

DEVELOPMENT OF MICROEMULSION-LOADED HYDROGEL FORMULATIONS FOR TOPICAL DELIVERY OF METOPROLOL TARTRATE: PHYSICOCHEMICAL CHARACTERIZATION AND *EX VIVO* EVALUATION

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Abstract

This study aimed to develop and assess microemulsion-loaded hydrogels (ME GEL) as novel topical drug delivery vehicles for a hydrophilic drug, metoprolol tartrate (MT). Solubility studies for metoprolol tartrate were conducted using various oils, non-ionic surfactants and cosurfactants for screening the microemulsion components. To define the microemulsion region, pseudoternary phase diagrams, created by a new method (Phase Diagram by Micro Plate Dilution method), were used. The selected MT loaded microemulsions were converted into gel form, using Carbopol EDT 2020 and were characterised for droplet size, polydispersity index and zeta potential. The permeation of MT through excised pig ear skin was studied using vertical diffusion cells; the permeation (flux, permeability coefficient, lag time) and release (release rate, diffusion coefficient) parameters of MT in the ME GEL formulations and hydrogel were calculated. The skin permeation profile of metoprolol tartrate from the experimental formulations followed both Korsmeyer-Peppas and Higuchi kinetics. Their stability, safety and therapeutic efficacy need to be further investigated by *in vitro* and *in vivo* studies.

Rezumat

Acest studiu a avut ca obiectiv dezvoltarea și evaluarea unor hidrogeluri încărcate cu microemulsii (ME GEL) ca noi vehicule pentru eliberarea topică a unei substanțe medicamentoase hidrofili, metoprololul tartrat (MT). În scopul alegerii componentelor microemulsiei, au fost efectuate studii de solubilitate a MT, folosind diferite uleiuri, surfactanți și cosurfactanți. Pentru a delimita regiunea de microemulsii, au fost utilizate diagrame ternare de fază, create printr-o metodă nouă, metoda diluției în microplacă. Microemulsiile selectate, încărcate cu MT, au fost aduse în formă de gel folosind Carbopol EDT 2020 și au fost caracterizate în ceea ce privește mărimea picăturii, indicele de polidispersie și potențialul zeta. Permeația MT prin piele excizată de pe urechea de porc, a fost studiată folosind celule de difuzie verticale; au fost calculați parametri de permeație (fluxul, coeficientul de permeabilitate, timpul de latență) și de eliberare (viteza de eliberare, coeficientul de difuzie) ai MT din formulările ME GEL și din hidrogelul de control. Profilul permeației prin piele a metoprololului tartrat din formulările experimentale a corespuns modelelor cinetice Korsmeyer-Peppas și Higuchi. Stabilitatea, siguranța și eficacitatea lor terapeutică trebuie investigate în continuare prin studii *in vitro* și *in vivo*.

Keywords: metoprolol tartrate, microemulsion, hydrogel, *ex vivo* skin permeation, topical delivery

Introduction

Metoprolol tartrate (MT), known as bis[(2RS)-1-[4-(2-metoxietil)fenoxil]-3-[(1-metiletil)amino]propan-2-ol](2R,3R)-2,3-dihidroxi-butandioat [25], is a

selective beta-blocker widely used as a first line treatment in mild to moderate hypertension and angina pectoris [6, 15]. Moreover, in the last five years, metoprolol and other beta-blockers were

under investigation for the treatment of infantile haemangiomas [17]. Unfortunately, metoprolol undergoes an intense hepatic first-pass metabolism, having a short half-life (3 - 4 hours) and its systemic bioavailability is low (40%), requiring frequent oral administration [15]. Due to these properties, MT is an ideal candidate for percutaneous application, which may be a successfully alternative treatment and explain the interest in formulating this drug as dermal and transdermal delivery systems [23, 2]. But the percutaneous penetration of MT is poor because it is a polar, hydrosoluble cationic molecule. In order to overcome this inconvenience and to improve the MT skin permeation, different strategies can be approached, such as developing nanostructured carriers including microspheres, solid lipid nanoparticles, polymeric nanoparticles, microemulsions, liposomes and niosomes. Microemulsions (ME) are defined as thermodynamically stable, fluid, transparent (or translucent) homogenous colloidal dispersions, consisting of four main components (oil phase, aqueous phase, surfactant and co-surfactant) at appropriate ratios, which form a single optically isotropic dispersion with a droplet diameter usually within the range of 10-100 nm [10]. As modern pharmaceutical dosage forms, microemulsions are useful for topical delivery of drugs due to their several advantages: excellent thermodynamic stability, high capacity to solubilise both hydrophilic and lipophilic compounds, facile and low cost preparation, optical clarity, and increased penetration of drugs through the skin [8]. Moreover, the low viscosity microemulsions, which are undesirable for skin application, can be overcome by adding gelling agents to obtain gelled microemulsions [1, 4, 5], without affecting the drug diffusion in the resulting system [7, 9, 13]. In the last ten years, microemulsions and gelled microemulsions were intensively investigated as vehicles for dermal and transdermal delivery of a wide variety of drugs [14, 20, 22, 24].

The aim of the present study was to develop microemulsion-loaded hydrogel (ME GEL) formulations to be used as vehicles for topical delivery of MT and to evaluate their potential *vs.* a traditional topical hydrogel. Thus, several ME GEL formulations and a hydrogel containing 3% MT were prepared using Carbopol EDT 2020 and hydroxypropylmethylcellulose (HPMC) as gelling agents. The quality control of the formulations, regarding the physico-chemical properties and stability, was performed. Also, in order to assess the formulations performance and consequently to identify the formulation with the highest delivery capacity of metoprolol tartrate, the *ex vivo* skin permeation through hairless pig ear skin was investigated.

Materials and Methods

Materials

Metoprolol tartrate was kindly donated by Vim Spectrum S.R.L. (Corunca, Romania). Solutol HS 15 (macrogol 15 hydroxystearate), Cremophor RH 40 (PEG-40 Hydrogenated Castor Oil) and Cremophor EL (macrogolglycerol ricinoleate) (BASF Chem Trade GMBH, Germany), Lauroglycol 90 (propyleneglycol monolaurate), Labrasol (caprylocaproyl macrogol-8 glycerides) and Labrafil M2125CS (linoleoyl macrogol-6 glycerides) (Gattefossé, France), Lansurf SML 20 (polyoxyethylene (20) sorbitan monolaurate), Lansurf SMO 80 (polyoxyethylene (20) sorbitan monooleate), Lansurf OA 10 (macrogol 400 monooleate) and Lansurf OA 14 (macrogol 600 monooleate) (Lankem L.t.d., UK), Captex 355 (caprylic/capric triglyceride), and Captex 500 (triacetin), Captex GTO (glyceryl trioleate), Capmul MCM (glyceryl caprylate/caprate) and Caprol MPGO (polyglyceryl-3 oleate and polyglyceryl-10 monodiolate) (Abitec Corporation, USA), isopropyl myristate (Cognis, Germany), hydroxypropylmethylcellulose (Methocel K4M, Colorcon Ltd., UK), Carbopol ETD 2020 (Lubrizol Advanced Materials, USA) and Natrosol 250 HR (hydroxyethylcellulose) (Ashland Spec. Ingredients G.P., Netherlands) were received as gift samples. Castor oil and oleic acid were supplied by S&D Chemicals (India) and Merck KGaA (Germany), propyleneglycol (PG) and tetraglycol (TG) were obtained from BASF Chem Trade GMBH (Germany) and Merck Schuchardt OHG (Germany), ethanol (96%) was purchased from Chimopar SA (Romania), methyl- and propyl- paraben were purchased from Stera Chemicals (Romania). Double distilled water was used throughout the study. All chemicals and reagents were of pharmaceutical or analytical grade and were used without further purification.

