ELSEVIER

International Journal of Pharmaceutics 244 (2002) 21–31

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

# Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability☆

Donatella Paolino [a], Cinzia Anna Ventura [b], Steven Nisticò [c], Giovanni Puglisi [a], Massimo Fresta [d,*]

[a] *Department of Pharmaceutical Sciences, University of Catania, Viale Andrea Doria 6, I-95125 Catania, Italy*
[b] *Pharmaceutical and Chemical Department, University of Messina, V.le Annunziata, I-98168 Messina, Italy*
[c] *Department of Dermatology, University of Rome, 'Tor Vergata', Viale Oxford 81, 00133 Rome, Italy*
[d] *Department of Pharmacobiological Sciences, Faculty of Pharmacy, University 'Magna Græcia' of Catanzaro, Complesso 'Ninì Barbieri', I-88021 Roccelletta di Borgia, Catanzaro, Italy*

Received 3 December 2001; received in revised form 24 May 2002; accepted 29 May 2002

**Abstract**

The potential application of highly biocompatible o/w microemulsions as topical drug carrier systems for the percutaneous delivery of anti-inflammatory drugs, i.e. ketoprofen, was investigated. Microemulsions were made up of triglycerides as oil phase, a mixture of lecithin and *n*-butanol as a surfactant/co-surfactant system and an aqueous solution as the external phase. To evaluate the percutaneous enhancing effect of oleic acid, this compound was used as a component of some o/w microemulsions. The topical carrier potentialities of lecithin-based o/w microemulsions were compared with respect to conventional formulations, i.e. a w/o emulsion, a o/w emulsion and a gel. Physicochemical characterisation of microemulsions was carried out by light scattering and zeta potential analyses. Microemulsions showed mean droplet size $< 35$ nm and a negative zeta potential, that is $-39.5$ mV for the oleic acid–lecithin microemulsion and $-19.7$ mV for the lecithin-based microemulsion. The percutaneous adsorption of the various topical formulations was evaluated through healthy adult human skin, which was obtained from abdominal reduction surgery. Ketoprofen-loaded microemulsions showed an enhanced permeation through human skin with respect to conventional formulations. No significant percutaneous enhancer effect was observed for ketoprofen-loaded oleic acid–lecithin microemulsions. The human skin tolerability of various microemulsion formulations was evaluated on human volunteers. Microemulsions showed a good human skin tolerability. 

*Keywords:* Microemulsion; Oleic acid; Ketoprofen; Percutaneous adsorption; Human skin tolerability

☆ Part of this research was presented at the XVII Symposium A.D.R.I.T.E.L.F. on Innovative Technological Research in drug Development. Catania (Italy), 4–7 Ottobre 2000

* Corresponding author. Tel.: +39-0961-391097; fax: +39-0961-391490
*E-mail address:* fresta@unicz.it (M. Fresta).

0378-5173/02/$ - see front matter 
PII: S0378-5173(02)00295-8

## 1. Introduction

Ketoprofen is a potent nonsteroidal anti-inflammatory drug (NSAID), that inhibits prostaglandin synthetase-cycloxygenase. This drug is used both systemically and topically for the treatment of several diseases such as tendinopathy (Rolf et al., 1997), acute and chronic arthritic conditions (Franchi et al., 1975), and knee osteoarthritis (Ottillinger et al., 2001). Unfortunately, the systemic administration of this drug, similar to other NSAIDs, presents gastrointestinal side effects that could be avoided by using a topical application (Valenta and Almasi-Szabo, 1995).

The topical treatment of several diseases is often limited by the poor percutaneous permeation through human skin. For this reason, the realisation of topical formulations, which are able to improve the percutaneous permeation of antinflammatory drugs, can be of particular importance for the success of topical therapeutic approaches.

The common method to improve drug permeation through the skin is to use penetration enhancers (Sugibayashi et al., 1992; Williams and Barry, 1992), i.e. organic solvents (ethanol, *N*-methylpyrrolidone) and fatty acids (oleic acid). Penetration enhancers can change the structure of skin lipids and alter the skin barrier function. These compounds, even if they increase the transdermal flux of several drugs, often generate skin irritation (Walters, 1989). Other methods have been proposed to increase the permeability of drugs through the skin, i.e. iontophoresis and ultrasound (Singh and Maibach, 1996; Boucaud et al., 2001), but these methods are not frequently used due to the requirement of qualified staff for their application. Recently, some authors (Kirjavainen et al., 1999) have proposed the use of substances endowed with low toxicity, i.e. phospholipids, as penetration enhancers. These substances present a notable affinity with cellular membranes thus leading to an increased absorption of several drugs (Henmi et al., 1994).

Various lecithin-based topical formulations have been proposed as dermal and transdermal drug delivery systems, i.e. liposomes (Foldvari et al., 1990; Fresta and Puglisi, 1996, 1997) and microemulsions (Benita and Levy, 1993 Osborne et al., 1991; Tenjarla, 1999). Microemulsions present some advantages over the liposomal carriers, namely higher storage stability, lower preparation cost, good production feasibility, absence of organic solvents, and no necessity of intensive sonication. These advantages are based on the fact that microemulsions are thermodynamically stable colloidal systems (clear or slightly opalescent isotropic systems), that can spontaneously be formed by mixing together an aqueous phase, a lypophilic phase and a surfactant/cosurfactant mixture.

