Layer-by-layer Assembled Films for Ocular Drug Delivery

Mónica Araújo[1], Jorge Morgado[1,2] and Quirina Ferreira[1]
[1]*Instituto de Telecomunicações, Av. Rovisco Pais, 1049-001, Lisbon, Portugal*
[2]*Bioengineering Department, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001, Lisbon, Portugal*

Keywords: Drug Delivery, Layer-by-layer Films, Glaucoma.

Abstract: In this paper we describe a simple and versatile method to prepare drug delivery films composed of an ocular drug used in glaucoma treatment, brimonidine, which was encapsulated in a polymer-beta cyclodextrin. The films were developed in order to allow a controlled sequential release during long periods of time. Here we show that by introducing barrier layers of graphene oxide between the drug delivery ones it is possible to delay the brimonidine release for a few days. The time interval between two dosages of drug release will be controlled by adjusting the number and/or thickness of the graphene layers.

1 INTRODUCTION

The layer-by-layer method is a simple and versatile tool for the controlled fabrication of thin films to a wide range of purposes (Raposo and Oliveira, 2000; Ferreira et al., 2014; Ferreira et al., 2012). This method was primarily introduced by Decher in 1992 as an assembly technique based on complementary chemical interaction (Decher et al., 1992). However, in theory, hydrogen bonding, Van der Waals forces and also biomolecular recognition (i.e. any complementary interaction) can be used (Seo et al., 2008; Ferreira et al., 2012; Ferreira et al., 2014). This technique is independent of the substrate type and due to its simplicity, versatility and robustness has been applied to biomolecules, biosensores, implantable materials and drug delivery systems. Several surfaces can be used to adsorb multilayers films such as metals, polymers, glasses and any kind of biomaterial (Tang et al., 2006; Ferreira et al., 2007; Ferreira et al., 2014).

The LbL technique enables the formation of complex multilayer films merely through the sequential adsorption of oppositely charged polymers, ceramics, nanoparticles and biological molecules. With this kind of deposition, it is possible to obtain ultrathin mono-, bi- or multilayers with precision at molecular scale. Varying the process parameters, such as concentration of the components, pH, ionic strenght and immersion time, it is possible to fine-tune the films (Halász et al., 2015; Oliveira, O.N.Jr.; He, J.-A.; Zucolotto, V.; Balasubramanian, S.; Li, L.; Nalwa, H.S.; Kumar, J.; Tripathy, 2002). A wide range of technologies, with different standard tools and procedures, can be applied for the production of LbL films depending on the intended application.

In this article we present drug delivery (DD) films fabricated by LbL method which can be used for glaucoma treatment. Glaucoma is an ocular degenerative disease caused by optical nerve inflammation and leads to an intraocular pressure (IOP) increase which can cause total loss of vision. Its treatment, at initial stage, is based on the prescription of eye drops composed of an α_2 adrenergic agonist such as brimonidine. However the non-compliance of the patients (Leitão et al., 2010; Nordstrom et al., 2005) for the auto-administration of the eye drops, as well as the low ocular bioavailability leads to the progress of the disease (European Glaucoma Society, 2014). The use of novel controlled drug delivery systems have proved to be particularly interesting since it increases the residence time of drugs in the eye.
In order to develop an autonomously system able to release brimonidine during long periods we develop multilayers and biocompatible films with DD function. Brimonidine was encapsulated in a polymer-β-cyclodextrin (PolyCD) (see Figure1) and the release was controlled by the presence of barrier layers composed of two materials: an hydrosoluble polymer - poly beta aminoester (PBAE) and charged graphene oxide(GO) layers (see Figure2). The PBAE was used to control de brimonidine release. PBAE is a cationic polymer, degradable by hydrolysis of the es-

