DESIGN, DEVELOPMENT AND CHARACTERISATION OF A NOVEL ANTIMICROBIAL SOLUBLE OCULAR INSERT

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To my family,

wherever we are, we are together.

To my better half,

the light dissipating all shadows.

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Research output

The work conducted underpinned the following research outputs.

Publications:

- *Differences in practitioner experience, practice type, and profession in attitudes toward growing contact lens practice.*

Published as: Thite, N., Desiato, A., Shinde, L., Wolffsohn, J. S., Naroo, S. A., Santodomingo Rubido, J., ..., Gil-Cazorla, R. (2022). Differences in practitioner experience, practice type, and profession in attitudes toward growing contact lens practice. Eye Contact Lens, 48(9), 369-376.

- *Opportunities and threats to contact lens practice: A global survey perspective.*

Published as: Thite, N., Desiato, A., Shinde, L., Wolffsohn, J. S., Naroo, S. A., Santodomingo-Rubido, J., ..., Gil-Cazorla, R. (2021). Opportunities and threats to contact lens practice: A global survey perspective. Cont Lens Anterior Eye, 44(6), 101496.

Conference contributions:

- *Optimisation and evaluation of a soluble ocular insert for sustained release of levofloxacin.*

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- *Design, development and evaluation of a bioerodible ocular insert for sustained release of levofloxacin*

At 2021 American Academy of Optometry Conference

Receiving the travel grant to the American Academy of Optometry 2021 by British Chapter of American Academy of Optometry and Bernard Gilmartin Optometry Scholarship by Optometry & Vision Science Research Group at Aston University,

- *Optimised drug content of bioerodible ocular inserts for sustained release of levofloxacin.*

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Receiving the Best Overall Poster Presentation prize by College of Optometrists'

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- *Opportunities in contact lens practice: a global perspective*

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Chapter 1 - Introduction

1.1 Topical delivery of pharmaceutical agents

The topical route of administration for ophthalmic drugs can be considered the simplest and least invasive modality to deliver drug to the eye. Eyedrops, which account for 90% of all the ocular marketed formulations, remain the principal therapeutical option for several ocular diseases, including infective, inflammatory, and allergic disorders (Jumelle *et al.*, 2020; Rodrigues *et al.*, 2018).

The efficacy of topical administration of pharmaceutical agents is dependent on patients' adherence to treatment regiment, and it can be strongly limited by the ocular anatomical and physiological barriers (Jumelle *et al.*, 2020; Yang & Lockwood, 2022).

Limited volume, rapid turnover and drainage of tear film can greatly limit the residence time of the drug on the ocular surface. Furthermore, the unique features of the cornea, and in particular the limited permeability of the epithelium, prevent the pharmaceutical agent to have ready access to the target site of action at an efficacious concentration (Agrahari *et al.*, 2016).

Thus, given the complex anatomical and physiological characteristics of the eye, topical administration of pharmaceutical agents is mainly deputed to treat conditions affecting the anterior ocular segment (Löscher *et al.*, 2022). Although the advancements in ocular drug delivery may provide less invasive topical treatment alternatives, the most valid current options for the posterior segment delivery have been identified in intravitreal injection, implants, or systemic intravenous injection (Cabrera *et al.*, 2019; Nayak & Misra, 2018).

Therefore, a better understanding of the challenges associated with the topical delivery of ophthalmic therapeutical agents to the anterior segment and the analysis of strategies that have been adopted to address those challenges will provide a useful background in the development of topical drug delivery systems.

1.2 Challenges in topical drug delivery to the eye

1.2.1 Ocular barriers to drug delivery

Among the unique features of the eye, they can be found multiple and diversified defensive mechanisms. Despite their presence is vital for the integrity of the ocular structures, anatomical and physiological barriers determine a huge challenge in designing topical drug formulations that can held sufficiently effective therapeutic outcomes.

Different ocular mechanisms are constantly active to prevent assaults from foreign substances and/or pathogenic organisms to the eye, which also affect the ocular bioavailability of pharmaceutical agents (Skalicky, 2016).

Considering topical application of ocular formulations, the major drawbacks limiting drug availability can be grouped into static and dynamic barriers, displayed in Figure 1.1.

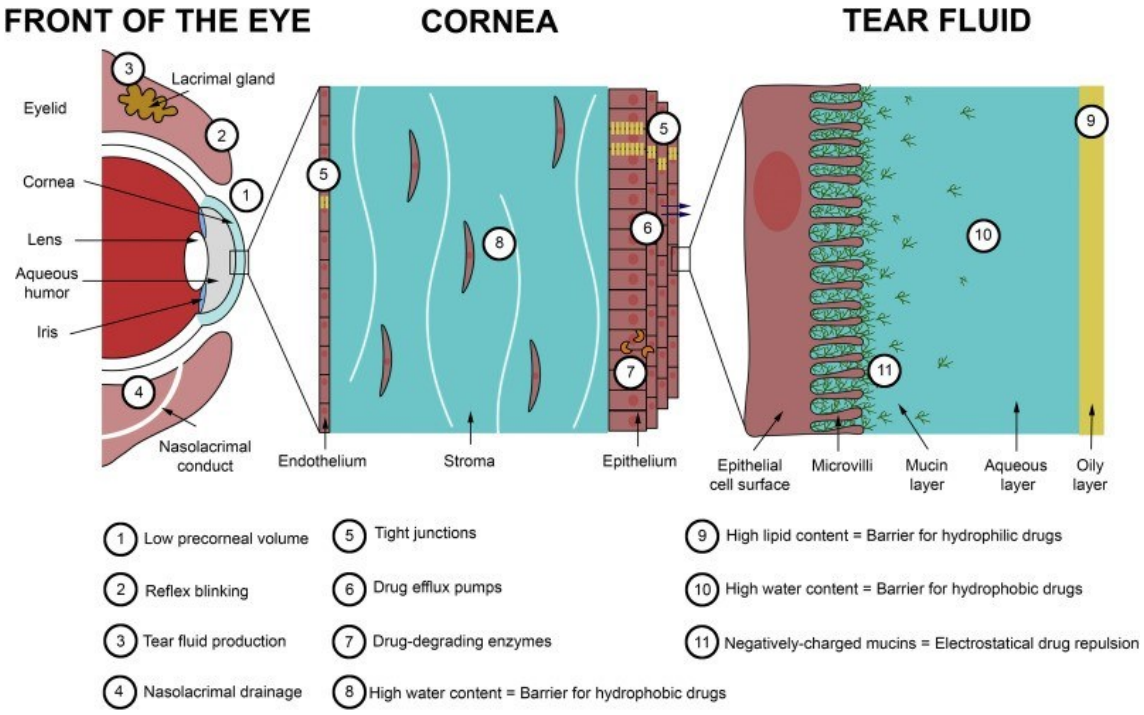

Figure 1.1 Main static and dynamic barriers for ocular drug delivery. Adapted from Jumelle et al. (2020)

The static barriers mainly relate to the anatomical characteristics of the cornea. Although all the different corneal structures contribute to the barrier function, the role of corneal epithelium can be considered the most relevant element in reducing drug penetration. In fact, the existence of an extremely complex arrangement of junctions impedes the paracellular diffusion of drug molecules in the tissue. The primary source of resistance is provided by the abundancy of tight junctions, predominantly located in the topmost apical epithelial layer. The presence of desmosomes in the wing cells and hemidesmosomes in the basal cells, along with adherens junctions dispersed throughout the various layers, offer structural stability and anchoring capabilities by ensuring adhesion to the underlying substrates, while gap junctions in the basal cell layers enable intercellular communication and significantly contribute to cell differentiation (Mantelli *et al.*, 2013). In addition, the presence of active efflux transporters in epithelial corneal cells results in reduced ocular bioavailability of the active substances (Karla *et al.*, 2009).

The dynamic barriers, instead, can be related to the tear film and its turnover. The average volume of the tear film has been estimated to be 6.5 µL, with lower average value in case of tear deficiency (Scherz *et al.*, 1974), and the lower conjunctival sac could act as a tear depot which can contain additionally 10 to 30 µL (Farkouh *et al.*, 2016). Furthermore, the normal tear film turnover has been reported to occur at rate of 16% per minute (Willcox *et al.*, 2017), thus the ocular surface can accommodate small volumes that are furtherly diluted upon administration.

Therefore, it can be estimated that the retention time of drops would be only of a few minutes (Figure 1.2) (Ipinazar Undurraga *et al.*, 2007; Lane *et al.*, 2009). Hence, the bioavailability of topical administered formulations can be assumed to be poor, and is has been approximated that only 5% of the pharmaceutical agents administered can reach the target site of action (Agrahari *et al.*, 2016). The remaining and prevailing fraction of instilled drops will conversely be discharged and/or absorbed by the body, potentially causing undesired side effects (Vaajanen & Vapaatalo, 2017).

1.2.2 Patients-related factors

To overcome the low bioavailability of eye drops, repeated administrations can be required to achieve the desired concentration of the drugs on the ocular surface, and this may give rise to different limitation in their use from the patients, mainly in terms of adherence and instillation technique (Urtti, 2006). Adherence to ophthalmic medications remains crucial in determining the effectiveness of ocular therapy, as well as the administration of pharmaceutical agents within suitable ranges (Figure 2.1)

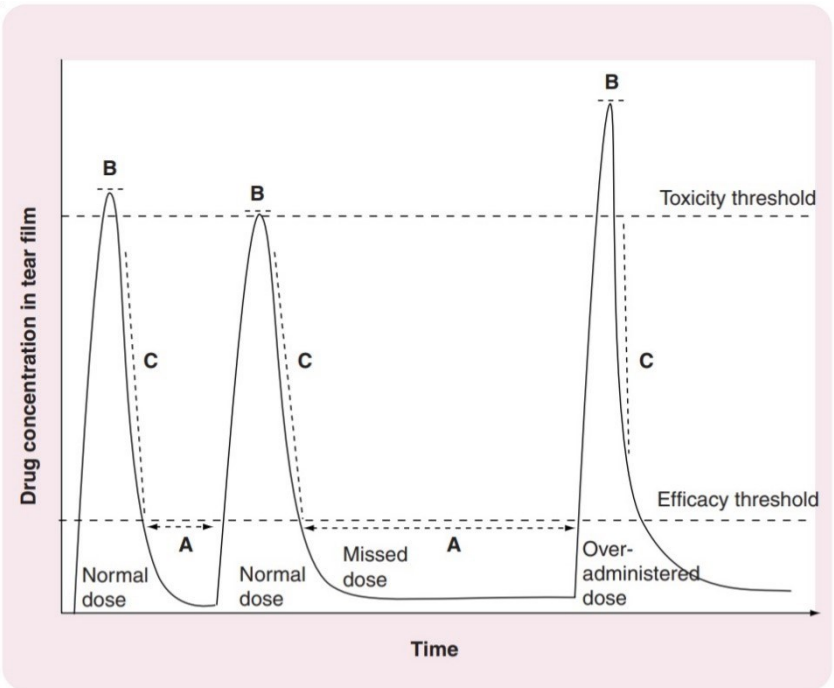

Figure 1.2 Hypothetical profile of topically applied drug concentration in the tear film over time. (A) Dosing intervals, (B) Dosing variability, (C) drainage of the administered solution. Adapted from Ali and Byrne (2008)

1.2.2.1 Dosing intervals

Although the level of adherence to ocular medication has been found difficult to evaluate, the impact of adherence rate has been related to clinical outcomes. In glaucoma patients, for example, lower rates of adherence were associated to a reduction of vision-related quality of life and to a progression of the visual field defect (Rossi *et al.*, 2011; Thompson *et al.*, 2018)

It has been estimated that for the treatment of chronic progressive diseases, the level of non-adherence ranged from 5% to 80% (Olthoff *et al.*, 2005). In spite of the difficulties in estimating the actual level of adherence, it could be reasonably considered that at least one-fourth to one-third of the patient are non-adherent (Frech *et al.*, 2018; Kim *et al.*, 2017; Robin & Muir, 2019; Schwartz & Quigley, 2008). Albeit the low rate of adherence was mainly associated to unintentional factors (forgetting to take medications) (Rees *et al.*, 2010), it is worth to consider that a need of a reduction of side effects, and of the number of administrations needed per day has been advocated by patients (Jampel *et al.*, 2003).

1.2.2.2 Dosing variability

The efficacy of administering liquid and semi-solid ocular formulations can also be influenced by the volume of formulation dispensed to the eye. The variability of the eye drops volume has been associated to factors related to the administration procedures and container characteristics. Bearing in mind that an optimum instillation of the medication may not be achieved by the patients (Gao *et al.*, 2018), the angle and rate of dispensing have been found significantly altering the volume administered (Sklubalová & Zatloukal, 2005). In addition, the design of the bottle and of the dispensing tip can influence the quantity of formulation administered, as they can do the physicochemical properties of the solution. Hence, the size of drops has been found to vary between 25 and 70 μL (Van Santvliet & Ludwig, 2004).

1.3 Strategies to enhance drug availability

Different strategies and drug delivery systems have been developed to address the limitations related to topical administration of drugs. The development of ocular topical formulations aims to increase the bioavailability of the therapeutic agents by extending the residence time in the ocular environment and/or favouring the penetration in the ocular tissues, mostly the cornea. The use of polymers, or excipients, in ocular formulations has been largely investigated, as they have been proved effective in modulating the retention and the absorption of pharmaceutical agents. In fact, many products including polymers have reached the market (Allyn *et al.*, 2021), while some other polymers have received the approval from regulatory agencies but they are not yet on the market (Kathuria *et al.*, 2021).

The following sections will present some general information about the potential strategies for topical drug delivery to the anterior segment of the eye, in particular, thin film inserts for the treatment of infections.

1.3.1 Liquid and semisolid ocular dosage forms

1.3.1.1 Viscosity enhancers

The main limitation of eyedrops can be identified in the rapid precorneal clearance time. Upon administration, the formulation will be rapidly diluted and removed from the eye surface, leaving a minor concentration of the drug available to the eye. To decrease the clearance, it can be possible to enhance the viscosity of the solutions by adding polymers that, by creating a three-dimensional network, will retain the drug in the tear film (Cassano *et al.*, 2021). Among those, they can be found cellulose derivatives or other biopolymers like alginate and hyaluronic acid, as well as synthetic polymers like polyacrylates and polyvinyl-alcohols (Grassiri *et al.*, 2021). Alternatively, *in situ* gelling agents can be embedded in ophthalmic solution/suspension. This formulation will extend precorneal residence time by modifying the viscosity of the solution in response to physicochemical characteristics of the ocular surface and tear film, such as temperature, pH, and ions. (Jumelle *et al.*, 2020; Wu *et al.*, 2019). Interestingly, some viscosifiers used as gelling agents in the formulation possess also *in situ* gelling properties (Wu *et al.*, 2019).

1.3.1.2 Mucoadhesive polymers

A further strategy to increase formulation residence time has been identified in the use of mucoadhesive polymers. Although the exact mechanism has not been entirely clarified, those molecules bond to the mucus layer, favouring persistence and absorption of the drug. Hence, the use of ionic polymers can further increment drug bioavailability (Boddupalli *et al.*, 2010; Ludwig, 2005).

1.3.1.3 Penetration enhancers

Penetration enhancers are a group of substances that modifies the permeability of the cornea, mainly by modifying the cellular structure of epithelial corneal cells and their junctions, or by altering the stability of the overlaying mucus and the tear film stability (Moiseev *et al.*, 2019).

Interestingly, some penetration enhancers, like the surfactant benzalkonium chloride and the chelating agent EDTA, can be found in ophthalmic solution as preservatives, thus it will be crucial to balance their efficacy to their toxicity for ocular cells (Burgalassi *et al.*, 2001)

1.3.1.4 Nanocarriers

Nanocarriers can be define as sub-micron sized structures that are used as drug vehicles to increase drug availability (Nagarwal *et al.*, 2009). They can be produced with a variety of different methods and compounds, which will determine the characteristics of the carries and the modality of drug loading and release (Akhter *et al.*, 2022; Vaneev *et al.*, 2021). In addition, depending on the features of the polymers employed, nanocarriers can enhance both retention and permeability of the pharmaceutical agents (Janagam *et al.*, 2017; Zhou *et al.*, 2013).

1.3.2 Ocular devices

1.3.2.1 Contact lenses

In the recent years, a growing attention has been dedicated to the use of contact lenses for drug delivery purpose, since they can extend residence time, favour penetration and increase bioavailability of pharmaceutical ingredients while being relatively safe, comfortable, and effective in aiding vision (Rykowska *et al.*, 2021; Toffoletto *et al.*, 2020).

In Table 1.1 are reported the main procedures to embed drugs into contact lenses, and their main advantages and disadvantages (Franco & De Marco, 2021). Among those techniques, the soaking method has gained great interest because, other than the simplicity of use, it can give the possibility to load drugs into matrices of commercially available contact lenses (Fan *et al.*, 2020), and that the ionic interaction produced by combining oppositely charged materials and drugs can enhance drug loading and release profile (Jones *et al.*, 2021).

Hence, the use of those materials showed that a significant amount of drug can be embedded and released. This has been assessed *in vitro* by statically incubating the CLs in specific volumes of saline (Hehl *et al.*, 1999), and *in vivo*, by using in rabbits' eyes as an animal model (Tian *et al.*, 2001). It should be considered that novel 3D-printed eye models, also include a simulation of blinking motion, have been recently introduced to provide a more biorelevant method in replicating the ocular surface conditions (Phan *et al.*, 2021).

Furthermore, it has been suggested that the use of contact lenses as a drug delivery system can be favourably seen by patients and health care practitioners, with the reduced dose frequency linked to the sustained release being the principal advantage (Ghazal *et al.*, 2019).

Finally, very recently the first drug-eluting contact lens received the approval from the pertinent national regulatory agencies and became available (only in a few countries). The contact lens has been proposed to prevent the symptoms of allergic conjunctivitis due to the sustained release of Ketotifen, an H1 histamine receptor antagonist, for up to 6 hours, with improvement of ocular symptoms lasting throughout the day (Ono & Toshida, 2022).

Table 1.1 Most commonly used methodologies investigated to embed drugs into contact lenses (CLs). Adapted from Franco and De Marco (2021)

DRUG LOADING Method	ADVANTAGES	DISADVANTAGES
Soaking method **Immersion of the lens in a solution/suspension/emulsion containing the drug to be loaded**	Easy, fast and low-cost method to load drugs into CLs	Massive use of solvents Low drug loadings, mainly due to a scarce penetration in the polymeric bulk High burst-effect in the release kinetics Rapid drug release
Solvent casting **Incorporation of drug-loaded films, generally produced directly by solvent casting, into the contact lens matrix**	Prolonged drug release Possible comfort and easy handling when thin and flexible films loaded with drugs are directly used as CLs	Possible degradation of active compounds due to the high process temperatures
Loading of vitamin E **Incorporating vitamin E into contact lenses as a diffusion barrier**	Prolonged drug release Additional therapeutic properties, mainly antioxidant activity Blocking of UV radiation, which damage eye tissues	Possible worsening of the lens' properties, as optical transparency, wettability, oxygen permeability A diffusion barrier mainly limited to hydrophilic compound
nanostructures or ring implants **Incorporation of nanocomposites or circular or ring implants loaded with ophthalmic drugs**	Prolonged drug release	Possible worsening of the lens' properties, as optical transparency, wettability, oxygen permeability Soaking method (with the related drawbacks) is often involved to incorporate drug-loaded particles
Molecular imprinting **Inducing a spatial arrangement of the monomers according to their ability to interact with the drug**	Formation of cavities into the CLs support with proper size/shape and high affinity for a specific drug High drug loadings Prolonged drug release	Possible undesired post-imprinting phenomena, like rearrangements of polymeric chains The selected drug as to be stable under the polymerization conditions
Supercritical technologies **Innovative supercritical fluid−assisted molecular imprinting method**	High drug loadings Prolonged drug release Preservation of polymeric structure	Possible worsening of the lens' optical properties, mainly due to a possible polymer foaming High operating costs due to high pressures

1.3.2.2 Rod-shaped devices

The administration of cylindrical devices can be an alternative strategy to increase availability of pharmaceutical agents, primarily by modulating the release and extending the residence time. Among those, it is possible to identify two topical devices that are commercially available, Mydriasert® (Thea Laboratories, Clermont-Ferrand, France) and Lacrisert® (Aton Pharma, Lawrenceville, USA) (Bertens et al., 2018).

Mydriasert® (4.3 mm length x 2.3 mm diameter) has been proposed for inducing immediate and lasting pre-operative mydriasis, by sustaining the release of tropicamide and phenylephrine after the insertion in the conjunctival fornix for few hours (Mouly et al., 2006). The use of this insoluble device has demonstrated a more effective and sustained mydriatic effect (Morgado *et al.*, 2010; Saenz-de-Viteri *et al.*, 2013), with an overall savings in healthcare costs (Shah *et al.*, 2015).

Lacrisert® (3.5 mm length x 1.24 mm diameter) is a resorbable drug-free device for the treatment of dry ere symptoms. The device has been designed as a small hydroxypropyl cellulose cylinder to be placed in at the bottom of the lower fornix after soaking in warm water. Upon gradual solubilisation, the constituent of the device acts as the therapeutic agent by increasing the viscosity of the tears, and conferring more stability to the tear film for up to 7 days (Nguyen & Latkany, 2011). The use of Lacrisert® has been associated to significant improvements in improved signs, symptoms, and quality of life associated with dry eye syndrome (Koffler *et al.*, 2010), also in contact lens wearer (McDonald *et al.*, 2009). Although some adverse events have been reported, with blurred vision being the most frequent (Luchs *et al.*, 2010), it has been reported that Lacrisert® has been also used for extended periods with a lower rate of adverse effects, suggesting safety and tolerability (Wander & Koffler, 2009).

1.3.2.3 Ocular film inserts

Ocular inserts can be defined as sterile, thin, multi-layered, drug-embedded devices with a solid or semisolid consistency whose size and shape are especially designed for ophthalmic application (Pelusi *et al.*, 2023; Rathore & Nema, 2009; Verma & Singh, 2014).

The development of thin films has gained increasing interest in recent times as a drug delivery form for different regions of the body (Karki *et al.*, 2016). The principal advantages of ocular inserts use have been identified in the increment of availability due to the sustained release and the extended retention of the drug, which can reduce systemic side effects and increase compliance of the patients (Rawas-Qalaji & Williams, 2012).

In addition, in respect of rod-shaped devices, thin films might be perceived as less invasive by the users. Also, the selection of formulation's components can tend toward a complete solubilisation of the device in the tear fluid, hence preventing the need of removal of the delivery device, which can be considered a limit of some of the insert already considered. Thus, they can be ideally designed to meet needs of patients and favour their compliance (Kumari *et al.*, 2010).

Among the large group of compounds that can be used to enhance the viscosity of ophthalmic formulation, it can be identified a number of polymers possessing also the ability to form films (Wafa *et al.*, 2021). After opportune solubilisation/melting and spreading, those polymers will rearrange and orientate to create a matrix characterised by a certain density of intermolecular bonding, depending on molecular structure of the compound (Yang *et al.*, 2023). The properties of the matrix can be modulated by the presence of plasticisers. The latter are low molecular weight compounds that can improve flexibility and processability of the films by increasing the intermolecular separation between the polymer chains (Snejdrova & Dittrich, 2012).

The combination of film-forming agents, plasticisers and drugs can confer to the film inserts specific physicochemical characteristics and determine the modalities by which the device is releasing the drug (Alrimawi *et al.*, 2021; Repka *et al.*, 1999).

Hence, the analysis of the evidence available about the successful production of thin film inserts designed for the ocular delivery of anti-infective agents can help to identify the formulations showing the most promising characteristics, in particular regarding the drug release profile, the acceptability and antimicrobial efficacy.

1.3.2.3.1 Thin films for ocular infections

This section reports the findings of a narrative review that investigated the characteristics of ocular antimicrobial film inserts, summarised in Table 1.2. The formulations have been grouped by the antimicrobial agent embedded in the device. Other than the shape and the dimension of the insert, they have been discussed the properties of the films to control the release of the drug. Also, if reported, the ability of the insert to meet and maintain the minimum inhibitory concentration (MIC) for contrast microorganism proliferation, as well as the potential tolerability/acceptability, were discussed.

Ciprofloxacin hydrochloride is an antibiotic drug, belonging to the class of quinolone, that has been mainly used and tested in thin film inserts manufactured with different polymeric formulation. Samantha & Gosal (2005) compared the proprieties of circular thin films (Ø: 4.5 mm) made of different concentrations of sodium alginate and hydroxypropylmethylcellulose (HPMC K4M), together with glycerol, as plasticiser. All the formulation proposed demonstrated a sustained release of the drug for several hours, up to 25 hours, and, *in vivo*, the amount of drug disperse in the tears was found to be above the estimated MIC for more than two days, in contrast with the conventional eye drop formulation, which disappeared after in 30 minutes. Finally, the authors identified a formulation based on sodium alginate alone (i.e., without HPMC) as the best insert formulation, because it gave the lowest peak of drug concentration and the more sustained release profile.

Charoo *et al.* (2003) have proposed to produce inserts by coupling a ciprofloxacin-loaded sodium alginate matrix with rate controlling membranes, fabricated with different species and concentrations of polymethacrylates and plasticised with diethylphthalate. They found that by increasing the amount of Eudragit RL100 and reducing RS100 it was possible to sustain the drug release over a period of 5 days, *in vitro* and *in vivo*. The inserts demonstrated the ability to produce and maintain a higher concertation of the drug in the aqueous humour during the testing animal models, which were also showing a more rapid recovery from a lab-induced bacterial conjunctivitis in respect of a three times daily administration of 0.3% ciprofloxacin treatment. Although they were not provided indications about the physical dimensions of the inserts, it can be assumed that they were showing suitable retention over days, while the persistence of ocular symptoms at the end of the treatment may suggest the need for further investigation about irritancy the tolerability of the device.

The same drug was embedded also in cross-linked gelatine inserts. Different gelatine concentrations and hardening/polymerisation times were tested. The circular thin film (Ø: 8 mm) were assessed *in vitro* and *in vivo* for 10 hours. The formulation with the highest percentage of gelatine (18%) and, more important, the longest hardening/polymerisation time (i.e., 60 minutes) was resulting in the best release pattern, giving a the linear, almost complete, delivery during the time tested. However, very little information was provided in terms of drug concentration and its potential clinical effectiveness. Hence, it was not possible to identify if the insert should be replaced (Mundada & Shrikhande, 2008).

Dawaba *et al.* (2018) have compared the use of different water-soluble polymers in the production of circular thin film inserts (Ø: 8 mm) for ciprofloxacin release, using propylene glycol to plasticise the films. They reported that the use of polyvinyl alcohol (PVA) (1.5%) was showing controlled release of the drug *in vitro* for 24 hours. Similar results were found for hydroxypropyl cellulose and xanthan gum (both at 1.5%). The fastest release rate, 6 hours, was reported for HPMC (K4M, 1.5%), while sodium carboxymethylcellulose (NaCMC) was releasing only a third of the drug at the end of the 24 hours testing. Close rates were found for the polysaccharides sodium alginate (1.5%) and pullulan (2%), which were releasing the drug in 8 hours. The PVA-based formulation was further tested on animal models, showing strong *in vitro* and *in vivo* correlation for the 8 hours testing reported. In light of this, together with good *in vitro* antimicrobial activity and no signs of irritation, the authors suggested that the device could be suitable for once-a-day administration. It would be interesting to determine the amount of pharmaceutical agent penetrating in the cornea, as the formulation showed one of the lowest mucoadhesive values among the polymers tested, and the authors reported that the insert could not be removed after the first 5 hours, which raises some doubts about how the final collections in the *in vivo* testing were performed.

Jain *et al.* (2010) proposed a different polymeric backbone. Circular thin films (Ø: 6 mm) were produced employing PVA and NaCMC (both water soluble polymers) after esterification of the polymers. This process conferred a slowed rate of dissolution to the inserts, leading to an *in vitro* sustained release profile of drug for up to 48 hours after initial burst release, in which half of the total drug amount was released within an hour. The formulation that guaranteed the longest release time *in vitro* was also tested *ex vivo*, for its ability to enhance permeation of sodium fluorescein in tissues of freshly excised goat eyeballs. The penetration was several-folds higher than the one registered for the eye drops formulation in cornea, iris, and retina, suggesting the efficacy of the insert in potentially conveying the drug more efficiently to deeper ocular structures. Furthermore, the study revealed good biocompatibility proprieties of the thin film insert, as well as its adhesive strength. However, it was not specified the time required for its complete dissolution, which could be estimated in more five days, thus a period longer than the effective drug release time.

The same group has blended PVA with gelatine to develop a biosynthetic hybrid polymer-based ocular insert (Ø: 6 mm) for ciprofloxacin release. The esterification (cross-linking) of the two compounds conferred to the formulation the ability to modify the characteristics of the inserts. The 10%-to-3% concentration ratio of the PVA-gelatin blend showed the more prolonged release rate of the antibiotic drug, together with the highest values of mechanical properties and mucoadhesion. It was also found active against the bacterial growth, while not being cytotoxic (*in vitro*) or irritant (*in vivo*). The final formulation, when loaded with sodium fluorescein, showed higher penetration of the dye in respect of eyedrop alternative (*ex vivo*). However, the cross-linking process determined that the inserts were less inclined to solubilisation in aqueous environment. Although the authors reported that the inserts were biodegradable, it remains unclear if the ocular residence time of the device would be comparable to the releasing period (Jain *et al.*, 2011).

Guillot et al (2021) have proposed a ciprofloxacin-loaded insert formulation obtained by combining PVA with Solupus®, a copolymer of polyvinyl caprolactam, polyvinyl acetate, and polyethylene glycol (Jin *et al.*, 2015). The inserts were completely dissolving in few minutes, but they were found able to increase permeability of the drug through the porcine cornea for the 6 hours in the *ex vivo* model. Although, as acknowledged by the authors, the drug release rate has to be furtherly investigated and developed, it can be still assumed that the formulation could find useful applicability for more immediate release.

Ciprofloxacin hydrochloride was used also in the poly (ethylene oxide)-based inserts, which were produced also with indomethacin and prednisolone sodium phosphate. The inserts were evaluated for the ability to enhance drug permeation into deeper ocular structures. The concentration of the model drugs in the eye tissues was compared with the one obtained after the instillation of the correspondent marketed eye drops formulation, after two hours from administration. Detectable concentrations of ciprofloxacin were found in all the tissues examined. The concentration in anterior eye structures was several times higher than what recovered for marketed aqueous formulation, whose administration was associated to non-detectable level in the posterior segment structures. Similar results were found also for the other drugs analysed. This, together with higher corneal permeation rate found in respect of the eyedrops, suggested that the poly (ethylene oxide)-based inserts could be further developed for drug delivery to the posterior segment of the eye (Balguri *et al.*, 2017).

Gatifloxacin is another antibiotic belonging to the fluoroquinolones drug class, which has also been used in manufacturing thin film inserts. Mishra & Gilhotra (2008) tested different concentrations of sodium alginate and chitosan, with constant amount of glycerine, to produce oval shaped inserts (area of approximately 77 mm^2). All the formulation demonstrated, *in vitro*, constant, and sustained release profiles for up to 12 hours. The formulation showing the longest release time (sodium alginate 2.0% and chitosan 1.0%) was further tested *in vivo* on rabbits, exhibiting very strong and positive *in vitro-in vivo* correlation. In addition, using higher percentages of chitosan was related to increased bioadhesive strength of the inserts and to a reduced swelling ability.

Aher & Nair (2004), manufactured gatifloxacin-loaded circular (area of 78.5 mm^2) bilayered inserts with different types of polymethacrylates and sodium alginate (each constituting one layer), which was used also in its thiolated variant. Thiolation is a chemical transformation of the polymers that favours crosslinking through the formation of disulphide intermolecular bonds, which has been demonstrated to enhance bioadhesive strength of sodium alginate (Bernkop-Schnurch *et al.*, 2001). *In vitro*, the insert with the thiolated variant of sodium alginate exhibited mucoadhesive strength two-times greater than those with the native polymeric form. The solubilisation times were also found different, with the insert including the thiolated from enduring for more than 12 hours, while the other dissolved in less than 30 minutes. Nonetheless, the release was found fast for both the native and the thiolated formulations (2 hours to complete diffusion), while sustained release profiles were achieved for both with the addition of the acrylate-based layer, reaching up to 80% release after 12 hours. Hence, although the use of sodium alginate in the thiolated derivative form was showing better mucoadhesion, the disparity between the release time and solubilisation time may discourage its use, while the addition of the polymethacrylate layer sustained the drug release (Aher & Nair, 2014).

Gorle & Gattani (2009), instead, combined chitosan with gellan gum. Different concentrations (2% or 3%) of the polymers were combined with different concentration (30% or 40%) of the plasticiser polyethylene glycol (PEG 400) to produce circular (Ø: 8 mm) inserts. The authors concluded that the films inserts made of chitosan can offer a more constant and linear gatifloxacin release for up to 24 hours, while the use of PEG 400 was associated to a slightly faster release of the drug. The same release profile was found *in vitro* and *in vivo*, showing a very strong correlation.

Furthermore, the same authors proposed a comparison between chitosan and gelatin-based film insert for the delivery of levofloxacin, a third-generation fluoroquinolone. The devices (Ø: 8 mm) were manufactured including also PEG, in different concentrations, and benzalkonium chloride. The formulation including 2% chitosan and 30% PEG showed the most linear release profile of the drug, which was found almost complete at the end of the 24 hours *in vitro* testing period. The same formulation was tested *in vivo*, exhibiting similar drug release profile and leading to determine a strong and positive correlation between the results of the testing methods. Nonetheless, as the *in vivo* drug release profile was determined by assessing the drug content remaining in the inserts after their removal from the animals eyes, it can be assumed that the device was persisting in the conjunctival sac at the end of the 24 hours testing time. Hence, it can be supposed that the inserts would require to be removed after it releases the amount of drug contained (Gorle & Gattani, 2010).

Bao *et al.* (2021) have proposed the use of hydrogel films to embed levofloxacin and dexamethasone. The inserts (Ø: 10 mm) were prepared combining a self-made derivative of chitosan grafted with dexamethasone and using different oxidation degrees of hyaluronic acid. The increment of the cross-linking density was corresponding to higher stability and hydrophobicity of the formulations. However, all the hydrogel film inserts were releasing the total amount of levofloxacin within 10 minutes, while only a small fraction of dexamethasone was release in the same period and not increasing over time. Hence, it was concluded that no significant effect of the high cross-linking density could be associated to the drug release rate.

Another fluoroquinolone tested in ocular inserts was ofloxacin. Tanwar *et al.* (2007) prepared films (area of 0.50 cm^2) of PVA, which were tested alone or in combination with rate-controlling membranes, made of ethyl cellulose or different types of polymethacrylates. The implementation of membranes greatly determined the characteristics of the inserts. In fact, along with minor physicochemical variations, the employment of membranes dramatically changed the release profile of the inserts. The uncoated reservoir showed complete release of the drug in 5 hours, whereas all the coated thin films demonstrated constant sustained release for more than 20 hours, irrespective of the polymer used. The insert coated with Eudragit RS100 showed a constant release of ofloxacin *in vitro* for 24 hours. It exhibited the same release profile when tested *in vivo* on rabbits, without any sing of irritation, suggesting its potential for being further tested as a once-per-day delivery system.

Kumar *et al.* (2012) investigated an ofloxacin-loaded insert (area of 0.50 cm^2), constituted by a mixture of guar gum, at different concentrations, with the addition of a glycerol. The use of increasing percentage of guar gum was associated to higher values of weight and thickness, and to a more sustained release. However, none of the formulations could release the drug completely, in the 24 hours *ex vivo* trans-corneal permeation study used, with the lowest guar gum concentration (0.5%) guaranteed the highest amount released (75.21%).

Reddy *et al.* (2017) investigated the possibility to release simultaneously ofloxacin and dexamethasone from thin film inserts made of HPMC and acrylates. The *in vitro* evaluation indicated that the inserts formulated were able to provide sustained release over the 24 hours testing time. Minor differences in the release profile curve could be identified in the rates of release recorded, with the formulations exhibiting different fractions of drug released within the first 4 hours (from 50% to 90%).

Moxifloxacin, on the other hand, was embedded in thin films (area of 0.50 cm^2) composed with different amounts of HPMC and polyvinylpyrrolidone (PVP K30). The formulation with the highest amount of HPMC (800 mg) and PVP K30 (1200 mg) was the only one found suitable for manufacturing the inserts. Those were then tested for drug delivery characteristics via *ex vivo* diffusion study, suggesting the ability to guarantee an estimated concentration of drug in the aqueous humour higher than the marketed eye drops. In addition, it was found that the insert converted into a gel within a period of 3 hours after the insertion, while the drug was not entirely released, suggesting that the transformation into gel was enduring the sustained release (Sebastian-Morello *et al.*, 2018).

A disc-shaped insert (area of 78.5 mm^2) was designed and optimised for the release of azithromycin, a macrolide antibiotic. The insert was designed to have a drug reservoir sandwiched between two rate-controlling membranes. The drug reservoir was made of different percentages of HPMC, while the membranes were fabricated with different amounts of Eudragit in the polymeric solution. The best formulation was identified in utilising 1.5% of HPMC and 3% Eudragit, which resulted in good physicochemical proprieties and to allow constant *in vitro* release profile over the 6 hours. Inserts were furtherly tested in rabbits for 12 hours, demonstrating good *in vitro-in vivo* correlation. In addition, the biocompatibility assessments did not highlight any irritancy or toxicity, in both *in vivo* and *in vitro* evaluations, and the size of the insert was also considered suitable (Thakur *et al.*, 2014).

Deshpande *et al.* (2010) proposed the use of cyclodextrins in preparing inserts loaded with acyclovir, an antiviral medication mainly to treat herpes simplex infection. Cyclodextrins have been used to increase bioavailability of active ingredients, by modifying their water solubility (Loftsson *et al.*, 2005). The acyclovir-cyclodextrins complex was prepared and added to polymeric solutions of HPMC and PEG to obtain the drug reservoir thin film. The reservoir was then sandwiched between two rate-controlling membranes of cellulose acetate phthalate. Formulations with different percentages of HPMC and cellulose acetate phthalate were tested. The release was satisfactory for the inserts combining 5% of cellulose acetate phthalate in the controlling membranes and 1.5% or 2% HPMC in the reservoir, which showed sustained and constant release for 20 hours. *In vivo*, both led to a detectable level of the drug in the aqueous humour for 20 hours, with a peak of concentration after 12 hours, in contrast with the ointment formulation not showing detectable concentrations after 4 hours. Therefore, it could be assumed that, even if the cyclodextrins had an impact on the solubility and the delivery of the drug, the composition of the membranes was crucial to reach a sustained release pattern (Deshpande *et al.*, 2010).

A drug-cyclodextrins complex was used also for fluconazole, an antifungal drug. The complex was mixed with different concentrations of HPMC, used as the film-forming agent, and a fixed amount of dibutyl phthalate, added with the dual purpose of acting as plasticiser and permeation enhancer. The membranes, instead, were made with different amounts of ethyl cellulose. The *in vitro* results showed similar trends across all the formulations, with inserts made of HPMC 1.5% and 2% in the reservoir and 5% of ethyl cellulose in the membranes giving the sustained and constant release for 20 hours. *In vivo*, the total amount of drug released in the same period was lower, but the release profile was still comparable. Thus, similar to what found for acyclovir-loaded inserts, it was confirmed the validity of cyclodextrins complex use, as it was cardinal the role of membranes in the sustaining the drug complex delivery (Abdul Ahad *et al.*, 2011).

Mirzaeei & Alizadeh (2017) developed an insert for the delivery of chloramphenicol, largely used antibiotic in optometric practice. Different kinds of acrylates were mixed with the drug and added to a blending of PVA and PVP, at different concentrations, with the addition of fixed amounts of glycerol and PEG. The formulation including Eudragit RL100 exhibited the best release profile, by achieving a complete delivery of the drug in sustained manner in 24 hours, *in vitro*. In addition, the insert was found to dissolve completely during that time. Nonetheless, this particular formulation produced inserts with thickness values close to 1 millimetre, debatably acceptable for thin films. Thus, it would be crucial to evaluate the effect of the physical dimensions on insert stability and tolerability.

Finally, a bilayered design was also proposed for chloramphenicol. Fixed concentrations of HPMC (K100) were prepared with and without PEG and including the drug. Those were alternatively coupled with water-insoluble films of Eudragit S100, made with different concentration of the plasticiser. The devices manufactured including PEG in both layers exhibited a longer durability in aqueous environment and, despite the use of water-insoluble polymethacrylates, all the inserts were completely disintegrated after 270 minutes. Even though the authors proposed the use of the system for ocular delivery and suggested it could sustain release for a minimum of 4 hours, the reported swelling characteristics need to be furtherly clarified. In fact, the inserts were found to expand their original volumes three to five times in respect of the dry state, prompting questions regarding their interaction with the tear film upon administration, which may become even more relevant in considering that the physical dimensions of the inserts were not specified. Comparing the two double layered films, the formulation with both layers plasticised remained intact for a longer period of time, representing an optimum ocular drug delivery system. In addition, all films, meaning that they are erodible and will be able to provide sustained drug release for at least 4 hours (Boateng & Popescu, 2016).

Table 1.2 Summary of antimicrobial topical thin film inserts reviewed

Drug(s)	Final formulation	Main Outcome	Release time [vitro/vivo]	Acceptance	Area [and/or diameter] and Thickness	Authors Year
Acyclovir + cyclodextrin	HPMC-K4M (1,5%-2%) and CAP (5%) + PEG (10%)	Aqueous humour (AH) concentration higher than ointment. 5-times longer (20h). 12h peak. *In vitro* efficacy doubled (24h)	20h [both]	No expulsion	[8] 0.151 to 0.171mm	Deshpande et al. 2010
Azithromycin	HPMC (1,5%), Eudragit RL100 (3%) + glycerol and dibutylphthalate (1%)	Time not satisfactory for daily use (>6h, no info on MIC).	6h [vitro], 12h [vivo]	Non-irritant and is well tolerated (up to 24 h)	78.5 mm^2 0.23 to 0.28 (coated) mm	Thakur et al. 2014
Chloramphenicol	2% w/v EUD + PEG (1.0% w/v) and HPMC 2% w/v + CHF (0.5% w/v)	Films released drug over 4 h, also expected to completely erode [removal not necessary]	6h [vitro]	N/A	20 × 20 mm strips *Depending on tests Between 12 and 17 µm	Boateng & Popescu 2016
Chloramphenicol	Eudragit R100 (1%), polyvinyl alcohol (4%), Polyvinyl pyrrolidone (2%) + glycerol (4%) and polyethylene glycol (4%)	24h dissolving, complete release. Suitable for once a day. No data on drug and efficacy level. Thickness to be monitored (about 1mm)	24h [vitro]	N/A	N/R 0.52 to 1.01mm	Mirzaeei & Alizadeh 2017
Ciprofloxacin Hydrochloride	Soluplus (3.3% w/v) + PVA (6.6% w/v) + PG (2%w/v) OR Soluplus (5% w/v) + PVA (10% w/v) + PG (3%w/v)	Increased permeability compared to eye drops	6h [*ex vivo*]	N/A	N/R 39.41 ± 0.60 to 28.54 ± 0.56 µm	Guillot et al. 2021

Ciprofloxacin hydrochloride	Not specified. PVA–gelatin blend: 10% (w/v) PVA + 1%, 2%, and 3% (w/v) for gelatin. 500 microliters of glycerol. Hydrochloric acid (50–100 µl) for esterification.	Increased contact time, prolonged drug release, improved trans-corneal penetration. Hydrophobic nature of the rate-controlling membrane plays a key role in releasing the drug from drug reservoir membrane.	24h [vitro], >3h [*ex vivo*, efficacy]	Excellent tolerability. No irritant	[6] 0.3 – 0.4 mm	Jain et al. 2011
Ciprofloxacin Hydrochloride	Sodium alginate (0.120 g) + Ciprofloxacin hydrochloride (0.75mg) dissolved in simulated tear fluid of ph 7.4 [reservoir]; Eudragit RL and RS + diethylphthalate OR Polyvinylacetate + diethylphthalate	Increasing the amount of Eudragit RL100 and reducing RS100, the rate of release of the drug was increased. Zero-order kinetics	5 days [both]	Sings: redness, lacrimal secretion, mucoidal, discharge, swelling of the eyelid, and response to ocular stimuli used as clinical evaluation of insert efficacy]	NK	Charoo et al. 2003
Ciprofloxacin hydrochloride	Sodium alginate (250mg), glycelrol (25mg), dissolved with distilled water (5ml) and Sorensen's PH (1ml)	Good retention. Single insert is sufficient to produce the necessary therapeutic action for at least two days.	24h [vitro], >2d [vivo, efficacy]	In place up to 3 days, minimum to no irritation	[4.5] N/R	Samanta & Ghosal 2005
Ciprofloxacin hydrochloride	1.5% PVA with 2.5% PG	Good bio-adhesion strength. Release of ciprofloxacin drug up to 24 h with no signs of eye irritancy. Good *In vitro-In vivo* correlation	24 h [vitro], 8 h [vivo]	Rabbits examined for eye irritancy for 7 days after the study: no rabbit showed any sign of irritancy	[8] 0.22 to 0.34 mm	Dawaba et al. 2018

Ciprofloxacin hydrochloride	Gelatin (18%) in glutaraldehyde solution (10%) [60 mins hardening time]	A specific formulation gave constant release up to 10h.	10h [both]	Draize: nontoxic and non-irritating. Not expelled	[8] 0.164 – 0.197 mm	Mundada & Shrikhande 2008
Ciprofloxacin hydrochloride	PVA (10%) nacmc (2%) + glycerol (500 microliters)	Permeation of fluorescein higher in cornea, iris and retina. Initial burst of drug.	48h [vitro], 3h [*ex vivo*, Na FL permeation]	Excellent tolerability. No irritant (Acute ocular toxicity study)	[6] 0.3 – 0.4 mm	Jain et al. 2010
Ciprofloxacin Hydrochloride, Indomethacin and prednisolone sodium phosphate	PolyOx™ and 10% drugs load	The corneal permeation was higher (not equally) for the three drugs. Level of drugs were higher in the ocular structures via the insert. Potentially improved by carriers	2h [vitro]	Non irritating (6h and 12h), not expelled	[4 x 2] 0.2 mm	Balguri et al. 2017
Fluconazole β-Cyclodextrin	HPMC-K4M (1,5% - 2%), EC (5%), Dibutyl phthalate (10%), Fluconazole β-CD (300mg)	Lower release % *in vivo*, sustained release. No info on drug concentration and effectiveness. Promising for once-a-day delivery	20h [both]	No expulsion. Absence of eye irritation and redness	[8-9] 0.16 – 0.17 mm	Abdul Ahad et al. 2011
Gatifloxacin	Sodium alginate (2%), Chitosan (1%), Glycerol (10%)	Chitosan crucial for bioadhesive strength and retention	12h [both]	N/R	77 mm^2 [13.2 x 5.4] 0.199 to 0.391 mm	Mishra & Gilhotra 2008
Gatifloxacin	Chitosan (2%-3%) or Gellan gum (2%-3%) + PEG-400 (20%)	Bioadhesive and plasticizer to increase bioavailability	24h [both]	No redness	0.5 cm^2 [8] 0.132 to 0.174 mm	Gorle & Gattani 2009
Gatifloxacin	Thiolated sodium alginate, Eudragit RL100 and Eudragit RS100 (75:25 ratio)	Thiolated higher mucoadhesion strength. Needed twice-per-day. Thiolation of SA does not contribute significantly towards improving the controlled release properties of polymer	12h [vitro]	Weighed amount inserts dispersed in PBS and administered to the cul-de-sac of conscious rabbits in order to quantify the *in vivo* interaction with the cornea	78.5 mm^2 0.054 [single] to 0.270 [coupled] mm	Aher & Nair 2014

Levofloxacin	Chitosan (2%) and PEG-400 (30%)	Zero order kinetics and show strong and positive *in vitro in vivo* correlation	24h [both]	No drag out + absence of redness in the rabbit eye	[8] 0.139 to 0.176 mm	Gorle & Gattani 2010
Levofloxacin	Succinated dexamethasone grafted glycol chitosan (adding Levofloxacin) + oxidized Hyaluronic acid [different oxidation times]	Lev rapidly released, reduced release of dexamethasone. Noncytotoxic against hcecs. Potent capacity to inhibit bacterial growth	1h [vitro]	Slight conjunctival irritation (e.g., hyperaemia) and minimal corneal irritation, but no intraocular irritation or IOP changes	[10] – *Depending on test N/R	Bao et al. 2021
Moxifloxacin	HPMC (800mg), PVP-K30 (1200mg), PEG (0,5ml) + glycerol (25mg)	Concentration in AH higher (estimated) then drops (short time tested)	3h [*ex vivo*]	N/A	0.5 cm^2/ 1 cm^2 0.05 mm	Sebastian-Morello et al. 2018
Ofloxacin	Ofloxacin (425 mg), Guar gum (0.5% w/v), Glycerol (10% w/w of polymer) in Purified water (100 ml)	Increasing percentage of guar gum led to higher thickness and weight, and more sustained release	24h [*ex vivo*, trans-corneal]	N/A	0.50 cm^2 N/R	Kumar et al. 2012
Ofloxacin	Polyvinyl alcohol (2%) [reservoir], Eudragit RS100 (+ Polyvinyl pyrrolidone K30, 3:1 ratio) (+ 30% Polyethylene glycol 400)	High *in vitro*/*in vivo* correlation, membranes crucial for physical proprieties and release pattern (Eudragit with best outcomes)	24h [both]	No irritant effects	0.50 cm^2 0.056 to 0.099 mm	Tanwar et al. 2007
Ofloxacin and dexamethasone	Undefined. Eudragit and HPMC K4M in different proportions.	Slight changes with formulation. Sustained slow (and complete) release at 24h, after initial burst. Cumulative effect, no data per single drug	24h [*ex vivo*, trans-corneal]	Non irritating (6h and 12h), not expelled	N/R 0.232 to 0.366 mm	Reddy et al. 2017

1.3.2.3.2 Considerations on thin films for ocular infections

A variety of polymers, polymeric blends, and designs have been used in the production of thin film inserts to sustain the ocular delivery of antimicrobial pharmaceutical agents. The polymeric composition determined the characteristics of the inserts tested. In terms of drug release time and profile, some useful information could be derived from the comparisons reported in the individual studies, but it was not possible to generalise any assumption about the effect of the excipients on the release properties of inserts manufactured. Similarly, neither the adoption of rate controlling membranes or of a preceding chemical transformation of the polymers were clearly demonstrating a superiority of the insert, if compared to the overall results reported. The drug release time was found ranging from few hours to 5 days, with most of the formulations releasing the drug in about 24 hours, suggesting a potential general once-a-day use of the antimicrobial inserts.

The antibiotics belonging to the class of fluoroquinolones were more often successfully embedded in the formulations proposed. It was not possible to identify any effect of the drug on the properties of the inserts, giving the lack of comparative studies specifically designed for the purpose and the variability of the strategies used to design the inserts. However, it was demonstrated that antimicrobial drugs could also be complexed or pre-loaded in carriers to produce ocular thin film inserts.

Across the study it was not possible to identify uniformity in the manufacturing and testing procedure for the inserts, whose descriptions were often found partial or missing. The drug dissolution testing could not refer to any standardised procedure and apparatus, making difficult to correlate the results reported in the studies. In addition, little to no information was given about the disintegration time and profile of the films, rendering difficult to predict any behaviour on the ocular surface after administration. Similarly, the procedures to test the tolerability of inserts greatly varied across the studies. Nonetheless, it could be seen that the inserts were rarely producing irritation or dislodgement. Furthermore, the antimicrobial efficacy was not always tested, and the testing protocols were not consistent across the studies.

Chapter 2 - Research aims and objectives

2.1 Background

Eyedrops are the most common vehicle used to treat ocular diseases affecting the anterior segment of the eye. The availability of the drug is strongly limited by the ocular barriers particularly if the drug is administered in a solution. The amount of drug that can reach the target site of action is strongly reduced by the restricted corneal epithelium permeability and the tear film dynamics, which result in in frequent drug administrations.

Different strategies have been adopted to overcome the ocular barriers to extend the retention of the formulation on the ocular surface and enhance the penetration of the drugs. For instance, drug availability can be increased by using excipients that enhance the viscosity of the drug and applying penetration enhancers.

On the other hand, sustained release drug delivery systems can enhance the availability of the drug in the eye. Contact lenses are emerging as alternative approach to topical eye drops due to their large residency time in the eye. Recently, the first drug releasing contact lens is available on the market, which will provide information on patients' and eye care practitioners' acceptability of this new contact lens application and will lead further development in this field. Also, rod-shaped ocular devices are an additional option to sustain drug release to the eye. However, these inserts have not been specifically designed for this purpose and their tolerability has been questioned.

Ocular thin film inserts have been proposed as an alternative sustained drug release system. Inserts have been embedded with a broad range of active pharmaceutical agents, including antimicrobial drugs.

2.2 Hypothesis

Although inserts have demonstrated the potential for antimicrobial drug delivery, the lack of consistency in manufacturing and testing methodologies, as well as the fragmentary and variable outcomes reported on their characterisation, offer the opportunity to further explore the development of ocular film inserts to deliver antimicrobial drugs to the eye.

2.3 Objectives

Therefore, the objectives of this work include:

- The evaluation of key elements in manufacturing procedures and testing methodologies for ocular anti-infective thin film inserts;
- The development of a novel blank soluble thin film insert for sustained ocular drug delivery;

- The development and the evaluation of a resorbable ocular antibiotic-loaded film insert;
- The optimisation of the manufacturing procedure for levofloxacin-loaded inserts manufacturing procedure, using a design of experiment approach;
- The optimisation of levofloxacin content and effects on drug release, trans-corneal permeability, and cytotoxicity of the inserts;
- Exploring the acceptability of novel applications of ocular devices by eye care practitioners, with a particular focus on contact lens opportunities.

