A review - polymer architectures *via* mass spectrometry and hyphenated techniques

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Abstract

This review covers the application of mass spectrometry (MS) and its hyphenated techniques to synthetic polymers of varying architectural complexities. The synthetic polymers are discussed as according to their architectural complexity from linear homopolymers and copolymers to stars, dendrimers, cyclic copolymers and other polymers. MS and tandem MS (MS/MS) has been extensively used for the analysis of synthetic polymers. However, the increase in structural or architectural complexity can result in analytical challenges that MS or MS/MS cannot overcome alone. Hyphenation to MS with different chromatographic techniques (2D $\times$ LC, SEC, HPLC etc.), utilization of other ionization methods (APCI, DESI etc.) and various mass analyzers (FT-ICR, quadrupole, time-of-flight, ion trap etc.) are applied to overcome these challenges and achieve more detailed structural characterizations of complex polymeric systems. In addition, computational methods (software: MassChrom2D, COCONUT, 2D maps etc.) have also reached polymer science to facilitate and accelerate data interpretation. Developments in technology and the comprehension of different polymer classes with diverse architectures have significantly improved, which allow for smart polymer designs to be examined and advanced. We present specific examples covering diverse analytical aspects as well as forthcoming prospects in polymer science.

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78	AUC	Analytical ultracentrifuge
79	AF4	Asymmetric flow field-flow fractionation
80	APCI	Atmospheric pressure chemical ionization
81	AROP	Anionic ring opening polymerization
82	ASAP	Atmospheric solid analysis probe
83	ATRP	Atom transfer radical polymerization
84	CCS	Collisional cross section
85	CE	Capillary electrophoresis
86	CI	Chemical ionization
87	CAD	Collision activated dissociation
88	CZE	Capillary zone electrophoresis
89	Đ	Dispersity
90	DESI	Desorption electrospray ionization
91	DMSS	Dimethylsilylstyrene
92	ECD	Electron capture dissociation
93	EI	Electron ionization
94	ELSD	Evaporative light scattering detector
95	EO	Ethylene oxide
96	ESI	Electrospray ionization
97	ETD	Electron transfer dissociation
98	FFF	Field-flow fractionation
99	FT-ICR	Fourier transform-ion cyclotron resonance
100	GC	Gas chromatography

101	HPLC	High-performance liquid chromatography
102	ICR	Ion cyclotron resonance
103	IM-MS	Ion mobility-mass spectrometry
104	IR	Infrared
105	ISD	In-source decay
106	LAC	Liquid adsorption chromatography
107	LACCC	Liquid adsorption chromatography at critical conditions
108	LC	Liquid chromatography
109	LDI	Laser desorption ionization
110	LS	Light scattering
111	MALDI	Matrix-assisted laser desorption/ionization
112	MD	Molecular dynamics
113	MS	Mass spectrometry
114	MS/MS	Tandem mass spectrometry
115	NMP	Nitroxide-mediated radical polymerization
116	NMR	Nuclear magnetic resonance
117	NP	Normal phase
118	PAA	Poly(acrylic acid)
119	PAMAM	Poly(amidoamine)
120	PB	Poly(butadiene)
121	PBS	Poly(butadiene succinate)
122	PBT	Poly(butylene terephthalate)
123	PCL	Poly(caprolactone)
124	PDMS	Poly(dimethylsiloxane)
125	PE	Poly(ethylene)

126	PEEK	Poly(ether ether ketone)
127	PEI	Poly(ethylenimine)
128	PEO	Poly(ethylene oxide)
129	PET	Poly(ethylene terephthalate)
130	PHEMA	Poly(hydroxyethylmethacrylate)
131	PI	Poly(<i>isoprene</i>)
132	PIB	Poly(<i>isobutylene</i>)
133	PLA	Poly(lactide)
134	PLGA	Poly(lactic- <i>co</i> -glycolic acid)
135	PLUMS	Polymer labeling using mass spectrometry
136	PMA	Poly(methyl acrylate)
137	PMAA	Poly(methacrylic acid)
138	PMMA	Poly(methyl methacrylate)
139	<i>Pn</i> BA	Poly(<i>n</i> -butyl acrylate)
140	PO	Propylene oxide
141	POM	Poly(oxymethylene)
142	PP	Polypropylene
143	PPG	Poly(propylene glycol)
144	PPI	Poly(propylene imine)
145	PPO	Poly(propylene oxide)
146	PS	Polystyrene
147	PSD	Post-source decomposition
148	PSS	Poly(styrene sulphonate)
149	<i>Pt</i> BA	Poly(<i>t</i> -butyl acrylate)
150	<i>Pt</i> BMA	Poly(<i>t</i> -butyl methacrylate)

151	PVAc	Poly(vinyl acetate)
152	PVAI	Poly(vinyl alcohol)
153	PVP	poly(<i>N</i> -vinyl pyrrolidone)
154	Py/GC	pyrolysis/gas chromatography
155	Q	Quadrupole
156	RAFT	Reversible addition-fragmentation chain transfer
157	RI	Refractive index
158	RP	Reversed phase
159	RP-TGIC	Reverse phase-temperature gradient interaction chromatography
160	SAXS	Small angle X-ray scattering
161	SEC	Size exclusion chromatography
162	SG1	<i>N</i> - <i>tert</i> -butyl- <i>N</i> -(1-diethylphosphono-2,2-dimethyl propyl)
163	SID	Surface-induced dissociation
164	SIMS	Secondary ionization mass spectrometry
165	SORI	Sustained off resonance irradiation
166	SL	Surface layer
167	SPME/GC	Solid phase microextraction/gas chromatography
168	TA-APGD	Thermal-assisted atmospheric pressure glow discharge
169	ThFFF	Thermal field-flow fractionation
170	TMPO	Trimethylolpropaneoxetane
171	ToF	Time-of-flight
172	UV	Ultraviolet
173		

1. Introduction

Wherever we look in everyday life, we see polymers in a multitude of forms, *e.g.* as simple plastic bags, as packing material, as molded forms in toys, computers, seats, pens, in cars, trains, bikes, in shampoo and clothes, various household furniture, windows and in the paint on the wall. Nonetheless, polymers do not exclusively satisfy basic requirements; but high-tech materials are designed from polymers to challenge societal problems in medicine, energy generation and storage nowadays.

Depending on the application of the material (for example, polystyrenes as applied in construction and foam material or polylactides for the use in drug delivery systems), structurally distinct polymers with different macroscopic properties are prepared from a large library of structurally diverse monomers. This first level of structural complexity is not only influenced by the chemical nature of the monomer, but also by the length of the macromolecules and their molar mass distribution, expressed in the dispersity ($\bar{M}_w/\bar{M}_n$), as well as their end groups. By using two or more monomers, a second degree of structural complexity is reached for copolymers. Tuning the molar ratio of the monomers and their organization, such as in block, gradient or random copolymers, plays an important role in the resulting material having different chemical and / or physical properties. A third stage of structural complexity is reached by the arrangement of the monomers into a cyclic, star-shaped, comb-shaped or dendritic structure (Figure 1). These different architectures enable the engineering of highly complex molecules for high-end applications, *e.g.* micelles, amphiphilic block copolymers, polyion complexes, which encapsulate or entrap agents for tumor targeting applications [1].

Figure 1. Schematic overview of various (co)polymer architectures.

However, by increasing the structural or architectural complexity of the polymeric system, the construction of the desired features results in far more demanding synthetic procedures, which increase the probability of defects. On the other hand, high-tech applications require well-defined and very reproducible products. To solve these opposing exigencies of synthesis reality and application requirements, a thorough and profound characterization of the polymer's architecture is required.

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) are extensively used to provide detailed information on structural properties of polymers [2-7]. Furthermore, many techniques that can be hyphenated to MS can provide greater insight on the polymer architectural information [8]. In particular, high-performance liquid chromatography (HPLC) techniques such as normal and reverse-phase HPLC and size exclusion chromatography (SEC) systems provide preseparation of complex mixtures and highly disperse samples for MS analysis [2,4,5,7,9]. Liquid adsorption chromatography at critical conditions (LACCC) is a specific HPLC condition, which enables the differentiation of end groups irrespective of the molar mass. First, the critical conditions are determined with a minimum of three polymer standards with the same end groups, but different molar masses and dissolved in a "good solvent". Then the "bad" solvent is added step by step, therefore promoting the elution of the

standards from SEC towards adsorption mode. The critical conditions are reached when all standards have the same retention time. In addition to one-dimensional separation, 2D-liquid chromatography (LC) is utilized by combining multiple chromatographic techniques to separate samples by their inherent chemical and physical properties prior to MS. Moreover, the shape of a polymer can be investigated by field-flow fractionation (FFF), and although it is ideal for the separation of higher molar mass polymers, its connection to MS is not exploited extensively [10]. A more recently applied technique to polymers is ion mobility-mass spectrometry (IM-MS), which shows great promise because of its ability to separate gas-phase ions depending on their shape, size and charge state [11]. The main advantages of IM-MS are the ability to rapidly separate and differentiate isobaric and isomeric species without the need for MS/MS. Finally, in addition to hyphenated techniques, computational methods are being employed increasingly in polymer science for detailed data interpretation essentially facilitating the spectral analysis considerably [12].

Within this feature, MS techniques and hyphenated MS setups that were used to acquire information on macromolecular architectures are discussed in view of their benefit and disadvantages, as well as the desired future developments needed for obtaining more detailed insight on the molecular composition and the structure of polymers.

2. First dimension of complexity: linear homopolymers with MS

The complexity of homopolymers is mainly determined by the chemical identity of the monomer, the length of the polymer chains, its $\overline{M}_n$ value and the α - and ω -end groups, which might show an immense diversity depending on the applied synthesis technique. Additionally, the analyses of homopolymers is seen as the fundamental starting point for studies striving to develop and improve methods for more complex structures, to create libraries, facilitate predictions and enable interpretations of more complex data sets. In essence, the lengths of the

polymer chains and $\bar{M}_n$ values can be acquired by a one-dimensional MS technique. By degradation or fragmentation of the polymer, information regarding the end groups as well as the chemical nature of the repeating unit can be obtained [3,13].

