INTRODUCTION

Osteoarthritis belongs to the most frequent chronic pain syndromes. Only in United States the osteoarthritis incidents have been estimated at 27 million (Plotnikoff et al., 2015). Clinicians as first-line medications suggest the use of acetaminophen. However, due to its ineffectiveness, nonsteroidal anti-inflammatory drugs (NSAIDs) are considered as the next step in therapy. Nonetheless, NSAIDs might present various side effects thus their topical administration instead of oral is preferred (Herndon, 2012). The topical administration of NSAIDs such as diclofenac and etofenamate, has been widely recommended for the localized pain and smaller, superficial joints. In fact, these formulations found to be efficacious in patients with osteoarthritis.

Etofenamate (ETF), 2-{{[3-(trifluoromethyl) phenyl] amino}benzoic acid 2-(2-hydroxy ethoxy)ethyl ester is a non-steroidal anti-inflammatory drug (NSAID) with antipyretic, analgesic as well as antirheumatic properties which non-selectively inhibits cyclo-oxygenase (COX). ETF exists as a viscous, yellow liquid with high lipophilicity. ETF marketed formulations are either injectable or topical gels whereas they are used for the treatment of conditions as lumbago, arthritis, joint and muscular pain (Marto et al., 2015; Peraman et al., 2013).

Topical or transdermal administration of drugs can be achieved via skin (Siafaka, Barmbalexis, Bikiaris, 2016a; Langasco et al., 2017; Tuncay, Özer, 2013). Human skin is a physical, immunological, and sensory barrier given that it protects human body from external hazardous materials such as chemicals, microorganisms, UV radiation and prevents loss of water and biological constituents (Brown et al., 2006; Okur et al., 2020b; Siafaka et al., 2016b, 2016a;
Telaprolu et al., 2016). The multilayered structure of skin, most particularly stratum corneum (SC)—the outer layer of the epidermis, plays an important role as main barrier (Prausnitz et al., 2012). Dermal drug administration could provide several benefits but also present some limitations. For instance, by applying dermal delivery carriers, the first pass metabolism can be avoided and side effects in association with systemic toxicity can be reduced. In further, a sustained and controlled release of the drug can be achieved via dermal delivery (Brown et al., 2006; Siafaka et al., 2020). Although dermal route is quite advantageous, it presents various limitations which should be regulated, in order to achieve a successful transportation of drug to the biological membranes. There are several dermal carriers, such as films, ointments, creams in situ gels or microemulsions. Microemulsions (ME) are among the most useful dermal carriers, since they present useful features as small droplet sizes as well as protection of the entrapped drug from degradation and hydrolysis. Moreover, some components of MEs could act as permeation enhancers.

MEs as promising vehicles for dermal and transdermal delivery, comprised from oil phase, aqueous phase, surfactant and co-surfactant. MEs present thermodynamically stability because of co-surfactant incorporation (Üstündağ Okur et al., 2011; Pillai et al., 2015). It has been proposed that low interfacial tension leads to MEs enhanced stability (Pillai et al., 2015). MEs can be easily prepared and they can load both hydrophilic and lipophilic substances. They also have long shelf-life due to their marvelous stability (Üstündağ Okur et al., 2011; Kajbafvala, Salabat, Salimi, 2018). These systems can be found in three main structures as swollen micellar (oil-in-water, O/W), reverse micelles (water-in-oil, W/O) and bicontinuous structures (Üstündağ Okur, Çağlar, Siafaka, 2020; Xavier-Junior et al., 2017).

In the past, the use of solid lipid nanocarriers for topical delivery of ETF has been reported in the literature. However, to the best of our knowledge this is the first time where microemulsions have been developed for the topical delivery of ETF. Thus, the aim of this study was the development and characterization of new ETF loaded microemulsions (\(M_{ETF}^{1}, M_{ETF}^{2}, M_{ETF}^{3}, M_{ETF}^{4}\)) as dermal delivery carriers. Therefore, in this study, the formulation, physicochemical characterization, \textit{ex vivo} penetration studies as well as anti-inflammatory activities of the novel MEs carried out, to explore the suitability of the carriers.

