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BRNO UNIVERSITY OF TECHNOLOGY

FAKULTA CHEMICKÁ
ÚSTAV CHEMIE MATERIÁLŮ

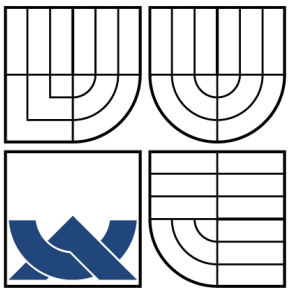
FACULTY OF CHEMISTRY
INSTITUTE OF MATERIALS SCIENCE

FUNCTIONALIZATION OF BIODEGRADABLE POLYMERS BY ITACONIC ANHYDRIDE

DIPLOMOVÁ PRÁCE
MASTER'S THESIS

AUTOR PRÁCE — Bc. LENKA MICHLOVSKÁ
AUTHOR

BRNO 2009



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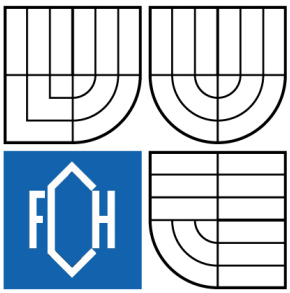

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MASTER'S THESIS

AUTOR PRÁCE — Bc. LENKA MICHLOVSKÁ
AUTHOR

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SUPERVISOR

BRNO 2009

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Master's thesis Assignment

Number of master's thesis:	**FCH-DIP0326/2008**	Academic year: **2008/2009**
Institute:	Institute of Materials Science	
Student:	**Bc. Lenka Michlovská**	
Study programme:	Chemistry, Technology and Properties of Materials (N2820)	
Study branch:	Chemistry, Technology and Properties of Materials (2808T016)	
Head of master's thesis:	**Ing. Lucy Vojtová, Ph.D.**	
Consultants of the master's thesis:		

Title of master's thesis:

Functionalization of biodegradable polymers by itaconic anhydride

Master's thesis assignment:

General goal of this work is synthesis and characterization of well-defined biodegradable polymers end-functionalized by itaconic anhydride (ITA) to bring reactive double bonds and functional carboxylic acid groups to the end of copolymer resulting in preparation of bioinductive polymers.
Literature searching:
1. Biodegradable polymers based on PLA, PGA
2. Itaconic anhydride and itaconic acid (properties, characterization, thermal stability)
3. Functionalization of polymers by itaconic anhydride

Experimental work:
1. Functionalization of PLGA-PEG-PLGA by ITA
2. Evaluation of influence of coupling conditions (temperature, time and solvent)
3. Optimization reaction conditions in order to obtain the highest amount of ITA bonded to polymer.
4. Characterization of products by GPC, FTIR and 1H NMR.
5. Conclusion

Deadline for master's thesis delivery: 29.5.2009

Master's thesis is necessary to deliver to a secreatry of institute in three copies and in an electronic way to a head of master's thesis. This assignment is enclosure of master's thesis.

Bc. Lenka Michlovská Student	Ing. Lucy Vojtová, Ph.D. Head of thesis	prof. RNDr. Josef Jančář, CSc. Head of institute

In Brno, 1.10.2008

doc. Ing. Jaromír Havlica, DrSc.
Dean

ABSTRACT

Presented diploma thesis describes preparation of biodegradable thermosensitive triblock copolymer based on poly(ethylene glycol), poly(lactic acid) and poly(glycolic acid) (PLGA-PEG-PLGA) that was subsequently modified by itaconic anhydride (ITA), which gives copolymer both reactive double bonds and functional carboxylic acid groups essential for the reaction with biological active material. The general goal was optimizing reaction conditions in order to reach the highest yield of ITA end-capped polymer resulting in ITA/PLGA-PEG-PLGA/ITA. Prepared functionalized copolymer as a component of heterogeneous composite e.g. with hydroxyapatite might be suitable for biomedical application in the field of tissue engineering as a temporary replacement or adhesive of hard tissues (bones).

In the theoretical part, hydrogels, their separation, crosslinking and degradation mechanism are generally described together with physico-chemical properties and the synthesis of the individual used biomaterials and their copolymers, itaconic anhydride and its functionalization.

The experimental part describes in details the synthesis of PLGA-PEG-PLGA copolymer via ring opening polymerization (ROP) using vacuum line and Schlenk's techniques. Kinetics of the ROP was measured and the optimization of polymerization conditions was suggested. Prepared thermosensitive copolymer was additionally modified by itaconic anhydride via catalytic ring-opening reaction. Optimization of ITA functionalization conditions were evaluated in terms of effect of temperature, time, ITA purification and presence of the solvent. Successful end-capping of PLGA-PEG-PLGA copolymer by ITA was precisely characterized by means of ^{1}H NMR, FT-IR and GPC methods.

Kinetics of PLGA-PEG-PLGA copolymerization from non-sublimated (neat) and sublimated (purified) D,L-lactide and glycolide were studied. In both cases the synthesis proceeded in a bulk at 130 °C for 3 hours with conversion approximately of 90 %. Prolonged polymerization period had no effect on the increase of conversion. In the case of ROP using unsublimated monomers, a rapid increase of monomer conversion was observed during first few minutes, followed by constant progress. Resulting copolymer displayed molecular weight of 7155 g·mol^{-1}and narrow polydispersity index of 1.26. Optimal conditions were reached when sublimated monomers were polymerized. First, increase of conversion up to 88 % was nearly linear (living polymerization) to 2.5 hours, after that a plateau was observed. Well-defined PLGA-PEG-PLGA copolymer with molecular weight of 7198 g·mol^{-1} and narrow polydispersity index of 1.20 was obtained.

Optimal conditions for synthesis of ITA/PLGA-PEG-PLGA/ITA copolymer were reached with sublimated itaconic anhydride in a bulk at the temperature of 110 °C with total reaction time of 1.5 hours. As a result 76.6 mol. % of ITA was end-capped to the original PLGA-PEG-PLGA copolymer. Resulting molecular weight of ITA/PLGA-PEG-PLGA/ITA copolymer (5881 g·mol^{-1}) with polydispersity index of 1.37 found by GPC correlated well with M_n calculated from ^{1}H NMR and a theoretical M_n ($M_{ntheor}/M_{nGPC}/M_{nNMR}$ = 1/0.89/0.96).

KEY WORDS: biodegradable polymers, itaconic anhydride, functionalization.

ABSTRAKT

Předložená diplomová práce se zabývá přípravou biodegradabilního termosenzitivního triblokového kopolymeru na bázi polyethylenglykolu, kyseliny polymléčné a polyglykolové (PLGA-PEG-PLGA) a dále pak především jeho modifikací anhydridem kyseliny itakonové (ITA), který dodá kopolymeru jak reaktivní dvojné vazby tak i funkční karboxylové skupiny důležité pro reakci s biologicky aktivními látkami. Hlavním cílem bylo optimalizovat reakční podmínky pro dosažení nejvyššího stupně navázání ITA na polymer za vzniku ITA/PLGA-PEG-PLGA/ITA. Uvedený kopolymer je po vytvoření heterogenního kompozitu např. s hydroxyapatitem vhodný pro biomedicínské aplikace především v oblasti tkáňového inženýrství jako dočasná náhrada či fixace tvrdých tkání (kostí).

V teoretické části uvedené práce jsou na základě literární rešerše obecně popsány hydrogely, jejich rozdělení, síťování a degradace. Stručně jsou rozepsány fyzikální a chemické vlastnosti a syntéza jednotlivých biomateriálů použitých při syntéze, anhydridu kyseliny itakonové a jejich kopolymerů.

Experimentální část popisuje detailně syntézu PLGA-PEG-PLGA kopolymeru polymerací za otevření kruhu pomocí vakuové linky a Schlenkových technik. Byla sledována i kinetika polymerace s navržením nejvhodnějších podmínek syntézy. Uvedený kopolymer byl následně modifikován anhydridem kyseliny itakonové opět katalytickou reakcí za otevření kruhu. V důsledku optimalizace reakčních podmínek byl sledován vliv teploty, rozpouštědla, času a čistoty vstupních látek. Výsledný ITA/PLGA-PEG-PLGA/ITA kopolymer byl charakterizován pomocí ^{1}H NMR, FT-IR a GPC metody.

Byly sledovány kinetiky polymerace PLGA-PEG-PLGA kopolymeru a to z přesublimované a nepřesublimované kyseliny polymléčné a polyglykolové. V obou případech probíhala kinetika reakcí bez přítomnosti rozpouštědla při 130 °C po dobu 3 hodin s konverzí asi 90 %. Delší čas neměl vliv na růst konverze. U kinetiky z nepřesublimovaných monomerů byl sledován během několika prvních minut prudký nárůst konverze a pak již byl průběh konstantní, na čase nezávislý. Výsledná polydisperzita kopolymeru byla 1,26 a molekulová hmotnost 7155 g·mol^{-1}. Optimálních podmínek bylo dosaženo u polymerace z přesublimovaných monomerů, kdy byl nárůst konverze do hodnoty 88 % získané během 2,5 hodin téměř lineární (polymerace byla živá) a poté byl progres konstantní. Byl získán přesně definovaný PLGA-PEG-PLGA kopolymer o molekulové hmotnosti 7198 g·mol^{-1} a polydisperzitě 1,20.

Nejlepších podmínek při syntéze ITA/PLGA-PEG-PLGA/ITA kopolymeru bylo dosaženo reakcí bez přítomnosti rozpouštědla při 110 °C po dobu 1,5 hodiny s přesublimovaným anhydridem kyseliny itakonové, kdy bylo na původní kopolymer navázáno 76.6 mol. % ITA. Výsledná molekulová hmotnost kopolymeru s polydisperzitou 1,37 stanovená pomocí GPC se shodovala s vypočítanou molekulovou hmotností z ^{1}H NMR i s teoretickou molární hmotností ($M_{nteor}/M_{nGPC}/M_{nNMR}$ = 1/0,89/0,96).

KLÍČOVÁ SLOVA: biodegradabilní polymery, anhydride kyseliny itakonové, funkcionalizace.

MICHLOVSKÁ, L.: *Functionalization of biodegradable polymers by itaconic anhydride*. Brno: Brno University of Technology, Faculty of Chemistry, 2009. 59 p. Supervisor Ing. Lucy Vojtová, Ph.D.

DECLARATION

I declare that the diploma thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the diploma thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

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student's signature

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I express my thanks to my dear mother for her lifelong support.

CONTENT

1 INTRODUCTION

A biomaterial can be defined as any material used to make devices to replace a part or a function of the body in a safe, reliable, economic, and physiologically acceptable manner. Thus a biomaterial is a synthetic material used to replace part of a living system or to function in intimate contact with living tissue. A variety of devices and materials is used in the treatment of disease or injury. Commonplace examples include sutures, tooth fillings, needles, catheters, bone plates, etc. In general three aspects of study on the subject of biomaterials can be envisioned; biological materials implant materials, and interaction between the two in the body [1].

Biodegradable synthetic polymers offer a number of advantages over materials for developing scaffolds in tissue engineering. The key advantages include the ability to tailor mechanical properties and degradation kinetics to suit various applications. Synthetic polymers are also attractive because they can be fabricated into various shapes with desired pore morphologic features conductive to tissue in-growth. Furthermore, polymers can be designed with chemical functional groups that can induce tissue in-growth [2].

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. These hydrogels are able to swell or deswell as a result of changing in the temperature of the surrounding fluid. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels. Thermoreversible biodegradable gels based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(D,L-lactic acid) (PLA) and poly(glycolic acid) (PGA) have been recently published [3]. While these thermoreversible gels increase the hydrogel applicability as injectable implants and biodegradable matrix for the controlled drug delivery systems without surgery, on the other hand, the reversible physical network, the phase-transition temperature range and low degree of functionality limit the application for other branches.

The aim of this diploma thesis is functionalization of poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) copolymer by itaconic anhydride (ITA) and optimization of reaction conditions. The effect of ITA purification, temperature, time and presence of solvent on the prepared samples properties will be evaluated. Resulting ITA/PLGA-PEG-PLGA/ITA copolymers can be cross-linked either chemically or physically in order to produce new functionalized hydrogel network.

2 THEORETICAL PART

The synthesis of new polymer multicomponent multifunctional materials with controlled life time mimicking to some extent natural materials such as wood, bone or tendon has become a major component of the current state-of-the-art in the science and engineering of advanced materials. In addition, low energy consumption in the course of production, using of raw materials from renewable resources and high degree of recycling or degradation are pivotal advantages for highly demanding applications.

2.1 Hydrogels

Efforts to emulate natural materials, which contain both hydrophilic and hydrophobic properties, have led to the development of amphiphilic biocompatible synthetic polymers [4,5]. This classification included hydrogels, made by cross-linking (chemical or physical) of polymer chains, which are significant for their special surface and physical properties with the ability to absorb more than 20% of water in proportion to their total weight [6, 7]. The hydrogel swelling ratio is influenced by the hydrophilic character and density of polymer network.

The use of hydrogels as biomaterials has recently gained great importance from the viewpoint of both low toxicity and high biocompatibility. The main hydrogel application areas include topical applications as wound dressings, drug delivery systems, transdermal systems, dental applications, injectable polymers, implants, ophthalmic applications, and stimuli-responsive systems [8, 9].

Originally, Wichterle and Lim introduced a type of hydrophobic gel for biological uses in the early 1960s [10]. Hydrogels are crosslinked, three-dimensional hydrophilic polymer networks, which swell but do not dissolve when brought into contact with water. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as –OH, –CONH–, $–CONH_2$, and $–SO_3H$ in polymers forming hydrogel structures. Due to the contribution of these groups and domains in the network, the polymer is thus hydrated to different degree depending on the nature of the aqueous environment and polymer composition. In contrast, polymeric networks of hydrophobic characteristics (e.g. poly(lactic acid) (PLA) or poly(lactic-*co*-glycolic acid) (PLGA)) have limited water absorbing capacities (<5-10%) [11, 12].

Hydrogels can be classified according to a variety of characteristics, including the nature of side groups (neutral or ionic), mechanical and structural features (affine or phantom), method or preparation (homo- or co-polymer), physical structure (amorphous, semicrystalline, hydrogen bonded, supermolecular and hydrocollodial), and responsiveness to physiologic environment stimuli (pH, ionic strength, temperature, electromagnetic radiation, etc.) [13].

Among the key properties that make hydrogels valuable in the field of their applications belong the degree of swelling, sorption kinetics, solute permeability, and their in vivo performance characteristics. The degree of crosslinking represents an important property directly influencing these properties. A swollen hydrogel is considered to contain three types of water. ‘Free water’ which freezes at normal freezing point, ‘intermediate freezing water’

which freezes below the usual freezing point and ‘unfrozen bound water’ which does not freeze [14].

2.1.1 Crosslinking

Crosslinked polyesters and polyanhydrides comprise most of the crosslinked resorbable biopolymers. The properties of the networks are governed by the properties of the constituent monomers, but also by the crosslinking density, which is related to the distance between the crosslinking points. Crosslinking has been used to obtain resorbable polymers with very high strength or with elastic properties. Elastic networks recover after deformation because the crosslinks resist creep effectively. Water-soluble precursors can be used in the preparation of resorbable hydrogels [14]. Polymers with hydrophobic domains can crosslink in aqueous environments via thermo-reversible sol-gel transitions, forming in situ hydrogels without injurious organic solvent or any chemical reactions. Fig. 1 schematically represents the thermo-reversible sol-gel transition of the triblock copolymers. The system can be loaded with drugs in aqueous phase at low temperature, below critical gelation temperature, where it forms a sol. At increase temperature to 37 °C, above critical gelation temperature, makes the injected sol to gel [15].

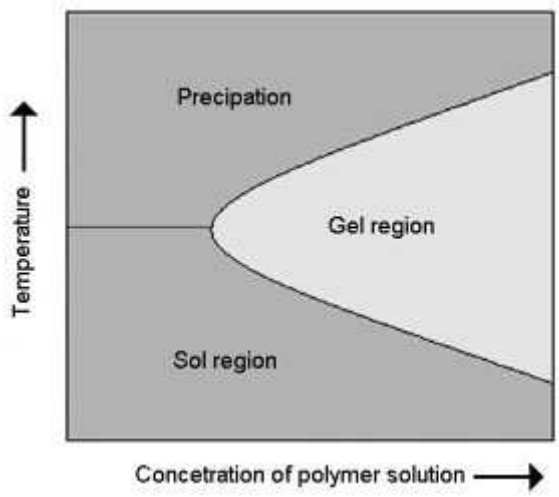


Fig. 1: Schematic representation of sol-to-gel transition in stimuli-sensitive polymers [16].

The preparation of the crosslinked hydrogels usually comprises several steps [14]. Crosslinked polyanhydrides have been synthesized from crosslinkable precursors that are either monomers or oligomers and contain labile anhydride bonds [17]. Novel crosslinking methods used in hydrogels are shown in Fig. 2 [18].

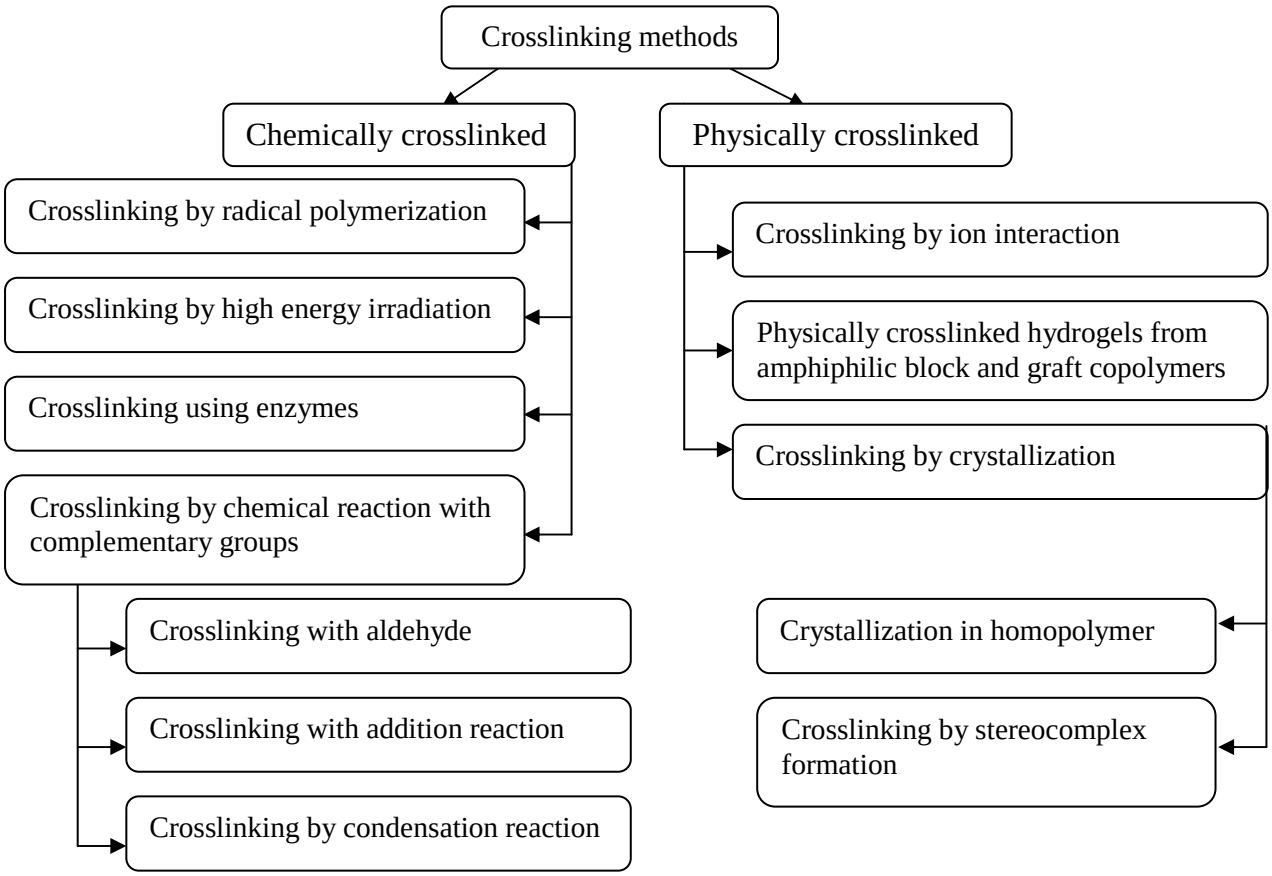


Fig. 2: Novel crosslinking methods used in hydrogels [17].