2.1. Thermal-MS techniques

Thermal-MS based techniques have the ability to decompose polymers by heat [13]. Subsequently, decomposed materials are analyzed by the mass spectrometer to obtain information regarding synthetic defects in the polymer chain and degradation products of the material. Several co-workers have used diverse techniques to investigate the polymer structure and pyrolysis products. For example, Zhang *et al.* utilized thermal-assisted atmospheric pressure glow discharge (TA-APGD) for structural characterization of polystyrene (PS) and poly(oxymethylene) (POM) [14]. The analysis of the degradation products by TA-APGD-MS provided information on the structural composition of the polymer without any sample pretreatment which can particularly be important for the analysis of polymer materials that are difficult to dissolve. Barton *et al.* used electrospray ionization (ESI)-MS in addition to matrix-assisted laser desorption/ionization (MALDI)-MS to investigate the degradation mechanisms of poly(propylene oxide) (PPO) by heating the polymer prior to MS analysis [15]. One of the notable advantages of thermal methods is that it allows insoluble polymers to be analyzed by mass spectrometry. Tsai *et al.* used stepwise pyrolysis/gas chromatography (Py/GC)-MS method to investigate the thermal degradation of poly(aryl-ether-ether-ketone) PEEK. This study shows that Py/GC-MS can be a very useful analytical tool to separate and characterize, even the isomeric temperature dependent pyrolysis products by applying a stepwise temperature program [16]. Another application of thermal methods with mass spectrometry was on lacquer films. Lu *et al.* analyzed and compared the laccol components of natural and

synthetic *Rhus succedanea* lacquer films, which also enabled the confirmation of the polymerization mechanism and structure of the macromolecule [17].

For linear homopolymers, thermal-MS techniques have mostly been used for determination of defects in microstructures and investigation of pyrolysis mechanisms since 1948 [18,19]. Characterization of the temperature dependent degradation products by thermal-MS techniques can provide information regarding both physical and structural properties, thus helping the manufacturing of robust products.

2.2. MS/MS techniques

MS/MS techniques involve fragmentation of analyte ions in the mass spectrometer to obtain more detailed structural and/or architectural information. An MS/MS analysis can be carried out by using diverse scanning modes such as: product ion scan, precursor ion scan, neutral loss scan and selected reaction monitoring. Out of all these modes, the product ion scan is one of the most common MS/MS modes used for structural characterization of various synthetic polymers. During this mode of MS/MS analysis, a precursor analyte ion is isolated and this is followed by its activation and fragmentation inside the mass spectrometer. Finally, all of the fragmentation products are scanned and analyzed for more detailed investigation of the precursor ion structure. Many activation methods have been developed, such as collision activated dissociation (CAD), surface-induced dissociation (SID), photodissociation, electron capture dissociation (ECD), electron transfer dissociation (ETD), and post-source decay (PSD). Although numerous fragmentation techniques exist and are applied to synthetic polymers, the most widely used technique is CAD, which is closely related to PSD and in-source decay (ISD) techniques. However, it does not always suffice for the full characterization of polymer architectures. Applications of MS/MS to a wide variety of

polymer types and their fragmentation mechanisms have been discussed in detail in a review by Wesdemiotis *et al.* and several representative examples of such work are highlighted below [20].

For example, aromatic side chain based polymers such as PS has numerous different applications due to their importance in life science, and poly(2-vinylpyridine) (P2VP), for instance, in membranes as well as in batteries, and features a similar structure to PS, with only a slightly different side chain [20]. Furthermore, poly(acrylate)s are also important for contact lenses, membranes [21] as well as nanoparticles for energy storage [22]; MS/MS studies have been reported on poly(methyl acrylate) (PMA) [20,23], poly(methyl methacrylate) (PMMA) [20,24,25], poly(*t*-butyl methacrylate) (PtBMA), poly(*t*-butyl acrylate) PtBA and poly(*n*-butyl acrylate) (PnBA) [20,26]. Other important polymers that have been investigated include poly(vinyl acetate) (PVA) [20,27], poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAA) [20,28], polyesters [29-34], poly(lactide) (PLA) [35-38], poly(ethylene oxide) (PEO) [20,39,40], and poly(oxazoline)s [41-44], all of which show remarkable promise in biodegradable and biocompatible materials [21,45-47]. Beyond these examples, many other polymer classes have been investigated under diverse ionization and fragmentation techniques, including poly(ethylenimine)s (PEI) [48,49], poly(dimethylsiloxane)s (PDMS) [20,39], complexes of PMMA with polyglycine [20], polyisocyanates [50], polyisobutylenes [51], and *N*-isopropylacrylamides [52], poly(ethylene/butylene terephthalate)s [29,30], polysulfide [53], polysulfones [54], polyesteramides [55], etc..

The above mentioned studies illustrate that, the differentiation between a pure polymer and its side products is a complex issue. MS/MS for homopolymers can be a very useful method for structural identification of the end groups and the sequence, *i.e.* the detection of defects in the polymer. Additionally MS/MS can also be used for determination of isobaric and isomeric species, and differences within a mixture of linear and nonlinear systems.

However, in such cases, preseparation by hyphenated techniques might be necessary. Despite the diversity of the fragmentation techniques that can be used, MS/MS might not be enough to provide the necessary information to fully distinguish architectural differences. The use of LC as an analytic technique in the liquid phase prior to ionization enables the separation of polymer mixtures due to differences in polarity or hydrophobicity, which can provide complementary information and simplify the MS/MS data obtained from mixtures. Also, ion mobility (IM) spectrometry can provide additionally gas-phase separation of polymers before and/or after fragmentation. The combination of MS/MS with either one of these techniques makes it possible to obtain more detailed information on the nature of the end groups, presence of isomeric architectures, monomer sequences and degree of substitutions. However, these hyphenated techniques are mostly applicable on oligomers with a m/z around 4000; thus, MS hyphenation techniques have to progress towards materials with higher molar masses and high dispersity.

3. First dimension of complexity: linear homopolymers with hyphenated techniques

3.1. LC-MS based techniques

Traditional analytical techniques for polymer characterization are pyrolysis MS, SEC and nuclear magnetic resonance (NMR) spectroscopy. The direct injection of a sample into the mass spectrometer without preseparation, known as direct MS, has been used as an analytical technique for polymers as well, but it has been proven that there are some cases where MS alone is insufficient for a comprehensive characterization of end groups, copolymer composition sequences, etc. Thus techniques involving hyphenation with HPLC either separating by polarity or size and 2D-LC have been developed to provide a more detailed polymer characterization [8]. The hyphenation to MS is a versatile tool and many different types of LC-MS techniques were introduced over the past 20 years making LC-MS

hyphenated methods important in polymer characterization and empowering them for sophisticated polymer analysis [8].

Many different 1D-LC systems hyphenated with MS are reported, particularly, for optimizing the transfer of the sample from a chromatographic system to the mass spectrometer. The main advantage of ESI is its compatibility with continuous hyphenation to diverse HPLC modes in comparison to MALDI, where most of the hyphenation techniques are carried out offline. Different hyphenations and different detectors are used to obtain extra knowledge regarding polymers, such as chemical heterogeneity and differentiation of (isomeric) architectures, thus enabling the chemist to improve the synthetic routes. A solvent free technique with an automated system was established for analyzing PEO under LACCC using a dry spraying technique [56]. PEO mixtures with different molar masses and different end groups were mixed and fractionated by using the LACCC technique prior to analysis of each fraction by solvent-free MALDI-MS. This tool allows an offline hyphenation of MALDI-MS with LACCC with much more convenient sample preparation procedure. Different spotting and simultaneous multisample deposition techniques were used to automate this hyphenation. Three different analytical methods have been combined to analyze PMMA homopolymers having different end groups [57]. LACCC-ESI MS with NMR spectroscopy and titration were used to investigate end group heterogeneity. Quantification of the components in the mixture was completed using an evaporative light scattering detector (ELSD) and identification of the different species was carried out *via* MS. Additionally, NMR spectroscopy was utilized for investigating the composition and titration for the quantification of OH groups. These complementary methods help to validate both structures and composition. The group of Barner-Kowollik hyphenated SEC with ESI-MS to analyze synthetic polymers that showed chromatographic broadening [58]. This hyphenation enables the determination of the structure *via* MS and of the molar masses *via* the refractive indices

(RIs), as shown for PMMA oligomers used for calibration standards. SEC is widely used for molar mass determination of synthesized polymers; in this case band broadening was corrected through an in-house algorithm built for both detectors. This led to several observations: for lower molar masses (<7 kDa), weak band broadening is present; however, with the algorithm, band broadening for 10 kDa PMMA is corrected successfully. The resulting values were also in fair agreement with the manufacturer's value within an error of 15%. In conclusion, the method shows consistency between the two detectors. Since conventional calibrations can be false, multi-detection is critical and important. Furthermore, in a follow-up contribution from the same group, important synthetic facts for tuning a polymerization, such as propagation rate coefficients (k_p), were elucidated through pulsed laser polymerization and further analyzed *via* SEC-ESI MS [59]. This was found to be an accurate way to determine the molar mass of a polymer of any class, provided the polymer is ionizable by ESI. Propagation rates can be measured by such experiments which are important determinants for polymer chain lengths. The reversible addition-fragmentation chain transfer (RAFT) polymerization technique is used widely by many polymer groups in the world due to its facile 'one pot' polymer synthesis ability and the availability of a wide range of monomers that are capable of reacting. A series of acrylates were prepared using different initiators, and the reaction rates of the systems were investigated *via* SEC-ESI MS. The results were also compared with the predictions of the polymer model PREDICI[®] [60]. Furthermore, CAD was used to analyze the intermediate reaction mixtures to obtain information concerning end groups and macromolecular structures. MS is essential for the investigation of polymer propagation and for establishing whether the proposed transformations occur. SEC-ESI MS followed by the application of the PREDICI[®] simulation tool is also used to determine the cross termination reactions in the RAFT polymerization of acrylates [61].

A 2D (RP-LC $\times$ SEC) chromatography system can also be coupled to MALDI and ESI.

