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

ISSN 2349-8870
Volume: 5
Issue: 10
414-420
Year: 2018

TRANSDERMAL DELIVERY OF KETOPROFEN FROM MICROEMULSION BASED SYSTEMS: OCCLUSIVE VERSUS OPEN APPLICATION

Noha W. El Khayat*, Ahmed A. Donia and Gamal M. El Maghraby

Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt.

***Corresponding Author: Dr. Noha W. El Khayat**
Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt.

Article Received on 20/08/2018 **Article Revised on 10/09/2018** **Article Accepted on 30/09/2018**

ABSTRACT
The use of microemulsion in transdermal drug delivery has gained interest recently with numerous studies recording promising results. The ability of such system to fill the micro-architecture of the skin surface was considered as the main mechanism for enhanced transdermal delivery in addition to the penetration enhancing activity of the components. The first mechanism depends on the fluidity of the formulation. Unfortunately, fluid systems are not widely accepted for topical application. Accordingly, the aim of this work was to compare transdermal delivery of ketoprofen from fluid and non-fluid microemuslion phase transition systems. The tested formulations were fluid ethanol free microemulsion, gel and liquid crystalline (LC) phase systems and ethanol containing microemulsions which thickens after evaporation of ethanol upon open application to skin. All formulations were better than saturated aqueous control. Fluid formulations were superior than gel and LC systems. Open application of ethanol containing systems delivered ketoprofen through the skin at higher rate than the corresponding gel or LC system. The study highlighted the possibility of open application of fluid microemulsion which contains volatile components. This system combines the stron penetration enhancing potential and feasibility of topical application due to thickening after evaporation of the volatile component.

INTRODUCTION
Transdermal drug delivery employs the most accessible organ in the body. It has many advantages compared to other drug delivery routes. However, successful transdermal drug delivery requires extensive work to breach the skin barrier.[1] Breaking the skin barrier is being achieved through various tactics including the use of chemical penetration enhancers which reversibly modulate the stratum conium.[2,3] The strategies also include controlling the thermodynamic activity of the active pharmaceutical ingredient[4] and application of weak electric current or ultrasonic waves.[5,6,7] Fluid nanostructures including vesicular drug delivery and microemulsions have been extensively investigated in the recent years.[8,9] Vesicular nanostructures have been shown to improve both dermal and transdermal drug delivery depending on the composition of the carrier.[10,11] Varying the composition of the vesicular drug delivery system resulted in development of different carriers including traditional liposomes, transfersomes, ethosomes and niosomes.[12,13,14] Despite of the success of vesicular delivery systems, the poor drug entrapment and difficulty of scaling up hampered their wide application in drug market. In addition, the success of sophisticated vesicular system depends on their fluidity which permits their influx into and through intact skin.[15] Microemulsion and self-microemulsifying systems provide another nanosized delivery system. Microemulsions have many advantages including high drug loading, ease of preparation in addition to the ability to deliver drugs into and through skin.[16,17,18,19] The critical parameter of microemulsions is their capacity to undergo thickening after mixing with aqueous environment.[20] This thickening is inhibited by the presence of high concentrations of cosurfactant like ethanol or isopropanol.[14] For example, mixing oleic acid with Tween 80 in presence and absence of propylene glycol provides a system which can be diluted with water forming fluid microemulsion at low water concentration. This fluid system undergoes thickening to form gel like preparation on increasing water content. The thickening process is abolished in presence of ethanol.[21] Taking these findings into consideration, it was concluded that systems like this can provide the pharmaceutical formulators with high versatility in selection of the final formulation for application into skin.[21,22] For example, open application of ethanol containing system will result in evaporation of ethanol leaving a gel like film on skin surface. Also direct application of the gel like system is possible. The difference between both strategies requires further investigations.

Accordingly, the objective of this work was to investigate the effects of composition of oleic acid based microemulsion and hence the phase behaviour on the transdermal delivery of ketoprofen (model drug). The

work was extended to test the effect of application technique.

MATERIALS AND METHODS

Materials

Ketoprofen was obtained from Sigma Pharmaceutical Company, Qwesna, Egypt. Oleic acid was obtained from LOBA Chemie, PVT LTD, Mumbai, India. Acetonitrile (HPLC grade) was purchased from BDH, Poole, England. Ethanol, propylene glycol, tween 80, sodium chloride, disodium hydrogen phosphate, potassium chloride and potassium dihydrogen phosphate were procured from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt.

