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Architectural Differentiation of Linear and Cyclic Polymeric Isomers by Ion Mobility Spectrometry-Mass Spectrometry

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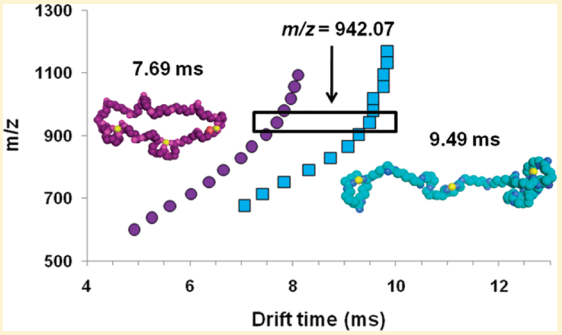
Supporting Information

ABSTRACT: An extra dimension of polymer analysis: ion mobility spectrometry-mass spectrometry (IMS-MS) separates ions according to their size in the gas phase, allowing differentiation of linear and cyclic polymeric isomers. This analytical technique is a rapid and sensitive method for assessing cyclic polymer purity in one step. As highly pure cyclic polymers are crucial for adequately assessing architecture dependent properties, IMS−MS offers great promise in the characterization of this unique class of polymers.



Recent synthetic improvements have enabled the more efficient preparation of cyclic polymers, which in turn should enable a better understanding of the unique behavior of this macromolecular topology.[1−3] The cyclic topology combined with the lack of chain ends imparts a unique set of physical properties on polymer macrocycles, including increased glass transition temperatures (T_g), smaller hydrodynamic volumes, and lower intrinsic viscosities when compared to their linear analogues.[4] Furthermore, cyclic macromolecules demonstrate unique degradation behavior[5] and biophysical properties which can be advantageous for drug delivery applications.[6,7] However, most preparative techniques are susceptible to the generation of linear byproducts, yet, well-defined, high purity polymeric species are crucial for adequately assessing architecture dependent biological and physical properties.[8] Unfortunately, no single analytical technique has been established to unambiguously characterize the purity of cyclic polymers (in particular to confirm the absence of trace amounts of linear polymer), however, tandem ion mobility spectrometry−mass spectrometry (IMS−MS) offers promise to successfully evaluate the architectural purity of polymers. Here, the contrasting conformational states of linear and cyclic polymers provide evidence for the effect that the cyclic confinement has on polymer conformation and can perhaps provide some insight into the behavior of cyclic polymer in solution phase as well as in bulk, especially in conjunction with detailed molecular modeling of cross-section values.

Traditional small molecule characterization techniques, such as NMR or IR spectroscopy, are limited in their ability to identify trace impurities because the signal of the polymer backbone often overwhelms that of the end groups, and the signal that is observed only measures the average structural features of a polymer. When linear analogues are available, size exclusion chromatography (SEC) has been used as one of the primary means for confirming cyclic polymeric architecture. The reduced conformational freedom of the repeat units in a cyclic polymer results in a smaller hydrodynamic radius and a longer elution time in SEC relative to linear species. However, the presence of trace structural impurities within a sample of polymer macrocycles can be obscured by the inherently low resolving power of this technique.

MS can provide complementary information that includes accurate mass measurement of each unique polymer chain length, detailed end group analysis, and fragmentation for structural characterization.[9] These analyses are enhanced by high mass resolution, mass accuracy, and superb sensitivity of the MS method. Differentiation of mixture composition, however, is only possible if the impurities exhibit different mass-to-charge (m/z) ratios, but it is not possible in this case where linear and cyclic polymers exhibit identical molecular weight (MW).[5,10−13]

While traditional SEC provides separation by size, and traditional mass spectrometry provides separation by mass, the 2-dimensional character of IMS-MS data enables direct correlation of each macromolecule's mass (m/z) with its size (drift time), enabling a more detailed characterization of architecturally diverse polymers. IMS separates ions in the gas-phase according to the number of charges and cross-section (size and shape).[14] Joining the IMS approach with traditional MS combines sensitivity and mass resolution for the analyses of the molecular composition of samples and imparts the ability to distinguish differences in shape for the same m/z. For example,

