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TEMPERATURE TRIGGERED *IN-SITU* GELLING SYSTEM FOR OCULAR ANTIVIRAL DRUG

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ABSTRACT: The bioavailability of conventional ophthalmic solutions is very poor due to efficient protective mechanisms of the eye, blinking, reflex lachrymation, and drainage, which remove rapidly various foreign substances, including drug, from the surface of the eye. Frequent installation of a drug solution is necessary to maintain a therapeutic drug level in the tear or at the site of action. The recent trends in ocular drug delivery are *in-situ* gelling systems, occuserts, dry drops, and gel foams. Acyclovir is a potent antiviral drug of low toxicity. Acyclovir has low oral bioavailability and a short half-life. In the present research work, Acyclovir *in-situ* gelling systems were prepared by using temperature triggered polymer. The formulations were evaluated for several parameters like drug-polymer interaction, clarity, pH measurement, drug content (%), gelling capacity, gelation temperature, viscosity, sterility, isotonicity, *in-vitro* drug release studies, eye irritation, and short term stability studies. At low temperature, the prepared formulations were in a liquid state, however as the Pluronic F-127 present in the prepared formulation comes in contact with the tear fluid, the solution gets transformed into a gel with high viscosity at body temperature 37 ºC. Increasing the viscosity of a drug formulation in the precorneal region will lead to increased bioavailability due to slower drainage from the cornea. In the developed formulation, F6 was selected, which exhibits 10 h release with non-irritating, sterile, and stable properties, thus increasing the residence time of the drug with better patient compliance.

INTRODUCTION: A major problem of ophthalmic drug delivery is not the lack of efficient drugs but at the attainment of their optimal concentration at the concentration of action [1]. The ocular bioavailability of drugs applied topically as eye drops is very poor.

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The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of eye and by other concomitant factors, for example- Drainage of an instilled solution, lacrimation and tear turnover, metabolism, tear evaporation, non-productive absorption/ adsorption, Limited corneal area and poor corneal permeability, Binding by the lacrimal proteins [2, 3].

This problem can be overcome by using *in-situ* forming gel as ophthalmic drug delivery systems [4]. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due

to rapid precorneal elimination of the drug may be overcome by the use of *in-situ* gel-forming systems that are instilled as drops into the eye and undergo a sol-gel transition. *In-situ* forming gels are liquid upon instillation and undergo a phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to the environmental changes. It has many advantages like-improved local bioavailability, reduced dose concentration, less total drug, improved patient acceptability, reduced dosing frequency [5-7].

A viral infection is characterized commonly by an acute follicular conjunctival reaction and preauricular adenopathy. Adenoviral conjunctivitis is the most common cause of viral conjunctivitis. Particular subtypes of adenoviral conjunctivitis include-epidemic keratoconjunctivitis (pink eye) and pharynx conjunctival fever. Transmission occurs through contact with infected upper respiratory droplets and contaminated swimming pools.

Primary ocular herpes simplex infection is common in children and usually is associated with follicular conjunctivitis. Infection usually is caused by HSV type I, although HSV type II may be a cause, especially in neonates. Recurrent infection, typically seen in adults, usually is associated with corneal involvement. Acyclovir is preferentially taken up by the virus-infected cells. Because of the selective generation of the active inhibitor in the virus-infected cell and its inhibitory effect on viral DNA synthesis, Acyclovir has low toxicity for host cells [8-10].

MATERIALS AND METHODS:

TABLE 1: LIST OF MATERIALS USED

Materials	Supplier
Acyclovir	Auribindo Pharmaceuticals Pvt.ltd
Pluronic acid F-127	Sigma Aldrich Chemicals Pvt. Ltd.
HPMC E 50LV	Remi chemicals
Benzalkonium Chloride	Merck specialities private limited, Mumbai
Sodium Chloride	SD Fine chemical Pvt.ltd
Sodium Bicarbonate	Ranbaxy Laboratories Limited
Sodium Hydroxide	SD Fine Chemical Pvt. ltd
Calcium Chloride dehydrate	SD Fine Chemical Pvt. ltd
Fluid Thioglycollate Medium	HiMedia Laboratories Pvt. ltd
Soyabean-Casein Medium	HiMedia Laboratories Pvt. ltd
Cellophane membrane	HiMedia Laboratories Pvt. ltd

TABLE 2: LIST OF INSTRUMENTS USED

Equipments/Instruments	Source
pH meter	Elico L1120.
FT-IR spectrophotometer	Shimadzu-IRFEINTY-1.
UV- Visible spectrophotometer	Shimadzu-UV-1700.
Brookfield Viscometer	Brookfield engineering laboratories, USA
Glassware	Borosil B-grade.
Stability chamber	Remi Instruments Pvt. Ltd, CHM-165
Hot air oven	Deenmak Instruments.
Magnetic Stirrer	Remi instrument pvt.ltd
Franz diffusion cell	Scientific works
Electronic weighing balance	Shimadzu, Japan
Refrigerator	Godrej

Preformulation Studies:

Study of Organoleptic Properties: A small quantity of pure Acyclovir powder was taken in a butter paper and viewed in well-illuminated place for appearance and color [11, 12].

Determination of Melting Point: The melting point of Acyclovir was determined by taking a small amount of drug separately in a capillary tube closed at one end and placed in a melting point apparatus and the temperature at which the drug melts was recorded. This was performed in triplicates, and the average value was reported [13].

UV- Determination of Absorption Maxima: Absorption maximum is the wavelength at which maximum absorption of radiation takes place. 50 mg of Acyclovir was taken in 50 ml volumetric flask and diluted with artificially stimulated tear fluid up to the mark. From this, a series of dilutions were taken and marked up with stimulated tear fluid to get the desired concentration and subjected for UV scanning in the range of 200-400 nm using UV-Visible spectrophotometer [14, 15].