Chapter 3 - Critical evaluation of key elements in manufacturing procedures and testing methodologies for ocular anti-infective thin film inserts

3.1 Introduction

There has been progressive interest in the development of thin films for ocular drug delivery in recent years, as these films have the potential to provide characteristics that may overcome the current limitations of conventional dosage forms – including the ability to sustain the release of the active pharmaceutical ingredients (APIs) (Karki *et al.*, 2016). This affords an opportunity to create a drug delivery system in the shape of a film, that can release the API over an extended period, whilst maintaining constant drug levels in the target tissue (FDA, 2009). Despite the presence of films formulations for various delivery routes (e.g., buccal, dermal) on the market, there still persists a considerable lack of regulatory guidance regarding the manufacturing procedures and testing methodologies of these films (Borges *et al.*, 2015).

Attention regarding the use of thin films has also involved the exploitation of this drug delivery system for ocular applications, including the administration of antimicrobial agents. In fact, to date, eye drops are the only available formulation option for treatment of topical ocular infections (NHS, 2022). Yet, eyedrop efficacy is greatly affected by the defensive mechanisms of the eyes, reducing the amount of drug available on the ocular surface to less than 5% (Urtti, 2006), accounting also for the rate of absorption by the ocular surface, which has been assessed by direct *in vivo* testing such as scintigraphy (Salminen, 1990) and *ex vivo* procedure carried out on enucleated animal eye tissues (Friedrich *et al.*, 1996).

Thus, the use of thin film inserts would be considerably beneficial in the administration of topical anti-infective agents, as this type of delivery system is intended to enhance the retention time of the API on the eye surface as well as increase drug availability, when compared with eye drops.

In the past two decades, several thin film inserts have been proposed for topical administration of anti-infective agents to the eye, but none has reached advanced developmental steps or clinical testing. However, a variety of adaptations and testing methodologies have been employed in manufacturing and characterising this drug delivery system. This is further worsened by the unavailability of monographs for thin films in the official compendia. Therefore, the aim of this review was to identify and evaluate the most relevant elements that have been reported in the delineation of the common strategies adopted in the manufacturing procedures and in the testing methodologies for ocular anti-infective thin film inserts, which can serve to delineate a common ground in the development of this device.

3.2 Manufacturing process

Although different manufacturing procedures have been successfully employed to produce film formulations, such as electrospinning, hot-melt extrusion and printing technologies (Musazzi *et al.*, 2020), the solvent-casting technique (and its modifications) is the most widely used method for production of films for ocular drug delivery. As such, anti-infective ophthalmic inserts have also been largely fabricated using this method. The extensive use of the solvent-casting technique has been linked to its relative simplicity, low cost/demand for equipment, and its suitability for a large number of polymers (Gupta *et al.*, 2022). Figure 3.1 summarises the most important steps and parameters to consider in the development of a solvent casting method. During the solvent casting manufacturing process, the adopted strategy should be primarily based on the therapeutic purpose, taking into consideration the expected onset and duration of drug effect (Patel *et al.*, 2013). The therapeutical purpose of the film insert should also direct the selection of the excipients, as they will determine the characteristics of the formulation. Upon selection, the polymers to be included in the formulation are weighed and solubilised in a suitable solvent(s) mixture. The active agent(s) can be either added during this step or solubilised separately and added at a later stage. After obtaining a homogeneous and degassed film forming solution, this solution will then be casted on the surface of choice or on a mold. Upon drying, the film can be removed from the casting surface and cut to the preferred shape/size (Morath *et al.*, 2022).

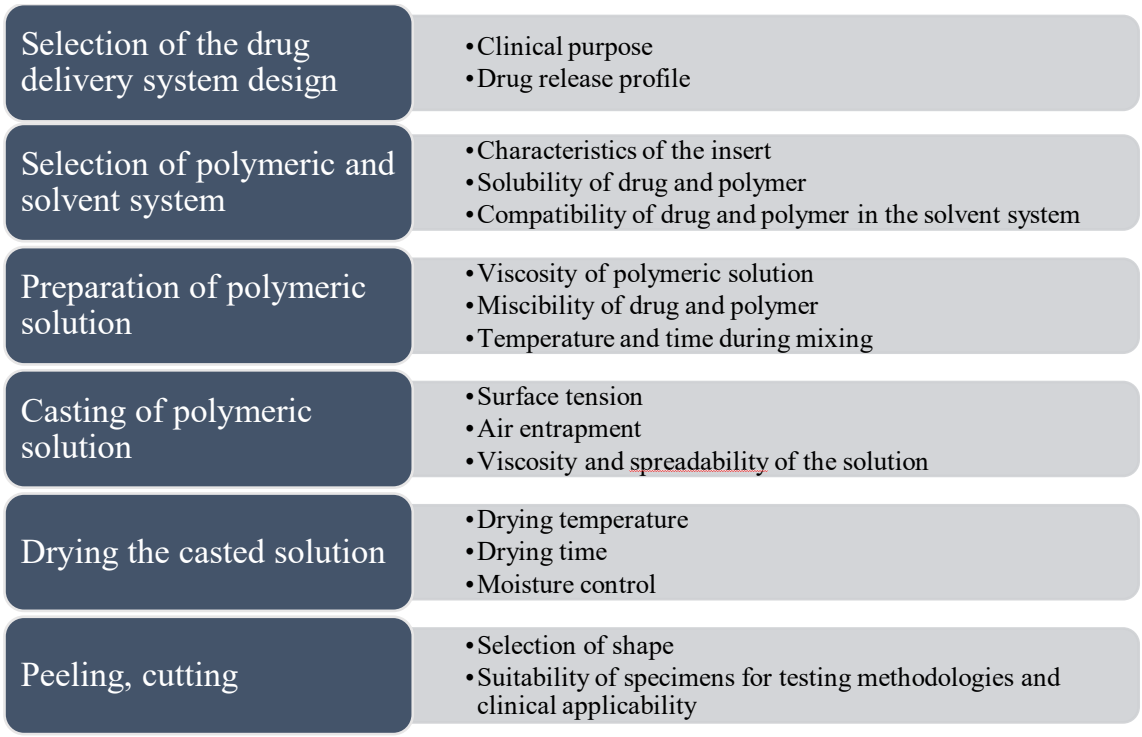

Figure 3.1 Steps and parameters in solvent casting method, adapted from (Karki et al., 2016)

The following sections report and discuss the key elements regarding the manufacturing of antibiotic-loaded film inserts that could be extracted from the current literature.

3.3 Design of antibiotic-loaded ocular films

In accordance with published studies, three main approaches have been identified for designing anti-infective ocular film inserts, namely: drug-loaded matrix systems, pre-processed polymer matrix systems, and multi-layered systems. Drug-loaded matrix systems is the prevalent design proposed and investigated. The manufacturing process for this system involves including the API(s) either before or after the addition of polymer (or mixture of polymers) into the solvent system. The drug can also be dissolved in a different solvent (Kumar *et al.*, 2012) or complexed (Abdul Ahad *et al.*, 2011; Deshpande *et al.*, 2010) before incorporation. Similarly, when combining soluble and insoluble polymers, the soluble polymers are solubilised separately and then added into the aqueous phase (Mirzaeei & Alizadeh, 2017).

Pre-processed polymer matrix systems are obtained by the same procedure used for the matrix design, but preceded by chemical transformation of the polymers. Modification of single components of the formulation (Aher & Nair, 2014; Bao *et al.*, 2021; Mundada & Shrikhande, 2008) or their combination (Jain *et al.*, 2010; Jain *et al.*, 2011) has been adopted to modulate inserts characteristics, particularly their water solubility and the associated drug release profile. The development of multi-layered systems was also proposed to ameliorate the drug release profile of infective ocular inserts. The systems include a water-soluble core sandwiched between two water-insoluble rate-controlling membranes. To obtain these three-layered systems, after the individual films have been manufactures and cut, they are sealed off using organic solvents or their vapours. Generally, the water-soluble layer is designed as a monolithic drug-loaded reservoir, while the insoluble membranes are manufactured without any API (Abdul Ahad *et al.*, 2011; Charoo *et al.*, 2003; Deshpande *et al.*, 2010; Tanwar *et al.*, 2007; Thakur *et al.*, 2014). However, bilayer designs have been proposed in which drug is present in the two coupled films and intended to provide both a loading and a maintenance dose (Aher & Nair, 2014); or added to the soluble component only, with the insoluble used as a rate controlling membrane (Boateng & Popescu, 2016).

3.4 Polymers in antibiotic-loaded films

The improvement of drug delivery systems has seen an extensive inclusion of polymeric compounds in ophthalmic formulations (Arribada *et al.*, 2022). To date, in fact, several polymers have been approved by FDA for use in the production of the ocular drug delivery systems, including eyedrops, intravitreal injections, implants, and inserts (Allyn *et al.*, 2021). These comprise both synthetic and biobased compounds. Biobased materials include polymers naturally present in nature and polymers synthesized artificially from natural resources (Caillol, 2020), and they are predominantly used in ocular drug delivery for their high biocompatibility and behaviour in aqueous environments (Allyn *et al.*, 2021). Furthermore, the combination of biobased and/or synthetic compounds, can offer opportunities to modify the characteristics of delivery systems to achieve suitable qualities for ocular application, and reducing the costs related to development of new molecules (Arribada *et al.*, 2022). During formulation of thin films, the film-forming polymers which constitute the backbone of the final delivery system have been identified as the principal elements (Cupone *et al.*, 2022). Upon solubilisation in appropriate solvents, film-forming agents create a solution/suspension or a gel in which the polymer chains interact with each other, creating the intermolecular bonds that lead to the eventual formation of a film upon evaporation of the solvent (Felton, 2013). Thus, their selection, and combination with other excipients, is crucial in determining the properties of the resulting films and in modulating the pattern of drug release (Karki *et al.*, 2016). These polymers are discussed below.

3.4.1 Biobased polymers

Polysaccharides, and in particular cellulose derivatives, can be considered the most widely used polymers in ocular formulations – used mainly as viscosity imparting agents in eyedrops (Dubashynskaya *et al.*, 2020). Interestingly, among the several attractive properties for ocular application, many polysaccharides also possess film-forming ability, and therefore, have been widely used in the development of thin film ocular inserts. Hydroxypropyl methylcellulose (HPMC) is a cellulose ether largely used in ocular formulations, because of the properties it can confer to specific delivery systems. In particular, HPMC has been employed as the backbone component of film matrices, in combination with other constituents (Tundisi *et al.*, 2021). In fact, it should be noted that utilisation of HPMC alone showed a faster *in vitro* drug release time compared to other water-soluble polymers used in the manufacturing of antimicrobial film insert, such as carboxymethyl cellulose and sodium alginate (Dawaba *et al.*, 2018). Also, the use of different molecular weights of HPMC mixed with water-insoluble components did not alter the drug release profile (Reddy *et al.*, 2017). This may be due to the hydrophilic and biodegradable nature of HPMC, which can induce a combined diffusive and erosive release of the API from the insert (Maderuelo *et al.*, 2011). As such, recently HPMC has been mainly used as a constituent of the hydrophilic element of multi-layered inserts (Abdul Ahad *et al.*, 2011; Deshpande *et al.*, 2010; Thakur *et al.*, 2014), and in bi-layered erodible ocular films for biocompatibility and mucoadhesiveness (Boateng & Popescu, 2016). Results have demonstrated that regarding extension of drug release rate, the presence of a rate controlling membrane is superior to combination with other water-soluble polymers (Sebastian-Morello *et al.*, 2018).

Among the various cellulose derivatives, other polysaccharides have been used in the manufacture of ocular films for antimicrobial delivery. Carboxymethyl cellulose (CMC) has been vastly employed as a viscosity enhancer in tear substitute and *in situ* gelling formulations for potential dry eye treatment (Gupta *et al.*, 2021). In antibiotic-loaded ocular films, CMC has been demonstrated to sustain the release of anti-infective agents, to promote their permeation in ocular tissue and to increase adhesiveness of the inserts (Dawaba *et al.*, 2018; Jain *et al.*, 2010; Jain *et al.*, 2011). Ethyl cellulose (EC) and cellulose acetate phthalate (CAP) are water-insoluble derivatives at ocular pH (Ahmadi *et al.*, 2022; Kim *et al.*, 2023). This insolubility, together with their film-forming properties, has enabled the use of both polymers in the rate-controlling membranes of multi-layered inserts, extending the release time of active ingredients (Deshpande *et al.*, 2010; Tanwar *et al.*, 2007).

Gums are another group of polysaccharides among the biobased polymers that have been used for antibiotic-loaded ocular films production. Gellan gum finds its main application in the food industry, in light of its gelling and film-forming properties (Wu *et al.*, 2021). Although its blending with other polymers has been advised to improve characteristics in manufacturing drug delivery systems (Zia *et al.*, 2018), gellan gum was used autonomously in the creation of films able to sustain ocular drug release (Gorle & Gattani, 2009). Similarly, guar gum has found increasing applications, also in diverse drug delivery systems (Thombare *et al.*, 2016). However, the high viscosity of guar gum solutions, even at low concentrations, should be considered carefully in relation to ocular film characteristics. The increment of the polymer concentration has been related to better mechanical properties, but with reduced total cumulative amount of drug released (Kumar *et al.*, 2012).

Alginate is a biobased polymer extracted from marine brown seaweeds that is largely used in the development of delivery systems for drugs and proteins, because of its biocompatibility and biodegradability, as well as its relative simplicity of use (Hariyadi & Islam, 2020). Alginates, and mostly the sodium salt derivative, are considered particularly attractive in topical ocular drug delivery application. Together with mucoadhesive properties, sodium alginate *in situ* gelling ability can be driven by calcium ions, naturally present in tears, triggering the crosslinking of the molecules and the consequent increment of formulation residence time and drug bioavailability (Karmakar *et al.*, 2022). Based on this principle, Samanta & Ghosal (2005) and Pawar *et al.* (2012) pre-treated a sodium alginate-based insert with calcium chloride solution and found that the procedure extended the release of the drug *in vitro*. Similarly to HPMC behaviour, drug release from sodium alginate formulations may be regulated by diffusion of the swollen matrix and the erosion of the superficial gelled layers (Mishra & Gilhotra, 2008); thus inter-molecular linkage may have an effect on both these release mechanisms. Regarding the manufacture of anti-infective ocular films, sodium alginate has either been used in drug-loaded matrix devices as the main component (Samanta & Ghosal, 2005) or mixed with other polymers (Mishra & Gilhotra, 2008). It has also been employed to constitute the core of multi-layered designs (Charoo *et al.*, 2003), and the use of its thiolated derivative form was suggested to increase the persistence of the matrix on the eye surface (Aher & Nair, 2014).

Chitosan is a biobased linear polysaccharide obtained by the deacetylation of chitin, which is a component of the exoskeletons of several living organisms, mainly sourced from crustaceans for pharmaceutical applications (Elieh-Ali-Komi & Hamblin, 2016). Chitosan possesses various characteristics that have made this polymer very interesting for ocular drug delivery (Mishra & Gilhotra, 2008). It is highly biocompatible and mucoadhesive, biodegradable by lysozyme (Li *et al.*, 2023), and has intrinsic antibacterial and permeation enhancing properties (Zamboulis *et al.*, 2020). Thus, chitosan has been vastly studied for various ophthalmic formulations including gels (Islam *et al.*, 2022), nanoparticles (Pal *et al.*, 2023), and films (Zamboulis *et al.*, 2020). However, chitosan has not yet been approved for ocular applications (Allyn *et al.*, 2021). The reason may be associated with the variability in its characteristics which depend on the degree of acetylation, and can therefore alter, for example, water solubility (Wu *et al.*, 2014) and mucoadhesive strength (Collado-González *et al.*, 2019). Chitosan is practically insoluble at physiological conditions (Bao *et al.*, 2021), and molecular characteristics of its derivatives are crucial in determining electrostatic interaction with mucus and hydrophilicity in the production of antimicrobial ocular films (Gorle & Gattani, 2010). Unfortunately, the degree of acetylation of chitosan has been only occasionally reported (Gorle & Gattani, 2009), despite the polymer being regularly dissolved in aqueous solutions (Gorle & Gattani, 2010; Mishra & Gilhotra, 2008).

Gelatin is a protein derived biobased polymer obtained by the hydrolysis of collagen (Elzoghby, 2013) – the main component of the cornea in terms of dry weight (Newsome *et al.*, 1982). Gelatin has a relatively reduced antigenicity in respect of original collagen because of its denatured molecular structure (Elzoghby *et al.*, 2012). As such, gelatin has been investigated for ocular drug delivery due to its biocompatibility and biodegradability, but also its gelling properties could provide suitable characteristics for topical formulations, including extended API bioavailability (Rana *et al.*, 2022) and bioadhesive properties (Gorle & Gattani, 2010). However, Jain *et al.* (2011) suggested that the use gelatin in manufacturing films can be associated with unsatisfactory mechanical properties of the device, and they opted to combine polyvinyl alcohol to gelatin to ameliorate mechanical properties. For these reasons, gelatin has been employed coupled with other polymers (Gorle & Gattani, 2010) or as its cross-linked derivatives (Jain *et al.*, 2011; Mundada & Shrikhande, 2008).

3.4.2 Synthetic polymers

Synthetic polymers are substances artificially produced by the repetition and/or combination of monomers, offering the possibility to make use of molecules with a variety of different properties. Synthetic polymers that have been employed in ocular drug delivery systems or their development are discussed below.

Polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) are water-soluble synthetic polymers that have gained increasing interest for ophthalmic applications in view of their biocompatibility, ability to increase drug bioavailability, thermal and chemical resistance, while being excellent film-forming agents (Franco & De Marco, 2020; Teodorescu *et al.*, 2019). Currently, other than a large use in eyedrops formulations, PVA has been mainly employed in the production of intravitreal implants (Allyn *et al.*, 2021), while PVP has been embedded into contact lenses (CLs) to enhance tear film stability (Reindel *et al.*, 2020). In the production of antibiotic-loaded films, the standalone use of PVA was associated with controlled drug release over 24 hours period, showing strong *in vitro-in vivo* correlation (Dawaba *et al.*, 2018). However, those results were not confirmed in other studies. Tanwar (2007) and Pawar (2012), for instance, found crucial to use rate-controlling membranes together with plain PVA inserts, which otherwise produced a reduced drug release time. Similarly, Jain *et al.* (2011) combined the polymer with gelatin to overcome the initial burst release of the antimicrobial agents and to maintain a sustained drug release, and the same results were found also for the addition of CMC (Jain *et al.*, 2010). Moreover, it was found that using higher PVA and Soluplus (solubiliser) concentrations could slow the release of the drug, but a further increase in their quantity produced very viscous solutions, making them inadequate to cast films (Guillot *et al.*, 2021). PVP instead has mainly been used for its bioadhesive properties, alone or blended with other polymers. PVP has been mixed with hydrophilic polymers to create a soluble insert enhancing drug penetration through cornea (Sebastian-Morello *et al.*, 2018), and has been combined with hydrophobic polymers to produce rate-controlling membranes (Tanwar *et al.*, 2007). The combined use of PVA and PVP has been advocated to exploit synergistic interaction of the polymers (Teodorescu *et al.*, 2019), and their blend has been associated with improved mechanical properties of films (Seabra & de Oliveira, 2004); while the effect on sustaining the drug release was not evident if compared to PVA alone (Mirzaeei & Alizadeh, 2017). Also, although they are regarded as biocompatible and non-toxic, it should be considered that the biodegradability of PVA and PVP has been limited to conditions not representative of the eye (Alves *et al.*, 2011; Vanharova *et al.*, 2017).

Polymethacrylates are a large group of synthetic polymers, comprising varying ratios of different methacrylic compounds (Rowe, 2020). Among those, two materials have been historically used in contact lenses: poly(methyl methacrylate) and poly(2-hydroxyethyl methacrylate) (Fan *et al.*, 2020). Eudragit polymers possibly constitute the largest and most representative family of polymethacrylates used as pharmaceutical excipients. The diversity of combinations of the individual substituents can confer peculiar properties to the polymers, and consequently to the delivery systems produced from them (Dos Santos *et al.*, 2021). For example, the solubility of the polymers in specific solvents or within distinct pH ranges offer the opportunity to tailor the attributes of drug release for system in line with the therapeutic purpose (Patra *et al.*, 2017). Although some Eudragit polymers are soluble at physiological conditions, those used in production of antimicrobial film inserts are insoluble in the ocular environment, in an attempt to extend drug release time. The inclusion of hydrophobic polymers has been achieved either by embedding them into an aqueous solution containing other hydrophilic compounds, or by creating separate rate-controlling membranes used in the multi-layered systems designs. Eudragit RL100 and Eudragit RS100 are polymers that can form films with different permeability to water. Charoo *et al.* (2003) combined these two polymers to create rate-controlling membranes for a sodium alginate reservoir containing an antibiotic and compared these to polyvinyl acetate membranes. Both the formulations extended drug release over a period of 5 days in *in vitro* release studies, and it was suggested that increasing the amount of Eudragit RL100 whilst reducing RS 100 increased the rate of drug released. A combination of Eudragit RL100 and Eudragit RS100 has been used to manufacture a membrane that was coupled to a sodium alginate hydrophilic film to create a bilayered insert, with both layers containing the antibiotic drug. Whether the hydrophobic layer was tested alone or coupled to the water-soluble component, the insert showed an initial burst followed by sustained release over a period of 12 hours, while the standalone hydrophilic layer released the drug in 2 hours (Aher & Nair, 2014). In an another bilayered film, Eudragit S100 was used to retard the release of the drug loaded only into the hydrophilic HPMC reservoir. The insert produced a biphasic profile, with complete release of the antibiotic 4 hours (Boateng & Popescu, 2016). These results suggest that to achieve higher extended release, the antimicrobial agents should be embedded in both layers. Tanwar *et al.* (2007) compared the efficiency of ethyl cellulose, Eudragit RL100 or Eudragit RS100 (combined with PVP) rate-controlling membranes, sandwiching a PVA drug-loaded core. All the membranes could extend drug release for greater than 20 hours, prolonging the release time of the hydrophilic core. In addition, it was found that the total amount of drug release was from Eudragit RL100, followed by Eudragit RS100 and ethyl cellulose. A drug reservoir layer of HPMC was sandwiched between rate-controlling membranes made with increasing concentrations of Eudragit RL100. The presence of the membranes increased the normal release period of 2-3 hours to over 12 hours. However, although using higher concentrations of Eudragit RL100 could retard the release of drug, the resulting inserts were thicker and less flexible (Thakur *et al.*, 2014). The drug release

profile of inserts was also sustained when Eudragit polymers were blended with hydrophilic polymers to produce monolithic drug reservoirs.

Reddy *et al.* (2017) combined HPMC, in different quantities and molecular weights, to Eudragit RL100 and Eudragit L100. They found that the highest linearity in the release profile was achieved by employing the highest ratio between hydrophobic and hydrophobic polymers, irrespective of which Eudragit specimen was leading (Reddy *et al.*, 2017). Yet, it was found that Eudragit RL100 produced the most extended drug release profile at the end of the 24 hours tested, over Eudragit L100 and Eudragit S100, if combined with a PVA and PVP mixture (Mirzaeei & Alizadeh, 2017). Eudragit RL100 was also found to provide to most sustained drug release in dip-coating a drug-loaded film made with PVA and sodium alginate. In this method, the inserts were immerged (and dried) several times into solutions of different Eudragit polymers. While dipping in Eudragit E100 did not produce any improvement to the 5 hours *in vitro* release time of original PVA and sodium alginate insert, Eudragit S100 and Eudragit RS100 could extend the release to 7 hours, and Eudragit RL100 showed a sustained release for 9 hours (Pawar *et al.*, 2012).

3.4.3 Plasticisers

Plasticisers are small molecules or polymers that are added to the formulations to lower the amount of intermolecular bonding between polymer chains and to promote their relative mobility, occupying the space among the polymers and changing their organisation (Turhan *et al.*, 2001; Vieira *et al.*, 2011). Plasticisers are generally used to prevent brittleness of the film and to facilitate handling and use (Oliveira *et al.*, 2020). Given that the use of plasticisers modifies the polymeric structure, it is crucial to identify compatibilities of the components used in the formulations to avoid plasticiser migration and to obtain suitable film properties (Šnejdrová & Dittrich, 2012). The addition of suitable plasticiser can lead to increased flexibility and toughness of the films (Lim & Hoag, 2013), increased film thickness, moisture content and solubility (Sanyang *et al.*, 2016), while the effect on drug release may not be significant (Jantrawut *et al.*, 2017) (Boateng & Popescu, 2016). Nonetheless, using higher concentrations of plasticiser has been linked to more rapid hydration and drug release from antibiotic-loaded ocular films, whilst reducing plasticiser concentration and increasing concentrations of other polymer(s) led to more prolonged drug release (Gorle & Gattani, 2009, 2010).

Yet, in the development of antimicrobial ocular films, few studies have reported results on the effects of plasticiser variations on the formulation, possibly because its optimisation is conducted at an early stage and subsequently kept constant (Deshpande *et al.*, 2010), or possible incompatibilities are inferred from the quality of films produced. Three plasticisers have been mainly used namely: polyethylene glycol (PEG), glycerol and dibutyl phthalate (DBP). PEG is a hydrophilic synthetic polymer available in different molecular weights. With very few exceptions, e.g., (Charoo *et al.*, 2003), PEG 400 has been the most widely used. It has been found that the mechanical behaviour of films could be altered by the molecular weight of the plasticiser, with the addition of higher grades of PEG significantly reducing the tensile strength and increasing the elongation at break of films (Laboulfie *et al.*, 2013). But surprisingly, some authors did not publish the molecular weight of PEGs used in formulating their films. The wide use of PEG with cellulose derivatives may confirm the strong interaction between these compounds (Turhan *et al.*, 2001). Despite its hydrophilic nature, PEG has occasionally been used to plasticise water-insoluble polymers, demonstrating its versatility (Kulkarni *et al.*, 2002). Glycerol, also referred to as glycerine, is a small natural hydrophilic molecule that has been used mainly in formulations containing PVA and sodium alginate, because of its strong ability to redefine the molecular organisation of those polymers (Gao *et al.*, 2017; Rahman *et al.*, 2010). Dibutyl phthalate is a lipophilic synthetic plasticiser commonly used in combination with water-insoluble polymers (Rowe, 2020). Although DBP could confer satisfactory mechanical and extended drug release properties to films, its utilisation should be dismissed, as the use of phthalates has been progressively restricted in recent years for being a potential risk to human health (Wang & Qian, 2021).

3.5 Film manufacture by solvent casting method

In manufacturing antimicrobial film inserts for ocular applications via solvent-casting technique, the ultimate goal is to combine the active ingredients and the excipients to create films exhibiting the desired pharmaceutical, physiochemical and mechanical properties (Karki *et al.*, 2016). Thus, various procedural specifications of the solvent-casting technique have been developed and reported to produce anti-infective loaded ocular thin film inserts, given the substantial inter-independence of formulation composition and the steps required to achieve an effective production protocol.

3.5.1 Solvent system

An understanding of the chemical and physical characteristics of the polymers is important to correctly adapt manufacturing methods to achieve adequate solubilisation of polymers, homogeneity of film forming solutions, and evaporation of the employed solvents. Once APIs and excipients have been selected, the identification of suitable solvents or solvent system can be considered the first crucial step in the production of films (Dixit & Puthli, 2009). An ideal solvent system should allow the generation of a uniform solution/suspension that can be casted, and evaporate the residual solvent (Morales & McConville, 2011), with the latter becoming of utmost importance to prevent undesired hazards associated with some substances, as hydrophobic polymers require the use of organic solvents (Barnhart, 2008). The rearrangement of molecular structures and the interactions among the ingredients are dependent on the ability of the solvent system to solubilise the components (Felton, 2013; Oliveira *et al.*, 2020). Hence, the selection of the solvent system was dependant on the solubility of the polymers, which was found different between the matrix and the multi-layered design elements. The manufacture of monolithic drug-loaded matrices has been mostly carried out using aqueous solutions, as a consequence of the large employment of hydrophilic polymers. Although it has not been clarified, it can be assumed that the use of buffered solutions has been preferred to pure water to elicit specific pH- and ion-dependant intermolecular bonding of the polymers (Samanta & Ghosal, 2005). However, it can be argued that the resulting increment in viscosity at the manufacturing stage may limit the production of the films (Guillot *et al.*, 2021), whilst gelation could be more usefully triggered after insert application (Dawaba *et al.*, 2018). Nonetheless, the solvent system selection should be best based on experimental outcomes. For example, in the production of drug reservoirs comprising both hydrophilic and hydrophobic polymers, the inclusion of organic solvents in the solvent system may require specific adaptation, and it can be crucial to mix solvents to achieve solubilisation of the compounds (Boateng & Popescu, 2016).

3.5.2 Mixing and degassing

To ensure uniformity of the final inserts, it is very important to achieve homogeneity of film forming solution before casting, thus appropriate mixing conditions and removal of bubbles from the formulation are needed (Buyukgoz *et al.*, 2021; Dixit & Puthli, 2009). Stirring time and stirring speed have generally been kept constant in the experimental setups of most researchers (Deshpande *et al.*, 2010), although variations of these parameters have been shown to modify drug content uniformity, (Buyukgoz *et al.*, 2021), and physico-chemical and mechanical properties (Masamba *et al.*, 2016; Santosa *et al.*, 2019). Despite that, the duration of stirring time used to homogenise the film forming solutions has been reported only occasionally (Mirzaeei & Alizadeh, 2017), and ranged from 30 minutes (Jain *et al.*, 2010; Jain *et al.*, 2011) to 12 hours (Mishra & Gilhotra, 2008). Also, whereas it has been described that specific times may be needed for the polymers to achieve complete solubilisation (Kumar *et al.*, 2012), it would be useful if values of stirring parameters were reported, to enhance appreciation of any modification induced by the blending of multiple molecules. Same considerations can be applied also to the value of stirring speed, which was found to range from 100 (Thakur *et al.*, 2014) to 700 revolutions per minute (rpm) (Guillot *et al.*, 2021), or just qualitatively described (Sebastian-Morello *et al.*, 2018). A further factor to consider in the homogenisation of the formulations is the addition of heating during the mixing process, because it can favour the solubilisation and distribution of the polymers (Dixit & Puthli, 2009). Nonetheless, mixing temperatures have been reported only for formulations including self-made hydrogel (Bao *et al.*, 2021) and PVA (Jain *et al.*, 2010; Jain *et al.*, 2011) (Dawaba *et al.*, 2018), as the polymer is known to require high temperatures for its solubilisation. However, most of the polymers used in the production of films are also used as viscosity enhancers or gelling agents in other ophthalmic formulations (Allyn *et al.*, 2021). Hence, while the use those compounds is probably considered to grant specific characteristics to the final film, it can also impose rheological properties to the precursory film forming solutions that may be challenging, which can be mitigated by increasing the mixing temperature. Similarly to the effect of plasticisers addition in films, providing heating to the polymeric solution can affect the intermolecular interactions among the chains of the compounds, lowering the viscosity of the fluid and favouring mobility of the molecules (Briscoe *et al.*, 2000). However, although polymers have a preferred temperature range for solubilisation (Rowe, 2020), often indicated by polymer manufacturers, it will be difficult to predict a suitable mixing temperature for the film forming solution, as requirement for the blending of polymers can modify those values (Nyamweya, 2021). Hence, it could be advisable to identify an optimal temperature range for the solubilisation of a specific combinations of polymers, as a way to maximise their miscibility without causing thermal degradation of the components, including heat-sensitive drugs (Amin *et al.*, 2015).

To obtain uniform films it is also important to remove air bubbles that may be trapped in the film forming solution (Morales & McConville, 2011). The prolongation of the stirring step, lowering the viscosity by heating the solution, and the use of more specialised equipment can all contribute to the deaeration of the blend (Dixit & Puthli, 2009). Nevertheless, for antimicrobial insert production, air removal has rarely been addressed, except occasionally by adding a resting step for the solutions, from 3 hours (Guillot *et al.*, 2021) to standing overnight (Kumar *et al.*, 2012; Mishra & Gilhotra, 2008), or by bath sonication (Dawaba *et al.*, 2018).

3.5.3 Casting surface

The final steps in solvent-casting method play a major role in the production of valid and uniform films. Either the formulations are casted as a single large film for cutting or the solutions are split into separate moulds to produce the final shape upon drying. Selection of the casting surface should aim for a smooth and stable surface, able to withstand effects of heating and not interact with drugs, polymers, and/or solvent systems (Perumal *et al.*, 2008). While in large-scale manufacturing, using continuous equipment is the most efficient option, in the early development of the formulations other options can be used (Dixit & Puthli, 2009). Petri dishes were a frequent choice for the casting of film forming solution for antimicrobial inserts. They were used as inert glass moulds (Boateng & Popescu, 2016; Kumar *et al.*, 2012) or prefilled with a mercury substrate (Mishra & Gilhotra, 2008). The use of mercury was found common also in the use plastic Petri dishes (Gorle & Gattani, 2009, 2010; Thakur *et al.*, 2014). This setup has been also implemented by the addition of rings on the substrate to serve as moulds for the final inserts (Tanwar *et al.*, 2007) (Aher & Nair, 2014; Deshpande *et al.*, 2010). Despite the common employment of mercury to ensure levelled casting surfaces, the use of such toxic materials has been criticised, together with high costs and limitations on films dimensions (Hansen & Taketomo, 1989). Also, the use of petri dishes forces the production of films with the same limited area, and the addition of small diameter rings may further decrease the effective surface. Petri dishes were also used after moistening/lubrication with glycerine to reduce sticking and facilitate peeling of the films (Abdul Ahad *et al.*, 2011; Dawaba *et al.*, 2018). The selection of metal casting surfaces, instead, appeared to be linked with a following heating exposure of the solutions for drying (Samanta & Ghosal, 2005) (Guillot *et al.*, 2021; Mirzaeei & Alizadeh, 2017). Merely selecting the right surface, however, cannot guarantee an adequate spreading of the formulations. Film forming solutions with higher viscosity will exhibit increased resistance to spreading on any surface (Roy *et al.*, 2009), preventing a good distribution and levelling of the exposed surface of the solution (Hansen & Taketomo, 1989). For such formulations, it would be advisable to adopt a casting setup involving an adjustable film applicator (Sebastian-Morello *et al.*, 2018), a device that allows a change to the distance between the spreading blade and the substrate using micrometre gauges (Dixit & Puthli, 2009).

3.5.4 Drying conditions

The drying conditions chosen depend on the characteristics of the film forming solution (Morales & McConville, 2011). The use of higher drying temperatures can favour volatilisation and maximise the removal of potentially harmful solvents from the final product, and can also reduce drying time (Karki *et al.*, 2016). However, increasing drying temperature can reduce the thickness and the flexibility of the films, potentially inducing brittleness to the films, as an excessive evaporation from the formulation can deprive the film of the plasticising effect of water (Bagheri *et al.*, 2019). Instead, drying films at 25°C resulted in better phisyco-chemical and mechanical properties than those dried at 45°C, which presented also a less uniform surface appearance (Al-Harrasi *et al.*, 2022). In drying film forming solutions for antimicrobial ocular inserts, a majority of the formulations underwent solvent dispersal at room temperature, while the adoption of higher drying temperatures mainly occurred for film forming solutions containing organic solvents (Thakur *et al.*, 2014), which were additionally exposed to a further drying step at room temperature (Abdul Ahad *et al.*, 2011; Aher & Nair, 2014; Deshpande *et al.*, 2010). This may confirm that for formulations including solvents the selection of higher temperatures is directed more towards solvent elimination than reducing drying time. Conversely, higher drying temperatures may have been used in sodium alginate-based formulations to promote water evaporation (Mishra & Gilhotra, 2008; Samanta & Ghosal, 2005), possibly in the attempt to force the release of water clusters trapped in the polymer chains during drying (Xiao, 2018).

3.5.5 General considerations

It is difficult to formulate a general “one size fits all” method for manufacturing films containing anti-infective agents, given the substantial inter-independence between formulation composition and the adaptations required for an efficient production protocol. In addition, the lack of consistency in reporting the specifications of some procedural steps restricts the opportunity to derive useful information in determining the causality of technique used.

3.6 Testing methodologies

The use of thin films for ocular drug delivery has gained increasing interest in recent years. Although some ophthalmic devices and contact lens have been approved and commercialised as drug delivery systems (Kim & Woo, 2021; Novack, 2023), the advancement of ocular films may be slowed by the lack of regulations and testing standards that are missing also the films designed for other routes of application (Khalid *et al.*, 2021). Hence, until standardised methods become available, the identification of the most suitable and consolidated methodologies applied in the evaluation of medicated thin films could provide a framework for the development of anti-infective ocular film inserts (Wasilewska & Winnicka, 2019).

3.6.1 Uniformity of inserts

Regardless of the manufacturing procedure, and specifically whether they are casted individually or cut from a large sheet, being a single-dose preparation, the dosage units should meet fundamental drug content uniformity criteria, which can be ascertained by different testing approaches (Manuel Martins & Farinha, 1998; Vranić & Uzunović, 2008). Although the amount of drug should be considered a key factor for the formulations (Subrizi *et al.*, 2019), the procedures used in the assessment of uniformity have been found inadequate in the characterisation of polymeric films (Perumal *et al.*, 2008).

Despite the fact that the measurement of thickness and weight have been considered routine tests (Nair *et al.*, 2013), their values can gain additional relevance in assessing film formulations because they are supposed to directly relate to the uniformity of drug distribution across the inserts (Bala *et al.*, 2013; Bhyan *et al.*, 2011; Salawi, 2022; Walicová *et al.*, 2016). Also, uniformity of physical characteristics of the inserts have been considered indicative of effectiveness and reproducibility of the manufacturing procedure (Kumar *et al.*, 2012) and of the formulation's clinical applicability (Boateng & Popescu, 2016; Reddy *et al.*, 2017).

3.6.1.1 Thickness

The values of thickness in anti-infective insert formulations have been mostly measured using callipers – either Vernier, gauge, or dead-weight (i.e., gauge equipped with a dead-weight probe, which can guarantee a more consistent pressure exerted on the samples). In literature, the thickness values of the formulations were mainly based on single insert measurements, although whole films have also been measured (Abdul Ahad *et al.*, 2011). For individual insert assessment, 3 to 10 randomly collected specimens were examined (Kumar *et al.*, 2012; Mundada & Shrikhande, 2008). The average values were calculated after repeated random measurements, in a number of varying repetitions ranging from 3 to 10 (Deshpande *et al.*, 2010; Jain *et al.*, 2011). In examining the uncut films, a similar number of readings was used to estimate the average thickness (Deshpande *et al.*, 2010), and the measurements were taken at random positions of the film or following a specific spatial pattern (e.g., centre and four corners) (Aher & Nair, 2014). Microscopical procedures have also been employed for average thickness values (Guillot *et al.*, 2021). However, for single insert evaluations were best represented by the thickness of the edge of the film (Kumar *et al.*, 2012).

3.6.1.2 Weight

Similar to thickness evaluation, the assessment of insert weight can provide information on the homogeneity of formulations and the repeatability of the manufacturing procedure, together with the uniformity of the API and excipient distribution across the film (Kumar *et al.*, 2012). The values of average weight of the formulations have been usually calculated from the reading obtained from 3 inserts, although higher numbers of specimens have been also used (Reddy *et al.*, 2017; Sebastian-Morello *et al.*, 2018). The number of specimens tested can become particularly important in considering the use of 20 samples. This value, in fact, would meet the number required for the pharmacopeial standard uniformity of weight testing for single-dose solid dosage forms (WHO, 2019b), potentially conferring increased robustness to the evaluation, and it can find additional relevance in testing blank films, because the inserts cannot be characterised for drug content (Walicová *et al.*, 2016). Nonetheless, the testing methodology was not applied in full in the evaluation of ocular anti-infective insert formulations even when 20 specimens were tested, as the weight values were not opportunely analysed (Mishra & Gilhotra, 2008).

3.6.1.3 Drug Content

The assessment of drug content and the verification of uniformity of drug distribution in films can be considered a mandatory requirement in the development of this drug delivery system (Subrizi *et al.*, 2019). The relevance of the testing methods is generally associated with the quality of the product manufactured (Vranić & Uzunović, 2008), but during developmental stages they can gain increased significance in evaluating the consistency of the manufacturing method and help to determine the rate of drug release from the inserts over time. Although they are intrinsically linked, the assessment of drug content and uniformity of drug distribution are two distinct methodologies, holding defined specifications to determine the acceptability of the formulations. The first evaluates the agreement between the expected drug content and the average of the actual amount present in the formulations of a batch, while the second measures the variability of the drug quantity found in the formulations of the batch, firstly assessed on 10 random dosage units (Bánfai *et al.*, 2007). In the assessment of anti-infective ocular inserts, considering the film as the batch and the dosage units as the inserts, the quantity of units assessed for content uniformity testing rarely met the threshold number of 10 (Guillot *et al.*, 2021), and it was found that inserts assessed were in a range from 3 to 5 (Gorle & Gattani, 2009; Tanwar *et al.*, 2007). The number of inserts tested for drug content was found to be in the same range (Abdul Ahad *et al.*, 2011; Dawaba *et al.*, 2018; Reddy *et al.*, 2017). Also, it should be noted that some researchers did not report these values while some evaluated uniformity qualitatively (Jain *et al.*, 2010).

These methodologies can assume specific relevance in relation to the manufacturing procedure. Low content uniformity results can be indicative, for example, that film casting solutions may possess variable levels of viscosity, which can make complete pouring and even spreading of the solution on the casting surface challenging. Interestingly, to measure drug content, the inserts have been solubilised in various solutions, with a prevalence of phosphate buffer saline and simulated tear fluid for monolithic matrices (Guillot *et al.*, 2021; Kumar *et al.*, 2012), and organic solvents for multi-layered inserts (Deshpande *et al.*, 2010; Thakur *et al.*, 2014). In addition, to enhance the solubilisation of the active ingredients, the inserts have also been crushed, shaken or sonicated (Abdul Ahad *et al.*, 2011; Mishra & Gilhotra, 2008; Reddy *et al.*, 2017).

3.6.2 Testing of physicochemical properties of inserts

The investigation of interaction between solid pharmaceutical dosage forms and moisture, especially water, is essential in the development of drug delivery systems, given that they may encounter each other during the production, storage and handling, potentially altering the device characteristics (British Pharmacopoeia Commission, 2023d). In manufacturing films, the interaction between solvents, drugs and excipients is the essence of the solvent-casting method (Ferretti & Cabral, 2016). Upon casting, the film forming solutions undergo a drying step, the conditions of which can influence the presence of residual solvents and characteristics of the final inserts (Karki *et al.*, 2016). It can be assumed, in fact, that the final film is obtained once the moisture level of the formulation has reached equilibrium with the drying environment, which can be represented by the stability of weight of the film over time (Sebastian-Morello *et al.*, 2018). Thus, at the end of the drying process, the inserts will still retain a certain level of moisture and the ability to incorporate moisture from the surroundings. These characteristics, namely moisture loss and moisture absorption, have been consistently measured in anti-infective ocular inserts in two specular modalities, by exposing the samples to high and low relative humidity conditions respectively.

3.6.2.1 Moisture loss

The quantity of moisture loss can be measured to assess the integrity of the inserts after film drying (Kumari *et al.*, 2010). The affinity of the polymers and plasticiser in the film to the solvents used can determine the residual moisture content of the inserts, which can be considered an important parameter in evaluating stickiness and mechanical properties of the film (Borges *et al.*, 2017; Preis *et al.*, 2014). In fact, the presence of residual solvents in the final films can favour molecular mobility in the matrix, acting as an additional plasticiser and determining insert flexibility (Janigová *et al.*, 2022; Owusu-Ware *et al.*, 2019). To test moisture loss of anti-infective ocular films, samples were collected and weighed individually in their original state, and then kept for three days in desiccators containing anhydrous calcium chloride, at a relative humidity of 30 ± 5 %. The values were reported as a percentage of the ratio between the original and the dried weight (Gorle & Gattani, 2009). This methodology, however, can have a limited efficacy in the determination of the total amount of solvents, in particular water, present in the insert. In fact, although film-shaped systems have reduced thickness, the addition of a further drying step to release solvents during film manufacture could decrease the evaporation rate at the surface, leading to a retention of the moisture in the bulk of the insert (Manzanarez *et al.*, 2021; Salmon *et al.*, 2017). Different gravimetric procedures are available in the determination of the residual solvent present in pharmaceutical formulations (British Pharmacopoeia Commission, 2023c). Among those, it was found that films for buccal delivery were widely tested via an oven-based technique: the inserts were pre-weighed and heated at a temperature above 100 °C until a constant mass was achieved (Wasilewska & Winnicka, 2019). Although it can be argued that the exposure of the formulations to higher temperature could degrade polymers in the films, the test can be adapted for temperature and duration to extract the solvents present also in the bulk of the inserts (Ahn *et al.*, 2014).

3.6.2.2 Moisture absorption

In testing anti-infective ocular film inserts, moisture absorption has been constantly assessed by pre-weighing and placing the samples for three days in desiccators at relative humidity of 75 ± 5%, obtained from saturated solutions of aluminium chloride or, more rarely, sodium chloride. The values were reported as a percentage of the ratio between the initial and the final weight (Deshpande *et al.*, 2010; Sebastian-Morello *et al.*, 2018). The ability of the formulations to assimilate moisture can be strongly altered by the relative humidity and the temperature, inducing a modification of the molecular structure and the mechanical properties of the films (Othman *et al.*, 2019). Thus, the evaluation of moisture absorption under specific conditions can be of use in determining the storage conditions for the inserts, more than predicting their behaviour upon administration to the eye (Attia & Rady, 2012). It can be speculated that the measurement of the capacity of inserts to incorporate moisture from the environment can provide information about the physical stability of the ocular films, and it should be interpreted as a degree of hygroscopicity, rather than a true determination of the amount of water the inserts can absorb (British Pharmacopoeia Commission, 2023a; Jethava & Jethava, 2014).

3.6.2.3 Water uptake and swelling index

The marked propensity of films to imbibe water-based solutions can be associated with the wide use of hydrophilic excipients in the production of these drug delivery devices (Karki *et al.*, 2016). The penetration of water in film matrices can produce a modification in the arrangement of polymeric constituents, which may alter the molecular structure of the inserts and lead to expansion and distortion of the device (Nair *et al.*, 2013). Thus, evaluating the ability of the film to uptake water can help in understanding how the inserts would interact with the application site and the modalities of drug release, potentially reflecting their behaviour after administration (Nair *et al.*, 2013). Different gravimetric methods have been proposed for determining water uptake of anti-infective ocular film inserts, usually estimated by comparing the initial weight of samples to the value obtained after soaking. To better represent the ocular environment, inserts were wetted with simulated tear fluid, although phosphate buffer and distilled water have also been used (Bao *et al.*, 2021; Guillot *et al.*, 2021; Kumar *et al.*, 2012). This specification can be crucial in interpreting the testing results, as it has been shown that the absorption can be modified by the soaking solution (Peh & Wong, 1999).

The testing of monolithic matrices has been mainly conducted using agar gel plates to provide fluid to the inserts, incubating the apparatus at a specific temperature, and weighing the samples at predetermined time periods until a constant weight was reached (Mishra & Gilhotra, 2008). Other than some variations in the incubation temperature, this methodology has been applied also with the implementation of pre-soaked filter paper, possibly to assist in the handling of the specimens during the procedure for formulations transforming into gels (Kumar *et al.*, 2012; Reddy *et al.*, 2017). However, it can be difficult to determine the amount of fluid available to the inserts, as little information was provided on the agar substrate processing (Mao *et al.*, 2017). The use of physical supports for the inserts has been employed also in testing multi-layered devices. However, their examination was carried out by directly placing the samples in a container with the soaking fluids, in volumes ranging from 5 to 25 millilitres, which may not be fully representative of the ocular characteristics (Abdul Ahad *et al.*, 2011; Aher & Nair, 2014).

While the water uptake of the inserts has been determined gravimetrically, the assessment of the swelling index, which is the analysis of the surface area expansion, can offer an additional opportunity to evaluate the retention properties of the inserts in the ocular cul-de-sac (Deshpande *et al.*, 2010; Samanta & Ghosal, 2005).

3.6.2.4 Surface pH

The evaluation of surface pH for film formulations can find its utility in determining any potential irritation or damage to the mucosal membrane (Salawi, 2022). Together with the APIs, the use of specific compounds and their concentrations can determine the surface pH of films (Patel *et al.*, 2007). Surface pH of anti-infective ocular film inserts has been measured by either pH paper or digital pH tester (Reddy *et al.*, 2017; Thakur *et al.*, 2014). The determination of pH can be obtained only after moistening of the samples and procedures adopted by researchers were not constant. The soaking conditions used ranged from two drops directly placed on the insert surface, to 5 millilitres (Kumar *et al.*, 2012; Thakur *et al.*, 2014). Also the moistening times differed greatly, with swelling allowed from 30 seconds for inserts directly soaked, to 5 hours for samples placed on agar plates (Dawaba *et al.*, 2018; Mishra & Gilhotra, 2008). Interestingly, distilled water was the solvent most commonly used to moisten the inserts, with simulated tear fluid rarely used (Mishra & Gilhotra, 2008). Nonetheless, it should be considered that, despite the low volume, the tear film holds buffering abilities capable of quickly restoring the physiological pH of the ocular surface, due to both lacrimal turnover and its inherent chemical composition (Yamada *et al.*, 1998). Although the lack of guidance in the assessment surface pH of ocular inserts must be taken into account, it may be proposed that testing methods can be adapted to use reduced soaking volumes, reduced moistening times and use soaking fluids possessing some buffering capacity.

3.6.3 Testing of mechanical properties

3.6.3.1 Tensile strength, elongation and Young's modulus

The assessment of mechanical properties of films can provide important information about the physical integrity and stability of the formulations (Morales & McConville, 2011). However, the identification of standardised tests or procedures to determine these characteristics of the inserts has been missing (Preis *et al.*, 2014). As such, various tools and methods have been applied, based on the testing standards applied to other materials (Wasilewska & Winnicka, 2019). The main elements in the determination of mechanical properties of film inserts are tensile strength, degree of elongation, and Young's modulus. They have been generally derived from the stress-strain curve, which represent the relation between the force applied and the deformation occurring to the film samples (Dixit & Puthli, 2009). Depending on the properties of the formulation, the curve can be characterised by an initial linear region that represents a reversible elastic behaviour of the inserts, followed by a non-linear plastic region that represent a permanent structural alteration of the samples, and terminating with the rupture of the specimens (Abramowitch & Easley, 2016).

The tensile strength is the maximum amount of pulling force that the insert can tolerate before undergoing a permanent deformation. Together with the elongation measured at this point, they define the plastic region of the formulation. The slope of this part of the curve is the Young's modulus of the sample, which can be also calculated by their ratio. The maximum elongation, instead, represents the ultimate stretching that the insert can undergo before rupture (Vaidya & Pathak, 2019). Young's modulus, also referred to as the modulus of elasticity, is indicative of the relative rigidity of formulations, with high module of elasticity linked to stiff films and low module to more flexible formulations (Dixit & Puthli, 2009; Vaidya & Pathak, 2019). Also, as elucidated in Figure 3.2, soft and weak inserts will present low values of tensile strength, Young's modulus, and maximum elongation at break, while a soft and strong film will exhibit a moderate tensile strength, low Young's modulus, and a high elongation at break (Morales & McConville, 2011). Although no reference values can be fully exploited, an ideal ocular film insert should possess concurrently, a good degree of stiffness to allow for proper handling and sufficient flexibility in the dried state to ensure ready adaptability to the ocular surface shape during application (Preis *et al.*, 2014).

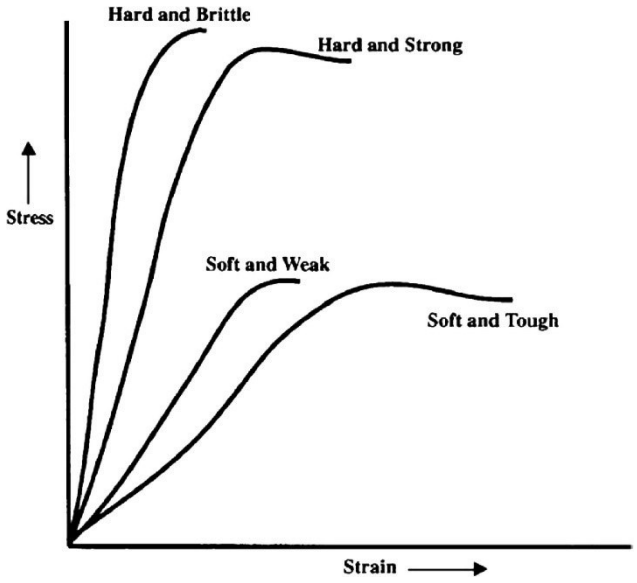

Figure 3.2 Examples of behaviours observed in stress–strain curves in polymeric film formulations. Adapted from Morales and McConville (2011)

The appraisal of these mechanical properties in anti-infective ocular inserts has been performed using various apparatuses, involving the use of computer-controlled testing equipment or self-made setups, used to determine the stress-strain curve (Jain *et al.*, 2010; Mishra & Gilhotra, 2008). Little and diverse information could be found regarding the specifications of the methodologies used. Among the few parameters reported, it can be seen that the specimen were shaped into rectangular strips, with dimensions of 50x10 mm (Mirzaeei & Alizadeh, 2017), while the pulling speeds were set from 30 to 50 mm/min, with an initial grip separation of 50 mm (Aher & Nair, 2014; Jain *et al.*, 2011).

3.6.3.2 Folding endurance

The determination of folding endurance value can offer an additional methodology to ascertain flexibility of film formulations. This test can supply important elements about potential inserts breakage during storage and administration (Wasilewska & Winnicka, 2019). This testing method was originally designated to test, in a standardised manner, the resilience of paper to repeated folding, which was provided by means of a specifically designed apparatus (Williams & Krasow, 1973). In anti-infective ocular inserts testing, folding endurance has been indicated as a quantitative measure of flexibility, or more commonly as an indicator of brittleness (Mirzaeei & Alizadeh, 2017) (Gorle & Gattani, 2010). The procedure has been performed by repeatedly folding the inserts along the same plane on the surface by overlapping specimen edges with two fingers (Reddy *et al.*, 2017; Thakur *et al.*, 2014). The procedure has also been performed by the help of forceps (Dawaba *et al.*, 2018). The folding process is repeated until visible cracks can be identified, or breakage has occurred. The total number of folding repetitions was considered the folding endurance value (Abdul Ahad *et al.*, 2011; Kumar *et al.*, 2012; Mishra & Gilhotra, 2008). Although lower values have been associated with adequate film flexibility, 300 repetitions has been suggested as the success threshold for the testing method (Aher & Nair, 2014). However, the intrinsic variability linked to the hand-made deformation of the inserts and the absence of clear indications about the determination of the procedural endpoint could raise concerns about accuracy and repeatability of the testing method, which can possibly find improvement by the implementation of automated devices, as it was firstly developed.

