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Original article

Thermoresponsive sol-gel improves ocular bioavailability of Dipivefrin hydrochloride and potentially reduces the elevated intraocular pressure *in vivo*

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ABSTRACT

The present study involves the development of Dipivefrin hydrochloride (DV) containing Poloxamers (P407 and P188)-Carbopol-934 (CP) based thermoresponsive-gels for the management of elevated intraocular pressure (IOP). Optimal formulation was evaluated for gelation temperature (T_{gel}), physicochemical and viscoelastic properties, *in-vitro* gel dissolution and drug release studies. The *in-vivo* safety, precorneal retention, ocular pharmacokinetics and efficacy in reducing IOP were also evaluated. T_{gel} of DV-containing thermoresponsive-gels were between 35.1 and 38.9 °C and it was Poloxamers and CP concentrations dependent. The optimal formulation (F8), composed of 20% P407, 5% P188 and 0.15% CP (w/v), had a T_{gel} of 35 °C. Its viscosity indicated good flow at room temperature and ability to convert to gel at ocular temperature and the rheology studies revealed favorable characteristics for its ocular use. In precorneal retention experiment, F8 indicated significantly higher area under concentrations curves as compared to DV-aqueous suspension (DV-AqS). *In-vivo* ocular pharmacokinetics indicated a significant improvement in ophthalmic bioavailability of epinephrine (active form of DV). F8 was non-irritant to the eyes and showed a successful, continuous and superior ability to reduce IOP compared to DV-AqS in rabbits. In conclusion, our developed system could be an appropriate substitute to the conventional DV eye preparations in the management of elevated IOP.
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1. Introduction

Ocular hypertension is characterized by an elevated pressure inside the eye (intraocular pressure, IOP) to levels higher than 10–21 mmHg. Elevated IOP might occur because of imbalance in the ratio of formation/ secretion and drainage of aqueous humor (AqH), and/ or an excessive formation of AqH. While ocular hypertension is not a disease, a patient suffering from it is considered glaucoma suspect. If the elevation of IOP persisted, glaucoma may damage optical nerves, which in turn may cause loss of vision (Hoyng and van Beek, 2000). All of these events slowly progress in patients without being aware of it, hence it is often termed as "silent thief of sight" (Abdull et al., 2016). The elevated IOP can be reduced to normal with therapeutic agents which act either by increasing the drainage or by decreasing the excess production of AqH (Khan et al., 2018).

Dipivefrin (DV), a prodrug of epinephrine (EP), is an adrenergic agonist and direct acting sympathomimetic agent that is used to reduce IOP in patients suffering from chronic open angle glaucoma (Anderson et al., 1980; Jarvinen and Jarvinen, 1996; Saxena et al., 2002; Taskar et al., 2017). This drug acts through decreasing production and increasing the outflow of AqH from the eye (Havener, 1983; Nakamura et al., 1993). A controlled study proved the usefulness of topically applied DV (0.1%, w/v) over EP (2%, w/v) in reducing the IOP in the patients who were intolerant to topically applied EP (Yablonski et al., 1977). In terms of safety, DV is associated with less systemic adverse effects (e.g. cardiovascular side effects) compared to EP, since it is only needed in very small dose. Thus, DV is considered more suitable for ocular application as compared to EP, especially in patients with cardiovascular disorders (Blondeau and Cote, 1984; Havener, 1983; Jarvinen and Jarvinen,

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1996; Kerr et al., 1982; Lee and Li, 1989; Liederer and Borchardt, 2006). In addition to the clinical benefits, DV has favorable physicochemical properties compared to EP. DV has an ideal lipophilicity and diffusivity across the lipophilic ocular dynamic and static barriers, due to the esterification of the two hydroxide (—OH) functional groups of EP, yielding dipivaloyl-EP. This chemical modification allows DV to avoid the unfavorable physicochemical and biopharmaceutical characteristics of the EP (Barot et al., 2012). Therefore, using DV in an ocular formulation will resolve the lipophilicity issue associated with EP and would provide a site specific delivery with a10-fold enhanced therapeutic efficacy compared to EP (Barot et al., 2012; Mandell et al., 1978; Niemi et al., 2005; Wei et al., 1978).

Delivering drugs via the ocular route is challenging due to the immediate tear-turnover rate and corneal impermeability, which restricts the ocular bioavailability of conventional topical eye drops or solutions (Alshamsan et al., 2019; Fangueiro et al., 2014; Kalam and Alshamsan, 2017; Knop and Knop, 2007). Therefore, there is a need for an appropriate ocular delivery system to achieve high transcorneal permeation, sustained and controlled delivery while providing sufficient ocular bioavailability (Huang et al., 2016). The goal of the present study is to formulate a new thermoresponsive sol-gel system for the ocular delivery of DV using Poloxamer-407 (P407), Poloxamer-188 (P188), and Carbopol-934 (CP). The formulation will be fully characterized and examined for its ability to reduce IOP compared to DV-AqS in rabbit eyes.

2. Materials and methods

2.1. Materials

Dipivefrin.HCl (DV), Poloxamer-407, and Poloxamer-188 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carbopol-934 was purchased from ALPHA CHEMIKA, India. Purified water was obtained from a Milli-Q® water purifier (Millipore, France). All chemicals and reagents used were of analytical grade.

2.2. Preparation of DV containing thermoresponsive gel

The sol-gels were developed by using the cold method on the basis of weight by volume (Qi et al., 2007). CP (0.1–0.15%, w/v), P407 (15–20%, w/v) and P188 (2.5–5%, w/v) were added to cold purified water (4 °C) with continuous magnetic stirring for 24 h. Mixtures were kept at 4 °C until the appearance of clear solution. An equivalent amount of DV (0.1%, w/v) was added to the polymer solution with continuous magnetic stirring for 1 h to obtain a clear solution. Benzalkonium chloride (0.01%, w/v) was incorporated to the polymer-drug solution as preservative and the pH of the system was adjusted to 7.2 ± 0.1 using 0.1 M NaOH solution while the osmolality of the solution system was maintained to 305 ± 5 $mOsmol{\cdot}L^{-1}$ (Stahl et al., 2012; Tomlinson et al., 2006) by means of 2.5%, w/v solution of mannitol. Compositions of each formulation (F1-F8) are shown in Table 1**.** The formulations were packed in amber colored tightly capped glass vials, terminally sterilization by UV–visible light at 254 nm and stored at 4 °C (Huang et al., 2016).

2.3. Determination of gelation temperature (T_{gel}) and physicochemical characteristics of sol-gels

T_{gel} of the sol-gels were measured through the magnetic stirring method as described previously (Li et al., 2014). The sol-gels (F1-F8) were diluted with simulated tear fluid (STF) at 6:1 (v/v) ratio. Around 5 mL of each diluted sol-gels was transferred to transparent glass vials and kept at 4 °C. After 2 h, a magnetic stir bar was placed in each sol-gels and temperature was increased at a rate of 1 $°C{\cdot}min^{-1}$. A thermometer was inserted into the sol-gels to monitor T_{gel}. The temperature at which the magnetic bar stopped stirring and sol-gels did not flow when the vials were inverted at an 180° angle, was considered as the gelation temperature (T_{gel}).

Transparency of the sol-gels was checked visually against a white and black background. Abbes' Refractometer (Precision Testing Instruments Laboratory, Germany) was used to evaluate the refractive index (RI) of the sol-gels at 25 °C. Osmolarity was checked by Osmometer (Fiske Associates, USA) and pH was measured by a calibrated pH meter (Mettler Toledo MP-220, Switzerland). The DV content in the sol-gels was determined by the reported HPLC-UV method (Jarho et al., 1997).

2.4. Rheological evaluation of thermoresponsive sol-gels

Viscosity of the sol-gels was evaluated by "Brookfield Viscometer (Brookfield Engineering Laboratories, Middleboro, MA)" as reported (Kalam et al., 2017). The viscosities of the sol-gels were determined at different shear rates (5 s^{-1} to 25 s^{-1}) under ocular physiological (35 ± 0.5 °C) and non-physiological (25 ± 0.5 °C) temperatures. The viscosities of DV-containing sol-gels were tested at 25 °C by keeping a constant shear rate at 5 s^{-1} before and after dilution with STF at a ratio of 6:1 (v/v) and re-adjusting pH to 7.2 ± 0.1 at 37 ± 0.5 °C (Qi et al., 2007; Wei et al., 2002).

