Mass spectrometry as a tool to advance polymer science

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Abstract | In contrast to natural polymers, which have existed for billions of years, the first well-understood synthetic polymers date back to just over one century ago. Nevertheless, this relatively short period has seen vast progress in synthetic polymer chemistry, which can now offer diverse macromolecules with varying structural complexities. To keep pace with this synthetic progress, there have been commensurate developments in analytical chemistry, where mass spectrometry has emerged as the pre-eminent technique for polymer analysis. This Perspective describes present challenges associated with the mass-spectrometric analysis of synthetic polymers, in particular the desorption, ionization and structural interrogation of high-molar-mass macromolecules, as well as strategies to lower spectral complexity. We critically evaluate recent advances in technology in the context of these challenges and suggest how to push the field beyond its current limitations. In this context, the increasingly important role of high-resolution mass spectrometry is emphasized because of its unrivalled ability to describe unique species within polymer ensembles, rather than to report the average properties of the ensemble.

Nature is a master of polymerization and can build highly complex yet extremely well-defined macromolecules that make up a series of intricate catalytic systems that enable life. Humanity has pioneered methods to construct synthetic polymers that are complex in structure and molecular diversity, yet are not very well defined. From a mass spectrometry perspective, biomacromolecules each exhibit narrow molar-mass distributions and are, in principle, simpler to analyse than synthetic polymers. The main difficulty in characterizing biomacromolecules instead arises because a complex living system features a myriad of different species. For example, there might be tens of thousands of different proteins in a proteome. Over the past decades, high-resolution mass spectrometry, a well-established tool in biomolecular analysis, has been increasingly applied to the analysis of complex polymer systems. The challenges in analysing polymers lie in desorbing and ionizing high-molar-mass polymers and in identifying the composition and structures of unique species, given the sheer number of species in most polymer samples. The most notable advances in polymer mass spectrometry came with the advent of soft ionization methods, specifically, the introduction of electrospray ionization by Fenn and matrix-assisted laser desorption by Tanaka, Karas and Hillenkamp. Fenn referred to biological macromolecules as ‘flying elephants’, but the identification of any polymer by mass spectrometry requires more than just making a ‘plastic elephant’ fly.

This Perspective describes some of the specific challenges that researchers face when performing mass-spectrometric analyses on synthetic polymers. We explore the strategies that might help overcome these challenges, thereby eliminating some of the limitations of mass spectrometry and making it an even more powerful tool for polymer analysis than it is today. The challenges are covered in five main sections that correspond to the different steps that are commonly performed or are necessary for a mass-spectrometric analysis. For example, even though pre-separation of an analyte mixture is not strictly necessary for every sample, it greatly facilitates the analysis of complex macromolecular materials. Therefore, the first section highlights the advantages of lowering the sample complexity prior to mass analysis. Next, macromolecules in a condensed phase must be converted into gas-phase ions, a task that is typically more challenging for synthetic polymers than it is for biopolymers. Thus, the second section highlights and compares three widespread ionization techniques for polymer analysis. Once ions are generated, they can be separated by an analyser in a mass spectrometer, which poses specific challenges for synthetic polymers, as we describe in the third section. Apart from the direct mass analysis of generated ions, fragmentation patterns arising from ion activation in tandem mass-spectrometry experiments can yield valuable information about the chemical structure of a polymer. Therefore, the fourth section elaborates on the use of mass spectrometry to obtain molecular information beyond the mass of the molecular ion. Finally, the acquired data must be analysed, which often proves to be a challenge on its own, especially when mass spectrometry is used for imaging applications. The final section, thus, provides a brief overview of how to handle the often vast amounts of raw data obtained from a mass-spectrometric analysis.

Beyond our general discussion of these five stages, readers searching for a more detailed overview of one or more of these are referred to dedicated reviews on these topics.

Separation and hyphenation | Synthetic polymers typically contain various unique macromolecular chain structures and are characterized by a molar mass distribution, rather than an exact molar mass, so even the simplest polymers often give complex mass spectra. For example, a homopolymer with an average molar mass ($M_n$) of 3,300 g mol$^{-1}$ and a dispersity ($D$) of 1.27 already contains molecules with 60 different masses, and the number of unique species will be higher if isomers are present. Consequently, if one were to link the above homopolymer to another equally simple homopolymer, the resulting block copolymer would exist as several thousands of different macromolecules, and that is only...
just in terms of nominal mass (FIG. 1a). The interpretation of the corresponding mass spectrum is, thus, far from trivial and is often hampered by partial overlap of isotopic envelopes of the reactants and the products (FIG. 1b). Although software tools can facilitate the interpretation to a certain extent, recent advances in polymer research allow for the synthesis of a wide variety of complex macromolecular architectures that test the limits of even state-of-the-art mass spectrometer hardware and software. Therefore, one of the main challenges in the mass spectrometric analysis of polymers is to lower the complexity of the spectrum in order to gain information about individual species.

