Degradation and analysis of synthetic polymeric materials for biomedical applications

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Chapter 1

1. Methods for the chemical analysis of degradable synthetic polymeric biomaterials

The performance of biodegradable polymeric systems strongly depends on their physical, as well as on their chemical properties. Therefore, the detailed chemical analysis of such systems is essential. Enzymatic and chemical hydrolysis are the primary biodegradation mechanisms for these materials. This review provides an overview of the strategies and analytical methods used for the structural and compositional chemical analysis of non-degraded, partially degraded and fully degraded synthetic polymeric biomaterials with an emphasis on modern solution-based techniques that yield large amounts of information. The degradation methods that facilitate the study of polymeric networks are also described.
1 Introduction

A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine [1]. Synthetic polymeric biomaterials are of great importance in the medical field due to an aging population and because of their potential to improve the quality of life [2]. There is a clear trend to replace non-degradable by degradable materials [3]. Biodegradable polymeric implants are intended to degrade gradually and their degradation products are meant to be excreted benignly by the body, so that they do not need to be surgically removed after their functional role (e.g. as drug-delivery carrier) has expired [4]. This causes increasingly strict demands on the design and synthesis of biodegradable polymeric materials for applications in drug-delivery devices, gene transfer, regenerative medicine, scaffolds for tissue engineering, and surgical implants, such as rods, sutures, pins and screws for fixation devices [5,6]. Degradation of biomaterials has many biological, physical, and chemical facets. Biological assessment involves cell tests or implantation. Morphology is important to understand degradation behaviour. For example, crystallinity plays a crucial role in the degradation of poly(lactic acid) [7].

This review is limited to the in vitro chemical analysis of synthetic polymeric biomaterials. The suitability of synthetic polymeric biomaterials for medical devices can be inferred from their chemical structure, mechanical properties, degradation kinetics, and the biocompatibility (tissue response) of the polymers and their degradation products [8]. The molecular weight, hydrophilic or hydrophobic nature, fractional composition sequence and (stereo-) regularity of the monomers in multi-block co-polymers, length of kinetic chains in photo-polymerized networks, nature and concentration of additives, shape and morphology of the specimen, and incubating media can all influence the degradation rate and mechanism in terms of surface erosion or bulk degradation. The biodegradation mechanism for such materials primarily involves enzymatic and chemical hydrolysis. Highly reactive species, such as peroxides, are produced in reaction of the human body to the biomaterial (foreign-body response). Such species may also degrade the polymer chain and contribute to the overall degradation of biomaterial [9].

Various reviews have been published on the synthesis and application of synthetic biomaterials. However, despite their increasing use in the biomedical industry, very few articles have reviewed selective characterization techniques. No review has been published
that summarizes in detail the analysis methods that lead to the identification and structural analysis of degradable polymeric biomaterials. Therefore, we set out to review different analytical methods used for the analysis of degradable polymeric biomaterials as a starting material, after partial hydrolysis under physiological conditions, and after complete hydrolysis. Methods involving chromatographic separation followed by spectroscopic or mass-spectrometric detection are discussed here. Degradation methods required to bring the complex copolymer and insoluble networks within the realm of chromatographic techniques and direct mass-spectrometric analysis are also emphasized.

2 Degradable biomaterials

Synthetic degradable polymeric biomaterials contain one or more functional groups, such as an ester, ether, amide, imide, thioester, anhydride, etc., in their chemical structure. This enables such materials to degrade gradually, either through chemical stress or through biological processes. The polymeric chains in a degradable polymer can differ in terms of their length, chemical structure, architecture, etc. On the basis of their chemical composition, they can be divided in homopolymers and copolymers. The sequence of the different monomers in polymeric chains further differentiates copolymers into block copolymers, alternating copolymers, random copolymers, graft copolymer, etc. The architecture of polymer molecules can be linear, branched, hyperbranched, or dendrimers. Polymers may also form three-dimensional chemically or physically cross-linked network. The arrangement of different fragments in polymeric chains not only determines their configuration (stereo-heterogeneity, such as isotactic, syndiotactic and atactic), but also their ability to rotate around a single bond (so-called conformational heterogeneity). All these parameters may directly (e.g. through chemical stability) or indirectly (e.g. through the crystallinity) influence the rate of degradation of biomaterials. Hence, all of them need to be investigated. Poly(2-hydroxyethyl methacrylate) (pHEMA), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly (lactide-co-glycolide) (PLGA), polycaprolactone (PCL), and functionalized cross-linked polyacrylates are the most extensively studied polyesters for biomaterials [10]. Polyurethanes have been investigated in the biomedical industry and their properties have been tailored by incorporation of ester and ether components to generate poly(ester urethane)s or poly(ether urethane)s. Poly(ester amides)s (PEAs), prefereably with natural amino acids, are attractive for biomedical applications such as drug-eluting stent coating [11]. Ulery et al. recently published a comprehensive review describing in detail the
biomedical applications of synthetic and natural biomaterials [12]. A few examples of different functionalities of synthetic polymeric biomaterials are tabulated in Table 1.

**Table 1** Selected synthetic biodegradable polymers and copolymers.

<table>
<thead>
<tr>
<th>Polymer types</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>[R_O_O_n]</td>
<td>[13]</td>
</tr>
<tr>
<td>Polyether</td>
<td>[R_O_n]</td>
<td>[14]</td>
</tr>
<tr>
<td>Polyamide</td>
<td>[R_O_N_n]</td>
<td>[15]</td>
</tr>
<tr>
<td>Polyimide</td>
<td>[R_2_N_N_O_O_n]</td>
<td>[16]</td>
</tr>
<tr>
<td>Polyurea</td>
<td>[N_N_O_R_1_R_1_O_O_R_2_R_2_n]</td>
<td>[17]</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>[N_N_O_O_R_1_R_1_O_O_R_2_R_2_n]</td>
<td>[18]</td>
</tr>
<tr>
<td>Polyanhydride</td>
<td>[O_O_O_n]</td>
<td>[19]</td>
</tr>
<tr>
<td>Polythioester</td>
<td>[R_S_S_n]</td>
<td>[20]</td>
</tr>
<tr>
<td>Polypophosphoesters</td>
<td>[R_1_O_P_O_n]</td>
<td>[21]</td>
</tr>
<tr>
<td>Polysiloxane</td>
<td>[O_Si_O_R_1_R_2_n]</td>
<td>[22]</td>
</tr>
<tr>
<td>Poly (ester amide)</td>
<td>[O_O_R_1_N_H_R_2_n]</td>
<td>[11,23]</td>
</tr>
<tr>
<td>Poly (ester urethane)</td>
<td>[O_R_1_N_H_O_O_R_2_n]</td>
<td>[10]</td>
</tr>
<tr>
<td>Poly (ester urea)</td>
<td>[O_R_1_N_H_O_R_2_n]</td>
<td>[24]</td>
</tr>
</tbody>
</table>
3 Analytical strategies

