Ana Isabel Dias Oliveira

STUDY OF BIOCOMPATIBLE MATERIALS FOR DELIVERY OF ANTI-GLAUCOMA DRUGS

Dissertation guided by Arménio C. Serra, PhD, and Jorge F. J. Coelho, PhD, and presented to the Faculty of Sciences and Technology of the University of Coimbra to obtain the Master's degree in Biomedical Engineering

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Study of biocompatible materials for delivery of anti-glaucoma drugs

Dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Biomedical Engineering

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Resumo

Estima-se que, atualmente, cerca 67 milhões de pessoas em todo o mundo sofram de glaucoma, doença esta que pode levar à cegueira quando não tratada. Os tratamentos disponíveis no mercado possuem efeitos secundários adversos e/ou necessitam de destreza manual por parte do paciente para serem administrados corretamente. Nesta perspectiva, e de forma a melhorar a biodisponibilidade do fármaco no olho através da administração de uma dose mais baixa de medicamento, diversos tipos de sistemas de libertação têm vindo a ser desenvolvidos.

Devido essencialmente à sua elevada biocompatibilidade e interesse para aplicações biomédicas, o poli(ácido láctico) (PLA) foi o polímero escolhido para o desenvolvimento deste trabalho. Contudo, sendo um material duro e quebradiço, a sua utilização em diversas aplicações tem sido limitada. Por forma a viabilizar a sua utilização na aplicação final, o uso de plastificantes tem-se mostrado como uma promissora alternativa. Assim, o presente trabalho tem como finalidade o estudo do PLA como material passível de ser utilizado na entrega de fármacos anti glaucoma. Para tal, numa primeira etapa, procedeu-se à síntese e caracterização de poliésteres saturados (SP) para plastificação do PLA e, utilizando os filmes de PLA desenvolvidos, estudou-se numa segunda fase a capacidade de libertação controlada de fármaco anti glaucoma dos mesmos.

Diferentes poliésteres saturados foram sintetizados por policondensação utilizando, para tal, diferentes glicóis e ácidos saturados. Após sintetizados, os diversos SPs foram caracterizados química, térmica e morfologicamente. Através das referidas análises verificou-se, com sucesso, a obtenção de quatro poliésteres: dois alifáticos (SP1 e SP3) e dois aromáticos (SP2 e SP4). De

seguida, por *solvent casting*, procedeu-se à formação de filmes de PLA com poliésteres, utilizando o clorofórmio como solvente. Para a obtenção do filme final, o solvente utilizado na sua preparação foi evaporado numa estufa a 50 °C. Através deste método todos os poliésteres mostraram ser capazes de formar filmes com o PLA. Contudo, apenas os poliésteres SP1 e SP3 mostraram ser capazes de formar filmes com o PLA e cujas propriedades mecânicas os tornam passíveis de ser estudados para libertação controlada de fármaco. Após caracterização, os filmes com melhores propriedades - SP1 PLA50 (formulação SP1/PLA na proporção de 50/50), SP3 PLA70 (formulação SP3/PLA na proporção de 30/70) e SP3 PLA50 (formulação SP3/PLA na proporção de 50/50) - foram revestidos com a solução polimérica de origem (PLA com SP) ou com um silicone reativo. Posteriormente foram realizados estudos de libertação de acetazolamida, um fármaco anti glaucoma. O filme SP3 PLA70, formulação de PLA com poliéster de ácido sebácico e 1,6-hexanodiol, provou ser o mais promissor dos três no que se refere aos perfis de libertação do fármaco. No que respeita aos revestimentos estudados, ambos mostraram ser eficazes na diminuição da libertação do fármaco. Paralelamente, e numa análise comparativa, o mesmo processo de secagem foi realizado à temperatura ambiente, avaliando assim o impacto da evaporação lenta do clorofórmio sendo obtidos os mesmos resultados quando em comparação com a evaporação na estufa.

Abstract

Currently, about 67 million people worldwide are affected by glaucoma. Without an appropriate treatment this disease can lead to irreversible blindness. The treatments available in the market present adverse side effects and/or require manual dexterity of the patient to be administered properly. In this regard, and in order to improve the bioavailability of the drug in the eye, a variety of drug delivery systems have been developed. Poly(lactic acid) (PLA) was chosen for the development of this work due to its high biocompatibility and interest for biomedical applications. However, this polymer is a brittle and stiff material, which restricts its use in a variety of applications, namely ocular drug delivery systems. The use of plasticizers aims to improve the mechanical properties in order to meet the specifications of the final application. Thus, the present work aims to study the PLA as a material that can be used in the delivery of anti glaucoma drugs. To this end, in a first stage, different saturated polyesters (SP) were synthesized and characterized in order to plasticize PLA. In a second stage, the developed SP/PLA films were studied by their ability for controlled release of acetazolamide, an anti-glaucoma drug.

Different saturated polyesters were synthesized by polycondensation using different saturated glycols and acids. Once synthesized, the different SPs have been characterized chemically, thermally and morphologically. Through these analyzes, it was verified that four polyesters, two aliphatic (SP1 and SP3) and two aromatic (SP2 and SP4), were successfully obtained. Then, using chloroform as solvent, the formation of SP/PLA films was performed by *solvent casting*. To obtain the final films, the solvent used in their preparation was evaporated in an oven at 50 ^{o}C. All the polyesters showed to be able

to form films with PLA. However, only SP1 and SP3 polyesters showed to be able to form films with the PLA with mechanical properties of interest for drug controlled release. After characterization, the films with improved properties - SP1 PLA50 (films containing 50/50 wt.% of SP1/PLA), SP3 PLA70 (films containing 30/70 wt.% of SP3/PLA) and SP3 PLA50 (films containing 50/50 wt.% of SP3/PLA) - were coated with the film main solution (SP PLA) or with a silicone.

Afterwards, acetazolamide release studies were performed. The SP3 PLA70 films proved to be the most promising of the three films used in this study, in what concerns the drug release profiles. As regards the studied coatings, both showed to be effective in lowering drug release. At the same time, the same drying process was performed at room temperature in order to evaluate the impact of slow evaporation of the chloroform in the film properties. The obtained results were similar to those obtained for the film prepared in the oven at 50 ^{o}C.

List of Abbreviations and Acronyms

ATR Attenuated Total Reflectance

ATR-FTIR Attenuated Total Reflectance Fourier Transform Infrared

AV Acid Value

CA Contact Angle

DDS Drug Delivery Systems

DEG Diethylene glycol

DLA D,L-lactic acid

DMTA Dynamic Thermal Mechanical Analysis

DSC Differential Scanning Calorimetry

DTG Derivative Thermogravimetry

DV Differential Viscometer

ECA Ethacrynic Acid

FTIR Fourier Transform Infrared

^{1}H NMR Proton Nuclear Magnetic Resonance

HEX 1,6-hexanediol

HPSEC High performance gel permeation chromatography

IOP Intra Ocular Pressure

IPhA Isophthalic acid

LA Lactic Acid

LALLS Low angle laser-light scattering

LLA L-lactic Acid

ROP Ring Opening Polymerization

SeA Sebacic Acid

SEC Size Exclusion Chromatography

SEM Scanning Electron Microscopy

SP Saturated Polyesters

SSI Supercritical solvent impregnation

PBS Phosphate Buffered Saline

PCL Poly(ε-caprolactone)

PDLA Poly(D-lactic acid)

PDLLA Poly(D,L-lactic acid)

PLA Poly(lactic acid)/Polylactides

PLGA Poly(lactide-co-glycolide)

PLLA Poly(L-lactic acid)

PTFE Polytetrafluoroethylene

RALLS Right angle laser-light scattering

RI Refractive Index

$\boldsymbol{T_g}$ Glass Transition Temperature

TGA Thermogravimetry

THF Tetrahydrofuran

THF-$\mathbf{d_8}$ Deuterated Tetrahydrofuran

$\boldsymbol{T_m}$ Melting Temperature

TMS Tetramethylsilane

UV/Vis Ultraviolet/Visible Spectrophotometry

WHO World Health Organization

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Chapter 1

Introduction

Currently, it is estimated that 67 million people worldwide suffer from glaucoma. If left untreated glaucoma can lead to irreversible blindness. The available medications are effective for a majority of cases but the treatments in the market have side effects and/or require manual dexterity to be administered correctly. [1] In this regard, and in order to improve the compliance of the treatment and the bioavailability of the drug in the eye, different types of controlled drug release systems have been developed.

Poly(lactic acid) is a synthetic polymer obtained from renewable sources, highly biocompatible and a promising material to biomedical applications, particularly drug delivery systems. However, this polymer is brittle and stiff which restricts its use in ocular drug delivery systems. The use of plasticizers aims to improve these properties in order to meet the specifications of the final application. [2]

Thus, the main goal of this work is the study of biocompatible materials for delivery of anti-glaucoma drugs. PLA was chosen for this work. Different polyesters were synthesized and characterized, for the plasticization of PLA. The mechanical properties of the resulting film were studied and the most

promising films were chosen. Acetazolamide was encapsulated within the films and the controlled release of the drug was studied.

Chapter 2

State of Art

2.1 Glaucoma

According to World Health Organization (WHO), glaucoma is an optic neuropathy determined by both structural changes and functional deficit. [3] In this disease, a specific type of retinal cells (the ganglion cells) die and, consequently, the connection between the eye and brain is progressively compromised. [1][4] Thus, with a damage in the optic nerve, an increasing of intra ocular pressure (IOP) is currently observed. The first symptom of glaucoma is the lost of peripheral vision and, then, a reduction in the central vision. The main cause of glaucoma is related to the individual, depending on age and genetic predisposition, being extremely difficult to prevent. [1][3] Without appropriate treatment, glaucoma leads to irreversible blindness. It is estimated that 67 million people worldwide are currently affected by glaucoma. For instance, up to 80 % of the world's blindness can be avoided and different initiatives have been carried out in this area. [1]

There are 2 major subtypes of glaucoma, open-angle and angle-closure glaucoma, characterized based on the status of the internal drainage system

of the eye. In both cases, high IOP has a tremendous responsibility in the progression of the disease but, alone, it is not enough for the diagnostic of glaucoma. IOP is the only modifiable risk factor that can avoid the progression of the disease and, therefore, different studies have been carried out over the years to develop a new medical therapy to prevent high IOP values. [1]

Commonly, IOP increases when the formation of aqueous humor is higher than the draining ability through the internal outflow system (see Figure 2.1). The trabecular meshwork is the major component of the outflow system and, in open-angle glaucoma, is the site of resistance to the draining of aqueous humor, leading to a slow and painless loss of peripheral vision. On the contrary, in angle-closure glaucoma, the trabecular meshwork is blocked by the iris. This fact leads to a rapid increase of IOP, which causes pain and sudden blurring of vision. [1]

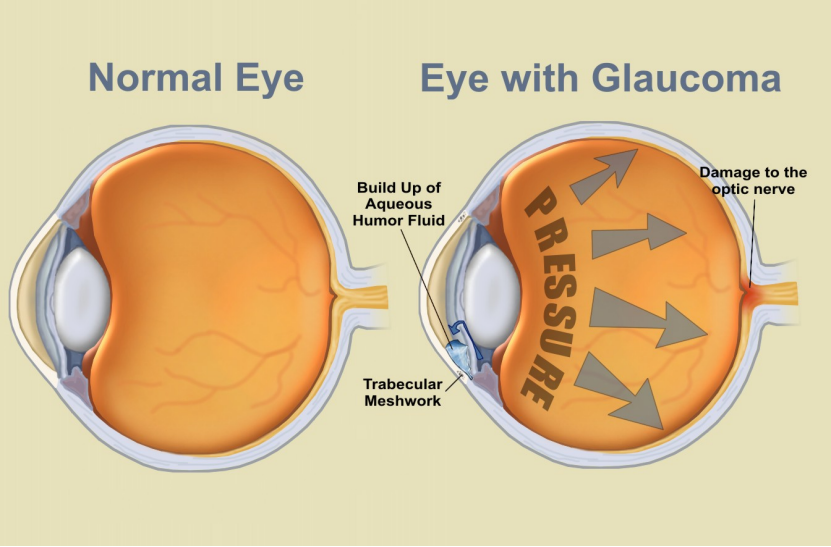


Figure 2.1: Normal eye versus eye with glaucoma, reprinted from [5].

As a disease that affects millions of people worldwide, glaucoma treatment is a major concern. Over the years different approaches have been studied

in order to slow down the rate of glaucomatous damage and, consequently, maintain adequate visual function. In the future, the early detection of the disease will be one of the main goals and new IOP-lowering agents, new surgical techniques and devices and different ways of drug delivery will be carefully developed. Specifically with drug delivery systems, the treatment compliance will be enhanced and the patient's dependence on self-administration will be reduced.

2.1.1 Glaucoma - The treatment

Currently, the therapy for glaucoma is focused on decreasing IOP by medical, laser and/or surgical therapy. In early stages, this disease is frequently treated with both medical and laser therapy. [1][3]

Medical therapy is based on daily administration of medication, usually used in early stages of the disease and also applied to control the IOP after surgery. However, glaucoma is difficult to diagnose and commonly affects old aged people. These facts can affect the effectiveness of the medical therapy which significantly depends on the treatment adherence. Another problem of this therapy is the measurement of the treatment effectiveness. Different patients have different reaction to therapy and, therefore, distinct degree of IOP reduction. Therefore, it is important to develop new modes of drug delivery to surpass the problems presented before. [1]

Laser therapy is often administered for the treatment of both open-angle and angle-closure glaucoma. This type of therapy can be used at any moment during the treatment and is frequently applied with medical therapy. Laser therapy is also used in medication intolerant patients. In the treatment of open-angle glaucoma, a laser is directed for the trabecular meshwork in order to increase the outflow of aqueous humor and, consequently, lower the IOP

in a process called trabeculoplasty. In angle-closure glaucoma, in a process called laser iridotomy, the laser is used to create a microscopic opening in the iris, allowing the outflow of aqueous humor. [1]

Finally, surgical therapy has proven to be a highly effective method to lower IOP as an alternative to medical and/or laser therapy. In the procedure, known as trabeculectomy, the aqueous humor is drained from the eye to the subconjunctival space and, consequently, the IOP is reduced. In a situation in which the eye has previous scarring and/or has been subjected to other surgeries a synthetic device, such as a tube shunt, is implanted to allow the flow of the aqueous humor to a reservoir. [1]

Over the years many drugs have been studied to reduce IOP by decreasing the aqueous humor in the eye. β-blockers and carbonic anhydrase inhibitors are specific inhibitors applied to decrease the aqueous humor formation. On the other hand, prostaglandin is applied to increase the outflow of aqueous humor and, consequently, also reduces the IOP. [1][6] Acetazolamide is an example of a carbonic anhydrase inhibitor used to treat glaucoma. This drug, commercially known as Diamox®, is currently the most effective drug to lower IOP and avoid blindness. [6][7] In the eye, carbonic anhydrase inhibitor prevents the action of the carbonic anhydrase enzyme (CA), responsible for the reversible catalysis of H_2O and CO_2 to form hydrogencarbonate (HCO_3^-) and hydrogen ion (H^+) (see Equation 2.1). [6]

$$H_2O + CO_2 \overset{CA}{\leftrightarrow} H^+ + HCO_3^- \tag{2.1}$$

The presence of HCO_3^- ion causes the movement of Na^+ and water into the eye. If this reaction does not occur, the water will not be able to move into the posterior chamber of the eye and form aqueous humor. Consequently, with low aqueous humor formation, lower IOP will be attained. [6]

Many other carbonic anhydrase inhibitors were studied over the years such as dorzolamide, also known as Trusopt®. [7][8] When comparing these two drugs, Maus and co-workers [8] concluded that acetazolamide is more efficient than dorzolamide in reducing aqueous humor flow. However, the first can have side effects, especially in long-term treatments such as diuresis and metabolic acidosis. [6][7]

Different efforts have been made to develop a topical preparation of acetazolamide. Indeed, some problems related with this drug have emerged due to its poor penetration coefficient and poor solubility in water. These facts lead to a limited ocular penetration of the drug and, consequently, insufficient amount of acetazolamide reaching the ciliary body. Despite the limitations presented by acetazolamide, this drug is still used for the treatment of acute glaucoma and post-surgical control of IOP. Acetazolamide is a well-established drug in the treatment of glaucoma, which makes it a cheap treatment. Furthermore, it reduces effectively the IOP without interfering in the outflow system of aqueous humor. [6][7]

