Nanostructured films for controlled release of drugs for glaucoma treatment

Mónica Araújo

Department of Bioengineering, Instituto Superior Técnico and Instituto de Telecomunicações, Lisbon, Portugal

May 2016

Abstract

Glaucoma is an ocular degenerative disease caused by optical nerve inflammation which leads to an intraocular pressure (IOP) increase which can lead to total loss of vision. Its treatment, at initial stage, is based on brimonidine administration which allows the decrease of IOP. However, this drug is applied by means of ocular drops and the patients noncompliance causes the disease worsening. This work allowed the development of biocompatible films that can work as coatings to an intraocular device in order to release the brimonidine autonomously and in-situ. The films, obtained by layer-by-layer technique, were composed of brimonidine monolayers encapsulated in poly (β-cyclodextrin) alternating with monolayers of a hydrossoluble polymer (poly (β-amino ester)) and/or graphene oxide monolayer that allow a precise drug release. The films growth and the drug release kinetics were monitorized by ultraviolet-visible spectroscopy, quartz crystal microbalance and atomic force microscopy. The obtained results showed that the films are stable and the drug release can be controlled by the presence of the hydrossoluble polymer and the graphene oxide. In particular, it was observed that the graphene oxide delays significantly the brimonidine release enabling the precise control of the amount of drug delivered over a specific interval of time.

Keywords: Glaucoma, controlled drug delivery, layer-by-layer, biodegradable polymers, nanostructured films, brimonidine, graphene oxide.

1. Introduction

The glaucoma is a degenerative ocular disease, considered by the World Health Organization as the second leading cause of blindness, with nearly 70 million cases worldwide [1]. It is related with high intraocular pressure (IOP), being its reduction the treatment applied for all types of glaucoma through the prescription of ophthalmic drugs, such as brimonidine [2, 3]. More severe cases of glaucoma require invasive surgeries without dispensing the use of eyedrops for life [4]. However, this conventional treatment methods, consisting on eye drops administration, reveal several inconveniences, such as the patient non-compliance (half of glaucoma patients do not use their ophthalmic medication properly) [5], and also because only 20% of the active drug in one droplet achieves the ocular anterior chamber, the remaining being drained through the nasolacrimal duct or running down the chin, and from these, only 1% to 3% of the topic dosage actually reaches the target site and penetrates the tissue [6].

The solution and objective of this research is the development of multilayers and biocompatible films with drug delivery (DD) function that can replace todays therapeutics. The developed films can coat an intraocular device and release the brimonidine in situ without human intervention. More specifically, the main goal was to prepare DD layer-by-layer (LbL) films composed by the drug already used in glaucoma treatment – brimonidine – able to be encapsulated in a poly β-cyclodextrin (poly-CD) that, for its turn, is alternated layer-by-layer with a monolayer that delay the drug release: poly (β-amino ester) (PBAE) and/or graphene oxide (GO).

The LbL method is a simple and versatile tool for the controlled fabrication of thin films for a wide range of purposes [7, 8, 9]. The LbL technique enables the formation of complex multilayer films merely through the sequential adsorption of polymers through hydrogen bonding. With this kind of deposition, it is possible to obtain ultra thin mono-, bi-, or multilayers precision at molecular scale.

The polymer used for drug encapsulation, poly-CD, is a cyclic oligosaccharid with seven α-D-glucopyranose units attached by α-(1, 4) glycosidic bonds. The conformation of the glucopyranose units originate a three-dimensional structure commonly described as a wreath-shaped truncated cone.
The polymerization of these monomers proceeds (99% purity, Alfa Aesar, CAS number 1070-70-8). was added to 2.87 mL of 1,4-butanediol diacrylate purity, Sigma Aldrich, CAS number 16898-52-5) [17].

