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Formulation and Evaluation of Ofloxacin Insitu Gel Using Sodium Alginate and Locust Bean Gum

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ABSTRACT

This study addresses the challenge of poor ocular bioavailability caused by rapid precorneal clearance by developing an ion-activated in situ gelling system for sustained delivery of Ofloxacin. In order to extend corneal healing duration, the technology permits use of natural polymers such as locust bean gum and sodium alginate. Five formulations (F1–F5) with different concentrations of sodium alginate were evaluated for their rheological traits like being drug content, physical and chemical properties, in vitro release kinetics, and antibacterial activity. FTIR spectroscopy and pre-formulation analyses verified both the medication and excipient were compatible and did not interact significantly as well. For accurate quantification, a validated technique of analysis showed strong linearity (R2 = 0.9908). Each formulation had a pH between 6.4 and 6.7 that was medically acceptable, was clear, and was light yellow in color. Viscosity rose with polymer concentration (14–48 cps) and the drug content had been extremely consistent (99.3–99.7%), which directly affected the drug release rate. A prolonged release profile that was inversely related to the concentration of the polymer was found by in vitro diffusion investigations. Formulation F3, which contains 1% sodium alginate, was found to be the best because it balances a controlled release of 74.9% over 8 hours with an acceptable viscosity of 28 cps. F3 was chosen due to its higher overall performance in rheology and release modulation, even though F4 confirmed a slightly better match in release kinetic models. According to the write up, the developed Ofloxacin in situ gelling system offers an attractive platform for ocular drug administration that maintains therapeutic effectiveness while strengthening precorneal retention and delivering prolonged release.

Keywords: In situ gel; Ofloxacin; Sustained release; Ion-activated; Bioavailability; Antibacterial activity.

INTRODUCTION

Ophthalmic drug delivery presents considerable therapeutic challenges, primarily due to the eye's sophisticated anatomical and physiological defense mechanisms.[1] The medication bioavailability of conventional topically applied therapies, such eye drops, frequently decreases below 5%, making them notably ineffective.[2] Rapid precorneal elimination of carried on by physiological mechanisms such lacrimal drainage, tear turnover, and the blink reflex is the cause of this inefficiency. As a result, regular administration is necessary, which compromises patient compliance and may eventually result in worse clinical results.[3]

In this regard, in-situ gelling technology have generated a lot of interest as an advanced method that could enhance ocular medication delivery.[4] These formulations are differentiated by their capacity to experience a solution-to-gel transition following instillation, which is dependent on by specialized physiological parameters such the tear fluid's ionic composition, temperature, or pH. Ion-activated systems are the most beneficial of them.[5] For example, a natural polysaccharide called sodium alginate promptly generates a hydrogel network by cross-linking with divalent cations (like Ca^{2+}) in the precorneal region. By significant resources extending the corneal contact duration and increasing formulation viscosity in situ, this phase transition enhances ocular bioavailability.[6]

Whereas sodium alginate by itself has good mucoadhesive and biocompatible qualities, incorporating additional polymers can improve its gel strength and rheological performance even further. Alginate and locust bean gum, a galactomannan polysaccharide, collaborate on projects to alter the gel's microstructure, increase its mechanical stability, and modulate the kinetics of drug release. Combinations like this proposal an efficient method to create strong, long-lasting ocular delivery systems.[7]

A standard treatment for bacterial conjunctivitis and additional disorders of the ocular surface is ofloxacin, a broad-spectrum fluoroquinolone antibiotic. Its traditional aqueous dosage dosage forms have the previously noted quick clearance limits, which need numerous daily instillations despite their strong antibacterial action.[8] By enhancing ocular concentration and permitting regulated drug release, an in-situ gelling formulation of ofloxacin could potentially enhance therapeutic efficacy and dosage convenience while addressing these negative aspects.[9]

