ORIGINAL ARTICLE

LORATADINE-LOADED MICROEMULSIONS FOR TOPICAL APPLICATION. FORMULATION, PHYSICOCHEMICAL CHARACTERIZATION AND *IN VITRO* DRUG RELEASE EVALUATION

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Abstract

The aim of this study was to develop and evaluate several microemulsion (ME) formulations as topical delivery systems for loratadine (LRT), a second-generation H1 antihistaminic drug, used for allergic skin manifestations treatment. The solubility of LRT in different oils, non-ionic surfactants and cosurfactants was determined to select the ME components. Establishment of pseudoternary phase diagrams, using a relatively new method (Phase Diagram by Micro Plate Dilution) for several systems, including the Captex 355/Cremophor Rh 40-Capryol 90/water system, was used to select the studied ME and gel-ME. The selected LRT-loaded ME were characterized for physicochemical properties and *in vitro* drug release through synthetic membrane. The results showed great impact of the ME components and their proportions on the mentioned characteristics. Three of the assessed ME, presenting permeation profiles best fitted with Korsmeyer-Peppas model, were suggested to be firstly evaluated for the *in vitro* drug release through a biological membrane model.

Rezumat

Scopul acestui studiu a fost de a dezvolta şi evalua mai multe formulări de microemulsie (ME) ca sisteme farmaceutice pentru administrarea locală a loratadinei (LRT), un antihistaminic H1 de a doua generaţie, utilizat pentru tratamentul manifestărilor alergice ale pielii. Solubilitatea LRT în diferite uleiuri, surfactanţi neionici şi cosurfactanţi a fost determinată pentru a selecta componentele ME. Stabilirea diagramelor pseudoternare de faze, utilizând o metodă relativ nouă (metoda diluţiei în microplacă) pentru mai multe sisteme, inclusiv sistemul Captex 355/Cremophor Rh 40-Capryol 90/apă, a fost utilizată pentru a selecta ME şi gel-ME studiate. ME cu LRT selectate au fost caracterizate în ceea ce priveşte proprietăţile fizico-chimice şi eliberarea *in vitro* a substanţei medicamentoase prin membrană sintetică. Rezultatele au arătat un impact mare al componentelor ME şi proporţiilor acestora asupra caracteristicilor menţionate. Trei dintre ME evaluate, prezentând profilurile de permeaţie care au fost fitate cel mai bine cu modelul Korsmeyer-Peppas, au fost propuse a fi primele evaluate privind eliberarea *in vitro* a substanţei medicamentoase printr-un model de membrană biologică.

Keywords: microemulsions, topical application, loratadine, pseudoternary phase diagrams, *in vitro* drug release

Introduction

In the last decade, the prevalence of allergic diseases, including asthma, rhinitis and skin allergies has increased worldwide in a great extent, in both developed and developing countries, with a greater burden in children. This increase is of great concern for the World Allergy Organization, which regards the allergic diseases as a major public healthcare problem [26]. The most common manifestations of skin allergies to foods, drugs or other allergens are eczema (commonly in form of atopic dermatitis), urticaria and angioedema. Referring to skin allergies, the data reported in the latest edition of White Book on Allergy, indicate the following aspects: i) their incidence increased by 2 - 3 fold, especially in industrialized countries in the last decades; ii) atopic dermatitis has a higher lifetime prevalence in children (15 - 30%) than in adults (2 - 10%), while the life-time prevalence of urticaria is above 20%. Thus, this publication outlines the great impact of skin allergies on the quality of life and their socio-economic burden [26]. In order to control the symptoms of allergic skin diseases and to improve the patient's quality of life, pharmacotherapy is the main approach. In modern pharmacologic treatment of urticaria and angioedema, H1-antihistaminic drugs (e.g. cetirizine, loratadine

and desloratadine) are used as first-line therapy, due to their effectiveness and safety [5, 10, 18].

Loratadine (LRT), a tricyclic piperidine derivative of the second-generation H1- antihistamines, intended for the treatment of urticaria, angioedema and other allergic skin manifestations, is currently administrated by oral route, although its oral bioavailability is poor and produces various adverse effects [11]. Consequently, in the treatment of skin disorders characterized by localized allergic reactions, the dermal route is more suitable for drug delivery than the oral one. LRT is a good candidate for dermal delivery, due to its low molecular weight (382.88 Da) and high lipophilicity (log P 5.2). Previously published data, showed that skin loratadine concentration is correlated to drug potency in inhibiting the clinical signs of urticaria and other allergic skin disorders [21]. However, the poor skin penetration and low water-solubility of LRT limit its topical application. To address these limitations, the effects of various penetration enhancers incorporated in the formulation of some hydrogels and emulgels were investigated in the recent years [6, 12, 22].

Another modern strategy to overcome the skin barrier is the use of new drug delivery systems, containing a colloidal phase as drug carrier, including micro-particulated systems (microemulsions) and nano-particulated systems (liposomes, nanoparticles, micelles, mixtes micelles and nanoemulsions).

Among them, microemulsions, as second-generation of colloidal carriers, have been extensively investigated for this purpose [9, 15, 20, 23, 24], due to their advantageous characteristics, including spontaneous formation and consequently facile preparation, excellent physical stability, high solubilisation potential for both hydrophilic and lipophilic substances and the ability to enhance the skin penetration of drugs [13, 14]. However, the microemulsions fluidity limit their residence time to the skin surface when used as dermal delivery systems, a disadvantage that can be averted by increasing their viscosity, using gelling agents, which will not significantly affect the drug diffusion from the obtained microemulsion gels.

The aim of the present work was to investigate the possibility of developing pharmaceutical oil in water (o/w) microemulsion and gel-microemulsion formulations for topical delivery of loratadine, by appropriate selection of their components, namely the oil phase, aqueous phase, surfactant and cosurfactant. The physicochemical properties and *in vitro* drug release of the developed loratadine-loaded micro-emulsions were also evaluated.

