Enhance transdermal delivery of flurbiprofen via microemulsions: Effects of different types of surfactants and cosurfactants

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**ABSTRACT**

Background and the purpose of the study: Microemulsions are thermodynamically stable, clear dispersions of water, oil, surfactant, and cosurfactant. This study was aimed to develop flurbiprofen microemulsion for enhanced transdermal delivery and investigate the effects of different surfactants and cosurfactants on its delivery and phase behavior.

Method: Various surfactant-cosurfactant mixtures in ratio of 2:1 (Smix) along with oleic acid (oil) were selected and phase diagrams were constructed. Six microemulsions each containing 5% drug, 5% oil, 56% Smix and 34% water, were prepared and compared for their permeation and phase behaviors to determine the effects of the type of Smix.

Results: In vitro transdermal permeation through rabbit skin of all microemulsions was high than saturated aqueous drug solution. Tween 20 and ethanol as Smix produced the highest flux amongst all the Smix, and were used to prepare formulations with different values of oil and Smix. While the type of surfactant did not affect the droplet size, propylene glycol as cosurfactant produced the largest droplets and highest viscosity. Decrease in oil or Smix concentration resulted in decrease of the droplet size and increase in permeation flux while decrease in viscosity also increased the permeation flux of microemulsions. Finally the selected microemulsion formulation comprising 5% flurbiprofen, 5% oleic acid, 46% Tween 20:ethanol (2:1) and 44% water, showed the highest transdermal flux and caused no skin irritation.

Conclusion: Type of surfactant and cosurfactant affect both the phase behavior and transdermal drug delivery of microemulsion; and results of this study showed that they are promising vehicles for improved transdermal delivery and sustained action of flurbiprofen.

**Keywords:** Permeation flux, Phase behavior, Microemulsion, Oleic acid, Tween 20.

**INTRODUCTION**

Flurbiprofen, a nonsteroidal anti-inflammatory drug, is frequently prescribed to treat gout, musculoskeletal disorders, rheumatic diseases, post-operative pain, dysmenorhoea and migraine. Oral administration of flurbiprofen is associated with severe gastrointestinal adverse events including abdominal discomfort, constipation, diarrhea, dyspepsia, nausea and vomiting (1). Moreover, it requires frequent dosing due to its short elimination half-life of 3.9 hrs (2). These facts raise a need for a long-term sustained delivery of flurbiprofen. Transdermal route of drug delivery has received increasing attention due to the advantages of avoidance of hepatic first-pass metabolism thereby reducing the dose, easier and convenient administration for patients, possibility of immediate withdrawal of treatment, and potential of long-term controlled release with steady-state plasma drug levels and ultimately improving the patient’s compliance (3). Flurbiprofen belongs to the Class II of the biopharmaceutical classification system (BCS) of drugs; and the aqueous solubility of drugs of this class limits their permeation flux (4). The physicochemical properties of flurbiprofen, i.e., lipophilicity (log $K_{ow} = 4.24$) (5), low molecular weight and short elimination half-life make it a suitable candidate for transdermal drug delivery but its aqueous solubility is very low (0.03 mg/ml) which hinders its skin penetration (6).

