Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.2024.21
Vol. 31, No. 3, 2024, 126-134

Original Article

Open Access
Publish Free

Effect of Various Herbal and Chemical Enhancers on Skin Permeability to Cetirizine: A Study of Changes in Rat Skin Cells

Saeed Mohammad Soleymani[1], Rahim Mombeini[2], Anayatollah Salimi[2,3*]

[1]Department of Clinical Pharmacy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

***Corresponding Author:** Anayatollah Salimi, Email: Anayatsalimi2003@yahoo.com

Abstract

Background: Cetirizine is a second-generation antihistamine with anti-allergy and anti-itching properties. The topical formulation of this medicine is used in androgenic alopecia treatment. Due to the hydrophilic nature of cetirizine, its skin absorption is negligible, so to increase its absorption, various enhancers were examined to see which can be used in the design of a topical formulation.

Methods: First, the skin was exposed to enhancers, including eucalyptus, menthol, Tween 80, propylene glycol, and oleic acid, for 1 or 2 hours. Then, the permeability parameters of the cetirizine solution and the structural changes of the skin after exposure to enhancers were analyzed by differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) techniques.

Results: The obtained results show that all used enhancers increased the permeability of the drug cetirizine compared to water. Various mechanisms, such as liquefaction of lipids, destruction of lipid structure, and irreversible denaturation of intracellular keratin, are involved in the increase in drug penetration caused by eucalyptus, mint, Tween 80, propylene glycol, and oleic acid.

Conclusions: The results showed that among the studied absorption enhancers, eucalyptus and Tween 80 had the strongest, and propylene glycol had the weakest absorption enhancement effect after 2- and 1-hour pre-contact, respectively.

Keywords: Enhanced absorption, Skin permeability, Cetirizine, DSC, FT-IR

Citation: Soleymani SM, Mombeini R, Salimi A. Effect of various herbal and chemical enhancers on skin permeability to cetirizine: a study of changes in rat skin cells. *Journal of Kerman University of Medical Sciences*. 2024;31(3):126–134. doi: 10.34172/jkmu.2024.21

Received: May 31, 2023, **Accepted**: August 20, 2023, **ePublished**: June 29, 2024

Introduction

Cetirizine is a second-generation antihistamine used to treat seasonal allergies, allergic rhinitis, hay fever, and allergic skin diseases, such as hives and insect bites. Cetirizine works by explicitly inhibiting H_1 receptors (a type of histamine receptor), and its difference with older antihistamines such as chlorpheniramine is that because it does not pass through the blood-brain barrier, it has a less soothing effect on the central nervous system and is less sleep-inducing (1-3). The topical application of cetirizine for treating androgenic alopecia has been mentioned in many articles (4). The hydrophilic nature of cetirizine can prevent its topical action (5,6).

The skin, the body's largest organ, is one of the most complex, interesting, and productive organs. It is a vital organ without which no creature can survive. The skin protects the body against mechanical damage, heat, and intense light. It also prevents the penetration of chemicals and the entry of microbes and microorganisms into the body. Moreover, it removes harmful substances from metabolic activities in the digestive system and liver (7).

The skin is a protective layer that performs various functions. The skin's primary barrier is the stratum corneum, the most superficial and outermost layer of the epidermis, consisting of 10 to 25 layers of dead cells. When a topical drug is placed on the skin, the active drug must pass through the stratum corneum and reach the living tissue. The limiting factor in this process is the slow penetration of the stratum corneum. In normal conditions, the main path is through intercellular spaces or bilayers. If the substance passes through the different layers of skin and enters the bloodstream, skin absorption has happened (8).

The skin's structure results in its high impermeability to foreign substances and drugs. Awareness of this structure guides us to optimize and increase drug delivery using prodrugs, chemical enhancers, iontophoresis, electroporation, and ultrasound waves, on which many practical arrangements and recent studies have been based (9).

© 2024 The Author(s); Published by Kerman University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Skin drug delivery has many advantages in comparison with other drug delivery methods, including elimination of the first hepatic metabolism, ease of use, and proper control over the speed of drug delivery. Unfortunately, the skin's natural structure acts as a significant barrier against the permeation of drugs (10,11). There are various strategies to increase skin drug delivery, which can be done using chemical enhancers, supersaturated drug delivery systems, electrical conduction of molecules into or through the skin using iontophoresis, physical destruction of the skin structure by electroporation or sonophoresis, or placement of drugs in viscous drug delivery systems (12).

Chemical enhancers are compounds that penetrate the skin. By interfering with the structures in the stratum corneum, they cause a reversible reduction of the skin's barrier effects. So far, nearly 400 chemical substances have been identified as enhancers, but due to their limitations, few are used in topical products (13).

Chemical enhancers have different mechanisms, which are mentioned below. For the first time, Barry et al. proposed the lipid protein-dispersibility (LPP) theory to justify the mechanism of the effect of chemical enhancers on the skin. According to this theory, enhancers will affect the skin permeation of drugs with three mechanisms:

a. Enhancers that affect the intercellular lipid structure.
b. Enhancers that affect the protein structure.
c. Enhancers that improve drug distribution in the stratum corneum (14).

Chemical enhancers can release skin fats and, as a result, create pathways for the penetration of drugs. They can also enter the lipid bilayer and, as a result, disrupt the lamellar fats and turn them into liquid. Removal of lipids or their conversion to liquid can be done by various mechanisms (15,16). Chemical enhancers increase the transfer of the drug through the skin by increasing the thermodynamic activity in the formulation (17).

Eucalyptus globulus is one of the most well-known medicinal plants. Its antimicrobial effects have long been noted. In the past, the leaves of this plant were used as a salve for all kinds of wounds and inflammations, and its decoction was used in edible products (18).

Menthol, a 10-carbon alcohol, is extracted from peppermint oil and used as a seasoning and aromatic substance. Its therapeutic class is hexahydrothymol (19).