In this paper, the preparation, characterisation and toxicity evaluation of lecithin-based microemulsions, as percutaneous drug delivery devices, are reported. In particular, lecithin microemulsions were compared with various conventional topical formulations: w/o and o/w emulsions, and a hydrophilic gel. A lecithin microemulsion containing oleic acid as penetration enhancer was also investigated. The percutaneous permeation profiles of various ketoprofen-loaded formulations through excised human skin are also reported.

## 2. Materials and methods

### 2.1. Chemicals

Soybean lecithin (SL), Epikuron 200 was a kind gift from Lucas Meyer Co. (Hamburg, Germany). Cetyl alcohol, *n*-butanol, propylene glycol, sodium EDTA and oleic acid (OA) were purchased from Sigma Chemicals Co (St. Louis, MO, USA). Carbomer 934 was purchased from BF Goodrich Speciality Chemicals Water Soluble Polymers Division (Italy). Triethanolamine, vaseline and liquid paraffin were obtained from Carlo Erba (Milan, Italy). Miglyol 812 N was obtained from Eigemann & Veronelli (Milan, Italy). Cetomacrogol BP 1000 was purchased from Cruciani Alberto Prodotti Crual, (Rome, Italy). Ketoprofen [(RS)-2-(3-benzoylphenyl) propionic acid] is a Sigma product.

### 2.2. Microemulsion preparation

An SL microemulsion was prepared by dispersing lecithin in the aqueous dispersion of ketoprofen (1% w/v) at 40 °C. This mixture was kept under constant agitation ( ~ 500 rpm) by means of a magnetic anchor until the complete dispersion of the lecithin component. The lipophilic phase made up of Mygliol 812 N was added under continuous agitation at room temperature, thus forming a milky emulsion that was stabilised for 24 h. This emulsion was transformed into a microemulsion by the addition of a suitable amount of *n*-butanol, that acts as the co-surfactant. Microemulsions were kept for 24 h at room temperature to allow the stabilisation of the colloidal phase. The OA-SL microemulsion was prepared using the same procedure but with the addition of oleic acid in the hydrophobic phase.

### 2.3. Preparation of topical formulations

Various ketoprofen-loaded topical formulations were prepared to be compared with the microemulsions, that is w/o and o/w emulsions, and a gel. The compositions of the various topical formulations are reported in Table 1.

The o/w emulsion was prepared by adding slowly the aqueous phase to the oily phase and to the blend of surfactants under continuous agitation: the phases were kept to 60 °C. The w/o emulsion was prepared by adding slowly the oily phase to the aqueous and to the surfactants at 60 °C. These mixtures were stirred until cool, thus forming the emulsion formulations.

The gel was prepared by dispersing Carbopol 934 in distilled water under continuous agitation. Ketoprofen (1 g) was solubilised in ethanol. The drug solution was added to the Carbopol dispersion. The water-ethanol solution was neutralised with triethanolamine. All the formulations were stored at room temperature for 24 h before use.

### 2.4. Dimensional analysis

Size analysis of microemulsions was carried out by photon correlation spectroscopy with a Zetamaster (Malvern Instruments Ltd, Sparing Lane South, Worcs, England). A solid state laser was used as the light source. This laser diode had a nominal power of 4.5 mW with a maximum output of 5 mW at 670 nm. The photon correlation spectroscopy measurements were carried out at a scattering angle of 90°. As a correlation function a third-order cumulant fitting (Chu, 1974; Berne and Pecora, 1976) with a dilation of 1.20 was applied to obtain mean particle diameter and polydispersity. Samples were suitably diluted with filtered water (Sartorius membrane filters 0.22 μm) to avoid multi-scattering phenomena and then placed in quartz couvettes. The real and imaginary refractive indexes were set at 1.59 and

Table 1
Composition of the various ketoprofen-loaded topical formulations

| Formulation | Oil phase | Aqueous phase | Surfactants and structurizing agents | Co-surfactant |
|---|---|---|---|---|
| w/o Cream | Liquid paraffin (15 g)<br>Vaseline (37.5 g) | Distilled water (24 g) | Cetyl alcohol (18 g)<br>Cetomacrogol (4.5 g) | – |
| o/w Cream | Liquid paraffin (6 g)<br>Vaseline (15 g) | Distilled water (69 g) | Cetyl alcohol (7.2 g)<br>Cetomacrogol (1.8 g) | – |
| Gel | – | Water (76 g)<br>Ethanol (20 g) | Triethanolamine (1.5 g)<br>Carbopol 934 (1.5 g) | – |
| SL-ME | Mygliol 812 N (6.3 g) | Water (48.1 g) | Soybean lecithin (19 g) | *n*-butanol (26.6 g) |
| OA-SL ME | Mygliol 812 N (6.3 g) | Water (47.1 g) | Soybean lecithin (19 g)<br>Oleic acid (1 g) | *n*-butanol (26.6 g) |

The drug was added in the aqueous phase at a concentration of 1 g.

