FORMULATION AND EVALUATION OF THERMO-RESPONSIVE OCULAR *IN SITU* GEL OF CIPROFLOXACIN AND OLOPATADINE HCL

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ABSTRACT

Ocular *in situ* gel (ISG) is a promising alternative to alleviate the shortcomings of conventional formulations due to their association with dose accuracy and effective administration with prolonged contact time. Therefore, present research aimed to develop a thermo-responsive *in situ* gel (TRISG) for ocular drug delivery (ODD) with different levels of Pluronic® F407 and Pluronic® F188 for ciprofloxacin HCl (CFH) and olopatadine HCl (OLH). The three optimal formulations were selected based on the physicochemical characterization of nine batches and were evaluated successfully. The batch F5 of CFH-OLH-TRISG explored the remarkable outcomes within acceptable limits in aspects of physicochemical characterization and other parameters. The TRISG has proven to release over 120 min, which was more significant than conventional drops (60 min), suggesting sustained release and better corneal penetration. A compressive finding explored the TRISG with combination might be a pragmatic choice for ODD with effective administration, enhanced ocular bioavailability, and sustained release.

Keywords: Thermo-responsive, *in situ* gel, ocular delivery, ciprofloxacin HCl, olopatadine HCl

INTRODUCTION

The design of a novel ocular drug delivery system (ODDS) has encountered numerous challenges and complications in managing several optic disorders[1]. Improving health care quality and treating a disease require developing and applying novel biomaterials[2]. There are potential benefits of *in situ gel* in, and various cellular, and enzymatic immobilization, researchers recently attracted attention in developing hydrogels in the forms of macro, micro, and nano-gels[3,4]. Formulation experts can modulate the proportion of stimuli-responsive gelling polymers without adding auxiliary solvents or co-polymerization accelerators to improve the extent of gelatinization, ocular retention time, and drug release rate[5–7]. Ciprofloxacin HCl (CFH) is a second-generation fluoroquinolone, a broad-spectrum antibiotic used in conjunctivitis. It is chemically *(S,S)* -1-cyclo-propyl 6-fluro-1,4 dihydro-4-oxo-7 [piperazine-1 yl]-3- quinolone carboxylic acid (Fig. 1a). Olopatadine HCl (OLH) is an antihistaminic used to treat allergic conjunctivitis by inhibiting the discharge of histamine from mast cells. It is chemically 11-[(*Z*)-3-(di-methyl-amino) propylidene]-

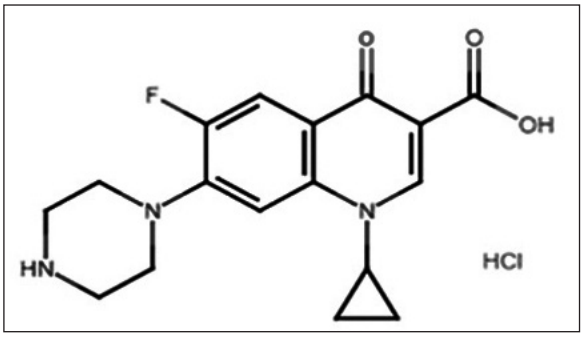

Fig. 1 a) Ciprofloxacin HCl

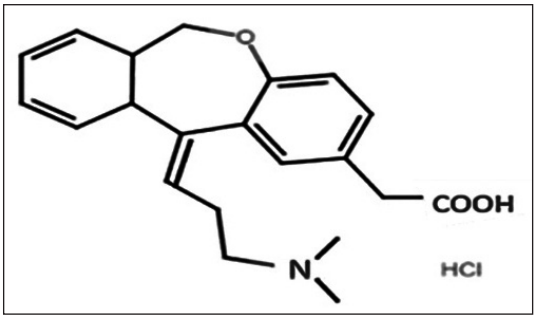

Fig. 1 b) Olopatadine HCl

Fig. 1: Chemical structures of CFH and OLH

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6,11-di-hydro-di-benzo [b,e] oxepin-2-acetic acid hydrochloride (Fig. 1b).

It is commercially marketed as eye drops [e.g. Ciplox D (Cipla), Olohyd (Oculent) etc.] and ointment [e.g. Ciplox D (Cipla), Zoxan (Apollo) etc.]. In the event of a severe infection, topical administration of CFH (0.3 %) and OLH (0.1 %, 0.2 % and 0.7 %) solutions are recommended for the patient[8,9]. Over the past decades, TRISG has gained enormous attention. Before administration, they are in a sol state but can gelate when exposed to various stimuli, including temperature, pH and incidence of ions[10].

They can be administered in a fluid form into the eye, similar to eye drops, to deliver a precise dose of medication. When *in situ* formulation converts into the gel at the eye's cul de sac, they eliminate the pitfalls associated with marketed eyes drops, such as quick nasolacrimal drainage and transitory corneal retention[11,12]. A substantial upsurge in the formulation's corneal retention time and bioavailability might be accomplished by *in situ* gelation compared to the traditional DDS. Thermo-responsive polymers can reveal dual-responsive properties when combined with other responsive moieties[13,14]. In micro/nano forms, temperature-triggered hydrogels demonstrate a volume phase transition at a specific temperature, leading to unexpected modification in the solvent state[15]. Numerous investigations have documented that CHF and OLH-containing temperature-triggered strategies can be employed to treat ocular ailments such as glaucoma and infections resulting from bacteria, fungi, and viruses[16–22]. The most frequently utilized polymers can be categorized into synthetic and natural polymers, such as poloxamers and poly (*N*-isopropyl acrylamide)[23–25]. The combination of two drugs is more effective than individual drugs; a single drug with a combination of two different polymers was previously developed, but CFH-OLH-TRISG has not been formulated using two polymer combinations. Hence in the present research, efforts have been taken to develop TRISG formulation using a thermo-sensitive polymer such as Pluronic® F407 (PNF 407) and Pluronic® F188 (PNF 188) for the effective administration of the CFH and OLH into the eye for treating ocular ailments, enhanced residence time and ocular bioavailability.

MATERIALS AND METHODS

Materials

CFH and OLH were procured from Aadhaar Life Sciences Pvt. Ltd, Solapur, India and Indoco Remedies Pvt. Ltd., Mumbai, India, respectively. Ciprofloxacin HCL eye drop (0.3% Cipla Ltd., CIPLOX) and Olopatadine HCl eye drop (0.1% Lupin Ltd., OLOBLU) were purchased from the local pharmacy. PNF 407 and PNF 188 were procured from Moly Chem. Pvt. Ltd., Mumbai, India. Benzalkonium Chloride (BKC) was bought from Loba Chemie Pvt. Ltd., Mumbai, India. The auxiliary chemicals and solvents used were all of analytical grade.

Methods

Pre-formulation studies

Determination melting point

The CFH and OLH powder's melting point was determined using a capillary glass technique. Both drugs were packed separately into glass capillaries, with one end sealed with a flame. The drug-containing capillary was immersed with an appropriate aperture in liquid paraffin inside Thiele's tube and heated with a silicon oil heater with a heating rate of 1 °C/minute. The melting points were monitored by analyzing the melting temperature at which they began to melt[26].

