

Meloxicam transdermal delivery: effect of eutectic point on the rate and extent of skin permeation

Soliman Mohammadi-Samani^{1, 2}, Gholamhossein Yousefi^{1, 2}, Farhad Mohammadi¹, Fatemeh Ahmadi^{1, 2*}

¹Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

²Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: May 6, 2013
Accepted: Oct 3, 2013

Keywords:
Differential scanning calorimetry
Eutectic mixture
Meloxicam
Skin permeation enhancer
Thymol

ABSTRACT

Objective(s): Drug delivery through the skin can transfer therapeutic levels of drugs for pharmacological effects. Analgesics such as NSAIDs have gastrointestinal side effects and topical dosage forms of these drugs are mainly preferred, especially for local pains. Meloxicam is one of NSAIDs with no topical form in the market. In this research, we attempted to quantify the skin permeation of a meloxicam topical preparation and to show how permeation would be increased by using thymol as an enhancer. The effect of eutectic point of drug and thymol mixture on rate and extent of skin permeation was also studied.

Materials and Methods: Different mixtures of thymol and meloxicam (2:8, 4:6, 5:5, 6:4, 8:2) were prepared and their melting point were obtained by differential scanning calorimetry. Then drug permeation was measured using diffusion cells and the Guinea pig skin.

Results: Mixtures in ratios 5:5 and 4:6 of meloxicam / thymol showed a new endotherm at 149 and 140°C in DSC thermograms. The permeability of meloxicam from the creams containing 6:4, 5:5 and 4:6 ratios of meloxicam to thymol were 4.71, 15.2, 22.06 µg/cm² respectively. This was significantly different from the cream of pure meloxicam (3.76 µg/cm²).

Conclusion: This study set out to determine that thymol plays as a skin permeation enhancer and increases the meloxicam skin absorption and this enhancement is significant at the eutectic point of drug-enhancer mixture.

► Please cite this paper as:

Mohammadi-Samani S, Yousefi GH, Mohammadi F, Ahmadi F. Meloxicam transdermal delivery: effect of eutectic point on the rate and extent of skin permeation. *Iran J Basic Med Sci*; 2014; 17:112-118.

Introduction

Patient compliance has always been one of the major concerns in development of pharmaceutical formulations. To overcome the limitations of the systemic administration of drug including oral and parenteral administration, alternative routes such as topical administration are considered. Transdermal drug delivery is one of these patient-friendly methods of drug delivery which provides several advantages over oral and injectable routes (1). These advantages include decreasing the food-drug interaction, premature metabolism in the gut wall and liver, avoiding needles and subsequent complications as well as the large surface area available for systemic and controlled drug delivery (2-4). Indeed, by transdermal delivery of drugs such as NSAIDs, risk of gastrointestinal (GI) adverse effects is reduced (5, 6). Meloxicam is one of NSAIDs which is reported to inhibit COX II more selectively than other NSAIDs. It is used as analgesic in treatment of osteoarthritis and rheumatoid arthritis and also acts as an antipyretic agent (5, 7). Meloxicam is less toxic than similar NSAIDs

and more people are tolerant to its side effects; however, the use of drug is still limited by its GI side effects and low aqueous solubility (8-10). Transdermal delivery of meloxicam can also provide steady plasma levels (11). Therefore, it could be an alternative route of administration for decreasing side effects and increasing patient compliance for local and systemic effects. Meloxicam presents lower skin irritation and toxicity than other NSAIDs (5), but the main obstacle for transdermal delivery of this drug is limited permeation through stratum corneum. Different approaches have been applied to modify the skin barrier properties including physical and chemical methods. Physical methods utilize magnetic, ultrasound, electric current or high velocity particles and some other physical energy input to change the permeation of drugs. Nevertheless, in chemical methods, chemical entities are basically involved, which are able to penetrate and disorganize the skin barrier (3, 12). Among different chemical enhancers tested, terpenes, terpenoids and essential oils are the most popular ones for transdermal delivery (3). The

*Corresponding author: Fatemeh Ahmadi. Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-711-2424128; Fax: +98-711-2424126; email: ahmadi_f@sums.ac.ir

effect of these compounds including menthol, thymol, cineol, etc has been examined on transdermal delivery of ibuprofen (13), tetracaine (14), testosterone (15) and zidovudine (16). Studies showed that terpenes exert these effects by changing the high regularity of the lipids of stratum corneum (17) as well as the formation of eutectic mixture with drugs which decreases their melting point and increases their thermodynamic activity in the skin (18). Eutectic mixture is a combination of two chemicals with lower melting point than each of the components and the higher transmembrane permeation (19).

