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Development of Microemulsion Dermal Products Based on Avocado Oil for Topical Administration

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Abstract

The research described in this study aimed at developing microemulsions for dermal application using avocado oil. Due to its composition, avocado oil helps maintaining the barrier function of the skin. It has a nutritional effect on the skin, and it reduces the intensity of the process of skin peeling. Various surfactant:cosurfactant systems were tested in the conducted studies. There were no significant differences between the diagrams generated by Tween 20 and the surfactant:cosurfactant system, Tween 20:PEG400, at a ratio of 1:1. Six formulations were selected from the dilution line 7 of the ternary phase diagrams obtained by using as a surfactant Tween 20 and Tween 20:PEG 400, respectively. The formulations were characterized by determining physicochemical properties specific. In the next phase of study, these six formulations were used as a vehicle for incorporating erythromycin in order to develop erythromycin incorporated formulations for topical administration. The quality control of microemulsions with erythromycin was performed by evaluating the physical chemical, organoleptic and sensorial properties. Microemulsions were pharmacotechnically characterized by assessing the *in vitro* and *ex vivo* release kinetics of erythromycin.

Keywords: microemulsion, avocado oil, dermal product

1. Introduction

Although microemulsions possess numerous advantages as drug delivery systems, these modern pharmaceutical formulations have a limited applicability in the pharmaceutical and cosmetic field, due to the quality conditions for raw materials imposed by current legislation. Lately, a special interest has been shown by the specialists of the pharmaceutical

community in formulation and optimization of microemulsions, as an alternative dosage form to conventional emulsions and to other delivery systems for various administration routes. [1, 2].

Avocado oil is a vegetable oil obtained by cold press extraction from the *Persea gratissima* tree fruit (*Lauraceae* fam.), and containing a high amount of saturated and unsaturated fatty acids and other ingredients such as fat-soluble vitamins, minerals, and oligoelements [3]. Due to these bioactive ingredients, avocado oil plays a role in maintaining the skin's barrier function, manifests a nurturing effect on the dermal tissue, and decreases the exfoliation process.

Acne is a common skin condition that affects about 30% of the world's population, and half of whom are patients aged between 30 and 50 years [4]. Topical antibiotic therapy is an integral part of the therapeutic process of acne. Erythromycin is a macrolide antibiotic used to treat acne for over 20 years [5, 6]. Although some studies have shown the emergence of erythromycin resistance of germs involved in the pathogenesis of acne and the presence of local irritative reactions, this antibiotic is still successfully used by dermatologists as the antibiotic of choice for the local treatment of this dermatosis [7–10]. Erythromycin for topical use is usually formulated as cream. Serdoz et al. studied the incorporation of erythromycin into microemulsions based on mono- and triglycerides of medium chain fatty acids using Tween 80 as a surfactant. The obtained results showed a doubling of ER bioavailability when it was orally administered as emulsion [11].

The aim of this study was to include erythromycin in microemulsion-based preparations obtained with avocado oil and various cosolvent systems designed to increase efficiency and skin tolerance to the antibiotic.

2. Materials and methods

2.1. Materials

Avocado oil (Natural Sourcing LLC, Oxford, England), isopropyl myristate (Sigma-Aldrich, Germany), polyethylene glycol 400 (Sigma-Aldrich, Germany), Tween 20 (Sigma-Aldrich, Germany), erythromycin 99.85% purity (Zhejiang Sanmen Hengkang Pharmaceutical Co., Ltd., China), chromatographically pure acetonitrile (Merck, Germany), disodium phosphate (Merck, Germany) were used. Purified water, double distilled water, and other reagents that conform to the quality requirements of the 10th Romanian Pharmacopoeia were used in preparing microemulsions and in the quantitative analysis.

2.2. Methods

2.2.1. Determination of the ternary phase diagram and preparation of microemulsions

In the studied systems, we used lipophilic phase at 1:1 ratio mixture of avocado oil and isopropyl myristate. The surfactant and cosurfactant of the systems were consequently Tween 20 and also a

1:1 ratio mixture of Tween 20/PEG 400. All formulations contained purified water as hydrophilic phase. The diagrams were built according to the method described by Fanun [12, 13].

2.2.1.1. Parameters involved in the physicochemical characterization of the microemulsions

Electrical conductivity (σ)—determined with Metrohm 712 conductometer (Herisau, Switzerland) with graphite electrode at room temperature ($25 \pm 2^\circ\text{C}$). The conductometric cell was calibrated with standard KCl solution.