**MATERIAL AND METHODS**

**Material**

Etofenamate was kindly gifted from Recordati, Turkey and Transcutol HP (diethylene glycol monooethyl ether) from Gattefosse, France. Span 80 and Tween 20 were purchased from Merck Company (Germany) whereas oleic acid, ethanol from Sigma-Aldrich (Germany) and Cremophor EL from Sigma (Germany). Acetonitrile and methanol were of High-pressure liquid chromatography (HPLC) grade and they were used for HPLC studies. All other chemicals and solvents were of analytical or HPLC grade. RAW 264.7 macrophage cell line (American Type Culture Collection-ATCC), DMEM (Gibco, UK), FBS (Gibco, USA), 1% streptomycin and penicillin (Gibco, USA), Griess reagent (1% sulfanilamide obtained by Sigma-Aldrich, USA, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride were obtained by Sigma-Aldrich, USA in 5% phosphoric acid which was purchased by Mettler, Switzerland). In addition, LPS (lipopolysaccharide, from \(E. coli\) 0111:B4, Sigma, USA), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, AppliChem, Germany), isopropanol (Sigma-Aldrich, Germany), sodium nitrite (Fluka Chemika, Germany) and microplate reader (Microplate photometer, Multiskan Ascent, Finland) were used for cell culture studies.

**Preparation of microemulsions**

Pseudo-ternary phase diagrams were fabricated to determine microemulsion regions and ideal formulation ratios. Various potential microemulsion concentrations which can form a single phase could be selected by using the aforementioned diagrams. The titration method was chosen in order to construct a pseudo-ternary phase. Oil, surfactant and co-surfactant mixtures were prepared and titrated by adding dropwise water at 25±5°C; the mixtures...
Solubility studies

The materials of formulations were chosen based on the solubilization capacity of ETF. The solubility of ETF was determined in several surfactants and solvents. Excess amount of ETF was added in 1 mL of selected surfactants, co-surfactants and oils and was shaken at 25 °C at 200 rpm for 24 h. Afterwards, the mixture was centrifuged at 15,000 rpm at 25 °C for 15 minutes. 10 μL of the supernatant parts were diluted to 1 mL in vials and analyzed by HPLC. Each experiment was performed in triplicate.

HPLC analysis

HP Agilent 1100 HPLC with pump and UV detector was used for the sample analysis. The used column was C18 (5μm, 150x 4.6 mm) whereas mobile phase consisted of methanol (MeOH)/acetonitrile (ACN)/purified water (H₂O) (45/35/20, v/v/v)). Flow rate set at 1.0 ml/min and 10 μL sample injection volume was chosen. The UV detector was applied to monitor, at 286 nm and retention time was 6.5 minutes. The temperature adjusted at 25 °C in the auto sampler chamber (Marto et al., 2015).

Determination of n-octanol/water partition coefficient

The n-octanol phase was soaked with double distilled H₂O for 24h to establish the n-octanol/water partition coefficient of ETF. ETF was weighted directly in assay tubes due to its low solubility. Afterwards 2 mL of organic phase and 2 mL of double distilled water were added. The tubes were shaken for 24h at 25±2 °C at 200 rpm and then centrifuged for 15 min at 25±2 °C at 3500 rpm. Water phases were collected and diluted from 50 μL to 10 mL with mobile phase in order to determine the concentration of ETF by HPLC (Üstündağ Okur, Yavaşoğlu, Karasulu, 2014c). The partition coefficient was calculated with the equation given below:

\[
\text{Partition coefficient} = \frac{\text{ETF concentration in octanol phase}}{\text{ETF concentration in aqueous phase}}
\]
Characterization of the microemulsions

Determination of pH

The pH values of the microemulsions were measured at room temperature using calibrated pH meter (Mettler Toledo, Switzerland). Each sample tested in triplicate and the average of these results was taken as pH of the formulations (Okur et al., 2019).

Drug content

100 μL of the formulations (M1_{ETF}-M4_{ETF}) were dissolved in mobile phase up to 10 mL and analyzed on HPLC to determine drug concentrations (Üstündağ Okur et al., 2019a).

Determination of viscosity of microemulsions

AND Vibro Viscometer was used to measure viscosity. The microemulsions were placed in the sampler tube and analyzed at 25 °C. Each sample tested in triplicate.

Determination of zeta potential, droplet size and PDI

Dynamic Light Scattering method (Nano ZS, Malvern Instruments, U.K.) was applied to calculate the average droplet size and polydispersity index (PDI). Experiments to measure the particle size and PDI values were rerun five times at 25 °C. The results were acquired by obtaining the average of five measurements at an angle of 173° by utilizing disposable cells (Üstündağ Okur et al., 2014b).

