Tomas Bata University in Zlín

Doctoral thesis

BIODEGRADABLE POLYESTERS AND POLYANHYDRIDES FOR ADVANCED APPLICATIONS

Biorozložitelné polyestery a polyanhydridy pro pokročilé aplikace

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TABLE OF CONTENTS

ABSTRAKT

Tato práce se zaměřuje na přípravu a charakterizaci nových typů biorozložitelných polyesterů a polyanhydridů . Mimo shrnutí současného stavu poznání v oblasti biorozložitelných polymerů jsou v této práci důkladně popsány přípravy kopolymerů polylaktidu-polyetylenglykolu včetně jejich aplikovatelnosti pro enkapsulační technologie. Další část této práce je věnována optimalizaci syntézy polyanhydridu kyseliny sebakové. Třetí část předkládané práce se zabývá přípravou a charakterizací polyester-uretanů na bázi polylaktidu a polyetylenglykolu za použití biokompatibilního diizokyanátu odvozeného od aminokyseliny lysinu. Součástí uvedených studií je i popis degradačního chování připravených polymerů s potenciálem využití v oblastech, kde je zapotřebí řízené uvolňování aktivních látek.

ABSTRACT

This work is focused on preparation and characterization of novel types of biodegradable polyesters and polyanhydrides. Besides the summary of the state of art in the field of biodegradable polymers, a detailed description of biodegradable polylactic acid-polyethylene glycol copolymers preparation is presented, including their applicability on encapsulation technologies. Further part of this work is dedicated to optimization of poly (sebacic anhydride) synthesis. Third part deals with preparation and characterization of polyester urethanes based on polylactic acid and polyethylene glycol linked with biocompatible diisocyanate derived from an amino acid – lysine. Degradation behaviour description of the prepared polymers is integral part of this thesis. Potential application of the compounds can be found in the fields where controlled release of bioactive compounds is required.

THEORETICAL BACKGROUND

Introduction

Synthetic biodegradable polymers possess a broad range of advantageous physicochemical properties, and thus they are used in many areas: industry, agriculture, packaging and medicine [1-3]. Specifically, the most widespread biodegradable polymers are polyesters, polyanhydrides, polyurethanes, polyamides and polyacetals, which exhibit various degradation behaviours the in terms of mechanism and period of degradation.

In the last decades a significant progress in biodegradable polymers occurred, because of an extensive effort devoted to the development of new technologies. Conventional use of biodegradable polymers includes e.g. mulch films, delivery systems for fertilizers, disposable dishes, food containers, hygiene products and others [4]. However, considerable potential of these materials was found in advanced medical applications, which are also placing greater demands on the properties in many aspects because of direct contact with living cells [3]. The main regulations concern toxicity or inflammatory effect eventually [5]. Thus the material properties during the degradation, low molecular degradation products and their interaction with organisms must be considered as well. With regard to the time of use, it is important to ensure sufficient mechanical and thermal properties and also consider pH of biological environment. For these purposes the biodegradable polymers are modified chemically or by the processing way to meet the current advanced applications requirements. These applications are e.g. sutures, drug delivery devices, tissue-engineering scaffolds, stents or implants and the main advantage is that they serve their function and concurrently they are being removed from body non-invasively.

In order to create a biodegradable polymeric system with ideal physicochemical and degradable properties, this study comes up with new approaches in the design of material syntheses conditions, catalysts and reactive components. Also the way of processing and final form (e.g. nanoparticles, nanofibres) of these materials can bring certain benefits and their investigation represents a supplementary task in this thesis.

The presented thesis is devoted to the preparation of biodegradable polymeric systems for advanced applications in medicine. The first part brings an overview of the current state in this area. It deals with introduction of biodegradable polymers, their properties, synthesis and modifications. That builds a foundation for the experimental part, where the attention will be paid to optimization of process of synthesis, processing and modifications of these polymers, in particular polyesters, polyester-urethanes and polyanhydrides.

1. Synthetic biodegradable polymers

Synthetic biodegradable polymers generally possess advantageous ability to have tailored predictable properties and batch-to-batch uniformity unlike natural polymers [6]. Opposite to natural polymers, synthetic biodegradable polymers show improved mechanical properties, which however decrease with degrees of derivation and the optimum compromise between them is the object of intensive studies (Fig. 1) [7]. Typical for biodegradable polymers is the presence of hydrolysable bonds (heterochains containing oxygen or nitrogen) within the backbone, such as ester, orthoester, anhydride, urethane or amide. At last they are involved in many processes in various environments and for further development it is important to entirely understand their interactions.

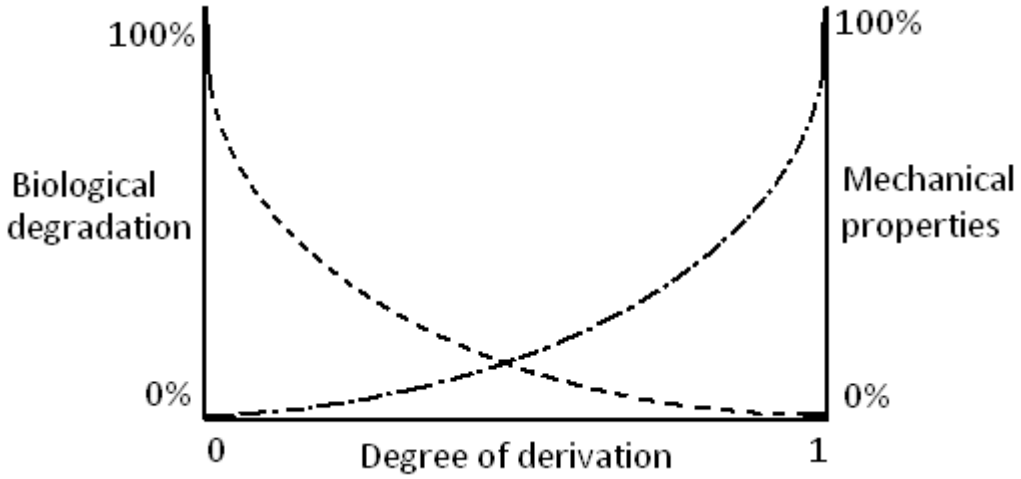

Figure 1 - Degree of derivation of biologic degraded material in relation with mechanical properties [7].

The ASTM standard D-5488-94d defines the term biodegradable as „*capable of undergoing decomposition into CO_2, methane, water, inorganic compounds or biomass in which the predominant mechanism is the enzymatic action of micro-organisms, that can be measured by standardized tests, in a specified period of time, reflecting available disposal conditions*" [8]. In polymer science the biodegradation can also be formulated as "*chemical process in which long chain polymers are cleaved in a biological environment, resulting in molecules with smaller sizes"* [9]. Nevertheless, due to many fields and specializations where the biodegradable polymers are employed, they can be considered within a much broader context, for example from the viewpoint of the manufacturing, environment, medicine or legislative [3, 10-12]. The biodegradable polymers in temporary surgical and pharmacological applications attract attention because of their promising possibilities.

Chemical structure-based classification of biodegradable polymers is discussed in the following section [3, 13].

1.1. Biodegradable polyesters

As mentioned before, polyesters represent very important group of polymers and they are among the most widely used polymers in medical applications [14]. Within the backbone, there are hydrolytically labile ester linkages and hence the polyesters are biodegradable. Beside that polyesters also show very good biocompatibility along with broad diversity of physicochemical and mechanical properties modifiable to current demands [15]. The most common synthetic routes are via step-growth polymerization (polycondensation) or ring opening polymerization of ester bond containing heterocyclic monomer [16].

Poly(glycolic acid) (PGA)

Poly(glycolic acid) is one of the oldest polyesters used by man. PGA was synthesized in 1893 and in 1960s the first totally synthetic biodegradable suture was produced, then in 1984 the PGA osteosynthesis implants for bone fixation were successfully applied [16-18]. PGA is the simplest aliphatic polyester and it can be synthesized by polycondensation or ring opening polymerization. Due to higher crystallinity (45-55%) the PGA exhibits elevated melting temperature of 220-226°C and lower solubility. Actually PGA is insoluble in most common organic solvents, it can be dissolved only in chlorinated solvents e.g. hexafluoroisopropanol [14]. Glycolide alone is very often copolymerized with other monomers like lactic acid [19, 20]. These copolymers are other important representatives of this group.

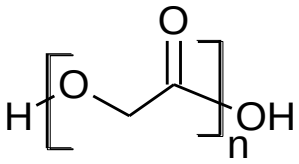

Figure 2 - Chemical structure of PGA.

Poly(lactic acid) (PLA)

PLA is synthesized from lactic acid or cyclic ester of lactic acid also called "lactide". Lactic acid (LA) exists in two optical isomers: L(+) and D(-), where the D form is not natural to be metabolized by human. Thus the use of L form is preferred; nevertheless, the combination of both isomers can appropriately modify some of PLA properties like crystallinity and therefore also the degradation rate. The PLA has earned increasing attention due to demonstration of its great biocompatible, (bio)degradable and bioresorbable properties. It is also a thermoplastic polymer which can be tailor-made into many forms - moulded plates, injected products, films, fibres, nanoparticles, etc. [21-23].

PLA is a solid, hard polymer, relatively brittle in nature and the elongation at break is very low. This fact can limit the use of PLA in certain applications.

Moreover, hydrophobic character of PLA can slow down the degradation process [24]. The physicochemical properties vary depending on PDLA presence as well. The pure PLLA has crystallinity about 37%, glass transition temperature between 55 - 65 °C and melting point around 170°C. It is insoluble in water and very well soluble in e.g. chloroform and acetone [25].

Despite some shortcomings the PLA is still the most promising polymer among other biodegradable materials. It is due to its versatility. LA has both a hydroxyl and a carboxyl functional group, thus the direct polycondensation is traditional and also the most economical procedure. Another way how to synthesize PLA is by the ring-opening polymerization (ROP) of ester of the acid [26, 27]. In an effort to reach qualities and make the PLA competitive with conventional plastics it is usually being modified. The most common strategies of modifications are copolymerization with e.g. other hydroxy acids, amino acids, lacton-type monomers, polyethylene glycol etc. [28]. Other attitude comes with the modification via chain extension reactions. This method offers both the improvement of physicochemical properties and also the introduction of functional groups onto chains, which is useful for further treatment or reactions [29]. In general, the process of synthesis can significantly affect properties of the final product. It will be discussed more specifically in a separate section along with functionalization or modifications of PLA.

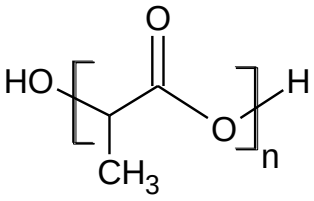

Figure 3 - Chemical structure of PLA.

Poly(lactic acid)/poly(glycolic acid) copolymers (PLGA)

PLGA is a widely investigated biodegradable polymer for its well-defined and controllable structure, which is so desirable in biomedical applications like sutures or drug delivery systems [30]. Both polymers build crystal structure, nevertheless the copolymers with compositions between 25-70% of GA are amorphous due to irregularity of polymer chains. Also the methyl group in PLA makes the copolymer less hydrophilic and thus the water uptake and hydrolysis rate are slower [31]. Therefore the degradation process can be effectively controlled by the ratio of both components, which can be important for drug release applications, although it was reported that structure of the incorporated drug can significantly contribute to the degradation rate of PLGA matrix [32].

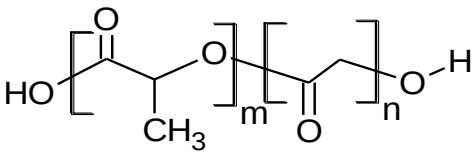

Figure 4 - Chemical structure of PLGA.

Polycaprolactone (PCL)

Polycaprolactone is typically hydrophobic, semi-crystalline aliphatic polyester. The low molecular weight polymers range from liquids to waxes, nevertheless the high molecular weight PCL has great mechanical properties, especially the elongation at break (>700%). The PCL is often used also as a modifier or an additive to obtain materials with unique features; it is mainly due to its excellent miscibility with other polymers (polyethylene, polystyrene, etc.). Beside that PCL is very often efficiently copolymerized with other polymers e.g. poly(lactic acid), polyethylene glycol, polystyrene, polyurethanes or grafted with cellulose, chitosan etc. This can extend and bring a brand new exploitation of PCL. Versatility like this also offers a wide range of various systems which can be created; pure PCL, its copolymers or blends can form e.g. micelles, hydrogels or dendrimers, which are suitable for biomedical applications.

PCL has also very good solubility in organic solvents in general, the melting point is 59-64°C and glass transition temperature about -60°C [33-35]. PCL is prepared by ring-opening polymerization of 3-caprolactone using catalyst such as stannous octanoate.

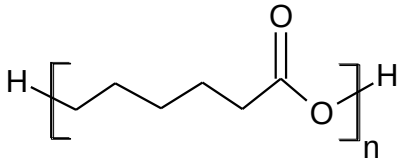

Figure 5 - Chemical structure of PCL.

Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (Fig. 6) are natural, biodegradable, biocompatible, non-toxic thermoplastic polymers, which also gained attention as potential medical materials [36, 37]. These polyesters are biosynthesized by some of the bacteria as energy storage compounds directly inside their cells. They are produced in both aerobic and anaerobic conditions .The amount of PHAs under the controlled fermentation conditions can be up to 70% of weight of a dry cell [38]. Beside that they can be also formed by synthetic route. The most widely naturally occurring is poly(β-hydroxybutyrate) (PHB) [39]. Despite the excellent biodegradability and biocompatibility (absolutely non-toxic by-products), this polymer possesses a high degree of crystallinity which makes it very brittle and also difficult to melt without degradation [40]. The poor mechanical properties

of PHB can be improved by copolymerization or addition of a plasticizer. As plasticizer was investigated e.g. oxypropylated glycerol (laprol), which positively affects the flexibility of PHB, however the degradation rate was slower than pure PHB [41].

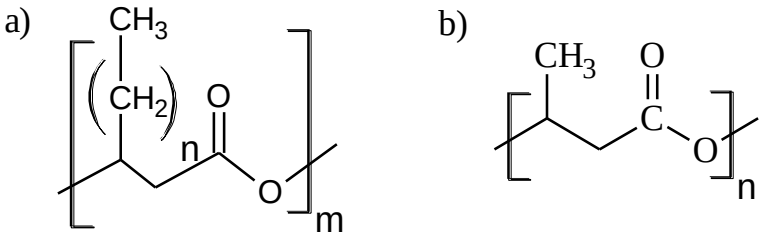

Figure 6 - Chemical structure of a) PHAs, b) PHB [42].

Polyanhydrides

Polyanhydrides are polymer compounds formed from carboxylic acids. They are usually very well available and low-cost. Polyanhydrides have also the advantage that they degrade into their diacid counterparts which are naturally occurring body constituents or metabolites [43]. In the structure, they have hydrolytically unstable anhydride bonds and it makes them very easily degradable. However, this can represent a problem within the meaning of storage, because they need to be kept in moisture free and frozen conditions [44]. On the other hand the hydrophobic character and the crystallinity (homopolyanhydrides crystallinity >50%) of the polyanhydrides does not allow the access of water molecules into polymer. These two properties result into surface erosion mechanism of polyanhydrides, which is undoubtedly an advantage for drug delivery systems [45]. Regarding the degradation behaviour, it can differ depending on the position of polyanhydride bond. The hydrolytic breakdown of anhydride bond which belongs to polymer backbone results in molecular weight decrease, whereas in case of anhydride as a side group this change does not occur; the example is poly(malic anhydride) [46]. Poly(sebacic anhydride) is the most frequently studied polyanhydride and also it was approved by Food and Drug Administration for the delivery of chemotherapy drugs [45]. Polyanhydrides have been synthesized by several techniques and some of them are discussed below.

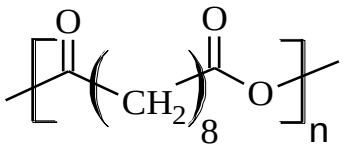

Figure 7- Chemical structure of Poly(sebacic anhydride) [47].

Polyurethanes (PUs)

Polyurethanes are very widespread in many fields. They can be prepared in various forms from foams to hard resins. PUs versatility is in their chemical structure consisting of alternately soft segment and hard segment, which can be in various ratio and length [48]. In addition, they usually undergo phase separation which can positively affect the mechanical strength [49]. PUs have proved good biocompatibility, but in long-term *in vivo* applications the biostability was not sufficient [50]. In effort to take advantage of PUs versatile properties the degradability was supported by introducing the hydrolytically sensitive bonds to form polyester- or polyether urethane [49]. Polyester urethanes consist of chemically different components: soft segment based on polyols (hydroxyl or amine terminated polyester or polyether), and diisocyanate and chain extender, which form the hard segment [51]. In general the hard segment provides the strength of material and also degrades more slowly than the soft segment because of hydrogen bonding in urethane linkage [48]. The most commonly used soft segments are based on poly(lactic acid), poly(glycolic acid) or poly(ε-caprolactone) [52]. Polyester urethanes prepared from oligomeric PLA prepolymers possess more rigid nature, whereas for example caprolactone as a co-monomer of LA can promote the elasticity of the material [53].

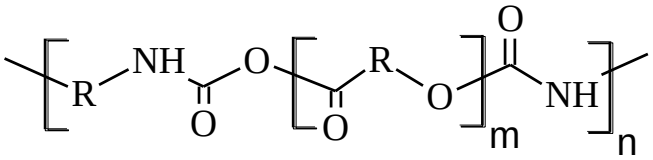

Figure 8: Chemical structure of PEU - Poly(ester-urethane).

Polyamides

Synthetic polyamides show great mechanical properties, excellent chemical and abrasion resistance. Due to nitrogen they are close to naturally occurring substances and, moreover, they have good hydrophilicity, therefore they are very suitable for medical applications [54]. The limitation of polyamides, however, is their low solubility and very high resistance to degradation; in fact they are mostly classified as non-degradable. The effort to make use of their beneficial properties leads to attempt to prepare them capable of biodegradation, e.g. by introducing substituents such as benzyl, hydroxyl and methyl. For example the degradability of copolymers, including both amide and ester bonds, grows with the increasing ester bonds contents.

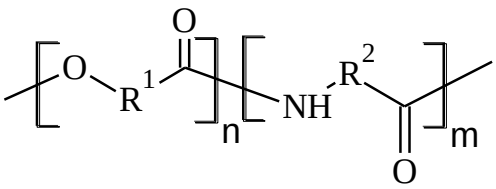

Figure 9 - Chemical structure of copoly(ester amide) [55].

To lack of degradation ability contributes also to their higher crystallinity and strong hydrogen bonds between chains of nylon [56, 57]. Polyamides can be synthesized from derivate of carbohydrates and amino acids, where the introduction of amino acids residues can form peptide bonds, which are susceptible to enzymatic degradation [58]. Several researchers reported degradation of nylon 6 oligomers by *Flavobacterium* sp. and *Pseudomonas* sp. microorganisms [59, 60] and degradation of nylon 4 in activated sludge, where the strains were identified as *Pseudomonas* sp. [61].

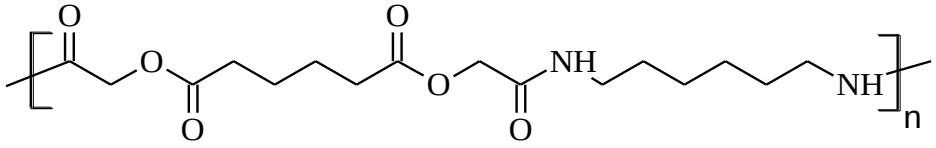

Figure 10: Chemical structure of nylon prepared from glycolic acid functionalized adipic acid and hexamethylenediamine [62].

1.2. Methodology of biodegradable polymers characterization

The characterization of composition, structure, morphology and behaviour of materials under certain condition is essential for framing their potential use and applications. This part is intended to describe the behaviour of biodegradable polymeric systems under the influence of abiotic and biotic factors of the environments. In addition, characterization of the interaction of polymers with the living cells is included.

The biodegradation is a very complex process. It can be considered as a sum of component contributions made by the polymer and the environment. In polymer, the chemical composition, structure of polymer and its surface, crystallinity and presence of impurities play significant role in degradation process [63, 64]. For example in the ISO definition, the biodegradable polymer is the one which undergoes the chemical change (oxidation) by acting of microorganism, while the CEN says that degradation residues have to be involved into metabolism of microorganisms and transformed into metabolites. From another point of view, the significant role can play the choice of testing method. There are three levels of biodegradability tests for polymeric material including the real time environment conditions testing, simulated environment

and laboratory testing. The real time biodegradation process brings the authentic results, however serious drawbacks in the form of controlling conditions and quantification of the disintegrated specimens occur. Also, according to the definitions above, the biodegradability is not clearly proved. In simulated environment the biodegradable tests are carried out in reactors containing compost, soil or water medium, which allows better treatment of samples and controlling of the conditions. Finally, the laboratory tests provide the most possibilities and good reproducibility. Biodegradable experiments can be accurately adjusted to specific polymer e.g. by defining the media or microorganism, regulation of the microbial activity or degradation rate, which can be eventually accelerated. This approach also allows the investigation of biodegradation mechanism [65].

In general the biodegradation process includes several steps [66]:

- Decomposition of polymer to small fractions - deterioration
- Reduction of molecular weight - depolymerisation
- Transport of molecules into the microorganism eventually - assimilation
- Exclusion of metabolites or simple molecules (CO_2, N_2, H_2O, CH_4) and their oxidation, also known as mineralization

The degradation process of certain polymer depends on many factors: the molecular weight, molecular architecture (crystallinity), as well as the size and porosity of sample and mainly on the type of bonding in the backbone. For example the polyanhydrides and poly(ortho-esters) are much more susceptible to hydrolysis unlike the polyesters or polyamides [67]. However, the reactivity can be considerably elevated using catalyst (acidic, alkaline media), modification of polymer substituents or adjustment of hydrophilicity.

1.3. Degradation factors

In the environment there are several initiators of degradation, which can be distinguished according to their character to biotic and abiotic. The main actors are water uptake, oxygen, light, temperature and pH [68]. Under abiotic conditions, biodegradable polymers mostly undergo passive hydrolysis, which can also be classified as chemical degradation [69]. The chemical and physical changes of polymers go along with this process. The cleavage of long main-chains to shorter - oligomers and monomers occurs and at the macroscopic scale it is accompanied by the loss of mechanical properties [70]. Therefore the monitoring of weight loss (standardized for in situ biodegradability test NF EN ISO 13432), decrease in the molecular mass or determination of residual monomers can be an important parameter in evaluation of degradation rate.

From the viewpoint of polyesters, the hydrolysis of ester bond is a common reaction, which can be supported by basic or acidic catalysts followed by RCOOH forming. Therefore, the polyesters cleavage is moreover autocatalysed by carboxyl end groups during the hydrolysis [71]. At a molecular level the water molecules diffuse into disordered amorphous regions, where the very first attack of ester bonds occurs, therefore the degradation products are formed both at surface and inside the sample; this mechanism of degradation is also called bulk degradation [72].

Photodegradation is another event affecting polymers in natural environment. This kind of experiment is easy to carry out in laboratory and standardize (ISO 4582, ASTM D5028-01). Further way how to estimate abiotic degradation is measuring of changes in thermal properties by DSC, TGA, TMA techniques and mechanical properties, which are all listed in ISO Standard 83.080.01: Plastics in general, in ASTM 1131 for TGA, ASTM D3418 for DSC and in ASTM D638 - 14 which covers measuring of tensile properties. For example within investigation of thermal changes it is possible to observe the increase of glass transition temperature as a result of the reduced mobility of polymer chains due to presence of residual phases of polymer, which may moreover create semicrystalline structures [73]. In case of semi-crystalline polymers, the degradation processes are facilitated above T_g, when disorganization of polymer chains allow better access of degradation agents. Specifications for assessment of polymer environmental performance in form of agricultural products in turn are defined in ASTM D6954 - 04, where there are the three tiers providing evaluation of loss of properties during abiotic degradation, measuring biodegradation and assessment of ecological impact. The visual assessment techniques are focused on monitoring of surface changes (cracks), e.g. scanning electron microscopy (SEM) or water contact angle method, the development of degradation can also be monitored by Fourier transform infrared spectroscopy (FTIR) and expressed by carbonyl index [74].

According to definition [8] the biodegradation is caused by biological activity, which may come from microorganisms or living cells in bodies, and both processes include enzymes, water, metabolites, ions, etc. thus the abiotic factors are inseparable part, or in other words, they effectively initiate environmental degradation process [66].

1.3.1. Degradation of polymers in biomedical applications

Many polymeric materials are used in various biomedical applications where there are several options available. The polymer device either needs to be stable with no integrity violation - the degradation is undesirable, or it remains of no importance or function and it needs to be surgically removed. Nevertheless, there are applications where the degradability is a necessity, for example the tissue engineered implants as scaffolds or stents and transport devices in the

form of nano-systems. Their time spent in a body should be compatible with completing their function there.

In biomedical devices, there are four main mechanisms of degradation being applied: hydrolysis, oxidation, enzymatic and physical degradation. The most common mechanism is hydrolysis. The mechanisms of oxidation and enzymatic degradation occur mostly because of the defensive system of organism, where the affected cells produce pro-oxidants or enzymes, which diffuse into implants to initiate the degradation. The physical - mechanical damage usually occurs after swelling and straining of implant [75].

1.3.2. Hydrolytic degradation

Hydrolysis is the reaction of water vulnerable chemical bonds, which in polymer results in chain cleavage and small molecules formation. This process depends on many factors, but generally, regarding the esters it is usually acid or base catalysed because water alone does not hydrolyse most of the esters (Fig. 11). Anhydrides are well known due to their hydrolytically labile bond and mostly the water is strong enough nucleophile (Fig. 13) [76].

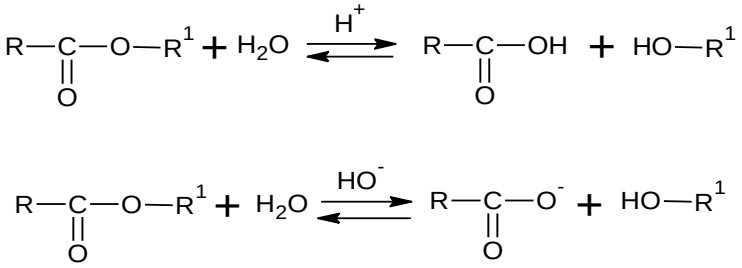

Figure 11 - Acid and basic catalysed hydrolysis of esters.

In the solid polymeric materials the hydrolysis rate given by hydrolytic constant is not controlled by the diffusion processes, which are related with mobility of molecules and volume of material and so that the kinetic constant is proportional to them. Also, what need to be considered is number of polymer chains what grows with progressing degradation because they elevate the hydrophilic character and therefore the absorption of water. After that, it could be assumed that the diffusion rate is being applied to affect the mechanism of degradation [77]. The theory providing the comprehensive view on molecular modelling of diffusion through polymer materials is described for example by Einstein–Smoluchowski diffusion equation [78]. Based on that, two main hydrolytic degradation mechanisms can be distinguished. It regards the bulk degradation mechanism and the surface erosion mechanism. According to the literature [75] the bulk degradation has three stages by which the polymer get through and these are depicted in dependence on molecular weight loss and mass loss (Fig. 12).

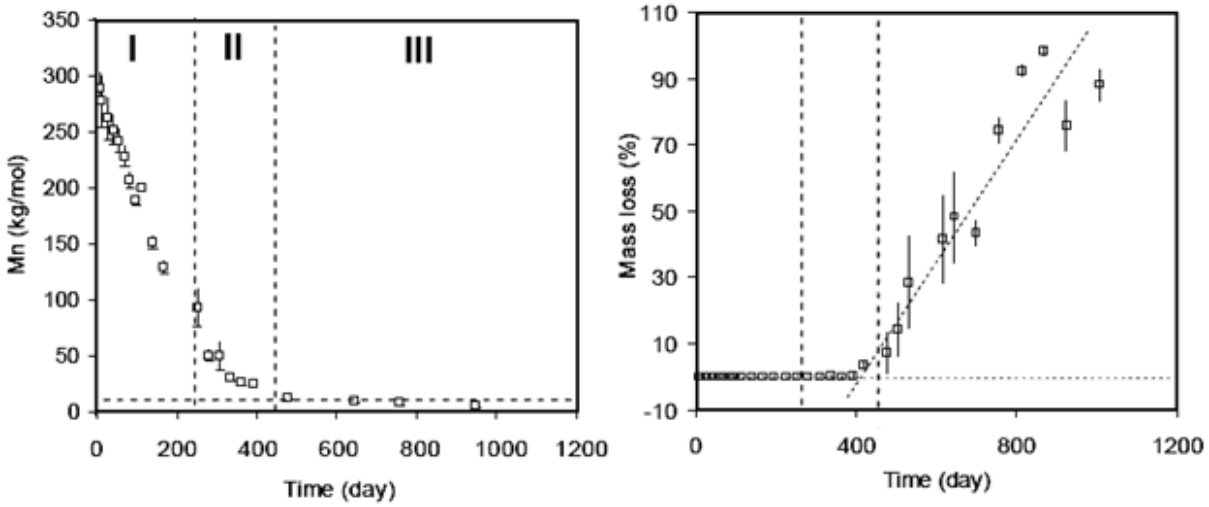

Figure 12 - Molecular weight and mass loss within the bulk degradation of solid PLA (70% L-lactide-co-30% D,L-lactide) at 37 °C in buffered medium [75].

In the first stage (I) the degradation follows the second order kinetics when the rate depends on concentration of hydrolysable bonds and water. A result is the increase of molecular ends and a decrease of molecular weight. After that the lowering of molecular weight is milder and short molecular chains are still inside the sample where they can catalyse further hydrolysis (stage II). Finally in the third stage (III) the polymer chains reach the molecular weight where they are soluble and thus the mass loss occurs [75]. This can also be affected by the size of sample where the thin sample allows better leaching out of short molecules as opposite to the thick samples. Thus, the sample would have certain thickness not to be affected by the size; nevertheless, the question is to what extent it would be significant for certain applications. [79].

Unlike the bulk degradation mechanisms in the surface erosion the degradation of polymer bonds is faster than the intrusion of water molecules into the bulk. It results in linear mass loss; the degradation independent on concentration of reactant(s) follows zero-order kinetics [70]. This phenomenon is the essential prerequisite for drug delivery systems, because it provides constants release of incorporated substances as opposed to bulk degradable system which can show decreasing profile of the release. This can be undoubtedly beneficial for enhancement of the therapeutic effect [80]. The surface erosion is a typical feature of polyanhydrides; their hydrolytic reaction is depicted in Fig. 13. This all implies that the choice of material for certain applications is crucial.

