

Study of the influence of antimicrobial modification of a polymer matrix on its biodegradability

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ABSTRAKT

Tato práce se zabývá studiem vlivu antimikrobiálního aditiva na vlastnosti degradabilní polymerní matrice s ohledem především na změnu jejich typických vlastností majících vliv na biologickou rozložitelnost v přírodních podmínkách.

V první části práce byla vypracována rešerše na téma biologicky odbouratelných polymerů, jejich aplikací a instrumentálních technik pro charakterizaci. Tato část byla doplněna informacemi o aktivních látkách v polymerních systémech, do kterých patří tepelné stabilizátory, antioxidanty a antimikrobiální aditiva.

V praktické části byly termoplasticky připraveny vzorky semikrystalického a amorfního typu polylaktidu s antimikrobiálními aditivy na bázi oxidu zinečnatého a stříbra. Následně byl studován efekt těchto aditiv na chemickou strukturu, fyzikální vlastnosti, antibakteriální aktivitu připravených systémů a degradaci polylaktidových vzorků dle normy ISO 20200:2015. Výsledky těchto testů byly diskutovány a porovnány s literaturou.

Klíčová slova: kyselina polyléčná, antimikrobiální modifikace, biologicky rozložitelný polymer.

ABSTRACT

This thesis aims to study the effect of antimicrobial additives on the properties of degradable polymer matrix concerning the change of their typical properties affecting biodegradability in the natural environment.

In the first part of the thesis, the research on biodegradable polymers, their applications, and instruments for characterization was carried out. This part was supplemented with a section on active compounds in polymer systems, including heat stabilizers, antioxidants, and antimicrobial additives.

In the practical part, samples of semicrystalline and amorphous type polylactide with zinc oxide and silver-based antimicrobial additives were thermoplastically prepared. Subsequently, the effect of these additives on the chemical structure, physical properties, the antibacterial activity of the prepared systems, and degradability of the polylactide samples was studied according to ISO 20200:2015 standard. The results of these tests were discussed and compared with the literature.

Keywords: polylactic acid, antimicrobial modifications, biodegradable polymer.

I would like to express my gratitude to my supervisor Ing. Martina Pummerová, PhD. for her guidance and valuable advice through the process of writing this thesis. Further, I would like to thank my family for their support and patience throughout the whole study.

I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

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INTRODUCTION

The first synthetic polymer was synthesized in the late 1860s; its name was celluloid. There was the need for synthetic material substitution of ivory, which was used for billiard balls etc. Celluloid development was considered a saviour of elephants and tortoises, as wild elephants were slaughtered in huge numbers for obtaining ivory [1,2].

Since the first synthesis in the late 1860s, polymers had been used in various fields because of their exceptional properties like lightweight, durability or flexibility, and economic viabilities. Due to the fact that most polymers are derived from petroleum, their biodegradability is inadequate or nonexistent. The problem of increasing waste started to be a concern in the 1970s. From 1950 to 2015, 8300 Mt of synthetic polymers were produced, of which 4900 Mt were landfilled or dumped in nature. In the 1980s, recycling started to be a solution, but it is still a very challenging and inadequate process [1, 2, 3, 4].

In addition to recycling, it is possible to replace existing non-biodegradable plastics with biodegradable substitutes. However, this substitution has some challenges: brittleness, low thermal stability, or even poor barrier properties [3].

One of the main benefits of plastics is their easy tailorability. The addition of additives impacts the property of the material and, in some cases, on its environment. One of the fields where this phenomenon is used is packaging, and it is called active packaging systems. One of the possible active systems is antimicrobial modification [5].

The antimicrobial activity of plastics is increased due to antimicrobial additives like silver, zinc oxide, or even polymer chitosan. As antimicrobial resistance is becoming a global challenge, it is essential to develop new composites. These composites have to be effective against bacteria and attack possible bacteria resistance [6].

This thesis aims to determine the changes in polymer matrix caused by antimicrobial additives. The practical part is focused on the preparation and characterization of its structural and chemical structure, thermal and antibacterial properties. The obtained information was confronted with the measured degradability rate of polymer matter.

I. THEORETICAL PART

1 BIODEGRADABLE POLYMERS

Biodegradable polymers are a group of polymers that can be decomposed by microorganisms to water and carbon dioxide. Biodegradable polymers can be divided into two main groups, natural and synthetic polymers. Another possible sorting is by the source from which the polymers are acquired (Figure 1) [4, 7].

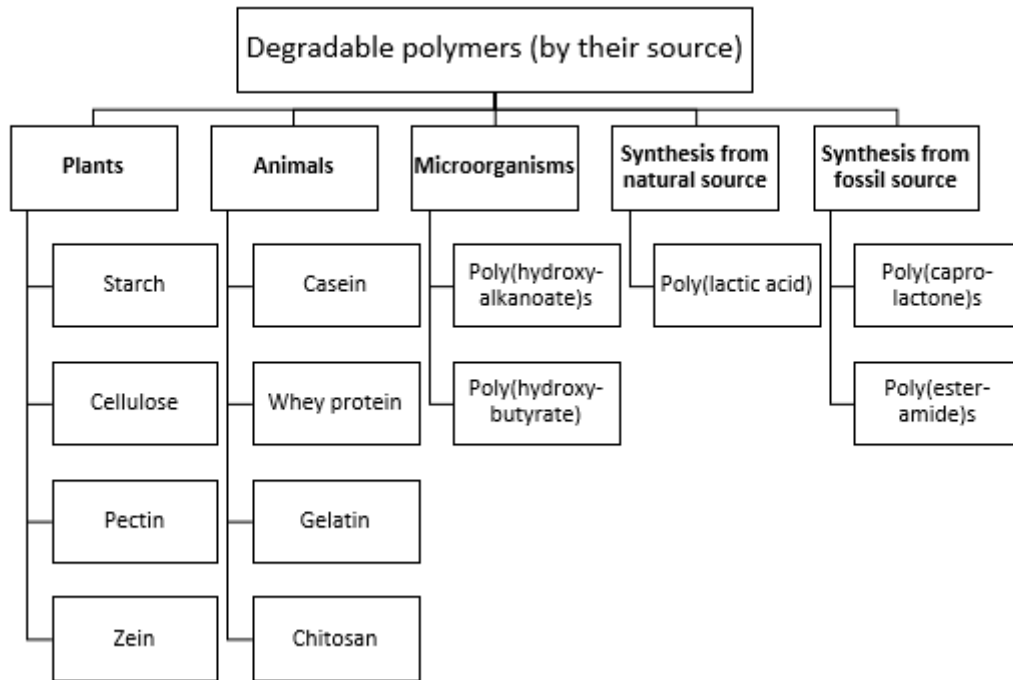


Figure 1 Sorting of biodegradable polymers, by their source [4]

1.1 Natural biodegradable polymers

Natural biodegradable polymers are also called natural biopolymers that usually occur in nature, synthesised by living organisms. Natural polymers are divided mainly into two groups, proteins, and polysaccharides. The disadvantages of natural polymers are vast diversity in qualities and an extensive polydispersity index. Natural biopolymers are materials divided into two main groups by their source. These groups are animal-based and plant-based. Examples of both animal and plant-based polymers can be seen in Figure 1. However, these materials cannot be used in their natural state. In order to complete successful application, it is necessary to convert it to functional polymers by chemical reactions, fermentations, purifications, and other modifications [7, 8].

1.2 Synthetic biodegradable polymers

Generally, synthetic polymers are widely used since they are fundamental to human life. Polymers are used in packaging, automotive, building, and textiles industries [9]. The main problem with synthetic polymers, such as polypropylene, is the recycling and long degradable time, which can be several hundred years. The new focus is on biodegradable polymers. When applying biodegradable polymers, the product left after degradation should be non-toxic and typically occurring in nature. The benefits of synthetic biodegradable polymers are the possibility to be modified and their tailorable designs [7, 9, 10].

The main target of this thesis is the study of poly(lactic acid), also called polylactide. Nevertheless, poly(ϵ -caprolactone), polyglycolide, poly(hydroxyalkanoates)s, and polylactide are introduced in the following chapter. These polymers are of the most used biodegradable synthetic polymers.

1.3 Poly(ϵ -caprolactone)

Poly(ϵ -caprolactone) (PCL) is a linear semicrystalline polymer. PCL is composed of units of hexanoate, illustrates in Figure 2. It was firstly synthesised in the 1930s and can be used in various forms such as film, fibres, or micro-particles. It can also be used for the fabrication of biodegradable 3D printed scaffolds [11, 12, 13].

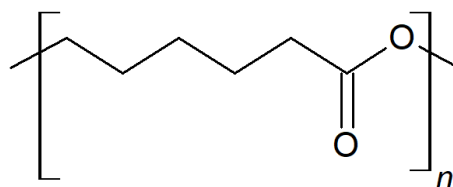


Figure 2 Structural formula of PCL [11]

1.3.1 Properties of PCL

PCL is a linear semicrystalline polymer with a melting temperature (T_m) around 63 °C and glass transition temperature (T_g) around -60 °C. Decomposition temperature starts at 250 °C. PCL has a broad crystallinity content, varying between 40 and 80 %, depending on molecular weight [10, 14].

PCL has a low density because of high olefin content. Another characteristic is low tensile strength, below 20 MPa, but high elongation at break, which can be up to 1000 %. PCL is considered a non-toxic polymer, so it is possible to use PCL for medical applications [11, 14].

PCL has a high ability to blend with an extensive range of various polymers. It is often combined with polylactide (PLA) or poly(lactic-co-glycolic acid) (PLGA), as with other biodegradable polymer matrices. Blending takes place because of some factors affecting biodegradability, for example, crystallinity. In many cases, the blends with PLA are known for increasing stiffness. PCL can also be mixed with non-biodegradable polymers, such as polyvinyl chloride (PVC), and can be used as a plasticizer instead of phthalates, which are harmful to human health, or adjunct to the biodegradability of these synthetic polymers [14, 15, 16, 17].

1.3.2 Synthesis of PCL

Synthesis of poly(ϵ -caprolactone) can be done using two different methods: polycondensation of 6-hydroxyhexanoic acid or the ring-opening polymerization of ϵ -caprolactone. The polycondensation reaction is not explored enough, and the value of the molecular weight of the final product is not sufficient [10, 14, 18].

PCL is mainly produced by the ring-opening method. The monomer is ϵ -caprolactone. Depending on the used catalyst, the reaction can proceed as anionic, cationic, or coordination-insertion mechanisms. All three mechanisms can be seen in Figure 3. The most used catalyst for the polymerisation is a stannous octanoate ($\text{Sn}(\text{Oct})_2$) [14].

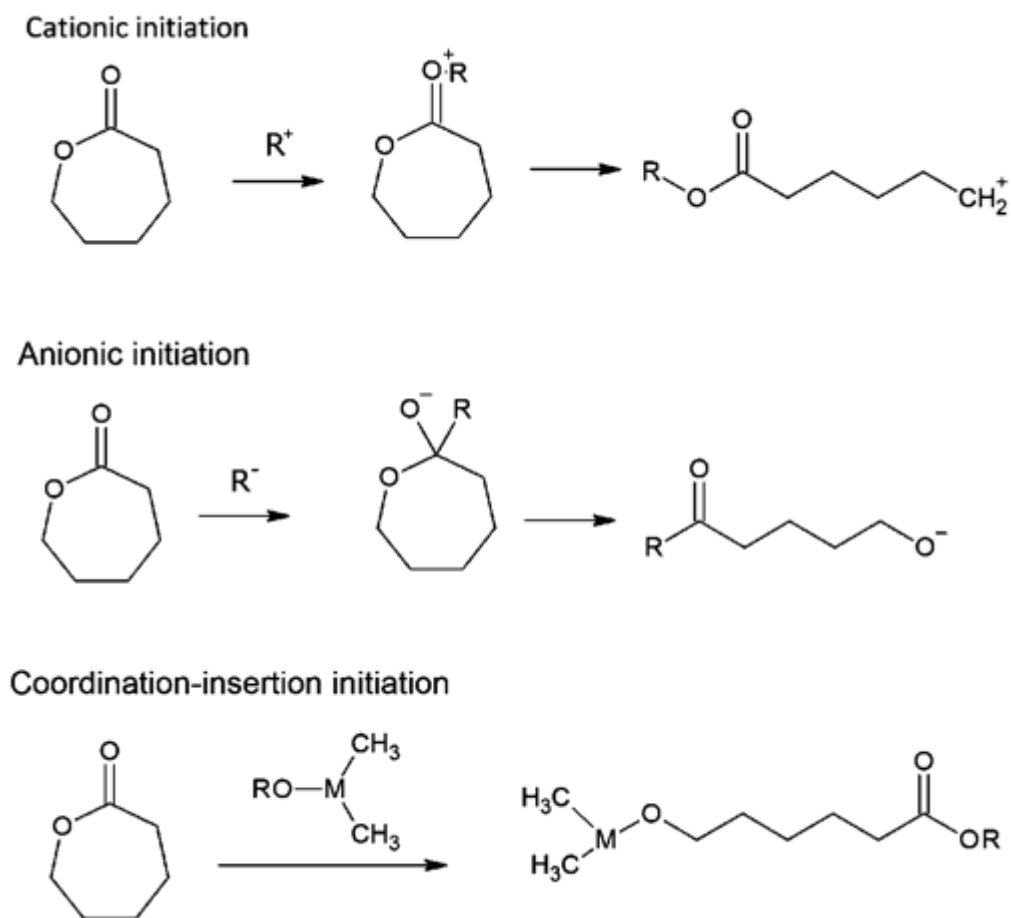


Figure 3 Mechanisms of initiation step for cationic, anionic, and coordination-insertion initiations [14]

The most frequently used mechanism for obtaining a high molecular weight of PCL is coordination-insertion polymerization, which is done under mild conditions and high conversions [14].

1.3.3 Degradation of PCL

PCL can degrade thermally, by enzymes and it is also prone to hydrolytic degradation. However, hydrolytic degradation is slower in comparison to polyglycolide, PLA, and PLGA because of its high crystallinity and hydrophobic nature. This hydrolytic degradation can take up several years, depending on the degradation conditions [10, 11, 13, 14].

1.4 Polyglycolide

Polyglycolide (PGA), also known as poly(glycolic acid), was one of the first biodegradable synthetic polymers (Figure 4). It is composed of repeating units of glutamic acid derived from α -hydroxy acids. Since the 1970s, it has been sold under the tradename DEXON.

PGA can be used in various forms such as fibres, pellets and films. Some of the innovative applications can be electrospinning fibres for bone scaffolds, blood vessels or packaging films [14, 19, 20, 21].

