In Vitro Release Kinetics Evaluation of Erythromycin in Microemulsions for Dermal Applications

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The purpose of this study was to develop and characterize the in vitro release kinetics of erythromycin from water-in-oil microemulsion preparations for dermal application. The results obtained confirm that the water-in-oil microemulsion systems based on avocado oil can be used as incorporation and delivery systems for the topical administration of ER in the treatment of skin disorders.

Keywords: erythromycin, drug release, microemulsions, dermal products

Although some studies show that there are germs involved in the etiopathology of skin dermatoses that have developed a resistance to erythromycin (ER) and that ER can induce local irritant reactions, this drug is still successfully prescribed by dermatologists as the antibiotic of choice in the topical treatment of skin diseases caused by ER-sensitive germs [1-3]. In general, for topical application, ER is formulated in creams. Serdaz F. et al studied the incorporation of ER in microemulsions based on mono- and triglycerides of medium chain fatty acids, using Tween 80 as a surfactant. The results obtained showed that ER doubled its oral bioavailability in these emulsions [4]. The objective of this study was to develop and characterize the *in vitro* release kinetics of erythromycin from water-in-oil microemulsion preparations for dermal application. The microemulsions were prepared by the method of the ternary phase diagram in which we used avocado oil as the oil phase, and Tween 20 and PEG 400 as surfactant and co-surfactant, respectively. Avocado oil is a vegetable oil used in dermocosmetology for its beneficial effects on the skin, among which moisturizing, cicatrizing, epithelisant and anti-wrinkle effects [5, 6]. The literature has presented results of research investigating the cicatrizing effect of avocado oil and its use in the treatment of psoriasis and skin scars [7, 8]. It was also shown that the unsaponifiable fraction of avocado oil has regenerative effects on the skin and improves the properties of scleroderma [9].

Experimental part

Materials

Materials used were*:* Avocado oil (Natural Sourcing LLC, Oxford, England), isopropylmyristate (Sigma Aldrich, Germany), polyethylene glycol 400 (Sigma Aldrich, Germany), Tween 20 ((Sigma Aldrich, Germany), erythromycin 99.85% purity (Zhejlang Sanmen Hengkang Pharmaceutical Co. Ltd. China); chromatographic purity acetonitrile (Merck, Germany); disodium phosphate (Merck, Germany). Purified water, double distilled water and other suitable reagents were used in the preparation of the microemulsions and the quantitative analysis, according to the quality requirements of RP 10th.

Preparation of micro-emulsions with avocado oil and erythromycin

Studies were carried out on 6 microemulsion formulations labeled as follows: three microemulsion formulas (F1 - F3) in the ternary phase diagram constructed with Tween 20, and 3 formulas (F4 - F6) in the diagram in which we used Tween 20: PEG 400 as surfactant: co-surfactant in the ratio of 1:1, according to the method described by Fanun [10-12]. The formulations were selected from the areas of microemulsion formation, according to the ternary phase diagrams shown in figure 1 and, respectively, (fig. 2). All microemulsions were used within 24 h of preparation.

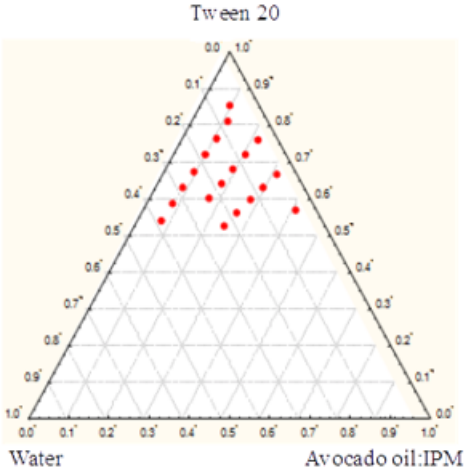

Fig. 1. Ternary phase diagram of the system avocado oil: IPM - Tween 20 - water

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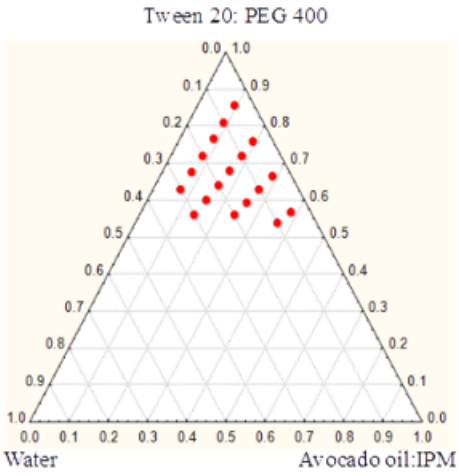

Fig. 2. Ternary phase diagram of the system avocado oil: IPM - Tween 20: PEG 400 - water

The determination of ER content in the microemulsions was performed by HPLC method developed and validated in house [13]*:* 0.50 g of sample (F1, F2, F3, F4, F5, F6) are dissolved separately in 25 mL of the mobile phase, are dispersed at warm temperature for about 5 min by magnetic stirring, and diluted to 10 mL with water. The resulting solution is centrifuged, filtered through a 0.45µm filter and chromatographed.