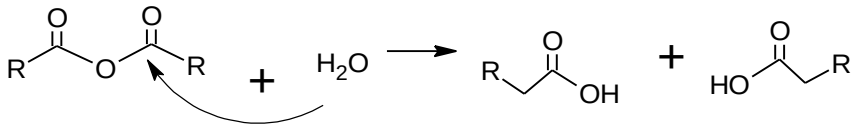

Figure 13 - Hydrolysis of anhydride bond.

1.3.3. Tissue/polymer integration

First it should be noted that it is a very complex and multifactorial issue that may not be fully understood yet. Implanted polymer can elicit a series of acute or chronic responses and the result can be the collagen capsule formation around the implant; this subsequently produces agents as enzymes or reactive forms of oxygen, which promote the degradation processes. In case of polymeric scaffolds it is about the pursuit of infiltration of cell into the polymer structure. A positive cellular response which can be facilitated and a support by using for example specific proteins attached to polymer surface functional group (the surface chemistry is then an important aspect) have to be ensured [81]. In general the implantation is characterized by a foreign body reaction; the detailed overview is reported by Anderson et al. [82] The main participants of this foreign body reactions are inflammatory cell population, monocytes/macrophages and foreign body giant cells (fused macrophages) [83]. Their production is induced by provisional matrix formed on the implant surface due to contact with blood protein [81]. In case of PLA production of the acidic ends within degradation, the inflammatory response may occur if the acid residues are not metabolized fast enough. Moreover depending on the level of response the further reactions as fibrosis can occur [84].

For polymeric scaffold and temporary implants, several important characteristics to stimulate cell proliferation and support of tissue function are considered: three-dimensional porous structure, biocompatibility, controllable degradation and resorption rate, suitable surface chemistry and sufficient mechanical properties. Regarding these aspects various strategies of treatment of this type of implant have been developed. An example is the strategy for bone transplant published by W. Hutmacher (2000), depicted in Figure 14. The graph describes progress of molecular weight and mass loss against the tissue formation divided into several phases. Firstly the scaffold is fabricated (A), thereafter the cell populations are seeded into scaffold in petri dish (static mode) (B), their growth in spinner flask follows (dynamic mode) (C) and subsequently there is the growth of mature tissue in bioreactor (D). Finally the implant is surgically transplanted into tissue (E) where the assimilation occurs (F). [85]

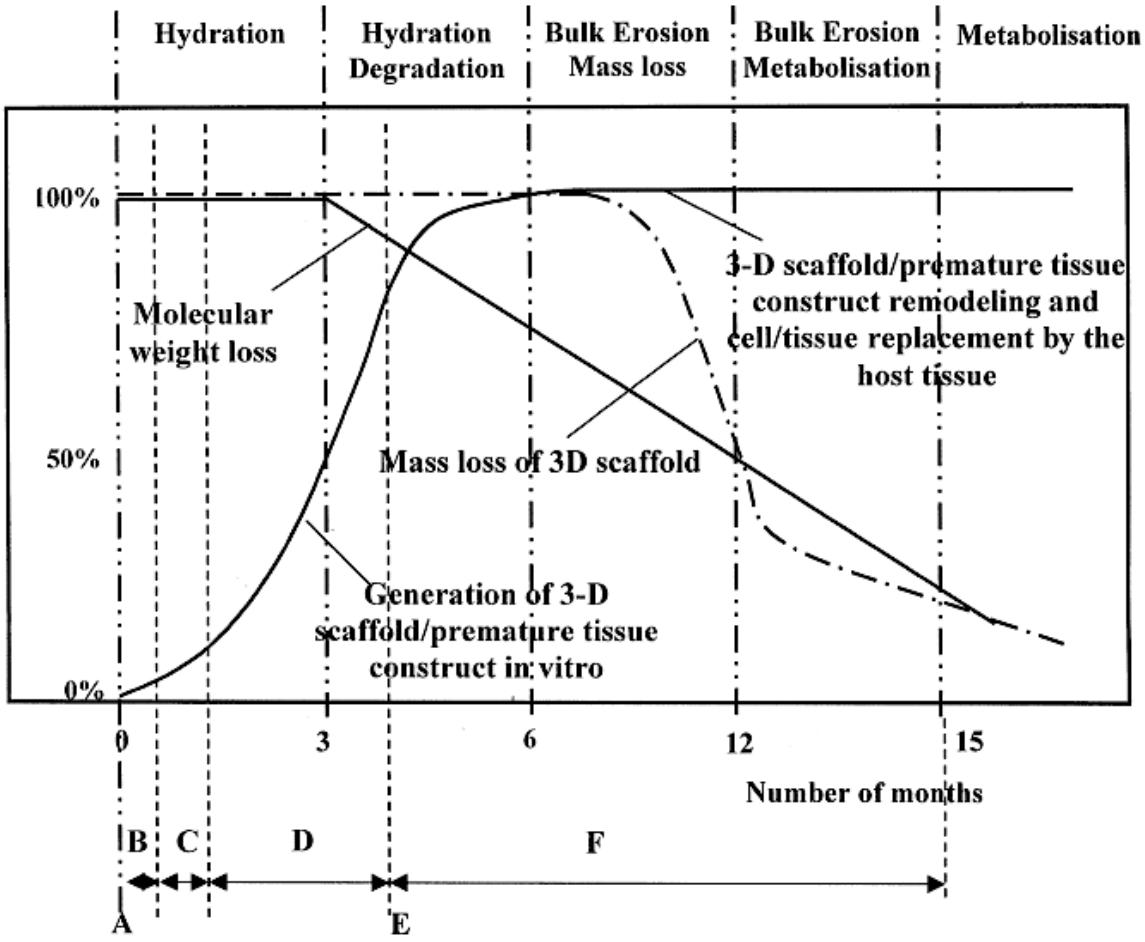

Figure 14 - Phases of scaffold degradation and cell proliferation [85].

1.4. Biocompatibility and cytotoxicity

As mentioned before, synthetic biodegradable polymers have a leading position in use as medical devices material. For these purposes there are particularly important criteria they have to prove, the biocompatibility and the bioresorbability. A frequently published definition of biocompatibility according to Williams defines biocompatibility as *"the ability of a material to perform with an appropriate host response in a specific application."*[86]. In other words the term biocompatibility includes the evaluation of ability of the material to elicit the response from tissue, namely toxic, inflammatory or infectious response [87]. These properties are usually clearly defined by relevant standard provided by International Organization for Standardization (ISO), namely ISO 10993, where are also references for testing methods. The ISO 10993 is a complex multi-part standard for evaluating effects of medical device material on the body and considers every aspect of biocompatibility. The first general part specifies the categories into which the devices are further classified. Based on this classification the number and manner of testing can be determined. According to Table 1 biocompatibility matrix it is obvious that demands on

device testing grow with the length of device contact with body. For example the permanent implant in contact with blood for more than 30 days shall be subjected to test according to ISO 10993: part 3 (genotoxicity, carcinogenicity, reproductive toxicity), 4 (interactions with blood) 5 (cytotoxicity), 6 (implantation), 10 (irritation, sensitization), 11 (systemic toxicity - acute and chronic). Additionally in case of polymeric materials it is necessary to identify and quantify degradation products from device - part 13. Deliberately degraded devices are further subjected to toxicokinetic study (part 16) and determination of leachates limits depending on health risks (part 17). Finally, for all materials the chemical, physicochemical and morphological properties characterization is obligation (part 18, 19). In the Czech legislation, the cornerstone of medical devices is the Act on Medical Devices (No. 268/2014) which has been amended with effect from 1 April 2015. The current form unifies previous legislative and takes into account the European legislative.

Appropriate biocompatibility testing is essential before any contact with human tissue of any kind in order to protect human being. Whole process comprises several stages starting from the less invasive, with the goal to eliminate animal tests. This means in practice that chemical analysis and characterization of material is firstly performed along with analysis of leachates in extracts obtained *in vitro* at elevated temperature usually of 37°C. Examples of standards dealing with regulations of testing *in vitro* are ISO 10993-4 and ISO 10993-5. Standards engaged with degradation of implantable materials are e.g. ISO 13781 – Poly(L-lactide) resins and fabricated forms for surgical implants - In vitro degradation testing ISO 15814 - Implants for surgery - Copolymers and blends based on polylactide - In vitro degradation testing.

Tissue permanent devices usually include *in vivo* tests. In vivo tests are supported by previous in vitro tests, due to which for example the duration of degradation of implant can be estimated. The comparison of in vitro and in vivo testing is reported in the study [88], which is furthermore dealing with development of a resorbable patch, based on poly(3-hydroxybutyrate). Regarding the *in vivo* testing, it can be considered quite controversial, thus the very strict conditions are established by organizations and ethic committees. The test period, animals, surgery, testing conditions and test specimens are precisely defined in ISO 10993-6 and animal welfare requirements are described in ISO 10993-2.

Synthetic materials which come into direct contact with the human body also relate to the biomaterials, which defined Williams (2009) "*A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine."* From the viewpoint of tissue interaction and tissue response the biomaterials can be classified into: active, which positively affect

tissue [89]; inert, which almost do not elicit response [90] and degradable or bioresorbable [91], which are tissue integrated and after period of time slowly replaced by new cells of tissue.

Cytotoxicity

The cytotoxicity assays are used in screening the viability of cells in presence of chemical compounds or foreign material and its residues; in other words, the cytotoxicity tests can prove the biocompatibility. The cytotoxicity tests are reproducible and cost-effective, while providing sufficiently convincing and reliable results [92]. Cytotoxicity assessment is conducted both in vitro and in vivo and it is employed before clinical use, which eliminates potential biological damage. Nevertheless there is a difference between sensitivity in vitro and in vivo, because of the concentration of substance and intrinsic sensitivity of cells. Moreover, the absorption of chemicals by cells in vivo is directly affected by other factors, such as distribution, biotransformation (metabolism), excretion and rate of absorption. Thus the in vitro cytotoxicity tests may appear to be more sensitive than in vivo and it can be difficult to extrapolate concentration which is toxic [93, 94].

In cytotoxicity assessment, there are many approaches how to estimate the viability of cells, which obviously also depends on the cell origin and nature [95]. Standard cytotoxicity (ISO) tests are: Direct Contact, Agar Overlay, minimum essential medium (MEM) Elution, 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) Cytotoxicity Test and Colony Formation, where the last two are quantitative and preferred by regulation institutions [96].

Table 1 - Biocompatibility test matrix according to ISO 10993.

Device categories			**Biological effect**											
Body contact		Contact duration A = Limited (≤ 24 h) B = Prolonged (24 h - 30 days) C = Permanent (> 30 days)	Cytotoxicity	Sensitization	Irritation of intracutaneous	Systemic toxicity [acute]	Subacute and subchronic toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic toxicity	Carcinogenicity	Reproductive and developmental	Biodegradation
Surface device	Skin	A	•	•	•									
		B	•	•	•									
		C	•	•	•									
	Mucosal membrane	A	•	•	•									
		B	•	•	•	D	D		D					
		C	•	•	•	D	•	•	D		D			
	Breached or compromised surface	A	•	•	•	D								
		B	•	•	•	D	D		D					
		C	•	•	•	D	•	•	D		D			
Externally Communi-cating devices	Blood path Indirect	A	•	•	•	•				•				
		B	•	•	•	•	D			•				
		C	•	•	D	•	•	•	D	•	D	D		
	Tissue/Bone dentin[1]	A	•	•	•	D								
		B	•	•	•	•	•	•	•					
		C	•	•	•	•	•	•	•		D	D		
	Circulating Blood	A	•	•	•	•		E		•				
		B	•	•	•	•	•	•	•	•				
		C	•	•	•	•	•	•	•	•	D	D		
Implant devices	Tissue/Bone	A	•	•	•	D								
		B	•	•	•	•	•	•	•					
		C	•	•	•	•	•	•	•		D	D		
	Blood	A	•	•	•	•	•		•	•				
		B	•	•	•	•	•	•	•	•				
		C	•	•	•	•	•	•	•	•	D	D		

The table provides appropriate evaluation of the biocompatibility of devices for certain use.

• - Tests per ISO 10993
D - Additional tests that may be required in the U.S.
1 - Tissue includes tissue fluid and subcutaneous spaces
E - For all devices used in extracorporeal circuits

1.5. Polymer characterization techniques

1. Chemical composition and structure:

Chromatography
- Gel permeation chromatography (GPC)
- High performance liquid chromatography (HPLC)
- Gas chromatography (GC)

Spectroscopy
- Fourier transform infrared spectroscopy (FTIR)
- Nuclear magnetic resonance (NMR)

Elemental analysis
- Total organic carbon (TOC)

2. Physical properties

Thermal analysis
- Differential scanning calorimetry (DSC)
- Thermogravimetric analysis (TGA)

Mechanical tests
- Stress/train test
- Hardness test
- Izod impact tests

In polymer science the crucial method for characterization of macromolecular chains is gel permeation chromatography (GPC). This method allows determining the number of average molecular weight (M_n), weight of average molecular weight (M_W) and fundamental molecular weight distribution (Đ). Additionally, this instrument is able to render information about linearity and branching of polymer chains. In principle, the separation of molecules occurs by their effective size in solution which flows through porous, rigid gel [97]. The Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) are used mainly for identifying chemicals. FTIR uses the ability of certain substances to absorb infrared light with characteristic wavelength [98]. NMR is a phenomenon which occurs after interaction of active atom nuclei with an external magnetic field and due to neighbouring nuclei interactions; the typical chemical shifts of molecules are measured [99]. For the description of thermal properties is generally used the differential scanning calorimetry (DSC). This technique is based on measuring the difference between heat quantity required to increase the temperature of the sample and standard to the same value. DSC analysis is associated with characterization of material transitions as function of time and temperature, and provides important material characteristics such as melting temperature, glass transition temperature,

crystallinity degree etc. [100,101]. Thermogravimetric analysis (TGA) is a tool for identification and characterization of thermal stability of polymers (e.g. oxidation, decomposition). Also the analysis of composition of copolymers or blends and additives can be performed. Principle of this method consists of observation of sample mass change as a function of the increasing temperature or time with constant temperature [102]. Static mechanical test provides important material characteristic values regarding tensile strength, Young's modulus, elongation at break and yield strength.

Table 2 - Typical properties of biodegradable polymers. (Ref. [103-105])

Polymer	**Tensile strength (MPa)**	**Modulus (GPa)**	**Elongation at break (%)**	**T_g (°C)**	**T_m (°C)**	**Degrada-tion time (months)**
PLLA	55 - 80	2.8 - 4.2	3 – 10	60 - 65	173 – 178	> 24
PDLLA	25 - 40	1.4 - 2.8	2 – 10	55 - 60	Amorph.	12 -16
PCL	20.7 - 42	0.2 - 0.4	300 – 1000	- 65 - 60	58 – 65	> 24
PGA	< 70	6 - 7	1.5 – 20	35 - 40	225 – 230	6 - 12
50/50 PLGA	~ 36	1.4 - 2.8	2 – 10	45 - 50	Amorph.	1 -2
PHB	~ 40	3.5 - 4.0	5 – 8	5 - 15	168 – 182	*

1.6. Polymers based on poly(lactic acid) - synthesis

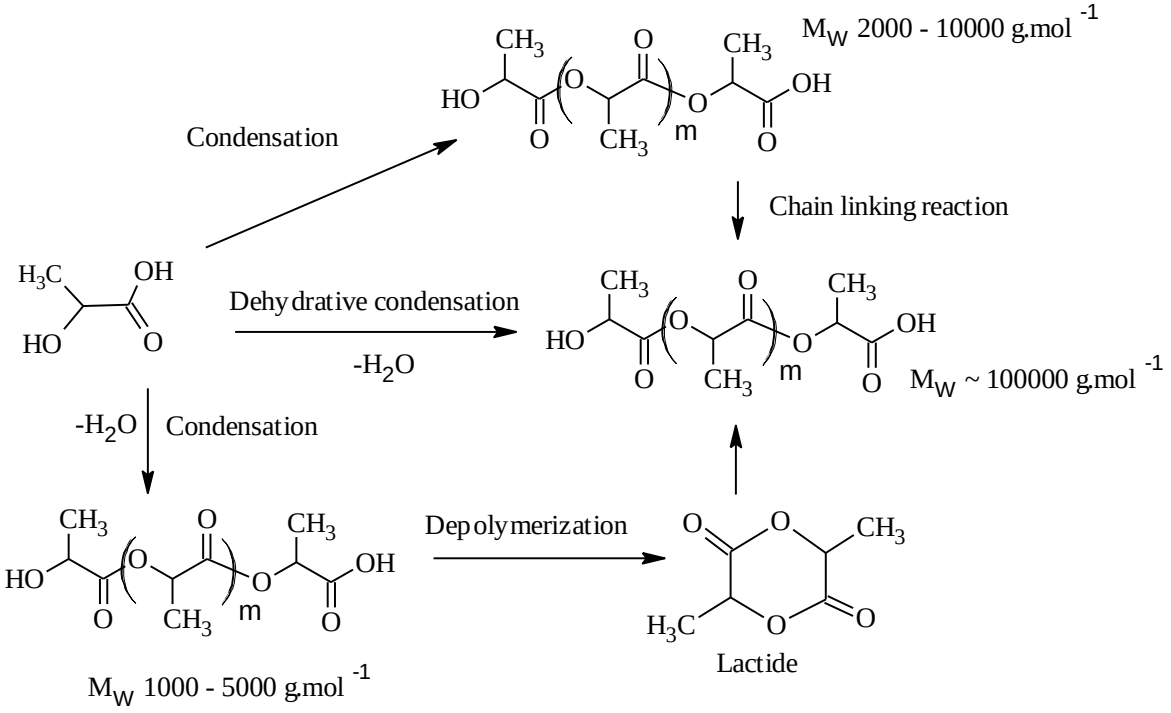

Figure 15 - Synthesis routes of high molecular weight PLA [106].

There are several different routes of synthesis poly(lactic acid) and lactic acid based polymers including direct polycondensation yielding low molecular weight product, azeotropic dehydrative polycondensation and ring-opening polymerization of lactide forming high molecular weight PLA or lastly the post-polymerization processing reactions [107, 108].

Ring-opening polymerization (ROP)

ROP is a mechanism of reaction of cyclic esters; it allows the preparation of high molecular weight polymers with high degree of stereoregularity. As opposed to polycondensation, at which polymerization temperature is usually in range of 100 - 190°C, the ROP in solution can be performed at 0 - 80°C, due to which the side reactions are minimized. Most polymers with medical importance prepared by ROP are based on glycolide, lactide, ε-caprolactone and 1,5-dioxepan-2-one.

ROP is very sensitive to any impurities like oxygen or water, thus the reactants preparation is mostly consuming time, and finally, it can be a demanding and expensive process. Polymerization of lactones and lactides can be classified according to the mechanism into anionic, cationic or coordination insertion [109-111]. The coordination insertion polymerization is the most studied mechanism [112-116]. The initiators used are various metal alkoxides

e.g. aluminium, magnesium, titanium, zirconium or tin alkoxides and carboxylates. Covalent initiators possesses important advance for control of molecular weight due to initiator/monomer ratio and therefore they can provide higher molecular weight polymers in comparison with the ion initiated reaction. Initiation in the coordination insertion way of polymerization is especially important in large scale production, in extrusion of poly(L-lactide), when higher temperatures up to 200°C are used and the racemization could occur, this event is significantly minimized due to it. [117] Nevertheless, for biomedical needs the presence of metal catalysts and initiators is inconceivable, because of the eventual toxicity, and also they have to be removed, which is another step in their processing. Thus for environmental friendly processes and material preparation purposes the enzyme-catalysed polymerizations were developed. The main advantages are mild reaction conditions and natural origin of enzymes. In nature the hydrolysis of ester bonds is catalysed by enzyme lipase. This reaction is reversible and vice versa in non-aqueous media the bond-formation can occur [118, 119].

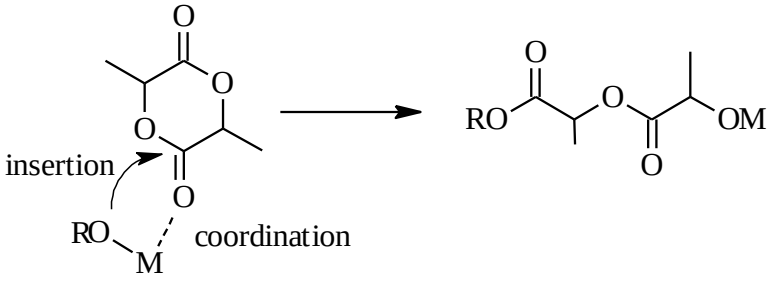

Figure 16 - Coordination-insertion mechanism for metal-catalysed ROP of lactide [120].

Polycondensation

Lactic acid is of difunctional nature; it contains both hydroxyl and carboxyl group. Equivalence of these functional groups provides intermolecular reactions due to which the macromolecular products are formed [121]. In general, the polycondensation is strongly affected by balance between polyester, free acid and water. The removal of water as by-product during this reaction is crucial, because the presence of water and increasing viscosity of the system negatively affect equilibrium shift toward the product and achievement of high molecular weight. Besides, the polycondensations usually become complicated by extensive side reactions, where the mono-functional (partially reacted, or degraded) oligomers, monomers or impurities participate in the reaction. Therefore, the products with low molecular weight (10,000 $g.mol^{-1}$) and indefinite molecular structure are obtained by simple direct polycondensation [122, 123]. According to the scheme, there is only one direct (one-step) method to prepare high molecular weight PLA denoted as Dehydrative condensation. This method was developed as an alternative to overcome shortcomings of melt

polycondensation. It is using mostly metal based catalysts and high boiling solvent (e.g. anisole, m-xylene, diphenyl ether, *o*-dichlorobenzene, *o*-chlorotoluene) to remove dissociated water by means of azeotropic distillation. [29] In literature, the molecular weight yields $6.7x10^4$ $g.mol^{-1}$ [106], $8.0x10^4$ $g.mol^{-1}$ [108] and even $3.0x10^5$ $g.mol^{-1}$ [113]gained by this method were reported. However the drawbacks as high reaction temperature, long reaction time and removal of solvent can be problematic in terms of complexity and economy of process.

Another method, solid state polycondensation (SSP), which is usually combined with melt polycondensation, was implemented in order to improve polycondensation efficiency. After interruption of melt polycondensation, PLA is in solid state heated under T_m but above T_g, (optimum 120, 130°C) [109] under the flow of inert gas and reaction proceeds between reactive ends in amorphous regions of polymer. Final SSP rate significantly depends on crystallinity and thus mobility of molecular chains, diffusion of by-products and processing properties as temperature etc. [110]. Regarding the properties, it was shown that due to polymer crystallization, chain end and catalysts are concentrated in amorphous phases, so that the SSP is suitable to carry out around the crystallization temperature [116]. According to the research [109] the 70% elevation of M_w is possible to attain; additionally, the solvent removal is eliminated and only simple equipment is required. Nevertheless, as a result of very slow reaction progress over a critical time reaction (20, 40 h) the decrease of M can occur. Research works have reported preparation of PLA by SSP with molecular weight M_W $1.0x10^5$ [114] or $2.66x10^5$ $g.mol^{-1}$ [115].

Beside the molecular weight, the polydispersity index (PDI), which is a measure of the width of molecular weight distribution (MWD), belongs to major concerns as well [124]. Polydispersity has a direct relationship to mechanical properties of polymers and very strong effect on the rheology of polymer. The broad molecular weight distribution makes the reduction of viscosity and shear stress of the melt, which can be beneficial for processability. Moreover, polydisperse polymers also show enhanced elastic effect in comparison with monodisperse polymers [125]. The PDI of unity is usually characteristic for controlled ROP methods unlike the polycondensations, which are not controlled and yield polymers with broad polydispersity. During the polycondensation firstly the oligomers are formed and then they are condensed together to create macromolecules. Beside the melt polycondensation other methods of PLA preparation are solid state polycondensation and solvent polycondensation, which are either time consuming or a difficult removal of solvent is needed [108, 114, 115, 126, 127]. The summary of polycondensation reactions using various catalysts is shown in Table 3.

Table 3 - Summary of some previous work on the synthesis of PLA prepared by polycondensation.

Monomer	**Catalyst**	**Molecular weight ($g.mol^{-1}$)**	**Ref.**
D-lactic acid	2-Naphthalenesulfonic acid	M_W = 47,000	128 (2013)
L-lactic acid	tin dichloride hydrate and p-toluenesulfonic acid	M_W = 500,000	129 (2001)
L, DL-lactic acids	no catalyst	M_W < 20,000	123 (1997)
L-lactic acid	tin chloride dihydrate	M_W = 23,000	130 (2002)
L-lactic acid	stannous oxide, tin chloride dehydrate	M_W < 30,000	131 (2000)
	tin chloride dihydrate activated by p-tolulenesulfonic acid monohydrate, boric acid, mphosphoric acid	M_W (TSA) ≥ 42,000 M_W (BA) ≤ 26,000 M_W (MPA) ≤ 32,000	
L-lactic acid	$SnCl_2 \cdot 2H_2O$ /TSA, $SnCl_2 \cdot 2H_2O$ /succinic anhydride, $SnCl_2 \cdot 2H_2O$ /maleic anhydride	M_W = 147,000 M_W = 160,000 M_W = 160,000	132 (2008)
L-lactic acid	Scandium triflate	M_n = 73,000	133 (2006)
Lactic acid	$SnCl_2 \cdot 2H_2O$	M_W = 198,000	134 (2010)
L-lactic acid	Creatinine	M_W = 26,000	135 (2014)
L-lactic acid	Germanium tetraethoxidee $SnCl_2 \cdot 2H_2O$/ Germanium tetraethoxidee	M_n = 29,000 M_W = 49,300 M_n = 37,000 M_W = 63,000	136 (2003)

Chain linking reactions

Chain linking reactions represent two-step method including polycondensation forming low molecular weight prepolymer followed by chain extending reaction (polyaddition) where there is no undesirable by-product. The chemical structure and properties of prepolymer are usually crucial for adjusting the final polymer properties regarding the degradation rate and mechanical performance [137].

Chain linkers or also extending agents are usually low molecular weight compounds, for example diisocyanates, bisoxazolines, acid chlorides and dianhydrides, which preferably react with either hydroxyl or carboxyl group

[25]. In order to obtain more efficient kinetics of reaction, high molecular weight yields and due to exclusivity of chain extender reactivity, the prepolymers with site-specific functionalities are prepared [138]. For this purpose the multi- or difunctional compounds terminated either hydroxyls or carboxyls are used [139]. Regarding the functionality, it should be pointed out that it is an important parameter. The reaction of the polymer with two end groups results in linear polymer which can show dramatically different properties in terms of melt rheology, mechanical behaviour, solubility and crystallinity compared to the branched polymers, which can moreover differ in the way of branching from random to regular. Branched structure is usually formed by polyfunctional or multifunctional monomers. Such a polymer shows enhanced solubility and viscosity and crystallinity lower than linear polymer formed from a difunctional compound [140].

To synthesize carboxyl terminated polymer, the maleic, succinic, adipic, citric acids [141, 142] or anhydrides of maleic, succinic acids can be employed [25]. Hydroxyl terminated PLA can be obtained through reaction with 1,4-butandiol [143] ethylene glycol [144, 145] etc. Due to broad diversity of reactants, it is possible to tailor a wide range of final properties of polymer directly to certain application. Molecular weight can be controlled by amount of difunctional compounds, which determine a number of molecules and thus their length. The amount of extenders can be derived from the number of functional groups which correspond to the number of average molecular weight of functionalized prepolymer. Important characteristic of prepolymer is acid number reflecting the amount of residual acid, which reduces the hydrolytic stability of product and negatively affect the catalysis of the reaction [146]. Final physicochemical properties (e.g. hydrophilicity, crystallinity) and degradation velocity of the resulting polymer considerably depend on the chemical nature of individual components (extender, functionalizing compound).

For carboxyl terminated PLA prepolymers, the bisoxazolines were found as effective extenders or chain-coupling agents leading to ester amide formation. Moreover they showed ability to reduce acidity of PLA polymer and thus increase the thermal stability [138]. Typical chain extender for hydroxyl terminated polyester prepolymers are diisocyanates forming polyester urethanes. A short review of works on use of chain extending reactions is summarized in Table 4.

Diisocyanates

In general, isocyanates very readily react with substances containing active hydrogen e.g. water, alcohols, phenols, amines and carboxylic acids; therefore the polyaddition can be accompanied by numerous competitive side reactions which can lead to branching or crosslinking of polymer. An example is the reaction of isocyanate towards the amino group resulting in the urea and biuret formation or towards the urethane forming allophanate structure. Side reactions can be initiated mostly by higher reaction temperature and excess of isocyanate. Amount of isocyanate is usually expressed as ratio between reactive groups, e.g. ratio NCO/OH in case of hydroxylated compound or NCO/NH_2 in case of amino compound [147]. Reactivity of diisocyanates is also affected by nature and position of their substituents. In general, electron withdrawing substituents increase the reactivity of diisocyanates; the ortho substituted aromatic diisocyanates are less reactive than para substituted analogue, because of steric hindrance. [148]

The structure of diisocyanate has also a significant impact on temperature properties of polymer. Aliphatic diisocyanate based polyurethanes in comparison with the aromatic show lower glass transition temperatures. It is due to higher flexibility and mobility of chains. The hard segment which is represented by diisocyanate part also contributes to stiffness of polymer and it was observed that aromatic diisocyanate imparts higher rigidity to polymer rather than aliphatic diisocyanates. The tensile stress is also elevated with excess of NCO group since the branched and crosslinked structure occurs and also due to strong intermolecular attraction of isocyanates. [149, 150]

According to literature, the typical diisocyanates used as chain linking agents are e.g. 1,6-hexamethylene diisocyanate, 4,4'-methylenediphenyl diisocyanate, isophorone diisocyanate, toluene diisocyanate etc.