The most common strategy to lower the complexity of a mass spectrum is to limit, using chromatography, the variety and number of molecules that enter a mass spectrometer at any given time. Size-exclusion chromatography (SEC), for example, is a routine liquid-chromatography-based approach.
...for polymer analysis that separates the macromolecules according to their hydrodynamic volume and, thus, indirectly according to their molar mass. Consequently, the hyphenation of SEC to a mass spectrometer represents a very powerful analytical tool that effectively allows for a slice-by-slice mass analysis of differently sized compounds in the mixture\textsuperscript{43,47} (Fig. 1b). This technique is typically used for structural confirmation and monitoring post-polymerization modifications\textsuperscript{46,48–51}. More advanced applications include the investigation of polymerization and photoinitiation kinetics\textsuperscript{52–55}, as well as the generation of extracted-ion chromatograms to map morphological changes\textsuperscript{56}. Finally, the quantitative information provided by the concentration-dependent detector (for example, refractive index detector) used in SEC can be combined with the measured masses to determine absolute molar-mass distributions\textsuperscript{42,43}.

An alternative or even complementary pre-separation technique to SEC is liquid adsorption chromatography (LAC), which is typically performed using a reversed (non-polar) stationary phase. When LAC is conducted under critical conditions (LACCC)\textsuperscript{46}, it differentiates macromolecules of different polarity, independent of the number of repeating units and, thus, molar mass. This allows for the separation of molecules with different backbones or even with the same backbone but different end groups\textsuperscript{56,47–50}. We again get a set of mass spectra with a lower complexity, allowing for a more straightforward identification of the different species in a polymer mixture\textsuperscript{51,53–57} (Fig. 1b).

Although both SEC and LAC can suitably lower sample complexity, co-elution of different species is still very common and might interfere with the interpretation of the resulting mass spectra\textsuperscript{1,10,51–56}. Therefore, these two techniques have been combined in a 2D chromatography set-up (LAC × SEC), which is a powerful and versatile tool that, for example, allows discrimination between (co)polymers only differing in their specific architecture\textsuperscript{51,54–56}. Individual species can subsequently be identified by hyphenation to a mass spectrometer\textsuperscript{48,49–51} (Fig. 1b).

Even though the above hyphenated techniques allow for comprehensive polymer quantification and identification, they are not routine analyses in research laboratories let alone industry, in which they are still considered specialized methods. This is largely because they are relatively time-consuming and, thus, very costly, especially in an industrial context. They typically require optimization (for example, there is no universal LAC × SEC-mass spectrometry (MS) set-up applicable to all samples) and, even then, a single run takes between tens of minutes (SEC-MS) to several hours (LAC-MS and LAC × SEC-MS)\textsuperscript{9,10}.

Rather than performing pre-separation in solution, the components of a mixture can also be separated in the gas phase. Every mass spectrometer separates ions based on their mass-to-charge (m/z) ratio in the gas phase, and it can do so with a certain resolving power (see the section Mass analysis). Ion-mobility spectrometry (IMS) is a technique, typically coupled to a mass spectrometer, in which the ionized analytes drift through a gas under the influence of an electric field. In this way, they are separated according to their charge and their collision cross-section, which depends on size and shape. Indeed, some analyte ions are more likely than others to collide with gas molecules, which slows their drift through the ion-mobility analyser. Although IMS is conceptually similar to SEC, a single scan can be performed on the order of milliseconds, which means that a reliable ion mobility-mass spectrometry (IM-MS) analysis (with multiple scans) should not take more than a few seconds. Moreover, since the pre-separation and mass analysis both occur in the gas phase, this hyphenation does not impose practical constraints on the ionization technique or the solvent for sample preparation. IM-MS has already proven to be a valuable tool for polymer analysis over recent years\textsuperscript{52–58}, and we are still now only developing our fundamental understanding of how IMS separation depends on polymer structure\textsuperscript{7,24}. Therefore, while LAC × SEC-MS might appear to be the ‘ultimate’ hyphenated technique, we believe that future developments should focus on IM-MS or LAC-IM-MS, which combine a lower complexity with an acquisition time attractive for academia and industry.

**Desorption and Ionization**

Polymer solutions or eluates from liquid chromatography can be amenable to mass spectrometry, in particular when using electrospray ionization. Analysis of solid polymer samples would be amenable to a broader range of samples but is associated with additional challenges. Such samples might include insoluble thermosetting resins, lacquers and membranes, surfaces being patterned or exposed to environmental degradation, composite materials ranging from laminated films to fibre-reinforced materials and other ‘real-world’ polymer applications, including stabilizers, release agents and other additives\textsuperscript{23}. The three most widespread techniques are discussed herein: electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) for solution samples and secondary-ion mass spectrometry (SIMS) for solid samples. The reader is referred to dedicated reviews for other ionization techniques, including sonic spray ionization, atmospheric-pressure chemical ionization, atmospheric-pressure photoionization, atmospheric-pressure plasma ionization (for example, direct analysis in real time) and desorption electrospray ionization (DESI) methods for imaging\textsuperscript{59–11,14,40–42}.

