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Editorial

There is Signal in Your Noise: A Case for Advanced Mass Analysis

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WHY WE NEED NEW ANALYTICAL TOOLS

Synthetic chemists often take modern characterization techniques for granted and do not appreciate the fortuitous process of analytical development. Imagine yourself as a 1950s chemist without easy access to spectroscopic instrumentation: how would you unambiguously assign chemical structures to small molecules? This question fueled an explosive growth of technologies that provide molecule-specific fingerprints. The advent of classical characterization tools (*e.g.*, NMR spectroscopy, mass spectrometry) accelerated the rate of discovery in organic chemistry as they provide sufficient information to deduce the identity and purity of a sample. In the context of polymer synthesis, however, these classical tools only provide insights related to bulk composition and often fail to fully capture terminal group speciation. As an unintended consequence, many graphical representations omit the chain-ends. Despite the lack of comprehensive knowledge, ambitious research programs have sparked a renaissance of renewed interest in developing new analytical methodologies. Post-polymerization reactions, for example, often exploit end-groups for practical applications (*e.g.*, upcycling,[1] dynamic network cross-linking[2]). A need, therefore, exists for new tools that uncover currently elusive structural details.

Advanced mass analysis has begun to reemerge as an effective solution to these problems. While the initial development of Kendrick analysis dates to the early 1960s,[3] only recent work from Fouquet and co-workers[4−8] developed the tools necessary for adaptation to polymer characterization. The power of this mass spectral method is readily apparent in Figure 1. Signals are elegantly pulled from the noise in a traditional mass spectrum by deconvoluting the data and rendering it across multiple dimensions. The resultant Kendrick plot extracts compositional information within a homologous series of polymers. Despite its obvious utility, Kendrick analysis has not yet received the attention it deserves nor is it a common-place technique. Indeed, synthetic polymer chemists are often unaware of its exis\tence despite routinely acquiring mass spectra (*e.g.*, MALDI). Indeed, we only learned of Kendrick analysis from an enlightening tutorial by Fouquet[7] entitled "The Kendrick analysis for polymer mass spectrometry".

Our serendipitous introduction to this technique occurred while we were characterizing complex mixtures of oligomers derived from dicyclopentadiene (DCPD).[9] Monomer resins with the second generation Grubbs catalyst (G2, [(SIMes)-Ru(=CHPh)(PCy_3)Cl_2]) produced short chain oligomers when in the presence of a chain-transfer agent (CTA; *e.g.*, styrene). Traditional characterization techniques approximated the molecular weights of the resultant materials but did not report on the chain-end speciation or fate of the pendent cyclopentene groups. While the MALDI pattern revealed the existence of multiple species, only Kendrick analysis provided definitive information as to their identities. At least seven types of species existed that varied by the number and type of chain-ends. Importantly, this technique revealed that cyclopentene ring-opening occurs; this is often invoked as, but rarely demonstrated to be, the cause for cross-linking in p(DCPD) thermosets. This technique also detected trace impurities within the monomer or CTA, oxidation products, and unusual monomer chain-transfer events (Scheme 1).[9]

SO, HOW DOES KENDRICK MASS ANALYSIS WORK?

In a seminal 1963 *Analytical Chemistry* report, Edward Kendrick[3] elegantly exploited mass referencing to extract detailed information about the composition of a homologous series of small organic molecules. In particular, Kendrick wanted to simplify the problem of computing, storing, and retrieving masses of common fragments (i.e., frequently occurring structural variants). Historically, ^{12}C and ^{16}O were competing mass definitions, with integer exact mass values of the reference isotope (i.e., 12.0000 and 16.0000, respectively). Prior to the advent of modern computers, internal calibration of mass-spectrometers required comprehensive tables of exact masses. For example, tables reported by Beynon[10] possessed ≈6000 distinct entries for a restricted subset of organic compositions (*e.g.*, m/z < 250, C/H/N/O only, <6 heteroatoms). Extrapolation to heavier species or compositions that include S and ^{13}C necessitates the calculation of exact masses for over 1.5 million distinct fragments.[3]