Even if this cannot be an online hyphenation, the procedure is still fully automated and very versatile. The RP-LC $\times$ SEC combination alone proves to be one of the most important analytical methods when specific conditions are applied in the first dimension, for example, LACCC. Linear poly(caprolactone) PCL with high and low molar masses were investigated by LACCC to identify end group heterogeneity, and the SEC dimension was used for molar mass separation [62]. As a result of hyphenating this technique to ESI and MALDI, the MS data served as the third dimension. High ionization efficiencies and multiply charged ion distributions were observed in ESI-MS spectra of the investigated PCL samples. Whereas in MALDI, prominently singly charged species, with low abundance, were observed because of the lower ionization efficiency and matrix interference. Pretorius *et al.* conducted a comprehensive RP-LC $\times$ SEC analysis to investigate the relationship between the chemical composition and molecular mass distributions of model phthalic anhydride / propylene glycol polyesters synthesized with a 30% molar excess of glycol [63]. Gradient RP-LC allowed the isolation of homogenous fractions based on their chemical composition, which were then transferred to SEC for separation by hydrodynamic volume (Figure 2). The 2D (RP-LC $\times$ SEC) contour plots show that the samples have different M_n and M_w values: s23 (135, 216), s25 (283, 837), s28 (569, 1520), respectively. The numbers shown are peaks associated with the one-dimensional gradient RP-LC analysis. Furthermore, each fraction was also analyzed with MALDI-ToF MS to elucidate the various stages of the polyesterification reaction in terms of molar mass, chemical composition, and end groups [63]**Error! Reference source not found..**

Figure 2. 2D (RP-LC $\times$ SEC) contour plots of polyester samples: s23, s25, s28. Reproduced from Ref. [63].

Depending on both chemical and physical properties of polymers, the RP-LC $\times$ SEC technique's ability of two-dimensional separation demonstrates its versatility, which makes it a very sophisticated and useful tool for polymer analysis. Therefore, its hyphenation to mass spectrometry can provide many advantages for polymer analysis, most importantly reducing the complexity of analysis of a highly heterogeneous polymer mixture having high dispersity: it will promote technology and insight for chemists to further develop smarter polymer designs.

3.2. Field-Flow Fractionation (FFF)-MS techniques

Field-flow fractionation is a separation technique, used mostly for aqueous solutions of disperse and charged or neutral species, dependent upon differing velocities in a field [64]. FFF is used for synthetic polymers but is less commonly applied in combination with the MS technique. Coupling this technique to a mass spectrometer is challenging due to the high molar masses of the investigated materials. Hassellöv *et al.* reported the analysis of low molar mass poly(styrene sulphonate) (PSS) and PEO standards by FFF-ESI MS [65]. After the low molar mass polymers were separated by FFF, they were analyzed by ESI MS to obtain compositional information. Further studies to obtain end group compositions were carried out by applying MS/MS. However, the technical online hyphenation was also highlighted and high salt concentrations caused clogging of the ESI-MS and high background signals for an efficient separation. Another example for the combination of FFF with MS was the coupling of a thermal field-flow fractionation (ThFFF) setup to MALDI-ToF MS for the analysis of high molar mass PS standards [10]. ThFFF enabled the separation and the fractionation of polymers that were further analyzed by MALDI-ToF MS. After the separation and analysis with both ThFFF and MALDI-ToF MS, it was shown that both techniques have correlating results; in addition, FFF confirmed the macromolecular conformations. The resolution obtained was < 15 kDa for the ThFFF separation and a maximum molar mass of 575 kDa by MALDI-ToF MS. To conclude, FFF is a promising technique for obtaining architectural information such as conformation and size of complex polymeric systems. Furthermore, the FFF-MS hyphenation shows that numerous dimensions expand the comprehensive characterization of polymers. This particular hyphenation needs further improvements and additional applications, specifically for more complex architectures of synthetic polymers where it is essential to characterize the composition and conformation.

3.3. Ion Mobility-Mass Spectrometry (IM-MS) techniques

Previously used for ‘omics’, IM-MS has recently been applied to polymers and metallo(supramolecular) materials [11], and its applications to polymer science continue to increase. IM spectrometry provides an additional dimension for elucidating different conformations or architectures present in an analyte. With IM-MS, gas-phase ions are separated according to their mobility and composition. Information obtained by IM separation can be used to render collision cross-sections, which relate directly to the macromolecular shape.

Isobaric ions, which are two different chemical species having the same nominal mass (mass difference at ppm level) but different elemental composition, are often encountered in polymer analysis and can only be resolved with high resolution spectra. If isobaric species are not resolved, obtaining a more detailed structural characterization with MS/MS experiments can be very difficult. This can result in very complicated MS/MS data, having fragment ions from both species in the same spectrum. Preseparation by a LC technique might be an option for a situation such as this. However, the most important advantage of IM over LC techniques is that the separation occurs within milliseconds in the gas phase, and does not require excessive solvent usage. IM separation is an ideal technique for hyphenation to MS since the separation takes place in the gas phase. Isobaric species can be separated depending on conformational or architectural differences at a much faster rate in the gas phase. After IM separation, these isobaric species can further be investigated separately by MS/MS for more detailed structural characterization, if needed. Furthermore, having higher resolution in IM-MS/MS (for example by employing Q/ToF instrumentation) enables better mass accuracy in MS/MS studies. For example, Hilton *et al.* were able to investigate a poly(ethylene glycol) (PEG)s mixture composed of two PEG chains having different end groups by IM-MS/MS [66]. In this particular example, these two isobaric PEG chains have diol and monooleate end

groups, and result in the same nominal m/z values. However, the structural difference at the end group of these two chains results in two distinct drift time values in the IM spectrum. Separation was therefore accomplished before MS/MS analysis. Figure 3 shows the IM separation of these two chains at an isolated m/z value of 553. Further MS/MS characterization of these separated PEG chains reveals the structural difference at the end groups (Figure 3). One can see that IM-MS could separate polymers having different end groups from both PEGs in the millisecond range when compared to LACCC, which requires a longer time approximately 1 to 2 hours to perform. As a result, IM-MS is highlighted as being fast, comparable in terms of separation to LC-MS and rich in image information, which is beneficial to both academia and industry.

Figure 3. Arrival time distribution (bottom) of m/z 553 from a mixture of PEG 1000 and PEG

monooleate and IM-MS/MS spectra (top) from the two peaks noted. Peaks in the IM-MS/MS spectra are partially annotated. IM-MS/MS spectra are very similar to those noted from MS/MS data (without IM separation) of the same oligomers, obtained from PEG 1000 and PEG monooleate separately. Reproduced from Ref. [66].

Synthetic inorganic polymers, such as polysiloxane and polyphosphazene based polymers, were investigated with ESI-IM-MS by Scionti *et al.* [67]. IM-MS provided information on the existence of higher order assemblies for the polysiloxanes. It also confirmed that the reaction of NH_4Cl with PCl_5 under aerobic conditions produces poly(dichlorophosphazene)s with both tadpole as well as linear architectures.

Waters being the manufacturer of the first commercial IM-MS instruments (Synapt line of models) published a report on the analysis of polymers with IM-MS and MS/MS. Compared to traditional MS techniques, added IM dimension, shown to facilitate the determination of physical properties and sequences [68]. Kim *et al.* applied ESI-IM-MS combined with molecular dynamics (MD) simulations on PLAs having different stereoregularities, formed from cyclic lactide dimers by ring-opening polymerization [69]. The gas-phase conformation of PLAs is affected by stereoregularity and architecture (linear or cyclic), both of which influence collision cross-section value trends. Poly-LD-lactide (PLDLA) has more structural flexibility in comparison to poly-L-lactide (PLLA), thus PLDLA is able to maximize intramolecular interactions, showing lower energies and higher degrees of weak hydrogen bond interactions, which lead to more compact structures for PLDLA with the exception of small macrocycles. These differences in stereoregularity result in different physical properties and structure. IM-MS in conjunction with computational analysis is a powerful tool to differentiate these structural differences. Therefore, it is expected that IM-MS analysis can be a promising technique to characterize polymers with different structures and

512 stereoregularities. Trimpin *et al.* used IM-MS to characterize PEO polymers of relatively high
513 molar masses using ESI as the ion source [70]. Simulated and experimental cross-sections
514 were compared and evaluated. High charge states were found to have an open and dynamic
515 conformation. The intrinsic limitation with ESI is the accessible molar mass range, which is
516 less restricted with MALDI. These two different sources coupled to IM-MS are becoming
517 increasingly more attractive for polymer chemists as a faster way to obtain dense and rich
518 information on polymer architectures. IM-MS has also been used to provide supportive
519 architectural information to ETD fragmentation patterns for linear polyester samples by
520 obtaining collision cross-section values of the major fragment series [36]. Another type of
521 polymer, poly(propylene) (PP), was investigated using pyrolysis with an atmospheric solid
522 analysis probe (ASAP) and IM-MS [71]. Pyrolysis was used to decompose polymers and IM-
523 MS used to determine their m/z values as well as to separate the polymer from any additives.
524 The pyrolysis products were established and compared to prior studies. These two methods
525 coupled to each other help to segregate species and confirm their structure. Barrère *et al.*
526 showed that ASAP-IM-MS can be used to successfully distinguish between polyester and
527 polyethylene blends [72]. ASAP as an ionization source is an effective technique for the
528 characterization of polymer blends with different polarities without the need of complex
529 sample preparation, which is particularly useful for samples that are difficult to dissolve.
530 Therefore, the combination of ASAP and the structural separation provided by IM allows the
531 clear identification of relatively large pyrolysis products from different polymers such as in
532 PLA-polyethylene (PE) blends. Song *et al.* reported a detailed study using ESI-IM-MS on a
533 PMMA homopolymer prepared by radical polymerization [73]. The small end group
534 differences in the resulting PMMA system, could be discriminated by IM without the need of
535 a time consuming LC separation. Finally, Hoskins *et al.* used IM-MS to differentiate between
536 linear and cyclic PCL polymers [74]. The study shows that the IM step is capable of

separating linear and cyclic architectures in a blend since the individual structures result in different drift time values (Figure 4). Figure 4c also displays that ions having same m/z values in mass spectrum were differentiated clearly according to their architectural differences.

Figure 4. Mass spectrum (a) and IM-MS 2-D plot (b) of a PCL blend having both cyclic and linear structures. The intensities of the ions detected are incorporated as a false color plot with red as the most abundant and blue as the least abundant ions. The inset (c) shows the drift time scale of a separated peak at 893.13 m/z . Reproduced from Ref. [74].

All the reported examples show that, as a hyphenated technique, IM-MS can be a very valuable analytical technique for polymer science as it already is for 'omics' fields. Its capability of differentiating architectural differences in the gas phase provides an advantage over other chromatographic techniques and makes it a very promising hyphenated technique for solving current and future analytical problems that can be faced during the characterization of complex polymeric systems.