Tween 80 is a non-ionic surfactant with the chemical formula $C_{64}H_{124}O_{26}$ (20).

Oleic acid is a fatty acid compound with a molecular formula of $C_{18}H_{34}O_2$ and a molar mass of 282.4614. It is a pale-yellow oily liquid. Its density is 0.895 g/cm^3, and its melting and boiling temperatures are 286 K and 633 K, respectively. Oleic acid is insoluble in water but soluble in methanol and organic compounds (21).

Propylene glycol is a chemical compound with the formula C3H8O2 and a molar mass of 76.9 g/mol. It has a melting point of 59 degrees Celsius and a boiling point of 188.2 °C, and it is soluble in water and ethanol (22). Propylene glycol is produced by distillation of propylene oxide (C_3H_6O). Among the uses of propylene glycol, we can refer to drug solvents, food additives, cleaners, and disinfectants in cosmetics and health industries (23).

In this study, the effect of five enhancers (oleic acid, eucalyptus oil, menthol, Tween 80, and propylene glycol) and their skin exposure time (1 and 2 hours) on increasing the skin permeability to cetirizine drug delivery was investigated. The obtained results can effectively achieve this drug's desired skin permeation and design a new topical formulation from it.

Materials and Methods

Materials

Cetirizine was obtained from Atrapharma, Iran, and eucalyptus oil was obtained from Barij essence, Kashan, Iran. Tween 80, oleic acid, menthol, propylene glycol, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Merck, Germany.

Animals

Adult male Wistar rats weighing 180–250 g and aged 10–12 weeks were purchased. The hair on the abdominal surface of the laboratory rats was removed without damaging the skin. The rats were euthanized by ketamine/xylazine (10.50 mg/kg), and then the skin of their abdominal area was separated. The sacrifice and preparation of the rats were done according to the protocols of the Research Center & Experimental Animal House of Ahvaz Jundishapur University of Medical Sciences and approved by the Research Ethics Committee of the Research Center & Experimental Animal House - Ahvaz Jundishapur University of Medical Sciences with the code of ethics IR.AJUMS.ABHC.REC.1400.107.

Cetirizine measurement method

It is necessary to use a reliable method to measure the drug to determine the suitable amount of the desired drug and the amount of drug that passes through the skin. In this study, the UV spectroscopy method was used at a wavelength of 230 nm. The selected wavelength is based on the optical absorption spectrum of cetirizine in phosphate buffer solution pH = 7, which is considered for permeability and release studies. At this wavelength, cetirizine has maximum absorption (24).

The method of investigating the permeation of cetirizine through the skin of rats using different enhancers

Cetirizine permeability measurement method

In this method, the permeability parameter of cetirizine drug was measured through the treated skin with or without enhancers. After preparation, the rat skin was placed on a Franz diffusion cell to investigate its cetirizine

permeation. First, the skin was pre-exposed to 1 gram of each absorption enhancer for 1 or 2 hours with (oleic acid, menthol, eucalyptus oil, Tween 80, and propylene glycol). After removing the absorption enhancer from the skin surface, the receiver phase was filled with 35 ml of phosphate buffer solution pH = 7, and the donor phase was filled with 5 ml of 1% drug solution (16,25).

The prepared cells were placed on the heater stirrer at 37 °C at around 200 rpm. At 0.5, 1, 2, …. 8, 24 hours, 2 milliliters of the receptor phase inside the cell were removed, 2 milliliters of phosphate buffer pH = 7 was replaced, and the absorbance of the sample was read in the UV device at a wavelength of 230 nm. This was done to obtain the wavelength that has the highest absorption. In the control permeability test, only distilled water was used in the donor phase, and phosphate buffer pH = 7 was used in the receiver chamber to use the extracted liquid to zero the absorbance of the UV spectrophotometer (25).

Study of the effect of enhancers on the skin by differential scanning calorimetry (DSC)

This device was used to check the skin's reaction to controlled temperatures, the reaction of the skin to the absorption of various enhancers, the penetration of each enhancer into the skin, and changes caused by each enhancer. Differential scanning calorimetry (DSC) is a thermal investigation technique that studies the thermal characteristics of the formulation structure. It involves to record the changes related to phase transition and chemical reactions due to temperature changes. In DSC experiments, the difference in the thermal absorption process of the sample and a reference at the same temperature is recorded as a thermal function. During the heating or cooling of the sample, endothermic (melting, sublimation, and chemical decomposition) or exothermic (crystallization) events are evaluated in the form of a thermogram. In this method, the sample and a reference are heated or cooled constantly and equally, and the heat flow required to keep the sample and reference at the same temperature is measured. Since the pressure is constant in DSC, the heat flow is equivalent to the enthalpy changes. The reference is an empty aluminum pan. Therefore, the graphs show the difference between the heating rate of the sample and the reference against the temperature, which is expressed in watts per second or joules per second. The surface area under the peak of a thermal event is proportional to the heat absorbed or released by the sample expressed in cal/(s.g) or J/(s.g) units (26).

A part of the skin of the abdominal region of each rat was exposed to the absorption agent for 2 hours and then dried in a vacuum (650 mm Hg) at a temperature of 25 ± 1 °C to remove the excess solvent. Following this, it was divided into small pieces, and the DSC spectrum was measured for it. With the DSC device, the changes of lipids and proteins are checked with the shift of temperature transitions (16).

Study of the effect of enhancers on the skin by Fourier transform infrared spectroscopy (FT-IR)

The working mechanism of this device is as follows. The target sample is placed facing the light, and the IR light hits it, causing the sample molecules to vibrate in different directions. These vibrations and their type represent the specific substances that constitute the sample. In this way, the functional groups can be identified. In FT (Fourier transfer), all wavelengths hit the sample as one. The absorption of visible or ultraviolet light by materials leads to the transfer of the electron energy level, and an electron absorption spectrum is obtained.

