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Development and evaluation of ocular antibiotic-loaded soluble film inserts

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ABSTRACT

Antibiotic eyedrops typically require frequent instillation due to the eye's defensive mechanisms limiting drugs from reaching target sites. This may risk patient non-adherence and treatment inefficacy. The aim of this study was to develop a biocompatible and fully soluble ocular film insert to enhance the delivery of levofloxacin, as well as the handling procedure for its administration; based on the anatomical dimensions and physiological conditions of the human eye.

Inserts were prepared by solvent casting method, using HPMC, sodium alginate, gelatin, PEG 400, and levofloxacin solution, and characterised for various physicochemical properties (e.g., uniformity of weight and thickness, loss on dryness, swelling index, water uptake and surface pH). Mechanical properties were assessed and compared against a commercially available buccal film formulation. Uniformity of content and release profile of inserts were assessed by means of a validated analytical method. Antibacterial effectiveness was studied by adapted disc diffusion method on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The formulation including HPMC E15 (1250 mg), low viscosity sodium alginate (750 mg), type A gelatin (250 mg) and PEG 400 (2.5 mL) and 0.1% levofloxacin solution, resulted in high quality inserts, exhibiting uniformity of mass, thickness, and levofloxacin content, that comply with Pharmacopeial standards. Inserts were able to withstand unilinear and repeated mechanical stresses, suggesting suitability for manipulation linked to eye administration. The fully soluble levofloxacin-loaded inserts exhibited good physicochemical and mechanical characteristics, indicating good compatibility with ocular environment and administration procedure. Consistent levofloxacin content and biphasic release pattern showed immediate and sustained antimicrobial efficacy, consistently above the minimum inhibitory concentrations for the model species tested. This work also presents an experimental framework that can be adapted for designing and testing ocular drug delivery systems accounting for anatomical and physiological characteristics of the eye.

1. Introduction

Corneal infections with subsequent stromal scarring are one of the major risks of blindness worldwide [1]. The use of antimicrobial agents remains the therapy of choice in the treatment of anterior eye infections [2,3]. Levofloxacin, a third generation fluoroquinolone, is a broad-spectrum antibiotic commonly used in the form of 0.5% eye drops [4], given its efficacy against different species of bacteria, including the most common causative pathogens of bacterial conjunctivitis and microbial keratitis [5–7]. Levofloxacin eye drops require frequent instillation, especially when starting treatment [8], with a typical dosage of 4 instillations per day [9]. This is to ensure drug concentration can be maintained above the minimum inhibitory concentration (MIC) of the causative agent(s) being treated [10]. In fact, the limited volume of eye drops the eye can accommodate and the constant tear turnover result in less than 5% of active ingredient in each drop administered reaching the target site [11], requiring elevated dosing frequency to ensure effective drug bioavailability [12,13], potentially increasing the risk of patient non-adherence and inadequacy of treatment regimen [14].

Drug delivery systems serve to uphold drug presence on the ocular surface, extend the efficacy duration of pharmaceutical agents, and decrease dosing frequency [15]. Soft contact lenses have been proposed as a means to achieve sustained drug delivery while concurrently correcting refractive errors [16]. Nonetheless, considering that the first

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drug-eluting contact lens was temporarily available on the market [17], its acceptance among eye care practitioners remains limited. This constraint may be due to its restricted availability in certain regions and a lack of collective experience or awareness regarding this technology [18]. Additionally, this contact lens is currently only approved for managing allergic conjunctivitis, leveraging the synergistic interplay between the released drug and the lens's presence on the ocular surface [19]. Further investigation is needed to determine its suitability for ocular conditions that typically require discontinuation of soft contact lens wear, such as microbial keratitis [20].

Ocular films have been proposed as an alternative therapeutic approach for drug release to treat various ocular diseases [21]. Most of these ocular film inserts have been developed with water-insoluble components or are entirely insoluble, mainly intended for lower fornix administration [22,23]. Although these systems may offer potential advantages in terms of sustained drug release efficacy [24], the inclusion of insoluble fractions in inserts may raise concerns about the necessity of removing depleted inserts, which could require professional intervention [25]. To develop biocompatible and soluble ocular inserts, various biobased polymers can be used. Hydroxypropyl methylcellulose (HPMC), a derivative of cellulose, has been widely used in ocular drug delivery, such as commercial eyedrops for dry eye disease [26]. Its viscosity properties and biocompatibility make it favourable for developing ocular film inserts, showing promising sustained drug release and good biocompatibility in devices designed for the treatment of glaucoma [27–32], corneal infections [22,24,33–35], inflammation [36–38], allergic conditions [39] dry eye disease [40] and anaesthesia [41]. Sodium alginate, a polysaccharide sourced from brown algae, has been used in numerous topical formulations due to its thickening [42], film-forming, and mucoadhesive properties [43]. It has shown promising applications in ophthalmic formulations for producing *in situ* gelling formulations, responsive to calcium ions in tears [44], thus enhancing bioavailability and improving drug release profile in delivery systems for a variety of pharmaceutical agents, either alone [45–47], or in combination with other polymers [48,49]. Gelatin is obtained from partial hydrolysis of collagen [42], which is a significant constituent of the cornea [50], conjunctiva and sclera [51]. While it has been used as the primary polymer in ocular film formulations [52,53] to extend drug bioavailability [54], its exclusive use is discouraged due to suboptimal mechanical properties of the final inserts [55].