Taking all of these into consideration, we aimed to design a formulation of meloxicam for transdermal delivery using thymol as the penetration enhancer. Differential scanning calorimetry (DSC) was applied to detect interaction of drug with thymol in different ratios. Then the optimum ratio was used for transdermal formulation and the *in vitro* skin permeation of the formulation was assessed using Franz-cell diffusion test.

Materials and Methods

Materials

Meloxicam was supplied by Iran Hormone Pharm Co (Tehran, Iran). Thymol, Tween 80, sodium hydroxide, sodium lauryl sulfate (SLS), acetic acid, chloroform and HPLC grade acetonitrile were purchased from Merck chemical company (Darmstadt, Germany). Carbopol was obtained from BF Goodrich (USA), liquid paraffin was received from Kian Kaveh pharmaceutical and chemicals industries (Iran). All other chemicals and materials were of analytical grade.

Preparation of meloxicam-thymol mixtures

To evaluate the interaction of drug with penetration enhancer, the two components should be mixed in molecular level (13). Two methods were applied for preparing meloxicam-thymol mixtures. In the first method, meloxicam and thymol in ratios of 8:2, 6:4, 5:5, 4:6, 2:8 were mixed in solid state by mortar and pestle. Total weight of the mixture was kept constant at 50mg. In the second method, solid mixtures of meloxicam and thymol was made by dissolving the components in 10 ml chloroform and keeping the mixture at room temperature for 24 hr until the solvent was fully evaporated. Then, the resulting solid residue was used as meloxicam thymol mixture.

To prevent thymol volatility, mixing of thymol and meloxicam was performed very slowly and in a room with controlled temperature. Following that, minimum pressure was applied during mixing to prevent heating of the mixture. Formulation of meloxicam-thymol mixture in cream also prevents the volatile content from sublimation.

Differential scanning calorimetry (DSC)

DSC studies were performed to determine the thermal behavior of meloxicam, thymol and their interaction upon mixing. DSC thermograms were recorded using DSC apparatus BÄHR Thermoanalyse GmbH, Type 302. Sample (5 mg) was sealed in an aluminum pan and DSC was run against a similar pan containing alumina as the reference material. Then, samples were heated from 20 to 270°C at heating rate 5°C/min. DSC was calibrated by indium (melting point, 156.6 ± 0.2°C) as a standard. All DSC runs were performed in triplicates.

Preparation of water in oil cream of meloxicam-thymol mixture

A w/o cream of meloxicam-thymol was prepared. Aqueous phase consisted of tween 80, distilled water and carbopol and oil phase contained liquid paraffin and meloxicam-thymol mixtures. The aqueous phase was added into the oil phase and mixed by turbine mixer and NaOH solution 0.2 N was added to provide the required consistency and viscosity by neutralizing carbopol. Details of cream formulations are presented in Table 1.

In vitro skin permeation test

Guinea pig abdominal skin was used for skin permeation test. Guinea pigs were euthanized with high dose of ether inhalation and the abdominal skin hair was shaved and the skin was cut and detached using a surgical knife. Skin was then fixed between the donor and acceptor compartment of a homemade Franz diffusion cell. Three cells with capacity 40 ml and diffusion area 5.72 cm² were connected serially to circulation pump to maintain the temperature of the diffusion cells at 34°C and receptor phase consisted of 1% SLS solution in water to preserve sink condition. For determining the solubility of meloxicam in receptor phase, a supersaturated solution of meloxicam in SLS solution was prepared. For this, excess amount of meloxicam was added to SLS solution on the stirrer and was stirred until no

Table 1. Composition of cream formulations prepared from different ratios of thymol-meloxicam