Rheological behavior—the dynamic viscosity (η) was determined by rotational rheoviscometer Rheolab MC120 (Stuttgart, Germany).

pH measurement—was performed using Thermo Orion pH meter (Thermo Fisher, Florida, USA).

Fourier transform infrared spectroscopy (FT-IR spectroscopy)—was done on a Vertex 70 FT-IR spectrophotometer (Bruker, Germany), through spectra measurement of each sample and respectively of the raw materials involved. The obtained spectra were compared standard spectra presented in the literature. All measurements were performed three times at room temperature ($25 \pm 2^\circ\text{C}$), with the results expressed as a mean of three determinations (\pm SD).

2.2.2. Preparation of microemulsions with avocado oil and erythromycin

Six microemulsion formulations were investigated. They were denoted as follows: three microemulsion formulations of the ternary phase diagram made with Tween 20 (F1—F3 and 3 formulae (F4—F6) of the diagram in which the surfactant:cosurfactant Tween 20:PEG 400 in the ratio of 1:1 was used. The formulations were selected from the regions of microemulsion formation based on the ternary phase diagrams, as shown in **Figures 1** and **2**, respectively. They were used within 24 h after preparation. Microemulsions were prepared by gentle warming and stirring of the oil phase, by adding the surfactant based on the ternary phase diagram and finally by including the aqueous phase, dropwise and with stirring at 200 rpm. Microemulsions were left at room temperature for 24 h, after which they were tested.

2.2.2.1. Quality control of microemulsions with erythromycin and avocado oil

Assessment of physical, chemical, and tactile properties—sensory properties of microemulsions, spreading ability, the emollient effect right after use (softness) and consistency were assessed in 10 volunteer patients, who rated products from 1 to 100 according to their satisfaction level, following international guidelines on testing of cosmetics [14, 15].

Evaluation of transepidermal water loss (TEWL) measurement—the measurements were performed using Tewameter TM 210 (Courage & Khazaka, Germany) on groups of six people, aged between 20 and 35 years. The epidermal water loss was measured immediately after applying the emulsion on the anterior surface of the palm (an area exposed to air, with a low

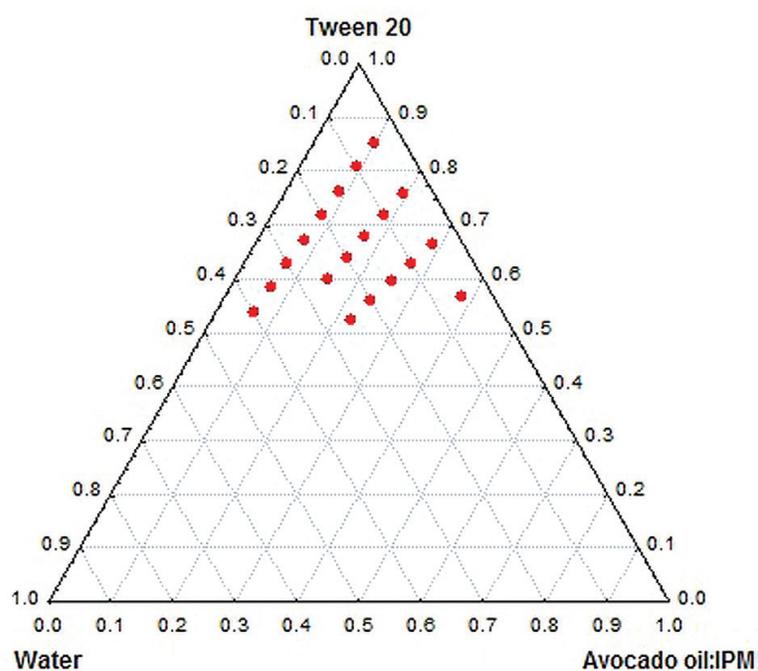


Figure 1. Ternary phase diagrams of the system avocado oil: IPM—Tween 20—water.