Preparation of tested formulations

The tested formulations included microemulsion, liquid crystalline and gel systems which were prepared according to the composition presented in Table 1. This composition was selected to formulate microemulsion containing oleic acid and Tween 80 and water (17.5: 70: 12.5). This system thickens upon titration with water to provide gel at a composition of 12.5: 50: 37.5 (oil/surfactant/water). The gel was gradually diluted with ethanol to form clear microemulsion at the composition presented in Table 1 (ETH TW ME). This microemulsion is expected to revert back to the gel upon open application and evaporation of ethanol. The same strategy was used to prepare formulations containing propylene glycol in absence and presence of ethanol. The developed formulations will comparison between the application of gel like formulation and the ethanol containing system which thickens upon open application. The formulations were prepared by mixing oil with the surfactant and cosurfcatant (if any) before addition of the required amount of water while mixing. Ketoprofen was added to each formulation to provide saturated solution with excess crystals added to maintain saturation after being equilibrated at 32^{o}C for about 72 hours.

Table 1: The composition of the selected formulations.

Formulation	Water	Oleic acid	Tween 80	Ethanol	PG	Ketoprofen solubility (mg/ml)
TW ME	12.5	17.5	70	-----	-----	72.09 (0.62)
TW GEL	37.5	12.5	50	-----	-----	ND
ETH TW ME	30	10	40	20	-----	144.63 (13.50)
PG TW ME	12.5	17.5	35	-----	35	115.58 (0.66)
PG TW LC	37.5	12.5	25	-----	25	ND
ETH PG TW ME	33.33	11.11	22.22	11.11	22.22	170.44 (0.92)

Values between brackets are S.D. (n=3).

Chromatography

The concentration of ketoprofen in test samples was quantified using high-pressure liquid chromatography (Waters^{TM} 600 controller, Milford, MA, USA). The system is controlled by computer and the samples are automatically injected and analyzed at 258nm using Waters (486) UV detector. The stationary phase employed a 150 mm x 4.6 mm ODS column (average particle size of 5µm, GI sciences, Tokyo, Japan). The mobile phase utilized a mixture of acetonitrile and acidified distilled water (adjusted to pH 3.5 with ortho-phosphoric acid) (60:40). This mobile phase was pumped at 1.3 ml/min.

Ethanolic stock solution of ketoprofen (1000 µg/ml) was prepared. This was used to prepare the standard concentrations (0.5, 1, 2, 3, 5, 10, 15 and 20 µg/ml) which were analyzed by the HPLC method. The calibration curves were constructed by plotting the area under the peak as a function of ketoprofen concentration. The method was validated for linearity, accuracy, precision, lower limit of detection (LOD) and lower limit of quantification (LOQ).

Solubility study

Excess amounts of ketoprofen were added to 10ml of the tested formulations. The resulting suspensions were left to equilibrate by continuous mixing in a thermostated water bath maintained at 32°C for 72 hours. The excess drug was removed by centrifugation. The concentration of ketoprofen in the clear supernatant was determined by HPLC after suitable dilution with ethanol.

Skin permeation studies

The procedures were approved by the ethical committee, College of Pharmacy, University of Tanta (approval number, 1606015). This employed rabbit ear model which is well documented in literature.[23,24] The study employed 24 male rabbits with an average weight of 2 kg. Full thickness inner side skin was pealed from of the underlying cartilage after cutting along the tips of the ears. The skin was cut into sections before mounting between the receptor and donor compartments of vertical glass diffusion cells which have surface area of 2.27 cm^2. The stratum corneum side was facing the donor compartment with the receptor compartment being filled with 40% (v/v) ethanol in phosphate buffered saline (pH 7.4). This receptor was selected to ensure existence of sink conditions throughout the permeation study. The whole assembly was equilibrated overnight and was maintained throughout the experiment in a water bath adjusted to maintain skin surface temperature of 32^{o}C. After equilibration, the tested formulations (2ml) were applied and the donor compartments were occluded with aluminium foil when required in the occlusive application protocol. Receptor samples were collected

periodically. These were analysed for drug content by HPLC. The cells were replenished with fresh receptor fluid to maintain constant receptor volume

In vitro drug release

These studies adopted the same skin permeation experimental setup but employed cellulose membrane (Cellulose tubing, Sigma diagnostics, St Louis, MO, USA) instead of skin.

