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Techniques useful in biodegradation tracking and biodegradable polymers characterization

Version 1

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Knowledge on biodegradable polymers' structures is essential for the selection of such materials for specific applications. In recent years, much attention has been given to organic recycling of biodegradable polymeric materials, enabling the controlled biological decomposition of these polymers to products, which are safe and friendly to the environment and human life and health. Products made from biodegradable polymers after their disposal are subjected to organic recycling processes. This significantly reduces the environmental charge with waste, and allows for their rational utilization by industrial composting.

The precise characterization of polymers plays a crucial role in the selection of the proper materials for specific applications and allows to predict the behaviour of the final product in the overall product life cycle.

In order to get their products certified as being biodegradable, producers have to perform biodegradation test of their products and/or materials in an accredited laboratory. These tests can be done at any stage of the product development process. The progress in (bio)degradation is determined by differential parameters such as macroscopic and microscopic changes in the polymer surface, changes in molar mass and weight loss and changes in microstructure. Appropriate measurements are performed using differential analytical techniques. The differential scanning calorimetry (DSC), the atomic force microscopy (AFM), the dynamic method of thermal analysis (DMA), gel permeation chromatography (GPC), and mass spectrometry (MS) as well as spectroscopy methods: nuclear magnetic resonance (NMR) and infrared absorption (IR), are used for observations of materials changes in the degradation process and complement each other. The NMR and IR spectroscopy allows to analyse polymeric materials' structures, and to track changes in the polymer chains microstructure due to the degradation process. Moreover the MS technique is used in the analysis of the oligomeric fraction formed during the degradation of the polymers.

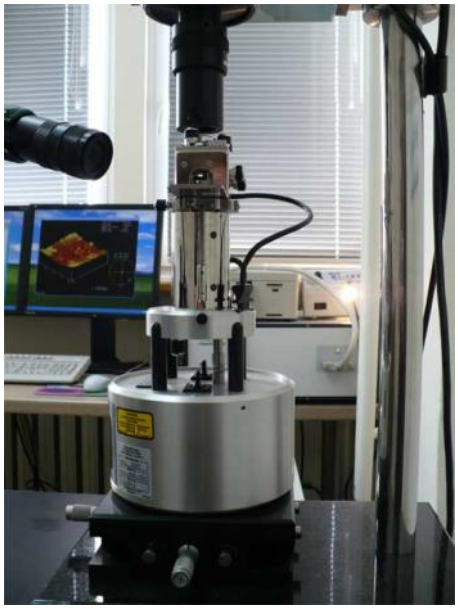
The below presented fact sheet gives an insight in the fundamentals of analytical techniques applied in biodegradable materials' analyses and has the aim to be a supportive document for companies planning to include biodegradable polymers in new product development.

Optical microscope

Microscopic observations of polymer surface changes are carried out by means of an optical microscope, equipped with a colour digital camera at different magnification.

Atomic force microscopy, AFM

The surface structure of biodegradable polymers is also examined by means of atomic force microscopy. AFM observations are based on interatomic forces and can be operated in standard fashion contact, allowing to obtain three-dimensional images. The maximum scanning area can be 130 microns x 130 microns or 2 microns x 2 microns, depending on the scanner used. Resolution in the plane x, y can be up to a tenth of a nanometer. The AFM method does not require special preparation of samples for testing, and the analysis does not damage the polymer surface.



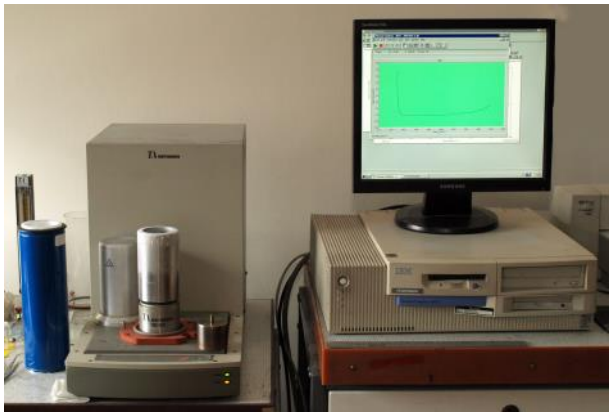
Dynamic mechanical analysis, DMA

The dynamic mechanical analysis is used to examine the visco-elastic properties of the materials, such as stiffness, modulus and loss modulus, viscosity, damping properties, and the glass transition temperature. DMA determines the modulus of elasticity and the damping values by applying an oscillating force to the sample. This method can also be used to study the crosslinking kinetics and the phenomenon of gelation and vitrification. A wide range ofmeasurement possibilities (under bending, tension, compression and shear condition) allows to receive a comprehensive characterization of the samples as a function of temperature, frequency, and stress or strain.

Gel permeation chromatography, GPC

Gel permeation chromatography is essential in polymer chemistry for measuring molar mass and their distribution. GPC is a kind of column liquid chromatography, and relies on separating the components of the mixture on silica gel or molecular sieves according to particle size. The GPC chromatograph is equipped with UV detectors and a differential refractometer, which allows to determine the polymers' dispersity and the average molar mass. Molar mass calculation is based on a calibration using narrow polystyrene or polymethylmethacrylate standards. The system can operate in a different solvent for example chloroform or tetrahydrofuran.