Many procedures and techniques can be applied to study the properties and degradation of biomaterials. Water-uptake (swelling-ratio) measurements provide useful information on the hydrophilic or hydrophobic nature of the materials. The results can be related to the degree of crystallinity of the structure. Monitoring the changes in the pH of media as a function of degradation indicates the acidic or basic nature of the released degradation products and their ultimate effect on the surrounding environment (cells, tissues, etc.). Weight-loss studies are almost universally performed to estimate any change in the mass of biomaterials during degradation. Changes in the specimen dimensions and surface morphology, such as crack, or micro channels, and changes inside the material can be highlighted by microscopy techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) or atomic-force microscopy (AFM). The surface chemistry of the biomaterial may alter or influence proteins and cells and may affect biocompatibility. The common methods to characterize the surface chemistry include contact-angle measurements, Fourier-transform infrared – attenuated-total-reflectance (FTIR-ATR) spectroscopy, X-ray photo-electron spectroscopy (XPS) and secondary-ion mass spectrometry (SIMS) [27]. Differential scanning calorimetry (DSC) and wide-angle x-ray diffraction (WAXS) are commonly used techniques to estimate changes in the crystallinity of a biomaterial during the degradation [28]. However, to tailor the properties of biomaterials, including physically or chemical cross-linked networks for a specific biomedical application and to estimate the compatibility of their degradation products with the surrounding biological environment, an in-depth knowledge of their chemical structure is mandatory [29]. This includes characterization of the starting material and the degradation products. Chromatographic separations, mass
spectrometry (MS), and FTIR and NMR spectroscopy can provide more insight in the nature and chemical structure of the degradation products.

There are three fundamentally different approaches to the structural characterization of biomaterials. The first approach is the analysis of biomaterials without degradation. If the biomaterials are soluble their identity and average molecular weights can be determined by NMR spectroscopy and and by size-exclusion chromatography (SEC), respectively. The compositional analysis of oligomers and low-molecular-weight polymers can be achieved by mass-spectrometric techniques, such as liquid chromatography coupled through an electrospray-ionization interface to a tandem mass-spectrometer (LC-ESI-MS/MS or LC-ESI-MS\(^n\)) or to a time-of-flight mass spectrometer (LC-ESI-ToF-MS), or matrix-assisted laser-desorption/ionization (MALDI) ToF-MS. However, all MS techniques have their limitations for high-molecular-weight synthetic polymers. When using ESI multiply charged ions swamp the spectrum, amplifying the number of different ions arising from the molecular-weight distribution. In MALDI both statistics and charge affinity may cause low-molecular-weight oligomers to dominate the spectrum.

In the second approach the polymer can be degraded at harsh conditions, such as high temperature or extreme pH, to complete degradation. This approach is suitable for the characterization of networks that lack solubility and thus cannot be subjected directly to chromatographic analysis [10]. When a polymer is being hydrolysed the degree of degradation can be monitored by NMR spectroscopy. The degradation products can be separated and quantified by, for example, LC with MS or UV-vis detection. The kinetic chain length of poly-addition backbones (-C-C-) can be determined by SEC.

The third approach to study prospective biomaterials involves a chemical or a specific enzymatic degradation under physiological conditions. This allows one to study the kinetics of degradation. First degradation under physiologically relevant conditions is performed, resulting in partially degraded material, the constituents of which may be identified [11]. Then complete and fast degradation of the products of the first step (oligomers, intermediates and other products) is performed, followed by quantitative analysis [30].

The collected information is helpful (i) to ascertain the composition of the original networks, (ii) to evaluate and optimize the synthesis of functional materials, (iii) to evaluate the toxicological of the degradation products at an early stage, (iv) to determine the rate of hydrolysis at different sites prone to attack, and (iv) for the rational design of new materials.
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Figure 1 Schematics for the degradation and analysis of synthetic polymeric biomaterials. This review concerns the green part of this scheme.

4 Degradation methods

Degradable polymeric materials contain moieties that are prone to chemical or enzymatic degradation (cf. Table 1). The degradation of such materials can be divided as follows.

4.1 Degradation under non-physiological conditions

This type of degradation involves harsh conditions, such as extreme pH values (both acidic and alkaline) and/or elevated temperatures. Such degradation methods can be used (i) to measure the kinetic chain length in photo-crosslinked polymeric networks, which lack solubility [31], (ii) to reduce the size of polymeric chains in complex multi-block copolymers to allow chromatographic separations, followed by sequence analysis with mass spectrometry [32], (iii) to estimate the composition of complex polymeric networks by quantifying each completely hydrolyzed building block [30], or (iv) to investigate the
stability of different chemical bonds in a copolymer under extreme conditions. Haken et al. reviewed the importance of vigorous chemical degradation of condensation polymers prior to their chromatographic analysis [33]. Matsubara et al. designed a novel set-up to study degradation, using supercritical methanol at 300°C and 8.1 MPa in a stainless-steel autoclave (placed in a GC oven (Figure 2a). They could selectively decompose the ester linkages in UV-cured acrylic esters [31]. Later, the developed set-up was successfully applied for the characterization of the network structure of radiation-cured resins of poly-functional acrylic ester and N-vinylpyrrolidone [34].

Peters et al. investigated the hydrolytic degradation of poly(D,L-lactide-co-glycolide 50:50)-di-acrylate network coatings. They followed a two-step degradation process, first degrading the coatings in PBS at 37°C and then further hydrolyzing the released products at 90°C in 10-M sodium hydroxide [30].

To tailor the performance of degradable synthetic polymeric biomaterials, it is important to understand their structure. High-molecular-weight polymers or complex copolymers are difficult to analyze by routine SEC or HPLC methods. The analyses usually involve time-consuming sample-preparation steps especially in case of networks. To assess the structure of such synthetic polymers, thermal degradation or pyrolysis can be a useful tool. In pyrolysis, the polymer samples (introduced in the form of a solution or as a solid) break down into small fragments (e.g. monomers or oligomers) by supplying thermal energy in an inert atmosphere or vacuum [35]. The small fragments can be separated and analyzed by chromatographic techniques such as GC or GC-MS [36].