Analysis of skin permeation and drug release data

The cumulative amounts of the drug permeated or released were plotted as a function of time to construct the permeation and release profiles, respectively. The permeation profiles were used to calculate the permeation parameters including the flux and lag time. The flux was calculated from the slope of the line fitted to the linear portion of the profile. Extrapolation of this line provides an estimate for the lag time. The release rates were computed from the slope of the release profiles.[9,25]

RESULTS AND DISCUSSION

HPLC analysis of ketoprofen

The retention time of ketoprofen was 4 minutes. The method was shown to be linear in the concentration range of 0.5 to 20 µg/ml. The method was precise as reflected from the calculated relative standard deviation which ranged from 0.35 and 2.94% (intra-day) and from 0.14 to 8.16%. The LOD was calculated to be 0.12 µg/ml and the LOQ was 0.35 µg/ml.

Saturation solubility of ketoprofen

The saturation solubility of ketoprofen was determined at 32^{o}C to simulate the experimental conditions of the skin. Table 1 presents the recorded solubility values. The saturation solubility of ketoprofen in water was 0.19 mg/ml. The microemulsion formulations were able to solubilize significantly higher amounts of ketoprofen, compared with water (Table 1). The solubility of ketoprofen in microemulsion formulations was increased in formulation containing propylene glycol and or ethanol. This is expected as microemulsions are known to have high drug loading due to large surface area. The presence of cosurfacatnt can further enhance the loading capacity by further reduction in the surface area and surface tension. In addition, the cosolvent power of ethanol and propylene glycol can contribute to the enhanced solubility. Similar findings have been published for other drugs with the authors reporting similar explanation.[14,26,27]

Release of ketoprofen from different formulation

The in vitro release of ketoprofen from different formulations was monitored both after occlusive and open application. The recorded release profiles are presented in Fig. 1. The release rates were calculated from the linear portion of the profiles. The calculated release rates are presented in Table 2. The rate of ketoprofen release depended on the composition of the formulation. In cosurfactant free system the release rate decreased by increasing water content due to thickening. So TW gel liberated ketoprofen at significantly lower rate ($P < 0.05$), compared to TW ME (Table 2). This can be attributed to the thickening of the formulation by increasing water content. The same trend was recorded for propylene glycol based systems at low and high water content. Addition of ethanol to the viscous formulation resulted in significant increase in the rate of drug release compared with the corresponding thick formulation (Table 2). Open application of the fluid formulation reduced of the rate of drug release due to evaporation of volatile components. Dependence of drug release from microemulsion on the water content has been recorded in other studies.[20,21]

Table 2: The in vitro drug release rate and the transdermal permeation parameters of ketoprofen obtained from different microemulsion formulations. The control was saturated aqueous solution of the drug.

Drug formulation	Occlusive application			Open application		
	Flux (µg cm^{-2} h^{-1})	Lag time (h)	Release rate (µg cm^{-2} h^{-1})	Flux (µg cm^{-2} h^{-1})	Lag time (h)	Release (µg cm^{-2} h^{-1})
TW ME	133.60 (17.37)	1.53 (0.76)	382.93 (3.22)	122.55 (7.22)	1.11 (0.22)	274.92 (8.3)
TW GEL	83.59 (2.89)	1.96 (0.31)	180.73 (15.58)	ND	ND	ND
ETH TW ME	212.75 (8.25)	0.78 (0.21)	597.67 (20.13)	190.99 (15.49)	0.59 (.26)	460.72 (38)
PG TW ME	185.18 (12.44)	1.52 (0.53)	434.19 (66.61)	147.9 (8.48)	1.36 (0.44)	389.97 (2.08)
PG TW LC	115.87 (1.4)	0.64 (0.24)	261.58 (12.68)	ND	ND	ND
ETH PG TW ME	369 (29.89)	1.6 (0.79)	626.16 (12.12)	234 (16.37)	1.38 (0.14)	574.65 (38.88)
Control	28.44 (5.1)	0.96 (0.53)	ND	28.02 (9.9)	1.56 (0.7)	ND

Values between brackets are S.D. (n=3). ND = not determined.

