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In Situ Gelling System Loaded with Lomefloxacin for Effective Management of Ocular Surface Bacterial Infections

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Abstract

In order to treat bacterial infections on the surface of the eye, this research study looks at the creation and improvement of a novel in situ gelling system for the controlled and prolonged release of the antibiotic memefloxacin, a fluoroquinolone. To expand the bioavailability of mefloxacin hydrochloride, the ongoing work set off to create and survey a polymeric visual in situ gel framework involving in situ polymers that show a reversible fluid gel stage progress. Poloxamer 407 and hydroxyl propyl methyl cellulose are utilized as mucoadhesive polymers in fluctuating proportions to make in situ gels. The medication fixation, clearness, pH, gelation temperature, thickness, antimicrobial movement, sterility testing, and visual eye aggravation test were undeniably surveyed for the in situ gels. The incompatibilities of drugs and polymers were determined using FT-IR spectroscopy. Between 92.04% and 99.33% of the drug was cumulatively released from the formulations at 10 hours, with the drug being released via the diffusion process. The outcomes showed that the concentration of polymers utilised affected the degree of gelation and medication release. It was found that the enhanced plan (PH5) had the ideal pH and gelation temperature required for an in situ gel drug conveyance framework. The consequences of this study are expected to give significant new data to the improvement of refined visual medication conveyance frameworks, giving a practical way to deal with the productive treatment of bacterial diseases of the visual surface.

Keywords: *Gelation temperature, , in situ gels, Lomefloxacin hydrochloride, poloxamer 407.*

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1. INTRODUCTION

The rise in bacterial infections on the surface of the eyes presents a major obstacle to the provision of ophthalmic healthcare, requiring the creation of novel therapeutic strategies to improve treatment results. The goal of this research is to address this problem by designing and optimising an in situ gelling system that is loaded with the powerful fluoroquinolone antibiotic lomefloxacin for the efficient treatment of bacterial infections on the ocular surface. If ocular infections are not promptly and effectively treated, they can result in serious problems. These infections are frequently caused by organisms including Staphylococcus aureus and Pseudomonas aeruginosa. The continuous release of drugs and the removal of obstacles to ocular medication absorption are two challenges faced by conventional eye drops and ointments. The suggested in situ gelling method provides a potential remedy for these issues, hoping to increase therapeutic efficacy and patient compliance.

This in situ gelling system is formulated using thermoresponsive polymers, which enable the liquid formulation to change into a gel upon contact with the ocular surface, extending the duration of drug release and retention. Lomefloxacin was selected as the antibacterial agent due to its broad-spectrum activity and demonstrated effectiveness against a variety of eye infections. The main goal of the study is to thoroughly characterise the in situ gelling system, taking into account variables such drug release kinetics, viscosity, and gelation temperature. Using this method guarantees the creation of a precise and optimised formulation that strikes a balance between prolonged medication delivery and simplicity of administration.

Apart from the physicochemical characterisation, the investigation also includes in vitro and in vivo assessments to evaluate the developed in situ gelling system's biocompatibility, antibacterial activity, and ocular tolerance. The research attempts to provide a thorough understanding of the system's performance by conducting a thorough assessment of these factors, leading its possible translation from the laboratory to clinical applications. In the end, the goal of this research is to bring new knowledge to the field of ocular medicine administration and promote developments that could greatly enhance the prognosis for patients with bacterial infections of the ocular surface.

2. LITERATURE REVIEW

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In order to treat glaucoma, Arora and Singh (2023) look at the formulation, characterization, and use of an in situ gel containing bimatoprost. One of the main causes of permanent blindness, glaucoma, necessitates consistent and accurate medication administration to the ocular tissues. The goal of the study is to optimise the formulation for increased therapeutic efficacy while examining the difficulties involved in incorporating bimatoprost into an in situ gel. The scientists employ a thorough methodology that incorporates a range of physicochemical characterizations, including as stability evaluations, in vitro drug release patterns, and rheological investigations. The discoveries of this study add to the improvement of visual medication conveyance frameworks by offering savvy data on the definition of in situ gels containing bimatoprost.

In their exploration of the various uses of chitosan-based in situ gelling systems for drug delivery, Das et al. (2022) highlight the systems' potential for use in ocular applications. Because of its mucoadhesive, biodegradable, and biocompatibility qualities, chitosan—which is derived from chitin—is a desirable substance for ocular medication administration. The paper provides a thorough analysis of the several chitosan-based in situ gelling systems and how they are used in medication delivery. Das et al. give a careful outline of the condition of chitosan-situated in situ gels in visual medication conveyance by summing up ongoing turns of events, troubles, and expected future applications. Researchers and practitioners who want to use chitosan's potential to create efficient and patient-friendly ocular medication delivery systems may find this review to be a useful resource.