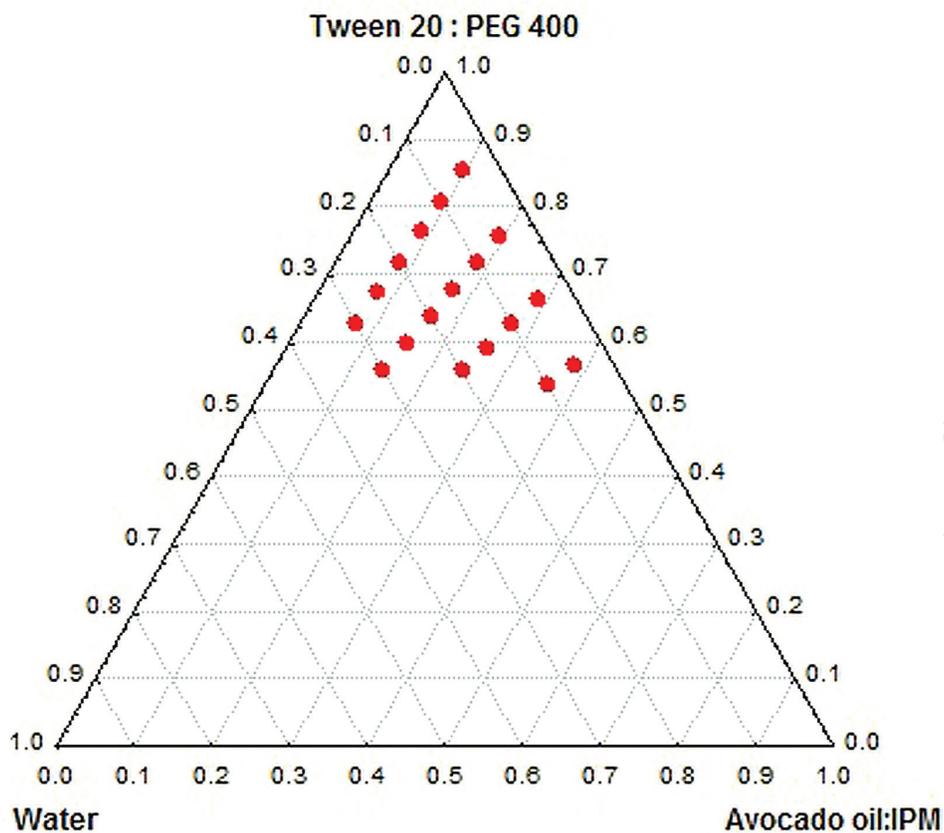


Figure 2. Ternary phase diagrams of the system avocado oil/IPM—Tween 20/PEG 400—water.

density of sebaceous glands). This was considered the starting point of measurements, and it was followed by successive measurements at 30-min intervals for 2 hours [16]. The experiment was carried out for three consecutive days, and the results were expressed as the mean of these three measurements.

Measurement of erythromycin (ER) content in avocado oil-based microemulsions—the measurement of ER content in microemulsions was made by using HPLC method developed and validated in-house [17]. Working method: 0.50 g of sample (F1, F2, F3, F4, F5, And F6) is dissolved separately in 25 mL of the mobile phase, dispersed at warm temperatures for about 5 min by magnetic stirring, and diluted to 10 mL with water. The resulting solution is centrifuged and filtered through a 0.45 μm filter and chromatographed.

2.2.3. Evaluation of *in vitro* and *ex vivo* release of erythromycin in avocado oil-based microemulsions

An *in vitro* dissolution test was carried out in a Franz cell with a diameter of 2.5 cm and the volume of the acceptor compartment of 15 mL, based on the following protocol: dissolution medium: phosphate buffer pH 7.4; mass of the sample: 0.5 g was processed for each studied formulation; cellulose membrane with a pore size of $\text{Ø} = 45 \mu\text{m}$ (Millipore, Merck Germany); temperature: $37 \pm 0.2^\circ\text{C}$; collection interval: 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h; an environment volume of 0.5 mL was harvested at each interval, and it was replaced with fresh medium; speed: 100 rpm.

An *ex vivo* dissolution test was carried out under similar conditions and the abdominal skin of female Wistar rats, weighing 200–250 g, was used as biological membrane. After harvesting, hair and adipose tissue were removed from the fraction of the skin and put into a buffer system pH 7.4 for 24 h prior to testing. The collected samples were chromatographed, and erythromycin spectrum was recorded at a wavelength $\lambda = 200 \text{ nm}$.

2.2.4. Measurement of the permeability coefficient of erythromycin in avocado oil-based microemulsions

The permeation rate of the substance in the stationary phase was measured from the slope, and the intercept was measured on the abscissa of the linear portion of the curves of the cumulative amount of drug substance that permeated with respect to time. The permeability coefficient was calculated according to Eq. (1):

$$K_p = J/C \times A \quad (1)$$

where:

K_p —permeability coefficient (cm/h); J —permeation rate of the drug substance or stationary phase flow ($\mu\text{g}/\text{h}$); C —concentration in the donor compartment ($\mu\text{g}/\text{mL}$); A —contact surface area (cm^2) [18].