Microemulsions are a good example of pharmaceutical nanotechnologic carrier systems which, during the recent decades, have increasingly attracted researchers as drug carriers for various therapeutic
applications having a potential of increasing the dermal drug delivery. Microemulsions are clear, thermodynamically stable, single, optically isotropic mixtures of water, oil, and surfactant, usually in combination with a cosurfactant (7). Microemulsions are considered as ideal liquid vehicles for drug delivery due to the ease of formulation, thermodynamic stability, high solubilization capacity, low viscosity, small droplet size and transport of drugs in a controlled manner (8). Several studies have revealed that microemulsions can markedly increase the transdermal delivery of both hydrophilic and lipophilic drugs compared to the conventional vehicles, e.g., micellar solutions, liposomes, macroemulsions, neat oil phases, surfactant-cosurfactant mixtures, aqueous solutions, creams and ointments (9, 10). The microemulsion properties and composition are well known to influence the percutaneous drug delivery. Several studies have discussed the effects of the type of oil (9), of cosurfactant (8) and of the surfactant-cosurfactant ratio (7, 11) on the phase behavior, in vitro drug release and transdermal permeation of the microemulsions. However, the influence of the type of different surfactants such as Tween 20 and 80 on the transdermal delivery of drug from flurbiprofen microemulsions has not so far been reported and needs to be studied. In this context, the first aim of this study was to investigate effects of the type and concentrations of surfactants and cosurfactants on transdermal delivery of the model drug (flurbiprofen). The second goal of the study was to develop an optimal, nonirritating stable transdermal microemulsion formulation of flurbiprofen to enhance its permeation through skin and to provide a sustained and prolonged delivery.

MATERIALS AND METHODS

Materials
Flurbiprofen was gifted by Hamaz Pharmaceuticals (Multan, Pakistan). Oleic acid (OA), Tween 80, Propylene Glycol (PG), Ethanol and Isopropyl Alcohol (IPA) were purchased from Merck (Germany). Isopropyl Myristate (IPM) was obtained from Panreac Quimica (Europe). Tween 20 was purchased from Fisher Scientific (UK). Water was purified by distillation using distillation apparatus (IRMESCO, Germany) and then membrane filtering. All other chemicals and solvents were of HPLC or analytical grade and used as received without further purification.

Determination of solubility of flurbiprofen
Excess of flurbiprofen was added to 2 ml of each of the selected oils (OA, and IPM), surfactants (Tween 20 and Tween 80), and cosurfactants (ethanol, IPA and PG) in 5 ml vial and mixed by slow magnetic stirring. After stirring for 48 hrs at 25 °C, the equilibrated samples were centrifuged for 15 min at 5,000 rpm and the supernatant was filtered through 0.45 µm membrane filter (Sartorius, Germany). Filtered samples were properly diluted with methanol and the concentration of flurbiprofen was determined by UV-VIS spectroscope (IRMESCO U2020, Germany) at 247 nm and compared with the standard curve of flurbiprofen in methanol (y = 0.0837x - 0.0031, R² = 0.9995) (12).

Construction of pseudo-ternary phase diagrams
OA was selected as an oil phase on the basis of results of solubility experiment results, while the aqueous phase was distilled and water filtered through 0.45 µm membrane filter. Water titration method (13) was used to construct pseudo-ternary phase diagrams.

Preparation of flurbiprofen-loaded microemulsions
From the constructed pseudoternary phase diagrams, six different microemulsion formulations were selected and prepared. The amount of drug (5% w/w), oil (5% w/w), Smix (56% w/w) and water (34% w/w) used in various formulations were the same (Table 1); the selected variables were the type of surfactant and cosurfactant. Oil and Smix were mixed vigorously under magnetic stirring and flurbiprofen was added to this oily phase and mixed; then water was incorporated in it. The microemulsions were compared with saturated aqueous solution of drug for transdermal drug delivery potential. Saturated aqueous solution was prepared by dissolving excess of flurbiprofen in distilled water and filtering through 0.45 µm membrane filter.

Characterization of microemulsions
The droplet size and polydispersity index of the microemulsions were determined by Zetasizer nano S (Malvern Instruments, UK) at 25 °C. Viscosity was determined at 25 °C ±0.2 °C using a Brookfield RVDD III ultra, Programmable rheometer (Brookfield, MA) with Rheocalc V2.6 software.
Electrical conductivity (σ) and pH were measured using a conductometer WTW Cond 197i (Weilhein, Germany), and a pH meter (WTW inolab, Germany), respectively, at 25 °C.

