Formulation and Characterization of pH-Dependent *In Situ* Gelling System for Ocular Drug Delivery of Ciprofloxacin Hydrochloride and Olopatadine Hydrochloride

SHAIKH DARAKHSHAN AFREEN* AND MOMIN MUNIRA[1]

Department of Pharmaceutics, Srinath College of Pharmacy, Aurangabad, Maharashtra 431136, [1]Department of Pharmaceutics, Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, Maharashtra 400056, India

Afreen *et al.*: pH-Dependent *In Situ* Gelling System for Ocular Drug Delivery

In ocular delivery, conventional drug administration systems have significant pitfalls, such as low bioavailability, low residence time and rapid precorneal drainage. An *in situ* gel is a novel drug delivery system with prolonged pharmacological action and simpler production techniques. This investigation aims to formulate and evaluate pH-responsive ophthalmic *in situ* gel of Ciprofloxacin hydrochloride and Olopatadine hydrochloride. The nine batches of ophthalmic *in situ* gel were prepared using carbapol-980 as a gelling agent and hydroxypropyl cellulose as a viscosity modifier. The designed formulations were examined for visual appearance, clarity, pH, gelling time, viscosity, drug content, *in vitro* drug release and stability study. The study's outcome explored that *in situ* gelling systems were translucent and clear, with a pH ranging from 4±0.26 to 7±0.27, suitable for ocular application. In gelling time study, F1, F2, and F3 batches required 34-39 min for gel formation and showed approximately a 4-5 times increase in the viscosity after gelation compared to all batches. In drug content analysis F3 batch exhibited the highest amount of drug content, 99.90±0.25 % for Ciprofloxacin hydrochloride and 101.45±0.93 % for Olopatadine hydrochloride; hence this batch was subjected to a drug release study. The kinetic study explored the resulting data best fitted to Korsmeyer-Peppas than zero order, first order, Higuchi models. A stability investigation exhibited no alteration in the formulation at 40°±2° with a relative humidity of 75 %-5 % RH after 3 mo. A compressive finding explored that ophthalmic pH-responsive *in situ* gel can be a potential approach for treating conjunctivitis.

Key words: pH-responsive *in situ* gel, ciprofloxacin hydrochloride, olopatadine hydrochloride, conjunctivitis

The term "conjunctivitis" refers to a broad spectrum of disorders characterized by conjunctival inflammation[1,2]. It is the most frequent consequence of red eyes, typically associated with viral or bacterial infection[3]. *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus sp., Pseudomonas aeruginosa*, and *Chlamydia trachomatis* are the most typical pathogens that aggravate bacterial conjunctivitis[4,5]. Ciprofloxacin Hydrochloride (CFH) is a second-generation fluoroquinolone, a broad-spectrum antibiotic utilized to treat eye disorders[6]. It is chemically 1-cyclopropyl-6-Fluro-1, 4-dihydro-4-oxo-7-(piperazine-1-yl)-3-quinoline carboxylic acid (fig. 1A)[7]. Olopatadine Hydrochloride (OLH) is an antihistaminic used to treat allergic conjunctivitis by inhibiting the discharge of histamine from mast cells[8]. It is chemically 11-[(Z)-3-(Dimethylamino) propylidene]-6, 11-dihydrodibenzo [b, e] oxepin-2-acetic acid hydrochloride (fig. 1B). It is commercially available in the form of 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 0.3 % CFH and 0.1 %, 0.2 %, 0.7 % OLH solutions are recommended for the patient[9]. The topical administration of eye drops in the lower cul-de-sac is the major systematic approach for managing ocular disorders[10].

*Address for correspondence
E-mail: darakhshan201992@gmail.com

Moreover, one of the primary complaints with eye drops is the quick and widespread removal caused by tear turnover, blinking and formulation drainage, resulting in a low precorneal residence period and ocular bioavailability[11,12]. Over the last decade, *In situ* Gel (ISG)-based Drug Delivery System (DDS) have received much attention[13]. Before administration, ISG is in a sol state and proficient in forming a gel in response to various extracellular stimuli such as temperature, pH and ion incidence[14,15]. These delivery systems are comprised of polymers that undergo a sol-to-gel phase transition in the eye due to advances in particular physiological circumstances[16,17]. Three kinds of systems are distinguished based on the approach used to establish a phase transition on the ocular surface pH-activated systems encompass hydrogen phthalate, cellulose acetate and carbopol, temperature-dependent systems involve pluronic and tetronics and ion-activated systems comprise gellan gum and sodium alginate[18,19]. Compared to traditional DDS, the sol-gel transition of this stimuli-responsive system provides a feasible method of drug delivery, sustained and prolonged drug distribution, high stability and biocompatibility[20,21]. When the tear fluid pH is raised to 7.4, pH-triggered systems undergo a sol-gel transformation; most pH-sensitive polymers are acidic polyanions with a low density[17-22]. Furthermore, when water is present, the polymer becomes ionised and surges as the pH rises to that of the body[16]. Existing studies developed several ISG formulations using single CFH and OLH for various eye disorders. In literature,