3. Results and discussion

Figures 1 and **2** display the ternary phase diagrams of the systems obtained with Tween 20 and Tween 20/PEG 400 (1:1), respectively. The analysis of these diagrams showed no significant differences of the microemulsion zones.

3.1. Physicochemical characterization of microemulsions

Stability assessment was done on six microemulsion formulations selected from the ternary phase diagram built with Tween 20 (1A, 1B, and 1C) and three formulations (2A, 2B, and 2C) selected from the diagram with Tween 20/PEG 400 as surfactant/cosurfactant (1:1 ratio). Formulations were selected on the dilution line 7 of the ternary phase diagrams and contained 5–15% lipophilic phase, with the results shown in **Table 1**.

Formulation	Concentration of hydrophilic phase (% w/w)	Electrical conductivity σ (mS/cm) (\pm SD)	Dynamic viscosity η (mPa·s) (\pm SD)	pH (\pm SD)
1A	5.00	11.98 (\pm 1.21)	40.25 (\pm 0.87)	5.02 (\pm 0.84)
1B	10.00	13.86 (\pm 1.08)	36.11 (\pm 2.01)	5.19 (\pm 0.54)
1C	15.00	17.51 (\pm 0.94)	33.32 (\pm 1.58)	5.21 (\pm 0.60)
2A	5.00	20.50 (\pm 1.42)	58.50 (\pm 1.74)	5.80 (\pm 0.63)
2B	10.00	25.35 (\pm 0.91)	55.01 (\pm 1.01)	5.11 (\pm 0.41)
2C	15.00	29.39 (\pm 1.92)	53.32 (\pm 1.81)	5.35 (\pm 0.31)

Table 1. Physicochemical parameters of microemulsions with avocado oil.

The electrical conductivity of the analyzed microemulsions increases proportionally with the concentration of the hydrophilic phase of the system. The viscosity values indicate a newtonian behavior and a pH value close to the physiological one.

The FTIR spectra of the samples prepared by the two methods are shown in **Figures 3a** and **4a**.

The FTIR spectra recorded for the raw excipients used for the preparation of microemulsions compiled with the reference spectra were presented in the literature [19]. Essentially, the FTIR spectra recorded for the microemulsion samples appear as a combination of the characteristic spectrum of avocado and that of the other three oily excipients. Regarding the resemblance with the spectra recorded for avocado oil, some spectral differences are obvious. First, a very intense and broad band is observed in the spectral range 3100–3700 cm^{-1} , which is the normal range of adsorption for aliphatic hydroxyl group, and it is associated with the water content of the sample. Indeed, this band becomes more intense as the amount of water used in the formulation increases. Secondly, few differences can be noticed in the spectral range 2750–3050 cm^{-1} also in the case of formulations encoded 1A, 1B, and 1C (shown in **Figure 3b**) and for those encoded as 2A, 2B, and 2C (shown in **Figure 4b**). In this area, it is notable the

fact that the band at 3009 cm^{-1} assigned to C-H stretching vibration of the *cis*-double bond disappears, while a decrease of the intensity of the band at 2852 cm^{-1} with the formation of a new band at 2874 cm^{-1} . The band at 2852 cm^{-1} is usually assigned to symmetric and asymmetric stretching vibration of the aliphatic $-\text{CH}_2$ group, and that from 2874 cm^{-1} corresponds to symmetric and asymmetric stretching vibration of the aliphatic $-\text{CH}_3$ group. These observations are supported also by the spectral changes in the region C=O region (1746 cm^{-1}) as it can be seen in **Figures 3c** and **4c**, respectively. Here, the study shows a shift of this band toward lower wavenumbers (1733 cm^{-1}). This may happen due to the formation of saturated aldehyde functional groups or other secondary products during the heating process. These results are in accordance with observations made by other authors on the FTIR study of edible oils [20]. Finally, a new band is observed at 1643 cm^{-1} . This band is associated with the bending vibrations of the covalent bond in water molecules. Its intensity increases with the amount of water used in the formulation (see **Figures 3c** and **4c**).