Methods

Solubility studies

The solubility of MT in water, in various oils (Captex 355, Captex 500, Capmul MCM, Captex GTO, Labrafil M2125CS, isopropyl myristate, oleic acid and castor oil), in surfactants (Solutol HS 15, Cremophor RH 40, Cremophor EL, Lansurf SML 20, Lansurf SMO 80, Lansurf OA 10, Lansurf OA 14, Caprol MPGO and Labrasol) and in cosurfactants (ethanol, propyleneglycol, tetraglycol and Lauro-glycol 90) was determined using the shake flask method. Briefly, an excess amount of MT was separately dispersed in 5 mL of each of the mentioned solvents, in 10 mL capacity stoppered vials and mixed for 10 min using a vortex mixer, in order to facilitate a proper mixing of MT with the vehicle. The mixture vials were then kept and shaken at $25 \pm 1^\circ\text{C}$ in an isothermal shaker bath (Memmert, Germany) for 98 h to get to equilibrium.

The resulting mixtures were then centrifuged at 12,000 rpm for 15 min and the supernatant was filtered through a membrane filter (0.45 µm, 25 mm, Teknokroma, Germany). The concentration of the MT in the filtrate, after an appropriate dilution with methanol, was determined by UV spectrophotometer (T70+, PG Instruments, U.K.) at the wavelength 276 nm. Each experiment was performed in triplicate.

The screening of formulations components

The screening of the oil. The oil phase for developing MEs of MT was selected based on its maximum solubilisation capacity for the drug.

The screening and selection of the surfactants. The surfactant used for developing o/w MEs of MT was selected based on its solubilisation capacity for MT and the oil phase (Capmul MCM). After performing the solubility studies, three different surfactants, Lansurf SMO 80, Lansurf OA 10, and Cremophor EL were screened. The solubilisation capacity of surfactants for Capmul MCM was determined using the technique described in some previous studies [1, 3, 21]. Briefly, to 2.5 mL of 15% (w/w) aqueous solution of surfactant, aliquots of 5 µL oil (Capmul MCM) were added with vigorous vortexing; if a one-phase clear solution was obtained, an additional quantity of oil was added until the solution became cloudy. The total amount of oil added before the appearance of the turbidity of the solution was considered the solubility. The solubility was calculated using Eq. (1):

$$\text{solubility of oil (\% w/w)} = \frac{a \cdot 0.995}{0.375} \cdot 100 \quad (1)$$

where *a* represents the volume (mL) of Capmul MCM added till the turbidity appearance, 0.995 is the density of Capmul MCM (g/mL), and 0.375 is the quantity of the surfactant contained in 2.5 mL of 15% (w/w) aqueous solution of the surfactant.

The screening and selection of cosurfactants. The selection criterion of the cosurfactant for developing o/w MEs was the area of the ME region. Cremophor EL was mixed with two of the solubilizers selected

as cosurfactants, namely ethanol and propylene-glycol. The pseudoternary phase diagrams were constructed at a fixed ratio S_{mix} of 1:1. The oil and S_{mix} were used in nine different weight ratios (from 9:1 to 1:9) so that the maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams.

The construction of the pseudo-ternary phase diagram

The pseudo-ternary phase diagrams were also used to obtain the concentration range of the components for the existing region of the microemulsions. The surfactant (Cremophor EL) and the cosurfactant (propyleneglycol) were blended in the weight ratios of 3:1, 2:1, 1:1 and 1:2. These S_{mix} ratios were chosen in decreasing concentration of the surfactant with respect to the cosurfactant and *viceversa* for detailed study of the phase diagrams. Different mixtures of oil and surfactant/cosurfactant mixtures were prepared at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The Phase Diagram by Micro Plate Dilution (PDMPD) method, a novel technique based on the water titration method, was used for the construction of the pseudo-ternary phase diagrams [19]. The microemulsion phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained.

The preparation of metoprolol tartrate micro-emulsion formulations

According to the microemulsion regions in the phase diagrams, four microemulsion formulations, at different component ratios were selected. The composition of metoprolol tartrate-loaded micro-emulsion formulations is given in Table I. MT was dissolved under stirring in the mixture of Capmul MCM, Cremophor EL and propyleneglycol, then the appropriate amount of water was added to the mixture drop by drop with continuous stirring. The final concentration of MT in microemulsion systems was 3% (w/w). All microemulsions were stored at $25 \pm 2^\circ\text{C}$.

Table I

The composition (g %) of metoprolol tartrate-loaded microemulsions

Microemulsion components	Weight (%) and formulation codes			
	ME MT 1	ME MT 2	ME MT 3	ME MT 4
Metoprolol tartrate	3.0	3.0	3.0	3.0
Capmul MCM	15.0	25.0	15.0	20.0
Cremophor EL – PG (3:1)	45.0	45.0	35.0	40.0
Methylparaben	0.028	0.020	0.035	0.028
Propylparaben	0.009	0.007	0.012	0.009
Distilled water	36.96	26.97	46.95	36.96

The preparation of microemulsion-loaded hydrogels of 3% metoprolol tartrate

Carbopol EDT 2020 was selected as suitable gelling agent to prepare the microemulsion-loaded hydrogel formulations. Carbopol EDT 2020 was

dispersed slowly in the microemulsion under stirring. After the complete dissolution of the carbomer, homogenous and transparent gelled-microemulsion systems were obtained. The carbomer concentration in the microemulsion-loaded hydrogel was 1.5% (w/w).

The preparation of 3% metoprolol tartrate hydrogel

The preservative mixture of methyl- and propyl-paraben (3:1) was dissolved in hot water under stirring. MT was dissolved under stirring in 10 g of preservative aqueous solution at room temperature. The hydrogel base was obtained by dissolving HPMC powder in the remaining aqueous solution of parabens, following the "hot/cold" preparation technique [26]. Afterwards, the propyleneglycol and the MT solution were added to the HPMC hydrogel base under gentle continuous stirring, avoiding as possible air entrapping.

