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SUSTAINED RELEASE OPHTHALMIC *IN-SITU* GELS OF DORZOLAMIDE HCl FOR GLAUCOMA-AN APPROACH TO FORMULATION AND *IN-VITRO* EVALUATION

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ABSTRACT: The objective of the present investigation was to formulate the thermosensitive ophthalmic *in-situ* gels of Dorzolamide HCl for the treatment of glaucoma. Dorzolamide HCl is presently available as conventional eye drops, which has demerits like pre-corneal elimination, lachrymal drainage and no sustained effect leading to poor bioavailability and hence there was a need to develop a better, stable and sustained drug release dosage form. Dorzolamide HCl loaded thermosensitive ophthalmic *in-situ* gels were prepared by cold method using poloxamers as thermo-sensitive polymers in different concentrations with HPMC K4M as viscosifier which was used in three different concentrations. A total of six formulations were made and evaluated for parameters, namely appearance, clarity, pH, gelling capacity, gelation temperature, rheological study, drug content, and *in-vitro* drug release studies. All the evaluation parameters were within limits. Among all six formulations, one (DF-5) showed the highest drug release, and this formulation was evaluated for sterility, isotonicity, and *in-vitro* ocular irritation study, where all these parameters were found to be satisfactory. The results of the current study conclude that Dorzolamide HCl *in-situ* gel is a better alternative approach providing sustained drug release for the management of glaucoma.

INTRODUCTION: A glaucoma is a group of disease which causes damage to the optic nerve in the eye, and if left untreated, the disease continues slowly over the years and can result in permanent vision loss. Glaucoma is referred to as the silent thief of sight and affects one in 200 people who are aged between 50 and younger and one in 10 over the age of 50 [1]. It occurs usually when there is an increase in intraocular pressure (IOP) where a normal eye cannot tolerate. Open-angle glaucoma is the most chronic and second-leading blindness disease worldwide [2].

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For treating glaucoma, drugs are available in conventional eye drops, which have several demerits like precorneal elimination, no sustained effect. Hence, a better alternative approach is needed to overcome this issue; one of the best approaches is ophthalmic *in-situ* gels. These are the dosage forms that are in liquid before administration and undergo sol-gel transition after administration in the cul-de-sac of the eye and release the drug in a sustained manner for a prolonged period for controlling glaucoma [3].

Ophthalmic *in-situ* gels can be formulated by three different approaches, namely pH triggered, temperature triggered, and ion activated systems, where each mechanism is comprising of suitable polymers [4]. Presently, temperature triggered mechanism is followed and polymers used were poloxamer 188 and poloxamer 407 which are crosslinked poly oxy polypropylenes responsible

for change of liquid to gel at a temperature of 35-37 °C during which micelle formation begins by an increase in the temperature from 20-25 °C to 35-37 °C [5].

MATERIALS AND METHODS: Dorzolamide HCl was obtained as a gift sample from Orbicular Pharmaceutical Technologies Pvt. Ltd. Hyderabad, India. Poloxamer 188, Poloxamer 407, HPMC K4M, sodium chloride, sodium citrate buffer, and benzalkonium chloride were procured from Hi media laboratories, Mumbai, India. Before the formulation design, a compatibility study was done for a pure drug with polymers to confirm for the presence or absence of chemical interaction. This was verified by FTIR and DSC studies.

FT-IR Spectroscopy: IR spectra of the pure drug of Dorzolamide HCl and physical mixture of the drug with polymers HPMC K4M, poloxamer 188 and poloxamer 407 was determined using ATR – Bruker alpha model spectrophotometer. From the interpretation of characteristic functional groups obtained with the physical mixture of drug and polymers, the compatibility was evaluated [6].

DSC Study: In this study, Dorzolamide HCl and equal amounts of Dorzolamide HCl, poloxamer 188 and poloxamer 407 as a physical mixture were used for the study. The samples were placed in a pan of Shimadzu and were heated at about 20- 380 °C at a rate of 10 °C/min using a nitrogen atmosphere. The compatibility of the drug with polymers was verified from the thermogram obtained at the standard melting point of drug and polymers used for study [7].