0.0, respectively. Five different experiments per sample were performed. Thirty measurements were carried out for each experiment. Data are expressed as mean value ± standard deviation (S.D.).

### 2.5. Electrophoretic mobility and zeta potential

Electrophoretic mobility and zeta potential distribution were measured with the Zetamaster particle electrophoresis analyzer set-up (Malvern) equipped with a 5 mW HeNe laser (633 nm). Zeta limits ranged from −120 to 120 V. Strobing parameters were set as follows: strobe delay −1.00, on time 200.00, off time 1.00. A Smoluchowsky constant $F$(Ka) of 1.5 was used to achieve zeta potential values from electrophoretic mobility. Ten different measurements for each sample were carried out.

### 2.6. In vitro permeation experiments through human skin

Samples of healthy adult human skin (mean age 35 ± 8 years) were obtained from abdominal reduction surgery. Membranes of the stratum corneum and epidermis (SCE) were isolated as previously reported in literature (Kligman and Christophers, 1963). Subcutaneous fat was surgically removed by means of a scalpel and the skin was immersed in distilled water at 60 ± 1 °C for 2 min. Then SCE were peeled off, dried in a dessicator at approximately 25% relative humidity, wrapped in aluminium foil and stored at 4 ± 1 °C (Swarbrick et al., 1982). At the moment of use, SCE membranes were rehydrated by immersion in distilled water for 1 h at room temperature. Skin permeation studies were carried out by using Franz-type diffusion cells (Franz, 1975). The cell had a diffusional surface area of 0.75 $cm^2$ and a 4.5 ml cell volume. Human skin was mounted horizontally with the stratum corneum side up, dividing the cell into two compartments: the donor and the receptor compartments. The donor compartment was covered with Parafilm® in order to achieve occlusive conditions. The receptor was filled with 33 mM phosphate-buffered saline (pH 7.4). The receptor fluid was constantly stirred with a small magnetic stirring bar in order to ensure its homogeneity. Each formulation (200 mg) was applied in the donor compartment of Franz's cells. The duration of experiments was 24 h. At time intervals, 100 μl of the acceptor phase were withdrawn and analysed by HPLC to determine the amount of permeated ketoprofen. The withdrawn volume was replaced with fresh medium and a correction for dilution was carried out. At the end of the experiments the receptor phase was withdrawn and analysed for the total amount of permeated ketoprofen. Each experiment was carried out six times.

### 2.7. Determination of ketoprofen

The concentration of ketoprofen in the receptor compartment of Franz's cells was determined by HPLC analysis. A Hewlett-Packard model 1050 liquid chromatographic system (Hewlett-Packard, Milan, Italy) equipped with a 20 μl Rheodyne model 7125 injection valve (Rheodyne, Cotati, CA) was used. The chromatographic analysis was carried out with a reverse phase Nucleosil 100-5 $C_{18}$ column (5 μm, 250 × 4.6 mm i.d.; Applied Biosystems) at room temperature. Ketoprofen detection was carried out at $\lambda_{max}$ 260 nm. The mobile phase was an acetonitrile/pH 5 phosphate buffer mixture (40:60 v/v) with a flow rate of 1 ml/min. The amount of ketoprofen was calculated by reporting the peak area of a sample on a standard calibration curve, that was built up by relating known drug concentrations with the respective peak areas. Standard solutions contained between 0.8 and 16 μg/ml of ketoprofen. Linear regression analysis of the peak areas of standard solutions gave a $r^2$ value of 0.9998. No interference of the other formulation components was observed.

### 2.8. Acceptability in humans

Skin acceptability of topical formulations was tested on human volunteers. A 48 h test for primary irritation was performed as previously reported (Friedman et al., 1995). Briefly, thirty screened volunteers comprised the test. An occlusive patch bearing the formulation was applied to

the upper outer arm for 23 h. One hour after removal, skin sites were assessed for signs of skin irritation, which were rated with a numerical scoring system. After assessment, an identical fresh patch was applied to the same skin area for a further 23 h. One hour after patch removal, skin sites were again assessed. The system of scoring took into account several different conditions of significance. In particular, the values assigned were as follows: vesicles, 5; oedema, 4; erythema, 3; flakiness, 2; dryness, 1; wrinkling, 1; glazing, 1. Each condition was scored according to the strength of observed reaction: 0, no visible reaction; 1, just present reaction; 2, slight reaction; 3, moderate reaction; 4, severe reaction. To obtain the value for the total reaction, the score for strength of reaction was multiplied by the corresponding condition rate and the resulting values were summed to provide a global score for the acceptability degree. Scores were calculated evaluating the mean values of the total reactions and dividing this value by the number of subjects showing a visible reaction for the sample in examination.