Cream formulations	Amount of ingredients (g)					
	Meloxicam	Thymol	Tween 80	Carbopol	Liquid paraffin	Water
Thymol-meloxicam (8:2)	0.2	0.8	0.2	0.1	4	16
Thymol-meloxicam (6:4)	0.2	0.3	0.2	0.1	4	16
Thymol-meloxicam (5:5)	0.2	0.2	0.2	0.1	4	16
Thymol-meloxicam (4:6)	0.2	0.13	0.2	0.1	4	16
Thymol-meloxicam (2:8)	0.2	0.05	0.2	0.1	4	16
Meloxicam	0.2	-	0.2	0.1	4	16

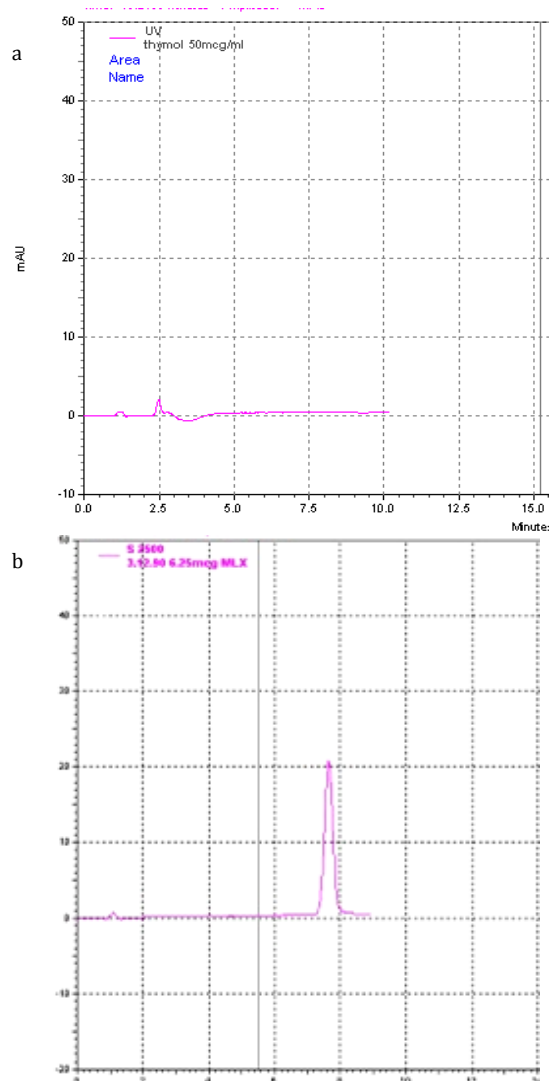


Figure 1. Representative chromatograms of solutions of a) thymol (50 µg/ml) and b) meloxicam (6.25 µg/ml) in acetonitrile

change in concentration determined by HPLC was observed. Then the mixture was filtered and drug solubility in SLS was measured by HPLC. This experiment was repeated three times.

After temperature equilibrium, 200 mg samples of the test cream were rubbed on the epidermal surface of the fixed skin. Five ml samples of the receptor phase were withdrawn at 15, 30, 60, 120, 240, 360 and 480 min and aliquot amounts of fresh buffer was substituted. The samples were kept in -20°C freezer and the concentration of the drug in the samples were analyzed using a Knauer HPLC.

High performance liquid chromatography (HPLC)

Determination of meloxicam was performed by HPLC. The chromatographic system used was Knauer (Smartline®, Model 1000, Germany) equipped with UV detector. Data was integrated using Eurochrome software for HPLC (Knauer, Germany). The HPLC method was developed according to previous studies

Table 2. Melting points of meloxicam and thymol in mixtures determined by DSC

Chemical composition	Melting points °C (T onset)	
	Mixing in dry state	Mixing in solution
Thymol	48.9	-
Thymol-meloxicam (8:2)	246.7	232.5
Thymol-meloxicam (6:4)	140.2	225
Thymol-meloxicam (5:5)	149, 211.9	209.9
Thymol-meloxicam (4:6)	223	211.6
Thymol-meloxicam (2:8)	210.9	219.9
Meloxicam	240.9	-

with some modifications (20-22). Mobile phase was consisted of acetonitrile: water 50:50 (pH 3) which pumped to the column at flow rate 1.2 ml/min. A C₁₈ column (150 mm×4.6 mm) was used and detection wavelength was set to 355 nm. Mobile phase was prepared freshly every day and degassed using vacuum filtration and ultrasonication.

