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Research paper

Optimization of intraocular lens hydrogels for dual drug release: Experimentation and modelling

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ABSTRACT

Topical administration of both antibiotic and non-steroidal anti-inflammatory drugs after cataract removal surgery is usually recommended to avoid infection and inflammatory process development. In this work, a HEMA/MMA based hydrogel was developed as a platform for simultaneous release of an antibiotic (moxifloxacin) and a non-steroidal anti-inflammatory drug (diclofenac). Initially, hydrogels with different HEMA/MMA compositions and cross-linking contents were produced and loaded separately with moxifloxacin and diclofenac. The *in vitro* release profiles of the drugs from the hydrogels were obtained and a mathematical model was employed to estimate the concentration *in vivo* induced by such systems. The most promising hydrogel was then sequentially loaded with diclofenac and moxifloxacin, and the same mathematical model was applied to the *in vitro* release results. The results suggest that the dual-drug loaded hydrogel could potentially release effective amounts of antibiotic and anti-inflammatory for three weeks. Nonetheless, adjustment of the concentration profiles can be achieved for example by tailoring of the loading conditions.

1. Introduction

Usually, a combination of antibiotic and nonsteroidal anti-inflammatory drugs (NSAIDs) is prescribed after cataract removal surgery [1,2], to prevent the development of infection, postoperative endophthalmitis (POE), and the progress of inflammatory response that can lead to cystoid macular edema (CME) development. POE may manifest after cataract removal surgery through patient complaints of decreased vision, pain, redness, and eyelid edema [3,4]. Studies report that in an acute phase, endophthalmitis could occur up to 13 days after surgery [5,6]. CME develops when excess fluid accumulates within the macular retina. It is thought to occur following disruption of the blood–retinal barrier that causes fluid to accumulate within the retina both intra- and extracellularly. When the edema persists for more than 6 to 9 months, chronic macular changes may occur, with permanent impairment of central vision [7].

Increasing evidence supports the use of antibiotics to reduce the bacterial load and risk of postoperative complications. Data from a survey carried out in 2014 among the members of the American Society for Cataract and Refractive Surgery show that 85% of the respondents used topical antibiotic prophylaxis preoperatively, and 97% of the respondents postoperatively [8]. Moreover, 83% of the respondents of the survey said that they would use intracameral antibiotics if an approved product was available. The major pathogens associated to post-operative endophthalmitis are coagulase-negative staphylococci, responsible for about 70% of the POE cases in the USA [9], *Staphylococcus aureus*, streptococci, other Gram-positive cocci, including enterococci and mixed bacteria, and Gram-negative bacilli [10]. Most of the coagulase-negative staphylococci associated with clinical disease belong to the normal skin flora, being *Staphylococcus epidermidis* the most predominant species [11].

Although it is a controversial topic, published studies suggest benefits to early visual recovery of topical application of NSAIDs, and also point to a decrease of likelihood of postoperative CME incidence. In a randomized clinical trial, 42 patients were given diclofenac eye drops after cataract extraction and 46, placebo. Eye drops were administered from 3 days before surgery until 3 months after, in four drops per day regime. Results showed that patients that applied diclofenac eye drops presented reduced ocular inflammation and the occurrence of angiographic CME after cataract surgery [12]. Kessel and co-workers

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performed a systematic literature search in four databases to identify randomized trials published from 1996 till 2014 comparing topical steroids with topical NSAIDs, to control inflammation and prevent CME in patients undergoing cataract extraction. They found low to moderate evidence that topical NSAIDs are more effective in controlling post-operative inflammation after cataract surgery [13].

Topical administration of ocular medication through eyedrops constitutes the most used form of treatment and prophylaxis of the eye diseases. It is estimated that this conventional dosage form accounts for approximately 90% of the commercial ophthalmic formulations [14–16]. Typically, only a small fraction of the administered dose (1–7%) is absorbed due to spillage from the eye, lacrimation and tear turnover, nasolacrimal drainage, metabolic degradation, and/or non-specific absorption. Intraocular lenses (IOLs) could potentially be used as alternative to topical administration of ophthalmic drugs with evident advantages (lower drug losses, less secondary effects, higher users comfort, etc), since they are implanted in situ, where infection and/or inflammation may develop [17].

The purpose of this work was to develop and optimize a dual drug release system able to deliver simultaneously an antibiotic and a NSAID to prevent POE and CME, based on hydrogels that could potentially be used for intraocular lenses manufacturing. To be effective, such systems should release therapeutic amounts of antibiotic for, at least, 1–2 weeks and of anti-inflammatories for, at least, 2–4 weeks [18,19].