Materials and Methods

Materials

Loratadine was kindly donated by S.C. Laropharm (Romania). Solutol HS 15 (macrogol 15 hydroxy-stearate), Cremophor RH 40 (PEG-40 hydrogenated Castor Oil) and isopropyl myristate (BASF Chem Trade GMBH, Germany), Lauroglycol 90 (propylene-glycol monolaurate), Capryol 90 (propyleneglycol monocaprylate) and Labrasol (caprylocaproyl macrogol-8 glycerides) (Gattefossé, France), Lansurf SML 20 and Lansurf SMO 80 (polyoxyethylene (20) sorbitan monolaurate and monooleate respectively), Lansurf OA14 (macrogol 600 monooleate) and Lansurf CO12 (castor oil 12 ethoxylate) (Lankem Ltd., UK), Captex 355 (caprylic/capric triglyceride), Captex 500 (triacetin) and Caprol MPGO (polyglyceryl-3 oleate and polyglyceryl-10 mono/dioleate) (Abitec Corp., USA) and Tagat S2 (PEG-20 glycerolstearate) (Evonik Industries AG Personal Care, Germany) were received as gift samples. Castor oil was supplied by S&D Chemicals (India). Ethanol, isopropylic alcohol, sodium chloride, potassium chloride, disodium phosphate and monopotassium phosphate were acquired from Chimopar, Romania, tetraglycol from Merck Schuchardt OHG, Germany and methyl p-hydroxybenzoate and propyl p-hydroxybenzoate were purchased from Stera Chemicals, Romania. All chemicals and reagents were of pharmaceutical or analytical grade and were used without further purification. Tuffryn HT synthetic hydrophilic membranes of polysulfone (0.45 μm, 25 mm) were supplied by Pall Corporation (USA). Double-distilled water was used throughout the study.

Methods

Solubility studies. The solubility of loratadine in different oils (Captex 355, Captex 500, isopropyl myristate and castor oil), various surfactants (Solutol HS 15, Cremophor RH 40, Lansurf SML 20, Lansurf SMO 80, Lansurf OA 14, Lansurf CO 12, Caprol MPGO, Labrasol Tagat S2) and six cosurfactants (96% ethanol, isopropanol, tetraglycol, propylene glycol, Capryol 90 and Lauroglycol 90) was determined by the conventional saturation shake-flask method, according to the following procedure: an excess amount of loratadine was added to each glass flask containing 5 g of the selected vehicle (oil, surfactant or cosurfactant). After closing, the flask was vigorously stirred for 10 minutes to facilitate proper dispersion of loratadine in the vehicle. Further, the mixtures were stirred for 98 hours at 25 ± 1ºC, then, due to the increased viscosity of some of the vehicles, the obtained fluid suspensions were centrifuged for 15 minutes at 12,000 rpm; the supernatant liquid was filtered through a filter membrane (0.45 μm, 25 mm, Teknokroma, Germany). The aliquots of each super-natant liquid were appropriately diluted with methanol and the LRT concentration in the sample was measured by UV spectrophotometry (T70+ spectrophotometer, PG Instruments, UK) at the wavelength of 250 nm. Three separate shake-flask measurements were performed in parallel for each reported solubility data.

Selection of formulation components

Selection of oil. In order to develop some LRT microemulsions, the oil phase was selected based on the maximum solubilisation capacity of the drug.

Selection of surfactant. The surfactant was selected based on its solubilisation capacity of LRT and oil phase (Captex 355). After conducting the solubility studies, two surfactants were screened, namely Solutol HS 15 and Cremophor RH 40.

The solubilisation capacity of the surfactants for oil phase was determined using the method described previously in literature [2, 3]. This method consists in adding aliquots of 5 µL of oil (Captex 355) to 2.5 mL of 15% (w/w) aqueous surfactant solution under vigorous stirring. If the obtained solution was clear, oil was added until the liquid became opalescent. The oil solubility in water in the presence of the surfactant was considered the total amount of the added oil until the solution becomes cloudy.

Selection of cosurfactant. The maximum area of the microemulsion region in the pseudoternary phase diagrams was considered the criterion for the cosurfactant selection. For this purpose, Cremophor RH 40 was mixed with two of the six cosurfactants investigated in the solubility studies, namely 96% ethanol and Capryol 90. Pseudoternary phase diagrams for the systems containing water, Captex 355 and surfactant-cosurfactant mixture (S_{mix}) at a set ratio 1:1 were constructed. The mixtures of oil and S_{mix} were prepared in nine weight ratios, namely 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, thus the maximum proportions were delineated to delimit the phase boundaries formed in the pseudoternary phase diagram.

Construction of pseudoternary phase diagrams

To determine the concentration of the components (oil phase, aqueous phase and surfactant/cosurfactant mixture) corresponding to the microemulsion region, pseudoternary phase diagrams were constructed. The surfactant (Cremophor RH 40) and the cosurfactant (Capryol 90) were mixed in three weight ratios (1:1, 2:1 and 3:1), chosen in ascending order of the surfactant concentration *versus* the cosurfactant and *vice versa*, for a detailed phase diagram study. Various mixtures of oil and S_{mix} were prepared in the following weight ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1.

Pseudoternary phase diagrams were constructed using a relatively new method, microplate dilution method, based on the conventional water titration method. Creating pseudoternary phase diagrams using the microplate dilution method has been recently described by Schmidts T. *et al.* [19] and has the advantage of saving time and materials. In the pseudoternary phase diagram, the microemulsion phase was designated by the region where the systems were clear, transparent and fluid.

Preparation of loratadine-loaded microemulsion and gel-microemulsion formulations

Six formulations, containing various oil proportions, S_{mix} and water, were chosen from the microemulsion and gel-microemulsion region of the pseudoternary phase diagram. The composition of the selected loratadine-loaded microemulsion (ME LRT) and loratadine-loaded gel-microemulsion (G-ME LRT) formulations is presented in Table I. LRT was dissolved in the mixture of Captex 355, Cremophor RH 40 and Capryol 90, under stirring. The appropriate amount of aqueous phase (parabens solution) was added dropwise and under continuous stirring to the resulting solution. The final concentration of LRT in microemulsion formulations was 0.5% (w/w). All prepared microemulsions were stored 24 hours at room temperature for equilibration before performing further tests.