In vitro skin permeation experiments
The animal study protocol was approved by Research Ethics Committee of the Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. Skin samples were obtained from male white rabbits weighing 1-1.25 kg by a method described previously (14). Tewameter™ (Courage & Khazaka, Germany) was used to measure the TEWL (Transepidermal Water Loss) and only those skin
pieces were introduced for testing whose TEWL levels were less than 15 g/m²/h. Vertical Franz-type diffusion cells (PermeGear, Bethlehem, PA) with surface area of 1.767 cm² and receptor compartment volume of 12 ml were used. The receptor chamber was filled with phosphate buffer of pH 7.4 maintained at 37±0.2°C and the skin was placed between the donor and receptor compartments. The donor compartment contained 1.0 g of test microemulsion containing 50 mg flurbiprofen. Samples (0.15 ml) were withdrawn at regular intervals up to 12 hrs, suitably diluted with the phosphate buffer and analyzed against a blank by UV-Vis spectrophotometer at 247 nm. Flurbiprofen concentration was determined using the standard curve prepared with phosphate buffer ($y = 0.0871x -0.0251, R^2 = 0.9882$). The aliquots of permeated formulation without the drug were used as a blank (12).

Concentration of the receiver compartment of flurbiprofen was corrected for sample removal by using the Hayton and Chen equation (15). Cumulative drug permeation per unit area of skin ($Q_n, \mu g/cm^2$) and steady-state flux ($J_{ss}, \mu g/h/cm^2$) were calculated as explained previously (12, 16). Apparent permeability coefficients ($K_p, cm/h$) and enhancement ratio ($E_r$) were calculated as:

$$K_p = \frac{J_{ss}}{C_d}$$  \hspace{1cm} (1)

$$E_r = \frac{J_{ss \text{ (formulation)}}}{J_{ss \text{ (control)}}}$$  \hspace{1cm} (2)

Selection of the final microemulsion formulation
On the basis of skin permeation data Tween 20 was selected as the surfactant and ethanol as the cosurfactant. Four different o/w microemulsions containing 5% flurbiprofen were prepared in which contents of OA and Smix were varied as 5, and 10%; and 46 and 56%, respectively (Table 2). These formulations were characterized for droplet size, viscosity, conductivity and pH. In vitro skin permeation experiments were conducted to evaluate the effects of the content of oil and Smix on the skin permeation of flurbiprofen; and to select the best permeated formulation.

Thermodynamic stability tests
The stability of the selected formulations was determined via visual clarity, phase separation and drug assay by applying stress tests including centrifugation, heating-cooling cycles and freeze thaw cycles (13). Long term stability studies were also carried out by storing the formulations at 40°C for 6 months.

Skin irritation study
The selected microemulsion M2 was tested for skin irritation by method described previously (7).

Statistical analysis
Statistical data of all skin permeation experiments was analyzed using SPSS 12.0 software, by one-way analysis of variance (ANOVA) followed by post hoc testing.

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Table 1. Different types of surfactant-cosurfactant mixtures in flurbiprofen microemulsions containing 5% drug, 5% oleic acid, 56% Smix and 34% water

| Microemulsion Code | Surfactant   | Cosurfactant    |
|--------------------|--------------|-----------------|
| T20P               | Tween 20     | Propylene Glycol|
| T80P               | Tween 80     | Propylene Glycol|
| T20I               | Tween 20     | Isopropyl Alcohol|
| T80I               | Tween 80     | Isopropyl Alcohol|
| T20E               | Tween 20     | Ethanol         |
| T80E               | Tween 80     | Ethanol         |

*Smix is surfactant-cosurfactant mixture (2:1)

Table 2. Flurbiprofen microemulsions of varying compositions used for optimization.

| Formulation | Flurbiprofen % w/w | Oleic Acid % w/w | Smix % w/w | Water % w/w |
|-------------|---------------------|------------------|-------------|-------------|
| M1 (T20E)   | 5                   | 5                | 56          | 34          |
| M2          | 5                   | 5                | 46          | 44          |
| M3          | 5                   | 10               | 56          | 29          |
| M4          | 5                   | 10               | 46          | 39          |

