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FAKULTA CHEMICKÁ

INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY
ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE

CROSSLINKING OF THERMOSENSITIVE FUNCTIONALIZED COPOLYMERS BY BLUE LIGHT
SÍŤOVÁNÍ TERMOCITLIVÝCH FUNKCIONALIZOVANÝCH KOPOLYMERŮ MODRÝM SVĚTLEM

BACHELOR'S THESIS
BAKALÁŘSKÁ PRÁCE

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Bachelor's Thesis Assignment

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Title of Bachelor's Thesis:

Crosslinking of thermosensitive functionalized copolymers by blue light

Bachelor's Thesis assignment:

1) Literature searching on thermosensitive copolymers and their functionalization
2) Synthesis of copolymers sensitive to light and heat
3) Copolymer crosslinking with blue light
4) Characterization of prepared hydrogels
5) Evaluation
6) Conclusion

Deadline for Bachelor's Thesis delivery: 24. 5. 2019

Bachelor's Thesis is necessary to deliver to a secretary of institute in the number of copies defined by the dean. This assignment is part of Bachelor's Thesis.

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In Brno, 31. 1. 2019

prof. Ing. Martin Weiter, Ph.D.
Dean

ABSTRACT

The aim of this thesis was to prepare a hydrogel with a hybrid network of only one type of biodegradable copolymer. The new degradable hydrogel, containing both physical interactions (arising at physiological temperature of 37 °C) and chemical bonds initiated by blue light could be used as a resorbable wound dressing or as an injectable carrier with a gradual and well controlled drug release.

Thermosensitive PLGA–PEG–PLGA copolymer synthesized by living ring-opening polymerization was subsequently functionalized with itaconic anhydride to form ITA/PLGA–PEG–PLGA/ITA light-sensitive and temperature-sensitive macromonomer. At 37 °C, the copolymer forms a micellar network due to hydrophobic interactions. Itaconic acid double bonds, which are attached to the ends of the copolymer chain, allow photochemical crosslinking of micelles with a view to increase the hydrolytic stability of novel hydrogel.

The synthesized copolymers were characterized by GPC and ^{1}H NMR methods. The formation of a physical network at physiological temperature was confirmed by rheological analysis. The physically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogel was then irradiated with blue light (430 – 490 nm) in the presence of a water soluble biocompatible photoinitiator LiTPO and chemically characterized by ATR-FTIR. The resulting hydrogel was transparent, flexible, absorbed up to 1176 % water, and was stable for 20 days in saline at 37 °C. The ITA/PLGA–PEG–PLGA/ITA hydrogel with hybrid network was also prepared in the presence of a crosslinker PEGDA, that significantly reduced the time required for crosslinking the hydrogel, but further analyses are needed to more fully understand the principles of the novel hydrogel types.

KEY WORDS

Hybrid network, photopolymerization, thermosensitive copolymer, blue light, hydrolytic stability

ABSTRAKT

Cílem mé bakalářské práce byla příprava hydrogelu s hybridní sítí pouze z jednoho typu biodegradovatelného kopolymeru. Nový degradabilní hydrogel, obsahující jak fyzikální interakce (vznikající při fyziologické teplotě 37 °C), tak i chemické vazby iniciované modrým světlem by mohl být využit jako resorbovatelný kryt ran nebo jako injektovatelný nosič s postupným a velice dobře řízeným uvolňováním léčiv.

Termocitlivý PLGA–PEG–PLGA kopolymer syntetizovaný živou polymerací za otevření kruhu byl následně funkcionalizován anhydridem kyseliny itakonové za vzniku ITA/PLGA–PEG–PLGA/ITA makromonomeru citlivého jak na světlo, tak i na změnu teploty. Při teplotě 37 °C tvoří kopolymer díky hydrofobním interakcím micelární síť. Dvojné vazby kyseliny itakonové, která je navázaná na koncích kopolymerního řetězce, umožňují fotochemické zesítění micel a zvýšení tak hydrolytické stability hydrogelu.

Syntetizované kopolymery byly charakterizované metodami GPC a ^{1}H NMR. Vznik fyzikální sítě při fyziologické teplotě byl potvrzen reologickou analýzou. Fyzikálně zesítěný ITA/PLGA–PEG–PLGA/ITA hydrogel byl následně, v přítomnosti hydrofilního fotoiniciátoru LiTPO, ozářen modrým světlem (o vlnové délce 430–490 nm) a chemicky charakterizován pomocí ATR-FTIR. Vzniklý hydrogel byl transparentní, ohebný, absorboval až 1176 % vody a ve fyziologickém roztoku při 37 °C byl stabilní 20 dní. ITA/PLGA–PEG–PLGA/ITA hydrogel s hybridní sítí byl rovněž připraven v přítomnosti síťovadla, které výrazně snížilo dobu potřebnou na zesítění hydrogelu, nicméně další analýzy jsou potřeba k podrobnějšímu pochopení principů nových typů hydrogelů.

KLÍČOVÁ SLOVA

Hybridní síť, fotopolymerizace, termocitlivý kopolymer, modré světlo, hydrolytická stabilita

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DECLARATION

I declare that the bachelor thesis has been worked out by myself and all the quotations from used literature sources are accurate and complete. The content of the bachelor 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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TABLE OF CONTENTS

1 INTRODUCTION

Thermosensitive polymers are embracing numerous biomedical and pharmaceutical applications. These polymers exhibit a sol-gel transition in response to a temperature change of the environment. Thermosensitive polymers with a free-flowing state (sol) at room temperature and a gel state at physiological temperature are of high interest in drug delivery and tissue engineering [1]. Mentioned polymers are injectable and undergo gelation at body temperature, thus forming hydrogel. Hydrogels may be used for drug delivery or as scaffolds. The delivery systems are applicable for targeted delivery as well as they can serve as a protection against hydrolysis and degradation of the encapsulated drug. Hydrogels formed by a temperature change are physically crosslinked and stabilized with hydrophobic interactions [2].

Chemical crosslinking of hydrogels provides better thermal and mechanical stability. Photopolymerization is a process of forming a chemically crosslinked network *in situ* by irradiation of the precursors with ultra-violet or visible light. Since the reaction occurs at physiological temperature and generates minimal heat, it can be carried out in the presence of living cells. Photopolymerization by blue light, harmless part of visible light spectrum, is already used in dentistry, printing, and for surface modifications [3].

The goal of the thesis is to prepare a hydrogel stabilized both with physical (by temperature) and chemical (by blue light) crosslinking, known as a hybrid network. Such hydrogels are potentially applicable in drug delivery and as wound dressings with a gradual release of drug. The release time of the medicament entrapped in the hydrogel depends on both the degradation of polymer network and diffusion and can be tailored either with the composition of the polymer or with the degree of chemical crosslinking [4].

2 THEORETICAL PART

2.1 Thermosensitive polymers

Hydrogels are three dimensional elastic networks formed with physical and/or chemical interactions. Chemical crosslinking is done by covalent bonds and provides high mechanical and thermal stability to the hydrogel. Physical crosslinking is reversible and is formed by non-covalent interactions, such as van der Waals forces, ionic interactions, hydrogen bonds and hydrophobic effect. Physical crosslinking is influenced by the properties of the surrounding environment (temperature, pH, pressure etc.) [5].

Thermosensitive polymers exhibit a simple method to form physically cross-linked hydrogels triggered by temperature change [6]. Thermosensitive polymers are typically amphiphilic molecules. Due to their amphiphilic properties they are able to self-assemble into micelles [7]. The polymer forms a free-flowing sol however when the temperature exceeds the critical gelation temperature (CGT), the polymer forms a gel. This is called a sol-gel transition [8]. The transition is influenced by parameters of the polymer, such as concentration, composition and molecular weight [9]. Typical phase diagrams of a micellar sol-gel transition are shown on Figure 1.

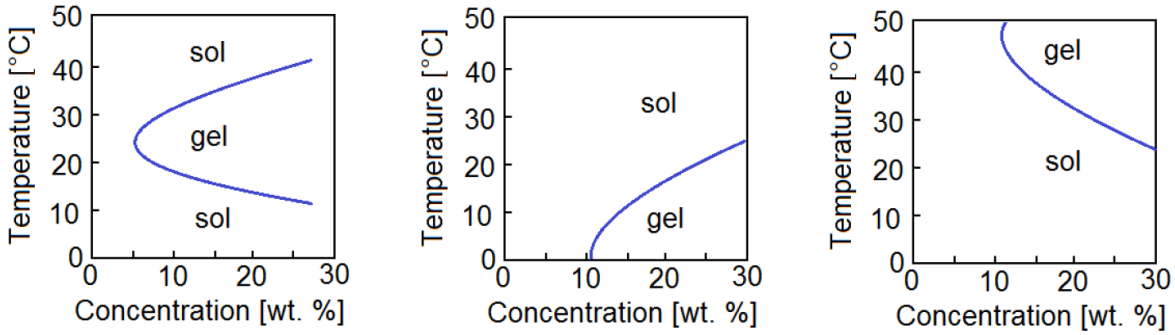

Figure 1: Phase transition diagrams. Sol-gel-sol (left), gel-sol (middle) and sol-gel (right) transition [10].

Thermosensitive polymers are of a high interest for biomedical purposes due to their advantages of forming a hydrogel without any additives, thus decreasing the potential toxicity. Thermosensitive polymers with a sol-gel transition close to body temperature can be used as drug-delivery carrier. For instance, a drug can be added to an aqueous solution of a thermosensitive polymer at room temperature, when injected into a body, the temperature of the environment rises and a hydrogel with the drug encapsulated inside is formed [7]. This type of carrier may be used for a targeted delivery of the drug to a specific site and as a protection against hydrolysis and enzymatic degradation of the encapsulated component [2]. Moreover, the controlled release of an active compound resolves in increased therapeutic activity compared to repetitive administrations of the medicament as the drug concentration maintains in the effective range, the so-called therapeutic window [11].

The first reported thermosensitive copolymer to form hydrogel at body temperature is a poloxamer, commercially known as Pluronic® (Figure 2). Pluronic® is a BAB type triblock copolymer, where B is a hydrophilic part (such as poly(ethylene oxide) (PEO)) and A a hydrophobic part (such as poly(propylene oxide) (PPO)), therefore PEO–PPO–PEO [12]. Despite having ideal sol-gel transition properties, the copolymer could not be used for drug delivery. Pluronic® is not biodegradable, has low mechanical stability and low molecular weight [1].

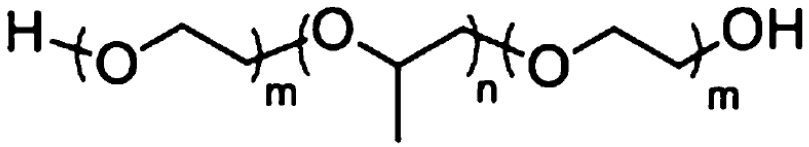

Figure 2: Structure of Pluronic® [13].

Copolymers based on poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), poly[(*R)*-3-hydroxybutyrate] (PHB), poly(glycolic acid) (PGA) and poly(ε-caprolactone) (PCL) represent an interesting alternative to Pluronic® due to their biocompatibility and biodegradability [13].

A triblock PEG-poly(*L*-lactic acid)-PEG (PEG–PLLA–PEG, Figure 3) copolymer, type BAB, showed gel-sol transition when heated. Therefore, the sol state occurs at higher temperatures. This characteristic is not ideal for the encapsulation of cells or bioactive molecules as they would have to be added to the sol at high temperatures which might cause their damage and further inactivity. Moreover, it may be also uncomfortable for the patients as the copolymer would have to be injected at higher temperatures [1].

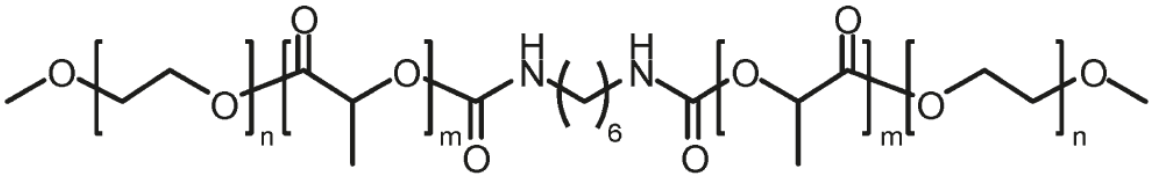

Figure 3: The structure of PEG-PLLA-PEG [13].

To avoid working at high temperatures a BAB copolymer PEG-poly(*D,L*-lactide-*co*-glycolide)-PEG (PEG–PLGA–PEG) can be used as an alternative. This triblock copolymer has a state of a free-flowing sol at room temperature and transforms to a gel at body temperature (sol-gel transition). Hydrophilic drugs incorporated into PEG–PLGA–PEG hydrogel are released by diffusion whereas the release of hydrophobic drugs depends on the degradation of the hydrogel [8][13][1].

