EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 11
63-70
Year: 2020

THE EFFECT OF CHEMICAL PENETRATION ENHANCERS ON THE PERCUTANEOUS ABSORPTION OF CARVEDILOL

Eskandar Moghimipour[1,2], Marzieh Mahmoodi[2] and Anayatollah Salimi[1,2*]

[1]Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

***Corresponding Author: Anayatollah Salimi**
Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Article Received on 02/09/2020 **Article Revised on 23/09/2020** **Article Accepted on 13/10/2020**

ABSTRACT
Carvedilol is a cardio-selective beta-blocker that is used to treat high blood pressure and heart failure. It has a protein binding of more than 98%. Its bioavailability is less than 23% during oral use, with a duration of action of about 6 hours. Transdermal drug delivery has advantages over oral dosing, which includes no first-pass liver metabolism and continuous drug release. In this study, the effect of chemical penetration enhancers on the permeability and skin absorption of carvedilol is investigated. Labrazol, labrafil, labrafac, Castor oil, and oleic acid were used as chemical penetration enhancers and rat skin was used as an animal model. The effect of enhancers on skin structure was studied by using DSC technique. The results of the permeability parameters showed that the highest permeation amount obtained from labrafac. The amount of flux and partitioning compared to control increased, as well as all chemical enhancers decreases the lag time compared to control. Castor oil had the least effect on increasing the drug's permeability from the skin. The result of the DSC technique also confirmed the same results. The use of chemical enhancers due to changes the structure of the skin to increase the permeability of the drug can be considered as suitable excipients in transdermal delivery of carvedilol.

KEY-WORDS: Carvedilol, transdermal, chemical enhancers, Percutaneous Absorption.

INTRODUCTION
Carvedilol is a racemic compound of two enantiomers, that block the activity of β and α_1 adrenergic receptors, which is why it is used as an auxiliary drug for patients with heart failure.[1]

Carvedilol is a white powder to pale yellow, practically insoluble in water. It is completely soluble in dimethyl sulfoxide. Soluble in methylene chloride and methanol and is dissolved in low amounts in ethanol and isopropyl alcohol. It strongly binds to proteins, as well as a small dependence on maladaptive and high endurance potentials.[2] Carvedilol has several specific features, such as low molecular weight, desirable logarithmic partition coefficient, and low dose tolerance.[3]

Carvedilol is one of the drugs prescribed in the long-term treatment of hypertension, which upon oral administration is rapidly absorbed in the stomach and intestine by about 80%. However, its bioavailability is less than 23% as this drug is significantly and rapidly expanded to the first course of metabolism in the liver by the enzyme cytochrome p450 and is excreted. It also found that the concentration of this drug in the urine is less than 0.3%.[4] The drug has a plasma half-life of about 6 hours.[3] Studies on the bioavailability of the carvedilol skin patches indicate that these patches can provide steady state levels of carvedilol with minimal fluctuations, and a bioavailability of about 70% as compared to oral administration.[3] The delivery of the drug is continuous and the plasma levels of the drug is constant and prolonged. This helps to prevent variability of the drug in patients undergoing long-term treatments. The treatment can also be discontinued at any time.[5,6] Several factors should be considered in the development of a dermatologic delivery system. These factors can be categorized into 5 groups: pharmacologic activity of the drug, skin characteristics, formulation, adhesion and design of the drug delivery system. Absorption is a key factor that increases the absorption of drug through skin or mucus barriers, although the exact mechanism of this delivery system is not clearly known yet.[7] Although the results of in-line experiments are very reliable for formulations, outsourcing studies also have many advantages. Study of several factors is possible with this method. Further, the conditions of the investigation are easily determinable and stable, and time to completion of experiment is less and at a lower cost.[8] In this study, the effect of absorption of chemical additives on the absorption of carvedilol is considered

MATERRIALS AND METHODS

MATERIALS

Carvedilol powder was purchased from Tolid daru pharmaceutical company (Tehran, Iran). Labrafil, labrafac and labrazol was obtained as gift samples (Gattefosse, Saint-Priest, France). Castor oil, oleic acid and potassium phosphate monobasic and dibasic were purchased from merck company.