Zeta potential

To measure the zeta potential of samples, disposable plain folded capillary zeta cells (Malvern Zetasizer Nano ZS) were used. The zeta potential was estimated by the electrophoretic mobility and via Helmholtz–Smoluchowski equation, under an electrical field of 40 V/cm. Software involved system was used for this process. The measurements were repeated five times at 25±2 °C (Üstündağ Okur et al., 2014b).

Conductivity measurement

Electrical conductivity measurement was performed in triplicate for each formulation at 25 ± 2 °C by using conductometer and conductometer probe (Milwaukee MW801, U.S.). The results are presented as mean ± SD (Üstündağ Okur et al., 2011; Üstündağ Okur et al., 2015).

Stability of ETF loaded microemulsions

M1_{ETF}, M2_{ETF}, M3_{ETF} and M4_{ETF} formulations were stored at 4±1 °C, 25±2 °C and 40±2 °C for 12 months, so as to examine their stability. ETF loaded microemulsions were examined in terms of their clarity, pH, viscosity, refractive index, phase separation, droplet size and electrical conductivity (Aksu et al., 2019). Centrifuge tests were performed, before their storing in the specified conditions, at 13,000 rpm for 30 min in order to determine the physical stability of microemulsions.

Besides, the heating-cooling cycle test was also carried out by storing formulations between 4±1°C and 40±1°C for 24h as one cycle. Six cycles were completed before investigating the formulations in terms of clarity, precipitation and phase separation (Üstündağ Okur et al., 2015).

Ex vivo studies (permeation and penetration studies)

Mice abdominal skin and diffusion cell of 0.785 cm² effective area was applied for ex vivo permeation evaluation of ETF loaded MEs. After mounting the skin samples on diffusion cells, receiver and donor compartments were prepared. 10 mL ethanol:PBS (ratio of 30:70) was used as a receptor compartment to ensure sink condition. 1 mL formulation was placed on a donor compartment and parafilm was used to prevent evaporation. Temperature was set at 37±1°C while magnetic stirring at 600 rpm was also performed during the experiment. 50 μL sample of the receiver compartment was withdrawn at a predetermined time and replaced with an equal volume of receptor solution. All samples were analyzed through HPLC (Üstündağ Okur et al., 2019b).
After 24h of the experiment, each skin was cut into pieces and placed in MeOH for penetration study. To determine the amount of ETF withheld in the skin, the tube containing methanol and skin was homogenized for 10 min, filtered through a membrane filter with 0.2 μm pore width. Samples from the solution were analyzed in HPLC.

Anti-Inflammatory activity and cytotoxicity of etofenamate formulations on RAW 264.7 macrophage cells

Cell cytotoxicity

RAW 264.7 cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin, at 37 °C in 5% CO₂. RAW 264.7 cell line was plated using a density of 1 × 10⁵ cells per well with 250 µL of growth medium. Cells were treated with M1ETF, M2ETF, M3ETF and M4ETF for 1 day in presence of LPS (1µg/mL). After the removal of cell medium, 100 µL of MTT solution (0.5 mg/ml) was added and incubated for 2 hours at 37°C. At the end of the incubation period, the medium was removed and 100 µL of isopropanol was added in each well and shaken gently to solubilize the dark blue crystals. Optical absorbance was assessed at 570 nm. The relative cell viability (%) was determined via the following formula, where OD depicts optical density (Aydin et al., 2018; Karadağ et al., 2019).

\[
\text{Cell viability} (\%) = \frac{\text{OD570 (sample)}}{\text{OD570 (medium control)}} \times 100
\]

% Inhibition = 100-\([N_1*100]/N_o\]

\(N_o\): Nitrite amount (µM) of LPS-treated medium control,
\(N_1\): Nitrite amount (µM) of MEs

Statistical analysis

The outcomes are shown as the mean ± SD of experiments. Statistical significance was determined via one-way ANOVA followed by Tukey’s test, using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). p < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Determination of ETF solubility