To address this problem, Kendrick[3] switched to a system indexed against methylene, which requires fewer than 100 000 discrete calculations to describe the same set of fragments. This so-called Kendrick mass relies on the definition of atomic mass; by IUPAC conventions, ^{12}C possesses an exact mass of 12.0000. Consequently, any fractional, noninteger values result from atom types other than ^{12}C (Figure 2, blue diamonds). Hydrogen and methylene, for example, possess exact IUPAC masses of 1.0079 and 14.0157 Da, respectively. Kendrick

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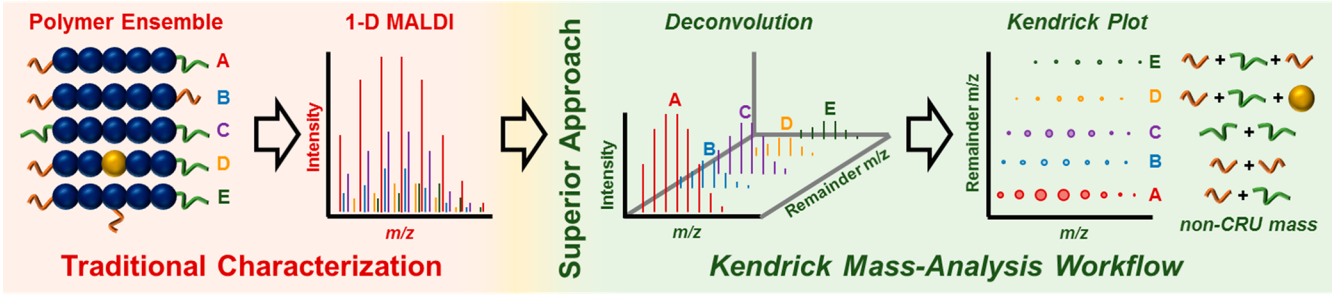
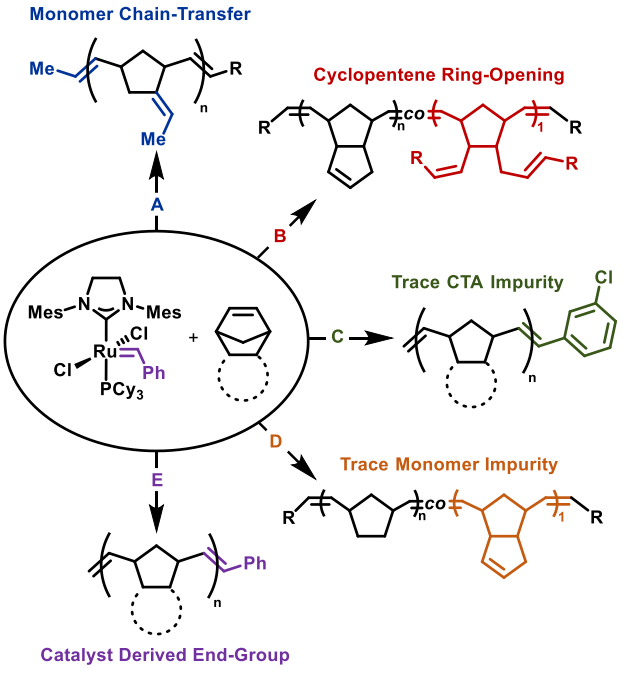


Figure 1. Workflow of Kendrick mass-analysis. Left: Traditional mass spectrometric characterization of polymers (i.e., MALDI-MS) provides a one-dimensional spectrum. Identification and assignment of all species in an ensemble of polymers with a distribution of molecular weights, chain-end types, and number of end groups per chain is challenging. Right: Application of a second-dimension resolves species with identical nonconstitutional repeating units (CRU; *e.g.,* chain-ends, adducting ions, comonomers). The diameter of each circle in the Kendrick plot is proportional to the intensity of the corresponding MALDI peak.

Scheme 1. Unusual Species Uncovered by Kendrick Analysis[a]



[a]Oligomers were generated by frontal ring-opening metathesis oligomerization (FROMO). Conditions. (A/B) FROMO of 5-ethylidene-2-norbornene (A) and DCPD (B) catalyzed by G2 with styrene as the CTA. (C) FROMO of DCPD catalyzed by G2 with 3-bromostyrene as the CTA. Trace 3-chlorostyrene impurities existed in the CTA, as reflected in this detectable species. (D) FROMO of norbornene catalyzed by G2 with styrene as the CTA. Trace DCPD impurities in the monomer source resulted in detectable quantities of this co-oligomer. (E) FROMO of DCPD catalyzed by G2 and terminated with either 3-bromostyrene or ethyl vinyl ether. This species is likely the first oligomer generated and contains a precatalyst derived end-group. Figure reprinted and adapted ref 9. Copyright 2022 American Chemical Society.

