IJPSR (2019), Volume 10, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 May 2018; received in revised form, 03 October 2018; accepted, 24 November 2018; published 01 January 2019

PREPARATION AND CHARACTERIZATION OF pH BASED CARBOPOL 934P *IN-SITU* HYDROGELS FOR THE TREATMENT OF HARMFUL BACTERIAL INFECTIONS

B. Anbarasan [1], R. Satiesh Kumar [1], J. Thanka [2], S. Ramaprabhu [4] and S. Shanmuganathan [*3]

Department of Pathology [2], Department of Pharmaceutics [3], Faculty of Pharmacy [1], Sri Ramachandra Medical College and Research Institute, Porur, Chennai - 600116, Tamil Nadu, India.
Department of Physics [4], Alternative Energy and Nanotechnology Laboratory, Indian Institute of Technology, Chennai - 600036, Tamil Nadu, India.

Keywords:

Carbopol 934P,
HPMC, Topical route,
HET- CAM study, Precorneal

Correspondence to Author:
S. Shanmuganathan

Professor & Head,
Department of Pharmaceutics,
Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai - 600116,
Tamil Nadu, India.

E-mail: shanmugganathan@gmail.com

ABSTRACT: The purpose of the present study describes the preparation of pH based *in-situ* gel formulations were applied for the harmful bacterial infections by using carbopol 934P and HPMC derivatives. The simple procedure was adopted for preparing these formulations. To eliminate or reduced adverse systemic effects produce from bacterial conjunctivitis, topical route of administration, such as eye drops was preferred for this type of infections and it should be installed into the eye's cornea. These eye drops are more effective to provide therapeutic index and also to achieve good bioavailability in the eye. Forthcoming, the developed *in-situ* gel formulations were characterized by gelling capacity, pH, clarity, appearance, viscosity determination, surface tension, drug content, *in-vitro* drug release, HET-CAM study, permeation study, and *in-vivo* ocular irritation study. The optimized formulation (F2) was set up to be non-irritant and well tolerable from the results both *in-vitro* HET-CAM study and ocular irritation study. The developments *in-situ* gel formulation forms good gelling capacity, clear/translucent in appearance, after administration in the eye. The developed *in-situ* gel formulations control the precorneal drainage, increased resistance time on the eye, improved patient compliance by increasing the bioavailability of drug concentrations to the eye.

INTRODUCTION: Globally, the human eyes are regarded by some bacteria and fungi which cause dangerous infections such as endophthalmitis, fungal endophthalmitis, and orbital cellulitis. Among the different infections, fungal endo-phthamitis is the dangerous infection, which is either exogenous or endogenous [1, 2, 3, 4, 5, 6]. The onset of activity of fungal infection is gradually delayed than bacterial infections.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.10(1).232-44

The article can be accessed online on
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(1).232-44

The fungi species such as *Fusarium* is mainly involved in causing different infections such as keratitis, scleritis, and intraocular infections. *Aspergillus keratitis* is a type of fungi that cause industrial trauma or surgery.

Bipolaris, *Curvulariala*, *Phialophora*, and *Lecytophora* are mainly concerned in keratitis and intraocular infections. *Purpureocillium lilacinum* is mainly responsible for the infection in contact lens and intra ocular lens implants. The fungal species such as *Candida species* is mainly involved in ocular infections [7, 8, 9, 10]. The severe infection of the cornea is mainly due to microbial keratitis. The acute and chronic effects of keratitis are chronic superficial eye disease, trauma, use of contact lenses and immune deficiency.

In the USA, it is reported that the patients having microbial keratitis infections owing to the usage of the soft contact lens are in the range of 40 to 210 per 100,000 per year. In most countries, the species like *Fusarium keratitis* is mainly responsible for causing the infection due to contamination in the contact lens solution. About 80% of healthy people have an eye infection due to *Acanthamoeba keratitis*. Panophthalmitis is a type of eye infection occurs, especially in ocular tissue layers including episclera. One of the specific characteristics of panophthalmitis is the severe pain during eye movement [11, 12, 13, 14, 15, 16, 17].

Uveitis is one of the eye infections and is found in 11.1% of instances which is caused primarily due to different clinical conditions caused by various usual fungi, bacteria, viruses, and fastidious bacteria. The bacterium, *Bartonella henselae* causes uveitis in 6.1% cases during the year 2001-2007.

The pharmaceutical scientist, facing more struggles in the area of ophthalmic drug delivery system to treat a disease in constant and conventional attacks. In human, an eye plays a vital role to see colorful things, and it is highly resistant to unknown (foreign) substances. The drug concentration at a specific site of action and their ensuing pharmacological effect are correlated [18].

The therapeutically effective concentration is administered through topical application and is successful in certain infections such as keratitis and bacterial conjunctivitis. During the usage of eye drops, bacteria and their antigens are washed away, and the adverse effects of drugs are either decreased or eliminated. The effective therapeutic drug concentration is achieved through frequent drug administration, varying drug concentration, the duration of the drug in the cornea, the lack of an intact corneal epithelium and the study of the lipophilic nature of the drug where the epithelium is intact.

During the topical administration of ocular drugs, merely 5% of the total drug is penetrating the cornea and contact the internal anterior eyes tissue [19]. The efficient and prompt drainage by the relative impermeability of cornea to hydrophobic and hydrophilic molecules, non-corneal absorption, nasolacrimal apparatus are resulting in deprived ocular bioavailability [20, 21]. During drug administration, the eye barrier must be protected without any tissue damage. One of the specific ways to improve drug bioavailability is to increase drug residence time inside the eye. An ample number of *in-situ* gelling systems has been developed to lengthen the pre-corneal drug residence time, better patient compliance and therefore improve ocular bioavailability [22].

Most of the available *in-situ* gelling systems are mainly as formulations in the form of eye solution, which could be dropped in the eye that further undergo transition into a gel. The transition of the sol-to-gel phase is mainly due to a change in pH, ions, and temperature in the cul-de-sac and hence lengthen the precorneal residence time. These types of *in-situ* gelling systems have been widely used to consider the precorneal drainage of artificial tear products, thermosetting gels, liposomal formulation of tropicamide, ophthalmic ointments, w/o microemulsions, an ion and pH-activated *in-situ* gelling system based on gellan gum and chitosan and alginate and HMPC based an ion activated *in-situ* gelling system [23, 24, 25, 26, 27].

Past 2 decades an ample number of literature has reported on the development of *in-situ* gel preparation in an aspect of the conversion of sol to stiff gel when it contacts with the eye. Due to this, the vision is blurred, and it could get normal after extended minutes. Whereas in this study, the prepared *in-situ* formulation is specifically in the form of intermediate gel when it contacts.

This is specifically prepared in such a way to avoid the blurred vision. The current study is mainly on the development of pH-based *in-situ* gelling systems of ofloxacin to treat severe bacterial eye infections. The formulation is prepared and optimized using various tests such as *ex-vivo* permeation study, HET-CAM membrane test, and *in-vivo* corneal irritation study. Carbopol 934P and HPMC derivatives were used to develop the pH-based *in-situ* gelling system. Among fluoro-quinolones, ofloxacin is preferred due to its pH 6 which is quietly bearable by eyes, maximum inherent solubility and utmost diffusion rate into ocular tissues. This drug is specifically considered due to their elevated diffusion rate into the tissues.

