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Do column frits contribute to the on-column, flow-induced degradation of macromolecules?

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ABSTRACT

Flow-induced, on-column degradation is a major hindrance to the accurate characterization of ultra-high molar mass macromolecules and colloids. This degradation is a direct result of the large shear rates which are generated within the column, which cause chain scission to occur both in the interstitial medium and, it has been postulated, at the packing particle pore boundary. An additional putative source of degradation has been the column frits, though little experimental evidence exists to either support or refute this claim. To this effect, the present experiments examine the role of the frits in the degradation of high molar mass macromolecules. Two narrow dispersity polystyrene standards, the molar mass of which differs by a factor of two, were analyzed on three different size-exclusion chromatography (SEC) columns, each with frits of different pore size, at various flow rates. In the smallest pore size column, which also contained the smallest frits and which was packed with the smallest diameter particles, the larger standard was forced to degrade by increasing the flow rate of the mobile phase. During the course of the latter portion of the study, the inlet and the outlet frits were removed from the column, in stepwise fashion. It was concluded that neither frit played any appreciable role in the degradation. Results of our studies were applied to explain previously observed degradation in ultra-high pressure liquid chromatography of polymers. The general conclusion arrived at herein is that *the column frits are likely to have a secondary role (as compared to interstitial and pore boundary stresses), or no role at all, in polymer degradation for cases where the frit radius is larger than or equal to the hydraulic radius r_c of the column.*

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1. Introduction

The size-exclusion chromatography (SEC) analysis of ultra-high molar mass (M) polymers and, even, of covalently bonded colloidal assemblies, is hindered by the degradation that can occur as the analytes traverse the packed, porous medium of the chromatographic columns [1–5]. This degradation is a direct result of the large shear rates to which the analytes are exposed within the columns. For a Newtonian fluid at laminar flow conditions, the on-column shear rate is given by the relation [1–6]:

$$\dot{\gamma} = \frac{4Q}{\varepsilon A r_c} \quad (1)$$

where $\dot{\gamma}$ is the shear rate, Q the volumetric flow rate through the column, ε the interparticle porosity of the column, A the cross-sectional area of the column, and r_c is the hydraulic radius of the column bed. The parameter r_c, which may be considered as the radius of a capillary with the same surface-to-volume ratio as the packed-column bed, may also be thought of as the average radius of the interstitial medium of the column (i.e., as half the average minimal distance between column packing particles), and is formally defined as [1,3,7]:

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

where d_p is the average diameter of a column packing particle. As seen from Eqs. (1) and (2), on-column shear rates increase linearly with flow rate and inversely with particle size. At a flow rate of 1 mL min^{-1}, shear rates in a 4 mm internal diameter (i.d.) column packed with 5 μm particles can reach ≈10,000 s^{-1}. The newest generation of ultra-high performance (or ultra-high pressure) size-exclusion chromatography (UHP SEC) columns touts particle diameters smaller than 2 μm [8]. According to the reported dimensions of these columns and conditions at which they are operated, a 4.6 mm i.d. column, packed with 1.7 μm particles, operating at 1 mL min^{-1}, and assuming a very reasonable value for ε of 0.36, generates on-column shear rates of ≈35,000 s^{-1}. At high flow rates (e.g., 10 mL min^{-1}), even 10 μm non-UHP columns can

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generate shear rates approaching or exceeding $10^5\ s^{-1}$. The shear rates to which polymers are exposed within a chromatographic column are thus comparable to the rates generated by such harsh industrial processes as pigment milling (10^3–$10^5\ s^{-1}$), blade coating (10^5–$10^6\ s^{-1}$), and gasoline engine lubrication (10^3–$10^7\ s^{-1}$) [9].

In light of the above, it is not surprising that ultra-high *M* polymers (generally described, in the case of linear polymers, as those with $M \geq 1 \times 10^6\ g\,mol^{-1}$) have been observed to degrade during SEC analysis. It should be noted that, in most cases where this type of degradation is reported, evidence for the latter is usually circumstantial and based on the chromatographic peaks shifting to larger retention volumes with increasing flow rate (elution in SEC being inversely related to size). However, for very large polymers, it has been shown that this shift in retention volume may be due, not to degradation (or not solely to degradation), but to a change in the mechanism of elution, from a size-exclusion mechanism to a slalom chromatography (SC) mode [10–12]. In SC, elution order is opposite that in SEC and smaller analytes elute earlier than do larger ones, as the latter have undergone critical flow-induced extension as a result of the high Deborah numbers generated at the higher flow rates (see Section 2.6 of Ref. [10]). The only way to unambiguously determine if degradation has occurred is by employing an on-line light scattering detector, which has the ability to determine the size and (depending of the type of light scattering) molar mass of the analyte and, thus, to determine if these properties change as a function of increasing or decreasing flow rate. Several studies, mostly from the 1980s, employing SEC with on-line low-angle static light scattering detection (LALS, which can measure molar mass, but not size) demonstrated the degradation of select polyolefins, polybutadienes, and polystyrenes as a function of increased chromatographic flow rate [13–18]. More recent work by the author has employed SEC with on-line multi-angle static light scattering (MALS, which can measure both *M* and radius of gyration R_G) to demonstrate the on-column degradation of the ultra-high *M*, hyperbranched polysaccharide alternan [1,2], and SEC with both MALS and quasi-elastic light scattering (QELS, from which the hydrodynamic radius R_H can be determined) detection to show that string-of-pearls colloidal silica degrades during analysis [4]. In the case of both alternan and string-of-pearls silica, the SEC analyses were optimized, i.e., the largest particle size and pore diameter columns that were commercially available for use with either organic (in the case of alternan) or aqueous (in the string-of-pearls case) solvent were employed, at the lowest flow rate at which the instruments could sustain a steady backpressure. In both cases, hydrodynamic chromatography [19,20] was employed as an alternative means of separation, as degradation in SEC was unavoidable.

