Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

COMBINATION STRATEGIES FOR ENHANCING TRANSDERMAL ABSORPTION OF THEOPHYLLINE THROUGH SHED SNAKE SKIN

ESKANDAR MOGHIMIPOUR[1*], SEYYED ABOLGHASSEM SAJJADI TABASSI [2], MARYAM KOUCHAK[3] AND HADI VARGHAEI [4]

[1]Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.[2]Department of Pharmaceutics, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.[3]Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.[4]Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, Email: moghimipour@yahoo.com

Received:16 November 2011, Revised and Accepted: 28 January 2012

ABSTRACT

Theophylline is a xanthine derivative mostly indicated for management of asthma and bronchitis. Due to dermal absorption benefits and its indication in infants, many studies have been performed to enhance its permeation through skin. The ability of bile salts and modifiers to modulate drug delivery without significant toxicity makes them useful to formulate topical theophylline. The aim of the present study was to characterize *in vitro* theophylline transdermal absorption through shed snake skin and to investigate the absorption enhancing effect of bile salts, sodium tauroglycocholate (STGC) and sodium deoxycholate (SDC), and skin modifiers such as oleic acid (OA) applied both individually and in combination. In vitro percutaneous absorption experiments were performed on snake skin using Franz diffusion cells and compared to the results from skin modifiers. Surface activity of the bile salts was also determined. Bile salts significantly enhanced the transdermal absorption of theophylline through treated skin model in comparison to control group, although the effect of STGC was more significant. Also, a reduction in enhancement effect was observed after mixed application of the bile salts. According to our findings, enhancing effect of bile salts depends not only on the nature and concentration, but also on the strategy and condition in which they have been used.

Keywords: theophylline, skin, permeation, bile salts, enhancer.

INTRODUCTION

Over the last two decades, the skin has gained importance as a means for the topical, regional and systemic application of drugs. Nevertheless, human skin is by nature a remarkably efficient barrier, thus, presenting difficulties for the transdermal delivery of therapeutic agents. Moreover, few drugs have the characteristics required to permeate sufficiently across the skin and achieve a therapeutic concentration in the blood.

Theophylline is a methylxanthine used as a bronchodilator. It relaxes bronchial smooth muscles by increasing levels of cyclic AMP through nonselective inhibition of phosphodiesterase. Theophylline is also an adenosine receptor antagonist [1]. Due to dermal absorption benefits and its indication in infants, many studies have been performed to enhance permeation of theophylline through skin. Recently, there has been considerable interest in the use of shed snake skin as a model membrane for in vitro diffusion studies monitoring the release of a number of drugs from different semisolid formulations [2-3]. Shed snake skin has also been suggested as a model membrane for the study of the effects of a number of penetration enhancers [4-6]. Although shed snake skin is not a mammalian integument, it has been reported that some compounds penetrate snake skin and human stratum corneum at similar rates [2-5].

Different methodologies, consisting of chemical and physical enhancers, have been investigated and developed in order to overcome the barrier properties of the skin and thus enhance drug transdermal absorption. Penetration enhancers have long been used to increase the range of drugs that can be effectively delivered via skin. To date, a vast array of chemicals has been evaluated as enhancers [11], yet their inclusion in topical or transdermal formulations are limited due to lack of knowledge surrounding their underlying mechanisms of action.

The aim of the present study was to characterize *in vitro* theophylline transdermal absorption through shed snake skin and to investigate the enhancing effect of bile salts, sodium tauroglycocholate (STGC) and sodium deoxycholate (SDC), and skin modifiers such as oleic acid (OA) applied both individually and in combination.

MATERIALS AND METHODS

The snake skin was kindly donated by Razi Institute, karaj, Iran. Theophylline (anhydrous) was purchased from Boehringer, Germany, STGC and Oleic acid from Merck, Germany, SDC from Sigma, Germany, and SLS from HW, UK.

Surface tension and CMC Determination

Using a Du-Nouy ring tensiometer (White, Germany) surface tension and critical micelle concentrations (CMC) of the aqueous samples were determined at room temperature.

