

## A Novel Pilocarpine Microemulsion as an Ocular Delivery System: *In Vitro* and *In Vivo* Studies

Iskender Ince<sup>1\*</sup>, Ercument Karasulu<sup>1,2</sup>, Halil Ates<sup>3</sup>, Altug Yavasoglu<sup>4</sup> and Levent Kirilmaz<sup>2</sup>

<sup>1</sup>Center for Drug R&D and Pharmacokinetic Applications, 35100, Bornova, Ege University, Izmir, Turkey

<sup>2</sup>Department of Biopharmaceutics and Pharmacokinetics, Ege University, 35100, Bornova, Izmir, Turkey

<sup>3</sup>Department of Ophthalmology, Ege University, 35100, Bornova, Izmir, Turkey

<sup>4</sup>Department of Histology & Embryology, Ege University, Faculty of Medicine, 35100, Bornova, Izmir, Turkey 35100

\*Corresponding author: Iskender Ince, Ph.D, Center for Drug R&D and Pharmacokinetic Appl., 35100, Bornova, Ege University, Izmir, Turkey, Tel: +902323392754; E-mail: iskender.ince@ege.edu.tr

Received date: Feb 09, 2015, Accepted date: Mar 25, 2015, Published date: Mar 30, 2015

Copyright: © 2015 Ince I, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

Despite several disadvantages like a rapid wash out and dilution of the formulation leading to a low bioavailability, eye drops are the most commonly used dosage form for the ocular route. Due to their properties and numerous benefits, microemulsions are promising systems for topical drug delivery.

The purpose of this work is to develop a suitable ocular microemulsion formulation with adequate physicochemical stability for enhancing the bioavailability of pilocarpine. Physicochemical characteristics of the microemulsion including the drug content, refractive index, conductivity, viscosity, intraocular pressure (IOP) and ocular tolerance were investigated. The ocular irritation test and the IOP lowering activity of the microemulsion were studied in New Zealand white rabbits.

The developed formulation showed good physicochemical properties and a beneficial stability for six months. After microemulsion instillation into the rabbit eyes, the intraocular pressure was reduced significantly. The ocular irritation test used suggested that microemulsion formulation did not cause any significant allergies to the eye.

**Keywords:** Pilocarpine; Intraocular; Microemulsion; Glaucoma; Bioavailability; Rabbit

### Introduction

Standard treatment of ocular diseases is mainly performed by topical application (90%), consisting of eye drops in the form of aqueous solutions. Due to the natural precorneal cleaning of the eye and high tear fluid turnover, these facts produce the major problems related to topical drug application to the eye. Only 1-5% of the applied drug penetrates into the cornea and reaches therapeutic concentrations in intraocular tissues. The challenging objective aimed at dealing with these problems is to develop topical drug delivery systems with improved ocular retention, increased corneal drug absorption and reduced systemic side effects [1]. In conventional ophthalmic dosage forms, water-soluble drugs are available as aqueous solutions, while water-insoluble drugs are available as suspensions, ointments or gels. Low corneal bioavailability and lack of efficiency in the posterior segment of ocular tissue are some of the serious handicaps related to these ocular systems. Recent research efforts have focused on the development of new and more effective drug delivery systems such as nanotechnology-based formulations like nanoemulsion/microemulsion, nanosuspension, solid lipid nanoparticle etc [2-4].

Microemulsions were preferred owing to their easy and cheap production and excellent stability. Microemulsions have emerged as a promising application form for ocular usage. They are clear, isotropic mixtures of oil, water and a surfactant frequently in combination with

a co-surfactant. Moreover, since they are composed of aqueous and oily components, they can accommodate both hydrophilic as well as lipophilic drugs [5]. Microemulsions of many ocular drugs like ofloxacin, timolol and prednisolone were successfully prepared with sustained effect and better bioavailability [6-8].

Glaucoma, which is the increased intraocular pressure, exhibits a group of eye diseases leading to the damage of optic nerves, leading to blindness as a worst-case scenario. Decreasing high IOP is the only effective approach that is currently available for treating this disease and protecting the eye from later consequences [9].