Solubility

Solubility testing was performed by following the consensus recommendations for vigorous shaking with slight alterations in the methodology reported by Bharate in 2015. The solubility of CFH was determined in double distilled water (DDW), methanol, and ethanol, while OLH's solubility was determined in DDW, formic acid and dehydrated alcohol. Each test tube containing solvent (5 mL) was laden with excess amount of CFH and OLH. Furthermore, the test tubes were covered using aluminium foil and the results documented based on complete or partial solubilization of the drug[27].

Fourier transformed infrared spectroscopy (FT-IR)

The FT-IR spectrophotometer (FTIR- 8400S, Shimadzu, Japan) was employed to discover the purity and identify drugs by detecting their functional groups. The samples of pure CFH and OLH (3 mg each) were precisely weighed, combined with IR-grade potassium bromide (50 mg), compressed into discs, and examined successfully. The scanning was done at a resolution of 0.48-1.93 cm^{-1} in 2000-400 cm^{-1} range[28].

Drug-excipient compatibility study

The drug-excipient compatibility was implemented to determine the chemical interaction between the drugs and excipients. The physical mixture of drugs [CFH (0.35 %); OLH (0.55 %)] and TRISG excipients was prepared by differing their concentrations (Table I) and stored for 30

days at a temperature 40 ± 2 °C and 75 ± 5 % relative humidity (RH). Afterwards, batches were analyzed by using RP-HPLC[29].

Table I: Excipient compatibility for TRISG

Sr. No.	Ingredient (%)	EC-1	EC-2	EC-3	EC-4	EC-5
1.	PNF 407	15.00	-	15.00	15.00	15.00
2.	PNF 188	15.00	15.00	-	15.00	15.00
3.	Sodium chloride (NaCl)	0.50	0.50	0.50	-	0.50
4.	BKC	0.02	0.02	0.02	0.02	-
5.	DDW	q.s.	q.s.	q.s.	q.s.	q.s.

Formulation and development of TRISG

The TRISG was prepared under aseptic conditions by dissolving varying concentrations of PNF 407 and PNF 188 in 50 mL DDW (A). The solution was placed in a refrigerator (4 ± 2 °C) for 24 h to obtain complete dispersion of the polymers. The CHF (0.35 %) and OLH (0.55 %) were added separately in deionized DDW (B). Afterwards, the drug and polymer solution were mixed inside the vials at room temperature (RT) (C) and stored in a refrigerator (4 ± 2 ºC) for 24 h (Fig. 2). All the prepared batches of TRISG formulations contain a constant concentration of BKC (0.02 %) and NaCl (0.50 %) as preservatives and tonicity agents, double distilled water (DDW) as a vehicle to stop the growth of microorganisms and irritation of the ocular membrane. Formulations were sterilized in an autoclave for 15 minutes at a temperature of 121°C and 15 PSI of pressure. The compositions of prepared TRISG comprising different gelling agents are given in Table II[30].

Physicochemical characterization of prepared CFH and OLH-TRISG formulations

The developed formulations of the TRISG were determined by demonstrating clarity, pH, gelation studies, viscosity, DC estimation, release kinetics and stability studies.

Visual appearance, clarity and pH

Clarity study is considered to be one of the most crucial parameters for ocular formulations. It was performed under visual inspection with adequate illumination. All formulations were observed against a black and white background for any indications of turbidity or scattered particles, with the components set in swirling motion. The pH of the formulated TRISG was estimated via a pH meter (Thermo Scientific Orion Star A211)[31].

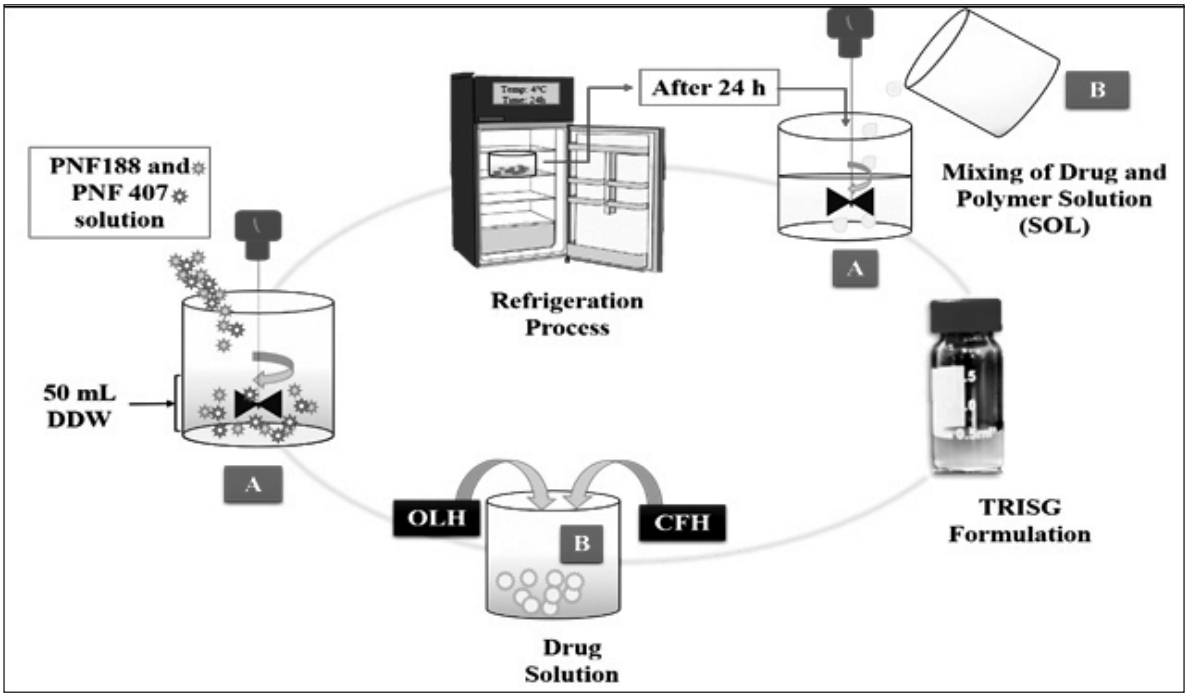

Fig. 2: Formulation of TRISG for ocular delivery

Table II: Quantitative data for polymers in TRISG formulation

Sr. No.	Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	PNF 407	5.00	7.50	10.0	10.0	15.0	20.0	15.0	22.5	30.0
2.	PNF 188	10.0	7.50	5.00	20.0	15.0	10.0	30.0	22.5	15.0

Evaluation of gelation temperature (GT)

The formulations' GT was assessed using the test tube inversion technique. A quantity of 2 mL of TRISG formulation was enclosed in a test tube and kept in a thermostatically controlled water bath. The temperature was gradually raised from 20 to 37 ºC with 0.5 ºC/minute; at each thermal point, the sample was left to stand for 60 seconds, and inverted at 90°. The temperature point at which no fluidity was detected that noted as the GT. The ideal characteristic of a TRISG is that it should be free-flowing at RT and form a gelate after being introduced into the eyes[32].

