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Artigo

# CHARACTERIZATION AND *IN VITRO* RELEASE OF CYCLOSPORINE-A FROM POLY(D,L-LACTIDE–CO–GLYCOLIDE IMPLANTS OBTAINED BY SOLVENT/EXTRACTION EVAPORATION

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Cyclosporine-A-loaded PLGA implants were developed intended for ocular route. Implants were prepared using solvent extraction/evaporation technique followed by casting of the cake into rods in a heated surface. XRD patterns showed that cyclosporine-A was completely incorporated into PLGA. FTIR and DSC results indicated alterations on drug molecular conformation aiming to reach the most stable thermodynamic conformation at polymer/drug interface. Implants provided controlled/sustained *in vitro* release of the drug. During the first 7 weeks, the drug release was controlled by the diffusion of the cyclosporine-A; and between 7-23 week period, the drug diffusion and degradation of PLGA controlled the drug release.

Keywords: cyclosporine-A; poly(D,L–lactide–co–glycolide) (PLGA); biodegradable intraocular implant.

## INTRODUCTION

The cyclosporine-A (CyA) is a selective immunosuppressive agent with antifungal and anti-inflammatory properties. The drug is commonly used to prevent rejection of transplanted organs such as kidney, liver and bone marrow, and in the treatment of auto--immune diseases such as uveitis, rheumatoid arthritis and early treatment of type I diabetes.[1-5]

The CyA presents a complex chemical structure characterized by a rigid cyclic undecapeptide, which contains eleven amino acids. The classic CyA structure is described as cyclo - [MeBmt$^{1}$-Abu$^{2}$--MeGli$^{3}$-MeLeu$^{4}$-Val$^{5}$-MeLeu$^{6}$-Ala$^{7}$-D-Ala$^{8}$-MeLeu$^{9}$-MeLeu$^{10}$ MeVal$^{11}$].[5] The CyA is a highly hydrophobic drug, and presents a relative high molecular weight and poor aqueous solubility.[6,7]

Due to the complex chemical structureand physico-chemical properties of the CyA, and the presence of natural ocular barriers, prior literature tends to focus on the difficulty in delivering an effective CyA concentration into the eye cavity through systemic and topical drug administration.[8-10] The intraocular implants represent a therapeutic alternative to release the drug directly into the eye cavity and to maintain intraocular concentration within a therapeutic range.[11,12] The intraocular implants consist of biodegradable or non-biodegradable polymers incorporated into active compounds.[12] The biodegradable polymeric matrices show advantages compared to non-biodegradable matrices as they are metabolized by the organism and no removal by surgical procedure is necessary.[12-15]

To date, most work has focused on the development and evaluation of implantable devices based on different biodegradable polymers for the treatment of severe diseases in the posterior segment of the eye.For example, intraocular implants prepared with poly(lactide-co-glicolide acid) (PLGA)and dexamethasone or cyclosporine-Awere evaluated in the treatment of uveitis, and the prevention of the post-cataract surgery diseases.[11-21] PLGA implants containing all-trans retinoic acid,cis-hydroxyprolinaor ganciclovirwere applied in the treatment of proliferative vitreo-retinopathy (PVR) and cytomegalovirus retinitis, respectively.[22-24] Additionally, ocular implantable devices based on poly(ortho ester), 5-fluorouracil and dexamethasone were prepared in order to treat experimental PVR induced in rabbit eyes.[25] Finally,intraocular implants containing poly(caprolactone)or biodegradable polyurethanesand dexamethasone were evaluated in the treatment of uveitis.[26-29] These biodegradable polymeric devices controlled the delivering of the drugs in the eye cavity through a prolonged period.