AraÃžjo M., Morgado J. and Ferreira Q.
Layer-by-layer Assembled Films for Ocular Drug Delivery.
DOI: 10.5220/0006320503950401

ter bonds of the backbone at physiological relevant pH (Zugates et al., 2007; Macdonald et al., 2008). It was designed by Langer and co-workers, produced through Michael addition polymerization of acrylate and amine monomers (Lynn and Langer, 2000; Smith, 2010). Studies *in vivo* showed that the hydrolysis of the polyester backbone of the polymer persists for several hours to a few days, but this property is largely affected by the polymer structure as well as the surrounding cellular condition (Deng et al., 2014). The GO is a single layer of two-dimensional carbon lattice tightly packed (Hong et al., 2012). GO is a graphene sheet funcionalized with oxygen-rich functional groups in the form of ether, hydroxyl, carboxyl, and epoxy groups (Choi et al., 2013). Graphene and GO layers have become an appelative field of study due to the promising biomedical applications revealed by these nanomaterials, such as in enzime adsorption, cell imaging, biosensors and drug delivery. Due to the fact that GO is highly hydrophilic, planar and chemically stable, it is possible to use these nanosheets as a temporary protective layer coating for the PBAE and poly-CD, delaying hydrolysis (Choi et al., 2013; Bosch-Navarro et al., 2012).
Results on the growth of films composed of brimonidine encapsulated in PolyCD and intercalated with layers of PBAE and GO are discussed in this article. The brimonidine release was followed under physiological conditions.

2 EXPERIMENTAL SECTION

2.1 Materials

The poly-β-cyclodextrin (polyCD) and the brimonidine (see figure 1) were purchase from Sigma Aldrich and used as received.

The poly-β-aminoester (PBAE) (see figure 2) was synthetized using the protocol described by Lynn *et al*. (Lynn and Langer, 2000), adding 3.28 g of 4,4′-trimethylenedipiperidine (S1) (97% purity, Sigma Aldrich, CAS number 16898-52-5) added to 2.87 mL of 1,4-butanediol diacrylate (S2) (99% purity, Alfa Aesar, CAS number 1070-70-8). The copolymerization of these monomers was carried out in THF (that was previously distilled) at a temperature of 50 °C, during 48 h. The final polymer PBAE, (Figure 2) was purified through repeated precipitation into diethyl ether. The precipitated polymer was vacuum filtrated with a Buchner funnel and left to dry in vacuum over night. The structure of the final product was confirmed by nuclear magnetic

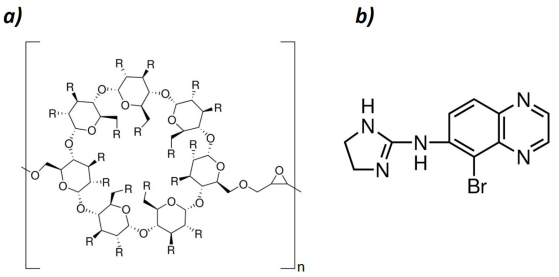

Figure 1: Chemical structure of a) β-cyclodextrin polymer and b) brimonidine. The polymer is composed of *n* rings of cyclodextrin. R corresponds to the hydroxyl group present in the molecule.

resonance spectroscopy. It was further characterized by gel permeation chromatography.

The negatively charged graphene (GO-COO^-) was purchased from Graphenea, as an aqueous dispersion with a concentration of 0.5 mg/mL (see Figure 2). The negative charges are due to the presence of carboxylic acids that deprotonate in low pH.

The positive graphene (GO-NH_3^+) was prepared in laboratory reducing the negative GO and linking amine groups to the carboxylic acids (Hwang et al., 2012). A method developed by Hwang, *et al.* was used to prepare positively charged GO. 50 mL of negative GO solution were mixed with 0.625 g of N-ethyl-N'-(3-dimethylaminopropyl)carbodiimede (EDC) (Sigma Aldrich) and with 5 mL of ethylenediamine (Sigma Aldrich). The solution was left stirring for 12 hours. EDC reacted with carboxylic groups activating the coupling of ethylenediamine. A dialysis of the final solution was performed in order to separate the functionalized graphene from the secondary products.