Preparation of Dorzolamide HCl ophthalmic *in-situ* gels: Ophthalmic *in-situ* gels of Dorzolamide HCl were prepared by adopting the cold method [8]. In this method, little quantity of purified water was taken in 100 ml beaker containing required amounts of poloxamer 188 and poloxamer 407, which were dissolved by sufficient stirring with a magnetic stirrer. Later required quantity of HPMC K4M was added to the polymer solution and stirred for 30 min. 0.6g of Dorzolamide HCl, 0.9% NaCl, 0.29% sodium citrate buffer, and 0.01% benzalkonium chloride were added and continued stirring for 30 min. Six formulations were prepared by varying the concentrations of HPMC K4M (0.10 g, 0.15 g & 0.20 g), and poloxamer 407 was used in two different concentrations of 16% and 17%, whereas poloxamer 188 was used in 5% concentration in all the formulations. All the formulated *in-situ* gels were kept in a refrigerator at 4 °C for overnight. On the next day, the volume was made up to 30 ml with purified water followed by 10 min of stirring, and the pH of all the formulations was brought in the range of 6.8-7.4 by dropwise addition of 0.1 N sodium hydroxide. 10 ml each of the formulations was filtered through sterile 0.22 µ syringe filters and filled into clean vials [9]. The formulation composition of Dorzolamide HCl ophthalmic *in-situ* gels is shown in **Table 1**.

TABLE 1: FORMULATION DESIGN OF DORZOL-AMIDE HCl OPHTHALMIC *IN-SITU* GELS

Ingredients	Formulation code					
	DF-1	DF-2	DF-3	DF-4	DF-5	DF-6
Dorzolamide HCl (gm)	0.6	0.6	0.6	0.6	0.6	0.6
HPMC K4M (gm)	0.10	0.15	0.20	0.10	0.15	0.20
Poloxamer 188 (%)	5	5	5	5	5	5
Poloxamer 407 (%)	15	15	15	16	16	16
Sodium chloride (%)	0.9	0.9	0.9	0.9	0.9	0.9
Sodium citrate dihydrate (%)	0.29	0.29	0.29	0.29	0.29	0.29
0.1N NaoH (ml)	q.s	q.s	q.s	q.s	q.s	q.s
Benzalkonium chloride (%)	0.01	0.01	0.01	0.01	0.01	0.01
Purified water q.s (ml)	30	30	30	30	30	30

Evaluation of Dorzolamide HCl Ophthalmic *In-situ* Gels: All the six formulated ophthalmic *in-situ* gels were evaluated for appearance, clarity, pH, rheological study, gelling capacity, drug content, and *in-vitro* drug release study. The optimized formulation was subjected to evaluate drug release kinetics, sterility test, isotonicity test and *in-vitro* ocular irritancy test (HET-CAM test).

The method and evaluation procedure for each of the above-said parameter is briefly discussed as follows.

Appearance, Clarity & pH: All the prepared ophthalmic *in-situ* gels were visually inspected for their physical appearance; clarity was checked for the presence of any dirt, foreign particles, and insoluble matter by observing the formulations under a fluorescent lamp against white and dark background surface. The pH of the *in-situ* gels was evaluated by digital pH meter to check the *in-situ* gels were within the ophthalmic pH range or not [10].

Gelling Capacity: This parameter is to check the sol-gel transition of the formulation, which becomes a gelling solution from the liquid state when administered to the cul-de-sac of the eye. Gelling capacity was evaluated by placing a drop of *in-situ* gel to 2 ml of simulated tear fluid (STF) in a vial, which was equilibrated on temperature controlling thermostat at 37 ± 2 °C. The gelling capacity was verified by observing the time taken for gelation and time taken to dissolve the gel [11].

The grading of gelling capacity was done as follows. (-) means no gelation, (+) means gelation started within a few seconds and dissolved immediately, (++) means gelation started immediately and retained for a few hrs**,** (+++) means gelation started immediately and retained for prolonged hrs.

Rheological Study: Since ophthalmic *in-situ* gels are liquid gelling systems, they should have desired viscosity, which has greater importance in flow patterns during administration into the eye. The viscosity of formulated *in-situ* gels was measured using Brookfield viscometer DV-II + pro model with spindle no. 61 at shear rates of 10, 20, 30, 50, and 100 rpm. Viscosity was measured both at 25 ± 2 °C and at 37 ± 2 °C. A plot of viscosity in cps versus shear rate in rpm was plotted to get rheological curves [12].

Gelation Temperature: It was evaluated by placing 10 ml of *in-situ* gel in a 50 ml beaker with a magnetic bead inside. The beaker is mounted on a hot plate magnetic stirrer, which was operated at a speed of 50 rpm, and the temperature of the stirrer was slightly increased. A thermometer was kept inside the beaker. As the bead was rotated and temperature increases, the solution becomes a gelling liquid with increased viscosity; rotation of the bead becomes slow and stops at a certain time. The temperature at which the bead stopped due to the transformation of liquid to gel is noted as gelation temperature [13].

