MASS SPECTROMETRY FOR THE ELUCIDATION OF THE SUBTLE MOLECULAR STRUCTURE OF BIODEGRADABLE POLYMERS AND THEIR DEGRADATION PRODUCTS

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Contemporary reports by Polish authors on the application of mass spectrometric methods for the elucidation of the subtle molecular structure of biodegradable polymers and their degradation products will be presented. Special emphasis will be given to natural aliphatic (co)polyesters (PHA) and their synthetic analogues, formed through anionic ring-opening polymerization (ROP) of β-substituted β-lactones. Moreover, the application of MS techniques for the evaluation of the structure of biodegradable polymers obtained in ionic and coordination polymerization of cyclic ethers and esters as well as products of step-growth polymerization, in which bifunctional or multifunctional monomers react to form oligomers and eventually long chain polymers, will be discussed. Furthermore, the application of modern MS techniques for the assessment of polymer degradation products, frequently bearing characteristic end groups that can be revealed and differentiated by MS, will be discussed within the context of specific degradation pathways. Finally, recent Polish accomplishments in the area of mass spectrometry will be outlined. © 2015 Wiley Periodicals, Inc. Mass Spec Rev 35:188–198, 2016.

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I. INTRODUCTION

Soft ionization mass spectrometry represents a powerful toolset for the structural characterization of polymers. Progress in this area is tremendous and covers the novel mass spectrometry (MS) characterization methods (Antoine, Lemoine J, & Dugourd, 2014) as well as the polymers that have been analyzed (Charles, 2014).

It is of importance in the case of biodegradable polymers and the polymeric materials made from them due to their potential applications in medicine, the cosmetics industry, agro chemistry, and environmental protection. MS can be successfully applied for the characterization of natural biodegradable polymers as well as their derivatives and may be also helpful in the synthesis of biodegradable (co)polymers from natural and nonrenewable sources (Montaudo, 2002). Moreover, MS is currently needed for the evaluation and understanding of the relationships between structure, properties, and behavior (before, during and after practical applications) of biodegradable polymer materials (Sikorska et al., 2014). The assessment of polymer degradation products is desirable due to the safety of these advanced polymer applications.

Some parts of the above mentioned MS studies have been recently reviewed in scientific journals and books (Li, Wesdemiotis, & Hoteling, 2014; Barner-Kowollik et al., 2011; Hakkarainen, 2012). Thus, in the current review, dedicated to the Central-European issue of MSR, we have focused on the most significant recent studies in this area published by Polish authors. Special emphasis has been given to natural aliphatic (co)polyesters (PHA) and their synthetic analogues, formed via anionic ring-opening polymerization (ROP) of β-substituted β-lactones. The application of MS techniques for the evaluation of the structure of biodegradable polymers obtained in ionic and coordination polymerization of cyclic ethers and esters as well as products of step-growth polymerization, in which bifunctional or multifunctional monomers react to form oligomers and eventually long chain polymers, has been also described. Furthermore, the application of modern MS techniques for the assessment of polymer degradation products has been discussed within the context of specific degradation pathways. Recent Polish accomplishments in the area of mass spectrometry have been also outlined.

II. MASS SPECTROMETRY FOR THE CHARACTERIZATION OF NATURAL BIODEGRADABLE POLYMERS AND THEIR DERIVATIVES

In current scientific literature, the term bioplastics is frequently used for polymeric materials which are biodegradable, bio-based or possess both such features. Thus, natural biodegradable polymers are polymers from renewable resources, i.e., made from biomass feedstock material and feature the property of biodegradation. The manufacturing of natural biodegradable polymers can include different procedures, which can be chemical or bio-technological (affected by microorganisms or enzymes). The most common procedures include the chemical synthesis of polymers from a monomer produced by the bio-technological conversion of a renewable resource (e.g., the use of lactic acid produced from the fermentation of sugars for the production of polylactic acid–PLA) (Mehtaa et al., 2005) or the production of polymers by bio-technological procedures based

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on a renewable resource (e.g., the fermentation of sugars or other organic carbon sources by microorganisms to thermoplastic aliphatic polyesters, such as polyhydroxyalkanoates–PHA (Koller, Niebelschüt, & Braunegg, 2013).

PHA represents an interesting group of natural biodegradable polymers that have recently received much attention, particularly as environmentally friendly materials, e.g., in packaging, agriculture, marine and medical fields (Kai & Loh, 2014). Apart from the poly(3-hydroxybutyrate) (PHB) homopolymer, PHA copolymers are even more interesting due to the possibility of regulating physical and thermal copolymer properties by varying molecular structure, average comonomer composition, and comonomer composition distribution (CCD) along with molecular mass distribution (Sudesh, Abe, & Doi, 2000; Doi, 1990; Inoue & Yoshie, 1992). It is important to determine these parameters accurately and precisely (Montaudo, 2002). Therefore, the electorpray ionisation-mass spectromety (ESI-MS) method has been applied for the determination of the comonomer unit composition and composition distribution in bacterial PHA copolymers based on the analysis of their oligomers obtained by controlled, partial, alkaline depolymerization (Montaudo & Lattimer, 2002; Adamus et al., 2000; Kowalczuk & Adamus, 2001). Using this approach, oligomers with the same composition and sequence distribution as the starting materials containing carboxylic and olefinic end groups were obtained as revealed by ^{1}H NMR and ESI-MS analysis (Adamus et al., 2000; Kowalczuk & Adamus, 2001; Adamus et al., 2003). Moreover, the multistage mass spectrometry (MS^n) technique can be additionally applied for the analyses of such oligomers and the molar masses and structural characteristic of mass-selected macromolecular ions have been determined, providing information about the structure of PHA copolymers at the molecular level. This relatively rapid approach, developed at our laboratories, has been recently adopted for the automated routine analysis of PHBV copolymers (Wei et al., 2014).

