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Development of dorzolamide hydrochloride in situ gel nanoemulsion for ocular delivery

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Abstract

Background: Several in situ gel-forming systems have been developed to prolong the precorneal residence time of a drug and to improve ocular bioavailability. Poloxamer 407 with its thermoreversible gelation and surface active properties was utilized to formulate a novel dorzolamide hydrochloride in situ gel nanoemulsion (NE) delivery system for ocular use. *Objective*: Improvement of both ocular bioavailability and duration of action for dorzolamide hydrochloride was the aim of this study. *Methods*: Physicochemical properties, in vitro drug release studies and biological evaluation of the prepared NEs were investigated. *Results*: The optimum formulation of in situ gel NE consisted of Triacetin (7.80%), Poloxamer 407 (13.65%), Poloxamer 188 (3.41%), Miranol C2M (4.55%), and water (70.59%). Biological evaluation of the designed dorzolamide formulation on normotensive albino rabbits indicated that this formulation had better biological performance, faster onset of action, and prolonged effect relative to either drug solution or the market product. The formula showed a superior pharmacodynamic activity compared to the in situ gel dorzolamide eye drops. This indicated the effectiveness of the in situ gel properties of poloxamer 407, besides formulating the drug in an NE form for improving the therapeutic efficacy of the drug. *Conclusion*: These results demonstrate the superiority of in situ gel NE to conventional ocular eye drops and in situ gels to enhance ocular drug bioavailability.

Key words: *Bioavailability; dorzolamide hydrochloride; in situ gel; nanoemulsion; ocular*

Introduction

Glaucoma is a serious eye disorder characterized by an increase in intraocular pressure (IOP), which results in damage to the optic disc[1], and thus leads gradually to loss of vision, usually without symptoms. Furthermore, it is the second leading cause of blindness worldwide[2,3]. It is believed that glaucoma is the result of an imbalance between aqueous humor secretion and drainage processes within the ocular chambers[4].

Dorzolamide hydrochloride is a carbonic anhydrase inhibitor used in the treatment of glaucoma. Carbonic anhydrase inhibitors reduce IOP by decreasing aqueous humor secretion through the inhibition of carbonic anhydrase isoenzyme II in the ciliary processes[5]. Topically effective aqueous dorzolamide eye drop solution (Trusopt®) has become one of the most widely used medications for the treatment of open-angle glaucoma since it became commercially available in 1995[6]. The concentration of dorzolamide HCl in Trusopt® is 2.2% (w/v), corresponding to 2.0% of the free base, pH 5.65. Hydroxyethyl cellulose is used to increase the viscosity of Trusopt® eye drops; this increased viscosity leads to increased corneal contact time and, consequently, to increased bioavailability. However, the relatively low pH and high viscosity have been shown to generate local irritation after topical administration of the eye drops[7] and may be consistent with transient epithelial alteration[8].

The anatomy, physiology, and biochemistry of the eye render it highly impervious to foreign substances. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye and by other concomitant factors[9]. Several in situ gel-forming systems have been

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developed to prolong the precorneal residence time of the drug and to improve ocular bioavailability.

In situ gels refer to polymer solutions that can be administered as liquids and undergo a phase transition to semisolid gels upon exposure to physiological environments. Because of its unique thermoreversible gelation properties, poloxamer 407 became one of the most extensively investigated temperature-responsive materials[10]. This polymer exhibits in situ gelling properties and can be instilled to the eye in a liquid form and shifts to a gel phase in the cul de sac[11]. The principal advantage of this formulation is the possibility of administering accurate and reproducible quantities in contrast to the already existing gelled formulations and, moreover, promoting precorneal retention[12,13].

Ocular microemulsions have been proposed to achieve sustained release of a drug applied to the cornea and higher penetration into the deeper layers of the ocular structure and the aqueous humor[14]. Furthermore, the possibility of prolonged release of drugs in microemulsions makes these vehicles very attractive for ocular administration because they can decrease the number of applications of eye drops per day[15]. The proposed mechanism is based on the adsorption of the nanodroplets representing the internal phase of the microemulsion, which constitutes a reservoir of the drug, on the cornea and should then limit their drainage[16]. Recently, because of the nanorange of the droplet size in the microemulsion, a new trend has appeared to describe the prepared system as nanoemulsion (NE)[17–19].