METHODS

Measurement of Carvedilol

With the help of a valid method for measuring the drug, the permeability of the drug from the skin was determined with the help of absorbance. In this research, UV spectrophotometer was used at a wavelength of 242 nm. The choice of the wavelength was based on the absorbance spectrum of carvedilol in buffer pH = 7.4 and methanol. It is this wavelength that gives maximum optical absorption of carvedilol with no interference observed from other materials. To prepare a standard curve, a concentrate 2mg/ml of the drug was prepared in pH 7.4 and methanol phosphate buffer, and 0.25, 0.5, 1, 1.5, 2, 2.5% was prepared at a concentration of 10 ml. And at a wavelength of 242 nm in three different days, each time three times the absorbance is read by the spectrophotometer and the standard curve is drawn using the numbers obtained.[9]

In vitro skin permeation study

Male wistar rats were used for the preparation of the skin. After killing them with high doses of thiopental sodium, their abdominal hair was shaved and entire skin of that area was separated. The skin samples were kept in the freezer until all skin absorption tests were completed. Before use, the skin samples are removed from the freezer and kept out in room temperature. The skin was then cut into small pieces and placed in the vertical glass diffusion cells fabricated in house(with an effective diffusion area of approximately 3.46 cm^2) at 37°C for 1 hour in contact with the receptor and receptor phase. After which, 1 g of the selective penetration enhancer was placed on the skin for a period of 4 hours then excess penetration enhancer was removed from the surface of the skin and 5 g of 1% aqueous solution of carvedilol was placed on the skin in the donor compartment, and the recipient phase was filled with phosphate buffer solution (pH = 7.4) and methanol as the drug solvent and stirred with a small magnetic bead at 200 rpm. The study was started and at specific times (0.5, 1, 2, 3,4,5.....,72 h), a 2 ml of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh receptor medium to maintain sink condition. The samples filtered and the permeated amount of carvedilol was determined by UV spectroscopy method at 242 nm.[11]

Permeation Data analysis and statistics

The cumulative amount of carvedilol permeated through unit area of the diffusion surface into the receptor was calculated and plotted as a 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 (Kp, cm/h) of carvedilol through the skin was calculated as in Equation (1):[12]

$Kp = Jss/C_0$ Equation (1)

where Jss is steady-state flux and C_0 the initial concentration of carvedilol in the donor compartment. Enhancement ratios(ER) were calculated from permeation parameters after enhancer treatment divided by the same parameters before enhancer treatment.

Statistical comparison was made using one-way ANOVA and $p < 0.05$ was considered statistically significant.

Pretreatment of skin samples with chemical penetration enhancers

For pretreatment of skin samples, fully skin samples were pre-treated with putting 2 ml of a chemical enhancer on the surface of skin in the donor phase for 4 hours. The donor and receptor compartments were then washed with water and filled with aqueous solution of carvedilol and pH 7.4 and methanol phosphate buffer, respectively. The effect of chemical enhancers was evaluated for carvedilol permeation through fully skin samples. Labrazol, labrafil, labrafac, Castor oil, and oleic acid were used as as chemical penetration enhancers. 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.[12]

Evaluation of the effect of penetration enhancers on Skin Structure Using DSC technique

To investigate the mechanism of enhancement effect on the lipid and protein structure of the skin, the rat skin was checked after exposure to each of penetration enhancer for 4 hours without any drug and then dried in a vacuum. The extra penetration enhancer was removed from the skin samples. The skin sample was divided into smaller portions and taken for DSC (Differential scanning calorimetery). Changes in the structure of the protein and lipid was compared to the non-treated skin for the penetration enhancer in terms of changes in the temperature and phase transition enthalpy of the lipid and protein in DSC.[10] The changes in structure of whole skin induced by chemical penetration enhancers were examined using a DSC (Mettler Toledo DSC^1 system) equipped. The fully hydrated skin samples were first immersed in a chemical penetration enhancer for 2 h and the excess of the enhancer was blotted out before they were hermetically sealed to avoid evaporation of water. Approximately 6– 10 mg of pre-treated skin samples were placed in hermetically sealed aluminium pans. Simultaneously an empty hermetically sealed pan was used as a reference. Skin samples were exposed to heat ranging from 20 to 200 ^{0}C (scan rate: 50C/min). In order to ensure accuracy and repeatability of data, DSC analyzer was calibrated and checked with indium standard.[13]

RESULTS AND DISCUSSION

Carvedilol Assay

The relationship between different concentrations of carvedilol in UV absorption is shown in figure 1. The correlation coefficient obtained ($R^2 = 0.9945$) indicates a linear correlation between different concentrations of carvedilol and their optical absorption, which means that 99.45% of the concentration values are estimated by optical absorption, which indicates the relationship between the concentration and optical absorption. According to the equation Abs = 2.1804x + 0.1062, the concentration of drug solution can be calculated by measuring optical absorption.

