Chitosan/alginate based multilayers to control drug release from ophthalmic lens

Diana Silva[1], Luís F. V. Pinto[2,3], Dimitriya Bozukova[4], Luís F. Santos[1], Ana Paula Serro[1,5], Benilde Saramago[1*]

[1]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

[2] Altakitin S.A., Rua José Gomes Ferreira, Arm. D, 2660-360 São Julião do Tojal, Lisboa, Portugal.

[3] CENIMAT/I3N, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal.

[4] PhysIOL sa/nv, Liège Science Park, Allée des Noisetiers 4, 4031 Liège, Belgium.

[5]CIIEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Quinta da Granja, Monte de Caparica, 2829-511 Caparica, Portugal.

Number of words: 5,923

Number of figures: 5

Number of tables: 2

*Corresponding author

b.saramago@tecnico.ulisboa.pt

Abstract

In this study we investigated the possibility of using layer-by-layer deposition, based in natural polymers (chitosan and alginate), to control the release of different ophthalmic drugs from three types of lens materials: a silicone-based hydrogel recently proposed by our group as drug releasing soft contact lens (SCL) material and two, commercially available, intraocular lens (IOL) materials (CI26Y and Definitive 50). The optimised coating, consisting in one double layer of (alginate - $CaCl_2$) / (chitosan+glyoxal) topped with a final alginate-$CaCl_2$ layer to avoid chitosan degradation by tear fluid proteins, proved to have excellent features to control the release of the anti-inflammatory, diclofenac, while keeping or improving the physical properties of the lenses. The coating leads to a controlled release of diclofenac from SCL material- for one week and, up to 120 h, for IOL materials. Due to its high hydrophilicity (water contact angle ≈ 0) and biocompatibility, it should avoid the use of further surface treatments to enhance the user´s comfort. However, the barrier effect of this coating is specific for diclofenac, giving evidence to the need of optimizing the chemical composition of the layers in view of the desired drug.

Key words: Controlled drug release; Ophthalmic lens; Layer-by-layer coatings; Alginate; Chitosan.

1. Introduction

The development of therapeutic ophthalmic lenses which ensure extended release of suitable drugs has been faced as a solution to avoid the tedious and inefficient eye drop administration. The efforts made by many researchers in the last decades to optimize drug delivery through soft contact lenses (SCLs) have been described in several recent reviews [1–3]. In the case of cataracts which are, nowadays, one of the most frequent eye diseases, other devices have been investigated to prevent postoperative infectious complications after surgery, namely, therapeutic intraocular lenses (IOLs) [4–6].

One of the main problems in the implementation of drug-loaded ophthalmic lenses to substitute the topical application of eye drops is the control of the drug release. In fact, the drug release from these devices is usually characterized by an initial burst which may be followed by decreasing the released drug to levels under therapeutic range, in the subsequent hours. Several approaches have been undertaken to ensure a continuous delivery of medication to the eye, during the required period, and at a controlled rate: (1) incorporation of chemical agents that can interact reversibly with the drug [7]; (2) the use of nanocarriers, such as micelles, liposomes or nanoparticles [8,9]; (3) introduction of diffusion barriers to the drugs, such as Vitamin E aggregates [10,11].

One possibility that, to our knowledge, has not been much investigated is the application of coatings which could be used in commercial lenses whose properties and production are already optimized. Coatings are presently applied to commercial SCLs to improve the surface wettability and lubricity, leading, as a result, to an increased comfort to the user. Other objective of this type of coatings is to avoid the adsorption of microorganisms and proteins from the ocular tear fluid which is one of the main causes of eye infections among the contact lenses users. The coatings applied to SCLs are,

essentially, based on polyelectrolyte multilayers obtained using layer-by-layer (LbL) deposition [12], on the adsorption/grafting of specific molecules [13,14], and on the immobilization of liposomes at the surface of the lens [15]. The LbL technique has been attracting great attention because it allows a high level of control over the composition and the physical properties of the coating material. It consists on the formation of a polyelectrolyte multilayer through the consecutive adsorption of polycation-polyanion layers by electrostatic interactions. The first LbL polyelectrolyte complexes were based on strong polyelectrolytes [16], but later, research attention shifted to multilayers of weak polyelectrolytes, e.g. poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) [17], which afford greater control of the properties of the LbL coatings. The use of LbL coatings to control drug release usually relies on specific interactions between the polyelectrolytes and the drugs loaded in the nanolayers which are weakened by structural changes of the coatings resulting from external stimuli, such as changes of pH, ionic strength, solvent, and applied external energy [18–20]. LbLs have also been used to build the shells of hollow capsules where solutes may be encapsulated and then released across the membrane in the presence of a concentration gradient [21,22].

Recently, the natural polymers alginate and chitosan have been under the attention of researchers in the field of LbL applications. Owing to the biocompatibility, non-antigenicity and non- toxicity of these biopolymers, they have been considered as an excellent choice for applications in drug delivery systems [23–26] and tissue engineering scaffolds [27, 28]. Chitosan is a cationic copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-d-glucopyranase and 1-amino-2-deoxy-d-glucopyranase. Due to its physicochemical characteristics, namely permeation enhancing properties, chitosan is an adequate material for drug delivery ocular devices [29]; however, it has very low mechanical integrity and enzymatically degrades, e.g, by the action of lysozyme [30].

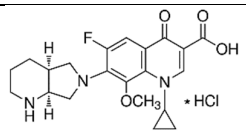
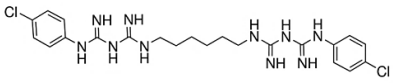
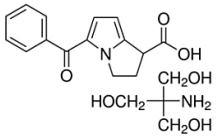
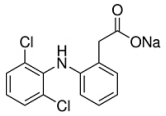
Alginate is an anionic linear polysaccharide containing -(1,4)-linked d-mannuronic acid and l-glucuronic acid residues which has the advantage of forming ionically crosslinked hydrogels [31].

The objective of the present work is to assess the performance of LbL coatings based in chitosan-alginate multilayers to control the drug release from hydrogels used as lens materials: 1) one silicone (3-tris(trimethylsilyloxy)silylpropyl 2-methylprop-2-enoate designated by TRIS)- based hydrogel which was recently investigated as drug releasing SCL material [32]; 2) two IOL materials commercially available under the names of CI26Y and Definitive 50 from Contamac Products (U.K.). CI26Y is a hydrophilic material composed by 2-hydroxyethyl methacrylate (HEMA) and methylmethacrylate (MMA), while Definitive 50 is a blend of fluorosilicone and hydrophilic monomers. CI26Y and Definitive 50 have water contents of 26% and 50%, respectively, while that of TRIS-based hydrogel is 41%.

Four drugs were tested using the TRIS-based hydrogel: an antibiotic, moxifloxacin, an antiseptic, chlorhexidine, and two anti-inflammatories, diclofenac and ketorolac. Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibiotic commonly used in the treatment of treatment of conjunctivitis, keratitis, keratoconjunctivitis, and bacterial endophthalmitis [33,34]. This molecule has a lipophilic character and its protonated form predominates in water at pH 5.6 [35]. Chlorhexidine is a hydrophilic, cationic drug with antibacterial properties which has long been known as a first line treatment for Acanthamoeba keratitis [36,37]. Diclofenac and ketorolac are non-steroidal, anti-inflammatory drugs which are mainly used to treat inflammation and to control pain in the postoperative period. Both are anionic and soluble in water as salts but, in their acidic form, they are hydrophobic drugs [38,39]. Diclofenac was further

tested in CI26Y and Definite 50**.** The characteristics of the drugs, in terms of structure, lyophilicity, ionicity and molecular weight (M_W), are summarized in Table 1.

Table 1 - Characteristics of the studied drugs.

Drug	Structure	Lyophilicity	Ionicity	M_w (g/mol)
Moxifloxacin hydrochloride		Lipophilic	Zwitterionic	401.43
Chlorhexidine diacetate		Hydrophilic	Cationic	643.57
Ketorolac tris salt		Hydrophilic	Anionic	376.4
Diclofenac sodium salt		Hydrophilic	Anionic	318.13

The formation of the chitosan/alginate layers was followed using a quartz crystal microbalance with dissipation and the topography, wettability and thickness of the LbL coatings were assessed. The effects of the coating on the transmittance and the refractive index of the samples were evaluated. Alterations in the drug release profiles induced by the LbL coating were studied.