The aim of this study was to develop a fully soluble ocular film insert to enhance the delivery of levofloxacin, based on the use of biocompatible excipients. Given the absence of specific regulatory guidance on the manufacturing processes and testing methods for films [56–58], the approach was based on anatomical considerations of the human eye and its physiological complexities, alongside attention to the procedural nuances governing its administration. Hence, this work not only aims to advance ocular drug delivery, but to also introduces an innovative lab protocol for designing delivery systems that address longstanding challenges in ocular therapeutic development based on the unique properties of the eye.

2. Materials and methods

2.1. Materials

Levofloxacin (98.0–102.0% anhydrous basis, HPLC grade), gelatin (from porcine skin, Type A), alginic acid sodium salt from brown algae (low and medium viscosities), polyethylene glycol (PEG) 400 (BioUltra), and glycerol (BioXtra $\geq$ 99%), as well as tryptic soy broth, sodium chloride, sodium bicarbonate, potassium chloride and calcium chloride were obtained from Sigma Aldrich (Gillingham, UK). HPMC E15 was purchased from JRS PHARMA (Rosenberg, Germany). Methocel™ E6, K4M and K100M were generously gifted from Colorcon (Dartford, UK). Setofilm (4 mg, orodispersible) was purchased from AAH Pharmaceuticals (Coventry, UK). Liquid chromatography reagents were purchased from Fisherbrand™ (Loughborough, UK). All reagents were used as received. Water was distilled with Purite Select Ondeo distiller.

2.2. Manufacturing process for the ocular inserts

Films were prepared by solvent-casting method. Film-forming solutions were made using a 0.1% levofloxacin solution, freshly prepared by dissolving the drug in ultrapure water and sonicating for 10 min. Desired amounts (Table 1) of HPMC, sodium alginate and gelatin powders were blended to obtain a uniform mixture, which was gradually added to the pre-heated solution including drug and plasticiser, stirred at 65°C for 60 min and then left to cool at room temperature (approximately 20°C). The resulting solutions were centrifuged, and emerging bubbles were removed. Film forming solutions were cast on glass plates and spread by motorised film applicator. Each formulation was used to manufacture a single film. Films were left to dry overnight at room temperature, and disc-shaped inserts were cut using a stainless-steel cork-borer.

2.3. Development of qualitative levofloxacin-loaded formulations

The ability of the formulations tested (Table 1) to translate into qualitative films and consistent inserts was monitored throughout the manufacturing process.

The assessing procedure followed a step-wise protocol: film forming solutions were first qualitatively inspected for homogeneity, viscosity and spreadability; produced films were then qualitatively inspected for uniformity of visual appearance, transparency/translucency, peelability and manoeuvrability, and surface texture on touch [59]; inserts were finally assessed for uniformity of mass, thickness, and drug content. Hence, the formulations exhibiting acceptable film-forming solution properties, the ability to produce qualitative films and consistently uniform inserts (according to the criteria reported in Table 2) were further characterised.

2.4. Uniformity of mass and thickness

Twenty-four disc-shaped inserts (diameter, Ø = 10 mm) were collected from each film. Samples were weighed using an analytical balance (Sartorius, Göttingen, Germany) and the thickness was measured with a digital micrometre screw gauge (Beslands, Jiaxing, China). The values were used to calculate mean, standard deviation and relative standard deviation (RSD), and compared against pharmacopoeia guidelines for uniformity of mass for single-dose preparations (i.e., no more than 2 individual masses out of 20 units tested deviate from the average mass by greater than 10% [60]). Same guidelines were used to assess the uniformity of thickness. Weight and thickness values were subsequently employed to determine the inter- and intra-day repeatability of the manufacturing process.

2.5. Mechanical properties

Tensile strength, elongation at maximum force and derived Young's modulus were determined using a tensiometer (Hounsfield Limited, Croydon, UK). The test was set with stress range 0.05 MPa, strain range 300%, speed 60 mm/min and without preload [36]. Folding endurance was tested by manually folding samples allowing opposite portions of the specimens to touch until visible cracking or rupture was observed [35,48,61], or completed 300 times [62]. Both testing procedures were performed in triplicate on larger disc-shaped specimens (Ø = 25 mm), to ensure sufficient surface was available for handling and, more importantly, to allow consistent and secure positioning/grip of the tensiometer claws. In addition, as there is currently no approved ocular film formulation available on the market, a rectangular (2.0 × 1.4 cm) buccal film (Setofilm®, LTS, Germany) was tested as a commercially available pharmaceutical reference formulation to verify the handling capability of the manufactured films.

Table 1
Formulations tested in film manufacturing.