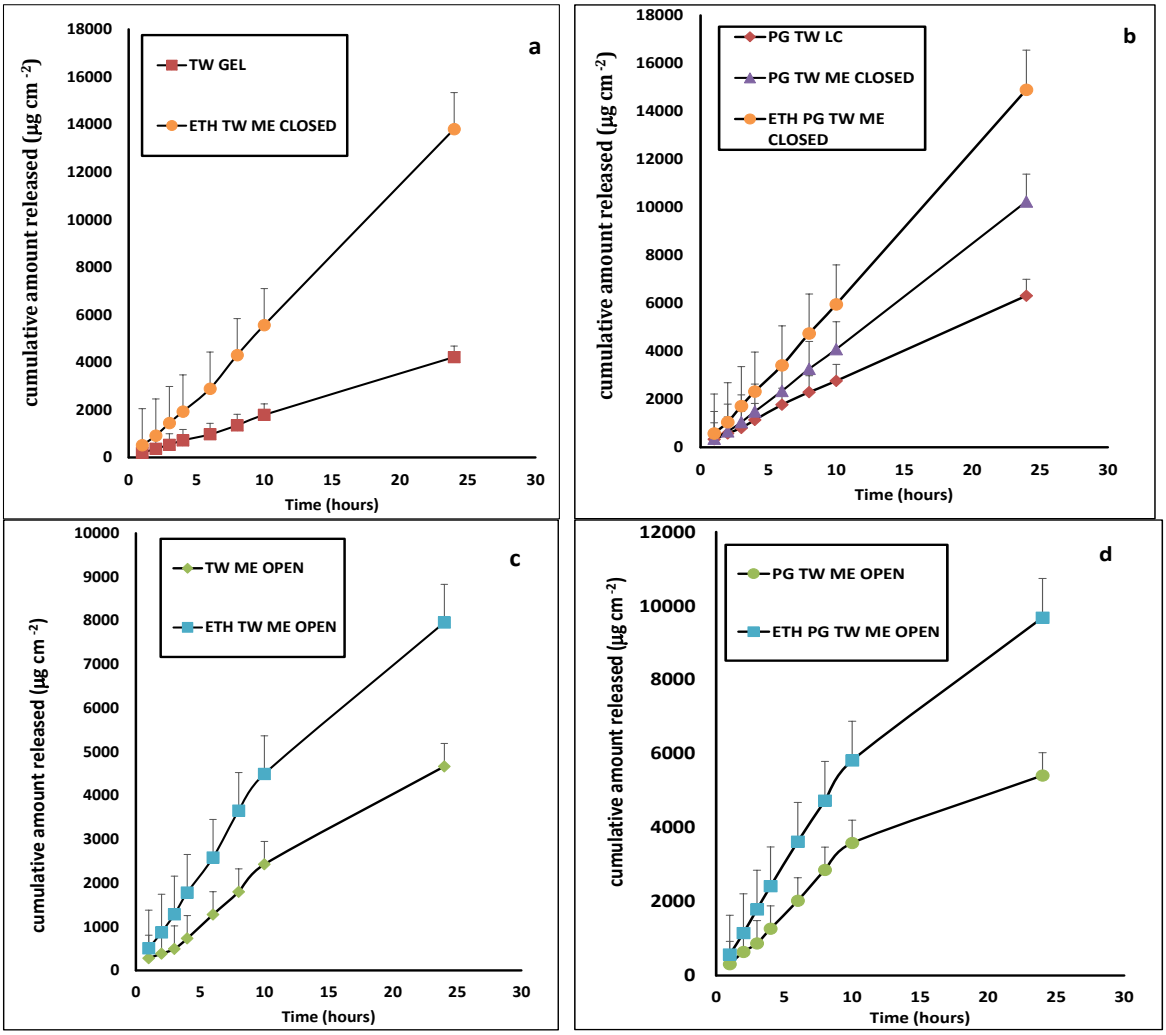

Fig. 1. In vitro release profiles of ketoprofen from microemulsion formulations. Fomulations details are in table 1.