different polymers are implemented to establish formulations, such as sodium alginate, chitosan, Hydroxypropyl Methylcellulose (HPMC), Carbapol-980 (CBL-980), and poloxamers[23-25]. Single drugs with a combination of polymers were previously developed, but neither CFH nor OLH has been formulated yet in conjunction using two polymer combinations[21-26]. Additionally, the combination of two drugs is more effective than an individual drug; hence in this present investigation, efforts have been taken to develop an ISG formulation incorporating a pH-dependant polymer such as CBL-980 and viscosity grade Hydroxypropylcellulose (HPC) for the effective administration of the CFH and OLH into the eye for prolonged drug release and increased ocular drug bioavailability. The detailed materials and methods employed in the investigation are explored in a subsequent section.

MATERIALS AND METHODS

Materials:

CFH and OLH were received as gift samples from Aadhaar Life Sciences Pvt. Ltd, Solapur, India and Indoco Remedies Pvt. Ltd, Mumbai, India, respectively. Carbopol 980 procured from Lubrizol India Pvt. Ltd, Navi Mumbai, India. Viscosity-grade HPC was obtained as a gift sample from Ashland India Pvt. Ltd. Mumbai, India. Sodium chloride procured from Thomas Baker, Pvt. Ltd, Mumbai, India. Benzalkonium chloride was purchased from Loba Chemie Pvt. Ltd, Mumbai, India. The other chemicals and solvents utilised in the research were of analytical grade.
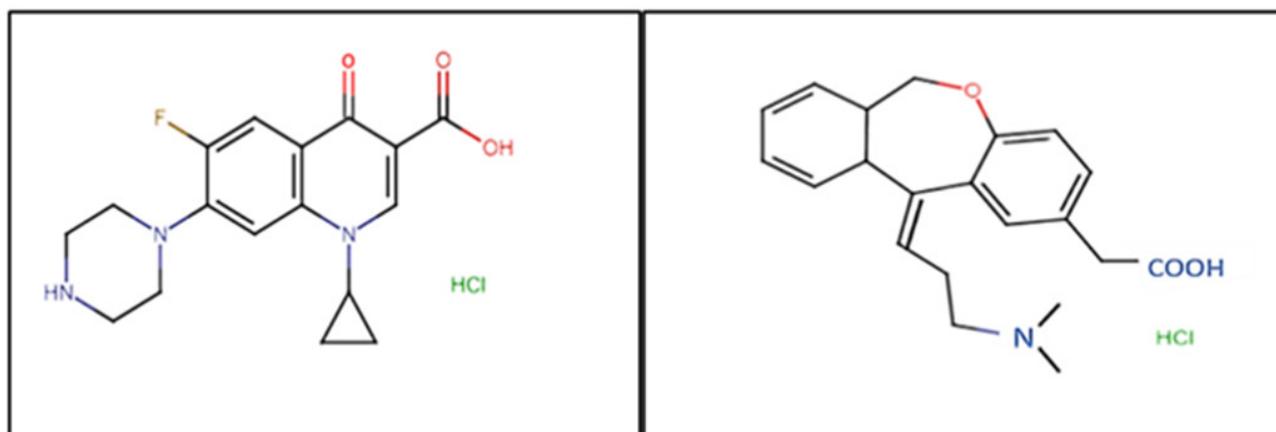
A: Ciproflxacin HCl   **B: Oloptadine HCl**

Fig. 1: Chemical structures of CFH and OLH

Methods:

Pre-formulation studies: Melting points of both drugs were determined using a glass capillary technique. CFH and OLH powders were packed separately into glass capillaries immersed in liquid paraffin containing Thiele's tube and heated with a silicon oil heater at a heating rate of 1°/min. The melting points were monitored by analysing the temperature at which drugs began to melt[27].

Solubility measurement was performed following the consensus recommendations for the vigorous shaking method with slight modifications in the methodology reported by Bharate *et al.*[28]. The solubility of CFH was examined in water, ethanol methanol and OLH in water, formic acid, and dehydrated alcohol. 5 ml of each tube was laden with excess CFH and OLH. Afterwards, the test tubes were covered with aluminium foil and the amount of drug solubilized was observed[29].

The functional groups existing in both drugs were identified by Fourier Transformed Infrared Spectroscopy (FTIR) spectroscopy. The CFH and OLH (3 mg each) were precisely weighed, combined with IR-grade potassium bromide, compressed into discs by applying hydraulic pressure, and examined on a (FTIR- 8400S, Shimadzu, Japan). The scanning was done at a resolution of 0.48-1.93 cm^{-1} in 2000-400 cm^{-1} wavelength[30].

