Universidad del País Vasco Euskal Herriko Unibertsitatea
FACULTY OF ENGINEERING BILBAO
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Degree in Industrial Technology Engineering

End-of-Degree Thesis

Effect of the addition of inorganic particles on the hydrolytic degradation of biodegradable polymers for biomedical applications

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Year: 2020-2021

June 27, 2021

Abstract

Poly(lactides) (PLAs) have demanded great attention in recent years for biomedical applications given their biocompatible and biodegradable behaviour. However, the raw properties are not good enough for its usage particular applications. In this way, novel reports suggest the addition of reinforcement particles to improve the system and deliver a feasible polymer capable of dealing with diverse applications with the advantage of avoiding an extraction surgery, sating socioeconomic needs. This project delivers the study on thermal and hydrolytic degradation of several poly(L-lactide) (PLLA) based-on polymers with the addition of inorganic particles like magnesium oxide (MgO), bioactive glass (BG) and barium sulphate ($BaSO_4$), conducted by means of Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Gel Permeation Chromatography (GPC) after their immersion in phosphate buffer saline (PBS) for different time intervals. The analysis has proved that MgO degrades more than BG the polymeric matrix due to fast degradation of the chains. Both systems, when subjected to a polydopamine (PD) coating, have delayed the degradation temperatures and recrystallization and melting temperatures (T_c and T_m), in some cases, behaving even better than pure PLLA. Results indicate that the addition of $BaSO_4$ does not have any practical effect on the hydrolytic degradation of the systems. These results enlighten the path for future research on particle reinforcement composites with the potential of altering the world of biomedical materials.

Keywords: PLLA, Inorganic reinforcement, MgO, BG, $BaSO_4$, Hydrolytic degradation, *in vitro*, DSC, TGA, GPC, Biomedical.

Resumen

Las polilactidas (PLAs) han demandado una gran atención en los últimos años para aplicaciones biomédicas debido a su comportamiento biocompatible y biodegradable. Sin embargo, las propiedades básicas no son suficientemente buenas para su uso en aplicaciones específicas. En este sentido, informes novedosos sugieren la adición de partículas de refuerzo para mejorar el sistema y proporcionar un polímero viable, capaz de tratar diversas aplicaciones con la ventaja de evitar una operación de extracción, saciando necesidades socieconómicas. Este proyecto proporciona el estudio en la degradación térmica e hidrolítica de varios polímeros basados en ácido poli(L-lactida) (PLLA) junto con la adición de partículas inorgánicas como óxido de magnesio (MgO), vidrio bioactivo (BG) y sulfato de bario ($BaSO_4$), realizado mediante Calorimetría Diferencial de Barrido (DSC), Análisis Termogravimétrico (TGA) y Cromatografía por Permeación de Gel (GPC) tras su inmersión en tampón fosfato salino (PBS) a diferentes tiempos. El análisis demuestra que el MgO degrada más que el BG la matriz polimérica debido a la rapida degradación de sus cadenas. Ambos sistemas, estando sujetos a un recubrimiento de polidopamina (PD), han retrasado las temperaturas de degradación y de recristalización y fusión (T_c y T_m), en algunos casos, incluso comportándose mejor que el PLLA puro. Los resultados indican que la adición de $BaSO_4$ no tiene ningún efecto práctico en la degradación hidrolítica de los sistemas. Estos

resultados iluminan el camino para futuras investigaciones acerca de polímeros con refuerzo de partículas con el potencial de cambiar el mundo de los materiales biomédicos.

Palabras Clave: PLLA, Refuerzo Inorgánico, MgO, BG, $BaSO_4$, Degradación hidrolítica, *in vitro*, DSC, TGA, GPC, Biomédico.

Laburpena

Azido poli-laktikoek (PLAs) arreta handia hartu dute azken urteotan aplikazio biomedikoetarako, portaera biobateragarria eta biodegradagarria dutelako. Hala ere, oinarrizko propietateak ez dira behar bezain onak aplikazio batzuetan. Ildo horretan, publikazio berritzaileek iradokitzen dute partikula sendotzaileak gehitzea sistema hobetzeko eta polimero bideragarri bat emateko, hainbat aplikazio tratatzeko gai izango dena erauzketa-eragiketa saihesteko abantailarekin, behar sozioekonomikoak asetuz. Proiektu honek azido poli-*L*-laktikoan (PLLA) oinarritutako hainbat polimeroren degradazio termiko eta hidrolitikoaren azterketa eskaintzen du, baita partikula inorganikoen gehikuntzarekin ere, hala nola magnesio-oxidoa (MgO), beira bioaktiboa (BG) eta bario sulfatoa ($BaSO_4$), Ekorketa Kalorimetro Diferentzialaren (DSC), Analisi Termograbimetrikoaren (TGA) eta Gel Iragazketa bidezko Kromatografiaren (GPC) bidez egina gatz-tanpoi fosfatoan (PBS) hainbat denboratan murgildu ondoren. Analisiak erakusten du MgOak matrize polimerikoa gehiago degradatzen duela BGarekin konparatuz, bere kateen degradazio azkarraren ondorioz. Bi sistemek polidopamina (PD) baten estalduri esker degradazio-tenperaturak eta birkristalizazio- eta fusio-tenperaturak (T_c y T_m) atzeratu egin dituzte, kasu batzuetan, PLLA hutsa baino hobeto portatzen ere. Emaitzen arabera, $BaSO_4$ gehitzeak ez du eragin praktikorik izan sistemen degradazio hidrolitikoan. Emaitza horiek bidea argitzen dute partikulek sendatutako polimeroei buruzko ikerketaren etorkizuna, material biomedikoen mundua aldatzeko potentzialarekin.

Hitz Gakoak: PLLA, Errefortzu Inorganikoa, MgO, BG, $BaSO_4$, Degradazio hidrolitikoa, *in vitro*, DSC, TGA, GPC, Biomediko.

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Acronyms

$BaSO_4$ Barium Sulphate

BG Bioactive Glass

BMP-2 Bone Morphogenetic Protein - 2

CH_2Cl_2 Dichloromethane

$CHCl_3$ Chloroform

DSC Differential Scanning Calorimetry

FESEM Field Emission Scanning Electron Microscope

GPC Gel Permeation Chromatography

H Entalhpy (H_c for crystalline and H_m for melting)

HA Hydroxyapatite

HCl Hydrochloric Acid

M_n Number Average Molecular Weight

M_w Molecular Weight

MBG Mesoporous Bioactive Glass

MC3T3-E1 Osteoblast Precursor Cell Line

MgO Magnesium Oxide

N_2 Nitrogen

$NH_2C(CH_2OH)_3$ Trizma Base Buffer

PBS Phosphate Buffered Saline

PD Polydopamine

PLA Poly(lactide)

PLLA Poly(L-lactide)

SEM/EDS Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy

$\mathbf{T}_c$ Crystallization Temperature

$\mathbf{T}_g$ Glass Transition Temperature

$\mathbf{T}_m$ Melting Temperature

TCP Tricalcium Phosphate

TGA Thermogravimetric Analysis

WBS Work Breakdown Structure

XRD X-Ray Diffraction

ZnO Zinc Oxide

1 Introduction

By means of the following document, the End-of-Degree Thesis conducted in the Department of Mining-Metallurgy Engineering and Materials Science at the Faculty of Engineering in Bilbao, belonging to the University of the Basque Country (UPV-EHU) is presented.

This report presents an analysis consisting of degradation studies carried out for different inorganic particles in a poly(L-lactide) (PLLA) matrix, composed by magnesium oxide (MgO), bioactive glass (BG) and barium sulphate ($BaSO_4$), covered with polydopamine (PD) or not. These materials are biocompatible and bioresorbable polymers widely used in the biomedical field, for which the correct analysis and assessment of the results is of utmost importance.

Firstly, to present the subject, a brief context shall be provided in order to reference the frame of study in the socio-medical field, followed up by the main objectives and the scope of the project itself.

Then, its impacts and benefitial effects on society will be presented, as well as on the technological and medical field, since it is expected to obtain evidence that will lead to an improvement of known composites as well as to the knowledge of its behaviour for future applications. A review of previous studies about the same subject that have been carried out, the state of the art, will be presented, considering all the technological advances made in recent years concerning these systems and their various applications. After having reviewed the information regarding the subject, a risk analysis will be carried out, measuring the influence the events that occurred in the development of the very project, for which the established plan of action is presented.

Subsequently, the used methodology for the obtention of results is explained, for the better understanding of the figures and results that will be exhibited and properly discussed. All this methodology had been planned in advance and the planning will be commented and exhibited by means of a Gantt diagram in its proper section. Furthermore, a budget covering all the expenses generated during this study will be exhibited.

Finally, the conclusions are drawn, as the main ideas extracted from the study. For a proper exposition of all the results, figures and tables will be included as well as appendixes if further explanation or complementary information on a certain subject is required.

2 Context

In recent years, biodegradable polymers have acquired a special interest in the biomedical field: with the increase of individuals with bone defects or injuries and internal implants due to fractures, amongst many, the use of these materials has stood out mainly thanks to the innecesary second surgery for their extraction. Amongst these polymers, the PLLA is the one which has received most of the interest, attributed to its good biocompatibility, biodegradability and bioreabsorption, together with its great processability; desired properties for bone repair by internal implants [1–3].

However, the existance of some drawbacks could limit the range of its applications: the low hemocompatibility being the main disadvantage, and being subjected to the response of each host, a thrombus may occur, leading to the failure of the implant in these biomaterials [4]. Another drawback is the initial acute inflammation [5] due to the acid-like degradation behaviour and its poor mechanical properties (inferior to those metallic implants used upon recently [6]), for which it would be convenient to adhere to its matrix an anti-inflammatory agent that would also enhance the hemocompatibility, providing a better cell-material interaction and a faster cell endothelialization. Furthermore, PLLA lacks of high bioactivity levels and is hydrophobic; all these disadvantages do not promote the proliferation and viability of bone cells.

In order to solve the aforementioned problems, it has been studied how to improve the properties of PLLA through the employment of different processing techniques [7] or the addition of inorganic reinforcements to the polymeric matrix [3, 4, 8–14]. This last path is on the rise since the search of new materials which have the potential to improve not only the mechanical properties, but also the thermal ones, is very promising. The particular study of MgO, as that of BG, as inorganic reinforcements has drawn the attention of several research groups. They both have an excellent bioactivity, proven by the *in vitro* formation of Mg^{2+} ions and the formation of hydroxyapatite, benefitial to the protein synthesis.

Moreover, since the systems under analysis are radiopaque, they could provide the composite on its various applications with a good monitoring under X-rays, for example, that until now had not been possible due to the lack of radiopacity of the PLAs. Nonetheless, although a small quantity of the inorganic particles (0.1-10% wt.) introduced in the matrix enhances the composites properties, an increase could trigger adverse consequences like brittleness or a more rapid degradation [11], undesired for certain applications. Other factors such as the polymer structure, its molecular weight, the reinforcements and their orientation within the matrix, the crystallinity degree and aging time of the polymer itself play a great role at establishing the behaviour of the study.

3 Objectives & Scope

In this section it is intended to expose the principal objectives of the project as well as its scope. Firstly, the different systems under analysis will be presented and the intended analysis undergone will be explained. Afterwards, a more thorough explanation will be given by means of a Work Breakdown Structure (WBS) to visualize all the main tasks that were carried out.

3.1 Objectives

The principal objective of this project was to study to effect of the addition of inorganic particles on the hydrolytic degradation of a biodegradable polymeric matrix; to undergo different thermal and structural analysis on the different systems in order to discuss the convenience of a certain system for a specific biomedical application, achieving a composite with high toughness and whose reabsorption is adequately controlled and monitorized.

The systems analysed consisted of a basic PLLA system (doubled: recent PLLA and old PLLA) in order to be able to compare results with, and the systems showed in Table 1.

Table 1. Systems under analysis.

MgO 0.1%	MgO 0.5%
MgO 1%	MgOPD 0.1%
MgOPD 0.5%	MgOPD 1%
MgOPD 5%	MgOPD 10%
MgO 1% $BaSO_4$ 5%	MgO 1% $BaSO_4$ 9%
MgOPD 1% $BaSO_4$ 5%	MgOPD 1% $BaSO_4$ 9%
MgOPD 3% $BaSO_4$ 7%	MgOPD 5% $BaSO_4$ 5%
BG 1% $BaSO_4$ 5%	BG 1% $BaSO_4$ 9%
BGPD 1% $BaSO_4$ 5%	BGPD 1% $BaSO_4$ 9%
BGPD 3% $BaSO_4$ 7%	BGPD 5% $BaSO_4$ 5%

The choice of these reinforcement particles was based on previous analysis, where an improvement in the mechanical properties of polymeric matrixes reinforced with MgO particles, amongst others, was evidenced. The analysis carried out has been mainly focused on the characterization of the evolution in hydrolytic and thermal behaviour of the composite after its hydrolysis in PBS for different immersion time periods.

3.2 Scope

The different executed tasks in order to fulfill the planned objectives will be extensively described in Section 8 Planning, but here below a summary of them is provided by means of a WBS.

The project has consisted of a series of tasks: from the gathering of information throughout its whole duration to the final analysis of the obtained results, through the preparation of samples by extrusion-injection molding and subsequent dissolution, testing of mechanical properties, immersion *in vitro*, submerged in phosphate buffer saline (PBS) and final thermal and compositional tests, composed of the following:

- Differential Scanning Calorimetry (DSC): it allows to determine the heat capacity, melting and crystallization temperatures and heat of fusion at constant heating rates by measuring them against a reference.
- Thermogravimetric Analysis (TGA): it allows to characterize the mass loss of the polymer as a function of temperature, yielding information such as composition of the sample and thermal stability.
- Gel Permeation Chromatography (GPC): it allows to separate polymer chains based on their size, further allowing to know the number average molecular weight and molecular weight (M_n and M_w).

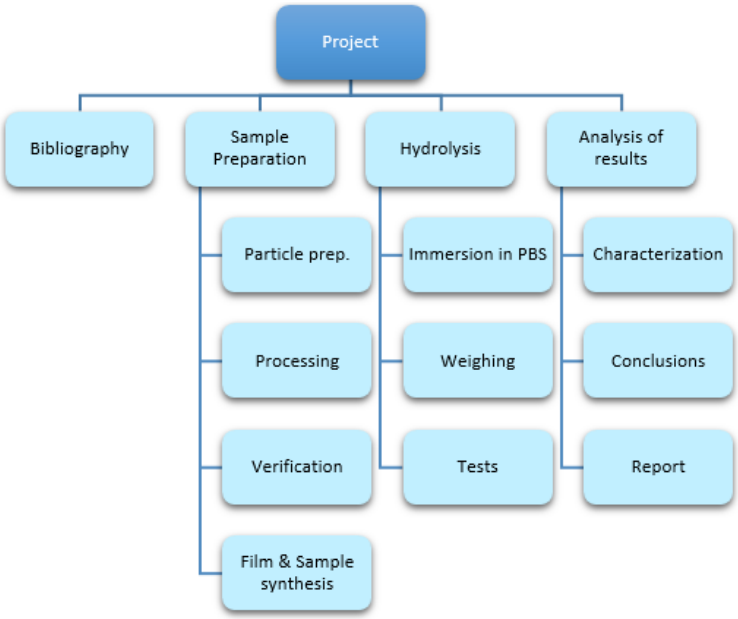


Figure 1. WBS of the project.

4 Benefits

In this section the diverse technological, medical and socioeconomical benefits are presented; all the stakeholders for whom this study results of certain interest.

(I) Technological Field

The study of these composites is not pioneer, several authors have studied aspects of specific composites similar to the ones analysed here. However, the tests undergone in this project are of great interest to further investigate the suitability of these biocompatible materials, as it is yet a long and uncharted path that seems to provide more benefits than metallic materials.

The project may open new and diverse possibilities, given that the more it is known, the more likely it is to find new applications in order to overcome current difficulties. As stated before, this reserch planned on providing the scientific field with a complete and convenient discussion of the suitability of a highly tough, radiopaque and with controlled monitoring and biodegradability composite that may complement current studies or help develop future ones.

(II) Medical Field

The main addressing of the study is to be able to implement the newly found improvements into biomedical applications, mainly bone implants and scaffolding. The metallic materials that have been governing these applications for centuries, now undergo a dethroning.

A renewal of the used materials in such applications is incresingly notorious, since these composites have proved to be suitable when metals have failed to, although having lower mechanical properties, which unnoticed, helps the subject recover better from the wounds as there is no stress-shielding.

(III) Socioeconomic Field

The main socioeconomical benefit of these composites is the comfort for the application user. Since they are biodegradable polymers, there is no need of a second surgical intervention for the extraction of the implant, thus, it reduces the risk at which the user is subjected, and the cost a second operation implies.

Furthermore, these polymers can be easily shaped and molded to the specific requirements of each patient and application without an increase in the costs. As a consequence, a more affordable and accessible treatment may be offered to whomever needs it.

5 State of the Art

With the development of polymers, specially biopolymers, great advances have been made in the technological, medical and socioeconomic fields, as aforementioned. Throughout many centuries, metallic and ceramic materials have been used in biomedical applications for their good mechanical properties and biocompatibility. However, nowadays, more and more applications are turning to biodegradable polymers; for instance, in implants. And one of the most used ones is the poly(lactide) (PLA).

PLA is an aliphatic[1] thermoplastic polyester derived from natural and renewable resources such as corn, yucca or sugar cane starch. It is usually obtained by ring-opening polymerization of lactide, the cyclic dimer of the basic repeating unit.

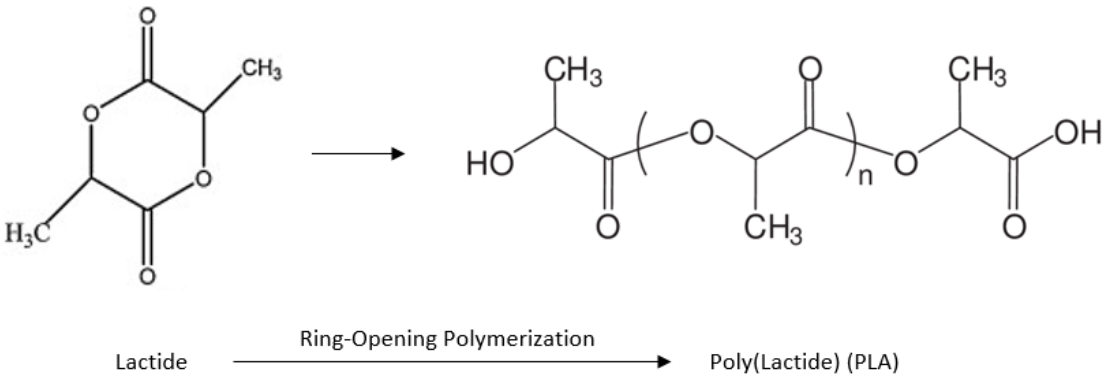


Figure 2. PLA synthesis.

Lactic acid is a chiral molecule: its molecular structure has 2 distinct mirror images, asymmetric carbons, called optical isomers. To distinguish between these, they are commonly referred to as L (laevorotatory) and D (dextrorotatory) based on the direction at which they polarize light. Both L- and D- are semicrystalline polymers, whereas their mixture, DL-, is completely amorphous. Controlling the processing is important as they have different characteristics.

The fact that PLA degrades by hydrolysis through de-esterification[2] into non-toxic products makes it suitable for sutures and implants meant to be absorbed by the human body, without any need of an extraction surgery. High-molecular weight PLLA is suitable for applications that need to maintain good mechanical properties over a long time period.

Poly(L-lactides) have relatively good mechanical properties, although much poorer than

[1]Related to the open chains formed by Carbon atoms, as opposed to aromatic (closed) ones.
[2]Conversion of ester via water into an acid and an alcohol.

those of metallic materials. They have a Young modulus of around 2 GPa with a tensile strength of 50-70 MPa [8]. However, their low fracture-elongation make these material have a brittle behaviour. Being such, it is interesting the search for strategies that would provide more toughness to the matrix, so as to be able to implement these materials into applications where the failure is not admissible, as bone fixation.

For fixation devices, historically, metallic plates have been used, firstly in 1895 [6]. However, it was proved that the high stiffness of these materials led to unsuccessful union of bone, refracture after removal and bone loss. This was due to the stress-shielding of the implants themselves, which bore the loads without any transfer to the tissue, preventing the body from healing properly. For this reason, composite plates with similar properties to those of the desired body part have been tried to be designed, to obtain adequate strain and load distributions at the site for a convenient cell regeneration.

After further research on the field, currently three main categories for biomaterials are defined: metals (although more adequate than initial ones), bioceramics and polymers. The future ahead relies on the bioceramics and polymers, on the grounds of their better corrosion resistance, biocompatibility and bioactivity. PLLA provides more new bone formation than empty control group[3] *in vitro*. However, adverse effects may take place: swelling at the site of implantation [15], initial acute inflammation [5, 16] or toxic residual products due to acidic degradation [8, 16].