It has been reported that the drug has to be dissolved in order to permeate the skin. Hence, solubility studies using different solvents and surfactants were carried out to identify the ideal ingredients of ETF microemulsion (Abd-Allah, Dawaba, Ahmed, 2010). It was revealed that ETF maximum solubility was obtained using EtOH and Transcutol HP at 253.108±13.590 mg/mL and 243.117±2.996 mg/mL, respectively. Thus, Transcutol HP was also selected as surfactant and EtOH as co-surfactant. Moreover, solubility on oleic acid was established at 134.547±2.520 mg/mL. This value was the greatest among the studied oils, so oleic acid was selected as oil phase for microemulsions. The results of solubility study are given in Table II.
### TABLE II - Solubility of ETF in different surfactants, co-surfactants, and oils

| Component       | Solubility (mg/mL) |
|-----------------|---------------------|
| Distilled water | 0.029 ± 0.8347      |
| EtOH            | 253.108 ± 13.590    |
| Span 80         | 125.351 ± 18.750    |
| Tween 20        | 176.047 ± 5.612     |
| Transcutol HP   | 243.117 ± 2.996     |
| Oleic acid      | 134.547 ± 2.520     |
| Cremophor EL    | 177.991 ± 8.852     |
| Tween 80        | 122.624 ± 11.345    |
| Span 20         | 2.445 ± 0.497       |
| Propylen glycol | 116.127 ± 1.709     |
| Labrafil        | 154.499 ± 3.703     |
| Labrasol        | 200.493 ± 1.879     |
| Capryol 90      | 215.483 ± 0.695     |
| Labrafac PG     | 217.588 ± 8.723     |
| Tween 60        | 173.631 ± 0.980     |
| Cremophor RH 40 | 46.553 ± 8.151      |
| Almond oil      | 111.340 ± 10.758    |
| Castor oil      | 134.022 ± 13.732    |

### Determination of n-octanol/water partition coefficient

The stratum corneum is the main lipophilic barrier of the skin which generally controls the molecules penetration according to their lipophilicity. Thus, drug lipophilicity is determined by logP of n-octanol/water. Herein, logP of ETF was calculated 4.21. This result shows that ETF is lipophilic compound and can permeate through the skin (Üstündağ Okur et al., 2011).

### Preparation of ETF loaded microemulsions

Pseudo-ternary phase diagrams were applied to calculate the combination of phases. The domains of the existing transparent, isotropic systems are similar to microemulsion phases. The ternary phase diagrams of all the ratios are shown in Figure 1. It can be said, that more than one stable microemulsions were obtained and their component ratios are shown in Table I. Accordingly, the ideal formulations were selected and 5% ETF (w/w) was dissolved in their oil phase. It has been reported that hydrophilic surfactants promote oil-in-water (O/W) microemulsion formation. In our study, Cre EL (HLB 12-14), TW20 (HLB 16,7) and SP80 (HLB 15) were used, so an O/W microemulsion was prepared (Lu, Gao, 2010).
The prepared MEs present sizes under 185 nm, revealing that can potentially deliver ETF delivery into the skin. PDI values measured between 0.257±0.047 and 0.429±0.044 determining homogeneity. Zeta potential values showed that all formulations were almost neutral as they were between 0.0028±0.0006 and 0.8886±0.4229. This means no precipitation was expected.

The refractive index measurement was carried out by refractometer and found to range between 1.3985±0.003 (M1ETF) to 1.4386±0.0008 (M4ETF). The viscosity measurement was carried out by AND Vibro Viscometer; viscosity calculated as 6.59±0.01 in case of M1ETF and 415.67±7.64 for M4ETF. Electrical conductivity has been widely utilized for the analysis of MEs microstructure (Abd-Allah, Dawaba, Ahmed, 2010). Herein, conductivity measurement was performed using Milwaukee MW801 conductometer and conductometer prob. It was found at 10.5±0.1 of M1ETF and 17.93±0.45 for M4ETF.

**Characterization of microemulsions**

The physicochemical characterization of microemulsions intended to be used in dermal applications is an important issue which should be considered during formulation stage (Çağlar et al., 2019). The characterization parameters and results of the microemulsions are listed in Table III. The pH values of the formulations can be criticized as appropriate for dermal application as they were between 4.84±0.01 and 5.42±0.01 (Ali, Yosipovitch, 2013; Okur et al., 2019). Nanotechnology is still the emerging application field for pharmaceutical technology. Nanomaterials are claimed to be promising in the medical field especially as imaging agents or local drug carriers due to their large surface area, improved tissue penetration, and controlled drug delivery (Siafaka et al., 2019). Thus, the droplet size of a carrier is significant value when topical drug delivery systems are studied considering that vehicles of sizes under 300 nm can deliver their ingredients to the deep skin layers.

