 DOI: 10.1002/masy.200750705

Degradation Process of Bioresorbable Copolyesters. Microstructure Investigation by NMR and ESI-MS

Joanna Jaworska, Janusz Kasperczyk, Piotr Dobrzyński*

Summary: On the basis of NMR and ESI-MS spectra completely different ways of degradation process for random and block polyesters copolymers have been observed. PGA/PLA copolymers containing more than 50% of glycolidyl units release short lactidyl microblocks more rapidly than long glycolidyl microblocks. In case of PGA/PCL copolymers chain structure before degradation and mechanism of degradation of two chosen samples are distinctly various despite of low content of GG units in copolymer chain (ca.10%) and lack of differences in NMR spectra. ESI-MS technique can be useful tool, complementary to NMR method in studying the chain microstructure.

Keywords: biodegradable polyesters; structure

Introduction

The aliphatic polyesters are one of the most widely utilized classes of biodegradable polymers in medicine and the poly(α-hydroxy acids), consisting of lactic and glycolic acid, are perhaps the best-known polymers in this broad class of materials. They are interesting because of their safety, biodegradability, availability and versatility. Lactide/glycolide polymers are now commercially available with different molecular weights and copolymer ratios. Devices prepared from poly(α-hydroxy acids) could be divided into the three major areas: wound closure, tissue repair and regeneration, and drug delivery.

The aim of wound closure materials is to pull the tissue around a wound together and maintain them in close proximity until the wound has healed. Sutures, surgical clips, surgical staples and adhesives- these are examples of materials which belong to the mentioned group. The idea of tissue engineering focus on maintaining the alignment of tissue by creating the scaffold, which takes part in a process of tissue regeneration. The delivery of drugs to the body might be conducted in a different ways depended on the kind of therapy. Drug delivery systems are alternative way of it. They are composed of therapeutic agents incorporated into a polymer matrix that undergo controlled degradation. The prospective applications include devices to treat cancer, drug addiction and infection, as well as drugs for contraception, vaccination and tissue regenerating. A number of products are commercially available such as Decapeptyl®, Lupron Depot®, Zoladex®, Adriamycin® and Capronor®. With all unique features of biodegradable polymers these materials can solve many important clinical problems.[1] However, influence of the chain microstructure on hydrolytic degradation of obtained bioresorbable materials were not fully described in the literature. Such knowledge seems to be very useful in designing different kind of bioresorbable systems used *e.g* in medicine. Hence, this study present microstructure investigations using NMR spectroscopy and mass spectromertry ESI-MS.

Centre of Polymer Chemistry, 34 Skłodowskiej-Curie St., 41-800 Zabrze, Poland
E-mail: jkasperczyk@wp.pl

Experimental Methods

Materials

Glycolide was purchased from Purac, and purified by recrystallization from dry ethyl acetate; ε-caprolactone was supplied by Fluka. It was distilled under argon before use. $Zr(acac)_4$ and $Sn(oct)_2$ (Aldrich Corp.) were used as received; L,L-lactide (Boehringer, Ingelheim, Germany) was purified by distillation in vacuum and several recrystallization from dry ethyl acetate; ε-caprolactone (Fluka, Buchs, Switzerland) was distilled under argon.

Copolymerization Procedure

Poly(glycolide-co-caprolactone) and poly-(glycolide-co-lactide) abbreviated as PGA/PCL and PGA/PLA in this paper, was synthesized by bulk ring-opening copolymerization of glycolide with ε-caprolactone and glycolide with L,L-lactide according to the procedure described earlier.[4] $Zr(acac)_4$ or $Sn(oct)_2$ was used as initiator with an I/M (initiator/monomers) molar ratio of 1/800. Polymerization paws performed under argon atmosphere at high temperature (100 to 150 °C) for predetermined periods of time. The obtained copolymers were ground, washed with methyl alcohol to remove unreacted traces of monomers, and then vacuum dried at 50 °C up to constant weight.