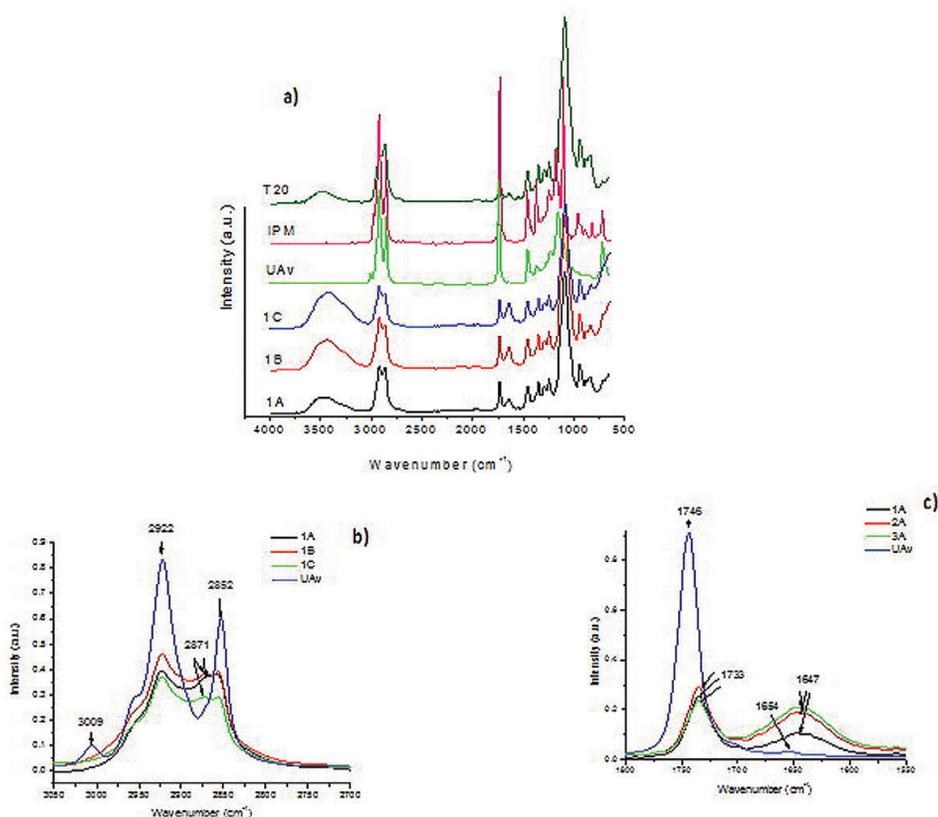


Figure 3. (a) FTIR spectra recorded for microemulsion samples 1A, 1B, 1C, and raw materials avocado oil (UAV), isopropyl miristate (IPM), Tween 20 (T20); (b) FTIR spectra recorded for samples 1A, 1B, 1C, and avocado oil in the $2700\text{--}3050\text{ cm}^{-1}$ spectral range; (c) FTIR spectra recorded for samples 1A, 1B, 1C, and avocado oil in the $1550\text{--}1800\text{ cm}^{-1}$ spectral range.

In the next phase of the study, these six formulations of avocado oil-based microemulsions were used as a vehicle for incorporating erythromycin in order to develop dermal preparations as microemulsions with erythromycin for topical administration.