Drug Content Estimation: It was done to find out the percentage amount of pure drug Dorzolamide HCl is prepared *in-situ* gels. The procedure involves a UV spectroscopic method; 1 ml of each formulation was diluted to 100 ml of simulated tear fluid (STF) to get a concentration of 100 µg/ml. The final dilutions were made with STF, and the solutions were scanned at 253 nm wavelength in UV spectrophotometer, and the absorbance was noted in percentage [14].

***In-vitro* Drug Release Study:** In this method, a dialysis membrane that was previously soaked in simulated tear fluid (STF) for 24 h was sandwiched between donor and receptor compartment of the modified Franz diffusion cell. The receptor compartment was filled with STF, and the *in-situ* gel formulation of 1 ml was placed on the donor compartment, which was in contact with the receptor compartment. The diffusion cell assembly was kept on a thermostat-controlled magnetic stirrer with a hot plate.

A magnetic bead was placed inside the receptor compartment, which was allowed to rotate at a speed of 50 rpm, and a temperature of 37 ± 0.5 °C was maintained. Aliquots of 1 ml were withdrawn at every 1 h up to 10 h from one open end of diffusion cell with the help of a 1 ml syringe, and this was diluted with 10 ml of STF. 1 ml of STF was replaced into the diffusion cell with the same syringe. All the diluted samples were scanned at 253 nm in a UV spectrophotometer to get a drug release amount. Finally, % cumulative drug release was calculated for all the formulations, and the one which shows the highest drug release for a prolonged time was optimized as best one and subjected for other following parameters [14].

Drug Release Kinetics: The optimized *in-situ* gel was subjected for different release kinetic models like zero order, first order, Higuchi, and kores-Peppas study. The best fit model was selected based on the highest regression value (R^2) obtained by plotting the desired data for the respective models [14].

***Ex-vivo* Drug Permeation Study:** This was performed to study the amount of drug diffused through biomembrane using goat eye cornea. In this procedure, the goat's eye corneal membrane was used as a dialysis membrane, and the study was carried with the Franz diffusion cell. Freshly collected goat eyeball from slaughterhouse was procured into the lab and was immersed in normal saline to avoid tissue degradation. The corneal membrane was carefully dissected with a scissor with 5-6 mm thickness, washed 2-3 times with normal saline. The corneal membrane was soaked in STF for about 12 h, and after that, drug permeation analysis was carried out in the same way as that of the *in-vitro* drug release procedure, and the release study was done up to 12 h [15].

Sterility Test: This test was done to verify the formulation is free of microbial attack or not. It was performed using fluid thioglycollate medium for bacteria and soya bean casein digest agar medium for fungi. The test was done as per Indian Pharmacopoeia 2014; the formulation was inoculated with both media, as mentioned above, along with positive and negative controls in a test tube. Test tubes containing fluid thioglycollate medium were incubated at 35-37 °C and soya bean casein digest agar media at 25-27 °C for 7 days. Growth in the test tubes was confirmed by the appearance of turbidity in the positive control, which was compared with positive and negative controls.

Isotonicity Test: Isotonicity test was conducted to verify whether the ophthalmic formulation is safe, non-irritant, and non-toxic with eye secretions. It was evaluated by adding a few drops of blood and mixed with few drops of the optimized formulation and observed under high-resolution Biovis particle size analyzer at the 45X objective lens. The same procedure was repeated with marketed eye drops for the comparison of RBC's, the RBC's were observed for any shrinkage and bulging of cells by comparing the RBC's optimized formulation and marketed eye drops [16].

***In-vitro* Ocular Irritancy Evaluation by HET-CAM Test:** Since, Drazie rabbit test has been banned in several countries, including India, a suitable *in-vitro* model can be used for checking the ocular irritancy. One of the most widely used OECD recommended test is Hen's chorioallantoic membrane test (HET-CAM). In this test, freshly collected chicken eggs (not older than 7 days) are obtained from chicken breeder form. Eggs weighed between 50-60 g were used, and those with physical damage, cracks were rejected. Three groups were made, each containing 3 eggs.

Standard Group: Here, the eggs were treated with 0.9% NaCl as a standard control.

