FABAD J. Pharm. Sci., 45, 3, 219-227, 2020

RESEARCH ARTICLE

Improvement of the Antimicrobial Activity of Moxifloxacin Using W/O Microemulsion System for Skin Infections

Gülşah EREL-AKBABA[*], İsmail ÖZTÜRK[**], Zeynep AY-ŞENYİĞİT[***°]

Improvement of the Antimicrobial Activity of Moxifloxacin Using W/O Microemulsion System for Skin Infections

SUMMARY

This article reports the preparation of moxifloxacin-loaded water in oil microemulsion systems and evaluation of their potential use in skin infections. According to the formulation development study, an optimal system consisted of 28% Peceol, 21% Lauroglycol 90, 7% Tween 80, 28% ethanol and 16% ultrapure water was selected for further studies using pseudo-ternary phase diagrams. Then, moxifloxacin was successfully loaded into developed microemulsions at two different concentrations of 1 mg/mL and 5 mg/mL. Moxifloxacin-loaded microemulsions showed nanometer size range (from 36.73 to 39.07 nm), low polydispersity index value (0.31 to 0.42) and negative zeta potential. In addition, these microemulsion formulations displayed low cytotoxicity on L929 mouse fibroblast cells. Moreover, the antibacterial activity of the formulations was evaluated by disk diffusion and microdilution tests. Results indicated that developed microemulsion formulations exhibited higher antibacterial activity in both Gram-positive and Gram-negative bacteria compared to solutions that include equal amount of moxifloxacin. In conclusion, the developed microemulsion formulation appears to be promising vehicle for topical delivery of moxifloxacin in skin infections.

Key Words: *Microemulsion, moxifloxacin, cytotoxicity, skin infection, antibacterial activity, antibiotic*

Moksifloksazinin Cilt Enfeksiyonları için Antimikrobiyal Aktivitesinin W/O Mikroemülsiyon Sistem Kullanarak Geliştirilmesi

ÖZ

Bu makale, moksifloksasin yüklü su/yağ mikroemülsiyon sistemlerinin hazırlanmasını ve geliştirilen formülasyonların cilt enfeksiyonlarında potansiyel kullanımlarının değerlendirilmesini bildirmektedir. Formülasyon geliştirme çalışmasına göre, % 28 Peceol, %21 Lauroglycol 90, % 7 Tween 80, % 28 etanol ve % 16 ultra saf sudan oluşan optimal sistem, sözde üçlü faz diyagramları yardımıyla daha sonraki çalışmalarda kullanılmak üzere seçilmiştir. Daha sonra, moksifloksasin, 1 mg/mL ve 5 mg/mL'lik iki farklı konsantrasyonda geliştirilmiş mikroemülsiyonlara başarıyla yüklenmiştir. Moksifloksasin yüklü mikroemülsiyonlar nanometre boyut aralığı (36.73 ila 39.07 nm), düşük polidispersite indeks değeri (0.31 ila 0.42) ve negatif zeta potansiyel göstermiştir. Ek olarak, bu mikroemülsiyon formülasyonları L929 fare fibroblast hücreleri üzerinde düşük sitotoksisite sergilemiştir. Ayrıca formülasyonların antibakteriyel aktivitesi, disk difüzyonu ve mikrodilüsyon testleri ile değerlendirilmiştir. Sonuçlar, gelişmiş mikroemülsiyon formülasyonlarının, eşit miktarda moksifloksasin içeren çözeltilere kıyasla hem Gram-pozitif hem de Gram-negatif bakterilerde daha yüksek antibakteriyel aktivite sergilediğini göstermiştir. Sonuç olarak, geliştirilen mikroemülsiyon formülasyonunun cilt enfeksiyonlarında moksifloksasinin topikal uygulanması için umut verici bir taşıyıcı sistem olduğu görülmektedir.