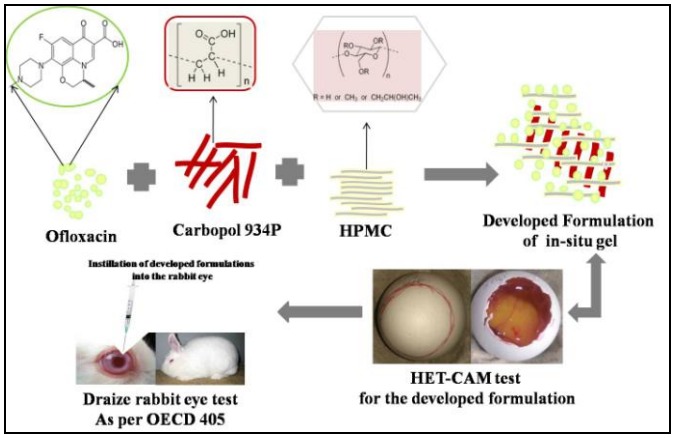

FIG. 1: SCHEMATIC REPRESENTATION OF THE DEVELOPED FORMULATION *IN-SITU* GEL

MATERIALS AND METHODS: The drug, polymers, and chemicals such as ofloxacin, HPMC (low and high Viscous), carbopol (4,000,000cPs) as carbopol 934P and benzalkonium chloride respectively were purchased from Sigma Aldrich Chemicals, Bangalore. The chemicals like *β*-cyclodextrin and sodium chloride were acquired from Fischer Chemicals Ltd., Chennai. Some of the chemicals such as calcium chloride, sodium bicarbonate, citric acid were obtained from Fischer Chemicals Ltd., Chennai. Analytical graded solvents were used in this study.

Method of Preparation of Formulation of *In-situ* Gel: *In-situ* gel formulation were made by dissolving HPMC of both low and high viscous cellulose derivatives and carbopol 934P in citro-phosphate buffer at pH 6, and the solution was permissible to hydrate. After hydration, the solution was mixed with chemicals such as sodium chloride, benzalkonium chloride, and *β*-cyclodextrin. Further, the prepared solution was mixed with ofloxacin drug solution and kept aside until the formation of an identical solution. Finally, the obtained identical solution was adjusted to the final volume using citro- phosphate buffer at pH 6. The different formulations were sterilized at 121 °C, 15 psi for 20 min and the samples were evaluated for further analysis [28].

XRD Spectrum of Drug and Excipient: XRD diffractometer analysis was carried out for both drug and polymer samples. XRD spectra were recorded based on 45 kV voltage, 30 mA power and at room temperature. The spectral data was measured using scattering angle (2θ) ranging from 10-90 °.

Fourier Transforms Infra-Red Spectrum (FTIR) Studies: To characterize the functional groups, FT-IR spectra of pure drug, polymers and prepared formulation were compared to each other. The samples were measured from 4000 to 400 cm^{-1} using Perkin Elmer FTIR spectrophotometer.

Differential Scanning Calorimeter (DSC): DSC analysis was performed for the prepared formulation using thermal analysis system (DSC-60, Shimadzu). About 5 mg of the sample was heated at 10 ºC/min at a constant rate in an aluminium pan under a nitrogen atmosphere.

Characterization of Prepared *in-situ* Gel: The presence of any particulate matter was observed by visual appearance. The determination of formulation clarity before and after gelling formations was done through visual examination under light against white or dark backgrounds. pH of the prepared formulation was measured using pH meter. *In-vitro* gelling capacity was ascertained using the visual method. The colored formulation of different concentrations in the range of 20µl - 50µl was added dropwise with the use of a pipette. The stiff gel like structure had been formed during the reactivity between the colored formulation and STF fluid, pH 7.4. The gelling capacity of the prepared formulation was assessed based on the stiffness of a stiff formed gel.

Drug content of the prepared formulation was done by diluting about 1 ml of prepared formulation in 0.1N HCl. Further, the sample was subjected to UV-Visible spectrophotometry at 294 nm [29]. The prepared *in-situ* gel formulations were taken in a sampling tube, and their viscosities were measured at 25 ºC using a Brookfield Viscometer DV2T model. The samples were measured at a rate of 15 rpm for 5 min [30]. The surface tension of prepared formulations was analyzed using a drop weight method. The surface tension was calculated based on Eq. (1).

$$\gamma_L = W_L / W_w \times \gamma_w \quad \text{.....(1)}$$

Where, γ_L - surface tension of liquid sample, W_L- Weight of 1 drop of liquid sample, W_w - Weight of 1 drop of water, γ_w - surface tension of water.

In-vitro release study of the prepared formulations was done using dialysis bag membrane method.

About 3 ml of sample was withdrawn on 1 h interval for about 8 h and substituted with fresh STF fluid. Further, the samples withdrawn for every hour were subjected to UV visible spectrophotometry analysis to measure the cumulative % drug release at 294 nm [31, 32, 33].

Bio-adhesion Measurement: The method was based on the measurements of the shear stress or tensile strength necessary to break the adhesive bond between test formulation and the model membrane. The prepared formulation was squashed in between two model membranes and set on the flexible support in the assemblies for 10 sec after the formation of the adhesive bond, the force required to separate the formed bond was measured and calculated as a bio-adhesive force.

Evaluation of *in-vitro* Release Kinetics: To evaluate *in-vitro* release kinetics, the data obtained were fitted using various kinetic models such as Zero-order reaction, first-order reaction, Higuchi's Kinetics model, Korsmeyer Peppas reaction and Hixson Crowell erosion equation methods.

***In-vitro* Permeation Study:** The freshly obtained goat cornea was obtained from the slaughtered house and mounted on the modified Franz diffusion cell (FDC) apparatus. In the receptor chamber, STF fluid was maintained at 37 ± 1 ºC and on continuous stirring. About 2 ml of test samples were taken at regular time intervals and replaced with fresh STF fluid. Each prepared formulation was allowed to permeate nearly for 0.5-8 h [34]. Further, the samples were subjected to UV-Visible spectrophotometer at 294 nm to analyze the concentration of ofloxacin. The permeated drug concentration at different time intervals was resolutely using the standard curve.

% Permeation = Amount of drug permeated in receptor / Initial amount of drug in donor × 100

Apparent Permeability Coefficient: The freshly obtained goat cornea was employed to assess apparent permeability coefficient using Eq. (2).

$$P_{app} = \Delta Q / \Delta t \times 1/ (A \times CO \times 60) \quad \text{..... (2)}$$

Where, $\Delta Q / \Delta t$ - is the flux across corneal tissue µg/mL, A - is the diffusion area (cm^2); CO - is the initial concentration of drug in donor compartment µg/cm^3 and 60 is taken as a factor to convert min into sec. The graph was plotted between the amount of permeated liquid and time.

***In-vitro* HET CAM Test:** HET-CAM test was done to assay severe ocular irritants and corrosives potential. The test substance was administered on embryonated hen's egg membrane, and their immediate effects were studied. The white Leghorn hen's fertile eggs were stored at a temperature and relative humidity of 37 ± 0.2 ºC and 58 ± 2% respectively for 8 days. The test substances and control were dissolved in 2.5% (w/v) agarose to achieve the final concentration of 25 µg/µL. After incubation, the air cell on the eggs was marked. The marked area on the eggs was sliced off without disturbing the chorioallantoic membrane in eggs. The generated liquids were placed directly on CAM, and the eggs were observed for 300 sec for the indication of any lysis reactions or hemorrhage on CAM. The negative and positive controls used in this study were dissolving 0.9% (w/v) sodium chloride (NaCl) and 1% (w/v) sodium hydroxide (NaOH) in distilled water respectively. Once test substance is applied on CAM, the capillaries and chorioallantoic blood vessels were observed for any irritants/corrosive effects such as hemorrhage, hyperemia, and clotting. Each prepared formulation was tested for any irritant effects at different time intervals.