### 4.2 Degradation under physiological conditions

The degradation of biomaterials under physiological conditions is studied to estimate their degradation rate and to investigate phenomena involving surface degradation or bulk degradation. Various other aspects, such as pH changes, degree of swelling, weight loss, surface chemistry and morphology, and toxicity of the released products can also be studied. These kinds of degradations are performed at 37°C, with different incubation media, such as phosphate-buffered-saline (PBS) solution, enzyme-containing buffer, serum, or simulated body fluids (SBF) at a suitable pH [28]. In conventional batch-mode analysis, the biodegradable polymers (films, coatings, 3D scaffolds, etc.) are immersed in the respective media followed by incubation at 37°C [40]. In vitro degradation conditions cannot mimic real physiological conditions. However, the selection of appropriate enzymes, incubation
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media, ratio’s of surface-to-mass of the specimen and surface-to-volume of the medium, duration of the experiment, and dynamic or static conditions during the degradation with respect to the site where the biomaterial is implanted may help to find conditions closer to the physiological ones [28].

Figure 2 (a) Schematic diagram of fast-degradation apparatus used for supercritical methanolysis (reprinted with permission from ref. [31]), (b) Schematic diagram of the reaction vessel used in the dynamic encrustation of urinary-tract devices based on polyurethanes, percuflex and silicone (reprinted with permission from ref. [37]), (c) Schematic showing the apparatus for studying the degradation of biodegradable scaffolds under dynamic conditions. Using a peristaltic pump, the scaffolds were subjected to a continuous flow (250 µl/min) of phosphate-buffered-saline (PBS) solution (pH 7.4) at 37°C (reprinted with permission from ref. [38]), (d) Schematic of dynamic flow simulation system used to study the effect of fluid flow on the degradation of poly(lactide-co-glycolide acid) (PLGA) for in vitro degradation of PLGA/b-TCP composite scaffolds (reprinted with permission from ref. [39]).

At the anatomical sites where there is minimal fluid flow, such as articular cartilage tissues, the mass-to-surface ratio may strongly influence the degradation kinetics [41]. The level and type of agitation (rotation, vibration, flow) during degradation may not only affect the
release of the degradation products from the bulk or the surface of the material to the surrounding media but also influence the contact between soluble reactants (e.g. enzyme) and the insoluble substrate [28].

Agrawal et al. demonstrated the effect of static and dynamic conditions on the degradation of scaffolds, fabricated from a copolymer of poly(lactic acid) and poly(glycolic acid), in PBS at 37°C for up to six weeks. Figure 2d illustrates the apparatus used by the authors to achieve dynamic conditions. They found that fluid flow decreased the degradation rate significantly [38]. Gorman et al. investigated the encrustation of urinary-tract devices based on polyurethanes, Percuflex and silicone in artificial urine under dynamic conditions. The same level of encrustation was observed under static and dynamic conditions and significantly higher levels of calcium and magnesium were found under static conditions [37]. In another study, the effect of fluid flow on the in vitro degradation of poly(L-lactic acid)/β-tricalcium phosphate (PLLA/β-TCP) composite in PBS was investigated. Significantly faster degradation was observed with a dynamic flow-simulation system [39].

Hooper et al. investigated the effects of SBF and PBS on the degradation of tyrosine-derived polymers. They noticed a good similarity between the in vitro degradation kinetics of the polymers in PBS and SBF and their in vivo results [42].

5 Chromatographic methods for degradable polymeric biomaterials

Novel degradable polymeric biomedical devices are developed using more-complex polymers, i.e. random, block and graft copolymers or polymer blends. The characterization of such polymers requires the use of chromatography. This involves the determination of the molar-mass distribution, which reflects the length distribution (dispersity) of the polymeric chain. Another important application of chromatographic systems is the separation of polymers on the basis of their chemical heterogeneity, functionality type and sequence lengths [43]. The size and the chemical nature of the degradation products determine the adoptability of degradable polymers by the in vivo environment. To estimate the toxicological nature of the degradation products sensitive and selective chromatographic methods are required [29]. Biomaterials that are soluble in water or common organic solvents can be analysed with common liquid-chromatographic (LC) methods. Some (but not all) degradable polymeric materials designed for biomedical applications are of very high molecular weight or based on insoluble polymeric networks. Such polymeric systems need to be degraded prior to their chromatographic analysis. For structural analysis, the
degradation methods involve chemical hydrolysis at harsh conditions, methanolysis, or partial degradation under mild conditions [10,30]. However, to estimate the degradation rate and release of degradation products, degradation experiments are carried out under physiological conditions, such as in PBS or enzyme-containing buffer at 37°C (see section 4.2).

5.1 Size-exclusion chromatography

During the polymerization process in which biodegradable polymers are formed, a large number of chains are grown. The length of the resulting chains may vary. Therefore, it is important to determine the molar-mass distribution (MMD; or molecular-weight distribution, MWD). Size-exclusion chromatography (SEC) (also called gel-permeation chromatography, GPC), is a popular analytical technique to separate polymer chains based on their size (hydrodynamic volume). Unlike other LC methods, entropic effects are dominant in SEC (TΔS >> ΔH) [43]. Mobile phases and packing materials are selected that minimize the enthalpic interactions of the polymeric chains, so that the partition equilibrium is essentially governed by the conformational entropy differences among the polymeric chains in the two phases [44]. The information related to peak-average, number-average, weight-average, and z-average molar masses (Mₚ, Mₙ, Mₚ, and Mₚ respectively) can be deducted from the position and shape of the peak. Differential refractometry (dRI), UV-visible spectrometry, and – to a lesser extent – evaporative laser-light scattering (ELSD) are concentration-sensitive detection methods that are widely used in SEC experiments. In such experiments the MMD and molar masses are typically calculated from a calibration curve, constructed using a set of narrowly dispersed polymer standards. Light-scattering detection methods, such as multi-angle laser-light scattering (MALLS) may provide useful information on the molecular size of polymers, as well as on chain branching, conformation, and aggregation [45]. A change in the shape and size of polymer molecules in solution influences the viscosity. Therefore, viscometric detection methods are also used to determine the MMD of polymers [43]. “Triple detection-methods” (typically dRI, light-scattering and viscometry) are used to determine “absolute” (i.e. accurate) molecular weights of branched and star-shaped polymers. Absorbance or fluorescence detection and MS may – often in combination with dRI detection – provide useful information on the distribution of specific fragments within the chains or end groups in a polymer [46].
Burdick et al. used aqueous SEC-dRI to characterize kinetic-chain-length distributions of poly(methacrylic acid) (PMAA) in the hydrolysates of highly cross-linked systems based on methacrylated sebacic acid, designed for orthopaedic applications [47]. The authors investigated the relationship between kinetic chain length and the structural evolution of the network. Themistou et al. determined the molecular weights and the molecular-weight distributions of the hydrolysis products and precursors of cross-linked star polymer model networks (CSPMNs) [48]. The linear and star polymers and their extractables were determined by SEC-dRI with tetrahydrofuran (THF) as an eluent. The CSPMSs studied were based on methyl methacrylate and the diacetal-based dimethacrylate cross-linker bis[(2-methacryloyloxy)ethoxymethyl] ether and designed for biomedical applications [48]. Mojsiewicz-Pieńkowska et al. reviewed the applications of SEC-ELSD for determining the molecular weights of linear polydimethylsiloxanes (PDMSs). These authors also highlighted the experimental conditions, such as calibration curve, mobile phase, flow rate and columns used to characterize the PDMSs and the precision and accuracy of the developed methods [22].