3.2.8 g of 4,4′-β-2.1.1 Poly 

3.2.8 g of 4,4′-β-2.1.1 Poly (2.1. Materials

2. Methods and characterization techniques

2.1. Materials

2.1.1 Poly (β-amino ester) synthesis

PBAE was synthesized in the Organic Electronics laboratory of the Instituto de Telecomunicaciones following the protocol described by Lynn, et al. [17]. 3.28 g of 4,4′-trimethylene dipiperidine (97 % purity, Sigma Aldrich, CAS number 16898-52-5) was added to 2.87 mL of 1,4-butadienedi diacrylate (99% purity, Alfa Aesar, CAS number 1070-70-8). The polymerization of these monomers proceeds in tetrahydrofuran, under a temperature of 50°C and during 48 h. The final polymer was purified through repeated precipitation into diethyl ether. The precipitated polymer was vacuum filtrated with a Buchner funnel and left to dry in vacuum environment over night. As for the structure of the yield product was confirmed by nuclear magnetic resonance spectroscopy and gel permeation chromatography.

2.1.2 Brimonidine encapsulation

Complexes of brimonidine encapsulated in poly-CD (poly-CD+Brim) were prepared by dissolving the drug into a solution of poly-CD at the proportion 0.8 mg/mL. The solution was stirred during about 17 hours until total brimonidine encapsulation, being ceased when no drug precipitation was seen in the bottom of the flask.

2.1.3 Preparation of graphene oxide positively charged

The negatively charged graphene oxide nanosheets (GO−) were purchased from Graphenea, as an aqueous dispersion with a concentration of 0.5 mg/mL. A method developed by Hwang, et al. [18] was used to prepare positively charged graphene oxide (GO+) by mixing 50 mL of (GO−) with 0.625 g of N-ethyl-N′,3-dimethylaminopropyl)carbodiimide methiodide (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.

To separate the GO+ from the by-products a cellulose membrane (12 000-14 000 Da, Sigma Aldrich) was used to perform the dialysis.

In order to obtain a similar pH of the polymers used to prepare the DD films, the pH of GO+ and GO− solutions was adjusted to pH=5 using a HCl solution (1.4 M) and NaOH (0.17 M) respectively.

2.1.4 Layer-by-layer technique

The DD films were prepared by immersive LbL technique. The adsorption was made at the solid/liquid interface sequentially immersing a charged substrate in different polymer solution. After each polymer submersion (PBAE and poly-CD in figure 1), the sample was washed in order to remove the physically adsorbed particles, ensuring homogeneous layers [19]. This procedure can be repeated as many times as necessary until the desired number of layers is achieved. The formation of one bilayer is composed by four steps schematized in figure 1.
2.2. Characterization techniques

2.2.1 Quartz Crystal Microbalance

The Quartz Crystal Microbalance (QCM) used, a QCM200 (Stanford Research Systems), has a resolution of 5 MHz and it is a simple, cost-effective, real-time and nanogram-sensitive mass sensing technique that uses the piezoelectric effect of a quartz crystal as the sensing element [20]. This method is based on the Sauerbrey model, which is described by a linear relationship, between frequency and mass variation per unit area at the crystal’s electrode surface. This variation is observed in the oscillation frequency of the crystal, described by equation 1 [21],

\[ \Delta f = -\frac{2f_0^2\Delta m}{A \cdot \sqrt{\rho_q \cdot \mu_q}} \]  

where \( \Delta f \) is the shift in frequency (Hz), \( f_0 \) is the unperturbed resonant frequency, i.e., without mass adsorption (Hz), \( A \) is the piezoelectrically active area of the excitation electrodes (cm²), \( \rho_q \) is the density of quartz (2.65 g.cm⁻³) and \( \mu_q \) is the shear modulus of quartz (2.95x10¹¹ dyn/cm²).

The frequency shift of the crystal is related with the amount of mass adsorbed and/or desorbed from the sensor surface in real-time [22].

QCM was used to monitor carried out for the films prepared by the LbL method, in order to control the adsorption of the DD films step-by-step.