Evaluation of in-vitro release of erythromycin from avocado oil-based microemulsions

In vitro dissolution tests were carried out on a Franz cell (diameter - 2.5 cm and volume of acceptor compartment - 15 mL) according to the following protocol:

-dissolution medium: phosphate buffer solution *p*H 7.4;

-sample weight: 0.5 g for every formula studied;

-cellulose membrane: pore diameter ∅ = 45 µm (Millipore, Merck Germany);

-temperature: 37 °C ± 0.2 °C;

-collection interval: 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h. A volume of 0.5 mL medium was collected at each interval and subsequently replaced with fresh medium;

-speed: 100 rpm.

Samples were chromatographed and erythromycin spectrum was recorded at a wavelength λ= 200 nm.

Results and discussions

The microemulsions obtained showed adequate macroscopic properties, with a homogeneous, transparent aspect, a greenish-yellow color and a weak odor characteristic of avocado oil. In assessing the ER content in the microemulsion formulations, a deviation within ± 3% from the declared value is allowed for the products containing 0.5% and more than 0.5% active substance [14]. The results presented in table 1 show a uniform distribution of ER in the microemulsions studied, with a content of active substance varying in the range 97.24 - 103.11%.

Table 1

ER CONTENT OF AVOCADO OIL-BASED MICROEMULSIONS

Formula	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

In vitro release test results showed a slow release of ER during the 6 h of the study. The cumulative analysis of data shows that formulations F1-F3 prepared with Tween 20 as a surfactant had a slightly accelerated release rate within the first two hours of the test, after which ER concentration reached a plateau (fig. 3). Formulations F4-F6 prepared with the co-surfactant system Tween 20:PEG400 had the highest percentage of ER release, in the range 26.98 - 35.18%.

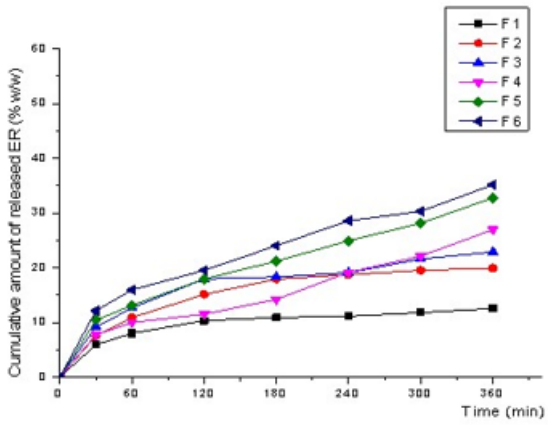

Fig. 3. Cumulative kinetic profile of *in vitro* release of ER in avocado oil-based microemulsions

This low percentage of release can be attributed both to ER insolubility in the dissolution medium and to the large size of the erythromycin molecule which may be an inhibitor factor to transmembrane permeability.

Models considerations

Ab initio, for a superior understanding of diffusional transport patterns, in the case of a (real) polymer chain dynamics in relationship with membrane's pores at various dimensional scales, we recommend reading a few articles in the field [15, 16].

In this study, the experimental results were analyzed by fitting on four mathematical models, according to the following equations:

i)Higuchi model expressed by the relation:

$$M_t = M_\infty \cdot K_H t^{1/2} \quad (1)$$

ii)Korsmeyer – Peppas model expressed by the relation:

$$M_t = M_\infty \cdot K_P t^n \quad (2)$$

where M_t is defined as the amount of drug released at time t, M_∞ the amount of drug released as time approaches infinity, equal, in most cases, with the initial amount of drug in microemulsions, M_o, K_o zero order release constant, K_H the Higuchi diffusion constant, K_p the Korsmeyer – Peppas constant, a kinetic constant, a measure of release rate and n the diffusional exponent that gives an indication of the mechanism of drug release and takes various values depending on the type of release device [17 - 19]. Let us note that the power type laws used in the classical theoretical models for drug release, i.e. relations (1) and (2), reflect the nonlinear character, both structural and functional, of the physical systems with controlled drug release. In our opinion, only a fractal type theory (for details, see [20 - 30]) could *cover*, in a unitary matter, the diversity of models applicable to drug release systems [31- 35].