A hygroscopic, odorless, bitter tasting drug in the form of white crystals or powder, pilocarpine hydrochloride (PHCl) is soluble in water and alcohol but virtually insoluble in most non-polar solvents. PHCl {(IUPAC: (3*S*-*cis*)-2(3*H*)-furanone-3-ethylidihydro-4-[(1-methyl-1*H*-imidazol-5-yl) methyl] monohydrochloride, Mr=244}. PHCl is a miotic drug used to control and reduce the IOP, if necessary. Ocular bioavailability of topically applied PHCl amounts to 0.1-3% if the drug is administered three to four times per day as eye-drops; and that impairs patient compliance. The poor bioavailability is attributed to the low lipophilicity of pilocarpine, to loss of the drug from the precorneal area via drainage and to very fast dilution of the formulation [10].

The aim of this work was to develop a novel microemulsion for eye drops for topical ocular administration, by using PHCl as a model drug and to evaluate its physicochemical characteristics, such as stability, permeation, ocular irritation and IOP lowering activity.

## Experimental

PHCl was kindly supplied by Bilim Pharmaceuticals (Turkey). Brij 35P, Span 80, and soybean oil were purchased from Fluca (Switzerland). 1-Butanol and sodium chloride were purchased from Riedel-de Haën (Germany),  $\alpha$ -tocopherol was kindly provided by Roche (Turkey). Phenol and cyclohexane were purchased from J. T. Baker (The Netherlands). Almond oil was purchased from Cagdas Laboratories (Turkey). All other chemicals used were of analytical grade.

### Preparation of the microemulsions

The microemulsion was prepared following a procedure called the titration method. Soybean oil was used as the oil phase, Brij 35P and Span 80 were the surfactants (S) and 1-butanol was used as the co-surfactant (CoS). Brij 35P/Span 80 ratio was 1:200 (m/m). First, Brij 35P was dissolved in 1-butanol at 25°C and then mixed with Span 80. Then, this mixture was added to an appropriate amount of soybean oil. The microemulsion formulation studies were carried out by titrating slowly with PHCl solution while stirring the mixture with a bar using a magnetic stirrer (IKA, Germany) (100 rpm) until a turbidity was observed. The final concentration of PHCl in our microemulsion was 2%.

Pseudo-ternary phase diagrams were constructed to obtain the concentration range of the components for the existing range of microemulsions. For each phase diagram, mixtures of soybean oil and surfactant/co-surfactant concentrations were prepared at mass ratios of 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2. These mixtures were titrated with water drop-by-drop, with continued stirring at 25 ± 2°C until the mixture became clear. The mixtures were assessed by visual characterization after being equilibrated. Typical microemulsion vehicles were selected and prepared at different component ratios after the microemulsion regions in the phase diagram were detected with the aid of phase diagrams drawn using a computer program developed by Ege et al. [11]. The composition of the microemulsion formulations is given in (Table 1). PHCl was dissolved in water and slowly incorporated into the microemulsion under stirring. After PHCl was entirely dissolved in the microemulsion, the clear microemulsion-based formulation was obtained. The final concentration of PHCl in microemulsion was 2% (m/m). PHCl microemulsions were sterilized using an aseptic membrane filtration technique.

	Best formulation components	
	% (w/w)	(w/w) /10 g
Soybean oil	37.12	3.64
Span 80	29.54	2.9
Brij 35P	0.15	0.02
1-butanol	29.69	2.91
Water	3.50	0.33
Pilocarpine hydrochloride	-	0.2

**Table 1:** Percentage weight and batch composition of microemulsion formulation in the presence or absence of PHCl.

### Characterization of the microemulsions

The microemulsions were analyzed for various physicochemical attributes. The average droplet size and polydispersity index (PDI) of microemulsions in the presence or absence of PHCl were measured by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, UK). The viscosities of microemulsions were measured at 25 ± 2°C using a viscosimeter (ULA spindle, equipped with a model ULA-40Y water jacket, DV-II-Pro Brookfield, USA). The refractive index of microemulsions was evaluated at 25 ± 2°C using a refractometer (Abbe Refractometer, Atago, Japan). Electrical conductivity of the microemulsions was studied at 25 ± 2°C using a conductometer and conductometer probe (Mettler Toledo, Switzerland). Experiments were carried out three times for each sample, and the results were presented as a mean ± SD.

### Stability of microemulsions

Microemulsion formulation was stored at 4 ± 1, 25 ± 2 and 40 ± 2°C in a dark setting for 6 months. The physical stability of microemulsions containing PHCl was determined via clarity, phase separation observation, droplet size, refractive index, viscosity and electrical conductivity. For the chemical stabilities, concentration of PHCl in the formulations was also investigated by HPLC analysis at 4 ± 1, 25 ± 2 and 40 ± 2°C for up to 6 months.