Differential Scanning Calorimetry, DSC



Differential scanning calorimetry monitors heat effects associated with physical transitions and chemical reactions as a function of temperature. In a DSC the difference in heat flow to the sample and a reference at the same temperature, is recorded as a function of temperature. Since the DSC experiment is at constant pressure, heat flow is equivalent to enthalpy changes. Measurements are carried out using a differential calorimeter with high and constant calorimetric sensitivity. Differential calorimeter measures temperature of the first and second order transition, enthalpy changes, and allows to the study the kinetics of physical processes and chemical reactions, heat capacity measurement, and the measurement of substance purity. Measurement temperature range for typical polymers is -180°C to 550°C.

The DSC analysis is used to study phase transitions or chemical reactions.

Nuclear Magnetic Resonance spectroscopy, NMR

Nuclear magnetic resonance spectroscopy is one of the most widely used spectroscopic techniques in chemistry and medicine. Spectroscopy is based on excitation of nuclear spins in a magnetic field by a quick change of the magnetic field, and then recording the electromagnetic radiation. NMR is therefore one of the emission spectroscopy methods assuring high accuracy and reproducibility. NMR spectra are obtained by using different types of nuclear magnetic resonance spectrometers, such as a Varian VXR 300 and Unity Inova 300 with high-resolution. They allow measurements over a wide frequency range (e.g.: ^{13}C, ^{14}N, ^{15}N, ^{17}O, ^{23}Na, ^{31}P, ^{39}K) with simultaneous processing and storage of data. Appropriate equipment spectrometer allows to measure relaxation times, carbon bond order determination of selective methods (e.g. DEPT) and two-dimensional measurement of 2D-NMR. NMR spectroscopy is used in research of optical properties of polymers, for potential use in optoelectronics, the analysis of the polymer chains structure and assigning resonance lines, present in the NMR spectrum, corresponding structural elements of the chain of test samples.

NMR spectroscopy is also used in studies of biodegradable polymers in terms of environmental protection also in studies of new polymers, which while preserving utility properties allows to obtain a film's ability to biodegradation. The nuclear magnetic resonance spectroscopy of high-resolution ^{1}H and ^{13}C NMR is used for analysis of the structure of biodegradable polymer chains. Objects of the study are lactide homopolymer chains or amorphous poly(3-hydroxybutyrate) chains, copolymers of lactide with glycolide, copolymers of lactide with caprolactone and copolymers glycolide with caprolactone. For the purpose of the analysis of the microstructure of biodegradable polymers' chains new methodologies are being developed.

Nuclear magnetic resonance spectroscopy (NMR) allows to get insight in the polymer chain structure as a result of which it will be possible to identify the proper structure for the application purposes.

Absorption spectroscopy

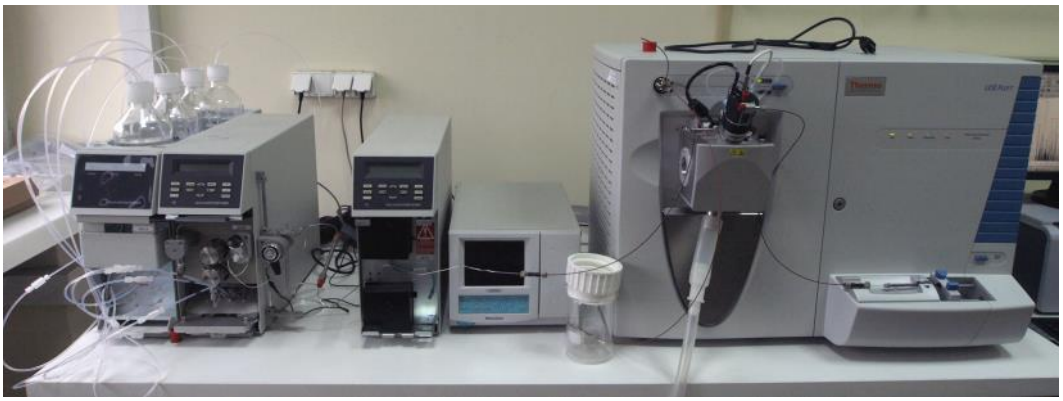
Light spectroscopy is a group of spectroscopic techniques, which use electromagnetic radiation in the range from deep ultraviolet to far infrared. The most widely used technique is the infrared absorption spectroscopy (IR). Light in the IR range has a length similar to the length of the chemical bond. Passing through the sample of the test substance, radiation is selective absorbed by vibrations excitation of the chemical bonds of a length corresponding to the length of the wavelength absorbed.

