Tutor/es

Dr. Elena Xuriguera Martin
Departamento de Ciencia de Materiales y Química Física

Dr. Oscar Castaño Linares
Departamento de Ingenieria Electrónica y Biomédica

Grau de Química

Treball Final de Grau

Controlled thermo-responsive hydrogels based on the stereocomplexed block of PLA-PEG-PLA for tissue engineering.

Hydrogeles termoresponables basados en el bloque esterocomplejado de PLA-PEG-PLA para ingeniería de tejidos.

Eisuke Comas Iwasita

June 2021

UNIVERSITAT DE BARCELONA

B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

El artista no es nada sin el don, pero el don no es nada sin el trabajo.

Emile Zola

Este trabajo no hubiera sido posible sin el apoyo de la gente que me rodea.

Principalmente, a toda mi familia, porqué sin ellos nada de esto hubiera sido posible. Por haberme mostrado un apoyo incondicional y que a pesar de la distancia que nos separa siempre los he sentido cerca. En especial, a mis padres, mi hermano y mi primo quienes me han hecho crecer como persona y me han hecho entender que con esfuerzo y constancia uno puede llegar donde se proponga.

A mis amigos, por haber hecho posible que desconecte en los momentos que más lo he necesitado, también por haberme escuchado y soportado infinitas veces cuando más estresado he estado.

A mis tutores, Dr. Oscar Castaño y Dra. Elena Xuriguera quienes me han ayudado durante esta etapa y me han aportado un amplio conocimiento sobre un tema totalmente nuevo para mí. Me gustaría agradecer también a Álex quien se ha portado muy bien conmigo y me ha echado un cable siempre que lo he necesitado.

REPORT

CONTENTS

1. SUMMARY

Nowadays one of the main causes of death is cardiovascular diseases, principally cardiac failure or myocardial infarction. As a result of these health disorders, scientists, especially those who specialize in bioengineering and tissue engineering, have carried out research to improve the lives of patients that suffer from heart problems.

In recent years, various therapeutic strategies have been proposed to put an end to these illnesses. Their objective is a full recovery of the cardiac cells and at the same time attempting to avoid the dangerous surgeries.

2011 saw the appearance of hydrogels which are capable of creating an environment rich in lactate, suitable for the regeneration of these cells. These hydrogels are formed from the micelle mixture of two enantiomeric triblock copolymers, PLLA-PEG-PLLA and PDLA-PEG-PDLA.

The stereo mixture of these copolymers displays some interesting characteristics such as biodegradable and biocompatible scaffolds, a high water-content, and mechanical features which are similar to soft muscle; these help to meet the desired need. In addition, these hydrogels exhibit a controllable sol-to-gel transition at a wide temperature range of 15-100˚C; hence, a transition to gel at body temperature can be achieved.

Consequently, the principal objective of this work is to achieve the synthesis of the triblock and the micelles by applying a temperature ramp to obtain the gel. A subsequent characterization of this gel is done by performing NMR and DLS, in order to obtain information that can further understand of its characteristics and its potential therapeutic use in the near future.

Keywords: Tissue engineering, scaffold, polylactic acid, polyethylene glycol, triblock, copolymer, hydrogel, gel, micelle, thermo-responsive.

2. RESUMEN

Hoy en día una de las principales causas de muerte son las enfermedades cardiovasculares. Principalmente las causas son la insuficiencia cardíaca o el infarto de miocardio. Como consecuencia de estos trastornos de salud, en el ámbito científico, en especial bioingeniería y la ingeniería de tejidos se han realizado diversos estudios para mejorar la vida de los pacientes que padecen problemas cardíacos.

Estos últimos años se han propuesto estrategias terapéuticas con el deseo de acabar con estas enfermedades, intentando una recuperación completa de las células cardíacas y al mismo tiempo intentando evitar las peligrosas cirugías.

En 2011, aparecieron algunos hidrogeles capaces de crear un ambiente rico en lactato adecuado para que se produzca la regeneración de estas células. Estos hidrogeles nacen de la mezcla de micelas de dos copolímeros enantioméricos, PLLA-PEG-PLLA y PDLA-PEG-PDLA.

La estereo mezcla de estos copolímeros presenta algunas características interesantes como que son andamios biodegradables y biocompatibles, también tienen un alto contenido en agua y características mecánicas similares al músculo blando pudiendo satisfacer así las necesidades deseadas. Además, estos hidrogeles exhiben una transición de sol a gel controlable en un amplio rango de temperaturas de 15-100°C, por lo tanto, se puede lograr una transición a gel a la temperatura corporal.

En consecuencia, el objetivo principal de este trabajo es la síntesis del tribloque y las micelas aplicando una rampa de temperatura para obtener el gel, de este se hará una posterior caracterización realizando RMN y DLS, con el fin de obtener información que permita comprender mejor sus características y su potencial uso terapéutico en un futuro próximo.

Paraules clau: Ingeniería de tejidos, andamio, ácido poli láctico, polietilenglicol, tribloque, copolímero, hidrogel, gel, micela, termo responsable.

3. INTRODUCTION

In the past decades, in the field of biomedical applications, the idea of making surgeries less invasive has grown. The idea consists of avoiding surgery wherever possible and making the rehabilitation process more effective.

Nowadays, one of the riskiest invasive procedures is heart surgery. The heart is the center of the circulatory system which includes the network of arteries and veins that make it possible to transport blood, a carrier of oxygen and other types of essential nutrients, to all parts of the body.

Cardiovascular disease (CVD) is one of the most known causes of death in the developed world. Principals CVDs are myocardial infarction and cardiac failure. Studies show that when a myocardial infarction takes place a part of the myocardium dies and is replaced by fibrotic tissue. This leads to a loss of contraction which stops the flow of blood through the system, finally leading to heart failure and death.

Knowing that it is impossible to live without this organ, when abnormal cardiac behavior is detected, clinicals will look for the cause, and recommend therapy. Initially this will be a non-invasive remedy but, in cases where this is not feasible or where non-invasive treatments haven't worked satisfactory, heart surgery will be carried out. However, cardiac regeneration and normal heart function are never recovered totally after surgery and, despite achieving many things, there are risks such as bleeding or infection. This is the main reason why bioengineers are looking for a different solution.

The engineering of tissues has put the focus on stem cell therapy, which is capable of repairing injured systems and of regenerating the muscle. Stem cells have the ability to recover tissue by functional repopulation. This has led tissue bioengineers to develop a 3D structure that can be used as scaffolding for the templating of tissue formation and ingrowth; these structures need to include certain chemical and biologicals substances necessary to guide the growth, differentiation, implantation, and distribution of the cells. [1][2]

Cardiomyocytes are the cells of the cardiac muscle. When heart disease is detected, cardiomyocytes will increase their production of lactate in order to improve the heart's performance. [3]

Hence the scaffold that is prepared in this work is a PLA-PEG-PLA with the final objective of providing a solution to all these heart issues, giving the patients a lower risk solution with a better medical recovery.

3.1. GEL

A gel is a state of matter that has the appearance of a solid and a density similar to that of a liquid. This state can be achieved, for example, when a colloidal solution rests in a liquid. The structure of the colloid is based on a liquid phase that is a continuous phase, and a discontinuous phase (scattered) which the case of this study is focused on a triblock copolymer. [4]

Within the structure of a gel, there are molecules which form a network within a liquid medium. Depending on the interaction between these parts the final product will acquire some peculiar qualities. For example, we can obtain two gels with the same continuous phase but different characteristics, one with an aqueous disperse phase (*hydrogel*) and the other one with an organic disperse phase (*organgel*). Both gels probably will have completely different properties. This work focuses on hydrogels.

3.2. HYDROGEL

Nowadays hydrogels are receiving special attention due to their high-water content and the related potential for many biomedical applications; this is because the elevated water content found in hydrogels means they have properties similar to those of human tissues.

Hydrogels are polymeric structures that are crosslinked for different causes: primary covalent crosslinks; ionic forces; hydrogen bonds; bio-recognition interactions; hydrophobic interactions; polymer crystallites; physical entanglements of individual polymer chains; or a combination of two or more of the above interactions. These types of links, which allow the polymer chains to be held together, provide a three-dimensional solid and these bonds can be classified into two categories: physical and chemical crosslinking.

There are further ways to classify hydrogels, one of which depends on the method of preparation. So, they may be:1) Homopolymer hydrogels; 2) copolymer hydrogels; 3) multipolymer hydrogels; and 4) interpenetrating network hydrogels.[4]

This work, focuses on copolymers that are made by cross-linking of chains formed by two monomer units, at least one of which must be hydrophilic to make them water-swellable.

3.3. COPOLYMER

In this work, the copolymer, is configured by two different polymers added one after the other in a A-B-A sequence that forms a triblock with amphiphilic features, which, in turn, can form micelles. This type of copolymer that has alternative sequences of chains of one monomer and another is called a triblock copolymer. One of the monomers for the 'A' polymer should include lactate. In this study, the chosen option is polylactic acid (PLA). It is not soluble in water and is unable to form a hydrogel on its own. For this reason, another water-soluble polymer is needed which, in this study, is polyethylene glycol (PEG) and it is the 'B' component. This combination forms the triblock. The structures are shown in **Appendix 1**.

3.3.1. Components of the copolymer

Polylactide (PLA)

PLA can be achieved through two mechanisms: condensation and polymerization. This study uses the polymerization process known as ring-opening polymerization (ROP). This method uses a metal catalyst blended with lactide to create the larger PLA molecules.

The polymer polylactide has an asymmetric carbon which is known as a chiral center, which gives two stereoisomers D(-)-PLA and L(+)-PLA. The structures are shown in appendix 1. Both of them form a regular stereocomplex with higher crystallinity favoring gel formation.

One of the fundamental attributes of PLA is that it is a biodegradable product solid that conforms to the hydrophobic part of the micelle. Hence in recent years, it has been studied for its utility in food handling and medical implants because, over time, it allows controlled growth of biological tissue during biodegradation within the body.