Transdermal delivery of ketoprofen from microemulsion after occlusive and open application

Fig. 2 shows the transdermal permeation profiles of ketoprofen after occlusive and open application of microemulaion formulations. The calculated permeation parameters are presented in Table 2. Occlusive application of ketoprofen in microemulsion systems or gel like formulations resulted in significant increase in the transdermal flux compared to the saturated aqueous solution of the drug (Table 2). The recoreded transdermal flux depended on the composition of each formulation with fluid formulations delivering the drug at higher rate compared to the corresponding thick system. Within the fluid formulation the presence of ethanol and/or propylene glycol enhanced the transdermal flux. These results are expected as propylene glycol and ethanol can act as cosurfactants which can reduce the interfacial tension and provide more intimate contact with the micro-architecture of the skin. In addition, ethanol can add to the penetration enhancing activity of the microemulsion components. Similar findings have been reported for ethanol and propylene glycol containing microemulsions.[9,14,28,29,30]

Moreover, the co-existence of propylene glycol with oleic acid is expected to provide synergistic effect in enhanced transdermal delivery. This synergism is attributed to the ability of propylene glycol to enter the skin modulating the characteristics of stratum corneum and allowing more oleic acid to enter the stratum corneum. This subsequently augments the skin penetration enhancement.[31]

The superiority of microemulsion formulations is mainly attributed to their ability to fill the micro-architecture of the skin surface due to very low surface tension and minute droplet size in addition to the penetration enhancing effect of the microemulsion components.[18,32,33] The first mechanism is more probable in case of fluid formulation.

