

Effects of the Various Solvents on the *In vitro* Permeability of Indomethacin through Whole Abdominal Rat Skin

Anayatollah Salimi^{1*}, Eskandar Moghimipour¹ and Faride Rahmani¹

¹Department of Pharmaceutics, Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AS and EM designed the study, wrote the protocol. Author AS wrote the first draft of the manuscript, performed preliminary data analysis and interpreted the data. Author FR performed the experiment. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2015/13410

Editor(s):

(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.

Reviewers:

(1) Chika J. Mbah, Pharmaceutical & Medicinal Chemistry, University of Nigeria, Nsukka, Nigeria.

(2) James Omale, Department of Biochemistry, Kogi State University, Anyigba, Nigeria.

(3) Anonymous, Dalhousie University, Canada.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=702&id=32&aid=6527>

Original Research Article

Received 16th August 2014
Accepted 26th September 2014
Published 16th October 2014

ABSTRACT

Purpose: The aim of the present study was to evaluate the effect of some solvents on *in vitro* dermal absorption of indomethacin.

Methods: The effects of different solvents such as Plurololeique, Labrasol, Tween 80, Isopropyl myristate and PEG 400 on indomethacin permeation through pretreated rat skin were evaluated. A specially designed Franz diffusion cell was used and the data were compared to the results of hydrated abdominal rat skin as a common control. The measured permeability parameters included permeability coefficient (Kp), diffusion coefficient (D) and flux (Jss). Enhancement mechanisms were studied by comparing changes in peak wave number position and their intensities of asymmetric (Asy) and symmetric (Sym) C-H stretching, lipid ester carbonyl stretching in SC, C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance using Fourier transform infrared spectroscopy (FTIR). Using differential scanning calorimetry (DSC), their mean transition temperature (Tm) and enthalpies (ΔH) were also compared.

Results: All the solvent materials significantly increased flux (Jss) ($p < 0.05$), with isopropyl myristate

*Corresponding author: E-mail: anayatsalimi2003@yahoo.com;

showing the greatest enhancement ratio (ER_{flux}) based on flux followed by Tween 80, plurololeique, polyethylene glycol 400 and labrasol. The ER_{flux} for all the solvents was higher than ER_D . Plurololeique, isopropyl myristate and Tween 80 significantly increased diffusion coefficient ($p < 0.05$) compared to hydrated rat skin. Lipid fluidization, lipid disruption structure and the irreversible denaturation of proteins in the SC layer of skin by isopropyl myristate, Tween 80 and plurololeique, as indicated by FT-IR and DSC, is the main factor for higher ER_{flux} and ER_D ratios compared to control.

Keywords: Indomethacin; percutaneous absorption; skin permeation; diffusion coefficient; flux; enthalpy.

1. INTRODUCTION

The skin, our body's largest organ, is generally considered as an impermeable protective barrier against mechanical, chemical, microbial and physical hazards. The success of a topical drug to be used for systemic drug delivery depends on the ability of the drug to penetrate via skin in sufficient quantities to achieve the desired effect [1]. Nowadays, transdermal route has become one of the most successful strategies for systemic drug delivery.

Transdermal delivery has many advantages among them, avoidance of first pass metabolism of drug, controlled and continuous drug delivery, reduction of dosing frequency, patient compliance enhancement, facilitation of drug localization at target site, avoidance of the risks of injections and reduction in toxic level of drugs [2-6].

Skin permeation of drugs is slightly section of percutaneous absorption. Permeation is the penetration from one layer into another layer of skin. The lipid matrix of stratum corneum layer plays a meaningful role in determining the permeability of substances through the skin. The factors that influence transdermal absorption include biological properties of skin, physicochemical properties of drug, vehicle and dosing concentrations. The biological factors consist of thickness of the skin, regional site, age, blood flow rate and skin conditioning can influence the skin permeation of drugs. Moreover, the physiological characteristics of the drug and vehicle play a significant role in determining percutaneous absorption. These factors include the molecular properties of the drug and vehicle. Skin permeation can be improved by several strategies: increasing the diffusivity in the skin by disturbance of SC lipid matrix, strengthening drug solubility in the skin, enhancing the degree of saturation of the drug in formulation [7,8].

Permeation of drugs through skin is the principle of transdermal delivery. Passive permeation of drug through skin often depends on two major physicochemical properties such as, drug solubility and partition coefficient in SC. Two possible strategies for improving the drug permeation are reversible enhancement of solvent and modification of the drug thermodynamic activity [7]. Hereby, drug transdermal permeation may be affected by solvent type and drug molecule thermodynamic activity. So, selection of a suitable solvent for preparation of topical formulations greatly improves drug delivery.

Enhancer solvents such as water, propylene glycol and oleic acid are known as permeation enhancer by different mechanisms, including disruption of the organized intercellular lipid structure of the stratum corneum, fluidizing the membrane lipids, altering cellular proteins, and extracting intercellular lipids by mostly non-polar solvents. Increase in the diffusivity of stratum corneum has been reported for polar solvent materials, e.g., dimethyl sulfoxide and dimethyl formamide [2,6-8]. Recently, the lipid organization and microstructure of the skin has been examined using various techniques including DSC and FTIR [6,9-10].

Indomethacin is a non-steroidal anti-inflammatory drug widely used as analgesic, antipyretic and for relief of dysmenorrheal pain symptoms. The most widely reported side effect of indomethacin as a member of NSAID drugs is gastrointestinal ulceration, accompanied by anemia due to bleeding. Administration of drug via dermal route may eliminate gastric irritation, minimizes the systemic toxicity and achieve a better therapeutic response [11]. The use of a topical drug for dermal and transdermal drug delivery depends on the ability of the drug to penetrate via skin in sufficient quantities to achieve the desired effect [1].