In this study, it was developed implants based on biodegradable PLGA and cyclosporine-Aintended for ocular route. These implantable devices were prepared by solvent evaporation method followed by casting of the cake into rods in a heated surface. The solvent extraction/evaporation technique was proposed as a technological alternative for preparing the polymeric implants. Then, the intraocular implants were characterized by thermal, structural and morphological techniques. The characterization seeks to define the drug distribution into the polymeric matrix, and the types of physical and chemical interactionsbetween drug and PLGA. It was also evaluated the long-term *in vitro* release profile of the CyA from the PLGA implants. This study was performed in order to investigate the controlled and prolonged release of the CyA from the implantable devices, which is essential to treat severe intraocular diseases.

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## EXPERIMENTAL

### Materials and reagents

Cyclosporine-A reference standard was purchased from Sigma Pharma (Brazil) (Molecular weight $M_w$=1202.61 g/mol, 98% of purity). Poly(D,L-lactide-co-glycolide) in ratio of 75:25 [PLGA (75:25)] was purchased from BoehringerIngelheim (Germany). All the solvents and reagents used in buffer solutions, in the preparation of the implants, and mobile phase were HPLC or analytical grade. Water was distilled, deionized and filtered through a 0.22 μm filter (Millipore, USA).

### Preparation of the intraocular implants

Initially, CyA-loaded PLGA microspheres were prepared by solventextraction/evaporation method. Briefly, 100 mg of PLGA 75:25 and 10 mg of CyA were dissolved in 10 mL of acetonitrile at room temperature. Then the solution was emulsified in 100 mL of polyvinyl alcohol (0.5%, w/w) with mechanical stirring at 50 g (1,000 rpm) for 3 min. The oil-in-water emulsion was magnetically agitated for 6 h at room temperature. The microspheres were collected by centrifugation at 50 g, washed three times with double--distilled water and placed in a freezer under – 80 ºC. Afterwards the frozen solution was lyophilized for 24 h (Christ Alpha 1-2 LD, Bioblock Scientific, France).The encapsulation efficiency of CyA--PLGA microspheres was 57.5% ± 3.0, and this result was obtained using the HPLC method described subsequently.[30] The obtained microspheres were then moulded into rods using Teflon® sheets heated on a hot plate from 100 to 120 °C in order to form CyA--loaded PLGA implants. According to the literature, the polymer and drug thermal stability was confirmed as no relevant alterations were detected in the system up to 200 °C based on the themogravimetric analysis performed.[18]

### Determination of the content of CyA incorporated into PLGA implants

Ten implants were selected and weighted. The implants were dissolved in 100 mL of a mixture of acetonitrile and distilled water (1:1). The amount of CyA was determined by high-performance liquid chromatography, using a Waters® apparatus with an Agilent C18 column (50, 4.6 x 250 mm) at 80 ºC. The HPLC system consists of Waters 510 pump and Waters 486 UV detector set at l = 210 nm, the mobile phase was acetonitrile/water (70:30, V/V) and the flow rate was 1.0 mL $min^{-1}$. The validation of the method has showed the absence of interference of the polymer with CyA retention time, discarding the risks of overestimation.[30]

### Characterization

*X-ray diffraction (XRD)*

X-ray diffraction (XRD) measurements were carried out using a Philips PW 1710 diffractometer with a copper target (l = 1.54 Å) and Ni filters. Scans were performed from 2q = 3.505 at rates of 0.01 $min^{-1}$.

*Fourier transform infrared spectroscopy (FTIR)*

Infrared spectra were collected in a Fourier transform infrared spectrophotometer (FTIR) (model Spectrum 1000; Perkin Elmer). Measurements were carried out using the attenuated total reflectance (ATR) technique. Each spectrum was a result of 32 scans with a resolution of 4 $cm^{-1}$.

*Scanning electron microscopy analysis (SEM)*

The morphology of the CyA-loaded PLGA implants was analyzed by scanning electron microscopy (SEM) (JEOL model JSM-6360LV). Prior to SEM analysis samples were gold-coated, then directly observed at an accelerating voltage of 15 kV.