When PLA is proposed as the hydrophobic part of the micelle, it is because this could be easily hydrolyzed into natural endogenous metabolites such as lactic acid, via a process of hydrolytic degradation as shown in **Appendix 2** (this takes place when the polymer enters an

aqueous medium). L-lactic acid can be metabolized as L-pyruvic acid, entering in the Krebs cycle and eliminated from the body in form of CO_2 and water. Several studies stated that, when the percentage of PLA in a copolymer is increased, there is a growth in the fluid index. This leads to a reduction in molecular weight which then makes it more susceptible to degradation. [5] [6]

Polyethylene glycol (PEG)

Nowadays polyethylene glycol is made using a polymerization reaction in which ethylene oxide interacts with oligomers. It is executed by forming ether links between monomers. This mechanism can be accelerated by the participation of an acidic or basic agent that catalyst the reaction.

The characteristics of polyethylene glycol are known to change in function of its molecular weight. In general, it is highly soluble in water and most organic solvents. Apart from this all-important hydrophilic feature, it is also flexible and non-degradable. PEG is also considered to be non-toxic, although it should be remembered that it contains ethylene glycol which is a potential carcinogen and a toxic substance.

It is well-known that the polymer PEG binds with proteins and enters the bloodstream. Part of it goes to the kidney and is expelled in urine. Another possibility is that PEGylated protein binds to a receptor, some PEG remains after protein catabolism and this polymer is eliminated via the liver in feces. In addition, a small proportion of PEG may be removed by oxidative metabolism.

What's more remarkable about the gel is that they are polyester hydrogels that degrade into natural, endogenous metabolites such as lactic, and the PEG block is then ejected through the kidneys. [7]

3.4. MECHANISM FORMATION OF TRIBLOCK

The triblock is created by following an opening ring polymerization (ROP) mechanism of an epoxide ring [8]. This type of polymerization is used to create linear polymers. This type of mechanism involves the ring of L or D lactide that is susceptible to being opened by an active species via a nucleophilic or electrophilic attack. To initiate the polymerization to obtain a polyester, it is necessary a Lewis acid that interacts with the lactone. In this study, the chosen acid to act as a catalyst is tin(II) 2-ethylhexanoate ($Sn(Oct)_2$. The catalyst mechanism is represented in **Appendix 3**. Some researchers assert that catalytic agents with primary and secondary alcohols produce a transfer reaction which forms new intermediate species of tin alkoxide; others assert that this species can also work without this request [9]. When the lactic acid is inserted to form PLA in a coordination-insertion mechanism, the monomer PEG links the PLA by esterification (figure 1). Indeed, PEG contains free alcohol groups that react with the acid groups of the PLA to obtain the desired copolymer.[10]

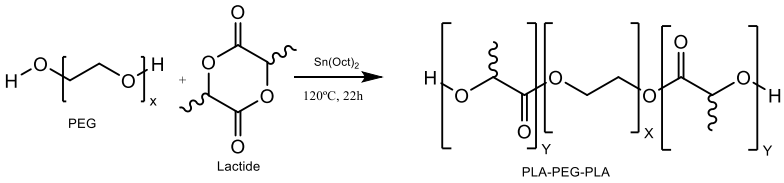


Figure 1. *Triblock formation reaction (PLA-PEG-PLA).*

3.5. THE MOLECULAR WEIGHT OF POLYMERS

Polymers are known to have high molecular weights. Looking at the ROP polymerization, it could be assumed that all the monomers join to form the copolymer; however, this is not the case. Some of them form a linkage but others do not and, for this reason, polymer chains can be seen to differ in length and molecular weight. For this reason, a molecular mass distribution appears. The two most common average molecular weight are the number average molecular mass (Mn), which is the statistical average molecular weight of all polymer chains in the sample, and weight average molecular weight (Mw), which is the molecular weight of a chain to determine the contributions of the average molecular weight. [4]

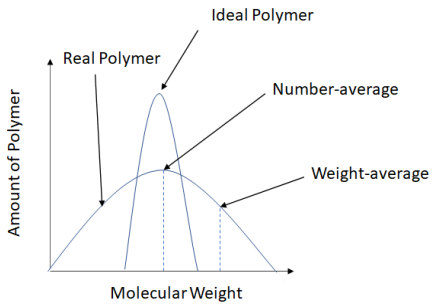


Figure 2. *Molecular weight Distribution of polymer.*

3.6. MICELLES AND COLLOIDS

Finally, a triblock is obtained that is arranged in a linear sequence with a central block of PEG and two outer blocks of PLA, which can be seen to be part hydrophilic and hydrophobic. [11]

Amphiphilic block copolymers can self-assemble into core-shell micelles allowing them to have a hydrophobic core surrounded by hydrophilic shells in an aqueous solution. Micelles are heterogeneous systems composed of a discontinuous phase (scattered) in which the micelles are found and the continuous phase (dispersant), the medium.

Studies have shown that when the interior part is the hydrophobic block and the exterior part is the hydrophilic block, the triblock produces a micelle with a flower-like shape (figure 3). This flower-like form can be fixed to others thanks to the central block that acts as a bridge. They are capable of transporting insoluble molecules in an aqueous medium. Consequently, they are known as amphipathic structures.

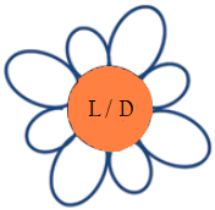


Figure 3. *Micelle with the shape of a flower-like.*

3.7. THERMO RESPONSIBILITY

Hydrogels have the ability to degrade via two mechanisms: the hydrolysis or enzymolysis of the main chain. This kind of hydrogel is indeed a great opportunity for applications in drug delivery and tissue engineering scaffolds.

Hydrolytically degradable hydrogels have been developed using triblock copolymers ABA structure that form the hydrogel held together by hydrophobic forces.

According to some papers, hydrogels usually exhibit swelling behavior in line with the external environment. Hence, significant changes in the swelling ratio of these types of gel have been studied in response to small changes in environmental conditions, such as pH, temperature, ionic strength, or light. The triblock that it is developed as a result is therefore known as a thermo-responsive hydrogel because its 3D conformation and structural interaction strongly change with temperature. [4]

Firstly, thermo-responsive hydrogels exhibit sharp temperature-sensitive swelling-deswelling behavior due to a change in the polymer/swelling agent compatibility over the temperature range of interest. In addition, this kind of gels exhibits a lower critical solution temperature (LCST), below which the polymer is soluble. Above this temperature, the triblock may lose its hydrophobically bounding water, and phase separate, causing the gel to collapse. Below the LCST, the cross-linked gel reswells to significantly higher degrees because of the increased hydrophobic bonding with water.

The temperature at which the sol transition to a gel shape is called the sol-gel transition temperature point (Ts-g), and this vary according to the application of the end product. In this study, a Ts-g slightly lower than the physiological temperature of 37 ºC is of interest so that the micelle sol can be injected into the body at low temperature 20 ºC for non-invasive injectable purposes which the heat of the body will then form into a gel for non-invasive injectable therapeutic purposes.

The class of thermo-responsive hydrogel is gels based on physical interactions of hydrophobic blocks of the triblock copolymers. These copolymers form in situ gels when injected subcutaneously and act as drug delivery depots, releasing entrapped drugs as they degrade.

3.8. CELL REGENERATION CARDIAC

Stem cell therapy has emerged as an option to regenerate injured tissues, for example heart tissue. During myocardial infarction it has been demonstrated that a part of the myocardium dies and is replaced by fibrotic tissue. As a result, the cardiac muscle that forms the heart walls, also known as cardiomyocytes, loses its main function of cardiac contractility leading to heart failure.

It is well-known that cardiomyocytes go through a cycle of differentiation that starts when they are progenitor stem cells. Then as a result of complex combinations of chemical substances and genetic signals they finally end up specializing. This means that they become cells with a specific function within the heart tissue. [12,13]

Studies show that these cells go through different changes during the human life-cycle. In early development, the fetus is situated in a low oxygen environment but is in a lactate-rich condition. For this reason, fetal cardiomyocytes tend to be proliferative and have poorly differentiated phenotypes. In contrast, after birth, the baby is in an oxygen-rich climate where cardiomyocytes acquire their final differentiation, but decreasing in proliferation. [3]

After cardiovascular disease, the cardiomyocytes increase their lactate, as a mechanism to compensate for the negative effects of hypertrophy and to improve the heart's performance. Some studies show that it is possible to improve proliferation, which contribute to the replenishment of heart muscle through sustained proliferation. This is the reason why the hydrogel must contain lactate because those cardiomyocytes will be more dependent on lactate.

4. OBJECTIVES

The main objectives of this work are the following:

1. The main objective is to obtain a thermo-responsive gel and to try to obtain a sol-gel transition point between the temperature range 15-100 °C and, in particular, a gelation temperature feasible for physiological use.
2. To synthesize a triblock formed by PLA-PEG-PLA in which the weight of central block PEG is varied.
3. To characterize the triblock and the preceding micelles.
4. To observe whether the characteristics of the gel are affected by the change of the triblock central block.

5. EXPERIMENTAL SECTION

5.1. MATERIALS

5.1.1. Solvents

Table 1. *Solvents with their purity and specification*

Solvent	Brand
Toluene. 99.8%	Sigma Aldrich
Dichloromethane, 99.5%	Panreac AppliChem
Ditehyl ether, 99.7%	Panreac AppliChem
Hexane, 98.5%	Panreac AppliChem
THF, 99.9%	Panreac AppliChem
H_2O	Deionized-standard

5.1.2. Reagents

Table 2. *Reagents with their purity and specification*

Reagent	Brand
L-lactide / D-lactide, 99.5%	Corbion Purac
PEG 2000 / PEG 3350, Pharmaceutical grade	Sigma - Aldrich
$Sn(Oct)_2$, 92.55%	Sigma - Aldrich

5.1.2. Wastes

Waters from the purification of the product that contained dichloromethane were decanted into the recipient of halogenated organic dissolvent.

5.2. PREPARATION METHOD

This part of the study contains a description of the following processes: 1) the synthesis of copolymer PLA-PEG-PLA, varying the molecular weight of the central block PEG 2000 or 3350, 2) the preparation of the micelle and hydrogels using different weight ratio of the central block.

5.2.1. Synthesis of Polylactide-Polyethylene glycol-Polylactide Triblock copolymers

The triblock composed by PLLA-PEG-PLLA and PDLA-PEG-PDLA was prepared following the method of Abebe & Fujiwara (2012) [11]. The chemicals were first processed using a mechanism known as ring-opening polymerization (ROP) which is a form of chain-growth polymerization in which the terminus of a polymer chain attacks the cyclic monomers to form a longer polymer. In this case, L or D-lactide was used in the presence of PEG-diol with the catalysis of $Sn(Oct)_2$ which was responsible for initiating the mechanism.