The aim of this research was to evaluate the effect of several hydrophilic and hydrophobic solvents on *in vitro* skin permeability of indomethacin with a view to designing and developing a suitable transdermal drug delivery system for indomethacin.

2. MATERIALS AND METHODS

Indomethacin was purchased from Darou pakhsh Company (Tehran, Iran). Plurololeique and Labrasol were obtained as gift samples (Gattefosse, France). Isopropyl myristate, Tween 80 and PEG 400 were obtained from Merck (Germany).

2.1 Solubility Tests

The solubility of indomethacin was determined in Plurololeique, Labrasol, Tween 80, Isopropyl myristate and PEG 400 (polyethylene glycol 400). An excess amount of indomethacin was added to 5 ml of the solvent. The mixture was submerged in a water bath for 24 h at 37°C and allowed to equilibrate. Then, suspension mixture was centrifuged for 10 min at 3000 rpm, filtered, diluted and the dissolved drug was measured by a validated UV spectrophotometric method at 321 nm [12].

2.2 Animal Studies

Male adult Wistar rats (weighing 200 - 250 g) and aged 10 - 12 weeks were purchased from Animals Laboratory, Jundishapur University of Medical Sciences, Ahvaz, Iran. The hair on the abdominal skin was removed with an electric clipper, taking care not to damage the skin. Prior to sacrificing the rats, they were anaesthetized with ether and abdominal full-thickness skin was removed and any extraneous subcutaneous fat was cleaned from the dorsal side using cooled pure acetone solution. Whole skin thickness was measured using a digital micrometer. The animals were treated according to the principles for the care and use of laboratory animals. The procedures followed comply with standard international guidelines [13].

2.3 Differential Scanning Calorimetry (DSC)

The solvents, which induced changes in structure of whole skin, were determined using a DSC instrument (Mettler Toledo DSC¹ system). Approximately 5 – 10 mg of skin samples were

placed in hermetically sealed aluminum pans. Simultaneously, an empty hermetically sealed pan was used as a reference. Skin samples were exposed to heat ranging from 20 to 200°C (scan rate: 5°C/min). All experiments were carried out in triplicate. Enthalpy (ΔH) values were measured from endothermic and exothermic transitions of the thermo grams [2,10].

2.4 FT-IR experiments

The excised abdominal rat skin samples were treated with Plurololeique, Labrasol, Tween 80, Isopropyl myristate and PEG 400 for 24 h, vacuum-dried (650 mm Hg, 25±1°C) for 1 h and stored in desiccators to evaporate traces of solvent [13]. The skin samples scanned in the range of 4000 to 500 cm⁻¹ using an FT-IR facility (Uker, Vertex70, Germany) [2].

2.5 *In-vitro* Permeation Studies

In-vitro permeation studies were carried out using vertical glass Franz diffusion cells with an approximated effective diffusion area of 5.73 cm². The receptor compartment volume contained 30ml. Whole skin sample was hydrated and then mounted between the donor and receptor compartments of the cell with the stratum corneum facing the donor medium. Indomethacin (1% w/v), dissolved in the test solvent, was in the donor compartment and the receptor cell was filled with methanol: phosphate buffer (pH 7) (2:1). The Franz diffusion cell was placed and clamped in a 37±0.5°C water bath with a magnetic stirrer. The receptor medium was stirred with a small magnetic bead at 200 rpm. At predetermined time intervals (0.5, 1, 2, 3,....., 56 h), a 2ml sample was withdrawn from the receptor medium and immediately exchanged for an equivalent volume of fresh methanol : phosphate buffer (2:1) to maintain sink condition. The samples were filtered and the permeated amount of indomethacin was analyzed by UV spectroscopy method at 321nm. Each of the test solvents was used as blank [2,14].

2.6 Data Analysis of Permeations

Indomethacin concentration was corrected for sampling effects according to Eq-1 [7,8].

$$C_{n1}^1 = C_n(V_T/V_T - V_S)/C_{n-1}^1/C_{n-1} \quad (1)$$

where C_n^1 is the corrected concentration of the n^{th} sample, C_n is the observed concentration of

indomethacin in the n^{th} sample, C_{n-1} is the obtained concentration of indomethacin in the $(n-1)^{\text{th}}$ sample, V_T is the total volume of the receptor fluid medium and V_S is the volume of the withdrawn sample. The corrected indomethacin concentration divided by the area of the skin exposed to donor solution to calculate the cumulative amount of indomethacin permeated per unit area which was plotted against time. Steady-state flux ($\text{mg}/\text{cm}^2/\text{h}$) was calculated from the linear portion of the slope of the permeation curve. Permeability coefficient (K_p , cm/h) of indomethacin through the skin was calculated as in Eq-2 [7,8]:

$$K_p = J_{ss}/C_v \quad (2)$$

Where J_{ss} is steady-state flux and C_v is the initial concentration of indomethacin in the cell diffusion donor compartment.

2.7 Statistical Methods

Statistical comparisons were made using one-way ANOVA and $p < 0.05$ was considered statistically significant. Correlation analysis was performed by least square linear regression method. All statistical analyses were conducted using Minitab software (version 16.0).

3. RESULTS

3.1 Solubility Study

The results of solubility study are shown in Table 1. The results indicate the highest solubility in Tween 80 followed by PEG 400, labrasol, isopropyl myristate and pluroleique in that order.

