Characterization of Randomly Branched Polymers Utilizing Liquid Chromatography and Mass Spectrometry

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Abstract

Branching in polymers is an important way to modify the materials properties, however the characterization of random branching in polymeric materials is a challenge in polymer analysis. In this work, liquid adsorption chromatography methods are developed for a commercially available hyperbranched polyester (Boltorn™). This chromatographic techniques was then coupled to offline MALDI-TOF MS analysis, a first in the analysis of randomly branched polymers. The coupling of these two techniques provides superior MALDI-TOF spectra, enabling the easy identification of structural subdistributons based on theoretical molecular weight. Detailed analysis of the MALDI-TOF spectra shows that these chromatographic conditions separate cyclic Boltorn polymers (with no core molecule) from non-cyclic polymers (with core molecule), and these are the only two architectures observed. MALDI MS also confirms that the chromatographic separation mode is adsorption, but further analysis is needed to determine if there is a separation by degree of branching.

Keywords: *hyperbranched polyesters, polymer mass spectrometry, polymer chromatography, liquid adsorption chromatography, MALDI-TOF MS*

1. Introduction

The incorporation of branching into linear polymers is recognized to significantly alter their materials properties. Specifically, increased branching lowers the crystallinity and rigidity of polymeric materials, as illustrated by the wide range of polyethylene applications which result from statistically varying the amount of branching. Furthermore, as branching increases, the amorphous regions and the number of end groups also increase. These two properties are important to consider for the biomedical application of polymers, as increasing the amorphous regions

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and the number of end groups has been shown to lead to an increase in the degradation rate of polyesters.[1] Branched polymers can be divided into two main categories: regularly branched polymers (stars, dendrimers, combs, etc.) and randomly branched polymers (hyperbranched polymers, polyethylene, polyacrylates, etc.). The main difference between these two categories is that randomly branched polymers (RBPs) contain a distribution in the number and/or size of branches, whereas regularly branched polymers do not. Additionally, in RBPs geometrical isomers can be formed, with identical molar mass and amount of branching.[2] Thus, RBPs present a greater challenge for polymer analysis, as precise characterization of both the amount and arrangement of branching (i.e. degree of branching (DB)) is difficult.

The most widely utilized characterization method to determine the DB of RBPs is NMR spectroscopy (^{1}H, ^{13}C, and/or ^{1}H COSY), with peak assignment generally aided by the synthesis of model compounds corresponding to each type of structural unit of the RBP (i.e. terminal, branched, or partially branched monomer units).[3] While this technique is relatively effective, it is both time consuming and potentially limited by the fact that the signal of the polymer backbone can quickly overwhelm that of the branching points, if only small degrees of branching are incorporated. This is an important consideration as only small degrees of branching are required to dramatically alter materials properties.

Another commonly utilized characterization technique is size-exclusion chromatography (SEC), which separates polymer chains according to hydrodynamic volume. When coupled to a multiangle laser light scattering detector (MALLS) and/or a viscosity detector, it is possible to obtain information about the number average molecular weight (M_n) or weight average molecular weight (M_w) during the elution chromatogram.[4-7] However, this does not overcome the fundamental problem of SEC for RBPs- namely, co-elution of different molecular species due to the heterogeneity of both molecular weight and structural topology of RBPs- and thus molar mass and size information again only represent averaged values at any time point during the elution.[7]

Mass spectrometry, specifically matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), can provide complementary information for polymer analysis, including accurate measurement of the degree of polymerization (DP), detailed end group analysis, and fragmentation to enable structural characterization,[8] however its utility for RBPs is largely unrealized to this point, as RPBs are typically polydisperse, and often high in molecular weight, and both of these factors can complicate MS analysis. Wolf and Frey report that it is possible to distinguish subdistributions in the MALDI-TOF spectra of hyperbranched poly(lactide) copolymer, with each subdistribution corresponding to a different number of incorporated branching units. For their samples this required fractionation by SEC prior to MALDI-TOF MS acquisition, due to the broad molecular weight distribution, and not all fractions yielded acceptable MALDI signal intensity.[9]

Chromatography techniques alternative to SEC, such as liquid adsorption chromatography (LAC) or liquid adsorption chromatography at the critical conditions (LACCC), have proven useful for the separation of complex polymers according to their chemical composition rather than their hydrodynamic volume.[10-12] LACCC and gradient chromatography were recently reported to separate aromatic hyperbranched polyesters with a correlation between degree of branching and elution volume.[13] However, these non-SEC chromatographic techniques have yet to be coupled to MS for the analysis of RBPs.