Peters et al. calculated the kinetic chain length of poly(acrylic acid) (PAA) backbone and the average lengths of chains between cross-links in UV-cured networks prepared from mixtures of di-functional (polyethylene–glycol di-acrylate) and mono-functional (2-ethylhexyl acrylate) acrylates after hydrolysis. They used aqueous SEC coupled on-line to reversed-phase LC with dRI and mass-spectrometric detection. The results were used to express the chemical network structure for the different UV-cured acrylate polymers in network parameters, such as the degree of cross-linking, the number of PAA units which were cross-linked and the network density [49]. In another study, the same group of authors used aqueous SEC-dRI to monitor the release of PAA chains during the hydrolytic degradation of cross-linked poly-(D,L-lactide-coglycolide 50:50)-di-acrylate film. An increase in the molecular weight with degradation time indicated that the release of these polyacrylate chains was controlled by the number and type of ester groups that had to be degraded hydrolytically to dissolve the chains [30].

Lin et al. used SEC with triple detection in chloroform to determine absolute molecular weights. They confirmed that the star architecture in their biodegradable star polymers consisted of hydrophilic hyperbranched poly-(ester amide) as core and hydrophobic PCL as shell [50].

SEC is often used in off-line combinations with information-rich detectors, such as MS, or FTIR or NMR spectroscopy. Rizzarelli et al. used matrix-assisted laser-desorption/ionization
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time-of-flight mass spectrometry (MALDI-ToF-MS) as an off-line detection method for the
detailed structural characterization of complex polydisperse copolyesters, such as
poly[(R,S)-3-hydroxybutyrate-co-L-lactic acid] and poly[(R,S)-3-hydroxybutyrate-co-ε-
caprolactone]. The results of compositional analysis were in good agreement with NMR
results [51]. Montaudo et al. demonstrated the use of NMR as an off-line detection method
for the compositional analysis of random copolymers with units of methyl methacrylate,
styrene, butyl acrylate and maleic-anhydride. They calculated the polydispersity index of the
copolymers by off-line MALDI-MS of the SEC fractions [52]. Nielen et al. explored the use
of electrospray-ionization – time-of-flight – mass spectrometry (ESI-ToF-MS) as a potential
detector for SEC analysis of polyesters. The absolute mass calibration of the SEC system
based on the polymer itself and determination of monomers and end groups from the mass
spectra were achieved [53].

Figure 3 (A) On-line SEC-1H NMR traces obtained by monitoring the methoxy proton resonance at 3.59 ppm
(a) and the α-methyl proton resonances at 0.86 ppm (………..) and 1.20 ppm (———) due to rr- and mm-triads,
respectively (b); NMR signals due to α-methyl protons of the PMMA eluted in the elution periods F1 (c), F2 (d)
and F3 (e) are also shown (reprinted with permission from ref. [55]) . (B) On-line SEC-NMR analysis of
PMMA-block-poly(n-BuMA) prepared with i-C₆H₅MgBr in toluene at -60°C (reprinted with permission from ref.
[56]).
The stereo-regularity of polymeric chains and the chemical composition of copolymers may affect their degradation rate and processing. On-line coupling of SEC with NMR (continuous-flow NMR spectroscopy) makes it possible to study directly the chemical composition and stereochemistry (isotactic, syndiotactic, atactic, etc.) of complex copolymers separated according to their molecular size [54]. Hatada et al. studied the tacticity of PMMA with on-line SEC-NMR. The results (Figure 3) showed a higher concentration of $rr$-triads (syndiotactic) in fraction F1 of the higher-molecular-weight range of the SEC chromatogram and a higher concentration of $mm$-triads (isotactic) in the lower-molecular-weight fraction F3 [55]. In another study, they investigated the chemical composition of block (PMMA-block-poly(n-BuMA)) and random (poly(MMA-ran-n-BuMA)) copolymers of methyl and butyl methacrylates as a function of their MMD by on-line SEC-NMR [56].

5.2 Adsorption liquid chromatography

Complex degradable polymeric systems are synthesized from (“telechelic”) oligomers and polymers possessing terminal functional groups. The nature and the number of functional groups on a chain may vary. Precursors for polymer synthesis, intermediate products, the produced polymer, and the degradation products after hydrolytic or enzymatic degradation can be separated based on different numbers of the same functionality or different functionalities in a polymeric chain by analytical technique, such as adsorption liquid chromatography (LC). The presence of side-reaction products and chiral impurities in the degradable synthetic polymer can strongly influence their degradation rate and biocompatibility [57]. Chromatographic methods with multiple detection methods are needed for the separation and characterization of such impurities. Adsorption LC involves enthalpic interactions between the stationary and mobile phases and the analyte molecules [58,59]. Interactions between flexible polymeric chains in solution and the surface of the stationary phase depend on the magnitude of the adsorption energy. The higher the adsorption enthalpy ($\Delta H$) the stronger is the adsorption to the packing materials [43]. Adsorption LC is used in the normal-phase (NP) mode (using a polar stationary phase) or in the reversed-phase (RP) mode (using a non-polar stationary phase).

Vu et al. reported on the use of LC with UV detection at 210 nm for determining the oligomeric distribution of concentrated lactic-acid solutions [60]. Ding et al. developed an LC method for the separation and quantification of water-soluble impurities and degradation
products in PLGA, to estimate changes in the polymer “micro-climate” (e.g. in pH). The released products containing ester groups were derivatized with a common chromophore to produce bromophenacyl esters prior to their gradient elution from a C18 column with UV-vis detection at 254 nm [61]. Al Samman et al. investigated the influence of the degree of branching on the retention behaviour of linear and branched aromatic polyesters in LC with UV and ELSD detection. The branched polyesters showed a stronger adsorptive interaction with the stationary phase than the corresponding linear molecules [58].