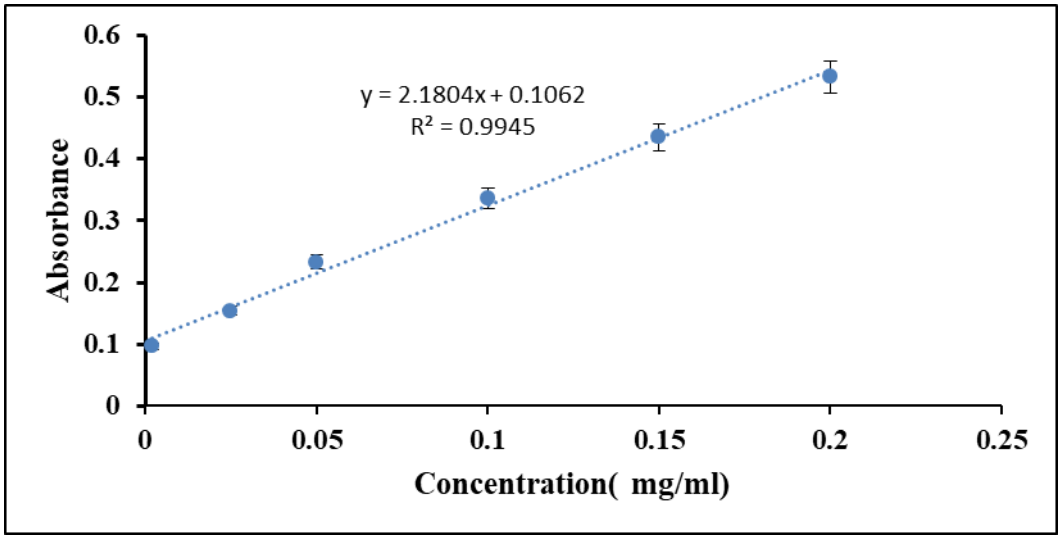

Figure 1. The relationship between different concentrations of carvedilol in phosphate buffer solution (pH 7.4) and methanol and their optical absorption (Mean ± SD).

Effect of the chemical penetration enhancers on carvedilol permeability

Permeability parameters after skin pretreatment for 4 hours with different penetration enhancers compared with control for carvedilol through the rat abdominal skin are presented in table 1, table 2 and figure 2, respectively. The results show that the highest absorption effect in terms of drug permeability is labrafac, and in terms of increasing the coefficient of distribution of oleic acid in relation to carvedilol. Treatment with chemical enhancers increases the amount of flux and distributability compared to control, as well as absorption increases the time of the munition compared to control.

Table 1: Skin permeability parameters of carvedilol after pretreatment with various penetration enhancers compared with control (Mean ± SD, n = 3).

Enhancer	J_{ss} (mg/cm^2.h)	D_{app} (cm^2/h)	P (cm/h)	T_{lag} (h)
Control (water)	0.1116 ± 0.0022	0.0178 ± 0.0017	0.0018 ± 0.0006	3.0494 ± 0.2898
Labrazol	0.2928 ± 0.0064	0.0345 ± 0.0018	0.0058 ± 0.0001	1.5691 ± 0.0827
Labrafil	0.3347 ± 0.0109	0.1073 ± 0.0032	0.0067 ± 0.0002	0.5047 ± 0.0153
Labrafac	0.7267 ± 0.0222	0.0362 ±0.0068	0.0145 ± 0.0004	1.5209 ± 0.2846
Oleic acid	0.6775 ± 0.0049	0.2540 ±0.0564	0.0135 ± 0.0009	0.2185 ± 0.0485
Castor oil	0.1453 ± 0.0156	0.1579 ±0.0319	0.0029 ± 0.0003	0.35 ± 0.0707

Table 2: Enhancement Ratios of skin permeability parameters of carvedilol after pretreatment with various penetration enhancers compared with control (Mean ± SD, n = 3).