Drug dual/simultaneous release from hydrogels has been focus of research in the last years, aiming the treatment of different healthcare problems. In a report published by Cheng and co-workers, doxorubicin and cisplatin, two chemotherapy drugs, were loaded into a dual delivery system, designed to be an in situ forming hydrogel for intratumoral treatment [20]. Murata et al. designed hydrogels that covalently contained polymeric micelles presenting different drug release properties, and successfully exhibited independent release behaviors of two compounds, rhodamine B and auramine O [21]. In the field of ocular drug delivery, Hsu and co-workers loaded commercially available contact lenses containing vitamin E with two drugs for glaucoma treatment, timolol and dorzolamide, and successfully increased the release duration [22]. A similar approach was followed by Rad and Mohajeri that studied the simultaneous loading and release of ciprofloxacin and betamethasone from vitamin E loaded silicone-based soft contact lenses [23]. White and co-workers engineered, via molecular imprinting strategies, silicone hydrogel contact lenses to simultaneously release up to four template molecules including hydropropyl methylcellulose, trehalose, ibuprofen and prednisolone [24]. As far as the authors know, dual drug release from IOLs has only been focus of study within our research group [25,26].

In the present study, a systematic work involving the optimization of the composition of home-made materials and of the drug loading conditions was carried out. Currently, intraocular lens composed of acrylic monomers studied in this work (HEMA and MMA) are available in the market as foldable IOLs. The main goal of the work was to evaluate how slight changes in the IOLs material composition could benefit drug release profiles. Moxifloxacin and diclofenac were the chosen antibiotic and anti-inflammatory drugs, respectively, as they are well studied for prevention of POE and CME and used in postoperative care. From individual drug release experiments, the most promising hydrogel was select and used as platform for simultaneous release. A mathematical model was applied to estimate the aqueous humor drug concentrations when if this system was implanted *in vivo*.

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobis(2-methylpropionitrile) (AIBN), methyl methacrylate (MMA), phosphate saline buffer (PBS, pH 7.4) were purchase from Sigma-Aldrich (USA). Moxifloxacin hydrochloride (MFX) and diclofenac sodium salt (DCF) were purchase from Carbosynth Limited (UK).

2.2. Hydrogels preparation

Two hydrogels with different HEMA:MMA ratios were prepared (HEMA80_MMA20 containing 80:20 %v/v, and HEMA90_MMA10 containing 90:10 %v/v). A third hydrogel (HEMA100) was prepared only with HEMA monomer, adding 20 %v/v of distilled and deionized (DD) water. To each of the reactional mixtures (HEMA80_MMA20: 1.69 g of HEMA + 0.37 g of MAA, HEMA90_MMA10: 1.94 g of HEMA + 0.19 g of MAA and HEMA100: 1.6 g of HEMA + 0.4 g of DD water), three different contents of cross-linker (EGDMA) were added to get final concentrations of 0.5, 2.5 and 5 wt%. After a mixing step all the preparations were bubbled with a gentle stream of nitrogen (30 min) and AIBN (initiator) was added to a final concentration of 0.4 wt%. After complete dissolution of the initiator the solution was injected into a mold consisting of two glass plates separated by a 0.254 mm Teflon spacer. The polymerization reaction was performed at 60 °C for 24 h. The obtained hydrogel sheets were washed over 5 days with DD water to remove unreacted monomers. The hydrated samples were cut with a leaker of diameter 1.5 cm, dried (dried masses ranged from 35 to 50 mg) and later used for all experiments except dual drug loading/release.

For the dual drug loading/release, HEMA90_MMA10 2.5 wt% CL hydrogel was produced following the description above, with a thickness approximated to that of the IOLs (0.5 mm). Samples were cut with 1 cm of diameter, and had weight values within the range 40–42 mg.

2.3. Swelling capacity

Determination of the equilibrium swelling capacity was performed by placing dried samples of each composition (in triplicates) in 10 mL of PBS at room temperature (20–24 °C). Several weight measurements were done until equilibrium was obtained. Swelling capacity, SC, was estimated as the relative weight gain during the hydration:

$$SC = \frac{W_\infty - W_o}{W_o} \times 100 \quad (1)$$

where W_o is the weight of the dry sample and W_∞ is the sample weight at equilibrium.

2.4. Drug loading procedure

Individual drug loading of the hydrogels was achieved through soaking in drug solutions in PBS (volume of 1.5 mL) for 7 days at room temperature (20–24 °C) with concentrations of 5 $mg\,mL^{-1}$ for MFX and 1 $mg\,mL^{-1}$ for DCF, which correspond to the drugs concentrations found in commercial eyedrops.