3.4. Computational methods

Computational methods are becoming more prominent in facilitating the analysis of MS and MS/MS spectra. For example, different poly(hydroxyethylmethacrylate) (PHEMA) backbone structures were analyzed *via* ESI by Jackson *et al.* The data analysis was done by the Polymerator software (Figure 5 (a)) [75]. End group analysis was performed, and different fragmentation series were obtained and analyzed with the Polymerator software to identify polymer fragments (Figure 5 (b)). The Polymerator enabled an easier and faster interpretation of the MS/MS data. Moreover, the information obtained can also support the formation of future libraries of fragments.

Figure 5. (a) Screenshot from Polymerator software of annotated ESI-MS/MS spectrum from the sodiated tetramer of PHEMA. (b) The details of the annotated fragment ions are displayed by the Polymerator software in the table. Reproduced from Ref. [75].

Another advantage of the Polymerator software is that it is dependent on the polymer class and the knowledge of individual fragments. This feature makes it possible to apply it to

different polymers to allow the validation of manual and computed fragmentations [76]. Jackson *et al.* also used this software to distinguish between end group cleavage and the fragmentation of the polymer chain, and performed detailed end group analysis of poly(propylene glycol) (PPG) by ESI with low energy MS/MS [77]. Williams *et al.* used the Polymerator software to analyze the CAD spectra of PEO ions having different end groups and different cationization agents, formed by desorption electrospray ionization (DESI) [78]. The authors emphasize the ability to determine microstructures for ‘polymers’. However, prior knowledge of the fragmentation mechanisms is a requirement for utilizing the software. On the other hand, software based analysis can benefit from polymer libraries and is quicker than manual interpretation. In addition to the Polymerator, the polymer labeling using mass spectrometry (PLUMS) software is another computational method developed for the interpretation of fragmentation spectra [79]. This software was used to determine the fragment series formed, knowing that no prior knowledge on the chemical behavior of the polymer class was required. Using PLUMS, practically all of the possible fragments were determined with an attached probability, in contrast to the Polymerator, where chemical parameters are pre-identified.

In conclusion, all discussed studies represent an immense step forward in polymer science in view of a detailed characterization of polymer architecture and conformation. The growth of these techniques will help chemists to evaluate complex polymers in a more in-depth manner by making them less time-consuming.

4. Second dimension of complexity: linear copolymers

4.1. Block copolymers

Block copolymers of linear architecture are composed of at least two segments/domains bound together. Block copolymers may undergo microphase separation due to differences in the solubility of the different segments in a specific solvent. This results in different morphologies in solution, for example, the formation of micellar structures. Furthermore, the morphologies of the formed nanostructures can be tuned *via* the relative length of the individual blocks. Such types of polymers are widely used in the fields of drug delivery, thin films, membranes etc. [1,80]. The use of MS and specific hyphenations allow the determination of block lengths and sequences, as well as the differentiation between different architectures/microstructures [81].

4.1.1. Direct MS techniques

Direct MS analysis of polymers helps to elucidate side products, end groups and the degree of polymerization of each block. For example, Houshia *et al.* analyzed high molar mass block copolymers comprising of EO and PO segments *via* MALDI-FTMS [82]. The authors use correlation functions and probability functions to evaluate diverse copolymer components overlapping isotopic patterns, which is required for polymers having high molar masses where the loss of resolution is predominant and the certitude of copolymer composition becomes weak.

MS analyses are generally more complex for linear copolymers than for homopolymers because the ionization efficiency can significantly differ as a result of the different lengths and co-monomers structures in each block. Nonetheless, direct MS can provide elements of a comprehensive characterization of copolymers, though additional methods such as thermal-MS, MS/MS and hyphenated techniques are needed for further elucidation of the architecture.

618

619 4.1.2. Thermal-MS based techniques

620 Similarly to homopolymers, thermal degradation techniques are useful for accomplishing
621 detailed evaluations of block copolymer structures. For example, Zhang *et al.* used thermal-
622 assisted atmospheric pressure glow discharge mass spectrometry (TA-APGDMS) to evaluate
623 block copolymer fragments [14]. The block copolymers were based on POM and
624 poly(butadiene succinate) (PBS) segments. Firstly, fragmentation pathways were established
625 for standard homopolymers, and later for the corresponding copolymers. Characteristic
626 degradation patterns from the POM homopolymers were also observed in the POM-
627 containing copolymers; however, this did not hold for PBS homopolymers and its block
628 copolymers. This study proved that thermal degradation is applicable to copolymers and helps
629 to elucidate their structure and chemical composition. PEO-*b*-(PPO)-*b*-PEO triblock
630 copolymers were also studied using thermal degradation [83]. SEC, NMR spectroscopy and
631 MALDI-MS were used to characterize poloxamer 407. The products of heat induced
632 degradation were analyzed by MALDI-MS and by solid phase microextraction/gas
633 chromatography (SPME/GC) MS. These complementary methods are essential in revealing
634 the oxidation mechanism of poloxamers and their structures. Ohran *et al.* have investigated
635 three kinds of block copolymers: PS-*b*-P2VP, P2VP-*b*-PMMA and poly(*isoprene*) (PI)-*b*-
636 P2VP *via* direct pyrolysis-MS [84]. This method allows the evaluation of backbone cleavages
637 and, as a consequence, the determination of the sequence and possible crosslinks formed.

638 Lattimer *et al.* investigated the pyrolysis products of segmented polyurethanes with
639 MALDI-MS after thermal degradation. In this study, different temperatures were used during
640 pyrolysis to investigate thermal degradation mechanisms by analyzing the pyrolysis products
641 (up to 10000 Da) with MALDI-MS [85]. Whitson *et al.* characterized commercial
642 polyurethanes by atmospheric pressure chemical ionization (APCI)-MS in combination with a

direct insertion probe (DP). By slowly increasing the temperature, the thermal degradation products and the other components in the blend, such as additives were successfully separated. The temporal separation that took place in this method was based on compound volatilities and bond stabilities. As a result, combining thermal method by using DP allowed these insoluble materials to be directly analyzed by MS [86]. Another application of this method was done on cross-linked amphiphilic conetworks composed by graft copoly(*N,N*-dimethyl acrylamide-*g*-dimethylsiloxane) (PDMAAm-*g*-PDMS) and their blends [87]. The different compositions of hydrophobic and hydrophilic components in these graft copolymer blends were distinguished by utilizing DP-APCI-MS. In addition to different comonomer compositions, information on the thermal stability of the different domains within the copolymer was obtained with the help of precise pyrolysis temperature control by DP [87].

In accordance with the studies discussed above, thermal-MS techniques have great potential for providing important information on structural (block lengths, defects in sequences) and physical properties such as thermal degradation characteristics. Additionally, utilizing a thermal based technique can be essential for MS characterization of polymers that are insoluble (for ESI) or too large to be desorbed (for solvent-free MALDI), and unable to form gas-phase ions due to the lack of functional groups to attract and bind a charged particle, such as a proton or metal cation.

4.1.3. MS/MS techniques

MS/MS techniques have proven to be very useful for the investigation of sequences and block lengths of different copolymer structures. Cerda *et al.* subjected [PPG-*b*-PEG]²⁺ to both ECD and CAD analysis, which allows for detailed structural characterization of this copolymer [40]. The product ions generated from the ECD experiments showed that all of the copolymers studied consist of diblock structures, and not triblock structures that are

designated by the polymer manufacturer. Furthermore, Baumgaertel *et al.* analyzed different poly(2-alkyl-2-oxazoline) block copolymers (variation of the alkyl side group), *via* ESI- and MALDI-MS using CAD for fragmentation [88]. The usage of both ionization methods revealed detailed information regarding the side products, monomer sequence and block length. The fragmentation patterns of block copolymers in such studies are mostly derived by using prior knowledge of the behavior of the analogous homopolymers under the same MS/MS conditions. Crecelius *et al.* studied *m*PEG-*b*-PS block copolymers prepared *via* atom transfer radical polymerization (ATRP) [89]. MALDI-ToF MS/MS was used for detailed structural characterization. For this reason, the homopolymers of each comonomer were investigated separately prior to the MS/MS analysis of the block copolymers to facilitate the interpretation of the fragmentation results. Detailed structural analysis by MALDI-MS/MS on these copolymer revealed accurate block lengths of PS and *m*PEG. The authors claimed to observe only fragments from both blocks individually, thus, concluding that a scission between the blocks takes place. The main fragmentation mechanisms observed were 1,4-hydrogen elimination and McLafferty rearrangement within the PEG chain.

In addition to CAD, different ion activation methods, such as ECD, can also provide useful MS/MS data for architectural analysis of copolymers. One of the earlier examples is the application of both CAD and ECD for sequence analysis of PEG-*b*-PPG-*b*-PEG copolymer mixtures. According to the results presented by Cerda *et al.*, CAD can lead to misleading rearrangements, however, as mentioned before, ECD causes minimal rearrangements thus preventing internal fragment formation [90]. Therefore, extensive structural details such as sequence information on complex mixtures of low abundance block copolymers can be obtained by using ECD as an ion activation method for MS/MS. Another study that involves the comparison between CAD and ECD fragmentation patterns was performed on random and block polyacrylate and polyether copolymers [91]. The study shows that CAD alone is able to

differentiate random and block copolymers. Random poly(methyl methacrylate)-*r*-poly(*n*-butyl methacrylate) (PMMA-*r*-P*n*BMA) oligomers tend to undergo random losses of MMA or BMA monomeric units, but block poly(methyl methacrylate)-*b*-poly(*n*-butyl methacrylate) (PMMA-*b*-P*n*BMA) oligomers lose BMA monomeric units preferentially [91].

Based on the examples portrayed, MS/MS provides essential details on structural properties of copolymeric systems such as comonomer sequences, microstructural differences, and block lengths. Accurate determination of such properties of copolymers is essential for the assessment of material's quality.

4.1.4. LC-MS and computational based techniques

As described previously, copolymers are rather heterogeneous and disperse systems. For copolymer structures of high complexity, analysis on additional dimensions may be essential for detailed characterization. Direct MS characterization is not always sufficient to elucidate the architecture of block copolymers. As a consequence, hyphenated analytical techniques have been employed to facilitate the analysis of such polymer systems.