*Differential scanning calorimetry analysis (DSC)*

Differential scanning calorimetry DSC-50, Shimatzu DSC was used. The samples were heated in sealed aluminum pans, and the first scan was measured at a heating rate of 10 ºC $min^{-1}$ from room temperature to 180 ºC. Subsequently, the samples were cooled to -100 ºC and heated to 400 ºC (second run) under helium atmosphere.

### *In vitro* release of CyA from the PLGA implants

The United States Pharmacopeia states in the general chapter 1092 the dissolution procedure: "sink conditions are defined as the volume of medium at least three times that required in order to forming a saturated solution of drug substance. When sink conditions are present, it is more likely that dissolution results will reflect the properties of the dosage form".[31]

The *in vitro* release of CyA from PLGA implants was carried out under sink conditions during 23 weeks. As the aqueous solubility of cyclosporine-A is 42 μg/mL ± 1.0 μg/mL at 37 ºC,[1] sink conditions are achieved with at least 3 mL. The intraocular implants were placed in different tubes containing 2 mL of balanced salt saline (n = 6). Those tubes were placed inside an incubator (Tecnal model TE 424) set at 37 ºC and stirring at 0.5 g (30 rpm)**.** At predetermined intervals, the entire medium was sampled and 2 mL of fresh phosphate buffered saline (PBS) was added to each tube. The release profile was evaluated as the cumulative percentage of cyclosporine-A released in the medium. The amount of CyA in the PBS solution was detected and quantified by validated high performance liquid chromatographic method previously described.[30]

## RESULTS AND DISCUSSION

### Preparation of the intraocular implants

The CyA loaded PLGA implants presented a planar shape with approximately 5.0 mm of length and 5.0 mm of diameter. The average weight of implants was 9.8 ± 0.1 mg (n = 10) and the average incorporation of CyA into PLGA was of 5.2 mg ± 3.0%. SEM micrographs of the intraocularimplants presented a reticular surface and the presence of a certain surface irregularities probably originated during the hot-molding process (Figure 1).

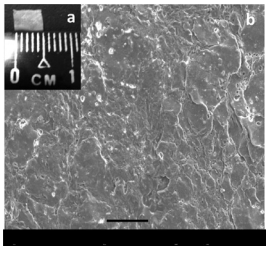


***Figure 1.*** *The photography of CyA-loaded PLGA implant (a) and scanning electron photomicrographs of the CyA-loaded PLGA implants at 200× magnification (b)*

### Characterization

The XRD was applied to generate information on the mode of inclusion of the CyA in the polymeric matrix of the implants (Figure

2). XRD patterns of the lyophilized CyA showed two broad peaks at 2θ = 9° and 20°, and characteristic narrow diffraction peaks, suggesting the existence of the semi-crystalline state of the drug due to the absence of long range three-dimensional order. The obtained XRD results are endorsed by those from Ballesteros *et al*.[3] who submitted the immunosuppressive drug to the spray drying technique. XRD patterns of the lyophilized PLGA 75:25 demonstrated the predominant amorphous state of the polymer. The narrow diffraction peaks of the lyophilized CyA were not observed in the XRD patterns of the CyA-loaded PLGA implants, indicating that the complete drug distribution within the polymeric chains changed its semi-crystalline state into afully amorphous state.

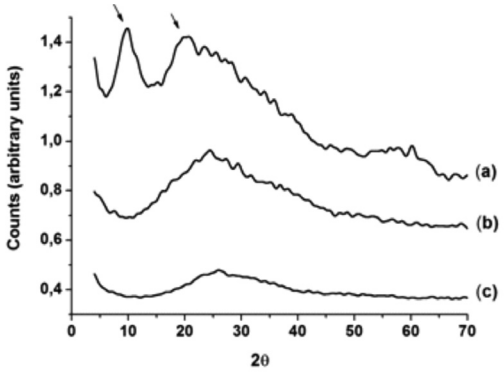


***Figure 2.*** *XRD patterns of: (a) lyophilized CyA, (b) lyophilized PLGA and (c) CyA-loaded PLGA implant. The arrows indicate the broad bands observed*