Over the years different approaches have been studied in order to overcome the problems associated with topical and oral glaucoma medications. For instance, oral medication, such as acetazolamide, has very low bioavailability in the eye and presents significant side effects. [6][7] On the other hand, topical medication used to treat glaucoma such as dorzolamide and timolol maleate, present some problems related with administration, compliance and also side effects. [8][9]

In an effort to surpass the limitations indicated above, controlled drug delivery systems (DDS) have been studied over the years. A DDS is "a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time,

and place of release of drugs in the body". [10] The use of DDS allows the use of smaller amounts of therapeutic drug, which makes them more economic when compared to the commercial available solutions. [11][12]

Currently, there are already some DDS developed for the treatment of eye diseases. Retisert®, Medidur® and Vitrasert® are polymeric DDS that have been used for long-term drug delivery in posterior segment of the eye. These approaches were studied for eye diseases, but not glaucoma. [9][12] Timoptic-XE® and Nyogel® are gel forming solutions, consisting of water soluble polymers that, when in the eye, increase the drug retention when compared to eye drops. [9][13] Other approaches have been studied such as liposomes, nanospheres and ocular inserts. It should be noted that the ocular inserts proved to be effective in the delivery of pilocarpine, an anti-glaucoma drug, for a period of 7 days. [9][12][13]

Nowadays, for the treatment of the glaucoma, the development of DDS able to release a drug for several days or weeks have been presented as a big challenge. With this purpose, implantable polymeric devices have been studied in the last years. Wang and co-workers [14] developed poly(lactide-co-glycolide) (PLGA) films in order to release ethacrynic acid (ECA) in the eye, a potential anti-glaucoma drug. Through *in vivo* studies, the authors found that the films were able to release the drug in a controlled manner, being observed a decrease in the IOP values for at least 10 days. In another work, Bertram and co-workers [15] developed microspheres of poly(D,L-lactic-co-glycolic acid) and PLA to deliver timolol maleate (a β-adrenergic receptor antagonist) in the subconjunctival of the eye. In their study, the developed material showed a sustained release of the drug for approximately 3 months. This treatment was the longest sustained delivery of timolol maleate described in the literature but its efficiency in lowering IOP was

still undetermined. In 2009, Schultz and co-workers [16] incubated commercial contact lenses in two different source solutions containing timolol maleate and brimonide tartrate, respectively, and studied the release kinetics. Three volunteers (with glaucoma and subjected to previous treatment) used impregnated contact lenses for 30 minutes a day for 2 weeks. IOP was maintained at levels equivalent to those obtained with other treatments. A similar approach was taken by Costa and co-workers [17], who used commercial silicone-based hydrogel contact lenses and impregnated them with two drugs, acetazolamide and timolol maleate. This was achieved using a discontinuous supercritical solvent impregnation (SSI) methodology. This method was proven ineffective since the release of the medications was complete after 4 hours in sink conditions. More recently in 2011, Natu and co-workers [12] prepared implantable films by blending poly(ε-caprolactone) (PCL) with Lutrol F 127 (poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)) and dorzolamide. The films were first prepared by solvent casting, with acetone. Then a dip-coating process, with a PCL-solution, was carried out in order to diminish the burst effect and improve the control the release of the drug from the discs. With this approach, the IOP of normotensive rabbits decreased by 13 % for a period of 10 days, for PCL-coated samples. Since the previous preparation mode is not reproducible, a new preparation method was developed by the same authors. [11] Melt compression proved to be an effective method to develop the Lu/PCL discs with a small polymer-to-drug ratio. The effect of PCL with different molecular weights was also studied. For disks with low molecular weight PCL, the release was complete after 1 month, as revealed by the *in vivo* experiments. On the other hand, high molecular weight PCL showed non-cumulative releases rates above the therapeutic level during 3 months *in vivo*, being a promising material. [11]

Further investigation needs to be carried out in order to increase the delivery of a drug by DDS (6 months to 2 years), with a more efficient treatment of glaucoma. As worth mentioning, polymeric materials such as poly(lactic-co-glycolic acid), PCL, polyanhydride and poly(orthoesther) have been used in the preparation of biodegradable implants to meet this objective. PLA is currently a material with a huge potential for biomedical applications, being a very promising material for biodegradable DDS. As mentioned above, PLA was used in the preparation of films and microspheres to treat glaucoma. But due to its inherent mechanical properties it has been copolymerized with monomers and other polymers in order to improve its mechanical properties. [18] More detailed information about PLA properties and application is presented next.

2.2 Poly(lactic acid)

Poly(lactic acid) or polylactide (PLA) is an extremely versatile and biodegradable aliphatic polyester, which has received significant attention in the past years. Obtained from a 100% renewable raw material, the lactic acid (LA), PLA is indicated as a sustainable alternative to petrochemical-based materials. Currently, it is one of the most remarkable polyesters, being used in a wide range of applications. [2][19][20][21]

PLA was firstly synthesized by Theóphile-Jules Pelouze, in 1845, by condensation of lactic acid. However, the synthesis of this polymer only gained interest in 1932 when Wallace Carothers polymerized lactide (a lactic acid cyclic dimer) to obtain PLA, being the process patented later by DuPont. Despite its promising properties, the use of PLA was initially limited for many applications due to its high cost and low molecular weight. In the

early 1990s, the breakthrough occurred when poly(L-lactide) (PLLA) with high molecular weight was synthesized by ring opening polymerization (ROP) of the lactide. Later, this polymer was commercialized by the same company, Cargill Inc. In 1997, a joint venture formed by Cargill and the Dow Chemical Company led to the emergence of the NatureWorks LLC, fully dedicated to the production of PLA. Currently, NatureWorks LLC is the main supplier of PLA, under the brand name Ingeo^{TM}, with a production capacity of 140 000 metric tons per year. [22][23]

In the last years, the main use of PLA was in food and food-related applications, such as candy wrap and packaging. However, due to its good biocompatibility and, namely, due to its biodegradability PLA has presented itself as a promising material in medical science, particularly in tissue engineering and DDS. [20][21]

As mentioned before, PLA is obtained from the monomer lactic acid (or 2-hydroxy propionic acid). LA is a chiral molecule, existing in two enantiomeric forms: the L-lactic acid (LLA) and D-lactic acid (DLA) (see Figure 2.2). [24]

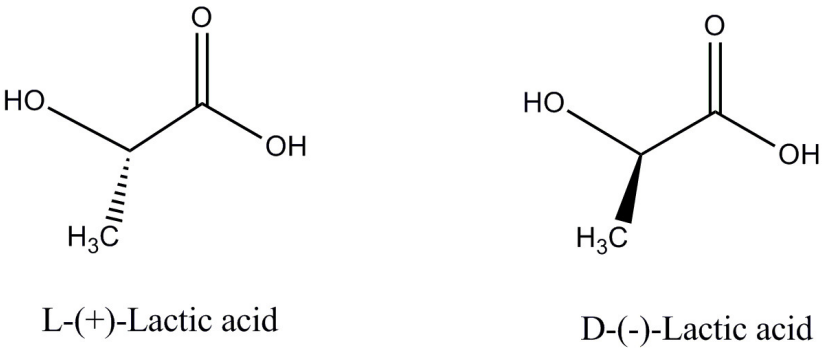


Figure 2.2: Chemical structure of lactic acid.

LA can be synthesized in two distinct pathways, by fermentation or by chemical route. The carbon source needed for the fermentation process can

be obtained from agriculture by-products such as sugarcane, potato, tapioca and wheat, making this method financially attractive. Different bacterial strains are used to obtain the two distinct forms (D(-) + L(+)) of LA. When comparing the two methods, the fermentation process is presented as more attractive due to the low energy and temperature required, low CO_2 emissions and, as indicated above, the low prices of substrate. Therefore, around 90% of lactic acid is produced by bacterial fermentation to obtain a product with high specificity. [2][19][21][24]

As indicated above, and being LA a chiral molecule, poly(lactic acid) refers to a family of polymers: pure poly(L-lactic acid) (PLLA), pure poly(D-lactic acid) (PDLA) and poly(D,L-lactic acid) (PDLLA). [2][20][21]

PLA is presently synthesized through two different methods: direct polycondensation of lactic acid and ROP of the lactide. The polycondensation of LA occurs in bulk by removal of water of condensation by the use of vacuum and temperature. Through this method, a low-molecular-weight polymer in the range of 10 000 to 20 000 $g.mol^{-1}$ is obtained. Thus, in order to increase the molecular weight of the polymer external coupling agents such as isocyanates, acid chlorides, anhydride and epoxides, are used. This constitutes the least expensive route to produce PLA. On the other hand, ROP process concerns three steps: polycondensation, depolymerization and ring opening polymerization of lactide. Low molecular weight LA oligomers are synthesized by polycondensation. This process is followed by depolymerization of the LA oligomers in order to produce a cyclic dimer, the lactide. Finally, PLA with controlled molecular weight is produced from the lactide by ring opening polymerization. Both of these methods use catalysts to obtain PLA. In direct polycondensation the catalysts used are boric and sulfuric acids, whereas in ROP stannous octoate is the catalyst used. [2][19][21][25] In the

future new and environmental friendly methods to produce PLA have to be developed.

2.2.1 Properties

PLA is a quite attractive polymer and, due to its properties, can be found on large-scale applications and also in biomedical and pharmaceutical industries. This polymer presents itself as a clear, colorless thermoplastic that can be processed into fiber and films. However, PLA is also a brittle and stiff material at room and body temperatures, presenting low ability to plastic deformation. [2][19][25][26]

For many applications an important characteristic is the degradation rate of the polymer. Degradation of polylactides, unlike most other biodegradable polymers, occurs in two distinct steps. First, in a hydrolytic degradation mechanism the scission of the ester groups occurs, with the consequent decrease of the molecular weight of the polymer. Only then, microorganisms are able to metabolize the low molecular weight PLA chains into lactic acid. In addition to temperature and moisture levels, crystallinity of the polymer is a parameter that also determines the degradation time of PLA. Highly crystalline polymer will take months or even years to degrade completely. On the contrary, an amorphous polymer can degrade in weeks. [21][25]

The *in vitro* degradation of polylactides is dependent on the pH of the solution. The *in vitro* degradation results can be used as a predictor of *in vivo* degradation of the polymer. For instance, a phosphate-buffered solution with pH of 7.4 at 37 ^{o}C can be used to mimic the conditions of the human eye and to understand the PLA degradation mechanism. Tsuji and co-workers [27] used these conditions to evaluate the degradation of PLLA. They found out that the hydrolysis of PLA occurs mainly due to bulk erosion and in

the amorphous areas of the polymer. The presence of enzymes can lead to formation of pores and fragmentation of the polymer which increases its degradation rate. [28]

In general, at room temperature, PLA is soluble in chloroform, dichloromethane, acetonitrile, methylene chloride, 1,1,2-trichloroethane and dichloroacetic acid. In contrast, solvents like ethyl benzene, toluene, acetone and tetrahydrofuran are only able to completely dissolve PLA at the boiling temperature. [2][21][25]

PLA can be easily processed by conventional techniques used for thermoplastics such as injection moulding and blow moulding extrusion. [26] However, for specific applications, PLA cannot compete with the conventional polymers. For instance, it presents inferior thermal stability and impact resistance to those of conventional thermoplastics. [2][25]

PLA is highly biocompatible and biodegradable polymer and, consequently, it's a promising material for biomedical applications. As mentioned above, its stiffness and brittleness are its major drawbacks, especially in applications such as ocular drug delivery systems. In the last years, different works have been carried out in order to change the properties of this polymer. The use of plasticizers to improve the PLA properties will be discussed below.

2.2.2 Plasticizers

Plasticizers have been used to improve the polymers processability, flexibility and durability. [29][30] By definition, a plasticizer is a small organic molecule that is added to a polymer in order to reduce its brittleness, improve its flexibility and toughness, to reduce the crystallinity and lower the T_m and T_g. Ideally, a plasticizer should be biodegradable, non-volatile and non-toxic,

with minimal leaching and phase separation, during storage and end-use applications. [30][31][32]

There are two types of plasticizers. Whereas internal plasticizers are part of the polymer (a second polymer is copolymerized into the polymer structure making the polymer chains more difficult to fit with each other), the external plasticizers are low volatility molecules which, without chemical reaction, interact with the polymer. [30]

In a plasticization of a polymer, the plasticizer increases the free volume between the polymeric chains, decreasing the number of polymer-polymer interactions. Thus, with lower T_g values, a polymer with lower rigidity and brittleness and improved mechanical properties will be obtained. [30][31][33][34] The effects of plasticizers in polymeric networks can be explained by three theories. The lubricity theory states that the plasticizers act as lubricants, contributing to an increase in the mobility of the polymeric chains. According to the gel theory, the plasticizer replaces polymer-polymer interactions that keep the polymer together and, consequently, there is an increase in the flexibility of the material. Finally, according to the free volume theory, the plasticizer increases and preserves the free volume of the polymer (internal space available for the movement of the polymeric chains) after processing. This theory helps to explain the effect of plasticizers in T_g. [30]

Over the years, different plasticizers have been studied in order to find an economical and easy method to modify PLA. Low molecular weight compounds such as oligomeric lactic acid and low molecular weight citrates have been used as plasticizers for PLA. [29][30][34] However, some of these plasticizers can exhibit leaching during the use, thermal instability and biocompatibility issues. Thus, a common way to reduce migration and leaching of plasticizers, during processing and storage, is to increase their molecular

weight, without comprising the miscibility with PLA. In this respect, polymers have been investigated as plasticizer for PLA. [32][35] For instance, Martino and co-workers [36] studied the use of three commercial polyadipates (Glyplast®) as plasticizers to modify PLA, which showed to be able to increase the elongation of the PLA and decrease its elastic modulus and tensile stress. Recently, Lapol® (polyester) has been introduced in the market as a bioplasticizer for PLA. It is claimed to be an efficient plasticizer for PLA at relative low concentrations (5-10 wt.%), promoting toughness and flexibility while minimizing the reduction of T_g. [32][35]

In general, polymeric plasticizers are less efficient that monomeric ones when it comes to improve elongation and reduce T_g of PLA. On the other hand, they provide better stiffness-toughness balance for PLA and are less prone to leaching. Thus, in order to obtain a polymer with improved properties and processing behavior for a specific application, an adequate choice of plasticizer type must be made. [30]

2.2.3 Applications

PLA has shown huge potential to be used as a bioplastic in a wide range of applications; packaging for food, in textile industry as disposable garments, feminine hygiene products, nappies, wipes and in medical care, and in agriculture, such as films and matrix for controlled release of fertilizers and herbicides. [21][25]

In recent years the use of PLA in biomedical fields, specifically in tissue engineering, has been extensively growing. This fact, related with its versatility, makes PLA a key material for biomedical applications. Some examples of its application in this so important area are degradable sutures, drug delivery systems (DDS) and porous scaffolds. [19][20][25]

Taking advantage of PLA characteristics, biodegradable implants have been developed. Thus, after the reparation of a tissue, a second surgery to remove the implant can be suppressed. In the last few years, PLA has also been used to produce microspheres and microcapsules for DDS. These devices are characterized by a controlled release of drugs over time. On the other hand, and due to its slow degradation rate, PLLA has been used to repair ligaments and tendons. In addition, due to its excellent mechanical properties, this polymer has also been used in scaffolds, for cell growth, and bone fixation devices. [21][25]

Nowadays, one challenge in the treatment of glaucoma is the development of a device to deliver anti-glaucoma medication in the eye, in a controlled manner.

Considering the indicated properties of PLA, namely its biocompatibility, DDS can be developed for the treatment of glaucoma. As exposed earlier, acetazolamide has side effects associated with its systemic administration, such as diuresis and metabolic acidosis. The use of DDS for the administration of this drug, allows the elimination of frequent dosing by the patient and, consequently, better treatment compliance, fewer side effects and lower doses. The main difficulty in the development of this type of DDS is, so far, the correct plasticization of PLA.