MS has been extensively exploited as an on-line detection method for the identification of oligomers and low-molecular-weight degradable polymers [18,25,62]. Elliott et al. isolated the degradation products of L-phenylalanine-based segmented polyester urethane ureas degraded with chymotrypsin on a solid-phase-extraction cartridge for subsequent LC separation and identification with LC-MS/MS. They observed the cleavage of urea, ester and urethane bonds [18]. In an interesting study, Tang et al. investigated the enzyme-mediated degradation of radio-labeled polycarbonate-polyurethanes (PCNUs). The water-soluble degradation products were separated by LC with diode-array UV detection. The radioactivity of the collected fractions was measured by a multi-purpose scintillation counter. The products were identified by LC-MS/MS. The profile of the released degradation products was in agreement with the structural analysis of synthesized polymers [62]. Deschamps et al. simulated the in vivo degradation of segmented poly(ether ester)s block copolymers based on poly(polyethylene glycol) and poly(butylene terephthalate) by their accelerated in vitro degradation in PBS. They demonstrated the potential of LC-UV-MS for the detailed analysis of the soluble degradation products. The results showed high amounts of the PEO fraction in the soluble degradation products, while a PEOT/PBT fraction was found to be insoluble. The results were confirmed with NMR [25].

Rizzarelli et al. found evidence for selective hydrolysis of aliphatic copolyesters, such as poly(butylene succinate-co-butylene adipate), P(BS-co-BA), and poly(butylene succinate-co-butylene sebacate), P(BS-co-BSe) induced by lipase. The water-soluble products, including co-oligomers with identical molecular weights, but different sequences, were separated and identified by on-line LC-ESI-MS/MS). The results showed a preferential cleavage of sebacic ester bonds in P(BS-co-BSe) and succinic ester bonds in P(BS-co-BA) [32]. Carstens et al. investigated the in vitro chemical and enzymatic degradation of monodisperse oligo(ε-caprolactone) (OCL) and its block copolymer with methoxy poly(ethylene glycol) (mPEG-b-OCL) by monitoring the water-soluble degradation products with LC-MS. The slow degradation of OCL ester micelles in phosphate buffer at pH 7.4 was accelerated by lipase
Pulkkinen et al. reported a fast analysis of the soluble degradation products of 2,2′-bis(2-oxazoline)-linked poly-ε-caprolactone (PCL-O), degraded in simulated intestinal fluid by LC-ESI-MS/MS. The polymer degraded primarily by ester hydrolysis, while amide bonds showed greater stability [23]. Peters et al. demonstrated the use of LC-MS to identify and quantify the various water-soluble oligomeric and polymeric degradation products released during the hydrolytic degradation of poly(D,L-lactide-co-glycolide 50:50)-di-acrylate networks. The products were analyzed directly after release and also after complete hydrolysis of the soluble fraction. They found a rapid release of residual photo-initiator followed by a gradual release of lactide/di-ethyleneglycol/glycolide oligomers with varying chain length and composition [64].

Figure 4 (1) Liquid chromatographic separation of PEG 1000 (chromatograms A and B) and soluble products derived from PEOT:PBT (71:29) copolymer during hydrolytic degradation at 100°C (chromatograms C and D) with UV detection at 251 nm (chromatograms A and C) and mass-spectrometric detection applying atmospheric-pressure chemical ionization in the positive-ion mode (APCI(+)) conditions (chromatograms B and D) recorded in scan mode (m/z=200–1600) (reprinted with permission from ref. [25]). (2) UV-absorbance and radioactivity chromatograms for the degradation products from radio-labeled polycarbonate-polyurethanes: (a) buffer incubation and (b) cholesterol esterase incubation (reprinted with permission from ref. [62]).
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The hyphenation of the most powerful spectroscopic technique \textit{i.e.} NMR (containing solvent suppression features) with HPLC is recently getting more attention for the online chemical composition and molar mass determination of oligomers and low MW polymers separated on reversed phase HPLC column according to their chemical structure. Pasch \textit{et al.} investigated the chemical structure, molar mass and end group analysis of poly(ethylene oxide) by an online HPLC-NMR setup \cite{65}.

\section*{5.3 Liquid chromatography at critical conditions}

Liquid chromatography at critical conditions (LCCC) is receiving increased attention for the separation of complex polymers. LCCC separates the polymers at the so-called critical conditions, \textit{i.e.} the chromatographic conditions where the enthalpy and entropy effects compensate each other (\(\Delta H = T\Delta S\)) \cite{66}. Under these conditions the retention of polymeric species becomes independent of their molecular weight \cite{66,67}. LCCC allows the separation of polymers on the basis of their functionality type distributions (FTDs). It has been applied for the separation of functional polymers, block copolymers, branched polymers, and polymer blends and to assess their stereo-regularity \cite{15,58,59,66,68}. The incorporation of hydrophilic and hydrophobic components and stereo-regularity of degradable copolymers for biomedical application control their degradation behaviour. Lee \textit{et al.} resolved oligomeric PLLA block species of poly(ethylene oxide)-\textit{block}-poly(L-lactide), (PEO-\textit{b}-PLLA) by RPLC at the critical conditions of PEO. They confirmed the composition of each species by off-line MALDI-MS analysis \cite{67}. In another study, they fractioned the LLA units in tri-block PLLA-\textit{b}-PEO-\textit{b}-PLLA copolymer by RPLC at the critical conditions of PEO and confirmed the results by off-line MALDI-MS. In tri-block copolymer, unlike in di-block (PEO-\textit{b}-PLLA) copolymer, they observed a splitting of the eluted peaks containing the same number of LLA units. They assigned this peak splitting to the different length distributions of PLLA blocks at the two ends of the PEO block \cite{14}. Mengerink \textit{et al.} developed a method for the separation of linear and cyclic oligomers of polyamide-6 by LCCC-ELSD. ESI-MS did not allow discrimination between the linear and cyclic products.\cite{15}. Peters \textit{et al.} reported the FTD of functional PMMA, obtained by LCCC-ELSD. The mono- and bifunctional PMMA peaks were identified by ESI- MS \cite{68}. Philips \textit{et al.} discussed novel developments in water-based LCCC. They varied the buffer concentration and the proportion of organic modifier in the mobile phase to approach the critical condition for two polymer systems, \textit{viz.} poly(styrene sulfonate) and poly(acrylic...
acid). The critical condition of poly-(acrylic acid) was then used to study the retention characteristics of a copolymer containing both acrylic acid and \( n \)-vinyl pyrrolidinone [69]. De Geus et al. utilized LCCC, with UV and ELSD detection, to separate PCL polymer samples with different end groups, in order to gain insight in the initiation process of enzymatic ring-opening polymerization. PCL chains with three different end groups were separated, \textit{i.e.} (i) linear carboxylic-acid end-functionalized species, (ii) linear hydroxyl-ester species, and (iii) cyclic species. The identity of each peak was confirmed by offline MALDI-MS [70].