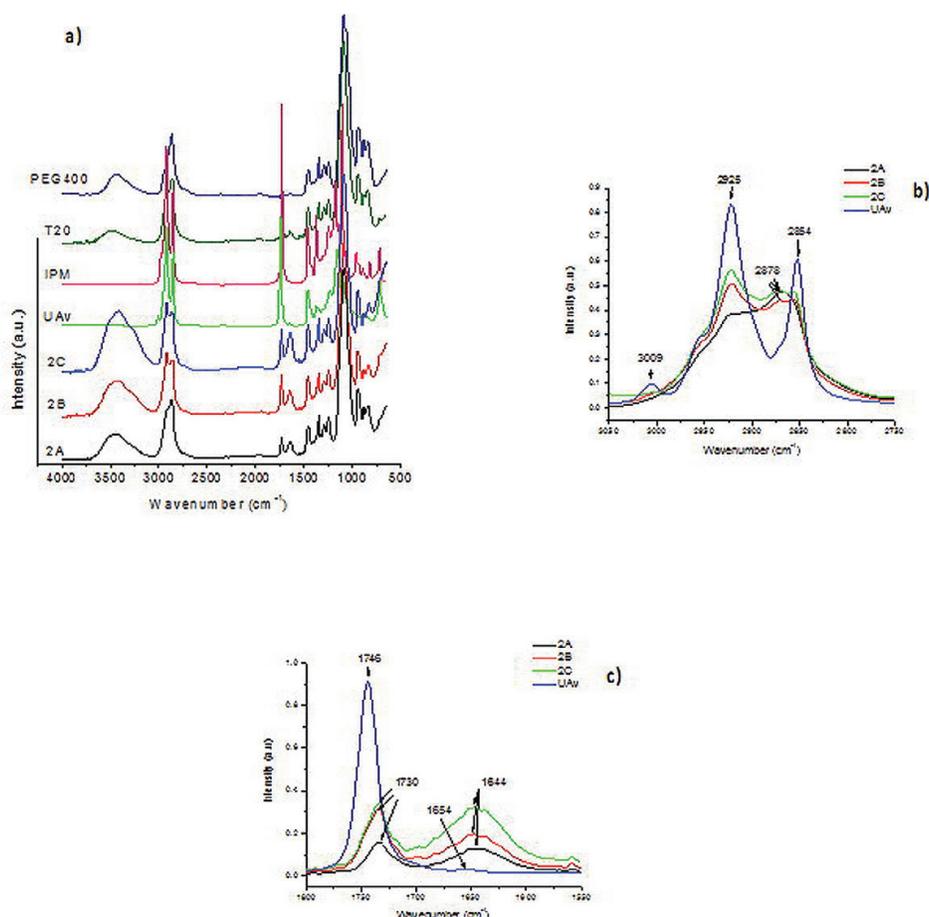


Figure 4. (a) FTIR Spectra recorded for microemulsion samples 2A, 2B, 2C, and raw materials avocado oil (UAV), isopropyl miristate (IPM), Tween 20 (T20); (b) FTIR spectra recorded for samples 2A, 2B, 2C, and avocado oil in the 2700–3050 cm^{-1} spectral range; (c) FTIR spectra recorded for samples 2A, 2B, 2C, and avocado oil in the 1550–1800 cm^{-1} spectral range.

The analysis of sensory properties showed that microemulsions prepared with a mixture Tween 20:PEG 400 as a surfactant had the best moisturizing and spreading properties (**Figure 5**).

This feature can be attributed to the presence of PEG 400 in the formulation, as it has a moisturizing effect on the skin. Although all microemulsion formulations were evaluated with a maximum score of 100 for the aspect, F1 and F3 formulations with the lowest percentage of the aqueous phase had the lowest score for spreading, as a greasy feeling was reported.

The TEWL measurement had the best values for the series of microemulsions prepared with the surfactant system Tween 20:PEG 400, as shown in **Figure 6**.

In order to assess the ER content of the studied microemulsion formulations, we compared the results with data from the monograph *Emulsiones* and *Unguenta* in the 10th Romanian Pharmacopoeia that admits a deviation of $\pm 3\%$ from the declared value for products containing 0.5% and more than 0.5% of active ingredient. The results shown in **Table 2** suggest a uniform distribution of ER in the studied microemulsions, with a content of active ingredient ranging from 97.24 to 103.11%. These values conform to the standards of the 10th Romanian Pharmacopoeia [21].

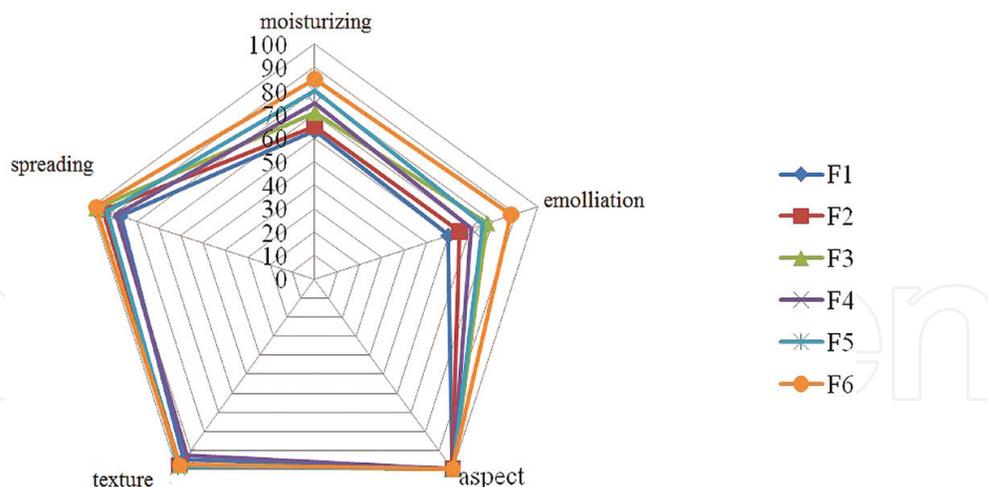


Figure 5. Representation of the sensory properties of avocado oil-based microemulsions with erythromycin.

