Bioactive oligomers from natural polyhydroxyalkanoates and their synthetic analogues

Grażyna Adamus[1)], Piotr Kurcok[1)], Iza Radecka[2)], Marek Kowalczuk[1), 2), *)]

DOI: dx.doi.org/10.14314/polimery.2017.317

This publication is dedicated to the memory of the scientist and friend Prof. Andrzej Duda

Abstract: Contemporary reports on the bioactive oligomers derived from natural aliphatic co-polyesters (PHA) and their synthetic analogues, formed through anionic ring-opening polymerization (ROP) of β-substituted β-lactones are presented. Synthetic routes for such oligomers, developed mostly by Polish authors, are discussed. The described approaches enable design of novel biodegradable and bioactive oligomers for diverse applications in medicine, cosmetic industry and agrochemistry.

Keywords: aliphatic polyester, anionic polymerization, bioactive oligomer, β-substituted β-lactone.

Otrzymywanie bioaktywnych oligomerów z naturalnych biopoliestrów i ich syntetycznych analogów

Streszczenie: Przedstawiono przegląd aktualnych wyników badań dotyczących bioaktywnych oligomerów otrzymywanych z naturalnych alifatycznych ko-poliestrów (PHA) oraz ich syntetycznych analogów, uzyskanych na drodze anionowej polimeryzacji z otwarciem pierścienia (ROP) β-podstawionych β-laktonów. Omówiono ścieżki syntezy tych oligomerów opracowane głównie przez polskich badaczy. Zaprezentowane metody umożliwiają projektowanie nowych biodegradowalnych, a zarazem bioaktywnych oligomerów mogących znaleźć różnorodne zastosowania w medycynie, przemyśle kosmetycznym i agrochemii.

Słowa kluczowe: poliester alifatyczny, anionowa polimeryzacja, oligomer bioaktywny, β-podstawiony β-lakton.

Polyhydroxyalkanoates (PHA) are a group of biopolyesters that have a wide range of applications. Extensive progress has been made in our understanding of PHA biosynthesis, and currently, it is possible to engineer bacterial strains to produce PHA with desired properties. Overview on the current carbon sources used for PHA production and the methods used to transform these sources into fermentable forms has been published recently [1]. It should be mentioned, that among various carbons sources we report also on the ability of bacteria to produce PHA from polyethylene *via* oxidized polyethylene wax as a novel carbon source [2]. The studies are also carried out by other laboratories on production of high levels of PHA in tobacco plants [3].

[1)] Centre of Polymer and Carbon Materials, Polish Academy of Sciences, 34 M. Curie-Skłodowskiej St., 41-119 Zabrze, Poland.

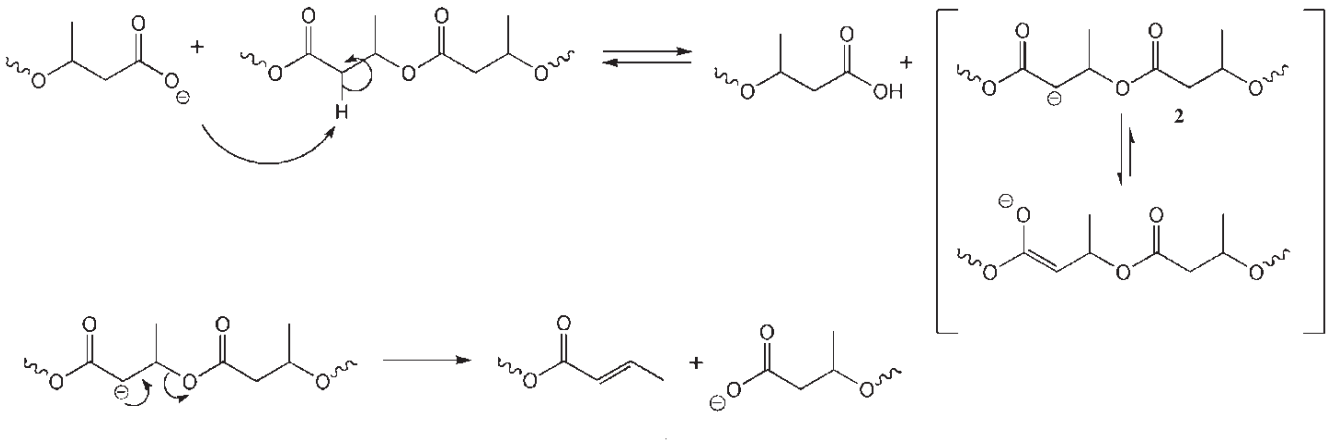
[2)] University of Wolverhampton, Faculty of Science and Engineering, School of Biology, Chemistry and Forensic Science, Wulfuna Street, Wolverhampton WV1 1LY, UK.