### 2.9. Statistical data analysis

Results are expressed as the mean value $\pm$ S.D. Statistical data analysis was performed using a one-way ANOVA with a posteriori Bonferroni's $t$-test. Data with $P < 0.05$ are considered significative. In the case of in vivo human acceptability experiments, the statistical analysis for the different scores was performed by using the nonparametric variable Kruskal–Wallis test. Also in this case a $P < 0.05$ was considered statistically significant. The Mann–Whitney test was applied for the analysis of individual differences.

## 3. Results and discussion

### 3.1. Physicochemical characterization

Microemulsions have been proposed as drug delivery vehicles. However, as most of the papers reported in literature used pharmaceutically unacceptable ingredients to prepare the microemulsion formulations, there is the necessity to investigate suitable microemulsion systems for pharmaceutical use. For this reason in this paper the various microemulsion components were chosen taking into account their biocompatibility, trying to improve as much as possible the acceptability of the final formulations. In particular, a mixture of short chain triglycerides (Miglyol 812 N) was used as the oil phase. Natural soybean lecithin, which has no toxic effect even at high concentrations (lecithins are normal constituents of biological membranes), was used as a surfactant to prepare microemulsions.

Due to the physicochemical properties of lecithin, i.e. high lipophilicity and tendency to form liquid crystalline structures, microemulsions are not generally formed with pharmaceutically acceptable oils, such as triglycerides, using lecithin as a primary surfactant (Shinoda et al., 1991; Schurtenberger et al., 1993; Aboofazeli et al., 1995). To obtain an optically clear, one-phase microemulsion using lecithin, there is a need for the presence of a short-chain alcohol as a cosurfactant (Aboofazeli and Lawrence, 1993). Therefore, in this study $n$-butanol was used to prepare the topical microemulsion formulations.

A common strategy to improve the transdermal passage of a drug is represented by the addition of substances that are able to disorganise in a reversible way the skin barrier properties (the so-called penetration enhancers) to the drug topical formulation (Barry, 1987). A component that is often present in topical formulations as a penetration enhancer is oleic acid (Ogiso and Shintani, 1990). To evaluate the effect of this compound on both the physicochemical and the percutaneous properties of microemulsions, oleic acid was added during the preparation of the colloidal system.

To characterise the physicochemical properties of various microemulsion formulations, size analysis and zeta potential of emulsion droplets were carried out by means of light scattering. Recently some authors (Friedman et al., 1995) observed a direct influence of the size of the droplets of the emulsion in which a drug is entrapped on its penetration into the skin. As shown in Fig. 1, both SL and OA-SL microemulsions are characterised by a very narrow size distribution as deter-

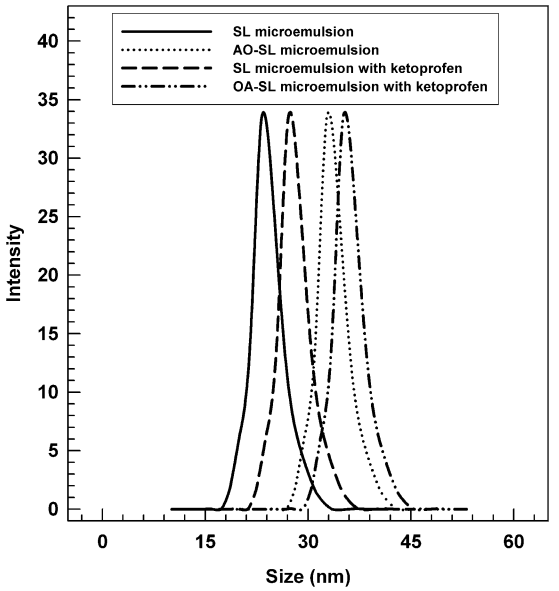


Fig. 1. Size distribution of SL and OA-SL microemulsions prepared both in the presence and in the absence of ketoprofen as determined by light-scattering cumulant analysis. For each sample thirty measurements were carried out at 25 °C. The various microemulsions showed lower polydispersity index values than 0.1.

mined by light scattering cumulant analysis. The presence of OA in the microemulsion formulation led to a significant ($P < 0.001$) increase of the microemulsion droplet size up to $32.7 \pm 0.9$ nm (Table 2). This finding may be due to the fact that the presence of OA at the level of the surfactant/cosurfactant film of the microemulsion droplet can elicit an electrostatic repulsion that leads to an increase of the microemulsion mean size. Proof of the fact that OA is able to change the surface electric charge properties of microemulsion droplets is achieved by zeta potential analysis. OA-SL microemulsions presented a zeta potential value of $-39.5 \pm 2.7$ mV, while SL microemulsions showed a zeta potential value of $-19.7 \pm 1.2$ mV (Table 2). Similarly to size distribution, both microemulsions were constituted by homogenous populations as regards to the surface charge properties (Fig. 2). The dilution of the two microemulsion systems with water did not significantly change their respective zeta potential values (data not reported), thus showing that the OA

