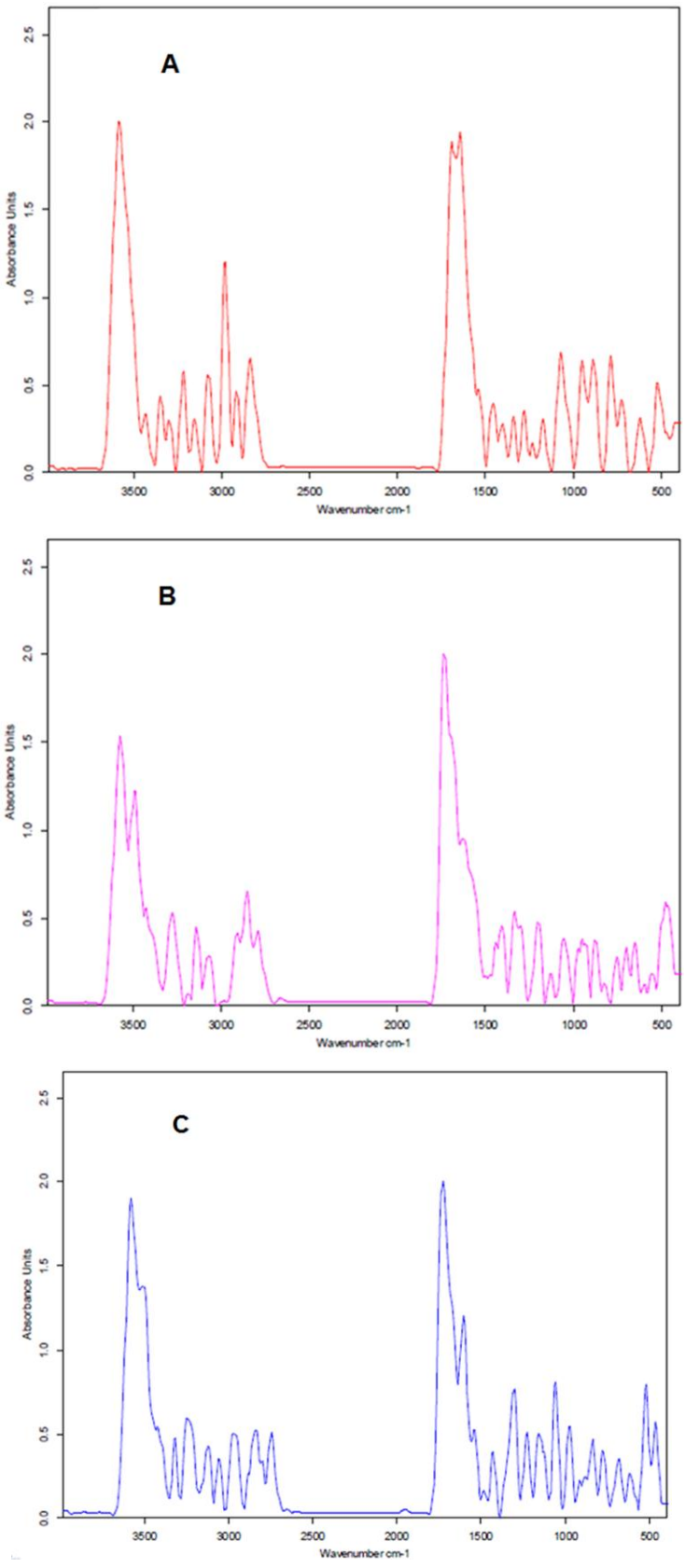


Figure 1. The amount of Minoxidil permeated from various pretreatment rat skins with various chemical enhancers.

and saturated aqueous solution of minoxidil as donor phase was utilized as control. The results indicate that eucalyptus oil, urea, and menthol significantly increased minoxidil flux