3.6.4 Drug Release testing

The determination of drug release over time is a crucial element in the characterisation of formulations that are designed to sustain the delivery of APIs. The identification and possible adaptation of *in vitro* methodologies to quantify the amount of drug released should provide a technique able to ensure sufficient levels of accuracy, robustness, reproducibility, and ability to identify modifications of the release profile due to changes in the formulations (Adrianto *et al.*, 2022). Whereas simplified models of the targeted tissue can offer advantages in the fabrication of the *in vitro* setup, they may not entirely represent the complexity of the tissues and to be tested (Kutlehria & Sachdeva, 2021). Different *in vitro* models for ocular drug delivery evaluation have been proposed, aiming to mimic specific districts of the eye, particularly the anterior surface (Phan *et al.*, 2016), anterior chamber (Liu *et al.*, 2022), and posterior pole (Adrianto *et al.*, 2022). The models designed to represent the anterior surface have been gaining great interest because they were developed to include a mechanism to reproduce blinking and to find primary application in the assessment of drug-eluting contact lenses (Phan *et al.*, 2021; Phan *et al.*, 2019). Analogously, the ocular model for the posterior eye has been developed to assess intravitreal implants performances (Adrianto *et al.*, 2022). However, the models proposed could find relatively reduced applicability in the evaluation of ocular film inserts, as they are not designed to include the conjunctival fornices, the preferred administration site for this kind of device.

The *in vitro* models for the determination of drug release for anti-infective ocular film inserts can be grouped into three main categories: vial methods, flow-through apparatus, and donor-receiver compartment models. The vial method is possibly the most straightforward *in vitro* device to be arranged. In this methodology, the inserts are placed in pre-warmed dissolution media, and the drug release is assessed by testing aliquots of the media collected directly from the container. The collected volumes of dissolution media have ranged from 3 to 30 millilitres (Aher & Nair, 2014; Bao *et al.*, 2021). The blinking interaction with the inserts has been simulated by oscillating the container (Abdul Ahad *et al.*, 2011; Deshpande *et al.*, 2010), or by stirring the media directly in the vial, while shielding the sample by the inclusion of a sieve over the film (Balguri *et al.*, 2017). A sieve, in form of a basket, has also been used in adapting the official pharmacopoeia methodology used to mimic drug release from oral solid dosage forms, but using lower volumes of dissolution media (Aher & Nair, 2014).

The flow-through apparatus design was set up by specifically implemented devices. The main characteristic of this model can be identified in the creation of a compartment within the apparatus that enables continuous irrigation of the insert with a constant flow of the dissolution media. The insert was either soaked in a fixed volume of dissolution media, continuously replaced (Tanwar *et al.*, 2007), or exposed to a constant flow rate, varying from 0.50 ± 0.10 mL/h to 10 drops per minute (Charoo *et al.*, 2003; Deshpande *et al.*, 2010), which was collected in a receiving chamber for further sampling.

The donor-receiver compartment models, on the other hand, comprised two distinct compartments, separated by a membrane through which the drug released by the insert passed from the apical/donor chamber to the receptor chamber (e.g., Figure 5.2). The Franz diffusion cells are a specific type of donor-receiver compartment model with advantages such as allowing the use of various membranes including excised corneal tissues; or being water jacketed for temperature control (Guillot *et al.*, 2021; Salamanca *et al.*, 2018). In fact, this kind of apparatus has also been used to test *in vitro*/*ex vivo* trans-corneal permeability studies on sheep, rabbit and porcine corneas (Guillot *et al.*, 2021; Kumar *et al.*, 2012; Reddy *et al.*, 2017; Sebastian-Morello *et al.*, 2018). Nonetheless, the membrane most commonly employed in the use of donor-receiver compartment models was a semi-permeable membrane of regenerated cellulose (dialysis tubing), which has also been claimed to mimic the corneal epithelial barrier (Mundada & Shrikhande, 2008). Interestingly, a great variety of dissolution media volumes have been used in receiver and donor compartments. For the receiver compartment, the quantity of media used was found in a range from 3 to 60 millilitres (Guillot *et al.*, 2021; Thakur *et al.*, 2014). For the donor chamber, however, reported information defined a range from 0.7 microlitres to 1 millilitre (Gorle & Gattani, 2010; Mirzaeei & Alizadeh, 2017). It has not been fully clarified, however, how the presence of such a small volume could be constantly maintained in the donor compartment, considering the propensity of the inserts to absorb water and the residual moisture present in the membrane after pre-soaking. Also, it remains uncertain if the inserts have been placed without any addition of media in the receiver compartment, in some instances where the value was not reported. Nonetheless, the values of drug released *in vitro* using donor-receiver compartment models adopting a semi-permeable membrane of regenerated cellulose have been most frequently found to demonstrate a strong and positive correlation with the *in vivo* evaluation of the same formulations (Gorle & Gattani, 2009; Mishra & Gilhotra, 2008; Mundada & Shrikhande, 2008).

However, it remains difficult to ascertain a preferential drug release *in vitro* testing method. In fact, although the *in vivo* testing methodology has been consistently carried out by examining the drug content remaining in the inserts after the administration in the cul-de-sac of rabbit eyes, a strong and positive *in vivo*-*in vitro* correlation has been found for the flow-through apparatuses (Charoo *et al.*, 2003; Tanwar *et al.*, 2007) and the vial method (Deshpande *et al.*, 2010). The concerns about the determination of an *in vitro* testing method able to represent the performance of topical drug delivery device for ocular administration have been previously raised for films (Franca *et al.*, 2019) and contact lenses (Wuchte *et al.*, 2021). Thus, the development of *in vitro* testing methodologies able to predict the *in vivo* performances of drug release remains of paramount importance to the advancement of sustained topical devices for the management of ocular conditions (Pereira-da-Mota *et al.*, 2022).

Table 3.1 In vivo and in vitro testing methodologies for anti-infective inserts.

***In vivo* Method**	**Dissolution Apparatus**	**Dissolution Method**	**Specifications**	**Dissolution Media**	**Dissolution Volumes**	**Time tested**	**Release Profile**	**Authors**
Inserts were removed	Donor-receiver	Donor-receptor (cellophane membrane)	Commercial semipermeable cellophane membrane, presoaked	STF	25 ml/ no media in donor?	Up to 12 h	Constant	Mishra & Gilhotra, 2008
Inserts were taken out carefully from the cul-de-sac of each rabbit and analysed for the remaining drug content	Donor-receiver	Bi-chambered donor receiver compartment with regenerated cellulose type (Sigma dialysis membrane)	Commercial semi-permeable membrane of transparent and regenerated cellulose type (Sigma dialysis membrane); mimic corneal epithelial barrier	PBS	25 ml/ 7 microlitres	10 h	Initially faster, then constant	Mundada & Shrikhande, 2008
Inserts were taken out carefully from the cul-de-sac of each rabbit and analysed for the remaining drug content	Donor-receiver	Bi-chambered donor receiver compartment with regenerated cellulose type (Sigma dialysis membrane)	Commercial semi permeable membrane of transparent and regenerated cellulose type (Sigma Dialysis Membrane)	PBS	25 ml/ 0.7 microlitres	24 h	Constant, various	Gorle & Gattani, 2009
Inserts were removed carefully and analysed for remaining drug content	Donor-receiver	Bi-chambered donor receiver compartment	Commercial semi-permeable membrane of transparent and regenerated cellulose type (Sigma Dialysis Membrane)	PBS	??? mL/ 0.7 microlitres	24 h	Linear	Gorle & Gattani, 2010

N/A	Donor-receiver	Modified Franz diffusion cell (sheep cornea)	Sheep cornea	STF	11 ml	24 h	Linear	Kumar et al., 2012
Inserts were taken out carefully from the cul-de-sac of each rabbit and analysed for the remaining drug content	Donor-receiver	Bi-chambered donor receiver compartment model	Commercial semi-permeable membrane of transparent and regenerated cellulose (dialysis membrane); 20 rpm simulate blinking	PBS	60 ml/ 5 microlitres	6 h	Similar slopes, different % at 6 h	Thakur et al, 2014
N/A	Donor-receiver	Donor-receptor	Dialysis membrane. Stirred continuously at 100 rpm maintained at 37°C. The receptor compartment was closed to prevent the evaporation	PBS	49 ml/1 ml	24h [vitro]	Initially faster (80% in 12h)	Mirzaeei & Alizadeh, 2017

N/A	Donor-receiver	Franz diffusion cell - Permeation experiment [Fresh cornea]	Sheep cornea	Simulated tear fluid (pH 7.4)	???/11 ml [data for receptor only]	24 [*in vitro*]	Initial burst, half in 4h	Reddy et al, 2017
Ocular inserts were removed carefully and analysed for amount of drug remaining in each ocusert	Donor-receiver	Donor-receptor [glass tube]	Cylindrical glass tube (Internal diameter 15 mm and length 100 mm). Shaking water bath was used to shake the contents of the receptor compartment continuously at constant temperature (37±0.5 °C).	PBS	25 ml / ???	24h [vitro], 8h [vivo]	Mostly linear. Different slopes depending on the polymer	Dawaba al, 2018
N/A	Donor-Receiver	Diffusion cells (Franz type) [*ex vivo* - transcorneal]	Rabbit corneas. 200 µL samples were taken manually from the receptor chamber at predetermined time intervals, every 15 min during the first hour and then every 30 min during the next 2 h for sink conditions	PBS	4.2 ml / ???	3 h [*in vitro*]	Linear	Sebastian-Morello et al, 2018
Ex vivo - Transcorneal Permeability + drug in tissues [cornea&sclera]	Donor-receiver	Franz-type diffusion cells	*In vitro* pectra/Por molecular porous membrane; *ex vivo* porcine cornea	PBS	3 ml / 150 µL	24 h [vitro], 6 h [vivo]	Non-Fickian diffusion mechanism	Guillot et al., 2021

Drug remaining in each ocular insert + Aqueous humour	Flow-through	Flowthrough apparatus	10 drops/min	STF	250/50 ml	120 h (both)	Linear	Charoo et al, 2003
Ocular inserts were removed carefully and analysed	Flow-through	Open flow through	No flow, Teflon disc	STF	2 ml	24 h	Constant, Prolonged	Tanwar et al, 2007
Ex vivo (goat eyeball) - with Sodium fluorescein	Flow-through	Flow-through apparatus	0.50 ± 0.10 ml/h	PBS	0.50 ± 0.10 ml/h flow	Up to 48 h (*in vitro*); 12 h (*ex vivo*, fluorescein)	Half of the drug released in the first hours	Jain et al, 2010
Ex vivo (goat eyeball) - with Sodium fluorescein	Flow-through	Continuous flow-through apparatus	0.60 ± 0.15 ml/h	PBS	0.60 ± 0.15 ml/h flow	24h [vitro], 3h [*ex vivo*]	Biphasic - 42–52% burst release, after prolonged till 24 hours	Jain et al., 2011
Tear samples	Vial	Vials	Closed with rubber stopper	Sorensen's	20 ml	Up to 57 h	Various durations, various peaks	Samanta & Ghosal, 2005
Ocular inserts were removed carefully and analysed + Aqueous humour	Vial	15 ml vials	Oscillating water bath at 32 ± 0.5°C with 25 oscillations per minute	PBS	10 ml	20 h	Constant	Deshpande et al, 2010

Ocuserts were removed carefully at 1, 2, 4, 8, 12, 16, and 20 hours and analysed for drug content	Vial	Vials	Oscillating water bath at 32 ± 0.5°C with 25 oscillations per minute	PBS	15ml vials containing 10ml of PBS	20h	Constant	Abdul Ahad et al, 2011
N/A	Vial	Modification of USP apparatus I with basket [beaker]	Basket, 50 rpm, 5 ml aliquots	STF	30 ml	Up to 12 h	Initial burst, 80% at 12 h	Aher & Nair, 2014
N/A	Vial	In a Petri dish	PBS in Petri dish	PBS (pH 7.4)	20 ml	6 h	Biphasic release, 60% within the first two hours - up to 70% final	Boateng & Popescu, 2016
Enucleated eyes. Ocular tissues separated and tested	Vial	Insert in vial + sieve over films + stir bar on the sieve [over a hot plate maintained at 34°C under stirring (spin bar)	Insert in vial + sieve over the films, 10 ml (pH 7.4) release/dissolution media. 34 C	5% w/v rmβcd (randomly methylated beta cyclodextrin) in isotonic PBS	10 ml	2h [vitro], 3h [vitro, eye], 2h [vivo]	No profile. Good release % at 2h (100, 93, 85)	Balguri et al., 2017
N/A	Vial	Vial placed in an air bath (37 °C)	Immersed in 3 ml PBS (pH = 7.4) and placed in an air bath (37 °C).	PBS	3 ml - total volume replacement	1 h (results) [vitro], 24 h [vivo]	Burst release (5 min)	Bao et al., 2021

3.6.5 Testing of antimicrobial efficacy

The aim of any anti-infective ocular insert is to provide a sufficient amount of API capable of combating the presence and the proliferation of unwanted microbes. Nonetheless, only a minority of proposed devices have assessed antimicrobial efficacy of the formulation. The antimicrobial efficacy of the inserts has been consistently evaluated by the measurement of the zone of inhibition created by the samples. For this method, an agar plate composed of suitable culture media must be prepared and inoculated with sufficient number (usually represented by a solution concentration) of the microorganisms of interest. The inserts are then placed on the substrate, and the plate incubated to allow the growth of microbes. If the drug present in the surroundings of the insert exceeds the minimum inhibitory concertation of the microorganism tested, the area will show no microbial growth (zone of inhibition). The diameter of the zone of inhibition can be compared against the control. The employment of blank insert as a control has been selected to determine, and eventually quantify, any inherited antimicrobial effect of the components of the inserts (Bao *et al.*, 2017; Dawaba *et al.*, 2018); as it is known that some polymers hold intrinsic antimicrobial properties (Yan *et al.*, 2021). The use of marketed eye drops (Mishra & Gilhotra, 2008) or eye ointment (Deshpande *et al.*, 2010) as controls was carried out to assesses the efficacy of an equivalent amount of drug present in the inserts; and the same has been used also with free drug solutions (Jain *et al.*, 2010). Also, the agar plate can be provided with both a negative and a positive control (Guillot *et al.*, 2021). Although some inserts produced zone of inhibition larger than those produced by the marketed formulations, it has not been clarified the mechanism underlying the results. If intrinsic antimicrobial properties are excluded, the dimension of the zone of inhibition should not exceed those created by the drug solutions, and, on the contrary, the embedding of the drug into a polymer matrix should reduce its spreading and penetration into the agar substrate. In fact, it has been reported that the increment in polymers concentration was associated to smaller zones of inhibition, and that the moistening of the inserts can play a role in determining *in vitro* antimicrobial efficacy (Guillot *et al.*, 2021).

To possibly overcome those limitations, a different testing method has been proposed to also consider the effectiveness of the inserts over time. Here, different concentrations of microorganisms were used to evaluate the efficacy of inserts at increasing levels of bacterial proliferation. The inserts, however, were able to prevent bacterial growth for the lowest concentrated inoculum, while little information could be derived by the sustained efficacy of the formulation (Tanwar *et al.*, 2007). Finally, the antimicrobial efficacy of inserts has been also theoretically assumed. Samanta & Ghosal (2005) and Mirzaeei & Alizadeh, (2017) compared the amount of antibiotic (chloramphenicol or ciprofloxacin) released to the minimum inhibitory concentrations (MIC). However, no specifications were provided about the microorganisms the drugs were supposed to act on.

3.7 Conclusion

The use of thin film inserts for ocular delivery of anti-infective agents can offer a valid alternative to the use of eye drops. Anti-infective ocular inserts have been mainly produced in the form of thin films by solvent casting method. The variety of excipients currently available can provide the opportunity to tailor insert characteristics, and the selection of manufacturing procedure parameters can determine properties of the final formulation. A recurrent lack of consistency and completeness of information was found in the evaluation of testing methodologies specifications reported. The characterisation of anti-infective ocular thin film inserts would greatly benefit from the inclusion of the procedural framework of reference standards currently used for other types of formulations. In addition, it would be of utmost importance to address the limitations associated with the existing methods to test *in vitro* drug release, and to create a model able to predict the *in vivo* performances of ocular inserts. Finally, a specifically designed antimicrobial testing methodology for the evaluation of thin sustained-release anti-infective drug delivery film inserts is needed.

Chapter 4 - Development and evaluations of a novel blank soluble thin film insert for ocular drug delivery

4.1 Introduction

The low bioavailability of eye drops, and the consequent need of repeated drug instillations, can lead to patient non-adherence and inefficacy of treatment regimens. A wide range of thin film inserts have been developed and proposed in recent years to address challenges in ocular drug delivery (Saettone, 1995). Most of these ocular film inserts have been formulated to include water-insoluble components (Karatas & Baykara, 2000), or to be fully insoluble (Lele & Hoffman, 2012). The latter, although potentially more promising in terms of drug release efficacy, can leave some uncertainty around removal of exhausted inserts, even necessitating the need for professional care. Such formulations can result in a reduced interest (amongst users, carers and prescribers), compared to formulations specifically designed to be completely soluble in the ocular environment, and therefore more suitable for self-administration.

To achieve inserts that are biocompatible and soluble upon placement on the ocular surface, various polymers can be used. Hydroxypropyl methylcellulose (HPMC), in combination with a variety of additives and plasticisers, is commonly used in ocular drug delivery due to its viscosity properties and compatibility; and is a component of commercial eyedrops – such as Tears Naturale® and GenTeal® for dry eyes (DeSimone, 2016).

HPMC has been widely used in developing film inserts for delivery of ocular hypotensive medications for the treatment of glaucoma (Kulkarni *et al.*, 2015; Pandey *et al.*, 2011, 2012; Rathod *et al.*, 2017; Ravindran *et al.*, 2014; Shivakumar *et al.*, 2007); to treat corneal infections (Abdul Ahad *et al.*, 2011; Deshpande *et al.*, 2010; Reddy *et al.*, 2017; Sebastian-Morello *et al.*, 2018; Thakur *et al.*, 2014), inflammation (Gilhotra *et al.*, 2009; Kulhari *et al.*, 2011; Tofighia *et al.*, 2017); allergic conditions (Sabale *et al.*, 2019); dry eye (Al-Saedi *et al.*, 2016) and for anaesthesia (Shukr, 2014). These demonstrate the favourable sustained drug release and good biocompatibility of the polymer.

Gelatin is an inclusive denomination of protein fraction extracted by partial hydrolysis of collagen (Sheskey *et al.*, 2017), which is one of the major components of eye tissues (Marshall *et al.*, 1993). Gelatin is the main constituent of the cornea (accounting for approximately the 70% of its dry weight) (Mannis & Holland, 2017), conjunctiva and sclera (Park *et al.*, 2016). Neat gelatin, alongside its cross-linked derivatives, has been employed as the main polymer in ocular formulations (Damodharan *et al.*, 2008; Mathurm & Gilhotra, 2011; Mundada & Shrikhande, 2008).

Sodium alginate is a salt derived from alginic acid that occurs in the cell wall of some brown algae. It has been employed in numerous topical formulations for its thickening proprieties (Sheskey *et al.*, 2017), its gel and film-forming ability, as well as for its mucoadhesive properties (Karki *et al.*, 2016). In ophthalmic topical formulations, sodium alginate has been used to produce stimuli responsive *in situ* gelling solutions that enhance the retention of eyedrops on the ocular surface and improve the drug release profile. This is achieved by a gelation process resulting from its interaction with calcium ions present in the tear film (Wu *et al.*, 2019). In ocular film formulation, sodium alginate has been used as the main component (Aburahma & Mahmoud, 2011; Gokce & Ustundag Okur, 2016; Samanta & Ghosal, 2005), or in combination with chitosan (Jethava & Jethava, 2014; Mishra & Gilhotra, 2008), and to produce cross-linked compounds (Szekalska *et al.*, 2017) to deliver hypotensive, anti-inflammatory and antimicrobial pharmaceutical agents.

Considering their versatility, HPMC in combinations with gelatin and sodium alginate, were chosen to formulate soluble ocular inserts, as a review of scientific literature produced no information on the concurrent use of these three polymers in the manufacture of soluble ocular thin film inserts. The aim of this study, therefore, was to initially screen available polymers for their film-forming ability; then optimise selected polymer combinations to produce soluble thin film inserts. The work would also evaluate the method for manufacture and testing of formulated films, and highlight the clinical implications of the tests.

4.2 Materials and Methods

4.2.1 Materials

HPMC E15 was purchased from JRS PHARMA (Rosenberg, Germany). Gelatin (from porcine skin, Type A), polyethylene glycol (PEG, average molecular weight 200), glycerol (BioXtra ≥ 99%), xanthan gum (from Xanthomonas campestris), arabic gum (from acacia tree), mucin (from porcine stomach, Type II), albumin (from bovine serum, minimum 96%), dibutyl phthalate (≥99%), chitosan (medium molecular weight) and general grade sodium alginate were purchased from Sigma Aldrich (Gillingham, UK). PolyOx^{TM} WSR-1105 LEO and WSR n-10 were generously gifted from Colorcon (Dartford, UK). Water was distilled with Purite Select Ondeo distiller (London, UK). All reagents were used as received.

4.2.2 Preliminary screening

The development of an ocular insert began with a preliminary screening of various polymers for their film-forming capabilities. The list of polymers to test was extrapolated from the review of the literature on ocular thin films (Chapter 1), focusing on the water-soluble compounds exhibiting promising outcomes in terms of dissolution profile and duration. Additionally, preliminary selection of polymers was based on any documented compatibility between the eye and the polymers, including evidence of non-ocular toxicity, and success in the development of ophthalmic topical formulations.

More than 100 formulations were screened for: film forming ability, qualitative appraisal, thickness, mechanical properties, and disintegration time. A list of formulations comprising HPMC is reported in Appendix II. The compounds constituting the most promising formulations during the preliminary screening were therefore selected for further investigation, and the results presented in this study. Other than the ability to produce qualitative films, the formulations were tested for their ability to form films with reduced thickness, to be better tolerated upon ocular administration; mechanical properties were assessed for estimate the ability to withstand the manipulation; disintegration time was assumed to be a precursor of the extent of residence time on the ocular surface.

4.2.3 Preparation of ocular films

Ocular films were prepared by solvent casting technique using HPMC as film forming agent, PEG 200 or glycerol as plasticiser, and gelatin and/or sodium alginate as additives. The list of formulations tested is presented in Table 4.1. All powders were accurately weighed using an analytical balance (Sartorius, Göttingen, Germany) and transferred into a 30 mL glass vial. Ten mL of distilled water was added to the powders mixture along with the plasticiser (both in liquid form). Vials were sealed and vortexed twice for 5 seconds to prevent powder clumping at the bottom of the vial, and to enhance adequate mixing. The mixture was sonicated for 30 minutes at 50˚C, then continuously stirred on a pre-heated magnetic stirring plate until a visually homogeneous solution was obtained. Thereafter, the solution was sonicated for a further 30 minutes (50˚C), to ensure complete elimination of entrapped gas. Finally, solutions were poured onto 9 cm Petri dishes using an ‘8-shaped motion’, to facilitate even distribution on dish. The plates were then left to dry at room temperature covered with blue roll, to favour loss of moisture without exposing them to inclusions. Upon drying, films were manually peeled using a spatula and tweezers. To prevent moisture transfer, any step including film handling was performed while wearing gloves.

The films were cut into 2x2 cm pieces using a chopping board and a specifically designed grid (Figure 4.1). During film preparation, any difficulties encountered such as inability to peel film off Petri dish, insufficient drying, inability to form a film or impossibility to generate a homogeneous film-forming solution were noted and used to assess the quality of films produced.

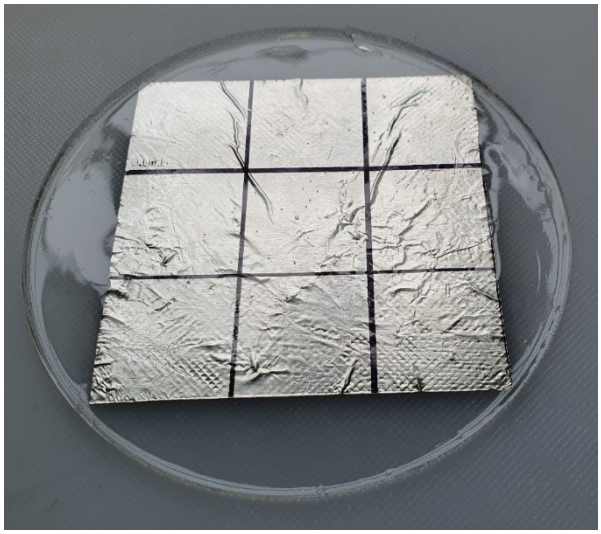

Figure 4.1 Reference grid used for film cutting – (squares 20x20 mm)

Table 4.1 Polymeric composition of the formulations tested, based on results from preliminary polymer screening (HPMC - hydroxypropyl methylcellulose; Na Al - sodium alginate; PEG - polyethylene glycol).

Formulation Number	HPMC (mg)	Gelatin (mg)	Na Al (mg)	PEG 200 (mL)	Glycerol (mL)	Water (mL)
1	500	-	-	0.5	-	10
2	500	-	-	1.0	-	10
3	500	-	-	-	0.5	10
4	500	-	-	-	1.0	10
5	500	100	-	0.5	-	10
6	500	100	-	1.0	-	10
7	500	100	-	-	0.5	10
8	500	100	-	-	1.0	10
9	500	200	-	0.5	-	10
10	500	200	-	1.0	-	10
11	500	200	-	-	0.5	10
12	500	200	-	-	1.0	10
13	500	-	300	0.5	-	10
14	500	-	400	0.5	-	10
15	500	-	500	0.5	-	10
16	500	100	300	0.5	-	10
17	500	100	400	0.5	-	10
18	500	100	500	0.5	-	10
19	500	100	300	1.0	-	10
20	500	100	400	1.0	-	10
21	500	100	500	1.0	-	10
22	500	100	300	-	0.5	10
23	500	100	400	-	0.5	10
24	500	100	500	-	0.5	10
25	500	100	300	-	1.0	10
26	500	100	400	-	1.0	10
27	500	100	500	-	1.0	10
28	500	200	300	0.5	-	10
29	500	200	400	0.5	-	10
30	500	200	500	0.5	-	10
31	500	200	300	1.0	-	10
32	500	200	400	1.0	-	10
33	500	200	500	1.0	-	10
34	500	200	300	-	0.5	10
35	500	200	400	-	0.5	10
36	500	200	500	-	0.5	10
37	500	200	300	-	1.0	10
38	500	200	400	-	1.0	10
39	500	200	500	-	1.0	10

4.2.4 Qualitative appraisal

Each sample was univocally coded and qualitatively inspected for colour, transparency, odour, gross surface appearance and feasibility to operate.

4.2.5 Physical characterisation

Weight. Each sample (2x2 cm) was weighed using an analytical balance in an antistatic diamond weighing boat. The weight of each formulation was calculated by averaging the weight of all samples (n=9).

Thickness. Using a digital Vernier calliper, the thickness of each sample was measured along the four lines symmetry. The four measures were recorded and averaged to estimate the thickness of each sample. The thickness value of the different formulations was derived by averaging the thicknesses of all the sample (n=9). The pressure exerted during the thickness measurement of the formulations can be affected by the specimens' compressibility. Nevertheless, the measurement process was meticulously executed to account for this potential error, with the least possible pressure being applied during the procedure.

4.2.6 Mechanical proprieties

The mechanical proprieties were evaluated using the Hounsfield Tensometer (Hounsfield Limited, Croydon, UK). Three samples (2x2 cm) from each formulation were tested. The squared pieces of film were placed in the grip to be equally covered by the upper and lower grip. The test parameters were set as follows (Gilhotra *et al.*, 2009): stress range 0.05 MPa; stress range 300%; speed 60 mm/min; gauge length 20 mm; no preload was set. Young modulus was derived three times from the measurements of tensile strength and elongation at maximum load (by dividing tensile strength by elongation) (Davis, 2004; Preis *et al.*, 2014). Results were reported as mean ± standard deviation.

4.2.7 Water uptake

To measure the water uptake properties, squared pieces of qualitative filter paper grade 413 (approximately 25x25 mm) were cut and coded to accommodate all the formulations samples. After weighing the filter paper and the film-paper system, 100 µL of distilled water at 36˚C were carefully poured in the centre of each sample using a pipette. After 2.5 minutes, the water laying on the film was gently removed from the specimen by tilting, and the device was weighed. The procedure was repeated every 10 minutes until film dissolved. To identify any erosion of the samples, the film-paper systems were placed on a blue tissue paper, to enhance visibility of water permeation from the apparatus. The water uptake was calculated using the formula:

$$Water\ uptake = \frac{(Wet\ weight - Dry\ weight)}{Dry\ weight}\ x100$$

4.2.8 Residual moisture content

The moisture content present in the films was evaluated as follows. Thirty-millilitres glass vials were coded and weighed to receive all the spare pieces of films remaining after cutting, as well as the singular components of the formulations. The filled vials were weighed and place in a vacuum oven at 105˚C (800 mBar). The systems were weighed after 12, 18 and 24 hours (Ahn *et al.*, 2014). The moisture content was calculated using the formula:

$$Moisture\ content = \frac{(Initial\ weight - Dried\ weight)}{Initial\ weight}\ x100$$

4.2.9 Surface imaging

Optical microscopy (AxioVision Software, AxioCam MRm, Zeiss, Jena, Germany) images were acquired to estimate the surface roughness of the films and to assess the presence and the extent of polymer aggregates and inclusions.

4.2.10 Disintegration time

For disintegration time testing, a 6-well cell culture plate was filled in each compartment with 10 mL of distilled water. In the wells was placed a specifically designed stainless steel meshwork support. The system was pre-heated to 36˚C, representative of the eye temperature (García-Porta *et al.*, 2019). The samples were marked with a permanent ink cross, representing the bisectors of the square, and carefully placed on the support with tweezers, ensuring that all the specimens were completely soaked in the water. The plates, covered with lid, were then placed in temperature controlled orbital shaker at 36˚C and 100 rpm. At each detection point, the plates were removed from the shaker and the samples were visually evaluated, photographed and inspected with the help of a contrast microscope (PrimoVert, Zeiss, Jena, Germany). The complete disappearance, or a massive disruption, of the cross present above the meshwork was assumed as the endpoint of the test. The test was performed in triplicate for each formulation.

4.3 Results and Discussion

4.3.1 Materials selection and film preparation

Overview of preliminary screening results. Among the film forming agents tested, HPMC resulted as the most versatile and demonstrated film forming ability in combination with different additives and plasticisers, in respect of sodium carboxymethylcellulose, chitosan and PolyOx™. Briefly, low molecular weight PolyOx™ required extended periods of drying and did not form films in combination with selected additives, higher molecular weight PolyOx™ formed films that were too brittle to be peeled. Although chitosan produced films on its own when dissolved in an acetic acid solution, the films were insoluble in water and exhibited a persistent acetic smell, suggesting an incomplete dispersal of the solvent. Therefore, chitosan films were not developed further. On the other hand, using water as the solvent produced a chitosan suspension that did not result in film formation. Furthermore, formulations containing xanthan gum, arabic gum, pectin, polyvinyl alcohol, and albumin exhibited film forming ability and, in most cases, acceptable thickness. Nonetheless, these films did not reveal adequate mechanical proprieties and/or sufficiently extended disintegration time and were not developed further. Mucin was also tested as an additive, but the films produced had a persistent unpleasant odour and were not characterised further. In terms of plasticiser screening, neither dibutyl phthalate nor propylene glycol, at the concentrations used, met the film forming abilities required when tested in combination with HPMC.

4.3.2 General appraisal on film forming ability of formulations

Almost all the formulations tested (Table 4.1) exhibited film-forming abilities. F14 and F15, containing HPMC, sodium alginate and 0.5 mL of PEG, were firmly attached to the Petri dish and disrupted during peeling. F19, F20 and F21, containing HPMC, sodium alginate and 1 mL of PEG were too dry and brittle to be peeled. This outcome was controversial, considering that PEG is a hygroscopic polymer (Baird *et al.*, 2010), that should have conferred flexibility to the films (Ghadermazi *et al.*, 2019). In addition, in F16, F17 and F18 blends, containing HPMC, sodium alginate and gelatin with 0.5 mL of PEG, formed peelable and handleable films. Moreover, F31, F32 and F33, containing the same components with 1 mL of PEG, (and double the amount of gelatin) formed acceptable films. F25 and F26 containing glycerol as plasticiser and no PEG were too soft and sticky, making it impossible to handle them properly. F27 which contained the highest amount of sodium alginate tested (500 mg), HPMC (500 mg), gelatin (100 mg) and glycerol (1 mL) produced an appropriate balance between film flexibility and film handleability.

The remaining formulations did form odourless films, either transparent or translucent, ranging from whitish to sandy colouration, and were characterised.

4.3.3 Physical characterisation

Weight. In Figure 4.2 are reported the averages of the weight for each formulation, all comprised within the range from 0.0367 to 0.1099 grams, belonging to F22 and F27 respectively, which were differentiated only by the concentration of glycerol. Even considering the low repeatability of handmade cutting procedure, it can be still noticed that the amount of plasticiser majorly impacted on the formulations weight. The formulations demonstrated good weight uniformity and low degree of variability, although the number of specimens did not meet the minimum required for tablets (within 10% range) by British Pharmacopoeia standard in testing mass uniformity for single-dose preparation (Pharmacopoeia, 2020b). It should be noted that till date, there is no official monograph for testing film inserts and characterisation relies on monographs for tablets and tests developed by individual researchers.

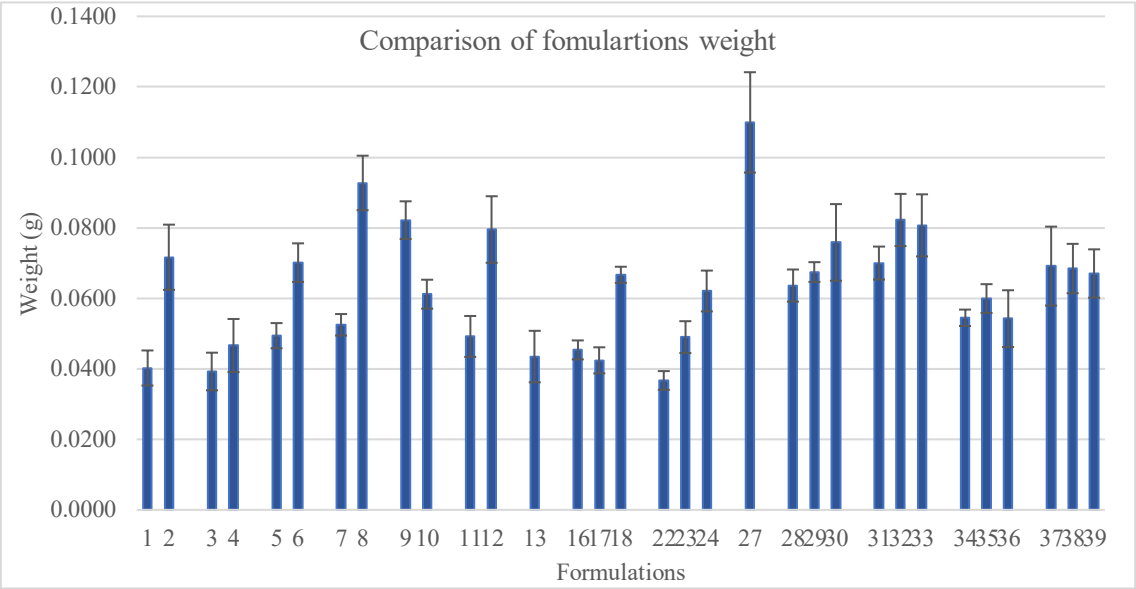

Figure 4.2 Average weight of film formulations (n=9), error bars indicate standard deviation.

Thickness. The averaged values of thickness for each formulation are reported in Figure 4.3. The thickness varied across a range of 0.0914 to 0.2764 millimetres, again belonging to F22 and F27, respectively. As with the weight values, the trend was confirmed that higher thickness values were associated with higher quantities of plasticiser used. The analysis of thickness can be also assumed to be an indicator of the wearability of the inserts, as well as its potential tolerability. Although the assessment of inserts in animal models can be considered an all-embracing evaluation of the formulations for any potential irritancy, useful information can still be derived from specifically considering insert thickness and therefore their tolerability. In previous researches conducted on animal models, HPMC-based formulations exhibiting thickness ranges similar to the highest values measured in this study were found non-irritating, well tolerated, and able to reside on the eye without being expelled (Reddy *et al.*, 2017; Thakur *et al.*, 2014). Furthermore, considering that formulations characterised by higher thickness values showed excellent tolerability (Jain *et al.*, 2010), it can be considered that all the formulations tested exhibited acceptable thickness values.

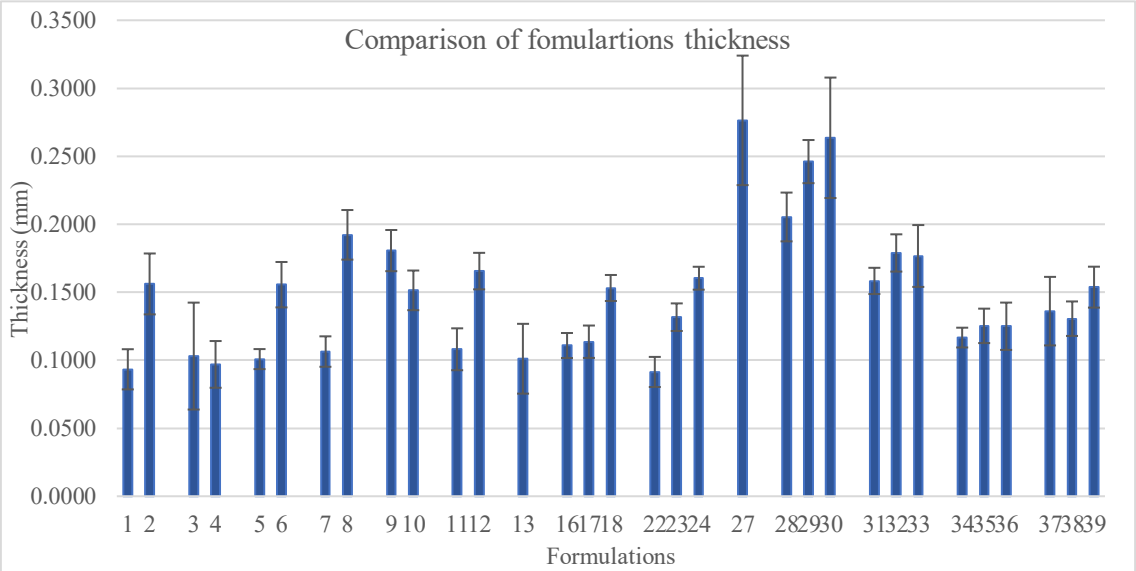

Figure 4.3 Average thickness for film formulations (n=9), error bars indicate standard deviation.

Nonetheless, further investigation may be needed to determine the effect of inserts thickness on ocular tolerability. It should be considered that the collection of thickness values was performed upon formulations drying, and not taking into account of any potential modification of this physical characteristic that could follow coming to contact with the tear film. Thus, it can be speculated that the analysis of thickness values could have a better clinical predictivity in considering predominantly insert tolerability at the time of insertion. On the other hand, it can also be assumed that the thickness values recorded for the formulations tested may be dependent on the manufacturing procedure, and that it can be possibly reshaped by altering the procedure and/or the characteristics of the drying surface.

4.3.4 Mechanical properties

Mechanical testing could provide data on material properties and the results could provide a guide to understanding the perspective behaviour of the film insert in physiological anatomies. The use of the tensometer for characterisation of specimens has been mainly employed to mimic the conditions present in mouth, nonetheless it could find a productive application for ocular applications as well. The device applies a unidirectional force to the sample under investigation to determine the tensile proprieties – useful in assessing flexibility and resilience (Thompson *et al.*, 1969). Similarly, this test provides a quantifiable insight to the physical compatibility among the components of mixtures. In addition, the stiffness of the films could be derived from the test, and is important during film storage and ensuring stability during administration.

In Table 4.2 are reported the tensile strength, the percentage of elongation at maximum load and the Young's modulus for each formulation.

Considering that some formulations could not be assessed because of poor mechanical characteristics (i.e., too brittle or too soft to be peeled) both plasticisers were found able to confer suitable properties to the formulations manufactured. Although a direct comparison between similar formulations was not always possible, the inclusion of PEG demonstrated more suitable mechanical performances than glycerol in the formulations including all the polymers, and this was found in line with a trend previously reported in other studies (Hong *et al.*, 2005) (Ghadermazi *et al.*, 2019). In considering the Young's modulus value as an overall index of mechanical performances of insert ductility, it can be seen that formulations including PEG instead of glycerol, having same quantities of the other polymers, demonstrated higher values of the modulus.

Likewise, it can be presumed that sodium alginate conferred better mechanical properties to the formulations, as suggested by Young's modulus recorded in F16, F17 and F18, if compared with F5, including HPMC with gelatin alone and the same quantity and type of plasticiser.

Different from the analysis of mechanical testing outcomes in use for other body districts, the testing performed was not intended to mimic the eye environment, but it was mainly employed to assess the characteristics of the inserts in terms of their administration, and to expand the understanding of components compatibility and stability of the formulations.

Figure 4.4 Tensometer for mechanical properties testing.

Table 4.2 Mechanical proprieties of the formulations tested (n=3).

	Tensile strength (Mpa)		Elongation at Max (%)		Young's modulus	
Formulation	Mean	Std	Mean	Std	Mean	Std
1	5.540	0.432	39.200	2.766	0.142	0.014
2	2.324	0.409	34.180	4.300	0.068	0.009
3	7.650	0.184	19.480	3.427	0.401	0.057
4	3.126	0.287	15.880	0.475	0.197	0.019
5	6.020	1.774	28.590	1.125	0.211	0.053
6	2.187	0.177	21.230	3.587	0.104	0.010
7	7.620	0.233	19.140	0.213	0.398	0.007
8	2.036	0.180	13.600	0.935	0.150	0.006
9	1.870	0.043	16.410	0.336	0.114	0.004
10	3.908	0.143	14.310	1.817	0.276	0.024
11	4.942	0.538	11.720	1.190	0.422	0.028
12	1.674	0.056	10.090	0.050	0.166	0.004
13	3.983	1.013	10.920	3.902	0.376	0.038
16	2.302	0.637	4.067	0.513	0.559	0.082
17	1.686	0.426	3.137	0.416	0.535	0.080
18	3.612	1.124	3.225	0.314	1.115	0.236
22	0.500	0.043	8.690	2.321	0.059	0.008
23	0.629	0.106	9.890	2.045	0.065	0.013
24	0.814	0.056	7.880	1.080	0.104	0.007
27	0.153	0.045	10.950	3.723	0.016	0.006
28	2.035	0.217	4.804	0.612	0.431	0.074
29	0.927	0.252	3.678	0.422	0.258	0.077
30	2.727	1.717	3.360	0.388	0.492	0.208
31	2.436	0.249	7.350	0.658	0.332	0.023
32	3.451	0.357	5.350	0.391	0.644	0.017
33	3.614	0.256	4.593	0.451	0.789	0.028
34	6.150	0.446	12.250	0.737	0.502	0.030
35	3.859	0.650	13.270	0.523	0.292	0.047
36	5.210	0.693	12.650	4.010	0.443	0.138
37	0.489	0.176	12.780	0.881	0.039	0.013
38	0.452	0.056	19.410	2.951	0.023	0.003
39	0.548	0.057	17.210	4.913	0.034	0.009

4.3.5 Moisture content & water uptake

In Table 4.3 are reported the water uptake and the moisture content values for the films in percentages. The moisture content of film formulations can be influenced by the nature and the quantity of the polymers used, and by the drying procedure (Silva *et al.*, 2012). Also, it has been linked to characteristics of the formulations, including mechanical properties, transparency, and stability of the films (Andrade-Mahecha *et al.*, 2012; Nyflött *et al.*, 2017; Prajapati *et al.*, 2011). Therefore, it has been considered useful to determine the moisture content of the formulations to ensure balanced and comparable values across the formulations. For the formulations tested in this study, the values of moisture content were between 10.09% and 16.73%.

The values of water uptake, instead, could give rise to some speculations regarding the clinical employability of films. Despite the lack of agreement about its precise value, the tear film volume can be grossly estimated at around 10 µL (Willcox *et al.*, 2017), and it has been suggested that the conjunctival sac can contain up to 30 µL of tear fluid (Gaudana *et al.*, 2010). Thus, in administering films with high propensity to uptake water, two main disadvantages can be produced: firstly, the tear film can be absorbed at a rate exceeding its secretion, leading to dryness of the ocular surface, and, secondly, the volume of the insert can expand to an unsustainable level for the eye, leading to increased interaction between the eye and the film that can cause dislodgment or even expulsion of the film. Although this specific aspect has not been specifically addressed, it might be still reasonable to consider those formulations presenting a very high water uptake value inadequate for ocular purposes.

The lowest water uptake value (177%) was recorded for F13 – the only film formed without gelatin in the blend. The highest rate was registered for F34, which included all the polymers tested and glycerol as plasticiser. The appearance of the film during the procedure (Figure 4.5) was suggesting an exorbitant volume expansion of the formulation.

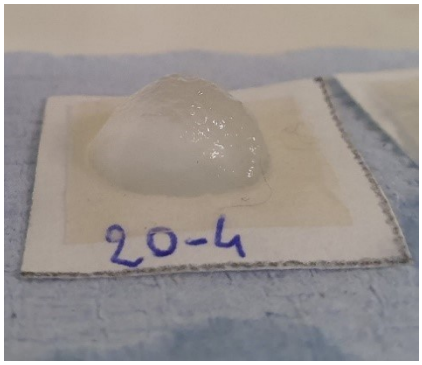

Figure 4.5 F34 appearance during swelling index testing.

*Table 4.3 Disintegration time, water uptake (%) and moisture content (%) of the films. [*Data for F28, F29 and F30 were obtained from a different batch, thus incomplete]*

Formulation	Disintegration	Water uptake	Moisture content
1	30 min	262	12.06
2	30 min	190	12.1
3	30 min	320	15.15
4	30 min	221	13.64
5	30 min	287	12.04
6	60 min	226	10.94
7	60 min	289	13.91
8	30 min	198	13.3
9	60 min	220	10.09
10	30 min	253	10.81
11	30 min	311	11.95
12	30 min	213	12.49
13	>14 h	177	12.27
16	14 h	437	13.52
17	14 h	348	14.57
18	14 h	398	12.57

Formulation	Disintegration	Water uptake	Moisture content
22	14 h	395	16.73
23	14 h	316	14.11
24	14 h	296	12.52
27	< 14 h	264	13.29
28	4-13 h*	-	-
29	4-13 h*	-	-
30	40-50 h*	-	-
31	6-14 h	239	13.48
32	6-14 h	578	12.15
33	6-14 h	347	13.65
34	6-14 h	994	14.37
35	6-14 h	478	14.39
36	14 h	326	14.87
37	6-14	441	15.35
38	6 h	414	14.28
39	14 h	447	16.15

4.3.6 Surface imaging

The appearance of the films produced varied greatly across the formulations. The presence of sodium alginate was associated to higher film surface irregularities and roughness, as can be recognised in F16 (Figure 4.7), which included sodium alginate, at the minimum concentration used in this study, in addition to the same components of F5 (Figure 4.8).

Although the role of the surface texture should be furtherly clarified, it could be speculated that if on one hand a higher surface roughness could provoke irritation and inflammation of the tissues, on the other, it might even favour the stability of the thin film after insertion, also considering that the presence of sodium alginate is supposed to increase formulation mucoadhesive strength (Kesavan *et al.*, 2010). Nonetheless, the irregular surface appearance of the films including sodium alginate can be to a suboptimal distribution of the compounds in the formulation, potentially linked to the manufacturing procedure.

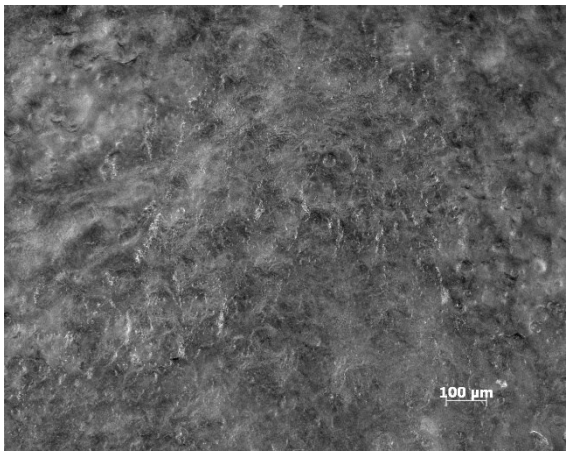

Figure 4.6 Optical microscopy imaging of F16 surface.

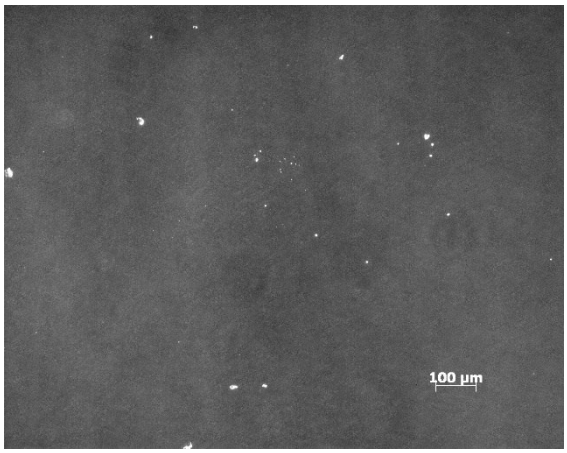

Figure 4.7 Optical microscopy imaging of F5 surface.

4.3.7 Disintegration time (test development and evaluation)

The significance of the disintegration time in potential clinical applications should not be overlooked. It was found that the evidence of ocular inserts effectiveness reported in literature was underpinned by the values of drug dissolution, but very little information was given with respect to the disintegration of the inserts. Although the way an insert can release the drug(s) can be considered primary, the information about the disintegration time may allow a more comprehensive evaluation of the films, and this, together with a lack of standard procedures, can justify the need for further development of the testing procedure (Gupta & Kumar, 2020).

In designing soluble inserts, the optimal clinical outcome could be reached if the complete disintegration/solubilisation of the film would occur at same time of the complete release of the pharmaceutical agent delivered. If this condition is met, there will be no need to remove the insert (outmost challenging if solubilisation will occur) or to wait for the excipients to be cleared form the eye if a new drug administration will be needed. In fact, the impact of excessive film residues on the ocular surface could affect vision by altering the optical quality of tear film (Koh *et al.*, 2018). Furthermore, an extended disintegration time, if associated with a sustained dissolution time, can reduce the number of drug administrations needed, and be beneficial for patients' adherence to treatment (Srivastava *et al.*, 2013).

In the British Pharmacopoeia (BP), the disintegration tests reported are designed for tablets and capsules, and defined as the time "in which any residue of the unit, except fragments of insoluble coating or capsule shell, […] is a soft mass having no palpably firm core" (Pharmacopoeia, 2020a). Given the relatively recent interest in thin films as ocular drug delivery systems (Karki *et al.*, 2016), some consideration could be drawn from orodispersible film formulation testing, as no specific testing has been approved. According to a recent review by Wasilewska & Winnicka (2019), the disintegration testing procedures for oral films can be grouped in two main categories: those employing the pharmacopoeia apparatus and those applying a force to the films. For the first group, the main limitation is the quantity of medium necessary to perform the test (900 mL), which is several orders of magnitude greater than tear film volume. For the second group, it must be noted that, despite the lower quantities of medium employed, the type of forces applied was still mimicking the buccal condition, thus not representative of the ocular environment.

For these reasons, a novel protocol for measuring the disintegration of the films was developed. Due to the transparent and colourless nature of the film inserts, it was difficult to visualise the occurrence or the end point of disintegration by the naked eyes. In a preliminary study, four specimens were compared: one transparent and colourless film sample, two samples with the same formulation and appearance characteristics of the former, to which they were added different percentages of the trypan blue dye, and one sample of an alternative formulation which was translucent and colourless. The samples were placed on hand-made stainless-steel meshwork supports, with a design specifically developed for the purpose (Figure 4.8). These setups were placed in 6-well cell culture plates. The support was designed to permit the films to be completely immersed in water, while having sufficient distance from the bottom well to appreciate film permeation through the meshwork.

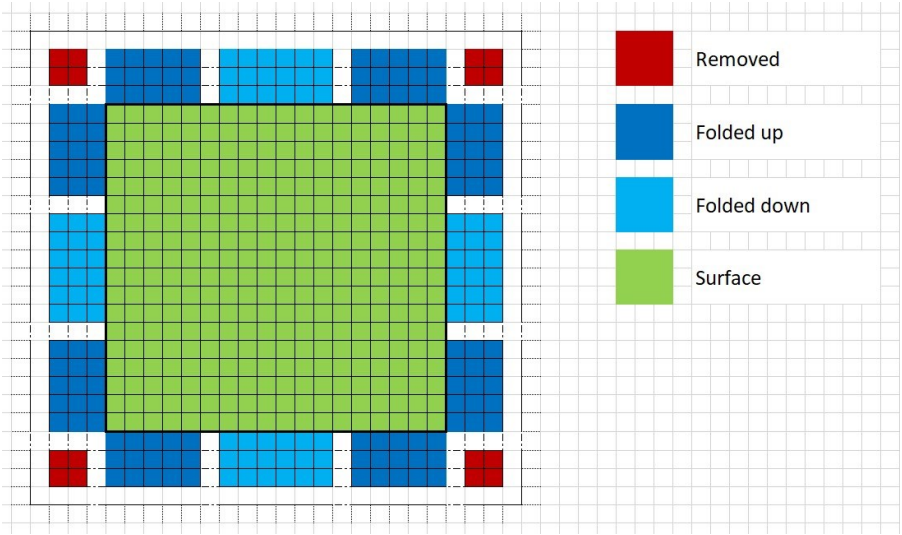

Figure 4.8 Design of the meshwork support for disintegration test.
(The sides of squares being approximately 1 mm)

Although difficult, the visual identification of the samples was still possible in dry conditions. Upon adding 10 mL of distilled water into the wells, the identification of the specimens was extremely challenging, even for the samples with dye embedded in the formulations – whether the appraisal was conducted by the naked eye or using phase contrast microscopy. For these reasons, a cross was drawn on each sample with permanent marker, given the water insolubility of the ink. The presence of the ink cross on the samples surface made it possible to roughly evaluate the specimen for its presence, position, integrity, and the passage of the film through the meshwork after transforming into a gel (Figure 4.9).

