International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 64-73 www.ijprajournal.com ISSN: 2456-4494

pH Triggering Ophthalmic In-situ Gel of Levofloxacin for the Treatment of Conjunctivitis

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Submitted: 01-09-2022 Accepted: 10-09-2022

ABSTRACT
In ocular delivery the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect. Thus with the use of these in situ gelling systems, residence time of the drug in the eye is increased. Continuous delivery of drugs in a controlled manner to the anterior chamber of the eye will eliminate the requirement for frequent drug administration, causing better patient compliance and resulting in extended duration of action. The present work describes the formulation and evaluation of an ophthalmic delivery system of an anti-bacterial drug Levofloxacin, based on the concept of pH triggered in situ gelation by using sodium alginate, In vitro release studies indicated Among the all formulations F8 shows better drug release when contacted with STF solution at 8 hrs study period. It shows antimicrobial, antibacterial efficacy with selected microorganisms. These results demonstrate that the developed system is an alternative to conventional ophthalmic drops, patient compliance, industrially oriented
and economical. The Prepared in situ gel biocompatibility was studied by Hen's Egg Test Chorioallantoic
Membrane. The formulations were evaluated for clarity, sterility, pH, viscosity, gelation temperature, gelation time, drug content, and drug release. The developed formulation is a viable alternatives to conventional eye drops by virtue of its ability to enhance the bioavailability through it longer precorneal residence time and ability to produce sustained drug release. This can be effectively used in the treatment of several eye diseases, infection caused by organisms and also used in the treatment of conjunctivitis.

Keywords: Ophthalmic delivery systems, in situ gelling, Levofloxacin, Sodium alginate , HET CAM Test.

I. INTRODUCTION

Ophthalmic preparations are the sterile product essentially free from foreign particles, suitably compounded and packaged for instillation in to the eye. Today topical ophthalmic application is considered as preferred way to achieve therapeutic level of drug agents used to treat ocular diseases because of smaller dose required compared to the system use and its rapid onset of action and free from systemic toxicity. The conventional preparation for this route falls in several categories: solution, suspension, semisolids and others. Bioavailability, particularly for ocular solutions ranges from 1 to 10% of the total administered dose. This is due in part to the rapid precorneal clearance kinetics resulting from reflex tears and blinking, where half life time of the instilled isotonic solutions approximate only 15 seconds in humans.

The eye drop dosage form is easy to instil but suffers from the inherent drawback that the majority of the medication it contains is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow, a process that proceeds more intensively in inflamed than in the normal eyes, and lacrimal-nasal drainage. Therefore, only a very small fraction of the instilled dose is absorbed into the target tissues, and relatively concentrated solution is required for instillation to achieve an adequate level of therapeutic effect. In the present work, an attempt has been made to formulate temperature triggering in situ gel as an ophthalmic drug delivery system.. They can be conveniently dropped as a solution into the conjunctival sac in the eye. Upon contact with the lacrimal fluid, the polymer changes its conformation to form a gel. This delivery system has the ease of administration similar to an ophthalmic solution and has a long retention time because of the gel formation. Several

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polymers have been used for preparing pH triggering in situ gels.