LCCC with on-line NMR analysis can provide detailed information on the end groups and chemical composition of polymeric chains. Hiller et al. demonstrated the use of on-line NMR detection for the analysis of complex mixtures of fatty alcohol ethoxylates (FAEs) by LCCC. The peaks were detected using an ELSD detector [71]. In another study, they investigated the separation of block copolymers of PS-\( b \)-PMMA and blends of PS and PMMA at the size-exclusion conditions for PS and critical conditions of PMMA with on-line \(^1\)H NMR detection [72]. Unfortunately, as the critical conditions are strongly dependent on the mobile-phase composition, but also on temperature and pressure [73], especially for high-molecular-weight polymers, LCCC is only rarely applied successfully to polymer systems with molecular weights exceeding 100 kDa.

5.4 Two-dimensional liquid chromatography

Complex polymers, including degradable synthetic polymers, exhibit several simultaneous distributions. For example, all functionalized polymers exhibit an MMD and a functionality-type distribution (FTD) and all copolymers exhibit an MMD and a chemical-composition distribution (CCD) [43,74]. Moreover, the different distributions in complex polymers tend to be mutually dependent [74]. SEC or HPLC by themselves may not reveal correct information on the MMD or the molecular heterogeneity of the polymeric chains [46]. To gain insight in multiple, mutually dependent distributions analytical techniques such as multi-dimensional separations are indispensable [74].

Kilz et al. provided a detailed description of the two-dimensional chromatographic techniques for polymers [75]. Pasch et al. reported on the two-dimensional separation of PEO-\( b \)-PPO block copolymers. In the first dimension, they separated copolymers with respect to the length of the PEO block by LCCC. The collected fractions were further separated in the second dimension, either by supercritical-fluid chromatography (SFC) or by
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SEC based on the length the PEO blocks [76]. Early two-dimensional LC techniques were based on “heart-cut” or fractionation methods and they were very specialized or time-consuming [74]. With the advent of modern technology, two-dimensional LC methods are becoming faster and more comprehensive.

Van der Horst et al. wrote a highly useful review on the advantages of comprehensive LC×LC for polymers over “heart-cutting” LC-LC [59]. Kok et al. reported on FTIR as an on-line detection method in comprehensive LC×SEC for the characterization of copolymers based on styrene and methacrylates [77]. The results were confirmed by UV detection. The generated functional-group contour plots showed a distinction between UV-active and non-UV-active groups of the polymer.

**Figure 5** LC×LC contour plots of (A) poly(2-ethylhexyl acrylate) P2EHA macro(RAFT agent), (B) copolymer (2-ethylhexyl acrylate and methyl acrylate)-1h, (C) copolymer-2h, (D) copolymer-4h, and (E) copolymer-8h. (F) is a rotation of 90° and inclination of 35° of the LC×LC chromatogram of the sample of copolymer-8h. 1st dimension: gradient LC with 0 to 70% THF in methanol in 200 min at 0.05 mL/min on PLRP-S 5 μm (Y-axis). 2nd dimension: SEC with THF at 1.5 mL/min on PL HTS-C (X-axis). Calibration: PMMA. Detection: ELSD (reprinted with permission from ref. [79]).
The hydrodynamic volume of a branched polymer of certain molecular weight may be identical to that of a linear polymer of lower molecular weight. Based on this principle Edam et al. demonstrated the use of comprehensive two-dimensional molecular-topology fractionation (MTF) × SEC for the separation of branched polymers based on their topology [78]. Raust et al. performed two-dimensional LC separations with a combination of LCCC and SEC to gain insight in the polymerization process of copolymers based on 2-ethylhexyl acrylate and methyl acrylate, (P2EHA-b-PMA), produced by reversible addition-fragmentation chain transfer (RAFT)-mediated polymerization in organic dispersion (Figure 5). The LC×SEC chromatograms revealed a certain heterogeneity of the polymer and allowed the precise characterization of the MA block length in the copolymer. For compositional analysis the results were confirmed by LC-1H NMR [79]. In summary, LC×LC methods can be useful for the characterization of complex degradable polymers in terms of several distributions (MMD, CCD, FTD, etc.) simultaneously. LC×LC can provide an efficient, reliable and comprehensive characterization of biodegradable polymers.

6 Gas chromatography

Gas chromatography (GC) is another powerful analytical tool for the identification and quantification of impurities, additives, and degradation products of degradable polymeric biomaterials. GC is most often applied in combination with flame-ionization detection (FID) and MS for the analysis of oligomers and low-molecular-weight polymers [36]. Barlow et al. reviewed the applications of GC in combination with pyrolysis for the analysis and characterization of polymer degradation [80]. Hakkarainen et al. investigated the nature of low-molecular-weight degradation products of PLA, PGA, and their copolymers. They reported a convenient and rapid solid-phase-extraction (SPE) – derivatization technique to improve the qualitative and quantitative GC-MS analysis of hydroxy acids released by the degradation of PLA and PGA in buffer solution [81-83]. The GC-MS analyses showed a difference in the patterns of degradation products released in biotic and abiotic media. In another study this group utilized GC-FID to explore single-drop micro-extraction (SDME) in combination with multiple-headspace (MHS) extraction for the quantitative determination of lactide in thermally oxidized polylactide [84]. During a study of the esterification reaction between lactic acid and different fatty acids, Torres et al. utilized GC-MS to estimate the degree of polymerization in polymerized fractions of commercial LA [85]. Vu et al. characterized the oligomeric
distribution of lactic acid in aqueous media by GC-MS [60]. Urakami et al. reported a rapid, precise and accurate compositional analysis of co-poly(DL-lactic/glycolic acid) (PLGA). This was performed by pyrolysis – gas chromatography – mass spectrometry (Py-GC/MS) combined with one-step hydrolysis and methylation in the presence of tetramethylammonium hydroxide (TMAH). They found good agreement of the analytical results with $^1$H NMR data [86]. Plikk et al. studied the chemical changes in porous scaffolds based on various L,L-lactide (LLA), 1,5-dioxepane-2-one (DXO) and ε-caprolactone (CL) copolymers after sterilization with electron beam and gamma irradiation [87]. The formation of low-molecular-weight degradation products was studied by GC-MS. Burford et al. described the rapid qualitative and quantitative analysis of polyester-based polyurethane elastomers by GC-FID after polymer cleavage into the corresponding glycol, dicarboxylic acid and diamine fragments by molten alkali fusion at high temperature [88]. All the carboxylic-acid products were reacted to dimethyl ester derivatives prior to their GC analysis [88]. Mallepally et al. investigated the enzymatic degradation of hyperbranched polyesters (HBPEs). The release of free fatty acids was studied using GC [89].

