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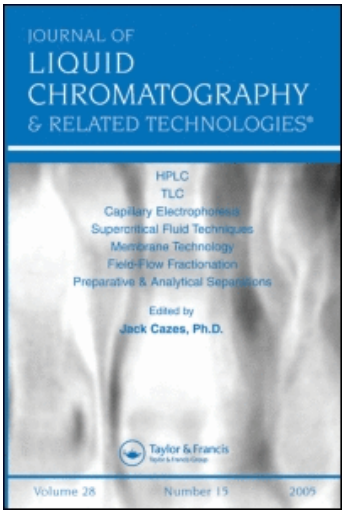

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Observations Regarding On-Column, Flow-Induced Degradation During SEC Analysis

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Abstract: The flow-induced degradation of ultra-high molar mass (M) polymers during their passage through a size-exclusion chromatography (SEC) column has been a subject of interest for several decades. Studies in this regard have been relatively few, however. Of interest is knowing whether degradation occurs in the interstitial medium, at the pore boundary, or both; whether the mechanism of degradation in the interstitial medium is the same as that at the pore boundary; and the types of flow fields involved in degradation, whether shear or extensional and, if the latter, what types of extensional flows are involved, transient or steady-state. Here, we attempt to shed some light on these topics by examining the SEC elution profiles of ultra-high M polystyrene standards. The standards have been analyzed at various flow rates, under conditions where the analyte either had access to both the interstitial volume and the pore volume or to only the interstitial volume. Results from these experiments were compared to each other, as well as to results from an ultrasonic degradation experiment where the analyte was depolymerized through the action of transient elongational flow fields. Results show that degradation occurs in both the interstitial medium and at the pore boundary and that the mechanisms of each of these are different from each other. While the degradation is almost assuredly due to extensional and not to shear flows, the former are either exclusively or predominantly steady-state, not transient.

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INTRODUCTION

Since its inception over 40 years ago, size-exclusion chromatography (SEC) has become the premier technique for characterizing the molar mass averages and distributions of both natural and synthetic macromolecules.[1] With the advent of multiple detection methods, the capabilities of the technique have been greatly enhanced, to provide a wealth of information regarding polymer architecture and dilute solution thermodynamics.[2–4] Likewise, the great advances in stationary phase synthesis and column packing methodologies have allowed analyses to extend both into the oligomeric as well as into the ultra-high molar mass (M) regions. Ultra-high molar mass generally refers to the condition where $M > 1 \times 10^6$ g/mol.[5] This includes a great many synthetic as well as natural polymers, archetypal examples of the latter being cellulose and amylopectin. While highly branched macromolecules of ultra-high M can be quite compact (e.g., PAMAM dendrimers with $G \geq 10$), this is not the case for lightly branched and linear polymers. Because highly extended polymers exposed to a large amount of mechanical stress tend to rupture, and because the shear rates generated during typical SEC analyses can be quite high, degradation of ultra-high M polymers occurs often in SEC. Unfortunately, in single detector SEC analysis this degradation often goes unnoticed, the results instead being attributed to the molar mass distribution (MMD) of the analyte being broader than originally expected. When molar mass sensitive detectors are used, such as a differential viscometer or a static multi-angle light scattering photometer, the degradation manifests itself by a decrease in viscosity, molar mass, or size, as these properties are measured across the elution profile of the sample.

Questions have arisen as to the type of flow-induced degradation that occurs on-column during SEC analysis. Namely, does the degradation occur in the interstitial medium, at the pore boundaries, or both? What type(s) of flow fields are involved in the degradation? Is it, indeed, shear degradation, as it is often called, or is the degradation actually extensional in nature? If the latter, what type of extensional flows are involved in the degradation? In this paper, some of these topics are addressed through a series of SEC experiments with ultra-high M, relatively narrow polydispersity polystyrenes. The topic of on-column degradation is quite broad and, as will be seen, more complex than might originally be suspected. While we cannot yet provide a definitive answer to all the questions formulated above (which may have different answers depending on analyte architecture, molar mass, dilute solution thermodynamics,

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or any combination of these), we believe the present study will help shed some light on the nature of the degradation, as well as assist those who work in the realm of ultra-high M polymers and, thus, need to exercise the utmost caution in characterizing their samples in order to avoid the types of degradation described herein.