Dual loading was done sequentially: in a first step DCF (5 $mg\,mL^{-1}$) was loaded through soaking for 6 days, and in a second step MFX (5 $mg\,mL^{-1}$) loading was performed either for 3, 7 or 10 days. The DCF concentration was increased relatively to the individual drug loading in order to load a higher amount of drug. Drug loading in materials such as those studied in this work occurs until equilibrium between the concentration of drug inside the material and the loading solution is achieved. Maximum drug loading (in drug mass) of the material is reached when this equilibrium is attained. The equilibrium is dependent on the intrinsic properties of the drug and of the material and is reflected by the partition coefficient of the drug into the material. In the dual drug loading stage of this work, we decided to include MFX loading periods inferior to those expectable for equilibrium to reduce the possibility of release of DCF previously loaded, during the MFX loading step.

After the loading period, all the hydrogel samples were gently

immersed in DD water and blotted, to remove residual drug solution from the samples surface.

2.5. In vitro drug release experiments

Drug release experiments ($n = 3$) were performed at room temperature (20–24 °C) and under mild shaking (150 rpm) in 15 mL of PBS to ensure sink conditions. At predetermined times, the concentration of the drugs in the supernatant solution was determined through absorbance measurements using a Thermo Scientific™ GENESYS™ 10S UV–Vis spectrophotometer, in the range 190–320 nm. For dual loaded samples, quantification of the released drugs was done following the reasoning reported by Kim and Chauhan [27]. Briefly, measured UV spectra may be considered a linear combination from the two individual drugs spectra:

$$Abs_\lambda = \alpha DCF_\lambda + \beta MFX_\lambda \quad (2)$$

where Abs_λ is the UV absorbance measured at wavelength λ, and DCF_λ and MFX_λ are the reference UV absorbance of diclofenac and moxifloxacin at the wavelength λ, respectively. A least square fit method was applied to calculate the optimum values of the constants α and β. The concentration of the drugs in the sample solution can be determined multiplying such constants by the concentration of the respective reference drug solution. The use of a wavelength range allows a more accurate result.

The partition coefficient (K, ratio between the drug concentrations in the gel and in the aqueous phase) was calculated based on the release data through the following equation:

$$K = \frac{V_r C_{f,r}}{V_{gel} C_{f,l}} \quad (3)$$

where $C_{f,r}$ is the final concentration of the release medium, V_r is the volume of the release medium, V_{gel} the volume of the fully hydrated gel sample and $C_{f,l}$ the equilibrium concentration in the loading solution.

Effective diffusivity coefficients, D_e, were calculated using the procedure described in detail by Pimenta et al. [28], which assumes that the drug diffusivity is independent of time and space. It was considered that the mass transfer from the material with a certain concentration of drug is described through the Fick's second law. The one-dimensional diffusion equation was fitted to the experimental release data in order to obtain D_e, since thickness ≪ diameter.

3. Results and discussion

3.1. Swelling capacity

In Fig. 1, the results of swelling capacity (SC) of water for the three polymeric compositions and three contents of cross-linking (CL) herein studied are shown. Dependence on mixing ratio of the hydrophilic (HEMA) and hydrophobic (MMA) monomer, and amount of added cross-linker was observed. As expected, a decrease of SC as consequence of increased MMA content and increased amount of cross-linker was obtained. The relation between cross-linking agent content and water absorption capacity has been studied by a number of authors. Khan and Ranjha [29] showed that the increase in the amount of cross-linking agent in the hydrogel composition resulted in strong physical entanglements between polymer chains and in lower swelling capacity. Perera and Shanks [30] investigated the effect of cross-linking agent content on the water capacity of UV-cured MMA and HEMA hydrogels. They concluded that for both hydrogels, the higher the cross-linker concentration, the lower the equilibrium water content. Concerning the effect of MMA, Muratore and co-workers [31] observed that the equilibrium water content of two MMA based hydrogels was dependent of the amount MMA added to the polymeric mixture: higher concentrations of MMA led to lower water contents. For HEMA100 hydrogels linear dependence of cross-linker wt% was observed within the studied range (0.5 to 5 wt%). For HEMA_MMA hydrogels a non-linear dependence was found. Results suggest that when more MMA is present in the hydrogel, SC become independent of the cross-linker amount, from a certain wt% up. In fact, HEMA80_MMA20 hydrogels with 2.5 and 5 wt % of cross-linker show similar SC. Further combination of monomer mixing ratio and amount of added cross-linker should be explored to confirm this tendency.

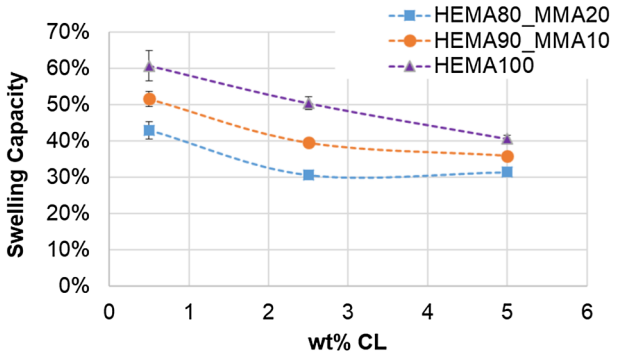

Fig. 1. Swelling capacity HEMA80_MMA20, HEMA90_MMA10 and HEMA100 in function of cross-linker (CL) content.