Test Group: In this group, eggs were tested with the optimized formulation.

Positive Control: In this, eggs were treated with 1% SDS (sodium dodecyl sulfate) as an irritant for comparison with standard and test.

Procedure: The eggs selected were kept on a tray which was placed in an incubator maintained at a temperature of 37 ± 0.5 °C and relative humidity of 58 ± 2 °C. Rotate the eggs manually with hand 5 times per day to prevent attachment of embryo, continue for about 8 days. Candle the eggs on the 8th day of incubation and remove the nonviable or defective eggs. After candling, eggs were replaced to the incubator without rotation by keeping the large end of eggs upward for one complete day. Remove the eggs on the 9th day; mark the air cell on top.

Make a hole on the air sac of eggshell without injuring the chorioallantoic membrane [17]. The eggs of the standard group were moistened with 0.3 ml of 0.9% NaCl and eggs were replaced into an incubator for 30 min. The eggs of the test group were treated with 0.3 ml of optimized formulation, and a positive control group was treated with 0.3 ml of 1% SDS.

Both the test and positive control groups were observed for the signs of hemorrhage, coagulation, and lysis of blood vessels for a time of 300 sec (5 min).

Evaluation: The irritation of test substance can be evaluated using the irritation score formula, and the grade of the irritation can be concluded by noting the score values after 5 min of treatment of the tested formulation [18]. The irritation score (IS) formula is given below, followed by irritation score value with inference shown in **Table 2**.

TABLE 2: IRRITATION SCORE VALUE WITH INFERENCE FOR HET-CAM TEST

Irritation score (IS)	Inference
0-0.9	No irritation
1-4.9	Weak irritation
5-8.9	Moderate irritation
9-21	Severe irritation

IS = (301-H) / 300 × 5 + (301- L) / 300 × 7 + (301- C) / 300 × 9

Where, H- Hemorrhage, L- Lysis of blood vessels, C- Coagulation

RESULTS AND DISCUSSION:

FTIR Study: The IR spectrum of Dorzolamide HCl was compared with standard FTIR and found to be the same. The physical mixture of dorzolamide HCl and HPMC K4M showed characteristic peaks at 3562.42 cm^{-1}, 3368.94 cm^{-1}, 3181.70 $cm^{-1,}$ and 1298.46 cm^{-1} exhibiting alcohol, amine, aromatic and sulphonamide groups. The physical mixture of Dorzolamide HCl with poloxamer 188 showed the peaks at 3670.54 cm^{-1}, 3479.58 cm^{-1}, 1348.24 cm^{-1} for alcohol, amine and sulphonamide groups, whereas the mixture of Dorzolamide HCl with poloxamer 407 showed IR peaks at 3674.39 cm^{-1}, 3290.56 cm^{-1}, 2742.78 cm^{-1} and 1348.24 cm^{-1} indicating the presence of alcohol, amine, aliphatic and sulphonamide groups. From the FTIR interpretation study, it was confirmed that there was no chemical interaction among the drug and polymers used for the present study.

DSC Study: From the DSC study, it was found that pure drug of Dorzolamide HCl showed a thermogram at 275.82 °C and the physical mixture of the drug with poloxamers showed a peak at 54.7 °C and 284.8 °C. There were no considerable changes when the drug was mixed with poloxamers since the melting point was within the standard range of drugs and polymers used for the study. The DSC thermograms for Dorzolamide HCl and physical mixture with poloxamers are shown in **Fig. 1**.

