Stop-Flow Methods to Monitor Broadening in Size-Exclusion Chromatography with Superficially Porous Particles

Alexander Toler, Joseph Naese, and James P. Grinias

Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08028

Abstract

Size exclusion chromatography (SEC) serves as an important method for characterizing petroleum-derived polymers. Yet, SEC methods typically suffer from poor efficiency and fairly long analysis times. However, with the introduction of superficially porous particles (SPPs) as a chromatographic stationary phase support, there is an opportunity to increase column efficiency for SEC. Knowing that longitudinal diffusion exists in all forms of chromatography and differs based on the diffusion coefficient (and thus, molecular weight) of the analyte, the effects of this band broadening mechanism were compared for compounds ranging between 100 Da and 50 kDa. Two bare silica columns (4.6 x 100 mm) containing 2.7 µm superficially porous particles with pore sizes of 90 Å and 160 Å were used in the study to also determine the effect of pore size. The effective size range for SEC separations was determined for each pore size, as well as the restriction to longitudinal diffusion. This data will inform the preparation of SEC columns for polymer separations in this molecular weight range.

Introduction & Theory

This project focuses on observing the effects of longitudinal diffusion on the separation efficiency of low-weight polystyrene standards using SEC. Unlike partition chromatography, separation is based from the difference in molecular size between the analytes. The superficially porous column packing consists of an exterior shell with pores of a predetermined size surrounding a solid core. As a mixture travels through the column, smaller analytes diffuse into the pores while larger analytes travel to the detector unimpeded. As mobile phase flows through the column, the smaller particles are freed from the pores and travel toward the detector.

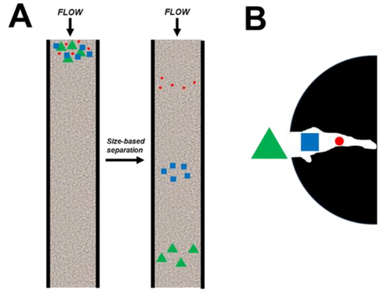

Figure 1. The general size-based separation mechanism for SEC is shown in A. The accessibility of different sized particles in the intraparticle mobile phase when stopped as a size-based process can be shown in B.

By utilizing stop flow chromatography in conjunction with SEC, the peak band broadening due to diffusion within the column can be determined, proportional to the time that flow is stopped. This longitudinal diffusion is caused by the random movement of particles within the column and is present within all separation modes. Larger particles have much smaller diffusion coefficients, and thus diffusion will have a lesser impact on the separation. For this phase, a solute band is loaded onto the column, and is allowed to progress to about halfway through the column length.

Experimental Set-Up

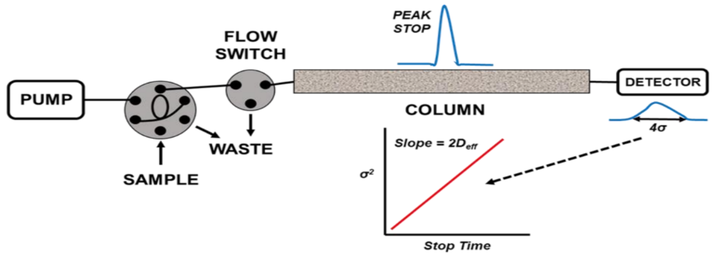

Figure 2. Instrument set-up needed to perform stop-flow. The analyte, shown in the form of a peak, travels halfway through the column. Flow is then abruptly stopped, and the peak is allowed to sit for a set amount of time. Flow is restarted to allow the peak to elute and reach the detector. A plot of the peak variances and their respective stop times can allow for the determination of the diffusion coefficient.

Method Validation

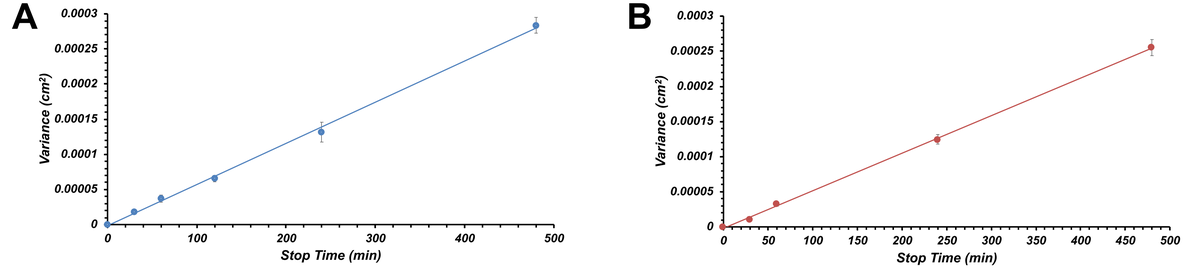

Figure 3. Plot of variances of 4.9 kDa polystyrene sample after different stop times taken from runs, allowing for the determination of the diffusion coefficient through the slope. Runs on the 90 Å column are represented in A, while runs on the 160 Å column are represented in B.