Measurements

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Varian Unity Inova spectrometer operating at 300 MHz, using dried dimethyl sulfoxide-d_6 as a solvent. Chemical shifts (δ) were given in ppm using TMS as an internal reference. The spectra were obtained at 80 °C with 32 scans, and 3.74 s acquisition time. Electron spray injection mass spectrometry (ESI-MS) experiments were carried out on degraded copolymers using a Finnigan LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA). Copolymers samples were dissolved in DMSO at a concentration of 0.5 mg/mL and inserted into the electrospray interface at a flow rate of 2 μL/min. Mass spectra were acquired over the range of m/z 50-2000 in negative-ion mode. The viscosity of the obtained copolymers was determined in 1,1,1,3,3,3-haxafluoro 2-propanol at 25 °C with Ubbelhode viscometer. The concentration of the solutions was 0,002 g/cm^3. Size-exclusion chromatography (SEC) measurements were performed for the copolymers soluble in chloroform with a Physics SP 8800 chromatograph apparatus equipped with a Shodex SE 61 detector. Chloroform was used as a mobile phase at a flow rate of 1.0 ml/min. The Styragel columns were calibrated with polystyrene standards (Polysciences, USA). Differental Scanning Calorymetry (DSC) was performed with a Parkin Elmer DSC 6 instruments, with heating rate of 10 °C/min.

X-ray diffraction spectra were registered with a Philips diffractometer composed of a Cu Kα ($\lambda = 0.154$ nm) source, a quartz monochromator and a goniometric plate.

Degradation

Films of the various copolymers were prepared by compression molding with a hydraulic press at 120–220 °C. Square specimens with dimensions of $10 \times 10 \times 0.5$ mm which weighed about 60 mg were then cut from the films. For degradation studies, each specimen was placed in a vial filled with 5 mL of 0.13 M phosphate buffer (pH 7.4) containing 1.0 mg of sodium azide to prevent bacterial growth. The vials were placed in an oven thermostated at 37 °C. At each degradation time, specimens were withdrawn from the degradation medium and washed with distilled water. After wiping, the specimens were weighed and vacuum- dried at room temperature for one week, weighed again, and subjected to analysis. Water uptake and weight loss values were also measured.

Results and Discussion

Influence of the chain microstructure on hydrolytic degradation were monitored for two groups of bioresorbable copolyesters: glycolide/lactide copolymers abbreviated

as: PGA/PLA and glycolide/caprolactone copolymers abbreviated as: PGA/PCL in this study.

PGA/PLA and PGA/PCL containing 8-70 mole % of glycolidyl units GG **[–O–CH_2–C(O)–O–CH_2–C(O)–]** in copolymer chain, have been obtained with various chain microstructure. Chain microstructure of the obtained samples was monitored by the high resolution NMR and characterized by the average lengths of caproyl and glycolidyl units, degree of randomness R and transesterification coefficient T.[2–4] The changes in copolymer chain microstructure during degradation process were monitored by measuring the intensity of lines from comonomeric sequences in ^{1}H and ^{13}C NMR spectra.

Two PGA/PLA copolymers samples with molar ratio:~70GG have been chosen for the presentation in this study. These copolymers have been obtained in: 100 °C using $Zr(acac)_4$ as initiator and in 150 °C using $MgBut_2$ and thus, have different degree of randomness and different chain microstructure. Figure 1 and 2 presents NMR spectra of chosen PGA/PLA copolymers.

In both cases disappearance of peaks no 4, 5, 6 is observable during degradation. Whereas, peaks no 7, which characterize transestrification processes increase. This is connected with degree of randomness. It is changing on every stage of degradation [Figure 3b]. Besides, content of GG units increases during the whole measured time. Long glycolidyl units are known to be less resistant to hydrolysis than long lactidyl units (LL units: **–O–CH(CH_3)–C(O)–O–CH(CH_3)–C(O)O–)**. Copolymers containing more than 50% of glycolidyl units release short lactidyl microblocks more rapid than long glycolidyl microblocks.

Two PGA/PCL copolymers samples with molar ratio ~10% of GG units have been chosen for the presentation in this study. These copolymers have been obtained in: 100 °C (Cop_{Zr100}) and in 150 °C (Cop_{Zr150}) using $Zr(acac)_4$ as initiator. Chain microstructure of the obtained samples and degradation process were monitored by the high resolution NMR. Figure 4a–4b present changes of the content of GG units and average length of blocks during degradation for Cop_{Zr100} and Cop_{Zr150}. All values of NMR parameters obtained from NMR spectra are similar for both copolymers. Only degree of randomness is slightly higher for Cop_{Zr150}.