Although LbL coatings based on chitosan and alginate have already been used to improve the surface properties of materials required for ophthalmic applications, they were never applied, to our knowledge, as a barrier to the drug diffusion. The novelty of this investigation is the successful application of an optimized chitosan/alginate LbL coating to combine a sustained drug release with adequate surface properties.

2. Materials and Methods

2.1 Materials

3-Tris(trimethylsilyloxy)silylpropyl 2-methylprop-2-enoate, 98% (TRIS), 2-hydroxyethyl methacrylate, ≥99% (HEMA), ethylene glycol dimethacrylate, 98% (EDGMA), 2,20-azobis isobutyronitrile, 98% (AIBN), acetic acid, ≥99.7%, alginic acid sodium salt from brown algae (alginate sodium) with mean molecular weight of 100-200 KDa, diclofenac sodium salt, ketorolac tris salt, ≥99%, glyoxal solution 40 % (w/w) in H_2O, polyethylenimine (PEI) with mean molecular weight of 750 KDa, were provided by Sigma–Aldrich. Chlorhexidine diacetate monohydrate, 98%, was supplied by AppliChem. N-Vinyl pyrrolidone, 98% (NVP), and sodium hydroxide, 99% (NaOH), were obtained from Merck. Sodium chloride, 99% (NaCl), sulfuric acid, 98% (H_2SO_4), and hydrogen peroxide, (H_2O_2), 30% (w/v), were purchased from Panreac. Moxifloxacin hydrochloride was purchased from Carbosynth and Hellmanex®II, from Hellma GmbH. Medical grade chitosan (high degree of acetylation), >90%, with mean molecular weight of 750 -1000 kDa, was supplied by Altakitin (Portugal), CI26Y and Definitive 50 (Contamac UK) were supplied by Physiol (Belgium). CI26Y contains a blue-light blocker which is proprietary to Physiol (patent WO2006074843; Yellow Chromophore Agent Composition for Intraocular Lenses and the thus Obtained Lenses). Polystyrene (PS) was synthesized and offered by Dra Clara Gomes from CQE-IST (Portugal). Lysozyme chicken egg white is from CalbioChem. Distilled and deionised (DD) water obtained from a Millipore system was used to prepare all solutions.

2.2 Preparation of polymeric samples

The silicone-based hydrogel (TRIS/NVP/HEMA) was synthesized, according to the procedure described elsewhere [40]. A mixture of 0.8 M of TRIS, 3.9 M of NVP, 1.8 M of HEMA, and 30 mM of EGDMA was prepared and degassed by ultrasounds (5 min) and bubbling with nitrogen (10 min). Then, AIBN was added to get a concentration of 15 mM, and the solution was homogenised by magnetic stirring. The final solution was poured into a mould constituted by two silanized glass plates separated by a Teflon frame with 0.3 mm of thickness. The silanization process reported in [41] was followed. Polymerization was done in an oven at 60°C for 24 h. In order to remove unreacted monomers and other impurities, the polymerized hydrogel was washed with DD water for 5 days, with renovation two times a day. Finally, the hydrated hydrogel sheets (thickness ≈ 300 μm) were cut in disks with 9 mm of diameter.

CI26Y and Definitive 50 samples were washed in a soxhlet extractor with DD water, for 60 cycles, according to the recommendations of the supplier and cut in disks with average thickness ≈ 1 mm and diameter of 6 mm. All samples were dried overnight in an oven at 36°C and stored inside closed flasks.

2.3 Drug loading and drug release tests

TRIS/NVP/HEMA dry samples (average weight 21 mg) were loaded with the drugs by soaking in 3 mL of drug solution with a concentration of 1 mg/mL for diclofenac and ketorolac, 2.5 mg/mL for chlorohexidine and 5 mg/mL for moxifloxacin. The loadings were done at 4°C for 38 h in the former two cases, and 72 h in the latter cases. The drugs were dissolved in a 130 mM NaCl solution, except chlorhexidine which was dissolved in DD water. The CI26Y (average weight 19.4 mg) and Definitive 50 (average weight mg) samples were loaded with diclofenac using the same conditions.

The release measurements were done by soaking the samples in a 3 mL saline solution (130 mM), at 36°C, with a 150 rpm stirring. Volumes of 200 µL were removed at scheduled times to measure the drug concentration, being replaced by the same volumes of new saline solution.

The concentration of the drugs in the collected solutions was determined by measuring the absorbance with a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific) at a wavelength of 255 nm for chlorohexidine, 276 nm for diclofenac, 290 nm for moxifloxacin and 323 nm for ketorolac.

2.4 LbL deposition

The drug loaded hydrogels were first coated with PEI which covalently bounds to the hydroxyl groups existing on their surface [42], by soaking the samples for 5 min in a PEI aqueous solution (20 mM). Meanwhile, the alginate and chitosan solutions with concentration of 10 mM each were prepared in DD water and in aqueous solution of acid acetic 1% (v/v), respectively. The pH of the alginate and chitosan solutions was 6.5 and 3.0, respectively. The pH of the chitosan solution was then adjusted to 5.0, with NaOH, in order to maintain its positive charge while avoiding depolymerisation of chitosan [43]. In some referred cases, 5% (w/w) of glyoxal solution (GL) was added to the chitosan solution. One double layer was achieved by dipping the hydrogel samples into the alginate solution for 1 min, subsequently immersing in 1 M $CaCl_2$ solution for 3 min, and finishing with chitosan solution for 1 min. To protect the chitosan layer from being enzymatically degraded by lysozyme [30,44], a final layer of alginate (crosslinked with $CaCl_2$) was deposited. After immersion in each solution, a rinsing with DD water was done. In the case of samples produced with chitosan solutions containing glyoxal,

the crosslinking of chitosan was achieved by drying the samples in the oven at 45ºC during 1 h, in the final stage.

In the meantime, drug loaded samples without coating (blank samples) were immersed in DD water during the time of the LbL formation (≈15 min) to mimic the process undergone by the coated samples. The blank samples prepared for comparison with those coated using glyoxal were finally placed inside an oven at 45°C for 1 h. With this procedure, it was ensured that the amount of drug lost during the LbL formation, which was experimentally determined through the analysis of the respective immersion solutions, was also lost by the blank samples.

The prepared samples were then immediately used for the drug release tests.

2.5 Evaluation of the LbL stability

The formation of the layers was studied using a quartz crystal microbalance with dissipation (QCM-D, E4 from Q-Sense). As previous described [40], the sensors were gold coated quartz crystals (5 MHz), which were previously covered with a polystyrene (PS) film by spin coating (2000 rpm, 30 s), and then coated with the (TRIS/NVP/HEMA) hydrogel forming reactional mixture (described in 2.2.) by spin coating (5000 rpm, 30 s).The hydrogel films were thermopolymerized at 60°C for 1 h. A nitrogen atmosphere was used for both deposition and polymerization steps to avoid the presence of oxygen which inhibits the crosslinking [45].

The experimental baseline was obtained with the hydrogel films pre-hydrated in DD water. Normalized frequency ($\Delta f/n$) and dissipation (ΔD) changes (where n = 1, 3, 5, 7, 9, and 11 corresponds to the number of the overtone of the fundamental frequency, 5 MHz) were monitored during the process of LbL deposition. After the addition of each solution a rinsing step was done with DD water.

The eventual degradation effect of the lysozyme on the LbL coating was studied by adding lysozyme solution after the stabilization of layers, followed by rinsing with NaCl solution. The concentration of lysozyme solution was 1.9 mg/mL to simulate that of the lacrimal fluid [46–48]. Eight independent experiments were made at 36°C.

After each experiment, the quartz crystals were cleaned by dipping for 5 s in piranha solution: H_2SO_4/H_2O_2 7/3 v:v (**Caution**: Piranha solution is highly corrosive and reacts violently with organic matter). Immediately afterwards, the crystals were washed in ultrasounds with a 2% (v/v) Hellmanex solution (15 min), followed by water (2x15 min). Then, they were rinsed with DD water, dried with a nitrogen flux and stored inside closed flasks.