[*)] Author for correspondence; e-mail: marek.kowalczuk@cmpw-pan.edu.pl

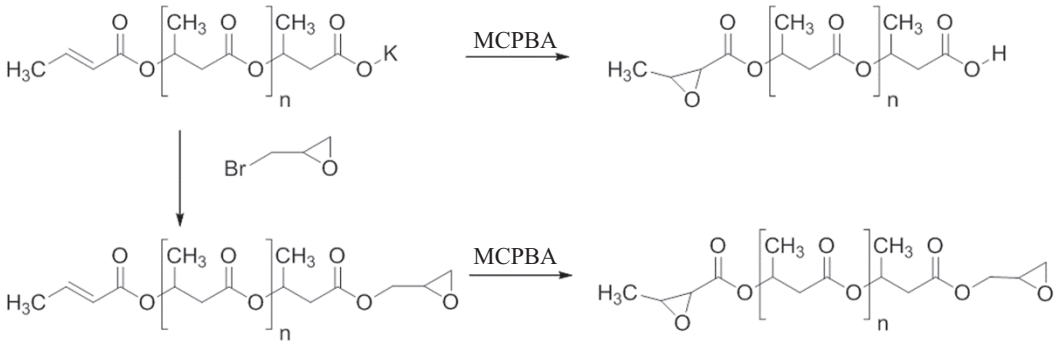
PHA polymers are thermoplastic and depending on their chemical composition they may differ in their properties. Blending of PHA with other biodegradable polymers tunes properties of polymeric materials suitable for packaging applications. Current trends in the packaging industry are towards compostable bio-based lighter weight materials for reduction of raw material use, transportation costs, minimizing the amount of waste and it may be expected that interest in sustainable materials combined with barrier improving additives will continue to growth [4].

OLIGOMERS FROM NATURAL POLYHYDROXYALKANOATES

PHA due to their *in vivo* and *in vitro* biodegradation as well as cell and tissue compatibility can be used for medical applications, especially as implants, including heart valve tissue engineering, vascular tissue engineering, bone tissue engineering, cartilage tissue engineering as well as nerve conduit tissue engineering. Moreover, PHA implants were found not to cause carcinogenesis during long-term implantation. Chemical modifications of PHA

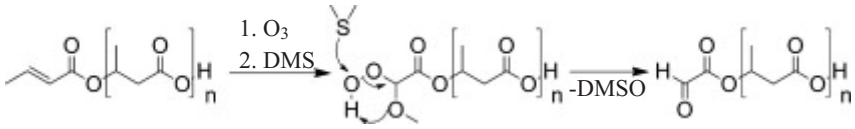


Scheme A



MCPBA – 3-chloroperoxybenzoic acid

Scheme B



Scheme C

in order to introduce functional groups, that cannot be easily achieved by bioconversion processes, are a valuable challenge since chemically modified PHA can be utilized as multifunctional biomaterials [5].

PHA oligomers contained unsaturated end groups

PHA can be reacted with a carbonate salts, yielding oligomers possessing a controlled molecular weight, which can be modulated in view of the specific application for which the products are intended [6–8]. The degradation reaction can be carried out as a continuous process, *e.g.*, by means of an extruder, with outstanding advantages for an industrial applications. The obtained oligomers are terminated by unsaturated and carboxylate end groups [as revealed by electrospray ionization mass spectrometry (ESI-MS^n) analyses]. They are formed according to E1cB elimination reaction mechanism of α-deprotonation of poly(3-hydroxyalkanoate)s, in the intermolecular process. The process of carboxylate induced degradation of PHA is shown in Scheme A [9].