Table 2
Physicochemical properties of SL and OA-SL microemulsions prepared both in the presence and in the absence of ketoprofen

| Microemulsion | Size (nm)[a] | PI[b] | Zeta potential (mV)[a] |
|---|---|---|---|
| *Without ketoprofen* | | | |
| SL | $23.2 \pm 1.1$ | 0.01 | $-19.7 \pm 1.2$ |
| OA-SL | $32.7 \pm 0.9$ | 0.02 | $-39.5 \pm 2.7$ |
| *With ketoprofen* | | | |
| SL | $27.1 \pm 1.3$ | 0.01 | $-24.1 \pm 1.6$ |
| OA-SL | $35.1 \pm 1.2$ | 0.01 | $-38.4 \pm 1.9$ |

Light scattering experiments were carried out at least 24 h after microemulsion preparation to allow the complete arrangement of the colloidal microstructure.

[a] Each value is the average of five different experiments ± S.D. ANOVA with a posteriori Bonferroni's $t$-test provided a $P < 0.05$ for various values. The difference of the zeta potential of OA-SL microemulsion with and without ketoprofen is not significative.

[b] Polydispersity index.

component is firmly anchored at the level of the surfactant/cosurfactant film of microemulsion droplets.

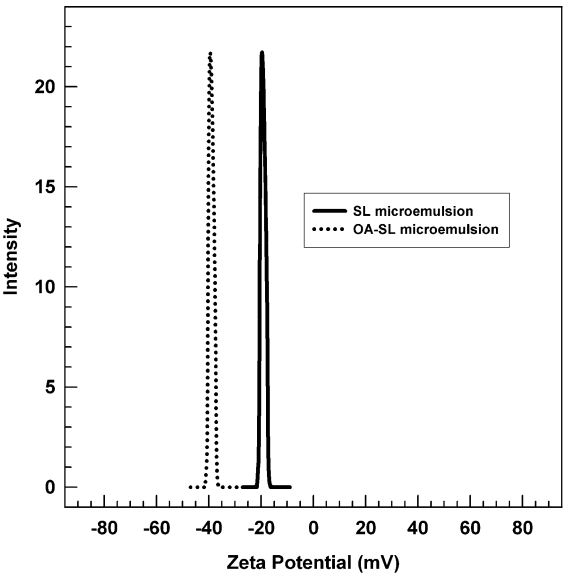


Fig. 2. Zeta potential analysis of SL and OA-SL microemulsions prepared in the absence of ketoprofen. The experiments were carried out at 25 °C. For Each experiment ten different measurement were carried out.

The morphological and surface charge characterisation was also carried out in the case of ketoprofen-loaded microemulsions. The incorporated drug can take part in the microstructure of the microemulsion system, thus influencing the microemulsion arrangement and properties via molecular interaction. This possibility can take place in the case of drugs showing amphiphilic and/or mesogenic properties, such as a variety of compounds belonging to the group of NSAIDs (Kriwet and Mueller-Goymann, 1993). In particular, ketoprofen caused a slight increase of the microemulsion droplet size in the case of both SL and OA-SL microemulsions (Table 2). The presence of this drug was also able to influence the surface properties of SL microemulsion by eliciting a change of the zeta potential from $-19.7$ to $-24.1$ mV. This finding shows that ketoprofen can be in part incorporated into the surfactant-cosurfactant film with the carboxylic group facing the aqueous continuous phase. In the case of the OA-SL microemulsion, ketoprofen did not significantly influence the zeta potential of microemulsion droplets, probably due to a competitive phenomenon with the OA molecules which could reduce the possibility for the drug to be inserted into the surfactant/cosurfactant film.

### 3.2. In vitro and in vivo experiments

To evaluate the ability of various topical formulations to delivery ketoprofen through the human skin, in vitro percutaneous permeation experiments through human skin stratum corneum were carried out.

Fig. 3 shows the permeation profiles of various ketoprofen-loaded topical formulations through human skin. OA-SL microemulsion is characterised by the highest ketoprofen permeation, while the hydrophobic w/o cream showed the lowest permeation profile. The gel presents an intermediate permeation profile, probably due to the penetration enhancing effect triggered by the presence of ethanol as a component of the topical formulation.

The presence of OA in the microemulsion formulation seems to poorly influence the permeation of ketoprofen through human skin. In fact, both

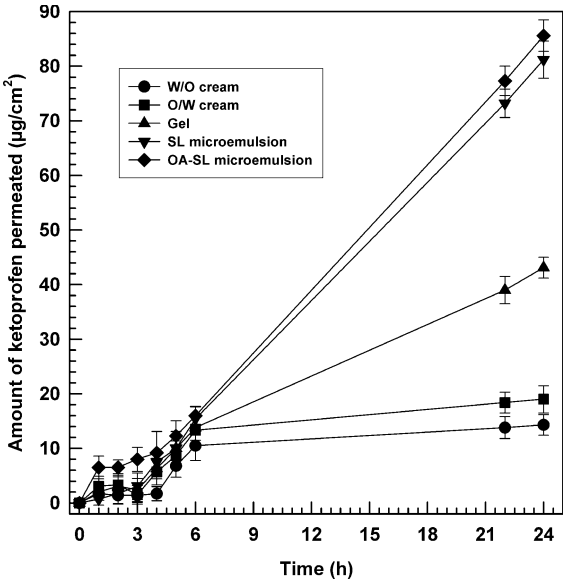


Fig. 3. Permeation profiles of ketoprofen through human skin from various topical formulations. Each value is the mean value of three different experiments ± S.D.