For the purpose of this master thesis, polymeric blends will be developed in order to create a DDS with improved drug release properties for the treatment of glaucoma. With this purpose, saturated polyesters will be synthesized and used to plasticize PLA. Acetazolamide will be also encapsulated within these structures and its release rate evaluated.

Chapter 3

Materials and Experimental Methodology

3.1 Materials

Sebacic acid (SeA, $\geq$ 95%), diethylene glycol (DEG, 99%), 1,6-hexanediol (HEX, 97%), isophthalic acid (IPhA, 99%), p-toluenesulfonic acid monohydrate (98.5%), acetazolamide and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, USA). Deuterated THF (THF-d_8, 99.5%) was purchased from Eurisotop (France). Chloroform, dichloromethane and tetrahydrofuran (THF) were supplied by Fisher Chemicals (United Kingdom). Potassium hydroxide solution (1N, in ethanol) was purchased from Acros Organic (Geel, Belgium) and n-butyl acetate and ethanol (96%) were supplied by Panreac (Spain). Phenolphthalein was obtained from Riedel-de-Häen (Germany). Poly-(lactic acid) Ingeo^{TM} 2500 HP was obtained from Nature Works LLC (Blair, USA). Acetone was purchased from JMGS Lda. (Odivelas, Portugal). All the reactants were used as received, unless otherwise stated.

3.2 Experimental Methodology

3.2.1 Synthesis of Saturated Polyesters

The saturated polyesters (SP1, SP2, SP3 and SP4) were prepared by bulk polycondensation (Figure 3.1). The monomers (glycols and acids) and the catalyst (p-toluenesulfonic acid monohydrate, 1 wt.%) were charged into the four-necked glass reactor (1), equipped with a mechanical stirrer (2), a nitrogen inlet (3) and a condenser (4) connected to a round-bottom flask (5).

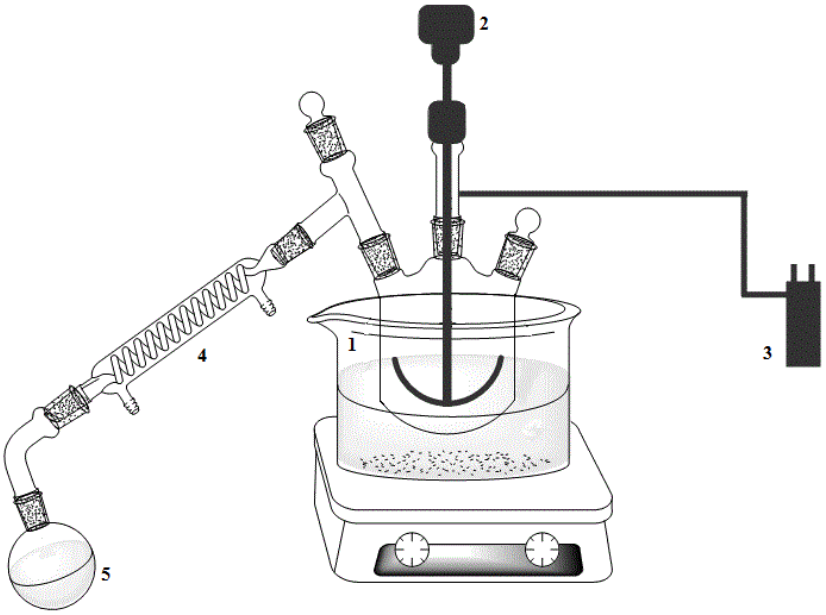


Figure 3.1: Illustration of a polycondensation reaction.

The polycondensations were carried out and water was distilled during reaction. The end of reaction was set when the acid value (AV) reached a value below 30 mg KOH/g (according to ASTM 109-01). Table 3.1 presents the molar percentages of the different compounds in the feed formulations and the synthesis conditions of the synthesized polyesters.

Table 3.1: Reaction conditions of the SPs.

Polyester	Saturated Acids (%)		Diols (%)		Temperature		
	SeA	IPhA	DEG	HEX	180°C	190°C	205°C
SP1	45,5	-	54,5	-	2h	4h	2h
SP2	22,5	22,5	55,0	-	2h	4h	-
SP3	45,5	-	-	54,5	2h	4h	2,75h
SP4	21,7	21,7	-	56,5	2h	4h	-

Saturated polyester can be obtained by polycondensation of a saturated acid and a diol. In this work, the synthesized SPs are based on SeA, a biobased monomer. SP1 has SeA and DEG, a diol, in its constitution. The use of DEG allows one to obtain linear saturated polyester with high flexibility. By its turn, SP2 has two saturated acids: SeA, as mentioned, and IPhA. The last is used in order to grant rigidity to polymer chains. Finally, with the purpose to study the influence of a diol with a long hydrocarbon chain, SP3 and SP4 were prepared using HEX. Similar to SP2, SP4 has in its constitution IPhA. The use of a catalyst (p-toluenesulfonic acid monohydrate) was required in the polycondensations.

3.2.2 Plasticization of PLA and film formation

After the synthesis of saturated polyesters (SP), the obtained materials were used to plasticize PLA, with the formation of films.

In order to improve the properties of PLA, blends of SP/PLA were prepared using chloroform as the solvent. PLA and a SP were dissolved in chloroform until a homogeneous solution was obtained. For each SP, two types of blends were prepared; a blend with 30/70 wt.% of SP/PLA and a blend with 50/50 wt.% of SP/PLA.

After this step, a fixed volume (2.5 ml) of the different solutions where placed in petri dishes and, in order to obtain the films, the chloroform was

left to evaporate following two different approaches. Some of the samples were placed in the oven at 50 °C until all the solvent evaporate or in a fume hood, at room temperature.

When dried, the flexibility of the obtained films was evaluated and the samples with the best properties were chosen.

3.2.3 Drug Encapsulation

The films with the most promising properties were selected to encapsulate acetazolamide in order to obtain a DDS for glaucoma treatment. The encapsulation was accomplished by mixing 25 mg of the drug in 25 ml of blends prepared in the previous step (SP/PLA dissolved in chloroform). Afterwards, a fixed volume (2.5 ml) of the different solutions with acetazolamide where placed in petri dishes and the chloroform was evaporated following the same two different approaches already mentioned. With this process, different films incorporating acetazolamide were obtained.

In the final step, for encapsulation studies films with acetazolamide were coated with a solution of polyester/PLA or with a silicone.

In order to monitor the drug release and the efficiency of the coatings, the films were submerged into 25 ml of PBS and placed in a shaker at 37 °C and 100 rpm, for two weeks. The amount of drug released over the days was quantified by UV/Vis spectroscopy, as explained above.

3.3 Characterization Techniques

3.3.1 Chemical Characterization

In order to obtain detailed information about the chemical structure of the synthesized polyesters, their monomers and the blends with PLA, a careful chemical characterization was carried out using different techniques.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Spectroscopy is an important technique used to identify and characterize functional groups presented in a sample. For the purpose of this project, the chemical structure of the synthesized polyesters was confirmed by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR). ATR-FTIR analysis was carried out with a Jasco FT-IR-4200 spectrometer, equipped with a Golden Gate Single Reflection Diamond ATR. Data collection was performed with wavelength ranging from 4000 to 500 cm^{-1}, with 4 cm^{-1} spectral resolution and 64 accumulations. The signals obtained were represented in spectrum where, the vertical and horizontal axis, correspond to the transmittance (%) and the wavelength (cm^{-1}), respectively.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR Spectroscopy provides information about the structure and chemical composition of a sample. This technique was applied to analyze the chemical structure of the synthesized polyesters. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is used to identify the carbon-hydrogen framework. The analysis was performed in a Bruker Avance III 400 MHz spectrometer, with a 5 mm TIX triple resonance detection probe. Deuterated tetrahydrofuran (THF-d_8) was used as solvent. Tetramethylsilane (TMS) was used as

the internal reference.

Size Exclusion Chromatography (SEC)

SEC is a technique currently used to determine the molecular weight distribution of polymers and to obtain M_n (number molecular weight) and M_w (average molecular weight). In this type of chromatography, the molecules of a sample are separated based on their size (hydrodynamic volume).

The molecular weight distribution of the samples was determined using high-performance gel permeation chromatography (HPSEC; Viscotek TDAmax) with a differential viscometer (DV), right-angle laser-light scattering (RALLS, Viscotek), low-angle laser-light scattering (LALLS, Viscotek), and refractive-index (RI) detectors. The column set consisted of one Viscotek Tguard column (8 μm), one Viscotek T2000 column (6 μm), one Viscotek T3000 column (6 μm) and one Viscotek LT4000L column (7 μm). Before the injection, the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.2 μm pore. The system was calibrated with narrow PS standards.

3.3.2 Thermal and mechanical characterization

The thermal and mechanical properties of the different polymers studied in this work were analyzed by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and by dynamic mechanical thermal analysis (DMTA).

Thermogravimetric analysis (TGA)

TGA allows the study of the thermal stability of a sample in which the mass of a sample is monitored as a function of the sample temperature and/or

time, when the sample is subjected to a specific temperature program. For the purpose of this project, this technique was used to study the synthesized polyesters and films of PLA/polyester.

The thermal stability of samples was evaluated by thermogravimetric analysis (TGA), using a TA Instruments Q500 thermogravimetric analyzer (thermobalance sensitivity: 0.1 μg). The equipment was calibrated within a temperature interval ranging from 25 °C to 1000 °C, at a heating rate of 10 $^{o}C \cdot min^{-1}$, running tin and leads as melting standards. The samples were analyzed using open alumina crucibles, in the temperature range 25 °C to 600 °C and at a heating rate of 10 $^{o}C \cdot min^{-1}$, under dry nitrogen purge flow of 100 $ml \cdot min^{-1}$.

Differential Scanning Calorimetry (DSC)

DSC is a thermal technique that measures the heat flow involved in materials transitions as a function of temperature. The sample in study and the reference sample are heated/cooled simultaneously using two independent furnaces. When transitions occur during heating/cooling, the heat flow versus temperature is registered.

The DSC studies of the polyesters and PLA/polyester films were performed in a TA Instruments Q100 model. The thermal history of the samples was erased by heating the samples until 110 °C and, then, their thermal behavior was measured in a temperature range -80 °C to 180 °C, at a heating rate of 10 $^{o}C \cdot min^{-1}$. The whole process took place under a 50 $ml \cdot min^{-1}$ flow of dry nitrogen.

Dynamic thermal mechanical analysis (DMTA)

DMTA is a technique that measures the stiffness and damping of materials. An oscillating load is applied to a sample and its elastic and viscous responses

are monitored against time, temperature or frequency of oscillation, over a temperature range. DMTA is currently an extremely popular technique due to its high precision in the determination of the glass transition temperature (T_g) of the polymeric materials.

The synthesized polyesters were placed in stainless steel material pockets and analyzed using a Tritec 2000 DMA, in single cantilever bending geometry. The tests were carried out from -150 °C to 300 °C, with a standard heating rate of 5 $^oC \cdot min^{-1}$, and in multifrequency mode (1 Hz and 10 Hz).

Tensile stress-strain test

In a tensile stress-strain test the mechanical resistance of materials is evaluated. The specimen was pulled until fracture, in a short time interval and constant velocity and an engineering stress-engineering strain diagram ($\sigma - \varepsilon$) is obtained.

With the purpose of studying the mechanical properties of the PLA/polyester films, samples with an average size of 6 × 2 cm were tested. The tests were performed at room temperature, with a crosshead speed of 10 $mm \cdot min^{-1}$ using the Chatillon TCD 1000 equipment. For each film, three measurements were done.

3.3.3 Morphologic Characterization

Scanning Electron Microscopy (SEM)

SEM is an image formation technique which provides information about morphology / topography of the surface of the material in study. A beam of electrons interacts with the atoms of the surface of a sample. The secondary electrons, emitted by these interactions, are used to form an image of the surface of the material.

First, the samples were treated in order to become conductive, which was accomplished by physical vapor deposition of gold particles. Finally, in order to analyze the surface of PLA/polyester films, a TESCAN VEGA3-SEM Analytical Scanning Electron Microscopy was used.

Contact Angle (CA)

The determination of the CA where a liquid meets a solid surface gives information about wettability of that material, being also an important technique to calculate the surface energy of a sample. As mentioned by Förch and coworkers [37], the CA is defined as the angle at which a liquid/vapor interface meets the solid surface.

The CA of polyester/PLA films was determined using OCA 20 equipment, from Dataphysics. To simulate the eye environment, water was the solvent selected for the measurements. For each test 10 μl of water was dropped, at a rate of 0.50 $\mu l/s$. The tests were performed using the static mode.

3.4 Drug release quantification

3.4.1 Ultraviolet/Visible (UV/Vis) Spectrophotometry

UV/Vis spectroscopy consists in the study of the interactions between the light and a sample. When a beam of light crosses a sample or solution, some of components are absorbed and some are transmitted (Figure 3.2).

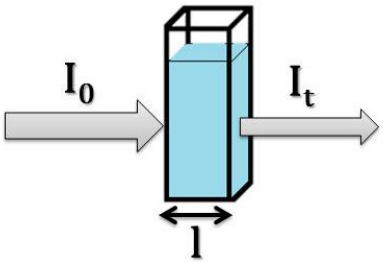


Figure 3.2: Illustration of the absorbance process in a spectrophotometer cuvette.

The ration between the intensity of the incident light (I_0) in the sample and the transmitted light (I_T) is known as transmittance (T) (see Equation 3.1).

$$T = \frac{I_T}{I_0} \tag{3.1}$$

The absorbance (A) can also be calculated, being related with the negative logarithm of transmittance, as represented in Equation 3.2.

$$A = -log_{10}T \tag{3.2}$$

According to Beer-Lambert Law, for an ideal solution, there is a linear relationship between the concentration (c) and the absorbance as long as the

path length (l) of the cuvette is kept constant. This law can be written as (see Equation 3.3) :

$$A = \varepsilon cl \tag{3.3}$$

where ε is the absorptivity at a specific wavelength.

In UV/Vis spectroscopy, the choice of the appropriate type of cuvette is crucial. Quartz cuvettes are probably the best choice due to their wavelength range and optical qualities. Nevertheless, they are expensive and, being frequently reused, contaminations may occur during the measurements. These make disposable cuvettes (plastic, UV-grade or glass cuvettes) advantageous when referring to costs and prevention of contamination. However, the last ones can only be used at wavelengths above 360 nm. For wavelengths below 300 nm UV-grade cuvettes are used, even though they present significant absorbance around 240 nm. Below that wavelength only quartz cuvettes can be used. A standard 10 mm cuvette has a maximum volume of 3.5 ml but, for limited volume assays, there are available other cuvettes with smaller volume. [38]

UV/Vis spectroscopy was used to measure the amount of drug released from different PLA/polyester films over time. A JASCO V-550 spectrophotometer with a deuterium and a halogen lamp was used for the purpose of this study. Two 10 mm UV High Precision Cells made of natural quartz crystals, with 3.5 ml capacity, from Hellman Analytics, were used during the whole process.

The first step of the measurements consists in determining the wavelength at which acetazolamide absorbs. Using as reference a phosphate buffered saline (PBS) solution (in order to mimic the physiological conditions of the eye) with pH 7.4, it was determined that the drug absorbed with a maximum

at 266 nm.

The second step was to prepare a calibration curve with solutions of known concentration. For which solution, the absorbance was registered using a PBS solution as reference. Afterward, the calibration curve was obtained by plotting the concentrations and respective absorbance (see Figure 3.3).

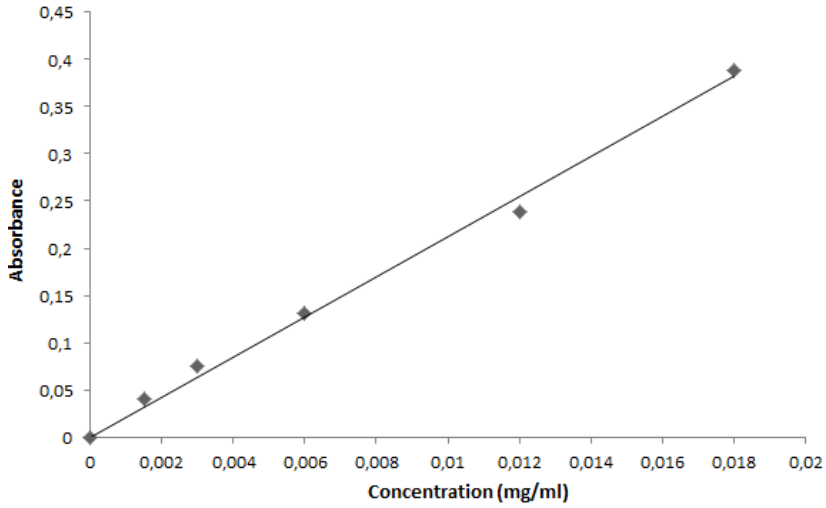


Figure 3.3: Example of a calibration curve.