A thorough review of new developments in ocular medication administration is given by Garg, Kumar, and Sharma (2019), with an emphasis on in-situ ophthalmic gels. The review talks about the benefits of in-situ gelling devices and the problems with conventional ocular medication delivery techniques. The impact of different polymers and formulation procedures on the effectiveness of in-situ ophthalmic gels is examined by the authors. This survey is a helpful instrument for specialists and professionals who need to keep awake to date with the changing field of visual medication conveyance since it sums up ongoing turns of events, obstructions, and potential open doors for what's to come. The inclusion of case studies and current advancements in the field improves the review's relevance for readers looking for a thorough grasp of the topic.

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Singh, Kurkure, and Anardi (2020) concentrate on the creation and evaluation of an in-situ gelling system for ciprofloxacin sustained release ocular drug administration. The purpose of the study is to solve the problems with traditional drug administration methods for eye illnesses. The researchers hope to improve patient compliance and therapeutic efficacy by achieving continuous medication release through the use of in-situ gel-forming polymers. Comprehensive evaluations, such as stability assessments, in vitro release experiments, and physicochemical characterizations, are part of this study. The findings of this study provide important new information about formulation techniques for ocular drug delivery with sustained release, especially with regard to ciprofloxacin.

The synergistic application of in-situ gelling polymers and nanomicellar technology as an ocular drug delivery system (ODDS) for cyclosporine-A is investigated by Terreni et al. (2021). By integrating two cutting-edge drug delivery technologies, this study takes a novel approach to improving the bioavailability and therapeutic effects of cyclosporine-A in ocular disorders. In vitro release kinetics, ocular penetration experiments, and a thorough examination of the formulation's physicochemical characteristics are all included in the research. The study intends to establish a platform for sustained drug release, increased corneal penetration, and improved treatment results by combining nanomicellar technology with in-situ gelling polymers.

3. MATERIALS AND METHOD

3.1. Materials

The lovely people at M/S Nakoda Pharmaceuticals (Hyderabad, India) provided memefloxacin hydrochloride. Sigma Aldrich (Gattefosse, India) provided hydroxyl propyl methyl cellulose (HPMC) and poloxamer 407. Analytical grade solvents, reagents, and other compounds were all utilised.

3.2. Preparation of in situ gels

Cold technique was used to prepare in situ gels containing methofloxacin hydrochloride. To put it briefly, a weighted quantity of poloxamer was dissolved in cold, ultrapure water and kept for 12 hours at 6˚C in the refrigerator. The soaked polymers solution was mixed with weighed amounts of HPMC, lomefloxacin hydrochloride, benzalkonium chloride, and sodium chloride

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(Table 1). The mixture was then agitated for 15 minutes at 300 rpm using a magnetic stirrer. A 0.1N sodium chloride solution was used to bring the formulations' pH down to 7.4. Using distilled water, the final volume was adjusted to 2 millilitres. Following preparation, every formulation was kept refrigerated until assessment.

3.3. Drug content

After precisely measuring 100µl of formulations in a test tube and appropriately diluting them with STF to achieve a concentration of 10µg/ml, the drug content was ascertained. A UV Visible spectrophotometer set at 281 nm was used to measure the drug's concentration. Every experiment was carried out three times.

3.4. Determination of pH

As soon as the formulations were prepared, the pH of each was measured with a digital pH metre that was calibrated. Every experiment was carried out three times.

3.5. Gelation temperature

One milliliter of the pre-arranged details was placed in a test cylinder to accomplish the ideal gelation temperature. The test tubes were set in a water shower at 4˚C subsequent to being parafilm-fixed. The water bath's temperature was raised by 1˚C increments, and each new temperature setting was given 15 minutes to acclimatise. The temperature was noted and the examples were checked for gelation, which happens when the test cylinder's meniscus becomes stationary when shifted more than 90˚ C. Every experiment was carried out three times.

3.6. In vitro drug release studies

Test samples were placed in 2 cm diameter cylinder test tubes that were open at both ends. One end of the glass cylinder was coupled with a dialysis membrane (cut off 12000 MW) that had been previously soaked for 12 hours in STF (pH 7.4). A precise weight and placement of the 0.5 ml volume of the formulation followed. Instead of a paddle, a glass cylinder was fastened to the USP apparatus II's shaft. After that, the cylinder was suspended in 50 millilitres of dissolving media that was kept at 34 ± 0.5°C and 25 revolutions per minute so that the dialysis membrane was barely touching the medium. Samples were taken out at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours, and were replaced with equivalent volumes of medium that

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were analysed for drug content at each time using a UV Spectrophotometer at 281 nm after being suitably diluted with STF. The drug's % release was calculated. The experiment was run three times.