The following selected examples highlight the successful characterization of block copolymers using 1D-LC-MS. Pyrolysis-GC-MS hyphenated to either a gradient reversed-phase (RP) LC or a SEC instrument were used in a study by Kaal *et al.* on PEG-*b*-PPO copolymers [92]. While SEC or RP-LC separated the copolymers according to their hydrodynamic volume or polarity, respectively, the quantitative information for different compositions was obtained by pyrolysis-GC-MS. Furthermore, this method also permits the calculation of monomer feed ratios, which is important for a validation of the composition, as well. Leeuwen *et al.* analyzed block copolymers composed of *m*PEO and PCL blocks *via* ESI-MS and APCI MS combined with RP-LC fractionation [93]. Mass spectrometric analysis was performed both in the positive and negative mode. Further, gradient elution was applied for

low molar mass copolymers and used to determine the block lengths as well as the comonomer composition. *m*PEO-*b*-PCL was investigated under APCI ionization conditions as a complementary method to ESI and for hyphenation to a HPLC system in order to evaluate the block length of both species present in this biocompatible polymer that can form micellar structures. Hayen *et al.* applied RP-LC-ESI MS to poly(ether-*b*-ester) block copolymers, *i.e.* PEO-*b*-poly(butylene terephthalate) (PBT) [94]. Application of RP-LC prior to MS analysis provided a separation of the side products having different segments, but similar molar masses. Girod *et al.* analyzed PEO-*b*-PS block copolymers by hyphenating LACCC to ESI-MS/MS [95]. The LACCC conditions were optimized by tuning the salt concentration in the mobile phase for better separation of the species with different end groups present in the sample, independent of their molar mass. Additionally, the length of the PS block was analyzed and also confirmed by CAD MS/MS. As a result, combination of these complementary methods allowed both the determination of the PS block length and end group analysis. A better understanding of RAFT polymerization of poly(*N*-vinyl pyrrolidone) (PVP) and poly(vinyl acetate) (PVAc) block copolymers (PVP-*b*-PVAc) and its byproducts was provided by Fandrich *et al.* who used gradient LC-MALDI and LC-Fourier transform-infrared (FT-IR) spectroscopy [96]. Hyphenation of various LC techniques to MALDI-MS and FT-IR made it possible to differentiate the main products from the by-products. Additionally, the results obtained throughout the study essentially helped to derive the possible changes in the reaction mechanism that could cause formation of copolymer blends containing long blocks of either PVP or PVAc.

Separation of compounds in complex mixtures can also be carried out by utilizing two different LC methods in combination to obtain two dimensional separation (2D-LC). 2D-LC is a time-consuming technique; however, it is a very informative method and can be effectively used for copolymer analysis. Baumgaertel *et al.* used 2D (LACCC $\times$ SEC) in

combination with MALDI- and ESI-MS/MS for the analysis of poly(2-ethyl-2-oxazoline-*b*-2-(2,6-difluorophenyl)-2-oxazoline) (p(EtOx-*b*-*o*DFOx)) block copolymers, which have potential medicinal applications. In this study, the application of LACCC allowed separation of the polymers containing different end groups with varying polarity as well as the series with different block lengths. Further analysis of the LC fractions with MALDI-MS and ESI-MS/MS allowed full structural characterization of PEtOx chain transfer and termination products. Finally, quantification of the separated components were carried out by addition of SEC dimension. The 2D contour plot in Figure 6 shows three fractions: fraction 1 corresponds to the PEtOx homopolymer with an ester end group (7.5 vol.%), fraction 2 corresponds to the copolymer (p(EtOx-*b*-*o*DFOx)) (87 vol.%) and fraction 3 corresponds to the PEtOx homopolymer with a hydroxyl end group (4.5 vol.%) [97]. As a result, combination of the essential information obtained from all three methods was used to ensure that all products are comprehensively characterized.

Figure 6. 2D contour plot of the (p(EtOx-*b*-*o*DFOx)) copolymer: 1st dimension LACCC for PEtOx (eluent: 2-propanol/H₂O = 91/9 (v/v)); 2nd dimension SEC (eluent: 0.07% triethylamine in THF). Reproduced from Ref. [97].

761

762 Other studies from the Weidner group involved imaging of similar copolymer
763 architectures by having specific RP-LC conditions hyphenated to a MALDI target *via* a spray
764 to separate and identify different compositions within one copolymer [98]. Weidner *et al.*
765 introduced this technique to study copolymers comprised of propylene oxide (PO) and
766 ethylene oxide (EO) blocks, which contained isobaric species or species with very similar
767 masses (within 2 Da). The separation of components differ in PPO and PEO content by
768 LACCC could differentiate compositions from a sample that has overlapping m/z values,
769 hence composition specific calibration curves were constructed based on the number of PO
770 and EO comonomers. Subsequently, more complex structures could be thoroughly separated,
771 and characterized, thereby setting the foundation for the next step: sequencing of copolymer
772 structures present in the blend.

773 Barqawi *et al.* used an automated system to deposit fractions of a triblock copolymers, that
774 were separated in 2D LC/SEC run, onto a MALDI target [99]. Firstly, the critical conditions
775 of poly(*isobutylene*) (PIB) homopolymers were identified, which was used as the first RP-LC
776 dimension for the identification of the end group heterogeneity. In the second dimension, a
777 SEC column was utilized to determine the molar mass of the polymer and to quantify the
778 products as well as the undesired side products. The 2D-LC eluents were mixed with the salt
779 and the matrix solutions and then sprayed onto a MALDI target with the help of an automated
780 transfer module. This whole automated system allowed a continuous MS data collection at the
781 various 2D-LC elution volumes, which essentially yielded numerous time-dependent spectra.
782 This system is a major achievement in automation and characterization of complex polymer
783 samples due to the fact that the first two dimensions are hyphenated to a MALDI-MS. It also
784 allows a facile way to interpret spectra due to less overlapping peaks, an estimation of
785 composition, chain length, and architecture of symmetrical and non-symmetrical triblock

786 copolymers. In a different offline technique 2D-LC was combined with MALDI-MS to
787 analyze EO based block copolymers [100]. LACCC was used as the first dimension to
788 separate polymers bearing different functionalities. The second dimension was either a SEC or
789 a LAC system which gave evidence for the molar mass of all products present with varying
790 functionality. The setup was hyphenated to MALDI-MS in an offline manner. Raw copolymer
791 samples are very complex to analyze due to high heterogeneity, however, advanced 2D
792 fractionation prior to MS analysis makes the characterization of architectural details possible.
793 It should also be considered that, this is a time-consuming method since optimization of
794 numerous factors (solvent, column, temperature etc.) for separation is needed.

795
796 Over the years, computational methods have grown and make the analysis of polymeric
797 materials faster and more straightforward. Weidner *et al.* have extensively studied PEO-*b*-
798 PPO-type block copolymers [101]. They have developed a software-based chromatographic
799 separation of block copolymers that are subsequently analyzed *via* MALDI-ToF MS. MALDI
800 mass spectra of complex copolymeric systems can result in overlapping isotope patterns of
801 ions comprising different monomeric compositions in the mass spectra. Therefore,
802 preseparation of these species prior to MS data collection become essential for more accurate
803 determination of monomer composition. In this case PEO-*b*-PPO-*b*-PEO was investigated
804 thoroughly, by first establishing the critical conditions of PPO which was followed by
805 separation of the components with LAC according to their monomer compositions. All of the
806 fractions obtained from the separation were then sprayed onto a MALDI target for MS
807 analysis of each component. These MS data were then processed by the MassChrom2D
808 algorithm and provided the number of EO and PO units present in each specific fraction. In
809 addition, the intensity obtained from chromatographic data was inserted in the 2D
810 composition plots formed by the MALDI spectra. The entire workflow of the protocol is

summarized in Figure 7. This method is a major step forward in quantifying and identifying the copolymers with different monomeric compositions present in complex mixtures.

Figure 7. Scheme of measurement procedure using MassChrom2D to combine chromatography MALDI data for determining the copolymer composition. Modified from Ref. [101].

Huijser *et al.* developed a software to establish the composition and topology of different linear chains of poly(lactide-*co*-glycolide) (PLLGA) which is used in drug delivery implants due to its advantageous properties as biodegradable and biocompatible materials [102]. The contour maps generated from the MALDI-MS analysis by using an in house developed software. The results show a significant difference in average composition between random and block copolymer chains. In the case of PLLGA, the line with a constant slope crossing the average composition do not pass through the origin, underlining a block-like structure, in contrast to PDLLGA which reveals a random like copolymer (Figure 8). Such analytical results are very useful for synthetic chemists, as they indicate an influence in the incorporation rate of a monomer between D-lactide and L-lactide. As it is shown in this study, 1-D MS can also have potential for the detailed analysis of polymer architectures with the help of a powerful data interpretation software.

Figure 8. Contour plots of a) PDLLGA and b) PLLGA for cyclic structures plotted with lactic acid units. Reproduced from Ref. [102].

In a contribution by Vivo-Truyols *et al.*, a PS-*b*-PI block copolymer was analyzed by SEC and, subsequently, MALDI-ToF MS [103]. The overall architecture was then determined from the combined analysis of each fractions. This approach can only be used for non-overlapping isotopic patterns, otherwise regression is used to deconvolute the overlap. The limitation of this method is that the whole spectrum cannot be fitted, and regression can only be applied to the center, which is not representative for the total polymer composition. Complications in both mass resolution and processing power of the computer have also to be taken into account. Nonetheless, this method can produce high-throughput data, offering a fast analysis and yielding kinetic information on copolymers – satisfying both analysts and synthetic chemists. In a related study, Willemse *et al.* portrayed the analysis and microstructure of PS-*b*-PI block copolymers [104]. The authors showed that MALDI-ToF MS can provide information on parameters such as chain length, composition, block properties or microstructures. It was also shown that overlapping peaks in a copolymer distribution can be

interpreted by using NMR as a feedback method if the integrals do not overlap excessively, and thus are not affecting the evaluation of the average composition. In addition, contour plots were produced revealing the number of monomer units of each monomer and their microstructures. Wilczek-Vera *et al.* analyzed PS-*b*-*am*PS by MALDI-MS and ¹H NMR spectroscopy, where both techniques were required for determination of the block lengths and chemical composition [105]. The Schulz-Zimm model correlates both experimental and theoretical distributions, and can also be applied to triblock copolymers. The model is a rapid method to obtain a complete composition of the copolymer. It was pointed out that anionic ring-opening polymerization (AROP) produces polymers with narrow Đ values. However, it should be noted that the narrowness does not mean that the copolymer is structurally less complex.