2.1.1 PLGA–PEG–PLGA copolymer

An ABA copolymer poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) showed a similar sol-gel transition to its BAB variation (Figure 4). The copolymer is composed of a hydrophilic PEG block in between two hydrophobic PLGA blocks (Figure 5). The sol-gel transition of PLGA–PEG–PLGA is dependent on the lactide to glycolide and also on the PLGA/PEG ratio. The CGT (marked C in Figure 4) decreases with the increasing LA/GA ratio [4] and the critical gelation concentration (CGC) also decreases with the increasing PLGA/PEG ratio [14].

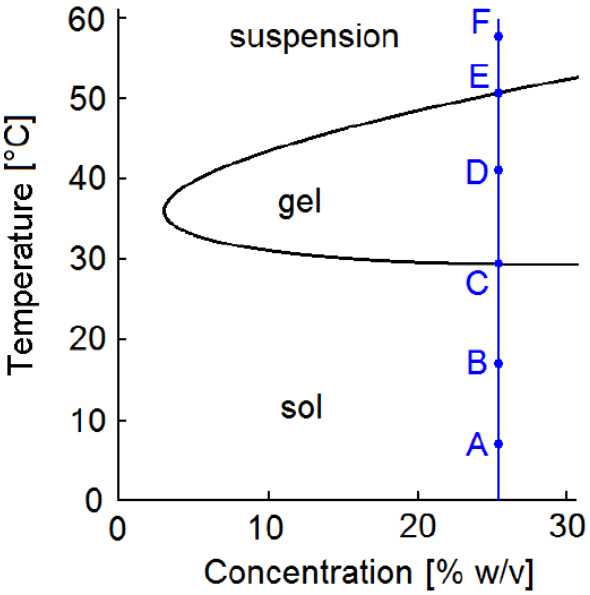

Figure 4: A sol-gel transition of PLGA–PEG–PLGA copolymer [1].

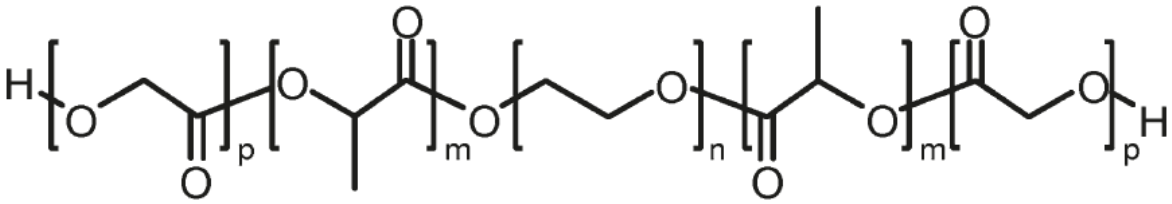

Figure 5: The structure of PLGA-PEG-PLGA copolymer [1].

A big advantage of biodegradable hydrogels is that there is no need for surgical removal of the carrier [15]. Aliphatic polyesters (such as PLGA) contain ester bonds which undergoes hydrolysis [16]. In the case of PLGA–PEG–PLGA the degradation products are lactic acid, PEG and glycolic acid [17]. Lactic acid is metabolized in the Krebs cycle to water and carbon dioxide. Glycolic acid is either excreted directly in urine or reacts to form glycine. Glycine may be used to synthesize serine and then transformed into pyruvic acid, which is metabolised in the Krebs cycle [18]. PEG is very soluble in water and has a small molecular weight therefore is not recognized by the immune system and is eliminated by urine. The copolymer degraded entirely *in vitro* at 37 °C after 6– 8 weeks [4].

Zentner et al. (2001) have used the PLGA–PEG–PLGA triblock copolymer as an injectable drug delivery system to carry paclitaxel. Paclitaxel is a hydrophobic drug used for the treatment of breast cancer. The combination of the ABA type copolymer and paclitaxel is called OncoGel™. The carrier provides a long gradual release up to 6 weeks depending on the degradation of the gel [17].

Kim et al. (2001) focused on the possibility of a prolonged release of insulin. The insulin was encapsulated in a PLGA–PEG–PLGA carrier. This resulted in a gradual release of insulin for up to 15 days, which would reduce the required number of injections to only twice a month [19].

Chen et al. (2005) used the PLGA–PEG–PLGA copolymer for delivery of testosterone. After increasing the PLGA block lengths, the testosterone was gradually released *in vitro* for 3 months [20].

Another application for the thermosensitive copolymer involves a treatment of chemical or thermal burns. In the experiment of Pratoomsoot et al. (2008) the PLGA–PEG–PLGA hydrogel, that was formed upon contact with the body surface, exhibited a porous network that could help the migration of the epithelial cells. The copolymer together with cellular adhesion peptides and antimicrobial agents offers a novel possibility of wound healing [21].

2.1.1.1 Synthesis of PLGA–PEG–PLGA

The copolymer is synthesized in bulk using stannous octanoate as a catalyst (Figure 6). Firstly, the catalyst reacts with reactive hydroxyl groups on both ends of PEG and forms an initiator complex. The PLA and PGA blocks are attached by ring-opening polymerization (ROP) [4].

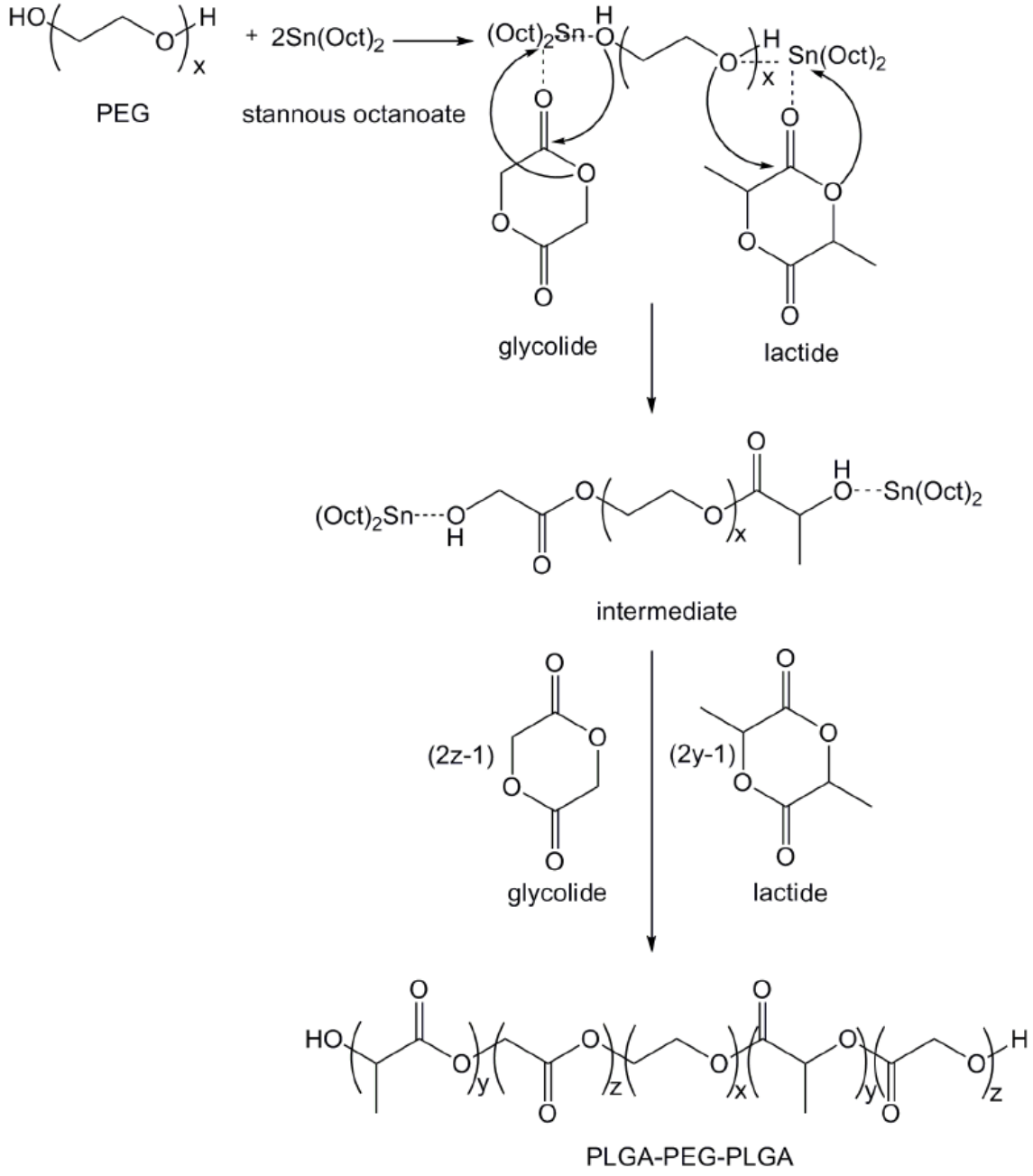

Figure 6: Mechanism of PLGA–PEG–PLGA polymerization [22].

2.1.2 Functionalization of thermosensitive polymers

The purpose to add functional groups to the polymer chain is to improve one or more properties of the copolymer. Thermosensitive polymers can be modified by introducing carboxyl, hydroxyl, amino groups or double bonds [23].

Yu et al. (2007) end-capped the PLGA–PEG–PLGA copolymer with small alkyl groups using acetyl chloride and acetic anhydride. The resulting copolymer was more hydrophobic and showed lower critical micellar concentration. However, the end-capped copolymer formed gel at lower temperatures than the body temperature. When the physiological temperature was reached, the copolymer precipitated from the solution [24].

Introducing double bonds to the polymer chain provides for chemical crosslinking. Chemically crosslinked hydrogels are more stable, therefore degrade more slowly. Michlovská et al. (2016) succeeded to modify PLGA–PEG–PLGA copolymer by itaconic anhydride. Itaconic anhydride (Figure 7) is a naturally occurring and biodegradable compound. It is produced by the fermentation of sugars with the bacteria *Aspergillus terreus* [25]. Itaconic anhydride connects to the polymer chain by ring-opening reaction forming itaconic acids at both ends of the chain.

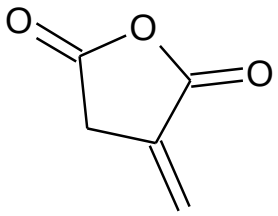

Figure 7: The structure of itaconic anhydride [26].

The resulting functionalized α,ω-itaconyl-PLGA–PEG–PLGA copolymer (ITA/PLGA–PEG–PLGA/ITA, Figure 8) is capable to form a hydrogel both with physical and chemical crosslinking (Figure 8). Physically crosslinked network may be achieved either with a pH or a temperature change of the environment. As a result of a pH change, the copolymer forms an ionic network, which is stabilized by ionic interactions of the carboxyl groups found in the structure of ITA/PLGA–PEG–PLGA/ITA. With the temperature change, the copolymer forms a micellar network. Due to the presence of double bonds at the ends of the copolymer chain, the copolymer is also suitable for chemical crosslinking, which results in an end-linked network [16].