Assessment of gelling time (GLT)

The gelation experiment was conducted in a cylindrical tube filled with 5 mL simulated tear fluid (STF). A quantity of 2 mL of the formulation was incorporated with a standard dropper into a tube containing STF and then the time for gelation was visually ascertained as GLT. The test was executed in triplicate and the average value calculated[33].

Viscosity

The viscosity of the TRISG was estimated by a viscometer (Brookfield, LVT model) at a shear rate of 10-200 N M^{-1} containing spindle no. 62 over a speed range of 0.3 to 30 rpm. The viscosity of TRISG was measured before the dilution of sol and after diluting the formulation by adding STF (40:7) at a temperature of 37 °C. The measurements were calculated using the average of three dial readings[34].

Drug content analysis (DC)

The DC was estimated for developed TRISG systems by using chromatographic techniques. The assay of these formulations was performed by significant dilutions equivalent to 100 µL of the CFH and OLH formulation to 25 mL with DDW in a sterilized volumetric flask. RP-HPLC was used to determine the content of drugs in the ISG by employing acetonitrile : 0.1 % TFA in water (40:60 V/V) as the mobile phase. The samples were collected and analyzed by RP-HPLC using UV detector[35].

In vitro drug release (IVRT) study

The IVRT study of selected TRISG formulations was accomplished using the Franz diffusion cell apparatus (orifice diameter 0.9 cm; Perme Gear Inc. Hellertown, PA, USA). A dialysis membrane (3.4 cm^2 diameter) was adapted to the terminal section of the donor compartment. The donor compartment was loaded with 1 mL of TRISG, sufficient to sustain constant sink conditions. To ensure sink conditions, the receptor chamber of the cell comprised (15 mL) of phosphate buffer solution (PBS) pH 7.4 that was agitated at 50 rpm and thermostated at 32±1°C. An aliquot of the sample (2 mL) was pipetted from the release media at specific time intervals (30, 60, 90, and up to 120 minutes) and immediately replaced with an equivalent quantity of fresh PBS pH 7.4. The concentration of CFH and OLH released was assessed by RP-HPLC analysis by measuring the absorbance at a detection wavelength of 260 nm[36].

Kinetics of drug release

The drug release was investigated using the goodness-of-fit technique for IVRT, and several kinetic models, including zero, first-order, Higuchi, and Korsemeyer-Peppas, were utilized to establish the kinetic modelling of drug release. The following equations were utilized for computing the release kinetics[37,38].

Zero-order kinetics: $dQ / dt = K_0$ ..... (1)

First-order kinetics: $dQ / dt = K_1 Q$ ..... (2)

Higuchi release model: $Q = KH\ t_{1/2}$ ..... (3)

Korsemeyer-peppas: $Mt/M\infty = KKP\ t\ n$ ..... (4)

Where Q = extent of drug release; K_0 = zero-order release rate constant; t = release time; K_1 = First-order release rate constant; $t_{1/2}$ = half-life of the drug; Mt/M∞ = drug release fraction; KKP = release rate constant for Korsemeyer-Peppas.

Effect of sterility testing

The sterility testing of three optimized formulations were performed to determine the influence of aerobic and anaerobic bacteria and fungi before and after putting the formulation in an autoclave for total sterilization. For

complete sterilization, the procedure was divided into four parts for better understanding. In the first part (A), both drugs and all preservatives mentioned previously were mixed thoroughly in separate beakers and filtered through a membrane filter (0.2 - μ). Furthermore, PNF 407 and PNF 188 were combined thoroughly in another beaker (B). Both mixtures A and B were combined to form the drug-polymer solution in a beaker named C and subjected to determine viscosity before the sterilization process. Afterwards, the obtained solution C was subjected to sterilization under an autoclave for 1 h and the viscosity calculated[39].

Accelerated stability study

To evaluate the stability of the drug and formulation, an accelerated stability study was conducted as per ICH Q1A standard (R2) at 4 different time points [(T0 - Zero Time point - Day 1), (T1 - 1 month from the initiation date), (T2- 2 months from the date of initiation), (T3- 3 months from the date of initiation)]. Optimized 3 formulations were packed separately in 5 mL white HDPE dropper bottles. The bottles were then placed in a stability chamber at 40 ◦C ± 2 ◦C and 75 % (±5 % RH for 3 months). The physical stability of the three formulations was evaluated by estimating pH, the viscosity of sol and gel, and chemical stability by estimating the percentage assay for DC of formulation[40].

RESULTS

Pre-formulation studies

Determination of melting point

The melting points of CFH and OLH were successfully measured by the glass capillary technique. The melting points of both drugs were reported as 256 ±2 °C and 248± 4 °C, respectively, within the standard limit (255-257 ◦C CFH) and (>240 °C OLH). In order to avoid degradation during the sterilizing process, the higher values of melting point play a vital role in the design of the ocular formulations.

Solubility

Solubility is one of the crucial parameters in the development of novel formulations. According to the solubility study performed for CFH and OLH, it was revealed that CFH was soluble in DDW and very soluble in methanol and ethanol, while OLH was very soluble in formic acid, sparingly soluble in DDW and slightly soluble in dehydrated alcohol. DDW is considered a potent solvent for effective ocular delivery, and the solubility profile for both the drugs in the present investigation explored significant DDW solubility; hence it was used as a solvent for ISG formulation.

Fourier transformed infrared spectroscopy (FT-IR)

FT-IR spectrum of CFH marked the presence of quinolones at 1650 to 1600 cm^{-1}. In contrast, the bands at 1750 to 1700 cm^{-1} were ascribed to carbonyl C = O stretching. The peaks revealed the bending vibrations of the O-H group at 1300 to 1250 cm^{-1}. The existence of carboxylic acid was observed between the FT-IR ranges 1450 to 1400 cm^{-1}. Furthermore, the C-F group gave an absorption peak between 1050 and 1000 cm^{-1}. The FTIR spectra of OLH showed that the band at 1750 cm^{-1} corresponds to the C=O bond, whereas the peak at 1600 cm^{-1} and 1000 cm^{-1} exhibit the C=C and C-H stretching, respectively.

Drug-excipient compatibility study

In the drug excipient compatibility study, the % assay for OLH and CFH was found to be between 98 % and 100 %. The study findings explored that all the batches (EC-1 to EC-5) did not exhibit any incompatibility; hence they were utilized in the TRISG formulation. Table III displays the quantitative data of excipient compatibility assay results in %, and Fig. 3 illustrates the chromatogram.

Table III: Quantitative data of excipient compatibility assay

Batch	% Assay for CFH	% Assay for OLH
EC-1	98.34 ± 0.14	99.93 ± 0.19
EC-2	99.98 ± 0.19	100.36 ± 0.21
EC-3	99.68 ± 0.11	99.43 ± 0.38
EC-4	100.16 ± 0.46	98.67 ± 0.67
EC-5	100.47 ± 0.93	100.24 ± 0.73

Formulation and development of TRISG

The TRISG formulations were developed successfully in aseptic conditions by employing various concentrations of PNF 407 and PNF 188. NaCl was used as an auxiliary material to improve drug release through the gel formulation. The nine batches were formulated successfully and subjected to physicochemical characterization. The outcomes of the characterization of developed TRISG are documented in the subsequent sections.