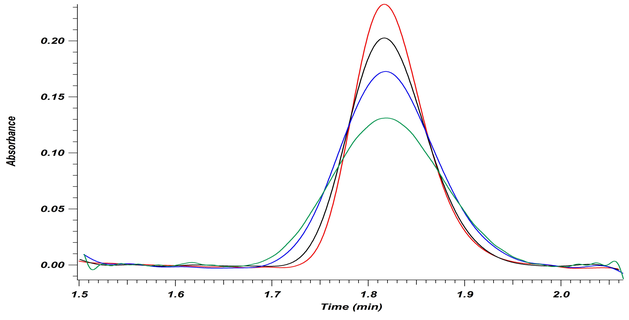

Figure 4. An overlay of the chromatograms of the 4.9 kDa polystyrene sample at various stop times, including 0 min (red), 60 min (black), 240 min (blue), and 480 min (green)

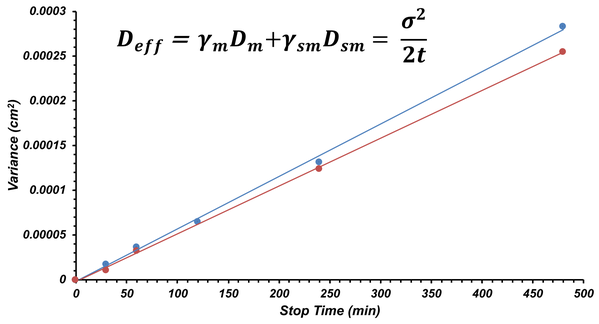

Figure 5. Plot of the 4.9 kDa polystyrene sample data from the 90 Å (blue) and 160 Å (red) columns. The column with the smaller pore size demonstrates a greater effective diffusion coefficient for this analyte size. This change can be shown through the combination of the diffusion coefficients in the flowing interparticle mobile phase and the stagnant intraparticle mobile phase [2].

Future Directions

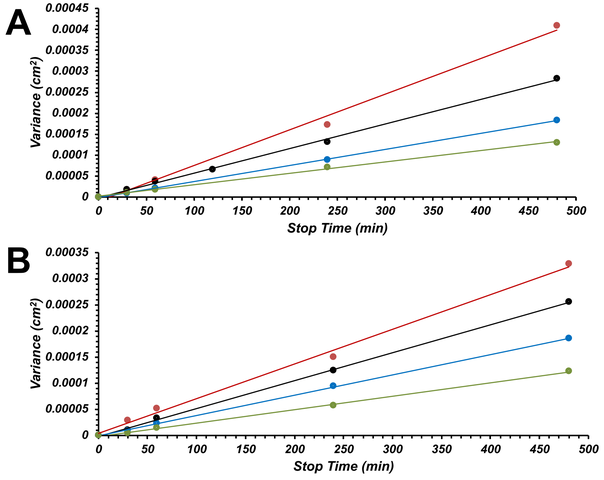

Figure 6. A collection of plots showing the varying diffusion coefficients of different molecular weight polystyrene samples. These plots include 1.9 kDa (red), 4.9 kDa (black), 13.0 kDa (blue), and 27.1 kDa (green). The curves on A represent data collected on the 90 Å column while B is from the 160 Å column.

As aforementioned, the diffusion coefficient for the polystyrene standards decreases with increasing molecular weight and larger particle pore sizes. The polystyrenes must fall under a given radius of gyration allowing for the analyte to fit into the pores of the column. Furthermore, these precursory tests will be further expanded by introducing a third mixed-pore column to these analytes, allowing for the observation of band broadening effects. This column theoretically can show the positives of both smaller and larger pore size columns by averaging the molecular weight range of the two pore sizes. Improving these methods for characterizing lower molecular weight polystyrenes can advance the measurement of antioxidants in plasticizers and lubricant oils as well as characterization of asphaltenes in crude oil, which is a prominent petroleum fuel source.

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References

[1] Mori, S.; Barth, H. G. *Size Exclusion Chromatography*; Springer-Verlag Berlin Heidelberg GmbH: Berlin, 1999.
[2] Neue, U. *HPLC Columns: Theory, Technology, and Practice*; Wiley-VCH, Inc.: New York, NY, 1997.