ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v20i2.7

Nig. J. Pharm. Res. 2024, 20 (2) pp 185-195
ISSN 0189-8434 e-ISSN 2635-3555

Available online at http://www.nigjpharmres.com

Formulation and Characterization of Stimuli-Responsive In Situ Gel For Treatment of Bacterial Keratitis

U.N. UBANI-UKOMA[A-F*], O.A. ENISENYIN[B,C,E,F], O.J. OYEWALE[B,C,E,F], P.A. NWANKWO[B,C,E,F]

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The aim of this study is to develop an in-situ gelling system for the effective delivery and sustained-release of levofloxacin in the treatment of anterior corneal infections.

Material and Methods: Sodium alginate (SA) and hydroxypropyl methylcellulose (HPMC) were used to formulate in-situ gels (ISGs) with formulation codes F1-F12 and 0.5% levofloxacin solution was used as a control. The formulations were evaluated for clarity, pH, gelling capacity, drug content and antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Staphylococcus aureus* using the Kirby-Bauer disk diffusion test. The irritability and toxicity of select formulations were assessed using the Hen's Egg Test, Chorioallantoic Membrane (HETCAM) assay.

Results: The formulated ISGs were within pH range of 5.86 and 7.60. Formulation F7 (0.5% SA + 1.5% HPMC) had the highest gelling capacity and all ISGs had comparable activity against the tested organisms. Formulation F6 (1% HPMC + 1% SA) had the slowest release with approximately 60% release after 4 h, formulation F11 (1.5% HPMC + 2% SA) had the fastest release of 72% after 4 h while LVF solution (control) released 70% in 1 h. There was no significant ($p = 0.101$) change in the concentration of levofloxacin ISGs after storage at 25° C for 60 days and the HETCAM test confirmed the non-toxicity and non-irritability of the formulations.

Conclusions: Levofloxacin *in-situ* gels formulated with SA and HPMC E5 LV are able to sustain the release of levofloxacin for 8 h and retain their effectiveness against relevant ocular bacterial infections.

Keywords: In-situ gel, Sustained-release, Ocular delivery, Levofloxacin, Antimicrobial efficacy

INTRODUCTION

Microbial keratitis is a major cause of visual impairment in the world and in the developing world, it is estimated that 1.5 to 2 million or more people are diagnosed with keratitis annually (Alshehri *et al.*, 2016). It can be caused by bacteria, viruses, fungi and parasites however, bacterial keratitis is the most common form of microbial keratitis (Stapleton, 2023). Infection by these organisms usually occurs when the epithelium is compromised (Farahani *et al*., 2017; Robertson *et al*., 2017). The main causative agents of bacterial keratitis are *Staphylococcus aureus*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa* with *P. aeruginosa* being the most prevalent causative agent (Robertson *et al.*, 2017). Treatment initially was with fortified antibiotics – a cephalosporin and an aminoglycoside to cover the gram-positive and gram-negative organisms respectively but in the last decade, fluoroquinolones are the mainstay of empirical treatment (Höfling-Lima *et al.*, 2014). The use of fortified antibiotics though

effective had some challenges that led to the change to fluoroquinolones. Treatment required half-hourly to hourly (Gokhale, 2008) administration of the two antibiotics which exposed the cornea to increased toxicity. Reflex tear secretion due to increased tonicity of the antibiotics will cause dilution of the dose and special mixing of each of the medications by a pharmacist will increase cost and the likelihood of contamination (Gangopadhyay *et al.,* 2000). Consequently, the use of a single antibiotic with broad-spectrum activity against most organisms was clearly preferred.

Fluoroquinolones are topoisomerase inhibitors which results in the inhibition of bacterial DNA. Levofloxacin is a fourth-generation, broad spectrum antibiotic fluoroquinolone indicated for the treatment of conjunctivitis, bacterial keratitis and keratoconjunctivitis *(*Gupta *et al., 2015;* Jain *et al., 2020)*. It is marketed as a 0.5% or 1.5% levofloxacin eye drop in Nigeria and is administered as 1 – 2 drops every 30 mins to 2 h in the first 2 days while awake, and subsequently, 1 – 2 drops every 4 h from days 3 – 7 while awake.

Medications for eye diseases are best administered locally to minimize adverse systemic effects. This requires the direct instillation or injection of the drug into the eye via topical dosage forms or parenteral formulations to the back of the eye. Some of the ocular routes are topical, intravitreal, intracameral and subconjunctival routes. Parenteral routes require a high level of expertise while the topical routes require frequent administration to overcome their low retention and low bioavailability (Chandra *et al*., 2022). Oral administration of medications for treatment of ocular diseases results in an ocular bioavailability of approximately 2% while topical formulations instilled in the eye is about 5% (Chandra *et al.,* 2022).

Eye drops are the most prescribed ocular formulation because of their ease of administration and high availability (Franco & De Marco, 2021). The ocular barrier to foreign bodies however had led to the low bioavailability of eye drops (Zafar *et al.,* 2022; Zhao *et al*., 2023). This has necessitated multiple instillations of the drops to increase its bioavailability and improve therapeutic efficacy. With the multiple instillations comes poor adherence to therapy which results mostly in poor prognosis. Also worrisome is the drug loss by rapid tear turnover and nasolacrimal drainage of instilled medications into the systemic circulation which may cause adverse effects (Kim *et al.,* 2023). To increase the retention time of drugs administered to the eye, solid and semi-solid dosage forms such as ointments, gels, inserts, contact lenses are employed and are currently being investigated as suitable alternatives to eye drops. Unfortunately, the issue of patient compliance with these alternatives remains because of the difficulty of drug administration compared to the ease of administration of eye drops.