In this way the spectrum is a series of sharp signals corresponding to vibrations set constraints. Another frequently used technique is absorption spectroscopy in the ultraviolet-visible spectral region, UV-VIS. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. Radiation in this range is absorbed by vibration excitation of larger molecular fragments. Spectroscopy does not provide too much information about the structure of molecules, but it is helpful in analyzing their potential electro-optical properties. On the other hand, light reflectance spectroscopy is rarely applied. However, it is useful to determine the chemical composition and electro-optical properties of the surface of a variety of substances. IR spectra (4000-400 cm^{-1}) was obtained using spectrometers such as BIO-RAD FTS-40A type equipped with dynamic alignment system and a temperature table (20°C to 250°C). The high quality of the spectra obtained by Fourier transformation and processing of computer data allows to further study the polymers' structure. Studies conducted using the infrared absorption spectroscopy technique through database spectra allows unambiguous identification of bonds present in the polymer sample.

Mass spectrometry, MS

Among the analytical techniques currently used for the characterization of polymers very important is mass spectrometry. This technique provides useful information about the molecular structure of the macromolecules and is a perfect complement to conventional spectroscopic methods such as nuclear magnetic resonance (NMR) and infrared absorption spectroscopy (IR). The recently significant instrumental progress in the techniques of mass spectrometry, in particular, the discovery of new methods of "soft" ionization, such as

matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) enabled the simplification of a long and complicated process analytical study of the polymers structure.

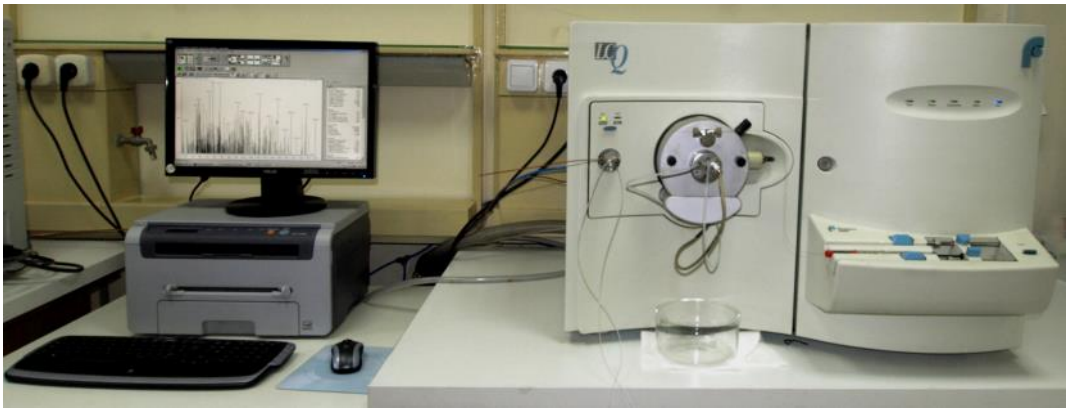


LCQ mass spectrometer (Finnigan MAT) fitted with snap enable ionization of samples by electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), is a universal connection LC/MS analysis allows to perform the combined techniques of liquid chromatography (LC) and mass spectrometry (MS). The use of the ion trap analyzer enables high sensitivity full-range mass spectra up to 2000 Da. It can register both positive and negative mass spectra of the test compounds and the execution of a multi-technique analysis of fragmentation (MS/MS and MS^n). These techniques are used to determine the individual masses of macromolecules constituting the tested polymer and the separation due to degree of polymerization. Information obtained by mass spectrometry techniques is essential to understand polyreaction mechanisms, in which may occur both linear macromolecules and cyclic oligomers (for example by an intramolecular transesterification). The use of modern "soft" ionization methods such as MALDI and ESI in combination with multi mass spectrometry makes a huge contribution to research development in the field of chemistry, pharmacy and biology. In recent years, more often "soft" ionization method is used in the structure study of the biodegradable synthetic polymer, comprising:

- verification of chemical homogeneity of the polymer and to determine the chemical structure of end groups,
- determination of the molar mass and dependent on the synthesis process the molar weight distribution,
- determination of the chemical composition and distribution of the sequence of units to individual macromolecules of copolymers.

Multistage mass spectrometry techniques (MS^n) are also used in studies of natural biopolymers, including the end-group analysis and to study the mechanism of the

fragmentation of selected molecular ions (obtained by electrospray ESI) individual macromolecular biopolymers from the group of poly (3-hydroxybutyrate), PHB.



Investigation of biodegradable polymers carried out using ESI-MS^n technique possible to determine the chemical structure of degradation products, and the course of their bioassymilation and, consequently, allow to establish the relationship between the structure of macromolecules and their behavior during biodegradation. Detailed analysis of the mass spectrum and its transformation into a "mapping" – showing the presence of oligomeric fractions with different structures and allowing quantitative assessment of the observed progressive degradation due to changes in the distribution of these oligomers – delivers enough information to propose appropriate degradation mechanisms.

Knowledge on the microstructure of biodegradable polymer chains is a precondition for designing appropriate chemical structures required for biodegradable materials and helps in controlling their mechanical properties, processability and time degradation.

Summary

All of the above presented analytical techniques are needed to monitor changes taking place in the material because of degradation. Of particular importance is the understanding of the relationship between the structure and properties of the polymers. Thanks to the application of these techniques companies will receive data on the complete characterization of the polymers, and thus will be able to control the properties of such materials, which makes it possible to directly control the speed and time of the degradation of biodegradable polymers.

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