

Thermogelling water solutions of multifunctional macromonomers based on PLGA-PEG-PLGA triblock copolymers

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Biodegradable thermosensitive triblock copolymers based on poly(ethylene glycol) and poly[(lactic acid)-*co*-(glycolic acid)] (PLGA-PEG-PLGA) with PLGA/PEG weight ratio in the range of 1.5–3.0 and LA/GA molar ratio equal to 2.4 or 3.0 were prepared via ring opening polymerization (ROP). Prepared copolymers were subsequently modified in “one pot” by itaconic anhydride (ITA) in order to functionalize both ends with carboxylic acid groups and reactive double bonds. Chemical structure was characterized by means of gel permeation chromatography and nuclear magnetic resonance.

Aqueous solutions of both modified and unmodified copolymers are able to form free flowing sol at room temperature and clear gel at temperature around 37°C. Therefore, sol-gel transitions of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers in aqueous solutions were investigated using test tube inverting method.

No matter if copolymer is modified or not, it was found that the critical gel temperature (CGT) increased when the PLGA/PEG weight ratio dropped from 2.5 to 2.0 and that the critical gel concentration (CGC) grew up with decreasing molar ratio of LA/GA from 3.0 up to 2.4. However, in all cases the ITA functionalization improved sol-gel characteristics of original PLGA-PEG-PLGA copolymer by approaching gel phase to body temperature.

As a result, aqueous solution of ITA/PLGA-PEG-PLGA/ITA having LA/GA = 3 and PLGA/PEG = 2 with concentration higher than 6 wt% might be suitable material for biomedical applications as injectable temporary implants.

Keywords: biodegradable polymers; poly(ethylene glycol); poly[(lactic acid)-*co*-(glycolic acid)], sol-gel transition.

Introduction

Among known biodegradable polymers, linear aliphatic polyesters especially those derived from lactic acid (LA), glycolic acid (GA) and their copolymers have met with increasing interest during the past two decades, particularly in the field of tissue engineering as controlled-release drug carriers for surgical implantation and wound treatment [1, 2]. These materials are biocompatible and after hydrolysis at physiological conditions to lactic acid and glycolic acids undergo degradation via the Krebs cycle to non-toxic products (water and carbone dioxide) [3, 4].

Thermoreversible block copolymers composed of poly(ethylene glycol) (PEG) (A) and biodegradable polyesters (B), such as poly(l-lactic acid) (PLLA), poly(d,l-lactic acid) (PDLA), poly[(lactic acid)-*co*-(glycolic acid)] (PLGA) and poly[(d,l-LA)-*co*-(ε-caprolactone)] (CL) can undergo a temperature-induced

reversible sol-gel transition upon heating or cooling of the aqueous solution [5–7]. Important role in the gelation of ABA- or BAB-type triblock copolymers play micelle formation and micellar behavior, which are dependent on the polymeric molecular structure, its hydrophobic-hydrophilic balance in water and concentration [8, 9]. Owing to their sharp phase transition these copolymers have potential as injectable drug delivery systems. High moldability (capable of filling irregular shaped defect) and delivering to the in vivo environment by limited surgical invasion (such as minimally invasive surgery) are their big advantages [10].

In this work, a series of BAB-type poly[(d,l-LA)-*co*-GA]-*b*-PEG-*b*-poly[(d,l-LA)-*co*-GA] triblock copolymers (PLGA-PEG-PLGA) were synthesized in which the LA/GA and PLGA/PEG ratios were varied. Subsequently, PLGA-PEG-PLGA copolymers were functionalized with itaconic anhydride (ITA), which brings reactive double bonds and functional carboxylic acid groups to the end

of copolymer resulting in preparation of ITA/PLGA-PEG-PLGA/ITA macromonomers. Some of these block copolymers showed a thermoreversible transition behavior where aqueous solution of these copolymers is a free flowing sol at room temperature but it becomes a gel at body temperature. Phase diagrams of these polymers in water were recorded in detail.

