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The effect of PLGA-PEG-PLGA modification on the sol-gel transition and degradation properties

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Abstract. This paper deals with the influence of an incubation medium pH on the hydrolytic degradation of a novel thermosensitive biodegradable triblock copolymer based on hydrophilic poly(ethylene glycol) and hydrophobic copolymer poly(lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA), consequently modified at α,ω-ends with itaconic acid (ITA) resulting in α,ω-itaconyl(PLGA-PEG-PLGA). Itaconic acid, obtained from renewable resources, delivers a reactive double bond and carboxylic functional group to the end of PLGA-PEG-PLGA copolymer: this is important for a reaction with biologically active substances. The suitability of the sample degradation was assessed depending on whether the copolymer formed a gel at 37 °C. Two reversible physical sol-gel-sol transitions from a sol (liquid phase) to a gel (solid phase) and back to a sol (suspension) were verified using the tube inverting method. The hydrolytical degradation was evaluated at a physiological temperature (37 °C) in the presence of phosphate solutions, at a pH of either 4.2 or 7.4 by monitoring the decrease of the number average molecular weight of copolymers by GPC. Moreover, the degradation kinetics was confirmed by the HPLC/DAD method, where the increasing amount of final degradation products (lactic and glycolic acids) was detected. The study demonstrated that the carboxylic groups modified copolymer (ITA/PLGA–PEG– PLGA/ITA) is more susceptible to hydrolytical degradation than the unmodified copolymer within first days of degradation at pH 7.4.

Keywords: *biodegradable polymers, degradation, itaconic acid, sol-gel transition, lactic acid*

1. Introduction

'Smart' polymers, based on synthetic linear aliphatic polyesters, have been known for many years. Over the years they have been used in numerous biomedical and pharmaceutical products. ReGel® is one such product, and is frequently cited. It contains tri-block copolymers, (based on poly(lactic acid), poly(glycolic acid) and poly(ethylene glycol) (PLGA-PEG-PLGA)) [1]. PLGA-PEG-PLGA (or ABA) hydrogel systems have played an important role in the development of controlled delivery devices for a variety of drug and food related bioactive ingredients [2]. The use of ABA example as a bioadhesive for the treatment of bone fractures has offered advantages. For example, copolymer which remains in the body during surgery does not cause chronic foreign body reaction. In addition, subsequent removal of the implant surgery is not necessary [3]. Furthermore, PLGA-PEG-PLGA gradually degrades to lactic and glycolic acids, which are natural metabolites of the human body. Gradual external heating of a solution of this copolymer forms a highly viscous gel. A crucial factor is the ratio of the hydrophobic (PLGA) and hydrophilic (PEG) components [4]. Reversible phase behaviors, i.e. the change from the liquid phase (sol) to the gel phase, and from the gel phase to sol phase (a suspension), were demonstrated for the ABA copolymer with a molecular weight of PLGA

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block from 900–1600 g·mol^{-1} [5]. This behavior depends on the concentration of the copolymer in the solution and an increase in the temperature, which can be easily verified using the inverting test tube method. This can be shown graphically using a sol-gel phase diagram [6–14]. 'Smart' hydrogels based on PLGA-PEG-PLGA have been developed primarily as stimuli-responsive materials, which can undergo volume changes in response to a change in temperature, when exposed to a biological target [1]. The use of PLGA-PEG-PLGA as drug delivery systems is, however, limited since the copolymer has a low degree of functionality (it contains only hydroxyl functional groups). For this reason, much attention is devoted to the PLGA-PEG-PLGA functionalization. Modification of the triblock copolymer end-chain structure by itaconic acid (α,ω-itaconyl (PLGA-PEG-PLGA) = ITA/PLGA–PEG–PLGA/ITA) was first reported by our group [18]. Itaconic acid belongs to the group of unsaturated organic carboxylic acid [15] and involving double bond in the structure forms macro monomers permitting easy copolymerization [16]. Ionized itaconic acid has two carboxyl groups with different *pKa* values (pKa_1 3.85 and 5.45): suitable for forming hydrogen 'bridges' [17]. Itaconic acid, derived from renewable sources like molasses and hydrolyzed starch, exhibits high biocompatibility [15] and biodegradability, the main degradation products are acetate, lactate and carbon dioxide [19]. Copolymers based on lactic and glycolic acids undergo hydrolytical degradation of unstable ester bonds in an aqueous environment either *in vitro* or *in vivo* [21]. Whereas the hydrolytic cleavage of the PLGA ester bond under basic conditions is irreversible and leads to hydroxyl group and acid salt, while acidic pH induces reversible hydrolysis resulting in one hydroxyl group and one carboxyl group capable of catalyzing the hydrolysis of other ester bonds [20]. Random chain cleavage results in a substantial reduction in molecular weight for high molecular weight polyesters in this stage, without a consequent loss of polymer mass. In the subsequent phase (erosion) reduction of the molecular weight is accompanied by a significant decrease in polymer weight, resulting in water soluble monomeric and oligomeric products which diffuse to the surface of the polymer material [21]. Numerous factors influence the degradation rate of polyester based copolymers. The most important factors include the size and shape of the object, temperature, pH and basic properties of the copolymer. It was confirmed that the degradation rate of these systems in an aqueous environment depends on the overall hydrophilicity of the copolymer [22]. The proposed work discussed the effect of PLGA-PEG-PLGA modification by bioactive renewable itaconic acid on the rate of copolymer hydrolytic degradation in different incubation media at 37 °C evaluated by the GPC method. The level and the rate of releasing degradation products (lactic and glycolic acid) was compared and interpreted.

2. Experimental section

2.1. Materials

Formic acid (puriss p.a., ≥98%) was obtained from Riedel-de Haën (Germany). 2,6-Di-tert-butyl-4-methylphenol, monopotassium phosphate, dipotassium phosphate, standard D,L-lactic acid 90% and chloroform-d ($CDCl_3$, 99.8 atom% D) were purchased from Sigma-Aldrich (Germany). Tetrahydrofuran (99.9%) and standard glycolic acid (puriss p.a.) were obtained Merck KGaA (Germany). Acetonitrile was procured from Penta (Czech Republic). Polystyrene standards EasyCal 580–377 400 g·mol^{-1} were purchased Polymer Laboratories (USA). All solvents were provided as HPLC grade.