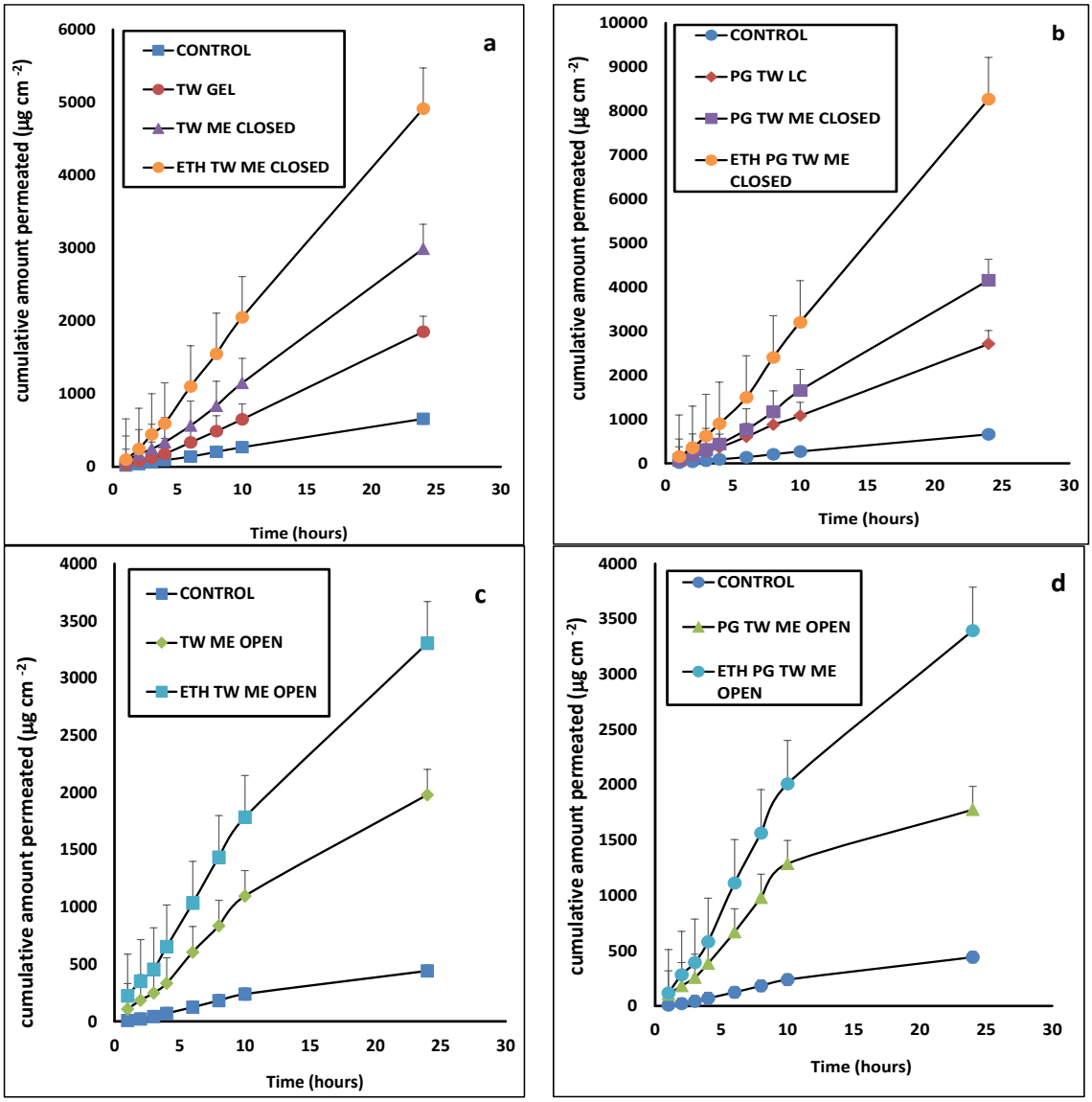

Fig. 2: Skin permeation profiles of ketoprofen from microemulsion formulations. Fomulations details are in table 1.

This explains the reduction in transdermal flux from the less fluid system particularly the liquid crystalline and gel phase systems compared to the corresponding fluid formulation. Unfortunately, Semisolid formulations are more pharmaceutically acceptable due to ease of application to form stable film which remains for longer time compared to the fluid system. This work monitored the feasibility of open application of fluid systems which thicken upon evaporation of volatile component. These systems were compared to the corresponding thick system which is applied under occlusion. Therefore, the gel and liquid crystalline formulations (Table 1) were diluted with ethanol to produce fluid microemulsion. This dilution has been shown to break the structured system.[14,34] Open application of ethanol containing fluid system produced transdermal drug flux higher than ($P< 0.05$) that obtained after occlusive application of the corresponding thick formulation. This is noticed by comparing the flux obtained after open application of ETH TW ME to that obtained after occlusive application of TW gel and comparing ETH PG TW ME to PG TW LC (Table 2). These finding suggests that formulation of such systems in the form of spray can provide better results than preparation of gel or liquid crystalline system. The recorded superiority of fluid system even after open application can be attributed to the beneficial effects of ethanol which provided further reduction in the surface tension and droplet size.[9] Moreover, evaporation of volatile components can lead to possible supersaturation.[21,35] However, this requires further investigations.

CONCLUSION

The study reflected the dependence of transdermal drug delivery potential on the composition of microemulsion. Open application of ethanol containing microemulsion which thicken after evaporation of ethanol is better than application of the corresponding thick formulation even under occlusion.

