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FORMULATION AND CHARACTERIZATION OF *IN-SITU* GEL FOR OPHTHALMIC FORMULATION CONTAINING CIPROFLOXACIN HYDROCHLORIDE

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Keywords:

In-situ gel, Ocular/ophthalmic, Ciprofloxacin hydrochloride, Carbopol-940, HPMC, *In-vitro* release, Antimicrobial efficacy

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ABSTRACT: Ophthalmic delivery for eye drops which currently cover to 90% of available ocular dosage forms are ideal for the treatment of eye diseases but having limitations of poor therapeutic response and low bioavailability. **Objectives:** The objectives of present research was to develop of formulation and characterize sustained release *in-situ* ocular gels containing ciprofloxacin hydrochloride using pH induced gelling polymers for increase drug retention time on eye surface which improved therapeutic response and patient compliance. **Methods:** *In-situ* gel formulations prepared by dispersion method using Carbopol-940 alone or in combination with hydroxypropyl methylcellulose (HPMC K4M). Formulations were evaluated for appearance, pH, viscosity, gelling capacity, and drug content and in vitro drug release. The optimized formulation was assessed for sterility and antimicrobial efficacy using disk diffusion technique. **Results**: The appearance of in situ gels were clear and free flowing in nature however, a viscous clear solution with flow was normal as solution state after gel conversion flow behavior was change. All formulations consisting 0.01%-0.09 %w/v of Carbopol-940 and 0.05% w/v HPMC K4M. PH of all the formulations was within the range of 5.8 to 6.9 before gelling condition. In situ gels with Carbopol-940 demonstrated higher viscosity compared to Carbopol-934 and drug release was sustained over a period of 8 hr. The selected formulation containing 0.03% w/v Carbopol-940 and 0.05% w/v HPMC K4M passed sterility test and demonstrated similar antimicrobial efficiency compared to commercial product. **Conclusion:** Carbopol-940 and HPMC K4-M based in situ gels have potential to improve patient's compliance by reducing the dosing frequency and can be an alternative to commercial product.

INTRODUCTION: Ocular drug delivery is one of the most interesting and challenging areas of pharmaceutical research. Topical delivery of eye drops which currently accounts for 90% of accessible ocular formulations is an ideal treatment for ocular diseases especially when the drug needs to produce a localized action.

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However, topical delivery is not without problems such as the poor bio availability and therapeutic response.

These challenges are attributable to the rapid pre corneal elimination due to tear secretion, non-productive absorption due to the bio logical barrier for drug penetration, absorption into the gastrointestinal tract due to drainage through nasal lacrimal duct and poor patient's compliance due to increasing number of instillations and difficulty in self-administration [1-4]. Thus, the concept of polymeric in situ gel forming system was introduced to alleviate these problems. *In-situ* gels are free flowing solutions at room temperature that

undergo phase transition from solution-gel (sol-gel) as a result of exposure to physiological temperature, pH or ionic compositions of lacrimal fluid [5-7]. The gel formed in the eye effectively prevents the rapid drainage of instilled drug from ocular site, improves the retention time of dosage form at the site of administration and sustains the drug release for a prolonged period of time. It aids to reduce the dosing frequency and to improve the therapeutic efficiency of drug. Furthermore, systemic side effects would also be reduced due to less systemic absorption. Therefore, it enhances patient's compliance and convenience [5].

Carbopol-940 is a water-soluble pH dependent in situ polymer. The formulations comprising of Carbopol-940 polymer remained as solution at acidic pH and forms a low viscosity gel when pH raised to alkaline. The pH difference between the formulations containing Carbopol and human tear fluid makes the sol-gel transition occurs almost instantly. Besides, Carbopol-940 has an excellent muco-adhesive property. Therefore, the polymer is responsible to increase the contact time of a drug in the eye by adhering to the ocular surface and thereby release the drug in a controlled fashion. However, acidic nature of Carbopol-940 may cause damage to surface of eye before being neutralized by the lacrimal fluid. Thus, HPMC, a viscosity enhancing polymer is usually added to Carbopol-940 contained formulations to overcome this problem, which resulted in pH induced polymeric mixture (Carbopol-940 and HPMC K4M). This polymeric mixture is in liquid state at its native formulated pH 4 to 6 at room temperature but rapid transition into gel phase occurs at the pH of tear fluid (pH 7.4) [3, 8-10].

Now days many of the ocular in situ forming gels have been investigated with a combination of Carbopol and cellulose derivatives [8, 11-13]. In addition, these two polymers are already listed in the FDA's Inactive Ingredient Guide (IIG) and widely used commercially for various drug applications including topical ophthalmic solutions [14-16]. Therefore, these polymers are considered to be safe to use. Ciprofloxacin hydrochloride is a pale yellow, crystalline powder which contains Fluoroquinolone group. Ciprofloxacin hydro chloride is used as an antibacterial agent in the treatment of corneal ulcers caused by susceptible strains of bacteria, including *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Strepto coccus* and *conjunctivitis,* bacterial (treatment*)* of conjunctivitis caused by *Haemophilus influenzae, S. aureus, S. epidermidis, S. pneumoniae,* and *Streptococcus* [17-18].

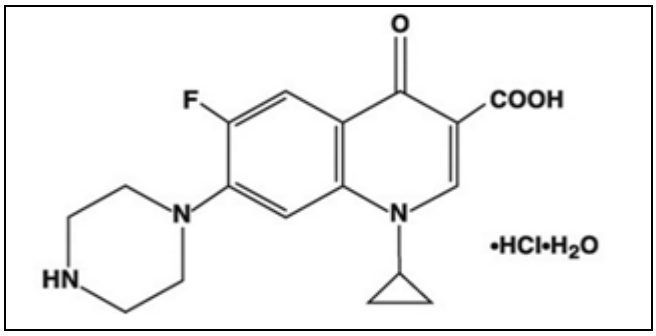


FIG. 1: STRUCTURE OF CIPROFLOXACIN HYDROCHLORIDE

Ciprofloxacin's bactericidal action is due to inhibition of DNA gyrase enzyme in bacteria, which is needed for the synthesis of bacterial DNA. It inhibits this enzyme hence will not allow multiplication of bacterial cell.

In situ gelling systems consist of polymer that exhibit sol-to-gel phase transitions in the cul-de-sac which improves patient compliance due to change in specific physio-chemical parameters like pH, temperature and ionic strength in the environment [19]. The sol-to-gel phase transition on the eye surface depending on the different methods employed which consist of thermo-sensitive, ion-activated and electric-sensitive, magnetic field-sensitive, ultrasonic-sensitive and chemical material-sensitive varieties. There are some type *In-situ* gelling system which as follows.

1. PH-triggered system (e.g., cellulose acetate hydrogen phthalate latex).
2. Temperature-dependent system (e**.**g. pluronics and tetronics).
3. Ion-activated system (eg. gelrite) [20-21].

The present work is based on the

pH Triggered System: *In-situ* gel-based drug delivery systems consist of active pharmaceutical ingredients, polymer, co-polymer and excipients. So, Carbopol-940, an ophthalmic gel forming

muco-adhesive polymer was chosen, as the polymer and Hydroxy Propyl Methyl Cellulose (HPMC) as copolymer.

Gel formation due to produce a specific temperature by virtue of its interaction with present in lachrymal fluid at (pH 7.4).

Hydroxy Propyl Methyl Cellulose (HPMC K4M) is incorporated as a viscosity enhancer to further aid in accomplishment of sustained drug delivery. HPMC is semisynthetic, inert, viscoelastic polymer which is non-ionic nontoxic, a good carrier for pharmaceutical application which exhibits high swelling capacity [22-24, 25].

MATERIAL AND METHOD: Ciprofloxacin hydrochloride was gift sample from Akums Drugs & Pharmaceuticals Ltd., SIDCUL, Ranipur, Haridwar, Uttarakhand by Akhilesh Sahooand Carbopol-940 & Hydroxy propyl methyl cellulose (HPMC K4M) were obtained from Akums Drugs & Pharmaceuticals Ltd., SIDCUL, Ranipur, Haridwar, Uttarakhand by Akhilesh Sahoo All other chemicals and reagents were of analytical grade procured from CDH chemicals.

Pre-Formulation and Characterization Studies: Pre-formulation scrutiny of the drug was executed to validate its character and legitimacy in addition to verifying that there are no critical restrictions in the development of designed formulation of the drug in association with selected in-situ gelling system. The studies supplementally confirm the composite identity and authenticity moreover displayed no relevant basic interaction of the drug with the chosen nano-carriers. The UV and FTIR spectra were found equivalent [26].

Identification of Drug: The sample of Ciprofloxacin HCl was Akums Drugs & Pharmaceuticals Ltd. It was identified and characterized as shown in the pre-formulation studies. The identification of the drug was carried out in the laboratory by observing its physical appearance, melting point determination, UV spectroscopy, IR spectroscopy [27, 28].

Physical Appearance and Odour: Physical appearance and melting point of the drug sample under investigation was found to be concordant with the reported values. Drug was found to be white in colour and odourless. Data see in **Table 1** [17-18].

Melting point of Ciprofloxacin HCl: The melting point of Ciprofloxacin HCl was studied in the mid of 290-300°C which was found to be within the limits of the declared scale i.e 273°C indicated the authenticity of drug. Data see in **Table 2** [29].

FT-IR Spectroscopy of Ciprofloxacin HCl: IR spectrum of Ciprofloxacin HCl, peaks at > 3000 cm^{-1} indicated that there is a typical carboxyl-structure. Many prominent peaks can be characterized for pure Ciprofloxacin HCl, such as the peaks at 1006 and 1268 cm^{-1} for cyclic ether, the peak at 1567 cm^{-1} for primary Amine, and the peak at 1716 cm^{-1} for conjugated ester. IR spectra match with the reported standard literature that confirms the identity and purity of the selected drug. Data see in **Table 3** and **Fig. 2** [29-30].

Partition Coefficient Determination: The partitioning behaviour of drug molecules plays an important role in lipid barrier transfer and loading in the lipophilic carrier systems. Partition coefficient estimation of the drug was performed in two different systems i.c distilled water (pH 7.4) and n-octanol. Thus found 20.1 and 2.15 mg/ml respectively indicates completely hydrophilic behavior of drug respectively. The partition coefficient was higher in case of distilled water. Data see in **Table 5** [30].

Identification of Ciprofloxacin by XRD: X-ray powder diffraction (XRD) is an analytical tool primarily used for phase identification of an amorphous material. The x-rays (Cu K-alpha) were produced using a sealed tube and the samples were scanned over a 20 range of 2°-50° with a scanning rate of 5/minute. The x-rays were detected using a fast-counting detector based on Silicon strip technology (Bruker Lynx Eye detector) Ciprofloxacin HCl, was found amorphous in nature as its XRD peaks were obtained at 20 values of at the range between 30.12-35.36 XRD pattern of Ciprofloxacin HCl revealed information of amorphous in nature. Data see in **Fig. 3** [31-35].