2.2. Materials for copolymer synthesis

Poly(ethylene glycol) (PEG, M_n =1500 g·mol^{-1}, Sigma-Aldrich Germany) was thoroughly degassed under vacuum for 3 hours at 130 °C. D,L-lactide (LA, 99.9%, Polysciences, Pennsylvania), glycolide (GA, 99.9%, Polysciences, Pennsylvania) and itaconic anhydride (ITA 97 , Fluka, Switzerland) were sublimated under reduced pressure (10 Pa) prior to use. Stannous 2-ethylhexanoate (95%, Sigma-Aldrich, Germany) was used as received.

2.3. Copolymer synthesis and purification

The PLGA-PEG-PLGA (ABA) and ITA/PLGA–PEG–PLGA/ITA (ITA-ABA-ITA) tri-block copolymers with different PLGA/PEG weight ratios and LA/GA molar ratios were synthesized in 'one pot' via the ring opening polymerization (ROP) method in bulk under a nitrogen atmosphere [18]. Shortly, PEG, LA and GA were homogenized at 130 °C followed by injecting Sn(II)2-ethylhexanoate. Reaction ran over 3 hours. In following step, ITA was added to the mixture and functionalization proceeded at 110 °C.

Table 1. Copolymer characterization

Sample	Type	$Ratio_{THEOR.}$ PLGA/PEG [wt/wt]	$Ratio_{THEOR.}$ LA/GA [mol/mol]	Theoretical M_n [g·mol^{-1}]
ABA–2.5/3	ABA	2.50	3.00	5250
ABA–2/3	ABA	2.00	3.00	4500
ABA-ITA–2/3	ITA-ABA-ITA	2.00	3.00	4500
ABA-ITA–2/2.5	ITA-ABA-ITA	2.00	2.50	4500

Copolymers were purified three times from unreacted monomers by dissolving in cold water and heating the solution up to 80 °C. Precipitated polymers were separated by decantation and dried in vacuum oven at 30 °C until the constant weight (for approx. 12 h). Purity of the synthesized copolymers was verified by ^{1}H NMR spectroscopy as described elsewhere (Michlovská *et al*. [18]).
Theoretical copolymer characterizations are shown in Table 1.

2.4. NMR characterization

Molecular weight and polymer composition (ratio of PLGA/PEG and GA/LA) were determined by ^{1}H NMR spectroscopy on a 500 MHz Bruker AVANCE III instrument with TMS as internal standard using 128 scans in $CDCl_3$ solvent. Sample concentration was 20 mg/mL in $CDCl_3$.

2.5. Gel permeation chromatography

Number average molecular weight (M_n) and polydisperisity index (PDI) of the copolymer were determined by gel permeation chromatography using an Agilent 1100 Series equipped with an isocratic pump (Agilent 1100 Series), and operated at a flow rate of 1.0 mL/min with tetrahydrofuran (THF), a vacuum degasser, an autosampler (Agilent 1100 Series), a column oven and a refractive index detector (Agilent 1100 Series) with an integrated temperature controller maintained at 30 °C. For molecular mass separation a guard column PLgel Mixed C 50×7.5 mm, 5 μm (Polymer Laboratories, USA) and a column PLgel Mixed C 300×7.5 mm, 5 μm (Polymer Laboratories, USA) were used in-line. The temperature of the column and the detector were set to 30 °C. The THF (HPLC grade) mobile phase was stabilized with 2,6-Di-tert-butyl-4-methylphenol. 50 μL of polymer samples and standards were injected. Number average molecular weight was calculated from a calibration curve constructed on the basis of a series of polystyrene standards EasyCal 580–377 400 g·mol^{-1} (Polymer Laboratories, USA).

2.6. High performance liquid chromatography

Phosphate solutions collected after certain degradation times were analyzed on an Agilent 1100 Series equipped with a gradient pump (Agilent 1100 Series) operated at a flow rate of 0.2 mL/min, a vacuum degasser, an autosampler (Agilent 1100 Series), a column oven and a UV-VIS detector of diode array type. For the separation of lactic and glycolic acids, an in-line guard column Restek Aqueous C18 4×2 mm, 5 μm (Restek, USA) and a column Restek Aqueous C18 250×4.6 mm, 5 μm (Restek, USA) were used. 5 μL of samples and standards were injected. The temperature of the column oven was set to 30 °C. The mobile phase was a mixture of acetonitrile (ACN) and 0.01 M formic acid. Gradient mobile phases were as follows: 0 min.:1% of ACN, 10 min.:1% of ACN, 12 min.: 50% of ACN, 30 min.: 50% of ACN, 30.01 min.: 1% of ACN. Glycolic and lactic acids were detected at a wavelength of 210 nm.
The analytes in the standard mixture and in the various samples were identified by comparing the retention time of the relevant peaks with those of the corresponding standards injected separately. Quantification was carried out by integration of the peak areas using the external standard chromatographic method.

2.7. Sample preparation for GPC analysis

Sample incubation was terminated after the collection of 400 μL of phosphate solution. Each sample was frozen to –30 °C after incubation. The frozen samples were subsequently subjected to lyophilization. The lyophilization was carried out at –80 °C and at a pressure of 15 Pa for 24 hours at Freezone 4.5 Freeze Dry System (Labconco, USA). From each lyophilized sample approximately 5 mg of degraded copolymer was collected. This amount of copolymer was dissolved in 1 mL of tetrahydrofuran and then analyzed by gel permeation chromatography.

2.8. Determining the critical gel concentration and critical gel temperature

A simple method of inverting test tubes is used for the preparation of the sol-gel phase diagram. The

advantage of this method is that it is possible to observe colour transitions and the turbidity of the solutions during the experiment. The disadvantage of the method, however, is the very subjective response of each observer.

A set of polymer samples with different concentrations in water was prepared. The aqueous copolymer solution for each concentration was prepared by dissolving the PLGA-PEG-PLGA or ITA/PLGA–PEG–PLGA/ITA in de-ionized water. For the test tube inverting method, the 4 mL vials (diameter 1.1 cm) contain 3 mL of copolymer solution. Dissolution was carried out in cool box at 4 °C by occasional stirring for a few days until the copolymer completely dissolved. After dissolution, the vials were equilibrated to room temperature. Experiments were performed in a water bath. A warming-up water bath was set at room temperature (23 °C), and thereafter the temperature was gradually increased by 1 °C steps to 60 °C. After 5 minutes the samples were removed from the water bath and the test tubes were turned upside down to observe the change in viscosity and color of each sample. The critical gelation concentration (CGC) and the critical gelation temperature (CGT) of the copolymer, were then determined from the measurement results. The accuracy of the sol-gel transition temperature was ±1 °C.