The unsaturated end groups can be subjected to subsequent modifications to obtain a wide variety of functional end groups, for instance hydroxyl, carboxyl or oxirane groups by oxidation of the above double bonds [10]. As example the synthesis of epoxy-functionalized oligo(3-hydroxybutyrate) (OHB) is presented in Scheme B.

Such unsaturated end groups of OHB may be also ozonolyzed to the aldehyde function and used as PHA-based carriers for drug delivery systems with pH-controlled release [11]. The example of such reaction can be synthesis of oligo(3-hydroxybutyrate) glyoxylate shown in Scheme C.

The controlled molecular weight and the presence of double bonds and/or other functional groups as terminal groups make the above oligomers or polymers particularly suitable as macromers (building blocks) for the synthesis and/or modification of polymers, particularly of biodegradable polymers for medical applications [12]. It was also found that oligomers consisted of 3-hydroxybutyrate 3-malic acid units can be synthesized *via* thermal treatment of poly(3-hydroxybutyrate) (PHB) in oxygen/ozone mixture. Nuclear magnetic resonance (NMR) and

multi-stage mass-spectrometry (MS) characterization revealed random distribution of 3-malic acid units in oligomeric products as well as content of the malic acid units dependent on oxidation conditions [13].

PHA oligomers contained hydroxyl end groups

A highly selective method for controlling the degradation of PHA *via* a reduction reaction that uses lithium borohydride was reported [14]. Using this method, oligo(hydroxyalkanoate)diols were obtained according reaction shown in Scheme D.

The structural characterization of the oligo(hydroxyalkanoate)diols was conducted using NMR and ESI-MS^n analyses, which confirmed that oligomers were terminated by two hydroxyl end groups. The reduction of the PHA occurred in a statistical way regardless of the chemical structure of the comonomer units or of the microstructure of the polyester chain. This method can be used to synthesize various PHA oligodiols that are potentially useful in the further synthesis of tailor-made biodegradable materials for medical applications.

PHA bioactive oligomers

The transesterification of PHA has been used as a tool for the preparation of delivery systems for selected bioactive compounds containing either carboxyl or hydroxyl functionalities [15]. The first synthetic strategy was designed for bioactive compounds within the carboxyl group, and these conjugates were obtained through the transesterification of natural PHA. Scheme E shows transesterification reaction of PHA by (4-chloro-2-methylphenoxy)acetic acid (MCPA).

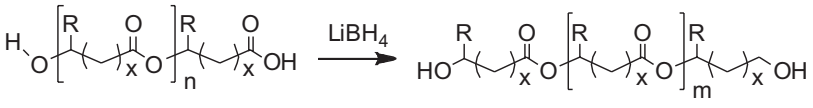
The second synthetic strategy was selected for bioactive compounds within the hydroxyl group [16]. The tyrosol-polymer conjugates were synthesized *via* transesterification reaction of tyrosol with selected PHA, presented in Scheme F.

The transesterification reaction of PHA with a bioactive compounds constitutes simple and economically favorable method for obtaining such conjugates. The bioactive PHA oligomers may be used in the area of controlled delivery systems in medicine, agrochemistry, in the cosmetic industry, in household products and in coating systems.