In a preceding communication[20], dorzolamide hydrochloride NE eye drops containing Tween 80 and/or cremophor EL as a surfactant were formulated and evaluated. The percentage of decrease in IOP of the formulated NE was investigated and compared to that of the drug solution and the market product. The biological studies revealed higher efficacy of the drug in NE eye drops.

In this work, we intended to make use of the advantages of both nonionic surface-active properties and in situ gelling properties of poloxamer 407 to formulate a novel dorzolamide hydrochloride in situ gel NE delivery system for ocular use, characterized by being nonirritant and highly therapeutically efficient.

Materials and methods

Materials

Dorzolamide hydrochloride was obtained from Hetero Drugs Ltd., Hetero House, Erragadda, Hyderabad, India. Poloxamer 407 (Plx 407), triacetin (glycerol triacetate), isopropyl myristate (IPM), and dialysis tubing cellulose membrane (molecular weight cutoff 12,000 g/mol) were obtained from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Poloxamer 188 (Plx 188) was purchased from MP Biomedicals, Inc., Illkirch, France. Miglyol 812 (caprylic/capric triglyceride) was kindly provided by Sasol Germany GmbH, Witten, Germany. Transcutol P (diethylene glycol monoethyl ether) was kindly supplied by Gattefossé, Saint-Priest, France. Miranol C2M Conc. NP (disodium cocoamphodiacetate) was donated by Rhodia, Inc., Martinez, CA, USA. Propylene glycol was purchased from BDH Laboratory Supplies, Poole, England and dorzolamide hydrochloride market product (Trusopt®, 2.2% dorzolamide hydrochloride) from Merck Sharp & Dohme B.V. (Haarlem, Netherlands). Potassium dihydrogen phosphate, disodium hydrogen phosphate, and sodium chloride were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Methods

Preparation of nanoemulsions

The pseudoternary phase diagrams of oil, surfactant, and cosurfactant were developed using water titration method[21]. For each combination of surfactant (S) and cosurfactant (CoS), three S/CoS weight ratios of 1:1, 2:1, and 3:1 were used. NEs were prepared by mixing oil, water, surfactant, and cosurfactant, then refrigerated at 4°C, and stirred periodically until homogeneous solutions were obtained.

In an effort to mimic physiological dilution process after ocular administration of the prepared NEs, the selected NEs were diluted 1:5 (v/v) with isotonic buffer solution (pH 7.4) and assessed visually for transparency for a period of at least 48 hours[22]. Diluted systems that showed transparency and no phase separation were considered as true oil-in-water NEs, maintaining their physical integrity, and thus were selected for further studies.

Accelerated physical stability studies

- Heating–cooling cycle: Six cycles at temperatures of 25°C and 4°C with storage at each temperature for not less than 48 hours were performed. Formulations that were stable at these temperatures were subjected to centrifugation test[23].
- Centrifugation: Passed formulations were centrifuged at 12,857 × *g* for 30 minutes and formulations that did not show any phase separation were taken for the freeze–thaw stress test[22].
- Freeze–thaw cycle: Three freeze–thaw cycles between –21°C and 25°C with storage at each temperature for not less than 48 hours were done for each formulation[23].

Physicochemical characterization

Particle size analysis was done using a Malvern Photon Correlation Spectrometer (Zetasizer 1000HS, Malvern

Instruments Ltd., Worcestershire, UK). Light scattering was monitored at 90° angle and 25°C.

The pH of the NEs was measured at 25°C using JENWAY model 350 (JENWAY Ltd., Essex, UK) and the refractive index was determined at 25°C using refractometer M 46.17/63707 supplied by Hilger and Watts Ltd., London, UK.

Rheological measurements were performed at 25 ± 0.1°C using a Bohlin rheometer (Model CS 100, Bohlin Instruments, Gloucestershire, UK) equipped with a cone/plate apparatus at 40 mm/4°. For each sample, continuous variation of shear rate γ (80–400 s^{-1}) was applied and the resulting shear stress σ was measured.