Enhancer	ER flux	ERD	ERp
Control(water)	-	-	-
Labrazole	2.6236± 0.0038	1.9412± 0.0822	3.4098±1.1081
Labrafil	3.0007±0.1584	6.0538± 0.7574	3.9345±1.4790
Labrafac	6.5150± 0.3310	2.0588± 0.5758	8.5397±3.1944
Oleic acid	6.0724±0.1674	14.4550±4.5336	7.9297±2.7940
Castor oil	1.3012±0.1136	8.9797±2.6422	1.6675±0.4043

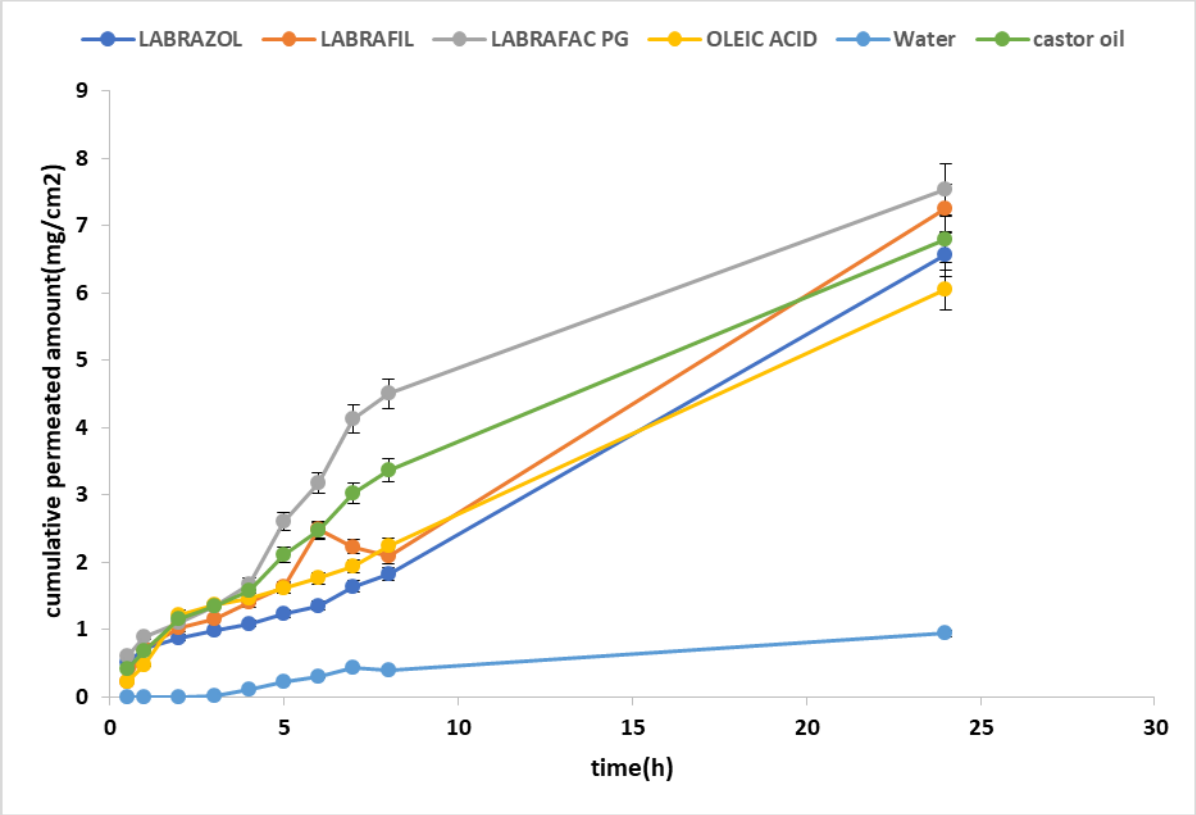

Figure 2: Cumulative permeability profile of carvedilol through a surface area with water, labrazol, labrafil, labrafac and oleic acid, and castor oil from the whole skin of the rat.

The Effect of Chemical penetration Enhancers On Rat Skin Using DSC technique

The DSC method is widely used to detect lipid melting points, phase transmission of lipid bilayers, and denaturation of proteins present in the horny layer. This method investigates the pre-invasive thermotropic behaviour of various proximal chemical enhancers in terms of average phase transition temperature (Tm) and their enthalpy. The displacement of the phase transition temperature to lower temperatures indicates that the lipid layer is impaired and the non-reversible denaturisation of the protein structure is in the horny layer. While the decrease in the enthalpy indicates lipid fluidization in the lipid bilayer and lipid-protein complex in the horn tissue.[14-16] Table 3 and figure 3-8 represented mean transition temperature and enthalpy values of different penetration enhancers and their thermograms, respectively.

Table 3: Mean transition temperature and enthalpy values in the skin of the rats after contact with water and various penetration enhancers (n = 3, mean ± SD).