FT-IR: Reagent: Potassium bromide IR-grade. Triturate about 1 mg of the sample with about 300 mg of potassium bromide. Record an IR-spectrum. Compare the spectrum with that obtained with the reference spectrum of Acyclovir [16].

Preparation of Buffer and Reagents:

Preparation of Simulated Tear Fluid: 0.670 g sodium chloride, 0.200 g sodium bicarbonate, and 0.008 g calcium chloride dihydrate was dissolved in deionized water and diluted to 100 ml in 100 ml volumetric flask.

Preparation of Calibration Curve of Acyclovir: 50 mg of Acyclovir was accurately weighed and transferred to 50 ml volumetric flask, and the volume was made up with artificial tear solution to get a standard stock solution of conc. 1 mg/ml. 5 ml of this solution was diluted to 50 ml to give a stock solution of 50 µg/ml in a 50 ml volumetric flask. The stock solution (0.2, 0.4, 0.6, 0.8, and 1.0) was further diluted to 10 ml in a 10 ml volumetric flask, the resulting solution gives the concentration of 2-20 µg/ml, and the absorbance of the resulting solution was measured spectrophotometrically at λ_{max} 252 nm.

Drug-polymer Compatibility Studies: Drug and excipients compatibility were carried out using FT-IR. Infrared spectrums of pure drug that is Acyclovir alone and along with excipients (HPMC E50 LV, Pluronic F-127) were taken. Based on compatibility with excipients, the formulation was selected.

Preparation of *in-situ* Gel Formulation: The formulation was prepared by the cold method. The different ratios of HPMC E50 LV and Pluronic F127 were prepared. HPMC E50 LV was dissolved in warm water and stirred for 1 h. Pluronic F-127 was dissolved in distilled water and cooled down to 4 °C. Then the drug is dissolved in polaxmer solution. Benzalkonium chloride was added as a preservative. This solution was filtered through a membrane filter and subjected to terminal sterilization (autoclave 15lb pressure, 20 min) after sealing into vials [17].

TABLE 3: FORMULATION CHART OF TEMPERATURE TRIGGERED *IN-SITU* GELLING SYSTEM OF ACYCLOVIR

Ingredient	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12	F 13	F 14	F 15
Acyclovir	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Pluronic F-127	15	15	15	18	18	18	20	20	20	22	22	22	25	25	25
HPMC-E50LV	0.5	.75	1.0	0.5	.75	1.0	0.5	.75	1.0	0.5	.75	1.0	0.5	.75	1.0
Benzalkonium Chloride	0.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02
Deionized Water	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Evaluation of Prepared *in-situ* Gelling System:

Clarity and pH: The formulation's general appearance was observed, which included color and clarity of the solution. The pH of the prepared formulations was checked by using a pocket pen pH meter.

Drug Content: Drug content estimation was done by pipette out 1 ml (0.3 mg) of 0.25% sample solution diluted to 100 ml with simulated tear fluid. From the stock 1 ml is pipette out diluted to 10 ml with simulated tear fluid. The absorbance of the resulting sample solution was measured at 252 nm.

Gelling Capacity: The gelling capacity of the prepared formulations were determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for their gelling was noted.

Gelling Temperature Determination: 10 ml of the sample solution and a magnetic bar were put in a transparent vial that was placed in a magnetic stirrer. A thermometer with an accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at a rate of 1 °C/min with continuous agitation (100 rpm). The temperature was determined as Gelling Temperature at which the magnetic bar stopped due to gelation. Each sample was measured in triplicate.

Isotonicity Evaluation: Isotonicity is an important characteristic of ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of the eye. F6 formulation was subjected to isotonicity testing since they exhibited good release characteristics and gelling capacity and the required viscosity. Formulations were mixed with few drops of blood and observed under a microscope at 45X magnification and compared with standard marketed ophthalmic formulation. The shape of the blood cell was compared with standard marketed ophthalmic formulation.

***In-vitro* Drug Diffusion Studies:** The *in-vitro* diffusion of Acyclovir from the formulations were studied through the cellophane membrane using a Franz diffusion apparatus. The diffusion medium used was freshly prepared Simulated Tear Fluid (STF). The cellophane membrane, previously soaked overnight in the diffusion medium (STF) was placed in between the donor and receptor compartment. 1 ml volume of the formulation was

accurately instilled into the donor compartment. STF was placed in the receptor compartment according to the capacity of it. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37 ± 0.5 °C. Aliquots, each 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with STF and analyzed by UV visible spectrophotometer at 252 nm [18].

Viscosity Determination: The viscosity measurements were done by using Brookfield DV-II+ viscometer using the LV-2 spindle. The developed formulations were poured into the adapter of the viscometer, and the angular velocity was increased gradually from 10 to 100 rpm. The angular velocity was reversed gradually. The average of the two readings was used to calculate viscosity. By adding STF the formulations were made into a gel form, and viscosity was determined as specified above using LV-3 spindle [19].

Test for Sterility:

Method/Procedure: Tests for sterility were performed for aerobic and fungi by using fluid Thioglycollate medium and soybean casein digest medium.

Test for Aerobic Bacteria: 20 ml each of sterile fluid Thioglycollate was transferred to 3 tubes aseptically. The tube labeled as a positive control was inoculated with the viable aerobic microorganism *Bacillus subtilis* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as a test. Then all three test tubes were incubated at 30-35 °C for not less than 7 days.