The drug excipient compatibility study determined the interaction between the drug and excipients utilized in the ISG. The physical combinations of drugs and excipients were developed by the same concentrations in five batches and stored for 30 d at 40°±2° and 75 %±5 % relative humidity. Afterwards, the percentage assays of all batches were determined using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)[31].

Formulation and development of pH-dependant ophthalmic ISG: The nine pH-dependent CFH and OLH ISG formulations were designed by employing differing concentrations of viscosity grade HPC, CBL-980, as shown in Table 1. To get a transparent dispersion, the HPC was gently added to the 70 ml of purified water at 30° with continuous stirring on a hot plate (Stuart, UK). The CBL-980 was then evenly dispersed over the HPC solution while being heated to approximately 50° and constantly stirred on the magnetic stirrer. Afterwards, a clear drug solution was obtained by dissolving the 0.35 % and 0.55 % CFH and OLH in 20 ml of purified water. This solution was combined with the polymer's dispersion, and the final volume was made using 100 ml of water. The formulation was filtered through a sterile membrane filter with a 0.22 μm pore size into final containers that had already been sanitised and sealed to exclude microorganisms. 0.50 % Sodium Chloride (NaCl) and 0.02 % BKC were employed as buffering agent and preservatives to prevent irritation of the ocular membrane and the growth of microorganisms[32,33].

Evaluations of ISG: The developed formulations of the ISG were evaluated by determining visual appearance clarity, pH, gelling time, viscosity, drug content evaluation, drug release studies, sterilization effect, and stability studies.

Visual appearance, clarity and pH: The clarity study was performed under visual inspection in adequate illumination to determine turbidity or the presence of any foreign particles. All established formulations were observed in contrast to a black and white background, with the components swirling in motion. ISG formulation's pH was evaluated employing a digital pH meter (Mettler Toledo, Greifensee, Switzerland)[34].

Gelling time: The gelation experiment was conducted in cylindrical tubes with 5 ml of synthetic, Simulated Lacrimal Fluid (SLF) phosphate buffer pH 7.4, which simulated the cation content. A 50 μl of each formulation was incorporated using a standard dropper into a tube comprising SLF and was then visually ascertained for the gelation time and gel dissolved time[35].

TABLE 1: FORMULA FOR pH-DEPENDENT ISG

S no.	Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	CBL-980	0.2	0.25	0.3	0.3	0.5	0.7	0.5	0.75	1
2	HPC	0.3	0.25	0.2	0.7	0.5	0.3	1	0.75	0.5

Viscosity: The viscosity of the ISG was examined by (Brookfield Viscometer LVT model) at a shear rate of 10-200 N/m. The viscosity measurements were conducted before the dilution of the sol and after diluting the formulation with SLF to quantify the gelation of the formulation after installation in the eye. The viscosity of the sample solution was examined using spindle number 62 over a speed range of 0.3 to 30 rpm. It was calculated using the average of the two dial readings[36].

Drug content estimation: The drug content of F1, F2 and F3 batches were determined by diluting 100 μl of CFH and OLH ISG to 25 ml with distilled water in the decontaminated volumetric flask. The RP-HPLC method was employed to determine the content of drugs in the ISG by employing Acetonitrile:0.1 % trifluoroacetic acid in water (40:60 v/v) as the mobile phase. The samples were collected and analysed by RP-HPLC method using an Ultra Violet (UV) detector[37].

In vitro **Release Test (IVRT) studies:** The IVRT experimentation was performed on an optimized single batch utilising Franz diffusion cells (orifice diameter 0.9 cm; Perme Gear Inc. Hellertown, PA, USA). The Polytetrafluoroethylene (PTFE) membrane was attached to one end of a primarily built glass cylinder (open at both ends) with an inner diameter of 3.4 cm^2. Before assembling in Franz-diffusion cells, the dehydrated membrane was rehydrated by immersing it in (20 ml) of pH 7.4 Phosphate Buffer (PB). A drug release diffusion study was conducted on ISG formulation. To ensure sink conditions, the receptor chamber of the cell comprised (5 ml) of SLF pH 7.4 that was agitated at 500 rpm and at 32°±1°. 3 g of the ISG was applied on membrane surfaces and wrapped with parafilm in the donor compartment to prevent evaporation. 0.5 ml sample was pipette out from release media at appropriate intervals of 30, 60, 90, 120, 180 and up to 240 min and replaced with an equivalent quantity of fresh SLF. CFH and OLH concentrations were assessed by the RP-HPLC method using a UV detector[38,39].