In-vitro penetration study

Shed snake skins of *Vipera labatina* were used as model membrane. The skin was hydrated by immersion in water at 40°C for 30 min before the experiments. Then it was mounted in a Franz-type diffusion cell. For the pretreatment study of enhancers, 1 mL of bile salt (or 200 μL oleic acid) solutions with different concentrations were applied to the shed snake skin 2 h before the experiment. Any remaining enhancer or solvent on the skin was blotted with a Kimwipe after pretreatment and the skin was mounted in the diffusion cell after hydration. Skin specimens were placed between 2 glass chambers with the stratum corneum side contacting the donor phase; and fastened using polymer washers and stainless steel clips. Drug solution containing 5 mg theophylline, alone or in combination with bile salts dissolved in PBS (total volume of 5 mL) was placed in donor compartment. 20 ml PBS (pH 7.4) was placed in the receptor compartment. Using a magnetic stirrer, the solution was stirred continuously during the experiment to assure sink condition. The cells were connected to a circulation water bath to adjust the temperature at 37°C. At 30 min intervals, 1-ml samples were removed from receiver phase and the volume of the sample removed was replaced with the same volume of PBS. The amount of drug contained in each sample was calculated to determine the accumulative amount of theophylline in the receptor compartment at each time point[12].

Analytical procedure

The samples were diluted with 3 mL PBS and Theophylline in the receptor fluid was determined by UV-spectrophtometric analysis at 272 nm in a double-beam spectrophotometer (JASCO Model 7850).

Data treatment

Steady-state fluxes for theophylline (J, nmol cm^{-2} h^{-1}) were calculated using linear regression analysis of the straight line

portion of the cumulative drug penetration vs. time plots. Data are presented as the mean ± SD. Statistical evaluation of the data was carried out using the Student T-test. The sampling protocol was identical in all the experiments performed. Also, using equation I, the enhancement factor (EF) for each enhancer was calculated.

Equation I:

$$EF = \frac{J_1}{J_0}$$

in which J_0 *is the permeation rate in absence of enhancer* and J_1 *is the permeation rate in presence of the enhancer.*The reported values are mean ratios from a minimum of 3 replicates [13].

RESULTS

Surface tension and CMC determination

Data from surface study are presented in Fig.1. It can be seen that the addition of bile salts has decreased the surface tension of water, although the effect of STGC is significantly ($p<0.01$) higher than that for SDC. While the critical micelle concentrations (CMCs) for both were almost the same (about 100 µg/ml in concentrations more than CMC) there was no decrease in surface tension for SDC, but the decrease continued in a relatively mild slope for STGC.

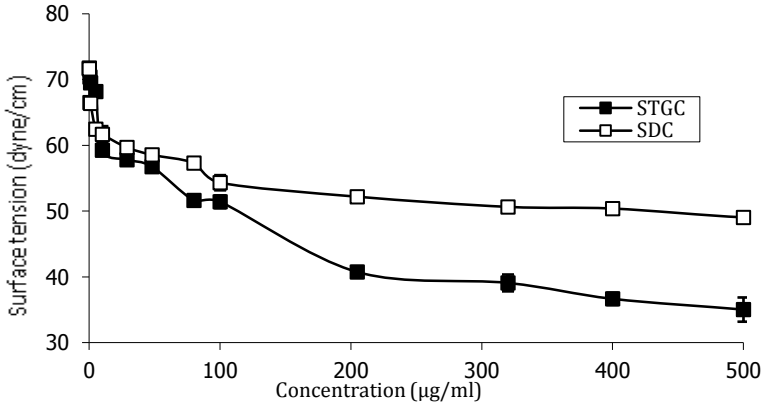

Fig 1: Surface tension decrease of STGC and SDC aqueous solutions as a function of the bile salts concentration at 21°C (n=6)
Combination strategies for enhancing transdermal absorption of theophylline through shed snake skin

Skin permeation

Skin permeation parameters

The flux, *J*, and enhancement factor, EF, for each enhancer according to equation I are tabulated in table 1. It can be seen that with the exception of STGC in the untreated state, there is a significant increase ($p<0.05$) in permeation for all of the treated samples in comparison to negative control group. Among STGC containing samples, the effect is more significant when higher than 1000 µg/ml is used for skin pretreatment. The results also show a considerable fluctuation in enhancing effect by increasing the concentration of enhancers, for example, the decrease in permeation rate and EF for 500 µg/ml STGC and 2000 µg/ml SDC. For SDC, it is observed that although the increasing of concentration significantly increases J and EF, but the increase due to the treatment process is much higher. The permeation rate of untreated skin in the presence of STGC is approximately equal to zero. Treatment of the skin led to considerable increase in J. The maximum permeation rate for treated skin is when combined with STGC 1000 µg/ml. Also the drug permeation rate for mixture of enhancers is significantly less than that of enhancers when used solely.

