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FORMULATION AND EVALUATION OF pH TRIGGERED *IN-SITU* OCULAR GEL OF OFLOXACIN

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ABSTRACT: Rapid precorneal elimination of the drug in conventional ophthalmic solution leads to poor bioavailability and less therapeutic response, which can be overcome by the use of *in-situ* gel forming system that is instilled into the eye and undergoes a sol-gel transition in the cul-de-sac. The aim of the present study was to formulate and evaluate pH-triggered *in-situ* gels for ophthalmic delivery of ofloxacin. Ofloxacin ophthalmic solution is commonly used in the treatment of bacterial eye infection. The *in-situ* gelling systems were prepared by using different concentrations of carbopol 934 as a gelling agent in combination with hydroxypropyl methylcellulose (HPMC) E15LV as a viscosity enhancing agent. The prepared formulations were then evaluated for visual appearance, clarity, pH measurement, gelling capacity, rheological studies, and drug content. The developed formulation was therapeutically efficacious and stable. Thus the developed system can be used as a viable alternative to conventional eye drops.

INTRODUCTION: The eye is considered to be a very sensitive organ. The perfect vision and ocular functionalities of eyes are generally performed by the visual cells and transparent tissues due to tight cellular membrane and barriers which control the fluid and solvent [1]. The delivery and targeting of ocular therapeutics are generally hindered by some barriers. The tear flow and blinking reflex help in maintaining a good environment and remove foreign material from the eye. The hindrance by barriers and tear flow lead to drainage of the drug from the eye when instilled into it. This leads to poor bioavailability of drug, thus reducing the desired therapeutic effect of the drug [2].

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But one of the advantages of the ocular route is that drug enters to the systemic circulation by eliminating hepatic first-pass metabolism [3]. Conventional ocular drug delivery generally consists of eye drops as the principal and widely used formulation. Eye drop can be manufactured easily and have better patient compliance, but their poor bioavailability is the major problem, which arises due to certain factors such as:

- Drainage of an instilled solution,
- Lacrimation,
- Non-productive absorption,
- Tear evaporation and permeability,
- Limited corneal area and poor corneal metabolism [4].

Ointments, suspensions and aqueous gels are few other conventional ophthalmic formulations developed to enhance ophthalmic bioavailability, but certain drawbacks of these formulations are:

- Poor patient compliance,
- Blurred vision,
- Difficulty in self-insertion,
- Premature release of the drug, and
- Instability of the formulation [4, 5, 6].

***In-situ* Ocular Gel System - Novel Ocular Drug Delivery System:** To overcome the problems of attainment and retention of optimum drug concentration at the site of action within the eye, various approaches of novel ocular drug delivery were studied. *The in-situ* ocular gel is considered to be one of the novels ocular drug delivery system. *In-situ* gel system comprises delivery vehicle composed of polymers (natural, semi-synthetic or synthetic) which has a special property of sol-gel conversion when influenced by some biological stimulus [7]. The few advantages of *in-situ* ocular drug delivery include:

- ✓ Easy administration like a conventional eye drop formulation,
- ✓ Ease of fabrication,
- ✓ Patient compliance,
- ✓ Sustained and controlled drug release due to the formation of the gel network,
- ✓ Enhancement of drug bioavailability, and
- ✓ Prolonged retentivity at the site of action [8, 9, 10].

Ofloxacin is a new fluorinated quinolone antibiotic with a broad spectrum of activity against a variety of gram-positive and -negative bacteria including *Enterobacteriaceae, Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus*. Also, ofloxacin has significant activity against *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycobacterium tuberculosis*, and this may give rise to new indications for the class of quinolone antibiotics [11].

METHODOLOGY:

Material: Ofloxacin was obtained as a gift sample from Ankur Drugs and Pharma Ltd., Baddi Unit I. Carbopol 934 was obtained from Oswald Scientific Store, Chandigarh. HPMC E15LV was obtained from Zone Scientific World, Ambala Cantt. All the chemicals used are of analytical grade.