Linearity of the method was tested by constructing calibration curve of the standard solutions in the concentration range 0.01-50 µg/ml. Reproducibility and precision of the method was also examined by calculating inter- and intra-day variations.

Results

HPLC analysis

The developed HPLC method was linear in the concentration range 0.01-50 µg/ml and the obtained regression factor (R^2) was 0.9989. The peaks of meloxicam and thymol were completely separated by the developed method without any interference (retention times of thymol and meloxicam were 2.5 and 7.5 min, respectively). Precision of the method was evaluated by repeated injections of the known concentrations of the drug and also calculating the inter- and intra-day variations. The results justify that for all concentrations injected to the system, the variation was less than 10%. Representative chromatograms of meloxicam and thymol are depicted in Figure 1.

DSC studies

Meloxicam and thymol melting point was found to be at 240.9 and 48.9 °C, respectively. By increasing the ratio of thymol to meloxicam in mixtures, the melting point of the drug was decreased. There was not a significant difference in the melting points between two mixing methods. Mixtures containing 2:8 and 4:6 ratios of meloxicam to thymol presented lowest melting point. DSC thermograms of the pure drug and the binary mixtures are shown in Figure 2 and the data are summarized in Table 2.

Three ratios 4:6, 5:5 and 6:4 of meloxicam-thymol which presented lower melting points and seems to provide higher skin permeation were selected for skin permeation tests.

Skin permeation test

Meloxicam is practically insoluble in water and

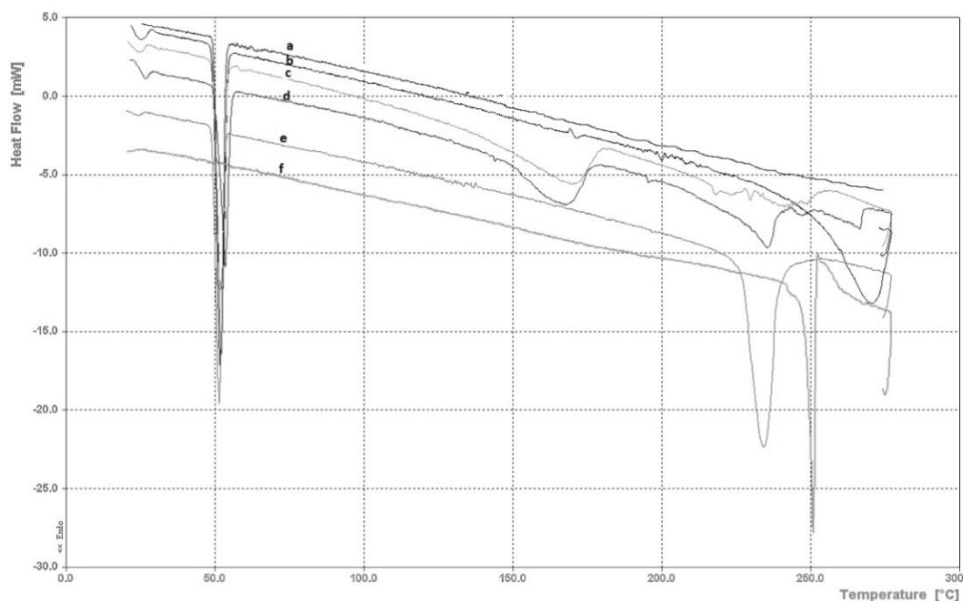


Figure 2. DSC thermograms of a) pure thymol, b) thymol-meloxicam 8:2, c) thymol-meloxicam 6:4, d) thymol-meloxicam 5:5, e) thymol-meloxicam 4:6 and f) pure meloxicam