Table 1: Theophylline skin absorption parameters in different strategies.

Enhancer Concentration (µg/ml)	Flux (*J*) (nmol/cm².h)	Enhancing Factor (EF)
Control	2.07	1
STGC		
5[(a)]	7.53*	3.64*
20[(a)]	0	0
100[(a)]	0.13	0.06
200[(a)]	0	0
600[(a)]	0	0
100[(b)]	21.73*	105.1*
500[(b)]	104.81*	50.64*
1000[(b)]	178.63*	86.30*
Eth 60° + 1000[(c)]	55.30*	26.72*
Eth 60° + 2000[(c)]	87.43*	42.24*
SDC		
600[(a)]	52.60*	25.41*
1000[(b)]	122.85*	59.35*
2000[(b)]	74.16*	35.83*
Eth 60° + 1000[(c)]	81.56*	39.40*
Eth 60° + 2000[(c)]	36.76*	17.76*
OA + 600[(d)]	55.90*	27.01*
Oleic acid		
200[(b)]	97.72*	44.58*

a. untreated skin, b. treated skin, c. skin treated with bile salt, donor compartment: Theophylline in ethanol,d. skin treated with oleic acid, donor compartment: Theophylline in PBS,

*** significantly differ from control group**

Among the SDC group, the maximum *J* is for the treated skin with SDC 1000 µg/ml. Also as for STGC, permeation rate decreases significantly when the enhancers were mixed. For STGC (1000 µg/ml and 2000 µg/ml), when combined with ethanol 60°, *J* is significantly more than that for SDC ($p<0.01$).

The profiles of permeation

The permeation profiles of theophylline in presence of bile salts are shown in Fig.2. The obtained data show an absorption enhancement effect due to the presence of bile salts. While, the enhancing ability of SDC is more considerable ($p<0.01$), STGC have also shown a significant enhancement in comparison to negative control group ($p>0.05$). Moreover, the enhancing effect of all of the enhancers begins in the 1st hour of the experiment.

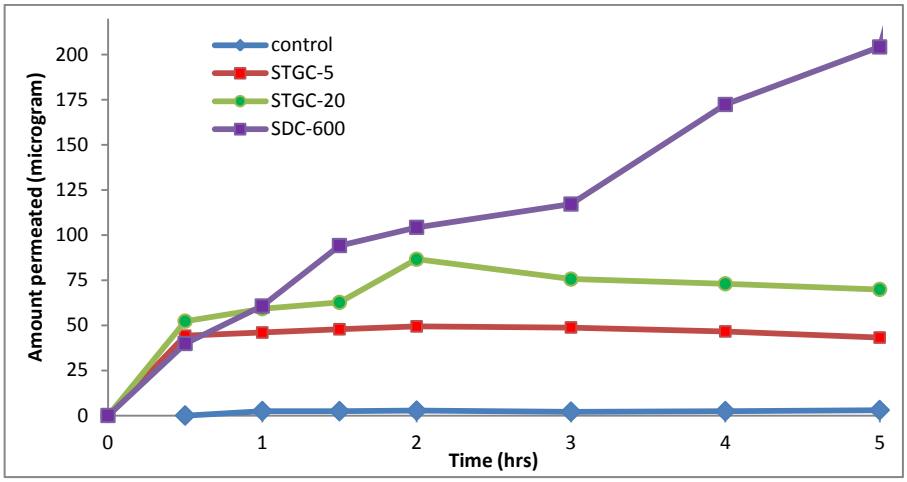

Fig 2: Amounts of Theophylline permeated through snake shed skin in the presence of different absorption enhancers at 37°C (n= 4-10)

Combination strategies for enhancing transdermal absorption of theophylline through shed snake skin

The permeation profile of theophylline regarding the effect of pretreatment with STGC is shown in fig.3. The enhancement effect began in the first hour of experiment and continued to the 5th hour. The obtained results show a significant difference ($p<0.001$) between pretreatment and control groups. Although the ethanolic solution of theophylline in the donor chamber considerably ($p<0.05$) raised the drug permeation during the experiment period, the effect was significantly less than that of aqueous solution of the drug.