Numerical modeling and data analysis

The parameters of the above models (1) – (2) obtained by fitting with the experimental data are presented in Table

Formula	Higuchi model		Korsmeyer – Peppas model		
	K_H	R^2	K_P	n	R^2
F1	5.8198	0.8537	7.8501	0.27	0.9879
F2	9.2341	0.9416	11.1017	0.36	0.9829
F3	10.1795	0.9257	12.4077	0.35	0.9846
F4	9.7818	0.9563	8.4282	0.61	0.9657
F5	12.8283	0.9928	12.8283	0.50	0.9928
F6	14.1812	0.9886	15.1546	0.45	0.9930

Table 2
PARAMETERS OF ER RELEASE KINETICS ANALYSIS IN THE AVOCADO OIL-BASED MICROEMULSION FORMULATIONS

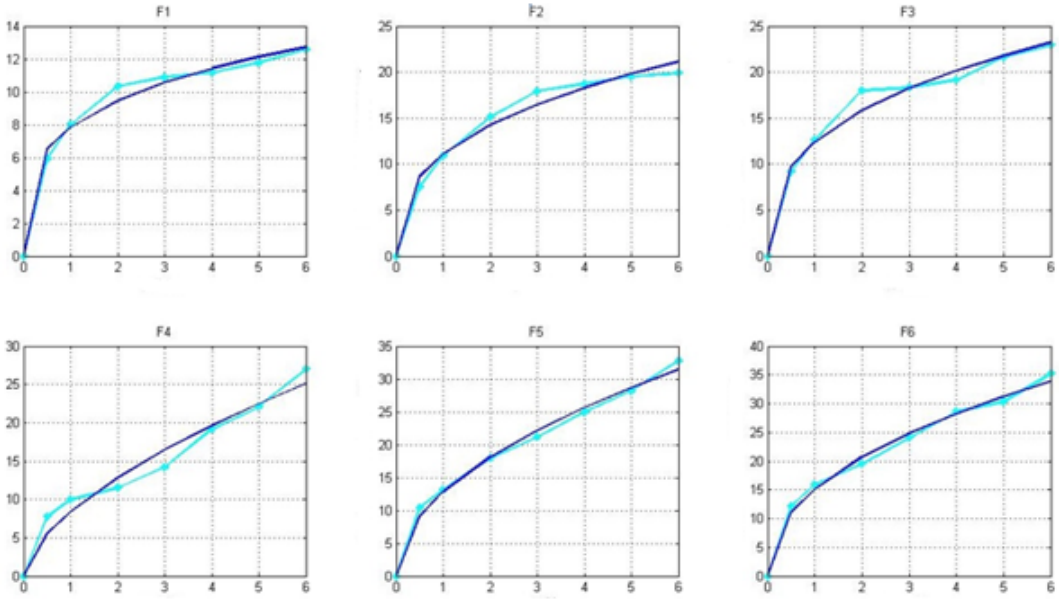

Fig. 4. Fitting of *in vitro* release profile of ER from avocado oil-based microemulsions on the Korsmeyer-Peppas model

2; data fitting was performed by linear or nonlinear regression using Matlab 7.1. For all samples, the correlation coefficients (R^2) are very good, higher than 0.85.

Referring to values, those up to 0.5 indicate a Fickian diffusion for samples F1, F2, F3, F6 and those between 0.5 and 1.0 reveal an anomalous (non-Fickian) transport (i.e. a mixed diffusion) for samples F4 and F5.

The theoretical plots and experimental release profiles are illustrated in figure 4.

Conclusions

The present study analyzed the *in vitro* release kinetics of ER from topical oil-in-water microemulsion systems prepared by the ternary phase diagram method. We analyzed six microemulsion formulations selected from dilution line 7 of the ternary phase diagrams. The microemulsions presented adequate macroscopic features and an even distribution of ER in the mass of the preparation. The *in vitro* release test results showed a release of ER below 40%. F4-F6 formulations prepared with Tween 20:PEG400 co-surfactant system had the highest percentage of ER release in the range of 26.98 to 35.18%. ER release from the avocado oil-based microemulsion formulations takes place by diffusion as a result of fitting on Korsmeyer-Peppas model. The results obtained confirm that the water-in-oil microemulsion systems based on avocado oil can be used as incorporation and topical delivery systems of ER for the treatment of skin disorders.

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