



LC–MS of Synthetic Polymers: The Parable of the Lame and the Blind

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A synthetic polymer is not a uniquely defined chemical compound. Not all molecules are identical. In ‘simple’ homopolymers the individual molecules vary (unavoidably) in size and (possibly) in end groups (functionality).*(*See footnote at end of article.*) Polymeric chains may be linear, branched to variable extents or even cyclic. In addition, some important polymers, such as polypropylene, polystyrene and polyacrylates exhibit variations in chain (stereo-) regularity or ‘tacticity’. In some other instances, such as PVC, head-to-head vs. head-to-tail, isomerism is a significant issue. Given all these possible variations between the individual molecules, we should stop calling homopolymers ‘simple.’ Molecules of copolymers enjoy several additional degrees of freedom. They show variations in their overall chemical composition (ratios of individual monomers present) and in the order in which the different monomers are connected in the chain (from fully random copolymers to block copolymers). Co-monomers may be present in the form of branches (grafts) connected to the main chain. Many other structural variations are possible between the molecules in samples of synthetic polymers. *Synthetic polymers are very complex mixtures.*

Liquid chromatography (LC) is eminently suitable for separating soluble polymers. A number of different mechanisms (size exclusion, adsorption, partition etc.) can be exploited. However, LC is just a separation technique. The separation may be performed to fractionate the sample, allowing off-line manipulation or characterization of the fractions. Alternatively, the objective of the separation may be quantitative analysis. This requires a detection device, the signal of which can be related to the mass (flow) or the concentration of the analyte(s). A third objective may be qualitative analysis. In combination with the above quantitative detector, complex mixtures can be characterized and sometimes identified by ‘fingerprint’ chromatograms. However, if detailed qualitative information is needed, such as structural information on the individual constituents of the sample, liquid chromatographers are essentially operating in the dark. *LC is blind.*

In mass spectrometry (MS) the molecules in a sample are first ionized, then separated according to their mass-to-charge ratio (m/z), and finally detected. Mass spectrometry provides

accurate information on the molecular weight of the separated and detected ions. This can often be translated into useful structural information. For example, the combined molecular weight of the end groups often indicates a specific molecular structure. However, accurate quantitative information is hard to obtain.^{1,2} The sensitivity of the complete MS system (ionization, separation, detection) varies with the chemical structure and molecular weight of the analytes. Sensitivity also usually varies between instruments and with time. For one or a few specific (‘target’) analytes this can be overcome by careful and frequent calibration of the response. However, for complex mixtures, containing a diverse range of molecules, the intensity axis of a mass spectrum should be considered with due scepticism. *MS is lame.*

For the detailed, qualitative analysis of synthetic polymers, either LC or MS are of limited use. Because LC is blind and MS is lame, it is obvious that their handicaps can be largely overcome if they can be put to use together (Table 1).

Liquid-phase separation techniques are the obvious way for separating solutions of polymers. The word solution is essential here. As long as a polymeric sample can be properly dissolved to yield a dilute solution of individually solvated molecules, the family of liquid-phase separations (that includes liquid chromatography, field-flow fractionation and electromigration methods) offers a vast spectrum of possibilities. A brief overview of techniques that may be combined with MS are listed in Table 2.

Mass spectrometry offers fantastic selectivity and sensitivity for the analysis of small molecules. In addition, its applicability to the analysis of synthetic polymers has been greatly enhanced in recent years, mainly thanks to the advent of two soft ionization techniques, which allow very large molecules to be ionized with good integrity. These two techniques, electrospray ionization (ESI) and matrix-assisted laser-desorption ionization (MALDI), will be discussed in the present article.

An example is shown in Figures 1–3. This involves the analysis of a series of hyperbranched polymers derived from 1,2-cyclohexanedicarboxylic acid anhydride (monomer C) and di-2-propanolamine (monomer D).^{3,4} Figure 1 shows the size-exclusion chromatogram of such a polymer, from which an



indication may be obtained about the size of the molecules in the sample. However, accurate determination of molecular weight using size-exclusion chromatography (SEC) is very difficult, especially for copolymers of variable chemical composition. The chromatogram is a useful fingerprint but we don't quite know what we are looking at.