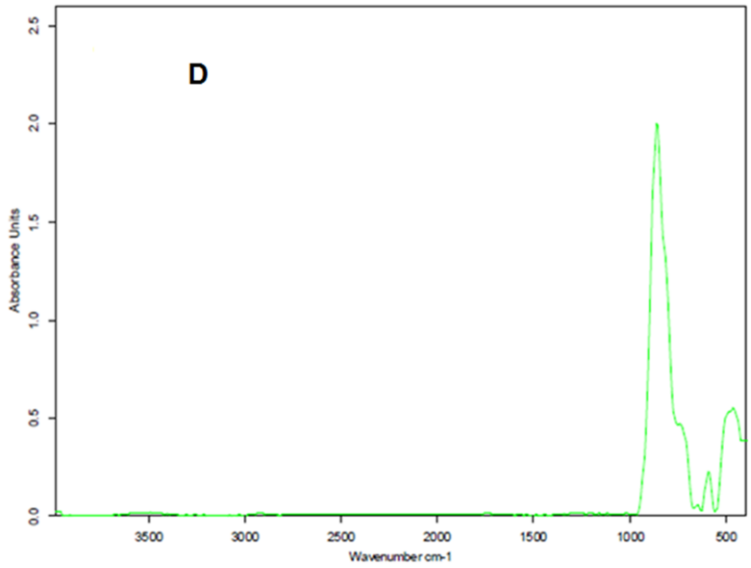


Figure 2. FTIR spectra of pretreatment skin rat with(A) water, (B)Urea, (C) menthol &(D) Eucalyptus oil.

and diffusion coefficient ($p < 0.05$). Urea provided the best enhancement of minoxidil flux, increasing it approximately up to 2.16-fold relative to control, followed by eucalyptus oil (1.86-fold) and menthol (1.74-fold). All of the chemical enhancers exerted significant effects on diffusion coefficient ($p < 0.05$), with eucalyptus oil showing the greatest enhancement effect on diffusion coefficient (up to 3.249 – fold) compared with control, followed by menthol (2.159-fold)and urea(1.344-fold).Also, all of the chemical enhancers showed significant effects on permeability coefficient($p < 0.05$), with urea showing the greatest enhancement effect on permeability coefficient (up to 2.163 – fold) compared with control, followed by eucalyptus oil l (1.863-fold)and menthol (1.474-fold). This observation indicates that the main mechanism of action of urea, menthol and eucalyptus enhancement activity is facilitation of drug partitioning and diffusion into skin.

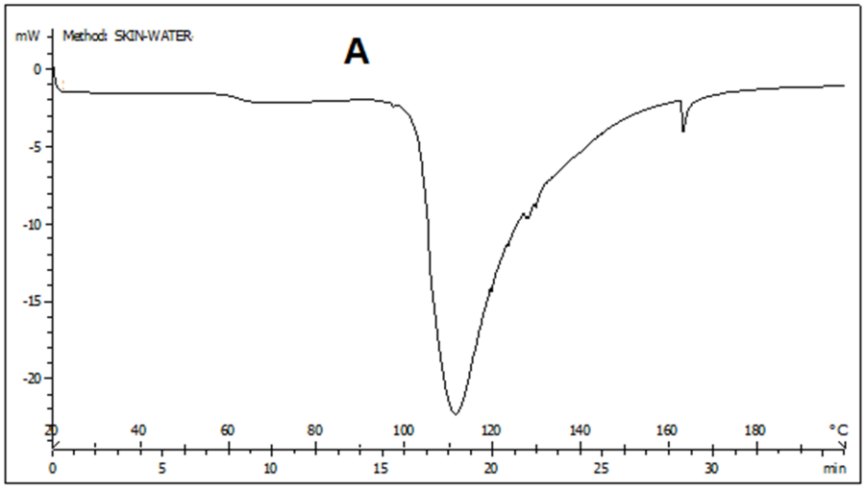
Eucalyptus oil consists of 75 % 1, 8-cineole. Cineole is a cyclic terpene that acts by creating liquid pools in stratum corneum and disrupting the lipid structure of the stratum corneum, thereby increasing the diffusion coefficient of polar and non-polar drugs in the membrane [23].