Measurement of gelation temperature of in situ gel nanoemulsions

For the determination of gelation temperature, NE samples of 2 mL were enclosed in glass tubes, heated at a rate of 2°C/min, and the temperature at which the physical state of the NE changed was regarded as the gelation temperature[24–26]. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after being tilted to an angle of 90°.

In vitro drug release studies

These studies were performed using USP dissolution apparatus (SR8 PLUS, Hanson dissolution tester, California, CA, USA). The release medium utilized was 900 mL phosphate buffer solution (pH 7.4), the temperature was set at 34 ± 0.5°C (the ocular surface temperature)[27,28], and the paddle revolution speed was 50 rpm. Release experiments were conducted for 6 hours as all tested preparations attained 100% release within this time period. A half milliliter of aqueous drug solution (pH 5.5) or the drug-loaded NE was instilled in a dialysis bag secured with two clamps at each end. At definite time intervals, 5 mL samples were withdrawn and replaced by fresh buffer; these samples were assayed for dorzolamide hydrochloride by Shimadzu UV Spectrophotometer, Kyoto, Japan, at 252.6 nm. Triplicate experiments were carried out for each release study and the mean value of release efficiency (RE) was calculated. RE was calculated from the area under the release curve at time *t*. It is expressed as the percentage of the area of the rectangle corresponding to 100% release, for the same total time, according to the following equation[29]:

$$\mathrm{RE} = \frac{\int_0^t y \times \mathrm{d}t}{y100 \times t} \times 100$$

where *y* is the percentage drug released at time *t*.

Ocular irritation studies

Four groups, each containing six New Zealand albino rabbits weighing 1.5–2 kg, were kept in an air-conditioned room at 25 ± 0.5°C with artificial fluorescent light providing a cycle of night and day for 12 hours and fed a standard pellet diet and water. All animals were healthy and free of clinically observable abnormalities. The experimental procedures conform to the ethical principles of the National Research Centre, Cairo, Egypt, applied for the use of experimental animals.

The right eye received 50 µL of the tested formulation, whereas the left eye was used as a control. Application of the tested formulation onto the rabbit's cornea was repeated every 2.5 hours through a period of 7.5 hours per day for 3 successive days and once on the fourth day[30]. After 1 and 24 hours from last instillation, the eyes were examined under general anesthesia (35 mg/kg ketamine and 5 mg/kg xylazine) utilizing Draize technique[31]. The eye lids, cornea, iris, conjunctiva, and anterior chamber were inspected for inflammation or toxic reaction. Furthermore, both eyes were stained with fluorescein and examined under UV light to verify possible corneal lesion.

After corneal examination, the corneas were separated, washed with saline phosphate buffer (pH 7.4), and immediately fixed in Bouin's solution [85 mL picric acid, 10 mL formalin (37–40%), and 5 mL acetic acid] for 24 hours. The corneas were then dehydrated with an ethyl alcohol gradient (70–90–100%) and xylene, put in melted paraffin, and solidified in block forms. Cross sections were cut, stained with hematoxylin and eosin, and microscopically examined for pathological modifications ($n = 3$)[30].

Biological studies

These studies were of a single-dose, cross-over design and were performed on in situ gel NE, in situ gel, drug solution, and the market product. Sterility of formulations was achieved by filtration through sterile 0.22 µm pore size pyrogen-free cellulose filters.

Male albino rabbits weighing 2–2.5 kg were used; the animals were housed as previously described and the experimental procedures conformed to the ethical principles of the National Research Centre, Cairo, Egypt, for the use of experimental animals. IOP measurements were performed with a Schiötz tonometer (Rudolf Riester GmbH & Co. KG, Jungingen, Germany). No more than three repeated readings for any eye were performed at each measurement. Only measurements in which two consecutive readings were identical were included. Animals showing a consistent difference of more than 2 mmHg between IOP of both eyes, any signs of irritation, or those which were agitated during handling were excluded.

Eye drops were instilled topically into the upper quadrant of the eye and the eye was manually blinked; one eye received 50 µL of the preparation and the other served as control. IOP was measured immediately before giving the drug and then at different time intervals following

the treatment. All measurements were done thrice at each interval and the mean values were used to calculate the percentage decrease in IOP.