Solubility Study of the Ciprofloxacin HCl: The solubility determination of Ciprofloxacin HCl was performed in different aqueous and organic solvent, Ciprofloxacin HCl was completely soluble in

distilled water acid and phosphate buffer, poorly soluble in Methanol**.** Data see in **Table 4** [36].

Calibration Curves of Ciprofloxacin HCl: The UV spectrum of Ciprofloxacin HCl was obtained by scanning 10µg/ml solution in water & PBS 7.4 pH between 200-400mm using Shimadzu-1700 UV Spectrophotometer. The Amax was found to be 273 nm in water/ PBS 7.4 pH. Data see in **Table 6** & **7** and **Fig. 4** & **5** [37].

Identification of Polymers:

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis of Carbopol-940 and HPMC K4M: FTIR spectra of Carbopol-940 and HPMC K4M showed the presence of different type of O-H stretching vibrations from oxygen functionalities and it was confirmed at 3263 cm^{-1}, whereas C-O stretching vibrations was observed at 1615 cm^{-1}. C-H stretching of CH3 group was observed at 2923 cm^{-1} and C-OH stretching vibrations were present as skeletal vibrations from un-oxidized graphitic molecule at 1383 cm^{-1} which was appeared sharp and may be due to traces of carboxyl groups in its chemical structure. However, stretching vibrations of C-0 at 1059 cm^{-1} was observed. Data see in table 3.8 and figure 3.6 of carbopol-940 and data see in **Table 9** and **Fig. 7.**

Characterization and Evaluation of Ciprofloxacin HCl- Loaded *In-situ* Gel:

Drug-polymer Interaction Studies: FTIR can be used to investigate and predict any physiochemical interaction between different excipients. IR spectra matching approach was used for detection of any possible chemical interaction between the drug and polymers. A physical mixture of drug, polymer and other excipients was prepared and mixed with suitable quantity. It was scanned from 4000 to 400 cm-1 in a FTIR spectrophotometer. FTIR studies revealed that there is no chemical interaction between the drug and *in-situ* gel polymers. Data see in **Table 12** and **Fig. 8, 9** & **10** [38-44].

Gelling Capacity: The gelling capacity of formulation was analysed by placing a drop of the formulation in a test tube which containing 2 ml of freshly prepared STF (7.4 pH) solution then it was visually observed for gelling time. Coding for the gelling capacity described in the table. According that B-5 F3 shows immediate gelation and for extended period all data was given in **Table 13** & **Fig. 11** [45].

Content of Drug [29]: The preparation of *in- situ* gel loaded with drug was carried out by the simple physi-sorption method. Ciprofloxacin HCl and *in-situ* gel polymer were mixed in the ratio of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 in the all-ratios drug content capacity of *in-situ* gel was calculated. After perceiving the concentration of the unbound drug in the supernatant with the assistance of UV- Vis spectrophotometry, outcomes predict that loading capacity of in-situ gel system for the ciprofloxacin HCl is as high as 1.82 mg/ml alongside the early ciprofloxacin HCl concentration. Data see in **Table 14.**

Clarity: The formulations B5 (F1-F6) were prepared by using various concentrations of Carbopol-940 along with HPMC K4M in different ratios. All the formulations prepared were clear without any turbidity and suspended particles or impurities. All data was show in the **Table 16** [45-46].

pH: The pH of in situ gel solution was found to be around (6.4–6.8) data see in the table. For all the formulations. The formulation batch-5 (F1-F6) post gelation pH were (7.4-8.0) which is an acceptable range for ophthalmic preparations. Data see in **Table 15** [47-49].

***In-vitro* Drug Release Study of Formulation (B5 F3):** Ciprofloxacin HCl releases slowly from *in-situ* gelling system at physiological pH 7.4 and only 80.17 % of the total bound drug was released from In-situ gel in 12 hours. However, in basic conditions, about of the drug were released respectively after 12 hours, which is much higher than that released at pH 7.4. As we discussed in the previous section, the hydrogen bonding interaction between ciprofloxacin HCl and gel polymer is strongest at the neutral pH, resulting in an efficient release. On the other hand, the higher amount of ciprofloxacin HCl released at pH 7-9 and 3-5 may be due to the partial dissociation of hydrogen bonding interaction. The reason for the high percent release amount of drug from the gel at pH 7.4 compared to pH 3- 5 may be due to the stronger hydrogen bonding interaction formed under basic conditions than that under acidic conditions [50].

The *in-vitro* release kinetics data of Ciprofloxacin HCl from the In-situ gelling system were fitted into suitable models, in order to understand the release mechanism see in the table. Ciprofloxacin HCl release from in-situ gel showed a better fit with the Zero order release and Higuchi model (R2 0.984, 0.964, and 0.981 at pH 3, 7.4, and 9 respectively) see in the table. These results suggest that the Ciprofloxacin HCl drug release from the in-situ gel is through diffusion. Furthermore, it occurs due to the diffusion of the dissolution medium into the gel. Dissolution medium solubilized the drug and releases it moderately. Data see in **Table 17** & **Fig. 12** and mathematical release order model Data see in **Table 18, 19, 20, 21** and **22** and **13, 14, 15, 3.16** & **17** [51].

Anti-microbial Activity: Anti-microbial activity of ciprofloxacin HCl loaded *in-situ* gel with free drug, Blank gel (polymeric solution), STF Solution Assessment of antimicrobial action of formulated ciprofloxacin HCl loaded In-situ gelling system was performed against microbial infection as a standard was used as a test organism. This test was conduct by Agar well diffusion method was used to test the antimicrobial efficacy of prepared formulations. The materials required for this test was muller hinton agar medium, nutrient broath and test organism. *Staphylococcus aureus* (ATCC 25923) were used as the test organisms to study the antimicrobial efficiency. Data see in **Table 1**

Muller Hintonagar Medium (1 L): The medium was formulated by dissolving 33.8 g of the commercially available MullerHinton agar medium in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs. pressure at 121°C for 15 min.

Nutrient Broth (1 L): One liter of nutrient broth was formulated by dissolving 13 g of commercially available nutrient medium in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs. pressure at 121° C for 15 min. Then Petri-disc was used for containing 20 ml Muller Hinton agar medium were seeded with bacterial culture of *Staphylococcus aureus*. Wells of approximately 15 mm was bored using a well cutter and different concentration of sample then it was incubated at 37°C for 24 h. It has been found that the zone of inhibition obtained by the ciprofloxacin HCl loaded In-situ gel after 48 ± 2 (hr.) weeks is higher (6.89 mm) as compared to marketed formulation of simple Ciprofloxacin HCl (free drug) solution (3.12 mm) all data see in table and figure. Data see in Table 25 & 26 and Fig. 19, 20, 21 [52].

Sterility: All formulation were conducting sterilization with the help of autoclave on the 121 °C for 15 minutes. The found a clear and transparent solution after sterilization process. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. All the test glass wares and syringes are sterilized by using hot air oven. The test liquid was aseptically transferred to soya bean - casein digest medium. The inoculated media were incubated not less than 7 days after check to formulation growth in microbial contamination or absence all data was show in table. Data see in **Table 30** and **Fig. 27** [53].

Rheological Studies: The viscosity was directly dependent on the polymeric content of the formulations. Addition of HPMC led to increase in the viscosity of formulations and exhibited more pseudo-plasticity (F1-F6) as compared to F6 prepared as higher concentration of carbopol-940 and HPMC K4M. F3 was optimized formulations which gives good results. The formulation which is in the solution form should have an optimum viscosity that will allow for easy instillation into the eye, which would undergo a rapid sol to gel transition. These results further confirmed that Ciprofloxacin HCl loaded *in-situ* gel. Produces strong antimicrobial effect due to the combined effect of drug loaded In-situ gel along with its superior release profile as compared to other formulations. Data see in **Table 27** & **28** and **Fig. 22** & **23** [51].

***Ex-vivo* Drug Permeation Studies:** The drug permeation was studied by bi-chambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium (freshly prepared) STF pH 7.4. The diffusion medium was filled in the receptor compartment and it was stirred at 100 rpm

at 37 ± 1º C. One end of the diffusion tube was covered by a cellophane membrane. The 2 ml formulation was spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium present in receptor compartment. The drug samples were withdrawn at the interval of 1 h for the period of 8 h from diffusion medium and analyzed by a UV spectrophotometer at 273 nm**.** Data see in **Table 24** & **Fig. 18** [52].

Stability Studies: All Data see in **Table 29** and **24, 25 & 26**

RESULT AND DISSCUSION:

Physiochemical Characterization of a Pure Drug (Ciprofloxacin HCl): To determine the genuine and pure profile of drug was characterized by using various techniques which includes:

Organoleptic Property: The physical appearance of the drug sample under investigation was found to be white powder and odourless which was similar as reported in the literature.

TABLE 1: ORGANOLEPTIC PROPERTY OF CIPROFLOXACIN HCL

S. no.	Characteristics	Observation
1.	Appearance	Amorphous
2.	Color	Whitecolor
3.	Odor	Odorless

Melting point of Ciprofloxacin HCl Drug: The melting point of the drug was determined by the capillary method**.** Average melting point was to be found 2900C.

TABLE 2: SHOWING MELTING POINT OF CIPROFLOXACIN HCL

S. no.	Mp ^{0}c(Mean± Sd) (Standard melting point)	Observed melting point
1.	293 ±3	290 (Average value)

Determination of λ max of Ciprofloxacin HCl by using UV-Visible Spectroscopy: The absorption maxima (λ max) of Ciprofloxacin HCl were found to be at 273 nm in distilled water previously described method by Kesarwani *et al* (2011) [55]. The measurements of the stock solution were taken by using a UV-Visible Spectrophotometer [shimadzu-1700, Japan] and the λmax was found to be 273 nm as shown in **Fig. 2.**

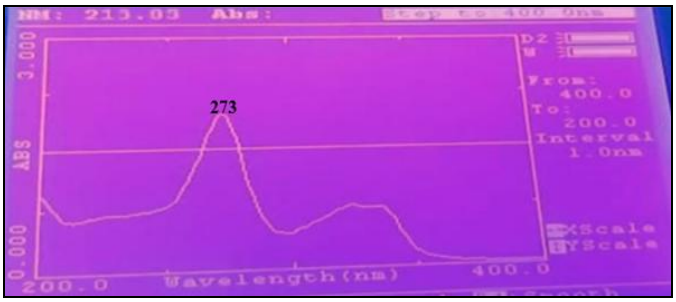


FIG. 2: ILLUSTRATION REPRESENTATION ABSORPTION MAXIMA (ΛMAX OF CIPROFLOXACIN HCL)

FTIR (Fourier Transform Infra-red spectroscopy) of Ciprofloxacin HCl: FTIR spectrum of the drug was obtained by KBr using (FT-IR Malvern, innovation center Bundelkhand University, Jhansi). The principle peaks of the drug were identified and matched with the standard FTIR of the drug confirming the identity and purity of the drug. In the FTIR spectra of Ciprofloxacin HCl, there are many peaks at 3308.51 cm^{-1} N-H stretching, 1646.70 cm^{-1} C=O stretching, 1237.46cm^{-1} C-N stretching, 1424 cm^{-1} C-H bending, 1381 cm^{-1} O-H phenol bending, 1276 cm^{-1} C-O Stretching Alkyl aryl ether, 1146.21 cm^{-1} C-O Stretching primary Alcohol, 689 cm-1 C=C Bending Alkene, 660 cm-1 C-Cl Stretching halo compound and 3067 cm-1 C=C stretching . The presence all of these groups in FTIR spectra which confirmed chemical structure of Ciprofloxacin HCl.