To overcome these problems, when combined with BG, the presence of hydroxyapatite (HA), or tricalcium phosphate (TCP), both inorganic, or organic substances as bone morphogenic protein-2[4] (BMP-2), enhance new bone formation and does not induce inflammatory tissue reactions *in vivo* [17, 18]. In the same way, permanent surface modification of PLLA with indomethacin[5] is able to minimize inflammatory problems in the host [5]. Recent studies have displayed common characteristic for ceramics and BG upon kinetic modification after implantation. The surface forms a biologically active HA [19], that, as stated before, improves the bonding interface and cell endothelization.

Deepening in the field of bioceramics and bioactive inorganic materials, mesoporous bioactive glass (MBG) has been showed to improve the mechanical properties of PLLA, hydroabsorption and degradability upon MBG content dependency, as well as promoting the attachment and proliferation of MC3T3-E1[6] cells as a result of large surface area to volume, while compensating the pH decrease from the acidic degradation of PLLA [9, 10, 18].

[3] Control group that does not receive any treatment.
[4] Protein capable of promoting bone production.
[5] Nonsteroidal anti-inflammatory drug used as pain killer.
[6] Cells derived from mouse skull's top, with potential for its usage as model in bone and tissue regeneration.

Similarly, the two most studied BG for tissue engineering are 45S5BG and 1393BG thanks to their good bioactivity and processability. An study from the University of Palermo [20], shows that the addition of 1393BG improves Young modulus while not affecting the morphology of the structure, unlike the 45S5BG one.

On the other hand, other authors have analysed the addition of inorganic oxides, such as zink oxide (ZnO) [21] or magnesium oxide (MgO) [8,11–14,21–27]. Several ways of introduction of these reinforcements can be employed into the composite-making, either in the form of whiskers (-*w*-) in a solution system with PLLA [25], particles themselves being incorporated in the injection molding [23] or even surface modified composites [12, 21, 24, 26, 27]. An illustration of these synthesis methods is shown in Figure 3.

They have shown a strong interface bonding between the matrix and the reinforcement, whilst being able to achieve a 170% increase of the yield strength [8] thanks to a correct load-bearing system. Moreover, the appearence of superficial roughness increases the toughness of the composite and the bioactivity is enhanced thanks to the Mg^{2+} ion, which promotes cell proliferation and osteogenic activity [11, 24], as shown in Figure 4.

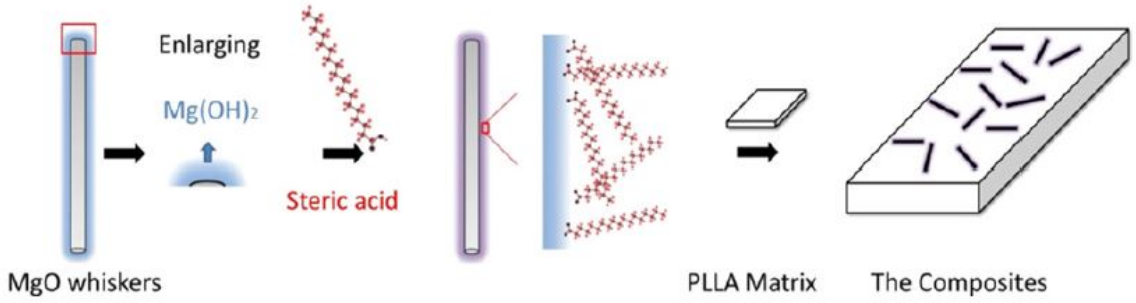


Figure 3. Schematic representation of composites reinforced with MgO whiskers and stearic acid surface modification [14].

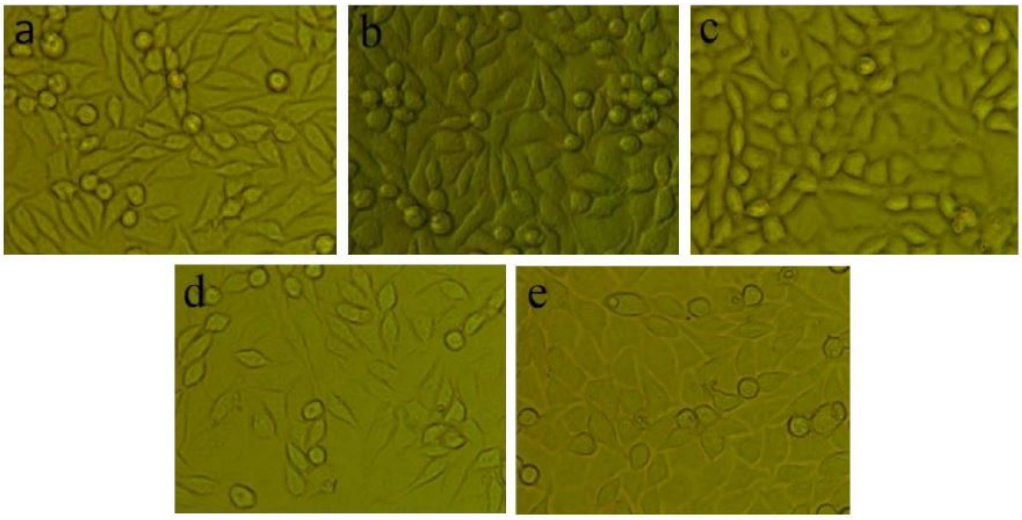


Figure 4. Cell morphology under leachate liquor for 2 days (x100): (**a**) Empty control, (**b**) PLLA, (**c**) PLLA 1% MgO-*w*, (**d**) PLLA 2% MgO-*w*, (**e**) PLLA 3% MgO-*w* [13].

Last but not least, recent studies conducted by researchers belonging to the Department of Mining-Metallurgy Engineering and Materials Science at the Faculty of Engineering in Bilbao [28] have concluded that with an addition of 10% wt. of $BaSO_4$ particles the base toughness of the polymer is multiplied 1647 times, achieving an elongation at fracture of 3338%, implying a net substantial improvement in medical applications where fragile fracture is not acceptable. Moreover, $BaSO_4$, together with MgO are radiopaque compounds, meaning they allow a correct monitoring of the use in the host with systems as X-rays, unavailable until now due to the lack of radiopacity of PLA.

Likewise, the methodology varies within the intended research; mainly, authors have used the phosphate buffer solution (PBS) to simulate body fluid conditions, whereas others have opted for computational analysis [8] or even the use of transcortical models in animals [17, 18, 24].

Thus, everything seems to point out that the addition of particles, mainly inorganic, to the polymeric matrix is the most outstanding strategy in order to improve its behaviour for biomedical applications. It is yet convenient to note that only a specific set of particle additions turns out to be benefitial, triggering a detrimental effect if a threshold is surpassed.

6 Risk Analysis

In this section, the awareness of the risks that have arisen throughout the project's development is raised. When the time to face the project comes, one must take into account the possible risks or unforeseen circumstances that may arise, triggering a delay in the overall length, an increase in the total costs or even any injury on the worker. Hereafter, such risks are exhibited, as well as what measures were taken to overcome them. More information regarding the initially identified risks and the action plan can be found in Appendix I: Identified Risks & Contingency Plan.

The main occurrence had been a bad batch of PLLA received. After having processed it, when it was time to test its mechanical properties in order to evaluate their proper state, it was discovered that the behaviour was not what it was supposed to be. This implied contacting with the supplier and having to wait a couple of weeks until a new correct batch was received, delaying the overall project, which is the reason for the duration of the processing in Section 8 being 4 weeks. This risk could not have been avoided, it was inherent to the product and until it reached the ascertaining of its properties nothing else could be done, except for accepting the risk of it happening.

At the same time, bad outcomes are intrinsic to any experimental project, and this was no exception. For the film and samples, the process of solvent casting had to be repeated several times. For such cases, the event was tried to be avoided being more cautious throughout the whole process, particularly in the critical activities. However, some outcomes were unavoidable, so they had to be passively accepted and repeated.

Last but not least, minor incidents of spilling of chemicals occurred, leaving no damaged people or sensitive equipment, although the chance of happening are always present and unpleasant. For that reason, to try to minimize this situations to zero, the courses "Good Practices in the Laboratory (I) and (II): Risk Prevention and Waste Management" had been simultaneously taken. Moreover, the first steps in the laboratory were taken under the surveillance of an experienced advisor, so as to properly learn, avoiding any issues.

This events pose a problem since they delay the project as a whole, while also increasing the expenses, due to the repetition of tests, synthesis of the samples and so on. It is for this reason that these events would need to be minimized as far as possible.

7 Methodology

This section aims to provide insight regarding the sequence of operations undergone to carry out the project. Beginning with the strategical search of related bibliography for a better understanding of the phenomena taking place, going through the description of the employed materials and methods, to end up with the description of the techniques followed for the analysis of the systems.

7.1 Search Strategy

A bibliographic search of the published literature was conducted on the following databases: Web of Science (WOS), ScienceDirect, PubMed, Europe PMC and Scopus. The following key words, separately or in combination were used for the main source of information: (PLLA AND MgO OR Polydopamine), (PLLA AND BG OR Bioactive Glass OR Polydopamine), (PLLA AND Implants OR Bone), (Composites AND Bone), (Bone Healing), (Polymer Analysis AND Thermal), (Polymer AND DSC), (Polymer AND TGA).

Within the identified literature, twelve studies about biodegradable polymers in similar applications were examined [1–6, 15–17, 19, 29, 30], while four additional ones were for the addition of BG [9, 10, 18, 20] and thirteen for the MgO particles [8, 11–14, 21–27, 31]. Also, articles from the Department of Mining-Metallurgy Engineering and Materials Science at the Faculty of Engineering in Bilbao with similar purposes were analysed [28, 32]. Finally, bibliography regarding fundamentals of processing of PLLA and thermal analysis of polymers and equipment was examined [7, 33–38].

7.2 Materials & Methods

The needed materials for the development of the project were the following:

- The raw PLLA was provided by PURAC BIOCHEM, whose datasheet indicated a molecular weight of 175,000 g/mol (175,000 Da), indicating it is a polymer with high molecular weight.
- MgO, $BaSO_4$ and dopamine (M_w=189.6 g/mol) were provided by Sigma Aldrich, whereas BG was provided by Novabone, for their mixing with pure PLLA at different proportions (Table 1).
- Trizma base buffer ($NH_2C(CH_2OH)_3$) and PBS were supplied by Sigma Aldrich, used for the PD coating preparation at 0.61 g/500mL and at 1 tablet every 200 mL of distilled water for the immersion fluid, respectively.
- Dichloromethane (CH_2Cl_2) (M_w=85 g/mol) with an assay greater than 99.95%, by Sigma Aldrich, to dissolve the processed probes into films with 20mL.

- Hydrochloric acid (HCl) 1M (M_w=36.5 g/mol) by Sigma Aldrich to adjust the pH level to the optimum (8.5) for the PD achievement.
- Chloroform ($CHCl_3$) (M_w=119 g/mol) stabilised with ethanol with an assay greater than 99.8%, by Sigma Aldrich, both for dissolving samples at 1mL every 10mg before GPC tests and throughout the GPC test (1mL/min flow).
- Distilled water (deionized) from iberiaAgua, for the PD synthesis and immersion fluid.

7.2.1 PD Coating

The MgO is a highly hygroscopic compound, and for such, it was stored inside a fridge in the laboratory. In total, 5 systems of MgO have been covered by polydopamine, whose contents have been 0.1, 0.5, 1, 5 and 10% in weight of MgO; in each injection molding a maximum of 12 grams can be inserted, thus, the total quantity of MgO required is 3.984g (regarding the percentages and wanting two set of samples). In case that some of the prepared samples lost quality or were considered of insufficient, it was decided to use 6g of MgO. The generic process to cover with PD is the following:

- Trizma base buffer ($NH_2C(CH_2OH)_3$) (0.61g) was added to 0.5L of distilled water under stirring in order to stabilise the pH (around 10.4)
- The ideal value of the pH so as to achieve PD is 8.5; for such, HCl (1M) was used in a controlled manner over a pH meter.
- 1g of dopamine was added and stirred for at least 30 minutes until the dopamine oxidized and acquired its characteristic black color.
- Finally, the corresponding quantities of the particles that were aimed to be covered were added and they were left stirring for the next 24 hours.

The necessary quantities in the process are displayed on Table 2.

Table 2. Quantities employed in the creation of MgOPD.

Element	Quantity (g/500 mL)	Employed quantity (g)
MgO	2	6
Dopamine	1	3
Trizma base buffer	0.61	1.83

The same process was followed for the coating of the BG samples. After the 24-hour stirring of the PD with the desired inorganic particles, the solution was filtered by using a Buchner funnel, a vacuum flask, a vacuum pump and 0.5 μm filtering paper. Next up, the filtered material needed to be dried for 3 days in a vacuum chamber at 50^{o}C to obtain

unmoisturized coated particles. Then, the particles were ready to be processed by injection molding.

7.2.2 Synthesis of Films & Samples

Firstly, the systems were synthesized by mixing and injection molding (see 7.3.1) in order to check the polymer was in good mechanical state by means of a tensile test (see 7.3.2) of some test pieces. After its checking, the remaining processed pieces were used for the synthesis of films by solvent casting.

For the films, it was desired that the samples had 100 μm of thickness. For it, since the final solution was poured into a petri, the diameter of it was measured, and noting an average density of PLLA of 1.24-1.25 g/cm^3 at 23°C, a simple calculation of the required mass of the system was carried out.

The required quantity for each petri was measured, then dissolved using 20mL CH_2Cl_2 into a vial and stirred until complete dissolution (around 30 minutes). Afterwards, the solution was poured into the petri and left in a well vented area to let the CH_2Cl_2 evaporate.

After 24h, the desired film is what was left in the petri, from which, with the aid of a cylindrical chisel and a hammer, 10mm round samples were extracted, ready to be subjected to the hydrolysis in PBS. The expected films are shown in Figure 5.

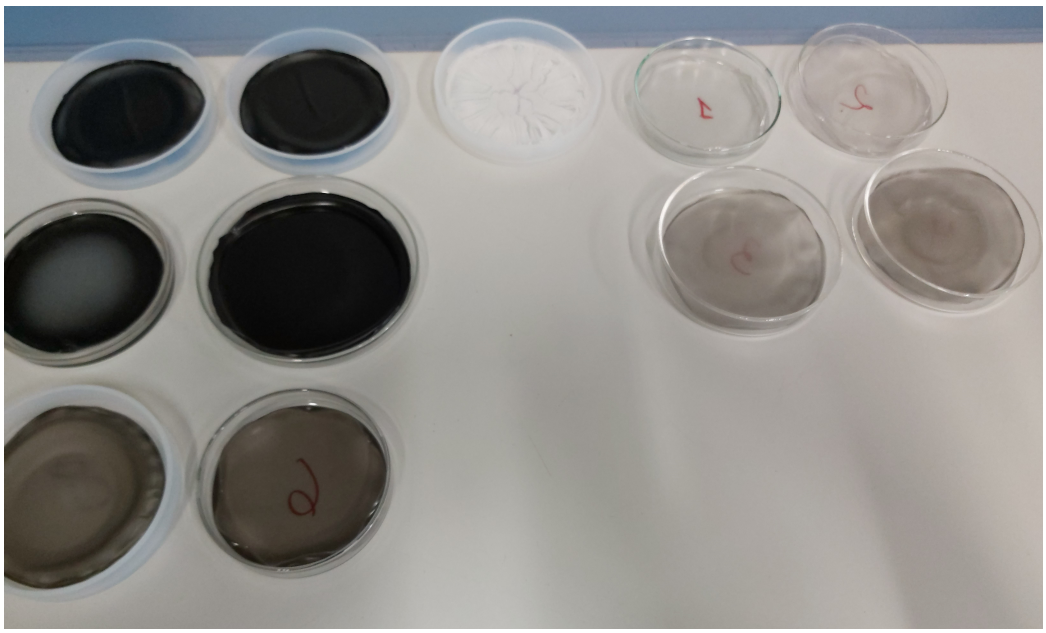


Figure 5. Films.

7.3 Techniques

The sequential order of operations and equipments used are described in this subsection, for a possible explanation of later on achieved uncharacteristic or unexpected behaviour of the sample. Hereafter, the schematic representation of the sequence of operations for the different systems is presented.

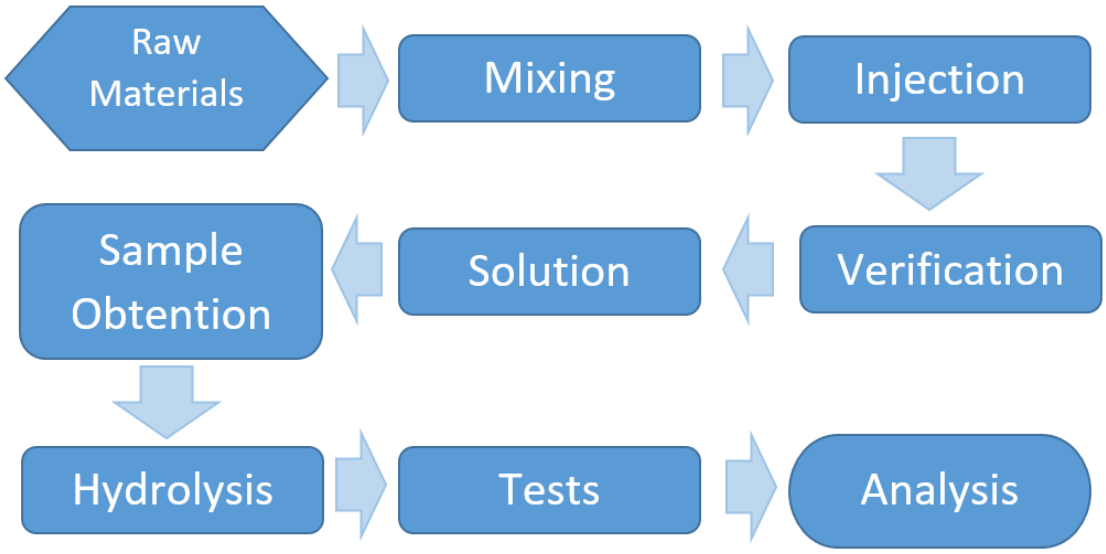


Figure 6. Sequence of operations.

In the case of PD coated samples, three additional steps have been needed to be carried out: PD coating preparation, filtration and drying of the coated particles. Henceforth, all the employed techniques in the sequence of operations will be described.

7.3.1 Processing

The mixing of the particles, PLLA and inorganic (coated with PD or not), was done thanks to a Vertical Xplore DSM model 5 mixing machine available in the basement of the university (Figure 7).

The mixing was done by inserting gradually the 12g of the system into a chamber at 210°C where two co-rotatory screws at 100 rpm were found. After having introduced all the particles, the inlet was sealed and the rotation speed was increased to 150 rpm for 2 minutes to ensure a good mixing (around 6000N of force); otherwise, agglomerations may have taken place. Meanwhile, the injection pistol was placed in the outlet of the mixing machine, ready to collect the viscous fluid that exited the mixer.

Figure 7. Vertical Xplore DSM mixing machine.

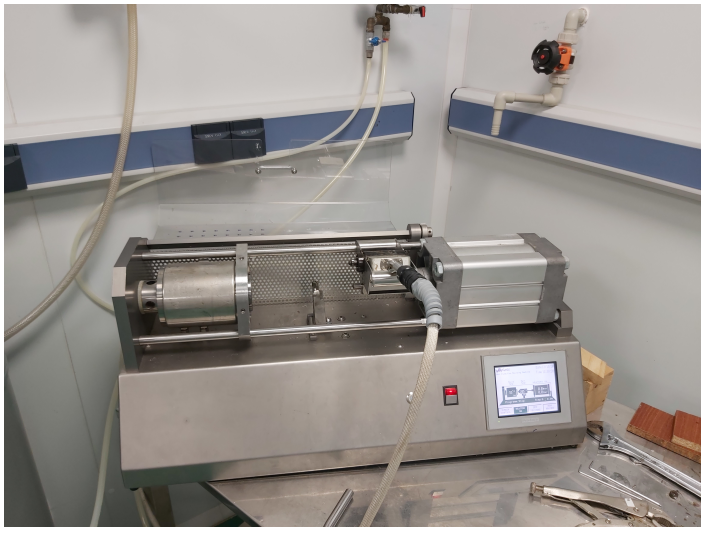


Figure 8. Micro Injection Moulding Machine 10cc.

The injection machine was a Micro Injection Moulding Machine, with a capacity of 10cc, (see Figure 8). The requirements for the injection were the following:

- Temperature of the pistol: 200°C
- Temperature of the mould: 45°C

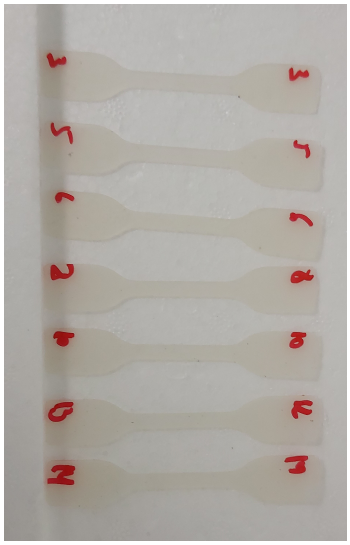
- Piston pressure: 16 bar during 8 seconds, staggered.

Afterwards, the test piece needed to be extracted quickly out of the mould, otherwise it would have got stuck when cooling and there was risk of breaking the sample. From each 12g mixing, between 6 and 8 test samples are extracted, so the process was repeated for each system in order to have enough material in the future without needing to head back to this stage.