### *In vitro* permeation studies

Diffusion cells were used for the permeability studies of PHCl. Synthetic membrane (cellulose, Mr 12,000) was mounted on a glass diffusion cell. Before starting the experiment, the cellulose membrane was first hydrated in an isotonic phosphate buffer solution (PBS) with pH 7.4 at room temperature for 30 min. These cells provided a diffusion area of 1.326 cm<sup>2</sup>. PBS pH 7.4 (10 mL, 600 rpm, 37°C) was used in the receptor compartment. The donor compartment contained 1 mL microemulsion (M) (containing 2% (m/m) PHCl) or commercial gel (G) (containing 4% (m/m), PHCl) formulation. Approximately 10 mL of the receptor medium was withdrawn at predetermined intervals (after 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min), and replaced immediately with an equal volume of receptor solution to maintain a constant volume. All samples were filtered through a membrane filter (0.2 micron, 25 mm Nylon, Millipore Millex-GN), and immediately injected into an HPLC system. Three replicates of each experiment were performed. All experiments were performed at 25 ± 2°C. Sink conditions were maintained in the receptor compartment during *in vitro* permeation studies.

### HPLC analysis of pilocarpine hydrochloride

The samples were analyzed using the HPLC (HP Agilent 1100 series) system that included a quaternary pump, 100  $\mu$ L loop, automatic electronic degasser, automatic thermostatic column and UV detector. The column was a Supelco-C18 column (4.6 mm × 150 mm, 5  $\mu$ m). The mobile phase contained acetonitrile and phosphate buffer (50+50 (V/V) (pH 7.4)). The flow rate was adjusted to 0.3 mL min<sup>-1</sup>, and the injection volume was 100  $\mu$ L.

### *In vivo* studies

Experimental glaucoma studies (n=20) and the tolerance test (n=6) were conducted using both male and female New Zealand albino rabbits weighing 1.5-2.5 kg. All animals were healthy and free of

clinical symptoms. The study was approved by the Animal Ethical Committee of Ege University, Turkey. Animals were housed in a room maintained at  $22 \pm 1^\circ\text{C}$  with an alternating 12 h light-dark cycle. Animals were orally fed daily with a normal diet in a standard laboratory chow. They were fed on balanced diet pellets. Tap water was also available ad libitum. The animals were transported to a quiet laboratory at least 1 h before the experiment. The rabbits were kept in restraining boxes throughout the course of each experiment. All tests were performed in an air-conditioned, illumination-controlled room ( $22 \pm 1^\circ\text{C}$ ).

### IOP lowering activity studies

In the rabbit eyes, the glaucoma was induced experimentally by means of subconjunctival injection of a sclerosing solution of 5% (m/V) phenol in almond oil, which caused an increase in IOP, but no apparent macroscopic or microscopic damages occurred in the eye. Glaucoma in rabbits is caused artificially by subconjunctival application of phenol (5%) in almond oil 3 times every 15 days [12,13]. When a measurement of the intraocular pressure of the right rabbit eye was repeated twice and the results were identical, the experiments were started.

The rabbits with induced glaucoma were divided into two groups each with ten rabbits. Each group was designated to receive one of the formulations: microemulsion (ME), commercial collyrium (C), or commercial gel (G). M, C and G contain 2% (m/V), 2% (m/m) and 4% (m/m) PHCl, respectively. M, C and G formulations were applied as a single dose (25  $\mu\text{L}$ ) into the right eyes of rabbits. No drug was administered to the contra-lateral eye (left), and they were considered as controls. The initial IOP (zero reading) of both eyes for each rabbit was measured. After the instillation of different formulation samples, the ocular bioavailability of PHCl was assessed by measuring IOP in both eyes after 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min. IOP of rabbit's eye was measured using a standardized Schiotz tonometer (Oculus, Optikgerate GmbH, Germany)

### Ocular tolerance test

This test was carried out in the eyes of six rabbits for both microemulsion without drug and physiological saline (NaCl) 0.9% (m/V). A volume of 25  $\mu\text{L}$  of the microemulsion was instilled in to the conjunctival sac of the right eye and 25  $\mu\text{L}$  of the physiological saline was instilled in to the conjunctival sac of the left eye of each rabbit. During the ocular irritation test on rabbit eyes, two times applications per day of one drop were carried out for 5 days for group one. On the other hand, eight-times applications of one drop every hour for 8 hours were carried out for 5 days for group two, resulting in a decreased reaction on the eye.