Formulation code	HPMC E15 (mg)	HPMC E6 (mg)	HPMC K100M (mg)	HPMC K4M (mg)	Sodium Alginate (low viscosity) (mg)	Gelatin (Type A) (mg)	PEG 400 (mL)	0.1 % Levofloxacin Solution (mL)
F1	1250	–	–	–	750	500	2.5	22.5
F2	1250	–	–	–	750	750	2.5	22.5
F3	1250	–	–	–	750	1000	2.5	22.5
F4	1250	–	–	–	500	250	2.5	22.5
F5	1250	–	–	–	750	250	2.5	22.5
F6	1250	–	–	–	1000	250	2.5	22.5
F7	1250	–	–	–	1250	250	2.5	22.5
F8	–	1250	–	–	750	250	2.5	22.5
F9	–	–	1250	–	750	250	2.5	22.5
F10	–	–	–	1250	750	250	2.5	22.5

Table 2
Desired properties for formulations tested, grouped by manufacturing stage.

Film forming solution	Film	Insert
Homogeneity	Peelability/maneuverability	Uniformity of mass
Pourability	Visual appearance regularity	Uniformity of thickness
Spreadibility	Transparency/translucency	Uniformity of drug content
	Surface smoothness	

2.6. Loss on drying

Disc-shaped samples (Ø = 25 mm) were randomly collected from the film, weighed and placed in a vacuum oven at 105°C for 24 h [63]. Upon cooling, three samples were individually weighed and moisture content was determined by calculating the ratio between weight difference and initial weight of the samples, reported as a percentage, in accordance with Eq. (1).

$$Loss\ on\ drying = \frac{Initial\ weight - Dried\ weight}{Initial\ weight}\ x\ \frac{100}{1} \tag{1}$$

2.7. Water uptake and swelling index

Water uptake and swelling index of formulations were tested by using paper support and a soaking chamber at controlled temperature. The supports (Whatman filter paper grade 1), were pre-soaked by accommodating them on sodden sponges placed in a lid-covered plastic box filled with 25 mL of ultrapure water, with temperature maintained at 37°C. Once weight equilibrium of the supports was reached, samples (Ø = 25 mm) were placed onto the support and the insert-support combinations were kept in the soaking chamber. Every hour, and until no further increments were recorded, each of the three insert-support combinations was weighed after blotting any excessive liquid from the support. At the same time points, the diameter of the samples was also recorded. Water uptake was calculated as the maximum ratio between the net weight difference of dry and soaked inserts (i.e., accounting for support weight) across time points and expressed as a percentage, according to Eq. (2) [64].

$$Water\ uptake = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}\ x\ \frac{100}{1} \tag{2}$$

Swelling index was derived from the ratio between the diameter variation upon swelling and the initial diameter of the dry insert (Ø = 25 mm), and expressed as a percentage, in accordance with Eq. (3) [65].

$$Swelling\ index = \frac{Swollen\ diameter - Dry\ diameter}{Dry\ diameter}\ x\ \frac{100}{1} \tag{3}$$

2.8. Surface pH

Surface pH of inserts was determined by sampling three random inserts (Ø = 10 mm). Thirty microliters of ultrapure water were pipetted onto the sample's surface, subsequently left to soak for 5 to 10 min [66]. Surface pH of inserts was estimated by pH indicator paper strips (Fisherbrand™, Loughborough, UK, pH range 1–14), and compared against the reference colour scale, after colouration equilibrium was achieved [67].

2.9. Drug content uniformity

Ten samples (Ø = 10 mm) from each film were collected and individually dissolved in 5 mL of ultrapure water for 12 h at 37°C, with gentle shaking. Approximately 3 mL of the suspension were collected and filtered. The aliquots were tested, and the drug concentrations were determined as per the chromatographic method described below. The quantity of drug recovered across the samples and its intra- and inter-day variability were compared against the uniformity of content pharmacopeial guidelines for single dose preparation (i.e., content of no more than 1 individual unit out of 10 units is outside 85%–115% of the average content) [60]. Note: Three batches of films prepared on the same day (F5a, F5b and F5c) were compared against a batch prepared on another day (F5), intra- and inter-day variability respectively.

2.10. Drug release study

The *in vitro* drug release study was carried out using a two chambered donor-receiver model, as it demonstrated a strong positive correlation with *in vivo* testing [22,37,48,68–70]. The apparatus was assembled using a layer of regenerated cellulose semi-permeable membrane (MEMBRA-CEL® MC18, Viskase, USA) tied to one side of an open-ended plastic tube used as donor compartment, while the opposite end was capped with a plastic stopper. This was pre-filled with 1 mL of (pre-heated at 37°C) simulated tear fluid (STF, composition: NaCl 6.8 g, $NaHCO_3$ 2.2 g, $CaCl_2$ 0.08 g, KCl 1.4 g and ultrapure water to final volume of 1000 mL [71]) and held in the receiver compartment such that the membrane was in contact with the dissolution media, consisting of 25 mL STF kept at 37 °C [72]. The apparatus also included slow speed stirring (approximately 30 rpm) to simulate blinking [35]. Three inserts (Ø = 10 mm) from each film were individually placed in the donor compartment, and the amount of drug released over time was evaluated by collecting 1 mL of suspension from the receiver compartment after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h, replacing with equal volume of warm STF. Aliquots collected were filtered and tested as described below. The values of cumulative drug released were calculated considering the effect of sampling procedure on instantaneous concentration recorded, and the percentage release was determined against average values obtained in content uniformity testing.