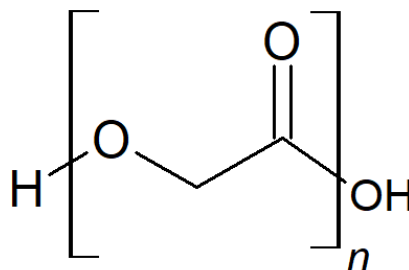


Figure 4 Struktur form of PGA [14]

1.4.1 Properties of PGA

PGA is an aliphatic crystalline linear polymer. Characteristic temperatures such as T_g and T_m are 35-40 °C and over 200 °C, respectively. PGA is insoluble in most organic solvents. Crystallinity can be around 45-55 %. Consequently, the tensile strength of PGA is exceptional in comparison with other synthetic biopolymers, and the modulus is about 12.8 GPa [19, 22, 23].

In the latest years, it is mixed with other biodegradable polymers because of the steep increase of glycolic acid, which can cause an inflammatory response or tissue damage. However, it is proven to have high biocompatibility [19, 24].

PGA production can be made by most common technologies such as extrusion, injection moulding or compression moulding. Although PGA is sensible to hydrolytic degradation, processing and the conditions must be carefully monitored [24].

1.4.2 Synthesis of PGA

Synthesis of PGA is carried out by polycondensation reaction of glycolic acid. Even though this way does not have sufficient molecular weight, for this reason, the ring-opening polymerization of glycolide is preferred, which is a cyclic diester. For this reaction, stannous octanoate, antimony, zinc, or lead can be used. It is possible to obtain monomer glycolide by heating glycolic acid under controlled conditions. This reaction is represented in Figure 5. Synthesis of PGA from glycolide depends on reaction mechanisms, reaction conditions and initiators [14, 25].

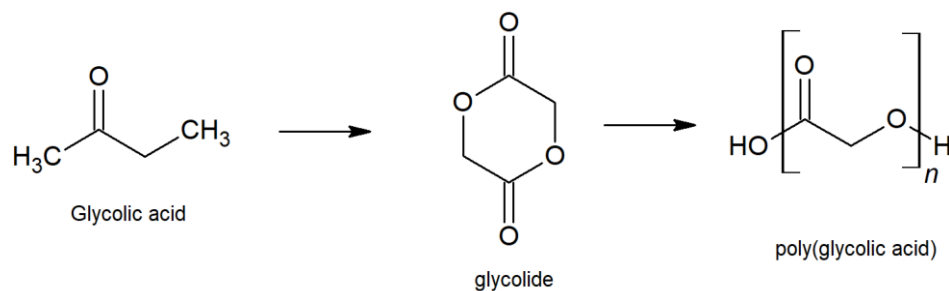


Figure 5 Synthesis of PGA [14]

1.4.3 Degradation of PGA

PGA is more likely to break down by hydrolysis rather than by enzymatic degradation. The hydrolysis is made via ester bond cleavage. Strong acid and alkaline media are accelerating the hydrolysis process. Nevertheless, the degradation is fast, PGA retains 50 % for 25 days, and it is fully degraded in 6-12 months. The product of degradation is glycolic acid, and although it is a natural metabolite, it can cause an inflammatory response in high concentrations and tissue damage. Several factors can determine the rate of degradation, such as porosity, morphology, crystallinity, molecular weight, and others [19, 23, 24, 26].

1.5 Poly(hydroxyalkanoate)s

Poly(hydroxyalkanoate)s (PHAs) are a group of polyesters produced directly by microorganisms. A general formula can be seen in Figure 6. PHA differs mainly in the length of the main back-bone and of the alkyl groups attached at the β position (R^1). PHAs are sorted into three groups divided by their length: short, medium or long chained structures [14, 27].

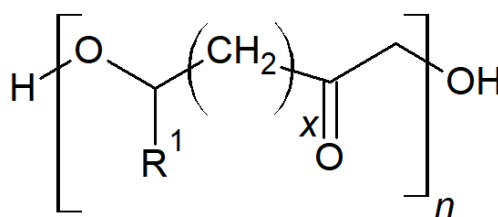


Figure 6 Structural formula of PHAs
 $x = 1, 2, \dots$, $\text{R}^1 = \text{H}$, methyl, ethyl...[14]

1.5.1 Properties of PHAs

Some properties of PHAs are similar to petroleum-based polymers, especially polypropylene, with similar properties such as good resistance to moisture and good barrier properties to gases. These polymers are insoluble in water but soluble in chloroform

or chlorinated solvents. Glass transition and melting temperatures can vary from -50 °C to -4 °C, and from 4 °C to 50 °C, respectively. Properties of different PHA types depend on the type of polymer and the amount of hydroxyalanoate units [14, 28].

PHAs can be blended with other biodegradable polymers, for instance, PCL and PLA. These blends may offer enhanced toughness and substantial elasticity [14, 28].

1.5.2 Synthesis of PHAs

PHAs are polymers synthesised directly by microorganisms (bacteria). These catalytic mechanisms involve different pathways linked to bacteria metabolic pathways, such as β -oxidation [27, 28].

Various microorganisms can produce different type of PHAs. The most known is poly(3-hydroxybutyrate) produced by *Bacillus megaterium*, *Pseudomonas fluorescent* strains accumulate medium chain length PHAs, containing 6-14 carbon atoms [27, 28].

The benefit of the synthesis of PHA is the possibility to obtain carbon source from sugar cane and out of water waste or vegetable oil waste. However, corn oil showed maximum PHAs of cell dry weight [27, 28].

1.5.3 Degradation of PHAs

Degradation depends on the type and composition of PHA and its crystallinity. Nevertheless, essential conditions are also environment and microorganisms. Hydrolysis is relatively slow; for example, poly(3-hydroxybutyrate) weight loss is only 2.8 % after 19 weeks of incubation in phosphate buffer solution at 37 °C. The rate of biodegradation can be altered by the crystallinity of PHAs, temperature and pH [14, 27, 29].

1.6 Polylactic acid

Poly(lactic acid) (PLA) is one of the most used synthetic degradable polymers. PLA is composed of repeating units of acid ester, as illustrates Figure 7. PLA has various applications, e.g. agricultural mulch foils, packaging, or medical scaffolds [20, 30].

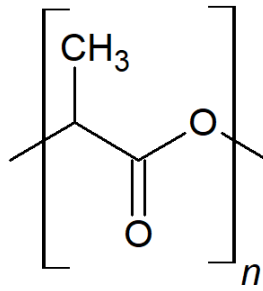


Figure 7 Structural formula of PLA [30]

1.6.1 Properties of PLA

PLA is a polymer with a monomer unit of lactic acid. This acid is a chiral molecule, so there can be three morphologically distinct polymers, D-PLA and L-PLA, which are semicrystalline, and amorphous racemate D, L-PLA. Lactic acid optical isomers can be seen in Figure 8 [10, 20, 30].

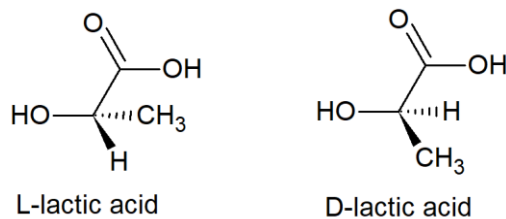


Figure 8 Optical isomers of lactic acid [30]

The glass transition temperature of semicrystalline materials is approximately 60 °C and the melting temperature around 180 °C. Amorphous racemate has T_g around 50-57°C. These temperatures are affected by the type of isomer and molecular number [10, 20, 30].

The mechanical properties of PLA can vary. It can be soft, elastic up to stiff high-strength materials. These properties depend on crystallinity, structure, molecular weight, material formula (plasticizers, fillers, additives etc.), and orientation. PLA has quite inferior strength and poor shear sensitivity. Tensile strength is in the range of 44-59 MPa [10, 30].

1.6.2 Synthesis of PLA

Production of PLA can be provided by three methods, types of polymerisation of lactic acid. These methods are direct condensation polymerisation of lactic acid, direct polycondensation in an azeotropic solution, and ring-opening polymerisation of lactide.

The monomer, lactic acid is synthesised by fermentation of carbohydrate, from natural sources as corn or cane sugar [10, 30].

The direct polycondensation of lactic acid is a single-step polymerisation. However, controlling molecular weight is poor, but the molecular weight is still higher than for the product of polymerisation by direct condensation reaction. For this reason, the ring-opening method is more favourable in industrial production [10, 30].

1.6.3 Degradation of PLA

Degradation of PLA is mainly direct hydrolysis of the ester bond in an aqueous environment. It is an autocatalytic reaction yielding carboxylic acid. The degradation process begins with the amorphous part of a polymer by random scission of hydrolysable ester bonds. After degradation of the amorphous part, degradation proceeds to the crystalline one. Within the degradation, crystallinity can increase, which can be caused by reorganising the remaining chains. Hydrolysis can be altered by the structure of PLA and additives presence, acting as a catalyst for the reaction. It can also be done by external factors such as pH, temperature, ionic strength and buffering capacity [10, 20, 30].

Moreover, PLA can degrade by enzymatic degradation, although it is not as prone to it as other polymers. Numerous enzymes had been isolated, such as pronase, bromelian and several esterase-type enzymes. The highest degrading activity toward PLA has savinase. Some enzymes can degrade L-PLA, but not D-PLA. For example, proteinase K. Enzymatic degradation occurs at the surface of a specimen. The particular reason for the circumstance is the inability of the enzyme to penetrate the sample [10, 20, 30].

Microbial degradability is the least susceptible process. In comparison with other biodegradable polymers, microbial degradability has a negligible effect. Although it is believed that microbial degradability mainly degrades by abiotic hydrolysis in the environment, it is also accelerated in the presence of microorganisms [10, 20, 30].

2 APPLICATIONS OF BIODEGRADABLE POLYMERS

In the last years, concerns about the harmful effects of petrochemical-derived plastics (like polyethene, polyethylene terephthalate, etc.) had aroused. As a result, the scientific community started to develop alternative biodegradable, eco-friendly polymers. For its high cost, biodegradable polymers are not used in massive quantity in comparison to petrochemical-derived ones. However, they can be found in specific fields, as can be seen in the following subchapters [27].

2.1 Biodegradable polymers in medicine

Biodegradable polymers used in medicine have to comply with these requirements: biocompatibility, non-toxicity, and non-immunogenicity. Also, they should not evoke an inflammatory reaction, and the product of degradation should be non-toxic and easily metabolized. For some medical purposes, natural (collagen, cellulose, chitosan) or synthetic biocompatible polymers (e.g. PLA, PCL, PHAs) can be used [20].

Biodegradable polymers are widely used as drug delivery systems. These systems can be utilized for short delivery applications, complete degradation in several hours or days, up to long term drug delivery, where the degradation rate can be two to three years. Polyanhydrides (synthetic polymers characterized by anhydride bonds that connect repeating units of the polymer backbone chain [26]) are used to release a drug quickly. PCL and PLA are applied for the long delivery drugs. Generally, slow-release systems are mainly used for the delivery of medication for long-term treatment, such as fibroids or cancer (brain, prostate, breast etc.) [10, 11, 13, 20].

Other usages of degradable polymers can be found in surgical implants. This category includes scaffolds, pins, tendons, surgical sutures, screws, or even nerve grafting. Over the years, several types of scaffold (e.g. PCL scaffold) that possess a calcium phosphate gradient have been developed. Scaffolds produced from L-PLA are another example. These scaffolds were used for cuff repairs in animal models as interposition devices for irreparable tears, reinforcement of suture repair line, and tender-bone repair. However, products made from PLA, PGA or their blend compound can lead to inflammatory response and bacterial infections. Although these issues are pretty unlikely (under 10 % of reported cases), there is still a potential danger. PHAs are also used for bone tissue engineering, and in contrast to PLA, they do not show any chronic inflammations [7, 11, 19, 27, 30, 31].

2.2 Biodegradable polymers in packaging

Packaging is the type of products that hold the item from producer to consumer. In the last years, biodegradable polymers started to be popular materials for this industry. It can be stated that it is a more environmentally friendly solution than conventional plastics [30, 32, 33].

Food packaging products have to accomplish several conditions, such as legislation regulations of a country (or European Union), safety, intoxicity, and its function and design [30, 32, 33].

Some examples of food packaging products are films, cups, bottles, bags, or food containers. Bottles are mainly produced out of PLA. However, these bottles need to be only for cold liquids, as the T_g of PLA is relatively low (around 50-60°C) [30, 32, 33].

These days, biodegradable packages are incorporated with antimicrobial additives, such as silver, for reducing, retarding or inhibiting the growth of fungi and microorganisms. These additives can be mixed with the polymer matrix in different forms: nanoparticles or microparticles incorporation or coating on product surface [30, 32, 33].

2.3 Biodegradable polymers in agriculture

Biodegradable materials used for agriculture purposes can be: synthetic polymers, such as PLA or PGA, or can be made as a composite with paper or natural-based fibres for required properties; microbial polymers, PHAs, e.g. poly(3)-hydroxybutyrate-hydroxyvalerate; natural polymers, mainly natural-based fibres as flax, hemp, paper, or different types of cellulose [30, 34, 35].

Generally, polymers in agriculture are mainly used as mulching films, low tunnels films and accessories. These products are primarily made out of polyolefins, most often polyethylene. Because of their non-degradable properties, it is hard to eliminate these products after their lifetime. The reasons for the switch to biodegradable polymers are: regulation of plastic waste by legislation; collecting, transporting and cleaning of the dirty plastic mulching films for its recyclation is financially demanding and ineffective; conventional plastics after use are creating visual pollution as same as physical pollution and high risk for fauna in the case of ingestion of these conservative plastics, which can cause the death of animals [30, 34, 35].

The solution could be to implement biodegradable polymers, which do not leave harmful residues in the soil after its lifetime. These days mulching foils can be already found

in utilization for protection against weed and plant diseases, reduce or eliminate the cost for pesticides, and eliminate moisture evaporation. Flower pots that are biodegradable in soil when it is time for the plant to be moved into the ground, and it is left with the planter can also be found in shops; nevertheless, these products are hardly available on the Czech market. As the pot degrades, it can also release valuable nutrients into the soil [30, 34, 35].

2.4 Special applications

Biodegradable polymers have potential on a broader spectrum, as mentioned in previous subchapters. Some other special applications are electrospinning, 3D printing of prototypes and designs.

Additive manufacturing, more known as 3D printing, is widely using PLA filaments as an extruded material. This method is used for so-called rapid prototyping, which is a quick process for creating prototypes and evaluating their functionality and product design. This technology also enables the printing of special products such as home decoration, arts or parts of cosplays. Additive manufacturing is a complex process consisting of design that is most of the time done in Computer Aided Design and Computer Aided Machining or using 3D scanners that scan the required object and gives a digital 3D copy [36, 37, 38].