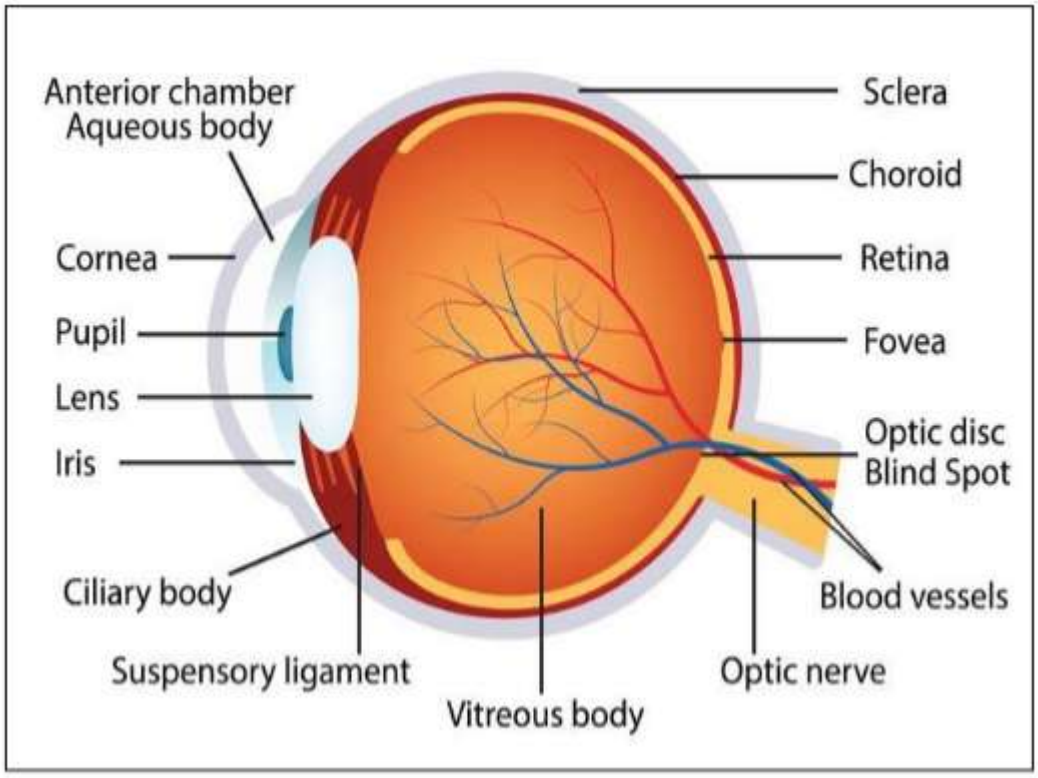

Fig.1Anatomical structureofthehumaneye

II. METHOD:

COLD METHOD

The buffer salts were dissolved in 50ml of purified water, HPMC E-50lv was added and allowed to hydrate, and carbopol was sprinkled over this solution and hydrated overnight. The solution was stirred with an overhead stirrer, and Levofloxacin was dissolved in a small quantity of water. Benzalkonium chloride (BKC) was added to this solution, and to add the drug solution to the polymer solution. Add purified water to make up the volume of 100ml of this solution and filter through 0-2mm filter paper. When the drug solution and polymer solution were mixed, immediate precipitation of carbopol occurred due to a decrease in pH brought about by carbopol. Therefore, the drug was incorporated in a sufficient quantity of 0.5M NaOH and then added to the polymer solution to get a clear solution of drug and polymer.[5]

PREFORMULATIONS STUDIES [6]

a) Identification of drug: The identification of levofloxacin was done by UV, DSC, and FTIR and confirmed as per monographs.

b)Solubility analysis: Solubility analysis of levofloxacin was carried out in various solvents and phosphate buffers. As a result, 10mg of levofloxacin was dissolved in water, pH 1.2, pH 4.8, pH 6.0, pH 7.4

c)Melting point determination: The capillary method has been used to determination of the melting point of levofloxacin. A small amount of compound was placed in a thin-walled capillary tube of about 10-15cm long and 1mm inside diameter and closed at one end. The capillary containing the sample and a thermometer is then suspended in an oil bath containing liquid paraffin. So, they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point

d)Fourier transform infra- red spectroscopy: The FT-IR of levofloxacin has been recorded with a nature of interacting forces that can be evaluated using the potassium bromide pellet method.

e) Thermal analysis by DSC: DSC can be conducted for in situ forming polymeric systems to quantify the hydrogel's water percentage. Differential scanning calorimetry is used to observe if there are any changes in thermograms as

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compared with the pure ingredients used, thus indicating the interactions.

Evaluation of prepared ophthalmic in situ gel:

i. Appearance and clarity

In this method the fluorescence light was used against the white and black back ground to check the appearance and clarity of the formulation. The formulation was examined for any particulate matter and turbidity.

ii. pH of gel

The pH of the prepared in situ gelling system after addition of all the ingredients was measured using pH meter.

iii. Viscosity determination

The viscosity measurements were carried out by using Brookfield viscometer with suitable spindle (no.5) at suitable speed (20/30/40/50 rpm). The instrument was equipped with the temperature control unit and the samples were equilibrated for 10 min before the measurement. The viscosity was measured (n=3) at two temperatures viz. 8±1°C and at 37±1ºC using a thermo stated water jacket.

iv. Gelation time

The gelation time was determined by test tube inverting method. Solution was taken in a thin-walled tube and kept at the respective gelation temperature on a water bath. The test tube was taken out every 1 min and inverted to observe the state of the sample. The gelation time was determined by flow or no-flow criterion with the test tube inverted.

v. Gelling capacity

In this method 2ml of freshly prepared stimulated tear fluid (STF) are taken in the vials to this add one drop of formulation and equilibrated at 37 0 C. By visual inspection time taken for the gelation was note.