2.5. In vitro drug release and data analysis using kinetic model equations

On the basis of rheological study and T_{gel}, F8 was selected for further evaluation since it shows characteristics mostly suitable for biological application. The *in vitro* release of DV from the sol-gels was performed using dialysis membrane method (Huang et al., 2016). The isotonicity of F8 was adjusted by adding mannitol and pH was adjusted to 7.2 using 0.1 M NaOH. One mL of F8 containing the equivalent amount of DV (0.1%, w/v i.e. 1.0 mg/mL) were placed in dialysis bags (MWCO 10–12 kDa) and sealed at both ends. Dialysis bags were placed in a beaker containing 50 mL of STF as a release medium. The whole assembly was placed in shaking water bath (50 rpm) maintained at 35 ± 0.5 °C to simulate the ocular surface temperature. At predetermined time points, 1 mL of each sample was withdrawn and an equal volume of release medium was replaced to maintain the sink conditions. Same procedure was followed for the *in vitro* release of drug from DV-AqS. Each experiment was conducted in triplicates. Withdrawn samples were then centrifuged at 13,000 rpm for 15 min at 4 °C, supernatant was collected and the concentration of DV was analyzed by Waters® HPLC system equipped with Waters® UV-detector, Waters® binary pump, Waters® automated sampling system. "Breeze (Waters®)" software was used to monitor the whole HPLC system. The system was equipped with RP-C_{18} column (Macherey-Nagel, 4.6 × 150 m m, 10 μm). The mobile phase composed of methanol and 0.02 M monobasic potassium phosphate buffer (pH 5) at 60:40 (v/v) ratio was pumped isocratically at 1 mL/min flow rate. DV was analyzed by injecting 30 μL of the supernatant at a detection wavelength of 215 nm and a column temperature of 40 °C (Jarho et al., 1997). A calibration curve of R^2 = 0.99 was used to calculate the DV concentration. The cumulative amounts (%) of DV released was calculated and plotted against time (h).

In vitro release data (obtained from F8 containing the 1.0 mg of DV) were fitted into different release kinetic models such as zero-order, first-order, Higuchi's square root plot, Korsmeyer-Peppas and Hixson-Crowell cube root plot (Huang et al., 2016; Peppas and Sahlin, 1989). The model that gave the highest value of correlation coefficient (R^2) approaching to 1.0, was considered as the

Table 1
Compositions of DV thermoresponsive gels.

Ingredients	Sol-gel formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Dipivefrin (mg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
P407 (%, w/v)	15.0	20.0	15.0	20.0	15.0	20.0	15.0	20.0
P188 (%, w/v)	2.5	2.5	2.5	5.0	2.5	2.5	5.0	5.0
CP (%, w/v)	0.1	0.1	–	0.1	0.15	0.15	–	0.15
Benzalkonium chloride (%, w/v)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mannitol (%, w/v)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Milli-Q® water qs to (mL)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

best fit model for the dissolution of DV from the sol-gel (Huang et al., 2016).

2.6. In vitro gel erosion study

The association between the erosion or dissolution of F8 and the release of DV was evaluated as per the reported methods (Desai and Blanchard, 1998; Huang et al., 2016). Briefly, 2 mL of F8 was transferred to a 5 mL glass vial (weighed with and without sol-gel) and placed in oven at 35 °C till gelation takes place. Differences in vial weights, pre and post addition of sol-gel, provided the initial weight of the gel. One milliliter of STF preheated to 35 °C was added to the gel underwent shaking in a shaking water bath at 50 rpm and maintained at 35 ± 0.5 °C. At predetermined time points the whole STF was removed and vials were reweighed. The differences in the vial weights between the adjacent time intervals provided the quantity of the dissolved gel during that time interval. Finally, the erosion or dissolution profile of the gel was obtained by plotting the dissolved weight of the gel *vs* time.

2.7. Animal study

New Zealand albino rabbits weighing 2.5–3.0 kg were provided by the College of Pharmacy, Animal care and use center, King Saud University, Riyadh, Saudi Arabia, for the *in vivo* studies. Animal studies were performed based on protocols approved by the "Experimental Animal Care Center, College of Pharmacy, King Saud University (Approval No. KSU-SE-18-25)". Rabbits were housed in light-controlled air-conditioned room at 70 ± 5% RH according to the recommendations of the Guide for the Care and Use of Laboratory Animals permitted by the center. All healthy rabbits were put on standard pellet diet and water *ad libitum*.

2.7.1. Ex vivo transcorneal permeation study

Freshly excised rabbit corneas were fixed between donor and receptor compartments of double jacketed automated Franz diffusion cells (sampling system-SFDC 6, LOGAN, New Jersey, USA) in such a way that the corneal epithelium faced the donor compartment. Receptor compartment was filled with STF and warm water (35 ± 1 °C) was circulated in outer jacket of diffusion cells. Air bubbles in the receptor compartment were expelled out by means of continuous magnetic stirring. Five hundred μL of F8 and DV-AqS were put into the donor compartment of diffusion cells. One mL of each samples from the receptor compartment were withdrawn at different time points and an equal volume of fresh STF (at 35 °C) were replaced. The content of DV in the withdrawn samples were analyzed by HPLC (Jarho et al., 1997). Each experiment was performed in triplicate. Transcorneal flux (J) and apparent permeability (P_{app}) of DV from the two formulations were estimated by plotting the permeated amount (μg/cm^2) of DV *vs* time. From the linear ascent of the plots the slope was obtained by using MS-Excel-2013. The J and P_{app} were evaluated by using the equations (1) and (2):

$$J\left(\mu g/cm^2/h^1\right) = (dQ/dt) \quad (1)$$

$$Papp\ (cm/h) = J/C_0 \quad (2)$$

where Q is the amount of DV ossed through the corneal area (dQ/dt, 0.636 cm^2), t is corneal contact time and C_0 is the initial concentration (μg/mL) of DV in the donor compartment of diffusion cell (Kalam and Alshamsan, 2017).

2.7.2. Ocular irritation study

Ocular irritation potential of F8 in comparison to 0.9% NaCl (control) was performed by following the Draize's rabbit eyes test (Draize et al., 1944; Kalam, 2016; Kalam and Alshamsan, 2017). Twelve rabbits were divided into two groups, each containing six animals (n = 6). Group-I received single instillation of F8 (50 μL) directly into the *cul de sac* of the right eye of each rabbit. An equal volume of NaCl (0.9%) was applied (as control) to the left eyes of each rabbit. One-hour of post dosing the animals were examined for the signs and symptoms of acute eye-irritation. The rabbits of group-II received the same treatment but three times a day, for seven days and they were examined at the end of the last dosing. Congestion or redness of the conjunctiva and any eye discharge were observed and recorded in terms of scores (Diebold et al., 2007; Draize et al., 1944). Score 0–3 was considered as non-irritating, score 4–8 slightly irritating, score 9–12 moderately irritating and score 13–16 severely irritating (Diebold et al., 2007; Kalam, 2016).

2.7.3. In vivo precorneal retention study

This study was performed to check the precorneal retention of DV containing F8 (indirectly it can be termed as precorneal drug kinetics). To quantify the presence of DV in the tear fluid of rabbit eye different time points, twelve rabbits were divided into two groups, each containing six animals (n = 6). First group received 50 μL of F8 (test group) and the second group received 50 μL of 0.1% DV-AqS (control group) into their left eyes. Tear fluids were collected into 1.5 mL Eppendorf tubes by following the non-invasive capillary tube methods as reported (Kalam, 2016; von Thun Und Hohenstein-Blaul et al., 2013). As different volumes of tear could be taken from different rabbit eyes, collected samples were normalized to the same volume. In each tube, methanol (500 μL) was added for protein precipitation. Samples were then vortexed for 2 min and centrifuged at 13,000 rpm for 5 min. The supernatant was collected and 30 μL of the supernatant was injected into the HPLC-UV for the quantification of DV.