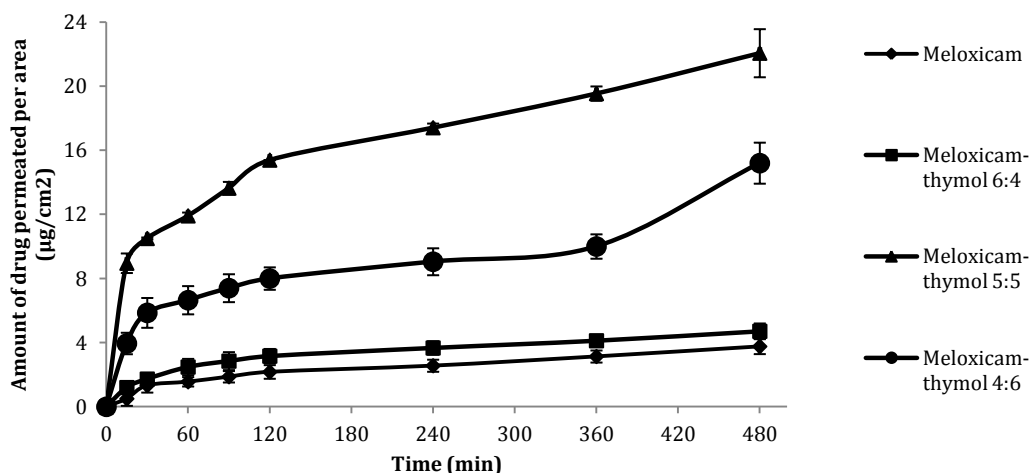


Figure 3. Permeation profile of the drug from the creams prepared by drug alone and different drug-thymol mixtures

its aqueous solubility has been reported to be 8 µg/ml (23). To preserve the sink condition in the receptor phase of diffusion cell, 1% solution of SLS in water was used as the receptor phase and the solubility of meloxicam in this medium was 46 µg/ml.

Results of skin permeation of the cream contained 1% meloxicam showed that the net amount of drug permeated through Guinea pig skin per area of diffusion was 3.76 µg/cm². Three different cream formulations were prepared from thymol-meloxicam mixtures in the ratio 4:6, 5:5 and 6:4 which were showing the higher melting point depression among the other ratios of meloxicam: thymol and were examined in skin permeation test. The permeation profiles of the drug in the creams prepared from drug alone and drug-thymol mixtures are presented in Figure 3. Results showed that by mixing the drug with thymol, the net amount of drug permeated through the skin was increased

significantly compared with the drug alone. Net amount of permeation per area of the diffusion was 4.71 µg/cm², 15.2 µg/cm² and 22.06 µg/cm² for 6:4, 5:5 and 4:6 ratios of meloxicam to thymol. The extent of this permeation enhancement was dependent on the amount of the absorption enhancer in the mixture and the melting point depression of the drug.

A histogram in Figure 4 represents the enhancement ratio of transdermal permeation of meloxicam per diffusion area in different ratios to thymol compared with the cream contained only meloxicam. The presence of thymol also decreased the lag time of permeation of drug and in ratio 5:5 of meloxicam to thymol, the extent of permeation at the first sampling time was 5-times higher than the drug alone. Figure states the effect of thymol as skin absorption enhancer in these formulations.

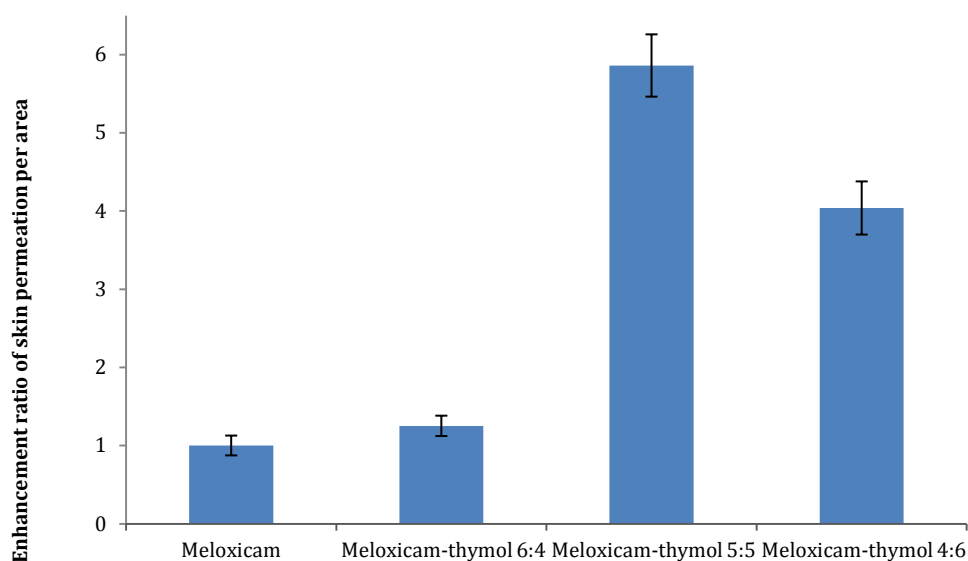


Figure 4. Histogram of enhancement ratio of transdermal permeation of creams containing meloxicam in mixtures with thymol compared with meloxicam alone (calculated from ratio of net flux of each formulation to the formulation containing meloxicam alone)