Experimental Part

Materials

Poly(ethylene glycol) (PEG, $M_n = 1500 \text{ g}\cdot\text{mol}^{-1}$, Aldrich Germany) was thoroughly degassed under vacuum for 8 hours at 130°C . D,L-lactide (LA), glycolide (GA), Polysciences, Pennsylvania) and itaconic anhydride (ITA 97%, Fluka, Switzerland) were sublimated under reduced pressure (10 Pa) prior the use. Sn(II)2-ethylhexanoate (95%, Sigma Germany) was used as received.

Copolymers Synthesis and Functionalization

The PLGA-PEG-PLGA triblock copolymers (BAB-type) with reactive feed weight ratio of PLGA/PEG = 1.5–3.0 and molar ratio of LA/GA = 2.4–3.0 were synthesized via ring opening polymerization (ROP) method in a bulk under nitrogen atmosphere as described elsewhere [6]. Shortly, PEG ($M_n = 1500 \text{ g}\cdot\text{mol}^{-1}$), LA and GA were homogenized at 130°C followed by injecting Sn(II)2-ethylhexanoate. Reaction ran over 3 hours. Functionalization proceeded with sublimated ITA (2.5 molar ratio to polymer) at 110°C for 3 hours in “one pot” reaction. Both PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers were purified (3 \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).

Characterization

The molecular structure and composition (such as PLGA/PEG and LA/GA ratios), molecular weights and amount of ITA bonding to original triblock copolymer were determined by proton nuclear magnetic resonance

(^1H NMR) on 700 MHz Bruker AVANCE III instrument using 128 scans in CDCl_3 solvent.

Number-average molecular weight (M_n) and polydispersity index (M_w/M_n) of the copolymers were measured using gel permeation chromatography (GPC) method using Agilent Technologies 1100 Series instrument equipped with isocratic pump, autosampler, RI and UV-VIS detector, fraction collector, column thermostat up to 80°C and $300 \times 7.5 \text{ mm}$ PLgel 5 μm MIXED B;C column with THF as the eluent at a flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$ against linear polystyrene standards. The sol-gel phase transitions of aqueous solutions of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers were evaluated by inverting test tube method in 4 ml vial over a temperature range of $20\text{--}50^\circ\text{C}$ at 1°C increase per 3 min. interval. The sol-gel transition temperature was determined based on the criteria of “flow” for sol and “no flow” for gel after the vial was inverted. Samples were kept at a constant temperature for 10 min to allow the establishment of equilibrium [11, 12].

Results and discussion

PLGA-PEG-PLGA triblock copolymers with PLGA/PEG weight ratio varied from 1.5 to 3 and LA/GA molar ratio equal to 2.4 or 3 were synthesized and subsequently functionalized with itaconic anhydride (ITA) in order to prepare ITA/PLGA-PEG-PLGA/ITA macromonomers. Since triblock copolymers having PLGA/PEG ratio higher than 2.5 are insoluble in water and copolymers with PLGA/PEG ratio lower than 2.0 are all soluble and do not form hydrogels in water, only sol-gel phase transitions of copolymers with PLGA/PEG ratios of 2.0 and 2.5 were studied.

The molecular weight and molecular weight distribution of the triblock copolymers, composition ratios of LA/GA or PLGA/PEG and the amount of bonded ITA determined by combining GPC and ^1H NMR results are listed in Table 1. PLGA-PEG-PLGA triblock copolymers (A1, B1, C1) display a single peak in the GPC trace and a polydispersity of less than 1.3 while samples modified by itaconic anhydride (A2, B2,

Table 1. Properties of PLGA-PEG-PLGA (BAB) and ITA/PLGA-PEG-PLGA/ITA (ITA-BAB-ITA) copolymers.

No.	Copolymer	PLGA/PEG ^{a)} (wt/wt)	LA/GA ^{a)} (mol/mol)	$M_n^a)$ $\text{kg}\cdot\text{mol}^{-1}$	$M_n^b)$ $\text{kg}\cdot\text{mol}^{-1}$	$M_w/M_n^b)$	$M_n^{\text{(theor)}/M_n^{\text{(GPC)}/M_n^{\text{(NMR)}}$	ITA ^{a)} (mol %)
A1	BAB	2.0	2.4	4.6	5.2	1.27	1/1.2/1	—
A2	ITA-BAB-ITA	2.0	2.4	4.7	4.7	1.33	1/1.1/1.1	66.8
B1	BAB	2.0	3.0	4.6	5.8	1.26	1/1.3/1	—
B2	ITA-BAB-ITA	2.0	3.0	4.7	5.6	1.35	1/1.2/1	70.2
C1	BAB	2.5	3.0	4.7	6.0	1.28	1/1.1/0.9	—
C2	ITA-BAB-ITA	2.5	3.0	5.3	6.3	1.40	1/1.2/1	72.4

^{a)} obtained from ^1H NMR, ^{b)} measured by GPC.