EXPERIMENTAL

Materials

The 1.92×10^6 g/mol PS standard, with $M_w/M_n = 1.03$, was from Polymer Laboratories; the 20×10^6 g/mol PS standard, with $M_w/M_n \leq 1.20$, was from Pressure Chemical Co. Uninhibited tetrahydrofuran (THF) was from Fischer Scientific.

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Size-Exclusion Chromatography

SEC analysis of the 1.92×10^6 g/mol PS standard was performed on a system consisting of a Waters 2695 separations module (which includes an on-line degasser and an in-line filter prior to the injector) and a Waters 410 differential refractive index (DRI) detector. For analysis of the 20×10^6 g/mol PS standard, the detector was a Wyatt ViscoStar differential viscometer. Separations occurred at flow rates described in the Results & Discussion section. The solvent and mobile phase were THF. For each analysis, 100 μL of unfiltered sample were injected onto either a PLgel 5 μm particle size, 50 Å pore size column or a PLgel 10 μm particle size, Mixed B column, both from Polymer Laboratories. The injector compartment, column compartment, and detector temperatures were all maintained at 35°C. In all cases, sample concentration was 0.1 mg/mL, well below the critical overlap concentration for PS in THF at 35°C.[6]

Ultrasonic Degradation

Ultrasonic degradation of a 0.1 mg/mL solution of the 1.92×10^6 g/mol PS standard was performed using a Branson 5200 ultrasonic bath operating at 47 KHz and 185 W. The water temperature of the bath was maintained at ~20°C via a home made recirculation system. The sample was analyzed by SEC/DRI prior to sonication and 4 mL aliquots were removed at discrete sonication times (see Figure 3) for continued analysis.

RESULTS AND DISCUSSION

Figure 1 shows the SEC elution profiles of the 20×10^6 g/mol PS standard, at various flow rates in the range 0.1–3.0 mL/min, obtained by SEC/viscometry. The increasing line thickness with decreasing flow rate is a result of the acquisition rate having been maintained constant during analysis. This analysis was carried out using a PLgel Mixed B column with a nominal exclusion limit of $\sim 10 \times 10^6$ g/mol (it should be noted that the exclusion limits given here for all columns were obtained by the manufacturer under experimental conditions almost identical to ours, i.e., using linear PS dissolved in THF and analyzed at either room temperature or 35°C). As can be seen in Figure 1, the elution profiles shift to larger retention volumes with increasing flow rate, indicative of the degradation of the analyte in solution (as retention volume in SEC is inversely proportional to molecular size in solution). Because the molar mass of this analyte greatly exceeded the exclusion volume of the column, on-column degradation can only have occurred in the interstitial medium, i.e., in the space between the column packing particles, as the analyte is too large to penetrate the pores of the column packing material.

Polymer degradation during SEC analysis has attracted sporadic attention over the years.[7–12] To avoid degradation of PS with