Block copolymers are very important in polymer science and have found many applications in diverse fields. Different block copolymer materials have successfully characterized by utilizing various analytical techniques (thermal techniques or LC methods) in combination with MS. Additionally, the urge to have more automated systems to produce data in a faster manner and understand block copolymer structures and architectures, has brought advanced informatics into polymer science.

4.2. Statistical copolymers

Statistical copolymers are composed of monomers, which form a sequence according to a statistical rule. Many copolymers form statistical sequences, such as poly(2-phenyl-2-oxazoline)-*stat*-(2(dec-9-enyl-2-oxazoline)). These are cationic polymers and due to their importance in biology and medicine, different analytical methods have been utilized in combination for their detailed characterization. The first example where statistical polymers

were studied using a 2D setup was reported by the Schoemakers group, who analyzed block and statistical copolymers *via* gradient elution liquid chromatography (GELC), Pyrolysis-GC MS, SEC and capillary electrophoresis-ultraviolet (CE-UV) [106]. All four of these separation techniques provided different complementary information, none of which were conclusive on their own, thus, showing that hyphenated techniques can be essential to characterize complex statistical copolymers. First, Py-GC–MS was used to determine the average chemical composition. Subsequently, GELC was applied to further study the chemical-composition distribution. Offline GELC of these statistical copolymers showed two separated fractions, which were both bimodal in nature. An addition of a SEC dimension revealed that separation of these fractions in GELC dimension was not based on differences in the molar mass (Figure 9). This was rationalized by the presence of an ionic fraction in the samples of statistical copolymers, resulting from either chain-transfer reactions or termination by addition of water. Confirmation of this rationale was obtained by further analysis with CE and the results revealed that cationically charged polymers were predominant with only a minority being neutral. All of these analyses show that the statistical copolymers are not monomodal in comparison to block copolymers, but had different end groups and probably a different chemical composition per polymer chain.

Figure 9. Off-line comprehensive 2D (GELC)/ $\times$ /SEC chromatograms of (polyPhOx₂₀-*stat*-DecEnOx₄₀) (A) and polyPhOx₄₀-*stat*-DecEnOx₂₀ (B). Reproduced from Ref. [106].

4.3. Random copolymers

Random copolymers are quite challenging to analyze – in particular when aiming for a sequence determination [81]. An example of MS/MS analysis of random copolymers was recently reported which involved MALDI-ToF-MS/MS on samples containing styrene and dimethylsilylstyrene (DMSS) repeating units [107]. MS/MS provided detailed structural information and sequence coverage by revealing comonomer localization along the copolymer chains. A polystyrene oligomer end-capped by a block DMSS was easily differentiated from the random samples by isolating and fragmenting individual lithiated oligomers. From the MS/MS data, it was found that the localization of the DMSS block differs depending on whether *p*-DMSS or *m*-DMSS is used during the synthesis. Sequence information could be obtained by following the mass shifts occurring on the different fragment series containing either the terminating or the initiating end of the precursor oligomer ion that was being fragmented.

Huijser *et al.* used MALDI-ToF MS spectra to determine the reactivity ratios of the employed monomers, even with a long chain length model, for random, gradient, alternating and block-like copolymers [108]. This study represents a way to establish kinetic analysis by simply acquiring mass spectra. It was shown that, if analyzed and used properly, a MALDI-MS method can reveal many details such as: reactivity ratios, composition, length of all chains and microstructures. Additionally, ESI-MS also shows a great promise for characterization of such complex copolymer mixtures with the addition of an IM dimension. Different blends of homopolymers and random copolymers have been studied by ESI-IM-MS [109]. Even from samples containing relatively low molar mass analytes, ESI produces ions in

many different charge states readily, making the spectra harder to analyze without the IM dimension. Furthermore, folding transitions of the polymer chain can also be detected in the IM-MS spectra of mobility separated charge states. One aspect to highlight is the investigation of random copolymers PEO-*r*-PPG and PEO-*r*-PPO and their blends. This demonstrates that ESI-IM-MS can be a cost effective analytical method where the component in a complex mixture can be separated and investigated according to their architectural differences. In addition, Engler *et al.* used copolymer composition numbering tool (COCONUT), a computational method to establish the compositional distribution from MS spectra and the microstructures of random/block copolymers of PS-*co*-PI via AROP [110]. Isobaric species and overlapping isotopes could also be resolved using a method based on linear programming. Nonetheless, this method is only semi-quantitative, which is a remaining challenge in polymer science and mass spectrometry.

Overall, different hyphenated analytical techniques, combined with computer-based data processing methods, provides an effective tool to determine the lengths of the blocks and distinguishing between different architectures and sequences of block, random or statistical copolymer chains. The goal of these techniques and hyphenations is to aid chemists in tuning their polymers for their industrial and/or biological applications, as well as understanding and optimizing their chemical reactions.

5. Third dimension of complexity: complex polymers

With increasing demand on advanced polymer architectures for diverse applications, polymers are becoming more and more complex in their architecture, and at the same time, more challenging to characterize. Star-shaped, graft 'like' and branched polymers in particular are in the focus of current research efforts. Even these non-linear architectures are analyzed by many of the established methods presented above.

943

944 **5.1. Graft ‘like’ polymers**

945 So far, only a few graft copolymers have jointly been investigated by MS and hyphenated
946 techniques. One such example was PEO-*g*-poly(vinyl alcohol) (PEO-*g*-PVAL), which was
947 used for instant-release tablet coatings due to its mechanical properties [111]. This particular
948 graft copolymer was analyzed by 2D (LACCC $\times$ SEC) experiments, which showed that no
949 free PEO was left after the radical polymerization. In addition, MALDI-ToF MS experiments
950 were performed and confirmed this finding. Both complementary methods yielded the same
951 basic results and furthermore, 2D-LC allowed the quantification of the degree of grafting.
952 Comb-like polymers are of wide interest due to their unique architecture, however, they have
953 not been analyzed widely either by MS or liquid chromatography. As an example, Adler *et al.*
954 showed that such copolymers based on hydrophilic PEO and PMAA units, could be
955 characterized *via* hyphenated techniques to MS [112]. Firstly, the LACCC conditions for
956 PEOs were established in order to identify the presence of possible different end groups,
957 followed by SEC for the molar mass measurement of PEO-*comb*-PMAA. The presence of the
958 PEO macromonomer was confirmed by this method, as complemented by FT-IR. To conclude
959 this analysis of hydrophilic copolymers, the authors showed that hyphenation and
960 complementary methods are of necessity to evaluate their architecture of polymers, thereby
961 quantifying the degree of grafting and, in addition, determining the monomer feed.

962 Graft ‘like’ copolymers have, to the best of our knowledge, hardly been analyzed *via* MS,
963 nonetheless, 2D chromatography has been performed fairly often. In this respect, the 2D-LC
964 based technique is very powerful for polymer analysis, especially with LACCC for one of the
965 employed monomers. Moreover, the offline analysis by MALDI-ToF MS allows an additional
966 dimension and, thus, provides further characteristics of the observed architectures. Both 2D-
967 LC and MS as hyphenated methods are versatile tools for the architecture characterization of

graft ‘like’ polymers. The major drawbacks of a 2D chromatographic system are its expense, time-requirement, and the lack of appropriate standards, leading to erroneous results.

5.2. Cyclic polymers

Cyclic polymers have very different physical properties when compared to their linear homologues and are applied in a wide range of fields, for example, commercial polycarbonates and polyesters for the perfume industry. In contrast to linear species, no chain ends are present as a result from ring closure. Many groups have studied the difference between linear and cyclic polymers using several different ionization methods, hyphenations and complementary methods to obtain as much knowledge and architectural information as possible. For example, Yol *et al.* have compared both linear and cyclic homopolymers of PS and poly(butadiene) (PB), which showed significant differences in their MALDI MS/MS spectra [113]. In particular, in the case of linear polymers, a noticeable “depolymerization” was observed, and low mass radical ions were predominant; in comparison, cyclic polymers of similar molar masses gave fragments of higher molar mass with higher relative abundance. In either case, MALDI-ToF MS/MS can determine the end group (for linear polymers) or the linker (for cyclic polymers) used, and is applicable to polymers prepared *via* different polymerization techniques. Maslinska-Solich *et al.*, was able to differentiate linear and macrocyclic oxazolidine-based polymers just by analyzing their MS spectra, and assigning specific peaks to polymer chains with and without end groups [114,115]. Wachsen *et al.*, used three complementary methods to compare linear and cyclic polymers [116]. PLA was used due to its importance as a synthetic polymer, since it is extensively used in biocompatible and biodegradable materials. SEC, LACCC, and MALDI-MS were used to perform a detailed characterization of PLAs. These complementary methods were essential to either determine the structure or separate the different architectures formed during various polymerization

techniques. LACCC is a very beneficial technique, however, it is very time consuming due to the many parameters that need to be optimized. Nevertheless, it allows the differentiation between the components with different end groups independently from their molar mass. Osaka *et al.* studied PLA by ESI- and MALDI-MS where differences between linear and cyclic architectures becomes apparent [37]. Different fragment ion series were observed in the ESI-MS/MS spectra: three series for the linear and one for the cyclic polymers. Compared to ESI-MS/MS results, MALDI-MS/MS spectra showed the same fragment series for cyclic architectures but differed for linear architectures (only one fragment series was formed). A less common technique was used by McDonnell *et al.* for the same purpose: sustained off resonance irradiation (SORI)-CAD-FT-ion cyclotron resonance (ICR). In contrast to the previous approaches, this method has very high mass resolution [117]. This allowed to carry out high accuracy mass measurements of the fragment ions which essentially helped to derive different fragmentation mechanisms observed for both linear and cyclic architectures.

Although MS techniques are widely used for the analysis of linear and cyclic polymers, quantification of cyclic and linear chains in an analyte mixture has hardly been addressed by using a hyphenated method. As an exception, Wang *et al.* applied surface layer (SL)-MALDI-ToF MS to quantify and determine different architectures (linear and cyclic) in PS films that were spin cast from blends [118]. Since the linear and cyclic polymers in the blend differ in mass, no MS/MS was required, but its use with either ESI- or MALDI-MS would make it possible to differentiate architectures and/or sequences that have the same mass. MALDI- and ESI-MS can cause chemical changes to labile polymers, leading to erroneous data when using an inappropriate solvent, salt or matrix is used. Such changes can be detected using IM-MS where charge states and/or overlapping components with different architecture can be deconvoluted [119].