The pharmacodynamic parameters taken into consideration were maximum percentage decrease in IOP (E_{max}), time for maximum response (t_{max}), and area under percentage decrease in IOP versus time curve ($AUC_{0–10\ hours}$). These parameters were calculated using WinNonlin® software (Pharsight Co., Mountain View, CA, USA).

Statistical analysis of the results was performed using one-way analysis of variance followed by the least-significant difference test. Statistical analysis was computed with the SPSS® software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Construction of pseudoternary phase diagrams

For this study, three different oils (IPM, Miglyol 812, and triacetin) were selected and used with different cosurfactants (propylene glycol, Transcutol, and Miranol C2M). Poloxamer 407 formed NEs only with the monoglyceride oil triacetin and was not able to form NEs with IPM or Miglyol. These results might be interpreted in terms of the law of chain length compatibility. Chain length compatibility of surfactant and oil is a very important factor regarding the formation of NEs[32,33]. Because of the fact that poloxamer 407 has very short hydrocarbon chain[34], it needs an oil with short hydrocarbon chain to form an NE. This could be explained on the basis that, when the chain length of adjacent hydrocarbon chains is matched, the resulting hydrocarbon layer will be more ordered and has tighter packing and consequently greater stability[35]. On the contrary, if chain length mismatching is present in a surfactant film, the excess hydrocarbon tails have more freedom to disrupt the molecular packing through conformational disorder, increased tail motion, or other factors. This disruption in the molecular packing leads to lower interaction energies and hence lower film stability relative to comparable scenarios in which the chain lengths are compatible[35].

Selection of nanoemulsion formulations

The ability of an NE to retain its integrity on dilution is essential for its use as a drug delivery vehicle because after ocular administration it will be diluted by eye tears. Eight NEs were formulated; in all these formulations, the S:CoS ratio was 3:1, whereas the water content was 77.78% (w/w). The prepared NEs were loaded with 2.22% (w/w) of dorzolamide hydrochloride and their compositions are shown in Table 1.

Stability studies

Stability of the selected NEs was monitored using stress tests of heating–cooling cycles, centrifugation, and freeze–thaw cycles. Formulated NEs did not show any phase separation or change in their transparency and thus they were considered stable and were subjected to further investigations.

Physicochemical characterization

Table 2 reveals the physicochemical properties of prepared dorzolamide hydrochloride NEs. All NEs had a mean droplet diameter within the nanorange (4.2–11.7 nm). The ideal pH for maximum comfort when an ophthalmic preparation is instilled into the eye should be in the order of 7.2 ± 0.2[36]. However, different pH values can be tolerated if the preparation is not or is only very slightly buffered, because in this case the limited buffering capacity of the tears is able to adjust the pH to physiological levels on administration[37]. The pH of therapeutic substances applied as eye drops can vary from 3.5 to 8.5[38]. The pH values of the prepared dorzolamide hydrochloride NEs varied from 5.3 to 6.8 that are within the acceptable range for eye preparations.

Table 1. Composition of dorzolamide hydrochloride-loaded nanoemulsions.

Component	Nanoemulsion (NE)							
(%, w/w)	1	2	3	4	5	6	7	8
Triacetin	2.00	4.00	2.00	4.00	6.00	2.00	4.00	6.00
Plx 407	13.50	12.00	13.50	12.00	10.50	13.50	12.00	10.50
PG	4.50	4.00						
Transcutol			4.50	4.00	3.50			
Miranol						4.50	4.00	3.50

Bidistilled deionized water was used as the aqueous phase (77.78%, w/w). Dorzolamide hydrochloride was used in a concentration of 2.22% (w/w).

Table 2. Physicochemical properties of prepared dorzolamide hydrochloride nanoemulsions.

	Nanoemulsion (NE)							
Physicochemical properties	1	2	3	4	5	6	7	8
Particle diameter (nm)	7.1	7.4	7.4	4.2	9.3	11	11.7	8.1
pH	5.98	5.44	5.92	5.51	5.26	6.82	6.37	6.14
Refractive index	1.357	1.356	1.357	1.356	1.353	1.356	1.356	1.356
Viscosity (mPa·s)	48.06	31.91	49.78	33.63	23.34	90.92	46.61	33.44

Refractive index measurements detect possible impairment of vision or discomfort to the patient after administration of eye drops[37]. Refractive index of tear fluid is 1.340–1.360[39]. It is recommended that eye drops should have refractive index values not greater than 1.476[40]. Dorzolamide hydrochloride NEs had refractive index values ranging from 1.353 to 1.357, which are within the recommended values to prevent impairment of vision or discomfort to the patient after instillation of eye drops[37].

