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Fast Gel Permeation Chromatography Using Large Gel Particles

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NOTE

Fast Gel Permeation Chromatography Using Large Gel Particles

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In two previous papers (*1,2*), we reported the effect of several operational parameters on separations by gel permeation chromatography (GPC). These studies included the effects of (a) solvent flow rate, (b) sample concentration, (c) sample molecular weight, (d) particle size, and (e) operating temperature. In this present study, we used extremely large particles (200–300 μ diameter) at flow rates up to 10 ml/min. This was done to magnify solute diffusion effects in hope of gaining a better understanding of the permeation process. By using the relatively large particles, interstitial spaces between particles will be wider and the gel pores deeper than with particles normally used (less than 75 μ) in GPC.

A modified Waters Associates Gel Permeation Chromatograph, Model 200, described previously (*1,2*), was operated at room temperature with toluene solvent. One 4 ft × 0.305 in. i.d. column of a special Styragel (exclusion limit, 10^3 Å), containing particles of 200–300 μ (50–80 mesh), was used for this study. A siphon calibration curve (*1*) was used to correct inaccuracies in the siphon dump volume. Figure 1 shows a calibration curve of the system determined at 1 ml/min. Samples were injected at a concentration of 0.5% (weight/volume) except for the 2×10^6 MW polystyrene standard which has 0.05% to decrease viscosity effects. A constant sample volume of 1 ml was used for all test samples.

Figure 2 shows a plot of elution volume vs flow rate. For small molecules, acetonitrile and *o*-dichlorobenzene (ODCB) elution volume was found to be invariant to flow rate. These small molecules, with diffusion coefficients of approximately 10^{-5} cm^2/sec, appear capable

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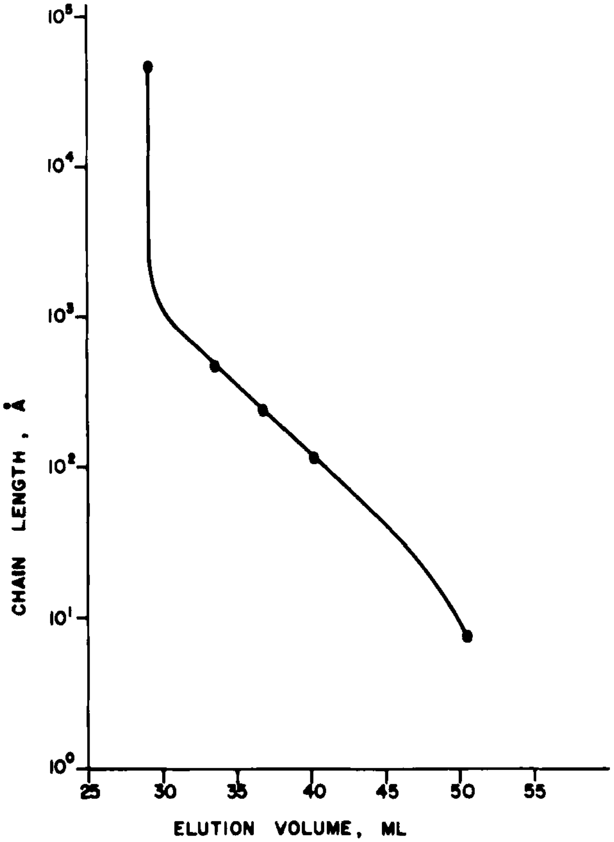

FIG. 1. Calibration curve of system.

of getting to all pore openings by convection and diffusion, even at flow rates up to 10 ml/min. Thus, their elution volume remains constant with flow rate (*1–3*).

For excluded molecules (2×10^6 MW polystyrene), elution volume remains constant with flow rate since the sample can only travel in the interstitial volume and elutes with the solvent front. It is irrelevant whether the excluded molecules contact fewer pore openings at the fast flow rates since they are unable to permeate.

For molecules in the selective permeation range, the elution volumes were found to decrease with flow rate. These molecules (5000 and 19 850 MW polystyrene standards) with diffusion coefficients of 1–2 $\times 10^{-6}$ cm^2/sec (*4*) are not as mobile as the small molecules. Hence, these molecules do not contact as many pore openings (by diffusion) at the higher flow rates, and therefore elute earlier. In our previous studies (*1,2*), we found this did not occur even at much higher flow rates since small particles (less than 42 μ) and consequently narrower interstitial spaces were used.

