Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 1, January - March 2014, p. 395 - 412

DEVELOPMENT AND EVALUATION OF MICROEMULSION-BASED HYDROGEL FORMULATIONS FOR TOPICAL DELIVERY OF PROPRANOLOL HYDROCHLORIDE

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The aim of the present investigation was to develop and evaluate microemulsion based hydrogels (MEH) for the topical delivery of propranolol hydrochloride (PRHCl). The solubility of PRHCl in oils, surfactants and cosurfactants was evaluated to identify the components of the microemulsion. The pseudoternary phase diagrams were constructed using the novel Phase Diagram by Micro Plate Dilution method. Carbopol EDT 2020 was used to convert PRHCl loaded microemulsions into gel form without affecting their structure. The selected microemulsions were assessed for globule size, zeta potential, and polidispersity index. Besides this, the MEH-PRHCl formulations were evaluated for drug content, pH, rheological properties and *in vitro* drug release through synthetic membrane. The optimized MEH-PRHCl formulations consisting of PRHCl 1%, Capryol 90 11% and 12% respectively as oil phase, Cremophor RH 40:propyleneglycol 49% and 53% respectively as surfactant:cosurfactant (2:1) and 1.7% Carbopol EDT 2020, showed high flux value, highest release rate values, shortest lag time values and lowest surfactant content. The *in vitro* PRHCl permeation through synthetic membrane from the studied MEH was found to follow the Korsmeyer-Pepas model ($R^2 > 0.99$) with a non-Fickian, "anomalous" release mechanism. The results suggest the potential use of developed MEHs as vehicles for topical delivery of PRHCl.

(Received January 8, 2014; Accepted March 21, 2014)

Keywords: Propranolol hydrochloride, Microemulsion, Hydrogel, Topical

1. Introduction

Microemulsions (ME) are defined as thermodynamically stable, fluid, transparent (or translucent) colloidal dispersions consisting of oil phase, aqueous phase, surfactant and cosurfactant at appropriate ratios, which constitute a single optically isotropic solution with a

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droplet diameter usually within the range of 10-100 nm [1-4]. These homogenous systems are useful for the topical delivery of drugs due to their several advantages, such as capacity to solubilise both hydrophilic and lipophilic compounds, frequently in high amount, excellent thermodynamic stability, facile and low cost preparation, optical clarity and increased penetration of drugs through the skin [4-8].

In the last decade, numerous studies have revealed the pharmaceutical importance of microemulsions as vehicles for dermal and transdermal delivery of a wide variety of drugs [9-32]. In order to explain the increase of drug penetration through the skin by microemulsions, several potential mechanisms have been proposed, including (1) increase the thermodynamic activity towards the skin due their high solubility potential; (2) the ingredients of microemulsions can act as permeation enhancers by reducing the diffusional barrier of the stratum corneum and increasing the permeation of drugs through the skin; (3) increase the permeation rate of the drug from microemulsions, by reducing the affinity of the drug to the internal phase of microemulsion and thus, favorising its partitioning into stratum corneum.

Propranolol hydrochloride (PRHCl), known as 2- propranolol ,1-[(1-methylethyl)amino]-3-(1-naphthalenyoxy)-,hydrochloride, (±)- or (±)-1-(Isopropyl amino)-3-(1-napthyloxy)-2-propranol hydrochloride [33], is a non-selective beta-blocker widely used in the treatment of hypertension, cardiac arrhythmias, angina pectoris and prophylaxis after recovery from myocardial infarction [34-36]. Moreover, in the last five years, oral [37-42] and topical [43, 44] propranolol has been reported to be an effective treatment for infantile hemangiomas. After oral administration, PRHCl is rapidly and almost completely (90-100 %) absorbed from the gastrointestinal tract (GIT), but has a short half–life (3-6 hours in man) [45] and a relatively low systemic bioavailability (of only 25-30 %) due to the significant hepatic first pass metabolism [46, 47], which required an increased dosing frequency. These properties of PRHCl make it an ideal candidate for percutaneous application, which explain the growing interest for developing systems delivery for dermal and transdermal delivery of this drug [48, 49]. But the percutaneous penetration of PRHCl is poor because it is a polar, hydrosoluble cationic molecule. Therefore, in order to improve the permeation of this drug in skin, several approaches have been investigated [50-54].

In view of all the above mentioned aspects, the aim of this study was to develop microemulsion-based hydrogel (MBH) formulations to be used as vehicles for topical delivery of PRHCl. Thus, several MBH formulations containing 1% PRHCl were prepared with Carbopol EDT 2020 as gelling agent, and their quality control, regarding physicochemical properties and stability, was performed. Also, the *in vitro* drug release and permeation through synthetic membrane was investigated in order to assess the formulations performance.

2. Materials and methods

Materials

Propranolol hydrochloride was kindly donated by S.C. Sintofarm S.A (Bucharest, Romania). Cremophor EL and Cremophor RH 40 (BASF Chem Trade GmbH, Germany), isopropyl myristate (Cognis, Germany), Capryol 90 and Labrasol (Gattefossé, France), Lansurf SML 20, Lansurf SMO 80 and Lansurf SMO 81 (Lankem L.t.d., UK), methylcellulose (Tylose MH 300, Fluka, Germany), carboxymethylcellulose sodium salt (Fluka, Germany), hydroxypropylmethylcellulose (Methocel K4M, Colorcon L.t.d., UK) and Carbopol ETD 2020 (Lubrizol Advanced Materials, USA) were received as gift samples. Castor oil was supplied by S&D Chemicals (India), oleic acid and Tween 65 were purchased from Merck KGaA (Germany) and propyleneglycol (PG) was obtained from BASF Chem Trade GmbH (Germany), ethanol (96%) and isopropyl alcohol (IPA) were purchased from Chimopar S.A. (Romania). Tuffryn HT synthetic hydrophilic membranes of polysulfone (0.45 μm, 25 mm) were supplied by Pall Coorporation (USA). 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 PRHCl in water, various oils (oleic acid, Capryol 90, isopropyl myristate and castor oil), surfactants (Cremophor EL, Cremophor RH40, Labrasol, Lansurf SML 20, Lansurf SMO 80, Lansurf SMO 81 and Tween 65) and cosurfactants (ethanol, isopropyl alcohol, propyleneglycol and PEG 400) was determined using the shake flask method. Briefly, an excess amount of PRHCl was dispersed in 2 mL of each of the solvents in 10 mL capacity stoppered vials separately and mixed for 10 min using a vortex mixer in order to facilitate proper mixing of PRHCl with the vehicles. The mixture vials were then kept and shaken at 37±1°C in an isothermal shaker bath (Memmert, Germany) for 72 h to get equilibrium. The resulting mixtures were then centrifuged at 5000 rpm for 15 min. The supernatant was filtered through a membrane filter (0.45 µm, 25 mm, Teknokroma, Germany). The concentration of the PRHCl in the filtrate was determined by UV spectrophotometer (T70+, PG Instruments, U.K.) at the wavelength 290 nm. Each experiment was performed in triplicate.