Drug release mechanism and kinetic modelling: The drug release mechanism was investigated using the goodness-of-fit procedure for IVRT release data. Different kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas were used to establish the kinetic modelling of drug release from an optimized single batch. The following equations were utilized for computing the release kinetics[22].

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

First-order kinetics: $dQ/dt=K_1Q$ (2)

Higuchi release model: $Q=KHt_{1/2}$ (3)

Korsmeyer-Peppas: $Mt/M_\infty=KKPtn$ (4)

Where Q is extent of drug release; K_0 is zero-order release rate constant; t is release time; K_1 is first-order release rate constant; $t_{1/2}$ is half-life of the drug; Mt/M_∞ is fraction of drug release and KKP is release rate constant for Korsmeyer-Peppas.

Effect of sterilization test: The sterility of F1, F2, and F3 optimised formulation batches were tested to determine the impact of aerobic, anaerobic bacteria and fungi before and after total sterilisation in an autoclave. For complete sterilization, the procedure was divided into four parts. In the first part of the sterilization process, both drugs and the aforementioned preservatives were mixed thoroughly in a separate beaker (A) and filtered through a 0.2-micron filter. Following that, CBL-980 and HPC were thoroughly mixed in a beaker (B). Before sterilisation, both mixtures (C) were combined and evaluated for viscosity. Afterwards, mixture (C) was subjected to the autoclave for 1 h, and viscosity was measured[35].

Accelerated stability study: To evaluate the drug and formulation stability, an accelerated stability study was performed according to ICH Q1A (R2) standards at 4 different time points ((T0-zero time point-d 1), (T1-1 mo from the initiation date), (T2-2 mo from the date of initiation), (T3-3 mo from the date of initiation)). Optimized F1, F2 and F3 formulations were packed in 5 ml white HDPE plastic dropper bottles separately and kept in a stability chamber at definite temperature and humidity (40o±2°/75 %±5 % RH) for 3 mo. The physical stability of the three formulations were evaluated by determining pH, osmolality, surface tension, the viscosity of sol and gel and chemical stability by evaluating the percentage assay of the formulation[40].

RESULTS AND DISCUSSION

The melting points of CFH and OLH were successfully ascertained by the glass capillary method. The melting points of both drugs were found to be 256°±2° and 238°±2°, which were in

the range of their standard values (255°-257° CFH) and (>240° OLH). Sterilization is an essential aspect of ocular formulation. The obtained higher melting points showed no degradation of drugs during the sterilization process.

The solubility study was performed for both drugs, and it was observed that CFH was soluble in water, very soluble in ethanol, methanol and OLH was very soluble in formic acid, sparingly soluble in water, and very slightly soluble in dehydrated alcohol. Water is a potent solvent for ocular drug delivery instead of organic solvents. The solubility study explored that both drugs were soluble in water; hence it was used as a solvent for ISG formulation.

FTIR spectrum of CFH marked the presence of quinolones at 1650-1600 cm^{-1}. In contrast, the bands at 1750-1700 cm^{-1} ascribed to carbonyl C=O stretching. The peaks revealed the bending vibrations of the O-H group at 1300-1250 cm^{-1}. The existence of carboxylic acid was explored between the FT-IR ranges 1450-1400 cm^{-1}. Furthermore, the C-F group exposed an absorption peak between 1050 and 1000 cm^{-1}. The FTIR spectrum 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 stretching vibrations of C=H and C-H, respectively. All observed functional groups in both drug's spectrums were found as per standard ranges; hence it's confirmed that both drugs were in pure form and represented in fig. 2.

In the drug excipient compatibility study, the percentage assay for CFH was found between 97 %-101 % and for OLH 98 %-100 %. The excipient compatibility study explored that all batches (EC-1 to EC-5) did not showed incompatibility; hence both drugs, in conjunction with excipients, were utilized in ISG formulation. The summary of the percentage assay excipient compatibility study is shown in the Table 2 and fig. 3 represents the study chromatograms. The nine batches of pH-dependant ISG formulation were developed successfully by employing CBL-980 and viscosity grade HPC and subjected to the following evaluation parameters.