![Pyrograms of UV-curable resins based on bifunctional poly(ethylene glycol)-diacrylate (PEDA). Pyrolysis at 400°C in the presence of TMAH. (a) prepolymer; (b) UV-cured resin. (reprinted with permission from ref. [91]).](image-url)
Eldäsäter et al. studied the degradation of poly (ester amide) and poly(butylene adipate-co-caproamide) in aqueous environment at 37°C, 60°C, and 80°C. GC-MS with SPE was used to investigate the nature of degradation products at different degradation conditions. Changes in the polymer composition were investigated by Py-GC-MS [90]. Matsubara et al. used pyrolysis GC (Py-GC) in the presence of tetramethylammonium hydroxide (TMAH) to characterize the network structure in UV-cured bifunctional poly(ethylene glycol)-diacrylate (PEDA) [91].

Kaal et al. developed a fully automated on-line SEC-Py-GC-MS method [92]. The polymer samples were separated based on molecular size and fractions were transferred on-line. The SEC solvent was evaporated in a programmable-temperature-vaporizer (PTV) injector prior to pyrolysis and GC-MS analysis. The scope of the method was extended to include aqueous SEC and RPLC by introducing a sintered liner, filled with sintered glass beads (60-100 µm) to approximately half of the cross sectional area [93]. The developed systems provided a great deal of quantitative insight in the composition of the on-line collected LC or SEC fractions. Recently, Chojnacka et al. investigated the effect of monomeric ratio of N-vinyl-2-pyrrolidone (VP) and vinyl acetate (VA) on the dissolution behaviour of their copolymers in water using Py-GC-MS. The compositional analysis of the fractions, collected at different time intervals during the dissolution study, revealed that copolymers with higher contents of VA dissolve considerably slower than the other copolymers [94].

### 7 Direct mass-spectrometric analyses

Mass spectrometry has emerged as a powerful analytical tool for the characterization of synthetic polymers and copolymers. A time-of-flight (ToF) mass spectrometer offers high sensitivity for multi-ion detection, a large mass range, and good mass resolving power. Therefore, ToF-MS is most commonly used as a mass analyzer for the characterization of polymers [95]. A ToF-MS can be conveniently combined with ESI or with MALDI. However, when using ESI multiply charged ions are usually formed, which complicates the interpretation of the spectra. In MALDI both statistics and “charge-ability” (a combination of several parameters, including affinity to charge and efficiency of transfer from solid to vacuum) may cause low-molecular-weight oligomers to dominate the spectrum. Several books and reviews have been published that describe the developments in the field of mass spectrometry of synthetic polymers [95-99].
ESI is a soft ionization technique, which produces multiply charged ions and little fragmentation. The analyte solution emerging from the column is nebulized, ionized and transferred to the vacuum of the mass analyzer and the MS detector [98]. Andersson et al. compared the degradation stability of stereo-complex poly(L-lactic acid)/poly(D-lactic acid) (PLLA/PDLA) with plain PLLA. The composition of degradation products was estimated semi-quantitatively by direct ESI-MS. The results showed a shorter hydrolysis time for PLLA/PDLA and more acidic degradation products [13]. Höglund et al. studied the effect of plasticizer (acetyl tributyl citrate) on the degradation of PLA. They investigated the water-soluble products and the plasticizer by ESI-MS [100]. In another study, this group studied the effect of surface modification on the hydrolytic degradation and investigated the degradation of PLA grafted with poly(acrylic acid) (PAA). The water-soluble degradation products were analyzed by ESI-MS [101]. Recently, Rizzarelli et al. developed a convenient direct ESI-MS method to determine concentrations of sebacic-acid (SA) and terephthalic-acid (TA) residues in biodegradable copolymers. The obtained results were in agreement with LC-UV data. The assay was proposed as a fast and sensitive alternative to currently employed methods for acid quantification [102].

MALDI is also a soft ionization technique. It allows the detection of large, non-volatile and labile molecules. The compounds of interest are desorbed and ionized by the combined influence of a laser beam and a chemical matrix, usually under vacuum. The resulting (predominantly singly charged) ions are directed to a (ToF) mass spectrometer by a continuous high voltage [99]. Burkoth et al. used MALDI-ToF-MS to characterize the molecular-weight distribution of (mostly) linear poly(methacrylic acid) degradation products as a function of the network evolution (i.e. double-bond conversion), rate of initiation, and monomer size during the degradation of cross-linked polymers based on PMA and sebacic acid [40]. Rizzarelli et al. employed MALDI with tandem mass spectrometry (MS^n) to investigate the fragmentation pathways of poly(butylene adipate) (PBAd) oligomers [103]. In another study, they applied post-source-decay (PSD) MALDI-ToF-MS^n for the sequence determination of aliphatic poly(ester amide)s synthesized from dimethyl sebacate or sebacic acid and 2-aminoethanol or 4-amino-1-butanol [104]. Luo et al. found a symmetric distribution in low-molecular-weight star polymers prepared by grafting poly(ethylene glycol) (PEG) arms onto a cholic acid core via anionic polymerization [105]. Weidner et al. performed fragmentation analysis by means of MALDI with collision-induced dissociation (CID) and MS^n to determine sequences and end groups of complex copolyesters based on hexanediol-neopentylglycol-adipic acid copolyesters [106].
During the course of the degradation process, the surface chemistry of the degradable polymeric device may influence the biological environment. Thus, it plays an important role in determining its biocompatibility [27]. Therefore, techniques for characterizing the surface of the biomaterials are gaining attention. Secondary ion mass spectrometry (SIMS) in combination with ToF can be used for surface characterization and (quantitative) analysis of synthetic copolymers and polymeric blends [96,107]. Belu et al. reviewed the application of ToF-SIMS for the structural characterization of biomaterial surfaces. They described the technique as a flexible and powerful surface-characterization tool [108]. Chen et al. used ToF-SIMS to study the in vitro hydrolytic degradation at the surface of different biodegradable polymers, including PLA, PGA, PLGA, poly(sebacic acid) (PSA), and two random copolymers of poly(fumaric-co-sebacic) acid (PFS) of different compositions. It was reported that useful information on reaction kinetics can be obtained from the ToF-SIMS spectra by analyzing the intensities of the molecular ions in the distribution [107].

Figure 7 (A) The 2 m drift-tube IMS-MS instrument design and operationa and (B) typical output for ions separated in the gas-phase detected by MS in different modes of operation (cf. details in ref. [110]). (reprinted with permission from ref. [110]).
Recently, the combination of ion-mobility spectroscopy (IMS) through CID with MS has been gaining recognition for the structural characterization of synthetic polymers [97]. In ion mobility, ions are separated on the basis of their conformational state (size and shape), as they drift through a gas (e.g. He, N2) under the influence of an electric field [109]. A major benefit of including IMS as an intermediate stage in LC with MS detection is the reduction of “chemical noise” due to the additional selectivity of IMS. This is especially important when determining trace amounts of compounds in complex mixtures such as body fluids. Trimpin et al. reported on the use of a multi-dimensional IMS-MS methodology that rendered a detailed view of molecular components in complex mixtures, based on the combined analysis of the three-dimensional geometries and masses of polymeric components adducted with metal cations in the gas phase [110]. The structure of PEGs with different functionalities, PPG, poly(tetramethylene glycol) (PTMEG), and several poly(alkyl methacrylate)s (PAMA)s (with alkyl = methyl, ethyl, butyl, etc.) was investigated using IMS-MS.