2.1.2 Degradation

Degradation is the irreversible process in which a material undergoes physical, chemical, and/or biochemical changes leading to an increase in entropy. Polymers for medical applications which are degraded in living tissues or in the environment of the living body are called also biodegradable since the degradation takes place in a biological environment.

Following part of this work deals with hydrolytic degradation mechanisms which do not involve enzymatic catalysis (chemical hydrolysis), because these mechanisms are very often solely abiotic. Degradation processes induces the subsequent erosion of the material which is defined as mass loss of material due to the process of polymer chain cleavage. Degradation can take place either in bulk (homogeneous) or polymer surface (heterogeneous erosion). The difference between bulk erosion and surface erosion is shown in Fig. 3 [19].

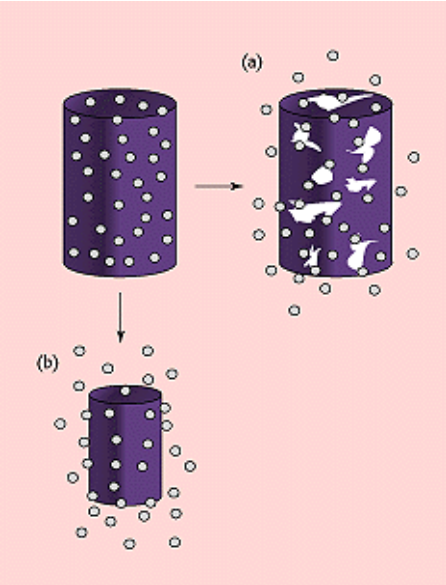


Fig. 3: Schematic illustrating a) bulk erosion and b) surface erosion [18].

Bulk eroding polymers degrade all over their cross-section because the penetration of water into the polymer bulk is faster than degradation of polymer. In surface-eroding polymers, degradation is faster than the penetration of water into the bulk. Best known examples of polymers that can be fabricated into surface eroding devices are polyanhydrides and poly (orthoesters) [20].

Degradation kinetics depends on many factors such as chemical and chain structure, crystallinity, copolymer ratio, molecular weight and molecular weight distribution, presence of low-molecular weight oligomers as well as properties of aqueous medium like pH, ionic strength, temperature, and buffering capacity [21, 22].

2.2 Biodegradable polymers

Generally polymers may be derived from natural sources or from synthetic organic processes. Naturally occurring biopolymers include plant materials (e.g. cellulose, sodium alginate, and natural rubber), animals materials (e.g. collagen, glycosaminoglycans (GAGs), heparin, and hyaluronic acid), and others (deoxyribonucleic acid (DNA), the genetic material of all living creatures). Synthetic polymeric biomaterials range from hydrophobic, non-water-absorbing materials (silicone rubber (SR), polyethylene (PE), polypropylene (PP), poly(ethylene terephthalate) (PET), polytetrafluoroethylene (PTFE), and poly(methyl metacrylate) (PMMA)) to somewhat more polar materials poly(vinyl chloride) (PVC), copoly(lactic-glycolic acid) (PLGA), nylons) to water-swelling materials (e.g. poly(hydroxyethyl methacrylate) (PHEMA)) and water-soluble materials (dextran, gelatin, poly(ethylene glycol) (PEG) etc.) [23].

Recent developments in the biomaterial field have been focused on biodegradable polymers. Significant progress has been made in the development and utilization of polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers poly(lactic-co-glycolic acid) (PLGA) namely because they were accepted by the U. S. Food and Drug Administration for internal use in human body [24]. Due to their ability to degrade by hydrolysis of ester

bonds to body non-toxic metabolites, i. e. to lactic and glycolic acid, these (co)polyesters embody high biocompatibility, non toxicity and easy process ability in different forms. The polymers and copolymers based on poly(lactic acid) and poly(glycolic acid) were originally developed and marketed as an industrial product as resorbable sutures. In comparison, many petroleum-based polymers are usually not easily recyclable and do not biodegrade [25].

2.2.1 Lactic acid and Polylactide (PLA)

Lactic acid (2-hydroxy propanoic acid) is easily obtained by either biotechnological process, usually based on the production of a strain of *lactobacillus*, or from inexpensive raw materials (e.g corn, wheat, barley and sugar cane) [26].

Polylactide (PLA) is a thermoplastic, high strength, high modulus polymer, which belongs to the family of aliphatic polyester. It has been known since 1932. Being biocompatible and biodegradable material PLA has been gaining rapidly in importance as an environmentally sustainable alternative to petrochemical-derived products [27, 28]. It undergoes scission of chains in the host body to monomeric units of lactic acid, which is a natural intermediate in carbohydrate metabolism. These characteristics make this polymer suitable for use in resorbable sutures, carries for the controlled release of drugs, implants for orthopaedic surgery or blood vessels, which finally can be replaced by living tissues [29]. The attraction of PLA as a biodegradable material is its ready availability from renewable resources such as corn starch (in the U. S.) and sugarcanes (rest of world). The PLA life cycle starts with corn starch. The plants are first milled to separate the starch, which is converted to lactic acid utilizing fermentation and a series of purification steps. It is fully compostable. It can be converted back to monomer and oligomer by enzymatic degradation, or it can be degraded into water, carbon dioxide and organic materials. Fig. 4 shows a closed life cycle of the PLA [26, 30]. The nomenclature of PLA prepared by different routes is full of contradictions in the literature, but polymers derived from lactic acid by polycondensation are generally referred as poly(lactic acid) and the ones prepared from lactide by ring opening polymerization (ROP) as polylactide. Both types are generally denoted by abbreviation of PLA.

Polymers and copolymers of PLA are transparent, colorless thermoplastics with a wide range of physical properties that mimic those of some conventional thermoplastics. When exposed to moisture or biological fluids, these modified PLA plastics hydrolyze slowly over a period of several months to natural, harmless materials such as lactic acid [31].

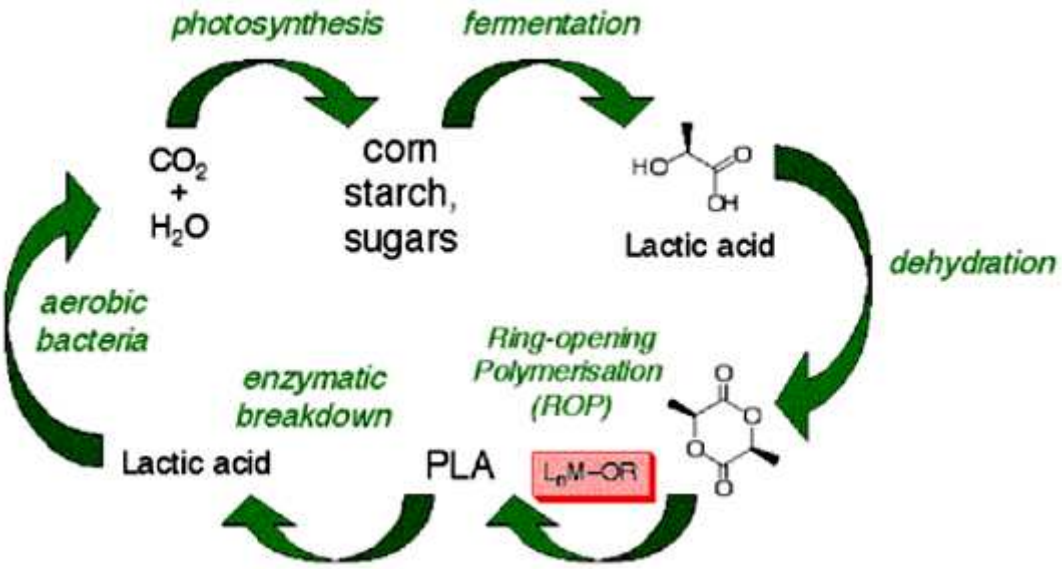


Fig. 4: Scheme of PLA life cycle including its biodegradation.

2.2.1.1 Properties and characterization

Lactic acid is the simplest hydroxy acid with an asymmetric carbon atom and exists in two optically active configurations. Both D- and L-enantiomers (Fig. 5) are produced in bacterial systems, thus lactic acid can be obtained by fermentation, selecting suitable microorganism, e.g. homo-lactic organisms. Various optimized or modified strains of *Lactobacilli* produce stereoregular L-lactic acid. However, lactic acid obtained by the chemical process is a racemic mixture of D- and L-isomers [23].

L – Lactic acid D – Lactic acid

Fig. 5: Two enatiomers of lactic acid [23].

Lactide exists in two optically active configurations: D-lactide, L-lactide and *meso*-lactide (Fig. 6). The properties of PLA, such as melting point, crystallinity, and mechanical strength are affected by the polymer architecture and its molecular weight [23, 32].

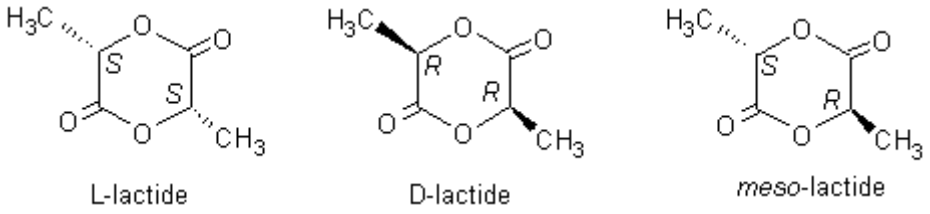


Fig. 6: Different isomeric forms of lactide [23].

PLA has glass transition temperature (T_g) in the range of 55 to 70 °C while the melting temperature (T_m) ranges from 130 to 180 °C. PDLA and PLLA form a highly regular stereo complex with increased crystallinity. The temperature stability is maximized when a 50:50 blend is used, but even at lower concentrations of 3 - 10% of PDLA, there is still a substantial improvement. In the latter case, PDLA acts as a nucleating agent, thereby increasing the crystallization rate. Because of higher crystallinity degree the biodegradation rate of PDLA is lower as compared to that of PLA. PDLA has the useful property of being optically transparent. The polymerization of a mixture of both L and D forms of lactic acid leads to the synthesis of poly-DL-lactide (PDLLA) which is amorphous. Other mechanical and physical properties of PLA are shown in Tab. 1 and Tab. 2. Copolymerization of lactide with other monomers like glycolide or caprolactone can significantly enhance the properties and broaden the use of polylactide [23, 33].

Tab. 1: Mechanical properties of PLA [33]

Properties	L-PLA	D,L-PLA
Yield Strength (MPa)	70	53
Tensile Strength (MPa)	66	44
Elongation at Break (%)	100 - 180	100 - 180
Flexural Strength (MPa)	119	88
Notched Izod Impact ($J \cdot m^{-1}$)	66	18
Vicat Penetration (°C)	165	52

Tab. 2: Physical properties of PLA [34]

Properties	PLA
Molecular Weight (kDa)	100 to 300
Glass Transition Temperature (°C)	55 - 70
Melting Temperature (°C)	130 - 180
Crystallinity	10 - 40 %
Surface Energy (dynes)	38
Solubility Parameters ($J^{0.5} \cdot cm^{-1.5}$)	19 - 20.5
Heat of melting ($J \cdot g^{-1}$)	8.1 - 93.1
Specific Gravity	1.25
Melt – Index range (g/10min)	2 - 20

2.2.1.2 Synthesis

Using lactic acid, which is produced on a large scale by fermentation [35], PLA can be prepared by different means. It is noteworthy to note that PLA is the second common

biopolymer which is produces by microbial fermentation. For production of PLA from the lactic acid monomer there are two major routes; direct polycondenzation of lactic acid and ring-opening polymerization through the lactide intermediate. The first route involves the removal of water by condensation. This route yields only from low- to intermediate-molecular weight polymers mainly because of the necessity of water removal and presence of impurities [27, 34]. The second route is a ring-opening polymerization (ROP) through the lactide intermediate and it is often non-solvent process.

The corn is converted via enzymatic hydrolysis into dextrose, which is fermented into lactic acid at near neutral pH. Low molecular weight prepolymer is catalytically depolymerized to form a cyclic intermediate dimer, referred to as lactide which is then purified by distillation to “polymer grade”. The purified lactide as a monomer is polymerized by ROP in a bulk (solvent free) and obtained polymer is processed into the pellets. Various routes of synthesis of PLA are shown in Fig. 7 [36, 37].

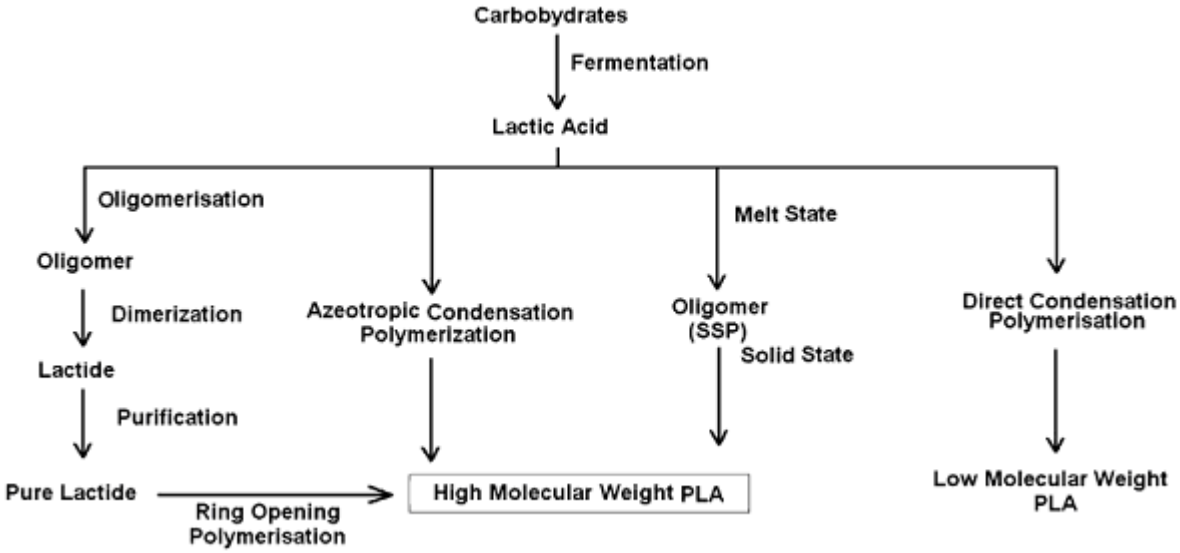


Fig. 7: Various routes of synthesis of PLA [36].

2.2.2 Glycolide and polyglycolide

Polyglycolide (Fig. 8) or polyglycolic acid (PGA) is a biodegradable, thermoplastic polymer and also the simplest linear, aliphatic polyester. It can be prepared starting from glycolic acid by either polycondensation or ring-opening polymerization. PGA has been known since 1954 as a tough fiber forming polymer, however, owing to its hydrolytic instability its use has been limited initially. Polyglycolide is a biodegradable polymer that degrades through hydrolysis of its ester bonds. Use of this material has been authorized for the production of implantable medical devices and resorbable sutures. Degradation of the polymer results in the production of glycolic acid which is not toxic for the organism and is resorbed from surrounding tissues, metabolized and excreted as water and carbon dioxide [38].

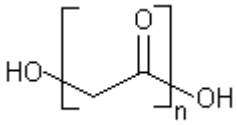


Fig. 8: Polyglycolide [30].

2.2.2.1 Properties and characterization

Polyglycolide $(C_2H_2O_2)_n$ is non-toxic aliphatic polyester with a melting point of 225-230 °C, glass transition temperature between 35-40 °C and density of 1.530 $g \cdot mL^{-1}$. Its high crystallinity (around 45-55 %) results in insolubility in water. The solubility properties of this polyester are somewhat unique; its high molecular weight form is insoluble in almost all common organic solvents (acetone, dichloromethane, chloroform, ethyl acetate, tetrahydrofuran), while low molecular weight oligomers sufficiently differing in their physical properties are more soluble. However, polyglycolide is soluble in highly fluorinated solvents like hexafluoroisopropanol and hexafluoroacetone sesquihydrate that can be used to prepare solutions of the high molecular weight polymer for melt spinning and film preparation [38, 39].

2.2.2.2 Synthesis

Polyglycolide can be obtained through several different processes starting with different materials as polycondensation of glycolic acid, ring-opening polymerization of glycolide, solid-state polycondensation of halogenoacetates and acid catalyzed reaction of carbon monoxide and formaldehyde. The most common synthesis used to produce a high molecular weight polymer is ring-opening polymerization of glycolide (see Fig. 9). Low molecular weight PGA can be prepared by heating under reduced pressure and, collecting the dieters by distillation. Ring-opening polymerization of glycolide can be catalyzed using different catalysts (e.g tin(II) 2-ethylhexanoate, antimony trioooxide, zinc lactate etc.) [40].

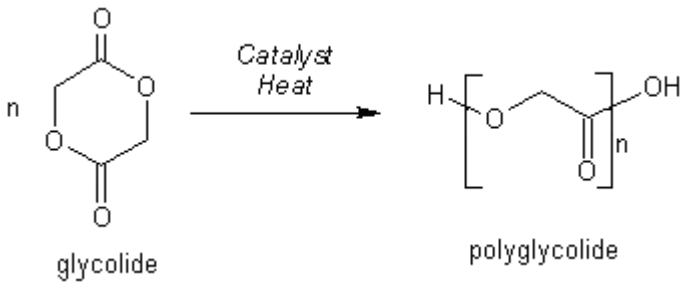


Fig. 9: Synthesis of PGA [41].

2.2.3 PLGA copolymers

Poly (lactic-*co*-glycolic acid) (Fig. 10) copolymers were among the few synthetic polymers approved for human clinical use in recent years since 1966. PLGA copolymers

can be easily processed into the desired configuration and their physical, chemical, mechanical, and degradation properties can be engineered to fit a particular need. The rate of polymer degradation may affect many cellular processes, including cell growth, tissue regeneration, and host response. The degradation products are endogenous compounds (lactic and glycolic acid) and as such are nontoxic. The presence of ester linkages in the polymer backbone allows gradual hydrolytic degradation - resorption. The rate of degradation can be controlled by the ratio of PGA and PLA.

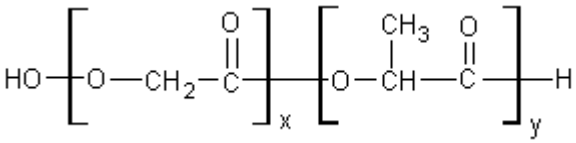


Fig. 10: Building units of poly(lactic-co-glycolic acid).