Visual inspection and clarity study of the developed pH-dependant ISG formulations revealed that all nine batches were translucent and clear in appearance, ensuring patient compliance. Additionally, it's indicated that the formulation did not contain any particulate matter, which may harm the ocular area. The pH of the F1-F3 batches were found to be 6.35±0.12 to 7±0.27, which correlated to the lacrimal fluid pH, whereas the pH of the F4-F6 batches were found to be 5±0.22 to 6±0.37, and F7-F9 batch showed 4±0.26 to 5.1±0.27 pH which was not suitable for ocular administration.
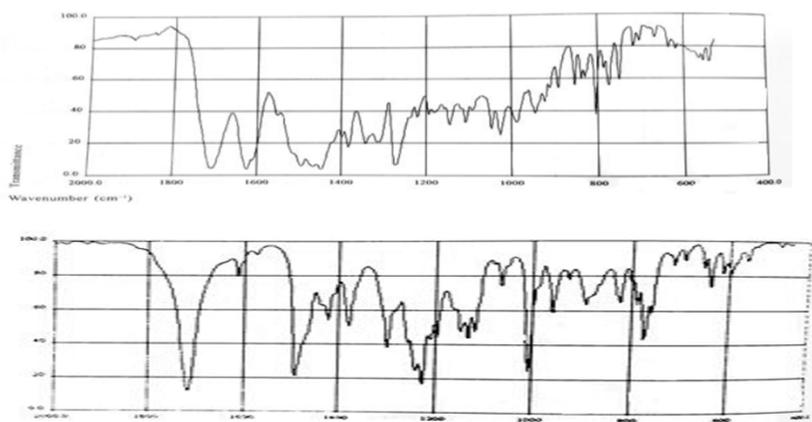
Fig. 2: FTIR of CFH and OLH, (A): FTIR of Ciproflxacin HCl and (B): FTIR of Oloptadine HCl

TABLE 2: SUMMARY OF EXCIPIENT COMPATIBILITY STUDY

Batches	% Assay for CFH	% Assay for OLH
EC-1	97.39±0.59	99.61±0.17
EC-2	98.21±0.25	98.07±0.42
EC-3	99.93±0.35	99.27±0.69
EC-4	100.49±0.27	99.63±0.48
EC-5	101.32±0.18	100.56±0.16
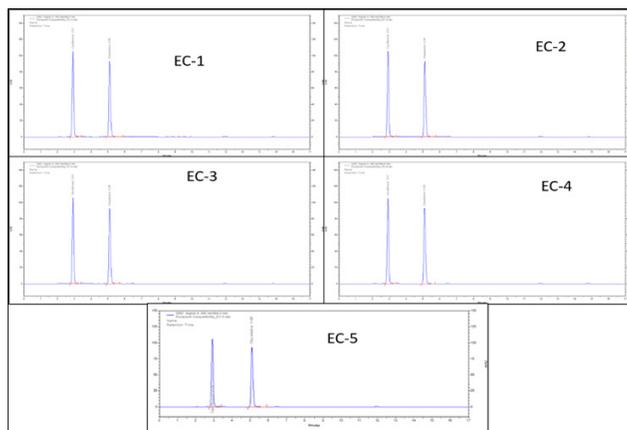
Fig. 3: Chromatograms for excipient compatibility study

The gelling time was studied by adding 50 µl of each formulation to the cylindrical fluid containing 5 ml of SLF (pH 7.4), and the time required to form the gel was noted in min. After adding a drop of SLF, the gel was formed in seconds and lasted for several min. The study outcomes showed that batches F1, F2 and F3 required 34-39 min to assemble the gel. Batch F4, F5 and F6 required 43-49 min to form the gel. Batch F7, F8 and F9 consist 38-53 min to form the gel. In all batches, F1, F2 and F3 showed a consistent gelling time suitable for ocular administration because a higher gelling time reduces formulation efficacy.

The viscosity of formulations was determined for sol and gel. Batch F1, F2 and F3 showed approximately a 4-5 times increase in the viscosity after gelation. In contrast, other batches showed an approximately 10 times increase which consisted of 0.5 %-1 % CBL-980, but the viscosity was significantly higher in comparison to what is required for ophthalmic preparation; hence 0.3 % concentration of CBL-980 and HPC were suitable for the ISG formulation as mentioned in fig. 4. The F1, F2 and F3 batches did not exhibit stiff form ISG hence the obtained viscosity was feasible for ocular DDS. The summary of overall evaluations is indicated in Table 3.

The three batches were selected for drug content estimation based on the aforementioned evaluations. In the visual appearance, clarity, pH, gelling time and viscosity study, it was observed that F1, F2, and F3 batches showed relevant results for ocular drug delivery; hence these batches were considered optimized batches subjected to drug content estimation. The drug content of all three batches for CFH was found in ranges of 98.89±0.49 to 100.88±0.29, respectively, and for OLH was found to be 98.37±0.54 to 101.45±0.93, respectively, as shown in fig. 5 and Table 4. F3 showed maximum drug content; hence this batch was subjected to an IVRT study. The minimal deviation in drug content implies the uniform distribution of CFH and OLH in the developed formulation.