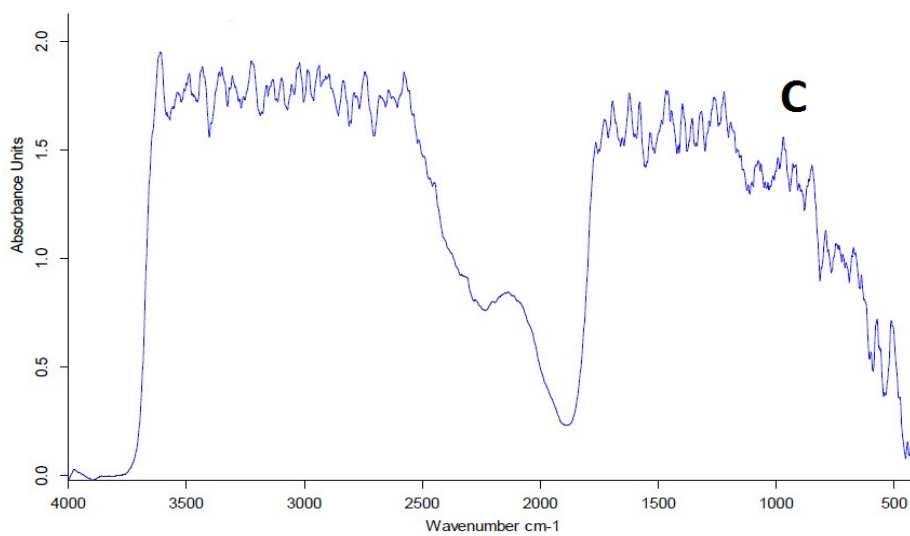
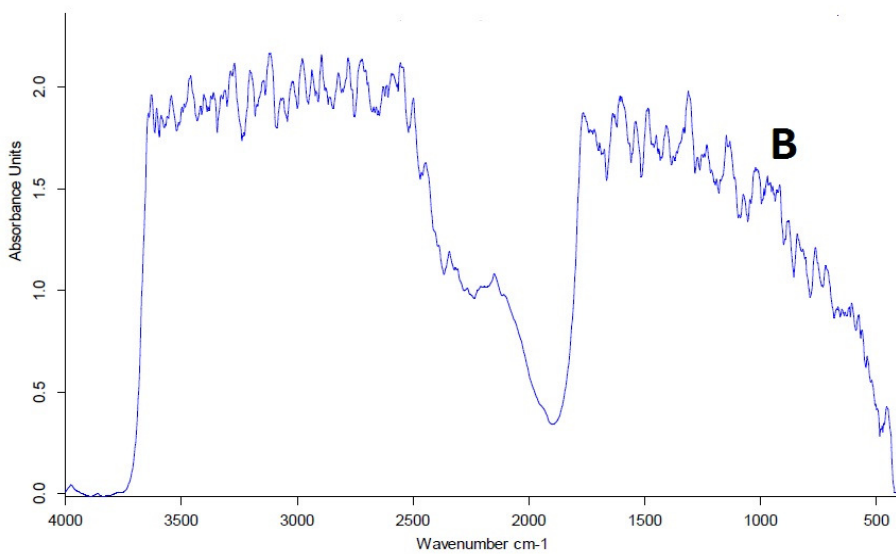
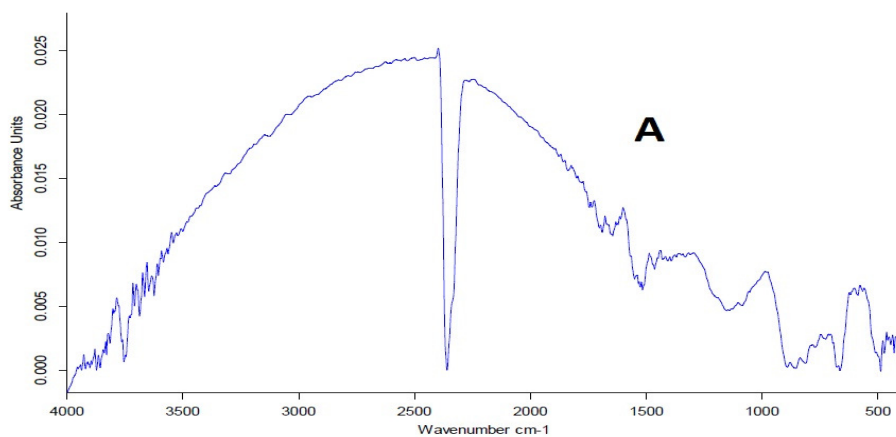
3.2 FT-IR

Spectral analysis involved examination of change in peak wave number position and their

intensities from 4000cm^{-1} - 500cm^{-1} (Fig. 1 and Table 2, Table 3, Table 4). The results indicated that hydrated whole rat skin (control) showed peak positions at 3610.46, 3417.59, 3310.20, 3255.39, 3200.08, 3105.44, 2948.02, 2821.56, 1723.62, 1652.78 and 1572.27cm^{-1} . The peak positions observed in the range of $3000 - 3600\text{cm}^{-1}$ display O-H and N-H stretching from lipid, protein and water while the peak positions near 2948.02 and 2821.65cm^{-1} represent asymmetric and symmetric stretching bands of the terminal methyl groups of lipids in rat skin [2]. The lipid ester carbonyl stretching in SC membrane showed at 1723.62cm^{-1} position while the bands observed at 1652.78 and 1572.27cm^{-1} represent amide I (C=O stretching) and amide II (C-N stretching) linkage of the helical secondary structure found in epidermal keratin [2,15-18]. The FT-IR spectra of PEG 400 treated whole abdominal rat skin represented significant decrease in peak height of the wave numbers (2952.9, 2475.62, 1724.34, 1677.75, 1539.97cm^{-1}) and any significant change in peak positions of wave number 2952.9, 1724.34 cm^{-1} . Also, the results showed red shift and blue shift at 2754.62, 1539.27cm^{-1} and 1539.97cm^{-1} , respectively. The IR spectra results of Tween 80 treated whole skin rat represented blue shifts and significant decreases in peak height of the wave numbers (3010.75, 2840.74, 1770.18, 1653, 1591.73cm^{-1}). The spectra for whole skin rat treated with labrasol presents red shifts and significant decrease in peak height of the wave numbers (2895.27, 2778, 1716.94, 1669.4 and 1591.82cm^{-1}). The FT-IR spectra of whole skin rat treated with pluroleique showed significant decrease in peak height of wave numbers (2936.97 , 2895.24, 1722.69, 1652.58, 1579.46cm^{-1}) and blue shifts at 2895.24, 1729.69, 1579.46cm^{-1} and red shift at 2936.97cm^{-1} .

