Department of Pharmaceutics[1], Faculty of Pharmacy, Al-Arab Medical Sciences University, Benghazi, Libya; Department of Pharmaceutics[2], Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi; New Drug Delivery System (NDDS)[3], Zydus Cadila Research Centre, Ahemdabad, India

Skin permeation mechanism of aceclofenac using novel nanoemulsion formulation

F. Shakeel[1], S. Baboota[2], A. Ahuja[2], J. Ali[2], S. Shafiq[3]

Received February 5, 2008, accepted February 27, 2008

Dr. Faiyaz Shakeel, Senior Lecturer, Department of Pharmaceutics, Faculty of Pharmacy, Alarab Medical Sciences University, Benghazi – 5341, Libya
faiyazs@fastmail.fm

Pharmazie 63: 580–584 (2008) *doi: 10.1691/ph.2008.8036*

The aim of the present study was to investigate the skin permeation mechanism of aceclofenac using a novel nanoemulsion formulation. An optimized oil-in-water nanoemulsion of aceclofenac was prepared by the spontaneous emulsification method. The optimized nanoemulsion contained 2% w/w aceclofenac, 10% w/w Labrafil, 5% w/w Triacetin, 35.33% w/w Tween 80, 17.66% w/w Transcutol P and 32% w/w distilled water. The skin permeation mechanism was evaluated by FTIR spectroscopy, DSC thermography, activation energy measurement and histopathological examination. FTIR spectra of skin treated with the nanoemulsion formulation indicated breaking of the hydrogen bond network at the head of ceramides. DSC thermograms indicated that intracellular transport could be a possible mechanism of permeation enhancement and that permeation occurred due to the extraction of SC lipids by the nanoemulsion. The significant decrease in activation energy for aceclofenac permeation across rat skin indicated that the SC lipid bilayers were significantly disrupted ($p < 0.05$). Photomicrography of skin showed disruption and extraction of lipid bilayers as distinct voids and empty spaces visible in the epidermal region. Overall these findings indicated that nanoemulsions can be successfully used to enhance skin permeation of drugs.

1. Introduction

Aceclofenac, a nonsteroidal anti-inflammatory drug (NSAID), has been given orally for the treatment of rheumatoid arthritis and osteoarthritis (Shakeel et al. 2007). Long term oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding (Yamazaki et al. 1997). It also causes anemia because of gastrointestinal bleeding. The transdermal route eliminates these side effects and maintains the plasma drug level for a longer period of time (Lee et al. 2005a; Shakeel et al. 2007). One of the most promising techniques to enhance transdermal permeation of drugs is nanoemulsion technology (Shakeel et al. 2007; Baboota et al. 2007a, b). Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm (Shafiq et al. 2007a, b, c). Nanoemulsions have improved transdermal permeation over conventional topical formulations such as emulsions (Gasco et al. 1991; Ktitis and Niopas 1998) and gels (Kriwet and Müller-Goymann 1995; Trotta 1999) for many drugs. Many researchers have investigated the mechanism of skin permeation for a number of drugs using chemical enhancers (Goodman and Barry 1989; Yamane et al. 1995; Zhao and Singh 1998; Stott et al. 2001; Vaddi et al. 2002; Cotte et al. 2004; Narishetty and Panchagnula 2004; Cole and Heard 2007). To our knowledge, the skin permeation mechanism of aceclofenac when formulated as a nanoemulsion using different methods has not been reported although nanoemulsions are known to enhance skin permeation of drugs (Shakeel et al. 2007; Baboota et al. 2007a, b). Therefore the aim of this study was to investigate the mechanism of skin permeation of aceclofenac as a nanoemulsion using various methods such as Fourier transform infrared (FTIR) spectral analysis, differential scanning calorimetry (DSC) spectral analysis, activation energy measurement and histopathological examination.