vi. In vitro drug release study

In vitro drug release study is done by using Franz diffusion cell. In the receptor compartment, freshly prepared artificial tear fluid (ATF) is placed. The dialysis membrane is placed in between receptor and donor compartments. The whole assembly is kept on the thermostatically controlled magnetic stirrer to simulate in vivo conditions, and the temperature of the medium is maintained at 37 o C ± 0.5 o C. Medium is continuously stirred at 20 rpm. 1ml of the formulation is placed in the donor compartment. Sample (0.5ml) is withdrawn at a predetermined time interval, and the ATF replaces same. Samples are analyzed either on a UV spectrophotometer or HPLC.

vii. Biocompatibility test:

Hen's Egg Test Chorioallantoic Membrane (HET-CAM) Development: Upon receipt, the eggs will be placed in commercial incubators. On day10 of development, the eggs will be removed from the incubator and candled to determine the viability of the embryo. A rectangular window will be removed from the shell directly over the air cell and the egg membrane will be carefully moistened with 2- 3 mL 0.9% saline and returned to the incubator. The eggs are then dosed and observed continuously for 5 minutes for the appearance of lysis, haemorrhaging and/or coagulation which is documented. In addition, the eggs are scored for severity at 1 and 5 minutes. The severity of each reaction after 1 and 5 minutes is recorded.

viii. X-ray diffractrometer (XRD)

X-ray diffraction design of pure drug Levofloxacin and its physical mixture were documented by utilizing (PROTO AXRD, benchtop system Canada) X- ray diffractrometer with a copper target, voltage 40.00 Kv, current 30.00 MA at a scanning speed of 0.30 0 C/min.

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III. RESULTS

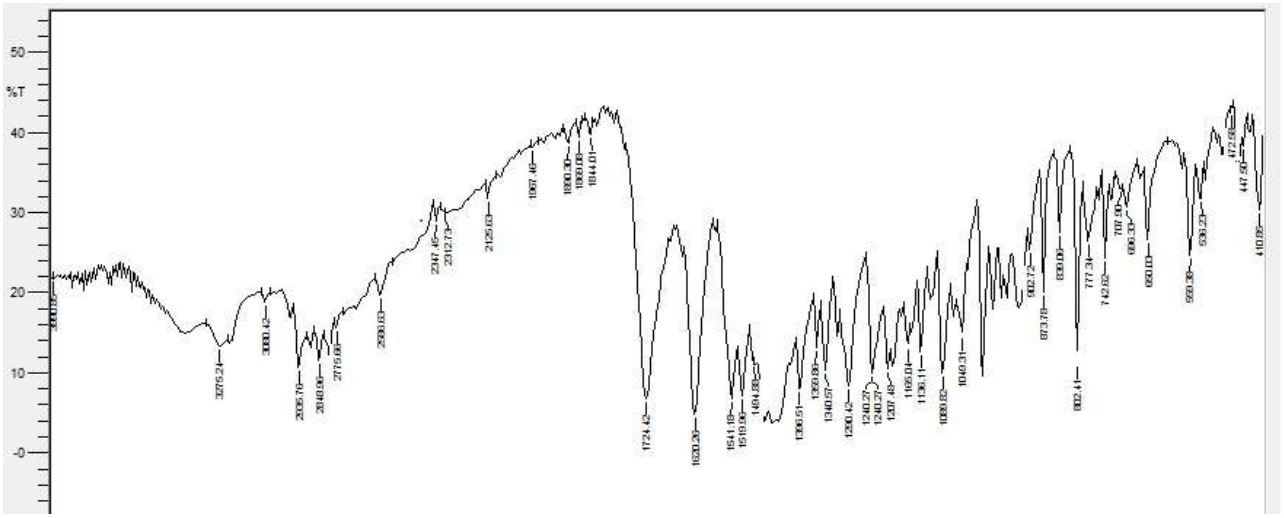
FORMULATION

Ingredients (grams)	F1	F2	F3	F4
Levofloxacin	0.5	0.5	0.5	0.5
Carbopol 940	1.5	1.5	1.5	1.5
HPMC E50LV	0.5	0.6	0.7	0.8
Sodium alginate	0.1	0.2	0.3	0.4
Benzylalkonium chloride	0.02	0.02	0.02	0.02
Gellan gum	0.5	0.6	0.7	0.8
Sodium chloride	0.9	0.9	0.9	0.9
Distilled water (q.s)	100	100	100	100