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RESULTS AND DISCUSSION
The solubility of flurbiprofen was found to be higher in oleic acid, Tween 80 and ethanol amongst the oils, surfactants and cosurfactants, respectively (Table 3). It was observed that increasing the chain length of cosurfactant as it moved from ethanol to IPA increased the area of microemulsion region in phase diagrams (Fig 1). However, increase in number of hydroxyl groups as it moved from IPA to PG reduced the microemulsion existence area. Tween 20 produced larger microemulsion regions compared to Tween 80 when IPA or PG were used as the cosurfactant which was reversed when ethanol was used as the cosurfactant. The type of surfactant did not considerably affect the droplet size, while PG containing microemulsions produced largest droplets as well as highest viscosity followed by ethanol and IPA containing formulations. Tween 80 containing formulations showed higher viscosity than those prepared with Tween 20 (Table 4). The microemulsions had appropriate pH (4.58 - 4.95) and due to high conductivities indicated o/w type microemulsions. All microemulsion formulations significantly increased the transdermal flux compared to the control (Figs 2a, b). In terms of skin permeation the formulations were ranked as T20E > T20P > T80E.

Table 3. Solubility of flurbiprofen in various excipients (mean±S.D., n = 3).

| Excipient         | Solubility (mg/ml) |
|-------------------|--------------------|
| Oleic Acid        | 75.25 ± 2.01       |
| Isopropyl Myristate| 46.52 ± 1.02       |
| Tween 80          | 517.5 ± 3.25       |
| Tween 20          | 495.5 ± 2.04       |
| Ethanol           | 503.50 ± 4.21      |
| Isopropyl Alcohol | 484.50 ± 3.14      |
| Propylene Glycol  | 133.47 ± 2.47      |

Figure 1. Pseudoternary phase diagrams indicating the area of existence of o/w microemulsion (shaded region) containing different Smix at surfactant-cosurfactant ratio of 2:1. (a) Smix = Tween 20-PG (b) Smix = Tween 80-PG (c) Smix = Tween 20-IPA (d) Smix = Tween 80-IPA (e) Smix = Tween 20-ethanol (f) Smix = Tween 80-ethanol.

analysis; paired sample t-test was performed for skin irritation study at the level of $P = 0.05$. 
Table 4. Physicochemical and permeation parameters of various microemulsion formulations with different surfactant-cosurfactant mixtures (mean ± S.D., n =3).

| Micro emulsion Code | Droplet Size (nm) | Polydispersity Index | Viscosity (cp) | Conductivity σ (µsiemens/cm) | Flux, Jss (µg/cm²/h) | Permeability Coefficient Kp × 10⁻⁵ (cm/h) | Enhancement Ratio Er |
|---------------------|-------------------|----------------------|---------------|-----------------|------------------|--------------------------------|-----------------|
| Control             | -                 | -                    | -             | -               | -                | -                                 | -               |
| T20P                | 98.6 ± 4.5        | 0.125 ± 0.09         | 186.67 ± 7.54 | 108.6 ± 2.2     | 1.32 ± 0.008     | 2.65 ± 0.017                     | 12.85 ± 0.50    |
| T80P                | 106.9 ± 5.3       | 0.230 ± 0.015        | 452.48 ± 13.56| 168.6 ± 5.4     | 1.12 ± 0.044     | 2.25 ± 0.088                     | 10.91 ± 2.60    |
| T20I                | 40.5 ± 2.1        | 0.252 ± 0.021        | 38.62 ± 4.62  | 111.5 ± 2.2     | 1.24 ± 0.007     | 2.48 ± 0.014                     | 12.06 ± 0.41    |
| T80I                | 42.8 ± 3.2        | 0.322 ± 0.023        | 41.04 ± 3.25  | 127.2 ± 3.5     | 0.98 ± 0.024     | 1.97 ± 0.048                     | 9.54 ± 1.40     |
| T20E                | 47.5 ± 3.1        | 0.210 ± 0.012        | 53.01 ± 5.5   | 143.9 ± 2.3     | 1.59 ± 0.036     | 3.18 ± 0.072                     | 15.45 ± 2.12    |
| T80E                | 45.8 ± 2.3        | 0.242 ± 0.021        | 97.25 ± 8.71  | 188.7 ± 5.6     | 1.25 ± 0.011     | 2.50 ± 0.022                     | 12.13 ± 0.65    |