Treatment	Transition temperature(°C)		Enthalpy(mj/mg)	
	Tm_1	Tm_2	ΔH_1	ΔH_2
Control	67.5±0.9	112±0.1	7.01±0.03	551.35±0.1
Labrazole	40±0.2	116±0.1	3.849±0.01	5.124±0.2
Labrafil	37±0.1	122±0.1	0.58±0.01	3.3±0.1
Labrafac	37.1±0.2	118±0.5	0.77±0.04	31.1±1.1
Oleic acid	36.1±0.1	124±0.5	4.29±0.3	5.89±0.4
Castor oil	35±0.2	123±0.4	1.86±0.1	2.67±0.2

Tm_1, mean transition temperature of lipids; Tm_2, mean transition temperature of irreversible denaturation of intracellular stratum corneum keratin; ΔH_1, transition enthalpy of lipid phase; ΔH_2, transition enthalpy of keratin phase.

The effect of increased absorption of labrazol on the skin of the rat shows a decrease in Tm1 and Tm2 and decrease of ΔH1 and ΔH2, indicating the effects of lipid imbalance and the irreversible denaturation of the protein structure on the horny layer of the skin as well as the fluidization of the lipid and lipid-protein complex in the horn tissue.

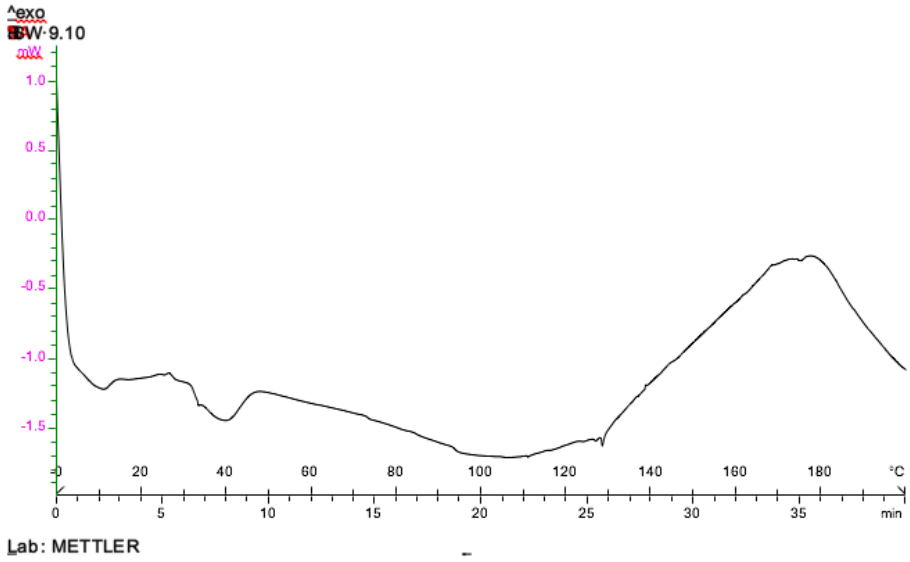

Figure 3: DSC thermogram of complete abdominal skin of the rat after contact with labrazol.

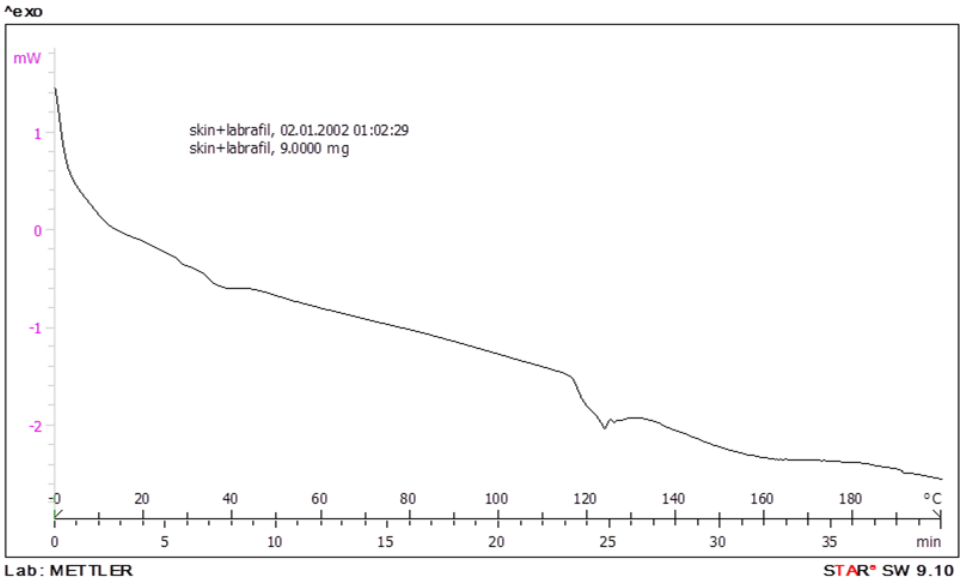

Figure 4: DSC thermogram of complete abdominal skin of the rat after contact with labrafil.