2.7.4. Ocular pharmacokinetics of DV

The ocular bioavailability of DV from the two formulations were determined by measuring the concentration of the drug in AqH of rabbits following their topical application. Six rabbits were divided in two groups (n = 3). Group-I received 50 μL of F8 once and group-II received 50 μL DV-AqS in the lower conjunctival sacs of their right eyes. Half an hour of post dosing, a mixture of ketamine.

HCl: xylazine (15: 3 mg/kg) was injected intravenously in the marginal ear vein to anesthetize the rabbits (Kalam, 2016; Kalam and Alshamsan, 2017). At different time points samples of AqH (around 25 μL) were collected using insulin syringe-needle (1 mL, 29-gauge) system. Collected samples were transferred to 2 mL Eppendorf tubes, 500 μL of acetonitrile was added, vortexed for 1 min and centrifuged at 13,000 rpm for 5 min at 4 °C to remove the proteins. Supernatants were collected and dried by N_2 gas at 25 °C. Obtained residues were dissolved in 250 μL of mobile phase (Water: ACN: TFA at 90: 10: 0.02 v/v) and quantification of EP (the active form of DV) was performed by using a modified UPLC method (Wang et al., 1999). Briefly, "Waters® Acquity H-Class UPLC system coupled with a Waters® TUV Detector by Acquity UPLC (Waters®, Milford, USA) was used. "The UPLC-system included quaternary solvent manager, sample manager (Acquity UPLC Waters®), 10 μL of injection capacity and column heater". Elution of EP was performed on Acquity UPLC BEH™ C_{18} column (1.7 μm, 2.1 × 50 mm, Waters®, USA) maintained at 25 °C. Mobile phase was pumped isocratically at 0.14 mL/min and UV-detection was done at a wavelength of 210 nm. The UPLC-UV system, data processing and acquisition were controlled by EMPOWER® software.

2.7.5. In vivo ocular pharmacodynamics

This study was performed to evaluate the IOP-lowering ability of DV-sol-gel in intraocular hypertensive animal model. Nine rabbits were divided into three groups (n = 3), group-I for F8, group-II for DV-AqS and group-III for 0.9% NaCl. Before starting the experiment, rabbits were examined to ensure that they were free from any ocular abnormality. Schiotz Eye Tonometer was used to measure the IOP in anesthetized rabbits. At the time of IOP measurement, few drops of proparacaine hydrochloride (0.5%, w/v) were also applied in the rabbit eyes (Huang et al., 2016; Reddy et al., 2001). Intra ocular hypertension was induced by reported method (Knepper et al., 1985; Pang et al., 2001). Briefly, 25 μL of 0.1%, w/v dexamethasone eye drop was administered topically four times per day for 2 weeks causing an increased IOP. The changes in the IOP were monitored on daily basis. Fifty microliters of F8, DV-AqS and 0.9% NaCl were instilled in the *cul de sac* of the left eyes of each rabbits of the respective groups mentioned above. The IOP of NaCl treated group was served as baseline values. To establish the baseline values, IOP values in the left and right eyes were measured three times during initial half hour before administrating the dosage (Qi et al., 2007). The IOP was measured initially (0 h) and at different predetermined time points under local anesthetic condition. To get the readings, the changes in IOP [ΔIOP = IOP of control eye (NaCl treated) - IOP of formulation treated eyes) were calculated. The effectiveness of F8 in comparison to DV-AqS was evaluated by the time required (T_{max}) to attain the peak ΔIOP (ΔIOP_{max}) and area under the ΔIOP *vs* time plot ($AUC_{0\text{-}12h}$), after ocular instillation of the two formulations. Data were evaluated, results were calculated and expressed as the mean ± SEM (n = 3).

3. Statistical data analysis

The data obtained from precorneal retention of F8 and ocular pharmacokinetics of DV were analyzed by linear trapezoidal method through the *PK* Solver (*V*2.0), Nanjing, China in MS-Excel (Kalam, 2016; Kalam and Alshamsan, 2017). Numerical values obtained through tonometer were transformed to IOP units of mmHg according to the Schiotz calibration scale **(Table S1**). Paired *t*-test (GraphPad Software, USA) was used for the comparison between the treated groups of animals by considering the * ($p < 0.05$) as statistically significant. All experiments were done in triplicate and data are represented as mean ± SD, unless otherwise indicated.

4. Results and discussion

4.1. Preparation of DV thermoresponsive gels

Polymer based *in situ* gelling carriers could be ideal for ocular applications as they are easy to apply in form of solution (sol). These sols then transform to a gel by external stimuli such as temperature, pH, and presence of ions (Agrawal et al., 2012; Huang et al., 2016; Li et al., 2014; Mundada and Avari, 2009; Wu et al., 2019). When a gel is responsive to changes in temperature (i.e. thermoresponsive gel), it goes through the sol-gel conversions after cooling or heating due to alterations in the intermolecular interactions (ionic, H-bonding and hydrophobic forces). Our developed system is composed of two Poloxamers, namely P407 and P188, and CP as a mucoadhesive material, formulated to enhance the ocular delivery of DV. It has been reported that P407 was successfully utilized as gelling agent in designing thermoresponsive, bioadhesive, and controlled-release ocular drug delivery system (Huang et al., 2016). P407 is a tri-block copolymer with a central polyoxypropylene (POP) hydrophobic chain and two adjacent polyoxyethylene (POE) hydrophilic chains that has concentration dependent gelation capability. Alongside P407, P188 solution is commonly added to the P407 solution to optimize gelation temperatures (T_{gel}). In addition to that, CP has an excellent binding characteristics that offer a prolonged ocular retention and controlled release property (Cao et al., 2010; Li et al., 2014). Furthermore, CP is suitable for pharmaceutical applications as it has an antioxidant property that reduces the need of toxic preservatives. CP also has pH triggered sol-gel transition property at neutral pH and at lower concentrations of 0.2–1.4% (Qi et al., 2007). DV was chosen in this study as a drug based on its superior physicochemical and clinical properties over EP. It was found that DV stays stable in aqueous solution if stored at 10–15 °C over 2 years (Yang et al., 2011). Esterase enzymes present in the eyes convert the ester-based DV to EP (Fig. 1**)** (Duvvuri et al., 2004), which act by stimulating the ocular α and β adrenergic receptors, triggering a decrease in ocular AqH production, an increase in the outflow facility and nasolacrimal drainage, thus reducing IOP (Nakamura et al., 1993).

It was reported that a thermoresponsive gel consisting of P407 (at 21% w/v) and P188 (at 5% w/v) was found to be most appropriate for ocular use based on the gelation temperature (T_{gel}), which is at 27.3 °C before diluting with STF and 34.8 °C after dilution with STF (Qi et al., 2006). The use of aqueous solution of P407 at 20–30% w/v alone did not show satisfactory sol to gel conversion at/or below 25 °C, while aqueous solution of P188 at same concentration has shown sol-gel conversion above 40 °C, suggesting a potential useful combination when used together (Asasutjarit et al., 2011; Soliman et al., 2019). Based on previous reports (Cao et al., 2010; Qi et al., 2007), and to improve the mucoadhesive property of the mixture, the concentrations of P407, P188, and CP chosen for our study were 15–20%, 2.5–5%, and 0.1–0.15%, w/v, respectively. We believe that this range of concentrations will deliver the desired sol-gel characteristics including gelling capacity, transparency, and appropriate T_{gel} to the formulations **(**Table 1**)**. In addition to the aforementioned system components, mannitol (2.5%, w/v) was used in the sol-gels to maintain isotonicity, increase the viscosity of the P407 and P188 solutions and also to decrease the IOP (Li et al., 2014).