Table I

The composition (g%) of loratadine-loaded microemulsion (ME) and gel-microemulsion (G-ME) formulations

Microemulsion components	Weight (%) and formulation codes					
	ME LRT 1	**ME LRT 2**	**ME LRT 3**	**G-ME LRT 4**	**ME LRT 5**	**G-ME LRT 6**
Loratadine	0.5	0.5	0.5	0.5	0.5	0.5
Captex 355	10.0	20.0	20.0	15.0	30.0	15.0
Cremophor RH 40-Capryol 90 (3:1)	70.0	60.0	50.0	50.0	50.0	40.0
Methylparaben	0.015	0.015	0.022	0.026	0.015	0.033
Propylparaben	0.005	0.005	0.008	0.009	0.005	0.011
Distilled water	19.48	19.48	29.47	34.47	19.48	44.46

Characterization of the loratadine-loaded microemulsions

The obtained microemulsions and gel-microemulsions containing 0.5% loratadine were characterized in regard to different physicochemical properties.

The *mean droplet size*, *polydispersity index (PDI)* and *zeta potential* of the LRT-loaded microemulsions and gel-microemulsions were measured in triplicate by photonic correlation spectroscopy using the Zetasizer Nano-ZS apparatus (Malvern Instruments, UK). Measurements were carried out at a fixed angle of 173° at 25°C. Microemulsion samples were diluted in a ratio of 1:5 with ultrapure water delivered by a Simplicity UV Water Purification System (Millipore SAS, France).

The pH of the experimental microemulsions was measured at 25 ± 2°C using a pH-meter (Sension™),

Hach Company, USA). Experiments were performed in triplicate for each sample.

The *loratadine content* of the studied microemulsions and gel-microemulsions was determined by accurately weighting 0.4 g of the microemulsion sample and dispersing it in 25 mL volumetric flask containing 0.1 M aqueous hydrochloric acid. The obtained dispersion was then filtered using a filter membrane (0.45 µm). 1 mL of the filtrate was suitably diluted with a 0.1 M HCl solution and analysed spectrophotometrically at 280 nm.

Rheological characterization consisted in viscosity and consistency measurements. The viscosity of the experimental ME formulations was determined at 25 ± 2°C, using a rotational viscosimeter equipped with SC4-25 and SC4-28 spindles (Brookfield DV-I+, UK). 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]. In addition, samples spreadability, a characteristic nearly related to consistency, was carried out by parallel-plate method using the Pozo Ojeda-Sune Arbussa extensometer [16]. All rheological measurements were performed in triplicate at 25ºC.

In vitro loratadine release studies. Drug release studies were performed on a six Franz diffusion cells system (Microette-Hanson system, 57-6AS9 model, Hanson, USA), using polysulfone hydrophilic synthetic membranes (HT Tuffryn membrane, Pall Corporation, USA). The diffusional area and the volume of the receptor compartment of the Franz diffusion cells were 1.767 cm^2 and 6.5 mL respectively. To ensure sink conditions throughout the test, the receptor chambers of the Franz diffusion cells were filled with freshly prepared phosphate buffered saline solution at pH 7.4 containing 30% ethanol (w/w). The synthetic membranes were firstly soaked for 30 minutes in the receptor medium and then mounted between the donor and receptor compartments of the Franz diffusion cells. Approximately 0.300 g of sample was weighed into the dosing capsule of each diffusion cell.

Throughout the experiment, the system was maintained at 32 ± 0.5ºC (to simulate existing *in vivo* conditions) and the receptor medium was constantly stirred at 600 rpm. At 1, 2, 3, 4, 5, 6, 7 and 8 h, 0.5 mL of receptor fluid were automatically withdrawn and replaced with an equal volume of fresh receptor medium to maintain a constant volume. The collected samples were analysed spectrophotometrically at the wavelength of 275 nm. The test was linear in the range of LRT concentrations of 5.6 - 56 µg/mL ($y = 0.1864x$, $R^2 = 0.9987$). The *in vitro* drug release experiments were conducted in triplicate and the data were expressed as mean ± SD.

Data analysis of the in vitro drug release studies. Steady-state flux (J_{ss}, µg/cm^2/h) and lag time (t_L, h) were calculated from the slope and respectively the *x*-axis intercept of the plots of the cumulative amount of drug (µg/cm^2) permeated *versus* time. Permeability coefficient (Kp, cm/h) was calculated by dividing the flux by the initial concentration of drug in the donor compartment. The release rate (k) values were calculated using the pseudo-steady-state slopes from the plots of the cumulative amount of LRT permeated through membrane (µg/cm^2) *vs.* square root of time. Diffusion coefficient (D) values were calculated from the release rate values.

To study the kinetic of LRT release from the experimental microemulsion formulations, four kinetic mathematical models, namely zero order model (cumulative amount of drug released *vs.* time), first order model (log cumulative percentage of drug remaining *vs.* time), Higuchi model (cumulative percentage of drug released *vs.* square root of time) and Korsmeyer-Peppas (log cumulative percentage of drug released *vs.* log time) were selected to process the *in vitro* drug release data. The goodness of fit for each model was expressed as the highest values of the determination coefficient.

Statistical analysis

The statistical analysis of the experimental data was carried out using Statistica software (version 7.0). The data were presented as mean ± standard deviation (SD) and the differences between the formulations were considered statistically significant when values of $p < 0.05$. In the graphs, ± SD are not presented for the graph clarity reason.

Results and Discussion

Selection of formulation components

Selection of oil. The solubility of LRT in different oils is indicated in Table II.