Eye drops formulated as in-situ gels retain its ease of administration and has the added benefit of reduced elimination by ocular barriers. This is because on storage at room temperature, in-situ gels remain as solutions just like eye drops but makes a sol-to-gel transition only when it comes in contact with the physiological fluid of the eye (Fathalla *et al*., 2022). This increased viscosity on contact with the eye is attributed to three stimuli of ion, temperature and pH. Dosing is reduced, drainage into the systemic circulation is eliminated and patient adherence to therapy is assured. In-situ gels are formulated using smart polymers that transform from solutions to gels in response to temperature, ion and pH changes. That is, prior to instillation, they remain as solutions but increase in viscosity in response to stimuli in the physiological environment (Gupta *et al.,* 2015). This means that the ease of administration and dosing associated with solutions is retained prior to instillation and the resident time on the eye is prolonged in response to the physiological stimuli. Prolonged resident time on the cornea leads to increased bioavailability because the active pharmaceutical ingredient(s) has enough time to be absorbed into the eye. Also, the increased viscosity of the formulation in contact with the eye reduces drainage into the systemic circulation where it may cause unwanted side effects. This also implies reduced loss of the administered drug by blinking and tear turnover.

Sodium alginate, a natural biodegradable and biocompatible polysaccharide, is used in in-situ gel preparation as a smart polymer that transforms into a gel in response to monovalent and divalent ions in the tear fluid (Frent *et al*., 2022; Gupta *et al.,* 2015). It is synthesized from brown algae and is used commercially in the food, pharmaceutical and cosmetic industries (Abka-khajouei *et al*., 2022; Frent *et al.,* 2022). Sodium alginate has been used in combination with different polymers to control drug release in paediatric (Abdelkader *et al*., 2023) and ocular formulations (Gupta et al., 2015) because of their safety profile and gelation properties. Hydroxypropyl methylcellulose (HPMC E5LV) a viscosity enhancer with good swelling properties has also been used with other polymers to prolong the release of formulations and so improve compliance to therapy.

In this study, we investigate the effectiveness of levofloxacin in-situ gels as controlled release formulations for the treatment of bacterial keratitis caused by two gram-negative bacteria *Pseudomonas*

aeruginosa and *Escherichia coli* and two gram-positive bacteria - *Staphylococcus aureus* and *Streptococcus pneumonia* using a combination of two smart polymers – sodium alginate (SA) and hydroxypropylmethyl cellulose (HPMC E5LV).

METHODOLOGY

Materials

Sodium Alginate and HPMC E5LV were obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Benzalkonium chloride, sodium chloride, and levofloxacin hemihydrate were acquired from by BDH laboratory supplies, England. Potassium chloride was purchased from Merck, 64271 Darmstadt, Germany, Calcium Chloride dihydrate 98% was purchased from Loba Chemie Pvt. Ltd., Mumbai, India, Sodium Bicarbonate was gotten from Josphine Ventures Lagos, Nigeria. Others are Tryptic Soy Broth and Mueller Hinton agar both made by Himedia™, Pennsylvania, USA. All other chemicals and solvents used are of analytical grade.

Method

Formulation of in-situ gels

In-situ gels were prepared by dissolving sodium alginate in 50 mL of distilled water and HPMC was added and stirred slowly with a magnetic stirrer, care was taken that no lumps were formed during stirring, the solution was allowed to hydrate overnight. Levofloxacin hemihydrate was dissolved in 10 mL of distilled water and benzalkonium chloride was added and the solution was filtered. The drug solution was added to the polymeric solution under constant stirring until a uniform mixture was obtained. The developed formulations were made up to 100 mL with deionized water. The formulations were filled in containers and sealed with fitting caps. Formulations obtained were tagged F1 to F12. Details of each formulation are as shown in Table 1.

Table 1: In-situ gel formulations F1 to F12

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Levofloxacin hemihydrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC E5LV	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5
Sodium alginate	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water to (mls)	100	100	100	100	100	100	100	100	100	100	100	100

Physicochemical characterization of formulated in-situ gels

The in-situ gels (ISGs) were characterized based on colour, clarity, pH, gelling capacity and gelling time. The pH of the formulations was measured using a digital pH meter Mettler Toledo FP20. The tip of the pH meter was dipped into the solution and allowed to stay for a few seconds to minutes until a constant reading was obtained.

The gelling capacity was determined by placing 3 mL of the formulation in a beaker containing 1 mL of freshly prepared 0.1% calcium chloride solution and was visually observed for gelling time (Makwana *et al*., 2016).

The clarity of the formulations was assessed visually in a white and black background when placed in a transparent vial. It was observed for turbidity, or any unwanted particles dispersed in the solution (Patil *et al.*, 2015).

Drug Content determination

The drug content of each of the formulations was determined by UV/Visible analysis. Briefly, 1 mL of the formulation was transferred to a 100 mL volumetric flask. Simulated tear fluid, STF (sodium chloride 0.68 g, sodium bicarbonate 0.22 g, calcium chloride dihydrate 0.008 g, potassium chloride 0.14 g) 50 mL was added to the flask and stirred. The solution was made up to the 100 mL mark with STF (pH 7.4) and filtered. Levofloxacin hemihydrate content was determined at a maximum wavelength of 294 nm using the UV/Visible spectrophotometer. All readings

were done in triplicate, and the results were expressed as the mean ± SD.