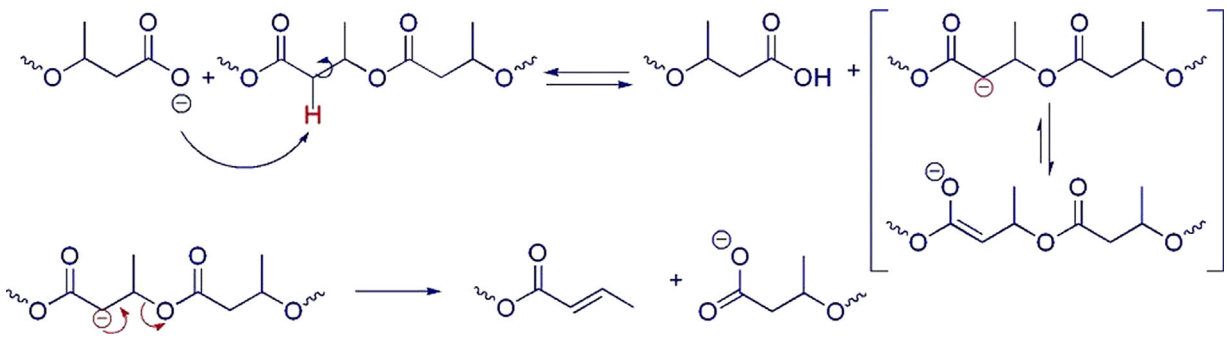
Another exploration of partial controlled degradation of PHA is concerned with their specific application in medicine, such as in controlled delivery systems, in tissue adhesion prevention films for surgical operations, and as scaffolds for tissue engineering (Chen & Wang, 2013). Therefore, systematic research has recently been conducted on the development of new polymeric biomaterials with required properties that are based on matrices or on structural segments derived from PHA biopolyesters (Laycock et al., 2014). Mentioned above, the partial saponification of PHAs, catalyzed by KOH/18-crown-6 or by the aqueous solution of tetrabutylammonium hydroxide, allows ones to obtain macroinitiators that contain carboxylate and unsaturated end groups, as revealed by MS analyses. The structure of macroinitiators containing the same end groups and prepared using a thermal method that is based on the random polymer-chain scission mechanism *via* intramolecular stereoselective cis elimination or *via* intermolecular decomposition was also established at the molecular level by the MS technique (Kawalec et al., 2007). Based on these studies, the E1cB elimination reaction mechanism of α-deprotonation of poly(3-hydroxyalkanoate)s, 3-PHA, was proposed for the intermolecular process. According to this mechanism, the degradation of 3-PHAs is induced by carboxylate end groups (Scheme 1).

The further extension of these studies was recently reported for bacterial poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P-(3HB4HB)) (Kwiecień et al., 2014). Thermal degradation of this natural copolymer has been investigated under mild reaction conditions in the presence of carboxylate salt. The chemical structure of the obtained oligomers was determined with the aid of ^{1}H NMR and ESI-mass spectrometry techniques. The exclusive formation of linear oligomers, which terminated on one end with unsaturated groups and on the other end with carboxyl groups, was noted in the case of base catalyzed degradation, contrary to oligomers produced *via* pyrolysis, which also contained macrocyclic structures and γ-butyrolactone. It is interesting to note that linear and cyclic oligomers were distinguished by ESI-MS analysis of the derivatized products formed after reaction with trimethysilyldiazomethane (Kwiecień et al., 2014).

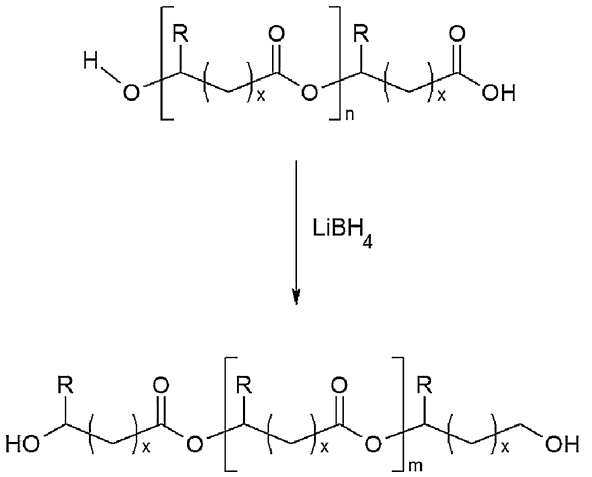
PHA oligomers that were terminated by unsaturated and carboxylate end groups (as revealed by ESI-MS^n analyses) were used as macroinitiators for the synthesis of block copolyesters containing segments derived from isotactic PHA biopolyesters and from atactic poly (*R*,*S*)-3-hydroxybutyrate (Adamus et al., 2012). Such block copolymers were found to be useful in cardiovascular engineering.

A highly selective method was also described for controlling the degradation of polyhydroxyalkanoates, PHA, *via* a reduction reaction that uses lithium borohydride (Scheme 2).