2.6 Topography

Topographic images of the surfaces of the hydrated hydrogels were obtained using an atomic force microscope (AFM) Nanosurf EasyScan 2. The analyses were done in tapping mode, at room temperature. Images of 20x20 μm^2 were obtained with silicon probes (resonance frequency: 204 – 497 kHz) at a scan rate of 0.7 Hz. The average roughness (R_a) of the surfaces was obtained for the total area of the images, using the software WSxM 5.0 develop 8.0.

2.7 Wettability

The wettability of the dry hydrogels was determined using the sessile drop method. The samples were previously dried for 72 h inside a vacuum oven, at room temperature. Drops of DD water were deposited with a micrometric syringe and on the substrate surface inside a temperature controlled chamber model 100-07-00 (Ramé-Hart, NJ, USA).The hydrated hydrogels were characterized by measuring the contact angles of

captive air bubbles lying underneath the substrates immersed in water. The images of drops and bubbles were obtained through a video camera (jAi CV-A50, Spain) mounted on a microscope Wild M3Z (Leica Microsystems, Germany) and analyzed by running the ADSA (Axisymmetric Drop Shape Analysis, Applied Surface Thermodynamics Research Associates, Toronto, Canada) software.

2.8 Optical properties

The transmittance of the hydrated hydrogel samples was determined with a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific). The wavelength interval of 400 to 700 nm was scanned with 1 nm intervals.

Ellipsometric functions tan Ψ and cos Δ were measured in the spectral range from 300 – 850 nm using a phase modulated spectroscopic ellipsometer (UVISEL, Horiba Jobin–Yvon), at 70^{o} incidence angle. The thickness and refractive index of the films/hydrogels were determined through suitable modelling using the DeltaPsi2 software package from Jobin-Yvon with a Cauchy dispersion function.

3. Results and discussion

3.1 Optimization of the LbL coating

The formation of the layers during the LbL deposition process was followed by QCM-D. The time course of $\Delta f/n$ and ΔD upon contact of the TRIS/NVP/HEMA hydrogel-coated quartz crystals with the polyelectrolyte solutions is shown in Figure 1.

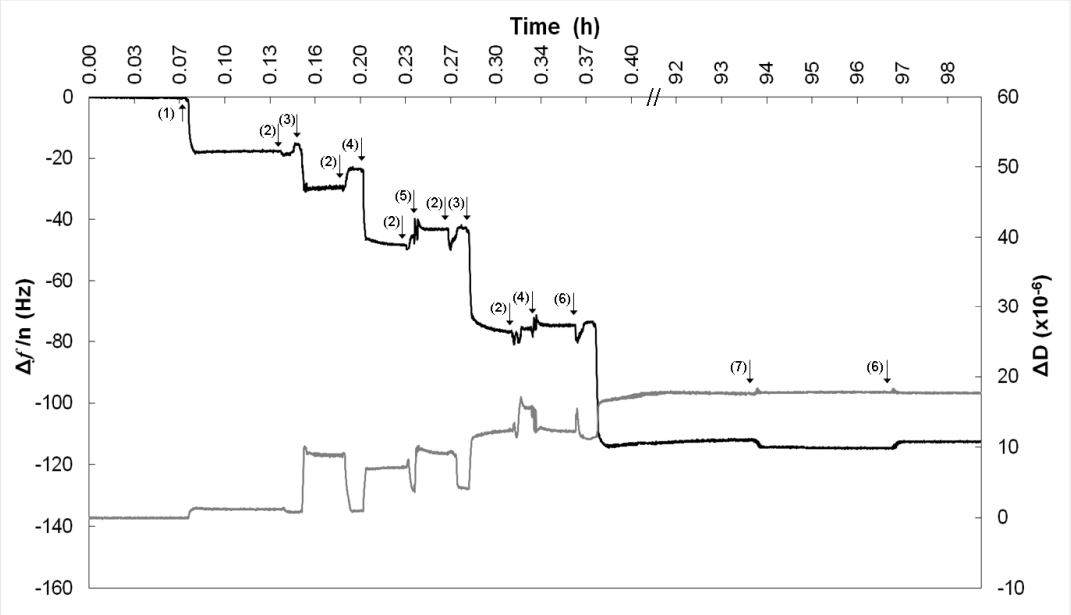


Figure 1

The baseline corresponds to the coated sensors equilibrated with water. After injection of the PEI solution, alginate, $CaCl_2$ and chitosan solutions were sequentially introduced, terminating with a second injection of alginate and $CaCl_2$ .The observed frequency and dissipation shifts indicate the formation of stable layers, where the rinsing steps performed after each injection had a small effect, demonstrating that the layers remained irreversibly adsorbed on the surface. The resistance of this coating terminated with a final layer of alginate and $CaCl_2$ against lysozyme was confirmed from the observation that the injection of the protein solution did not induce any change in the frequency and dissipation of the adsorbed layers for several hours (> 4 h). At this point, it is important to refer that the toxicity of PEI should not be a problem because it was used as the deepest layer which remained attached to the surface. Moreover, there are several reports in the literature about the reduction of the cytotoxicity of PEI in presence of chitosan and alginate [49,50]. However, cytocompatibility tests would be necessary before in vivo application.

In order to test the effect of the number of layers, 1, 2 and 4 (alginate-$CaCl_2$)/chitosan double layers were deposited on top of the TRIS/NVP/HEMA surface always terminated by an alginate-$CaCl_2$ layer. The cumulative release profiles of diclofenac from TRIS/NVP/HEMA hydrogels are presented in Figure 2.

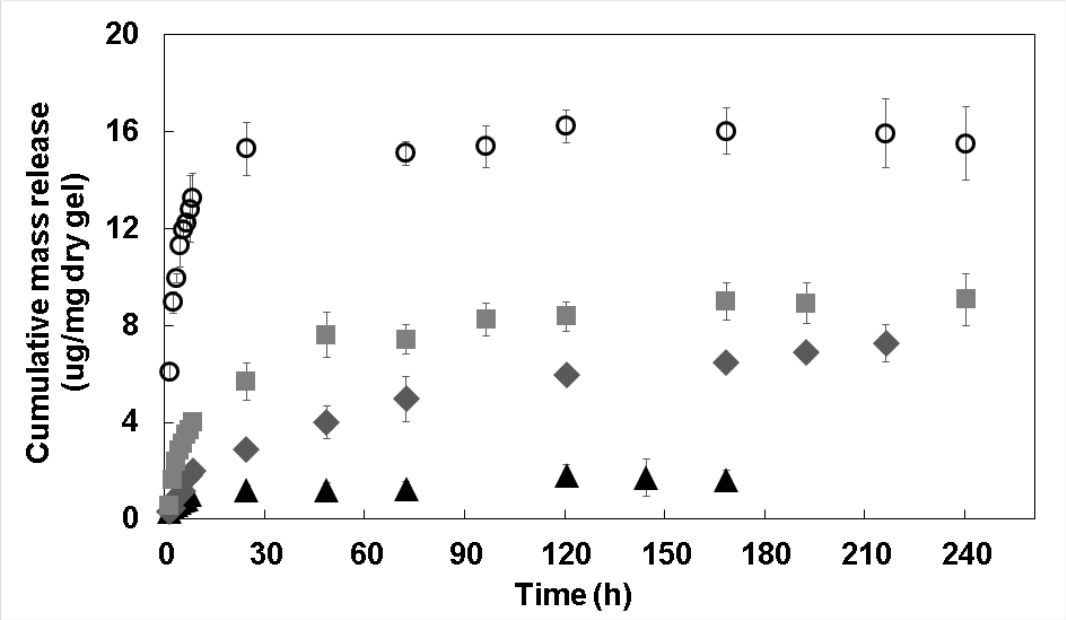


Figure 2

Analysis of the figure shows that deposition of 1 and 2 double layers decreases the initial rate of drug release and extends the release duration, thus, significantly improving the release profile. However, 4 double layers almost hinder the release of the drug which led us to abandon this number of layers. Then, the wettability, roughness, transmittance and coating thickness of the hydrogels with 0, 1 and 2 double layers were determined and the measured values are presented in Table 2 (three first columns). The thickness of 1 double layer could not be measured apparently due to the heterogeneous nature of this coating. The contact angles of water on the hydrated sample (captive bubble) with 1 double layer and the dried samples (sessile drop) with 1 and 2 double layers were not measured.