Table1. Solubility of indomethacin indifferent solvents at 37 °C (mean±SD, n=3)

Solvent	Solubility(mg/ml)
Tween 80	9.61±0.13
PEG 400	8.42±0.22
Labrasol	7.37±0.09
Isopropyl myristate	6.53±0.06
Pluroleique	2.53±0.07



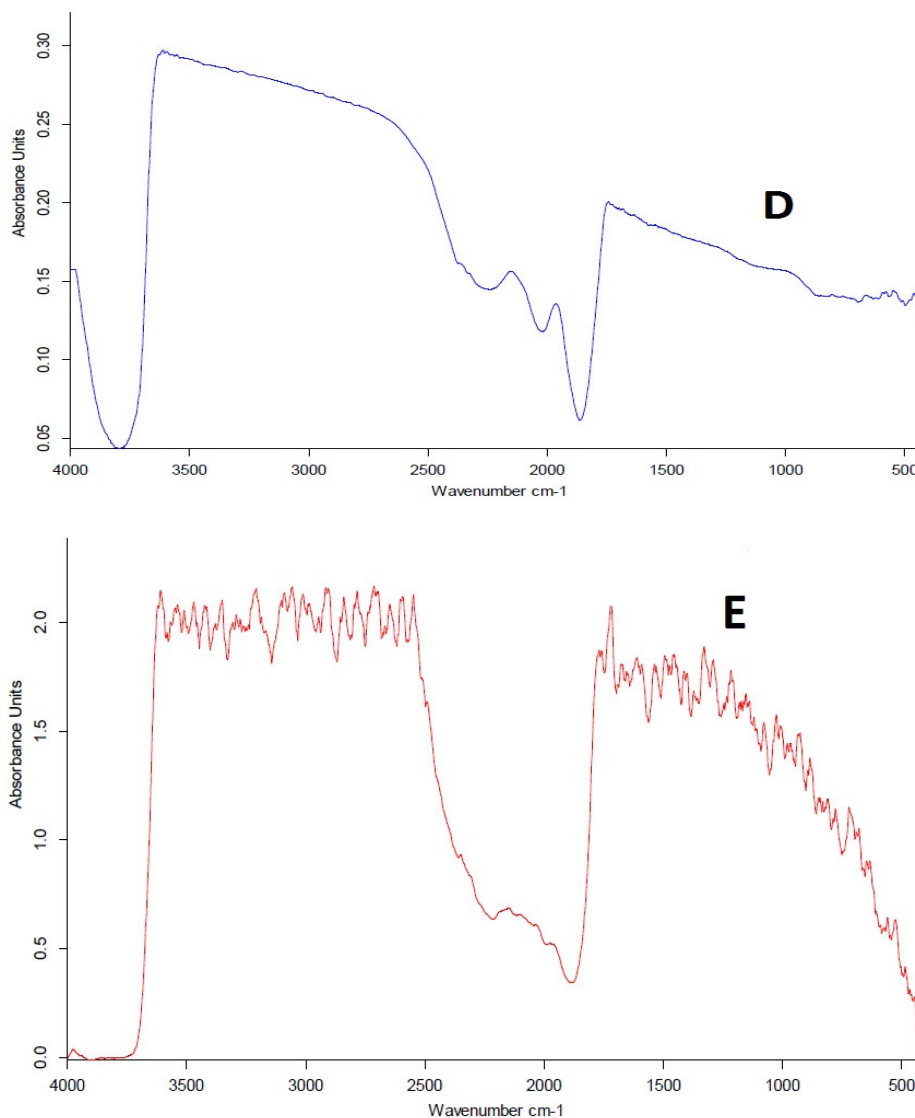


Fig. 1. FT-IR spectra of whole skin abdominal rat after treatment with (A) Polyethylene glycol 400 (B) Isopropyl myristate (C) Pluerololeique (D) Labrasol and (E) Tween 80

Table 2. Decrease in mean peak height (\pm SD), compared to control (hydrated skin) of asymmetric (Asy) and symmetric (Sym) C-H stretching and C=O stretching absorbance of abdominal hydrated whole skin rat following treatment with different solvents (mean \pm SD, n=3)

Solvent	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester	
	Peak height	%D	Peak height	%D	Peak height	%D
Control	4.877 \pm 0.0015	-	5.026 \pm 0.0026	-	4.999 \pm 0.0065	-
PEG 400	0.021 \pm 0.002	99.56 \pm 1.05	0.023 \pm 0.0041	99.54 \pm 1.547	0.013 \pm 0.002	99.73 \pm 1.41
Isopropyl myristate	0	100	1.95 \pm 0.041	61.2 \pm 1.004	1.874 \pm 0.018	62.5 \pm 1.015
Tween 80	2.129 \pm 0.066	56.34 \pm 1.84	2.115 \pm 0.055	57.91 \pm 1.804	1.872 \pm 0.025	62.55 \pm 1.215
Labrasol	2.092 \pm 0.008	57.1 \pm 1.93	1.999 \pm 0.078	59.01 \pm 2.11	1.944 \pm 0.045	61.1 \pm 1.312
Plurololeique	1.888 \pm 0.012	61.28 \pm 2.01	1.850 \pm 0.114	63.19 \pm 2.29	1.640 \pm 0.051	67.19 \pm 1.493