Discussion

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that is used in a wide range of inflammatory diseases. Side effects of meloxicam limit the use of drug and designing topical formulations can limit meloxicam adverse effects. Meloxicam and thymol are lipophilic materials and have proper solubility in non-polar solvents. Therefore, in the present study it was decided to use oil in water cream with minimum portion of oil phase to maximize the loading of lipophilic drugs in the cream as well as decreasing the risk of retention of drug in the oil phase. For designing the receptor phase for skin permeation test, the low aqueous solubility of meloxicam was a limiting factor. The skin permeation of meloxicam may be enhanced with enhancers that change skin lipids regularity and conformation. Moreover, mixing these chemicals such as terpenoids and essential oils with drugs provide opportunity of formation of eutectic mixture and lowering melting point (3, 17). Among different terpenoids, thymol was used as the permeation enhancer, as there are some reports on the efficacy of this terpene for increasing transdermal delivery along with the use of thymol as an ingredient in topical lotions, creams and solutions. In another study on percutaneous absorption of tamoxifen, it was reported that thymol can increase the partitioning of drug in stratum corneum (24). It is also a lipophilic terpene ($\text{Log } P \ 3.28 \pm 0.20$) which is a good option for increasing permeation of lipophilic drugs such as meloxicam (25). Eutectic mixture is a combination of two or more chemicals which forms a composition with lower melting point (26).

According to DSC data in Figure 2 and Table 2, thymol is melted at 48-49°C and this melting endotherm is not displaced significantly in any of the mixtures with meloxicam. Meloxicam in pure form is melted at 241°C, but by adding thymol to meloxicam, melting point gradually decreased to 238, 223, 212, 223 and 211°C for meloxicam-thymol ratios of 8:2, 6:4, 5:5, 4:6 and 2:8, respectively. The interesting point in DSC thermograms, is new endothermic peaks at 149 and 140°C which were observed in DSC results of mixtures containing ratios 5:5 and 4:6 of meloxicam to thymol. This pattern is very similar to eutectic composition with excess of one of the chemicals in the mixture (here thymol) (13, 27). The ratio 5:5 is very near to the eutectic point, with almost complete elimination of the melting endotherm at 223°C. There was no considerable difference between mixing meloxicam and thymol in dry state and in solution, just for the intensity of thymol melting endotherm, which is probably related to the evaporation of highly volatile thymol.

Net amount of drug permeated through Guinea pig skin per area of diffusion from the cream contained 1% meloxicam was 3.76 $\mu\text{g}/\text{cm}^2$. This extent of permeation was much higher than meloxicam suspension and was almost similar to meloxicam-loaded transfersomes studied by Duangjit *et al* (8). This basic amount of permeation is related to the solubility of meloxicam in oil phase and emulsifier of the cream and permeation through the lipids of the skin. Relatively low permeation of the drug resulting from this formulation is related to the oily nature of the formulation and viscosity of the vehicle which

reduces the diffusion rate of the drug (28). High solubility in oil phase which results from high value of partition coefficient of meloxicam could also decrease the chemical potential of the drug (29).