Table 2- Properties of uncoated and coated TRIS/NVP/HEMA hydrogels: water contact angles on hydrated and dried samples, average roughness (R_a), transmittance, coating thickness, and refraction index. The errors are standard deviations (in all cases n=3, except the contact angles with n=10).

	Uncoated samples	**1 Double layer**	**2 Double layers**	**1 Double layer with glyoxal**
Contact angle (°) (captive bubble)	35±5	-	42±2	≈ 0
Contact angle (°) (sessile drop)	83±6	-	-	27±5
Average roughness, (nm)	20±9	26±7	16±8	33±2
Transmittance (%)	98±0.5	92±1	80±1	94±2
Thickness (nm)	-	-	60±3	40±1
Refraction index	1.46±0.02	1.41±0.02	1.48±0.01	1.50±0.01

The roughness increased after the first double layer but then decreased when the second double layer was introduced; the refraction index lowered with the first double layer, but recovered for the second one, which may be attributed to different contents of water in these samples. The wettability of the hydrated sample with the 2 double layers coating slightly decreased.

The AFM images showing the topography of the samples are presented in Figure 3 a), b) and c). The porous structure, which is typical of these hydrogels and has already been reported in a previous work [32], is clearly identified on the uncoated hydrogel. It completely disappears on the sample coated with 2 double layers. In the 1 double layer case, only a few pores are still visible. Although 2 double layers seemed to yield a more

adequate coating from the point of view of physical homogeneity and drug release control, the reduction of the transmittance to 80 % did not allow pursuing this route.

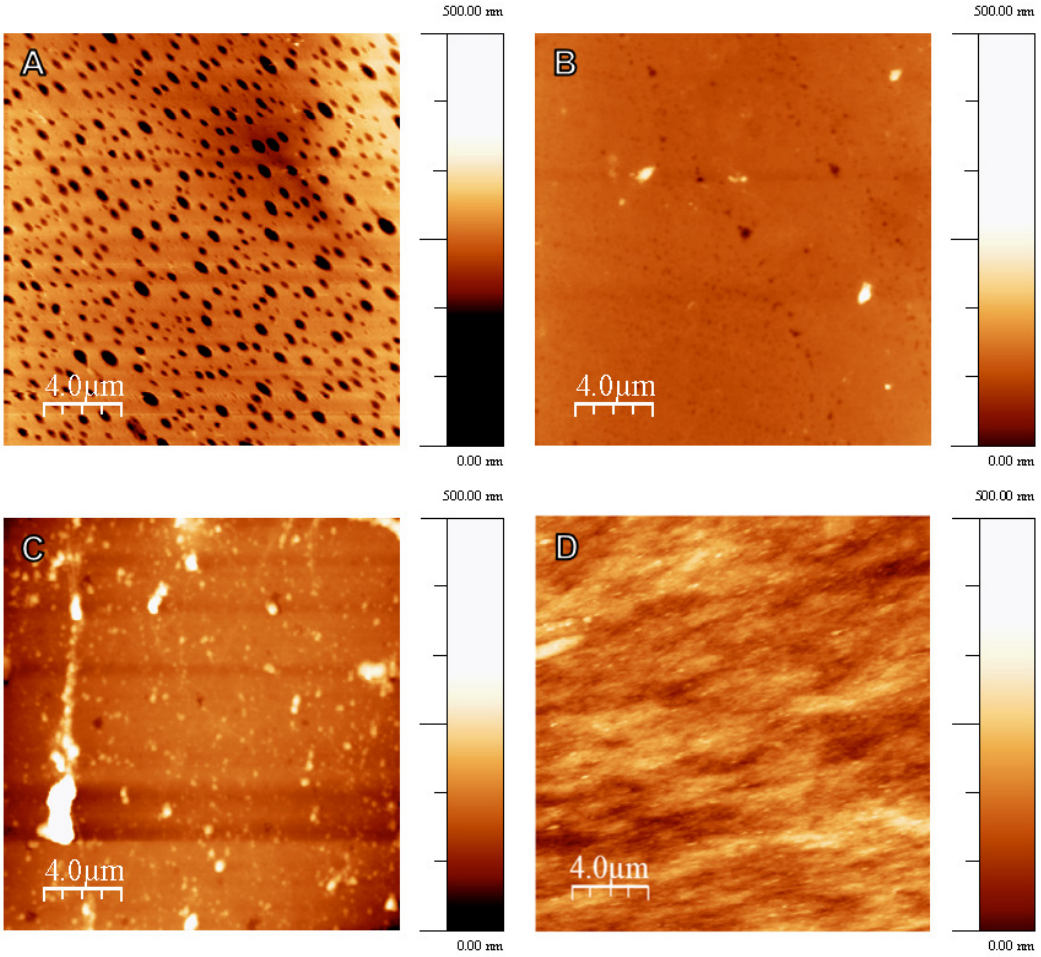


Figure 3

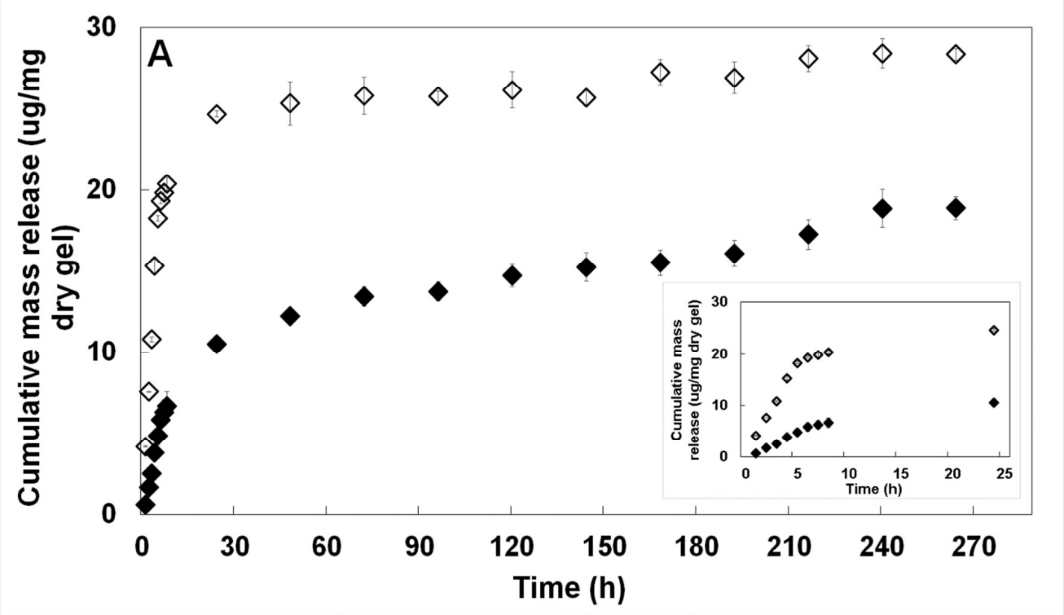
We then focused our attention on the 1 double layer coating. In order to achieve a consistent uniform coating, the crosslinking agent glyoxal, which is a small aldehyde known to be biocompatible [51], was added to the chitosan solution. Since the coated sample had to be dried at 45 °C, it was not possible to follow the LbL deposition with the QCM-D. The wettability, roughness, transmittance, coating thickness and refraction index of these new coated samples were determined and are shown in Table 2 (right column). This coating has very interesting properties: it is very hydrophilic, even in the dry state; it is quite homogeneous (the ellipsometric measurements easily converged in