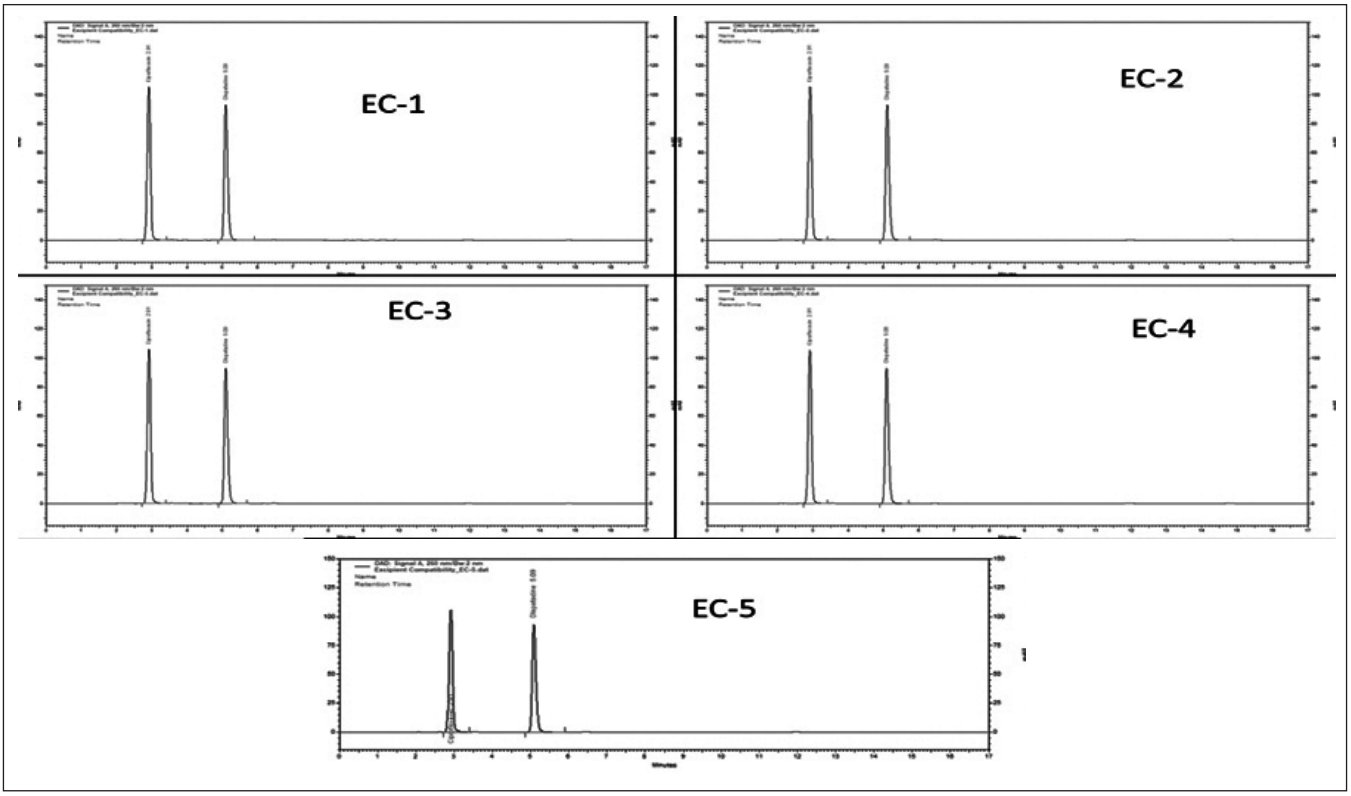

Fig. 3: Chromatogram of EC-1 to EC-5

Table IV: Physicochemical characterization of TRISG

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance / Clarity	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear	TLT / Clear
Viscosity of SOL (cps)	37 ± 0.17	45 ± 0.21	66 ± 0.54	72 ± 0.27	83 ± 0.67	92 ± 0.51	112 ± 0.91	138 ± 0.19	156 ± 0.35
Viscosity of GEL (cps)	177 ± 0.28	283 ± 0.37	475 ± 0.57	691 ± 0.57	913 ± 0.81	764 ± 0.69	414 ± 0.44	538 ± 0.53	670 ± 0.67
GLT (minutes)	17	32	55	35	33	39	21	34	56
GT (℃)	32.1	34.5	35	30	32.5	33.12	37.2	37.6	38.8
pH of SOL	6.79	6.59	7.49	7.13	7.22	7.37	8.17	8.46	8.72

Physicochemical characterization of CFH and OLH-TRISG formulations

Visual appearance, clarity and pH

The visual inspection of the prepared TRISG formulations exhibited a clear to translucent (TLT) appearance. The pH of the F1–F6 batches were found to be within an acceptable limit (6.8–7.4) and did not irritate when administered into the eye. The batches F7 to F9 demonstrated significantly higher pH (above 8), which was not suitable for ocular administration.

Evaluation of gelation temperature and time

The primary prerequisites of the gelling system are GT and GLT. Furthermore, to enable sustained release of ISG to the ocular tissue, its integrity should be maintained without dissolving for an extended period. The GLT was studied *in vitro* by adding one drop of each formulation to the glass vial enclosing 2 mL of STF (pH 7.4); batch F1 to F3 exhibited the gelation within 17-55 minutes at GT 32.1 to 35°C. Batches F4 to F6 took 33-39 minutes for gelation at GT 30 to 33.12 °C with a consistent GLT for

the same polymer concentration. However, the batches F7, F8 and F9 required 21-56 minutes to form the ISG at GT range 37.2 to 38.8°C, significantly higher than the standard reported values for GT.

Viscosity

The effectiveness of ISG for ODD depends on their post-administration properties. It is also essential that they should exhibit low viscosity for consistent dose administration and easy administration into the eye. Batches F4 to F6 containing 30 % Pluronic® concentration showed approximately 10 times increased viscosity after gelation (Table I). Hence, it can be concluded that the 30 % Pluronic® concentration is suitable for the TRISG formulation. The viscosity profile of formulated sol and ISG is represented graphically in Fig. 4. The qualitative and quantitative data associated with the physicochemical characterization of TRISG are indicated in Table IV.

Drug content (DC) analysis

The DC analysis findings reported for the physicochemical characterization of TRISG demonstrated the best results for F4 to F6 batches in all aspects of characterization; hence they were selected for further DC analysis. The % assay between 98.49 to 100.61 % was explored for the CFH, whereas the % assay between 98.54 to 99.76 % was observed for OLH within TRISG. Furthermore %DC of 99.63 for CFH and 98.54 for OLH, respectively, indicating minimal deviation in the findings and subjected to further IVRT analysis. The comprehensive data associated with DC analysis are indicated in Table V and illustrated in Fig. 5.