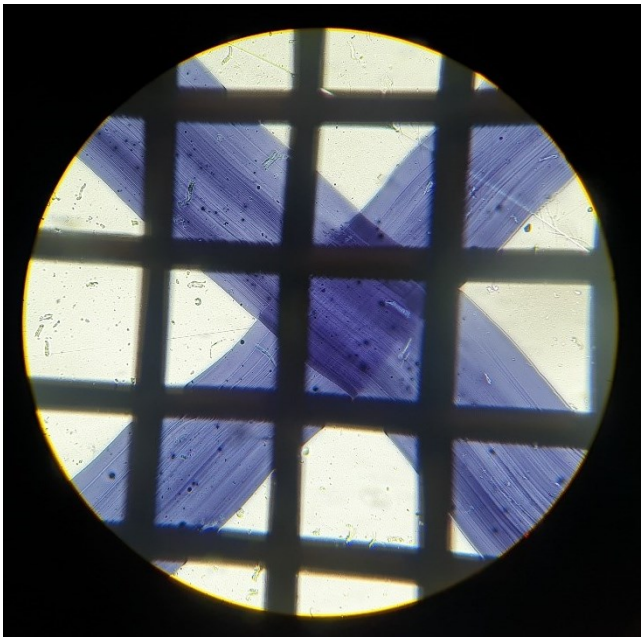

Figure 4.9 Inverted microscopy appearance in testing samples marked with the cross.

The disintegration times of the formulations estimated as previously described, are reported in Table 4.3. It was highlighted that the disintegration time showed a bimodal trend, where some inserts (not containing sodium alginate) disintegrated rapidly within-one-hour (F1 to F12), while the remaining (containing sodium alginate) disintegrated within-several-hours (F13 to F39). Thus, it can be inferred that the presence of sodium alginate in the blend was crucial in determining a longer disintegration time, suggesting that the polymers can confer this characteristic.

4.4 Conclusions

The results obtained in the study suggested that the combination of HPMC, gelatin and sodium alginate, together with PEG, can lead to the production of soluble films suitable for ocular use, in terms of physical characteristics and mechanical proprieties. In particular, F31, which comprised 500 mg of HPMC, 200 mg of gelatin, 300 mg of sodium alginate, and 10 mL of PEG, demonstrated the most compelling equilibrium among physical size, mechanical characteristics, hydrophilic nature, and disintegration duration out of all the samples tested.

From this exploratory study, it can be assumed that the identification of the water-soluble polymers constituting the formulations produced could serve as the basis to manufacture drug-loaded inserts for ocular application. Further experiments should be conducted to upgrade the film manufacturing efficacy and robustness. In addition, a more comprehensive variety of testing methodologies should be employed to further characterise the inserts manufactured.

Although the disintegration test proposed would require further ameliorations toward objective results (e.g., by digital imaging analysis) and test repeatability, it could be effectively used in differentiating between faster (within-one-hour) and more extended (several hours) thin film disintegration times. The extended disintegration times for tested formulations indicate their potential application for sustained ocular drug release.

Chapter 5 - Development and evaluation of soluble ocular antibiotic-loaded film insert

5.1 Introduction

The ocular disorders that affect cornea are the second primary cause of blindness globally, following only cataract for importance. Although the causes of corneal disorders are widely different, the development of infections that will result in presence of corneal scarring are among the sources of risk (Whitcher *et al.*, 2001). The use of antimicrobial agents remains the therapy of choice in the treatment of bacterial keratitis, and they are effective also in bacterial ulcers and bacterial conjunctivitis (Austin *et al.*, 2017; Chen *et al.*, 2023).

Levofloxacin is an antibiotic drug that belongs to the third generation of fluoroquinolones. It has broad-spectrum antibacterial activity and is commonly used for external ocular infections in the form of 0.5% eye drops (Keating, 2009). In fact, levofloxacin has been found effective against different species of bacteria, including most common causative pathogens of bacterial conjunctivitis and microbial keratitis (Hwang *et al.*, 2003; Li *et al.*, 2020).

To meet antimicrobial efficacy, levofloxacin eye drops require very frequent instillation, in particular at the beginning of pharmacological treatment (Szaflik *et al.*, 2009). In fact, to remain effective, the drug concentration available on the ocular surface should be maintained above the minimum inhibitory concentration (MIC90) of the causative agent(s) being treated (Herbert *et al.*, 2022). For this reason, the dosing frequency of levofloxacin has usually been set to 4 instillations per day (Kanda *et al.*, 2012).

The need of elevated dosing interval frequency in administration of antibiotic eye drops is necessary to compensate for the low bioavailability of active ingredient in the ocular environment, limited by the presence of ocular barriers (Agrahari *et al.*, 2016). The limited volume of eye drops the eye can accommodate and the constant tear turnover determine that less than 5% of active ingredient in each drop administered can reach the target site (Gaudana *et al.*, 2010). Also, the frequent instillations of antibiotic eye drops that are required can expose to the risk of patient non-adherence with consequent inefficacy of treatment regimen (Rossi *et al.*, 2011).

Drug delivery devices can sustain the presence of the drug on the ocular surface and increase the residence time of the antimicrobial agents. Therefore, as no drug delivery device for ocular delivery of levofloxacin is available, it can be useful to formulate a thin film insert for ocular delivery of this drug.

Hence, the aim of this study was to produce soluble levofloxacin-loaded film inserts using hydrophilic polymers as excipients, in the attempt to sustain the drug release and consequently overcome the limitations linked to the use of levofloxacin eye drops currently available.

5.2 Materials and methods

5.2.1 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 viscosity), polyethylene glycol 400 (BioUltra), and glycerol (BioXtra ≥ 99%), as well as tryptic soy broth, sodium chloride, sodium bicarbonate, potassium chloride and calcium chloride were purchased 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.

5.2.2 Methods

5.2.2.1 Film manufacturing process

All the films were prepared by solvent-casting method. The film-forming solution were made after a 0.1% Levofloxacin solution, freshly prepared by dissolving the drug in ultrapure water and sonicating for 10 minutes. Target amount of plasticiser and drug solution were added in a glass beaker and heated to 65˚C under slow stirring. After accurate weighing, desired amounts of HPMC, sodium alginate and gelatin powders (Table 1) were blended to obtain a uniform mixture and gradually added to the solution under vigorous stirring. The solution was stirred under medium velocity at 65˚C for 60 minutes and left to cool at room (approximately 20˚C) temperature under low speed stirring. The resulting solutions were transferred in tubes, centrifuged for 5 minutes at 1000 rpm and emerging bubbles were removed with disposable pipettes. Film forming solutions were casted on glass plates and spread by motorised film applicator. Each formulation was used to manufacture a single film. Films were left at room temperature to dry and cut into ten millimetres diameter disc-shaped inserts using stainless-steel cork-borer. Twenty-five millimetres diameter inserts were also collected and used to best suit specific testing methodologies (e.g., tensile properties, water uptake).

5.2.2.2 Pre-formulation study and film quality

The formulations tested were assessed for their ability to translate into film forming solution and their ability to produce qualitative film. During manufacturing process, the film forming solutions were qualitative inspected, monitoring solution homogeneity, viscosity and spreadability. Of the suitable formulations, produced films were then inspected for uniformity of visual appearance and transparency/translucency, peelability and manoeuvrability, and surface texture on touch. The formulations exhibiting the ability to produce qualitative films were further characterised.

Table 5.1 Formulation tested in film manufacturing. Concentrations of excipients were reported as percentage of film forming solution (weight/volume or volume/volume).

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

5.2.2.3 Uniformity of mass and thickness

Twenty-four disc-shaped inserts of 10 mm diameter were cut from the films. The samples were weighed using analytical balance (Sartorius, Göttingen, Germany) and the thickness was measured with digital micrometre gauge (Beslands, Jiaxing, China). The values were used to calculate mean, standard deviation and relative standard deviation (RSD) and compared against the International pharmacopoeia guidelines for uniformity of mass for single-dose preparations (British Pharmacopoeia Commission, 2023b). The values of weight and thickness were also used to determine inter- and intra-day repeatability in film manufacturing.

5.2.2.4 Mechanical properties

The capability of films to be handled was estimated by assessing the mechanical properties of the inserts. The tensile strength, elongation at maximum force, and derived Young's modulus, were determined using Hounsfield Tensometer (Hounsfield Limited, Croydon, UK). The testing was performed on disc-shaped specimen (Ø = 25 mm), with grips placed at equal distance from insert edges, along a theoretical diameter line of the insert. The test was set with stress range 0.05 MPa, strain range 300%, speed 60 mm/min and without preload (Gilhotra *et al.*, 2009). Folding endurance of the film was tested by folding disc-shaped samples of the same dimension in the centre with fingers allowing opposite portions of the film to touch. The procedure was repeated until visible cracking or rupture of the insert was observed or completed 300 times (Aher & Nair, 2014). Both testing procedures were performed in triplicate. (Jain *et al.*, 2010). As no ocular film formulations were found approved/marketed, the buccal rectangular (2.0 x 1.4 cm) film Setofilm® (LTS, Germany) was tested to determine mechanical properties reference values of a commercially available pharmaceutical formulation.

5.2.2.5 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 hours (Ahn *et al.*, 2014). Upon cooling, the samples were individually weighed afresh, and the moisture content was determined by calculating the ratio between the weight difference and the initial weight of the samples, reported as a percentage, according with the formula:

$$Loss\ on\ drying = \frac{original\ weight - dried\ weight}{original\ weight}$$

5.2.2.6 Water uptake and Swelling Index

The characteristic of inserts to absorb water and to modify physical dimension upon swelling were studied with a modified version of the method proposed by Bertram & Bodmeier (Bertram & Bodmeier, 2006) for characterisation of ocular film inserts. The sponge method was set up placing two pre-soaked sponges (8 x 5.5 x 2.2 cm) next to each other in a plastic box (12 x 8 x 3 cm) covered with a lid, filled with 25 mL of ultrapure water to ensure constant moistening of the sponges. The apparatus was constantly kept at 37˚C during the procedure. Paper supports were cut from Whatman filter paper (grade 1) and placed on the sponges until equilibrium in weight was reached (Figure 5.1). Dry weight of inserts was recorded, and the samples placed on the soaked supports. After every hour, each insert-support combination was weighed after blotting excessive water on the inferior surface of the support. The water uptake was then calculated as the maximum ratio between the dry and soaked inserts weight (determined after deduction of wet support weight) across time points and expressed as a percentage, according with the formula:

$$Water\ uptake = \frac{wet\ weight - dry\ weight}{dry\ diameter}$$

The same experimental setup was used to determine the swelling index. As previously described by Labib *et al.* (2013), the diameter of the samples was recorded hourly and compared against the original dry measure. The swelling index was derived from the proportion between the diameter variation upon swelling and the initial diameter of the insert (Ø = 25 mm), and expressed as a percentage, according with the formula:

$$Swelling\ Index = \frac{swollen\ diameter - dry\ diameter}{dry\ diameter}$$

Figure 5.1 Experimental setup used in determination of water uptake and swelling index of the film manufactured.

5.2.2.7 Surface pH

The surface pH of the films was determined by randomly sampling three disc-shaped inserts (Ø = 10 mm). Thirty microliters of ultrapure water were carefully pipetted onto samples surface and the samples were left to soak for 5 to 10 minutes (Abdelkader *et al.*, 2014). The surface pH of the films was estimated by placing pH indicator paper strips (Fisherbrand™, Loughborough, UK, pH range 1-14) on the moistened films and compared against the reference colour scale, after equilibrium of colouration was achieved (Shah *et al.*, 2018).

5.2.2.8 Drug Content Uniformity

The constancy of drug quantity present in the inserts was assessed. Ten samples from each film were collected and dissolved in 5 mL of ultrapure water for 12 hours at 37˚C. Approximately 3 mL of the suspension was collected, filtered, and tested as per the high-performance liquid chromatography procedure described below. The concentration values were adjusted to account the volume of solubilisation media used. The quantity of drug recovered across the samples and its variability were compared against the uniformity of content of the International pharmacopoeia guidelines for single dose preparation (WHO, 2019a).

5.2.2.9 Dissolution testing

The release of drug from the thin film inserts was tested to determine the amount of active ingredient over time. No definitive test or apparatus for dissolution study for ocular film inserts has been established. Hence, the two chambered donor-receiver model was selected, because it was found demonstrating a strong positive correlation with *in vivo* testing, as discussed in section 3.6.4 (Gorle & Gattani, 2009, 2010; Kulhari *et al.*, 2011; Mishra & Gilhotra, 2008; Mundada & Shrikhande, 2006; Thakur *et al.*, 2014). The donor-receiver apparatus was designed 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 other was capped with a plastic stopper to reduce evaporation. The donor compartment was filled with 1 mL of pre-heated at 37˚C simulated tear fluid (STF, composition: NaCl 6.8 g, NaHCO3 2.2 g, CaCl2 0.08 g, KCl 1.4 g and ultrapure water to final volume of 1000 mL (Abdou & Kandil, 2017)) and placed in the receiver compartment by a support, ensuring that the membrane was entirely in contact with the media, which consisted of 25 mL of STF at 37˚C (Figure 5.2). The simulated tear fluid used was employed to replicate the pH, osmolarity and buffering capacity of the tears, as those characteristics could produce an effect in the performances of the formulations manufactured. In addition, the media in the receiver compartment was continuously stirred at slow speed (approximately 30 rpm) to simulate blinking. Stirring action and heating were achieved using multi-position hot plate stirrer. Three inserts from each film were carefully placed in the donor compartment and separately tested for amount of drug released by collecting 1 mL of suspension from the receiver after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours, replaced with equal volume of warm STF. The dissolution media was replaced to maintain sink conditions, thus preventing drug concentration in the medium to reach saturation level and the consequent potential decrease in the release rate occurring at that stage. The media replacement has been then factored into the computation of cumulative drug released, and considered for both the amount and the percentage. The aliquots collected were filtered (0.45 µm syringe filters) before high-performance liquid chromatography analysis. The values of cumulative drug release were calculated considering the effect sampling procedure on instantaneous concentration recorded. The values found in drug content uniformity for each film were used to determine the percentage of drug released from the inserts tested.

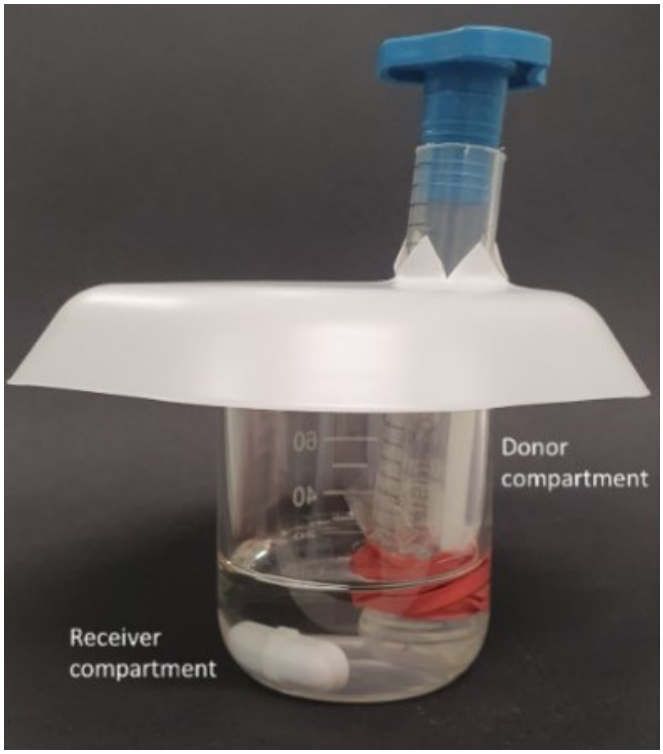

Figure 5.2 Donor-receiver model used. The donor (tube) and the receiver (beaker) compartments were separated by dialysis membrane. Both compartments were filled with simulated tear fluid.

5.2.2.10 Effect of residual levofloxacin content of inserts on bacterial growth

The donor-receiver model was employed to test the efficacy of residual levofloxacin content in film inserts on bacterial growth curve. Staphylococcus aureus and Pseudomonas aeruginosa were used as bacterial models for testing. For both bacteria, suspensions were prepared by mixing aliquots of bacteria collected from pure cultures in tryptic soy broth and left at 37˚C overnight. The bacterial cultures were diluted to a final optical density OD600 = 0.1 in tryptic soy broth. At each time point of dissolution testing (0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours), 20 µL were collected twice from the donor compartments containing the inserts and replaced with 40 µL of warm simulated tear fluid. The samples collected at each time point from the different inserts were placed in two separate 96-wells plates. In each well, the sample collected was mixed with 180 µL of OD600 = 0.1 bacterial suspension. One plate was used for Staphylococcus aureus, while the other for Pseudomonas aeruginosa. 200 µL of both OD600 = 0.1 bacterial suspension were used as positive control. The plates were tested with monochromator-based UV/VIS spectrophotometer (Multiskan® GO, Thermo Scientific™, Waltham, USA) and the OD600 of all samples was recorded every 30 minutes for 20 hours, while plates were kept at 37˚C under continuous medium shaking. The OD600 readings were then used to plot the growth curve of Staphylococcus aureus and Pseudomonas aeruginosa while exposed to the residual drug content of the inserts collected during dissolution and compared against bacterial cultures (Smith *et al.*, 2003).

5.2.2.11 High performance Liquid Chromatography Analytical Method

The high performance liquid chromatography analytical method (HPLC) for levofloxacin detection was developed, validated according with ICH validation of analytical procedures (Borman & Elder, 2017), and used for content uniformity and dissolution of levofloxacin. 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 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 reference levofloxacin solutions were prepared by sonicating for 10 minutes the target amount of levofloxacin (HPLC grade) in approximately 80% of ultrapure water, which was filled up to final volume after cooling. The method was developed and validated in accordance with International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures (Borman & Elder, 2017).

5.2.2.12 Statistical analysis

The 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 (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.

5.3 Results and Discussion

5.3.1 Pre-formulation study and film quality

The identification of suitable formulations aimed to identify those presenting the desired characteristics summarised in Table 5.2. Screening of formulations demonstrated that four of the formulations F1, F4, F5 and F6 (Table 5.1) tested produced consistent films, easy to peel and handle. For formulations including higher percentage of gelatin (F2 and F3) and sodium alginate (F6 and F7), similarly to those made with HPMC of higher viscosity (F9 and F10), it was possible to obtain homogeneous film forming solution when heated, which became a thick gel at room temperature, making impossible to pour and spread them. Excipient polymers were processed at higher temperature to favour their solubilisation and to produce homogeneous solutions. However, although gelatin and sodium alginate viscosity/gelation at body temperature was desired (Lee & Mooney, 2012; Osorio *et al.*, 2007), the gelation of formulations including higher concentrations of those excipients at room temperature impeded the production of films. Similar behaviour was noticed also for HPMC of higher viscosity. The film prepared with HPMC E6 (F8) was too brittle, hence difficult to peel and handle. The ruptures provoked during peeling and the brittleness of the formulation prevented any processing, thus it was not analysed further. The remaining formulations (F1, F4, F5 and F6) resulted in qualitative films holding various characteristics, which are reported in Table 5.2.

Table 5.2 Attributes of the formulation assessed throughout the manufacturing process.

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

Although all four formulations produced films easy to peel and handle, their appearance varied, depending on the amount of sodium alginate and gelatin used. In line with that found about the film forming solutions, formulations including higher percentage of sodium alginate and gelatin resulted in increased opacity of the film (Figure 5.3). In formulation F1, including 500 mg of gelatin, opaque spots were randomly present in the film, while in formulation F6, including 1000 mg of sodium alginate, marked opacity was entirely characterising the film manufactured. The use of higher percentage of gelatin in film production has been associated to reduced transparency of the films (Nur Hanani *et al.*, 2013), which can be modified by the amount of plasticiser (Elango *et al.*, 2014). In addition, the reduction of transparency of films made with sodium alginate combined with other polymers was associated to reduced molecular compatibility between the materials in rearranging into a matrix, giving rise to heterogeneous film structure (Yoo & Krochta, 2011). Thus, given the fixed amount of plasticiser used, the appearance of films produced with formulation F1 and F6 could indicate the inhomogeneous arrangement of the polymers chains at concentrations used. Instead, formulation F4 and F5 (with 500 mg and 750 mg of sodium alginate, respectively) produced translucent, even appearance of the films, and considered more interesting for ocular application, because they might be perceived less invasive than opaque formulations. Aside, the translucency (i.e., not transparent) of the film could be even facilitating the administration of insert in the eyes, because they can be seen more easily throughout the procedure, in respect of transparent devices. In addition, as the target administration site was identified in the lower fornix, the lack of transparency of the inserts could be intended to not directly obstruct vision. The effects of insert hydration and erosion on transmittance of visible light of a solubilisation media (e.g., STF) should be further investigated to provide additional information on potential optical interference.

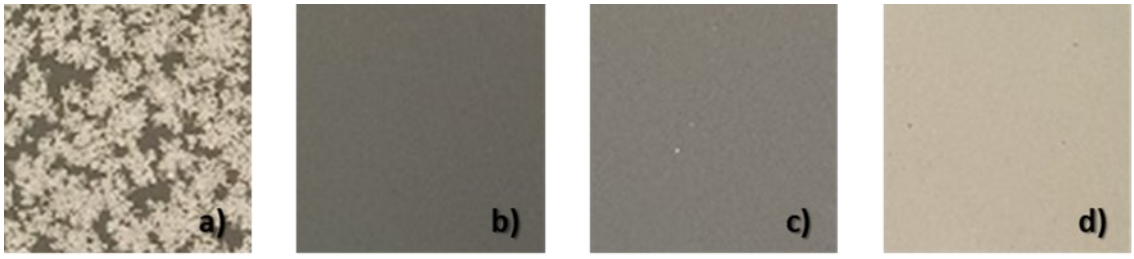

Figure 5.3 Appearance of film produced. The images represent a 2 x 2 cm section of photographs acquired with digital camera against dark background. Formulations 1, 4, 5 and 6 are respectively reported in pictures a, b, c, and d.

All the films produced demonstrated good physical uniformity, exhibiting reduced variability in both weight and thickness distribution, and they were complying with the International Pharmacopoeia guidelines for uniformity of mass (WHO, 2019b). It should be noted that specific guidelines for ocular insert formulations are not yet available, thus the specification for tablet single-dose preparations (average weight below 80 milligrams) was used, which states that the deviation of individual masses from the average mass should not exceed the limits of ±10% in 18 out of the 20 specimens tested and no more than 2 specimens can exceed the ±20% limit. The same guidelines were used also to assess the uniformity of thickness of inserts.

Table 5.3 Characterisation of successful film. 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 2) Uniformity of content (2).

	Gross 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]

The quantity of levofloxacin recovered from inserts showed characteristics of the films similar to those for weight and thickness, as all the formulations met the requirement for uniformity of content delineated by the International Pharmacopoeia (WHO, 2019a). For the uniformity of content, each single unit should contain an amount of the active ingredient within ±15% of the average content of ten randomly collected samples. Thus, it can be assumed that the manufacturing process utilised was able to confer satisfactory uniformity to the films produced. Formulation F4 demonstrated higher variability in weight, thickness, and levofloxacin content, suggesting that the formulation can be more susceptible to constancy variation. Hence, among the formulations tested, formulation F5 including 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.

5.3.2 Levofloxacin-loaded film characterisation

In light of the results obtained, the composition of formulation F5 was used to prepare three separate batches of film forming solution, each used to manufacture an independent film (named F5a, F5b, and F5c), using the manufacturing process previously described and characterised.

All three films, produced with independent batches, were found of good quality, translucent and easy to peel and to process. In Table 5.4 are summarised the average values for physical and mechanical properties of films.

The distribution of weight and thickness values demonstrated that the average values found in film F5c were significantly higher than film F5a and F5b (one-way ANOVA, $p < 0.05$), while between F5a and F5b, the differences were not significant ($p>0.05$). However, in considering the samples collected from the films altogether (3x24=72 samples), the values of weight and thickness fell within the ±10% range dictated by International Pharmacopoeia guidelines, suggesting that adequate repeatability could be achieved. Similar results were obtained in including values collected during preliminary test from the same formulation, on a different date. The statistical analysis revealed a significant difference ($p < 0.05$) in terms of weight and thickness average, but the values were found within the allowance range. Thus, it can be presumed that the combination of formulation and manufacturing process exhibited inter and intraday repeatability.

The range of thickness values found for the formulation tested, coupled with the diameter selected for the inserts (10 mm), led to estimate that the volume of the inserts can be approximately equivalent to 10 microliters of tears. In considering that the conjunctival sac can accommodate up to 30 microliters (Gaudana *et al.*, 2010) and that average depth of lower fornix was found higher than 10 mm (Jutley *et al.*, 2016; Khan *et al.*, 2014), it can be assumed that insert dimension can allow cul-de-sac administration.

The determination of mechanical properties of films can provide information on the ability of the insert to be manipulated during insertion in the eye. As reported in Table , the tensile strength of the films manufactured varied between 2.6 and 3.0 MPa, while elongation of the specimens at those forces did not exceed 16% of original length. Thus, the elastic modulus was found approximately 0.19 MPa and lower than values associated to other formulations proposed, in particular because of reduced elongation (Gilhotra *et al.*, 2009; Jain *et al.*, 2010). Nonetheless, the values of elongation found in formulation tested was still higher than those found in the commercially available buccal film Setofilm® (6.0 ± 0.6), which held instead higher tensile strength (9.0 ± 0.5), which suggests that the inserts produced can tolerate higher dimensional deformations (e.g., pulling) without losing their plasticity. Aside, the films were found able to undergo more than 300 repeated folding without fracture, assumed to be indicative of satisfactory film properties (Sabale *et al.*, 2019). It can be assumed that this test could better represent the perspective solicitations the inserts would endure during manipulation. In this case, Setofilm® (approximate thickness 90 µm) specimens could resist to a reduced number of folding (247 ± 29) than the ocular films manufactured, suggesting that the inserts can withstand mechanical strain during insertion procedure when administered to the eyes.

*Table 5.4 Physical and mechanical properties of film tested. Films F5a, F5b and F5c were independently produced with same formulation. Weight and thickness values were averaged from 24 samples (Ø=10mm), while mechanical tests were performed on 3 samples (Ø=25mm). Total is the average from 72 or 9 samples. *St. Dev. could not be calculated for foldability.*

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

The films produced were also assessed for those characteristics that may impact the eye environment (Table 5.5). The method for determining water uptake and swelling index of the insert was selected to guarantee constant moisture availability, as presumably provided by tear production, at eye temperature. In those condition, the films were shown to absorb up to three times their weight in water, ranging from 258% to 292% increments. As such, some concerns can be raised about the potential behaviour of the insert upon ocular administration. The rate of water absorption can be linked to potential interference with tear film, which can lead to discomfort and visual disturbances. Nonetheless, the impact of absorption should consider also the rates at which tear film is produced and the inserts subtract water from the ocular surface. According to the initial and the fully hydrated weight values, approximately 30 µL of (simulated) tear fluid were absorbed by the insert, in a few hours. Tear film turnover, instead, has been indicated to be 1-3 µL per minute (Dartt & Willcox, 2013), and it would take up to 30 minutes to replace the full volume absorbed. Hence, the rate at which tear film is produced should compensate for the amount loss in the insert. In addition, the tear production has been suggested to increase during infections (Asbell & Torres, 1991; Ormerod *et al.*, 1986). Moreover, the swelling index oscillated between 6.7±2.3% and 9.3±2.3% of the linear expansion of the inserts' diameter. As previously mentioned, the dimension of the lower fornix depth could accommodate such a variation. In addition, in combining both values, it appeared that the larger fraction of volume expansion was dependant on thickness increment, which could be contained by the lower lid. In terms of potential irritancy of the formulation, pH values were found in the range between 6 and 7. Similar pH range values were found non-irritant and well tolerated in animal model testing (Thakur *et al.*, 2014).

Table 5.5 Characterisation of films produced after formulation F5.

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

5.3.3 HPLC method validation

The HPLC analytical method validation aimed to provide reliable levofloxacin detection and quantification in a range that could comprise concentrations of drug that were expected to be recovered for the inserts and during dissolution testing. In Table 5.6 are reported the parameters of the calibration curve linking the chromatographic values provided by the photodetector and the concentration of levofloxacin in the samples, while Figure 5.4 shows the appearance of the chromatogram peak for a 0.0075% levofloxacin concentration in deionised water.

Table 5.6 HPLC calibration curve parameters

Linearity range (µg/mL)	1.95 - 62.5
Slope	53.001
Intercept	16.935
Correlation coefficient	0.9999
RSD (%)	0.42
Limit of Detection (µg/mL)	0.068
Limit of Quantification (µg/mL)	0.228

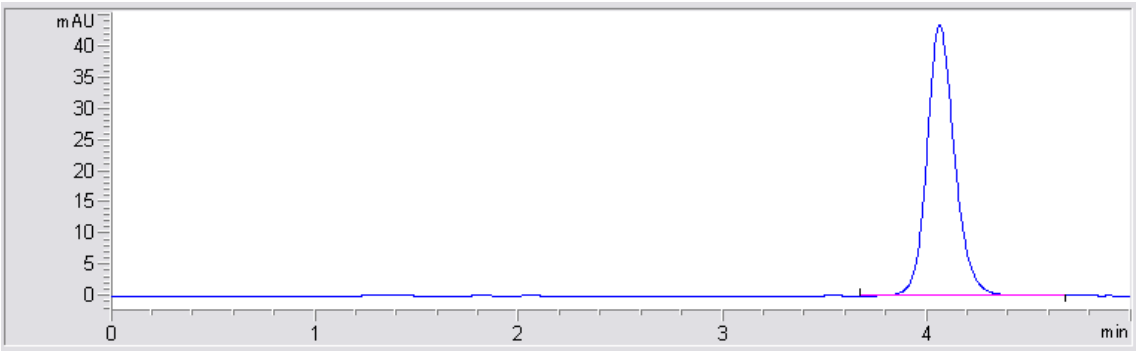

Figure 5.4 Chromatogram produced by running 0.0075% levofloxacin in deionised water solution.

The method was tested for intraday accuracy, precision, and intraday repeatability. In Table 5.7 are reported the parameters found for the method in performing the analysis on separate days.

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.

Table 5.7 Intra- and inter-day accuracy and precision of HPLC method. Values represent average and standard deviation from triplicate testing.

Drug concentration target (µg/mL)	***Drug concentration recovered (µg/mL)***	***RSD (%)***	***Drug recovery (%)***	***Drug concentration recovered (µg/mL)***	***RSD (%)***	***Drug recovery (%)***
	Day n			Day n+1		
1.95	1.98 ± 0.02	0.76	101.59 ± 0.77	1.96 ± 0.03	1.61	100.19 ±1.61
3.91	3.90 ± 0.03	0.79	99.97 ± 0.79	3.82 ± 0.07	1.77	97.75 ±1.73
7.81	7.79 ± 0.03	0.41	99.70 ± 0.41	7.61 ± 0.12	1.57	97.44 ±1.53
15.63	15.69 ± 0.02	0.12	99.87 ± 0.12	15.39 ± 0.22	1.46	98.49 ±1.44
31.25	31.26 ± 0.02	0.08	100.04 ± 0.08	30.85 ± 0.26	0.83	98.72 ± 0.82
62.50	62.50 ± 0.08	0.12	100.00 ± 0.12	61.68 ± 0.34	0.56	98.69 ± 0.55
	Average	0.38	100.20 ± 0.70	Average	1.30	98.55 ± 0.96
	INTRA-DAY			INTER-DAY		

5.3.4 Levofloxacin content of inserts

To assess the uniformity of levofloxacin distribution in the films manufactured, 10 inserts were collected at random locations from each film and dissolved, following International Pharmacopoeia guidelines for this testing. As found per uniformity of mass evaluation, the values of levofloxacin present in the samples were complying with International Pharmacopoeia guidelines, within the ±15% range from average value. In assessing films individually, levofloxacin content of the inserts did not exceed the ±3% average value of the film tested, while, in assessing all the samples collected from films F5a, F5b and F5c (n=30) altogether, the values range was found ±14%. The average amount of drug present in film F5c was 42.1 ± 0.4 micrograms, which was significantly higher than the amount recovered in film F5a and F5b ($p < 0.05$), respectively 37.5 ± 0.2 and 37.7 ± 0.3 micrograms, which were not significantly different from each other ($p > 0.05$). The higher average quantity of levofloxacin recovered in samples collected from film F5c followed the findings of weight and thickness of the same specimens. In fact, the correlation between drug content and weight was found very strong (Pearson's $r = 0.926$, $p < 0.001$), suggesting that the drug was uniformly distributed in film forming solution and that the spreading of the latter was crucial not only for the constancy of inserts physical characteristics, but also for amount of drug present across the samples. In addition, although the quantity of levofloxacin recovered during pre-formulation evaluation (F5) on the corresponding film (38.7 ± 0.7 µg) was significantly different from films F5a and F5c, all the values fell within the ±15% range indicated by uniformity of content guidelines, meaning a reproducible insert manufacturing process also in terms of levofloxacin content.

5.3.5 Dissolution testing

The release pattern of levofloxacin from ocular inserts was also evaluated and related to the average drug amount found in content uniformity testing. Three inserts from each film were placed in 1 mL of simulated tear fluid at the temperature of 37˚C, kept constant throughout the procedure. The temperature value was set to better represent that ocular conditions the inserts will be presumably used. In fact, research has shown that the values of eye temperature recorded in non-central assessments and in case of inflammation (Carracedo *et al.*, 2016) were found higher than central corneal temperature, which was estimated around 34˚C (Konieczka *et al.*, 2018). At each time point, the collection of the dispersion from receiver compartment was replaced by fresh simulated tear fluid, and that was taken into account in calculating the cumulative release of levofloxacin form the inserts.

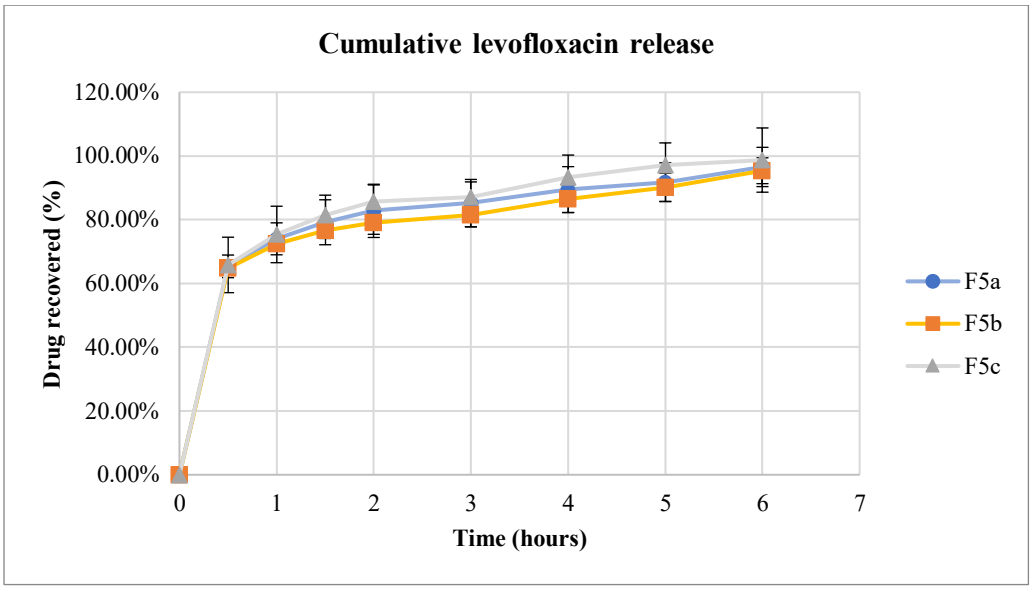

Figure 5.5 Cumulative release of levofloxacin from inserts collected from films produced. Values represent mean percentage of samples (n=3) against the average amount of the film. The error bars represent standard deviation of the mean.

In Figure 5.5 is reported the percentage of levofloxacin recovered after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours. The release pattern of levofloxacin showed that more than half of the drug amount (65%) present in the inserts was released within the first thirty minutes, while the remaining quantity was gradually released up to 6 hours, consistently across the film tested. The biphasic release pattern can be interpreted in light of the high hydrophilicity of excipients used. Insert polymeric matrix can initially be characterised by drug release linked to diffusion and surface erosion of insert upon water absorption and swelling (Shah *et al.*, 2018), while the gradual release can be modulated by the gelling properties associated with presence of gelatin and sodium alginate (Kesavan *et al.*, 2010; Liu *et al.*, 2010). Those findings can suggest prompt efficacy of the insert in releasing the levofloxacin in the ocular environment, followed by gradual diffusion of smaller fraction of the drug over next hours. In addition, the employment of donor-receiver model for dissolution testing can provide useful indication on the insert potential behaviour *in vivo*. The use of the model has showed repeated strong and positive correlation with the testing in animal models, suggesting that it may provide releasing pattern data that also include drug drainage caused by tear film turnover. Furthermore, in considering that levofloxacin can be effective in inhibiting the majority of bacterial growth (MIC90) from concentrations of 8 µg/mL for both Staphylococcus aureus (Metzler *et al.*, 2004) and Pseudomonas aeruginosa (Dave *et al.*, 2020), the immediate effectiveness of inserts can be assumed because the drug amount released within the first 30 minutes was found higher to be than 24 µg/mL. However the potential use over time necessitated further assessment, as the concentrations of drug recovered between the following time collections was estimated to be 4 µg/mL or less (Table 5.8).

Table 5.8 Drug release study results, including levofloxacin concentration from receiver compartment at time points and cumulative release. Release was reported as drug amount (µg) and percentage of drug released compared against the average amount found for each film. Values represent average from triplicate testing.

	Conc. (µg /ml)		Volume Adj.	Cum. Release (µg)		Drug content (µg)
F5a						37.471 ± 0.220
Time (h)	Avg.	St. Dev.		Avg.	St. Dev.	
0	0.000	0.000	0.000	0.000	0.000	
0.5	0.980	0.277	1.000	24.497	1.334	
1	1.065	0.295	0.960	27.729	1.876	
1.5	1.094	0.299	0.922	29.678	2.642	
2	1.098	0.289	0.885	31.021	3.129	
3	1.085	0.262	0.849	31.925	2.788	
4	1.093	0.235	0.815	33.515	2.700	
5	1.077	0.192	0.783	34.399	2.258	
6	1.087	0.171	0.751	36.178	2.308	
F5b						37.739 ± 0.251
Time (h)	Avg.	St. Dev.		Avg.	St. Dev.	
0	0.000	0.000	0.000	0.000	0.000	
0.5	0.977	0.030	1.000	24.419	0.752	
1	1.051	0.027	0.960	27.369	0.716	
1.5	1.066	0.035	0.922	28.918	0.947	
2	1.056	0.049	0.885	29.839	1.372	
3	1.045	0.048	0.849	30.768	1.421	
4	1.064	0.052	0.815	32.628	1.579	
5	1.065	0.053	0.783	34.008	1.680	
6	1.082	0.046	0.751	35.990	1.527	
F5c						42.113 ± 0.350
Time (h)	Avg.	St. Dev.		Avg.	St. Dev.	
0	0.000	0.000	0.000	0.000	0.000	
0.5	1.109	0.147	1.000	27.720	3.666	
1	1.223	0.139	0.960	31.746	3.732	
1.5	1.270	0.090	0.922	34.343	2.598	
2	1.281	0.071	0.885	36.074	2.209	
3	1.250	0.061	0.849	36.672	2.010	
4	1.286	0.092	0.815	39.276	2.960	
5	1.286	0.086	0.783	40.912	2.925	
6	1.254	0.125	0.751	41.571	4.240	

5.3.6 Effect of residual levofloxacin content of inserts on bacterial growth

The efficacy of residual levofloxacin content in film inserts on Staphylococcus aureus and Pseudomonas aeruginosa was tested by evaluating the growth curve of bacterial cultures after mixing with unreleased levofloxacin content from inserts. The samples were collected from the donor compartment of the donor-receiver model used for dissolution testing, as the model was assumed to mimic ocular conditions in determining levofloxacin release pattern from the inserts. Thus, testing the effect of residual levofloxacin content of inserts on bacterial growth was supposed to be a suitable method to indirectly evaluate the sustained efficacy of the inserts in releasing drug. The effect of unreleased levofloxacin fractions on bacterial growth was assessed by comparing the concentrations of bacteria in the solutions prepared using samples collected after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours against the pure bacterial cultures, by virtue of optical density (OD600) monitoring. The procedure was separately conducted on Staphylococcus aureus and Pseudomonas aeruginosa.

The effect of unreleased content of levofloxacin exhibited different levels of efficacy on Staphylococcus aureus (Figure 5.6) and Pseudomonas aeruginosa (Figure 5.7). For Staphylococcus aureus it appeared that the quantity of levofloxacin not yet released from the donor compartment at collection times from 1 to 6 hours was able to inhibit bacterial growth, causing a lag time in bacterial replication (> 15 hours) that was found longer than the total dissolution time tested (6 hours) (Peleg & Corradini, 2011). Thus, it can be assumed that the fraction of drug unreleased from inserts within the first hour can still be sufficient in inhibiting the growth of Staphylococcus aureus. On the other hand, according to the drug releasing testing results, the quantity of unreleased drug after 30 minutes should be found higher than the following testing times. However, upon insertion in the donor compartment, the inserts settled at the bottom of the chamber, directly in contact with the semi-permeable membrane, while the solutions sampled were collected from the top of the dissolution media (1 mL of STF) present in the chamber. As such, it can be speculated that levofloxacin distribution in the donor compartment may have followed diffusion mechanism during the early stage of the testing, inducing a delayed peak of the drug concentration in apical part of the donor solution, while the concentration in the receiver compartment could be made homogeneous by the stirring motion.

The efficacy of unreleased levofloxacin fractions in reducing Pseudomonas Aeruginosa growth (Figure 5.7) was not proven, and it was found lower than what found in Staphylococcus aureus growth curves. For all the samples tested, the quantity of levofloxacin recovered from donor compartment was not sufficient to retard the exponential growth phases of Pseudomonas Aeruginosa, which overlapped the pure culture behaviour. Although the samples collected from 0.5 to 4 hours appeared to slightly reduce the concentration of bacteria during the stationary phase (Wang *et al.*, 2015), with maximum efficacy found after 1.5 hours, the potential antimicrobial efficacy was not demonstrated. Thus, given the reduced effect of unreleased levofloxacin on Pseudomonas aeruginosa, it should be further investigated if to increase the total amount of drug in the inserts can provide extended capability in preventing the growth of this bacteria.

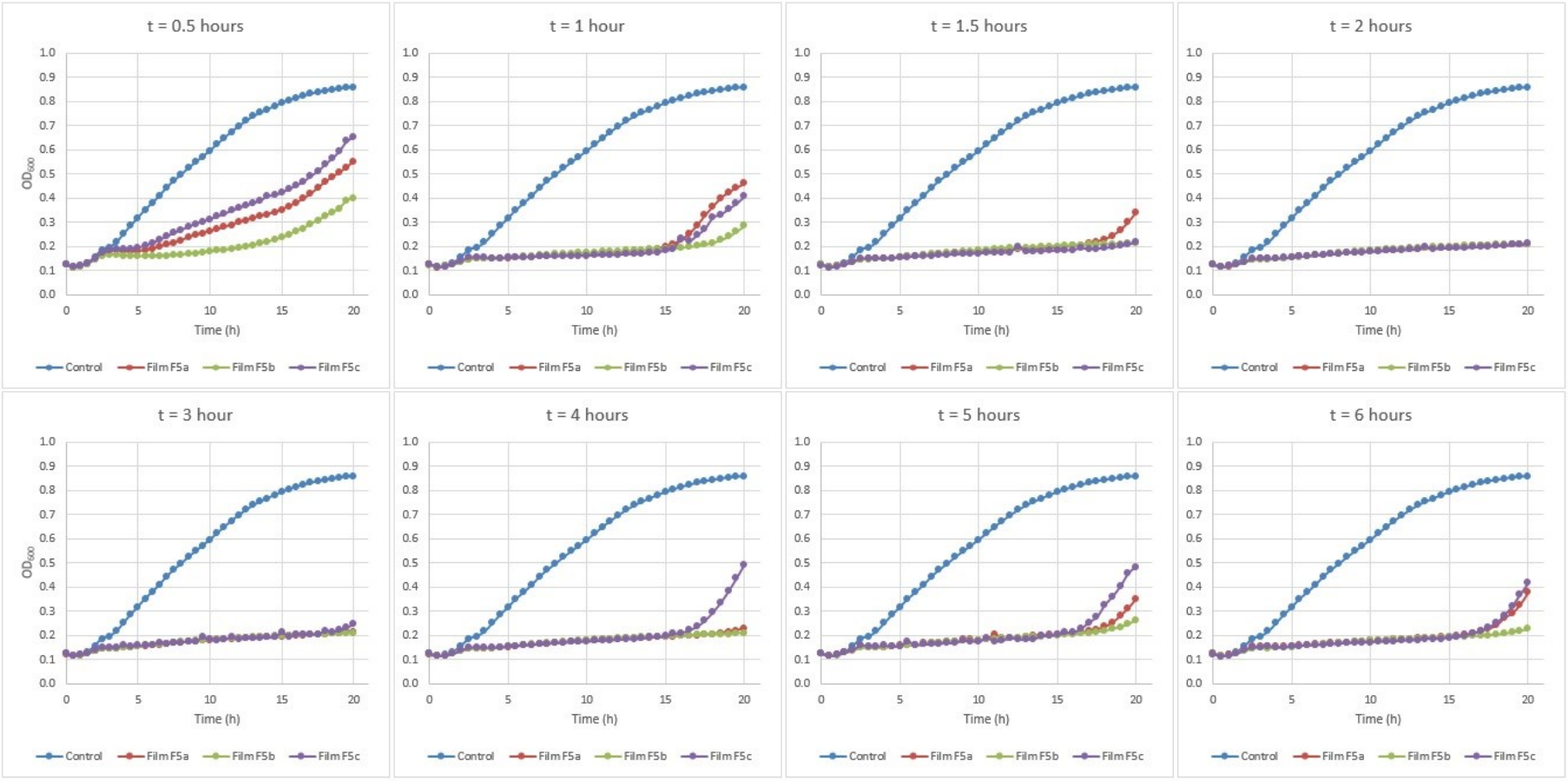

Figure 5.6 . Effect of inserts residual drug content on Staphylococcus Aureus growth. The control curve represents the OD_{600} of pure bacterial cells in TSB over time. For film A, B and A, values represent the mean OD_{600} of three samples. In each graph is reported the effect of levofloxacin fraction collected after the specified time.

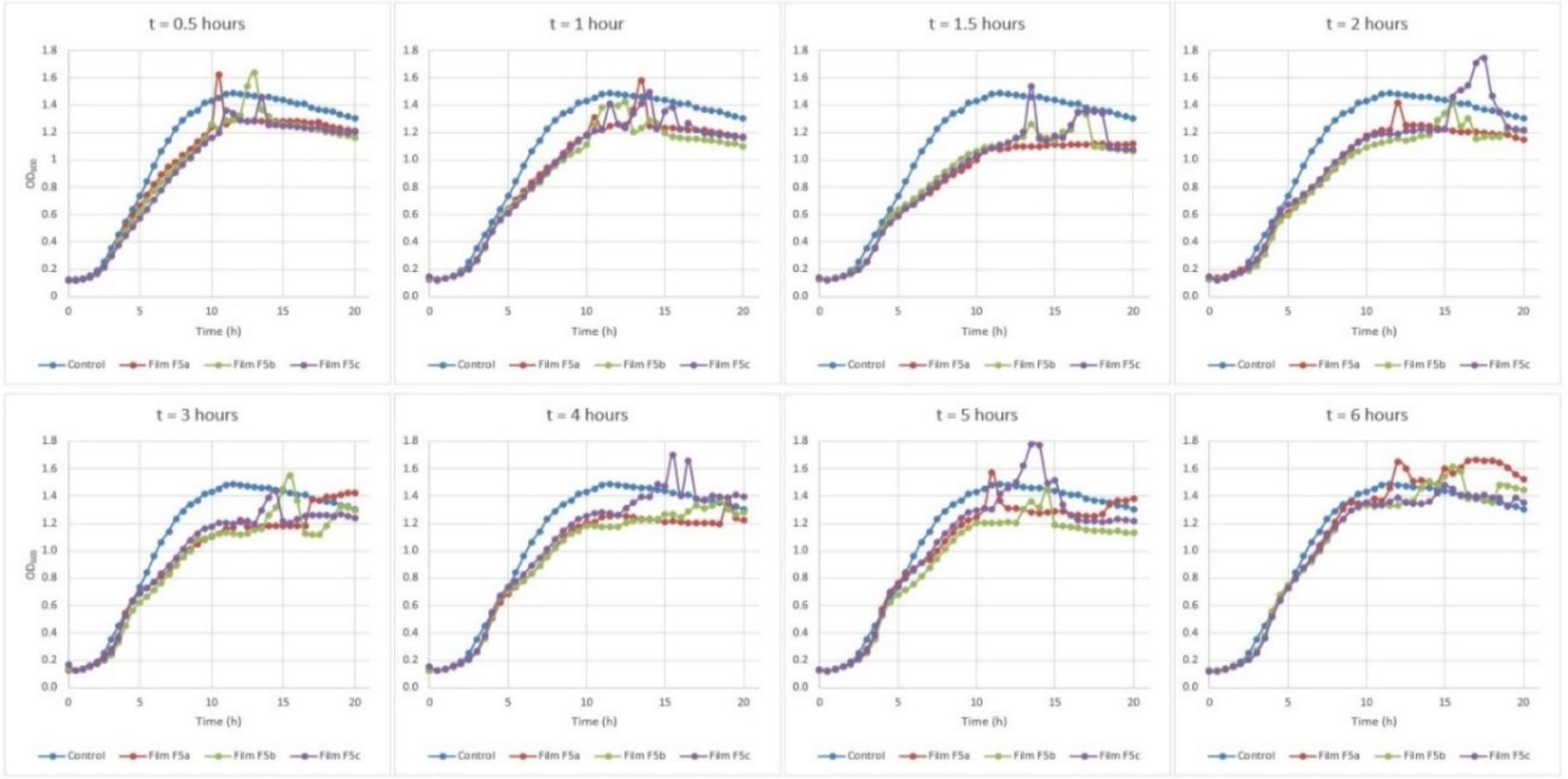

Figure 5.7 . Effect of inserts residual drug content on Pseudomonas Aeruginosa growth. The control curve represents the OD_{600} of pure bacterial cells in TSB over time. For film A, B and C, values represent the mean OD_{600} of three samples. In each graph is reported the effect of levofloxacin fraction collected after the specified time.

5.4 Conclusions

The levofloxacin-loaded films produced with formulation F5, which included 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. Although additional investigations are required, in particular regarding the effect of water uptake, the inserts showed promising characteristics for ocular use. The physical and chemical characterisation of the inserts appeared to respect the ocular anatomy and physiology, while the mechanical properties suggested that they can successfully comply with the insertion procedure. The content of levofloxacin was found consistent across the inserts manufactured. The drug release pattern showed overlapping curves for the inserts tested, indicating consistency in the modality of drug release. The majority of levofloxacin present in the inserts was released within the first 30 minutes, while the complete release was found after 6 hours. However, the biphasic release pattern demonstrated by the inserts left uncertainty regarding the potential sustained efficacy of the formulation. The findings were partially in line with desired profile, as the minimum inhibitory concentration of the drug was reached in a short time, while the fractions of levofloxacin released over time were not necessarily ensuring effective levels of pharmaceutical agent. The fraction of levofloxacin unreleased from inserts after the first 30 minutes was found sufficient to prevent Staphylococcus aureus growth, while the efficacy on Pseudomonas aeruginosa was not proven, suggesting that the formulation should be further developed to meet satisfactory antimicrobial efficacy on different bacterial species. Future studies will focus on formulation optimisation to improve drug release profile and antibacterial efficacy over time, as well as establishing stability and biocompatibility of the inserts.

Chapter 6 - Optimisation of the manufacturing procedure for levofloxacin-loaded inserts – a design of experiment approach

6.1 Introduction

Ocular thin film insert designs can be included in either matrix or reservoir-based drug delivery systems, which both aim to target a definite region for sustained dosing of active APIs through embedding of the drug in polymer complexes. Other than the properties of the drug, the characteristics of the polymers play a major role in determining the way the active substance is made available to the target site (Yang & Pierstorff, 2012). Nonetheless, the manufacturing method can be deemed a fundamental part of defining the properties of these film formulations and, even when dealing with similar/same compositions, the optimisation of the production process should be considered to achieve final products that are robust and that exhibit desired features (Borges *et al.*, 2017).

In the development of pharmaceutical products, the Quality-by-Design (QbD) concept encompasses a systematic approach for establishing the associations between the parameters (a.k.a. independent factors) of the manufacturing process and the attributes (a.k.a. responses) of the final drug delivery system, in order to confer repeatability and robustness to the procedure and design quality attributes into the final products (Yu, 2008). Design of Experiments (DoE), being a vital part of QbD, can be represented as the mathematical approach describing the relationships between the critical process parameters (CPP) used in the manufacturing, and critical quality attributes (CQA) of the delivery system. DoE provides a systematic analysis of the factors applied in the production and the mathematical models representing their relationship to the responses found in the final formulations (Fukuda *et al.*, 2018; Politis *et al.*, 2017).

The CQAs of the products can include a variety of characteristics of the device, and they can be regarded as the properties that allow for prediction of the performance of the system (Rathore & Winkle, 2009). It has also been suggested that CQAs can become the benchmark for future development of ocular formulations (Belenos *et al.*, 2023). Nonetheless, the identification of critical attributes for thin film products, including ocular, has been influenced by the lack of regulations and the paucity of testing standards for these devices, as well as the absence of specifications they should meet (Borges *et al.*, 2017). In particular, there has been advocacy highlighting the inadequacy of testing methods in guaranteeing bioequivalence and the absence of validated methodologies for testing ophthalmic products (Gore *et al.*, 2014; Rahman *et al.*, 2014). However, some critical attributes of the final formulations can be identified for ocular films.