Figure 3 shows a plot of peak width vs elution volume. The peak widths of acetonitrile and ODCB were found to increase gradually with flow rate, similarly to what we reported earlier (*1,2*). The peak width of the excluded molecules (2×10^6 MW) remains virtually constant with flow rate, as reported by others (*3–5*).

An interesting finding of this study is the peak width behavior of partially permeating molecules with increasing flow rate. Since the elution volume of the 19 850 standard decreases with flow rate, the molecules necessarily get to fewer pore openings, resulting in less peak spreading. At the highest flow rate (10 ml/min), the 19 850 standard is almost excluded from the gel pores and a peak width only slightly greater than that of excluded molecules is observed (Figs. 2 and 3). The elution volume of the 5000 standard also decreases with flow rate, but even at the highest flow rate (10 ml/min) there is significant permeation of the gel (elution volume = 36.5 ml). The net result of the two opposing effects (decreased elution volume and increased flow rate) is a slight increase in the peak width of the 5000

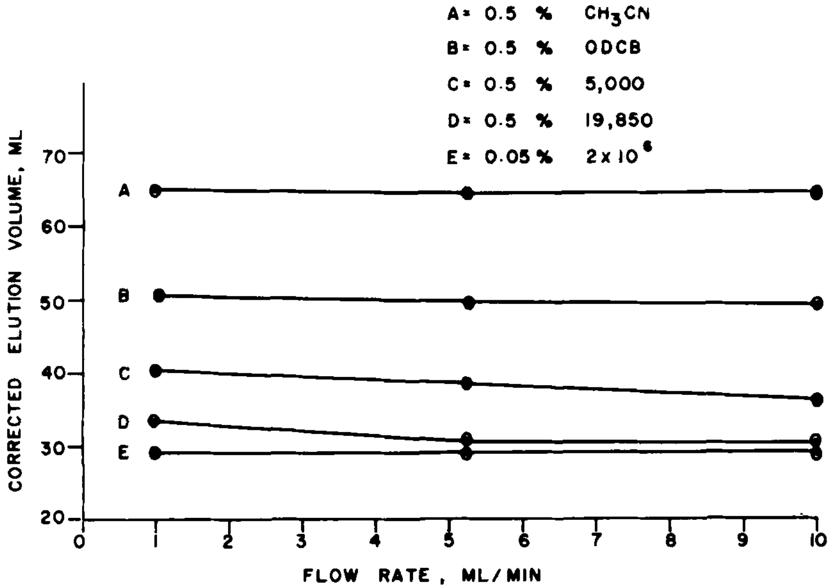

FIG. 2. Elution volume as a function of flow rate.

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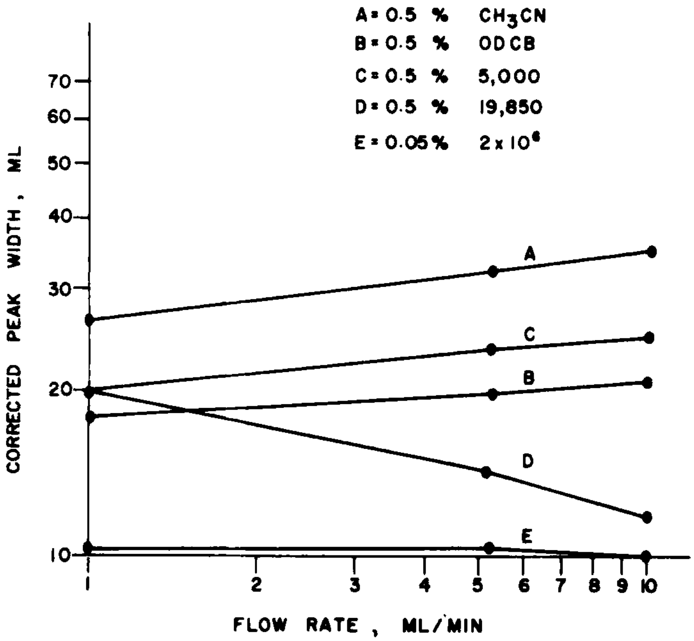

FIG. 3. Peak width as a function of flow rate.

standard with flow rate. In fact, the increase in peak width with flow rate for the less mobile 5000 standard is no greater than that of the smaller and more mobile solutes (acetonitrile and ODCB).

In summary, we have found by using very large gel particles that a decrease in elution volume with flow rate is observed for polymer molecules in the permeation range. In one case, we report a decrease in peak width with flow rate.

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