2.2.2 Ultraviolet-visible spectroscopy

Ultraviolet-visible (UV-Vis) absorption was used to characterize the solutions and the DD films. A Cecil Aquarius CE 7200 spectrophotometer was used in all experiments. With this technique, absorption spectra of the DD film were obtained after the formation of each bilayer and also to quantify the brimonidine release.

2.2.3 Atomic Force Microscopy

The surface morphology of DD films was characterized by AFM using Nano-Observer AFM from CSIInstruments.

2.2.4 Pharmacokinetics characterization

To analyse the drug release, the Korsmeyer-Peppas equation (see equation 3) was used, by which the dissolution rate of the drug from the film was determined [24]:

\[ \left( \frac{M_t}{M_\infty} \right) = K t^n \]  

where \( M_t \) is the amount of drug released at time \( t \), \( M_\infty \) corresponds to the total amount of drug present, \( K \) is the kinetics constant; and \( n \) is the diffusion value. In this model, the kinetics is determined by the diffusion exponent (\( n \)). 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 diffusion. Values of \( n \) in the range of \( 0.5 < n < 1 \), indicate that Fickian diffusion and Case II transport occur simultaneously, and in this case the drug release is diffusion-controlled, but also erosion-controlled, respectively. 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) [25, 26, 27].

3. Results and discussion

The multilayer films were prepared using the LbL technique, where layer adsorption was assessed by QCM and UV-Vis spectroscopy. The following sections describe the results obtained for the prepared multilayer drug delivery films.

3.1. Quartz crystal microbalance measurements

3.1.1 Adsorption time determination

Multilayer films of (PBAE/poly − CD)ₙ were prepared by the LbL technique using reported adsorption times of about 3 and 10 minutes [28, 29]. However, the results suggested a random mass adsorption as function of the number of layers and
films presented visible salt agglomerates that suggest mass accumulation in some regions.

In order to overcome these unsuccessful results, several experiments were carried out to determine the optimum adsorption time of PBAE and poly-CD. Each polymer was studied individually, where small amounts of mass were adsorbed with an accurate control and optimization of the assembly process (figure 1), periodic checkpoints were performed to follow the progress of the process. The substrate was left in the polymer solution for one and half minutes, followed by washing with sodium acetate and dry with nitrogen gas. The mass added to the substrate was inferred from the frequency value obtained by the QCM through the Sauerbrey equation (equation 1). After immersion of the substrate in the polymer during short intervals of time, the frequency value was registered. The same process was repeated until the frequency obtained by the QCM showed some stability, evidencing that no more molecules of polymer could be adsorbed at the substrate, suggesting that the surface was fully covered by the polymer.

From these experiments it was possible to conclude that 6 minutes are required for PBAE to fully cover the substrates surface. AFM technique was used to analyze the surface of PBAE in order to confirm if indeed the polymer covers all substrate. The topography and phase images of figure show that the PBAE has a granular morphology and fully covers the substrate, once the phase image only reveals one type of material (figure 3).

The same method was used to determine the adsorption time of poly-CD on PBAE and for PBAE on poly-CD. Figure 1 shows the results obtained for each monolayer and it is possible to observe that the poly-CD needs 11 minutes to cover the first PBAE monolayer (figure 1b) and the PBAE needs about 7 minutes to cover the poly-CD surface (figure 1c).

After the determination of the optimal adsorption time for each polymer it was possible to proceed to the self-assembly of the monolayers using QCM for the process characterization. The multilayer films were prepared with and without brimonidine in order to compare both kinetics. The following sections describe the obtained results.

3.1.2 Growth of layer-by-layer films
A multilayer film with six (PBAE/poly-CD) alternating layers without brimonidine was prepared using the LbL technique. Figure 4 shows the variation of mass of each PBAE and poly-CD layer obtained by QCM.

As for the mass values, arithmetic average from three different experiments were taken into consideration and the error bars represent the standard deviation of the average values. It was possible to conclude that standard deviation is very significant.
that is due to QCM sensitivity. This technique is very sensitive for instance to the floor vibrations that can influence the measurements. In spite of the large error bars, the growth is linear as indicated by the linear fit (red dotted line of the figure) with an average adsorbed mass approximately constant between layers.