Table 5. Physicochemical and permeation parameters of the microemulsions with different compositions used for optimization (mean ± S.D., n =3).

| Formulation | Droplet Size (nm) | Polydispersity Index | Viscosity (cp) | Conductivity σ (µsiemens/cm) | Flux, Jss (µg/cm²/h) | Permeability Coefficient Kp × 10⁻⁵ (cm/h) | Enhancement Ratio Er |
|-------------|-------------------|----------------------|---------------|-----------------|------------------|--------------------------------|-----------------|
| M1 (T20E)  | 47.5 ± 3.1        | 0.210 ± 0.012        | 53.01 ± 5.5   | 143.9 ± 2.3     | 1.59 ± 0.036     | 3.18 ± 0.072                     | 15.45 ± 2.12    |
| M2         | 30.8 ± 1.9        | 0.306 ± 0.028        | 48.54 ± 3.25  | 152.7 ± 3.1     | 2.21 ± 0.037     | 4.43 ± 0.075                     | 21.50 ± 2.19    |
| M3         | 57.7 ± 4.3        | 0.254 ± 0.015        | 61.96 ± 2.64  | 149.2 ± 2.4     | 1.13 ± 0.020     | 2.25 ± 0.039                     | 10.93 ± 1.16    |
| M4         | 52.6 ± 3.3        | 0.154 ± 0.012        | 51.21 ± 4.83  | 170.2 ± 3.2     | 1.98 ± 0.023     | 3.95 ± 0.047                     | 19.19 ± 1.37    |

> T20I > T80P > T80I > control (Table 4). Tween 20 produced a significantly greater enhancement in transdermal flux compared to Tween 80. A comparison of cosurfactants showed that ethanol had the maximum permeation rate followed by PG and then IPA. Short chain alcohols, like ethanol, IPA and PG are small solvent molecules that, in their own right, permeate into the skin. Their presence in the skin improve the partitioning of the drug between the donor vehicle and the skin and thereby have influence on the flux (17). The physicochemical characteristics of microemulsions (M1-M4) are given in table 5. pH values ranged from 4.56-4.94 and high conductivity values proved their o/w type. All the selected formulations remained stable and no phase separation or drug degradation was observed over the period of stability testing; they significantly (P< 0.05) increased the transdermal flux in comparison to the control (Fig 3). In terms of skin permeation these formulations were ranked as M2 > M4 > M1 > M3 (Table 5). M2 formulation which consisted of 5% flurbiprofen, 5% oleic acid, 46% Tween 20-ethanol (2:1) mixture and 44% water produced the highest flux (2.21 µg/cm²/h). An increase in oil concentration from 5% to 10% reduced the flux with both 56% and 44% water produced the highest flux (2.21 µg/cm²/h). This might be due to an enhanced thermodynamic activity of drug in microemulsions with lower content of surfactant mixture (9). Increase in water content from 29% (M3) to 44% (M2) also significantly increased the permeation flux. This may be due to the increase in hydration of stratum corneum which in turn increased the permeation rate. Thus, formulation M2 was selected on the basis of permeation experiments as the final formulation for the transdermal delivery of flurbiprofen. M2 was also nonsensitizing and nonirritating to the skin; the difference between erythema values of control and the formulation was insignificant (P = 0.458).