Ultraviolet-visible spectroscopy is among the most widely used qualitative and quantitative analysis techniques investigating the interaction of light and matter. In spectroscopy, a beam of light (ray) is irradiated to the desired substance, and data is collected by examining the reflected, absorbed, or emitted light. The electromagnetic spectrum contains a range of wavelengths that make differentiating the different substances possible (27).

FT-IR consists of different optical and electronic parts. The radiation source, which can be a tungsten lamp for producing wavelengths in the visible region and hydrogen or deuterium for the ultraviolet region, provides a continuous spectrum of radiation. This radiation spectrum is separated by a monochromator, and a narrow band of wavelength is irradiated by optical instruments to the cell. The transmitted light is then focused by a mirror and measured by a detector (28).

Statistical methods and calculation of permeability parameters

All the studies were repeated thrice, and the values are expressed as mean and standard deviation.

In this research, the permeability to cetirizine was investigated in the whole skin of laboratory rats, and permeability parameters such as the rate of permeation in the equilibrium state (*Jss*), permeability coefficient (p), residence time (T_{lag}), and apparent diffusion coefficient (D_{app}) were calculated. In order to calculate the permeability parameters, the graph of the cumulative amount of drug passing through the surface unit was drawn against time. The permeability coefficient (p) was calculated using the equation $JSS = PC$, in which C is the drug concentration in the donor phase (16).

T_{lag}, or the amount of incubation time, is obtained from extending the equilibrium line to the time axis in the drug's accumulation curve passing through the skin. The value of D is also obtained with the help of the equation $D = h^2 / 6T_{lag}$. Because h does not represent the actual length of the drug's permeation, the D calculated from this formula is also an apparent D (29).

Results

Effects of different enhancers on cetirizine's skin permeability

The effect of enhancers with different excipients in 1-hour and 2-hour contact with the skin on the permeability of cetirizine is shown in Table 1. ER_{flux} indicates the ratio of drug penetration after treated by enhancer to the ratio of drug penetration without using the enhancer. Also, ER_D indicates the increase of the drug diffusion coefficient after using the enhancer compared to not using it, and ER_P indicates the ratio of the permeability coefficient after using the enhancer to before using it (30). Also, the cumulative concentration of cetirizine that passed the skin after 1 and 2 hours of exposure to the enhancers is shown in Figures 1 and 2, respectively.

Among the enhancers, the highest and lowest fluxes are oleic acid and propylene glycol respectively, the highest and lowest *D* values belong to menthol and propylene glycol respectively. The highest and lowest T belong to propylene glycol and Tween 80, respectively. ER_{flux} and ER_P are also similar to T. Eucalyptus has the highest permeability in one hour, and the highest ER_D in one hour belongs to menthol.

Enhancers' effect on rat skin structure

FT-IR and DSC methods have been used to investigate the mechanism of the enhancers' effect on rat skin structure.

FT-IR

FT-IR spectrum analysis of skin before or after enhancer's exposure is a practical method to study the interaction between chemicals and particles in the skin, as shown in Tables 2 to 3.

DSC

The rat's skin was treated with different enhancers, and the phase transition temperature in the change values (ΔH) corresponding to each absorption agent is shown in Table 4. DSC thermograms resulting from the effect of different enhancers on the whole skin of rats are shown in Figure 3.

Discussion

The effect of enhancers on the permeation of the drug through the skin of rats compared to the control was obtained by calculating ER_{Flux}, ER_P, and ER_D. The results have shown that all absorption of eucalyptus oil, Tween

Table 1. The results of the effect of different enhancers on different parameters of permeability to cetirizine in the rat skin ($n=3$, Mean ± SD)

Enhancer	Flux (mg.cm^{-2}.h^{-1})	*D* (cm^2.h^{-1})	T_{lag} (h)	*P*(cm/h)	ER_P	ER_D	ER_{flux}
Control (water)	0.00095 ± 0.0008	0.011 ± 0.0004	4.875 ± 0.18	0.00002 ± 0.000016	-	-	-
PG (1 hour)	0.00295 ± 0.00007	0.023 ± 0.002	2.302 ± 0.23	0.00006 ± 0.000001	4.716 ± 3.936	2.132 ± 0.292	4.716 ± 3.936
PG (2 hours)	0.00520 ± 0.0001	0.257 ± 0.124	0.239 ± 0.116	0.00010 ± 0.000003	8.325 ± 6.965	23.342 ± 12.067	8.325 ± 6.965
Menthol (1 hour)	0.0045 ± 0.0006	0.664 ± 0.889	0.929 ± 0.850	0.00009 ± 0.00001	13.391 ± 11.113	3.664 ± 0.302	13.391 ± 11.113
Menthol (2 hours)	0.0082 ± 0.00028	0.110 ± 0.002	0.494 ± 0.008	0.000164 ± 0.000006	26.275 ± 21.884	18.642 ± 0.275	26.275 ± 21.884
Tween80 (1 hour)	0.0084 ± 0.0001	0.0407 ± 0.0019	1.333 ± 0.062	0.000168 ± 0.000003	6.758 ± 4.937	58.329 ± 77.871	6.758 ± 4.937
Tween80 (2 hours)	0.016 ± 0.00035	0.207 ± 0.00445	0.261 ± 0.00562	0.00033 ± 0.0000071	13.166 ± 11.078	9.866 ± 0.190	13.166 ± 11.078
Eucalyptus Oil (1 hour)	0.0082 ± 0.0004	0.0260 ± 0.0013	2.088 ± 0.107	0.00016 ± 0.000008	12.708 ± 9.958	2.334 ± 0.035	12.708 ± 9.958
Eucalyptus Oil (2 hours)	0.0288 ± 0.001	0.108 ± 0.0016	0.500 ± 0.0074	0.00058 ± 0.00002	46.241 ± 38.902	9.745 ± 0.209	46.241 ± 38.902
Oleic acid (1 hour)	0.0073 ± 0.001	0.024 ± 0.001	2.225 ± 0.089	0.00015 ± 0.00002	12.2 ± 11.030	2.190 ± 0.008	12.200 ± 11.030
Oleic acid (2 hours)	0.0098 ± 0.0005	0.106 ± 0.0001	0.510 ± 0.0006	0.00020 ± 0.00001	15.883 ± 13.600	9.554 ± 0.335	15.883 ± 13.600

$$ER = \frac{\text{Cetirizine permeability parameter of treated skin}}{\text{Cetirizine permeability parameter of untreated skin}}$$