Pre-exposure and post-exposure evaluations of the eye lids, conjunctiva, cornea and iris were performed by external observation with proper illumination. Additional information was provided by slit-lamp biomicroscopy [14]. The evaluations were made 72 and 120 h after exposure to the sample. A scale of weighted scores for grading the severity of ocular lesions was used. Afterwards, one drop of 2% m/V fluorescein sodium (in water) was applied to the treated eyes of the rabbits and the surface of the cornea was observed by using the slit-lamp.

### Histological studies

Then the rabbits were sacrificed at the end of ocular tolerance test and the eyes were enucleated. Each eyeball was fixed in a glutaraldehyde-formalin solution for approximately 24 h, and washed with tap water for a night. Calcium deposits or ossified region of eyeballs were decalcified with a decalcification solution (20% sodium citrate+45% formic acid, 1:1, V/V) for 3 days, and washed with tap water during night. Otherwise, each globe of the eye was dehydrated through an increasing ethanol series, immersed in xylene and was finally fixed in paraffin wax at  $56^\circ\text{C}$ . Paraffin blocks were cut serially in 5  $\mu\text{m}$  slices using a rotary microtome (RM 2145, Leica Co., Germany). Sections were stained with haematoxylin and eosin (H&E) and examined by a light microscope (Olympus BX-51, Japan).

### Statistical analysis

Repeated measure analysis of variance was used for the evaluation of the pharmacodynamics response with regard to treatment groups. Difference between the groups was determined as statistically significant for 0.05 significant level. Tukeys's LSD procedure was used for post-hoc analyses.

## Results and Discussion

### Preparation of microemulsions

The aim of the construction of pseudoternary phase diagram was to find out the existence range of microemulsions. Pseudoternary phase diagrams were with various mass ratios of soybean oil, Span 80, Brij 35P, 1-butanol and water. Optimum formulation was found surfactant to the co-surfactant ratio 1:8 (m/m) for W/O microemulsion (Figure 1).

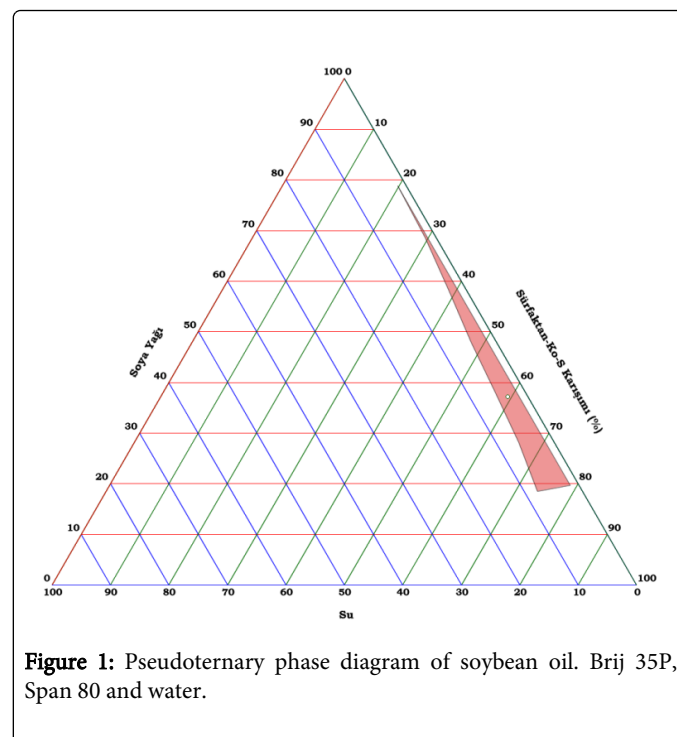


Figure 1: Pseudoternary phase diagram of soybean oil, Brij 35P, Span 80 and water.

The area of W/O ME became enlarged and the optimum formulation was obtained at its highest level. The exact composition according to oil, S, coS and aqueous phases was shown (Table 1).

### Characterization of the microemulsions

The characterization parameters of microemulsions are listed in (Table 2). In the absence of PHCl, the average droplet size of microemulsion was 0.709 nm PDI 0.219. However, in the presence of 2% m/m PHCl, the average microemulsion droplet size was 0.695 nm PDI 0.432. These findings support a recent study that found the mean droplet size was significantly decreased after loading the drug [15]. The PDI value described the homogeneous of the droplet size. All PDI values were smaller than 0.5 pointing to the fact that droplets were homogeneous. On the other hand, viscosity of microemulsions was found to be  $2,184.10^{-3} \pm 1.10^{-5}$  cP. The average refractive index of microemulsions was  $1.442 \pm 0.002$ . Incorporation of PHCl into microemulsion increased refractive index.