The synthesis was carried out using a 2-neck spherical reaction flask to which was added 2.5g of PEG, either 2000 or 3500 according to the desired hydrogel, and the 2g of PLA. The latter was either L or D stereoisomer, chosen again in function of the desired hydrogel. The necks of the flasks were closed with a septum and the vessel was equipped with a reflux condenser and a stir bar.

The assembly equipment was connected to a compensated Schlenk line (see figure 4) and the whole system was sealed and evacuated to remove moisture, then converted to N_2 gas, in order to create an inert atmosphere. Following standard recommended procedure, the vacuum was applied for 10 minutes and then the N_2 gas was introduced. The process was repeated a minimum of 3 times until it was completed. The N_2 atmosphere was maintained by equipping the apparatus with a nitrogen-filled balloon.

After homogenizing the reagents by stirring, the flask was heated at 100 ºC and stirring continued for a further 30 minutes. Subsequently, a needle was connected to a positive pressure of inert gas which was injected into in the round spherical bottom of the suspension in order to degas the molten mixture. Another needle was also necessary to remove the overpressure; this step was carried out for 15 minutes. The needles were removed and the suspension was allowed to homogenize for 15 minutes.

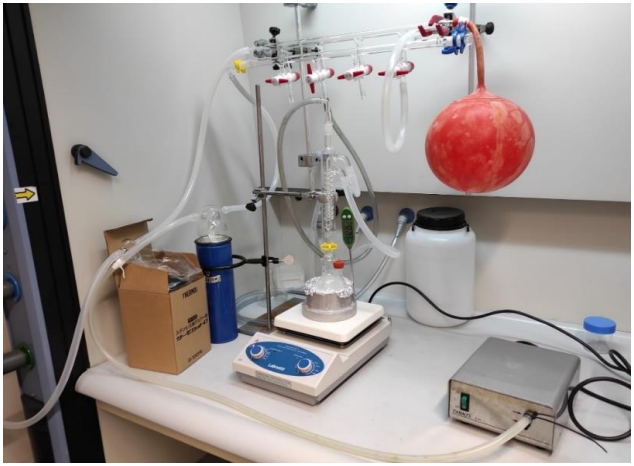


Figure 4. *Synthesis assembly*

The next step was to add 0.05g of $Sn(Oct)_2$ to the solution as a catalyst and 0.42g of toluene. Later, the reaction temperature was raised to 120 ºC and the solution was allowed to react overnight. The following day, the solution was cooled to room temperature at which point the mixture solidified to a cloudy solid, as seen in figure 5.

The next step, was the purification process to eliminate unwanted impurities and to prompt recrystallization. This process was carried out first by using dichloromethane (25ml) to dissolve the product. Then, a solution of 1:1 diethyl ether/hexane (60ml of each solvent) was added dropwise to reprecipitate the product. The precipitate was recovered by vacuum filtration using a Buchner flask. Initially, two filters of 0.2 microns were used to recover the product but the product was not retained. Finally, the best option was to add a piece of cotton cloth that allowed the triblock for successful retention of the triblock. The process was repeated three times to remove all the impurities. The last copolymer was dried at 60 ºC overnight.

All the synthesis, of both of PEG 2000 or 3350, were completed in the same way.

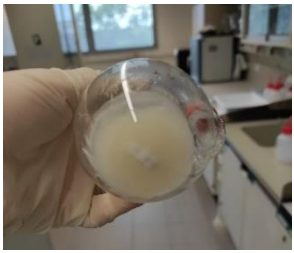


Figure 5. *Triblock with impurities it looks like a cloudy solid.*

5.2.2. Micelle's preparation

The micelle solutions of PLLA-PEG-PLLA and PDLA-PEG-PDLA triblock copolymers with the same size PEG were prepared by adding 0.2g of the copolymer synthesized in the previous stage to a plastic test tube. The triblock was dissolved in the minimum amount (roughly 1ml) of THF, a water-miscible solvent. The blend was joined dropwise to 2ml of deionized water, then the solvent tetrahydrofuran was extracted by applying compressed air over the surface of the mix. To yield a homogenous product, the outcome was sonicated for 10 minutes at 4 ºC to reach the desired temperature before ice was added to the ultrasonic bath.

The copolymers composed of the same PLA stereoisomer but different lengths of PEG were also combined following the same steps. For example, 50% mix micelle was composed of PLLA-PEG2000(50%)/PLLA-PEG3350(50%). This was achieved by adding 0.05g of the copolymer that contains PEG2000 and 0.05g of the copolymer that contains PEG3350.

Figure 6 exhibit the preparation procedure to do the micelles and table 3 shows the different combinations that were carried out to form the micelles.

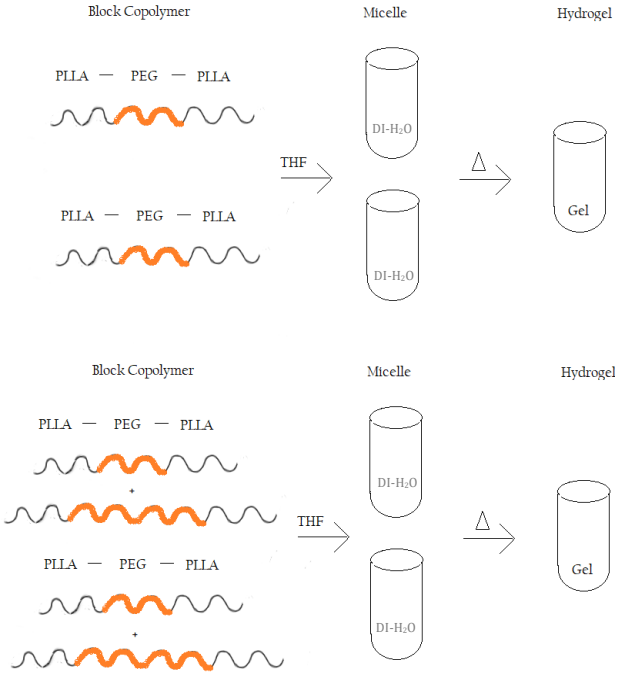


Figure 6. *Micelle and hydrogel preparation procedure.*

Table 3. *Triblock weight used on micelle sols*

Try	Copolymers (weight ratio, %)	Tared Weight	ml THF	Sol-gel Transition
1	PLLA-PEG_{2000}-PLLA	0.1001g	1ml	15ºC
	PDLA-PEG_{2000}-PDLA	0.1006g	1ml	
2	PLLA-PEG_{3350}-PLLA	0.1001g	1ml	Exceeds 100ºC
	PDLA-PEG_{3350}-PDLA	0.1003g	1ml	
3	PLLA-PEG_{2000}-PLLA (75%) PLLA-PEG_{3350}-PLLA (25%)	0.0752g 0.0253g	1ml	55ºC
	PDLA-PEG_{2000}-PDLA (75%) PDLA-PEG_{3350}-PDLA (25%)	0.0749g 0.0254g	1ml	
4	PLLA-PEG_{2000}-PLLA (50%) PLLA-PEG_{3350}-PLLA (50%)	0.0501g 0.0500g	1ml	59ºC
	PDLA-PEG_{2000}-PDLA (50%) PDLA-PEG_{3350}-PDLA (50%)	0.0499g 0.0500g	1ml	
5	PLLA-PEG_{2000}-PLLA (40%) PLLA-PEG_{3350}-PLLA (60%)	0.0400g 0.0605g	1ml	61.5ºC
	PDLA-PEG_{2000}-PDLA (40%) PDLA-PEG_{3350}-PDLA (60%)	0.0399g 0.0604g	1ml	
6	PLLA-PEG_{2000}-PLLA (25%) PLLA-PEG_{3350}-PLLA (75%)	0.0247g 0.0750g	1ml	99ºC
	PDLA-PEG_{2000}-PDLA (25%) PDLA-PEG_{3350}-PDLA (75%)	0.0257g 0.0751g	1ml	

5.2.3. Hydrogel Preparation

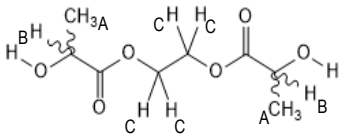
Controlled hydrogel formation was achieved by a combining equal volume of the PLLA-PEG-PLLA and PDLA-PEG-PDLA micelles.

Before obtaining the hydrogel, an equal volume of the micelle solutions PLLA-PEG-PLLA and PDLA-PEG-PDLA were added to a vial and next immersed in 4º C ultrasonic bath before being subjected to ultrasonic agitation. The combination was allowed to sonicate for 10 min or until a homogeneous solution was obtained.

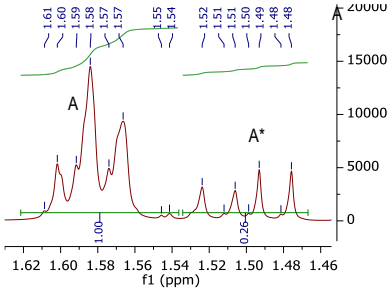
Subsequently, the vial was transferred to a temperature-controlled circulating water bath in which the temperature was gradually ramped. The physical state of the mixture was reported at each temperature interval by tilting the vial. If the mixture flowed, then it was reported as a solution. If this did not flow for at least 8s, it was reported as a gel the temperature for obtaining sol-gel transition.