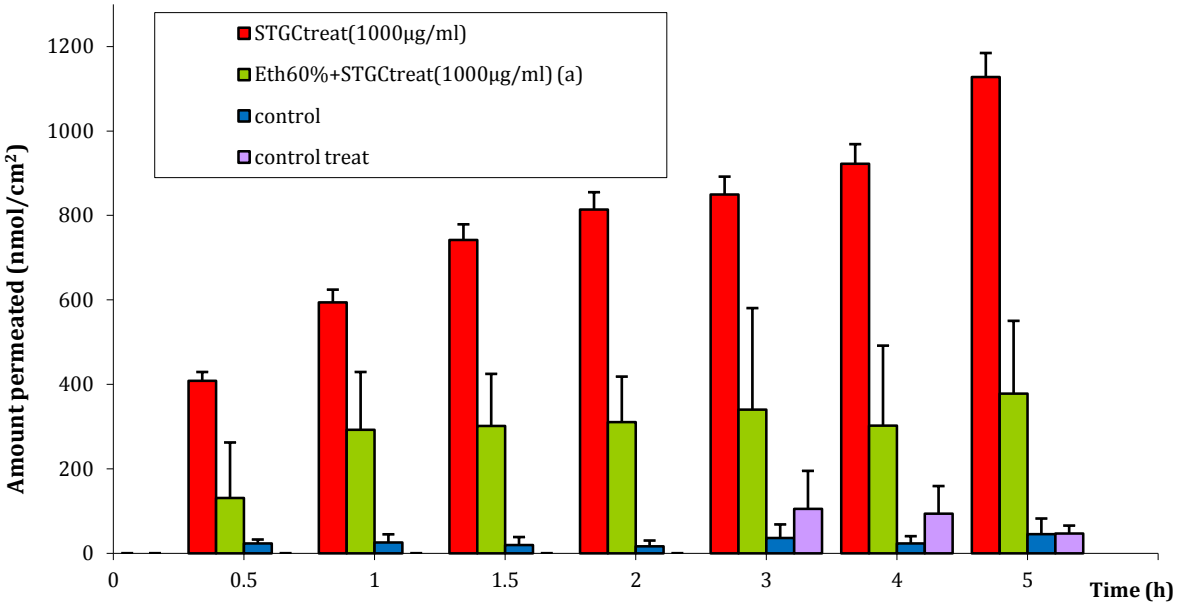

Fig. 3: Rate of theophylline permeability through pretreated and untreated shed snake skin at 37°C (n= 4-10)

skin treated with bile salt, donor compartment: Theophylline in ethanol

The results from simultaneous application of skin treatment and enhancer addition are shown in Fig.4. The results indicate that the combination of skin treatment with ethanol 60° and 1000 or 2000 µg/ml STGC or 2000 µg/ml SDC are similar, but there is a lower rate of permeation for SDC when combined with ethanol 60°. The results showed maximum rate for STGC (2000 µg/ml) and SDC (both concentrations) after 3rd hour of experiment, while it was at the first hour of experiment for STGC (1000 µg/ml). Permeation enhancing effect of oleic acid with 600 µg/ml SDC begins in the 4th hour of application. The maximum drug permeated is 618.2 nmol/cm² for the combination of treated with ethanol 60° and 2000 µg/ml STGC after 6 hours of application. The results for all of the test groups were significantly different ($p<0.05$) from the negative control group.