Anahtar Kelimeler: *Mikroemülsiyon, moksifloksasin, sitotoksisite, cilt enfeksiyonu, antibakteriyel aktivite, antibiyotik*

Received: 29.05.2020
Revised: 28.06.2020
Accepted: 16.07.2020

[*] ORCID: 0000-0003-3287-527, Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Izmir Katip Celebi University, 35620, Izmir, Turkey
[**] ORCID: 0000-0002-2669-3090, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Izmir Katip Celebi University, 35620, Izmir, Turkey
[***] ORCID: 0000-0002-4920-2469, Department of Pharmaceutical Technology, Faculty of Pharmacy, Izmir Katip Celebi University, 35620, Izmir, Turkey

[°] Corresponding Author; Zeynep Ay-Şenyiğit, Ph.D.
Tel: +90 232 329 35 11 / 6106, GSM: +90 533 649 10 18, Fax: +90 232 325 40 42, e-mail: zeynep.senyigit@ikcu.edu.tr

INTRODUCTION

The skin and underlying soft tissues are frequent sites of bacterial infection and they are one of the most common reasons for administering antibiotic therapy. Skin infections range from relatively benign, uncomplicated conditions (e.g. carbuncles, impetigo) to complicated ones (e.g. major abscesses, traumatic wounds, and diabetic foot infections) (Bogner, Kutaiman, Esguerra-Alcalen, Heldner, & Arvis, 2013; Dryden, 2010). The most common pathogens involved with skin infections are Gram-positive cocci, *Staphylococcus aureus*, and streptococci. On the other hand, complicated infections often involve with enteric Gram-negative bacilli and anaerobic bacteria, especially for patients with underlying risk factors (diabetic, immunocompromised, vascular compromise). Common Gram-negative pathogens causing skin infections include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas aeruginosa* (DiNubile & Lipsky, 2004; Giordano, Song, Pertel, Herrington, & Kowalsky, 2005; Rennie, Jones, & Mutnick, 2003)

The investments in the pharmaceutical industry and biotechnology offer new generations of antibiotics for different infectious diseases (Årdal et al., 2020). Moxifloxacin (MOX) is a novel fourth-generation fluoroquinolone family antibiotic. It has a broad-spectrum showing greater *in vitro* activity against Gram-positive aerobic pathogens than earlier fluoroquinolones (e.g.,ciprofloxacin). It has also potent activity against Gram-negative bacteria (Guay, 2006)

Water in oil (w/o) microemulsions are optically isotropic, thermodynamically stable monophasic systems made from oil, water and at least a surfactant, with droplet size in the range from 10 to 200 nm. These formulations offer advantages such as spontaneous formation, manufacture simplicity, solubilization of both lipophilic and hydrophilic compounds and the capacity to increase the bioavailability of hydrophobic drugs (Akkuş Arslan, Inceçayir, & Tirnaksiz, 2010; Volpe, Nascimento, Insausti, & Grünhut, 2018). Furthermore, microemulsion components can act as permeation enhancers in topical drug delivery. These components may reduce the diffusional barrier of the stratum corneum and promote the penetration of the drug into skin (Shukla et al., 2018; Volpe et al., 2020). Moreover, the dispersed phase of microemulsions may act as a reservoir, which makes it possible to maintain a constant concentration in continuous phase (Butani, Yewale, & Misra, 2014). It has been reported in the literature that enhanced skin delivery has been demonstrated by microemulsions compared with conventional emulsions and gels. The obtained results has been attributed to the action of their components on the skin as well as their phase structure and particle size (Çilek, Türkyilmaz, & Çelebi, 2002; Nastiti et al., 2017).

In present study, w/o microemulsion formulations of MOX were developed using pseudo-ternary phase diagrams. According to our findings, there is no report available in literature about topical microemulsion formulations of MOX for the treatment of skin infections. The prepared microemulsions were characterized by their particle sizes, polydispersity indexes and zeta potential values. Cytotoxicity evaluation of formulations was performed on L929 mouse fibroblast cells. The antibacterial activity of the developed microemulsions was examined by disk diffusion and microdilution methods according to CLSI criteria. The comparative antibacterial activity of moxifloxacin loaded microemulsions was investigated using negative and positive controls.

MATERIALS AND METHODS

Materials

MOX was purchased from Bayer (Turkey). Peceol and Lauroglycol 90 was kindly donated by Gattefosse (France). Tween 80 and ethanol were obtained from Merck-Co. (Germany). Phosphate-buffered saline (PBS) tablets (pH: 7.4) were purchased from AppliChem GmbH (Darmstadt, Germany). Alamar Blue Cell Viability Reagant was obtained from Invitrogen (USA). Murine fibroblast cell line (L929) cells were kindly provided by Ege University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology Laboratories (Izmir, Turkey). Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, L-glutamine and penicillin-streptomycin were obtained from Thermo Fisher Scientific (USA). All other chemicals were of analytical grade and used as received. Ultrapure water was used in all stage needed.