2.9. Degradation experiment

PLGA-PEG-PLGA (ABA) copolymer and copolymer modified with itaconic acid (ITA-ABA-ITA) are very viscous, 'honey-like' materials. For sample preparation, it was necessary to weigh certain quantities of these copolymers for the weight concentration. For a certain amount of the copolymer, the calculated quantity of de-ionized water was added. The prepared samples were kept in a cool box. Dissolution was carried out with occasional stirring for a few days until the copolymer completely dissolved.

Different concentrations of PLGA-PEG-PLGA and ITA/PLGA–PEG–PLGA/ITA copolymer were prepared in order to determine the degree of degradation. 300 μL of stock copolymer solution was pipetted sol into 2 mL vials. The vials were kept subsequently in an incubator (Nüve cooled incubator EC 110, Turkey) at 37 °C until the solution became a gel. The resulting gel was then added to 700 μL of phosphate solution, which was preheated to 37 °C. Degradation was carried out in two environments: an acidic environment at a pH of 4.2; and a neutral environment at a pH of 7.4. 400 μL of phosphate solution was collected in the first day at the beginning of incubation. The collected solution was subsequently analyzed by high performance liquid chromatography with UV-VIS detection of diode array type. The rest of the hydrogel was frozen and lyophilized. Lyophilized rests samples were measured by GPC.

3. Results and discussion

3.1. Copolymer synthesis and characterization

Molar ratio of synthesis was selected based on a literature review about PLGA-PEG-PLGA triblock copolymers [4]. It was assumed that aqueous solutions of these copolymers will change to gel in the vicinity of the body temperature.

PEG, LA and GA were homogenized at 130 °C followed by injecting Sn(II)2-ethylhexanoate. Sn(II)2-ethylhexanoate was chosen for its low toxicity, approved by FDA. Molar ratio of catalyst to initiator was 0.029. Reaction ran over 3 hours. Yield of synthesized copolymers was in a range from 75 to 87%. In following step, ITA was added to mixture and functionalization proceeded at 110 °C.

The ITA/PLGA–PEG–PLGA/ITA triblock copolymers with weight ratio of PLGA/PEG equal to 2 and 2.5 and molar ratio of LA/GA equal 3 were synthesised in 'one pot' reaction (Figure 1) via the ring opening polymerization (ROP) method in bulk under a nitrogen atmosphere as described elsewhere (Michlovská *et al*., [18]).

The amount of end-capped ITA to the both ends of PLGA-PEG-PLGA and real M_n were determined by ^{1}H NMR spectroscopy. Spectra of PLGA-PEG-PLGA and ITA/PLGA–PEG–PLGA/ITA copoly-

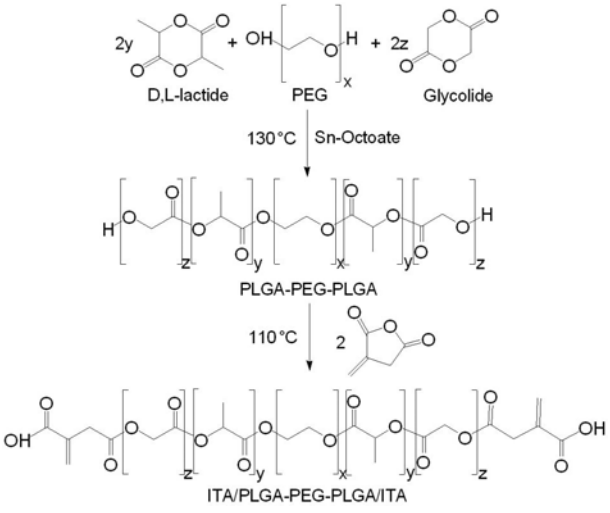

Figure 1. Scheme of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA synthesis

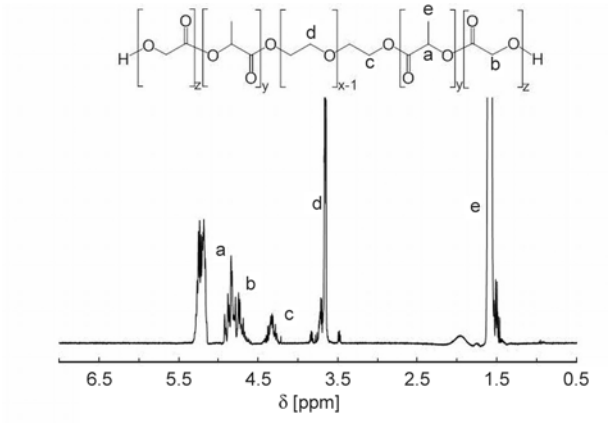

Figure 2. Proton NMR spectrum of PLGA-PEG-PLGA Sample ABA-2.5/3

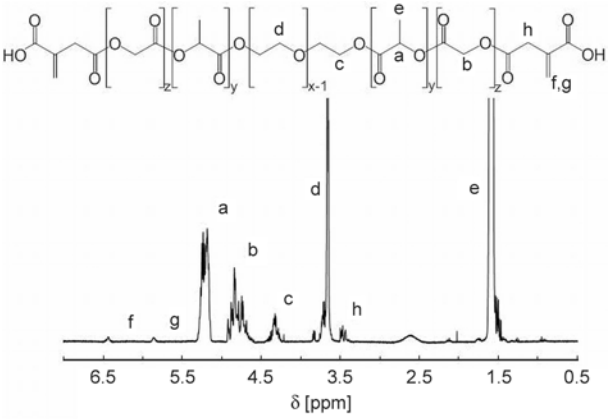

Figure 3. Proton NMR spectrum of ITA/PLGA–PEG–PLGA/ITA Sample ABA-ITA-2/3

mers are shown in Figure 2 and in Figure 3. From integrals of characteristic proton intensities of itaconic acid double bond ($OC(CH_2)CCH_2COOH$) at δ = 5.7–5.8 ppm (g), δ = 6.35–6.5 ppm (f), itaconic acid backbone ($OC\underline{H_2}(C=O)$) at δ = 3.40–3.44 ppm (h), lactic acid ($C–C\underline{H_3}(C=O)$) at δ = 1.5–1.65 ppm (e), glycolic acid ($OC\underline{H_2}O$) at δ = 4.6–4.9 ppm (b) and PEG ($OC\underline{H_2}C\underline{H_2}O$) at δ = 3.55 - 3.75 ppm (d).