Test for Fungi: 20 ml each of sterile soyabean-casein digest medium was transferred to 3 tubes aseptically. The tube labeled as a positive control was inoculated with *Candida albicans* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as a test. Then all three test tubes were incubated at 20-25 °C for not less than 7 days. The sterility testing of the ophthalmic drug delivery system was performed for aerobic bacteria and fungi by using fluid Thioglycollate medium and soybean casein digest medium as per the IP Procedure.

Eye Irritation Studies: The ethical committee of the Institution had permitted the ocular irritation studies. Two albino rabbits of both sexes weighing 2.0 to 2.5 kg were used for the study. 0.1 ml of the selected formulation was instilled in the conjunctival sac of the right eye of each rabbit and readings were observed at 1, 24, and 48 h. The eye was evaluated for injuries to the cornea, conjunctiva, and the iris were scored separately. In the above studies, the left eye was served as control (without drug-placebo), and the right eye was served as a test (sterile formulation). The scoring was given according to the Draize irritancy scale.

Stability Studies: Stability studies were carried out on most satisfactory formulations (F6, F11, and F12) as per ICH Guidelines. The sterile gel-forming ophthalmic solution was filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures, and sealed with aluminum foils. The formulations were kept in a stability chamber of at 40 ± 2 °C & 75 ± 5% RH for 2 months. Samples were evaluated for drug content, pH and Clarity, gelling capacity, Isotonicity, and *in-vitro* diffusion [20].

RESULTS AND DISCUSSION:

TABLE 4: DESCRIPTION DATA OF ACYCLOVIR

Name	ACYCLOVIR
Formula	$C_8H_{10}N_5O_3Na$
Molecular Weight	247.19 g/mol
Appearance	White Crystalline Powder
Colour	White
Odor	Odorless
Taste	Characteristic taste
Solubility	Soluble in water, alkali oxides

TABLE 5: MELTING POINT OF ACYCLOVIR

S. no.	Actual Melting point (°C)	Observed Melting point (°C)
1	255 °C - 259 °C	257 °C
2	255 °C - 259 °C	257 °C
3	255 °C - 259 °C	257 °C

Absorption Maxima: The solution showed absorption maxima at about 252 nm.

Drug Excipient Compatibility Study: Few mg of sample was triturated with about 300 mg of Potassium bromide. The pellets were prepared, and IR-spectrum was recorded at 4000 to 400 cm^{-1}. The spectrum was compared with the reference spectrum of Acyclovir.