The results of DSC related to the effect of absorbing labrafac on the skin also showed a decrease in Tm_1 and Tm_2, and decrease in ΔH_1 and ΔH_2 levels as compared to control. These results also indicate a lipid layer disorder and interference with the keratin of the horn layer, as well as the fluidization of lipids and lipid-protein complexes.

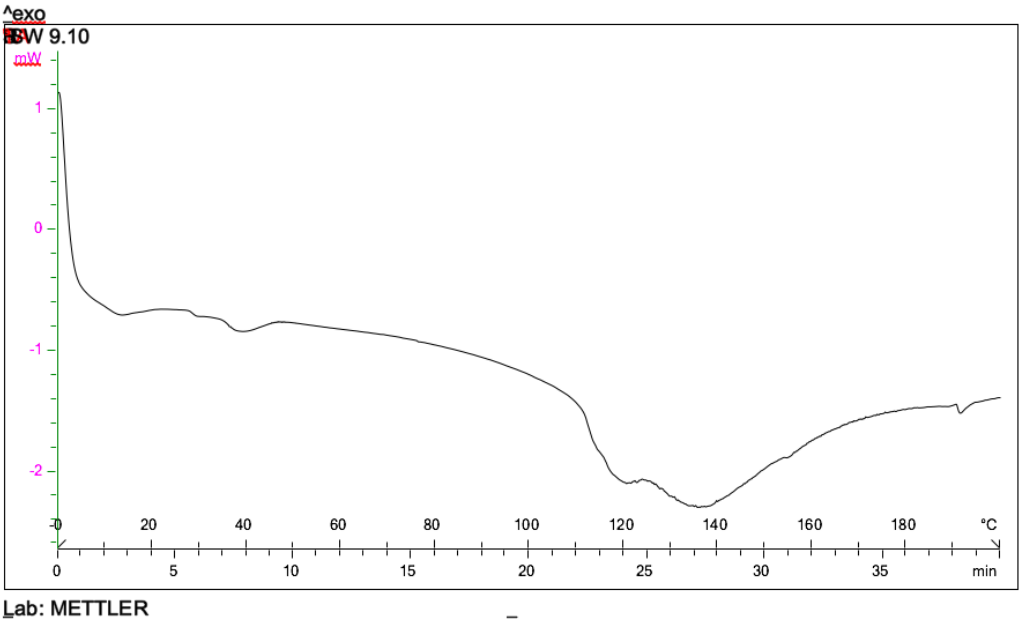

Figure 5: DSC thermogram of complete abdominal rat skin after contact with the labrafac.

DSC results from the effect of high oleic acid enhancers on the skin show a decrease in Tm_1 an increase in Tm_2, and decrease in ΔH_1 and ΔH_2 levels. Which indicates a denaturation in the lipid structure and fluidization of the lipid bilayer which has previously been proven by the researchers.