ESI is a common interface between a chromatography system and a mass spectrometer, in which parts of the eluate are passed through a needle held at a few kV potential relative to the vacuum inlet (Fig. 2a). Flowing from the needle tip is a spray consisting of charged droplets, the dispersion of which is often assisted by a sheath gas flowing around the needle. Solvent evaporation is achieved by the droplets passing a zone of hot inert gas or...
of these cations can be added to polymer samples but, particularly in the case of Na⁺, the adduct ions often appear anyway because the metal is a typical synthetic impurity. Thus, adding a salt to an analyte solution is not always required, but it does suppress cross-ionization of multiply-charged ions, thereby simplifying the resulting mass spectrum. For example, the analysis of poly(ethylene oxide) commonly affords [M+2NH₄⁺]²⁺, [M+NH₄⁺Na⁺]²⁺, [M+NH₄⁺K⁺]²⁺, [M+2Na⁺]²⁺ and [M+Na⁺K⁺]²⁺ ions when no salt is added. Moreover, metals often have a distinct isotope pattern that ‘tags’ an organic polymer and facilitates the assignment of ESI mass spectra. Although the choice of salt is not crucial for successful ionization of most polar polymers such as poly(ethylene oxide), it does affect the analysis of more apolar polymers, such as polystyrene and polybutadiene.

As an alternative to conventional ESI, in which droplets undergo desolvation and then enter the high-vacuum region of separation, solvent droplets can be sprayed onto a solid surface, from which the sample components are desorbed and then enter the high vacuum as usual. This DESI method is nowadays mainly applied to biological and pharmaceutical samples, but also has some environmental and food applications, as well as uses in polymer science.

MALDI became a very versatile technique in polymer science, as evidenced by the development of the NIST Synthetic Polymer MALDI Recipes Database, a large repository of protocols and reference data. This ionization technique is popular because it enables the detection of large polymer fragments up to 30 kDa and the determination of a molar mass distribution. MALDI relies on the combination of the analyte with a matrix molecule, which can participate in photoreactions that afford charged analyte species. The sample preparation can involve mixing the matrix with the analyte in solution and then spotting it on a target plate and drying it. Alternatively, one might spray the matrix solution onto a solid polymer or simply grind the solid matrix and sample together and press the powder on a tape. Matrix molecules have a low molar mass and feature ultraviolet chromophores, such that they are readily volatilized, along with the analyte, by an impinging laser. The analyte might now receive H⁺ from an additional exogenous Brønsted acid like CF₃CO₂H or an acidic residue on the matrix itself. Aside from the resulting [M+H⁺]⁺ ions, one might also observe [M+Na⁺]⁺ or [M+K⁺]⁺ ions.

a heated capillary. As the volume of the droplets decreases by evaporation, the charge density increases until the droplets undergo fission to form even smaller droplets by Coulomb explosion. This process can result in the direct ejection of individual analyte ions, thereby lowering the charge density in the remaining droplets by what is referred to as an ‘ion-evaporation mechanism.’ Alternatively, the droplets might participate in the ‘charged-residue mechanism,’ wherein every droplet contains a single analyte molecule that inherits the charge after desolvation. The latter mechanism is believed to be prevalent in the ionization of analytes with high molar mass. Regardless of how analyte ions form, they will subsequently pass a skimmer/orifice set-up and a differential pumping stage to enter the high-vacuum part of the mass spectrometer.

ESI of polymers commonly affords ions that bear different charges, which often results in an m/z envelope for ions of a given charge (partially) overlapping with the envelope of the next charge state (Fig. 2a).

Polymer ions in ESI often take the form of protonated species [M+nH⁺]ⁿ⁺ but can also be obtained when charge-neutral polymers form adducts with NH₄⁺ or metal cations, such as Na⁺, K⁺, Ag⁺, Zn²⁺, Co²⁺ or Ni²⁺. Salts of these cations can be added to polymer samples but, particularly in the case of Na⁺, the adduct ions often appear anyway because the metal is a typical synthetic impurity. Thus, adding a salt to an analyte solution is not always required, but it does suppress cross-ionization of multiply-charged ions, thereby simplifying the resulting mass spectrum. For example, the analysis of poly(ethylene oxide) commonly affords [M+2NH₄⁺]²⁺, [M+NH₄⁺Na⁺]²⁺, [M+NH₄⁺K⁺]²⁺, [M+2Na⁺]²⁺ and [M+Na⁺K⁺]²⁺ ions when no salt is added. Moreover, metals often have a distinct isotope pattern that ‘tags’ an organic polymer and facilitates the assignment of ESI mass spectra. Although the choice of salt is not crucial for successful ionization of most polar polymers such as poly(ethylene oxide), it does affect the analysis of more apolar polymers, such as polystyrene and polybutadiene.