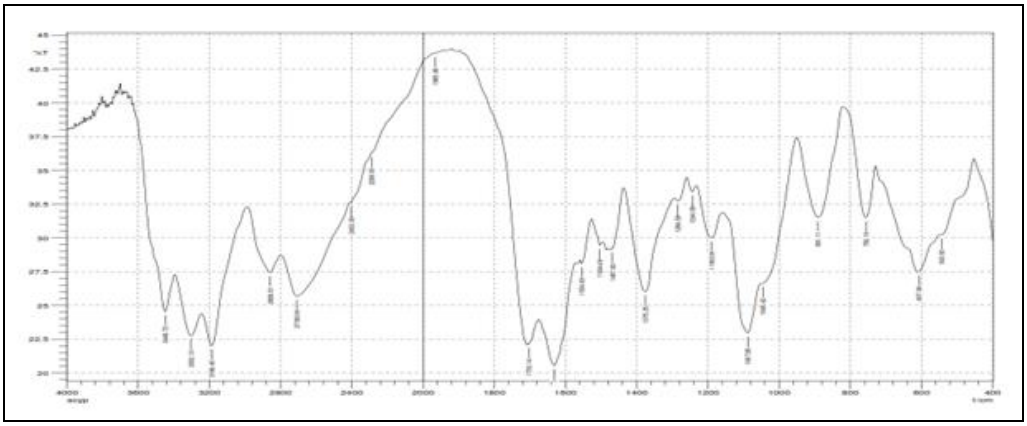

FIG. 1: IR SPECTRUM OF PURE ACYCLOVIR

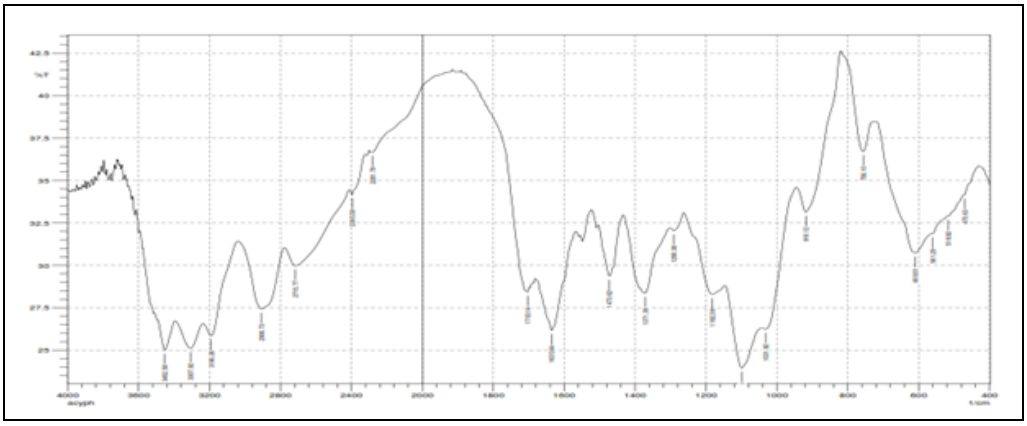

FIG. 2: IR SPECTRUM OF ACYCLOVIR AND HPMCE 50 LV

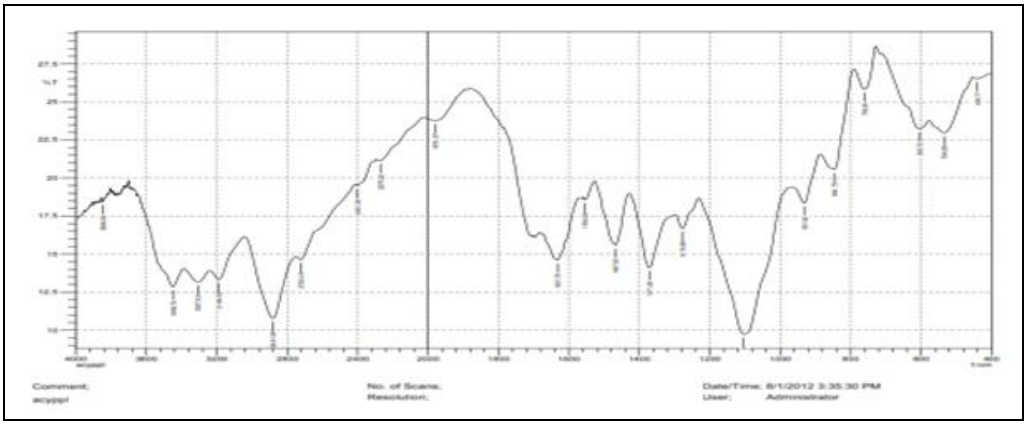

FIG. 3: IR SPECTRUM OF ACYCLOVIRAND PLURONIC F-127

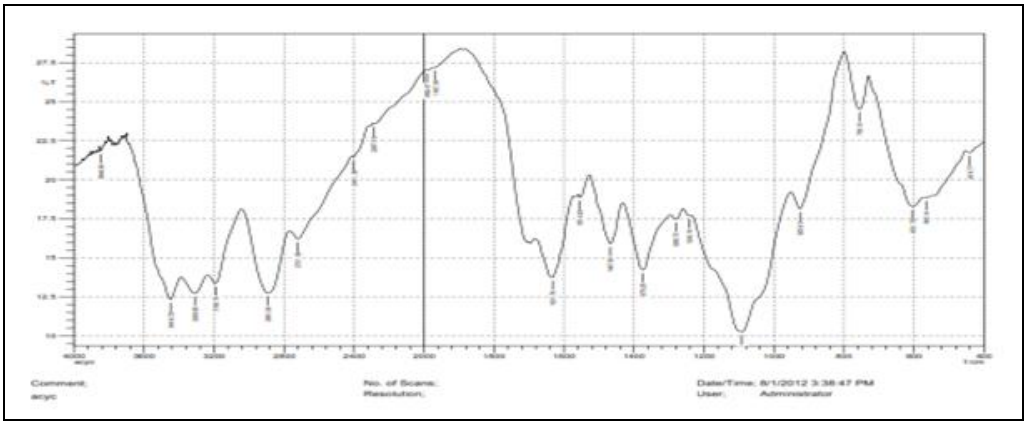

FIG. 4: IR SPECTRUM OF ACYCLOVIR+HPMC E50 LV+ PLURONIC F-127

TABLE 6: INTERPRETATION OF FT-IR DATA

Functional groups	IR range (cm^{-1})	Std. value
NH_2 (stretching)	3520 - 3439	3500 - 3100
-NH (stretching)	3185	3300 - 3000
C-H (stretching)	3028	3000 - 2850
C=N (stretching)	2268	2260 - 2240
C=O (Bending)	1705	1850 - 1630

The interpretation of Acyclovir values is in range. So, the FT-IR study showed that there is no interaction between drug and polymer; hence the drug and polymer are compatible.

Calibration Curve of Acyclovir: The resulting solution was measured spectrophotometrically at λ_{max} 252 nm.

TABLE 7: CALIBRATION CURVE OF ACYCLOVIR

Concentration (µg/ml)	Absorbance
0	0
2	0.171±0.0025
4	0.345±0.0109
6	0.550±0.028
8	0.774±0.014
10	0.912±0.055

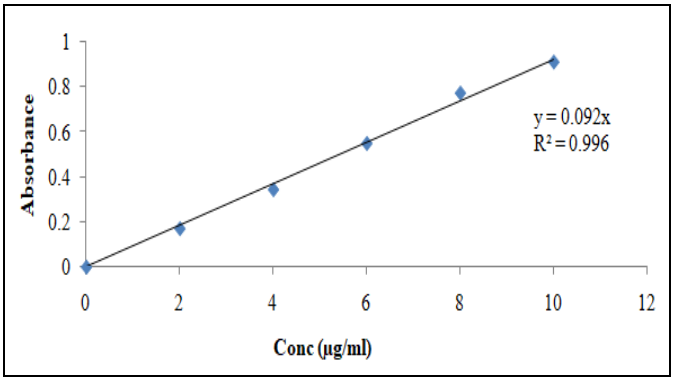

FIG. 5: CALIBRATION CURVE OF ACYCLOVIR

Acyclovir showed maximum absorption at wavelength 252 nm in simulated tear fluid. The standard calibration curve in simulated tear fluid obeyed Beer's law in the concentration range of 2-20 µg/ml.

The correlation coefficient for the standard curve was closer to 1 at the range 2-20 µg/ml; the regression equation generated was slope = 0.0924 and R^2 = 0.9965.