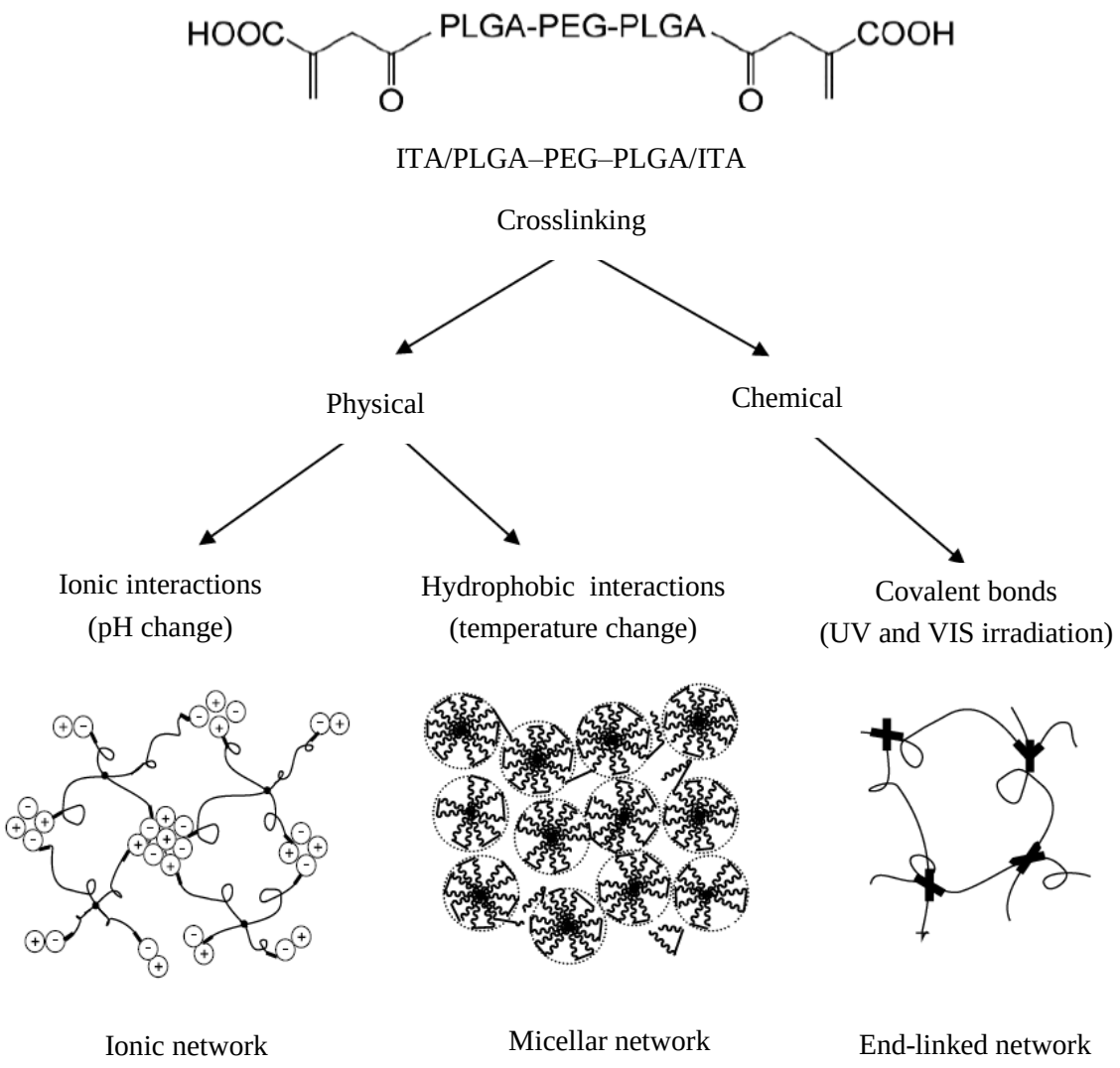

Figure 8: Crosslinking possibilities of ITA/PLGA–PEG–PLGA/ITA [4].

2.1.3 Irreversible crosslinking of micelles – hybrid network

Hydrogel network formed both with physical interactions and chemical bonds is called a hybrid network [27]. Photo-induced chemical crosslinking of micelles is an interesting method to stabilize the micellar structure of copolymers and so to prevent the disintegration of micelles. The micellar stabilization may be achieved via crosslinking of the core or the shell, depending on where the photo-sensitive group is found [28][29]. The core-crosslinked micelles proved to have improved *in vivo* performance (e.g. biodistribution, therapeutic efficacy and tolerability).

Moreover, drugs may be covalently bonded in the core, which allows tight control over the release kinetics of the drugs entrapped inside [30]. In the study of Talelli et al. (2010) a doxorubicin methacrylamide derivative was covalently crosslinked inside a polymeric micelle. Doxorubicin is a drug commonly used in treatment of cancer. The prepared drug-micelle complex showed decreased toxicity and increased therapeutic efficacy as the drug was released gradually over time and close to the target [31].

2.2 Photopolymerization of macromers

The formation of crosslinked networks *in situ* may be achieved with illumination of monomers or macromers by ultra-violet or visible light. Photopolymerization occurs at physiological temperature and produces minimal heat. Due to the mild manner of the creation of hydrogel, it may be carried out in the presence of living cells. The aqueous precursors may be injected into the body and illuminated transdermally. Photopolymerized hydrogels polymerize into a defect of any shape, which results in greater control of the location and shape of the hydrogel. The initiation of polymerization by light is used widely for dental restorations, as well as in printing, optical or electronic materials, coatings and surface modifications. Photopolymerizable hydrogels have been additionally investigated for biomedical purposes, for instance as barriers on wounds to improve the healing process, localized drug delivery depots or cell encapsulation materials [3]. Another advantage of photopolymerized hydrogels is producing layered matrices. The matrices are prepared layer by layer with each layer adhering firmly to another one. As layers are prepared separately, each layer can contain different concentrations of the drug according to the requirement [32].

Photopolymerization of monomers cannot usually take place in the presence of living tissue as most of the monomers are cytotoxic. Therefore, photopolymerized hydrogels for biomedical applications are formed from macromonomers (macromers). Additionally, in comparison with monomers, macromers can be usually photopolymerized using VIS light, rather than UV light. Examples of photopolymerizable macromers include PEG derivates, poly(vinyl alcohol) (PVA), photo crosslinkable poly(propylene fumarate) (PPF), oligo(poly[ethylene glycol] fumarate) (OPF), alginate, chitosan and hyaluronic acid (HA) [3].

2.2.1 Blue light

Blue light is a part of visible light spectrum with the wavelength range approximately of 430–490 nm. A common blue light source is a quartz-tungsten halogen bulb. However, this source produces a broad spectrum, so a filtration is needed. Whereas light-emitting-diode (LED) curing units emit light with a narrow range of wavelengths [33]. The application of blue light for biomedical purposes is preferred over the UV light as it has been proved that UV light causes DNA damage, which may lead to carcinogenesis [34]. Furthermore, while irradiating transdermally, the blue light transmits better through the skin as it has longer wavelengths than UV light. Blue light is commonly used in dentistry for polymerization of light cure resin-based composites [3]. And in the experiment of Papageorgiou et al. (2000) it has been used to treat acne vulgaris for its antibacterial properties [35]. Another application of blue light is in phototherapy for the treatment of neonatal hyperbilirubinemia [36].

2.2.2 Photoinitiators

When exposed to the visible or UV light, light-sensitive compounds (photoinitiators) form free radicals which then initiate a process of crosslinking, therefore a formation of a hydrogel. Photoinitiators (PIs) have a high absorption at a specific wavelength of light. When the light is absorbed, PIs produce radicals which attack double bonds of monomers or macromers. The ideal photoinitiator for biomedical purposes would be biocompatible, water soluble, stable and would absorb photon at visible light range [13][32].

PIs are divided by the mechanism of initiating photopolymerization into two groups. As it is shown in Figure 9, type I PIs cleave into two radicals after irradiation, whereas type II PIs undergo a bimolecular reaction. After the light irradiation of PIs type II, they abstract a hydrogen atom from a different molecule, called a co-initiator, to create radicals [38].

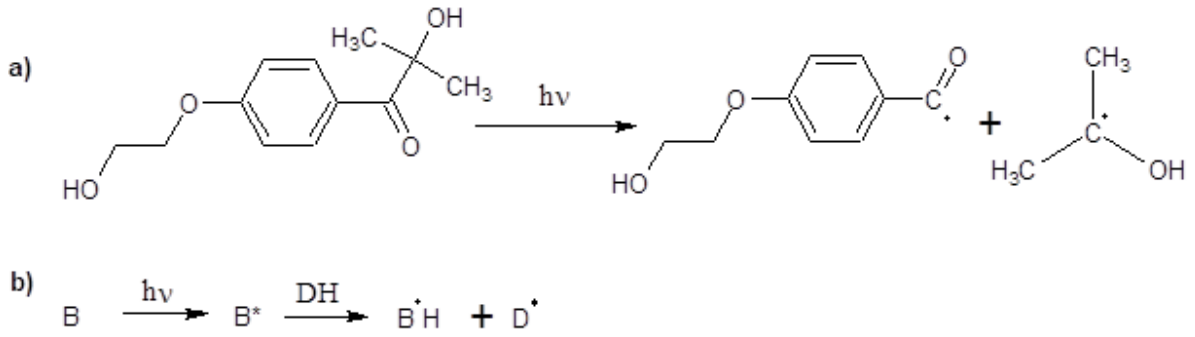

Figure 9: Cleavage of type I photoinitiator (I2959) following photon absorption (a) [38] and an example of formation radicals with type II photoinitiators and co-initiators (b) [13].

2.2.2.1 Photoinitiators soluble in organic solvents

A PI widely used in dentistry is camphorquinone (CQ). It is a yellow chemical with an absorbance of light with wavelengths between 390 and 510 nm [39]. CQ is a PI type II, for the photopolymerization to occur a co-initiator has to be present. In the case of CQ, a tertiary amine is commonly added [40].

As the colour of CQ affects the dental product's aesthetics, alternative PIs in dentistry are becoming more common, e.g. phenylpropanedione (PPD) or 2,4,6-trimethylbenzoyldiphenylphosphine oxide (Lucirin TPO). These alternatives have solved the aesthetic problem however they are still insoluble in water. This attribute limits their further application [39].

2.2.2.2 Photoinitiators soluble in water

One of the most frequently used photoinitiator in tissue engineering is a type I initiator Irgacure 2959 (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propanone). It is biocompatible and has a high reactivity however it is poorly soluble in water, which is a problem when a higher concentration is needed. Furthermore, Irgacure 2959 cleaves into radicals when exposed to undesirable UV light [3]. In order to improve the properties, the structure of Irgacure 2959 was modified. The modified Irgacure 2959, which is called APi-180®, showed higher water solubility, however UV light still have to be used [41].

The monoacylphosphineoxide MAPO (Na-TPO, LiTPO) and the bisacylphosphineoxide BAPO (BAPO-ONa, BAPO-OLi) salts are important groups of water-soluble type I initiators. In the study of Benedikt et al (2016) these four salts were tested and compared to already commercially known PIs – Irgacure 2959, APi-180® and Quantacure BPQ. The observed properties were solubility, storage stability, UV-VIS absorption, cytotoxicity and reactivity. All salts showed high water solubility, only

APi-180® and Quantacure BPQ showed comparable values. Both MAPO and BAPO salts were stable in acidic, basic and neutral environment and have strong absorption in the visible light spectrum. BAPO-OLi displayed highest reactivity for visible light irradiation. Furthermore, LiTPO (Figure 10) achieved the best biocompatibility [41].

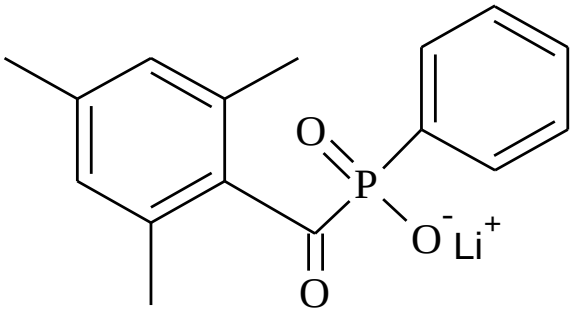

Figure 10: Structure of LiTPO [41].

3 MAIN GOAL OF THE WORK

The goal of this work is to synthesize and characterize a thermosensitive PLGA–PEG–PLGA copolymer. Subsequently to functionalize the copolymer by the itaconic anhydride and produce a α,ω-itaconyl-PLGA–PEG–PLGA copolymer sensitive to both light and heat. The aim is to prove the hybrid network formation in the presence of a water soluble photoinitiator LiTPO when irradiated by blue light and to further characterize the prepared hydrogels.

- Synthesis of PLGA–PEG–PLGA copolymer via ring-opening polymerization
- Functionalization of PLGA–PEG–PLGA copolymer by itaconic anhydride
- Characterization of the copolymers using GPC, ^{1}H NMR and rheology
- Photopolymerization of physically crosslinked α,ω-itaconyl-PLGA–PEG–PLGA copolymer with blue light
- Characterization of prepared hydrogels with hybrid network using ATR-FTIR and determination of hydrolytic stability and water uptake in physiological solution at 37 °C

4 EXPERIMENTAL PART

4.1 Chemicals

- Acetone (p.a.) was purchased from Lach-Ner, s.r.o. (Czech Republic).
- Chloroform-d (99.8 atom % D) was purchased from Sigma Aldrich (USA).
- D,L-lactide (LA, 99.9%) was purchased from Polysciences, Inc. (Pennsylvania).
- Glycolide (GA, 99.9%) was purchased from Sigma-Aldrich (USA).
- Itaconic anhydride (98%) flushed with nitrogen was purchased from Acros Organics.
- Liquid nitrogen was purchased from Linde Gas, a.s., Czech Republic.
- Lithium phenyl-2,4,6-trimethylbenzoylphospinate (LiTPO) was prepared under collaboration at the Technical University of Vienna.
- Physiological solution (0.9% NaCl) was purchased from B. Braun (Germany).
- Poly(ethylene glycol) (PEG) with M_n = 1500 g·mol^{-1} was purchased from Fluka (Germany).
- Poly(ethylene glycol) diacrylate (PEGDA) with M_n = 6000 g·mol^{-1} was purchased from Sigma-Aldrich (USA).
- Tin octanoate (SnII 2-ethylhexanoate, 95%) was purchased from Sigma-Aldrich (USA).
- Tetrahydrofuran (THF, p.a.) was purchased from PENTA (Czech Republic).
- Ultra-pure water type I was prepared on a water purification system Milipore Direct-Q® 3 UV equipped at CEITEC BUT.