Using this method, oligo(hydroxyalkanoate) diols derived from P(3HB4HB) were obtained and characterized using NMR and ESI-mass spectrometry analyses. Unfortunately, in the case of the P(3HB4HB) copolyester oligomers, the molecular masses of the 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) units are the same, and these two units are therefore indistinguishable by mass spectrometry. However, they were determined based on the proton NMR spectra. Nevertheless, the NMR and ESI-MS analyses confirmed that the degradation



SCHEME 1. The E1cB mechanism of α-deprotonation of poly(3-hydroxyalkanolate), 3- PHA (Kawalec et al., 2007).



SCHEME 2. Reduction reaction of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) by lithium borohydride: R=CH_3 and x=1 for 3HB monomer units; RH and x=2 for 4HB monomer units (Kwiecień at al., 2013a).

process of high molar mass P(3HB4HB) biopolyester *via* the reduction reaction using lithium borohydride is highly selective and enables the formation of uniform oligomers with defined end groups (Kwiecień, Adamus, & Kowalczuk, 2013a).

Recent developments in PHA technologies have spawned an industrial value chain ranging from biofermentation to the exploration of new, high value added applications. New biofunctional (co)polymers can be obtained by the valorization of microbial PHA. The synthesis and MS structural characterization of conjugates in which bioactive compounds are covalently bonded to the oligomers obtained from natural PHA was recently demonstrated (Polish Patent Application P-404770, 2013; Kwiecień et al., 2015). 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. The first synthetic strategy was designed for bioactive compounds within the carboxyl group, and these conjugates were obtained through the transesterification of natural PHAs (Scheme 3).

The second synthetic strategy was selected for bioactive compounds within the hydroxyl group. Using this strategy conjugates were synthesized using a two-step method: cyclic PHA oligomers were obtained from aliphatic biopolyesters and were subsequently employed in enzymatic transesterification in the presence of lipase (Scheme 4).

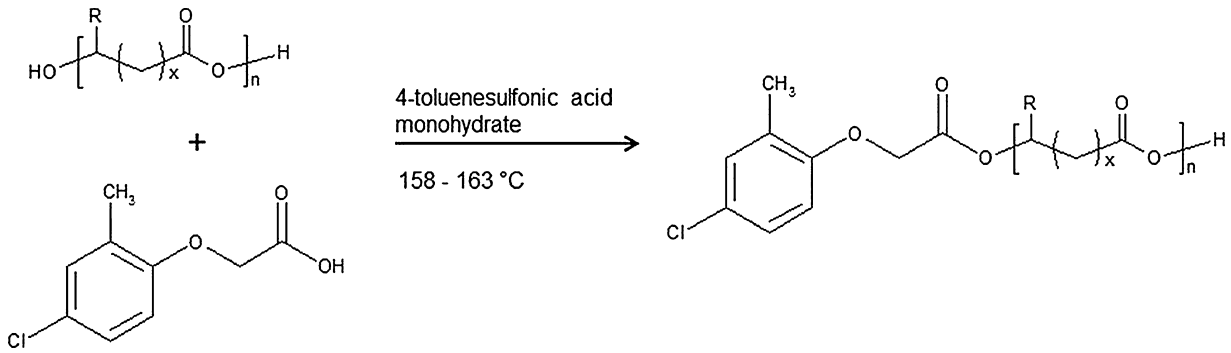
The above mentioned recent examples of ESI-MS^n application for the characterization of natural biodegradable polymers and their derivatives indicate that deeper insight into the structure of such homopolyesters and copolyesters can be achieved by means of mass spectrometric fragmentation techniques. The principal advantage of using this technique is that more information is available than can be obtained by direct analysis using conventional (single stage) mass spectral methods (Yol et al., 2014). It is of particular interest for controlled release systems based on PHA that enable the combination of biofunctionality and biomimetic structure that bear the promise of successful new applications (Shrivastav, Kim, & Kim, 2013).

III. MASS SPECTROMETRY HELPS IN THE SYNTHESIS OF BIODEGRADABLE (CO)POLYMERS

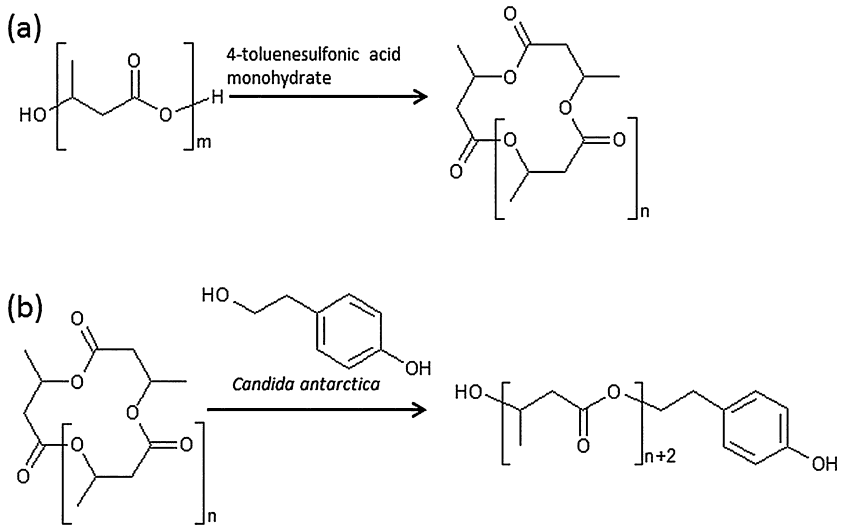
After the revolutionary development of the MALDI and ESI ionization, mass spectrometry may be currently considered a routine technique for the characterization of synthetic polymers. This trend was also observed in the publications on the ring-opening polymerization (ROP) of cyclic esters and ether as well as in research devoted to polycondensation and polyaddition processes, which are typical routes for biodegradable synthetic (co)polymer preparations.