In applying the QbD approach to film manufacturing through solvent-casting method, various aspects of the process can be considered. The homogeneity of the film, for example, remains a crucial factor in determining the quality of the film inserts, and it can be demonstrated by diverse critical attributes. Nevertheless, it can be altered by several process parameters in both dissolution and casting steps; hence, by means of qualitative analysis, preliminary trials can be performed to define the CPPs to test (Dalal *et al.*, 2021). In exploring solvent-casting method for film manufacturing, other than the analysis of formulation components, the main process parameters that have been tested include stirring time and temperature, cooling time, casting volume and drying settings (Ain *et al.*, 2022; Foo *et al.*, 2018; Vo *et al.*, 2020; Xu *et al.*, 2015), with the aim of simplifying the manufacturing process and ameliorating the CQAs of the films (Zhang *et al.*, 2018).

However, despite DoE being recently used for studying the formulation and manufacturing process of an ocular insert made by hot-melt extrusion (Alzahrani *et al.*, 2023a; Alzahrani *et al.*, 2023b), the implementation of this systematic approach has not yet been reported in evaluating the solvent-casting manufacturing process for ocular thin film inserts, including those loaded with antimicrobial agents. Thus, the aim of this work was to optimise the solvent casting manufacturing process of ocular thin film inserts by screening the factors (CPP) that may impact the production of the ocular film the most, and to implement the design of experiment method to delineate the effects of variations in manufacturing parameters on the quality of the resulting formulations.

6.2 Materials and Methods

6.2.1 Materials

Levofloxacin (HPLC grade), gelatin (from porcine skin, Type A), alginic acid sodium salt from brown algae (low viscosity), polyethylene glycol 400 (BioUltra), and 50 mL polypropylene centrifuge tubes (Corning®), were purchased from Sigma Aldrich (Gillingham, UK). HPMC E15 was purchased from JRS PHARMA (Rosenberg, Germany). Liquid chromatography reagents were purchased from Fisherbrand™ (Loughborough, UK). All reagents were used as received.

6.2.2 Methods

6.2.2.1 Preliminary studies to determine experimental framework/design space

6.2.2.1.1 Preparation of film-forming solution

Diverse methods were explored to achieve the production of film-forming blending, for the ability to provide homogeneous and repeatable solutions, in suitable volumes to obtain uniform films with increased dimensions. The following mixing procedures were operated to obtain a solution of 1250 mg of HPMC, 750 mg of sodium alginate (low viscosity), 250 mg of gelatin (type A), and 2.5 mL of PEG400 in 22.5 mL distilled water (optimised formulation from previous chapter).

Sonication - Deionised water at room temperature was added to the powder mixture in a 30 mL glass vial, followed by plasticiser addition; the solution was sonicated for 30 minutes at 50˚C before and left stirring overnight on a pre-heated magnetic stirring plate at 60˚C.

Homogenisation – The film forming solutions were made with the same method described for the sonication method above, but the sonication steps were replaced by mixing the blend with a homogeniser before stirring overnight.

Split-beaker solubilisation – For this procedure, separate solutions were prepared for the compounds at different temperatures. Part of the water was mixed with HPMC under vigorous stirring at 70˚C, while the remaining water (to match the final concentrations) was mixed in separate glass beakers with the powder blend of sodium alginate and gelatin at 60˚C. The resulting solutions were thereafter added together.

Single-beaker solubilisation – The compounds were separately weighed and added consecutively during stirring. HPMC was added first to part of the total water previously heated to 70˚C under vigorous stirring. The remaining water was then added, and the temperature was reduced to 60˚C before adding sodium alginate and gelatin to the solution one after the other.

Single-beaker solubilisation of powders mixture – The polymers were separately weighed and carefully mixed afterward to create a powder blend. The mixture was gradually added to water previously heated to 60˚C. After 60-90 minutes, the heating source was switched off and the film forming solution were left to cool to room temperature, before plasticiser addition.

Removal of bubbles – Distinct procedures and equipment were employed to identify a sufficiently effective method to remove bubbles from the film forming solutions. Hand removal – A spatula was used to remove bubbles on the surface while a disposable Pasteur pipette was used to remove bubbles present within in the solution. Centrifugation – Incremental time span length centrifuge steps were added to the hand removal procedure. The bubbles on the surface were first removed from the top of the solution and the fluid was then transferred into a centrifuge tube. Samples were centrifuged for up to 5 minutes at 1000 rpm at room temperature. The bubbles emerging after centrifugation were carefully removed from top of the solution with a disposable Pasteur pipette.

6.2.2.1.2 Film casting and drying procedure

The selection of the casting surface aimed to identify a support allowing a large casting surface area to be used in conjunction with an automatic film applicator and a micrometre applicator blade. Stainless steel plates, aluminium plates, Teflon-coated trays, PVC foils, Petri dishes, and glass plates were tested as casting surfaces for their ability to produce qualitative films. Also, upon the selection of the casting surface, the parameters of film applicator traverse speed and blade height were optimised, and the use of distinct volumes of film-forming solution volumes were tested. The effect of different drying temperature on the final films was also tested.

6.2.2.2 Design of experiment

6.2.2.2.1 Design space identification

Experimental design was outlined with MODDE Pro (Sartorius, v12) from Umetrics. The evaluation of manufacturing process focussed on five CPPs, reported in Table 6.1 alongside their value ranges, supposed to potentially induce significant effects on films/inserts.

Table 6.1 DoE factors

Factor	*Abbreviation*	*Unit*	*Type*	*Range*
Stirring temperature (X_1)	STe	˚C	Quantitative	40 to 60
Stirring time (X_2)	STi	h	Quantitative	1 to 3
Stirring cooling time (X_3)	SCT	h	Quantitative	1 to 3
Centrifuge rpm (X_4)	CSp	rpm	Quantitative	200 to 1000
Centrifuge time (X_5)	CTi	min	Quantitative	1 to 5

Also, the CQAs of the films/inserts investigated were selected to evaluate physical characteristics (weight and thickness), potential clinical efficacy (drug content), and mechanical properties (tensile strength and elongation at maximum load), reported in Table 6.2.

Table 6.2 Responses

Response	*Abbreviation*	*Unit*
Weight (Y_1)	Wei	mg
Thickness (Y_2)	Thi	mcm
Drug Content (Y_3)	Con	mcg
Tensile Strength (Y_4)	Ten	MPa
Elongation at Max Load (Y_5)	Elo	%

A central composite face (CCF) design was selected, as it allows to create design space for products optimisation with a reduced number of experiments, compared to the full factorial design. In Table 6.3 are reported the experimental conditions used for film production, according to the quadratic model generated by the software.

*Table 6.3 Specifications of experiments performed according to the central composite face design selected. *Factor related to plate used for the runs was included after preliminary data analysis.*

Experiment code	*Stirring temperature (˚c)*	*Stirring time (h)*	*Stirring cooling time (h)*	*Centrifuge speed (rpm)*	*Centrifuge time (min)*	*Plate code**
N1	40	1	1	200	5	P3
N2	60	1	1	200	1	P1
N3	40	3	1	200	1	P2
N4	60	3	1	200	5	P2
N5	40	1	3	200	1	P2
N6	60	1	3	200	5	P2
N7	40	3	3	200	5	P1
N8	60	3	3	200	1	P4
N9	40	1	1	1000	1	P4
N10	60	1	1	1000	5	P1
N11	40	3	1	1000	5	P3
N12	60	3	1	1000	1	P1
N13	40	1	3	1000	5	P1
N14	60	1	3	1000	1	P1
N15	40	3	3	1000	1	P4
N16	60	3	3	1000	5	P3
N17	40	2	2	600	3	P1
N18	60	2	2	600	3	P3
N19	50	1	2	600	3	P2
N20	50	3	2	600	3	P2
N21	50	2	1	600	3	P1
N22	50	2	3	600	3	P3
N23	50	2	2	200	3	P4
N24	50	2	2	1000	3	P1
N25	50	2	2	600	1	P2
N26	50	2	2	600	5	P2
N27	50	2	2	600	3	P3
N28	50	2	2	600	3	P3
N29	50	2	2	600	3	P4

6.2.2.2.2 Film manufacturing procedure

All the films were manufactured by solvent-casting method, with an individual film forming solution prepared for each run. The 0.2% Levofloxacin solutions were freshly prepared by dissolving the drug in ultrapure water (Purite Select Ondeo distiller – London, UK) and sonicating for 10 minutes. For each batch, 22.5 mL drug solution was placed in a beaker and heated to the target stirring temperature. After accurate weighing, 1.25 g of HPMC, 0.75 g of sodium alginate and 0.5 g of gelatin powders were blended to obtain a uniform mixture, and gradually added to the solution under vigorous stirring (powders had been stored overnight in a desiccator to minimise environmental variations). The solutions were then placed on a magnetic stirring plate, already set to the desired temperature, stirred at 300 rpm for the designated time and then allowed to cool, with continued stirring. To minimise plasticiser degradation, 2.5 mL of PEG400 was added to the film forming solution once the temperature dropped below 40˚C and stirring continued for designated cooling time. Following this, surface bubbles were removed, and the solutions were centrifuged according to the experimental design parameters, and emerging bubbles were removed with disposable pipettes. Film forming solutions were subsequently poured onto glass plates and spread using an Elcometer® 4340 Motorised/Automatic Film Applicator (Manchester, UK). Films were placed in an incubator chamber at 25˚C overnight, and until completely dry. Twenty-four disc-shaped inserts of ten millimetres diameter were cut using a stainless-steel cork-borer. In addition, six inserts of twenty-five millimetres diameter were also collected and used to perform mechanical testing. All samples were collected in a consistent pattern across the films (Figure 6.1).

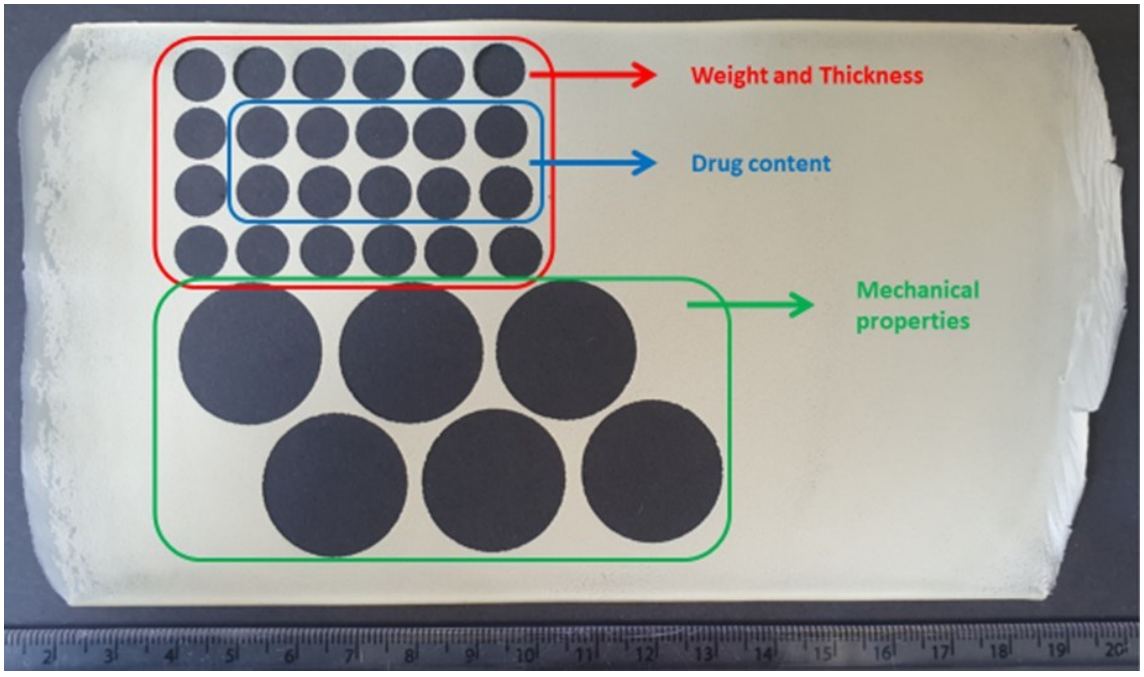

Figure 6.1 Sample collection scheme. 24 samples (10 mm) for testing weight and thickness uniformity, 10 samples (10 mm) for testing content uniformity and six samples (25 mm) for testing mechanical properties

6.2.2.2.3 Characterisation of samples

The values of weight and thickness were computed by averaging the gauge calliper measurements performed on 24 samples (Ø = 10 mm). Of those, 10 samples, consistently collected across the films (Figure 6.1), were further tested for levofloxacin content. Samples were placed in glass vials pre-filled with 5 mL ultrapure water and left to dissolve overnight (14-16 hours) using an orbital incubator at 37˚C and shaking motion speed of 100 rpm. Samples were then vortexed for 5 seconds before being collected from the vials to favour homogenous distribution of levofloxacin; and filtered. For the analysis, an Agilent 1200 HPLC system, 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% 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, and wavelength for UV detector of 288 nm.

The mechanical testing was performed on six disc-shaped samples (Ø = 25 mm) using a Hounsfield Tensometer (Hounsfield Limited, Croydon, UK), with grips placed at equal distance from insert edges, along a theoretical diameter line of the inserts. The test was set with stress range 0.05 MPa, strain range 300%, speed 60 mm/min and without preload. The distribution of the data collected for the different characteristics were independently tested for normality by appropriate statistical testing, in order to identify the values best representing the datasets, to be further used in the design of experiment worksheet.

6.2.2.2.4 DoE model construction and evaluation

A statistical model including all factors and their interactions (Table 6.4) was used to assess the effects of the CPP on the CQA and the regression equation is shown below:

$$\boldsymbol{Y_i} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1} + \boldsymbol{\beta_2 X_2} + \boldsymbol{\beta_3 X_3} + \boldsymbol{\beta_4 X_4} + \boldsymbol{\beta_5 X_5} + \boldsymbol{\beta_{12} X_1 X_2} + \boldsymbol{\beta_{13} X_1 X_3} + \boldsymbol{\beta_{14} X_1 X_4} + \boldsymbol{\beta_{15} X_1 X_5} + \boldsymbol{\beta_{23} X_2 X_3} + \ldots + \boldsymbol{\beta_{11} X_1^2} + \boldsymbol{\beta_{22} X_2^2} + \boldsymbol{\beta_{33} X_3^2} + \boldsymbol{\beta_{44} X_4^2} + \boldsymbol{\beta_{55} X_5^2} \quad \text{Eqn. 6.1}$$

Where Y_i is the targeted response; β_0, is the arithmetic mean of each response; β_1, β_2, β_3, β_4, β_5 and β_6 are the estimated coefficients of each of the factors respectively; the main effects (X_1, X_2, X_3, X_4, X_5 and X_6) depict the average effect obtained when one factor is ranged from high to low value while all other factors are maintained at their mid-point; the interactive terms (X_1X_2, X_1X_3, X_2X_3 etc.) represent the mean values obtained when factors are varied concurrently; and the quadratic terms (X_1^2, X_2^2, X_3^2, X_4^2, X_5^2 and X_6^2) assessed any non-linear relationships within the model.

Multiple linear regression (MLR) and partial least squares (PLS) regression were used to fit the data and the better model chosen. Data was checked for presence of outliers, and separate models were identified for each response, with coefficients selected based on their significance to the model, the importance of variables, and the hierarchy of the terms. Model performance was evaluated using R2 (variation of the response), Q2 (variation of the response predicted by the model according to cross validation), model validity and reproducibility. The models were then tested for statistical significance (using ANOVA) and lack of fit. The final significant models were used to determine the effect of the factors employed in the experimental design on the responses investigated, and to identify potential strategies and procedure specifications to optimise the film manufacturing process.

Table 6.4 Terms selected for models best fitting the responses. In each column are reported all the factors and the interactions included in the best mathematical models identified in the determination of the effects of the critical process parameters (CPP) on the critical quality attributes (CQA) of the formulation.

Weight	*Thickness*	*Drug Content*	*Tensile Strength*	*Elongation at Max*
Constant	Constant	Constant	Constant	Constant
Stirring Temperature	Stirring Temperature	Stirring Temperature	Stirring Temperature	Stirring Temperature
Stirring Time	Stirring Time	Stirring Time	Stirring Time	Stirring Time
Stirring Cooling Time	Stirring Cooling Time	Stirring Cooling Time	Stirring Cooling Time	Stirring Cooling Time
Centrifuge Speed	Centrifuge Speed	Centrifuge Speed	Centrifuge Speed	Centrifuge Speed
Centrifuge Time	Centrifuge Time	Centrifuge Time	Centrifuge Time	Centrifuge Time
Plate	Plate	Plate	Stirring Temperature * Stirring Temperature	Stirring Temperature * Stirring Temperature
Centrifuge Speed * Centrifuge Speed	Stirring Time * Stirring Time	Stirring Temperature * Stirring Temperature	Centrifuge Speed * Centrifuge Speed	Stirring Cooling Time * Stirring Cooling Time
Centrifuge Time * Centrifuge Time	Centrifuge Speed * Centrifuge Speed	Centrifuge Speed * Centrifuge Speed	Centrifuge Time*Centrifuge Time	Centrifuge Time * Centrifuge Time
Stirring Temperature*Stirring Cooling Time	Centrifuge Time * Centrifuge Time	Centrifuge Time * Centrifuge Time	Stirring Temperature * Stirring Cooling Time	Stirring Temperature * Stirring Time
Stirring Temperature * Centrifuge Time	Stirring Temperature * Stirring Time	Stirring Temperature * Stirring Cooling Time	Stirring Temperature * Centrifuge Speed	Stirring Temperature * Stirring Cooling Time
Stirring Time * Stirring Cooling Time	Stirring Temperature * Stirring Cooling Time	Stirring Temperature * Centrifuge Speed	Stirring Temperature * Centrifuge Time	Stirring Temperature * Centrifuge Speed
Stirring Time * Centrifuge Speed	Stirring Temperature * Centrifuge Speed	Stirring Time * Stirring Cooling Time	Stirring Time * Stirring Cooling Time	Stirring Time * Stirring Cooling Time
Stirring Time * Centrifuge Time	Stirring Temperature * Centrifuge Time	Stirring Time * Centrifuge Time	Stirring Time * Centrifuge Speed	Stirring Time * Centrifuge Speed
Stirring Cooling Time*Centrifuge Speed	Stirring Time * Stirring Cooling Time	Stirring Cooling Time * Centrifuge Time	Centrifuge Speed * Centrifuge Time	Stirring Cooling Time * Centrifuge Speed
Stirring Cooling Time * Centrifuge Time	Stirring Cooling Time*Centrifuge Speed	Centrifuge Speed * Centrifuge Time		Centrifuge Speed * Centrifuge Time
Centrifuge Speed * Centrifuge Time	Stirring Cooling Time * Centrifuge Time			
	Centrifuge Speed * Centrifuge Time			

6.3 Results and Discussion

6.3.1 Preliminary studies to determinate experimental framework/design space

6.3.1.1 Film-forming solution preparation

The determination of mixing and degassing procedures are crucial requirements in the manufacturing of homogeneous films (Buyukgoz *et al.*, 2021; Dixit & Puthli, 2009). The order of addition of the excipients and the APIs can also play a role in obtaining a qualitative film forming solution (Dalal *et al.*, 2021). Among the procedures tested to prepare the film forming solutions, the single-beaker solubilisation of powders mixture method was found to be the most suitable. This procedure, in fact, did not present major issues, with gradual powder addition being the most crucial step in prevention of lumps. The mobility of the fluid during stirring was found consistent in the beaker, and the solution appeared homogeneous upon visual inspection. Although the presence of bubbles crust could not be prevented, it was found consistently and partially fading over time. The other methods presented major limitations. Sonication resulted in the formation of polymers/gel lumps in both solutions and films, with the top fraction of film forming solution scarcely moved during mixing and thus, not completely homogenised. Also, the addition of 2 x 30 second vortexing steps before stirring showed similar results. The substitution of sonication with the use of a homogeniser, instead, produced dense presence of bubbles in the solution that were not eliminated by the stirring, with the top fraction of the foam still scarcely moving under stirring. Furthermore, the separate solubilisation of the polymers favoured the solubilisation of the compounds, but the solutions held distinct features preventing proper blending when combined. The HPMC solution was characterised by the presence of a thick and firm bubble crust on top of the fluid; the sodium alginate and gelatin solutions presented bubbles, but in lower quantity, randomly trapped in the mix, while being more viscous. The presence of the bubbles crust and the high viscosity of the solutions led to reduced mixing of the fluids, with residues remaining into the beakers – so final concentrations could not be ascertained. Finally, the addition of the single polymers in the same beaker during stirring prevented sufficient mixing because the solution underwent a rapid change in viscosity and created surface bubbles, impeding the inclusion of the other compounds. Hence, single-beaker solubilisation of powders mixture was taken as the optimal method for film forming solutions production and homogenisation.

The removal of bubbles with a spatula, after the solubilisation of compounds directly in the beaker used for mixing, dramatically reduced the presence of surface bubbles, but had a minor effect on those trapped deeper in the solution. Implementing the use of a disposable Pasteur pipette to further remove bubbles directly in the beaker reduced the bubbles count, which, however, remained present. The addition of a centrifugation step for bubbles removal improved the overall effectiveness of the procedure. Overall, given that the results were dependant on centrifuge settings, those were selected as a critical parameter and evaluated in the DoE.

6.3.1.2 Film casting and drying procedure

The selection of the casting surface aimed to identify a support able to expand the surface area covered by the film-forming solution, in the attempt to produce larger films allowing the collection of a higher number of specimens, compared to the Petri dishes that are most commonly used at the developmental laboratory stage (Dixit & Puthli, 2009). Stainless steel, aluminium and Teflon-coated trays could not guarantee a satisfactory surface smoothness, due to an uneven distribution of the film forming solution, roughness of the film, and focal thinning of the film in presence of micrometric protrusions on the support, causing inhomogeneity of film thickness and ruptures during peeling. Also, because of their intrinsic flexibility, the use of PVC foil was dependent on employment of a support. The lack of rigidity of this support was particularly crucial in manageability of the formulations and during film peeling. For PVC foil, it was observed that folds occurred easily during the procedure, altering the surface regularity and compromising film casting consistency. On the contrary, the selection of tempered glass plates (300x300x10mm) fulfilled the need for a smooth and stable casting surface. The stiffness of the material, coupled with the smoothness of the surface, provided superior benefits in respect of the aforementioned supports while well complying with the stability requirements for film peeling. Moreover, the use of glass plates showed a significant reduction in the maximum coefficient of variation in mass of samples collected, when compared to Petri dishes (8% vs 21%, $p < 0.05$).

Upon selection of the glass plate as the casting surface, the parameters of the automatic film applicator were evaluated. The height of the micrometric film applicator blade was set to 0.5, 1.0 and 1.5 millimetres and the apparatus was used to spread 10 mL of film forming solution. At 1.5 mm, the blade could intercept only the bulging areas of the film- forming solution, not ensuring even fluid distribution. At 0.5 mm, the procedure led to homogeneous spreading of solution, but the resulting dried films could not be peeled because of repeated ruptures, due to suboptimal thickness. At 1.0 mm height, the film applicator flattened the solution evenly, contributing to produce manageable dried films that could be easily peeled. The speed of the apparatus was gradually incremented. From mid-low speed steps on, it was noticed that air bubbles were incorporated into the portion of the film where the applicator first encountered the film forming solution. Moreover, for mid-high speed settings, a relevant fraction of the solution was displaced by the film applicator to the distal portion of the plate, not contributing to the final film.

Hence the final casting method was identified as the use of an automated film applicator set at lowest speed, and with the micrometre casting blade set at 1.0 mm height. The use of glass plates was furtherly implemented by the addition of silicon strips to aid the spreading of the solution by the apparatus, favouring a more even distribution of volume of the film forming solution across the surface of the support, as shown in Figure 6.2.

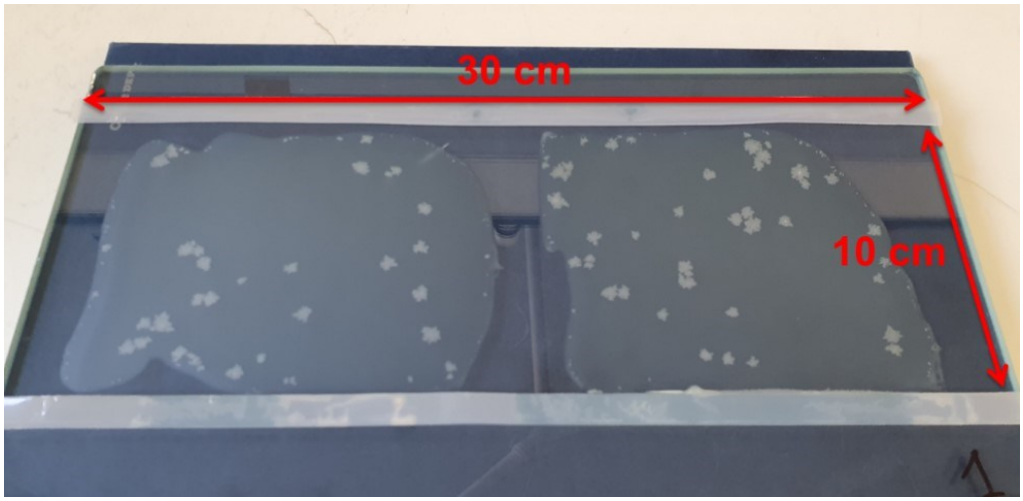

Figure 6.2 Casting surface. Glass plate implemented with silicon strips (shown in white).

Increasing drying temperatures were used to favour solvent evaporation from the formulations. However, the casted solutions could not form a peelable film if the drying temperature was 40˚C or above. In fact, although the exposure of casted film-forming solutions to temperature higher than the room has been suggested to reduce the film drying time (Bagheri *et al.*, 2019), this was found to cause excessive evaporation of the solvents and cause film brittleness and fragility. Hence, drying temperature was set to 25˚C.

6.3.2 Design of experiment

According to the design matrix selected, 29 films were manufactured after the same number of individual batches of film forming solution prepared in line with the manufacturing process specifications delineated by the central composite face design (Table 6.3). All film forming solutions appeared as yellowish (due to levofloxacin) opaque fluids, which could be poured and spread easily. After drying, all the solutions produced translucent, consistent, and flexible films, which were easy to peel, handle and cut into the desired shape. The Shapiro-Wilk tests of normality indicated that data collected in the different testing, with very few exceptions, were not normally distributed ($p > 0.05$). Thus, the average values from the data were reported as medians and used in the design of experiment evaluation as in Table 6.5.

Table 6.5 Median values of inserts/films manufactured.

Run code	*Plate code*	*Weight*	*Thickness*	*Drug content*	*Tensile strength*	*Elongation*
N1	*P3*	10.80	149.5	69.47	0.99	7.03
N2	*P1*	11.95	171.5	82.56	0.85	6.48
N3	*P2*	10.32	139.0	66.95	1.20	5.20
N4	*P2*	10.14	148.0	72.77	0.94	7.03
N5	*P2*	10.56	146.0	68.06	0.96	7.70
N6	*P2*	10.49	145.5	73.57	0.91	7.13
N7	*P1*	11.48	161.0	75.37	1.14	10.24
N8	*P4*	11.92	175.0	83.70	1.10	8.31
N9	*P4*	11.10	157.5	75.34	0.90	11.05
N10	*P1*	11.77	160.5	83.96	1.10	8.83
N11	*P3*	10.87	150.0	71.19	1.02	6.61
N12	*P1*	11.65	170.0	81.84	1.02	7.10
N13	*P1*	12.06	171.5	79.26	1.12	11.05
N14	*P1*	11.63	167.5	79.97	1.18	8.23
N15	*P4*	11.85	166.0	78.89	0.73	9.50
N16	*P3*	11.70	168.0	81.91	1.09	7.20
N17	*P1*	12.68	177.0	91.62	1.07	7.29
N18	*P3*	11.46	163.0	80.96	1.11	7.15
N19	*P2*	10.63	149.0	77.54	1.04	6.53
N20	*P2*	10.57	144.0	75.90	1.19	7.60
N21	*P1*	11.52	162.5	80.00	1.25	7.05
N22	*P3*	11.28	148.0	82.40	1.23	6.48
N23	*P4*	12.10	172.5	84.26	1.18	6.07
N24	*P1*	11.97	175.0	83.94	1.29	6.69
N25	*P2*	10.17	145.0	70.28	1.14	6.16
N26	*P2*	10.52	148.0	71.69	1.38	6.50
N27	*P3*	11.35	161.5	78.35	1.21	7.12
N28	*P3*	11.29	159.5	79.03	1.35	5.95
N29	*P4*	11.95	169.5	82.95	1.32	6.70

To verify the model, the summary fit plot was generated and is presented in Figure 6.3. The indexes are computed as percentage values, hence a value of 1 would represent the highest possible score. R2 is the variation of the responses explained by the model, in other words, how well the model fits the data. Considering that a value below 0.5 is assumed to represent a rather low significance, the R2 values found in the analysis indicates that the models constructed fit the data collected closely. Q2 value indicates the capability of the model in predicting new data, and, for good models, it should be above 0.5 and not exceeding 0.3 difference from R2 (Dennison *et al.*, 2016). From figure 6.3, a good predictive power was achieved for all models.

Model validity index represents the relation between the model error and the pure error. For values above 0.25, the model error is in the same range of the pure error, and the presence of lack of fit can be excluded (Dennison *et al.*, 2016). Thus, the values found in the analysis indicated that all the models were valid. Reproducibility values, instead, represent the variability of data collected from replicates, and they should be above 0.5 (Dennison *et al.*, 2016). It can be noticed that reproducibility values for all models met this criterion. Values for mechanical testing were markedly lower than the others, and this can be associated with the main limitation of low repeatability the testing methodologies.

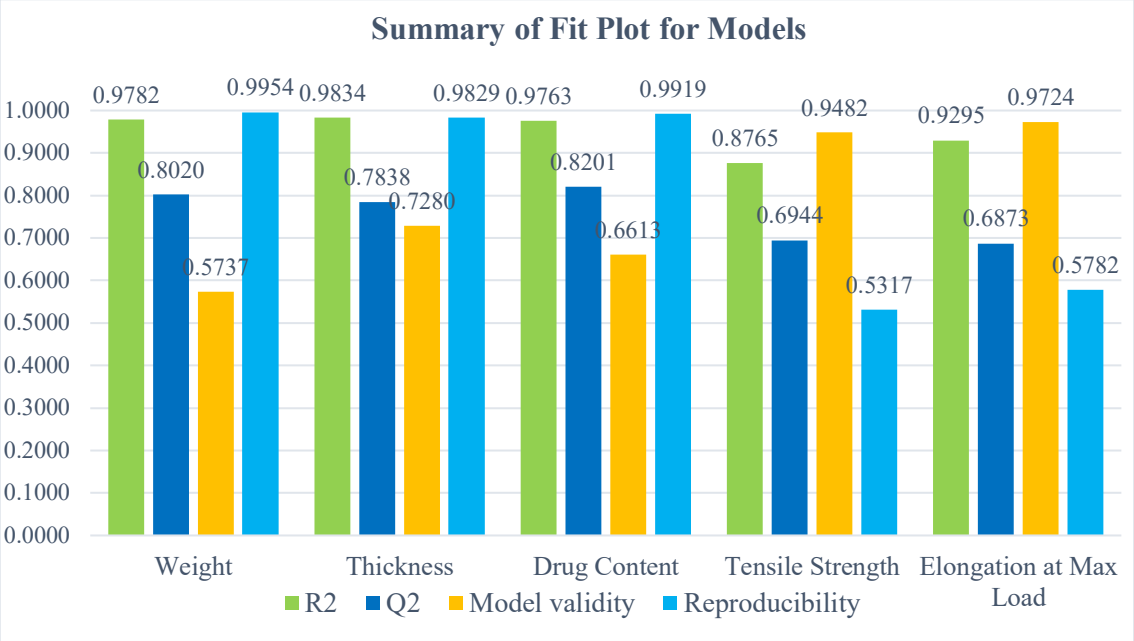

Figure 6.3 Summary of fit plot for the models constructed for each response.

In addition, ANOVA was used to check the statistical significance of the models. As reported in Table 6.6, for all the models, the probability of the regression was significant ($p < 0.05$), while the probability of lack of fit was not significant ($p > 0.05$), indicating that the models were statistically significant with no lack of fit (Dahmash *et al.*, 2018).

Table 6.6 ANOVA test results for the models constructed. Significance of model and lack of fit are in bold.

	Degree of freedom	*Sum of squares*	*Mean square (variance)*	*F value*	*P value*	*Standard deviation*
			Weight			
Total	27	3412	126.37			
Constant	1	3401.79	3401.79			
Total corrected	26	10.2095	0.392672			0.626635
Regression	18	9.98733	0.554852	19.9817	0.000	0.744884
Residual	8	0.222144	0.027768			0.166637
Lack of fit (model error)	7	0.220344	0.0314777	17.4874	0.182	0.17742
Pure error (replicate error)	1	0.00180003	0.00180003			0.0424267
			Thickness			
Total	26	665974	25614.4			
Constant	1	663042	663042			
Total corrected	25	2931.56	117.262			10.8288
Regression	19	2882.98	151.736	18.7406	0.001	12.3181
Residual	6	48.5799	8.09665			2.84546
Lack of fit (model error)	5	46.5799	9.31598	4.65799	0.337	3.05221
Pure error (replicate error)	1	2	2			1.41421
			Drug Content			
Total	28	169258	6044.94			
Constant	1	168501	168501			
Total corrected	27	757.25	28.0463			5.29588
Regression	17	739.329	43.4899	24.2674	0.000	6.59469
Residual	10	17.9211	1.79211			1.3387
Lack of fit (model error)	9	17.6937	1.96596	8.64255	0.258	1.40213
Pure error (replicate error)	1	0.227475	0.227475			0.476943
			Tensile Strength			
Total	28	34.0487	1.21603			
Constant	1	33.4633	33.4633			
Total corrected	27	0.585426	0.0216825			0.14725
Regression	14	0.513097	0.0366498	6.58723	0.001	0.191441
Residual	13	0.0723289	0.00556377			0.0745907
Lack of fit (model error)	12	0.0621758	0.00518132	0.510318	0.813	0.0719814
Pure error (replicate error)	1	0.0101531	0.0101531			0.100763
			Elongation at Max Load			
Total	28	18.1199	0.647139			
Constant	1	17.897	17.897			
Total corrected	27	0.222933	0.00825677			0.0908668
Regression	15	0.207218	0.0138145	10.5487	0.000	0.117535
Residual	12	0.0157151	0.00130959			0.0361883
Lack of fit (model error)	11	0.0122323	0.00111203	0.319293	0.896	0.0333471
Pure error (replicate error)	1	0.00348278	0.00348278			0.0590151

6.3.2.1 Effect of CPPs on CQAs for film manufacture

After elimination of non-significant/non-hierarchical terms, the following regression equations depicting the model terms that had a significant effect on the responses are shown below. It should be noted that a positive (+) effect coefficient value indicates that an increase in the factor resulted in an increase in the response, and vice versa. Similarly, a higher value indicates a higher effect of the term on the response, and vice versa. These equations summarise the relationships between the factors and responses and will be discussed in the following sections.

$$Y_1 = 11.38 + 0.27X_1 + 0.36X_3 - 0.39X_3^2 \quad \text{Eqn. 6.2}$$

$$Y_2 = 159.97 + 7.59X_1 + 4.81X_3 - 15.87X_3^2 + 11.84X_4^2 \quad \text{Eqn. 6.3}$$

$$Y_3 = 80.31 + 6.68X_1 + 2.90X_3 - 3.72X_5^2 \quad \text{Eqn. 6.4}$$

$$Y_4 = 1.24 + 0.13X_5 - 0.44X_2^2 - 0.14X_2X_4 + 0.1X_4X_5 \quad \text{Eqn. 6.5}$$

$$Y_5 = 0.83 - 0.05X_1 - 0.05X_2 + 0.08X_3 + 0.05X_4 - 0.06X_1X_3 + 0.06X_2X_3 - 0.08X_2X_4 - 0.05X_4X_5 + 0.15X_1^2 \quad \text{Eqn. 6.6}$$

Where Y_1 is film weight, Y_2 is film thickness, Y_3 is drug content, Y_4 is film tensile strength and Y_5 is film elongation at maximum load. X_1 is stirring temperature, X_2 is stirring time, X_3 is stirring cooling time, X_4 is centrifuge rpm and X_5 is centrifuge time.

6.3.2.1.1 Effect of stirring temperature, X_1

In the experimental design, the temperature used to solubilise the powdered polymers was varied between 40˚C and 60˚C. As shown in Figure 6.4, which reports the main effect of stirring temperature on all responses, an increment in stirring temperature induced a significant increment in weight, thickness and drug content of the films manufactured (Eqn. 6.2 - Eqn. 6.4). Even though the beakers were mainly covered with lids, the stirring procedure was not performed under air-tight conditions. Furthermore, during the addition of polymeric powders, the drug solution was left uncovered, thus potentially enhancing the rate of water evaporation at higher temperatures. Consequently, the film forming solutions with higher rates of evaporation may have been more concentrated and more viscous, resulting in more polymer and drug fractions being deposited in the same area of the glass plate during spreading.

In terms of mechanical properties, tensile strength and elongation demonstrated opposite trends against temperature increment. Tensile strength increased up to 50˚C and then declined, while elongation decreased until 50˚C and then began to increase with increasing temperatures respectively. For both responses, a reversal of trend occurred at 50˚C. However, the effect on tensile strength was not deemed significant (Eqn. 6.5). It should be considered that the polymers in the formulation have different temperature ranges of solubilisation and degradation. As the temperature rises above 40 ˚C, in fact, gelatin may begin to undergo a slow depolymerization in aqueous solutions (van den Bosch & Gielens, 2003). Also, gelatin can present a temperature-dependant modification of the helices that can influencing the ability to entrap and retain water molecule after gelation (Djabourov *et al.*, 1988). Hence, the mechanical properties of the films could have reflected the interaction between the compounds up to 50˚C, followed by degradation of their bonds or of the polymers at higher temperatures.

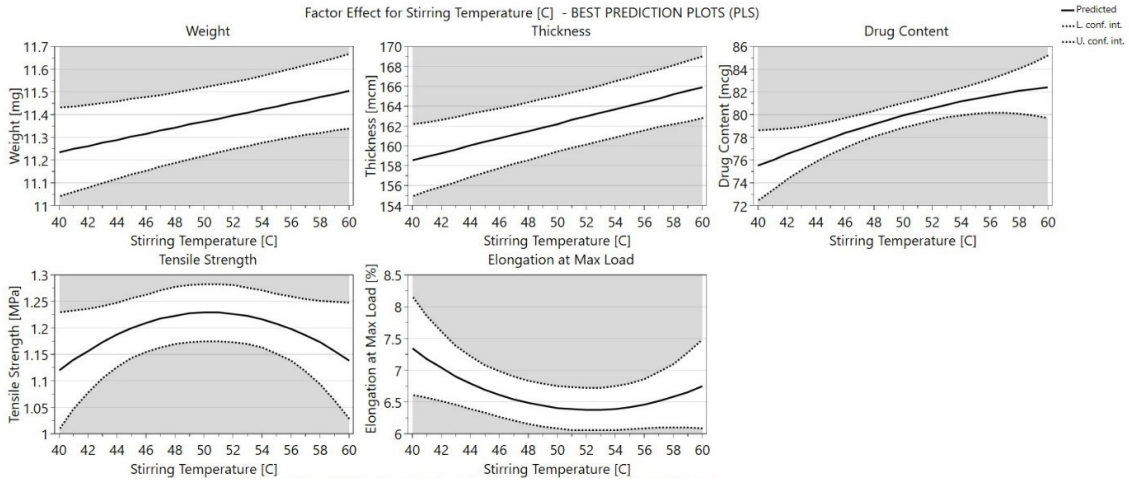

Figure 6.4 Main effect plots of stirring temperature on film characteristics.

6.3.2.1.2 Effect of stirring time, X_2

Overall, the main effect of stirring time on film characteristics was found insignificant for weight, thickness and drug content of the formulation (Figure 6.5; Eqn. 6.2 - Eqn. 6.4). Instead, stirring time showed a significant direct proportionality with tensile strength (Eqn. 6.5) and an inverse quadratic proportionality with elongation (Eqn. 6.6), suggesting that increased stirring time can favour the formation of polymer bonds without impacting on casting solution concentration.

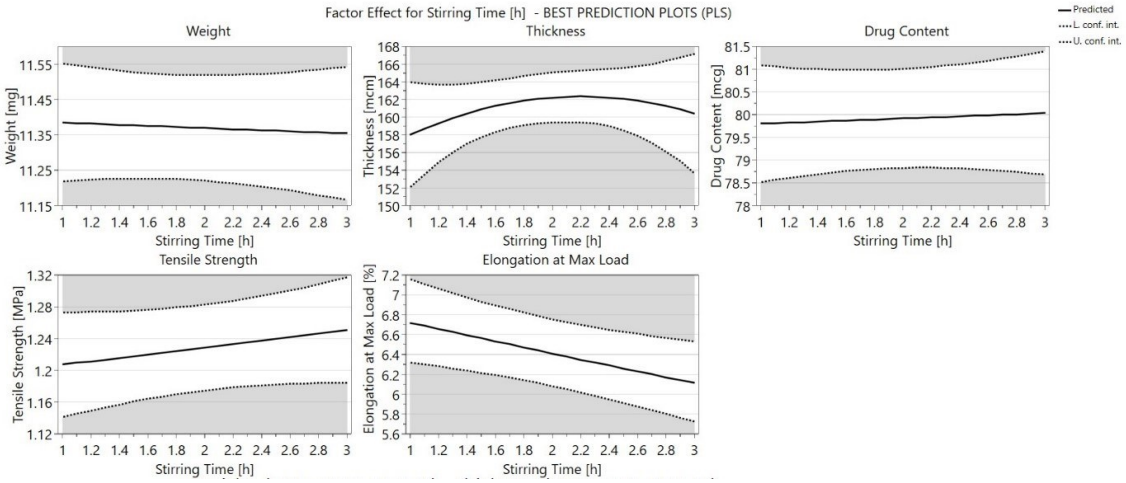

Figure 6.5 Main effect plots of stirring time on film characteristics.

6.3.2.1.3 Effect of stirring cooling time, X_3

An extended duration of stirring cooling time significantly increased the values for weight, thickness and drug content (Figure 6.6; Eqn. 6.2 - Eqn. 6.4). Upon cooling, the film-forming solution gradually transforms into a gel. Although favourable gelation conditions, in terms of temperature and time, can been identified for single polymer used, it was not possible to predict the conditions for the polymeric blend (Balaghi *et al.*, 2014; Erdem & Ak, 2021; Mad-Ali *et al.*, 2017). According to the results, it can be assumed that the increment of stirring (cooling) time determined a denser structure in the gel. This led to an increased viscosity of film forming solution, which can be responsible for reduced spreading rate of polymeric fraction on the glass plates – hence increased weight, thickness and drug content. The effect on tensile strength was not significant but for elongation, although a significant increase in elongation was observed with longer stirring cooling time, an interactive effect between this factor and stirring temperature was observed (Eqn. 6.6). It can be hypothesised that the effect of plasticiser can be more evident after sufficient time for it to penetrate the matrix, once the matrix has become more structured. However, it should be considered also that the prolonged exposure of the matrix to the shear forces of the stirring could have weakened the polymeric network, favouring molecular mobility (Baudonnet *et al.*, 2004; Vo *et al.*, 2020).

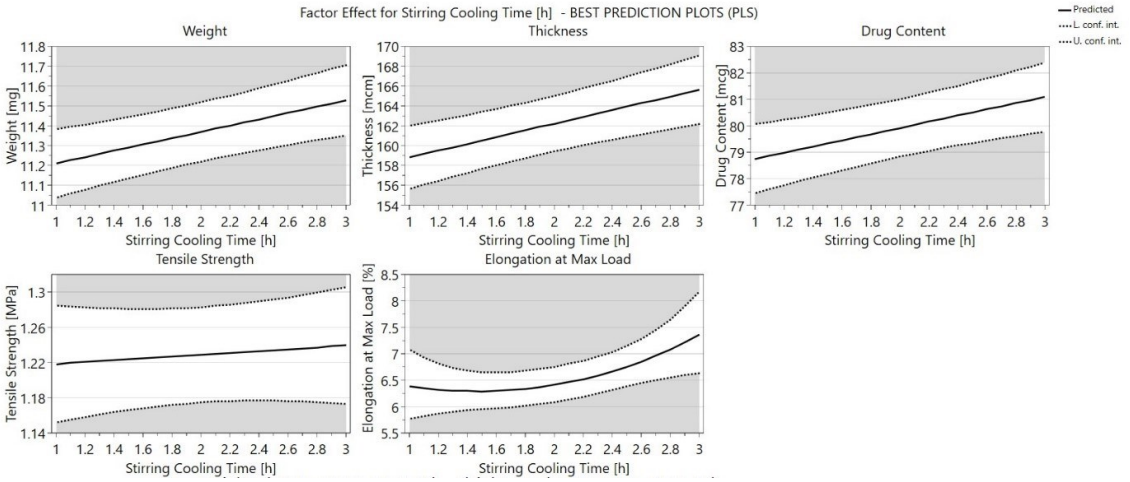

Figure 6.6 Main effect plots of stirring cooling time on film characteristics.

6.3.2.1.4 Effect of centrifugation speed (X_4) and centrifugation time (X_5)

Figure 6.7 depicts the main effects of centrifugation speed (a) and time (b) on the responses. Although centrifugation of the film forming solutions was included in the manufacturing procedure to minimise the quantity of air bubbles entrapped in the fluids, it appeared that this step could modify final film properties. In particular, the centrifuge speed of 600 rpm produced the lowest values of weight, thickness and drug content, the highest value of tensile strength. Centrifugal speed was significantly directly proportional to elongation values across all values (Eqn. 6.6). In terms of centrifugation time, processing the fluids for 3 minutes was associated with the highest values of weight, thickness, drug content, and tensile strength, while a negligible effect on elongation was recorded. Albeit, as no evident deposit could be observed upon visually inspection of the centrifugation tubes, it can be speculated that the speed and the time of spinning could have generated a differential centrifugation-like effect, altering the composition of the polymeric network in the fractions of polymers, plasticisers and water present. Nonetheless, further investigation will be needed to clarify the effect of this degassing procedure on the attributes of the inserts.

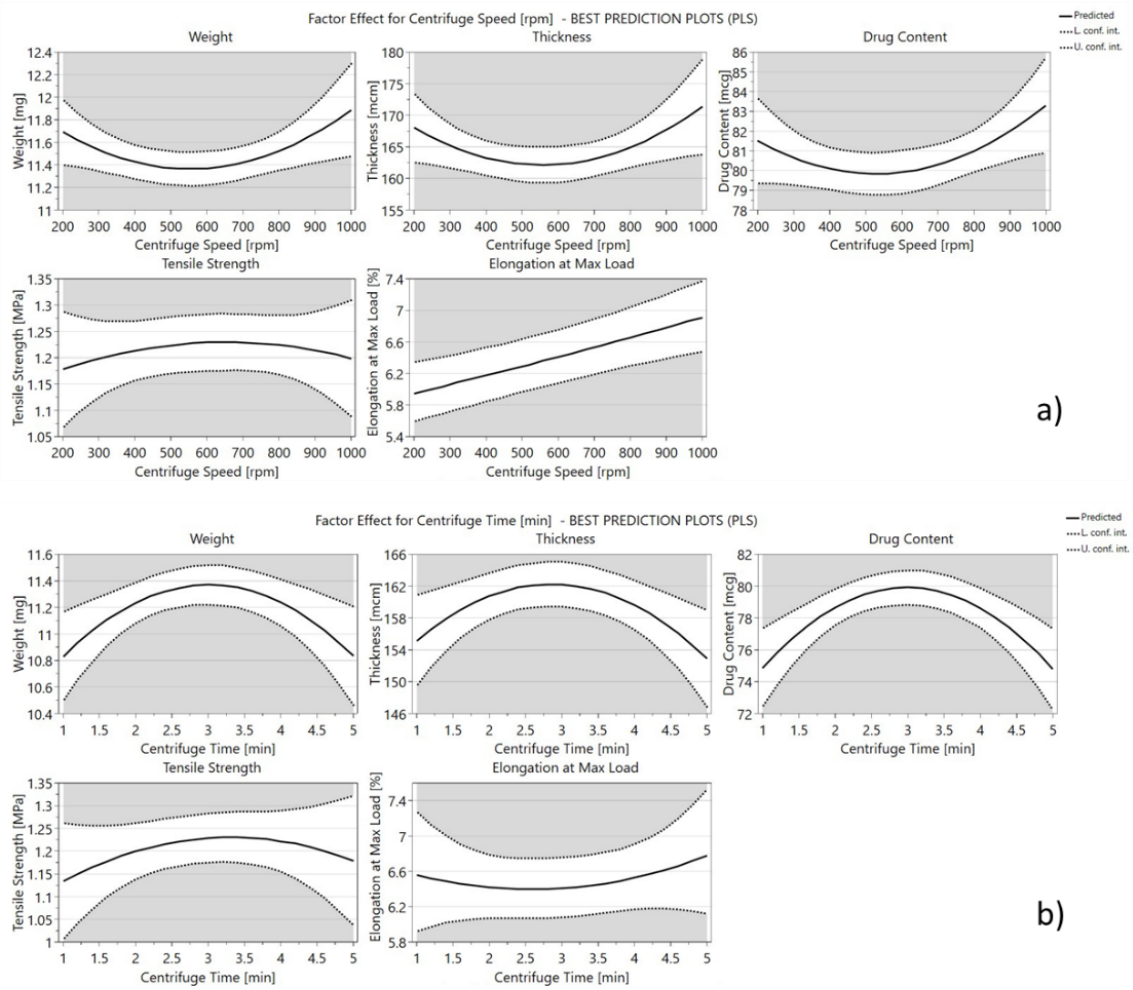

Figure 6.7 Main effect plots of centrifuge speed (a) and centrifuge time (b) on film characteristics.

6.3.2.1.5 Effect of film casting surface

Although the effect of glass plates was not included in the initial definition of the design space, the data analysis revealed that this factor could have induced an effect on the responses, hence altering the quality of the models derived from the experiments run. MODDE allowed for the inclusion of a further factor, therefore type of glass plate (designated as P1, P2, P3 and P4) was included as a qualitative factor in the analysis after completion of data collection. However, despite the design being modified by including this qualitative factor, the inclusion led not only to determination of valid models from the analysis, but to even ameliorate performance of the models.

The main effects of using different glass plates in film manufacture is graphically represented in Figure 6.8. As can be appreciated from the plots, the use of different plates altered the physical characteristics (weight, thickness and drug content) of the films/inserts. It can be inferred that an inconsistent spreading of the film forming solutions may have led to a variation in thickness of fluid layers casted, which can be linked directly with films thickness and, by assuming homogeneity of the solution, and indirectly with weight and drug content. On the contrary, mechanical properties of the films were not dependent on the glass plate used. Although the reason behind this variability requires further investigation, it appears evident that not all the glass plates can be interchangeably used, and this may be due to microstructural differences on the surfaces, retaining dissimilar volume fractions of film-forming solutions surface per surface area unit. Therefore, during formulation of thin film inserts, it is important that the same casting surface is used, to reduce variability in insert functionality.

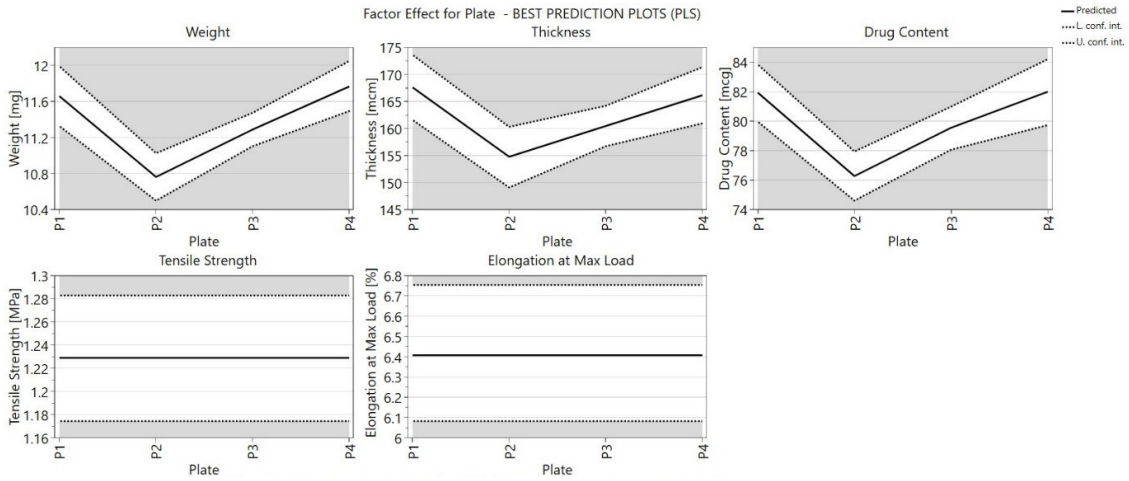

Figure 6.8 Main effect plots of glass plates on film characteristics.

6.3.2.2 Optimisation of film manufacturing process

The construction and evaluation of models can also give the opportunity to optimise the procedure according to the responses desired. In this case, the drug content of inserts was identified as the objective of the optimisation, as it was considered the most relevant/predictive element in terms of clinical efficacy of the drug delivery system. The MODDE optimiser was set to identify optimal factor specifications to maximise levofloxacin amount in the inserts, because loading a higher amount of drug in the insert can be crucial in maintaining the anti-infective agent concentration in tears above the minimum effective concentration (Subrizi *et al.*, 2019). The best setpoints, i.e. factor combinations, produced by the optimiser are reported in Table 6.7. These factor combinations were predicted to increase drug loading of inserts by a minimum of 10% above the average drug amount found across the experiment runs. Setpoint R was further refined after simulation to identify factors combination holding maximum distance from acceptance boundaries.

Table 6.7 Best factors combination from MODDE optimiser for drug content maximisation.