4.2 Equipment

- Common laboratory equipment and glassware, e. g. beakers, flasks, spatulas etc.
- OHAUS Adventurer Pro, analytical Balance (Germany)
- All glass high-vacuum line (hand-made at CEITEC BUT)
- CHRIST Epsilon 2-10D LSCplus, lyophilizator (Germany)
- BLUEDENT LED smart – BG LIGHT LTD (Bulgaria)
- Thermoblock HLC (DITABIS AG, Germany)
- Rheometer TA Instruments AR-2 (TA Instruments, USA)
- GPC, Agilent 1260 Infinity, Wyatt Technology (Germany) equipped with MALS photometer HELEOS-II and RI Optilab T-rEX detector.
- Incubator CO2cell (MMM group, Germany)
- 700 MHz NMR spectrometer Bruker AVANCE III (Bruker Co., Germany)
- ATR-FTIR, Vertex 70v (Bruker Co., Germany)

4.3 Methods

4.3.1 Synthesis of PLGA–PEG–PLGA

The PLGA–PEG–PLGA copolymer with a theoretical PLGA/PEG weight ratio of 2.5 and a LA/GA molar ration of 3 was synthesized via ROP method according to Michlovská (2009) [26].

The copolymer was synthesized in a two-neck reaction flask using an all glass high-vacuum line (Figure 11). Firstly, the flask and the manifold were purged three times by nitrogen and vacuum. Prior to the synthesis PEG (M_n = 1500 g·mol^{-1}) was added into the flask against the nitrogen flow together with a magnetic stirrer and purified under vacuum at 130 °C for three hours.

After the purification, the flask was let to cool down and the calculated amount of lactide and glycolide were added to the vacuum dried PEG against the nitrogen flow. The flask and the manifold were purged again three times by nitrogen and vacuum. The mixture was then placed in an oil bath with a set temperature of 130 °C. When homogenized, a catalyst Sn(II)2-ethylhexanoate was injected. The synthesis ran for three hours.

Figure 11: High-vacuum line

In order to separate the prepared copolymer from unreacted monomers, the product was dissolved in ultra-pure water (approximately 10 wt. %) at 3 °C. The solution was then heated to 80 °C and the copolymer precipitated from the solution. The solution with the dissolved monomers was poured out. The decantation was repeated three times.

4.3.1.1 Functionalization by itaconic anhydride

The PLGA–PEG–PLGA copolymer was synthesized as stated in paragraph 4.3.1. After the synthesis the flask was let to cool down. The itaconic anhydride was added into the flask against a nitrogen flow. The flask and the manifold were again purged three times by nitrogen and vacuum. The copolymer was functionalized under nitrogen atmosphere and intense stirring at 110 °C for one hour. The ITA/PLGA–PEG–PLGA/ITA copolymer was purified as it is stated above (4.3.1).

4.3.2 Characterization of the copolymer

The synthesized PLGA–PEG–PLGA and functionalized ITA/PLGA–PEG–PLGA/ITA were further characterized.

4.3.2.1 Proton Nuclear Magnetic Resonance (^{1}H NMR)

The molecular weight, PLGA/PEG, LA/GA ratios and the amount of bonded ITA were confirmed using ^{1}H NMR spectroscopy on 700 MHz Bruker AVANCE III HD instrument using 128 scans in deuterated chloroform solvent at 25 °C. Measurement was provided by dr. Humpa from Masaryk University in Brno. Spectra were evaluated using ACD/1D NMR Processor.

4.3.2.2 Gel Permeation Chromatography (GPC)

The number average molecular weight (M_n) and polydispersity index (M_w/M_n) of the copolymers were determined by GPC. The instrument was equipped with multi-angle light scattering photometer HELEOS-II and refractive index Optilab T-rEX detectors (Wyatt Technology, Germany). Two columns PLgel 5 μm Mixed-C were used for separation. Tetrahydrofuran (THF) served as the mobile phase at a flow rate of 1 ml/min against polystyrene standard (M_w = 30 000 g/mol, polydispersity index 1.06). The measurement was performed by dr. Brtníková from CEITEC. The received data were evaluated using Astra software.

4.3.2.3 Rheology

The sol-gel transition and rheological properties of aqueous solutions of PLGA–PEG–PLGA and ITA/PLGA–PEG–PLGA/ITA were studied using rheometer TA Instruments AR-2. A cone-plate geometry with diameter 40 mm and 2° angle was used for the measurement. The copolymer solution (600 μl) was transferred to the Peltier and a geometry gap was set to 60 μm. The measurements were carried out with the frequency set to 1 rad/s and with the temperature increasing from 20 to 60 °C at rate 0.5 °C/min. During the measurements the solvent trap was filled with water to prevent water evaporation. The measurement was performed by Ing. Valová from CEITEC.

4.3.3 Proof of chemical crosslinking

The 20 wt. % aqueous solution of ITA/PLGA–PEG–PLGA/ITA was prepared. The polymer was weighed into a vial and a calculated amount of ultra-pure water was added. Then it was stirred on a magnetic stirrer for 4–6 days at 8 °C. Afterwards, the water soluble PI LiTPO was added to the solution (1 wt. %). The concentrations of the copolymer and the PI were adopted from the master thesis of Habánková [32]. After the addition of LiTPO to protect it from the light all manipulations with the

solution either took place in a yellow lab or the sample was protected with the aluminium foil. The sample was loaded onto a small Petri dish and put on a thermoblock heated at 37 °C. The physical network was formed.

Chemical crosslinking was achieved by irradiation of the physically crosslinked hydrogels with BLUEDENT LED Smart lamp, emitting light with wavelengths from 430 to 490 nm and intensity around 1200 mW/sq.cm. The lamp was attached so the distance between the tip and the Petri dish was 5 cm.

In order to proof the formation of chemical network, the prepared sample was put into acetone. Non-irradiated copolymer dissolves rapidly in acetone however chemically crosslinked hydrogel does not.

4.3.3.1 Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR measurements before and after irradiation by blue light were performed on the spectrometer Vertex 70v. Prior to the measurement all samples were dried in lyophilizator. Spectra were recorded using 128 scans from 700 to 4000 cm^{-1} at resolution 4 cm^{-1} on a Germanium crystal.

4.3.3.2 Water uptake

The irradiated samples were immersed in physiological solution (0.9% solution of NaCl) and left in the incubator at 37 °C. At certain time intervals the excess of physiological solution was removed with filter paper and the swollen hydrogel was weighed.

5 RESULTS AND DISCUSSION

5.1 Characterization of PLGA–PEG–PLGA and ITA/PLGA–PEG–PLGA/ITA

The thermosensitive PLGA–PEG–PLGA copolymer was synthesized via ring-opening polymerization and further functionalized with ITA to form a temperature and light sensitive ITA/PLGA–PEG–PLGA/ITA copolymer. Theoretical values and results from GPC and NMR are shown in the Table 1. Both copolymers exhibit a very low polydispersity index. The ratios of PLGA/PEG and LA/GA calculated from the NMR spectra differs a bit from the theoretical values. However, this difference is acceptable. The number average molecular weight (M_n) obtained from GPC was at both measurements higher than the one obtained from NMR spectroscopy. The M_n calculated from NMR spectrum was in a good agreement with the theoretical value. The degree of modification by ITA was around 54 %.

Table 1: Characterization of PLGA–PEG–PLGA (ABA) and ITA/PLGA–PEG–PLGA/ITA (ABA/ITA). [1] theoretical values, [2] obtained from GPC, [3] obtained from NMR spectroscopy.

Sample	M_w/M_n[2]	PLGA/PEG[1] [wt/wt]	PLGA/PEG[3] [wt/wt]	LA/GA[1] [mol/mol]	LA/GA[3] [mol/mol]	M_n[1] [g/mol]	M_n[2] [g/mol]	M_n[3] [g/mol]	ITA[3] [mol.%]
ABA	1.080	2.50	2.53	3.00	2.99	5 250	5 767	5 425	-
ABA/ITA	1.084		2.54		2.96		6 016	5 371	54.4

The ^{1}H NMR spectrum of PLGA–PEG–PLGA is shown in Figure 12. The characteristic peaks of lactide were found in the range of 5.28 – 5.12 ppm, representing the CH group (a), and 1.62 – 1.51 ppm, representing the CH_3 group (e). The peaks in the interval of 4.91 – 4.64 ppm (b) stand for the CH_2 group in glycolide. The peak (c) around 4.3 ppm represents the CH_2 group between PEG and lactide. The characteristic peak of PEG is in the range of 3.7 – 3.5 ppm.

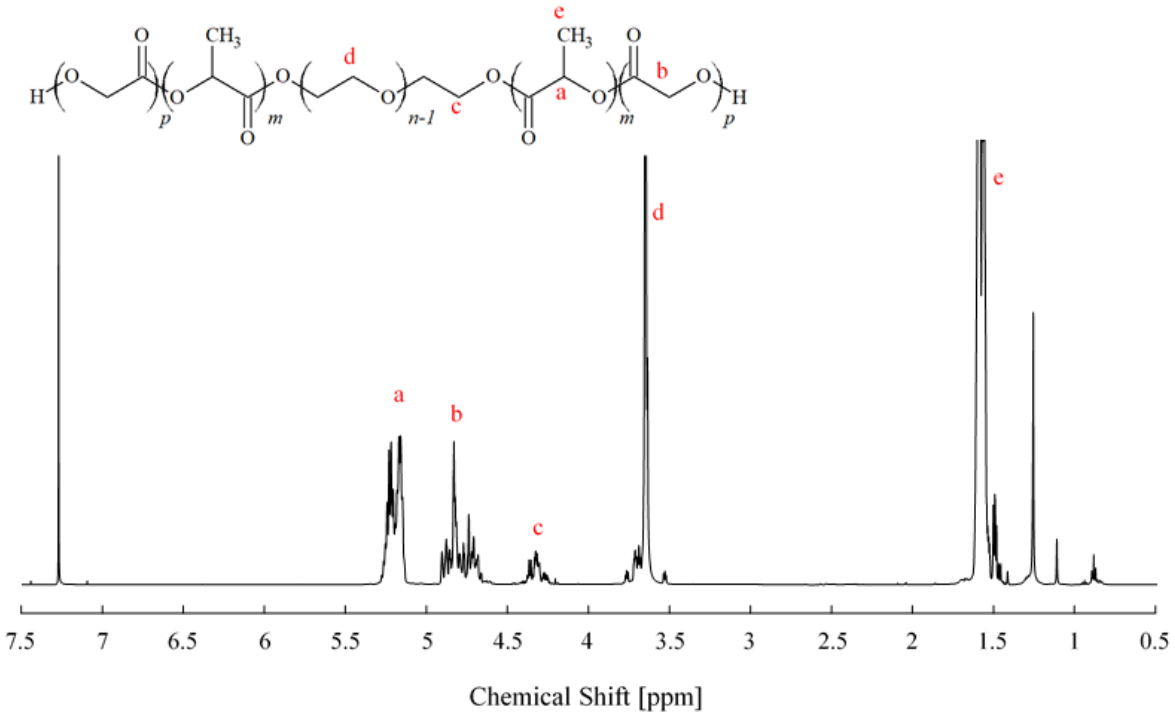

Figure 12: ^{1}H NMR spectrum of PLGA–PEG–PLGA.

The ^{1}H NMR spectrum of modified ITA/PLGA–PEG–PLGA/ITA is shown in Figure 13. All the characteristic peaks from above can be also seen in this spectrum. Additionally, there are two small peaks (f, g) at around 6.43 and 5.84 ppm. These peaks stand for protons in itaconic acid double bonds. The amount of linked ITA was determined from integrals of the peak intensities.