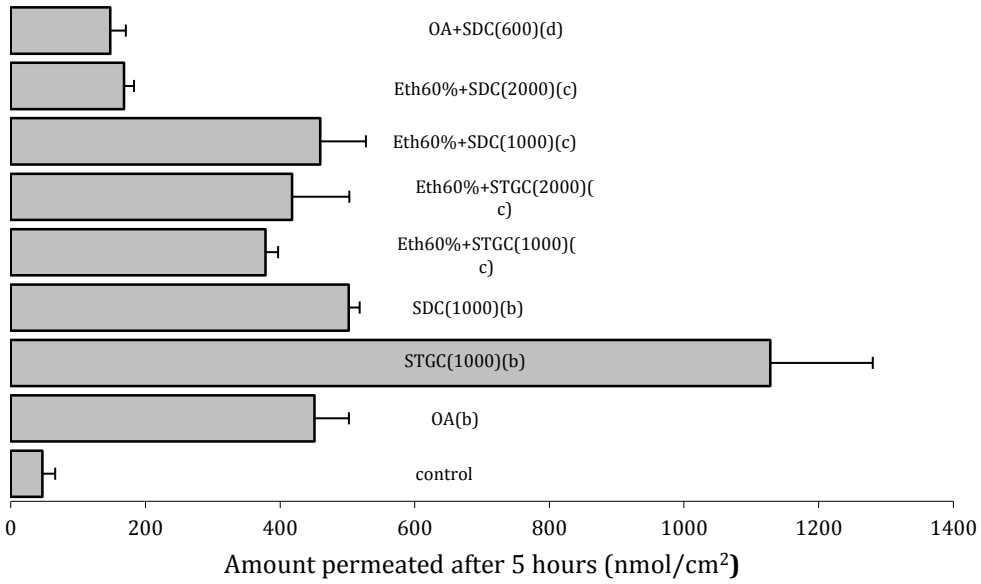

Fig. 4: Amount of theophylline permeated through shed snake skin with different strategies at 37°C (n= 4-10)

a. untreated skin, b. treated skin, c. skin treated with bile salt, donor compartment: theophylline in ethanol, d. skin treated with oleic acid, donor compartment: theophylline in PBS

DISCUSSION

Although there have been many efforts to enhance buccal and nasal absorption of drugs [14-16], there are only few researches on their application in transdermal drug delivery. Ogiso et. al. increased transdermal absorption of alkatonin through rat skin using bile salts [17]. The bile salts have shown ability to enhance drug permeation, specifically focused on nasal permeation. In general, bile salts are considered as nasal, rectal, vaginal, buccal, oral and dermal permeation enhancers [18]. The active site of those enhancers is thought to be intercellular lipids as confirmed using laser scanning confocal microscopy [19]. According to the results, enhancer free samples showed almost no absorption through the skin model. The highest permeation rate was obtained with ethanol. Also, the results indicate that the enhancing ability of ethanol is a concentration-dependent effect, so that by increasing the degree of ethanol from 40 to 60, enhancing factor increases significantly ($p<0.05$) from 117.3 to 156.1. The results are in accordance with Takahashi et. al. reports who applied ethanol (20, 40 and 60 percent) for enhancing absorption of ondanesterone through snake skin. They showed that enhancing effect of ethanol arise from 40% concentration and suggested that the drug thermodynamic activity depends on the concentration of the enhancer in donor compartment [13].

According to our findings, enhancing effect of bile salts depend not only on their nature and concentration, but also on the strategy and condition in which they have been used. The main difference was seen when the skin was treated before the experiment. There was a considerable increase in theophylline permeation upon pre-treatment with bile salts. It should be mentioned that in untreated samples, bile salts were combined with the drug in donor chamber, while in pretreatment strategy bile salts were not directly in contact with theophylline and have been removed from skin surface prior to permeation study. Therefore, the significant difference of drug absorption between two strategies may be related to the enhancement mechanism of the bile salts. Three main mechanisms for transdermal absorption of drugs have been suggested. The first is related to change in thermodynamic activity of drug which leads to modulation of drug-solvent interaction and so enhancement of drug absorption. Micelle formation and change in drug solubility are considered as indices of thermodynamic changes. The second suggested mechanism refers to the ability of enhancer to change the nature of horny layer [20]. Both mechanisms are suggested for bile salts [21]. The third mechanism suggests that these enhancers induce permeation enhancement in transdermal transport of a lipophilic compound by alteration of the polarity and/or microviscosity of the transport rate limiting pathway in subcutaneous lipid domain [22]. Considering low absorption in presence of different concentrations (under and above of CMC) in untreated skin, it seems that the first mechanism is not involved. The results from pretreated skin study are in accordance with the second mechanism.