FTIR spectroscopy was conducted aiming at evaluating possible chemical interactions between the CyA and the polymer network by focusing the analysis at the amide I and amines (CyA) and ester regions (PLGA). These are important characteristic chemical groups present in each molecule used as precursors for preparing the polymer-drug systems. Therefore, changes at these absorption bands could be utilized as supporting evidence about chemical interactions occurring in the system.[32] Figure 3 shows the full range of the infrared spectra of the lyophilized CyA (Figure 3a) and PLGA (Figure 3b), and the CyA-loaded PLGA implants (Figure 3c). The presence of the specific infrared absorption bands at 1630-1680 $cm^{-1}$ related to amide groups of CyA and at 1725-1750 $cm^{-1}$, corresponding to ester groups of PLGA matrix were clearly observed in the spectra (Figures 3a and 3b), respectively (detailed region-I, from 1500 to 2000 $cm^{-1}$).[33,34] The spectrum of the implant (polymer-drug, Figure 3c) has shown a relatively small shift in this ester region suggesting that the absorption bands of both molecules have been overlapped. Additionally, the spectra ranging from 2600 to 4000 $cm^{-1}$ (Figure 3, detailed region-II) characterized the presence of stretching of –alkyl groups (nCH, ~2850-2950 $cm^{-1}$) and N-H stretching modes of primary and secondary amines (~3200-3600 $cm^{-1}$).[32,34-37] Duplet at 3320 and 3370 $cm^{-1}$ associated with N-H stretching mode were identified for lyophilized CyA and CyA-loaded PLGA implants (Figures 3a and 3c). Vibrational modes related to the alkyls (-CH) were detected in all three the spectra. Also, it was observed in the FTIR spectra the typical C-N stretching band of amines (~1020-1300 $cm^{-1}$, CyA, Figure 3a) and the band due to the –CO- groups (PLGA, ester, 1000-1250 $cm^{-1}$, Figure 3b). One again, the spectrum of the PLGA-CyA implant has showed the contribution from both components with relative small band shifts at these characteristic chemical groups when compared to the spectra of each component separately. Nevertheless, it cannot be discarded that perhaps some minor chemical interactions involving the components may also have occurred. It is reasonable to assume that the system based on mixture of the polyester (PLGA) and macrocyclic molecule (CyA) has searched for a more stable thermodynamic three--dimensional conformation by lowering the overall free energy. That means chemical interactions like van der Waals forces and hydrogen bonds are likely to have occurred among the polymer chains and the cyclosporine molecules. A deeper investigation of such interactions is beyond the scope of this study and would require different resolution of the FTIR method used in the experiments performed.