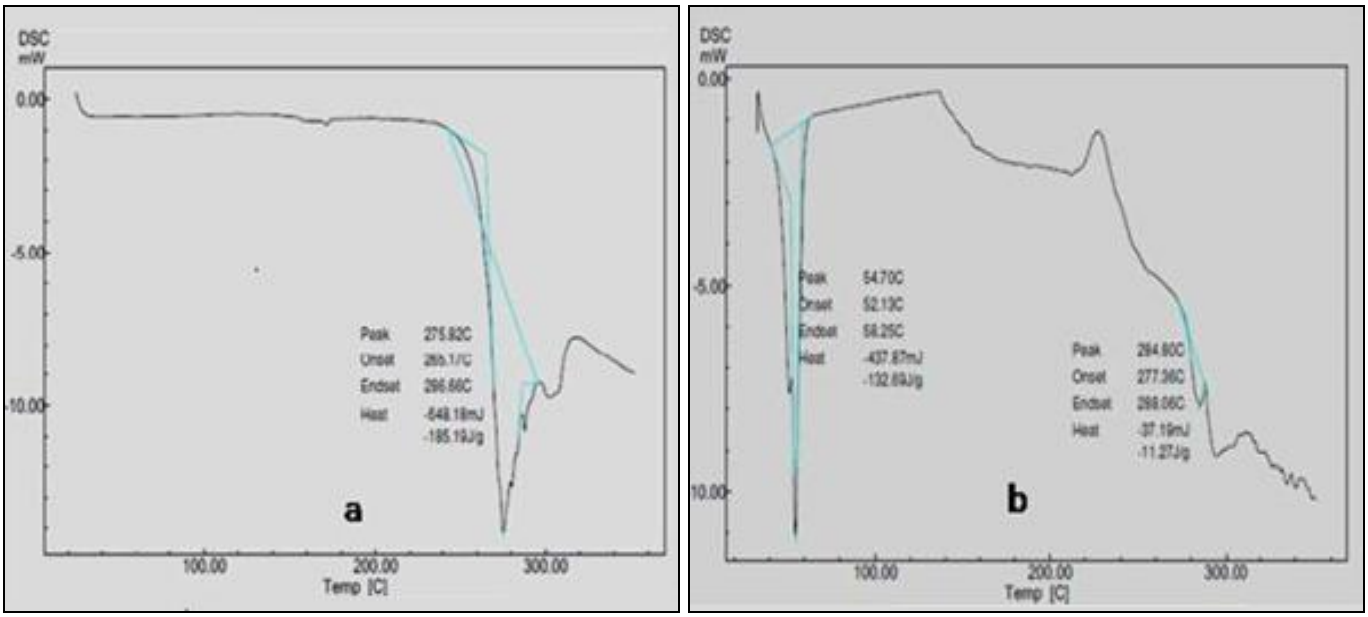

FIG. 1: DSC OF DORZOLAMIDE HCl (A) AND DSC OF PHYSICAL MIXTURE (B)

Appearance: The formulated Dorzolamide HCl *in-situ* gels were found to be clear, free- flowing liquids. The results of the appearance were discussed in **Table 3**.

Clarity: Clarity of formulated in situ gels revealed that there was no particulate matter found. The results of the clarity test were reported in **Table 3**.

pH: The pH of all the formulations was found to be 7-7.4, which was within the physiological range of eye; hence would not cause any irritation upon administration. The results of the pH were depicted in **Table 3**.

Gelling Capacity: The ophthalmic *in-situ* gels should have an optimum gelling capacity, so that after installation into the cul de sac of the eye as a liquid, it would undergo a rapid sol to gel transition and preserve its integrity without dissolving or eroding for a prolonged time. The results of the gelling capacity were discussed in **Table 3**.

TABLE 3: EVALUATION OF APPEARANCE, CLARITY, PH AND GELLING CAPACITY OF *IN-SITU* GELS

Formulation code	Appearance	Clarity	pH (mean ± SD)	Gelling capacity
DF-1	Free-flowing liquid	Clear	7.2 ± 0.25	++
DF-2	Free-flowing liquid	Clear	7.1 ± 0.05	++
DF-3	Free-flowing liquid	Clear	7.0 ± 0.14	++
DF-4	Free-flowing liquid	Clear	7.4 ±0.65	+++
DF-5	Free-flowing liquid	Clear	7.4 ± 0.37	+++
DF-6	Free-flowing liquid	Clear	7.3 ± 0.22	+++

Rheological Study: The viscosity of formulations was found to increase with a decrease in shear rate (rpm). The viscosity curves for the *in-situ* gels at 25 ± 2 °C and 37 ± 2 °C were depicted in **Fig. 2** and **3**. Based on the results it was found that formulations showed the viscosity ranging from 6.22 to 80.64 cps at 25 ± 2 °C and 247.27- 2538.37 cps at 37 ± 2 °C which was within the acceptable range. There was a drastic change in viscosity of *in-situ* gels at 37 ± 2 °C; this might be due to the fact that the *in-situ* gelling system undergoes gel formation by the formation of micelles which make them more viscoelastic liquids with an increase in temperature.