Screening of formulations components

Screening of oil

The selection of the oil phase for developing MEs of PRHCl was based upon the maximum solubilising capacity for drug.

Screening and selection of surfactants

The surfactant for developing o/w MEs of PRHCl was selected based on its solubilisation capacity for PRHCl and Capryol 90. After performing the solubility studies, four different surfactants, including Lansurf SMO 81, Lansurf SMO 80, Lansurf SMO 20 and Cremophor RH 40 were screened. The solubilisation capacity of surfactants for Capryol 90 was determined using technique described in some previous studies [25, 55, 56]. Briefly, to 2.5 mL of 15% (w/w) aqueous solution of surfactant aliquots of 5 µL of oil (Capryol 90) was added with vigorous vortexing; if a one-phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy.

Screening and selection of cosurfactants

The selection criterion of cosurfactant for developing o/w MEs was the area of ME region. Cremophor RH 40 was mixed with three types of solubilizers selected as cosurfactants, namely ethanol, IPA and PG. At a fixed ratio S_{mix} of 1:1 the pseudoternary phase diagrams were constructed. The oil and S_{mix} were used in nine different weight ratios (from 9:1 to 1:9) so that maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams.

Construction of 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 microemulsions. Surfactant (Cremophor RH 40) and cosurfactant (PG) 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 surfactant with respect to 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 [57]. In brief, the individual oil-emulsifier mixtures (oil, surfactant and cosurfactant) were gradually diluted with water in a microtitre plate (96 wells, 350 µL volumes each). The microtitre plates were filled by microsyringe according to the filling scheme: the oil-emulsifier phase was added starting at A1 with 200 µL up to D4 with 5 µL, decresing 5 µL each well, and the water phase was then added from A2 with 5 µL up to D5 with 200 µL, increasing 5 µL each well. The wells E1 up to H5 were filled with the next batch using the same procedure. The plates filled in this way were then sealed with adhesive storage films and shaken on the temperature controlled thermomixer at 25°C in order to ensure adequate mixing and temperature adjustment of the system. Subsequently, each plate was evaluated visually regarding the isotropy and the boundary between the homogeneous or

the heterogeneous system. The microemulsion phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained.

Preparation of PRHCl microemulsion formulations

According to microemulsion regions in the phase diagrams, ten microemulsion formulations were selected at different component ratios. The composition of propranolol hydrochloride-loaded microemulsion formulations is given in Table 1. PRHCl was dissolved under stirring in mixture of Capryol 90, Cremophor RH 40 and PG. Then the appropriate amount of water was added to the mixture drop by drop with continuous stirring. All microemulsions were stored at 25±2°C. The final concentration of PRHCl in microemulsion systems was 1% (w/w).

Preparation of microemulsion-based hydrogel of PRHCl

Carbopol EDT 2020 was selected as suitable gelling agent to prepare the microemulsion-based hydrogel formulations. Carbopol EDT 2020 was dispersed slowly in the microemulsion under stirring. The concentration of carbomer in microemulsion-based hydrogel was 1.7% (w/w).

Characterization of PRHCl microemulsions

The obtained microemulsions were evaluated regarding various physicochemical characteristics.

The average droplet size, polydispersity index and zeta potential of the PRHCl microemulsions were measured in triplicate by photon correlation spectroscopy using a Zetasizer Nano-ZS (Malvern Instruments, UK) instrument. Measurements were carried at a fixed angle of 173° at 25°C. Microemulsions were diluted in ratio of 1:3 with ultrapure water delivered by a Simplicity UV Water Purification System (Millipore SAS, France). The refractive indexes and the viscosities of formulations were determined using a refractometer (Digital ABBE Mark II-Reichert, Depew, USA) and a rotational viscosimeter (Brookfield DV-I+, UK) respectively. The pH of the microemulsions was detected at 25±2°C using a pH-meter (Sension™1, Hach Company, USA). Experiments were performed in triplicate for each sample.

Characterization of PRHCl microemulsion-based hydrogels

Determination of drug content and pH

To determine the drug content, about 1 g of MBH was weighted in a 100 mL volumetric flask, and dissolved in methanol; 1 mL of filtered solution was diluted appropriately and PRHCl content was analyzed spectrophotometrically, at 290 nm. The pH values of aqueous solutions containing 5% (w/w) *PRHCl MBH* were determined at 25°C using the Sension™1 digital pH-meter (Hach Company, USA). Each experiment was performed in triplicate.

Rheological characterization

The rheological studies were conducted to determine the viscosity and the consistency of samples. 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 3.1 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 pharmacopoeias. Also, the spreadability of the hydrogels was determined, as this characteristic is nearly related to consistency. The spreadability of the samples was carried out using the parallel-plate method. In brief, 1 g hydrogel was placed within a circle of 1 cm diameter premarked on the centre of a glass plate over which a second glass plate was placed and the diameter was measured after 1 minute. Subsequently, every 1 minute standardized weights (50 g, 100 g, 200 g, 250 g, 500 g and 750 g) were placed on the upper glass plate and the spread diameters were recorded each time. Then, the areas of respective circles were calculated and the obtained values, expressed as mean ± SD, were plotted versus corresponding standardized weight. All rheological tests were performed in triplicate at 25ºC.

In vitro drug release studies

The *in vitro* release of PRHCl from selected MBH formulations was determined to evaluate the effect of the formulation variables on preparations performance. The release experiments were performed on a system of 6 Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA) with an effective diffusional area of 1.767 cm^2 and 7 mL of receptor cell capacity. The synthetic membrane (HT Tuffryn membrane, Pall Corporation, USA) was mounted between donor and receptor compartments of Franz diffusion cells. The receptor chambers were filled with freshly prepared phosphate buffer saline pH 7.4 to ensure sink conditions. It was constantly stirred at 600 rpm and the diffusion cells were maintained at 32±1°C throughout the experiment. 300 mg of tested formulation was placed into each donor compartment. 0.5 mL sample of the receptor medium were withdrawn at predetermined time (30, 60, 120, 180, 240, 300, 360, 420 and 480 min) and replaced with an equal volume of fresh receiver medium to maintain a constant volume. Collected samples were analyzed for PRHCl content by UV spectrophotometric method, at 290 nm. The assay was linear in the PRHCl concentration range of 10-130 μg/mL ($y = 0.097x$, $R^2 = 0.9998$). Three replicates of each experiment were performed.

Data analysis of in vitro drug release studies

Cumulative amount of PRHCl permeated through the membrane (μg/cm^2) was plotted as a function of time (t, min). The permeation rate of drug at steady-state (flux, J_s, μg/cm^2/min) and the lag time (t_L, min) 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/min) was calculated by dividing the flux with initial concentration of drug in donor compartment.