2. Investigations, results and discussion

The FTIR spectrum of untreated stratum corneum (SC) showed various peaks due to molecular vibration of proteins and lipids present in the SC. The absorption bands from 3000 to 2700 cm^{-1} seen in untreated SC are due to the C−H stretching of the alkyl groups present in both proteins and lipids (Fig. 1a). The signal associated with proteins was a broad band, rather weak as compared to the absorption of lipids which exhibits four peaks at around 2955 cm^{-1}, 2920 cm^{-1}, 2870 cm^{-1} and 2850 cm^{-1} (Hori et al. 1991; Cumming and Winfield 1994; Zhao and Singh 1998; Panchagnula et al. 2001; Cotte et al. 2004; Krishnaiah et al. 2006). The bands at 2920 cm^{-1} and 2850 cm^{-1}

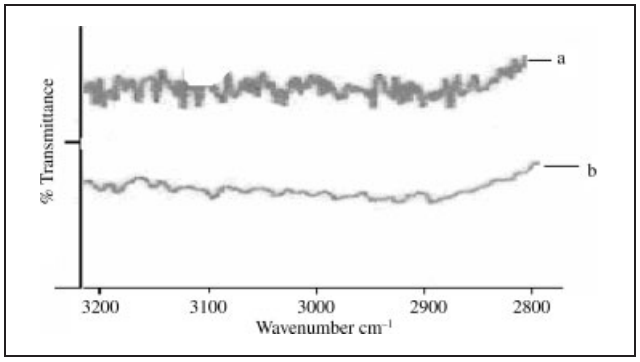


Fig. 1: FTIR spectra of rat SC. Change in lipid C−H stretching (2920 cm^{-1}) vibrations after 24 h treatment with (a) control (b) nanoemulsion

were due to asymmetric $-CH_2$ and symmetric $-CH_2$ vibrations of long chain hydrocarbons of lipids respectively. The bands at 2955 cm^{-1} and 2870 cm^{-1} were due to asymmetric and symmetric CH_3 vibration respectively (Babua and Pandit 2005). These narrow bands were attributed to the long alkyl chains of ceramides, cholesterol and fatty acids, which are the major components of the SC lipids.

The two strong bands at 1650 cm^{-1} and 1550 cm^{-1} were due to the amide I and amide II stretching vibrations of SC proteins (Fig. 2a). The amide I bands arise from C=O stretching vibration and the amide II bands from C−N bending vibration. The amide I band consists of component bands, representing various secondary structures of keratin.

There was a clear difference between the FTIR spectra of untreated and nanoemulsion treated SC with a marked decrease of peak height and area in asymmetric and symmetric CH– stretching (Fig. 1b).

The rate limiting step or main barrier to transdermal drug delivery is the lipophilic part of the SC in which lipids (ceramides) are tightly packed as bilayers due to the high degree of hydrogen bonding. The amide I group of ceramide is hydrogen bonded to the amide II group of another ceramide forming a tight network of hydrogen bonding at the surface of ceramide. This hydrogen bonding lends strength and stability to lipid bilayers and thus imparts the barrier property to the SC (Panchagnula et al. 2001; Jain et al. 2001). When the skin was treated with a nanoemulsion formulation, ceramides were loosened because of competitive hydrogen bonding leading to breaking the hydrogen bond network at the ceramide surface due to permeation of the nanoemulsion into the lipid bilayers of the SC. The tight hydrogen bonding between ceramides

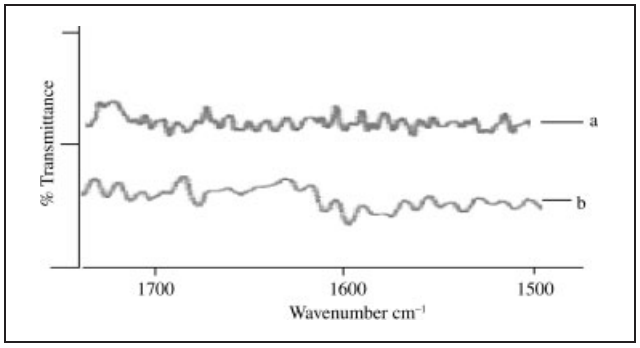


Fig. 2: FTIR spectra of rat SC. Change in amide I (1640 cm^{-1}) and amide II (1550 cm^{-1}) stretching vibrations after 24 h treatment with (a) control (b) nanoemulsion