3.2. The effect of ITA modification on sol-gel transition of PLGA-PEG-PLGA

Since the tested copolymers are not of commercial origin, first they were initially tested using a test tube inversion method to determine critical gelation temperature (CGT) and critical gelation concentration (CGC). For its use as a drug carries, it is necessary for the PLGA-PEG-PLGA copolymer to form a gel at a body temperature of 37 °C. Copolymers fulfilling this condition were subsequently subjected to degradation at 37 °C in phosphate solution at pH of either 7.4 or 4.2.

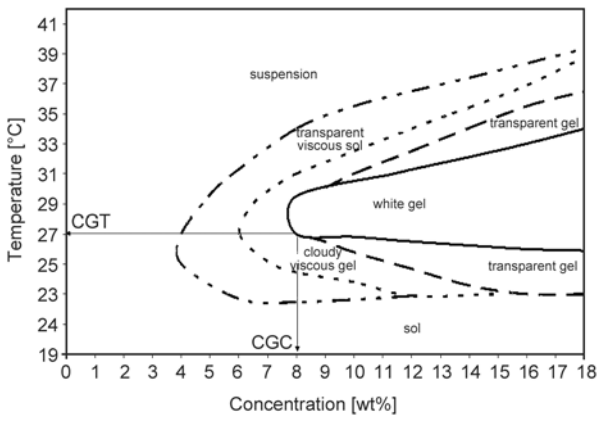

Figure 4. Phase diagram of the ABA-2.5/3

Overview of tested copolymers and their chemical compositions, CGC and CGT are described in Table 2.

The phase diagram of the aqueous solutions of ABA-2.5/3 determined by a test tube inverting method is shown in Figure 4. As it can be seen, several phase transitions and color changes during the gelation process have been observed. The critical gel concentration (CGC) above which white gel phase appears was about 8 wt%. The entire set of concentrations of the copolymer in water formed sol up to room temperature (23 °C). Lower concentrations from 4 to 12 wt% passed the more viscous sol when the temperature gradually increased. However, the solutions of concentration above 15 wt% passed straight to the transparent gel when temperature was higher than 23 °C. The transparent gel became turbid between 27 and 32 °C, indicating higher micelle aggregation. Further increase in temperature resulted in syneresis of the gel, and the system flows by gel-to-sol transition. The upper sol is a two-phase suspension at the temperature above the gel-to-sol transition.

Table 2. Experimental characteristics of tested copolymers

Sample	Type	$M_{n\ NMR}$ [g·mol^{-1}]	$Ratio_{REAL}$. PLGA/PEG [wt/wt]	$Ratio_{REAL}$. LA/GA [mol/mol]	$M_{n\ GPC}$ [g·mol^{-1}]	PDI	CGC [wt%]	CGT [°C]
ABA-2.5/3	ABA	4876	2.25	2.83	5949	1.28	8	27
ABA-2/3	ABA	4758	2.17	3.06	5845	1.26	6	38
ABA-ITA-2/3	ITA-ABA-ITA	4697	2.13	2.96	5557	1.35	6	36
ABA-ITA-2/2.5	ITA-ABA-ITA	4739	2.16	2.41	5209	1.27	18	40

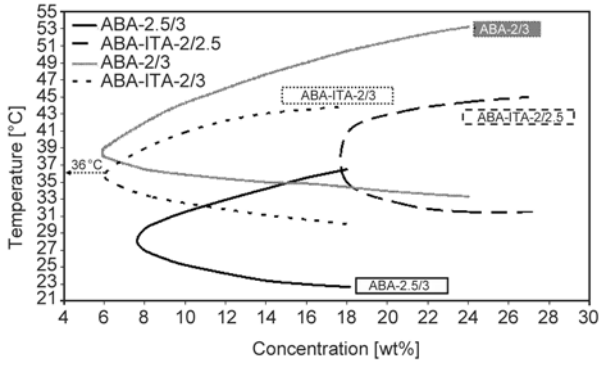

Figure 5. Phase diagrams of all samples

Sol-gel phase diagrams (describing just the area of both transparent and white gel) of all samples are shown in Figure 5.

From Figure 5 it is evident that decreasing the ratio of PLGA/PEG makes the entire copolymer less hydrophobic allowing poor micelles interaction and thus resulting in increasing CGT. By reducing ratio of LA/GA from 3 to 2.5 the CGC significantly increased which might be caused by PLA. PLA is more hydrophobic due to the methyl group than PGA. With decreasing LA/GA ratio the hydrophobicity of the whole copolymer is reduced (see Table 2).

3.3. The effect of ITA modification on hydrolytical degradation of PLGA-PEG-PLGA

The copolymers forming a gel in the temperature range from 36 °C to 38 °C meet our requirements and were selected for the use in the degradation experiment. Therefore, ABA-2.5/3 and ABA-2/3 (both type of PLGA-PEG-PLGA) and ABA-ITA-2/3 – copolymer (modified with itaconic acid) were selected for the degradation experiment. These samples safely fulfill the condition of gel formation – form a gel at a temperature of 36–38 °C. As for ABA-2.5/3 solution of 16 wt% was prepared. Dealing the ABA-2/3 and ABA-ITA-2/3 solutions of 16, 20 and 24 wt% were prepared for degradation study. Stock solutions of the copolymers were prepared in deionized water. Degradation was carried out in an incubator at 37 °C. Degradation media of both phosphate solution of pH value equal to 4.2 and phosphate buffer solution of pH value of 7.4 were used. The pH value of 7.4 was chosen as it is the pH of human blood. But all human organs do not have the same pH as the bloodstream. For example human vagina exhibits a slightly acidic pH (4±0.2). Linear polyesters degradation however is still pH dependent. It was tempting to try degradation of such copolymers in the mildly acidic and neutral environment and after that compared degradation of origin copolymer and modified copolymer.

3.3.1. Change in polymer length

Degradation of chains of the copolymer is proportional to the number average molecular weight (M_n) decrease. Gel permeation chromatography with a refractive index detector was used to determine the change in molecular weight and polydispersity index of the copolymer after its degradation.