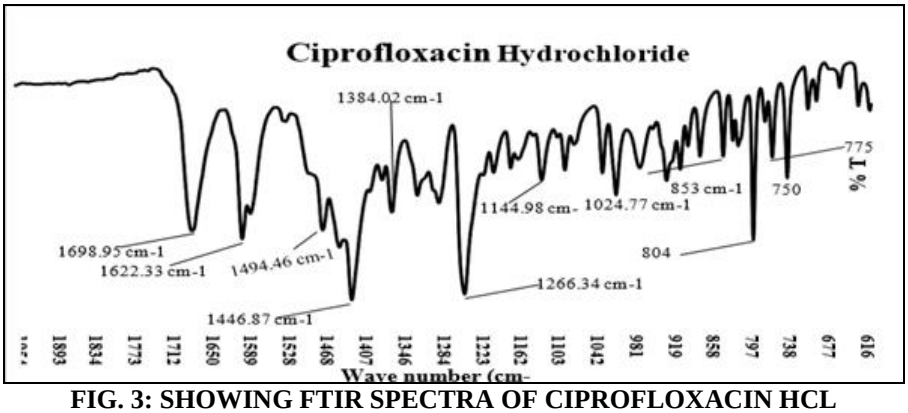


FIG. 3: SHOWING FTIR SPECTRA OF CIPROFLOXACIN HCL

TABLE 3: OBSERVED FUNCTIONAL GROUP WITH STANDARD RANGE OF FUNCTIONAL GROUP

S. no.	Characteristic, functional, group	Standard Range (cm^{-1})	Observed Peaks (cm^{-1})
1.	C=Stretching	1850-1680	1698.95cm-1
2.	Aromatic¬C=C	1680-1450	1622.33cm-1,1494.46cm-1
3.	CH&CH_2Aliphaticbending	1440-1350	1446.87,1384.02 cm-1
4.	Alkylketone group	1325-1215	1307.02,1266.34 cm-1
5.	N-CH_3stretching (Alkylamine)	1250-1000	1144.98,1105,1045,1024.77cm-1
6.	C-O,C-C	1000-800	985.05,853,804cm-1
7.	CH, Out of planede formation	760-735	775,750cm-1
8.	C-O-H twist broad	680-650	666.05 cm-1

X-Ray Diffraction (XRD) of Ciprofloxacin HCl: XRD powder diffraction is very fast analytical method for phase identification of a crystalline material that can provide information on unit cell dimension. This analyses to the fine group ,homogenized and average bulk composition is determined by XRD techniques which carrying out using a Bruker D8 advance X-ray diffractometer which produce X-ray (Cu K alpha) using on a sealed tube and sample were scanned over a 2θ .this scanning range of 2o-5o and rate of scanning was o/minutes. The X-rays were detected using a fast detector on silicon strip technology (Bruker Lynx Eye detector).

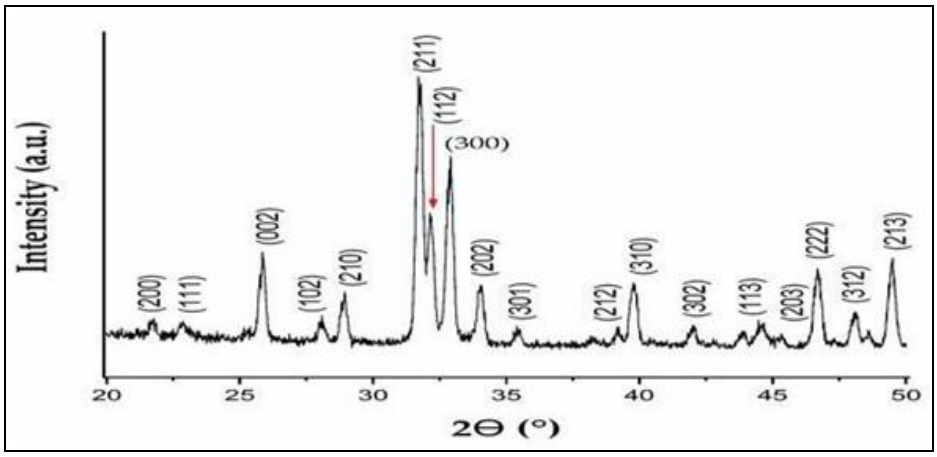


FIG. 4: CIPROFLOXACIN HCL BY X-RAY DIFFRACTION (XRD)

Solubility Determination of Ciprofloxacin HCl: The solubility study of the drug Ciprofloxacin HCl was performed in take different solvents (e.g. distilled water, Phosphate buffer pH6.8, ethanol, methanol, *etc*.). A predetermine quantity of the drug was transferred in a series of different solvents having volume of 5ml in four different test tubes. An excess amount of drug was added to different solvents till the solution became saturated and these test tubes were shaken by a mechanical shaker (Jyoti Scientific Industry,) for 1-hour at fixed vibration & temperature.

After this period the solution was centrifuged and collected supernatant then analyzed by UV spectrophotometer (Shimadzu-1700, Japan) at 273 nm with appropriate dilution. There determinations were carried out before each sample to calculate the solubility of drug Ciprofloxacin HCl in different solvents.

TABLE 4: VALUE OF SOLUBILITY (MG/ML) OF CIPROFLOXACIN HCL IN DIFFERENT SOLVENTS

S. no.	Media	Solubility(mg/ml)
1.	Distilled water	Completely soluble
2.	PhosphateBufferpH6.8	Freely soluble
3.	Ethanol	Very slightly soluble
4.	Methanol	Poorly soluble

Partition Coefficient of Drug Ciprofloxacin Hydrochloride: The partition coefficient is defined as the ratio between unionized drugs distributed between the organic and aqueous phases in medium. This ratio provide a means of characterizing the Lipophilic/Hydrophilic nature of the drug. It directly influences the permeability of the drug through the bio- membrane and could be approximated by measuring the partition coefficient of the drug in n- octanol/water. The partition coefficient of the drug was determined by allowing 10.0 mg of the drug to equilibrate in a mixture of n-octanol/water containing 10.0 ml of

each or (1:1) kept in separating funnel and this mixture was shaken or agitated until the drug was completely dissolve by and then storing it for 24 hours at 25°C± 2°C in a separating funnel. The two layers were separated and the concentration of the drug in the two layers was determined by UV spectrophotometer the partition coefficient was determined by using the formula as given below.

Partition coefficient = Concentration of drug in organic phase / Concentration of drug in aqueous phase

= 0.574/2.525 = 0.22

TABLE 5: PARTITION COEFFICIENT VALUE OF CIPROFLOXACIN HCL IN DIFFERENT SOLVENT SYSTEM

S. no.	Solvent system	Partition coefficient Values	Nature of the drug
1.	n-octanol: Water	0.22	hydrophilic

Preparation of Calibration Curve of Drug:

Preparation of Calibration Curve of Ciprofloxacin HCl in distilled Water:

Preparation of Stock Solution: Ciprofloxacin Drug (10 mg) was accurately weighed and transferred in 100 ml volumetric flask and dissolved in a small amount of distilled water by shaking gently and volume was made up to 100 ml distilled water in volumetric flask. The resultant solution was concentration 100 µg/ml was formed. Then 10 ml of this solution was taken in 100 ml of volumetric flask and volume made u with water to from stock solution.

Determination of λ max of Ciprofloxacin HCl: The λ max of the drug sample was determine by scanning 10 µg/ml standard stock solution in the range from 200-400 nm by using shimadzu 1700-U.V. spectrophotometer.

Preparation of Calibration Curve: The calibration curve of Ciprofloxacin HCl in distilled water was carried out by using a UV Visible Spectrophotometer [shimadzu-1700, Japan]. The results of absorbance for all the prepared concentrations were plotted i.e. Concentration vs. Absorbance the method was found to be linear over the prepared concentration range with the standard equation y = 0.097x + 0.0144 and the Regression value was found to be 0.9987 as shown in the **Fig. 5.**

TABLE 6: SHOWING STANDARD CALIBRATION DATA OF CIPROFLOXACIN HCL

S. no.	Conc.(µg/ml)	Abs.(Mean±SD)
1.	0	0.00 ±0.00
2.	1	0.12±0.01
3.	2	0.21±0.02
4.	3	0.31±0.030
5.	4	0.38±0.045
6.	5	0.51±0.056
7.	6	0.60±0.068
8.	7	0.68±0.079
9.	8	0.77±0.089
10.	9	0.89±0.095
11.	10	0.989±0.098

NOTE: In this table Showing Standard Calibration data of Ciprofloxacin HCl All data are represented as Mean ± SD (n = 3)

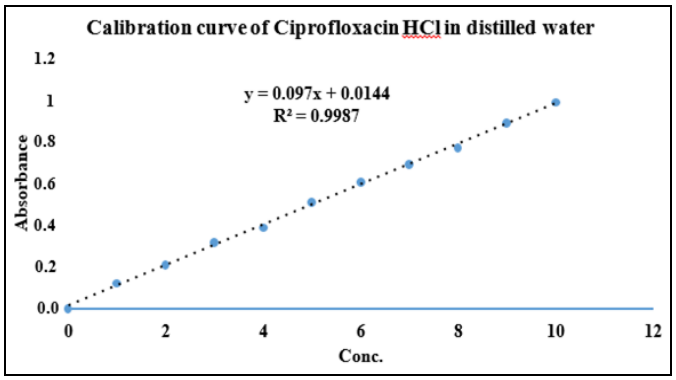


FIG. 5: STANDARD CALIBRATION CURVE OF CIPROFLOXACIN HCL IN DISTILLED WATER

Standard Calibration Curve of Ciprofloxacin HCl in PBS pH 6.8:

Preparation of Stock Solution: Ciprofloxacin Drug (10 mg) was accurately weighed and transferred in 100 ml volumetric flask and dissolved in a small amount of PBS 6.8 pH by shaking gently and volume was made up to 100 ml PBS 6.8 pH in volumetric flask. The resultant solution was a concentration 100 µg/ml was formed. Then 10 ml of this solution was taken in

100 ml of volumetric flask and volume made up with PBS 6.8 pH to from stock solution.