After the extraction of all test samples, they were labelled with a number in a bag corresponding to the system, and the samples were subjected to measuring their tensile strength tests to verify that their mechanical properties corresponded to the theoretical ones. If any disturbance or incoherent behaviour would have been observed, either the process would have been needed to be repeated or the supplier contacted due to problems with the raw materials.

7.3.2 Mechanical Properties

Two dimensions of the test pieces were measured with a micrometer: neck thickness and head width. Afterwards, some representative samples out of the total system were chosen to be tested (Figure 9a). The testing machine was a Universal Tensile Testing Machine (Figure 9b), which used compressed air to restrain the clamps. The test was carried out based on a program with elongation control of 5mm/min until fracture.



(a) Samples.

(b) Tensile testing machine.

Figure 9. Tensile test.

From the analysis of the curves obtained, the Young modulus of the test samples could be calculated to verify it matched the theoretical one of PLLA in order to discard any future behaviour problems due to any initial mismatch. The initially obtained results for uncoated PLLA-MgO systems can be seen below.

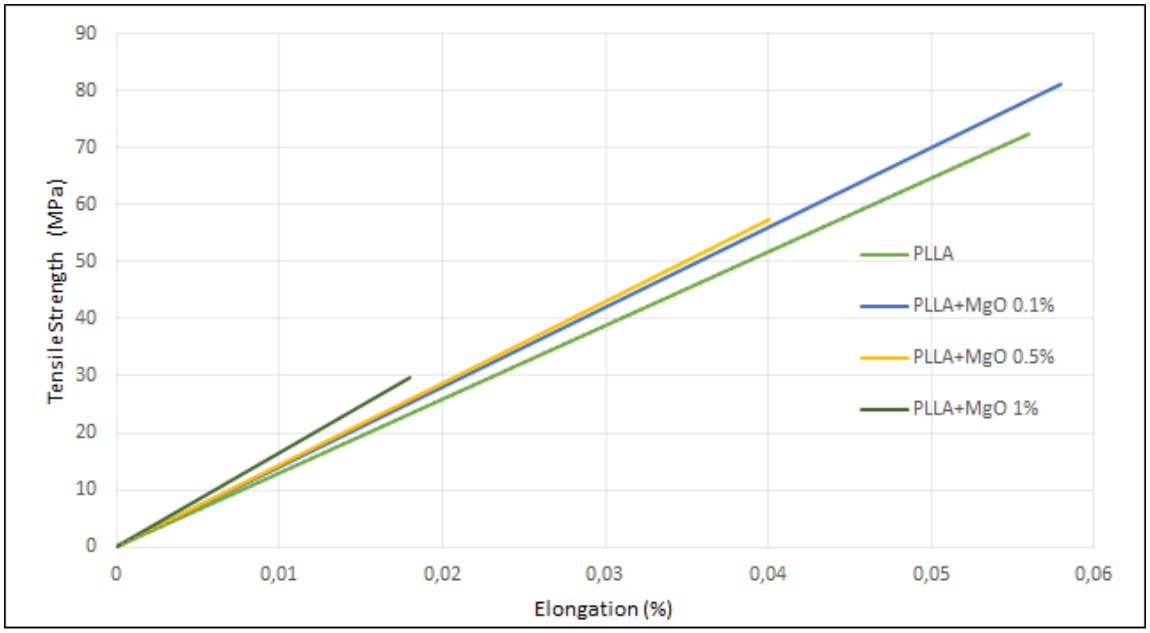


Figure 10. Tensile test results.

Table 3. Tensile test results for uncoated PLLA systems.

System	Young modulus (GPa)	Elongation at break (%)
PLLA	1.3	0.056
PLLA+MgO 0.1%	1.4	0.058
PLLA+MgO 0.5%	1.4	0.040
PLLA+MgO 1%	1.6	0.018

7.3.3 Differential Scanning Calorimetry (DSC)

DSC is the technique in which the heat flow rate difference into a substance is measured against a reference as a function of temperature [34]. The most important variable in this machine is the temperature; it is the only measured quantity. The remaining variables are calculated from the changes in temperature between the sample and the reference.

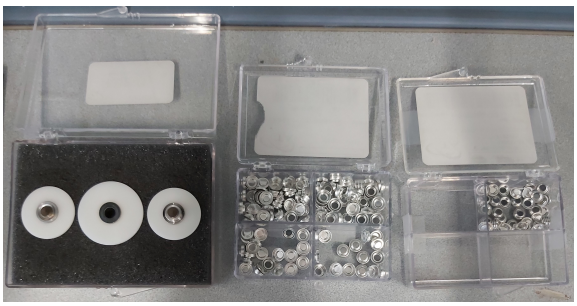
Both the purge gas and encapsulation of the sample are vital: the DSC cells are continuously purged with nitrogen (N_2), inert gas, in order to prevent condensation of water on the cell and sample at subambient temperatures, to carry away possible present contaminants, to prevent oxidation at high temperatures or to increase heat transfer.

Figure 11. DSC machine.

The program of the DSC was established as follows:

- Heating from -20°C to 200°C at 20°C/min to see rightafter hydrolysis behaviour.
- Cooling to -20°C.
- Reheating from -20°C to 200°C at 20°C/min to see the behaviour of the polymer after having erased its thermal history during the previous scan.

On the other hand, from each sample, 6 mg were encapsulated into hermetically sealed pans to withstand the pressure for a convenient heat transfer. This encapsulation was done by means of a punching station, as shown in Figure 12. The pan and lid were first punched together using the die on the left, and then the one of the right to provide an adequate sealing of the capsule, ensuring it would not break during the DSC test.



(a) Pans and lids.

(b) Punching station.

Figure 12. Encapsulation.

From this tests, mainly, the thermal transitions of the composite are visualized, while at the same time, it is possible to characterize completely its behaviour thanks to other variables such as the melting temperature (T_m), enthalpy (H) and so on.

7.3.4 Thermogravimetric Analysis (TGA)

TGA is a technique where the mass of a polymer is measured as a function of temperature while the samples is under a controlled atmosphere of inert gas (N_2) [34]. This technique provides information about components present in the polymer, which degrade at different temperatures. Thus, it yields information on the composition, cure extension and thermal stability of the polymer.

The program used for the obtention of curves was the following:

- Equilibrate at 25°C.
- Heating to 500°C at 10°C/min.
- Cool and equilibrate to 100°C.

Unlike the DSC, in the TGA there was no need of encapsulating the polymer. Instead, a circular sample was cut so as to make it easier to introduce it in an alumina capsule previously tared to provide the correct weight of the sample introduced. The equipment is sensitive to several physical factors, so it is important to be cautious when using not only this, but all 3 equipments (DSC, TGA and GPC).

Figure 13. TGA machine

7.3.5 Hydrolytic Degradation

Previous to the immersion of the samples, the fluid had to be prepared. For that, phosphate buffer saline (PBS) was used in a proportion of 1 tablet every 200mL of distilled water. Taking into account that the samples were submerged in Falcon tubes, whose capacity is 15mL, and that the different time periods were: 0, 7, 14, 21, 28 days and 2 and 3 months, and that for each time period (except for the 0 day samples) 3 Falcon of each of the 22 systems under study were prepared, 396 Falcon tubes were needed. Thus, 5.94 L of distilled water with PBS.

PBS was used due to its osmolarity and ion concentrations being very similar to the extracellular fluid of mammal. The phosphate group is responsible for maintaining the pH level neutral, around 7.4, and it is not toxic for cells. Thus, since the fluid simulates

corporal conditions, the Falcon tubes were introduced in an oven at 37°C to completely establish normal *in vitro* conditions. Each sample was weighed before the hydrolysis once and after each period twice: both wet and dry, to establish the weight loss and the water absorption capacity.

For the characterization of the hydrolytic degradation, mainly a GPC equipment was used, for the analysis of the amount of chains that were detected. On the other hand, the weight loss of the systems after the immersion times was characterized as well, by means of a precision scale.

7.3.6 Gel Permeation Chromatography (GPC)

GPC is a technique in which polymers in the presence of high purity diluents have a fine-mesh form suitable for its distribution in chromatographic columns [38]. Columns are capable of separating high molecular weight chains from the low molecular weight ones, providing a qualitative image of the topology of the polymer. The columns firstly trap high molecular weight chains into their gaps, giving a Gaussian distribution.

In order to conduct a GPC test, the polymer had to be previously dissolved in high-purity chloroform ($CHCl_3$) and filtered with a 0.2 μm filter since the equipment is very sensitive to non pure, big particles.

Figure 14. GPC machine.

For its preparation, 10mg of each system were dissolved with 1mL of $CHCl_3$ and then filtered in the table next to the GPC system. Previosuly to the injection (for which a glass needle was used), it is vital to ensure that no bubbles were left inside the needle. Otherwise, the instrumentation undergoes problems. Furthermore, a continuous flow (1mL/min) of high purity $CHCl_3$ is constanly needed for the correct flow of the polymer and data processing.

7.3.7 Weight Loss Characterization

As previously explained, the hydrolytic degradation of the polymer and its decomposition was characterized, together with the GPC tests, by measuring the weight loss of the different systems. After their extraction from PBS, they were weighed while still wet, to determine their capacity of water absorption. Then, they were placed into an oven for 3 days under vacuum to dry the samples completely. Finally, they were weighed again, with a precision scale in order to accurately determine the weight loss suffered by the decoupling of the adjacent chains after their progressive shortening.

8 Planning

This section gathers the planning of the project. On the basis of the WBS (see Figure 1), taking into account the initial bibliography-gathering phase and the experimental procedure with its corresponding tests and characterization, a list containing the primary activities is materialized.

8.1 Primary Phases

1. **Bibliographic Research Phase**
   This initial phase has covered almost the entire duration of the project, since one does not stop seeking for additional information at any point, looking for a deeper comprehension of the phenomena taking place. Its duration has been of 185 days, until the characterization of all systems, as well as the conclusions, had already been finished and the report was going to be carried out.

2. **Sample Preparation**
   It covers the whole process of preparing the necessary materials for the sample obtention; from the preparation of the coated particles to their solution and casting into films for sample extraction. A milestone was established for the achievement of the samples, ready for immersion. This phase finished on 1^{st} December, taking 53 days for its completion.

3. **Hydrolysis**
   This phase has lasted long since the systems have been immersed in PBS up to 3 months. After extraction in the corresponding time lapses, several tests aforementioned were conducted to each system, leading to the last phase, the analysis of results. A milestone had been established for when all the hydrolytic degradation process finished.

4. **Analysis of Results**
   This phase has been the longest lasting phase after the bibliographic research, since it started early on, with the characterization of samples that had not undergone hydrolysis (0 day samples), and lasted 2 more months than the hydrolysis. This was due to the ingent amount of data that needed to be processed. A milestone was established to check its completion before extracting the conclusions of the project and writing this report supporting all the work done.

According to the Gantt diagram in Figure 15, the project's duration has been of 200 days. It is possible to identify the critical tasks of the project, whose local delay might have triggered an overall delay in the whole process. Being such, it is clearly observable how vital the sample preparation was: if the major risk, as established (in Appendix I: Identified Risks & Contingency Plan) to be the possibility of lockdown, had occurred, preventing the samples from being prepared, the whole project would have been vastly delayed. And perhaps it would not have been finished in the current dates, taking into account that 3 months were necessary to compel with the proposed objectives.

Nonetheless, the most important phase has been the correct characterization of the systems, by the processing of the DSC, TGA and GPC data throughout the whole degradation, since it does not only influence the duration of the project, but also the quality of it. In the next page, a table showing each phase and their subordinate tasks is presented, also visible in the Gantt diagram.

Table 4. Phases and tasks present in the planning of the project.

WBS	TASK NAME	DURATION	START	END
Phase I	**Bibliographic Research**	**37 weeks**	**17 Sep. 2020**	**02 June 2021**
Phase II	**Sample Preparation**	**11 weeks**	**17 Sep. 2020**	**02 Dec. 2020**
Task 2.1	Coating Preparation	1 week	17 Sep. 2020	21 Sep. 2020
Task 2.2	Filtering & Drying	1 week	22 Sep. 2020	24 Sep. 2020
Task 2.3	Processing	4 weeks	25 Sep. 2020	06 Oct. 2020
Task 2.4	Test	1 day	09 Oct. 2020	09 Oct. 2020
Task 2.5	Film & Sample Obtention	3 weeks	10 Nov. 2020	02 Dec. 2020
Milestone 1	Preparation Completed			
Phase III	**Hydrolysis**	**18 weeks**	**03 Dec. 2020**	**30 Mar. 2021**
Task 3.1	7 days	7 weeks	09 Dec. 2020	16 Feb. 2021
Subtask 3.1.1	Extraction & Wet weighing	1 day	09 Dec. 2020	09 Dec. 2020
Subtask 3.1.2	Dry Weighing	2 days	10 Dec. 2020	14 Dec. 2020
Subtask 3.1.3	DSC	6 weeks	14 Dec. 2020	12 Feb. 2021
Subtask 3.1.4	TGA	5 weeks	14 Dec. 2020	04 Feb. 2021
Subtask 3.1.5	GPC	1 week	11 Feb. 2021	16 Feb. 2021
Task 3.2*	14 days	6 weeks	16 Dec. 2020	19 Feb. 2021
Task 3.3*	21 days	8 weeks	23 Dec. 2020	12 Mar. 2021
Task 3.4*	28 days	9 weeks	30 Dec. 2020	30 Mar. 2021
Task 3.5*	2 months	4 weeks	09 Feb. 2021	19 Mar. 2021
Task 3.5*	3 months	3 weeks	03 Mar. 2021	30 Mar. 2021
Task 3.6**	0 days	7 weeks	03 Dec. 2020	11 Feb. 2021
Milestone 2	Hydrolysis Completed			
Phase IV	**Analysis of Results**	**29 weeks**	**03 Dec. 2020**	**24 June 2021**
Task 4.1	Characterization	17 weeks	03 Dec. 2020	20 Mar. 2021
Milestone 3	Characterization Completed			
Task 4.2	Conclusions	2 weeks	20 May 2021	03 June 2021
Task 4.3	Report	2 weeks	03 June 2021	24 June 2021
Milestone 4	Report Completed			

*: Same subtasks as Task 3.1. **: Same subtasks as Task 3.1 except for Subtask 3.1.1.

8.2 Gantt Diagram

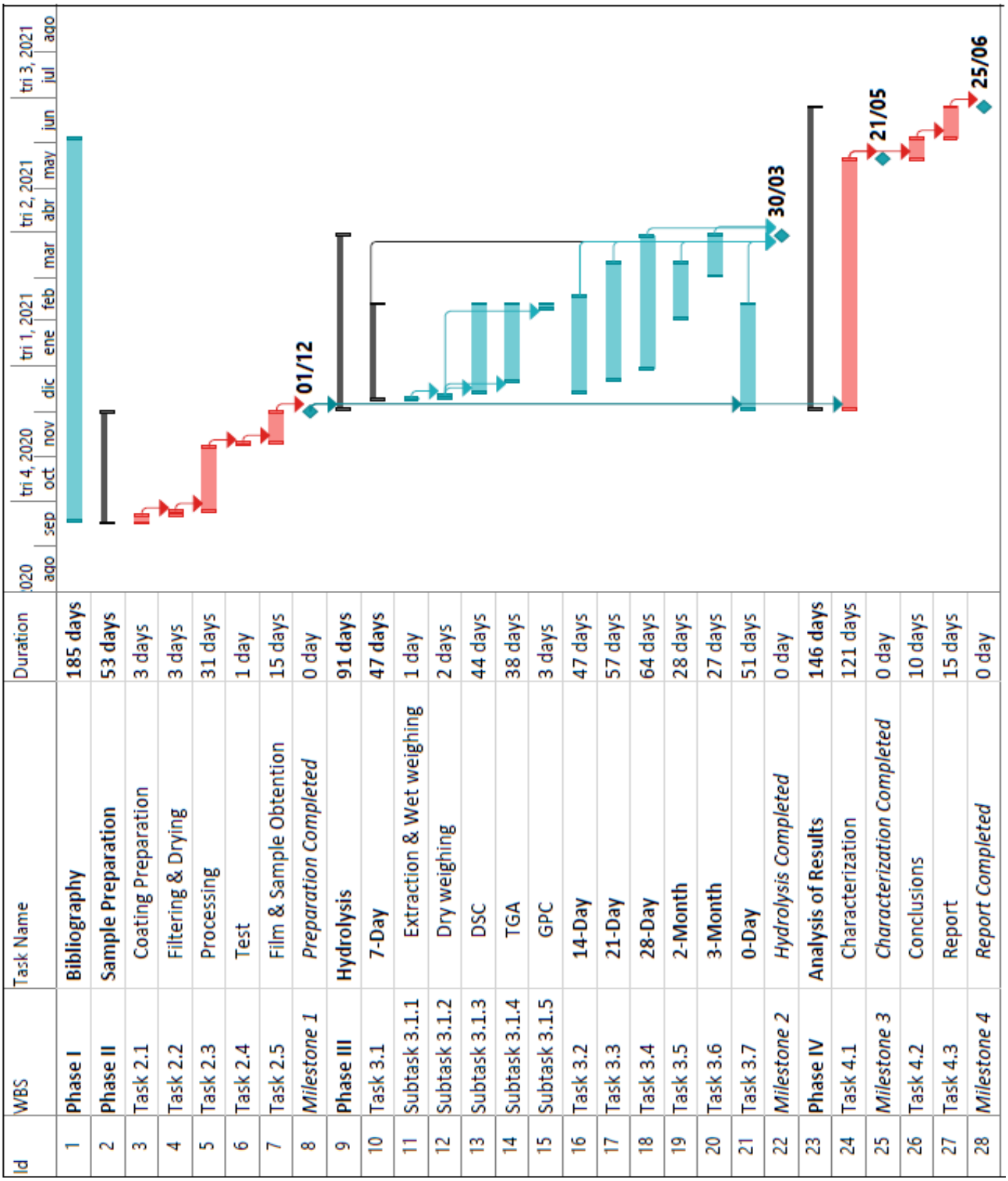


Figure 15. Gantt diagram.

9 Results & Discussion

This section aims to present and properly discuss the behaviour of the different systems (Table 1) that have undergone the specified hydrolytic degradation for different time periods, in order to determine whether the addition of inorganic particles affects the polymeric matrix or not, and qualitative and quantitatively determining how much.

For the sake of organization, this section will be divided into several segments to clearly approach each effect. This will allow a better explanation of the phenomena taking place that will later on be used to justify what type of system is more adequate regarding its specific application.

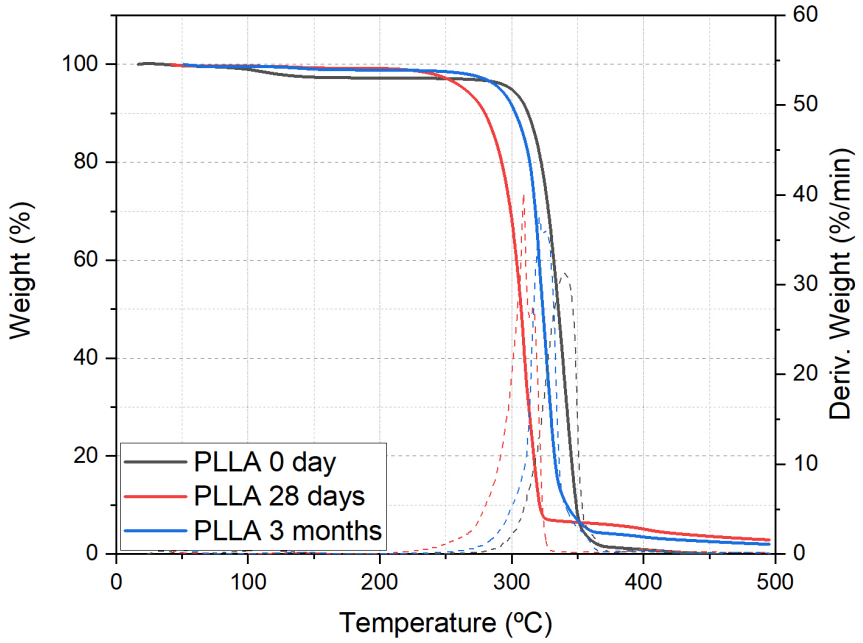
Main variations and changes will be commented on. However, so as not to overwhelm this document with all the processed data in this section, complementary information concerning the specific systems not commented here shall be found in the different appendixes, if it is worth denoting it.