In Vitro Drug Release studies

In vitro drug release studies were done using the Franz diffusion cell. ISG 100 µl was carefully placed on a cellophane membrane of dimension 0.98 cm in the donor chamber. The receptor chamber was filled with approximately 30 mL STF of pH 7.4. The temperature of the receptor chamber was maintained at 37 ± 1 ºC. while constantly stirred with a magnetic stirrer. The drug release was monitored by removing 1 mL of the STF at predetermined intervals and this was replaced with equal volume of fresh STF. The concentration of drug released was determined at a maximum wavelength of 294 nm using the UV/Vis spectrophotometer and a previously obtained calibration curve. The above procedure was repeated for all formulations including the levofloxacin hemihydrate solution (0.5%w/v) which served as the control.

The release kinetics of all the formulations were determined by model dependent methods. The release profiles were fitted to zero-order kinetics by plotting cumulative drug release against time; first-order kinetics by plotting log cumulative percentage of drug remaining against time; Higuchi by plotting cumulative percentage release against the square root of time and Korsemeyer-Peppas model by plotting log cumulative percentage drug release against log of time. The release exponent, n, was obtained by calculating the slope using release data that falls below 60% levofloxacin release. The n-value characterizes the release pattern of the formulation with an n-value < 0.5 corresponding to release by fickian diffusion, n-value of 0.45<n<0.89 corresponds to non-fickian transport and n-value > 0.89 depicts release by super case transport (Dash et al., 2010).

Drug content and stability test

Formulated ISGs (F1 to F12) (5 mL samples) were stored in airtight containers at room temperature for a period of 60 days away from direct sunlight. Drug content studies were repeated to assess any change in concentration after storage. The contents were also observed for clarity or cloudiness.

Antimicrobial assay

The antibacterial efficacy of the formulations was tested against ocular infection causing organisms such as *Staphylococcus aureus, Escherichia coli, Streptococcus pneumonia* and *Pseudomonas aeruginosa* using the Kirby-Bauer disk diffusion method (Hudzicki, 2009). These organisms were cultured and isolated using Tryptic soy agar (TSA). Test suspensions of 1 x 10^6 colony forming units of each of the organisms were prepared in saline solution and their turbidity compared to a previously prepared 0.5% McFarland solution. Pour-plate method was used to inoculate the bacteria in Mueller-Hilton (MH) agar. After solidification, 0.1 mL of each of the ISGs were placed in a hole bored using a sterile cork borer in the MH agar petri dishes. The same procedure was repeated with 0.5% Levofloxacin solution as positive control and simulated tear fluid as negative control. The plates were incubated at 37 ºC for 24 h. After Incubation, the inhibition zone diameter was measured to the nearest millimetre. All procedure was carried out under lamina flow to prevent contamination.

In vitro ocular toxicity and irritability test

Due to the similarity of rabbit eyes to human eyes, rabbits are widely used in ophthalmic research (Ahn *et al.*, 2016) and most times large number of these animals are required. This has become an ethical issue leading to the concept of reduction, refinement and replacement. Consequently, scientists use validated *in vitro* and *ex vivo* models for ocular research studies (Pinnock *et al*., 2017; Ubani-Ukoma *et al*., 2020, Ubani-Ukoma *et al*., 2022).

The hen's egg test-chorioallantoic membrane (HET-CAM) assay is an alternative and less expensive laboratory method of assessing the toxicity of ocular formulation using fertilized viable hen's eggs (Kalweit *et al*., 1990). Freshly fertilized eggs obtained from the farm were kept in a humified incubator for nine days. The eggs were rotated five times a day at 38ºC and 60% humidity. At 7 days incubation, the eggs were candled to determine their viability by illuminating their blunt ends with a candling lamp.

After confirmation of viability, the eggshell around the air sac was gently removed and sodium chloride dropped on the membrane surface and allowed to stand for 30 minutes. The sodium chloride was gently decanted and 0.3 ml of each of the formulations was applied to the CAM surface. The egg was observed for haemorrhage, lysis or coagulation over a period of 5 mins. An irritation score (0 – 21) was calculated based on Equation 1.

$$\text{Score} = \left(\frac{301-\sec H}{300}\right) \times 5 + \left(\frac{301-\sec L}{300}\right) \times 7 + \left(\frac{301-\sec C}{300}\right) \times 9 \quad (1)$$

Where H – Haemorrhage, L – Lysis, C – Coagulation and sec is starting second. From the total score, each formulation is classified as non-irritant (0 – 0.9), slightly irritant (1 – 4.9), moderately irritant (5 – 8.9) and strong irritant (9 – 21). Sodium hydroxide (0.1M NaOH) was used as a positive control.

Statistical analysis

Data were analysed using MS Excel 365 software to determine the release profiles and MS Excel Student T-test was used to compare the drug content before and after stability studies. Significance value was set at $p \leq 0.05$. Drug release studies were conducted in triplicates and the values expressed as mean ±SD.

RESULTS AND DISCUSSION

The human eye is a very important and sensitive organ with barriers to entry of foreign bodies (Abdi *et al*., 2023; Sarmout *et al*., 2023). These barriers include the blood retinal barrier, the blood aqueous barrier and the rapid tear turnover process that causes the dilution of instilled medications into the eye. Consequently, conventional dosage forms such as eye drops are rapidly lost only a few minutes after instillation. This results in only about 5% of the administered dosage being absorbed for therapeutic efficacy. Despite these drawbacks, eye drops remain the most frequently used eye medication because of its ease of administration.