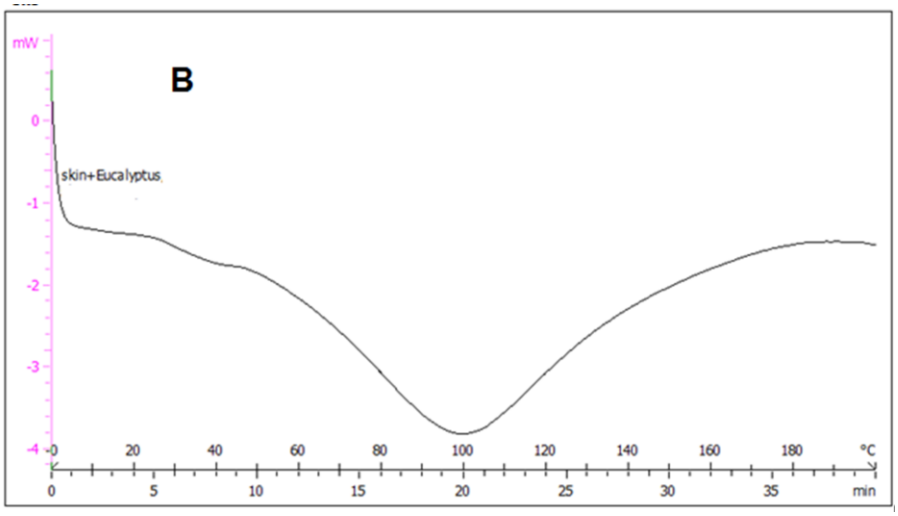
The keratolytic properties of urea and its derivatives are one reason for the modest penetration enhancement achieved by use of these compounds. The other reason for the enhancing effects of urea derivatives is the increase in the stratum corneum water content by these moisturizing agents. This may lead to hydrophilic diffusion channels within the barrier [24].

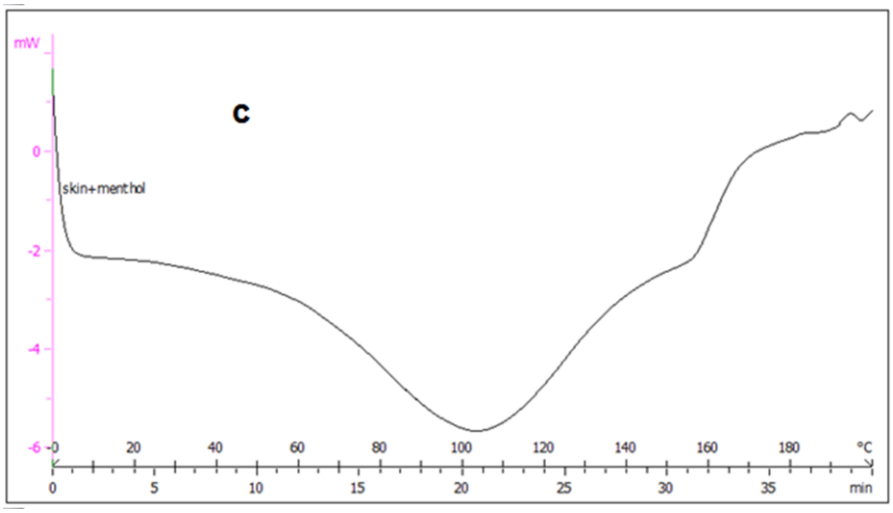
Urea and its analogues, which fall under this category, are usually used as permeation enhancers in solvents where they can have different effect on the skin based on the

solvent system chosen, but generally act by disrupting the skin lipids [25].

Urea promotes transdermal permeation by facilitating hydration of the stratum corneum and by the formation of hydrophilic diffusion channels within the barrier. Cyclic urea







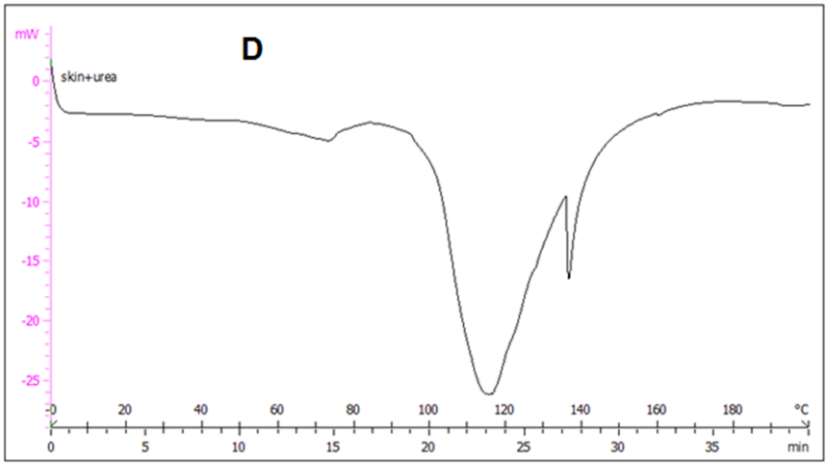


Figure 3. DSC Thermograms of pretreatment skin rat with (A) water,(B) Eucalyptus oil, (C) menthol &(D) Urea.