9.1 PLLA

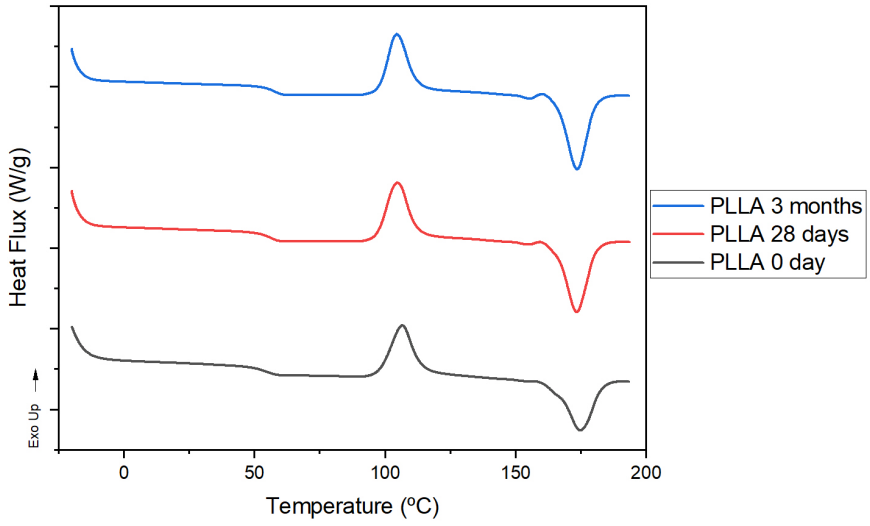
Pure PLLA will be the reference system for comparison. The rest of the systems will be proyected over this one, to clearly see the changes that have taken place regarding the hydrolytic and thermal behaviour.

First of all, its degradation will be presented and commented, for a basic understanding of its behaviour. Next figures will present DSC and TGA tests carried out for days 0, 28 and 3 months, to visualize major variations over time. Together with its thermal degradation, the molecular topology of the polymer, the amount of chains and their molecular weight will be displayed, to possibly justify any visible changes.

From Figure 16, it can be easily seen that when the sample is subjected to hydrolytic degradation, its thermal stability decreases, as can be seen in Figure 16a, where after weeks of degradation the sample degrades at lower temperatures and higher rates. Nonetheless, a certain recovery is appreciated after 3 months. And regarding the DSC tests, no significant changes can be seen, apart from denoting small exothermic peaks before melting, due to a complex reorganization of the molecular structure [33]. PLLA is characterized for having its T_g at 60-65°C and a melting temperature at 165-180°C, while a recrystallization peak appears at 100-110°C.



(a) TGA PLLA.



(b) DSC PLLA.

Figure 16. Thermal analysis of PLLA.

In all cases it cannot be seen any important difference for these times of degradation, since PLLA takes a long time to degrade. However, these DSC curves correspond to the second scanning, for which we previously had heated and cooled the polymer in order to erase any thermal history it may have. The first scanning displays what the actual state of the polymer was, as shown in Figure 17.

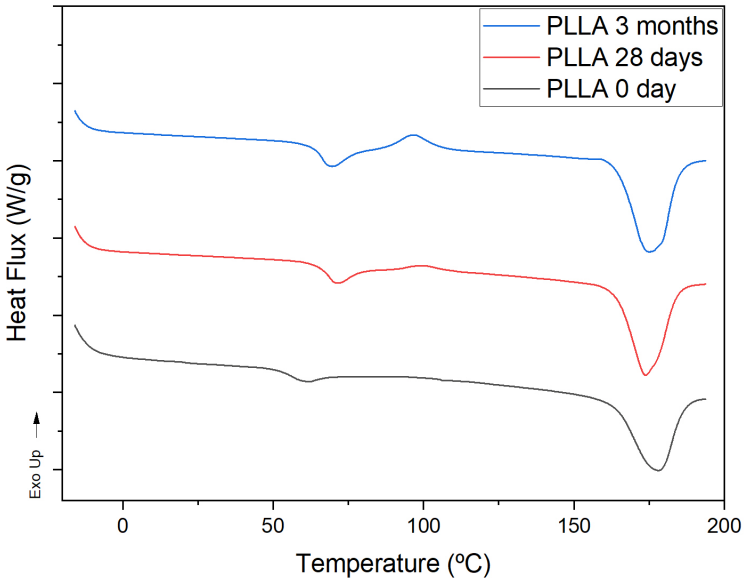


Figure 17. DSC PLLA (1^{st} sweep).

Additionally, the distribution of molecular chains is extracted by means of GPC tests, from where it can be found that the chains have 113,900 and 40,708 g/mol of M_w and M_n for day 0 and 108,049 and 42,860 g/mol for 3 months, respectively. This would mean that when degrading, larger chains begin to decompose, forming shorter chains, more easily crystallized and whose crystals demand less energy to rearrange themselves. That is why we should be expecting displacements of the thermal curves towards the left, indicating faster decomposition, and lower thermal stability after having undergone hydrolytic degradation. In biodegradable polyesters, the main mechanism responsible for the molecular weight loss is the random chain scission [39].

9.2 Influence of MgO

The first systems to be implemented are MgO systems, at 0.1, 0.5 and 1% in weight content. Although higher content of MgO has proved to substantially improve its mechanical characteristics [8,14,22,23], it was not feasible to conduct their processing without any coatings.

To begin with, as stated, it is known that MgO helps the matrix bear larger loads more easily by increasing the overall strength. However, it degrades faster the matrix as can be suggested in Figure 18, by the displacement of the melting well towards the left. The more content of MgO, the more degraded the matrix will be, that is why the worst case scenario is shown (complementary data can be found in Appendix III: Additional MgO systems).

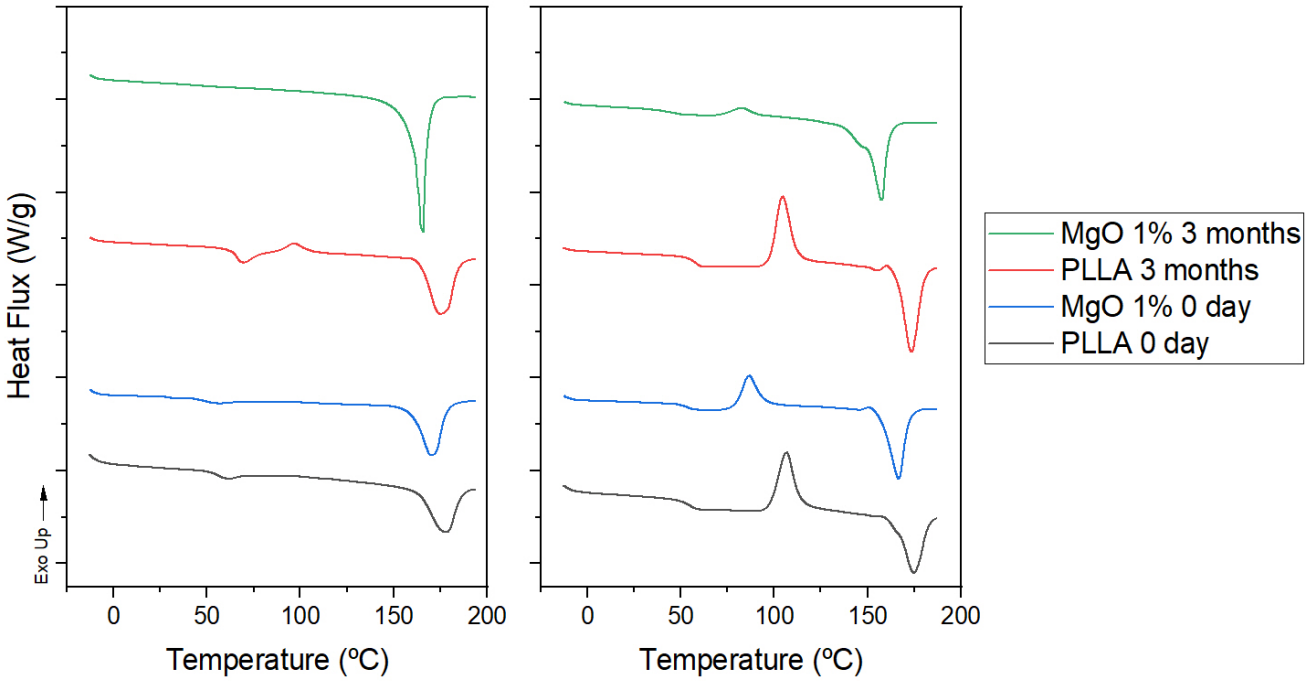


Figure 18. DSC comparison of pure PLLA with MgO 1%. Left: 1^{st} scan (actual state of the polymer). Right: 2^{nd} scan.

The most notable change with the addition of MgO particles is the faster degradation of the matrix (reported by several authors based on weight loss [11, 27]), as all temperatures are displaced further to the left, indicating less heat and energy required for its decomposition. Moreover, the polymer with 1% MgO after 3 month degradation does not undergo any glass transition process (straight line in the 1^{st} scan), indicating that the polymer is mainly crystalline with overall shorter chains, while it has some capability, as low as it might be, of doing it according to the 2^{nd} scan. This is very likely due to the conversion of larger chains into smaller ones, which require less energy for their rearrangement, and have greater capacity of creating crystals, thus, displaces the curves towards the left (Table 5).

Table 5. Chain distributions of MgO 1% and pure PLLA.

System	Time	M_w (g/mol)	M_n (g/mol)
MgO 1%	0 day	53,483	23,739
	3 months	18,110	6,646
PLLA	0 day	113,900	40,708
	3 months	108,049	42,860

Table 5 displays numerically the difference in molecular weights between the systems, from where it is possible to see that the mere addition of MgO in the processing already degrades the matrix, without even undergoing any hydrolysis (see Figure 19).

This information supports the aforementioned explanation that shorter chains or chains with lower M_w have greater mobility and crystallize more easily, with less energy requirements, degrading faster. In order to verify the higher degradation rate due to the addition of the MgO 1%, the TGA analysis is displayed (Figure 19). The addition of MgO is making the system degrade faster thermally.

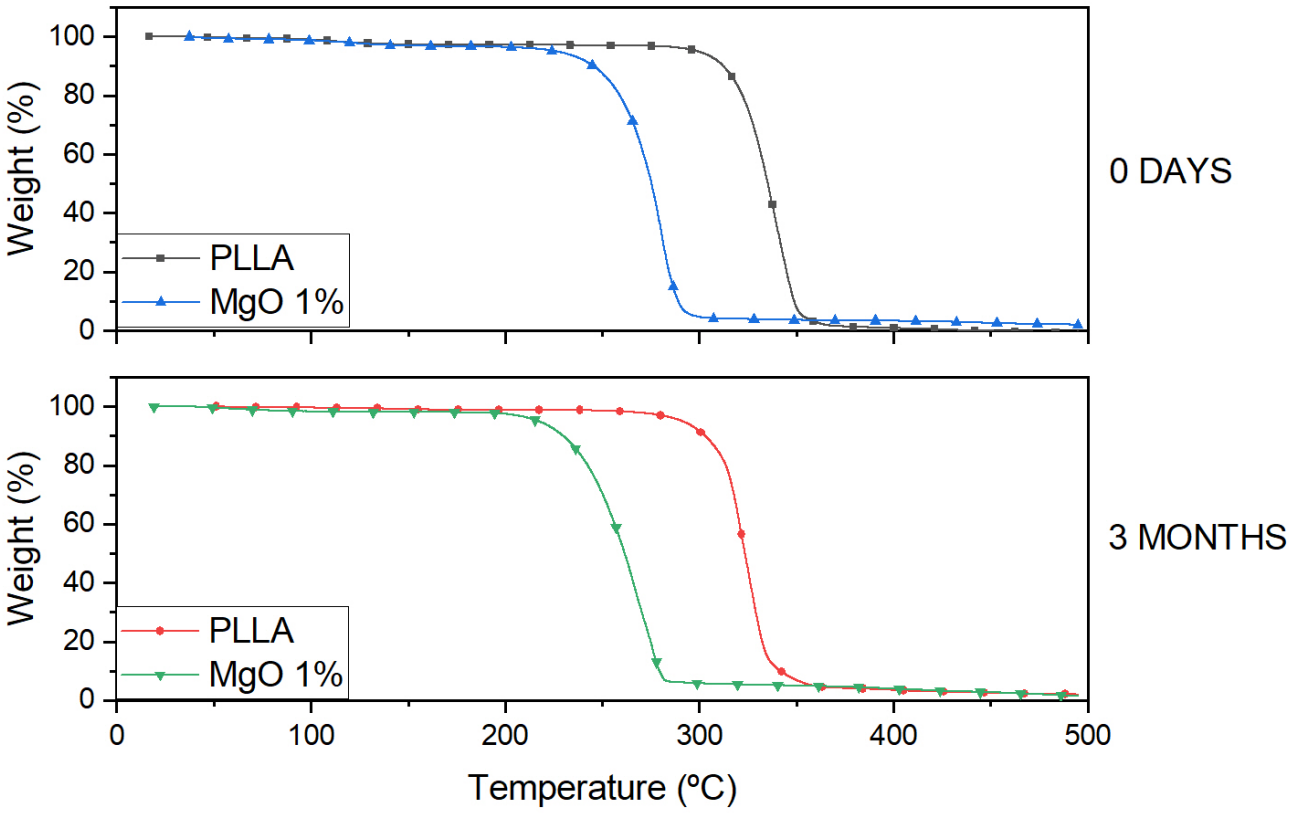


Figure 19. TGA comparison of pure PLLA with MgO 1%.

Regarding Figure 20, it can be seen how, as a whole, the MgO 1% polymer loses weight at a higher rate than pure PLLA. Nonetheless, there is not much weight loss record due to the relatively low degradation periods for these systems, since PLLA takes up to 2 years to fully degrade. It is obvious that this effects are proportional to the quantity of MgO added as reinforcements. Lesser weight percentage of MgO will make the matrix degrade slower (although faster than PLLA) with a cost implied of lower mechanical resistance.

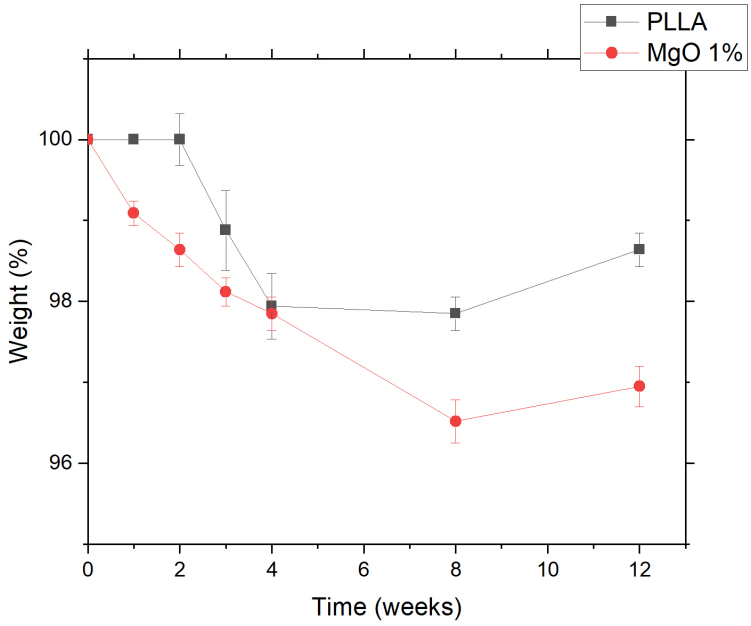


Figure 20. Weight loss comparison of pure PLLA with MgO 1%.

9.3 Influence of coated MgO

For greater percentages without compromising the stability during the processing of the systems, a coating of PD was used. As PD acts as a coating, carrying inside MgO particles, the toughness given to the matrix will be greater than in previous cases, since more MgO is able to be introduced as reinforcement. What has to be determined is whether the coating acts as a retardant or a "catalyst" (if it would accelerate) in the degradation of the system in comparison with pure PLLA and without any coating.

Figure 21 clearly shows that a coating of PD further protects the beginning of its decomposition, although greater hydrolytic degradation than the uncoated sample can be seen. Nonetheless, the DSC and GPC tests should be checked in order to know its physicochemical properties, if it has crystallized or not, and how are the chains of the polymer.

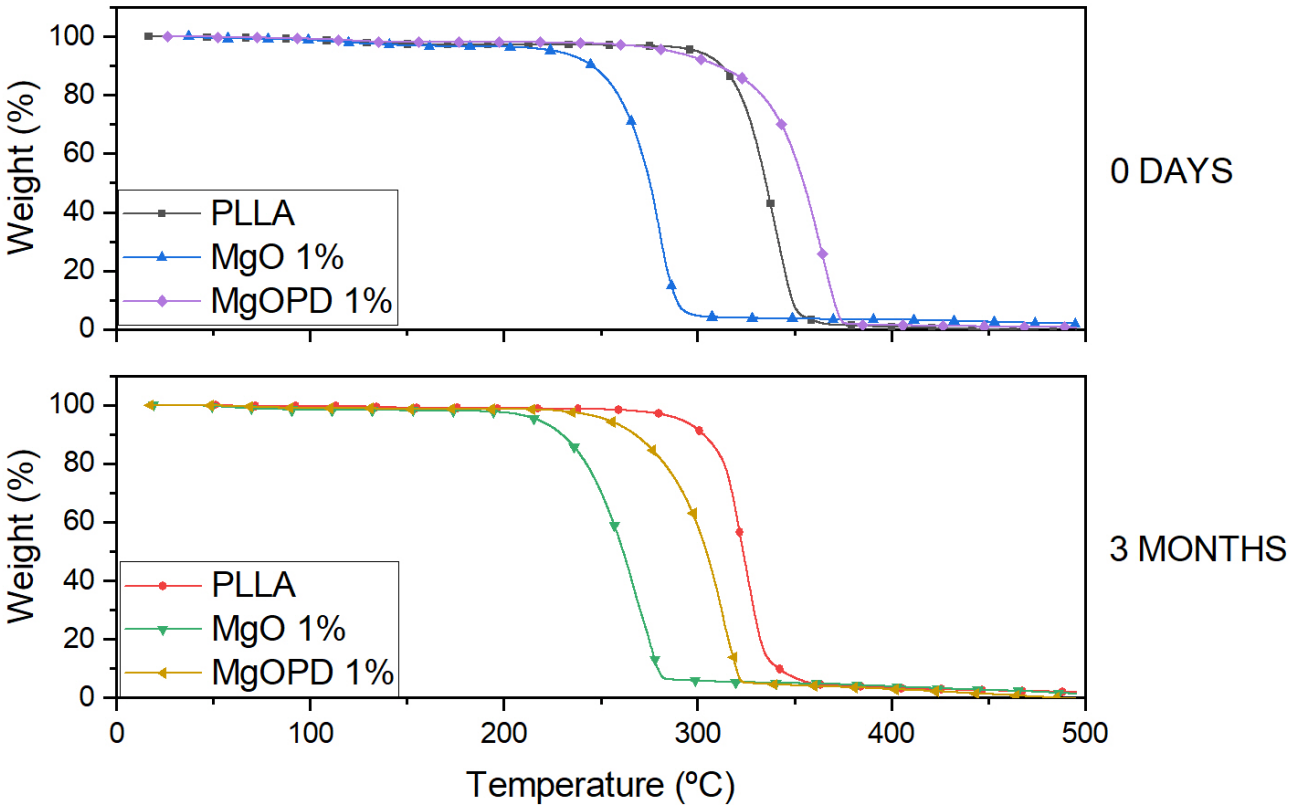


Figure 21. TGA comparison of pure PLLA vs MgO 1% vs MgOPD 1%.

Figure 22 shows how after hydrolytic degradation, the state at which the composite is left is less degraded than the uncoated sample. Moreover, when deleting its thermal history, it reveals that the coating substantially improves its capacity of crystallizing, and how it holds back the T_m, even better than pure PLLA. The numerical data can be extracted from the following table.

Table 6. Characteristic values of the DSC test for PLLA, MgO 1% and MgOPD 1% at day 0 and after 3 months.

	PLLA				MgO 1%				MgOPD 1%			
	1^{st} scan		2^{nd} scan		1^{st} scan		2^{nd} scan		1^{st} scan		2^{nd} scan	
	0 Day	3 Months	0 Day	3 Months	0 Day	3 Months	0 Day	3 Months	0 Day	3 Months	0 Day	3 Months
T_g (°C)	53.17	66.00	53.67	58.50	47.30	41.11	52.94	44.82	54.03	62.69	51.15	58.49
T_c (°C)	-	87.18	97.81	98.05	-	-	79.44	71.24	87.41	-	100.20	89.61
T_m (°C)	162.06	164.48	165.27	166.19	159.13	143.88	157.41	148.86	162.73	163.34	163.92	164.83

From this table it can be seen that the T_m of the coated sample remains somehow equal to that of the pure PLLA, while the uncoated one melts at a lower temperature. It is also worth noting that similarly happens with the T_c; for the coated sample a crystallization peak is found at a higher temperature than that of the reference.