Parameter	Microemulsion (mean $\pm$ SD)
Refractive index	$1.442 \pm 0.002$
Conductivity (mS)	$4.50 \pm 0.08$
Viscosity (mPas)	$21.84 \pm 0.01$
Particle size PDI	0.695 nm pdi; 0.432

**Table 2:** Physicochemical properties of the developed microemulsion (n=3).

The electrical conductivity of microemulsions was found to be  $4.50 \pm 0.008$ . There was a strong correlation between the specific structure of the microemulsions and their electrical conductivity behavior [16]. According to the conductivity measurements, the investigated microemulsions could be divided into W/O and O/W. The conductivity of microemulsion samples showed that PHCl microemulsion is of (W/O) type. If there was no significant difference in the conductivity after storage, it indicated that the external phase is oily and that this phase did not change during the storage.

Incorporating the co-surfactant into the microemulsion resulted in a significant reduction in the viscosity of the formulations, with the flow changing to a simple Newtonian flow. Because of a direct relation between the shear stress and shear rate, it could be proven that our microemulsion is a Newtonian fluid. The Newtonian property of PHCl microemulsions is compatible with reports in the literature [17].

The refractive index of a substance at a certain temperature and density reflects its purity. A microemulsion stored at three different temperatures up to 6 months showed no significant differences of the refractive index, indicating a constant structure of the microemulsion (Table 3). The refractive index of the tear fluid is about 1.34-1.36 [7]. PHCl containing microemulsion had a refractive index of 1.441-1.442. Both refractive indices were similar, which ensured an ocular application without adverse effects to the eye capacity.

Zurowska et al. [18] have reported about the good chemical stability of a submicron emulsion containing PHCl prepared at pH 5 and stored at 4°C for 6 months. In this study, it was found that PHCl degraded with first-order kinetics. With the help of the Arrhenius equation  $t_{90}$  values at three different temperatures were calculated as 455 days for 4°C, 227 days for 25°C and 152 days for 40°C. The best

conditions for preparing PHCl containing eye drops were pH of 4-5.5, concerning its chemical stability. The microemulsion used in this study was prepared at a pH of 4.6.

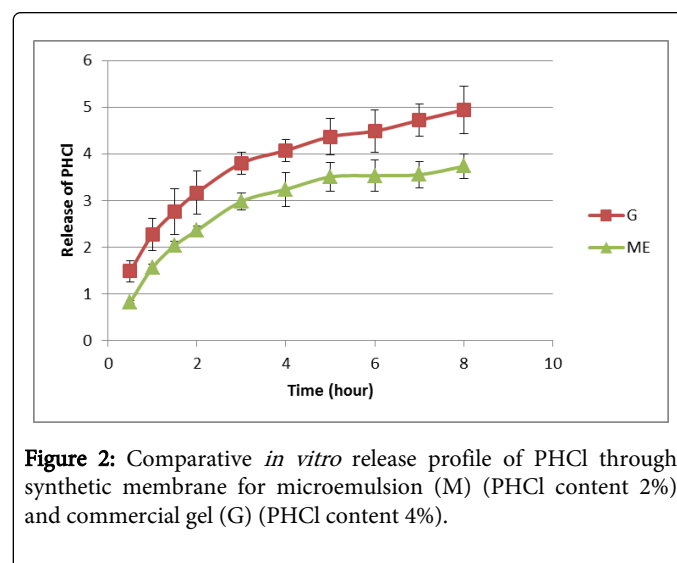
Parameter	Time	Temperature		
		4°C	25°C	40°C
Drug content (%)	1 month	$99.68 \pm 0.15$	$99.34 \pm 0.13$	$97.01 \pm 0.17$
	2 months	$99.37 \pm 0.21$	$98.64 \pm 0.10$	$95.23 \pm 0.20$
	3 months	$98.85 \pm 0.19$	$97.37 \pm 0.19$	$92.92 \pm 0.27$
Electrical conductivity (mS)	1 month	$4.51 \pm 0.08$	$4.52 \pm 0.07$	$4.50 \pm 0.06$
	3 months	$4.52 \pm 0.08$	$4.52 \pm 0.07$	$4.51 \pm 0.09$
	6 months	$4.51 \pm 0.06$	$4.52 \pm 0.08$	$4.50 \pm 0.10$
Refractive index	1 month	$1.442 \pm 0.001$	$1.441 \pm 0.001$	$1.442 \pm 0.000$
	3 months	$1.442 \pm 0.002$	$1.442 \pm 0.003$	$1.442 \pm 0.001$
	6 months	$1.441 \pm 0.002$	$1.442 \pm 0.001$	$1.442 \pm 0.001$
Viscosity (mPas)	1 month	$22.23 \pm 0.06$	$21.66 \pm 0.23$	$21.94 \pm 0.02$
	3 months	$22.05 \pm 0.09$	$22.11 \pm 0.06$	$22.17 \pm 0.08$
	6 months	$22.43 \pm 0.08$	$22.45 \pm 0.10$	$22.60 \pm 0.06$