Method:

Formulation of *in-situ* Gels: The detailed procedure for preparing the *in-situ* ocular gel of ofloxacin is described below:

Buffers salts were dissolved in 75 ml of distilled water. Hydroxypropyl methyl cellulose E15LV (HPMC E15LV) was added to the buffer solution and allowed to hydrate. Then carbopol 934 was sprinkled over the solution and allowed to hydrate overnight. The solution was then stirred on a magnetic stirrer. Ofloxacin was dissolved in a small amount of 0.1 N NaOH, and 0.002 gm of benzalkonium chloride was added to this solution. The drug solution was then added to the polymer solution with constant stirring, and volume was makeup to 100 ml using distilled water. The formulation will then subject to autoclaving at 121 °C for 20 min. Eight batches were selected with varying concentration of carbopol 934 and HPMC E15LV as described in **Table 1**.

TABLE 1: FORMULATION OF *IN-SITU* OCULAR GELS OF OFLOXACIN

Formulation	F1	F2	F3	F4	F5	F6	F7	F8
Ofloxacin (gm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Carbopol 934 (gm)	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.5
HPMC E15LV (gm)	1	1.5	1	1.5	1	1.5	1	1.5
Citric acid (gm)	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Sodium dihydrogen orthophosphate (gm)	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125
Sodium chloride (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Preparation of Stimulated Tear Fluid: 0.67gm of NaCl, 0.20 gm of $NaHCO_3$ and 0.008 gm of $CaCl_2$-H_2O was weighed and dissolving it in a 100 ml volumetric flask and making the volume up to mark using distilled water [12].

Determination of λ_{max} of Ofloxacin: For the determination of absorption maxima, stock solution (100 µg/ml) was prepared by weighing 10 mg of drug and dissolving in 100 ml volumetric flask and making the volume up to mark with phosphate

buffer 7.4. Serial dilutions with concentration 1, 2, 3 up to 10 µg/ml were prepared by transferring a specific amount of stock solution in 10 ml of volumetric flask and make up the volume with phosphate buffer 7.4 up to the mark. The resu1lting solution was scanned between 200 to 400 nm using UV Visible Spectrophotometer (Shimadzu UV 1800) [12].

FT-IR Studies: The IR spectra were recorded using FT-IR spectrometer (Perkin Elmer). Spectra of a mixture of carbopol 934, HPMC E15LV and the drug (Ofloxacin) will be obtained. Spectral scanning will be in the range between 4000 and 450 cm^{-1}.

Evaluation of *in-situ* Ocular Gel:

Physical Examination: Physical evaluation or test of appearance and clarity is very important. It includes observation of color, odour, and presence of suspended particulate matter. The clarity test should be performed against black and white background [13].

Gelling Capacity: To check the gelling capacity of the prepared formulation, few ml of the formulation was taken in a beaker and pH was raised to 7.4 using 0.5N NaOH and observed visually [14, 15].

pH Measurement: The pH of the ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using Portable pH meter (pHep, Hanna) [16].

Rheological Studies: Viscosity of the formulation will be checked by using Brookfield viscometer (Cole Parmer), before and after gelation using spindle no. L3. [4, 17] The angular velocity of the spindle was increased 10 to 100, and the viscosity of the formulation was measured before and after gelation [17, 18].

Drug Content Estimation: Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 100 ml with STF solution (pH 7.4). Aliquot of 1 ml was withdrawn and further diluted to 10 ml with STF. Ofloxacin concentration was then determined at 287.37 nm by using UV-Vis spectrophotometer (Shimadzu UV 1800) [16].

RESULTS AND DISCUSSION:

Calibration Curve of Ofloxacin at pH 7.4: The value of λ_{max} of ofloxacin was found to be 287.37 nm. The spectrum of all dilutions, *i.e.* 1 to 10 µg/ml, is shown in **Fig. 1** and the calibration curve in **Fig. 2**, along with absorbance values of different concentration at 287.37 nm in **Table 2**.