2.2 Methods

The films were prepared by layer-by-layer technique (Ferreira et al., 2014). Quartz substrates were used to adsorb the films that were previously submitted to oxygen plasma and immersed in a piranha solution in order to clean all organic residues and to negatively charge the surfaces. A quartz crystal lamella was immersed into a solution of PBAE. After remaining in this solution during 5 minutes, the substrate was rinsed with sodium acetate (with pH adjusted to 5.0) in order to remove all the molecules that are not adsorbed, or only physically adsorbed and then dried with nitrogen gas. After this sequence of steps a monolayer of PBAE formed. Then the substrate was immersed, one more time, but now in

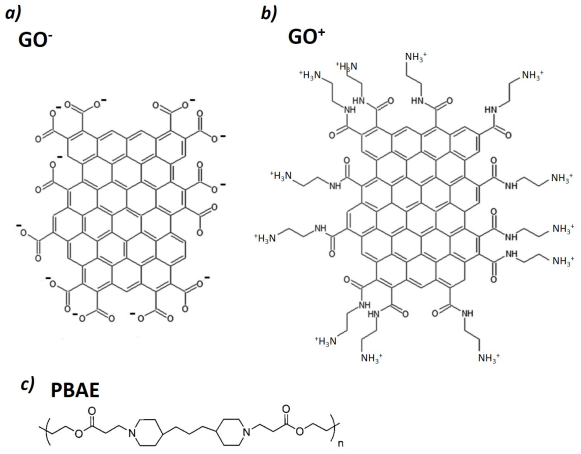

Figure 2: Schematic representation of chemically modified graphene oxide a) GO-COO^- and b) GO-NH_3^+. c) Chemical structure of poly(β-amino ester) (PBAE).

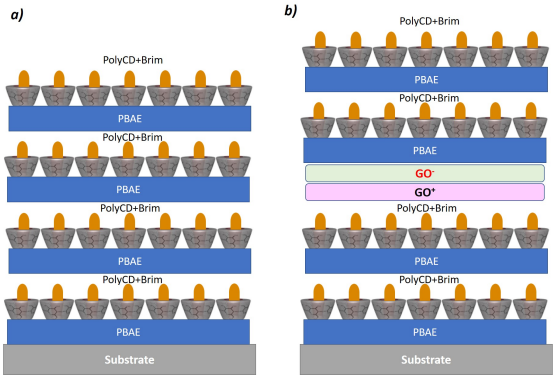

Figure 3: Schematic representation of drug delivery layer-by-layer (DD LBL) films. a)DD LBL film composed of 4 bilayers of $(PBAE/PolyCD+Brim)_4$. b)DD LBL film composed of a graphene bilayer of charged graphene between DD bilayers - $((PBAE/PolyCD+Brim)_2/GO-COO^-/GO-NH_3^+/(PBAE/PolyCD+Brim)_2$.

the polymeric solution of polyCD. The quartz substrate was left in the polyCD solution during 5 minutes and then was washed in sodium acetate (pH=5.0) and dried with nitrogen. This process completes one cycle of the LbL assembly, forming one bilayer of (PBAE/polyCD). The deposition cycle was repeated the number of times equivalent to the number of bilayers intended.

3 RESULTS

The DD LbL films were prepared using the LbL technique, where each layer adsorption was followed by UV-Vis spectroscopy. Two types of films were performed: films with polymeric bilayers of PBAE and PolyCD+Brim and films with charged GO layers intercalated between the polymeric ones, see the schematic illustration of Figure 3.

Figure 4 shows the absorption spectra of each (PBAE/PolyCD) bilayer of a film with 4 bilayers. The film has an almost linear growth, established by an increase in the absorbance of brimonidine, which means that more molecules are being added to the film. The brimonidine release was also followed by UV-Vis spectroscopy. The LbL films were immersed in a Phosphate Buffer Saline (PBS) solution that has properties similar to those of biological fluids, in terms of pH (pH=7.4, equal to the physiologic pH) and concentration of salts. A phosphate buffered saline solution consists on a phosphate buffer with a concentration of 0.01M and a sodium chloride concentration of 0.154 M. The experiments were done at 37 °C in order to mimic the physiological conditions where a glass beaker with PBS was maintained inside an oven at this temperature. The PBS solution is changed at the end of each immersion. After a specific period of time the substrate was removed, dried with a nitrogen flux and its absorption spectrum was recorded. The Figure 5 shows the absorption spectrum of $(PBAE/polyCD+Brim)_4$ and the absorption spectra of the same film, after immersion into a PBS solution at 37 °C, after determined periods of time up to a maximum of 14 minutes and 30 seconds. It is possible to see that the absorbance of the film immersed in to the PBS solution decreases in time demonstrating the brimonidine desorption. In particular, it is possible to observe that after 30 seconds of immersion, the film has lost one bilayer because its absorption spectrum is similar to that of the film with 3 bilayers. This means that it takes 30 seconds for the 4^{th} bilayer to be released to the PBS solution. It was also observed that after 1 minute and 30 seconds in PBS solution the same film has the same spectrum as that obtained for two bilayers revealing that the third bilayer was released. The immersion of the film in PBS solution continued up to 14 minutes and 30 seconds. However, after the 10 minutes of immersion, salt deposition was observed on the top of the film that affected the absorption spectra. The kinetics of brimonidine was only quantified up to 10 minutes of film immersion.