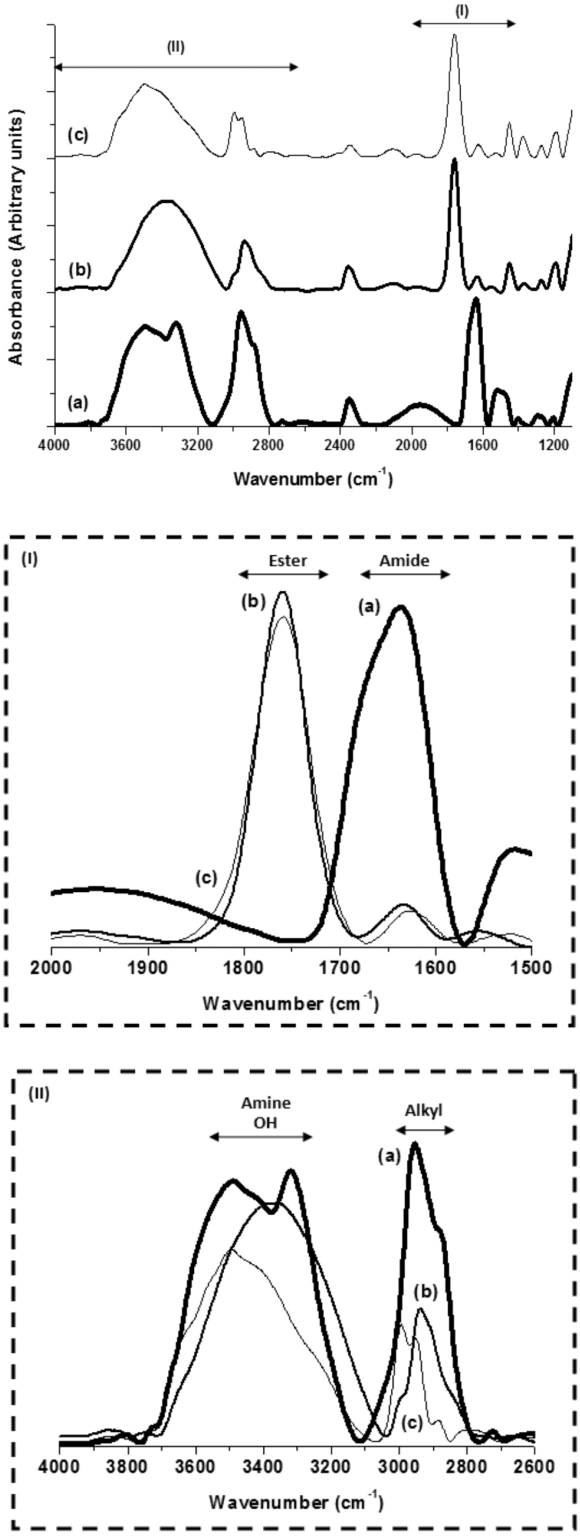


***Figure 3.*** *FTIR spectra of: (a) lyophilized CyA, (b) lyophilized PLGA and (c) CyA-loaded PLGA implant in the wavelength ranges of 1200-4000 $cm^{-1}$*

The glass transition temperature (Tg) of the polymer can be used as a measurement for the mobility of the macromolecules,[36] and also give information about the CyA molecular mobility and changes at its semi-crystalline state. Figure 4 represented the DSC curves of lyophilized CyA and PLGA, and the intraocular implants. In this work, the lyophilized polymer glass transition was identified from 51 to 55 °C. The same result was obtained in the DSC curve of CyA--loaded PLGA implants. The absence of variation in the lyophilized polymer and polymer of the device indicated an unchanged mobility from polymeric chains, and suggested the absence of detectable interactions between polymer and drug. Additionally, the lyophilized CyA meltingwas observed at approximately 124 ºC; but the same pattern was not verified in the DSC curve of the intraocular implants.

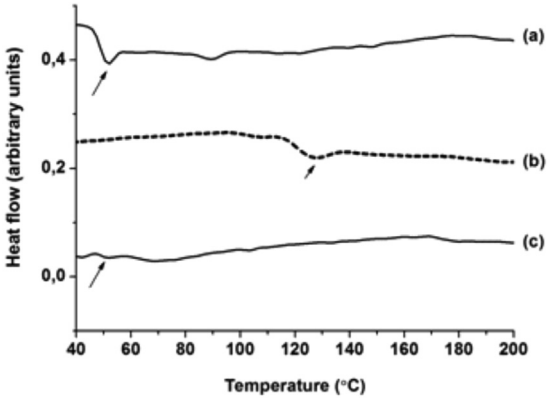


***Figure 4.*** *DSC curves of: (a) lyophilized CyA, (b) lyophilized PLGA and (c) CyA-loaded PLGA implant***.** *The arrows indicate the different endothermic transition observed*