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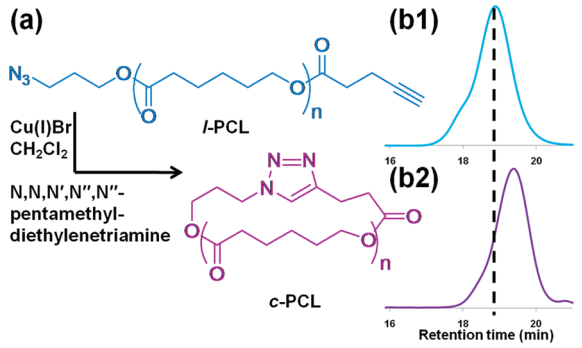

Figure 1. (a) Structurally isomeric *l*-PCL and *c*-PCL and synthetic conditions (b) SEC traces for (b1) *l*-PCL and (b2) *c*-PCL showing a shift to longer retention time for the *c*-PCL species.

IMS−MS has been used to differentiate between a protein in its native folded state and its intact, but denatured analogue.[15] In the case of synthetic polymers, IMS has been used to detect low abundance oligomers,[16] differentiate end groups,[17] analyze different side chain isomers,[18] elucidate different block copolymer ratios,[18] and separate polymer blends.[18] Linear and cyclic hexacadmium complexes can also be distinguished, offering precedence for distinguishing these macromolecular architectures.[19]

Cyclic poly(ε-caprolactone), (*c*-PCL, $M_{n(MALDI)}$ = 2300 Da) and its linear precursor (*l*-PCL) of the same MW were prepared using a click cyclization approach and their structure confirmed via GPC, MALDI-TOF MS, and NMR (Figure 1 and Supporting Information).[5] IMS−MS characterization was performed using a Waters SYNAPT G2 mass spectrometer with polymer ions produced by electrospray ionization (ESI) with the aid of LiCl as the cation. The IMS−MS data of the two isomeric samples show drastically different behavior both in terms of the number of charges accommodated by each architecture and their cross-sectional area in the gas phase.

The 2-dimensional (2-D) plot of drift time versus *m*/*z* (Figure 2, right) shows that the charge state families (assigned via data extraction in DriftScope software, see Supporting Information) are well separated in the drift time dimension for the *l*-PCL but not for the *c*-PCL. Notably, singly charged species are not observed. Singly charged ions can be produced in ESI,[18] but ionization is size dependent.[16] The polymer systems studied here are large enough to accommodate two and more charges. With increasing number of charges, PCL travels more slowly in the IMS dimension, especially in the case of *l*-PCL (Figure 2, a2). While it is typical for macromolecules, especially proteins, to exhibit faster drift times with respect to increased charge, slower drift times with respect to charge have also been reported for PEG.[18] A "beads-on-a-string" model was used to explain the charge inversion phenomena with flexible linear PEG in which charge repulsion yielded a more extended structure and thus a slower drift velocity with an increase in charges.[16,18] Similar to these previous reports for linear PEG, *l*-PCL shows distinct folding transitions for charge states +2 to +5 (Figure 2, a2). These folding transitions are attributed to a conformational collapse of the structure, resulting from changes in the preferred conformation of the polymer with respect to increasing molecular weight, while stabilizing the multiple associated cations.

Conversely, *c*-PCL in charge state +2 and +3 maintains a relatively constant ratio of drift time versus *m*/*z* over the same mass range indicating a more consistent conformation regardless