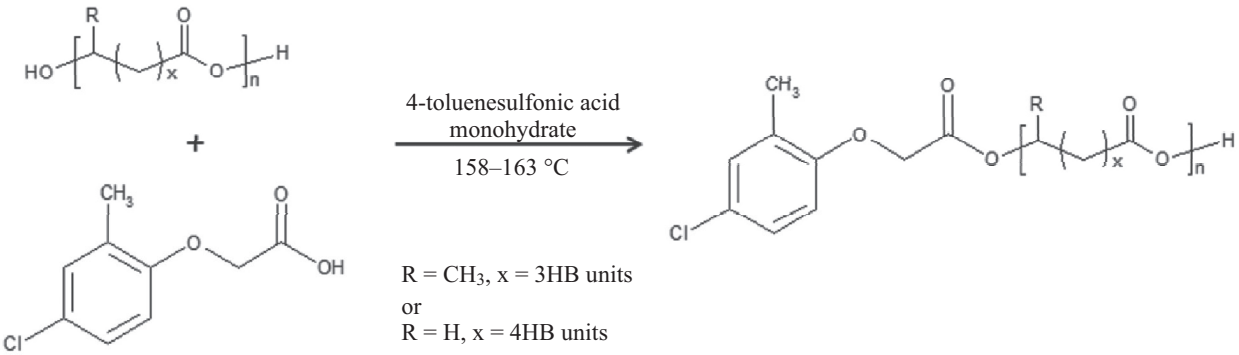
BIOACTIVE OLIGOMERS FROM SYNTHETIC ANALOGUES OF POLYHYDROXYALKANOATES

Bioactive oligomers of PHB analogue

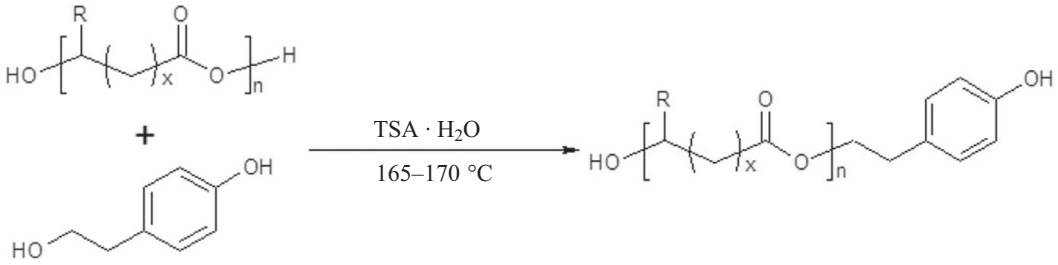
Developed at our laboratories, and independently confirmed by Duda the anionic ring-opening polymerization (ROP) of β-substituted β-lactones seems to be a perfect



Scheme D



Scheme E



Scheme F

tool for the preparation of PHA analogues with the desired molecular structure, including the structure of the end groups [17–19]. In contrast to unsubstantiated four membered β-propiolactone, β-butyrolactone (BL) is not polymerized by common anionic initiators. However, these initiators, when activated by the addition of macrocyclic ligands such as crown ethers or cryptands, are able to initiate the polymerization of BL to PHB, *i.e.*, the simplest PHA analogue [20, 21]. The same effect may be achieved by using bulky counterions or suitable highly polar aprotic solvents, *e.g.*, DMSO [22, 23]. The polymer chain growth proceeds regioselectively and stereoselectively entirely *via* carboxylate anions [24]. The anionic ROP reaction of BL to PHB is shown in Scheme G.

Propagation on carboxylate active centers (much less sensitive to impurities than any other anionic species) enables the scaling up of the anionic ROP polymerization process [25, 26].

Also other catalysts were used for ROP reaction of BL, including discrete rare earth and cationic systems [27–30].

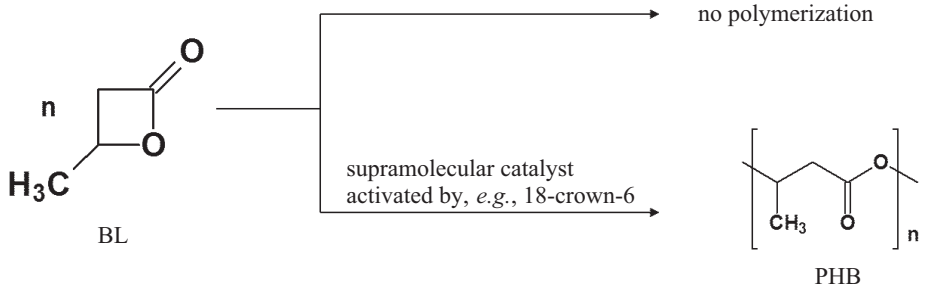
Synthetically prepared OHB were found to be nontoxic and they may be used as carriers covalently bounded to suitable bioactive compounds [31]. In our studies regarding this area, several bioactive PHB oligomers suitable for medical, cosmetic, agrichemical and functional packaging applications have been prepared and characterized at the molecular level using the ESI-MS^n technique. The drug delivery systems were focused on penicillin G, acetylsalicylic acid, and ibuprofen [32–34]. For perspective applications in cosmetology the OHB conjugates with α-lipoic acid (LA), *p*-coumaric acid, *p*-anisic acid (AA) and vanillic acid (VA) have been obtained and characterized [35, 36]. Synthetic pathway to OHB conjugate with selected antioxidants (AA and VA) is shown in Scheme H.