Another result of our study was the considerable decrease in enhancement activity of enhancers when applied in combination. The enhancing abilities of the combination of ethanol 60° and STGC or SDC, and oleic acid in combination with SDC are significantly lower than that for each compound when applied solely. These findings are not in accordance with previous studies in which ethanol (or oleic acid) has had synergetic effect on other enhancers. It has been shown that, when compared with separately applied, ethanol promotes the effect of oleic acid or lauryl ether in absorption enhancement of ondanesterone through snake skin [13]. Lee et. al. has shown a promotion in absorption enhancement effect of surfactants on rat skin when combined with ethanol [23]. It seems that the decrease resulted in the present study is due to the interaction of ethanol and oleic acid with drug partition in horny layer [24]. An 18 hrs pretreatment with ethanol or oleic acid may affect the nature of horny layer and so drug partition may be affected. Further investigations are needed to determine the exact mechanism of such interactions.

Our findings showed a non-significant difference in surface and enhancing activity of STGC when compared with SDC. According to the chemical structure of bile salts, deoxycholates and taurocholates are among dihydroxy and trihydroxy bile salts, respectively. The difference could be related to the number of their hydroxyl groups. There have been several efforts to increase transdermal absorption of theophylline, using ethanol and surfactants [22], calcium glycocholate [25] and N-methyl-2-pyrrolidone [26]. Kadir et al. have studied the enhancing effect of propionic, hexanoic and octanoic acid on theophylline absorption through human skin. According to their results, there has been a significant increase in the absorption up to 3-6 μmol/cm² during 5 to 7 hours of experiment[27]. A comparison of their results with ours shows a higher degree of enhancement for the alkan carboxylic acids. Due to the difference between the methods and origin of skin specimens, an exact conclusion does not seem rational. It can be suggested that in addition to the nature and concentration of enhancer and the duration of pretreatment, the nature and molecular size of drug molecule also have determining roles in transdermal absorption procedure. These factors should be specifically considered for designing a drug-enhancer combination. On the other hand, the results from the study of a drug should not be generalized for the others, unless in the presence of proved

similarity in their molecular structure and size or physical properties. Microscopic observation and the study of changes in transepithelial electric resistance (TEER) are additional requirements to understand the exact mechanism of bile salts effect on horny layer and differentiate between inter- and intra-cellular pathways.

CONCLUSION

It can be concluded that for untreated skin, when compared with negative control results, SDC and STGC are potent absorption enhancers for theophylline through shed snake skin. In addition, it can be concluded that their activity directly correlates with their surface activity; and the treatment procedure is a reliable method for increasing the permeation of some drugs through the skin.

ACKNOWLEDGEMENT

"The paper is issued from Pharm.D. thesis of Hadi Varghaei and financially supported by Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran". We gratefully thank Razi Institute for the preparation and donation of skin samples.