2.11. High-performance liquid chromatography analytical method

The high-performance liquid chromatography (HPLC) analytical method for levofloxacin detection was developed and validated according to International Council for Harmonisation (ICH) guidelines

[73], and used to determine content uniformity and drug release. The HPLC system used was an Agilent 1200, equipped with reverse phase C18 column Phenomenex Kinetex XB-C18 (Phase C18, ID 4.6 mm, Length 150 mm, Particle Size 3.5 μm, Pore size 100 Å). The mobile phase was made of a mixture of 0.1% trifluoroacetic acid (TFA) in ultrapure water: acetonitrile (70:30, v/v), with measured pH 2.0, vacuum filtered (0.45 μm hydrophilic polypropylene filters) and sonicated under vacuum. The analysis was performed in a thermostatic column compartment at 20°C and under isocratic conditions. The flow rate was set at 0.7 mL/min, while the wavelength for UV detector was set at 288 nm. The analytical method developed for levofloxacin quantification met the specifications of ICH guidelines for validation in linearity (> 0.9), accuracy (100 ± 2 %) and both intraday and inter-day precision (< 2%). In addition, the limit of quantification of levofloxacin was found 0.228 μg/mL, hence suitable for the analysis of the inserts.

2.12. In vitro antimicrobial efficacy

The antimicrobial efficacy of developed inserts was tested *in vitro* on static overlay plate by adaptation of the disc diffusion method [74]. *Staphylococcus aureus* (NCTC8325) and *Pseudomonas aeruginosa* (PA01), were used as representatives of gram-positive and gram-negative bacteria, respectively. Aliquots from pure cultures were collected and independently dissolved in 10 mL of Tryptone Soya Broth (TSB), previously prepared and sterilised by autoclaving, and left to grow in a shaking incubator chamber (37C) overnight. One hundred microliters aliquots of either *S. aureus* or *P. aeruginosa* solutions, previously diluted in TSB (OD_{600} = 0.6), were used to inoculate 10 mL of Tryptone Soya Agar (TSA), allowed to solidify in petri plates under sterile conditions. To maintain consistent moisture levels on each side of the specimens, 25 μl of TSB were first dispensed onto the centre of the agar plates, inserts (Ø = 10 mm) were then positioned on the moistened area, and an additional 25 Âμl of TSB were pipetted directly onto the specimens. Loaded plates were incubated overnight at 37°C, and the zones of inhibition were measured by the help of image-assisted digital analysis for both *S. aureus* and *P. aeruginosa* [74,75].

2.13. Statistical analysis

Statistical analysis was performed using SPSS (V 26, IBM, New York, USA). As the data showed normal distribution (Kolmogorov–Smirnov $p < 0.05$), parametric comparisons (*t*-test, one-way ANOVA) and correlations (Pearson correlation coefficient) were performed. Tukey's honestly significant difference (HSD) post hoc test was used. The statistical significance was taken for p-values lower than 0.05.

3. Results

3.1. Development of qualitative levofloxacin-loaded formulations

The detection of suitable formulations aimed to identify those presenting the desired characteristics, according to the criteria selected (Table 2). Formulations including higher quantities of gelatin (F2 and F3) and sodium alginate (F7), as well as those made with HPMC of higher viscosity (F9 and F10), did not result in satisfactory film-forming solutions. Although they produced homogeneous film forming solutions on heating, they resulted in thick gels at room temperature. Thus, they were not processed further for not reaching adequate levels of pourability and spreadibility. The film prepared with HPMC E6 (F8) was found too brittle upon drying, and not matching the peelability/manoeuvrability criterion, hence it was not tested further. Formulations F1, F4, F5 and F6 produced consistent films, which were easy to peel and handle (Table 3). However, their appearance varied depending on the amount of sodium alginate and gelatin used (Fig. 1). Formulations including higher percentage of both excipients resulted in increased opacity of the film: F1 (500 mg of gelatin) opaque spots were randomly present in the film; F6 (1000 mg of sodium alginate) marked opacity characterised the manufactured film. Hence, F1 and F6 did not meet the criteria of visual appearance regularity and transparency/translucency, respectively.

Formulations F1, F4, F5 and F6 demonstrated good physical uniformity, exhibiting minimal variability in both weight and thickness distribution (maximum RSD 5.2% and 5.0%, respectively), and all complying with the uniformity of mass limits [60]. Similarly, all the formulations exhibited reduced RSD for levofloxacin content, and were within pharmacopeial limits (85–105%) [60].

However, as F4 demonstrated a slightly higher variability in weight, thickness, and levofloxacin content, suggesting an increased susceptibility of the formulation to inconsistency, it did not completely meet the uniformity criteria.

Hence, among the formulations tested, F5 – incorporating HPMC E15 (1250 mg), low viscosity sodium alginate (750 mg), type A gelatin (250 mg) and PEG 400 (2.5 mL), exhibited the most promising characteristics, with satisfactory manoeuvrability, appearance, and uniformity (Table 3), and was further characterised.