Bacterial strains

American type culture collection (ATCC) and Refik Saydam Culture Collection (RSKK) strains including three Gram-positive and three Gram-negative strains were used in antibacterial activity experiments. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* RSKK 02021, *Escherichia coli* ATCC 25922, *Salmonella enterica* RSKK 04059 and *Pseudomonas aeruginosa* ATCC 27853 strains were stored in brain-heart infusion broth (Merck) with 10% glycerine at -80°C.

Formation of microemulsion delivery system

In order to prepare water in oil (w/o) microemulsion system, Peceol was used as oil phase; Lauroglycol 90 and Tween 80 as surfactants (3:1, w/w) and ethanol as co-surfactant . Surfactant to co-surfactant ratio was kept constant at 1:1 (w/w). To determine the w/o microemulsion formation area, pseudo-ternary phase diagram was constructed by titration of the oil/surfactant/co-surfactant mixture with ultrapure water under constant stirring rate (500 rpm) at room temperature. The points where the appearance of the system is homogenous and transparent used for the construction of the phase diagram (Akbaba, Erel Akbaba, & Kantarcı, 2018). According to the phase diagram study, optimal oil, surfactant/co-surfactant and ultrapure water ratios were determined as to be used for the further studies. To form MOX loaded microemulsions (MOX-ME), MOX were incorporated to the aqueous phase of the microemulsion for the concentration 5 mg/mL or 1 mg/mL, as the final concentration. Blank microemulsion (ME) containing only ultrapure water as the aqueous phase was also prepared as control formulation.

Characterization studies

The particle size and polydispersity index (PDI) measurements of ME and MOX-MEs with different MOX concentrations were performed by Dynamic Light Scattering (DLS, Zeta sizer Nano ZS, Malvern Insturements Ltd., UK) using non-invasive back scattering mode with the detector positioned at 173° from the incident beam within the disposable polystyrene microcuvettes (Mutlu Ağardan, Değim, & Yilmaz, 2018).

To measure the electrophoretic mobility of the microemulsions, formulations were diluted 10 fold with 1 mM NaCl. The measurements were performed in standard zeta cuvettes (Malvern) at 25°C (Bhardwaj et al., 2006). Zeta potential was calculated by the software using Smoluchowski equation. Each measurement was performed at least in triplicate and mean value (± SD) was presented.

pH value of each formulations was measured using pH meter (SevenGo Duo SG 23, Mettler Toledo Ohio, USA) which was calibrated using buffers of pH 4, pH 7 and pH 10 at 25°C.

***In vitro* cytotoxicity assay**

Cell culture studies was performed on L929 mouse fibroblast cell line, which is a standard cell line for cytotoxicity assays according to ISO10993-5 guidelines (Li et al., 2019). Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and penicillin/streptomycin was used as culture medium. The cells were seeded into 96 well plate at the concentration 1 x 10^5 cells/mL at 100 µL per well and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h. Then, the medium was replaced by the fresh medium containing different amount of microemulsions such as 4, 8, 12,16 and 20 µL. Cytotoxicity assay was performed at 24 h after the addition of formulations. Following the incubation period, the medium was removed and the cells washed twice with PBS (pH = 7.4). The proportion of viable cells was evaluated according to the Alamar Blue cell viability assay kit (Thermo Fisher Scientific, USA) by using the manufacturer's instructions (Erel-Akbaba et al., 2019). Florescence was read using a fluorescence excitation wavelength of 560 nm and an emission of 590 nm by Varioskan Flash microplate reader (ThermoFisher Scientific, USA). Untreated cells were used as control group. Cell viability was calculated by normalizing the fluorescence of media from treated cells to untreated cells. Experiments were carried out at least in triplicate.

Antibacterial activity experiments

Inhibitory effects of the formulations were examined by disk diffusion and microdilution methods. Quality control of the experiments were performed according to the criteria of Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2014).