The bioconjugate hydrolytic degradation studies allowed gaining thorough insight into the hydrolysis process and confirmed the release of bioactive species. *In vitro* studies demonstrated that all of the conjugates studied were well tolerated by KB and HaCaT cell lines, as they had no marked cytotoxicity, while conjugates with a relatively short OHB carrier are optimal to support keratinocyte function [37].

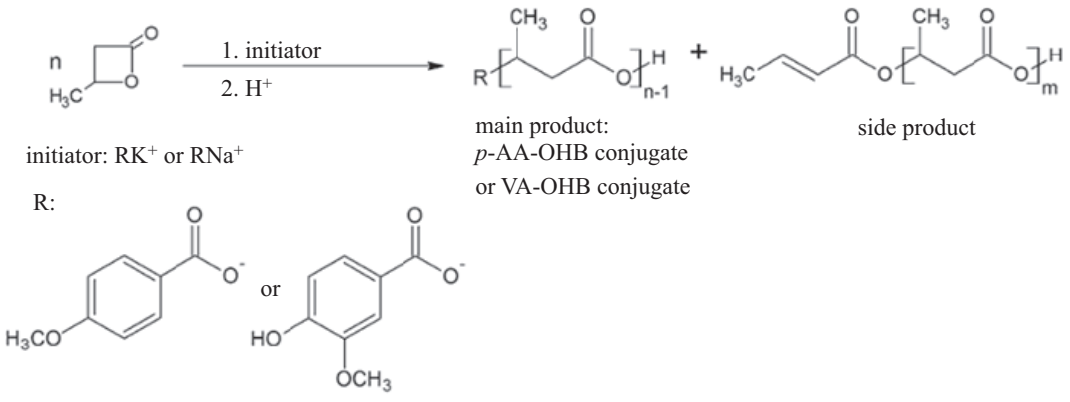
For potential agricultural applications novel phenoxycarboxylic acid-OHB conjugates have been synthetized, and OHB conjugates with sorbic acid and benzoic acid, designed for food active packaging systems, have been also reported and the structures of the resulting conjugates have been established at the molecular level by electrospray ionization multi-stage mass spectrometry [38]. The same strategy has also been applied for the synthesis and characterization of a novel polypyrrole material grafted with biodegradable OHB pendants. The obtained OHB functionalized pyrroles were found to be promising candidates for the preparation of biodegradable conductive polymers [39, 40].

Bioactive oligomers of PHA analogues

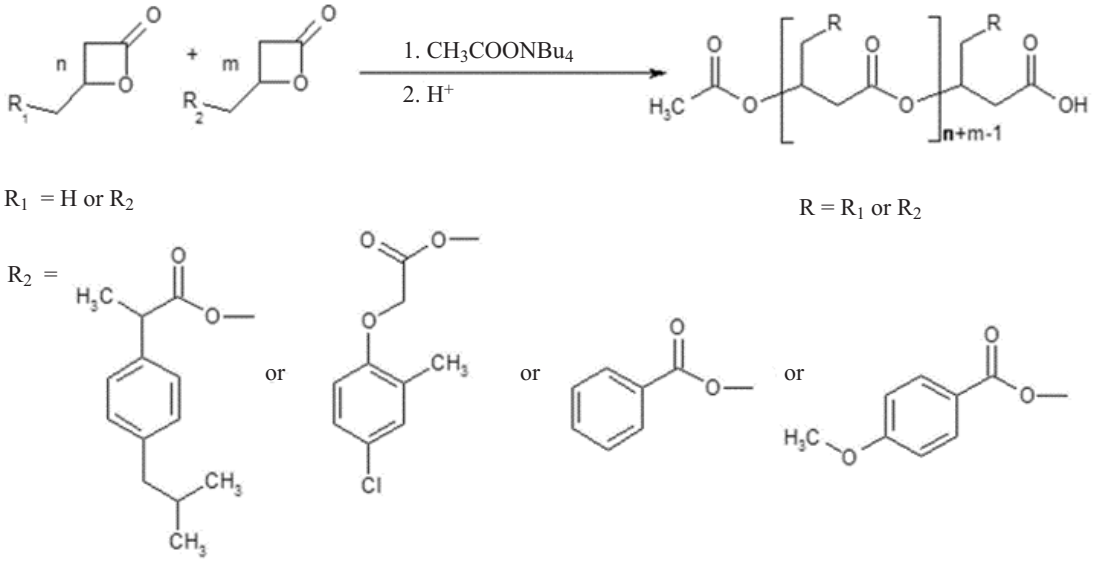
The specific synthetic method based on the carbonylation of the respective epoxides under CO at ambient pres-