OA-SL and SL microemulsions are characterised by similar ketoprofen permeation profiles with a total amount of ketoprofen permeated after 24 h that is not significantly different. These data can be due to two different factors: (i) the penetration enhancer effect of OA is masked by the percutaneous enhancing effect exerted by the phospholipid constituents of the various microemulsions; (ii) the oleic acid is strongly bound to the microemulsion structure, thus hampering the disorganization effect on skin lipids that is responsible for the increase in drug percutaneous permeation.

The significant difference in ketoprofen percutaneous permeation between conventional formulations and the two microemulsions was probably due to the mean size of the internal phase droplets, which are noticeably smaller in the case of microemulsions. Besides the improved colloidal properties of the microemulsion dispersed phase, other factors, that can contribute to the increased permeation of ketoprofen through human skin stratum corneum can be both a solubilizing effect of the drug elicited by the microemulsion lecithin matrix and a penetration enhancer effect mediated by the lecithin component (Henmi et al., 1994).

In the case of the solubilizing effect, OA-SL and SL microemulsions in drug delivery from aqueous vehicle systems can act as true carriers. Namely, microemulsions solubilise the poorly water soluble drug and deliver it to the membranes where the drug molecules are released from the microemulsion systems to the skin surface, thus increasing the drug skin permeation. While, the penetration enhancer effect of lecithin can be mediated by the high affinity of lecithins for epidermal tissue, being able to mix with the skin lipid components (Barry, 1987). This behaviour can induce a change of the skin lipid fluidity, thus leading to an enhanced percutaneous adsorption of drugs (Nishihata et al., 1988).

In addition, the lecithin-mediated improvement of drug percutaneous adsorption can also be elicited by the increased hydration of the stratum corneum determined by various lecithin-based formulations (Valenta and Almasi-Szabo, 1995).

The steady-state flux (Js), the lag time ($T_L$) and the area under the curve (AUC) of permeation profiles are summarised in Table 3. It is evident that SL and SL-OA show a higher flux of ketoprofen respect to conventional formulations: w/o and o/w creams, and hydroalcoholic gel. The use

Table 3
Percutaneous permeation parameters of ketoprofen through human stratum corneum from various topical formulations

| Formulation | AUC[a] | $L_T$[b] | J (μg/cm$^2$ h) |
|---|---|---|---|
| W/O emulsion | 240.75 ± 50.35 | 3.61 ± 0.25 | 0.795 ± 0.001 |
| O/W emulsion | 319.95 ± 53.30 | 2.73 ± 0.22 | 1.005 ± 0.005 |
| Gel | 535.20 ± 52.15 | 2.40 ± 0.19 | 2.395 ± 0.001 |
| SL microemulsion | 893.92 ± 56.25 | 2.02 ± 0.15 | 4.511 ± 0.001 |
| OA-SL microemulsion | 959.81 ± 53.24 | 1.71 ± 0.11 | 4.750 ± 0.006 |

Each value is the mean of six experiments ± S.D.

[a] One-way ANOVA analysis with a posteriori Bonferroni's $t$-test provided $P<0.001$ for various formulations. Only W/O vs. O/W emulsion and SL vs. OA-SL microemulsion are not significative.

[b] $P<0.02$ for various formulations.

Table 4
Human skin irritancy test of various topical formulations after 24 h of treatment

| Sample | Irritation evidence at 24 h | | | | | | | Score[b] |
|---|---|---|---|---|---|---|---|---|
| | Number of cases[a] | | | | | | | |
| | Vesicles | Edema | Erythema | Flakiness | Dryness | Wrinkling | Glazing | No of visible reaction |
| OA 1% | – | – | 3 | 7 | – | 2 | – | 18/30 | 10.17 ± 2.08 |
| W/O | – | – | – | – | – | 2 | 3 | 25/30 | 6.20 ± 2.77 |
| O/W | – | – | – | – | – | 2 | 1 | 27/30 | 4.67 ± 2.52 |
| Gel | – | – | – | – | – | 1 | 2 | 27/30 | 4.33 ± 1.15 |
| SL ME | – | – | – | – | – | 1 | 1 | 28/30 | 3.50 ± 1.39 |
| OA-SL ME | – | – | – | – | – | 2 | 1 | 27/30 | 4.67 ± 2.08 |

[a] The value reported in each column represents the number of subjects who showed the skin reaction symptom.