Disk Diffusion Test

Disk diffusion test was examined to determine inhibitory zone diameters on agar media according to CLSI criteria (Clinical and Laboratory Standards Institute, 2014). Bacterial strains were grown on Mueller-Hinton agar (MHA) (Merck, Germany) for 24 h at 35°C. Fresh colonies were suspended in sterile physiological saline, and suspensions were adjusted to 0.5 McFarland turbidity by densitometer device (Den-1, Biosan, Turkey). The surface of MHA in petri dishes was covered with bacterial suspensions (100 µl) using sterile swabs, and the plates were air dried for 15 min. Sterile disks (6 mm in diameter, Oxoid, United Kingdom) were put on the surface of MHA, and 10 µl of the formulations [ME, MOX-ME (1 mg/mL), MOX-ME (5 mg/mL)] were added to the diss. MOX solutions were also prepared, and added for the same concentrations, as controls. Petri dishes were incubated at 35°C for 16-20 h, and inhibition zone diameters on the media were measured in millimetres. Negative control group (ME) was also investigated. Ciprofloxacin disk (5 µg, Oxoid, United Kingdom) was used as reference antibiotic, and the quality control ranges

were evaluated according to CLSI criteria (Clinical and Laboratory Standards Institute, 2014). Each formulation was tested in triplicate, and mean inhibition zone diameters ± standard deviations (SD) were reported. Quality control ranges (Inhibition zone diameters) for ciprofloxacin are between 22-30 mm for *S. aureus*, 29-37 mm for *E. coli* and 25-33 mm for *P. aeruginosa* according to CLSI.

Microdilution method

Minimum inhibitory concentrations (MIC) of the compounds were determined by microdilution method in 96-well microplates according to CLSI criteria (Clinical and Laboratory Standards Institute, 2014). Bacterial strains were grown on MHA at 35°C for 24 h. Fresh colonies were suspended in physiological saline solution, and cell density was adjusted to 0.5 McFarland turbidity. Bacterial suspensions were diluted 100 fold. 50 µL of cation adjusted Mueller-Hinton broth (Merck) were pipetted into the wells of plate. 50 µL of each formulation and solution was added into the first wells and 1/2 serial dilutions were performed. Bacterial suspensions (50 µL) were added to the wells and incubated at 35°C for 16-20 h. Lowest concentrations that inhibited the growth of bacteria was defined as MIC values. Each sample was tested in triplicate. MOX was used as a reference agent for antimicrobial activity, and quality control ranges were evaluated. Quality control ranges (MICs) for MOX are between 0.016-0.12 µg/ml for *S. aureus*, between 0.016-0.5 µg/ml for *E. faecalis*, between 0.06-0.008 µg/ml for *E. coli* and between 1-8 µg/ml for *P. aeruginosa* according to CLSI.

Statistical analysis

GraphPad Prism 6.0 (GraphPad Software, Inc., USA) was used for statistical analysis of all data. Results are expressed as means ± SEM. For analysis between multiple groups, a one- way ANOVA was performed followed by Sidak's multiple comparison test to compare differences between two groups. An unpaired two- tailed student t test was used for the comparison of two samples. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Pseudo-ternary phase diagram for microemulsion system

Pseudo-ternary phase diagram constructed by titration of water into the Peceol (oil), Lauroglycol 90: Tween 80 (Sur) and Ethanol (CoSur) mixture was given in Figure 1. Following construction of the phase diagram, a microemulsion consisting of 28% Peceol, 21% Lauroglycol 90, 7% Tween 80, 28 % Ethanol and 16 % ultrapure water was selected for MOX loading as to be used in the further studies based on its clear appearance and having good stability (Karasulu et al., 2015). An important criteria for the selection of surfactant is that the required hydrophilic lipophilic balance (HLB) value to form the water in oil microemulsion system (Patel, Patel, Bhatt, & Patel, 2013). In this context, it was presumed that the adjustment of HLB value to 7.5 by Lauroglycol 90 and Tween 80 caused the aimed w/o microemulsion formation (Lawrence & Rees, 2012)

In our microemulsion formulations, ethanol was used as co-surfactant and Peceol was used as oily phase. Peceol is oily vehicle based on a mixture of non-toxic polar lipids comprising mono,di and tri-olein for topical formulations. In the literature, the binary mixture of Peceol and ethanol was studied (Mouri et al., 2014). It was shown that the addition of ethanol to pure Peceol has a significant fluidifying and disordering effect on the Peceol supramolecular structure with an enhancement in water solubilization. Therefore, it can be concluded that the addition of ethanol improved the water solubilization and microemulsion formation capacity of Peceol.