Scheme G



Scheme H



Scheme I

sure opens up new opportunities for exploring the utility of β-lactones (and in particular precursors of synthetic analogues of natural PHA, *i.e.*, β-substituted β-lactones) as monomers for the synthesis of new polymers with desired properties. Thus, block and random synthetic PHA copolymers were prepared by anionic ROP [41–43].

Incorporation of bioactive compounds into the β-lactones structure may lead to homo- and co-oligoesters with a bioactive moiety covalently linked as pendent groups along an oligomer backbone. This synthetic strategy was applied by us for preparation of the PHA synthetic analogues with ibuprofen pendant groups [44], pesticide moieties [45] and recently antioxidants used in cosmetics [46]. The respective synthetic pathway, presented on Scheme I shows synthesis of bioactive co-oligoesters *via* copolymerization of BL with β-substituted β-lactones containing covalently bonded selected bioactive moieties [*e.g.*, ibuprofen, (4-chloro-2-methylphenoxy)acetic acid, and anisic acid].

Novel delivery systems obtained *via* the elaborated synthetic strategy contain a larger loading of biologically active substances per polymer macromolecule and their amount as well as position at the (*co*)polymer chain can be controlled.

CONCLUSIONS

Biodegradable polymers have become materials of hope for the future and knowledge on the relationships between their structure, properties, and function is essential for prospective safe applications of such materials in the areas of human health and the environment.

When the development of biodegradable polymers was in its infancy the most crucial features were concentrated on the effect of macromolecular architecture, new monomer systems, polymerization mechanisms, and different polymerization techniques on final biodegradable properties. Significant efforts have been directed towards specific areas, such as mechanisms of biodegradation, biocompatibility and processing conditions. However, especially for potential applications in medicine such aspects like bio-safety of biodegradable polymers or nano-safety of their composites were and still are frequently neglected. The diverse applications of biodegradable polymers require case specific characterization and optimization of the material properties, its preparation, processing, and recycling. Pulling these different elements together under the common thread of forensic engineering of advanced polymeric materials (FEAPM) provides a central driving force for the otherwise disconnected works and constitutes the novelty of our recent research [47]. Such an approach helps to design novel biodegradable polymeric materials and to avoid failures of the commercial products manufactured from them.

This work was supported by the National Science Centre projects: DEC-2012/07/B/ST5/00627 (MK), UMO-2013/11/B/ST5/02222 (GA), 2015/17/B/ST5/01086 (PK) and by the European Regional Development Fund: contract No. POIG.01.03.01-00-018/08. The outcomes of this research will be used to implement the UE PLASTiCE project (3CE368P1, "Innovation value chain development for sustainable plastics in Central Europe", CENTRAL EUROPE Programme, cofinanced by ERDF.

REFERENCES

[1] Jiang G., Hill D.J., Kowalczuk M. *et al.*: *International Journal of Molecular Sciences* **2016**, *17*, 1157. http://dx.doi.org/10.3390/ijms17071157

[2] Radecka I., Irorere V., Jiang G. *et al.*: *Materials* **2016**, *9*, 367. http://dx.doi.org/10.3390/ma9050367

[3] Bohmert-Tatarev K., McAvoy S., Daughtry S. *et al.*: *Plant Physiology* **2011**, *155*, 1690. http://dx.doi.org/10.1104/pp.110.169581