Evaluation of Prepared *in-situ* Gelling System:

TABLE 8: CLARITY, pH, GELLING CAPACITY, GELATION TEMPERATURE AND DRUG CONTENT

Formulation code	Drug content (%)	Visual appearance	Clarity	Gelling capacity	Gelation temp. (°C)	pH
F1	89.98±1.68	Transparent	Clear	+	28.5±0.4	7.4
F2	95.53±1.55	Transparent	Clear	++	26.5±0.71	7.4
F3	94.0±2.68	Transparent	Clear	+++	29.1±0.23	7.4
F4	94.62±1.33	Transparent	Clear	+	26.9±0.49	7.4
F5	95.49±1.55	Transparent	Clear	+++	26.4±0.45	7.4
F6	97.58±1.85	Transparent	Clear	+++	25.6±0.26	7.4
F7	91.52±1.33	Transparent	Clear	++	28.6±0.32	7.4
F8	93.49±1.64	Transparent	Clear	+++	27.5±0.27	7.4
F9	95.13±1.11	Transparent	Clear	+++	26.8±0.45	7.4
F10	99.46±1.53	Transparent	Clear	++	26.4±0.45	7.4
F11	90.92±1.78	Transparent	Clear	+++	28.6±0.32	7.4
F12	88.81±1.98	Transparent	Clear	+++	26.9±0.49	7.4
F13	90.84±1.54	Transparent	Clear	++	27.5±0.27	7.4
F14	95.16±1.69	Transparent	Clear	+++	26.8±0.45	7.4
F15	88.82±1.01	Transparent	Clear	+++	25.6±0.26	7.4

+: Gels after few min, remains for up to 2-3 h. ++: Gelation immediate remains for up to 4-6 h. +++: Gelation immediate remains for up to 7-9 h.

The formulations from F1 to F15 were transparent. The pH of all the formulations was within the acceptable range and hence would not cause any irritation upon administration. The drug content of all the formulations was in the range, except for the formulations F1 and F4, all other formulations gelled instantaneously with a transparent matrix on an addition to STF. The drug content of all the formulations lies in the range of 88.82% to 97.58%, indicating the greater uniformity of the dosage in the formulations.

Isotonicity Evaluation: Formulation F6 was subjected to Isotonicity testing since it exhibited good release characteristics and gelling capacity and the required viscosity.

The formulation was mixed with few drops of blood and observed under a microscope at 45X magnification and compared with standard marketed ophthalmic formulation. The shape of the blood cell was compared with standard marketed ophthalmic formulation.

In-vitro Diffusion Studies:

TABLE 9: *IN-VITRO* DIFFUSION STUDIES

Formulation Code	% Cumulative Drug Diffused									
	1H	2H	3H	4H	5H	6H	7H	8H	9H	10H
F1	22.22 ±1.24	26.42 ±1.05	27.61 ±1.02	39.93 ±1.12	94.76 ±0.92					
F2	28.26 ±1.05	34.54 ±1.22	47.41 ±1.23	68.96 ±1.25	79.55 ±0.58	95.76 ±1.22				
F3	31.81 ±0.55	38.10 ±1.22	40.90 ±2.02	43.70 ±1.02	84.93 ±1.43	96.06 ±1.21				
F4	61.61 ±0.32	67.61 ±0.54	76.17 ±1.02	77.21 ±1.01	86.84 ±0.33	94.28 ±0.25				
F5	16.16 ±1.08	24.86 ±1.29	35.64 ±1.23	44.48 ±1.52	70.05 ±1.21	73.57 ±1.32	87.29 ±0.23	94.39 ±0.54		
F6	14.64 ±0.23	19.29 ±0.53	31.55 ±0.33	40.86 ±0.43	43.68 ±0.55	54.09 ±0.09	61.50 ±0.04	71.56 ±0.21	82.19 ±0.45	97.88 ±0.66
F7	22.22 ±0.32	40.05 ±0.54	43.37 ±1.66	46.70 ±1.76	52.08 ±0.58	64.57 ±0.54	80.68 ±0.37	90.84 ±0.59	96.01 ±1.44	
F8	24.74 ±0.33	35.50 ±0.43	43.35 ±0.54	52.25 ±0.72	80.79 ±0.81	86.9 ±0.26	95.09 ±1.23			
F9	4.5 ±0.23	7.1 ±0.56	8.1 ±1.22	8.7 ±1.54	10.8 ±1.17	21.9 ±1.14	33.2 ±2.01	47.1 ±0.033	52.6 ±1.21	69.4 ±0.21
F10	38.88 ±1.22	39.62 ±1.38	42.98 ±1.54	45.81 ±1.44	53.70 ±1.35	64.68 ±1.22	80.29 ±1.32	94.48 ±1.34		
F11	23.23 ±1.02	25.92 ±1.2	30.14 ±1.32	31.37 ±1.12	34.11 ±1.06	37.38 ±1.02	47.74 ±1.23	62.21 ±0.008	74.26 ±0.35	85.38 ±0.68
F12	20.70 ±1.04	31.96 ±1.28	33.70 ±1.08	49.55 ±0.005	52.44 ±0.009	56.85 ±0.021	74.41 ±0.11	87.05 ±1.17	96.75 ±0.22	96.99 ±0.87
F13	32.32 ±0.07	39.11 ±0.078	53.53 ±0.224	75.63 ±0.456	81.21 ±0.543	83.80 ±0.21	94.49 ±1.23			
F14	32.31 ±0.21	48.21 ±0.87	67.24 ±0.58	74.63 ±0.62	95.64 ±1.65					
F15	29.29 ±0.22	81.52 ±0.43	91.64 ±0.65	94.87 ±0.72						