3.7. Ex vivo corneal permeation

Studies on the ex vivo corneal penetration of both the standard medication solution and the optimised formulations were conducted. Goat corneas were used as the permeation membrane to simulate in vivo environments. The modified USP apparatus II paddle method was used to conduct ex vivo drug release experiments for formulations, and STF (pH 7.4) was used as the dissolution medium. After being isolated and soaked in STF (pH 7.4), the cornea was attached to one finish of the glass chamber and loaded up with 0.5 milliliters of the definition. As mentioned in the in vitro drug release approach, penetration investigations were carried out. The experiment was run three times.

3.8. Autoclaving sterilization

The United States Pharmacopeia's (USP) recommended autoclaving sterilisation settings were applied to the chosen formulations in order to investigate the impact of this process on the physicochemical properties of in situ gels. In outline, 20 minutes of autoclaving at 121˚C and around 15 psi pressure were applied to screw cap test tubes holding 10g of medication stacked in situ gel. Prior to being put through autoclaving, the plans were surveyed for physicochemical qualities, for example, stream capacity, rate marked amount, pH, sol-gel change temperature, and in vitro drug discharge.

3.9. Release mechanisms

The drug release mechanism of synthesised in situ gels was investigated by fitting in vitro and ex vivo permeability data to several models: the zero-order model, the first-order model, the Higuchi release model, and Korsemeyer and Peppa's model. The model with the most noteworthy relationship coefficient was considered to be the most proper for this reason.

4. RESULTS AND DISCUSSION

4.1. Preparation of in situ gels

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A combination of polaxomer 407 and HPMC was used in the cold procedure to produce the in situ gels of lomefloxacin hydrochloride. The fact that the created in situ gels were transparent indicated that all of the components had been fully dissolved in the vehicle at both room temperature and refrigeration. It was discovered that every formulation was transparent, clear, and devoid of any suspended particle matter.

Table 1: Formulas for chitosan in situ gel formulations with Poloxamer 407

Formulation code	Poloxamer407 (%w/v)	Chitosan (% w/v)
PH1	16	0.7
PH2	16	1.2
PH3	16	1.7
PH4	18	0.7
PH5	18	1.2
PH6	18	1.7
PH7	20	0.7
PH8	20	1.2
PH9	20	1.7

Table 1 lists the in situ gel formulations that include chitosan and Poloxamer 407; the codes corresponding to these formulations are PH1 through PH9. The concentrations of chitosan (%w/v) and poloxamer 407 (%w/v) for each formulation are shown in the table. Notably, there are three distinct concentrations of Poloxamer 407(16%), 18%, and 20%), and there are three increasing amounts of chitosan content (0.7%, 1.2%, and 1.7%). This systematic variation provides a useful basis for comprehending the formulation's impacts on drug delivery and possible treatment results by enabling a thorough investigation of the influence of these components on the in situ gel characteristics.

4.2. Drug content, pH and Gelation temperature

Involving reproduced tear liquid as a clear, the medication content for the delivered plans was estimated utilizing an UV-Noticeable spectrophotometer at 281 nm. There was a drug content ranging from 85 to 94 percent.The pH of the different formulations varied between 8.43±1.04 and 8.39±1.06, and the gelation temperature varied between 28.2±1.15˚C and 39.7˚±1.14˚C. Table 2 displays the corresponding data.

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Table 2: Analysis of in situ gels using chitosan and poloxamer 407

Code	Drug Content (%)	pH	Gelation Temperature
PH1	86.5±2.7	8.39±1.04	39.7^0C±1.73
PH2	84.2±2.26	8.6±1.09	39.2^0C±1.16
PH3	88.8±2.7	8.34±1.03	38.7^0C±1.17
PH4	93.2±2.88	8.42±1.04	36.7^0C±1.77
PH5	88.5±2.34	8.43±1.03	35.7^0C±1.16
PH6	88.2±2.80	8.40±1.07	36.7^0C±1.19
PH7	87.8±3.06	8.44±1.07	28.7^0C±1.74
PH8	93.2±3.58	8.42±1.14	29.2^0C±1.05
PH9	92.5±2.87	8.43±1.05	28.2^0C±1.78

The assessment of chitosan and Poloxamer 407 in situ gels (PH1 to PH9) is presented in Table 2. The medication content varies between 84.2% and 93.2% in the formulations, but the pH levels remain appropriate (8.34 to 8.6) for topical application. The gelation temperatures of the formulations range from 28.2°C to 39.7°C, demonstrating that they can gel at physiologically relevant temperatures. The selection of the best in situ gel formulations for prospective ocular drug delivery applications is guided by these data, which provide essential insights into the formulation features.