permeation enhancers are biodegradable and non-toxic molecules consisting of a polar parent moiety and a long chain alkyl ester group. As a result, enhancement mechanism may be a consequence of both hydrophilic activity and lipid disruption mechanism [26].

3.2. Differential Scanning Calorimetry (DSC)

Mean transition temperature (Tm) and their enthalpies (ΔH) were used to evaluate thermotropic behavior of treated skin. Table 5 shows transition temperatures and enthalpies while figure 3 shows thermograms. Tm_1 andTm_2 resulted from hydrated rat skin were 67.5 and 112°C, respectively, indicating melting of lipids and intracellular keratin irreversible denaturation. Any transition in Tm to lower degrees may be due to lipid disruption in bilayer and irreversible protein denaturation in SC. While, enthalpy decrease is generally related to lipid fluidization in lipid bilayers and protein–lipid complexes [15]. Kaushik *et al*. reported human dermal DSC graphs and observed three endothermic transition peaks at temperatures 59-63°C (Tm_1), 75-82°C (Tm_2), and 99.5-120°C (Tm_3)[16] .

They suggested that Tm_1 was due to transformation of lipid forms from a lamellar to disordered state, Tm_2 corresponds to

Table 3. FT-IR Peak wave numbers (cm^{-1}) changes compared to control (untreated skin) and abdominal hydrated whole skin rat following treatment with different enhancers. (mean±SD, n=3).

Permeation Enhancer	**C-H stretching Asy**	**C-H stretching Sym**	**C=O stretching of lipid ester**	**Amide I**	**Amide II**
Water	2914.94±0.11	2840.39±0.15	1691.38±0.2	1642.65±0.1	1460.63±0.14
Urea	2852.5±0.1	2791.86±0.09	1733.09±0.1	Deleted	Deleted
Menthol	2972.81±0.16	2838.36±0.1	1727.58±0.22	1603.87±0.21	1538.91±0.12
Eucalyptus oil	Deleted	Deleted	Deleted	Deleted	Deleted

Table 4. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (Mean ± SD, n = 3).

Permeation Enhancer	C=O stretching of keratin		C-N stretching of keratin	
	Peak height	%D	Peak height	%D
Water	1.94±0.11	-	0.414	-
Urea	0	100	0	100
Menthol	1.202±0.001	38	0.534±0.004	N.D
Eucalyptus oil	0	100	0	100

Table 5. Effect of permeation enhancer on the thermal properties of Exised rat skin (mean ± SD, n = 3).

Permeation Enhancer	Penetration Enhancer	Transition temperature (°C)	Transition enthalpy (mj/mg)	
	Tm1	Tm2	*ΔH1*	*ΔH2*
Water	67.5±2.1	112±6.5	7.01±0.3	521.35±10.5
Urea	71±0.1	113±0.2	22.1±0.1	397.5±2
Menthol	deleted	102±0.10	0	150.98±2.2
Eucalyptus oil	37±0.2	98±0.1	8.7±0.01	120.76±2.2

Tm1 *= mean transition temperature of lipids; SC* ***Tm2*** *= mean transition temperature of irreversible denaturation of intracellular stratum corneum keratin;* ***ΔH1*** *= transition enthalpy of lipid phase SC* ***ΔH2*** *= transition enthalpy of keratin phase SC*

protein-lipid [16] or the disruption of polar head groups of lipids and Tm_3 is known to occur during the proteins irreversible denaturation, respectively [16].

The thermograms of skin pretreated with menthol are presented lower Tm_2 and ΔH_1, ΔH_2 decreased in comparison with hydrated rat skin. In addition, Tm_1 was disappeared by menthol.