Thus, knowing the absorbance of the samples, their concentrations can be obtained through the calibration curve.

Finally, the absorbance of samples was measured over time. For each type of film a reference solution, containing the films but no drug, and three solutions with films containing acetazolamide were prepared. Two cuvettes were placed in the spectrophotometer, one containing the reference liquid and another with the solution with the drug.

Chapter 4

Results and Discussion

4.1 Synthesis and characterization of Saturated Polyesters

As mentioned in 3.2.1, four different saturated polyesters were synthesized for plasticization of PLA. Saturated polyesters can be obtained by polycondensation of a saturated acid and a glycol. Figures 4.1 to 4.4 show the chemical structures of the synthesized SPs in this work.

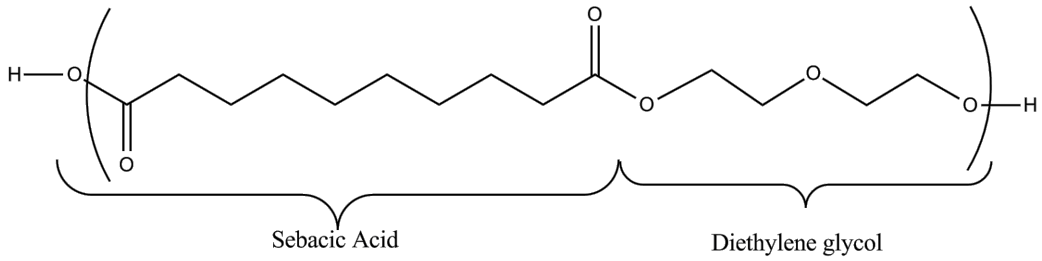


Figure 4.1: Chemical structure of SP1.

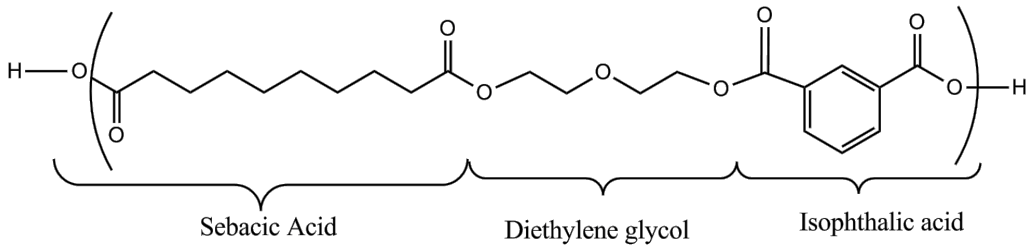


Figure 4.2: Chemical structure of SP2.

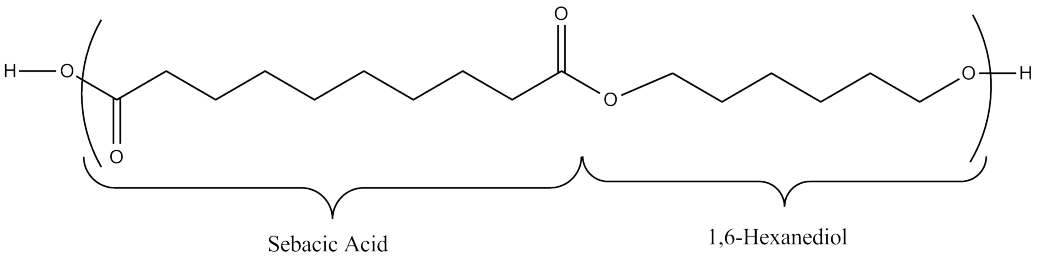


Figure 4.3: Chemical structure of SP3.

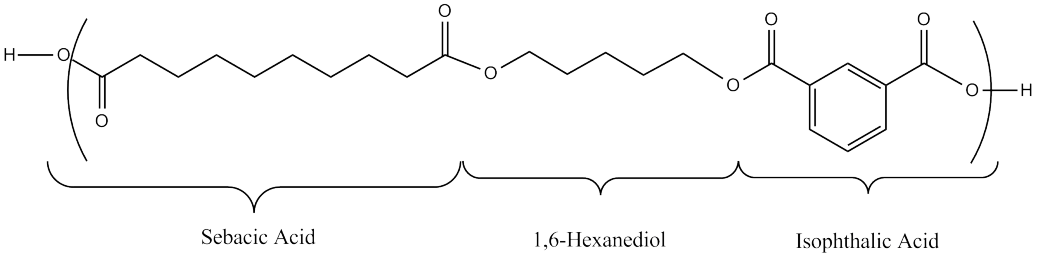


Figure 4.4: Chemical structure of SP4.

The main objective of the different syntheses was to determine the effect of the composition of the SP in the plasticization of PLA. To understand this effect, the chemical characterization of the obtained SPs is essential. The chemical structure of the synthesized polyesters was analyzed by ATR-FTIR (Figure 4.5).

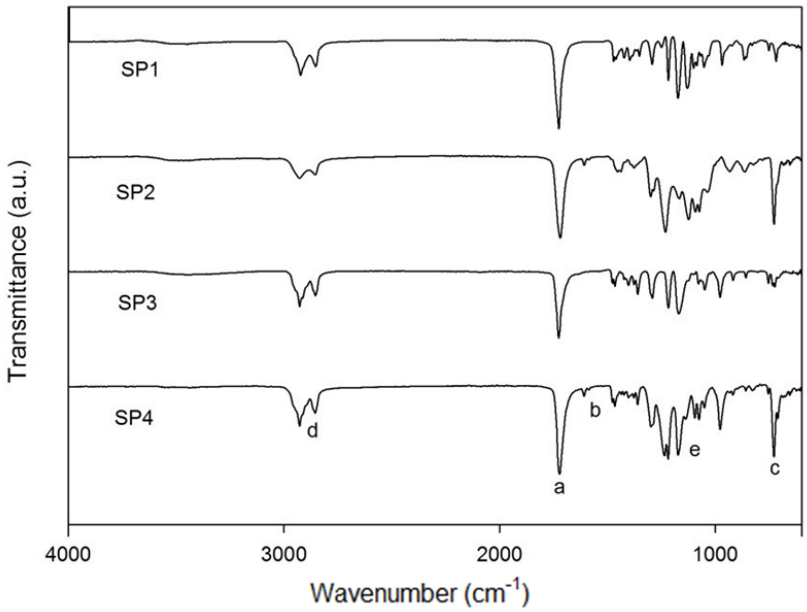


Figure 4.5: ATR-FTIR spectra for SP1, SP2, SP3 and SP4.

As expected, the spectra presented in Figure 4.5 show bands corresponding to the stretching vibration of the carbonyl group of the ester linkage (a) (1750-1725 cm^{-1}). [39][40][41] The bands in the 1650-1580 cm^{-1} and 1510-1450 cm^{-1} (b) regions are characteristic of the aromatic ring of isophthalic acid present in SP2 and SP4. In the same polyesters, it was observed a distinct peak at 725 cm^{-1} (c), ascribed to the aromatic C-H out-of-plane bending vibrations.[39] The presence of absorption peaks at ca. 2860 cm^{-1} (d) can be attributed to the vibrational modes from the CH_2 groups. In all samples, the peaks in the characteristic region 1150-1050 cm^{-1} (e) can be attributed to the C-O group. The absence of peaks in the region 3600-3200 cm^{-1}, ascribed to the hydroxyl group, indicates that all the monomer reacted. [39] As expected, no bands were observed in this region.

Further insights onto the chemical structure of the SPs were given by ^{1}H NMR. $THF - d_8$ was used as solvent for the analysis of the samples.

The SP1 spectrum (Figure 4.6) exhibits three distinct peaks at $\delta = 1.32$ ppm (a), 1.59 ppm (b) and, 2.28 ppm (c) which are ascribed to the internal $-CH_2$ protons of the SeA. As expected, all the SPs present all the three peaks, proving the incorporation of the SeA in the structure of the polyesters. For formulations containing isophthalic acid (IPhA), SP2 and SP4, three peaks at $\delta = 7.5$ ppm (i), 8.2 ppm (h) and 8.63 ppm (j) can also be assigned to the protons characteristics of the aromatic ring of the acid.

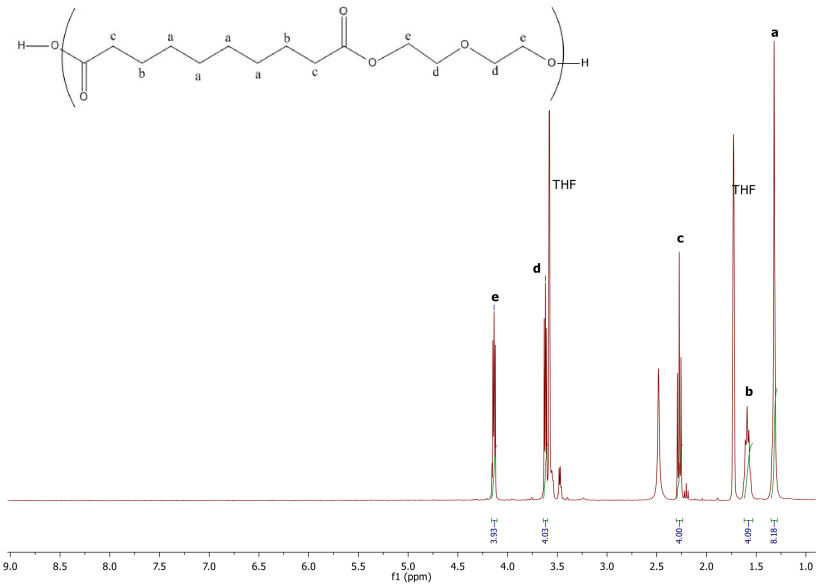


Figure 4.6: ^{1}H NMR of SP1.

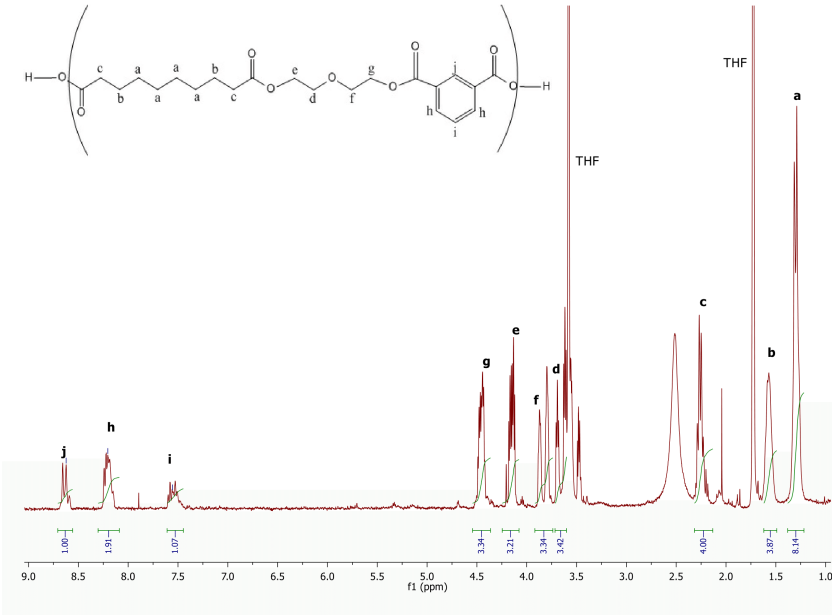


Figure 4.7: ^{1}H NMR of SP2.

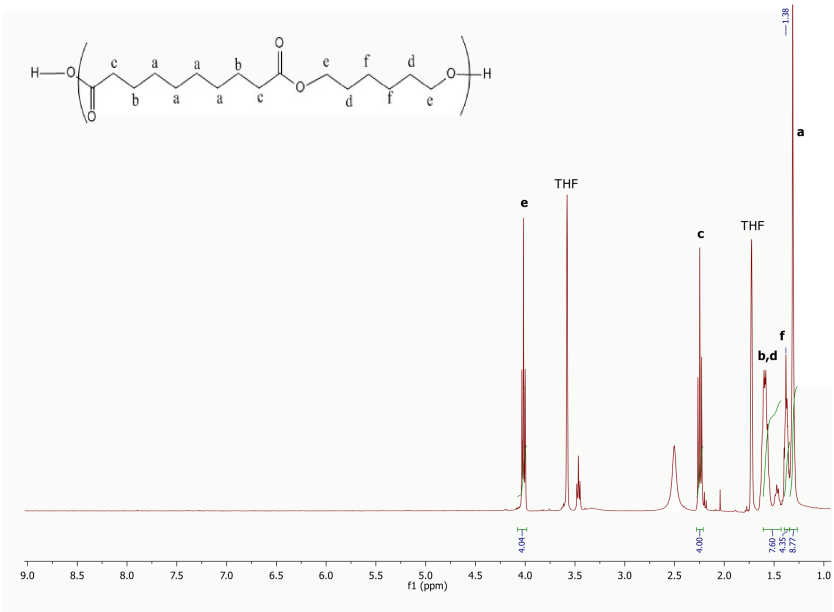


Figure 4.8: ^{1}H NMR of SP3.

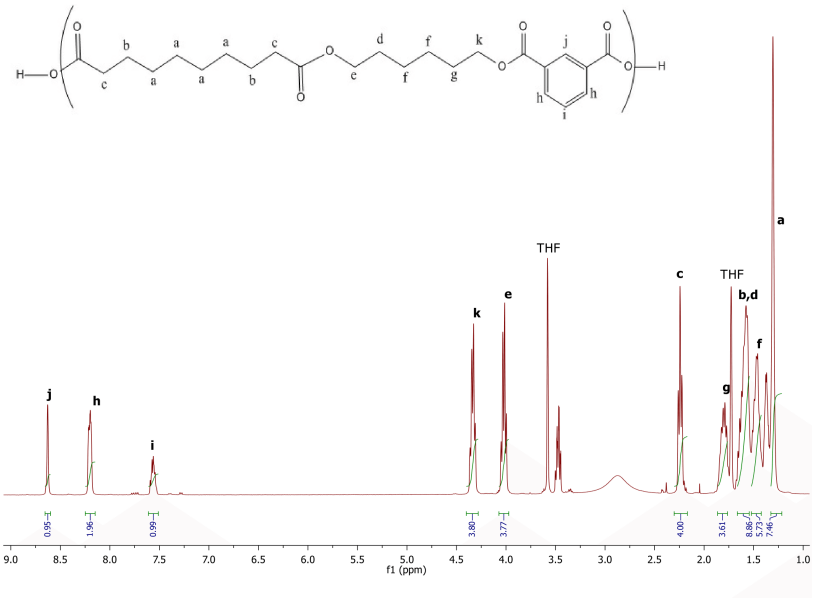


Figure 4.9: ^{1}H NMR of SP4.

As can be seen in Figures 4.1 to 4.4, two different diols were selected for the synthesis of SPs, diethylene glycol (DEG) and 1,6-hexanediol (HEX). In formulations with DEG, SP1 and SP2, the signals of the $-CH_2$ protons are presented between 3.6 and 4.5 ppm. On the other hand, the CH_2 protons of the HEX can be identified in the chemical structure of SP3 and SP4. [42] In SP3 spectrum (Figure 4.8), the internal CH_2 protons are located between 1.4 (f) and 1.6 ppm (d) while the peak at 4.0 ppm (e) corresponds to the $-CH_2$ bonded to the ester groups. On the other hand, and due to the presence of the IPhA, in SP4 spectrum (Figure 4.9) it is also possible to identify $-CH_2$ protons at 1.8 and 4.3 ppm.