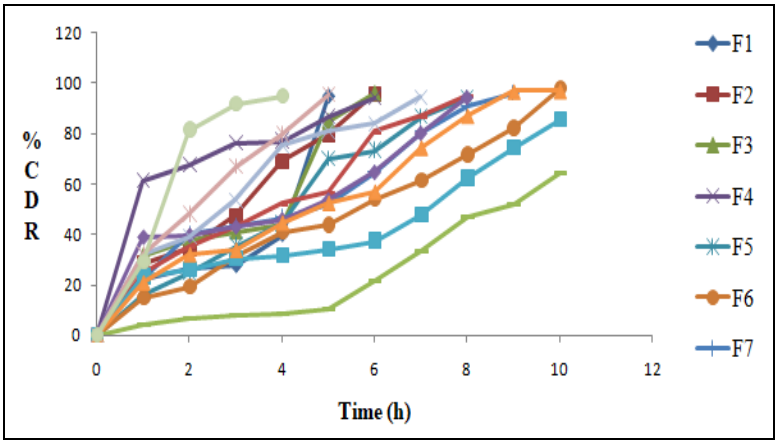

FIG. 6: *IN-VITRO* DIFFUSION PROFILE FOR F1 TO F15 FORMULATIONS

The *in-vitro* release studies indicated that amongst all the formulations, F6 showed the maximum % cumulative drug diffusion and sustained drug release for 10 h, which may be due to the best combination concentration of Pluronic F-127 and HPMC E50 LV.

TABLE 10: REACTION KINETICS FOR BEST FORMULATION

Order	Graphs
Zero Order	Time *vs*. Drug Release
First Order	Time *vs*. log % Drug Remaining
Higuchi Model	SQRT Time *vs*. % Drug Release
Peppas Model	log Time *vs*. log % Drug Release

TABLE 11: RELEASED KINETICS FOR BEST FORMULATION

Time (h)	Log Time	SQRT Time	% Cumulative Release	Log % Release	% Drug remaining	Log % Drug remaining
0	0	0	100	2		
1	0	1	14.64	1.166	85.36	1.931
2	0.301	1.414	19.29	1.2853	80.71	1.907
3	0.477	1.732	31.55	1.499	68.45	1.835
4	0.602	2.0	40.86	1.6113	59.14	1.772
5	0.699	2.236	43.68	1.6403	56.32	1.751
6	0.778	2.449	54.09	1.7331	45.91	1.662
7	0.845	2.646	61.5	1.7889	38.5	1.585
8	0.903	2.828	71.56	1.8547	28.44	1.454
9	0.954	3.0	82.19	1.9148	17.81	1.251
10	1.0	3.162	97.88	1.9907	2.12	0.326

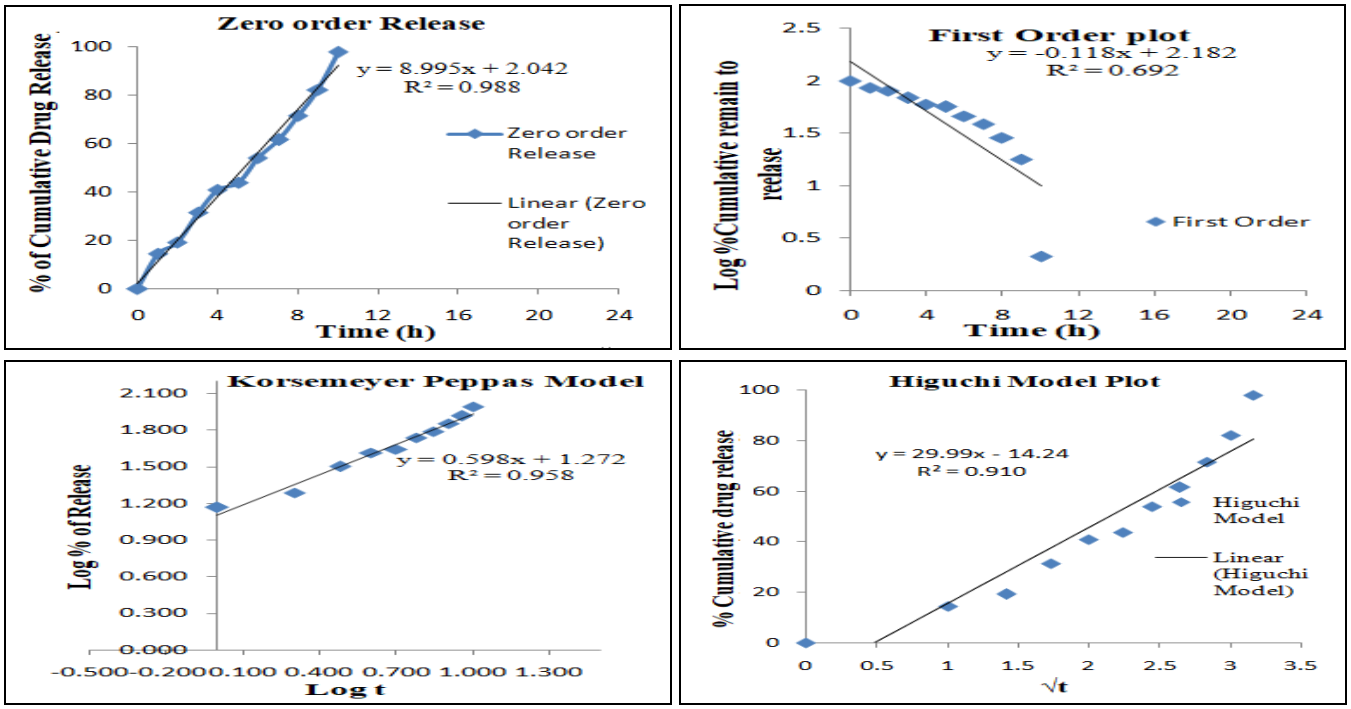

FIG. 7: RELEASE KINETICS FOR BEST FORMULATION

Kinetics Modeling of Drug Dissolution Profiles: *In-vitro* release study data of optimized formulation F6 is fitted into various mathematical models, *i.e.*, Zero order, First order, Higuchi model, Korsmeyer Peppas to determine the best-fit model. The release was found to follow zero-order with a regression coefficient value of 0.988.