In conclusion, 2D chromatography methods, MS, MS/MS and/or IM-MS have been used to characterize cyclic synthetic (co)polymers. Direct ESI- and MALDI-MS can be enough to differentiate between linear and cyclic oligomers, if there is high enough resolution. However, this is difficult to achieve for high molar masses. CAD is also used for this specific differentiation, however, the poor fragmentation efficiency at higher m/z values, typically limits its application to lower molecular weight polymers. IM-MS, on the contrary, is a much faster technique that can be used to differentiate linear and cyclic polymers.

5.3. Star-shaped polymers

Star-shaped polymers are synthesized *via* different techniques (for example “core-first”, “arm-first” or “graft-onto” methodologies) and are attractive materials for diverse applications, such as drug delivery or material science. However, their characterization is not facile due to their chemical structure. Several analytical techniques have been used to establish a full characterization of star-shaped polymers.

The RAFT polymerization technique is one of the commonly used methods for the preparation of star-shaped polymers [61,120,121]. Star-shaped polymers of *para*-acetoxystyrene and its by-products obtained during RAFT polymerization was analyzed by Chaffey-Millar *et al.* with MS techniques [120]. In this example, ESI-MS proved to be a powerful tool to differentiate between “star-star couples”, terminated with initiator fragments and linear polymers. In addition to ESI-MS analysis, a kinetic model for the polymerization had to be utilized to aid the interpretation of the MS data. Based on these facts, one can conclude that complementary methods are vital to explain experimental observations.

A number of other polymerization techniques are also available for the synthesis of star-shaped copolymers, depending on the employed monomer class and the (targeted) molar

mass. PB-based star-shaped polymers were synthesized by Allgaier *et al.* and the products were analyzed with MALDI-ToF MS [122]. The investigation was limited to low molar masses, however, it was still sufficient to confirm the star-shaped architecture of the polymers. The results were extrapolated to high molar masses through the use of a theoretical modeling. A four-arm PEO-based star-shaped polymer was obtained from AROP and subsequently transformed into an eight-shaped polymer *via* end group modification and two-fold intramolecular ring closure [123]. The final products as well as all intermediates are unambiguously identified by MALDI-MS. IM-MS was also used to examine the conformations of linear PCLs and star shaped PCLs having different topologies [124]. By using theoretical molecular dynamics calculations, two major conformations were found for such polymers (near-spherical and elongated) depending on the charge state and the length of the polymer. Furthermore, these findings were supported by CAD experiments. In light of the results that were obtained by both experiments and simulations, it was concluded that the final conformations depend on the degree of polymerization, charge state, and the branching ratio of the polymer.

Star-shaped block copolymers can also be prepared through the coupling to a core, and are of great interest for drug delivery applications. Li *et al.* showed that characterization of such star-shaped polymers is difficult [125]. Star-shaped PCL-*b*-PEGs were characterized *via* SEC and MALDI-ToF MS, where MALDI analyses provided information on both the molar masses and the structural identity. The SEC analyses provided additional confirmation and complementary information on the molar masses. One has to be cautious when a star is composed of two different monomers due to differences in the ionization behavior. In this case, a study with a 2D (LACCC $\times$ SEC) system would be appealing, taking the critical conditions of both homopolymers (PEG and PCL) into account to ensure that the star is not missing an arm. Furthermore, Rudolph *et al.* showed that MALDI, static light scattering

(SLS) and 2D-LC are essential to obtain absolute molar masses, and to confirm the architecture of their star-shaped [PEO₂₈-*b*-PEtOx]₈, which was synthesized by the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC). These techniques are necessary considering that NMR spectroscopy and SEC are not satisfactory to fully characterize these systems [126].

Another type of architecture, branched-based star polymers, was investigated by Yu *et al.* by MALDI-MS, even at high molar masses [127]. The monomer employed here was EO, which – due to its neutral properties and high ionization efficiency – enables an investigation of polymers even at high molar masses and non-linear architectures. Here, MALDI-MS proves to be a powerful tool to identify stars with different numbers of arms.

Despite the successful characterizations of star-shaped polymer systems in the above examples, analysis of such materials still remains complex in nature. From the diverse examples portrayed, it is clear that star-shaped polymers and their possible side products *e.g.* star-star couples, are distinguishable by MS only. However, IM-MS and 2D-LC can provide additional information to differentiate topologies, and moreover this can be supported by MS/MS or other complementary techniques such as LS or small angle X-ray scattering (SAXS), to determine branching ratios, charge states and the degree of polymerization. The analytical potential of IM-MS is increasingly being appreciated and its speed in analysis is being remarkably acknowledged. Although some methods are still more suitable for high molar masses *e.g.* LS, asymmetric flow field-flow fractionation (AF4) and analytical ultracentrifuge AUC. It is envisaged that complementary techniques such as IM-MS, 2D-LC, LS, AF4 should further support the characterization of these polymer systems.

5.4. Branched polymers

The analysis of dendritic materials with MALDI-ToF MS has been carried out utilizing different matrices, doping agents, solvents, delayed extractions and polymer concentrations. Many examples based on different types of polymers and different numbers of dendritic generations as well as branched or hyperbranched polymers have been characterized *via* direct MALDI MS and also by a range of hyphenated techniques.

5.4.1. Amidoamines and imines

Numerous methods are used to analyze and evaluate different architectures of such compounds. FT-ICR MS was utilized to characterize poly(amidoamine) (PAMAM) based dendrimers – their trade name is Starburst – which exhibit good biocompatibility [128]. High-resolution spectra can be used to determine the *D* values of dendrimers, at least for those of low molar masses. Moreover, this specific instrumentation offers resolving power for high molar masses even of rather disperse samples. MS/MS is useful in this particular case to determine the faults introduced in the synthetic route and to identify the detailed architecture by the specific fragmentation reactions of defect and non-defect dendrimers. This can be done using fragments resulting from competitive *retro*-Michael additions on the PAMAM: from multiply charged dendrimer ions, two major types of fragments are formed during MS/MS experiments due to the separation of charges [129]. Amide-based dendrimers have been used widely in studies designed to fully understand the ECD mechanism [130]. Unexpected results were discovered such as *b'*/*y* cleavages (cleavage after C=O towards the outer layer), *S*/*E* dissociations (direction of the cleavage start, end respectively) and minor *c*/*z'* (cleavage after N-H towards the outer layer) fragmentations. The fragmentation patterns were compared to poly(propylene imine) (PPI) dendrimers, which lack the amide groups. The comparison showed that macromolecular properties, intramolecular charge-solvation and energy barriers

are important determinants for the fragmentation pathways observed, and thus, for the architecture deduced from them. In addition, IM-MS has been used to separate dendrimer constituents and turned out to be much faster than LC or capillary zone electrophoresis (CZE) [131]. Unique cross-sections would be assigned to ‘perfect and ‘imperfect’ dendrimers, thus unveiling their architectures and complementing the MS/MS data. Furthermore, the results correlated with NMR spectroscopy and SAXS, thus emphasizing the versatility of this technique. Another application of IM-MS in combination with molecular modeling on PAMAM dendrimers concerns the dependence of conformation on the degree of protonation. The results obtained by theoretical calculations show that changes in the conformation are dictated by electrostatic repulsion, which is in agreement with the experimental IM-MS results. On the basis of these findings, IM-MS supported by computational chemistry is a promising tool for future studies on the architectures of higher generation dendrimers [132]. PAMAM dendrimers of higher molar mass were also studied by MALDI-MS and ISD [133]. Different matrices were found to result in different fragmentation patterns. The fragments obtained from ISD of PAMAM dendrimers correlate well with those from ECD of such materials.

Dendrimers of the PPI-type have been studied using different fragmentation techniques to elucidate their architectures. For example, Adhiya *et al.* examined the conformation of such dendrimers in solution and the gas phase by ESI-CAD and MALDI-PSD-MS [134]. Solutions in different solvents were used and diverse fragmentation pathways, such as the cleavage of outer branches from singly protonated ions, were monitored as a function of the solvent (polar/protic *vs.* non-polar). Lower intensities were observed with non-polar solvents, indicating differences in the dendrimer architectures and the conformations probed. Meijer and co-workers have reviewed the same type of dendrimers in detail by using ESI-CAD, and similar pathways were observed [135]. Further, low energy ESI-MS has been applied to

monitor the growth, fragmentations and shape of such dendrimers [136,137]. In addition, HPLC was hyphenated to ESI-MS and used to detect defects in the dendrimers, however, this represents a demanding task due to co-elution of components and isomerism of structures. Adding MS/MS experiments should at least partly resolve these problems by providing information on the different architectures eluting [138]. Branched PEI was investigated under ESI- and MALDI-MS conditions with CAD. These polymers were widely studied due to their reputation as a “golden standard” for gene delivery applications [49]. Three low abundant fragmentation series are detected, arising from a C-N bond cleavage. Their relative abundances provide useful insight on the branching architectural features of the examined PEI samples.

5.4.2. Ethers and esters

Ether based dendrimers have several application domains and MALDI-ToF MS is the traditionally used method for their characterization. MALDI-MS has been utilized to determine the structure and end groups of hyperbranched systems and, thus, differentiate cyclic structures of 3-[2-[2-(2-hydroxyethoxy)ethoxymethyl]-3'-methyl-oxetane (PHEMO), which is used as polymer electrolyte for reinforcement of polyelectrolyte membranes [139]. FT-ICR combined with liquid secondary ion mass spectrometry (SIMS) was used to evaluate, the fragmentation behavior of poly(ether)-type dendrimers with lateral terpyridine moieties and their complexes with iron(II) ions, for an architectural investigation [140]. Hyperbranched 3-ethyl-3-hydroxymethyloxetane trimethylolpropaneoxtane (TMPO)-based polyethers of low molar masses were investigated with ToF-SIMS [141]. The use of this technique with dendrimers of early generations allowed the determination of the degree of branching. However, high molar masses are not accessible with this method.