High-molecular weight PLGA copolymer is commonly prepared by ring-opening polymerization of the cyclic dimeric selfcondensation products of lactic and glycolic acids, i.e., dilactide and diglycolide in the presence of catalysts [42].

PLGA has been shown to undergo bulk erosion through hydrolysis of the ester bonds and the rate of degradation depends on a variety of parameters including the PLA/PGA ratio, molecular weight, and the shape and structure of the matrix. The major popularity of these copolymers can be attributed for use in humans, its good processability which enables fabrication of a variety of structures and forms and controllable degradation rates. PLGA demonstrates good cell adhesion and proliferation making it a potential candidate for tissue engineering. Various studies have been performed so far using micro- and nano-fabrication techniques to form three-dimensional scaffolds based on PLGA [43].

2.2.3.1 Properties and characterization

Polyglycolide has high crystallinity, a high melting point, and very poor solubility. If we copolymerize glycolide and lactide, the product PLGA should exhibit better properties than PLA and PGA. Summarization of properties and applications of PGA, PLA and PLGA is shown in Tab. 3.

PLGAs usually exhibit lower crystallinities and T_m values. The degradation characteristics of the PLGA could be adjusted by controlling the ratio of LA to GA in the feeding dose. For example, a copolymer of 50 % glycolide and 50 % D,L-lactide degrades faster than either homopolymer. The degradation rate of PLGA is higher than that of PLA and can be increased by increasing the PGA content. However, the solubility and toughness of the copolymer is limited by the composition [15].

Tab. 3: Properties and applications of PGA, PLA and PLGA [15]

Polymer	Crystallinity	T_g (°C)	Degradation Rate[a]	Typical applications
PGA	Highly crystalline (T_m = 225-230 °C)	35 - 40	2-3 months	Suture, Soft anaplerosis
PLA (L form)	Semicrystalline (T_m = 173-178 °C)	60 - 65	> 2 years	Fracture fixation, Ligament augmentation
PLA (D, L form)	Amorphous	55 - 60	12-16 months	Drug Delivery System
PLGA	Amorphous	45 - 55	1-6 months [b]	Suture, Fracture fixation, Oral implant, Drug delivery microsphere

[a] Rate depends on molecular weights of the polymers.
[b] Rate may change according to the ratio of LA and GA.

2.2.4 Polyethylene glycol

Poly(ethylene glycol) (PEG), also known as poly(ethylene oxide) (PEO), poly (oxyethylene) (POE) and polyoxirane, is a hydrophilic, non-ionic polymer. It is chemical structure is shown in Fig. 11. It is soluble in water via hydrogen bonding interactions. PEG is known for its biocompatibility and low toxicity. It has been approved by the FDA for internal consumption [44]. It can make a surface highly resistant to biological fouling, and can reduce protein adsorption and resistance to bacterial and animal cell adhesion. It is also apparently not readily recognized by the immune system. Since it is very well soluble in water and many organic solvents, it can also be readily eliminated from the body by body fluids. Modifying proteins with PEG have been shown to reduce the immunogenicities and antigenicities of these proteins and to increase circulation times [23, 45].

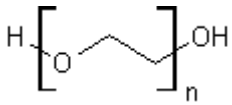


Fig. 11: The chemical structure of poly(ethylene glycol) (PEG).

Since PEG has two hydroxyl groups as reactive ends, we chose to use it as the macromonomer to improve the hydrophilicity and the biocompatibility of our synthesized copolymer.

2.2.4.1 Properties and characterization

PEGs having molecular weight less than 1000 g·mol^{-1} are viscous, colorless liquids; higher molecular weight PEGs are waxy, white solids. The melting point of the solid is proportional

to molecular weight, approaching a plateau about 67 °C. The molecular weights commonly used in biomedical and biotechnical applications range from a few hundred to approximately 20 000 $g \cdot mol^{-1}$. In general usage, PEG refer to polyols of molecular weight below about 20 000, PEG refers to higher molecular weight polymer, and PEO and polyoxirane are not specific in this regard [46].

2.2.4.2 Synthesis

PEGs are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weight from 300 to 10 000 $g \cdot mol^{-1}$. It is produced by the interaction of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers. The reaction is catalyzed by acidic or basic catalysts. Ethylene glycol and its oligomers are preferable as a starting material instead of water, because they allow the preparation of polymers with a low polydispersity index. Polymer chain length depends on the ratio of reactions components.

High-molecular PEG is synthesized by suspension polymerization. It is necessary to maintain the growing polymer chain in solution in the course of the polycondensation process. The reaction is catalyzed by magnesium-, aluminium- or calcium-organometallic compounds. To prevent coagulation of polymer chains from solution, chelating additives such as dimethylglyoxime are used. Alkali catalysts such as NaOH, KOH or Na_2CO_3 are used to prepare low-molecular PEG [47].

2.2.5 PLGA-PEG copolymers

Block copolymers consisting of a hydrophobic polyester segment and a hydrophilic PEG segment have attracted large attention due to their biodegradability and biocompatibility. A wide variety of drug formulations, such as micro/nano-particles, micelles, hydrogels, and injectable drug delivery systems have been developed using PLGA-PEG block copolymers. The biodegradation rate and hydrophilicity of block copolymers can be modulated by adjusting the ratio of its hydrophilic and hydrophobic constituents. Usually, PLGA-PEG block copolymers have shown quite different properties when compared to each constituting polymer. Various kinds of block copolymers can be classified according to their block structure as AB diblock, ABA, or BAB triblock, multi-block, branched block, star-shaped block, and graft block copolymers, in which A is a hydrophobic block (e.g. PLGA) and B is a hydrophilic PEG block [15].

Amphiphilic PLGA-PEG block copolymers from micelles composed of a hydrophobic PLGA core and hydrophilic PEG shell in water shown in Fig. 12. Hydrophobic blocks are segregated from the aqueous exterior to form an inner core surrounded by a palisade of hydrophilic segments. Block copolymer micelles are water soluble, biocompatible nanocontainers in the size of 10-100 nm with proven efficacy of delivering hydrophobic drugs. The size and morphology of block copolymer micelles can be easily changed by adjusting the chemical composition, total molecular weight, and ratio of the block lengths.

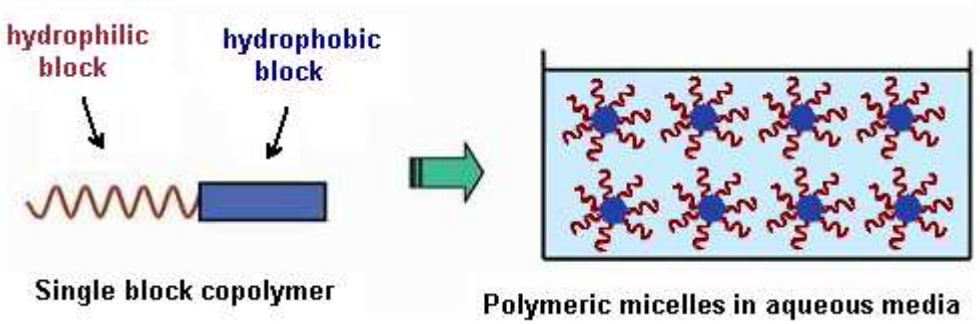


Fig. 12: Self-assembly of amphiphilic block copolymers to a micellar structure in aqueous solution [15].

2.2.6 Itaconic acid and itaconic anhydride

Originally itaconic acid (Fig. 13a)) was synthesized in 1836 by Baup as a thermal decomposition product of citric acid. Itaconic anhydride (Fig. 13b)) was prepared by heating itaconic acid in 1880. Itaconic acid has been prepared by the distillation of citric acid. The name itaconic was devised as an anagram of aconitic [48]. It can copolymerize with other monomers to prepare synthetic latex, emulsion coating, leather coating and other electrical appliances to improve adhesion, color and weather resistance. Because of its unique structure and characteristics, itaconic acid and its ester are useful materials for bioindustry and are frequently used in medicine.

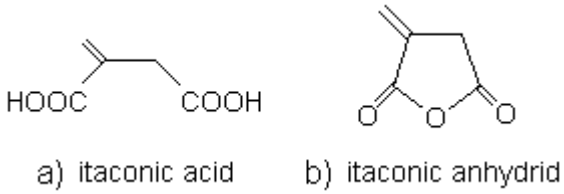


Fig. 13: a) Itaconic acid (IA), b) Itaconic anhydride (IAH).

2.2.6.1 Properties and characterization

Itaconic acid ($C_5H_6O_4$), with synonym name methylenesuccinic acid, is an unsaturated dicarboxylic acid, white crystalline, relatively non-toxic compound with a melting point of 167 - 168 °C, boiling point 268 °C and density of 1.632 $g \cdot mL^{-1}$. The property that makes itaconic acid as uniquely valuable compound is the conjunction of the two carboxyl groups by methylene group. These ionisable groups, with different pKa values, can form hydrogen bonds. The methylene group is able to take part in additional polymerization giving rise to polymers with many free carboxyl groups that confer advantageous properties.

Itaconic acid (IA) is very hydrophilic and is expected to show high biocompatibility because of its natural source. IA easily copolymerizes and provides polymer chains with carboxylic side groups, which are highly hydrophilic and able to form hydrogen bonds with corresponding groups. Small amounts of itaconic acid comonomer in the gel network generally introduce pH sensitivity and increase the degree of swelling. In addition,

incorporation of comonomers which can contribute to H-bonding can increase the mechanical strength of the hydrogel [49].

Itaconic anhydride, with synonym name 2,5-furandione, crystallizes from benzene as monoclinic crystals with T_m of 68-69 °C. Four molecules are in the unit cell, which has the dimensions: a, 7.54 Å; b, 5.16 Å; c, 12.19 Å; α and γ, 90°; β, 103°6'. Dissociation constants in water at 25 °C are: K_1, 1.40×10^{-4}; K_2, 3.56×10^{-6} [50].

The extent of ionization of itaconic acid below pH = 3 is negligible. Concentration of the monoanion is at a maximum pH of 4-5. Above pH = 8 the acid is completely ionized. Itaconic anhydride hydrolyzes completely at 25 °C in water at a rate comparable to the rate reported for succinic and methyl succinic anhydride under the same conditions [50].

2.2.6.2 Synthesis

Itaconic anhydride can be made by heating itaconic acid and by distillation of citric acid monohydrat at 175-190 °C with the yield of 37-47 % of the theoretical amount. This reaction is shown in Fig. 14 [48].

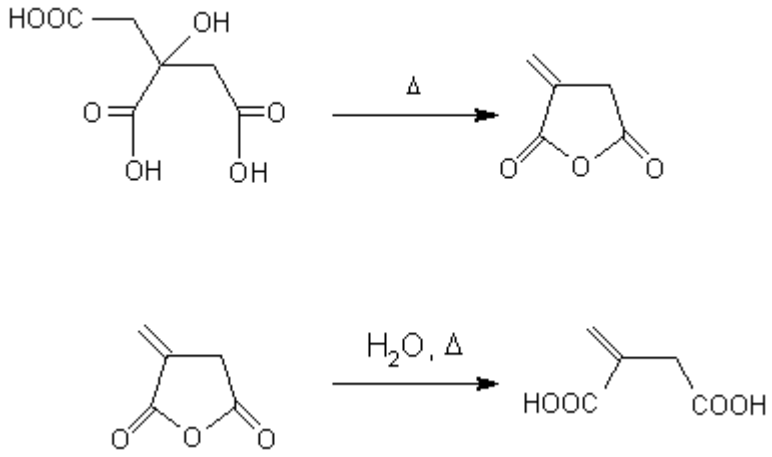


Fig. 14: Synthesis of itaconic anhydride and itaconic acid [48].

Itaconic anhydride can be synthesized in good yield from itaconic acid by reaction of the later with thionyl chloride, phosphorous pentoxide, acetyl chloride or acetic anhydride. In turn itaconic acid has been prepared commercially by fermentation of carbohydrates by *Aspergilus terreus.* Using molasses, glucose, or raw cane as source the of the carbon framework, the yield of the reaction depends on pH, strain of *A. terreus*, and temperature. It has been proposed that the citric acid undergoes dehydration forming the aconitic acid, which then forms the itaconic anhydride by decarboxylation. This reaction is shown in Fig. 15.

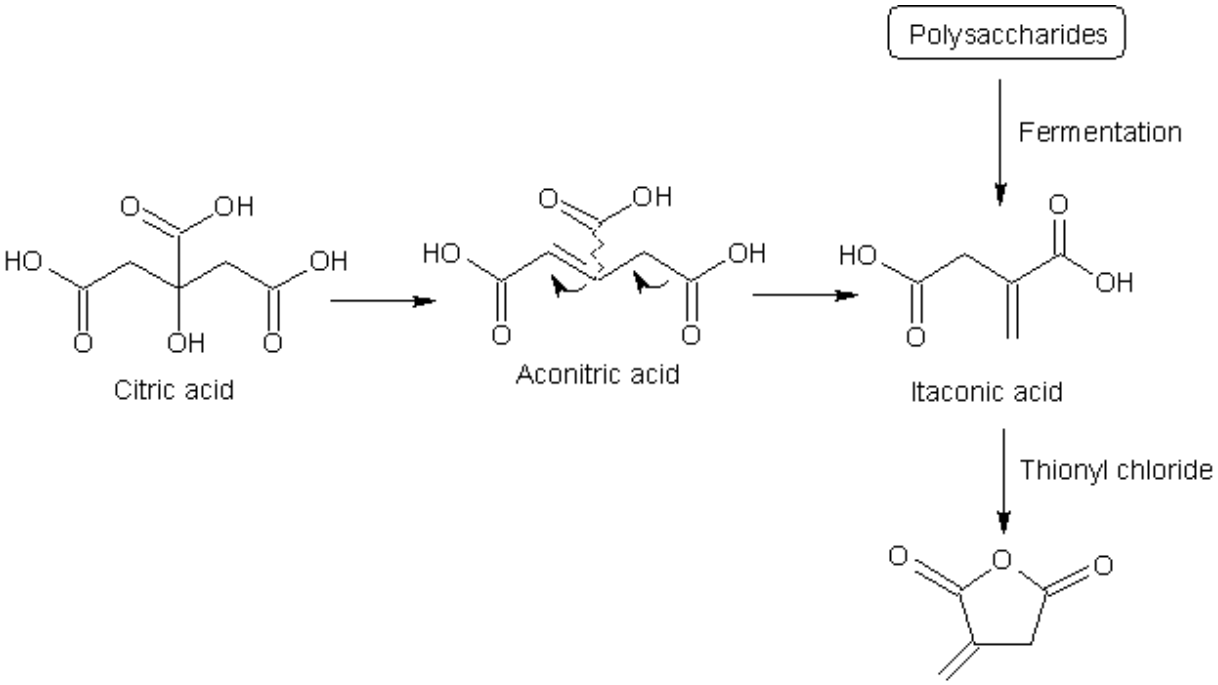


Fig. 15: Deriving itaconic anhydride from renewable sources.

Next way to prepare of itaconic anhydride is exchange between itaconic acid and acetic anhydride using the conventional processes of transanhydridation. This process has the disadvantage of using a raw material which is expensive, namely acetic anhydride, with the formation of a by-product which must be eliminated, i.e., acetic acid. The reaction is further limited as to the temperature which can be used to avoid initiating a polymerization reaction. A temperature below 75 °C is essential. The yields obtained by this process do not exceed 90 % of itaconic anhydride [51].

Itaconic acid can by prepared by the distillation of citric acid, of aconitic acid, and of itamalic acid; by heating citric acid with dilute sulfuric acid in a closed tube; by treating aconitic acid with water at 180° C; by heating citraconic acid with sodium hydroxide; by heating citraconic anhydride with water at 150 °C; by heating a concentrated solution of citraconic acid at 120 - 130 °C in a sealed tube. Itaconic acid is also produced by starch hydrolysate by *Aspergillus terreus* and by the action on fungus *Aspergillus itaconicus* on cane sugar [52, 53].

2.2.6.3 Toxicity

Itaconic acid (IA) and itaconic anhydride are relatively innocuous materials. However, studies observing toxicity of itaconic acid prove dependence on decrease in growth rate of the weight of rats after the intake of IA. The toxic effects of continued oral administration of itaconic acid were studied by the drug-diet method (Fig. 16). In the chronic feeding experiments there was a define decrease in growth rate as the intake of itaconic acid was increased, compared to the controls. The rats were fed diets containing varying amounts (range from 9.7 to 10.9 g of the acid per day) for a period of 210 days and showed no significant toxic effects other than restricted growth [54].

Only in the dose 0.125 and 0.25 % itaconic acid there was a divergence. With increasing dose the body weight slightly rose. In rats ingesting diet containing 1.0 or 2.0 % of itaconic acid there was a statistically significant inhibition of growth. Postmortem examination for gross pathological changes did not reveal any specific abnormalities in heart, lung, liver, spleen, kidney, adrenal, pancreas, thyroid and ovary. Other than a few isolated inflammatory lung changes, no significant lesions were found [54].

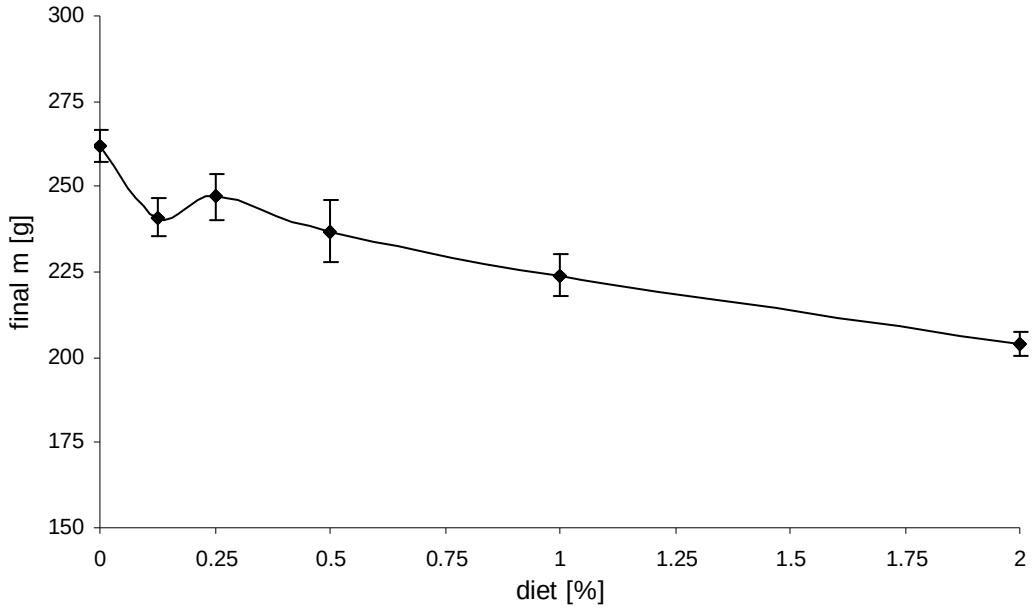


Fig. 16: Food consumption and statistical evaluation body weights of rats.

2.2.6.4 Thermal stability

Thermal stability of itaconic anhydride and itaconic acid was not overly studied. Gawron O. and Mahajan K. P described facile thermal isomerization of itaconic anhydride to citraconic anhydride [46]. Itaconic anhydride at 210 - 215 °C isomerizes and distills out as methylmaleic anhydride (Fig. 17) [55].