Table 2. Peak height and percentage of reduction in symmetric and asymmetric CH-CH peak height, stretchable C=O, and stretchable C-N group due to the effect of enhancers ($n=3$, mean ± SD)

Enhancer	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester		C=O stretching of keratin		C-N stretching of keratin	
	Peak height	*D*%	Peak height	*D*%	Peak height	*D*%	Peak height	*D*%	Peak height	*D*%
Control (water)	1.835	_	1.95	_	2.061	_	2.111	-	2.151	-
PG	0.224	87.79	0.13	93.33	0.212	89.71	0.96	54.52	0.141	93.44
Menthol	0.527	71.29	0.512	73.74	2	2.96	1.2	43.15	0.534	75.17
Tween 80	2.129	56.34	2.115	57.91	1.872	62.55	1.774	64.17	1.806	62.68
Eucalyptus oil	0	100	0.001	99.58	0.038	N. S	0.02	83.3	0.01	98.6
Oleic acid	0.382	79.18	0.229	88.25	0.19	90.72	0.404	80.86	0.258	88

NS: Not seen.

$$\% = \frac{(\text{Peak height in skin without pretreatment} - \text{Peak height in skin pretreatment with the drug})}{\text{amount of decrease in the height of the peak in the skin without pretreatment}} \times 100$$

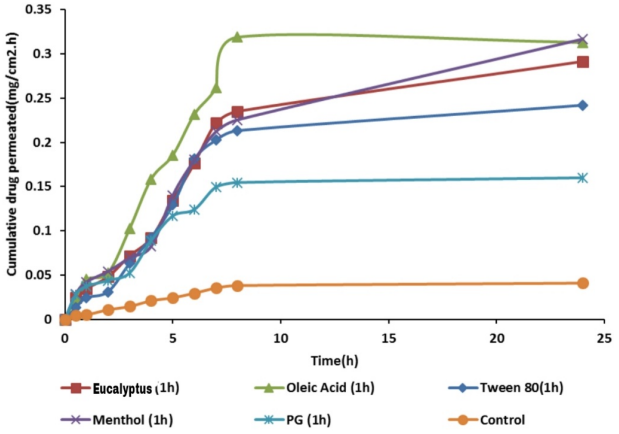

Figure 1. Cumulative diagram of cetirizine passing through the rat skin surface unit after one hour of skin contact with eucalyptus, menthol, PG (propylene glycol), Tween 80, and oleic Acid

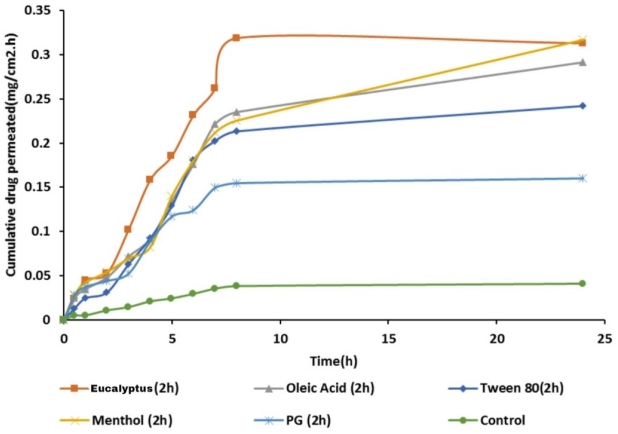

Figure 2. Cumulative diagram of cetirizine passing through the surface unit after two hours of skin contact with eucalyptus, menthol, PG (propylene glycol), Tween 80, and oleic acid through the rat's skin

Table 3. Wavelengths related to symmetric and asymmetric -CH and C=O stretching group of amides I and II in hydrated skin and skin pretreatment with enhancers ($n=3$, mean±SD)

Enhancer	C-H stretching asymmetric	C-H stretching Symmetric	C=O stretching of lipid ester	Amid I	Amid II
Control (water)	2918.77±0.16	2856.34±0.16	1731.68±0.14	1667.04±0.12	1547.67±0.11
PG	2921.20±0.22	2850.21±0.13	1740.84±0.21	1539.60±0.96	1454.915±0.14
Menthol	2838.06±0.15	2747.53±0.11	1728.58±0.11	1603.87±0.16	1538.91±0.20
Tween 80	3010.00±1.02	2840.00±0.27	1770.18±0.19	1653.00±0.90	1591.73±0.58
Eucalyptus oil	-	2856.57±0.1	1725.03±0.1	1631.07±0.10	1546.34±0.10
Oleic acid	2921.40±0.38	2955.43±0.23	1745.12±0.19	1632.12±0.40	1564.81±0.26

Table 4. The shift of average transfer temperatures and enthalpy values in rat skin with enhancers ($n=3$, mean±SD)

Enhancer	Tm_1	Tm_2	ΔH_1	ΔH_2
Control (water)	67.5±2.1	112.0±6.6	-7.0±0.4	-551.3±19.5
PG	59.0±0.9	153±0.9	0.9±0.0	2.7±0.3
Menthol	-	124±0.1	-	2.714±0.3
Tween 80	-	-	-	-
Eucalyptus oil	37.5±0.1	120.1±0.1	-0.8±0.0	26.6±0.8
Oleic acid	63.0±1.1	124.0±1.1	0.9±0.0	2.2±0.1

80, menthol, propylene glycol, and oleic acid in one-hour and two-hour contact with the skin has caused a significant ($P<0.05$) increase in ER_{Flux}, ER_P, and ER_D. The results show that increasing the pre-contact time increases the amount of drug that passes through the skin.