Meltblown and spunbond are other possible technologies for products made out of biodegradable polymers. Those technologies make nonwovens materials: polymer melt (meltblowing) or polymer solution (spunbond) extruded throughout an extrusion die tip (spinneret), and high-velocity air blows the material onto a conveyor to form an opaque fine-fibre net. The product of meltblowing is utilized as diapers, feminine hygiene products, air and liquid filter or surgical masks. The most used polymer for this technology is polypropylene, but it is possible to use some biodegradable polymers for this technology. Those polymers can be PLA or polyesteramide and also some natural polymers like silk or cellulose acetate. These technologies manufacture fibres of diameter 1-5 μm [39, 40, 41].

Electrospinning is a technology for non-woven materials. The difference between this technology and the technology mentioned above is the filament diameter. The filament diameter made by electrospinning is 3-5000 nm. It is a favourite technology because of its cheap and straightforward setup, and thus over 100 synthetic and natural materials can be utilized. Electrospinning is classified into two types, depending on the state of the used polymer: solution electrospinning and melt electrospinning. It has three elementary components, a capillary tube, a high-voltage power supply (the voltage is usually between

5-15 kV), and a metallic collector. The high voltage is applied between the collector and the capillary needle end, the polymer solution/melt is electrically charged and deformed from spherical pendant droplets to a conical shape. Biodegradable polymers used for electrospinning can be of a natural or synthetic character. Natural polymers used for electrospinning include collagen, gelatin, elastin, chitosan, silk fibroin or alginate; synthetic polymers involve PGA, PLA, PCL, polyvinyl alcohol or polyethylene glycol. These fibres are used in medicine as nanofibers for nerve and skin tissue engineering or electrochemical sensors [10, 42, 43].

3 ACTIVE SYSTEMS IN POLYMERS

Active systems are widely used for packaging products. Active packaging is a type of products that involves interaction between the package and stored food or internal atmosphere. These changes are extending shelf-life or improve safety [5].

3.1 Thermal stabilizers

The thermostability of biodegradable polyesters (mainly PLA) is relatively poor. This is caused by a methane-based structure of the backbone chain of polyester. For example, the carboxyl group of PLA is produced by breaking the ester bond throughout processing, which causes the thermal degradation of PLA. The thermal decomposition depends on different factors such as crystallinity, purity, temperature, pH, additives. Due to these factors, the decomposition temperatures can vary. For example, PLA decomposition temperature is usually in the range of 230-260 °C, but it is losing structural properties around 60 °C. Different modifications and additives are used for improving thermostability [30, 44, 45].

The nucleation agents incorporation into a matrix is often used to increase the crystallinity and thus hinder the chain mobility, leading to improved heat resistance [44].

Another possibility of improving heat resistance is fibre reinforcement. Fibres can supplant the subsidiary role of the polymer matrix. The heat resistance is enhanced because the movement of chains is restricted. For this purpose, natural plant fibres, like hemp or bamboo, natural animal fibres, silk, mineral fibres, basalt, or chemical fibres, such as glass fibres, or carbon fibres (including carbon nanotubes), can be used. Carbon fibres are also implemented as flame retardants [44, 45, 46].

Compounding is a technological process required for thermal modification. Inorganic particles dispersed in a polymer matrix are most frequently used as modifications. A homogenous dispersion is essential in order to achieve the desired properties. Composite films have lower dimensional changes in comparison to neat material and higher characteristic temperatures. Some of the used additives are different clays such as halloysite nanotubes, smectite rich clay, organoclay or even titanium dioxide [44, 47, 48, 49, 50].

Also, blending with another polymer could bring the required results. A blending of polymers is the most often used method, thanks to its simplicity. Although blending of biodegradable and high-resistant engineering plastics (like polymethyl methacrylate, polyethylene, or polycarbonate) have evident improvements of heat resistance, it is not very

researched because these polymers are not biodegradable, which is a desirable property for applications of biodegradable polymers. The focus is on blends of biodegradable polymers like PLA, PCL, hyperbranched polyglycerol and others [44, 51, 52, 53, 54, 55].

Lastly, it is possible to use crosslinking of the molecules to immobilize them. This method improves not only the heat resistance but also strength and toughness [44].

3.2 Antioxidants

Antioxidants are compounds used for inhibition or prevention of oxidation caused by free radicals. As a result, antioxidants are included as stabilizer agents. And thus, antioxidants intercept the destruction of material and alteration of properties. It is also used as active packaging to enhance the performance of the packaging system [26, 56].

Some of the polymers already have antioxidant properties. Many natural-based polymers like chitosan, chitin, alginates, or synthetic ones like polyaniline, polypyrrole and poly(3,4-ethylenedioxythiophene) have natural antioxidant properties. The rest of the materials, thermoplastics the same as elastomers, have to use additives to obtain antioxidant properties. For plastic materials are used two types of antioxidants. These antioxidants differ in the mechanism: primary or secondary antioxidants. The most often used primary antioxidants are hindered phenols or hindered aromatic amines. The most frequent utilized secondary antioxidants are phosphides or thioesters. It is desirable to use both types of antioxidants for the synergic effect. On the other hand, elastomers are required to withstand harsh environments. For this reason, elastomers have different antioxidants for individual mechanisms of decomposing, such as metal deactivators, light absorbers, peroxide decomposers, antioxidants against oxidation and ozonation [56].

In recent years natural antioxidants started to be also used. Some of the reasons are consumer demand and that certain synthetic antioxidants are dangerous to human health, such as butylated hydroxytoluene, tert-butylhydroquinone and butylated hydroxyanisole. This problem is mainly for materials utilized in the food packaging industry. These natural antioxidants are located in spices, flavonoids, leaves and stems of some shrubs. A few of those natural antioxidants are rosemary, green tea, grapefruit seed, barley husks, curcumin, or essentials oils like lavender essential oil, or biopolymer chitosan, as polymer itself as well as a polymer additive. In most of them, polyphenolic and other natural antioxidant compounds typically occur [57, 56].

3.3 Antimicrobial additives

By definition, according to Merriam-Webster dictionary, the word ‘antimicrobial’ means ‘destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms’ [58]. In current years a problem with antimicrobial resistance to the treatment of bacterial infections mainly caused by overusing antibiotics has emerged. This antimicrobial resistance compromises the ability to treat infectious diseases and has resulted in increased mortality and morbidity [59].

Antimicrobial polymers have to have the ability to reduce or inhibit the growth of bacteria, fungi or yeast. These materials exhibit antimicrobial activity through their chemical structure, or their matrixes serve as backbone or carrier for the existing antibiotics and active antimicrobial compound. Antibacterial polymers with antimicrobial activity can also serve as antimicrobial additives or can be blended [6].

3.3.1 Organic compounds

Antimicrobial polymers

Progressive development and understanding of chemistry lead to the possibility to design and synthesize antimicrobial organic compounds. Polymers with natural antimicrobial activity often have in their backbone quaternary nitrogen groups, halamines or polylysine. For example, ultraviolet irradiation can create the antimicrobial activity of nylon by exciting its structure [6, 60].

The most utilised natural antibacterial polymer is chitosan. Chitosan is derived out of chitin, and it is approved as a food ingredient by the US Food and Drug Administration (FDA). The long-chain polymer is not as effective as short or medium-long chitosan. One of the problems is to utilize production with consistent properties. Chitin has to pass through several chemical reactions involving deproteinization, demineralization, deacetylation, and decolourization - these processes lead to the growth of the final price of chitosan [59, 60].

When natural polymers are blended into biodegradable polymers, for example, chitosan into PLA matrix, it affects PLA structure. Chitosan can increase the hydrophilicity of the blend. This hydrophilicity can have the effect of favouring the interaction with moisture and the attack of microorganisms during biodegradation. Although the weight loss was small, changes in mechanical, thermal, and surface properties were significant, as described in the literature [61]. The antimicrobial effect of chitosan in PLA films is proven to increase, which

is confirmed in other studies [62, 63]. Chitosan can be used in active antimicrobial packaging and systems in biomedical applications, like tissue engineering, wound dressing or cancer diagnosis [59].

Non-volatile compounds

Non-volatile compounds are primarily solid at room temperature and are widely used in the chemical industry. Some of those compounds also have antimicrobial properties. These compounds can be peptides or polyphenols [64, 65].

Several peptides have been reported to exhibit antimicrobial, antifungal, and antiviral properties. Those compounds have typically short-chain, up to 50 amino acids. The incorporation of peptides into polymer matrices have been studied for several applications like food packaging, medicine, pharmacy and personal care. The mixing of peptides into the polymer matrix shows an inhibitory effect against microorganisms already at low concentrations. Because of the high thermal stability of peptides, the incorporation into the matrix can be done by extrusion and other high-temperature methods. The isolation of pure natural peptides is a low yield process alongside high production cost [65, 66, 67].

Nisin is one of those antimicrobial peptides. Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*. It has been used as a food preservative for over 60 years, and US FDA classifies it as safe for use. Nisin incorporated in polyethylene film is effective in inhibiting against *Micrococcus luteus*. When it is incorporated into PLA film, nisin can reduce *Escherichia coli* and *Salmonella enteritidis*. Mersacidin, staphylococcin C55, lacticin 3147 and 481 are examples of other researched antimicrobial peptides [65, 66, 67].

Polyphenols are organic compounds occurring naturally in herbs, spices, and generally in plants. They contain at least one aromatic ring, bearing hydroxyl groups. Curcumin and resveratrol are the two most profound and researched polyphenols. Curcumin is naturally found in rhizome of *Curcuma longa*. For centuries, it had been used as a drug for its antiinflammatory, antiapoptotic and antioxidant properties or as a spice in a meal because of its characteristic colour and taste. It can increase microbial inhibition of PLA packaging product against *Escherichia coli* and *Bacillus cereus* when used as an antimicrobial agent. Resveratrol is naturally found in almost a hundred plants, like grapes, cranberry or peanuts. It has diverse biological activities, including phyestrogenic, antitumor, antioxidant and antiviral function. When used as an antimicrobial agent in polymer, e.g. cellulose acetate [68], it increases the inhibition of bacterial strains growth [65, 69, 70, 71, 72].

Volatile organic compounds

Volatile organic compounds are chemical compounds that are capable of evaporation at room temperature. Essential oils are classified as volatile organic compounds [73].

Essential oils are proven to have antimicrobial properties. The incorporation of essential oils into a polymer matrix is becoming more popular. Antibacterial activity is proven against *Staphylococcus aureus*, *Enterobacteriaceae*, *Escherichia coli* or *Listeria monocytogenes*. Essential oils can also be used as antioxidants. The disadvantage of essential oils is their great aromatic character. However, this trait does not impact the food when used in packaging materials (this applies mainly to oregano essential oil). Some of those oils are *Mentha piperita*, *Bunium persicum* essential oil or oregano essential oil [65, 74].

3.3.2 Inorganic compounds

Metals and their oxides have been employed as antimicrobial agents for centuries. These metals are, for example, silver, gold, and oxides of metals such as zinc oxides or titanium oxide [59, 75].

Silver (Ag)

The antimicrobial properties of silver are known since ancient history. Silver for its antimicrobial properties was used till the discovery of antibiotics. It was applied on wounds and burns. Silver is not as toxic as other metals for human cells. Antimicrobial activity is mainly by biocidal silver ions [59].

Antimicrobial activity depends on the concentration of silver in the mixture. At low concentrations, the damaged bacteria can survive by re-establish homeostasis. Nonetheless, with higher concentration, the bacterial cell damage is irreversible and leads to the death of the microorganism. It is a complicated process to establish breakpoints for the inactivation of bacteria. The silver ions can be drawn to chemical substances and interact with them, which can be, for example, sulfur groups or halides. This activity also depends on strains of a species [59]. Silver nanoparticles are proven to have an intense antimicrobial activity to gram-negative bacteria, *Escherichia coli* and *Vibro parahaemolyticus*, and gram-positive *Staphylococcus aureus* up to 30 wt%. Incorporation of Ag nanoparticles into PLA is mainly done via a chemical reduction method in diphasic solvent [76, 77].

Copper

Copper is another traditional material used for its antimicrobial properties. In the past, fewer studies on antibacterial properties of copper had been done than on silver, but in recent years, it is on uprisings (over 4 000 studies in 2020 alone). The mechanism of the activity is not fully explored. Generally, it can be said that the antimicrobial activity is, however, lower than for silver. Because of this reason, it is applied in combination with silver [59, 78]. It was proved that the antimicrobial activity against bacteria *Escherichia coli* and *Staphylococcus aureus* of silver/copper is higher than for just copper, but silver had higher antimicrobial activity [78].

Gold

The antimicrobial activity of gold appears to be lower than copper and silver [59, 78]. This activity is relatively low and can be improved by mixing with copper, silver, or both. Gold has high biocompatibility, polyvalent effect, is easily modified, and is photothermally stable. Also, for gold, the performance mechanism is not well known, and for that, the improvement of its antimicrobial activity is difficult. However, gold nanoparticles (Au-NPs) were employed in applications with biological systems involved. Theoretically, Au-NPs should improve cell wall penetration properties and be capable of delivering higher concentrations of antibiotics [59, 75, 79].

Zinc oxide

In recent years, zinc oxide (ZnO) received more considerable attention because of its stability under challenging processing conditions and generally non-toxic to humans. To further investigate the property of ZnO, further studies should be made. The exact mechanism of antibacterial activity is not entirely understood; however, it can be improved by morphology, concentration, particle size, etc. [59, 80]. Previous studies conclude that *Escherichia coli* and *Listeria monocytogenes* colonies constantly grow on PLA matrix with ZnO, up to several hours, and then it starts to decrease [81].

Titanium dioxide

Titanium dioxide (TiO₂) is a titanium compound occurring in nature in three crystallographic phases: brookite, anatase and rutile. In commonly employed concentrations, it is non-toxic. The mechanism of antibacterial activity of TiO₂ is a photocatalytic process. It can lead to the peroxidation of the polyunsaturated phospholipid component of the microbial lipid membrane. This is causing the death of the cell, which is triggered by the loss of respiratory

activity. TiO₂ incorporated into PLA, PCL, and cellulose acetate matrixes has proven antimicrobial activity of used polymers. This activity was tested against *Escherichia coli* [59, 82].

4 CHARACTERISATION OF DEGRADABLE POLYMERS

In this chapter, some of the instruments for the characterisation of degradation of polymers are listed. Not all of these methods were used in the practical part of this thesis.