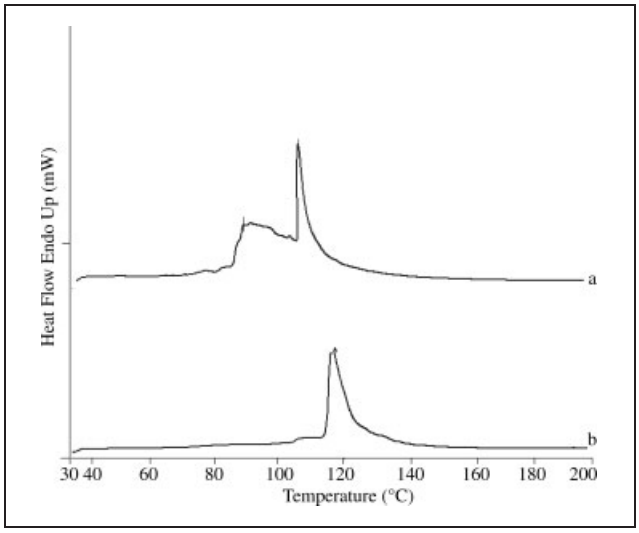


Fig. 3: DSC thermogram of control SC and SC treated with nanoemulsion formulation for 24 h (a) control (b) nanoemulsion

caused a split in the peak at 1650 cm^{-1} (amide I) as shown in the control skin spectrum (Fig. 2a). Treatment with nanoemulsion resulted in either a double or single peak at 1650 cm^{-1} (Fig. 2b) which suggests breaking of hydrogen bonds by the nanoemulsion. The mechanism of skin permeation enhancement by the nanoemulsion being investigated was further elucidated by DSC studies.

The DSC thermogram of untreated rat epidermis presented four endotherms (Fig. 3a). The first three endotherms were obtained at 34 °C (T_1), 82 °C (T_2) and 105 °C (T_3) respectively, whereas the fourth endotherm (T_4) produced a very sharp and prominent peak at 114 °C which is attributed to SC proteins. The first endotherm (having the lowest enthalpy) is attributed to the sebaceous section (Lee et al. 2005b) or surface contamination and to minor structural rearrangement of the lipid bilayer (Xiong et al. 1996). The second and third endotherms T_2 and T_3 appear as a result of the melting of SC lipids and the fourth endotherm T_4 has been assigned to intracellular keratin denaturation (Goodman et al. 1989). The thermogram of the skin specimen treated with nanoemulsion formulation was compared with the above mentioned control SC thermogram. It was observed that both T_2 and T_3 endotherms completely disappeared or shifted to lower melting points in the thermogram of SC treated with nanoemulsion formulation (F1). This indicated that the components of the nanoemulsion enhanced skin permeation of drugs through the extraction of SC lipids. The nanoemulsion formulation also decreased the protein endotherm T_4 to a lower melting point, suggesting keratin denaturation and a possible intracellular permeation mechanism in addition to the extraction of lipid bilayers (Fig. 3b). Thus it was concluded that intracellular transport is a possible mechanism of permeation enhancement. Permeation occurred due to the extraction of SC lipids by nanoemulsion formulations. Another observation was that T_4 increased to 122 °C with the nanoemulsion formulation with broadening of the peak. The shift to a higher transition temperature (T_m) and peak broadening has been attributed to dehydration of SC (Vaddi et al. 2002) as another mechanism of permeation enhancement in addition to lipid extraction resulting in higher permeation of the active medicament (aceclofenac).

The value of activation energy (E_a) for the diffusion of a drug molecule across skin depends on its route of diffusion and physicochemical properties. Moreover, the value

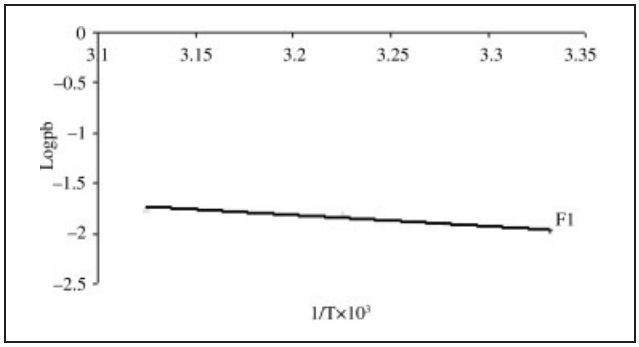
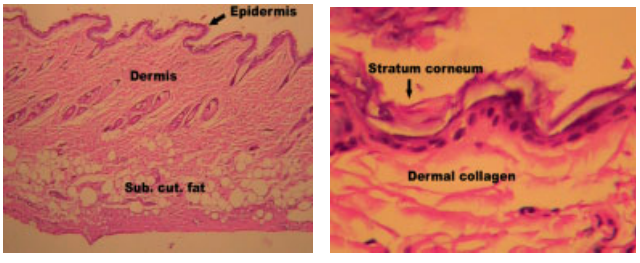