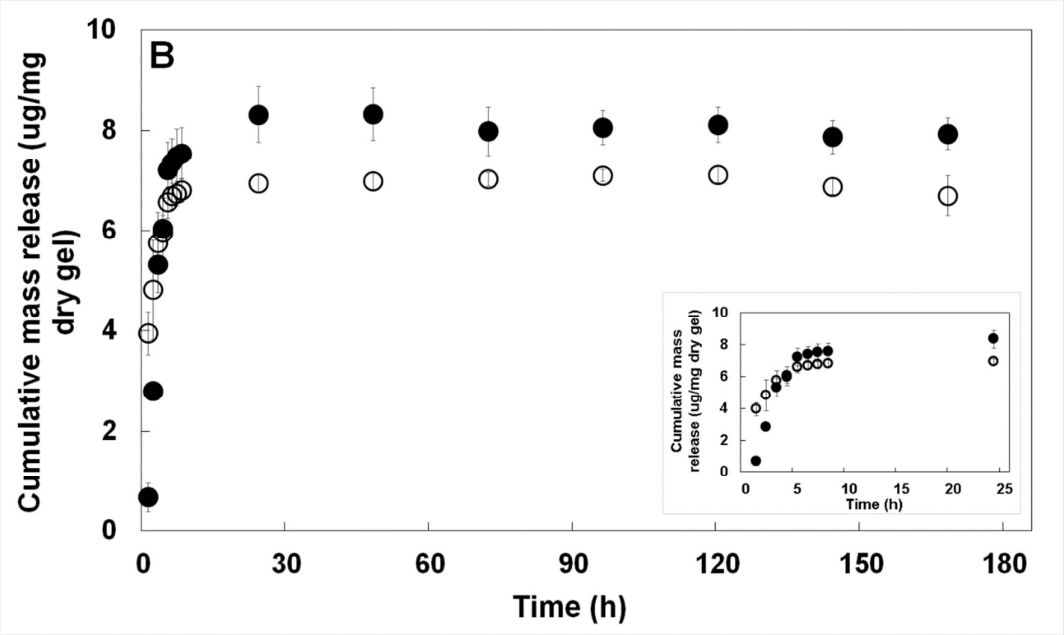
a precise value of thickness); it ensures a transmittance value above 90% which is the minimum required for ophthalmic lenses [52,53]; it has a small effect on the bulk refraction index of the hydrogel; it should resist to degradation by lysozyme (as demonstrated by QCM-D data, in Figure 1, for the coating without glyoxal). The contact angle of the hydrated sample, measured with the captive bubble technique, was considered to be null due to the bubble instability which derives from the rather hydrophilic nature of this surface. The AFM image, shown in Figure 3D, reveals a dense coating which completely covers the underlying hydrogel. Thus, the use of this type of LbL coating seems to be very promising for drug control release in ophthalmic lenses and its effectiveness was tested in different drug/lens systems.

3.2 Drug release profiles of coated ophthalmic hydrogels

The release profiles of diclofenac, ketorolac, moxifloxacin, and chlorhexidine from TRIS/NVP/HEMA coated with 1 double layer (alginate-$CaCl_2$)/(chitosan+glyoxal) terminated with alginate-$CaCl_2$ are presented in Figure 4. Comparison of the obtained profiles reveals that diclofenac stands out as leading to the most efficient release. Apparently, the uptake of diclofenac by the hydrogel is large and reversible yielding a cumulative mass release about three times larger than that of the other drugs. In a recent study by our group on drug partitioning and diffusion [54], the release of diclofenac and chlorhexidine in TRIS/PVP/HEMA were compared. Although both drugs had similar partition coefficients, chlorhexidine showed a much stronger adsorption on the polymeric fibers than diclofenac which was attributed to the interaction of positively charged amine groups with the acrylate groups in the HEMA monomers. Moxifloxacin, being lipophilic, should adsorb on the hydrophobic sites of the chains (TRIS monomers). The difference between diclofenac and ketorolac is difficult to explain.

This means that the uptake and release of diclofenac should be preferentially determined by the aqueous phase of the hydrogel and that bulk diffusion essentially determines the release profile.





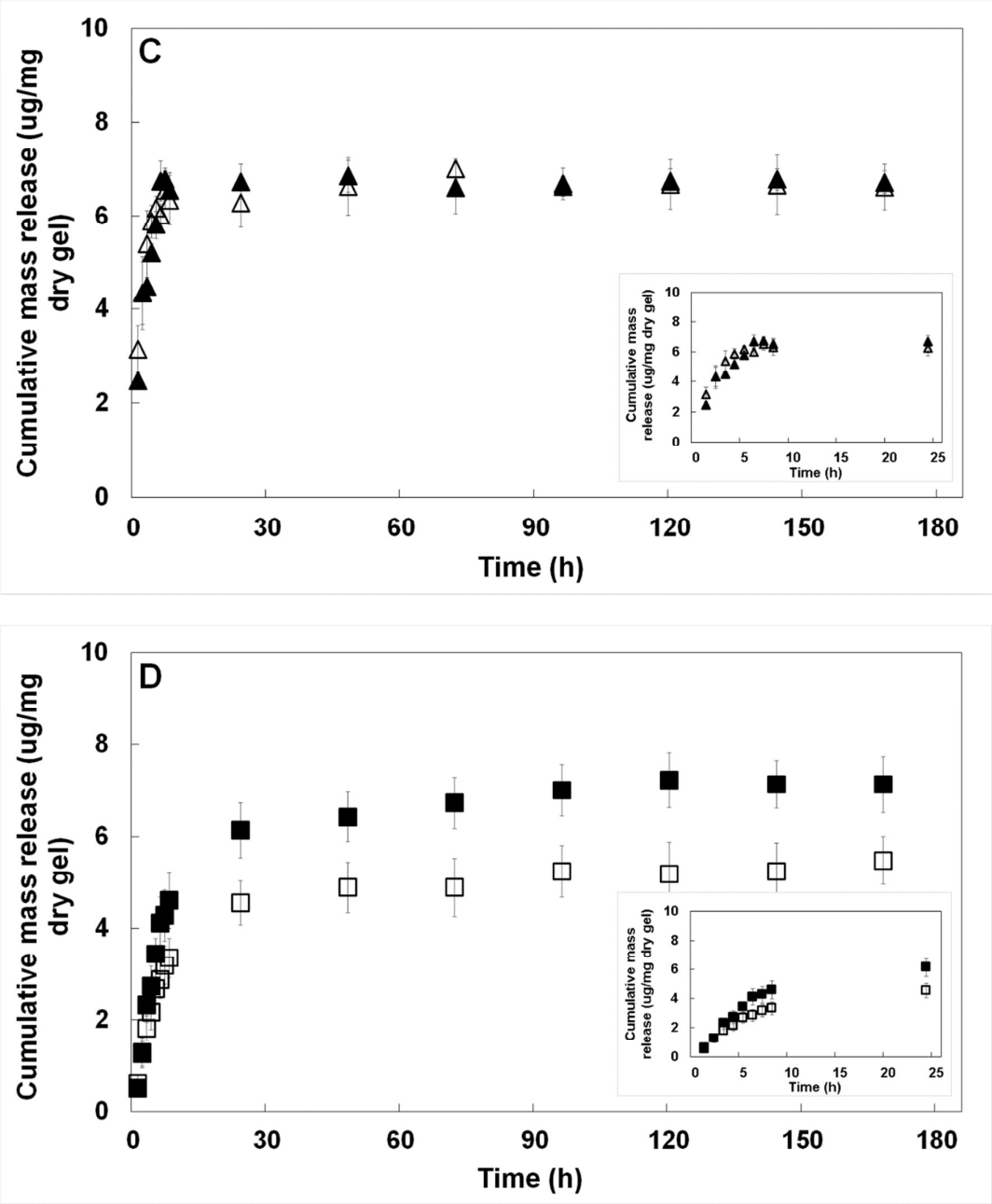


Figure 4

The barrier effect of the LbL coating is clearly most effective for diclofenac. In the case of moxifloxacin, the profile was not affected, while for ketorolac and chlorhexidine the released amount increased but the rate of release did not improve. Diclofenac is the smallest molecule among the studied drugs. Taking into consideration only its size, the special efficiency in the release control demonstrated by the LbL coating is unexpected.