In order to investigate the release kinetics of the PRHCl from MBH formulations, the data obtained from *in vitro* drug release studies were fitted into various mathematical models, as follows:

- Zero order model: $M_t = M_0 + K_0 t$, where M_t is the amount of drug dissolved 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: $logC = logC_0 - K_1 t/2.303$, where C_0 is the initial concentration of drug, K is the first order rate constant, and t is the time.
- Higuchi model: $M = K_H t^{1/2}$, 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 drug released at time t, 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 ± standard deviation (SD) and were considered statistically significant at $P < 0.05$.

3. Results

Screening of formulations ingredients

Screening of oil and water

The solubility of PRHCl in different oils as well as in distilled water is listed in Table 1.

Table 1. The solubility of PRHCl in water, oils, surfactants and cosurfactants at 25±2°C

Component	Solubility (mg/mL)
Water	8097.876±0.032
Oleic acid	337.488±0.256
Capryol 90	485.226±0.347
Isopropyl myristate	46.260±0.073
Castor oil	335.180±1.597
Cremophor EL	1177.285±1.326
Cremophor RH40	1343.490±2.075
Labrasol	1154.201±0.046
Lansurf SML 20	1371.191±0.832
Lansurf SMO 80	2396.122±0.041
Lansurf SMO 81	3855.032±0.017
Tween 65	1154.201±0.028
Ethanol	5401.662±0.014
Isopropyl alcohol	2954.755±0.203
Propyleneglycol	8753.463±0.316

Screening of surfactants

The results of the solubility study involving the surfactants and cosurfactants are also presented in Table 1.

Figure 1 shows the solubilisation behaviour of the selected oil (Capryol 90) into seven types of surfactant solutions.

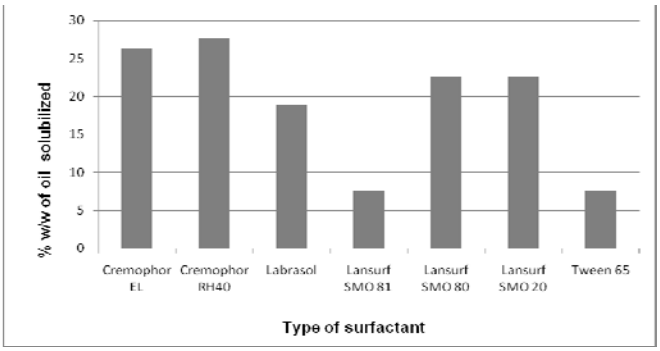

Fig 1. Oil (Capryol 90) solubilized by different surfactants.

Screening of cosurfactants

Addition of cosurfactants provides further reduction in the interfacial tension and increase the fluidity of interfacial surfactant film which can take up different curvatures and thus expanding the area of existence of microemulsion system [1, 2]. Consequently, ethanol, isopropyl alcohol and propylene glycol were selected as cosurfactants.

The microemulsion area in the pseudo-ternary phase diagrams was used to assess the emulsification potential of these cosurfactants. Figure 2 presents the pseudo-ternary phase diagrams constructed for Capryol 90 (oil phase), water, Cremophor RH 40 and cosurfactant at a fixed ratio S_{mix} 1:1.

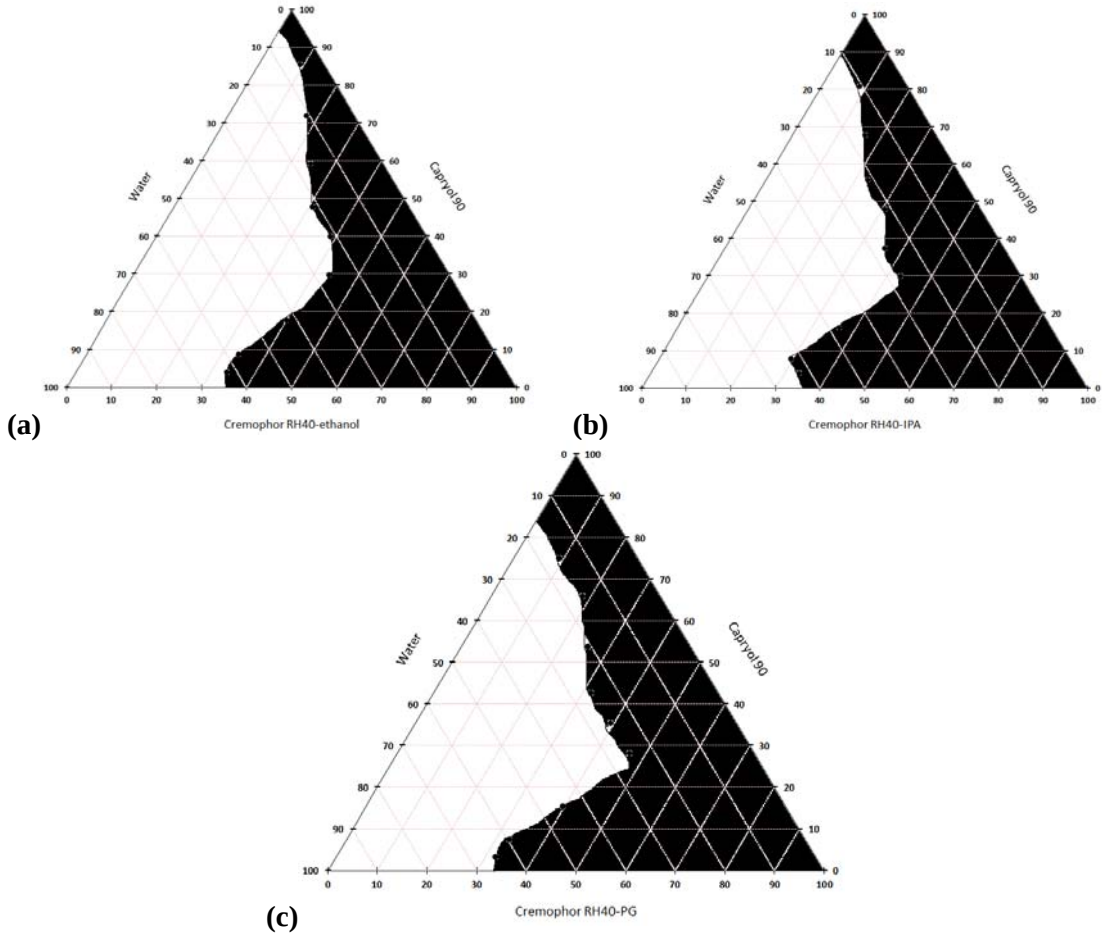

Fig. 2. Pseudo-ternary phase diagrams of systems composed of Capryol 90, Cremophor RH 40, water and different cosurfactants (a ethanol, b isopropyl alcohol, c propylene glycol) at S_{mix} 1:1.

Construction of pseudo-ternary phase diagram

The construction of pseudo-ternary phase diagrams was used to determine the appropriate concentration ranges of components (aqueous phase, oil phase, surfactant and cosurfactant) in the regions of forming microemulsions. Figure 3 presents the pseudo-ternary phase diagrams of Capryol 90, Cremophor RH 40, water systems in the presence of cosurfactant (propylene glycol) with various weight ratios of Cremophor RH 40/propylene glycol.