8 Nuclear-magnetic-resonance spectroscopy

Nuclear-magnetic-resonance (NMR) spectroscopy is an extensively used analytical technique in the field of synthetic polymers. The microstructure, region-isomerism, stereochemical isomerism, geometric isomeric, branches and end groups, copolymer composition, number-average molecular weight ($M_n$), chain conformation, and intermolecular association of the polymers are among the parameters that can be investigated by high-resolution NMR and 2D NMR experiments [111]. LeMaster et al. studied the effect of $T_1$ and $T_2$ relaxation on the 2D $^1$H-$^{13}$C correlation spectra of linear commercial polymers. The results were used to estimate the concentration of end groups in polyester urethanes ($M_n$ 40 kDa). They estimated an uncertainty in $M_n$ of 6-7% (r.s.d.) [112]. Two-dimensional homo-nuclear correlation spectroscopy ($^1$H-$^1$H COSY) was used to confirm the formation of poly($\alpha$-peptide) in the protease-catalyzed polymerization of L-glutamic acid diethyl ester hydrochloride [113]. Pergal et al. synthesized polyurethanes-siloxane copolymers containing high contents of PCL-PDMS-PCL segments [114]. The structure of copolymers, the lengths of hard and soft segments, and the connectivities between homonuclear or heteronuclear atoms with single or multiple bond were investigated by $^1$H, $^{13}$C NMR and 2D NMR experiments, such as $^1$H-$^1$H COSY, HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple-bond correlation). In an interesting study, the generation of
hyper-branched poly(amine-ester)s was confirmed by $^{13}$C-, DEPT-135 NMR and 2D NMR techniques [115]. Shaver et al. attached six arms of poly(lactic acid) to dipentaerythritol cores. $^1$H NMR experiments provided useful information on the tacticity of the synthesized star polymer [116]. To investigate branching, Cooper et al. performed $^1$H-, $^{13}$C-, COSY, and HSQC NMR experiments and SEC to determine the number of end groups and repeating units in the backbone of poly(lactic acid)-polyurethane functionalized with pendent carboxylic-acid groups [117].

9 Conclusions

Separation methods based on chromatography are essential analytical tools to estimate the FTD, CCD, and MMD of complex degradable polymeric systems. The analysis of the chemical nature of the degradation products not only highlights the stability of different degradable bonds, but also reflects the toxicological nature and the biocompatibility of biomaterials. SEC with dRI, UV-vis or ELSD detectors yields molecular-weight distributions. Light-scattering or viscometry may provide additional information, such as molecular size and absolute molar masses. LC separates polymers on the basis of their functionality and chemical composition. LCCC is a method of choice to separate low-molecular-weight functional polymers, copolymers and polymer blends at critical conditions. NMR spectroscopy as an on-line detector for SEC, LC, or LCCC provides useful information about the functionality, chemical composition, and tacticity of the polymeric chains along their MMD. Coupling SEC on-line with MS also broadens its scope. Comprehensive two-dimensional LC techniques, such as LC×LC, LC×SEC, LCCC×SEC, MTF×SEC, etc. are developing into promising analytical tools for the detailed analysis of, for example copolymers, branched polymers, and polymer blends. Gas chromatography is extensively used to separate and identify degradation products. For complex polymers and networks, Py-GC-MS provides more insight in the chemical composition by pyrolyzing the liquid or solid samples. Direct mass spectrometric methods, such as ESI-ToF-MS, MALDI-ToF-MS, and SIMS can provide rapid analysis of the chemical composition of oligomers or low-molecular-weight polymers. MALDI and SIMS allow studying the surface chemistry before and after degradation. IMS-MS promises to contribute to utilize the 3D structure of the polymers for additional selectivity. Despite the limitations associated with each analytical technique, a combination of selective and sensitive methods can usually be devised for the analysis for different classes of polymeric biomaterials.
10 Scope of the thesis

The objective of this thesis is to develop new analytical methods by exploring different analytical techniques. The thesis deals with the degradation and analysis of synthetic polymeric biomaterials. The performance of degradable polymeric biomaterials depends on their chemical structure and on the chemical nature of their degradation products. Therefore, Chapter 1 reviews the different strategies and analytical techniques for the chemical analysis of degradable polymeric biomaterials, in particular those based on chromatographic separations.

In Chapter 2 of this thesis, the quantitative structural analysis of polymeric networks that are insoluble under normal physiological conditions is described. An *in vitro* method is developed that is well suited for the quick and complete hydrolytic degradation of poly(2-hydroxyethyl methacrylate) (pHEMA), poly(lactide-coglycolide50:50)$_{1550}$-diol (PLGA(50:50)$_{1550}$-diol) and polyester-urethane-acrylate-based networks, using a microwave set-up. The microwave glass vials are coated internally with Teflon (PTFE) to avoid contact of the alkaline medium with the inner surface of the glass vessel and thus prevent the formation of residues. The degree of hydrolysis is monitored by NMR spectroscopy. The hydrolyzed components can be separated by liquid chromatography and quantified by mass spectrometry or UV-vis detection. The kinetic chain length of poly-addition backbones (-C-C-) can be determined by SEC.

Chapter 3 describes an *in vitro* enzymatic degradation of multi-block PEA with $\alpha$-chymotrypsin and proteinase-K at 37°C. The release of different monomeric and oligomeric products is monitored by LC-ESI-ToF-MS. Semi-quantitative analysis of water-soluble degradation products reveals the protease and/or amidase activity and provides indications of the relative fragment (bond) stabilities. The polymer does not degrade by chemical degradation under physiological conditions. The structure of the polymer is characterized by NMR spectroscopy.

In Chapter 4 the development of a miniaturized and automated system is reported that can be used for the fast, on-line investigation of the *in-vitro* enzymatic degradation of PEA coatings. The system can be used under both static and dynamic (flow) conditions and includes on-line LC-ToF-MS analysis of the hydrolysate (containing enzyme and degradation products). The system is investigated with respect to different injection volumes (pulses) of an $\alpha$-chymotrypsin ($\alpha$-CT) solution, flow rates of injected $\alpha$-CT band or $\alpha$-CT solution through the coated capillary, concentration of $\alpha$-CT, and lengths of coated capillary.
The versatility of the system makes it easy to follow the course of degradation and to differentiate between primary and secondary degradation products.

11 References


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