Figure 2 shows the (off-line) positive-mode ESI-MS spectrum of the (entire) polymeric sample. The masses belonging to the individual peaks can be used to assign them to specific oligomers, after subtracting the mass of the adduct ion (in this instance predominantly sodium, which was added to the mobile phase). Very small ions, such as $[CD_2 + Na]^+$ ($m/z = 425$), $[C_2D_3 + Na]^+$ (694), and $[C_3D_4 + Na]^+$ (963) are dominating the spectrum, suggesting either that the sample predominantly contains such small molecules or that excessive fragmentation occurs.

Figure 3 shows the (off-line) positive-mode ESI-MS spectrum of a specific fraction of the sample collected from the

SEC effluent (indicated by the vertical bar at around 32 mL in Figure 1). The top half of this figure mainly shows triply charged ions. Several series of peaks can be identified.**(*See footnote at end of article.*) The lower half of the figure shows similar patterns arising from doubly charged oligomer ions. The inevitable conclusions from this figure are that

- the molecules eluting at the time of the vertical bar in Figure 1 are very much larger than those corresponding to the ions observed in Figure 2,
- excessive fragmentation is not observed in Figure 3 (which was recorded under the same ESI conditions as Figure 2), and thus that
- Figure 2 provides an incorrect and misleading image of the entire (polydisperse) sample.

LC Separation of Polymers

In Table 2, three families of liquid-phase separations are distinguished. Liquid chromatography (LC) is the first and

Table 1: Characteristics of liquid chromatography and mass spectrometry for separating and analysing macromolecules.

Characteristic	LC	MS
Separation	<ul style="list-style-type: none"> • High resolution for small molecules • Limited resolution across a broad MW range 	<ul style="list-style-type: none"> • Very high (or extremely high) resolution
	<ul style="list-style-type: none"> • Immense versatility (many different mechanisms, mobile and stationary phases) 	<ul style="list-style-type: none"> • ESI: Limited range of eluents (no non-volatile additives) • MALDI: Higher tolerance to buffers and additives
Applicability	<ul style="list-style-type: none"> • All soluble polymers 	<ul style="list-style-type: none"> • MALDI: Polar or moderately polar polymers • ESI, MALDI: Polar polymers
Identification	<ul style="list-style-type: none"> • Retention times (of limited use) • Fingerprinting of complex samples • Otherwise relying on (informative) detectors 	<ul style="list-style-type: none"> • Molecular-weight information • Structural formulae (high-resolution MS) • Structural information (mainly from MS-MS)
Quantitation	<ul style="list-style-type: none"> • Depending on detector (often good to excellent) 	<ul style="list-style-type: none"> • Selective (different response factors for different classes of components); • Specific (no response for certain classes); • Discriminative (varying response within a class). • Short-term repeatability of response factors is acceptable; long-term repeatability and reproducibility are poor. • (Frequent) calibration is a must, but suitable standards are hard to obtain.

most important of these. SEC is the most commonly applied technique for separating polymers. The separation mechanism is based on the size of polymer molecules in solution and the extent to which they are excluded from porous packing particles. Genuine SEC (i.e., when secondary retention mechanisms, such as adsorption effects, are absent) has a finite retention window, which stretches from total exclusion (retention volumes somewhere around 40% of the empty-column volume) to total permeation (up to around 80%). By choosing packing materials with different pore-size distributions SEC selectivity can be tuned to a specific range of molecular weights. SEC typically makes use of wide-bore columns, with diameters between 4.6 and 8 mm. Although the linear velocities in SEC are usually two to five times lower than in LC, this still means that the volumetric flow-rates are quite high (typically between 0.2 and 1 mL/min).

'Interactive' LC, which is based on molecular interactions between the polymer molecules and the mobile and stationary phases in the column, can be used to separate polymers based

on chemical composition or functionality (functional groups or end groups). As in conventional LC techniques, the composition of the mobile phase is used to achieve the desired separation. Gradient elution is often needed to elute a variety of polymer molecules within a reasonable time. However, there is a specific mode of isocratic LC, in which retention is independent of molecular weight and solely influenced by the chemical composition or functionality of the molecules. These critical conditions are hard to achieve and maintain, but they are extremely useful for separating analyte molecules according to the number of functional groups present.