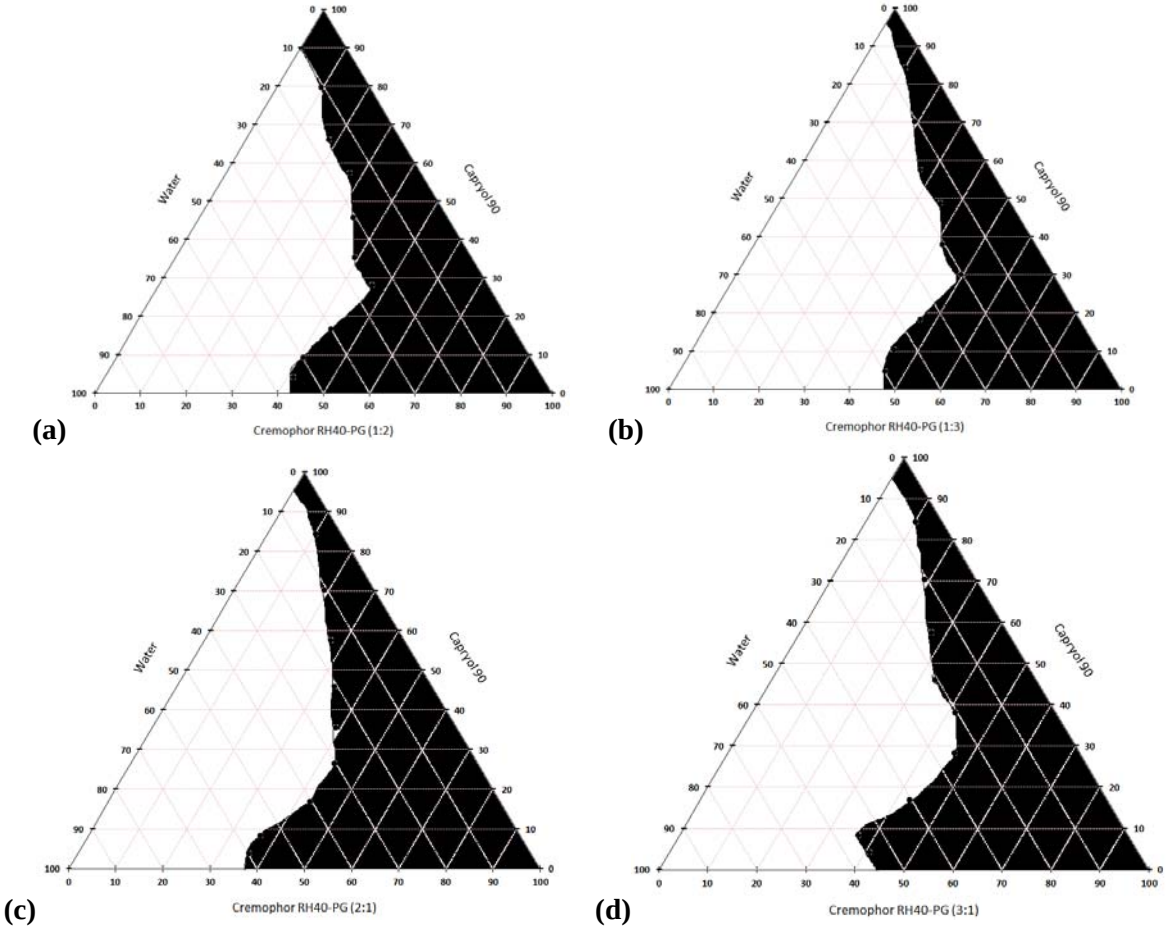

Fig. 3. Pseudo-ternary phase diagrams of systems composed of Capryol 90 (oil phase), Cremophor RH 40 (surfactant), propylene glycol (cosurfactant) and water at different S_{mix} (a 1:2; b 1:3; c 2:1; d 3:1).

Formulation and preparation of PRHCl microemulsions

From the microemulsion region of pseudo-ternary phase diagram constructed for the systems containing Capryol 90, Cremophor RH 40/propylene glycol in 1:1 weight ratio and water, ten mixtures (formulations) along the water dilution line of oil: S_{mix} mass ratio 2:8 have been selected (Figure 3a). This selection will thus permit to study the effect of formulation components on the microemulsion characteristics. The composition of the studied formulations is shown in Table 2.

Table 2. Composition of propranolol hydrochloride-loaded microemulsions.

Microemulsion components	Weight (%) and formulation codes									
	ME-PRHCl 1	ME-PRHCl 2	ME-PRHCl 3	ME-PRHCl 4	ME-PRHCl 5	ME-PRHCl 6	ME-PRHCl 7	ME-PRHCl 8	ME-PRHCl 9	ME-PRHCl 10
Propranolol hydrochloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Capryol 90	17.0	16.5	16.0	15.0	14.0	13.5	13.0	12.5	12.0	11.0
Cremophor RH 40 – Propylene glycol (2:1)	78.0	74.0	72.0	67.0	64.0	60.0	58.0	56.0	53.0	49.0
Methylparaben	0.003	0.006	0.009	0.012	0.015	0.018	0.021	0.024	0.027	0.030
Propylparaben	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.100
Distilled water	3.996	8.492	10.988	16.984	20.98	25.476	27.972	30.468	33.964	38.87

Characterization of PRHCl microemulsions

The results of tests evaluating the physical characteristics of developed PRHCl microemulsions are shown in Table 3.

Table 3. Mean droplet size, polydispersity index, viscosity, refractive index and zeta potential of the PRHCl microemulsion formulations.

Formulation code	**Droplet size (nm)**	**Polydispersity index**	**Viscosity (mPa)**	**Refractive index**	**Zeta potential (mV)**	**pH**
ME PRHCl 1	6.089±0.82	0.099	115.0±0.82	1.4423±0.01	2.34±0.06	5.87±0.11
ME PRHCl 2	6.986±1.05	0.073	112.5±0.94	1.4387±0.01	3.24±0.04	5.83±0.08
ME PRHCl 3	6.529±0.97	0.039	109.0±1.25	1.4361±0.03	3.33±0.12	5.78±0.01
ME PRHCl 4	7.001±1.34	0.037	106.0±1.34	1.4310±0.02	4.63±0.08	5.76±0.02
ME PRHCl 5	7.023±1.59	0.018	103.5±0.98	1.4272±0.05	4.28±0.13	5.76±0.01
ME PRHCl 6	6.472±0.77	0.039	98.5±0.77	1.4221±0.02	4.80±0.03	5.72±0.03
ME PRHCl 7	6.789±1.46	0.030	96.0±1.36	1.4198±0.01	6.35±0.14	5.71±0.07
ME PRHCl 8	6.965±0.92	0.032	97.5±0.88	1.4170±0.04	5.66±0.09	5.69±0.02
ME PRHCl 9	12.31±1.85	0.168	95.0±1.46	1.4135±0.03	6.65±0.17	5.67±0.01
ME PRHCl 10	12.97±2.13	0.117	102.5±1.53	1.4073±0.06	7.56±0.12	5.66±0.04

Characterization of PRHCl microemulsion-based hydrogels

The PRHCl content of microemulsion-based hydrogels and their pH and viscosity and values are indicated in Table 4. Also, the results of penetration measurements are presented in Table 4.