From these assignments it is possible to calculate the approximate composition of the polyesters comparing with the feed composition as well as their AV values of the obtained polyesters (Table 4.1).

Table 4.1: Synthesis conditions of the synthesized SPs from diethylene glycol (DEG), sebacic acid (SeA), 1,6-Hexanediol (HEX) and isophthalic acid (IPhA).

Polyester	**Composition**	**Initial Molar Ratio (%)**	**Acids/Alcohols Final Molar Ratio**	**AV (mg KOH/g)**
SP1	DEG/SeA	50/50	50/50	24.3
SP2	DEG/SeA/IPhA	50/25/25	35/35/30	26.4
SP3	HEX/SeA	50/50	48/52	21.9
SP4	HEX/SeA/IPhA	50/25/25	30/28/42	28.9

As can be seen in Table 4.1, the molar amount of monomers in the feed and the final composition of SPs calculated by NMR has some differences. These differences can be justified by the loss of diols during the polycondensation reaction, by the reaction conditions and by the different reactivity of the monomers.

The thermal stability of the SPs was studied by termogravimetric analysis (TGA). Figure 4.10 presents the TGA curves for the different SPs.

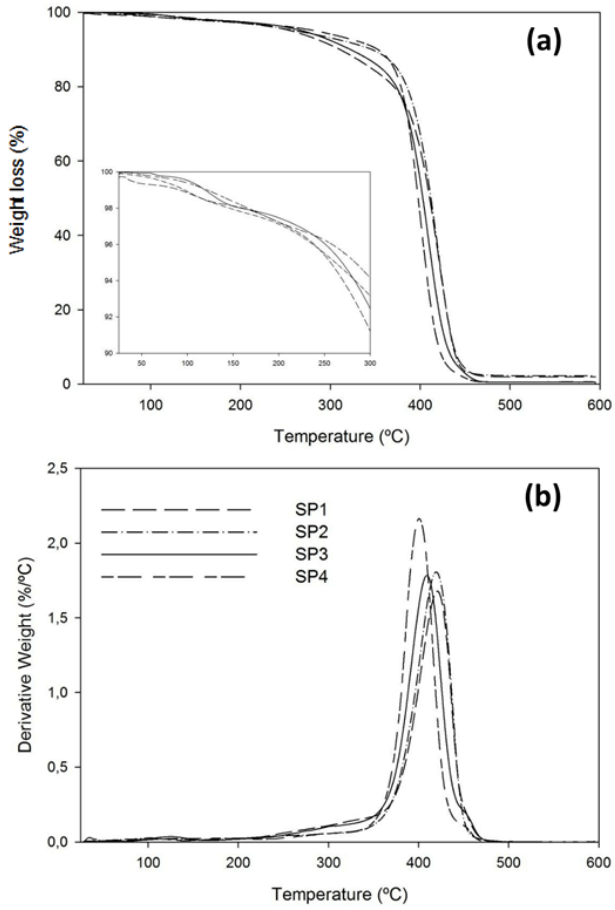


Figure 4.10: Thermogravimetric curves of SPs (a) TG and (b) DTG.

The synthesized SPs present a single stage of weight loss (Figure 4.10 (a)), which can be confirmed by the presence of a single peak in the derivative thermogravimetric (DTG) curve (Figure 4.10 (b)), presenting a similar thermogravimetric profile. All the studied polyesters are stable until high temperatures, starting to decompose at approximately 300 ºC. However, a closer look reveals that SP1 is the less stable polyester which is explained by the absence of an aromatic ring in its structure as well as the chain length of the glycol. In fact, the HEX presented in the structure of SP3 has a higher number of carbons in the main chain and, consequently, this polyester has

higher thermal stability when compared to SP1. On the other hand, polymers with IPhA presented the highest thermal stability. According to Scheirs [43], IPhA can be used to improve the rigidity of the polymers, proving the obtained results. As already indicated, and confirmed by the results presented in Table 4.2, the thermogravimetric profile of the SPs is similar. SP1 has lower values of $T_{5\%}$ and $T_{10\%}$, being the less stable of all four polyesters. By its turn, SP4 has higher $T_{5\%}$ and $T_{10\%}$ which makes it the more stable of the synthesized polyesters.

Table 4.2: Characteristic temperatures of the SPs. $T_{5\%}$: temperature corresponding to 5% of mass loss; $T_{10\%}$: temperature corresponding to 10% of mass loss.

Polyester	$T_{5\%}$ **(°C)**	$T_{10\%}$ **(°C)**
SP1	255.67	310.87
SP2	260.58	346.54
SP3	268.39	322.58
SP4	282.49	353.04

The thermal events of the synthesized polyesters were studied by DSC and DMTA. The thermal history of the samples was erased by heating the samples until 125 °C. Then, their thermal behavior was measured by cooling the samples until -80 °C and, finally, they were heated until 200 °C. The heat flow curve from the cooling cycle (a) and the second heating cycle (b) are represented in Figure 4.11.

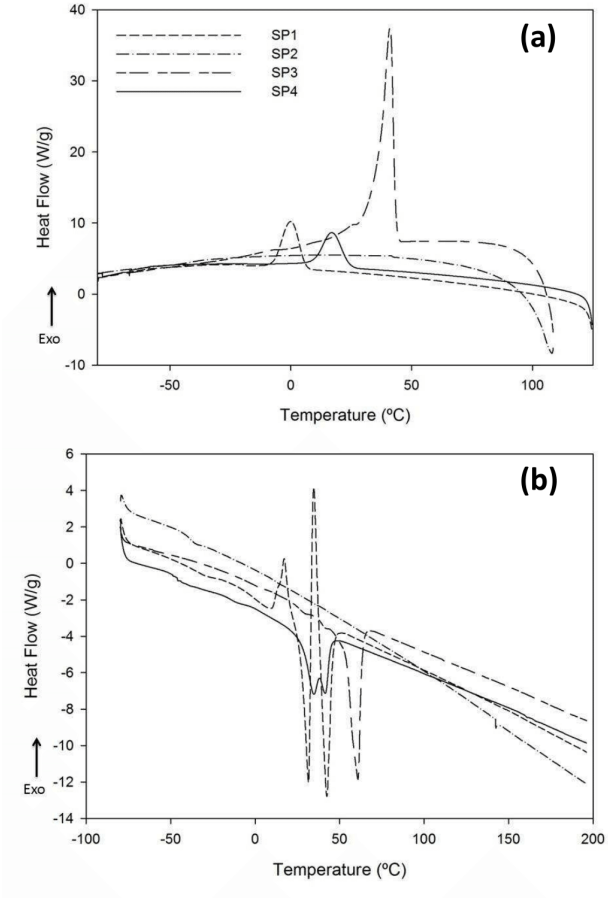


Figure 4.11: DSC curves of the synthesized SPs obtained at the cooling cycle (a) and at the second heating cycle (b).

According to Figure 4.11 (b), SP1, SP3 and SP4 have crystalline and amorphous regions which is proved by the presence of an endothermic peak matching the melting temperature of the polyesters. On the other hand, SP2 is an amorphous material proved by the absence of T_m. By its turn, SP4 presents two T_m, which can be explained by two distinct crystalline areas in the polymer. Finally, SP1 curve holds two endothermic events interposed by an exothermic event. This type of events, for the specific case of this polyester, is not described in the literature. It's important to note that

DSC analyzes were repeated for both polyesters (SP1 and SP4) in order to understand if the obtained results were correct. The results of a second analysis proved to be consistent with the first analysis.

Table 4.3 presents the data obtained by differential scanning calorimetry for the synthesized polyesters and PLA. SP4 presents two T_m both inferior to the T_m of SP3. SP1 and SP3 are the simplest polyesters synthesized. As a consequence of the lack of information concerning the T_m of SP1 it's not possible to compare these two polyesters. However, the T_c of SP1 is lower than the value obtained for SP3 which can be due to the presence of DEG in its structure.

Table 4.3: DSC of the SPs and PLA (T_c: Crystallization Temperature; T_m: melting temperature).

Polyester	T_c **(ºC)**	T_m**(ºC)**
SP1	0.00	—
SP2	—	—
SP3	41.05	60.86
SP4	16.85	34.57/41.89
PLA	—	175.08

Table 4.4 presents the T_g values obtained from DSC and DMTA for all synthesized SPs and PLA. The differences observed between the values obtained by these two techniques can be ascribed to differences in the principles used to determine its value. [39] With exception of SP1, the T_g of the samples can be obtained by DSC. However, DMTA is a technique with higher precision in the determination of the glass transition temperature of the polymers.

Table 4.4: Glass transition temperature obtained from DMTA and DSC.

Polyester	**Tg (°C)**		**Tan δ(max)**
	DMTA	**DSC**	
SP1	-24.7	——	0.07
SP2	51.9	-38.1	0.02
SP3	-53.0	-39.8	0.07
SP4	-9.2	-14.6	0.05
PLA	77.6	60.4	0.22

SP1 presents lower value of T_g when compared to SP2. This result is expected due to the absence of an aromatic ring in its structure. On the other hand, the lowest T_g value was observed for SP3. Indeed, this polyester has no aromatic ring and has a glycol with longest carbon chain, giving higher flexibility. As a consequence, lower T_g was expected. Finally, the values of loss tangent (tan δ) reveal that all synthesized SP have little capacity to dissipate energy.

As mentioned before, SPs were synthesized to plasticize PLA. The choice of a plasticizer depends on the desired final properties of the material. For that, it is important to understand the compatibility between the plasticizer and the polymer. Chemical structure of the plasticizer (including chemical composition and molecular weight) is one of the parameters that can affect the solubility of the plasticizer in the polymer. [31][32] Thus, the synthesized SPs were studied by SEC in order to determine their molecular weight. All the samples have a molecular weight inferior to 4000 $g.mol^{-1}$, which makes them low molecular weight plasticizers. Another parameter to take into account is the T_m of the plasticizer. Lower T_m values can lead to increases solubility of the plasticizer in the material since less energy is required to fuse with the polymer. Thus, it is expected that SP4 is able to plasticize PLA more easily that SP3, since it has lower T_m values.

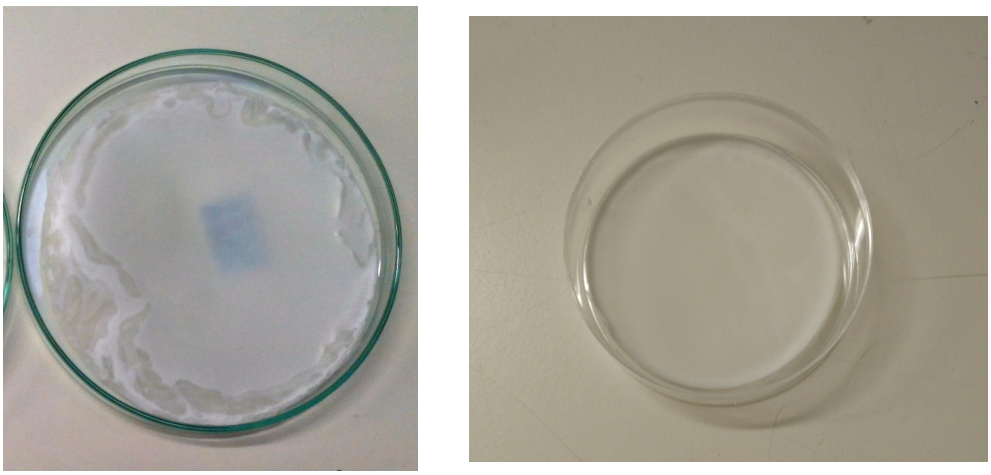
After synthesis and charactherization of the polyesters, they were used to form films. These films were characterized in order to understand if plasticization occurred. Finally, the most promissing films were used to study the drug release of acetazolamide.

4.2 Film formation and characterization

To obtain a plastified film of PLA, it is important to identify the suitable solvent to dissolve this polymer. As mentioned by several authors, PLA is soluble in chloroform and dichloromethane. However, the use of dichloromethane revealed to be less efficient. In fact, due to its lower boiling temperature ($T_{boiling}$ = 39.6 ºC) when compared to chloroform ($T_{boiling}$ = 61.2 ºC), the films prepared with this solvent presented an heterogeneous structure with some bubbles.

On the other hand, different proportions of SP/PLA (20/80 wt.% , 30/70 wt.% and 50/50 wt.%) were studied for the formation of films. However, as can be seen in Figure 4.12 (a), the combination of 20/80 wt.% of SP1/PLA showed to be unable to form a homogenous film by *solvent casting*. Figure 4.12 (b) shows a 50/50 wt.% SP1/PLA film.

Thus, taking these results into consideration, two types of blends were prepared; a blend with 30/70 wt.% of SP/PLA and a blend with 50/50 wt.% of SP/PLA. Table 4.5 shows the composition of each film.



(a) (b)

Figure 4.12: Film containing 20/80 wt.% of SP/PLA (a) and a film containing 50/50 wt.% of SP/PLA (b).

Table 4.5: Composition of the SP/PLA films obtained in the oven.

Films	SP/PLA ratio (%)
SP1 PLA70 O	SP1/PLA 30/70
SP1 PLA50 O	SP1/PLA 50/50
SP2 PLA70 O	SP2/PLA 30/70
SP2 PLA50 O	SP2/PLA 50/50
SP3 PLA70 O	SP3/PLA 30/70
SP3 PLA50 O	SP3/PLA 50/50
SP4 PLA70 O	SP4/PLA 30/70
SP4 PLA50 O	SP4/PLA 50/50

After the film formation (as explained in 3.2.2) it was possible to understand that SP1 PLA70 O as well as SP2 and SP4 (with both SP contents) dried in the oven at 50 °C were unable to form films. Nevertheless, SP1 PLA50 O, SP3 PLA70 O and SP3 PLA50 O formed films able to be used for drug delivery studies.

It is known that a plasticizer should decrease the T_g of PLA and enhance tensile toughness. [32] In order to understand if SP1 and SP3 were able to plasticize PLA, the thermal and mechanical properties of the resulting films

were studied.

The thermal events of the synthesized films, specifically the T_g values, were studied by DSC. The heat flow curve from the cooling cycle (a) and the second heating cycle (b) are represented in Figure 4.13.

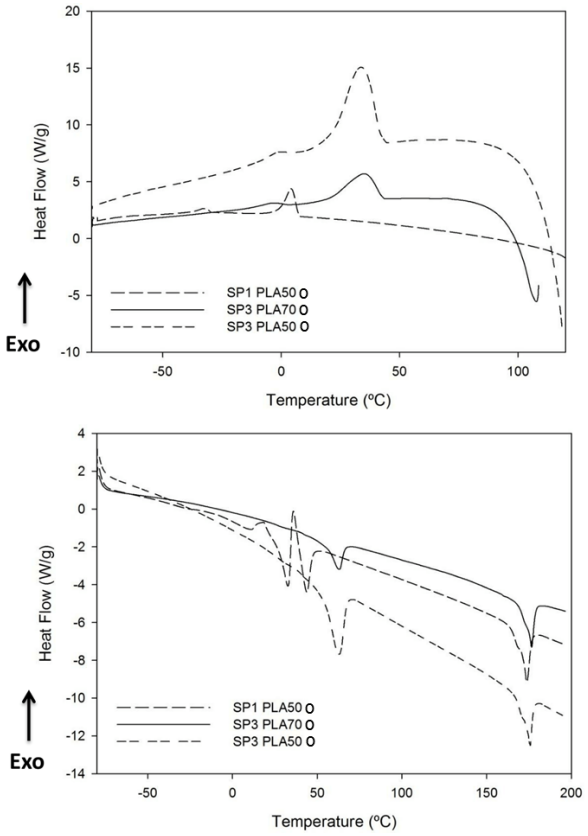


Figure 4.13: DSC curves of the SP/PLA films obtained at the cooling cycle (a) and at the second heating cycle (b).