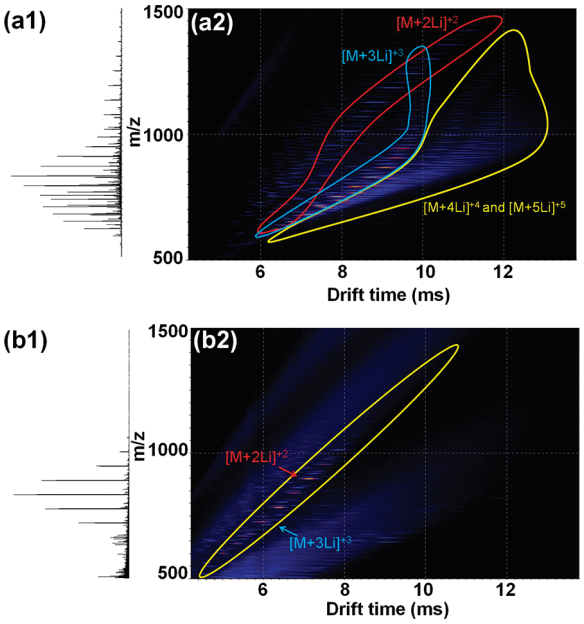

Figure 2. IMS−MS 2-D plot of (a) *l*-PCL and (b) *c*-PCL. The intensity of the ions detected are incorporated as a false color plot with red as the most abundant and blue as the least abundant ions. The respective charge state families, determined by the extracted mass spectra (Figures S1−S10, Supporting Information) are indicated in the 2-D plots for both samples.

of the number of charges (Figure 2, b2). Likewise, the consistent linear trend despite increasing MW suggests that the folding transitions observed for *l*-PCL do not occur in the same mass range for their cyclic analogues.

To enable direct comparison of these two data sets, the extracted mass spectral and drift time data from equivalent charge states of *c*-PCL and *l*-PCL are plotted in the same graph (Figure 3). *c*-PCL exhibits shorter drift times relative to *l*-PCL for both the +2 and +3 common charge state families. This is due to a more compact structure introduced by the covalent confinement in *c*-PCL and is in agreement with conventional IMS theory.[20] The +3 charge state occurs for *l*- and *c*-PCL in the *m*/*z* range of 600 to 1092 and therefore significantly larger in MW (1785 to 3267 Da) than the PCL architectures ionized by two charges (*m*/*z* range 611 to 1296, MW range 1215 to 2584 Da). As previously reported, the smaller oligomers can only accommodate two cations, while the larger oligomers bind to three and more depending on the flexibility of the polymer topology.[16]

A more detailed explanation of the gas-phase separation of isomeric architectures using an IMS−MS approach must take into account the competing forces of Coulombic repulsion,[16,18,21,22] and complexation (solvation) of the cations. In the case of more than one cation, Coulombic repulsion drives the cations apart but this force is dampened by cations that are well solvated by the ester oxygens. For smaller oligomers, the repulsion between nearby cations is particularly strong (being inversely proportional to square of the distance between the charges) favoring a Bretzel-like conformation[16] of the polymer bound to two cations. In the case of low MW cyclic polymers, structural confinement prevents the extended conformation, resulting in shorter drift times. For example, +2 the *l*-PCL 11-mer at *m*/*z* 725 exhibits a longer drift time (7.06 ms) than its cyclic isomer (5.89 ms) (Figure 3a). However, as the length of the polymer chain increases, the strength

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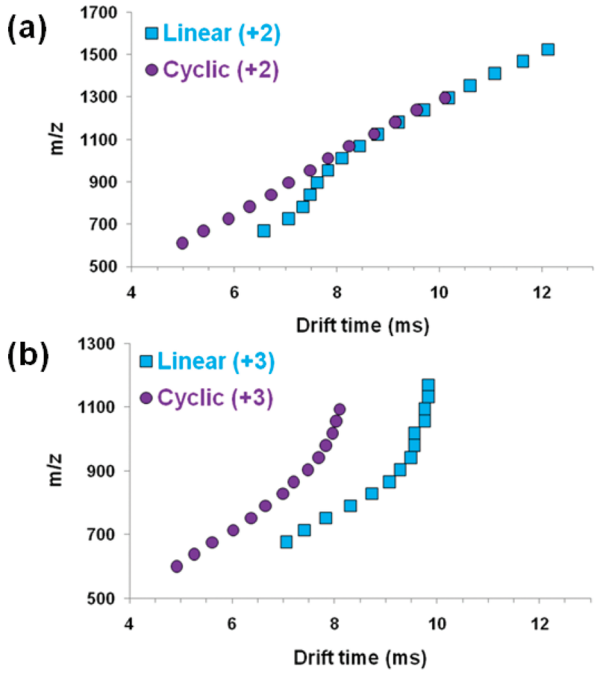

Figure 3. Comparison of equivalent charge state families (a) +2 and (b) +3 for *l*-PCL (square blue markers) and *c*-PCL (purple circular markers).