Table 4. Drug content, pH, viscosity and penetration value of the PRHCl microemulsion-based hydrogel formulations.

Formulation code	Drug content (%)	pH	Viscosity (Pas)	Penetration value (mm)
MEH PRHCl 1	99.85±0.25	4.63±0.52	1.31±0.08	164±0.85
MEH PRHCl 2	99.21±0.64	4.48±0.21	1.38±0.12	176±1.75
MEH PRHCl 3	98.45±0.63	4.07±0.48	1.45±0.06	183±2.06
MEH PRHCl 4	98.74±0.15	4.11±0.30	1.52±0.14	128±2.15
MEH PRHCl 5	99.53±0.84	4.52±0.27	1.58±0.09	131±1.23
MEH PRHCl 6	100.55±0.38	4.68±0.71	1.61±0.20	138±0.92
MEH PRHCl 7	99.13±0.56	4.36±0.12	1.64±0.17	113±1.83
MEH PRHCl 8	101.42±0.76	4.79±0.25	1.69±0.08	118±1.67
MEH PRHCl 9	102.13±0.28	4.87±0.41	1.71±0.19	114±0.72
MEH PRHCl 10	101.30±0.45	4.72±0.38	2.69±0.24	107±1.42

The results of spreadability measurements are presented as extensiometric curves in Figure 4.

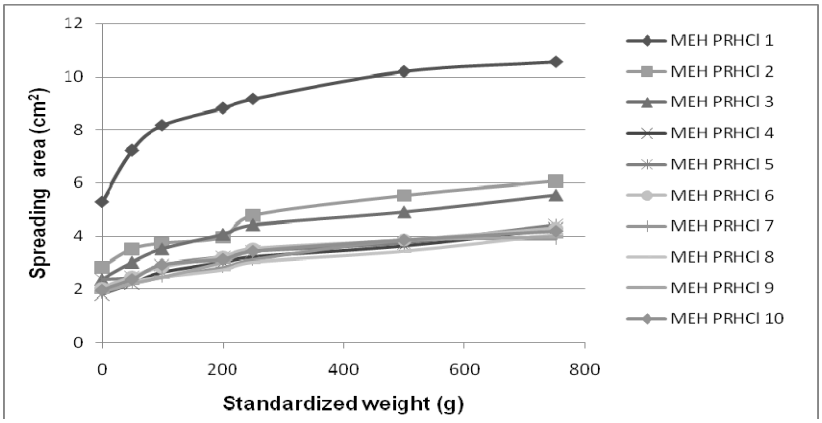

Fig. 4. Extensiometric curves of the studied propranolol hydrochloride microemulsion-based hydrogel formulations. Data shown as mean ±SD, which was less than 2% and is not presented in the interest of clarity.

In vitro drug release studies

In order to assess the formulations performance, the propranolol hydrochloride loaded microemulsion-based hydrogels were studied for *in vitro* drug permeation and release through synthetic membrane. The results are listed in Table 5, and illustrated in Figures 5 and 6.

Table 5. The permeation and release parameters of the propranolol hydrochloride-loaded microemulsion-based hydrogels through synthetic membrane.

Formulation code	Permeation parameters			Release parameters	
	J x10^{-2} (μg/cm^2/min)	K_P x 10^{-6} (cm/min)	t_L (min)	k (μg/cm^2/min$^{1/2}$)	D x 10^{-5} (cm^2/min)
MEH PRHCl 1	1.69±0.21	1.69	5.36±1.38	35.77±0.40	1.00
MEH PRHCl 2	1.89±0.07	1.89	3.22±2.44	30.34±0.02	0.72
MEH PRHCl 3	1.91±0.08	1.91	5.71±1.47	33.59±0.40	0.89
MEH PRHCl 4	1.79±0.12	1.79	4.15±0.15	39.42±0.39	1.22
MEH PRHCl 5	2.42±0.21	2.42	7.29±0.90	34.50±0.68	0.93
MEH PRHCl 6	2.16±0.06	2.16	4.72±0.87	38.76±0.05	1.18
MEH PRHCl 7	1.96±0.26	1.96	2.74±1.36	37.4±0.73	1.10
MEH PRHCl 8	2.05±0.10	2.05	4.45±1.15	42.66±0.52	1.43
MEH PRHCl 9	2.19±0.09	2.19	1.62±1.30	42.67±0.28	1.43
MEH PRHCl 10	2.19±0.07	2.19	2.28±1.27	42.53±0.37	1.42

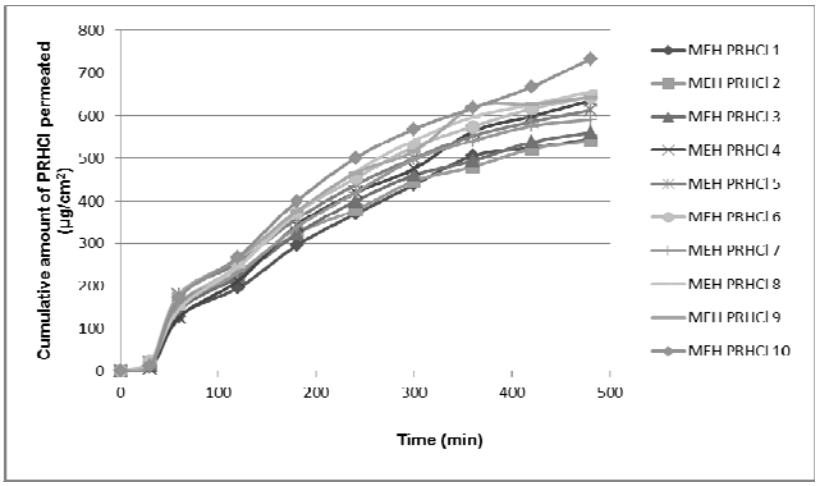

Fig. 5. In vitro propranolol hydrochloride permeation profile through synthetic membrane from microemulsion-based hydrogels (mean ± SD, n = 3).