Table II

The solubility of LRT in oils, surfactants and cosurfactants at 25 ± 2°C

Component	Solubility (mg/mL)
Captex 355	463.55 ± 2.68
Captex 500	33.45 ± 0.21
Isopropyl myristate	13.56 ± 0.42
Castor oil	41.66 ± 0.85
Solutol HS 15	216.55 ± 3.04
Cremophor RH 40	366.27 ± 2.52
Tagat S2	171.72 ± 1.13
Caprol MPGO	145.07 ± 0.91
Lansurf SML 20	40.86 ± 1.37
Lansurf SMO 80	106.07 ± 2.15
Lansurf OA 14	45.17 ± 0.62
Lansurf CO 12	90.51 ± 1.28
Labrasol	47.62 ± 0.84
Ethanol	105.74 ± 1.35
Isopropanol	70.21 ± 0.93
Propyleneglycol	20.39 ± 0.72
Tetraglycol	29.78 ± 0.54
Lauroglycol 90	142.62 ± 1.83
Capryol 90	180.68 ± 1.52

Loratadine exhibited the highest solubility in Captex 355, whereas in castor oil, Captex 500 and isopropyl myristate its solubility was much lower (11.13 fold, 13.86 fold respectively 34.19 fold). This can be assigned to the more pronounced hydrophobic character, consequently to a higher lipophilic nature of Captex 355 compared to Captex 500, due to an increased content in capric acid (C10) (22 - 45%). Compared to castor oil and isopropyl myristate, the higher solubilisation capacity of Captex 355 can be attributed to its higher fluidity and shorter C-chain fatty acid residues in its composition [4]. Moreover, medium chain triglycerides are more easily emulsified than long chain ones [8] and formulating a micro-emulsion using an oil in which the drug has a high solubility, will require a smaller amount of oil to dissolve the desired drug amount, which further leads to the use of the surfactant in a lower concentration for the oil phase solubilisation, thus increasing the safety and tolerability of the formulation. Therefore, Captex 355 was selected as the oil phase for the development of oil-in-water loratadine microemulsions.

Selection of surfactant. In the formulation of the topical pharmaceutical microemulsions, which require high concentration of surfactant and/or cosurfactant, the surfactant selection is challenging, because the following criteria are to be considered: i) its ability to stabilize the microemulsion, in correlation to an appropriate HLB value; ii) good tolerability, associated with low toxicity and low skin irritation potential; iii) high solubilisation ability for the drug and the oil phase; iv) pharmaceutical acceptability [3].

Among the surfactants used in the solubility study (Table II), Cremophor RH 40 showed the highest dissolution capacity of loratadine, followed by Solutol HS 15 and Tagat S2, for which the solubility coefficient was 1.7 - 2 times lower than that obtained for Cremophor RH 40. Also, compared to Cremophor RH 40, Caprol MPGO showed a good solubilisation capacity of loratadine, but 2.5 fold lower, and polysorbate 80 and Lansurf CO 12 led to close values of the solubility coefficient for loratadine, but 3.45 - 4 fold lower. The drug was poorly soluble in polysorbate 20, Lansurf OA 14 and Labrasol, surfactants for which the lowest values of solubility were measured.

Consequently, the following three surfactants were selected for screening: Cremophor RH 40, Solutol HS 15 and Tagat S2.

The surfactant selection was mainly governed by its potential to dissolve the selected oil phase (Captex 355), also its dissolution capacity for loratadine was considered as an additional advantage. The solubilized amounts of Captex 355 by Cremophor RH 40, Solutol HS 15 and Tagat S2 were 25.2%, 12.6% and 3.18% (w/w), respectively. Of the three tested surfactants, Cremophor RH 40 (macrogol-40-glycerol hydroxystearate) was found to be the best solubilizer for Captex 355, most likely due to the presence of hydroxystearate chain and the 40 moles of ethylene oxide in its structure. This explanation is also supported by the fact that Solutol HS 15 (macrogol-15 hydroxystearate), having a structure which contains both entities (except that there are only 15 moles of ethylene oxide), dissolved Captex 355, but 2 fold less than Cremophor RH 40, while Tagat S2 (macrogol-20-glycerol monostearate), which does not contain the hydroxystearate chain, was practically ineffective. Also, it is worth noting, that in this case the differences between the three surfactants with respect to their solubilizing potential for Captex 355, cannot be attributed to their HLB values, which are very similar (12 - 14 for Cremophor RH 40, 14-16 for Solutol HS 15 and 15 for Tagat S2).

Because Cremophor RH 40 has solubilized the maximum amount of Captex 355, it was selected as the surfactant for developing the microemulsions; moreover, this surfactant also has the greatest solubilisation capacity of loratadine.

Selection of cosurfactant. Adding cosurfactants in the microemulsion formulation provides further reduction in the interfacial tension and increases the fluidity of the surfactant film, which can be curved in various ways, thereby expanding the existence field of the microemulsion system. Therefore, six solvents commonly used as cosurfactants in the pharmaceutical microemulsion formulations, were investigated in the loratadine solubility study; based on the obtained results (Table II), ethanol, Capryol 90 and Lauroglycol 90 were selected for the further cosurfactant screening.

In order to evaluate the emulsifying capacity of these cosurfactants, the area of the microemulsion region in the pseudoternary phase diagrams constructed for Captex 355, water, Cremophor RH 40 and cosurfactant (ethanol, Capryol 90 or Lauroglycol 90) systems in a fixed ratio of S_{mix} 1:1 (Figure 1) was used. Comparative analysis of the size of the microemulsion areas in these pseudoternary phase diagrams indicated that ethanol (a short chain aliphatic alcohol) did not favour the formation of the microemulsion systems, fact revealed by the very small area of the microemulsion region (2.58%) in the diagram (Figure 1a). In contrast, the two esters of propylene glycol with fatty acids caprylic and lauric (Capryol 90, respectively Lauroglycol 90), known as lipophilic surfactants ($HLB_{Capryol\ 90}$ = 5 - 7, $HLB_{Lauroglycol\ 90}$ = 3 - 5), developed much higher microemulsion regions (8.23% and 7.19% respectively) (Figures 1b and 1c), which highlights the favourable effect of the presence of a secondary surfactant as cosurfactant on the micro-emulsion phase behaviour. In addition, the region of the microemulsion produced by Capryol 90, was slightly higher than that obtained for Lauroglycol 90, most likely because of the slightly higher HLB value of the first surfactant, i.e. the shorter chain of caprylic acid (C8) compared

to that of the lauric acid (C12). This also reveals the influence of the type of the secondary surfactant on the formation of the micro-emulsion systems.

Based on these results, propylene glycol mono-caprylate (Capryol 90) was selected as cosurfactant for the development of the microemulsion formulations.

The selection of Capryol 90 as cosurfactant for the loratadine microemulsion formulations was also sustained by its higher solubilizing ability for loratadine, compared to that of the others studied cosurfactants (Table II).