Considering the initial concentration of 2% w/v as 100%.

**Table 3:** Stability results of the monitored parameters for the developed microemulsion (mean  $\pm$  SD n=3).

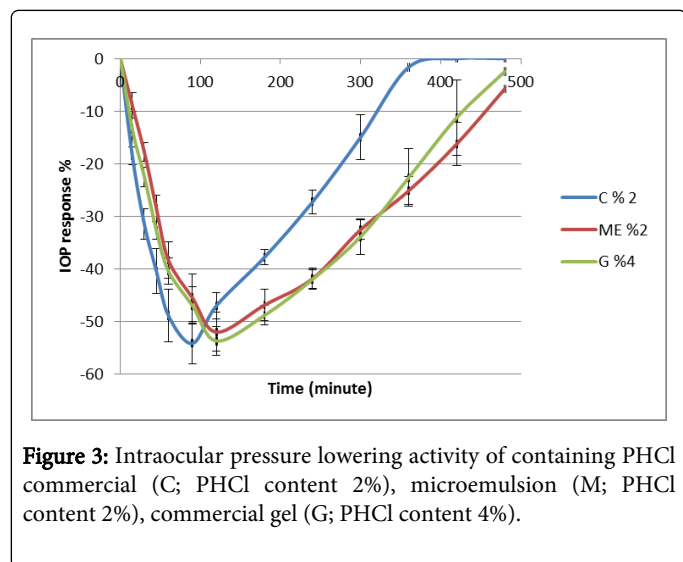
### *In vitro* permeation studies

The permeation of M, and G, both containing 4% (m/m) PHCl, were compared *in vitro* using a cellulose membrane. 4% PHCl containing gel had a higher and faster drug release compared to that of microemulsion containing 4% PHCl (Figures 2 and 3).



**Figure 2:** Comparative *in vitro* release profile of PHCl through synthetic membrane for microemulsion (M) (PHCl content 2%) and commercial gel (G) (PHCl content 4%).

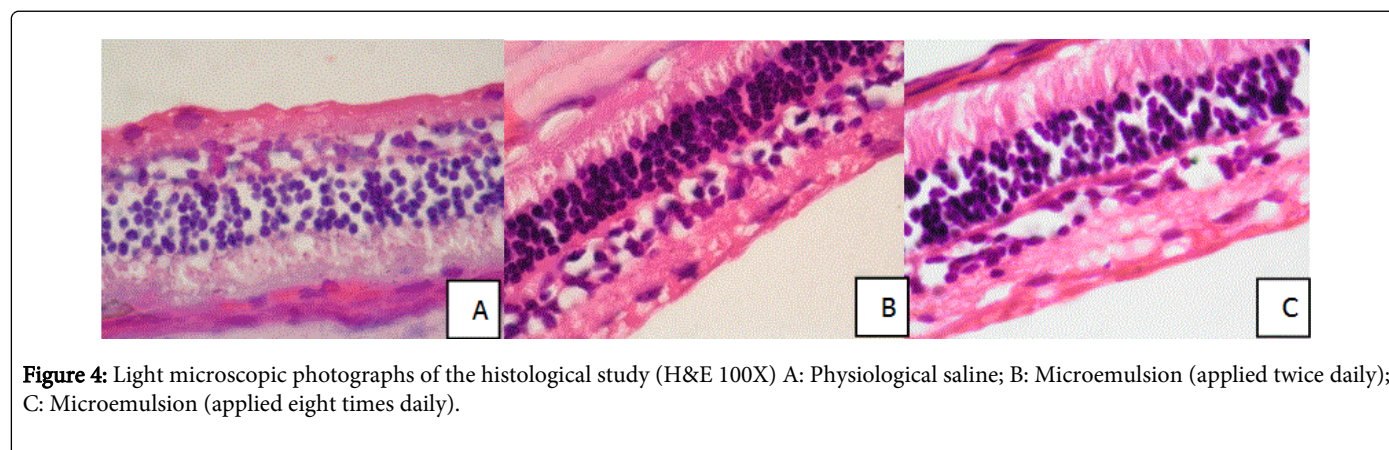
The method of diffusion cell is not representative of the real situation *in vivo* because cellulose membrane cannot mimic the barriers of corneal membrane and the constant volume of diffusion cell will not be able to eliminate the drug released by tear fluid turnover.