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Fig. 2 | Principles behind the three most widespread ionization techniques in polymer MS. a | Electrospray ionization (ESI) commonly gives rise to multiply-charged ions, affording partially overlapping envelopes corresponding to the different charge states. b | The formation of multiply-charged ions is less common in matrix-assisted laser desorption/ionization, which affords mainly quasimolecular species. However, the presence of matrix fragment ions and agglomerates hampers the interpretation of the low-m/z region. c | In contrast to matrix-assisted laser desorption/ionization, secondary-ion mass spectrometry (MS) does not require the addition of a matrix. A disadvantage of this technique is that it is more destructive to analytes and, in the case of polymers, mainly affords a low-m/z distribution of fragments, rather than intact molecular ions. This can partly be addressed by using metal-assisted cationization and polyatomic primary ions, which allow for improved generation of larger secondary ions and, thus, detection of intact macromolecules. All mass spectra reflect data simulated for poly(ethylene oxide).
adducts if salts are present (see above). MALDI is commonly used for both natural and synthetic polymers, and, in contrast to ESI, it predominantly generates singly-charged ions and, thus, affords a ‘cleaner’ mass spectrum (Fig. 2b). However, a disadvantage of MALDI is that analytes experience substantial ion suppression, whereby easily ionized species suppress the ionization of other molecules. Additionally, mass discrimination can occur, which means that, assuming no ion suppression, lighter species desorb and ionize better than heavier ones. Hence, the low-mass region (typically m/z < 500) is dominated by fragments and agglomerates of the matrix molecules desorbing in high quantities and is commonly excluded from data evaluation. An interesting development for MALDI is a technically challenging laser post-ionization, similar to what is applied in secondary neutral mass spectrometry, in which the process of evaporation is decoupled from ionization. However, the necessary probabilities of analytes to undergo photoionization for quantitative analysis are not clear a priori, so examples of this post-ionization are scarce and appear only in adjacent fields of application.

Compared with ESI and MALDI, the combined desorption and ionization process is more destructive in SIMS. SIMS involves bombarding a solid sample surface with highly energetic (∼25 keV) primary ions. The top few nanometres of the surface release predominantly charge-neutral fragments, but secondary ions can also be sputtered off (Fig. 2c). These ions are typically singly charged and can be separated using a magnetic sector or time-of-flight (TOF) analyser or, in the latest-generation instruments, an orbitrap analyser. Although SIMS typically generates low-mass ions, we have observed that physisorbed oligopeptides can be detected as quasimolecular secondary ions or Na⁺ adducts in the 2-kDa range. This is enabled in most state-of-the-art spectrometers using a Bi liquid metal ion gun, which affords beams comprised of Bi⁺ and higher clusters. More important to note is that primary-ion beams from such point-like field-emission tips can be focused on sub-100-nm spots, with a clear advantage over ion sources relying on the impact of electrons with gas molecules such as Cs⁺ or Ar clusters, which have a lower spatial focus. The latter cluster sources, however, make perfect ion beams for depth profiling because they can erode organic matter while preserving molecular structures in the erosion zone to be probed with the Bi beam. Moreover, Ar cluster ion sources can also serve as primary-ion sources for TOF set-ups. Although they lack the very fine focus of liquid-metal ion guns, Ar clusters are often superior in the high-molecular-weight range and are very useful in combination withorbitrap mass analysers, which allow for very high mass resolution and accuracy for large molecular species (see the section Mass analysis). Although there are also other approaches improving the yield of high-molar-mass species in SIMS, the quest for making elephants fly with SIMS without using Ar cluster sources attracted limited attention. Several studies demonstrate that a dedicated sample preparation is a key prerequisite for metal-assisted cationization of large molecules, while no clear advantage over a corresponding — and much simpler — MALDI approach exists.

In addition to structure elucidation, MALDI and especially SIMS are often used for molecular imaging (or mapping), in which spatial resolution in 2D or 3D is as important as molecular information from a detailed mass spectrum. By scanning a laser spot (MALDI) or primary-ion beam (SIMS) in space, both methods can image the sample composition and provide a full mass spectrum for each pixel. The advantages of MALDI are showcased when imaging metabolites in tissue sections where one distinct target species has to be found in a biological specimen containing a plethora of other compounds. A lateral resolution of 1–150 μm has been reported in MALDI-MS/MS, with a value of ~70 μm, suggested in a round-robin study using demanding samples, being a good reference. In comparison, SIMS imaging can achieve a higher lateral resolution of 100–400 nm, depending on the required mass resolution and sensitivity, which are typically limited by the smaller sampling volume. SIMS is, thus, the method of choice when high spatial resolution is required and the sample complexity is not very troublesome. Thus, one would not use SIMS primarily for biological samples but instead for insoluble plasma-polymer layers, surface-modified polymers, polymer blends, copolymers undergoing surface segregation, 2D patterned samples or arrays, 3D layered samples and polymer nanoparticles. Despite their technological differences, MALDI and SIMS are today converging with respect to their applications. Indeed, MS/MS coupling for SIMS increases its discrimination power, while the development of charge-compensation systems for MALDI allows for a less demanding sample preparation for polymer samples that are electrical insulators. Thus, we expect that the gap between these methods will close as each of them becomes similarly applicable for (industrial) polymer samples and biological questions.