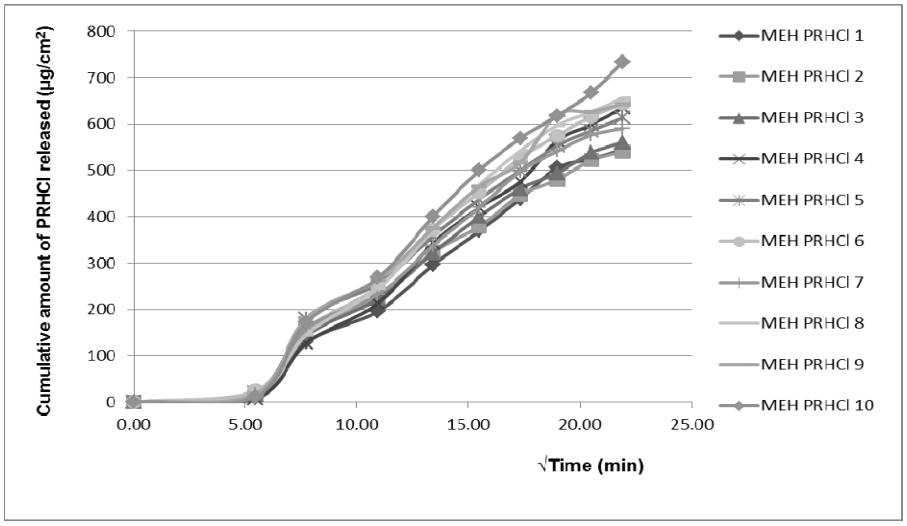

Fig. 6. In vitro propranolol hydrochloride release profile through synthetic membrane from microemulsion-based hydrogels (mean ± SD, n = 3).

In order to predict and evaluate the *in vitro* propranolol hydrochloride permeation behaviour from the studied microemulsion-based hydrogels through synthetic hydrophilic membrane, fitting into a suitable mathematical model is required. Data obtained from the *in vitro* drug permeation of the *MEH PRHCl* formulations were kinetically evaluated by various mathematical models like zero-order, first-order, Higuchi and Korsmeyer-Pepas model. The results of curve fitting into above mentioned mathematical models were evaluated by the highest correlation coefficient, and are presented in Table 6.

Table 6. Results of kinetic analysis of the in vitro permeation data of propranolol hydrochloride loaded microemulsion-based hydrogels.

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Pepas	
	K_0 (min^{-1})	R^2	K_1 (min^{-1})	R^2	K_H (min^{-1})	R^2	*n*	R^2
MEH PRHCl 1	0.0698	0.9548	0.0008	0.9703	1.3195	0.9290	0.5878	0.9999
MEH PRHCl 2	0.0660	0.9357	0.0008	0.9566	1.3874	0.9483	0.5002	0.9966
MEH PRHCl 3	0.0693	0.9421	0.0008	0.9621	1.4309	0.9430	0.5424	0.9952
MEH PRHCl 4	0.0798	0.9644	0.0010	0.9814	1.5555	0.9220	0.6336	0.9937
MEH PRHCl 5	0.0749	0.9363	0.0009	0.9603	1.5843	0.9516	0.4905	0.9997
MEH PRHCl 6	0.0798	0.9466	0.0010	0.9685	1.6442	0.9443	0.5625	0.9956
MEH PRHCl 7	0.0743	0.9411	0.0009	0.9614	1.5337	0.9416	0.5397	0.9994
MEH PRHCl 8	0.0828	0.9461	0.0010	0.9671	1.6757	0.9343	0.6092	0.9942
MEH PRHCl 9	0.0809	0.9356	0.0010	0.9580	1.6873	0.9427	0.5200	0.9976
MEH PRHCl 10	0.0894	0.9531	0.0012	0.9775	1.8050	0.9367	0.5597	0.9955

4. Discussion

Screening of formulations ingredients

Screening of oil and water

The solubility of PRHCl was found to be highest in Capryol 90, followed by oleic acid and castor oil and that in isopropyl myristate was relatively low. This may be attributed to the surfactant properties and low molecular volume of Capryol 90, a lipophilic product from novel semisynthetic medium chain derivatives category, having a great ability to dissolve large amounts of lipophilic and hydrophilic drugs. Further, formulation of microemulsion with oil of high drug solubility would require incorporation of less oil to incorporate the desired drug dose, which in turn would require lower surfactant concentration to achieve oil solubilization, which might increase the safety and tolerability of the system. Therefore, Capryol 90 was selected as the oil phase for the development of microemulsions containing PRHCl.

Screening of surfactants

Selecting of the surfactant is critical for the development of MEs, as it consider the surfactant effectiveness and toxicity. The effectiveness of surfactant is related to proper HLB value leading to the spontaneous formation of a stable ME formulation, while the toxicity is another important factor because the MEs formation usually requires large amounts of surfactants, which may cause skin irritation when administered topically. Therefore, it is clearly crucial to select the surfactant with proper HLB value, determine the surfactant concentration properly and use the minimum concentration in the formulation. Other important criteria for surfactant selection are the drug solubility and solubilization capacity of oil respectively. It is not necessary that the surfactant that has good solubilizing power for drugs would have equally good affinity for the oil phase [55].

In the present study, seven nonionic surfactants, namely Cremophor EL, Cremophor RH 40, Labrasol, Lansurf SML 20, Lansurf SMO 80, Lansurf SMO 81 and Tween 65 were chosen for screening. Nonionic surfactants were selected because of their low toxicity and irritation potential, stability and low sensibility on pH changes or in the presence of electrolytes or charged macromolecules. On the other hand, selection of surfactant was primarily governed by its solubilization efficiency for selected oil phase and its solubility potential for PRHCl was considered as an additional advantage.

The results of the solubility study involving the surfactants (Table 1) showed that Lansurf SMO 81 has the highest solubilizing potential for PRHCl, followed by Lansurf SMO 80, Lansurf SMO 20 and Cremophor RH 40. However, after selection of Capryol 90 as oil phase, the surfactant was selected based on the highest solubilization capacity for the oil phase (Capryol 90). Cremophor RH 40 and Cremophor EL solubilized similarly amounts of Capryol 90 (27.63% and

26.38% respectively, w/w), followed closely by Lansurf SMO 80 and Lansurf SMO 20 (22.61%), and Labrasol (18.84%), whereas Tween 65 and Lansurf SMO 81 (7.54%) appear to be poor solubilizers for Capryol 90 (Figure 1). The differences between surfactants in terms of ability to solubilize and emulsify Capryol 90, can be explained by HLB values. The surfactants having HLB values in the range of 14 to 16.7, namely Cremophor EL, Cremophor RH 40, Lansurf SMO 80, Lansurf SMO 20 and Labrasol were more effective than Tween 65 and Lansurf SMO 81 with lower HLB values (10.5 and 10 respectively). As Cremophor RH 40 solubilized the maximum amount of Capryol 90, it was selected as the surfactant for microemulsions development.

Screening of cosurfactants

Comparing the size of the microemulsion region in the phase diagrams obtained at a fixed ratio S_{mix} (1:1), keeping the surfactant the same but replacing the cosurfactant, it was observed a very slight enhancement in the microemulsion area when the chain length was increased from ethanol (Figure 2a) to isopropyl alcohol (Figure 2b). Also, increasing the number of hydroxyl groups from isopropyl alcohol to propylene glycol further enhanced the size of microemulsion region (Figure 2c). Propylene glycol further improved the microemulsification ability of Capryol 90 with added advantage of good solubilization potential for PRHCl over other two cosurfactants, and therefore was selected as cosurfactant.