The composition of the final metoprolol tartrate hydrogel was: 3% (w/w) MT, 10% (w/w) propyleneglycol, 2.5% (w/w) HPMC, 0.1% (w/w) mixture of methyl- and propyl-paraben (3:1), and water. All the samples were stored at 5°C for 24 hours before testing.

The characterization of metoprolol tartrate micro-emulsions

The obtained microemulsions were evaluated regarding various physicochemical properties. The average droplet size, polydispersity index and zeta potential of the MT microemulsions were measured in triplicate by photon correlation spectroscopy using a Zetasizer Nano-ZS (Malvern Instruments, UK) instrument. Measurements were carried out at a fixed angle of 173° at 25°C. Microemulsions were diluted in a ratio of 1:30 with ultrapure water delivered by a Simplicity UV Water Purification System (Millipore SAS, France). The refractive indexes and the viscosities of formulations were determined at $25 \pm 2^\circ\text{C}$, using a refractometer (Digital ABBE Mark II-Reichert, Depew, USA) and a rotational viscosimeter equipped with a SC4-25 spindle (Brookfield DV-I+, UK) respectively. The pH of the microemulsions was measured at $25 \pm 2^\circ\text{C}$ using a pH-meter (Sension™1, Hach Company, USA). Experiments were performed in triplicate for each sample.

The characterization of microemulsion-loaded hydrogels and hydrogel containing 3% metoprolol tartrate

The determination of drug content and pH. To determine the drug content, about 1 g of formulation (ME GEL or hydrogel) was weighted in a 100 mL volumetric flask, and dissolved in methanol; 1 mL of the filtered solution was diluted appropriately and the MT content was spectrophotometrically analysed, at 276 nm, against a blank, prepared in the same way, using a plain ME GEL or hydrogel.

The pH values of aqueous solutions containing 5% (w/w) ME GEL MT or GEL MT were determined at 25°C using the Sension™1 digital pH-meter (Hach Company, USA). Each experiment was performed in triplicate.

Rheological characterization. In order to determine the viscosity and the consistency of the samples,

rheological studies were conducted. Viscosimetric measurements were performed using a stress-controlled rheometer (RheoStress 1, HAAKE, France) equipped with a cone-plate geometry (1/60) and data analysis was carried out by HAAKE RheoWin 4.3 software. Measurement of consistency was performed by penetrometry using a penetrometer (PNR 12, Petrolab, Germany) equipped with a micro-cone and suitable container, following the procedure described in the European Pharmacopoeia [25]. The spreadability of the samples was carried out using the parallel-plate method [16]. All rheological tests were performed in triplicate at 25°C.

Ex vivo skin permeation studies

Preparation of the skin. Dermatomed pig ear skin with a surface area of 1.767 cm² was used to carry out the *ex vivo* permeation studies. The skin was excised from 4-month-old, female or male domestic pig ears, obtained from a local slaughterhouse. Immediately after excision, the pig ears were cleaned up with tap water, their outer region was clipped of bristles and then the skin was dermatomed to a thickness of around 500 µm. The dermatomed skin samples were immediately used for the permeation experiments or stored at -20°C for a maximum period of 2 months. Before use, the dermatomed pig skin was removed from the freezer and allowed to thaw at room temperature. The integrity of the skin was evaluated by visual examination for physical damage, excluding unsuitable samples. The thickness of each sheet was measured with a micrometer and then squares of skin 2 to 2.2 cm² were cut from the sheet.

Ex vivo permeation study. To evaluate the effect of the formulation variables on preparations performance, the *ex vivo* permeation of metoprolol tartrate from selected ME GEL and GEL formulations was determined, using a system of 6 Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA). The Franz diffusion cells presented an effective diffusional area of 1.767 cm² and 6.5 mL of receptor cell capacity. To ensure sink conditions, the receptor chambers were filled with freshly prepared phosphate buffered saline solution (PBS) at pH 7.4. The skin pieces were mounted carefully on the Franz diffusion cells, between the donor and receptor compartments, with *stratum corneum* facing the donor chamber, and allowed to rest in contact with PBS at pH 7.4, 1 h prior the application of the samples. 300 mg of tested formulation was placed into each donor compartment. The receptor compartment was constantly stirred at 600 rpm and the diffusion cells were maintained at $32 \pm 1^\circ\text{C}$ throughout the experiment. 0.5 mL sample of the receptor medium were withdrawn at predetermined time (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22 and 24 h) and replaced with an equal volume of

fresh receiver medium to maintain a constant volume. The collected samples were analysed for MT content by UV spectrophotometric method, at 265 nm. The assay was linear in the MT concentration range of 38.4 - 384 µg/mL ($y = 0.0285x + 0.011$, $R^2 = 0.9986$). Three replicates of each experiment were performed.

Data analysis of ex vivo drug release studies. A cumulative amount of metoprolol tartrate permeated through the membrane (µg/cm²) was plotted as a function of time (t, h). The permeation rate of drug at steady-state (flux, J_s , µg/cm²/h) and the lag time (t_L , h) were calculated from the slope and the x intercept of the linear portion of the plots of cumulative amount of drug permeated *versus* time in steady state conditions, respectively. Permeability coefficient (K_p , cm/h) was calculated by dividing the flux with initial concentration of drug in the donor compartment. The release rate (k) values were calculated using the pseudo steady-state slopes from plots of cumulative amount of MT permeated through membrane (µg/cm²) *vs.* square root of time. Diffusion coefficient (D) values were calculated from the release rate values.

In order to investigate the release kinetics of the MT from ME GEL formulations and conventional hydrogel, the data obtained from *ex vivo* drug release studies were fitted into various mathematical models, as follows: *Zero order model*: $M_t = M_0 + K_0t$, where M_t is the amount of drug delivered in time t , M_0 is the initial amount of drug in the solution (it is usually zero), K_0 is the zero order release constant expressed in units of concentration/time, and t is the time; *First order model*: $\log C = \log C_0 - K_1t/2.303$, where C_0 is the initial concentration of drug, K_1 is the first order rate constant, and t is the time; *Higuchi model*: $M = K_H \times t_{1/2}$, where M is the amount of drug released in time t and K_H is the Higuchi release constant; *Korsmeyer-Peppas model*: $M_t/M_\infty = K_p t^n$, where M_t/M_∞ represents the fraction of the drug released at time t (M_∞ being the equilibrium concentration of the drug in the release solution), K_p is the Korsmeyer-Peppas release rate constant, and n is the diffusion coefficient. In this case, the first 60% drug release data were incorporated.

The following plots were made: cumulative percentage drug released *vs.* time (zero-order kinetics), log cumulative percentage of drug remaining *vs.* time (first-order kinetics), cumulative percentage drug released *vs.* square root of time (Higuchi model) and log cumulative percentage drug release *vs.* log time (Korsmeyer-Peppas model).