### *In vitro* release of CyA from the PLGA implants

Figure 5 showed the *in vitro* release of CyA from the PLGA implants. It was considered that the CyA release profile presented two defined stages. The first stage occurred until 7 weeks, and it was characterized by the controlled and prolonged CyA release due to the diffusion of the drug through the polymeric matrix. The second stage occurred between 7 and 23 weeks. In this stage, a greater CyA concentration was released due to probably the faster degradation of the PLGA, providing the formation of new channels and porous in the polymeric matrix, which has facilitated the dissolution and diffusion of the drug. The intraocular implants evaluated in this work did not present an initial burst of drug release, representing an advantage of this system compared to other similar systems.[16]

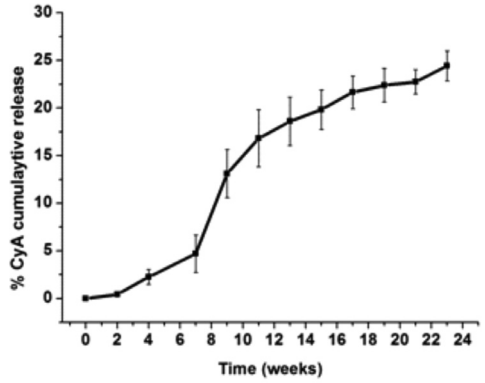


***Figure 5.*** *Cumulative release of CyA from PLGA implants in PBS buffer 7.4. The values are shown as mean ± standard deviation (s.d., n = 3)*

The SEM image showed in Figure 6 confirmed the erosion of the PLGA due to the presence of pores on the surface of the implants subjected to the *in vitro* release study. The CyA-loaded microspheres presented a relative uniform surface before the *in vitro* release study (Figure 6a). After 7 days of *in vitro* study, the degradation of the implant matrix was observed associated with the presence of scattered pores on the surface, which characterizes the polymeric matrix erosion behavior (Figure 6b).

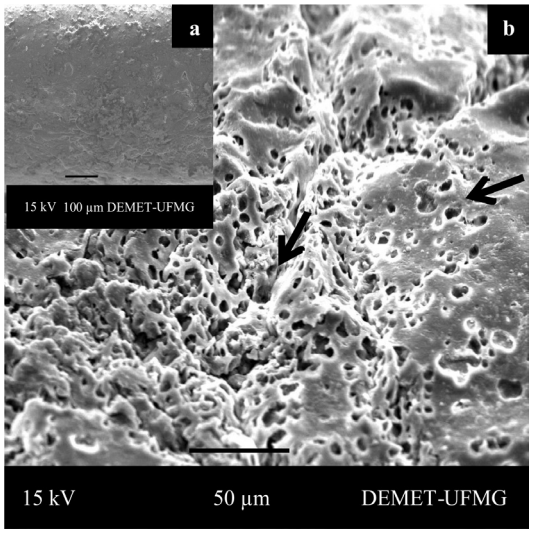


***Figure 6.*** *Scanning electron photomicrographs of the CyA-loaded PLGA implants after in vitro incubation. (a) 200x magnification of the molded implant; (b) 500x magnification after 7 days of in vitro incubation. The arrows highlight the pores in the polymeric matrix*

During 23 week period, the cumulative CyA released from the PLGA implants was 24.4%. It is interesting that the implants present a slow drug delivery profile once the majority of the ocular inflammatory diseases require a long-term drug treatment. Additionally, the CyA concentration detected in the medium, during the 23 week period, was higher than 200 ng/mL. We achieved in acceptor compartment the concentration which reflects the needed concentration of CYA in the place of application.[18]

## CONCLUSION

In this work, implants based on PLGA and cyclosporine-Awere developed intended for intraocular route. These implants were prepared by solvent evaporation method followed by casting of the cake into rods in a heated surface. The solvent extraction/evaporation technique was proposed as a technological alternative for preparing the polymeric implants. XRD patterns indicated the complete distribution of the cyclosporine within the polymeric matrix. FTIR results have indicated some minor interactions between the cyclosporine and the PLGA perhaps associated with the presence weak forces at polymer/drug interfaces. These results were complementary with the XRD patterns and DSC resultsas no relevant changes in the polymer-drug systems were observed. The biodegradable implants were able to control and sustain the cyclosporine release for 23 weeks.In summary, the cyclosporine-loaded PLGA implants produced in this work may be a promise therapeutic alternative for the treatment of inflammatory eye diseases.