The pseudo-ternary phase diagrams of the microemulsion (a) The pseudo-ternary phase diagram of the microemulsion composed of OA, SP80, TW20, ethanol and water (w/w). (b) The pseudo-ternary phase diagram of the microemulsion composed of OA, Cre EL, EtOH and water (w/w). (c) The pseudo-ternary phase diagram of the microemulsion composed of OA, Cre EL, Transcutol HP and water (w/w). (d) The pseudo-ternary phase diagram of the microemulsion composed of OA, SP80, TW20, Transcutol HP and water (w/w).
Ex vivo studies (permeation and penetration studies)

M1 ETF, M2 ETF, M3 ETF and M4 ETF microemulsions were prepared for ex vivo penetration and permeation study. Mice abdominal skins were used. The permeation percentage of ETF through the mice skins into the receptor phase and penetrated ETF in the skin after 24 h is shown in Table IV. After 24 hours, the highest penetration detected for M1 ETF was 63% and for M2 ETF was 47%.

Stability studies of microemulsions

Blank and ETF loaded microemulsions were examined at 4±2°C, 25±2°C and 40±2°C to check their stabilities. It has been reported that physical stability of MEs is dependent on particle size and particle size distribution which are critical boundaries on in-process control and especially in quality assurance (Vicentini et al., 2011). It was revealed that there was not a significant difference in droplet size of tested MEs for the duration of 12 months storage at 4±2°C and 25±2°C. ETF concentration was still above 75% during 12 months of storage. The concentration change is given in Figure 2. The value of viscosity was also stable. In addition, ETF loaded microemulsions showed no changes in physical appearance after centrifugation and freeze-thaw cycles.

![Figure 2 - ETF amount change for 3, 6 and 12 months storage at 4 ± 2°C, 25 ± 2°C and 40 ± 2°C.](image-url)

**TABLE III** - Characterization of the developed blank and ETF-loaded microemulsion formulations (mean ± SD, n=3)

| Formulation/parameters | pH (mean ± SD) | Droplet size (nm) (mean ± SD) | PDI (mean ± SD) | Zeta potential (mV) (mean ± SD) | Refractive index (mean ± SD) | Conductivity (mS/cm) (mean ± SD) | Viscosity (cP) (mean ± SD) |
|------------------------|----------------|-------------------------------|-----------------|---------------------------------|-----------------------------|----------------------------------|-----------------------------|
| M1 ETF                 | 4.84 ± 0.006   | 7.523 ± 0.375                | 0.429 ± 0.044   | 0.0084 ± 0.0018                 | 1.395 ± 0.003               | 17.93 ± 0.451                    | 6.59 ± 0.0058                |
| M2 ETF                 | 5.22 ± 0.000   | 8.183 ± 0.65                 | 0.351 ± 0.03    | 0.261 ± 0.0041                  | 1.403 ± 0.001               | 16.87 ± 0.306                    | 113.67 ± 1.5275             |
| M3 ETF                 | 5.17 ± 0.012   | 109.8 ± 1.572                | 0.314 ± 0.046   | 0.0554 ± 0.0036                 | 1.4095 ± 0.009              | 15.13 ± 0.208                    | 76.87 ± 0.1528               |
| M4 ETF                 | 5.36 ± 0.000   | 162 ± 1                      | 0.334 ± 0.0076  | 0.8886 ± 0.4229                 | 1.4237 ± 0.001              | 11.73 ± 0.153                    | 213.33 ± 0.5774              |
The penetrated ETF amount was found at 63% for M1_{ETF} and 25% for M2_{ETF}. It can be said that these were the highest and lowest value. It can be concluded that M1_{ETF} performed better in permeation and penetration study.

According to Mura’s study (2011), the permeation percentage difference between M3_{ETF} and M4_{ETF} occurred due to Transcutol HP amount in the microemulsion. It is believed that as Transcutol HP concentration is increased, the drug could reach deeper in the skin layers (Mura et al., 2011). The main mechanism of Transcutol HP as permeation enhancer is to increase the partition parameter of the drug into the skin. This could be due to the close solubility parameter of Transcutol HP with skin (Haque, Talukder, 2018). In another study, researchers proved that Cre EL enhanced permeation of active compound (Abd-Elsalam, El-Zahaby, Al-Mahallawi, 2018). The same correlation was also found between M1_{ETF} and M2_{ETF}.