[b] Nonparametric variable Kruskal–Wallis test provided: $P<0.001$ for OA (1% w/w) aqueous dispersion vs. all other samples; $P<0.05$ for W/O cream vs. all other samples; $P<0.05$ for SL microemulsion vs. O/W cream, gel and OA-SL microemulsion. This value was calculated according to the procedure reported in Section 2.

of lecithin in both microemulsions increased the flux of the drug and decreased the $T_L$ respect to others formulations. This finding may be due to the fact that lecithin can influence the permeability of the skin by lipid-fluidization, which leads to decreased barrier function in a reversible way. The $T_L$ value of SL and OA-SL is significantly decreased with respect to other formulations probably, because lecithin interacts with the stratum corneum rapidly, disorganises the ordinate structure (penetration enhancing effect) and ketoprofen may permeate through human skin.

The potentiality of a topical formulation to be used as a transdermal drug delivery device should be evaluated not only in terms of carrier capacity and percutaneous drug adsorption, but also in terms of tolerability and toxicity of the proposed formulation. For this reason, the microemulsion tolerability on healthy human volunteers was evaluated and compared with that of conventional topical formulations.

In Tables 4 and 5, experiments on human skin tolerability are reported. Microemulsion formulations showed the highest skin tolerability. It is interesting to note the higher tolerability of OA-SL microemulsion with respect to OA aqueous dispersions containing the same amount of OA. Also in this case, the incorporation of OA in the microemulsion structure and the impossibility to strongly perturb the stratum corneum skin lipids can be the reason for the reduced skin irritation.

Statistic analysis shows a significantly different tolerability between SL and OA-SL microemulsions. This finding may be due to the capacity of lecithin to enhance the percutaneous permeability of various components of formulations, including molecules of oleic acid that may create irritancy in the skin.

## 4. Conclusion

The percutaneous absorption of ketoprofen from topical formulations depends both on the mean size of the organic phase droplets (dispersion degree) and on the vehicle constituents. In fact, a suitable choice of the components is essential to minimise the irritancy effect and to determine an improvement of the percutaneous permeation of the drug through the stratum corneum. SL microemulsions significantly increased the skin percutaneous permeation of ketoprofen with respect to conventional formulations, i.e. creams and gel. Interestingly, the presence of oleic acid in the constitution of microemulsions, as a percutaneous penetration enhancer, seems to exert no significant effect on the percutaneous permeation of ketoprofen. These findings on mi-

Table 5
Human skin irritancy test of various topical formulations after 48 h of treatment

| Sample | Irritation evidence at 48 h | | | | | | | | Score[b] |
|---|---|---|---|---|---|---|---|---|---|
| | Number of cases[a] | | | | | | | | |
| | Vesicles | Edema | Erythema | Flakiness | Dryness | Wrinkling | Glazing | No visible reaction | |
| OA 1% | – | – | 6 | 7 | – | 1 | 1 | 15/30 | $16.27 \pm 2.89$ |
| W/O | – | – | – | 1 | 2 | 2 | 2 | 23/30 | $8.71 \pm 3.15$ |
| O/W | – | – | – | 1 | 1 | 2 | 2 | 24/30 | $6.17 \pm 1.83$ |
| Gel | – | – | – | – | – | 2 | 2 | 26/30 | $4.75 \pm 2.06$ |
| SL ME | – | – | – | – | – | 2 | 2 | 26/30 | $4.00 \pm 1.83$ |
| OA-SL ME | – | – | – | 1 | 1 | 2 | 1 | 25/30 | $5.60 \pm 2.07$ |

[a] The value reported in each column represents the number of subjects who showed the skin reaction symptom.

[b] Nonparametric variable Kruskal–Wallis test provided: $P < 0.001$ for OA (1% w/w) aqueous dispersion and W/O cream vs. all other samples; $P < 0.01$ for O/W cream vs. gel and SL microemulsion; $P < 0.005$ for SL microemulsion vs. OA-SL microemulsion. This value was calculated according to the procedure reported in Section 2.

croemulsions made up of biocompatible constituents (high percutaneous effect and low human skin irritation) prompt their use as topical delivery systems both in pharmaceutical and cosmetic fields.

## Acknowledgements

This work was financially supported by Italian MURST 60%. The authors are very grateful to Dr Antony Bridgewood for his revision of the language of this paper.

## References

Aboofazeli, R., Lawrence, M.J., 1993. Investigation into the formation and characterization of phospholipid microemulsions. I. Pseudo-ternary phase diagrams of systems containing water–lecithin–alcohol–isopropyl myristate. Int. J. Pharm. 93, 161–175.

Aboofazeli, R., Patel, N., Thomas, M., Lawrence, M.J., 1995. Investigation into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water–lecithin–oil and cosurfactant. Int. J. Pharm. 125, 107–116.

Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. J. Control. Rel. 6, 85–97.

Benita, S., Levy, M.Y., 1993. Submicron emulsions as colloidal drug carriers for intravenous administration: comprehensive physicochemical characterization. J Pharm. Sci. 82, 1069–1079.

Berne, B., Pecora, R., 1976. Dynamic Light Scattering. Wiley, New York.