Several authors proposed the use of chitosan matrices to sustain the release of diclofenac from tablets or nanoparticles [55]. Sabnis et al [56] found that, in acidic media, the release of diclofenac from chitosan matrices was slowest for chitosan of high degree of deacetylation, meaning that the number of amino groups present in the polymer backbone is an important factor to control the drug release via (1) the formation of a chitosan gel barrier and (2) ionic interactions between ionized amino groups and anionic diclofenac. González-Rodríguez et al [57] prepared alginate-chitosan microspheres for diclofenac release and claimed that drug release was controlled by the interactions between diclofenac and the polycation chitosan, in competition with alginate. However, other authors claimed that although chitosan matrices efficiently entrap diclofenac, the ionic interaction between them is low; in other words, the complexes formed between these two molecules should have low stability [58,59].The formation of these unstable complexes of chitosan-diclofenac may offer an explanation for the retardation effect felt by the diclofenac when crossing the surface layer during the release process. There are also reports in the literature on the use of chitosan-alginate nanodispersions for ocular sustained delivery of ketorolac but the initial burst was not avoided [60]. In our case, the chitosan/alginate based coating decreased the initial burst but did not reduce the following release rate. The increase in the amount of ketorolac and chlorhexidine released in the presence of the chitosan/alginate coating is difficult to explain. One possibility could be a decrease in the density of the coating caused by the interaction with the drug. Abruzzo et al [61] reported a decrease in the density of chitosan/alginate matrices loaded with chlorhexidine, suggesting some extension of the polymeric chains in presence of drug.

In view of the efficient control of the release of diclofenac achieved with this LbL coating on TRIS/NVP/HEMA samples, other substrates were tested. The release

profiles of diclofenac from IOL materials (CL26Y and Definitive 50), shown in Figures 5A and 5B, respectively, reveal that the amount of drug released is much higher for the more hydrophobic silicone-based Definitive 50 hydrogel (Figure 5B). Furthermore, comparison of the IOL materials with the TRIS/NVP/HEMA (Figure 4A) confirms that the diclofenac release is directly correlated with the water content of the hydrogel which is minimum for CL26Y. The barrier effect of the LbL coating exists for both IOL materials but it is more striking in the case of Definitive 50, where the initial release rate is considerably reduced and the release duration increased, at least, up to 120 h.

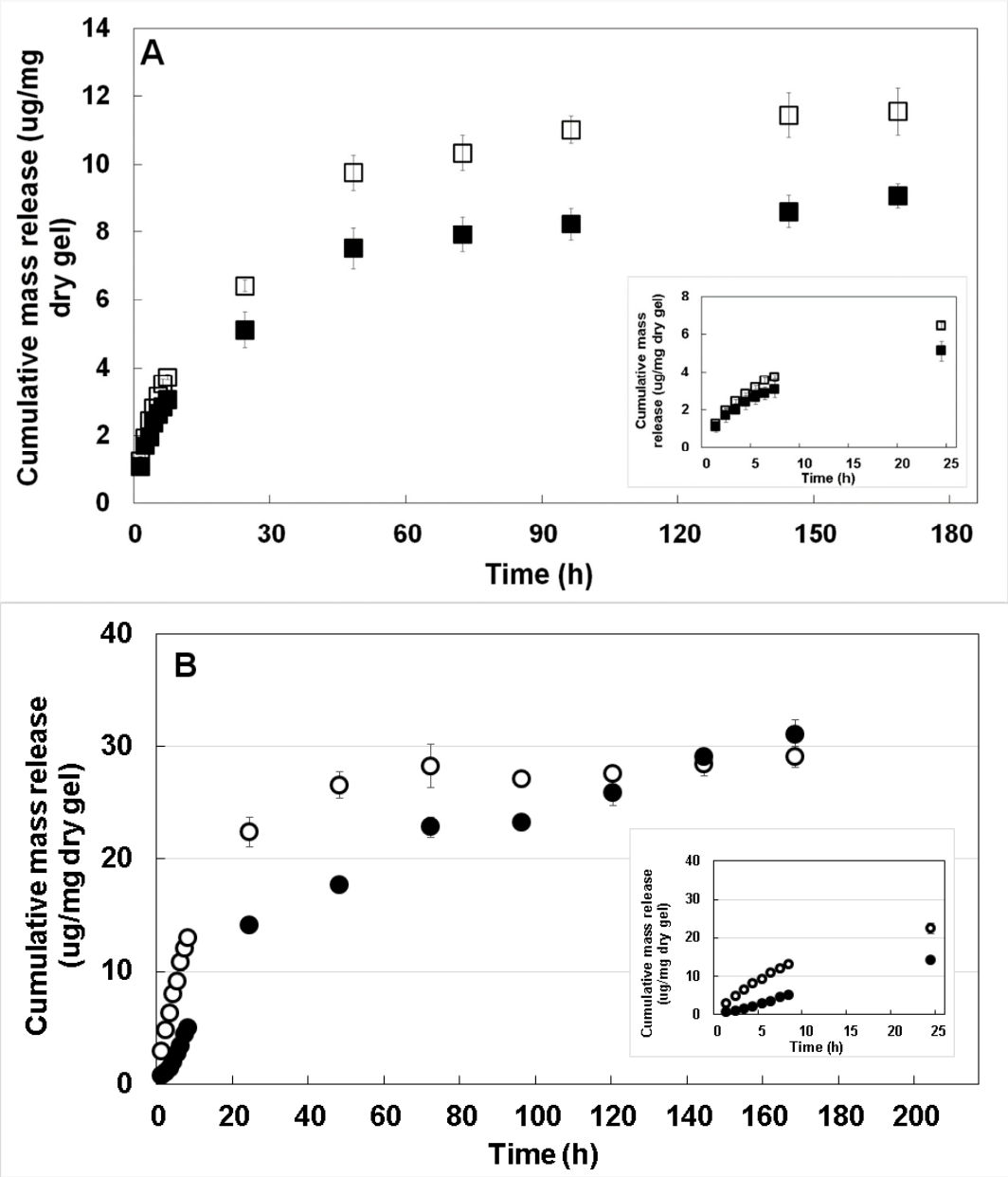


Figure 5

Although, further tests are needed to confirm the feasibility of using these alginate-chitosan based LbL coatings on ophthalmic materials, namely their mechanical properties, resistance to blinking and cytocompatibility, our study suggests that they may offer a valuable solution to control the release of diclofenac from different lens materials. Besides their ability to avoid the initial burst, typical of drug loaded lenses,

these coatings increase dramatically the hydrophilicity of the silicone-based material, thus avoiding extra surface treatments usually performed to ensure high comfort to lens wearers and to minimize deposits of lipids and proteins from the tear fluid. The barrier effect of the coating seems to be independent of the size of the drug molecule, but it is strongly determined by its chemical nature. The coating was not efficient for the control of the release of the other studied drugs, even the anionic ketorolac. At this point, we are not able to give a reasonable explanation for the different behaviours. However, in view of the promising results obtained with diclofenac, it would be important to pursue with this investigation, looking for other types of functionalized chitosan and/or crosslinking agents in order to optimize the reversible interactions between drug and coating needed to ensure a sustained release.

4. Conclusions

This work describes an investigation about the use of coatings obtained with the LbL deposition of alginate/chitosan-based layers to control the drug release from ophthalmic lens materials. Optimization of the properties of the coated samples taking into consideration the requirements for their application as ophthalmic lens materials was first attempted using TRIS/NVP/HEMA hydrogel and diclofenac. Very good results were obtained with the double layer (alginate-$CaCl_2$)/(chitosan+glyoxal) topped with a final alginate-$CaCl_2$ layer to avoid chitosan degradation by tear fluid proteins. The coating is dense, homogeneous, and very hydrophilic; it does not affect the bulk refraction index, slightly reduces the light transmittance and leads to a controlled release of diclofenac for more than one week. Such a promising behaviour led us to investigate its performance with other drugs (ketorolac, moxifloxacin and chlorhexidine) and two

IOL materials (CI26Y and Definitive 50). The barrier effect of the coating revealed to be strongly affected by the characteristics of the pair hydrogel/drug: it existed for the three tested hydrogels but was more prominent for TRIS/NVP/HEMA; surprisingly, only diclofenac, which is the smallest molecule, was effectively controlled. Further studies using adequately functionalized chitosan should be done to optimize the release control of each specific drug.