Construction of pseudo-ternary phase diagram

The microemulsion region decreased slightly in size with the increasing of surfactant concentration of S_{mix} from 1:1 (Figure 3a) to 2:1 (Figure 3c) and 3:1 (Figure 3d). It might be due to insufficient cosurfactant concentration required at O/W interface in order to form microemulsion systems. In contrast, when cosurfactant concentration with respect to surfactant was increased to the S_{mix} 1:2 and 1:3, it was observed that the microemulsion area decreased as compared to S_{mix} 1:1. This slightly, but progressively reduction of microemulsion region was most likely due to a decrease in surfactant concentration by the increased amount of propylene glycol. Briefly, the largest microemulsion area was observed in S_{mix} 1:1 as compared to the other ratios, indicating that surfactant and cosurfactant weight ratio (S_{mix}) have marked effect on phase properties. i.e. size and position of microemulsion region.

Preparation of microemulsion-based hydrogel of propranolol hydrochloride (MEH-PRHCl)

Different gelling agents namely methylcellulose (Tylose MH 300), carboxymethylcellulose sodium salt, hydroxypropylmethylcellulose (Methocel K4M) and Carbopol ETD 2020 were evaluated for their potential to thick the PRHCL microemulsions. Selection of the suitable gelling agent was made on the basis of compatibility with microemulsions components. It was observed that cellulose derivatives were not able to gel the propranolol hydrochloride-loaded microemulsions. This inefficiency could be attributed to their susceptibility to coagulate in the presence of high concentrations of surfactants. Further, carboxymethylcellulose sodium being an anionic polymer is incompatible with propranolol hydrochloride which is a cationic drug. Similarly, in the case of Carbopol ETD 2020 it was noticed that the thickening activity of microemulsions could not be achieved after neutralization, i.e. adding triethanolamine, as is generally recommended. This abolition of carbomer gelling ability could be explained by the fact that neutralization ionizes the polymer and generates negative charges which interact with propranolol hydrochloride (cationic in nature) leading to the formation of an insoluble complex. However, a clear gel could be obtained if the neutralization was not performed.

Characterization of PRHCl microemulsions

The mean droplet size of propranolol hydrochloride microemulsions was found in the range of 6.089-12.97 nm (Table 3). For the formulations *ME-PRHCl 1-8* containing 4-30.5% water, the mean droplet size ranged between 6.089 and 7.023 nm, with no significant differences. The mean droplet size was lowest (formulation *ME-PRHCl 1*) when the concentration of both oil and S_{mix} were 4.25 and respectively 19.5 fold higher than water concentration. The mean droplet size doubled when the water concentration was higher than 30.5%. Hence, the formulation *ME-*

PRHCl 10 containing 39% water, 11% oil and 49% S_{mix} presented the highest average droplet size, followed closely by formulation *ME-PRHCl 9* having a similar composition (34% water, 12% oil and 53% S_{mix}). However, in all formulations the ratio between oil and S_{mix} remained constant. Due to the very small average droplet size of all studied microemulsions, their surface areas are assumed to be high; therefore, a better contact between the oil droplets and the membrane/skin can be accomplished, thus providing high concentration gradient and improved permeation of propranolol.

The values of polydispersity index observed for all the formulations (Table 3) were very low (0.030 to 0.168) which indicated that the microemulsion droplets were homogenous and had narrow size distribution.

The viscosity of microemulsion formulations (Table 3) tends to decrease with increase of the water content, but the differences between formulations were very small. Moreover, the viscosity of all formulations was low, which is expected as one of the properties of microemulsions is low viscosity.

The refractive index indicates the isotropy of the microemulsions, the mean values of refractive index ranged between 1.4073±0.06 – 1.4423±0.01 (Table 3). As water content was increased from 4 to 39%, the refractive index decrease from 1.4423 to 1.4073, due to the lower refractive index of water compared with that of other components of the formulations, i.e. oil or S_{mix}.

Zeta potentials of the studied microemulsion formulations were found in the range of 2.34±0.06 to 7.56±0.12 mV (Table 3). These small values indicated the stability of systems, as the globules aggregation is not expected to take place.

The pH values of all formulations were found in the range of 5.66±0.04 to 5.87±0.11 (Table 3), falling within the limits stipulated by pharmacopoeia.

Characterization of PRHCl microemulsion-based hydrogels

Determination of drug content and pH

The drug content evaluation of the microemulsion-based hydrogel formulations considered the range of 90-110% of the claimed drug content required by most pharmacopeial monographs of topical semisolid preparations. The PRHCl content of microemulsion preparations (Table 4) ranged from 98.45±0.63 to 102.13±0.28% of the theoretical value (1%, w/w), which complies with the pharmacopeial specifications for drug content. The obtained data indicated the uniform distribution of drug within the hydrogels.

The developed microemulsion-based hydrogels had pH values varying from 4.07±0.48 to 4.87±0.41, slightly lower than those of propanolol hydrochloride 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.

Rheological characterization

The viscosity values of microemulsion-based hydrogels were in the range from 1.31±0.08 Pas to 2.69±0.24 Pas, as shown in Table 4, indicating a slight increase with the water content. It was also observed that the viscosities of microemulsion-based hydrogel formulations increased significantly compared with those of microemulsions, due to the addition of 1.7% Carbopol EDT 2020, which made the preparations more suitable for topical administration.

Formulations *MBH-PRHCl 7*, *8*, *9* and *10* presented lower penetration values, indicating a higher consistency; in contrast, formulations *MBH-PRHCl 1, 2* and *3* had the highest penetration values, therefore the lowest consistency (Table 4).

Spreadability is a very important property of topical semisolid formulations since it indicates the facility of formulations applying on the skin or mucosa. It was found that higher spreading areas were obtained for *MEH PRHCl 1*, *2* and *3*, whereas the spreading areas of all other tested formulations were lower and almost the same (Figure 4). However, all formulations presented good spreadability, proved by relatively high values of spreading areas. Data were in accordance with the results of the penetration measurements.

The differences in consistency of the studied systems 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, loosed the gel matrix nature of microemulsion based hydrogel formulations (case of formulations *1*, *2* and *3*), while the increase of water content improved the gelling ability of carbomer, particulary in the case of formulations *7*, *8*, *9* and *10*.

In vitro drug release studies

As shown in Figure 5 and Table 5, lower permeation flux values in the range 1.69±0.21 to 1.91±0.08 μg/cm^2/min were observed in case of formulations *1*, *2*, *3* and *4*, which contained higher amounts of oil (15-17%) and S_{mix} (67-78%) and lower amount of aqueous phase (4-17%). All other formulations showed slightly higher permeation flux values, between 2.05±0.10 to 2.42±0.21 μg/cm^2/min, most probably due to the increase in the amount of aqueous phase in their composition. Among these microemulsion-based hydrogels, the higher permeation flux of 2.42±0.21 μg/cm^2/min was observed in case of formulation *MEH PRHCl 5*, followed by formulations *MEH PRHCl 9* and *10*.