In this study, in-situ gels which retain the ease of administration of eye drops were successfully prepared and characterized.

Physicochemical characterization of formulated in-situ gels

The physicochemical properties of formulated in-situ gels are shown in Table 2 below. The results show a minimum pH of 5.86 and a maximum pH of 7.60. This falls within the expected pH range of 3.5 – 8.5 for ophthalmic solutions which can be buffered to pH 7.4 by the tear fluid on instillation (Obiedallah *et al*., 2018).

Table 2: Physicochemical Properties of formulated in-situ gels

Formulation	pH	Gelling time (sec)	Gelling Capacity	Clarity
F1	6.09	20	++	Clear
F2	6.14	15	+++	Clear
F3	6.22	10	+++	Clear
F4	6.39	10	+++	Clear
F5	5.86	5	+	Clear
F6	7.40	10	++	Clear
F7	6.85	5	+++	Clear
F8	6.90	15	+++	Clear
F9	6.80	11	++	Clear
F10	6.95	15	++	Clear
F11	6.05	11	+++	Clear
F12	7.60	15	+++	Clear

+++ gelation is rapid and retained over time, ++ gels and retains gelation, + gels but disappears.

After preparation, the formulations were clear and free of particles as expected of an elegant pharmaceutical formulation. The pH of the tear fluid is 7.4 and to avoid irritability and ensure comfort on administration, the pH of eye formulations ideally should fall within the expected range of 3.5 to 8.5 avoiding both extremes; this means that they would not be irritable on instillation. The gelling capacity of the formulations vary based on the concentration of the SA used and the viscosity of the HPMC. The ISGs with the fastest gelling times were F5 and F7 containing HPMC:sodium alginate at ratios 1.0:0.5 and 1.0:1.5, respectively. Gelation was observed in 5 seconds on addition of calcium chloride ion. However,

the F5 quickly reversed to its solution form while the gelation of F7 retained its gel form over 24 h. Other ISGs that showed high gelation include formulations F2, F3, F4, F8, F11 and F12 (Table 2). The fast gelation and subsequent disappearance of the gelation in F5 can be attributed to the viscosity enhancing effect of the HPMC at 1% concentration but low concentration of the SA, thus, the gelation could not be sustained. Jain *et al* reported that the fast gelation properties of HPMC E15 and SA used in the study informed the choice of polymers in the formulation of levofloxacin in-situ gel (Jain *et al*., 2020). Though the type of HPMC differs from that used in this study, the results obtained confirm the combination of SA and HPMC is appropriate for optimal gel formation.

Drug content and stability test

The drug content study revealed no significant change ($p = 0.101$) in drug concentration after storage at 37 ± 2^{o}C and 50% RH for 60 days. The graph of drug content on day 0 and day 60 is shown in Figure 1.