Viscosity Determination:

TABLE 12: VISCOSITY OF FORMULATIONS IN SOLUTION FORM

Formulation Code	Number of rotations per minute					
	10	20	30	50	60	100
	Viscosity(cps)					
F6	9.4±0.24	22.76±0.55	37.42±0.54	55.73±0.72	78.29±0.12	99.25±0.l3
F11	10.24±1.21	18.84±1.32	27.84±0.43	35.72±0.62	62.13±0.25	89.25±0.16
F12	12.5±0.34	16.7±0.55	29.98±0.82	56.91±0.54	75.74±0.26	85.54±0.34

TABLE 13: VISCOSITY OF FORMULATION IN GEL FORM

Formulation Code	Number of rotations per minute					
	10	20	30	50	60	100
	Viscosity(cps)					
F6	104.24±0.22	98.75±0.34	79.44±0.43	68.24±0.25	35.15±0.36	12.24±0.43
F11	82.16±0.32	74.82±0.36	66.72±0.44	56.2±0.15	25.3±1.23	10.5±1.72
F12	98.3±0.23	80.2±0.43	56.14±0.54	35.7±0.57	26.7±0.67	13.5±0.82

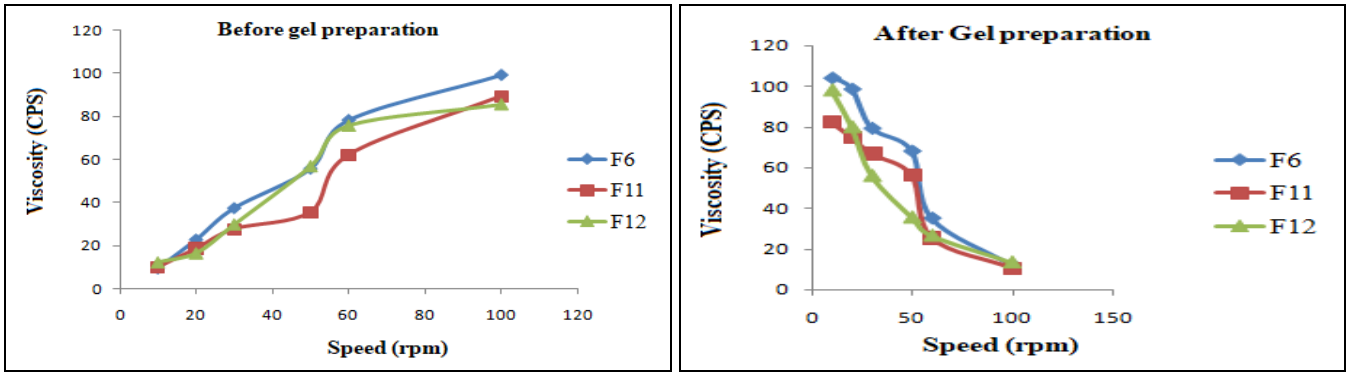

FIG. 8: VISCOSITY OF FORMULATIONS IN SOLUTION AND GEL FORM

The viscosity of the formulations F6, F11, and F12 ranged from 105-9 cps. All the formulations exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity with increased angular velocity.

Test for Sterility:

TABLE 14: OBSERVATIONS OF STERILITY TESTING

Sterility Tests	Results Obtained																				
	Negative control							Test							Positive control						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Test for aerobic bacteria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
Test for Fungi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

(-) sign suggests negative results (No growth of microorganisms)
(+) sign suggests positive results (Formation of colonies of microorganisms)

The formulation passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth. The overall results of the sterility test showed that the prepared ophthalmic formulation was sterile.

Eye Irritation Studies: The ethical committee of the institution had permitted the ocular irritation studies. Two albino rabbits of both sexes weighing 2.0 to 2.5 kg were used for the study. 0.1 ml of the selected formulation- F6 was instilled in the conjunctival sac of the right eye of each rabbit, and readings were observed at 1, 24, and 48 h. The eye was evaluated for injuries to the cornea, conjunctiva, and the iris were scored separately.

TABLE 15: DRAIZE IRRITANCY SCALE

Section	Tissues	Total scores	Total maximum scores
Section-1	Cornea	0	80
Section-2	Iris	0	10
Section-3	conjunctiva	0	20

In the above studies, the left eye was served as control (without drug-placebo), and the right eye was served as test (sterile formulation). The scoring was given according to Draize irritancy scale.

The result of ocular irritation studies indicated that the formulations were non-irritant. Excellent ocular tolerance was observed. No ocular damage or abnormal clinical signs to the Cornea, Iris or Conjunctivae were observed.

Stability Studies: Stability studies were carried out on most satisfactory formulations (F6, F11, and F12) as per ICH Guidelines. Sterile gel-forming ophthalmic solution was filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures and sealed with aluminum foils. The formulations were kept in stability chamber of at 40 ± 2 °C & 75 ± 5% RH for 2 months. Samples were evaluated for drug content, pH and Clarity, gelling capacity, Isotonicity, and *in-vitro* diffusion.