Table V: Qualitative data of DC

Batch	% Assay for CFH	% Assay for OLH
F4	98.49 ± 0.41	99.76 ± 0.27
F5	99.63 ± 0.33	98.54 ± 0.67
F6	100.61 ± 0.76	99.20 ± 0.38

In vitro drug release (IVRT) study

The IVRT study of CFH-marketed eye drops showed 99.54 % drug release for 60 minutes, which confirms the rapid release of the drug. Similarly, OLH-marketed product shows 97.12 % drug release for 60 minutes. The summary of the results is given in Table VI and Fig. 6.

Table VI: Summary of drug release study of the marketed product

Time (min)	Marketed CFH eye drop	Marketed OLH eye drop
	% Drug release	% Drug release
0	0	0
10	36.22 ± 0.25	24.31 ± 0.09
20	53.67 ± 0.15	48.76 ± 0.15
30	71.13 ± 0.12	76.88 ± 0.42
45	87.97 ± 0.05	85.33 ± 0.33
60	99.54 ± 0.02	97.12 ± 0.21

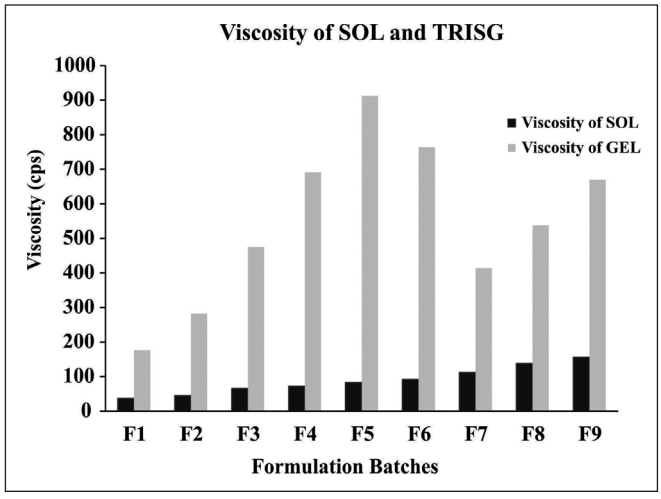

Fig. 4: Viscosity of SOL and TRISG

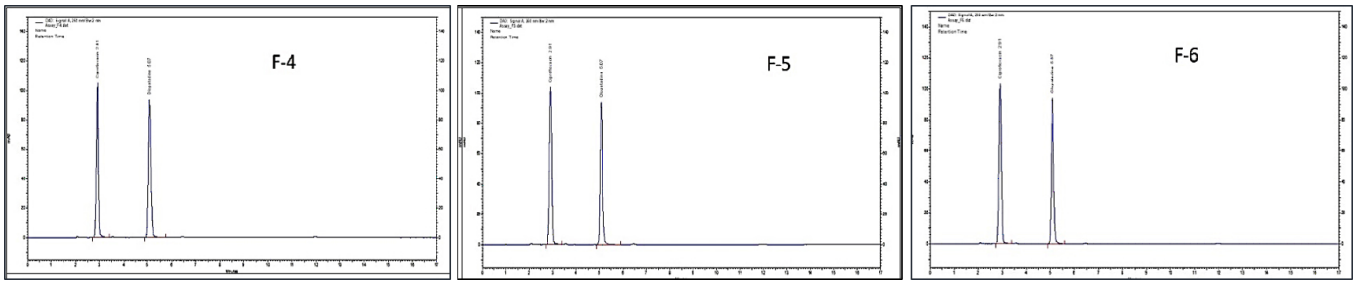

Fig. 5: Chromatograms of DC F4 to F6

Table VII: IVRT data of CFH and OLH for TRISG formulation

CFH				
Time	**Log of time**	**Square root of time**	**% Drug release**	**Log % drug release**
30	1.48	5.48	19.34 ± 0.11	1.29
60	1.78	7.75	41.19 ± 0.05	1.61
90	1.95	9.49	67.76 ± 0.28	1.83
120	2.08	10.95	94.67 ± 0.17	1.98
OLH				
30	1.48	5.48	22.45 ± 0.13	1.35
60	1.78	7.75	38.34 ± 0.21	1.58
90	1.95	9.49	64.23 ± 0.54	1.81
120	2.08	10.95	98.76 ± 0.72	1.99

The efficacy of the drug release from developed gel employing synthetic membrane was compared by using adopting an *in vitro* Franz diffusion system. When developing ocular drug delivery methods, IVRT is used as a screening method to evaluate the efficacy characteristics of multiple prototype formulations. In DC estimation, it was observed that from three selected batches, F5 batch showed the highest amount of DC; hence this batch was selected for the IVRT study. The percentage of CFH and OLH from the F5 TRISG formulation released through the synthetic membrane was evaluated for 120 minutes and plotted successfully (Fig. 7 and Table VII). The resulting data revealed that TRISG formulation showed 1.98 % log drug release of CFH and 1.99 % log drug release for OLH, which was satisfactory for ocular application.

Table VIII: Viscosity before and after sterilization

Batch	Viscosity (cps)	
	Pre-sterilization	**Post-sterilization**
F4	815 ± 7.5	837 ± 3.2
F5	952 ± 10.2	949 ± 7.4
F6	897 ± 2.6	890 ± 5.6

Kinetics of drug release

To discover the release pattern and mechanism of the optimised formulation, the consequential data were incorporated into the preceding mathematical models (F5). The drug release data from the ISG was plotted utilizing several kinetic models such as zero order, first order, Higuchi and Korsemeyer-Peppas. The best fit model of the release was estimated by zero and first maximal regression (R^2). Higuchi model R^2 values revealed that dispersion functions were analogous to specify the release mechanism. The slope value 'n' for the Korsemeyer-Peppas authenticates the mechanism and pattern shown in Fig. 8.

Effect of sterility testing

The effect of sterilization was studied on three batches before and after putting the formulation in an autoclave for total sterilization, and viscosity was measured and is reported in Table VIII. No significant viscosity changes

Table IX: Stability study data for developed ISG formulations

Time		**0 month (T0)**			**1 month (T1)**			**2 months (T2)**			**3 months (T3)**		
Parameter		F4	F5	F6	F4	F5	F6	F4	F5	F6	F4	F5	F6
pH		7.13	7.22	7.37	7.16	7.19	7.35	7.19	7.04	7.28	7.29	7.08	7.20
Viscosity (cps)	Sol	72 ± 0.57	83 ± 0.78	92 ± 0.81	75 ± 0.76	86 ± 0.54	93 ± 0.86	84 ± 0.91	94 ± 0.17	96 ± 0.21	79 ± 0.13	91 ± 0.19	99 ± 0.21
	Gel	691 ± 1.6	913 ± 2.2	764 ± 5.4	645 ± 2.9	937 ± 8.1	719 ± 7.7	781 ± 0.27	957 ± 0.67	868 ± 0.91	809 ± 0.21	948 ± 4.3	901 ± 2.1
% Assay	CFH	98.49 ± 0.41	99.63 ± 0.33	100.61 ± 0.76	99.57 ± 0.21	98.12 ± 0.37	99.34 ± 0.96	98.97 ± 0.22	99.84 ± 0.30	98.25 ± 0.04	98.71 ± 0.21	99.87 ± 0.27	98.38 ± 0.96
	OLH	99.76 ± 0.27	98.54 ± 0.67	99.20 ± 0.38	98.67 ± 0.64	101.34 ± 0.54	100.19 ± 0.24	99.01 ± 0.51	100.09 ± 0.57	99.99 ± 0.11	98.54 ± 0.07	100.11 ± 0.21	99.18 ± 0.35