Field-flow fractionation (FFF) has hardly been used in combination with MS, which is perhaps as a result of the fact that this family is not quite as mature as the much more common LC. A more fundamental reason is that, unlike MS, FFF is most suitable for very-high-MW molecules (and particles). However, recently one group⁵ has begun to combine thermal FFF off-line with MALDI. FFF has been demonstrated to yield potentially very high selectivity, so that very narrow

Table 2: Overview of selected liquid-separation techniques for synthetic polymers and their characteristics for coupling with MS.¹

Family	Technique ²	Separation according to	On-line ESI-MS	Off-line MALDI-MS
LC	SEC	Molecular size	⊗ Large columns (i.d. ≥ 4.6 mm) are typically used	⊗ Very narrow MW fractions
	Gradient LC	Chemical composition Chemical functionality	⊗ Separation mechanism very different from that of MS ('orthogonal') ⊗ Great variations in mobile-phase composition (polar and apolar)	⊗ Fractions vary mainly in molecular weight ⊗ (Optimum) deposition conditions vary with time
	Isocratic ('critical') LC	Chemical functionality	⊗ Constant mobile-phase composition ⊗ Typically short retention times ⊗ Limited resolution	⊗ Constant deposition conditions ⊗ Complex fractions are obtained (in which MW varies) ⊗ Retention times may vary with fluctuations in mobile-phase composition and temperature
FFF	HDC ³	Molecular size	⊗ Very fast separations ⊗ Used mainly for very large macromolecules (MW ≥ 10 ⁵)	⊗ Very narrow MW fractions
	Thermal FFF	Molecular size and chemical composition (confounded)	⊗ Used mainly for very large macromolecules (MW ≥ 10 ⁵)	⊗ Very narrow MW fractions
	(Hollow-fibre) flow FFF	Molecular size	⊗ Miniaturization and low flow-rates possible ⊗ Used mainly for very large macromolecules (MW ≥ 10 ⁵)	⊗ Very narrow MW fractions
CE	CE	Molecular size relative to charge	⊗ Very small (electro-osmotic) flow-rates	⊗ Very small amounts of sample ⊗ Deposition not straightforward
	CGE	Size of (similarly charged) ions	⊗ Buffer ions present	
	CEC	Chemical composition Chemical functionality	⊗ Neutral molecules (must still be ionized)	⊗ Very small amounts of sample ⊗ Deposition not straightforward
	SEEC	Molecular size	⊗ Very small (electro-osmotic) flow-rates ⊗ Ions present	

¹ Liquid-phase separation techniques that are not (or not yet) considered for use in combination with MS are not listed in this table.

² SEC = size-exclusion chromatography; HDC = hydrodynamic chromatography; FFF = field-flow fractionation; CE = capillary electrophoresis; CGE = capillary gel electrophoresis; CEC = capillary electro-chromatography; SEEC = size-exclusion electro-chromatography.

³ Packed-column HDC (using very small packing particles (≤ 2 μm) is considered here. This is a practical proposition for separating polymers (see G. Stegeman, *On hydrodynamic chromatography in packed columns*, PhD Thesis, University of Amsterdam 1994), unlike micro-channel HDC, in which open columns or channels of similarly small dimensions must be employed.

fractions of high-MW polymers may be obtained. This latter aspect is vital if good (MALDI-) MS results are to be obtained for such large molecules.⁶ Thermal FFF, in which a lateral thermal field is used to force polymers towards a collection wall, has long been considered as the most useful FFF technique for separating synthetic polymers. However, recent developments regarding the use of organic solvents in flow FFF (in which the lateral field is created by a cross flow through a semi-permeable membrane) are creating new possibilities. Hollow-fibre flow FFF now appears to be an ideal candidate for studying the applicability of MS in combination with liquid-phase separations for the analysis of very large polymers. It is a conceptually simple FFF technique that allows low flow-rates to be used.⁷

Electromigration techniques, of which capillary electrophoresis (CE) is the leading exponent, are much more commonly applied to natural (bio-)macromolecules than to synthetic polymers. Their on-line coupling to MS with an ESI interface is now quite common, but their off-line coupling with MALDI-MS is unexplored territory. This has everything to do with the difficulties associated with collecting fractions after electromigrative separations. In contrast, MALDI-MS is such a sensitive technique that its combination with microscale separations is not fundamentally difficult.

MS Techniques for Polymers

MS hardware: Time-of-flight (TOF) has quickly established itself as the preferred type of mass analyser for the characterization of (synthetic) macromolecules, both in combination with ESI and with MALDI. TOF combines a high sensitivity (because of a favourable duty cycle) with a broad mass range and a high spectral resolution and accuracy. In addition, TOF-MS instruments have become much more accessible and much more affordable in recent years. A TOF spectrometer is now the default instrument in our field. This kind of instrument has been used in the experiments described below, unless otherwise specified.