3.2. Levofloxacin-loaded film characterisation

Three separate batches of F5 film forming solution were prepared to manufacture independent films (coded as F5a, F5b, and F5c), as per manufacturing process previously described. The three films and their relative solutions were of good quality (as per Table 2), translucent, and easy to peel and process. In Table 4 are summarised the average values for physical and mechanical properties of the films.

The weight and thickness distribution showed that average values of F5c were higher than F5a and F5b (one-way ANOVA, $p < 0.05$), with no significant difference between F5a and F5b ($p > 0.05$). Considering all samples (3 x 24 = 72), both weight and thickness individual values fell within pharmacopeial limits, suggesting a robust intra-day repeatability of insert manufacturing. This consistency was also upheld when including values of F5, obtained in the earlier developmental phase of the formulation. Despite statistical differences ($p < 0.05$) in average weight and thickness, all values remained within the acceptable pharmacopeial limits, exhibiting also satisfactory inter-day manufacturing repeatability.

The mechanical properties of the films (Table 4) indicated suitability

Table 3
Characterisation of successful films. For weight and thickness, the values were averaged from 24 samples, while for drug recovery 10 samples were tested: according to International Pharmacopoeia guidelines for Uniformity of mass[1] and Uniformity of content[2]. Data reported as Mean ± St. Dev.

	Qualitative appraisal		Weight		Thickness		Drug content	
Formulation code	Appearance	Surface Texture	Mean and St. Dev. (mg)	RSD (%) [Out of 10 % range[1]]	Mean and St. Dev. (μm)	RSD (%) [Out of 10 % range]	Mean and St. Dev. (μg/mL)	RSD (%) [Out of 15 % range[2]]
F1	Opaque inclusions	Smooth	10.8 ± 0.2	1.7 % [0]	151 ± 3	2.1 % [0]	36.9 ± 0.4	1.2 % [0]
F4	Translucent	Smooth	10.0 ± 0.5	5.2 % [1]	124 ± 6	5.0 % [1]	40.4 ± 1.8	4.5 % [0]
F5	Translucent	Smooth	10.5 ± 0.3	2.9 % [0]	129 ± 2	2.0 % [0]	38.7 ± 0.7	1.9 % [0]
F6	Opaque	Smooth	10.4 ± 0.2	2.2 % [0]	133 ± 3	2.3 % [0]	37.1 ± 0.6	1.5 % [0]

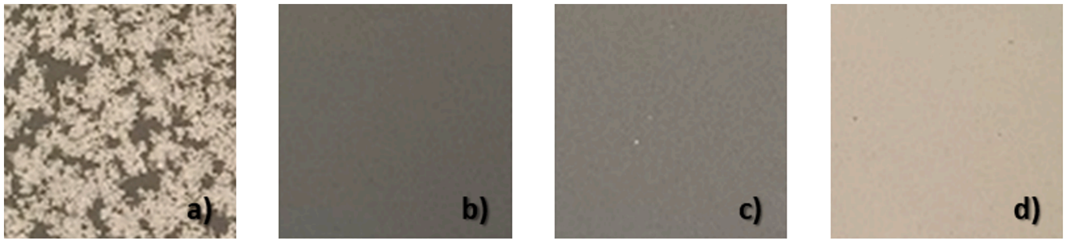

Fig. 1. Appearance of film produced with formulations F1 (a), F4 (b), F5 (c) and F6 (d), highlighting opaque spots and low transparency/translucency. The images represent a 2 cm × 2 cm section of photographs acquired using digital camera against dark background.

Table 4
Physical and mechanical properties of drug loaded films tested. Films F5a, F5b and F5c were independently produced with same formulation. Weight and thickness values were averaged from 24 samples (Ø=10 mm), while mechanical tests were performed on 3 samples (Ø=25 mm). Total is the average from 72 or 9 samples respectively. Data reported as Mean ± St. Dev. *St. Dev. could not be calculated for foldability.

Film Code	Weight (mg)[Out of 10 % range]	Thickness (μm)[Out of 10 % range]	Tensile Strength (MPa)	Elongation at Max (%)	Young's modulus (MPa)	Foldability*
F5a	10.0 ± 0.3 [0]	130 ± 4 [0]	2.6 ± 0.2	13.9 ± 2.0	0.19 ± 0.02	>300
F5b	9.9 ± 0.1 [0]	129 ± 2 [0]	2.9 ± 0.2	14.6 ± 1.9	0.20 ± 0.04	>300
F5c	11.0 ± 0.1 [0]	138 ± 2 [0]	3.0 ± 0.1	15.9 ± 0.5	0.19 ± 0.01	>300
Total	10.3 ± 0.5 [0]	132 ± 5 [0]	2.9 ± 0.2	14.8 ± 1.7	0.20 ± 0.04	>300

of the formulation for manipulation. The tensile strength ranged from 2.6 to 3.0 MPa, with a maximum elongation of 16% from the original length, resulting in an elastic modulus of approximately 0.19 MPa. Compared to the commercial buccal film Setofilm® (6.0 ± 0.6 % elongation; 9.0 ± 0.5 MPa tensile strength), the developed formulation exhibited higher elongation, but lower tensile strength ($p < 0.05$). Additionally, the films could endure over 300 folds without breaking, surpassing Setofilm® (thickness ≈ 90 μm) foldability (247 ± 29).