Code	*Stirring Temp.*	*Stirring Time*	*Stirring Cooling Time*	*Centrifuge Speed*	*Centrifuge Time*	*Plate*	*Drug Content Predicted*	*Probability of failure*
15	57.420	2.9999	2.9996	968.67	3.4146	P4	88.203	0.82%
7	58.617	2.9992	2.9983	997.72	3.2123	P1	88.712	0.87%
R	**58.666**	**3**	**3**	**1000**	**3.1333**	**P1**	**88.757**	**0.92%**

Finally, the setpoint R factors values were used to predict films/insert characteristics in utilising the different glass plates, in Table 6.8. It should be noticed that the use of plates P2 and P3 were not able to overcome the drug content threshold selected.

Table 6.8 Prediction of films/inserts characteristics for different glass plates in using most robust setpoint found (R).

Plate	*Weight*	*Thickness*	*Drug Content*	*Tensile Strength*	*Elongation at Max Load*
P1	12.5258	184.425	88.757	1.1591	7.0373
P2	11.6304	171.549	83.1188	1.1591	7.0373
P3	12.1586	177.225	86.3732	1.1591	7.0373
P4	12.6392	182.945	88.8169	1.1591	7.0373

6.4 Conclusion

In this chapter, design of experiment (DoE) was used to optimise the film manufacturing procedure. All the conditions comprising the design space led to the production of qualitative films. Median values collected during film characterisation across the runs allowed to delineate valid and significant models for all the responses. All the analysed factors demonstrated an effect on at least one of the responses, although the effect of degassing procedure on insert characteristics should be clarified. The implementation of an additional factor for the type of glass plate used permitted the construction of better models, even considering that no effects on mechanical properties were found. Drug content was selected as the cardinal element to refine the procedure. To maximise the drug quantity in the inserts, stirring temperature and time, stirring cooling time, and centrifugation speed should be set at or close to the maximum values of the ranges that were tested, while centrifugation time should be 3 minutes. Finally, it was found that selection of glass plates used could determine the amount of levofloxacin in the inserts. Nonetheless, although different strategies should be adopted if larger variations of the drug may be needed, it was found that the optimisation of the procedure offers the opportunity to tweak levofloxacin content.

Chapter 7 - Optimisation of levofloxacin content and effects on drug release, trans-corneal permeability and cytotoxicity of the inserts

7.1 Introduction

A great variety of active pharmaceutical agents (APIs) is currently available, and constantly evolving, for the treatment of the various infectious ocular disorders. With diverse mechanisms of action, these anti-infective agents can find preferential use in the treatment of specific diseases for their ability to inhibit the proliferation of the causative microorganisms. Thus, selection anti-infective drug should be based on efficacy against the pathogens and their safety for the ocular environment (Tabbara, 2014).

The efficacy of antibiotics against bacteria, including levofloxacin and the others in the groups of fluoroquinolones, relies on reaching a concentration of the drug in the target tissue capable of inhibiting pathogen proliferation. Thus, the concentration and the dose frequency of the antibiotic should be selected to attain and maintain the minimum inhibitory concentration (MIC90), depending on the causative agent being treated (Herbert *et al.*, 2022).

Levofloxacin, in the form of 0.5% ophthalmic solution, has found extensive and regular clinical use for its efficacy and safety in the treatment of bacterial infections, given its activity against numerous species of microorganisms, belonging to both Gram-positive and Gram-negative classification, with a preferential dose frequency of 3 or 4 administrations per day (Kanda *et al.*, 2012). Also, to reach deeper ocular tissues in useful amount, higher concentrations of the drug would be required to eliminate the infection (Bucci *et al.*, 2016; Puustjärvi *et al.*, 2006). Hence, the use of levofloxacin can be considered a valid option in the treatment of external ocular infections, along with preoperative prophylactic use (Keating, 2009).

The administration of levofloxacin at increased concentrations can enhance drug penetration into ocular tissues and improve antimicrobial efficacy of the formulations (Li *et al.*, 2020). However, although the use of incremental doses of levofloxacin has shown to increase the susceptibility of bacterial cells, on the other hand, it also reduced the viability of ocular cells (Kim *et al.*, 2007). In fact, the topical use of fluoroquinolones on the ocular surface at high concentration and frequency of administration has been associated with increased risk of corneal epithelial damage (Yang *et al.*, 2020).

The aim of the experiments reported in this chapter was to optimise the levofloxacin content of the inserts, primarily to overcome the limited antimicrobial efficacy demonstrated during its early development, with the objective to evaluate also the ability of the formulations to sustain drug corneal penetration and to exclude any increase potential cytotoxicity. Considering that variations of the drug content can alter the qualities of the films (Alrimawi *et al.*, 2021), the inserts were also characterised for their uniformity, physicochemical and mechanical properties.

7.2 Materials and Methods

7.2.1 Thin film inserts manufacturing

Inserts were manufactured in two distinct experimental groups. All the formulations were manufactured by solvent-casting method. The desired concentrations of levofloxacin in the solutions (HPLC grade, Sigma Aldrich, Gillingham, UK) were obtained by solubilising appropriate amounts of the drug in distilled water (Purite Select Ondeo distiller, London, UK), and sonicating the solutions for 10 minutes. For both the groups of films produced, 22.5 mL of the drug solution were used in combination with 1250 mg of HPMC (E15, JRS PHARMA, Rosenberg, Germany), 750 mg of alginic acid sodium salt (from brown algae, low viscosity, Sigma Aldrich), 250 mg of gelatin (from porcine skin, Type A, Sigma Aldrich), and 2.5 mL of polyethylene glycol (PEG 400, BioUltra, Sigma Aldrich).

The manufacturing procedures for the film-forming solutions were non-identical for the two experimental groups. The first set of inserts was manufactured after the procedure previously described in chapter 5. Briefly, levofloxacin solutions with concentration of 0.2%, 0.3% and 0.4% were prepared. PEG 400 was added to the drug solution and heated to 65˚C under slow stirring. The rest of the excipients were gradually added to the solution under vigorous stirring, after accurate blending of the powders. The film-forming solutions were stirred for 60 minutes at the same temperature, and then left to cool down to room temperature (approximately 20˚C). The casting solutions were cleared of air bubbles by a combination of hand removal and centrifugation at 1000 rpm for 5 minutes, after transfer into centrifuge tubes.

The second experimental set of inserts was produced following results from optimisation of the film manufacturing procedure via the DoE, reported in chapter 6. Drug solutions were prepared to concentrations of 0.2%, 0.5% and 1.0% of levofloxacin, and heated to 50˚C before the gradual addition of the powder mixtures. The solutions were stirred at 300 rpm for 3 hours at constant temperature and allowed to cool down to a temperature of 40˚C before the addition of the plasticizer. The gelation time was set to 3 hours, after which air bubbles were removed from the solutions by hand removal and centrifugation at 1000 rpm for 3 minutes.

For both groups, the film-forming solutions were casted on glass plates using an automated film applicator (Elcometer®, Manchester, UK) and left to airdry overnight. Finally, the samples of different sizes were collected by cutting the films with stainless steel cork borer. All the film-forming solutions were prepared in triplicate, generating three distinct films for each drug concentration tested in the different batches. Given that two distinct sets of films were prepared using a 0.2% levofloxacin solution belonging to separate experimental groups, and for the sake of clarity, those prepared with the first procedure described were named 0.2-a, while films produced after DoE findings were named 0.2-b.

7.2.1 Characterisation of the inserts

To determine any potential modifications of the insert properties derived from the variation of drug amount used in the manufacturing process, the characterisation of the formulations was conducted primarily following or adapting the testing methodologies adopted in the early development of the inserts (summarised below), while specific experimental protocols have been added for permeability and cytotoxicity studies on immortalised corneal epithelial cells.

7.2.1.1 Uniformity of inserts

The uniformity of mass and thickness was assessed on 24 samples (Ø = 10 mm) collected from each film (n=72 per formulation). The values of weight were measured with an analytical balance (Sartorius, Göttingen, Germany), while thickness was measured by digital micrometre gauge (Beslands, Jiaxing, China). The number of specimens to be tested was selected to meet the number required for the pharmacopeial standard uniformity of weight testing for single-dose solid dosage forms (WHO, 2019b).

7.2.1.2 Mechanical properties of inserts

Mechanical properties were evaluated by use of a Hounsfield Tensometer (Hounsfield Limited, Croydon, UK) on three specimens (Ø = 25 mm), assessing tensile strength, elongation at maximum force and Young's modulus. The testing parameters were: stress range 0.05 MPa, stress range 300%, and crosshead speed 60 mm/min and without preload (Gilhotra *et al.*, 2009).

The folding endurance of formulations was tested by repeatedly folding three inserts of same dimensions along the same plane, putting opposite edges in contact with two fingers, until visible cracks could be identified or the procedure completed 300 folds (Aher & Nair, 2014).

7.2.1.3 Physicochemical properties

The property of formulation to absorb water and the effect of the imbibition on inserts linear dimensions were assessed by evaluating the increment of weight and diameter of samples (Ø = 25 mm, n=3) pre and post soaking. The water absorption was induced at 37˚C by placing the specimens onto pre-soaked sponges, using filter paper (grade 1, Whatman®, Chalfont St. Giles, UK) as a support for the sample (Bertram & Bodmeier, 2006).

The residual moisture content in the dried films was assessed by comparing the weight samples before and after placing the specimens in a vacuum oven at 105˚C for 24 hours (Ahn *et al.*, 2014). The pH of insert surface was evaluated by pH indicator paper strips (pH range 1-14, Fisherbrand™, Loughborough, UK) on moistened samples (Ø = 10 mm, n=3) after colouration of the strip was found stable (Abdelkader *et al.*, 2014; Shah *et al.*, 2018). The coloration was compared against the reference colour scale using a graphics painting program, sampling from photos taken of the formulations.

7.2.2 Drug content variation, release profile and antimicrobial efficacy

7.2.2.1 Drug content uniformity

To test the uniformity of drug content in the manufactured films, 10 samples (Ø = 10 mm) per film (n=30 per formulation) were collected and dissolved individually in 5 millilitres of simulated tear fluid (STF) for 12 hours at 37˚C, with gentle shaking motion (British Pharmacopoeia Commission, 2023b). STF was freshly prepared with 6.8 g of sodium chloride (Sigma Aldrich), 2.2 g of sodium bicarbonate (Sigma Aldrich), 0.08 g of calcium chloride (Sigma Aldrich) and 1.4 g of potassium chloride (Sigma Aldrich) per litre of ultrapure water solution (Abdou & Kandil, 2017). Aliquots of the samples were filtered (hydrophilic nylon membrane, 0.45µm pore size, Fisherbrand™, Loughborough, UK) and measured by validated analytical HPLC method described earlier.

7.2.2.2 Dissolution testing

To determine the amount of drug released from inserts over time, the development of a donor-receiver dissolution testing apparatus was implemented, on the basis of the recurrent strong *in vitro-in vivo* correlation that has been reported for this methodology (Gorle & Gattani, 2009; Mishra & Gilhotra, 2008; Mundada & Shrikhande, 2008). The apparatus (Chapter 5, Figure 5.2) included an open-ended tube (donor compartment), presenting regenerated cellulose semi-permeable membrane (MEMBRA-CEL® MC18, Viskase, USA) attached on the side immersed in a beaker (receiver compartment). Before use, the membrane was thoroughly washed, rinsed and soaked overnight in STF. The receiver compartment was pre-filled with 25 mL of STF heated to 37˚C under gentle stirring (60 rpm) to simulate the blinking reflex (Thakur *et al.*, 2014). The donor compartment, on the other hand, was pre-filled with 0.5 mL of STF at 37˚C before insertion of the insert. For the formulations produced in the first experimental set of films (including 0.2%, 0.3% and 0.4% levofloxacin solutions) the testing was conducted for 6 hours, while for the second set (including 0.2%, 0.5% and 1.0% drug solutions) the testing time was extended to 24 hours. At each time point tested, 1 mL aliquots were collected and replaced with equal volume of warm STF. The samples were filtered (0.45µm pore size, Fisherbrand™) and measured by validated HPLC method. The cumulative drug release was calculated, and the percentage of release was determined against the amount of drug identified during drug content testing.

7.2.2.3 Antimicrobial efficacy

The use of the donor-receiver model described above allowed for estimation of the antimicrobial effectiveness of the formulations prepared. Testing the residual amount of levofloxacin present in the inserts can represent the effect of the formulations on bacterial growth when administered in the conjunctival sac. Thus, two aliquots of 20 µL were collected from the donor compartments and replaced with 40 µL of warm STF at each of the time points for dissolution testing. The aliquots were placed into two separate 96-well microplates (flat bottom, Corning®, Somerville, USA). Upon completion of the dissolution testing and sterilization by UV exposure for 15 minutes (Krishnamurthi *et al.*, 2021), the wells were filled, under aseptic conditions, with 180 µL of bacterial suspension, previously diluted to an optical density of OD600 = 0.1, assessed by scanning spectrophotometer (Jenway®, St Neots, UK). One plate was used to test Staphylococcus aureus, and the other for Pseudomonas aeruginosa. Both bacterial species were cultured in sterile Brain Heart Infusion Broth (NutriSelect® Plus, Merck, Darmstadt, Germany), prepared as indicated by the manufacturer, to promote similar growth of the bacteria (Wijesinghe *et al.*, 2019). For the control, 20 µL of sterile phosphate buffered saline (pH 7.4, from tablets, Sigma Aldrich) was used. The plates containing the aliquots from inserts and bacterial suspensions were tested with a microplate spectrophotometer (Multiskan® GO, Thermo Scientific™, Waltham, USA). The testing was run for 24 hours, with the plates kept at 37˚C under continuous shaking. The growth curve of Staphylococcus aureus and Pseudomonas aeruginosa were determined by the OD600 readings, recorded every 30 minutes, and compared against the pure bacterial cultures (positive control) to determine the efficacy of levofloxacin fractions released over time (Smith *et al.*, 2003).

7.2.3 Corneal permeability and cytotoxicity

In vitro models based on corneal tissues can find effective application in studying drug permeability and safety of ophthalmic drug delivery devices (Kaluzhny *et al.*, 2018). Among those, the use of epithelial corneal immortalised cell line is reputed as a reliable alternative to *in vivo* models, particularly convenient in formulation development. The epithelial cell-based model mimics the corneal epithelial barrier and it has been employed to evaluate toxicity and penetration of pharmaceutical substances (Agarwal & Rupenthal, 2016).

Human corneal epithelial cells (HCE-2, 50.B1, CRL-11135, American Type Culture Collection, Manassas, USA) were grown at 37°C in humidified air with 5% CO2, in suitable standard culture medium. The standard medium comprised keratinocyte serum free medium kit, including L-glutamine 5 ng/mL human recombinant epidermal growth factor, and 0.05 mg/mL bovine pituitary extract (Gibco™, Thermo Scientific™, Waltham, USA), which was supplemented with 500 ng/mL hydrocortisone (BioReagent, Sigma Aldrich), 100 IU penicillin-streptomycin (BioReagent, Sigma Aldrich), 10 µg gentamycin (BioReagent, Sigma Aldrich), amphotericin B (BioReagent, Sigma Aldrich), and 5 ng/mL insulin (from bovine pancreas, BioReagent, Sigma Aldrich). The culture medium was replaced twice per week, according to manufacturer instruction, and the cells were cultured on surfaces previously coated with 100 µL/cm2 collagen solution (type I from rat tail, BioReagent, Sigma Aldrich) (Mencucci *et al.*, 2022; Toropainen *et al.*, 2001).

7.2.3.1 Corneal epithelial cytotoxicity

The *in vitro* cytotoxicity assay was performed on HCE-2 cells by MTT (Thiazolyl Blue Tetrazolium Bromide, BioReagent, Sigma Aldrich) assay. The testing method relies on the chemical transformation of MTT in living cells, determining the rate of mitochondrial activity within the cell population, and, thus, the number of viable cells (van Meerloo *et al.*, 2011). Cell culture was resuspended, seeded into 24-well plates (100,000 cells/ cm2 density), and incubated at the growing conditions previously described. After overnight growth and 70-80% confluence, the cells were incubated with the inserts and control drug solutions for 24 hours, based on the duration of permeability experiments and the expected residence time of the inserts on the eye. For insert formulations, specimens tested were collected from the films made with 0.2%, 0.5% and 1.0% levofloxacin film forming solutions (n = 6 for each formulation). A serial dilution of levofloxacin in the standard media was prepared to determine the effect of the neat drug on cell viability, with concentrations ranging from 1.25% to 0.078%. The drug samples (n = 12) concentration range was based on results of actual drug content found in the inserts. Untreated wells containing the standard media, were used as control. To avoid any potential interference of cells viability caused by the physical contact between the cells and the inserts to be tested, a novel permeability study setup was developed. Hence, to create a spatial separation between the cells and the insert, a silicon ring (9mm internal diameter, 2 mm width) was carefully laid on the cells, and a round corner square piece of stainless-steel mesh (40-mesh, USP Apparatus 1 standards (Sirasitthichoke *et al.*, 2021)) was placed on top of the ring after adequate sterilisation by soaking in absolute ethanol and UV light exposure overnight. To obviate any potential differences in cell viability due to the ring and mesh, the same set up was employed for the cytotoxicity tests using the drug solution. After 24 hours incubation, the plates were carefully emptied and cell layers washed with HBSS (Hanks Balanced Salt Solution, without Calcium Magnesium and Phenol Red, Corning®, Somerville, USA). The cells were further incubated with MTT in standard medium (to final concentration of 1mg/mL) for 2 hours. Upon removal of MTT solution, cells were lysed by exposure to dimethyl sulfoxide (DMSO, Thermo Scientific™, Waltham, USA) for 30 minutes at 37°C under shaking. The absorbance of MTT was determined at 590nm wavelength by microplate reader (Spark®, Tecan, Männedorf, Switzerland). The viability of cells after exposure to levofloxacin solutions or drug-loaded inserts was expressed as a percentage of the value recorded for control cells cultured in the standard medium (Elhabal *et al.*, 2023; Mencucci *et al.*, 2020; Mencucci *et al.*, 2022).

7.2.3.2 Transepithelial corneal permeability

For permeability studies, HCE-2 cells suspensions with a density of 100,000 cells/ cm2 were seeded on previously collagen-coated polyester membranes (0.4µm pore size, 1.12 growth area cm2, Transwell®, Corning®, Somerville, USA) and grown in incubator for 28 days as per the protocol described above, with 1.5 mL of the standard medium in the basolateral well and 0.5 mL in the apical chamber replaced twice per week (Toropainen *et al.*, 2003). The study was designed to determine the permeation of levofloxacin from the apical chamber, acting as the donor, to the basolateral chamber, acting as the receiver, through the corneal epithelial cell membrane. The inserts were placed in the apical chamber containing the cells, thus, as for the permeability study, the setup of the experiment was implemented using silicon rings and the stainless-steel mesh on the cell layer grown on the apical chamber membrane, as displayed in figure 7.1.

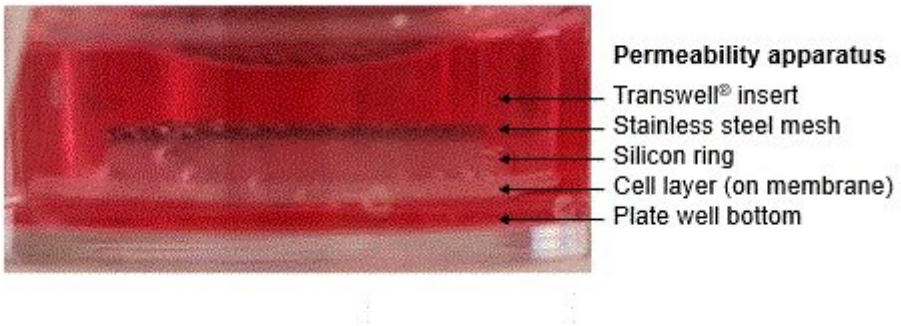

Figure 7.1 A novel adapted transepithelial permeability apparatus developed for testing thin film inserts.

The apical chamber was prefilled with 0.5 mL of standard media to guarantee that the whole setup was immersed, while 1.5 mL were placed in the basolateral chamber. The film inserts were carefully placed on top of the mesh, ensuring complete covering of the media, at the start of the study. At each time point, specifically after 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours, the apical chambers containing the inserts were transferred into a new basolateral chamber, prefilled with fresh media at 37°C, and the plates were placed in incubator during the intervening times. The experiment was conducted at optimal cell growth conditions to minimise any cells loss and consequent alteration of the membrane permeability. To determine the quantity of levofloxacin permeated, the content of basolateral chambers was collected, filtered (0.45µm pore size, Fisherbrand™), and measured by HPLC. The cumulative amount of drug permeated was computed, and percentage of permeated drug was derived from the drug content previously ascertained, with all formulations tested in triplicate

7.2.4 Statistical analysis

The statistical analysis was conducted using SPSS (V 26, IBM, New York, USA). As the data showed normal distribution (Kolmogorov–Smirnov $p<0.05$), parametric comparisons (one-way ANOVA) and correlations (Pearson's correlation coefficient) were performed. Tukey's wholly significant difference (WSD) post hoc test was used. The statistical significance was taken for p-values lower than 0.05.

7.3 Results and Discussion

7.3.1 Characterisation of inserts

Levofloxacin-loaded thin film ocular inserts were successfully manufactured. Regardless of the initial drug concentration and of the manufacturing procedure adopted, all the tested formulations resulted in qualitative films, which were easy to peel and to manipulate. The films appeared smooth and translucent, yet crystal-like inclusions were noticed randomly disseminated across the surface of the films. Whereas films manufactured in the first experimental set (containing 0.2%, 0.3% and 0.4% drug solutions) did not exhibit a coherent variation in the number of inclusions, it appeared that the films produced in the second group presented an increased number of inclusions with higher drug concentrations used. Although the relationship between the appearance of the films and the quantity of drug used should be clarified, it can be assumed that the presence of inclusion was related to levofloxacin concentration, as it can be present in a crystalline anhydrous form (Gaspar *et al.*, 2015; Gorman *et al.*, 2012). It has been suggested that increasing drug concentration in the formulation can induce higher amounts of drug crystals embedded in the films, possibly because the drug molecules would exceed the available bonding sites offered by the long-chain polymers in the formulation (Chan *et al.*, 2019). An additional factor in support of this hypothesis is the fact that levofloxacin is sparingly soluble in water, and it comprised floating particulates during solubilisation at higher concentrations (Buntrock, 2013).

In analysing the singular films tested from the same formulation, uniformity of mass reached acceptable levels of homogeneity across all inserts tested (Table 7.1), as their weight values were characterised by reduced variability, with none of the samples exceeding the pharmacopeial threshold of 10% difference from the mean value (British Pharmacopoeia Commission, 2023b). However, films manufactured with 0.3% levofloxacin solution showed the highest coefficient of variance when tested (6.5%, n=72). For this formulation, in fact, the average weight of one of the films (n=24) was also found significantly different from the others ($p < 0.05$), suggesting that the variability was probably not related to the drug quantity used. This discrepancy in weight, instead, could be attributed to a certain degree of inconsistency in the manufacturing procedure, as films containing higher concentrations of levofloxacin (0.5%, 1%) showed minimal coefficient of variance.

Surprisingly though, the variability of thickness was higher and more frequent, compared to the findings on weight. Particularly interesting, in this regard, was the comparison between formulations 0.2-a and 0.2-b. Despite being produced using non-identical procedures, but with the same amount of drug, films from both formulations exhibited comparable values of weight. Nonetheless, the thickness average values and their variability were found different. One possible explanation may be derived from the random presence of inclusions in the films, as previously discussed. Focal protrusion of drug crystals on the film surface could partially explain the inhomogeneity of thickness isolated from alteration of the weight distribution (Boateng *et al.*, 2013). Nonetheless, it remains to be clarified the contribution of the manufacturing procedure to this occurrence, as it was found more prevalent in the second set of films manufactured. Thickness measurements of single films (n=24) for all formulations were found to be within the pharmacopoeia specifications.

Table 7.1 Physical characteristics of the inserts, n=24; (rows in bold, n=72); [shows no. of films outside 10% range]

			Weight		*Thickness*	
	Drug solution (Conc.)	**Film code**	**Mean and SD (mg)**	**RSD (%) [Out of 10% range[1]]**	**Mean and SD (µm)**	**RSD (%) [Out of 10% range]**
Experimental Group 1	0.2-a	1	11.3 ± 0.2	1.8% [0]	151 ± 3	1.8% [0]
	0.2-a	2	11.3 ± 0.1	0.9% [0]	150 ± 2	1.1% [0]
	0.2-a	3	11.2 ± 0.1	0.8% [0]	146 ± 3	1.2% [0]
	0.2-a	**Tot.**	**11.3 ± 0.2**	**1.4% [0]**	**149 ± 3**	**1.9% [0]**
	0.3	1	11.2 ± 0.1	1.2% [0]	148 ± 4	2.7% [1]
	0.3	2	9.8 ± 0.1	1.0% [0]	128 ± 1	0.9% [0]
	0.3	3	9.8 ± 0.2	2.3% [0]	128 ± 3	2.2% [0]
	0.3	**Tot.**	**10.3 ± 0.7**	**6.5% [1]**	**135 ± 10**	**7.3% [6]**
	0.4	1	10.8 ± 0.1	1.1% [0]	146 ± 9	5.9% [0]
	0.4	2	10.9 ± 0.1	0.7% [0]	141 ± 1	0.7% [0]
	0.4	3	11.0 ± 0.1	0.8% [0]	141 ± 1	1.1% [0]
	0.4	**Tot.**	**10.9 ± 0.1**	**1.2% [0]**	**143 ± 5**	**3.8% [3]**
Experimental Group 2	0.2-b	1	11.5 ± 0.2	1.7% [0]	172 ± 13	7.3% [3]
	0.2-b	2	11.4 ± 0.3	2.4% [0]	157 ± 11	6.8% [1]
	0.2-b	3	11.1 ± 0.2	2.0% [0]	158 ± 6	3.6% [0]
	0.2-b	**Tot.**	**11.4 ± 0.3**	**2.5% [0]**	**163 ± 12**	**8.8% [5]**
	0.5	1	11.4 ± 0.2	1.9% [0]	163 ± 8	5.2% [2]
	0.5	2	11.1 ± 0.2	1.6% [0]	158 ± 11	6.9% [1]
	0.5	3	10.9 ± 0.1	1.3% [0]	160 ± 12	7.4% [2]
	0.5	**Tot.**	**11.1 ± 0.3**	**2.4% [0]**	**160 ± 11**	**6.6% [5]**
	1.0	1	11.4 ± 0.2	1.7% [0]	171 ± 7	4.0% [0]
	1.0	2	11.5 ± 0.2	1.8% [0]	170 ± 11	6.2% [2]
	1.0	3	11.1 ± 0.2	1.7% [0]	160 ± 10	5.9% [2]
	1.0	**Tot.**	**11.4 ± 0.3**	**2.2% [0]**	**169 ± 10**	**5.7% [4]**

The manufacturing protocol appeared to be a more determinant factor in the modification of mechanical properties of the inserts compared to the concentration of drug in the film forming solution. It has been reported that, in the presence of specific film-forming agents and plasticisers, the inclusion of drug can produce an 'antiplasticisation effect' on the final film formulations, that can be amplified with increasing drug load (Alrimawi *et al.*, 2021). This effect is proposed to originate from intermolecular hydrogen bonding between drug and film-forming polymer(s), which can compete with plasticisers to create bonds at the same site of action, thus leading to higher tensile strength and lower elongation of the film (Lin *et al.*, 1995). However, in evaluating the mechanical properties of the films manufactured, it was found that the tensile strength was constant within and across the formulations present in the two manufacturing groups, hence not demonstrating the 'antiplasticisation effect' with the increment of levofloxacin used (Table 7.2). Instead, a noticeable difference was found in the elongation and the derived Young's modulus, with a minor, yet significant, difference in the tensile strength values between the two production methods. Hence, it can be hypothesised that the combination of excipients and plasticiser used in the formulations prevented the mechanical properties of the film from being altered by the addition of levofloxacin, within the range of concentrations tested. Also, the consistency of finding for foldability testing (Table 7.2) can support the mechanical solidity of the inserts to the potential drug-polymers interaction.

Table 7.2 Mechanical properties of the inserts.

	Drug solution (Conc.)	*Film code*	*Tensile Strength (MPa)*	*Elongation at Max (%)*	*Young's modulus (MPa)*	*Foldability*
Experimental Group 1	0.2-a	1	2.3 ± 0.3	12.7 ± 2.4	0.18 ± 0.03	> 300
	0.2-a	2	2.8 ± 0.1	14.0 ± 1.1	0.20 ± 0.01	> 300
	0.2-a	3	2.5 ± 0.3	10.8 ± 1.9	0.24 ± 0.01	> 300
	0.2-a	**Tot.**	**2.5 ± 0.3**	**12.5 ± 2.2**	**0.21 ± 0.03**	
	0.3	1	2.7 ± 0.2	13.4 ± 1.0	0.20 ± 0.02	> 300
	0.3	2	2.3 ± 0.1	12.2 ± 3.2	0.20 ± 0.05	> 300
	0.3	3	2.9 ± 0.3	10.7 ± 1.5	0.28 ± 0.06	> 300
	0.3	**Tot.**	**2.6 ± 0.3**	**12.1 ± 2.2**	**0.23 ± 0.06**	
	0.4	1	2.5 ± 0.2	12.2 ± 2.5	0.21 ± 0.03	> 300
	0.4	2	2.5 ± 0.3	11.5 ± 1.0	0.22 ± 0.04	> 300
	0.4	3	2.5 ± 0.1	12.2 ± 1.1	0.21 ± 0.02	> 300
	0.4	**Tot.**	**2.5 ± 0.2**	**12.0 ± 1.5**	**0.21 ± 0.03**	
Experimental Group 2	0.2-b	1	2.0 ± 0.1	24.0 ± 0.4	0.08 ± 0.01	> 300
	0.2-b	2	2.1 ± 0.1	26.4 ± 1.1	0.08 ± 0.01	> 300
	0.2-b	3	2.0 ± 0.1	27.1 ± 1.4	0.07 ± 0.01	> 300
	0.2-b	**Tot.**	**2.0 ± 0.1**	**25.8 ± 1.7**	**0.08 ± 0.01**	
	0.5	1	2.2 ± 0.1	25.5 ± 0.5	0.09 ± 0.01	> 300
	0.5	2	1.9 ± 0.1	25.3 ± 3.3	0.08 ± 0.01	> 300
	0.5	3	2.0 ± 0.1	25.7 ± 1.6	0.08 ± 0.01	> 300
	0.5	**Tot.**	**2.0 ± 0.2**	**25.5 ± 1.9**	**0.08 ± 0.03**	
	1.0	1	1.9 ± 0.2	20.9 ± 1.3	0.08 ± 0.01	> 300
	1.0	2	2.1 ± 0.1	22.0 ± 3.6	0.10 ± 0.02	> 300
	1.0	3	2.1 ± 0.1	22.6 ± 1.1	0.09 ± 0.01	> 300
	1.0	**Tot.**	**2.0 ± 0.1**	**21.8 ± 2.2**	**0.09 ± 0.01**	

The addition of drugs to polymeric film-forming blends and the variation of their concentrations has been related to a modification of the final film behaviour in aqueous environments. It has been reported that the presence of drug in films can reduce the ability of the inserts containing hydrophilic polymers to absorb water and swell (Boateng *et al.*, 2013). Although a similar trend was found when testing polysaccharide-based formulations, Alrimawi *et al.* (2021) additionally reported that increasing the quantity of drug resulted in an unexpected and isolated increment in water absorption rate for one of the formulations tested. As with the mechanical and physicochemical characterisation of the inserts, Table 7.3 revealed that some differences in the film attributes could be ascribed to the experimental group. The water uptake values were found higher in the second set, but it was not possible to delineate any trend related to the drug concentration of the formulations. The residual water present in the inserts, instead, appeared to decrease with increasing drug content. Although the findings were in line with a proposed trend, the formulations did not show the associated increment in water absorption that has been proposed by the same authors (Chan *et al.*, 2019).

Table 7.3 Physicochemical properties of the inserts.

	Drug solution (Conc.)	*Loss on Drying (%)*	*Water Uptake (%)*	*Swelling Index (%)*	*Surface pH*
Exp. 1	0.2-a	4.9 ± 0.4	80.6 ± 2.2	7.1 ± 3.2	6-7
	0.3	3.7 ± 0.6	85.5 ± 24.3	7.1 ± 3.2	6-7
	0.4	3.5 ± 0.1	86.1 ± 7.3	8.9 ± 3.3	6-7
Exp. 2	0.2-b	4.8 ± 0.2	121.1 ± 12.7	8.0 ± 3.5	6-7
	0.5	4.0 ± 0.3	107.4 ± 3.9	6.2 ± 3.3	6-7
	1.0	3.2 ± 0.4	107.9 ± 2.5	6.2 ± 3.5	6-7

7.3.2 Drug content variation and release profiles

The determination of drug content and the verification of drug distribution is an essential procedure in the development of drug delivery systems (Subrizi et al., 2019). Nonetheless, it has been claimed that the verification of those parameters has been too often overlooked in production of film formulations (Perumal et al., 2008). The relevance of assessing drug quantity and distribution can be primarily associated with the quality of formulation (Vranić & Uzunović, 2008). Yet, the significance of those attributes can assume increased importance during the refinement of drug content optimisation, particularly if the manufacturing procedure has not been designed to target a specific quantity of the API in the final product. Hence, the drug content and the homogeneity of drug distribution was ascertained for all the films manufactured (Bánfai et al., 2007), to determine the variation of levofloxacin content in the inserts arising from the selection of different initial drug loading concentrations, and to evaluate the possible modifications to content uniformity of the formulations due to the selection of different quantities of drug.

In Table 7.4 are reported the average values of drug content for each of the films manufactured, as well as the indexes of homogeneity of levofloxacin distribution, calculated after the analysis of 10 samples. In addition, the value of content and uniformity were calculated for all the formulations, treating all samples a formulation (3 films each, n=30) as one batch. Whether the films were tested singularly or as constituting a unitary formulation, the distribution of the drug was found uniform and consistent across the samples analysed, suggesting that all the formulations, generated with the two manufacturing procedures, resulted in consistent and repeatable production of anti-infective inserts. All the individually tested films and the formulations showed reduced variability, with maximum relative standard deviations of 2.2% and 6.1%, respectively, and to comply with pharmacopeial guidelines of content uniformity, with no samples exceeding the 15% variation range from the average content values.

Thus, it can be assumed that varying the initial drug loading content did not produce a deterioration of the content uniformity of the inserts, while the manufacturing repeatability of the single films can impact this characteristic of the formulations. In fact, it can be appreciated that for inserts produced with 0.3% levofloxacin solutions, again the values of variability of the single films were found lower than the combined value, derived from the formulation when considered as a whole, as seen in the variability of weight results. Thus, as expected, the drug content variability of the formulation was most possibly related to the differences in the physical dimensions of the inserts, as also suggested by the very strong positive correlation found between the weight and the drug content of the inserts (Pearson's $r = 0.996$, $p < 0.001$).

Table 7.4 Drug content of the inserts.

	Formulation		Film 1 (n = 10)	Film 2 (n = 10)	Film 3 (n = 10)	Total (n = 30)
Experimental Group 1	**0.2-a**	Average (µg)	86.6	87.6	87.9	*87.3*
		SD (µg)	0.7	0.5	1.1	*0.9*
		RSD	0.8%	0.6%	1.2%	*1.1%*
		Out 15% range[2]	0	0	0	*0*
	0.3	Average (µg)	133.8	117.8	118.8	*123.5*
		SD (µg)	0.9	0.4	1.3	*7.5*
		RSD	0.7%	0.3%	1.1%	*6.1%*
		Out 15% range[2]	0	0	0	*0*
	0.4	Average (µg)	172.3	172.8	176.6	*173.9*
		SD (µg)	0.9	1.8	0.8	*2.3*
		RSD	0.5%	1.0%	0.5%	*1.3%*
		Out 15% range[2]	0	0	0	*0*
Experimental Group 2	**0.2-b**	Average (µg)	99.7	97.1	95.8	*97.5*
		SD (µg)	1.2	1.2	1.5	*2.0*
		RSD	1.2%	1.2%	1.5%	*2.1%*
		Out 15% range[2]	0	0	0	*0*
	0.5	Average (µg)	237.2	232.5	228.5	*232.8*
		SD (µg)	2.7	2.0	2.8	*4.4*
		RSD	1.1%	0.8%	1.2%	*1.9%*
		Out 15% range[2]	0	0	0	*0*
	1.0	Average (µg)	454.6	453.9	445.0	*451.2*
		SD (µg)	5.1	10.0	6.3	*8.4*
		RSD	1.1%	2.2%	1.4%	*1.9%*
		Out 15% range[2]	0	0	0	*0*

The assessment of levofloxacin quantity present in the final inserts revealed excellent correlation with the initial concentration of drug used in the production of the film-forming solution (Pearson's $r = 0.998$, $p < 0.001$). This finding may be suggestive of a negligible alteration of drug loading ability of the formulations within the range tested, implying that the inserts can easily accommodate up to 1% concentration of levofloxacin.

In addition, this can be interpreted as an indicator of robustness of the solvent-casting procedure adopted with the polymeric blend selected, which was able to comply with the variation of drug quantity used and to minor modifications of manufacturing procedure.

The adoption of DoE-based modification to the insert manufacturing, as expected, increased the final drug content of the inserts, as proved by the different quantities recovered from formulation 0.2-a (87.3 ± 0.9) and 0.2-b (97.5 ± 2.0). On the contrary, the use of two different methods did not alter the overall ability to modulate the levofloxacin content of the inserts arising after selection of initial drug concentration. The analysis, in fact, demonstrated the interesting possibility to predict the drug loading of the final inserts, according to the model reported in Figure 7.2, as suggested by the high value of the coefficient of determination and the small intercept found (Chicco *et al.*, 2021). Additionally, the final drug loading of the inserts can be easily adjusted to the desired amounts by opportune selection of the levofloxacin loading concentration used in the production phase, despite reports that achieving desired drug content levels during the production of thin films is challenging (Boateng *et al.*, 2010). Furthermore, it should be considered that, in conjunction with the release profile, the content can determine the level and the duration of insert efficacy of the drug at the target site (Subrizi *et al.*, 2019).

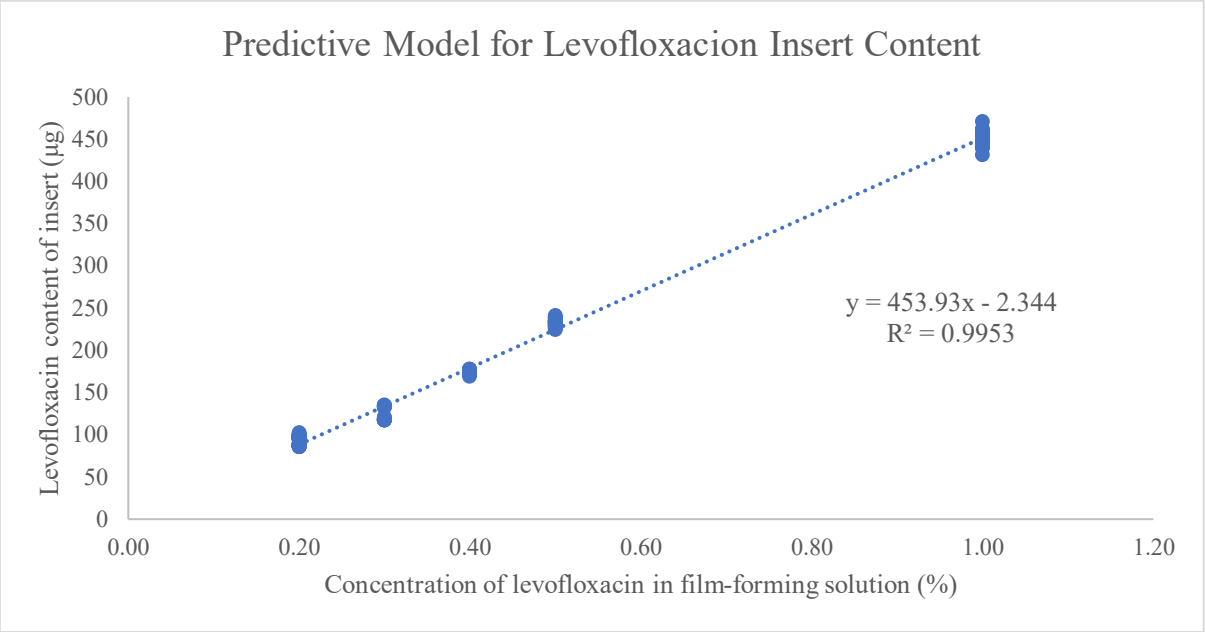

Figure 7.2 Scatter plot with linear regression curve between levofloxacin inserts content and levofloxacin concentration in the film forming solutions

The evaluation of drug release profiles of the formulations was conducted separately for the experimental groups. The samples of first set were tested for up to 6 hours, while the testing of second group was extended to 24 hours. In figure 7.3 and figure 7.4 are reported the drug release profiles of the inserts manufactured with 0.2%, 0.3% and 0.4% levofloxacin loading concentrations, in terms of cumulative amounts and its percentage calculated against the average content of the film found during drug content testing.

In considering the percentage of cumulative drug released by the inserts, it can be noticed that the dissolution profiles of the formulations overlapped, showing similar trend to the results found in the first development of the levofloxacin loaded inserts (Chapter 5, Figure 5.2). Hence, it can be assumed that the variation of drug content in the inserts did not alter the release mechanisms of the formulations. These findings may confirm that the release mechanism of the inserts was characterised by an initial burst release, possibly due to the relaxation of the polymeric matrix upon absorption of the media and favouring the diffusion of drug from the insert surface, followed by the gelation of the formulation which sustained the release of the remaining drug by erosive mechanism (Bruschi, 2015; Kesavan *et al.*, 2010; Liu *et al.*, 2010).

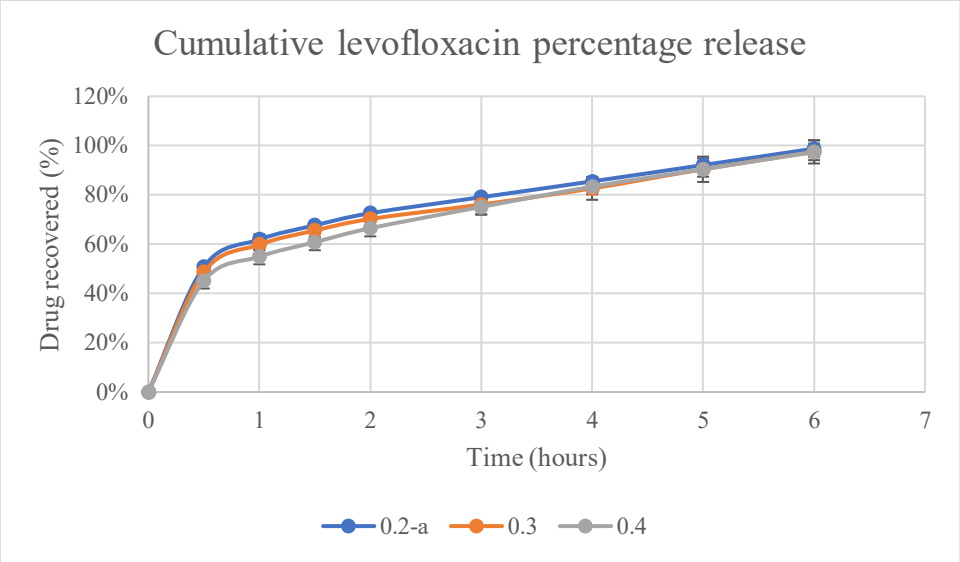

Figure 7.3 Cumulative percentage of levofloxacin released (Group 1)

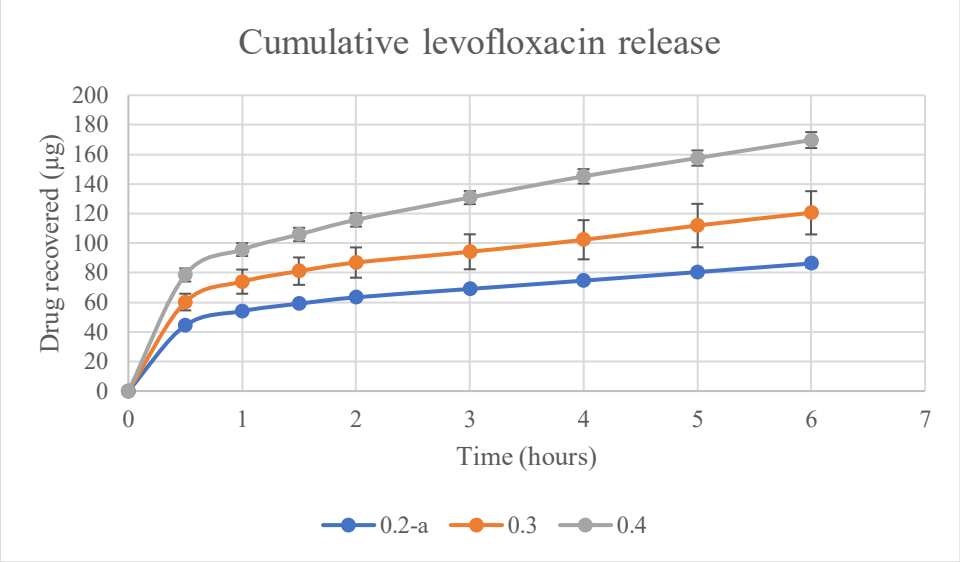

Figure 7.4 Cumulative amount of levofloxacin released (Group 1)

The patterns of cumulative amount of drug released, on the other hand, were modified by the initial levofloxacin quantity. Assuming that the release followed a biphasic modality, with an initial burst followed by an (almost) linear profile, it can be seen that the initial concentration of levofloxacin determined both the amount released in the first 30 minutes and the slope of the following release profile curve. Hence, it could be presumed that the drug fractions released hourly after the initial burst were proportional to the initial quantity of drug, and they could be used to tailor the release profile of the drug (Lin *et al.*, 1995).

To further investigate the possibility to adapt the release profile, an additional set of films was analysed, including 0.2%, 0.5% and 1.0% levofloxacin concentration, which were tested for a more extended period. As reported in Figure 7.5, inserts showed to follow similar profiles in respect of percentage release. In addition, they confirmed the possibility to modulate the amount of drug diffused in the first minutes and the fractions of levofloxacin released in the remaining testing time (Figure 7.6).

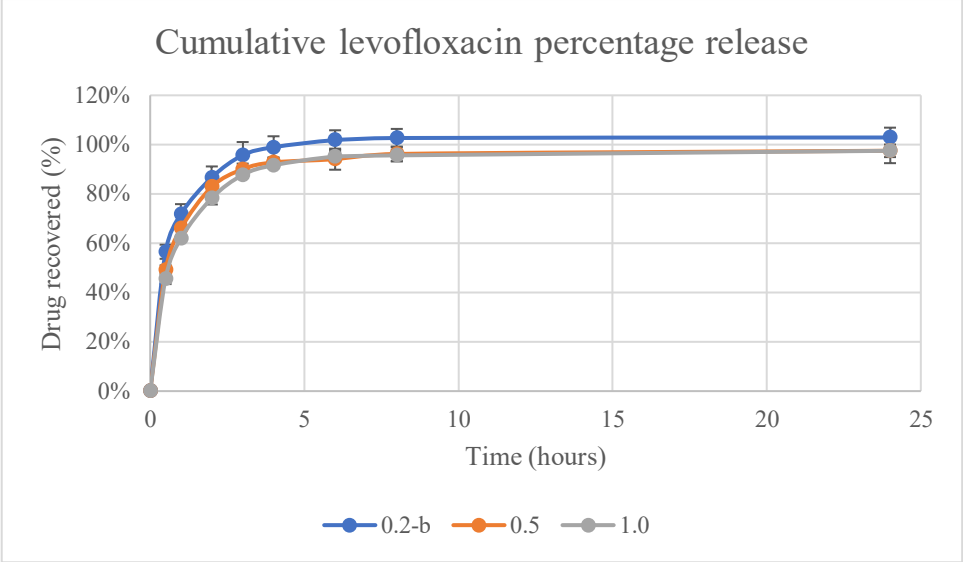

Figure 7.5 Cumulative percentage of levofloxacin released (Group 2)

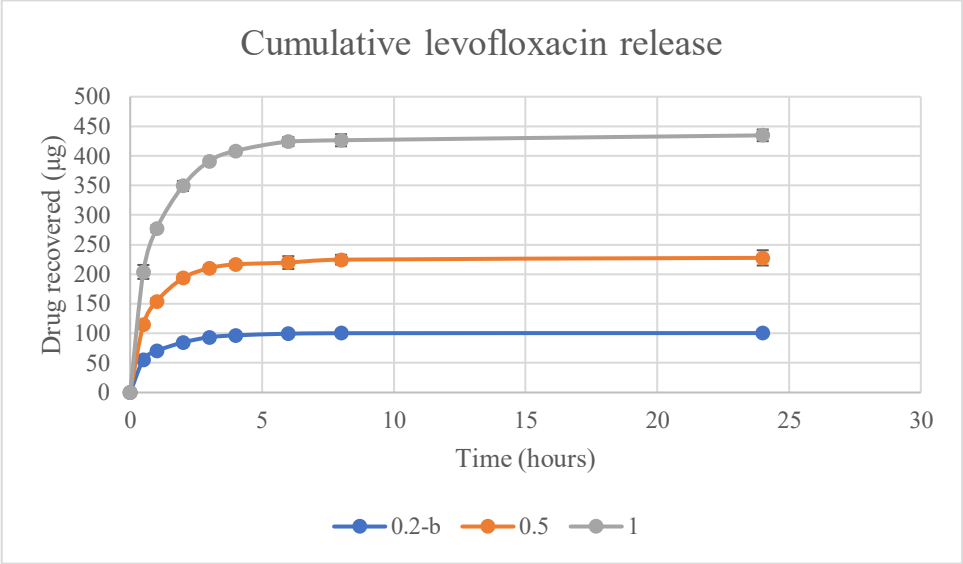

Figure 7.6 Cumulative amount of levofloxacin released (Group 2)

Nonetheless, some differences could be appreciated in the release profiles described by the inserts belonging to the two experimental groups. In particular, as reported in Table 7.5, it can be seen that, despite containing similar quantities of levofloxacin, formulations 0.2-a and 0.2-b showed a different rate of drug release. Bearing in mind that for the second experimental set of formulations, where process parameters were adopted from the DoE to maximise the drug content, it remains to be clarified if this could have impacted also on the release mechanisms of the inserts. In fact, the ability of water uptake between the groups was also found different, and this characteristic of the formulations was found to play a relevant role in the determination of drug release profile (Mali *et al.*, 2018).

Table 7.5 Dissolution testing data for inserts manufactures with two procedures and differing drug loading concentrations. (in top half cumulative percentages released, in bottom half cumulative amounts)

Release Time (h)	0.2-a	0.3	0.4	Release Time (h)	0.2-b	0.5	1.0
0.5	50.76 ± 0.0161%	48.69 ± 1.01%	45.08 ± 3.06%	**0.5**	56.54 ± 2.86%	49.13 ± 1.9%	45.7 ± 2.28%
1	61.88 ± 2.15%	59.81 ± 2.2%	54.94 ± 3.11%	**1**	71.93 ± 3.93%	66.02 ± 2.71%	62.05 ± 0.56%
1.5	67.64 ± 1.3%	65.54 ± 2.66%	60.79 ± 3.25%	**2**	86.71 ± 4.44%	82.87 ± 3.11%	78.32 ± 2.61%
2	72.56 ± 0.55%	70.22 ± 3.11%	66.46 ± 3.28%	**3**	95.7 ± 5.35%	90.03 ± 2.28%	87.7 ± 0.6%
3	79.05 ± 0.85%	76.07 ± 3.94%	75.12 ± 3.16%	**4**	98.94 ± 4.45%	92.79 ± 2.06%	91.61 ± 1.02%
4	85.47 ± 1.82%	82.64 ± 4.63%	83.34 ± 3.12%	**6**	101.83 ± 3.99%	94.1 ± 4.27%	95.12 ± 1.98%
5	92.1 ± 2.69%	90.38 ± 5.14%	90.45 ± 3.06%	**8**	102.69 ± 3.69%	96.22 ± 3%	95.64 ± 2.36%
6	98.75 ± 3.44%	97.41 ± 4.67%	97.43 ± 3.33%	**24**	102.9 ± 3.99%	97.51 ± 5.03%	97.49 ± 2.56%
Release Time (h)	**0.2-a**	**0.3**	**0.4**	**Release Time (h)**	**0.2-b**	**0.5**	**1.0**
0.5	44.33 ± 1.27	60.18 ± 5.65	78.49 ± 4.43	**0.5**	55.17 ± 1.39	114.66 ± 4.99	203.83 ± 11.75
1	54.06 ± 2.13	73.98 ± 8.17	95.67 ± 4.35	**1**	70.18 ± 2.03	154.02 ± 5.42	276.71 ± 5.86
1.5	59.09 ± 1.51	81.08 ± 9.26	105.87 ± 4.52	**2**	84.6 ± 2.2	193.34 ± 6.55	349.14 ± 8.55
2	63.38 ± 0.94	86.89 ± 10.23	115.74 ± 4.55	**3**	93.37 ± 2.84	210.07 ± 5.18	391.04 ± 2.33
3	69.05 ± 0.81	94.16 ± 11.84	130.83 ± 4.46	**4**	96.55 ± 1.87	216.52 ± 5.48	408.47 ± 3.77
4	74.66 ± 1.36	102.31 ± 13.24	145.17 ± 4.89	**6**	99.39 ± 1.39	219.59 ± 10.74	424.1 ± 7.57
5	80.44 ± 1.98	111.9 ± 14.68	157.55 ± 5.14	**8**	100.23 ± 1.05	224.55 ± 8.17	426.42 ± 10.2
6	86.25 ± 2.64	120.54 ± 14.61	169.71 ± 5.38	**24**	100.43 ± 1.43	227.57 ± 12.86	434.66 ± 9.83

7.3.3 Antimicrobial efficacy of inserts

Levofloxacin eyedrops, in the concentration of 0.5%, can be considered a safe and effective treatment option for external ocular infections and for preoperative antibiotic prophylaxis (Kanda *et al.*, 2012; Keating, 2009). Nonetheless, the antimicrobial efficacy of the treatment is dependent on overcoming the minimum inhibitory concentration (MIC) of the causative pathogen to be contrasted, which can determinate the concentration and frequency of application of the drug (Herbert *et al.*, 2022). *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively belonging to Gram-positive and Gram-negative classification, were the most isolated bacterial genres in ocular infections (Teweldemedhin *et al.*, 2017), and these were selected to test antimicrobial efficacy of the inserts.