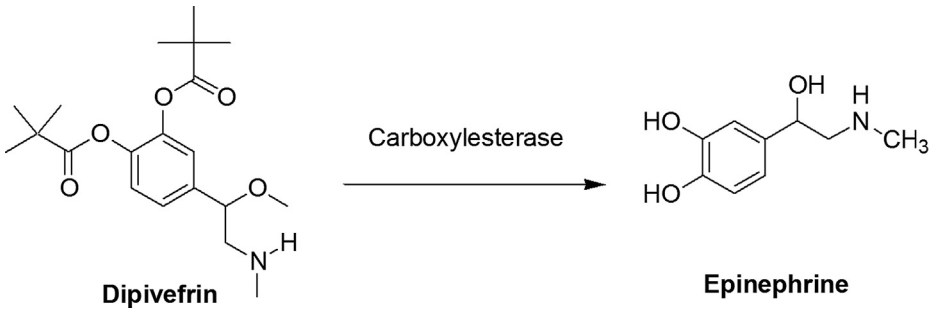

Fig. 1. The activation of Dipivefrin to epinephrine through carboxyl esterase.

4.2. Physicochemical characteristics and T_{gel} of DV-thermoresponsive gels

We evaluated the clarity and transparency of sol-gels at ocular physiological conditions (pH 7.2 and 35 °C) and observed that formulations containing low percentages of P407 (15%), P188 (2.5%), either with or without 0.1% of CP were slightly translucent before reaching 35 °C. This might be due to the presence of —COOH groups in the matrix of 0.1% CP that induced the formation of H-bonding with the hydrophilic blocks (POE) of P407 and P188, which would consequently lower the hydrophilicity of P407 and P188 (Cao et al., 2010; Wei et al., 2002) and might reduce the aqueous solubility of the Poloxamers that lead to the translucent appearance of sol-gel. Moreover, in this aqueous environment and at mentioned temperature and concentrations of Poloxamers, they may self-arranged themselves to form spherical micellar structure where the hydrophilic POE-chains surrounded the hydrophobic polyoxypropylene-core. On the other hand, sol-gels were transparent when the higher percentage of P407 (20%), P188 (5%) with 0.15% of CP were used. This could be attributed to the higher concentration of CP, which interferes with H-bonding formation between the —COOH groups in the polyacrylic acid in CP and the POE-blocks of P407 and P188. Additionally, a slight increase in the percentages of P407 and P188 would increase the POE ratio, which causes an increase in the T_{gel}. This yields a relatively more hydrophilic system, due to the increased population of hydrophilic POE-chains, compared to using lower concertation of P407 and P188. This may lead to the increased aqueous solubility of the Poloxamers and might be a possible reason for formation of transparent sol-gel at the higher concentrations of the polymers.

A thermoresponsive sol-gel would be considered optimal for ocular delivery when it remains as a solution at 25 °C and undergo gelation in the *cul de sac* of eyes at 35 °C (Qi et al., 2007; Wei et al., 2002), after being mixed with tear fluids. In addition, *in situ* gels should maintain their integrity for a prolonged time with gradual erosion and dissolution in the eye. On the basis of above facts, the concentration ranges of P407, P188 and CP was optimized while considering the T_{gel} to be more than 25 °C but less than 35 °C. The transparency, drug content, RI, osmolarity, pH, and T_{gel} of the developed sol-gels were found satisfactory as listed in Table 2**.** Out of the tested formulations, F8 showed the most suitable T_{gel} which was 26.7 ± 0.2 °C before and 35.1 ± 0.4 °C after dilution with the STF. Osmolarity of the sol-gels were in the range of 294 to 307 mOsmol/L, which was approximately similar to the osmolarity of tear fluid (302 mOsmol/L) in normal eye conditions (Tomlinson et al., 2006).

4.3. Flow rheology of the DV-thermoresponsive gels

Flow properties of the developed sol-gels of varying polymer compositions under ocular physiological (35 °C with STF) and non-physiological (25 °C) conditions were evaluated. At non-physiological conditions, all eight sol-gels remained liquid, while at physiological conditions, formulations F1-F3, F5 and F7 remained as gel-like liquid but still able to flow (Table 2). Viscosity of gels increased with increasing the concentrations of Poloxamers in presence of higher percentage of CP (Table 2 and Fig. 2**A).** Formulations F4, F6, F7, and F8 were found to be suitable for biological application because of their gel-formation characteristics at around 35 °C and a pH of 7.2. Among these formulations, F8 was the most suitable for biological application as it also showed a significant increase in viscosity (≈7.85 Pa·s) prior to the addition of STF at 28 °C and (≈1.61 Pa·s) post addition of STF at 37 °C **(**Fig. 2**B).** This increase in viscosity was due to thermoresponsive characteristics of P407 and P188, and the pH-sensitive properties of CP at the pH of STF. Thus, F8 was selected for further evaluation. Alongside a decrease in viscosity at 35 °C, the enhancement of shear rate of the formulation as shown in Fig. 2**C,** indicate that the formulation will be easily distributed on the ocular surface without causing a noticeable discomfort to the patient during blinking because of the high viscosity. Plotting shear stress (Pa) *vs* shear rate (s^{-1}) revealed that F8 exhibited a non-Newtonian pseudoplastic flow behavior **(**Fig. 2**D**). Moreover, shear stress was higher at ocular physiological temperature and it was lower at non-physiological temperature. The shear thinning behavior with increasing shear rate exhibited by F8 indicates that viscosity decreases with increasing shear and at the time of application, thus F8 is convenient and easy to apply.

4.4. In vitro drug release and polymer dissolution

The cumulative *in vitro* release percentages of DV from F8 and DV-AqS as a function of time are represented in Fig. 3**A.** DV release from DV-AqS was faster and immediate, 95% in 2 h, compared to 75% released from F8 for the same time period. The 75% drug released from F8 within 2 h was high, which might be due to the immediate release of the dispersed drug molecules within the polymer matrix (present in extramicellar channels). This might be considered as a good indication about the sol-gel formulation from the therapeutic point of view. Sometimes an initial high dose might have needed to exert the therapeutic potential of the drug. Within 4 h, almost 99% of DV was released from the DV-AqS, while it took 8 h for around 89% of DV to be released from F8. These results come in agreement with previous reports confirming sustained release of drugs when P407 was used as sol-gel system, either with or without P188 (Xuan et al., 2010), resulting in an improved ocular bioavailability of many drugs (Almeida et al., 2014; Ricci et al., 2005). Moreover, the sustained release of DV from F8 could also justified through Higuchi's square root release model as represented in Fig. 3**A'.** It shows the release rate plot for diffusion of DV from F8 and DV-AqS, where the fraction of drug release (*y*-axis) was plotted against the square root of time (*x*-axis). In case of F8, the increase in the percentage of DV release was

Table 2
Physicochemical characteristics, gelation temperatures, and flow behaviors of DV thermoresponsive gels (mean ± SD, n = 3).

Sol-gels	Clarity (Transparency) at 25 °C	Drug content (%)	Refractive index (RI)	pH	Osmolarity (mOsmol·L^{-1})	GT (°C) without STF**	GT (°C) diluted with STF**	Flow behavior at 25 °C	Flow behavior at 35 °C with STF** (pH 7.2)
F1	Clear transparent	98.2 ± 0.7	1.328	6.98 ± 0.14	298 ± 4	25.7 ± 0.3	37.4 ± 0.5	+	++
F2	Clear transparent	99.2 ± 0.6	1.341	7.06 ± 0.13	305 ± 6	26.4 ± 0.1	35.8 ± 0.7	+	++
F3	Clear transparent	98.2 ± 0.2	1.329	6.58 ± 0.05	294 ± 5	26.1 ± 0.2	38.9 ± 0.6	+	++
F4	Clear transparent	99.4 ± 0.4	1.332	7.08 ± 0.04	302 ± 7	27.5 ± 0.3	36.3 ± 0.5	+	+++
F5	Clear transparent	98.8 ± 0.2	1.340	6.58 ± 0.05	300 ± 2	25.4 ± 0.1	37.1 ± 0.2	+	++
F6	Clear transparent	99.1 ± 0.8	1.346	7.12 ± 0.09	306 ± 4	26.8 ± 0.5	35.6 ± 0.7	+	+++
F7	Clear transparent	99.1 ± 0.8	1.343	6.82 ± 0.15	307 ± 3	26.9 ± 0.4	35.2 ± 0.3	+	++
F8	Clear transparent	99.4 ± 0.3	1.347	7.02 ± 0.06	305 ± 5	26.7 ± 0.2	35.1 ± 0.4	+	+++

**"+" indicates the clear slightly viscous liquid and flow easily, "++" indicates gel like liquid still flowable, "+++" indicates sols converted to gel with good consistency but pourable.
** The simulated tear fluid (STF) was composed of 0.68 g NaCl, 0.22 g $NaHCO_3$, 0.008 g $CaCl_2{\cdot}2H_2O$ and 0.14 g KCl in 100 mL Milli-Q water.