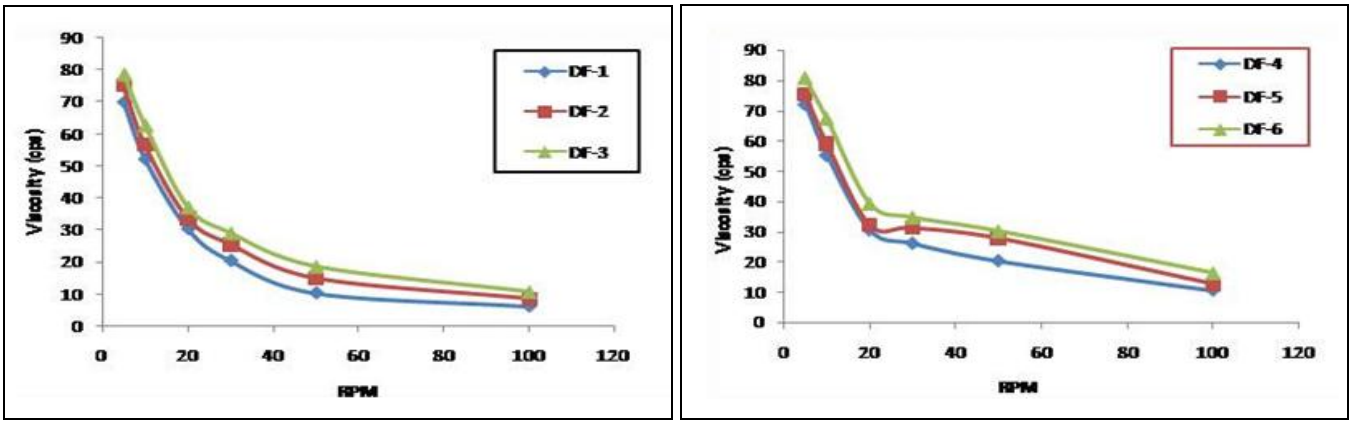

FIG. 2: VISCOSITY OF DORZOLAMIDE HCL *IN-SITU* GELS AT 25 ± 2 °C

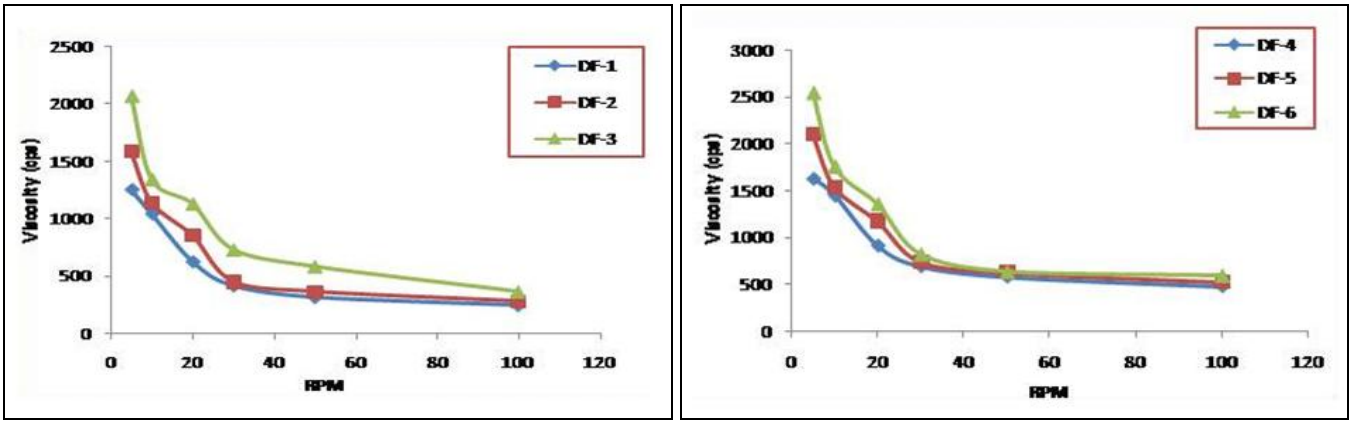

FIG. 3: VISCOSITY OF DORZOLAMIDE HCL *IN-SITU* GELS AT 37 ± 2 °C

Gelation Temperature: The gelation temperature of the formulated *in-situ* gels was found be in the range of 35-38 °C. It was observed that there were no unacceptable changes in the gelation temperature of the formulated *in-situ* gels.

Drug Content Estimation: The drug content of Dorzolamide HCl **in-situ** gels was in the range of 95.96-98.34% and was found to be within the standard limits.