3.1.3 Growth of layer-by-layer films with brimonidine

Following the study of the system without encapsulated drugs it was used the same strategy, but this time, with poly-CD carrying brimonidine inside of the cavity. Figure 5 shows the evolution of mass measured with QCM of a film composed by 16 alternating layers of PBAE and poly-CD with brimonidine (poly-CD+Brim). The mass values are averaged from two different experiments and the error bars represent the standard deviation. The growth of the films is linear as it was obtained for the film without brimonidine. The presence of the drug does not influence the integrity of the film as shown in figure 5.

The surface of the first and fourth (PBAE/poly-CD+Brim) bilayers was analyzed by AFM, as it is shown in figure 6.

The topographic image in figure 7a corresponds to a surface with a Rms of 3.149 nm that is lower compared with the sample of (poly-CD+Brim) (shown in figure 6). This data suggests that the surface becomes smoother upon increase of the number of bilayers adsorbed.

3.2. Ultraviolet-visible spectroscopy characterization

Ultraviolet-visible spectroscopy was used to support the results obtained by QCM, where an absorption spectra was obtained for each bilayer of (PBAE/poly-CD+Brim). The figure 8 shows the evolution of absorption spectra for a film with 14 bilayers. The films have an almost linear growth, established by an increase in the absorbance of brimonidine, which means that more molecules of brimonidine were bonded in the film.

3.3. Drug Release Kinetics

The brimonidine release kinetics was followed by UV-Vis, recording the absorption spectra of the substrate after its immersion in a PBS solution at 37°C, (where a glass beaker with PBS was maintained inside an oven at this temperature) in order to mimic the physiological conditions (where a glass
beaker with PBS was maintained inside an oven at this temperature. The PBS solution was changed after each immersion. After a specific period of time the substrate was removed, dried with a nitrogen flux and its adsorption spectrum was recorded. Figure 9 shows the absorption spectrum of a film with 4 bilayers of (PBAE/poly-CD+Brim) and the absorption spectra of the same film, after immersion in a PBS solution at 37 °C, during 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 after exposure to the PBS solution decreases in time, demonstrating the brimonidine desorption.

Figure 9: Absorption spectra of the 4 (PBAE/poly-CD+Brim) layers (symbols), and the spectra obtained after the film immersion in PBS, during 14 minutes and 30 seconds (blue spectra).

In particular, it is possible to observe in figure 10 that after 30 seconds of immersion in PBS solution, the film has lost the (PBAE/poly-CD+Brim)4 layer because its absorption spectrum is similar to spectrum of the film with 3 bilayers. This means that it takes 30 seconds for the 4th 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 shows a spectrum similar to the spectrum obtained with the film with two bilayers adsorbed ((PBAE/poly-CD+Brim)2), revealing that the (PBAE/poly-CD+Brim)3 layer was released to the medium.

Figure 10: Absorption spectra of the film with (PBAE/poly-CD+Brim)4 and (PBAE/poly-CD+Brim)2 layers and the spectrum of the film after immersed in PBS solution during 30 seconds.

The graphic of figure 11 shows the spectra of the film with 2 and 3 bilayers and the spectrum after immersion in PBS solution. It is possible to observe that the third bilayer is released after 1 minute and 30 seconds.

Figure 11: Absorption spectra of the film with (PBAE/poly-CD+Brim)3 and (PBAE/poly-CD+Brim)2 layers and the spectrum of the film after immersed in PBS solution during 1 and half minutes.

Figure 12 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 kinetics shows that after 9 minutes immersion time in PBS, 30% of the drug was released to the medium. That could correspond to the two outer (PBAE/poly-CD+Brim) bilayers. The kinetics represented in figure 12 was fitted with Korsemeyer-Peppas equation (eq. 3). 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).

Figure 12: Percentage of released brimonidine from (PBAE/poly-CD+Brim)4 up to 8 minutes in immersion time in PBS solution.