*Decrease Percent 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

Table 3. Decrease in mean peak height (\pm SD), compared to 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 solvents (mean \pm SD, n = 3)

Solvent	C=O stretching of keratin		C-N stretching of keratin	
	Peak height	%D	Peak height	%D
Control	4.952 \pm 0.021	-	4.840 \pm 0.045	-
PEG 400	0.012 \pm 0.002	99.75 \pm 1.211	0.007 \pm 0.0003	99.85 \pm 2.18
Isopropyl myristate	1.725 \pm 0.089	65.16 \pm 1.15	1.953 \pm 0.053	59.64 \pm 1.71
Tween 80	1.774 \pm 0.011	64.17 \pm 2.05	1.806 \pm 0.007	62.68 \pm 1.92
Labrasol	1.806 \pm 0.026	63.52 \pm 1.88	1.857 \pm 0.013	61.63 \pm 1.58
Plurololeique	1.555 \pm 0.064	68.598 \pm 2.014	1.719 \pm 0.037	64.48 \pm 1.71

Table 4. FT-IR Peak wave numbers (cm^{-1}) changes compared to control (untreated skin) and abdominal hydrated whole skin rat following treatment with different solvents. (mean \pm SD, n=3)

Solvent	C-H stretching Asy	C-H stretching Sym	C=O stretching of lipid ester	Amide I	Amide II
Control	2948.02 \pm 0.115	2821.65 \pm 0.42	1723.65 \pm 0.55	1652.78 \pm 0.35	1572.27 \pm 0.22
PEG 400	2952.9 \pm 0.6	2754.62 \pm 0.53	1724.34 \pm 0.45	1677.75 \pm 0.47	1539.97 \pm 0.45
Isopropyl myristate	deleted	2860.64 \pm 22	1764.86 \pm 0.42	1694.84 \pm 0.38	1599.9 \pm 0.62
Tween 80	3010.75 \pm 1.02	2840.74 \pm 0.27	1770.18 \pm 0.19	1653 \pm 0.9	1591.73 \pm 0.58
Labrasol	2895.27 \pm 0.62	2778 \pm 1.2	1716.94 \pm 0.62	1669.4 \pm 0.56	1591.82 \pm 0.72
Pleuroleique	2936.97 \pm 0.35	2895.24 \pm 1.4	1729.69 \pm 0.48	1652.58 \pm 0.81	1579.46 \pm 0.63

3.3 Differential Scanning Calorimetry (DSC)

Thermotropic states of skin treated with different solvents evaluated by comparing for mean transition temperature (T_m) and their enthalpies (ΔH). Table 5 shows the temperature transition and enthalpy amounts after solvent treatment and the thermograms are displayed in Fig. 2. Two endothermic transitions as obtained from the thermogram of hydrated whole rat skin were observed around 67.5 (T_{m1}) and 112°C (T_{m2}) were obtained in thermogram of hydrated whole rat skin. T_{m1} and T_{m2} transitions appeared to be due to melting of lipids and irreversible denaturation of intracellular keratin, respectively. Vaddi et al. reported three endothermic transition at 62, 79, and 95°C in the thermotropic behavior of rat skin [19], while Shakeel et al. [20], observed 4 endothermic transitions at 34, 82, 105, and 114°C.

Kaushik et al. studied the SC of human skin and observed three endothermic transition peaks at temperatures 59 - 63°C (T_{m1}), 75 - 82°C (T_{m2}) and 99.5 - 120°C (T_{m3}) [20]. It has been shown that T_{m1} corresponded to lipid transformation from a lamellar to disordered state, T_{m2} is due to the melting of lipid - protein (keratin) complex [22], or disruption of polar head groups of lipids

[20-23], T_{m3} is known to occur during the irreversible denaturation of proteins in the SC [20]. It was observed that T_{m1} was shifted by isopropyl myristate, labrasol and plurololeique to lower melting points and ΔH_1 decreased when compared to control. In addition, T_{m1} was removed by Tween 80, while T_{m1} was shifted by PEG400 to higher melting point and ΔH_1 increased in comparison with control. The results indicated that all of the solvents caused T_{m2} shift to higher melting points (with the exception of labrasol) and lower enthalpy that suggest possible denaturation of protein [2].