The apparatus employed to determine the drug release profile of the formulations was used also to ascertain the efficacy of residual levofloxacin content in the inserts on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Bearing in mind that a strong *in vivo-in vitro* correlation associated with the testing methodology was obtained by comparison with the amount of drug remaining in the inserts upon administration (Chapter 3.6.4), it could be assumed that the quantity of drug accumulated in the receiver compartment represented, *in vivo*, the sum of drug permeating through ocular tissues and excreted from the eye. In addition, the volume of dissolution media present in the receiver compartment was generally three orders of magnitude higher than the actual volume of tears, thus allowing theoretical appraisal of inserts efficacy but not permitting to use aliquots directly for antimicrobial testing. Thus, the use of residual fraction of levofloxacin present in the donor compartment of the dissolution apparatus was hypothesised to better represent the availability of the drug on the corneal surface and was employed in determination of insert antimicrobial efficacy.

In figures 7.7 & 7.8 are reported the growth curves of *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, after exposing the bacteria to residual fractions of levofloxacin collected at the first (30 minutes), after 2 hours, and at the last release time (6 or 24 hours, depending on the experimental set). While the first and the last time points were selected to verify the immediate and the sustained efficacy of the inserts, the collection at 2 hours was selected because of the general use of the levofloxacin eyedrops. In fact, the ophthalmic solution has been estimated to have an efficacy of 2 hours (Walters *et al.*, 2010), thus requiring a dosing frequency of 4 times per day (Kanda *et al.*, 2012).

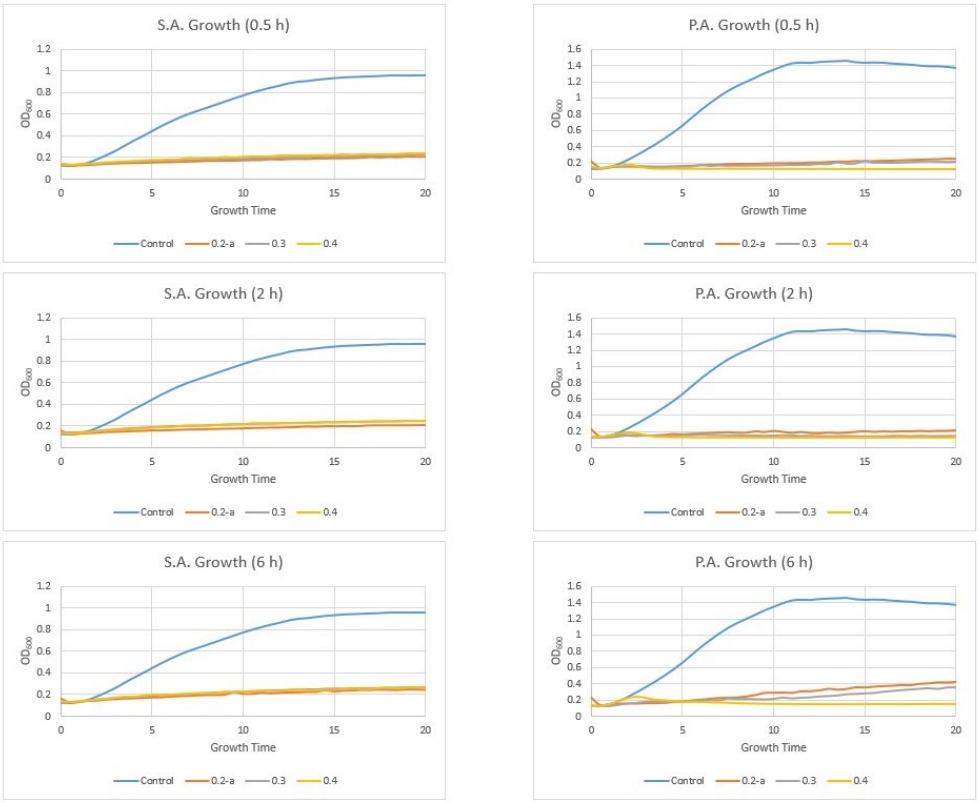

Figure 7.7 Bacterial growth curves from insert drug release over 6 hours (Group 1): Staphylococcus aureus (S.A.), Pseudomonas aeruginosa (P.A)

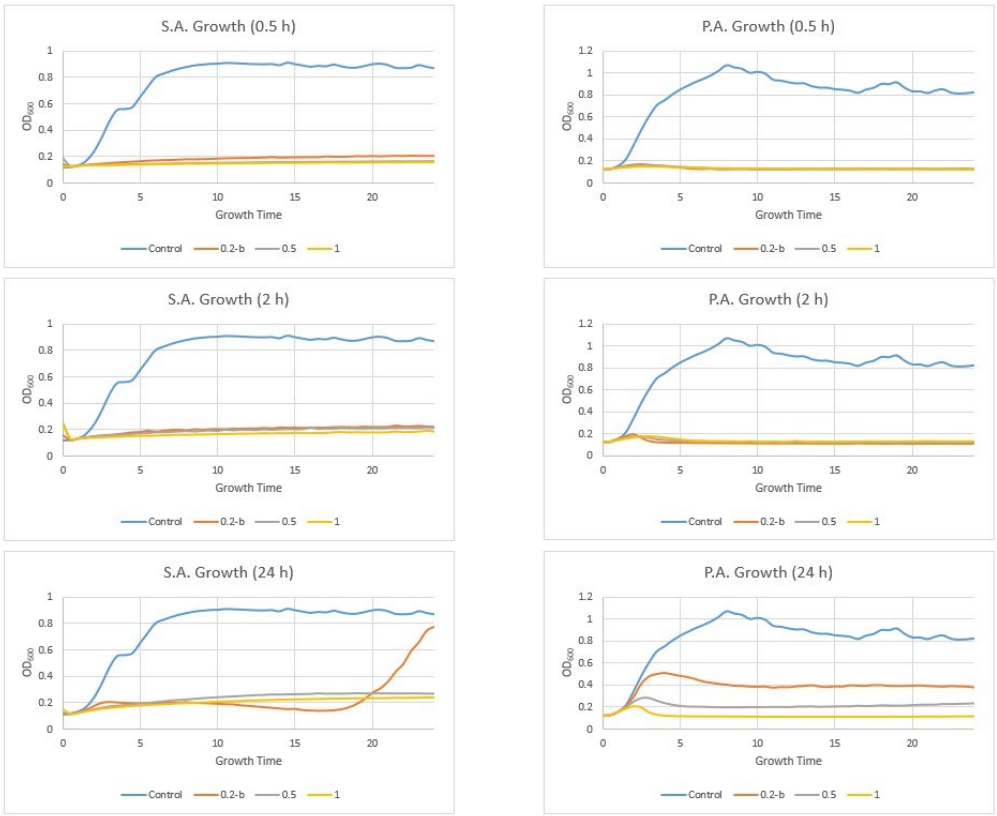

Figure 7.8 Bacterial growth curves from insert drug release over 24 hours (Group 2): Staphylococcus aureus (S.A.), Pseudomonas aeruginosa (P.A.)

The results indicated that for all the formulations tested, irrespective of the drug concentration and of the experimental set, the residual fractions collected after 30 minutes and after 2 hours exhibited the ability to prevent the growth of both bacteria tested. The analysis of the supposed sustained efficacy, instead, revealed different properties of the inserts. The growth curves associated with the formulations belonging to the first group, whose last time collection point was set at 6 hours, exhibited the potentiality to reduce bacterial proliferation over time, but not to prevent the growth, which was more evident in particular for *Pseudomonas aeruginosa* in formulation 0.2-a and 0.3. The reduced efficacy at low drug concentration became more evident in testing the second experimental set. For samples collected after 24 hours release, in fact, the formulation 0.2-b could only delay the proliferation of *Staphylococcus aureus* and to partially reduce the number of bacteria during the stationary phase for *Pseudomonas aeruginosa*, demonstrating a mild bacteriostatic behaviour (Krishnamurthi *et al.*, 2021). More similar were found the effects of formulation 0.5 and 1.0, exhibiting a better efficacy in modulating the bacterial growth, overcoming the exponential growth phase and the number of bacterial cells at a level similar to the inoculum during the stationary phase. In addition, it should be considered that, if it assumed that the inserts would be following a once-a-day dose frequency scheme, the administration of a new insert on the ocular environment should be able to combine the effect with the previous application and guarantee sufficient antibiotic coverage.

The ability of the ophthalmic drug delivery systems to achieve a satisfactory antimicrobial efficacy is dependent on the characteristics of the formulation to ensure a sufficient level of the drug penetrating through ocular tissues and reaching the target site of action (Li *et al.*, 2020). For topically applied levofloxacin formulations, it has been shown that the increment of drug concentration in the solution administered favoured the penetration of the antibiotic (Puustjärvi *et al.*, 2006). Also, the use of higher drug concentration, and the consequent enhancement of its permeation, can lead to a greater potency in contrasting bacterial presence (Bucci *et al.*, 2016).

7.3.4 Corneal cytotoxicity and permeability

The effect of levofloxacin content variation in the inserts was evaluated to determine the permeation of the antibiotic through *in vitro* cultured corneal epithelial cells, a widely used ocular model in pharmaceutical research and development (Shafaie *et al.*, 2016). For cell-based evaluations, the formulations tested belonged only to the second experimental set, with inserts manufactured from film-forming solutions containing 0.2%, 0.5% and 1.0% levofloxacin concentrations. The permeability studies conducted employed an epithelial corneal tissue model that had been proven to provide good reproducibility and *in vivo* correlation in assessment of topical ocular formulations (Kaluzhny *et al.*, 2018). In addition, the adaptation applied (Figure 7.1) was relatively easy to implement and appeared to achieve the aim of creating a physical separation between the formulations tested and the cells.

Results of permeability studies (Figure 7.9) showed that the amount of drug present in the inserts did not modify the profile of the relative drug percentages passing through the cellular model, but influenced the amounts of levofloxacin permeating (Figure 7.10) over the testing period, similar to results from dissolution testing. The concentrations of levofloxacin recovered after permeation in this *in vitro* experiment were found higher the values reported in *in vivo* aqueous humour sampling (Puustjärvi *et al.*, 2006). However, they were comparable to values detected within the stromal tissue, suggesting that the model may be most suitable for superficial ocular surface tissues, rather than deeper ocular structures (Healy *et al.*, 2004).

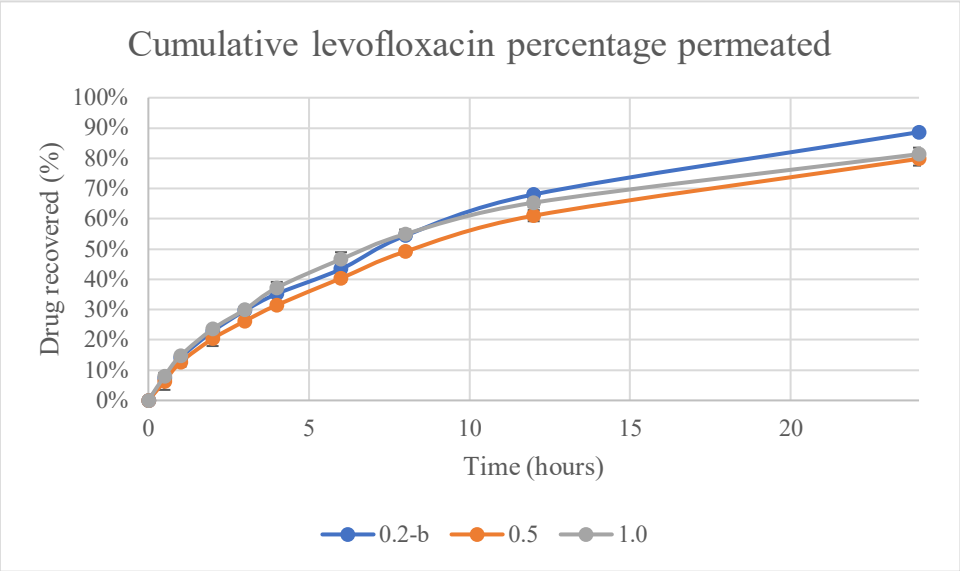

Figure 7.9 Cumulative percentage of levofloxacin permeated from inserts (Group 2)

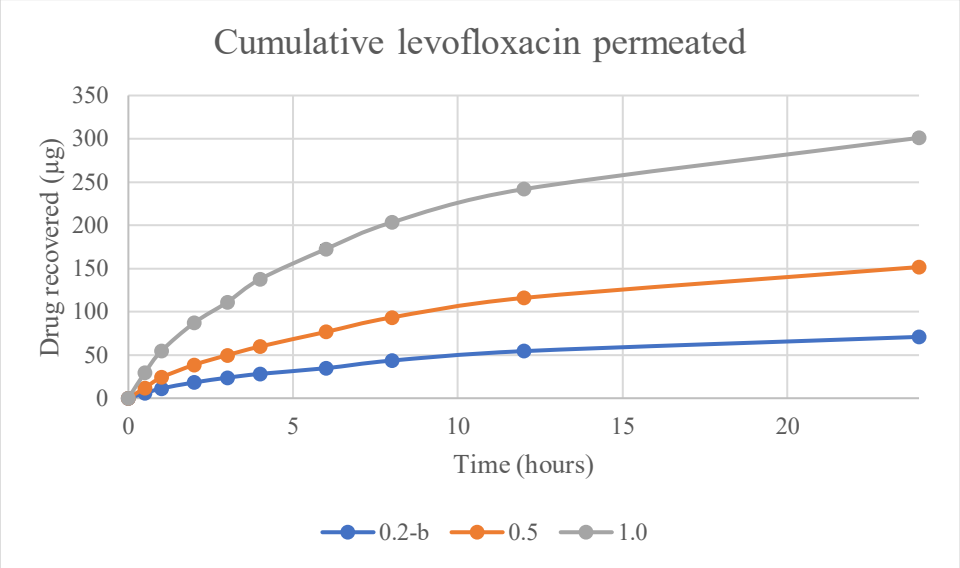

Figure 7.10 Cumulative amount of levofloxacin permeated from inserts (Group 2)

Nonetheless, the analysis of levofloxacin fractions permeated through the epithelial model suggested that the concentrations of 0.5% and 1.0% levofloxacin in the initial solutions conferred to the inserts the ability to allow the immediate passage of a sufficient quantity of drug to overcome the MIC_{90} of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Dave *et al.*, 2020; Metzler *et al.*, 2004), and to reach corneal concentration levels that, *in vivo*, would require multiple instillation of eyedrops (Herbert *et al.*, 2022). Moreover, in comparing the amounts of levofloxacin found at the different time points, reported in Table 7.6, it can be noticed that both the insert formulations made with 0.5% and 1.0% drug were able to maintain a rate of permeation above the MIC_{90} for both bacteria, throughout the length of the testing period.

Table 7.6 Permeability study data for levofloxacin loaded inserts (Group 2).

	Levofloxacin Amount (µg)				**Levofloxacin Percentage (%)**		
Time (h)	**0.2-b**	**0.5**	**1.0**	**Time (h)**	**0.2-b**	**0.5**	**1.0**
0.5	5.77 ± 0.6	11.96 ± 2.25	29.43 ± 1.72	**0.5**	7.21 ± 0.75	6.3 ± 2.81	7.95 ± 0.9
1	11.25 ± 0.35	23.88 ± 1.12	54.49 ± 1.11	**1**	14.06 ± 0.44	12.57 ± 1.4	14.73 ± 0.58
2	18.23 ± 0.99	38.64 ± 1.9	87.18 ± 1.36	**2**	22.79 ± 1.24	20.34 ± 2.38	23.56 ± 0.72
3	23.7 ± 0.44	49.74 ± 0.56	110.95 ± 1.65	**3**	29.62 ± 0.55	26.18 ± 0.7	29.99 ± 0.87
4	28.16 ± 0.05	59.83 ± 0.8	137.68 ± 3.68	**4**	35.2 ± 0.06	31.49 ± 1	37.21 ± 1.94
6	34.72 ± 0.65	76.66 ± 0.94	172.51 ± 4.42	**6**	43.4 ± 0.81	40.35 ± 1.18	46.63 ± 2.33
8	43.54 ± 0.53	93.39 ± 0.77	203.28 ± 2.88	**8**	54.42 ± 0.66	49.15 ± 0.97	54.94 ± 1.52
24	54.43 ± 0.85	115.94 ± 1.46	241.71 ± 2.89	**24**	68.04 ± 1.07	61.02 ± 1.83	65.33 ± 1.52

However, although the employment of higher concentrations of levofloxacin can produce an increment in ocular penetration and efficacy of the drug, it should also be considered that the use of such concentrations can increase the risk of ocular toxicity (Kim *et al.*, 2007).

Hence, the corneal epithelial cell model was employed, together with the implementation for spatial distancing of the samples, to evaluate the effects of insert compositions on cell viability, in comparison with the neat levofloxacin solutions (Ubels & Clousing, 2005).

As represented in Figure 7.11, there was a high correlation between drug content and cell viability – with the inserts apparently demonstrating a higher epithelial cell survival rate than the drug solutions. However, the differences in cell viability were not found statistically significant ($p > 0.05$), preventing a direct inference about their superiority in terms of lowering the risk of damage to the ocular tissues in levofloxacin administration. Nonetheless, it can still be assumed that the polymeric composition of the inserts did not add any further hazard to ocular surface in respect of the levofloxacin solutions, whose cell viability values were found in agreement to previous studies (Sosa *et al.*, 2008; Tsai *et al.*, 2010).

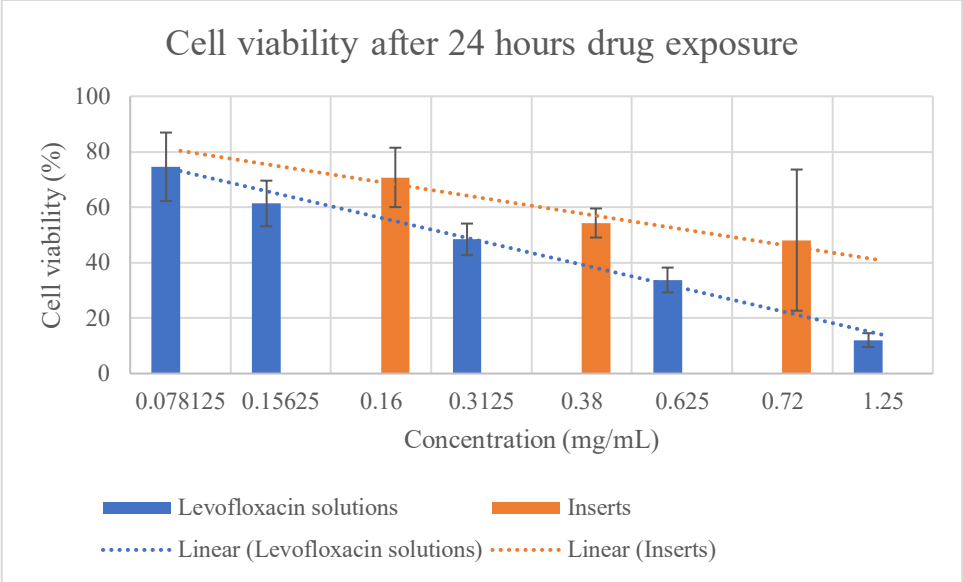

Figure 7.11 Cell viability after exposure to levofloxacin in solutions and in inserts.

7.4 Conclusions

This chapter explored the possibility of employing higher drug loading in the inserts and demonstrated that the formulations tested were able to accommodate up to 1% levofloxacin in qualitative films. Although some minor modifications of the film properties were recorded, the insert qualities did not deteriorate upon increasing the drug loading. The inserts showed acceptable content uniformity and repeatability of production. Therefore, final levofloxacin content in thin film inserts could be predetermined by the concentration of the drug used in film manufacturing, due to a strong positive correlation exhibited.

Modifications of the levofloxacin content did not alter the % drug release profile, hence demonstrating the potential ability to tailor the released drug quantity above the MIC90. In addition, the antimicrobial efficacy of the inserts could be ameliorated by increasing the antibiotic loading. The inserts could sustain the penetration of levofloxacin through *in vitro* corneal epithelial model above the minimum concentration to contrast the most common causative agents of ocular infections. Finally, the embedding of the drug into the inserts did not increase the risk of cytotoxicity in comparison of the neat drug solution. This work provides a robust and efficacious patient-friendly alternative formulation for treatment of superficial ocular bacterial infections.

Chapter 8 - Exploring the acceptability of novel applications of ocular devices by eye care practitioners: a focus on contact lens opportunities

8.1 Introduction

According with the most updated data, the current number of Contact Lens (CL) wearers worldwide has been estimated around 175 million (Akerman, 2018), revising the figures of 140 million used as reference for more than a decade (Stapleton *et al.*, 2007). Despite this, it could be argued that the diffusion of CLs among potential wearers is more likely showing a flattening of the market penetration curve rather than a genuine growth, considering the increasing prevalence of refractive errors and the need for refractive correction among public (Hashemi *et al.*, 2017).

The growth of the CL market currently reported by CL manufactures (Nichols & Starcher, 2020) may be the result of the expansion of specific sectors, such as silicone hydrogel surpassing hydrogel in soft daily CLs market share (Morgan, 2020) and the resurgence of scleral lens fittings (Vincent, 2018). In addition, contact lens dropout can be included among the current relevant factors limiting CLs expansion and, as recently reported (Van Der Worp, 2020), has remained constant over the last decades, despite the numerous innovations in CL industry and practice. Thus, it can be supposed that practitioners are fittings different types of CLs more than managing a higher number of CL wearers.

The current availability of new materials and technological advancements offers valid tools in the management of CL fittings. Despite this, the rate of CL discontinuation that eye care practitioners (ECPs) face has remained constant over the past few decades (Dumbleton *et al.*, 2013), mainly because of role of CL discomfort (Pucker *et al.*, 2019) and the clinical challenges linked to its multifactorial nature (Nichols *et al.*, 2013). Even considering that a tiny reduction of CL drop out can produce a dramatic increase in CL wearers (Van Der Worp, 2020), it is still advisable to focus on engaging potential new wearers to ensure the CL market grows.

The large proportion of world population requiring correction (Hashemi *et al.*, 2017), and the estimates of increasing prevalence of myopia (Holden *et al.*, 2016) and presbyopia (Wolffsohn & Davies, 2019), indicates an extraordinary growth opportunity for CL diffusion. Proactivity is a key strategy for increasing number of CL wearers among the public. For instance, providing CLs during frame selection was associated to an increased interest in wearing CLs and to new fittings (Mayers *et al.*, 2019). Conventional recommendation (proposing and discussing CLs use) was found even more efficient in terms of conversion rate from first trial to a final CL prescription in new wearers (Thite *et al.*, 2018), in line with what previously reported (Jones *et al.*, 1996), suggesting that proactive approaches can raise the number of CL wearers.

Therefore, as for proactivity, it could be thought that there are additional elements potentially determining the expansion of CL market and an additional understanding of those factors could highlight effective strategies to increment the number of CL wearers worldwide.

Innovative uses of CLs have increasingly attracted more attention in recent years, because of the appealing prospect of combining the correction refractive errors to diagnostic and therapeutic purposes. Among those, the possibility to benefit from CLs as a vehicle to administer drugs to the eye has been largely investigated, since they can extend residence time, favour penetration and increase bioavailability of pharmaceutical ingredients while being relatively safe, comfortable, and correct refractive error (Rykowska *et al.*, 2021; Toffoletto *et al.*, 2020). Moreover, it has been suggested that the use of contact lenses as a sustained drug delivery device can be favourably seen by patients and eye care practitioners (Ghazal *et al.*, 2019). Therefore, it can be interesting to evaluate how open are the ECPs toward the new role of CLs as ocular drug delivery device, contextualising this opportunity among the other applications of CLs.

Therefore, the aims of this study were to understand the views of contact lens practitioners regarding what they perceive as opportunities in contact lens practice, and to enable interested stakeholders to design targeted strategies to enhance contact lens practice and address the perceived threats. To evaluate the characteristics of CL practices, practitioners' attitudes, and their effect on rate of new CL fittings, together with the potential interventions felt more relevant by CL practitioners to help delineate promising strategies to favour CL practice growth. Finally, to discuss the perception of ECPs on the role of contact lens as drug delivery device.

8.2 Materials and Methods

8.2.1 Design and Survey Distribution

A self-administered, anonymised survey was developed to explore ECPs point of view of the future of their CL practice in the next five years, including questions on demographic, features and attitudes of CL practitioner and their CL practice. In addition, the opinion of practitioners on potential opportunities (Table 2.2), together with interventions and threats (Table 2.3), to help CL practice growth was investigated through a ten-points rating scale, from 'not at all' to 'maximum'. The survey was first formulated to capture informal discussions with eye care experts, which highlighted varying degrees of optimism about the future of contact lens practice, and subsequently critiqued and refined by the investigators.

The questionnaire (Appendix I) was originally constructed in English and then translated into different languages (Spanish, Italian, French, Korean, Russian and Simplified Chinese). To ensure meaning equivalence a forward-backward translation method was adopted involving native dual linguists and independent reviewers. The survey was distributed online via social media platforms and mailing lists involving reputed international professional bodies. A paper-based version was used in Russia. The online survey could only be completed once from any device to reduce accidental bias from multiple completion. The survey was circulated between November 2019 and March 2020 (it should be noted that this was before the global COVID-19 pandemic). Ethical clearance was obtained for the survey from Aston University, Birmingham, UK.

The development of the survey and the collection of responses were conducted and coordinated by the International Association of Contact Lens Educators (IACLE). The responses dataset was received and analysed after preliminary exploration of the questionnaires, in collaboration with the IACLE coordinators of the survey.

8.2.2 Statistical analysis

The statistical analysis was performed using SPSS (V 26, IBM, New York, USA). Following samples distribution appraisal (Shapiro-Wilk; Kolmogorov–Smirnov), non-parametric comparisons tests (Mood's Median test) were performed. Only responses from countries with 30 or more replies were included in the analysis (Wolffsohn *et al.*, 2016). Statistical significance was set for p-values lower than 0.05, adjusting for multiple comparisons with Bonferroni correction. Only relevant and significant comparisons have been reported for the sake of conciseness. Unless diversely specified, all the average scores have been reported following as medians (and interquartile ranges).

Generalised linear mixed model (GLMM) was used to verify the authenticity of differences in scores attributed to opportunities, interventions, and threats across the geographical areas, while controlling for effect of demographics (age, sex, profession, type of practice and years prescribing CLs). Ordered logistic regression models were constructed with geographical areas as fixed effect, either or not including demographics as random effects for each of the question. Significance was checked for the models and for the interactions of random effects.

8.3 Results

8.3.1 Responses

The total number of 2408 valid surveys were received and analysed. Responses were located all over the world, being the distribution by geographical areas of: Africa 3.6% (n = 87), Asia 32.1% (n = 773), Australasia 2.5% (n = 60), Europe 35.2% (n = 848), Middle East 10.6% (n = 256), North America 7.0% (n = 169) and South America 8.9% (n = 215). Table 2.1 reports the number of responses from each Country, grouped in the geographical areas aforementioned.

Number of females (52%) and number of males (48%) were found similar. The median age was 37.0 years, ranging from 16 to 82 years old, with distribution skewed toward lower values. The median working experience was 11.0 years (IQR: 18.0, 4.0 – 22.0).

Table 8.1 Number of replies received from each Country, grouped in geographical areas

Africa	87	Australasia	60	Middle East	256
Algeria	1	Australia	41	Bahrain	3
Botswana	4	New Zealand	19	Iraq	2
Ghana	4	Europe	848	Israel	2
Kenya	37	Belgium	7	Jordan	86
Mauritius	1	Bulgaria	1	Kuwait	3
Morocco	1	Czech Republic	1	Lebanon	1
Namibia	1	Denmark	1	Oman	46
Nigeria	1	Finland	12	Qatar	4
South Africa	33	France	60	Saudi Arabia	47
Tunisia	1	Germany	11	United Arab Emirates	62
Uganda	1	Greece	2	North America	169
Zimbabwe	2	Ireland	1	Canada	113
Asia	773	Italy	87	USA	56
China	197	Latvia	2	South America	215
Hong Kong	102	Netherlands	47	Argentina	43
India	159	Norway	1	Bolivia	2
Indonesia	56	Portugal	1	Brazil	1
Malaysia	67	Russia	112	Caribbean	1
Nepal	40	Slovenia	1	Colombia	49
Pakistan	1	Spain	436	Ecuador	30
Philippines	20	Sweden	1	Grenada	1
Singapore	53	Switzerland	10	Guatemala	3
South Korea	47	Ukraine	3	Mexico	65
Sri Lanka	18	United Kingdom	51	Peru	18
Taiwan	12			Uruguay	1
Thailand	1			Venezuela	1
				Total	2408

8.3.2 Opportunities in contact lens practice

8.3.2.1 Demographics

Most responses came from Optometrists, 82.1% (n = 1977), followed by Contact lens specialists, 7.6% (n = 184), Ophthalmologists, 5.2% (n = 126), Opticians, 4.0% (n = 96) and other operators involved in CL practice, 1.0% (n = 24).

Of the professionals interviewed, 48.5% (n = 1167) reported to be employed in stand-alone/Independent practices, three-times the values of the Hospital-based ECPs, 16.2% (n = 389), the second most represented sub-group, similar to the number of questionnaires sent by those working in local, 13.4% (n = 322), and national/regional retail chains, 12.0% (n = 289).

8.3.2.2 Potential Opportunities

Overall, multifocal CLs for presbyopia, CLs for myopia control, the use of daily disposable (DD) CLs by occasional wearers and the availability of biocompatible materials to improve comfort (Median: 8/10 for all precedents) were perceived by ECPs more promising. In contrast, fitting coloured/cosmetic CLs was perceived less favourable opportunity (Median: 5/10). Similarly, the innovative uses of CLs for diagnostic and therapeutic purposes, such as drug delivery, received the lowest score (Median: 5/10).

The employment of DD CLs for occasional wearers received the highest score in each geographical area, with lowest variability across those regions. Nonetheless, ECPs in Australasia, North America and Europe valued the option moderately more favourable (Median: 9/10 for all) in respect of colleagues in Asia (Median: 8/10, $p < 0.001$), South America (Median: 8/10, $p < 0.01$) and Africa (Median: 8/10 $p < 0.01$). Furthermore, scores from Middle East (Median: 9/10) were higher than from Asia ($p = 0.005$).

In terms of biocompatible CL materials to improve comfort, practitioners that better evaluated this item were in Europe, North America, and South America (Median: 8/10 for all precedents), with higher scores than African colleagues (Median: 6/10, $p < 0.05$). The use of multifocal CLs for presbyopia was perceived as a better opportunity by practitioners in North America and Europe (Median: 9/10 for both), as well as in Australasia (Median: 8/10), in comparison to Asia, Africa and Middle East (for all Median: 6/10, $p < 0.001$). In addition, scores from South America (Median: 8/10) were higher than from Asia ($p = 0.001$) and Middle East ($p < 0.001$), but lower than North America and Europe ($p < 0.001$).

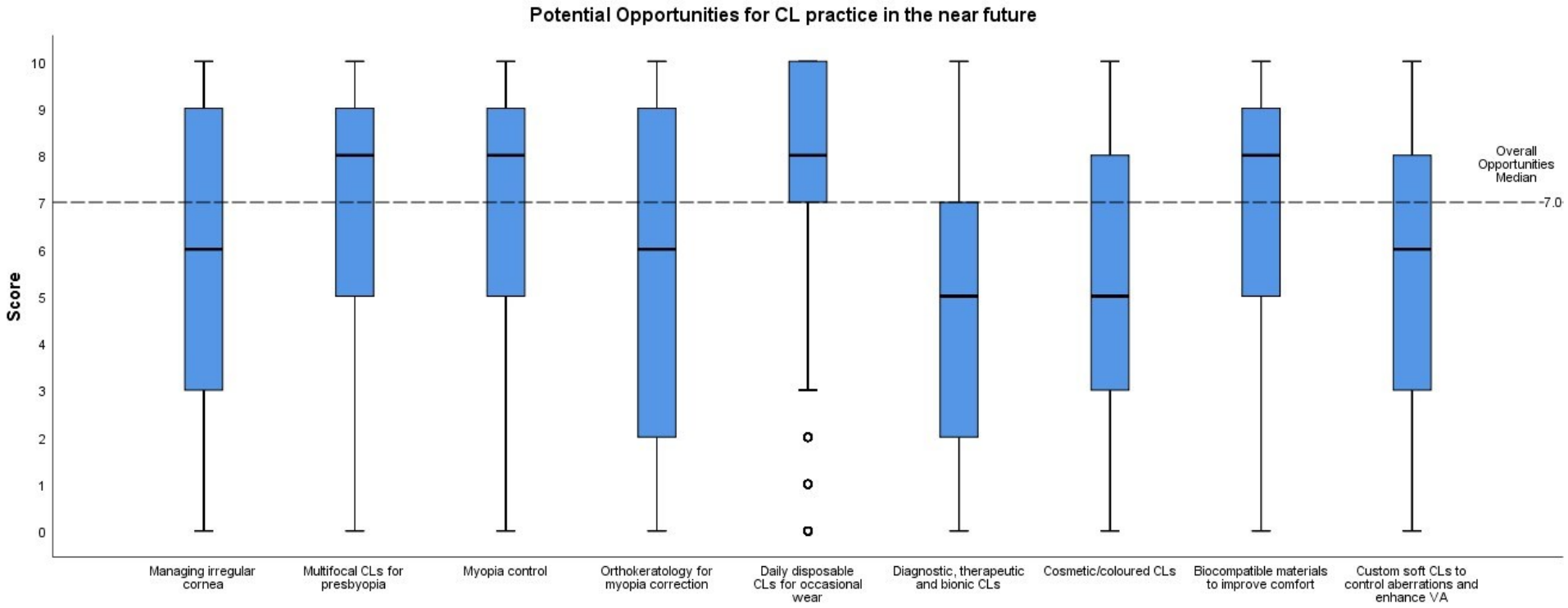

Figure 8.1 Global average scores for potential opportunities to CL practice in near future (expressed as median and interquartile range) Range from 0 to 10

For CL management of myopia control, responses with greater grades came from Australasia (Median: 9/10), North America and Europe (Median: 8/10 for both). In respect of those, lower scores were recorded in Asia (Median: 7/10, $p < 0.005$), South America (Median: 7/10, $p < 0.05$), Middle East (Median: 6/10, $p < 0.001$) and Africa (Median: 6/10, $p < 0.05$).

Greater diversity of opinion was recorded in regard of orthokeratology for myopia correction. The highest value was registered in Australasia (Median: 9/10), higher than in Europe (Median: 7/10, $p < 0.01$) and Asia (Median: 7/10, $p = 0.001$). For all the precedent, the values were higher than in Middle East (Median: 5/10, $p < 0.001$), North America (Median: 4/10, $p < 0.001$) and Africa (Median: 3/10, $p < 0.001$). Values from Australasia ($p < 0.001$) and Europe ($p < 0.05$) were also significantly higher than in South America (Median: 5/10).

The practitioners' opinions about the coloured/cosmetic CLs showed marked variability across the geographical areas, with highest scores received from practitioners working in Middle East (Median: 8/10, $p < 0.001$), followed by those in Asia (Median: 7/10, $p < 0.001$), whilst lowest scores were expressed by Australasian ECPs (Median: 2/10, $p < 0.05$).

Table 8.2 Global and regional average scores (expressed as median and interquartile range) of potential opportunities, interventions and threats in CL practice, on a scale from 0 to 10.

OPPORTUNITIES	Global	Africa	Asia	Australasia	Europe	Middle East	North America	South America
Managing irregular cornea	6.0 (3.0-9.0)	7.0 (3.0-9.0)	6.0 (3.0-8.0)	7.0 (3.3-10.0)	6.0 (3.0-9.0)	7.0 (5.0-8.0)	7.0 (3.0-10.0)	8.0 (6.0-10.0)
Multifocal contact lenses for presbyopes	8.0 (5.0-9.0)	6.0 (2.0-8.0)	6.0 (4.0-8.0)	8.0 (7.0-10.0)	9.0 (7.0-10.0)	6.0 (3.0-8.0)	9.0 (7.0-10.0)	8.0 (5.0-9.0)
Myopia control	8.0 (5.0-9.0)	6.0 (4.0-8.0)	7.0 (5.0-9.0)	9.0 (7.0-10.0)	8.0 (5.8-10.0)	6.0 (3.0-8.0)	8.0 (6.0-10.0)	7.0 (3.0-9.0)
Orthokeratology for myopia correction	6.0 (2.0-9.0)	3.0 (1.0-7.0)	7.0 (3.0-9.0)	9.0 (6.3-10.0)	7.0 (3.0-9.0)	5.0 (2.0-7.0)	4.0 (0.0-8.0)	5.0 (1.0-8.0)
Daily disposable CLs for occasional wear	8.0 (7.0-10.0)	8.0 (6.0-9.0)	8.0 (6.0-9.0)	9.0 (8.0-10.0)	9.0 (7.0-10.0)	9.0 (7.0-10.0)	9.0 (8.0-10.0)	8.0 (6.0-10.0)
Diagnostic, therapeutic and bionic lenses	5.0 (2.0-7.0)	5.0 (2.0-7.0)	5.0 (2.0-7.0)	4.0 (2.0-7.0)	5.0 (2.0-7.0)	5.0 (3.0-7.0)	5.0 (1.5-8.0)	7.0 (5.0-9.0)
Cosmetic lenses	5.0 (3.0-8.0)	6.0 (3.0-9.0)	7.0 (5.0-9.0)	2.0 (1.0-4.8)	4.0 (2.0-6.0)	8.0 (6.0-10.0)	4.0 (2.0-6.0)	5.0 (3.0-8.0)
Biocompatible materials to improve comfort	8.0 (5.0-9.0)	6.0 (5.0-8.0)	7.0 (5.0-9.0)	7.5 (6.0-9.0)	8.0 (5.0-9.0)	7.0 (5.0-9.0)	8.0 (6.0-9.0)	8.0 (6.0-10.0)
Custom soft CLs to control aberrations and enhance VA	6.0 (3.0-8.0)	6.0 (3.0-8.0)	6.0 (3.0-8.0)	5.0 (2.0-7.0)	6.0 (3.0-8.0)	6.0 (4.0-8.0)	6.0 (3.0-8.0)	8.0 (6.0-10.0)

8.3.2.3 Potential Interventions and Threats

The most relevant actions perceived by practitioners were the need for constant updating of knowledge/skills and to become competent in managing CL-related complications (Median: 9/10 for both). Instead, the employment of social media marketing campaigns was assigned the lowest score (Median: 7/10). Overall, South American ECPs generally expressed significant higher scores in comparison to other geographical areas.

ECPs expressed concerns about CL practice with respect to the availability of CLs online without ECPs supervision and the access to CL prescriptions via digital devices in absence of direct involvement of the professionals (Median: 9/10 for both). Refractive surgery and the improvement in spectacles' industry, as well as the risk of infection related to CLs were reputed less concerning among the options proposed (Median: 5/10). Practitioners based in Europe and South America expressed equivalent levels of concern on lack of regulation in CL sales (Median: 9/10), availability of CLs online (Median: 10/10) and their prescription through digital devices (Median: 10/10) ($p > 0.05$). While in other geographical areas practitioners showed similar level of worry about those topics, slightly less concerned appeared ECPs in Middle East (Medians: 6, 8 and 7/10, respectively). The latter expressed minor concern also in regards of level of competency of colleagues (Median: 6/10), alike practitioners in Australasia (Median: 5.5/10) and North America (Median: 6/10) ($p > 0.05$), while higher scores were found in South America (Median: 9/10) and in the other areas (Median: 8/10 for all remaining). The threat of infections related to CL use was indicated as less concerning by practitioners in North America and Australasia (Median: 4/10 for both), in comparison with those working in South America and Middle Est (for both, Median: 6/10, $p < 0.001$), as in Asia and Europe (Median: 5/10, $p < 0.005$).

Table 8.3 Global and regional average scores (expressed as median and interquartile range) of potential interventions and threats in CL practice, on a scale from 0 to 10.

INTERVENTIONS	Global	Africa	Asia	Austra lasia	Europe	Middle East	North America	South America
Continuously updating knowledge/skills of practitioners	9.0 (8.0-10.0)	8.0 (7.0-10.0)	8.0 (7.0-10.0)	8.0 (7.0-8.0)	9.0 (8.0-10.0)	8.0 (7.0-10.0)	8.0 (6.5-10.0)	10.0 (9.0-10.0)
Educating the parents about children to wear CLs	8.0 (7.0-10.0)	8.0 (7.0-9.0)	8.0 (6.0-9.0)	9.0 (8.0-10.0)	9.0 (7.0-10.0)	8.0 (6.0-9.0)	8.0 (7.0-10.0)	10.0 (8.0-10.0)
Being competent in managing CL-related complications	9.0 (8.0-10.0)	8.0 (7.0-10.0)	8.0 (7.0-10.0)	9.0 (8.0-10.0)	9.0 (8.0-10.0)	8.0 (6.0-9.0)	9.0 (7.0-10.0)	10.0 (9.0-10.0)
Making CLs more affordable to patients (especially DD CLs)	8.0 (6.0-9.0)	8.0 (7.0-10.0)	8.0 (6.0-9.0)	7.0 (5.0-9.0)	7.0 (5.0-9.0)	8.0 (6.0-9.8)	7.0 (6.0-9.0)	9.0 (8.0-10.0)
Marketing CL practice on social media	7.0 (5.0-9.0)	8.0 (6.0-9.0)	8.0 (5.0-9.0)	7.0 (5.0-8.0)	7.0 (5.0-9.0)	7.0 (5.0-9.0)	7.0 (5.0-9.0)	8.0 (5.0-10.0)

THREATS	Global	Africa	Asia	Austral asia	Europe	Middle East	North America	South America
Lack of regulation	8.0 (6.0-10.0)	9.0 (7.0-10.0)	8.0 (5.0-10.0)	8.0 (5.0-10.0)	9.0 (7.0-10.0)	6.0 (4.3-8.0)	8.0 (7.0-10.0)	9.0 (7.0-10.0)
CLs available online without professional supervision	9.0 (7.0-10.0)	9.0 (7.0-10.0)	9.0 (7.0-10.0)	9.0 (7.0-10.0)	10.0 (8.0-10.0)	8.0 (5.0-10.0)	10.0 (8.0-10.0)	10.0 (8.0-10.0)
CL prescriptions available via digital devices	9.0 (7.0-10.0)	8.0 (6.0-10.0)	8.0 (6.0-10.0)	8.5 (7.0-10.0)	10.0 (8.0-10.0)	7.0 (5.0-9.0)	9.0 (8.0-10.0)	10.0 (7.0-10.0)
Clinics without proper instrumentation	8.0 (5.0-10.0)	8.0 (5.0-10.0)	8.0 (5.0-9.0)	7.0 (5.0-9.0)	8.0 (6.0-10.0)	7.0 (4.0-8.0)	8.0 (5.0-10.0)	9.0 (7.0-10.0)
Commoditization of CL	8.0 (5.0-10.0)	7.0 (5.0-9.0)	7.0 (5.0-8.5)	8.0 (5.0-10.0)	8.0 (5.0-10.0)	6.0 (5.0-8.0)	8.0 (7.0-10.0)	8.0 (6.0-10.0)
Drop out due to discomfort and/or dryness	7.0 (5.0-8.0)	6.0 (5.0-8.0)	6.0 (5.0-8.0)	6.0 (5.0-8.0)	7.0 (5.0-8.0)	6.0 (5.0-8.0)	7.0 (5.0-8.0)	7.0 (5.0-9.0)
CL related infections	5.0 (3.0-7.0)	5.0 (3.0-6.0)	5.0 (4.0-8.0)	4.0 (2.0-6.0)	5.0 (3.0-7.0)	6.0 (4.0-7.0)	4.0 (2.0-5.0)	6.0 (4.0-8.0)

8.3.3 Practitioners' attitudes to favour CL practice growth

The largest part of responses came from Optometrists, 81.9% (n = 1820), followed by Contact lens specialists, 7.7% (n = 170), Ophthalmologists, 5.5 % (n = 123), Opticians, 3.9% (n = 86) and other operators involved in CL practice, 1.0% (n = 22). In particular, the responding ophthalmologists were mostly located in Russia (n = 72) and China (n = 42), respectively accounting for 58.5% and 34.1% of the overall replies for that profession. Of the professionals responding, 47.6% (n = 1057) reported to be employed in stand-alone/independent practices, 13.6% (n = 302) in local retail chains and 11.7% (n = 289) in national/regional retail chains. Hospital-based ECPs were 17.0% (n = 378), while 8.5% (n = 188) of the responders were based in university settings. The median working experience in CL prescribing was 11.0 years (IQR: 18.0, 4 – 22 years), grouped in categories representing the duration of professional experience: 10.0% (n = 222) less than 2 years; 20.8% (n = 462) from 2 to 5 years; 18.1% (n = 403) from 6 to 10 years; 33.3% (n = 741) from 11 to 25 years and 17.3% (n = 384) more than 25 years.

ECPs were also requested to indicate the type of CL fitted in their practice, with the possibility to select multiple options among soft spherical, soft toric, soft multifocal, any kind of rigid gas permeable (RGP), scleral and other types of CL. The options chosen by ECPs were subsequently grouped into three categories, distinguishing fitting level of practice between: basic, exclusively soft CL, without any distinction among spherical, toric and multifocal CLs; advanced, any RGP CLs, either exclusively or in association with soft lenses; and speciality, scleral CLs and any other type of CLs alone or combined to the ones already mentioned CL. Accordingly, 52.2% (n = 1159) of the practitioners were counted in basic, 24.3% (n = 540) in the advanced and 22.9% (n = 509) in the speciality group. Further 0.6% (n = 14) reported to not to fit CLs at all.

According to the breakdown analysis of CL types (Table 2.3), among the professions the highest rate of CL practitioners fitting solely basic CLs was found among optometrists (55.3%, n = 1006), while contactologist/CL specialists reported the highest rate of both advanced CLs (any RGP) (42.9%, n = 73), and speciality CLs fittings (e.g., scleral) (39.4%, n = 67). Basic CL fittings were more frequently reported by ECPs working in chains, with both national/regional (70.3%, n = 182) and local (73.8%, n = 223) diffusion, while among professionals working in hospital settings was found the highest rate of advanced (38.1%, n = 144) and speciality (33.3%, n = 126) CL fittings. In addition, the majority of novice practitioners reported to manage basic CL fittings (65.3%, n = 145) and those with longest working experience - i.e., more than 25 years - were found more frequently fitting advanced (28.6%, n = 110) and speciality CLs (32.0%, n = 123).

Table 8.4 Distribution of ECPs on type of CL fitted categories, expressed by profession, type of practice and CL practice length groups. [Values are reported as percentage (and number) within the groups]

Profession

	Basic		*Advanced*		*Speciality*		*None*		*Total*	
Optometrist	55.3%	(1006)	22.4%	(407)	21.8%	(396)	0.6%	(11)	81.9%	(1820)
Ophthalmologist	43.9%	(54)	31.7%	(39)	22.0%	(27)	2.4%	(3)	5.5%	(123)
Contactologist/CL specialist	17.6%	(30)	42.9%	(73)	39.4%	(67)	0.0%	(0)	7.7%	(170)
Optician	72.1%	(62)	15.1%	(13)	12.8%	(11)	0.0%	(0)	3.9%	(86)

Type of Practice

	Basic		*Advanced*		*Speciality*		*None*		*Total*	
Stand-alone/Independent	52.0%	(550)	22.4%	(237)	25.4%	(268)	0.2%	(2)	47.6%	(1057)
National/ regional retail chain	70.3%	(182)	17.0%	(44)	12.7%	(33)	0.0%	(0)	11.7%	(259)
Local retail chain	73.8%	(223)	16.6%	(50)	9.6%	(29)	0.0%	(0)	13.6%	(302)
Hospital based	27.5%	(104)	38.1%	(144)	33.3%	(126)	1.1%	(4)	17.0%	(378)
University based	43.6%	(82)	30.3%	(57)	22.3%	(42)	3.7%	(7)	8.5%	(188)

Years of CL Practice

	Basic		*Advanced*		*Speciality*		*None*		*Total*	
Less than 2	65.3%	(145)	15.8%	(35)	14.4%	(32)	4.5%	(10)	10.0%	(222)
From 2 to 5	56.9%	(263)	24.2%	(112)	18.8%	(87)	0.0%	(0)	20.8%	(462)
From 6 to 10	51.1%	(206)	26.6%	(107)	21.8%	(88)	0.5%	(2)	18.1%	(403)
From 11 to 25	52.1%	(386)	23.8%	(176)	24.0%	(178)	0.1%	(1)	33.3%	(741)
More than 25	39.1%	(150)	28.6%	(110)	32.0%	(123)	0.3%	(1)	17.3%	(384)
Total	**52.2%**	**(1159)**	**24.3%**	**(540)**	**22.9%**	**(509)**	**0.6%**	**(14)**	**100%**	**(2222)**

8.3.3.1 Practitioners' attitudes

Practitioners were asked to indicate the frequency at which they encourage the use of CL to subjects not demonstrating evident contraindications to CL wear. Most of the practitioners (61.6%, n = 1483) reported always encouraging CL wear, followed by responders who reported to propose CLs sometimes (36.6%, n = 881) and never (1.8%, n = 44). Furthermore, the reasons underlying a non-systematic encouragement of CL wear was investigated, requesting respondents to select one or more alternatives among the options provided (reported in Figure 2.2). Notably, the responses were received also from a fraction of the practitioners (4.9%, n = 108) who indicated to always promote CL wear. The most frequent reason was assuming patients are not interested in CL wear (n = 336, 15.1% of the total responders), while the least selected option was the discomfort felt by ECPs in counselling patients to start CL wear (n = 55, 2.5% of the total).

The viewpoint of the professionals about the next future of their own CL practice was also explored, by requesting them to select the option best representing their feeling on a 5-items scale from very hopeful to very worried. Of the ECPs responding, 22.9% (n = 509) declared themselves to be very hopeful, 45.1% (n = 1002) hopeful, 21.6% (n = 500) unsure, 7.7% (n = 184) worried and 2.7% (n = 61) very worried. Aside, the level of practitioners' proactivity was tested by asking responders to report on a scale from 0 (not at all) to 10 (highly) the level at which they proactively recommend CLs in their practical settings. Additionally, the scores were used to identify three profiles of the responders: proactive (self-reported scores of 8 or more), active (scores between 5 and 7) and inactive/reactive (scores of 4 or below). The median value of proactivity was 7.0 (IQR: 2.0, 6.0 – 8.0). According with the categorisation described, 46.7% (n = 1037) of the ECPs were identified as proactive, 41.6% (n = 925) as active and 11.7% (n = 260) as inactive/reactive.

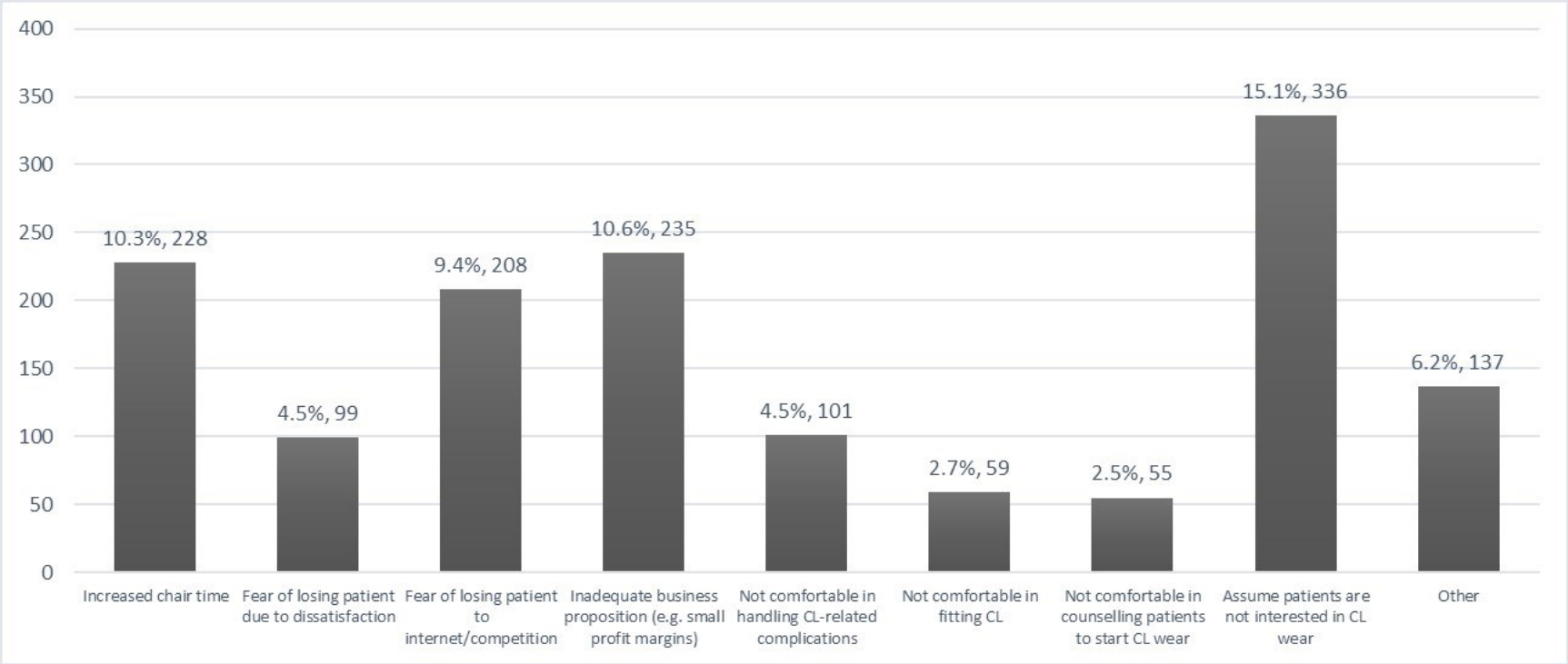

Figure 8.2 Reasons reported for not always encouraging CLs to potential wearers (percentage is referred to the total number of replies)

8.3.3.2 New CL fittings

Practitioners were asked to report an estimate of the average number of new CL fittings performed and the overall median was found to be 5.0 (IQR: 7.0, 3.0 – 10.0) new fittings per month. A similar number of new fittings was reported by optometrists and opticians (Median: 5.0), both significantly lower than values reported by ophthalmologist (Median: 10.0) and CL specialists (Median: 15.0) (all $p < 0.001$). In hospital settings the average number of new fittings (Median: 10.0) was higher than in independent practices (Median: 5.0, $p < 0.001$), universities (Median: 5.0, $p < 0.001$), local chains (Median: 5.0, $p < 0.001$) and in national retail chains (Median: 7.0, $p < 0.05$). Average fitting number in national retail chains was also higher than independent practices ($p < 0.01$). The rate of new CL fittings varied for novice professionals, with significant differences between the value reported by ECPs working from less than 2 years (Median: 4.0) and those in practice from 2 to 5 years (Median: 5.0), from 6 to 10 years (Median: 7.0), from 11 to 25 years (Median: 5.0) and those working for more than 25 years (Median: 6.0) (all $p < 0.001$). The average number of new fittings were higher in the Speciality cluster (Median: 10.0) than in the Advanced (Median: 7.0) and in the Basic (Median: 5.0) groups. The difference between Advanced and Basic fitters was also significant (all $p < 0.001$).