Figure 4.13 (a) shows the crystallization peak for each film. According to Okamoto [29], PLA has no cristalization peak in the cooling step. On the other hand, as presented in Table 4.3, SP1 and SP3 have crystalization peaks at 0 °C and 41.05 °C, respectively. Thus, in the presented films, only the crystalization of SPs is observed. Figure 4.13 (b) shows the endothermic peaks associated with the T_m values for the three films, being these values

close to the T_m value for PLA. Moreover, for the films with SP3, it was also identified the endothermic peak characteristic of the polyester, at around 61 ºC. In addition, the curve of SP1 PLA50 O film presents the two endothermic events identified in the DSC study of SP1. The DSC analysis was repeated for this film, which proved to be consistent with first analysis. A similar profile was found for films left in the fume hood to evaporate the chloroform.

According to Okamoto [29] , it is expected the presence of a crystallization peak in the heat flow curve of the second heating (Figure 4.13 (b)). However, this peak is not observed for any film, being ascribed probably due to the rearrangement of the polymeric chains during cooling, after the thermal history of the polymer was erased during the first heating.

Table 4.6 presents the data obtained by this thermal analysis.

Table 4.6: DSC of the SP/PLA films (T_c : Crystallization Temperature; T_m : melting temperature; ΔH_m : fusion enthalpy).

Films	T_c **(ºC)**	T_m **(ºC)**	$\Delta H_m(J \cdot g^{-1})$
SP1 PLA50 O	4.12	173.66	26.31
SP3 PLA70 O	35.30	176.60	22.27
SP3 PLA50 O	33.66	175.76	19.85

The T_m values of the films showed to be similar to each other. The fusion enthalpy (ΔH_m) of the films was determined in order to understand if there was a variation in the crystallinity of the PLA caused by the incorporation of SP. When compared to neat PLA($\Delta H_m = 47.34\ J \cdot g^{-1}$), the films presented lower ΔH_m values indicating a decrease of nearly 50% of crystallinity.

In addition to the described thermal events, no other event was detected in the studied films. Indeed, it was not identified any T_g value by DSC. Thus, the T_g of each film was determined by DMTA. However, this technique proved to be ineffective in determining the T_g of the films by tension mode, the geometry currently applied to analyse this type of samples. Consequently,

with respect to the plasticization of PLA no conclusion can be drawn based on the T_g of the films. However, it is important to note that assuming that the films are plasticized a reduction of T_g is expected.

The mechanical properties of the films prepared in the oven and in the fume hood were evaluated by a tensile stress-strain test. It is important to note that the samples used for these tests do not have a standard shape. Consequently, no quantitative analyses were performed. The obtained results for the SP/PLA films formed in the oven proved to be inconclusive. Therefore, they are not presented in this work. Figure 4.14 shows the obtained results for the SP/PLA films formed in the fume hood. The PLA films (without any SP) were also prepared in order to be used as a reference material.

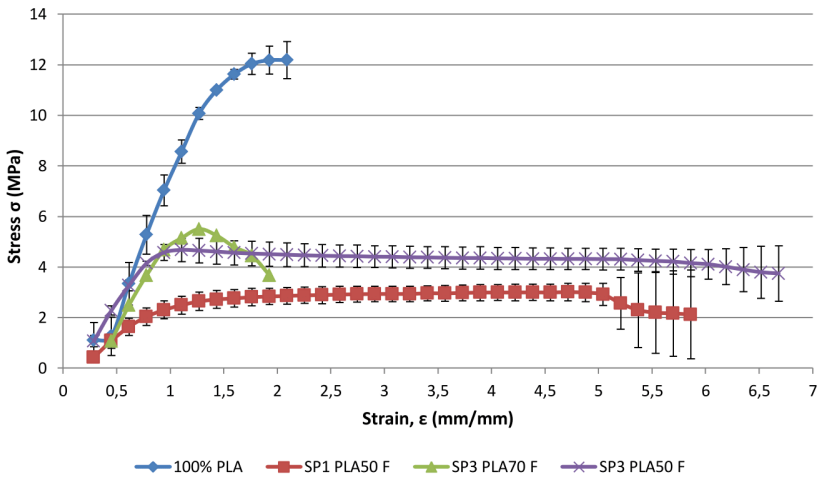


Figure 4.14: Stress vs strain curves from tensile testing for SP/PLA films prepared in the fume hood.

As can be seen in Figure 4.14, all the three blends showed a decrease in yield strength (σ_y) when compared to PLA films. However, SP1 PLA50 F and SP3 PLA50 F show a clear increase in the elongation at break (ε_b) which indicates a ductile behaviour with formation of necking. By its turn, SP3 PLA70 F show a brittle behaviour. By the data obtained through the tensile testing it is possible to observe that the SP1 PLA50 F and SP3 PLA50 F seem to be plasticized.

Despite being an exact technique to measure the yield strength, elongation at break and Young modulus of a material, the use of a non-standard geometry for the tests and the non-homogeneity of the films can lead to erros in the data obtained.

To understand how water interactes with the surface of the films, the contact angle (CA) of the films were also studied and the results are presented in Table 4.7.

Table 4.7: Contact angle of SP/PLA films.

Sample	**Evaporation Process**	**CA (º)**	**Error (º)**
PLA 100% O	Oven	75	3
SP1 PLA50 O		59	3
SP3 PLA70 O		79	2
SP3 PLA50 O		92	2
PLA 100% F	Fume Hood	77	2
SP1 PLA50 F		58	2
SP3 PLA70 F		91	3
SP3 PLA50 F		87	2

The CA for PLA is similar by the films obtained in the oven and in the fume hood, presenting a partially wetting surface. [37] By their turn, SP1 PLA50 films have a decrease in contact angle when compared to PLA films, which can indicate that this blend is more prone to suffer degradation due to

interaction with water. SP3 PLA70 and SP3 PLA50 have an increased CA when compared to PLA films, which makes them more hydrophobic, and non wetting. Thus, it is expectable that the drug delivery systems using these type of films present lower release of the drug in the first days. The CA of both sides of the films were measured and proved to have identical CA.

4.3 Drug Releasing Tests

To understand the relationship between the concentration of the drug and the absorbance measured in the spectrophotometer, the calibration curve of acetazolamide was established. As mentioned in 3.4.1, a full wavelength scan was run in order to determine the maximum absorbance peak of acetazolamide. The results of such measurement determined that the drug absorbed with a maximum at 266 nm. Thus, all the measurements performed in this work were carried out in a fixed wavelength of 266 nm.

To obtain the calibration curve (presented in Figure 4.15), a main solution was prepared by dissolving acetazolamide in a saline solution, with a final concentration of 0.06 mg/ml. Different volumes of this main solution were distributed for different flasks obtaining five different concentrations of acetazolamide. Table 4.8 present the absorbance obtained in the spectrophotometer for each solution.

Table 4.8: Details of the prepared dissolutions to build the acetazolamide curve.

Concentration (mg/ml)	**Absorbance**
0.06	0.9114
0.018	0.3890
0.012	0.2393
0.006	0.1316
0.003	0.0756
0.0015	0.0411

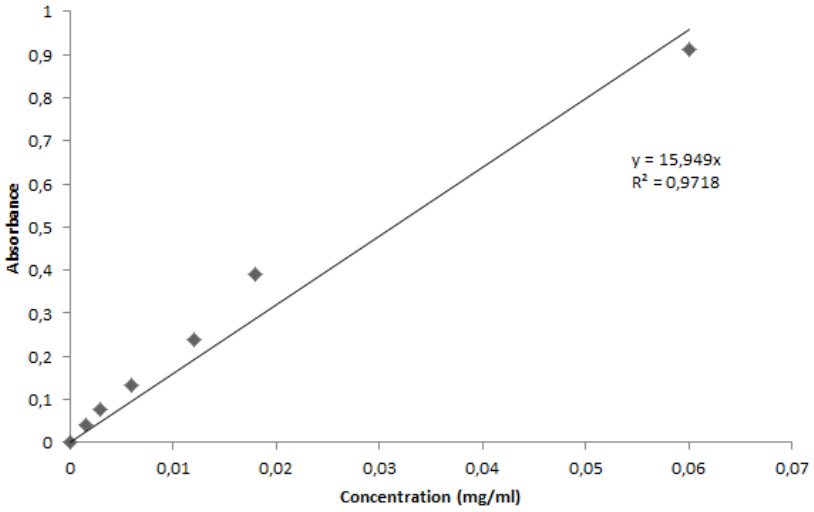


Figure 4.15: Calibration curve of acetazolamide, relating the measured absorbance and the concentration of the solutions.

With the concentrations and respective absorbance (Table 4.8), the calibration curve for the drug was obtained and is presented in Equation 4.1, where y is the absorbance and x the concentration:

$$y = 15.949 \cdot x \tag{4.1}$$

4.3.1 Experiment number 1

As indicated above, three films were selected for drug encapsulation: SP1 PLA50 O, SP3 PLA70 O and SP3 PLA50 O. As explained before, 25 mg of acetazolamide were dissolved in 25 ml of film solution. Afterwards, a fixed volume (2.5 ml) of the different solutions was placed in petri dishes and, in order to obtain the final films, the chloroform was left to evaporate in an oven at 50 ºC. Three discs with a diameter of 2 cm were obtained for each sample, containing 1 mg of acetazolamide each. In addition, as reference, a film without the drug was also prepared. The four films were next submerged into 25 ml of PBS and placed in a shaker at 37 ºC and 100 rpm. Then, the drug delivery behavior of the films was studied.

After 4 hours of immersion and in the following days, for a period of 8 days, the absorbance was measured for all samples. Using the calibration curve (Figure 4.15), the concentration of the drug in PBS solution for each film was calculated. For each reading, the PBS solution was returned to the vials until next measurement. The results of the average release for these three samples are shown in Figure 4.16, where 100% of released drug corresponds to the total 1 mg of acetazolamide.

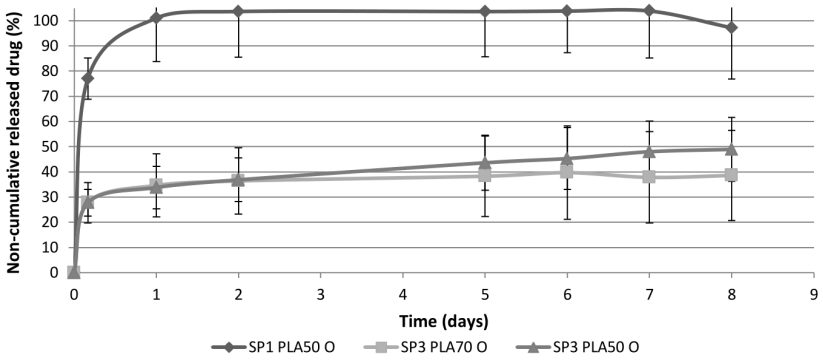
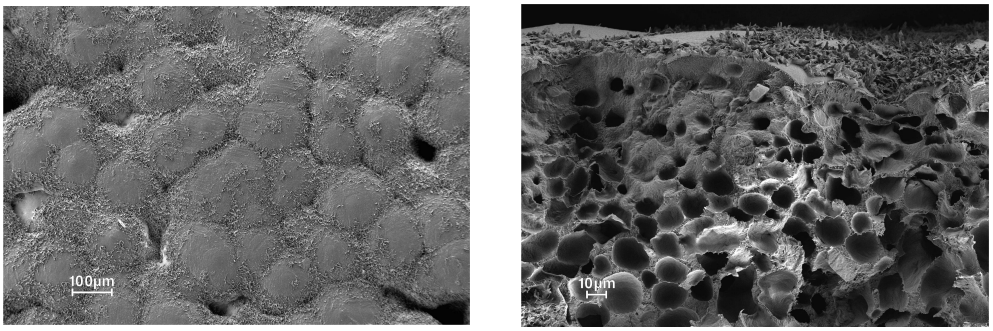


Figure 4.16: Comparison between the acetazolamide releasing profile by SP1 PLA50 O, SP3 PLA70 O and SP3 PLA50 O films over the course of 8 days.

In all three samples (with drug) it is clear that a burst release takes place during the first 4 hours of immersion. With a total release of the drug in the first 24 hours, SP1 PLA50 O films were eliminated of this study. On the other hand, SP3 PLA70 O and SP3 PLA50 O showed similar drug release during the first 8 days. After this period, SP3 PLA70 O released $38.6 \pm 17.8\%$ of the drug whereas SP3 PLA50 O released $48.9 \pm 12.6\%$. It is clear that SP3 PLA70 O films showed lower release when compared to SP3 PLA50 O films, but taking into account the associated errors, this difference is not significant. The release profile of these two films may also indicate that acetazolamide is stable inside and outside of the films, since after a period of 5 days the release of the drug tends to stabilize. It is also important to note that after this study, the films showed no signs of degradation.

To evaluate the surface properties of the films, SEM imaging was performed. Figure 4.17a shows the surface of a SP1 PLA50 O film. The heterogeneity of the surface can be due to phase separation of the PLA and the plasticizer. Figure 4.17b shows an image of the cross section of the same film, which allows to understand that the film is porous. The surface morphology

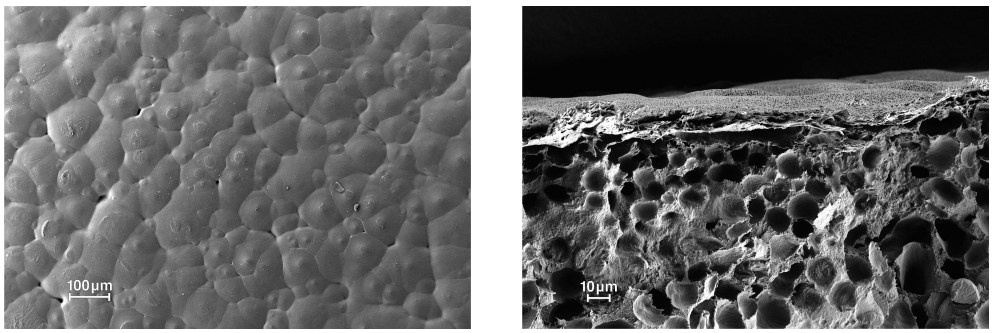
of the films is also visible. Thus, the irregularities observed in the surface and the porosity in the material support the high releasing rates obtained for this type of films.



(a) (b)

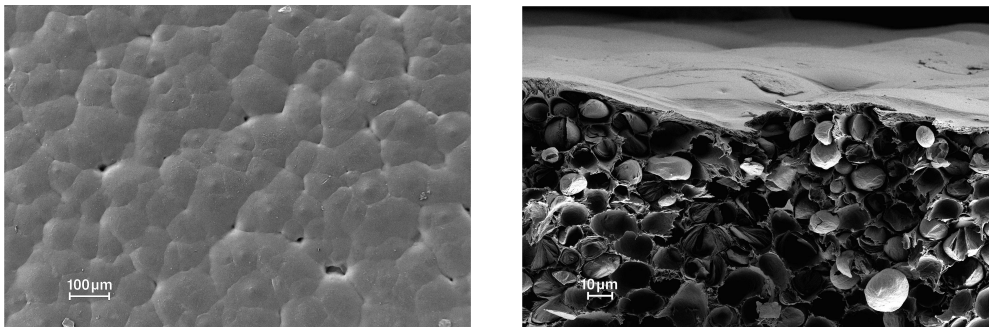
Figure 4.17: SEM image of (a) the surface (with a magnification of 100X) and (b) the cross section (with a magnification of 500X) of SP1 PLA50 O films.

By their turn, SP3 PLA70 O and SP3 PLA50 O films present similar surfaces, as can be seen in Figure 4.18a and Figure 4.19a, respectively. Both films present a morphology similar to neat PLA (see Appendix A), but they present phase separation. The spherical structures in their surface are smaller than the ones in the surface of SP1 PLA50 O films. Also, their homogeneity when compared to the SP1 PLA50 O films can explain the better results of drug release obtained by these films. When analyzing the cross section of SP3 PLA70 O (Figure 4.18b) and SP3 PLA50 O (Figure 4.19b) it is possible to understand that the films are porous, as seen for SP1 PLA50 O films (Figure 4.17b).



(a) (b)

Figure 4.18: SEM image of (a) the surface (with a magnification of 100X) and (b) the cross section (with a magnification of 500X) of SP3 PLA70 O films.



(a) (b)

Figure 4.19: SEM image of (a) the surface (with a magnification of 100X) and (b) the cross section (with a magnification of 637X) of SP3 PLA50 O films.