Using sodium alginate and locust bean gum, the present moment work was intended to develop, manufacture, and analyze an ion-activated in-situ gelling system for ofloxacin. Key physicochemical the requirements, such as clarity, pH, drug content homogeneity, rheological behavior, in-vitro release profile, release kinetics, and antibacterial effectiveness, were comprehensively assessed for the formulations. Establishing an ideal formulation that could sustain drug release while maintaining Ofloxacin antibacterial efficacy was the key objective in order to provide an acceptable alternative for traditional ocular drops.[10]

MATERIALS AND METHODS

Materials

ARTEE Life Science (India) provided the ofloxacin hydrochloride. Pioneer Chemicals Co. contributed locust bean gum, while Fine Chemicals (India) issued sodium alginate. The preservative utilized was benzoalkonium chloride. Analytical grade sodium hydroxide (0.1 N) and glacial acetic acid were present. The investigation was conducted using distilled water. The remaining chemicals and reagents were all analytical grade and were used precisely as supplied.

Preparation of Ofloxacin In-Situ Gel

Table 1 lists the specific ingredients of the five in-situ gelling formulations (F1–F5) loaded with ofloxacin. With the goal of ensuring solution homogeneity and polymer hydration, the preparations were performed out in order of procedure. Initially, the designated amount of sodium alginate was dissolved in distilled water while continually being agitated by a magnetic stirrer and heated to a moderate temperature (40–45 °C) until a transparent, completely hydrated solution was achieved. In parallel, a locust bean gum solution was made by dissolving the polymer in distilled water and then heating it to 80–85 °C while stirring constantly to accomplish total dissolution. Following cooling to room temperature (25 ± 2 °C), both polymer solutions were mixed together with moderate stirring to create a homogenous polymeric mixture. To improve water solubility, ofloxacin hydrochloride, which is corresponding to 0.3% w/v of the base drug, was dissolved in a small amount of diluted acetic acid (1% v/v). Using 0.1 N sodium hydroxide, the pH of this medication solution was systematically adjusted down to 6.5 ± 0.1. As a preservative, benzoalkonium chloride (0.02% v/v) was added and well mixed. In order to make certain a uniform dispersion, this drug-preservative solution was then gradually added to the polymeric mix while constantly stirring the mixture. Each formulation's final volume was increased by 100 mL using distilled water, and stirring was preserved until a transparent and clear solution was obtained. The final formulations were autoclaved for 15 minutes at 121 °C (15 pressure) to achieve terminal sterilization after being aseptically wrapped into sterile amber-colored glass vials.

Table No.1: Composition Of Ophthalmic In-Situ Gel Containing Ofloxacin

S.No	Ingredients	F1	F2	F3	F4	F5
1	Ofloxacin	0.3	0.3	0.3	0.3	0.3
2	Sodium Alginate(gm)	0.5	0.75	1	1.25	1.5
3	Locust bean gum	0.1	0.1	0.1	0.1	0.1
4	NaCl (gm)	0.9	0.9	0.9	0.9	0.9
5	Benzalkonium chloride (gm)	0.02	0.02	0.02	0.02	0.02
6	Distilled water	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml

Analytical Method Development

UV–Visible Spectrophotometric Method for Estimation of Ofloxacin

Determination of λmax

To define an identifiable analytical wavelength for quantification, UV-visible spectrophotometry was used for determining the absorption maximum (λ-max) of ofloxacin. Ofloxacin was precisely weighed, dissolved in methanol, and then diluted with distilled water to create a standard stock solution (100 µg/mL). To create a working solution with a concentration between 10 and 20 µg/mL,

this stock solution was then diluted with a methanol–water combination. Using a solvent blank as a reference, the spectra of this solution was taken captive using a UV-Visible spectrophotometer between 200 and 400 nm. A clear and significant absorption peak at 295 nm was found in the resulting spectra, and this was chosen as the λ-max for all spectrophotometric analyses that followed.[11]

Construction of Calibration Curve

A confirmed HPLC technique was used for developing a calibration curve for ofloxacin. Five concentration levels (50–150 µg/mL), which correspond to 50–150% of the 100 µg/mL working standard, have been obtained by serially diluting a 1000 µg/mL stock solution made by dissolving 100 mg of the Ofloxacin standard in the mobile phase. Three injections of each concentration were made. Plotting the mean peak area compared to concentration revealed excellent linearity throughout the range, with a correlation coefficient (r^2) satisfying predetermined validation specifications.[12]