The films were evaluated for characteristics potentially interfering with anterior eye equilibrium (Table 5). Water uptake and swelling index testing were selected to mimic tear presence by guarantying constant moisture availability at eye temperature. Inserts were found to absorb up to three times their weight in water, with uptake values ranging from 258% to 292% recorded after 2 or more hours, and to linearly expand to a maximum of less than 10% in respect of the initial diameter upon imbibition, with average swelling index values oscillating between 6.7% and 9.3%. Moreover, the surface pH values of the formulation were within the range of 6 to 7.

3.3. Drug content uniformity

The average amount of levofloxacin present in film F5c (42.1 ± 0.4 μg) was higher than what recovered in film F5a (37.5 ± 0.2 μg) and F5b (37.7 ± 0.3 μg) ($p < 0.05$), which, instead, were not different from each other ($p > 0.05$). The three formulations exhibited individual RSD within 3%, which increased to 14% in considering samples collected from F5a, F5b and F5c ($n = 30$) altogether. The additional inclusion of F5 data (38.7 ± 0.7 μg) in the analysis revealed that, despite the significant differences found with F5a and F5c ($p < 0.05$), the values of all the individual inserts ($n = 40$) fell within pharmacopeial limits for

Table 5
Characterisation of films produced after formulation F5. Data reported as Mean ± St. Dev. Surface pH reported as range.

Film Code	Loss on drying (%)	Water uptake (%)	Swelling Index (%)	Surface pH range	Drug content (μg) [Out of 15 % range]
F5a	5.4 ± 0.6	258 ± 46	6.7 ± 2.3	6–7	37.5 ± 0.2 [0]
F5b	4.8 ± 0.2	280 ± 29	8.0 ± 4.0	6–7	37.7 ± 0.3 [0]
F5c	4.2 ± 0.4	292 ± 19	9.3 ± 2.3	6–7	42.1 ± 0.4 [0]
Total	4.8 ± 0.6	277 ± 32	8.0 ± 2.8	6–7	39.1 ± 2.2 [0]

uniformity of content [60]. Hence, the assessment of drug content confirmed the intra- and inter-day repeatability of the dosage form, as, whether the formulations F5a, F5b and F5c were analysed separately or cumulatively with F5, none of the specimens was outside the required range.

3.4. In vitro drug release

The release pattern of levofloxacin from ocular inserts was evaluated through donor-receiver apparatus testing. Fig. 2 shows the amount of levofloxacin recovered after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h. The release of levofloxacin from the manufactured inserts showed a biphasic profile, with more than 24 μg of the drug amount in inserts (accounting for approximately 65%) released within the first 30 min, and the remaining quantity gradually released up to 6 h, with consistent results across the samples tested.

3.5. In vitro antimicrobial efficacy

The formulation F5 was tested *in vitro* for antimicrobial effectiveness against *S. aureus* and *P. aeruginosa*, respectively representing Gram-positive and Gram-negative bacteria. As illustrated in Fig. 3, the levofloxacin-loaded inserts showed antimicrobial action against both bacterial species. The analysis revealed that the zones of inhibition produced by films were approximately 3.8 cm diameter for *S. aureus* and 2.9 cm for *P. aeruginosa*, with a significantly larger zone for the Gram-positive compared to the Gram-negative bacteria ($p < 0.05$).

4. Discussion

The development of the levofloxacin-loaded formulations aimed to identify the most suitable biobased polymer for manufacturing qualitative thin film soluble inserts intended for ocular application into the lower fornix. The lack of regulatory and standardised guidelines for the development and testing of films has been widely recognised [56–58]. In response to this gap, this study proposes a laboratory protocol designed to replicate and assess eye-specific characteristics under experimental conditions, providing a framework that can be applied to the manufacturing and evaluation of ocular dosage forms. By considering the anatomical and physiological complexities of the human eye, as well as the procedural nuances of administration, this work aims to advance

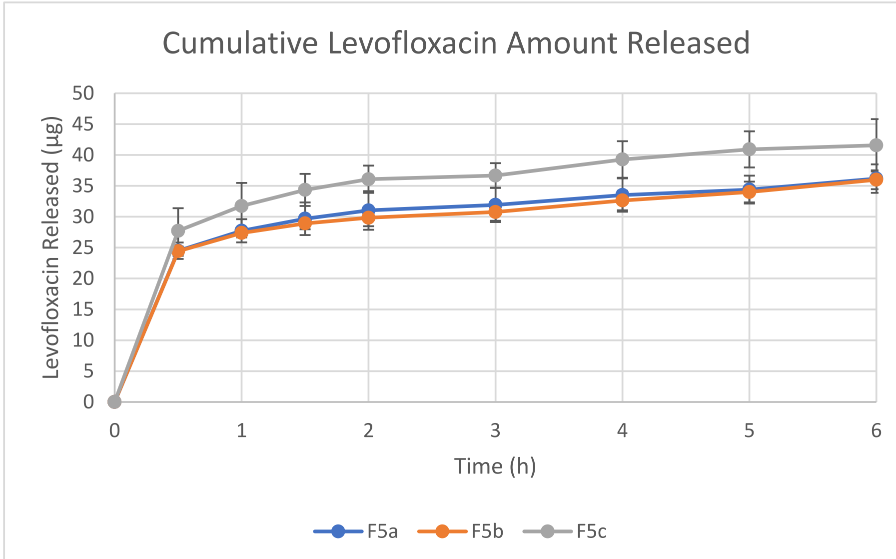

Fig. 2. Cumulative release of levofloxacin from inserts collected from films produced. Values represent mean percentage of samples (n = 3) against the average amount of the film. The error bars represent standard deviation from the mean.