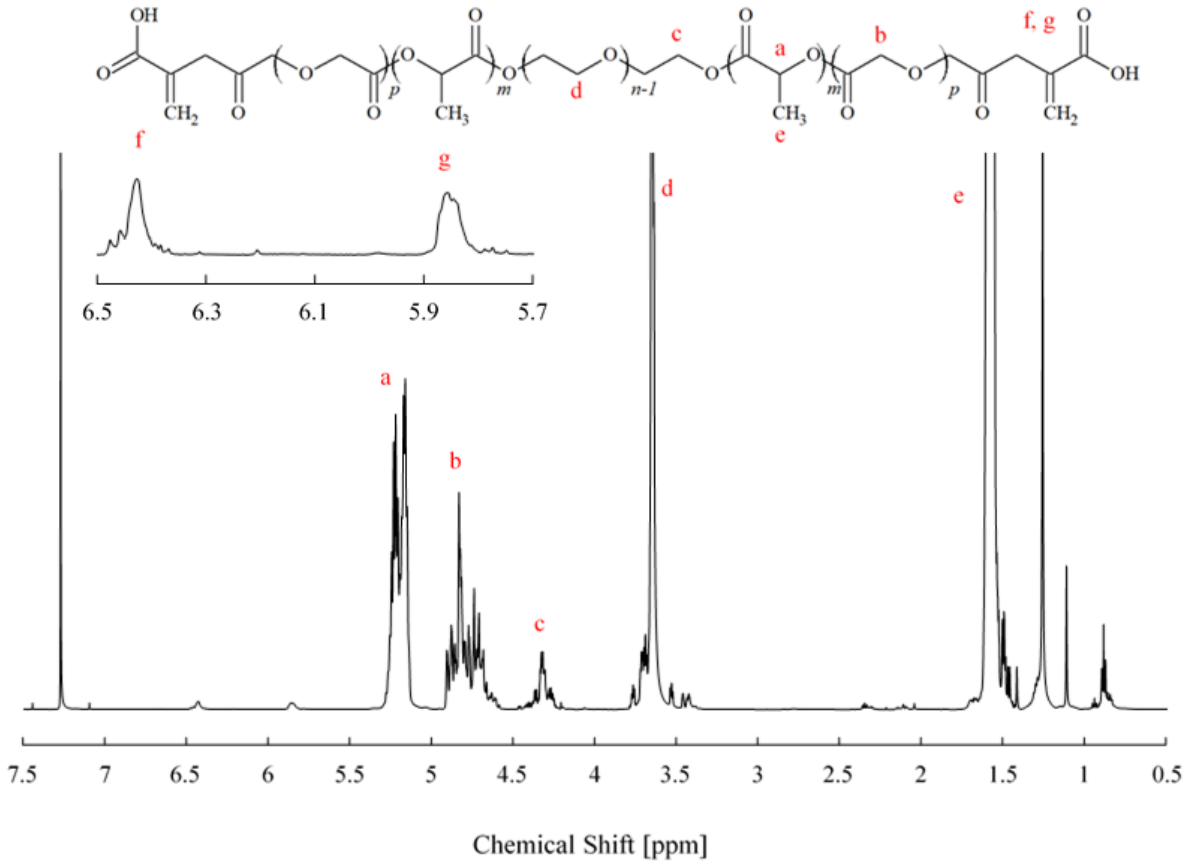

Figure 13: ^{1}H NMR spectrum of ITA/PLGA–PEG–PLGA/ITA.

5.2 Physical crosslinking

Thermosensitive triblock ITA/PLGA–PEG–PLGA/ITA and PLGA–PEG–PLGA copolymers form a free-flowing solution below the CGT. Due to their amphiphilic properties they generate micelles. They have a hydrophobic core, formed by PLGA blocks, and a hydrophilic shell containing PEG blocks. With the increasing temperature the PLGA blocks diffuse into other micelles and form a bridge. PLGA bridges stiffen the structure. When the CGT is reached a gel is formed (sol-gel transition). The number of PLGA bridges grows rapidly with the rising temperature. With even higher temperatures the gel structure starts to break apart. The PLGA blocks inside the micelles start to shrink and the PEG blocks undergo dehydration, which results in formation of suspension (gel-suspension transition) [4]. The transitions are captured on the Figure 14. In the case of the ITA/PLGA–PEG–PLGA/ITA and PLGA–PEG–PLGA copolymers the sol phase occurs at laboratory or lower temperatures. The gel is formed at a physiological temperature (37 °C). When the temperature is increased the copolymer precipitates from the solution and the white suspension is formed.

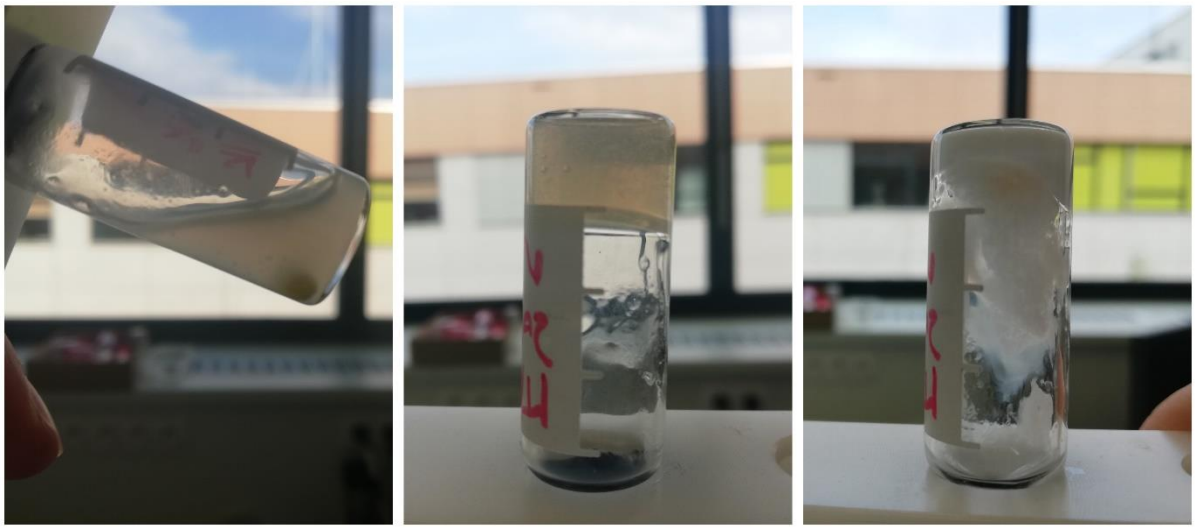

Figure 14: Visual representation of the phase transitions. The photo on the left shows a sol, the middle one a gel (at 37 °C) and the right one a suspension.

The effect of the concentration of aqueous solutions and modification by itaconic anhydride on physical crosslinking has been investigated. The most important studied feature was the temperature when the copolymer exhibits the highest stiffness. The temperature should be as close to the body temperature as possible.

The rheological analysis of 20 wt. % aqueous solution of PLGA–PEG–PLGA is shown on Figure 15. The temperature of the first cross-point of storage (G′) and loss (G′′) moduli represents the sol-gel transition (T_{SG}). The temperature of the second cross-point of G′ and G′′ represents the gel-suspension transition (T_{GS}). The temperature when the G′ reaches the maximum (G'_{max}) is when the gel has the highest stiffness. The exact temperatures are written in the Table 2. The PLGA–PEG–PLGA copolymer was in a form of gel in the temperature interval 33.4 – 40.3 °C. Therefore, it can be stated that, when injected into a human body, the copolymer will form a gel.

Table 2: Cross-points of PLGA–PEG–PLGA (ABA) copolymer with concentration 20 wt. % from Figure 15.

Sample	T_{SG} [°C]	T (G'_{max}) [°C]	T_{GS} [°C]
ABA	33.4	38.2	40.3

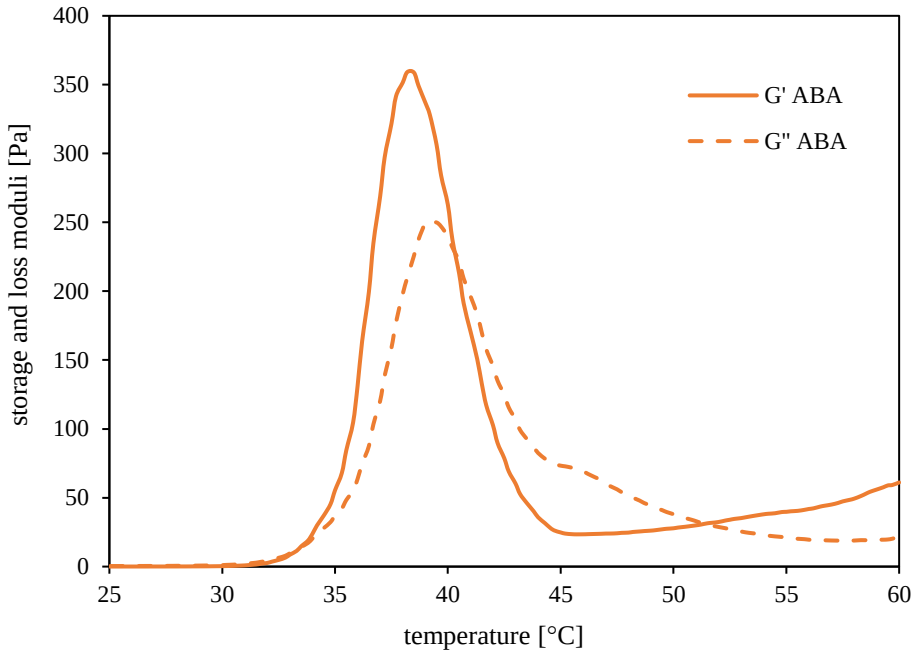

Figure 15: Rheological properties of PLGA–PEG–PLGA (ABA) copolymer.

The influence of the functionalization by itaconic anhydride on the rheological properties has been investigated. On Figure 16 the storage moduli of PLGA–PEG–PLGA and ITA/PLGA–PEG–PLGA/ITA copolymers is shown. There is a visible shift of G'_{max} as the functionalized copolymer displays the highest stiffness at around 37 °C and the PLGA–PEG–PLGA copolymer at around 38.5 °C.

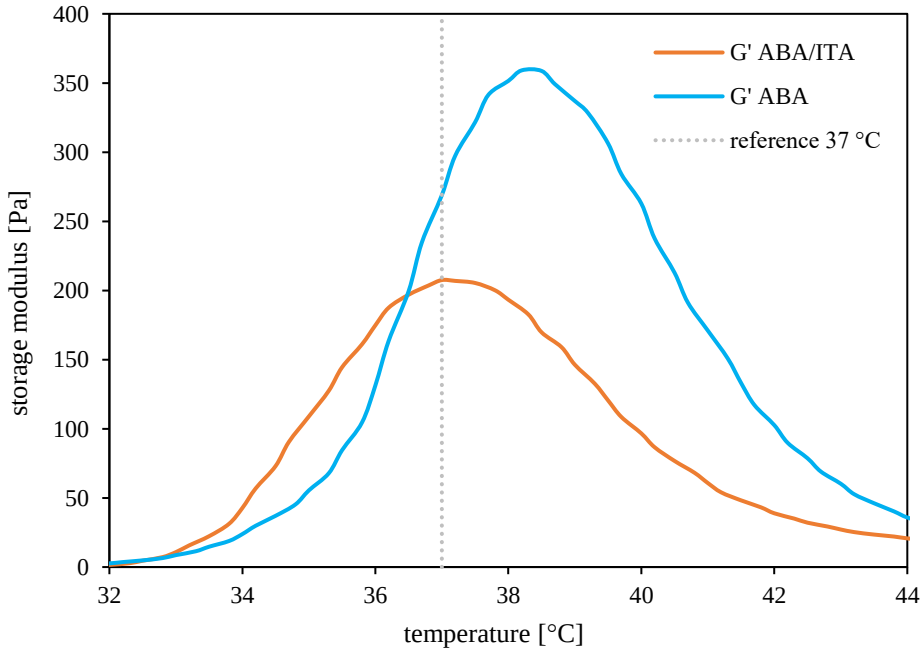

Figure 16: Comparison of storage moduli of PLGA–PEG–PLGA (ABA) and ITA/ PLGA–PEG–PLGA/ITA (ABA/ITA) copolymers solutions with concentration 20 wt. %.

The rheological properties of series of ITA/PLGA–PEG–PLGA/ITA copolymers with different concentrations (5, 10, 15 and 20 wt. %) in ultra-pure water were measured. The results are shown in Figure 17. The gel stiffness detected by G´ increases with the increasing concentration of the copolymer. Moreover, the G'_{max} moves to 37 °C (body temperature) with the increasing concentration of the copolymer.

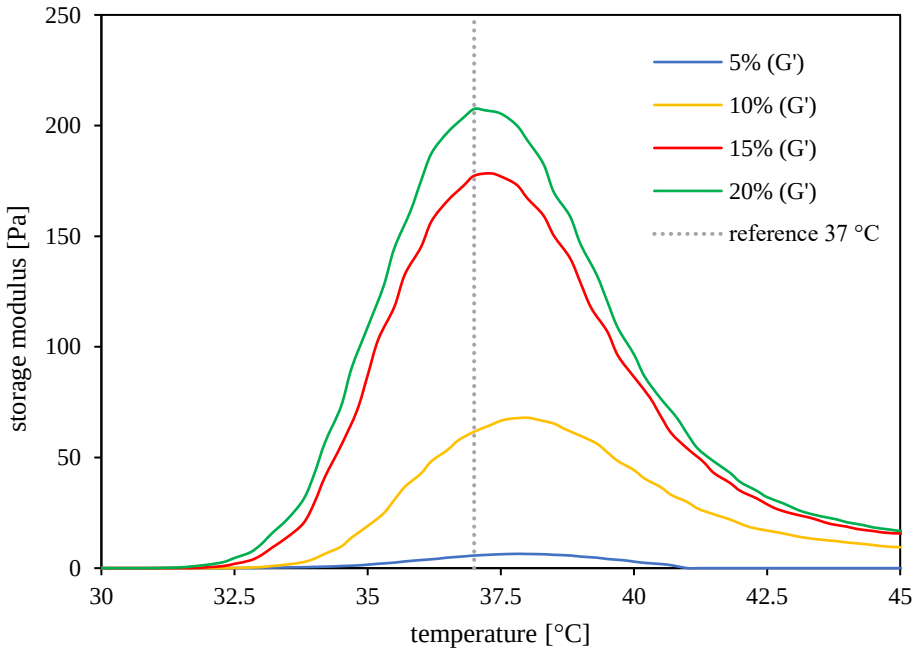

Figure 17: The effect of concentration on rheological properties of the copolymer ITA/ PLGA–PEG–PLGA/ITA.