3.2. Individual drug release

3.2.1. Experimental release profiles

The partition and effective diffusivity values (K and D_e, respectively) of MFX and DCF in the hydrogels are presented in Fig. 2 as function of the CL content. K and D_e were obtained through experimental data fitting as described in Pimenta et al. [28]. The fractional cumulative masses released of MFX and DCF are provided as Supplementary Information, for all the nine hydrogels herein produced, with the model fitting for D_e determination.

Partition of MFX, a hydrophilic drug, in the hydrogels decreases with increased hydrophobic monomer content, and with increased wt% CL, whereas for diclofenac these relations do not occur for all cases (Fig. 2A). As expected, both MFX and DCF release kinetics are affected by the different hydrogel compositions. Dependence of D_e follows the same tendency as SC, with decrease of D_e as consequence of increased MMA content or increased amount of cross-linker, which could indicate that the aqueous phase plays a decisive role on the mechanism of transport (Fig. 2B). In general, with increased amount of added cross-linker, lower values of D_e are observed, with smaller D_e values for MFX when compare to those of DCF in the same material, except for the HEMA80_MMA20 when D_e for both drugs present similar values. Since a detailed characterization of the hydrogel-drug interactions was not the main focus of this work, no further studies were conducted.

3.2.2. Concentration predictions with mathematical model

A mathematical model for estimation of the concentrations profiles of the drug released from a soaked IOL in the aqueous humor, was used to predict the expected *in vivo* efficacy of IOLs. In this model, previously described in detail [28], the aqueous humor is represented as a film bounded by a non-deformable cornea and a non-deformable IOL. A two-dimensional geometry was assumed. Considering that the diffusion of the drug through the IOL gel matrix is a purely diffusive process, the Fick's second law with proper boundary conditions between both the vitreous-IOL and the aqueous humor-IOL, as discussed in Pimenta et al. [28], enables to describe the drug concentration in the aqueous humor $C_{aq}(t)$ through the following equation:

$$V_{aq}\frac{dC_{aq}}{dt} = A_{surface} D_e \frac{\partial C}{\partial y}\Big|_{y=h} - (k_{cornea} A_{cornea} + \not{\ell}) C_{aq} \quad (4)$$

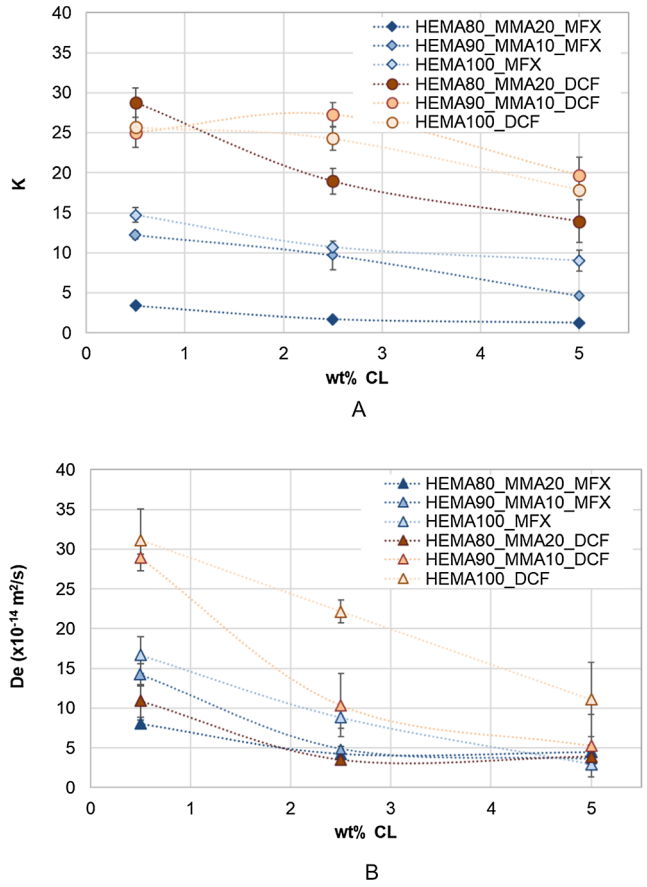

Fig. 2. (A) Partition and (B) effective diffusivity of moxifloxacin and diclofenac released from HEMA80_MMA20, HEMA90_MMA10 and HEMA100 in function of cross-linker (CL) content.

in which V_{aq} is the volume of the aqueous humor, $A_{surface}$ the permeation area of the lens, D_e, the effective diffusivity, $\not{d}$ the renovation rate of the aqueous humor and ($k_{cornea}A_{cornea}$) the drug permeation into the cornea. In the simulations here presented for the nine hydrogels loaded with MFX or DCF, we assumed lens dimensions of 6 mm of diameter and 0.6 mm of thickness (reference dimensions of a commercial IOL with + 20 diopters) and a period of 15 days of loading in 5 mg mL^{-1} solutions of either MFX or DCF.