The easy release of the two outer bilayers com-
pared with the first ones can be due to the morphology of the surface. AFM results showed that the first layers present a granular morphology (see figure 3) while the surface of the 4th bilayer is smoother. These differences in morphology are influenced by the conformation of the polymeric chains, having the last bilayers a possible conformation that allows the desorption of these layers.

Another sample, with fourteen layers was also studied using the same method, aiming to investigate how the number of layers influences the desorption kinetics from the films. After 1 minute of immersion it was observed that the last 3 bilayers were released from the film, as shown in figure 13, where the absorption spectrum of the film after this time is almost coincident with the spectrum of the film with 11 bilayers (PBAE/poly−CD+Brim)11.

In accordance with figure 14, the film needs about 10 minutes to release the 10th bilayer. This is a significant difference compared with the time observed for the last 3 bilayers. This behavior could be explained by the polymeric chains arrangement in the film, whose interaction of the last bilayers could be weaker and influencing the brimonidine release.

The film was maintained in solution for more 56 minutes. However, it was impossible to monitoring the film absorbance from the 15 minutes onwards due to salt accumulation that occurred on the top of the film. This effect can be explained by the scattering effect: salts lead to light dispersion that reduces the light transmission and appears as absorption.

To analyse the percentage of released brimonidine, during the first 11 minutes and half, the film absorption at 220 nm, after each immersion was plotted as function of the number of bilayers as it is shown in figure 15. The red line corresponds to the fitting and it is possible to conclude that 25% of the brimonidine was released from the film after 11 minutes and 30 seconds. The fitting is very poor, yields an n value obtain was n = 0.50 ± 0.05 , and a K value of 6.9 ± 0.1, which means that this film displays a mechanism of drug release that follows the Fickian diffusion, i.e., the brimonidine release is diffusion-controlled [24].

For the film with 14 bilayers of figure 13 it was concluded that the last 3 bilayers are released during 1 minute as the spectrum is almost coincident with the film with 11 bilayers. This result is similar to that observed for the film with 4 bilayers.

The desorption proceeded until 11 minutes and 30 seconds of immersion in PBS solution. The absorption spectrum of the film has an intensity between that of the 9th and of the 10th bilayers, as shown in figure 14.

Comparing the brimonidine released from the film with 14 bilayers with the film with 4 bilayers, it is possible to conclude that in both cases the amount of brimonidine released in approximately 12 minutes is about 25%. The number of bilayers seems not to influence the drug release. However, the kinetic study was only conducted up to 15 min immersion time, which we attribute to the accumulation of salts.
3.4. Drug delivery films with graphene oxide grown by layer-by-layer

We found that it is possible to delay the brimonidine release introducing graphene oxide layers between (PBAE/poly-CD+Brim) layers.

The film growth followed by UV-Vis is shown in figure 16 where it is possible to observe that the film presents a stable, continuous growth of all layers.

![Figure 16: Absorption spectra of a (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ / (PBAE/poly-CD+Brim)$_2$ film.](image)

The surface of GO and the outer polymeric bilayers were analyzed by AFM. Figure 17 shows a topographic and phase images of (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ surface. The images display straight domains that we attribute to GO nanosheets overlapping due to the $\pi-\pi$ stacking interactions. The phase images do not show evidences of the underlying polymeric layer, suggesting that the bilayer of graphene totally covers the surface of the film. Also, the surface seems to be substantial smoother comparing with the samples without GO adsorbed, as proven by the Rms that decreases from 3.149 to 2.766 nm.

![Figure 17: Topography (a) and phase (b) images of (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ with 1.0 $\mu$m scanning length.](image)

3.4.1 Drug release kinetics of films with graphene oxide

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 detailed previously.