Figure 6 represents the percentage of brimonidine released to the PBS solution as function of time that was calculated subtracting the absorbance at 220 nm after the film immersion to the absorbance at the same wavelength before film immersion. The brimonidine kinetic shows that after 9 minutes of immersion time in PBS, 30% of the drug was released. That could correspond to the two outer (PBAE/polyCD+Brim) bilayers. The kinetics of brimonidine released, repre-

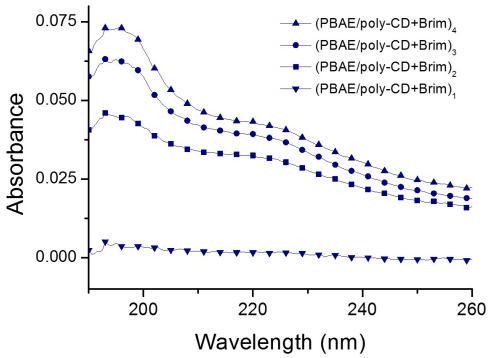

Figure 4: Absorption spectrum of each of the 4 $(PBAE/PolyCD+Brim)$ bilayers.

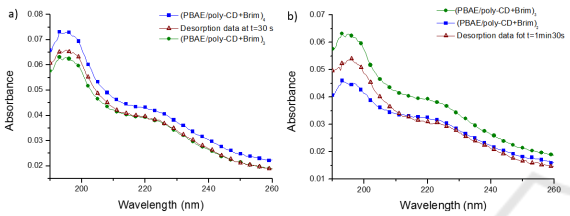

Figure 5: Absorption spectra of a)$(PBAE/polyCD+Brim)_4$ and $(PBAE/polyCD+Brim)_3$ layers and the spectrum obtained after immersion in PBS solution of the film with 4 bilayers during 30 seconds. b)$(PBAE/polyCD+Brim)_3$ and $(PBAE/polyCD+Brim)_2$ layers and the spectrum of the film obtained after immersion in PBS solution during 1 minute and 30 seconds.

sented in Figure 6 was fitted with Korsemeyer-Peppas model.

The majority of drugs reveal a first-order release (or "burst release") from the substrate followed by a continuous decrease in drug concentration in the PBS solution. The ideal pharmacokinetic system is represented by a zero-order kinetic response over time, since it minimizes the variation of drug concentration, allowing a constant release rate of drug. To analyse the release, the Korsemeyer-Peppas equation (equation 1) (Holowka and Bhatia, 2014) was used, by which the dissolution rate of the drug from the matrix was determined:

$$\left(\frac{M_t}{M_\infty}\right) = Kt^n \qquad (1)$$

where M_t is the amount of drug released at time t, M_∞ corresponds to the total amount of drug present, K is the kinetic constant; and n is the diffusion value. In this model, the kinetics is determined by the diffusion expoent value (n). Values of n=0.5 imply classic Fickian diffusion, *i.e.* the main mechanism that controls the release of the drug in the system is pure diffusion. In diffusion-controlled systems, the drug release process occurs due to aqueous stimuli through polymer swelling, causing an uniform volume expan-