Acute Eye Irritation / Corrosion Study: The optimized formulation was evaluated *in-vivo* in an animal model, Male New Zealand white rabbits. The protocol used in this study was according to Draize technique and also approved by the Animal Ethical Committee (IAEC/51/SRU/550/2017). The rabbits were housed in cages at 27 ºC and fed with standard diet and water. The left and right eyes of white rabbits were marked as control and test respectively. The control eye has no sample mentioned as blank whereas the test eye was injected with 0.1 ml of formulation. Further, the eyes were observed at different time intervals such as 1, 24, 48, 72 h and 1 week after having an exposure with the optimized formulation. The ocular changes were graded using a scoring system which incorporates score rating and any alteration on conjunctiva, eyelids, cornea, and iris. Besides, the eyes of white rabbits were also observed regularly for redness, swelling, and watering of the eye [35, 36, 37, 38].

Stability Studies: To study the stability of different prepared formulations, the guidelines offered by International Conference on Harmonization guidelines were adopted for this study. The stored *in-situ* gel was maintained at a temperature, relative humidity and refrigerator condition of 25 ± 2 ºC, 60 ± 5% and 5 ± 2 ºC respectively [39, 40]. The stored vials were analyzed for every 30 days to study their gelling effect, appearance, drug content and pH.

Antibacterial Activity: Activity was determined by agar diffusion test employing a cup plate technique. The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC 2 µg/mL) of control and developed formulations containing *in-situ* gel was prepared. A total of 100 ml of nutrient agar media was prepared and sterilized at 15 lb/sq-inch pressure for 20 min in an autoclave; 0.5 ml of microorganism suspension was poured into the above medium which is maintained at a temperature of 50 °C to 58 °C. This will be done in an aseptic condition. Immediately 20 ml of the microbial agar suspension was poured into each petri plate. After solidification of the media, sterile solutions of ofloxacin (standard solutions) and the developed formulations diluted suitably with sterile distilled water (test solutions) were poured into the cup of sterile nutrient agar Petri plates. This was previously seeded with test organisms (*Escherichia coli* and *Bacillus subtilis*). After allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37 °C for 24 h. The Zone of inhibition (ZOI) was measured around each cup and compared with that of control. The entire operation was carried out in a laminar airflow unit. Each formulation solution was tested in triplicate.

Sterility Test: The aerobic, anaerobic bacteria and fungi of the optimized best formulations underwent sterility test using thioglycolate and soya bean casein that was made by dissolving 500 mg of peptic digest of animal tissue. To obtain 100 ml so, as to adjust the pH 7.1 ± 0.2. Then was a drained, centrifuged, and disseminated into flask 7 of 10 ml quantities and was allowed to sterilize at 121 ºC for 20 min. The positive and negative control tests for growth promotion and sterility were performed respectively. The microorganisms like *Bacillus subtilis* (aerobic), *Bacteroides vulgaris* (anaerobic) and *Candida Albicans* (fungi) respectively were used as test organisms. The incubation was done, and growth was observed [41].

RESULTS:

Characterization of the Optimized Formulation: The developed *in-situ* gel formulations were optimized and adopted in the simple procedure and composition was given in **Table 1**.

TABLE 1: OPTIMIZATION OF FORMULATIONS (F1-F5)

Formulation code	Ofloxacin conc. (g)	Carbopol 934P (g)	HPMC (g)			pH	Clarity	Gelling time
			Low viscosity	High viscosity	Combination			
F1	0.3	0.5	0.1	0.1	(0.1 + 0.1)	6.18	C	++
F2	0.3	0.5	0.1	0.2	(0.1 + 0.2)	6.3	C	+++
F3	0.3	0.5	0.1	0.3	(0.1 + 0.3)	6.27	C	+++
F4	0.3	0.5	0.1	0.4	(0.1 + 0.4)	6.28	C	+++
F5	0.3	0.5	0.1	0.5	(0.1 + 0.5)	6.24	C	+++

XRD Spectrum Analysis: XRD spectral peak positions of the drug, excipient and the mixture of excipients were obtained. XRD spectrum of ofloxacin indicated peak position (2θ) at 20.77º, 22.10º, 24.05º, 26.24º, 26.97º, 27.68º and 29.75º. XRD spectrum of carbopol 934P showed peak position (2θ) at 27. 21º, 36.21º, 53.54º and 70.93º. XRD spectrum of the physical mixture of the drug and polymer showed only two sharp peaks at 20.53º and 27.16º.

The remaining peak positions in the physical mixture were not sharp which is mainly due to the peak positions present in drug and polymer were merged and is indicated in **Fig. 1**. XRD peak signals indicated that the prepared formulation was in amorphous state than the pure drug.

FT-IR Spectra: The presence of functional groups was confirmed by using FT IR (Instrument-JASCO 4100). The readings were obtained between 400 cm^{-1} to 4000 cm^{-1} using a KBr pellet technique. FT-IR study was carried out for drug and various excipients. The results showed that various stretching, bending and rocking vibration based on the groups present.

TABLE 2: INTERPRETATION OF FT-IR SPECTRUM OF DRUG, POLYMER AND PHYSICAL MIXTURES

S. no.	Fig. 3	Wave number (cm^{-1})	Vibrations
1.	(a)	3042, 2787, 1709, 1620, 1520, 1454, 1287, 198, 1137, 1048, 1009, 954, 876, 798 and 704	N-H, C=H, C=O and C=N are stretching, N-H, C-H, C-N, C-H & C-C bending and N-H rocking
2.	(b)	2936, 2361, 1703, 1451, 1415, 801 and 618	C-H, C-C, and C-H=O are stretching, C-C, C-H, O-H are bending, and C-H rocking
3.	(c)	3407, 2927, 2386, 1710,1619, 1521, 1399, 1050, 949 and 774	O-H, C-H, C=O-H, C=O, C=O, C-C and C=C are stretching, N-H is bending, and C-H rocking
4.	(d)	3434, 2925, 2362, 1709, 1619, 1399, 1242, 1197, 1050, 961 and 708	O-H, C=O-H, C=O, C-O, C-C, and C=C are stretching, N-H and O-H are bending and N-H rocking
5.	(e)	3429, 2914, 1709, 1620, 1401, 1197, 105, 949 and 707	O-H, C=O-H, C=O, C-O, C-C, and C=C are stretching, N-H and O-H are bending, N-H and C-H are rocking

Fig. 3 showed FT-IR spectra of pure drug, polymer and their physical mixture. The interpretations of FT-IR spectra for the respective compounds were given in **Table 2**.

DSC Studies: The physical state of the drug in the formulation was examined through differential scanning calorimetry (DSC).

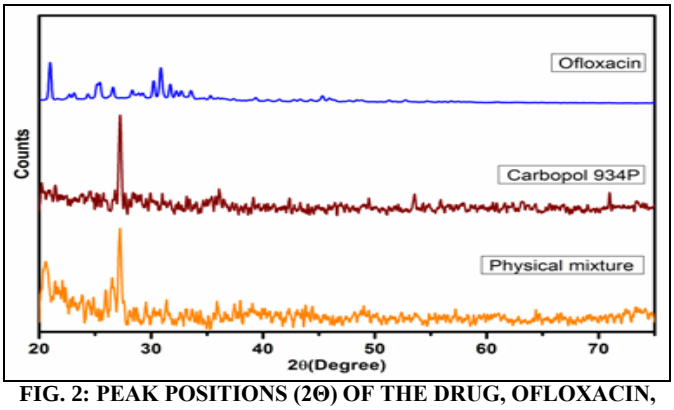

FIG. 2: PEAK POSITIONS (2Θ) OF THE DRUG, OFLOXACIN, POLYMER, CARBOPOL 934P AND THE MIXTURE OF OFLOXACIN AND CARBOPOL 934P