Degradation mechanism was also monitored using mass spectrometry ESI-MS as a complementary tool in describing changes

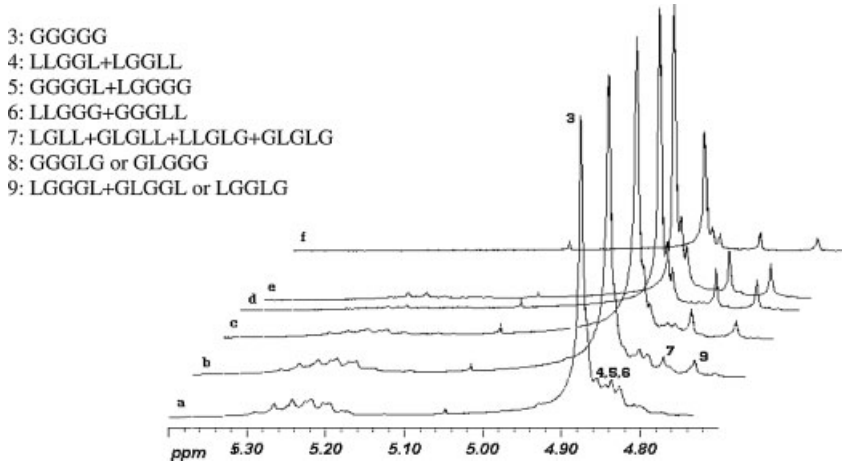

Figure 1.
NMR spectra of PGA/PLA (71GG, 100 °C, $Zr(acac)_4$): **a**- before degradation, **b**- 2nd week of degradation, **c**- 4th week of degradation, **d**- 8th week of degradation, **e**- 16th week of degradation, **f**- 36th week of degradation.

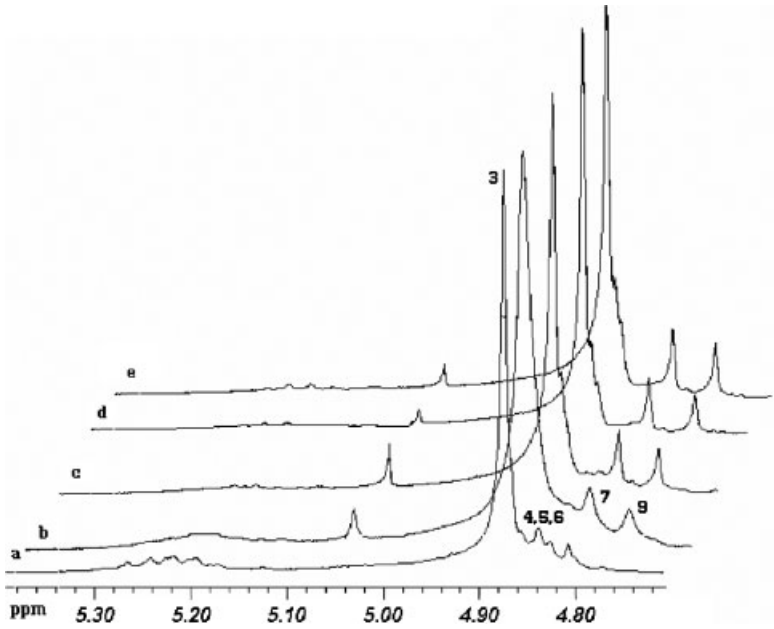

Figure 2.
NMR spectra of PGA/PLA (75GG, 150 °C, $MgBut_2$) **a**- before degradation, **b**- 2nd week of degradation, **c**- 4th week of degradation, **d**- 8th week of degradation, **e**- 16th week of degradation.

in chain microstructure. Figure 5 presents ESI-MS spectrum of PGA/PCL. The negative ESI mass spectrum of oligocopolymers consists of numerous ions with general chemical structures shown on the Figure 5. Peaks (m/z values) observed in the ESI-MS spectra have been assigned to the chemical composition of the ions. Assignments for peaks are reported in the Table 1. Oligocopolymers are observed in whole region of recorded spectra (up to 2000 m/z) during degradation as a function of intensity. On the basis of ESI-MS spectra types of oligomers presented in the chain on every stage