Statistical data analysis

Statistical analysis was performed using Statistica 7.0 software. Data were shown as mean $\pm$ standard deviation (SD) and were considered statistically

significant at $p < 0.05$. In the graphs, $\pm$ SD are not presented for the graph clarity reason.

Results and Discussion

The screening of formulations ingredients

The screening of oil and water. The solubility of MT in different oils is listed in Table II.

Table II
The solubility of MT in oils, surfactants and cosurfactants at $25 \pm 2^\circ\text{C}$

Component	Solubility (mg/mL)
Captex 355	35.04 $\pm$ 0.063
Captex 500	44.63 $\pm$ 0.37
Capmul MCM	21167.44 $\pm$ 9.82
Caprol MPGO	4971.74 $\pm$ 5.16
Labrafil M2125CS	2852.91 $\pm$ 2.04
Isopropyl myristate	109.49 $\pm$ 0.92
Oleic acid	14389.69 $\pm$ 7.48
Castor oil	1353.46 $\pm$ 1.35
Solutol HS 15	1336.78 $\pm$ 2.19
Cremophor RH 40	1086.53 $\pm$ 3.91
Cremophor EL	2469.19 $\pm$ 2.57
Lansurf SML 20	1351.38 $\pm$ 1.46
Lansurf SMO 80	15328.15 $\pm$ 7.24
Lansurf OA 14	7264.33 $\pm$ 1.35
Lansurf OA 10	10427.31 $\pm$ 8.05
Labrasol	7716.18 $\pm$ 4.08
Ethanol	4654.92 $\pm$ 1.16
Propylenglycol	28779.38 $\pm$ 7.42
Tetraglycol	3441.01 $\pm$ 2.77
Lauroglycol 90	681.95 $\pm$ 0.86

Regarding the solubility of MT in oils, the highest was found for Capmul MCM, followed by oleic acid (1.5 fold lower). Compared to Capmul MCM, Labrafil M 2125CS, castor oil and isopropyl myristate showed a much lower ability to dissolve MT (7.4 fold, 15.3 fold and 193.3 fold respectively). The lowest MT solubility was found to be in Captex 355 and Captex 500 (Table II), most probably due to their more pronounced hydrophobic character. It is generally accepted that the formulation of o/w microemulsion using an oil with high drug solubility would require less oil to incorporate the desired drug dose, which in turn would require lower surfactant concentration to achieve oil solubilisation, increasing the safety and tolerability of the system. Therefore, Capmul MCM was selected as the oil phase for the development of o/w microemulsions containing MT.

The screening of surfactants. The results of the solubility study involving the surfactants and cosurfactants are also presented in Table II. The surfactant selection is critical for the development of MEs, as it considers the surfactant effectiveness and also their toxicity. The surfactant effectiveness is related to the proper HLB value leading to the spontaneous formation of a stable ME formulation. The toxicity is important because the MEs

formation usually requires large amounts of surfactants, which may cause skin irritation in topical administration. Therefore, it is crucial to select the surfactant with a proper HLB value, but with a minimum necessary concentration in the formulation. Other important criteria for surfactant selection are the drug solubility and solubilisation capacity for oil, respectively. It is not necessarily true that the surfactant with good solubilizing potential for drugs would have equally good affinity for the oil phase [3]. In the present study, several non-ionic surfactants were chosen for screening, because of their stability and low sensitivity to pH changes in presence of electrolytes or charged macromolecules, low toxicity and irritation potential. On the other hand, the selection of surfactant was primarily governed by its solubilisation efficiency for the selected oil phase; its solubility potential for MT was considered an additional advantage.

The results of the solubility study involving the surfactants (Table II) showed that Lansurf SMO 80 has the highest solubilizing potential for MT, followed by Lansurf OA 10, Labrasol and Lansurf OA 14 (1.47 - 2.11 fold lower compared to Lansurf SMO 80). Also, compared to Lansurf SMO 80, the Cremophor EL ability to dissolve MT was 6.2 fold lower. The lowest MT solubility values were measured for Solutol HS 15, Lansurf SML 20 and Cremophor RH 40. However, after the selection of Capmul MCM as oil phase, the surfactant was chosen based on the highest solubilisation capacity for this oil phase. Because the experimental method commonly used to determine the solubilisation

potential of a surfactant for a certain oil could not be applied in the case of Lansurf OA 10 and Lansurf OA 14 because of their low aqueous solubility, only Lansurf SMO 80 and Cremophor EL were tested. The solubility of Capmul MCM in Lansurf SMO 80 and Cremophor EL was 3.32% (w/w) and 36.48% (w/w) respectively. It can be observed that Cremophor EL was a better solubilizer for Capmul MCM than polysorbate 80, which can be attributed to its higher affinity for this monoglycerides, most probably due to the similarity regarding the total number of carbon atoms in the fatty acid chain. Therefore, Cremophor EL was selected as the surfactant for microemulsions development.

The screening of cosurfactants. In the presence of cosurfactants, the area of existence of microemulsion system is expanded, due to further reduction in the interfacial tension and increased fluidity of the interfacial surfactant film [10]. Consequently, ethanol, propylene glycol, tetraglycol and Lauroglycol 90 were selected as cosurfactants. However, based on the results of the solubility study, only ethanol and propyleneglycol were selected for the cosurfactant screening, as they showed the highest solubilizing potential for MT (Table II).

The microemulsion area in the pseudo-ternary phase diagrams was used to assess the emulsification potential of these cosurfactants. Figure 1 presents the pseudo-ternary phase diagrams constructed for Capmul MCM (oil phase), water, Cremophor EL and cosurfactant at a fixed ratio S_{mix} 1:1.

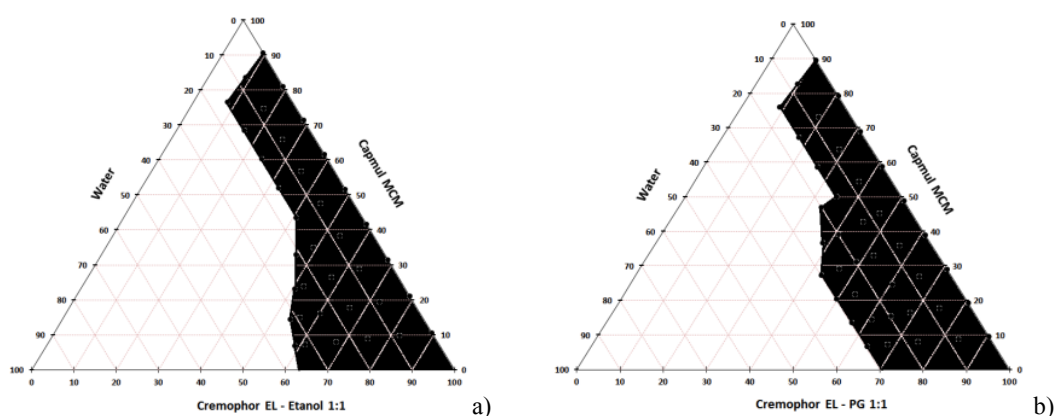


Figure 1.