1.7. Applications

Current development in biomedical materials is concentrated on tissue engineering aims and delivery devices, which are represented by various nanostructured and mostly high-value applications. Basic forms in general are scaffolds and nanoparticles. Scaffolds are components of many strategies in tissue engineering. They provide temporary support for tissue and cell growth or provide the delivery of cells; simultaneously they can serve also as a vehicle for drug delivery [151]. Typical for scaffolds, contrary to nanoparticles is their porous structure which enables them to fulfil their functions. Nanoparticles can occur in the form of nanocapsules, nanotubes, nanospheres, etc. and the substance to be delivered can be loaded inside the particle or absorbed on the sphere functionalized surface [152]. Main characteristics of delivery systems are particle size, loading efficiency, binding affinity, the burst effect and

releasing profile. Additionally, assessed qualities of nanostructured systems as scaffolds are sufficient mechanical properties, acceptable biocompatibility results, adherence of cells on surface, zero immunogenicity and stability [153]. In the future, the research could be focused on development of active nanostructured biomedical devices, which could be more adaptable and due to biosensing functionalities provide adequate response *in vivo.*

A recently published study revealed the use of poly(L-lactide-co-glycolide) copolymer with fluorescence labelling for preparation of nano and micro particles for treatment of the inflammatory bowel disease (IBD). The study is unique in size-dependent targeting of particles tested on humans. Example of these particles is in Figure 17 [154].

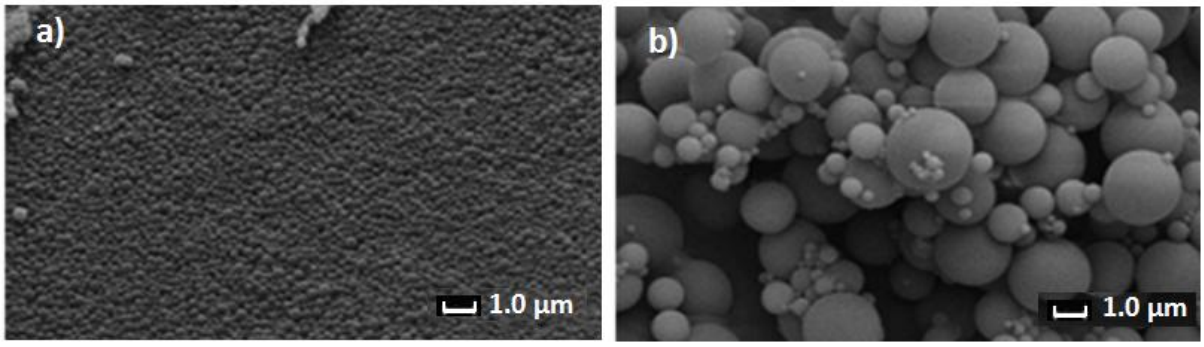

Figure 17 - SEM pictures of a) freeze-dried and re-suspended FA-PLGA nanoparticles and b) freeze-dried and re-suspended FA-PLGA microparticles [154].

Table 4 - Summary of some previous work on chain extending reactions of PLA based polymers.

Monomer 1	**Monomer 2**	**Chain extender**	**Molecular weight [g.mol^{-1}]**	**Ref.**
L-lactic acid	-	1,6-Hexamethylene diisocyanate	M_n = 33,000	155 (1995)
L-lactic acid	-	Methylenediphenyl Diisocyanate	M_n = 15,000 M_W = 57,000	156 (1999)
L-Lactic acid	1,4-Butanediol	1,6-hexamethylene diisocyanate	M_n = 27,500 M_W = 116,900	157 (2008)
L-lactic acid	Succinic anhydride	2,2-bis(2-oxazoline)	M_n = 48,800 M_W = 130,400	158 (2002)
L-Lactic acid	1,4-Butanediol	Toluene-2,4-diisocyanate	M_W = 170,000	159 (2005)
L-Lactic acid	Succinic acid, 1,4-Butanediol	Toluene-2,4-diisocyanate	M_n = 134,000 M_W = 326,000	160 (2009)
L-Lactic acid	Poly(ethylene glycol)	2,6-diisocyanato ethyl caproate	M_n = 5,990 M_W = 10,340	161 (2011)
PDLLA diol	1,4-Butanediamine	Hexamethylene diisocyanate	M_n = 5.153 M_W = 6.905	162 (2011)
L-Lactic acid	Poly(ethylene oxide)	Methylenediphenyl Diisocyanate Hexamethylene diisocyanate	M_W = 39,000 M_W = 300,000	163 (2013)
L-Lactic acid	1,4-Butanediol	IPDI 4,4-dicyclohexyl methane diisocyanate	M_W = 38,500 M_W = 37,400	164 (1996)

1.8. Synthesis and modifications of polyanhydrides

The most common method is melt polycondensation (Fig. 18), which is both inexpensive and provides good yields. Nevertheless, polycondensation is usually necessary to be performed at higher temperatures, which can be a problem for temperature-sensitive monomers [165]. In the two-step process the acetyl terminated prepolymer is formed in reaction of dicarboxylic acid in an excess of acetic anhydride. Next, the temperature is elevated (180°C) and reaction carries on under high vacuum. However, the product is of quite low molecular weight character, which can be a reason for low physico-mechanical properties [166]. The optimization of polymerization process conditions as reaction temperature, time, presence of catalyst or the purity of reactants can be an effective strategy to overcome polyanhydrides limits [47].

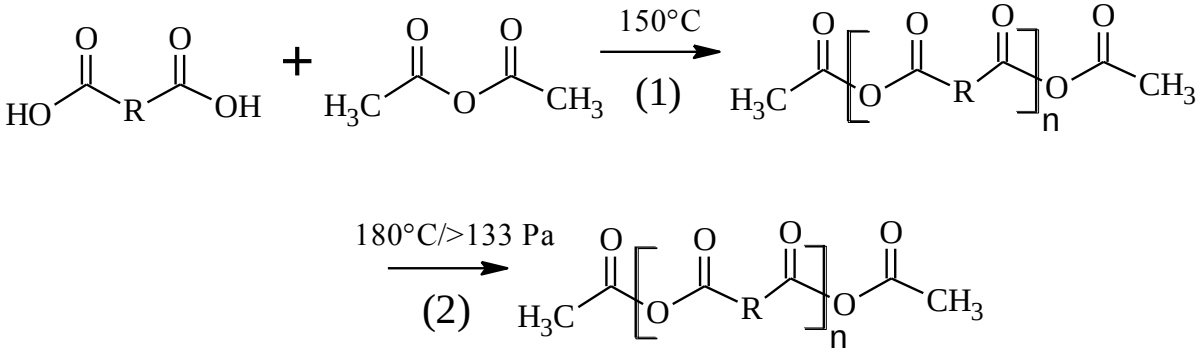

Figure 18 - Synthesis of polyanhydrides - melt polycondensation [45].

An alternative for melt polycondensation is the solution polymerization, which can be performed at ambient temperature. Typically the dehydrochloration (Fig. 19) between a dicarboxylic acid and dichloride acid provides a polyanhydride [16]. The ring-opening polymerization of cyclic anhydrides can be also employed, but the reaction is demanding for the high purity of reactants [47]. Structurally there are several types of polyanhydrides: saturated aliphatic, unsaturated aliphatic, aromatic and aliphatic-aromatic polyanhydrides, amino acids based polyanhydrides, fatty acids based polyanhydrides and poly(ester anhydrides). Aliphatic polyanhydrides are crystals with melting temperature under 100°C and they degrade relatively fast within months compared to aromatic ones, whose degradation rate is extremely slow [167].

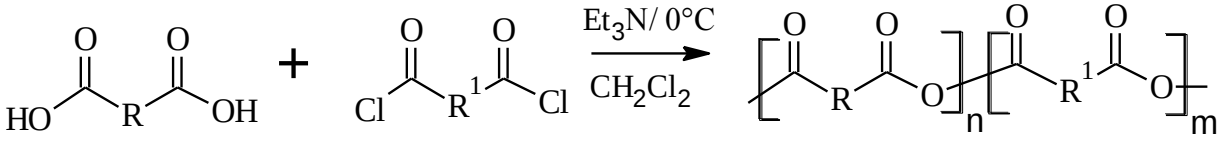

Figure 19 - Synthesis of polyanhydrides - polymerization in solution (dehydrochloration) [45].

Poly(sebacic anhydride) was shown to be the most appropriate for medical devices, in the research [168] it was synthesized in different molecular weights (13000 $g.mol^{-1}$) and used as pills for drug delivery purposes. It was shown that the release profile and burst effect decreased with higher M, which also brought the higher T_m and smoother surface morphology. In effort to enhance the release profile, the aliphatic poly(sebacic anhydride) was modified by using glycol chain extender forming poly(ester anhydride) copolymer. As a result of this modification, the release rate of model substance was slower for PSAG system than for PSA [169]. In other published research the hydrophobicity of PSA was used for preparation of micellar systems with PEG targeted to drug delivery applications [170]. In contrast to the aliphatic polyanhydrides, the aromatic show much slower degradation rate and they are insoluble in common organic solvents. The solubility issue is examined in the work [171], which proved the

enhanced solubility after change of the substitution pattern of phenyl ring from para to ortho of poly(1,3 bis(carboxyphenoxy) propane anhydride). The very thorough knowledge base providing comprehensive view regarding the polyanhydrides is founded by Abraham J. Domb in [172-174].

Fatty acids have the great potential in preparation of biodegradable polyanhydrides because they are naturally occurring hydrophobic compounds. Normally they possess only one functional group, thus they are used as chain terminators of polyanhydrides. To obtain sites that are more reactive they can be converted into a diacid monomer by derivation from dimers of unsaturated fatty acids [175, 176]. Example of preparation of fatty acid based polyanhydride is synthesis of random copolymer of sebacic acid and ricinoleic acid, which was found due to presence of both hydroxyl and carboxyl group to be the most suitable to obtain low molecular weight injectable polymer [177].

Despite the many various approaches in preparation and tailoring of polyanhydrides, the melt polycondensation can appear to be the most effective technique and along with that the polymerization conditions and catalytic systems are the main variables affecting qualitative properties of polyanhydrides. In study reported by Domb and Langer [167], the use of heterogenic coordination catalysts: cadmium acetate, $ZnEt_2$-H_2O (1:1), barium oxide, calcium oxide, and calcium carbonate is disclosed. The highest M they reached was 245,000 $g.mol^{-1}$. In the study [167] the aliphatic polyanhydrides poly(adipic anhydride) (PAA), poly(sebacic anhydride) (PSA) and poly(dodecanoic anhydride) (PDA) were prepared by melt polycondensation to yield molecular weight up to 33000 $g.mol^{-1}$ (PDA) and T_m = 90°C. They also found out that the longer diacid the higher molecular weight and polymerization rate. Cadmium acetate as catalyst of melt polycondensation was used to synthesize the poly(fumaric anhydride-co-isophothalic anhydride) in [178 The resulting polymer was intended for preparation of microparticles with controlled drug release properties. They reached M of 17,000 $g.mol^{-1}$ and good solubility in dichloromethane and chloroform, which allowed the preparation of microparticles by solvent method.

Polyanhydrides are suitable materials for programmed delivery systems due to surface accessible to hydrolysis. For example a polyanhydride of sebacic acid was used as a component in implantable delivery system consisting of sebacic acid (SA) 1,3-*bis*(*p*-carboxyphenoxy) propane (CPP) and poly(ethylene glycol) (PEG) in study [179] for pulsatile administration of parathyroid, hormone for regulation of calcium metabolism (Fig. 20).

For the biodegradable polymers in biomedical applications which are determined to be in contact with body the polymer structure and temperature properties affecting the degradation rate are crucial and the catalytic system used can influence them significantly. More extensive study of these connections (catalytic system, molecular weight and temperature properties) could bring new relevant insights into this issue.

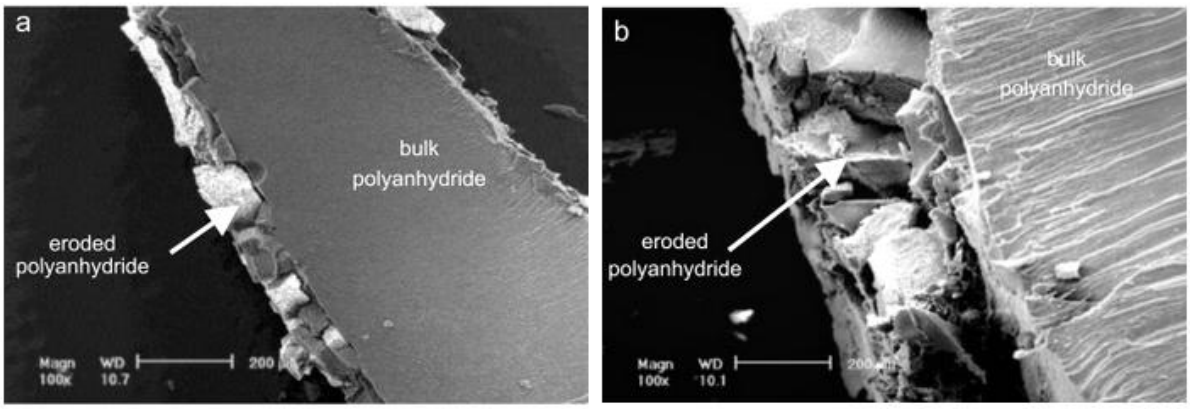

Figure 20 - SEM micrographs of polyanhydrides specimens with different compositions after erosion in 0.1 M PBS at 37 °C for 24 h. a) PEG/SA/CPP= 0/20/80; b) PEG/SA/CPP=2.5/20/80 [179].

SUMMARY OF THEORETICAL PART

Synthetic biodegradable polymers are currently widely used in various applications across sectors with relatively low requirements for the material properties, such as agriculture or industry, to biomedical applications, which are directly in contact (short or long-term) with the human organism and therefore they have to meet special requirements mainly related their biocompatibility.

Polylactides and polyanhydrides based polymers are due to their excellent biocompatibility one of the most promising biodegradable materials for advanced biomedical applications. The tailoring and enhancement of their physico-chemical properties could extend their utilization and knowledge regarding the degradation behaviour.

The simple and low-cost method for synthesis of PLA is the direct polycondensation. The copolymerization with PEG increases the hydrophilicity and also provides the functionalization of PLA by hydroxyl groups which allows the effective elevation of molecular weight by reactions with structurally different diisocyanates. Relating to potential applications, this part moreover deals with processing of these materials into nanoparticles and nanofibres.

Sebacic acid polyanhydride has been widely studied for medical use, nevertheless, the investigation of effects of various catalysts on molecular weight and thermal degradation behaviour details were not mentioned in the literature so far.

Despite the wide research done in the field of PLA and polyanhydrides, the following areas remain to be investigated in detail to obtain a complex view of these materials:

- synthesis of chain extended PLA based on copolymers prepared by polycondensation of LA and PEG and optimization of the synthesis of sebacic acid polyanhydrides
- nanofabrication possibilities of polyester urethanes
- biodegradation behaviour study of polyester urethanes under various conditions (abiotic, biotic)

AIMS OF WORK

The doctoral thesis is devoted to development and characterization of the modified synthetic biodegradable polymers based on PLA and polyanhydrides. The main framework has been focused on accomplishment of the following partial aims:

- Synthesis and characterization of chain linked PLA/based polyester urethanes

  - PLA/PEG chain linking reaction conditions optimization

  - Investigation of the effect of various diisocyanates on molecular weight and hydrolytic degradability

  - Preparation and characterization of nanoparticles and characterization of releasing profile of model substance

- Synthesis of sebacic acid based polyanhydrides

  - focused on optimization of reaction conditions with emphasis on catalytic system

  - Characterization and the thermal degradation study of polyanhydrides

- Degradation of chain linked PLA/PEG copolymers

  - Description of relationship between degradation process and molecular structure

  - Detailed study of degradation behaviour of polymers under various conditions

  - Investigation of processability of prepared materials by electrospinning and characterization of fibres properties

- Characterization of non-toxic PLA/PEG based polyester urethane

  - Preparation and testing of polymers with respect to chemical composition and toxicity

2. EXPERIMENTAL PART

Practical part is divided into sections according to predefined objectives based on theoretical research.

First part is devoted to investigation of synthesis of novel chain linked copolymers of PLA/PEG and their potential application in nano-encapsulation. Two structural various types of diisocyanates - HMDI, MDI were used in various ratio (NCO/OH = 2.3, 2.7, 3.2) of functional groups to prepolymer and their impact on efficacy of reaction was observed. Also the comparison of material properties including structure characterization, molecular weight, polydispersity, transition temperatures change of prepolymer and polymers were performed. Another part of this experimental work was preparation of nanoparticles by emulsion evaporation method and encapsulation of metazachlor (herbicide). The diameter, distribution of particles, encapsulation efficiency and releasing profile, which are crucial parameters for evaluation of effectivity of encapsuled substance were also studied. Moreover the molecular weight characterization was employed for characterization of hydrolysis process of prepared polyester urethanes. Biodegradability was demonstrated by means of composting test and expressed in the percentage of mineralization. Beside the nanoparticles, this material, due to its primarily linear structure can show great assumptions for nanofibres fabrication and utilization of this material for this purpose has not been reported yet. Moreover nanofibres structures are base of implantable scaffolds, which are being created from biodegradable materials. Therefore investigation of morphology and fibres diameter distribution was included. Finally, additional application of these materials can be as matrix of biocomposites. Hence they were compounded with natural flax fibres to obtain improved mechanical performance of this degradable material, however comprehensive results are beyond the scope of this study.

Next section was focused on optimization of melt polycondensation parameters for sebacic acid polyanhydride, promising material for applications as drug delivery device for instance. Selecting the catalyst is a key aspect that affects the final properties of the material, especially the molecular weight achieved and the reaction rate. Only a few papers have been produced on the catalysed synthesis of polyanhydrides, while almost no information is available on the effect of the applied catalyst on the thermal properties of the resultant polymer. For instance, Domb and Langer in their work used various transesterification catalysts derived on metal salts, earth metal oxides, alkoxy metals, and organometallic (iron) compounds for producing aliphatic and cyclic polyanhydrides in addition to their copolymers [47]. In this part the most attention was paid to investigation of effect of various catalysts on the polyanhydride structure when about 20 catalyst of different chemical character was examined. Also the development of molecular weight during reaction was

recorded and considered with respect to the specific polymerization progress. The polyanhydrides were further tested for thermal stability and thermal degradation products were analysed.

Final part was devoted to polyester urethanes and it was targeted on preparation of high molecular weight polymer with maximally eliminated potential toxicity. For this purpose the hydrochloric acid as catalyst and L-lysine diisocyanate as chain extender were chosen. The preparation of prepolymer was performed with PEG of various molecular weights which was expected to affect flexibility of polymer and thus mechanical testing was performed. Also the hydrolytic degradation rate of this type of material was investigated along with cytotoxicity.

Statistical evaluation of data

To obtain relevant data some measurements were performed at least three times in parallel and results were processed using statistical analysis. For the identification and rejection the outliers Dean-Dixon test (Q-test) was used. Confidence level of α was 0.95. After eliminating outliers, the arithmetic mean (x_i) and standard deviation (s) was calculated. [180]

$$\overline{x} = \frac{1}{N}\sum_{i=1}^{N} x_i \qquad (1)$$

N – number of measurements, x_i – measured values

$$s = \sqrt{\frac{1}{N}\left(\sum_{i=1}^{N} x_i^2 - N\overline{x^2}\right)} \qquad (2)$$

2.1. Novel poly(lactic acid)-poly(ethylene oxide) chain-linked copolymer and its application in nano-encapsulation

Introduction

Poly(lactic acid) has been broadly studied due to its variability in properties and degradability, which is beneficial for some applications ranging from conventional thermoplastic to biomedical sector devices [25, 181]. It can be prepared from lactide by ring opening polymerization, however this method is demanding for equipment, skills and thus it is rather expensive [182]. Second method is performed via polycondensation of lactic acid, and economically it can be advantageous, nevertheless, the product is usually of lower molecular weight and therefore of insufficient mechanical performance [183, 184].

The chain linking reactions represent the way how to prepare PLA based polymer with high molecular weight and others PLA inherent beneficial properties as biodegradability and biocompatibility. Besides, the reaction could bring new variabilities due to broad diversity of reactants [185]. Chain linking reactions (extending or coupling) occur via linking of oligomeric prepolymers, which is provided with reactive terminal groups. Efficient chain linkers are diisocyanates, which are very reactive with substances consisting of active hydrogen [186]. For this purpose, the prepolymer is usually prepared to be terminated by hydroxyl groups and in reaction with diisocyanate the urethane bonds are formed [138]. Nevertheless, the product is not a homopolymer [187].

Significant research papers dealing with chain-linking PLA are summarized in Table 5 where the information regarding the components and mechanism of polymerization are given. Table includes also important comparison of key material properties. It can be seen that the most common used compound providing hydroxyl groups is 1,4-butanediol likely due to its stability and availability [188]] and the highest molecular weights are provided by HMDI as a chain-linker. Although it seems that according to the table the use of PEG can provide prepolymers only with lower molecular weight, it has not affected the molecular weight of the final product, which is comparable to product of prepolymer with BD. One of the motivations of using PEG in our research was the intention to prepare prepolymer for chain linking by polycondensation, which can be more cost-effective in contrast to ROP employed by other researchers. Besides this component brings elevated hydrophilicity and lower glass transition temperature which both accelerate the degradation velocity [161].

The aim of the research presented here was to prepare diisocyanate chain-linked PLA, containing PEG as the hydroxyl terminating agent, through the simple polycondensation process. The resultant materials were analysed by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy

(FTIR)-attenuated total reflectance, differential scanning calorimetry (DSC), and water uptake experimentation. The prepared materials were further subjected to testing degradability rate (hydrolysis) and biodegradability (composting). The effect of chemical composition was also correlated with mechanical materials properties. Additional study was conducted on utilizing the newly developed material in advanced techniques for nano-encapsulation with metazachlor as a model compound and for electrospinning where the morphology and diameter distribution were observed.

Table 5 - Summary of previous work on chain linking of PLA with diisocyanates.

Chain extender	Prepolymer synthesis				PEU synthesis							
	OH termination	**L or D**	**M_w or (M_n) (10^3 g.mol^{-1})**	**Mechanism**	**Temp. (°C)**	**M_w (10^3 g.mol^{-1})**	**Đ**	**T_g (°C)**	**T_m (°C)**	**Tensile strength (MPa)**	**Elongation (%)**	**Ref.**
HMDI	1,4-BD	L	7.9 (M_n)	DP	170	20-390	1.8-6.8	45-54	n.a.	n.a	n.a	(143)
HMDI	1,,4-BD	L	8.9-11.7	DP	180	75-185	2.2-3.5	50	n.a	n.a	n.a	(189)
HMDI	1,4-BD	L	12.9	DP	150	300	4	n.a	n.a	55	11	(146)
HMDI IPDI DES	1,4-BD	L	5.5-26	DP	150-180	13-200	1.7-6.1	45-59	n.a.	n.a	n.a	(164)
HMDI+ BOX	n.t	L	25	DP	150	300	n.a	n.a	n.a	n.a	n.a	(138)
MDI	n.t	L	100**	-	25	n.a	n.a	53-64	170*	5	1.15-1.4	(192)
HMDI	1,4-BD	L	13	DP	180	72-115	3-4.3	50	150	12-29	1.5-3.7	(157)
MDI TDI	PEG1500	DL	1.1 (M_n)	SOL	90-160	<30	<2	n.a	n.a	n.a	n.a	(191)
HMDI	PDMS	L	n.a	SOL	60	5.2	1.8	n.a	n.a	1.2-1.4	22-33	(192)
LDI	PEG1500	L	2.5 (M_n)	ROP	140	<10	1.5-2.6	-35	Am.	n.a	n.a	(161)
HMDI	PEG1000-100000	DL	n.a	ROP	100	105 (Mn)	<2	n.a	n.a	n.a	n.a	(193)
MDI TDI IPDI	1,4-BD	DL	3.2(M_n)	ROP	70	83-233	1.5-2.1	50-60	n.a.	35-51	6-25	(194)
MDI	n.t	L	9.8	DP	175	57	3.8	68	149	n.a	n.a	(156)
HMDI	n.t	L	6-11	DP	160	14-76	<2.5	55	140	n.a	n.a	(155)

n.t - not OH terminated; n.a – not available; DP, direct polycondensation; SOL, solution polycondensation; ROP, ring opening polymerization

Materials

For preparation of prepolymer L-lactic acid (LA) 80% water solution and PEG (M_W = 380 - 420 g.mol-1) sourced from Merc were used. As catalyst Tin(II) 2-ethylene hexanoate ($Sn(Oct)_2$) ~95% was employed, diisocyanate components were HMDI (hexamethylene diisocyanate) 98%, and MDI 4,4-methylenebis(phenyl isocyanate) 98% all purchased from Sigma-Aldrich, Germany. Solvents chloroform, acetone, methanol and ethanol were purchased from IPL Petr Lukes, Czech Republic; chloroform and tetrahydrofuran (HPLC grade) were from Chromspec, Czech Republic. As the active substance for encapsulation was chosen herbicide metazachlor (2-chloro-N-(2,6-dimethylphenyl-N-(1H-pyrazol-1ylmethyl)acetamide (MTZ) purchased from Chemos (Czech Republic) in the monoclinic and triclinic form: off-white powder of molecular weight 277.75, water solubility 430 $mg.L^{-1}$, purity 98%, melting point 74 – 78 °C, and density 1.19 $g.cm^{-3}$. Phosphate buffer (PB, 0.1 $mol.L^{-1}$, pH = 7, NaH_2PO_4 adjusted with NaOH was sourced from Chromspec, Brno, Czech Republic.

Synthesis of prepolymers

Prepolymers were prepared via melt polycondensation. L-LA (100 mL) was added into 250 mL two-neck flask equipped with stirrer placed in oil bath and dehydrated under condenser for 4h at 160°C and reduced pressure of 20 kPa. After the PEG (7.5wt.%) and catalyst ($Sn(Oct)_2$) (0.5wt%) were added and reaction proceeded for 6 h at 10 kPa, then the pressure was lowered to 3 kPa and reaction was conducted for a further 10 h. Final product was cooled and stored in desiccator.

Synthesis of PLA/PEG chain-linked polymers

Before the reaction started the prepolymer of 30 g was melted in two-neck flask at 160°C under flow of inert gas – nitrogen while stirring. The chain linking reaction alone was initiated by adding of MDI or HMDI in calculated amount, which was expressed as ratio of reactive groups of diisocyanate and PEG (NCO/OH). Reaction was conducted for 30 min when the melt become viscous and amber coloured. Final polymer was purified by dissolving in acetone and precipitating into cooled methanol/water (1:1). The precipitate was filtered and dried in vacuum for 24 h. Beside the 2.3 and 2.7 NCO/OH the highest concentration of NCO to OH was founded to be 3.2 when the polymer was still soluble.

Sample preparation

Material was moulded into a plate (60 x 60 mm, thickness 1.5 mm) in a manual press at 140°C for 4 min and cooled down in second cold press to ambient

temperature and the specimens for tensile testing and degradation experiment were cut and conditioned before testing.

METHODS

Molecular weight determination by gel permeation chromatography

Gel permeation chromatography is technique broadly used in polymer chemistry to separate molecules basis on their size. It provides comprehensive information about molecular weights and chromatograph can suggest also the linearity or branching in polymer structure, which is substantial technical information.

GPC analysis was conducted using the Waters chromatographic system. Samples were dissolved in $CHCl_3$ (~2 $mg.mL^{-1}$) and filtered. Separation and detection took place on a PL gel-mixed-D bed column (300 × 7.8 mm, 5 μm particles) with an RI response detector (Waters 2414). Analyses were carried out at 30°C, flow rate 1.0 $mL.min^{-1}$ and injection volume was 100 μL. The GPC system was calibrated with narrow polystyrene standards (Polymer Laboratories Ltd., UK). The weight average molar mass M_w, number average molar mass M_n and molar-mass dispersity ($Đ = M_w/M_n$) of the tested samples were determined from their peaks corresponding to the polymer fraction, and expressed as "polystyrene relative" molecular weights. All data processing was carried out using Empower software.

Structural analysis by FTIR

For investigation of chemical structure Fourier transform infrared spectroscopy in attenuated total reflectance mode was used. The analysis was carried out using Nicolet iS10 equipped with diamond crystal at resolution 4 cm^{-1} and number of scans 64.

Structural analysis by NMR

Proton nuclear magnetic resonance measurements were performed using a Varian Unity Inova 400nspectrometer. Chemical shifts of signals in spectra were referenced to the solvent peaks ($CDCl_3$ $-_1$H NMR (400 MHz, $CDCl_3$): δ = 7.25 ppm. First order analysis was used to evaluate all the NMR spectra received.

Thermal properties by DSC

Thermal characteristics as melting point temperature (T_m), crystallization temperature (T_c) and enthalpies (ΔH_m, ΔH_c) were measured on a Mettler Toledo DSC1 STAR testing machine (Mettler Toledo), over the temperature range from 0°C to 190°C at a heating/cooling scan rate of 10°C.min^{-1} under nitrogen flow (30 ml.min^{-1}). Two temperature cycles were recorded where in second heating

cycle the glass transition temperature (T_g) was obtained at the mid-point stepwise increase of the specific heat associated with glass transition.

Determination of acidity number

The concentration of terminal carboxyl groups was expressed as an acid number (AN), which represents the amount of KOH (in milligrams) needed to neutralize 1 g of a substance. AN was determined by titration of a sample in methanol/dichloromethane (1:1 v/v) with 0.01 M KOH ethanol solution. Bromothyol blue was used as an indicator.

SEM analysis

Characterization of nanoparticles and nanofibres was carried out by scanning electron microscopy on (VEGA IILMU, TESCAN). Prior to microscopy of nanoparticles, samples were freeze dried for 48 h. All specimens were coated with a thin layer of Au/Pd. The microscope was operated in high vacuum mode at an acceleration voltage of 5kV.

Water uptake behaviour

The specimens were pre-dried (at 30 °C and p= 2 kPa up to constant weight) prior to further investigation. Then they were immersed in distilled water (25mL) and incubated at 25 °C under static conditions. The specimens were removed, wiped with a paper towel, and immediately weighed after predetermined time intervals. Water uptake, WU (%), was calculated via Equation (3) as follows:

$$WU = \frac{w_1 - w_0}{w_0} 100 \quad (3)$$

where, w_0 is the initial weight of the specimen after pre-drying and w_1 is the wet mass of the specimen at the given time. Three samples were investigated in parallel.

Water contact angle

Water contact angle (CA) measurements were performed on an optical video contact angle instrument (Model OCA 40, Dataphysics, Germany) at room temperature, using the sessile drop method. A 5-µL water droplet was utilized for experimentation by the aforementioned sessile drop method; the reported values of CA were the averages of at least 10 measurements at different positions on the sheet prepared through the compression molding technique described in the preceding text.

Mechanical properties

Tensile properties investigations were carried out on universal tensile testing machine M350-5 CT Materials Testing Machine (Testometric Company, Lancashire, UK) at a crosshead speed of 1 $mm.min^{-1}$. Rectangle form specimens with dimensions of (50 x 7 x 1.5) mm (length, width, thickness) were cut from the compression moulded plates.