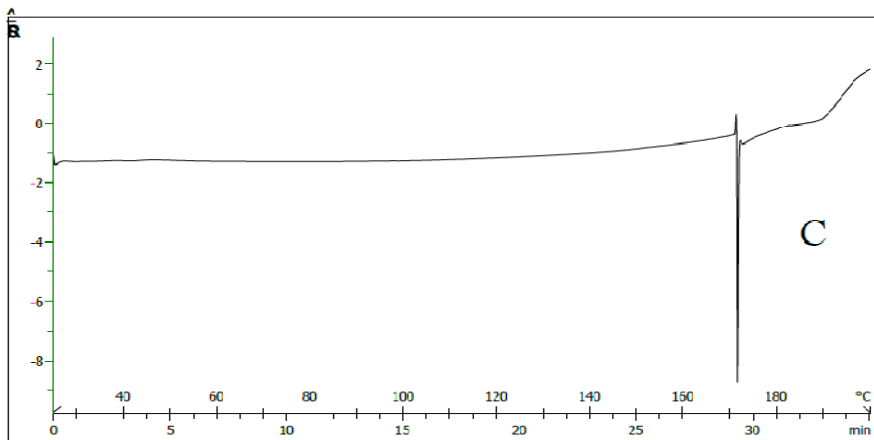
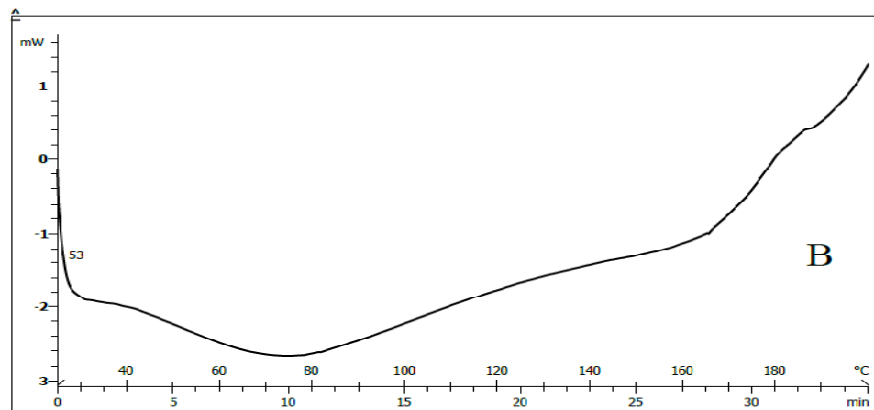
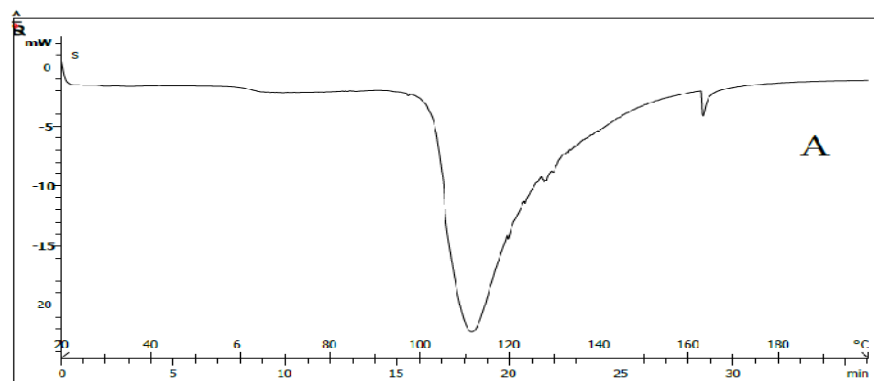
3.4 In vitro Skin Permeation of Indomethacin

The effect of solvents on indomethacin permeability with hydrated skin as control is expressed in Table 6 in which ER_{flux} is ratio of drug flux after and before skin pretreatment with solvent and ER_D is drug diffusion coefficient after and before skin pretreatment with solvents. All solvents increased permeability across rat skin compared to control. Isopropyl myristate provided the best effect on indomethacin flux, followed by Tween 80, plurololeique, PEG 400, and labrasol in that order.

Table 5. Effect of solvents on the transition temperature (°C) and transition enthalpy (j/g) of hydrated whole abdominal rat skin (mean ± SD, n = 3)

Treatment	Transition temperature (°C)		Transition treatment enthalpy (j/g)	
	Tm_1	Tm_2	ΔH_1	ΔH_2
Hydrated skin*	67.5±2.1	112±6.6	7.01±0.4	552.1±19.5
PEG 400	71±0.5	171.5±1.3	44.1±1.15	22.55±1.87
Isopropyl myristate	38±0.5	117±1.1	0.416±0.006	49.65±1.92
Tween 80	deleted	-	deleted	-
Labra sol	41±0.8	102±2.3	1.559±0.75	19.58±1.25
Plurololeique	39.7±0.6	121±0.5	1.191±0.24	2.757±0.710