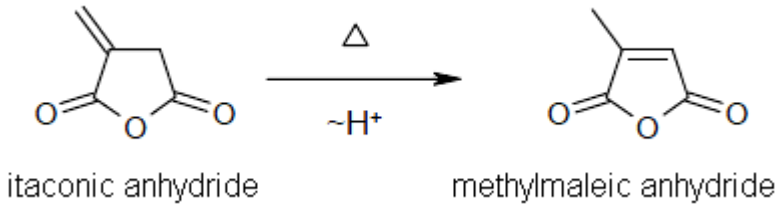


Fig. 17: Transformation itaconic anhydride to methylmaleic anhydride.

2.2.6.5 Copolymers functionalized by itaconic anhydride

A few years ago, introduced multicomponent biodegradable amphiphilic hydrogels based on PEG and polycaprolactone were modified by itaconic acid (ITA) to achieve end-functional reactive double bonds and carboxylic acid groups [50, 56].

Recently, PLGA-PEG-PLGA triblock copolymers functionalized with itaconic anhydride have been prepared by our group [57]. While the synthesis of ITA/PLGA-PEG-PLGA/ITA (Fig. 18) was successful the degree of functionalization together with optimal reaction conditions were not studied.

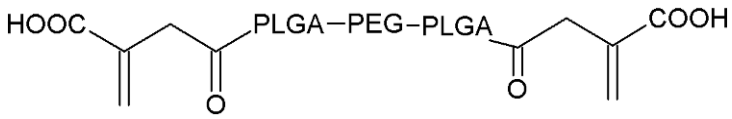


Fig. 18: Copolymer of ITA/PLGA-PEG-PLGA/ITA.

Resulting biodegradable ITA/PLGA-PEG-PLGA/ITA copolymers are light, temperature and pH sensitive and can be cross-linked either chemically by covalent bonding though the double bonds or physically by ionic interactions in order to produce new functionalized hydrogel network. Moreover, functional carboxylic groups can be used as coupling sites for increasing hydrogel's biocompatibility, bioinductivity, adhesion or other physical properties and hence can be applied as injectable polymer drug delivery systems, tissue implants or resorbable bone adhesives.

3 EXPERIMENTAL PART

3.1 Chemicals

- Itaconic anhydride (> 97 %) were purchased from Fluka, Switzerland and used:
  a) As received.
  b) Sublimated under vacuum at 70 °C and dissolved in dry distilled THF in order to get concentration of 2708 mmol·L^{-1}.
  c) Sublimated under vacuum at 70 °C and used in solid state.
- Poly(ethylene glycol) (M_n = 1500 g·mol^{-1}) was purchased from Sigma – Aldrich, Germany.
- D, L – lactide (≥ 99.9 %) was purchased from Polysciences inc., Pennsylvania and used:
  a) As received.
  b) Sublimated under vacuum at 70 °C.
- Glycolide (≥ 99.9 %) was purchased from Polysciences inc., Pennsylvania and used:
  a) As received.
  b) Sublimated under vacuum at 60 °C.
- Tetrahydrofuran (THF, p.a.) were purchased from Lach-Ner s. r. o., CZ and vacuum distilled prior the use from sodium/benzophenone.
- Sn(II)2-ethylhexanoate (95 %) (Sn-octoate) were purchased from Sigma – Aldrich, Germany and used:
  a) As received.
  b) Dissolved in dry distilled THF in order to get 17 mmol·L^{-1} solutions.
- MilliQ water was prepared by Milipore S.A.S. equipment at FCH BUT, CZ.
- KBr for IR sp. was purchased from Lach-Ner s. r. o., CZ.
- Acetone p. a. was purchased from Lach-Ner, s.r.o., CZ.
- Chloroform-d 99.8 Atom % D for NMR spectroscopy was purchased from ISOSAR GmbH, Germany.
- Gaseous nitrogen (99.999 %, SIAD Czech spol. s r.o.) was refined by drying column filled with molecular sieves and Cu catalyst.

3.2 Equipments

- All glass high-vacuum line (hand-made at BUT, Faculty of Chemistry)
- 500 MHz NMR spectrometer Bruker AVANCE III (Masaryk University, Faculty of Science)
- IR spectrophotometer Nicolet iS10
- Gel permeation chromatograph Agilent Technologies 1100 Series
- High performance liquid chromatograph Agilent Technologies 1100 Series
- Glove bag (Aldrich, Germany)
- Analytical scales (Mettler Toledo classic AB204S)
- Desiccators

3.3 Methods

Polymers were synthesized using vacuum line and manifold (Fig. 19) in glass reactors by Schlenk's technique under the nitrogen atmosphere.

Fig. 19: Vacuum line and manifold.

3.3.1 Synthesis of PLGA-PEG-PLGA copolymer

The PLGA-PEG-PLGA triblock copolymers (ABA type) were prepared via typical ring opening polymerization (ROP) method in a bulk. Polymer was prepared under nitrogen atmosphere in glass reactor, which was purged by nitrogen and vacuum for three times prior the reaction.

In a typical reaction, PEG (M_n = 1500, 4 mmol) as macromonomeric initiator was added to the two-neck round-bottom reaction vessel (25 mL) equipped with egg-shape stir bar. Prior the polymerization, the PEG was both degassed and dewatered at 120 or 130 °C for 6.5 up to 8 h under the vacuum. After cooling down sublimated or unsublimated D,L-lactide (83 mmol) and glycolide (28 mmol) monomers were added against the nitrogen outflow and the reaction flask was placed in an oil bath thermostated at either 120 or 130 °C. After the homogenization by stirring, Sn(II)2-ethylhexanoate (Sn-octoate, 0.1 mmol) as organic catalyst was injected by the gas-tight glass syringe in order to start up the copolymerization. Reaction proceeded for 3 up to 9 h. Different conditions and labeling of PLGA-PEG-PLGA samples are described in Tab. 4.

Tab. 4: Conditions of PLGA-PEG-PLGA copolymers synthesis.

Polymer	Temperature [°C]		Time [h]	
	PEG purification	polymerization	PEG purification	polymerization
P-1	130	130	4.5	8
P-2	130	130	6.5	9
P-3*	120	120	7.5	8
P-4	130	130	8	3
P-5*	130	130	8	3
P-6	130	130	8	3

* Sublimated glycolide and D,L-lactide.

3.3.1.1 Kinetics of polymerization

Kinetics of copolymerization was studied at runs P-2, P-4 and P-5. The conversion of monomer was determined both gravimetrically and by HPLC method. An initial aliquot (time approximately 0 min) was taken out immediately when catalyst was added. Other aliquots were withdrawn during the polymerization after particular time intervals (average weight of 165 mg for gravimetric method and 56 mg for HPLC). Total weight of withdrawn samples could not exceed 10 wt. % of the polymerization mixture amount otherwise the internal polymerization conditions changed. Samples for gravimetric method were purified prior the weighing, on the other hand, samples for HPLC were not purified just 1mL of acetonitrile was added to each sample and analyzed.

3.3.1.2 Samples purification

PLGA-PEG-PLGA copolymers were purified from unreacted monomers by dissolving in cold MilliQ water (approx. 10 wt. %) before analysis. Copolymers were separated from water solution by heating up to 80 °C, decanted and dried in vacuum oven at 30 °C until the constant weight (for approx. 12 h). Purifying was repeated three times. Prepared PLGA-PEG-PLGA triblock copolymers were precisely characterized by means of ^{1}H NMR, FT-IR and GPC analyses.

3.3.2 Synthesis of ITA/PLGA-PEG-PLGA/ITA

PLGA-PEG-PLGA copolymer was functionalized by ITA end-capping either in solution using THF as solvent or in a bulk without solvent.

3.3.2.1 ITA functionalization in a solution

The PLGA-PEG-PLGA copolymer was modified by adding an excess of ITA (2.5 molar ratio to polymer) under nitrogen atmosphere. Purified PLGA-PEG-PLGA copolymer (103 μmol) was added into two-neck glass reactor (25 mL) with stir bar and degassed by vacuum at room temperature, 60 or 75 °C for 1 hour. ITA (257 μmol) was placed into reaction vessel under the nitrogen, degassed and back-filled with nitrogen three times and left under vacuum for 30 min. Dry THF (5 mL) was added into reactor via nitrogen-purged syringes and stirred. Subsequently, the reaction flask was placed in an oil bath thermostated at 23 - 75 °C. After the homogenization Sn-octoate (2.3 μmol) was added and the reaction proceeded for 24 hours under the nitrogen atmosphere and condenser (reflux), if needed. Different conditions and labeling of ITA/PLGA-PEG-PLGA/ITA samples are described in Tab. 5. Prepared ITA/PLGA-PEG-PLGA/ITA copolymers were purified and characterized as mentioned above (3.3.1.2).

Tab. 5: Conditions of ITA functionalization in solution.

Polymer	ABA type	ITA	Temp. [°C]	Time [h]	Note
I-23	P-1	as received	23	24	
I-23S	P-1	sublimated	23	24	
I-40	P-1	as received	40	24	
I-40S	P-1	sublimated	40	24	
I-60	P-1	as received	60	24	reflux
I-60S	P-1	sublimated	60	24	reflux
I-75	P-2	as received	75	24	reflux
I-75S	P-2	sublimated	75	24	reflux

Sample's labeling: I = ITA, number = temp. of reaction, S = sublimated.

3.3.2.2 ITA functionalization in a bulk

Functionalization of the PLGA-PEG-PLGA copolymer with ITA in a bulk proceeded in two ways. One way was the same as the ITA functionalization in THF solution (see 3.3.2.1) just without the solvent addition at the temperature of 155 °C in a vacuum or under the nitrogen atmosphere ("two-pot" reaction). Different conditions and labeling of these ITA/PLGA-PEG-PLGA/ITA samples are described in Tab. 6.

The second way of ITA functionalization proceeded as "one-pot" reaction in two steps. Firstly, the ABA copolymer was prepared as mentioned in 3.3.1 and subsequently modified in second step by ITA after the cooling down the reaction temperature. The sublimated ITA (2.5 molar ratio to polymer) was added under the nitrogen atmosphere. Since there has already been Sn-octoate catalyst in the reactor from ABA copolymerization, no extra catalyst was added. Functionalization proceeded without solvent at 110 - 155 °C for 2 - 8 h. Different

conditions and labeling of these ITA/PLGA-PEG-PLGA/ITA samples are described in Tab. 7.

All prepared ITA/PLGA-PEG-PLGA/ITA copolymers were purified and characterized as mentioned above (3.3.1.2).

Tab. 6: Conditions of ITA functionalization in a bulk as “two-pot” reaction.

Polymer	ABA type	atmosphere	Temperature [°C]	Time [h]
I-155S(vac)	P-1	vacuum	155	8
I-155S	P-1	nitrogen	155	8

Tab. 7: Conditions of ITA functionalization in a bulk as “one-pot” reaction.

Polymer	Temperature [°C]		Time [h]	
	Polymerization	Functionalization	Polymerization	Functionalization
1-I-110S	120	110	8	8
2-I-110S	130	110	3	2
I-115S	120	115	8	8

Sample 1-I-110S (sublimated ITA, 110 °C, 8 h) was studied in term of functionalization kinetics by ^{1}H NMR method. The procedure was same as described in 3.3.1.1 except that an initial sample (time 0 min) was taken out immediately when ITA was added.

3.4 Characterization of copolymers

3.4.1 Nuclear Magnetic Resonance (NMR)

Molecular weight and polymer characterization results were confirmed using ^{1}H NMR spectroscopy on 500 MHz Bruker AVANCE III instrument using 128 scans in $CDCl_3$ solvent.

3.4.2 Fourier Transformed Infra-Red spectroscopy (FT-IR)

IR spectra of triblock copolymers and chemical functionalization with ITA were confirmed by FT-IR using Nicolet iS10 FTIR Spectrometer in range of 4000 - 400 cm^{-1} via common KBr pellets technique.

3.4.3 Gel Permeation Chromatography (GPC)

Number average molecular weights (M_n) and polydispersity index (M_w/M_n) of the copolymers were determined by GPC method using Agilent Technologies 1100 Series instrument equipped with isocratic pump, autosampler, RI and UV-vis detector, fraction

collector, column thermostat up to 80 °C and 300 x 7.5 mm PLgel 5 μm MIXED B;C column with THF as the eluent at a flow rate of 1 mL·min^{-1} against linear polystyrene standards.

3.4.4 High Performance Liquid Chromatography (HPLC)

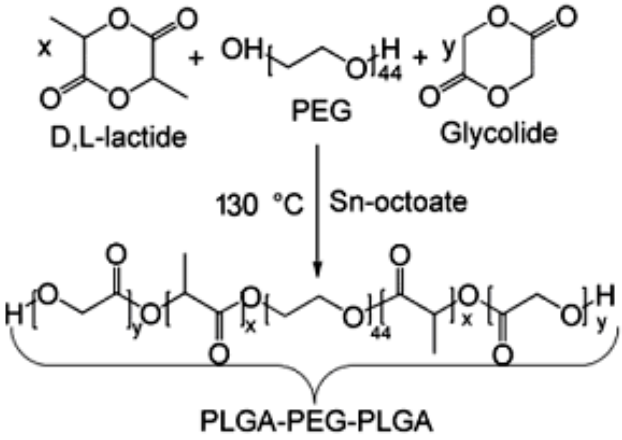
Monomer conversion was determined by HPLC method using Agilent Technologies 1100 Series instrument equipped with isocratic pump, autosampler, UV-VIS diode array detector, fraction collector, column thermostat up to 80°C and 150 x 4.6 mm; 5 μm Eclipse XDB C18 column with isocratic H_2O : acetonitrile (80:20) eluent as the mobile phase at a flow rate of 1 mL·min^{-1}, temperature of 30 °C and wavelength of 210 nm.

4 RESULT AND DISCUSSION

In this work, the novel approach for synthesis of biodegradable thermosensitive PLGA-PEG-PLGA copolymers both-end terminated with itaconic acid bringing both carboxyl groups (-COOH) and double bonds (-CH=CH_2) was utilized. Firstly, PLGA-PEG-PLGA copolymer was synthesized via ring-opening polymerization in a bulk followed by ITA functionalization. Synthesis of PLGA-PEG-PLGA copolymer was carried out in a range of different temperatures and times of polymerization in order to optimize the physical conditions with a view of first-order kinetics, predicted M_n, narrow polydispersity and high initiator efficiency. Functionalization of PLGA-PEG-PLGA copolymer with ITA proceeded either in solvent or in a bulk at different temperatures in order to find optimal conditions for linking as much as higher amount of ITA to the end of ABA copolymer. The effect of monomer and ITA purification prior the use on the polymer characteristics was monitored as well.

4.1 Synthesis of PLGA-PEG-PLGA copolymer

PLGA-PEG-PLGA triblock copolymers were synthesized via ring-opening polymerization of D,L-lactide with glycolide using poly(ethylene glycol) as macroinitiator and Sn-octoate as organometallic catalyst (Scheme 1). Reaction proceeded in a bulk and the prepared copolymers after purification were characterized by GPC, ^{1}H NMR and FT-IR.



Scheme 1: Synthetic route to PLGA-PEG-PLGA triblock copolymers.

4.1.1 Characterization by GPC

GPC results were used as a qualitative tool to check the peak shape and size distribution of polymers. Fig. 20 shows GPC chromatograms of PLGA-PEG-PLGA copolymers prepared in a bulk. All obtained elution curves were symmetrical and unimodal. There was found almost little change among the polymer samples from neat monomers. On the other hand samples (P-3, P-5) from sublimated monomers displayed some unique characteristics; sample P-3 (120 °C for 8 h) shows the highest M_n (8680) and the sample P-5 (130 °C for 3 h) has the lowest polydispersity index (1.20).

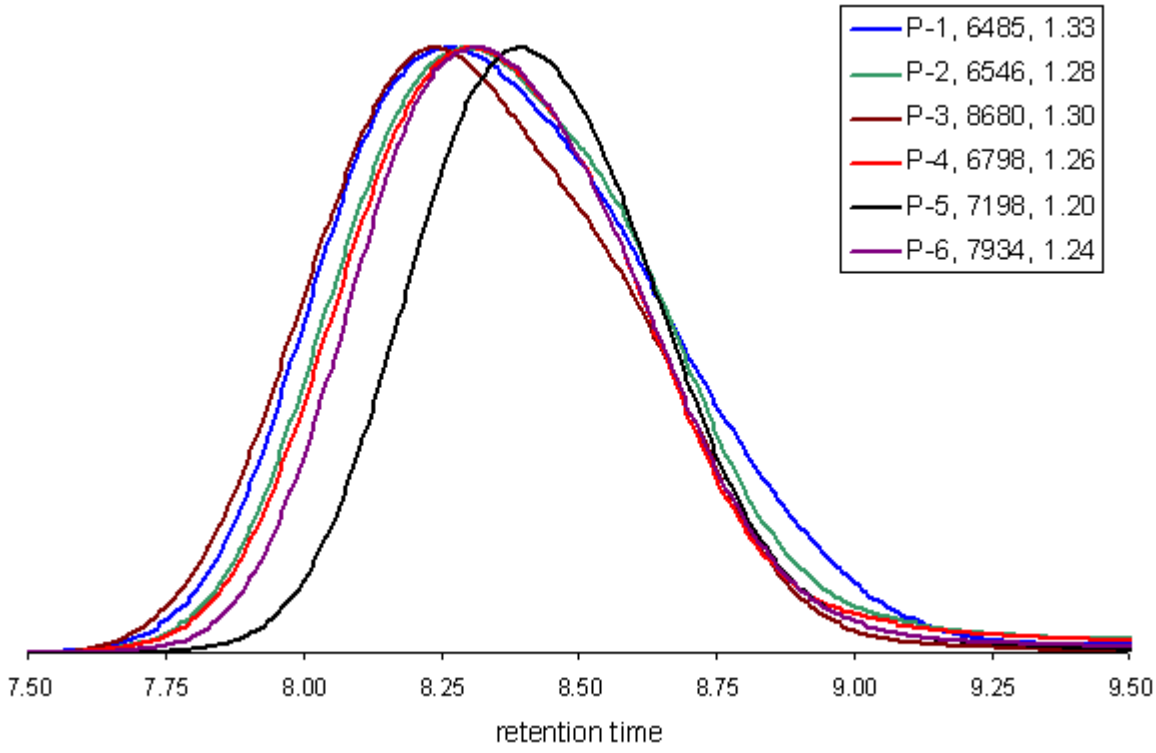


Fig. 20: GPC diagram of PLGA-PEG-PLGA copolymers.

Molecular weight's properties of prepared copolymer are shown in Tab. 8. Individual values of molecular weight's and polydispersity index are approximately identical. Just samples polymerized for only 3 h (P-4, P-5 and P-6) show somewhat lower polydispersity index in comparison with other samples prepared for 8 h. However, the time of polymerization had no effect on the M_n of samples.

The M_n calculed from ^{1}H NMR spectra was in a very good agreement with M_n found by GPC as well as with theoretical M_n of copolymer showing the initiator efficiency ($M_{n(theor)}/M_{n(GPC)}$) higher than 60 % in all cases.

Tab. 8: Molecular weight's properties of prepared PLGA-PEG-PLGA copolymers.