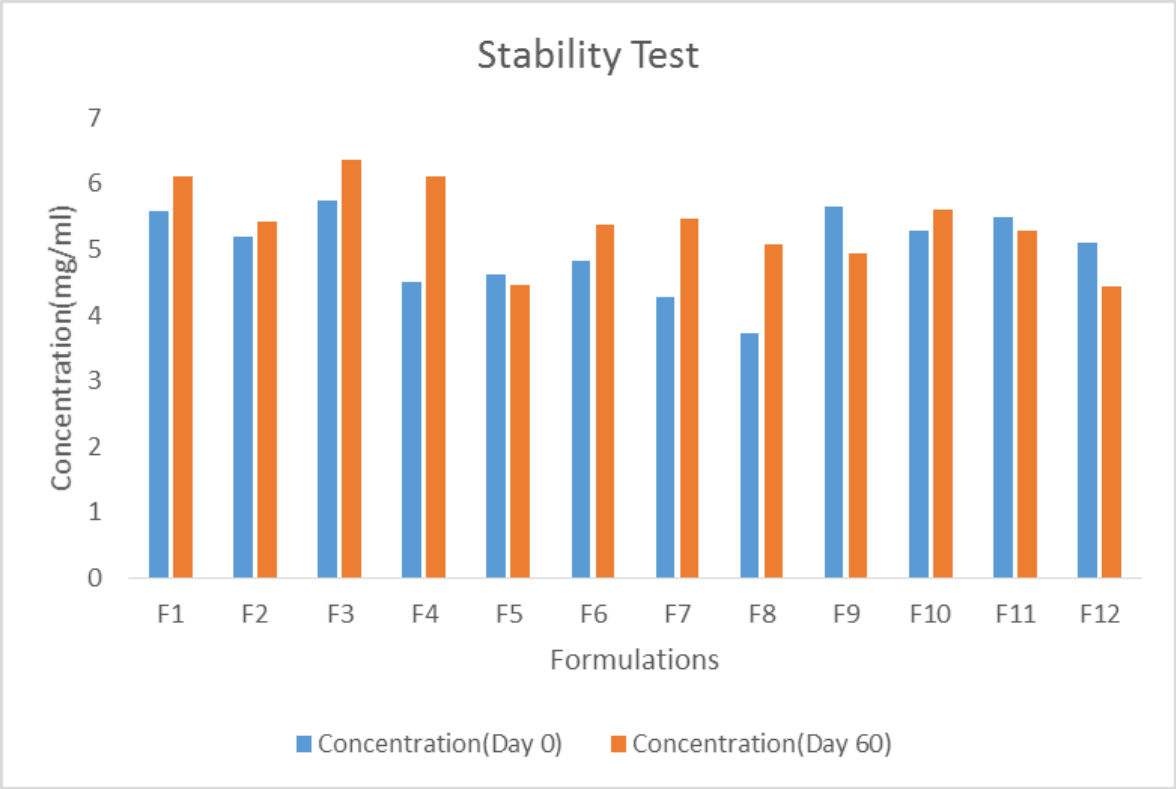

Figure 1: Bar chart of drug content of in situ gels on Day 0 and Day 60

In vitro release analysis

The cumulative percentage drug release from the formulations was plotted against time in hours. The result of the release study showed a burst release of the drug from the eye drop solution compared to the ISGs. One major advantage gels have over solutions is their viscosity which prolongs their resident time on the cornea after instillation (Nayak & Bera, 2019). Prolonged contact on the anterior surface of the eye increases the time for drug absorption and therefore the bioavailability of the drug. Increased bioavailability will increase the therapeutic efficacy of the medication. Due to the fluidity of solutions, eye drops are made with high concentrations of active pharmaceutical ingredients (APIs) and instilled multiple times to ensure a steady state concentration. Despite these measures, the challenge of nasolacrimal drainage, uneven dosing and poor compliance to dosage regimen remains. With ISGs, drug release from the formulations is prolonged and instillation of medication is reduced to once or twice daily. Therefore, patient adherence to therapy is improved and healing occurs. Fig. 2 shows the drug release profiles of the in-situ gels in comparison with the conventional LVF solution. The LVF release profile shows a burst release; in just 1 h, 70% of the drug was released. Compared to the LVF solution, most of the formulations had a more gradual release with 70% of the drugs being released after 4 or 5 h. The slowest release was from F6 which showed about 61% release after 4 h and just about 36% release in an hour. Burst release may lead to toxicity though it could be

advantageous for quick reduction of the microbial load in the eye. This however can be safely achieved with the ISGs as majority of the formulations released between 40 and 50% API in the first hour. Increasing the concentration of the polymers will most likely have a higher prolonged release effect on the formulations because of the longer resident time of a more viscous formulation.

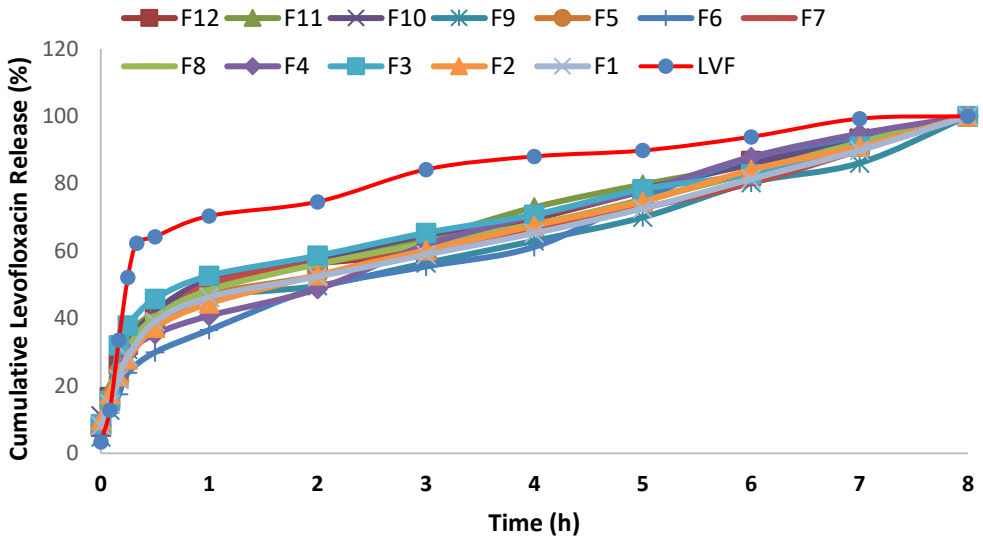

Figure 2: Cumulative levofloxacin release plot against time (h) from formulations F1 to F12 and levofloxacin solution as control

In vitro release kinetics study.

The release profile for formulations F4, F8 and F12 were fitted to different kinetic models to determine the mechanism of levofloxacin release. These ISGs were chosen because they have the highest concentration of ion and temperature sensitive polymers and showed high gelling capacity. After analysis using the dependent release models – zero order, first order, Higuchi and Korsemeyer-Peppas graphs, Table 3 shows the regression values of the formulated gels and the n-value for the Korsemeyer-Peppas graph.

Table 3: *In vitro* release kinetics data

Formulations	Zero order	First order	Higuchi model	Korsemeyer-Peppas model	
				R^2	n
F4	0.9438	0.8783	0.9907	0.9759	0.40
F8	0.9629	0.969	0.9831	0.9629	0.45
F12	0.9069	0.9171	0.9749	0.9656	0.36

The release mechanism for formulations F4, F8 and F12 fits the Higuchi model as the plots show high linearity compared to the other models based on the correlation coefficient (R^2) values of 0.991, 0.983 and 0.975 respectively. The KorseMeyer-Peppas exponent n-value were all below 0.5 confirming drug release from the polymeric gels is by drug diffusion (Dash *et al.*, 2010)

Antimicrobial assay

The antimicrobial efficacy of the formulated ISGs and LVF solution against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia* are shown in Table 4.