A. MALDI-TOF MS in Synthesis of Biodegradable (Co) Polymers

Several research groups in Poland have successfully used MALDI-TOF MS for elucidation of the mechanisms of cyclic ester ROP, mostly by checking the chemical structure of the end groups of the biodegradable polymers obtained from them. The authors used MALDI-TOF mass spectrometry for structural studies of poly(ϵ-caprolactone) prepared via ring- opening polymerization of ϵ-caprolactone initiated with stannous octoate $Sn(Oct)_2$ in the presence of butyl alcohol (BuOH) or water as the co-initiator. It was assumed that for successful MS detection of the Sn(II)-containing species connected with the polymer chains, at least two prerequisites should be fulfilled. A high starting concentration of $Sn(Oct)_2$ is needed to have macromolecules with M_n sufficiently low to detect Sn in isotopic profiles of individual macromolecules. On the other hand, if there is too much water in the system, the Sn(II)-O bonds will hydrolyze before the MALDI experiment is conducted. The identification of the tin-containing macromolecules was not only based on the agreement between the observed m/z and the



SCHEME 3. Transesterification of natural PHAs (Polish Patent Application P-404770).



SCHEME 4. Two-step synthetic method of PHA conjugates.

calculated molar mass values but also on the particular isotopic distribution provided by the tin atom (Kowalski, Duda, & Penczek, 2000a). In further studies the authors demonstrated that $Sn(Oct)_2$ itself also does not play an active role in the ring opening polymerization of L,L-dilactide and that the L,L-dilactide/$Sn(Oct)_2$ system is mechanistically similar to the ϵ-caprolactone/$Sn(Oct)_2$ system (Kowalski, Duda, & Penczek, 2000b). It was also demonstrated, based on the analysis of MALDI-TOF mass spectra, that the mechanism of the ϵ-caprolactone or lactide polymerization by a $Sn(Oct)_2$/primary amine (RNH_2) system does not differ significantly from that of one co-initiated with alcohol (ROH) (Kowalski et al., 2005).

The solvent-free modification of matrix-assisted laser desorption/ionization time-of-flight (SF MALDI-TOF) mass spectrometry in the analysis of biodegradable synthetic polymers was also investigated. In conventional solvent- based dried droplet (DD) sample preparation, the sample and matrix are not uniformly distributed over a spot. This limitation can be overcome to some extent by the use of a solvent-free sample preparation method (Sroka-Bartnicka et al., 2010).

The cationic copolymerization of racemic-β-butyrolactone with L,L-lactide, initiated by alcohol and catalyzed by trifluoromethanesulfonic acid, was recently studied. For the β-butyrolactone prepolymers with degree of polymerization close to theoretical values, the presence of only one population of macromolecules as well as the structure of the end groups was confirmed by MALDI-TOF spectra analysis (Basko et al., 2013).

The influence of a catalyst and the reaction conditions on the synthesis of oligocarbonate diols by the transesterification of propylene or ethylene carbonates with aliphatic diols was investigated by Rokicki and Kowalczyk, (2000). Based on the information obtained from the MALDI-TOF-MS analysis of oligomeric products, it was found that during the transesterification of cyclic propylene or ethylene carbonates with aliphatic diols (in the presence of a coordination catalyst, e.g., tin carboxylate), only relatively small amounts of oxyethylene fragments were inserted into the oligocarbonate diols. Such information is valuable for the discussion of this reaction mechanism. These authors also investigated the mechanistic aspects of the coordination polymerization of six-membered cyclic carbonates (5,5-dimethyl-1,3-dioxan-2-one, DTC) initiated by a tin(II) alkoxide-based catalyst. The MALDI-TOF mass spectrometric analysis of the polymerization products revealed that predominantly macrocyclic oligocarbonates are formed in the presence of a stannane catalyst. The lack of mass spectrometric signals corresponding to cyclic oligocarbonates containing a residue diol originating from the catalyst used indicated that only one kind of Sn-O bond in the dimeric catalyst was active in the coordination-insertion of the cyclic carbonate monomer (Rokicki & Piotrowska, 2002).

The structure of multi- and tri-block copolymers synthesized *via* the ROP copolymerization of 1,5-dioxepan-2-one (DXO) with ϵ-caprolactone (CL) initiated by cyclic tin-alkoxide has been investigated using MALDI (Adamus et al., 2009a). MALDI-TOF mass spectra of different DXO/CL copolymers showed that regardless of the copolymer type or composition, the main series of peaks in the mass spectra corresponded to sodium-cationized linear chains terminated by hydroxyl and carboxylic end groups. The high abundance of macromolecules terminated by hydroxyl and carboxylic acid end groups as well as cyclic oligomers indicated that side reactions could take place during the synthesis as well as during the storage of the poly(ether-ester) samples studied.