**Mass analysis**

The advances in desorption and ionization approaches we have so far described have underpinned the extension of mass spectrometry to be applicable to a wider range of polymers of large size and molecular complexity. To fully exploit these advancements, we have also seen concomitant performance improvements in the mass-analysis capabilities of modern instrumentation, developments that have been in response to market demands, particularly from the biomolecular science community. Notably, the mass range and resolving power of commercial analysers have increased substantially over the past 10 years.

The increased mass-resolving power of state-of-the-art mass spectrometers has resulted in a shift in polymer mass spectrometry away from using instruments based on quadrupole and quadrupole-ion-trap analysers, which have low m/z ranges and resolving powers. Polymer chemists now routinely use high-resolution and ultrahigh-resolution analysers such as reflectron and multipass (for example, spiral) TOF, orbitrap and magnetic resonance (also called ion-cyclotron resonance) instruments. High-resolution instrumentation has empowered the exploration of higher-molar-mass polymers, the deconvolution of more complex mixtures and the identification of subtle modifications to macromolecular structures (see below). Although there exists a measurement trade-off between sensitivity and mass-resolving power (R = m/Δm), gains in the latter are proving particularly important in empowering macromolecular science to develop more highly functional materials and exact greater control over side-chain and end-group chemistries. Nevertheless, synthetic polymers are usually available in greater amounts and, therefore, little trade-off exists in accessing high or even ultrahigh mass-resolving power, with the resulting benefits for molecular fidelity. In some contexts, for example, when a high spatial resolution in direct desorption/ionization experiments (namely, SIMS and MALDI) is required, the amount of analyte per pixel is extremely limited. Therefore, maximizing...
R may need to be sacrificed in favour of sensitivity.

Let us emphasize the importance of resolving power in polymer analysis by considering a dithioester-terminated poly(methyl methacrylate) that might undergo hydrolysis to the corresponding thiol or, instead, be treated under oxidative conditions to form the desired isobaric hydroperoxide30 (Fig. 3a). The mass difference between these distinct elemental compositions is too small ($\Delta m = m(132) - 2m(16O) = 0.018$ Da) to be distinguishable at unit mass resolution. It is illustrative to model the instrument performance required to resolve these two chemically distinct species at the high-mass and low-mass ends of the polymer distribution to explore how much resolving power is sufficient. These simulations reveal that, for a singly-charged 15-mer, $R$ of nearly 400,000 is required to achieve discrimination of thiol-terminated and peroxide-terminated chains, while for the 60-mer with a nominal $m/z$ of 6,130, even this resolving power will afford only a modest separation (~80% valley definition; Fig. 3b). This exemplifies the frontier challenge for mass analysis of polymers, in which achieving even $R = 400,000$ in the mass range of interest is challenging using contemporary instrumentation. The resolving power of the three major classes of high-resolution and ultrahigh-resolution analysers each exhibit a distinct dependence on $m/z$ (Fig. 3c). Noting that higher performance has been achieved for each category of analyser, the trends depicted here represent typical benchmarks for present commercially available instruments, which all struggle to meet an $R = 100,000$ benchmark across the $m/z$ range 1,000–10,000. The optimal choice of mass spectrometer for a particular polymer analysis will, thus, be the one that provides the best $R$ over the $m/z$ range of interest. Substantial increases in $R$ over the higher $m/z$ range would greatly add to the flexibility of mass spectrometry for polymer analysis, and recent studies indicate that such increases are being realized for each of the three analyser types166.