4.3.2 Experiment number 2

In a second experiment, the films SP3 PLA70 O and SP3 PLA50 O were coated with the same polyester solution used in their preparation (SP3/PLA) in order to decrease their release rates. Three films containing acetazolamide were coated using a paint-brush and placed, as before, in a vial with 25 ml of PBS. Being the reference, one film without any drug also coated. Two types of measurements were made: one in which the solution of PBS was returned

to the vial after the reading, as done for the first experiment, and one in which the solution of the vial was replaced by new PBS each time a reading was performed.

Figure 4.20 shows the releasing profiles of SP3 PLA70 O and SP3 PLA50 O coated films when the PBS solution is kept the same. The absorbance was measured 4 hours after the immersion and in the following days, for two weeks.

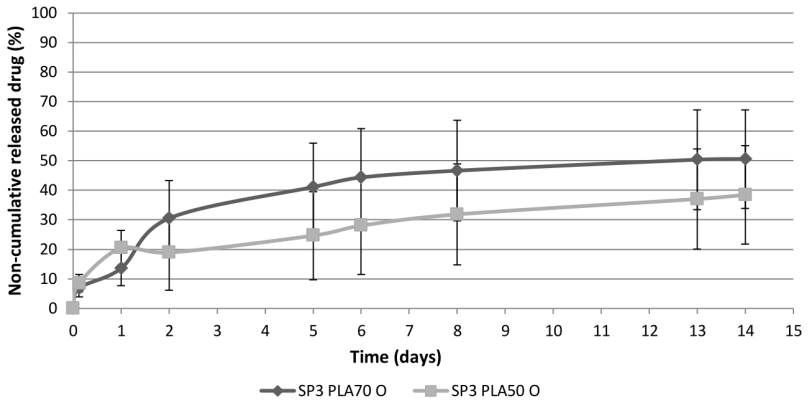


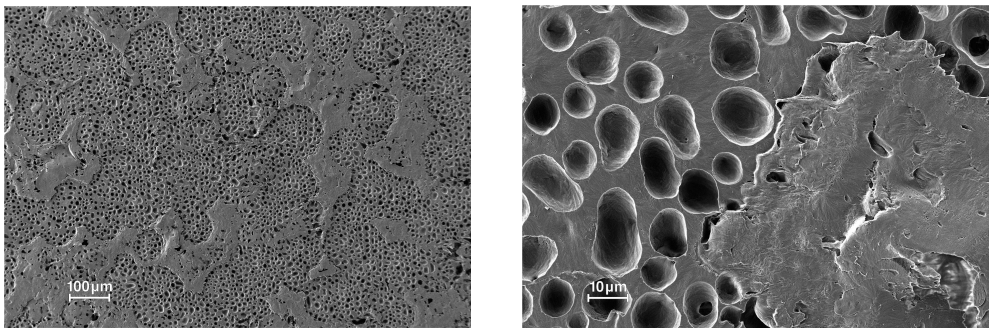
Figure 4.20: Comparison between the non-cumulative acetazolamide releasing profile by SP3 PLA70 O and SP3 PLA50 O coated films over the course of 14 days.

The presence of the coating proved to be effective in decreasing the prompt release during the first 4 hours of immersion. SP3 PLA70 O films showed a decrease in its release from $27.8 \pm 5.3\%$ to $6.8 \pm 3.0\%$ in the first 4 hours, being that for sample SP3 PLA50 O the release decreased from $27.8 \pm 8.1\%$ to $8.5 \pm 4.5\%$. Unlike the results of the first experiment, where SP3 PLA70 O showed a lower drug release than SP3 PLA50 O, in the second experiment SP3 PLA50 O shown lower drug release when compared to SP3 PLA70 O after 24 hours.

The coating proved its efficiency in decreasing the release of acetazolamide

from SP3 PLA50 O films. After 8 days, SP3 PLA50 O coated films have released $31.8 \pm 13.6\%$ of its drug content when compared to $48.9 \pm 12.6\%$ released by SP3 PLA50 O films without coating. The final values of drug release after 14 days were $50.6 \pm 16.7\%$ for SP3 PLA70 coated films and $38.4 \pm 16.1\%$ for SP3 PLA50 coated films.

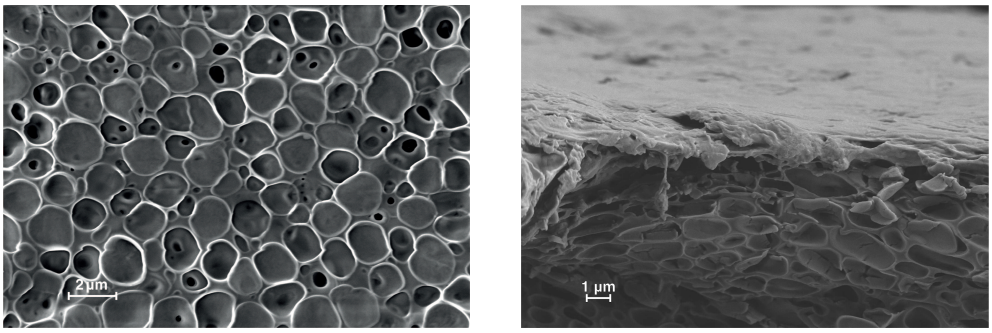
In Figure 4.21 it is presented the SEM image of the SP3 PLA70 O film coated with its main solution. It is clear that the coating was not evenly distributed across the film, exposing some areas of the film. These observations confirm the release profile obtained for SP3 PLA70 coated films. During the coating, the films showed a tendency to dissolve due to the presence of the solution with chloroform. The change in the surface of the coated films can be due to the evaporation of chloroform of the solution used for coating.



(a) (b)

Figure 4.21: SEM image of the surface SP3 PLA70 O film coated with its main solution with a magnification of 100X (a) and with a magnification of 1.000X (b).

SEM images of the SP3 PLA50 O film coated with its main solution are presented in Figure 4.22. The coating seems to be more evenly distributed through the surface of the film when compared to SP3 PLA70 O coated films. This can explain the lower releasing rates associated with SP3 PLA50 O coated films when compared to SP3 PLA70 O coated films.



(a) (b)

Figure 4.22: SEM image of (a) the surface (with a magnification of 6000X) and (b) the cross section (with a magnification of 6100X) of SP3 PLA50 O coated films.

On the other hand, Figure 4.23 shows the releasing profiles of SP3 PLA70 O and SP3 PLA50 O coated films when the surrounding solution was replaced by PBS solution each time a reading was performed. Thus, the amount of acetazolamide released in each reading is added to the total amount that was released until that moment, becoming cumulative results.

The absorbance was measured 4, 16 and 18 hours after the immersion and in the following days, for two weeks. An increased number of measurements was made in the first hours of release in order to understand if by replacing the surrounding medium an intense burst in drug release was observed.

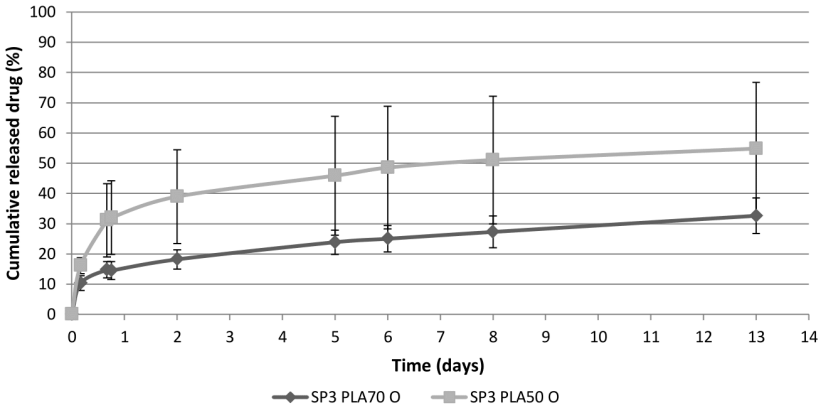


Figure 4.23: Comparison between the cumulative acetazolamide releasing profile by SP3 PLA70 O and SP3 PLA50 O coated films over the course of 13 days.

SP3 PLA70 O coated films show lower drug release over two weeks when compared to SP3 PLA50 O coated films. However, the errors associated with the readings from SP3 PLA50 O coated samples were high, which can be due to errors in the experimental methodology and irregular coating of the films.

Similar to the previous experiment, the coating has proved to be efficient in decreasing the first 4 hours prompt release. The values after 4 hours were $10.3 \pm 2.4\%$ for SP3 PLA70 O coated samples and $16.1 \pm 2.7\%$ for SP3 PLA50 O coated samples. After 13 days, the total release for SP3 PLA70 O coated samples was $32.6 \pm 5.9\%$ and $54.8 \pm 22.0\%$ for SP3 PLA50 O coated samples.

4.3.3 Experiment number 3

A third experiment was performed in order to understand the changes in drug release when the films were left in the fume hood to evaporate the chloroform. The three solutions studied in the first experiment were used to form films. After all the chloroform has evaporated, the discs with film

were cut and coated with their main solutions. Figure 4.24 shows the release results for the three films.

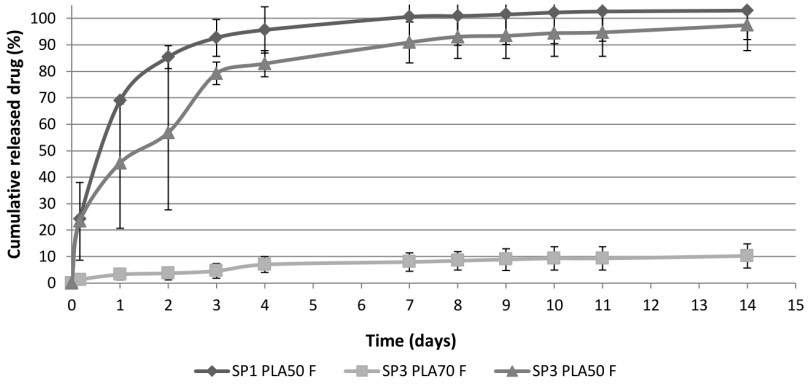


Figure 4.24: Comparison between the cumulative acetazolamide releasing profile by SP1 PLA50 F, SP3 PLA70 F and SP3 PLA50 F coated films over the course of 14 days.

As seen in the first experiment, SP3 PLA70 F is a promissing formulation to develop drug delivery systems in order to release acetazolamide over time. Besides its total released drug after 14 days was only $10.2 \pm 4.6\%$, the initial burst observed in other formulation it's not present for SP3 PLA70 F coated films (Figure 4.24). Since chloroform evaporated slowly, when compared to the films obtained in the oven at 50 °C, the polymeric chain had time to form an organized polymer, which can influence the releasing properties of the final material. Figure 4.25 shows the three different films obtained in the fume hood, before being coated. It is visible that all the films present a porous surface but the phase separation present in the films obtained in the oven (Figure 4.17 to 4.19) is not observed, which can indicate a better plasticization of PLA.

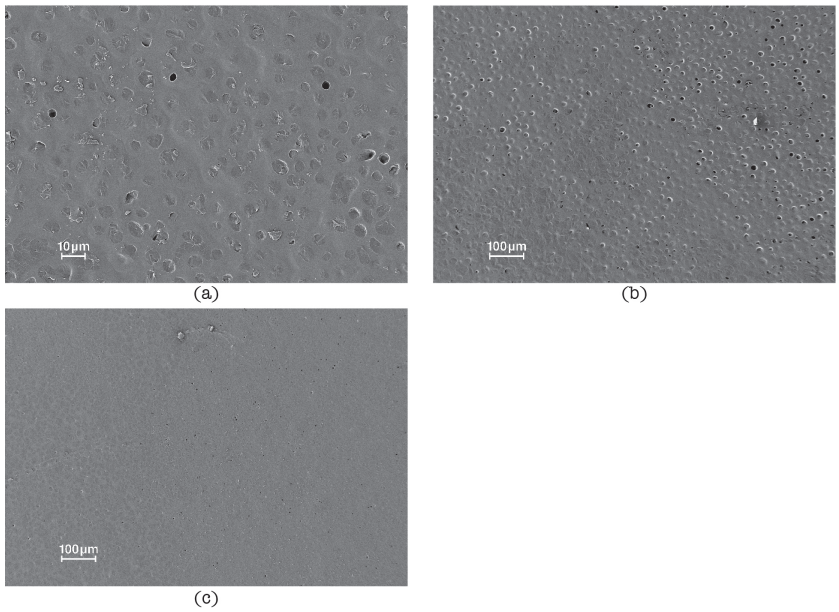


Figure 4.25: SEM images of SP1 PLA50 (a), SP3 PLA70 (b) and SP3 PLA50 (c) films formed in the fume hood with a magnification of 100X.

On the other hand, SP1 PLA50 F and SP3 PLA50 F coated films showed a fairly similar behavior over 14 days. Both samples showed a brust release in the first 4 hours, being that $24.3 \pm 0.6\%$ of the drug was release to the saline solution by SP1 PLA50 films and $23.4 \pm 14.7\%$ by SP3 PLA50 films. After 14 days, the total released values were $103.0 \pm 11.0\%$ for SP1 PLA50 coated samples and $97.4 \pm 9.6\%$ for SP3 PLA50 F coated samples. Ideally, a drug delivery system should be capable of release drugs for long periods of time (months), consequently these two films show no promising results for this application.

With the purpose to overcome this problem, a new coating was tested. The coating was performed by dip-coating technique. The films were submerged in a 30/70 wt. % n-butyl-acetate/silicone solution.

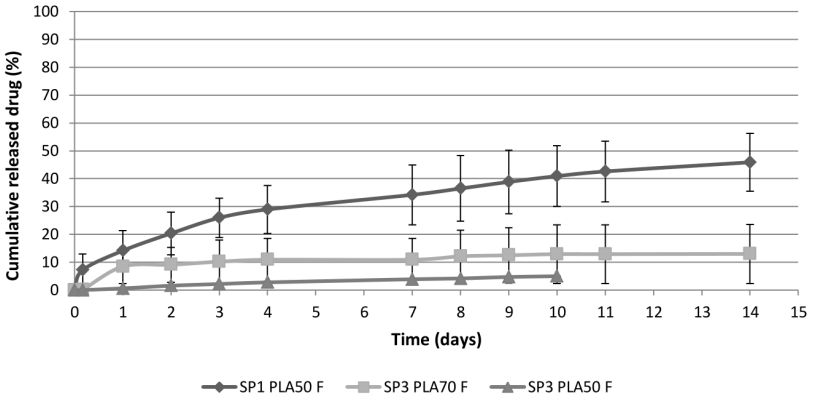
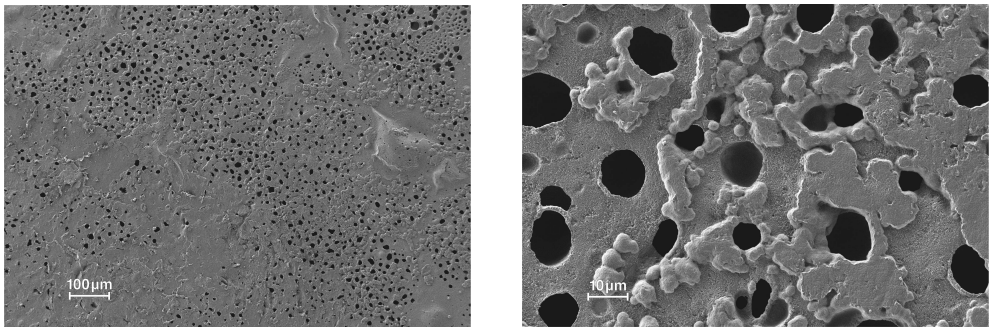


Figure 4.26: Comparison between the cumulative acetazolamide releasing profile by SP1 PLA50 F, SP3 PLA70 F and SP3 PLA50 F films with silicone coating over the course of 14 days.

The silicone coating proved its efficiency in decreasing the drug release after 4 hours of immersion (Figure 4.26) and, for SP1 PLA50 F and SP3 PLA50 F samples, it proved to be more effective than the previous coating in decreasing the release of the drug. The releasing profiles for each film can be found attached to this work (See Appendix B).