The efficacy of the drug release from developed gel employing synthetic membrane was compared by adopting an *in vitro* Franz cell system. When developing ocular drug delivery formulation, IVRT is used as a screening method to evaluate the efficiency characteristics of multiple prototype formulations. In drug content estimation, it was observed that F3 showed the highest amount of drug content from three selected batches, 99.56±0.42 for CFH and 99.89±0.51 for OLH; hence this batch was selected for the IVRT study. The percentage of CFH and OLH from the F3 batch released through the PTFE synthetic membrane was evaluated for up to 240 min and plotted as a function of time, as shown in Table 5 and fig. 6. The resulting data explored that pH-dependant formulation showed 1.80 % log drug release of CFH and 1.77 % log drug release for OLH within 240 min, which was satisfactory for ocular application and sustained drug delivery.

The resulting data were fitted into the preceding mathematical models to establish the optimised F3 batch release pattern and mechanism. The ISG drug release data was plotted utilizing several kinetic models, including zero order, first order, Higuchi and Korsmeyer-Peppas. Table 6 summarises the R^2 values of the drug-loaded ISG formulation. The best fit model of the release pattern was estimated with zero and first-order maximum regression values (R^2). Higuchi model regression values showed diffusion functions were comparable to specifying

the release mechanism. The slope value 'n' of the Korsmeyer-Peppas model further demonstrates the release mechanism shown in fig. 7.

The effect of sterilization was studied on F1, F2 and F3 batches before and after putting the formulation in an autoclave for total sterilization. The viscosity of formulations was measured and reported in Table 7. It observed no changes in viscosity after pre-sterilization and post-sterilization. Therefore, indicating that the product can be sterilized before filling during manufacturing.

The stability of the F1, F2 and F3 batches were evaluated at 40°±2° with a relative humidity of 75 %±5 % RH for 6 mo, as displayed in Table 8 and fig. 8. The sample exhibited outstanding physical characteristics under the circumstances specified each time. Besides, the results showed no remarkable changes in all investigated parameters, pH, viscosity, and percentage assay. In addition, the gelling properties and appearance remained unchanged, stating that the formulation was stable and effective for 3 mo.
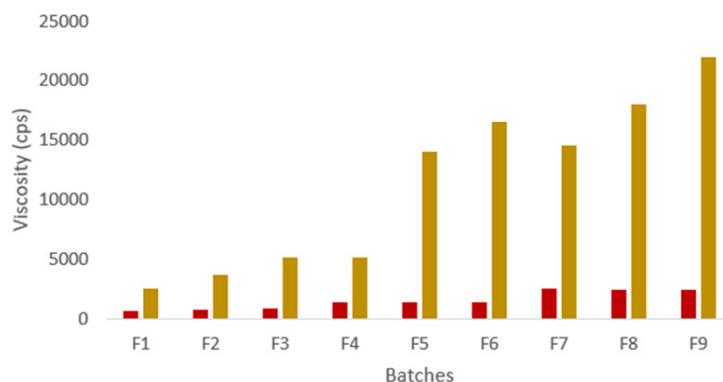
Fig. 4: Graph for the viscosity of sol and pH-dependent ISG
Note: (■): Viscosity of SOL and (■): Viscosity of GEL

TABLE 3: SUMMARY OF pH-DEPENDENT *In Situ* GEL EVALUATIONS

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity of sol (cps)	631±2.2	783±1.7	861±5.4	1389±3.54	1362±6.9	1331±9.25	2475±11.57	2460±21.08	2438±19.84
Viscosity of gel (cps)	2500±17	3700±23	5100±27	5100±1.5	14000±131	16500±100	14500±126	18000±119	22000±185
Gelling time (min)	39	36	34	55	43	49	38	48	53
pH of sol	6.35	6.75	6.47	5.74	5.88	5.66	4.75	4.33	4.76
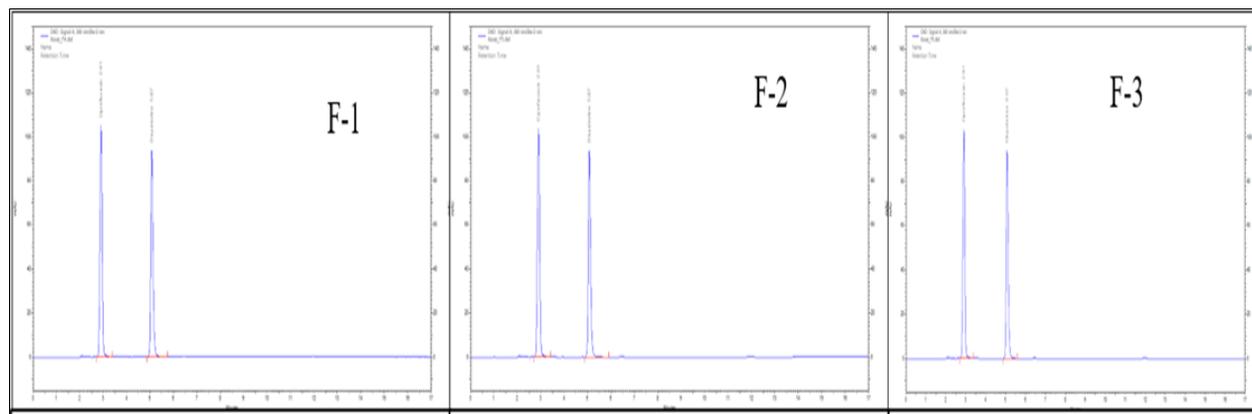
Fig. 5: Chromatogram for drug content estimation