Encapsulation

The amount of released MTZ was determined by HPLC (Waters, 2487) under following conditions: column (Xselect CSH, C18, 5μm, 250x4.6 mm; Waters), mobile phase (acetonitrile:water - 60:40), detection: UV 220 nm and 266 nm. Two important parameters, encapsulation efficiency (EE %) and herbicide loading (HL %) were calculated. The EE was defined as a ratio between the weight of MTZ encapsulated and its total weight at the beginning of the process, Equation 3:

$$EE = \frac{weight\ of\ encapsulated\ MTZ}{initial\ weight\ of\ particles} 100 \quad (4)$$

Herbicide loading (HL, %) was defined as the amount of MTZ encapsulated divided by the final weight of particles with encapsulated MTZ at the end of the process, Equation 4:

$$HL = \frac{weight\ of\ encapsulated\ MTZ}{weight\ of\ particles} 100 \quad (5)$$

Once the chloroform was evaporated and suspension produced MTZ occurred in three forms, as it is depicted in Fig. 21-A: untrapped in water phase, encapsulated inside nanoparticles and captured on the surface of the nanoparticles and the sum of these three forms is assumed to be equal to the initial amount of MTZ and the process of determination the weight of encapsulated MTZ necessary for calculation of EE and HL was as follows.

Of the obtained suspension 1 mL was centrifuged (Hettich Universal 320) at 10 000 rpm for 10 min to separate out the water phase containing untrapped MTZ (Fig. 21 - B) from the particles, then each of them was handled in a different way. The water phase containing untrapped MTZ was analysed via HPLC.

The centrifuged particles were re-suspended in 40 mL of distilled water (Fig. 21 - C). The total time of the contact of particles with fresh water phase was exactly 60 s. This should represent a sufficient time to dissolve non-encapsulated MTZ on particle surface but a short enough time for the release of encapsulated MTZ from the particles [195]. The 2 mL of this mixture was then immediately filtered through a 0.22 μm poly(tetrafluoroethylene) (PTFE)

syringe filter and in filtrate the concentration of surface MTZ was measured in filtrate (Fig. 21- D).

The weight of encapsulated MTZ was calculated as the difference between its initial amount and combined amounts of MTZ in the initial suspension water phase and the readily soluble fraction on the surface of the particles.

Release experiment

Re-suspended particle suspensions of 5 ml were transferred into 100 mL of phosphate buffer (20 mmol·L^{-1}, pH=7) containing 0.2% sodium azide to prevent undesirable microbial degradation (Fig. 21 - E). All this was done in triplicate. Suspensions were shaken (120 rpm) at 25 °C. Subsamples of 1.5 mL were taken in time intervals (0-720h), centrifuged at 14 000 rpm for 10 min, and filtered through a 0.22 μm syringe PTFE filter to remove any remaining particles. MTZ in samples was determined by the HPLC method described in encapsulation part.

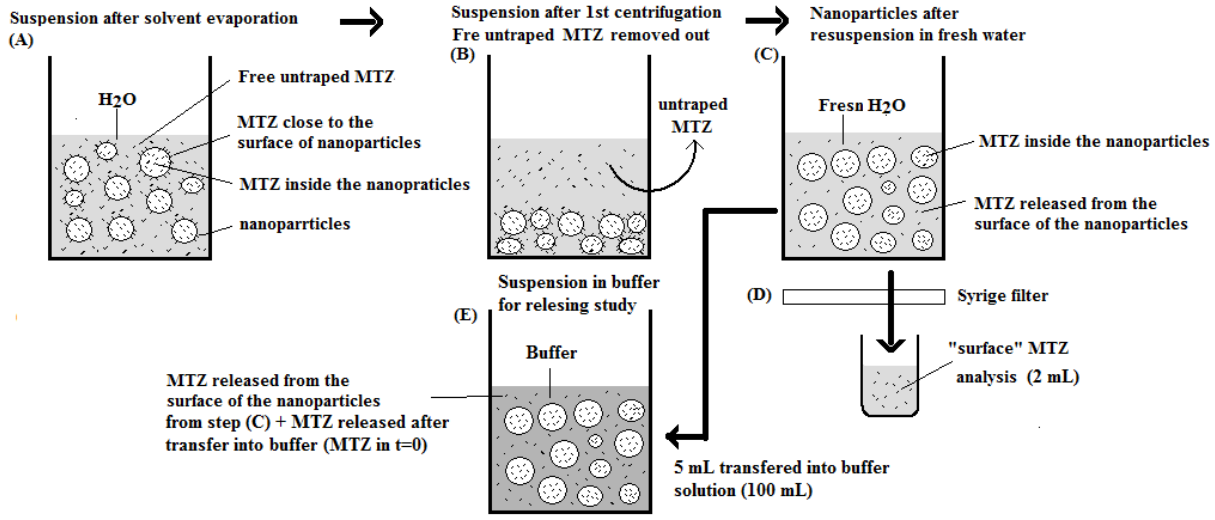

Figure 21 - Treatment of nanoparticles and release experiment.

Hydrolysis test

The test was performed in liquid buffered medium (pH=7) on samples having round shape dimension (diameter 3.4 mm and thickness 1.5 mm). The specimens were placed in 25 mL glass bottles fully immersed in hydrolysis medium and shake. In each follow-up time one specimen was removed and analysed by GPC. The hydrolysis was studied under temperatures 37 °C and 55 °C. The weight changes during hydrolysis were monitored under the same condition just the diameter of cut specimen was 10 mm.

Electro spinning

Electrospinning process was carried out on apparatus consisting of jet and target with separation distance 18 cm at 23 °C. The PEU solution (12 wt. % in DMF) was charged by DC 75 kV. Flow rate of the polymer solution was 0.086 ml . min^{-1}. The conductivity of polymer solution was adjusted with citric acid and sodium tetraborate (3:1, w/w) to 59.5, 107.9 and 150.9 $\mu S.cm^{-1}$.

Mechanical testing

Mechanical properties are based on chemical composition, structure and molecular weight of polymer. Further parameters external character as temperature, time, strain-rate frequency and moisture content significantly contribute to mechanical performance [196]. In polymer mechanical testing is widely used tensile test which output is the stress-strain curve (in tension). Tensile stress is the force per unit of cross-sectional area applied on to the material:

$$\sigma = \frac{F}{A} \tag{6}$$

where the F is force and A is area.

Tensile properties investigations were carried out on universal tensile testing machine M350-5CT Materials Testing Machine (Testometric Company, Lancashire, UK) at a crosshead speed of 1 $mm.min^{-1}$. Rectangle form specimens with dimensions of (50 x 7 x 1.5) mm (length, width, thickness) were cut from the compression moulded plates. Prior to testing the specimens were conditioned according the standard ISO 291 at 25°C, 50% humidity for 10 days. Measurements were performed five times and the average value was calculated.

RESULT AND DISCUSSION

Analysis of prepolymer and polymers

The ^{1}H NMR spectrum of prepolymer is depicted in Fig. 22. Typical resonance for PLA-PEG copolymer can be found at 1.5–1.6 ppm (CH_3, PLA), 5.1–5.27 (CH, PLA), 3.6–3.7 (CH_2, PEG), and 4.2–4.4 (CH_2 from PEG bonded to PLA). [191,197] Prepolymer composition was discerned by the LA/EG ratio and degree of polymerization of PEG (DPPEG) and PLA (DPPLA) segments, as well as the number average molecular weight of prepolymer according to information obtained from specific peak areas, in adherence to the procedure described by Ren et al. [197] Calculations show that DPPEG = 9 (LA/EG ratio is 4.71) and DPPLA = 42. It should be noted that the initial feed LA/EG ratio was 5.21. This discrepancy is contributed to partial conversion of LA in form of lactide during synthesis of prepolymer. On the basis of this, the value of M_n = 3400 $g.mol^{-1}$ is calculated. It is in general accord with GPC results (M_n = 3300 $g.mol^{-1}$). The qualitative analysis of polymers after the chain-linking

reaction is also shown in Fig. 22 (^{1}H NMR spectra of 2.7MDI and 2.7HMDI). The presence of a urethane bond can be seen at 3.12 ppm (2.7HMDI), which indicates a successful reaction between OH and NCO groups. The same signal in MDI chain linked samples can be seen at 8.45 ppm. Furthermore, the presence of aromatic rings originating in the MDI structure can be noticed at 7.0ppm (CH) and 3.8 (CH_2) [191].

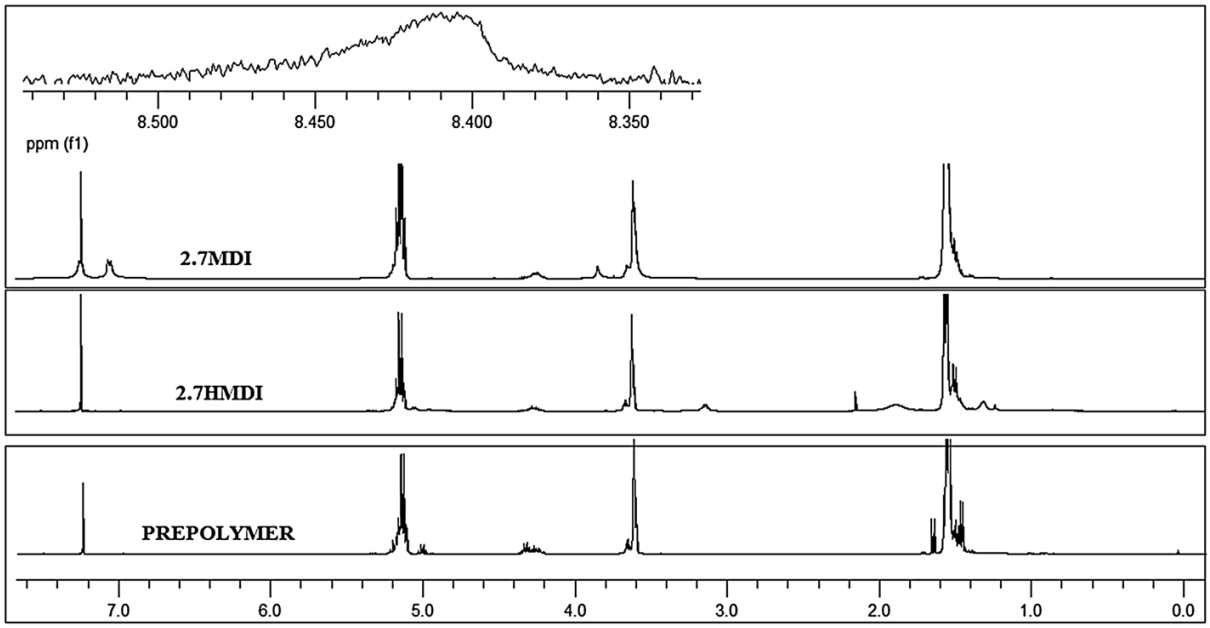

Figure 22 - Hydrogen-1 nuclear magnetic resonance spectra of prepolymer and selected chain-linked copolymers.

Polymer synthesis - molecular weight and structure characterization

Changes in molecular weight after a chain-linking reaction, along with their distribution, are depicted in Fig. 23. It can be seen that molecular weight was significantly enhanced by adding a chain-linker. However, a noticeably higher increase of M_W was observed with HMDI when the maximum value of nearly 300,000 g.mol^{-1} was achieved at the NCO/OH ratio of 3.2, while the initial M_W of prepolymer was 5200 g.mol^{-1} (Tab. 6). In the case of MDI, this effect was remarkably less visible, and the maximum achieved M_W was only 39,000 g.mol^{-1} at the same NCO/OH ratio (3.2). The increase of M_W was caused by a successful reaction between NCO groups and prepolymer end groups. Due to distinctly higher M_W achieved with HMDI rather than MDI, it could be concluded that HMDI was more reactive with prepolymer. The value of the M_W of the prepolymer was similar to that achieved by other authors, [146, 164, 189] and the M_W of chain-linked products was comparable with that reported elsewhere, [143, 146, 157, 164, 194] despite 1, 4-BD being used as a chain terminating agent in all these works. Therefore, it can be stated that, from the perspective of molecular weight, comparable results can be achieved even

with PEG as the terminating agent instead of 1,4-BD. The polydispersity value (Fig. 23, solid line) revealed remarkable differences between HMDI and MDI content systems. While prepolymer exhibited narrow Đ (1.68), this became broader after the chain-linking reaction. The highest Đ = 13 was achieved with the HMDI chain-linking agent at the NCO/OH ratio of 2.7. Generally, broader distribution brought about materials containing HMDI. All the values of Đ are considerably higher than those presented in works by other authors [53, 146,155,156,164,190,194, 198], which could be attributed to extensive chain-branching during reaction, especially when allophanate and biuret bonds form [199]. However, it was expected that differing prepolymer structures, reaction conditions, and determination methods would also be responsible for relatively broad Đ.

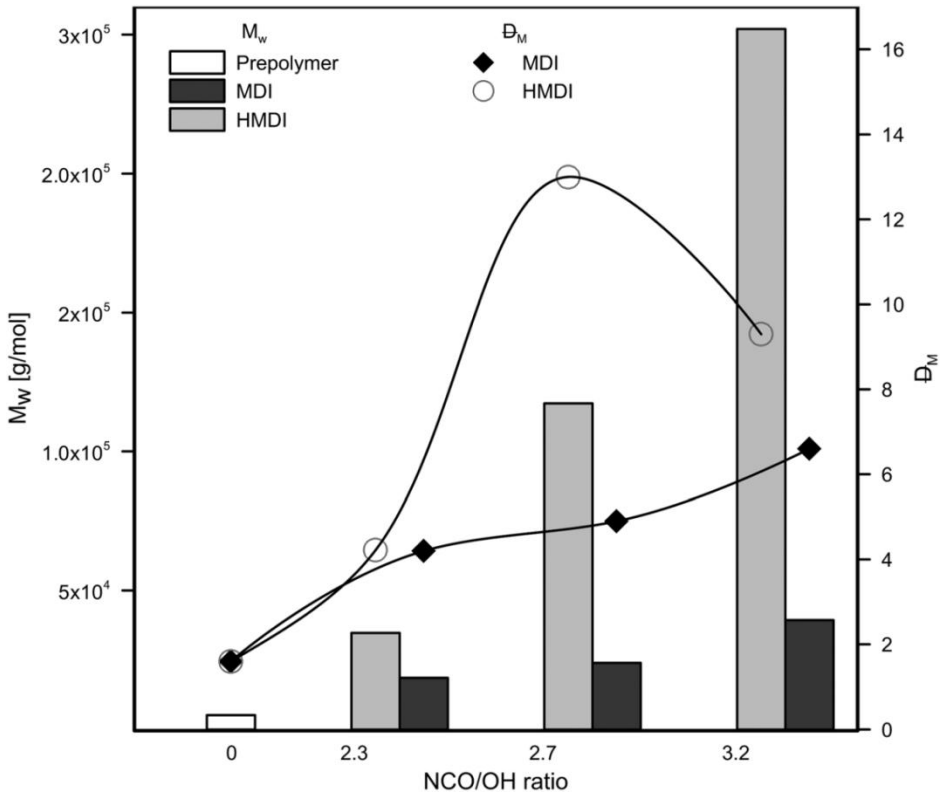

Figure 23 - M_W and polydispersity of poly(lactic acid)–poly(ethylene oxide) chain-linked copolymers.

Qualitative analysis of the chain-extending reaction was evaluated by FTIR spectroscopy; the spectra thus obtained are depicted in Fig. 24. In the cases of pure HMDI and MDI, the most intense signal was discerned at 2285–2250 cm^{-1}, which was assigned to the NCO band, while the peaks between 3000 and 2800 cm^{-1} belong among aliphatic CH stretching deformations. Furthermore, in the case of MDI signals between 3100 and 3000, and 1600 and 1500 cm^{-1}, these are related to C–H and C=H; C aromatic ring deformation, respectively. For PEG, the most intensive peaks were detected in the region

1200–1000 cm^{-1} assigned to the C–O bond, while 3600–3200 cm^{-1} related to end OH groups. As for the prepolymer, five main groups were distinguished, these being between 3600 and 3300 cm^{-1} (OH), 3000 and 2800, 1400 and 1330 (C–H), 1840–1680 (C=H;O), 1490 and 1420 ($–CH_3$), and 1200 and 1000 cm^{-1} (–C–O–). Neither the HMDI nor MDI content samples gave off signals at 3600–3200 and 2285–2250 cm^{-1}, leading to the conclusion that all the NCO groups had successfully reacted with OH end groups during chain extension. Two new peaks appeared at 3300–3450 cm^{-1} (N–H) and 1580 1490 cm^{-1} (N–H amide II), which confirmed a newly formed urethane bond [194]. Moreover, a broader –C=H-O signal to 1640 cm^{-1} showed with the presence of an amide I bond, arising from a reaction of NCO with unreacted COOH groups [192]. These observations confirmed that chain-extending reactions between PLA-PEG prepolymer groups and chain-linkers had taken place and are in general accord with the spectra published in other works [138,146].

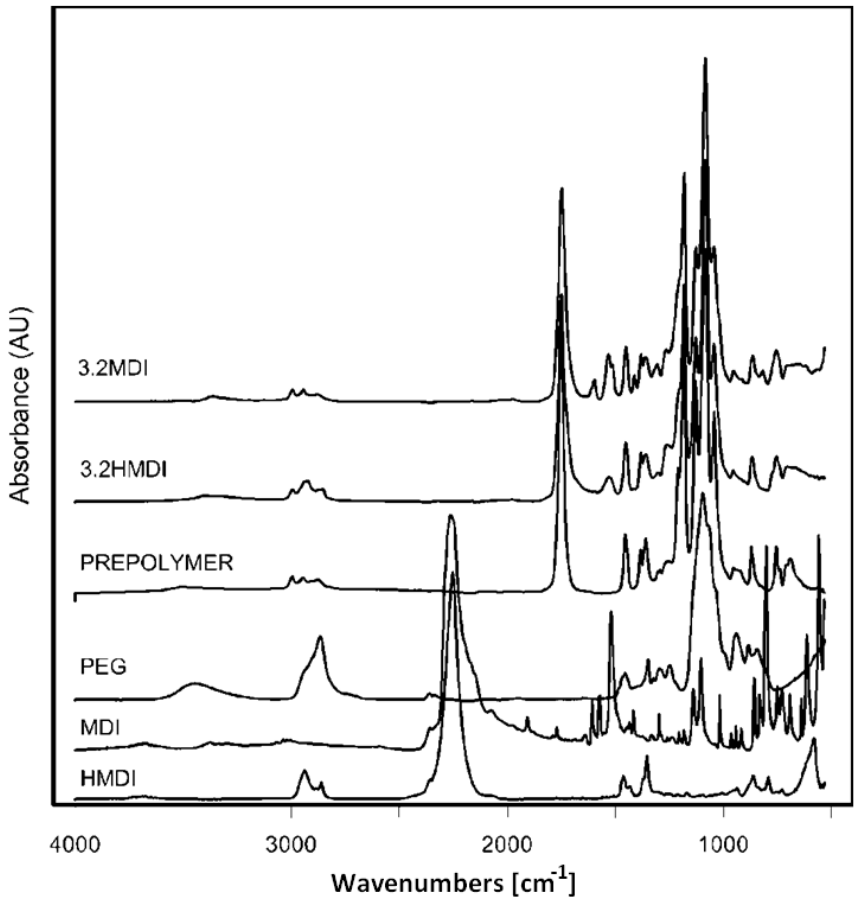

Figure 24 - Fourier transform infrared spectroscopy–attenuated total reflectance spectra of pure components, prepolymer, and poly(lactic acid)–poly(ethylene oxide) copolymers.

It can be expected that physical–chemical features (chemical structure, state, etc.) of the chain-linkers used in this study influence the Mw of the resultant PLA PEG products. Nevertheless, the effect of the presented COOH groups should not be overlooked. Quantitative analysis of free COOH groups is presented in Fig. 25. It is noticeable that prepolymer exhibited relatively high AN, equalling 26.2 mg KOH/g. These free COOH groups originate either from unreacted LA or PLA chains, which are not terminated by PEG. It can also be discerned that AN decreased after the chain-linking reaction with both MDI and HMDI. However, AN reduction was more significant for HMDI. It is known that NCO groups can react with COOH-forming amide bonds. This reaction may lead to chain-linking, too. Nevertheless, the kinetics of this reaction is slower than that for OH [31]. The results in Figure 25 clearly show that the reactivity of MDI with COOH is slower than that with HMDI, and this is probably one of the reasons for the noticeably lower M_W obtained when the MDI chain-linking agent was used (Fig. 23).

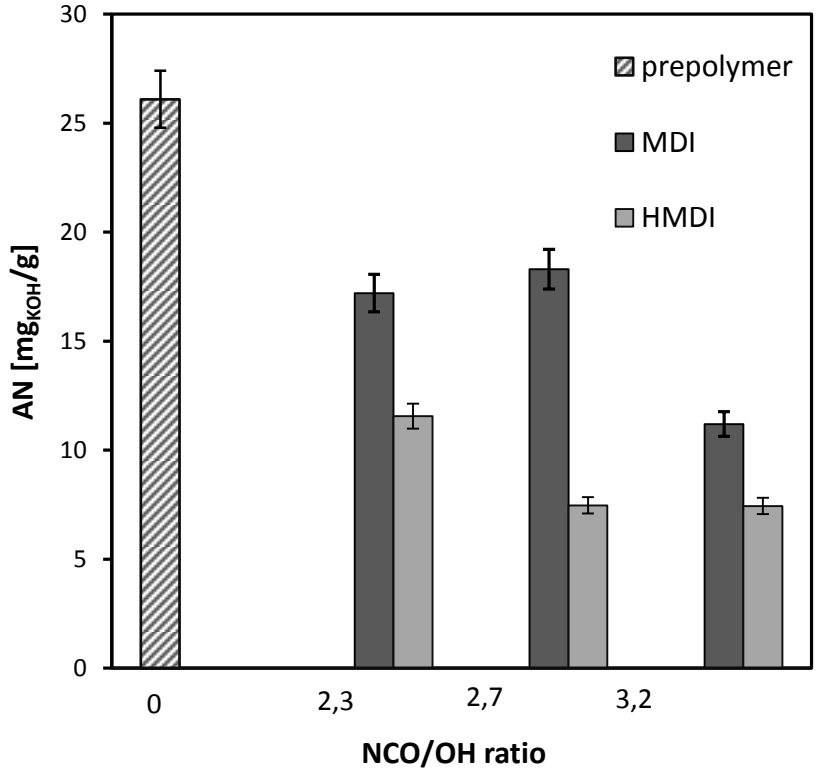

Figure 25 - Effect of NCO/OH groups ratio in reaction feed on acid number (AN) of prepolymer and poly(lactic acid) poly(ethylene oxide) copolymers.

Table 6 – The summary of the properties of prepared PLA-PEG copolymers with HMDI.

Sample Label	LA/EO ratio*	NCO/ OH ratio**	DP PEG***	DP PLA*	M_w a [g.mol^{-1}]	Đa	T_g b [°C]	T_m b [°C]	AN c [mg_{KOH} /g]
2.3HMDI	4.77	2.3	9	42	36700	4.2	30	130	11.6
2.7HMDI		2.7			117000	13.0	33	n.f.	7.4
3.2HMDI		3.2			300000	9.2	34	n.f.	7.5

** measured by ^{1}H NMR; ** calculated based on concentration of OH groups (from PEG) added to reaction feed; *** degree of polymerization of PEG (based on calculation DP=400/44); a – values obtained from GPC (PS relative calibration), b – measured by DSC, c – acid number (determined by titration), d – after 140h at 25°C, e – based on measuring weight on air and in water; n.f. – not found in the first heating scan*

Thermal properties characterization

Differential scanning calorimetry thermograms are depicted in Fig. 26, and all data are summarized in Table 7. It can be seen that prepolymer exhibited strong double melting peaks at 101 and 126 °C, which could indicate the presence of different types of crystals with different stabilities, or the presence of less perfect crystals that had had enough time to melt and then rearrange into crystals of a more ideal structure, which then re-melted at a higher temperature [200, 201]. On the contrary, HMDI chain-linked products displayed different behaviour in the first heating scan. It was found that only samples with the NCO/OH ratio 2.3 were able to crystallize, although it should be noted that the enthalpy of melting was considerably depressed (Tab. 7). The values of T_g were obtained from a second heating scan (Tab. 7). It can be seen that prepolymer exhibited low T_g at 15.3 °C, although after a chain-linking reaction this value had remarkably increased above 30 °C for all the samples. Generally, the increase of T_g is attributed to enhanced molecular weight and a decreased number of chain-end groups, which tends to decrease T_g. Nevertheless, MDI content samples show T_g at over 40 °C, which may be connected with the presence of an aromatic ring in the polymer chain that conducts as a hard and low mobility segment. This is likely to be caused by the presence of a flexible PEG molecule in the structure. Although the samples were initially amorphous, or only minor melting behaviour was observed in the first DSC run, in the second heating run all HMDI chain-linked samples exhibited a melting temperature of around 130 °C and a cold crystallization peak at ~105–110 °C.

This suggests that, despite the highly amorphous state of the material after preparation, it is possible to induce the crystallization process through annealing the copolymer at temperatures above T_g. On the contrary, MDI content samples remained completely amorphous even after the second scan. This could be connected with the presence of aromatic rings in the structure that decrease the uniformity and mobility of polymeric chains. In terms of glass transition temperature and melting temperature, the result achieved in this work differs from that presented by other research groups. Gu et al.,[157] who followed a similar procedure, reported T_g at around 50 and a T_m of 150 °C, which is greater than the figures given here. Similarly, other authors [143,155,156,164,189,194] measured values higher than 30 and 130 °C. The main reason for this discrepancy could relate to the type of chain extender. While all the papers mentioned in the preceding text deal with 1,4-BD, the researchers here used PEG and consequently a lower T_g was anticipated.

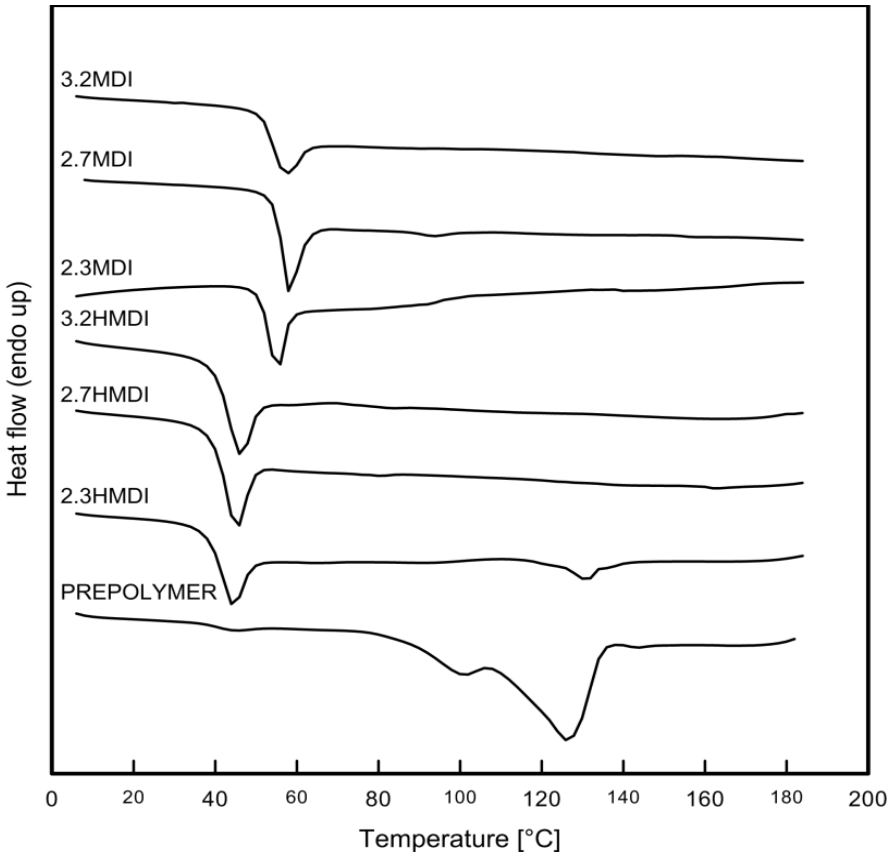

Figure 26 - 1st heating scan of prepolymer and PLA/PEG diisocyanate chain linked products.

Table 7 - Summary of differential scanning calorimetry results from heating and cooling scans.

	1st Heating scan						**Cooling**		**2nd Heating scan**					
Sample	**T_g [°C]**	**c_p [J/g.K]**	**T_c [°C]**	**dH_c [J/g]**	**T_m [°C]**	**dH_m [J/g]**	**T_g [°C]**	**cp [J/g.K]**	**T_g [°C]**	**c_p [J/g.K]**	**T_c [°C]**	**dH_c [J/g]**	**T_m [°C]**	**dH_m [J/g]**
Prepolymer	38.9	0.064	*	*	101.1	-32.33	*	*	15.3	0.504	83.2	13.11	*	*
2.3HMDI	39.0	0.423	112.1	1.03	131.1	-1.26	28.9	-0.558	30.4	0.532	105.1	3.17	129.6	-1.12
2.7HMDI	39.5	0.452	*	*	*	*	30.2	-0.550	32.8	0.490	108.2	0.7	129.7	-0.51
3.2HMDI	40.8	0.492	*	*	*	*	31.9	-0.603	33.9	0.476	110.1	0.23	129.7	-0.15
2.3MDI	50.8	0.424	*	*	*	*	37.8	-0.596	40.5	0.436	*	*	*	*
2.7MDI	54.6	0.513	*	*	*	*	41.0	-0.611	42.8	0.482	*	*	*	*
3.2MDI	52.8	0.477	*	*	*	*	39.1	-0.515	44.3	0.485	*	*	*	*

Water uptake and water contact angle measuring

Water uptake experimentation is depicted in Fig. 27. It can be seen that all the samples exhibited considerably higher water uptake than reported for pure PLA (<1%) [202]. It is due to the presence of hydrophilic PEG, which effectively increased the amount of water absorbed. However, the experiment also revealed significant differences between HMDI and MDI chain-linked products. It was found that MDI content samples absorbed nearly twofold less volume of H_2O than HMDI ones. Similar behaviour was observed when measuring the water contact angles (Fig. 28), where the MDI content samples exhibited considerably higher values, which contributed to the higher hydrophobicity of these polymers. The explanation for the higher hydrophobicity of MDI based PLA-PEG samples lies in the chemical nature and quantity of the chain-linker used. Due to the presence of two aromatic rings in the MDI structure, it can be expected to be more hydrophobic than HMDI.