Pseudo-ternary phase diagrams of systems composed of Capmul MCM, Cremophor EL, water and different cosurfactants (a) ethanol and b) propylene glycol) at S_{mix} 1:1

Comparing the size of the microemulsion region in the phase diagrams obtained at a fixed ratio S_{mix} (1:1), keeping the same surfactant, but replacing the cosurfactant determined a slight enhancement in the microemulsion area from 37.82% to 39.32%, when the chain length and the number of hydroxyl groups

were increased from ethanol (Figure 1a) to propyleneglycol (Figure 1b) respectively. Further, the solubility of MT was 6-fold higher in propyleneglycol than in ethanol. Taken these into consideration and the fact that propyleneglycol is less volatile

than ethanol, the former was selected as co-surfactant for formulating metoprolol tartrate MEs.

The construction of pseudo-ternary phase diagram

In order to determine the appropriate concentration ranges of components (aqueous phase, oil phase, surfactant and cosurfactant) in the regions of forming

microemulsions, the construction of pseudo-ternary phase diagrams was used. Figure 2 presents the pseudo-ternary phase diagrams for Capmul MCM, Cremophor EL, and water systems in the presence of cosurfactant (propyleneglycol) with various weight ratios of S_{mix} .

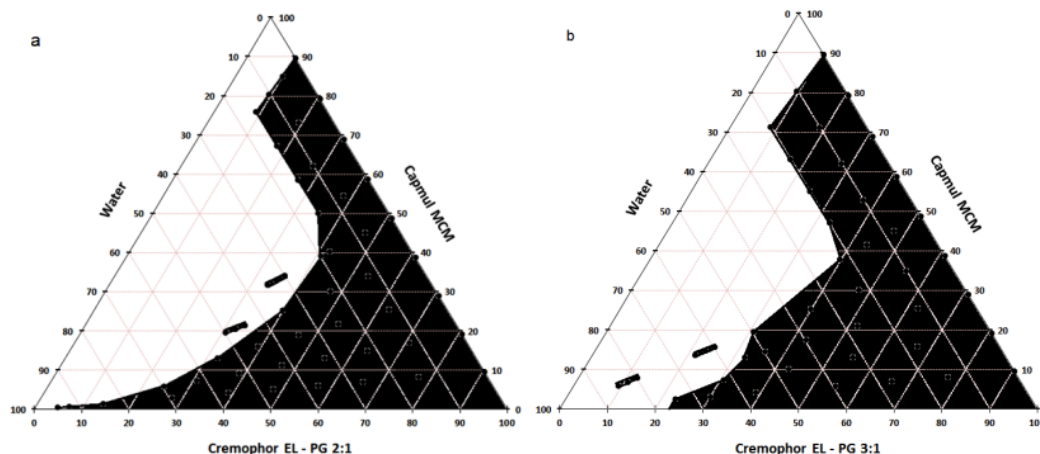


Figure 2.

Pseudo-ternary phase diagrams of systems composed of Capmul MCM (oil phase), Cremophor EL (surfactant), propyleneglycol (cosurfactant) and water at different S_{mix} (a 2:1 and b 3:1)

In the pseudo-ternary phase diagrams, the microemulsion region increased in size from 55.30% to 58.65% with the increasing of surfactant concentration of S_{mix} from 1:1 (Figure 1b) to 2:1 (Figure 2a) and 3:1 (Figure 2b). This increment, more pronounced for the first step, can be attributed to the progressive reduction of the interfacial tension and indicates that the surfactant and cosurfactant weight ratio (S_{mix}) have a marked effect on phase properties (i.e. size and position of microemulsion region). Because a slightly larger microemulsion area was observed in S_{mix} 3:1 as compared to S_{mix} 2:1, the studied formulations were selected from the o/w microemulsion zone of the pseudo-ternary phase diagram constructed at S_{mix} 3:1.

Formulation of metoprolol tartrate microemulsions

For the studied o/w microemulsion formulations, the following selection criteria were considered: i) the amount of drug to be dissolved in the internal oil phase; ii) the oil phase in the selected concentration has to completely dissolve the drug; iii) if possible, for each selected concentration of the oil phase, a minimum concentration of a S_{mix} will be established in order to obtain stable and well tolerated formulations. This last selection criterion has been established also based on the observations reported in the literature according to which, for dermal delivery where enhanced skin permeation is the aim, the maximum flux is usually not obtained with formulations that contain the highest amount of surfactant [3]. The composition of the studied formulations is shown in Table I.

The preparation of microemulsion-based hydrogels of metoprolol tartrate

Different gelling agents namely hydroxypropylmethylcellulose (Methocel K4M), hydroxyethylcellulose (Natrosol 250 HR) and Carbopol ETD 2020 were evaluated for their thickening potential of the MT microemulsions. The selection of the suitable gelling agent was made on the basis of its compatibility with the microemulsions components. It was observed that cellulose derivatives were not able to gel the metoprolol tartrate-loaded microemulsions. This inefficiency can be attributed to their susceptibility to coagulate in the presence of high concentrations of surfactants. It is necessary to remark that in case of metoprolol tartrate hydrogel, without surfactant, the gelation easily occurred in the presence of hydroxypropylmethylcellulose.

In the case of Carbopol ETD 2020 it was noticed that the microemulsions thickening could not be achieved after neutralization, i.e. adding triethanolamine, as it is generally recommended, because the resulting carbomer salt, anionic in nature, is incompatible with metoprolol tartrate, which is a cationic compound. However, a clear gel could be obtained if the neutralization was not performed, as the carbomer in its acidic form is compatible with both the drug and the surfactant in high concentrations.

The characterization of metoprolol tartrate microemulsions

The measured physical characteristics of the developed MT microemulsions are listed in Table III.