TABLE 4: SUMMARY OF DRUG CONTENT

Batches	% Assay for CFH	% Assay for OLH
F1	99.90±0.25	101.45±0.93
F2	98.89±0.49	98.37±0.54
F3	100.88±0.29	99.35±0.62

TABLE 5: IVRT DATA OF CFH AND OLH FOR pH-DEPENDENT FORMULATION

Time (min)	Log of time	CFH			OLH		
		The square root of time	% drug release	Log % drug release	square root of time	% drug release	Log % drug release
30	1.48	5.48	1.21±0.012	0.08	5.48	2.15±0.015	0.33
60	1.78	7.75	6.25±0.023	0.8	7.75	8.45±0.013	0.93
90	1.95	9.49	14.95±0.17	1.17	9.49	15.93±0.17	1.2
120	2.08	10.95	24.78±0.15	1.39	10.95	28.34±0.23	1.45
180	2.26	13.42	45.24±0.26	1.66	13.42	40.33±0.31	1.61
240	2.38	15.5	62.45±0.23	1.8	15.49	58.64±0.12	1.77
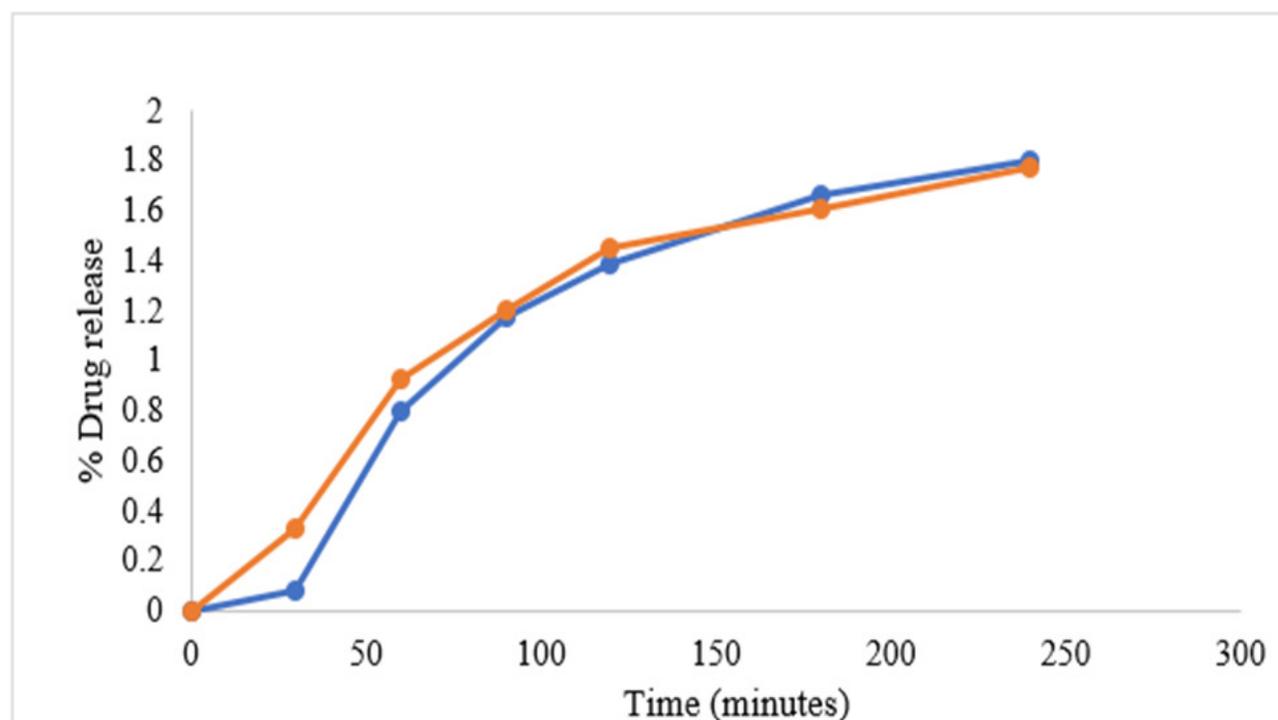
Fig. 6: IVRT profile of CFH and OLH ISG
Note: (━━): CFH and (━━): OLH