Therefore, the goal of this work is to develop non-SEC chromatographic methods for the structural resolution of hyperbranched RPBs, and couple this chromatographic separation to MALDI-TOF MS analysis, in order to enable detailed structural characterization. Commercially available aliphatic hyperbranched polyesters prepared from the polycondensation of 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) with an ethoxylated pentaerythritol core (PP50) (marketed as Boltorn™ polymers) were chosen for these initial studies.[14] These polymers are biomedically relevant, as they are biocompatible, biodegradable, nontoxic, and highly soluble, and have been investigated for applications such as encapsulation, controlled release, gene delivery, and the formation of unimolecular micelles.[2]

2. Experimental Procedures and Methods

High performance liquid chromatography (HPLC) was performed on an Agilent system (Agilent Technologies GmbH, Boeblingen, Germany) consisting of a 1200 series degasser (G1322A), 1100 series quaternary pump (G1311A), 1200 series autosampler (G1329A), 1100 series column oven (G1316A), and 1100 series variable wavelength detector (G1314A). The HPLC system was coupled to an evaporative light scattering detector (PL-ELS 1000, Polymer Laboratories), with a nitrogen flow rate of 1.0 SLM, nebulizer temperature of 40°C, and evaporator temperature of 80°C. Data collection and processing was performed with Agilent Chemstation for LC 3D systems (Rev.B.04.01). Chromatography was performed on a single Nucleosil column (5 μm, 100 Å, 4.6 mm ID, Macherey-Nagel, Düren, Germany). For chromatographic conditions, the injection volume was 25 μL, sample concentrations were 2-10 mg/mL, the column temperature was 45°C, and the flow rate was 0.5 mL/min. Acetone (Merck, Darmstadt, Germany) and n-hexane (Carl Roth, Karlsruhe, Germany) were HPLC grade and used as received.

MALDI-TOF MS was performed on a Bruker Autoflex III (Bruker Daltonik GmbH, Bremen, Germany) equipped with a Smartbeam™ laser (356 nm, frequency 200 Hz). Polymer samples were prepared at approximately 2 mg/mL, the matrix (dithranol) was prepared at 10 mg/mL, and sodium trifluoroacetate (3 mg/mL) was used for ionization, with all solutions prepared in tetrahydrofuran. The polymer (20 μL), matrix (50 μL), and counterion (3 μL) solutions were mixed, and 3 μL was spotted on the MALDI target using the dried droplet technique. MALDI

spectra were calibrated with an external PEG standard. The instrument was operated in reflector mode with the following parameters: IS1 19.00 V, IS2 16.60 V, Lens 8.60 V, R1 21.00 V, R2 9.70 V, and extraction delay 0 ns. The laser power was generally set to 25-30%, and each spectrum was a total of at least 10000 laser shots. The data was acquired in FlexControl (Version 3.0), and the data processed with FlexAnalysis (Version 3.0) (Bruker Daltonik GmbH, Bremen, Germany).

Boltorn™ hyperbranched polyesters (H20. H30, and H40 premium grade) (see Figure 1) were obtained from Polymer Factory Sweden AB, Stockholm, Sweden. The numbers (20, 30, or 40) represent the "pseudo-generation" of the polymer, and the average number of hydroxyl groups is 16, 32, and 64, respectively.