Table III

Mean droplet size, polydispersity index, viscosity, refractive index, zeta potential and pH of the metoprolol tartrate microemulsion formulations

Formulation code	Droplet size (nm)	Polydispersity index	Viscosity (mPa)	Refractive index	Zeta potential (mV)	pH
ME MT 1	69.62 ± 0.15	0.490	166.7 ± 1.86	1.4170 ± 0.02	-0.752 ± 0.21	4.76 ± 0.05
ME MT 2	194.0 ± 1.54	0.297	33.0 ± 0.34	1.4278 ± 0.01	-0.988 ± 0.13	4.77 ± 0.01
ME MT 3	136.1 ± 0.56	0.447	33.3 ± 0.69	1.4020 ± 0.07	-1.360 ± 0.25	4.80 ± 0.03
ME MT 4	178.0 ± 0.47	0.295	33.3 ± 0.52	1.4160 ± 0.05	-0.887 ± 0.18	4.75 ± 0.01

The mean droplet size of metoprolol tartrate microemulsions was found in a relatively wide range of values, namely 69.62 ± 0.15 and 194.0 ± 1.54 nm (Table III), which were in most of the cases, higher than the upper limit of the range generally accepted for this type of dispersed systems (10 - 100 nm). However, these formulations can be considered microemulsions, primarily because they are liquid systems that comply with all other basic characteristics defined above for a microemulsion. Secondly, the particle size of the studied MT formulations was less than 200 nm, as it was reported in a previous detailed study reported by Prajapati *et al.*, for the microemulsion phase formed in Capmul MCM/ Cremophor EL/water systems [18]. The formulation ME MT 2 containing 25% Capmul MCM, 45% S_{mix} and 30% water presented the highest average droplet size (194.0 ± 1.4 nm), followed by formulation ME MT 4 (178.0 ± 0.47 nm) having in composition 5% less oil, 5% less S_{mix} and 10% more water than formulation ME MT 2. The mean droplet size was lower when the oil content was 15% and the concentration of S_{mix} was the same or 10% less than that of formulation ME MT 2. Hence, the formulation ME MT 1 containing 15% Capmul MCM, 45% S_{mix} and 40% water presented the lowest average droplet size, while a 2- fold higher mean droplet size was measured for the formulation ME MT 3, having the same oil concentration, but lower S_{mix} concentration (35%) and higher water content (50%). These results showed that the droplet diameter increased with increasing content of oil phase and with decreasing content of S_{mix} , most probably because of the reduction of surfactant effects (namely condensation and stabilization) on the interfacial film formed along with an oil fraction. Due to the small average droplets size of the studied microemulsion systems, especially of the ME MT 1 formulation, their surface area is assumed to be high; therefore, a better contact between the oil droplets and the skin can be accomplished, thus providing high concentration gradient and improved permeation of metoprolol tartrate.

The values of polydispersity index observed for all the formulations (Table III) were relatively high (0.295 to 0.490), indicating the size heterogeneity of the o/w microemulsion droplets and, possibly,

the existence of larger shape heterogeneity. It can also be observed that the polydispersity values measured for the ME MT 1 and ME MT 3 formulations (which presented lower mean droplet size) were 1.5 - 1.6 fold higher than those obtained for the formulations ME MT 2 and ME MT 4, containing increased size droplets. These results can be attributed to formulation variables, namely the content in oil phase, S_{mix} and water, influencing the particularly mechanism of microemulsion formation in which may both the surfactant (Cremophor EL), but also the lipid (Capmul MCM) participate, as both species are surface active in nature and differ in regard of HLB values (Capmul MCM has HLB 4.7, being less hydrophilic than Cremophor EL with HLB 13) [18].

The viscosity of formulations ME MT 2, ME MT 3 and ME MT 4 was similar (Table III), while the formulation ME MT 1 was 5 fold more viscous, most probably due to the lower mean droplet size and the content in oil phase, S_{mix} and water. Moreover, the viscosity of all formulations was low, which is expected, as one of the properties of microemulsions is the low viscosity.

The refractive index indicates the isotropy of the microemulsions. The values of refractive index ranged between 1.4020 ± 0.07 and 1.4278 ± 0.01 (Table III). As water content was increased from 30% (formulation ME MT 2) to 50% (formulation ME MT 3), the refractive index decrease from 1.4278 to 1.4020 due to the lower refractive index of water compared with that of other components of the formulations, i.e. oil or S_{mix} .

The zeta potential values of the studied microemulsion formulations were negative in the range of -0.752 ± 0.21 to -1.360 ± 0.25 mV (Table III). These small values revealed the stability of systems, as the droplets aggregation is not expected to take place, due to the presence of Cremophor EL which acts through sterical stabilization. Moreover, it is known that ethoxylated surfactants give slightly negative zeta potential value, which is assigned to ions adsorption. Also, the negative zeta potential values can help improve the MT skin permeation after topical administration of the microemulsions, due to electrostatic repulsion between the oil droplets surface charge and the same negative skin surface charge.

The pH values of all microemulsion formulations were found in the range of 4.76 ± 0.05 to 4.80 ± 0.03 (Table III), being very close to the natural skin surface pH.

The characterization of metoprolol tartrate hydrogel and microemulsion-loaded hydrogels

The determination of drug content and pH. The MT content of microemulsion-loaded hydrogels and

traditional hydrogel (Table IV) ranged from $96.46 \pm 0.25\%$ to $103.12 \pm 0.44\%$ and $100.75 \pm 0.61\%$ respectively of the theoretical value (3%, w/w), which complies with the pharmacopoeial specifications for drug content. The obtained data revealed the uniform distribution of the drug within all studied hydrogels.

Table IV

Drug content, pH, viscosity and the penetration (consistency) of the metoprolol tartrate hydrogel formulations

Formulation code	Drug content (%)	pH	Viscosity (Pas)	Penetration value (mm)
ME GEL MT 1	96.46 ± 0.25	4.59 ± 0.07	4.25 ± 0.22	180.0 ± 0.82
ME GEL MT 2	103.12 ± 0.44	4.55 ± 0.09	2.25 ± 0.36	247.3 ± 1.69
ME GEL MT 3	98.81 ± 0.73	4.58 ± 0.01	2.73 ± 0.15	297.0 ± 5.71
ME GEL MT 4	99.64 ± 0.35	4.56 ± 0.03	1.87 ± 0.39	231.3 ± 1.89
GEL MT	100.75 ± 0.61	5.89 ± 0.02	1.45 ± 0.12	267.7 ± 4.23

The developed microemulsion-loaded hydrogels had pH values varying from 4.55 ± 0.09 to 4.59 ± 0.07 , slightly lower than those of the MT microemulsion formulations. This decrease of the pH can be attributed to the presence of the gelling agent Carbopol EDT 2020, a compound with acidic character. On the other hand, the pH value of the MT conventional hydrogel was 5.89 ± 0.02 , hence higher than those of the ME GEL MT formulations, because it was obtained by using HPMC as gelling agent.

Rheological characterization. As shown in Table IV, the viscosity values of the MT microemulsion-loaded hydrogels were in the range from 1.87 ± 0.39 Pas to 4.25 ± 0.22 Pas, the differences possibly being determined by the changes in oil, S_{mix} and water concentrations of the formulations. Thus, the formulation ME MT 1 was the most viscous and, compared to ME MT 3 formulation (with the same oil content, but 10% less S_{mix} and 10% more water), its viscosity value was 1.56 fold higher, which reveals the influence of the other two components. The effect of decreasing viscosity with increasing oil content was more evident in the case of ME GEL MT 2 and ME GEL MT 4 formulations (containing 25% and respectively 20% oil), which showed the lowest viscosity values, despite their high S_{mix} content. It was also observed, that the viscosities of all ME GEL MT formulations increased significantly compared with those of the micro-emulsions, due to the addition of 1.5% Carbopol EDT 2020, which made the preparations more suitable for topical administration.