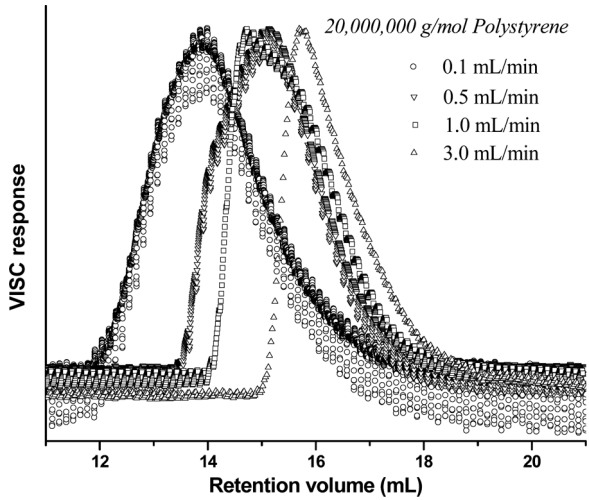

Figure 1. Degradation of a 20×10^6 g/mol PS standard during its passage through an SEC column with exclusion limit of $\sim 10 \times 10^6$ g/mol. VISC: Differential viscometer. Ordinates have been scaled to allow for visual comparison. See text for experimental details.

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$M > 5 \times 10^6$ g/mol, Aust et al. recommended using flow rates of $\leq$0.2 mL/min, packing material of 20 μm diameter, injection volumes of ~100 μL, and sample concentrations of $\leq$0.1 mg/mL. Except for the diameter of the packing material, all of these agree with our conditions of analysis. Our flow rates, of course, oftentimes exceeded 0.2 mL/min, but in these cases we were purposefully performing flow-induced degradation. We note, however, that while a flow rate of 0.1 mL/min seemed sufficiently low to avoid degradation under the present conditions, based on the fact that elution profiles at 0.1 mL/min were identical to the profiles at flow rates of <0.1 mL/min (the latter not shown in the figures to avoid clutter), a flow rate of 0.2 mL/min was observed to cause degradation of the 1.92×10^6 g/mol PS standard, as seen in Figures 2a and 2b.

Following the reasoning in Reference [13], for a Newtonian fluid under laminar flow, the shear rates generated during SEC analysis can be calculated from Equation (1):

$$\dot{\gamma} = \frac{4Q}{\varepsilon A r_c} \tag{1}$$

where $\dot{\gamma}$ is the shear rate (in sec^{-1}), Q is the volumetric flow rate, ε is the porosity of the packed column, A is the cross-sectional area of the column, and r_c is the hydrodynamic radius of the bed. This radius, r_c, is related to both the porosity, ε, as well as to the diameter of the column packing material, d_p, via Equation (2):

$$r_c = \frac{d_p \varepsilon}{3(1-\varepsilon)} \tag{2}$$

From Equation (1), we see that shear rate increases linearly with flow rate. For a column packed with 5 μm particles, for example, at 0.1 mL/min shear rates of ~1,000 sec^{-1} are generated. At 1 mL/min, γ has now increased to ~10,000 sec^{-1}. At higher flow rates, or when using packing material with d_p of 2–3 μm, analytes can experience shear rates of ~10^5 sec^{-1} during their passage through a packed column, comparable to the shear rates encountered in such industrial processes as gasoline engine lubrication, blade coating, or pigment milling.[14]

During flow through a chromatographic column, velocity gradients are generated. These gradients can cause the macromolecule to become stretched. It was once believed that velocity gradients transverse to the direction of flow (i.e., shear fields) could degrade polymers. This is now generally considered untrue. While polymers can, under extreme conditions, degrade as a result of exposure to a shear field, it is now generally agreed that flow-induced polymer degradation is almost always