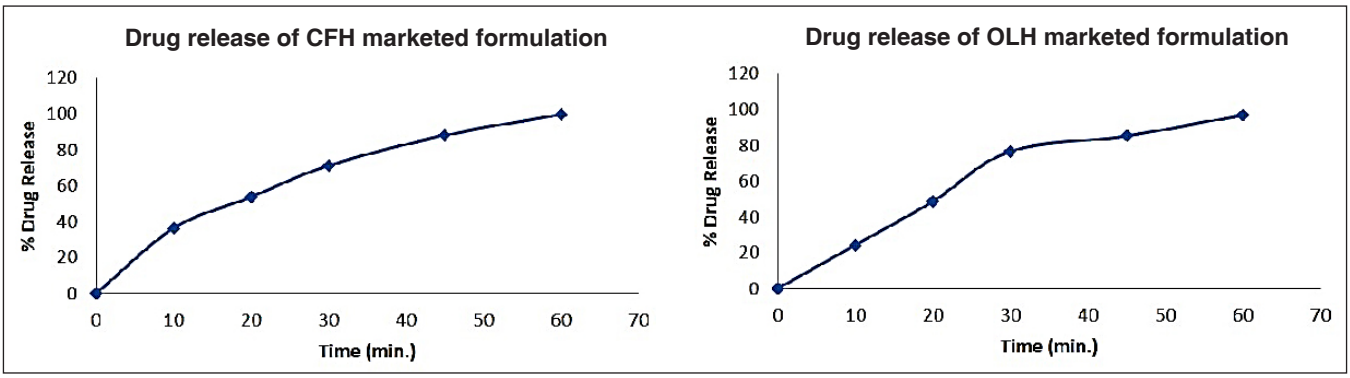

Fig. 6: Percent drug release of CFH and OLH marketed products

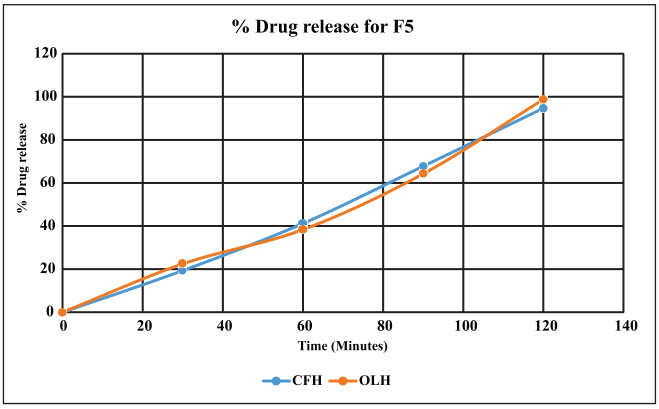

Fig. 7: IVRT profile of CFH and OLH- TRISG

were observed after sterilizing the final formula, thus indicating that the product can be sterilized before filling during manufacturing.

Accelerated stability study

The stability of the F1, F2 and F3 batches was evaluated at 40 ± 2 °C with a 75 % ± 5 % RH for three months, as displayed in Table IX and Fig. 9. The sample exhibited outstanding physical characteristics under the circumstances specified each time. Furthermore, the results showed no remarkable changes in all investigated parameters, pH, viscosity, and % assay. In addition, the gelling properties and appearance remained unchanged, stating that the formulation was stable and effective for 3 months.