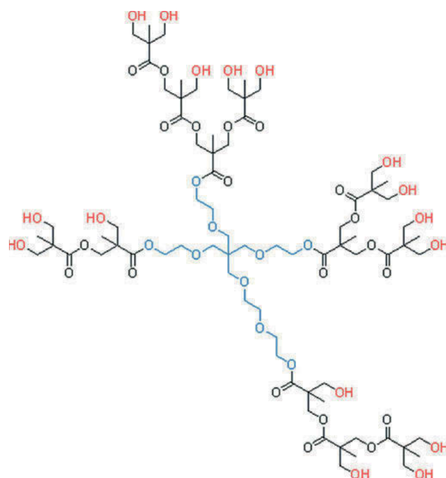

Figure 1. *Representative structure of Boltorn H20. This particular structure represents the average number of hydroxyl groups (16) and the average number of ethylene oxide groups (5) present on the PP50 core molecule, however many structural isomers are possible even with this particular (average) chemical formula.*

3. Results and discussion

3.1 Liquid Chromatography

After initial investigations into reverse phase and gradient chromatographic conditions for Boltorn™ polymers (not presented), a LAC chromatographic system was chosen instead. In LAC, enthalphic interactions between the analyte and the stationary phase govern retention, and analytes are typically eluted from low

molecular weigh to high molecular weight (i.e. opposite the order of elution in SEC separations). Similarly to work reported for LACCC of poly(lactide),[12] the hydroxyl groups of the Boltorn samples were expected to have strong adsorptive interactions with the stationary phase. Thus, a polar silica column was chosen as the stationary phase, and a moderately polar solvent (acetone) was selected as the mobile phase, with the polarity modified as necessary with small amounts of n-hexane. Figure 2 displays the chromatograms of all three Boltorn samples under these LAC conditions.

As seen in Figure 2, all three samples elute as relatively broad peaks with the same general retention time. The sharp peaks within the overall distribution, particularly observed between 5 and 10 min retention time, were consistent between runs for each sample and indicated the possibility of some sort of structural resolution, perhaps by DB, DP, or architecture.

In order to further investigate this effect, fractions were collected from the chromatographic runs. Initial chromatographic experiments were performed with a 2 mg/mL sample concentration for all Boltorn polymers. However, further investigations indicated that it was possible to increase the sample concentration to 6 mg/mL for Boltorn H30 and 10 mg/mL for Boltorn H40 with no loss of resolution in the resulting chromatogram (with these concentrations largely determined by the solubility of the each polymer in acetone). Thus, these higher sample concentrations were utilized for the fraction collection in order to minimize the number of runs needed to obtain sufficient material for MALDI-TOF MS analysis. Fractions were collected every 2 minutes, beginning from 6 min and ending at the end of the elution for each sample. For H20, H30, and H40 the number of HPLC runs performed was 20, 10, and 5, respectively, with the solvent removed by evaporation prior to re-solvation in 40 μL of THF for MALDI-TOF MS.

3.2 Mass Spectrometry

While, as mentioned previously, MALDI-TOF MS analysis of RBPs can be complicated by high molecular weight and polydispersity (PDI), all three Boltorn samples have relatively low PDIs (<1.4) and low molecular weights (<10 kDa theoretical). Thus, the total MALDI-TOF spectra of each sample are easily obtained with dithranol as the matrix and sodium trifluoroacetate to assist in ionization. However, as is shown in the example spectrum for Boltorn H30 (Figure 3), the spectra are quite complicated and contain numerous (>15) subdistributions. All of the subdistributions (each marked with a different color in the Figure 3 inset) are separated by a mass of ~116 Da (corresponding to the molecular weight of the bis-MPA monomer), but are offset from one another, indicating a possible difference in chemical structure. Ionization studies indicate that all species are singly charged $[M+Na]^+$ adducts, and thus these numerous subdistributions do not seem

to result from ionization with different cations (e.g. H^+ of K^+) or the presence of multiply charged species.

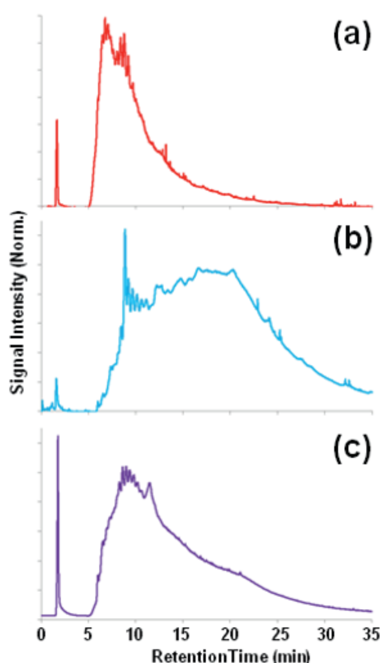

Figure 2. *LAC chromatograms for (a) Boltorn H20 (b) Boltorn H30 and (c) Boltorn H40. For (a) and (b) the mobile phase was 99% acetone/1% n-hexane (v/v), and for (c) the mobile phase was 100% acetone. The sharp peaks within the overall broad distribution (especially between 5 and 15 min in all samples) were consistent between runs and indicated the possibility of structural resolution.*