Determination of max of Ciprofloxacin HCl: The λ max of the drug sample was determine by scanning 10 µg/ml standard stock solution in the range from 200-400 nm by using Shimadzu 1700-U.V. spectrophotometer.

Preparation of Calibration Curve: The calibration curve of Ciprofloxacin HCl in PBS pH 6.8 was carried out by using a UV Visible Spectrophotometer [shimadzu-1700, Japan]. The results of absorbance for all the prepared concentrations were plotted i.e. Concentration vs. Absorbance the method was found to be linear over the prepared concentration range with the standard equation y = 0.098x - 0.003 and the Regression value was found to be R^2 = 0.9991 as shown in the figure:

TABLE 7: SHOWING STANDARD CALIBRATION DATA OF CIPROFLOXACIN HCL IN PBS

S. no.	Conc.(µg/ml)	Abs.(Mean±SD)
1.	0	0.0±0.0
2.	1	0.11±0.01
3.	2	0.19±0.02
4.	3	0.29±0.03
5.	4	0.38±0.04
6.	5	0.49±0.05
7.	6	0.57±0.056
8.	7	0.67±0.060
9.	8	0.78±0.070
10.	9	0.89±0.080
11.	10	0.98±0.095

NOTE: Ciprofloxacin HCl in PBS 6.8pH all data are represented as mean± SD (n=3)

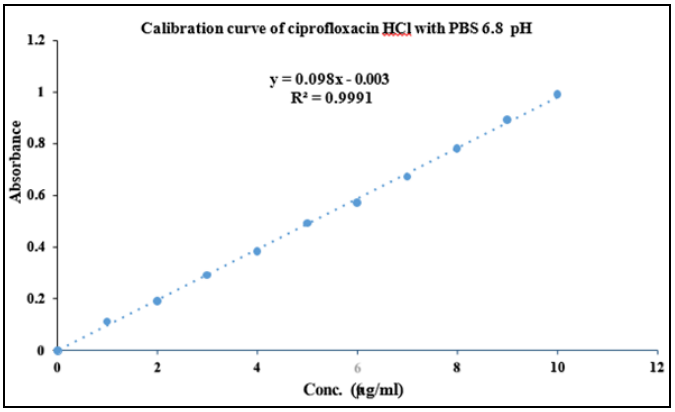


FIG. 6: STANDARD CALIBRATION CURVE OF CIPROFLOXACIN HCL IN PBS 6.8 PH

Characterization of Polymers:

Carbopol-940:

Organoleptic Property: The physical appearance of the Carbopol-940 sample under investigation was found to be white powder and odorless which was similar as reported in the literature.

TABLE 8: ORGANOLEPTIC PROPERTY OF CARBOPOL-940

S. no.	Characteristics	Observation
1.	Appearance	Amorphous
2.	Color	white-color
3.	Odor	odorless

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis of Carbopol 940: The range 400-4000 cm^{-1}. This figure show different types stretching groups and functionality which confirm qualitative analysis of polymer.

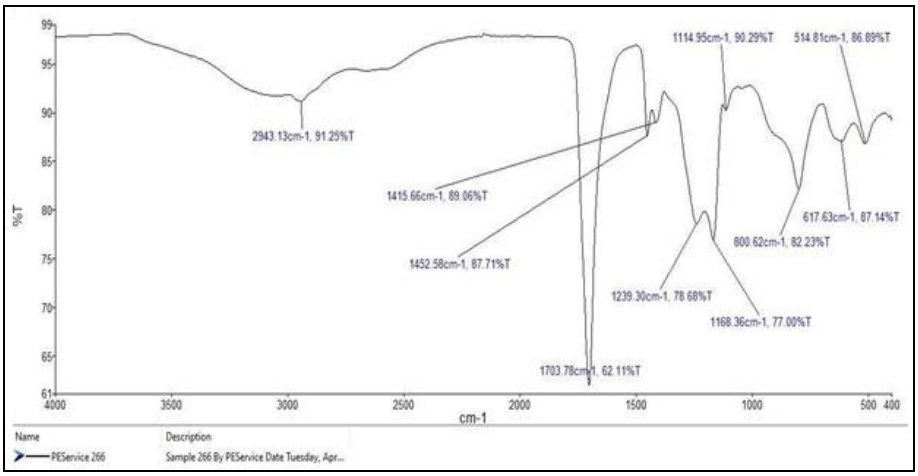


FIG. 7: FTIR SPECTRA OF CARBOPOL-940

HPMCK4M:

Organoleptic Property:

TABLE 9: ORGANOLEPTICPROPERTY

S. no.	Characteristics	Observation
1.	Appearance	Amorphous
2.	Color	white-color
3.	Odor	odorless

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis of HPMCK4M:

FIG. 8: FTIR SPECTRA OF HPMCK4M

Characterization of Ciprofloxacin HCl Loaded *In-situ* Gel:

Development of Conjugate (Cipro-*In-situ* gel)

Preparation of *In-situ* gel: The polymeric solution was prepared by simple physio-sorption method dispersing required quantity of carbopol-940 as main polymer and HPMC- K4M as co-polymers in water using a magnetic stirrer until the polymers completely dissolve. Aqueous solution of ciprofloxacin hydrochloride was added in to the polymeric solution with continuous stirring [4]. The pH of the solution was adjusted to pH6.8 using 0.1NNaoH/0.1NHCl. The *in-situ* gel formulations are given in **Table 10.**

TABLE 10: FORMULATION DESIGNED OF *IN-SITU* GEL

S. no.	Name of Ingredients	Quantity (gm)					
	Batch-5	F1	F2	F3	F4	F5	F6
1.	Ciprofloxacin HCl	0.03	0.03	0.03	0.03	0.03	0.03
2.	Carbopol-940	0.01	0.02	0.03	0.05	0.07	0.09
3.	HPMCK4 M	0.05	0.05	0.05	0.05	0.05	0.05
4.	0.1 N NaoH/0.1N HCl	asperreq.	asper req.	asperreq.	asperreq.	asperreq.	asperreq.
5.	Distilled water	100ml.	100ml.	100ml.	100ml.	100ml.	100ml.

TABLE 11: COMPOSITION OF STF

S. no.	Ingredients	Qty.
1.	Sodium chloride	0.67 g
2.	Sodium bicarbonate	0.2 g
3.	Calcium chloride dehydrate	0.008 g
4.	Water q.s to	**100 ml**

Evaluation of Ciprofloxacin Loaded *In-situ* gelling System: This includes various physicochemical parameters for evaluation of prepared *in-situ* gel.

FTIR Study for Drug and *In-situ* Gel Polymers Interaction: The drug-In situ gel polymer interaction study was carried out by FTIR spectroscopy. The FTIR spectrum of a composite of drug and In situ gel polymer to be liquid in the formulation was acquired using an FTIR spectrophotometer (Perkin Elmer, USA, model BX2) and matched with the spectra of polymers with drug and without drug.

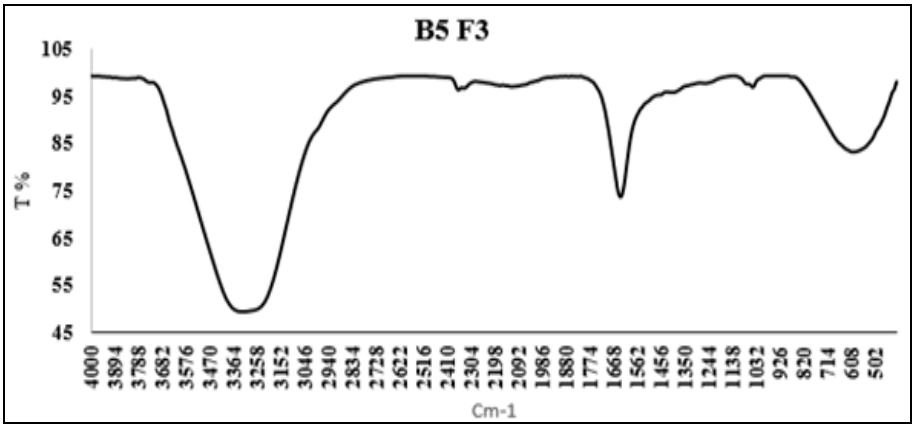


FIG. 9: FTIR SPECTRA (AT GEL STATE) OF CIPROFLOXACIN LOADED IN SITU GEL FORMULATION

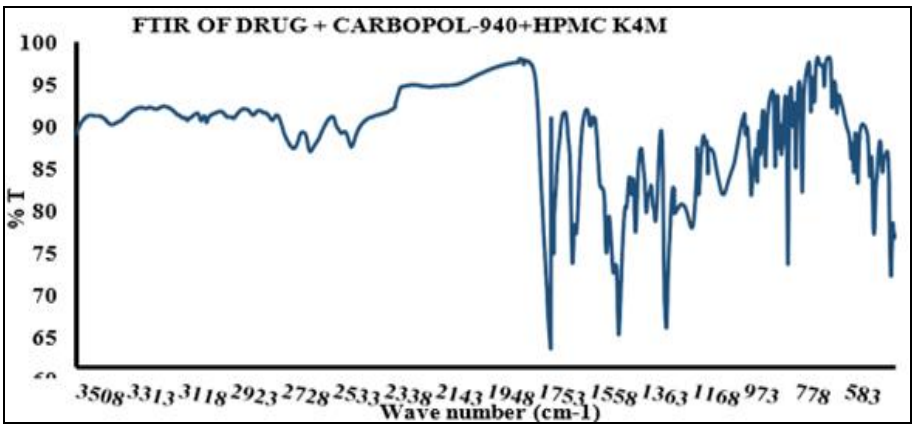
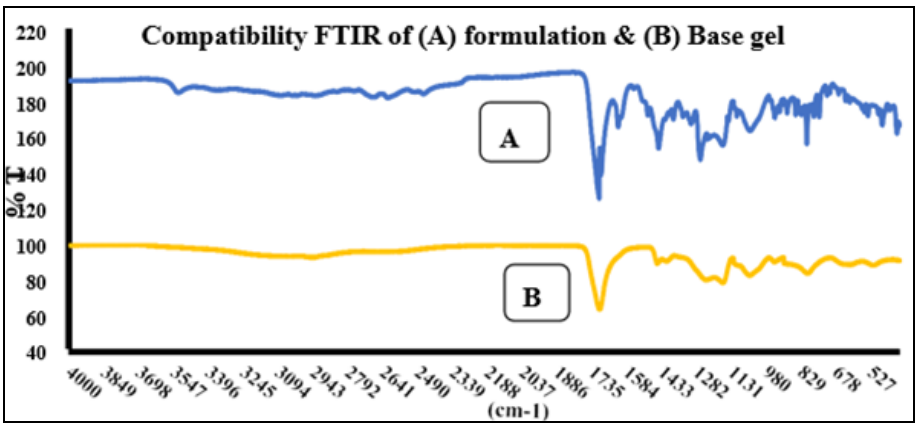