In contrast to MALDI, the charge imparted on an analyte by ESI is greater for analytes with greater mass, such that ESI is amenable to mass analysis of biomolecules and even biomolecular assemblies in the MDa regime156,160. Thus, species with large $m$ also have $z > 1$, such that $m/z$ falls in the effective range of the mass analysers, a principle that, of course, is also applicable to synthetic polymers. There are disadvantages to generating more highly charged species, however. A given family of species can be present in multiple charge states, effectively dividing the total ion current of the species across multiple charge states. This diminishes the signal-to-noise ratio161 and increases the signal density within a given $m/z$ window. It follows, therefore, that establishing control over the charge state of polymer ions is intimately linked to the twin goals of achieving greater mass range and resolution in analysis. Recent efforts to establish this control have involved adding reagents to analytes to effect ‘supercharging’30,161 or, conversely, exploiting gas-phase ion–ion reactions to shift the population of an analyte ion to lower charge states162,163. While these approaches serve to modulate the number of charges carried by an ion ensemble, an alternative strategy uses IMS to effectively separate the different charge states of a polymer and, thus, to reduce mass-spectral complexity. Given the achievements in biomolecular mass spectrometry, it is reasonable to conclude that contemporary instrumentation enables us to generate, transmit and analyse ions with exceedingly high $m/z$ ratios164. Nevertheless, it is notable that ESI of polymers typically only samples the low-molar-mass fraction of the mixture and not the entire distribution of chains. The mechanistic explanation for these observations remains to be fully elucidated165. Frontier challenges for polymer mass spectrometry, therefore, lie in better harnessing existing mass-analyser technologies by introducing innovative desorption and ionization strategies for polymers that enable on-demand control of charge state(s). Such progress necessarily leads to new challenges in describing the composition, structure and distribution of high-molar-mass polymers and requires new ways of controlled fragmentation and mobilization164.

Structure elucidation

No matter how high the resolution and accuracy with which we measure $m/z$ of an ionized polymer, the data will always reflect the overall composition and not the molecular structure of individual species. This stands at odds with the present drive towards more highly functional materials, for which it is the structure that ultimately defines the intended function. Tandem mass spectrometry is, therefore, increasingly being applied to the structural interrogation of new polymer materials.

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Fig. 3 | The required resolving power and the optimum choice of mass analyser are highly sample dependent. a | Let us consider a poly(methyl methacrylate) chain initiated by azobisisobutyronitrile (AIBN) and terminated with a dithioester end group. Depending on the reaction conditions, the dithioester can be homolytically removed and the chain oxidized to give a hydroperoxide30 and/or the dithioester can undergo hydrolysis to give a thiol. b | Spectra simulated for an equimolar amount of both products indicate how a larger resolving power is required to discriminate between two polymer species of similar $m/z$ if the polymer has a high $m/z$. c | Time-of-flight analysers have a relatively constant $R$ over a wide $m/z$ range, while the $R$ of an orbitrap or a magnetic resonance analyser is strongly dependent on $m/z$. The curves are based on reported $m/z$ dependencies and manufacturer specifications of commercially available high-performance devices (scan frequency >1 Hz)165.
In a tandem mass-spectrometry experiment, ions in a narrow m/z range are selected (or filtered) in an initial discrimination event and are then subjected to some form of activation that raises the ion internal energy to facilitate cleavage of chemical bonds. The charged products of these activation–dissociation events are then separated in a second m/z analysis and measured at a detector. The most common ion-activation method in tandem mass spectrometry is collision-induced dissociation (CID), whereby mass-selected precursor ions are subjected to an energetic collision with a neutral gas, such as N₂, Ar or He. The ion internal energy (estimated as the centre-of-mass collision energy, \( E_{\text{cm}} \)) is, then, a function of its mass (\( m \)) and charge (\( z \)), applied electrical potential energy (\( V \), typically <100 eV) and the mass of the collision gas (\( m_g \)) according to \( E_{\text{cm}} = z V m_g / (m_z + m_g) \). Thus, typical centre-of-mass collision energies range from ~12 eV for a small molecule (\( m/z \sim 300 \)) undergoing collision with Ar with a (laboratory-frame) collision energy of 100 eV, down to just ~1 eV for an ionized polymer (\( m/z \sim 3,000 \)) under the same conditions. Under CID conditions, ions dissociate through low-energy unimolecular mechanisms typically associated with the nature and location of the charged residue within the ion. The most widely celebrated example of this technique is CID of cationized peptides, fragmentation of which occurs at the amide bonds following migration of the charge to these sites.[166] This predictable phenomenon allows the sequencing of peptides and is the mainstay of contemporary proteomics technologies.[167]

The ability to sequence synthetic polymers in the same manner as peptides is highly desirable and can be achieved for suitably labile polymer structures in which the monomers are linked by bonds that can be broken under CID conditions. For example, ESI of poly(selenoureas) affords ions of poly(selenoureas) and ions of which can be established using CID, of which can be established using CID, where a putative mobile H⁺ mechanism facilitates unimolecular dissociation at the selenourea moieties, thereby allowing individual chains to be sequenced.[168] Moreover, labile polymers have been specifically engineered to exploit CID of monomer linkages. These polymers include sequence-defined nitroxides, poly(triazole amide)s, and amide-linked and carbamate-linked polymers,[169–171] with the latter example being ionizable as a 5-mer that undergoes cleavage of its carbamate bonds to afford the respective fragment ions in its tandem mass spectrum (Fig. 4). If one observes a predictable fragmentation pattern, it becomes possible to directly read the defined sequence of a polymer from its mass spectrum.[172]