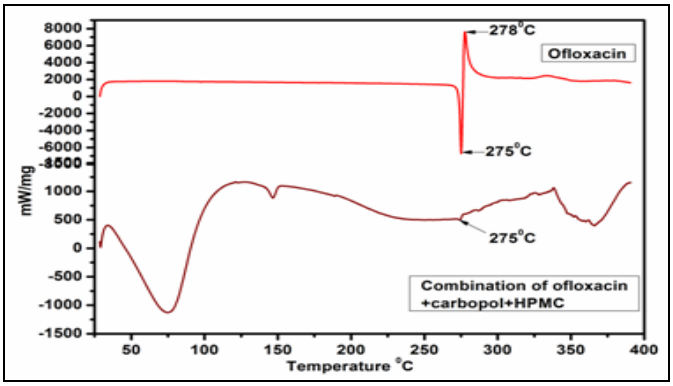

FIG. 4: THERMOGRAM OF (a) PURE DRUG, OFLOXACIN AND (b) PHYSICAL MIXTURE OF OFLOXACIN, CARBOPOL 934P, AND BOTH HIGH AND LOW VISCOUS HPMC

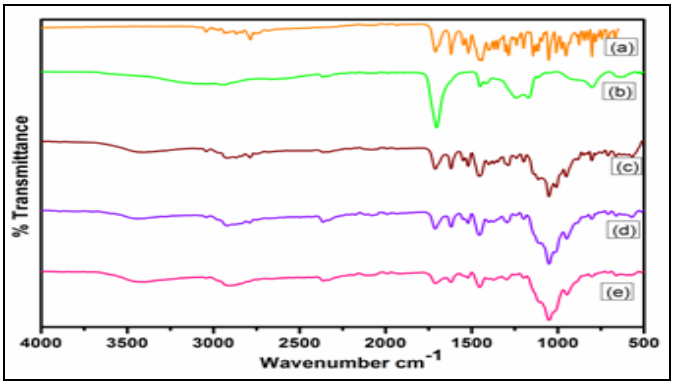

FIG. 3: FT-IR SPECTRA OF (a) OFLOXACIN, (b) CARBOPOL 934P, (c) THE MIXTURE OF OFLOXACIN, LOW VISCOUS HPMC AND CARBOPOL 934P, (d) THE MIXTURE OF OFLOXACIN, HIGH VISCOUS HPMC AND CARBOPOL 934P, AND (e) OFLOXACIN, BOTH HIGH AND LOW VISCOUS HPMC AND CARBOPOL 934P

The endothermic melting transition of pure ofloxacin was observed at 275 ºC and 278 ºC which was very close to its reported melting point. In the thermogram of formulation, the melting endothermic peak appeared near the melting temperature of ofloxacin at 275 ºC and it's slightly weak which was shown in **Fig. 4**. Hence, the thermogram studies revealed the physical nature of drug was found to be stable. DSC studies of the pure drug, polymer and the mixture of formulations showed that they form thermally good stability **Fig. 4**.

Gelling Capacity: The pH, visual appearance, gelation studies of the formulations F1-F5 were

shown in **Fig. 5**. All the developed formulations were found to be slightly pale white dispersion in the pH range of 6.20 to 6.30, and after adding STF fluid pH 7.4 immediate stiff gelation were found for formulations, F1-F5 were represented in **Fig. 5a**, **b**, **c,** and **d**.

Fig. 5 showed the optimized formulations F1-F5. After the addition of 20-50 µl formulation to STF fluid, the transition of sol to gel formation occurs. The formed gel remained nearly for 8-10 h. The indications of gelling time are as follows. (a) (+) Gelation occurred within few minutes, and the formed gel-like structure dissolved rapidly, (b) (++) Gelation remains for 6-8 h and (c) (+++) Gelation will be retained for more than 8-10 h. Based on the gelation time, the gelling capacities of the prepared formulations were analyzed and used for further analysis. The combination of ofloxacin, carbopol 934P, and both high and low viscous HPMC have maximum gelling capacity than other formulations.

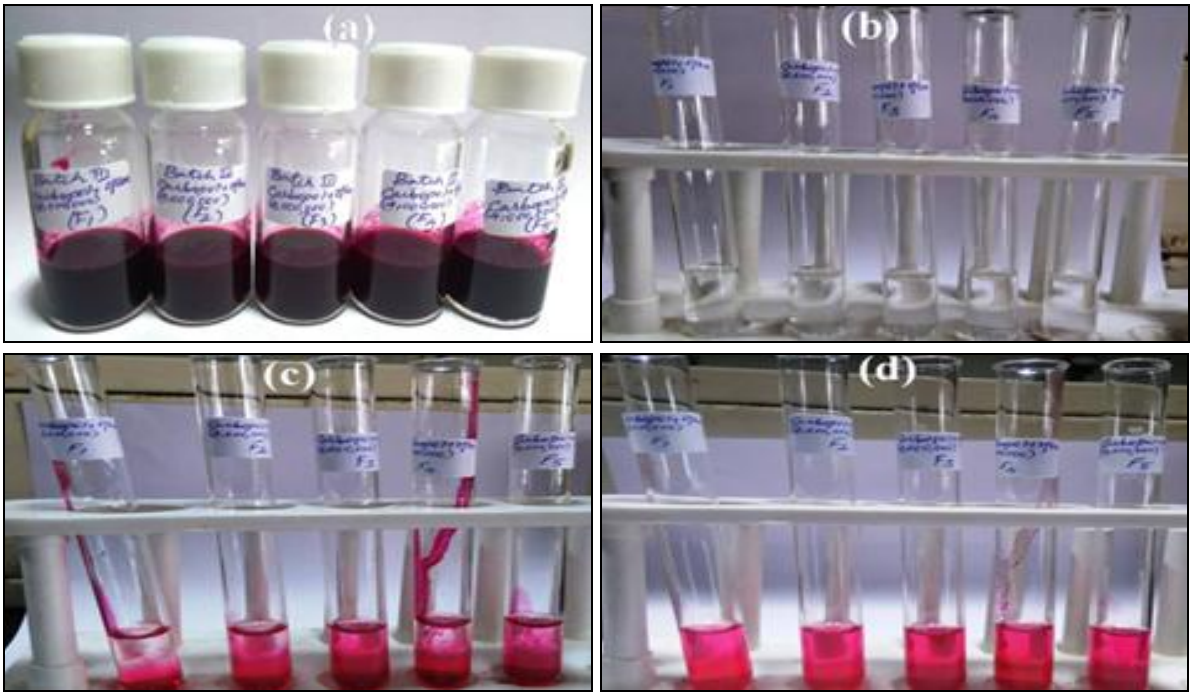

FIG. 5: OPTIMIZED FORMULATIONS F1-F5 (a) COLOURED SOLUTION CONTAINS FORMULATION AND AMARANTH DYE, (b) STF FLUID, pH 7.4, (c) TRANSITION OF SOL TO GEL AND (d) GEL LIKE STRUCTURE FORMATION

Surface Tension: The surface tension of the prepared formulations F1-F5 was analyzed through drop weight method of analysis using Stalagmometer. The surface tension showed trivial differences between different prepared formulations.

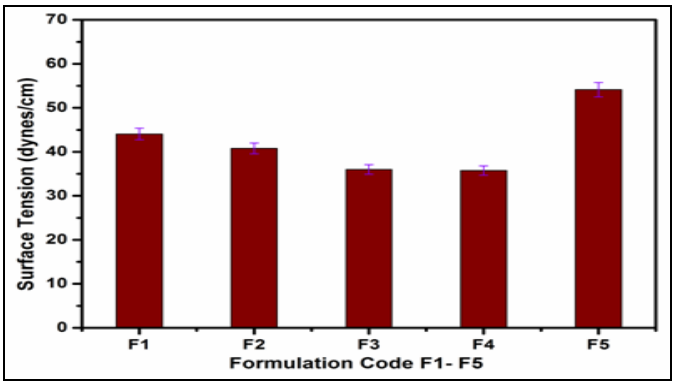

FIG. 6: SURFACE TENSION OF THE PREPARED FORMULATIONS F1-F5

The results revealed that the surface tension of the prepared formulations F1-F5 was in the range of 36.5-54.12 dynes/cm. Among all the formulations, formulation F5 showed the maximum surface tension of 54.12 dynes/cm. Next, to F5, F1 showed the highest value of 44.05 dynes/cm **Fig. 6**.