6. TRIBLOCK CHARACTERIZATION

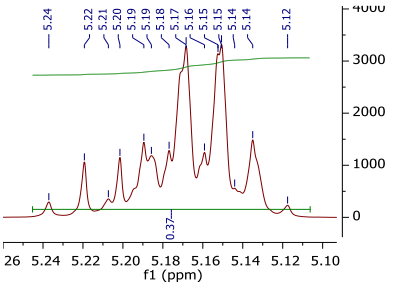
In order to confirm the structure of the copolymers and the absence of impurities, a ^{1}H NMR characterization was carried out. Four main bands are observed (figure 7), two of which are associated with PLA blocks and is located at 1.5 ppm and 5.2 ppm; these are assigned to methyl and methine protons. A third band at 3.5-3.7 ppm is attributed to the methylene units of PEG blocks. The final band appeared at 4.3 ppm, this is characteristic of the PEG methylene proton that links PEG and PLA. These results confirm the formation of the desired triblock. [14]



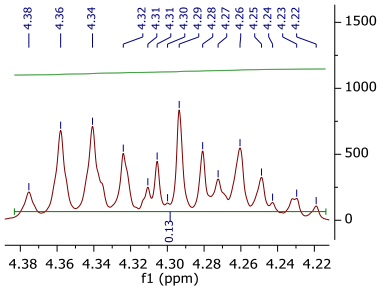
Proton	Chemical Displacement
A	1.5 ppm
B	5.2 ppm
C	3.5 ppm
D	4.3 ppm



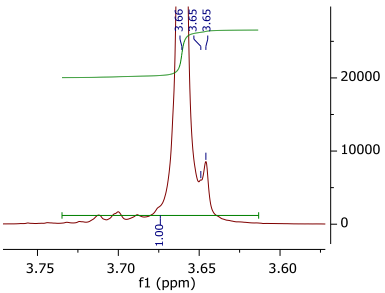
Methyl PLA proton
Internal A
Terminal A*



Methine PLA proton B



Methylene PEG proton D



Methylene PEG proton C

Figure 7. *Characteristic bands of PLA and PEG RMN's*

The NMR's obtained for all synthesized compounds are shown in **Appendix 4**, the ^{1}H-RMNs (example figure 8) are recorded with a Bruker 400 MHz Avance III NMR Device with a cryocouple.

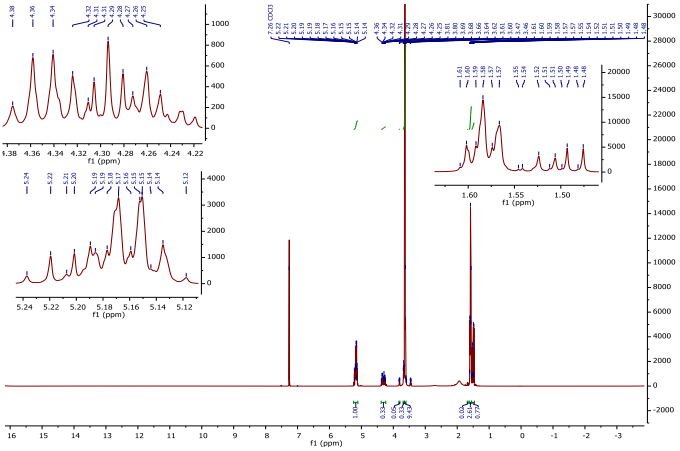


Figure 8. *Characteristic bands of PLA and PEG RMN's*

In order to calculate the molecular weight of copolymer, José E. Báez in his article [8] proposes using the integration of dissimilar protons of the PLA monomer and then comparing it with a distinctive proton of the PEG chain, since the molecular weight of this is known, with the goal of obtaining a relation and acquiring the PLA monomer number. Thus, if the number of PLAs that have been joined to the copolymer and the molecular weight of the PEG is known, the only one needs to do is add everything to obtain the molecular weight.

To calculate the PLA monomer number that is cited above, the Equation 1 is used. In the denominator, the relation of the special proton of each chain is used; 3.64 ppm correspond to the 4 protons characteristics of the PEG chain that links PLA with PEG and the 5.2 ppm corresponds to the single proton of PLA methine. So, if the numerator of the expression is observed, the PEG monomer number is used. It is known that in the different tries of synthesis of the triblock PEG (2000) and PEG (3350) are used, then this number can be calculated by dividing the total molecular weight by the molecular weight of a monomer obtaining that the number of PEG varies between 45 or 76 in function of the molecular weight of the PEG used.

$$n\,PLA = \frac{1}{2}\; x\; \frac{n\,PEG}{\frac{\int \frac{3{,}64}{4\;protons}}{\int \frac{5{,}2}{1\;proton}}}$$

Equation 1.

To finally obtain the copolymer molecular weight, only Equation 2 need to be applied.

$$Mw = 2\,x\,(n\,PLA\,x\,72) + mw\,PEG$$

Equation 2.

Table 3 shows the number of PLA monomers obtained, thus obtaining the molecular weight of the triblock. It is worth highlighting that the copolymers that contain a central PEG with a higher molecular weight average form triblock with a greater number of PLA monomers, maybe it is due to that the probability to find a center of PEG to do the linkage with PLA is more probable.

The article presented by Daniel G. Abebe argues that to obtain a successful triblock more than 7 repetitions of PLA monomer units are needed. The results in table 4 support this assertion. [11]

Table 4. *PLA monomers number and the total copolymer Mn obtained by ^{1}H-RMN*

Try	Block Copolymer	PLA monomers number	Triblock Mn (g/mol)
1	PLLA-PEG_{2000}-PLLA	13.85	3994.45
1	PDLA-PEG_{2000}-PDLA	19.08	4747.52
1	PLLA-PEG_{3350}-PLLA	28.09	7395.59
1	PDLA-PEG_{3350}-PDLA	30.28	7709.63

7. MICELLE CHARACTERIZATION

Some studies have pointed out that the micelle sols state could be one of the most relevant factors in the formation of the hydrogel; hence, it is said that gels should be associated with the primary micellar formation and secondary intermicellar aggregation features. Therefore, research into these micelles gives key information to understanding the hydrogel mechanism formation, for example, why micelles with higher PEG form micelles smaller than those that have lower PEG.

In order to complete the characterization of micelles an instrument called Dynamic Light Scattering (DLS) Zetaister nano-ZS is used. With this, data such as the size of the micelles, zeta potential, and the polydispersity index (PdI) is obtained. Summarizing, it is a technique that, thanks to the analysis of the trajectory of the colloidal particles in suspension by laser scattering, correlates this trajectory with the size of particle using a model based on the Strokes-Einstein equation.

In this study, the hydrodynamic diameters of micelles with a varied composition of the polymer PEG are measured by DLS at 25 ºC and are listed in table 5. All measurements are done at a concentration of 0.1% $w_{micelle}/w_{water}$ concentration.

Table 5. *Hydrodynamic Diameter Zeta Potential and Polydispersity Index of the micelles represented in the appendix 4 by DLS (0.1wt %)*

Samples	**Micelle Size** (nm)	**Zeta Potential** (mV)	**Polydispersity Index**
PLLA-2000	187,60	-6,53	0,03
PDLA-2000	289,00	-5,10	0,33
PLLA-3350	192,60	0,01	0,13
PDLA-3350	169,60	-6,78	0,06
PLLA-2000(50%) PLLA-3350(50%)	502,90	-1,81	0,01
PDLA-2000(50%) PDLA-3350(50%)	151,20	-5,96	0,07
PLLA-2000(75%) PLLA-3350(25%)	209,20	-3,58	0,07
PDLA-2000(75%) PDLA-3350(25%)	151,00	-4,55	0,34
PLLA-2000(25%) PLLA-3350(75%)	164,40	-9,69	0,17

PDLA-2000(25%) PDLA-3350(75%)	247,90	-6,59	0,07
PLLA-2000(40%) PLLA-3350(60%)	226,80	-1,32	0,05
PDLA-2000(40%) PDLA-3350(60%)	179,90	-5,00	0,04

Figure 9 depicts the hydrodynamic diameters of micelles formed by different composition. The micelle size does not follow an observable trend, however, there is a sample PLLA-2000(50%)/PLLA-3350(50%) that stood out from the others. One possible explanation could be that when the ultrasonic agitation was applied, this sample wasn't stirred for enough time. In fact, all the values are higher than expected. A low absolute value of the Z-potential may explain this behavior. Overall, these outcomes of mixing different copolymers and resulting micelles with intermedium size match other studies like, those made by Daniel G. Abebe and Tomoko Fujiwara.[11]

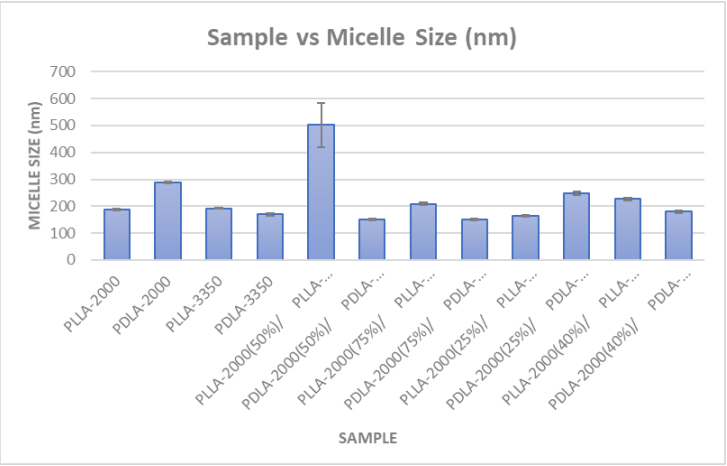


Figure 9. *Plot of sample vs micelle size (nm) of all the micelles represented in the table 5.*

Figure 10 shows the micelle size vs polydispersity index. This final set of data describes the degree of heterogeneity of the size distribution of the particles and provides information about the width of molecular weight distribution (MWD) of the polymer. Specifically, that samples with values close to 0 in PdI are more uniform samples. [15]

However, the polydispersity index of varied micelles points to the tendency that the smaller the micelles composed of one stereoisomer (L or D), the lower the PdI, whereas those that are formed by hybrid micelles (mixture of two different micelles) and are huge, maintain an increase in their PdI, this makes it more difficult for them to form a hydrogel since the binding centers to form the gels are further apart.