Because of the solid nature of the used surfactant, the prepared NEs had high viscosity values ranging from 23.3 to 90.9 mPa·s.

In vitro drug release studies

The release efficiency values of dorzolamide hydrochloride from NEs containing triacetin, poloxamer 407, and different cosurfactants (propylene glycol, Transcutol, and Miranol; NEs 1–8) are presented in Table 3. It is observed that the release of the drug from NEs was lower ($P < 0.0001$) than that from the drug solution that attained 100% after 45 minutes.

It is evident that the release efficiency of NEs 4, 6, 7, and 8 was significantly lower than that of the other NEs ($P < 0.05$). NE 8 was selected for further investigations because it contains lower surfactant and cosurfactant concentrations (Table 1).

In situ gel nanoemulsion formulations

Poloxamers consist of poly(oxyethylene) (PEO) and poly(oxypropylene) (PPO) units, with the general formula PEO_x–PPO_y–PEO_x[41]. PEO is predominantly hydrophilic, whereas PPO is hydrophilic at low temperatures and becomes more hydrophobic at higher temperatures. Once blocks of PEO and PPO are combined, one can expect amphiphilic characteristics and aggregation phenomena at higher temperatures[42]. That is, when the polymer concentration and the characteristic temperature are above a critical point, this triblock copolymer forms micelles[43,44]. The formation of micelles may increase the viscosity of vehicles and thus leads to gelation at high temperatures above a critical point[45–47].

The phenomenon of thermogelation is perfectly reversible and is characterized by a gelation temperature. Thermogelation results from interactions between different segments of the copolymer[48]. As temperature increases above a critical value, poloxamer 407 molecules aggregate into spherical micelles with a dehydrated PPO core surrounded with an outer shell of hydrated swollen PEO chains[49,50]. This micellization is followed by gelation for sufficiently concentrated samples. This gelation is attributed to the ordered packing of micelles. According to Liu and Chu[51], a face-centered cubic structure is obtained for poloxamer 407 concentrations in water ranging between 20% and 40%. At higher concentrations (50%), a body-centered cubic packing of micelles is observed. These micellar cubic structures and possible micellar entanglements produce high viscosity, partial rigidity, and slow dissolution of the gels. Such properties facilitate the incorporation of both hydrophilic and hydrophobic drugs.

It is known that the PPO poloxamer unit, which is hydrophobic, decreases the gelation temperature, whereas the PEO unit is hydrophilic and increases the gelation temperature[52]. Accordingly, different PEO : PPO ratios will lead to different gelation temperatures[53]. Poloxamer 407 and poloxamer 188 possess different PEO : PPO ratios and it was found by Choi et al.[54] that the gelation temperatures of poloxamer solutions containing 18–25% of poloxamer 407 or 30% of poloxamer 188 alone were 13–25°C and 48°C, respectively, and concluded that the optimal gelation temperature can be reached by mixing different amounts of poloxamer 407 and poloxamer 188 in the preparation.

The optimum ophthalmic thermosensitive in situ NE should have a gelation temperature higher than room temperature and a shift to gel at the conjunctival sac temperature (35°C)[11,53].

NE 8 with the highest release efficiency had a gelation temperature at 24°C that makes it in a gel form at room temperature. If this preparation was stored in refrigerator to make administration easier, the potential irritation of low temperature to the sensitive ocular tissues must be taken into consideration. If a cold ophthalmic preparation is instilled on the cornea surface, tear production and blinking frequency will increase, and then the gel will be diluted and more quickly eliminated from the eye[50]. Therefore, to increase the gelation temperature of NE 8, we decreased its content of poloxamer 407 by increasing the water concentration from 70% to 75% (Table 4). This resulted in two NEs (12 and 13), which are able to have gelation temperature within

Table 3. Release efficiency of dorzolamide hydrochloride-loaded nanoemulsions.