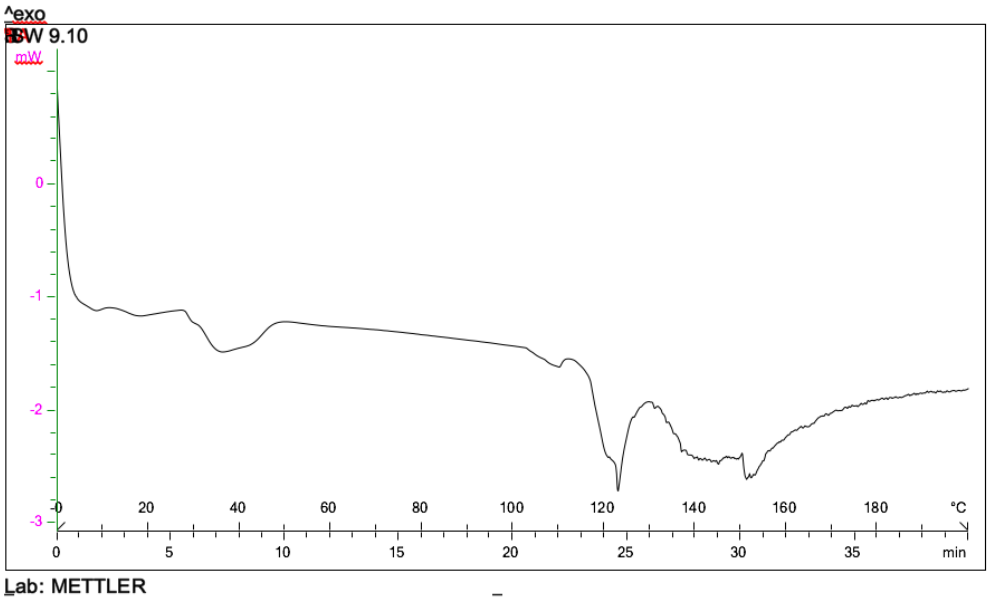

Figure 6: DSC thermogram of complete abdominal rat skin after contact with oleic acid.

DSC results of the effects of chemical enhancers of castor oil indicate a decrease in Tm_1 and Tm_2, and decrease in ΔH_1 and ΔH_2 levels, indicating impaired lipid layer and lipid fluidization in the bilayer and lipid-protein complexes.

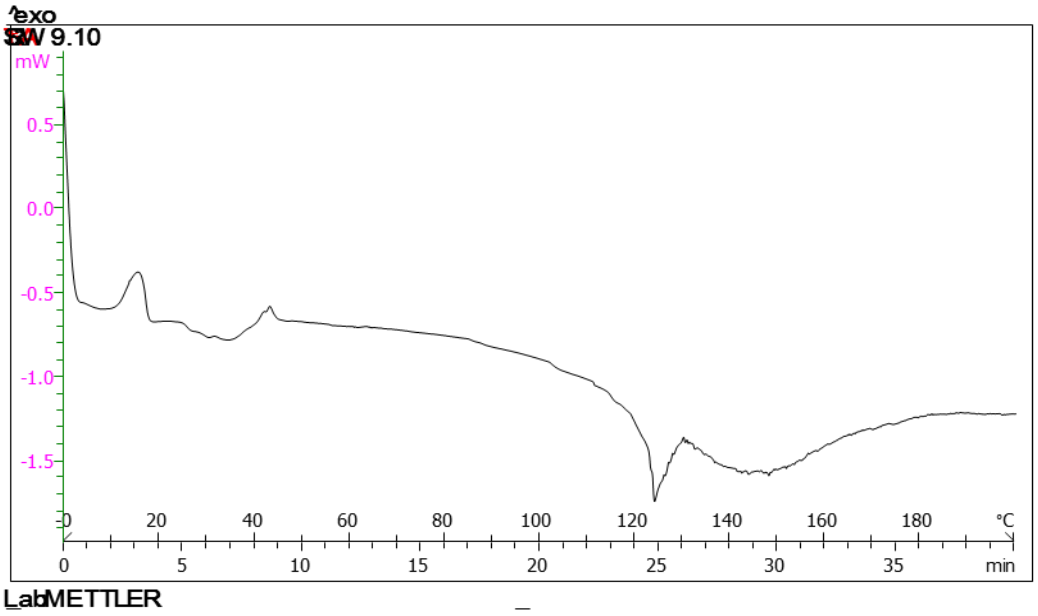

Figure 7: DSC thermogram of complete abdominal rat skin after contact with castor oil.

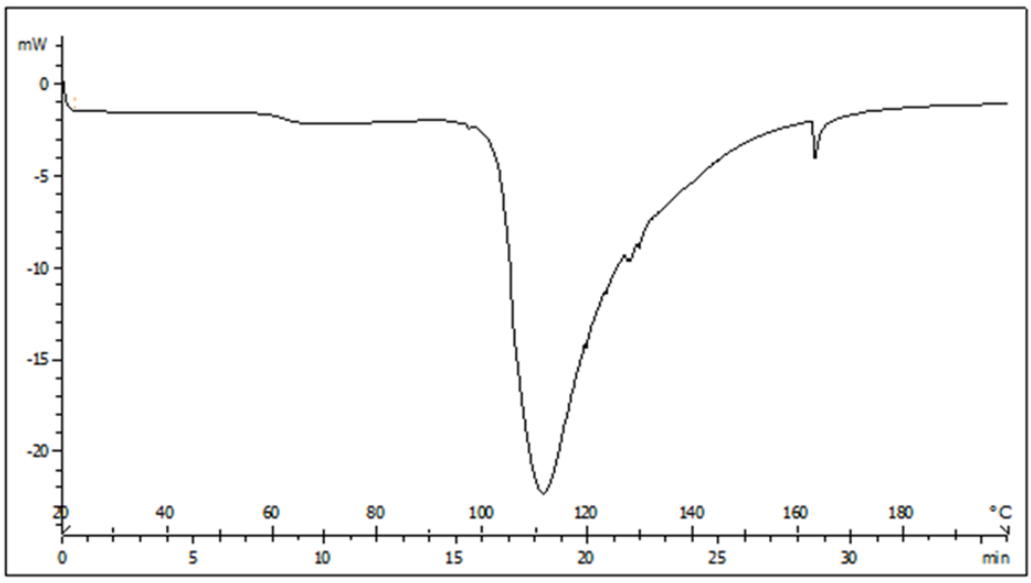

Figure 8: DSC thermogram of complete abdominal rat skin after contact with water.