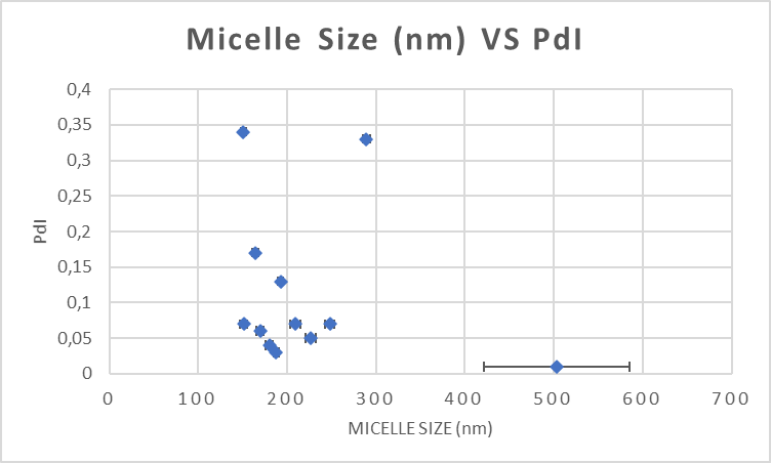


Figure 10. *Plot of micelle size (nm) vs PdI of all the micelles represented in the table 5.*

In figure 10, it is observed that in the range of 100 to 300 nm most of the samples are found, this is due to the fact that they form aggregates that would act as a driving force for the formation of the gel.

Figure 11 presents the information about micelle size in relation to zeta potential. Zeta potential gives an idea of the degree of repulsion or agglomeration between the charged particles in the dispersion. A high absolute value indicates colloidal stability.

As can be seen from figure 11, the majority of the tests involved negative values of zeta potential. This means that the micelles had a negatively charged surface. Moreover, there is an observable although not totally consistent pattern: the triblock with PEG3350 usually tends to have a higher zeta potential considering the absolute value. So, the micelles that hold longer PEG have more functional groups on the surface and this allows them to captivate more negative charge.

The negative feature of these samples is discussed in some studies, specifically Gang Ruan and Si-Shen [16] said that it is caused by the presence of polylactide acid because this contains carboxyl groups which, due to the folded conformation that the polymer chain adopts, means that a negative value is observed.

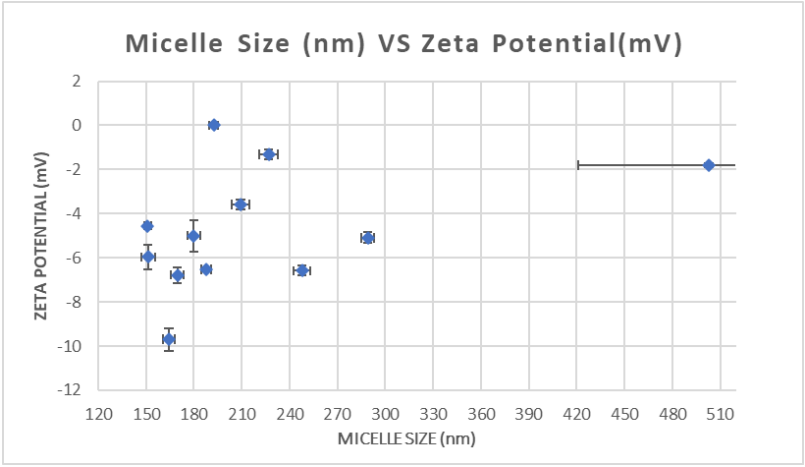


Figure 11. *Plot micelle size (nm) vs zeta potential (mV) of all the micelles represented in the table 5.*

Figure 12 shows the representation of PdI vs Zeta potential (mV). A slight grouping can be observed which suggests that if the polydispersity index is lower, this implies an increase in the zeta potential; this offers the idea of the Stern layer.

The charges on a Stern layer are the ones that condition the level of aggregation or agglomeration of the samples during the analysis. This surface is a layer of ions charged oppositely to the surface to which they are linked. From the data in figure 11, it can be concluded that most of the samples in this study have a surface which is positive and a Stern layer of negative ions.

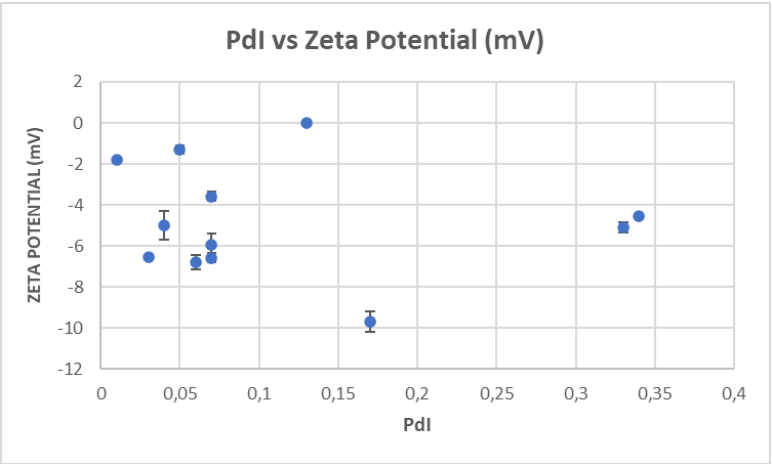


Figure 12. *Plot polydispersity index vs zeta potential of all the micelles represented in the table 5.*

The last set of data, figure 13, shows the triblock Mn(g/mol) vs micelle size. The results indicate that the micelles with elevated content in PEG 3350 usually have a smaller micelle size, in contrast to the copolymers formed by PEG 2000 that they have a higher micelle size. This outcome corroborates the paper of Daniel G. Abebe and Tomoko Fujiwara [11] which asserts that when PEG is increased a smaller micelle is found, attributable to the lower number of polymers associated in the micelle core due to the large corona volume. The mechanism of association is represented in figure 14.

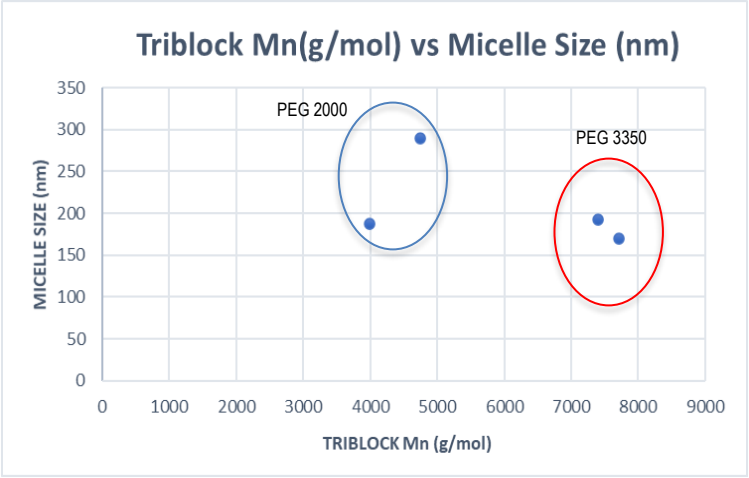


Figure 13. *Triblock number-average Mn (g/mol) vs micelle size (nm), the samples are those that the triblock differ in the central weight of PEG (2000 blue circle or 3350 red circle) representing both*

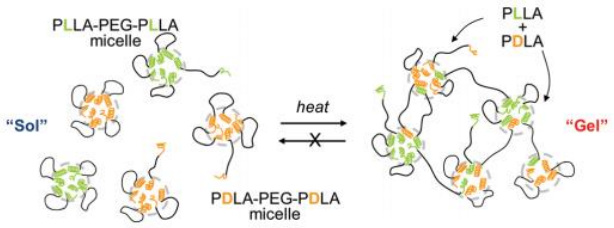


Figure 14. *Stereocomplexed hydrogel mechanism. (Figure adopted from [11]).*

8. GEL CHARACTERIZATION

One of the main interests in this work is to obtain gels that have a transition temperature between 15-100˚C so different micelle sols were prepared different which mix different percentages of distinct triblock. To observe the sol-gel shift a temperature ramp was applied to trigger the micelle sol to gel. It was noticed that in an initial phase the vial looked translucent, however, when the temperature started to increase a finally white solid gel was reported.

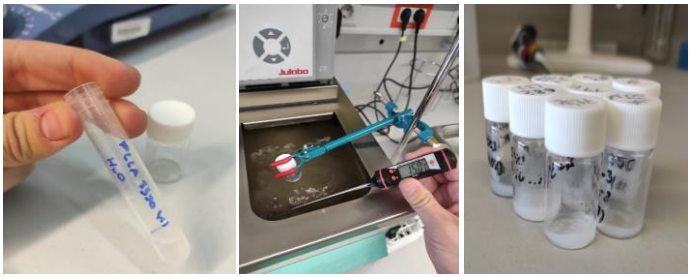


Figure 15. *Hydrogel obtained by the mixture of enantiomeric PLA-PEG-PLA from micelle solutions.*

The physical gelation one of its main driving force is a network of intermicellar bridging. The key factor to obtain the desired gel is to use a stereocomplex crystallization which, according to some papers, speeds up the gelation rate. With regard to mixing the same PLA configuration, no gel was observed, reinforcing the idea of stereo complexation as a driving force.

Furthermore, the critical temperature gelation (CGT) according to the results is influenced by the composition of the samples. Figure 16, represents the sol-to-gel phase diagram, and looking at the results, a linear response in the sol-gel transition temperature can be observed which supports the work done by Daniel G. Abebe and Tomoko Fujiwara. [11] Gelation temperature is directly affected by the composition of the micelles; as the Mw of PEG is increased, the stability of the micelles increases obtaining hydrogels with a higher sol-gel transition. This is due to fact that the PEG acts as a bridge between micelles by chain exchange, and hence If the PEG is longer, the micelles are less compact, obtaining a higher gel transition temperature.

In an attempt to reach the idoneous temperature, different micelles were mixed without following the standard pattern of putting the same concentration of the copolymers in the L stereoisomer and in the D stereoisomer. For example, PLLA (2000) 75% / PLLA (3350) 25% were mixed with PDLA (2000) 50% / PDLA (3350) 50% i.e., changing the ratio of the PEG in the L stereoisomer and the D stereoisomer led to a gel transition of 71,3 ºC, a temperature far away from the desired one.

The most interesting outcome of this research is that, by adjusting the copolymer ratio, a sol-gel transition of around 30ºC can be obtained which is similar to body temperature. This gel will acquire properties which could be used as a thermosensitive injectable hydrogel. Looking at the figure 15, it is demonstrated that this gel needs more PEG 2000 in proportion to PEG 3350.