4.1 Scanning electron microscopy

Scanning electron microscopy (SEM) is a microscopic instrument that works on an electron beam principle. This beam is of relatively low energy in a range of a few hundred eV to 40 keV. The image of the specimen is formed pixel by pixel as the beam scanning across the sample. Compared to optical microscopes, the advantages of SEM are their greater depth of field and higher resolution. However, the sample must be conductive. If it is not conductive, it is necessary to coat the specimen before inserting them into the SEM instrument. The utilization is the examination of the morphology or distribution of contained particles. The study of morphology is also possible after degradation, which gives a better understanding of the process and its conditions [83, 84, 85].

4.2 Transmission electron microscopy

Transmission electron microscopy (TEM) is a microscopic instrument presenting significant information about the size, shape, structure, and morphology of studied material. The principle of this technique is the transmission of a beam of electrons, generated out of an electron gun, through a thin sample, interacting with it. The image is formed from the interactions of the electrons transmitted through the specimen. Different types of sensors can detect it. TEM can be used to study a structure during degradation, and after degradation, for differences between samples, a similar way as SEM [86].

4.3 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is an analytic device used to characterise the thermal properties of a polymer. It can be used to measure the melting temperature (T_m), the crystallisation temperature (T_c), the glass transition temperature (T_g), the denaturation temperature, or heat capacity. This instrument can get similar curves as in Figure 9, where the figure shows characteristic temperatures for polymers [87, 88].

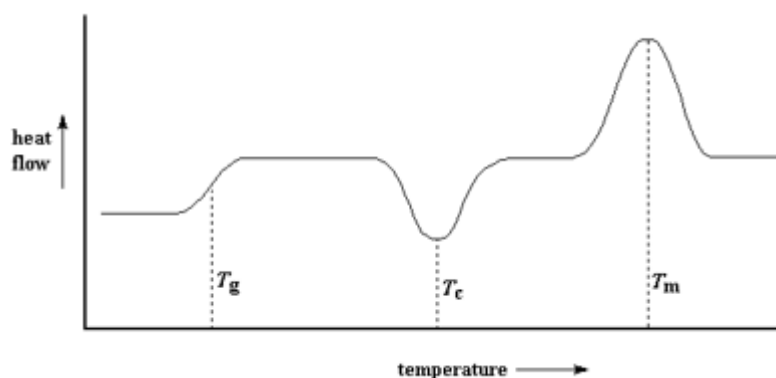


Figure 9 Scheme of DSC plot [88]

It is necessary to know the theoretical enthalpy of fusion for a 100% crystalline studied polymer to calculate the relative degree of crystallinity. This theoretical enthalpy is put into a ratio with the enthalpy of fusion of the polymer sample obtained from the DSC thermograph [84].

4.4 Dynamic mechanical testing

Dynamic mechanical testing (DMA) is an instrument that applies a small amount of deformation to a sample in a cyclic manner. In some cases, DMA can be called dynamic mechanical thermal analysis. This method investigates the response of the material to deformation, stress, temperature, frequency, and others. DMA is quite sensitive, and it can detect the secondary thermal transition. While studying the degradability of polymers, it is used to investigate mechanical changes during and after degradation [84, 89].

4.5 Gel permeation chromatography

Gel permeation chromatography (GPC) is a method applicable for determining the molecular weight distribution of polymers. The principle of this method is to separate particles by their size, thence, with their molar mass. The substance is dissolved in an appropriate solvent which is transferred between two phases: stationary and mobile [84, 90].

This method can also be used to study the interaction between macromolecules or be used as a purification technique. In addition, it can be applied to acquire information about the number average molecular weight (M_n), weight average molecular mass (M_w), viscosity average molecular weight, and average molecular weight [84, 90].

4.6 Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is an analytical instrument for defining the functional group in the system. Irradiation of the material by FTIR light absorption of the occurred infrared (IR) frequency coincides with the specific bond's vibration frequency in the sample. Every absorbed frequency is characteristic of a particular chemical group and its environment. FTIR is used to identify an unknown specimen and contributes to an understanding of chemical changes during degradation [84, 91].

4.7 Antimicrobial activity tests

Just very few polymer materials have sufficient antimicrobial behaviour. For this reason, the antimicrobial property is achieved by mixing or coating with antimicrobial agents. Nowadays, there is a growing interest in antibacterial packaging. It is applicable in the food industry, medicine or in healthcare [92].

Antimicrobial activity can be measured by several different methods. This chapter introduces two methods: disk diffusion method and antibacterial activity on plastic surfaces according to the standard ISO 22196:2011.

4.7.1 Disk diffusion method

The disk diffusion method, known as the Kirby-Bauer disk diffusion susceptibility test, was the first used method to determine the resistance or sensitivity of pathogenic aerobic and facultative anaerobic bacteria to diverse compounds in the medical environment for testing the efficiency of antibiotics [92].

Although this method is based on antibiotics testing, it is also hugely enhanced to determine the diffusion of the antimicrobial agent from polymer matter. The critical factor of effectiveness is the migration rate and velocity properties [92].

This method is done with a plastic disk or film on a culture suspension swabbed on a solid medium agar plate. The concentration of biocide must be determined in advance. Most often, it is made with a control cellulose disc on one Petri dish. After 18-24 hours of incubation, the results can be read by the naked eye or by computer software, such as Scan 500 Interscience. This is a quick and relatively cheap method of antimicrobial efficiency [92, 93].

4.7.2 Measurement of antibacterial activity on plastics surfaces

Standard ISO 22196:2011 is used for the specification of evaluating the antibacterial activity of antibacterial-treated surfaces of plastic products. The test is designed to examine the ability of investigated film to eliminate or prevent the growth of microorganisms over a period of contact, 24 hours. *Staphylococcus aureus* and *Escherichia coli* are specified bacteria strains, but other species can be used if required. The difference can appear because of the particular application of examined plastic material. Other conditions can be introduced to this standard, e.g. UV exposure to the films. The procedure and expression of the results of this method are introduced more closely in chapter 7.5 [94].

4.8 Biodegradation of plastic materials

It is possible to determine the biodegradation of materials by several methods. This chapter introduces two of those methods. The first one is the determination of the ultimate aerobic biodegradability of plastic materials under controlled composting condition. The second one is a preliminary evaluation of the disintegration of plastic under the simulated composting condition in a laboratory scale test [95, 96].

4.8.1 Biodegradation under controlled conditions

This standard ISO 14855-1 is a method that is applicable for determining the ultimate aerobic biodegradability of plastic materials by measuring the amount of evolved carbon dioxide and the tested material disintegration degree at the end of the test [95].

The principle is to measure the amount of evolved carbon dioxide by gas chromatography from examined samples in flasks and control flask. Out of these data it can be calculated the per cent of biodegradation, which needs a theoretical amount of carbon dioxide produced in each flask. This theoretical value is calculated out of the total weight of the dry examined plastic material. At the end of the test, the loss in mass of the tested polymer can be examined [95].

4.8.2 Disintegration test

The disintegration test is done in synthetic compost under controlled conditions. The standards, which can be followed are CSN EN 14806 and CSN EN ISO 20200. The examined material compostability is evaluated as the disintegration of the sample specimen to be smaller than 2 mm. This test is more closely described in chapter 7.6 [96].

II. PRACTICAL PART

5 AIM OF THE WORK

This thesis aimed to evaluate the influence of antimicrobial additives on the biodegradability of PLA films. Two types of PLA (type for thermoplastic extrusion and fibre processing) with antimicrobial additives addition in concentration 0 wt%, 0.5 wt%, and 1 wt% were studied.

For achieving this goal following sub-tasks were set:

- Thermoplastic preparation of samples;
- Characterization of used materials, with the focus on changes caused by added additives: structural and chemical changes, a shift in characteristic temperatures;
- Determination of antibacterial activity;
- Assessment of the effect of antimicrobial additives on degradability.

In the end, the samples biodegradability was discussed in relation to the characteristic properties of materials.

6 MATERIALS

The practical part of this thesis is focused on film preparation from two different PLA matrices with two inorganic additives in concentration 0, 0.5, and 1.0 wt%, respectively. The following materials were used for this purpose.

The first utilized polymer material was PLA Ingeo 2003D, named as Ingeo™ Biopolymer 2003D provided by a producer NatureWorks LLC, USA. It is material for conventional extrusion purposes. It is recommended for dairy containers, food service ware, transparent food containers, hinged-ware, and cold drink cups. Endorse processing temperatures are in the range of 190-210 °C [97].

The second used material was PLA Luminy LX175U, full name Luminy® LX175U, by producer Corbion, The Netherlands. It is amorphous compostable material with high viscosity. Potential processing methods are fibre (spinning) extrusion, film extrusion, or thermoforming. Its density is 1.24 g·cm⁻³ (similar to PLA Ingeo 2003D type). Endorse processing temperatures are in the range of 170-210 °C [98].

Nano ZnO is a nano zinc oxide product of the company BOCHEMIE a.s., Czech Republic. Accordingly to the datasheet, it is toxic for the water environment. The purity is 99.9 %, with manganese oxide as an impurity. It is a white powder without odour and a density of 5.61 g·cm⁻³. It is still widely used in various industries like plastics, rubbers, paints, filters or cosmetics. There is no limit applying to a concentration in a polymer matrix [99].

The additive “Sanitized Ag” was kindly supported by the production of Sanitized company, Switzerland. This fine white powder based on silver is the commercially available antimicrobial additive for biodegradable polymers [101].

6.1 Preparation of films

Firstly, both kinds of PLAs were dried at 60 °C for 24 h before usage. Secondly, the PLA matrix was mixed with antimicrobial additive (Nano ZnO or Sanitized Ag) in concentration 0, 0.5, 1.0 wt%, respectively. (The designation of the samples are shown in Table 1). The mixing process was done by melt-compounding in a Brebender mixer (Plastograph EC plus W 50 EHT), equipped with two stainless steel screws and a bypass valve to allow continuous material recirculation. Samples were thermoplastically melted at temperature 180 °C at an operating speed of 50 rpm for 10 minutes. Subsequently, the obtained products were

compression-moulded at 180 °C for 5 minutes in a manual press into a film (approximately 1 mm thick) and cooled under pressure until cold.

Table 1 Samples designation and their labelling

PLA matrix	Type of additive	Content of additives (wt%)	Labelling
PLA Ingeo 2003D	-	0	PLA D
	Sanitized Ag	0.5	D 0.5 Ag
		1	D 1 Ag
	Nano ZnO	0.5	D 0.5 ZnO
1		D 1 ZnO	
PLA Luminy LX175U	-	0	PLA L
	Sanitized Ag	0.5	L 0.5 Ag
		1	L 1 Ag
	Nano ZnO	0.5	L 0.5 ZnO
1		L 1 ZnO	

7 METHODS AND DEVICES

This chapter introduces methods applied for the characterisation of the materials, their antibacterial activity and degradability.

7.1 Scanning electron microscopy

SEM was used to characterise the fractured surface, focusing on dispersion and distribution of additive in the matter. Phenom Pro microscope (Phenom-World BV, The Netherlands) with magnifications 140x and 1000x was used. The microscope was operated in vacuum mode at an acceleration voltage of 5 kV. Analysis was performed on the cryo-fractured parts of the neat PLA films and samples with additives to evaluate the degree of homogeneity and gain insight into the internal structure of the films.

7.2 Differential scanning calorimetry

The thermal properties of samples were obtained by differential scanning calorimetry. The test was carried out using Mettler-Toledo DSC 1 STARe System. The samples, which weight was between 5-8 mg, were loaded into aluminium pans.

Two heatings were done for each specimen. The first heating stage from 25 °C to 180 °C, followed by cooling to -30 °C, and finally heating up to 250 °C. Afterwards, it was cooled to room temperature to remove the samples from the instrument. All scans were done at 10 °C·min⁻¹ under a nitrogen atmosphere. The first heating was proceeded to remove prior processing history and is not included in the results. The temperatures T_g , T_m and the cold crystallization temperature (T_{cc}) were determined during the second heating scan. The degree of crystallinity (X_c) was calculated accordingly to the following equation (1) [101]:

$$X_c = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0 \cdot w} \cdot 100\% \quad (1)$$

where ΔH_m is melting enthalpy, ΔH_{cc} is cold crystallization enthalpy, ΔH_m^0 is the heat of fusion associated with pure crystalline PLA (93.1 J·g⁻¹), and w is the weight fraction of PLA in the sample [102].

7.3 Gel permeation chromatography

GPC examination was carried out on an HT-GPC 220 chromatographic system, equipped with a dual detection system (viscometric response and refractive index detectors).

Overnight 25 mg of each sample was dissolved in 10 ml of tetrahydrofuran (THF) in triplicate. After that, samples were filtered through a 0.45 μm PTFE syringe filter. The detection and selection took place on PL gel-mixed bed columns (1x Mixed-A, 300 \times 7.8 mm, 15 μm particles + 1x Mixed-B, 300 \times 7.8 mm, 10 μm particles + 1x Mixed-D, 300 \times 7.8 mm, 5 μm particles) at 40 $^{\circ}\text{C}$ in THF; the flow rate of mobile phase equalled 1.0 $\text{m}\cdot\text{min}^{-1}$, and the injection volume was 100 μl . Molecular weights M_w and M_n were established. All processed data were carried out using Cirrus software.

The following equations (2) and (3) defining molecular weights (M_n , M_w) [84]:

$$M_n = \frac{\sum_{i=1}^x N_i M_i}{\sum_{i=1}^x N_i} \quad (2)$$

$$M_w = \frac{\sum_{i=1}^x N_i M_i^2}{\sum_{i=1}^x N_i M_i} \quad (3)$$

where N_i is the number of polymer chains, characterise by M_i .

The polydispersity index (PDI), which means the heterogeneity of chains, was calculated by equation (4) [84]:

$$PDI = \frac{M_w}{M_n} \quad (4)$$

7.4 Infrared spectroscopy

The influence of antimicrobial additives on PLA samples' chemical surface properties was assessed by spectroscopic analysis, carried out using an ATR-FTIR spectrometer Nicolet Si5, with germanium crystal and resolution 4, by 64 scans. The spectra were recorded in the range of 4000 – 650 cm^{-1} .

7.5 Antibacterial activity

Antibacterial activity was evaluated according to the ISO 22196:2011 standard. The methodology was performed as follows.

The specimens were disinfected with 70% ethanol before testing. Two bacteria strains suspensions were used for utilization. As a gram-positive bacteria strain, *Staphylococcus aureus* was suspended in a density of $5.6 \cdot 10^5$ CFU $\cdot\text{ml}^{-1}$. *Escherichia coli* in density

$2.3 \cdot 10^5$ CFU·ml⁻¹ was selected as a gram-negative bacteria. These strains were prepared in 1/500 nutrient broth. The bacteria suspension was applied to the surface of the specimen at a volume of 100 µl. Specimens had dimensions of 25x25 mm. Each sample was covered with inert polypropylene foil, with dimensions of 20x20 mm. Poised samples were cultivated at 35 ± 2 °C and min. 90% relative humidity for 24 hours [94].