4.3. In vitro drug release method

To investigate the release mechanism, the in vitro drug release data was fitted into various release kinetics. The factors taken into consideration to assess which model best fits the drug's release kinetics were K and r2. The best model was determined to be Peppa's, with a r2 value of 0.99. portion of the drug release from in situ gel may come from the gels diffusing the drug, and portion may come from the gels dissolving simultaneously in the surrounding dissolving environment (Table 3). Conditions for drug release in the eye can differ significantly between in vitro and in vivo settings. Nonetheless, the in vitro findings unequivocally show that the gels can hold onto the medication for an extended amount of time. As a result of the shearing activity of the eyelids and movements of the eyeball, the gel in the circular drive might break down more rapidly.

Table 3: Release kinetics of in situ gels in vitro

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Formulation	ZERO ORDER		FIRST ORDER		HIGUCHI		PEPPA'S	
	r^2	K	r^2	K	r^2	K	r^2	K
PH1	0.953	23.32	0.941	4.094	0.992	10.452	0.993	0.347
PH2	0.983	18.39	0.910	4.152	0.976	14.58	0.974	0.347
PH3	0.963	20.23	0.962	4.091	0.991	13.78	0.996	0.235
PH4	0.943	20.04	0.956	4.114	0.987	15.83	0.991	0.119
PH5	0.982	16.27	0.877	4.234	0.994	19.17	1.000	0.086
PH6	0.961	13.48	0.981	4.092	0.985	21.93	0.980	0.115
PH7	0.979	11.138	0.973	4.097	0.985	23.37	0.983	0.139
PH8	0.991	7.565	0.940	4.134	0.969	26.64	0.978	0.202
PH9	0.984	7.490	0.935	4.132	0.966	27.37	0.983	0.239
PH5(ex vivo)	0.980	8.490	0.975	3.992	0.971	6.400	0.981	0.373

The in vitro release kinetics of the PH1 through PH9 in situ gel formulations are shown in Table 3. With high correlation coefficients (r2), the results show good fitting to a variety of models. Release rate constants (K) differ amongst formulations, suggesting different drug release behaviours. For the purpose of customising and improving medication delivery methods in ocular applications, this data is useful.

4.4. Ex vivo corneal permeation

For the optimised formulations, in vivo investigations were carried out (PH5). By keeping the artificial eye conditions constant, the amount of medication that entered the cornea's 10 mm diameter was determined. For the optimised formulation, the cumulative percent released during corneal permeation was 60.96% in 10 hours, while for the pure medication, it was released approximately 83.76% in 2 hours. To investigate the release mechanism, the ex vivo permeation data was fitted into various release kinetics. The best model was determined to be Peppa's, with a r2 value of 0.99 (Table 3). Diffusion was thought to be the primary mechanism of the release based on the n value.

4.5. Autoclaving sterilization

We compared the drug content and pH of the optimised formulation before and after autoclaving it at 121ºC and 15 lb pressure for 20 minutes to see how the process affected the formulation. Table 4 shows that there was no substantial change in the drug content or pH following autoclaving, suggesting that the formulation was stable in these respects.

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Table 4: Autoclave's impact on in situ gels (PH5)

	Before Autoclaving (n=3)	**After Autoclaving (n=3)**
Assay (%)	89.5±2.34	87.8±2.7
pH	9.43±1.03	9.26±1.05

The effect of autoclaving on in situ gel formulation PH5 is shown in Table 4. The initial drug content was shown by the test %, which was 89.5±2.34 prior to autoclaving and reduced marginally to 87.8±2.7 following autoclaving. After autoclaving, the pH values, which were originally 9.43±1.03, slightly decreased to 9.26±1.05 and were nevertheless within an acceptable range for ocular applications. With careful attention to drug concentration and pH as crucial criteria, these results support the in situ gels' potential application in clinical settings by indicating that they maintain stability following autoclaving.

5. CONCLUSION

Using an in situ gelling system that has been loaded with omefloxacin is a viable and successful approach to treating bacterial infections of the ocular surface. Notable characteristics of the designed gels included improved ocular residence duration, increased bioavailability, and sustained drug release. Poloxamer 407 and chitosan were used to create a new pH and thermoreversible in situ gel formulation of Lomefloxacin Hydrochloride. Compatibility investigations were conducted and the prepared formulations assessed. The results of the in vitro and ex vivo investigations showed that the created formulation maintains concentrations for a longer period of time by releasing for more than 10 hours. Because of its capacity to increase patient compliance and bioavailability, the developed in situ gel presents a viable substitute for traditional eye drops. Overall, the results highlight the exciting potential of this novel ocular drug delivery system, providing an effective and regulated method for treating bacterial infections of the ocular surface, thus meeting a vital demand in ophthalmic therapies. To confirm these results and prepare the path for the in situ gelling system's translational application in ocular healthcare, more clinical research is necessary.

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