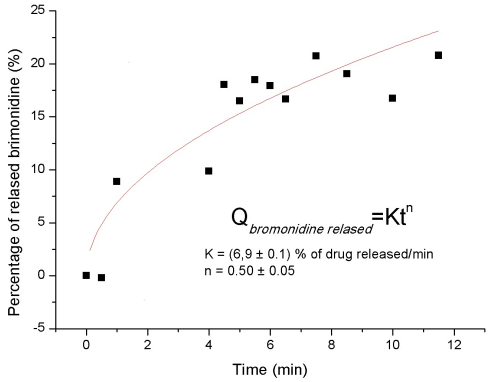

Figure 6: Percentage of released brimonidine for the $(PBAE/polyCD+Brim)_4$ after 8 minutes of immersion time in PBS solution. The fitting curve was calculated using equation (1).

sion of the bulk material. Ultimately, this will lead to pore opening of the matrix structure. Values of n in the range of $0.5 < n < 1$, indicate that the drug release occurs by Fickian diffusion and Case II transport, i.e., in this regime the drug release is both diffusion-controlled, and erosion-controlled, respectively. In erosion-control systems the mechanism of drug release relies on the attack of the covalent bonds in the polymer matrix by the components present in the release solution, allowing the drug to escape. It can occur due to volume decrease of the matrix, where its density remains constant; or due to decrease in the matrix density, while the volume remains constant. In these cases the diffusion obeys the Fick's law (Fick, 1995). If the diffusion exponent is n=1, it suggests Case II transport (or zero-order release) with constant release rate and controlled by polymer relaxation. At last, cases with $n > 1$, indicate Super Case II transport (or release that is erosion-controlled) (Holowka and Bhatia, 2014; Siegel and Rathbone, 2012). The value of diffusion exponent for this system is $n = 0.49 \pm 0.04$ with $K = 12.0 \pm 0.1$, which means that, in this case, the mechanism of drug release follows the Fickian diffusion (the driving force behind the brimonidine release in this film is diffusion).

Due to the fast brimonidine release to the biological medium, layers of graphene oxide were introduced between the polymeric (PBAE/PolyCD+Brim) bilayers (see schema of Figure 3). The multilayer film growth and subsequent release kinetics were monitorized by UV-Vis absorption. The films were also prepared by LbL method. Figure 7 shows the absorbance spectra of all layers of a film composed of 4 bilayers of $(PBAE/PolyCD+Brim)_4$ followed by one bilayer of charged graphene (GO^+/GO^-) and with more four outer polymeric bilayers of $PBAE/PolyCD+Brim)_4$.

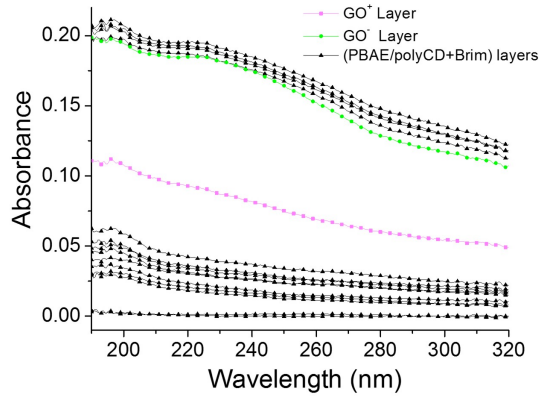

Figure 7: Absorption spectra of LbL film as a function of the growth step, up to the final structure composed by $(PBAE/polyCD + Brim)_{10}/(GO^{+}/GO^{-})/(PBAE/polyCD+Brim)_{4}$.

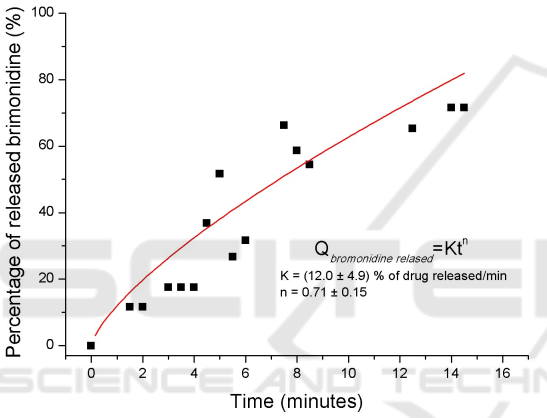

Figure 8: Percentage of released brimonidine for the $(PBAE/polyCD + Brim)_{10}/(GO^{+}/GO^{-})/(PBAE/polyCD + Brim)_{4}$ after 15 minutes immersion film in PBS solution.