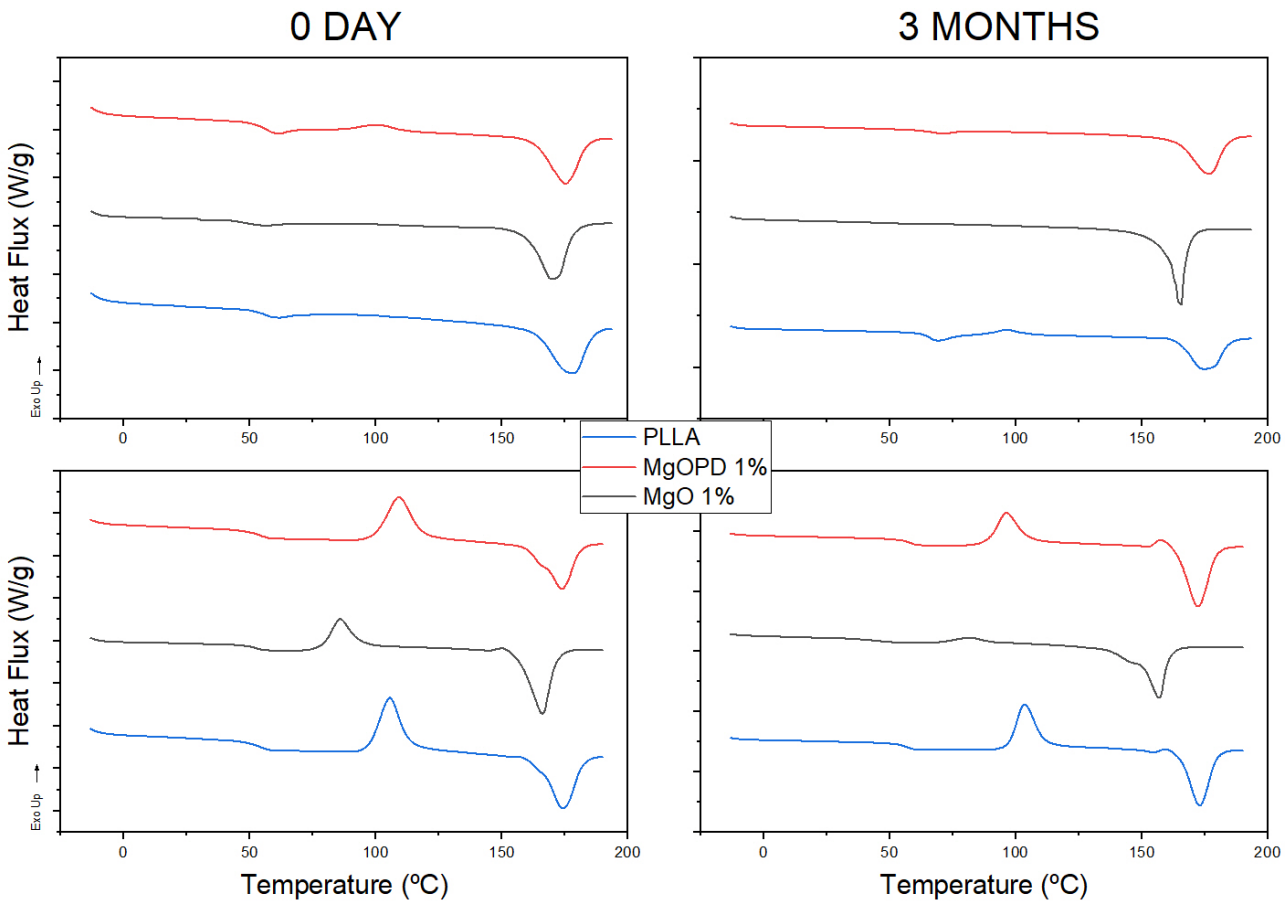


Figure 22. DSC comparison of pure PLLA vs MgO 1% vs MgOPD 1%. Top: 1^{st} scan. Bottom: 2^{nd} scan.

Figure 23 shows the weight loss after the different degradation periods between PLLA, MgO at 1% and MgOPD at 1 and 10%. It is observable how the PD coating initially stabilizes more than no coating (except for a rapid weight loss between 3-4 weeks) and that the more MgOPD content, the less weight is lost at initial weeks.

It would have been more interesting conducting such a register if the systems had undergone longer periods of hydrolysis, possibly being able to see further changes in the weight loss, because with the data registered up to 3 months, no greater weight loss than 5% is appreciated in any of the systems.

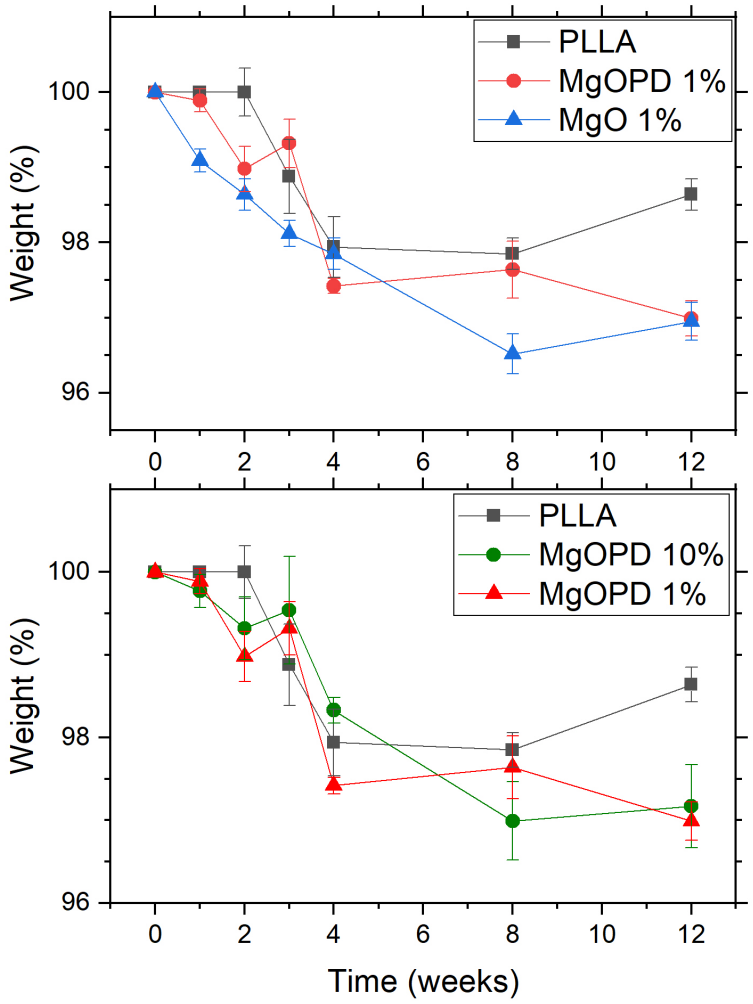


Figure 23. Weight loss comparison of pure PLLA with MgO 1% and MgOPD 1 and 10%.

Thus, from these figures it can be concluded the the coating delays the degradation, it takes longer time and greater energy to degrade a sample coated with PD. Moreover, as it allows to introduce more MgO as reinforcements, the mechanical properties would be greatly improved as well.

9.4 Influence of $BaSO_4$

For this section, particles of $BaSO_4$ have been introduced, together with MgO and coated MgO, as well as with the BG systems, which will be commented later on.

$BaSO_4$ is known to highly improve the toughness and elongation at break of PLLA [28]. It has been introduced with other inorganic particles for this reason, so as to take advantage of the synergy between the different compounds: MgO on the one side improves

mechanical properties and promotes cell proliferation thanks to the Mg^{2+} ion released in the hydrolysis, whereas BG is known to form HA and enhances osteogenic activity while avoiding any possible inflammatory reaction.

However, another important factor when designing implants is the capacity to be able to track and monitor the implant *in vivo*, in the host patient. For this reason, radiopacity is looked for with great interest, since with a simple X-rays device, the progress could be easily monitored. Such desired radiopacity can be achieved by 3 ways with the systems in this study:

1. Introducing the highest amount possible of $BaSO_4$ into the matrix, since $BaSO_4$ is radiopaque.
2. Introducing MgOPD 10%, since MgO is radiopaque as well.
3. Mixing both compounds (MgO and $BaSO_4$) in different proportions, 1/9, 3/7 or 5/5%, respectively, in order to achieve maximum radiopaque charge.

The contents proposed are the highest as possible since MgO at 1% would also provide radiopacity, but perhaps not enough for a correct tracking. And having such other systems in hand, that characteristic shall not be omitted. So, firstly, how does this compound affect the overall stability of the polymer will be commented, to be able to extract ideas that lead to and may justify its introduction in specific systems, depending on the desired customization.

Regarding how does it withstand the degradation as a function of temperature, in Figure 24, it is noticeable how little does the thermal degradation of the polymer vary with the addition of $BaSO_4$, either at 5% or 9%. Moreover, the distribution of their chains is somehow similar to that of the MgO 1%, despite the addition of $BaSO_4$. This lower amount of larger chains would explain the already crystalline behaviour of these systems, without a noticeable amorphous region (Figure 25).

Attending to the Figure 25, it can be stated that an increase of $BaSO_4$ does not really affect the matrix, it degrades somehow similar to that systems of MgO 1% without it. Together with the TGA test (Figure 24), it is observed that the degradation behaviour does not change. Similarly, the weight loss over time remains practically unaffected by the addition of $BaSO_4$. Thus, this system provides toughness and greater elongation at break, as well as an appropriate radiopacity for the tracking of the device, at no expense of a faster degradation than that of MgO.

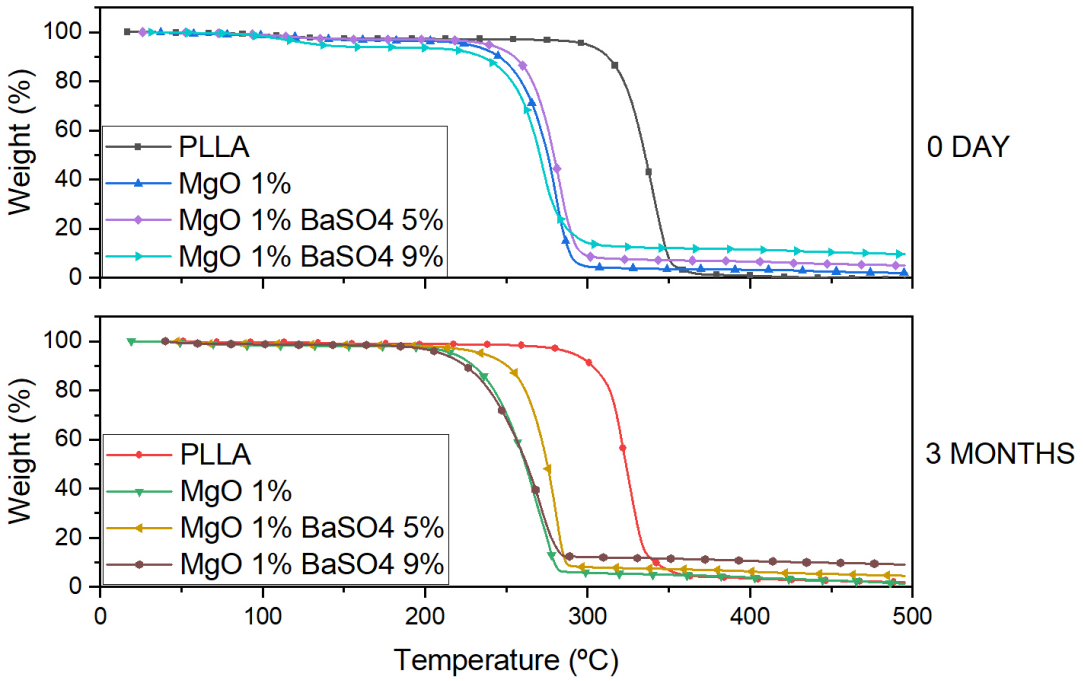


Figure 24. TGA of pure PLLA vs MgO 1% vs MgO 1% $BaSO_4$ 5% and 9% before and after degradation.

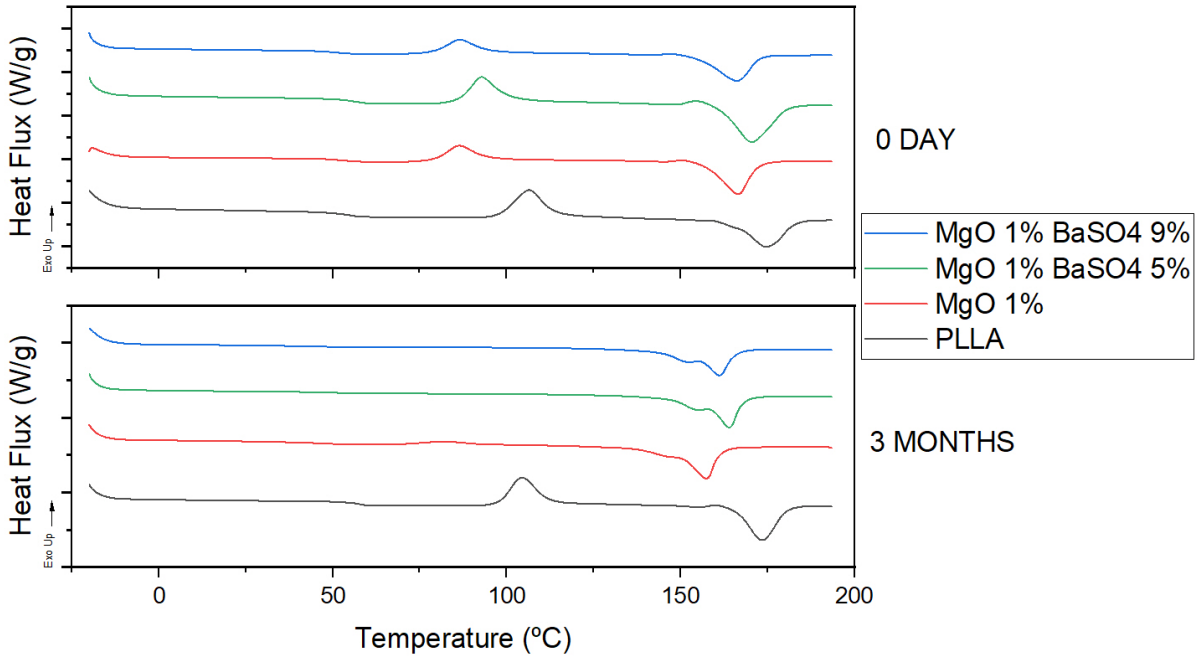


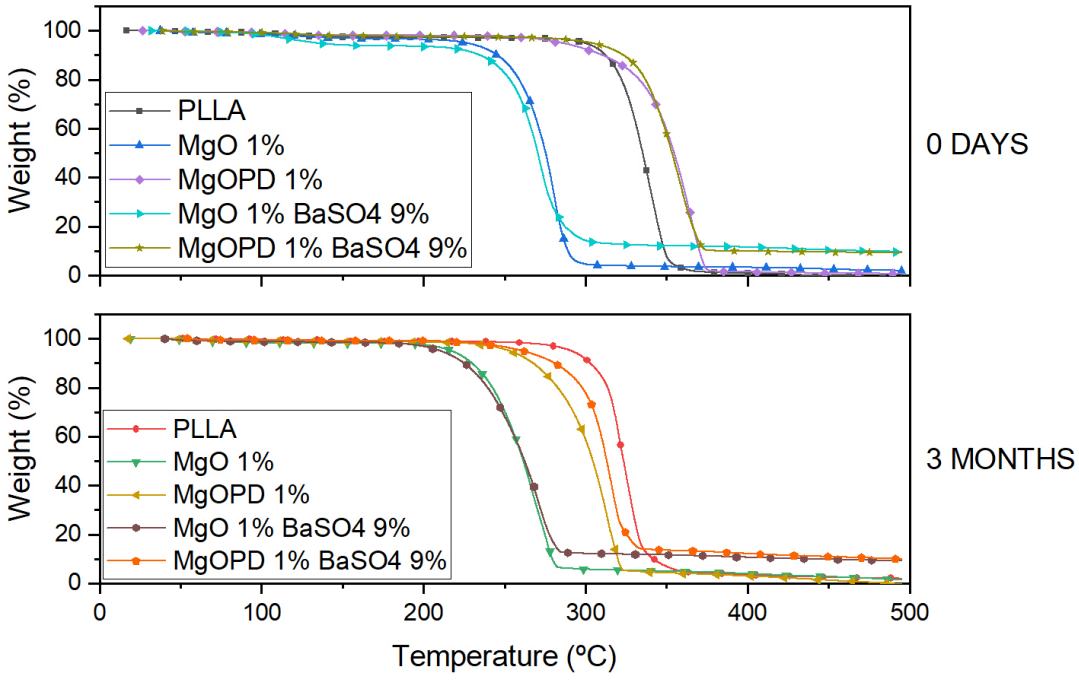
Figure 25. DSC of MgO 1% with $BaSO_4$ at 5% and 9% vs MgO 1% vs PLLA.

It has already been showed that $BaSO_4$ has no practical effect on the degradation of uncoated MgO systems. So, similarly, within the PD coated MgO systems, it would be expected that the $BaSO_4$ did not influence the degradation of the polymer over temperature. For its illustration, Figure 26 is exhibited.

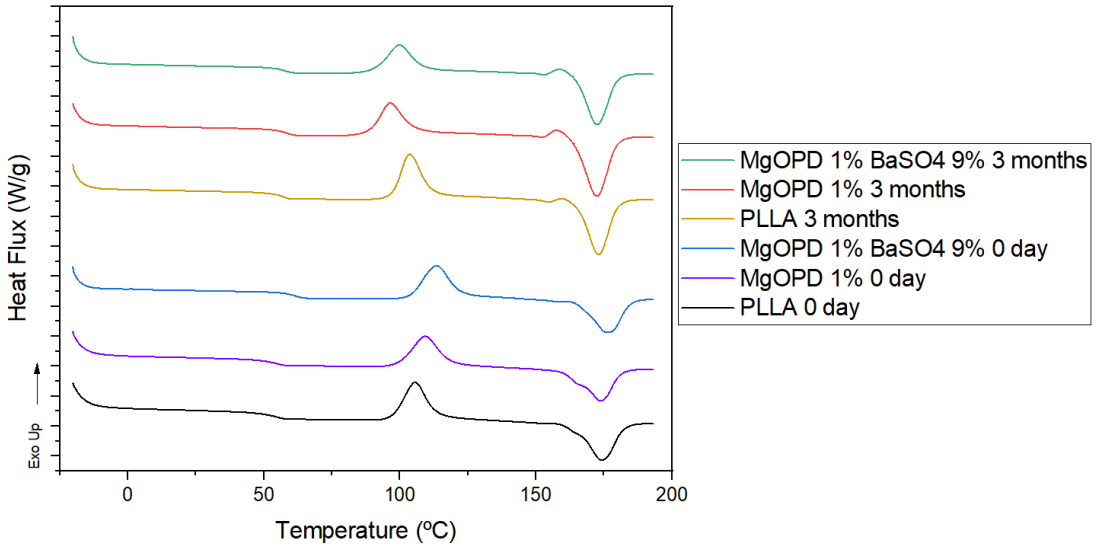
As it can be seen in Figure 26b, in the presence of PD, the $BaSO_4$ stabilizes thermally the polymer even more. It stabilizes the system even more than pure PLLA, without any more noticeable differences than their molecular organization.

Table 7. Chain distributions of MgOPD 1% with and without $BaSO_4$ 9% and pure PLLA at different times.

System	M_w (g/mol)		M_n (g/mol)	
	0 day	3 months	0 day	3 months
PLLA	113,900	108,049	40,708	42,860
MgOPD 1%	106,776	88,403	39,798	38,562
MgOPD 1% $BaSO_4$ 9%	126,337	104,090	48,253	42,653



(a) TGA of coated and uncoated MgO with and without $BaSO_4$ vs PLLA.



(b) DSC Coated MgO with and without $BaSO_4$ vs PLLA.

Figure 26. Thermal analysis of coated $BaSO_4$ systems.

From Table 7, we extract that initially, the coated sample with $BaSO_4$ has greater M_w than the PLLA itself, reason for which such systems resist better the degradation. However, after 3 month degradation, mainly due to the presence of the MgO, the crystalline region begins to degrade, which is the reason to the decrease in M_w, responsible for the reduction of T_m.

As a summary of this subsection, $BaSO_4$ seems to improve in every sense the degradation behaviour of the polymer, if we consider a lower degradation rate benefitial. For instance, there may be implants desired only for a short period of time for which other particle combinations shall be chosen. Also, some of its mechanical properties, although not studied here, are known to improve, such as the toughness and elongation at fracture [28].

9.5 Influence of BG

The remaining systems are composed of BG, either coated or uncoated, but mixed together with $BaSO_4$ for the achievement of the priorly explained radiopacity, which cannot be achieved with pure BG. Even so, the presence of BG under degradation conditions, favours the appearence of HA, which has been proved to improve osteogenic activity and promote MC3T3-E1 cell proliferation and endothelization [9, 10, 19], as well as preventing any initial inflammatory response of the site of implantation [17, 18].

In order to comment on the influence of the BG (BG+$BaSO_4$), comparisons between these systems and MgO ones in the same proportions will be exhibited, always with pure

PLLA as reference. From Figure 27, it is observable how initially, the BG system is more stable thermally than the MgO. However, after 3 month hydrolysis, the system is more degraded, although it is worth mentioning that still a T_g and T_c are observable, whereas not in the MgO system. This shows that these systems had greater amorphous region capable of crystallizing, given by larger chains in the molecular structure degrading over time, as shown in Table 8.

Table 8. Chain distributions of MgO and BG 1% with $BaSO_4$ 9% and pure PLLA at different times.