Also, the results show a significant difference between the J_{ss} ($P<0.05$) of the absorption enhancer sample used in both one and two-hour modes compared to that of the control sample, which indicates an increase in permeability with the use of the studied enhancer.

The results have shown that all enhancers lead to an increase in the release of drug (D_{app}) from the skin and the rate of drug penetration (flux). In the meantime, Tween 80 and eucalyptus have the greatest effect in increasing the rate of penetration from mouse skin after one hour and two hours.

The results of the present study show that the maximum and minimum increases in drug permeation speed are as follows:

Two hours: propylene glycol > menthol > oleic acid > Tween 80 > eucalyptus

One hour: propylene glycol > menthol > oleic acid > eucalyptus > Tween 80

The present study shows that any change in the pretreatment time of the enhancer's exposure with rat skin can change the drug permeability parameters.

FT-IR

FT-IR absorption spectrum bands represent the vibrations of lipid and protein molecules in the stratum corneum (31). The molecular vibrations of lipids are an excellent indicator for evaluating lamellar lipid microstructures in the intercellular region of the skin's stratum corneum. In investigating the mechanism of drug effect on the skin structure using the FT-IR method, the displacement and shift in the position of the absorption bands towards higher and lower wavelengths and the change in the intensity of the resulting signal at that absorption position are usually considered (32).

Suppose the absorption band is transferred towards higher wavelengths. This would indicate the liquefaction of the bilayers in the stratum corneum membrane, disruption of the barrier properties, and possibly an increase in drug permeation through the stratum corneum. On the other hand, if the absorption band is shifted to a

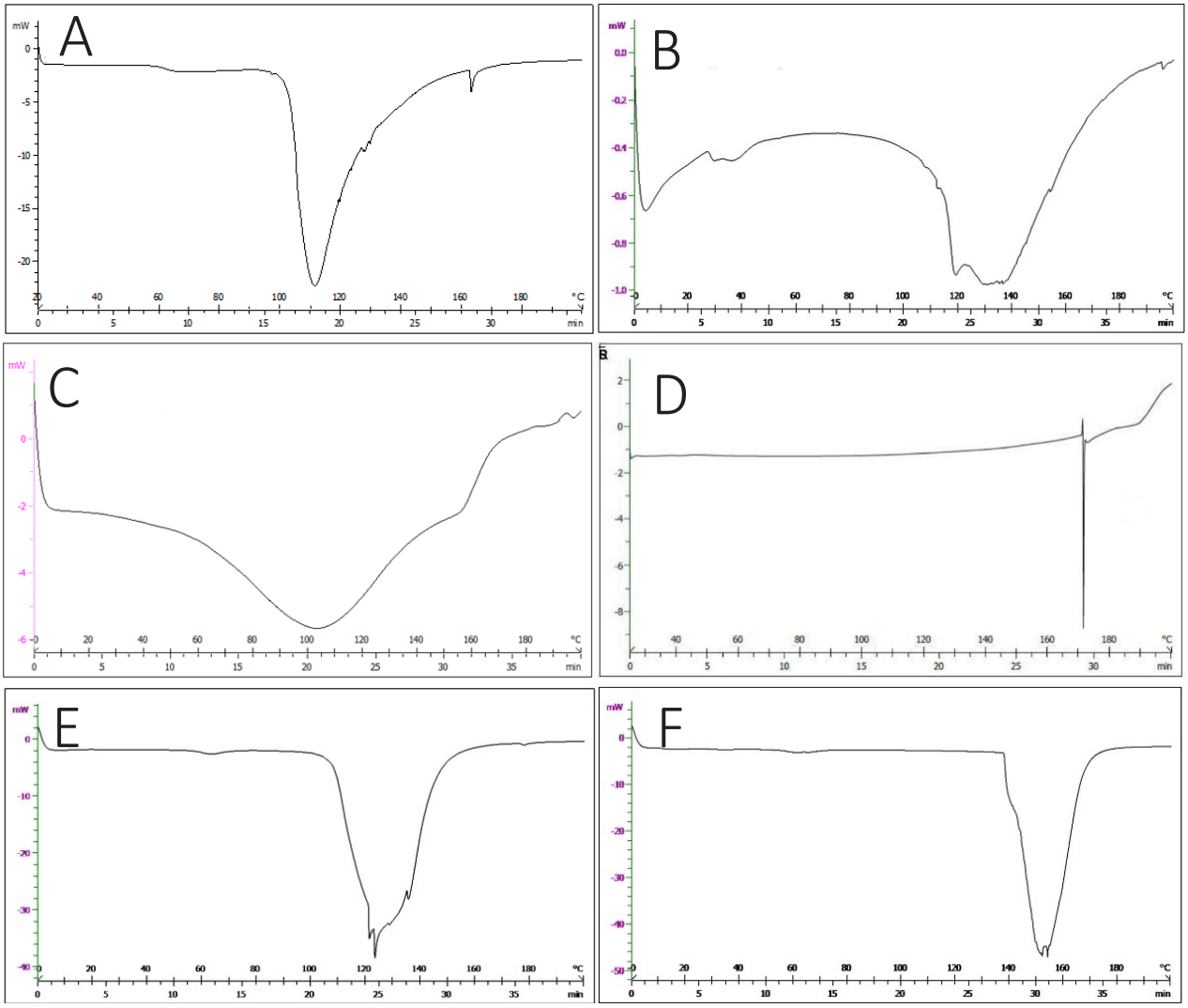

Figure 3. DSC thermograms of the whole abdominal skin of a rat after pretreatment with A: water, B: eucalyptus, C: menthol, D: Tween 80, E: oleic acid, and F: propylene glycol

lower wavelength, it indicates the reorientation of the lipid bilayer groups of the stratum corneum, which ultimately prevents the drug from entering the skin (33).