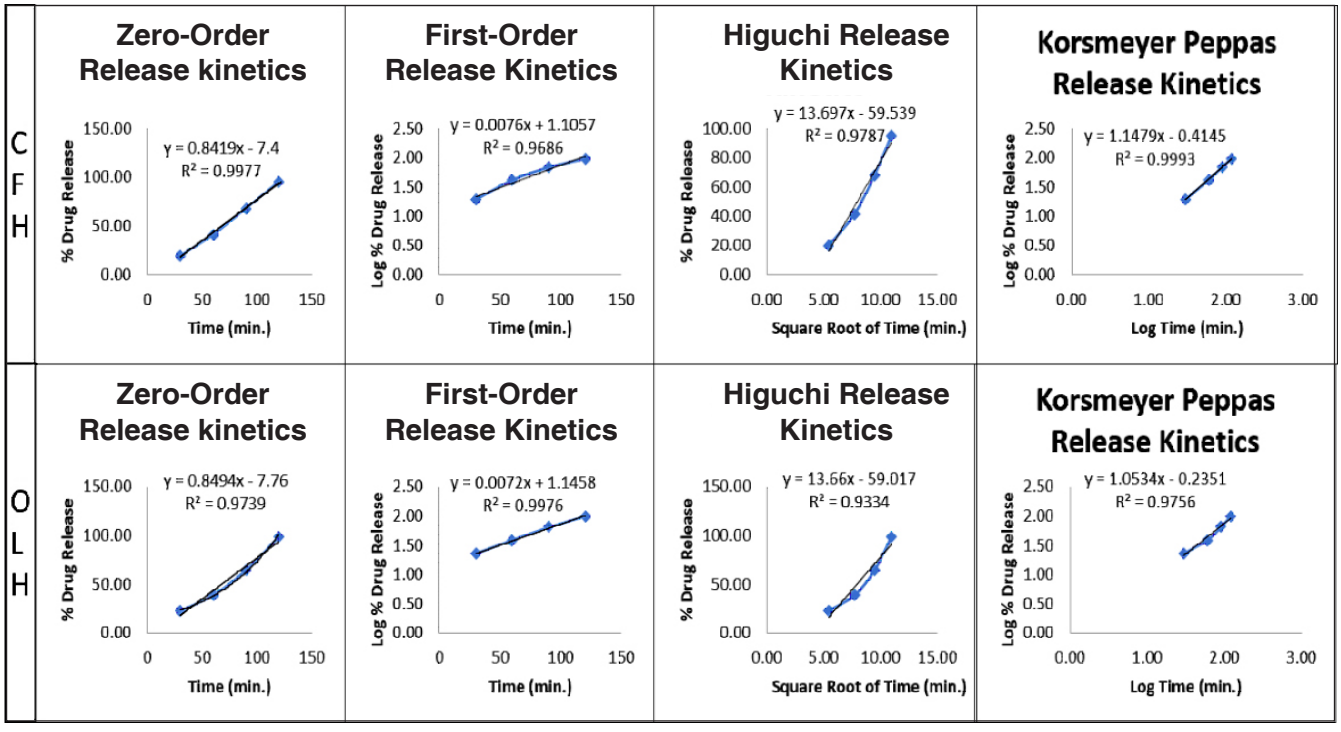

Fig. 8: Kinetics of drug release for TRISG

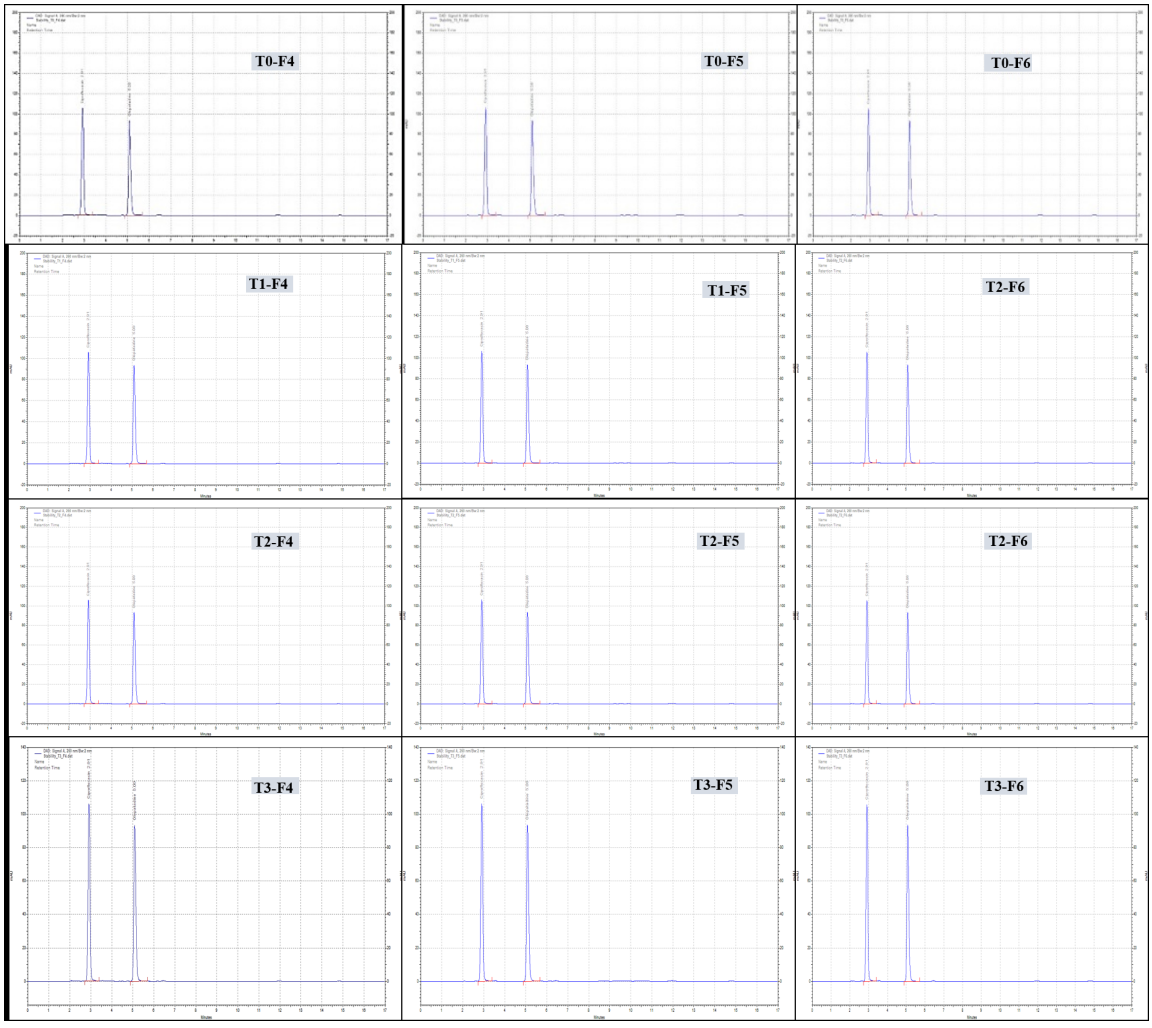

Fig. 9: Chromatograms of stability study

DISCUSSION

The ODD of TRISG formulation employing PNF 407 and PNF 188 for the effective administration of CFH-OLH in combination has been reported for the first time in this investigation. Overall, an ODDS embedding CFH and OLH in a TRISG has been developed by the cold method and characterized. The physiochemical characterization demonstrated that the CFH-OLH-TRISG delivery system is safe and non-irritating. The developed TRISG formulation not only extends the drug release, but also reduces tear elimination and improves aqueous humour bioavailability. This formulation shows great potential in ODD due to its easy administration and patient compliance, which leads to a decrease in dose frequency. More prominently, the effective development of TRISG with two different drugs in combination can provide a novel approach to ODDS.

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REFERENCES

1. Bhattacharjee A., Das P. J. and Adhikari P., et al. Novel drug delivery systems for ocular therapy: With special reference to liposomal ocular delivery. **Eur. J. Ophthalmol.,** 2019, 29(1), 113-126.
2. Todros S., Todesco M. and Bagno A.: Biomaterials and their biomedical applications: from replacement to regeneration. **Processes,** 2021, 9(11).
3. Lăcrămioara P., Mihaela V. G., Cristina-Elena D.-P.: Hydrogels - Smart Materials for Biomedical Applications. **Intech**., 2019.
4. Chyzy A., Tomczykowa M., Plonska-Brzezinska M. E.: Hydrogels as potential nano-, micro- and macro-scale systems for controlled drug delivery. **Materials (Basel),** 2020, 13(1), 188.
5. Gupta H., Jain S., Mathur R., Mishra P., Mishra A. K., Velpandian T.: Sustained ocular drug delivery from a temperature and pH triggered novel *in situ* gel system. **Drug Deliv.,** 2007, 14(8), 507-515.