FIG. 10: FTIR SPECTRA (AT SOLID STATE) OF CIPROFLOXACIN LOADED *IN-SITU* GEL FORMULATION

TABLE 12: OBSERVED FUNCTIONAL GROUP OF FORMULATION

S. no.	Characteristic functional group	Standard Range (cm^{-})	Observed Peaks (cm^{-1}) of drug	Observed peaks (cm-1) of polymers
1.	C=O Stretching	1850-1680	1698.95cm-1	1704cm-1 (Carbopol-940)
2.	Aromatic ¬C=C	1680-1450	1622.33cm-, 1494.46cm-1	
3.	CH & CH_2, Aliphatic bending	1440-1350	1446.87,1384.02cm-1	
4.	Alkylketone group	1325-1215	1307.02,1266.34cm-1	
5.	N-CH_3 stretching (Alkyl$_{amine}$)	1250-1000	1144.98,1105,1045,1024.77cm-1	1170,1235cm-1(Carbopol-940),1054,1108cm-1(HPMC K4 M)
6.	C-O, C-C	1000-800	985.05,853,804cm-1	
7.	CH, Out. of plane deformation	760-735	775,750cm-1	
8.	C-O-H twist broad	680-650	666.05 cm-1	

Compatibility Study by FTIR of Formulation between Blank Gel (polymers) and Drug:



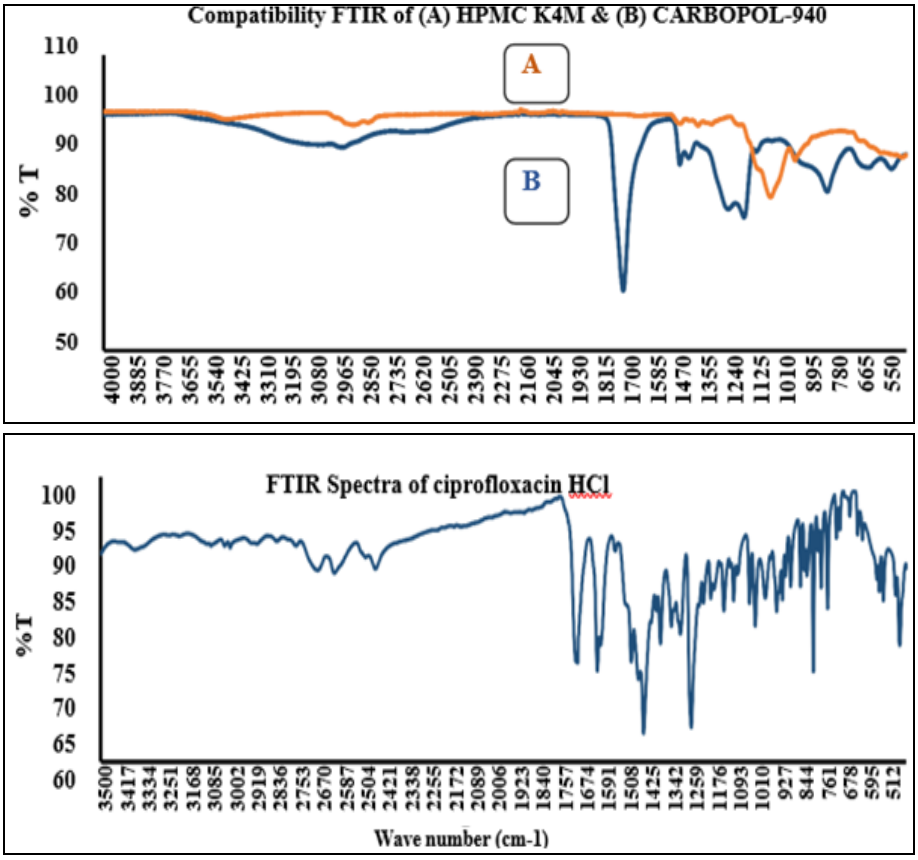


FIG. 11: COMPATIBILITY STUDY OF FORMULATION, G-BASE, DRUG FTIR SPECTRA

Determination of Gelling Capacity: The gelling capacity was analyzed by of the prepared formulation was 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 its gelling was noted

FIG. 12: PREGELATION AND POST GELATION CONDITION OF FORMULATION

TABLE 13: CODING OF GELLING CAPACITY

S. no.	Observation	Coding
1.	No elation	-
2.	Gelation occurred in few minutes and remained for few hour	+
3.	Gelation occurred in immediate, remained for few hour	++
4.	Gelation immediate, and for extended period	+++
5.	Very stiff gel	++++

Note: + indicates gelation occurred after few minutes and dissolved rapidly**,** ++ indicates immediate gelation and remained up to few hours**,** +++ indicates immediate gelation and remains for extended period.

Determination of Drug Content Estimation: The drug content estimation was carried out by diluting 1 ml of prepared formulation in 100 ml of fresh prepared STF (standard tear fluid pH7.4) then

stirred with magnetic stirrer for 1 hr. then filter by whatman filter paper after collect 5ml. solution and further diluted with 25 ml. STF and Conc. determine by analyzed using UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 273 nm.

TABLE 14: DRUG CONTENT ESTIMATION

S. no.	Formulations	%Drug Content
1.	F1	89.26
2.	F2	90.05
3.	F3	92.41
4.	F4	91.56
5.	F5	90.78
6.	F6	89.36

Determination of pH: The pH of the gels was determined by using a digital pH meter [Jyoti scientific] at room temperature after calibrating the pH meter using standard buffers of pH 4, 7 for this purpose, accurately pH was recorded of formulation pre-gelation and post gelation during my work.

TABLE 15: FORMULATIONS OF pH

S. no.	Formulations	Pre-gelation pH	Postgelation pH
1.	F1	5.8	7.8
2.	F2	6.2	7.9
3.	F3	6.7	7.5
4.	F4	6.6	7.9
5.	F5	6.5	7.6
6.	F6	6.9	8.0

Determination of Visual Appearance Clarity: Clarity test was observed by visual inspection under a good light, viewed against a black and white background, with the contents set in motion with a swirling action. Also it was observed for formation of turbidity or any unwanted particles dispersed in the solution 3, 18, and 19. All the formulations (F1-F6) prepared were clear without any turbidity and suspended particles or impurities.

TABLE 16: FORMULATIONS OF CLARITY

S. no.	Formulations	Appearance	Clarity
1.	F1	White Color	Clear
2.	F2	White Color	Clear
3.	F3	White Color	Clear
4.	F4	White Color	Clear
5.	F5	White Color	Slightly clear
6.	F6	White Color	Slightly clear

***In-vitro* Drug Release:** Dialysis technique is a separation method which involve the removal of small, unwanted compounds or macromolecules remove form solution through passive diffusion by semi- permeable membrane. This dialysis bag have a pore size is very small (2.2-2.4 nm), has a certain porosity size rated as Molecular Weight Cut off (MWCO).

This is very crucial for consider the molecular shape, ionic charge, degree of hydration, and polarity along with particle size while choosing an appropriate membrane. Some different factors such as dialysis buffer volume, buffer composition, time, temperature, particle size, and pore size affect the dialysis process. In the method, the drug-loaded dosage form is placed in the dialysis bag (cellulose membrane, molecular weight cut off 12,000 D), hermetically sealed, and immersed into 50 ml of STF (pH 7.4).

The entire system was kept at 37 ± 0.5°C with continuous magnetic stirring at 100 rpm/min. Samples were withdrawn from the receptor compartment at predetermined time intervals and replaced by a fresh medium. The amount of drug dissolved was determined with UV spectrophotometry at 273nm.

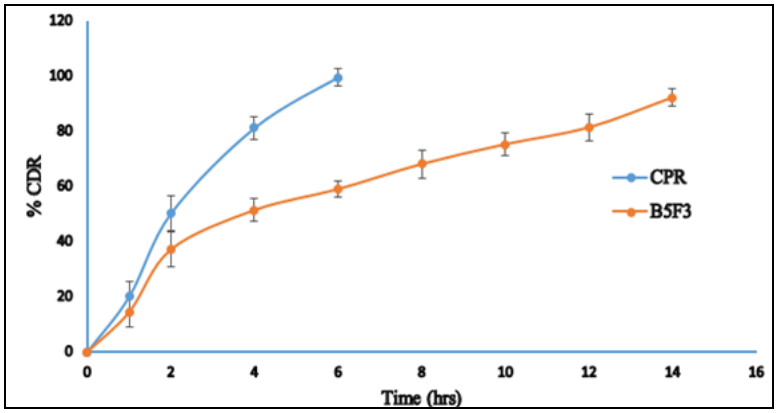


FIG. 13: *IN-VITRO* DRUG RELEASE OF PURE DRUG SOLUTION AND IN SITU GEL FORMULATION AT STF 7.4 PH

TABLE 17: RELEASE OF CIPROFLOXACIN HYDROCHLORIDE AT PURE DRUG SOLUTION AND IN SITU GEL FORMULATION

S. no.	Time (hr.)	Pure Drug solution (%CDR)	B5F3 (% CDR)
1.	0	0.0	0.0±0.00
2.	1	20.0	14.45±5.62
3.	2	50.2	37.11±6.24
4.	4	81.2	51.37±4.035
5.	6	99.56	58.99±3.091
6.	8		68.07±5.038
7.	10		75.23±4.026
8.	12		81.35±5.024
9.	14		92.23±3.016

***In-vitro* Release Kinetics Models:** *In-vitro* release kinetic studies, the percentage drug remaining was calculated using the cumulative percentage of the drug released, and the rate constant for zero-order and the first order for *in-vitro* release studies were calculated after each time interval standard deviation, and coefficients were also calculated. The four mainly exercised mathematical models were used i.e.