Table 4: Disk diffusion test Inhibition Zone Diameter

Formulations	Average Zone of Inhibition (mm) (n = 3)			
	E.coli	***S. aureus***	***P.aeruginosa***	***S. pneumonia***
F1	21.3 ± 2.30	48.0 ± 2.00	30.7 ± 1.15	40.0 ± 2.00
F2	16.7 ± 1.15	47.3 ± 1.15	36.7 ± 1.15	47.3 ± 1.15
F3	24.0 ± 2.00	54.7 ± 1.15	30.0 ± 2.00	42.0 ± 2.00
F4	24.6 ± 1.15	49.3 ± 1.15	31.3 ± 1.15	36.6 ± 1.15
F5	16.7 ± 3.06	47.3 ± 1.15	23.3 ± 1.15	42.7 ± 1.15
F6	16.7 ± 1.15	51.3 ± 1.15	26.7 ± 1.15	42.7 ± 3.06
F7	15.3 ± 2.31	60.7 ± 1.15	20.0 ± 4.00	28.7 ± 1.15
F8	23.3 ± 1.15	47.3 ± 1.15	24.0 ± 2.00	36.7 ± 3.06
F9	17.3 ± 1.15	46.0 ± 4.00	22.0 ± 2.00	34.0 ± 2.00
F10	16.3 ± 1.15	47.3 ± 1.15	38.7 ± 1.15	40.7 ± 1.15
F11	17.3 ± 1.15	47.3 ± 1.15	38.7 ± 2.31	34.0 ± 2.00
F12	12.0 ± 2.00	56.7 ± 1.15	25.0 ± 0.82	38.0 ± 2.00
LVF Solution	30.0 ± 2.00	41.0 ± 1.15	21.3 ± 1.15	38.0 ± 2.00

The antimicrobial assay shows that the efficacy of levofloxacin in the ISGs against the gram positive and gram-negative bacteria that causes ocular infections are comparable to that from the conventional eye drops (Table 1). Formulation F7 has the highest IZD against *S. aureus* – 60.7 ± 1.15, F11 has the highest IZD against *P. aeruginosa* – 38.7 ± 2.31, F2 had the highest IZD against *S. pneumonia* – 47.3 ± 1.15 while LVF solution had the highest IZD against *E. coli* – 30 ± 2.00. Generally, it was observed that the organisms tested were all sensitive to the incorporated drug regardless of the type of formulation. Therefore, it can be confirmed that the sustained release property of the ISGs did not compromise the efficacy of the drug against the gram-negative and gram-positive organisms.

In vitro ocular toxicity and irritability test

The irritability and/or toxicity profile of the formulations were investigated using fertilized egg as shown in Fig. 3. The ISGs with the highest polymer content – F4 (HPMC 0.5 and SA 2.0), F8 (HPMC 1.0, SA 2.0) and F12 (HPMC 1.5, SA 2.0) – showed good characteristics such as high gelling capacity and acceptable pH range 6.39, 6.90 and 7.60 respectively. These were subjected to ocular irritation studies which confirmed the safety and non-irritability of the formulations compared to the positive control - 0.1M NaOH (Fig.3). The positive control clearly shows haemorrhage from the veins after 5 mins of instillation while the ISGs F4, F8 and F12 remained the same at 0 min and 5 mins after instillation of the formulations into the air sac of the fertilized eggs. This shows that the formulations were non-irritant and non-toxic.

The irritation score of 0.1M NaOH after 5 mins of instillation into the chorioallantoic membrane (CAM) of the fertilized egg was calculated to be 10.59 using Equation 1. Within a few minutes of contact with the CAM, haemorrhage, lysis and coagulation of the vesicles were observed. On the contrary, there were no changes or observed irritation when the formulations were placed on the CAM as shown in Fig. 3. After incubation of fertilised eggs for 9 to 10 days, the embryonic egg has well-developed arteries, veins and capillaries which undergo inflammatory processes similar to that of the rabbit's eye in response to injury (Cazedey *et al.*, 2009). The HETCAM test is a validated alternative *in vitro* test to Draize test (Steiling *et al.*, 1999).

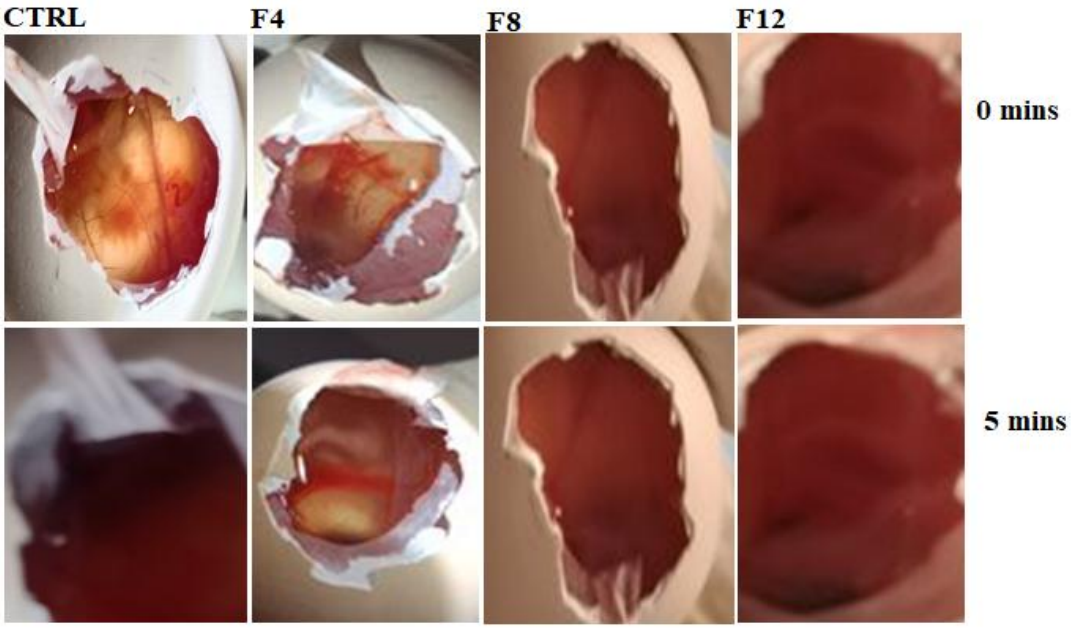

Figure 3: HETCAM test images at 0 mins and 5 mins after instillation of the control (0.1M NaOH), F4, F8 and F12.