[4] Bugnicourt E., Cinelli P., Lazzeri A. *et al.*: *eXPRESS Polymer*

Letters **2014**, *8*, 791. http://dx.doi.org/10.3144/expresspolymlett.2014.82
[5] Kai D., Loh X.J.: *ACS Sustainable Chemistry and Engineering* **2014**, *2*, 106. http://dx.doi.org/10.1021/sc400340p
[6] Kawalec M., Sobota M., Scandola M. *et al.*: *Journal of Polymer Science Part A: Polymer Chemistry* **2010**, *48*, 5490. http://dx.doi.org/10.1002/pola.24357
[7] *Eur. Pat.* EP 2 346 922 (2014).
[8] Kwiecień M., Kawalec M., Kurcok P. *et al.*: *Polymer Degradation & Stability* **2014**, *110*, 71. http://dx.doi.org/10.1016/j.polymdegradstab.2014.07.028
[9] Kawalec M., Adamus G., Kurcok P. *et al.*: *Biomacromolecules* **2007**, *8*, 1053. http://dx.doi.org/10.1021/bm061155n
[10] Michalak M., Kawalec M., Kurcok P.: *Polymer Degradation & Stability* **2012**, *97*, 1861. http://dx.doi.org/10.1016/j.polymdegradstab.2012.05.007
[11] Michalak M., Marek A., Zawadiak J. *et al.*: *European Polymer Journal* **2013**, *49*, 4149. http://dx.doi.org/10.1016/j.eurpolymj.2013.09.021
[12] Adamus G., Sikorska W., Janeczek H. *et al.*: *European Polymer Journal* **2012**, *48*, 621. http://dx.doi.org/10.1016/j.eurpolymj.2011.12.017
[13] Michalak M., Kwiecień M., Kawalec M. *et al.*: *RCS Advances* **2016**, *6*, 12 809. http://dx.doi.org/10.1039/c5ra27041c
[14] Kwiecień M., Adamus G., Kowalczuk M.: *Biomacromolecules* **2013**, *14*, 1181. http://dx.doi.org/10.1021/bm400141s
[15] Kwiecień I., Radecka I., Kowalczuk M. *et al.*: *PLoS ONE* **2015**, *10*, e0120149. http://dx.doi.org/10.1371/journal.pone.0120149
[16] Kwiecień I., Radecka I., Kwiecień M. *et al.*: *Materials* **2016**, *9*, 307. http://dx.doi.org/10.3390/ma9050307
[17] Jedliński Z., Kowalczuk M., Główkowski W. *et al.*: *Macromolecules* **1991**, *24*, 349. http://dx.doi.org/10.1021/ma00002a002
[18] Duda A.: *Journal of Polymer Science Part A: Polymer Chemistry* **1992**, *30*, 21. http://dx.doi.org/10.1002/pola.1992.080300103
[19] Adamus G., Kowalczuk M.: *Biomacromolecules* **2008**, *9*, 696. http://dx.doi.org/10.1021/bm701077v
[20] Kawalec M., Śmiga-Matuszowicz M., Kurcok P.: *European Polymer Journal* **2008**, *44*, 3556. http://dx.doi.org/10.1016/j.eurpolymj.2008.09.008
[21] Jedliński Z., Kurcok P., Lenz R.W.: *Macromolecules* **1998**, *31*, 6718. http://dx.doi.org/10.1021/ma980663p
[22] Kurcok P., Śmiga M., Jedliński Z.: *Journal of Polymer Science Part A: Polymer Chemistry* **2002**, *40*, 2184. http://dx.doi.org/10.1002/pola.10285
[23] Juzwa M., Jedliński Z.: *Macromolecules* **2006**, *39*, 4627. http://dx.doi.org/10.1021/ma0602369
[24] Jedliński Z., Kowalczuk M., Kurcok P. *et al.*: *Macromolecules* **1996**, *29*, 3773. http://dx.doi.org/10.1021/ma951888s
[25] Kowalczuk M.: *Polymer Science Series* **2009**, *A 51*, 1229. http://dx.doi.org/10.1134/S0965545X09110078
[26] Kawalec M., Coulembier O., Gerbaux P. *et al.*: *Reactive & Functional Polymers* **2012**, *72*, 509. http://dx.doi.org/10.1016/j.reactfunctpolym.2012.04.013