Minimum inhibitory concentration (MIC) of MFX against susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* [32], and DCF half maximal inhibitory concentration (IC_{50}) values for the inhibition of the cyclooxygenase (COX) enzyme and the consequential reduction in prostaglandin synthesis [33] were considered to obtain information about the expected *in vivo* efficacy potential of our hydrogels (see Table 1).

Table 1
Minimum inhibitory concentration for moxifloxacin and half maximal inhibitory concentration for diclofenac [32–34].

	MIC (μg mL^{-1})
	Moxifloxacin
S. aureus	0.06
S. epidermidis	0.03
	IC_{50} (μg.mL^{-1})
	Diclofenac
COX-1	0.038 to 0.302
COX-2	0.010 to 0.029

In Fig. 3, the estimated concentrations for both drugs released from the nine hydrogels at day 21 after implantation, assuming the continuous clearance of the aqueous humor, are presented. In fact, after cataract removal surgery antibiotic and anti-inflammatory eyedrops are usually applied for 3 weeks. Considering the maximum IC_{50} assumed in this work for DCF, four hydrogel compositions are predicted to release enough DCF to remain above this concentration value during the referred period. As for MFX, only two compositions (HEMA100_2.5%CL and HEMA90_MMA10_0.5%CL) are below the required concentrations for inhibition of *Staphylococcus aureus* and *Staphylococcus epidermidis*. With the results from individual MFX and DCF release, and the *in vivo* concentration predictions, HEMA90_MMA10 2.5 wt% CL was the chosen composition for further study as platform for dual release of MFX and DCF. This was one of the compositions that released both MFX and DCF for 3 weeks above the considered MIC and IC_{50} values. The other factor considered for this choice was that in a clinical context it may not be positive to have an IOL releasing drugs for prolong periods, since it can lead to undesired secondary effects. Hence, the hydrogels that were predicted to release DCF for long periods were discarded (HEMA80_MMA20_2.5%CL, HEMA80_MMA20_5%CL and HEMA90_MMA10_5%CL).

3.3. Dual drug release

3.3.1. Experimental release profiles

When dissolution of DCF and MFX was attempted in the same solution, precipitation of drug was visually observed, therefore a sequential drug loading strategy was decided instead of simultaneous drug loading. Loading through soaking of HEMA90_MMA10 2.5 wt% CL hydrogel samples with DCF was first performed for 6 days. After this period, the hydrogel samples were loaded in MFX solution either for 3, 7 or 10 days.

In Fig. 4, photographs of hydrogel samples after loading of DCF and MFX, or only MFX are shown. Although due to the high concentration of the drug loading solution and the small amount of drug loaded into each sample, it has not been possible to determine differences in drug concentration before and after the loading step, it was possible to qualitatively access MFX uptake differences as consequence of the loading period. It is known that moxifloxacin presents a yellowish coloration [35], and therefore the samples with stronger yellow coloration (10 days of loading) are assumed to have higher MFX uptake.

The dual loaded samples partially loose transparency most probably due to the precipitation of drug molecules that occurs inside the hydrogels during final MFX loading. However, after placing the dual loaded samples in fresh PBS, light transmittance in the visible range (400–700 nm) is recovered, assuming values above 90% in all cases, after 30 min. It should be noted that the volume of solution used in the drug release tests is superior to the aqueous humor volume (≈0.25 mL), whereby longer times for transparency recovery would be expectable *in vivo*. In a normal, non-eventful cataract surgery, the patient will have a convalescence period where the operated eye will be shielded from 1 to 3 days. Therefore, patient visibility shall not be affected, since full recovery of transparency of the material should be completely reached within the convalescence period, due to the eye's aqueous humor turnover (turnover period 2 h)."

The release profiles of DCF and MFX are plotted in Fig. 5. In Table 2 partition and effective diffusivity coefficients of the drugs released simultaneously are presented and compared to those obtained for individual release from the same hydrogel.

The total mass of DCF released (see Fig. 5A) is independent of the loading period of MFX. DCF release attains plateau equilibrium after approximately 14 days of release, for the three loading sequential conditions.