The Haddleton group investigated aryl ester dendrimers by MALDI-ToF MS. Their low homogeneity, disperse nature and the high laser power requirement for their ionization made the analysis of the spectra of such dendrimers intricate [142]. Fragmentations are induced in-source due to the high laser intensity, indirectly affirming the architecture by the presence of a series of cleaved off branch units. In a different study, MALDI-MS was applied to verify the synthetic route to such dendrimers [143]. The findings pointed out that traditional solution-based methods, such as NMR spectroscopy and SEC, are not powerful enough, and that hyphenated techniques and/or direct MS is required. Polyesteramides, as biodegradable polymers, were studied with MALDI- and ESI-MS giving different results, such as OH groups being present in the ESI spectra but not in the MALDI spectra [144]. This study demonstrated the need for using more than one ionization technique in order to determine architecture through direct MS. The other observation was that ISD is observed with MALDI and not with ESI; thus the analysis of the MALDI data should be carried out carefully due to fragments that lead to erroneous architecture deduction. Finally, Koster *et al.* used both ECD and low energy CAD to analyze hyperbranched oligomers and concluded that ECD produces more fragments, because it deposits higher internal energies to cause consecutive fragmentations; however, ECD did not produce complementary sequence information, because the new fragments were internal [145]. Thus one has to be aware, when using the data of different fragmentation techniques, that the fragment production mechanisms permit the derivation of the correct architecture.

Hyperbranched polyacrylates, in which the branches grow out of the ester groups, have been prepared by ATRP of acrylate inimers (an inimer can react as an initiator or monomer) [26]. Their architectures were analyzed by MS/MS. Fragmentation occurs by sigmatropic 1,5-H rearrangements over the ester groups, but not by rearrangement of H atoms in the polyacrylate chain. The rearrangements over the side chain provide information about the

branching architecture. The fragments produced reveal branching sites and branch sizes, and are completely different from fragments generated by the isomeric linear architecture.

5.4.3. Aromatic ring based

Aromatic monomers with a variety of structures have been polymerized to form dendrimers. Such dendrimers, or branched systems, were analyzed *via* MS techniques (as well as other methods). For example, the Fréchet group applied MALDI-MS to study the functionalities at the surface and the focal point of dendritic aromatic poly(ether)s [146,147]. This method allowed monitoring of the purity and the growth unlike other standard polymer characterization methods. Aromatic poly(ether)s and block-like dendrimers with potential biological applications were characterized by MALDI-ToF MS, which enabled confirmation of the architecture generated using different monomers [148]. In this study, the choice of matrix and the sample preparation method were critical for obtaining informative mass spectra. Aromatic polyester dendrimers were analyzed by MALDI and laser desorption ionization (LDI) in the presence of different metal salts, which enabled the polymer to be ionized by cation attachment, so that the different generations formed could be identified and verified [149]. In a more recent study, branched and linear poly(aramide)s were compared by MALDI-IM-MS. Synthesized poly(aramide)s were compared to commercial Kevlar[®] and was found that in Kevlar[®] both linear and branched species are present [150]. Furthermore, IM separation enabled the distinction between branched *meta-para* aramide and *para* aramide (Kevlar[®]). In addition, CAD of both branched and linear products was performed and distinct fragments appeared from each sample, thus enabling differentiation between different architectures. This example demonstrates that how a fast and facile these techniques can be to control the production and quality of the material and how polymer characterization can improve in the future. A highly branched PS was synthesized by linking polystyrene anions

with 4-chlorodimethyl-silylstyrene and analyzed by MALDI-MS and HPLC [151]. Reverse phase temperature gradient interaction chromatography (RP-TGIC) was used first to fractionate the polymer according to the number of PS branches. The fractions were then characterized by MALDI-ToF MS. Resolution was higher with a RP column in comparison to a normal phase (NP) one, and increased with the number of branches. LACCC resulted in lower resolution than RP-TGIC. Non-aromatic branched polymers were investigated by Liu *et al.* who analyzed the compositions, sequences and architectures of small oligomers of a hyperbranched glycopolymer, prepared by atom transfer radical copolymerization of an acrylate monomer (A) and an acrylate inimer (B), both carrying mannose ester pendants [152]. MALDI-MS and/or ESI-MS data confirmed the incorporation of multiple inimer repeat units, which lead to formation of a hyperbranched material. MSⁿ studies elucidated the atomic connectivity in various n-mers and specific sequences were distinguished from isomers based on the characteristic elimination of a bromomethane molecule. This provided definitive information about the comonomer connectivity in the copolymeric AB₂ trimer and A₂B₂ tetramer isomers. Consistent with the MS/MS data, IM-MS studies confirmed that only one of the possible A₂B₂ structures was formed (Figure 10). A comparison of the experimentally determined collision cross-section (CCS) of the detected isomer with the theoretical predictions for the two sequences conclusively showed that BBA₂ is the prevalent tetrameric architecture.

Figure 10. (a - c) ESI-IM-MS drift time distributions of sodiated, $[M+nNa]^{n+}$, glycopolymer samples having different comonomeric ratios. Three peaks are observed for all three samples, corresponding to ions with +1 to +3 sodium charges. The A_2B_1 trimer and A_3B_1 tetramer can only have the sequence shown, as they contain only one inimer unit. Based on MS/MS, the A_2B_2 tetramer loses CH_3Br , which is compatible with the two sequences shown. Only the first, BBA_2 , has a simulated collision cross-section that matches the measured one. Reproduced from Ref. [152].

MALDI-ToF MS is extremely beneficial for characterizing branched polymers. By utilizing a higher laser power and fragmentation in MALDI-MS are suitable for samples with high branching and the determination of branching points. Therefore, various ionization techniques with different fragmentation methods should be used to support hypotheses. In some of the cases, CAD, ECD or ISD were used to establish possible defects in architectures. Specific cleavages enable the verification of the synthetic routes *e.g.* outer layer cleavages of specific bonds. Therefore, tandem mass spectrometry remains an important technique in

characterizing the structural features in branched polymers. Furthermore, addition of IM dimension and molecular modeling to the analysis are useful for confirming their respective results regarding conformation changes and for providing complementary information on architectural differences.

6. Conclusion

The field of polymer science includes a very broad range of macromolecular species, with diverse structural complexity and molar masses. In the past 20 years, many applications involved synthetic polymers, which experience growing interest in the fields of biology and medicine. Many properties of synthetic polymers are important such as heterogeneity, topology, composition, functionality and/or molar mass. ESI-MS and MALDI-MS have been extensively used in the last two decades and have provided a great magnitude of information on the above-mentioned properties. Numerous developments have arisen recently in MS concerning high-resolution mass analyzers and higher sensitivity, which will boost MS applications in polymer science. Hyphenation of other analytical techniques to MS, for example LC, facilitates the determination of chemical heterogeneity and molar mass, and thus, enhances MS analysis. Furthermore, IM-MS is found to be of potential equivalence to LC, by separating molecules in the gas-phase relatively quickly according to their architectural differences without the need of excessive amounts of solvent consumption. In the future, further improvement and application of all these potential tools will help to investigate more complex architectures of different polymeric materials, which is still a challenging topic in terms of characterization. Moreover, automated software are being developed and continue to be implemented to support complicated data interpretation. MS remains to be a versatile technique and has a promising future in combination with other analytical and computational

methods for an improved analysis, and a better understanding of polymer systems with different architectures.

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1752 **Graphical Abstract**

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1763 **Sarah Crotty** was born in Ambilly (France) in 1987. She studied chemistry and analytical
1764 chemistry in Loughborough (B.Sc. in 2010) and Warwick (M.Sc. in 2011) (both UK). She
1765 worked in India for one year in R&D investigating the synthesis and characterization of
1766 potential drugs for tropical diseases. She joined in 2012 the research group of Prof. Ulrich S.
1767 Schubert as a Ph.D. student at the Friedrich Schiller University Jena. Her research focuses on
1768 the characterization of synthetic polymers by the use of diverse techniques 2D-LC, liquid
1769 adsorption chromatography at critical conditions (LACCC), spotting, mass spectrometry
1770 (MALDI-, ESI- & MS/MS) and software algorithm.

1771
1772 **Selim Gerişlioğlu** was born in Izmir (Turkey) in 1989. He studied chemistry at Hacettepe
1773 University (B.Sc. in 2010) (Ankara, Turkey). He completed his M.Sc. degree on physical
1774 chemistry in 2012 at Hacettepe University where he investigated ionization mechanisms in
1775 MALDI-MS. He moved to Akron, USA and joined the research group of Prof. Chrys
1776 Wesdemiotis as a Ph.D. student at The University of Akron in 2013. His current research
1777 focuses on investigation of fragmentation mechanisms and structural characterization of
1778 copolymers by utilizing different tandem mass spectrometric techniques such as electron
1779 transfer dissociation and collisionally activated dissociation.

1780
1781 **Kevin Endres** was born in Addison, IL (U.S.) in 1992. He studied Specialized Chemistry at
1782 the University of Illinois at Urbana-Champaign (B.Sc. in 2014), and conducted synthetic
1783 research on self-assembling nanoparticles for potential self-healing applications. He joined the
1784 research group of Prof. Chrys Wesdemiotis as a Ph.D. student at the University of Akron. His
1785 research focuses on the characterization of synthetic polymers with mass spectrometry (ESI
1786 and MALDI), polymer and supramolecular structures with IM-ESI-MS, and polymer blended
1787 films using SL-MALDI-ToF.

1788 **Chrys Wesdemiotis** was born in Thessaloniki (Greece) in 1952. He completed his B.Sc.,
1789 M.Sc. and Ph.D. at Technische Universität Berlin (1979). After a postdoctoral fellowship with
1790 Fred W. McLafferty at Cornell University (1980) and military service in Greece (1981-1983),
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1793 Science, and Integrated Bioscience. Wesdemiotis is AAAS fellow, Editor of the European
1794 Journal of Mass Spectrometry, and member of the Editorial Boards of Mass Spectrometry
1795 Reviews, International Journal of Mass Spectrometry, and the Journal of the American
1796 Society for Mass Spectrometry. Research in the Wesdemiotis group focuses on the
1797 development and applications of multidimensional mass spectrometry methodologies for the
1798 characterization of new synthetic polymers and polymer-biomolecule interfaces.

1799
1800 **Ulrich S. Schubert** was born in Tübingen (Germany) in 1969. He studied chemistry in
1801 Frankfurt and Bayreuth (both Germany) as well as in Richmond (USA). His Ph.D. studies
1802 were performed at the Universities of Bayreuth and South Florida/Tampa (USA). After a
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1805 2000 he was Professor at the Center for NanoScience, University of Munich (Germany), and
1806 from 2000 to 2007 Full-Professor at Eindhoven University of Technology (The Netherlands).
1807 Currently, he holds a chair at the Friedrich Schiller University Jena with research interest in
1808 nanoparticle systems as sensor and drug delivery devices, supramolecular chemistry, inkjet
1809 printing of polymers, polymers for energy storage, and self-healing materials.

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