The superior transdermal delivery from microemulsions may be due to the high drug loading capacity, penetration enhancing effect of the individual components, entry of the components in the skin as monomers thereby increasing the solubility of drug in the skin, possible formation of supersaturated vehicle during application thus increasing the thermodynamic activity and driving force for the transdermal drug delivery; and lower viscosity than creams and gels thereby higher thermodynamic activity (8, 9, 16). The study proves that a decrease in viscosity of microemulsion enhances its permeation flux. The formulation with
Figure 2. Permeation profiles of flurbiprofen through excised rabbit skin from microemulsions containing Tween 20 (a) and Tween 80 (b) as the surfactant (mean ± S.D., n = 3).

Figure 3. Permeation profiles of Flurbiprofen through excised rabbit skin from microemulsions of varying compositions (mean ± S.D., n = 3).
the highest viscosity, M3, produced the lowest flux while M2 which had lowest viscosity produced the highest transdermal flux. Thus, it can be inferred that surfactants and cosurfactants have a considerable effect not only on the skin permeation of drug but also on the droplet size, viscosity and microemulsion existence area. Further, the developed microemulsions produced up to 21 times greater flux than the control. Therefore, it may be concluded that the developed microemulsions have a great potential as a highly bioavailable non-irritant transdermal drug delivery system with sustained release action and minimum side effects.

REFERENCES

1. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale’s Pharmacology. 6th Ed., Elsevier, 2007; 227-237.
2. Heyneman CA, Lawless-Liday C, Wall GC. Oral versus topical NSAIDs in rheumatic diseases: A comparison. Drugs, 2000; 60: 555-574.
3. Barry BW. Transdermal drug delivery. In: Aulton ME, ed. Aulton's Pharmaceutics: The design and manufacture of medicines. Elsevier, 2007; 565-597.
4. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceuticals drug disposition classification system. Pharm Res, 2005; 22:11-23.
5. Li Q, Tsuji H, Kato Y, Sai Y, Kubo Y, Tsuji A. Characterization of the transdermal transport of flurbiprofen and indomethacin. J Control Release, 2006; 110: 542-556.
6. Fang JY, Leu YL, Chang CC, Lin CH, Tsai YH. Lipid nano/submicron emulsions as vehicles for topical flurbiprofen delivery. Drug Deliv, 2004; 11: 97-105.
7. Kantarci G, Ozgoney I, Karasulu HY, Arzik S, Guneri T. Comparison of different Water/Oil microemulsions containing diclofenac sodium: Preparation, characterization, release rate, and skin irritation studies. AAPS PharmSciTech, 2007; 8: E1-E7.
8. El Maghraby GM. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants. Int J Pharm, 2008; 355: 285-292.
9. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliver Rev, 2002; 54: S77-S98.
10. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interfac, 2006; 123: 369-385.
11. Aboofazeli R, Mortazavi SA, Khoshnevis P. Phase diagrams of lecithin-based microemulsions containing sodium salicylate. Daru, 2000; 8: 1-7.
12. Ozgoney IS, Karasulu HY, Kantarci G, Sozer S, Guneri T, Ertan G. Transdermal delivery of diclofenac sodium through rat skin from various formulations. AAPS PharmSciTech, 2006; 7: E1-E7.
13. Shafiq-un-Nabi S, Shafeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, et al. Formulation development and optimization using nanoemulsion technique: a technical note. AAPS PharmSciTech, 2007; 8: E1-E6.
14. Pellett MA, Watkinson AC, Brain KR, Hadgraft J. Synergism between supersaturation and chemical enhancement in permeation of flurbiprofen through human skin. In: Brain KR, James VJ, Walters KA, ed. Perspectives in Percutaneous Penetration. Cardiff, STS Publishing. 1997; 86-89.
15. Hayton WL, Chen T. Correction of perfusate concentration for sample removal. J Pharm Sci, 1982; 71: 820-821.
16. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterisation and transdermal drug delivery potential of microemulsion systems. J Control Release, 2000; 69: 421-433.
17. Guy RH, Hadgraft J. Feasibility assessment in topical and transdermal delivery: Mathematical models and in vitro studies. In: Guy RH, Hadgraft J, ed. Transdermal Drug Delivery. 2nd Ed., Marcel Dekker. 2003; 1-23.