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New vistas in mass spectrometry for sequence analysis of natural and synthetic biodegradable macromolecules

KEYWORDS: Biodegradable polymers, PHA, mass spectrometry, sequencing.

Abstract 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. Examples are given of the uses of mass spectrometry (MS) for structural studies of biodegradable (co)polymers along with the use of multi-stage electrospray mass spectrometry (ESI-MSⁿ). Specifically they concern applications of MS for the characterization of natural biodegradable polymers and their derivatives, ESI-MSⁿ in the synthesis of biodegradable copolyesters, MS for forensic engineering of advanced biodegradable polymeric materials as well as ESI-MSⁿ for identification of selected biodegradable polymers on the way of molecular labelling.

INTRODUCTION

Biodegradable polymers play an important role in human life, increasingly becoming having an irreplaceable role. This concerns in particular medical products, including for example bioresorbable sutures or implants. It also concerns environmental issues in the area of compostable polymeric packaging materials of food, which could reduce the amount of currently generated cumbersome packaging waste from conventional plastics. When the development of biodegradable (co)polymers was in its infancy research focussed upon 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, processing conditions and potential applications in medicine, protection of environment and agro chemistry. However, such aspects like bio-safety of biodegradable polymers or nano-safety of their composites were, and still are, frequently neglected. In recent years there has been a rapid increase in the number of publications on biodegradable polymers with some reports indicating limitations and complications in their applications (particularly in the area of biomaterials). Consequently there is a urgent need to examine and minimize any potential setbacks related to their future importance for civilization and social function (1). In the future it is extremely important to design such biodegradable polymers that would be safe for human

health and the environment, and to indicate responsibly and sustainably new areas where their unique properties could be adopted.

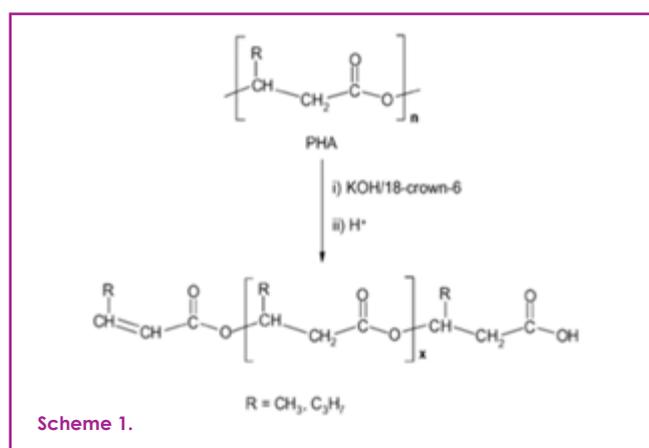
In the endeavour to safeguard biodegradable (co)polymers their identification at the molecular level should be explored. For this purpose mass spectrometry methods are of particular importance in (co)polymer analyses due to their high sensitivity, selectivity, specificity and speed. Polymer scientists have been unfamiliar with the advances made in the field of modern mass spectrometry for a long time. However, thanks to pioneering works, including amongst others the Professor Giorgio Montaudo Italian school of polymer mass spectrometry, today mass spectrometry complements in many ways the structural data provided by NMR, IR and other polymer characterization methodologies (2).

Development of soft ionization techniques in mass spectrometry have helped to solve the difficult question regarding the molecular structure of (co)polymer. Taking part in the "Electrospray Revolution", we have concentrated on multistage MS in polymer chemistry evaluating ring opening polymerization (ROP) mechanisms of selected oxacyclic monomers, on molecular level characterization of aliphatic biopolyesters by multistage MS, and recently on application of MS in forensic engineering of advanced polymer materials (3). In this mini review MS sequence analysis of natural and synthetic biodegradable macromolecules will be discussed with the special emphasis on the specific area of applied research on aliphatic biopolyesters and their synthetic analogues.

SEQUENCE ANALYSIS OF NATURAL BIODEGRADABLE COPOLYESTERS WITH ESI-MSⁿ

Among biodegradable polymers polyhydroxyalkanoates (PHA) offer a range of processing and performance improvements since they exhibit thermal and mechanical properties that are similar to petroleum-based plastics. Being bio-based and biodegradable PHA are commercially-valuable materials with many potential industrial applications. PHA properties may be adjusted by varying molecular structure, average co-monomer composition and co-monomer composition distribution along with molecular mass distribution.

The electrospray ionisation-mass spectrometry (ESI-MS) method has been originally applied for the determination of the co-monomer unit composition and composition distribution in bacterial PHA copolymers based upon the analysis of their oligomers obtained by partial alkaline depolymerisation, as presented in Scheme 1 (4).