System	M_w (g/mol)		M_n (g/mol)	
	0 day	3 months	0 day	3 months
PLLA	113,900	108,049	40,708	42,860
MgO 1% $BaSO_4$ 9%	51,033	22,197	21,042	6,858
BG 1% $BaSO_4$ 9%	69,498	29,937	23,444	7,922

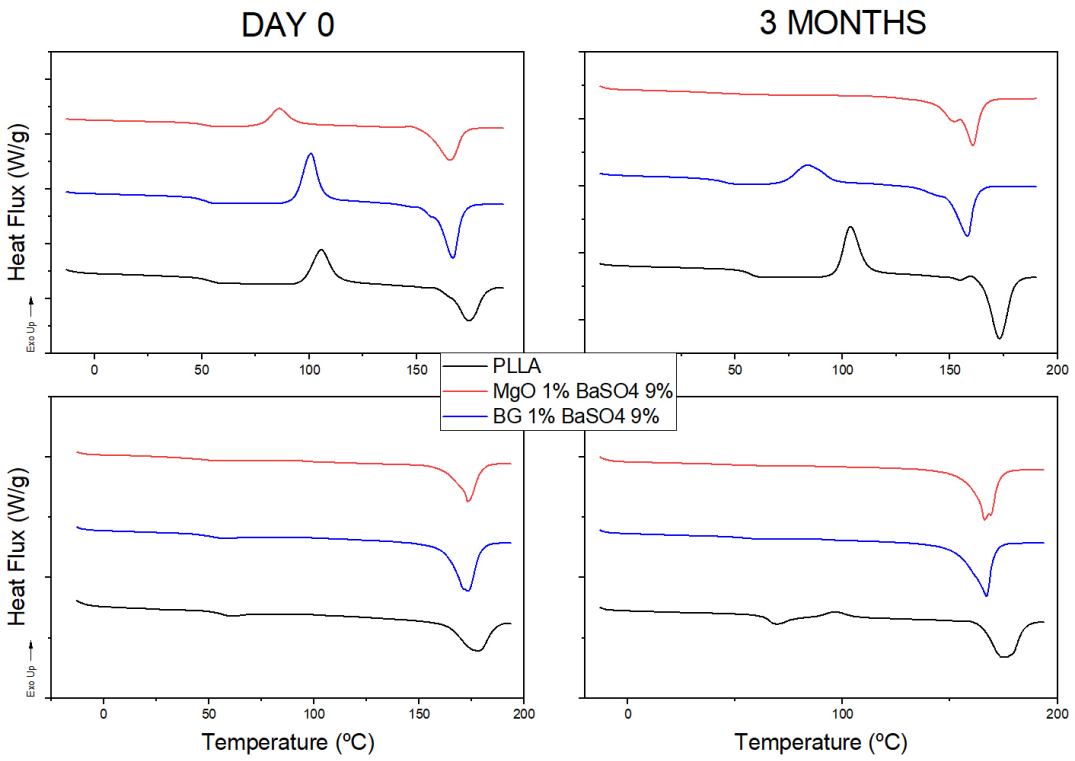


Figure 27. Top: 2^{nd} scan. Bottom: 1^{st} scan (actual state).

Attending to the degradation of the particle as a function of temperature, Figure 28 shows, as observed before, the greater stability of the BG system. However, over time, it

suffers greater degradation than the rest of the systems, so it can be concluded that the presence of BG makes the polymer more sensitive to hydrolytic degradation.

Specific 28-day and 2-months data for the BG system have been added to Figure 28 to exhibit the degradation recovery behaviour of this system: it reaches its lowest temperature-widthstanding point before weight loss at 28 days. However, for the 2-months sample, a recovery is observable, being capable of taking more heat until the beginning of its decomposition.

From here, it is possible to propose this system for mid-term implant applications, between 6 and 9 months from the processed data, since it undergoes a recovery of thermal stability over time, which may not be exclusive to a 3 month hydrolytic degradation.

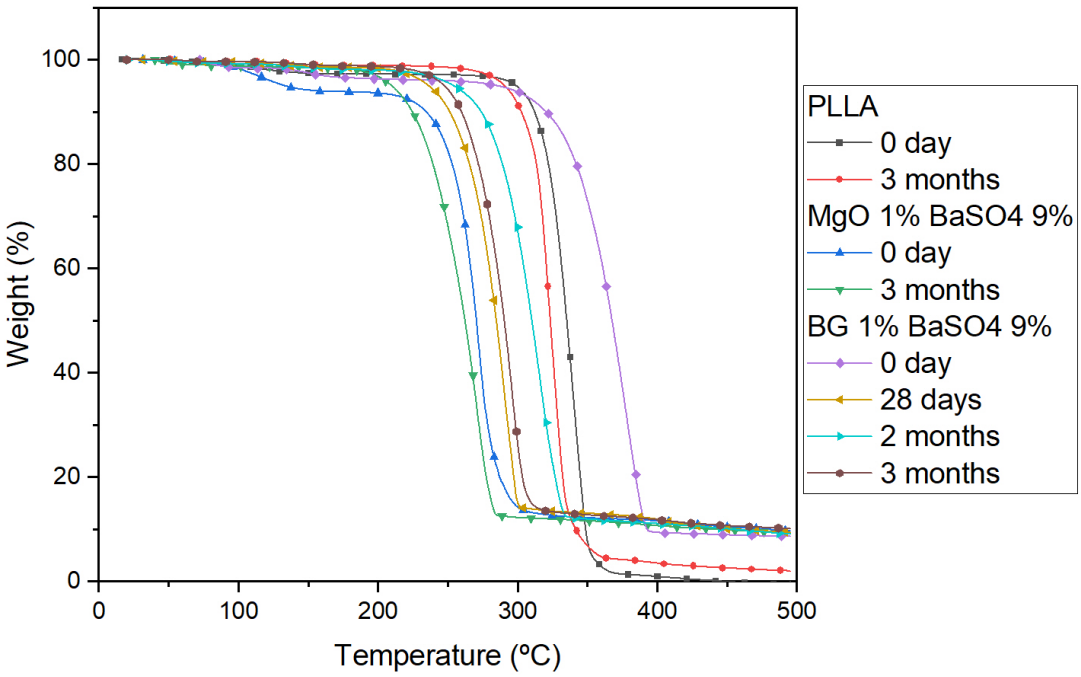


Figure 28. TGA of MgO and BG at 1% with $BaSO_4$ at 9% vs PLLA.

9.6 Influence of coated BG

And to end with, it remains to check whether the PD coating acts in BG systems in the same way it does for the MgO ones: protecting the polymer and delaying its degradation, making the system more stable. By coating the BG systems, as it happened with previous systems, the PD coating protects and prevents major degradation, enhancing the thermal stability of the polymers (Figure 30a).

Figure 29 exhibits the more stable behaviour of BG 1% with $BaSO_4$ 9% at initial weeks than the coated system with 5% $BaSO_4$, even better than pure PLLA between weeks 2-4, whereas for later weeks, the coated system proves greater stability in weight loss.

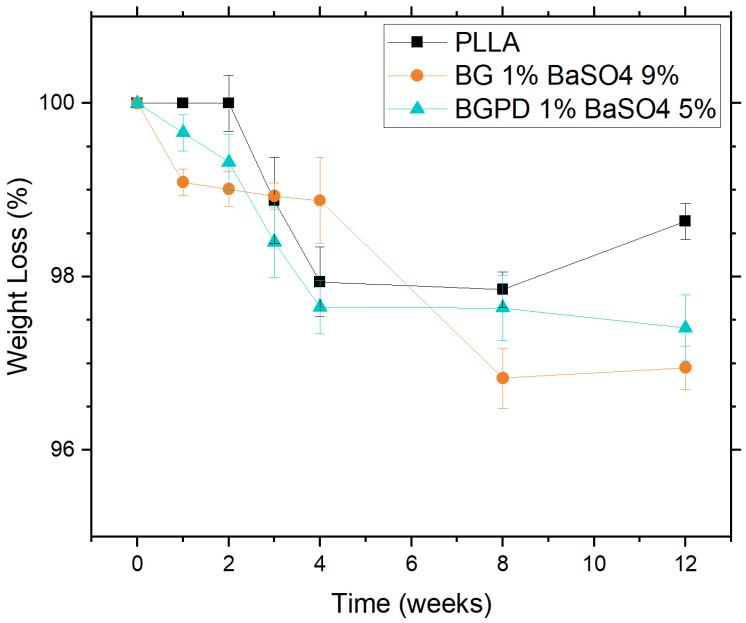
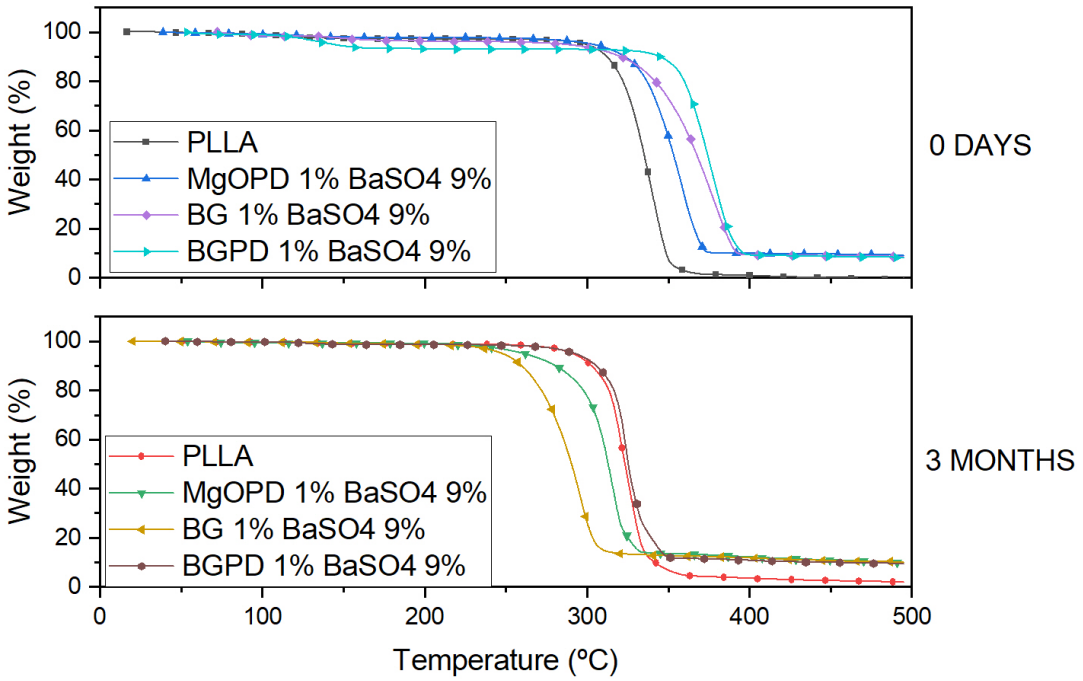
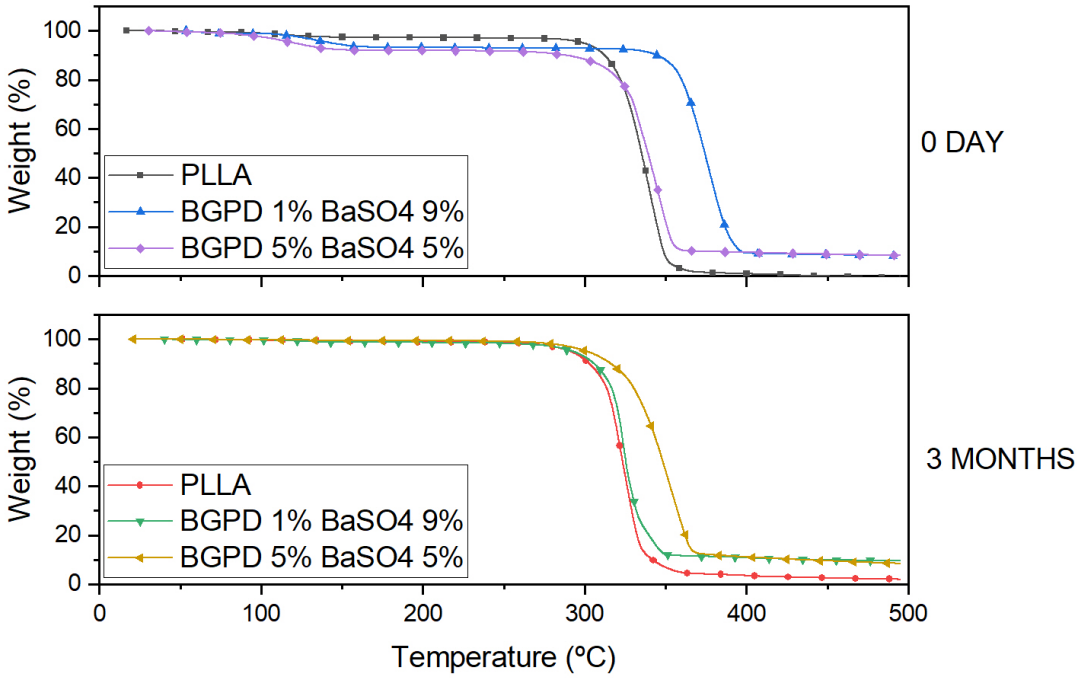


Figure 29. Weight loss of coated and uncoated BG$BaSO_4$ (1 and 5% and 1 and 9%, respectively) vs pure PLLA.

Although not shown in Figure 30a, it still exists some recovery behaviour in the presence of BG after 28 days of hydrolysis. However, all degradation curves are more compacted thanks to the coating. The coated BG systems exhibit better thermal resistance than MgOPD and even pure PLLA. However, it is worth mentioning that both main systems, MgO and BG ones (with $BaSO_4$), when coated, undergo a small thermal degradation at 75-125°C, more prominent with increasing particle proportions (see Appendixes III and IV), until 3 months, when this effect dissappears. Furthermore, for the 5/5% system of BG and $BaSO_4$, the thermal stability is better after hydrolysis for 3 months than at day 0. On the other hand, the DSC test proved no additional influence in the crystalline behaviour of the polymer, except for its delay in T_g, T_c and T_m temperatures, thanks to the mere coating of PD. So, as expected, the coating provides better stability and degradation behaviour for these systems as well.



(a) TGA comparison of coated and uncoated systems with $BaSO_4$ vs PLLA.



(b) TGA Coated BG at 1 and 5% with $BaSO_4$ at 9 and 5% (respectively) vs PLLA.

Figure 30. Thermal analysis of coated BG+$BaSO_4$ systems.

9.7 Summary

The following tables aim to present a summary of all the benefitial and detrimental effects of the addition of the different inorganic particles to a PLLA polymeric matrix, with the objective of visually helping the reader absorb all the main ideas, as well as to propose useful time-lapses for their applications based on the gathered data.

Table 9. Summary of the effect of the addition of inorganic particles.

SYSTEM	PROPORTION	BENEFITS	DETRIMENTS	APPLICATIONS
PLLA	100%	-	-	Reference polymer
MgO	1%	Improvement of mechanical properties[b] Remanence	High initial degradation Rapid weight loss Brittle	High load-bearing[b]
MgOPD	1%	Improvement of mechanical properties[b] Initial protection of reinforcements Radiopacity More stable weight loss (vs MgO)	-	Good mech. props.[b] Reduced initial degradation
MgO + $BaSO_4$	1% + 5% 1% + 9%	Increased toughness[b] Improved elongation at break[b] Radiopacity High initial degradation	Partial degradation at low T	Radiopaque Critical implants[b]
MgOPD + $BaSO_4$	1% + 9%	Improvement of mechanical properties[b] Initial protection of reinforcements Improved elongation at break[b] Radiopacity	Partial degradation at low T	Good mech. props.[b] Reduced initial degradation Radiopaque Critical implants[b]
BG + $BaSO_4$	1% + 9%	HA formation[b] Better biological performance[b] Certain recovery over time Stable initial weight loss (<4 weeks)	Fast degradation Faster weight loss after >4 weeks	Cell/Osteogenic activity[b]
BGPD + $BaSO_4$	1% + 9% 5% + 5%	HA formation[b] Better biological performance[b] Certain recovery over time Initial protection of reinforcements Improved elongation at break[b] Radiopacity Stable weight loss after >4 weeks	Partial degradation at low T Fast initial weight loss	Reduced initial degradation Radiopaque Cell/Osteogenic activity[b] Critical implants[b]

[b]: extracted from references.

Together with the summary of the main extracted attributes of each system, it has been proposed the following estimated duration for applications, attending to the results obtained from this project. It does not exist enough evidence supporting these proposals, nonetheless, a personal proposal for the viability of each system is desired to be addressed, following Table 9.

Table 10. Estimated duration of each system for applications.

SYSTEM	**ESTIMATED TIME-LAPSE**
PLLA	24 months
MgO	6-9 months
MgOPD	9-12 months
MgO + $BaSO_4$	6-9 months
MgOPD + $BaSO_4$	10-16 months
BG + $BaSO_4$	6-9 months
BGPD + $BaSO_4$	12-18 months

10 Expense Discharge - Budget

This section gathers the information concerning all the expenses of the project. Preliminar calculations of the hourly fees for the machinery are made. Afterwards, the costs for the 22 systems under analysis are calculated and finally, the overall expenses are displayed.

From a series of tables located in Appendix V: Expense Discharge - Initial Data, the necessary data is collected:

- Table 12 shows important data regarding the machinery (cost, power consumption and lifespan).
- Table 13 shows additional information for the calculation of the expenses, such as the time price of researchers and the electricity cost.
- Table 14 reflects the hourly fee of the diverse equipment.

Attending to these tables, and adding other expenses, as may be considered the use of computer and additional materials, such as flasks, vials, petris and so on, which are englobed in the miscellaneous section of the table of expenses, the overall expense discharge is exhibited in Table 11. Additionally, indirect costs attributed to the project itself, the maintenance of facilities, cleaning service, light and water and so on are included, assuming them to be around a 7% of the total direct costs.

It is worth noting that almost 50% of the costs are due to internal hours. This is due to the fact that the large amount of samples have required a lot of testing time and data post-processing time, entailing many hours, with their subsequent cost. Besides, the duration of the project has not been reduced, since the hydrolysis of the systems has required long periods of time (up to 3 months), involving great costs. Moreover, falling back to the Planning section, it is a project whose duration has been of almost the whole course. As a consequence, the expenses incurred have been elevated.

Table 11. Complete expense discharge.

CONCEPT	**UNIT**	**QUANTITY**	**UNITARY COST (€)**	**TOTAL COST (€)**
MATERIALS				3,756.55
PLLA	g	498.7	6.00	2,992.2
MgO	g	15.456	1.00	15.456
BG	g	2.88	1.00	2.88
PD	g	8.4	3.00	25.20
$BaSO_4$	g	19.2	0.60	11.52
Distilled water	L	8.94	0.23	2.06
$CHCl_3$	mL	6560	0.04	239.05
CH_2Cl_2	mL	640	0.0016	1.02
PBS	pad	30	1.782	53.46
$NH_2C(CH_2OH)_3$	g	10.176	0.364	3.70
N_2	L	30	3.00	90.00
Pans & Lids	-	160	2.00	320.00
AMORTISATIONS				758.34
Magnetic shaker	h	22	0.10	2.30
Vacuum pump	h	435	0.07	31.15
Oven	h	432	0.31	135.06
DSM	h	8	1.57	12.54
Injector	h	4	1.31	5.23
Tensile machine	h	1	1.24	1.24
DSC	h	80	2.19	175.11
TGA	h	160	1.92	307.31
GPC	h	82	1.08	88.39
INTERNAL HOURS				8,500.00
Undergraduate engineer	h	300	25.00	7,500.00
Senior engineer	h	20	40.00	800.00
MISCELLANEOUS				4,000.00
Computer	-	-	-	1,000.00
Other	-	-	-	3,000.00
DIRECT COSTS				17,014.89
INDIRECT COSTS			7%	1,191.04
TOTAL				**18,205.93 €**

11 Conclusions

Thanks to the monitoring of different systems under hydrolytic and thermal degradation, this work has been able to display and analyse the behaviour of the addition of inorganic particles to a reference polymer at different time intervals in order to guarantee their suitability for different biomedical applications. This study broadens the current knowledge of these polymer-reinforcement systems for an adequate choice of specific applications.

Magnesium oxide systems, although they are known to greatly improve mechanical properties [11], have exhibited lower degradation endurance than the rest of the systems. For this reason they may result in suitable for high load-bearing applications between 6-9 months. By coating MgO with PD, it is possible to achieve improved degradation resistance and allow its processing with a higher reinforcement content in the matrix, achieving radiopacity and better mechanical properties than PLLA. Moreover, the addition of $BaSO_4$, unvarying the degradation behaviour, provides increased toughness and elongation at break [28] for critical applications.

On the other hand, bioactive glass systems are known to improve cell proliferation and osteogenic activity thanks to the presence of biologically active hydroxyapatite [9]. It has shown good initial thermal stability and recovery over time. When coating, such degradation is reduced, making it suitable for application between 12-18 months. However, it exhibits partial degradation at low temperatures. But thanks to the combination of BG and $BaSO_4$, better mechanical performance is achieved, with unvarying conditions under hydrolytic degradation.

Hence, by determining the correct selection of the different requirements a particular application ought to have in order to achieve the desired behaviour, a thorough assessment of the diverse characteristics (benefits and detriments) each of the particles endow the polymer with shall be carried out to reach the optimal system for such application.