REFERENCES

1. Ruben Bunag R. xPharm: The Comprehensive Pharmacology Reference, Elsevier Inc. (2008) 1-5.
2. Craane-van Hinsberg WHM, Verhoef JC, Bax LJ, Junginger HE and Boddé HE. Role of appendages in skin resistance and iontophoretic peptide flux: human versus snake skin. Pharm. Res. (1995) **12**:1506–1512.
3. Kuramoto M, Tanaka T, Makita H, Nakamura Y and Yata N. Characteristics of shed snake skin permeability to indomethacin and fatty alcohols. J. Pharm. Pharmacol. (1996) **48**: 680–684.
4. Hirvonen J, Kontturi K, Murtomaki L, Paronen P and Urtti A. Transdermal iontophoresis of sotalol and salicylate; the effect of skin charge and penetration enhancers. J. Control. Release. (1993) **26:** 109–117.
5. Turunen TM, Buyuktimkin S, Buyuktimkin N, Urtti A, Paronen P. and Rytting JH. Enhanced delivery of 5-fluorouracil through shed snake skin by two new transdermal penetration enhancers. Int. J. Pharm. (1993) **92**: 89–95.
6. Suh H and Jun HW. Effectiveness and mode of action of isopropyl myristate as a permeation enhancer for naproxen through shed snake skin. J. Pharm. Pharmacol. (1996) **48**: 812–816.
7. Itoh T, Xia J, Magavi R, Nishihata T and Rytting JH. Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. (1990) **7**:1042–1047.
8. Rigg PC and Barry BW. Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. J. Invest. Dermatol. (1990) **94**: 235–240.9.
9. Harada K, Murakami T, Kawasaki E, Higashi Y, Yamamoto S and Yata N. In vitro permeability to salicylic acid of human, rodent and shed snake skin. J. Pharm. Pharmaco. (1993) **4**: 414–418.
10. Takahashi K, Tamagawa S, Katagi T, Rytting JH, Nishihata T and Mizuno N. Percutaneous penetration of basic compounds through shed snake skin as a model membrane. J. Pharm. Pharmacol. (1993) **45**, pp. 882–886
11. Williams AC and Barry BW. Penetration enhancers. Adv Drug Deliv Rev. (2004). 56(5):603-18.
12. Moghimipour E, Jalali A, Sajjadi Tabassi SA and Löbenberg R. The Enhancing Effect of Sodium Glycocholate and Sodium Salicylate on Rats Gastro-intestinal Permeability to Insulin, Iranian Journal of Pharmaceutical Research. (2004) 2: 87-91.
13. Takahashi K and Rytting JH. Novel approach to improve permeation of ondansetron across shed snake skin as a model membrane. JPP. (2001) 53:789-94.
14. Moghimipour E, Sajjadi Tabassi SA, Ramazani M, Loebenberg R. Enhanced permeability of gentamicin sulfate through shed snake skin and liposomal membranes by different enhancers. Iranian Journal of Basic Medicinal Sciences. (2003) 6(1): 9-19.
15. Kirshnaiah YS, Satyanarayana V and karthikeyan RS. Effect of the solvent system on the in vitro permeability of nicardipine hydrochloride through excised rat epidermis. J Pharm Pharmaceut Sci. (2002) 5(2): 123-30.
16. Senel S and Hincal AA. Drug permeation enhancement via buccal route: possibilities and limitations. J Cont Rel. (2001) 72(1): 133-44.
17. Ogiso T, Iwaki M, Yoneda I, Horinouchi M and Yamashita K. Percutaneous absorption of elcatonin and hypocalcemic effect in rat. Chem Pharm Bull. (1991) 39:449-53.
18. Swarbrick J and Boylan JC. editors. Encyclopedia of pharmaceutical technology. 2nd ed. New York: Marcel Dekker. (2002) 1: 1-6, 20, 3:3127.
19. Obata Y, Utsumi S, Watanabe H, Suda M, Tokudome Y, Makoto Otsuka M and Takayama K. Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers. International Journal of Pharmaceutics (2010) 389: 18–23.
20. Ranade VV. Drug delivery systems.6. Transdermal drug delivery. J Clin Phormacol. (1991) 31:401-18.
21. Hsieh DS. (ed.) Drug permeation enhancement theory and applications. Marcel Dekker, New York. (1994) 10, 27-31, 59-63, 149-69.
22. Ibrahim SA and Li SK. Chemical enhancer solubility in human stratum corneum lipids and enhancer mechanism of action on stratum corneum lipid domain. International Journal of Pharmaceutics, (2010) 383: 89–98.
23. Lee CK, Kitagawa K, Uchida T, Kim NS and Goto S. Transdermal delivery of theophylline using an ethanol / panasate 800-ethyl cellulose gel preparation. Biol Pharm Bull. (1995) 18(1): 176-80.
24. Martin A. Physical pharmacy.4th ed. Lea and Febiger, Philadelphia. (1993) 148,371-76, 396.
25. Kushida K, Matsumura M, Ohshima T, Yoshikawa H, Takada K and Muranishi S. Application of calcium thioglycolate to improve transdermal delivery of theophylline in rats. Chem Pharm Bull. (1984) 32(1): 268-74.
26. Tarantino R, Bishop E, Chen FC, Iqbal K and Malick AW. . N-methyl- 2- pyrrolidone as a cosolvent: relationship of cosolvent effect with solute polarity and the presence of proton- donating groups on model drug compounds. J Pharm Sci. (1994) 83(9): 1213-6.
27. Kadir R, Stempler D, Liron Z and Cohen S. Delivery of theophylline into excised human skin from alkanoic acid solutions: a push-pull mechanism. J Pharm Sci. (1987) 76(10): 774-8.