Polymer	M_n (GPC) [g·mol^{-1}]	M_w/M_n (GPC)	M_n (^{1}H NMR) [g·mol^{-1}]	M_n (theoret.) [g·mol^{-1}]	$M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$
P-1	6485	1.33	4641	5250	1/0.81/1.13
P-2	6545	1.36	5143	5250	1/0.81/1.02
P-3	8680	1.30	5357	5250	1/0.60/0.98
P-4	6798	1.26	5200	5250	1/0.77/1.01
P-5	7198	1.20	4711	5250	1/0.73/1.11
P-6	7934	1.24	5021	5250	1/0.66/1.05

4.1.2 Characterization by ^{1}H NMR

All prepared PLGA-PEG-PLGA copolymers were characterized by ^{1}H NMR spectroscopy with a typical spectrum shown in Fig. 21. Characteristic peaks of lactic acid (O-(C<u>H</u>$_3$)CHO) protons were found in range between δ = 1.5 - 1.75 ppm (e) and (O-(CH$_3$)C<u>H</u>O) proton was found in range between δ = 5.1 - 5.35 ppm (a). A characteristic peak of glycolic acid (OC<u>H</u>$_2$O) proton was found in range between δ = 4.6 - 4.9 ppm (b) and peak of PEG (OC<u>H</u>$_2$C<u>H</u>$_2$O) proton in range between δ = 3.55 - 3.75 ppm (d). Molecular weight was calculated from integral of peak (a) belongs to PLA and from integral of peak (b) of PGA.

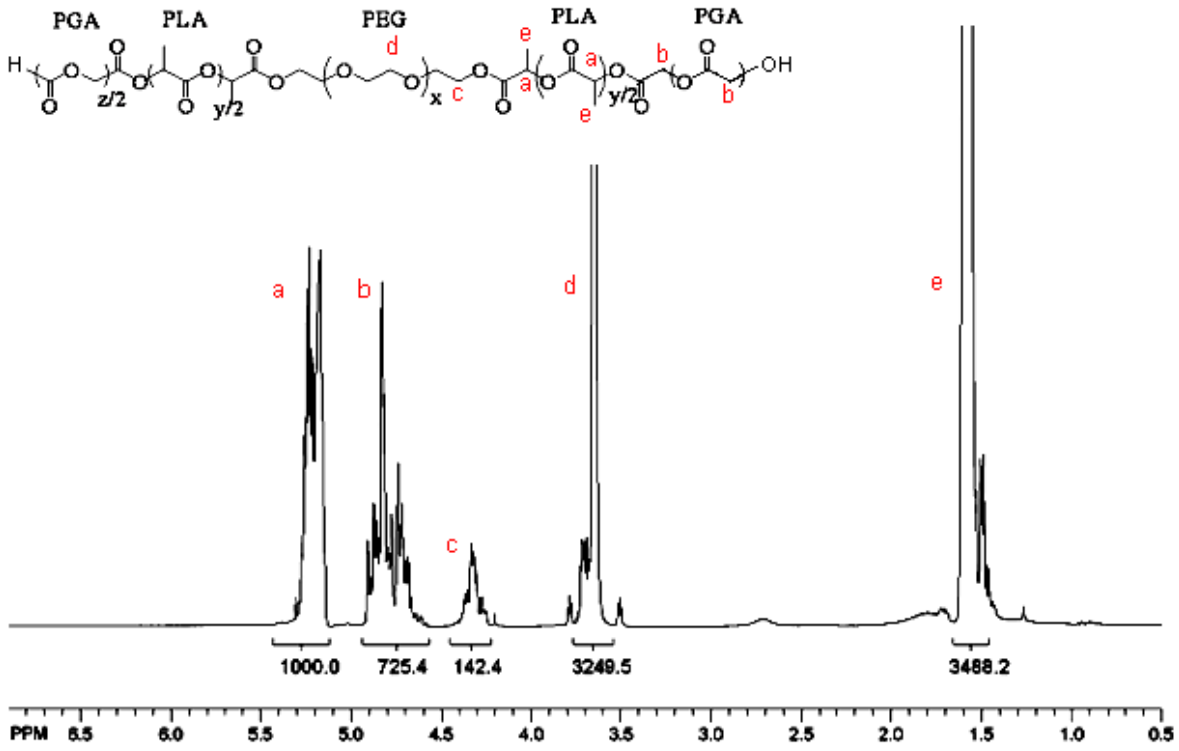


Fig. 21: Typical ^{1}H NMR spectrum of P-4 triblock copolymer.

4.1.3 Characterization by FT-IR

Infrared spectrum of PLGA-PEG-PLGA copolymer (see Fig. 22) included several characteristic groups, e.g. hydroxyl bond, ester bond, acidic and alkyl bonds. We observed above all, presence of hydroxyl functional end-groups of PLGA-PEG-PLGA at 3530 cm^{-1}.

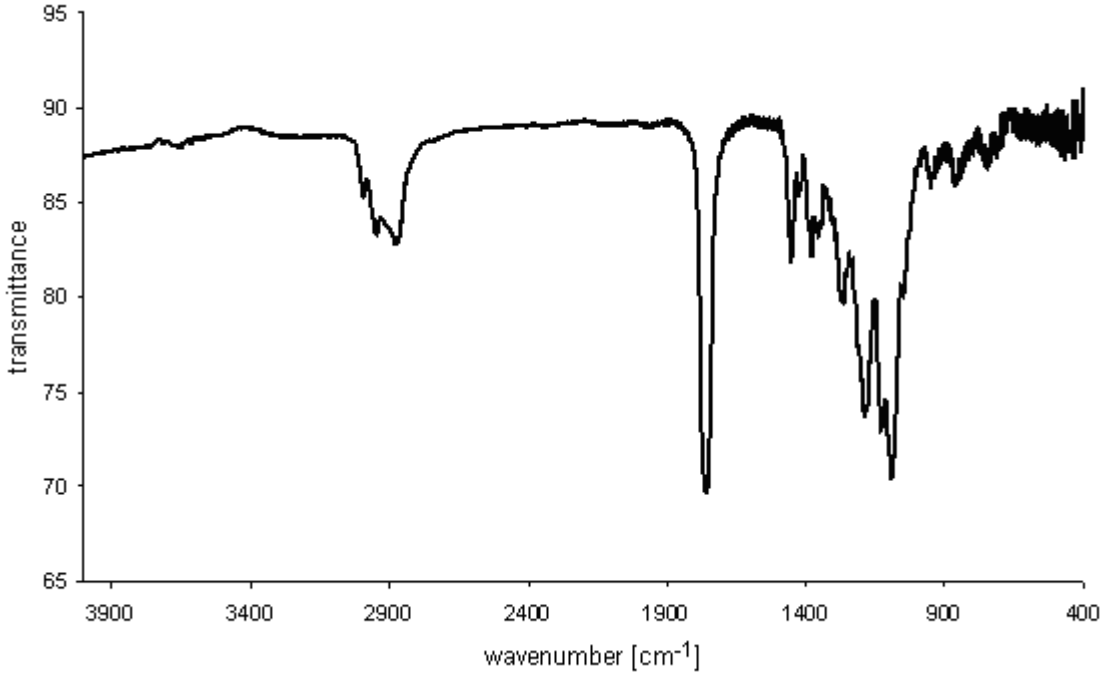


Fig. 22: Typical FT-IR spectrum for PLGA-PEG-PLGA copolymer.

4.1.4 Kinetics of PLGA-PEG-PLGA copolymer

Kinetics of polymerization of PLGA-PEG-PLGA copolymer from unsublimated (P-2, P-4) and sublimated (P-5) monomers of D,L-lactide and glycolide was investigated by both gravimetric and HPLC method. The M_n and M_w/M_n were measured by GPC of purified samples taken out for gravimetrical method.

Fig. 23 shows GPC chromatograms of aliquots taken out during synthesis of PLGA-PEG-PLGA copolymer from neat (unsublimated) monomers. As the molecular weight increases with time, the polydispersity decreases at the beginning of the reaction until 35 min up to 1.16 followed by peaks broadening. The obtained polydispersity index of synthesized copolymer in a bulk at 130 °C in 3 h was 1.26 and molecular weight was 6798 $g \cdot mol^{-1}$.

Fig. 24 shows GPC chromatograms of aliquots taken out during synthesis of PLGA-PEG-PLGA copolymer from sublimated LA and GA. Molecular weights of copolymers increased with increased time of kinetic measurement in contrast to polydispersity index, which was reduced at the beginning of the reaction and stayed very narrow until the end. The obtained polydispersity of synthesized copolymer in a bulk at 130 °C in 3 h starting from purified monomers was 1.19 and molecular weight was 7320 $g \cdot mol^{-1}$.

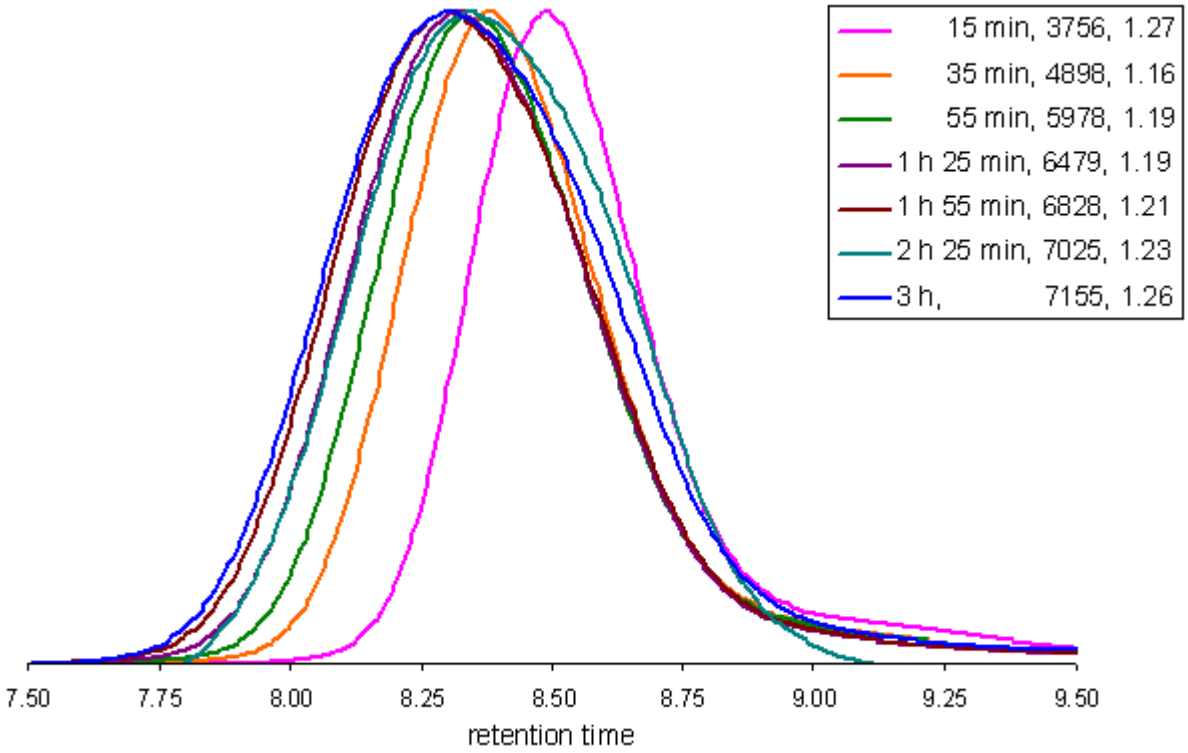


Fig. 23: GPC of PLGA-PEG-PLGA copolymer (P-4) from unsublimated PLA and PGA at each time interval proceeded for 3 h.

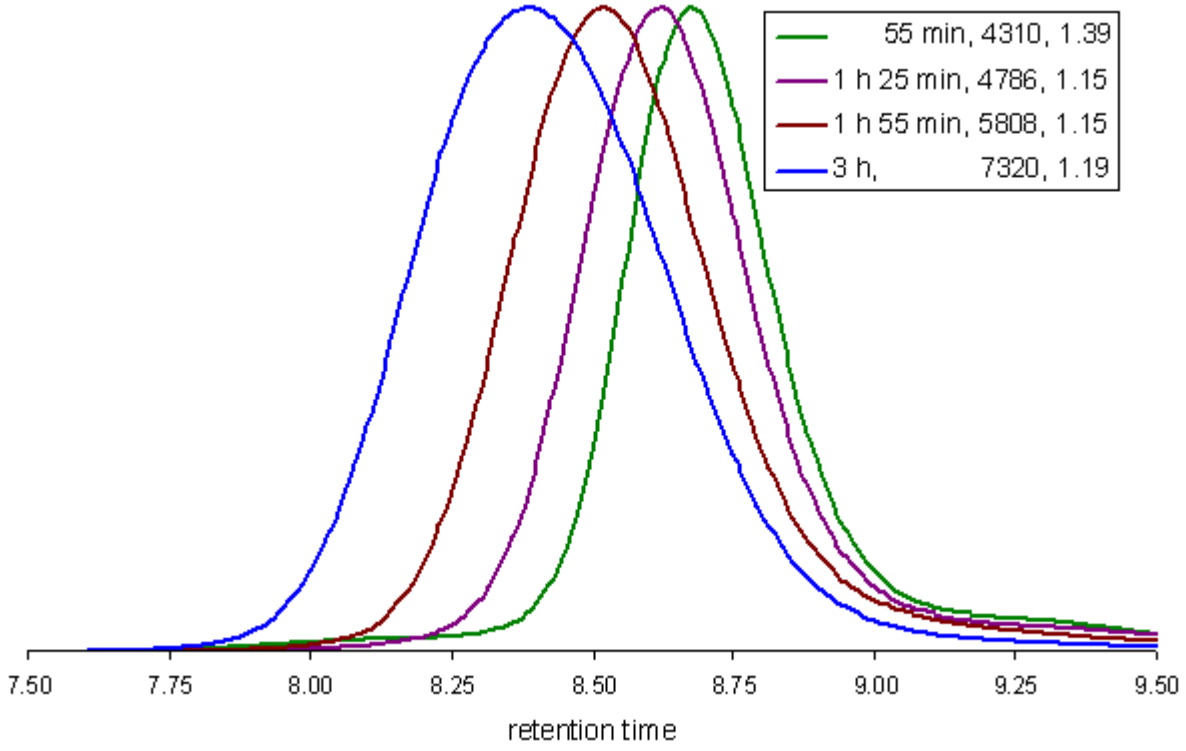


Fig. 24: GPC of PLGA-PEG-PLGA copolymer (P-5) from sublimated PLA and PGA measured after individual time intervals measured for 3 hours.

Fig. 25 shows dependence of molecular weight on conversion during polymerization of PLGA-PEG-PLGA copolymer from both unsublimated (sample P-4) and sublimated (sample P-5) LA and GA. In both cases, molecular weight increasing with conversion was almost identical and came up to 7155 g·mol^{-1} with conversion of 87 % for P-4 and 7198 g·mol^{-1} with conversion of 91 % for P-5 copolymers.

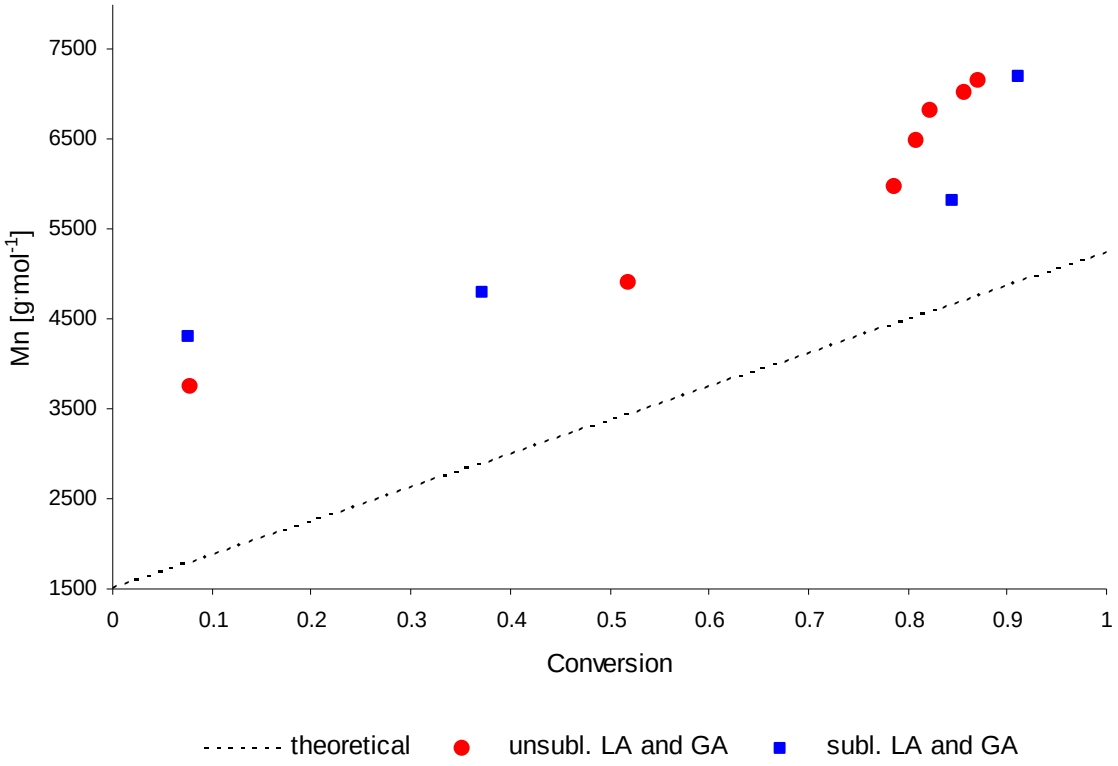


Fig. 25: Dependence of M_n on conversion for kinetic of copolymerization of unsublimated and sublimated LA and GA.

As for dependence of polydispersity on the conversion (Fig. 26) for both unpurified (P-4) and purified (P-5) monomers, it can be seen that M_w/M_n remained almost constant and low up to the conversion of 84.5 % in comparison with unsublimated monomers where the polydispersity index decreased at the beginning of polymerization and started increase from the conversion of 52.0 % resulting in possibility of some side reactions.

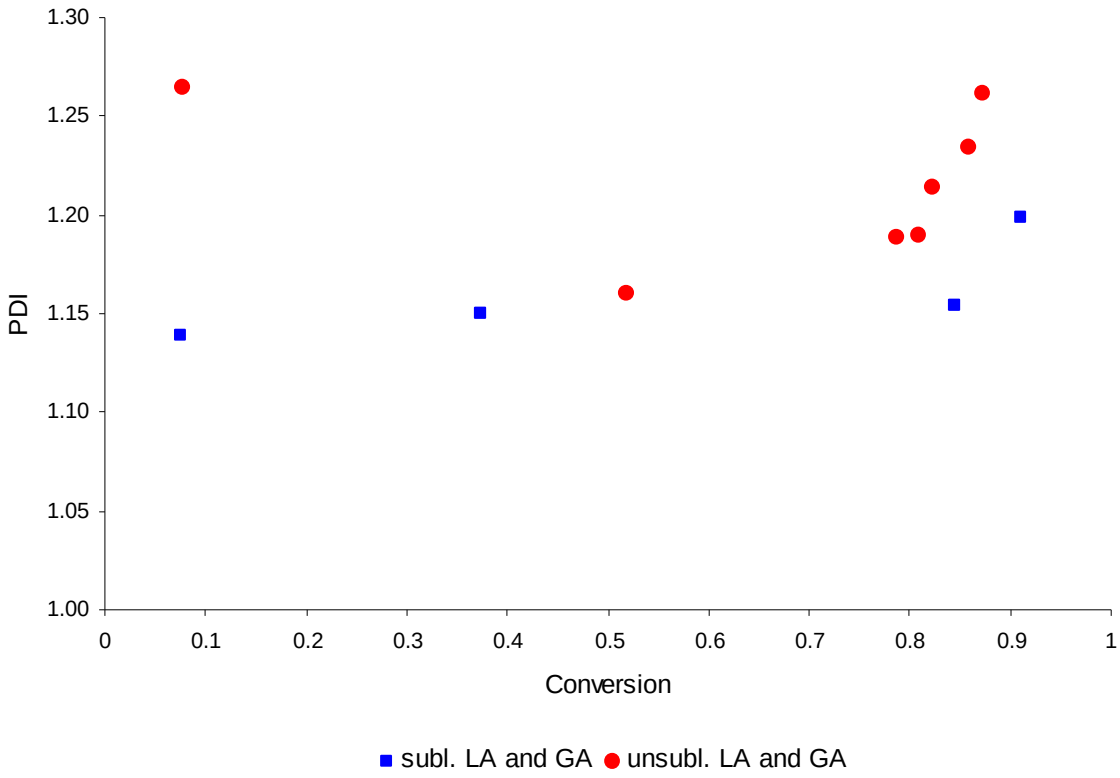


Fig. 26: Dependence of PDI on conversion for kinetic of copolymerization of unsublimated and sublimated LA and GA.