Since the height (intensity) or the position of the absorption bands shows the amount of lipid or protein in the stratum corneum, the increase in the intensity of the peak indicates the strengthening of the lipid structure and the retardation effects of the drug. In contrast, the decrease in the peak intensity indicates the weakening of the lipid structure. It is the stratum corneum that increases the permeability of the drug (34).

Sapra et al. have proven that menthol causes the lipid extraction of corneal tissue; menthol is a cyclic terpene compound that increases the absorption of some drugs, such as ibuprofen and nicardipine (35).

The FTIR results obtained from the effect of eucalyptus on rat skin show that it has a blue-shift from the areas of 2915.13 cm^{-1} and 2840.7 cm^{-1}, which indicates the liquefaction of the stratum corneum (bi-layer), disruption of the barrier properties, and increase in drug permeation. These results also show that eucalyptus has caused a significant reduction in the height of the desired peaks. Obata et al. demonstrated that the cineole, a terpene compound in eucalyptus oil, increases the release of polar and non-polar compounds by creating liquid pools in the stratum corneum and disrupting its lipid structure (36).

The results of FT-IR of the skin pretreated with menthol show that asymmetric C-H and symmetric C-H have moved towards the lower absorption band position (2747.53 cm^{-1} and 2838.63 cm^{-1}, respectively), which indicates a red-shift. It shows that menthol has caused the orientation of bilayer lipid groups and ultimately increased the barrier effect of drug entry.

Also, the FT-IR results of this peak show that menthol has shifted the absorption band in the region of C = O stressing amide I and amide II towards the lower wavelengths (1728.58 cm^{-1}, 1603.87 cm^{-1}, and 1538.91 cm^{-1}, respectively), which indicates the orientation of protein groups in the bilayer of keratinized tissue. Also, the intensity of the resulting signals in the desired

absorption positions shows that menthol has caused a decrease in the height of the peak in the symmetrical and asymmetrical C-H regions and C=O stressing of amide 1 and 2 and has caused a decrease in the percentage of these intensities.

The results of FT-IR of the skin pretreatment with Tween 80 show a blue-shift and a significant decrease in the height of peaks 3010.75 cm^{-1}, 2840.74 cm^{-1}, 1770.18 cm^{-1}, 1653 cm^{-1}, and 1591.73 cm^{-1}, which shows the effect of this absorption enhancer on the lipid part and the stratum corneum is proteinaceous and causes differences in the barrier properties of this layer.

The results of FT-IR of the skin pre-exposed to propylene glycol show that asymmetric C-H and symmetric C-H shifted towards the absorption band (2921.201 cm^{-1} and 2850.21 cm^{-1}, respectively), which indicates a red-shift and shows that propylene glycol affects the orientation of bilayer lipid groups and ultimately prevents the drug from entering the skin.

Also, the results showed that propylene glycol caused the shift of the absorption band in the C=O region and amide I and II towards lower wavelengths (1740.837 cm^{-1}, 1539.592 $cm^{-1,}$ and 1456.915 cm^{-1}), which indicates the orientation of protein groups in the stratum corneum bilayer.

The results of FT-IR of pretreatment skin with oleic acid enhancer show that it increases the absorption wavelength in the C-H asymmetric and C-H symmetric regions (2921.403 cm^{-1} and 2955.426 cm^{-1}, respectively), indicating a blue-shift and liquefaction of bilayers in the stratum corneum. There is a disturbance in the barrier properties and an increase in the permeation of the drug through the stratum corneum. These enhancers increase the C=O stress wavelength (1745.121 cm^{-1}), which indicates the weakening of hydrogen bonds between lipid molecules. Oleic acid also decreases the wavelength number of amide I. It increases the wavelength number of amide II, changes that increase and decrease the barrier effects of the stratum corneum protein part, respectively.

DSC

The DSC method is used to a significant extent in detecting the melting points of lipids, phase transition of lipid bilayers, and denaturation of proteins in the stratum corneum. This method examines the temperature transfer of the skin pretreatment with different enhancers in terms of the average phase transition temperature (Tm) and their enthalpy. The shift of the phase transition temperature to lower temperatures indicates the disruption of the lipid layer and the irreversible denaturation of the protein structure in the stratum corneum. At the same time, the decrease in enthalpy indicates the liquefaction of lipids in lipid bilayers and lipid-protein complexes in the corneal tissue (37).

In this study, Tm_1 was attributed to the temperature of lipid transition from lamellar to disordered state, Tm_2 to the melting temperature of the keratin-lipid complex or disorder in the polar head groups of lipids, and Tm_3 to the temperature of irreversible protein denaturation in the stratum corneum.

The spectrum of the DSC thermogram of water-hydrated skin is two temperature averages of endothermic phase transition at temperatures of 67.5 °C (Tm_1) (lipid transition temperature) and 112 °C (Tm_2) (irreversible protein denaturation temperature in the stratum corneum) (38).

The DSC results related to eucalyptus show the displacement of Tm_1 to a lower temperature and the decrease of ΔH_1 and ΔH_2 to lower values than the control. Based on the decrease in phase transition temperature, it can be concluded that eucalyptus disrupts the lipid layer of stratum corneum. Also, considering the reduction of ΔH_1 and ΔH_2, eucalyptus has caused the liquefaction of bilayer lipids and the lipid-protein complex in the corneal tissue.

The DSC results related to the effect of propylene glycol on the abdominal rat skin show that propylene glycol decreases the Tm_1 phase transition temperature and increases the Tm_2 phase transition temperature. This compound also shows that it causes a significant decrease in ΔH_2. This indicates propylene glycol disrupts the lipid layer and liquefies the stratum corneum's lipid-protein complex. Therefore, the results obtained from the DSC of pre-exposed skin are largely consistent with the FT-IR results.