Zero Order Drug Release Model: When the data is plotted as cumulative "% drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0. Zero-order release would be predicted by the following equation:-

$$A_t = A_0 - K_0 t$$

Where, A_t = Drug release at a "time", A_0 = initial drug concentration, K_0 = Zero-Order rate constant (hr-1).

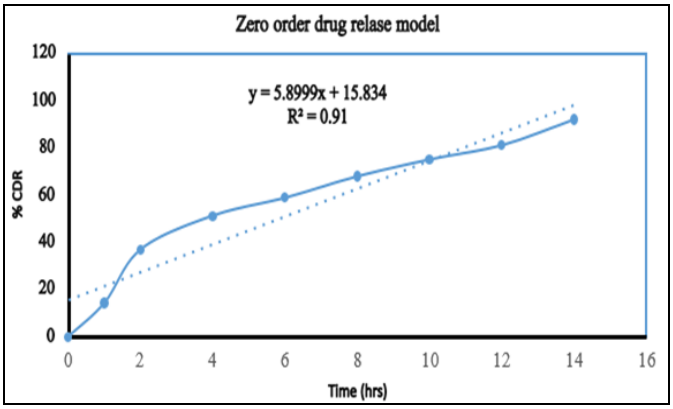


FIG. 14: ZERO ORDER RELEASE MODEL

TABLE 18: ZERO ORDER DRUG RELEASE OBSERVATION

S. no.	Time(hr.)	B5F3 (%CDR)
1.	0	0.0±0.0
2.	1	14.45±0.010
3.	2	37.11±0.023
4.	4	51.37±0.035
5.	6	58.99±0.0.49
6.	8	68.07±0.058
7.	10	75.23±0.065
8.	12	81.35±0.078
9.	14	92.23±0.089

First Order Release Model: When the data is plotted as log cumulative % drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant "K" can be obtained by multiplying 2.303 with the slope values. The first-order release would be predicted by the following equation:-

$$\text{Log } C = \log C_0 - K_t / 2.303$$

Where, C=Amount of drug remained at the time "t", C_0- Initial concentration of drug, K= first-order rate constant (hr-1).

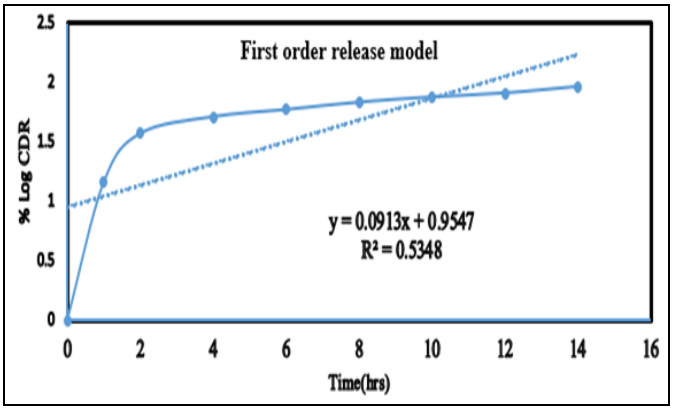


FIG. 15: FIRST ORDER RELEASE MODEL

TABLE 19: OBSERVATION TABLE OF FIRST ORDER RELEASE MODEL

S. no.	Time(hr.)	B5F3	%Log(CDR)
1.	0	0.0±0.0	0.00
2.	1	14.45±0.010	1.159868
3.	2	37.11±0.023	1.569491
4.	4	51.37±0.035	1.71071
5.	6	58.99±0.0.49	1.770778
6.	8	68.07±0.058	1.832956
7.	10	75.23±0.065	1.876391
8.	12	81.35±0.078	1.910358
9.	14	92.23±0.089	1.964872

Higuchi Model: When the data is plotted as cumulative drug release versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to "K" Drug release from the formulation by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\,\varepsilon / \varepsilon\,(2A-CS)CSt]_{1/2}$$

Where, Q= Amount of drug released at a "time", D = Diffusion coefficient of the drug in the matrix, A = total amount of drug in the unit volume of a matrix, CS = Solubility of the drug in the matrix, ε = porosity of the matrix, T = Tortuosity.

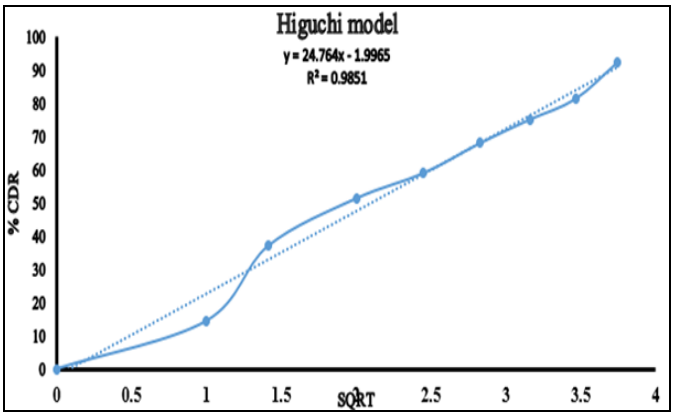


FIG. 16: HIGUCHI RELEASE MODEL

TABLE 20: OBSERVATION TABLE OF HIGUCHI RELEASE MODEL

S. no.	Time(hr.)	B5F3	SQRT
1.	0	0.0±0.0	0.0
2.	1	14.45±0.010	1.0
3.	2	37.11±0.023	1.414214
4.	4	51.37±0.035	2.36
5.	6	58.99±0.0.49	2.44949
6.	8	68.07±0.058	2.828427
7.	10	75.23±0.065	3.162278
8.	12	81.35±0.078	3.464102
9.	14	92.23±0.089	3.741657

Hixon-Crowell Model: Hixson Crowell model describes the releases from the systems where the change in surface area and changes diameter of the particles.

$$W_0\ 1\ 3 - W_t\ 1\ 3 = \kappa\ t$$

Where, W0 = initial weight of the drug, Wt = weight of drug at time t, KHC = Hixson-Crowell's constant relating surface area.

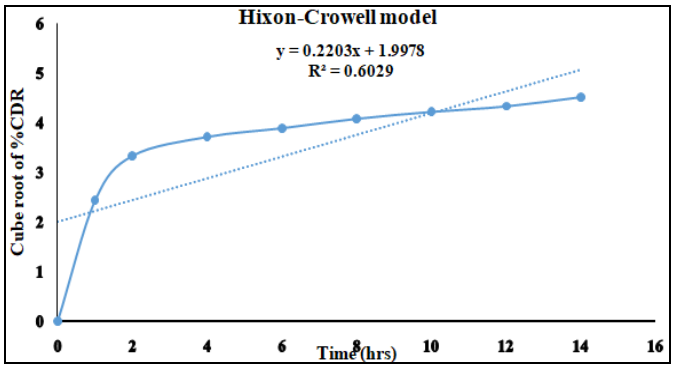


FIG. 17: HIXON-CROWELL MODEL

TABLE 21: OBSERVATION TABLE OF HIXON-CROWELL MODEL

S. no.	Time (hr.)	B5F3	Cube root of % CDR
1.	0	0.0±0.0	0.0
2.	1	14.45±0.010	2.435693
3.	2	37.11±0.023	3.335521
4.	4	51.37±0.035	3.717376
5.	6	58.99±0.0.49	3.892776
6.	8	68.07±0.058	4.083055
7.	10	75.23±0.065	4.22147
8.	12	81.35±0.078	4.332972
9.	14	92.23±0.089	4.518116

Korsmeyer-Peppas Model: When the data is plotted as a log of drug released versus time, yield a straight line with a slope equal to "n" and the "K" can be obtained from the y-intercept. To study the mechanism of drug release, the drug release data were also fitted to the well- known exponential equation (Korsmeyer equation/Peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t/M_a = K_{tn}$$

Where, M_t/M_a = is the amount of drug released at time, K = is the release rate constant, and n is the release exponent, n = value is used to characterize the different release mechanisms.

TABLE 22: OBSERVATION TABLE OF KORSMEYER - PEPPAS MODEL

S. no.	Time (hr.)	%Log (CDR) (Time)	B5F3	%Log (CDR) (formulation)
1.	0	0	0	**0**
2.	1	0.30103	14.45	1.159868
3.	2	0.60206	37.11	1.569491
4.	4	0.778151	51.37	1.71071
5.	6	0.90309	58.99	1.770778
6.	8	1.0	68.07	1.832956
7.	10	1.079181	75.23	1.876391
8.	12	1.146128	81.35	1.910358
9.	14	1.216589	92.23	1.964872

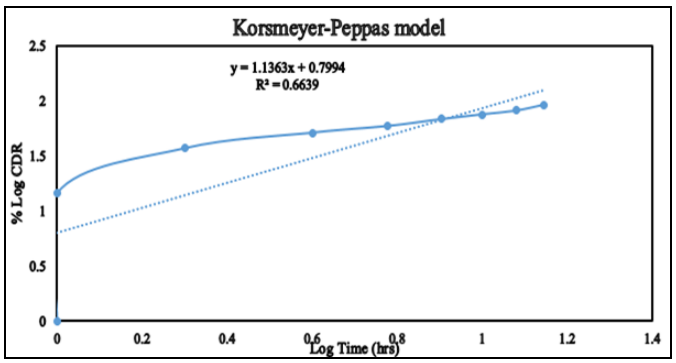


FIG. 18: KORSMEYER- PEPPAS MODEL

TABLE 23: RELEASE RATE CORRELATION COEFFICIENT CALCULATED AFTER FITTING THE RELEASE PROFILE OBTAINED BY USING THESE DIFFERENT MATHEMATICAL MODELS

S. no.	zero-order R^2	First-order R^2	Higuchi order R^2	Korsmeyer-Peppas R^2	Hixon-Crowell R^2
B5 F3	0.91	0.5348	0.9851	0.6639	0.6029

***Ex-vivo* Permeability Study:** The *ex-vivo* permeability study was carried out by using cellophane membrane purchased from Gupta Agencies Ambala followed by protocols described by (47- 50) to ensure the amount of drug permeate from cellophane membrane. The membrane was sandwiched between donor and acceptor compartment and mounted on Franz diffusion cell. The acceptor compartment filled with STF pH 7.4 (Volume 50 ml fresh prepared STF), stirring speed of 50 rpm, at a temperature of 37±0.5°C. The formulation was kept at donor compartment and 1 ml of media was withdrawn from receptor compartment at different time point intervals of 1, 2, 4, 6, 8, 10, 12 and 14 hr. I was replaced with same amount of fresh volume from receiver compartment. The sample was analyzed by UV visible spectrophotometer at of 273 nm in order to quantify content of drug permeate from cellophane membrane. An apparent permeability coefficient was calculated by using following equation; (3) by calculating steady state flux (*J*ss) from slope of liner regression line obtained by plotting cumulative permeated amount of formulation (µg/cm2) vs time (hr).

$$Papp = Jss / Cd$$

Where, *Papp* = Apparent permeability coefficient (cm/s), *Jss* = Steady state flux.