Estimation of Drug Content and In-Vitro Drug Release

When required, collected aliquots were diluted with simulated tear fluid for the drug content and in-vitro diffusion experiments. At the specified analytical wavelength, the absorbance associated with each diluted sample was measured. Applying the sample's absorbance to the linear regression equation obtained from the calibration curve allowed for the determination of the appropriate Ofloxacin concentration. The proportion of the measured drug in relation to the formulation's theoretical quantity was used for representing the uniformity of drug content. The concentration measured at each predefined time interval was used to gradually compute the cumulative percentage of medication released for the release experiment.[13]

Drug–Polymer Compatibility Studies

Fourier Transform Infrared (FTIR) Spectroscopy

Ofloxacin and the formulation excipients have been evaluated for significant chemical interactions using Fourier Transform Infrared (FTIR) spectroscopy. Using the potassium bromide (KBr) pellet methodology, samples of pure Ofloxacin, individual polymers, and their physical mixes were made. Every sample was crushed onto a clear the hard drive after being evenly mixed with KBr powder. Spectral scans have been carried out between 4000 and 400 cm^{-1}. A careful comparison was made between the physical mixture's final spectrum and the spectra of its pure constituents. The lack of significant modifications such as the disappearance of distinctive drug peaks, notable changes in their wavenumber, or the formation of new absorption bands, which demonstrated no detrimental drug–polymer interactions, evidenced compatibility.[14]

Evaluation of In-Situ Gel Formulations[15]

Visual Appearance and Clarity

Every manufactured formulation was visually inspected for the presence of particle matter or undissolved residue against both black and white backgrounds in both ambient as well as focused light. In order to confirm both transparency and physical homogeneity, they were evaluated for color and clarity both before and after gelation, which has been developed by synthetic tear fluid.

pH Measurement

Using a calibrated digital pH meter, the pH of each formulation has been determined three times at room temperature (25 ± 1 °C). Using 0.1 M sodium hydroxide and moderate to high stirring, the formulations' pH were adjusted within the acceptable ophthalmic range (about 6.5) in order to ensure physiological compatibility while minimizing eye discomfort. For every single batch, the mean values were noted.

Drug Content Uniformity

By taking care to remove 1 mL of the in-situ gel formulation and diluting it with 100 mL of simulated tear fluid (STF) in a volumetric flask, the drug concentration was ascertained. STF was used to further dilute 1 mL of this solution to 10 mL. A UV-visible spectrophotometer was used to measure the absorbance of the resultant solution at 295 nm using STF as a blank. Using the previously created calibration curve, the drug concentration was computed, and the % drug content was ascertained.

Viscosity Measurement

In order to evaluate viscosity, a Brookfield DV-III Programmable Rheometer was used. To figure out rheological behavior, the viscosity of each formulation was evaluated at various spindle speeds. To evaluate the change in viscosity under physiological circumstances, simulated tear fluid was implemented. All formulations had a sol-to-gel transition when the pH was educated to 7.4. Analysis of the rheological behavior revealed that the formulations had pseudo-plastic (shear-thinning) properties, as evidenced by a drop in viscosity as the shear rate progressed.

Gelling Capacity

A small portion of the formulation was added to a test tube holding two milliliters of freshly produced simulated tear fluid that was kept at 37 °C in order to evaluate the gelling capability. The gelation time was noted and the gel formation was visually observed. Furthermore recorded was the amount of time the gel remained intact before dissipating.

In-Vitro Diffusion Study

In-vitro drug release from the formulations was evaluated using an egg membrane diffusion technique. A Franz diffusion cell's donor and receptor compartments got separated by a freshly made egg membrane. In order to replicate ocular surface conditions, the receptor compartment was filled with chemical-based tear fluid (pH 7.4) and kept at 37 ± 0.5 °C while being constantly stirred with a thermostatic magnetic stirrer. Each formulation was placed in the donor chamber in a defined volume. To maintain sink conditions, 1 mL aliquots were taken out of the receptor compartment at scheduled hourly intervals for 8 hours and immediately replaced with an equivalent volume of brand-new, pre-warmed medium. Using spectrophotometry, the amount of ofloxacin in samples that have been taken was measured at 295 nm. A pre-established standard calibration curve was used to figure out the cumulative percentage drug release (%CDR) compared with time.