### Cell cytotoxicity

When novel drug carriers are developed, their potential to be cytotoxic should be examined by various methods. In vitro cell cytotoxicity assay can predict the in vivo possible toxicity of the developed carriers. Herein, the MEs were tested for their cytotoxicity against RAW 264.7 cell lines via MTT assay (Table V). The concentration of MEs, which did not significantly affect cell viability, were further chosen and used for the subsequent nitrite assay. IC_{50} values of formulations, which stands for the inhibition of cell growth by 50%, were as follows: Formulation M1_{ETF}:2.10±0.04‰ (v/v), Formulation M2_{ETF}:0.52±0.01‰ (v/v), Formulation M3_{ETF}:1.03±0.02‰ (v/v). As it was expected, the developed MEs were not quite cytotoxic in desirable dilution, and they can be safely applied for dermal delivery. The obtained data were expected since the used chemical substances are non-toxic. Similar results were reported by Okur et al. who studied the possible cytotoxicity of ME based gels loaded with fusidic acid on RAW 264.7 cell lines and examined them as dermal burn wound healing systems (Okur et al., 2020a).

#### TABLE V - Effects of formulations (M1_{ETF}, M2_{ETF}, M3_{ETF} and M4_{ETF}) on the viability of LPS-treated RAW 264.7 macrophages at different concentrations (‰ v/v)

| Formulation Codes | Cell viability % (Relative to medium control) (Mean ± SD) |
|-------------------|--------------------------------------------------------|
| M1_{ETF}          | 105.7 ± 5.4                                           |
| M2_{ETF}          | 107.9 ± 4.3                                           |
| M3_{ETF}          | 109.4 ± 7.9                                           |
| M4_{ETF}          | 23.74 ± 5.5                                           |

#### TABLE IV - Ex vivo permeation and penetration studies result of M1_{ETF}, M2_{ETF}, M3_{ETF} and M4_{ETF} formulations

| Formulation Codes | % Permeation from skin (after 24 h) | % Penetration to skin |
|-------------------|--------------------------------------|----------------------|
| M1_{ETF}          | 42.894 ± 3.96                        | 63.963 ± 0.012       |
| M2_{ETF}          | 47.884 ± 2.30                        | 25.912 ± 0.201       |
| M3_{ETF}          | 24.856 ± 3.85                        | 30.399 ± 0.170       |
| M4_{ETF}          | 35.466 ± 0.73                        | 26.812 ± 0.111       |
Nitrite assay

MEs were studied for their inhibitory properties opposed to LPS-induced nitrite production in RAW 264.7 cell lines. The comparison of anti-inflammatory activities of the formulations with the reference molecule, indomethacin (100 µM), are shown in Figure 3. Nitrite inhibition (%) of formulations $M_1^{ETF}$, $M_2^{ETF}$, $M_3^{ETF}$ and $M_4^{ETF}$ at their highest studied concentrations, was calculated at 51.3%, 73.7%, 55.9% and 52.1% respectively. Formulation $M_2^{ETF}$ exhibited the highest anti-inflammatory activity compared to the indomethacin treated group (57.6%). It can be said that the developed MEs present anti-inflammatory effects in LPS induced RAW 264.7 macrophage in a dose dependent manner. Similar data reported by Okur et al. (Okur et al., 2020a).

CONCLUSION

The present study demonstrates that the microemulsions can be applied as an alternative system for the enhancement of poorly water-soluble drugs like ETF. Solubility assays exhibited that oleic acid, almond oil, and castor oil led to the greatest solubilization of ETF, and consequently oleic acid was chosen as oil phase. From the constructed ternary phase diagrams, the optimal microemulsions were selected for further analysis. The optimized microemulsion containing oleic acid, Cremophor EL, ethanol and distilled water was transparent with low viscosity, appearing the droplet size of 109.8±1.572 nm. From the stability studies, it was confirmed that the optimal microemulsion was stable for 12 months. As a result of ex vivo studies, the optimized formulation showed the maximum permeation through the skin and showed the minimal penetration amount to the skin. At last, $M_2^{ETF}$ was found to be the most effective to inhibit nitrite production. As it is explained before, this result indicates that $M_2^{ETF}$ presents the highest anti-inflammatory effect, among others. In conclusion, this study showed that etofenamate could be delivered successfully via dermal administration route when it is loaded to microemulsion formulation.