Acknowledgements

The authors thank Fundação para a Ciência e a Tecnologia for Diana Silva PhD Grant (PD/BD/114088/2015), and for funding through the projects UID/QUI/00100/2013 and M-ERA.NET/0005/2012. Dr Clara Gomes from CQE-IST is greatly acknowledged for providing polystyrene.

References

[1] C. González-Chomón, A. Concheiro, C. Alvarez-Lorenzo, Soft contact lenses for controlled ocular delivery: 50 years in the making, Ther. Deliv. 4 (2013) 1141–1161.

[2] P.W.J. Morrison, V. V. Khutoryanskiy, Advances in ophthalmic drug delivery, Ther. Deliv. 5 (2014) 1297–1315.

[3] A. ElShaerl, B. Ghatoral, S. Mustafal, R.G. Alany, Contact lenses as drug reservoirs & delivery systems: the successes & challenges, Ther. Deliv. 5 (2014) 1085–1100.

[4] C. González-Chomón, A. Concheiro, C. Alvarez-Lorenzo, Drug-Eluting Intraocular Lenses, Materials (Basel). 4 (2011) 1927–1940.

[5] Y. Masmoudia, L.B. Azzouka, O. Forzanob, J.M. Andreb, E. Badensa, Supercritical impregnation of intraocular lenses, J. Supercrit. Fluids. 60 (2011) 98–105.

[6] A. Shukla, R.C. Fuller, P.T. Hammond, Design of multi-drug release coatings targeting infection and inflammation, J. Control. Release. 155 (2011) 159–166.

[7] C. Lokendrakumar, A. Chauhan, Extended delivery of an anionic drug by contact lens loaded with a cationic surfactant, Biomaterials. 34 (2013) 2814–2821.

[8] D. Gulsen, A. Chauhan, Ophthalmic drug delivery through contact lenses, Invest. Ophthalmol. Vis. Sci. 45 (2004) 2342–2347.

[9] I.M. Carvalho, C.S. Marques, R.S. Oliveira, P.B. Coelho, P.C. Costa, D.C. Ferreira, Sustained drug release by contact lenses for glaucoma treatment-a review., J. Control. Release. 202 (2015) 76–82. doi:10.1016/j.jconrel.2015.01.023.

[10] J. Kim, C.C. Peng, A. Chauhan, Extended release of dexamethasone from silicone-hydrogel contact lenses containing vitamin E, J. Control. Release. 148 (2010) 110–116.

[11] C.C. Peng, M.T. Burke, A. Chauhan, Transport of Topical Anesthetics in Vitamin E Loaded Silicone Hydrogel Contact Lenses, Langmuir. 28 (2012) 1478–1487.

[12] Y. Qiu, C. Winterton, J.M. Lally, Y. Matsuzawa, Method for Applying an LbL Coating onto a Medical Device, 2005.

[13] J.L. Court, R.P. Redman, J.H. Wang, S.W. Leppard, V.J. Obyrne, S.A. Small, et al., A novel phosphorylcholine-coated contact lens for extended wear use, Biomaterials. 22 (2001) 3261–72.

[14] W. Cheng, C. Yang, X. Ding, A. Engler, J. Hedrick, Y. Yan, Broad-Spectrum Antimicrobial/Antifouling Soft Material Coatings using Poly(ethylenimine) as a Tailorable Scaffold, Biomacromolecules. 16 (2015) 1967–1977.

[15] A. Danion, I. Arsenault, P. Vermette, Antibacterial activity of contact lenses bearing surface-immobilized layers of intact liposomes loaded with levofloxacin, J Pharm Sci. 96 (2007) 2350–63.

[16] Y. Guo, W. Geng, J. Sun, Layer-by-layer deposition of polyelectrolyte-

polyelectrolyte complexes for multilayer film fabrication, Langmuir. 25 (2009) 1004–10.

[17] M. Berg, Biological applications of weak polyelectrolyte multilayers, Massachusetts Institute of Technology, 2005.

[18] A.B. Scranton, B. Rangarajan, J. Klier, Biomedical Applications of Polyelectrolytes, in: N. Peppas, R. Langer (Eds.), Adv. Polym. Sci., Springer-Veflag Berlin, 1995: pp. 1–54.

[19] X. Su, B. Kim, S.R. Kim, P.T. Hammond, D.J. Irvine, Layer-by-Layer-Assembled Multilayer Films for Transcutaneous Drug and Vaccine Delivery, ACS Nano. 3 (2009) 3719–3729.

[20] P. Chen, X. Wang, Y. Dong, X. Hu, Development of a Layer-by-Layer Assembled Film on Hydrogel for Ocular Drug Delivery, Int. J. Polym. Sci. (2015) 1–9.

[21] G. Berth, A. Voight, H. Dautzenberg, E. Donath, H. Möhwald, Polyelectrolyte Complexes and Layer-by-Layer Capsules from Chitosan/Chitosan Sulfate, Biomacromolecules. 3 (2002) 579–590.

[22] J. Kang, L. Dähne, Strong Response of Multilayer Polyelectrolyte Films to Cationic Surfactants, Langmuir. 27 (2011) 4627–4634.

[23] H. Lu, Z. Chen, X. Yang, L. Cen, X. Zhang, P. Gao, Layer-by-layer self-assembly of minocycline-loaded chitosan/alginate multilayer on titanium substrates to inhibit biofilm formation, J. Dent. 42 (2014) 1464–1472.

[24] Q. Wang, Z. Dong, Y. Du, J.F. Kennedy, Controlled release of ciprofloxacin hydrochloride from chitosan / polyethylene glycol blend films, Carbohydr. Polym. 69 (2007) 336–343.

[25] Y. Yang, S. Wang, Y. Wang, X. Wang, Q. Wang, M. Chen, Advances in self-assembled chitosan nanomaterials for drug delivery, Biotechnol. Adv. 32 (2014) 1301–1316.

[26] F. Ding, H. Deng, Y. Du, Q. Wang, Emerging chitin and chitosan nanofibrous materials for biomedical applications, Nanoscale. 6 (2014) 9477–9493. doi:10.1039/C4NR02814G.

[27] C.R. Wittmer, J.A. Phelps, C.M. Lepus, W.M. Saltzman, M.J. Harding, P.R. Van Tassel, Multilayer nanofilms as substrates for hepatocellular applications, Biomaterials. 29 (2008) 4082–4090.

[28] Q. Wang, S. Jamal, M.S. Detamore, C. Berkland, PLGA-chitosan/PLGA-alginate Nanoparticle Blends as Biodegradable Colloidal Gels for Seeding Human Umbilical Cord Mesenchymal Stem Cells, J Biomed Mater Res B. 96 (2012) 520–527.

[29] A. Bernkop-Schnürch, S. Dünnhaupt, Chitosan-based drug delivery systems, Eur. J. Pharm. Biopharm. 81 (2012) 463–469.

[30] L. Wei, C. Cai, J. Lin, L. Wang, X. Zhang, Degradation controllable biomaterials constructed from lysozyme-loaded Ca-alginate microparticle/chitosan composites, Polymer (Guildf). 52 (2011) 5139–5148.

[31] L. Grøndahl, G. Lawrie, A. Jejurikar, Alginate-based drug delivery devices, Biointegration Med. Implant Mater. (2010) 236–266. doi:10.1533/9781845699802.2.236.

[32] P. Paradiso, R. Galante, L. Santos, A.P. Alves de Matos, R. Colaço, A.P. Serro, et al., Comparison of two hydrogel formulations for drug release in ophthalmic lenses, J Biomed Mater Res B. 102 (2014) 1170–1180.

[33] D. Miller, Review of moxifloxacin hydrochloride ophthalmic solution in the treatment of bacterial eye infections, Clin Ophthalmol. 2 (2008) 77–91.

[34] E. Vasil'eva, S. Kidalov, V. Sokolov, G. Klimov, J. Puguang, Properties of copper-detonation nanodiamond composites obtained by spray drying, Tech. Phys. Lett. 39 (2013) 137–139. doi:10.1134/S1063785013010410.