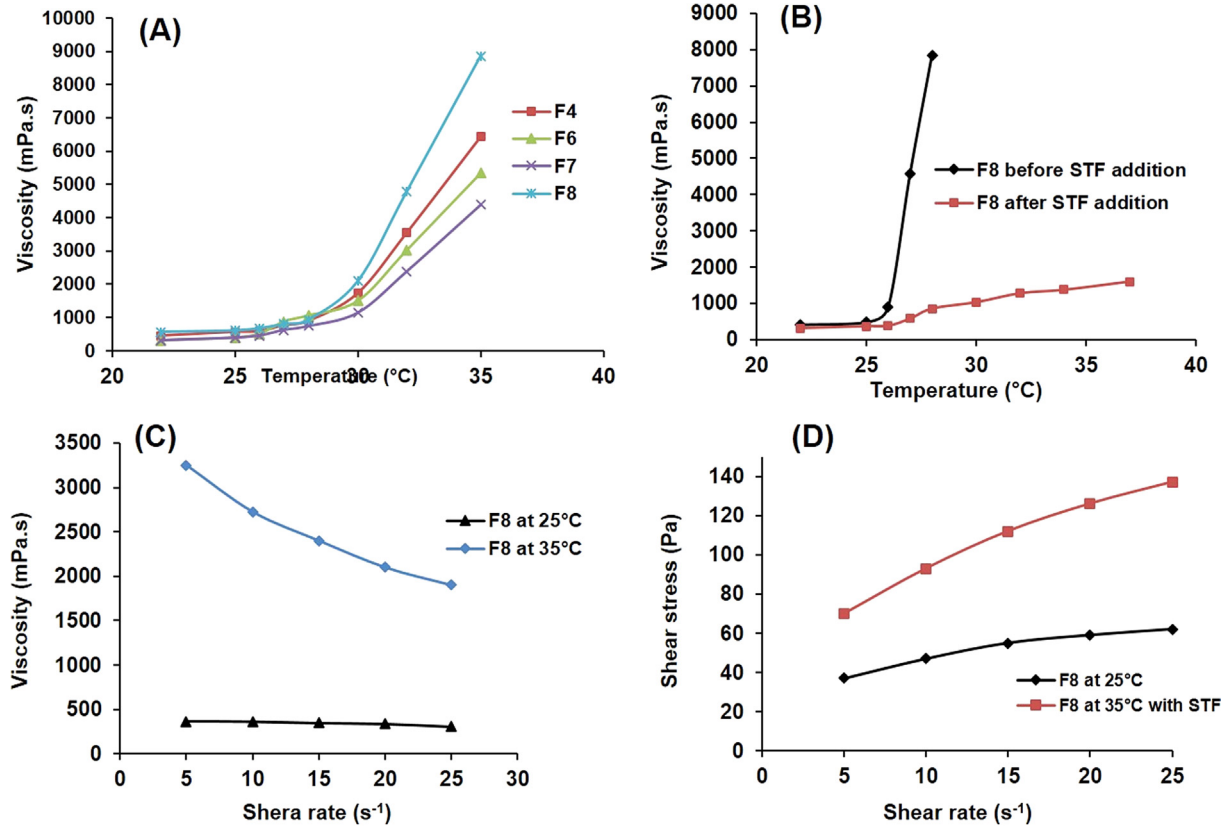

Fig. 2. Rheological evaluation of DV thermoresponsive gels: Viscosity *vs* temperature profiles for F4, F6, F7 and F8 **(A);** Viscosities of F8 at different temperatures before and after STF dilution **(B)**; Viscosities of F8 at 25 °C and 35 °C at different shear rates (s^{-1}) **(C)**; Shear rate *vs* shear stress profile of F8 in non-physiological (25 °C) and ocular physiological (35 °C with STF) conditions **(D).**

found almost linear with respect to the square root of time with a coefficient of correlation (R^2 = 0.988), which indicates the sustained drug release property of F8 as compared to DV-AqS.

The DV released data were fitted into different release kinetic models (Table 3**).** The cumulative amount of DV released was proportionate to the square root of time, and linearity was found with the correlations coefficient approaching 1.0. These results were in agreement with the results of drug release seen with other polymeric systems (Huang et al., 2016; Liu et al., 2010; Qi et al., 2007). By comparing the values of correlation coefficient (R^2), the drug release curve of F8 was best fitted into the Higuchi's-matrix (M_0-M_t = $kt^{1/2}$) kinetic model. Based on the higher magnitude of R^2, the diffusion exponent (n-value) which was 0.067 fell between 0 and 0.5, specifying the drug release kinetics from F8 was primarily by Fickian diffusion type following polymer matrix erosion.

In physiological conditions, drug release from sol-gel is typically affected by the presence of tears in *cul-de-sac*, and shear stress caused by blinking of eyelids (Bother and Waaler, 1990; Kalam et al., 2008). To investigate drug release in such conditions, we adopted an analysis method reported by Huang et al. to elucidate the correlation between gel dissolution and the release of DV (Huang et al., 2016). For F8 in STF at 35 °C in, we observed that amount of gels eroded with time had a direct relationship with the amount of drug released (Fig. 3**B**). A linear plot between the cumulative release of DV (%) *vs* the dissolution of F8 (Fig. 3**C**) confirmed that the release of DV from gel was unconditionally related to the dissolution of the gel. The cumulative release of DV and the dissolution of the polymers of F8 with time (Fig. 3**D**) demonstrated that the release of DV was well controlled by the dissolution of gel only. On the basis of above findings, the release of DV from poly-