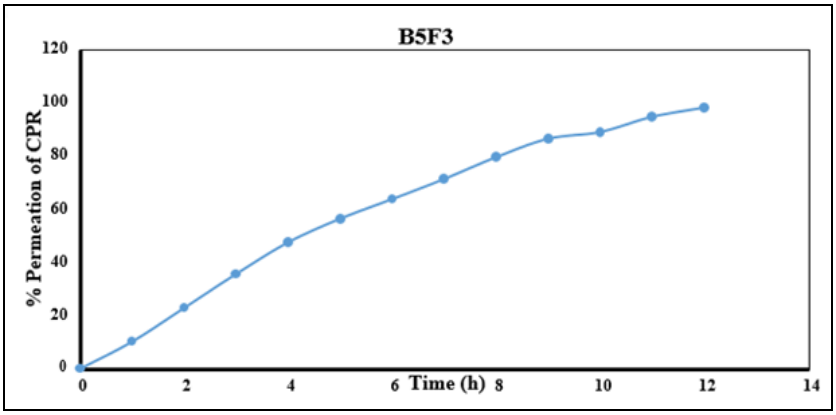


FIG. 19: *EX-VIVO* PERMEABILITY GRAPH OF FORMULATION USING CELLOPHANE MEMBRANE

TABLE 24: OBSERVATION DATA OF FORMULATION (B5 F3) DURING PERMEABILITY

S. no.	Time(hr.)	(% CDR) Permeation of formulation (B5F3)
1.	0	0.00
2.	1	10.23
3.	2	22.89
4.	3	35.69
5.	4	47.58
6.	5	56.48
7.	6	63.84
8.	7	71.41
9.	8	79.63
10.	9	86.57
11.	10	89.13
12.	11	94.16
13.	12	98.29

Antimicrobial Activity: Anti-microbial activity was conduct on the pure free drug solution, formulation (B5 F3), B-Gel and fresh prepared 7.4 pH STF. This study was conduct by using the agar well diffusion method with slight modification. It was culture into the Dextrose (SD) broth media at pH 7.4 for antimicrobial activities in term of the ZOI. Agar media was used as nutrient for prepared in distilled water and sterilized using autoclave at 121°C for 15 min. The nutrient agar media was then allowed to cool at room temperature aseptically. One milliliter of culture streptococcus aureus, was mixed with the 25 ml of SD agar media. The mixture was slowly poured into the sterile Petri- plates under the laminar chamber to get solidified. The wells were created using the sterilized borer and filled with the test sample followed by incubation for 48 h at 37 ±1°C. The ZOI around the well was measured in mm using scale and expressed as in mean value.

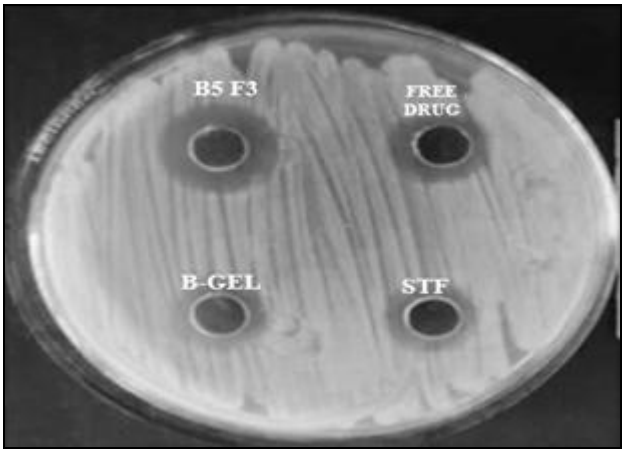


FIG. 20: ANTIMICROBIAL ACTIVITY OF DIFFERENT FORMULATION ON SINGLE PETRI DISC

TABLE 25: OBSERVATION VALUE OF ZONE IHIBITION AREA IN (MM)

S. no.	B5 F3	B-Gel	Free drug	STF
1.	6.8	1.09	3.01	0.21
2.	0.11±	0.31±	0.29±	0.05±

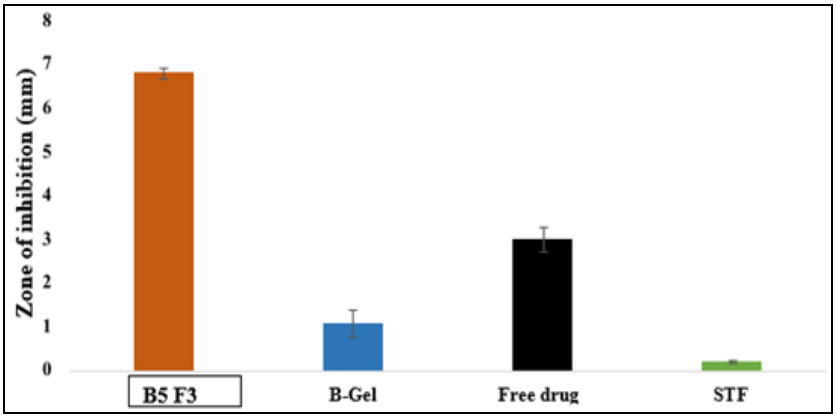


FIG. 21: ZONE OF INHIBITION OF FORMULATION, BASE GEL, FREE DRUG SOLUTION, AND STF

TABLE 26: OBSERVATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

S. no.	B5 F3	B-Gel	Free drug	STF
1.	6.8	1.09	3.01	0.21
2.	0.11±	0.31±	0.29±	0.05±

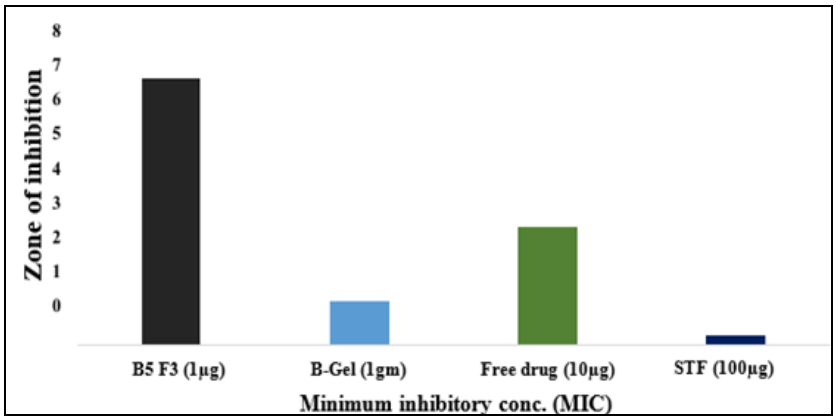


FIG 22: MINIMUM INHIBITORY CONCENTRATION OF FORMULATION, B-GEL, FREE DRUG AND STF

Rheological Studies: The viscosity measurements were carried out using Brookfield viscometer LVDV-E model. The *in-situ* gel formulations were placed in the sampler tube. The samples were analyzed at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each

measurement [7–10]. The angular velocity of the spindle was increased 1 to 4 and the viscosity of the formulation was measured.

1. Pre gelatin
2. Post gelation

TABLE 27: OBSERVATION OF VISCOSITY DATA OF FORMULATION (TEMP VS VISCOSITY) (PRE & POST GELATION)

S. no.	Temp.[°C]	Viscosity[mPa·s]	S. no.	Temp [°C]	Viscosity[m Pa·s]	S.N.	Temp [°C]	Viscosity[m Pa·s]
1.	20.45	432.88	29.	34.06	1548.45	56.	43.06	1375.36
2.	21.25	445.36	30.	34.56	1575.45	57.	43.56	1356.36
3.	22	458.23	31.	35.05	1658.58	58.	44.06	1325.65
4.	22.23	465.36	32.	35.55	1680.47	59.	44.56	1298.36
6.	22.45	470.36	33.	36.06	1725.56	60.	45.05	1265.36
7.	22.98	520.36	34.	36.56	1765.98	61.	45.56	1215.36
8.	23.1	535.65	35.	37.06	1800.2	62.	46.06	1065.56
9.	24.04	625.36	36.	37.56	1807.45	63.	46.55	1032.65
10.	24.54	674.23	37.	38.06	1756.36	64.	47.06	1010.32
11.	25.05	712.56	38.	38.56	1665.45	65.	47.56	950.36
12.	25.55	768.36	39.	39.06	1635.2	66.	48.06	923.32
13.	26.05	812.63	40.	39.56	1610.25	67.	48.56	889.56
14.	26.55	865.12	41.	40.05	1565.45	68.	49.06	825.65
15.	27.05	912.36	42.	40.56	1535.15	69.	49.56	790.23
16.	27.55	978.65	43.	41.06	1499.45	70.	50.06	742.23
17.	28.05	1010.36	44.	41.56	1456.65	71.	51.02	642.36
18.	28.55	1065.36	45.	42.06	1429.45	72.	51.63	598.36
19.	29.06	1111.36	46.	42.56	1410.25	73.	51.98	532.15
20.	29.55	1152.36	47.	43.06	1375.36	74.	52.36	501.36
21.	30.06	1205.56	48.	43.56	1356.36	75.	52.45	456.36
22.	30.56	1254.65	49.	44.06	1325.65	76.	52.69	401.36
23.	31.02	1310.02						
24.	31.46	1386.10						
25.	32.23	1405.20						
26.	32.89	1425.30						
27.	33.24	1478.45						
28.	33.85	1510.15						

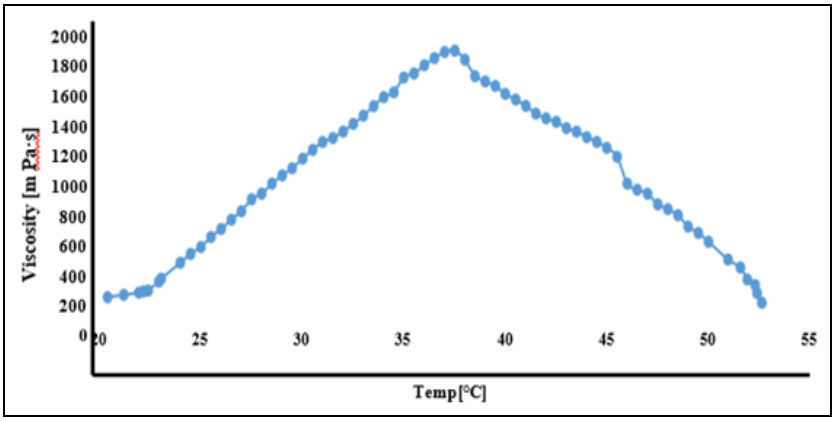


FIG. 23: OBSERVATION SHOW VISCOSITY OF FORMULATION (B5 F3) BY USING BROOK FIELD VISCOMETER

TABLE 28: OBSERVATION PRE-GELATION AND POST-GELATION VISCOSITY STUDYDATA OF FORMULATION (TORQUE (RPM) VS VISCOSITY)