***In-vitro* Drug Release Study:** The formulations DF-1, DF-2 and DF-3 containing 5% poloxamer 188 and 16% poloxamer 407 showed the drug release of 85.05-89.37% up to 8 h since the gelling capacity of these formulations was retained for 8 hrs, and the formulations DF-4, DF-5 and DF-6 containing 5% poloxamer and 17% poloxamer 407 showed the release of drug from 86.32- 92.67% up to 10 h since the gelling capacity of these formu-

lations was retained for an extended period of 10 h. The *in-vitro* drug release profile curve is shown in **Fig. 4**.

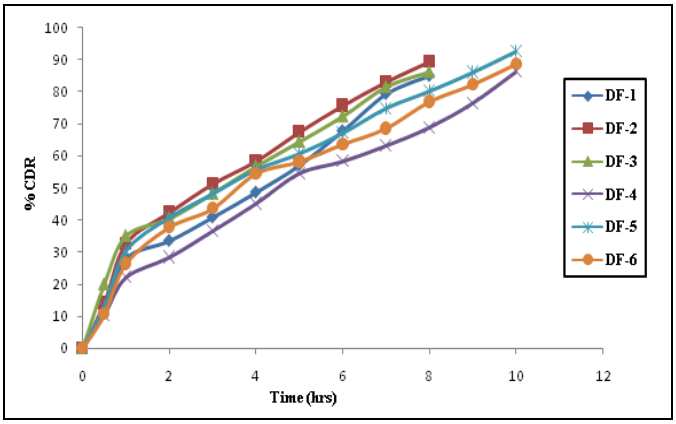

FIG. 4: DRUG RELEASE PROFILE OF DORZOLAMIDE HCL *IN-SITU* GELS

The formulation DF-5 which showed highest drug release of 92.67% was optimized as the best formulation and it was evaluated for remaining parameters.

Drug Release Kinetics: The Higuchi release kinetics study revealed the higher regression value and the formulation followed diffusion mechanism for sustaining the drug release. The Higuchi release mechanism plot was shown in **Fig. 5**.

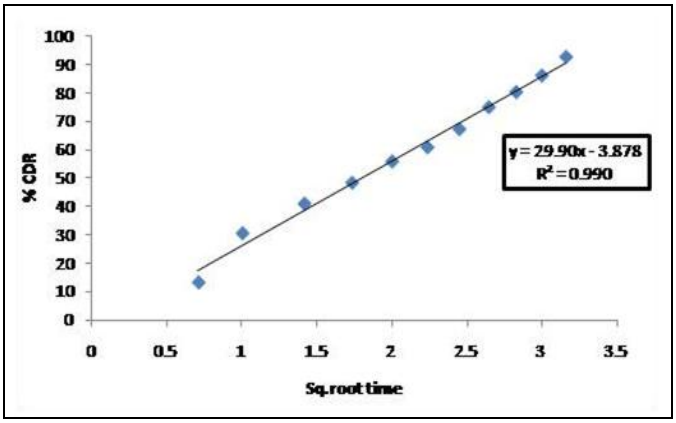

FIG. 5: HIGUCHI RELEASE KINETICS OF DF-5 FORMULATION

***Ex-vivo* Drug Permeation Study:** The variation in *in-vitro* and *ex-vivo* permeation study may be due to the biological variance between thee corneal membrane and dialysis membrane. The release plot of *ex-vivo* drug permeation is depicted in **Fig. 6**.

Sterility Test: From the sterility test, it was ensured that the formulation DF-5 did not showed any signs of microbial growth during the incubation period and the formulation was found to be sterile.

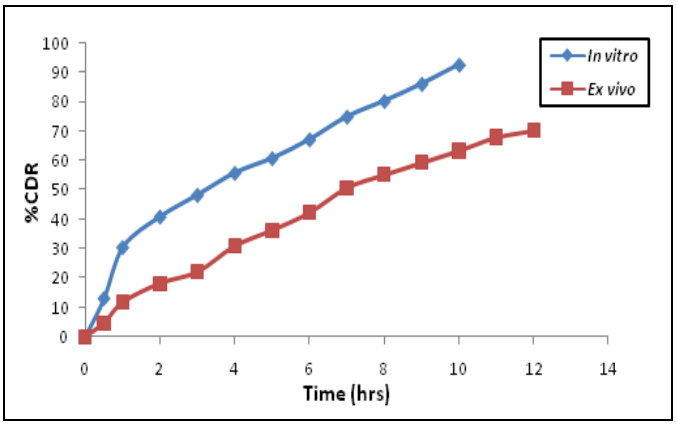

FIG. 6: COMPARISON OF *IN-VITRO* AND *EX-VIVO* DRUG RELEASE FOR DF-5

Isotonicity Test: From the isotonicity test, it was confirmed that the optimized formulation, DF-5 was isotonic with blood, since there was no change in the shape of RBCs when compared with marketed eye drops. The Isotonicity test images for DF-5 and marketed formulation were depicted in **Fig. 7** and **8**.