[35] S. Lemaire, P.M. Tulkens, F. Van Bambeke, Contrasting Effects of Acidic pH on the Extracellular and Intracellular Activities of the Anti-Gram-Positive Fluoroquinolones Moxifloxacin and Delafloxacin against Staphylococcus aureus, Antimicrob. Agents Chemother. 55 (2011) 649–658.

[36] M. Elder, J.G. Dart, S. Kilvington, D.V. Seal, J. Hay, C.M. Kirkness, Chemotherapy for acanthamoeba keratitis, Lancet. 345 (1995) 791–793.

[37] W. Mathers, Use of higher medication concentrations in the treatment of acanthamoeba keratitis, Arch Opthalmol. 124 (2006) 923.

[38] A. Fini, G. Fazio, I. Rapaport, Diclofenac⁄N-(2-hydroxyethyl) pyrroli-dine: a new salt for an old drug, Drugs Exp Clin Res. 19 (1993) 81–8.

[39] A.K. Gupta, S. Madan, D.K. Majumdar, A. Maitra, Ketorolac entrapped in polymeric micelles: preparation, characterisation and ocular anti-inflammatory studies, Int J Pharm. 209 (2000) 1–14.

[40] D. Silva, A.C. Fernandes, T.G. Nunes, R. Colaço, A.P. Serro, The effect of albumin and cholesterol on the biotribological behaviour of hydrogels for contact lenses., Acta Biomater. 26 (2015) 184–194. doi:10.1016/j.actbio.2015.08.011.

[41] R. Vazquez, R. Nogueira, M. Orfao, J. Mata, B. Saramago, Stability of triglyceride liquid films on hydrophilic and hydrophobic glasses, J. Colloid Interface Sci. 299 (2006) 274–282.

[42] L. Xinming, C. Yingde, A.W. Lloyd, Polymeric hydrogels for novel contact lens-based ophthalmic drug delivery systems: a review, Contact Lens Anterior Eye. 31 (2008) 57–64.

[43] C.K.S. Pillai, W. Paul, C.P. Sharma, Chitin and chitosan polymers: Chemistry, solubility and fiber formation, Prog. Polym. Sci. 34 (2009) 641–678. doi:10.1016/j.progpolymsci.2009.04.001.

[44] D. Mawad, C. Warren, M. Barton, D. Mahns, J. Morley, Lysozyme depolymerization of photo-activated chitosan adhesive films, Carbohydr Polym. 121 (2015) 56–63.

[45] J. Teichroeb, J. Forrest, L. Jones, J. Chan, K. Dalton, Quartz crystal microbalance study of protein adsorption kinetics on poly(2-hydroxyethyl methacrylate), J Colloid Interface Sci. 325 (2008) 157–64.

[46] S. Jadi, M. Heynen, D. Luensmann, L. Jones, Composition of incubation solution impacts in vitro protein uptake to silicone hydrogel contact lenses, Mol. Vis. 18 (2012) 337–347.

[47] E.R. Berman, Biochemistry of the Eye, Elsevier, 1991.

[48] R.J. Fullard, C. Snyder, Protein levels in nonstimulated and stimulated tears of

normal human subjects, Investig. Ophthalmol. Vis. Sci. 31 (1990) 1119–1126.

[49] S.-H. Min, K.C. Park, Y.I. Yeom, Chitosan-mediated Non-viral Gene Delivery with Improved Serum Stability and Reduced Cytotoxicity, Biotechnol. Bioprocess Eng. 19 (2014) 1077–1082.

[50] S. Patnaik, A. Aggarwal, S. Nimesh, A. Goel, M. Ganguli, N. Saini, et al., PEI-alginate nanocomposites as efficient in vitro gene transfection agents, J. Control. Release. 114 (2006) 398–409.

[51] L. Wang, J.P. Stegemann, Glyoxal crosslinking of cell-seeded chitosan/collagen hydrogels for bone regeneration, Acta Biomater. 7 (2011) 2410–2417.

[52] C. Peng, J. Kim, A. Chauhan, Extended delivery of hydrophilic drugs from silicone hydrogel contact lenses containing Vitamin E diffusion barriers, Biomaterials. 31 (2010) 4032–4047.

[53] N. Efron, C. Maldonado-Codina, Development of Contact Lenses from a Biomaterial Point of View – Materials, Manufacture, and Clinical Application, Comprehensive Biomaterials. Elsevier, 2011.

[54] A.F.R. Pimenta, J. Ascenso, R. Colaço, A.P. Serro, B. Saramago, Controlled drug release from hydrogels for ophthalmic lenses: drug partitioning and diffusion, (n.d.).

[55] S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, J. Control. Release. 100 (2004) 5–28.

[56] S. Sabnis, P. Rege, L. Block, Use of Chitosan in Compressed Tablets of Diclofenac Sodium: Inhibition of Drug Release in an Acidic Environment, Pharm. Dev. Technol. 2 (1997) 243–255.

[57] M.A. González-Rodrígueza, M.L. Holgadoa, C. Sánchez-Lafuentea, A.M. Rabascoa, A. Finib, Alginate/chitosan particulate systems for sodium diclofenac release, Int. J. Pharm. 232 (2002) 225–234.

[58] Y. Boonsongrit, A. Mitrevej, B. Mueller, Chitosan drug binding by ionic interaction, Eur. J. Pharm. Biopharm. 62 (2006) 267–274.

[59] A. Bani-Jaber, D. Anani, I.I. Hamdan, B.A. Alkhalidi, Investigation of Drug Polymer Interaction: Evaluation and Characterization of Diclofenac-Chitosan Co-precipitate, Jordan J. Pharm. Sci. 2 (2009) 140–149.

[60] N. Morsi, D. Ghorab, H. Refai, H. Teba, Preparation and evaluation of Alginate/Chitosan Nanodispersions for Ocular Delivery, Int J Pharm Pharm Sci. 7 (2015) 234–240.

[61] A. Abruzzoa, F. Biguccia, T. Cerchiaraa, B. Saladinib, M.C. Galluccic, F. Cruciania, et al., Chitosan/alginate complexes for vaginal delivery of chlorhexidine digluconate, Carbohydr. Polym. 91 (2013) 651–658.

Figure Captions

Figure 1. Normalized shift in the frequency, $\Delta f/n$, (dark grey line) and shift in the dissipation, ΔD, (light grey line) for the third harmonic of the resonant frequency of a quartz crystal sensor after being coated with a TRIS/NVP/HEMA hydrogel film, during successive additions of solutions of PEI (1), rising with DD water (2) alginate (3), $CaCl_2$ (4), chitosan (5), rising with NaCl (6) and lysozyme (7) as a function of the time, to form a double layer of (alginate- $CaCl_2$) / chitosan topped with a final layer of alginate-$CaCl_2$.

Figure 2. Cumulative release profiles of diclofenac from TRIS/NVP/HEMA hydrogels uncoated (○), and coated with 1 (■), 2 (♦) and 4 (▲) (alginate-$CaCl_2$)/chitosan double layers, terminated by an alginate-$CaCl_2$ final layer. The error bars are ± the standard deviations (n=7). The uncoated hydrogels (blank samples) are different from those presented

Figure 3. AFM images of the surface of TRIS/NVP/HEMA: uncoated (A); coated with 1 double layer (B); coated with 2 double layers (C); coated with 1 double layer containing glyoxal (D).

Figure 4. Cumulative mass release of diclofenac (A), ketorolac (B), moxifloxacin (C), and chlorhexidine (D) from TRIS/NVP/HEMA coated with 1 double layer of (alginate-$CaCl_2$)/(chitosan+glyoxal) terminated with alginate-$CaCl_2$ (closed symbols).The open symbols refer to the drug release from uncoated lenses. The error bars are ± the

standard deviations (n=7).The inserts represent the release data obtained during the first 24 hours.

Figure 5. Cumulative mass release of diclofenac from CI26Y (A) and Definitive 50 (B) coated with 1 double layer of (alginate-$CaCl_2$)/(chitosan+glyoxal) terminated with alginate-$CaCl_2$ (closed symbols).The open symbols refer to the drug release from uncoated lenses (blank samples). The error bars are ± the standard deviations: A (n=6), B (n=7). The inserts represent the release data obtained during the first 24 hours.