Figure 6 shows the change in number molecular weight measured from 1st up to 10th day of degradation in pH 7.4 for 16 wt% water solution of ABA-ITA-2/3 at 37 °C. The measurement was provided until the gels were destroyed (11th day).

Figure 8 shows the change in number molecular weight measured from 1st up to 10th day of degradation in pH 7.4 for 16 wt% water solution of ABA-2/3 at 37 °C. The measurement was provided until the gels were destroyed (11th day) too.

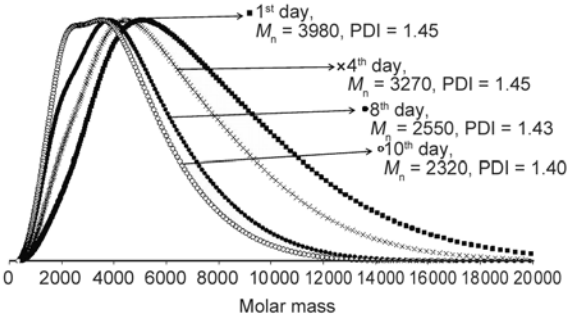

Figure 6. Molecular weight shift in the hydrolytic degradation in phosphate buffer solution pH value 7.4 for 16 wt% ABA-ITA-2/3

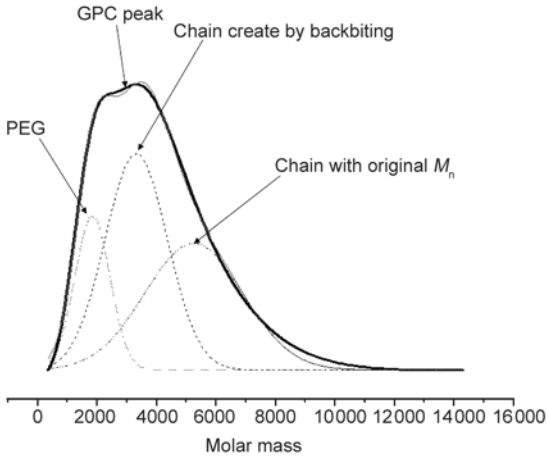

Figure 7. ABA-ITA-2/3 peak deconvolution for 10th day of degradation

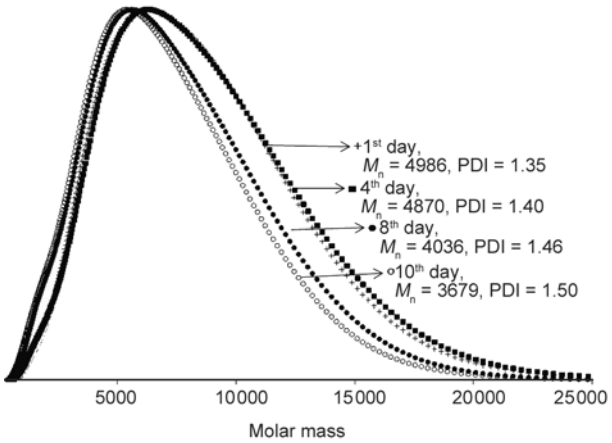

Figure 8. Molecular weight shift in the hydrolytic degradation in phosphate buffer solution pH value 7.4 for 16 wt% ABA-2/3

Decrease of number average molecular weight is evident in Figure 6. There is shown a noticeable shift peak top from right side (higher molecular weight) to left side (lower molecular weight). Formation of two maxima is possible to observe in 8th day although total M_n still decreases but PDI is still almost same. Degradation-mediated increase of acidic chain-ends causes the ester bond-scission to be faster in the chain-ends than in the internal bonds copolymer chain. Such a phenomenon is due to the short distance between the carbonyl and the alkoxy groups in the main chain making the cleavage of the ester bonds non-random under acidic conditions. Chain-end responsible for this backbiting mechanism is the hydroxyl-terminated chain-end within oligomers based on lactic acid. If all copolymer chains are cleaved by backbiting mechanism thus number average molecular weight of system is decreasing but chain distribution is still same (same PDI). Deconvolution of the peak for 8th day was performed using the software OriginPro 7.5. This deconvolution demonstrated that the peak contains three different long chains. This peak includes original copolymer chain and chain created by backbiting and polyethylene glycol chain. Two maxima are very perceptible in 10th degradation day. 10th day of degradation confirms this assertation (Figure 7).

M_n decrease is evident for ABA-2/3 in Figure 8. M_n decrease of ABA-2/3 is minimal for the first 4 days. ABA copolymer is more hydrophobic at pH 7.4 than ITA-ABA-ITA. The higher hydrophobicity of ABA decreases copolymer hydrolysis for the first days of degradation experiments.

3.3.2. The effect of degradation media pH

Influence of degradation media pH on the degradation progress of the copolymers was observed as a decrease of number average molecular weight M_n of the copolymer with time of degradation. Degradation time was evaluated in 5th and 10th day for all samples. For objective assessment of the overall course of the degradation most appropriate was to calculate the percent decrease in M_n because the initial molecular weight of the dissolved copolymer has never been the same at the start of experiments. The percentage decrease in copolymer molecular weight was interspersed as linear regression line. The percentage decrease of copolymer molecular weight for the selected time of degradation was calculated from the regression equation.

M_n decrease of ABA-2/3 in phosphate buffer solution having pH value 7.4 is shown in Figure 9. Influence the time on copolymer degradation was evaluated for each tested copolymer concentration as shows Figure 9. Time influence on copolymer degradation was confirmed for pH value 4.2 as well as. Further it is evident from Figure 9, that all tested sample concentrations are degraded in range of 16–18% until 5th day. This difference is almost insignificant. Degradation was found to be from 29 to 36% until 10th day in PBS (pH 7.4). Decrease in M_n behaved similar for medium pH value 4.2, where until 5th day the M_n degreased of about 10–15% and until 10th day in a range between 31–43%. It follows that there is no significant influence of copolymer weight concentration on hydrolytic degradation. Figure 9 shows too 5th degradation day for ABA-2/3 for incubation media pH value 4.2 and for all tested weight concentrations. It is evident that pH influence has not been con-