Evaluation of Mucoadhesive Strength: During *in-situ* preparation, long retention and bio-adhesion are important characteristics to evaluate mucoadhesive strength. Both carbopol and HPMC function as a permeation enhancer and mucoadhesive agent and the study of adhesion force proves the mucoadhesive nature of carbopol and HPMC. Due to abundant hydrogen bond forming groups in carbopol, it is rich in hydrogen property and thus has specific bio-adhesive property. When carbopol is mixed with widely used

bio-adhesive material like HPMC, the synergic effect of bio-adhesive property is more predictable. To improve the physical property of the formulation such as mucoadhesive strength, the mucoadhesive polymer is added and is vital for the formulation to remain at the site of action for the effective amount of time. The results were found to be around (F1 - 3.421 ± 104 dynes/cm^2, F2 - 12.157 ± 127 dynes/cm^2, F3 - 13.019 ± 075 dynes/cm^2, F4 - 16.947 ± 023 dynes/cm^2, F5 - 19.142 ± 035 dynes/cm^2)

Drug Content: The drug content present in the prepared formulations F1-F5 was analyzed using UV visible spectrophotometer at 294 nm. The drug content was in the range of 96.87% to 98.67% for all the developed formulations F1-F5 **Fig. 7**.

Viscosity: The viscosities of all the prepared formulations F1-F5 were measured using Brookfield Viscometer DV2T model and are reported in **Fig. 8**. The results showed that when there are increases in the concentration of polymer, viscosities of the respective formulations were found to be increased. Viscosities of the prepared formulations F1-F5 were in the range of 94.6-102.12cPs. Among all the formulations, formulation F5 showed the maximum viscosity of 102.12cPs **Fig. 8**.

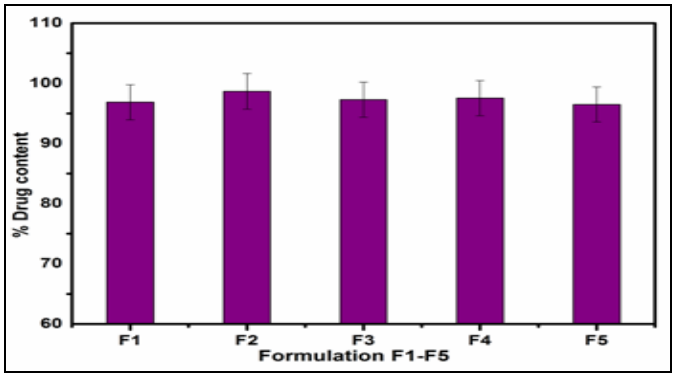

FIG. 7: DRUG CONTENT OF THE PREPARED FORMULATIONS F1-F5

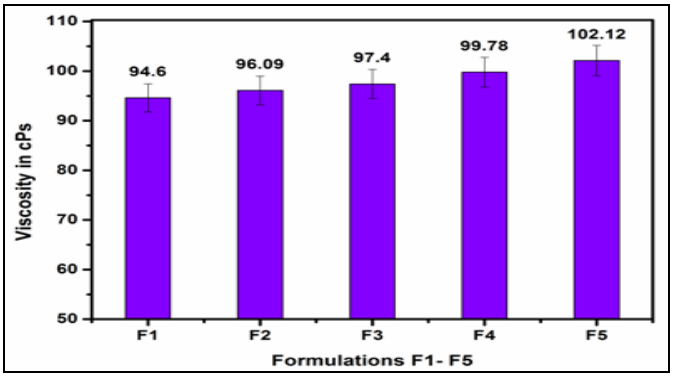

FIG. 8: VISCOSITY OF THE DEVELOPED FORMULATIONS F1-F5

***In-vitro* Release Study:** The result of *in-vitro* drug release of *in-situ* gel formation of all the formulations was shown in **Fig. 9**.

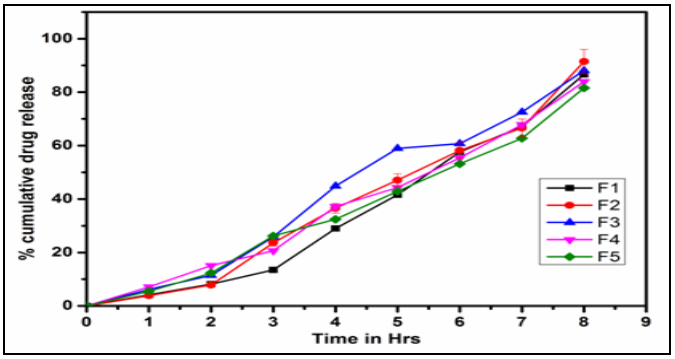

FIG. 9: CUMULATIVE DRUG RELEASE OF DEVELOPED FORMULATIONS F1-F5

The cumulative drug release percent of all formulations are as follows F1-89.38%, F2-95.28%, F3-89.85%, F4-90.03%, and F5-94.32%. The drug release patterns of all the formulations are shown to be increased concerning time. Among all formulations, F2 showed the maximum percent cumulative drug release of 95.28% **Fig. 8**. The pharmacokinetic studies revealed that F2 follows Higuchi kinetic model (R^2 value = 0.9988) and the release mechanism confirms through the diffusion mechanism.

***In-vitro* Transcorneal Approach - Permeation Experiment:** *Ex-vivo* study was done for the optimized formulation, F2 by plotting cumulative percent drug release concerning time and is shown in **Fig. 10**.

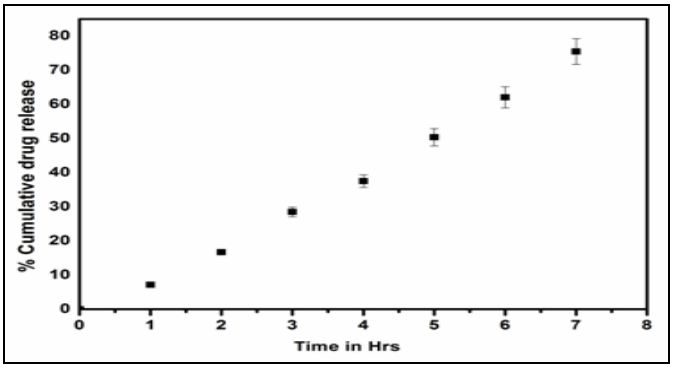

FIG. 10: *EX-VIVO* STUDY OF CUMULATIVE PERCENT DRUG RELEASE FOR THE OPTIMIZED FORMULATION F2

The formulation F2 showed the maximum percent drug release of 95.01% at the end of 7 h **Fig. 10**. The permeability coefficient of the developed formulation F2 was found to be Kp = 0.0065 cm/h.

Hen's Egg Test on Chorioallantoic Membrane (HET-CAM): In substituting to animal experimentation, HET-CAM test is an alternative method to assay corrosives/severe ocular irritants on CAM of the fertilized eggs. This test is helpful to analyze any damages caused by test substance on CAM.

HET-CAM (Hen's Egg Test - Chorioallantoic Membrane) study was carried out for F2 with suitable positive and negative controls and is shown in **Fig. 11**. The result showed that there is no significant damage to CAM membrane during the application of formulation F2 on CAM and no irritations were observed in the conjunctiva **Fig.11** and in **Table 3**.