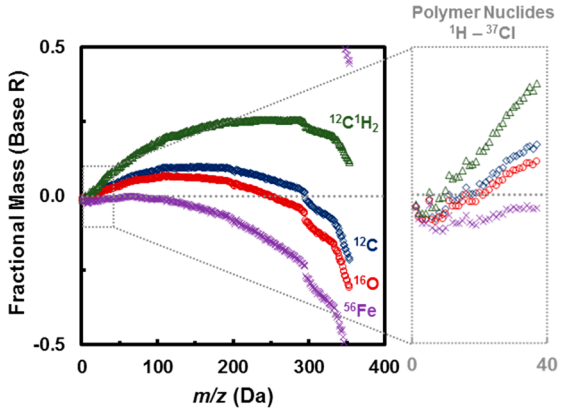


Figure 2. Fractional masses of stable nuclides derived from different Kendrick mass scales (≈ 350, m/z reported by the National Institute of Standards and Technology[11]). Kendrick masses were determined using eq 2, and the corresponding reference isotope is provided next to each curve. Fractional masses were calculated with eq 3. Inset: Common, low-mass nuclides most relevant for polymer chemistry (i.e., ^{1}H through ^{37}Cl).

redefined methylene's monoisotopic mass (i.e., $^{12}C^{1}H_2$) to 14.0000 and adjusted other mass fragments by the ratio described in eq 1,

$$m_k = m/z \times \frac{14.0000}{14.0157} \quad (1)$$

where m_k and m/z are the Kendrick and IUPAC masses of the species of interest, respectively. With this new scale, decimal mass values arise from non-methylene units (Figure 2, green triangles). In fact, mass analysis requires a nonquantized definition of m/z. If atomic masses equaled the sum of the corresponding proton and neutron numbers (i.e., integers), then every nuclide would lose its unique mass fingerprint and Kendrick analysis would lose its usefulness. Chemical insight with this method derives from a nuclide's noninteger mass!

For polymer analysis, one may imagine that any constitutional repeat unit (CRU) acts as a convenient reference point. Specifically, the mass of a monomer (or repeat unit mass more generally) serves as an ideal base unit. The general expression in eq 2 defines the Kendrick mass relative to any CRU with an IUPAC mass of R,

$$m_k(m/z, R, x, Z) = m/z \times Z\frac{x}{R}$$
$$Z = \{1, \ldots, z, z+1, \ldots\}$$
$$\text{floor}\left(\frac{2}{3}R\right) < x = \frac{z}{Z}\left(\text{floor}\left(\frac{2}{3}R\right) + n\right) < 2R; \quad n \in \mathbb{N} \quad (2)$$

where m_k and m/z retain their definitions from above, Z is a scaling integer associated with the charge of the molecular

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fragment,[12] x is a bounded variable integer,[13] and n is an element of the natural numbers.[14]

The true power of Kendrick analysis, however, comes from pulling signal from the noise; mass spectral data is projected onto multiple dimensions, as highlighted Figure 1.[4−8,15,16] For most homopolymers, a series of evenly spaced peaks separated by the CRU mass is observed. As an example, see the interactive Excel file included in our recent *Macromolecules* article (DOI: 10.1021/acs.macromol.2c01654);[9] readers are encourage to adapt and apply this "code" to their own polymer analyses. It is also not uncommon to identify multiple sets of species that share the same CRU fragment, but vary by the number and type of chain-ends. These series are mass shifted with respect to one another by these non-CRU groups. In many cases, one set is more predominant than the others. While typical one-dimensional MALDI spectra enable facile identification of the most intense series, detection of minor species is challenging for several reasons. First, differentiation of true signals from random noise introduces bias from the researcher to answer the following question: does a specific peak "fit" with an obvious molecular composition or does it result from some random impurity? Second, manual peak by peak assignment is often laborious, which creates a practical barrier for thorough analysis. These difficulties often encourage researchers to ignore subtle complexities buried within their data. Indeed, it is all too common for low intensity secondary series to be dismissed or unnoticed in many published MALDI data. With Kendrick analysis, however, signals are easily differentiated from noise. Lone peaks that do not correlate with any series are easily assigned as a nonpolymeric species (*e.g.*, matrix-derived small molecules). In great contrast, homologous series of peaks separated by the CRU mass result solely from polymerization reactions, even if the peak intensities are much lower than the primary sequence. We argue that these secondary series contain highly useful information about the polymer architecture and specific details involved in the reaction itself (*e.g.*, chain-transfer pathways).