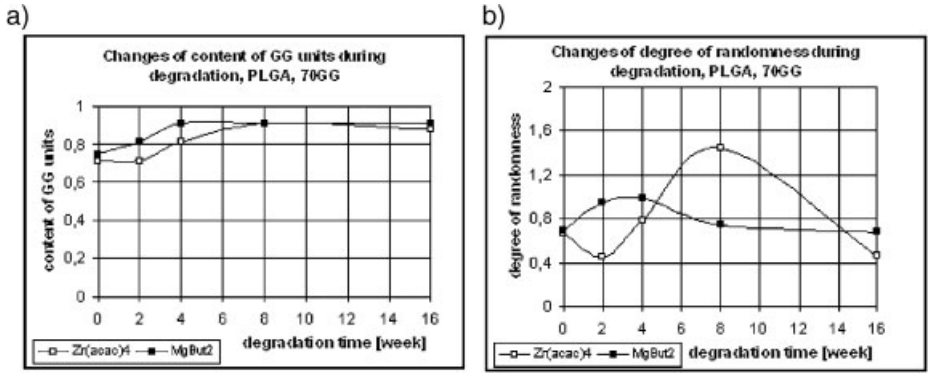

Figure 3.
a. Changes of content of GG units during degradation, PGA/PLA 70GG (left) 3b. Changes of degree of randomness during degradation, PGA/PLA 70GG (right).

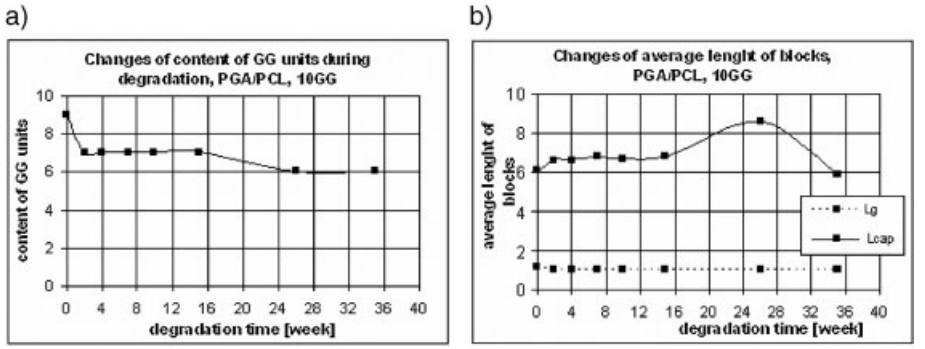

Figure 4.
a. Changes of GG units content during degradation, PGA/PCL (left) 4b. Changes of average length of blocks during degradation, PGA/PCL (right).

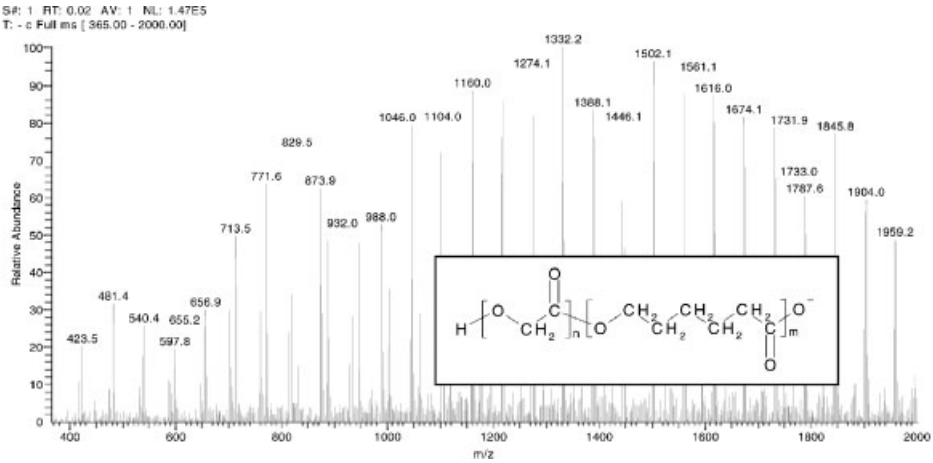

Figure 5.
ESI-MS spectrum for copolymer with 10 mole % of glycolidyl units.