The lag time values did not varied in all the cases as was expected, namely longer lag time values in case of slow diffusion. Thus, longer lag time values (from 4.15±0.15 min to 5.71±1.47 min) were observed in case of formulations *1*, *3* and *4*, which presented lower flux values, but also in case of formulations *5*, *6* and *8*, characterized by faster diffusion (Table 5); the highest lag time value (7.29±0.90 min) was obtained for the formulation *MEH PRHCl 5*. The shortest lag time was calculated for the formulation *MEH PRHCl 9* (1.62±1.30), followed by the formulation *MEH PRHCl 10*, which presented higher but identical flux values. Also, for the hydrogels *2* and *7* higher lag time values were calculated, although these formulations showed a slow diffusion.

As was expected, the values of release rate of PRHCl from the microemulsion-based hydrogels were found to range between 30.34±0.02 and 42.67±0.28 μg/cm^2/min$^{1/2}$, indicating that the release was significantly affected by their composition. The release rate of hydrophilic *PRHCl* increased with water content, displaying the highest values in case of formulations *8*, *9* and *10* with considerable amounts of water (30.4-38.8%); at water content of 3.99-10.98%, *MEH* formulations *1*, *2* and *3* resulted in lower release rate values (35.77±0.40, 30.34±0.02 and 33.59±0.40 μg/cm^2/min$^{1/2}$ respectively). The differences observed between release profiles of *MEHs PRHCl* revealed that apart from the contribution of water content in enhancing drug release, the varying oil and surfactant content might be responsible for improved drug permeation.

In order to correlate the *in vitro* permeation results with the release results, a ranking of formulations was made, based on the flux values (*MEH PRHCl 5 > MEH PRHCl 9 > MEH PRHCl 6 > MEH PRHCl 10 > MEH PRHCl 8 > MEH PRHCl 7 > MEH PRHCl 3 > MEH PRHCl 2 > MEH PRHCl 4 > MEH PRHCl 1*) and the release rate (*MEH PRHCl 9 > MEH PRHCl 8 > MEH PRHCl 10 > MEH PRHCl 4 > MEH PRHCl 6 > MEH PRHCl 7 > MEH PRHCl 1 > MEH PRHCl 5 > MEH PRHCl 3 > MEH PRHCl 2*). As can be observed, in both ranks among the first five formulations are situated *MEH PRHCl 8*, *9* and *10* respectively, presenting high flux values (2.05±0.10 – 2.19±0.07 μg/cm^2/min) and also the highest release rate values (42.53±0.37 – 42.67±0.28 μg/cm^2/min$^{1/2}$). Similarly, the formulations *1*, *2* and *3* with low flux values (1.69±0.21 – 1.91±0.08 μg/cm^2/min) and low release rate values (30.34±0.02 – 35.77±0.40 μg/cm^2/min$^{1/2}$) are situated at the end of this rank. Another kind of flux-release rate relationship, namely inverse variation, was observed in case of formulations *4* and *5*. Thus, the flux value for *MEH PRHCl 4* was low (1.79±0.12 μg/cm^2/min) and very closed to those of formulations *1*, *2* and *3*, but the release rate presented high value (39.42±0.39 μg/cm^2/min$^{1/2}$) situated among the first five in above mentioned rank. Formulation *5* showed the highest flux value, but lower release rate value (34.50±0.68 μg/cm^2/min$^{1/2}$) than those of formulation *2* and *3*.

Furthermore, the release rate was greater than transmembranar flux in all cases. Taking all these into consideration, it is evident that the delivery of propranolol hydrochloride from microemulsion-based hydrogels through synthetic hydrophilic membrane is dependent on the rate of its release from the formulations. In addition, the deviations from linear flux-release rate relationship highlighted that all formulation variables (oil, S_{mix}, water and gelling agent content) significantly influenced the processes governing the *in vitro* permeation and release of propranolol hydrochloride from the studied microemulsion-based hydrogels through synthetic hydrophilic membrane (eg. partitioning of hydrophilic drug both in the phases of the L/H microemulsion based

hydrogel and from this system to the membrane surface, diffusion of drug through the vehicle and membrane).

The results of kinetic analysis (Table 6) showed that propranolol hydrochloride release from all formulations best fitted to Korsmeyer-Pepas model ($R^2 > 0.99$), suggesting that the main drug release mechanism is diffusion. Moreover, the analysis of the first 60% of drug release data using again the Korsmeyer-Pepas model was perform 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 n values for all *MEH* formulations ranged between 0.4905 and 0.6092, the propranolol hydrochloride release from these systems followed non-Fickian, "anomalous" transport, which appears to be driven by a combination of two processes, diffusion and erosion.

4. Conclusions

In summary, in this research paper several microemulsion-based hydrogels were developed and evaluated for their potential as topical delivery systems for PRHCl, a hydrophilic drug presenting extensive first-pass metabolism and short elimination half-life after oral administration, but also a poor percutaneous penetration. The results showed that the content of microemulsion based hydrogels components (oil, S_{mix} and water) had significant effect on their physical, rheological and *in vitro* drug release characteristics.

It were considered as most desirable formulations the microemulsion-based hydrogels *9* and *10* containing Capryol 90 (12% and 11% respectively) as oil phase, S_{mix} (2:1) Cremophor RH 40-propyleneglycol (53% and 49% respectively) as surfactant-cosurfactant, Carbopol EDT 2020 (1.7%) as gelling agent, and water (33.96% and 38.87% respectively), since they exhibited high flux value (2.19 μg/cm^2/min), highest release rate values (42.67±0.28 μg/cm^2/min$^{1/2}$ and 42.53±0.37 μg/cm^2/min$^{1/2}$ respectively), shortest lag time values (1.62±1.30 min and 2.28±1.27 min respectively) and lowest surfactant content. These formulations also possessed the globule size of 12.31 nm and 12.97 nm respectively, the polidispersity index of 0,117 and 0.168 respectively, and zeta potential of 6.65 mV and 7.56 mV respectively.

However, further *in vitro* and *in vivo* studies need to be performed for developing commercially viable topical microemulsion based hydrogel formulation of propranolol hydrochloride.

Acknowledgement

The authors are thankful to S.C. Sintofarm S.A (Bucharest, Romania) for the gift sample of propranolol hydrochloride, BASF Chem Trade GmbH (Germany) for providing Cremophor EL and Cremophor RH 40 as gift samples, Gattefossé (Lyon, France) for providing the free samples of Capryol 90 and Labrasol, Cognis GmbH (Düsseldorf, Germany) for the gift of isopropyl myristate, Lankem L.t.d. (UK) for providing gift samples of surfactants (Lansurf SML 20, Lansurf SMO 80 and Lansurf SMO 81), Colorcon L.t.d. (UK) and Lubrizol Advanced Materials (USA) for providing hydroxypropylmethylcellulose (Methocel K4M) and Carbopol ETD 2020 respectively as free samples.

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