The complexity of the total MS of these polymer samples stems from the complexity of the samples themselves. Of course, as for all polymers with polydispersity, a Gaussian distribution of signals is expected for each distribution, which results directly from the number of monomers on each molecule (i.e. DP). Additionally, however, the PP50 core used in the polycondensation is itself a polydisperse molecule. Thus, while the PP50 core has an average of 5 ethylene oxide units, variations of this number in either direction are possible, so that a normal Boltorn molecule with a DP=10, for example, will result in multiple signals due to the presence of multiple core species. Furthermore, Boltorn polyesters are known to form cycles through intramolecular esterification or etherification, either with or without a core molecule. Additionally, intermolecular esterification or etherification can occur, which can result in two core species on one molecule. Finally, it is also reported in the literature that the bis-MPA molecules can self-condense with no core molecule at all.[15]

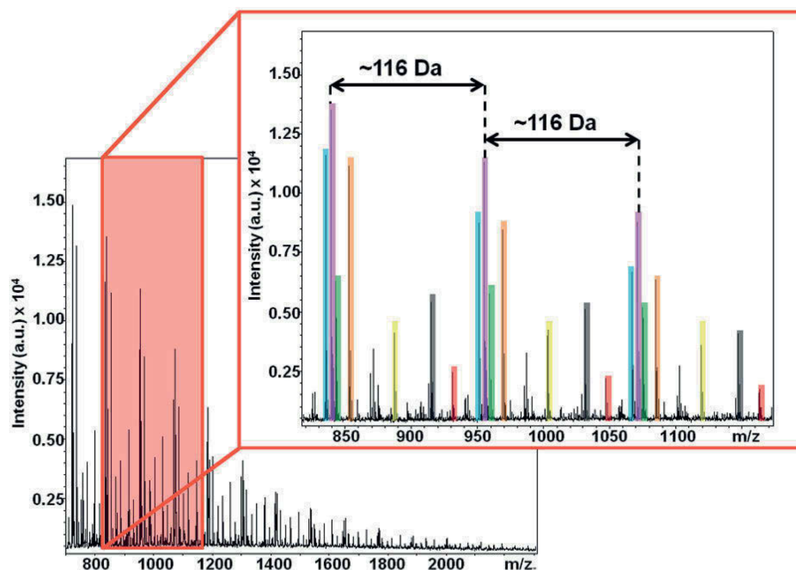

Figure 3. *MALDI-TOF MS spectra for Boltorn H30. On the left is the total spectrum of the sample, from m/z 800 to 2200. The box on the left highlights the region which is expanded in the inset on the right (from m/z 850 to 1150). In the inset, the different shaded boxes each correspond to a different subdistribution, separated by ~116 Da. Only seven major distributions are highlighted to enhance clarity, but eight more are easily visible, for a total of at least 15 different structures in this one sample.*

The MALDI data for the Boltorn H30 fractions, as a representative example, is displayed in Figure 4. Confirming that the separation mechanism was indeed adsorption, for any given subdistribution the molecular weight increases with retention time. This effect can be clearly observed by examining the signals marked by an asterisk (*) in the first four fractions in Figure 4. With careful MALDI-TOF calibration, the observed *m/z* values can be accurately compared to theoretical (calculated) values for the possible Boltorn structures discussed previously. For example, the calculated monoisotopic mass $[M+Na]^+$ for an intramolecularly cyclized Boltorn molecule (DP=10) with no core is 1183.46 Da. This corresponds closely to the observed peak in the (*) distribution at 1183.55 Da, indicating that cyclized Boltorn elutes first. The next distribution, marked by triangles, appears in fraction 2, offset from the first distribution by +4 Da. It is probable that this is also a molecule without a core, as there is only one such distribution and not several, as would be expected if the PP50 molecule was incorporated. However, it could not be conclusively identified as one of the structures known to exist in Boltorn samples, and thus perhaps is the result of an unknown starting material impurity in the bis-MPA monomer or core molecule.