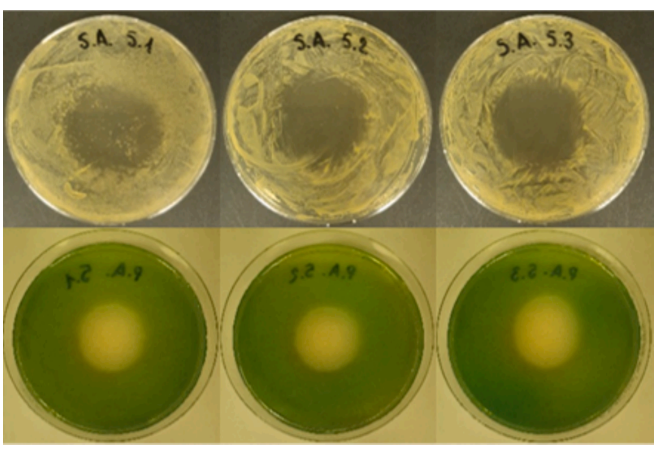

Fig. 3. Zone of inhibition produced by F5 (from Table 1) on Staphylococcus aureus (top) and Pseudomonas aeruginosa (bottom) cultures on agar plates.

ocular drug delivery and introduces an innovative approach to addressing the longstanding challenges in ocular therapeutic development.

The consistent uniformity observed in the films produced showed robustness of the manufacturing process, with individual RSD for the individual content found below 3%. Additionally, results from assessing the inserts indicate both intra- and inter-day repeatability of the procedure when applied to the proposed formulation. In fact, the analyses of the uniformity of mass evaluation findings and of the levofloxacin content recovered from the samples indicated that the inserts consistently met pharmacopeial standards, whether assessed individually or collectively.

Polymers were subjected to heat to facilitate their solubilisation and create homogeneous solutions. However, the advantageous viscosity and gelation characteristics of high-viscosity HPMC, sodium alginate and gelatin at body temperature [76,77] posed challenges at room temperature. At these concentrations, they hindered the formation of qualitative films. Regarding the visual appearance of the film, increasing concentrations of gelatin and sodium alginate have been associated with reduced transparency, potentially due to a more heterogeneous molecular arrangement and reduced compatibility of the excipient [78,79]. In contrast, formulation F5 produced translucent films with even appearance, which could be more appealing for ocular application, as they may be perceived as less obstructive than opaque formulations. Moreover, translucency (rather than transparency) might even facilitate insert administration into the eyes, potentially aiding the procedure similar to contact lens handling tints. This characteristic could offer advantages without directly impeding vision, especially when considering the inserts are intended for administration into the lower fornix.

The ability of the insert to be manipulated during administration to the eye could be assessed by determining the mechanical properties of the films. Despite exhibiting reduced tensile strength, the developed formulation showed higher values of elongation at maximum strength compared to those observed for the commercial film. This suggests that the produced inserts can endure greater dimensional deformations, such as pulling without losing their plasticity. Additionally, the insert formulations demonstrated resistance to 300 repeated folds without fracture, indicating of satisfactory film properties [39]. This resistance was higher than the commercial buccal formulation, confirming their ability to withstand mechanical strain during insertion into the eyes. During evaluation of mechanical properties, the tested specimens were of a larger size to better match accommodate the requirement to conduct the testing procedures. Nonetheless, the tests could be considered representative of the smaller diameter alternative, as, for example, the values of tensile strength and elongation are computed using the dimensions of the samples, hence uninfluenced by shape/size.

Additional testing methodologies were used to assess the potential impact of the insert following ocular administration. The methodology employed to determine water uptake and swelling index of the insert ensured a consistent moisture environment at eye temperature, simulating tear presence. While concerns may arise regarding the potential influence of water absorption rate on tear film, it should be noted that the rate at which the insert absorbs tears and the rate at which tears are replaced provide a more accurate depiction of the insert's behaviour upon ocular administration. The inserts reached maximum absorption (approximately 30 µL of STF) within a few hours. Tear film turnover, however, estimated in 1–3 µL per minute production [80], would take up to 30 min to replace the full volume absorbed. Therefore, while *in vivo* studies are necessary to determine the actual interaction between the insert and the tear films, it can be speculated that the rate of tear film production should compensate for the amount lost from the insert, minimising discomfort and visual disturbances associated with reduced tear presence, especially considering increased tear production during infections [81,82], which are the primary therapeutic applications of the inserts. Furthermore, it remains important to verify the balance between the amount of tear produced and absorbed by the insert, as if one end the presence of excipients that concentrate the tears can help retain a clinically useful amount of drug distributed on the anterior eye, this may also reduce the bacterial washout for the eye surface/fornix. Hence, the administration of regular eye drops concurrent with the insert may offer the opportunity to equilibrate anterior eye environment and modulate tear dynamics and insert effectiveness.