The dependence of the conversion on the time obtained under conditions mentioned above are given in Fig. 27 for unsublimated and sublimated LA determined by HPLC and in Fig. 28 for unsublimated and sublimated monomers determined by gravimetric method. In kinetics with sublimated LA determined by HPLC there was observed a nearly linear progress up to conversion of 87.2 % during first 2.5 hours followed by constant conversion independent on the time. As for kinetics with unsublimated lactide determined by HPLC, a swift increase of conversion during first 15 minutes occurred with slight growing progress up to 95.8 % within next 1 hour. Conversion was time independent after 1.5 h of polymerization.

Regarding the kinetics with sublimated monomers determined by the gravimetric method a very slight growth of conversion (up to 2 %) within first 30 min was observed followed by nearly linear growth of conversion up to 85 % (Fig. 28). After 1.5h the conversion was not changed.

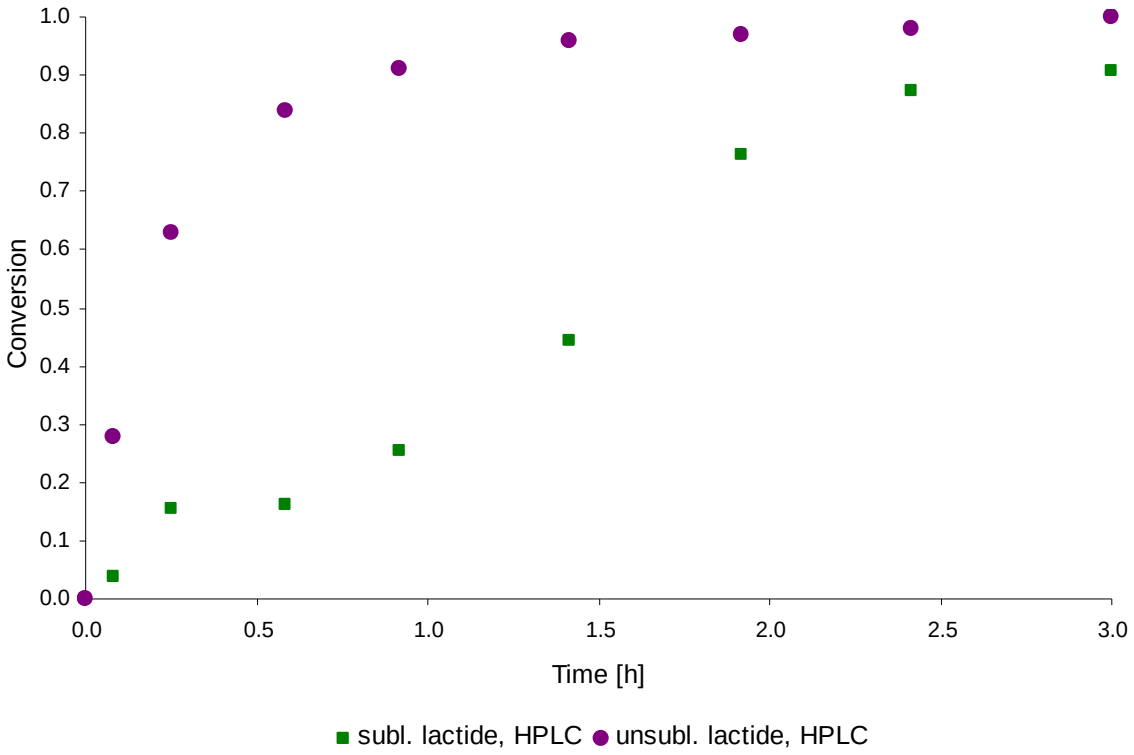


Fig. 27: Dependence of conversion on time for kinetic of copolymerization of unsublimated and sublimated LA measured by HPLC.

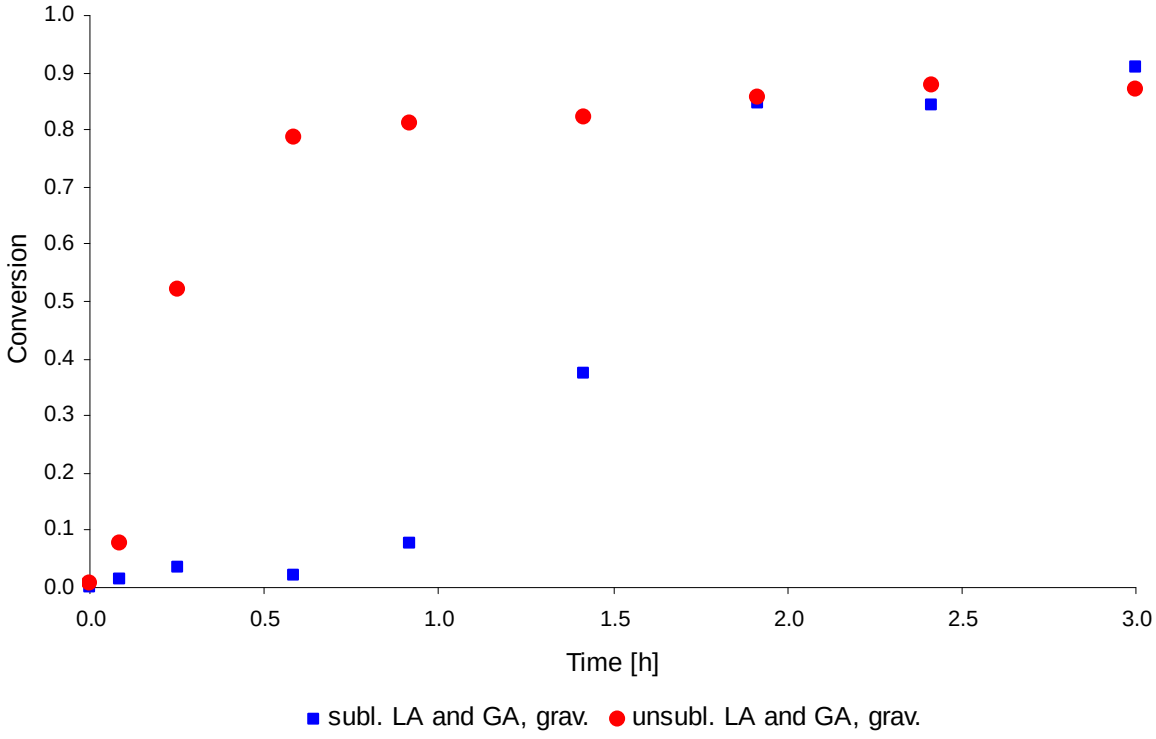


Fig. 28: Dependence of conversion on. time for kinetic of copolymerization of unsublimated and sublimated LA and GA measured by gravimetric method.

In order to see the better firs-order kinetics for both sublimated and unsublimated monomers with respect to the measured method, the logarithm of monomer concentration at the t = 0 h to monomer concentration at certain time ratio was plotted in dependence on time. There is a significant difference in the kinetics rate between the sublimated and unsublimated monomers proved almost identically by both HPLC (Fig. 29) and gravimetric (Fig. 30) method. In both cases the polymerization with unpurified monomers was much faster, however samples from sublimated monomers showed predicted molecular weight and narrow molecular weight distribution within all kinetic measurement proved elimination of side reaction and living character of copolymerization reaction.

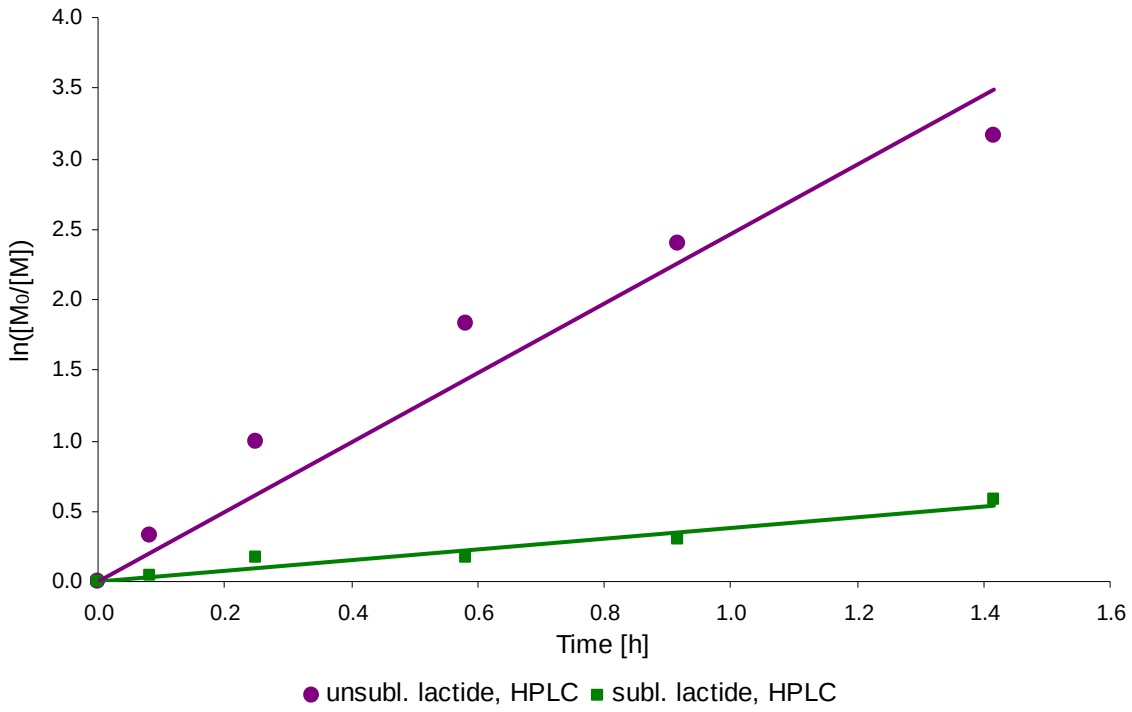


Fig. 29: Dependence of ln ($[M]_0/[M]$) on time for kinetic of copolymerization of unsublimated and sublimated LA measured by HPLC.

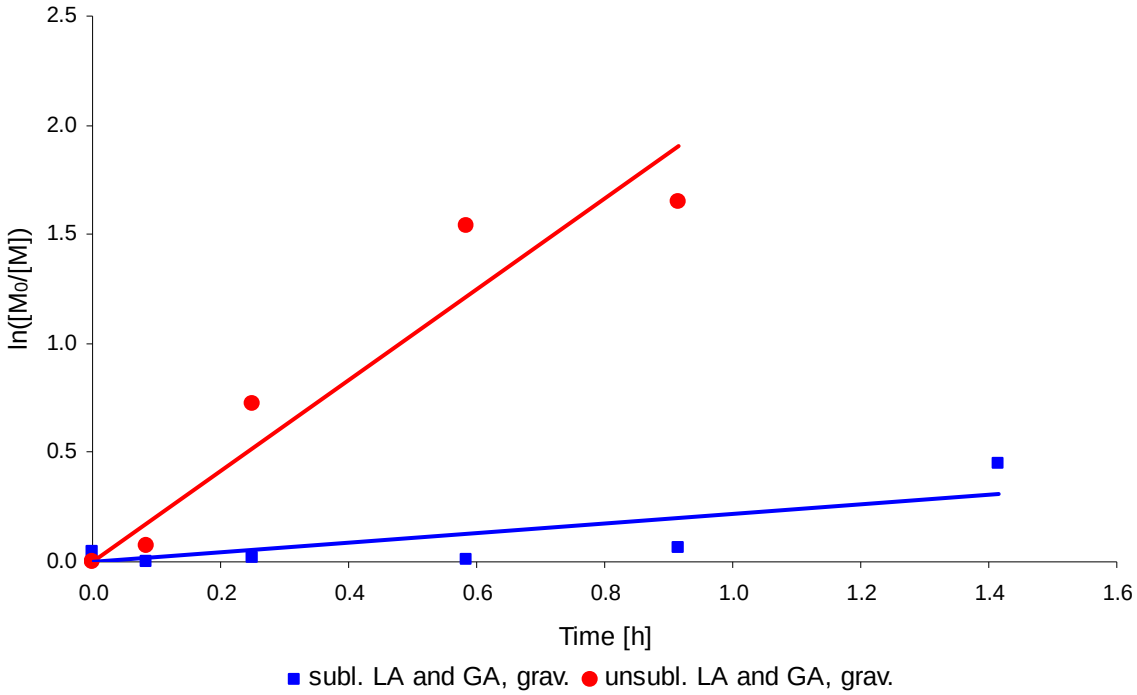


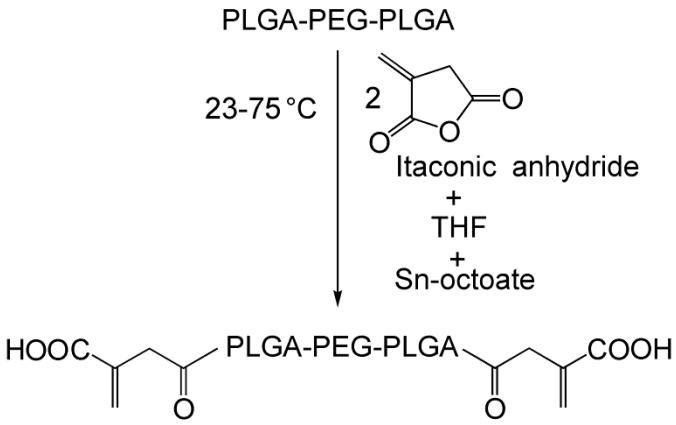
Fig. 30: Dependence of ln ($[M]_0/[M]$) on time of unsublimated and sublimated LA and GA measured by gravimetric method.

4.2 Synthesis of ITA/PLGA-PEG-PLGA/ITA

ITA/PLGA-PEG-PLGA/ITA copolymers were prepared by modification of PLGA-PEG-PLGA copolymers with itaconic anhydride via catalytic ring-opening reaction in both solution using THF as solvent or in a bulk without solvent.

4.2.1 ITA functionalization in a solution

PLGA-PEG-PLGA triblock copolymers were modified by unsublimated or sublimated itaconic anhydride (Scheme 2) in a solution with a view of finding out the ITA purification effect since unpurified ITA is only 97 %.



Scheme 2: Schema of PLGA-PEG-PLGA functionalization with ITA in solution.

4.2.1.1 Characterization by GPC and ^{1}H NMR

Fig. 31 shows GPC curves of PLGA-PEG-PLGA copolymers modified by sublimated (purified) and unsublimated (unpurified) ITA in a solution at different temperature. In this case purity of ITA had impact on progress of curves. GPC curves were identical at temperatures 23 - 60 °C. Moreover, polydispersity index were not changed markedly and ratio $M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$ was identical (Tab. 9). Again the ratio of molecular weights ($M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$) and polydispersity index of copolymers from sublimated and unsublimated ITA were approximately same.

At samples I-40S, I-60S and I-75 the content of ITA determined by ^{1}H NMR was only 1.39, 1.44 and 13.75 mol. %, respectively.

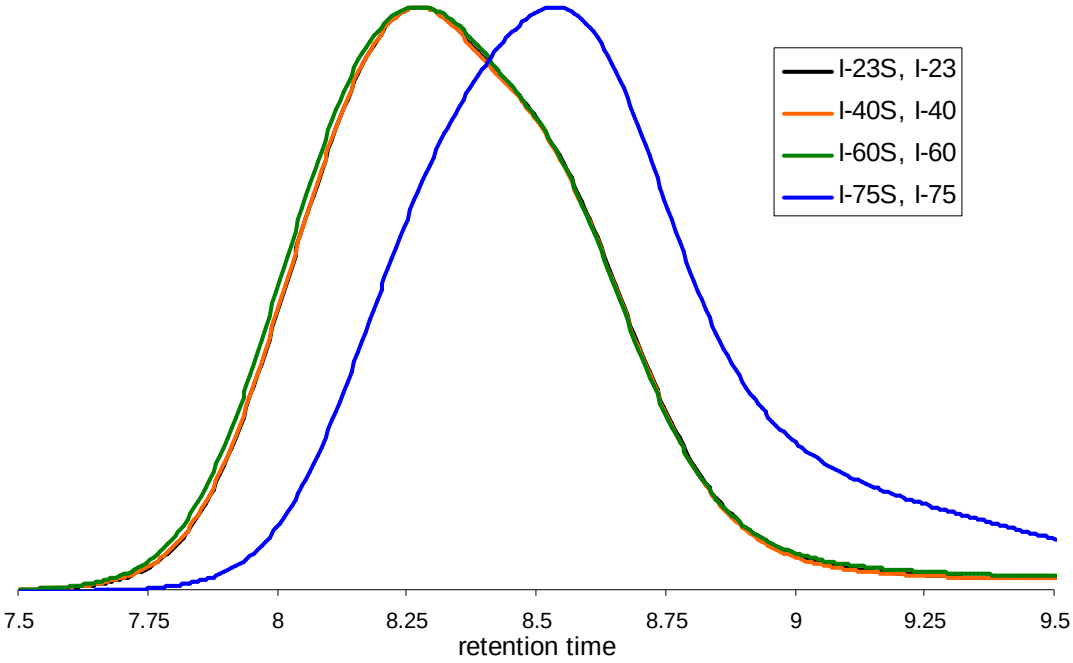


Fig. 31: GPC chromatograms of ITA/PLGA-PEG-PLGA/ITA copolymers synthesis in a solution.

Tab. 9: Molecular weight's properties of prepared ITA/PLGA-PEG-PLGA/ITA copolymers in a solution.

Polymer ITA-ABA-ITA	M_n (GPC) [g·mol^{-1}]	M_w/M_n (GPC)	M_n (^{1}H NMR) [g·mol^{-1}]	M_n (theoret.) [g·mol^{-1}]	$M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$
I-23	6782	1.266	5175	5250	1/0.77/1.01
I-23S	6808	1.273	5166	5250	1/0.77/1.02
I-40	6925	1.251	5168	5250	1/0.76/1.02
I-40S	6879	1.265	5130	5250	1/0.76/1.02
I-60	6583	1.265	5135	5250	1/0.79/1.02
I-60S	6949	1.274	5252	5250	1/0.79/1.05
I-75	4876	1.362	5556	5250	1/1.13/0.99
I-75S	4350	1.366	5831	5250	1/1.27/0.95

4.2.1.2 Characterization by FT-IR

The FT-IR spectroscopy confirmed the ITA functionalization with changing the absorption of –OH group of PLGA-PEG-PLGA copolymer to –COOH group. At infrared spectrum of copolymer synthesized in a solution at different temperature with unsublimated ITA (Fig. 32) we can observe only presence of hydroxyl functional group at the end of polymer in infrared region of 3400-3590 cm^{-1} (in magnification screen).