As indicated in DSC thermograms in Figure 2, melting point of ratio 6:4 of meloxicam to thymol was lower than pure meloxicam, but for ratios 5:5 and 4:6 a new endothermic peak was observed which is due to the new composition of the eutectic mixture. In addition, a 4-5 fold increase in the skin permeation of these creams was observed. Similar data has been reported on the skin permeation of ibuprofen-thymol mixture (13). It is evident that by increasing the amount of thymol, drug permeation was increased until the ratio of 5:5 is achieved. Following that, increasing the ratio of thymol to 6 presented negative effect on drug permeation. When increasing the amount of thymol, meloxicam is decreasing and the drug is mainly in liquid form. Therefore, amount of solid is decreased. Disappearance of the solid drug in the mixture and therefore lower thermodynamic activity and driving force for permeation is the reason of lower permeation of this ratio (6:4, thymol to meloxicam) compared to the 5:5 ratio. Similar results have been observed in study on skin permeation of thymol-ibuprofen mixtures (13). It was shown in their study that pretreatment with thymol increased the permeation of a saturated solution of ibuprofen by two folds; however, the eutectic mixture provided a much higher increase in skin permeation. Prior studies proved that decrease in melting point of eutectic mixture is the reason of higher permeability of these mixtures in lipid structure of skin. It has been stated for ibuprofen-menthol and lidocaine-prilocaine mixtures that the main reason of enhancement in absorption is increasing lipid solubility of the mixture (13, 30, 31). Certainly, the effect of other factors like excipients of formulations for example surfactants or volatile solvents should not be ignored because these factors could also affect the integrity of the skin structure. Volatile solvent can even influence eutectic point of mixtures. SLS has important effects on integrity of the cell membrane and can therefore change the results of permeation, however, study of effect of 1% SLS on skin penetration of diazepam has shown that this concentration is not effective for changing the skin permeation of the drug (32). In order to limit the effect of these factors, in this study it was tried to keep the composition of all formulations the same. Furthermore, the only determining variable was the ratio of drug to permeation enhancer. Other explanations that may be considered for enhancement of skin permeation are ability of terpenes to fluidize the lipids of the membrane and disorganize them (33), higher distribution of eutectic mixtures in cellular membrane because of lower melting point and higher thermodynamic activity

(34), higher access of molecules of eutectic mixture to the membrane (30) and hydrogen bonding between thymol and meloxicam that make them more lipophilic (17).

Conclusion

One of the significant findings to emerge from this study was that the rate and extent of skin permeation of cream containing 5:5 ratio of meloxicam to thymol was highest amongst all other ratios. This is resulted from higher amount of permeation enhancer, thymol together with the lower melting point of the mixture as stated by DSC studies and higher thermodynamic activity near the eutectic point.

Acknowledgment

The present article was extracted from a Pharm D thesis (No. 483) written by Farhad Mohammadi and was financially supported by School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

References

1. El-Kattan AF, Asbill CS, Kim N, Michniak BB. The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. *Int J Pharm* 2001; 215:229-240.
2. Cui LL, Hou XM, Jiang J, Li GD, Liang YY, Xin X. Comparative enhancing effects of electret with chemical enhancers on transdermal delivery of meloxicam *in vitro*. *J Phys Conf Ser* 2008; 142:12-15.
3. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. *Biochim Biophys Acta* 2009;1788:2362-2373.
4. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: overcoming the skin's barrier function. *Pharm Sci Technol Today* 2000; 3:318-326.
5. Ah Y-C, Choi J-K, Choi Y-K, Ki H-M, Bae J-H. A novel transdermal patch incorporating meloxicam: *In vitro* and *in vivo* characterization. *Int J Pharm* 2010; 385:12-19.
6. Cordero JA, Alarcon L, Escribano E, Obach R, Domenech J. A comparative study of the transdermal penetration of a series of nonsteroidal antiinflammatory drugs. *J Pharm Sci* 1997; 86:503-508.
7. Bae JW, Kim MJ, Jang CG, Lee SY. Determination of meloxicam in human plasma using a HPLC method with UV detection and its application to a pharmacokinetic study. *J Chromatogr B* 2007; 859:69-73.
8. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Characterization and *in vitro* skin permeation of meloxicam-loaded liposomes versus transfersomes. *J Drug Deliv* 2011; 2011:418316.
9. Martin RM, Biswas P, Mann RD. The incidence of adverse events and risk factors for upper gastrointestinal disorders associated with meloxicam use amongst 19 087 patients in general practice in England: cohort study. *Br J Clin Pharmacol* 2000; 50:35-42.
10. Ruiz Martinez MA, López-Viota Gallardo J, de Benavides MM, de Dios García López-Duran J, Gallardo