The cover foils were retrieved, and each sample was cleansed with SCDLP (Soybean Casein Lecithin Polysorbate 80) broth at the end of the incubation period. To determine the viable bacteria, the pour plate method was used, followed by another incubation period at 35 ± 2 °C of 24 hours. After that, the number of viable bacteria recovered per cm² per test specimen was estimated accordingly to equation (5) [94]:

$$N = \frac{100 \cdot C \cdot D \cdot V}{A} \quad (5)$$

where N is the number of viable bacteria recovered per cm² per test specimen, C is the average plate count for the duplicate plates, D is the dilution factor for the plates counted, V is the volume of SCDLP added to the specimen in ml, and A is the surface area of the cover foil in mm² [94].

The efficacy of the tested films was expressed as antibacterial activity (R), calculated via equation (6) [94]:

$$R = U_t - A_t \quad (6)$$

where R is an antibacterial activity, U_t is the average of the common logarithm of the number of viable bacteria (CFU·cm⁻²) recovered from the untreated sample (blank); A_t represents the common logarithm of the number of viable bacteria (CFU·cm⁻²) recovered from the treated test specimens after 24 h [94].

7.6 Test of degradability

Testing the degradability of samples was performed according to standard ISO 20200:2015 Plastics — Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test. The analysis conditions were the following: in every reactor were 22-24 pieces of samples of dimensions 15x15x1 mm with the total weight 5-7 g; the set temperature during the process was 58 °C and humidity 90%. The test was in duplicate [96].

The synthetic solid waste, in which the samples were biodegraded, had a specific number of components, as shown in Table 2. Solid waste was put into reactors of dimensions 30x20x10 cm. Into the reactor was put 450 g of synthetic waste and 550 g of water [96].

Table 2 Amount of components in synthetic solid waste

Material	Weight in a dry state [%]	Description
Sawdust	40	Local joinery workshop - a mixture of hardwood and softwood
Food for rabbits	30	Versele-Laga Country's best
Compost	10	Central composting plant Brno
Starch	10	Ruf Corn Starch (Kaufland)
Saccharose	4	TTD sugar (Kaufland)
Oil	4	Refined Sunflower Oil (Kaufland)
Urea	2	PENTA Chemicals, Czech Republic
In total	100	

Based on Table 3, the inspection of each reactor was performed; pH, temperature, and water loss were measured. Water was added up to 100% of the original weight [96].

Termination of the test has been carried out after the disintegration of the samples. The disintegration degree was calculated by the following equation (7) [96]:

$$D = \frac{M_i - M_r}{M_i} \cdot 100\% \quad (7)$$

where M_i is the initial dry mass of plastic material and M_r corresponds to the dry mass of the recovered plastic material after composting and sieving [96].

In order to validate the disintegration results, the volatile solids decreasing (R) and the variability of the disintegration degree were calculated by the following equation (8) [96]:

$$R = \frac{(M_{SW} \cdot DM_{SW} \cdot VS_{SW}) - (M_C \cdot DM_C \cdot VS_C)}{(M_{SW} \cdot DM_{SW} \cdot VS_{SW})} \cdot 100\% \quad (8)$$

where M_{SW} denotes the initial mass of the wet synthetic material before composting, DM_{SW} is the initial dry mass of synthetic waste (as % of total mass), and VS_{SW} represents the volatile solids of the initial synthetic material (as a percentage of DM). The term M_C corresponds to the final dry mass of the obtained compost, DM_C represents the final dried

mass of compost (as % of total mass), and VS_C is the value of the volatile solid of the obtained compost (as % of DM) [96].

Table 3 Composting procedure (thermophilic incubation period) [96]

Time from start days	Operation
0	Record initial mass of the reactor.
1,2,3,4,7,9,11,14	Weight reactor and add water to restore the initial mass, if needed. Mix the composting matter.
8,10,16,18,21,23,25,28	Weight reactor and add water to restore the initial mass, if needed. Do not mix the composting matter.
30,45	Weight reactor and add water to restore the mass to 80 % of the initial mass, if needed. Mix the composting matter.
From 30 till 60, twice a week	Weight reactor and add water to restore the mass to 80 % of the initial mass, if needed. Mix the composting matter.
From 60 onward, twice a week	Weight reactor and add water to restore the mass to 70 % of the initial mass, if needed. Mix the composting matter.

8 RESULTS AND DISCUSSION

The films with an approximate thickness of 1 mm were successfully prepared by thermoplastic processing. The films were mate and milk-coloured.

The following chapter discusses the results of the tested materials and their changes depending on antimicrobial additive incorporation during thermoplastic processing.

8.1 Scanning electron microscopy

The effect of Nano ZnO and Sanitized Ag particles, respectively, on PLA matrix morphology was investigated by SEM, as shown in Figure 10 -13.

Figure 10 depicted the PLA 2003D matrix and identical matrix with additives, respectively, at a magnification of 140x. A slight separation of the external layer (red circles) can be observed for this matrix. But this is only followed by lower concentrations of both additives Figure 10B, Figure 10D, and neat matrix Figure 10A. This phenomenon is not observed in materials with matrix PLA Luminy LX175U (Figure 12).

The fracture on the edge of samples with the PLA Luminy LX175U matrix is getting less rough with increasing additives concentration (Figure 12). The surface of samples is smoother with enhanced additive concentration for both matrices.

All samples have a homogenous distribution of additives (Figure 11 and Figure 13) in the whole structure with visible aggregation of particles.

Studies of degradation of PLA/ZnO and PHBV(poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/ZnO composites prepared by melt processing and effects of Ag, ZnO and TiO₂ nanoparticles at low contents on the crystallization of PLA showed similar trends when incorporating ZnO and Ag particles into the PLA matrix [103, 104].

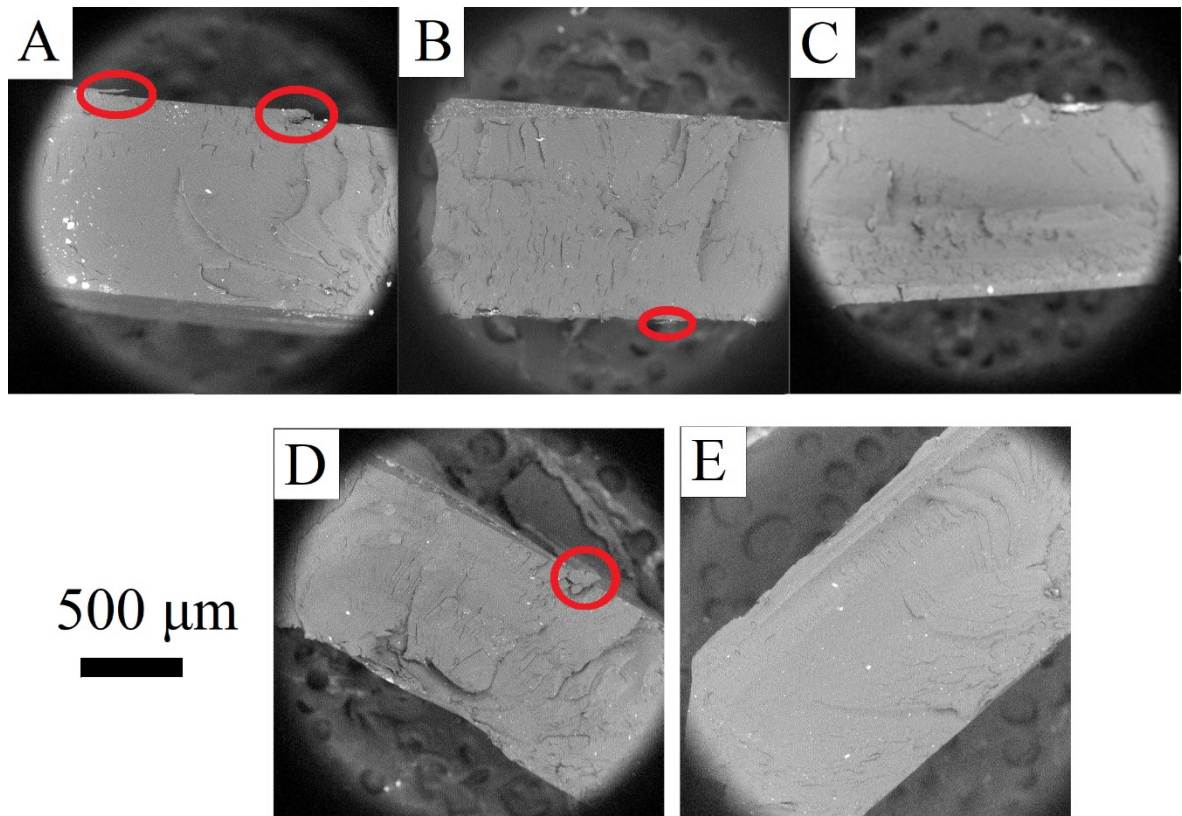


Figure 10 Fractured surface of PLA Ingeo 2003D matrix, 140x, magnification (A – PLA D, B – D 0.5 Ag, C – D 1 Ag, D – D 0.5 ZnO, E – D 1 ZnO)

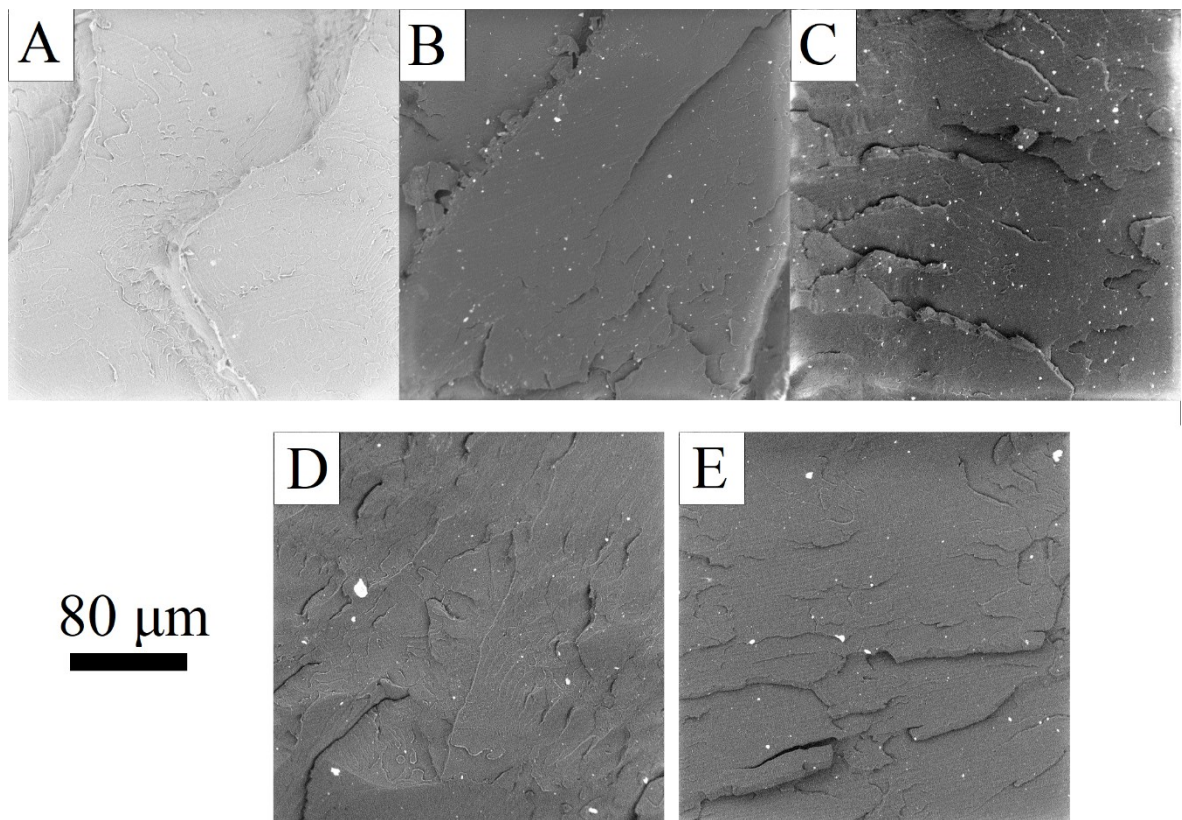


Figure 11 Fractured surface of PLA Ingeo 2003D matrix, 1000x magnification (A – PLA D, B – D 0.5 Ag, C – D 1 Ag, D – D 0.5 ZnO, E – D 1 ZnO)

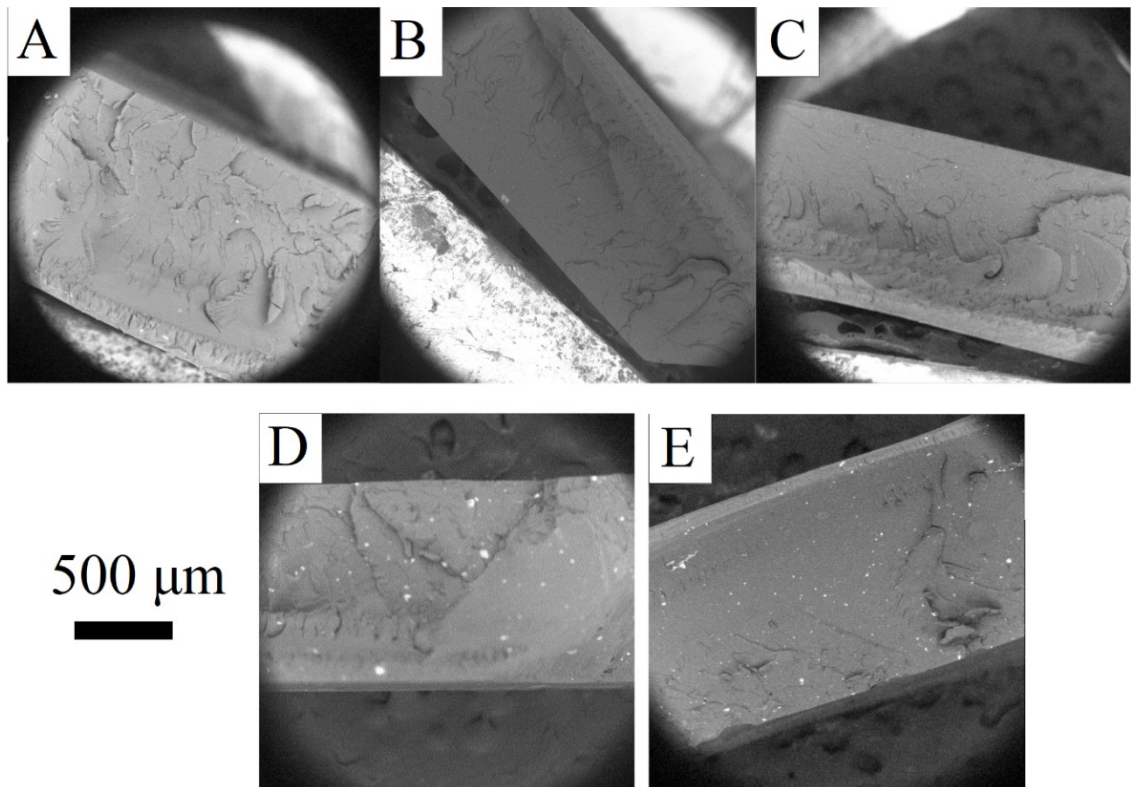


Figure 12 Fractured surface of PLA Luminy LX175U matrix, 140x magnification
(A – PLA L, B – L 0.5 Ag, C – L 1 Ag, D – L 0.5 ZnO, E – L 1 ZnO)