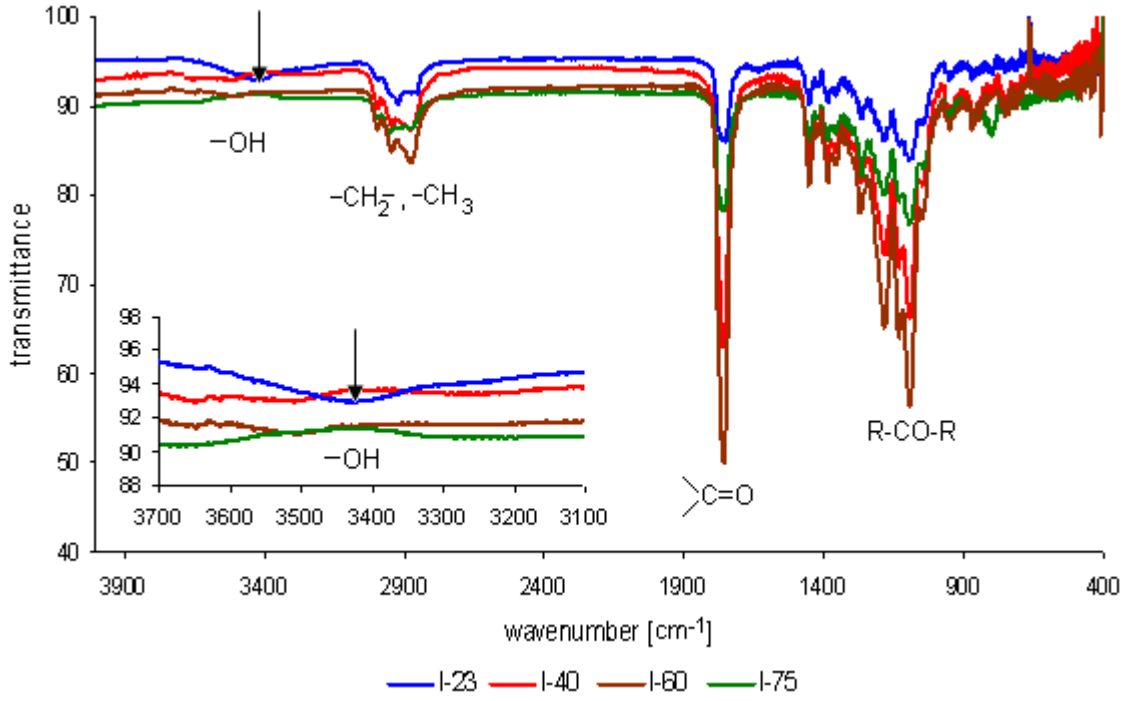


Fig. 32: FT-IR spectra of copolymers with non-sublimated ITA in a solvent.

We can observe at sample I-40S and I-60S minimal contain of carboxylic functional group at the end of polymer at 3450 cm^{-1} at infrared spectrum of copolymer synthesized in a solution at different temperature with sublimated ITA (Fig. 33).

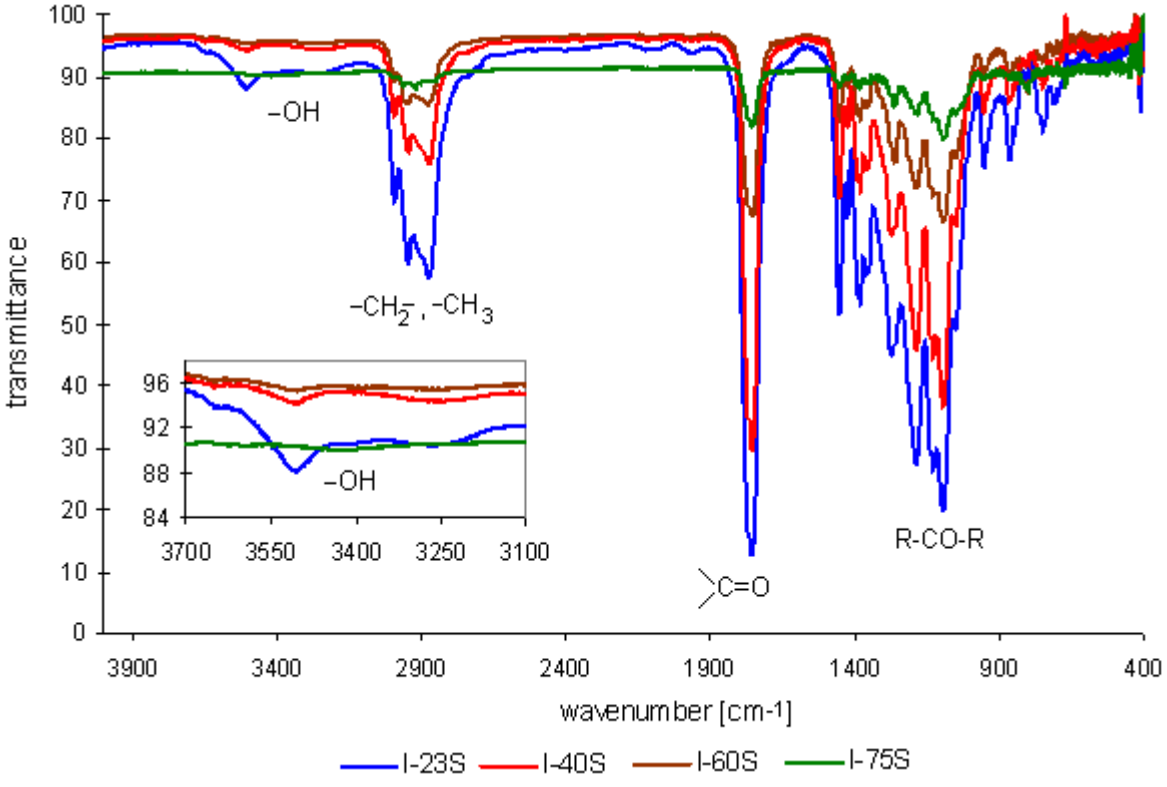
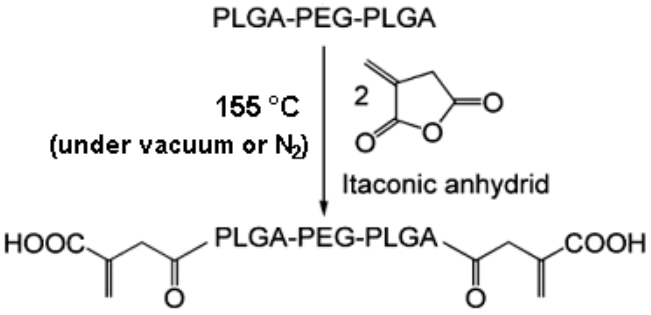


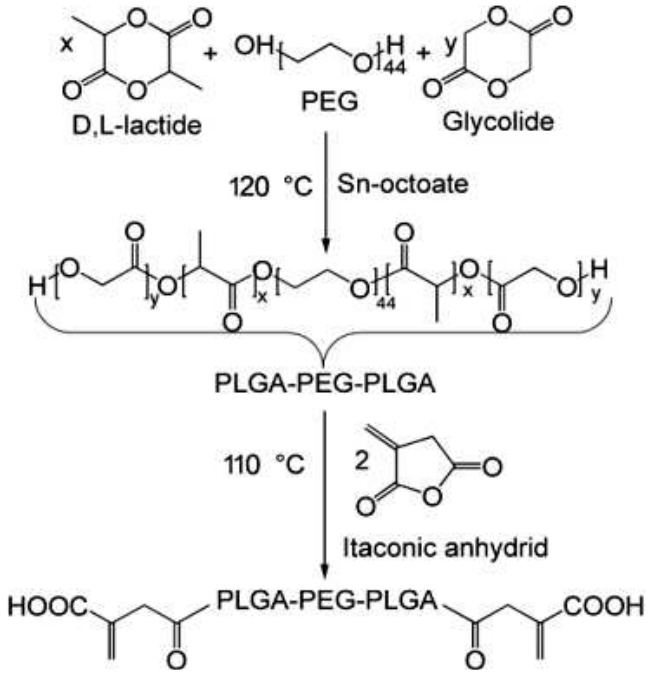
Fig. 33: FT-IR spectra of ITA/PLGA-PEG-PLGA/ITA copolymers with sublimated ITA in a solvent.

4.2.2 ITA functionalization in a bulk

PLGA-PEG-PLGA triblock copolymers were modified only by sublimated itaconic anhydride in a bulk. Functionalization proceeded in both "two-pot" (Scheme 3) and "one-pot" reaction (Scheme 4). In "two-pot" reaction the already prepared and purified PLGA-PEG-PLGA copolymer was used and functionalized by ITA at 155 °C under vacuum or nitrogen. For comparison, in "one-pot" reaction the PLGA-PEG-PLGA copolymer was freshly prepared and immediately without purification functionalized by ITA at 110 °C or 115 °C.



Scheme 3: Schema of ITA functionalization in "two-pot" reaction.



Scheme 4: Schema of ITA functionalization in "one-pot" reaction resulting in ITA/PLGA-PEG-PLGA/ITA macromonomer.

4.2.2.1 Characterization by GPC and ^{1}H NMR

Fig. 34 shows GPC chromatograms of ITA/PLGA-PEG-PLGA/ITA copolymers from sublimated ITA synthesized in a bulk at different temperatures for 8 hours under nitrogen atmosphere, in the case of sample I-155S(vac) under vacuum atmosphere. All curves except

the blue one were symmetrical and their progress was not differentiated. We can see a substantial difference from Tab. 10. Polydispersity index grew with the increasing temperature and molecular weight changed considerably. Copolymer 1-I-110S had the best result from the ratio of $M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$. Degradation of copolymer I-155S(vac) occurred as an asymmetrical curve, high polydispersity index and lower molecular weight determinated by GPC.

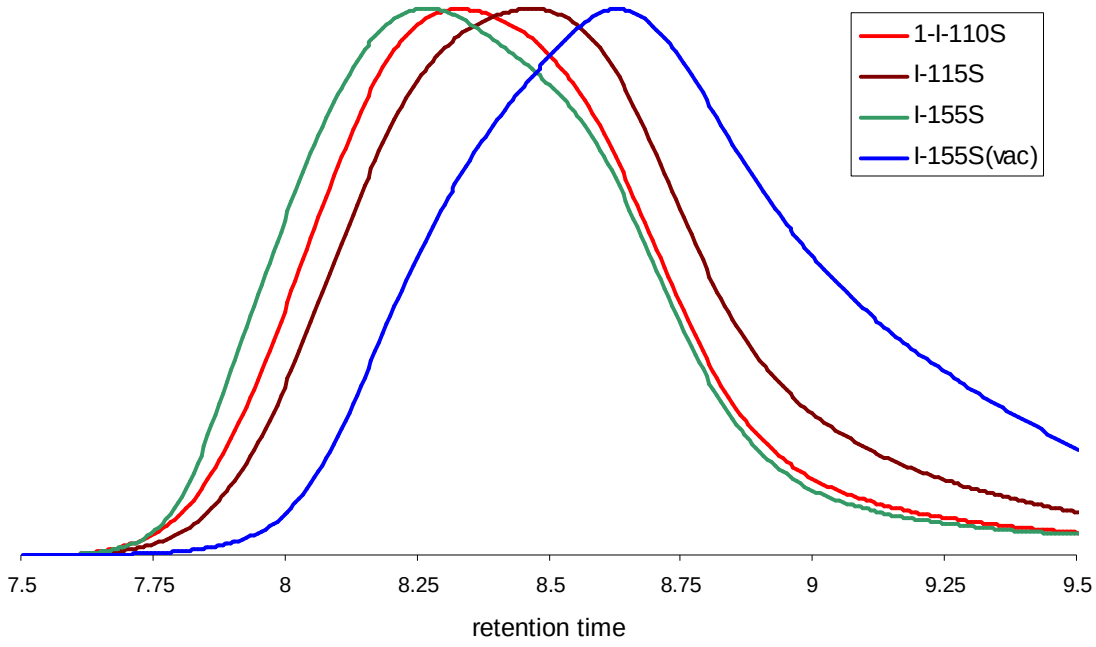


Fig. 34: GPC spectra of PLGA-PEG-PLGA copolymers modified with sublimation ITA at different temperature in a bulk for 8 hours.

Tab. 10: Molecular weight's properties of prepared ITA/PLGA-PEG-PLGA/ITA copolymers in a bulk.

Polymer ITA-ABA-ITA	M_n (GPC) [g·mol^{-1}]	M_w/M_n (GPC)	M_n (^{1}H NMR) [g·mol^{-1}]	M_n (theoret.) [g·mol^{-1}]	$M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$
1-I-110S	5862	1.38	5461	5250	1/0.90/0.96
2-I-110S	8025	1.34	5737	5250	1/0.65/0.91
I-115S	4920	1.42	5957	5250	1/1.07/0.88
I-155S	6226	1.38	5016	5250	1/0.84/1.05
I-155S(vac)	3334	1.46	5903	5250	1/1.58/0.89

At all samples synthesized in a bulk content of end-capped ITA were determined by ^{1}H NMR, which represents Fig. 35. Even the sample I-115S contained higher amount of end-capped ITA (72 %) than sample I-110S (66 %) the polydispersity index had higher.

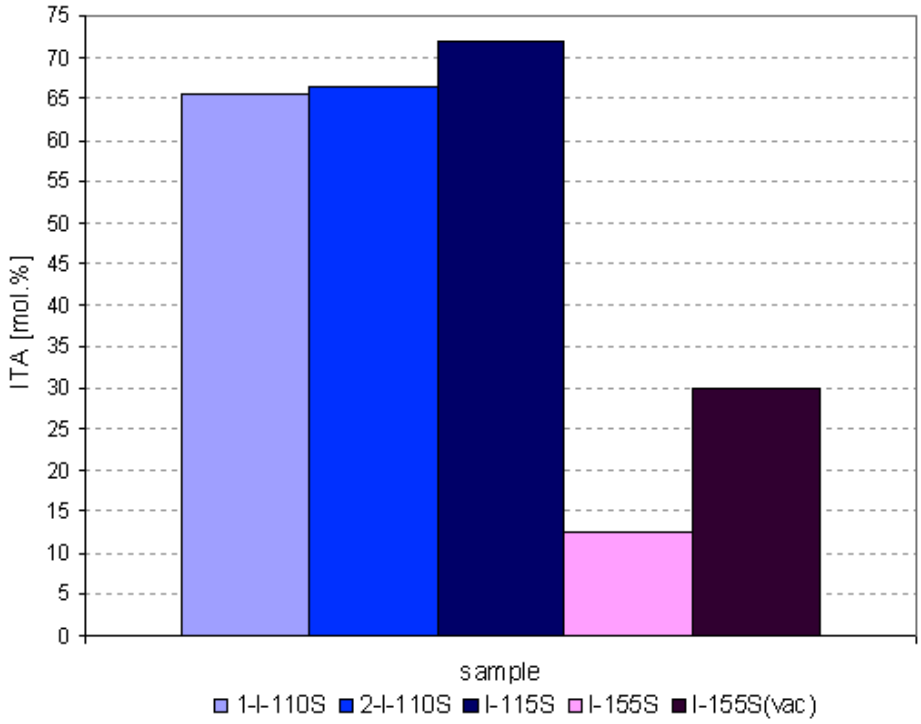


Fig. 35: Amount of sublimated ITA end-capping to PLGA-PEG-PLGA copolymer.

4.2.2.2 Characterization by FT-IR

At infrared spectrum (Fig. 36) of copolymer synthesized in a bulk for 8 hours at different temperature with sublimated ITA under nitrogen atmosphere, the samples I-110S (at 110 °C) and I-115S (at 115 °C) under nitrogen atmosphere embodied peaks of carboxylic functional group at the end of polymer at 3450 cm^{-1}.

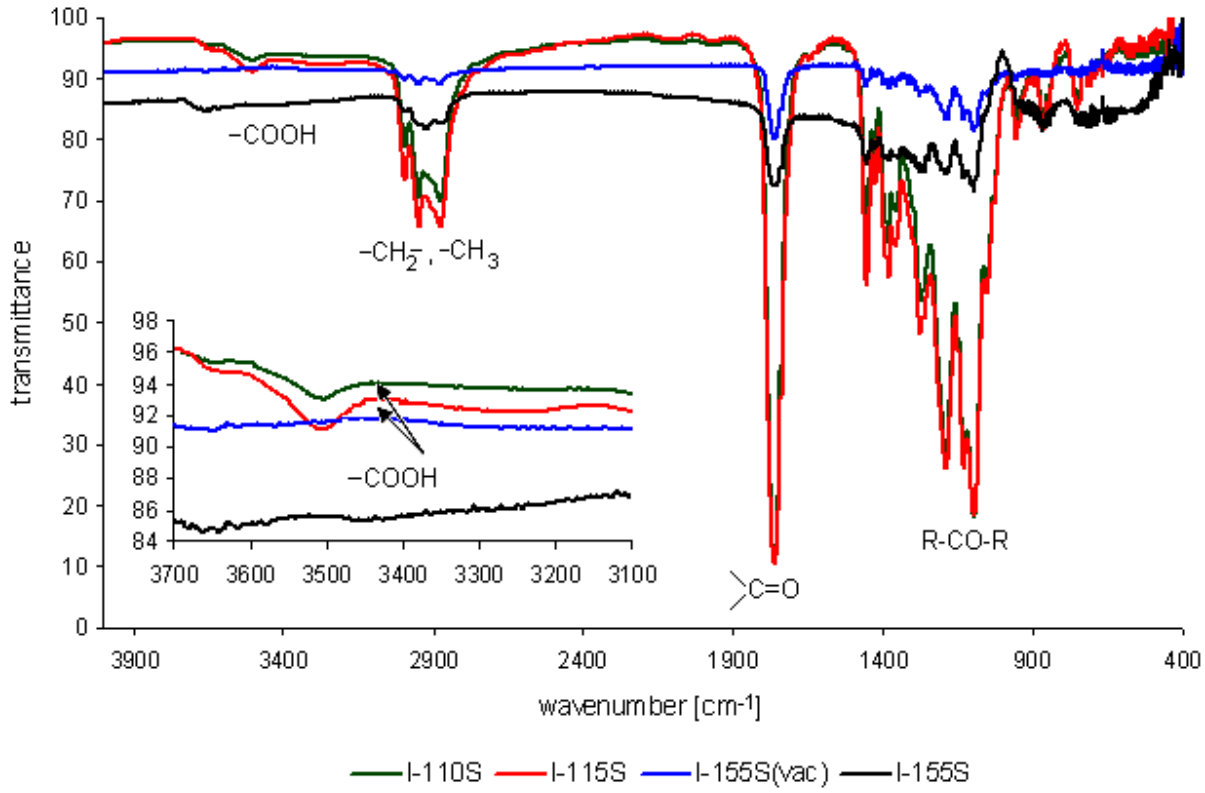


Fig. 36: FT-IR spectra of ITA/PLGA-PEG-PLGA/ITA copolymers synthesized with sublimated itaconic anhydride in a bulk.