However, the total mass of MFX released depends on the loading time period: MFX mass release is proportional to the loading time (see Fig. 5B). Samples loaded for 3 days released ≈0.18 mg of MFX, samples

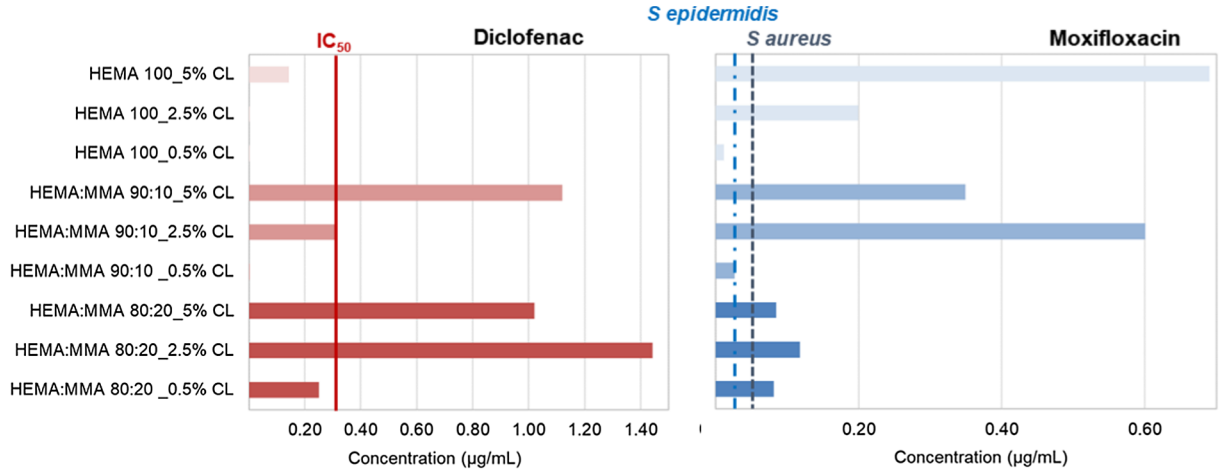

Fig. 3. Estimated concentrations of DCF and MFX released from the studied hydrogels at day 21 after implantation, assuming the continuous clearance of the aqueous humor. MICs of MFX for *S. aureus* and *S. epidermidis*, as well as the maximum value reported for IC50 of COX-1 for DCF are also presented.

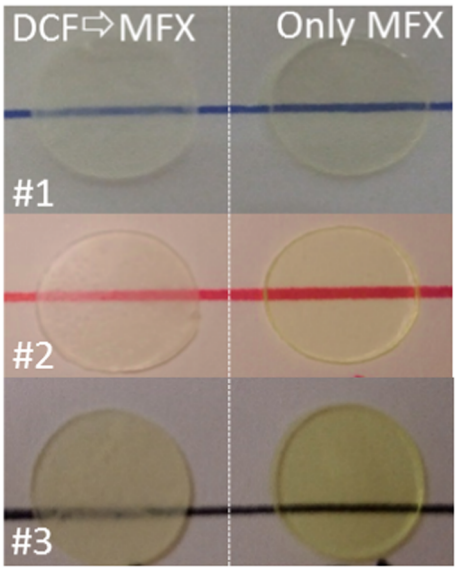

Fig. 4. Hydrogels dual loaded with DCF (6 days) and MFX (left side) and single loaded with MXF (right side). #1, #2, and #3 correspond to 3, 7 and 10 days of MFX loading, respectively.

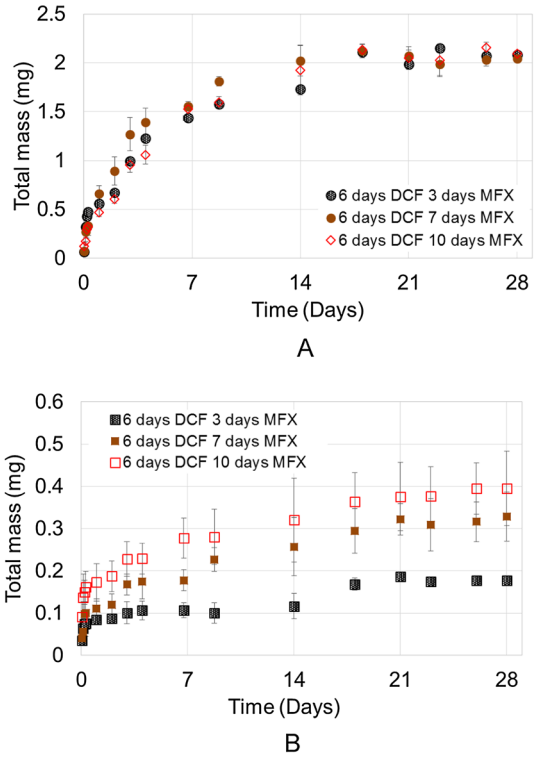

Fig. 5. (A) Diclofenac and (B) moxifloxacin total cumulative mass released from dual loaded HEMA90_MMA10 2.5 wt% CL samples.

loaded for 7 days released ≈0.33 mg of MFX and samples loaded for 10 days released ≈0.4 mg of MFX. Although for MFX, release plateau equilibrium is difficult to identify, by the 21st day of release it was achieved for all the three conditions.