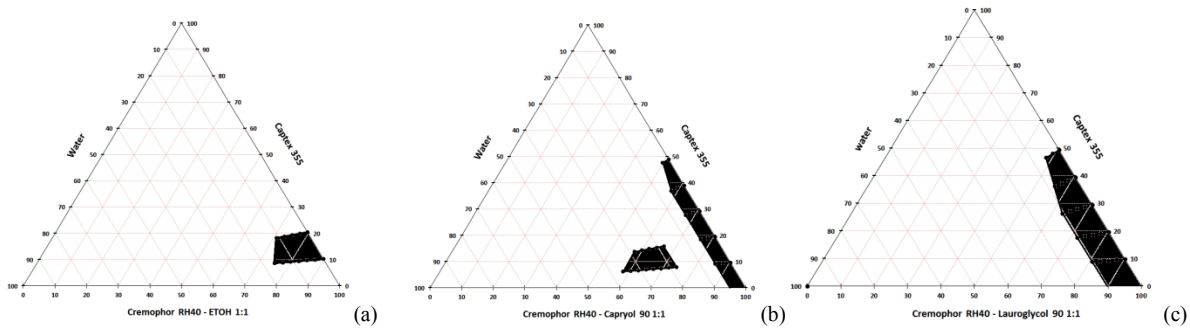

Figure 1.
Pseudo-ternary phase diagrams of systems composed of Captex 355, Cremophor RH 40, water and different cosurfactants (a) ethanol, (b) Capryol 90 and (c) Lauroglycol 90 at S_{mix} 1:1

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams were used to obtain the concentration range of the components (aqueous phase, oil phase, surfactant and cosurfactant) from the formed microemulsion regions. Figure 2 shows the pseudoternary phase diagrams constructed for the systems with Captex 355, Cremophor RH 40, water and Capryol 90 (as cosurfactant) containing different Cremophor RH 40/Capryol 90 weight ratios (2:1 and 3:1).

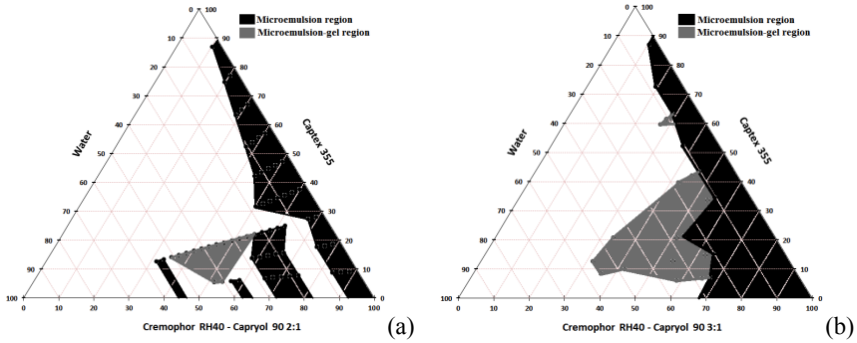

Figure 2.
Pseudo-ternary phase diagrams of systems composed of Captex 355 (oil phase), Cremophor RH 40 (surfactant), Capryol 90 (cosurfactant) and water at different S_{mix} (a) 2:1 and (b) 3:1

In both pseudoternary phase diagrams, both micro-emulsion and microemulsion-gel regions have been observed, regions specific to many Cremophor RH 40-water-oil ternary systems, as shown in other published studies [17]. The size of the microemulsion region in the pseudoternary phase diagrams increased drastically from 8.23% to 22.79% and slightly from 22.79% to 25.93%, with the increase of the surfactant concentration in the S_{mix}, from 1:1 (Figure 1b) to 2:1 (Figure 2a) and from 2:1 to 3:1 (Figure 2b). The progressive depression of the interfacial tension, produced by increasing the surfactant concentration, is mainly responsible of this increment, and reveals the pronounced effect of S_{mix} ratio on the size and position of the microemulsion domain in the pseudo-ternary phase diagram. Also, increasing the surfactant proportion in S_{mix} from 1:1 to 2:1 and 3:1 clears the microemulsions transformed into gels, as it is indicated in the respective pseudoternary phase diagrams (Figures 2a and 2b). Similar to the microemulsion region, the gel phase region of S_{mix} 3:1 (Figure 2b) was larger than that of S_{mix} 2:1 (Figure 2a), indicating the marked effect of the surfactant/cosurfactant (S_{mix}) weight ratio on the gel phase properties (size and position in the pseudoternary phase diagram).

Based on these observations, the studied microemulsion and gel-microemulsion formulations were selected from the L/H microemulsion regions and L/H gel phase microemulsion regions of the pseudoternary phase diagram obtained at S_{mix} 3:1.

Formulation of loratadine-loaded microemulsions and gel-microemulsions

The selection criteria of the experimental formulations were as follows: i) the internal oil phase, in the selected concentration, able to dissolve completely the drug in the required concentration; ii) obtaining stable and well-tolerated formulations, a minimum concentration of S_{mix} should be selected to solubilize each of the selected oil phase concentrations. The second criterion for the selection of the amounts of components in the studied microemulsion formulations was also established considering previously reported results, according to which, when dermal administration of a drug aims to increase its permeation through the skin, for the formulations containing the highest surfactant amount, the maximum transfer rate is usually not achieved [17].

From the L/H microemulsion and L/H gel-microemulsion regions of the pseudoternary phase diagram constructed for the systems containing Captex 355, water and Cremophor RH 40/Capryol 90 in a 3:1 weight ratio (Figure 2b), six formulations were selected (Table I). The selection of these formulations will allow studying the effect of the formulation components on the microemulsion characteristics.

Characterization of loratadine-loaded microemulsions and gel-microemulsions

The results of the physico-chemical properties evaluation of the experimental microemulsions and gel-microemulsions formulations containing 0.5% loratadine are shown in Table III.