	Drug solution	Nanoemulsion (NE)						
		1	2	3	4	5	6	7
Release efficiency	97.02 ± 0.48	89.63 ± 0.39	88.79 ± 0.58	89.42 ± 2.68	86.77 ± 2.57	89.88 ± 1.35	82.65 ± 0.98	85.62 ± 1.17
		8	12	17	22	29	36	
Release efficiency		85.57 ± 1.99	85.05 ± 0.49	77.33 ± 0.18	79.86 ± 1.90	84.57 ± 1.91	81.04 ± 2.40	

Table 4. Liquid–gel nanoemulsions containing combinations of poloxamer 407 and poloxamer 188.

	Nanoemulsions containing poloxamer 407 : poloxamer 188 in weight ratios of																	
	(1 : 0)					(4 : 1)						(2 : 1)						
Component (%, w/w)	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Triacetin	9.00	8.40	8.10	7.80	7.50	9.00	8.40	8.10	7.80	7.50	7.20	8.40	8.10	7.80	7.50	7.20	6.90	
Plx 407	15.75	14.70	14.18	13.65	13.13	15.75	14.70	14.18	13.65	13.13	12.60	14.70	14.18	13.65	13.13	12.60	12.07	
Plx 188						3.94	3.68	3.54	3.41	3.28	3.15	7.35	7.08	6.83	6.56	6.30	6.04	
Miranol	5.25	4.90	4.72	4.55	4.37	5.25	4.90	4.72	4.55	4.37	4.20	4.90	4.73	4.55	4.37	4.20	4.02	
Water	70.00	72.00	73.00	74.00	75.00	66.06	68.32	69.45	70.59	71.71	72.85	64.65	65.91	67.17	68.44	69.70	70.96	
Gelation temp. (°C)	24.0	26.0	29.3	30.0	34.0	26.0	28.0	30.3	32.0	35.0	39.5	27.5	29.0	32.3	34.0	36.8	45.5	
	(4 : 3)									(1 : 1)								
	26	27	28	29	30	31	32	33	34	35	36	37	38					
Triacetin	8.40	8.10	7.80	7.50	7.20	6.90	8.10	7.80	7.50	7.20	6.90	6.60	6.30					
Plx 407	14.70	14.18	13.65	13.13	12.60	12.08	14.18	13.65	13.13	12.60	12.08	11.55	11.03					
Plx 188	11.03	10.63	10.24	9.84	9.45	9.06	14.18	13.65	13.13	12.60	12.08	11.55	11.03					
Miranol	4.90	4.72	4.55	4.37	4.20	4.02	4.72	4 .55	4.37	4.20	4.02	3.85	3.67					
Water	60.97	62.37	63.76	65.16	66.55	67.94	58.82	60.35	61.87	63.40	64.92	66.45	67.97					
Gelation temp. (°C)	25.8	28.0	30.0	32.8	34.5	39.5	24.5	27.0	29.8	30.5	32.8	37.8	41.8					

the required range for ocular preparation. However, the gelation temperature for NE 12 (30°C) is close to the ambient temperature, which could make it in a gel form before administration. On the contrary, NE 13 has gelation temperature (34°C) close to that of the eye. This will induce slow gelation rate to the NE, and thus it will be diluted with eye tears before gelation. Therefore, we intended to add poloxamer 188 to have a gelation temperature between these gelation temperatures (30–34°C).

The composition of NE 8 (Table 1) was modified by using both poloxamers 407 and 188 in different concentrations to obtain compositions possessing in situ gelling temperature within the acceptable range. Combinations of poloxamers 407 and 188 were used in ratios of 4 : 1, 2 : 1, 4 : 3, and 1 : 1 w/w, respectively (Table 4).

Based on gelation temperature evaluations (Table 4), NEs 12, 17, 22, 29, and 36 were loaded with 2.22% (w/w) dorzolamide hydrochloride selected for further investigations.

Accelerated physical stability studies

Stability of the selected NEs was monitored using stress tests of heating–cooling cycles, centrifugation, and freeze–thaw cycles. All in situ gel NEs were found to be stable.

Physicochemical characterization

The data presented in Table 5 reveal that the investigated in situ gel NEs exhibit mean particle diameter within the nanorange and pH and refraction index values within the acceptable range for eye preparations[37,38].