REFERENCES

1. Barry BW. Dermatological Formulations: Percutaneous Absorption, Marcel Dekker, New York and Basel, 1983.
2. Goodman M, Barry BW. Action of penetration enhancers on human skin as assessed by permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J Invest Dermatol, 1988; 91: 323–327.
3. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev., 2004; 56: 603-18.
4. Megrab NA, Williams AC, Barry BW. Oestradiol permeation across human skin and silastic membranes: effects of propylene glycol and supersaturation. J Control Rel, 1995; 36: 277–294.
5. Miller LL, Kolaskie CJ, Smith GA, Rivier J. Transdermal Iontophoresis of gonadotropin releasing hormone and two analogues. J Pharm Sci., 1990; 79: 490–493.
6. Kost J, Pliquett U, Mitragotri S, Yamamoto A, Langer R, Weaver J. Synergistic effect of electric field and ultrasound on transdermal transport. Pharm Res., 1996; 13: 633–638.
7. Banga AK, Bose S, Ghosh TK. Iontophoresis and electroporation: comparisons and contrasts. Int J Pharm, 1999; 179: 1–19.
8. El Maghraby GM., Williams AC. Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin. Expert Opin Drug Deliv, 2009; 6: 149-63.
9. El Maghraby GM, Arafa MF, Osman MA. Microemulsion for simultaneous transdermal delivery of benzocaine and indomethacin: in vitro and in vivo evaluation. Drug Dev Ind Pharm, 2014; 40: 1637-44.
10. Mezei M, Gulasekharam V. Liposomes--a selective drug delivery system for the topical route of administration. Lotion dosage form. Life Sci., 1980; 26: 1473-7.
11. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta, 1992; 1104: 226-32.
12. Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J Control Rel, 1994; 30: 1–15.
13. El Maghraby GM, Williams AC, Barry BW. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. J Pharm Pharmacol, 1999; 51: 1123-34.
14. El Maghraby GM. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: effects of cosurfactant. Int J Pharm, 2008; 355: 285–292.
15. El Maghraby GM, Williams AC, Barry BW. Can drug-bearing liposomes penetrate intact skin? J Pharm Pharmacol, 2006; 58: 415-29.
16. Boltri L, Morel S, Trotta M, Gasco MR. In vitro transdermal permeation of nifedipine from thickened microemulsions. J Pharm Belg, 1994; 49: 315-20.
17. Delgado Charro MB, Iglesias Vilas G, Blanco Mendez J, Lopez Quintela MA, Marty JP, Guy RH. Delivery of a hydrophilic solute through the skin from novel microemulsion systems Eur. J. Pharm. Biopharm, 1997; 43: 37-42.
18. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev, 2002; 54: 77-98.
19. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interface Sci, 2006; 123-126: 369-85.
20. Chan J, Maghraby GM, Craig JP, Alany RG. Phase transition water-in-oil microemulsions as ocular drug delivery systems: in vitro and in vivo evaluation. Int J Pharm, 2007; 328: 65-71.
21. El Maghraby GM. Microemulsions as Transdermal Drug Delivery Systems. Curr Nanosci, 2012; 8: 504-511.
22. El Maghraby GM. Self microemulsifying and microemulsion systems for transdermal delivery of indomethacin: Effect of phase transition. Colloids Surf B, 2010; 75: 595-600.
23. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Rel, 2000; 65: 403–418.
24. El Maghraby GM, Ahmed AA, Osman MA. Transdermal Delivery of Nisoldipine: Refinement of Vehicles. Int J Pharm Compd, 2015; 19: 152-60.
25. El Maghraby GM, Williams AC, Barry BW. Skin delivery of oestradoil from lipid vesicles: importance of liposome structure. Int J Pharm, 2000; 204: 159-69.
26. Danielsson I, Lindman B. The definition of microemulsion. Colloids Surf, 1981; 3: 391-392.
27. Malcolmson C, Lawrence MJ. A comparison between nonionic micelles and microemulsions as a means of incorporating the poorly water soluble drug diazepam. J pharm pharmcol Suppl, 1990; 42: 6P.
28. Yamane MA., Williams AC., Barry BW. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol, 1995; 47:978–989.
29. Narishetty ST. Panchangnula R. Effect of L-menthol and 1,8-cineole on the phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J. Control. Rel, 2005; 102: 59–70.
30. Liu CH, Chang FY, Hung DK. Terpene microemulsions for transdermal curcumin delivery: effects of terpenes and cosurfactants. Colloids Surf B, 2011; 82: 63-70.
31. Barry BW. Mode of action of penetration enhancers in human skin. J Control Rel, 1987; 6: 85-97.
32. Dreher F, Walde P, Walther P, Wehrli E. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J Control Rel, 1997; 45: 131–140.

33. Peltola S, Saarinen Savolainen P, Kiesvaara J, Suhonen TM, Urtti A. Microemulsions for topical delivery of estradiol. Int. J. Pharm, 2003; 254: 99–107.
34. Alany RG, Tucker IG, Davies NM, Rades T. Characterizing colloidal structures of pseudoternary phase diagrams formed by oil/water/amphiphile systems. Drug Dev Ind Pharm, 2001; 27: 31-8.
35. Leichtnam ML, Rolland H, Wüthrich P, Guy RH. Impact of antinucleants on transdermal delivery of testosterone from a spray. J Pharm Sci., 2007; 96: 84–92.