## SUPPLEMENTARY MATERIAL

Available on the http://quimicanova.sbq.org.br, in pdf file, with free access. Figure 1S. Chemical structures of cyclosporine-A (a)

and PLGA (b); Figure 2S. SEM image of the CyA-loaded PLGA implants (1,000x magnification).

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## REFERENCES

1. Malaekeh-Nikouei,B.; Jaafari, M. R.; Tabassi, S. A. S.; Samiei, A.; *J. Colloid Interface Sci*. **2007**, *67*, 238.
2. Zhou, T.; Lewis, H.; Foster, R. E.; Schwendeman, S. P.; *J. Controlled Release* **1998**, *55*, 281.
3. Ballesteros, D. L.; Lechuga-Ballesteros, D.; Abdul-Fattah, A.; Stevenson, C. L.; Bennett, D. B.; *J. Pharm. Sci. Technol*. **2003**, *92*, 1.
4. Abendroth, D.; *Transplant. Pro.* **2004**, *36*, 426.
5. Donnefeld, E.; Stephen, C.; Pflugfelder, C.; *Surv. Ophthalmol*. **2009**, *54*, 321.
6. Velez, G.; Whitcup, S. M.; *Br. J. Ophthalmol.* **1998**, *83*, 1225.
7. Loor, F.; Tiberghien, F.; Wenandy, T.; Didier, A.; Traber, R. J.; *Med. Chem.* **2002**, *45*, 4612.
8. Amo, E. M.; Urtti, A.; *Drug Discov. Today* **2008**, *13*, 135.
9. Bochot, A.; Lajavardi, L.; Camelo, S.; Bourges, J. L.; Behar-Cohen, F.; DeKozac, Y.; Fattal, E.; *Ann. Pharm. Fr.* **2011**, *1*, 1.
10. Peyman, G. A.; Ganiban, G. J.; *Adv. Drug Delivery Rev.* **1995**, *16*, 107.
11. Fialho, S. L.; Cunha, A. S.; *Drug Delivery* **2005**, *12*, 109.
12. Lee, S. S.; Hughes, P.; Ross, A. D.; Robinson, M. R.; *Pharm. Res*. **2010**, *2*, 2043.
13. Choonara, Y. E.; Pillay, V.; Danckwerts, M. P.; Carmichael, T. R.; Du Toit, L. C.; *J. Pharm. Sci*. **2009**, *99*, 2219.
14. Athanasiou, K. A.; Niederauer, G. G.; Agrawal, C. M.; *Biomaterials* **1996**, *17*, 93.
15. Merkli, A.; Tabatabay, C.; Gurny, R.; Heller, J.; *Prog. Polym. Sci.* **1998**, *23*, 563.
16. Fialho, S. L.; **Rêgo**, M. B.; Siqueira, R. C.; Jorge, R.; Haddad, A.; Rodrigues, A. L.; Silva-Cunha, A. J.; *Curr. Eye Res.* **2006**, *31*, 1.
17. Kuppermann, B. D.; Blumenkranz, M. S.; Haller, J. A.; Williams, G. A.; Weinberg, D. V.; Chou, C.; Whitcup, S. M.; *Arch. Ophthalmol.* **2007**, *25*, 309.
18. Saliba, J. B.; Faraco, A. A. G.; Yoshida, M. I.; Vasconcelos, W. L.; Silva-Cunha, A. J.; Mansur, H. S.; *Mater. Res.* **2008**, *11*, 207.
19. Tan, D. T.; Chee, S. P.; Lim, L.; Theng, J.; van-Ede, M.; *Ophthalmology* **1999**, *106*, 223.