Practitioners that "always" suggested CLs to their patients reported a higher rate of new fittings per month (Median: 7.0) than the value reported by ECPs proposing CLs sometimes (Median: 5.0, $p < 0.001$), and by those never promoting CLs (Median: 3.0, $p < 0.05$). The most proactive practitioners also had a higher number of new CL fittings per month (Median: 8.0), than those defined as the active group (Median: 5.0), which in turn was higher than the reactive/inactive practitioners' group (Median: 3.0) (all $p < 0.001$). Finally, ECPs expressing that they were "very hopeful" reported a higher number of new CL fittings per month (Median: 10.0), than those "hopeful", "unsure", "worried" and "very worried" (all median 5.0, $p < 0.001$).

8.4 Discussion

The opinion of ECPs involved in CL fitting was collected from 72 Countries around the world to outline which CL practice facets were perceived more relevant by the professionals. Responders were mainly optometrists, of which 3 out of 4 worked in optical shops, either in independent practices or chains.

8.4.1 Opportunities in contact lens practice

As indicated by the data collected in this survey, higher scores were expressed by practitioners about opportunities in CL practice intersected areas that have been intensively empowered with new products and technologies by CL manufacturing companies in the recent years, with highest scores assigned to potential opportunities offered by daily disposable lenses, new biocompatible materials, multifocal CLs for presbyopia and CL management of myopia control.

According to the results, it was confirmed that the use of daily disposable (DD) CLs was greatly evaluated by practitioners all around the world, even if in this case it was tested specifically for occasional wear. The use of DD CLs is constantly increasing in recent years possibly, from practitioners' perspective, because of the fast adaptation in neophytes (Wolffsohn *et al.*, 2020), the deflection of detrimental factors linked to CL cases and care regimens (Wu *et al.*, 2015), and the reduction of clinical (Chalmers *et al.*, 2015) and subclinical (Saliman *et al.*, 2020) adverse ocular reactions. In addition, albeit not immediately after its advent in CLs production (Efron & Morgan, 2008), silicone hydrogel has gained prominent positioning in the sector, now accounting for almost two thirds of daily disposable CL market (EUROMCONTACT, 2020; Morgan, 2020). The use of silicone hydrogel CLs with daily replacement schedule has been indicated to offer more benefits to wearers (Sulley & Dumbleton, 2020), and reputed as the best option by ECPs in terms of safety and comfort, with the perceived higher cost being the main barrier (Orsborn & Dumbleton, 2019) - in line with the opinion of ECPs collected in this survey about the need to make CLs more affordable to patients, especially DD CLs -, but it should be reasoned that a sporadic DD CLs use can compensate the more expensive unit price (Efron *et al.*, 2010a; Efron *et al.*, 2012). Hence, occasional use of daily disposable of silicone hydrogel CLs can be considered a solid mainstream part of CL practice, and it is sensible to expect that to be a valid opportunity for expansion in next years.

As seen, the impact of silicone hydrogel on CLs has produced significant changes, in terms of both CLs' performance and perception. Nonetheless, a major issue associated to even permanent CLs discontinuation can be still identified in ocular discomfort and dryness during CL wear (Dumbleton *et al.*, 2013) and the responding professionals identified that as relevant potential threats to CL practice future (Global median: 7/10). Accordingly, practitioners highly valued the positive impact on CL practice offered by the possibility to adopt new materials, in the direction of reducing discomfort by the help of biocompatible materials, whereas it was suggested that CLs related discomfort can be associated to geometrical characteristics and mobility of CLs, other than the material (Nichols *et al.*, 2013; Stapleton & Tan, 2017; Tighe, 2013). Thus, ECPs might have expressed the willing of material(s) able to guarantee readiness of use, in terms of clinical performance, with the aim of reducing the chance of CL drop out caused by discomfort/dryness.

In recent years, major companies have brought to the market new DD multifocal CLs that have replenished the interest in this type of compensation for presbyopia. Nonetheless, the percentage of multifocal CL fittings was found to be a relatively small part of the CL market (Nichols & Starcher, 2020), in particular if compared against the large part of population who can benefit from presbyopia corrections alternative to spectacles. Data analysis revealed that higher scores overlapped older median age of the specific geographical areas (even considering the variability imposed by South America Countries), suggesting that that potential area of expansion for CL practice is more presumably going to take place in regions denoted by larger portions of population suitable for this compensation. On the other hand, it can be assumed that the positive opinion attributed to multifocal CLs for presbyopia were prospective and demanded new and more efficient products and designs, in particular in view of the rate of multifocal CLs discontinuation due to vision related problems (Sulley *et al.*, 2017; Sulley *et al.*, 2018).

Aside the bright future delineated by ECPs on daily disposable and multifocal CLs, the fear of losing the centrality of professionalism in CL fitting may act as a counterpoint to the 'simplicity' of use of CLs reached by current technical achievements. In light of this view, it can be interpreted the high and widespread (with the exception of few regional differences) levels of concern regarding the availability of CLs online and their prescription via digital devices without professionals' involvement or supervision (Median: 9/10 for both), and, to the same extent, they might be included also the worries linked to the lack of regulation in CL sales and the commoditization of contact lenses, in practitioners' opinion (Median: 8/10 for both). The factors associated to unsupervised CL selection and supply (Young *et al.*, 2014) can be related to higher risk rate of CL related problems (Dumbleton *et al.*, 2011; Sorbara *et al.*, 2018), a fortiori in consideration of controversial compliance level demonstrated by CL wearers (Morgan *et al.*, 2011). In particular, regarding the inadequate frequency of aftercare visits (Wu *et al.*, 2010), the role practitioners' intervention is vital to identify and prevent CL related complications (Chen *et al.*, 2020).

The opportunity offered by the CL management of myopia progression was positively evaluated by practitioners. During the last years, the world of CLs has seen an increase interest in the topic, considering the evidence suggesting that specific types of CL fittings can contribute to slow/reduce the progression of myopia (Prousali *et al.*, 2019; Walline *et al.*, 2020). Furthermore, even if it was advocated to discard single vision CLs in favour of more effective fitting types (Bullimore & Richdale, 2020), almost two thirds of suitable wearers were single vision spectacles and/or CLs, with increased costs and inadequate information as main deterrent (Wolffsohn *et al.*, 2020). Geographical variations in the evaluation of this potential opportunity may be based on differences in myopia prevalence across the world. The lowest score came from African practitioners, who deal with one of the lowest prevalence of myopia in school children in the World, and they may be more focused on the large portion of undiscovered refractive errors in children (Atowa *et al.*, 2017). Lower rates of myopia prevalence in school children, and relevant proportion of uncorrected refractive errors, were identified also in Middle East (Al Wadaani *et al.*, 2012) (Aldebasi, 2014; Khoshhal *et al.*, 2020) and South America (Carter *et al.*, 2013; Moraes Ibrahim *et al.*, 2013), which may explain the similarity of scores for the item proposed. Data obtained from Asian practitioners can be interpreted to an equivalent extent (Median: 7/10), with ECPs expressing more interested in myopia control in China (Median: 9/10) and South Korea (Median: 8/10), where myopia prevalence in school children was found higher (respectively 65% (Li *et al.*, 2017) and 52% (Rim *et al.*, 2016)), in respect of India (Median: 5/10) and Indonesia (Median: 7/10), which held lower myopia prevalence in school children (respectively 13% (Saxena *et al.*, 2015) and 33% (Mahayana *et al.*, 2017)). European and North American practitioners expressed comparable high scores on this potential opportunity (Median: 8/10), while characterized - apart from National variability across regions - by analogous myopia prevalence (42.7% (Matamoros *et al.*, 2015) vs 42.2% (Hrynchak *et al.*, 2013)). In Australasia, instead, even if not in presence of one on top prevalence rates (French *et al.*, 2013), myopia management by CLs was considered a great opportunity (Median: 9/10) by ECPs. Thus, in conjunction with the estimates on increasing myopia prevalence in the forthcoming decades (Holden *et al.*, 2016), data depicted the need to set new standard in CL practice on the modalities ECPs deal in myopia management, for example, by educating parents about myopia management with CLs (Median: 8/10).

Among the potential interventions, the continuously updating of knowledge and skills was felt more urgent by practitioners (Median: 9/10), in particular, the management of CL related complications (Median: 9/10). The combination of those potential interventions with the criticism raised by ECPs regarding the availability of adequate instrumentation (Median: 8/10) could explain why the other potential opportunities tested were rated below the grand median of the group (Figure 2.1).

The items regarding the management of irregular corneas, orthokeratology and custom aberration-controlled designed soft CLs have in common the requirement of supplementary knowledge and experience (Gill *et al.*, 2010), as well as additional equipment in the clinical settings (Ortiz-Toquero & Martin, 2017). Thus, it can be assumed that ECPs did not view the aforementioned opportunities as valuable as they could, possibly because they felt not sufficiently confident to engage in more challenging fittings without adequate preparation/experience and equipment.

Orthokeratology has been evaluated as an effective, safe, and well accepted correction modality to compensate myopia (Hiraoka *et al.*, 2009; Nichols *et al.*, 2000). Nonetheless, the distribution of this type of fitting was attributed to a reduced group of practitioners devoted to orthokeratology, dedicated to myopia control and mainly based in Europe and Australasia (Morgan *et al.*, 2019). Our findings show that orthokeratology was considered a more valuable opportunity in regions where this modality was already well established.

The opinions about the coloured/cosmetic CLs were extremely variable across the geographical areas. Australasian practitioners were very sceptic about this opportunity (Median: 2/10), in contrast to ECPs in Middle East (Median: 8/10) and Asia (Median: 7/10), confirming that this type of CLs can be more appreciated in specific areas around the world, as it has been previously reported (Rah *et al.*, 2013). Noticeably, practitioners in North America (Median: 4/10) reported cosmetic CLs more connected to non-legal purchase, in particular via online sources, and to development of CL related complication, despite FDA regulated this type of CLs (Gaiser *et al.*, 2017).

8.4.2 Practitioners' attitudes to favour CL practice growth

The analysis revealed the presence of factors that may influence the growth of CL practice among those characterising practitioners' attitudes and the nature of CL practice performed, as well as promising potential interventions reputed by practitioners' valuable strategies to support CL practice growth.

The percentage of replies received from optical shops (independent, local, and national chains) accounted for almost 75% of the total responders, suggesting a reliable portrait of day-by-day practice, even though higher levels of CL practice might have been overrepresented in this survey. In fact, in comparing characteristics of survey sample against published data, discrepancies were identified. The overall number of practitioners fittings soft CL percentage was 87.5%, in line with the figures reported on number of fittings (Nichols & Starcher, 2020), however those declaring to fit toric and multifocal soft CLs were 75.3% and 55.8%, respectively, either alone or associated with other CLs types. Those figures differed with the number of toric and multifocal soft CLs fitted, which were estimated to account for 26% and 13% (Nichols & Starcher, 2020). Similarly, the ECPs reporting to fit CLs other than soft in this survey were almost half of the responders, whereas in the literature the fraction of non-soft CLs rarely exceeded 30% of the total fittings (Efron *et al.*, 2010b; Woods *et al.*). Thus, if on one side it can be drawn that advanced and speciality CL fittings distributions are fragmentary, on the other side, it might be assumed that, overall, the survey intercepted practitioners of higher profile.

In the same direction can be interpreted the comparison of types of CLs fitted across professionals, type of practice and working experience. Most opticians reported to dedicate their CL practice exclusively to soft CL fittings (72.1%), while most of CL specialists reported to devote their practice also to advanced and speciality CL fittings, with a small group fitting soft CLs only (17.6%). Aside, the relative proportion of CL fitting type categories was similar between optometrists and ophthalmologists: practitioners conducting basic fittings was the larger fraction (respectively 55.3% and 43.9%), followed by lower percentages of those fitting advanced (22.4% and 31.7%) and speciality (21.8% and 22.0%) CLs. Thus, although national differences in legislation and training should be considered, it appeared that the distribution of the level of CLs fitted was related to the degree of education and engagement in CL field. Supplemental training permits opticians to extend the management of CL fittings to more advanced lens types. In fact, the figures of CL specialists appeared complementary to those coming from opticians, suggesting that ECPs investing on knowledge are more frequently able to translate it into everyday practice. Aside, similarities between optometrists and ophthalmologists may indicate that comprehensive CL educational paths allow practitioners to spend their competences according to the needs typifying their workplace.

Clinical settings can determine the nature of CL practice conducted by ECPs. Practitioners working in national and local retail chains were found more often involved in fitting basic lenses only (70.3% and 73.8%, respectively), denoting that companies frequently request employees to focus just on soft CL fittings, even though the availability of multiple practices still allows chains to offer a complete service, by redirecting wearers needing advanced and/or speciality CLs to dedicated practitioners/stores. The referral for specific fitting requirements can also explain why practitioners working in hospitals frequently manage also advanced (38.1%) and speciality (33.3%) CLs, particularly in those countries where the management of advanced and speciality CLs is mostly provided in hospital settings (e.g., in the UK). To the same extent, it can be assumed that the figure characterising independent practices may reflect the central role of those practices in offering to public CL management at all levels, especially in areas where referral system is not yet definitely established. The relation between types of CLs fitted and length of working experience suggested that novice practitioners dedicate more time fitting more basic CLs and, by increasing the amount of experience and knowledge, they can broaden the variety of CLs fittings during their career progression over years.

A relatively large portion of practitioners firstly reporting to always promote CLs still felt the need to express reason(s) underpinning a non-constant proposition of the device. This can be interpreted as a latent uncertainty in recommending CLs to public which, together with the assumption that potential wearers are not interested in CLs was the most frequent reason for not always encouraging CLs, can explain the efficacy of a more proactive approach. In particular, if it is considered the presence, in some areas, of unawareness of CLs as refractive correction aid (Abokyi *et al.*, 2017) and a lack of information about the device (Thite *et al.*, 2015) were major limitations in diffusion of CLs. Thus, while some practitioners might improperly assume the people are not interested in something completely or partially unknown, other colleagues can take advantage of promoting CLs just by increasing knowledge about this type of correction. According to this view, it can be interpreted that the group of practitioners expressing highest level of confidence in their CL practice future was formed by ECPs performing more new CL fittings per month (Median value: 10.0). However, no significant differences were found in the number of new fittings across the remaining categories (Median: 5.0 for all), suggesting that number of new fittings may not be a sensitive indicator of practitioners' perception on their CL practice future.

Overall, practitioners fitting exclusively soft CLs appeared to attract a lower number of new wearers (Median: 5.0 new fittings per month) in respect of ECPs managing advanced (RGP) CLs (Median: 7.0) and those fittings also speciality CLs (Median: 10.0). Those figures may suggest that by offering a wider service in CL field ECPs can aggregate the numbers coming from each of the fitting types and consequently facilitate the growth of their practice. Additionally, it can be supposed that managing a broader selection of CL types, practitioners' professionalism may be better perceived by the public, placing them in reference position in CL practice in those areas. The lower average value of new fittings found in the less experienced practitioners' group, although small, may be seen as a warning, indicating that the enthusiasm of newly qualified CL practitioners may not be supported by an educational path able to prepare them in full on proposing and managing CLs confidently at the beginning of their careers.

The average number of new fittings per month was used as reference to evaluate the effect of level of proactiveness and frequency in counselling CL wear on the growth rate of CL practice. Proactive attitude towards CLs has been associated to an increment of new CL wearers rate in ametropes, if compared to a mere reactive approach from practitioners (Jones *et al.*, 1996). As well, either a conventional proactive recommendation or the tangible experience of trying CLs during spectacles selection were evaluated as potentially effective proposition of CLs to new wearers (Atkins *et al.*, 2009; Thite *et al.*, 2018). In this survey, based on self-reported scores, most the practitioners were be identified as proactive (46.7%) and active (41.6%), indicating a common enthusiastic approach to CLs. In addition, ECPs reporting to adopt a proactive attitude in regards of CL practice and those reporting to always counsel CLs to potential wearer held higher average value of new CLs fittings per month (Medians: 8.0 and 7.0, respectively). In comparing the values of new CL fittings found in groups defined by proactivity and frequency in counselling CLs against the grand median of the sample, it appeared evident that the number of new fittings was closely related to the level of engagement in CLs and their proposition. While middle levels of proactiveness and suggesting CLs frequency (i.e., 'active' and 'sometimes' groups) overlapped the overall average number of new fittings per month (Median: 5.0), more committed approaches resulted in higher average number of new CL fittings. Thus, a robust and consistent CL practice growth can be expected in result of more prepositive approaches, such as proactivity and constant proposition of CLs to potential wearers

The responding ECPs expressed high level of appreciation in regards of the potential intervention proposed, with scores global medians of 7/10 or above. The interventions felt more pressing by practitioners were to continuously update practitioners' knowledge/skills and to develop competency in managing CL related complications (Median overall score 9/10 for both). The willing of increased knowledge in CL field was found extremely comprehensive, embracing positive reactions across working experience length, profession and CL practice setting groups, suggesting that the importance of seeking for wider knowledge is broadly shared among all CL practitioners.

In profession-based analysis, ophthalmologists did not value proactiveness as high as other professionals (6/10 vs 8/10), probably because ophthalmologists usually work in hospital eye departments where patients are referred for contact lens fitting. In terms of different clinical settings, proactiveness was one of the potential interventions better evaluated by practitioners in larger retail chains, along with educating parents about children wearing CLs (9/10), development of improved referral system (9/10) and increased competencies in managing CL related complications (9/10). Those findings, together with the higher frequency of ECPs fitting only soft CLs, potentially suggest the will of increased CL practice level among practitioners working in chains. Even more marked can be considered the desire of enhanced CL practice among proactive practitioners, which enthusiasm towards CL practice might constitute the basis of the diffuse higher agreement to the potential interventions tested in the survey. In addition, besides the newly qualified professionals who may not be fully aware of CL practice during their settling period, CL practitioners with shorter working experience are more attentive to CL affordability and less to CL complications management in respect of those with greater experience, denoting a shift towards additional attentiveness to quality of CL service, rather than its financial implications.

8.4.3 Innovative use of CLs

Innovative uses of CLs have been proposed including devices able to monitor ocular specificities (intraocular pressure, glucose level) and ocular drug deliver (Jones *et al.*, 2016). For the latter, different delivery modalities were evaluated (Gote *et al.*, 2019) and, despite some limitation, ocular drug delivery through CLs can be still considered promising. In line with those results, in a study recently conducted in the UK, it was found that almost 60% of the responders stated that they would prescribe/dispense CLs to treat ocular disease (Ghazal *et al.*, 2019). However, those results were based on a survey conducted in hospital setting on a cohort mainly constituted by pharmacists, and it may not be fully representative of the views of professional that are more directly involved in prescription and management of contact lenses. This survey, instead, can be considered more plural, and representative of the various modalities by which the eyecare services are provided worldwide. The ECPs, overall, described a dynamic and enthusiastic approach to CL practice, and appeared willing to take on new challenges. However, the innovative use of CLs like drug delivery was given one of the lowest scores (Global median: 5/10). Nonetheless, it is worthwhile to recall that professionals responding from different countries held various scopes of practice - often not including diagnosis and pharmacological management of ocular pathologies - and that opportunity might be considered out of their scope of practice even more considering the overall lack of knowledge and experience with those innovative devices, also because their distribution on the market was absent or very limited at the time of the survey. In fact, it must be noticed that this study preceded the publication of the Contact Lens Evidence-Based Academic Reports (CLEAR) (Wolffsohn *et al.*, 2021), which is intended to serve as a new basis to expand knowledge in CL field, with the section regarding the pioneering technologies leading CL advancements particularly promoting the awareness of innovative uses of CLs (Jones *et al.*, 2021). Furthermore, only very recently the first drug-eluting contact lens received the approval from the pertinent national regulatory agencies and became available only in a few countries, allowing to access published data from clinical setting (Ono & Toshida, 2022). The use of this lens has been proposed to prevent the symptoms of allergic conjunctivitis due to the release of Ketotifen, an H1 histamine receptor antagonist, while correcting the refractive errors. At the same time, the effectiveness of this device can benefit from a synergistic effect between the drug released and the presence of the lens on the ocular surface. In fact, it has been suggested that the use of CLs per se can act as a barrier and reduce symptoms accompanying exposure to antigens (Wolffsohn & Emberlin, 2011). Nonetheless, the existing availability of a drug-eluting contact lens remains pivotal in the development of this ocular drug delivery device. Although further evidence will be needed to confirm the applicabity of drug-eluting contact lenses in the management of different ocular conditions, it can still be assumed that there can be a potential expansion of the contact lens sector associated with the increased understanding and promotion of this device.

8.5 Conclusions

Practitioners all around the world recognised the multifaceted nature of CL practice and indicated potential areas of expansion of their CL practice. Overall, the most appealing opportunities for CL practice growth were identified in the occasional use of DD CLs, the employment of biocompatible materials to reduce CL discomfort, the implementation of multifocal CLs for presbyopia correction, and the management of myopia control with CLs. In addition, the ECPs strongly expressed the desire to develop and update their professional knowledge and skills, particularly the management of CL related complications. Regarding threats, the lack of regulation in CL sales, especially online, seems to be a threat.

The responses collected from practitioners helped to delineate the variety of elements characterising CL practice worldwide. The nature of practice settings and the educational paths of CL practitioners influenced the level of CL service provided. The widespread desire to increase the level of knowledge/skills in CL practice could be interpreted considering the higher number of new fittings monthly managed by practitioners providing also advanced and speciality CLs to wearers. Thus, it can be speculated that well educated and trained practitioners, able to manage multiple types of CLs, can be more likely conducting successful CL practices. At the same time, the willingness to improve the management of CL related complications can be interpret in the direction of reducing CL dropout and, consequently, enhance the retain higher number of wearers. Aside, to adopt more enthusiastic approaches in proposing CLs can lead to a relevant increment of CL wearers. Constantly encouraging the use of CLs to suitable potential wearers, especially in overcoming the idea that patients may not be interested in wearing CLs, can be a readily modifiable factor to favour CL practice growth. The overall level of proactivity and the diffuse agreement to the potential interventions proposed in the survey indicated a promising level of engagement of practitioners in CL practice, which can serve as a basis to tailor strategic interventions at national and international level.

The innovative uses of CLs, including ocular drug delivery, were not seen as favourably by the ECPs at the time of the survey. However, it can be speculated that the most recent advancements and the current/imminent availability of drug-eluting contact lenses can potentially reverse this trend, and even lead the expansion of CL, although the applicability this device to different ocular conditions requires further investigation.

Chapter 9 – Thesis summary, conclusions and future work

9.1 Thesis Summary

Topical instillation can be considered the simplest and least invasive route of administration of ophthalmic drugs. Eyedrops, which account for 90% of all the ocular formulations, remain the principal therapeutical option for several ocular conditions such as glaucoma, corneal ulcers, anterior uveitis and allergic conjunctivitis (Jumelle *et al.*, 2020). On the other hand, the efficacy of topical administration of pharmaceutical agents is strongly limited by the ocular structure and physiological barrier properties. Limited volume, rapid turnover and continuous drainage of tear film can significantly limit the residence time of the drug on the ocular surface. Additionally, the unique features of the cornea, and in particular the limited permeability of the epithelium, prevent the pharmaceutical agent to have immediate access to the target site of action (Agrahari *et al.*, 2016). Thus, the development of topically administered drug delivery systems should be directed towards the increase of drug bioavailability at the target site. This can be achieved through the increase of pre-ocular residence time and/or by enhancing the drug permeability (Ali & Byrne, 2008). Penetration enhancers, instead, are a series of compounds that alter the homeostasis of ocular surfaces tissues favouring the drug passage through the ocular barriers. (Moiseev *et al.*, 2019), On the other hand, micro/nano-scaled carrier systems, previously loaded with the pharmaceutical agent, can help the drug to circumvent the ocular barriers (Vaneev *et al.*, 2021). However, drug retention time can be increased by loading the drug in systems that increase tear viscosity or mucoadhesiveness, or using devices that gradually release the drug on the ocular surface (Grassiri *et al.*, 2021).

The use ocular inserts can offer a valid alternative for topical delivery of anti-infective agents to the eye. Those have been produced primarily in the form of thin films, adopting solvent casting manufacturing methods. A variety of different polymers, and polymeric blends, have been proposed in the production of this kind of device. The composition of the film formulations and the selection of specific manufacturing process parameters can determine the properties of the final products, offering the opportunity to tailor the characteristics of final insert produced. However, the information available in literature with reference to manufacturing process and testing methodologies of the inserts has been found partial and inconsistent. Hence, it would be beneficial for the development of this type of antimicrobial ocular drug delivery device to be provided with shared reference standards and procedural testing framework. Among those, it will require particular attention the definition of testing methodologies for *in vitro* drug release and antimicrobial efficacy.

The screening of hydrophilic polymers for the production of blank ocular films revealed that the combination of HPMC, gelatin and sodium alginate, associated with PEG, lead to the production of qualitative soluble inserts, which could subsequently serve as the basis to manufacture drug-loaded inserts for ocular application. The group of formulations including the aforementioned excipients demonstrated favourable physical characteristics and mechanical proprieties. On the other hand, it found that identifying a specific procedure on the basis of the characteristics of the used compounds was needed to upgrade the efficacy and robustness of film manufacturing process. Furthermore, as no drug was present in the inserts, it was proposed a disintegration test, which was found able to differentiate between faster (within one hour) and more extended (several hours) inserts disintegration times.

Hence, levofloxacin-loaded inserts were produced starting from a film-forming solution constituted by 0.1% drug solution, HPMC E15 (1250 mg), low viscosity sodium alginate (750 mg), type A gelatin (250 mg) and PEG 400 (2.5 mL). The inserts exhibited good qualitative properties and manufacturing repeatability. The overall physical and chemical characterisation of the inserts appeared to respect the ocular anatomy and physiology, but the ability to uptake water of the formulation was requiring additional investigations. The values collected in testing the mechanical properties suggested that inserts can successfully comply with the insertion procedure.

The content of levofloxacin was found uniform across the inserts manufactured, and to comply with uniformity references. The drug release profiles were found consistent across the samples tested, with overlapping patterns. The majority of levofloxacin present in the inserts was released within the first 30 minutes, followed by extended release of the drug for up to 6 hours. The drug release profile was initially found suitable with potential antibiotic use, as the drug concentration was overcoming the theoretical minimum inhibitory concentration of the bacteria in a short time, which was furtherly incremented over time. However, the residual fraction of levofloxacin present in the inserts was found sufficient to prevent after the first 30 minutes Staphylococcus aureus growth, while the inserts were not found effective on Pseudomonas aeruginosa for the drug content used. Thus, it was identified the need of further developments fir the inserts to meet satisfactory antimicrobial efficacy on different bacterial species, and to investigate the opportunity to modulate the drug release profile.

The possibility to optimise film manufacturing procedure was through design of experiment (DoE) approach. Different process parameters were investigated in relation to important qualities of the inserts. All the variations of the film-forming solution parameters (stirring time, stirring temperature, and gelation stirring time) and the centrifuge-based degassing method specifications (centrifuge time and centrifuge speed) that were delineating the design space led to the production of qualitative films. The median values of weight, thickness, drug content, tensile strength and elongation collected during film characterisation across the runs allowed to delineate valid and significant models for all the responses. All the analysed factors demonstrated an effect on at least one of the responses, although the effect of degassing procedure on insert characteristics should be clarified. The implementation of an additional factor for the glass plates used permitted the construction of better models, even considering that no effects on mechanical properties were found. To maximise the drug quantity in the inserts, stirring temperature and time, stirring cooling time, and centrifugation speed should be set at or close to the maximum values of the ranges that were tested, while centrifugation time should be 3 minutes. Finally, it was found that selection of glass plates used could determine the amount of levofloxacin in the inserts. The predicted potential increase of levofloxacin content was found at around 10%, implying the need of a higher initial concentration to meet drug content increment above that value. Nonetheless, it was found that the optimisation of the procedure offers the opportunity to tweak levofloxacin content.

As a consequence of the previous outcomes, it was tested the opportunity to variate the levofloxacin content in the inserts. It was found that the formulation developed was able to accommodate up to 1% levofloxacin in qualitative films. Although some minor modifications of the film properties were appreciated, the inserts were not presenting fatal deterioration of their qualities at increased drug loadings. The inserts were showing acceptable content uniformity and repeatability of production, with the additional possibility to predetermine the final levofloxacin content by selecting appropriate concentration of the drug in film manufacturing procedure. In addition, the modifications of the levofloxacin content were not producing an alteration of the drug release profile, allowing to suppose that it could be possible to tailor the drug quantity to be release over time. As expected, in increasing the levofloxacin loading the inserts were showing an enhanced antimicrobial efficacy against the bacterial model tested, with inserts produced after 0.5% and 1.0% levofloxacin concentrations showing the ability to allow the immediate passage of a sufficient quantity of drug to overcome the MIC90 of Staphylococcus aureus and Pseudomonas aeruginosa (Dave *et al.*, 2020; Metzler *et al.*, 2004), and to reach corneal concentration levels that, *in vivo*, would require multiple instillation of eyedrops (Herbert *et al.*, 2022). Finally, the inserts that were manufactured after levofloxacin solution at concentrations of 0.5% and 1.0% could sustain the penetration of levofloxacin through *in vitro* corneal epithelial model, in a way that the drug permeated was exceeding the minimum inhibitory concentration of the two most common causative agents of ocular infections, without increasing the risk of cytotoxicity associated to the pure drug solution.

Soft contact lenses have been suggested as an innovative drug delivery system, not only to prolong and sustain drug delivery but also to correct refractive errors (Jones *et al.*, 2021). Very recently, the first drug-eluting contact lens has started to become available on the market, allowing also to access published data from clinical setting collected from the experience of general population (Ono & Toshida, 2022). However, this innovative use of SCLs has not captivated the interest of eye care practitioners yet, possibility because of the limited collective experience/awareness regarding this opportunity (Thite *et al.*, 2021). Also, it should be considered that the use of SCLs for drug delivery is currently limited to allergic conjunctivitis treatment, for which the efficacy of the system can benefit from a synergistic effect between the drug released and the presence of the lens on the ocular surface (Wolffsohn & Emberlin, 2011). It remains to be furtherly investigated, instead, the extent of their employability in ocular conditions which would generally require to halt SCLs use (i.e., infections) (Carnt *et al.*, 2017).

9.2 Conclusions

The research presented in this thesis focused on reviewing the features of existing ocular topical drug delivery methods and proposing a potential alternative for delivering antibiotic medications to the eye.

The findings of this study can be summarised into the following categories:

- Eyedrops are the most common topical administration method for ophthalmic drugs, but they face challenges due to ocular structure and physiological barriers. Among ocular inserts, thin films can offer a promising alternative for the topical delivery of anti-infective agents, with properties that can be tailored by varying the composition and manufacturing processes of the formulations.

- The study of hydrophilic polymers for blank films found that a combination of HPMC, gelatin, sodium alginate, and PEG resulted in quality soluble inserts displaying favourable physical and mechanical properties, and prolonged disintegration time, making it suitable for ocular drug-loaded inserts production.

- Levofloxacin-loaded inserts were created using 22.5 mL 0.1% drug solution, 1250 mg of HPMC E15, 750 mg of sodium alginate, 250 mg of gelatin, and 2.5 mL of PEG 400. The inserts displayed good qualitative properties and consistent manufacturing, compatibility with ocular anatomy and physiology, and to mechanically withstand the insertion procedure. Although promising, the results collected for drug content and release, and antimicrobial efficacy identified the need for further development of the formulation.

- The optimization of the film manufacturing procedure identified robustness of the formulation and indicated that the variation of process parameters, such as stirring time and temperature, and centrifuge specifications, provides the opportunity to tweak inserts' attributes, including the levofloxacin content. Additionally, it was highlighted the importance of casting surface selection in determining final inserts characteristics.
- The optimisation of inserts drug content indicated that formulation developed could accommodate up to 1% levofloxacin. Inserts showed acceptable content uniformity and production repeatability, and the ability to tailor the content and the quantity of drug released over time by adjusting its concentration in the manufacturing process. Inserts produced with 0.5% and 1.0% levofloxacin concentrations showed enhanced antimicrobial efficacy against bacterial models, and allowed sustained penetration through *in vitro* corneal epithelial models without increasing cytotoxicity risks, thus potentially able to replace multiple instillations of eyedrops.
- Soft contact lenses (SCLs) have been proposed as an innovative drug delivery system to extend and sustain drug delivery and correct refractive errors. Recently, the first drug-eluting contact lens has reached the market, with some clinical data available. However, this innovative application has not yet gained traction among eye care practitioners, possibly due to limited experience or awareness of this opportunity. Currently, the use of SCLs for drug delivery is restricted mainly to treating allergic conjunctivitis, where the system's efficacy may benefit from a synergistic effect with the lens on the ocular surface. The potential use of SCLs for other ocular conditions, such as infections that typically require halting SCLs use, needs further investigation.
- Soft contact lenses are emerging as a potential system to deliver drugs to the eye, offering sustained release and vision correction. Despite the first drug-releasing lens is now available, eye care professionals have shown limited interest, possibly due to inexperience or unfamiliarity. Currently, the use of SCLs for drug delivery is limited to allergic conjunctivitis treatment, hence further research is needed to assess a potential broadening of their applications, including eye infections.

9.3 Future work

Many experiments have been left for future work due to lack of time. Future work concerns the areas that need further development to enhance this novel ocular drug delivery system.

9.3.1 Additional characterisation

Despite the strong and positive *in vivo-in vitro* correlations between the dissolution systems and the animal models used to test ocular drug release, a certain degree of uncertainty can persist in accepting dissolution testing data (Franca *et al.*, 2019; Wuchte *et al.*, 2021). Especially, when the values of correlation found were similar across all the different *in vitro* methodologies tested (Charoo *et al.*, 2003; Tanwar *et al.*, 2007). Therefore, the addition of an *in vivo* evaluation of the manufactured inserts could provide further information about the ability of the delivery system to maintain a sustained drug release the antimicrobial efficacy and the drug toxicity in more biorelevant experimental conditions. Moreover, successful outcomes obtained in the *in vivo* assessment of the proposed formulation could function as preclinical testing for the new device, thereby establishing the initial phase in the regulatory journey toward its approval.

9.3.2 Expanding the drug selection

Levofloxacin has been selected as the model drug based on its safety and good efficacy. However, the antimicrobial potency of the anti-infective agents can decrease after their first development and introduction in general use (Scoper, 2008). Two newer generations of fluoroquinolones are currently available, therefore, it could be sensible to test the ability of the inserts to deliver also other pharmaceutical agents belonging to the same family, given the known affinity to the model drug already tested.

9.3.3 Implementing formulation with rate controlling membrane

The use of water-insoluble rate controlling membranes (RCMs) to sandwich the hydrophilic matrix core to produce multi-layered inserts has been shown to generate significant improvement on the extent of drug release. Bearing in mind that the duration of drug release can be determined by the nature and the quantity of polymers used in the formulation of the RCMs (Tanwar *et al.*, 2007), it appeared that the use of blank synthetic polymethacrylates (Eudragits) membranes was found effective with reservoirs made of either sodium alginate or HPMC (Charoo *et al.*, 2003; Tanwar *et al.*, 2007), two of the polymers used in development of the final formulation identified in the experiments here reported. However, although it may appear obvious to implement the current formulation with Eudragit-based RCM, it should be also considered that those membranes would not be soluble or degradable. Thus, they can dramatically affect the simplicity of use of the soluble inserts manufactured, which would then require to be removed. Hence, a biocompatible and biodegradable polymer (e.g., chitosan) can be considered as an option to load the drug in both the hydrophilic matrix and in the rate controlling membrane (Aher & Nair, 2014).

9.3.4 Embedding drug-loaded nanocarriers

The use of nanocarriers for ocular drug delivery has been associated to improve efficacy of the ophthalmic formulations, as they can increase bioavailability by enhancing corneal permeability and residence time of the pharmaceutical agents. As such, they can be currently found among the FDA approved formulations or at later clinical trials stages (Afarid *et al.*, 2022). However, it has been claimed that additional evaluations are needed, in particular to understand their exact mechanism of action, and to define also the links between nanocarriers attributes and their actual efficacy (Zhang *et al.*, 2021). Mirzaeei (2022) have successfully embedded nanocarriers into films, which were produced in different batches by adding various polymers to the nanoparticulated solutions. Although it was shown that the nanocarriers could be embedded in different polymeric matrices without altering overall quality or mechanical properties of the films, it was not possible to derive any conclusions about expanding the sustained release in presence of nanoparticles, as they were present in all the formulation and no pure drug solution control was used (Mirzaeei *et al.*, 2022). Nonetheless, the potential loading of drug carriers within the inserts remains be very interesting and a potential further development of the formulation refined in this study, in particular if the two elements would demonstrate good compatibility and exhibit synergistic effects on ocular drug bioavailability.

9.3.5 Ocular insert shape

The selection of shape is a factor that has been surprisingly left unaddressed in the development of ocular inserts. This characteristic, in fact, will be a critical factor in the drug loading capacity of the delivery device. In addition, it can have a great impact on inserts facility of administration and, together with the adhesive properties, on potential dislocation and retention after insertion, which can determine its perceived comfort (Bertens *et al.*, 2018). In this series of experiments, it was found that the combination of inserts polymeric formulation and the manufacturing procedure allowed to produce consistent and predictable drug content in the inserts. This combination of properties could become useful in redefining inserts shape. Thus, it will be possible to vary the shape of inserts based on ocular anatomical features, therapeutic purposes and patients' and practitioners' opinions.

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Appendix I

Questionnaire text

SUPPLEMENTARY QUESTIONAIRE

Opportunities and Threats to Contact Lens Practice- A Global Perspective

Dear Colleague,

Contact lens (CL) practice across the world is at critical juncture with some potential opportunities like contact lenses for myopia control, multifocal lenses for presbyopia, specialty lenses etc and few looming threats such as increasing competition from online business. Anecdotal discussions with eye care professionals have revealed different level of optimism regarding the future of CL practice. Hence, we wish to conduct this study to understand the views of contact lens practitioners regarding what they perceive as opportunities and threats to CL practice. The findings of this study will help the stakeholders to design targeted strategies to enhance CL practice and address the perceived threats.

We would appreciate it, if you could complete the attached brief survey; completion of which is expected to take about 5 minutes. The questions are quite general and there are no known or anticipated risks to participation in this study. Your participation is voluntary and anonymous. All information you provide will be kept confidential. If you need more information, here is a URL linked to the participant information sheet (PIS):

https://aston.box.com/s/aey2jg4wdtwax9853zrt5ssmhrzhdysp

If you are an eye care practitioner who fits contact lenses and wish to participate in this survey, kindly proceed.

Age: _____ years

Sex: Female / Male / Prefer not to mention

Country (wherein you primarily practice): _____

Profession:

Optometrist

Ophthalmologist

Contactologist/Contact lens specialist

Optician

Other (Please specify)

Type of practice:

Stand-alone practice/Independent practice

Hospital based

University based

Local retail chain

National or regional retail chain

Other (Please specify)

For how many years have you been prescribing contact lenses? _____

A) In an average month, how many new CL (first time CL wear) fits do you perform? (approximate number)

B) In an average month, which of the following types of lenses do you fit? (you can select multiple answers)

Spherical soft

Toric soft

Multifocal soft

RGP (Any type of RGP)

Scleral

Other (Please specify)

C) How 'proactive' would you consider YOUR contact lens (CL) practice in terms of recommending
contact lenses on a scale from 0 (not at all) to 10 (highly)?

Which one of the following reflects your view about the near future (next five years) of YOUR CL practice?

Very hopeful

Hopeful

Unsure

Worried

Very worried

D) How often do you encourage potential CL patients who have no apparent contraindications to consider CL wear?

Always

Never

Sometimes

E) If your answer to the above question is 'Never' or 'Sometimes,' pls state the reason/s why you do not always encourage them (you can select multiple answers):

Increased chair time

Fear of losing patient due to dissatisfaction

Fear of losing patient to internet/competition

Inadequate business proposition (e.g. small profit margins)

Not comfortable in handling CL-related complications

Not comfortable in fitting CL

Not comfortable in counselling patients to start CL wear

Assume patients are not interested in CL wear

Other (pls specify)

Please rate the following potential OPPORTUNITIES for YOUR contact lens practice in the near future (i.e. next five years) on a scale from 0 (not at all) to 10 (maximum)

1. Managing irregular cornea (i.e. keratoconus, keratoglobus etc)
2. Multifocal contact lenses for presbyopes
3. Myopia control (i.e. specially designed single vision lenses, OrthoK, multifocal soft lenses)
4. Orthokeratology for myopia correction
5. Daily disposable CL for occasional wear
6. Diagnostic, therapeutic and bionic lenses
7. Cosmetic / coloured lenses
8. Biocompatible materials to improve comfort
9. Custom made soft contact lenses to control ocular aberrations and enhance visual acuity

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How much do you expect the following INTERVENTIONS to help YOUR contact lens practice grow in near future (next five years) on a scale from 0 (not at all) to 10 (maximum)?

1. Creating awareness among public about safety and utility of CL
2. Continuously updating knowledge/skills of practitioners
3. Training the support staff (counsellor, sales team)
4. Proactively recommending CL to potential patients
5. Educating the parents about the opportunities for children to wear CL
6. Establishing a referral system with fellow eye and health care professionals
7. Creating an efficient recall system for follow up examinations
8. Being competent in managing CL-related complications
9. Making CLs more affordable to patients
10. Marketing CL practice on social media

Please rate the following potential THREATS for YOUR contact lens practice in the near future (i.e., next five years) on a scale from 0 (not at all) to 10 (extreme)

1. Lack of regulation (i.e. over the counter sale)
2. Lenses being available online without adequate eye care professional supervision
3. CL prescriptions being available via digital devices without practitioner's involvement
4. Clinics without proper instrumentation for fitting and dispensing CL
5. Incompetent practitioners
6. Refractive surgeries
7. Negative myths about CL among public
8. Advances in spectacle industry (e.g. better materials, optics, designs etc)
9. Commoditization of CL (i.e. not considered as a medical device)
10. Drop out due to discomfort/dryness
11. CL related infections
12. Unfavourable industry policies (e.g. reduced profit margin, unavailability of lenses and trials etc)

Please use this space for any other comments.

Thank you for taking to complete this survey. In case you wish to receive a summary of the results, please contact the authors at nilesh_thite@yahoo.com OR J.S.W.Wolffsohn@aston.ac.uk. You will receive the summary after May 2020.

Questionnaire ethical approval

Aston University
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United Kingdom

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www.aston.ac.uk

Date: 13 February 2020

Professor James Wolffsohn

School of Life and Health Sciences

Dear James,

Study title:	Opportunities and Threats to Contact Lens Practice
REC REF:	#1623

Confirmation of Favourable Ethical Opinion

On behalf of the Committee, I am pleased to confirm a favourable opinion for the above research on the basis of the application described in the application form, protocol and supporting documentation listed below.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows.

Document	*Version*	*Date*
Participant Information Sheet	1	
Questionnaire	1	

After starting your research please notify the University Research Ethics Committee of any of the following:

- Amendments. Any amendment should be sent as a Word document, with the amendment highlighted or showing tracked changes. The amendment request must be accompanied by a covering letter along with all amended documents, e.g. protocols, participant information sheets, consent forms etc. Please include a version number and amended date to the file name of any amended documentation (e.g. "Ethics Application #100 Protocol v2 amended 17/02/19.doc").

  Amendment requests should be outlined in a "Notice of Amendment Foem" available by emailing research_governance@aston.ac.uk.

- Unforeseen or adverse events e.g. disclosure of personal data, harm to participants.

- New Investigators

- End of the study

Please email all notifications or queries to research_governance@aston.ac.uk and quote your UREC reference number with all correspondence.

Wishing you every success with your research.

Yours sincerely

Professor Richard Booth

Vice Chair, University Research Ethics Committee

Appendix II

Preliminary screening of film formulations

In the table below are reported the findings of the preliminary screening of film formulation. Thickness and Young's modulus were compared to reference contact lens threshold values (details in paragraph 2.2). [The formulations in red did not form films]

Formulation	Thickness (mm)		Young's Modulus (MPa)		Disintegration	
	Mean	St. Dev.	Mean	St. Dev	Time	Temp.
HPMC 2%	0.0308	0.0055	30.8303	8.2722	N.A.	
HPMC 3%	0.0404	0.0053	2.2416	0.3220	N.A.	
HPMC 4%	0.0525	0.0172	2.6481	1.1098	N.A.	
HPMC 2% + PEG 10%	0.1613	0.0097	0.0111	0.0009	N.A.	
HPMC 3% + PEG 10%	0.2304	0.0349	0.0208	0.0005	N.A.	
HPMC 4% + PEG 10%	0.3433	0.0500	0.0238	0.0024	N.A.	
HPMC 4% + PEG 2.5%	0.0617	0.0064	0.4973	0.0531	5-10 min	Room
HPMC 4% + PEG 5%	0.1038	0.0092	0.1134	0.0168	5-10 min	Room
HPMC 4% + PEG 7.5%	0.1288	0.0049	0.0990	0.0027	5-10 min	Room
HPMC 4% + Gly 2.5%	0.0533	0.0113	1.3634	0.4409	5-10 min	Room
HPMC 4% + Gly 5%	0.1338	0.0186	0.3200	0.0947	5-10 min	Room
HPMC 4% + Gly 7.5%						
HPMC 4% + PEG 10%	0.1746	0.0207	0.0877	0.0524		
HPMC 4% + PEG 20%	0.3579	0.0252	0.0100	0.0021		
HPMC 4% + PEG 30%						
HPMC 4% + DBP 10%						
HPMC 4% + DBP 20%						
HPMC 4% + DBP 30%						
HPMC 4% + PG 10%	0.1892	0.0293	0.0231	0.0110	N.A.	
HPMC 4% + PG 20%	0.2433	0.0516	0.0036	0.0019	N.A.	
HPMC 4% + PG 30%	0.4658	0.0177	N.A.		N.A.	
HPMC 4% + Gly 10%	0.1759	0.0302	0.1522	0.0225	N.A.	
HPMC 4% + Gly 20%	0.4179	0.0654	0.0341	0.0125	N.A.	
HPMC 4% + Gly 30%	0.5696	0.0258	0.0097	0.0023	N.A.	
HPMC 4% + PEG 10% + AG 0.5%	0.2658	0.0281	0.0235	0.0074	30-60 min	Room
HPMC 4% + PEG 10% + AG 1.0%	0.2083	0.0248	0.0209	0.0030	30-60 min	Room
HPMC 4% + PEG 10% + AG 1.5%	0.3142	0.0127	0.0229	0.0015	30-60 min	Room
HPMC 4% + PEG 10% + XG 0.5%	0.1579	0.0174	0.0094	-	30-60 min	Room
HPMC 4% + PEG 10% + XG 1.0%						
HPMC 4% + PEG 10% + XG 1.5%						
HPMC 4% + Gly 10% + AG 0.5%	0.1654	0.0163	0.2539	0.0344	30-60 min	Room
HPMC 4% + Gly 10% + AG 1.0%	0.1454	0.0271	0.1327	0.0188	30 min	Room
HPMC 4% + Gly 10% + AG 1.5%	0.2050	0.0041	0.1952	0.0093	5-10 min	Room
HPMC 4% + Gly 10% + XG 0.5%	0.1742	0.0248	0.6351	0.5604	30-60 min	Room
HPMC 4% + Gly 10% + XG 1.5%						
HPMC 4% + Gly 10% + XG 1.0%						

Continued on next page.

Formulation	Thickness (mm)		Young's Modulus (MPa)		Disintegration	
	Mean	St. Dev.	Mean	St. Dev.	Time	Temp.
HPMC 4% + PEG 10% + Pec 0.5%	0.2950	0.0305	0.0482	0.0066	5-10 min	Room
HPMC 4% + PEG 10% + Pec 1.0%	0.2233	0.0344	0.0644	0.0017	5-10 min	Room
HPMC 4% + PEG 10% + Pec 1.5%	0.2200	0.0158	0.0731	0.0044	10-15 min	Room
HPMC 4% + PEG 10% + Gel 0.5%	0.2225	0.0268	0.0481	0.0051	< 72 h	Room
HPMC 4% + PEG 10% + Gel 1.0%	0.2954	0.0344	0.0491	0.0007	> 72 h	Room
HPMC 4% + PEG 10% + Gel 1.5%	0.2713	0.0185	0.0717	0.0151	> 72 h	Room
HPMC 4% + Gly 10% + Pec 0.5%	0.2342	0.0440	0.0944	0.0046	60-90 min	Room
HPMC 4% + Gly 10% + Pec 1.0%	0.2267	0.0326	0.7903	0.5900	60-90 min	Room
HPMC 4% + Gly 10% + Pec 1.5%	0.2158	0.0120	0.2060	0.0030	60-90 min	Room
HPMC 4% + Gly 10% + Gel 0.5%	0.2621	0.0331	0.1552	0.0619	> 72 h	Room
HPMC 4% + Gly 10% + Gel 1.0%	0.1658	0.0286	0.7184	0.7968	> 72 h	Room
HPMC 4% + Gly 10% + Gel 1.5%	0.2775	0.0207	0.0953	0.0199	> 72 h	Room
HPMC 4% + PVA 10% + PEG 10%						
HPMC 4% + PVA 10% + Gly 10%	0.3017	0.0286	0.1008	0.0402	5-10 min	36°C
HPMC 5% + Gel 1% + PEG 5%	0.1042	0.0152	0.2431	0.0569	15-30 min	36°C
HPMC 5% + Gel 1% + PEG 10%	0.1788	0.0202	0.1066	0.0202	15-30 min	36°C
HPMC 5% + Gel 2% + PEG 5%	0.1317	0.0116	0.4498	0.1012	15-30 min	36°C
HPMC 5% + Gel 2% + PEG 10%	0.1471	0.0081	0.1011	0.0134	>60 min	36°C
HPMC 5% + Gel 1% + Gly 5%	0.0829	0.0087	0.9255	0.1715	30-60 min	36°C
HPMC 5% + Gel 1% + Gly 10%	0.1588	0.0109	0.2520	0.0251	30-60 min	36°C
HPMC 5% + Gel 2% + Gly 5%	0.0879	0.0098	0.4635	0.0059	30-60 min	36°C
HPMC 5% + Gel 2% + Gly 10%	0.1608	0.0086	0.2072	0.0072	30-60 min	36°C
HPMC 5% + Alb1 2% + PEG 10%	0.0550	0.0122	5.9049	1.7359	< 30 min	36°C
HPMC 5% + Alb1 3% + PEG 10%	0.0917	0.0459	4.3453	1.0679	< 30 min	36°C
HPMC 5% + Alb1 4% + PEG 10%	0.0756	0.0082	3.2351	0.4351	<30 min	36°C
HPMC 5% + Alb2 2% + PEG 10%	0.0513	0.0054	6.5738	2.9196	< 30 min	36°C
HPMC 5% + Alb2 3% + PEG 10%	0.0838	0.0259	2.3418	0.2542	< 30 min	36°C
HPMC 5% + Alb2 4% + PEG 10%	0.1167	0.0400	1.1072	0.5248	<30 min	36°C
HPMC 5% + Muc 2% + PEG 10%	0.1679	0.0070	0.1091	0.0105	30-60 min	36°C
HPMC 5% + Muc 3% + PEG 10%	0.2188	0.0130	0.1097	0.0039	30-60 min	36°C
HPMC 5% + Muc 4% + PEG 10%	0.2200	0.0174	0.1041	0.0099	30-60 min	36°C
HPMC 5% + Gel 1% + PEG 5% + Na Al 1%	0.1614	0.0319	0.2932	0.0761	15-30 min	36°C
HPMC 5% + Gel 1% + PEG 5% + Na Al 2%	0.2000	0.0329	0.3826	0.1088	15-30 min	36°C
HPMC 5% + Gel 1% + PEG 5% + Na Al 3%	0.2054	0.0179	0.4311	0.0738	4-13 h	36°C
HPMC 5% + Gel 1% + PEG 5% + Na Al 4%	0.2461	0.0159	0.2577	0.0771	4-13 h	36°C
HPMC 5% + Gel 1% + PEG 5% + Na Al 5%	0.2636	0.0443	0.4918	0.2082	40-50 h*	36°C
HPMC 5% + Gel 1% + Alb 1% + PEG 10%	0.2859	0.0197	0.0498	0.0014	< 30 min	36°C
HPMC 5% + Gel 2% + Alb 2% + PEG 10%	0.3791	0.0306	0.0631	0.0008	< 30 min	36°C
HPMC 5% + Gel 3% + Alb 3% + PEG 10%	0.3984	0.0170	0.0506	0.0051	< 30 min	36°C
HPMC 5% + Gel 1% + Alb 1% + Gly 10%	0.2941	0.0180	0.1093	0.0007	< 30 min	36°C
HPMC 5% + Gel 2% + Alb 2% + Gly 10%	0.3514	0.0155	0.1628	0.0217	< 30 min	36°C
HPMC 5% + Gel 3% + Alb 3% + Gly 10%	0.3675	0.0183	0.1316	0.0037	< 30 min	36°C