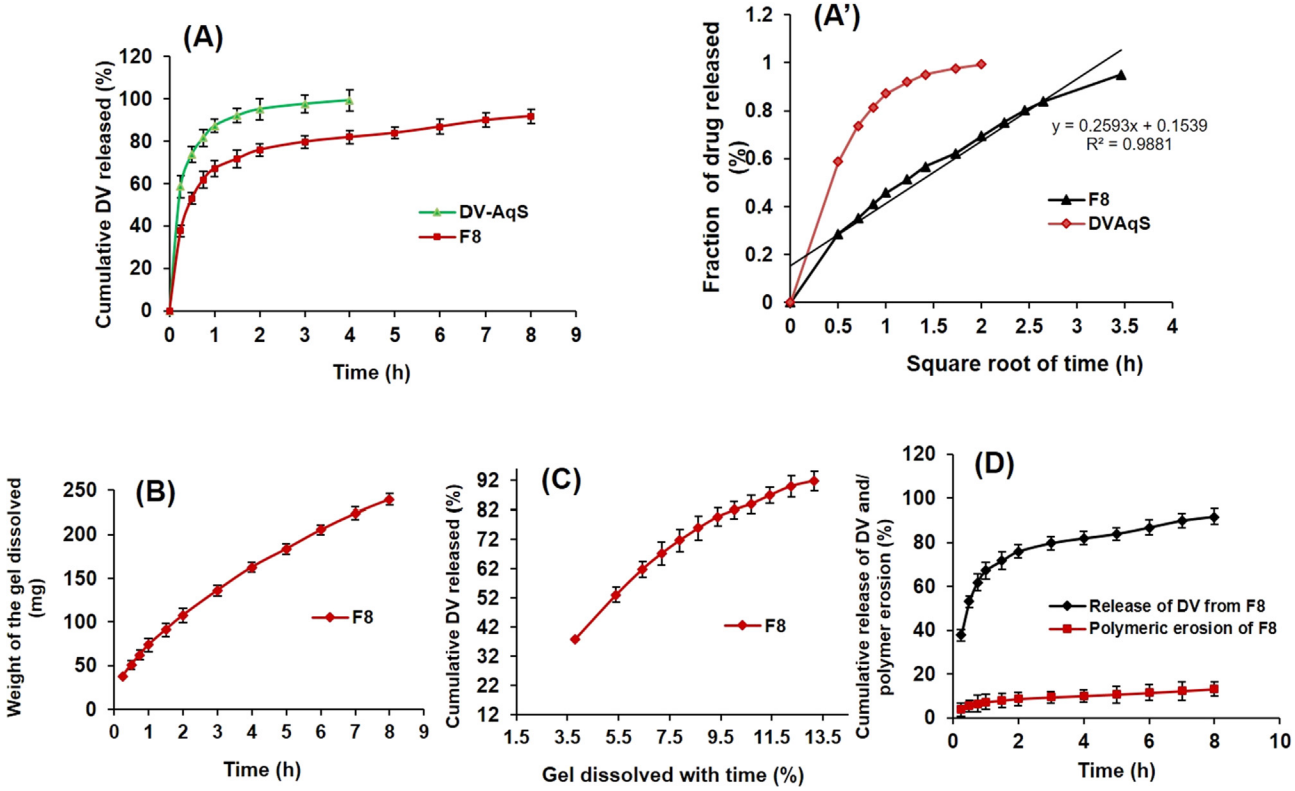

Fig. 3. *In vitro* release profiles of DV (1.0 mg) from F8 and DV-AqS at 35 ± 0.5 °C in STF **(A);** *In vitro* polymer erosion of F8 with time at 35 ± 0.5 °C in STF **(B);** Cumulative DV released (%) and polymer erosion (%) for F8 with time **(C);** Link between gel dissolution with time *vs* cumulative DV released from F8 **(D)**. All the values were represented as mean with ± SD, n = 3.

Table 3
Fitting *in vitro* release data of DV (1.0 mg) from the F8 thermoresponsive gel into different release kinetics models.

Release model equations	Sol-gel (F8)	
	R^2 value	n-value
Zero order (M_0-M_t = kt)	0.8144	
First order (ln M_t = ln M_0 + kt)	0.9061	
Higuchi's square root plot (M_0-M_t = $kt^{1/2}$)	0.9881	0.067
Korsmeyer-Peppas (log (M_0-M_t) = log k + n log t)	0.9514	
Hixson-Crowell ($M_0^{1/3}$-$M_t^{1/3}$ = $k_s t$)	0.8514	

"*M_0 is the initial drug amount (100%, when represented as percentage); M_t the amount of drug remaining at particular time (t); k the rate constant and 'n' being the diffusion exponent".

meric gel matrix was mainly affected by the dissolution of the gel. This was the reason for the absence of initial burst release of DV rather than a prolonged release with time. The results of *in vitro* drug release and polymer dissolution, infer the suitability of F8 with obvious sustained and prolonged-release of DV for ocular use.

4.5. Ex vivo transcorneal permeation of DV

According to the literature, DV has a molecular mass of 378.90 g/mol and a LogP value ≈1.7, which is suitable for a relatively stress-free trans-epithelial passage of the drug through the lipophilic corneal epithelium. Additionally, the solubility ratio of DV for octanol/PBS at pH 7.2 was found to be 4.89, as compared to 0.0081 ratio of the parent molecule (Wei et al., 1978). Hence, the partition coefficient of DV could be 603 fold higher compared to epinephrine. Moreover, the ionization constant (p*K*a) of DV is 8.4, suggesting that a larger fraction of DV is available in the unionized form at 7.2 pH, which could enhance the transcorneal flux of DV from DV-AqS at 0.5 and 1 h (Wei et al., 1978).

To measure the flux (J) and apparent permeability co-efficient (P_{app}) of DV, we used 6.9 mL of release medium, transcorneal permeation area of 0.636 cm^2, and initial DV concentration of 500 μg/mL. The pH values for DV-AqS and F8 were 6.86 ± 0.21 and 7.02 ± 0.06, respectively. These pH values are subject to change slightly due to the good buffering capacity of tears. Fig. 4**A** and Table 4 shows that F8 enabled the sustained permeation of DV by diffusion in a time dependent manner through the cornea into the receiver compartment. The cumulative amount of DV permeated from DV-AqS across the excised cornea was 115.5 μg/cm^2 in the 1st h and then almost constant at an approximate rate of 119.39 μg/cm^2 for the next 4 h. On the other hand, DV permeation from F8 was 31.57 μg/cm^2 in the 1st h and has progressively improved with time, reaching 136.33 μg/cm^2 after 4 h **(**Fig. 4**A).**

The flux (J) and apparent permeability (P*app*) were interpreted by exploiting the obtained plots of permeated amount of drug (μg/cm^2) against time (h). From the linear ascent of these plots the slope (dQ/dt) was obtained by using MS-Excel-2013. Now, considering the involved corneal area (0.636 cm^2) during permeation study, J was calculated by dividing the slope with 0.636. Then, P_{app} was calculated by dividing the obtained value of J with initial drug concentration (C_0). The difference in the apparent permeability of the drug between the F8 and DV-AqS can be easily interpreted from the mentioned values of P*app* in Table 4**,** which are (7.8 ± 0.5) × 10^{-2} cm/h and (1.2 ± 0.1) × 10^{-2} cm/h for F8 and DV-AqS, respectively. Here, we can observe that, at the equal concentration of the drug and equal area of cornea involved in this experiment for the two formulations, resulted around 6.5-times higher P*app* for F8 as compared to DV-AqS.

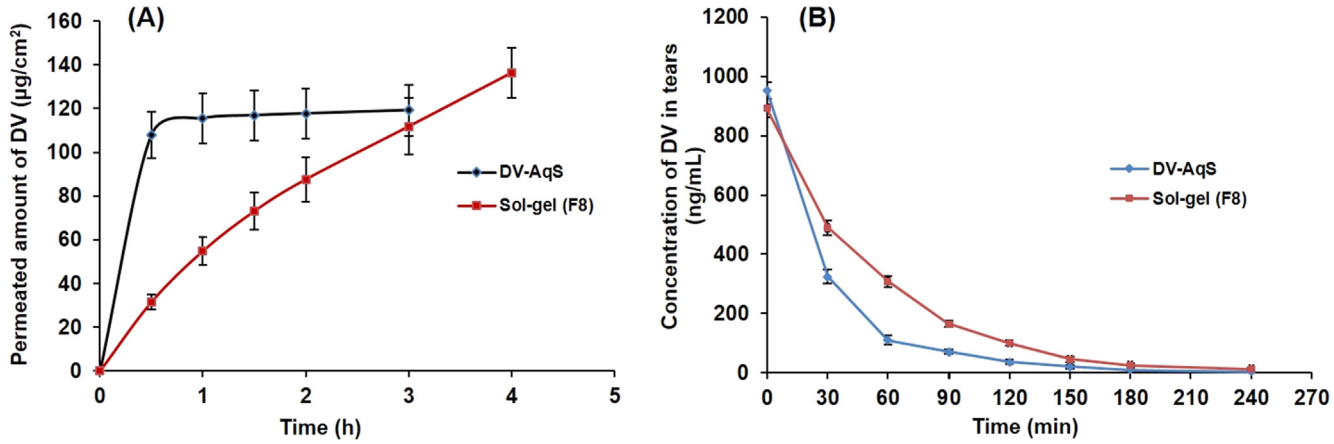

Fig. 4. *Ex vivo* corneal permeation of DV from F8 and DV-AqS **(A);** DV concentrations in tear fluids after ocular application of F8 and DV-AqS in to rabbit eyes **(B).** All the values were represented as mean with ± SD n = 3.

Table 4
Corneal permeation of DV after the application of F8 or DV-AqS (mean ± SD, n = 3).