ACKNOWLEDGEMENTS

The authors would like to thank the Recordati and Gattefosse for providing the etofenamate and Transcutol HP. Authors wish to thank Prof. Dr. Dilek Telci of Yeditepe University, Faculty of Engineering, Department
of Genetics and Bioengineering (İstanbul, Turkey) for providing the used RAW 264.7 cell line.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abd-Allah FI, Dawaba HM, Ahmed AM. Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug. Drug Discov Ther. 2010;4(4):257–66.

Abd-Elsalam WH, El-Zahaby SA, Al-Mahallawi AM. Formulation and in vivo assessment of terconazole-loaded polymeric mixed micelles enriched with Cremophor EL as dual functioning mediator for augmenting physical stability and skin delivery. Drug Deliv. 2018;25(1):484–92.

Aksu NB, Yozgatlı V, Okur ME, Ayla Ş, Yoltaş A, Üstündağ Okur N. Preparation and evaluation of QbD based fusidic acid loaded in situ gel formulations for burn wound treatment. J Drug Deliv Sci Technol. 2019;52:110–21.

Ali SM, Yosipovitch G. Skin pH: From basic science to basic skin care. Acta DERM Venereol. 2013;93(3):261–7.

Aydin A, Reis R, Sipahi H, Zeybekoğlu G, Çelik N, Kırızlıkçam B, et al. Hydroxytyrosol: The Phytochemical Responsible for Bioactivity of Traditionally used Olive Pits. Euroasian J Hepato-Gastroenterology. 2018;125:214–20.

Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects. Drug Deliv. 2006;13(3):175–87.

Karadağ AE, Demirci B, Çaşkurlu A, Demirci F, Okur ME, Orak D, et al. In vitro antibacterial, antioxidant, anti-inflammatory and analgesic evaluation of Rosmarinus officinalis L. flower extract fractions. South African J Bot. 2019;125:214–20.

Koksal M, Ozkan-Dagiyian I, Ozyazici T, Kadioglu B, Sipahi H, Bozkurt A, et al. Some Novel Mannich Bases of 5-(3,4-Dichlorophenyl)-1,3,4-oxadiazole-2(3H)-one and Their Anti-Inflammatory Activity. Arch Pharm (Weinheim). 2017;350(9):1–8.

Lu GW, Gao P. Emulsions and microemulsions for topical and transdermal drug delivery. handb. Non-Invasive Drug Deliv. Syst., Elsevier; 2010, p. 59–94.

Marto J, Baltazar D, Duarte A, Fernandes A, Gouveia L, Militão M, et al. Topical gels of etofenamate : in vitro and in vivo evaluation. Pharm Dev Technol. 2015;20(6):710–5.

Mura S, Manconi M, Valenti D, Sinico C, Vila AO, Fadda AM. Transcutol containing vesicles for topical delivery of minoxidil. J Drug Target. 2011;19(3):189–96.

Okur ME, Ayla Ş, Batur Ş, Yoltaş A, Genç E, Pertek S, et al. Evaluation of In Situ Gel Containing Pycnogenol for Cutaneous Wound Healing. Medeni Med J. 2019;34(1):67–75.

Okur ME, Ayla Ş, Yozgatlı V, Aksu NB, Yoltaş A, Orak D, et al. Evaluation of burn wound healing activity of novel fusidic acid loaded microemulsion based gel in male Wistar albino rats. Saudi Pharm J. 2020a;28(3):338–48.

Okur ME, Karantas ID, Şenyiğit Z, Üstündağ Okur N, Siafaka PI. Recent trends on wound management; new therapeutic choices based on polymeric carriers. Asian J Pharm Sci. 2020b;15(6):661-684.

Peraman R, Nayakanti D, Theja Dugga HH, Kodikonda S. Development and validation of a stability-indicating assay of etofenamate by RP-HPLC and characterization of degradation products. Sci Pharm. 2013;81(4):1017–28.

Pillai AB, Nair J V., Gupta NK, Gupta S. Microemulsion-loaded hydrogel formulation of butenafine hydrochloride for improved topical delivery. Arch Dermatol Res. 2015;307(7):625–33.