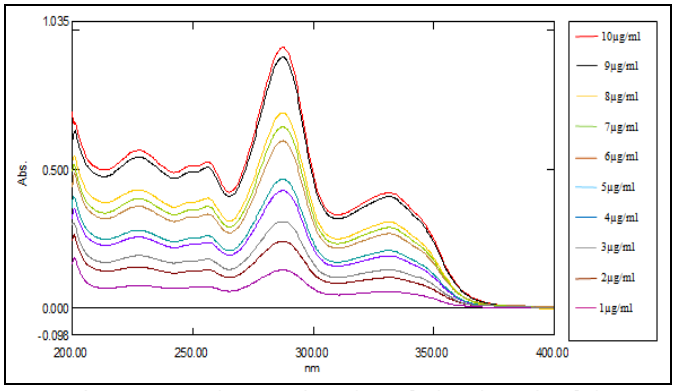

FIG. 1: UV VISIBLE SPECTRA OF OFLOXACIN AT 287 nm

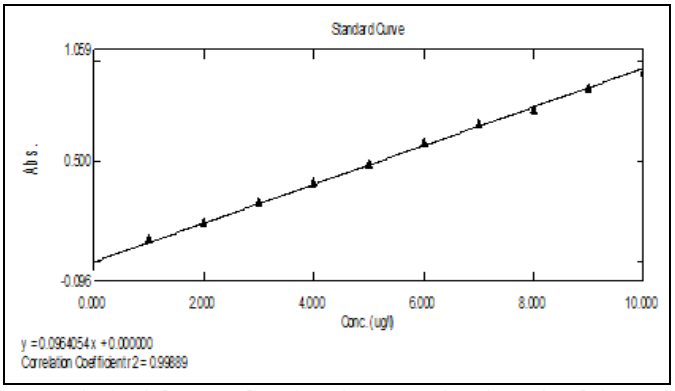

FIG. 2: CALIBRATION CURVE OF OFLOXACIN AT 287.37 nm

TABLE 2: ABSORBANCE AT 287.37 nm

Concentration (µg/ml)	1	2	3	4	5	6	7	8	9	10
Absorbance	0.12	0.197	0.295	0.399	0.485	0.594	0.690	0.760	0.863	0.943

FT-IR Studies: Interaction studies were carried out by FT-IR spectra to check possible interaction among the ingredients in the formulations. **Fig. 3**, **4** and **5** compare the IR spectra of ofloxacin, ofloxacin with carbopol 934 and physical mixture of ofloxacin with all excipients respectively no new

bands were detected in spectra of physical mixtures when compared with drug and individual polymers. This revealed that the ingredients are compatible with each other, and no interaction took place between drug and polymer mixture.