The nature of the added dimension depends on the minimum spectral resolution (i.e., $\Delta m_{50\%} = |m_2 - m_1|$) and resolving power ($m/\Delta m_{50\%}$) of the employed mass analyzer.[17] High-resolution multipass time-of-flight (TOF) instruments, for example, typically possess resolving powers of $\approx 10^4$ to 10^5, whereas modern FTICR mass analyzers can have resolving powers > 10^6. In cases with $m/\Delta m_{50\%} > 10^4$, individual isotopomers are easily distinguishable due to the excellent accuracy and reliability of the decimal significant figures.[17] Kendrick analysis of such data, therefore, employs the fractional mass (FM). The FM of a given m_k is defined by eq 3 and bounded as a result of a discontinuity created at the switchover between rounding up vs down (i.e., 0.5). Species with identical non-CRU elements (*e.g.*, chain-ends, adducting ions, isotopomers, etc.) exhibit nonzero FM values and horizontally align in a Kendrick plot of FM vs m_k (or m/z), as highlighted in Figure 1. The excellent resolving power, however, comes at the expense of signal intensity at high m/z values.

$$-0.5 < \mathrm{FM}(m_k) = \mathrm{round}(m_k) - m_k \leq 0.5 \tag{3}$$

Kendrick analysis has historically employed high-resolution data sets, particularly in the context of small-molecule identification and fragmentation. In contrast, the needs of a polymer chemist generally do not require high resolutions, as it is often more desirable to access a broad range of m/z values. Developments in modern hardware (particularly linear-mode MALDI-TOF/TOF) overcome this challenge, and provide lower resolution data without a theoretical m/z upper limit.[17] In the low-resolution regime, the limited accuracy and reliability of the FM values necessitates a different variable to project onto. The remainder of the Kendrick mass (RKM) provides sufficient discriminating power to differentiate species of nonidentical CRUs. As defined in eq 4, the RKM represents the remaining, noninteger values after dividing m_k by R (eq 5).[18] Moreover, the RKM relates to the mass sum of non-CRU elements as described in eq 6. For exceedingly small values of R or non-CRUs with large mass values, an aliasing problem arises; the actual mass of non-CRU units may equal the RKM plus multiple R values. As with the FM, species with identical non-CRU components horizontally align in an analogous Kendrick plot of RKM vs m/z.

$$0 \leq \mathrm{RKM}(m_k, R) = m_k \bmod R < R \tag{4}$$

$$\frac{m_k}{R} = m + \frac{\mathrm{RKM}}{R};\ \ m \in \mathbb{N}^0 \tag{5}$$

$$\mathrm{RKM} + pR = \sum m/z(\text{non-CRUs});\ \ p \in \mathbb{N}^0,\ p \leq m \tag{6}$$

An added benefit of the Kendrick workflow relates to the identification of signal from noise and reduction in researcher bias. Low intensity peaks within a series are easily differentiated from random noise as they are evenly spaced by R. In contrast, nonpolymeric peaks (*e.g.*, matrix-derived) exist as separate, unaligned singular entities. In sum, Kendrick analysis effectively boosts the resolving power within a data set without the need for state-of-the-art equipment. As a result, there no longer exists a need for a conservative peak-picking algorithm in data preprocessing to "weed-out" superfluous noise signals.