S. no.	R.P.M	Viscosity in (cps) Pre-gelation	Viscosity in (cps) Post-gelation
1.	3.0	498	1948
2.	4.0	415	1903
3.	5.0	401	1853
4.	9.0	368	1707
5.	13.0	303	1698

6.	21.0	295	1654
7.	32.0	238	1609
8.	50.0	107	1558
9.	70.0	97	1532
10.	100.0	92	1501

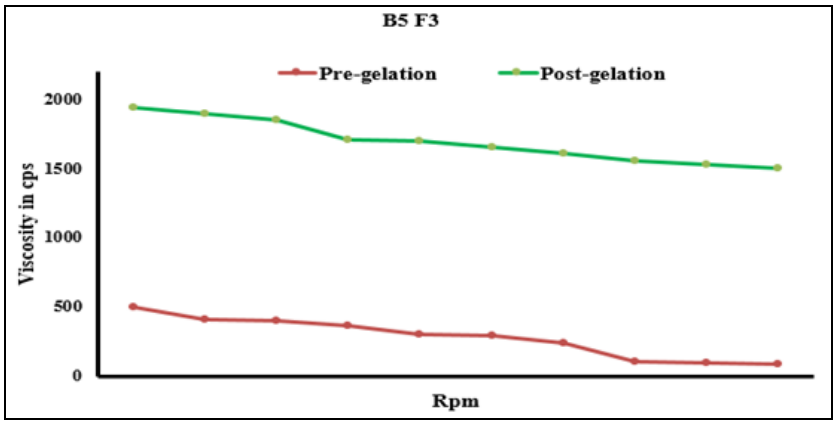


FIG. 24: PRE AND POST GELATION VISCOSITY OF FORMULATION (B5 F3) BY USING BROOKFIELD-VISCOMETER BY SPINDLE 62

Stability Study: Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage, the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Optimized formulation was subjected to stability studies at ambient humidity conditions at 2˚C to 8˚C, ambient temperature and 40±1˚C and 75±5% RH as per modified ICH guidelines for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for drug content. The ophthalmic formulations are placed in amber colored vials.

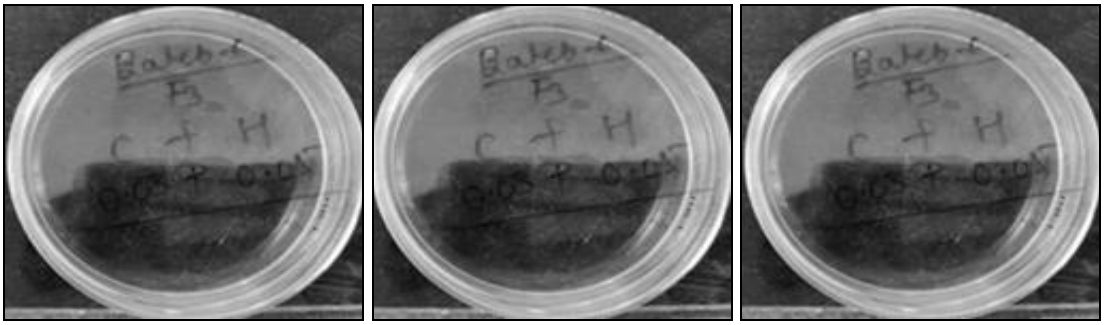


FIG. 25: AFTER 7 DAYS AFTER 15 DAYS AFTER 30 DAY

Stability Study of Formulation with Different pH:

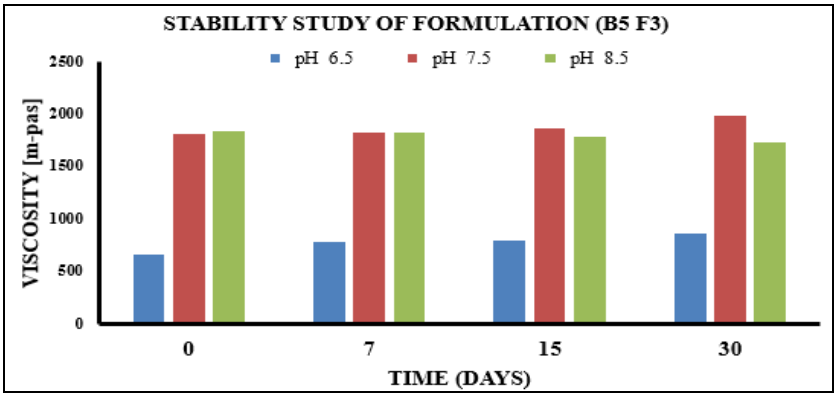


FIG 26: STABILITY STUDY OF FORMULATION AT VARIOUS PH

TABLE 29: STABILITY DATA OF FORMULATION AT DIFFERENT pH

S. no.	Time (Days)	pH 6.5	pH 7.5	pH 8.5
1.	0	653.36	1810.29	1840.5
2.	7	779.75	1827.45	1818.23
3.	15	789.89	1860.45	1779.56
4.	30	856.26	1987.45	1732.58

Stability Study of Formulation with Different Temperature:

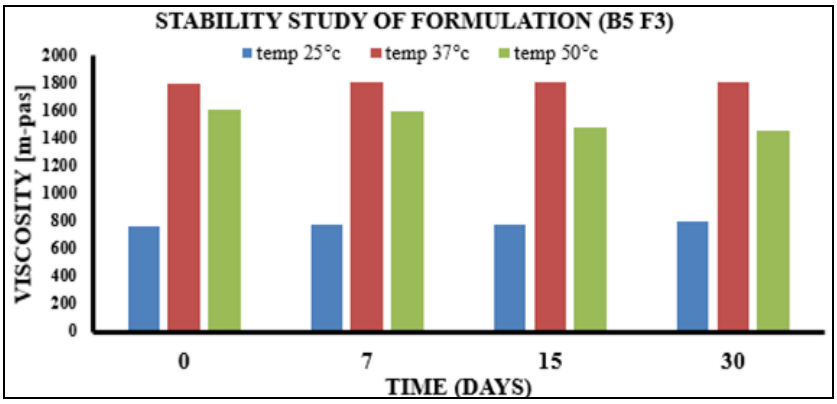


FIG. 27: STABILITY STUDY OF FORMULATION AT VARIOUS TEMPERATURES

TABLE 30: STABILITY DATA OF FORMULATION AT DIFFERENT TEMPERATURE

S. no.	Time (Days)	Temp (25°C)	Temp (37°C)	Temp (50°C)
1.	0	768.36	1800.2	1609.5
2.	7	770.75	1807.45	1595.56
3.	15	773.89	1810.45	1479.56
4.	30	798.26	1813.45	1462.58

Sterility: Sterility testing was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate and soybean casein digest medium respectively as per the Indian Pharmacopoeia. All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30˚C to 35˚C in the case of fluid thioglycolate medium and 20˚C to 25˚C in the case of soya bean casein digest media. Both positive and negative controls were maintained the study.

FIG. 28: PRE AND POST AUTOCLAVING OF FORMULATION

There was not any microbial growth in formulation after autoclaving 121 for 15 minutes. After this formulation was placed in petri dish and agar media for microbial growth for given as incubators with 37±5 temperature in BOD Incubator for storage in one week with these suitable environments for microbial growth.

TABLE 31: STERILITY TEST DATA OF PREPARED FORMULATIONS

S. no.	Formulation Code	Incubation Days	
		1-7	7-14
1.	F1	-	-
2.	F2	-	-
3.	F3	-	-
4.	F4	-	-
5.	F5	-	-
6.	F6	-	-

Note: -sign indicates no growth.

RESULTS: The appearance of in situ gels were clear and free flowing in nature however, a viscous clear solution with flow was normal as solution state after gel conversion flow behavior was change. All formulations consisting 0.01%-0.09 %w/v of Carbopol-940 and 0.05% w/v HPMC K4M. PH of all the formulations was within the range of 5.8 to 6.9 before gelling condition. In situ gels with Carbopol-940 demonstrated higher viscosity compared to Carbopol-934 and drug release was sustained over a period of 8 hr. The selected formulation containing 0.03% w/v Carbopol-940 and 0.05% w/v HPMC K4M passed sterility test and demonstrated similar antimicrobial efficiency compared to commercial product.

SUMMARY AND CONCLUSION: Highly water-soluble ciprofloxacin HCl belongs to a class of drugs called quinolone antibiotics. It works by stopping the growth of bacteria. Ciprofloxacin hydrochloride is a BCS Class IV drug which is low soluble with low permeable. This antibiotic treat only bacterial infections. It will not work for virus infections (such as common cold, flu). Using any antibiotic. Calcium rich foods, including dairy products (such as milk, yogurt) or calcium-enriched juice, can also decrease the effect of this medication. Take this medication at least 2 hours before or 6 hours after eating calcium-rich foods.

Present study is based on the Formulation and characterization of *in-situ* gel for ophthalmic formulation containing Ciprofloxacin hydrochloride using a gelling agent like Carbapol-940 and HPMC K4M used as viscosity enhancer agent. In this formulation *in-situ* gel is used as carrier for ocular drug delivery system. Optimized all batch shows transparent solution in appearance. pH of all six batches was found between 5-5.6. pH before gelling after gelling pH of all batch formulation was range between 7.4 - 8 pH. Optimized batch-5 F3 was found 5.5 which lies in the normal PH of eye. Viscosity, clarity, pH, gelling capacity, is an important parameter for characterizing the *in-situ* gels as it affects s. extrudability and release of the drug, all the formulated batches should increase viscosity as the concentration of gelling agent increased optimized batch-5 F3 shows ideal viscosity.

All the prepared groups indicate consistency in drug content. Optimized batch-5 F3 shows

92.13 % drug content which shows uniform drug dispersion in *in-situ* gel. *In-vitro* release studies were carried out by using STF pH 7.4 release of ciprofloxacin HCl from optimized batch-5 F3 prepared *in-situ* gel formulations was found to be satisfactory. In optimized batch- 5 F3, drug diffusion occurs through the fluid phase and hence they offer little resistance to drug diffusion and release.

Overall, the study objectives are fulfilled based on the experimental results. It can be inferred that the formulation of *in-situ* (pH based) gel that can be hold the drug for long time on eye surface as compare to eye solution (eye drop) to attain applicable which can be very effective against conjunctivitis and also decrease to dosing frequency & increase to patient compliance.

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