When directly compared to the individual DCF and MFX released from HEMA90_MMA10 2.5 wt% CL (see Supplementary Data) dual release presents longer time duration. It must be stressed that the samples sequentially loaded presented double thickness comparatively to the individually loaded, to approximate to the real IOL thickness. In fact, in the first part of this work, the individual loading of MFX and DCF was done in the nine studied hydrogels to determine partition and diffusion coefficients and to evaluate the potential of the different materials for the intended use. Thinner hydrogel sheets were used to reduce the experiment time, since diffusion and partition coefficients are independent of the thickness of the material and only depend on the drug and material intrinsic properties. After choosing the best material for dual drug loading and delivery, the thickness of the sheets used on the drug loading and release experiments was increased to approximate the test samples to real conditions.

Since the amount of released DCF does not vary significantly for different MFX loading periods, the precipitation visible in the photographs could be attributed to DCF, indicating that until 7 days of MFX loading it is possible to retain DCF inside the hydrogel during for the propose of the sequential loading.

As for MFX partition values, they slightly increase with MFX loading time increasing, suggesting that, even if already loaded DCF impacts the efficiency of MFX loading, it is still possible to increase the amount of MFX inside the hydrogel, by extending the loading time period.

Effective diffusivity values of DCF are reduced in the dual loaded samples, when compared to the individual loaded, which could be related to drug-drug interactions inside the gel. MFX effective diffusivity does not present such meaningfully variation between the studied conditions.

Table 2
MFX and DCF partition (K) and effective diffusivity (D_e) coefficients from dual loaded HEMA90_MMA10 2.5 wt% CL samples: individual and dual release. Dual loaded samples soaked 6 days in DCF solution and 3, 7 or 10 days in MXF (#1, #2, and #3, respectively).

		Moxifloxacin	
		K	D_e ($\times 10^{-14}$ $m^2 s^{-1}$)
Individual release		9.70 ± 1.78	4.88 ± 0.31
Simultaneous release	#1	1.05 ± 0.06	3.89 ± 1.57
	#2	1.86 ± 0.23	3.75 ± 0.59
	#3	2.19 ± 0.5	5.37 ± 1.28
		Diclofenac	
		K	D_e ($\times 10^{-14}$ $m^2 s^{-1}$)
Individual release		27.26 ± 1.48	10.4 ± 0.40
Simultaneous release	#1	16.82 ± 0.50	4.31 ± 1.82
	#2	17.55 ± 0.76	7.04 ± 1.12
	#3	17.45 ± 1.55	4.03 ± 0.14

3.3.2. Concentration predictions with mathematical model

In vivo concentration predictions were done to estimate the efficacy of dual loaded IOLs made from the hydrogel. Model assumptions and parameters (drug corneal permeability, volume and turnover of the aqueous humor) described in Pimenta *et al* [28] were kept, but, since the model does not predict the simultaneous release of two different species, loading and release was simulated for each of the drugs using the values presented in Table 2. The effective diffusion coefficients (D_e) of DCF and MFX molecules determined when drugs are simultaneously released account for eventual interactions [36–38] impacting the overall drugs release when compared to individual release rate. Therefore, computational results obtained using experimental input data from simultaneous release are able to provide insight about the herein studied drug release system.

Simulation results for the first two days of implantation are plotted in Fig. 6. It is possible to observe the expected initial drug concentration burst. Maximum expected concentrations during the initial burst are approximately 70 μg mL^{-1} for DCF and 8 μg mL^{-1} for MFX. A report published by Lee and co-workers shows that a DCF commercial eye drops formulation (Ofenac®) presented toxic effects on human corneal epithelial cells, which were proportional to the drug concentration and to the exposure time. They report significant differences on cell cytotoxicity between 12 and 24 h exposure to 20 or 100 μg mL^{-1}, with no significant toxic effect for the lower DCF concentration [39]. In our case, we observe that an expected DCF *in vivo* concentration above 20 μg mL^{-1} could occur for periods of time inferior to 24 h. Nonetheless, Lee et al. results were obtained with a commercial formulation that contains preservatives, which are associate to toxic effects, particularly in long-term treatments [40,41]. To further infer about the possible toxic effect of DCF released from our preservative free IOL, *in vitro* cytotoxicity studies should be conducted.

MFX burst concentration is small when compared to the concentration of antibiotic delivered through the use of intracameral antibiotic injection during the surgical procedure [42]. Hence, a toxic effect it is not expected and, in fact, a complementary intracameral antibiotic injection should still be applied to reduce the initial bacterial load present in the eye, since a 8 μg mL^{-1} concentration value is not projected to be sufficient.