The drug release kinetics study was developed with the immersion of the film with DD layers into a PBS solution (diluted in milli-Q water 1/10, pH=7.4) at 37 °C as described previously. During the first 15 minutes, spectra were recorded every 30 seconds of immersion in PBS, and the PBS solution was changed after each measurement. After that, the spectra were obtained with 30 minutes interval up to a total of 3 hours and 15 minutes of immersion (changing the PBS solution after each 15 minutes). Afterwards the immersion time was extended to 1 hour, until the total desorption time reached 6 hours and 15 minutes (with fresh PBS solution after each half hour). Concluding this period, the desorption time has been extended to an average of 12 hours of continuous desorption, followed by spectral analysis.

Between the beginning of the experiment until the 15^{th} minute the outer polymer bilayers were desorbed from the substrate. The release of the brimonidine from the outer bilayers was quantified using the Korsemeyer-Peppas equation (described in equation 1). It is possible to observe that about 80% of brimonidine presented in the outer two bilayers is release during 14 minutes. The red line is the result of the fitting, where it was obtained a n greater than 0.5 ($n = 0.71 \pm 0.15$) and $K = 12.0 \pm 4.9$, leading to the conclusion that this system exhibits a drug release that is both diffusion-controlled and erosion-controlled. By definition, in controlled released systems with $0.5 \leq n \leq 1.0$, the drug release is a combination of Fikian diffusion and Case II transport of drug molecules through the polymeric film (Enscore et al., 1977; Ritger and Peppas, 1987). The monitorization of the kinetics continued during more than 5 days. At t=110 h (more than 4 and a half days after the desorption began) the adsorption has undergone an impressive decrease in its intensity. The spectrum of the film was almost coincident with the spectrum of the GO^{+} layer as we can see in (figure 9). After this immersion time, the absorbance spectrum indicated that only the layers $(PBAE/polyCD+Brim)_{4}/(GO^{+})$ remained in the film.

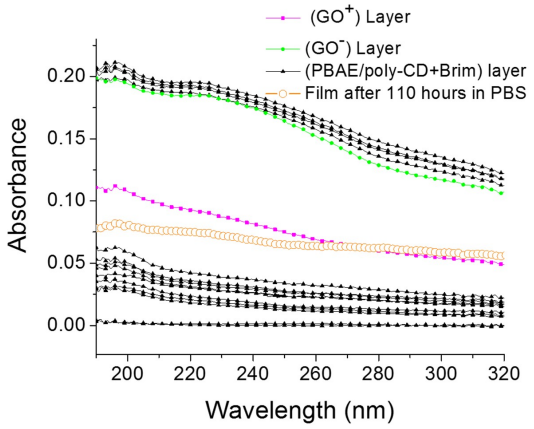

Figure 9: Absorption spectra of a film with all layers: $(PBAE/polyCD + Brim)_{10}/(GO^{+}/GO^{-})/(PBAE/polyCD + Brim)_{4}$. After more than 4 and a half days, the outer polymeric layers and the GO^{+} layer were desorbed remaining only the layer of GO^{+}. The absorbance spectrum of this desorption data is almost coincident to the GO^{+} layer.

After 5 days in immersion (approximately t=121 h) the last GO layer was desorbed. The obtained absorbance spectrum is almost coincident with the $(PBAE/polyCD+Brim)_{4}$ film, as it is possible to conclude from the spectra of Figure 10.