Table 1.
Assignments for peaks observed in the ESI-MS spectra.

m/z value observed in the ESI-MS spectra	Chemical structure	m/z value observed in the ESI-MS spectra	Chemical structure
423,5	G_7	1274,1	$Cap_{10}G_2$
481,4	G_8	1332,2	$Cap_{10}G_3$
540,4	G_9	1388,1	$Cap_{11}G_2$
655,2	G_{11}	1446,1	$Cap_{11}G_3$
713,5	G_{12}	1502,1	$Cap_{12}G_2$
771,6	G_{13}	1561,1	$Cap_{12}G_3$
829,5	G_{14}	1616,0	$Cap_{13}G_2$
873,9	Cap_7G	1674,1	$Cap_{13}G_3$
932,0	Cap_7G_2	1731,9	$Cap_{14}G_2$
988,0	Cap_8G	1787,6	$Cap_{15}G$
1046,0	Cap_8G_2	1845,8	$Cap_{15}G_2$
1104,0	Cap_8G_3	1904,0	$Cap_{15}G_3$
1160,0	Cap_9G_2	1959,2	$Cap_{16}G_2$

of degradation have been described. Figure 6–7 show types of oligocopolymers in planar projection, which remain in the material subjected to degradation. Changes of the chain microstructure during degradation are noticeable (changes of Cap units: **–O–$(CH_2)_5$–CO–** and G units: **–O–CH_2–CO–** in oligocopolymers of **H–[Cap_m–G_n]–O^-** type).

Figure 6 presents changes in the chain microstructure of Cop_{Zr100} during degradation. After 7th week of degradation, only mixed oligocopolymers built of G and Cap units are observed i.e Cap_6G, Cap_9G_2 etc. After 10, 35 weeks of degradation this mixed oligocopolymers are still visible in the ESI-MS spectra. It suggests that degradation proceed evenly. Long glycolidyl units are probably presented in the copolymer chain, but they are degraded in a later period of time. Thus, they are not observed on the ESI-MS spectra. Caproyl units are released together with glycolidyl units.

Different situation is in the case of Cop_{Zr150}. Figure 3 presents changes in the chain microstructure of Cop_{Zr150}. Apart from mixed sequences consisted of G and Cap units i.e. $Cap_{10}G_2$, Cap_9G_3 etc. which are observable on every stage of degradation, other oligocopolymers additionally appear. After 7th week of degradation longer sequences consisted only of units of one kind (-$(G)_n$-) are visible. They do not appear in the later period of time.

All these observations suggest that in both cases: Cop_{Zr100} and Cop_{Zr150} there are a lot of mixed sequences presented in the copolymer chain. They remain in studied copolymers for a whole measured period of time. However, ESI-MS analysis leads unequivocally that copolymer chain structure before degradation and mechanism of degradation are distinctly various for copolymers obtained in 100 °C and 150 °C in the presence of zirconium initiator despite of low content of GG units in

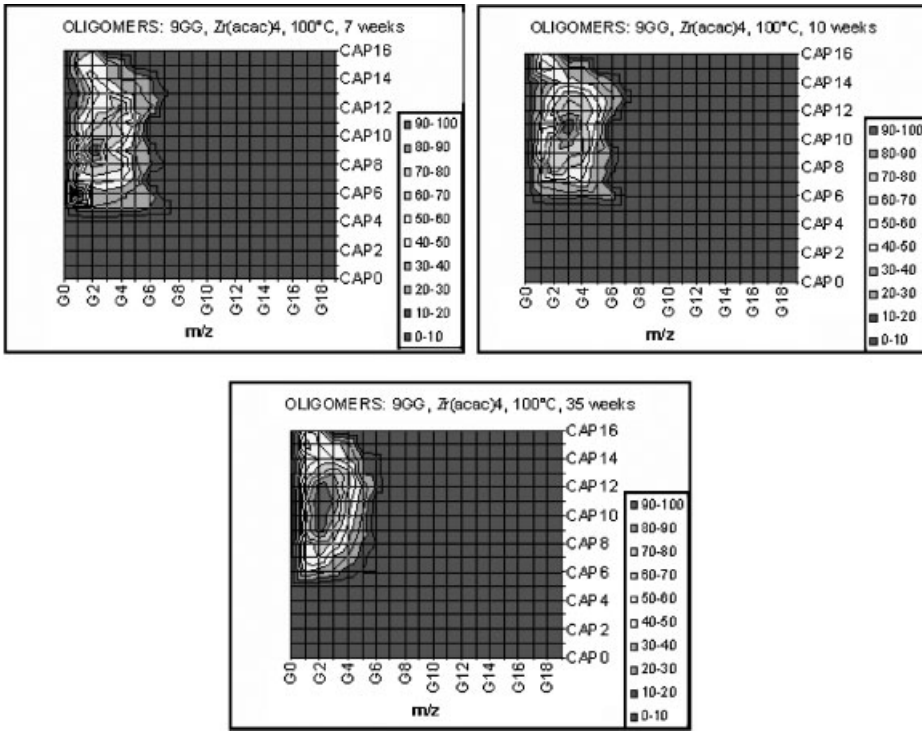

Figure 6.
Changes in chemical structure of oligocopolymers during degradation of Cop_{Zr100}.