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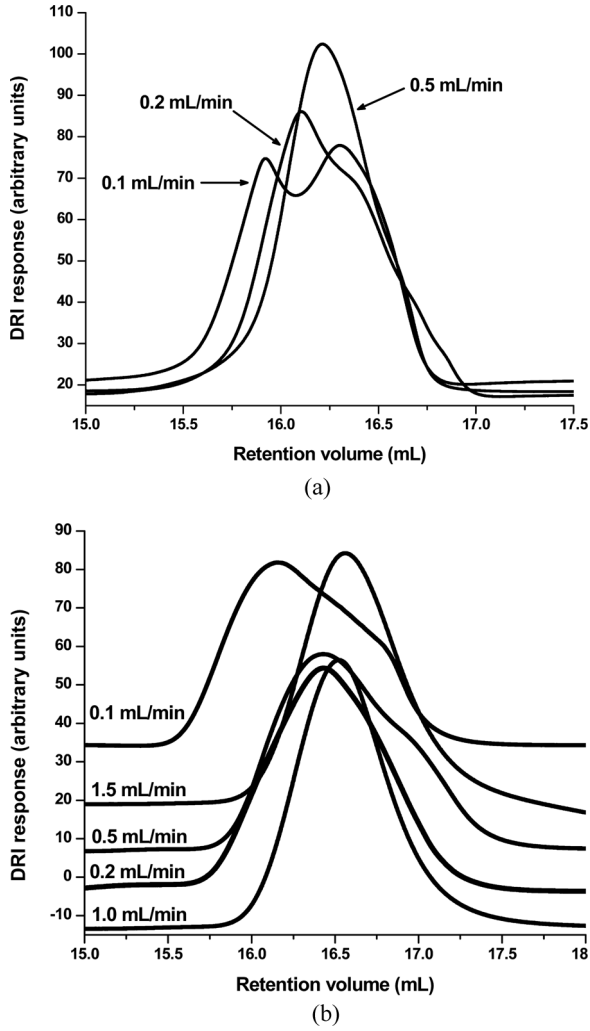

Figure 2. Degradation of a 1.92×10^6 g/mol PS standard during SEC analysis: (a) Degradation in the interstitial medium only. (b) Degradation in both the interstitial medium and at the pore boundary. Flow rates corresponding to each chromatogram are given in the figures. DRI: Differential refractive index detector. See text for experimental details.

due to extensional flows, where the velocity gradients are parallel to the direction of flow and which are capable of inducing a large degree of macromolecular coil extension.[15] The term "shear degradation" is therefore a popular misnomer for what is almost certainly extensional degradation. Because there are different types of extensional flows, the

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question of which type of flow is involved in polymer degradation during SEC analysis has not been answered. We will return to this topic when we discuss the results of our ultrasonication experiments.

Figure 1 shows that an ultra-high molar mass polystyrene, with $M = 20 \times 10^6$ g/mol, can degrade in the interstitial medium of an SEC column and that degradation increases as a function of flow rate. This is also seen to occur for a polystyrene one order of magnitude smaller in molar mass, with $M = 1.92 \times 10^6$ g/mol, shown in Figure 2a. Here, we used a PLgel 5 μm particle size, 50 Å pore size column with an exclusion limit of $\sim 2 \times 10^3$ g/mol. Because the molar mass of the analyte is much greater than the exclusion volume of the column, the observed on-column degradation is the result of processes occurring exclusively in the interstitial medium. When this same polystyrene was analyzed using the Mixed B column (exclusion limit $\sim 10 \times 10^6$ g/mol), the polymer was also observed to degrade, as seen in Figure 2b. Again, degradation increased as a function of increasing flow rate, the latter varying from 0.1 to 1.5 mL/min at discrete intervals shown in the figure. When using the Mixed B column, however, the analyte samples both the interstitial volume and the pore volume. Interestingly, the patterns in the chromatograms in Figure 2b are different from those in Figure 2a, indicating that a different or, more likely, an additional mechanism of degradation is present at the pore boundaries as compared to when degradation occurs solely in the interstitial medium. The bimodality observed at 0.1 mL/min is likely due to the polydispersity of the sample in combination with a hydrodynamic chromatography mechanism operating in the interstitial medium.[16,17]