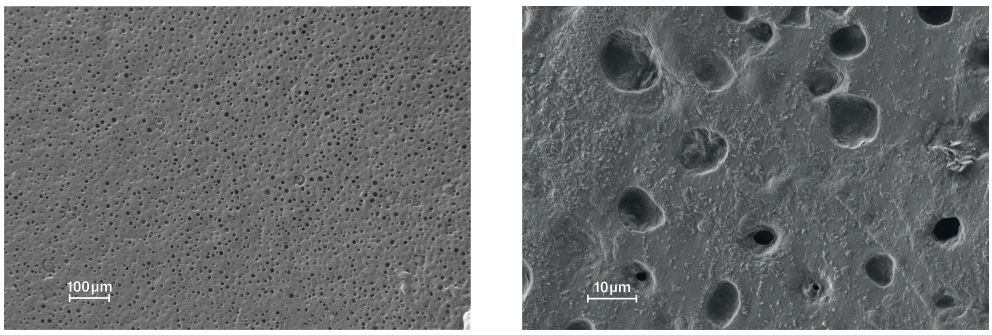
After 14 days, the total release of drug for SP1 PLA50 F sample was $45.9 \pm 10.4\%$ and for SP3 PLA70 F sample it was $13.0 \pm 10.6\%$. However, observing the results of the release values from SP3 PLA50 F it is possible to understand that these particular films have values close to 0. In order to understand if acetazolamide was encapsulated inside the films, they were cut and placed in a PBS solution for 48 hours. Yet, no increase in the absorbance was registered. Thus, drug release from SP3 PLA50 F coated films must be assessed in the future.

The efficiency of the silicone in coating the films was evaluated by SEM. Figure 4.27 to 4.29 show SP1 PLA50 F, SP3 PLA70 F and SP3 PLA50 F films, respectively. It is visible that the silicone was more efficient in coating SP3 PLA70 F and SP3 PLA50 F films, respectively (Figure 4.28 and Figure 4.29). These observations are in line with the releasing profiles of each coated film.



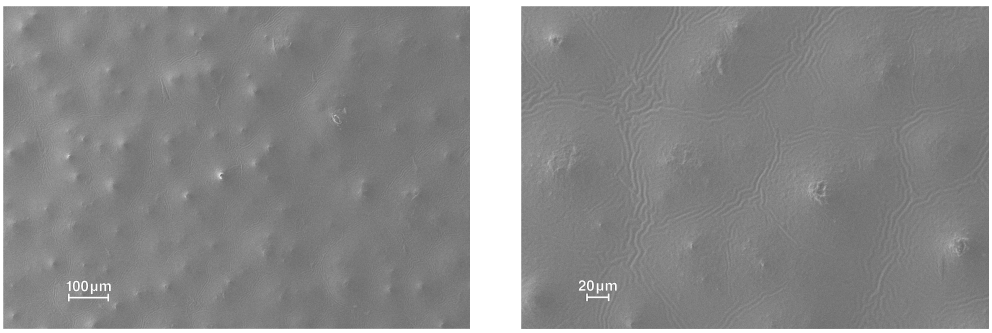
(a) (b)

Figure 4.27: SEM image of the surface SP1 PLA50 F film coated with silicone with a magnification of 100X (a) and with a magnification of 1.000X (b).



(a) (b)

Figure 4.28: SEM image of the surface SP3 PLA70 F film coated with silicone with a magnification of 100X (a) and with a magnification of 1.230X (b).



(a) (b)

Figure 4.29: SEM image of the surface SP3 PLA50 F film coated with silicone with a magnification of 100X (a) and with a magnification of 283X (b).

In a final analysis of the data collected through the different experiments, a comparison between the films prepared in the oven at 50 °C and the ones prepared in the fume hood at room temperature (the films were formed between June and November 2015) was carried out. It is important to note that the room temperature varied depending on the time of the year which can have an impact in the final properties of the obtained films.

Figure 4.30 compares SP3 PLA70 coated films left in the oven and in the fume hood to evaporate the chloroform.

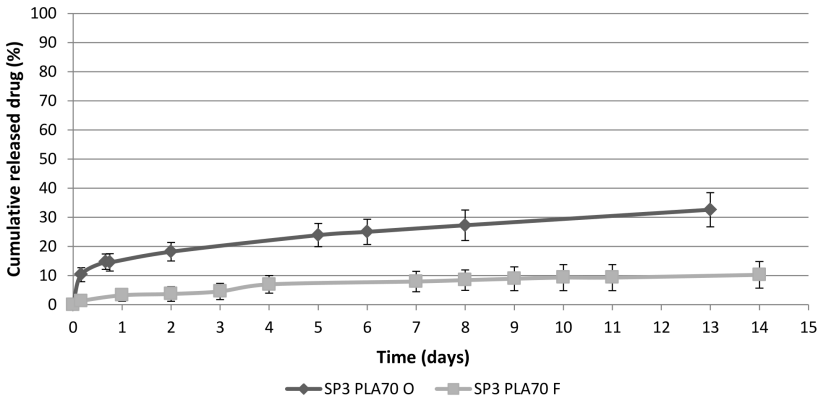


Figure 4.30: Comparison between the cumulative acetazolamide releasing profile by SP3 PLA70 films prepared in the oven and in the fume hood over the course of 14 days.

By comparing the release between the films prepared by the two methods, it is visible that the film prepared in the fume hood shows a lower release than films prepared in the oven (Figure 4.30). This can be attributed to the arrangement of the polymeric chains, as mentioned before.

On the other hand, Figure 4.31 shows the differences between the SP3 PLA50 coated films. Contrary to SP3 PLA70 coated films, the films prepared in the oven present a lower release of acetazolamide over time.

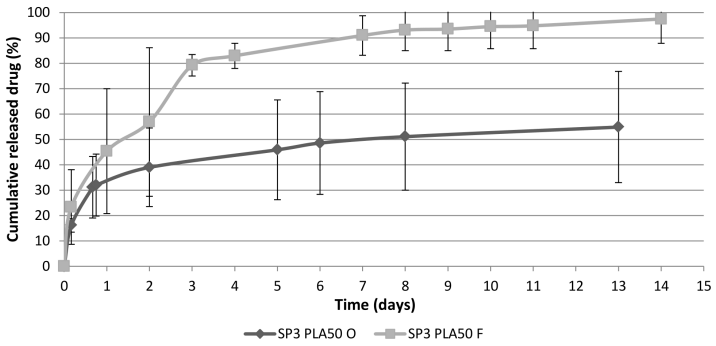


Figure 4.31: Comparison between the cumulative acetazolamide releasing profile by SP3 PLA50 films prepared in the oven and in the fume hood over the course of 14 days.

Thus, SP3 PLA70 films seem to be the most promising films to release drug in a controlled manner since they require smaller amounts of plasticizer when compared to the other two films, show improved drug release when coated and when the chloroform is evaporated in the fume hood, at room temperature.

Chapter 5

Conclusions and Future Work

5.1 Conclusions

Concerning the present work, saturated polyesters (SP) were synthesized and characterized in order to plasticize poly(lactic acid) (PLA). The obtained materials were studied as drug delivery systems for acetazolamide.

Four SP were successfully obtained, two aliphatic polyesters (SP1 and SP3) and two aromatic polyesters (SP2 and SP4), by polycondensation of saturated acids and a diol. Different proportions of the synthesized polyesters were combined with PLA and, using the solvent casting process, different films were obtained. When the chloroform solution of the polymers was evaporated in an oven, all the polyesters showed to be able to form films with PLA. However, only SP1 and SP3 polyesters showed to be able to form films with the PLA with mechanical properties suitable for drug release systems. The films with improved properties (SP1 PLA50 O, SP3 PLA70 O and SP3 PLA50 O) were studied by DSC and DMTA in order to determine the T_g value of each film. Both techniques proved to be ineffective in determining this transition. However, assuming that the films are plasticized, a reduction

of T_g is expected. The mechanical properties of the films were evaluated by a tensile stress-strain test. This test proved to be inconclusive for the films obtained in the oven. At the same time, and in a comparative analysis, the same drying process was performed at room temperature, in order to evaluate the impact of slow evaporation of chloroform in the final properties of the developed materials. Through tensile testing, SP1 PLA50 F and SP3 PLA50 F showed an increased elongation at break.

After characterization, the films were studied by their ability to release acetazolamide. All the films showed a burst release in the first 4 hours. Thus, in order to solve this problem, the films were coated with the polymer solution (SP PLA) or with a silicon based material. The subsequently acetazolamide release studies were performed. The SP3 PLA70 films obtained in the oven and in the fume hood, PLA film with polyester constituted by sebacic acid and 1,6-hexanediol, proved to be the most promising film in what concerns the drug release profiles. Both coatings proved to be effective in lowering drug release.

5.2 Future Work

This work was focused primarily in the synthesis and characterization of saturated polyesters in order to plasticize PLA. As mentioned above, the polyesters were successfully synthesized and characterized by FTIR, ^{1}H NMR, TGA and SEC. However, in DSC analysis, it was not possible to separate the thermal events of SP1 and SP4. In order to overcome this limitation, the use of modulated DSC is presented as a solution. It is expected that, using this technique, the identification of the endothermic peak corresponding to the T_m of the polyester will be possible. In addition, in order to remove the thermal

history of the sample, the first heating step should be performed in temperature range from -80 to 200 °C. Thus, it will be possible to understand if the first cycles were responsible for the reorganization of the polymeric chains and, consequently, for the absence of the cold crystallization peak in second heating cycle.

It is also noted that, the DSC technique proved to be inefficient in determining the T_g value of the SP/PLA films. The T_g value was determined by DMTA analysis. However, this technique also proved to be inefficient in determining the T_g of the films by tension mode. As mentioned, the T_g value is an important reference of plasticization. Thus, in future work, films with appropriate geometry and thickness should be formed in order to carry out the DMTA analysis.

Another important analysis for plasticized films is the tensile testing. In order to obtain conclusive information from the stress-strain curves of the films it is important to obtain standard samples for the analyses. This can be achieved by injection molding of the films.

Furthermore, the use of other plasticizers should be taken into account. Polyester containing phthalic acid, as an example, and a new method to form the films may prove useful in order to improve PLA properties.

The promising results obtained to this point can be used to encapsulate other drugs. Additionally, cytotoxicity of the films and coating should be evaluated, as well as, the degradation behavior of the former.

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Appendix A

SEM image of the surface of a PLA film.

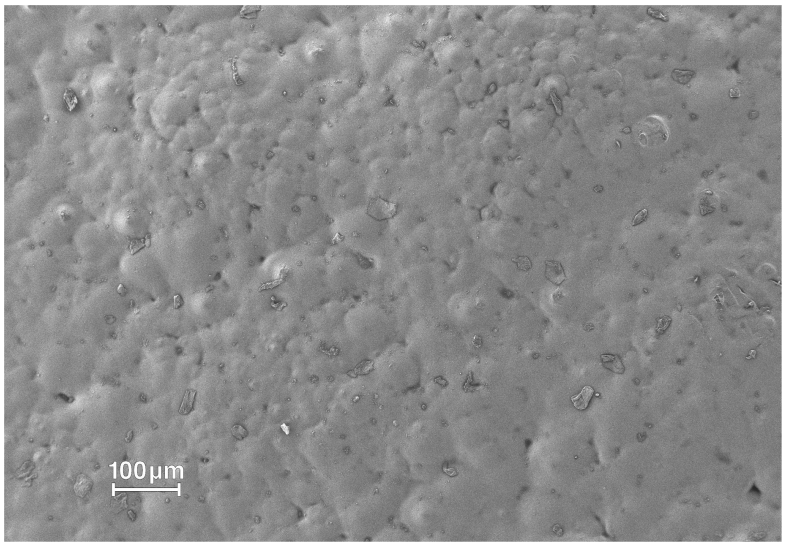

Figure A.1: SEM image of the surface of a PLA film with a magnification of 100X.

Appendix B

Drug Releasing Tests

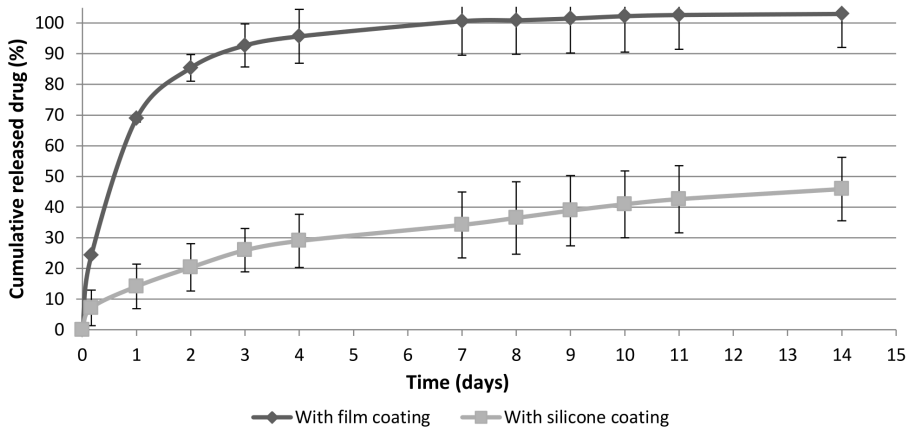

Figure B.1: Comparison between the acetazolamide releasing profile by SP1 PLA50 F films with coating and with a silicone solution over the course of 14 days.

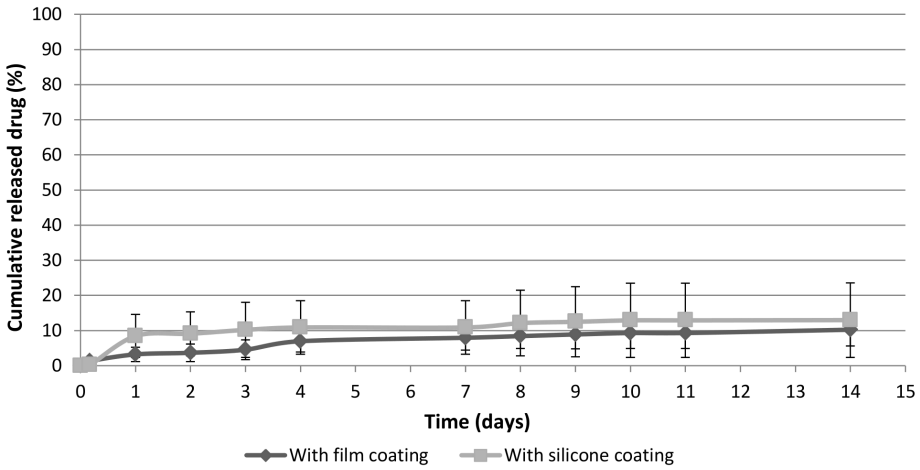

Figure B.2: Comparison between the acetazolamide releasing profile by SP3 PLA70 F films with coating and with a silicone solution over the course of 14 days.

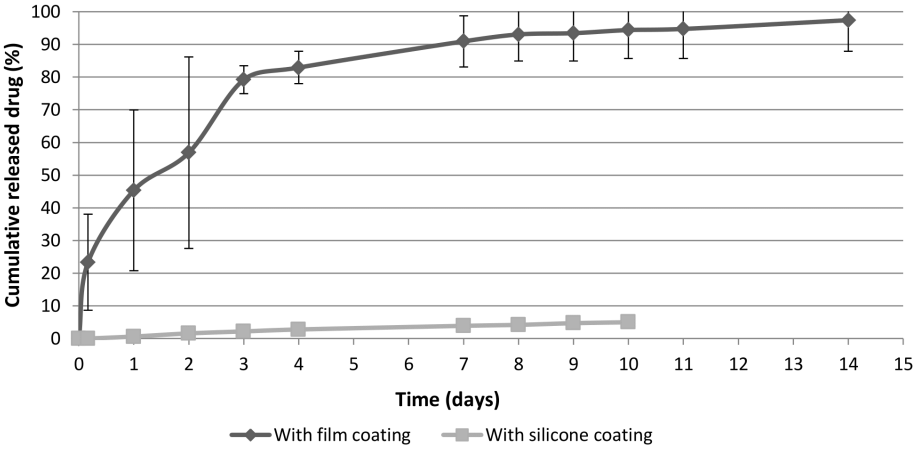

Figure B.3: Comparison between the acetazolamide releasing profile by SP3 PLA50 F films coated with film solution and with a silicone solution over the course of 14 days.