It is also worth considering the limitations of Kendrick analysis to ensure proper implementation. As with any mass spectrometric technique, *the absence of evidence is not the evidence of absence*. Only easily ionized species that reach the mass detector are observed. Highly cross-linked materials, for instance, typically do not ionize. Similarly, large polymers (m/z > 10 kDa) are often difficult to detect. Second, the ionizabilities of wildly different species are not necessarily identical; the relative signal intensities do not always accurately reflect concentration differences within a sample. With linear-mode low resolution data, isotopic drift occurs at higher m/z values, as the peaks correspond more closely with MW than with the monoisotopic exact mass. We have previously discussed these limitations in greater detail in the Supporting Information of our recent *Macromolecules* article.[9]

FUTURE DIRECTIONS IN POLYMER SYNTHESIS: COPOLYMERS

While the conventional wisdom in polymer chemistry advises against analysis of copolymer MALDI spectra, Kendrick analysis provides a straightforward workflow to dissect copolymers. Fouquet and colleagues[5,8] adapted Kendrick analysis to short-chain ($\bar{M}_n$ < 1.5 kDa) copolymers of the type p(ethylene-*r*-vinyl acetate). Specifically, the authors derived m_k, FM, and RKM values using R values associated with both monomers. In this way, a degree of polymerization plot was constructed, which enumerates the monomer composition of all copolymer species within the material. This example, however, is limited by the high resolution

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required for FM determination. Ongoing efforts from our laboratory are underway to extend this methodology to low-resolution data sets.

WHY MASS ANALYSIS IS NOT YET UBIQUITOUS

Despite the enormous potential for Kendrick analysis, it has yet to break out into the mainstream. While many reasons for this may exist, we speculate that this technique lacks a synthetic champion; for analogy, consider the development and widespread implementation of NMR spectroscopy.[19,20] In 1939, Rabi and co-workers[21,22] first observed NMR in isotopes of Li and F, as well as in H_2. A decade later in 1950, Gutowsky and Hoffman[23] introduced the concept of chemical shift in the context of ^{19}F magnetic resonance. Widespread usage in organic chemistry, however, only began to occur in the mid-1950s. Classic work in 1958 by Shoolery and Rogers[24] demonstrated that NMR spectroscopy on low-field magnets could identify the structure of steroidal compounds. Shortly thereafter in 1959, Karplus[25] demonstrated the NMR *J*-values associated with three-bond vicinal coupling were angle dependent. In the same year, two key textbooks by Jackman[26] and Roberts[27] paved the way for the ubiquitous usage of NMR spectroscopy in organic synthesis. By the late 1960s, physical-organic chemists utilized NMR spectroscopy to deduce rate constants associated with chemical exchange processes.[28] Fast-forward to the present; chemistry departments typically have several high-field instruments. Work by Fulmer et al.,[29] which catalogued the chemical shifts for common laboratory solvents and impurities, highlights the routine nature of modern NMR spectroscopy. This work has >2300 citations and >662 000 article views according to metrics gathered by ACS as of October, 2022.[29]

In contrast to NMR spectroscopy, we believe that Kendrick analysis has yet to escape the mass spectrometry and analytical chemistry communities despite its obvious utility. Indeed, our earlier work highlighted the benefits of this method to study thermoset fabrication *via* frontal ring-opening metathesis polymerization. While analytical limitations associated with cross-linked thermosets complicate detailed understanding of the chemistry involved in curing, Kendrick analysis of oligomeric models provided a clear path to overcome this challenge.[9] It is our hope that our recent example and this editorial will spark permeation of Kendrick analysis into the broader synthetic organic and polymer fields. Perhaps one day, Kendrick analysis will be a common technique in a synthetic chemist's analytical toolkit.

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Notes

Views expressed in this editorial are those of the authors and not necessarily the views of the ACS.

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(12) For purely hydrocarbon polymers, *Z* typically equals 1; introduction of charged pendant fragments (or chain-ends), however, can result in multicharged species that necessitate other *Z* values.
(13) A floor function rounds a value down to the nearest whole integer. Consider the following example where *R* = 132.0939 and *Z* = *z* = 1. In this case, the lower *x* boundary is defined as floor ((2/3)*132.0939) = floor(88.0626)=88. The upper *x* boundary in this example is 2*132.0939 = 264.1878. Hence, *x* can equal 89 through 264.
(14) $\mathbb{N}$ denotes the set of natural numbers.
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