In the literature, ethanol was commonly used in dermal microemulsion formulations as co-surfactant at different ratios. In our formulations, ethanol was used in a concentration of 28%. Similarly, there are topical microemulsion formulations that has ethanol concentration around 20% or higher has been reported (Üstündağ Okur, Çağlar, Pekcan, Okur, & Ayla, 2019; Üstündağ Okur, Er, Çağlar, Ekmen, & Sala, 2017).

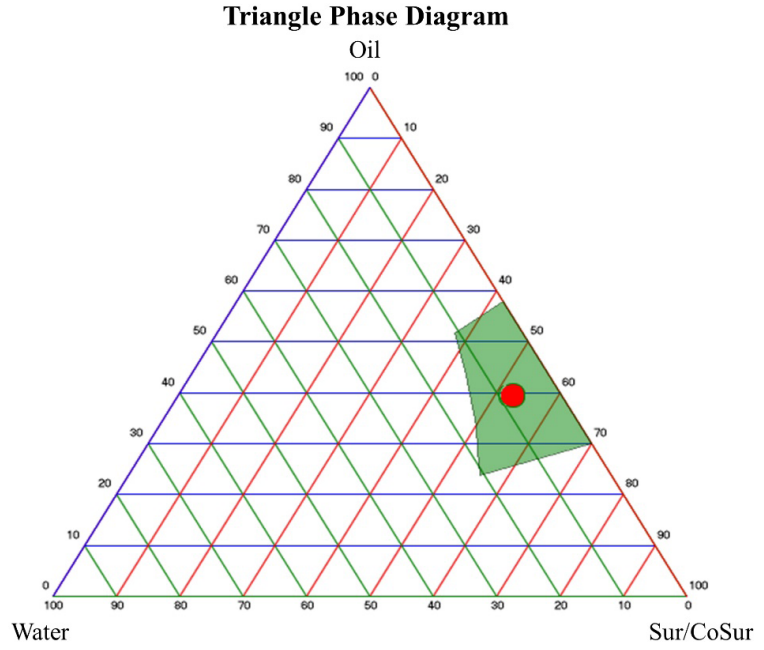

Figure 1. Pseudo-ternary phase diagram for the system Peceol, Lauroglycol 90, Tween 80, ethanol and water. Grey area represents transparent w/o microemulsion formation region.

Characterization studies of obtained microemulsions

To determine the particle size, PDI and zeta potential of microemulsions, DLS measurements were performed. As displayed in Table 1, the droplet size of microemulsions are in the nanometer size range (from 36.73 to 39.07 nm) and had low values of PDI (0.31 to 0.42) indicating the uniformity of the systems (Gaur, Mishra, Bajpai, & Mishra, 2014). Size distributions graphs of formulations supports this fact as well. Even the small amount of micelles which have particle sizes of <10 nm has formed, more than 85% of the droplets show uniformity in terms of particle sizes between 35 to 40 nm. The zeta potential of the empty microemulsion was measured as -3.26. After the MOX loading, although the zeta potential of the system approached zero, it has remained negative. pH value of empty microemulsion was determined as 5.18. By addition of MOX into the inner phase of microemulsion, pH values increased to 5.41 and 5.43 for the formulations ME-MOX (1 mg/mL) and ME-MOX (5 mg/mL), respectively. It can be said that pH values of microemulsions are appropriate for topical application route and did not significantly affected by MOX loading ($p > 0.05$) .

Table 1. Particle size, PDI and Zeta potential measurement results of microemulsion formulations (n=3)

Formulation	Particle Size (nm, ±SD)	PDI (±SD)	Zeta Potential (mV, ±SD)
ME	39.07 ± 1.65	0.42 ± 0.07	- 3.26 ± 0.38
MOX-ME (1 mg/mL)	38.71 ± 7.49	0.31 ± 0.15	- 2.60 ± 0.50
MOX-ME (5 mg/mL)	36.73 ± 2.38	0.44 ± 0.19	-1.19 ± 0.20

Evaluation of *in vitro* cytotoxicity

Cytotoxicity evaluation of formulations was performed on L929 mouse fibroblast cells. The results obtained from Alamar Blue cell viability assay presented in Figure 2. No significant cytotoxicity was observed on L929 cells for the doses 2 and 4 µL/well for all formulations. More than 80% cell viability was observed for these doses compared to the untreated group (control). Besides, all formulations showed a concentration dependent cell viability while there is no significant difference in their cytotoxic properties. All excipients used in this study were selected due to their low-cytotoxic properties. These results were attributed to this low-cytotoxic behaviour of components.