Moreover, the linear expansions observed in the swelling index testing indicated that the maximum diameter reached by the inserts in their fully hydrated state increased by approximately 9%. This, for the intended dosage form design of 10 mm, could potentially lead to a maximum diameter of 10.9 mm after administration. Notably, this dimension aligns with the average value of lower fornix depth [83], suggesting that the insert can be dimensionally suitable for cul-de-sac administration even upon swelling. Furthermore, the majority of insert expansion upon imbibition could be attributed to thickness increment, further indicating that the device can be fully contained by the lower lid, as the conjunctival sac can accommodate up to 30 μl [11]. Additionally, in terms of potential irritancy of the formulation, pH values were found within the same range values of tear film present in the lower fornix [84], and in line with values observed in other formulations that were found non-irritant and well tolerated in animal model testing [22].

The release pattern of levofloxacin from ocular inserts was evaluated under conditions chosen to replicate eye characteristics. The use of donor-receiver apparatus for drug release testing can provide valuable indications of the potential behaviour of the insert *in vivo*, as these apparatus have consistently shown strong and positive correlation with testing in animal models [48,69,85]. In addition, temperature value (37°C) was selected to better mimic the administration site, as this temperature has been recorded in non-central ocular assessments and in cases of inflammation [86]. The biphasic release profile can be explained by the characteristics of the biobased polymers used in the formulation. These polymers contribute to the formation of a polymeric chain arrangement in dried state, which begins to relax and erode upon hydration and swelling. This process leads to rapid diffusion of the drug from the polymeric network, especially from its superficial portions, as observed in the initial release phase [67]. Subsequently, in a fully hydrated state, the gelling properties of the polymers induce a specific three-dimensional arrangement and porosity of formulation microstructure [87]. Furthermore, anionic polysaccharides like sodium alginate have been extensively studied as ocular in situ gelling systems due to their ion-dependent gelation, facilitated by polymer interactions with cations naturally present in the tear film (e.g., Na^+, Ca^{++}, etc) [88]. Therefore, the intrinsic and ion-triggered gelling properties of the excipients could have determined the gradual drug release recorded in the second phase [89,90]. Consequently, findings from the drug release study suggest a rapid release of levofloxacin in the anterior eye environment, followed by the gradual diffusion of remaining drug fraction over the subsequent hours from a gelling system formed by insert solubilisation. Thus, it can be assumed that the presence of gelling agents in the formulation leads to prolonged availability of the antibiotic agent and sustained therapeutic efficacy of the inserts [91].

The optimised formulation was tested *in vitro* for antimicrobial effectiveness against *S. aureus* and *P. aeruginosa*, which are the predominant bacterial species in ocular infections [5]. The levofloxacin-loaded inserts showed varying degrees of antimicrobial efficacy against the bacterial types. The inhibition zones differed between the two bacteria, with the insert displaying a strong antimicrobial activity against *S. aureus*, while a reduced effect was found against *P. aeruginosa* [74,75]. The ability of the insert to produce inhibition zones was consistent with the drug release tests, where the initial permeated antibiotic concentration (24 μg/mL), retrieved from the receiver chamber, was higher than the MIC_{90} for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (1 μg/mL and 8 μg/mL, respectively) [92,93]. Furthermore, it should be considered that the presence of the outer membrane in gram-negative bacteria like *P. aeruginosa* can provide additional protection and influence antibiotics effectiveness [94].

5. Conclusion

This study proposes a framework for applying insights into the anatomy and physiology of the eye to the design and testing of novel ocular dosage forms, such as film inserts, as an alternative to eye drops. The levofloxacin-loaded films produced with formulation F5, which incorporated HPMC E15 (1250 mg), low viscosity sodium alginate (750 mg), type A gelatin (250 mg) and PEG 400 (2.5 mL) as excipients, exhibited good qualitative properties and manufacturing repeatability (intra- and inter-day) and showed promising characteristics for ocular application. The physical and chemical characterisation of the inserts aligned with the ocular anatomy and physiology, while their mechanical properties suggested successful compliance with the insertion procedure. Levofloxacin content remained consistent across the manufactured inserts. The drug release pattern showed overlapping curves for the inserts tested, indicating consistency in the modality of drug release. Approximately 65% of levofloxacin was released within the first 30 min, with complete release observed after 6 h. Moreover, the inserts demonstrated potent inhibition of bacterial growth across different bacterial strains, with released drug levels exceeding the minimum inhibitory concentrations for these bacteria. Although the insert developed will require further *in vivo* characterisation, the *in vitro* results reported suggest that this innovative combination of biobased polymers holds tremendous potential for the development of effective, sustained, and fully soluble ocular drug delivery systems for antibiotics, offering both compatibility and simplicity of use.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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