The formulations ME GEL MT 2, 3 and 4 presented higher penetration values, indicating a lower consistency; contrastingly, formulation ME GEL MT 1 had the lowest penetration value, therefore the highest consistency (Table IV).

The conventional MT hydrogel presented a low viscosity value (1.45 ± 0.12 Pas), close to that of

ME GEL MT 4 formulation and consequently, a similar penetration level (267.7 ± 4.23 mm).

The spreadability is a very important property of topical semisolid formulations since it indicates the facility of applying the formulations on the skin or mucosa. The results of the spreadability measurements are depicted as extensiometric curves in Figure 3. It was found that the highest spreading area was obtained for ME GEL MT 3, followed by that of the formulations GEL MT and ME GEL MT 4. The other two ME GEL formulations showed lower spreadability, indicated by the significantly lower spreading areas (Figure 3).

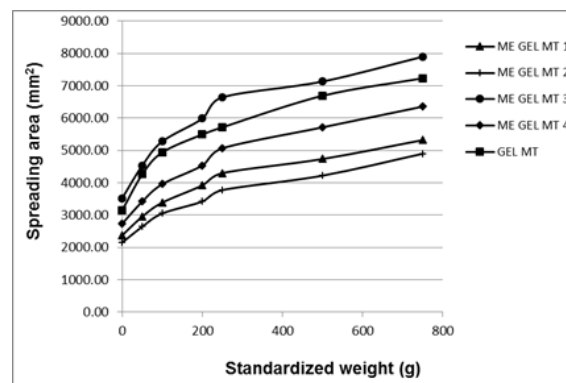


Figure 3.

Extensiometric curves of the studied MT hydrogel formulations

However, all formulations presented good spreadability, proved by relatively high values of the spreading areas. The differences between the studied systems in terms of consistency, expressed as penetration value and spreadability, were most likely due to formulation variables, namely the concentration of oil, S_{mix} and water, which modifies the gelling potential of Carbopol EDT 2020. Thus, high concentrations of oil and S_{mix} and consequently low water content, loosened the gel matrix nature of microemulsion based hydrogel formulations (case

of formulations 2, 3 and 4), while in the presence of low oil content and increased water concentration, the gelling ability of carbomer was improved (case of formulation ME GEL MT 1).

Ex vivo drug release studies. The *ex vivo* drug permeation through skin is an alternative technique to *in vivo* studies in humans for evaluating the bioavailability of a newly developed topical formulation. In the present study, the skin permeation experiments used excised pig ear skin, which is extensively used as alternative model to human skin, due to the lack of availability of the last one. The results of the *ex vivo* permeation and release of metoprolol tartrate through porcine ear skin are illustrated in Figure 4 and summarized in Table V.

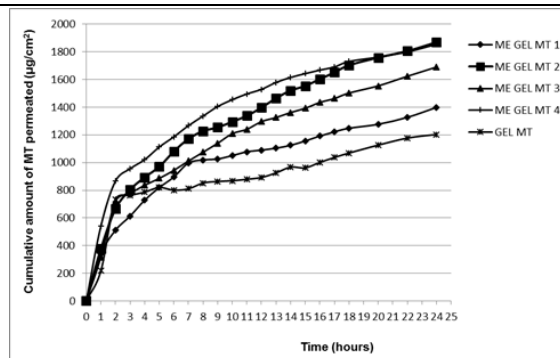


Figure 4.

Ex vivo metoprolol tartrate permeation profiles through pig ear skin from microemulsion-based hydrogels and conventional hydrogel

Table V

The permeation and the release parameters of the MT-loaded formulations (microemulsion-loaded hydrogels and hydrogel) through pig ear skin^a

Formulation code	Permeation parameters			Release parameters	
	J_s ($\mu\text{g}/\text{cm}^2/\text{h}$)	$K_p \times 10^{-6}$ (cm/h)	t_L (h)	k ($\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$)	$D \times 10^{-5}$ (cm^2/h)
ME GEL MT 1	105.38 ± 4.35 (1 - 7 h)	35.13	0.138	391.80 ± 8.28	13.39
	23.21 ± 7.31 (7 - 24 h)	7.74	-	191.37 ± 6.27	3.19
ME GEL MT 2	111.81 ± 4.64 (1 - 8 h)	37.27	0.055	455.34 ± 8.42	18.08
	42.81 ± 4.09 (8 - 24 h)	14.27	-	332.47 ± 9.12	9.64
ME GEL MT 3	360.04 ± 6.73 (0 - 2 h)	120.01	0.039	473.70 ± 15.42	19.60
	43.64 ± 3.54 (3 - 24 h)	14.55	-	290.49 ± 16.04	7.36
ME GEL MT 4	433.19 ± 19.85 (0 - 2 h)	144.40	0.022	599.35 ± 15.28	31.31
	50.92 ± 2.34 (3 - 18 h)	16.97	-	316.08 ± 12.605	8.71
GEL MT	366.99 ± 11.85 (0 - 2 h)	122.33	0.135	463.44 ± 12.57	18.73
	21.14 ± 6.28 (2 - 24 h)	7.05	-	132.02 ± 8.15	1.52

^a J_s : steady-state flux; K_p : permeability coefficient; t_L : lag time; k : release rate; D : diffusion coefficient

The permeation of metoprolol tartrate through excised pig ear skin over 24 hour period (Figure 5) presented a biphasic profile. The initial phase was faster for all studied formulations, but its length varied significantly from two hours (ME GEL MT 3, ME GEL MT 4 and GEL MT formulations) to 7 - 8 hours (ME GEL MT 1 and ME GEL MT 2 formulations). The flux values calculated for this initial phase (Table V) indicated a 3.2 to 3.9 fold improvement in metoprolol tartrate permeation through pig ear skin achieved with formulations containing 15% or 20% oil, 35% or 40% S_{mix} and 47% or 37% water (ME GEL MT 3 and ME GEL MT 4) and conventional hydrogel (without oil and S_{mix}) in comparison to the ME GEL MT 1 and ME GEL MT 2 formulations with higher S_{mix} content and the same or lower water concentration. This improvement can be attributed to increased MT thermodynamic activity with the decrease in S_{mix} content, and consequently to the increased fraction of solubilised MT in external aqueous phase, which was the determinant factor of the initial faster phase of MT skin transfer.