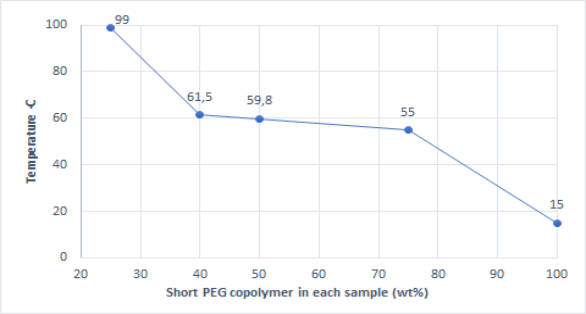


Figure 16. *Sol-gel transition temperature of enantiomeric blends from micelles. The x axis marks the weight fraction of the short PEG copolymer in the different gels.*

8.2. RHEOLOGICAL PROPERTIES OF HYDROGELS

To obtain the rheological properties of hydrogels were characterized. A rheometer was used with the objective of measuring fluids flow behavior and deformation. This method of characterization is also referred to as dynamic mechanical analysis (DMA).

It has been demonstrated that, depending on the inner structure, fluids with long space between the molecules flow easier, whereas the molecules which are more compacted remain closer. Another factor to consider is the external forces that stress the material and that bestow properties such as deformation or flow.

Viscosity refers to the resistance that some fluids have during their flow and deformation. The factors of higher influence in the viscosity of the hydrogel are principally temperature (the greater the temperature, the lower the viscosity) and time. In order to measure the degree of flow resistance, the gel is stocked between two plates. The lower plate stands still the upper plate moves along very slowly, causing laminar flow. By using this method, the shear stress and shear strain (deformation) is obtained.

DMA allows assessing the storage modulus (G') which relates to the material's ability to store energy elastically. Also, the loss modulus (G''), which is the energy consumed during the friction process, this energy is expressed in the form of heat due to the movement of the molecules. By last, once the yield point is passed, some fraction of the deformation will be permanent. [17] [18]

All the samples were measured using Haake Mars (figure 17) (Modular Advanced Rheometer System). Figure 18 shows the storage modulus (G') and the loss modulus (G'') of the stereocomplexed hydrogels obtained in the previous section as a function of shear stress, the loss factor (tan (δ)) is also presented in a secondary shaft. The yield point can also be observed, which is that point where G' and G'' intersect. Measurements were done at 37°C and at 1 Hz due to the gel is considered stable under these conditions.

Figure 17. *Haake Mars device used in this study.*

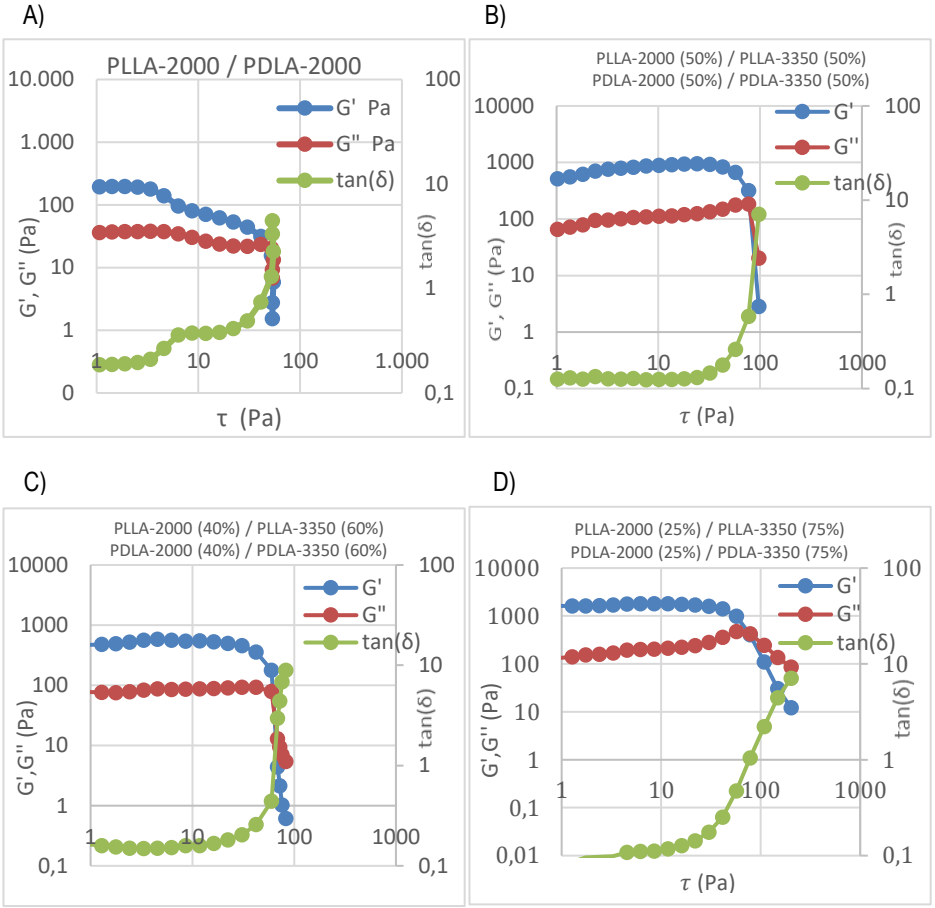


Figure 18. *Plots of storage modulus (G') and loss modulus (G") as function of stress in x axis and in a secondary shaft is represented the loss factor (tan(δ)) at 37ºC for stereomixture of gels obtained: a) PLLA-PEG2000 / PDLA-PEG2000 b) PLLA-PEG2000 (50%) / PLLA-PEG3350 (50%) + PDLA-PEG2000 (50%) / PDLA-PEG3350 (50%) c) PLLA-PEG2000 (40%) / PLLA-PEG3350 (60%) + PDLA-PEG2000 (40%) / PDLA-PEG3350 (60%) d) PLLA-PEG2000 (25%) / PLLA-PEG3350 (75%) + PDLA-PEG2000 (25%) / PDLA-PEG3350 (75%)*

Rheological tests were performed on the following samples (figure 18a) PLLA-PEG2000 / PDLA-PEG2000 (figure 18b) PLLA-PEG2000 (50%) / PLLA-PEG3350 (50%) + PDLA-PEG2000 (50%) / PDLA-PEG3350 (50%) (figure 18c) PLLA-PEG2000 (40%) / PLLA-PEG3350 (60%) + PDLA-PEG2000 (40%) / PDLA-PEG3350 (60%) (figure 18d) PLLA-PEG2000 (25%) / PLLA-PEG3350 (75%) + PDLA-PEG2000 (25%) / PDLA-PEG3350 (75%), however, two more were performed which were PLLA-PEG3350 / PDLA-PEG3350 y PLLA-PEG2000 (75%) / PLLA-PEG3350 (25%) + PDLA-PEG2000 (75%) / PDLA-PEG3350 (25%), these were discarded because there wasn't enough gel to fill the plates well which meant that the shear stress was not exerted well, obtaining anomalous results.

Looking the sample PLLA-PEG2000 / PDLA-PEG2000, (figure 18a), which is a mixture with the shortest PEG, this has one of the lowest values of G' indicating it has the lowest elastic behavior away from the characteristics of a solid. In other words, when the shear process is applied to the sample, the quantity of this energy is stored and used as a driving force for the reformation process. The conclusion then is that this sample will have a liquid-like, microstructure.

On the other hand, the measurement of the sample PLLA-PEG2000 (50%) / PLLA-PEG3350 (50%) + PDLA-PEG2000 (50%) / PDLA-PEG3350 (50%), (figure 18b), which is a mixture of 50/50 of both PEGs, it is observable a value of G' higher than G'', which indicates that this hydrogel possesses a more solid-like, rigid microstructure. Furthermore, the yield point is located over 100 Pa.

Comparing the previous result with the sample PLLA-PEG2000 (40%) / PLLA-PEG3350 (60%) + PDLA-PEG2000 (40%) / PDLA-PEG3350 (60%), (figure 18c), there is a noticeable tendency in the values of G' and G'' increase as the percentage of PEG 3350 increases. Obtaining more elastic gels, that has solid-like features. It is also observed that the yield point continues to be around 100 Pa.

Focusing on last sample PLLA-PEG2000 (25%) / PLLA-PEG3350 (75%) + PDLA-PEG2000 (25%) / PDLA-PEG3350 (75%), (figure 18d), with elevated content in PEG-3350 shows that a higher value of loss modulus and storage modulus is translated in solid-like characteristics, reinforcing the previously observed trend that the more PEG3350 the larger the elastic values. In this case, the yield point is displaced, so it could also be said that the more PEG3350 also displaces the yield point.

A further factor that can be examined is the loss factor, which represents the gap which exists between effort and deformation or, in other words, the relation between the lost and dissipated energy. Furthermore, it reveals the ratio of viscous and the elastic portion, represented by equation 2:

$$\tan(\delta) = G''/G' \qquad 0 \leq \tan(\delta) \leq \infty$$

Equation 2.

There are three options. The first is an ideal elastic material when stress and strain are in phase obtaining tan (δ) = 0. A majority content of PEG3350 presents a more elastic behavior. The second option is to have a loss factor that tends to infinity, this behavior leads to an ideal viscous material and is the contrary of the previous case because the stress and strain are out of phase. The final option is the state in which the sample is viscoelastic tan (δ) = 1 which is the value of all the samples at the beginning. This study showed that all the samples behave like this.[17]

To sum up, when the PEG portion has increased the storage modulus and the loss modulus have increased, as well as the yield point, therefore the length of the central block changes the rheological properties of the hydrogel.

10. CONCLUSIONS

- Thermo-responsive gels have been obtained complying with the desired temperature ranges (15-100°C).
- More proportion of PEG2000 in front of PEG3350 lowers the gelation temperature. Then it is possible to obtain a further gel with sol-gel transition feasible to work at physiological temperature.
- Triblock copolymers with higher PEG 3350 contents ensure higher PLA MWs.
- Gels with a higher content in PEG3350 form smaller micelles and, higher Z-potentials.
- Regarding the rheological properties, are also affected by the change of the central PEG. The higher the PEG3350 content, the higher the values of G', G'' and yield point.

11. REFERENCES AND NOTES

[1] Engler, A. J., Sen, S., Sweeney, H. L., & Discher, D. E. (2006). Matrix Elasticity Directs Stem Cell Lineage Specification. In *Cell* (Vol. 126, Issue 4, pp. 677–689).