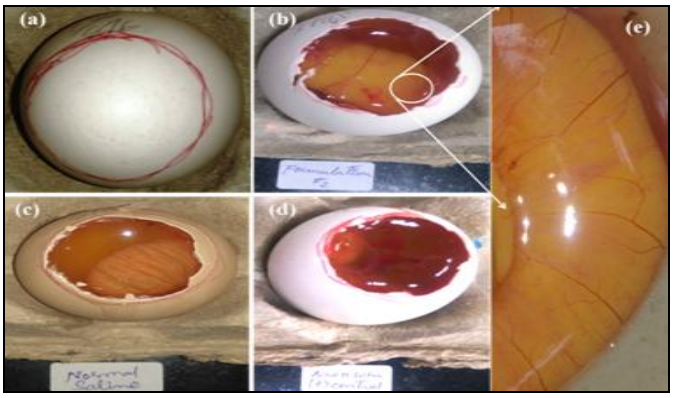

FIG. 11: (a) FERTILIZED EGGS, (b) HET-CAM STUDY USING THE OPTIMIZED FORMULATION F2, (c) NEGATIVE CONTROL USING SODIUM CHLORIDE, (d) POSITIVE CONTROL USING SODIUM HYDROXIDE AND (e) THE CAM MEMBRANE SHOWS THAT THEY HAVE NO DAMAGE OR REDNESS

TABLE 3: SCORE OBTAINED IN HET – CAM TEST

Sample	Score Formulations							
	Time (in Min)							
	0	**5**	**15**	**30**	**60**	**120**	**240**	**300**
Sodium hydroxide and normal Saline as Control (positive and negative)								
Egg 1	0	0.5	0	0	0	0	0	0
Egg 2	0	0.5	0	0	0	0	0	0
Egg 3	0	0.5	0	0	0	0	0	0
Average mean (n=3)	0	0.5	0	0	0	0	0	0
Developed *in-situ* gel formulations (F2)								
Egg 1	0	0	0	0	0	0	0	0
Egg 2	0	0	0	0	0	0	0	0
Egg 3	0	0	0	0	0	0	0	0
Average mean (n=3)	0	0	0	0	0	0	0	0

Score test – 0-3 is normal, 3-5 is a mild irritant, 5-9 is a moderate irritant, and 9-11 is a severe irritant.

Acute Eye Irritation / Corrosion Study: This study was carried out to observe any irritations in the conjunctiva of the eyes and the score was tabulated in **Table 2**. While applying the test substance on the eyes of white rabbits, the eyes were observed regularly for redness, swelling and watering of the eye were examined by the use of light source from a standard ophthalmoscope. From **Table 4**, it is inferred that there is no ocular irritation on white rabbit eyes and hence it is confirmed that the optimized formulation, F2 was found to be non-irritant.

According to Draize score test, the results of the ocular irritation studies indicated that the formulation has no average score, Zero. The excellent ocular tolerance was noted. There was no ocular damage or abnormal clinical signs in cornea, iris, conjunctiva, and chemosis. During initial and confirmatory tests, the animals showed no microscopic lesions in both eyes **Table 4**.

TABLE 4: MEAN SCORE FOR EYE IRRITATION

Initial and Confirmatory Tests			
A. no	**1**	**2**	**3**
Ocular Region	**Control (Left Eye)**		
	Mean Score for 24, 48 and 72 h		
Cornea	0	0	0
Iris	0	0	0
Conjunctivae	0	0	0
Chemosis	0	0	0
Ocular Region	**Treated (Right eye)**		
	Mean Score for 24, 48 and 72 h		
Cornea	0	0	0
Iris	0	0	0
Conjunctivae	0	0	0
Chemosis	0	0	0

#A. no. – Animal Number, Cornea (0 – no ulceration or opacity), Iris (0 - normal), Conjunctiva (0 - normal), Chemosis (0 - normal)

Based on the above observations, the prepared *in-situ* gel formulation was found to be non-irritant to the rabbit white eyes.

Antibacterial Activity: Antimicrobial efficacy study was performed on F2 formulation using gram +ve *Bacillus subtilis* and gram -ve *E. coli* organism. The zone of inhibition of F2 ophthalmic formulation found to be 30.33 and 29.33 mm, respectively, for gram +ve *S. aureus* and gram –ve *E. coli* organism. The results of antimicrobial activity are as shown in **Fig. 12** and **Table 5**. The study indicated ofloxacin retained its antimicrobial activity when formulated as gel-forming ophthalmic system against both selected *B. subtilis* and *E. coli*.

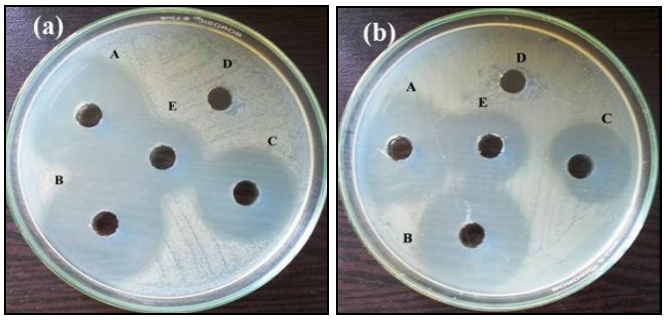

FIG. 12: ANTI-BACTERIAL ACTIVITY OF FORMULATION F2 USING (a) *BACILLUS SUBTILIS* AND (b) *ESCHERICHIA COLI*

TABLE 5: ANTI-BACTERIAL ACTIVITY OF FORMULATION F2

S. no.	Micro-organisms	Zone of Inhibition in mm				Ofloxacin 10 µg
		1000 µg	500 µg	250 µg	Distilled water	
1	*Escherichia coli*	31	30	27	-	20
2	*Bacillus subtilis*	30	30	31	-	20

Sterility Test: To obtain or for best formulation seven days of sterility test was done which brought out that the negative group showed no signs of precipitation which in turn indicated that there were no microorganisms.

TABLE 6: STERILITY TEST FOR *IN-SITU* GEL FORMULATION

Sterility test	Results obtained		
	Negative control	Test sample	Positive control
Test for Aerobic bacteria (*Bacillus subtilis*)	-	-	+
Test for Anaerobic bacteria (*Bacteriodes vulgaris*)	-	-	+
Test for Fungi (*Candida albicans*)	-	-	+

(-) The absence of microorganisms (+) Presence of microorganisms

In the positive control, the microorganisms were introduced in the growth medium showing a milk-white precipitate which was significant. The tubes with F2 formulation *in-situ* gel was clear confirming that they are sterile and free from micro-organisms as shown in **Table 6**.

Stability Study: Stability study was carried out for formulations as per ICH guidelines. Formulations were stored in tightly closed amber colored glass vials sealed with an aluminium foil at room temperature 25ºC ± 2ºC and 40ºC ± 75% RH for 0-6 months. The samples were withdrawn at regular intervals and were vortexing in deionized water for 3-5 min. The stability study was performed, and it is indicated that the prepared formulations were found to be most stable at room temperature than at higher temperatures. About 90% of the drug content was stable as shown in **Table 7**.

Further, the formulation kept for stability studies were undergone the parameters of the test like the appearance, the pH, gelling capacity and drug content were shown good results after 6 months as shown in **Table 8**.