Fig. 4: Arrhenius plot of nanoemulsion (F1) permeation across rat skin

of E_a may be modified due to changes in skin composition. Nanoemulsions may change the E_a by virtue of their action on SC lipids. The Arrhenius plot of the logarithm of permeability coefficient (log P_b) vs 1/T was found to be linear in the selected temperature range between 27–47 °C (Fig. 4), indicating no significant structural or phase transition changes within the skin membrane. The E_a values for permeation of aceclofenac across rat skin were calculated from the slope of the Arrhenius plot (Fig. 4). The E_a of aceclofenac from nanoemulsion formulation F1 was found to be 2.616 kcal/mol. The significant decrease in E_a for aceclofenac permeation across rat skin indicates that the SC lipid bilayers were significantly disrupted ($p < 0.05$).

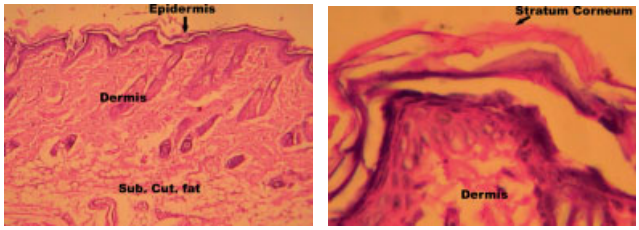
The activation energy for ion transport has been reported as 10.7 kcal/mol across the phosphatidylcholine bilayer (Monti et al. 1995) and 4.1 kcal/mol across human epidermis (Pagano and Thompson 1968). It is well established that ion transport across skin occurs mainly via aqueous shunt pathways (Cullander and Guy 1991). In view of these reports it can be anticipated that if a molecule moves via polar pathways across human cadaver epidermis then the E_a value would be similar to that of ion transport across skin. In our study, the E_a of aceclofenac from formulation F1 was 2.616 kcal/mol. Therefore it was interpreted that nanoemulsions create pathways in the lipid bilayers of SC resulting in enhanced transdermal permeation of aceclofenac (Clarys et al. 1998).

The photomicrographs of untreated rat skin (control) showed normal skin with well defined epidermal and dermal layers. The keratin layer was well formed and lay immediately adjacent to the topmost layer of the epidermis. The dermis was devoid of any inflammatory cells. Skin appendages were within normal limits (Fig. 5a, b). When the skin was treated with nanoemulsion formulation (F1) for 24 h, definite changes were observed in skin morphology. Low power photomicrography of a skin sample showed epidermis with a prominent keratin layer, and a normal dermis and subcutaneous tissues. High power



(a) (b)

Fig. 5: Photomicrographs of skin sample from control group animal showing normal epidermis, dermis and subcutaneous tissues at (a) low power view (HE × 100) (b) high power view (HE × 400)



(a) (b)

Fig. 6: Photomicrographs of skin sample from nanoemulsion treated animal at (a) low power view (HE × 100) (b) high power view (HE × 400)

photomicrography of the skin sample showed a thickened and reduplicated stratum corneum with 3–4 distinct layers. The uppermost layer however still has a normal basket weave appearance. The epidermis shows an increase in its cellular layers to 4–6 cells. The dermis does not show any edema or inflammatory cell infiltration. The disruption and extraction of lipid bilayers was clearly evident as distinct voids and empty spaces visible in the epidermal region (Fig. 6a, b). The extraction of SC lipids might cause dehydration of SC with significant loss of moisture. These observations support the *in vitro* skin permeation data.