Sterility Testing

The agar plate technique was used to check the autoclaved formulations' sterility. Three experimental sets were implemented in the analysis: (i) a negative control, which confirmed the media's sterility by using sterile agar medium alone; (ii) a positive control, which involved inoculating sterile agar medium with Staphylococcus aureus ATCC 25923 in order to verify the medium's ability for encouraging growth; and (iii) a test sample, in which the sterilized formulation was aseptically transferred onto the sterile agar medium. Following 48 hours of aerobic incubation at 37°C, each plate was examined for microbial growth. The absence of any microbial colonies indicated that the test formulation was sterile, whereas the positive control demonstrated strong growth.

RESULT AND DISCUSSION

All of the manufactured Ofloxacin in-situ gel formulations (F1–F5) showed homogeneous polymer dispersion, no apparent particle matter, and a light yellow, clear appearance. Both before and after gel formation, the formulations remained clear, demonstrating adequate physical stability and uniformity.

Determination of Absorption Maxima for Ofloxacin

The solvent used to create a standard solution of Ofloxacin (10–20 µg/mL) was a combination of distilled water or a methanol–water compound. With a 1 cm quartz cuvette and a UV-visible spectrophotometer, the solution was scanned between 200 and 400 nm. The absolute maximum absorbance (λmax) was detected at 295 nm, and the recorded wavelength spectrum displayed a distinct peak. For the spectrophotometric study that followed, this wavelength was used for the quantitative estimation of ofloxacin.

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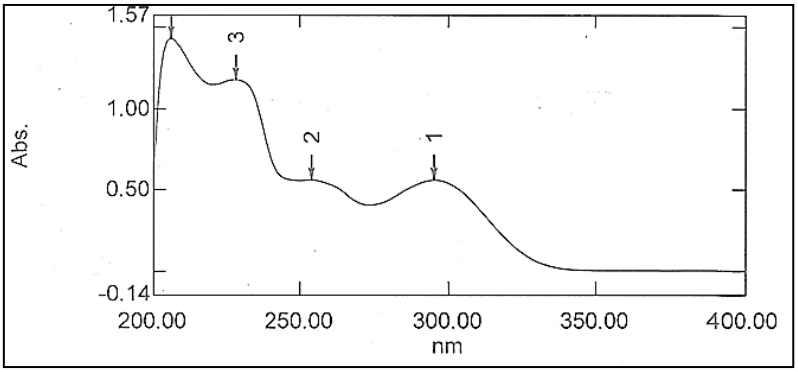

Figure No.1: λmax Scan of Ofloxacin

Calibration curve for Ofloxacin

To make sure precise quantification, a calibration curve for ofloxacin was created using chromatographic analysis using a high-performance liquid chromatography (HPLC) technology. After preparing a primary stock standard solution, it was meticulously serially diluted. As a result of this entire process, five different concentration levels - 50%, 80%, 100%, 120%, and 150% of the desired operating concentration—were produced, providing a thorough linear range for sample analysis and technique validation.

Table No.2: Linearity and Range

Sample ID	Concentration,in mcg/ml	Area obtained
50% of operating concentration	55	1266716
80% of operating concentration	88	1974256
100% of operating concentration	110*	2538484
120% of operating concentration	132	2983254
150% of operating concentration	165	3839230