In order to reconstruct the sequence of the un-degraded copolymer from the examination of a partially degraded sample the ESI-MSⁿ technique was subsequently used for the analyses of such oligomers. The structural characteristics of mass-selected macromolecular ions have been determined, providing information about the structure of PHA copolymers at the molecular level. For example, in order to verify the structure of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) biopolyester (PHBH), oligomers obtained by partial alkaline depolymerisation of this natural copolymer that contained 13–14 mol% of hydroxyhexanoate (HH) units were investigated, and its microstructure was assessed up to the level of 28 repeat units (5). The subtle structural details of the PHBH were evaluated based upon sequencing of individual quasimacromolecular ions thus showing, that both HH and hydroxybutyrate (HB) units of the PHBH copolymer were randomly distributed (Figure 1).

Some of the possible combinations of the co-monomer distribution for the sodium adduct ion of HB₃HH₂ oligomer are presented in Figure 1. The MS² experiment conducted for this ion indicated two sets of fragment ions with a 28 Da spacing. The fragment ion at m/z 423 corresponds to the HB₂HH₂ oligomer formed by the loss of crotonic acid (86 Da), and the ion at m/z 395 corresponds to the HB₃HH₁ oligomer formed by the expulsion of 2-hexenoic acid (114 Da) from the HB₃HH₂ sodium adduct ion. The fragment ion at m/z 281 (HB₃) is formed due to the expulsion

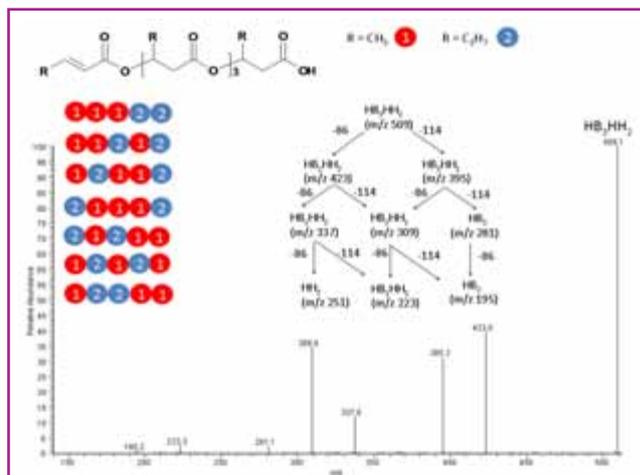


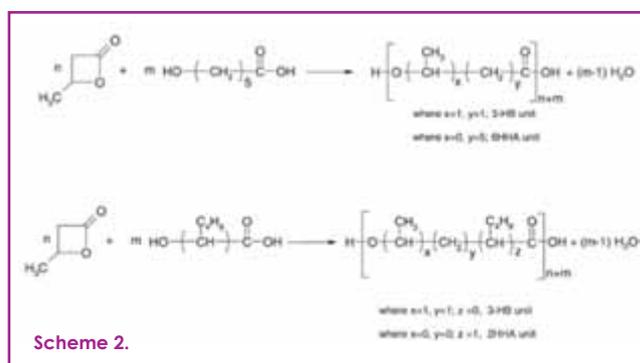
Figure 1. MS² fragment ion spectrum obtained for the molecular sodium adduct ion of HB₃HH₂ at m/z 509, selected from the 5-mer cluster of PHBH oligomers.

of 2-hexenoic acid from fragment ion m/z 395. However, the fragment ion at m/z 309 (HB₂HH₁) may be formed from fragment ion m/z 395 due to the loss of crotonic acid or from the fragment ion m/z 423 by the expulsion of 2-hexenoic acid. Thus, the MS² fragment ion spectrum indicates the random distribution of HB and HH units within copolymer chains.

Sequence distribution in microbial PHA revived by ESI-MSⁿ was also assessed by NMR measurements and both techniques gave results that were in good agreement for all investigated samples (6). Recently, this relatively rapid ESI-MSⁿ approach, developed at our laboratories, has been adopted for the routine analysis of PHA copolymers by other authors (7).

SEQUENCE ANALYSIS OF SYNTHETIC BIODEGRADABLE COPOLYESTERS WITH ESI-MSⁿ

The ESI-MSⁿ was successfully applied for the sequencing of copolyester oligomers with a composition ranging from 34–77 mol% of (R,S)-3-HB units, obtained via polytransesterification reactions conducted in bulk between racemic (R,S)-β-butyrolactone and 6-hydroxyhexanoic acid or (R,S)-2-hydroxyhexanoic acid, respectively as presented in Scheme 2 (8).



The arrangements of co-monomer structural units along the oligocopolyester chains were determined by sequencing with the aid of ESI MSⁿ and investigation of the respective fragmentation pathways. The complete structural and

molecular characterization of the low molecular weight copolyesters by ESI MSⁿ is particularly suitable when NMR, the conventional technique for copolymer sequence determination, requires spectral assignments for the reliable interpretation.