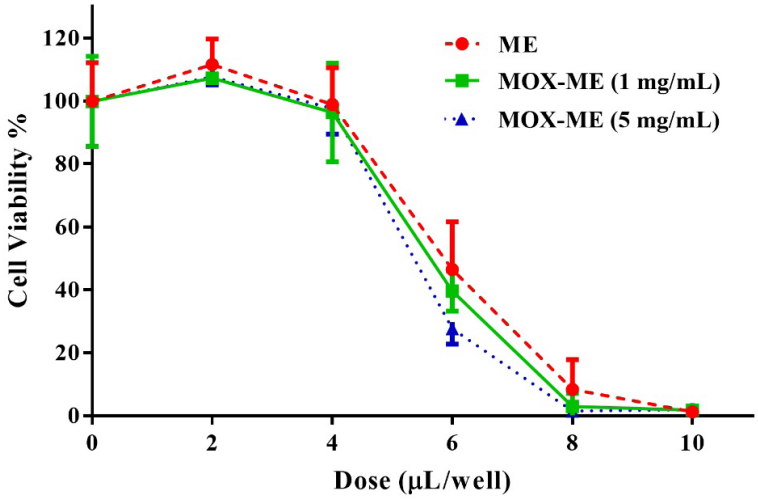

Figure 2. Cell viability of L929 cells after incubation with different doses ME, MOX-ME (1 mg/mL), and MOX-ME (5 mg/mL); data shown as mean ± SD (n = 4).

Antibacterial activity experiments

Disk diffusion test

Antibacterial activity of the formulations was evaluated by disk diffusion test against bacteria. Mean inhibition zone diameters and standard deviation values in Gram-positive and Gram-negative bacteria were demonstrated in Table 2. According to the results of disk diffusion test, microemulsions that include ME-MOX were found to be more effective than solutions that include equal amount of MOX Solution in both Gram-positive and Gram-negative bacteria. For the ME-MOX (1 mg/mL), the measured zone diameters was significantly bigger than MOX solution (1 mg/mL) for *S. aureus*, *E. faecalis*, *E.coli*, *S. enterica* and *E. faecalis* ($p < 0.05$). Although not statistically significant higher potency was observed on E. coli, ME-MOX (1 mg/mL) exhibited slightly bigger inhibition zone diameter comparing MOX solution (1 mg/mL) ($p > 0.05$). Blank formulation was found to be ineffective against all studied bacteria. Inhibition zone diameters of ciprofloxacin disk were in quality control ranges against *S. aureus*, *E. coli* and *P. aeruginosa* according to the CLSI criteria.

Similarly in the literature the antifungal or antibacterial activity of topical microemulsion formulations were evaluated with in vitro disk diffusion test. The obtained results showed that the activity of ME formulations were significantly higher compared with plain drug solution. It is assumed that plain drug solution showed the lowest zone of inhibition due to its less penetration effect than ME formulations (Butani et al., 2014).

The reason of improved bioactivity of prepared microemulsion formulations compared to solution is microemulsions may affect the stratum corneum structure and reduce the diffusional barrier by acting as a permeation enhancer (Changez, Chander, & Dinda, 2006; Changez, Varshney, Chander, & Dinda, 2006). The non-ionic surfactants (Lauroglycol 90 and Tween 80) reportedly emulsify sebum and extract skin lipids that enhancing the thermodynamic coefficient of the drug allowing it to penetrate into the cells more effectively (Hathout, Mansour, Mortada, Geneidi, & Guy, 2010; Rigg & Barry, 1990). In addition, the water content of MEs may enhance the permeation, because hydration of stratum corneum leads to the development and widening of channels in the keratin layer and distortion of lipid bilayer (Mullen, Carter, & Baillie, 1997; Yuan, Ansari, Samaan, & Acosta, 2008).