Considering the MIC values of MFX for the reference bacteria and IC_{50} range for DCF (Table 1) it is possible to estimate the efficacy of our dual drug releasing IOLs. Fig. 7 shows the estimated concentrations at day 21 after implantation. Only the hydrogel loaded for 6 days with DCF and 3 days for MFX is expected to be ineffective *in vivo* for 3 weeks.

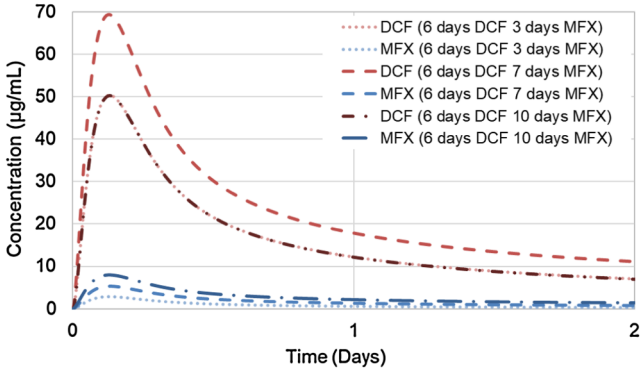

Fig. 6. Prediction of DCF and MFX concentration in the aqueous humor released from dual loaded HEMA90_MMA10 2.5 wt% CL for different loading conditions. (The curves of DCF (6 days DCF 3 days MXF) and DCF (6 days DCF 10 days MXF) are almost superimposed due to the similarity of the K and D_e values.

4. Conclusions

A system based on the use of IOLs that could simultaneously deliver effective doses of antibiotic and an anti-inflammatory over a significant period of time, is viewed as an added value for postoperative cataract removal prophylaxis. In this work, we designed and optimized a hydrogel and a dual loading strategy for the delivery of MFX and DCF to meet the described features.

Different hydrogel compositions were produced, and, through individual loading/release of both moxifloxacin and diclofenac, release kinetics was evaluated. Partition and diffusion coefficients of drugs in the different studied materials presented direct proportional dependence on the water content capacity of the materials. In general, the water content capacity of the materials decreased proportionally to the increase in the amount of the hydrophobic monomer and with the increase of the cross-linker agent. With a mathematical model, it was possible to screen the prospective *in vivo* efficacy of the hydrogels and the most promising composition was used as platform for dual drug

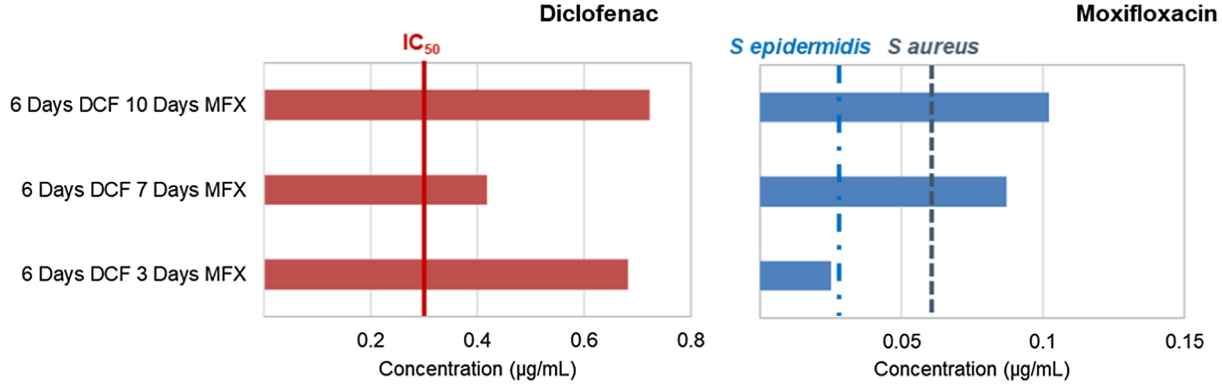

Fig. 7. Estimated concentrations of DCF and MFX released from dual loaded hydrogel at day 21 after implantation, assuming the continuous clearance of the aqueous humor.

release.

Our results show that an IOL manufactured with the proper hydrophilic/hydrophobic monomer ratio and amount of cross-linker, loaded with an antibiotic and an anti-inflammatory, following a sequential drug loading strategy, could be an effective controlled drug delivery system for cataract removal surgery postoperative prophylaxis. Moreover, it is possible to tailor the loading conditions (e.g. time, concentration) to pursue expectable *in vivo* concentration profiles that meet the desired therapeutic recommendations. Further characterization of relevant properties of the materials (e.g. mechanical, optical and other physicochemical properties) shall be performed in order to optimize the *in vivo* IOĹs performance.

Declaration of Competing Interest

A.F.R. Pimenta, A.P Serro, R. Colaço and A. Chauhan declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.05.016.

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