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, and the co-monomeric β -lactones may be obtained by carbonylation of the respective epoxides under CO at ambient pressure (9-11). In systematic studies on the molecular level structures of synthetic analogues of PHA, the ESI-MSⁿ fragmentation technique has been preferentially used because under such experiments each co-monomer shows distinct fragmentation pathways along the chain. It was originally reported that diblock copolyesters may be differentiated from random ones by the fragmentation patterns (12). As an example, the ESI-MS² spectrum of the sodiated precursor ion m/z 1033 selected from the simple ESI mass spectrum of diblock copolyester is presented on Figure 2.

Further studies in this area are oriented on the bio-functional PHA analogues containing bioactive compounds incorporated into the polyester backbone. In this research, the ESI-MSⁿ sequencing revealed that fragmentation of selected ions of potentially bioactive (co)oligoesters proceeded by random breakage of ester bonds along the oligomer chain as well as of the ester bonds of the bioactive pendant groups (13).

ESI-MSⁿ FOR FORENSIC ENGINEERING OF ADVANCED BIODEGRADABLE POLYMERIC MATERIALS

Forensic engineering of advanced biodegradable polymeric materials (FEAPM) deals with the evaluation of the relationships between their structure, properties and behaviour before, during and after practical applications (14, 15). The diverse applications of biodegradable polymers

requires case specific characterization and optimization of the material properties, its preparation, processing and recycling. Pulling these different elements together under the common thread of FEAPM provides a central driving force for the otherwise disconnected works and constitutes the novelty of this research. 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 mass spectrometry in FEAPM.

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-MSⁿ revealed that PLA degradation occurred not only in the presence of polar organic solvents (ethyl alcohol, glycerine, propylene glycol) but also in the presence of paraffin because of the residual moisture content. Furthermore, the degree of erosion of PLA-based rigid films was observed to be strongly dependent on their blend composition with synthetic (R,S)-PHB and the miscibility of these two components (14).

ESI-MSⁿ was successfully applied in the prediction study dedicated to evaluation of the effect of the solvent-free non-woven fabrics formation method on the release rate of lactic and glycolic acids from the tin-free poly(lactide-co-glycolide), PLGA, nonwovens (15). The results of the ESI-MSⁿ sequencing of the PLGA samples remaining after hydrolytic degradation indicated that the hydrolysis of ester bonds reduces the degree of ordering of the macromolecules of the PLGA copolymer (16).

FUTURE TRENDS - ESI-MSⁿ FOR DECODING OF BIODEGRADABLE POLYMERIC MATERIALS MOLECULAR LABELING

In general, the design of synthetic macromolecules containing information can be inspired by the biological codes. In such an approach the monomers could be used

as coding units to implement information in the polymer (17). This concept is very difficult to be achieved in the case of high molecular weight copolymers. Nevertheless, it seems to be real at the level of synthetic oligomers as it was demonstrated recently for monodisperse sequence-encoded poly(alkoxyamine amide) oligomers by Roy et al (18).

The complete decoding of a sequence-defined macromolecule may be achieved by MS/MS sequencing. However, the MS/MS dissociation patterns strongly depend on the chemistry of the polymer backbone (19-20). As mentioned above, the ESI-MSⁿ technique was successfully applied for sequencing of some PHA oligomers and thus it provides information about the structure of respective PHA copolymers at the molecular level. Therefore, with the understanding of the respective fragmentation patterns, the basis for decoding of labeled biodegradable polymer materials will have been created. Currently in our laboratories the libraries of monodisperse sequence-encoded PHA oligomers are under development. The