B. ESI-MS^n in the Synthesis of Biodegradable (Co) Polyesters

Mass information alone may be insufficient to elucidate macromolecular structures, especially in the case of new (co) polymerization methods and/or polymerization processes proceeding through complex and unknown mechanisms. In these cases, multistage mass spectrometry (MS^n) can be applied in order to determine how a polymer's constituents are connected to each other and to characterize individual end groups of

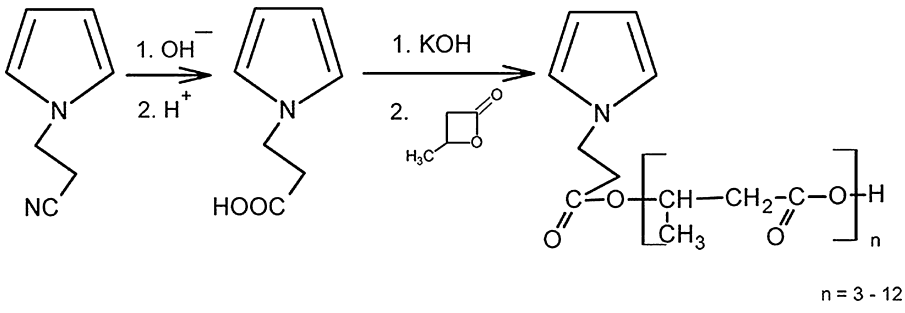
polymers as well as to identify macromolecular sequences and architectures, especially of copolymers. The collision energy necessary to drive the fragmentation of polymers decreased in the order of polyethers > polymethacrylates > polyesters > polysaccharides. These results indicate that polyesters fragment relatively easily (Nasioudis et al., 2010). However, the mechanisms of fragmentation are strongly dependent on the subtle chemical structure of the polyesters studied. Therefore, a combined theoretical and experimental study on the mechanisms of fragmentation active for synthetic poly(3-hydroxybutyrate) oligomers by multistage mass spectrometry has been performed (Bednarski et al., 2011).

The direct evidence of the ROP mechanism of β-lactones initiated with strong nucleophiles was provided by ESI-MS^n over 15 years ago (Jedliński et al., 1998). The evaluation of the structure of synthetic poly(3-hydroxybutyrate) at the molecular level supported the proposed addition-elimination mechanism of the ROP of β-butyrolactone initiated by strong nucleophiles. Only macromolecules with either hydroxy or crotonate end groups were identified and poly(3- hydroxybutyrate) prepared *via* the anionic polymerization of β-butyrolactone using potassium alkoxide/18-crown-6 as an initiator did not contain end groups derived from the initiator. In further studies, on the basis of the end group analysis as well as the distribution of polyester macromolecules with hydroxyl and unsaturated end groups, the authors postulated that the ROP of α,β-dialkylsubstituted-β-lactones follows the addition-elimination mechanisms previously proposed for simple β-lactones, and potassium hydroxide acts as a real initiator in both systems (Arkin et al., 2001).

In contrast to unsubstantiated four membered β-propiolactone, β-butyrolactone is not polymerized by common anionic initiators. However, these initiators, when activated by the addition of macrocyclic ligands such as crown ethers or cryptandes, are able to initiate the polymerization of β-butyrolactone to a PHB analogue. The same effect may be achieved by using bulky counterions or suitable highly polar aprotic solvents, e.g., DMSO. The polymer chain growth proceeds regioselectively and stereoselectively entirely *via* carboxylate anions. 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 (Kowalczuk, 2009). Synthetically prepared oligomers of PHB were found to be nontoxic and they may be used as carriers covalently bounded to suitable bioactive compounds. 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 (Adamus & Kowalczuk, 2000; Juzwa et al., 2008; Zawidlak-Wegrzynska et al., 2010). For perspective applications in cosmetology, we reported the ESI-MS/MS characterization at the molecular level of the oligo(3-hydroxybutyrate) conjugates with α-lipoic acid and *p*-coumaric acid (Maksymiak et al., 2013; Maksymiak, Kowalczuk, & Adamus, 2014). For potential agricultural applications, molecular-level structural characterization of novel phenoxycarboxylic acid–oligo(3-hydroxybutyrate) conjugates was performed with the aid of ESI-MS^n (Kwiecień, Adamus, & Kowalczuk, 2012). The synthesis and structural studies of oligo(3-hydroxybutyrate) conjugates with sorbic acid and benzoic acid, designed for food active packaging systems, have been recently reported and the structures of the resulting conjugates have been established at the molecular level by electrospray ionization multistage mass spectrometry (Kwiecień et al., 2013b). The same synthetic strategy has also been applied for the synthesis and characterization of a novel polypyrrole material grafted with biodegradable oligo-3- hydroxybutyrate pendants (Scheme 5).

The ESI-MS fragmentation experiments performed for selected macromonomer precursor ions confirmed that the initiator moiety, i.e., 1-(2-carboxyethyl)pyrrole, was covalently bonded with the oligo-3-hydroxybutyrate chain. The obtained oligo-3-hydroxybutyrate functionalized pyrroles were found to be promising candidates for the preparation of biodegradable conductive polymers (Domagala et al., 2014).

Developed at our laboratories, the anionic ROP of β-substituted β-lactones seems to be a perfect tool for the preparation of PHA analogues with the desired molecular structure, including the structure of the end groups (Adamus & Kowalczuk, 2008). The specific synthetic method based on the carbonylation of the respective epoxides under CO at ambient pressure opens up new opportunities for exploring the utility of β-lactones (and in particular precursors of synthetic analogues of natural poly(3- hydroxyalkanoate)s, 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 of β- butyrolactone with comonomeric β-substituted β-lactones containing aliphatic or aromatic moieties. It was originally reported that random and diblock copolyesters, derived from the same comonomeric units (with equal ratio), may be differentiated by comparing the fragmentation patterns obtained by both types of copolyester molecular ions. The model copolyesters with designed architecture were obtained *via* the anionic ROP of β-butyrolactone with



SCHEME 5. Synthetic pathway to oligo(3-hydroxybutyrate) *N*-functionalised pyrrole (Domagala et al., 2014).