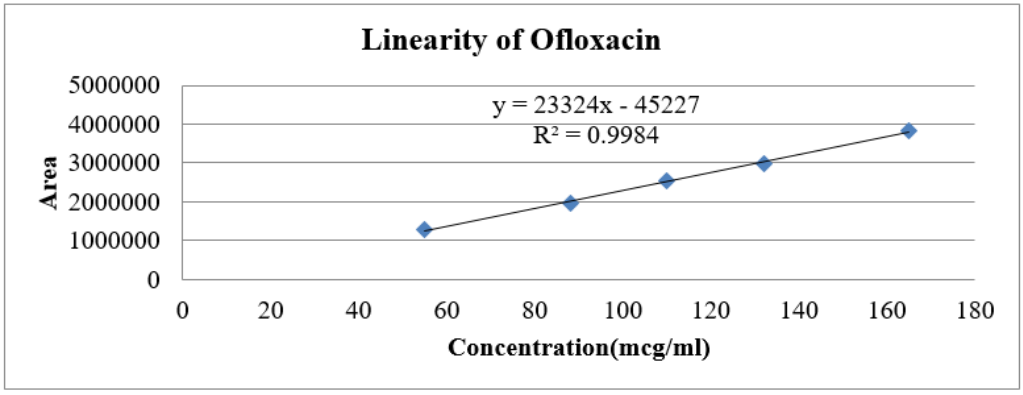

Figure No.2: Linearity of Ofloxacin

Report: The Correlation coefficient and y-intercept for the plot between concentration and response is found to be within limits.

Drug–Polymer Compatibility (FTIR Analysis)

The medication and formulation polymers were analyzed for any potential interactions using Fourier-transform infrared (FTIR) spectroscopy. Pure Ofloxacin's spectra showed its distinctive absorption bands. Without any noticeable changes, widening, or the emergence of additional peaks, the spectrum of the physical mixture including ofloxacin, sodium alginate, and locust bean gum displayed the compatible peaks of each component. The stability and physicochemical compatibility of the drug-polymer combination inside the developed in-situ gel system are demonstrated by this spectrum superimposition, which proves the lack of chemical interactions.

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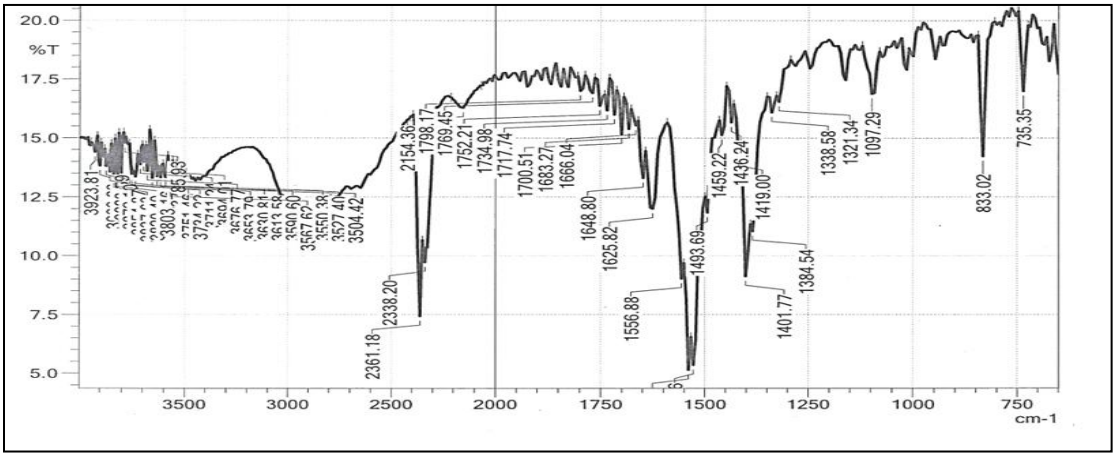