4.2.3 Kinetics of PLGA-PEG-PLGA functionalization with sublimated ITA

Fig. 37 shows chromatograms of ITA/PLGA-PEG-PLGA/ITA copolymer (I-110S) during functionalization of PLGA-PEG-PLGA copolymer with sublimated ITA in a bulk at 110 °C for 8 hours. All curves were symmetrical, identical and unimodal for all period of modification The ratio of molecular weight $M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$) and polydispersity index of copolymers were partially identical (Tab. 11).

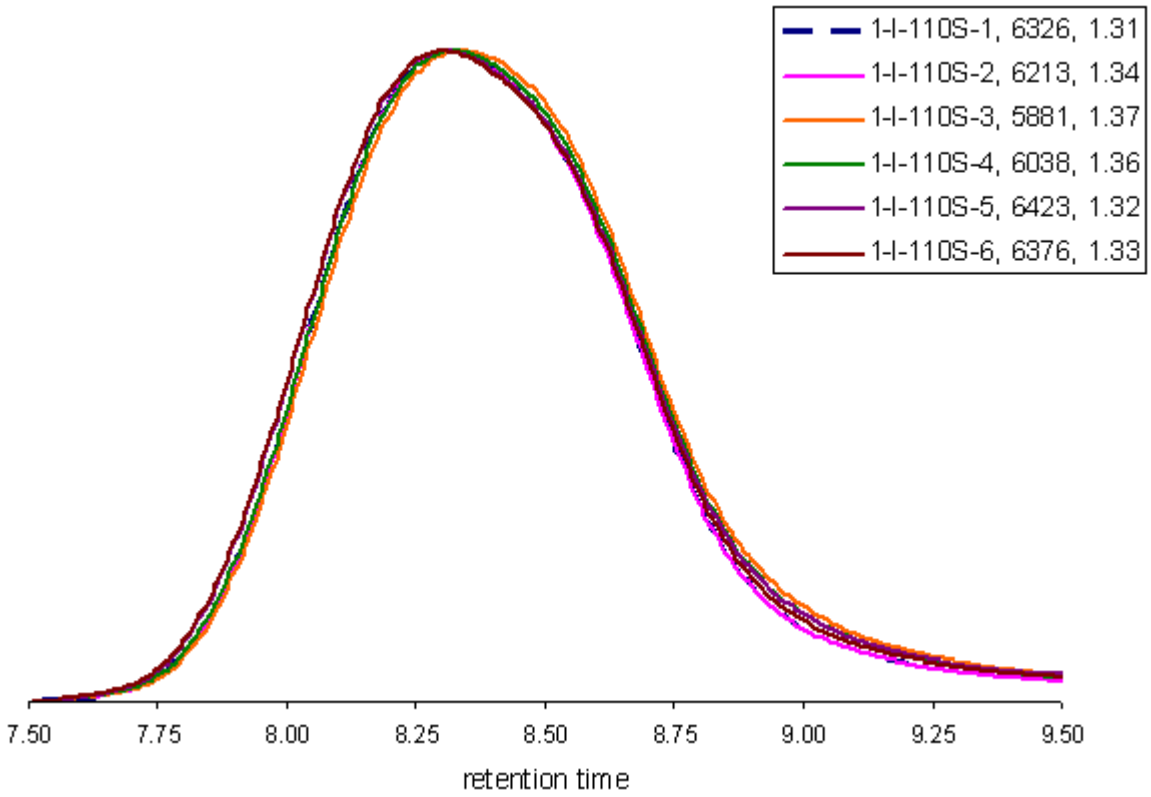


Fig. 37: GPC spectra of ITA/PLGA-PEG-PLGA/ITA copolymers during functionalization PLGA-PEG-PLGA copolymer with sublimated itaconic anhydride.

Tab. 11: Molecular weight's properties of ITA/PLGA-PEG-PLGA/ITA copolymers during kinetic.

Polymer	*M*n (GPC) [g·mol^{-1}]	M_w/M_n (GPC)	M_n (^{1}H NMR) [g·mol^{-1}]	M_n (theoret.) [g·mol^{-1}]	$M_{ntheor}/M_{nGPC}/M_{nNMR}$
1-I-110S-1	6326	1.31	5511	5250	1/0.83/0.95
1-I-110S-2	6213	1.34	5495	5250	1/0.85/0.95
1-I-110S-3	5881	1.37	5488	5250	1/0.89/0.96
1-I-110S-4	6038	1.36	5465	5250	1/0.87/0.96
1-I-110S-5	6423	1.32	5455	5250	1/0.82/0.96
1-I-110S-6	6376	1.33	5454	5250	1/0.82/0.96

The amount of ITA linked to the end of PLGA-PEG-PLGA copolymer was studied by ^{1}H NMR. NMR spectrum (of sample I-110S-3) with the highest yield of ITA end-capped to polymer is shown in Fig. 38. Characteristic peaks of itaconic acid double bond (OC(CH2)CCH2COOH) protons were found in a range between of δ = 5.7 - 5.8 ppm (g) and δ = 6.35 - 6.5 ppm (f). Characteristic peaks of lactic acid incorporated to the end of polymer (O-(C$\underline{H}_3$)CHO) protons were found in range between of δ = 1.5 - 1.75 ppm (e) and (O-(CH$_3$)C$\underline{H}$O) proton was found in range between of δ = 5.1 – 5.35 ppm (a). A characteristic peak of glycolic acid (OC$\underline{H}_2$O) proton was found in range between of δ = 4.6 - 4.9 ppm (b) and peak of PEG (OC$\underline{H}_2$C$\underline{H}_2$O) proton in range between of δ = 3.55 - 3.75 ppm (d). Molecular weight was calculated from integral of peak (a) belongs to PLA and from integral of peak (b) of PGA.

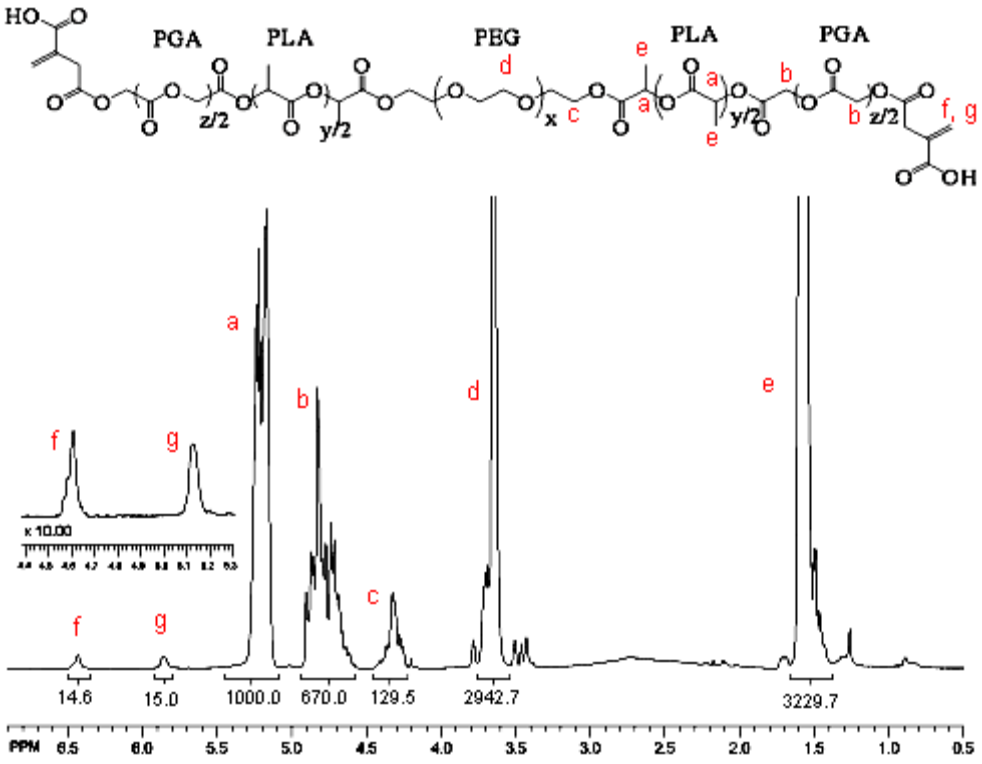


Fig. 38: NMR spectrum of ITA/PLGA-PEG-PLGA/ITA copolymer took during functionalization in 1.5 hours.

Fig. 39 shows molar amount of sublimated ITA end-capped to the original PLGA-PEG-PLGA copolymer during modification calculated from 1H NMR. Optimal conditions for synthesis of ITA/PLGA-PEG-PLGA/ITA copolymer were reached with sublimated itaconic anhydride in a bulk, at the temperature of 110 °C with total time of 1.5 hours, when 76.6 mol. % of ITA was end-capped to the original PLGA-PEG-PLGA copolymer. Longer time of modification produced reduction in the amount of end-capped ITA of about 10 % in 8 h of total time.

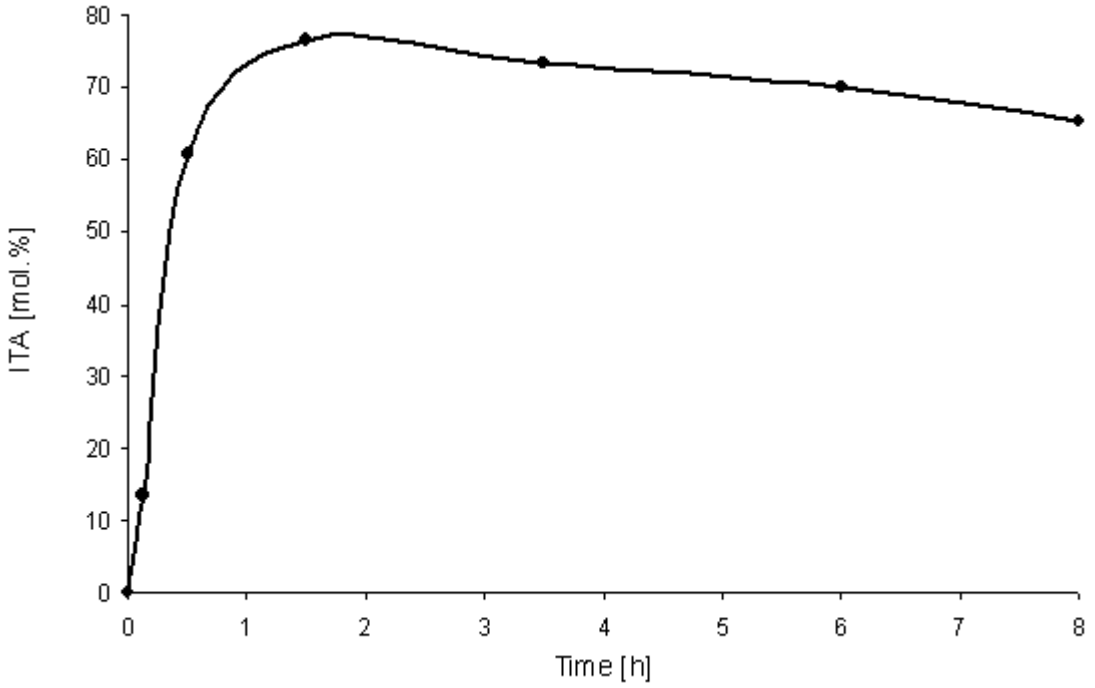


Fig. 39: Among of ITA end-capped to PLGA-PEG-PLGA copolymer.

Fig. 40 shows FT-IR spectra of ITA/PLGA-PEG-PLGA/ITA copolymers during functionalization of PLGA-PEG-PLGA copolymer with sublimated itaconic anhydride. We can observe an increase amount of carboxylic functional group with increased time of modification. Carboxylic functional groups were found in range of wavenumer 1703-1725 cm^{-1} and 1600-1900 cm^{-1} for dimmer form and 2500-2700 cm^{-1} for characteristic line.

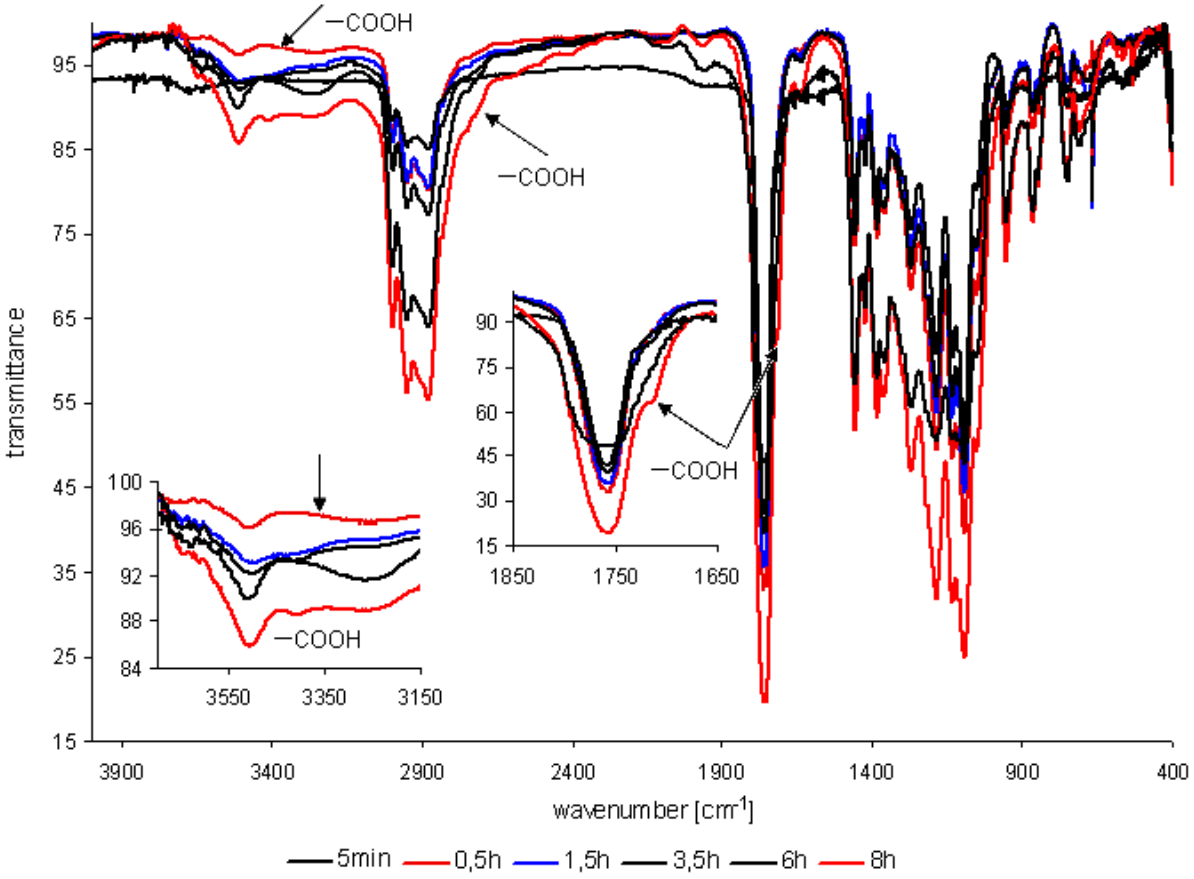


Fig. 40: FT-IR spectra of ITA /PLGA-PEG-PLGA/ITA copolymers during functionalization of PLGA-PEG-PLGA copolymer with sublimated itaconic anhydride.

5 CONCLUSION

The main aim of the presented work was functionalization of biodegradable thermosensitive PLGA-PEG-PLGA copolymer with itaconic anhydride (ITA) via catalytic ring opening reaction and optimization of ITA functionalization conditions in terms of temperature, time, ITA purification and presence of the solvent. ITA brings reactive double bonds and functional carboxylic acid groups to the both ends of copolymer resulting in synthesis of bioinductive macromonomers.

Kinetics of PLGA-PEG-PLGA copolymer synthesis from unsublimated (unpurified) and sublimated (purified) lactide and glycolide monomers were studied. In both cases the synthesis proceeded in a bulk at 130 °C for 3 hours with conversion of approximately 90 %. Longer time had no effect on the growth of conversion. As for kinetics from unsublimated monomers, a rapid increase of conversion during first few minutes occurred followed by the constant progress. Resulting molecular weight and polydispersity were 7155 $g \cdot mol^{-1}$ and 1.26, respectively. However, optimal conditions were reached when monomers were purified by sublimation, when increase of conversion up to 88 % was nearly linear (living polymerization) up to 2.5 hours and then remained constant. Well-defined PLGA-PEG-PLGA copolymer with molecular weight and polydispersity index of 7198 $g \cdot mol^{-1}$ and 1.2, respectively, was obtained.

Functionalization of PLGA-PEG-PLGA copolymer with unsublimated itaconic anhydride (unpurified) and sublimated itaconic anhydride (purified) in presence of THF as solvent at different temperature (23, 40, 60 and 75 °C) for 24 hours under nitrogen atmosphere was studied. At PLGA-PEG-PLGA copolymers modified by unsublimated ITA no amount of ITA end-capped to polymer was detected. At PLGA-PEG-PLGA copolymers modified by sublimated ITA a little amount of ITA at the ends of PLGA-PEG-PLGA copolymer was detected, when 1.4 mol % for 40 and 60 °C and 13.8 mol. % for 75 °C were end-capped to the original PLGA-PEG-PLGA copolymer. Since there were no progressive results obtained from the solution reactions next functionalizations proceeded in a bulk with no solvent added under higher temperatures.

Influence of nitrogen or vacuum atmosphere on the modification of PLGA-PEG-PLGA by sublimated ITA at 155 °C was studied. By GPC and FT-IR methods it was detected that synthesis under vacuum was not satisfied. Anyhow, both prepared ITA/PLGA-PEG-PLGA/ITA copolymers contained very low amount of itaconic anhydride.

Finally, optimal conditions for synthesis of ITA/PLGA-PEG-PLGA/ITA copolymer were reached with sublimated itaconic anhydride in a bulk, at the temperature of 110 °C with total time of 1.5 h, when 76.6 mol. % of ITA was end-capped to the original PLGA-PEG-PLGA copolymer. Resulting molecular weight of ITA/PLGA-PEG-PLGA/ITA copolymer (M_n = 5881 $g \cdot mol^{-1}$) with polydispersity index of 1.37 found by GPC was in a very good agreement with M_n calculated from ^{1}H NMR and a theoretical M_n ($M_{ntheor}/M_{nGPC}/M_{nNMR}$ = 1/0.89/0.96) proving high initiator efficiency (89 %).

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7 LIST OF SHORTCUTS

DNA	deoxyribonucleic acid
FDA	Food and Drug Administration
FT-IR	Fourier Transformed Infrared
GA	glycolic acid
GAGs	glycosaminoglycans
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
IR	infrared
ITA	itaconic anhydride
LA	lactic acid
NMR	nuclear magnetic resonance
PDI	polydispersity index
PE	polyethylene
PEG	poly(ethylene glycol)
PET	poly(ethylene terephthalate)
PGA	poly(glycolic acid)
PHEMA	poly(hydroxyethyl methacrylate)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PMMA	poly(methyl metacrylate)
PP	polypropylene
PTFE	polytetrafluoroethylene
PVC	poly(vinyl chloride)
ROP	ring-opening polymerization
SR	silicone rubber
THF	tetrahydrofuran