[2] Jiang, Z., Shaha, R., Jiang, K., Mcbride, R., Frick, C., & Oakey, J. (2019). Composite Hydrogels With Controlled Degradation in 3D Printed Scaffolds. *IEEE Transactions on Nanobioscience*, *18*(2), 261–264.

[3] Ordoño, J., Pérez-Amodio, S., Ball, K., Aguirre, A., & Engel, E. (2020). *Lactate promotes cardiomyocyte dedifferentiation through metabolic reprogramming.*

[4] Babensee, J. E. (2020). Inflammation, Wound Healing, the Foreign-Body Response, and Alternative Tissue Responses. In *Biomaterials Science*.

[5] Estupiñán, H. A., Vásquez, C., Peña, D. Y., & Ardila, L.F. (2011). Degradation of polylactic acid / hydroxyapatite and polyglycolic acid in simulated body fluid. *Revista UIS Ingenierías, 10*, 145-150.

[6] Medina, J., Roche, Y., Maldonado, O., Hernandez, J., & Zapata, C. (2018). Degradación hidrolítica y biodegradación de mezclas binarias de ácido poliláctico (PLA) con residuos plásticos. In *Revista ingeniería UC* (Vol. 25, Issue 2, pp. 248–258).

[7] Baumann, A., Piel, I., Hucke, F., Sandmann, S., Hetzel, T., & Schwarz, T. (2019). Pharmacokinetics, excretion, distribution, and metabolism of 60-kDa polyethylene glycol used in BAY 94-9027 in rats and its value for human prediction. *European Journal of Pharmaceutical Sciences*, *130*(August 2018), 11–20.

[8] Báez, J. E. (2010). Cómo obtener un polímero degradable en el laboratorio: síntesis de la poli(D, L-lactida) y caracterización por RMN 1 H. *Educación Química*, *21*(2), 170–177.

[9] Kaihara, S., Matsumura, S., Mikos, A. G., & Fisher, J. P. (2007). Synthesis of poly(L-lactide) and polyglycolide by ring-opening polymerization. *Nature Protocols*, *2*(11), 2667–2671.

[10] Ghasemi, R., Abdollahi, M., Emamgholi Zadeh, E., Khodabakhshi, K., Badeli, A., Bagheri, H., & Hosseinkhani, S. (2018). MPEG-PLA and PLA-PEG-PLA nanoparticles as new carriers for delivery of recombinant human Growth Hormone (rhGH). *Scientific Reports, 8*(1),1-13.

[11] Abebe, D. G., & Fujiwara, T. (2012). Controlled thermoresponsive hydrogels by stereocomplexed PLA-PEG-PLA prepared via hybrid micelles of pre-mixed copolymers with different PEG lengths. *Biomacromolecules, 13(6),* 1828-1836.

[12] Pérez de Prada, M. (2009). Las células madre o progenitoras. *Libro De La Salud Cardiovascular Del Hospital Clinico* (pp. 321–326).

[13] Herreros, J., Chachques, J. C., Trainini, J., Pontón, A., Sarralde, A., & Genovese, J. (2011). Regeneración celular cardíaca. *Cirugía Cardiovascular*, *18*(3), 207–215.

[14] Li, Feng, Li, S., & Vert, M. (2005). Synthesis and rheological properties of polylactide/poly(ethylene glycol) multiblock copolymers. *Macromolecular Bioscience*, *5*(11), 1125–1131.

[15] Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier systems. *Pharmaceutics*, *10*(2), 1–17.

[16] Ruan, G., & Feng, S. S. (2003). Preparation and characterization of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomaterials, 24*(27), 5037-5044.

[17] Mezger, T. G. (2010). *The rheology handbook* (Vol. 59, Issue 4).

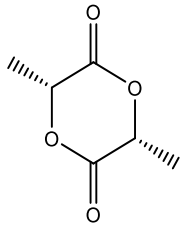
[18] Ahmed, A. S., Mandal, U. K., Taher, M., Susanti, D., & Jaffri, J. M. (2017). PVA-PEG physically cross linked hydrogel film as a wound dressing: experimental design and optimization. *Pharmaceutical Development and Technology*, *23*(8), 751–760.

12. ACRONYMS

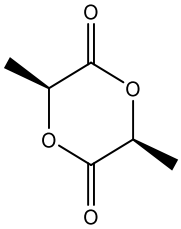
PLA	Polylactic acid
PLLA	Polylactic acid (Stereoisomer L)
PDLA	Polylactic acid (Stereoisomer D)
PEG	Polyethylene glycol
$Sn(Oct)_2$	Tin (II) 2-ethyl hexanoate
ROP	Ring-opening polymerization
LCST	Lower critical solution temperature
TS-G	Sol-gel transition temperature point
^{1}H-NMR	Proton Nuclear Magnetic Resonance
SLS	Static Light Scattering
DMA	Dynamic mechanical analysis
Mn	Number-average molecular weight
Mw	Weight-average molecular weight
PdI	Polydispersity Index
DLS	Dynamic Light Scattering
G'	Storage modulus
G''	Loss modulus