Melting point of Levofloxacin

Reported	Method	Observed
224-227 ℃	Thiel's tube method	224 ℃
	DSC	227℃

FTIR OF LEVOFLOXACIN



DSC THERMOGRAPH OF LEVOFLOXACIN

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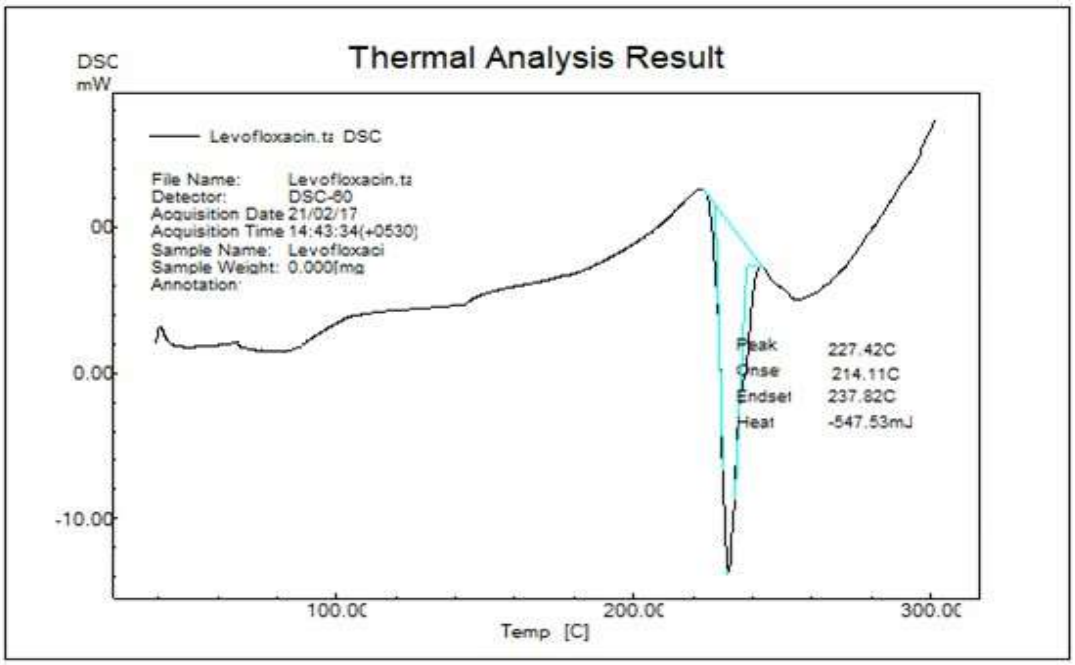

Appearance and Clarity Test

(A) **(B)**

(A) Clarity tested under black background **(B)** Clarity tested under white background

pH of the Gel

Formulation code	**pH**
F1	6.86±0.03
F2	6.55±0.03
F3	6.39±0.03

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F4	6.65±0.04

Viscosity of formulations F1-F4

Sl.No.	FoFFormulation code	Viscosity(cps)	
		8°C*	**37°C***
1	F1	359±3.25	22426±5.12
2	F2	583±4.56	25468±4.95
3	F3	301±5.66	20154±6.18
4	F4	496±2.15	23426±5.41

Gelationtime of formulations F1-F5

Sl.No.	Formulationcode	Gelation time(min) Mean±SD*
1	F1	2±0.985
2	F2	1±0.545
3	F3	2±0.655
4	F4	3±0.891

Gelling Capacity of Gel

Formulations	Gelling Capacity
F1	+++
F2	+++
F3	+++
F4	+++

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Visual Appearance

Formulations code	Visual Apperance
F1	Transparent
F2	Transparent
F3	Transparent
F4	Transparent

In vitro Diffusion

TIME	F1	F2	F3	F4
0	0	0	0	0
1	15.55252	22.10095	9.276944	5.729877
2	25.10232	34.37926	23.73806	17.73533
3	33.28786	51.02319	33.01501	26.73943
4	50.20464	60.30014	48.02183	36.28922
5	61.93724	75.03411	61.93724	53.75171
6	69.84993	77.48977	69.84993	64.93861
7	77.48977	82.40109	78.30832	72.30559
8	84.85675	95.2251	90.31378	83.76535