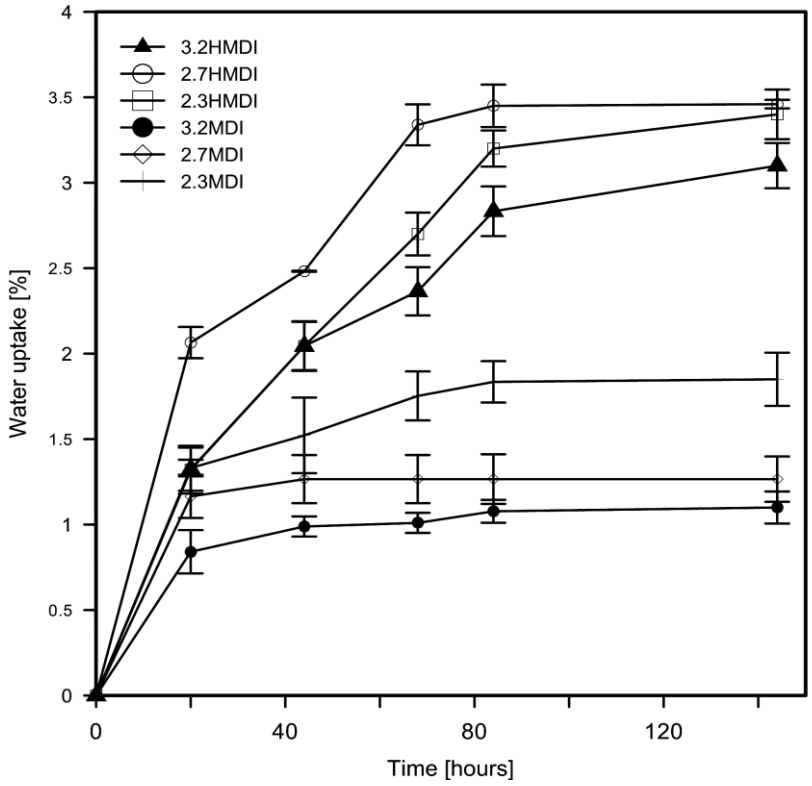

Figure 27 - Water uptake behaviour of PLA/PEG diisocyanate chain linked polymers.

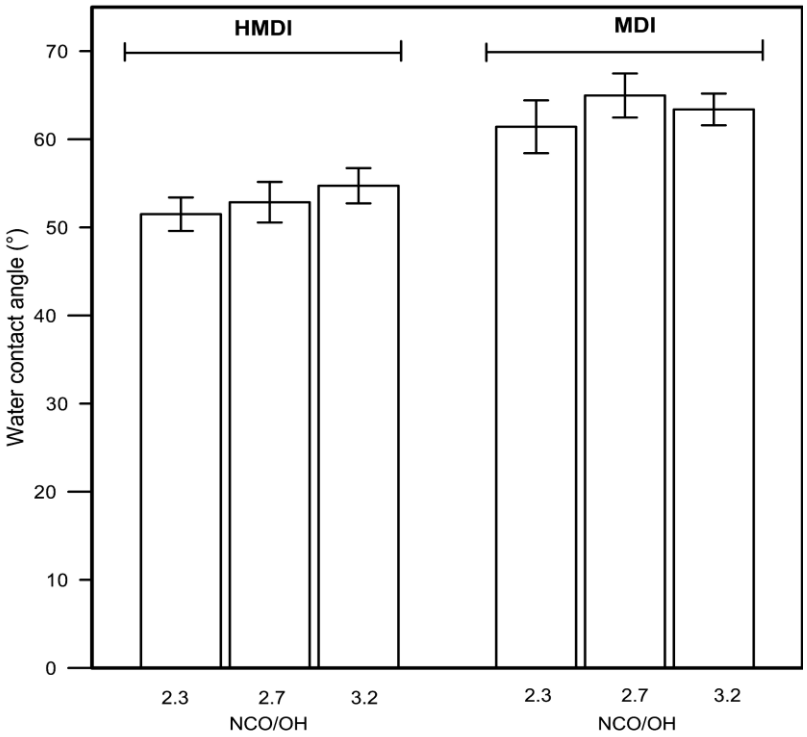

Figure 28 - contact angle values (right) of PLA/PEG diisocyanate chain linked polymers.

Preparation of nanoparticles, encapsulation

The PLA-PEG copolymers produced were used to prepare nanoparticles, and their characteristics are summarized in Table 8. It can be seen that, in all cases, the diameter ranged between 290 and 320 nm with narrow polydispersity ~ 0.2. These results show that all materials can successfully be utilized for nanoparticle formation, and this research has not found any significant link between structure and particle diameter. When preparing the particles, no self emulgating properties were observed, as are typical for simple PLA/PEG block copolymers. This suggested the theory that the physicochemical structure of the chain-linked products is more complex. Evaluation of the MTZ encapsulation is detailed in Table 8. As can be seen, the MTZ loading achieved was about twofold lower than that initially dissolved, and total encapsulation efficiency varied between 45% and 65%. The best result was achieved with sample 3.2MDI, when EE reached nearly 65%. This might have been caused by the low hydrophilicity of MDI content products as measured by the water uptake experimentation, which supports the entrapment of hydrophobic metazachlor. In the previous work by the authors [203] low molecular weight PLA was used for MTZ encapsulation and EE of around 60% was achieved under the same preparation conditions, which is comparable with results obtained from the MDI chain-linked copolymer. The release experiment (Fig. 30) revealed that initially some MTZ

was released into the surrounding medium, a so-called burst effect [204]. which is generally considered undesirable. For HMDI samples, the rate of occurrence equalled 40–51% and only 10% in case of the MDI chain extended one. Releasing profiles displayed first order kinetics, and it could be seen that a relatively high portion of MTZ still remained in the nanoparticles after completing the experiment. This was probably trapped inside the nanoparticle, therefore, diffusion out was restricted. Scanning electron microscopy micrographs of the prepared particles are shown in Fig. 29. It is clear that the particle diameter is greater than that measured through observation by the light scattering technique, which is probably caused by particle agglomeration during freeze-drying.

Table 8 - Characteristics of the nanoparticles obtained and encapsulated metazachlor.

Sample	**Diameter [nm]**	**s.d [nm]**	**PDI**	**s.d**	**EE [%]**	**s.d [%]**	**Theore-tical HL[%]**	**HL [%]**	**s.d [%]**
3.2HMDI	320	2	0.21	0.009	51.0	2.2	20	9.3	0.4
2.7HMDI	290	2	0.17	0.014	52.4	3.1	20	9.5	0.6
2.3HMDI	310	4	0.21	0.012	52.6	2. 1	20	9.5	0.4
3.2MDI	320	4	0.18	0.015	64.7	1.4	20	11.5	0.3
2.7MDI	310	3	0.17	0.023	55.7	1.0	20	10.0	0.1
2.3MDI	290	2	0.13	0.005	45.9	0.3	20	8.4	0.1

PDI, polydispersity of particles; s.d., standard deviation from three parallel samples.

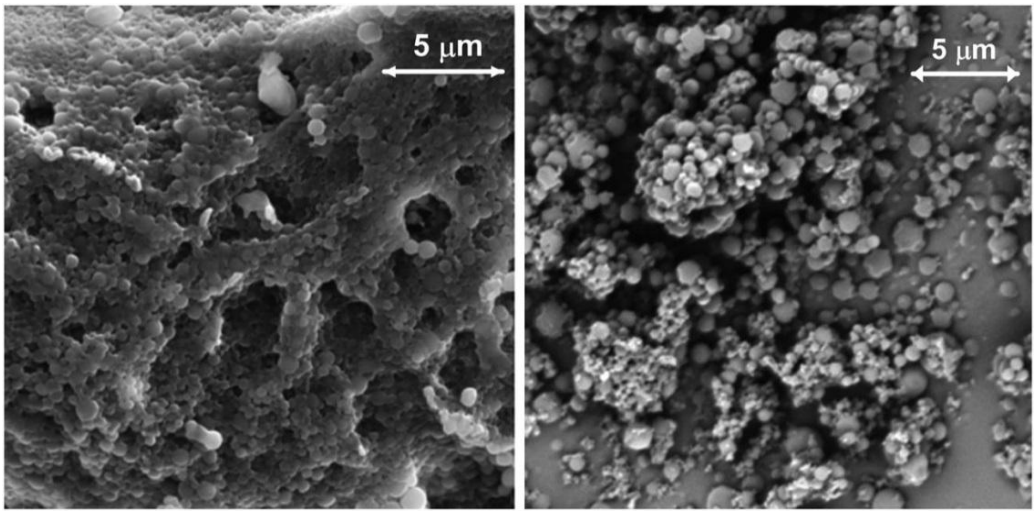

Figure 29 - Scanning electron microscopy images of poly(lactic acid)–poly(ethylene oxide) nanoparticles based on 3.2HMDI (left) and 3.2MDI (right) copolymer samples.

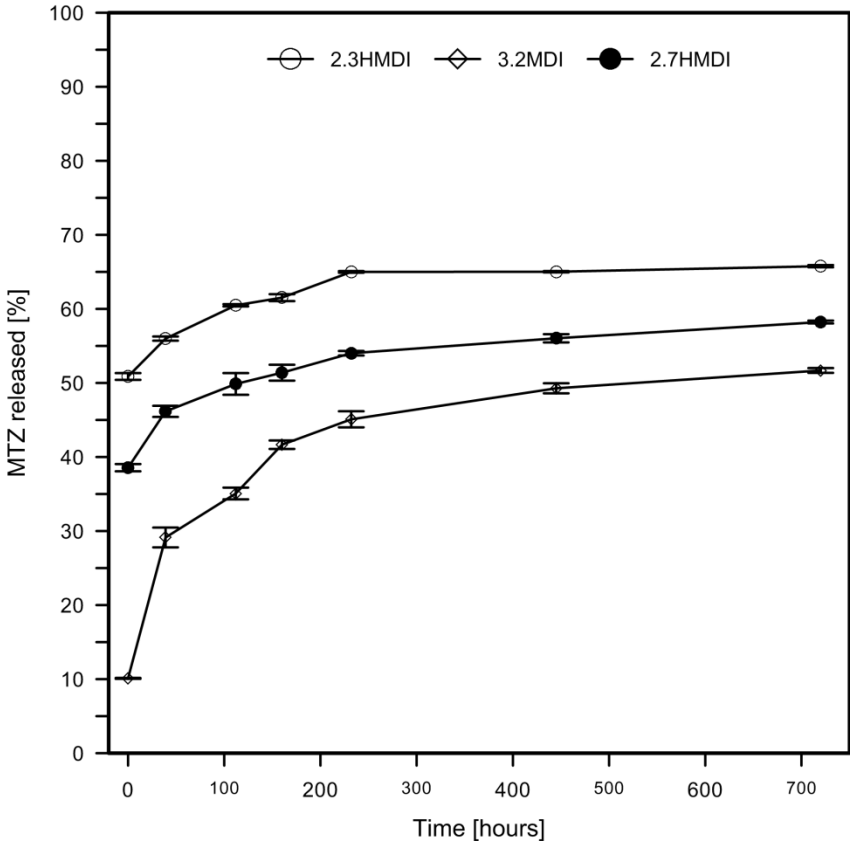

Figure 30 - Releasing profile of MTZ from selected PLA/PEG diizocyanate matrixes.

Mechanical testing

Mechanical properties of prepared samples are summarized in Table 9. It can be seen that tensile strength grow with increased NCO/OH ratio and the highest value of 21 MPa was reached with 3.2HMDI. In case of E-modulus, there was no clear trend observed with NCO/OH ratio and all values varied between 1000-1400 MPa. Strain at break was very poor for all samples (~1%) and only 3.2HMDI exhibited elongation beyond 3 %, which was comparable with commercially available PLA [205]. Increased tensile strength is connected with increased molecular weight, while the low tensile strain was probably attributed to amorphous nature of the products and rigid molecule of diisocyanate in polymer chain. It is also probable that chain branching or even partial crosslinking which might occurs in this type of materials and broad polydispersity contributed to poor elongability. The similar values of tensile properties were reported by Gu et al. [157], which used similar concept for chain extending but instead of PEG, butanediol was used in their case. It should be noted that their product exhibited more or less similar M_w and polydispersity.

On the other hand, in work of Kylma at al. [146] which used HMDI for the chain linking of PLA prepolymer nearly the two folds higher values of tensile strength and strain were reported. However, in this case it was probably caused

by different molecular structure like low polydispersity (<3) and presence of 1,4-butanediol in main chain. Also, the amount of rigid HMDI segments used was much lower and synthesis was based on solution polymerization in that case.

Table 9 - Mechanical properties of the chain linked products.

Sample	Tensile strength [MPa]	SD	Tensile modulus [MPa]	SD	Strain at break [%]	SD
2.3HMDI	5.7	1.1	1130	40	1.0	0.2
2.7HMDI	15.4	2.5	1350	140	2.0	0.2
3.2HMDI	21.0	1.1	1140	140	3.2	0.1

Hydrolytic degradation

The profile of molecular weight loss is depicted in Figures 31 and 32. As can be seen, at 55°C a sharp drop of M_n in all samples was observed, which was attributed to sensitivity of hydrolysis of ester bond to elevated temperature. Similarly, the significant decline of M_n was observed at 37°C, however the decrease was slower than in previous case and constant M_n was observed after 25 days. The decrease of M_n was accompanied by narrowing of ~~Đ~~, which was caused by random cleavage of long molecular chains to shorter segments. The similar hydrolysis experiment (°C and 55°C) was performed by Hiltunen and co-workers but noticeably longer time was necessary to obtain the same constant molecular weight. The reason for much slower hydrolysis of their material laid probably in its narrower polydispersity (~2) and higher T_g (~45°C) [16].

The process of weight loss in dependence on time, temperature and amount of HMDI was presented in Figures 33 and 34. The temperature effect is obvious at first sight. It can be seen that at 55°C the first weight loss occurred already after 7th day of experiment and further continued with time. The sample 2.3HMDI disintegrated after 14th day whereas the other two samples disintegrated after 24th days. The faster disintegration of 2.3HMDI sample was likely attributed to its low molecular weight. On the contrary, weight loss at 37°C was considerable slower than at 55°C. The first weight change occurred in 21st day (2.3HMDI, 2.7HMDI) and at 37th day (3.2HMDI) which reflected the molecular weight of the samples. At the end of the experiment (60days) all samples exhibited compact structure with weight loss between 65-72% and no disintegration was observed. This behaviour is typical for bulk hydrolysis mechanism during which samples can retain their compact structure till the last stage of hydrolysis [195]. Furthermore, no mass change immediately after beginning of the experiment show on fact that no unreacted PEG was presented in the samples since that would leached out in the very first stage.

Through the observing of M_n when the first weight loss took place the lowest M_n of fragments necessary for leaching out could be determined. It can be seen, that at both temperatures this value was about 3000 g.mol^{-1}. This was similar as observed in case of neat low M_w PLA where approximately 3000 g.mol^{-1} were necessary to achieve prior to diffusion can take place [206]. This similarity shows that although PLA/PEG chain linked polymer was more hydrophilic, the chemically more complicated fragments created during hydrolysis were not more soluble than simple PLA oligomers.

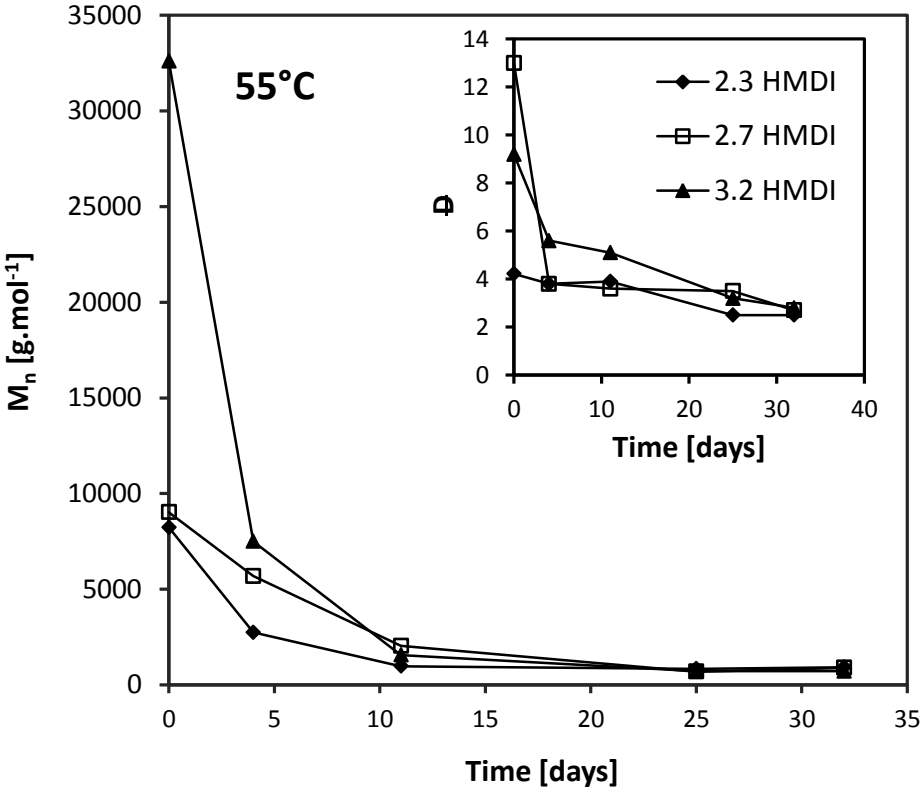

Figure 31 - Number average molecular weight loss of PLA-PEG copolymers with HMDI as a function of hydrolysis time at 55°C.

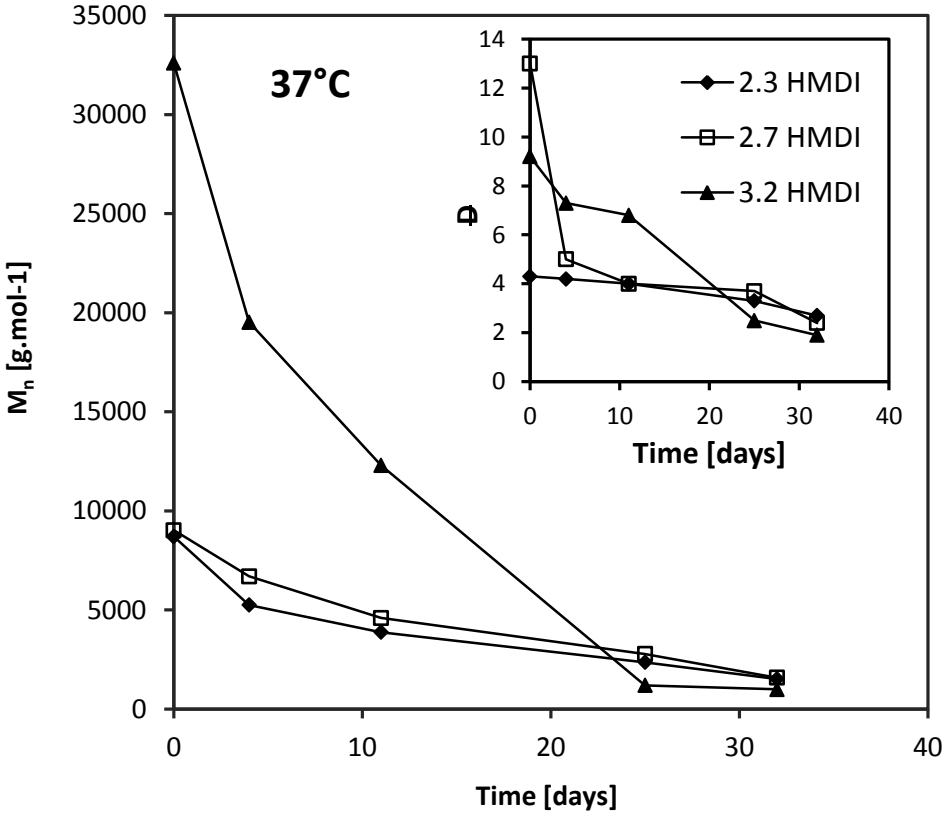

Figure 32 - Number average molecular weight loss of PLA-PEG copolymers with HMDI as a function of hydrolysis time at 37°C.

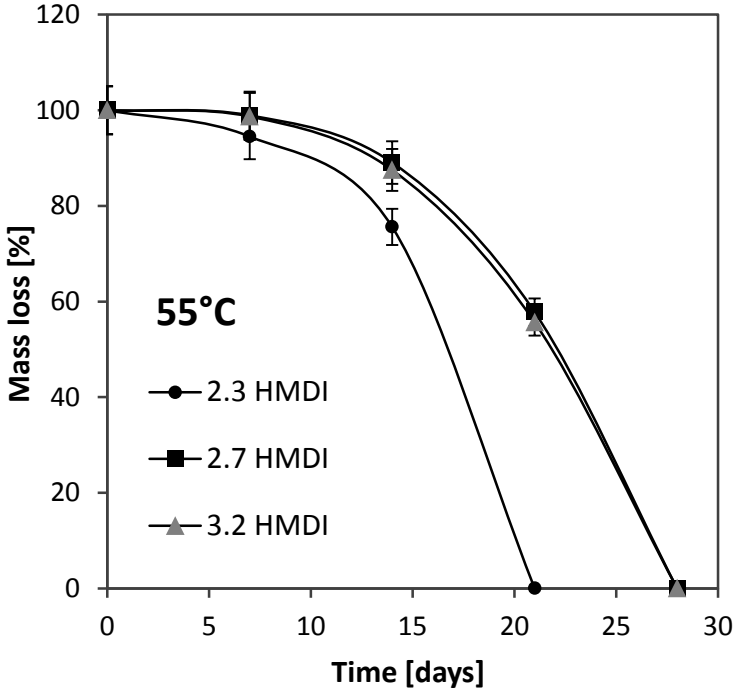

Figure 33 - Mass loss degradation profile of PLA-PEG copolymers with HMDI as a function of time at 55°C.

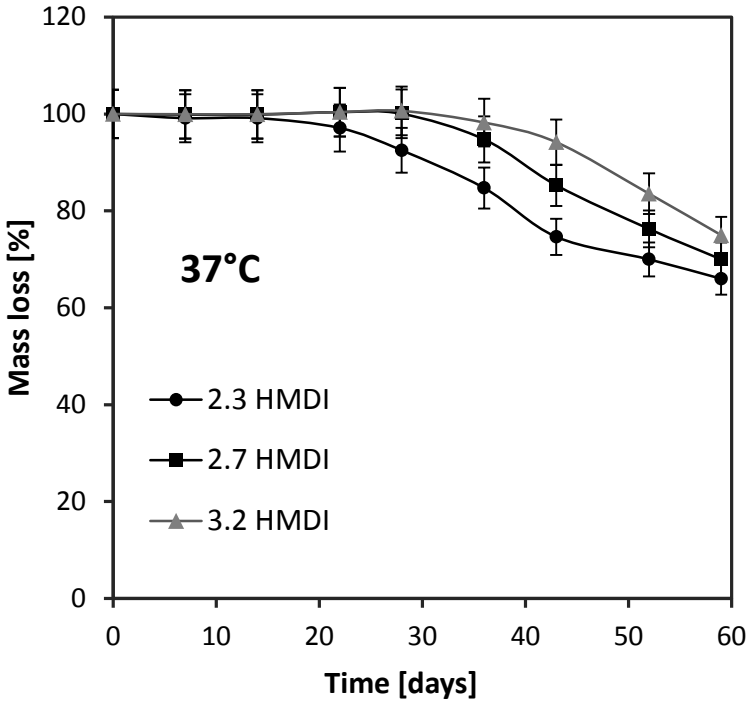

Figure 34 - Mass loss degradation profile of PLA-PEG copolymers with HMDI as a function of time at 37°C.

Electrospinning – preparation of nanofibres

The electrospun fibres obtained from MDF solution can be seen in Figures 35 - 37. Electrospinning process was performed on industrial scale equipment where large amount of solution (polymer) was needed and thus the work was focused on behaviour of this material during electrospinning process and did not deal with optimization of all processing parameters. In this work, only sample 3.2HMDI was used due to its high molecular weight character.

Firstly, the polymer was dissolved in DMF in concentration of 40% but the solution was rather inhomogeneous and exhibited gel-like structure. Therefore the solution was continuously diluted to 12% where homogenous solution was obtained. At this concentration the electrospinning was performed, however the process was not successful and only bead formations instead of fibres were formed and high volume of solution was "spluttered" from jet. This phenomenon occurs usually due to either low concentration of polymeric solution or due to its low conductivity [207, 208]. To overcome this complication it was decided to modify the conductivity.

The initial conductivity (G) of untreated 12% polymer solution was 9.8 $\mu S.cm^{-1}$ which was further increased with solution of citric acid and sodium tetraborate (3:1) to value 59.5 $\mu S.cm^{-1}$. Under these conditions the process was partially successful. It can be seen, that a combination of beads and fibres with broad diameter distribution and non-uniform density were created (Fig. 35).

On the basis of that, the conductivity was increased to 107.9 and 151 μS.cm^{-1}, respectively. It can be seen, that better dispersion of fibres was obtained, however there were still places with too dense and sparse layers. Finally, at G = 150.9 μS.cm^{-1} (Fig. 37) the coating was sufficiently homogenous and also the losing of polymer solution at the beginning of the electrospinning process was reduced, nevertheless not uniform diameters of fibres were obtained but combination of nano and semi-micro fibres was formed what can be clear also from histogram (Fig. 37). This was not observed in any work dealing with electrospinning of PLA or PLA/PEG copolymers [209 - 211] and the reason of this was not clearly explained. However, it might be speculated, that this was connected with broad molecular weight distribution of sample, because it is known, that higher molecular weight polymers tends to create fibres with higher diameters. Such structured material could be beneficial for preparation of highly porous scaffolds for tissue engineering.

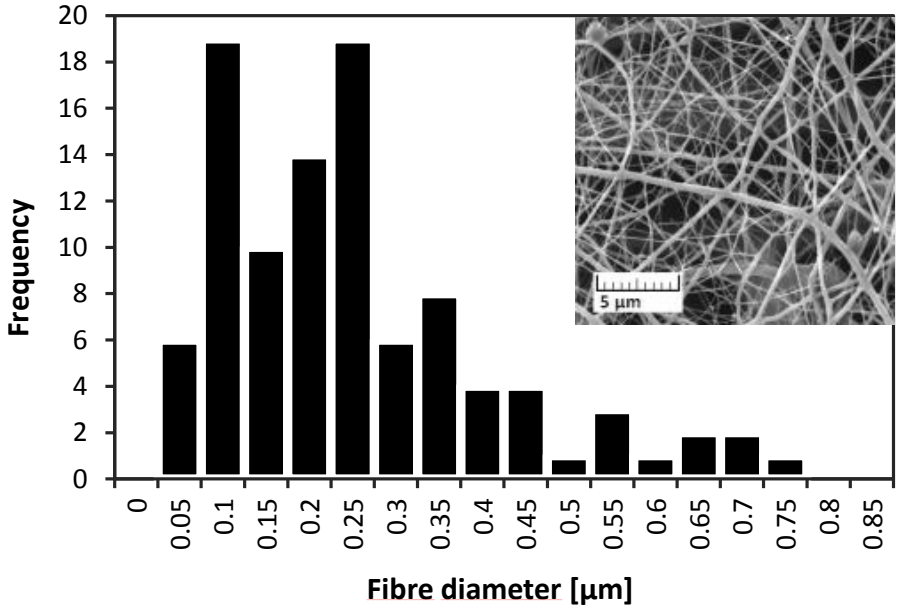

Figure 35 - Fibre diameters distribution of PLA-PEG copolymers with HMDI (59.5 μS.cm^{-1}) and their SEM micrographs.

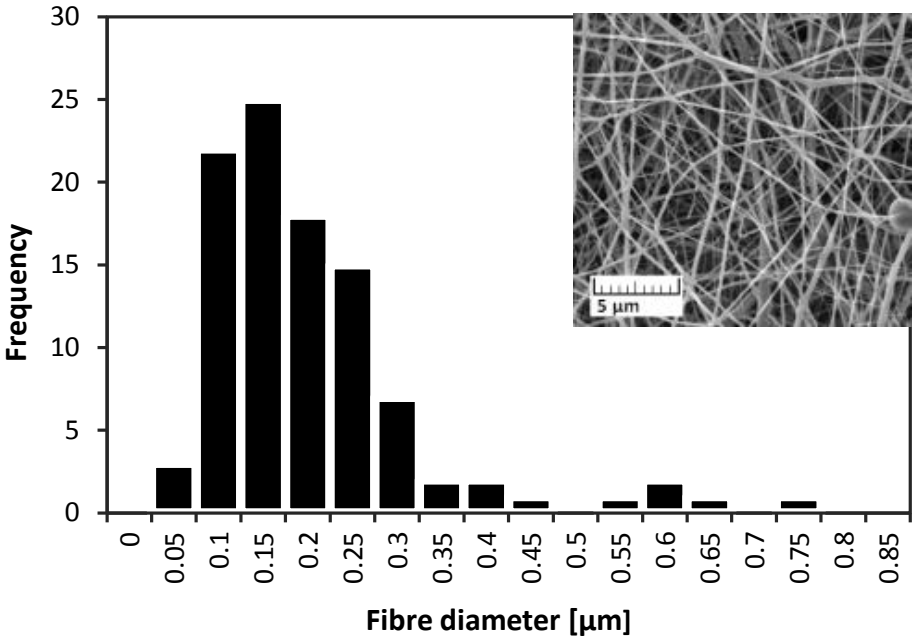

Figure 36 - Fibre diameters distribution of PLA-PEG copolymers with HMDI (107.9 µS.cm^{-1}) and their SEM micrographs.