- Lara V. Rheological behavior of gels and meloxicam release. *Int J Pharm* 2007; 333:17-23.
11. Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of meloxicam. *Bull Pharm Sci* 2010; 3:131-140.
 12. El Maghraby GM. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants. *Int J Pharm* 2008; 355:285-292.
 13. Stott PW, Williams AC, Barry BW. Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen. *J Control Release* 1998; 50:297-308.
 14. Liu Y, Ye X, Feng X, Zhou G, Rong Z, Fang C, *et al*. Menthol facilitates the skin analgesic effect of tetracaine gel. *Int J Pharm* 2005; 305:31-36.
 15. Kaplun-Frischoff Y, Touitou E. Testosterone skin permeation enhancement by menthol through formation of eutectic with drug and interaction with skin lipids. *J Pharm Sci* 1997; 86:1394-1399.
 16. Narishetty ST, Panchagnula R. Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. *J Control Release* 2004; 95:367-379.
 17. Sinha VR, Kaur MP. Permeation enhancers for transdermal drug delivery. *Drug Dev Ind Pharm* 2000; 26:1131-1140.
 18. Benson HAE. Transdermal drug delivery: penetration enhancement techniques. *Curr Drug Deliv* 2005; 2:23-33.
 19. Fiala S, Brown MB, Jones SA. Dynamic in-situ eutectic formation for topical drug delivery. *J Pharm Pharmacol* 2011; 63:1428-1436.
 20. Bandarkar FS, Vavia PR. A stability indicating HPLC method for the determination of meloxicam in bulk and commercial formulations. *Trop J Pharm Res* 2009; 8:257-264.
 21. Mahmood K, Ashraf M. A simple, specific and precise HPLC method for the measurement of meloxicam in biological fluids. *Pak J Sci* 2008; 60:85-89.
 22. Mahmood K, Khan B, Ashraf M, Haq I. Specific and simple hplc assay of ecofriendly meloxicam in pharmaceutical formulations. *J Pharm Sci Res* 2010; 2:878-883.
 23. Awasthi SS, Kumar TG, Manisha P, Preeti Y, Kumar SS. Development of meloxicam formulations utilizing ternary complexation for solubility enhancement. *Pak J Pharm Sci* 2011; 24:533-538.
 24. Gao S, Singh J. *In vitro* percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. *J Control Release* 1998; 51:193-199.
 25. El-Kattan AF, Asbill CS, Michniak BB. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. *Int J Pharm* 2000; 198:179-189.
 26. Gohel MC, Nagori SA. Resolving issues of content uniformity and low permeability using eutectic blend of camphor and menthol. *Ind J Pharm Sci* 2009; 71:622-629.
 27. Stott PW, Williams AC, Barry BW. Mechanistic study into the enhanced transdermal permeation of a model β -blocker, propranolol, by fatty acids: a melting point depression effect. *Int J Pharm* 2001; 219:161-176.
 28. Yener G, Dal Ö, Üner M. Effect of vehicles on release of meloxicam from various topical formulations. *Open Drug Deliv J* 2009; 3:19-23.
 29. Jantharaprapap R, Stagni G. Effects of penetration enhancers on *in vitro* permeability of meloxicam gels. *Int J Pharm* 2007; 343:26-33.
 30. Fiala S, Jones SA, Brown MB. A fundamental investigation into the effects of eutectic formation on transmembrane transport. *Int J Pharm* 2010; 393:68-73.
 31. Yong CS, Oh Y-K, Jung SH, Rhee J-D, Kim H-D, Kim C-K, *et al*. Preparation of ibuprofen-loaded liquid suppository using eutectic mixture system with menthol. *Eur J Pharm Sci* 2004; 23:347-353.
 32. Shokri J, Nokhodchi A, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar Jalali M. The effect of surfactants on the skin penetration of diazepam. *Int J Pharm* 2001; 228:99-107.
 33. dos Anjos JLV, Alonso A. Terpenes increase the partitioning and molecular dynamics of an amphipathic spin label in stratum corneum membranes. *Int J Pharm* 2008; 350:103-112.
 34. Yuan X. Enhanced transdermal drug delivery of NSAIDs using eutectic formation and a two-phase liquid system. 2003. PhD thesis, Graduate Faculty of The University of Georgia.