Thanks to novel techniques, capable of processing composites of diverse interest at nanoscales while preserving the overall integrity of the matrix, improvements in the near future are awaiting, and may as well diverge from the original purpose to fulfill any need in other scientific or technological fields. With additional techniques, such as FESEM, XRD or SEM/EDS, further characterization corresponding to the morphology of the systems, the evolution of crystalline phases and the presence of different elements with the capacity to grant the polymer with diverse characteristics could have been conducted.

In conclusion, the project overcomes both socioeconomic and scientific needs, and enlightens a new path for a deeper research of these biocompatible materials so as for the patient to bear its suffering confortably and achieve a faster wound/fracture recovery. In this way, it has been proved, regardless of the journey's remaining distance, the suitability of different such polymers, and their promising future that lies ahead.

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Appendix I: Identified Risks & Contingency Plan

This 1^{st} appendix is shown to complement the information and action plans that would have been taken in case some of the such identified risks had taken place, complementing what explained in Section 6.

A1: Identified Risks

The main identified risks that could have had a major impact on the project were the following, labelled in decreasing order of importance to the development of the project.

A) **Lockdown**
Due to the difficult and so easy-changing times we have been living in, this risk was one of the major problems that may arise in an experimental thesis. Last year, a scholarship research of the same characteristics was ruined because of this, so it was of utmost importance to advance as quicly as possible to try to avoid getting caught in the preparation steps in such a situation.

B) **Problems with the supplied material**
The raw materials received might have had some imperfections and not meet the requirements, and a major delay might have happened (in October a 2-3 weeks of delay due to PLLA supplied in bad conditions, whose characteristics did not correspond with the required ones, was faced).

C) **Problems with the testing machines**
It might happen that the equipments measured some undesired noise during some tests, or that the equipments may have suffered some sort of inaccuracies that led to the repetition of the test, as well as a failure of the machine itself. The former consequence should not take a considerable delay. However, if the machine broke of some sort, from the call to the technician to the fixing of the problem, depending on the gravity of the problem, it may have taken an additional couple of weeks.

D) **Bad Outcomes**
Bad outcomes in experimental projects are intrinsic to the very project. With the covering of PD for example, there were some problems that made it necessary to repeat the whole process. Similarly happened with the preparation of films, where several samples had to be redissolved and casted again in order to be able to extract films.

E) **Laboral Accident**
The work was carried out in a chemical laboratory with several hazardous chemical compounds that present a risk to human health, thus, it was a risk that should have been taken into account, as low as it might have been.

A2: Contingency Plan

In the process of the management of risks, the next step is to develop an answer to all these previous risks. There exist four different possibilities: to avoid the risk, to accept it (actively or passively), to reduce either the probability or the impact of the risk and to transfer its responsibilities. Regarding the different risks, the following ideas had been proposed:

A) **Lockdown**

The situation was not something we were able to control or do anything to reduce its chances of happening. For that reason, it was only possible to accept such risk or at least try to minimize its impact by advancing at the early stages of the project as fast as possible. If a lockdown had taken place while preparing samples, nothing could be extracted and all the time would be wasted. However, if the samples were already inmersed *in vitro*, afterwards some valuable data could be extracted.

B) **Problems with the supplied material**

Considering a fixed contract with the supplying company, and not being feasible to have established relationships with other enterprises or ordering equipment and material to other suppliers, no other measure than to passively accept such risk was left.

C) **Problems with the testing machines**

This risk could have been slightly minimized by having its proper periodic maintenance done and having used the equipment correctly, under supervised or experimented users. Nevertheless, some errors that would lead to repeating the test are inherent to the nature of the equipment and the facilities surrounding it.

D) **Bad Outcomes**

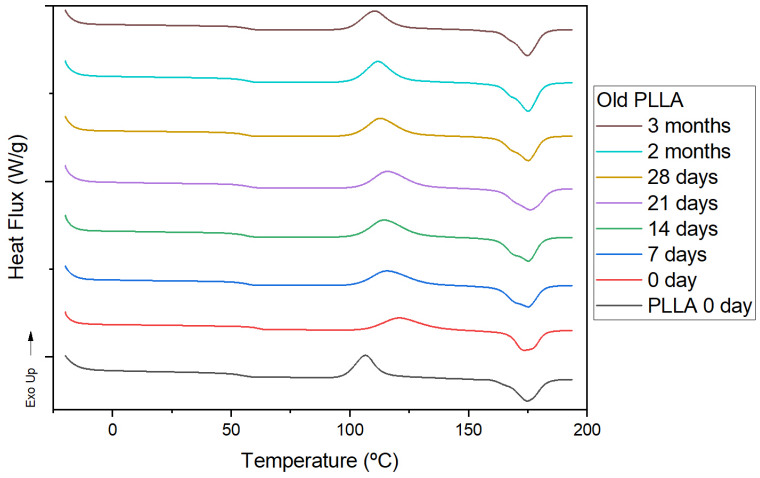
Bad outcomes such as incorrectly binded PD or getting nonsuccessful films were attached to the process itself and depended on incountless factors. For such cases, one could only accept, passively, the risk, regardless of how cautiously the process may have been carried out.

E) **Laboral Accident**

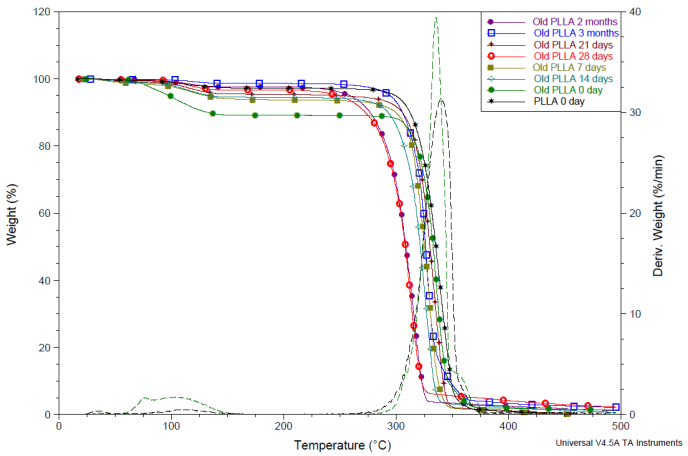
The risk of suffering any chemical injury or laboratory accident by spilling compounds or by not following the adequate protocol was greatly reduced thanks to the laboratory courses the university provides its students with. The courses "Good Practices in the Laboratory (I) and (II): Risk Prevention and Waste Management", as well as learning under the surveillance of an experienced advisor, were useful to avoid all sort of problems and undesired accidents.

Appendix II: Additional PLLA data

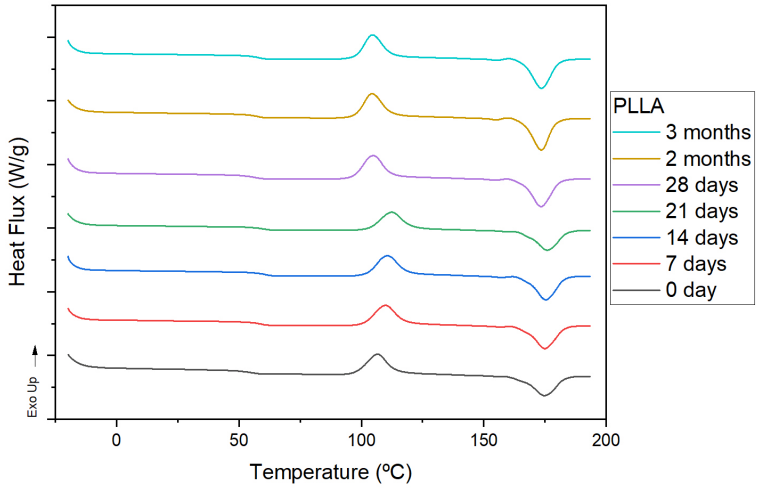
This 2^{nd} appendix shows additional data in figures about the reference PLLA system used for comparison in Section 9, as well as old PLLA, likewise analyzed, and so, its behaviour is displayed here.



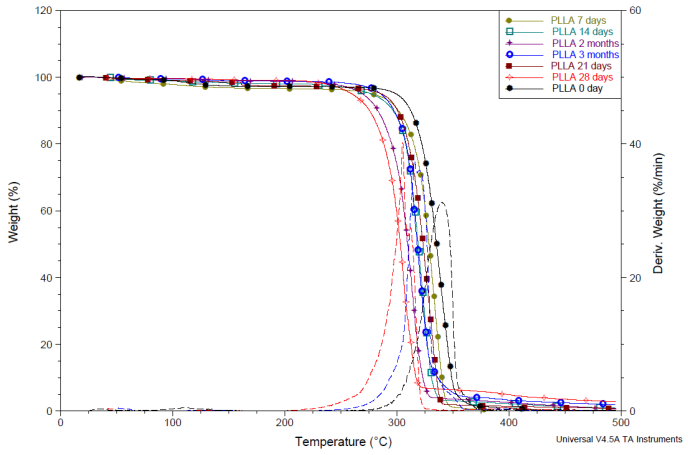
(a) DSC old PLLA.



(b) TGA old PLLA.



(c) DSC PLLA.



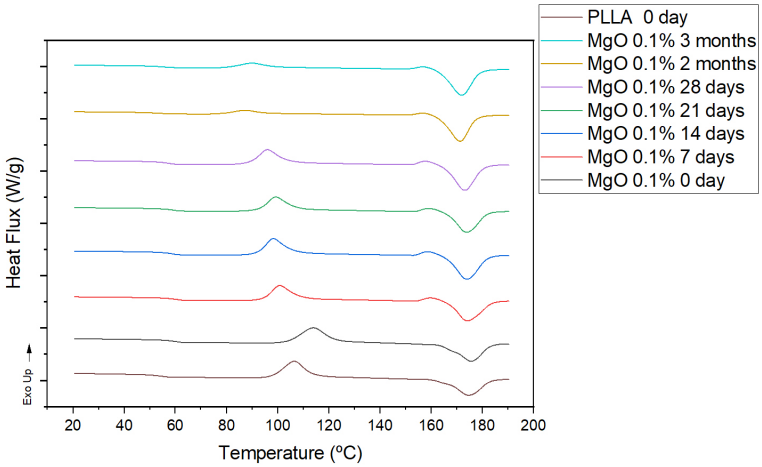
(d) TGA PLLA.

Figure 31. Thermal analysis of old and recent PLLA.

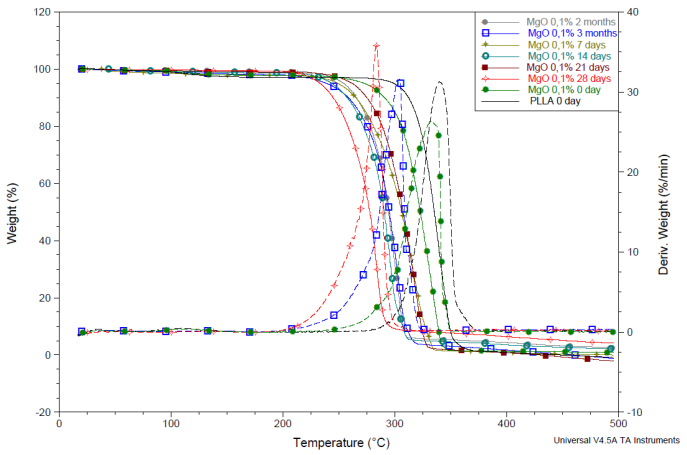
Appendix III: Additional MgO systems

This 3^{rd} appendix showcases additional information of the systems of MgO, coated, uncoated or with $BaSO_4$ for complementing what has been explained on Section 9.

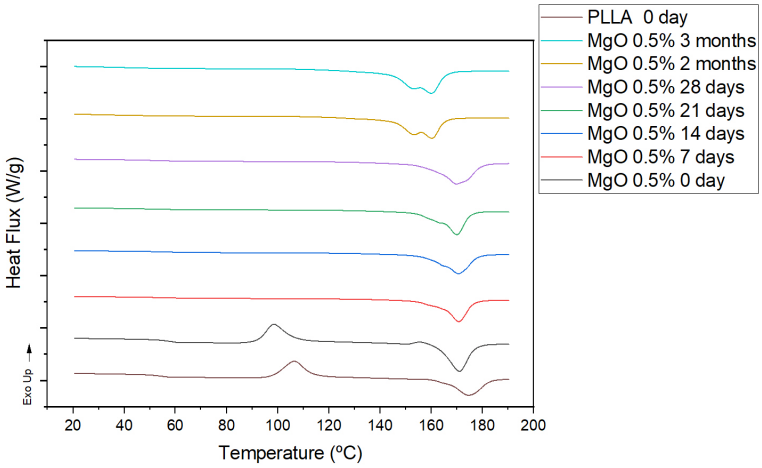
C1: Uncoated MgO Systems



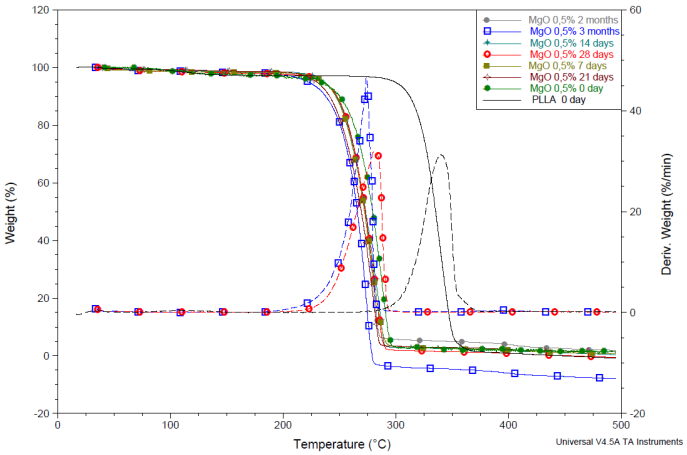
(a) DSC MgO 0.1%.



(b) TGA MgO 0.1%.



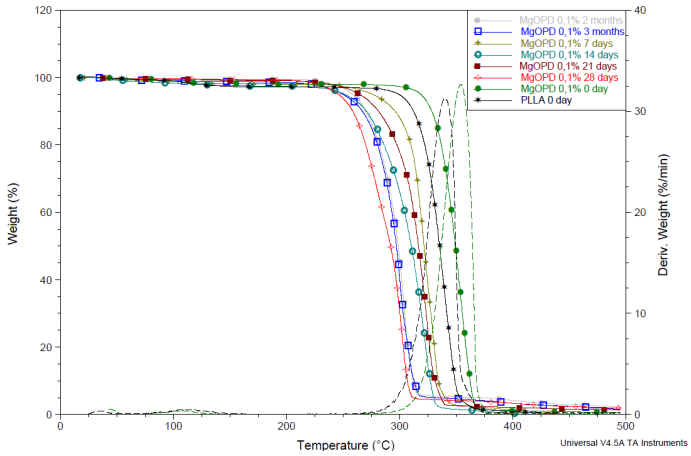
(c) DSC MgO 0.5%.



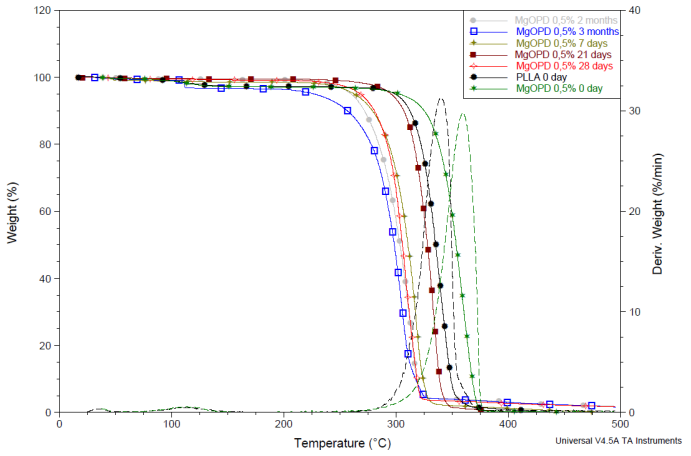
(d) TGA MgO 0.5%.

Figure 32. Thermal analysis of MgO 0.1% and 0.5%.

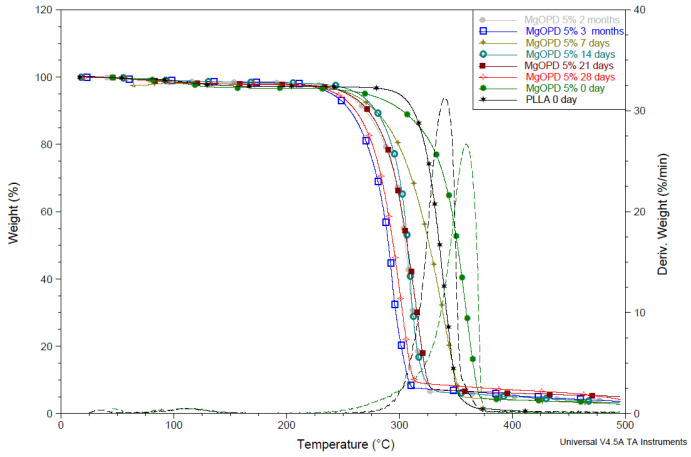
C2: Coated MgO systems



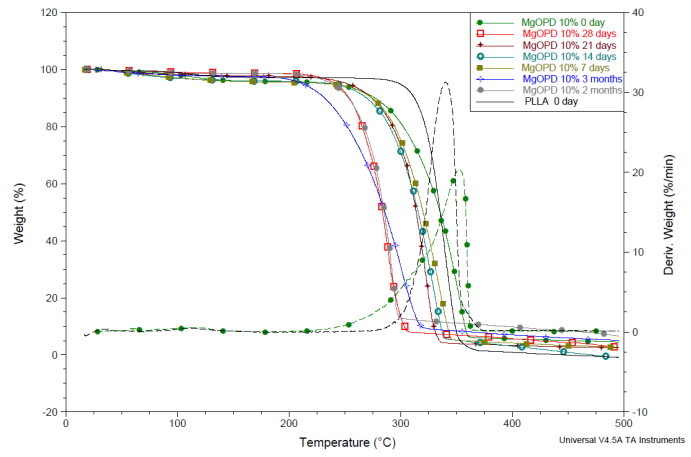
(a) TGA MgOPD 0.1%.



(b) TGA MgOPD 0.5%.



(c) TGA MgOPD 5%.



(d) TGA MgOPD 10%.

Figure 33. TGA of MgOPD.