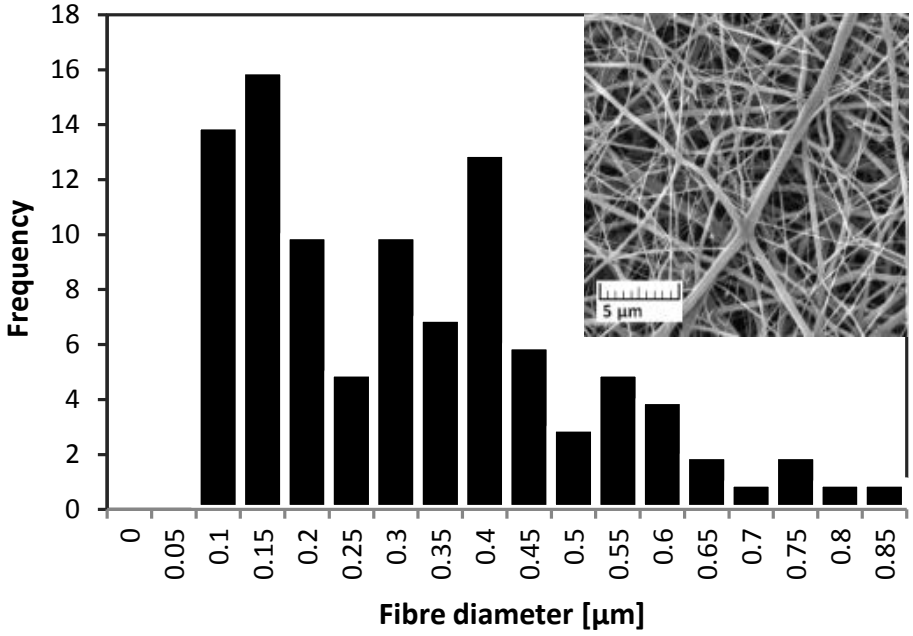

Figure 37 - Fibre diameters distribution of PLA-PEG copolymers with HMDI (150.9 µS.cm^{-1}) and their SEM micrographs.

Conclusions

First part of work was focused on preparing and characterizing biodegradable PLA-based copolymers for nano-applications, such as the controlled delivery of bioactive agents and fabrication of nanofibres. Consequently, introducing PEG segments into the polymer chains and their subsequent linking by using various diisocyanates was deemed a suitable method for relevant optimization of the physicochemical properties of the copolymers and enhancement of their chain length.

The obtained results indicate that molecular weight was successfully enhanced but considerably better results were achieved with HMDI (up to 300,000 $g.mol^{-1}$) rather than MDI. The products exhibited relatively high polydispersity, which can probably be attributed to the presence of chain-branching reactions typical for diisocyanates. The glass transition temperatures of the prepared materials varied between 30–44 °C, while higher values were exhibited by samples containing MDI. It was also found that MDI chain-linked samples possessed a highly amorphous quality, while HMDI ones tended to crystallize at least during the second heating scan.

The nanoparticles were successfully prepared via the solvent evaporation technique, with diameters of around 300 nm, exhibiting no strong reliance on the concentration of the chain-linking agent. The encapsulation efficiency of the model compound metazachlor was constant approximately 51% in the case of the HMDI samples - but varied between 46% and 65% for MDI chain-linked samples. The variance in EE was explained by noticeable differences in the hydrophilicity of both types of material. Following the release experiment, it was concluded that the most hydrophobic sample (3.2MDI) exhibited a minimal burst release effect with the slowest release of MTZ. All the investigated samples exhibited first order release kinetics.

The degradation and electro spinning of PLA/PEG diisocyanate chain linked copolymers has been investigated as well. It was found that newly prepared copolymers easily undergo hydrolytic degradation. Molecular weight decrease started immediately after exposure to degradation environment but weight loss occurred with time delay, therefore in was concluded, that random bulk hydrolysis mechanism took place. In electrospinning experiment the nanofibres were fabricated while optimizing conductivity of solution. Elevation of conductivity resulted into reduction of bead formations and unique combination of micro and nano-fibres was obtained.

2.2. Synthesis of poly(sebacic anhydride): effect of various catalysts.

Introduction

Polyanhydrides are a class of polymeric materials that possess reasonable biocompatibility, biodegradability and bioresorbability [45]. It is primarily their smooth degradation mechanism, in the form of hydrolytic surface erosion that makes them desirable, especially in drug delivery applications and other biomedical areas [168, 212]. Recently, series of advancements have led to dozens of forms of polyanhydrides and their copolymers with polyesters or polyimides are being prepared [45, 213, 214].

Polyanhydrides usually possess high crystallinity, their melting points ranging from 70°C- 300°C depending on the nature of the monomer(s) used [45]. The rate of hydrolysis could be extremely fast in contrast with, for instance, linear polyesters such as polylactide (PLA) or polycaprolactone (PLC), due to the presence of unstable anhydride bonds in the polymer main chain. Just as for all biodegradable polymers, their degradation speed depends especially on factors such as molecular weight, chemical composition, polymer crystallinity and the presence of catalyst residues or impurities [16, 215]. Optimizing all such aspects is very important for further application. In terms of medical applications, the material utilized must not cause an inflammatory reaction and should be easily eliminated from the organism. At present, the environmental compatibility of the resulting metabolites excreted from a living body is crucial to tailoring any such drug-delivery system [3].

Aliphatic polyanhydrides represent one of the best choices for biological and medical application and the suitable properties possess polyanhydrides of sebacic acid (PSAs) - naturally occurring dicarboxylic acid. It is taken from castor oil, which is extracted by pressing the Castor plant (*Ricinus Communis*) [216] and a great attention has recently been paid to synthesizing, applying and degrading this promising material. Polyanhydrides can be prepared by various techniques such as melt condensation, ring opening polymerization, interfacial condensation, dehydrochlorination, and by a dehydrative coupling agent [217, 218]. Of these, the most important appears to be melt polycondensation, which is studied extensively due to its simplicity, widely available and inexpensive monomers and its ability to create products of high molecular weight. However, its limitation lies in the fact that reaction is usually performed at high temperatures (>150 °C) which is close to the degradation temperature for the monomers used [176]. The second drawback worthy of mention is the lack of control over the reaction. The course of said reaction is not living; consequently, overall tailoring of the reaction's product proves quite difficult [219-223].

In this study the catalyst system choice as a key factor affecting the polyanhydride yields was investigated. In the literature a several research regarding the catalyst systems are reported. The most effective catalyst for

prepolymer polymerization was found to be cadmium acetate (CdAcet); in this case M_w = 240 000 g.mol^{-1} was reached after 31 minutes [47]. There are also other catalytic systems described in this pioneering paper, e.g. diethylzinc/water-$ZnCl_2/H_2O$ - 1:1, BaO, CaO and $CaCO_3$. A similar catalyst (CdAcet , BaO and $CaCO_3$) was also used in a paper by Hanes et al. This research highlighted the synthesis of poly[trimellitylimidoL-tyrosineco-sebacic acid-co-1,3 bis(carboxyphenoxy)propane] amorphous polyanhydride copolymer [224]. The M_w of the polycondensation product achieved was approximately 78 000 g.mol^{-1} after 30 minutes. Cadmium acetate was additionally used in the work by Domb, in which aromatic polyanhydride copolymers were synthesized, reaching a molecular weight of up to 35 000 g.mol^{-1} [225]. Zinc chloride as a catalyst was investigated by Yoda and Miyake as long ago as 1959. In this work, the monomers were not simple diacids but their chlorides and methyl esters. However, the molecular weights achieved were not stated [226].

Returning to the work presented here, the authors report on the synthesis of polysebacic anhydride (PSA) as one of the simplest forms of polyanhydride utilized in biomedical applications. The PSAs were prepared using a wide range of catalysts (22 types), the afore-mentioned compounds, in addition to previously unapplied—yet potentially promising— substances. To the best of the authors' knowledge, such an extensive study has never been published before. The reaction was performed as a two-step process. Low molecular weight prepolymer was prepared in the first stage of the procedure. The second stage was performed in the molten state. Herein, the influence of various types of catalyst, used in the second stage of reaction, on molecular weight and thermal properties was investigated. The resultant products were analysed via methods of gel permeation chromatography (GPC), differential scanning calorimetry (DSC), Infrared spectroscopy (FTIR-ATR), nuclear magnetic resonance spectroscopy (H-NMR, NMR), thermogravimetry (TGA) and pyrolysis gas chromatography coupled with mass spectroscopy (Py-GC-MS).

Materials

Acetic anhydride and chloroform, both in analytical grades, were purchased from PENTA Svec (Praha, Czech Republic); toluene, diethyl ether and petroleum ether were purchased from IPL Lukes, (Uhersky Brod, Czech Republic); sebacic acid was supplied from Sigma Aldrich, (Steinheim, Germany). Chloroform - HPLC grade - was provided by Chromservis (Czech Republic). The catalysts used, along with their physicochemical properties, are detailed in Table 1. All of the chemicals were used as received without further purification except the molecular sieves 5A (MS), which were activated before reaction at 120°C for 4 hours and cooled under vacuum conditions.

Polymer synthesis

The preparation of polyanhydride prepolymer was carried out in two stages. The first (synthesis of prepolymer) consisted of heating the sebacic acid monomer in excess acetic anhydride (1:10 (w/v)) at 140°C for 40 min under reflux and flow of inert gas (N_2) in an oil bath. Then the excess acetic anhydride was removed on a rotary vacuum evaporator (RVO) (Heidolph) at 60°C under reduced pressure (30 kPa). The residue was dissolved in toluene and stored at -20°C for 24 h. The resulting precipitate was filtered off and washed three times with a mixture of cold anhydrous diethyl ether and petroleum ether (1:1 (v/v)). The prepolymer was dried for 24 h (30 kPa) and stored at -20°C.

Polymers were synthetized by melt polycondensation of the prepolymers at 180°C (without solvent) under a vacuum of 1 kPa, in a method similar to that described by Domb and Langer [22]. The prepolymer, and relevant proportion of the catalyst (2% wt.), were added into a flask equipped with a Teflon stirrer. Polycondensation was left to occur for 90 min. Analytical pick-outs were performed during the course of the reaction at selected times (15, 30, 45, 60, 75 minutes). The resultant polymer gained from the main reaction sample was dissolved in chloroform and precipitated into cold anhydrous diethyl ether. The latter step was repeated three times to remove traces of unreacted monomer.

Table 10 - List of catalysts used in the study with their basic properties.

Type	Catalyst name	Label	Form / assay [%]	T_m [°C]	T_b [°C]	Average particle size [µm] / form	Supplier
Oxides	Tin oxide	SnO_2	Solid / 99	1127	1800	0.28 / spheres	d
	Lithium oxide	Li_2O	Solid / ≥ 97.0	1438	n.k.	5x4 / irregular	e
	Zinc oxide	ZnO	Solid / 97	≥ 390	1975	<50nm[a]	e
	Titanium oxide	TiO2	Solid / ≥ 99	1843	2972	0.25 / spheres	d
	Antimony trioxide	Sb_2O_3	Solid / 99.0	656	1425	3.4 / rods	d
	Copper (II) oxide	CuO	Solid / 97%	1326	2000	<50nm[a]	e
	Calcium oxide	CaO	Solid / 99.99	2580	2850	12x11 / cubic	e
	Phosphorus pentoxide	P_2O_5	Solid / ≥ 99	340	360	n.m	d
Carbonates	Lithium carbonate	Li_2CO_3	Solid / ≥ 98	723	1310	19.5 / aggregates	e
	Sodium bicarbonate	$NaHCO_3$	Solid / ≥ 99.5	300	851	30x100 / aggregates[b]	e
	Calcium carbonate	$CaCO_3$	Solid / ≥ 99.0	1300	n.k.	8 / cubes	e
Chlorides	Tin(II) chloride dihydrate	$SnCl_2$	Solid / 98	38	652	12x3 / rods	d
	Zinc(II) chloride, anhydrous	$ZnCl_2$	Solid / ≥99.995	293	732	n.m.	e
	Aluminium chloride	$AlCl_3$	Solid / 99.99	192	n.k.	60 / porous spheres	e
Organometals	Diethylzinc	DEZ	liquid / 1M in hexanes	-28[f]	117[f]	0.8 / spheres[g]	e
	Tin(II) 2-ethylhexanoate	SnOct	Liquid / 95	-20	228	-	e
	Sodium ethoxide	C_2H_5ONa	Solid / 95	260.0	91.0	1.5 / aggregates	e
	Titanium(IV) butoxide	TBO	Liquid / 97	-55	310	-	e
NHC compounds	1,3-Bis(2,4,6-trimethylphenyl)-1,3-dihydro-2H-imidazol-2-ylidene	TDI	Solid / 97	140	n.k.	-[c]	e
	1,3-Bis(2,6-di-i-propylphenyl)imidazolidin-2-ylidene	DII	Solid / n.k.	170	n.k.	-[c]	e
Acetate	Cadmium acetate dihydrate	CdAcet	solid / 98	254	n.k.	60 / cubes	e
Minerals	Molecular sieves 5A	MS	Solid / n.k	2572	n.k.	3 / cubes	e

n.k. not known, n.m. not measurable; **a** Information from producer; **b** Containing wires with dimension about 10×2 µm; **c** Not measured due to low melting point; **d** IPL Petr Lukes, Uhersky Brod, Czech republic; **e** Sigma Aldrich, Steinheim, Germany **f** Data for solid DEZ; **g** After evaporation of hexanes; Average particle sizes/forms were received by electron microscopy

Analytical methods

GPC measurements

Evaluating of the weight and number-average molecular weight and distribution was carried out under the same conditions as reported in chapter 2.1.

FTIR-ATR spectroscopy

FTIR spectral transmission was measured on a Nicolet iS5, using the infrared spectroscopy mode ATR, range measurement was 530-4000 cm^{-1}, the measuring crystal was Ge, 64 scans and the resolution of 4 cm^{-1} were maintained in all cases.

NMR spectroscopy

^{1}H NMR and ^{13}C NMR measurements were performed using a Varian Unity Inova 400 spectrometer. Chemical shifts of signals in spectra were referenced to the solvent peaks ($CDCl_3$ – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 ppm; ^{13}C NMR (100 MHz, $CDCl_3$: 77.23 ppm). First order analysis was used to evaluate all the NMR spectra received.

SEM analysis of catalyst structure

The structures of catalysts were assessed on a TESCAN VEGA/LMU scanning electron microscope (Czech Republic). The microscope was operated in high vacuum mode at an acceleration voltage of 5 kV and all samples were coated with an Au/Pt layer. The results obtained for all catalysts (the size and shape of catalyst particles) are presented in Table 1.

Py-GC-MS measurements

GC/MS was carried out on a GCMS-QP 2010 MS (Shimadzu) gas chromatograph coupled with a pyrolysis system. In order to delineate pyrolysis components a capillary DB-5 column was used (30 m x 0.25 mm i.d. x 0.25 μm film thickness); the flow rate of inert gas (He) was set up at 1 mL/min and the split ratio 1/250. Samples of approximately 1 mg were pyrolysed using the double-shot method at the temperatures 300°C for 2 min and 460°C. GC analysis occurred under the following conditions: $T_{initial}$ = 50°C and holding temperature was T_{final} = 370°C for 30 min with ramp rate 10°C/min. The mass spectrometer setting was 250°C for an ion source, with the resolution from 45 to 800 and at scan speed 10 000.

TGA analysis

The thermal stability of PSA samples was investigated using a thermogravimeter (SETARAM TG-GA 12) under dynamic conditions at the heating rate 10°C.min^{-1}, with temperature ramped from 25°C to 500°C in a helium atmosphere at the constant flow rate of 100 mL.min^{-1}. The amount of sample was between 10-15 mg in all cases. The decomposition temperature (T_{dec}) was taken as a temperature corresponding to 5% weight loss and each degradation step (T_p) was described on the basis of the peak position from TGA derivative curve (DTG).

Results and discussion

NMR analysis

Typical ^{1}H NMR spectra of the selected polymer (catalysed by TiO_2) and its assignments is depicted in Figure 38. The spectra show a characteristic pattern related to PSA [227]. As can be seen, there was no peak observed at 21.8 ppm in the ^{13}C NMR spectrum (Fig. 39) related to the terminal CH_3 group. This reveals that the polymer reaction was successful [228]. In ^{1}H NMR spectra (Fig. 38) there is an observable triplet signal (a') at 2.33 belonging to the CH_2 groups (a) at chain ends, in the example picture.

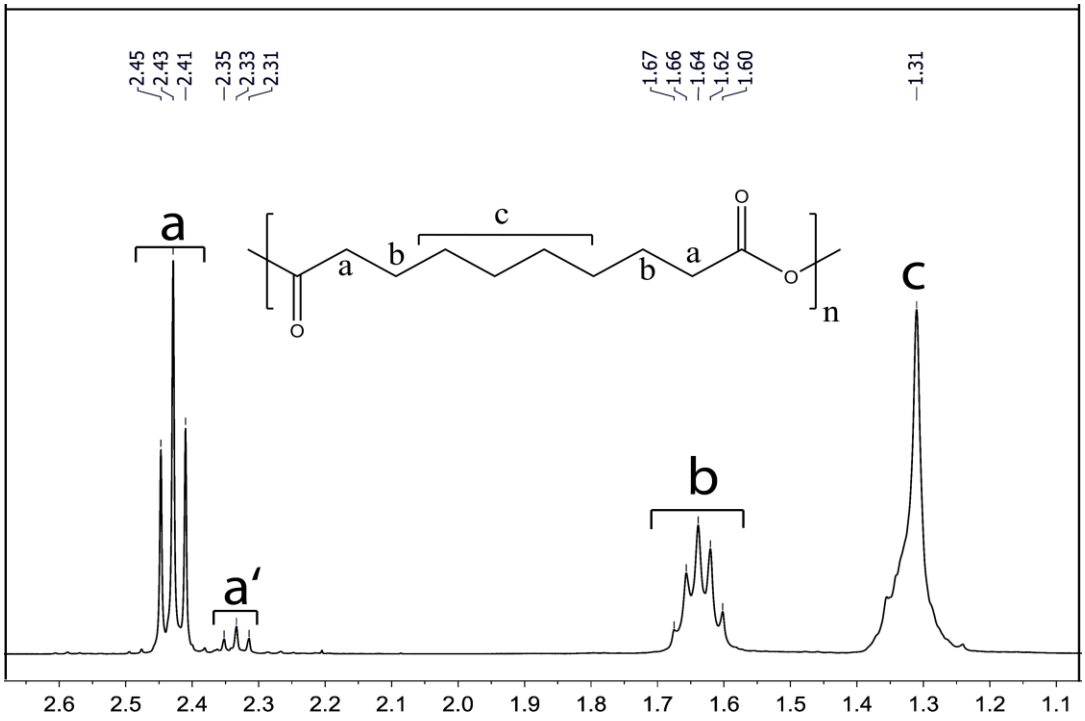

Figure 38 - ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of PSA prepared using TiO_2 catalyst.

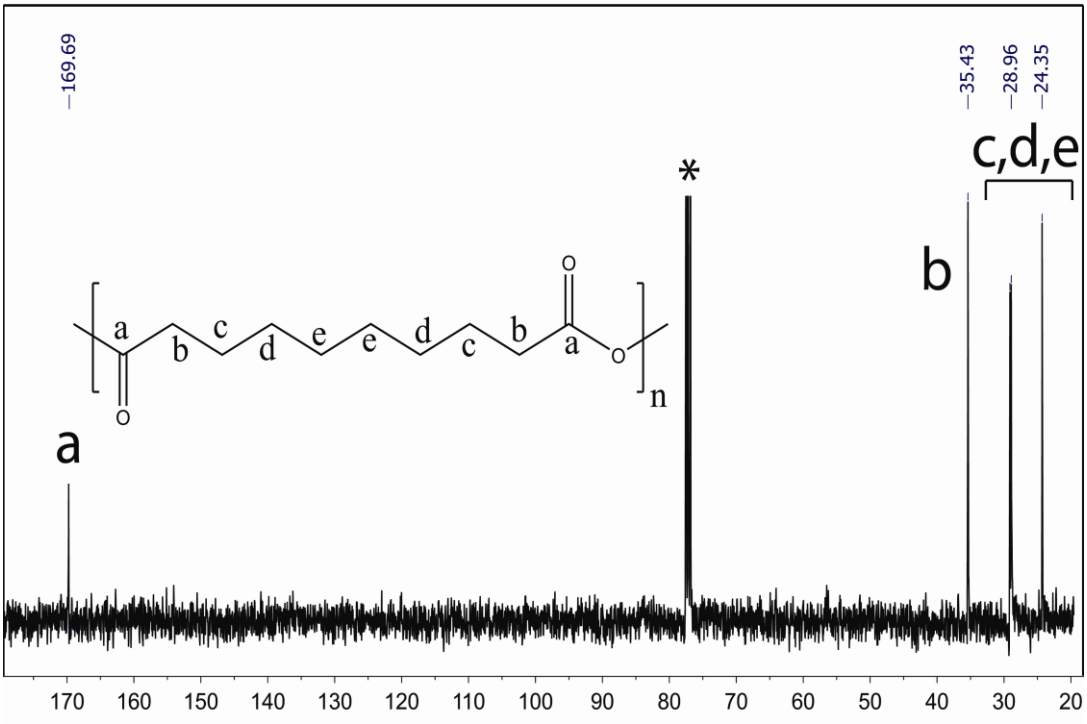

Figure 39 - ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of PSA prepared using TiO_2 catalyst.

FTIR structural analysis

FTIR spectroscopy analysis of PSA prepolymer and prepared polymers is presented in Figure 40. Absorptions at 2914 and 2851cm^{-1} correspond to methyl groups (-CH_2-, -CH_3). A typical double anhydride carbonyl (C=O) peaks at 1800 and 1740 cm^{-1}, which confirms the presence of anhydride bonds [170]. Other typical peaks for symmetrical stretching vibrations of anhydride segments lay at 1065 and 1041 cm^{-1} (C-O-C stretching). The signal at 1705 cm^{-1}, which was visible in samples after polymerization, was assigned to carboxylic acid groups formed due to thermal degradation of the samples during reaction. The peak located at 3400 cm^{-1}, when using a C_2H_5ONa prepared sample, is typical for hydroxyl group stretching (-OH), and it probably occurs due to absorption of water by the hygroscopic catalyst. In comparison with polymer prepared only thermally, without the presence of a catalyst, there is another peak in the 1550 - 1610 cm^{-1} wavenumber region. This could be attributed to the presence of corresponding carboxylic acid salts. That acid salts were detected only in products catalysed by C_2H_5ONa, and CdAcet FTIR spectroscopy proved that the functionality of PSA can significantly be affected by the catalyst used. It is obvious that the presence of functional groups affects the final properties of the product (especially degradation behaviour).

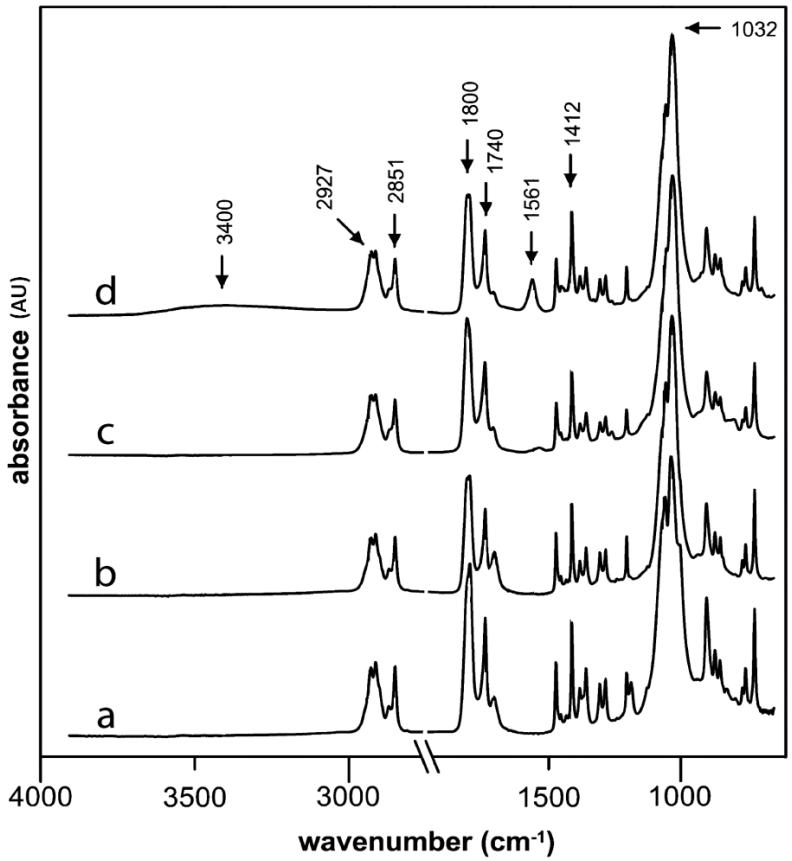

Figure 40 - FTIR-ATR spectra of (a) prepolymer and sebacic acid polyahydride: (b) sample prepared without catalyst and samples prepared using (c) CdAcet and (d) C_2H_5ONa.

Synthesis and molecular weight characterization by GPC

Detailed study was made on how the molecular weight evolved of the polymer formed during the reaction, using the GPC technique. The values of weight averages (M_W) were measured at selected time reaction intervals (15, 30, 45, 60, 75, 90 min); the effect of catalysts on apparent Mw development during the polymerization process is shown in Table 11. It can be seen that the prepared prepolymer was of low M_W (3 kg.mol^{-1}), with the relatively narrow distribution of 2.2. This is in general accord with results presented elsewhere [224, 227]. The catalysts used in this work are typically used for transesterification, ring-opening polymerization and related polymerization reactions, and they are known to exhibit good effectiveness at polymerizing the monomers presented. It is a recognized fact that earth metal oxides, carbonates and coordination catalysts enhance the nucleophilicity of carbonyl carbon [229, 230]. Moreover, $ZnCl_2$, CaO and $CaCO_3$ were used for comparison with previously published results [47]. In this study a reference experiment was performed without the presence of a catalyst. In this instance there was strong increase (from 30 to 49 kg.mol^{-1}) in M_W observed for the sample prepared without the presence of catalyst in the first 15 min of reaction (Tab. 11). An initially rapid Mw increase was followed by an

M_W reduction of up to 60 min, when Mw= 26 kg.mol^{-1} was observed. Analyses of the samples taken from further reaction stages (75 and 90 min) showed increased M_W values (52 kg.mol^{-1} after 90 min) This rise in M_W is connected with the increase in the dispersity index that signifies the consequence of random chain scissoring and an exchange reaction. Generally, the catalysts studied in this paper can be divided into three groups according to their efficiency:

i) the compounds Li_2O, CuO, P_2O_5, $ZnCl_2$, 1,3-Bis(2,4,6- trimethylphenyl)-1,3-dihydro-2H-imidazol-2-ylidene (TII) and diethyl zinc (DEZ) did not result in high M_W PSA (M_W <13 kg.mol^{-1}). This is caused by predominant degradation reaction during the synthesis.
ii) TiO_2, $NaHCO_3$, C_2H_5ONa, CdAcet and MS resulted in PSA showing considerably higher M_W than a sample prepared without the presence of a catalyst. The best result was achieved with CaO (M_W = 270 kg mol^{-1}).
iii) SnO_2, Li_2CO_3, $CaCO_3$ and $AlCl_3$ could potentially be more effective concerning molecular weight quantity, after optimizing some of the reaction conditions, such as catalyst content, temperature and the time of the reaction.

As regards reaction time, it is obvious that M_W increased during the first 30 min in most cases (Tab. 11). In conjunction with a rise in reaction time, M_W values either increased (CaO, MS) or fluctuated around the M_W detected after 30 min, the latter trend probably being caused by redistribution of chain length, which is connected with varied competitive degradation, and/or cyclization, and/or chain transfer reactions that can occur under high temperatures [230]. This assumption was supported by changes in the dispersity index (Đ).
Typical examples of M_W profiles determined at various concentration times are presented in Figure 41. While the sample prepared without a catalyst shows unimodal M_W distribution (Fig. 41 - a), the reaction products of catalysed reactions are characterized by bimodal M_W distributions (Fig. 41 - b), which can be observed in the case of specific step-growth polymerizations. It is also noticeable that any increase in Mw value is connected with the occurrence of a second peak relating to long polymer chains. Similar results have been reported by Domb et al. [47]. The catalyst used can be formally categorized under the groups:

i) dehydrating agents: CaO, MS, P_2O5, TBO
ii) compounds able to donate/accept a proton (i.e. Broesnted acids and bases): CaO, P_2O_5, Li_2CO_3, $NaHCO_3$, $CaCO_3$, EtONa, ZnO (amphoteric), SnO2 (amphoteric). LiO_2 (amphoteric), Sb_2O_3, CuO (amphoteric)
iii) compounds able to accept a free electron pair (i.e. Lewis acids): $ZnCl_2$, $AlCl_3$, $SnCl_2$
iv) Lewis bases—carbon-donating compounds of carbene characterizations TII, DII

v) Transeterification of catalysts, or those catalysts used for polyester preparation: DEZ, SnOct, $SnCl_2$, CdAcet

Given that this categorization of the catalysts is purely for the purpose of orientation, such catalyst classification does cause overlap, mainly for entries i) and ii). To the best of the authors' knowledge a direct mechanism of an anhydride exchange reaction is not known from the literature. However, the exchange process typically takes the form of a competitive reaction, and there is equilibrium between polymer chain growth and its degradation under the given reaction conditions (temperature, time and composition of the reaction mixture).

Most of the catalysts from groups (i) and ii) used in the present work have a positive influence on Mw characteristics after 15 min of reaction. Interesting results were obtained in the case of CaO, $NaHCO_3$ and MS. These catalysts display a moderate proton donating/accepting ability, and thus the highest value of M_W was achieved by CaO after 60 min of reaction time (280 $kg.mol^{-1}$). Further course of reaction leads to a decrease in M_W. This behaviour is typical for all the catalysts utilized in the given group. The only exception is NaHCO3, when a strong Mw increase is observed at 90 min of reaction. However, this rise is accompanied by an increase in dispersity (Tab. 11). ZnO, SnO_2, LiO_2 Sb_2O_3 TiO_2 and CuO represent compounds of amphoteric character capable of accepting and donating a proton. The influence of these catalysts is prevailingly negative or indifferent. ZnO, Li_2O, Sb_2O_3 and CuO compounds might serve as bases. The M_W values reached are significantly lower compared to the system without a catalyst. SnO_2 is almost indifferent in comparison with the system lacking the catalyst. An exception in this group is TiO_2, where the M_W values recorded were slightly improved compared to the system without a catalyst. There is also a slight broadening of the molecular weight distribution represented by higher Đ values. Titanium butoxide reacts with carboxylic acids and serves as a dehydration agent [231]. The catalysts utilized from group (iii) (Lewis acids) do not provide reaction products with reasonable values of Mw. For example, $ZnCl_2$ caused strong polymer degradation in comparison with $AlCl_3$, which has no influence on the Mw values reached even after 60 min of reaction time (Table 11). Only slight changes in the dispersity index are observable in the case of $AlCl_3$. This observation might correspond to the afore-mentioned thermal $AlCl_3$ stability. Tin chloride is known to form complexes and catalyses a transesterification reaction [232, 233]. In the first reaction stage, M_W reached a relatively high value (see Table 11). The M_W remained unchanged over greater reaction times. It is likely that the catalyst decomposed due to the high reaction temperature. An interesting group of catalysts are the carbon-donating ligands TII and DII, which are of carbene characterization. They formally belong to the group iv) catalysts with the ability of donate an electron pair. Their influence on the course of the reaction is similar in both cases. After the initial rise in M_W (after 15 min) all molecular weight characteristics

remained unchanged. Therefore, it may be concluded that the presence of the TDI and DII compounds positively influences the initial stages of the reaction. The carbene probably decomposes at a high temperature and no catalytic activity is observed in later reaction stages. The last group of catalysts is also represented by $Sn(Oct)_2$, which is a typical catalyst utilized in ring-opening polymerization of D,L-lactide or ε-caprolactone. Diethyl zinc is a strongly pyrophoric compound that reacts vigorously with water. The catalysis of 12-hydroxystearic acid (derived from castrol oil) has been reported. It is known to catalyse ring polymerization of 12-hydroxystearic acid, which is also created from castor oil [234]. In this study, a significant increase in M_W was observed after 15 min of reaction, followed by degradation of the polymer chains formed during further course of the reaction.