Table 2. Mean inhibition zone diameters and standard deviation values of formulations in Gram-positive and Gram-negative bacteria

	Mean diameter (mm)					
	Gram-positive bacteria			*Gram-negative bacteria*		
	S. aureus	***E. faecalis***	***B. subtilis***	***S. aureus***	***E. faecalis***	***B. subtilis***
ME-MOX (1 mg /mL)	29.00±0.00	26.00±0.00	38.66±0.58	29.33±0.58	34.33±0.58	30.00±0.00
MOX Solution (1 mg /mL)	23.00±0.00	23.33±0.58	38.00±0.00	28.00±0.00	30.00±0.00	28.33±0.58
ME-MOX (5 mg /mL)	32.66±0.58	28.00±0.00	41.00±0.00	34.00±0.00	37.00±0.00	35.00±0.00
MOX Solution (5 mg /mL)	30.00±0.00	24.66±0.58	40.00±0.00	31.00±0.00	34.00±0.00	33.66±0.58
Blank ME	-	-	-	-	-	-

ME: Microemulsion, MOX: Moxifloxacin, (-): no inhibition zone

Microdilution method

MIC values of the formulations [ME-MOX (1 mg/mL) and (5 mg/mL)] and alteration of MIC values compared to the control groups [MOX Solution (1 mg/mL) and (5 mg/mL)] were presented in Table 3. According to the results of microdilution method, the MIC values of ME-MOX (1 mg/mL) were found to be 2-fold lower than the MIC values of MOX Solution (1 mg/mL) in *S. aureus*, *B. subtilis*, *S. enterica* and *P. aeruginosa*. In addition, MIC value of ME-MOX (1 mg/mL) against *E. faecalis* was found to be 4-fold lower than the MIC value of MOX Solution (1 mg/mL) although no alteration was observed in MIC value against *E. coli* when compared the MIC value of ME-MOX (1 mg/mL) and MOX Solution (1 mg/mL). The MIC values of ME-MOX (5 mg/mL) were found to be 4-fold lower than the MIC values of MOX Solution (5 mg/mL) in *S. aureus*, *E. faecalis* and *P. aeruginosa*. Moreover, MIC value of ME-MOX (5 mg/mL) against *B. subtilis* was found to be 8-fold lower than the MIC value of MOX Solution (5 mg/mL). In parallel to the findings on alteration of MICs in *E. coli* for the dose 1 mg/mL, no alteration was observed in MIC value against *E. coli* when compared the MIC value of ME-MOX (5 mg/mL) and MOX Solution (5 mg/mL). Blank formulation was found to be ineffective against bacterial strains. MICs of MOX were in quality control ranges against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* according to CLSI criteria.

Table 3. Minimum inhibitory concentration values (µg/ml) of ME-MOX (1 mg/mL) / (5 mg/mL) and MOX Solution (1 mg/mL) / (5 mg/mL) with the alterations (n-fold change) on reducing MIC values in bacteria

	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	***E. faecalis***	***B. subtilis***	***E. coli***	***S. enterica***	***P. aeruginosa***
ME-MOX (1 mg /mL)	0.03	0.03	0.015	0.015	0.008	0.98
MOX Solution (1 mg /mL)	0.06	0.12	0.03	0.015	0.015	1.96
n-fold change for 1 mg /mL	2-fold	4-fold	2-fold	-	2-fold	2-fold
ME-MOX (5 mg /mL)	0.019	0.038	0.005	0.019	0.010	0.31
MOX Solution (5 mg /mL)	0.076	0.15	0.038	0.019	0.019	1.22
n-fold change for 5 mg /mL	4-fold	4-fold	8-fold	-	2-fold	4-fold

MIC: Minimum inhibitory concentration, ME: Microemulsion, MOX: Moxifloxacin, (-): No alteration

CONCLUSION

From the results obtained in the present work, it can be concluded that, the developed water in oil microemulsion formulation increased the bacterial activity of MOX on important bacterial strains such as *S. aureus*, *B. subtilis*, *S. enterica*, *P. aeruginosa* and *E. faecalis*. This improved antibacterial activity could contribute to an alternative treatment regimen for bacterial skin infections that require high antibiotic dose. Moreover, the developed microemulsion formulation may act as a promising and effective carrier system for skin delivery of other fluoroquinolone class of antibiotics.

ACKNOWLEDGEMENTS

This study was supported by İzmir Kâtip Çelebi University, Scientific Research Projects Coordinatorship (Project Number: 2018-ÖNAP-ECZF-0002).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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