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We now return to the discussion of the types of flow fields involved in the degradation. Figure 3 overlays the SEC/DRI chromatograms of the same 1.92×10^6 g/mol PS as shown in Figure 2. This time, solutions of the polystyrene standard were sonicated for varying periods.[18,19] The cavitational bubble collapse characteristic of ultrasonication produces elongational flow fields that are termed "fast transient" or "transient elongational."[15,20,21] In transient elongational flow, the velocity time scale of a volume element of fluid is several orders of magnitude greater than the relaxation time of a macromolecule, whereas in "steady state" elongational flows, the velocity of a volume element of fluid is of the same order of magnitude as the polymer's relaxation time. The chromatograms shown in Figure 3 were obtained at the same solvent/temperature conditions as those in Figure 2, using the same Mixed B column as used to obtain Figure 2b. The flow rate for Figure 3 was 0.1 mL/min, where no or negligible on-column flow-induced degradation was observed, a conclusion reached by analyzing the sample at flow rates <0.1 mL/min and noting that these chromatograms appeared identical to the chromatograms obtained at 0.1 mL/min. The 1.92×10^6 g/mol polystyrene is seen to degrade more and more as a function of increasing exposure to

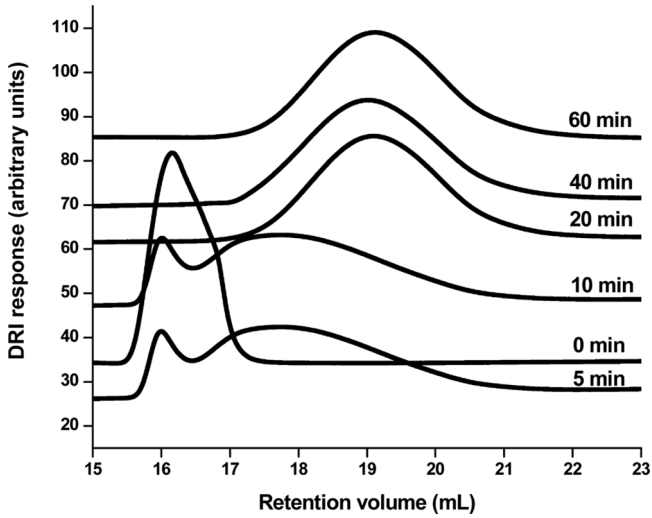

Figure 3. SEC/DRI analysis of ultrasonically-degraded 1.92×10^6 g/mol PS standard. Analyte and experimental conditions same as in Figure 2b. Flow rate: 0.1 mL/min. Times above each chromatogram correspond to times of exposure to ultrasonic irradiation (see Experimental for details). Chromatograms are offset from each other on the *y*-axis for viewing convenience.

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ultrasonication, again evidenced by the shift in the primary mode of the elution profiles to larger retention volumes. Even a brief 5 minutes of sonication are seen to noticeably degrade the polymer. The pattern observed in the overlays in Figure 3 is quite different from the patterns observed in Figures 2a and 2b. This indicates that on-column, flow-induced degradation, whether it occurs solely in the interstitial medium or in a combination of interstitial medium and pore boundary, cannot be caused solely or, perhaps, even primarily by transient elongational flow fields. If it were, the patterns in Figure 2 would be expected to resemble those in Figure 3.

CONCLUSIONS

We have investigated the on-column degradation of ultra-high molar mass polymers during SEC analysis. Analytes that were too large to fit into the pores of the column packing material were observed to degrade in the interstitial medium of the column. When the column characteristics were changed so that the analytes now had access to the pores, degradation was still observed. In this latter case, however, the SEC elution profiles of the degraded material were different from the profiles of material that had degraded exclusively in the interstitial medium of the

column. This indicates that a different or an additional mechanism of degradation operates at the pore boundary vis-à-vis in the interstitial medium.

High molar mass polymer was also degraded ultrasonically. The flow fields produced during ultrasonic bubble collapse are transient elongational in nature. Monitoring the results of degradation as a result of increased exposure to ultrasonic irradiation, and comparison of these degradation results to those from on-column degradation, yielded one important insight: The on-column, flow-induced degradation of high-M analytes during SEC analysis is not caused solely, if at all, by transient elongational flow fields. Because shear fields can generally be ruled out as being able to cause macromolecular degradation, the on-column degradation of high-M polymers in SEC must be caused solely or primarily by steady state elongational flows.

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