Figure No.3: FTIR Spectra of pure Ofloxacin

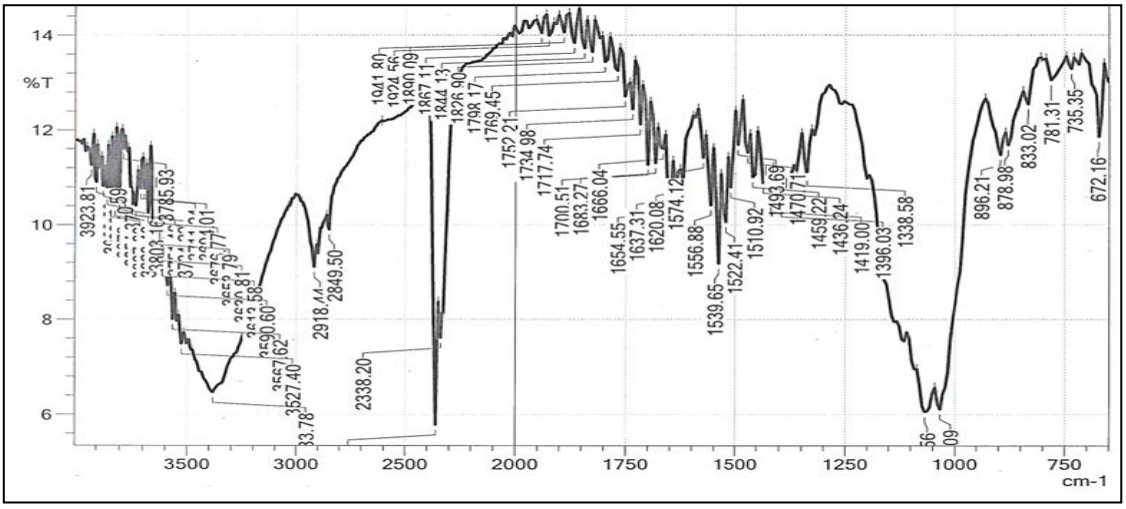

Figure No.4: FTIR Spectra of physical mixture of ofloxacin insitu gel

Evaluation of Physicochemical Properties

The best physical stability has been proven by the clear, light yellow, and particle-free created in-situ gelling formulations (F1–F5). There was a lesser possibility of ocular inflammation during delivery considering the measured pH varied from 6.4 to 6.7, which is well within the physiologically appropriate range for ophthalmic usage and compatible with the pH of lacrimal fluid. With a range of 99.3% to 99.7%, the drug content homogeneity was consistently high across batches, confirming both the manufacturing process's repeatability and the uniform drug distribution inside the polymeric technology.

Visual Appearance and Clarity

All of the manufactured in-situ gelling formulations (F1–F5) had a uniform, pale yellow look and maintained their optical clarity without showing any signs of turbidity or particle contamination. The full solubilization of Ofloxacin and the even dispersion of the polymeric components have been identified as by the constant maintenance of this clarity in both the pre-gelation solution and post-gelation phases. The formulations' physical stability and efficacy for ocular administration are further supported by the lack of precipitation or cloudiness during the study.

pH of the Formulations

The produced formulations' measured pH values fluctuated between 6.4 and 6.7 (Table 3). Patient acceptance and ocular compatibility can be processed by these values, which nearly match the normal pH of human lacrimal fluid and ensure that the formulations won't cause irritation or pain when administered ocularly.

Drug Content Uniformity

Drug content analysis showed values ranging from 99.3% to 99.7% (Table 3), indicating uniform distribution of Ofloxacin within the polymeric matrix and high reproducibility of the formulation process. This small range shows low batch-to-batch alterations or processing loss, as well as constant and effective drug integration into the polymeric matrix. The outcomes validate the stability and compatibility of the system of locust bean gum and sodium alginate, which successfully maintained ofloxacin without loss of effectiveness. Additionally, the high level of content homogeneity confirms that the analytical process used is reliable.

Viscosity and Rheological Behavior

As shown in Table 3, viscosity research showed an established relationship between formulation viscosity and sodium alginate content. Formulation F5, which had the most significant concentration, had a much higher viscosity of 48 cps, indicating a more extensive polymer network and superior cross-linking density, whereas Formulation F1, which had the lowest polymer content, had a viscosity of 14 cps. A fundamental property for ocular distribution, pseudoplastic (shear-thinning) rheology was demonstrated by all formulations. The unique feature makes it easier to be embedded as a low-viscosity fluid throughout blinking, and it quickly recovers to a higher-viscosity gel when the shear stops, extending the corneal contact duration. Although F5 had the strongest gel, its high viscosity might cause blurred vision and adversely impact patient comfort. Formulations F3 (28 cps) and F4 (36 cps), on the other hand, offered the best balance between ocular tolerability and viscosity, providing enough for prolonged drug release.