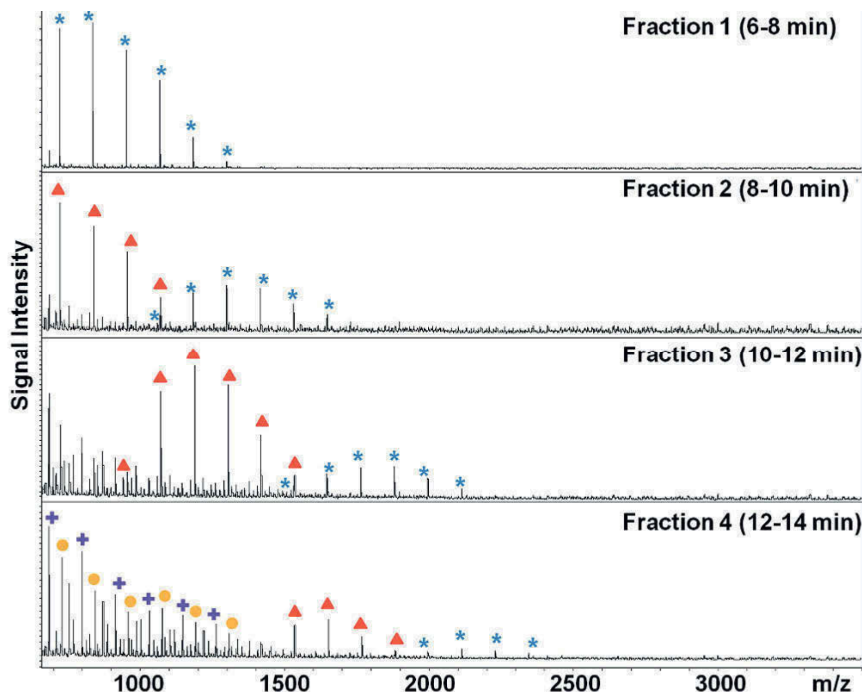

Figure 4. *MALDI-TOF MS spectra for the first 4 fractions collected for Boltorn H30. Each symbol corresponds to a different structure, or subdistribution. The asterisk distribution is a cyclized Boltorn with no core molecule, while the circle and cross distributions are hyperbranched Boltorn with PP50 core molecule. The triangle distribution could not be identified.*

Beginning in fraction 3 (Figure 4), the lower molecular weight region of the spectrum becomes much more complicated. This is the start of the elution of the "normal" hyperbranched Boltorn with a PP50 core molecule (that is, variations of the non-cyclic structure in Figure 1). In fraction 4, two of these distributions are marked. The distribution marked with the circles corresponds to the Boltorn with the PP50 core molecule as drawn (with 5 ethylene oxide groups), while the cross distribution corresponds to Boltorn with the PP50 core molecule with only 4 ethylene oxide groups. The other two main distributions in fraction 4 (not marked) correspond to Boltorn with a PP50 core molecule with either 6 or 3 ethylene oxide groups. Boltorn with the PP50 core with 3 through 6 ethylene oxide groups remain the primary distributions for fractions 5-8 (14-22 min retention time), with the additional appearance of a PP50 core with 7 ethylene oxide units beginning in fraction 6. As observed for the early eluting cyclic distribution, the Boltorn with PP50 core distributions likewise increase in molecular weight with increasing elution time. There was no evidence of intermolecular dimerization or cyclic molecules containing a core in any of the mass spectra. These same general trends were observed in the fractions collected for Boltorn H20 and H40.

4. Conclusion

LAC conditions have been established which allow the separation of Boltorn hyperbranched polyesters based on architecture (cyclic versus non-cyclic). Furthermore, this LAC separation enables better MALDI-TOF MS analysis, as the fractionation increases signal intensity for each subdistribution relative to the total mass spectrum. As the same Boltorn structure (non-cyclic) is eluted in the latter half of each spectrum, it is possible that this broad elution is governed by degree of branching, however further investigations are needed. Only cyclic Boltorn with no core and non-cyclic Boltorn with a PP50 core were observed in the mass spectra.

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