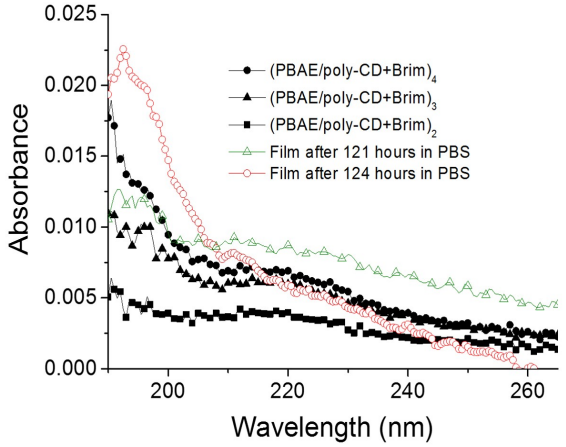

Figure 10: Absorption spectra of a film with all layers: $(PBAE/polyCD + Brim)_4/(GO^+/GO^-)/(PBAE/polyCD + Brim)_4$ and the spectrum obtained after more than 5 days immersion time.

4 CONCLUSIONS

A new drug delivery system based on multilayer films fabricated by the layer-by-layer technique was presented. This versatile method allowed the fabrication of a time controlled system introducing in the composition of the film an hydrosoluble polymer - poly (β-amino ester) - and charged graphene oxyde layers. Both materials are able to control the release of the studied drug (brimonidine) encapsulated in a poly β-cyclodextrin but the presence of graphene oxide can delay the brimonidine release up to 5 days. This is an important result for the study of time-controlled drug delivery systems since it allows adaptation of the number of layers and the film architecture in order to delay the film desorption, stopping the release of brimonidine in the eye, in the way that only the needed dose will be administrated.

This work developed the first DD LbL films for glaucoma treatment using biocompatible and biodegradable materials for the release of precise amounts of an anti-IOP drug, at determined periods of time. The high non-compliance level in glaucoma treatment leads to thousands of individuals to go blind every year. However, the latest developments on drug delivery of drugs, with the most varied carriers have revolutionized the ophthalmic treatments offering new, improved systems that can control the glaucoma condition but also substitute the current treatments with daily eye drop application.

ACKNOWLEDGEMENTS

The authors thank Fundaçao para a Ciência e Tecnologia-Portugal for financial support under the project UID/EEA/50008/2013 and Post-Doc grant SFRH/BPD/75338/2010.

REFERENCES

Bosch-Navarro, C., Coronado, E., Martí-Gastaldo, C., Sánchez-Royo, J. F., and Gómez, M. G. (2012). Influence of the ph on the synthesis of reduced graphene oxide under hydrothermal conditions. *Nanoscale*, 4(13):3977.

Choi, W., Choi, J., Bang, J., and Lee, J. H. (2013). Layer-by-layer assembly of graphene oxide nanosheets on polyamide membranes for durable reverse-osmosis applications. *ACS Applied Materials and Interfaces*, 5(23):12510–12519.

Decher, G., Hong, J., and Schmitt, J. (1992). Buildup of ultrathin multilayer films by a self-assembly process: Iii. consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. *Thin Solid Films*, 210-211:831–835.

Deng, X., Zheng, N., Song, Z., Yin, L., and Cheng, J. (2014). Trigger-responsive, fast-degradable poly(β-amino ester)s for enhanced dna unpackaging and reduced toxicity. *Biomaterials*, 35(18):5006–5015.

Enscore, D., Hopfenberg, H., Stannett, V., and Berens, A. (1977). Effect of prior sample history on n-hexane sorption in glassy polystyrene microspheres. *Polymer*, 18(11):1105–1110.

European Glaucoma Society (2014). *Terminolology and guidelines for glaucoma*. European Glaucoma Society, 4th edition edition.

Ferreira, Q., Braganca, A. M., Alcacer, L., and Morgado, J. (2014). Conductance of well-defined porphyrin self-assembled molecular wires up to 14 nm in length. *Journal of Physical Chemistry C*, 118(13):7229–7234.

Ferreira, Q., Gomes, P., Maneira, M., Ribeiro, P., and Raposo, M. (2007). Mechanisms of adsorption of an azo-polyelectrolyte onto layer-by-layer films. *Sensors and Actuators B: Chemical*, 126(1):311–317.

Ferreira, Q., Ribeiro, P. A., Oliveira, O. N., and Raposo, M. (2012). Long-term stability at high temperatures for birefringence in pazo/pah layer-by-layer films. *ACS Applied Materials & Interfaces*, 4(3):1470–1477.