Gelling Capacity

Following interacting with simulated tear fluid, all formulations showed an ion-activated sol-to-gel transition. Gelling capability was evaluated qualitatively, and Table 3 shows the results of the investigation. In addition to exhibiting instantaneous gelation (+++), formulations F3, F4, and F5 continued to exhibit structural integrity throughout time. F1 and F2, on the other hand, produced gels of a moderate strength (++). Increased ionic cross-linking of sodium alginate chains in the presence of cations in the tear fluid is responsible for the improved gelling performance seen at higher polymer concentrations. despite the fact that formulation F3 gelled quickly, it struck the ideal balance between gel strength and consistency, encouraging prolonged ocular development without adding too much viscosity that could be uncomfortable for patients.

Table No.3: Physicochemical Evaluation of Ofloxacin In-Situ Gel Formulations

S. No	Formulation	pH	Drug Content (%)	Viscosity (cps)	Gelling Capacity
1	F1	6.7	99.62	14	++
2	F2	6.6	99.66	21	++
3	F3	6.4	99.43	28	+++
4	F4	6.5	99.38	36	+++
5	F5	6.5	99.76	48	+++

In-Vitro Diffusion Studies

All formulations showed a sustained release profile over an 8-hour period, corresponding to the cumulative drug release data (Table 15). A clear inverse correlation was observed between polymer concentration and the extent of drug release. As a result of its lower viscosity and less thick gel matrix, which allowed for comparatively unencumbered drug diffusion, formulation F1, which had the lowest polymer concentration, had the highest cumulative release (78.6%). As a result of its high viscosity and the creation of a very cohesive gel network that significantly impeded molecular diffusion, formulation F5, which had the greatest polymer content, on the other hand, had the most controlled release (71.4%).

Formulation F3 (1.0% sodium alginate) carried the best and most controlled release profile among the studied variations, attaining 74.9% cumulative release at the 8-hour endpoint. The needs for long-term therapeutic availability at the ocular surface and less time spent of an early burst impact are well balanced by this intermediate release rate. The amount of effective design of an ion-activated in-situ gelling platform for extended ocular administration is supported by these collective release kinetics. Formulation F3 was chosen as the best option because to its regulated release profile, constant gelation, as well as beneficial rheological attributes.

Table No.4: In-Vitro Diffusion Studies of F1- F5

S.No	Time (h)	F1	F2	F3	F4	F5
1	1	12.9	12.4	12	11.3	10.6
2	2	26.1	25.4	24.7	23.5	22.4
3	3	37.9	36.6	35.3	34.1	32.9
4	4	49.1	47.7	46.4	44.9	43.5
5	5	58.3	56.9	55.6	53.7	51.9
6	6	66.7	65.1	63.5	61.8	60.2
7	7	73.3	71.7	70.2	68.5	66.8
8	8	78.6	76.7	74.9	73.1	71.4