C2) show slightly higher polydispersity index probably due to the inter- or intramolecular transesterification side reactions occurring at longer polymerization time or enhanced temperature [13]. However, results indicate that structure-controlled triblock copolymers were synthesized.

The amount of end-capped ITA and real M_n were determined by ^1H NMR spectroscopy (Fig. 1) from integrals of characteristic proton intensities of itaconic acid double bond ($\text{OC}(\text{CH}_2)\text{CCH}_2\text{COOH}$) at $\delta = 5.7\text{--}5.8$ ppm (g), $\delta = 6.35\text{--}6.5$ ppm (f), itaconic acid backbone ($\text{OCH}_2(\text{C}=\text{O})$) at $\delta = 3.40\text{--}3.44$ ppm (h), lactic acid ($\text{C}-\text{CH}_3(\text{C}=\text{O})$) at $\delta = 1.5\text{--}1.65$ ppm (e), glycolic acid (OCH_2O) at $\delta = 4.6\text{--}4.9$ ppm (b) and PEG ($\text{OCH}_2\text{CH}_2\text{O}$) at $\delta = 3.55\text{--}3.75$ ppm (d).

The M_n calculated from ^1H NMR spectra was in very good agreement with theoretical M_n of copolymers showing the PEG initiator efficiency ($M_{n(\text{theor.})}/M_{n(\text{GPC})}$) on the average of 96.8%. The amount of bonded ITA to the original copolymer was approximately 70 mol%. It is assumed that probably cyclic products of the side transesterification reactions suppress more ITA coupling, since there are less -OH end-groups than presupposed.

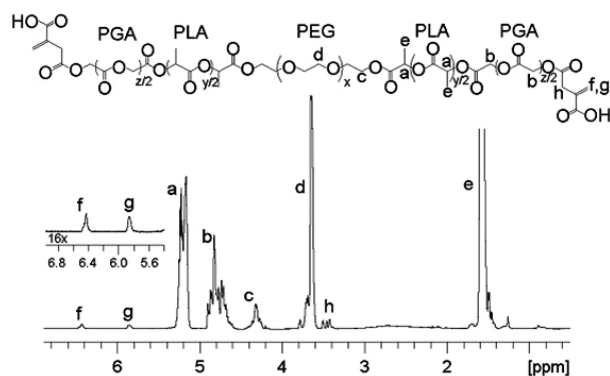


Fig. 1. ^1H NMR of ITA/PLGA-PEG-PLGA/ITA copolymer.

The thermosensitive triblock copolymers are soluble in water forming free-flowing solution (sol) that spontaneously gels as the temperature increases generating a water-insoluble physical hydrogel (first sol-gel transition). Further increasing the temperature causes the micelle structure separation from the water and above the second gel-sol transition the polymer is separated from the water (suspension). The phase diagrams of investigated PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers are shown in Fig. 2 and 3, respectively. These phase diagrams demonstrate three basic areas separated by each sol-gel curve: sol, gel and suspension (as is described above). All traces show two phase transitions (first-lower and second-upper) above critical gel concentration (CGC). Below the CGC the samples do not gel.

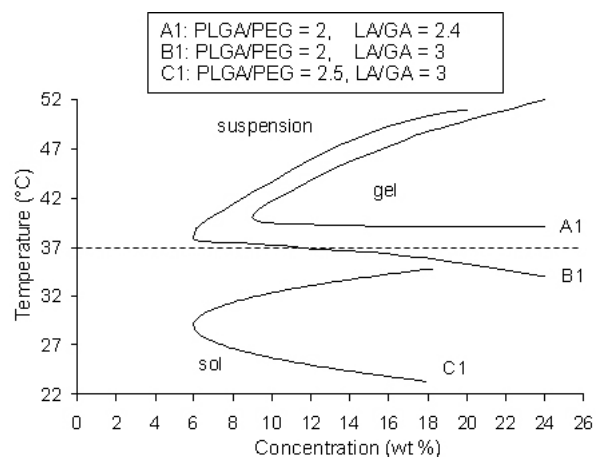


Fig. 2. Phase diagrams of PLGA-PEG-PLGA copolymers with various ratios of PLGA/PEG and LA/GA in water.