TABLE 7: STABILITY STUDIES OF THE FORMULATIONS F1-F5 AS PER ICH GUIDELINES FOR 0-6 MONTHS

Formulation code	Drug content (%) at 0 month (n=3) ± S.D	Drug content (%) at 3 month (n=3) ± S.D		Drug content (%) at 6 month (n=3) ± S.D	
	25ºC	25ºC	40ºC ± 75%RH	25ºC	40ºC ± 75%RH
F1	96.87 ± 0.25	96.51 ± 0.17	95.88 ± 0.18	96.19 ± 0.16	95.37 ± 0.48
F2	98.67 ± 0.11	97.59 ± 0.15	96.65 ± 0.43	97.17 ± 0.13	96.24 ± 0.37
F3	97.28 ± 0.09	97.09 ± 0.28	96.24 ± 0.16	96.89 ± 0.22	95.74 ± 0.29
F4	97.54 ± 0.41	97.23 ± 0.21	96.15 ± 0.08	96.92 ± 0.36	95.49 ± 0.14
F5	96.49 ± 0.26	96.15 ± 0.30	95.79 ± 0.29	96.01 ± 0.25	95.17 ± 0.26

TABLE 8: AFTER STABILITY DATA STUDIES TEST PARAMETERS WERE CARRIED OUT

Months	Formulation F2 at 25 ± 2ºC/ 5%RH			Formulation F2 at 40ºC ± 2ºC/ 75%RH		
	0	3	6	0	3	6
Appearance	Translucent	translucent	Translucent	Translucent	translucent	translucent
pH	6.05	6.01	6.04	6.09	6.08	6.06
Drug content	98.67 ± 0.11	97.59 ± 0.15	96.65 ± 0.43	97.57 ± 0.21	96.89 ± 0.19	95.51 ± 0.27
Gelling studies	+++	+++	+++	+++	+++	+++

DISCUSSION: The optimization of formulation indicated that the combination of carbopol 934P, HPMC (both low and high viscosity) showed the maximum period of gelling capacity when compared to other formulations as shown in **Table 1**. XRD signals indicated that the formulation was amorphous in condition when compared with the pure individual drug as shown in **Fig. 2**. FTIR spectrum indicated that the drug-polymer showed no interaction with each other. No interaction between the molecules of drug and polymer 934P confirms that the drug and polymer molecule binds with each other by physical bond or Vander Waal's force. So, that the bond can easily break and bind with any other molecule as shown in **Fig. 3** and **Table 2**.

DSC studies of the pure drug, polymer (carbopol 934P) and the mixture of formulations showed that they form thermally good stability **Fig. 4** the gelling capacity of the formulations were carried out and shown in **Fig 5**. The surface tension of the formulations F1-F5 was calculated by the drop weight method by using Stalagmometer. The results revealed that the surface tension of the prepared formulations F1-F5 was found to in the range of 36.5 - 57.14 dynes/cm as shown in **Fig. 6**.

Drug content of the formulations F1-F5 was calculated and analyzed by using UV visible spectrophotometer at 294 nm. The drug content was found to be in the range of 96.87% to 98.67% for all developed formulations F1-F5 as mentioned in **Fig. 7**. Brookfield viscometer DV2T model analyzed the viscosity of all formulations (F1-F5) by using S18 spindle at 15 rpm for the duration of 5 min. Results depicted that as the concentration increased the viscosities of the formulations were also found to be increased. The viscosities of all formulations F1-F5 were 94.6-102.12 cPs plotted in **Fig. 8**.

In-vitro release studies of the developed formulations F1-F5 showed the % drug release of F1- 89.38%, F2-95.28%, F3-89.85%, F4-90.03% and F5-94.32% as in increased order as shown in **Fig. 9**. The pharmacokinetic studies revealed that the optimized formulation F2 obeyed the Higuchi kinetic model (R^2 value = 0.9988) and followed the diffusion mechanism. *Ex-vivo* studies were performed for the optimized formulations (F2) which showed the maximum percent of drug release 95.01% at the end of 7 h as shown in **Fig. 10**. HET-CAM studies were performed for the optimized formulations F2, and it doesn't show any significance redness or damage to this CAM membrane, and also no irritations were observed as shown in **Fig. 11** and scoring test as mentioned in **Table 3**.

The ocular irritation studies revealed that no irritation in the rabbit's eye. Hence the developed formulation *in-situ* gel was found to be non-irritant. The results of the ocular irritation studies indicated that the formulation has no average score, *i.e*. (Zero) according to the Draize score test. Excellent ocular tolerance was noted; no ocular damage or abnormal clinical signs in the cornea, iris, conjunctiva, and chemosis were visible. In initial and confirmatory test animals showed no microscopic lesions were observed in both eyes from control (left) and treated (right) as shown in **Table 4**. Based on the above observations under tested conclusion, the *in-situ* gel formulation was found to be non-irritant to the eye.

The antibacterial activity shows the zone of inhibition for the formulation F2 good inhibition activity as shown in **Fig. 12** and **Table 5**. The tubes with F2 formulation *in-situ* gel was clear confirming that they are sterile and free from micro-organisms as shown in **Table 6**.

Stability studies were carried out for the developed formulations (F1-F5) as per the ICH guidelines for 0-6 months. The drug content of the formulations F1-F5 was calculated by UV-visible spectro-photometer at 294 nm for 0 months, 3rd month and 6th month at room temperature (25ºC) and 40 ºC ± 75% relative humidity as shown in **Table 7** and **8**.

The formulations were found to be stable in both the temperature (25ºC ± 2ºC and 40ºC ± 75% Rh) and were above T90% as per the ICH guidelines.

CONCLUSION: From the above study work, it was accomplished that the developed *in-situ* gel formulations system is a feasible choice when compared to conventional drops by its ability to enhance bioavailability through its long precorneal residence time, ability to sustain drug release.

Also, it is important in case of administration affords, decreased frequency of administration and resulting in better patient acceptance. Our wish is that this research will improve the quality of life likely, there by developing professional functioning by way of fall in the frequency of administration of dosage form, biodegradable polymers provide prolong action and enhanced bioavailability could be achieved by a single instillation of eye drops. Hence, to conclude it is one of the future novel delivery system in the field of ocular therapeutics for the effective treatment of bacterial infections.

ACKNOWLEDGEMENT: The authors would like to thank Sri Ramachandra Medical College and Research Institute, Porur for providing the lab facilities.

CONFLICT OF INTEREST: There is no conflict of interest for all authors.

REFERENCES:

1. Baum J: Infections of the eye. Clinical Infectious Diseases 1995; 21: 479-86.
2. Blake FA, Siegert J, Wedl J, Gbara A and Schmelzle R: The acute orbit: etiology, diagnosis, and therapy. Journal of Oral and Maxillofacial Surgery 2006; 64: 87-93.
3. Callegan MC, Engelbert M, Parke DW 2nd, Jett BD and Gilmore MS: Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. Clinical Microbiology Reviews 2002; 15: 111-24.
4. Thomas PA: Current perspectives on ophthalmic mycoses. Clinical Microbiology Reviews 2003; 16: 730-97.
5. Todokoro D, Yamada N, Fukuchi M and Kishi S: Topical voriconazole therapy of *Purpureocillium lilacinum* keratitis that occurred in disposable soft contact lens wearers. International Ophthalmology 2014; 34: 1159-63.
6. Lopez MR, Perez MA and Fuster FC: Eye infections caused by *Purpureocillium lilacinum*: A case report and literature review. Revista Iberoamericana de Micología 2015; 32: 111-4.
7. Saghrouni F, Saidi W, Ben SZ, Gheith S, Ben SM, Ranque S and Denguezli M: Cutaneous hyalohyphomycosis caused by *Purpureocillium lilacinum* in an immunocompetent patient: case report and review. Medical Mycology 2013; 51: 664-8.
8. Khan Z, Ahmad S, Al-Ghimlas F, Al-Mutairi S, Joseph L, Chandy R, Sutton DA and Guarro J: *Purpureocillium lilacinum* as a cause of cavitary pulmonary disease: a new clinical presentation and observations on atypical morphologic characteristics of the isolate. Journal of Clinical Microbiology 2012; 50: 1800-4.
9. Luangsa AJ, Houbraken J, van DT, Hong SB, Borman AM, Hywel-Jones NL and Samson RA: *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiology Letters 2011; 321: 141-9.
10. Samra W, David CR, David SG, Jeffrey ML, John AS and Robert R: New patterns of infecting organisms in late bleb-related endophthalmitis: A ten year review. Journal of Medical and Veterinary Mycology 1976; 14: 155-70.
11. Kredics L, Varga J, Kocsube S, Doczi I, Samson RA, Rajaraman R, Narendran V, Bhaskar M, Vagvolgyi C and Manikandan P: Case of keratitis caused by *A. tamarii*. Journal of Clinical Microbiology 2007; 45: 3464-7.
12. Chang DC, Grant GB, O'Donnell K, Wannemuehler KA, Noble-Wang J, Rao CY, Jacobson LM, Crowell CS, Sneed RS, Lewis FM, Schaffzin JK, Kainer MA, Genese CA, Alfonso EC, Jones DB, Srinivasan A, Fridkin SK and Park BJ: *Fusarium keratitis* investigation team. Multistate outbreak of *Fusarium keratitis* associated with use of a contact lens solution. Journal of the American Medical Association 2006, 23(296): 953-63.
13. Bullock JD: Root cause analysis of the *Fusarium keratitis* epidemic of 2004-2006 and prescriptions for preventing future epidemics. Transactions of the American Ophthalmological Society 2009; 107: 194-204.
14. Gaujoux T, Chatel MA, Chaumeil C, Laroche L and Borderie VM: Outbreak of contact lens-related *Fusarium keratitis* in France. Cornea 2008; 27: 1018-21.
15. Ma SK, So K, Chung PH, Tsang HF and Chuang SK: A multi-country outbreak of fungal keratitis associated with a brand of contact lens solution: the Hong Kong experience. International Journal of Infectious Diseases 2009; 13: 443-8.
16. Tu EY and Joslin CE: Recent outbreaks of atypical contact lens-related keratitis: what have we learned? American Journal of Ophthalmology 2010; 150: 602-608.
17. Solky AC, Laver NM, Williams J and Fraire A: *Prototheca wickerhamii* infection of a corneal graft. Cornea 2011; 30: 1173-5.
18. Indu P, Kaur AG, Anil KS and Deeepika A: Vesicular systems in ocular drug delivery an overview. International Journal of Pharmaceutics 2004; 269: 1-14.
19. Le Bourlais CL, Acar L, Zia H, Sado PA Needham T and Leverge R: Ophthalmic drug delivery systems-recent advances. Progress in Retinal and Eye Research 1998; 17: 33-58.
20. Le Bourlais C, Chewanne F, Ropert P, Bretagne G, Losa, Acar L, Zia H, Sado PA, Needham TE and Leverge R: Release kinetics of liposome encapsulated ganciclovir after intravitreal injection in rabbits. Journal of Micro-encapsulation 1996; 13: 473-480.
21. Lee VHL and Robinson JR: Topical ocular drug delivery: recent developments and future challenges. Journal of Ocular Pharmacology and Therapeutics 1986; 2: 67-108.
22. Lin H and Sung KC: Carbopol/pluronic phase change solutions for ophthalmic drug delivery. Journal of Controlled Release 2000; 69: 379-388.
23. Greaves JL, Wilson CG and Brimingham AT: Assessment of the precorneal residence of an ophthalmic ointment in healthy subjects. Journal of Clinical Pharmacology 1993; 35: 188-192.

24. Nagarsenker MS, Londh VY and Nadkarni GD: Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. International Journal of Pharmaceutics 1999; 190: 63-71.
25. Alany RG, Rades T, Nicoll J, Tucker IG and Davies NM: W/O microemulsions for ocular delivery: evaluation of ocular irritation and precorneal retention. Journal of Controlled Release 2006; 315: 12-17.
26. Wei G, Xu H, Ding PT, Li SMa and Zheng JM: Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies. Journal of Controlled Release 2002; 83: 65-74.
27. Liu Z, Li J, Nie S, Liu H, Ding P and Pan W: Study of an alginate/HPMC-based *in-situ* gelling ophthalmic delivery system for gatifloxacin. International Journal of Pharmaceutics 2006; 315: 12-17.
28. Gupta H, Velpandian T and Jain S: Ion and pH-activated novel *in-situ* gel system for sustained ocular drug delivery. Journal of Drug Targeting 2010; 18: 499-505.
29. Jovita K, Kanchan S, Manisha P, Kymonil KM and Saraf SA. Formulation and Characterization of a novel pH-triggered in-situ gelling ocular system containing Gatifloxacin. International Current Pharmaceutical Journal 2012; 1:43-49.
30. Abraham S, Furtado S, Bharath S, Basavaraj BV, Deveswaran R and Madhavan V: Sustained ophthalmic delivery of ofloxacin from an ion-activated *in-situ* gelling system. Pakistan Journal of Pharmaceutical Sciences 2009; 22: 175-179.
31. Patel PR, Dadhani B, Ladani R, Baria AH and Patel J: Formulation, evaluation and optimization of stomach-specific *in-situ* gel of clarithromycin and metronidazole benzoate. International Journal of Drug Delivery 2010; 2: 141-153.
32. Lin HR and Sung KC: Carbopol/ pluronic phase change solutions for ophthalmic drug delivery. Journal of Controlled Release 2000; 69: 379-88.
33. Desai SD and Blanchard J: *In-vitro* evaluation of Pluronic F127 based controlled release ocular delivery systems for pilocarpine. Jou of Pharmaceutical Sci 1998; 87: 226-30.
34. Mohan EC, Kandukuri JM and Allenki V: Preparation and evaluation of *in-situ* gels for ocular drug delivery. Journal of Pharmacy Research 2009; 2: 1089-1094.
35. Malhotra M and Majumdar DK: *In-vitro* transcorneal permeation of ketorolac tromethamine from buffered and unbuffered aqueous ocular drops. Indian Journal of Experimental Biology 1997; 35: 941-947.
36. Shastri DH, Prajapati ST and Patel LD: Thermo-reversible mucoadhesive ophthalmic in-situ hydrogel: Design and optimization using a combination of polymers. Acta Pharmaceutica 2010; 60: 349-360.
37. Indrajeet DG and Vrushali SK: *In-situ* gelling system of ofloxacin *in-vivo* precorneal drainage study. Der Pharmacia Lettre 2012; 4: 1541-1546.
38. Jike S, Hongsheng B, Xiaofeng X, Junguo G, Xingrong W and Damei L: Preparation and evaluation of sinomenine hydrochloride *in-situ* gel for uveitis treatment. International Immunopharmacology 2013; 17:99–107.
39. Patel P, Javia A and Sheth AK: Formulation and evaluation of sustained release chlorhexidine *in situ* gel for periodontitis. Die Pharmazie 2013; 4:465-78.
40. Sonjoy M, Manjunath KM, Prabhu shankar GL and Geetha MS: Formulation and evaluation of an in-situ gel forming ophthalmic formulation of moxifloxacin hydrochloride. Int Journal of Pharmaceutical Investigation 2012; 2: 78-82.
41. Shastri DH, Prajapati ST and Patel LD: Design and development of thermo-reversible ophthalmic in-situ hydrogel of moxifloxacin HCl. Current Drug Delivery 2010; 7: 238-43.

How to cite this article:
Anbarasan B, Kumar RS, Thanka J, Ramaprabhu S and Shanmuganathan S: Preparation and characterization of pH based carbopol 934P *in-situ* hydrogels for the treatment of harmful bacterial infections. Int J Pharm Sci & Res 2019; 10(1): 232-44. doi: 10.13040/IJPSR.0975-8232.10(1).232-44.