The skin permeation of MT in the second phase was slower and longer than the first phase for all

studied hydrogels and might be related to the MT release from the oil droplets of the microemulsion and to the MT diffusion through the gelling polymer network. Comparing the obtained flux values (Table V), it was found that the formulation ME GEL MT 4 provided the highest rate of permeation ($50.92 \pm 2.34 \mu\text{g}/\text{cm}^2/\text{h}$), followed closely by ME GEL MT 2 and ME GEL MT 3 ($42.81 \pm 4.09 \mu\text{g}/\text{cm}^2/\text{h}$ and $43.64 \pm 3.54 \mu\text{g}/\text{cm}^2/\text{h}$). The lowest flux values, ranged between $21.14 \pm 6.28 \mu\text{g}/\text{cm}^2/\text{h}$ and $23.21 \pm 7.31 \mu\text{g}/\text{cm}^2/\text{h}$, produced the ME GEL MT 1 and the conventional hydrogel.

These results were consistent with previous reports suggesting that the skin permeation rate of a hydrophilic drug from an oil-in-water microemulsion is more pronounced with higher water content, which influences the internal structure of the microemulsion and improves the hydration of the skin [12]. It must be also considered the effects of the type and concentration of oil phase and surfactant on the system internal structure, as well as on the skin barrier, acting as penetration enhancers [11]. In the present case, not only the surfactant (Cremophor EL) and cosurfactant (propyleneglycol) have the penetration enhancing effect, but also the oil phase

Capmul MCM, which has been shown to be an effective permeation enhancer. As it was already highlighted, the oil phase-surfactant ratio is of great importance for the formation of o/w microemulsions containing Capmul MCM/Cremophor EL/water. In such systems loaded with a hydrophilic drug, increased Capmul MCM concentration improves the microemulsion loading capacity (through drug solubilization) and skin permeability. Contrastingly, increased Cremophor EL concentrations (above 40%) produced the decrease of MT skin permeation by improving the packing of interfacial layer, thus affecting the microemulsion ability to release the drug. Furthermore, the contribution of the systems viscosity on the MT skin permeation should not be underestimated, since the increased values of this parameter are generally correlated with decreased values of permeation parameters. Thus, the values of MT skin permeation rates were in accordance with this assumption in all four cases of ME GEL formulations. In turn, the conventional hydrogel produced the lowest flux value, although it was the less viscous (about 4-fold lower than ME GEL MT 1 with a similar flux value), which demonstrate once more the microemulsion enhancing effect on the MT percutaneous penetration.

The calculated release rates of MT for all ME GELs were found to range between $191.37 \pm 6.27 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$ (for ME GEL MT 1) and $332.47 \pm 9.12 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$

(for ME GEL MT 2), being considerably higher than that of the corresponding fluxes, as it can be observed (Table V). The lowest release rate of MT was produced by the conventional hydrogel ($132.02 \pm 8.15 \mu\text{g}/\text{cm}^2/\text{h}^{1/2}$). Ranking the studied formulations according to this release parameter, a similar hierarchy with that based on flux values was obtained, indicating once more that apart from the contribution of water content in enhancing MT release, the varying oil and surfactant content might be responsible for improved drug release.

From Table V, it can be observed that in all cases the lag time values vary as expected, namely longer lag time values in case of slow diffusion. Correlating these results with the values of flux and release rate, it is clear that the lag time (a permeation parameter) of the studied formulations depends not only on the diffusivity of the drug through the membrane, but also on the drug release, both phenomenon being influenced by the systems composition and their viscosity.

Data obtained from the *ex vivo* MT permeation of all hydrogel formulations through pig ear skin, were fitted to various mathematical models like zero-order, first-order, Higuchi and Korsmeyer-Peppas model. The results of curve fitting into above mentioned mathematical models are presented in Table VI and were correlated with the value of the highest determination coefficient.

Table VI

Results of kinetic analysis of the *ex vivo* permeation data through pig ear skin obtained for 3% metoprolol tartrate loaded hydrogels^a

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0 ($\mu\text{g}/\text{h}$)	R^2	K_1 (h^{-1})	R^2	K_H ($\text{h}^{-0.5}$)	R^2	K_P (h^{-n})	n	R^2
ME GEL MT 1	0.8536	0.8019	0.0103	0.8354	5.9836	0.9265	0.8579	0.4522	0.9691
ME GEL MT 2	1.2382	0.8771	0.0160	0.9179	7.7930	0.9827	0.9261	0.4825	0.9747
ME GEL MT 3	1.091	0.8614	0.0137	0.9011	7.0925	0.9669	0.9029	0.4691	0.9218
ME GEL MT 4	1.1314	0.7982	0.0148	0.8494	8.3118	0.9066	1.0652	0.3839	0.9793
GEL MT	0.6201	0.6892	0.0072	0.7246	5.0654	0.7368	0.8603	0.3803	0.6388

^a K_0 : zero order release constant; K_1 : first order rate constant; K_H : Higuchi release constant; K_P : Korsmeyer-Peppas release rate constant; n : diffusion coefficient in Korsmeyer-Peppas model; R^2 : determination coefficient

ME GEL MT 1 and ME GEL MT 4 followed the Korsmeyer-Peppas model ($R^2 > 0.96$), whereas ME GEL MT 2 and ME GEL MT 3 followed the Higuchi model ($R^2 > 0.96$) over a period of 24 hours. Moreover, in the case of the first group of formulations (fitting to Korsmeyer-Peppas model), the analysis of the first 60% of drug release data using this model was performed to determine the values of diffusion exponent (n), an indicative of drug release mechanism: Fickian diffusion when $n \leq 0.5$, non-Fickian transport when $0.45 < n < 0.89$, case II transport when $n = 0.89$, and super case II transport when $n > 0.89$. As the calculated values of diffusion exponent, n , were 0.3839 and 0.4522 (Table VI), it can be suggested the MT permeation from the

respective systems followed the Fickian (nonsteady) diffusional mechanism.

Conclusions

In this study, four microemulsion-loaded hydrogel formulations, as potential carriers for topical delivery of metoprolol tartrate, were prepared and physico-chemically characterized, and their *ex vivo* drug release properties through porcine skin were also evaluated. The obtained results suggested the important effect of oil, S_{mix} and water, as microemulsion-loaded hydrogel components, on the physical, rheological and *ex vivo* drug release characteristics of the studied formulations. Differences between the formulations in terms of *ex vivo* MT

permeation through porcine skin could be attributed to the drug solubility and its partition into different phases (Capmul MCM, Cremophor EL/propyleneglycol and water) of the microemulsion-loaded hydrogel, in correlation with their various proportions in the system.

Based on the results, our conclusion is that the best microemulsion-loaded hydrogel formulation for topical delivery of metoprolol tartrate is ME GEL MT 4, containing 20% Capmul MCM as oily phase, 40% S_{mix} (3:1) Cremophor EL-propyleneglycol as surfactant-cosurfactant, 1.5% Carbopol EDT 2020 as gelling agent, and 37% water.

However, further *in vitro*, *ex vivo* and *in vivo* studies are necessary in order to establish the stability, safety and therapeutic efficacy of the new developed metoprolol tartrate loaded microemulsion-hydrogel, as potential topical drug delivery system.

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