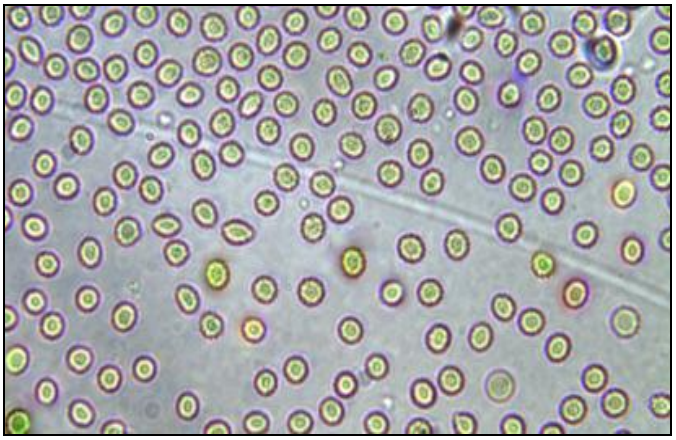

FIG. 7: RBCs WITH MARKETED EYEDROPS

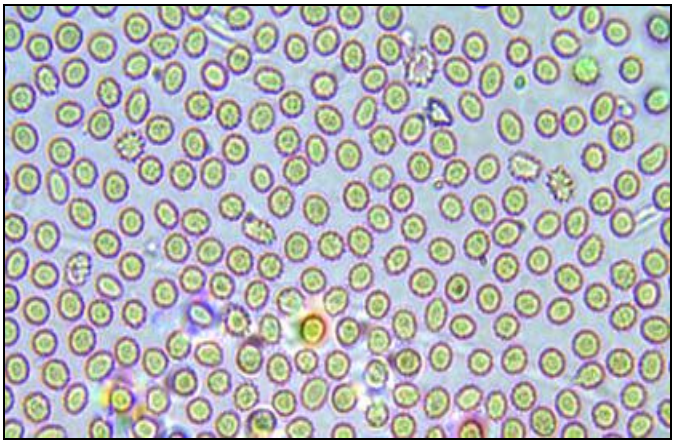

FIG. 8: RBCs WITH OPTIMIZED *IN-SITU* GEL (DF-5)

***In-vitro* Ocular Irritancy Test by HET-CAM Method:** The results of the HET-CAM test revealed a greater difference between the test and positive control for the ocular irritancy in the chorioallantoic membrane of hen's eggs. The results showed that 1% SDS induced major damage by producing lysis of blood vessels followed by little hemorrhage, whereas test solution of optimized formulation did not show any signs of irritation for the 5 min time duration. 0.9% NaCl standard solution was used as a reference for comparing the effects of irritation in the embryo. After noting the time in sec for calculating the mean irritation value, the 1% SDS treated Eggs showed the mean score of 11.4 indicating severe irritation, and the optimized formulation showed 0.04 score revealing there was no ocular irritation which was found to be non-toxic and non-irritant. The images of the chorioallantoic membrane treated with 0.9% NaCl, optimized formulation, and 1% SDS were represented in **Fig. 9**.

0.9% NACL TREATED **OPTIMIZED *IN-SITU* GEL TREATED (DF-5)**

1% SDS TREATED (EFFECT OF BLOOD VESSEL LYSIS) **1% SDS TREATED (EFFECT OF HEMORRHAGE)**

FIG. 9: HET-CAM TEST FOR OPTIMIZED DORZOLAMIDE HCL *IN-SITU* GEL (DF-5)

CONCLUSION: Thermosensitive ophthalmic *in-situ* gels of Dorzolamide HCl were successfully prepared with poloxamers as a better alternative approach with sustained drug release manner, which had greater compliance than conventional eye drops for the management of Glaucoma. From the results obtained by present research, it can be concluded that Thermosensitive ophthalmic *in-situ* gel will be best, promising formulation approach for Dorzolamide HCl for the control of Glaucoma.

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CONFLICTS OF INTEREST: The authors declare there are no competing conflicts of interest.

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