Permeation parameters	F8	DV-AqS
Cumulative amount permeated (µg/cm² at 4 h)	136.3 ± 11.6	119.4 ± 10.1
pH	7.1 ± 0.1	7.2 ± 0.1
Steady-state flux, J (µg/cm²/h)	39.4 ± 2.4	6.1 ± 0.6
Apparent permeability, P_{app} (cm/h)	(7.8 ± 0.5) × 10^{-2}	(1.2 ± 0.1) × 10^{-2}

4.6. Ocular irritation study and in vivo precorneal retention studies

The acute and long-term eye irritation tests by following Draize's test (Draize et al., 1944) and scoring system of Diebold et al. (2007). Any score that is between 0 and 3 is considered acceptable (Alshamsan et al., 2019; Huang et al., 2016). Twelve rabbits were divided into two groups each containing six animals (group-I for single dosing and group-II for multiple dosing of F8). The right eye of each rabbits of group-1 received single instillation of F8 while the left eyes (as control) of each rabbit received single instillation of 0.9% NaCl solution. After 1 h of dosing the animals were examined for signs of acute eye-irritation. Group-II animals received the same treatment but three times a day, for seven days and they were examined at the end of the treatment. Congestion or redness of the conjunctiva and any eye discharge were observed and recorded in terms of scores. The results of irritation test indicated that during the single dosing of F8, mild redness was observed in the eyes of 4 out of 6 rabbits, which might be due to the dilation of blood vessels in the conjunctiva. The average score value recorded was 0.666 which was <3 **(**Table 5**).** In the second group however, all the six rabbits showed mild redness and slight congestion in the conjunctiva, while one rabbit showed watery ocular discharge as well. The recorded average score value for second group was 1.166, a bit higher than the first group but still less than 3 **(**Table 5**).** The NaCl treated eyes of all animals of both the groups were found to be normal. The observed discharge might be attributed to the thermoresponsive gelation of the frequent instillation of F8 and might be also associated with nonionic-surfactant property of the Poloxamers, which is less likely to happen with single dosing. In fact, F8 at single dosing did not cause any discharge and the recorded score was 0.666. Thus, the results have shown that F8 was well tolerated with minor watery discharge with its frequent administration that was resolved in the next 24 h of last dosing and the treated eyes were found normal at visual observation.

Table 5
Weighted scores for eye irritation after F8 ocular application.

Rabbit No.	Scores for the congestion/ redness of the conjunctiva	
	After single dosing of F8	After multiple dosing of F8
1st	1	1
2nd	0	1
3rd	1	1
4th	0	1
5th	1	2
6th	1	1
Average score	4/6 = 0.666 (falls between 0 and 3)	7/6 = 1.166 (falls between 0 and 3)

Score 1 and 2″ were designated for congestion/redness of conjunctiva and ocular discharge, respectively

In vivo precorneal pharmacokinetic study was designed to evaluate the potential benefit of utilizing F8 to prolong residence at ocular surfaces to yield an optimum C_{max} (not very high as compared to DV-AqS) so as to reduce the lacrimal elimination of DV (Bhatta et al., 2012). The concentration of DV in tears *vs* time profile as shown in Fig. 4**B**, indicates similarity in the kinetics of DV-AqS and F8. While at time zero tears DV concentration from the AqS was slightly higher than F8, the concentrations of DV at 30 min all the way towards 240 min was higher in tears of rabbit receiving F8. This result suggested that a larger portion of DV-AqS was washed out initially, whereas F8 had good corneal retention yielding relatively elevated concentrations of DV. The values of area under the curve, $AUC_{0\text{-}240\ min}$ and $AUC_{0\text{-}inf}$, as well as $AUMC_{0\text{-}inf}$ and $MRT_{0\text{-}inf}$ values F8 and DV-AqS are summarized in Table 6. The values of $AUC_{0\text{-}240\ min}$, $AUC_{0\text{-}inf}$, $AUMC_{0\text{-}inf}$ and $MRT_{0\text{-}inf}$ after ocular application of F8 were respectively 1.5, 1.5, 2.4 and 1.6-times higher than DV-AqS, and were found to be statistically significant (p < 0.05). AUC and MRT values of F8 indicated that a larger fraction of DV persisted in the pre-corneal region (up to 240 min) and clearance was significantly decreased (around 0.6-fold). The better ocular retention of F8 could be a good indicator of therapeutic efficacy owed to the good gelation ability of Poloxamers and mucoadhesive property of CP, that lead to prolong ocular retention time (Gratieri et al., 2010; Qi et al., 2007; Shastri et al., 2010).

4.7. Ocular pharmacokinetics of DV

After topical instillation and ocular absorption, DV rapidly and extensively undergoes hydrolysis to EP by acetylcholinesterase,

Table 6
Area under the curve of DV and mean residence time from F8 and DV-AqS in rabbits tear fluids (mean ± SEM, n = 3).

Parameters with units	F8	DV-AqS
$AUC_{0\text{-}240\ min}$ (ng/mL·min)	42566.5* ± 1142	30390.3 ± 1430.6
$AUC_{0\text{-}inf}$ (ng/mL·min)	43332.8* ± 1242.4	30605.3 ± 1430.7
$AUMC_{0\text{-}inf}$ (ng/mL·min^2)	2,246,764* ± 122696.3	1073555 ± 50369.5
$MRT_{0\text{-}inf}$ (min)	51.8* ± 1.6	35.1 ± 0.2
Cl/F ((ng)/(ng/mL)/min)	1.02 ± 0.05	1.6* ± 0.04

* $p < 0.05$ versus DV-AqS.

carbonic anhydrase, and pseudo-cholinesterase in the cornea, conjunctiva and AqH. This was reported by Wei *et al.*, where they were able to detect EP and metanephrine in the AqH of rabbit eyes within 30 min post treatment with either DV or EP (Mandell et al., 1978; Wei et al., 1978). Thus, here we focused on the quantification of EP after ocular application of DV.

The concentration of EP following the application of DV-AqS peaked after roughly 1 h, then it rapidly decreased to very low concentration at 6 and 12 h (Fig. 5**).** On the other hand, the concentration of EP following the application of F8 peaked at 2 h, and it decreased in a sustained manner with significantly higher concentrations at 2, 4, 6, and 12 h compared to DV-AqS. In addition, a significant improvement in the ocular bioavailability of EP was achieved with F8 system where there was a 2.6, 2.7, 5.6 and 2.1 times increase in $AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$, $AUMC_{0\text{-}inf}$ and $MRT_{0\text{-}inf}$, respectively, in comparison to DV-AqS (Table 7**A**). Although, there was no significant difference in C_{max} of EP between F8 and DV-AqS, $AUC_{0\text{-}12h}$ and $AUC_{0\text{-}inf}$ of F8 and DV-AqH were found to have significant differences. This discrepancy can be explained on the basis of MRT and clearance of F8 as compared to DV-AqS (Endrenyi et al., 1991; Lin et al., 2016). Despite the fact of being same drainage rate and tear turn over, around 2.1-fold higher MRT of F8 indicated its prolonged retention on ocular surfaces, which provided prolonged ocular absorption of the drug from F8. Moreover, 2.8-times faster clearance (Cl/F) of DV-AqS as compared to F8, indicated that the absorption phase of both were almost similar to reach an approximately same C_{max}, but the elimination of the drug from F8 was around 2.8-times slower than that occurred from DV-AqS (Endrenyi et al., 1991; Lin et al., 2016), that too supported from the measured concentration of EP, which was well detected even at 12 h, indicating the adherence of F8 on ocular surfaces and slower uptake of EP by the ocular tissues. On other hand, the administered DV-AqS was almost eliminated at 6 h and very small concentration (4.1 ng/mL) of EP was detected at 12 h, which was also explained in previous report (Kang Derwent and Mieler, 2008). The significant improvement in the ocular pharmacokinetics of EP indicates a higher transcorneal flux of the drug from F8 as compared to DV-AqS, which in turn is due to the prolonged ocular retention of F8 and sustained release of the DV.