5.3 Hybrid network

In the case of the ITA/PLGA–PEG–PLGA/ITA copolymer, the chemical crosslinking is enabled by the double bonds contained in the itaconyl group in the copolymer. The scheme of the process of forming a hydrogel stabilized with hybrid network is drawn on Figure 18. Firstly, a physical (micellar) network was formed in aqueous solution heated to 37 °C. This physically crosslinked hydrogel was subsequently irradiated by blue light, producing radicals which attack double bonds presented in the copolymer structure and forming new covalent bonds. As the double bonds are found in the core of the micelles, the micelle structure of this copolymer is secured by chemical crosslinking of the core.

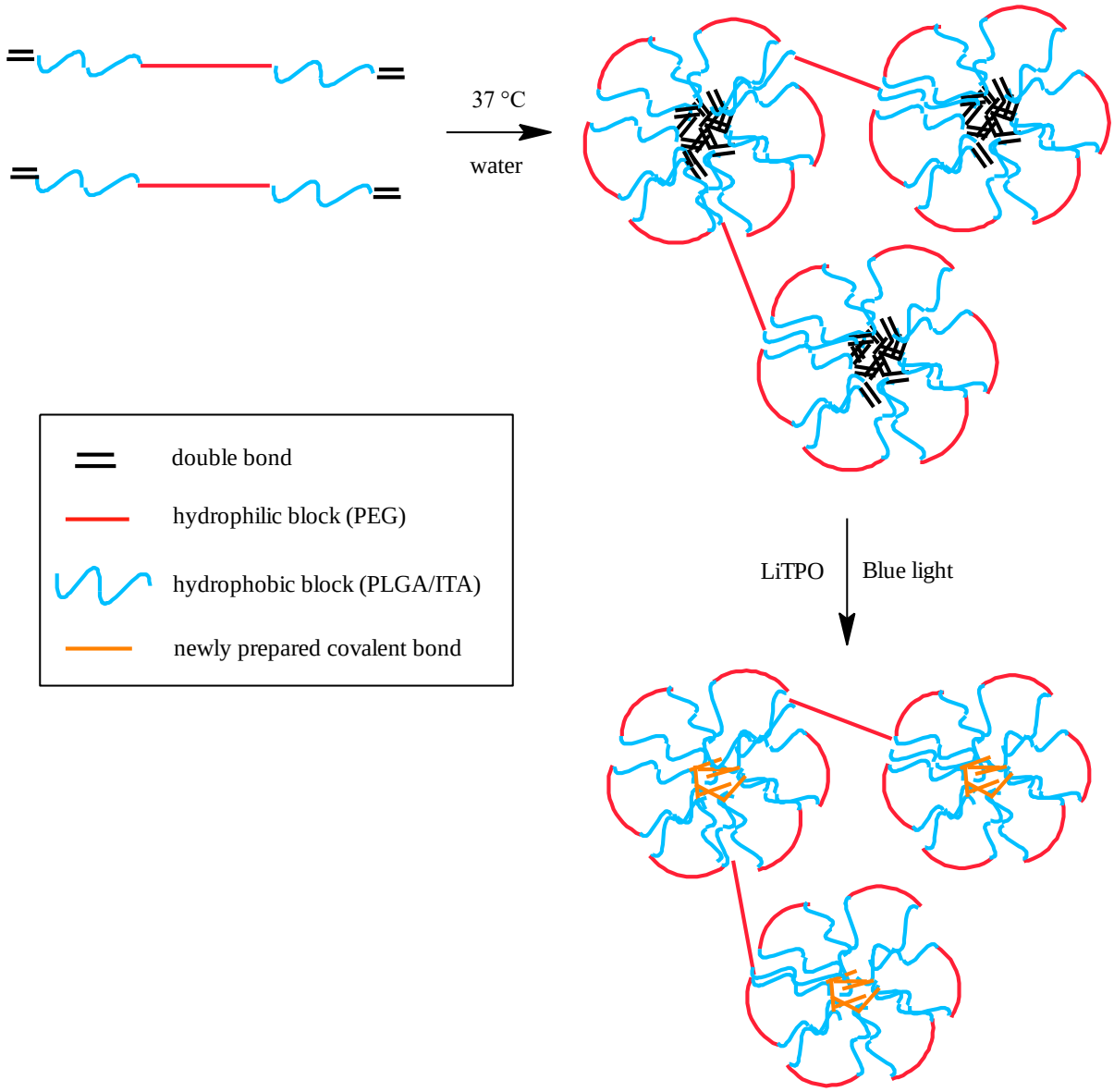

Figure 18: Photo-induced crosslinking of micelles of the ITA/PLGA–PEG–PLGA/ITA copolymer.

In the first place, the minimal time of irradiation for the chemically crosslinked hydrogel to be formed had to be determined. The samples were irradiated for different time and the products were immersed in ultra-pure water and acetone. The uncrosslinked or physically crosslinked hydrogel would dissolve rapidly in acetone however the chemically crosslinked hydrogel would not. It was found out that when irradiating with blue light (430 – 490 nm, intensity of 1200 mW/sq.cm) the minimal irradiation time required to form chemically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogel in ultra-pure water in the presence of 1 wt. % LiTPO at 37 °C is 6 min. This time of irradiation was further used in the experimental part. The proof of chemical crosslinking can be seen on the Figure 19. On the left, the prepared hydrogel is immersed in ultra-pure water and on the right, it is immersed in acetone. It is visible that the hydrogel is insoluble in acetone. The resulting hydrogel with hybrid network is sticky and flexible. As it can be seen on Figure 20, the hydrogel is transparent.

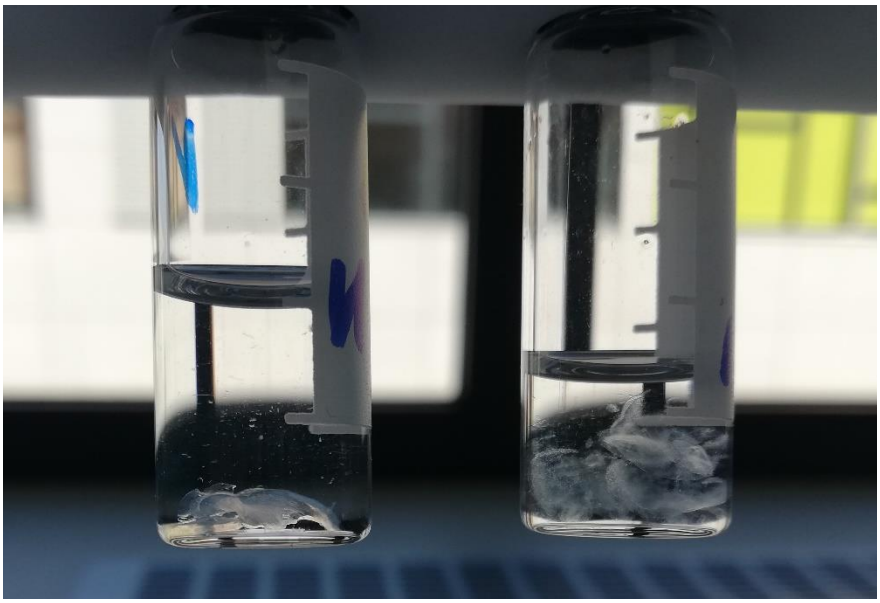

Figure 19: Hydrogel stabilized with hybrid network immersed in water (left) and in acetone (right).

Figure 20: Hydrogel with hybrid network.

5.3.1 Photopolymerization with crosslinker PEGDA

To compare, 20 wt. % ITA/PLGA–PEG–PLGA/ITA solution was crosslinked in the presence of 1 wt. % LiTPO with the addition of crosslinker poly(ethylene glycol) diacrylate (PEGDA). The concentration of PEGDA was 5 mol. % per double bonds. The concentration of PEGDA was adopted from the master thesis of Habánková [32]. After the addition, the solution was homogenised in ultrasonic bath for 1 hour. Otherwise the hydrogels were prepared the same way.

To determinate the minimal irradiation time, same steps were followed as for the samples without the crosslinker. When irradiated for 6 min (Figure 21, left) the hydrogel was not as solid as the chemically

crosslinked hydrogels without the crosslinker. It was impossible transfer it from the Petri dish. As the acrylates react very fast, the minimal irradiation time required to form chemically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogel with the addition of crosslinker PEGDA in ultra-pure water at 37 °C is 15 s. The minimal irradiation time of samples with PEGDA is much lower than of those without the crosslinker. This indicates that in the sample with PEGDA irradiated for 6 min has led to β-scission and partial recombination of double bonds. It would also explain why the sample did not maintain its shape. The hydrogel with crosslinker irradiated for 15 s was flexible and maintained its shape. For the next experiments the samples with the addition of crosslinker were irradiated for 6 min in order to be comparable to the samples without any crosslinker.

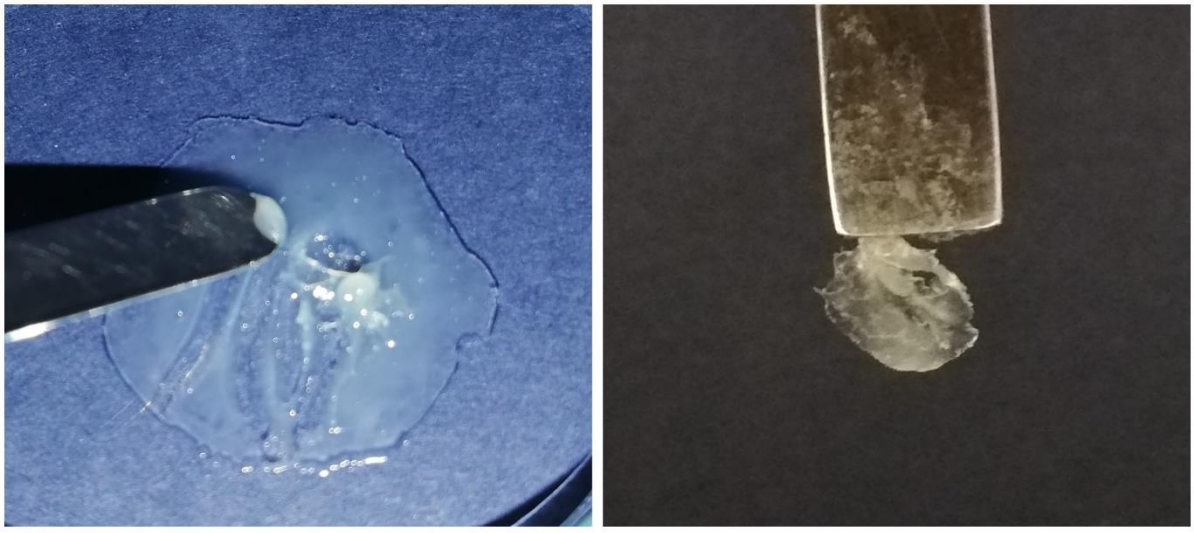

Figure 21: Hydrogels formed with crosslinker PEGDA. Irradiated for 6 min (left) and 15 s (right).

The hydrogel prepared with the addition of crosslinker PEGDA and irradiated for 6 min was less transparent than the hydrogel without crosslinker irradiated for the same time. This might be due to the β-scission and partial recombination of double bonds as the irradiated hydrogel with crosslinker looks similar to the unirradiated hydrogel with only micellar network.

Figure 22: Comparison of hydrogel without (left) and with crosslinker PEGDA (right).

5.3.2 ATR-FTIR

The samples before and after photocrosslinking, with and without PEGDA were measured on infrared spectroscopy (Figure 23). The peaks at wavenumber 3500 cm^{-1} (a) correspond to –COOH groups. The peaks at wavenumber 2900 cm^{-1} (b) stand for $–CH_2–$ and in the samples with PEGDA the peak (b) and the peak at wavenumber 1342 cm^{-1} (e) correspond also to $–O–CH_2$ groups. Peak at 1750 cm^{-1} (c) stand for C=O group and peak at 1100 cm^{-1} (f) to R–CO–R. When compared to the IR spectrum of PEGDA, the peaks at 950 (g) and 840 cm^{-1} (h) of samples with crosslinker correspond to double bonds in the structure of PEGDA. The decrease of double bonds in irradiated ITA/PLGA–PEG–PLGA/ITA hydrogel at 1640 cm^{-1} (d) and the formation of new bonds at 840 cm^{-1} (h) were observed (Figure 24). The spectra of samples with crosslinker irradiated and unirradiated were the same due to the β-scission of newly prepared covalent bonds back to double bonds, this was caused by the long irradiation time.