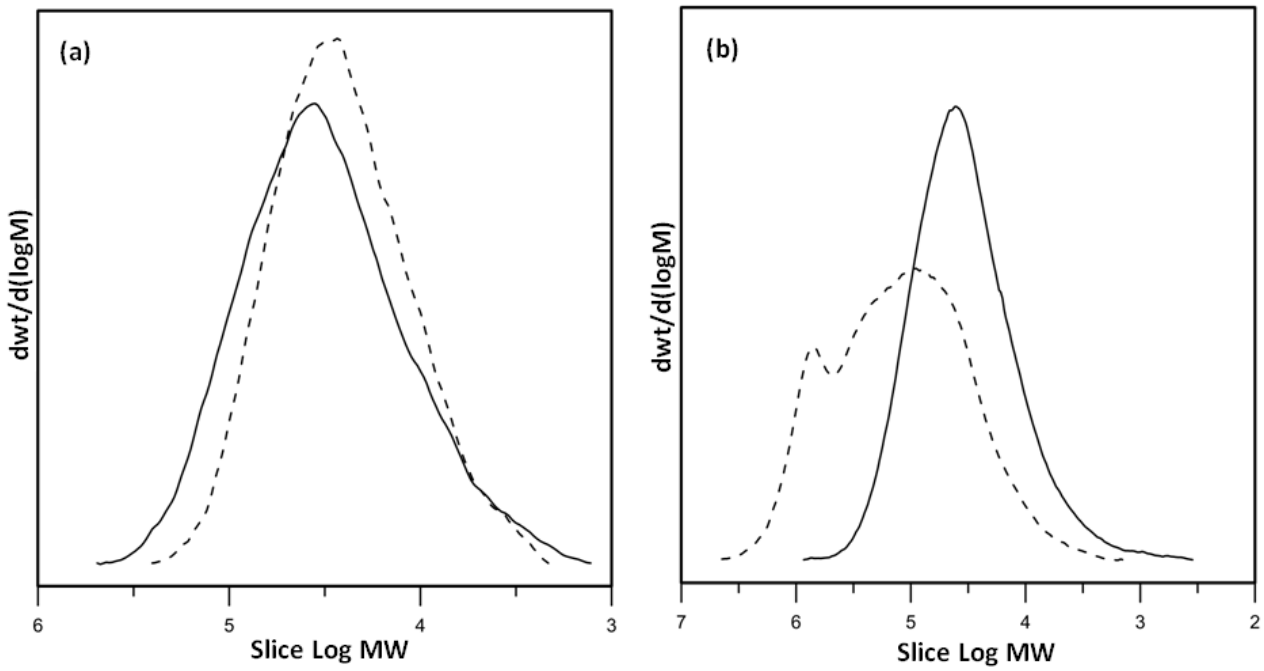

Figure 41 - Changes in molecular weight profile during polycondensation reaction: (a) sample without catalyst and (b) sample with catalyst CaO after 15 min (solid lines) and 60 min (dashed lines) of the reaction.

Table 11 - The effect of catalysts on M_w development during synthesis.

	Catalyst	Time (15 min)		Time (30 min)		Time (45 min)		Time (60 min)		Time (75 min)		Time (90 min)	
		$M_w/10^2$ [g.mol^{-1}]	~~Đ~~	$M_w/10^2$ [g.mol^{-1}]	~~Đ~~	$M_w/10^2$ [g.mol^{-1}]	~~Đ~~	$M_w/10^2$ [g.mol^{-1}]	~~Đ~~	$M_w/10^2$ [g.mol^{-1}]	~~Đ~~	$M_w/10^2$ [g.mol^{-1}]	~~Đ~~
	Prepolymer	300	2.2	-	-	-	-	-	-	-	-	-	-
	No catalyst	490	2.6	370	2.7	330	2.4	260	2.2	440	2.4	520	3.2
Oxides	**SnO_2**	290	2.5	370	2.5	390	2.4	230	3.0	380	2.6	410	2.6
	Li_2O	83	2.5	92	2.5	100	3.2	79	3.6	80	3.9	49.0	3.2
	ZnO	290	1.8	92	9.7	130	9.5	640	6.5	80	7.6	92	8.5
	TiO_2	640	6.2	720	3.2	620	3.0	810	3.6	840	3.2	570	5.2
	Sb_2O_3	170	4.0	170	4.8	140	4.6	130	4.9	110	4.3	80	6.4
	CuO	8.0	2.7	19	4.3	13	3.4	27	5.5	15	3.5	20	3.8
	CaO	550	2.5	640	2.8	1700	3.6	2800	4.9	1600	4.2	1600	4.4
	P_2O_5	88	5.2	73	4.9	13	2.3	-	-	-	-	-	-
Carbonates	**Li_2CO_3**	510	2.3	-	-	570	2.6	-	-	320	2.2	200	2.5
	$NaHCO_3$	-	-	820	2.9	600	2.6	830	3.1	580	2.6	1200	4.3
	$CaCO_3$	320	2.4	540	2.7	590	2.3	560	2.6	390	2.4	460	2.5
Chlorides	**$SnCl_2$**	200	3.2	250	3.5	230	3.0	-	-	-	-	-	-
	$ZnCl_2$	-	-	47	5.4	44	4.1	-	-	78	5.7	98	5.2
	$AlCl_3$	590	3.3	590	3.3	580	3.7	590	3.7	-	-	-	-
Organometallic compounds	**DEZ**	200	3.8	150	3.9	110	3.6	100	3.6	170	3.9	130	4.0
	SnOct	380	2.9	460	3.5	380	3.5	350	3.1	330	4.3	290	5.4
	C_2H_5ONa	800	3.1	1100	3.4	1200	3.3	1000	3.0	810	8.6	1100	3.8
	TBO	190	2.4	180	3.2	180	2.6	200	3.0	170	3.2	160	2.9
NHC	**TII**	1300	5.6	140	5.8	140	6.2	130	7.3	110	4.3	48	5.9
	DII	380	2.7	340	3.0	300	3.1	300	3.1	250	3.3	230	3.6
Acetate	**CdAcet**	-	-	1900	4.9	1500	5.4	1500	5.8	1700	6.3	1900	7.1
Minerals	**MS**	480	2.1	760	2.6	1000	3.4	1100	4.3	790	3.5	950	4.4

Thermal properties and stability

Investigating the thermal stability of the selected PSA samples was conducted by TGA. TGA curves and their derivatives (DTG) are shown in Figure 42. The DTG curves were graphically shifted along the Y axis to make them more readable. The thermal degradation of PSA is connected with decarboxylation and decarbonylation of the polymer backbone, becoming significant at temperatures above 130 °C [218]. It was also discerned that a twostep degradation mechanism occurred under all observations. The first DTG peak was situated between 315 and 336 °C, while the second was stable for all samples around 461 °C. The low thermal stability of the sample prepared without a catalyst can be attributed to its low molecular weight. Whereas in the case of the sample prepared using C_2H_5ONa, this was probably connected with the accelerated decomposition caused by this type of catalyst. The other samples did not show any significant differences, and their levels of thermal stability might be considered as more or less similar. A small degradation peak was detected at around 100 °C for solely the MS catalysed sample, which could be attributed to the presence of water entrapped by molecular sieves. Two-step degradation is typical when two components are present in the sample (e.g. copolymers or blends). However, in this case it is believed that the second degradation peak was connected with the final decomposition of the primary degraded products. Qualitative analysis of the gaseous degradation products evolved was performed by a pyrolysis experiment, coupled with mass spectroscopy.

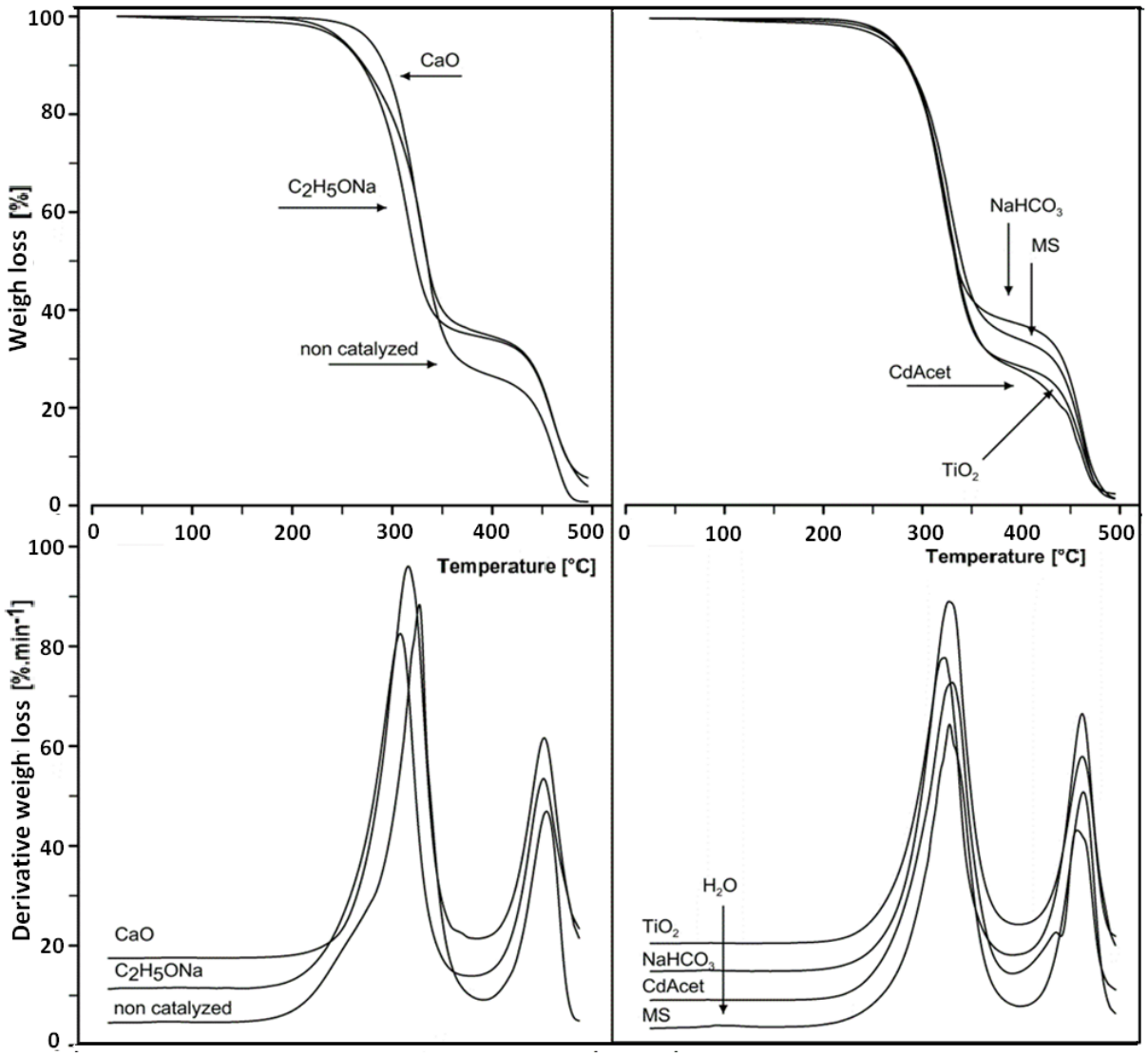

Figure 42 - Weight loss curves (left) and derivative weight loss curve (right) measured by TGA.

Pyrograms of the PSA sample prepared without a catalyst are depicted in Figure 43. The pyrolysis temperatures of 300 °C and 460 °C were selected on the basis of TGA measurements, where these temperatures corresponded to values of the first and second DTG peaks, respectively. Various degradation products could be observed in the course of the pyrolysis experiment (at around 300ºC). The most dominant of these is sebacic acid. Further chemical moiety observed concerned traces of acetic acid originating from acetylation of the monomer. At 460 °C, when the second degradation step was taking place, various degradation products were detected (some of them are labelled in Figure 43). In the second thermal degradation step, no primary components (sebacic acid) were detected, and the presence of linear and cyclic carbon-hydrogen compounds was proven. This observation supports the assumption that in the

second thermal degradation step, the products of primary decomposition are degraded even further.

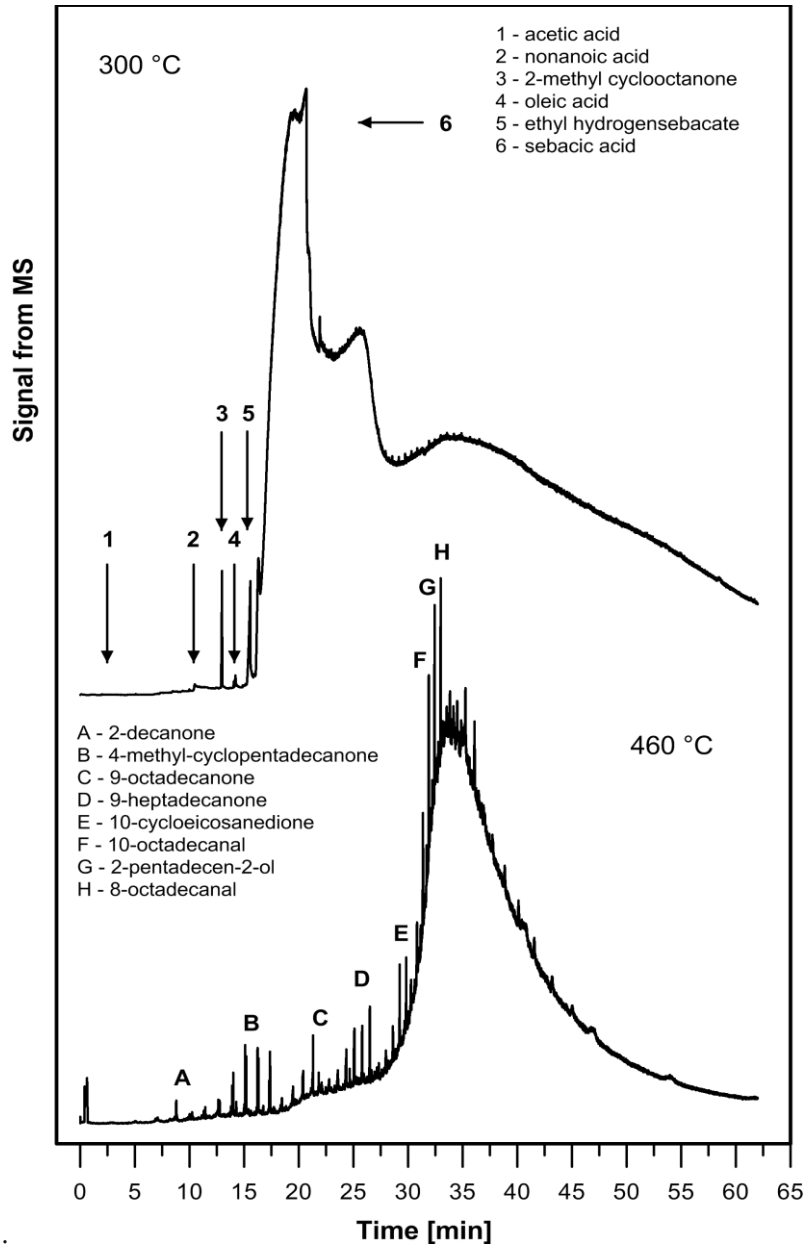

Figure 43 - Pyrograms of PSA prepared without catalyst (after 15 min of reaction time) at 300 °C (upper curve) and 460 °C (lower curve).

Conclusions

This paper focuses on the synthesis of polysebacic anhydride (PSA) via melt polycondensation of low molecular weight acetylated sebacic anhydride prepolymer, in the presence of various catalyst systems under various reaction temperatures and times.

The results show that the catalyst selection deeply influences the molecular weight characteristics of the resultant polymer. In summary, CaO was found to be the most effective catalyst for PSA synthesis, providing a high molecular weight product (M_w~275 000 g.mol^{-1} after 60 minutes of reaction time). Additionally, TiO_2, $NaHCO_3$, C_2H_5ONa, CdAcet and MS based catalysts were found to be suitable for this type of polymerization. The values of the M_w reached for the resulting PSAs ranged from 60 000 to 150 000 g.mol^{-1}. Thermal degradation of PSA proceeded in a two-step process. It was found that the thermal stability of PSA polycondensates reduces in conjunction with decreased length in the macromolecular chain.

2.3. Characterization of biocompatible non-toxic polyester urethanes based on poly(lactic acid)-poly(ethylene glycol) and L-Lysine diisocyanate

Aliphatic polyester based polymers are promising materials for high valuable applications in medical sector. They dispose properties as biocompatibility and biodegradability which represents their main assets [235]. Poly(lactic acid) and PLA based polymers are already frequent in medical applications as transport devices for drug delivery (nanoparticles, nanocapsules) or scaffolds and stents [236-239]. Qualities of PLA based polymers have been already shown; nevertheless, some limitations in mechanical properties - lack of flexibility, may still be an obstacle for certain applications [240]. To overcome this shortcoming the copolymerization is one of the effective compromises.

Copolymerization of PLA with poly(ethylene glycol) (PEG) can bring benefits as flexibility and also hydrophilicity, which may affect the degradation characteristics. Moreover, due to reaction with PEG, the hydroxyl terminated product enables further modifications. In this particular way the chain linking reactions are widely employed to create high molecular weight products [138, 164, 189]. To the hydroxyl-terminated prepolymers, the diisocyanates represent the most effective chain extenders. Their overview is summarized in the chapter 2.1. Broadly used diisocyanates are for example HMDI (hexamethylene diisocyanate), MDI 4,4-methylenebis(phenyl isocyanate) and TDI (toluene diisocyanate); however, the toxicity of these components can be objected in connection with the biomedical applications [241]. Diisocyanates reacting with hydroxyl-terminated polyesters form polyester urethanes, which undergo hydrolytic degradation more easily. Products of degradation of these polymers incorporating aromatic diisocyanates can be toxic and carcinogenic compounds such as aromatic diamines. An example is MDI, which hydrolyses into 4,4-methylenedianiline causing hepatitis [242]. Therefore the aliphatic diisocyanates which mitigate the toxic risk are preferable [243].

As alternatives to biomedical devices, the use of HMDI, lysine methyl ester diisocyanate (LDI) or 1,4-diisocyanatobutane (BDI) in synthesis of polyester urethanes have been reported [155, 244]. However, the main focus is on LDI. Its degradation product is lysine, endogenous amino acid. Skarja et al. published preparation of biodegradable segmented polyurethanes based on polycaprolactone diol and polyethylene oxide chain-linked with 2,6-diisocyanato methyl caproate - lysine methyl ester diisocynanate (LDI) and they investigated mainly mechanical and surface properties [245]. In the study [246] the research was conducted to prepare segmented poly(urethane urea)s with hard segment of LDI and various soft segments formed by D,L-lactic acid, e-caprolactone, trimethylene carbonate and 1,4-butandiol as initiator. They also studied biocompatibility *in vivo*, which proved foreign body reaction after 1 and 6 weeks. The research published in 2009 described synthesis of LDI with hydroxyl terminated poly(ε-caprolactone) in presence of 1,4- butanediol (BD),

this time as a chain extender. Final polyurethane was tested on hydrolytic stability of films and also the processing of this material by electrospinning to tubular scaffold was investigated [247]. Work conducted on use of biodegradable PLA/PEG copolymer chain-linked with LDI was published in 2010 where authors examined hydrolysis degradation behaviour of PLA/PEG/LDI polyurethane chain extended with BD, but the cytotoxicity was not tested in this research [161].

This part of the thesis was focused on preparation of metal-free catalysed synthesis of polyester-urethanes (PEUs), which could be useful for a wide range of biomedical applications. Copolymers based on poly(lactic acid) and poly(ethylene glycol) with different molecular weight were prepared by polycondensation reaction catalysed by hydrochloride acid and afterwards the chain extension reaction with L-lysine ethyl ester diisocyanate (LDI) was employed to obtain polyester-urethanes with elevated molecular weight and mechanical properties as well. The molecular weights of polyester-urethanes were characterised by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FTIR) was used for investigation of products and differential scanning calorimetry (DSC) was used for characterization of thermal properties. Also tensile strength of the prepared polyester-urethanes was tested. Furthermore, the hydrolytic degradation examination was performed in buffer solution (PBS) at 37°C. Degradation of prepared PEU was characterized by total organic carbon (TOC) and GPC methods over ten weeks. Moreover cytotoxicity assays of the samples were also performed.

Experimental

Materials

L-lactic acid (LA), 80% water solution was sourced from Merck, PEG (M_n = 1000, 1450, 2000 $g.mol^{-1}$) and HCl 30% (purity for trace analysis) were purchased from Sigma-Aldrich, Steinheim, Germany. Solvents— acetone and methanol (analytical-grade)—were obtained from IPL Petr Lukes, Uhersky Brod, Czech Republic. L-*Lysine diisocyanate ethyl* ester 95% (LDI) was purchased from Chemos (Czech Republic).

Preparation of poly(lactic acid)-poly(ethylene glycol) low molecular weight copolymer

The dehydration of 100 ml of L-LA was carried out in round-bottom flask equipped with Teflon stirrer, which was placed in oil bath. The reaction was performed at 160°C and reduced pressure of 20 kPa for 6 h. Then the PEG (1.0 or 2.0 mol%) was put in and melted and afterwards the catalyst hydrochloride acid (0.2 mol%) was added. The reaction was conducted for another 20 h at

160°C and reduced pressure of 10 kPa. The resulting copolymer was dissolved in acetone (200 ml), filtered and precipitated in water/methanol (1:1) solution. White powder was obtained after centrifugation of precipitate and drying under vacuum at room temperature for 48 h. By changing PEG with different molecular weight and its amount, six samples of prepolymers were prepared.

Synthesis of polyester-urethanes based on PLA/PEG using L-Lysine diisocyanate

Prepolymer (10 g) was melted in two-neck flask equipped with a mechanical stirrer at 180°C under nitrogen atmosphere and after that the LDI was added. The volume of isocyanate component was calculated as molar ratio of NCO/OH groups and it was set on 1.1:1. The reaction took place for 30 min. The polymer melt was cooled down in desiccator and then dissolved in acetone and precipitated in water/methanol (1:2) solution to extract low molecular weight residues. Finally, the product was dried at 30°C in vacuum for 24 h.

Preparation of samples

The powder was moulded for 2 min at 140°C (60 x 60 x 1 mm) and slowly cooled down in second manual press. The samples (50 x 7 x 1.5 mm) for mechanical testing and round shape samples (diameter 3.4 mm) for hydrolysis were cut-out from moulded plates and conditioned at desiccator at room temperature for 72 h before testing.

Characterization

The average molecular weight and molecular weight distribution were determined by gel permeation chromatography (HT-GPC 220 system, Agilent, refractive index and viscosimetric detector) with respect to polystyrene standards. Samples were dissolved in THF at concentration ~ 3 $g.L^{-1}$ and separation was performed on PL gel-mixed-D bed column at 40°C in THF with flow rate 1.0 $ml.min^{-1}$.

Fourier transform infrared spectroscopy was performed under conditions in section 2.2., tensile properties and thermal properties evaluated using DSC were measured according to description in the section 2.1.

Hydrolysis test

The specimens were placed in 25 mL glass bottles fully immersed into buffered medium (pH=7). In each follow-up time one specimen analysed by GPC. The hydrolysis test was performed at 37 °C. The buffered media of samples were analysed for dissolved organic carbon (TOC 5000A Analyzer, Shimadzu). The percentage of hydrolysed polymer for the given time point was

calculated from the amount of dissolved carbon and the initial amount of the material.

MTT assay

To prove biocompatibility the cytotoxicity tests were performed and expressed via scaling of the cell viability. As the cell line the mouse embryonic fibroblast (ATCC CRL-1658 NIH/3T3, USA) was used and cultured in the ATCC–formulated Dulbecco's Modified Eagle's Medium (PAA Laboratories GmbH, Austria) containing 10% of calf serum (BioSera, France) and 100 U mL^{-1} Penicillin/Streptomycin (GE Healthcare HyClone, United Kingdom). Cells (seeding concentration $1x10^5$ cells per mL) were seeded in the microtitration test plates (TPP, Switzerland) and pre-incubated for 24 hours. After this period of time the medium was replaced by individual extracts. Extracts were prepared according to ISO standard 10993-12; in ratio of 0,2g/1ml of culture medium. The extractions were carried out in chemically inert closed container for 24±1 hours at 37±1°C under stirring. The parent extracts (100 %) were then diluted in culture medium to obtain a series of dilutions with concentrations of 75, 50, 25, 10, 5 % and used up to 24 hours. The ability of cells to respond to cytotoxic substances was verified by sodium dodecyl sulphate (Sigma) with the final concentrations of 1, 10, 20 μg/mL in DMEM. The cell viability was evaluated by MTT assay based on the metabolisation of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) . The absorbance of light was measured at 570 nm by Infinite M200PRO multimode reader (Tecan, Switzerland). The cell viability was expressed as percentage of present cells relatively to cells cultivated in pure medium - reference (100 % viability). All the tests were performed in quadruplicates. For removal of the outliers was used Dixon's Q test.

RESULTS AND DISCUSSION

Synthesis and molecular weight characterization

Polymers were obtained by two-step polymerization method including polycondensation catalysed by HCl yielding prepolymer PLA terminated by hydroxyl groups followed by polyaddition using lysine diisocyanate. Polycondensations were performed with 1 and 2 mol % content of PEG varying in molecular weight. Chain linked polyester urethanes were synthesized in equimolar ratio of isocyanate to hydroxyl groups (NCO/OH) with constant excess of 10% of isocyanate component. The molecular weights and molecular weight distributions of prepolymers and polymers are summarized in Table 12. The effect of chain extender (diisocyanate) was significant. The molecular weight was rapidly elevated and the molecular weight distribution increased as well. This phenomenon occurs as a result of connection of hydroxyl and

isocyanate groups forming urethane bonds. However, the molecular weight was many times higher than just two OH-terminated chains connected with diisocyanate. This fact and also broad polydispersity suggests that other reactions provided by isocyanates took place. In this view, reactions with COOH groups shall be also considered. Their presence was proved by acid number determination, reactions of isocyanate with urethane resulting in allophanate formation occur and their direct consequence can be branched structure and additional crosslinking of polymer. [157, 199]. Various molecular weight PEGs were employed for investigation of their effect on macroscopic properties of chain extended copolymers. In general, higher concentration of PEG has effect on elevation of molecular weight; however, in series P1-P3 the higher absolute amount of PEG could bring into reaction more impurities or water molecules and thus lower the reaction yields. Nevertheless, molecular weights, which are significantly higher than in the related work were reached [161].

Table 12 - Molecular weights provided by GPC.

Pre-polymer	**M_n PEG**	**M_n [g.mol^{-1}]**	**M_w [g.mol^{-1}]**	***Đ***	**Acid number [mg$_{KOH}$/g]**	**Polymer**	**M_n [g.mol^{-1}]**	**M_w [g.mol^{-1}]**	***Đ***
A1-1	1000	3300	4500	1.4	11.2	P1	53600	711200	13.3
A2-2	1450	2800	3500	1.3	19.3	P2	31700	324100	10.2
A3-3	2000	2600	3600	1.4	21.6	P3	33100	303500	9.2
B1-4	1000	2200	2800	1.3	19.2	P4	14100	171000	12.1
B2-5	1450	2100	3100	1.5	15.3	P5	22400	203500	9.1
B3-6	2000	2700	4000	1.5	17.4	P6	24300	212000	8.7

A - 2 mol. % PEG, B - 1 mol. % PEG

Structure characterization of PLA based polyester urethanes

Qualitative analyses were performed using FTIR presented in Figure 44. Prepolymer consists of poly(lactic acid) and polyethylene glycol; their FTIR bands, methyl of PLA and methylene of PEG partially overlap at 2800 - 3000 cm^{-1}. Prepolymer is characterized by presence of end hydroxyl groups which appear typically at 3550–3230 cm^{-1}. Intense signals from 1050 to 1200 cm^{-1} are attributed to the C-O-C stretching bands in copolymers and peaks located at 1400 - 1500 cm^{-1} are related to CH_2 bands of PEG and CH_3 band of PLA. The spectra of products show new absorption peaks at 3300 - 3400 cm^{-1} characteristic for N-H linkage in urethane bond and also at 1540 cm^{-1} which signalizes contribution of N-H and C-N stretching vibrations of amide II. Also the absorption peaks arose at range 1600 - 1760 cm^{-1} which are associated to the C=O stretching vibrations as a result of formed urethane bond and carbonyl ester group of PLA which are overlapping at 1755 cm^{-1}. After hydrolysis an increase

of total area of peak at 1755 cm^{-1} was observed, which is in accordance with growth of COOH end groups [248, 249].