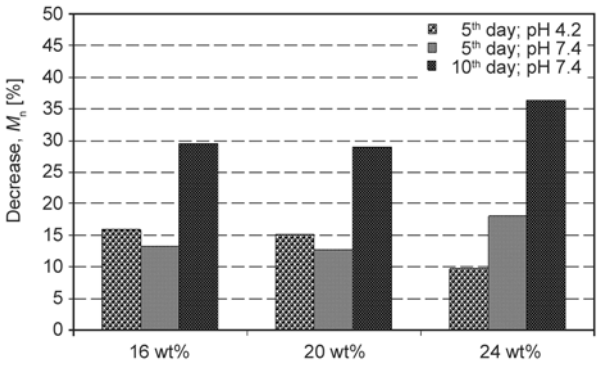

Figure 9. The effect of time, copolymer concentration of ABA-2/3 and pH of incubation media on the decrease of M_n during the degradation until 5th and 10th day

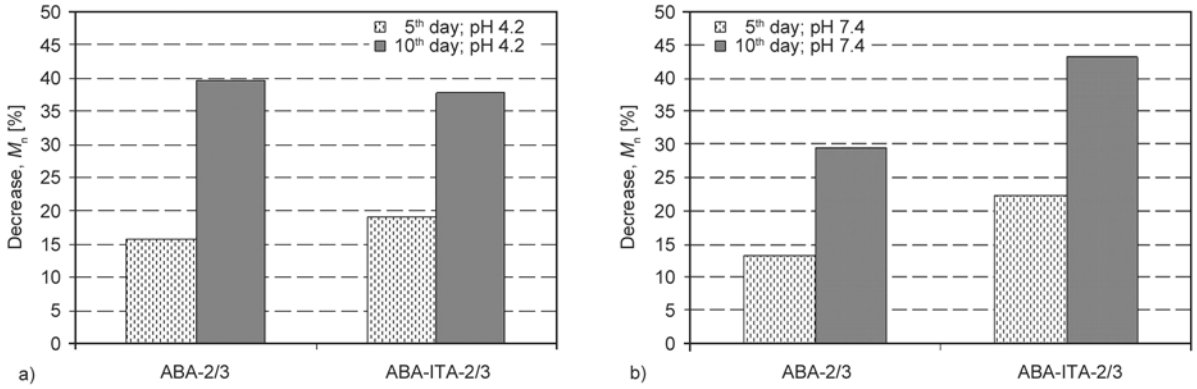

Figure 10. (a) Comparative degradation of 16 wt% ABA-2/3 versus 16 wt% ABA-ITA-2/3 at pH 4.2, (b) comparative degradation of 16 wt% ABA-2/3 versus 16 wt% ABA-ITA-2/3 at pH 7.4

firmed for unmodified PLGA-PEG-PLGA copolymer degradation.

The influence of the ratio of PLGA/PEG on decrease number average molecular weight of copolymer for samples ABA-2.5/3 (PLGA/PEG = 2.5) and ABA-2/3 (PLGA/PEG = 2.0) has not been confirmed. This low difference in degradation of both samples might be caused by little difference in PLGA/PEG ratio where for ABA-2.5/3 the real ratio is 2.25 and for ABA-2/3 the real ratio is 2.17.

Further influence of copolymer modification by itaconic acid is seen in Figure 10 where 16 wt% ABA-2/3 and 16 wt% ABA-ITA-2/3 are compared. It is evident that ABA-ITA-2/3 degraded faster at pH 7.4 (Figure 10b). Dissociation of carboxyl groups reduces the whole copolymer hydrophobicity in the aqueous environment at pH 7.4 and thus it leads to hydrophobic bonds decomposition. This causes more rapid degradation of the copolymer at pH 7.4. Conversely, carboxyl groups are not ionized at low pH. These carboxyl end groups are able of forming hydrogen bonds and thus the whole copolymer behaves more hydrophobic. Therefore, samples of the ABA-2/3 and ABA-ITA-2/3 degrade almost equally at pH 4.2 (Figure 10a).

3.3.3. Lactic and glycolic acid release

The release of lactic and glycolic acids into the incubation medium within the degradation process was quantitatively determined by High Performance Liquid Chromatography (HPLC) with UV-VIS detection of diode-array type (DAD). Increasing the concentration of lactic acid and glycolic acid in the solution was monitored during degradation. Individual analytes were evaluated using the calibration dependence of peak areas to concentrations of individual acids. The calibration solutions were prepared as a mixed standard of glycolic acid and lactic acid. Standards concentrations in calibration solution was ranged between 0.01–7.0 mg·mL^{-1}. For the standard curves, good linearity was observed with correlation factors typically above 0.99.

The limit of detection (LOD) and limit of quantification (LOQ) was calculated from the noise of the baseline. Limit of detection is the concentration the response of the detector will have a signal to noise ratio greater than 3. LOD was calculated according to the Equation (1). Limit of quantification is the concentration the response of the detector will have a signal to noise ratio greater than 10. LOQ was calculated according to the Equation (2). The limit of detection was determined 0.0044 mg·mL^{-1} of glycolic acid the limit of quantification was determined 0.0146 mg·mL^{-1} of glycolic acid. *LOD* was determined 0.0048 mg·mL^{-1} of lactic acid. *LOQ* was determined 0.0162 mg·mL^{-1} of lactic acid (Equations (1) and (2)):

$$LOD\,[\mathrm{mg{\cdot}mL^{-1}}] = 3\left(\frac{c[\mathrm{mg{\cdot}mL^{-1}}]}{\frac{S}{N}}\right) \quad (1)$$

$$LOQ\,[\mathrm{mg{\cdot}mL^{-1}}] = 10\left(\frac{c[\mathrm{mg{\cdot}mL^{-1}}]}{\frac{S}{N}}\right) \quad (2)$$

Glycolic acid is not detected during the first days of degradation especially in samples with low weight concentration. This is due to the actual ratio of glycolic acid and lactic in copolymer structure. The theoretical ratio of PLA/PGA was 3.0 for tested the samples. Lactic acid was nearly always detected after the first day of degradation in contrast to glycolic acid. This is probably due to the beginning of the copolymer degradation during preparation of samples –

xerogel dissolution in de-ionized water. Glycolic acid probably should be released in this way, but due to its low ratio in the copolymer, its concentration was below the limit of detection. In order to be compared release of both acids.