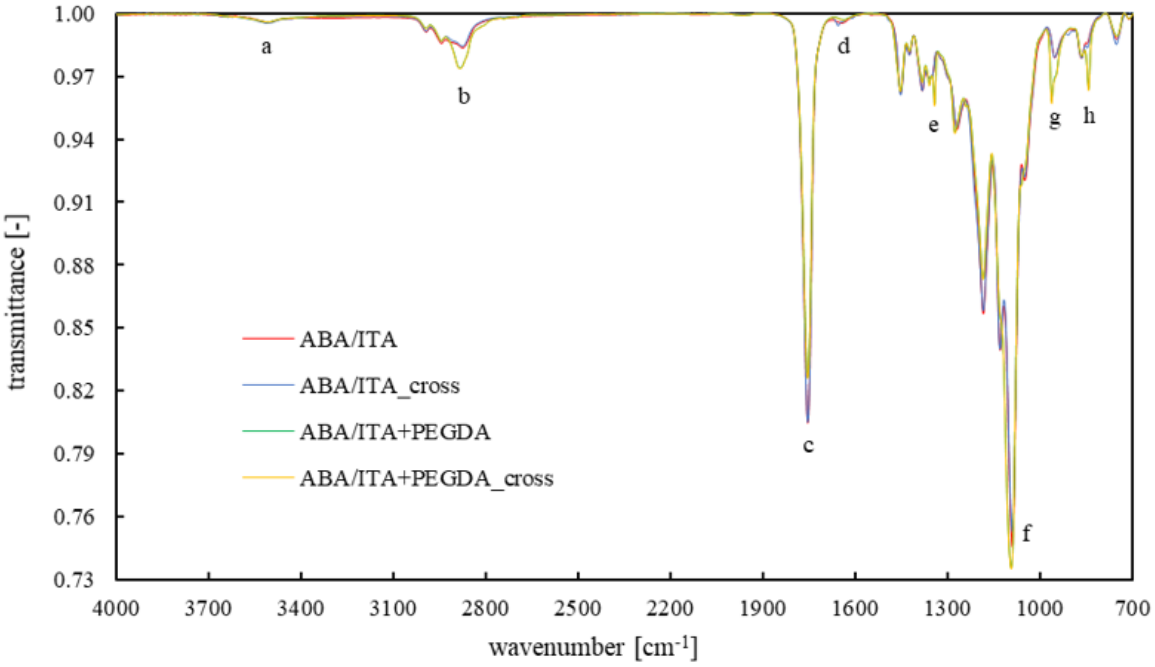

Figure 23: Infrared spectra of samples before and after photocrosslinking, with and without PEGDA.

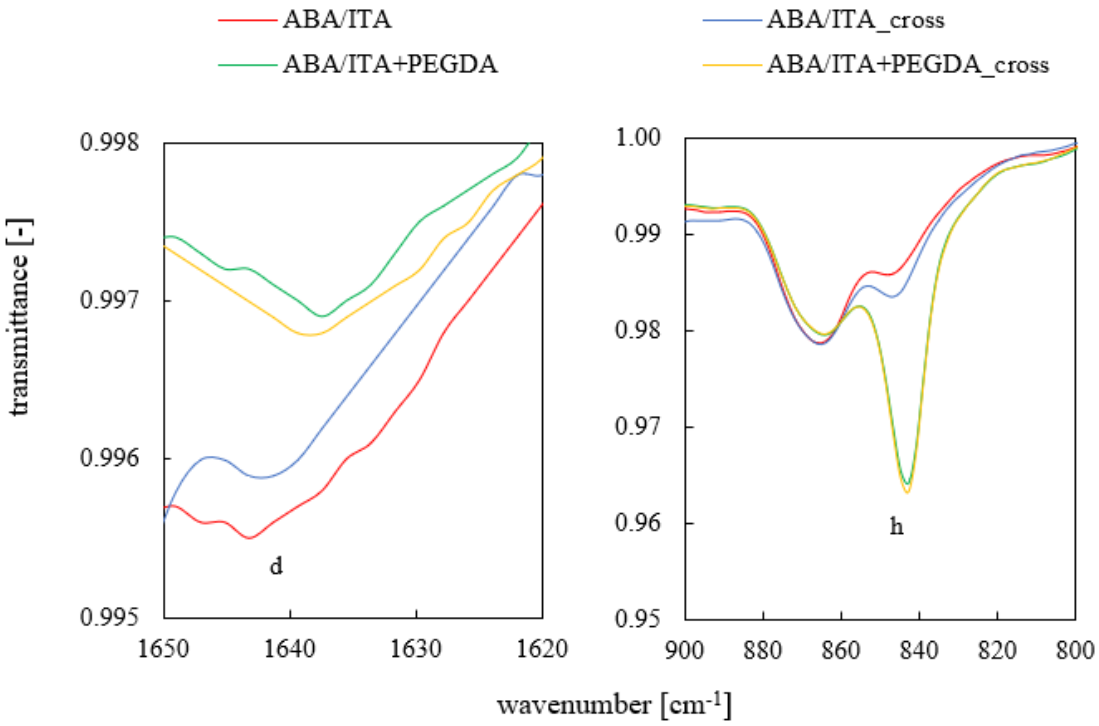

Figure 24: Zoom of spectra in the range 1650–1620 and 900–800 cm^{-1}.

5.3.3 Water uptake

Water uptake and stability of hydrogels with hybrid network with and without the addition of crosslinker were measured in physiological solution at 37 °C.

From the graph (Figure 25) it is apparent that the water content of hydrogels without crosslinker increased more gradually than in the case of hydrogels with PEGDA and reached maximum after nearly 4 days. The average maximal water uptake of samples without crosslinker was 1176 % (weight increased in average 12 times). Subsequently to reaching the peak of water content, the network nodes broke and the PLGA ester bonds hydrolysed. Lactic and glycolic acid were released from the hydrogel. The degradation curve is nearly linear (Figure 26). The hydrogels without crosslinker dissolved after 20 days.

The hydrogel with crosslinker absorbed water rapidly and reached maximum in the first 8 hours of swelling (Figure 27). The samples reached an even higher average maximal water uptake, 1339 % (weight increased in average 14 times). However, after reaching the maximum the hydrogels with PEGDA started to degrade dramatically and after only 2 days the water content dropped to 883 %. Afterwards the degradation moderated. The samples completely degraded after 15 days. The rapid absorption and shorter lifetime of the samples with crosslinker is probably due to the β-scission of new covalent bonds which causes less dense chemical network (5.3.1).

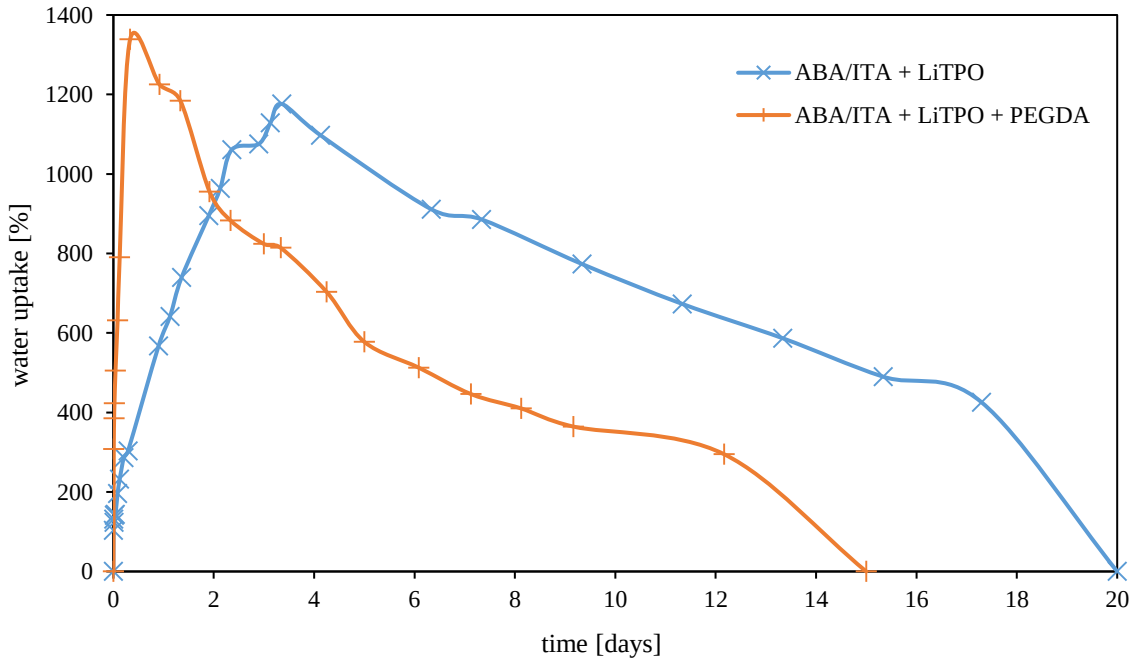

Figure 25: Water uptake of chemically and physically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogel with and without the addition of a crosslinker.

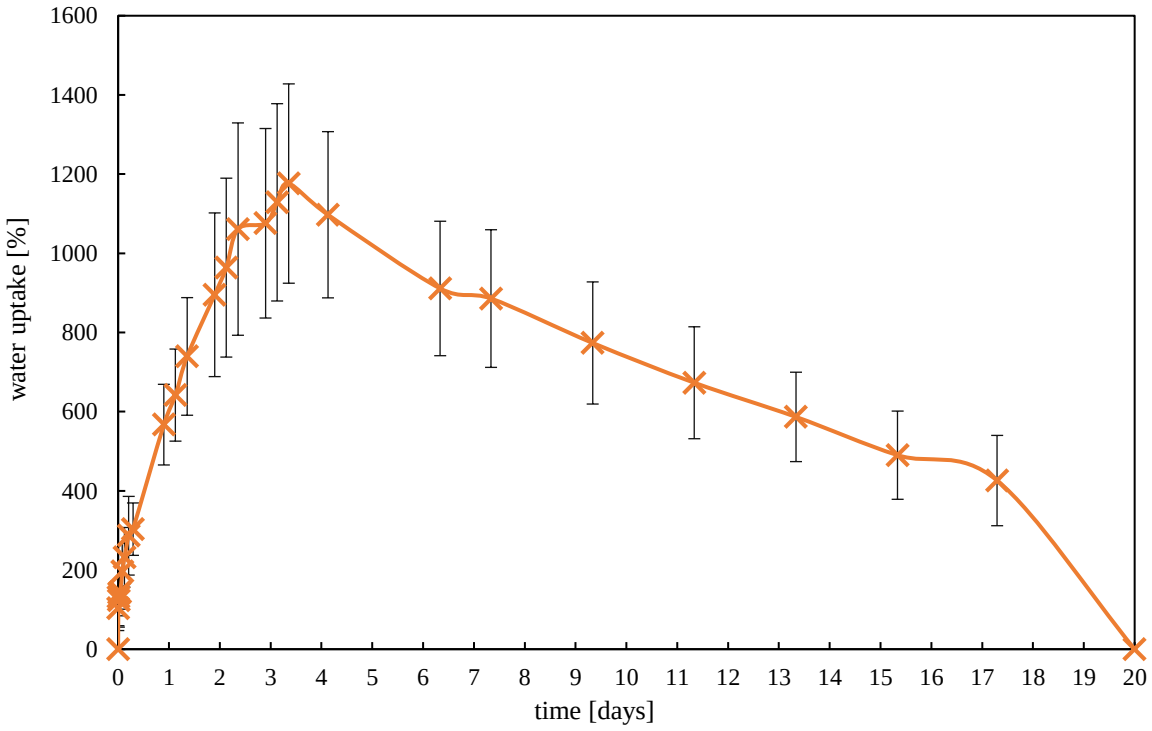

Figure 26: Water uptake of ITA/PLGA–PEG–PLGA/ITA hydrogel stabilized with hybrid network.