Electrospray ionization (ESI): In electrospray a flow of a liquid is dispersed into very fine droplets, while being subjected to a strong electric field. This ultimately leads to the association of intact molecules with one or more small cations or anions. Electrospray is a very soft ionization technique, that is, it yields virtually no fragment ions. This greatly simplifies the resulting spectra. Alternatively, multiple charges often occur on polar polymers, such as polyglycols. This complicates the spectra. Multiple ionization also allows larger molecules to be studied by ESI-MS. However, the technique is typically applied to study relatively low-molecular-weight polymers (or the low-molecular-weight fraction of a polydisperse sample).

When applying ESI-MS for the characterization of polymers, a high spectral resolution is beneficial. This allows the isotope pattern of multiply ionized peaks to be resolved. Even after a separation by, for example, SEC copolymers may still give rise to very complex ESI-MS spectra, because many different molecules may elute at the same retention time. Again, high-resolution MS is desirable. MS-MS is also an interesting option.

While it is legitimate to perform ESI-MS separations off-line after separation and fraction collection, it is quite feasible to perform LC-ESI-MS on-line and this will doubtlessly be the way forward. Optimum flow-rates for an electrospray are in the order of 30 $\mu\text{L}/\text{min}$ or less. This allows direct coupling

(without the need of postcolumn splitting) to LC columns with inner diameters of 1 mm or less.

To form a sufficiently large number of ions, ESI is most suitable for studying polar macromolecules. Fully organic (non-aqueous) eluents can be used, in which case a solution of a salt (often containing a polar solvent, such as isopropanol) is added between the column and the MS.

Electrospray is a form of atmospheric ionization, as is atmospheric-pressure chemical ionization (APCI). The latter technique is not quite as relevant for large (polymeric) molecules because vaporization is required to a larger extent. Because of the greater analyte volatility required, APCI requires higher temperatures than ESI. This can lead to thermal degradation of polymers but also of low-molecular-weight compounds, such as additives.

Matrix-assisted laser-desorption ionization (MALDI): MALDI was developed in 1970 (see reference 8). However, it is still a

Figure 1: Size-exclusion chromatogram of a polymer sample arising from the polymerization of 1,2-cyclohexanedicarboxylic acid anhydride (monomer C) and di-2-propanolamine (monomer D).^{3,4} SEC conditions: HP 1090 liquid chromatograph with refractive-index detection; four PL-gel mixed-C columns; dichloromethane containing 0.1 M ethylamine as eluent and sample solvent; flow-rate 1 mL/min; sample volume 200 μL .

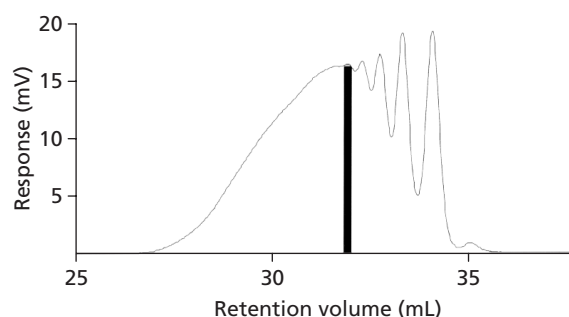
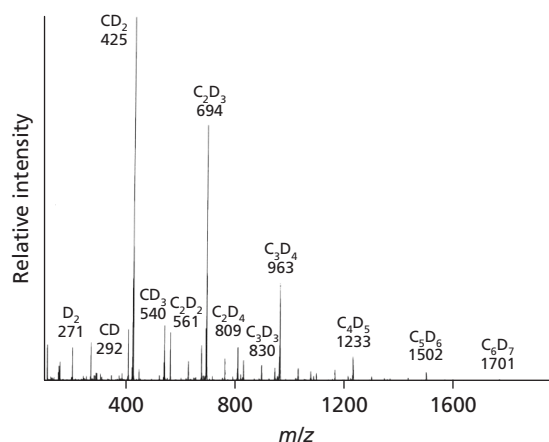


Figure 2: Off-line positive-ion ESI-MS spectrum obtained for the same sample as that of Figure 1. Instrument: PE Sciex API 150 single-quadrupole MS; infusion solvent: THF containing 20 mM sodium iodide at 5 $\mu\text{L}/\text{min}$; capillary tip at 5 kV; orifice at 30 V; mass spectra recorded in full-scan mode from $m/z = 200$ –2000 Da in 30 s.