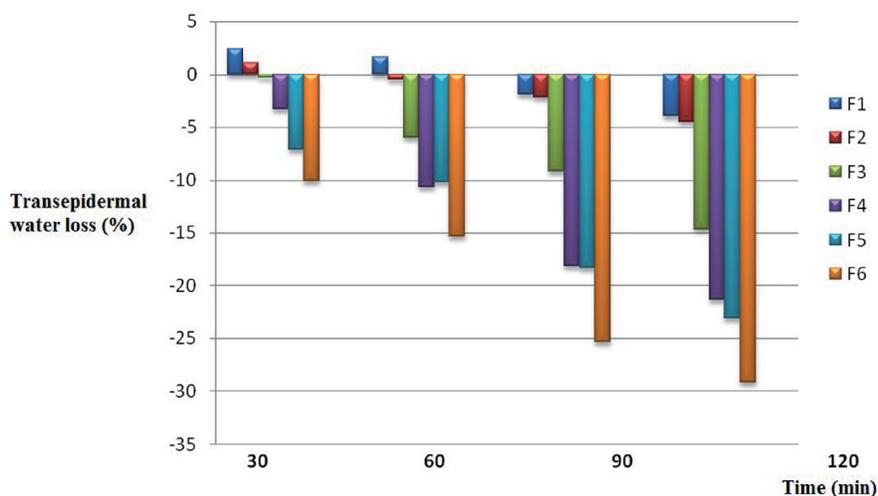


Figure 6. Representation of the TEWL measurement of avocado oil-based microemulsions with erythromycin.

Formulation	The ER content (%)
F1	101.13 ± 0.54
F2	99.25 ± 0.71
F3	97.24 ± 1.04
F4	99.85 ± 0.96
F5	103.11 ± 0.46
F6	100.53 ± 0.87

Table 2. The content of the active ingredient in avocado oil-based microemulsions with erythromycin.

The results of the *in vitro* and *ex vivo* ER release tests in avocado oil-based microemulsions are shown in Figures 7 and 8.

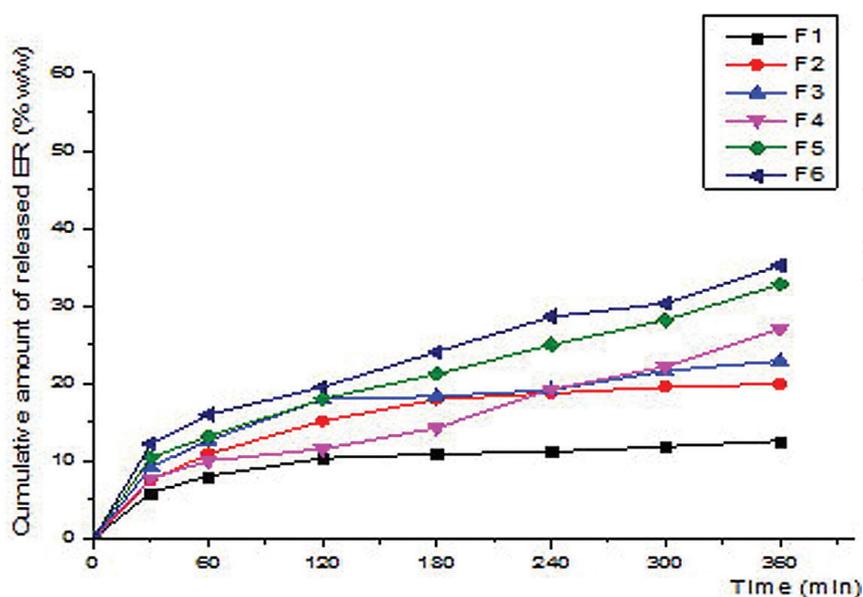


Figure 7. Cumulative kinetic profile of *in vitro* release of ER in avocado oil-based microemulsions.