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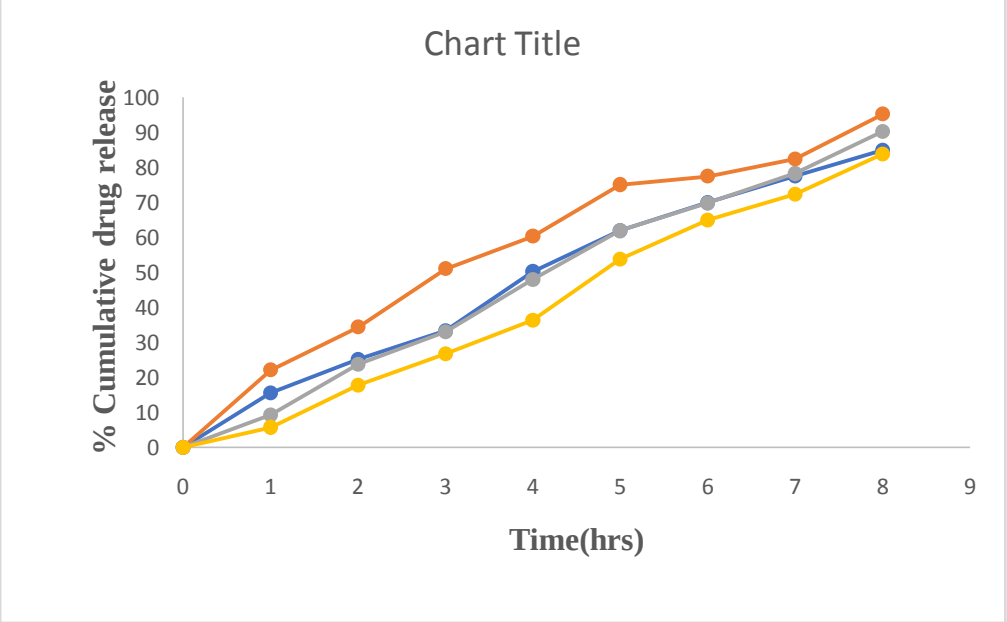
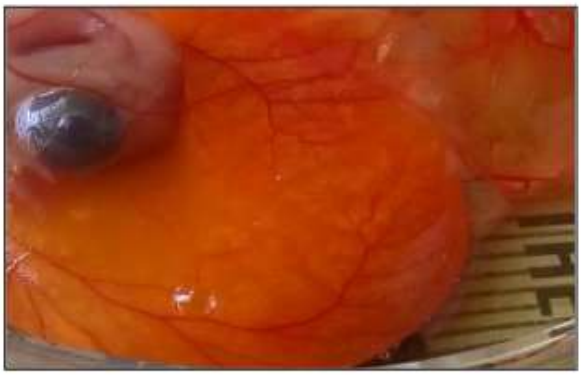
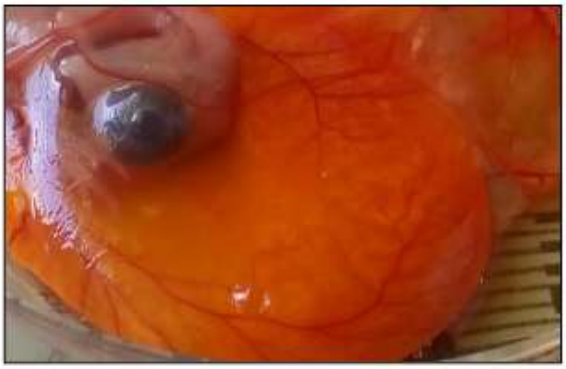


Biocompatibility test: - Hen's Egg Test Chorioallantoic Membrane (HET-CAM)test:



Controlled before application



Controlled after application

Test before application

Testafter application

International Journal of Pharmaceutical Research and Applications
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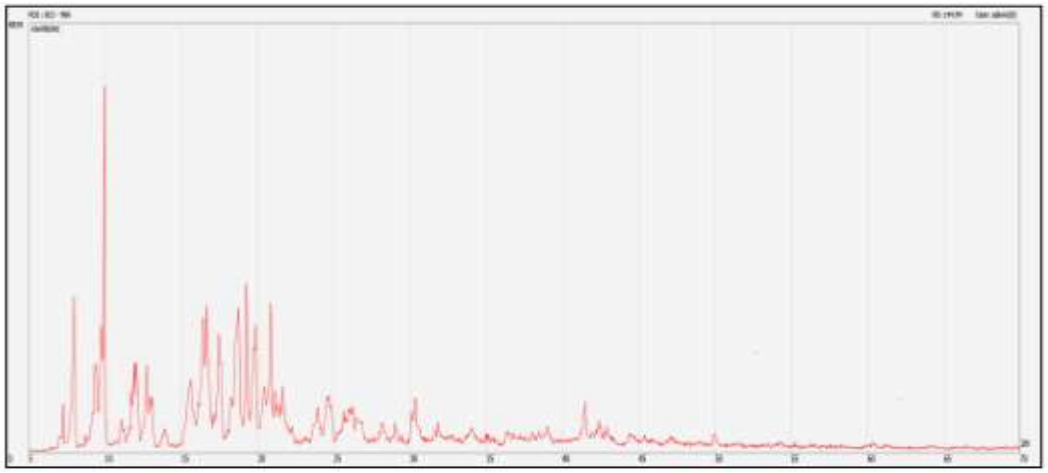
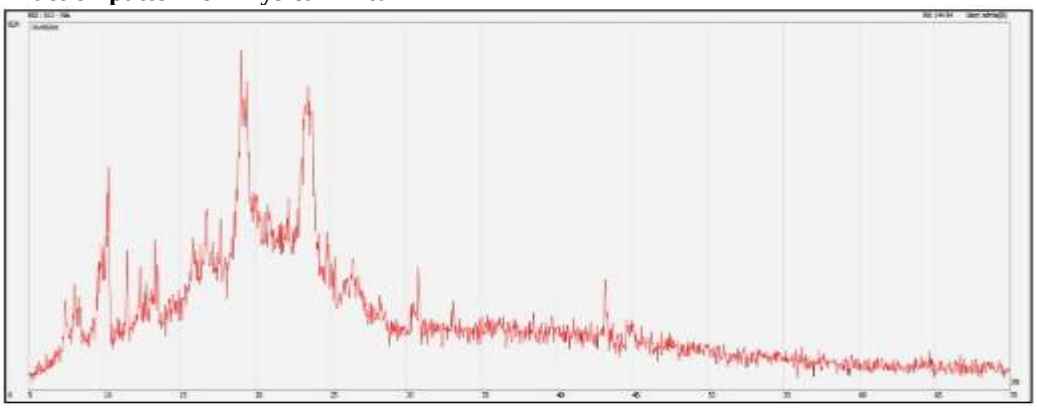
Scoring forirritationtestingwiththeHET-CAMtest method

Effect	Time(minutes)		
	0.5	**2.0**	**5.0**
Hyperaemia	0.0	0.0	0.0
Haemorrhage	0.0	0.0	0.0
Coagulation	0.0	0.0	0.0

X-Ray diffraction Pattern
X-ray diffraction pattern of Lev of loxacin



X-ray diffraction pattern of Physicalmixtur



IV. DISCUSSION AND CONCLUSION

- This research aimed to develop an ophthalmic in situ drug delivery system of levofloxacin to improve its poor ocular bioavailability.
- The ocular in situ levofloxacin gel was prepared using carbopol 940 and HPMC (E50LV) by pH-triggered in situ gelling technique.
- The broad-spectrum antibacterial agent used in treating ocular infections like conjunctivitis was successfully formulated in situ gelling system using 0.5%W/V of levofloxacin.
- The formulated in situ gelling systems were characterized for appearance, colour, pH, gelling capacity, rheological character, and in vitro release in simulated tear fluid.

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- The viscosity results revealed that F-2 showed better pseudo-plastic behaviour in the highest concentration of Carbopol 940 (0.6%) than in other formulations.
- The in vitro release studies revealed that the increase in polymer concentration retards the drug release and the decrease in polymer concentration increases the drug release.
- Preformulation studies of Azithromycin were carried out by determination Melting point, solubility and λmax. The obtained results complied with IP standards, thus indicating the purity of drug.
- FT-IR, DSC and XRD study revealed that there was no interaction between the drug and polymers (Sodium alginate, HPMC and Carbopol).
- Biocompatibility test: - Hen's egg test Chorioallantoic membrane test showed a compatible to the optimum formulation there in no irritant effect was found.
- In situ gels were clear and transparent with good appearance.
- All formulation of in situ gel was found to be sterile.
- The pH of all formulation was found within range. Then it is suitable for the administration.
- The viscosity of formulations increased with increase in temperature and the gelation time decreases with increases in the polymer concentration.
- The highest drug content was observed in F2 (97.92 ±0.809) because of high drug loading.
- The in vitro release of drug from ophthalmic in situ gelling system was found to vary according to the type and % of polymer used. The F2 formulation with 1.5gm HPMC, 0.6gm Sodium alginate, 0.2gm carbopol showed the sustained drug release among all the formulations because of its viscosity properties when compared to the other formulations.

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