The DSC results of the effect of oleic acid show that this compound has caused a decrease in Tm_1, an increase in Tm_2, and a significant decrease in ΔH_1 and ΔH_2. It seems that this compound affects the protein structure through disruption and irreversible denaturation of the lipid layer in the stratum corneum. The significant decrease in enthalpy causes the liquefaction of lipids in the lipid bilayer and the lipid-protein complex in the stratum corneum. The results are consistent with the FT-IR results of propylene glycol.

The DSC results related to the effect of menthol on the abdominal rat skin show that menthol has caused the removal of the phase transition temperature (Tm_1) and the increase of the phase transition temperature (Tm_2). Also, this compound caused a significant decrease in ΔH_2. This indicates that menthol disrupts the lipid layer and liquefaction of the lipid-protein complex in the stratum corneum. Therefore, the DSC results of the skin pre-exposed to menthol are largely consistent with its FT-IR results.

The effect of Tween 80 on the abdominal skin of the rat in the DSC thermogram shows that this enhancer caused the destruction of all the peaks in the mentioned areas and therefore this enhancer caused the complete destruction of the lipid layer and the liquefaction of the bilayer and denaturation of protein in the stratum corneum. This

finding can be an important reason for the increased drug permeation rate in the skin pre-exposed to this absorption enhancer.

Conclusion

The permeation of cetirizine through the skin is very low due to its hydrophilic nature. Enhancers such as propylene glycol, menthol, oleic acid, eucalyptus, and Tween 80 can significantly increase the rate and speed of skin absorption of cetirizine. Using these acids in the topical formulation of cetirizine or their pretreatment use before taking the topical formulation of the drug can help its local effectiveness. The amount and speed of cetirizine permeation through the rat skin increased with the increase in enhancer pretreatment time.

Acknowledgements

This article was extracted from a General Pharm.D thesis (R. Mombeini), number 1364, approved by the Research Committee of Jundishapur University of Medical Sciences, Ahvaz.

Authors' Contribution:

Conceptualization: Anayatollah Salimi, Saeed Mohammad Soleymani.
Data curation: Rahim Mombeini.
Formal analysis: Anayatollah Salimi.
Funding acquisition: Rahim Mombeini.
Investigation: Anayatollah Salimi, Saeed Mohammad Soleymani.
Methodology: Anayatollah Salimi.
Project administration: Saeed Mohammad Soleymani.
Resources: Anayatollah Salimi, Saeed Mohammad Soleymani.
Software: Anayatollah Salimi.
Supervision: Anayatollah Salimi.
Validation: Saeed Mohammad Soleymani.
Visualization: Saeed Mohammad Soleymani.
Writing–original draft: Rahim Mombeini.
Writing–review & editing: Anayatollah Salimi, Saeed Mohammad Soleymani.

Competing Interests

There is no conflict of interest.

Ethical Approval

The ethics committee of Jundishapur University of Medical Sciences (IR.AJUMS.ABHC.REC.1400.107) on 28.12.2021 provided ethical approval for this study.

Funding

None.

References

1. Spencer CM, Faulds D, Peters DH. Cetirizine. A reappraisal of its pharmacological properties and therapeutic use in selected allergic disorders. Drugs. 1993;46(6):1055-80. doi: 10.2165/00003495-199346060-00008.
2. Campoli-Richards DM, Buckley MM, Fitton A. Cetirizine. A review of its pharmacological properties and clinical potential in allergic rhinitis, pollen-induced asthma, and chronic urticaria. Drugs. 1990;40(5):762-81. doi: 10.2165/00003495-199040050-00009.
3. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012;37(1):106-26. doi: 10.1016/j.progpolymsci.2011.06.003.
4. Chen X, Xiang H, Yang M. Topical cetirizine for treating androgenetic alopecia: a systematic review. J Cosmet Dermatol. 2022;21(11):5519-26. doi: 10.1111/jocd.15309.
5. Pagliara A, Testa B, Carrupt PA, Jolliet P, Morin C, Morin D, et al. Molecular properties and pharmacokinetic behavior of cetirizine, a zwitterionic H1-receptor antagonist. J Med Chem. 1998;41(6):853-63. doi: 10.1021/jm9704311.
6. Vitorino C, Sousa J, Pais A. Overcoming the skin permeation barrier: challenges and opportunities. Curr Pharm Des. 2015;21(20):2698-712. doi: 10.2174/1381612821666150428124053.
7. Lever WF. Histopathology of the Skin. J. B. Lippincott & Co; 1949.
8. Pillion DJ, Recchia J, Wang P, Marciani DJ, Kensil CR. DS-1, a modified *Quillaja* saponin, enhances ocular and nasal absorption of insulin. J Pharm Sci. 1995;84(11):1276-9. doi: 10.1002/jps.2600841104.
9. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci. 2001;14(2):101-14. doi: 10.1016/s0928-0987(01)00167-1.
10. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interface Sci. 2006;123-126:369-85. doi: 10.1016/j.cis.2006.05.014.
11. Salimi A, Mohammad Soleymani S, Mohammad Soleymani H. The use of nano-carriers in transdermal drug delivery. Jundishapur Sci Med J. 2020;19(4):435-64. doi: 10.22118/jsmj.2020.211687.1918. [Persian].
12. Dreher F, Walde P, Walther P, Wehrli E. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J Control Release. 1997;45(2):131-40. doi: 10.1016/s0168-3659(96)01559-3.
13. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev. 2012;64 Suppl:128-37. doi: 10.1016/j.addr.2012.09.032.
14. Aboofazeli R, Zia H, Needham TE. Transdermal delivery of nicardipine: an approach to in vitro permeation enhancement. Drug Deliv. 2002;9(4):239-47. doi: 10.1080/10717540260397855.
15. Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S. Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci U S A. 2005;102(13):4688-93. doi: 10.1073/pnas.0501176102.
16. Salimi A, Moghimipour E, Kogani P, Mohammad Soleymani S. The effect of various penetration enhancers on the octyl methoxycinnamate permeability: mechanisms of action study. Iran J Pharm Sci. 2020;16(2):87-104.
17. Kim YC, Park JH, Ludovice PJ, Prausnitz MR. Synergistic enhancement of skin permeability by N-lauroylsarcosine and ethanol. Int J Pharm. 2008;352(1-2):129-38. doi: 10.1016/j.ijpharm.2007.10.031.
18. Mulyaningsih S, Sporer F, Reichling J, Wink M. Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens. Pharm Biol. 2011;49(9):893-9. doi: 10.3109/13880209.2011.553625.
19. Chey WD, Lacy BE, Cash BD, Epstein M, Shah SM. Efficacy of caraway oil/L-menthol plus usual care vs placebo plus usual care, in functional dyspepsia patients with post-prandial distress (PDS) or epigastric pain (EPS) syndromes: results from a US RCT. Gastroenterology. 2017;152(5):S307. doi: 10.1016/s0016-5085(17)31314-8.
20. Cheng M, Zeng G, Huang D, Yang C, Lai C, Zhang C, et al. Advantages and challenges of Tween 80 surfactant-enhanced technologies for the remediation of soils contaminated with hydrophobic organic compounds. Chem Eng J. 2017;314:98-113. doi: 10.1016/j.cej.2016.12.135.