β-ethoxymethyl-β-propiolactone, initiated by tetrabutylammonium acetate. The ESI-MS^n analysis demonstrated that the resulting copolyesters contained predominantly linear macromolecular chains terminated by acetate end groups derived from the used initiator. The significant differences in molecular level structures of diblock and random copolyesters studied were discerned by the application of the ion trap ESI-MS/MS method. The ESI-MS/MS experiments performed revealed that the sequence distribution presented in the fragmented individual copolyester macromolecular ions influenced their fragmentation patterns (Fig. 1).

Thus, arrangements of comonomer structural units along the diblock and random copolyester chains studied were determined based on the investigation of their fragmentation product patterns (Adamus, 2009b).

In our systematic studies on the molecular level structures of synthetic analogues of PHA, the ESI-MS fragmentation technique is preferably used because under this experiment each comonomer shows distinct fragmentation pathways along the chain. However, in some cases tandem MS (MS/MS) may be difficult due to the peak overlapping or low intensity of the parent ions. Recently the ESI tandem mass spectrometry and the sequence distribution analysis (performed based on the relative peak intensities of the detected molecular anions in the mass spectra) were applied for novel copolyesters obtained by anionic ring opening copolymerization of β-substituted β-lactones. Detailed analysis of these copolyesters, including molecular chain architecture as well as chemical structure of the end groups, was established based on the ESI-MS/MS collision induced dissociation (CID) spectra. The random arrangement of co-monomer structural units along the copolyester chains was demonstrated by a comparison of ESI-MS/MS fragmentation spectra and the respective fragmentation pathways. Moreover, sequence distribution analysis of the comonomeric units confirmed the random copolymer structure (Adamus et al., 2014). Further studies in this area are oriented on the biofunctional PHA analogues containing the bioactive compounds incorporated into the polyester backbone, and will be published soon.

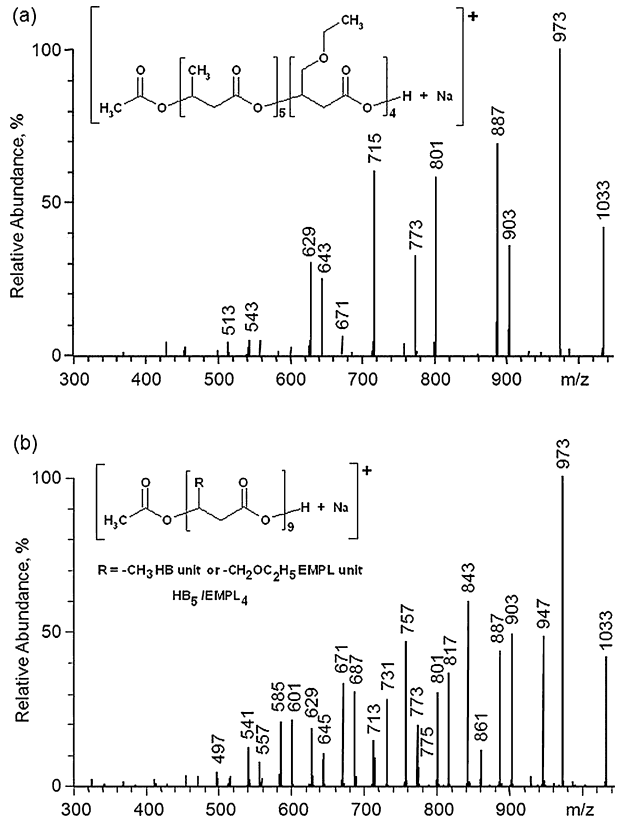


FIGURE 1. ESI-MS/MS spectra of the sodiated precursor ions [HB5/EMPL4 + Na]$^+$, m/z 1033, selected from the simple ESI mass spectra of: (a) diblock copolyester; (b) random copolyester samples. For theoretical fragmentation pathways of the selected ions see Adamus, 2009b.

IV. MASS SPECTROMETRY FOR FORENSIC ENGINEERING OF BIODEGRADABLE POLYMER MATERIALS

Forensic engineering of biodegradable polymer materials (FIBPM) deals with the evaluation of the relationships between their structure, properties and behavior before, during and after practical applications. Classical forensic engineering is considered to be the investigation of materials, products, structures or components that fail or do not function as intended. However, there is a need to detect problems before they arise. Thus, *ex-ante* investigations as well as *ex-post* studies are needed in the area of FIBPM in order to define and minimize the potential failure of novel biodegradable polymer products before and after specific applications. In the FIBPM studies, testing in simulated environments is needed before they are rolled out. Due to the wide spectrum of their potential applications, e.g., in medicine, in the field of compostable polymer packages (especially of long-shelf life products such as cosmetics or household chemicals) as well as in agrichemical formulations, the FIBPM can provide basic knowledge and a valuable service by increasing understanding and helping prevent future problems. Such an approach helps to design novel biodegradable polymeric materials and to avoid failures of the commercial products manufactured from them. It also opens up wide opportunities for biodegradable polymer mass spectrometry.