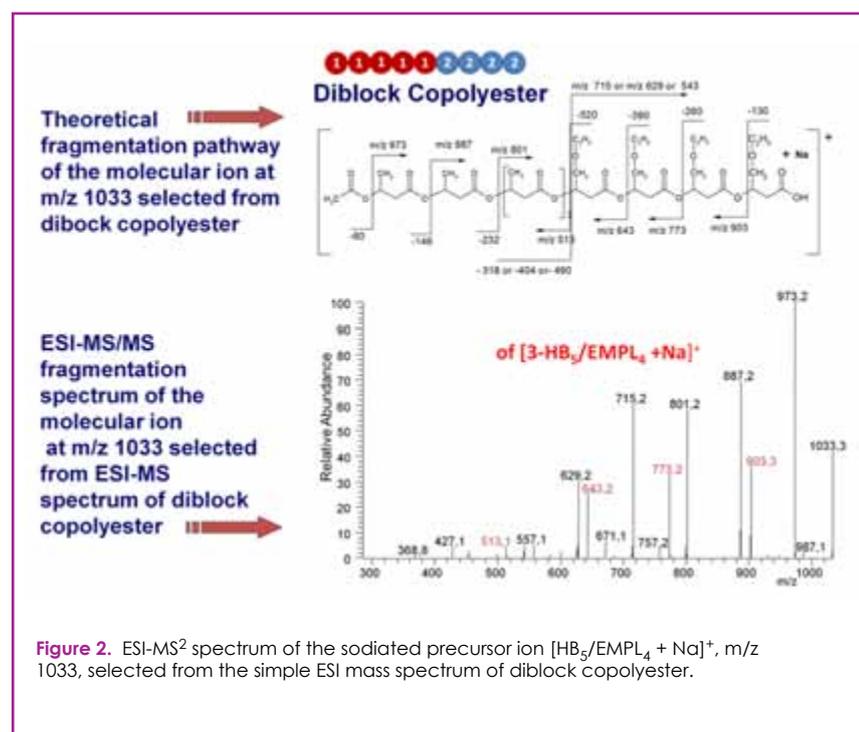


Figure 2. ESI-MS² spectrum of the sodiated precursor ion $[HB_2/EMPL_4 + Na]^+$, m/z 1033, selected from the simple ESI mass spectrum of diblock copolyester.

monodisperse sequence-encoded PHA oligomers after their MS/MS sequencing will be used for reading of binary-coded information. The bases of molecular labelling thus generated should enable identification of biodegradable polymeric materials at the molecular level. Introducing easily detectable oligomeric markers is an endeavour that constitutes an important opportunity for identification of e.g. biodegradable polymer manufacturer, product serial number or year of production.

CONCLUSIONS

Application of ESI-MS "soft" ionization method, which generate predominantly quasimolecular ions, enables sequence analysis of natural and synthetic biodegradable macromolecules because it permits the production of gas-phase ions from a wide variety of polymers, with little or no fragmentation during ionization. Moreover, multistage mass spectrometry permits the structural analysis of mass-selected macromolecular ions of (co)polymers at the molecular level. It may be therefore expected that ESI-MSⁿ becomes the routine and accurate analytical technique of biodegradable macromolecules for the years to come.

ACKNOWLEDGEMENT

This work was partially supported by the Polish National Science Centre (Decision No. DEC-2012/07/B/ST5/00627). The author is indebted to Professor David Hill from University of Wolverhampton for stimulating discussion.

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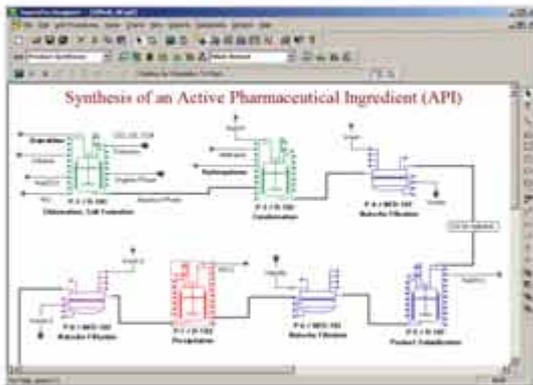
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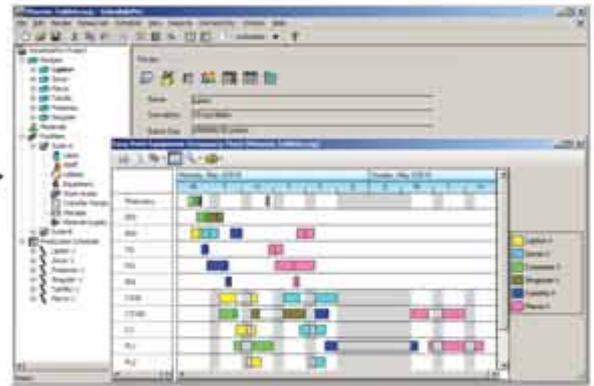
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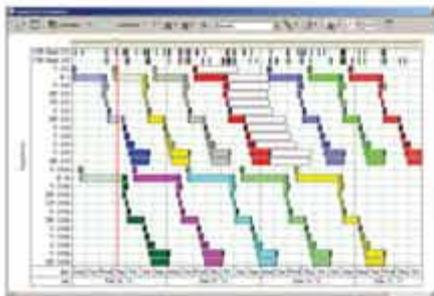


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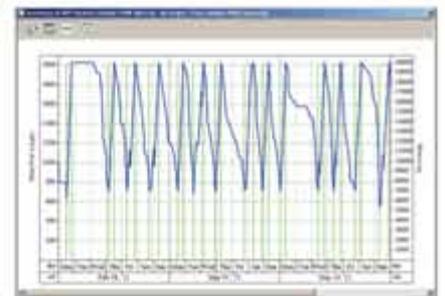
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