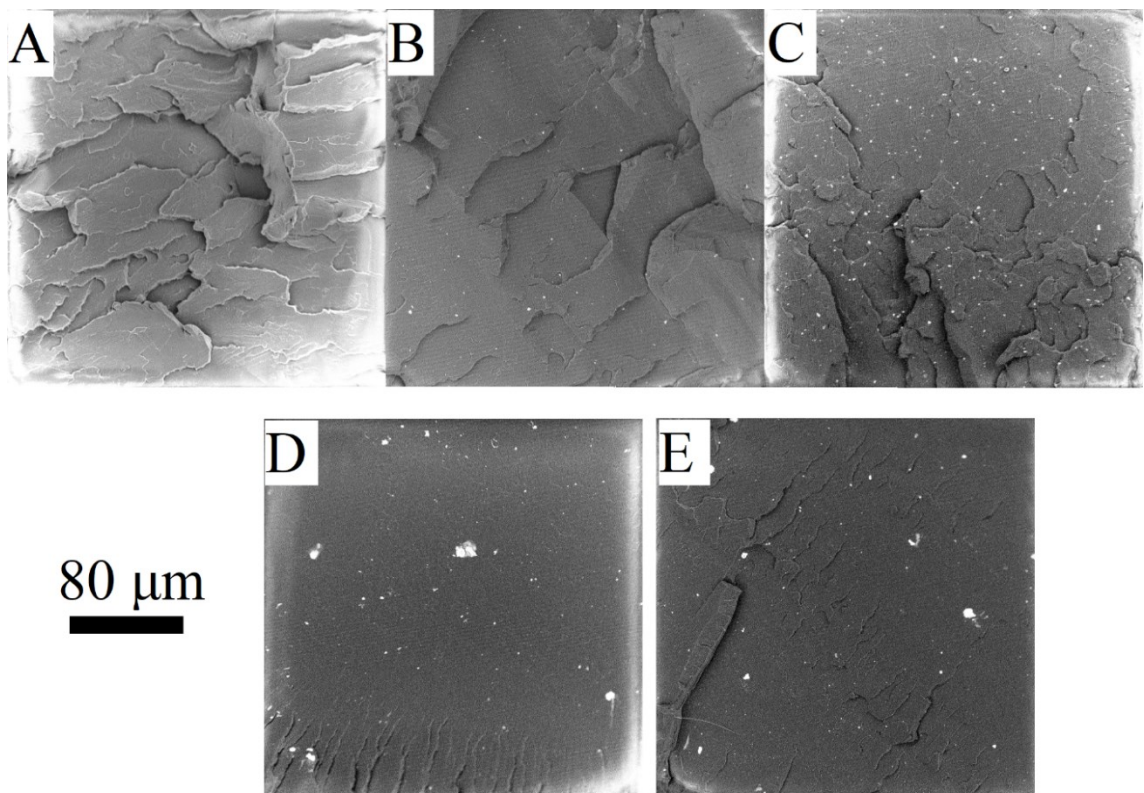


Figure 13 Fractured surface of PLA Luminy matrix LX175U, 1000x magnification
(A – PLA L, B – L 0.5 Ag, C – L 1 Ag, D – L 0.5 ZnO, E – L 1 ZnO)

8.2 Differential scanning calorimetry

DSC was used to define characteristic polymer temperatures, glass transition temperature, melt temperature, and cold crystallisation temperature. According to equation (1), relative crystallisation for every sample was calculated. These results are shown in Table 4.

Table 4 Results of DSC analysis

		T _g (°C)	T _{m1} (°C)	T _{m2} (°C)	T _{cc} (°C)	X _c (%)
PLA Ingeo 2003D	PLA D	60.58	150.39	-	117.59	0.67
	D 0.5 Ag	61.40	151.48	-	119.14	1.00
	D 1 Ag	61.17	150.90	-	118.92	0.56
	D 0.5 ZnO	59.91	152.36	-	128.66	0.91
	D 1 ZnO	59.50	152.20	-	131.77	0.25
PLA Luminy LX175U	PLA L	59.59	158.41	166.63	110.43	1.42
	L 0.5 Ag	60.79	158.93	167.20	110.53	3.30
	L 1 Ag	60.61	158.60	166.84	110.56	4.51
	L 0.5 ZnO	60.61	160.33	168.12	114.26	1.48
	L 1 ZnO	60.03	158.02	165.77	111.52	7.84

DSC results revealed that the glass transition of PLA Ingeo 2003D samples with additives had changed insignificantly. To specify, glass transition temperatures for this type of PLA, incorporating Sanitized Ag in both concentrations, had slightly increased approximately by 1 °C. In contrast, T_g of PLA mixed with ZnO moderately decreased. The study of degradation of PLA/ZnO and PHBV/ZnO composites also reported a meagre decline in glass transition temperature [103].

Thermal analysis outcomes of the glass transition temperature of PLA Luminy LX175U with additives did not report significant changes compared to neat material. The glass transition temperature of all samples increased slightly, with the highest value of 1.20 °C for the sample L 0.5 Ag.

Melting temperatures of samples based on PLA Ingeo 2003D did not report significant changes. The temperature for PLA with Sanitized Ag raised approximately by 1 °C. Meanwhile, T_m of PLA with ZnO enlarged by 2 °C. Similar results of T_m reported in a study by Pantani et al. [105].

All samples containing PLA Luminy LX175U expressed bimodal melting behaviour, which is observed quite often for the conduct of PLA. Several possible reasons for bimodal melting behaviour include: (a) formation of the disordered alpha phase of PLA caused by low

crystallisation temperature; (b) polymer is containing more than one crystal structure, α and β crystals of PLA; (c) melting of the original crystals, recrystallisation, and remelting the recrystallised crystals during the heating scan; (d) distinct lamellae morphologies formed before the heating scan are present in crystals; (e) it can be caused by processing conditions and molecular weight distribution. This bimodal melting behaviour of PLA is recorded in a study by Manju et al. [106] and Yuzay et al. [107].

In comparison with neat PLA Luminy LX175U, all specimens have slightly higher both T_{m1} and T_{m2} , except sample L 1 ZnO. The sample with the highest value of T_{m1} and T_{m2} is L 0.5 ZnO; these temperatures are higher than neat material by 2 °C and 1.5 °C, respectively. The sample L 1 ZnO has an indistinguishably lower T_{m1} by 0.3 °C and T_{m2} by 1 °C.

The cold crystallisation temperature of the samples based on PLA Ingeo 2003D compared with neat PLA, all samples have risen. The highest T_{cc} had sample D 1 ZnO, 14.59 °C higher than PLA D. T_{cc} of D 0.5 ZnO had also increased significantly by 11.07 °C. Higher cold crystallization temperature may be caused by decreased crystallinity. Samples with additive Sanitized Ag had risen on average by just 1 °C.

Thermal analysis outcomes of cold crystallization temperature of PLA Luminy LX175U with additives had notably changed just for sample L 0.5 ZnO by 3.83 °C, the lowest change of T_{cc} was for the sample L 0.5 Ag (0.10 °C). T_{cc} of all samples with additives were higher than T_{cc} of neat material.

PLA Ingeo 2003D crystallinity of both samples containing 0.5 wt% of additive was vaguely higher than the crystallinity of neat material. The crystallinity value of specimens D 0.5 Ag and D 0.5 ZnO was 0.33% and 0.24 % higher than the crystallinity of neat material, respectively. On the other hand, the crystallinity of samples containing 1 wt% of additive, D 1 Ag and D 1 ZnO, was subtly lower, by 0.11 % and 0.42 %, respectively. A similar trend was concluded in the study by Črešnar et al. [104].

The crystallinity of PLA Luminy LX175U specimens with additives raised more in comparison with neat PLA material. The highest crystallinity value for the sample L 1 ZnO is 7.84%, which is 6.43% higher than PLA L. The rising crystallinity can be caused by the nucleation effect, which can occur in additive presence, serving as nucleation agent. Similar results were concluded in the study of Nanocomposites of PLA containing ZnO nanofibers made by solvent cast 3D printing [108].

Interactions between additive and matrix cause the differences between the crystallization values of concentrations of additives in PLA Ingeo 2003D and PLA Luminy LX175U matrices. If the interaction is weak or absent, the additive has the role of nucleation agent. On the other hand, when the polymer is strongly interacting with additive particles, the nucleating role is lacking. The decrease in crystallinity of PLA with additives, silver and ZnO, reported study of effects of Ag, ZnO and TiO₂ nanoparticles at low contents [104].

A look at the comparison between both materials, PLA L and PLA D, T_g did not obtain significant differences. Both matrices with additive Sanitized Ag increased their glass transition temperature, a modest rise in melting temperature, and a lower increase with a 0.5 wt% concentration [104]. The same trend showed results in a study by Črešnar et al. [104]. Generally, for samples of neat material and samples of materials with additives, T_{cc} is higher for PLA D samples. However, crystallinity is lower for both additives of 0.5 wt%, caused by strong interactions between matrix and additive, as mentioned above.

8.3 Gel permeation chromatography

The samples were analyzed by GPC for the characterization of M_w, M_n, and PDI, which are summarized in Table 5.

Table 5 Number average molecular weight, weight average molecular mass and polydispersity index of prepared films

Material	Sample	M _n [g·mol ⁻¹]	M _w [g·mol ⁻¹]	PDI [-]
PLA Ingeo 2003D	PLA D	46,000	123,000	2.7
	D 0.5 Ag	49,000	130,000	2.7
	D 1 Ag	47,000	124,000	2.6
	D 0.5 ZnO	23,000	55,000	2.4
	D 1 ZnO	17,000	40,000	2.4
PLA Luminy LX175U	PLA L	23,000	54,000	2.3
	L 0.5 Ag	25,000	59,000	2.4
	L 1 Ag	30,000	76,000	2.5
	L 0.5 ZnO	26,000	74,000	2.4
	L 1 ZnO	31,000	64,000	2.1

The additive Sanitize Ag incorporation into PLA Ingeo 2003D has increased both M_w and M_n in both concentrations. Samples containing 0.5 wt% of this additive have a more significant rise than samples containing 1 wt% Sanitized Ag. M_w and M_n of D 0.5 Ag increased by 5 % and 6 %, respectively. The M_w and M_n for sample D 1 Ag grew by 1 %

and 3 %, respectively. A similar trend in the number average molecular weights change was observed in PMMA/silver nanocomposites studied by Siddiqui et al. [109].

On the contrary, samples containing ZnO in both concentrations decreased molecular weight compared to PLA D. M_w reduced by 55 % for D 0.5 ZnO and 67 % for D 1 ZnO. The decrease of M_n was not as steep; D 0.5 ZnO declined by 50 %, and D 1 ZnO by 63 %. This reduction can be caused by the catalytic effect of the Nano ZnO additive causing the matrix degradation during melt mixing and compression moulding of the samples. The same conclusion was reached in the study of Accelerated disintegration of compostable Ecovio polymer by using ZnO particles as filler [110].

Specimens containing matrix PLA Luminy (see Table 5) show increased molecular weight for both additives and their concentrations compared to neat material. The growth of molecular weights was more significant for samples containing 1 wt% of the additive, except for M_w of L 0.5 ZnO had increased by 36 %, which is more than L 1 ZnO (rise by 18 %). The increase of molecular weights of samples containing Nano ZnO can be caused by the property of ZnO, which is in the industry used as a polymerization catalyst. A similar assumption was made in the study of efficient ZnO aqueous nanoparticle catalysed lactide synthesis for poly(lactic acid) fibre production from food waste [111].

The polydispersity index (PDI) determines the heterogeneity of a sample based on size. In polymer science, the PDI index indicates the distribution of polymer chain molecular weights in a given polymer [112]. All PDI were over 2, the lowest value was for L 1 ZnO (2.4), and the highest one is for PLA D and D 0.5 Ag (2.7). So it can be concluded that prepared samples exhibit a broad size distribution of particles in the matter.

8.4 Infrared spectroscopy

FTIR technique was used to characterise the interaction between two concentrations of Sanitized Ag and Nano ZnO in the two types of PLA, respectively. Measured curves are depicted in Figure 14 and Figure 15. The first peak (PLA D/PLA L) at $757/755\text{ cm}^{-1}$ can be assigned to the polymer crystalline phase and $874/870\text{ cm}^{-1}$ to the amorphous phase of PLA. The peaks at $1086/1090\text{ cm}^{-1}$ showed asymmetric modes of C-O-C, and the peaks at $1185/1182\text{ cm}^{-1}$ were associated with the symmetry stretching of a -C-O-C stretch of the ester group. The CH deformation and asymmetric band were found at $1381/1385\text{ cm}^{-1}$ and $1362/1364\text{ cm}^{-1}$, respectively. The absorption for asymmetric -CH₃ deformation vibration

was observed at $1453/1453\text{ cm}^{-1}$. Peaks at $1754/1756\text{ cm}^{-1}$ were attributed to the C=O vibration of the ester group in the PLA. The bands at $2936/2936\text{ cm}^{-1}$ and $2994/2991\text{ cm}^{-1}$ were assigned to the symmetric and asymmetric stretching vibration of the CH₃ group, respectively [81, 113, 114].