No apparent signs of skin irritation (erythma and edema) were observed on visual examination of skin specimens treated with nanoemulsion formulation indicating absence of any skin irritation as a consequence of nanoemulsion treatment. Moreover, the components used to produce nanoemulsions have been listed as generally regarded as safe (GRAS) excipients.

3. Experimental

3.1. Materials

Aceclofenac was donated by Ranbaxy Research Labs (Gurgaon, India). Oleoyl macroglycerides-EP (Labrafil) was obtained as a gift sample from Gattefossé (Cedex, France). Glycerol triacetate (Triacetin), diethylene glycol monoethyl ether (Transcutol-P) and ethanol were purchased from E-Merck (Mumbai, India). Tween-80 was purchased from Sigma Aldrich (St. Louis, MO). All other chemicals used in the study were of analytical reagent grade. Animals (rats) were obtained from our institute (Jamia Hamdard, India). Approval to carry out studies on rats was obtained from the animal ethics committee of Jamia Hamdard, India and their guidelines were followed for the studies.

3.2. Preparation of aceclofenac nanoemulsion

The aceclofenac nanoemulsion was prepared by the spontaneous emulsification method.

Details of its preparation, characterization and optimization are given in our previous paper (Shakeel et al. 2007).

Aceclofenac 2% w/w was dissolved in 15% of a mixture of Labrafil and Triacetin (2 : 1). Then 35.33% w/w of Tween-80 and 17.66% w/w of Transcutol-P were added with vortex mixing. The final preparation was made up to 100% by slow addition of distilled water.

3.3. FTIR spectral analysis of nanoemulsion treated and untreated rat skin

Skin was prepared and stratum corneum (SC) was cut into small circular discs of approximate diameter 1.5 cm. 0.9% (w/v) sodium chloride was prepared and 0.01% sodium azide was added as an antibacterial and antimycotic agent (Jain et al. 2001). Equal volumes of 0.9% sodium chloride solution were placed in different conical flasks and SC of approximate 1.5 cm diameter was suspended over it for 3 days. After 3 days of hydration, these discs were thoroughly blotted over filter paper and FTIR spectra (Perkin Elmer, Germany) of each SC disc were recorded before nanoemulsion treatment (control) in the frequency range of 400 to 4000 cm^{-1} with resolution of 2 cm^{-1}. After taking FTIR spectra, the same discs were dipped into the aceclofenac nanoemulsion formulation in 4 ml of ethanolic phosphate buffer saline (PBS) pH 7.4 (20 : 80). This was kept for a period of 24 h (equivalent to the permeation studies) at 21 ± 1 °C. Each SC disc was washed, blotted dry, and then air dried for 2 h after treatment. Sam-

ples were kept under vacuum in desiccators for 15 min to remove traces of formulations completely. FTIR spectra of the treated SC discs were recorded again. Each sample served as its own control.

3.4. DSC studies of nanoemulsion treated and untreated rat skin

Approximately 20 mg of freshly prepared SC was taken and hydrated over saturated potassium sulphate solution for 3 days. Then the SC was blotted resulting in hydration between 20–25%. Percent hydration was calculated using the formula:

$$\% \text{ hydration} = \frac{\text{weight of hydrated SC} - \text{weight of dry SC}}{\text{weight of dry SC}} \times 100 \quad (1)$$

A hydrated SC sample was dipped into nanoemulsion formulation in 4 ml of ethanolic PBS pH 7.4 (20 : 80). This was kept for 24 h (equivalent to the permeation studies) at 21 ± 1 °C. After treatment, SC was removed and blotted to attain hydration of 20–25%, cut (5 mg) and sealed in aluminum hermatic pans, and equilibrated for 1 h before the DSC run. Then, the SC samples were scanned on a DSC6 Differential Scanning Calorimeter (Perkin Elmer, Germany). Scanning rate was 5 °C/min over the temperature range of 30 to 200 °C (Cumming and Winfield 1994; Panchagnula et al. 2001; Vaddi et al. 2002).