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



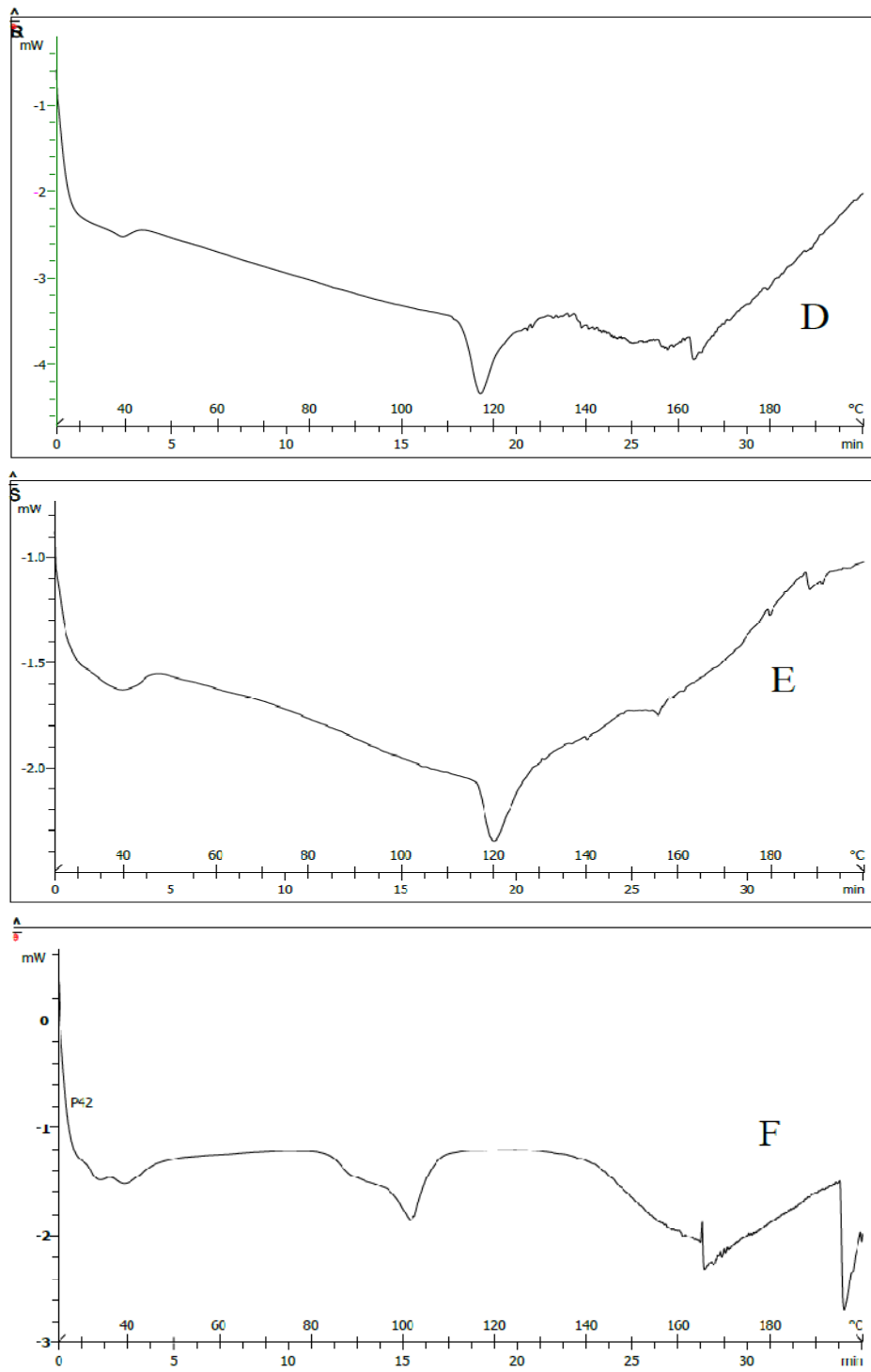


Fig. 2. DSC Thermograms of whole skin abdominal rat after treatment with (A) water (B) Polyethylene glycol 400 (C) Tween80 (D) Isopropyl myristate (E) Plurololeique (F) Labrasol

Table 6. Effect of solvents on derived permeability parameters for indomethacin (mean \pm SD, n = 3)

Solvent	ER _{flux}	ER _D	ER _p
PEG 400	4 \pm 0.33	1.050 \pm 0.660	0.045 \pm 0.003
Isopropyl myristate	18.37 \pm 0.61	2.089 \pm 0.494	0.1857 \pm 0.006
Tween 80	17.77 \pm 1.25	1.931 \pm 0.080	0.153 \pm 0.004
Labra sol	3.166 \pm 0.39	1.483 \pm 0.473	0.0386 \pm 0.001
Plurololeique	17.33 \pm 0.47	6.656 \pm 0.701	0.133 \pm 0.007

ER_{flux} = ratio of flux after and before treatment with solvent

ER_D = ratio of diffusion coefficient after and before treatment with solvent

ER_p = ratio of permeability coefficient after and before treatment with solvent

Significant increase in diffusion coefficient ($p < 0.05$) resulted with plurololeique with the highest ER_D followed by Isopropyl myristate, Tween 80, labrasol and PEG 400. ER_{flux} of all solvents were higher than their ER_D, indicating that the solvents increased flux more than they did diffusion coefficient. The solvents showed significant decrease in permeability coefficient ($p < 0.05$) compared to water solution of indomethacin.

4. DISCUSSION

The results show that all solvents increased drug flux through skin more than they did diffusion. Indomethacin falls in Biopharmaceutical Classification System (BCS) class II, so that the low solubility of indomethacin in water suggests that partitioning from stratum corneum to vital epidermis the rate-limiting step. All solvents with different lipophilic properties increased partitioning and flux from stratum corneum to vital periderm. The results of this study indicate significant correlation between drug solubility in various solvents and ER_D (ER_D = 9.7-1.07 drug solubility amount in solvents, $p = 0.036$) so that with decrease of drug solubility in solvent, ER_D value is increased. The low solubility of drug in solvents might have led to higher thermodynamic activity and hence less resistance of skin, thus resulting increase in diffusion coefficient. ER_D data after skin treatment with PEG400 correlate with FT-IR and DSC results as they indicate that PEG400 interacts mostly with SC keratins (ΔH_2 of skin treated with PEG400 less than control), and does not alter significantly SC lipid organization. The shift of 2821.65 cm^{-1} and 1572.27 cm^{-1} bands to a lower wave numbers (2754.62 cm^{-1} and 1539.97 cm^{-1}) provided red shift which indicates partial orientation of lipid groups leading to strengthening of SC barrier properties. Treatment with penetration modifiers may have led to extraction of lipids from SC membrane as usually occurs with enhancers. Increasing another probable mechanism is the intensity at the particular band representing retardation in

the case of retardants [2,15]. Lipid fluidization, lipid disruption and the irreversible denaturation of proteins in the SC layer of skin by isopropyl myristate, Tween 80 and plurololeique, as indicated by FT-IR and DSC, are the main factors for higher ER_{flux} and ER_D ratios in comparing to control. Group Increase in enthalpy and transition T_{m1} by PEG 400 may be due to bilayer cohesion, which is in contrast to lipid extraction and fluidization. The shift in T_m to lower temperatures can be interpreted as disruption of the lipid bilayer and the irreversible denaturation of proteins in the SC layer of skin, while decrease in ΔH is related to fluidization of lipid in lipid bilayer and lipid – protein complex [2,19-26].

The spectra for whole skin rat treated with labrasol obtained the relative red shift in 1716.94 cm^{-1} band that indicates formation of strong hydrogen bonds within the lipid molecules leading to strengthening of SC barrier properties, thus resulting in decrease in diffusion coefficient. Isopropyl myristate is hydrophobic and hence miscible with lipid, thus resulting in decrease in the melting point of the stratum corneum lipids. Plurololeique is a water-in-oil surfactant with HLB equal to 6 and form water-in -oil emulsion and can act as oily phase and hence miscible with lipid. Therefore, it may cause lipid disruption, lipid fluidization in the SC layer of skin.