**Figure 3:** Intraocular pressure lowering activity of containing PHCl commercial (C; PHCl content 2%), microemulsion (M; PHCl content 2%), commercial gel (G; PHCl content 4%).

### HPLC analysis of pilocarpine hydrochloride

The detection wavelength was 215 nm and the retention time was 6.6 min. No interference of the other formulation components was observed. The peak area was correlated linearly with PHCl concentration in the range of 0.2-2.0 mg mL<sup>-1</sup> with the limit of detection (LOD) at 0.008 mg mL<sup>-1</sup>, and the average correlation coefficient was 0.999.



**Figure 4:** Light microscopic photographs of the histological study (H&E 100X) A: Physiological saline; B: Microemulsion (applied twice daily); C: Microemulsion (applied eight times daily).

### Ocular tolerance test

Physiological saline served as control and caused no irritation for both doses. Surfactants often cause a local irritation resulting from an ocular application. Due to these facts, the surfactants must be chosen carefully. Furrer et al. [19] examined the potential ocular irritation of several surfactants on rabbit and rat eyes in comparison to physiological serum. They showed that 0.5% Brij 35P caused almost no irritation, just like physiological saline. That's why it was decided that it could be used as a suitable surfactant.

### *In vivo* studies

There are several anatomic and physiological similarities between human and rabbit eye. The results of this investigation were expressed by decreases in percent IOP (Figure 4). Each time point results were calculated in relation to the IOP before the therapy started (time 0) for prevents or minimizes errors caused by intra-day deviations.

Considering pharmacodynamic properties of PHCl containing commercial C, G and ME formulations, it reveals that the maximum effect of C was achieved after 90 min, in contrast to a G and ME with the maximum effect observed after 120 min. After the application of C to the eye, the IOP reverts 6 hours later to the original value, whereas using G or ME that time accounts two hours longer, namely 8 hours. That means that the effect of ME and G lasts much longer compared to commercial collyrium, in contrast to the microemulsion with 2% (m/m) PHCl.

Sznitowska et al. [10] prepared submicron emulsions containing pilocarpic acid mono- and di-ester as a prodrug. They determined the miotic effect on rabbit eyes, and compared them with solutions of 0.5 and 2% PHCl. Using the solutions, C<sub>max</sub> was achieved after 30 min. and for the submicron emulsion with the prodrug after 90 min. The miotic effect of the two solutions decreased after 6 hours while the same effect of the submicron emulsion was for the same time. C<sub>max</sub> observed for PHCl microemulsion after 120 min. After the application of microemulsion PHCl to the eye, the IOP reverts 8 hours later to the original value. The obtained results of PHCl microemulsion were very similar to the results of the submicron emulsion [13].

The pharmacological effects of ME and commercial gel were found delayed and lasted longer than commercial collyrium because both of them could improve retention and prolonged release of incorporated drug.

### Histological studies

There were no histopathological observations on the eyes treated with physiological saline as the control group (Figure 4A). When the prepared microemulsion formulation was applied twice a day, every morning and evening (low dose) for 5 days (Figure 4B), just like on the control eyes treated with physiological saline, there were no histological pathology so this application is non-irritant. However, when the same formulation was applied 8 times with 1-hour intervals for 5 days (high dose) (Figure 4C), edema, bleeding and related flattening were observed on the pigment epithelium cells at the retina.

In addition to these, leukocyte infiltration and inflammation were observed at the ciliary body and cornea. Second application of the microemulsion caused irritation in the eye.

### Statistical analysis

According to post-hoc analysis, it was determined that the difference between “microemulsion” group and “gel” group was not statistically significant. On the other hand, the differences between the “commercial collyrium” and both “microemulsion” and “commercial gel” groups were significant (Tables 4 and 5).