3.5. Determination of activation energy

An *in vitro* permeation study of aceclofenac across rat skin was carried out at 27, 37, and 47 °C in the vehicle (ethanolic PBS pH 7.4). 1 ml of formulation (containing 20 mg of aceclofenac) was placed in the donor compartment. The receiver compartment contained the vehicle only. Permeability coefficients were calculated at each temperature and the activation energy of aceclofenac was then calculated from the Arrhenius relationship as follows (Golden et al. 1986; Narishetty and Panchagnula 2004).

$$P = P_o\, e^{-Ea/RT} \quad \text{or} \quad (2)$$

$$\log P = Ea/2.303\, RT + \log P_o \quad (3)$$

Where Ea is the activation energy, R is the gas constant (1.987kcal/mol), T is the absolute temperature in K, P is the permeability cofficient, and Po is the Arrhenius factor.

3.6. Histopathological examination of skin specimens

Abdominal skin of Wistar rats was treated with the optimized aceclofenac nanoemulsion in ethanolic PBS at pH 7.4. After 24 h, the rats were sacrificed and skin samples were taken from treated and untreated (control) areas. Each specimen was stored in 10% formalin solution in phosphate buffer saline (pH 7.4). The specimens were cut into sections vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. These samples were then observed under a light microscope (Motic, Japan) and compared with control samples. For each skin sample, three different sites were scanned and evaluated to elucidate the mechanism of penetration enhancement (Fang et al. 2003; Aqil et al. 2004). These slides were interpreted by Dr. Ashok Mukherjee, Professor, Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, India.

In conclusion, FTIR spectra of skin treated with the nanoemulsion formulation indicate breaking of the hydrogen bond network at the ceramide surface due to entry of nanoemulsion into the lipid bilayers of the SC. DSC thermograms indicated that intracellular transport is a possible mechanism of permeation enhancement and that permeation occurred due to the extraction of SC lipids by the nanoemulsion. The significant decrease in activation energy for aceclofenac permeation across rat skin indicates that the SC lipid bilayers was significantly disrupted ($p < 0.05$). Photomicrographs of skin samples showed the disruption and extraction of lipid bilayers as distinct voids and empty spaces visible in the epidermal region. There were no apparent signs of skin irritation (erythma and edema) observed on visual examination of skin specimens treated with the nanoemulsion formulation indicating an absence of any skin irritation as a consequence of nanoemulsion treatment. Overall these findings indicate that nanoemulsions can be successfully used to enhance skin permeation of drugs.

Acknowledgement: The authors are grateful to Dr. Ashok Mukherjee, Professor, Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, India for examination and interpretation of photomicrographs of skin samples.

References

Aqil M, Ali A, Sultana Y (2004) Matrix type transdermal drug delivery system of metoprolol tartrate: skin toxicity and *in vivo* characterization. Ethiop Pharm J 22: 53–60.

Baboota S, Shakeel F, Ahuja A, Ali J, Shafiq S (2007a) Design development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm 8: 315–332.

Baboota S, Alazaki A, Kohli K, Ali J, Dixit N, Shakeel F (2007b) Development and evaluation of a microemulsion formulation for transdermal delivery of terbinafine. PDA J Pharm Sci Technol 61: 276–285.

Babua RJ, Pandit JK (2005) Effect of penetration enhancers on the release and skin permeation of bupranolol from reservoir-type transdermal delivery systems. Int J Pharm 288: 325–334.

Clarys P, Alewaeters K, Jadoul A, Barel A, Manadas RO, Préat V (1998) *In vitro* percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. Eur J Pharm Biopharm 46: 279–283.

Cole L, Heard C (2007) Skin permeation enhancement potential of Aloe vera and a proposed mechanism of action based upon size exclusion and pull effect. Int J Pharm 333: 10–16.

Cotte M, Dumas P, Besnard M, Tchoreloff P, Walter P (2004) Synchrotron FT-IR microscopic study of chemical enhancers in transdermal drug delivery: example of fatty acids. J Control Release 97: 269–281.

Cullander PA, Guy RH (1991) Sites of iontophoretic current flow into the skin: identification and characterization with the vibrating probe electrode. J Invest Dermatol 97: 55–64.

Cumming KI, Winfield AJ (1994) *In vitro* evaluation of a series of sodium carboxylates as dermal penetration enhancers. Int J Pharm 108: 141–148.