5. CONCLUSION

The results obtained indicate that all solvents tested increased flux and diffusion coefficient across abdominal rat skin. Lipid fluidization, disruption lipid structure, lipid extraction and the irreversible denaturation of proteins in the SC membrane by isopropyl myristate, Tween 80 and plurololeique are the main factors for higher ER_D in comparison to polyethylene glycol 400 and labrasol.

ACKNOWLEDGEMENTS

This paper is derived from the pharm D thesis of one of the authors (Faride Rahmani). Ahvaz Jundishapur University of Medical Sciences is acknowledged for providing financial support. We wish to gratefully thank the Dr. Abdolghani Ameri who helped us to manuscript editing and review. The authors are very thankful to the editors and four anonymous reviewers for their useful comments that greatly improved this paper.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Mission L. Carriers/vesicles based approaches for penetration enhancement in transdermal drug delivery system, Indian Pharm Industrial Directory. 2009;9:1-9.
- Moghimipour E, Salimi A, Sharif Makhmalzadeh B. Effect of the Various Solvents on the *In vitro* Permeability of Vitamin B12 through Excised Rat Skin. Trop J Pharm Res. 2013;12(5):671-677.
- Snigdha B, Vipin Kumar G, Sharma P K, Bansal M, Kumar N. Recent advancement in transdermal drug delivery system. Inter J Pharma Professional Res. 2011;2(1):247-254.
- Rosado C, Monteiro Rodrigues L. Solvent effects in permeation assessed *in vivo* by skin surface biopsy. BMC Dermatol. 2003;3:1-6.
- Trommer H, Neubert RHH. Overcoming the stratum corneum: The modulation of skin penetration. Skin Pharmacol Physiol. 2006;19:106-121.
- Kumar R, Philip A. Modified transdermal technologies: Breaking the barriers of drug permeation via the skin. Trop J Pharm Res. 2007;6(1):663-644.
- Salimi A, Sharif Makhmal Zadeh B, Safavi B. Effect of formulation components on the *In vitro* skin permeation of micro emulsion drug delivery system of piroxicam. Int. Res J Pharm. App Sci. 2013;3(4):152-160.
- 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):103-112.
- Schulman JH, Hoar TP. Transparent water-in-oil dispersions: The oleopathic Hydromicelle. Nature. 1943;152:102.
- Moghimipour E, Salimi A, Eftekhari S. Design and characterization of microemulsion systems for naproxen. Advanced Pharmaceutical Bulletin. 2013;3(1):63-71
- Abhay S. Chauhan , Sridevi S, Kishore B. Chalasani , Akhlesh K. Jain ,Sanjay K. Jain, Jain NK, Prakash V. Diwan. Dendrimer-mediated transdermal delivery: Enhanced bioavailability of indomethacin. Journal of Controlled Release. 2003;90:335–34313.
- Salimi A, Sharif Makhmal Zadeh B, Moghimipour E. Preparation and characterization of cyanocobalamin (Vit B12) microemulsion properties and structure for topical and transdermal application. Iran J Basic Med Sci. 2013;6(7):865-872.
- National Institutes of Health, USA. Public Health Service Policy on Human Care and Use of laboratory animals; 2002.
- Salami A, Moghimipour E, Tavakolbekhoda N. Transdermal delivery of celecoxib through rat skin from various micro emulsions. Int Res J Pharm. App Sci. 2013;3(4):173-181.
- Obata Y, et al. Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers. Int J Pharm. 2010;38:18-23.
- Boncheva M, Damien F, Nomand V. Molecular organization of the lipid matrix in intact stratum corneum using ATR-FTIR spectroscopy. Biochim Biophys Acta (BBB) - Bio membranes. 2008;1775(5):1344-1355.
- Izhar A. Ansari, Faiyaz HM Vaid, Iqbal Ahmad. Spectral study of photolysis of aqueous cyanocobalamin solutions in presence of vitamins B and C. Pakistan J Pharma Sci. 2004;17(2):93-99.
- Sohonon TM, Bouwstra JA, Urtti A. Chemical enhancement of percutaneous absorption in relation to stratum corneum. J Control Release. 1999;59:146-161.
- Vaddi HK, Ho PC, Chan SY. Terpens 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):1639-1651.

20. Shakeel F, Boboota S, Ahuja A, Javed A, Sheikh S. Skin permeation mechanism and bioavailability enhancement of celecoxib from transdermally applied nanoemulsion. *J Nanobiotech.* 2008;6(8):1-11.
21. Kaushik D, Michniak-Kohn B. Percutaneous penetration modifiers and formulation effects: Thermal and spectral analyses, *AAPS Pharm Sci Tech.* 2010;11(3):1068-1083.
22. Kaushik D, Michniak-Kohn B. Percutaneous penetration modifiers and formulation effects: Thermal and spectral analyses, *AAPS Pharm Sci Tech.* 2010;11(3):1068-1083.
23. Golden GM, Guzek DB, Harris RR, Mickie JE, Potts RO. Lipid thermotropic transition human stratum corneum. *J Invest Dermatol.* 1986;86:255-259.
24. Al-Saidan SM, Barry BW, Williams AC. Differential scanning calorimetry of human and animal stratum corneum membranes. *Int J Pharm.* 1998;168:17- 22.
25. Barry BW. Mode of action of penetration enhancers in human skin. *J Control Release.* 1987;6:85-97.
26. Vaddi HK, Ho PC, Chan WY, Chan SY. Terpenes in ethanol: Haloperidol permeation and partition through human skin and stratum corneum changes. *J Control Release.* 2002;81:121-133.

© 2015 Salimi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=702&id=32&aid=6527>