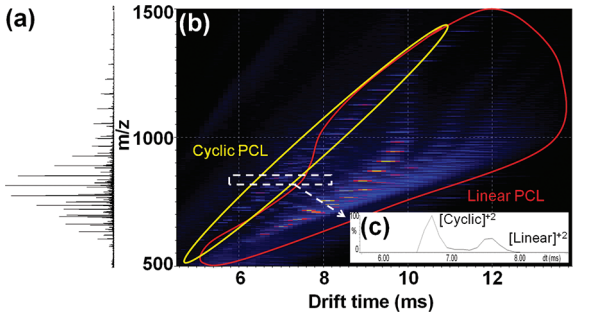

Figure 4. Mass spectra (a) and IMS−MS 2-D plot (b) of an intentional mixture of *l*-PCL and *c*-PCL. The intensity of the ions detected in the 2-D plot are incorporated as a false color plot with red as the most abundant and blue as the least abundant ions. The same charge state families are for each species in the mixture as are observed running each sample separately. Inset c shows the drift time spectrum for the selected exact mass peak at 893.13 *m*/*z*, corresponding to the +2 13-mer for both species. The drift time distribution is distinctly bimodal, indicating the two different isomeric architectures present at 839.13 *m*/*z*.

of the Coulombic repulsion decreases, enabling polymer chains to fold in order to stabilize the two cations via additional intermolecular interactions. For +2 *l*-PCL, this folding is evident from *m*/*z* 750 to 1000 as increasingly larger polymers shift to a more compact structure, very similar to that observed for the *c*-PCL. Notably, *c*-PCL exhibits a consistent ratio of *m*/*z* to drift time, suggesting it is not susceptible to folding in the studied MW range.

In the +3 charge state, the larger MW of the PCL ionized along with the additional Coulombic repulsion of a third Li cation results in similar distinction in drift time between *l*-PCL and *c*-PCL. Folding transitions are observed for both *l*- and *c*-PCL (initiated around *m*/*z* 900, Figure 3b), however it is important to note that the drift time distributions do not converge as they did in the +2 charge state, indicating distinct gas phase conformations across the studied molecular weight range.

Additionally, in purposefully mixed samples of *l* -PCL and *c*-PCL, the isomeric architectures remain distinct below the folding transition of *l* -PCL in the +2 charge state and throughout the +3 charge state (Figure 4). For example, in the +2 charge state at *m*/*z* 839 the two isomers are baseline separated, with the *l*-PCL 13-mer exhibiting a longer drift time (7.61 ms) than its cyclic isomer (6.73 ms) (Figure 4c). Both of these drift times in the mixture agree closely with the drift times observed when the samples are analyzed separately (Figures S11−S13, Supporting Information). This confirms the ability of IMS−MS to isolate and unambiguously identify polymers that are structural isomers, providing an invaluable technique for both confirming the cyclic architecture of *c*-PCL, and probing its architectural purity.

In conclusion, IMS−MS is a powerful, rapid, and sensitive tool to clearly distinguish between cyclic and linear polymeric architectures. The unique structural signatures of linear and cyclic polymers are expected to be useful in verifying the "cyclic purity" of polymers, though additional studies with alternative backbones will need to be pursued to understand the general trends independent of backbone chemistries.

■ ASSOCIATED CONTENT

Supporting Information. Full experimental procedures and characterization, ESI−IMS−MS sample preparation and acquisition parameters, and ESI−IMS−MS data extraction procedures, including extracted mass spectra corresponding to each charge state. This material is available free of charge via the Internet at http://pubs.acs.org/.

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