APPENDICES

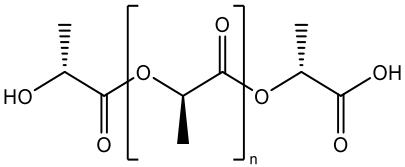
APPENDIX 1: MOLECULAR STRUCTURES



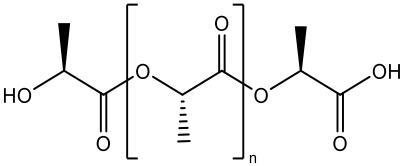
D-lactide



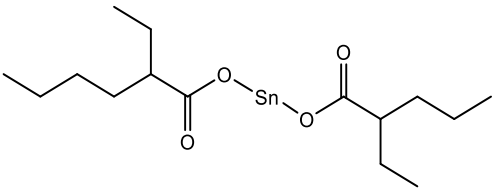
L-lactide



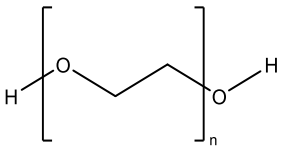
D-PLA



L-PLA



$Sn(Oct)_2$



PEG

Figure 1 Appendix. *Molecular structures*

APPENDIX 2: HYDROLYSIS OF PLA

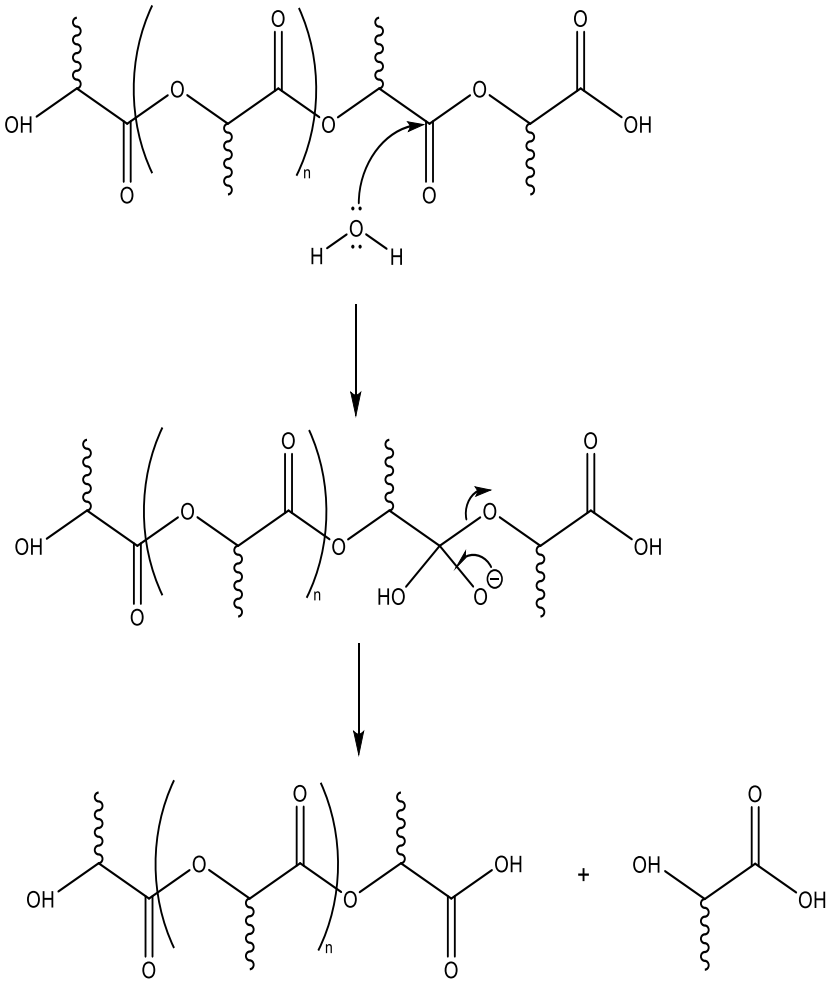


Figure 2 Appendix. *Mechanism Hydrolysis of PLA*

APPENDIX 3: CATALYSIS OF LACTIDE

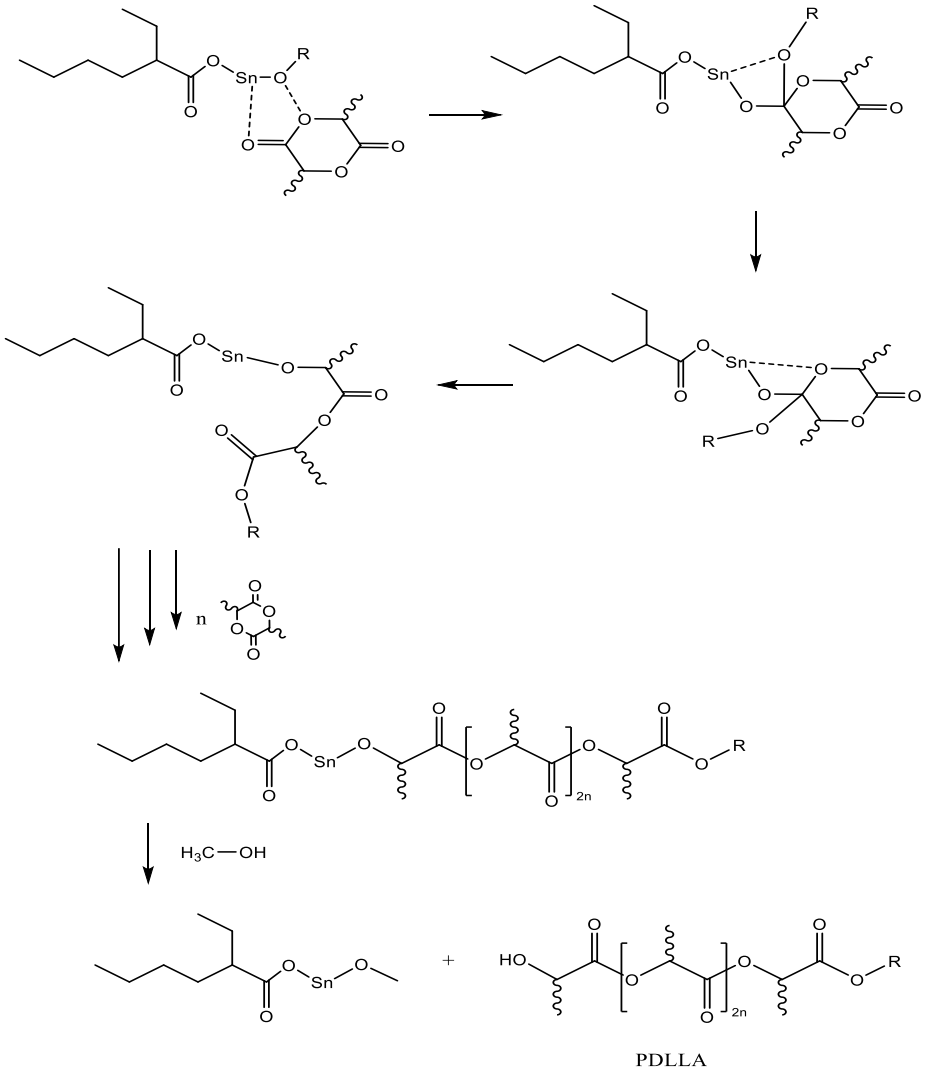


Figure 3 Appendix. *Catalysis of lactide*

APPENDIX 4: TRIBLOCK ^{1}H-NMR'S

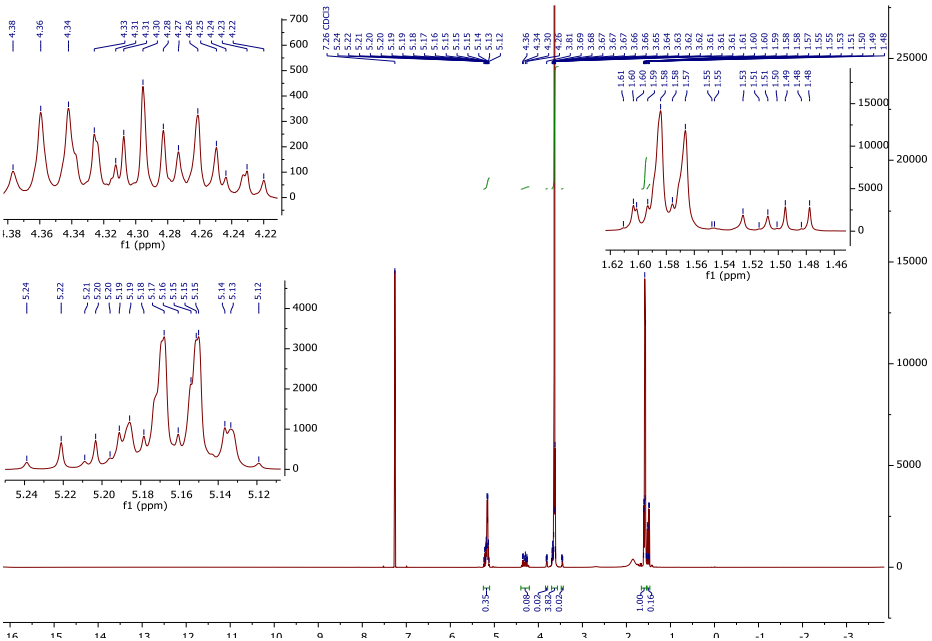


Figure 4 Appendix. *^{1}H-NMR of the sample PLLA(2000)-PEG(3350)-PLLA(2000)*

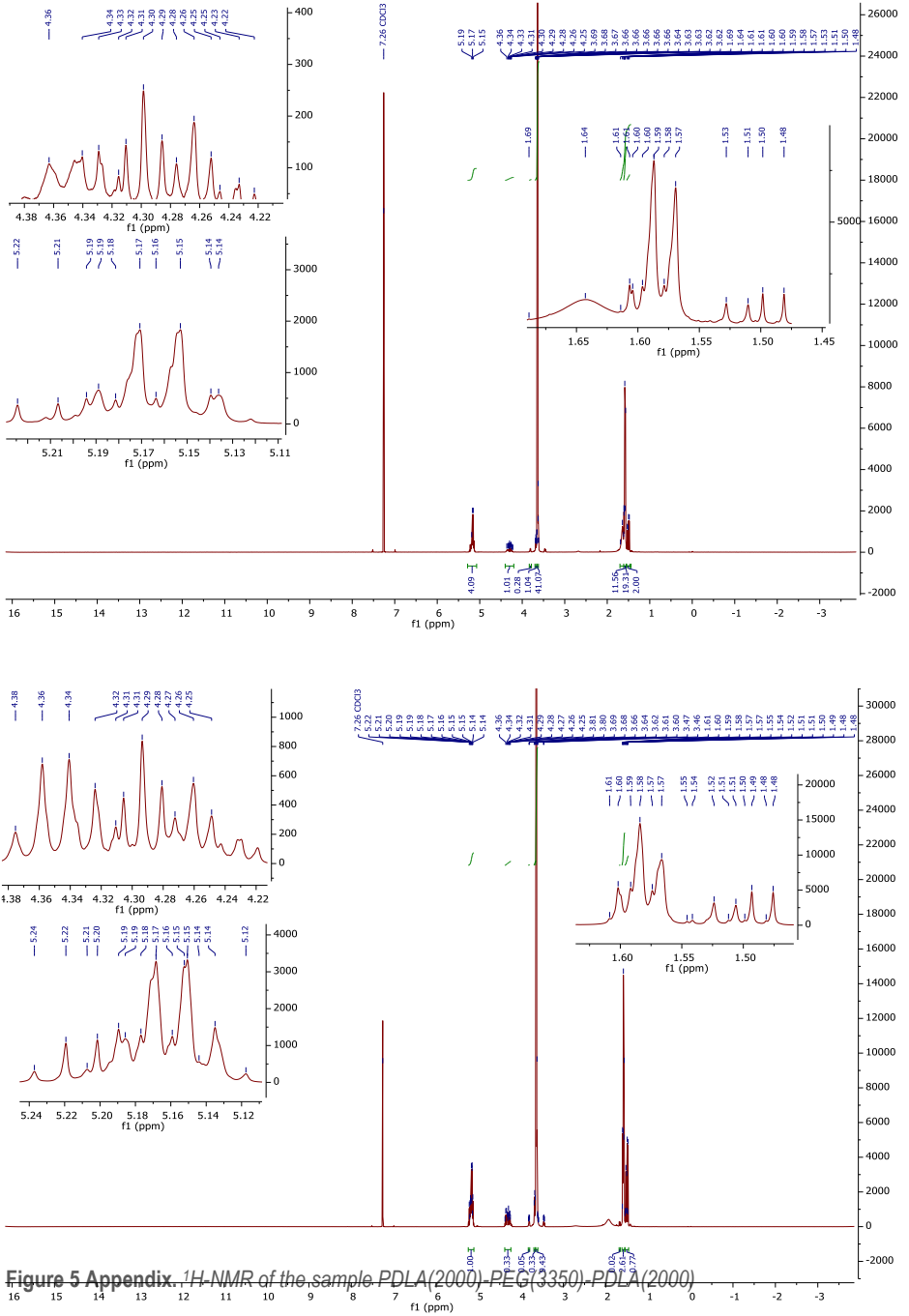


Figure 5 Appendix. *1H-NMR of the sample PDLA(2000)-PEG(3350)-PDLA(2000)*

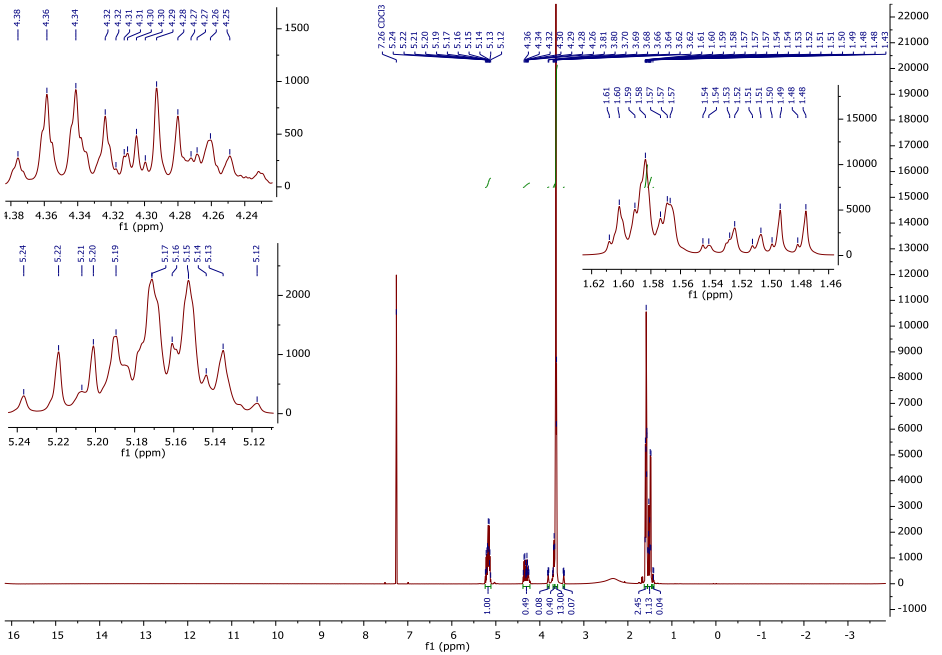


Figure 7 Appendix. *^{1}H-NMR of the sample PLLA(2000)-PEG(2000)-PLLA(2000)*