Studies on the biodegradation of polyesters by MS are a relatively new field and several MS techniques have been tested to develop methodologies in order to evaluate the biodegradation of polymeric materials. It is known that aliphatic polyesters may be degraded under natural conditions through the cleavage of ester bonds. The process may take place by chemical or enzymatic hydrolysis or in both ways. At the final stage the bioassimilation of the degradation products should take place. Water-soluble oligomers of atactic PHB (from dimer to dodecamer), synthesized by anionic oligomerization of (*R*,*S*)-β-butyrolactone and characterized by ESI-MS^n, were found to be utilized by not only two PHB-degrading bacteria (*Alcaligenes faecalis* T1 and *Comamonas* sp.) but also by a non-PHB-degrading bacterium (*Ralstonia eutropha* H16). The mineralization of these oligomers demonstrated the total biodegradability of synthetic high molecular weight atactic poly [(*R*,*S*)-3-hydroxybutyrate] (Focarete et al., 1999). In further studies, a new type of thermoalkalophilic hydrolase of *Paucimonas lemoignei* with a high specificity for amorphous polyesters of short chain- length hydroxyalkanoic acids was isolated and found (also using the ESI-MS technique) to degrade atactic poly ((*R*,*S*)-3-hydroxybutyrate), synthetized by anionic ROP (Handrick et al., 2001). The ESI-MS was used in ecotoxicological

studies of polyester blends containing atactic poly(3-hydroxybutyrate) as well as poly[(1,4- butylene terephthalate)-co-(1,4-butylene adipate)] (Ecoflex, BTA) (Rychter et al., 2006; Rychter et al., 2010). Regarding this latter polymer, LC–ESI-MS^n analysis was also performed in order to get detailed information on its structure through an analysis of the products of partial degradation under alkaline conditions (Song et al., 2011).

As mentioned above, the biodegradable and biocompatible conjugates of oligo- 3-hydroxybutyrate with lipoic acid were obtained *via* the anionic ring-opening oligomerization of (*R*,*S*)-β-butyrolactone initiated by lipoic acid potassium salt. The structure of their water-soluble hydrolytic degradation products was established at the molecular level by electrospray ionization mass spectrometry (ESI-MS^n) supported by ^{1}H NMR analyses (Maksymiak et al., 2013). The ESI-MS/MS was applied in hydrolytic degradation studies of biodegradable homopolyesters and copolyesters of 1,5-dioxepan-2-one (DXO), CL and of cross-linked polyester-ether networks. In particular, ESI-MS revealed the influence of hydrophilicity on the water-soluble degradation products of homo- and copolyesters of DXO and CL (Hakkarainen et al., 2008; Höglund et al., 2008). The use of environmentally friendly polymers as packaging materials for long shelf-life applications such as cosmetic packages is the new trend for production. However, ESI-MS revealed that PLA degradation occurred not only in the presence of polar solvents (ethyl alcohol, glycerine, propylene glycol) but also in the presence of paraffin, thus limiting its application in the packaging of cosmetics (Rydz et al., 2013).

V. MS RESEARCH IN POLAND

The scientific interest of Polish authors in mass spectrometry may be reflected by the number of paper published in this area. During the last 40 years, over 6,000 publications have been indexed by the Web of Science database, where the criteria of mass spectrometry and authors from Poland have been combined. Tremendous progress has been observed in the current century (Fig. 2).

MS research is conducted predominantly at the institutes of the Polish Academy of Sciences, universities and industrial research institutes. The subjects of MS studies are related with global trends, and it would be rather difficult to report all of the respective accomplishments in this review. Therefore, only several recent illustrations are given below in the area of bio and organic chemistry, mostly from research groups cooperating with the authors of this review.

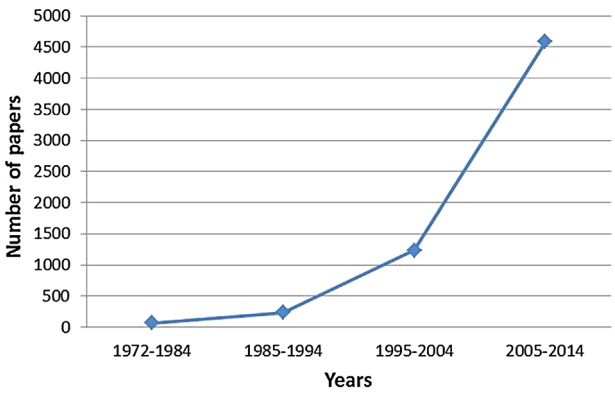


FIGURE 2. Number of mass spectrometry papers of Polish authors published in the years 1972–2014 (based on the Web of Science).

The discovery of an endogenous inhibitor of a dynorphin-converting enzyme from human cerebrospinal fluid (CSF, bikunin) was reported by Silberring et al. This protein is present together with its target enzyme in the same body fluids. The finding indicated that bikunin could play a significant role as a regulatory mechanism of neuropeptides, where one bioactive peptide is converted to a shorter sequence, which in turn can affect the action of its longer form (Suder et al., 2006). Recently, the development of a method for the detection of the use of isobaric tags for relative and absolute quantification labeling (iTRAQ) for quantitative, proteomic analysis using typical, widely available ion trap devices and manufacturer's software has been published as well as development of an ambient plasma ion source for quick identification of psychoactive drugs (Drabik et al., 2013; Smoluch et al., 2012). Using primary neuronal cell culture assays, combined with 2-D gel electrophoresis and capillary LC-MS, the differences in proteomes between control and morphine-treated cells were identified. Statistically significant differences were observed among 26 proteins. It is expected that further investigations may lead to the discovery of new proteome-based effects of morphine on living organisms (Bodzon-Kulakowska et al., 2009).