Table III

Values of physicochemical parameters of the experimental microemulsion and gel-microemulsion formulations containing 0.5% loratadine

Formulation code	Droplet size (nm)	Polydispersity index	Zeta potential (mV)	Drug content (%)	pH	Viscosity (Pa x s)	Penetration value (mm)
ME LRT 1	228.3 ± 0.13	0.221	-1.02 ± 0.05	100.3 ± 0.23	4.81 ± 0.03	0.176 ± 0.35	-
ME LRT 2	59.9 ± 0.18	0.255	-0.87 ± 0.08	99.5 ± 0.38	4.60 ± 0.01	0.212 ± 0.08	-
ME LRT 3	67.6 ± 0.42	0.234	-1.49 ± 0.12	97.6 ± 0.52	4.77 ± 0.02	0.102 ± 0.16	579.3 ± 2.05
G-ME LRT 4	33.3 ± 0.38	0.290	-1.57 ± 0.09	102.3 ± 0.75	4.70 ± 0.03	0.555 ± 0.22	284.0 ± 1.63
ME LRT 5	117.8 ± 0.51	0.274	-1.00 ± 0.06	107.1 ± 0.61	4.61 ± 0.01	0.149 ± 0.39	508.0 ± 6.68
G-ME LRT 6	32.9 ± 0.20	0.252	-2.72 ± 0.14	101.3 ± 0.40	4.63 ± 0.01	1.267 ± 0.51	135.3 ± 1.69

The mean droplet diameter of the studied microemulsions loaded with loratadine ranged from 32.9 nm to 228.3 nm (Table III). The ME LRT 1 formulation, containing the smallest amount of oil (10%) and the highest percentage of S_{mix} (70%), showed the highest mean droplet diameter (228.3 ± 0.13 nm). This parameter was approximately two fold lower in the case of ME LRT 5 formulation, which contained the highest percentage of oil (30%), 50% of S_{mix} and a small amount of water (19.5%). Lower values of the mean droplet diameter (59.9 ± 0.18 nm and 67.6 ± 0.42 nm) were measured for ME LRT 2 and ME LRT 3 formulations, having a similar composition (20% oil, 50 - 60% S_{mix} and 19.5 - 29.5% water). The mean droplet diameter was the lowest (G-ME LRT 4 and G-ME LRT 6 formulations) when the oil concentration was reduced to 15%, the water concentration increased to 34.5 - 44.5%, and the S_{mix} concentration was close to that of the water (40 - 50%). These results indicated that the droplet diameter decreased significantly when the proportions of the oil phase and S_{mix} were between 15 - 20% and 40 - 50% respectively, which may support the affirmation that the effects of the surfactant/cosurfactant mixture (e.g. condensation and stabilization) on the interfacial film are the most intense at those proportions. Due to the small average droplet size of the ME LRT 2, ME LRT 3, G-ME LRT 4 and G-ME LRT 6 formulations, it is assumed that their specific surface area is large and therefore a better contact between the skin and the drug-loaded oil droplets is possible, thus leading to a higher concentration gradient and an increased permeation of loratadine. It is known that the reduced droplet size is, to a large extent, a necessary condition for an appropriate topical drug delivery, most probably because the oil droplets tend to fusion on the skin surface, generating a channel for drug transport.

The *PDI* values of the experimental ME formulations were relatively low, from 0.221 to 0.290 (Table III), indicating the uniformity of the droplet size and their distribution within a narrow range of values.

The measured zeta potential values of the experimental loratadine-loaded microemulsions were negative, ranging from -0.87 ± 0.08 to -2.72 ± 0.14 mV (Table III). These values indicated the systems stability, since droplet aggregation is not expected due to their negative surface charge. In addition, it is known that ion adsorption produce negative zeta potential values, which was also reported for ethoxylated surfactants.

The *pH* values of all the studied formulations ranged from 4.60 ± 0.01 to 4.81 ± 0.03 (Table III), being very close to the physiological pH of the skin surface.

The loratadine content of the experimental *ME* formulations ranged from 97.6 ± 0.52% to 107.1 ± 0.61% (Table III) of the theoretical value (0.5%, w/w), falling within the limits recommended by the pharmacopoeias for the declared content of the drug

in pharmaceutical preparations (90 - 110%). The obtained data showed the uniform distribution of loratadine in all the studied ME formulations.

Rheological analysis. The viscosity of the loratadine-loaded microemulsion formulations varied within a wide range of values, from 0.102 to 1.267 Pa.s (Table III), which reveals the significant influence of the system's composition on this characteristic. The most viscous was the G-ME LRT 6 formulation, followed by the G-ME LRT 4 formulation, whose viscosity was 2.3 fold lower. Compared with the G-ME LRT 6, the other ME LRT formulations produced viscosity values 6 - 12 fold lower, being more fluid. It can be observed that the viscosity increased mainly by increasing the water content of the formulation, most probably because water favours the gel phase formation (in the case of formulations *G*-ME LRT 6, G-ME LRT 4 and ME LRT 2, containing 44.5%, 34.5% and respectively 29.5% water).

Also, another formulation variable that influenced the viscosity of the systems was the S_{mix} content, whose effect was more evident in the case of fluid formulations (ME LRT 1, ME LRT 3 and ME LRT 5). In this group, the viscosity decreased with a decrease of the S_{mix} concentration from 70% to 50%, when the water content was the same (19.5% for formulations ME LRT 1 and ME LRT 5) or increased 1.5 times (ME LRT 3 formulation). It is worth noting that the gel phase formation in the case of the G-ME LRT 4 and G-ME LRT 6 formulations is advantageous, as it makes these preparations more suitable for topical application.

Considering the obtained penetration values, the measurement of the consistency of the studied microemulsion formulations showed that G-ME LRT 4 and G-ME LRT 6 had the lowest penetration rates, indicating their higher consistency (Table III), most likely due to the presence of water in high concentration, which results in gel phase formation. In contrast, the ME LRT 3 and ME LRT 5 formulations produced the highest penetration rates, correlated with a much lower consistency, whereas the ME LRT 1 and ME LRT 2 formulations could not be subjected to this test, being too fluid. The four experimental formulations tested by penetrometry were also evaluated regarding their spreadability, an important characteristic of topical preparations, as it highly influences not only the delivery of the correct drug dose to the target site, but also the ease of their application on the skin or mucosa. The extensiometric curves presented in Figure 3, showed that G-ME LRT 4 produced the largest spreading areas, followed by ME LRT 3 and ME LRT 5, while the G-ME LRT 6 formulation exhibited the lowest spreading capacity, indicated by the much smaller values of the spreading areas. These results were consistent with those obtained from penetrometric measurements. However, the high extensiometric values of all analysed ME formulations support their appropriate spreadability.