Plotnikoff R, Karunamuni N, Lytvyak E, Penfold C, Schopflocher D, Imayama I, et al. Osteoarthritis prevalence and modifiable factors: a population study. BMC Public Health. 2015;15:1195.

Prausnitz MR, Elias PM, Franz TJ, Schmuth M, Tsai J-C, Menon GK, et al. Skin Barrier and Transdermal Drug Delivery. Dermatology. 2012;2065–73.

Siafaka PI, Barmbalexis P, Bikiaris DN. Novel electrospun nanofibrous matrices prepared from poly(lactic acid)/poly(butylene adipate) blends for controlled release
formulations of an anti-rheumatoid agent. Eur J Pharm Sci. 2016a;88:12–25.

Siafaka PI, Bülbül EÖ, Mutlu G, EvrenOkur M, Karantas ID, Üstündag Okur N. Transdermal drug delivery systems and their potential on Alzheimer’s disease management. CNS Neurol Disord - Drug Targets. 2020;19(5):360-373.

Siafaka PI, Okur ME, Ayla Ş, Er S, Çağlar EŞ, Üstündag Okur N. Design and characterization of nanocarriers loaded with Levofloxacin for enhanced antimicrobial activity; physicochemical properties, in vitro release and oral acute toxicity. Braz J Pharm Sci. 2019;55:1–13.

Siafaka PI, Zisi AP, Exindari MK, Karantas ID, Bikiaris DN. Porous dressings of modified chitosan with poly(2-hydroxyethyl acrylate) for topical wound delivery of levofloxacin. Carbohydr Polym. 2016b;143:90–9.

Telaprolu K, Mohammed YH, Grice JE, Roberts MS. Skin models for the testing of transdermal drugs. Clin Pharmacol Adv Appl. 2016;8:163–76.

Tuncay S, Özer Ö. Investigation of different emulsion systems for dermal delivery of nicotinamide. Pharm Dev Technol. 2013;18(6):1417–23.

Üstündag N, Özdemir Dİ, Görgülü Ş, Şenyiğit ZA, Aşıkoglu M, Genç L, et al. Assessment of aprotinin loaded microemulsion formulations for parenteral drug delivery: Preparation, characterization, in vitro release and cytotoxicity studies. Curr Drug Deliv. 2015;12(6):668–79.

Üstündağ-Okur N, Ege MA, Karasulu HY. Preparation and characterization of naproxen loaded microemulsion formulations for dermal application. Int J Pharm. 2014a;4(4):33–42.

Üstündağ-Okur N, Gökçe EH, Eğrilmez S, Özer Ö, Ertan G. Novel Ofloxacin-Loaded Microemulsion Formulations for Ocular Delivery. J Ocul Pharmacol Ther. 2014b;30(4):319–32.

Üstündağ Okur N, Apaydın S, Karabay Yavaşoğlu NÜ, Yavaşoğlu A, Karasulu HY. Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. Int J Pharm. 2011;416(1):136–44.

Üstündağ Okur N, Çağlar EŞ, Siafaka PI. Novel ocular drug delivery systems: An update on microemulsions. J Ocul Pharmacol Ther. 2020;36(6):342-354 - jop.2019.0135.

Üstündağ Okur N, Hökenek N, Okur ME, Ayla Ş, Yoltaş A, Siafaka PI, et al. An alternative approach to wound healing field; new composite films from natural polymers for mupirocin dermal delivery. Saudi Pharm J. 2019a;27(5):738–52.

Üstündağ Okur N, Yavaşoğlu A, Karasulu HY. Preparation and evaluation of microemulsion formulations of naproxen for dermal delivery. Chem Pharm Bull. 2014c;62(2):135–43.

Üstündağ Okur N, Yozgatlı V, Okur ME, Yoltaş A, Siafaka PI. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. J Drug Deliv Sci Technol. 2019b;49:323–33.

Vicentini FTMC, Vaz MMOLL, Fonseca YM, Bentley MVLB, Fonseca MJV. Characterization and stability study of a water-in-oil microemulsion incorporating quercetin. Drug Dev Ind Pharm. 2011;37(1):47–55.

Xavier-Junior FH, Vauthier C, Morais ARV, Alencar EN, Egito EST. Microemulsion systems containing bioactive natural oils: an overview on the state of the art. Drug Dev Ind Pharm. 2017;43(5):700-14.

Received for publication on 10th March 2020
Accepted for publication on 13th July 2020