TABLE 6: KINETIC STUDY DATA FOR pH-DEPENDENT ISG

Kinetic models	CFH (R^2)	OLH (R^2)
Zero-order	0.9947	0.9937
First order	0.8261	0.8302
Higuchi release kinetics	0.959	0.9699
Korsmeyer-Peppas model	0.9827	0.9826
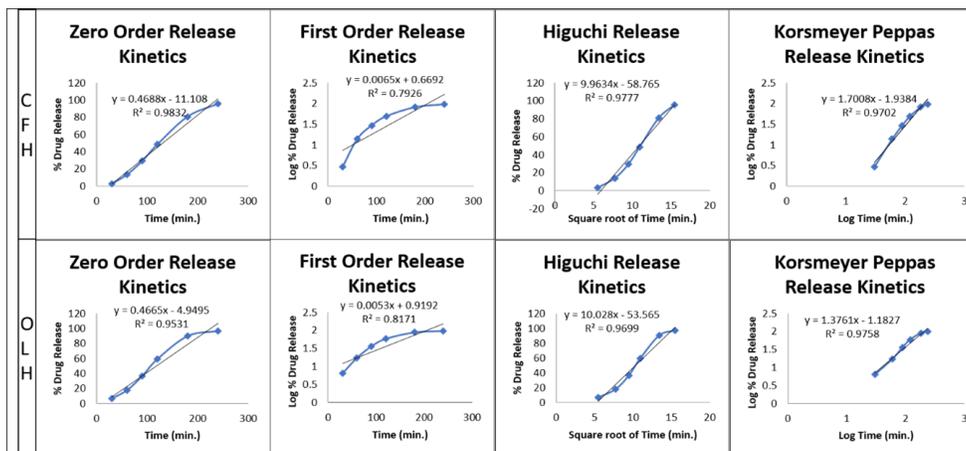
Fig. 7: Graphs of kinetic study for the pH-dependent formulation

TABLE 7: SUMMARY OF STERILIZATION EFFECT STUDY

Batches	Viscosity	
	Pre-sterilization	Post-sterilization
F1	2609±5.8	2578±17.2
F2	3803±4.6	3817±28.4
F3	5091±16.5	5231±47.3

TABLE 8: STABILITY STUDY DATA FOR pH-DEPENDENT ISG FORMULATIONS

Time	Para-meters	0 mo (T0)			1 mo (T1)			2 mo (T2)			3 mo (T3)		
		F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
pH		6.35	6.75	6.47	6.49	6.88	6.91	7.34	7.01	7.31	7.21	7	7.14
Viscosity (cps)	Sol	631± 2.5	783± 5.4	861± 7.3	644± 3.3	749± 2.1	852± 5.4	59± 0.14	784± 0.29	8177± 0.58	601± 0.11	817± 0.17	7915± 0.25
	Gel	2500± 12.5	3700± 28.3	5100± 17.6	2600± 17	3780± 15	5000± 23	2704± 0.32	3715± 0.22	5102± 0.57	2617± 2.6	3745± 2.4	5101± 3.6
% assay	CFH	99.90± 0.25	98.89± 0.49	100.88± 0.29	97.31± 0.52	98.11± 0.67	99.44± 0.51	97.87± 0.67	99.01± 0.51	99.41± 0.52	97.90± 0.11	99.15± 0.25	99.00± 0.14
	OLH	101.45± 0.93	98.37± 0.54	99.35± 0.62	98.16± 0.84	99.76± 0.75	98.17± 0.34	98.41± 0.57	99.81± 0.65	98.01± 0.71	98.56± 0.34	99.89± 0.25	98.18± 0.31
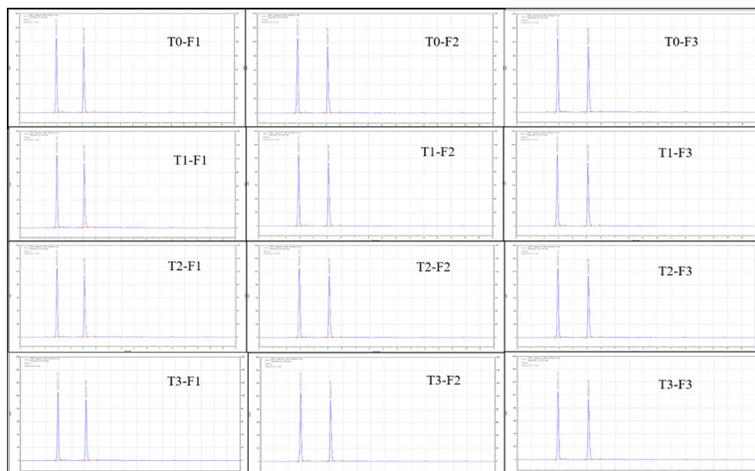
Fig. 8: Chromatogram for the stability study

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