CONCLUSION

This study has shown that levofloxacin in-situ gels formulated with low viscosity sodium alginate and HPMC E5LV is non-toxic and well tolerated based on the *in vitro* result obtained from the HETCAM test. The formulations all show high activity against the tested gram-negative and gram-positive bacteria. These ISGs are easy to make, they retain the ease of administration of eye drops and have the added advantage of prolonged release. These formulations should be further investigated as potential sustained release medications and commercialized for use in the treatment and prevention of bacterial ocular infections. Though the results look promising, it is important that preclinical studies are carried out to confirm the efficacy of the formulations in an animal model.

REFERENCES

Abdelkader, H., Abdel-Aleem, J. A., Mousa, H. S., Elgendy, M. O., Al Fatease, A., and Abou-Taleb, H. A. (2023). Captopril Polyvinyl Alcohol/Sodium Alginate/Gelatin-Based Oral Dispersible Films (ODFs) with Modified Release and Advanced Oral Bioavailability for the Treatment of Pediatric Hypertension. Pharm. J, 16(9): 1-13 https://doi.org/10.3390/ph16091323

Abdi, B., Mofidfar, M., Hassanpour, F., Kirbas Cilingir, E., Kalajahi, S. K., Milani, P. H., Ghanbarzadeh, M., Fadel, D., Barnett, M., Ta, C. N., Leblanc, R. M., Chauhan, A., and Abbasi, F. (2023). Therapeutic contact lenses for the treatment of corneal and ocular surface diseases: Advances in extended and targeted drug delivery. Int. J. Pharm 638:1 - 18. Elsevier B.V. https://doi.org/10.1016/j.ijpharm.2023.122740

Abka-khajouei, R., Tounsi, L., Shahabi, N., Patel, A. K., Abdelkafi, S., and Michaud, P. (2022). Structures, Properties and Applications of Alginates. Marine Drugs 20(6)122740. MDPI. https://doi.org/10.3390/md20060364

Ahn, S. J., Hong, H. K., Na, Y. M., Park, S. J., Ahn, J., Oh, J., Chung, J. Y., Park, K. H., and Woo, S. J. (2016). Use of Rabbit Eyes in Pharmacokinetic Studies of Intraocular Drugs. J Vis Exp, 113: 1–8. https://doi.org/10.3791/53878

Alshehri, J. M., Caballero-Lima, D., Hillarby, M. C., Shawcross, S. G., Brahma, A., Carley, F., Read, N. D., and Radhakrishnan, H. (2016). Evaluation of Corneal Cross-Linking for Treatment of Fungal Keratitis: Using Confocal Laser Scanning Microscopy on an Ex Vivo Human Corneal Model. IOVS 57(14): 6367–6373. https://doi.org/10.1167/iovs.16-20110

Cazedey E.C.L., Carvalho, F. C., Fiorentino, F. A. M., Gremiao, M. P. D., and Salgado, H. R. N. (2009). Corrositex ®, BCOP and HET-CAM as alternative methods to animal experimentation Braz. J. Pharm. Sci., 45(4) 759 - 766.

Chandra, N. S., Gorantla, S., Priya, S., and Singhvi, G. (2022). Insight on updates in polysaccharides for ocular drug delivery. Carbohydr. Polym. (Vol. 297). Elsevier, Netherlands Ltd. https://doi.org/10.1016/j.carbpol.2022.120014

Dash, S., Murthy, P. N., Nath, L., and Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol. Pharm 67(3): 217–223.

Farahani, M., Patel, R., and Dwarakanathan, S. (2017). Infectious corneal ulcers. In Disease-a-Month (Vol. 63, Issue 2, pp. 33–37). https://doi.org/10.1016/j.disamonth.2016.09.003

Fathalla, Z., Mustafa, W. W., Abdelkader, H., Moharram, H., Sabry, A. M., and Alany, R. G. (2022). Hybrid thermosensitive-mucoadhesive in situ forming gels for enhanced corneal wound healing effect of L-carnosine. Drug Deliv, 29(1): 374–385. https://doi.org/10.1080/10717544.2021.2023236

Franco, P., and De Marco, I. (2021). Contact lenses as ophthalmic drug delivery systems: A review. Polymers, 13(7): 1 - 22. https://doi.org/10.3390/polym13071102

Frent, O. D., Vicas, L. G., Duteanu, N., Morgovan, C. M., Jurca, T., Pallag, A., Muresan, M. E., Filip, S. M., Lucaciu, R. L., and Marian, E. (2022). Sodium Alginate—Natural Microencapsulation Material of Polymeric Microparticles. Int. J. of Mol. Sci. 23(20): 1 - 24. MDPI. https://doi.org/10.3390/ijms232012108