Fick, A. (1995). On liquid diffusion. *Journal of Membrane Science*, 100(1):33–38.

Halász, K., Hosakun, Y., and Csóka, L. (2015). Reducing water vapor permeability of poly(lactic acid) film and bottle through layer-by-layer deposition of green-processed cellulose nanocrystals and chitosan. *International Journal of Polymer Science*, 2015:1–6.

Holowka, E. P. and Bhatia, S. K. (2014). *Drug Delivery*, volume 197 of *Handbook of Experimental Pharmacology*. Springer New York.

Hong, J., Shah, N. J., Drake, A. C., DeMuth, P. C., Lee, J. B., Chen, J., and Hammond, P. T. (2012). Graphene multilayers as gates for multi-week sequential release of proteins from surfaces. *ACS Nano*, 6(1):81–88.

Hwang, H., Joo, P., Kang, M. S., Ahn, G., Han, J. T., Kim, B.-S., and Cho, J. H. (2012). Highly tunable charge transport in layer-by-layer assembled graphene transistors. *ACS Nano*, 6(3):2432–2440.

Leitão, P., Amaral, A., Pinto, L. A., Ferreira, J., Magriço, A., Trincão, F., Silva, J. P., and M., R. (2010). Avaliação do Conhecimento , Adesão Terapêutica e Repercussão de uma Sessão Educativa sobre o Glaucoma numa População de Doentes seguidos na Consulta de Especialidade num Hospital Central. *Oftalmologia*, 34:295 – 300.

Lynn, D. M. and Langer, R. (2000). Degradable poly (beta-amino esters): Synthesis , characterization , and self-assembly with plasmid dna. *J Am Chem Soc*, 122(10):10761–10768.

Macdonald, M., Rodriguez, N. M., Smith, R., and Hammond, P. T. (2008). Release of a model protein from biodegradable self assembled films for surface delivery applications. *Journal of Controlled Release*, 131(3):228–234.

Nordstrom, B. L., Friedman, D. S., Mozaffari, E., Quigley, H. A., and Walker, A. M. (2005). Persistence and Adherence With Topical Glaucoma Therapy. *American Journal of Ophthalmology*, 140(4):598.e1–598.e11.

Oliveira, O.N.Jr.; He, J.-A.; Zucolotto, V.; Balasubramanian, S.; Li, L.; Nalwa, H.S.; Kumar, J.; Tripathy, S. (2002). Layer-by-layer polyelectrolyte-based thin films for electronic and photonic applications. *Handbook of Polyelectrolytes and Their Applications*, 1:1–38.

Raposo, M. and Oliveira, O. N. (2000). Energies of adsorption of poly(o -methoxyaniline) layer-by-layer films. *Langmuir*, 16(6):2839–2844.

Ritger, P. L. and Peppas, N. A. (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release*, 5(1):37–42.

Seo, J., Lutkenhaus, J. L., Kim, J., Hammond, P. T., and Char, K. (2008). Effect of the layer-by-layer (lbl) deposition method on the surface morphology and wetting behavior of hydrophobically modified peo and paa lbl films. *Langmuir*, 24(15):7995–8000.

Siegel, R. A. and Rathbone, M. J. (2012). *Fundamentals and Applications of Controlled Release Drug Delivery*. Springer US, Boston, MA.

Smith, R. C. (2010). *Toward a Drug Delivery Coating for Intraocular*. PhD thesis, University of North Carolina - Massachussets Institute of Technology.

Tang, Z., Wang, Y., Podsiadlo, P., and Kotov, N. A. (2006). Biomedical applications of layer-by-layer assembly: From biomimetics to tissue engineering. *Advanced Materials*, 18(24):3203–3224.

Zugates, G. T., Tedford, N. C., Zumbuehl, A., Jhunjhunwala, S., Kang, C. S., Griffith, L. G., Lauffenburger, D. a., Langer, R., and Anderson, D. G. (2007). Gene delivery properties of end-modified poly(beta-amino ester)s. *Bioconjugate chemistry*, 18(6):1887–1896.