4.8. In vivo intraocular pressure lowering effect

Relative ocular hypotensive effect of F8 and DV-AqS (both containing 0.1%, w/v DV) were tested in dexamethasone induced ocular hypertensive rabbits. The average IOP values of the control group (0.9%, NaCl) served as baseline values **(**Fig. 6**A).** Both formulations, F8 and DV-AqS, have significantly ($p < 0.05$) reduced and maintained lower IOP until the end of experiment at 12 h. However, effect was more pronounced with F8 reaching lowest IOP values of 14.63 ± 1.25 mmHg at 4 h post-treatment compared to around 20 mmHg found with DV-AqS. These results were further confirmed when the changes in IOP (ΔIOP, mmHg) were plotted against time (h) as shown in Fig. 6**B.**

It was found from the ocular pharmacodynamics in terms of IOP changes (Table 7**B)** that although it takes longer to reach T_{max} with F8, the $t_{1/2}$ was 1.3 times longer compared to DV-AqS. F8 was able to induce a 1.3 times more change in ΔIOP_{max}, as well as a 1.42, 1.67 and 1.99 times increase in $AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$ and $AUMC_{0\text{-}inf}$ respectively. Finally, the *in vivo* pharmacodynamic experiments indicated that the sol-gel system consisting P407, P188 and CP polymers prolonged the ocular retention and improved the ocular bioavailability of EP, which possibly caused a prolonged stimulation of ocular α and β_2-adrenergic receptors in the trabecular meshwork, triggering a decrease in aqueous production, an increase outflow facility and nasolacrimal drainage. So, a more change in the IOP. Thus, F8 was found to have better efficacy than DV-AqS.

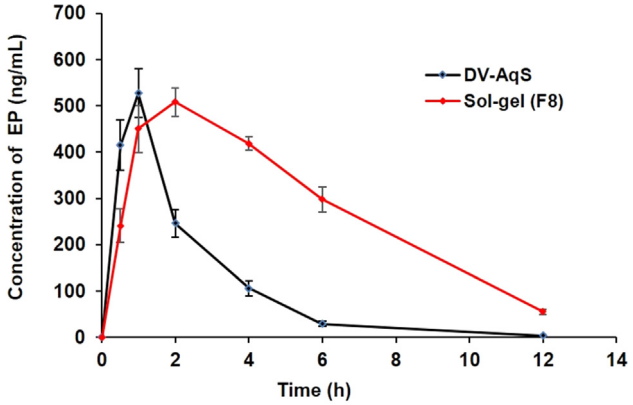

Fig. 5. Aqueous humor drug concentrations versus time profiles after topical application of DV-containing formulations in to rabbit eyes. All the values were represented as mean with ± SEM, n = 3.

Table 7
Pharmacokinetic (a) and pharmacodynamics (b) parameters of DV and EP after the ocular application of F8 or DV-AqS in rabbit (mean ± SEM, n = 3).

Pharmacokinetic parameters	F8	DV-AqS
A. Ocular pharmacokinetics in terms of EP quantification in aqueous humor		
$t_{1/2}$ (h)	2.6* ± 0.1	1.7 ± 0.1
T_{max} (h)	2.0 ± 0	1.0 ± 0
C_{max} (ng/mL)	507.9 ± 3	527.4 ± 5
$AUC_{0\text{-}12h}$ (ng/mL·h)	3414.1* ± 223.4	1316.4 ± 109
$AUC_{0\text{-}inf}$ (ng/mL·h)	3625.8* ± 248.6	1328 ± 107.6
$AUMC_{0\text{-}inf}$ (ng/mL·h^2)	17758.4* ± 1528.1	3123.8 ± 294.4
$MRT_{0\text{-}inf}$ (h)	4.8* ± 0.2	2.4 ± 0.1
Cl/F ((ng)/(ng/mL)/h)	13.8 ± 0.9	39.6* ± 1.9
B. Ocular pharmacodynamics (in terms of changes in IOP)		
$t_{1/2}$ (h)	11.2* ± 3.4	8.4 ± 2.4
T_{max} (h)	4.3* ± 0.6	3.3 ± 0.6
ΔIOP_{max} (mmHg)	19.6* ± 2.4	15 ± 3.1
$AUC_{0\text{-}12h}$ (mmHg*h)	193.4* ± 20.1	135.4 ± 17.9
$AUC_{0\text{-}inf}$ (mmHg*h)	415.4* ± 26.9	249.3 ± 80.3

* $p < 0.05$ versus DV-AqS.

5. Conclusion

Poloxamers (407 and 188) with Carbopol-934 based thermoresponsive sol-gels for ocular delivery of Dipivefrin.HCl was successfully developed. The optimized sol-gel (F8) consisting of 20% P407, 5% P188 and 0.15%, CP (w/v) exhibited suitable rheology, good gelling ability at 35 °C and sustained release of DV for 8 h. F8 could be instilled easily in the eyes at room temperature and is expected to have good spreadability for its pseudoplastic flow-behavior at ocular temperature. F8 provided a prolonged precorneal retention, better apparent permeability and absorption of the drug. In general, F8 retains superior bioavailability of DV in rabbits as com-

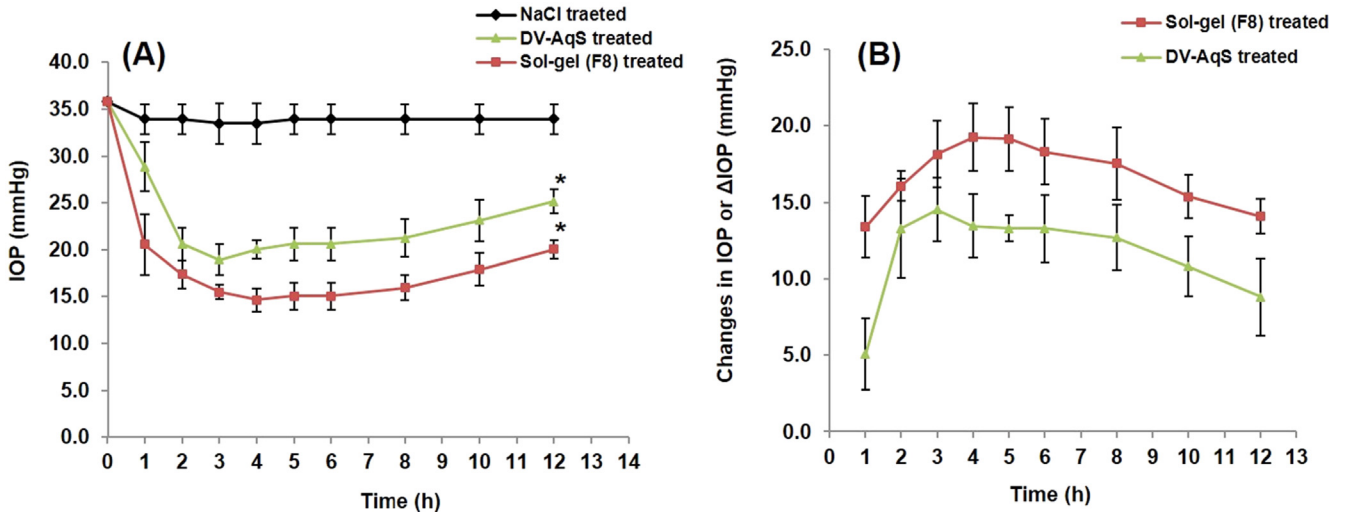

Fig. 6. Intraocular pressure of the rabbits with ocular hypertension versus time profiles **(A);** Changes in intraocular pressure (ΔIOP) *vs* time profiles **(B),** after topical application of F8 and DV-AqS in to rabbit eyes. All the values were represented as mean with ± SEM, $n = 3$ *($p < 0.05$) *vs* DV-AqS.

pared to DV-AqS as demonstrated by the improved values of ocular pharmacokinetic parameters ($AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$, $AUMC_{0\text{-}inf}$ and $MRT_{0\text{-}inf}$). *In vivo* ocular irritation experiment revealed that F8 is non-irritant to rabbit eyes and is relatively safe. *In vivo* efficacy study indicated that F8 has a better IOP reduction ability than DV-AqS which lasted 12 h. Thus, F8 could be a promising new formulation for the ocular delivery of Dipivefrin.HCl to minimize an elevated IOP.

CRediT authorship contribution statement

Musaed Alkholief: Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Mohd Abul Kalam:** Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Aliyah Almomen:** Methodology, Visualization, Data curation, Formal analysis. **Abdullah Alshememry:** Methodology, Investigation, Formal analysis. **Aws Alshamsan:** Conceptualization, Methodology, Resources, Data curation, Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2020.07.001.

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