Gangopadhyay, N., Daniell, M., Weih, L. A., and Taylor, H. R. (2000). Fluoroquinolone and fortified antibiotics for treating bacterial corneal ulcers. Brit. J. Ophthalmol. 84(4): 378–384. https://doi.org/10.1136/bjo.84.4.378

Gokhale, N. (2008). Medical management approach to infectious keratitis. Indian J. Ophthalmol, 56(3): 215–220. https://doi.org/10.4103/0301-4738.40360

Gupta, H., Aqil, M., Khar, R., Ali, A., Bhatnagar, A., and Mittal, G. (2015). An alternative in situ gel-formulation of levofloxacin eye drops for prolong ocular retention. J. pharm. bioallied sci, 7(1): 9–14. https://doi.org/10.4103/0975-7406.149810

Höfling-Lima, A. L., Bandeira, F., and Md, S. (2014). Management of acute bacterial keratitis: Fortified antibiotics or fluoroquinolones? Pan-Am, 13(3): 70–72.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. ASM December 2009, 1–14.

Jain, P., Jaiswal, C. P., Mirza, M. A., Anwer, M. K., and Iqbal, Z. (2020). Preparation of levofloxacin loaded in situ gel for sustained ocular delivery: in vitro and ex vivo evaluations. Drug Dev. Ind. Pharm. 46(1): 50–56. https://doi.org/10.1080/03639045.2019.1698598

Kalweit, S., Besoke, R., Gerner, I., and Spielmann, H. (1990). A national validation project of alternative methods to the Draize rabbit eye test. In Vitro Toxicol 4(4–5): 702–706. https://doi.org/10.1016/0887-2333(90)90147-L

Kim, T. Y., Lee, G. H., Mun, J., Cheong, S., Choi, I., Kim, H., and Hahn, S. K. (2023). Smart contact lens systems for ocular drug delivery and therapy. Adv. Drug Deliv. Rev. (Vol. 196). Elsevier, Netherlands B.V. https://doi.org/10.1016/j.addr.2023.114817

Nayak, A. K., and Bera, H. (2019). In situ polysaccharide-based gels for topical drug delivery applications. Polysaccharide Carriers for Drug Del. pp. 615–638. Elsevier, Netherlands. https://doi.org/10.1016/B978-0-08-102553-6.00021-0

Obiedallah, M. M., Abdel-Mageed, A. M., & Elfaham, T. H. (2018). Ocular administration of acetazolamide microsponges in situ gel formulations. SPJ, 26(7): 909–920. https://doi.org/10.1016/j.jsps.2018.01.005

Pinnock, A., Shivshetty, N., Roy, S., Rimmer, S., Douglas, I., MacNeil, S., and Garg, P. (2017). Ex vivo rabbit and human corneas as models for bacterial and fungal keratitis. Graef Arch. for J. Clin. Exp Ophthalmol. 255(2):333–342. https://doi.org/10.1007/s00417-016-3546-0

Robertson, D. M., Rogers, N. A., Petroll, W. M., and Zhu, M. (2017). Second harmonic generation imaging of corneal stroma after infection by Pseudomonas aeruginosa. Scientific Reports, 7(April): 1–10. https://doi.org/10.1038/srep46116

Sarmout, M., Xiao, Y., Hu, X., Rakhmetova, A., and Koole, L. H. (2023). A novel approach to achieve semi-sustained drug delivery to the eye through asymmetric loading of soft contact lenses. Heliyon, 9(6) 1 - 14. https://doi.org/10.1016/j.heliyon.2023.e16916

Stapleton, F. (2023). The epidemiology of infectious keratitis. Ocul. Surf., 28: 351–363. https://doi.org/10.1016/j.jtos.2021.08.007

Steiling, W., Bracher, M., Courtellemont, P., and Silva, O. De. (1999). The HET±CAM, a Useful In Vitro Assay for Assessing the Eye Irritation Properties of Cosmetic Formulations and Ingredients. In Vitro Toxicol., 13: 375–384.

Ubani-Ukoma, U., Chauhan, A., Schultz, G., and Gibson, D. J. (2020). An ex vivo cornea infection model. MethodsX, 7. https://doi.org/10.1016/j.mex.2020.100876

Ubani-Ukoma, U., Egbo, J. I., Ezimoha, J. C., Oluwole, T. O., Ilomuanya, M. O., and Silva, B. O. (2022). Evaluation of the therapeutic effectiveness and sustained release capacity of levofloxacin loaded silicone hydrogel contact lenses. AJOPRED 14(2): 119–127.

Zafar, A., Alsaidan, O. A., Imam, S. S., Yasir, M., and Alharbi, K. S. (2022). Formulation and Evaluation of Moxifloxacin Loaded Bilosomes In-Situ Gel Optimization to Antibacterial Evaluation. Gels, 8(418): 1–21. https://doi.org/https://doi.org/10.3390/ gels8070418

Zhao, L., Song, J., Du, Y., Ren, C., Guo, B., and Bi, H. (2023). Therapeutic applications of contact lens-based drug delivery systems in ophthalmic diseases. Drug Deliv., 30(1): 1 -14. https://doi.org/10.1080/10717544.2023.2219419

*Address for correspondence: Uloma N. Ubani-Ukoma
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy,
Faculty of Pharmacy,
University of Lagos,
Nigeria
Telephone: +234 813 135 4110
E-mail: uubani-ukoma@unilag.edu.ng

Conflict of Interest: None declared
Received: Oct 16, 2024
Accepted: December 2, 2024