Boucaud, A., Machet, L., Arbeille, M.C., Sournac, M., Mavon, A., Patat, F., Vaillant, L., 2001. In vitro study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin. Int. J. Pharm. 228, 69–77.

Chu, B., 1974. Laser Light Scattering. Academic Press, New York.

Foldvari, M., Gesztes, A., Mezei, M., 1990. Dermal drug delivery by liposome encapsulation: clinical and electron microscopic studies. J. Microencapsul. 4, 479–489.

Franchi, R., Viara, M., Liverta, C., Pollioni, C., Pontirolo, A.E., 1975. Ketoprofen and indomethacin in the treatment of rheumatoid arthritis: a double-blind cross-over trial. Eur. J. Clin. Pharmacol. 8, 205–208.

Franz, T.J., 1975. Percutaneous absorption: on the relevance of in vitro data. J. Invest. Dermatol. 64, 190–195.

Fresta, M., Puglisi, G., 1996. Application of liposomes as potential cutaneous drug delivery systems. In vitro and in vivo investigation with radioactively labelled vesicles. J. Drug Target. 4, 95–101.

Fresta, M., Puglisi, G., 1997. Corticosteroid dermal delivery with skin-lipid liposomes. J. Control. Release 44, 141–151.

Friedman, D.I., Schwarz, J.S, Weisspapir, M., 1995. Submicron emulsion vehicle for enhanced transdermal delivery of steroidal and nonsteroidal antiinflammatory drugs. J.Pharm. Sci. 84, 324–329.

Henmi, T., Fuji, K., Kikuchi, K., Yamanobe, N., Matsumoto, M., 1994. Application of an oily gel formed by hydrogenated soybean phospholipids as a percutaneous absorption-type ointments base. Chem. Pharm. Bull. 42, 651–655.

Kirjavainen, M., Monklonen, J., Saukkosaari, M., Valjakka-Koskela, J., Urtti, A., 1999. Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers. J Control. Release 58, 207–214.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human skin. Arch. Dermatol. 88, 702–705.

Kriwet, K., Mueller-Goymann, C.C., 1993. Binary diclofenac diethylamine water systems: micelles, vesicles and lyotropic liquid crystals. Eur. J. Pharm. Biopharm. 39, 234–243.

Nishihata, T., Kamada, A., Sakai, K., Takahahu, K., Matsumoto, K., Shinozaji, K., Tabat, Y., Keigami, M., Miyagi, T., Tatsumi, N., 1988. Percutaneous absorption of diclofenac in rats and humans: aqueous gel formulation. Int. J. Pharm. 46, 1–7.

Ogiso, T., Shintani, M., 1990. Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propronolol. J. Pharm. Sci. 79, 1065–1071.

Osborne, D.W., Ward, A.J., O'Neill, K.J., 1991. Microemulsions as topical drug delivery vehicles: in-vitro transdermal studies of a model hydrophilic drug. J. Pharm. Pharmacol. 43, 450–454.

Ottillinger, B., Gomor, B., Michel, B.A., Pavelka, K., Beck, W., Elsasser, U., 2001. Efficacy and safety of eltenac gel in the treatment of knee osteoarthritis. Osteoarthr. Cartilage 9, 273–280.

Rolf, C., Movin, T., Engstrom, B., Jacobs, L.D., Beauchard, C, Le Liboux, A., 1997. An open, randomised study of ketoprofen in patients in surgery for Achilles or patellar tendinopathy. J. Rheumatol. 24, 1595–1598.

Schurtenberger, P., Peng, Q., Leser, M.E., Luisi, P.L., 1993. Structure and phase behaviour of lecithin-based microemulsions: a study of the chain length dependence. J. Colloid Interf. Sci. 156, 43–51.

Shinoda, K., Araki, M., Sadaghiani, A., Khan, A., Lindman, B., 1991. Lecithin-based microemulsions: phase behaviour and microstructure. J. Phys. Chem. 95, 989–993.

Singh, P., Maibach, H.I., 1996. Iontophoresis: an alternative to the use of carriers in cutaneous drug delivery. Adv. Drug Deliv. Rev. 18, 379–394.

Sugibayashi, K., Nakayama, S., Seki, T., Hosoya, K., Morimoto, Y., 1992. Mechanism of skin penetration-enhancing effect by laurocapram. J. Pharm. Sci. 81, 58–64.

Swarbrick, J., Lee, G., Brom, J., 1982. Drug permeation through human skin: I. Effect of storage conditions of skin. J. Invest. Dermatol. 78, 63–66.

Tenjarla, S., 1999. Microemulsions: an overview and pharmaceutical applications. Crit. Rev. Ther. Drug Carrier Syst. 16, 461–521.

Valenta, C., Almasi-Szabo, I., 1995. In vitro diffusion studies of ketoprofen transdermal therapeutic systems. Drug Dev. Ind. Pharm. 21, 1799–1805.

Walters, K.A., 1989. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Dekker, New York, pp. 197–246.

Williams, A.C., Barry, B.W., 1992. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 9, 305–353.