[27] Carpentier J.: *Macromolecular Rapid Communications* **2010**, *31*, 1696. http://dx.doi.org/10.1002/marc.201000114
[28] Basko M., Duda A., Kazmierski S. *et al.*: *Journal of Polymer Science, Part A: Polymer Chemistry* **2013**, *51*, 4873. http://dx.doi.org/10.1002/pola.26916
[29] Dove A.P.: *Chemical Communications* **2008**, 6446. http://dx.doi.org/10.1039/B813059K
[30] Guillaume S.M., Annunziata L., del Rosal I. *et al.*: *Polymer Chemistry* **2013**, *4*, 3077. http://dx.doi.org/10.1039/C3PY00056G
[31] Piddubnyak V., Kurcok P., Matuszowicz A. *et al.*: *Biomaterials* **2004**, *25*, 5271. http://dx.doi.org/10.1016/j.biomaterials.2003.12.029
[32] Adamus G., Kowalczuk M.: *Rapid Communications in Mass Spectrometry* **2000**, *14*, 195. http://dx.doi.org/10.1002/(SICI)1097-0231(20000229)14:4<195::AID-RCM864>3.0.CO;2-X
[33] Juzwa M., Rusin A., Zawidlak-Węgrzyńska B. *et al.*: *European Journal of Medicinal Chemistry* **2008**, *43*, 1785. http://dx.doi.org/10.1016/j.ejmech.2007.11.004
[34] Zawidlak-Węgrzyńska B., Kawalec M., Bosek I. *et al.*: *European Journal of Medicinal Chemistry* **2010**, *45*, 1833. http://dx.doi.org/10.1016/j.ejmech.2010.01.020
[35] Maksymiak M., Kowalczuk M., Adamus G.: *International Journal of Mass Spectrometry* **2014**, *359*, 6. http://dx.doi.org/10.1016/j.ijms.2013.11.009
[36] Maksymiak M., Dębowska R., Jelonek K.: *Rapid Communications in Mass Spectrometry* **2013**, *27*, 773. http://dx.doi.org/10.1002/rcm.6509
[37] Maksymiak M., Dębowska R., Bazela K. *et al.*: *Biomacromolecules* **2015**, *16*, 3603. http://dx.doi.org/10.1021/acs.biomac.5b01065
[38] Kwiecień I., Adamus G., Bartkowiak A. *et al.*: *Designed Monomers & Polymers* **2013**, *17*, 311. http://dx.doi.org/10.1080/15685551.2013.840505
[39] Domagała A., Maksymiak M., Janeczek H. *et al.*: *Journal of Material Science* **2014**, *49* (*14*), 5227. http://dx.doi.org/10.1007/s10853-014-8241-0
[40] Domagała A., Domagała W., Ledwoń P. *et al.*: *Polymer International* **2016**, *65*, 1395. http://dx.doi.org/10.1002/pi.5190
[41] Jedliński Z., Kowalczuk M., Kurcok P. *et al.*: *Makromolekulare Chemie* **1987**, *188*, 1575. http://dx.doi.org/10.1002/macp.1987.021880704
[42] Adamus G.: *Macromolecules* **2009**, *42*, 4547. http://dx.doi.org/10.1021/ma900349u
[43] Adamus G., Kwiecień I., Maksymiak M. *et al.*: *Analytica Chimica Acta* **2014**, *808*, 104. http://dx.doi.org/10.1016/j.aca.2013.09.001
[44] *Pol. Pat. App.* P-409 509 (2014).
[45] Kwiecień I., Bałakier T., Jurczak J. *et al.*: *Rapid Communications in Mass Spectrometry* **2015**, *29*, 533. http://dx.doi.org/10.1002/rcm.7133
[46] Maksymiak M., Bałakier T., Jurczak J. *et al.*: *RSC Advances* **2016**, *6*, 57 751. http://dx.doi.org/10.1039/c6ra09870c
[47] Sikorska W., Adamus G., Dobrzyński P. *et al.*: *Polymer Degradation and Stability* **2014**, *110*, 518. http://dx.doi.org/10.1016/j.polymdegradstab.2014.09.019