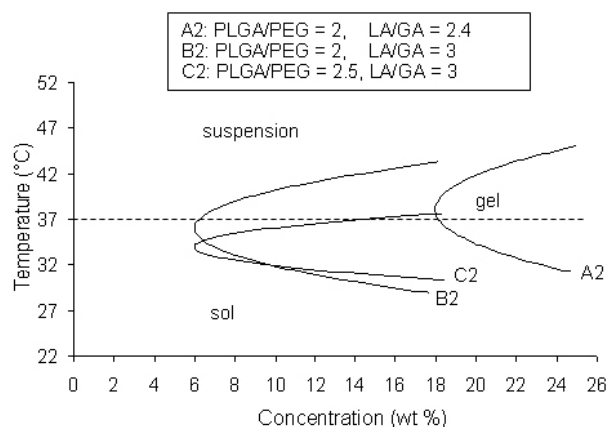


Fig. 3. Phase diagrams of ITA/PLGA-PEG-PLGA/ITA copolymers with various ratios of PLGA/PEG and LA/GA in water.

Phase diagram of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers with the same PLGA/PEG ratios displays similar critical gel temperature (CGT). With reducing the ratio of LA/GA critical gel concentration increased together with narrowing the phase curve showing smaller gel zone since an increase in the GA content in PLGA increases the hydrophilicity of PLGA blocks (Table 2). Gel zone in phase diagrams for sample A1, B1 and C1 widened with higher PLGA/PEG ratio, whereas at modified copolymers (A2, B2, C2) widened with lower PLGA/PEG ratio and higher LA/GA ratio. In both cases, copolymers have the same CGC due to the same LA/GA ratio. As we can see from Fig. 2 and Table 2, CGT lowers for PLGA-PEG-PLGA aqueous solutions with increasing the PLGA/PEG ratio, because hydrophobic PLGA blocks can easily locate into different micelles, leading to bridging connections which facilitates aggregation [14].

Phase diagrams of ITA/PLGA-PEG-PLGA/ITA aqueous solutions occurred more around depicted 37°C

Table 2. Critical gel temperature and critical gel concentration for individual copolymers.

No.	Copolymer	PLGA/PEG (wt/wt)	LA/GA (mol/mol)	CGT (°C)	CGC (wt %)
A1	BAB	2.0	2.4	40	9
A2	ITA-BAB-ITA	2.0	2.4	38	18
B1	BAB	2.0	3.0	38	6
B2	ITA-BAB-ITA	2.0	3.0	36	6
C1	BAB	2.5	3.0	29	6
C2	ITA-BAB-ITA	2.5	3.0	34	6

than gel phases of original copolymers. In a studied systems, CGT of ITA modified copolymers is situated between 34–38°C while that of unmodified copolymers is in the range of 29–40°C. It indicates that the presence of –COOH end-groups allow for interaction between each other depending on certain temperature rather than on the PLGA/PEG ratio.

Conclusion

Results indicated that the critical gel concentration of PLGA-PEG-PLGA triblock copolymer-water systems is mainly determined by the LA/GA ratio while the critical gel temperature is more affected by changing the PLGA/PEG ratio. All itaconic acid functionalized copolymers displayed better sol-gel characteristics than original unmodified copolymers by approximating CGT to body temperature.

The best gelation behaviors exhibited ITA/PLGA-PEG-PLGA/ITA copolymer with PLGA/PEG weight ratio of 2 and LA/GA molar ratio equal to 3 having CGC = 6 wt % and CGT = 36°C. Water solutions of this functionalized copolymer at concentrations higher than 6 wt% might be a suitable material for biomedical applications as injectable temporary implants.

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