Acids release is controlled by first-order kinetics. Acid release kinetics is described by Equations (3)–(6). Rate constant (k) was calculated from the linear regressions. Example for 16 wt% sample ABA-2/3 and releasing for lactic acid at pH 4.2 shows Figure 11.

$$-\frac{\mathrm{d}C_{\mathrm{acid}}}{\mathrm{d}t} = k \cdot C_{\mathrm{acid}} \tag{3}$$

$$-\frac{\mathrm{d}C_{\mathrm{acid}}}{\mathrm{d}t} = k \cdot (C_0 - C_{\mathrm{release}}) \tag{4}$$

$$\frac{\mathrm{d}C_{\mathrm{acid}}}{(C_0 - C_{\mathrm{release}})} = k \cdot \mathrm{d}t \tag{5}$$

$$\ln\frac{\mathrm{d}C_0}{(C_0 - C_{\mathrm{release}})} = -k \cdot t \tag{6}$$

where the following notation was used (Equations (7)–(11)):

$$C_0 = \frac{n_{\mathrm{acid}}}{V_{\mathrm{copolymer\ solution}}} [\mathrm{mol \cdot L^{-1}}] \tag{7}$$

$$n_{\mathrm{acid}} = \frac{C_{\mathrm{max}}\mathrm{Acid}}{M_{\mathrm{acid}}} [\mathrm{mol}] \tag{8}$$

$$C_{\mathrm{max}}Acid = \frac{m_{\mathrm{mer}}}{M_{\mathrm{mer}}} \cdot M_{\mathrm{acid}} [\mathrm{g}] \tag{9}$$

$$m_{\mathrm{mer}} = \frac{m_{\mathrm{PLGA}} \cdot M_{\mathrm{mer}}}{\frac{M_{\mathrm{lactide}} + M_{\mathrm{glycolide}}}{\mathrm{ratio\ LA/GA}}} [\mathrm{g}] \tag{10}$$

$$m_{\mathrm{PLGA}} = \frac{m_{\mathrm{copolymer\ in\ 300}\mu\mathrm{L}}}{\mathrm{ratio\ LA/GA}} \cdot \mathrm{ratio\ PLGA/PEG\ [g]} \tag{11}$$

where C_{acid} lactic or glycolic acid concentration, k rate constant [h^{-1}], t time [h], C_0 acid initial concentration in copolymer, C_{release} acid actual molar concentration in phosphate solution, n_{acid} acid molar amount, M_{acid} acid molar mass, C_{max}Acid maximal acid concentration when copolymer fully degraded, m_{mer} amount of lactide or glycolide, M_{mer} lactide or glycolide molar mass, m_{PLGA} PLGA amount.

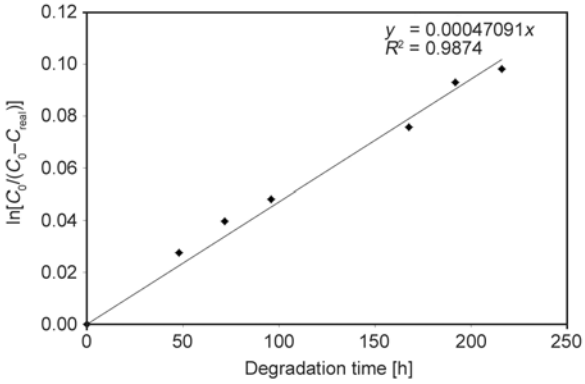

Figure 11. Linear regression and rate constant for lactic acid, sample 16 wt% ABA-2/3, pH 4.2

Figure 12 shows ABA-2/3 and its released acids. It is obvious that influence of copolymer weight concentration on released acids has not been confirmed.

Rate of release is faster for glycolic acid compared to lactic acid both tested pH. Major reason for faster releasing of glycolic acid that glycolic acid is more hydrophilic than lactic acid. Further lactic acid required more time for releasing 5% acid at buffer solution pH value 4.2. This was not confirmed with regard to the calculated rate constants k, R^2 linear regressions for glycolic acid were 0.8. The release of glycolic acid does not take place first-order reaction (ideal model). The acidic hydrolysis takes place at pH 4.2 but low pH environment causes that the cleaved acids create quite stable dimers. Therefore, the kinetics of acids release is slower than at pH 7.4 which take place by backbiting mechanism.

Figure 13 shows ABA-2/3 and glycolic acid releasing. The data within the interval from 100 to 160 hours were not recorded but 3 points are enough for linear regression. Glycolic acid is released steeper for phosphate buffer solution pH value 7.4. Glycolic acid is released within the range 40–48 % for 264 hours in

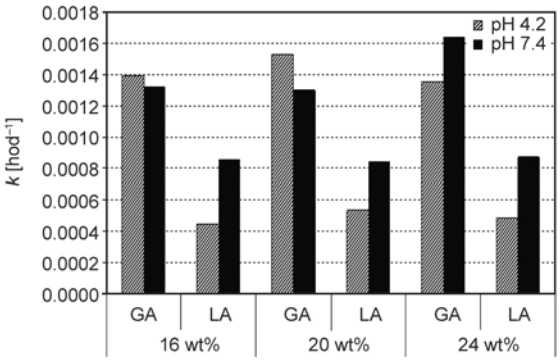

Figure 12. The effect of weight concentration ABA-2/3 and pH influence to acids release

phosphate buffer solution pH value 7.4. Opposite glycolic acid is released within the range 20–22 % only for the same time in phosphate solution pH value 4.2.