Table 5. Physicochemical properties of prepared dorzolamide hydrochloride in situ gel nanoemulsions

	In situ gel nanoemulsion (NE)				
Physicochemical properties	12	17	22	29	36
Particle diameter (nm)	8.1	7.0	6.9	5.0	9.0
pH	7.40	7.45	7.49	7.49	7.53
Refractive index	1.356	1.356	1.355	1.356	1.352
Viscosity (mPa?s)	19.6	22.0	42.2	57.2	49.4

Graphical representation of shearing stress as a function of shearing rate for dorzolamide hydrochloride in situ gel NEs reveals that these NEs exhibit a Newtonian behavior at 25°C (Figure 1). The viscosity values of these in situ gel NEs are presented in Table 5.

In vitro drug release studies

A prolonged release of the drug from the investigated in situ gel NEs was observed compared to that of the drug solution ($P < 0.001$) (Table 3). NE 17 exhibited the highest retardation of drug release ($P < 0.05$); accordingly, this NE was chosen for ocular irritation and biological studies. It was stated that dispersion of the drug into the oil phase of the NE could increase its release time, because the drug will slowly diffuse into the continuous aqueous phase, as it releases from the system[37].

Ocular irritation studies

Ocular irritation test revealed that the selected in situ gel NE (NE 17) was nonirritant and could be tolerated by the rabbit eye (average total score 0.33). Cross sections from the corneas of rabbit eye after application of the tested formulation showed that both corneal structure and integrity were unaffected.

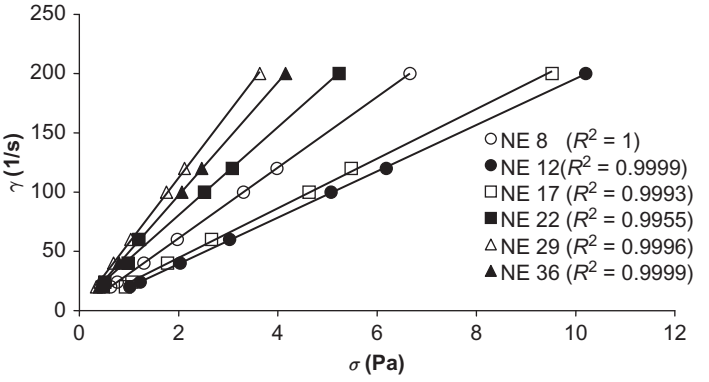

Figure 1. Flow curves of dorzolamide hydrochloride in situ gelling nanoemulsions.

Triacetin used as the oil phase in our study is well tolerated by the eye[55–57]. It has been reported that an injectable intraocular lens formulation containing 25% poloxamer 407 produced no inflammatory response or toxicity in the conjunctiva, iris, vitreous, or retina using New Zealand white rabbits[58]. Miranol C2M Conc. NP, used as a cosurfactant in the present study, is known as common emulsion excipient suitable for dissolving or dispersing lipophilic drugs in ocular preparations[59].

Taking into consideration that rabbit eye is more susceptible to irritant substances than human eye[60], our results regarding ocular tolerability would be considered very promising.

Biological studies

Figure 2 demonstrates the percentage decrease in IOP of normotensive rabbits after instillation of a single dose (50 µL) of the designed dorzolamide hydrochloride in situ gel NE, drug solution, and the market product. It is observed that the in situ gel NE, in contrast to the drug solution or the market product, induced a pronounced decrease in IOP half an hour postinstillation of the eye drops. This indicates that formulation of dorzolamide hydrochloride as an NE led to a faster onset of drug action compared to that of either the drug solution or the market product.

It is also observed that the mean maximum percentage decrease in IOP (E_{max}) occurred 1.1–1.6 hours after instillation of the NE, drug solution, or the market product (Figure 2). There was no significant difference between the value of this parameter for the in situ gel NE and that for the drug solution or the market product ($P > 0.05$).

With respect to duration of drug action, it is evident that the effect of in situ gel NE was continued for up to 8 hours. On the contrary, the effects of drug solution and the market product were continued only for 3 and 4 hours, respectively. This would indicate that dorzolamide hydrochloride in situ gelling NE exhibited a more prolonged effect compared to either the drug solution or the market product.