The results showed that the highest absorption effect in terms of drug permeability has been obtained by labrafac and in terms of increasing the coefficient of distribution of oleic acid in relation to carvedilol. Treated chemical enhancers increases the amount of flux and distributability as compared to control, as well as absorption increases the time of the munition as compared to control. Among the chemical enhancers, castor oil has the weakest outcomes in increasing the permeability of the drug from the skin.

According to the results, it was found that labrafac had the highest rate of drug, 6.5 times higher than control group and castor oil which was 1.30 times of the control had the lowest rate of drug permeability.

The present study showed that the different chemical enhancers can alter the physicochemical and permeability parameters of the drug during permeation to rat skin. Although all the enhancers increased and improved permeability through the control, the best results was for labrafac and oleic acid, and the weakest effect was on castor oil.

REFERENCES

1. Moffat AC, Osselton MD, Widdop B, Galichet LY. Clarke's analysis of drugs and poisons. 2004.
2. Rizwan M, Aqil M, Azeem A, Talegaonkar S, Sultana Y, Ali A. Enhanced transdermal delivery of carvedilol using nanoemulsion as a vehicle. Journal of Experimental Nanoscience, 2010; 5(5): 390-411.

3. Ubaidulla U, Reddy MV, Ruckmani K, Ahmad FJ, Khar RK. Transdermal therapeutic system of carvedilol: effect of hydrophilic and hydrophobic matrix on in vitro and in vivo characteristics. AAPS Pharm Sci Tech., 2007; 8(1): E13-E20.
4. Möllendorff Ev, Reiff K, Neugebauer G. Pharmacokinetics and bioavailability of carvedilol, a vasodilating beta-blocker. European journal of clinical pharmacology, 1987; 33(5): 511-3.
5. Salimi A, Sharif Makhmal B, Hemati AS, Akbari Birgani A. Design and Evaluation of Self-Emulsifying Drug Delivery System (SEDDS) Of Carvedilol to Improve the Oral Absorption. Jundishapur J Nat Pharm Prod, 2014; 9(3): e16125.
6. Godin B, Touitou E. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Advanced drug delivery reviews, 2007; 5(11): 1152-61.
7. Pagliara A., et al., Evaluation and prediction of drug permeation. Journal of Pharmacy and Pharmacology, 1999; 51: 1339-1357.
8. Megrab NA, Williams A, Barry B. Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation. Journal of controlled release, 1995; 36(3): 277-94.
9. Schäfer-Korting, M., Mehnert, W., Korting, H. C. "Lipid Nanoparticles for Improved Topical Application of Drugs for Skin Diseases", Advanced Drug Delivery Reviews, 2007; 59: 427-443.
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. Mohammad Soleymani S, Salimi A. Enhancement of Dermal Delivery of Finasteride Using Microemulsion Systems. Adv Pharm Bull, 2019; 9(4): 584-592.
12. Salimi A, Sharif Makhmal Zadeh B, Godazgari S, Rahdar A. Development and Evaluation of Azelaic Acid-Loaded Microemulsion for Transfollicular Drug Delivery Through Guinea Pig Skin: A Mechanistic Study. Adv Pharm Bull, 2020; 10(2): 239-246.
13. Williams AC, Barry BW: Penetration enhancers. Adv Drug Deliv Rev., 2004; 56: 603–618.
14. Salimi A, Fouladi M, EFFECT OF THE VARIOUS PENETRATION ENHANCERS ON THE IN VITRO SKIN PERMEATION OF MELOXICAM THROUGH WHOLE RAT SKIN. ejbps, 2015; 2(3): 1282-1291.
15. MoghimipourE, Salimi1A, Changizi S. Preparation and Microstructural Characterization of Griseofulvin Microemulsions Using Different Experimental Methods: SAXS and DSC. Adv Pharm Bull, 2017; 7(2): 281-289.