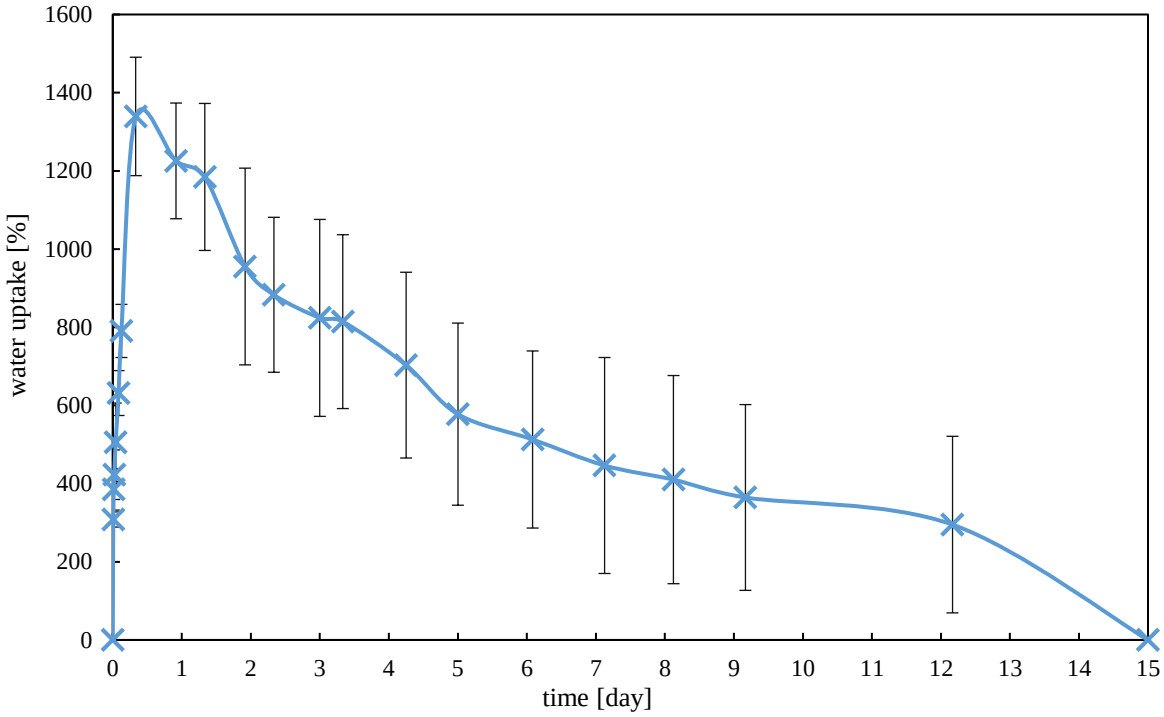

Figure 27: Water uptake of hydrogel with hybrid network with the addition of crosslinker PEGDA.

On Figure 28 photos of a hydrogel with hybrid network during swelling are shown. Photo 1 shows the hydrogel before swelling, photo 2 is the hydrogel after 6 days of swelling in physiological solution and photo 3 shows the sample after 18 days. The hydrogel before swelling is transparent. As the hydrogel absorbs water it becomes less transparent (Figure 28, photos 2 and 3). Pores in the hydrogel structure are visible on the photo of the hydrogel after 18 days in physiological solution (Figure 28, 3). The photo of the dissolved hydrogel after 20 days is captured on Figure 28 (photo 4). The hydrogel has degraded to oligomers.

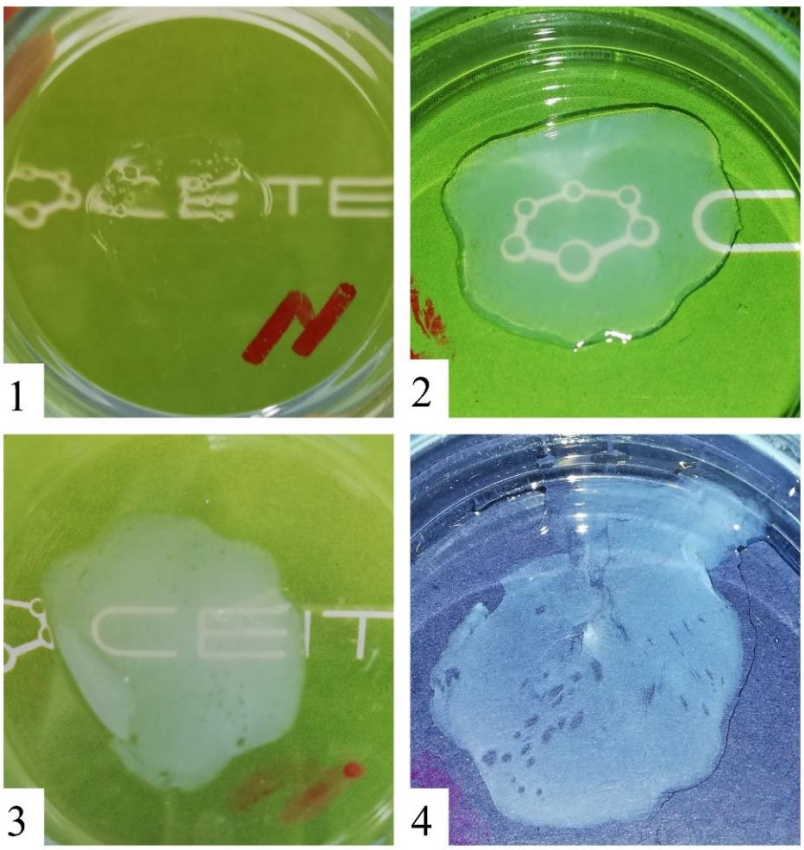

Figure 28: Chemically and physically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogels before water uptake (1), after 6 days (2), 18 days (3) and dissolved (4).

On Figure 29 hydrogels with hybrid network formed with the addition of crosslinker PEGDA are captured. On the left, there is the hydrogel without any absorbed water. The hydrogel after 1 day in physiological solution has apparently grown in size (Figure 29, middle) as it has absorbed more than 1200 % of water. The hydrogel with crosslinker has degraded in a different manner than the hydrogel without crosslinker. While immersed in physiological solution small parts were cleaved from the hydrogel with PEGDA. In the end, it had not dissolved as the hydrogel without crosslinker did (Figure 28, 4) however it had cleaved into small pieces.

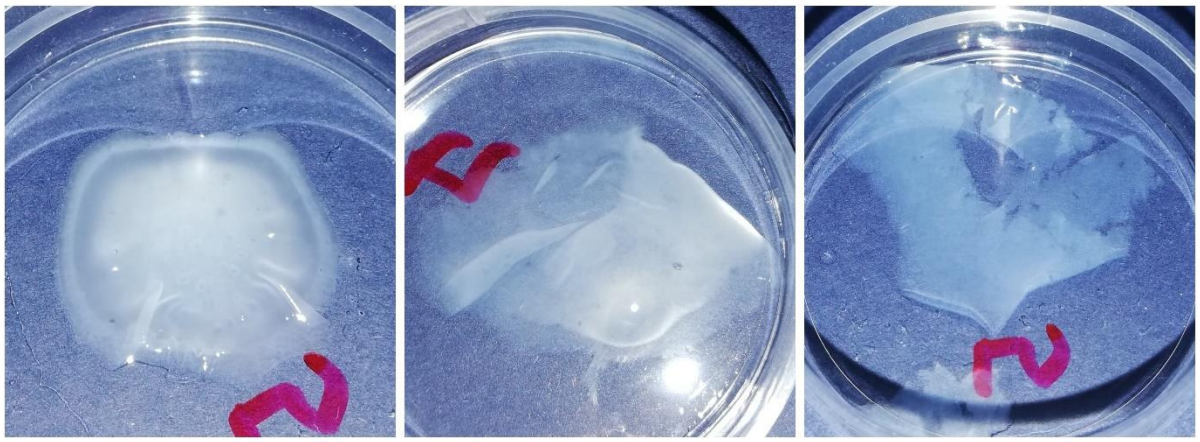

Figure 29: Swelling of hydrogels formed with crosslinker PEGDA. Before water uptake (left), after 1 day (middle) and 6 days (right).

6 CONCLUSION

The thermosensitive PLGA–PEG–PLGA copolymer was synthesized via living ROP. The copolymer was further functionalized with itaconic anhydride to prepare temperature and light sensitive ITA/PLGA–PEG–PLGA/ITA copolymer. The structure and molecular weight of the prepared copolymers were confirmed in terms of ^{1}H NMR spectroscopy and GPC. The PLGA/PEG and LA/GA ratios as well as the molecular weight measured by ^{1}H NMR were in a good agreement with the theoretical values. The calculated molar amount of polymer chains modified by itaconate groups was equal to 54 %. The polydispersity index of both copolymers was around 1.08 which indicates a uniformity of lengths of the copolymers.

Furthermore, rheological properties of the synthesized copolymers were studied in relation to the temperature. It was confirmed that synthesized copolymers form a physical network at physiological temperature. The effect of functionalization by itaconic anhydride and concentration of aqueous solutions on the physical network was investigated. ITA-modification reduced temperature of gelation by its shifting towards physiological temperature. The 20% aqueous solution of functionalized ITA/PLGA–PEG–PLGA/ITA copolymer exhibited G'_{max} exactly at body temperature (37 °C). As expected, increased copolymer concentration improved the hydrogel stiffness (G´).

The physically crosslinked ITA/PLGA–PEG–PLGA/ITA hydrogel was photocrosslinked by blue light in the presence of a water soluble LiTPO (1 wt. %) photoinitiator to form a hydrogel stabilized with hybrid network. In order to prove whether the chemical network was formed, samples were extracted in acetone. If hydrogels were chemically crosslinked, they were insoluble in acetone. The minimal irradiation time required to form a hybrid network for ITA/PLGA–PEG–PLGA hydrogel was 6 min resulting in transparent and flexible hydrogel. Due to the long crosslinking time, photoreactive PEGDA has been added in the amount of 5 mol. % PEGDA (per double bonds of ITA-modified copolymer) thus decreasing the irradiation time to only 15 s. As a qualitative measurement of irradiated and unirradiated samples with or without a crosslinker, the ATR-FTIR method was applied.

The water absorption and stability in physiological solution of photocrosslinked hydrogels with and without the addition of PEGDA was observed. The hydrogel prepared in the presence of PEGDA and irradiated for 6 min absorbed water up to 1339 % and degraded after 15 days in physiological solution at 37 °C. The ITA/PLGA–PEG–PLGA/ITA hydrogel with hybrid network irradiated for the same time absorbed less water (1176 %), degraded at a slower rate and dissolved after 20 days. It shows better crosslining efficiency without PEGDA, probably because 6 min of irradiation copolymers with PEGDA is too long when crosslinked copolymer started to degrade. Therefore, comparison of the ITA/PLGA–PEG–PLGA/ITA hydrogels with and without the PEGDA irradiated for only 15 s would help to better understand the PEGDA efficiency.

The proposed bachelor thesis proved photocrosslinking abilities of ITA/PLGA–PEG–PLGA/ITA hydrogel in aqueous environment that might be applicable for example as a wound dressing with a controlled and gradual release of an active compound.

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8 LIST OF ABBREVIATIONS AND SYMBOLS

ABA	PLGA–PEG–PLGA
ABA/ITA	ITA/PLGA–PEG–PLGA/ITA
BAPO	Biacylphosphineoxide
BAPO-OLi	Lithium biacylphospineoxide
BAPO-ONa	Sodium biacylphospineoxide
CGC	Critical gelation concentration
CGT	Critical gelation temperature
CQ	Camphorquinone
DNA	Deoxyribonucleic acid
GPC	Gel permeation chromatography
G′	Storage modulus
G′′	Loss modulus
HA	Hyaluronic acid
^{1}H NMR	Proton nuclear magnetic resonance
IR	infrared
ITA	Itaconic acid
ITA/PLGA–PEG–PLGA/ITA	α,ω-itaconyl-poly(D,L-lactic acid-*co*-glycolic acid)-b-poly(ethylene glycol)-b-poly(D,L-lactic acid-*co*-glycolic acid)
LiTPO	Lithium phenyl-2,4,6-trimethylbenzoylphosphinate
MAPO	Monoacylphosphineoxide
M_n	Number average molecular weight
M_w	Weight average molecular weight
Na-TPO	Sodium phenyl-2,4,6-trimethylbenzoylphosphinate
OPF	Oligo(polye[ethylene glycol] fumarate)
PCL	Poly(ε-caprolactone)
PEG	Poly(ethylene glycol)

PEGDA	Poly(ethylene glycol) diacrylate
PEO	Poly(ethylene oxide)
PGA	Poly(glycolic acid)
PHB	Poly[(*R*)-3-hydroxybutyrate]
PI	Photoinitiator
PLA	Poly(lactic acid)
PLGA	Poly(D,L-lactic acid-*co*-glycolic acid)
PLGA–PEG–PLGA	Poly(D,L-lactic acid-*co*-glycolic acid)-b-poly(ethylene glycol)-b-poly(D,L-lactic acid-*co*-glycolic acid)
PLLA	Poly(L-lactic acid)
PPF	Poly(propylene fumarate)
PPO	Poly(propylene oxide)
PVA	Poly(vinyl alcohol)
ROP	Ring-opening polymerization
T_{GS}	Gel-suspension transition temperature
THF	tetrahydrofuran
T_{SG}	Sol-gel transition temperature
UV	Ultra-violet
VIS	visible

9 LIST OF FIGURES

10 LIST OF TABLES