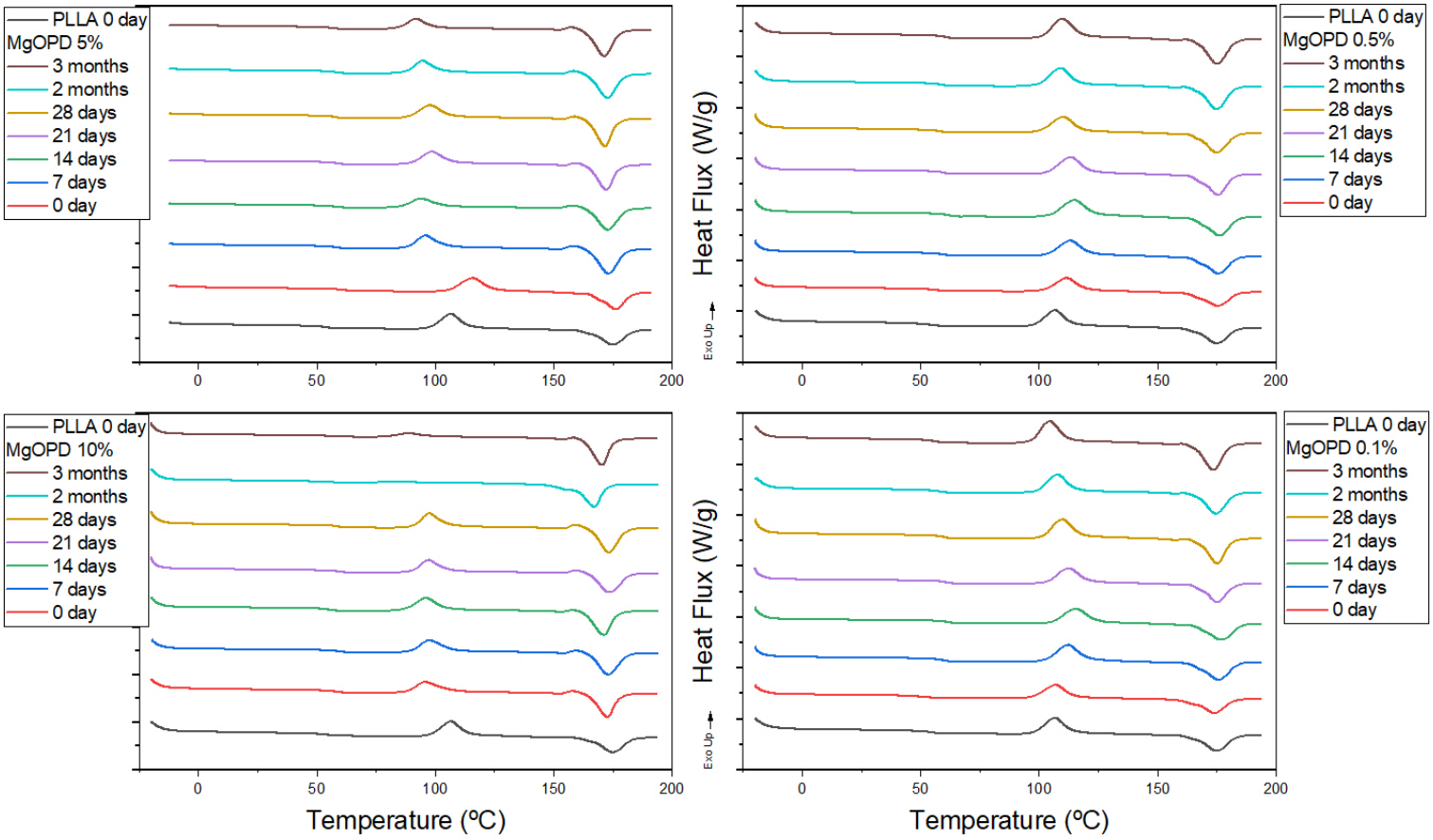
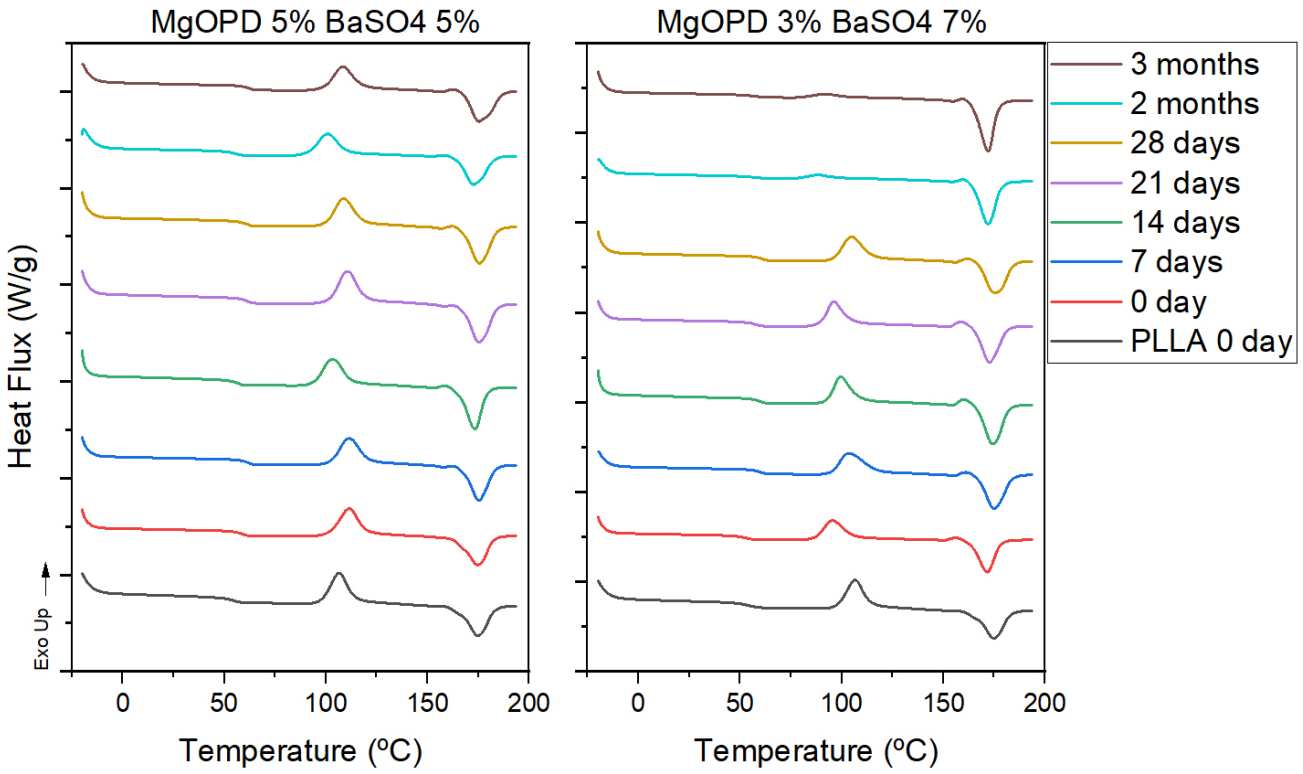
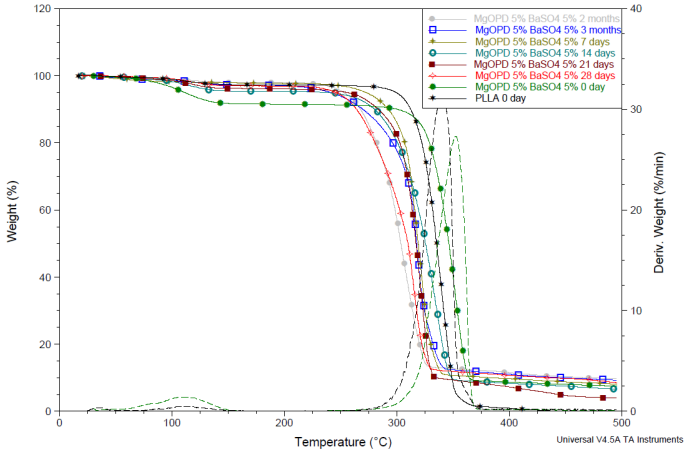


Figure 34. DSC analysis of MgOPD systems.

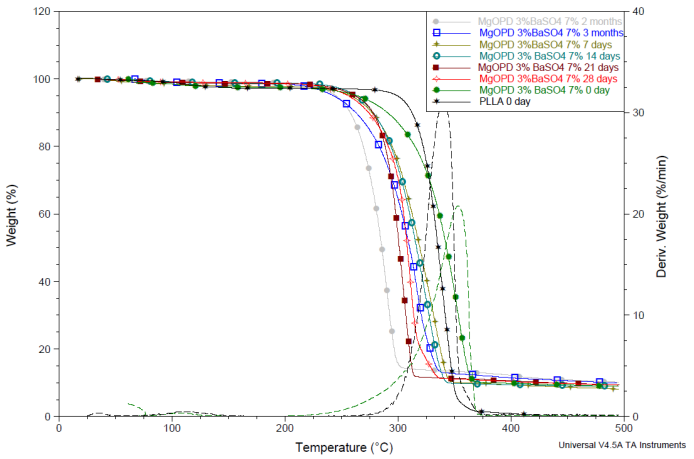
C3: MgOPD + $BaSO_4$ Systems



(a) DSC MgOPD+$BaSO_4$.



(b) TGA MgOPD 5% $BaSO_4$ 5%.



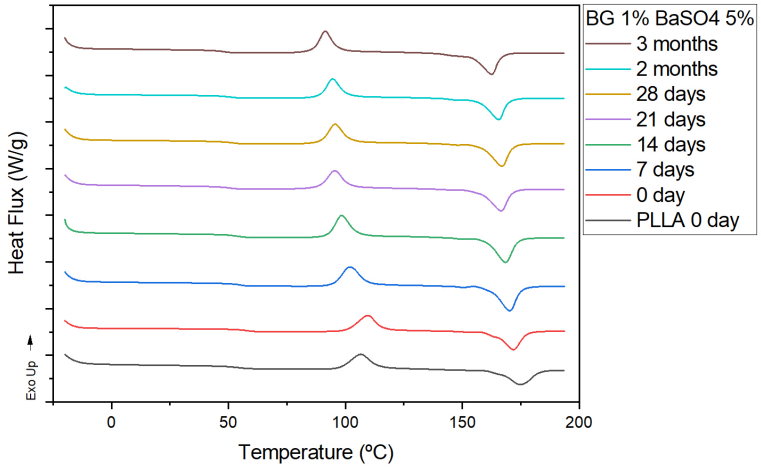
(c) TGA MgOPD 3% $BaSO_4$ 7%.

Figure 35. Thermal analysis of MgOPD+$BaSO_4$.

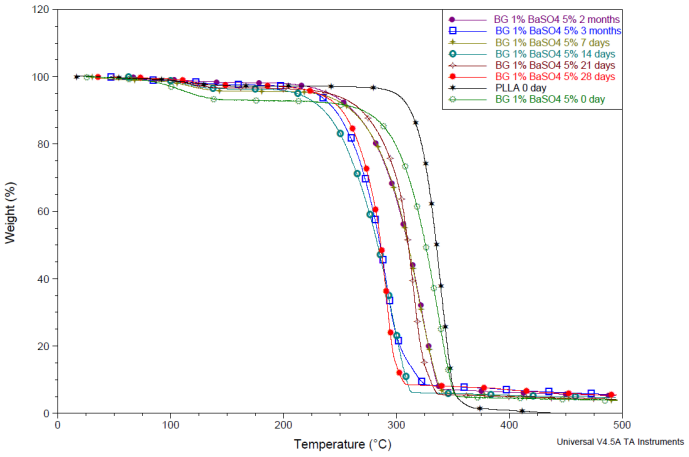
Appendix IV: Additional BG systems

This 4^{th} appendix showcases additional information of the BG systems with $BaSO_4$, coated and uncoated, for general interest to complement what has been explained on Section Results & Discussion.

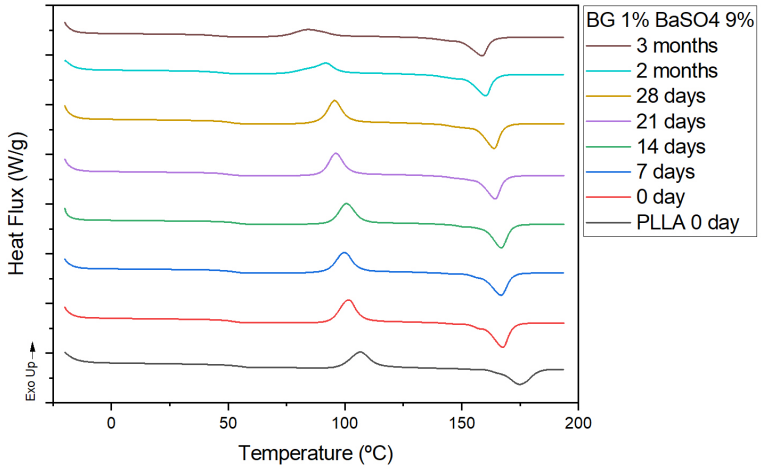
D1: Uncoated BG systems



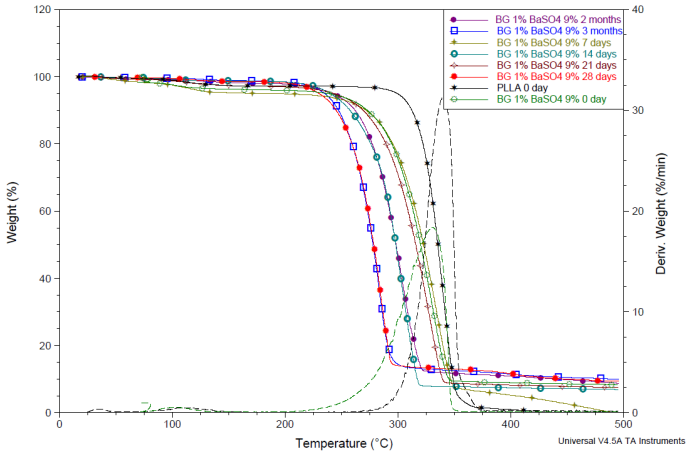
(a) DSC BG 1% $BaSO_4$ 5%.



(b) TGA BG 1% $BaSO_4$ 5%.



(c) DSC BG 1% $BaSO_4$ 9%.



(d) TGA BG 1% $BaSO_4$ 9%.

Figure 36. Thermal analysis of BG 1% $BaSO_4$ 5% and 9%.

D2: Coated BG systems

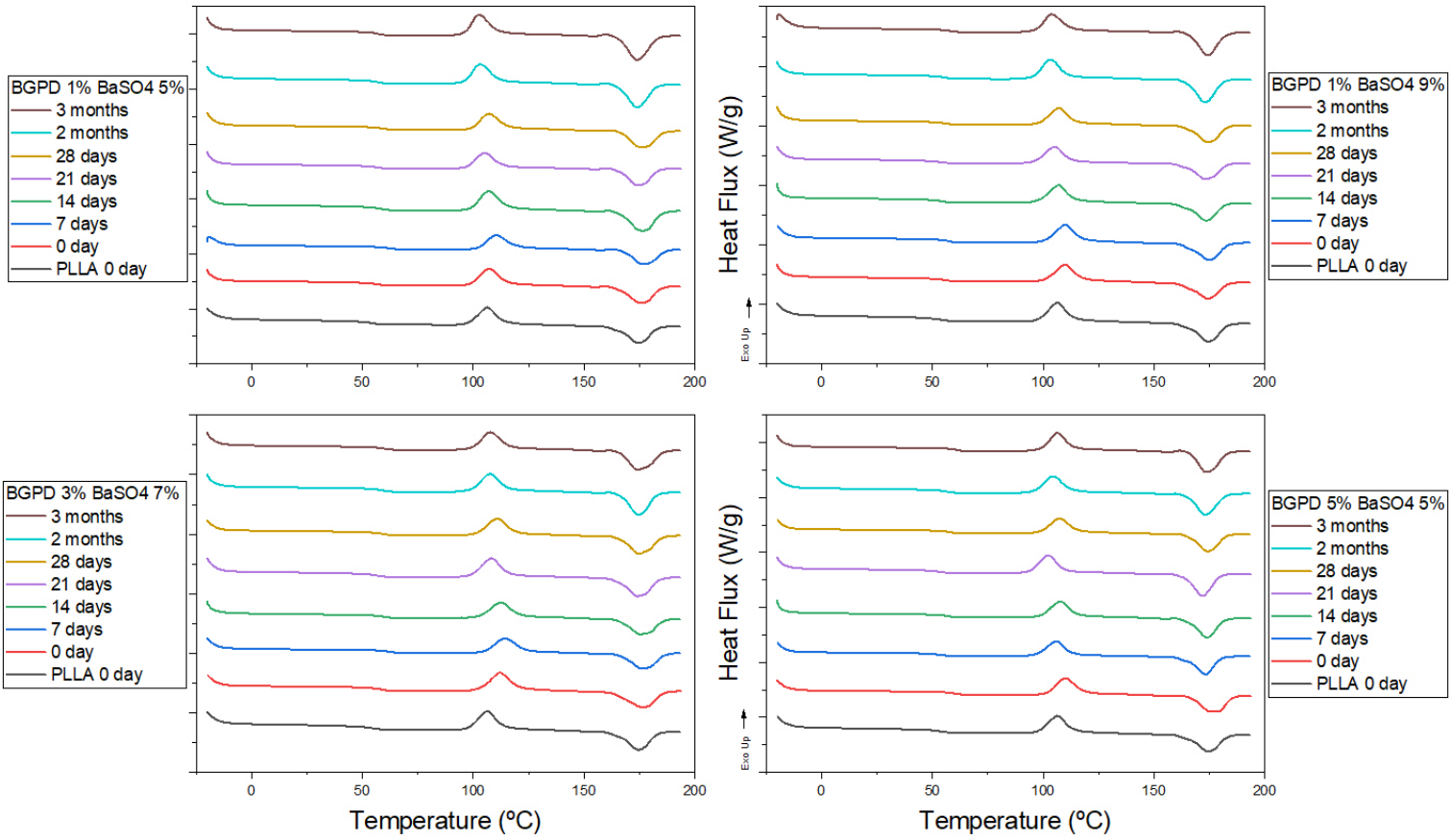
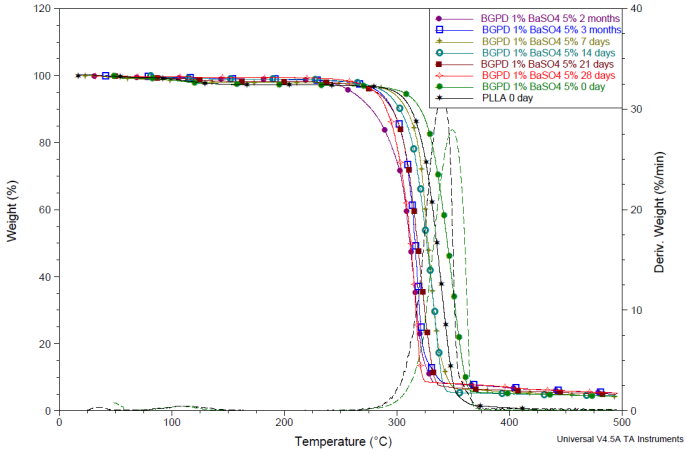
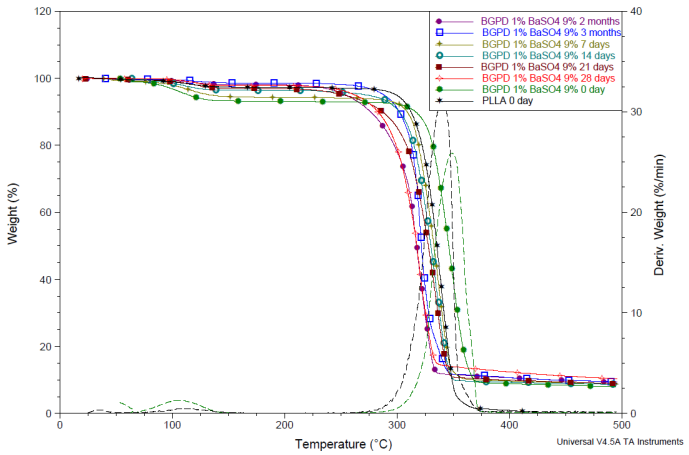


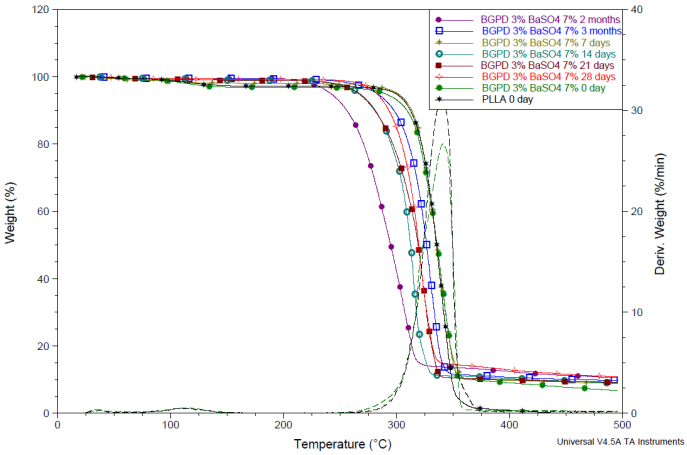
Figure 37. DSC Analysis on BGPD+$BaSO_4$ systems.



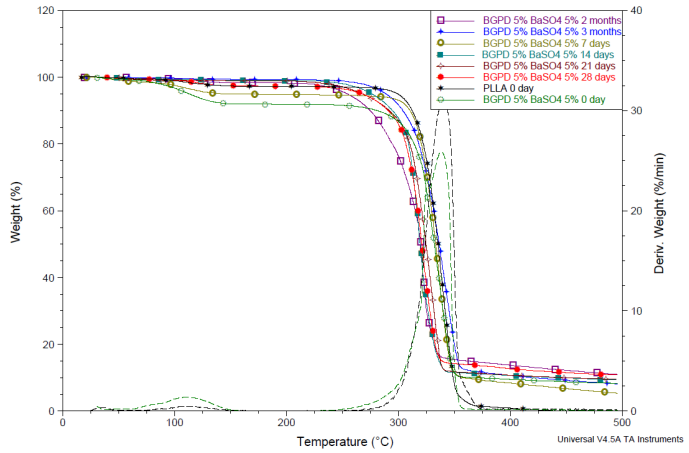
(a) TGA BGPD 1% + $BaSO_4$ 5%.



(b) TGA BGPD 1% + $BaSO_4$ 9%.



(c) TGA BGPD 3% + $BaSO_4$ 7%.



(d) TGA BGPD 5% + $BaSO_4$ 5%.

Figure 38. TGA of BGPD+$BaSO_4$.

Appendix V: Expense Discharge - Initial Data

This last appendix provides the necessary information to fully comprehend the expense discharge displayed in Section 10.

Table 12. Machinery cost, consumed power and lifespan.

Machinery	**Cost (€)**	**Power (kW)**	**Lifespan (years)**
Magnetic shakers	350.00	0.7	15
Vacuum pump	500.00	0.4	15
Oven	3,000.00	1.5	15
DSM	35,000.00	1.5	15
Injector	30,000.00	1	15
Tensile machine	30,000.00	0.5	15
DSC	35,000.00	1	10
TGA	30,000.00	1.2	10
GPC	20,000.00	0.75	12

Table 13. Complementary data.

Undergraduate engineer	25.00 €/h
Senior engineer	50.00 €/h
Electricity cost	0.13 €/kWh

Table 14. Hourly fee of machinery.

Machinery	**Repayment (€/h)**	**Electricity (€/h)**	**Hourly fee (€/h)**
Magnetic shakers	0.013	0.091	0.105
Vacuum pump	0.02	0.052	0.072
Oven	0.12	0.195	0.313
DSM	1.37	0.195	1.568
Injector	1.18	0.13	1.306
Tensile machine	1.18	0.065	1.241
DSC	2.06	0.13	2.189
TGA	1.76	0.156	1.921
GPC	0.98	0.75	1.078