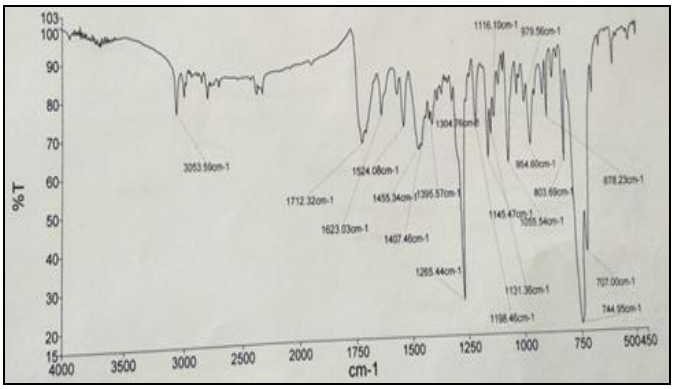

FIG. 3: FT-IR SPECTRA OF OFLOXACIN

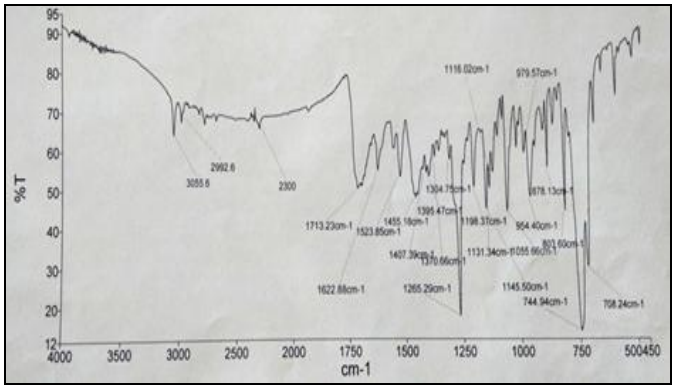

FIG. 4: FT-IR SPECTRA OF OFLOXACIN WITH CARBOPOL 934

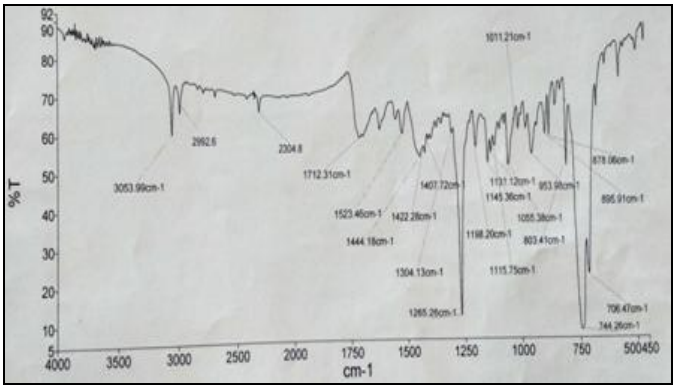

FIG. 5: FT-IR SPECTRA OF OFLOXACIN WITH ALL EXCIPIENTS

Physical Examination: All the formulations were translucent in color and were found to be clear without any turbidity and suspended particles or impurities. The pH of the formulations was found to be in the range of 6.3 to 6.7. All these observations are listed in **Table 3**.

Gelling Capacity: Gelling capacity is coded as described in **Table 4**.

Formulation F1 and F2 shows no gelation, and while formulation F8 shows immediate gelation and remained for a longer time.

TABLE 3: PHYSICAL APPEARANCE, pH, 5 DRUG CONTENT AND GELLING CAPACITY OF *IN-SITU* OCULAR GEL OF OFLOXACIN

Formulation	Appearance	Clarity	pH	% Drug content	Gelling capacity
F1	Translucent	Clear	6.3	91.63	-
F2	Translucent	Clear	6.5	90.56	-
F3	Translucent	Clear	6.7	89.90	+
F4	Translucent	Clear	6.5	94.17	+
F5	Translucent	Clear	6.4	93.01	+
F6	Translucent	Clear	6.6	98.20	++
F7	Translucent	Clear	6.7	93.70	++
F8	Translucent	Clear	6.6	98.61	+++

TABLE 4: CODING FOR THE GELLING CAPACITY

Coding	Observation
-	No Gelation
+	Gels after a few minutes dissolves rapidly
++	Gelation immediate remains for few hours
+++	Gelation immediate, remains for extended period

Drug Content Estimation: The % drug content was found to be in the range of 89.90% to 98.61%.

Rheological Studies: Viscosity values of all prepared *in-situ* ocular gel of ofloxacin were

obtained using Brookfield viscometer (Cole Parmer), using spindle no. L3 at various rpm and the values of viscosity before and after gelation are enlisted in **Table 5** and **6** respectively. All the formulation exhibit Newtonian flow before gelling (at 6.6 pH) and exhibit pseudoplastic flow after gelling (at 7.4 pH) as shown in **Fig. 6** and **7,** respectively. The data shows that viscosity was directly dependent on the polymeric content of the formulations. The viscosity increased with increasing concentration of carbopol 934 and HPMC E15LV.