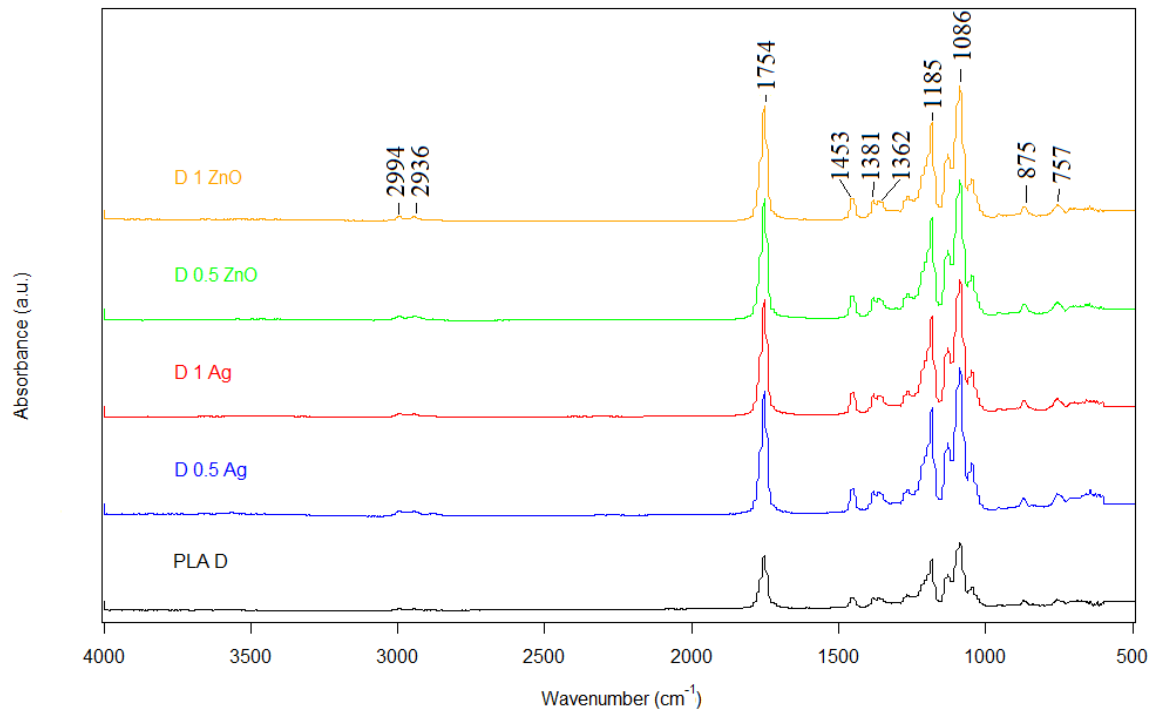


Figure 14 FTIR spectra of samples with PLA Ingeo 2003D matrix (PLA D, D 0.5 Ag, D 1 Ag, D 0.5 ZnO, D 1 ZnO)

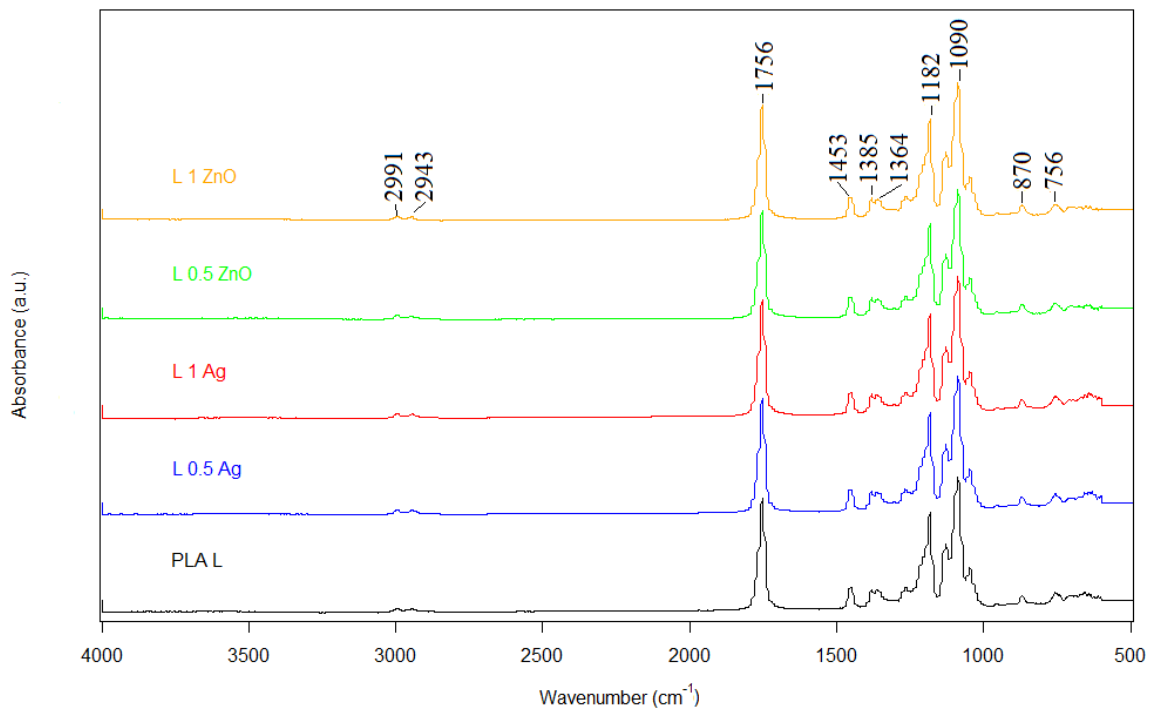


Figure 15 FTIR spectra of samples with PLA Luminy LX175U matrix (PLA L, L 0.5 Ag, L 1 Ag, L 0.5 ZnO, L 1 ZnO)

In conclusion, all samples have bonds that are characteristic of PLA. No bond had appeared, and no bond had disappeared. Infrared spectra of PLA samples and modified specimens with additives were quite identical. The addition of additives did not change samples with both polymer matrices, PLA Ingeo 2003D and PLA Luminy LX175U. These results indicate that only a weak intermolecular bond had formed between PLA and additives [81].

Similar trends of FTIR spectra for PLA samples with silver were also observed in the study of silver/poly(lactic acid) nanocomposites [76] and the research of the effect of Ag, ZnO and TiO₂ nanoparticles at low contents on the crystallization, semicrystalline morphology, interfacial phenomena and segmental dynamics of PLA [104]. A previous study aimed to investigate ZnO and silver particles, which outcomes also correlated with the results of this thesis. The paper incorporation of zinc oxide nanoparticles improved the mechanical, water vapor barrier, UV-light barrier, and antibacterial properties of PLA-based nanocomposite films additionally showed similar results for PLA with ZnO particles [81].

8.5 Antibacterial activity

The *in vitro* antibacterial activity analysis of prepared films was done accordingly to the test method based on ISO 22196:2011. The antibacterial activity of the samples against used

bacteria strains, *S. aureus* and *E. coli*, were expressed as values N and R, summarized in Table 6.

Table 6 Evaluation of ISO 22196:2011

Material	Sample	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		N (CFU·cm ⁻²)	R (-)	N (CFU·cm ⁻²)	R (-)
PLA Ingeo 2003D	PLA D (blank)	2.2·10 ⁵	-	1.1·10 ⁶	-
	D 0.5 Ag	2.2·10 ¹	3.4	5.4·10 ¹	2.0
	D 1 Ag	1.6·10 ²	2.5	7.4·10 ²	0.8
	D 0.5 ZnO	4.8·10 ³	1.0	1.1·10 ²	1.7
	D 1 ZnO	2.4·10 ³	1.3	4.1·10 ⁵	0
PLA Luminy LX175U	PLA L (blank)	1.1·10 ⁵	0	3.3·10 ⁴	0
	L 0.5 Ag	1.1·10 ²	2.7	<1	≥3.7
	L 1 Ag	<1	≥4.7	<1	≥3.7
	L 0.5 ZnO	<1	≥4.7	<1	≥3.7
	L 1 ZnO	<1	≥4.7	<1	≥3.7

The antimicrobial activity of samples with PLA 2003D matrix is shown in Table 6. PLA D and PLA L (neat materials) have no antibacterial properties, as expected for both bacteria strains. Antibacterial activity was heavily dependent on the homogeneity of the sample surface, the presence of antimicrobial additive near the surface of the specimen, and the aggregation of the particles. Results showed that all samples with Sanitized Ag additive had reduced the growth of colonies. These outcomes correlate with the study results by Zhang et al. [115] and Shameli et al. [76]. The reduction of bacteria growth presented that all antibacterial modifications were more effective against *S. aureus* than against *E. coli*, except for the D 0.5 ZnO sample. The study of ZnO-PLA nanocomposite coated paper for antimicrobial packaging application proved antimicrobial activity of ZnO incorporated into PLA matrix to be effective against *S.aureus*; however, in their case, only samples with 3 wt% of ZnO could be classified as antimicrobial. In the same study, the *E. coli* bacteria strain was also tested, as a gram-negative individual, and it was concluded that *E.coli* was more susceptible to ZnO nanoparticles than *S. aureus*. The antimicrobial activity against *E.coli* was notated already at 0.5 wt% of ZnO nanoparticles. [116]. The results of this study are consistent with this thesis.

The D 1 ZnO showed as ineffective against *E.coli*. It seems as a higher concentration of Nano ZnO has lower activity against *E. coli*. This could be provoked by the aggregation of particles, thus reducing the surface area of particles, which was confirmed by SEM

micrographs. It means that the aggregates of ZnO particles are enclosed in a polymer structure and therefore cannot come into direct contact with the bacteria cell. Another reasoning for this inactivity could be a controversial objection to the antimicrobial activity of ZnO. These conclusions were reached by the authors of the study of the improvement of mechanical properties and antibacterial activity of electrospun poly(D, L-lactide)-based mats by incorporation of ZnO-graft-poly(D, L-lactide) nanoparticles [117].

Research results, obtaining activity against *E. coli* is a controversial subject, and their explanation is not the aim of this thesis. There is inconsistency for antimicrobial activity of ZnO in different testing methods, method of incorporation of ZnO particles into a matrix, surface modifications and size of ZnO particles.

The only sample based on PLA Luminy LX175U with detected *S. aureus* colonies after incubation is L 0.5 Ag. There were no visible colonies of *S. aureus* and *E. coli* after incubation on the PLA Luminy samples with 1 wt% Ag, 0.5 wt% ZnO and 1 wt% ZnO. The sample L 0.5 Ag showed no *E. coli* colonies after incubation. It means that these concentrations of additives are highly effective against tested microorganisms. Moreover, it can be concluded that polymer matrix PLA Luminy LX 175U (amorphous type appropriate for fibres) is more suitable for antimicrobial modifications in comparison with PLA Ingeo 2003D (semicrystalline extrusion type). It is caused by crystal and amorphous parts in the structures.

8.6 Test of degradability

The disintegration of tested materials was conducted as introduced in chapter 7.6. This test was implemented to investigate the influence of antimicrobial additive incorporation into biodegradable polymer on the change of decomposition time and the comparison of degradation rate between samples. For the verification of the correctness of the procedure, pH values for each reactor were measured at the predefined time. These pH values can be seen in Figure 18, representing PLA Ingeo 2003D and Figure 19 illustrates PLA Luminy LX175U samples. The equation (7) was not used in any cases because no specimen residue of samples was found after the termination of the test.

Accordingly to standard ISO 20200:2015, the result of R can be considered valid as all the calculated values for the 20 reactors were greater than 30 %.

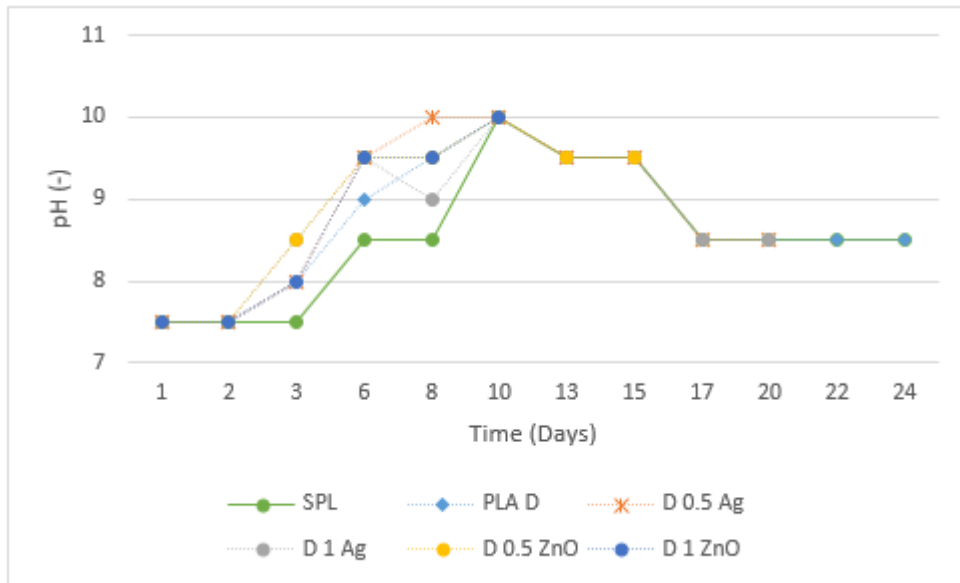


Figure 16 Compost pH variation during the composting period with samples containing PLA Ingeo 2003D matrix

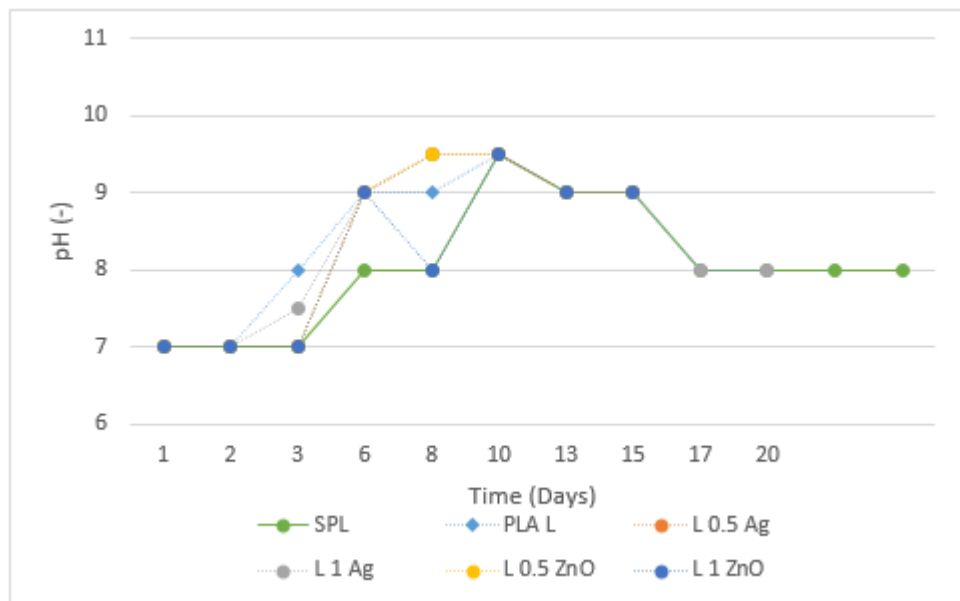


Figure 17 Compost pH variation during the composting period with samples containing PLA Luminy LX175U matrix

Figure 16 and 18 shows the average mean pH of two reactors for each specimen over time. The SPL line indicated the process in the reference reactors, which contained synthetic waste only. The reactors with specimens deviate from the blind trial in the 3-8th day (samples containing PLA Ingeo 2003D matrix) and 6-8th day (samples containing PLA Luminy

LX175U matrix). This aberration was caused by an ongoing decomposition reaction of the polymer samples. The standard deviations were under 5 %.