This finding indicates that menthol induced structural changing, increased lipid fluidity in intercellular region, lipid disruption in bilayer and irreversible protein denaturation in SC. The DSC results obtained from skin pretreated with eucalyptus oil shows lower Tm_1,Tm_2 and ΔH_1, ΔH_2 decreased in comparison with hydrated rat skin. This finding indicates that eucalyptus oil can increase skin permeation by more of mechanisms i.e.; lipid disruption in bilayer and irreversible protein denaturation in SC layer. The thermograms of skin pretreated with urea indicated $Tm_1$and Tm_2 shifted to higher melting points and ΔH_1 increased compared with control. This findings shows that urea induced lipid bilayer reorientation in SC layer. In addition, ΔH_2 decreased in comparison with control that represents lipid-protein complex fluidity in DC layer.

3.3. FT-IR Spectroscopy

Figure 2, table 2, table 3, and table 4 show spectral analysis of samples, regarding any change in position of peaks and also their intensities from intensities from 4000cm^{-1}-500cm^{-1}.If the shift is to higher wave number (blue shift), it indicates SC membrane (lipid bilayer) fluidization that in change contribute to disruption of the barrier properties that

probably causes the substance permeation enhancement through the SC [27].

On the other hand, lipid groups are oriented again, a phenomenon that causes a shift to lower wave number (e.g. red shift) and strengthening of subcutaneous barrier properties which finally slows down the entrance of permeant through the skin [11].

The spectra of menthol pretreated rat skin show changes in peak height and wave numbers. Red shift was observed skin pretreated with menthol at wave number (2972.81 cm^{-1}), indicating reorientation within the lipid groups that causes strengthening of SC barrier properties. Relatively red shift in 1727.580cm^{-1} band was observed in skin pretreated with menthol, indicating formation of strong hydrogen bonds within the lipid structures. Blue shift in which height of peak number (1603.87 cm^{-1}) was observed by skin pretreated with menthol, indicate irreversible denaturation of proteins in the SC layer. A 38% decrease in mean peak height of C=O stretching (Amide I) absorbance of Pretreated skin rat with menthol indicate interacts mainly with proteins in stratum corneum layer.

The FT-IR spectra of skin pretreated with eucalyptus oil is indicated disappearance at wave numbers and significant decreases (100%) in height of peaks in the 2914.94, 2840.39, 1691.38, 1642.65, and 1460.63 cm^{-1} wave numbers. The results show that eucalyptus oil interacts mainly with lipids and proteins in stratum corneum layer. ER_D results after skin pretreatment with eucalyptus oil correlate with FT-IR and DSC observations so that with eucalyptus oil interacts mainly with lipids and proteins in SC layer.

The FT-IR spectra for skin pretreated with urea indicates blue shifts with lowering of in peak height (23%) of the wave number (2791.86cm^{-1} ,) disappearance at wave numbers (1642.65, 1460.63 cm-1), and significant decreases(100%) . These findings show that urea interacts mainly with proteins and intracellular keratin irreversible denaturation in SC.

4. Conclusion

The results obtained indicate that the used permeation enhancers increased the drug permeability through the excised rat skin. Different mechanisms including lipid fluidization, disruption lipid structure, and also intracellular keratin irreversible denaturation in stratum corneum by Eucalyptus oil, Menthol, Urea are the main probable mechanisms for higher ER_{flux}, ER_D, and ER_P ratios compared with water skin hydrated.

Acknowledgement

This paper was derived from the Pharm D thesis (Talebpour, A) for which financial support was provided by Student Research Committee, Ahvaz Jundishapour University of Medical Sciences.

References

[1] Hadgraft J, Lane ME. Skin permeation: The years of enlightenment. Int J Pharm. (2005) 305:2-12.

[2] Singh P, Roberts MS. Skin permeability and local tissue concentrations of non-steroidal anti-

inflammatory drugs after topical application. J. Pharmacol. Exp Ther. (1994) 268: 144-151

[3] Ghosh TK, Banja AK. Methods of enhancement of transdermal drug delivery: part IIA, c.hemical permeation enhancers. Pharm Tech. (1993) 18: 62-90.

[4] Ogiso T, Shintani M. Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. J Pharm Sci. (1990) 70: 1065-1076.