20. Wadood, A. C.; Armbrecht, A. M.; Aspinall, P. A.; Dhillon, B.; *J. Cataract. Refract. Surg.* **2004**, *30*, 761.
21. Seah, S. K.; Husain, R.; Gazzard, G.; Lim, M. C.; Hoh, S. T.; Oen, F. T.; Aung, T.; *Am. J. Ophthalmol.* **2005**, *139*, 927.
22. Dong, X.; Shi, W.; Yuan, G.; Xie, L.; Wang, S.; Lin, P.; *Invest. Ophthalmol. Vis. Sci*. **2006**, *244*, 492.
23. Yasukawa, T.; Kimura, H.; Tabata, Y.; Miyamoto, H.; Honda, Y.; Ogura, Y.; *Graefes Arch. Clin. Exp. Ophthalmol.* **2002**, *240*, 672.
24. Kunou, N.; Ogura, Y.; Yasukawa, T.; Kimura, H.; Miyamoto, H.; Honda, Y.; Ikada, Y.; *J. Control. Rel.* **1998**, *37*, 143.
25. Bourges, J. L.; Bloquel, C.; Thomas, A.; Froussart, F.; Bochot, A.; Azan, F.; Gurny, R.; Behar-Cohen, F.; *Adv. Drug Delivery Rev.* **2006**, *58*, 1182.
26. Fialho, S. L.; Behar-Cohen, F.; Silva-Cunha, A. J.; *Eur. J. Pharm. Biopharm.* **2008**, *68*, 637.
27. Silva-Cunha, A. J.; Fialho, S. L.; Behar-Cohen, F.; *Invest. Ophthalmol. Vis. Sci*. **2009**, *50*, 2312.
28. Da Silva, G. R.; Moura, S. A. L.; Carmona, D.; Ayres, E.; Oréfice, R. L.; Silva-Cunha, A. J.; *J. Drug. Target* **2009**, *17*, 374.
29. Da Silva, G. R.; Silva-Cunha, A. J.; Ayres, E.; Oréfice, R. L.; *J. Mater. Sci. Mater. Med.* **2009**, *20*, 481.
30. Saliba, J. B.; Faraco, A. A. G.; Yoshida, M. I.; Vasconcelos, W. L.; Silva-Cunha, A. J.; Mansur, H. S.; *Quim. Nova* **2011**, *34*, 140.
31. *The United States Pharmacopeia*; 31th ed., United States Pharmacopeial Convention: Rockville, 2008.
32. Mansur, H. S.; Machado, L. J. C.; Vasconcelos, W. L.; Lobato Z. I. P.; *Spectroscopy* **2002**, *16*, 351.
33. Coates, J. Em *Encyclopedia of Analytical Chemistry;* Meyers, R. A., ed.; John Wiley & Sons Ltd: Chichester, 2000, p. 10815.
34. Andrade, G.; Barbosa-Stancioli, E. F.; Mansur, A. P.; Vasconcelos, W. L.; Mansur, H. S.; *Bio-Med. Mater.* **2006**, *4*, 221.
35. Jayasuriya, A. C.; *Surf. Technol.* **2006**, *200*, 6336.
36. Meyres, R. A.; *Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation*, John Wiley & Sons*:* Chicester, 2000.
37. Källkrot, M.; Edlund, U.; Albertsson, A.; *Biomaterials* **2006**, *27*, 1788.
38. Elkharraz, N.; Faisant, N.; Guse, C.; Siepmann, F.; Arica-Yegin, B.; Oger, J. M.; Gust, R.; Siepmann, J.; *Int. J. Pharm.* **2006**, *314*, 127.