TABLE 5: RHEOLOGICAL STUDY OF *IN-SITU* GELS OF OFLOXACIN BEFORE GELATION (AT 6.6 pH)

RPM	F1	F2	F3	F4	F5	F6	F7	F8
10	22.6	26.4	35.2	38.1	42.5	45.8	51.8	56.6
12	24.5	27.9	36.9	40	44.6	47.1	53.4	58.2
20	26.4	30.6	41.2	43	48.2	51.1	58	63.1
30	30.8	34.7	44.7	46.2	52.3	54	60.9	65.7
50	35.8	39	47.6	49.9	56	58.4	64.5	69
60	36.6	43	50.1	51.4	57.8	60.9	66.3	69.3
100	40.4	46.8	54.8	57.2	61.6	65.9	70.5	75.2

Viscosity in cps

TABLE 6: RHEOLOGICAL STUDY OF *IN-SITU* GELS OF OFLOXACIN AFTER GELATION (AT 7.4 pH)

RPM	F1	F2	F3	F4	F5	F6	F7	F8
10	200.4	328	463.5	529.5	846.5	944.1	1005.9	1263.5
12	140.5	268.3	458.7	506.1	752.1	870.2	931.5	1258.7
20	130.8	198.4	421.7	497.3	670.4	750.5	787.4	921.7
30	119.5	150.2	377.2	405.7	498.7	604.1	645.5	777.2
50	116.2	142.6	369.8	328.4	405.8	556.8	550.8	669.8
60	111.2	139.2	253.3	280.3	398.5	494.2	454.8	575.6
100	105.9	120.4	176.3	200.4	296	404.2	320.2	476.4

Viscosity in cps

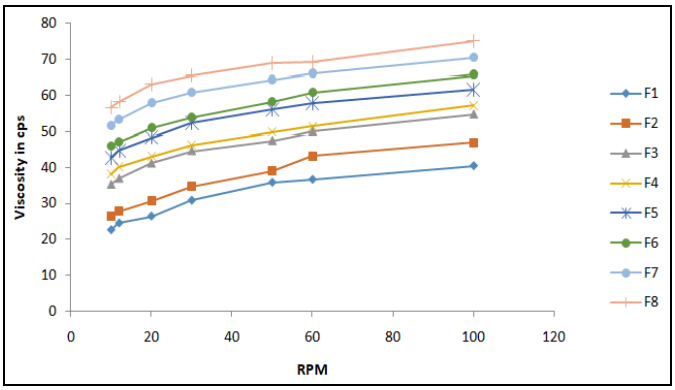

FIG. 6: RHEOLOGICAL STUDY OF *IN-SITU* GELS OF OFLOXACIN BEFORE GELATION (AT 6.6 pH)

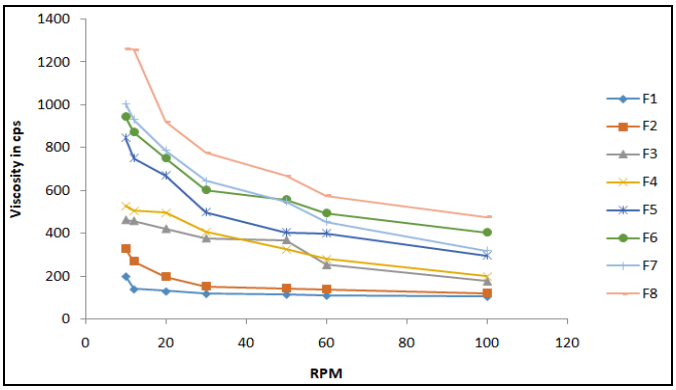

FIG. 7: RHEOLOGICAL STUDIES OF *IN-SITU* GELS OF OFLOXACIN AFTER GELATION (AT 7.4 pH)

CONCLUSION: *In-situ* ocular gel of Ofloxacin was successfully formulated by using pH-triggered gelation method and was developed to a satisfactory level, in terms of physical appearance pH, gelling capacity, viscosity, and drug content. All formulations are clear and translucent in appearance. Formulation F8 with 0.5% carbopol 934 and 1.5% HPMC E15LV shows immediate gelation and remained for extended period. Formulation F8 also shows the highest % drug content of 98.61%. All the formulation exhibit Newtonian flow before gelation and pseudoplastic flow after gelation. Thus, the developed formulation is a viable alternative to conventional eye drops.

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CONFLICT OF INTEREST: None

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