6. Chowhan A. and Giri T. K.: Polysaccharide as renewable responsive biopolymer for *in situ* gel in the delivery of drug through ocular route. **Int. J. Biol. Macromol.,** 2020, 150, 559-572.
7. Al-Kinani A. A., Zidan G., Elsaid N., Seyfoddin A., Alani A. W. G. and Alany R. G.: Ophthalmic gels: Past, present and future. **Adv. Drug Deliv Rev.,** 2018, 126, 113-126.
8. Uchio E.: Treatment of allergic conjunctivitis with olopatadine hydrochloride eye drops. **Clin. Ophthalmol.,** 2008, 2(3), 525.
9. Dubald M., Bourgeois S., Andrieu V. and Fessi H.: Ophthalmic drug delivery systems for antibiotherapy- A review. **Pharmaceutics,** 2018, 10(1).
10. Vigani B., Rossi S., Sandri G., Bonferoni M. C., Caramella C. M. and Ferrari F. Recent advances in the development of *in situ* gelling drug delivery systems for non-parenteral administration routes. **Pharmaceutics,** 2020, 12(9), 1-29.
11. Langer R. and Peppas N. A.: Advances in Biomaterials, Drug Delivery, and Bionanotechnology. **AIChE J.** 2003, 49(12), 2990-3006.
12. Jumelle C., Gholizadeh S., Annabi N. and Dana R.: Advances and limitations of drug delivery systems formulated as eye drops. **J. Control. Release,** 2020, 321, 1-22.
13. Musarurwa H. and Tavengwa N. T.: Stimuli-responsive polymers and their applications in separation science. **React. Funct. Polym.,** 2022, 175, 105282.
14. Pandey M., Choudhury H. and Aziz A. B. A., et al.: Potential of stimuli-responsive *in situ* gel system for sustained ocular drug delivery: Recent progress and contemporary research. **Polymers (Basel),** 2021, 13(8).
15. Karimi M., Sahandi Z. P. and Ghasemi A., et al.: Temperature-responsive smart nanocarriers for delivery of therapeutic agents: applications and recent advances. **ACS Appl. Mater. Interfaces,** 2016, 8(33), 21107-21133.
16. Cao Y., Zhang C., Shen W., Cheng Z., Yu L. and Ping Q.: Poly(*N*-isopropylacrylamide)-chitosan as thermosensitive *in situ* gel-forming system for ocular drug delivery. **J. Control. Release,** 2007, 120(3), 186-194.
17. Qi H., Chen W. and Huang C., et al. Development of a poloxamer analogs/carbopol-based *in situ* gelling and mucoadhesive ophthalmic delivery system for puerarin. **Int. J. Pharm.,** 2007, 337(1-2), 178-187.
18. Yassir Al-Bazzaz F. and Al-Kotaji M.: Ophthalmic *in situ* sustained gel of ciprofloxacin, preparation and evaluation study. **Int. J. Appl. Pharm**., 2018, 10(4), 153-161.
19. Varshosaz J., Tabbakhian M. and Salmani Z.: Designing of a thermosensitive chitosan/poloxamer *in situ* gel for ocular delivery of ciprofloxacin. **Open Drug Deliv. J.,** 2008, 2(1), 61-70.
20. Irimia T., Dinu-Pîrvu C. E. and Ghica M. V. et al. Chitosan-based *in situ* gels for ocular delivery of therapeutics: A state-of-the-art review. **Mar. Drugs,** 2018, 16(10), 373.
21. Kurniawansyah I. S., Rusdiana T., Sopyan I. and Subarnas A.: A review on poloxamer and hydroxy propyl methyl cellulose combination as thermoresponsive polymers in novel ophthalmic *in situ* gel formulation and their characterization. **Int. J. Appl. Pharm.,** 2021, 13(1), 27-31.
22. Kumar D., Nagaich U., Gulati N. and Jain N.: Controlled ocular drug delivery of ofloxacin using temperature modulated *in situ* gelling system. **J. Sci. Soc.,** 2013, 40(2), 90.
23. Sarwan T., Kumar P., Choonara Y. E. and Pillay V.: Hybrid thermo-responsive polymer systems and their biomedical applications. **Front Mater.,** 2020, 7(3).
24. Sahoo S. R., Mothilal M., Priyadharshini B. and Damodharan N.: Thermosensitive hydrogels–a potential carrier for the delivery of drugs and macromolecules. **Int. J. Appl. Pharm**., 2021, 13(2), 102-109.
25. Vasile C., Pamfil D., Stoleru E. and Baican M.: New developments in medical applications of hybrid hydrogels containing natural polymers. **Molecules**, 2020, 25(7).
26. Osonwa U. E., Ugochukwu J. I., Ajaegbu E. E., Chukwu K. I., Azevedo R. B. and Esimone C. O.: Enhancement of antibacterial activity of ciprofloxacin hydrochloride by complexation with sodium cholate. **Bull. Fac. Pharmacy, Cairo Univ.** 2017, 55(2), 233-237.
27. Bharate S. S. and Vishwakarma R. A.: Thermodynamic equilibrium solubility measurements in simulated fluids by 96-well plate method in early drug discovery. **Bioorganic Med. Chem. Lett.,** 2015, 25(7), 1561-1567.
28. Douglas A., Skoog F. and James Holler S. R. C.: **Principles of Instrumental Analysis,** 2016, 7.
29. Patel P.: Preformulation studies: An integral part of formulation design. In: **Pharmaceutical Formulation Design - Recent Practices.** IntechOpen; 2020. doi:10.5772/intechopen.8286
30. Gugleva V., Titeva S. and Ermenlieva N. et al. Development and evaluation of doxycycline niosomal thermoresponsive *in situ* gel for ophthalmic delivery. **Int. J. Pharm.,** 2020, 591.
31. Kesarla R., Tank T., Vora P. A., Shah T., Parmar S. and Omri A.: Preparation and evaluation of nanoparticles loaded ophthalmic *in situ* gel. **Drug Deliv.,** 2016, 23(7), 2363-2370.
32. Lou J., Hu W. and Tian R. et al.: Optimization and evaluation of a thermoresponsive ophthalmic *in situ* gel containing curcumin-loaded albumin nanoparticles. **Int. J. Nanomed.,** 2014, 9(1), 2517-2525.
33. Chaudhari P. D. and Desai U. S.: Formulation and evaluation of niosomal *in situ* gel of prednisolone sodium phosphate for ocular drug delivery. **Int. J. Appl. Pharm.,** 2019, 11(2), 97-116.
34. Franzesi G. T., Ni B., Ling Y. and Khademhosseini A.: A controlled-release strategy for the generation of cross-linked hydrogel microstructures. **J. Am. Chem. Soc.,** 2006, 128(47), 15064-15065.
35. Dasankoppa F., Solankiy P., Sholapur H., Jamakandi V., Sajjanar V. and Walveka P.: Design, formulation, and evaluation of *in situ* gelling ophthalmic drug delivery system comprising anionic and nonionic polymers. **Indian J. Heal Sci. Biomed. Res.,** 2017, 10(3), 323.
36. Huang W., Zhang N. and Hua H., et al.: Preparation, pharmacokinetics and pharmacodynamics of ophthalmic thermosensitive *in situ* hydrogel of betaxolol hydrochloride. **Biomed. Pharmacother.,** 2016, 83, 107-113.
37. Eram F. V.: *In vivo* evaluation and characterization of novel *in situ* gelling system as controlled delivery system containing ciprofloxacin for ocular drug delivery. **J. Drug Deliv. Ther.,** 2020, 10(5-s), 32-39.
38. Dash S. P., Lilakanta N. and Chowdhury P.: Kinetic modeling on drug release from controlled drug delivery systems. **Acta Pol. Pharm. ñ Drug Res**., 2010, 67(3), 217-223.
39. Ranch K. M., Maulvi F. A., Naik M. J., Koli A. R., Parikh R. K. and Shah D. O.: Optimization of a novel *in situ* gel for sustained ocular drug delivery using Box-Behnken design: *In vitro*, *ex vivo*, *in vivo* and human studies. **Int. J. Pharm.,** 2019, 554, 264-275.
40. Muthu M. S. and Feng S. S.: Pharmaceutical stability aspects of nanomedicines. **Nanomedicine,** 2009, 4(8), 857-860.