21. Sales-Campos H, de Souza PR, Peghini BC, da Silva JS, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev Med Chem. 2013;13(2):201-10.
22. De Cock RF, Allegaert K, Vanhaesebrouck S, de Hoon J, Verbesselt R, Danhof M, et al. Low but inducible contribution of renal elimination to clearance of propylene glycol in preterm and term neonates. Ther Drug Monit. 2014;36(3):278-87. doi: 10.1097/ftd.0000000000000003.
23. Jacob SE, Scheman A, McGowan MA. Propylene glycol. Dermatitis. 2018;29(1):3-5. doi: 10.1097/der.0000000000000315.
24. El Walily AF, Korany MA, El Gindy A, Bedair MF. Spectrophotometric and high-performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets. J Pharm Biomed Anal. 1998;17(3):435-42. doi: 10.1016/s0731-7085(97)00213-6.
25. Ermer J, Miller JH. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. John Wiley & Sons; 2006.
26. Kunieda H, Hasegawa Y, John AC, Naito M, Muto M. Phase behaviour of polyoxyethylene hydrogenated castor oil in oil/water system. Colloids Surf A Physicochem Eng Asp. 1996;109:209-16. doi: 10.1016/0927-7757(95)03456-0.
27. Álvarez Á, Yáñez J. Screening of gunshot residue in skin using attenuated total reflection Fourier transform infrared (ATR FT-IR) hyperspectral microscopy. Appl Spectrosc. 2020;74(4):400-7. doi: 10.1177/0003702819892930.
28. Raja M, Raj Muhamed R, Muthu S, Suresh M. Synthesis, spectroscopic (FT-IR, FT-Raman, NMR, UV–visible), NLO, NBO, HOMO-LUMO, Fukui function and molecular docking study of (E)-1-(5-bromo-2-hydroxybenzylidene) semicarbazide. J Mol Struct. 2017;1141:284-98. doi: 10.1016/j.molstruc.2017.03.117.
29. Salimi A, Emam M, Mohammad Soleymani S. Increase adapalene delivery using chemical and herbal enhancers. J Cosmet Dermatol. 2021;20(9):3011-7. doi: 10.1111/jocd.13960.
30. Salimi A, Moghimipour E, Kashani S, Mohammad Soleymani S. The effect of various vehicles on the in vitro skin permeability of minoxidil. J Kermanshah Univ Med Sci. 2022;26(3):e128920. doi: 10.5812/jkums-128920.
31. Salimi A, Sharif Makhmalzadeh B, Davood Baharvandi G, Mohammad Soleymani S. The effect of vegetable and volatile oils on the piroxicam skin permeability: a mechanistic study. J Kermanshah Univ Med Sci. 2022;26(2):e126619. doi: 10.5812/jkums-126619.
32. Constantinides PP, Lancaster CM, Marcello J, Chiossone DC, Orner D, Hidalgo I, et al. Enhanced intestinal absorption of an RGD peptide from water-in-oil microemulsions of different composition and particle size. J Control Release. 1995;34(2):109-16. doi: 10.1016/0168-3659(94)00129-i.
33. Kawakami K, Yoshikawa T, Moroto Y, Kanaoka E, Takahashi K, Nishihara Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs: I. Prescription design. J Control Release. 2002;81(1-2):65-74. doi: 10.1016/s0168-3659(02)00049-4.
34. Salimi A, Mohammad Soleymani H, Mohammad Soleymani S. Altered skin permeation of finasteride using clove oil, urea, and lyophilized powder of grape seed extract. Adv Pharm Bull. 2023;13(1):96-103. doi: 10.34172/apb.2023.010.
35. Sapra P, Allen TM. Ligand-targeted liposomal anticancer drugs. Prog Lipid Res. 2003;42(5):439-62. doi: 10.1016/s0163-7827(03)00032-8.
36. Obata Y. Relation between aroma and chemical constitution. Journal of Synthetic Organic Chemistry Japan. 1959;17(2):55-78. doi: 10.5059/yukigoseikyokaishi.17.55.
37. Aoyagi T, Terashima O, Suzuki N, Matsui K, Nagase Y. Polymerization of benzalkonium chloride-type monomer and application to percutaneous drug absorption enhancer. J Control Release. 1990;13(1):63-71. doi: 10.1016/0168-3659(90)90075-5.
38. Lin SY, Duan KJ, Lin TC. Simultaneous determination of the protein conversion process in porcine stratum corneum after pretreatment with skin enhancers by a combined microscopic FT-IR/DSC system. Spectrochim Acta A Mol Biomol Spectrosc. 1996;52(12):1671-8. doi: 10.1016/0584-8539(96)01722-9.