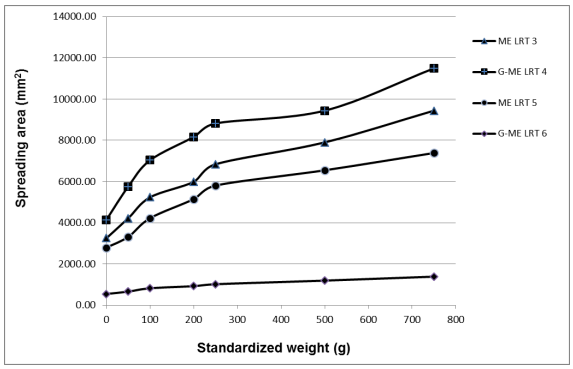

Figure 3.
Extensiometric curves of the studied ME formulations containing 0.5% loratadine

The differences in consistency of the studied ME systems can be attributed, as in the case of viscosity, to the combined effects of the water and S_{mix} concentration in the formulations.

In vitro release study of loratadine through synthetic membrane

The *in vitro* drug release is nowadays recognized as a simple, reliable, reasonable and reproducible method to assess the performance of a dosage form, and the obtained *in vitro* release profile of the drug is regarded as a valuable quality control parameter for a topical formulation. Accordingly, in our study, a static Franz diffusion cell system and a hydrophilic synthetic membrane were used to evaluate the effect of microemulsion formulation variables on the performance of the studied preparations. The results of the *in vitro* loratadine release and permeation, from the experimental ME formulations, are illustrated in Figure 4 and listed in Table IV.

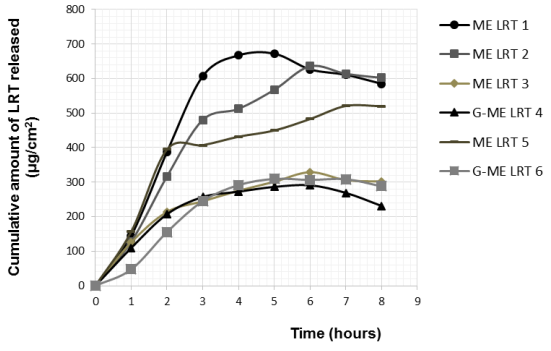

Figure 4.
In vitro loratadine permeation profiles through synthetic membrane from experimental ME formulations

Table IV

The permeation and release parameters of the loratadine-loaded formulations through synthetic membrane

Formulation code	Permeation parameters			Release parameters	
	J_{ss} (μg/cm^2/h)	K_P x 10^{-6} (cm/h)	t_L (h)	k (μg/cm^2/h$^{1/2}$)	D x 10^{-5} (cm^2/h)
ME LRT 1	203.35 ± 1.40 (0 - 3 h)	406.70	0.12 ± 0.12	628.78 ± 8.72	1241.44
ME LRT 2	163.01 ± 4.84 (0 - 3 h) 52.16 ± 3.25 (3 - 6 h)	326.02 104.32	0.08 ± 0.24 -	480.82 ± 3.71 216.10 ± 6.02	725.90 146.64
ME LRT 3	41.25 ± 0.87 (1 - 5 h)	82.50	-	136.04 ± 4.33	58.11
G-ME LRT 4	86.89 ± 1.34 (0 - 3 h) 11.56 ± 1.79 (3 - 6 h)	173.79 23.12	-	203.26 ± 6.95 48.78 ± 8.23	129.73 7.47
ME LRT 5	197.46 ± 5.20 (0 - 2 h) 24.98 ± 5.69 (2 - 7 h)	394.92 49.96	0.07 ± 0.36 -	574.66 ± 8.43 99.96 ± 9.26	1036.94 31.40
G-ME LRT 6	78.10 ± 1.21 (0 - 4 h)	156.19	0.11 ± 0.05	249.34 ± 4.71	195.20

J_{ss} – steady-state flux, K_p – permeability coefficient, t_L – lag time, k – release rate, D – diffusion coefficient

Comparing the total amount of LRT released from the studied microemulsions, the ME LRT 1 and ME LRT 2 formulations released the maximum amount of LRT (93.06 ± 1.58% and 92.65 ± 0.83% respectively) after 3 or 5 hours of testing. Also, high total drug amounts, ranging from 68.33 ± 0.42% to 78.53 ± 1.17%, were released from systems ME LRT 3, G-ME LRT 4 and ME LRT 5 after 5 - 7 hours of testing. The lowest amount of LRT (40.5 ± 0.76%) was released after 5 hours from the G-ME LRT 6 formulation.

For the formulations ME LRT 2, G-ME LRT 4 and ME LRT 5 the plots of the cumulative amount of LRT released *per* unit surface area of membrane *vs.* time (Figure 4) presented two linear portions: before the steady state (the first 2 - 3 hours) and during steady state (from 2 - 3 hours to 6 - 7 hours). During the first 2 - 3 hours, a higher and faster transfer of loratadine was observed for the ME LRT 1 and ME LRT 5 formulations, followed by the ME LRT 2 formulation. In contrast, in the same period of time, the flux and release rate of loratadine for formulations G-ME LRT 4 and G-ME LRT 6 were significantly lower ($p < 0.05$) compared to those of formulations ME LRT 1 and ME LRT 5, whereas the ME LRT 3 formulation produced the lowest flux and release rate values. During the steady state, from the three ME formulations (ME LRT 2, G-ME LRT 4 and ME LRT 5), the highest flux value (52.16 ± 3.25 μg/cm^2/h) was observed for formulation ME LRT 2, followed by ME LRT 5, which produced a twofold lower flux. As shown in Figure 4 and Table IV, the lowest flux and release rate values over this period of time were obtained for formulation G-ME LRT 4.