Figure 18 and Figure 19 displayed the visual appearance of composite films retrieved at different times of disintegration in compost. It can be seen that modified specimens started to be yellow already after one day of testing compared to samples of neat materials (PLA D and PLA L). This signalled the water absorption and hydrolytic degradation process, causing the formation of low molecular weight compounds [118]. Over the composting, the materials started to have a dark colour. It was impossible to clean the samples without devaluation of the samples, as shown in Figure 18 and Figure 19. The samples have also begun to be slimy, with advanced hydrolysis.

All samples with additives degraded prior to neat materials. Both neat materials degraded after 24 days of composting. The first sample that disintegrated was D 1 ZnO in 10 days (see Figure 18). This additive lowered T_g , M_w and M_n , as showed DSC and GPC outcomes, indicating degradation already during the mixing and compression moulding process. ZnO particles accelerated the degradation, and a similar conclusion was published by Anžlovar et al. They suggested minimalizing the melt processing time as much as possible [103]. Another possible explanation could be larger ZnO particles in the composite structure caused by aggregation. Campo et al. reached a similar conclusion that larger the size of the incorporated particles, the higher rate of decomposition occurs [110].

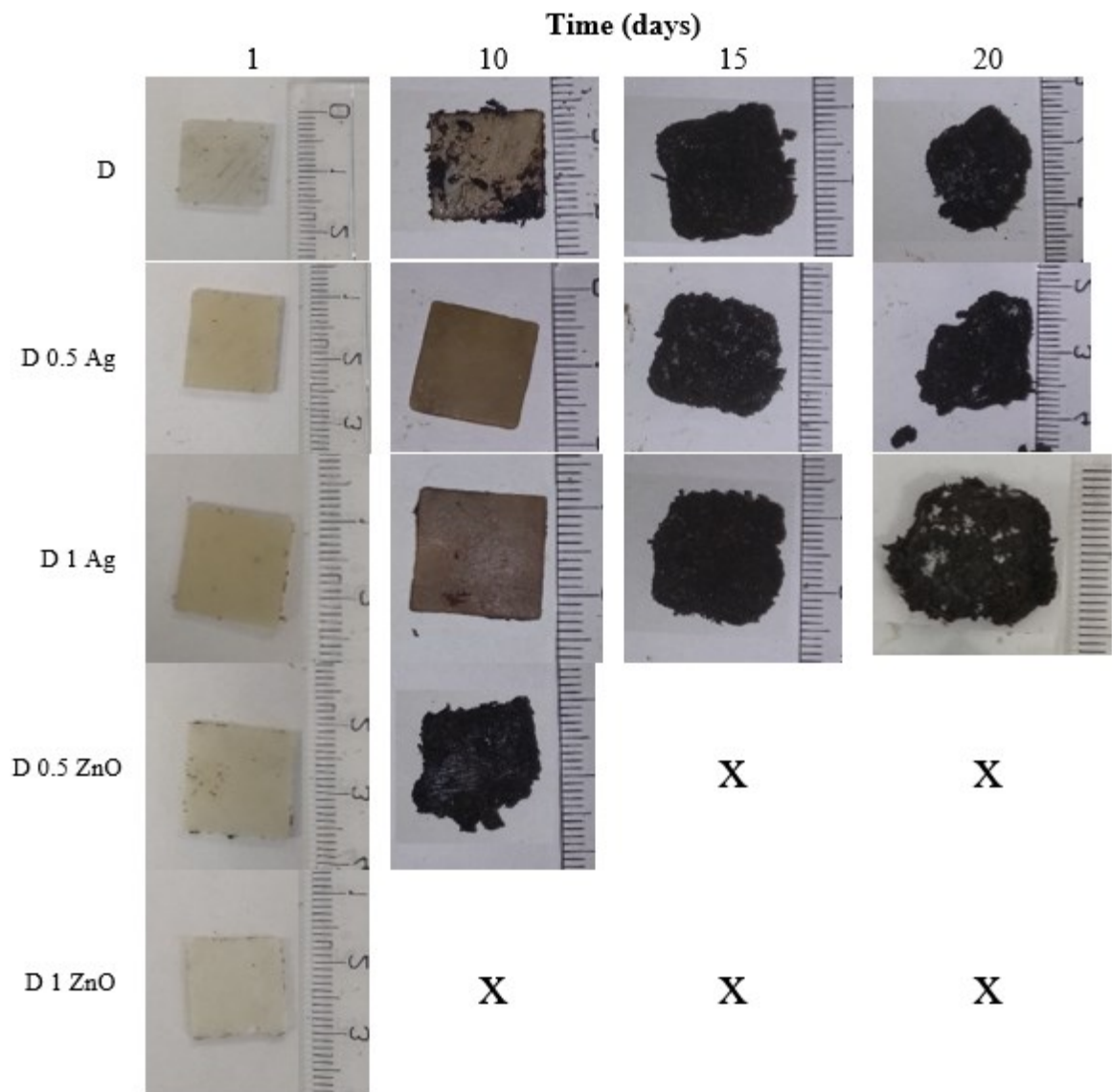


Figure 18 Process of degradation of samples containing PLA 2003D matrix, X means that no samples were found in monitored days (scale is an office ruler)

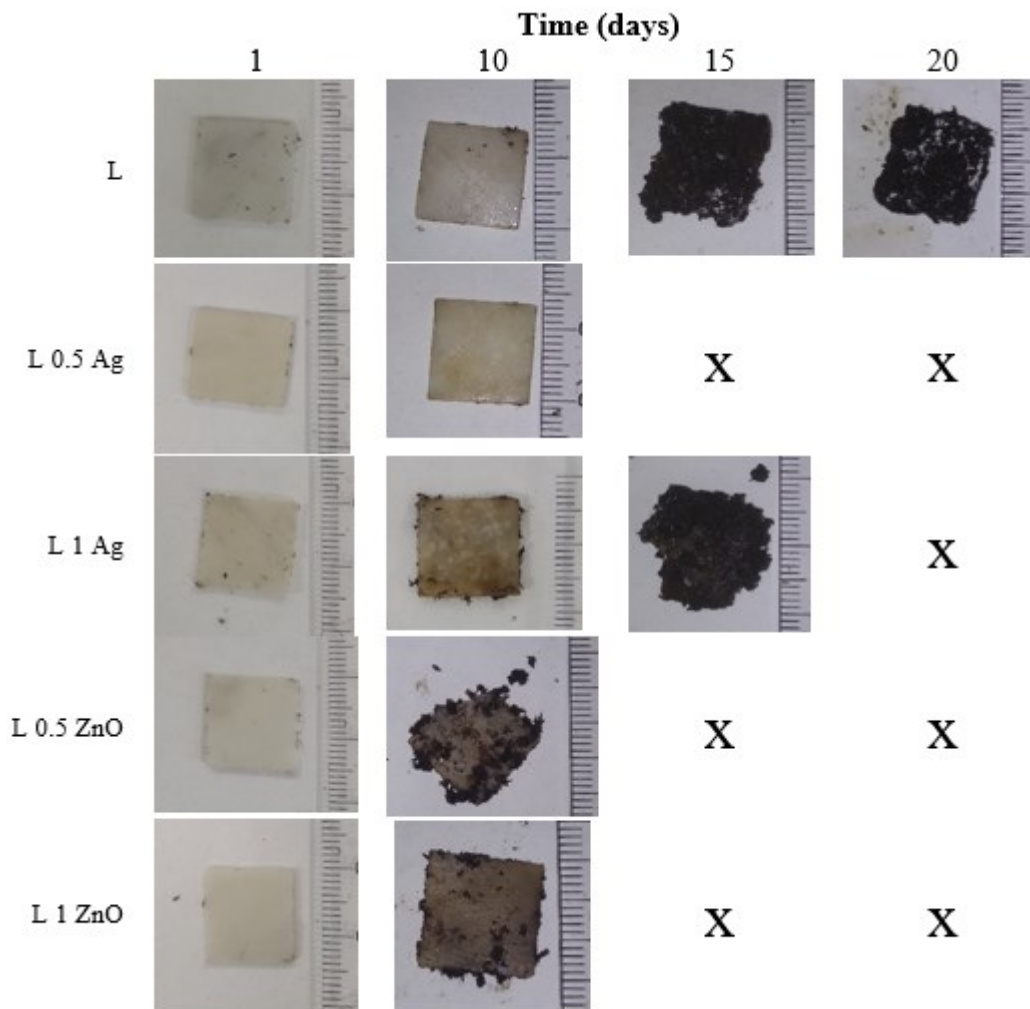


Figure 19 process of degradation of samples containing PLA Luminy matrix, X means that no samples were found in monitor days (scale is an office ruler)
 Specimens D 0.5 ZnO, L 0.5 Ag, L 0.5 ZnO and L 1 ZnO degraded after 15 days. After 20 days, L 1 Ag disappeared, and finally, after 22 days, D 0.5 Ag and D 1 Ag degraded, as can be seen in Figure 18 and Figure 19.

Based on the established values of average molecular weights (M_n and M_w), the different degradation time of D 1 ZnO and L 1 ZnO were found. Weight average molecular mass and the number average molecular weight of L 1 ZnO are 60 % and 85 % higher than D 1 ZnO specimen, respectively.

The study of the influence of thymol and silver nanoparticles on the degradation of poly(lactic acid) based nanocomposites verified that silver particles could improve the degradation of PLA with a higher percentage of silver nanoparticles [119]. This statement, however, contradicts the result of this thesis for samples containing the PLA Luminy LX175U matrix. This contradiction could be caused by higher M_w and M_n of L 1 Ag than

L 0.5 Ag by 27 % and 20 %, respectively. However, it is hard to decide whenever it has a similar effect for samples D 1 Ag and D 0.5 Ag, as these specimens degraded at the same rate. This test describes the influence of additives Sanitized Ag and ZnO on the degradation under a simulated composting condition on a laboratory scale.

CONCLUSION

The influence of antimicrobial additives on the degradability of two types of PLA matrix was studied. These matrices were PLA Ingeo 2003D and PLA Luminy LX175U. PLA biodegradable polymer was melt mixed with two types of antimicrobial additives in concentration 0, 0.5, and 1.0 wt%, respectively. Those additives were inorganic zinc oxide and additive on an organic base containing silver particles (Sanitized Ag). These composites were compression moulded into thin films.

Prepared films were tested to characterize the chemical and physical changes of composites. Scanning electron microscopy was used to determine the homogenous distribution of mixed films. Aggregation of particles was also noticed, especially for samples containing PLA Ingeo 2003D matrix with ZnO particles, which was more noticeable with one per cent of this additive.

Differential scanning calorimetry technique was used for evaluation of characteristic temperatures changes. The characteristics temperature changed 3 °C on average, except for samples constructed on matrix PLA Ingeo 2003D and ZnO additive. These samples cold crystallisation temperature rose around 10 °C. The incorporation of zinc additive provoked drastic changes in thermal degradation of PLA Ingeo 2003D matrix, which was proved by gel permeation chromatography. The rest of the samples reported a rise in both molecular weights.

Fourier infrared spectroscopy was used for determining chemical changes in studied composites. No new bond has appeared; only weak secondary bonds have formed between PLA matrixes and particles of additives.

For the reason that these additives are used as antimicrobial agents, a test to determine the antimicrobial activity of prepared films was carried out. Samples containing the PLA Ingeo 2003D matrix showed gradually growing antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, compared to neat material, except for samples with 1 % of ZnO particles. On the other hand, samples containing the PLA Luminy LX175U matrix presented expected antibacterial activity, except samples containing a 0.5 wt% Sanitized Ag where the colonies were detected after incubation. PLA Luminy LX175U is more suitable for antimicrobial modifications due to the amorphous character.

The disintegration of the composites was evaluated in a simulated composting environment at a laboratory scale level. All samples were utterly degraded. It was found that antimicrobial

additives significantly impact the disintegration of the samples, which degraded faster than neat PLA materials. The disintegration degree for all samples was 100 %. This degradation also depends on the molecular weight of the tested specimens and the size of incorporated particles (agglomerates).

Accordingly to all these results, it can be deduced that the application of composites containing PLA Luminy LX175U has a promising future due to the profitable antimicrobial activity and disintegration under control conditions.

Moreover, these additives could also have potential technological applications as an accelerator in decomposing compostable polymers.

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LIST OF ABBREVIATIONS

Ag	silver
Au-NPs	gold nanoparticles
CS	chitosan
DCS	Differential scanning calorimetry
DMA	Dynamic mechanical testing
e.g.	exempli gratia
et al.	et allia
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
GPC	gel permeation chromatography
IR	infrared
M_n	number average molecular weight
Mt	megatons
M_w	weight average molecular mass
nZnO	nanoparticle zinc oxide
PCL	Poly(ϵ -caprolactone)
PDI	polydispersity index
PGA	Polyglycolide
PHAs	Poly(hydroxyalkanoate)s
PHBV	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PLA	polylactide
PLGA	poly(lactic-co-glycolic acid)
PMMA	polymethylmethacrylate
PTFE	polytetrafluorethylene
PVC	polyvinyl chloride
SCDLP	Soybean Casein Lecithin Polysorbate 80
SEM	Scanning electron microscopy
Sn(Oct) ₂	stannous octanoate
Subsp.	subspecies
T_c	crystalization temperature
T_{cc}	cold crystallisation temperature
TEM	transmission electron microscopy
T_g	glass transition temperature

THF	tetrahydrofuran
TiO ₂	titanium dioxide
T _m	melting temperature
T _{m1}	melting temperature 1
T _{m2}	melting temperature 2
US	United States of America
UV	ultraviolet
X _c	degree of crystallinity
ZnO	zinc oxide

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