The AUC value of the in situ gel NE was 4 times higher than that of the drug solution and more than

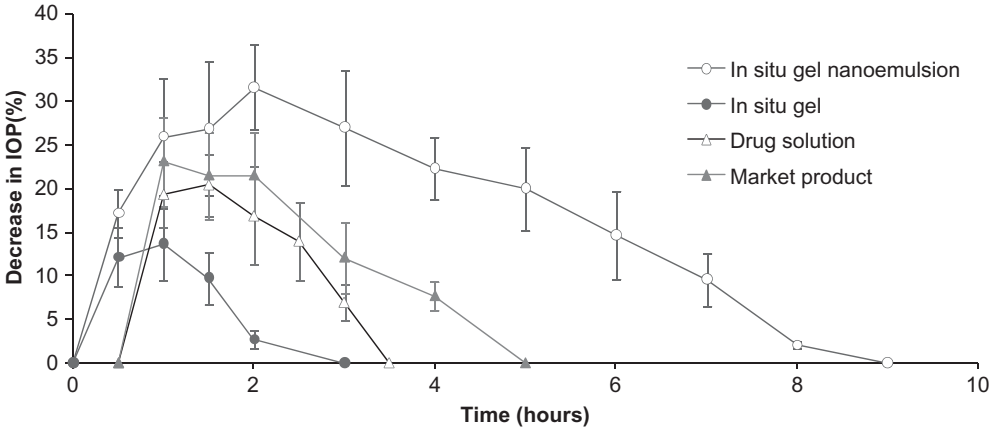

Figure 2. Therapeutic efficacy of different dorzolamide hydrochloride formulations.

Table 6. Pharmacodynamic parameters for dorzolamide hydrochloride in situ gel nanoemulsion, in situ gel, drug solution, and the market product (mean ± SD).

Parameter	In situ gel nanoemulsion	In situ gel	Drug solution	Market product
E_{max} (%)	31.58 ± 4.88	14.32 ± 3.40	20.93 ± 4.17	23.11 ± 5.11
t_{max} (hours)	1.6 ± 0.5	0.8 ± 0.3	1.2 ± 0.4	1.1 ± 0.2
$AUC_{0-10\ hours}$	154.29 ± 25.88	19.82 ± 3.81	38.73 ± 8.38	58.20 ± 10.90

double that of the market product indicating higher drug bioavailability from the in situ gel NE formulation (Table 6).

The above-mentioned results show that dorzolamide hydrochloride in situ gel NE exhibited, thus, high therapeutic efficacy relative to that of drug solution or the market product. This NE possessed high $AUC_{0-10\ hours}$ value indicating high drug bioavailability and induced a marked decrease in IOP of normotensive rabbits' eyes already 0.5 hours postinstillation of the NE which continued for up to 8 hours. This might be due to long precorneal residence time for in situ gel-forming systems. Furthermore, NEs act as penetration enhancers by removing the mucous layer and disrupting tight junctional complexes to facilitate corneal drug delivery[61,62]. The submicron particles were also reported to penetrate into the corneal epithelium cells by endocytosis[63].

To elucidate whether drug therapeutic efficacy—enhancing effect—of the in situ gel NE is due to utilizing poloxamers 407 and 188 in an NE form or just due to their presence as in situ gelling polymers, a system containing the same composition of this NE except the oil triacetin, which was replaced by water, was prepared and monitored for drug efficacy. Biological studies on the two formulations revealed that the in situ gel NE showed superior therapeutic efficacy (Table 6). This indicates that formulation of dorzolamide hydrochloride in an in situ gel NE form by the use of poloxamers rather than inclusion of these polymers as in situ gel polymers led to the enhancement of therapeutic efficacy of the drug.

Conclusion

Formulating dorzolamide hydrochloride in an in situ gel NE form successfully enhanced the therapeutic efficacy of this drug relative to either simple drug solution or the market drug product. This might be due to greater penetration of the drug from NEs because of the presence of surfactants and cosurfactants that increase the membrane permeability, thereby increasing drug uptake. Furthermore, the presence of the in situ gelling polymers in this NE leads to longer residence in the eye because of transformation of this NE into gel form. Such NE formulation offers a more intensive treatment of glaucoma, a decrease in the number of applications per day, and a better patient compliance.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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