[5] Salimi A, Moghimipour E, Rahmani F.Effects of the Various Solvents on the *In vitro* Permeability of Indomethacin through Whole Abdominal Rat Skin. Annual Research and Review in Biology. (2015) 5(4): 335-346

[6] Nikhil K, Dilip D, Jeetendra N. Effect of permeation enhancers on the release and permeation kinetics of meloxicam gel formulations through rat skin. Asian Journal of Pharmaceutical Sciences. (2008) 3(5): 193-199

[7] Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. European Journal of Pharmaceutical Sciences. (2001) 14(2): 101-114.

[8] Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: an update review. Current Drug Delivery. (2007) 4(4): 297-305.

[9] Barrie CF, Timothy M. Transdermal penetration enhancers: Applications, limitations, and potential. J Pharm Sci.(1999) 88(10) :955-958.

[10] Salimi A, Hedayatipour N, Moghimipour E. The Effect of Various Vehicles on the Naproxen Permeability through Rat Skin: A Mechanistic Study by DSC and FT-IR Techniques. Adv Pharm Bull. (2016) 6(1): 9-16.

[11] Boncheva M, Damien F, Normand V. Molecular organization of the lipid matrix in intact stratum corneum using ATR-FTIR spectroscopy. Biochimica et Biophysica Acta (2008) 1778(5):1344-55.

[12] Moghimipour E, Salimi A, Sharif Makhmal Zadeh B. Effect of the various solvents on the *in vitro* permeability of vitamin B_{12} through excised rat skin. Trop J Pharm Res. (2013) 12(5): 671-677.

[13] Kumar R, Philip A. Modified transdermal technologies: Breaking the barriers of drug permeation via the skin. Trop J Pharm Res. (2007) 6(1):663-44.

[14] Salimi A, Sharif Makhmal Zadeh B, Safavi G. Effect of formulation components on the *in vitro* skin permeation of microemulsion drug delivery system of piroxicam. Int Res J Pharm Appl Sci. (2013) 3(4):152-60.

[15] Kaushik D, Michniak-Kohn B. Percutaneous penetration modifiers and formulation effects: Thermal and spectral analyses. AAPS Pharm Sci Tech. (2010) 11(3):1068-83.

[16] Obata Y, Utsumi S, Watanabe H, Suda M, Tokudome Y, Otsuka M, *et al.* Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers. Int J Pharm. (2010) 389(1-2): 18-23.

[17] Moghimipour E, Salimi A, Eftekhari S. Design and characterization of microemulsion systems for naproxen. Adv Pharm Bull. (2013) 3(1):63-71.

[18] Sharif Makhmal Zadeh B , Salimi A , Tolabi H. Preparation and characterization of minoxidil loaded niosome carrier for effective follicular delivery. Int. J. Curr.Res.Chem.Pharma.Sci. (2014) 1(9): 203–210

[19] Moser K, Kriwet K, Naik A, Kalia YN, Guy RH. Passive skin penetration enhancement and its quantification *in vitro*. Eur J Pharm Biopharm. (2001) 52(2):103-112.

[20] Williams AC, Barry BW. Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. Pharm Res. (1991) 8(1): 17-24.

[21] Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. (2001) 14:101-114.

[22] Green PG, Guy RG, Hadgraft J. *In vitro* and *in vivo* enhancement of skin permeation with oleic and lauric acids. Int. J. Pharm. (1988) 48:103-111.

[23] Vaddi HK, Ho PC, Chan SY. Terpenes in propylene glycol as skin-penetration enhancers: Permeation and partition of haloperidol, fourier transform infrared spectroscopy, and differential scanning calorimetry. J Pharm Sci. (2002) 91(7): 1639-651.

[24] Williams AC, Barry BW: Penetration enhancers. Adv Drug Deliv Rev. (2004) 56:603–618.

[25] Karande P, Mitragotri S, Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochimica et Biophysica Acta (BBA) - Biomembranes. (2009) 1788(11) :2362–2373.

[26] Singh PB, Choudhury PK. Penetration enhancers for transdermal drug delivery of systemic agents. J Pharm Res. (2007) 6: 44-50.

[27] Songkro S. An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. Songklanakarin J Sci Technol. (2009) 31(3): 299-321.