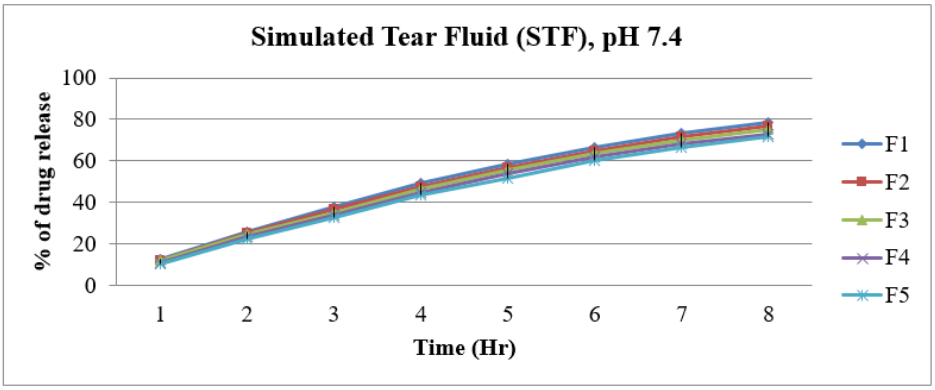

Figure No.5: In-Vitro Diffusion Studies of F1- F5

Kinetic Modeling of Drug Release

Higuchi, Korsmeyer–Peppas, zero-order, and first-order kinetic models were used to evaluate the in-vitro release profiles. With correlation coefficients (R2) above 0.98 for all models, all formulations showed excellent model fitting (Table 16). First-order (≈0.998–0.999) and Korsmeyer–Peppas (≈0.998–0.999) kinetics demonstrated the strongest concurrence with the release data, suggesting a primarily concentration-dependent release mechanism mediated by anomalous (non-Fickian) diffusion. In particular, a dual release mechanism involving both drug diffusion and polymer relaxation was confirmed by the enhanced the formulation (F3), which showed a strong match to these models. The present study confirms that the drug concentration gradient and the ion-activated gel matrix's swelling and erosion dynamics both control the sustained-release behavior at the same time.

Table No.5: Kinetic Modeling of Drug Release

S. No	Formulation	Zero-Order R^2	First-Order R^2	Higuchi - Order R^2	Korsmeyer–Order R^2
1	F1	0.9807	0.997	0.9983	0.9993
2	F2	0.9811	0.9977	0.9981	0.9987
3	F3	0.9817	0.998	0.9977	0.9982
4	F4	0.983	0.9982	0.9977	0.9987
5	F5	0.984	0.9983	0.9976	0.9989

Antibacterial Activity

The antibacterial efficacy of the optimized Ofloxacin in-situ gel formulations was assessed using a standard agar well diffusion assay against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). All tested formulations produced well-defined zones of inhibition, the diameters of which were statistically comparable to those generated by a pure Ofloxacin reference standard. These findings demonstrate that the antimicrobial activity of Ofloxacin was not compromised by its encapsulation within the ion-sensitive alginate-locust bean gum polymeric matrix. The results confirm that the developed in-situ gel system successfully retains the drug's intrinsic antibacterial potency against ocular pathogens from both Gram classifications.

Table No.6: Antibacterial Activity

Microorganism	Zone of Inhibition(mm)					
	Standard (Pure Drug)	F1	F2	F3	F4	F5
S. aureus	29	28	28	28.6	26.5	27.5
E. coli.	30	27.5	28.5	29.4	27.5	29.5

Summery and Conclusion

An ion-activated ophthalmic in-situ gelling system of Ofloxacin was successfully formulated using Sodium Alginate and Locust Bean Gum to address the rapid precorneal clearance associated with conventional eye drops. A robust formulation stability and batch-to-batch repeatability have been demonstrated by the favorable physicochemical features of all generated formulations, which included physiologically suitable pH (6.4–6.7), optical clarity, and uniform drug content (99.3–99.7%). Rheological analysis verified pseudoplastic flow, a desired property that enables simple instillation followed by quick gelation upon contact with tear fluid, and an appropriate increase in viscosity was reported with increasing polymer content. The in-situ gelling technique was confirmed by ion-triggered gelation in simulated tear fluid. Because a gradually denser hydrogel matrix forms, in-vitro release experiments showed sustained drug diffusion over an 8-hour period, with release kinetics inversely corresponding to polymer concentration. Based on its balanced rheological profile, sufficient gel strength, as well as controlled drug release (74.9% over 8 hours), formulation F3, incorporating 1% sodium alginate, was determined to be the best. First-order and Korsmeyer–Peppas models provided the greatest description of drug release kinetics, confirming a diffusion-coupled, non-Fickian release mechanism. Importantly, testing for bacteria against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) strains proved that the improved gel maintained antimicrobial potency on rate with pure Ofloxacin solution. By spreading corneal residence time, maintaining therapeutic drug levels, modifying dosing frequency, and ultimately improving clinical efficacy and patient adherence, these outcomes collectively establish that the developed in-situ gelling system is a promising vehicle for ocular delivery of Ofloxacin.

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