The derivatization of peptides as quaternary ammonium salts (QAS) is a promising method for the enhancement of ionization in ESI-mass spectrometry. It has been demonstrated that incorporation of the quaternary nitrogen atom into a peptide molecule allows for detection at subfemtomole to attomole levels in the multiple reaction-monitoring mode. This approach has found application in the mass spectrometric deconvolution of combinatorial libraries of the "one compound-one bead" type. Identification of derivatized peptides requires efficient fragmentation. Therefore, how the structure of QAS influences collision-induced dissociation of the labeled peptide has been investigated. For the majority of quaternary ammonium salts, the fragmentation pattern is more complex than for regular peptides because of the Hofmann elimination. In addition, peptides derivatized with *N*,*N*,*N*-trialkylglycine residue are susceptible to base-catalyzed hydrogen- deuterium exchange at the α-carbon atom. This reaction dramatically slows down at a lower pH, therefore incorporated deuterons are stable during LC-MS analysis and may be utilized in the quantification of peptides by the isotopic dilution method (Bąchor et al., 2012; Bąchor et al., 2013; Bąchor et al., 2014; Cydzik et al., 2011a; Cydzik et al., 2011b; Rudowska et al., 2012; Rudowska et al., 2013).

The products of protein glycation are formed in a reaction between reducing sugars and amino groups. The proteolytic fragments of glycated proteins could be used as diabetes markers. Based on developed methods for regioselective synthesis of analytically pure peptide-derived Amadori products, new methods for selective detection of glycated peptides have been proposed. The sequencing of Amadori products by CID is difficult, because these compounds are susceptible to the elimination of water and formaldehyde molecules as well as the whole hexose moiety. Therefore, the fragmentation spectrum is complex and because of poor sequence coverage rather difficult to interpret. It has been demonstrated that electron capture dissociation (ECD) provides a comprehensive spectrum suitable for the sequencing of peptides. However, further studies

performed on glycated proteins suggested that this method does not provide quantitative information on the relative glycation of particular lysine residues in proteins (Stefanowicz et al., 2007; Stefanowicz et al., 2009a; Stefanowicz, Kijewska, & Szewczuk, 2009b; Stefanowicz et al., 2010a; Stefanowicz et al., 2010b; Kijewska et al., 2011; Stefanowicz, Kijewska, & Szewczuk, 2014).

In the Chair of Analytical Chemistry at the Faculty of Chemistry, Warsaw University of Technology, mass spectrometry has been used as an analytical tool since 2001. The first research concerned the examination of structures of metal-organic ligand complexes through the use of atmospheric pressure chemical ionization- and electrospray ionization-MS. Further studies, on the identification of color substances–constituents of natural dyes used by ancient artists–performed with ESI-MS with a simple quadrupole analyzer as well as triple quad and ToF, began in 2003 and are still continued today. Inductively coupled plasma–mass spectrometry (ICP-MS) usually coupled with HPLC or capillary electrophoresis, is widely used for the investigation of: interactions of metalocomplexes (Pt, Ga, and Ru), potential anticancer drugs, with components of the blood stream as well as cancer cells (since 2004), mechanisms of phytoremediation and phytoextraction (since 2005) and food analysis (since 2005) (Oszwałdowski, Witowska, & Jarosz, 2001; Witowska-Jarosz et al., 2002; Witowska- Jarosz et al., 2003a; Ackacha, Połeć-Pawlak, & Jarosz, 2003; Timerbaev et al., 2004; Ruzik et al., 2005; Połeć-Pawlak et al., 2005; Połeć-Pawlak et al., 2007; Lipiec et al., 2011; Aleksenko et al., 2013; Lech et al., 2013; Matczuk et al., 2014; Miszczak et al., 2013; Rybak & Ruzik, 2013; Lech, Witkoś, & Jarosz, 2014).

Scientists working in the area of mass spectrometry in Poland have established the Polish Mass Spectrometry Society (http://ptsm.ibch.poznan.pl/). This is a non-profit organization established "by the people and for the people interested in all aspects of theory and practice in mass spectrometry and related topics". The main goals of the Society are: (i) dissemination of knowledge about mass spectrometry in the Polish scientific community, (ii) improving the information exchange between mass spectrometry laboratories in Poland, (iii) cooperation with the international mass spectrometry community. These goals are accomplished through the organization of seminars, conferences, information exchange between the members and promoting domestic and international scientific cooperation. The Polish Mass Spectrometry Society is a member of the European Society for Mass Spectrometry and International Mass Spectrometry Foundation.

VI. ABBREVIATIONS

BTA	poly[(1,4- butylene terephthalate)-co-(1,4-butylene adipate)]
CCD	comonomer composition distribution
CL	ϵ-caprolactone
DD	dried droplet method
DMSO	dimethyl sulfoxide
DTC	5,5-dimethyl- 1,3-dioxan-2-one
DXO	1,5-dioxepan- 2-one
E1cB	Elimination Unimolecular conjugate Base mechanism
FIBPM	Forensic engineering of biodegradable polymer materials
iTRAQ	isobaric tags for relative and absolute quantification labeling
PHA	natural aliphatic (co)polyesters, polyhydroxyalkanoates
3- PHA	poly(3-hydroxyalkanoate) s
PHB	poly(3-hydroxybutyrate)
PHBV	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
P (3HB4HB)	poly(3-hydroxybutyrate-co-4-hydroxybutyrate)
PLA	polylactic acid
QAS	quaternary ammonium salts
ROP	ring-opening polymerization
SF MALDI -TOF	solvent-free matrix- assisted laser desorption/ ionization time-of- flight

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