Besides the columns, other parts of an SEC instrument have been identified as potential sources of degradation, due to the elevated shear rates encountered therein [5]. These sources include the injection valve and any connecting capillary tubing (including that inside the detectors). The shear rates produced in common injection valves are on the order of a few thousand s^{-1}, while in connecting capillary tubing (i.d. ranging from 0.01 in. to 0.04 in.), shear rates can range from $10\ s^{-1}$ to $10^3\ s^{-1}$. The average shear rates $\left|\dot{\gamma}_{ave}\right|$ generated in cylindrical capillaries of radius *R* at laminar flow conditions can be calculated via [21]:

$$\left|\dot{\gamma}_{ave}\right| = \frac{8Q}{3\pi R^3} \tag{3}$$

Reed employed the above relation to calculate the average shear rates within the most commonly employed on-line viscometers (Wheatstone bridge-type) and MALS photometers [21,22]. In the former, shear rates less than $1000\ s^{-1}$ are generated at $1\ mL\,min^{-1}$ while in the latter, at the same flow rate, the shear rates are less than $60\ s^{-1}$ (with the shear rate sampled by the laser beam in the cell being about one-third this value, or lower). Intradetector shear rates are, thus, low. While degradation in other parts of the system (e.g., the injector) is possible, these can be eliminated from consideration when attempting to ascertain whether the column is the culprit, by maintaining a constant instrumental set-up, save for the column, when performing degradation studies. Degradation can also be caused by outside agencies such as sample filtration, sonication, vortexing, excessive heating/freezing, etc., though this is a topic beyond the scope of the present discussion [10].

Regarding degradation occurring within the chromatographic column, it should be noted that in SEC separations this appears to proceed predominantly by a steady-state extensional mechanism (is caused by steady-state extensional flow fields), which results in random scission of the polymer chain [3]. This is contrary to fast transient or transient extensional degradation which, for linear homopolymers, results in non-random, near-midchain scission [23–26], and which does not appear to be responsible (or, at least, chiefly responsible) for the on-column, flow-induced degradation of macromolecules (the term "shear degradation" is a misnomer, as degradation of polymers by the action of shear fields is an exceedingly rare phenomenon). Degradation has been found to occur in both the interstitial medium and at the pore boundaries, for pores sufficiently large to allow penetration of the polymer. In the latter case, it appears that a portion of the macromolecule is "hooked" within the pore and, thus, stagnant while the rest of the chain, the portion of the macromolecule which remains outside the pore, is pulled away by extensional forces the strength of which is sufficient to effect chain scission [3]. (This type of "hooking" or "anchoring" mechanism is akin to that invoked by Podzimek to explain the abnormal elution of certain long-chain branched polymers in SEC but not in asymmetric flow field-flow fractionation [27].)

An additional source of column degradation has been postulated to be the column frits. These are, in most cases, sintered steel porous discs, ≈1 mm thick and having an ≈5 μL dead volume, with a porosity of 30–40%, that are placed at the column inlet and outlet (a spreader usually precedes the inlet frit [10]) [28]. Recently, these have been implicated in the degradation of ultra-high-*M* polystyrene (PS) in ultra-high-pressure (also termed ultra-high performance) liquid chromatography (UHPLC), where very small diameter (0.2 μm) inlet and outlet frits are employed (contrary to the case of the "non-UHP" SEC separations discussed above, on-column, interparticle degradation in UHPLC appears to be predominantly the result of transient, rather than steady-state, extensional flows, which cause near-midchain scission of linear homopolymers) [12]. In this study, however, we concern ourselves experimentally with non-UHP SEC where, historically, the frits have a venerable history of being touted (usually anecdotally) as a possible source of polymer degradation [28–32]. The implications of our results for polymer UHPLC (which includes UHP SEC, UHP HDC, and UHP SC) are addressed, though.

In a 2003 SEC/LALS/DRI study, Aust concluded that the column frits did not influence degradation [18]. His reasoning followed the following logic: The peak shifts of narrow dispersity PS standards toward larger retention volumes, as a function of increased flow rate, must be the result of degradation within the column, as these shifts would not occur if the outlet frit had caused the degradation. Having ruled out the outlet frit, the inlet frit was ruled out as well, as both frits have the same geometry and pore diameter. There are two concerns with this line of reasoning, however. First, the analyte solution is more concentrated at the inlet frit than at the outlet frit, due to the dilutory effects of the separation, and more concentrated solutions generally degrade more readily than do more dilute ones. Second, if degradation occurred within the column *and also* at the