TABLE 16: STABILITY STUDY READINGS AFTER 3 MONTH AT 40 ± 2°C & 75 ± 5% RH

Formulation code	Drug content (%)	Clarity	pH of the solution	Gelling capacity	Isotonicity
F6	97.52±1.35	clear	7.4	+++	Isotonic
F11	90.0±1.87	Little turbid	7.4	+++	Isotonic
F12	89.92±1.32	Little turbid	7.4	+++	Isotonic

TABLE 17: *IN-VITRO* DRUG DIFFUSION STUDIES AFTER 3 MONTH AT 40 ± 2°C & 75 ± 5% RH

Formulation Code	% Cumulative drug diffused									
	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h
F6	13.5 ±0.24	18.78 ±0.26	24.55 ±0.54	35.8 ±0.43	43.68 ±0.65	50.84 ±0.72	61.52 ±0.54	70.82 ±0.32	81.2 ±0.44	96.92 ±0.54
F11	20.20 ±0.24	25.4 ±0.44	31.4 ±0.33	32.37 ±0.53	39.88 ±0.54	45.27 ±1.32	63.023 ±0.32	72.21 ±1.21	78.35 ±0.25	81.70 ±0.43
F12	20.7 ±0.23	31.3 ±0.42	34.35 ±0.54	36.3 5±0.43	41.72 ±0.23	52.45 ±0.43	55.86 ±0.32	75.19 ±0.65	87.19 ±0.75	88.19 ±0.43

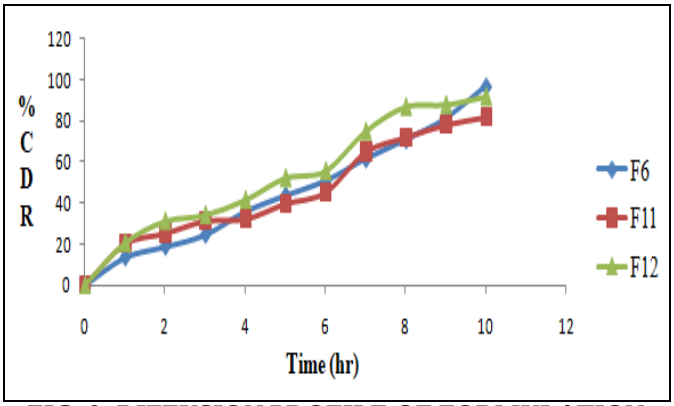

FIG. 9: DIFFUSION PROFILE OF FORMULATION F6, F11, F12

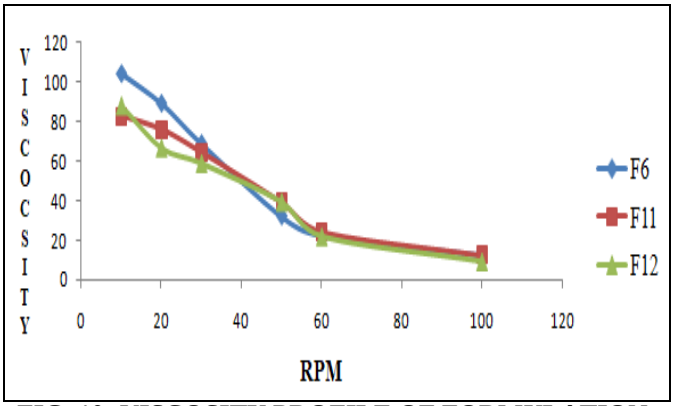

FIG. 10: VISCOSITY PROFILE OF FORMULATION F6, F11, F12

TABLE 18: VISCOSITY FOR BEST FORMULATIONS AFTER 3 MONTH

Formulation Code	Number of rotations per minute					
	10	20	30	50	60	100
	Viscosity (cps)					
F6	104.2±1.23	89.1±1.01	68.7±1.23	31.9±1.23	22.4±1.54	12.4±1.23
F11	82.7±1.03	75.9±1.23	64.3±1.02	39.1±0.54	24.3±0.54	12.5±0.28
F12	88.1±0.54	68.7±0.54	58.9±0.67	39.2±0.64	21.8±0.54	09±0.44

Stability studies of the formulations were carried out as per the ICH guidelines. Gelling capacity, Drug content, pH, and clarity of F6 formulation did not show any significant change as compared to the F11 and F12 formulation, which developed haziness when stored for 90 days at 40 ± 2 °C & 75 ± 5% RH. So F11 and F12 batches were discarded. The results showed that there were no significant changes in the *in-vitro* drug diffusion studies of the F6 batch.

The study taken up was very interesting amidst facing and solving lots of challenges that came across during the period of my project work. Further research work can be taken up to produce a product of Acyclovir *in-situ* gel, which can be done by combination methods like temperature and ion triggered method, and all the combinations can be carried out for treating the herpes virus. The pH triggered method alone can be carried out for the treatment of herpes virus comparison studies, *in-vivo* studies and *in-vitro* and *in-vivo* correlation studies can be extended.

CONCLUSION: A novel drug delivery system by using Acyclovir entrapped in an *in-situ* gel-forming system was formulated in a solution form such that the Acyclovir drops when instilled into the eye, undergo a solution-gel transition in a cul-de-sac. The advantage of this drug delivery system improves patient compliance since only one drop is needed twice a day. The loss of drug is overcome due to the immediate gel formation between the eye membrane and the drug getting entrapped simultaneously in solution-gel transition in the cul-de-sac.

The best formulations selected by keeping in focus the key parameters such as gel formation, gel retention, and *in-vitro* drug diffusion studies, out of which the most suitable batches falling under all the basic criteria's involved *in-situ* gel drug delivery system batches F6 and F11 and F12 were taken up for the final study.

In the last part of the study, after identification of good formulation, stability study as per ICH

guidelines and eye irritation studies on animals (Rabbits) were carried out to select the ideal formulation (F6).

Further research work can be taken up to produce a product of Acyclovir *in-situ* gel, which can be done by combination methods like temperature and ion triggered method, and all the combinations can be carried out for treating Herpes virus.

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