The applicability of CID to sequence a wide variety of polymers is limited by the polydispersity of the analytes themselves, which is in stark contrast to sequence-defined structures. Moreover, CID of many synthetic polymers is challenging because the monomers are often linked by strong bonds, such as C–O ether bonds in poly(ethylene oxide) and C–C bonds in polyolefins. Furthermore, the dramatic difference in mass between ionized polymers and molecular (or atomic) collision gases limits the centre-of-mass collision energy imparted in a conventional CID experiment: consider a flying elephant colliding with a mosquito! These challenges have motivated the deployment of alternative ion-activation modalities for the structural characterization of polymers. These modalities include high-energy CID (\( >100 \text{ eV} \)) in beam-type instruments (including implementation in SIMS instruments), surface-induced dissociation and electron-capture and electron-transfer dissociation and photodissociation.[167,172] All of these activation modalities are now (or can readily be) implemented on commercially available mass spectrometers of varying
geometries and provide a broad range of options for the polymer chemist to tailor activation conditions to their analytes. The potential of each of these tandem methods has recently been critically evaluated, and it appears that there is a wide scope for further developing and optimizing these technologies to elucidate sequence, side-chain and end-group structures across the pantheon of polymer chemistry. It is particularly attractive to consider matching the ion-activation modality to the chemistry and intended application of the polymer itself. For example, in the emerging field of photoactive polymers, where materials undergo designed changes in response to different colours of light, the interrogation of mass-selected polymer ions with radiation of a tunable wavelength could yield both structural information of the polymer while also predicting the function of the material in its intended application.

The behaviour of ions within IM analysers is dependent both on m/z and geometry. We have outlined the advantages of IM for spectral deconvolution in the section Separation and hyphenation and should note here that the method had also been used to identify the structures of ionized polymers. Exploiting the geometry dependence in IMS enables the tantalizing prospect of discriminating between isomeric polymers, such as the products of wavelength-gated-cycloaddition reactions or linear-cycloaddition reactions processes. As is the case with m/z resolution, the ‘mobility resolution’ and, thus, the ability to separate ions with a similar ion mobility, is improved with every generation of IM instrumentation, which uses analysers based on cyclic-ion mobility, trapped-ion mobility and structures for lossless ion mobility.

Increasing mobility resolution may bring with it new insights into the structural complexity of polymers with identical overall composition, such as the isomeric example above. Further, careful extraction of collision cross-section values, after appropriate standardization, allows elucidation of the structure and conformation of ionized polymers in the gas phase. Indeed, a recent analysis of polyacrylamides using a high-mobility-resolution cyclic IM device revealed evidence that polymer compositions, when in a given charge state, can exist in multiple discrete conformations, as is the case with ionized peptides. It would be desirable to combine an increase in resolving power of IMS with different ion-activation modalities, such that the different shapes and sizes of ionized polymers could be directly correlated to the sequence or side-chain structures.

Data handling
The final task in a mass spectrometric analysis is data processing, which can be rather laborious, given the complex datasets often obtained, especially in imaging applications. For example, dynamic SIMS can afford datasets with up to five dimensions: ion counts as a function of m/z and three spatial coordinates. The visualization of these data is achieved using software packages — both proprietary tools by instrument manufacturers, as well as open-source alternatives. These visualization tools can afford 2D cross-sections through a spatial dataset that map the ion intensity associated with any m/z value (or range) on the fly without time-consuming reprocessing of raw data. Moreover, such tools typically include algorithms to correct measurement artefacts, including shifts in the m/z distribution caused by differential charging.

Multidimensional data can be visualized using multivariate statistical analysis, which is, moreover, commonly applied to reduce the dimensionality of dataset and to extract information from a large number of channels that each has a poor signal-to-noise-ratio. For DESI and MALDI, this analysis is commonly used in biological or pharmaceutical studies. Researchers using SIMS for more complex studies also apply multivariate data analyses, such as principal component analysis or negative matrix factorization or multivariate curve resolution. Although these approaches are becoming increasingly popular for both biological and polymer applications, critical and careful chemists have observed and reported possible pitfalls that the reader should be aware of.

The lessons regarding standardization and data availability learned from mass-spectrometry-imaging applications in modern clinical settings will no doubt help advance ‘regular’ mass spectrometry in polymer science as well. However, being unsupervised and purely statistical, the multivariate methods cannot take advantage of the repetitive nature of synthetic polymer sequences. Indeed, in polymer chemistry, any pattern in a mass spectrum represents useful information that is completely disregarded by the statistical data analysis. As early as the 1960s, Kendrick sought to address this by introducing a chemical-substructure-based approach that was used as high-resolution mass spectrometry data became commonly available. This approach used building blocks to simplify mass spectra by rescaling the m/z scale to a ‘Kendrick mass’ scale, so that the m/z of the building block becomes its nominal mass. Initially, CH₄ was used as a building block, which means that each m/z is multiplied by a factor of 14.0000/14.01565. The deviation from unit mass in this new mass scale was defined as the ‘Kendrick mass defect’ (KMD) and allows grouping of peaks derived from compounds that differ only in their number of CH₄ groups. For example, peaks corresponding to a homologous series of compounds, a size distribution of polyethylene or a series of fragments obtained from one single parent compound all share the same KMD.