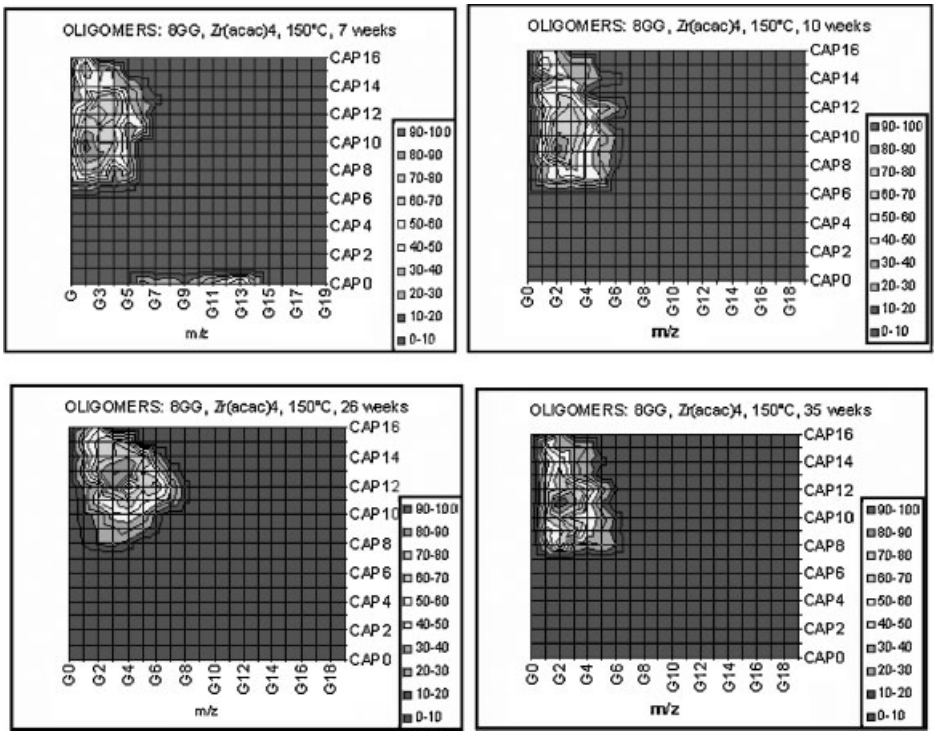

Figure 7.
Changes in chemical structure of oligocopolymers during degradation of Cop_{Zr150}.

copolymer chain (ca.10%) and lack of differences in NMR spectra.

Conclusions

On the basis of NMR and ESI-MS spectra completely different ways of degradation process for random and block polyester copolymers have been observed. The hydrolytic degradation of the copolymer chains with higher degree of randomness proceeds faster than block copolymer chains. For PGA/PLA copolymers long glycolidyl units are known to be less resistant to hydrolysis than long lactidyl units Copolymers containing more than 50 % of glycolidyl units release short lactidyl microblocks more rapid than long glycolidyl microblocks. ESI-MS analysis of degradation products leads distinctions in copolymer chain structure of PGA/PCL samples despite of low content of GG units in copolymer chain (ca.10%) and lack of differences in NMR spectra.

It have appeared that ESI-MS technique can be useful tool, complementary to NMR method in studying the chain microstructure since it provide a lot of information not observed in nuclear magnetic resonance experiment.

Acknowledgements: Regional Scholarship Fund for PhD Students EFS-2.6 ZPORR No. Z/2.24/II/2.6/17/04 RFSD

[1] R. L. Dunn, *Biomedical Applications of Synthetic Biodegradable Polymers*, J. O. Hollinger, Ed., CRC Press, Boca Raton, FL **1995**, p. 17–31.
[2] J. Kasperczyk, *Polymer* **1996**, *37*, 201.
[3] S. Li, P. Dobrzynski, J. Kasperczyk, M. Bero, C. Braud, M. Vert, *Biomacromolecules* **2005**, *6*, p. 489–497.
[4] P. Dobrzynski, S. Li, J. Kasperczyk, M. Bero, F. Gasc, M. Vert, *Biomacromolecules* **2006**, *6*, p. 483–488.