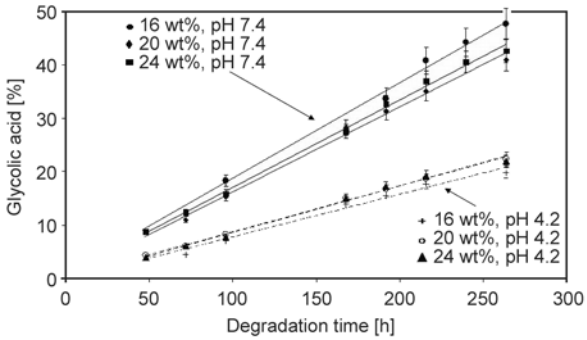

Figure 13. The influence pH value on releasing glycolic acid for ABA-2/3

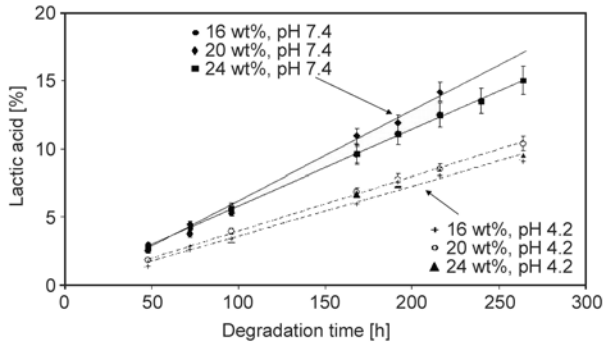

Figure 14. The influence pH value on releasing lactic acid for ABA-2/3

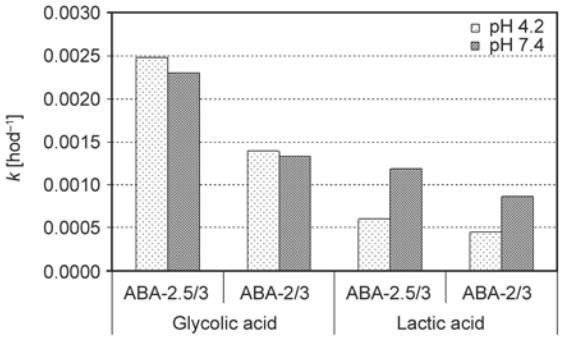

Figure 15. The influence ratio PLGA/PEG for 16 wt% ABA-2.5/3 (PLGA/PEG = 2.5) and 16 wt% ABA-2/3 (PLGA/PEG = 2.0)

Figure 14 shows ABA-2/3 and lactic acid releasing. Lactic acid is released steeper for phosphate buffer solution pH value 7.4. Lactic acid is released 15% for 264 hours in phosphate buffer solution at pH 7.4. Opposite lactic acid is released 9% only for the same time in phosphate solution at pH 4.2.

Figure 15 shows 16 wt% ABA-2.5/3 in compared 16 wt% ABA-2/3. At first sight copolymers are degraded 'worse' in the environment of phosphate solution at pH value 4.2 for lactic acid again. The release kinetics of acids is again influenced by the formation of oligomers at pH 4.2. However assumption that more hydrophobic ABA-2.5/3 will require more time for releasing acids, it wasn't confirm. It could be caused that acids are released during samples preparing and create a strongly acidic interior to the copolymer which can auto-catalyze degradation.

Figures 16 show ABA-2/3 and ABA-ITA-2/3 in environment at pH 4.2. The influence of modification by itaconic acid is observed on released glycolic acid and lactic acid. It is evident that ABA-ITA-2/3 needs more time for release of acids than unmodified ABA-2/3. ABA-ITA-2/3 contains carboxyl end groups are not ionized at low pH (3±1). The unionized carboxyl groups may create hydrogen bonding

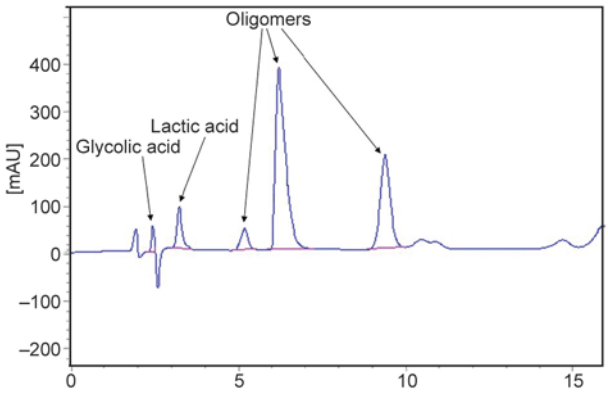

Figure 17. HPLC chromatogram of sample taken at degradation of the 24 wt% ABA-ITA-2/3 at pH 4.2

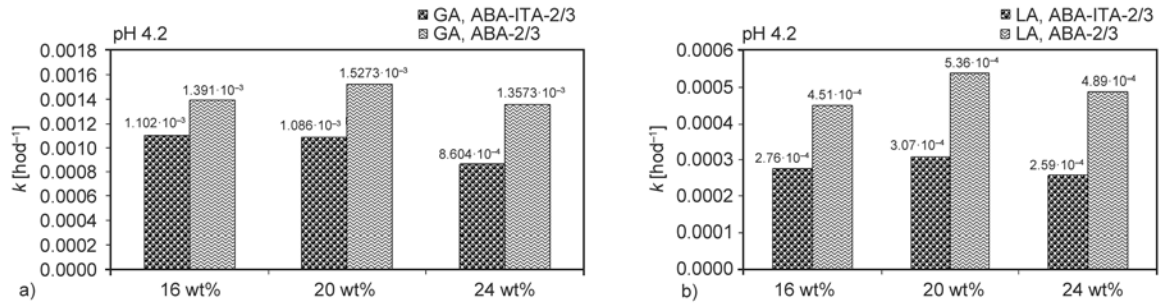

Figure 16. (a) The comparison of ABA-ITA-2/3 and ABA-2/3 for glycolic acid kinetics at pH 4.2 (b) the comparison of ABA-ITA-2/3 and ABA-2/3 for lactic acid kinetics at pH 4.2

and thus the whole copolymer behaves more hydrophobic. ABA-ITA-2/3 degradation and acids release are slower. Degradation occurs by acidic hydrolysis. The low pH environment supports the formation of oligomers, of course. This is shown in Figure 17. At pH 7.4 the release of acids is fast and very similar for both types of samples.

4. Conclusions

Functionalization of PLGA-PEG-PLGA copolymer by both carboxyl groups and double bonds coming from itaconic acid for bioactivity enhancement maintains reversible sol-gel transition behaviors and at certain composition keeps gelation at 37 °C. Their hydrolytical degradation at 37 °C was affected by the incubation medium. In phosphate solution at physiological pH equal to 7.4 the modified copolymer degraded significantly faster than its unmodified copolymer. This is due to the presence of carboxyl end groups, which are ionized at this pH thus increasing the hydrophilic nature. Conversely, at more acidic pH 4.2 carboxyl end groups are not ionized but create hydrogen bonding and thus the whole copolymer behaves more hydrophobic and the ITA-ABA-ITA degradation proceeded a bit slower than that of ABA. Anyhow, the hydrolytical stability of all samples has not exceeded 11 days.

The overall release of glycolic acid from the copolymer hydrogels was higher than the release of lactic acid due to the more hydrophilic character; however at pH 4.2 the acid release was slower since low pH supports the formation of oligomers (dimers, etc.).

Prepared ITA functionalized copolymers having carboxyl groups for bioactive molecules grafting and double bonds supporting chemical crosslinking are proved to be suitable materials for injectable drug delivery carriers, cells and gene scaffolds for tissue engineering as well as biodegradable hydrogels for tissue regeneration.

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