Fig. 5 | Ion-mobility spectrometry exploits the m/z, size and shape dependence of ion diffusion to separate analytes. a | Travelling-wave ion mobility uses ring electrodes to confine the ions axially, on top of which is applied a travelling potential wave that propagates ions at different rates through a buffer gas, depending on their m/z, size and shape. b | For example, travelling-wave ion mobility can reveal the different shapes of linear and cyclized isomers of a polymer with the same composition and sequence. Of the analyte ions formed, the linear isomer adopts a more compact structure in the gas phase than the cyclized form, which cannot adopt such a collapsed structure. The cyclized form experiences more collisions with the buffer gas and emerges from the ion-mobility analyser at a later drift time. The ionogram is based on raw data from REF.©.

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Kendrick’s novel approach was later applied to common polymers that have more complex building blocks, such as ethylene oxide, propylene oxide and lactic acid groups\(^{19}\). Consequently, the mass spectra of polymer mixtures, in which each polymer type has its own molar-mass distribution, became much easier to interpret because only components sharing a repeating unit also share a KMD\(^{194}\). It is also possible to perform the reverse approach: calculating the KMD from the composition of the building block. This allows one to analyse a polymer and come up with a possible model for a repeating unit, for example, by using a predefined list defining the occurring elements or the number of unsaturations\(^{195}\).

Synthetic polymers typically afford mass spectra with predictable patterns and, aside from the KMD, this has also afforded an alternative approach to help assign the multitude of ions observed. This strategy involves ab initio generation of a library of macromolecules based on knowledge of the monomers and end groups. From this hypothetical library, one can generate an initial approximation of a calculated mass spectrum, which can be compared with experimental data to reveal the identities and relative abundances of detected species. These data enable one to calculate parameters defining molar-mass distribution (\(M_g\) and \(D\)), within the limitations of a mass spectrometric analysis. It should be noted that, although the masses of various species can be determined with high accuracy, quantitative mass spectrometry remains challenging because the relative abundances of the ions measured is influenced by mass-discrimination effects during ionization (see the section Desorption and ionization), ion transmission and detection.\(^{17}\) The ab initio approach for spectral assignment was first implemented in an algorithm to analyse low-resolution mass spectra of homopolymers\(^{196}\). More recently, the availability of high-performance tools for the calculation of high-resolution mass spectra\(^{197}\) has enabled the automated interpretation of complex high-resolution mass spectra of copolymers\(^{198}\).

Conclusions and outlook

Mass spectrometry has evolved into a highly useful tool for materials science, enabling polymer chemists to essentially assess the fate of macromolecules chain by chain. In contrast to other common tools for polymer characterization, such as SEC, mass spectrometry of polymers offers absolute mass determination with unrivalled accuracy. Herein, we have highlighted the current strength of mass spectrometry for polymer analysis, yet critically pointed towards the developments that are required to elevate the technology to greater levels of utility. For example, what many polymer chemists would wish for is a means to entirely replace SEC with a rapid, direct-infusion mass-spectrometry method that yields highly accurate molar-mass averages for a polymer distribution, along with structural information associated with each chain in the ensemble.

If we were to ask ‘can we make wings for plastic elephants?’ the answer would be ‘yes and no’. A myriad of ionization methods emerged from the pioneering work of Tanaka and Fenn, so the success of a measurement often relies on choosing the correct method for a given sample (or analysis). Then, the challenges or issues of the mass spectrometric analysis are associated with the processes before and/or after the ionization phase. For example, the field of hyphenated techniques needs to move beyond the present standard of SEC/LAC-MS and focus on improving IM-MS or LAC-1M-MS. This would provide a good balance between lowering spectral complexity and analysis time, and would make the methods more generally applicable in academia and industry. Furthermore, there is huge untapped potential in applying tandem mass spectrometry to polymer analysis, thereby allowing structural interrogation of individual macromolecular chains to determine the sequence, side-chain and end-group functionalities, and, through IMS, even complex tertiary structures. Instead of describing a polymer by its distribution, tandem mass spectrometry allows us to characterize each species from which it is composed.

The main challenge of polymer mass spectrometry is to extend the mass range accessible to conventional analyses. Even though an \(m/z\) range up to multiple thousands is nowadays readily analysed, the ions detected represent a fraction of only a few percent of the molar-mass distribution of many (industrial) polymers. Solving this problem will require an integrated approach that encompasses improved ionization techniques to simplify the input of the mass spectrometer, a deeper understanding of desorption/ionization mechanisms (including modulation of charge states) to optimize the generation of high-mass ions and fully exploiting advances in the mass range and resolving power of mass analysers.

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