relatively recent addition to the popular family of ionization techniques for MS. In MALDI, the sample is in contact with a matrix, the functions of which are (1) to absorb laser light and (2) to donate a charge to the analyte molecules. The laser can be directed at a specific spot. In our case this may be the location where a fraction of the LC effluent is deposited. This deposition can take place manually after fraction collection (off-line), using an automated deposition interface (facilitated off-line), or even on-line (see below). The matrix can then be added before (using a preconditioned well plate), during (by postcolumn addition of a matrix solution), or after the sample deposition. Concomitant application of the sample and the matrix is likely to give the best results, because 'co-crystallization' with the analyte is desirable.⁹

Remarkable results have been obtained with MALDI of synthetic polymers. It can be used for macromolecules with high polarities (such as proteins), but also with moderate polarities (such as polystyrene) or even low polarities, such as polyisobutylene.¹⁰ Useful spectra can be obtained from very high-MW samples, provided that the polydispersity is sufficiently low.⁶ However, MALDI-MS should not be considered a mature technique. Several aspects must still be improved in the coming years.

- The repeatability of MALDI-MS experiments is generally poor. Some 'shots' (laser pulses) may give no spectral sensitivity, while other shots on the same spot may yield beautiful spectra. In between these two extremes, the sensitivity of the MALDI-MS experiment is highly variable. Local variations in spot composition and morphology are likely to play a role, so that more-homogeneous spots may lead to greater precision.
- The selection of a suitable matrix for MALDI-MS experiments is still essentially based on trial-and-error. Generally, matrices that have been used successfully by others are suitable — but not necessarily optimal. The number of possible choices is increasing, enhancing the need for generally applicable matrix-selection rules.
- The lameness of MS can be particularly striking when using MALDI. Larger ions are discriminated against (or small

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molecules are favoured). In addition, molecules present in large concentrations may suppress the ionization of trace constituents, an effect known as ion suppression. In a sample of a polydisperse polymer, all sample components are trace constituents.

Recent Achievements

Several groups have studied the on-line coupling of MALDI^{11–14} and liquid chromatography. In most instances, peptides are the target analytes.^{11,14} However, the application of on-line SEC-MALDI-TOF-MS for the separation of synthetic poly(ethylene glycols) has been discussed in reference 13. The authors of this latter paper describe a seemingly simple interface that allows on-line interfacing of LC and MALDI. The effluent from the HPLC column is mixed with a solution of the matrix in a T-piece, the third leg of which is connected to the MALDI ionization chamber by a capillary tube, at the end of which a stainless-steel frit is glued. The LC effluent crystallizes together with the matrix on the MS side of the frit. A laser beam is used to effectuate the MALDI on the crystallized effluent. The idea is to continuously regenerate the interface through the combined actions of solvent flushing and laser ablation. However, so far the interface of reference 13 has only been used for short periods of time (5–10 s). This interface looks very promising, but the capacity of the vacuum system was said to constitute a limiting factor. The system will also need some changes before it can be applied in conjunction with non-aqueous eluents. For example, the PEEK tubing and epoxy resin may have to be replaced.¹

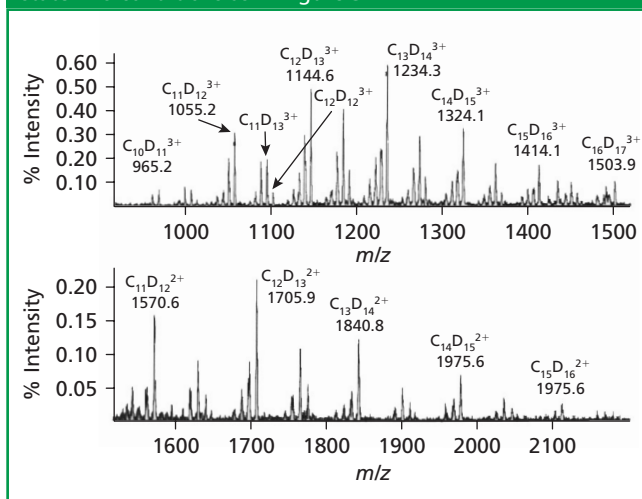
Advances in the direction of on-line LC-MALDI-MS are obviously desirable and research is to be strongly encouraged. However, because the (off-line) application of MALDI for the analysis of synthetic polymers is still immature (poor repeatability and robustness), the on-line coupling of SEC and MALDI is arguably premature.