Fang JY, Hung CF, Chiu HC, Wang JJ, Chan TF (2003) Efficacy and irritancy of enhancers on the *in-vitro* and *in-vivo* percutaneous absorption of curcumin. J Pharm Pharmacol 55: 593–601.

Gasco MR, Gallarate M, Pattarino F (1991) *In vitro* permeation of azelaic acid from viscosized microemulsions. Int J Pharm 69: 193–196.

Golden GM, Guzek DB, Harris RR, McKie JE, Potts RO (1986) Lipid thermotropic transition in human stratum corneum. J Invest Dermatol 86: 255–259.

Goodman M, Barry BW (1989) Action of penetration enhancers on human stratum corneum as assessed by differential scanning calorimetry. In: Bronaugh RL, Maibach HI (eds) Percutaneous absorption. Marcel Dekker, New York and Basel, 567–593.

Hori M, Satoh S, Maibach HI, Guy RH (1991) Enhancement of propranolol hydrochloride and diazepam skin absorption *in vitro*: effect of enhancer lipophilicity. J Pharm Sci 80: 32–35.

Jain AK, Thomas NS, Panchagula R (2001) Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J Control Release 79: 93–101.

Krishnaiah YS, Al-Saidan SM, Jayaram B (2006) Effect of nerodilol, carvone and anethole on the *in vitro* transdermal delivery of selegiline hydrochloride. Pharmazie 61: 46–53.

Kriwet K, Muller-Goymann CC (1995) Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. Int J Pharm 125: 231–242.

Ktistis G, Niopas I (1998) A study on the *in-vitro* percutaneous absorption of propranolol from disperse systems. J Pharm Pharmacol 50: 413–419.

Lee J, Lee Y, Kim J, Yoon M, Choi YK (2005a) Formulation of microemulsion systems for transdermal delivery of aceclofenac. Arch Pharm Res 28: 1097–1102.

Lee PJ, Langer R, Shastri VP (2005b) Role of n-methyl pyrrolidone in the enhancement of aqueous phase transdermal transport. J Pharm Sci 94: 912–917.

Monti D, Saettone MF, Giannaccini B, Galli-Angeli D (1995) Enhancement of transdermal penetration of dapiprazole through hairless mouse skin. J Control Release 33: 71–77.

Narishetty STK, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95: 367–379.

Pagano R, Thompson TE (1968) Spherical bilayer membranes: electrical and isotopic studies of ion permeability. J Mol Biol 38: 41–57.

Panchagnula R, Salve PS, Thomas NS, Jain AK, Ramarao P (2001) Transdermal delivery of naloxone: effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin. Int J Pharm 219: 95–105.

Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M (2007a) Design and development of oral oil in water ramipril nanoemulsion formulation: *In vitro* and *in vivo* assessment. J Biomed Nanotech 3: 28–44.

Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M (2007c) Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 66: 227–242.

Shafiq S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, Khar RK, Ali M (2007b) Formulation development and optimization using nanoemulsion technique: a technical note. AAPS PharmSciTech 8: E28.

Shakeel F, Baboota S, Ahuja A, Ali J, Aqil M, Shafiq S (2007) Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS PharmSciTech 8: E104.

Stott PW, Williams AC, Barry BW (2001) Mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acids: a melting point depression effect. Int J Pharm 219: 161–176.

Trotta M (1999) Influence of phase transformation on indomethacin release from microemulsions. J Control Release 60: 399–405.

Vaddi HK, Ho PC, Chan SY (2002) Terpenes in propylene glycol as skin-penetration enhancers: permeation and partition of haloperidol, Fourier transform tnfrared spectroscopy and differential scanning calorimetry. J Pharm Sci 91: 1639–1651.

Xiong GL, Quan D, Maibach HI (1996) Effects of penetration enhacers on *in vitro* percutaneous absorption of low molecular weight heparin through human skin. J Control Release 42: 289–296

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47: 978–989.

Yamazaki R, Kawai S, Mastsuzaki T (1997) Aceclofenac blocks prostaglandin E2 production following its intracellular conversion into cyclooxygenase inhibitors. Eur J Pharmacol 329: 181–187.

Zhao K, Singh J (1998) Mechanisms of percutaneous absorption of tamoxifen by terpenes: eugenol, D-limonene and menthone. J Control Release 55: 253–264.