The drug solubility in the components of the experimental formulations and the different proportions of these components, influencing the loratadine partition among the microemulsions phases (the internal oil phase, the external aqueous phase and the surfactant micelles) and the systems viscosity, can explain the differences in loratadine release and permeation from the studied microemulsions through the synthetic membrane. It is generally accepted that the release of the drug from oil-in water microemulsion is restricted by the diffusion of the active compound from the oil and micellar phases into the external aqueous phase, where the drug molecules are available for release. In addition, a partial disruption of the surfactant micelles can occur at the drug transfer from the micellar phase and, consequently, the solubilisation of the surfactant molecules in the aqueous phase of the microemulsion takes place. Moreover, due to the fact that the transfer of the drug from the micellar phase to the aqueous phase takes place at a faster rate than that from the oil phase to the aqueous phase, decreasing the drug concentration in the aqueous phase (attributed to the drug permeation through the membrane) causes initially the drug transfer from the micellar to the aqueous phase and then the alteration of the micelle structure. Therefore, in the case of poorly water soluble drugs, the rupture of the micelles containing the drug increases the drug solubility in the external aqueous phase of the micro-emulsion, which is the force behind an increased release [1, 7]. On the other hand, because the drug solubility in the external phase of an aqueous micro-emulsion depends on the water proportion of the system, the solubility of the incorporated drug decreases by increasing the water content, forming a temporary supersaturated solution with a high thermodynamic activity.

Due to a higher solubility of loratadine in S_{mix} than in the oil and aqueous phases, the drug molecules would be mainly present at the oil-water interface, from where they diffuse more rapidly in the aqueous phase. Therefore, the increased flux values for the ME LRT 1 and ME LRT 2 formulations may be due to the presence of a higher amount of loratadine dissolved in the aqueous phase, the oil content being lower and the S_{mix} higher than in the other formulations. In contrast to what was expected, G-ME LRT 4 and G-ME LRT 6 formulations, with larger amounts of aqueous phase and smaller proportions of oil and S_{mix}, presented lower flux values than those of ME LRT 1, ME LRT 2 and ME LRT 5, most likely because the surfactants micelles

stability was higher and the systems viscosity was increased.

The ME LRT 1, ME LRT 2 and ME LRT 5 formulations, characterized by a faster diffusion, showed a lag time (0.07 - 0.12 h), similar to that of formulation G-ME LRT 6, from which loratadine diffused much slower (Table IV). Therefore, it can be noted that the lag time values did not vary according to expectations, namely a longer lag time in case of a slow diffusion. The other formulations presented different transmembranar permeation profiles and without lag time. Similar results were obtained in our previous study of several microemulsion formulations with fluconazole, also a poorly water soluble drug [7]. Thus, it can be assumed that also in the case of loratadine experimental microemulsions, the calculated lag time depends both on the drug release from the vehicle, but also on its diffusion through the synthetic membrane; in both processes, the systems composition and viscosity had an important role.

Four mathematical models (zero order, first order, Higuchi and Korsmeyer-Peppas models) were fitted to the data obtained from the *in vitro* drug release study in order to evaluate the LRT release mechanism from the experimental microemulsion formulations through synthetic membrane. The results of curves fitting (the values of release constant and determination coefficient) are listed in Table V.

Table V

Results of kinetic analysis of the *in vitro* permeation data through synthetic membrane obtained for 0.5% loratadine-loaded microemulsion formulations

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0 (μg/h)	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-0.5}$)	R^2	K_P (h^{-n})	n	R^2
ME LRT 1	10.942	0.6248	0.3872	0.4264	39.512	0.8183	1.4159	0.9613	0.9129
ME LRT 2	12.499	0.8308	0.6083	0.9174	38.459	0.9362	1.3724	0.9393	0.9442
ME LRT 3	8.1017	0.7504	0.1669	0.8295	30.149	0.9239	1.504	0.5322	0.965
G-ME LRT 4	6.2619	0.5575	0.1066	0.5532	26.821	0.7622	1.4529	0.6	0.9282
ME LRT 5	8.372	0.759	0.1742	0.8896	30.135	0.917	1.4485	0.626	0.804
G-ME LRT 6	5.0216	0.7614	0.0659	0.7761	15.813	0.8786	0.865	1.1888	0.943

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

Comparing the obtained values, except for ME LRT 5, all the other formulations fitted best with the Korsmeyer-Peppas model. Further, analysing the first 60% of drug release data by this kinetic model, the values of the diffusion exponent (n) were determined, which allowed to indicate the drug release mechanism: Fickian (non-steady) diffusion when $n \leq 0.5$, non-Fickian ("anomalous") transport when $0.45 < n < 0.89$, case II transport when $n = 0.89$ and super case II transport when $n > 0.89$. The calculated values of the diffusion exponent, n (Table V), indicated two different loratadine release mechanisms from the experimental microemulsion formulations through the synthetic membrane: non Fickian transport for formulations ME LRT 3, G-ME LRT 4 and ME LRT 5, and super case II transport for formulations ME LRT 1, ME LRT 2 and G-ME LRT 6.

Conclusions

In the present work, loratadine-loaded oil in water (o/w) microemulsion and gel-microemulsion formulations for topical drug delivery were successfully developed using caprylic/capric triglyceride (Captex 355) as oil phase, Cremophor RH 40 as surfactant and propylene glycol monocaprylate (Capryol 90) as cosurfactant. In the formulation step, the selection of these components was performed using two valuable tools, namely the solubility study and the construction and evaluation of pseudoternary phase diagrams. Six microemulsion formulations, including two gel-microemulsions, were prepared and evaluated for physico-chemical characteristics (mean droplet size, polydispersity index, zeta potential, pH, drug content, viscosity and consistency) and *in vitro* drug release through synthetic hydrophilic membrane. The obtained data of the experimental tests showed the considerable effect of the microemulsion components (oil, S_{mix} and water) and their proportions on the above mentioned characteristics.

Based on the results of the *in vitro* drug release study using synthetic hydrophilic membrane, the formulations for incorporating 0.5% loratadine to be firstly evaluated for *in vitro* drug release through a biological membrane model (animal and/or human skin), can be considered the microemulsions 1, 2 and 5 containing Captex 355 (10%, 20% and 30% respectively) as oil phase, S_{mix} (3:1) Cremophor RH 40 - Capryol 90 (70%, 60% and 50% respectively) as surfactant-cosurfactant, and 19.5% water, as they produced the highest flux and release rates values. Also, additional *in vitro* and *in vivo* studies are necessary to assess the stability, safety and therapeutic efficacy of these experimental preparations, which may be recommended for further development as potential vehicles for topical delivery of loratadine.

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