Outlook

Arguably the most appropriate application of LC-MALDI-MS in the field of synthetic polymers is for calibration of a SEC system, that is, to convert SEC retention times into MW data. In case of homopolymers, MALDI allows accurate calibration to be performed without the need for standards. In case of copolymers it is virtually impossible to obtain suitable standards, but MALDI spectra are difficult to interpret because molecules of different molecular weight and composition may be eluted simultaneously.

There exist several established SEC detectors that allow absolute molecular-mass distributions to be obtained (see Table 3). In comparison with MS, such detectors yield additional information (intrinsic viscometry, degree of branching). They are particularly useful for high-MW polymers and (especially in the case of light scattering) for the characterization of suspended particles. MS is more suitable in the lower MW range, while it yields additional information on functional groups and chemical composition. It is clear from Table 3 that MS techniques are complementary to existing SEC detection systems.

Figure 3: Off-line positive-ion ESI-MS spectrum obtained for a narrow fraction of the SEC effluent of Figure 1 (indicated by the vertical bar in Figure 1). (a) 3+ charge state, (b) 2+ charge state. MS conditions as in Figure 3.



Both spectral interpretation and quantification are still challenging issues in the situation of copolymers. Comprehensive two-dimensional separations (e.g., SEC×LC) in combination with MS may ultimately be the way forward. Another important development in this context is the emergence of LC–NMR. The viability of this improbable combination is no longer in question. Now the question is not *whether* this combination will become a major tool for polymer analysis — but *when*. However, it is good to realize that NMR can only provide average (composition) data on polymer solutions or separated fractions, while MS — as a separation technique — may provide (qualitative or semi-quantitative) information on the distributions present within a fraction. Already, NMR and (MALDI-)MS have been used in tandem after SEC fractionation.^{15,16}

Complex polymers feature several simultaneous distributions. To characterize these, several simultaneous separations are indispensable. LC is a separation method. MS is both a separation method and a characterization method. As a two-dimensional separation method, LC–MS (or LC×MS) is a technique of immense value in polymer analysis. In a comprehensive two-dimensional separation system the characteristics of the separation methods should be selected such as to match the structural properties that distinguish between the different components (molecules) in the sample. The result of such a judicious selection may be order in the resulting two-dimensional chromatograms.¹⁷ The overall picture makes it much easier to distinguish, for example, between the different ionization states in LC–ESI–MS.¹⁸ In LC–MS we must learn to look at this entire forest, before barking up any given tree.

LC has got no eyes. The blindness of LC is incurable. Liquid chromatographers need friends to show them what they have achieved. The lameness of MS is not a fundamental deficiency. It will not simply be outgrown in time, but it may be cured with great effort. At least, the seriousness of the condition may be reduced. It takes a pessimist to sing that mass spectrometrists will never walk alone. However, until they do, they can rely on their LC friends to lean on.

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* The extent to which molecules vary in molecular weight is often underestimated. The width of polymer distributions is usually expressed in terms of the polydispersity ($D = M_w/M_n$, i.e., the ratio between the weight-average and number-average molecular weights). A polymeric sample with $D = 1.05$ is considered to be approximately monodisperse (a 'narrow standard'). Yet, the relative standard deviation of the molecular-weight distribution is equal to $\sqrt{(1 - D)} = 22\%$. From the perspective of an analytical chemist and, especially, that of a chromatographer, narrow polymer standards represent broad peaks. A distribution with $D = 1.05$ is as broad as a chromatographic peak with $(1/(D - 1)) = 20$ plates!

** The peaks at $m/z = 965, 1055, 1144, 1234, 1324$ and 1411 are associated with the cationization with three sodium ions (i.e., $[C_nD_{n+1} + 3Na]^{3+}$ ions). The second series of peaks at $m/z = 1002, 1091, 1181, 1271, 1361$ and 1451 are assigned to $[C_nD_{n+2} + 3Na]^{3+}$ ions. Finally, the peaks at $1099, 1189, 1278$ and 1368 originate from triply sodicated cyclic $C_nD_n - H_2O$ oligomers. Other peaks in the spectrum are the result of ionization by one or more protons instead of sodium cations, i.e., $[M + 2Na + H]^{3+}$, $[M + Na + 2H]^{3+}$ and $[M + 3H]^{3+}$.