Treatment group	Mean
Colyr	35.015
Gel	33.268
Microemulsion	33.655

**Table 4:** The mean values of treatment groups.

Parameter	Score
Significant level (a)	0.05
Degrees of freedom	16
Sum of Squares	6.338
Least significant difference	0.849

**Table 5:** Results of the comparison between the microemulsion and the gel using the TUKEY LSD method.

### Conclusions

In conclusion, microemulsion formulation containing PHCl decreased the IOP, proven by a glaucoma rabbit model. It showed that a satisfying stability and low dose applications caused no irritation and no pathological effects on the eye. According to the obtained results, it can be concluded that microemulsion formulation may be a suitable and serious possibility for ocular application of drugs in the future.

### Acknowledgments

This project was supported by the Research Fund of Ege University and performed in Ege University Center for Drug R&D and Pharmacokinetic Applications.

### References

1. Alany RG, Rades T, Nicoll J, Tucker IG, Davies NM (2006) W/O microemulsions for ocular delivery: evaluation of ocular irritation and precorneal retention. *J Control Release* 111: 145-152.

2. Sahoo SK, Dilnawaz F, Krishnakumar S (2008) Nanotechnology in ocular drug delivery. *Drug Discov Today* 13: 144-151.

3. Diebold Y, Calonge M (2010) Applications of nanoparticles in ophthalmology. *Prog Retin Eye Res* 29: 596-609.

4. Parveen S, Misra R, Sahoo SK (2012) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine* 8: 147-166.

5. Lawrence MJ, Rees GD (2000) Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev* 45: 89-121.

6. Üstündag-Okur N, Gökçe EH, Eğrilmez S, Özer Ö, Ertan G (2014) Novel ofloxacin-loaded microemulsion formulations for ocular delivery. *J Ocul Pharmacol Ther* 30: 319-332.

7. Hegde RR, Bhattacharya SS, Verma A, Ghosh A (2014) Physicochemical and pharmacological investigation of water/oil microemulsion of non-selective beta blocker for treatment of glaucoma. *Curr Eye Res* 39: 155-163.

8. El Maghraby GM, Bosela AA (2011) Investigation of self-microemulsifying and microemulsion systems for protection of prednisolone from gamma radiation. *Pharm Dev Technol* 16: 237-242.

9. Wang Y, Challa P, Epstein DL, Yuan F (2004) Controlled release of ethacrynic acid from poly(lactide-co-glycolide) films for glaucoma treatment. *Biomaterials* 25: 4279-4285.

10. Sznitowska M, Zurowska-Pryczkowska K, Janicki S, (1999) Miotic effect and irritation potential of pilocarpine prodrug incorporated into a submicron emulsion vehicle. *Int J Pharm* 184: 115-120.

11. Ege MA, Karasulu HY, Güneri T (2004) Triangle phase diagram analysis software. The 4th International Postgraduate Research Symposium on Pharmaceutics (IPORSIP-2004), 36, Istanbul.

12. Luntz MH (1966) Experimental glaucoma in the rabbit. *Am J Ophthalmol* 61: 665-680.

13. Monem AS, Ali FM, Ismail MW (2000) Prolonged effect of liposomes encapsulating pilocarpine HCl in normal and glaucomatous rabbits. *Int J Pharm* 198: 29-38.

14. Fialho SL, da Silva-Cunha A (2004) New vehicle based on a microemulsion for topical ocular administration of dexamethasone. *Clin Experiment Ophthalmol* 32: 626-632.

15. Cui J, Yu B, Zhao Y, Zhu W, Li H, et al. (2009) Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. *Int J Pharm* 371: 148-155.

16. Yue Y, San-ming L, Pan D, Da-fang Z, (2007) Physicochemical properties and evaluation of microemulsion systems for transdermal delivery of meloxicam. *Chem Res Chin Univ* 23: 81-86.

17. Moulik SP, Paul BK, (1998) Structure, dynamics and transport properties of microemulsions *Advances in Colloidal and Interface Science*. 78: 99-195.

18. Zurowska-Pryczkowska K1, Sznitowska M, Janicki S (1999) Studies on the effect of pilocarpine incorporation into a submicron emulsion on the stability of the drug and the vehicle. *Eur J Pharm Biopharm* 47: 255-260.

19. Furrer P, Plazonnet B, Mayer JM, Gurny R (2000) Application of in vivo confocal microscopy to objective evaluation of ocular irritation induced by surfactants *Int J Pharm*. 207: 89-98.