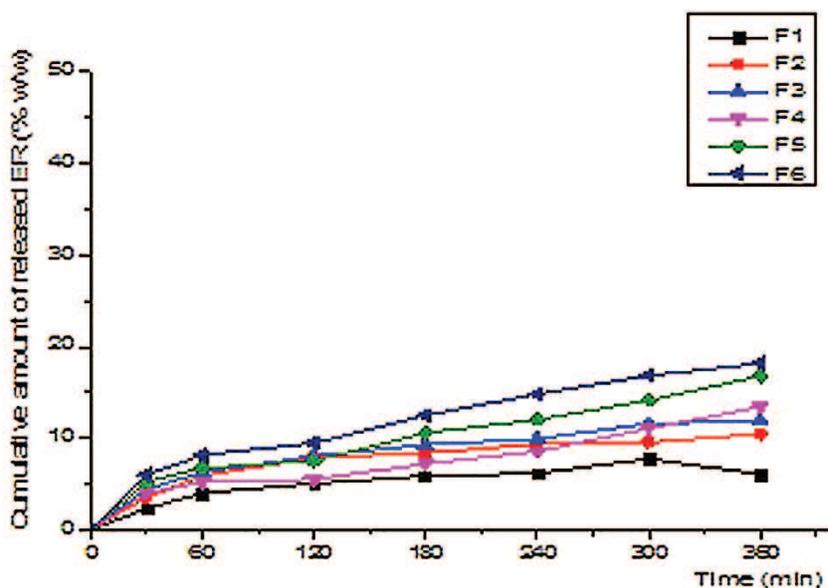


Figure 8. Cumulative kinetic profile of *ex vivo* release of ER in avocado oil-based microemulsions.

The results of the *in vitro* release through the synthetic membrane and *ex vivo* release through the biological membrane showed an unsatisfactory release of ER during the 6 hours of the study. Cumulative analysis of data suggests that F1-F3 formulations prepared with the Tween 20 surfactant had a slightly accelerated release rate within the first 2 hours of testing, after which the concentration of ER remains at a plateau level. We have found that F3 and F6 formulations that

had the highest water content showed the highest percentage of release both in testing through biological membrane (F3 = 11.90%, and F6 = 18.18%) and in testing through synthetic membrane (F3 = 22.90 and 35.18%, respectively). F4-F6 formulations prepared with the cosurfactant system Tween 20:PEG 400 showed the highest percentage of ER release, ranging from 26.98 to 35.18% for the *in vitro* test and from 13.45 to 18.18% for the *ex vivo* test. This low percentage of release can be attributed to possible interactions between erythromycin and certain substances found in the microemulsion formulations, to ER insolubility in the dissolution medium, and to the large size of the erythromycin molecule.

Formulations prepared with the cosolvent system Tween 20:PEG 400 had the best coefficient of penetration, due to the presence of PEG 400 in the formulation (**Table 3**).

Formulation	Permeation parameters	
	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	$K_p \times 10^{-6}$ (cm/h)
Synthetic membrane (<i>in vitro</i>)		
F1	30.4559 \pm 7.2197	7115.86
F2	30.3371 \pm 7.5029	7561.58
F3	25.0658 \pm 7.7158	5424.43
F4	30.3625 \pm 7.7720	7882.27
F5	28.2829 \pm 7.7888	6926.99
F6	26.9587 \pm 7.4907	6156.37
Biological membrane (<i>ex vivo</i>)		
F1	25.0234 \pm 7.1224	5181.90
F2	26.8229 \pm 7.3379	5922.48
F3	25.3714 \pm 7.3734	5466.80
F4	26.6616 \pm 7.1809	5839.17
F5	25.0998 \pm 7.2846	5387.38
F6	25.2441 \pm 7.5083	5465.27

Table 3. Parameters specific to ER permeation in avocado oil-based microemulsions.

4. Conclusions

Microemulsions prepared with the surfactant system Tween 20:PEG 400 had the best sensory properties and the best transepidermal water loss measurement. ER was evenly distributed in the studied microemulsions that had a content of active ingredient ranging from 97.24 to 103.11%. The results of the *in vitro* release tests through synthetic membrane and the results of the *ex vivo* release tests through biological membrane showed an ER release of less than 40%. F4–F6 formulations prepared with the cosurfactant system Tween 20:PEG 400 had the high-

est percentage of ER release, ranging from 26.98 to 35.18% for the *in vitro* test and from 13.45 to 18.18% for the *ex vivo* test. The permeation degree of erythromycin was influenced by the presence of PEG 400, which led to a significant increase in this parameter.

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