Between the beginning of the experiment until the 15th minute the two outer polymer bilayers were desorbed from the substrate as it is shown in the figure 19. The spectra of the film recorded during the 15 minutes of immersion are similar to the spectrum of the film with (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ layers. Note that, the spectra are not coincident, but at t=10 min, the value at 230 nm is almost coincident suggesting that the two outer bilayers were desorbed. This result is similar with the observed for the film of (PBAE/poly-CD+Brim) with 14 bilayers (figure 8) where the last two bilayers needed about 10 minutes to be released for the PBS solution.

![Figure 18: Topography (a) and phase (b) images of (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ / (PBAE/poly-CD+Brim)$_2$ with 1.0 $\mu$m scanning length.](image)

![Figure 19: Absorption spectra of the (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ film, (PBAE/poly-CD+Brim)$_4$/(GO$^+$/GO$^-$)$_2$ / (PBAE/poly-CD+Brim)$_2$ film and absorption spectra of the film immersed in PBS solution up to 15 minutes.](image)
The release of the brimonidine from the outer bilayers was analysed using the equation 3 (figure 20). 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 fit, yielding $n$ 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 between Fikian diffusion and Case II transport of drug molecules through the polymeric film [30].

![Figure 20: Percentage of released brimonidine for the (PBAE/poly – CD + Brim)$_4$/GO$^+/GO^-$$_2$/ (PBAE/poly – CD + Brim)$_2$ after 15 minutes immersed in PBS solution.](image)

The monitoring of the kinetics continued but it was observed that the film began to adsorb PBS salts. However, more than 4 and half days after the desorption began, the absorption has undergone an impressive decrease in its intensity. The spectrum of the film was almost coincident with the spectrum of the first layer of $GO^+$ as shown in figure 21. After this time, the absorbance spectrum indicated that only the layers (PBAE/poly – CD + Brim)$_4$/GO$^+$ remained in the film.

![Figure 21: Absorption spectra of a film with all layers: (PBAE/poly – CD + Brim)$_4$/GO$^+/GO^-$$_2$/ (PBAE/poly – CD + Brim)$_2$ and the spectrum of the film after immersion in PBS for more than 4 and half days.](image)

After 5 days, all graphene oxide layers were desorbed. The absorbance spectrum was almost coincident with that of (PBAE/poly – CD + Brim)$_4$ film (figure 22).

![Figure 22: Absorption spectra of a film with all layers up to (PBAE/poly – CD + Brim)$_4$/GO$^+/GO^-$$_2$/ (PBAE/poly – CD + Brim)$_2$ and the spectrum of the film obtained after 5 days in immersion.](image)

The absorption spectrum of the film after 5 days in PBS ($t=124$ h 40 min) is similar to that of the film with (PBAE/poly – CD + Brim)$_2$ layers indicating that the film has only 2 bilayers of (PBAE/poly-CD+Brim), as shown in figure 23.

![Figure 23: Absorption spectra of a film with (PBAE/poly – CD + Brim)$_2$, (PBAE/poly – CD + Brim)$_3$ and (PBAE/poly – CD + Brim)$_4$ layers and the spectrum of the film obtained after more than 5 days in immersion.](image)

4. Conclusions

New therapeutics that can replace the daily application of eye drops are imperative, in order to provide more life quality to the patients but also to overcome the limitations that cause the poor IOP control leading to the advancement of the disease.

With this work it was possible to develop a new biocompatible DD system with PBAE, poly-CD and charged graphene oxide layers able to carry drugs and release them at controlled periods of time. The developed films can coat ocular devices...
in order to release the drugs \textit{in situ} and without human intervention.

\textbf{Acknowledgements}

The author would like to thank Prof. Jorge Morgado for all the guidance and support, Dr.\textsuperscript{a} Quirina Ferreira for the tireless work and profound knowledge, and Dr.\textsuperscript{a} Ana Charas for all the valuable help and expertise shared.

All the support from the team and colleagues at Organic Electronics laboratory, which made this work possible is acknowledged.

Lastly, the author wants to thank the Bioengineering department and the Instituto of Telecomunicações for the facilities, and for the financial support of the project UID/EEA/50008/2013 (STMImage).

\textbf{References}