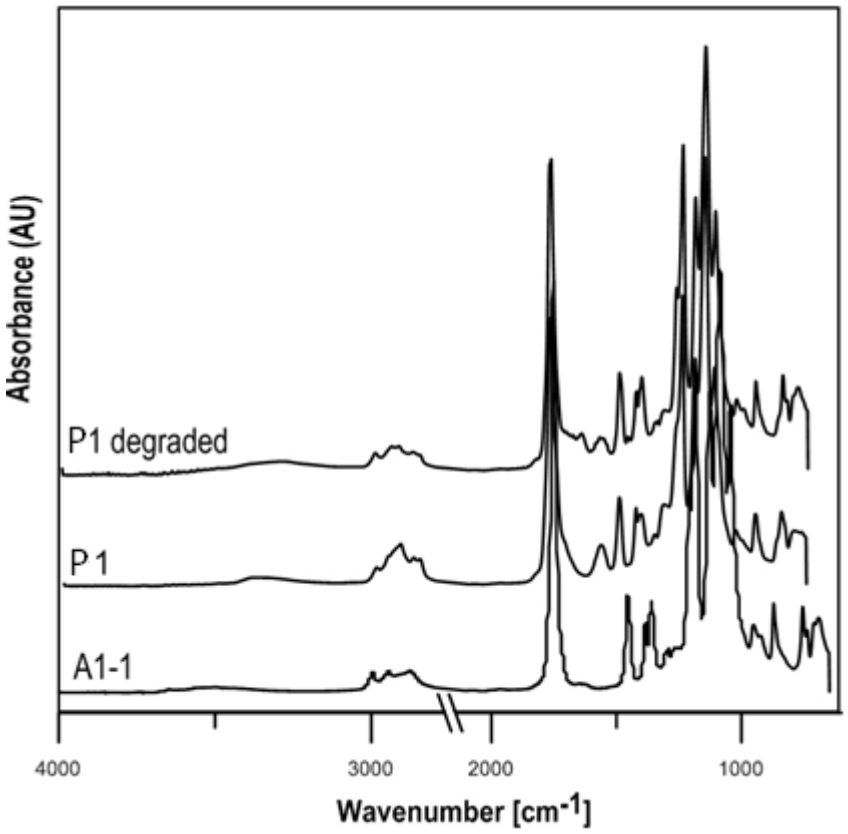

Figure 44 - Fourier transform infrared spectroscopy–attenuated total reflectance spectra of prepolymer, and chain linked poly(lactic acid)–poly(ethylene oxide) copolymers before and after degradation.

Mechanical properties

Results of tensile testing are shown in Figures 45 - 47. Mechanical performance appears to be consistent with composition of the prepared polyester urethanes. As it was expected, polymers with higher content of PEG (2%) show lower Young modulus than samples of 1% PEG which is in accordance with the elongation at break, which demonstrates very clear decrease at lower amount of PEG. Varying in PEG molecular weights was also reflected in mechanical performance mainly at concentration of 2% when obvious decrease of Young modulus occurred at M_w (PEG) = 2000 $g.mol^{-1}$ and this polymer (P3) also appears as more flexible in comparison with other samples. Unlike polyester urethanes consisting 1% PEG, the dependence of molecular weight of PEG was not proved. Compared to previously synthesized PEUs with HMDI, similar values have been reached; however, this PEU showed overall better flexibility.

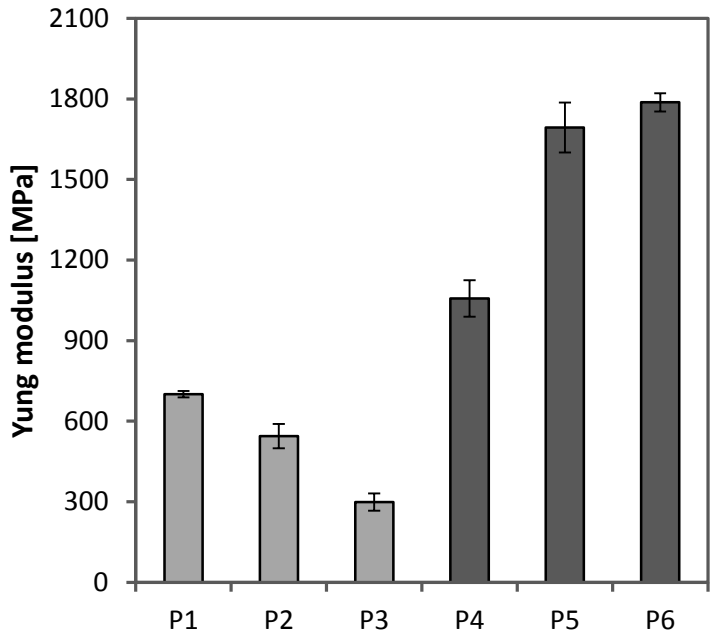

Figure 45 - Young modulus of PEUs (for sample designation see Table 12).

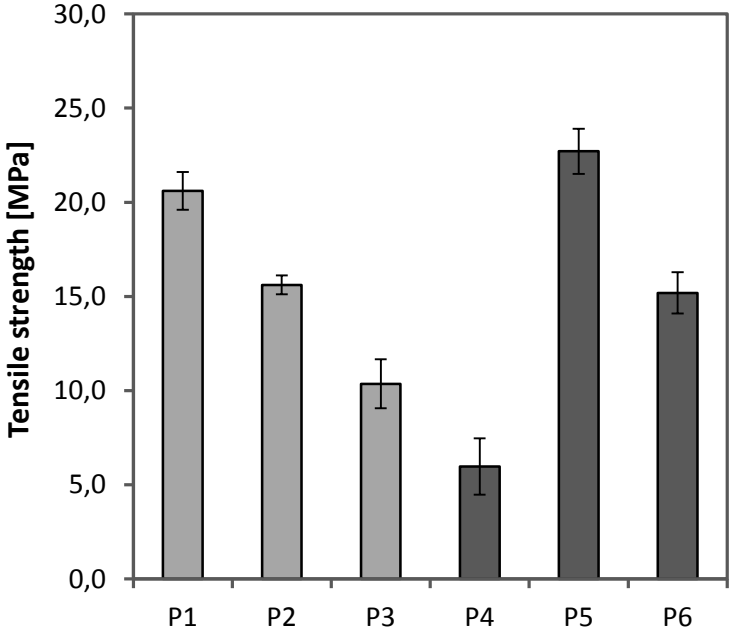

Figure 46 - Tensile strength of PEUs (for sample designation see Table 12).

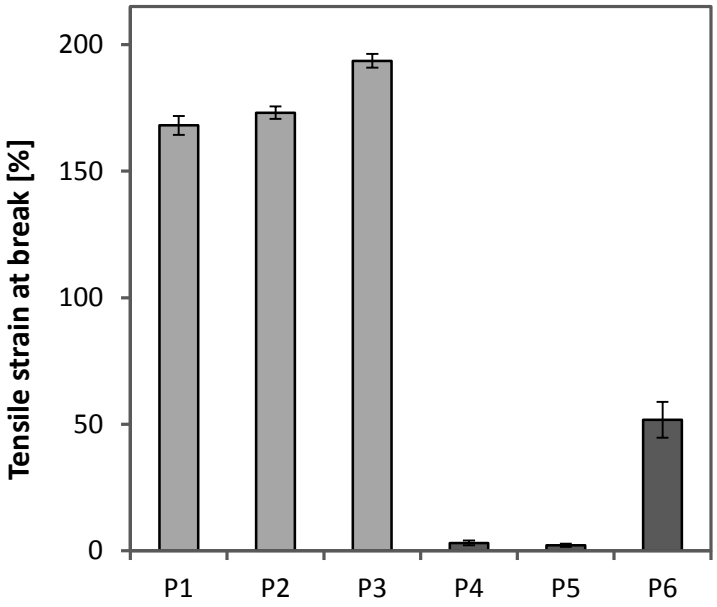

Figure 47 - Tensile strain at break of PEUs (for sample designation see Table 12).

Hydrolytic degradation testing

Hydrolytic degradation progress is depicted in Figure 48. It shows a relationship between $1/M_n$ and time. The degradation course follows the theoretical model of bulk degradation; nevertheless, due to elevated hydrophilicity by introduction of PEG the initiation and run of degradation is steeply growing rather than for example pure PLA [250]. It can be assumed that diffusion of water molecules already occurs and thus promotes degradation processes by the autocatalysis in this early stage. In later stage the degradation rate is slower, which can be a result of decrease of hydrolysable bond concentration. Moreover, the autocatalysis phenomenon likely do not contribute any more, which it is due to better access of water molecules into the samples and thus equalization of pH. Thus in comparison with theory [75], we could say that this type of polymer goes through two bulk degradation stages.

Considering that from the graphs the effect of molecular weight is not very clear and in later stage the degradation process is more or less random, it can be assumed that further factors affect its extent. For example, the restricted mobility of chains due to or high entanglement can impede the diffusion of water.

The level of hydrolysis was also monitored by measuring the total soluble organic carbon released to buffered medium; its progress is depicted in Figure: . The sharp increase of soluble carbon at the beginning of the experiment is in agreement with fast hydrolysis ($1/M_n$) in the first stage. The highest hydrolysis degree at the end of experiment showed sample P4 which had both the lowest

molecular weight and also high molecular weight distribution (*Đ*), whereby the leaching low molecular polymer chains out of bulk was facilitated [251]. In hydrolysis graphs the fluctuations in $1/M_n$ and carbon concentrations can be seen, which could be connected with presence of some impurities or inhomogeneous of samples coming from some processing difficulties. In addition, it can be noted that no evident difference in hydrolysis occurred in dependence on volume and molecular weight of PEG; however, its introduction could affect the final temperature properties of polymers which is connected directly with mobility of polymer chains and thus the degradation rate.

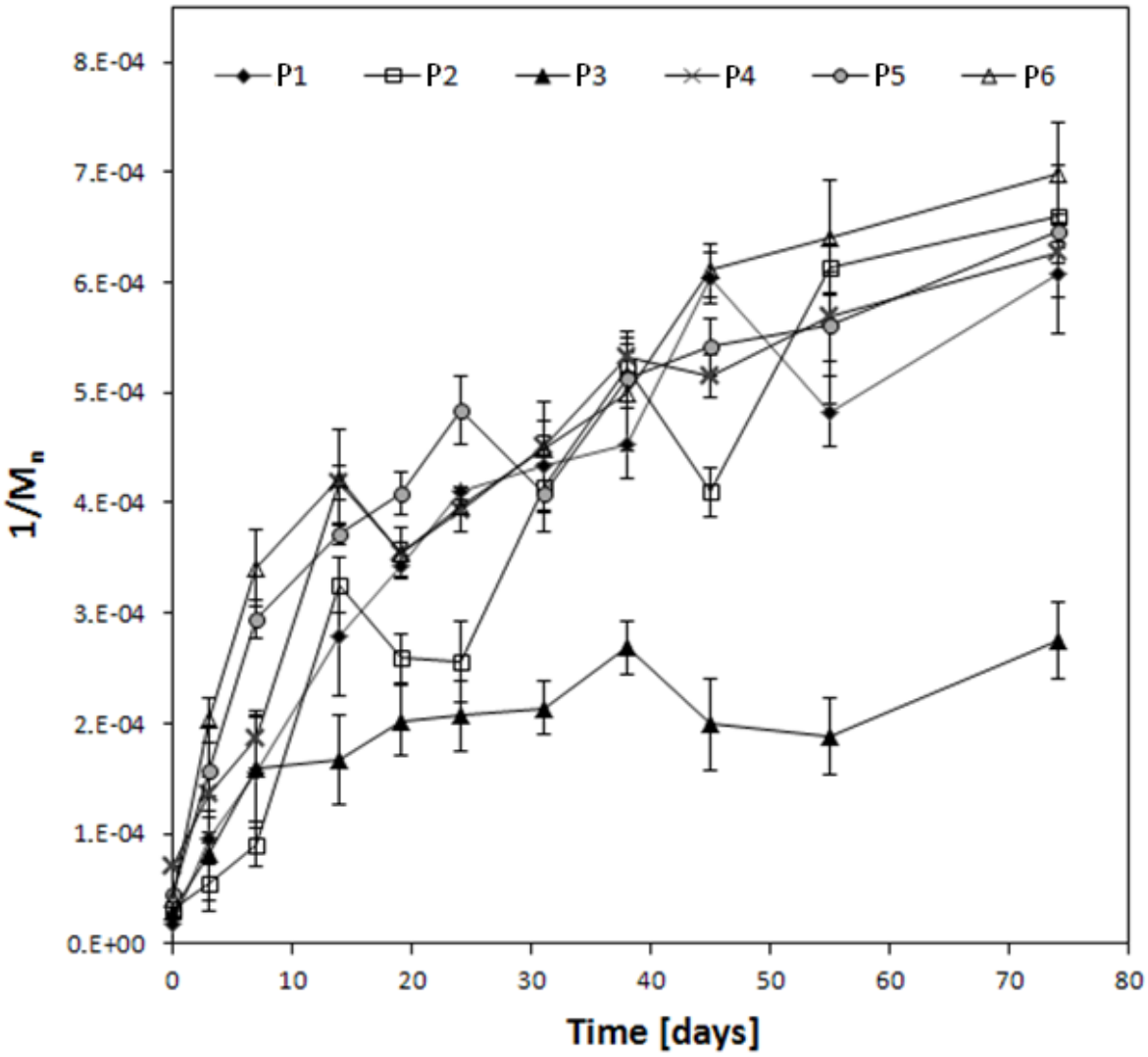

Figure 48 - Results of molecular weight loss during the hydrolytic bulk degradation of PEUs at 37°C.

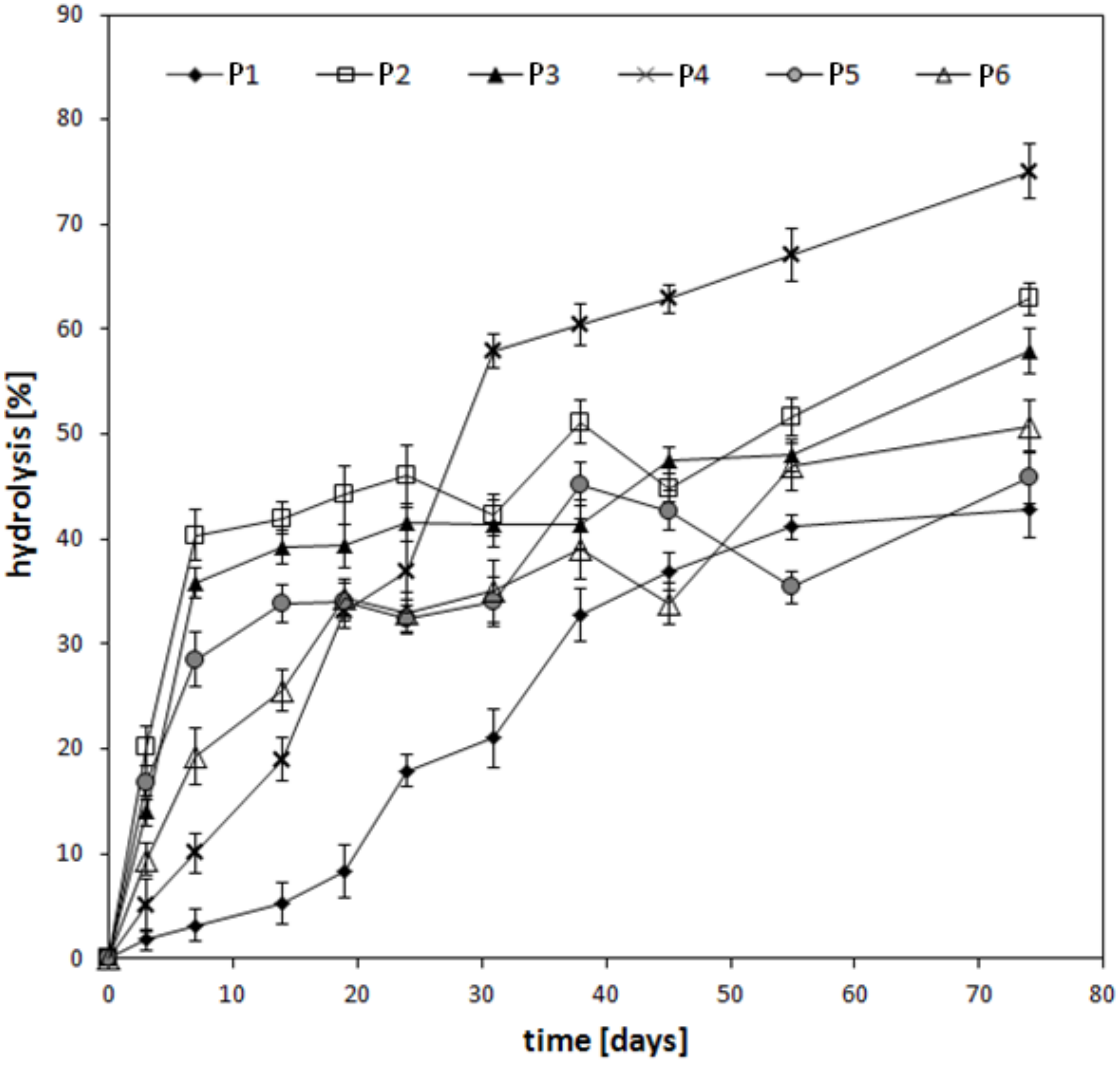

Figure 49 - Hydrolylsis of PEUs in buffered medium at 37°C.

Thermal characteristics

Thermal properties are presented in Table 13. T_g of polymer series P1-P3 showed lower values than samples of series P4-P5 and for samples P1 and P2 the melting peaks at 62 and 59°C were observed, which are the melting points related to PEG [252]. The lower T_g can also reflect the high polydispersity which introduces into polymer more free volume thereby facilitate the chain mobility. The contribution of high polydispersity can be showed mainly for polymer P1. These findings could be also connected with higher tensile strain of P1-P3 samples, because the tests were performed at temperature close to T_g of these polymers at which the elevation of chain mobility is supported [253].

Table 13 - Thermal characteristics of PEUs provided by DSC.

Sample	T_g [°C]	T_m [°C]	ΔH[J/g]
P1	16.6	62.2	7.44
P2	20.2	59	4.3
P3	25.6	*	*
P4	26.8	*	*
P5	31.2	*	*
P6	26.6	*	*

Cytotoxicity assays

The cytotoxicity was assessed via scaling of cell viability after application of PEUs extracts. The cytotoxic effect was evaluated according to EN ISO 10993-5 standard. The reference sample represents 100% of cell viability, above 80% to samples was attributed zero cytotoxicity, between 80% and 60% mild cytotoxicity, between 60 and 40 % moderate cytotoxicity and below 40% severe cytotoxicity. It can be seen that the higher extract concentration the lower viability of cells (Fig. 50, 51). However, none of the samples elicited severe cytotoxic effect even at 100% concentration. The samples prepared with 2% content of PEG showed an overall higher viability and almost complete absence of cytotoxicity at low concentration of parent extract of sample P1.

The observed cytotoxicity could be lower considering the character of reactants. Hence, the analysis of one individual extract of PEUs was performed additionally by GC/MS and the presence of lactic acid was proved. Taking into account the possibility of biomedical applications of the prepared PEUs, the further purification treatment could reduce the cytotoxicity more.

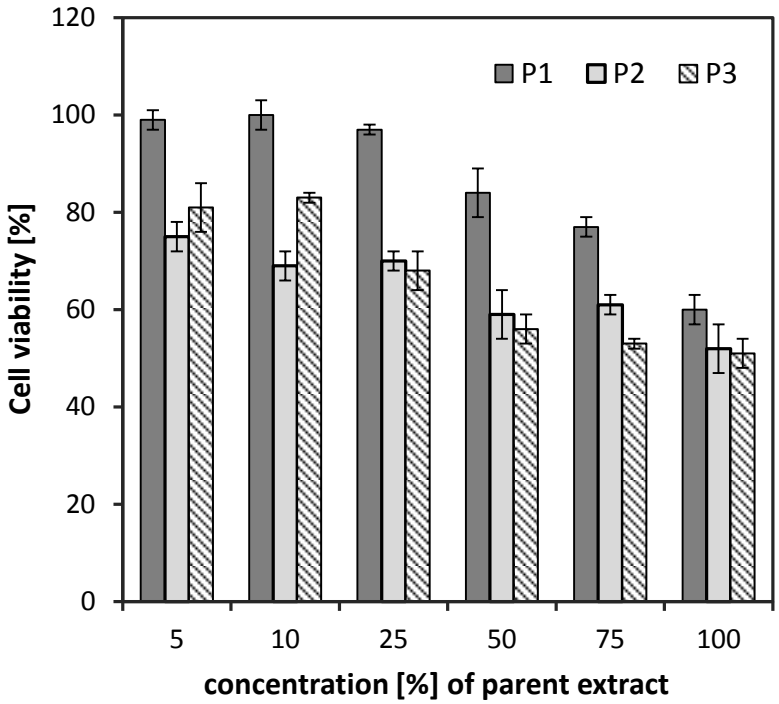

Figure 50 - Viability of NIH/3T3 *cells in polyurethanes extracts (P1-P3).*

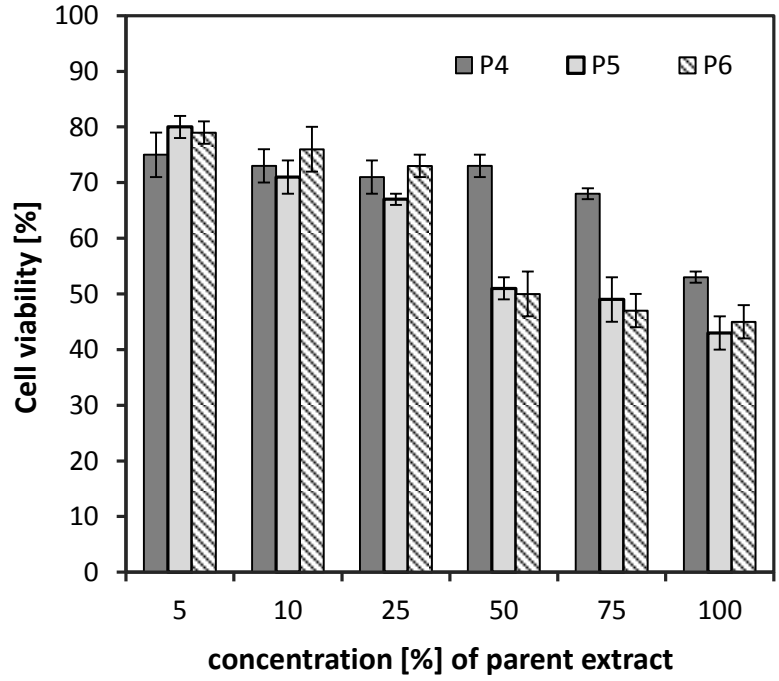

Figure 51 - Viability of NIH/3T3 *cells in polyurethanes extracts (P4-P6).*

Conclusions

Biodegradable polyester urethanes based on PLA/PEG were synthesized by using non-toxic initiator (hydrochloric acid) and diisocyanate (L-Lysine diisocyanate). Effect of PEG molecular weight (1000, 1450 and 2000 $g.mol^{-1}$) and concentration (1 and 2 mol. %) in the reaction feed was studied in detail.

Polymers based on PLA/PEG copolymers by means of chain extension reaction with L-Lysine diisocyanate reached molecular weight about 200 - 300 $kg.mol^{-1}$. One of the prepared samples reached even 700 $kg.mol^{-1}$. However, the polymers possessed also broad polydispersity (over 10).

It was proved that the increasing molecular weight of PEG has a positive effect on mechanical properties of the resultant polyester urethanes at concentration of 1 mol. % PEG in the feed. On the contrary, opposite trend was observed at 2 mol. % PEG. The samples prepared with higher PEG content (2 mol. %) possessed semicrystalline structures. All samples were accessible to hydrolytic degradation. Cytotoxicity testing revealed moderate cytotoxicity in all cases. Reduction of the cytotoxicity parameter will be a subject of future research activities.

SUMMARY OF WORK

This thesis is focused on synthesis and characterization of biodegradable polymers based on polyesters and polyanhydrides, which possess excellent prerequisites for their use in biomedical applications either as transport mediators or tissue (growth) support.

In this regard the PLA and polysebacic acid based polyanhydrides have a great potential and they are still broadly investigated for further possibility of usage in this field, even though they are already present in a variety of biomedical applications. Among the properties which are being modified or improved there are for example mechanical properties, degradation rate, releasing profile or biocompatibility with tissue, etc.

According to the literature and current state of knowledge the overview of this issue was drawn in the theoretical part. On the basis of this, specific research aims of work were defined.

In the first part the work was devoted to preparation of novel PLA/PEG based polyester urethanes using various diisocyanates as chain extenders and study of their degradability. Resulting polymers were also investigated for their fabrication on nanoparticles and nanofibres and other characteristics of these nano-systems were studied. Results showed that the high molecular weight polymers can be obtained, which can degrade in frame of weeks. Moreover, they are able to create well-defined nanoparticles and fibres.

Secondly, the sebacic acid polyanhydrides were prepared via melt polycondensation. This time the comprehensive study was focused on the investigation of newly possible catalysts for this reaction with relation to the molecular weight of polymer depending on the reaction time. Also the thermal stability and degradation products were characterized. Several important findings were revealed. The efficient catalyst was found to be CaO and the optimal reaction conditions were described. The thermal stability is reduced for the short molecular chains polymers.

The final part was dealing with synthesis of biocompatible polyester urethane using non-toxic reactants and metal free catalysts. Effect of PEG molecular weight (1000, 1450 and 2000 $g.mol^{-1}$) and concentration (1 and 2 mol. %) in the reaction feed was studied in detail. The results revealed significance of both studied parameters that affect the structure, mechanical and thermal properties, and described the degradation behaviour of the prepared samples.

CONTRIBUTIONS TO SCIENCE AND PRACTICE

Presented work brings new insights into the field of biodegradable polymers based on poly(lactic acid) and polyanhydrides, which are highly potential for biomedical applications.

This thesis represents multidisciplinary oriented research work considering field of macromolecular and analytic chemistry, material engineering, biology and microbiology. Besides, preparation and characterization of new materials is accompanied by practical applicability testing in most cases. The subject of interest was nano-structured products in form of nanoparticles or nanofibres.

The results presented in this thesis were (or will be) published in reputed international journals. Moreover, specialization of this thesis laid the foundations for further development of biodegradable polymers research at the Centre of Polymer Systems at Tomas Bata University in Zlín.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AN	Acid number
BA	Boric acid
BOX	2,2-bis(2-oxazoline)
BD	1,4-Butanediol
CdAcet	Cadmium acetate dehydrate
DES	4,4-dicyclohexylmethane diisocyanate
DEZ	Diethyl zinc
DII	1,3-Bis(2,6-di-i-propylphenyl)imidazolidin-2-ylidene
DP	Degree of polymerization
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
IBD	Inflammatory bowel disease
IPDI	Isophorone diisocyanate
LA	Lactic acid
M_n	Number average molecular weight
M_W	Weight average molecular weight
MEM	Minimum essential medium cytotoxicity test
MPA	m-Phosphoric acid
MS	Molecular sieves
MTT	3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide cytotoxicity test
MWD	Molecular weight distribution (Đ)
NMR	Nuclear magnetic resonance
PCL	Polycaprolactone
PHAs	Polyhadroxyalcanoates
PDI	Polydispersity index
PDMS	Polydimethylsiloxane
PEG	Poly(ethylene glycol)
PEO	Polyethylene oxide
PEU	Polyester urethane
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLGA	Poly(lactic acid)/poly(glycolic acid) copolymer
PSA	Poly(sebacic anhydride)
PUs	Polyurethanes
ROP	Ring opening polymerization
SA	Succinic acid
HMDI	1,6-Hexamethylene diisocyanate
MDI	4,4'-methylenediphenyl diisocyanate
MTZ	Metazachlor

TBO	Titanium(IV) butoxide
TDI	Toluene-2,4-diisocyanate
LDIm	2,6 diisocyanato methyl caproate
LDIe	2,6 diisocyanato ethyl caproate
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TMA	Thermomechanical analysis
TSA	p-Tolulenesulfonic acid

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- **GJ15-08287Y** Immobilization of specific bioactive natural substances in functionalized biodegradable polymer matrices (2015-2017), provider: Czech Science Foundation. Member of research team

- **TE02000006** Centre for alternative environment friendly high effective polymer antimicrobial agents for industrial applications (2014-2019), provider: Technology Agency of the Czech Republic (TA ČR). Member of research team.

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- **IGA/FT/2014/012** Research of antimicrobial biodegradable polymer systems, (2014), provider: Internal Grant Agency of Tomas Bata University in Zlín. Principal investigator.

- **IGA/FT/2013/004** Preparation and characterization of novel biologically active polymeric systems based on natural substances, (2013), provider: Internal Grant Agency of Tomas Bata University in Zlín. Member of research team.

LIST OF PUBLICATIONS

Journal articles:

1. PAVELKOVA, A., KUCHARCZYK, P., ZEDNIK, J., SEDLARIK., V. Synthesis of poly (sebacic anhydride): effect of various catalysts on structure and thermal properties. *Journal of Polymer Research*, 2014, 21.5: 1-13. DOI: 10.1007/s10965-014-0426-3.
2. PAVELKOVA, A., KUCHARCZYK, P., STLOUKAL, P., KOUTNY, M., SEDLARIK, V. Novel poly (lactic acid)–poly (ethylene oxide) chain-linked copolymer and its application in nano-encapsulation. *Polymers for Advanced Technologies*, 2014, 25.6: 595-604. DOI: 10.1002/pat.3241.
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4. PAVELKOVA, A., KUCHARCZYK, P., SEDLARIK, V. Electrospinning of Biodegradable Polyester Urethane: Effect of Polymer Solution Conductivity, *Materiali in Tehnologije/Materials and Technology*, submitted 2015.
5. PAVELKOVA, A., KUCHARCZYK, P., KUCEKOVA, Z., ZEDNIK, J., SEDLARIK, V. Characterization of biocompatible non-toxic polyester urethanes based on poly(lactic acid)-poly(ethylene glycol) and L-Lysine diisocyanate, to be submitted in 2016.

Conference proceedings:

1. PAVELKOVA, A., KUCHARCZYK, P., SEDLARIK, V., Biodegradable polyester urethane based matrices for manofibres fabrications, 23rd International conference on materials and technology, Portorož, 2015, Slovenia.
2. PAVELKOVA, A., ZEDNIK, J., SEDLARIK, V., Synthesis caprolactone copolymers and methoxypolyethylene intended for the preparation of polymeric micelles with a defined hydrophilic lipophilic balance, Plastko 2014, Zlín Czech Republic
3. KUCHARCZYK, P., PAVELKOVA, A., STLOUKAL, P., SEDLARIK, V., Preparation of nanoparticles based on biodegradable chain linked PLA/PEG polymer for controlled release of herbicide Metazachlor. Nanocon 2013, Brno, Czech Republic

4. KUCHARCZYK, P., STLOUKAL, P., KOUTNÝ, M., PAVELKOVÁ, A., SEDLAŘÍK, V., Synthesis of PLA-PEG copolymer and its chain extending with di-isocyanate compounds. Structural properties, degradation and potential utilization. 7th MoDeSt conference, 2012, Prague, Czech Republic, ISBN 978-80-85009-74-3.