Gradient

Forensic –

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molecular weight lower than 10^4 and is observed ever at phenomenon is almost improbable for polymers with a weights than for those with lower molecular weights. This more pronounced for polymers with a higher molecular magnitude of the peak shift to higher retention volume is a well-known phenomenon and the sample solution. The concentration dependence of the polymers increase with increased concentration of the injection variables in SEC, because the retention volumes of because the retention volumes of polymers increase with increased concentration of the sample solution. The concentration dependence of the retention volume is a well-known phenomenon and the magnitude of the peak shift to higher retention volume is more pronounced for polymers with a higher molecular weights than for those with lower molecular weights. This phenomenon is almost improbable for polymers with a molecular weight lower than 10^4 and is observed ever at a low concentration, such as 0.01%, although the peak shift is smaller than that at a higher concentration.

In this sense, this concentration dependence of the retention volume should be called the “concentration effect,” not “overload effect” or “viscosity effect.” If a large volume of a sample solution is injected, an appreciable shift in retention volume is observed, even for low-molecular-weight polymers; this is called the “overload effect.”

The retention volume increases with increasing concentration of the sample solution and the magnitude of the increase is related to the increasing molecular weight of the sample polymers. The reason for the increase in retention volume with increasing polymer concentration is considered to result from the decrease in the hydrodynamic volume of the polymer molecules in the solution.

Molecular-weight averages calculated with calibration curves of varying concentrations may differ in value. As the influence of the sample concentration on the retention volume is based on the essential nature of the hydrodynamic volume of the polymer in solution, it is necessary to select experimental conditions that will reduce the errors produced by the concentration effect.

By rule of thumb, the preferred sample concentrations, if two SEC columns of 8 mm inner diameter (I.D.) × 25 cm in length are used, are as follows. The sample concentrations should be as low as possible and no more than 0.2%. For high-molecular-weight polymers, concentrations less than 0.1% are often required, and for low-molecular-weight polymers, concentrations of more than 0.2% are possible. The concentrations of polystyrene standards for calibration should be one-half of the unknown sample concentration. For polystyrene standards with a molecular weight over 10^6, it is preferable that they are one-eighth to one-tenth and for those with a molecular weights between 5 × 10^5 and 10^6, a quarter to one-fifth of the sample concentration.

The retention volume of a polymer sample increases as the injection volume increases. In some cases, the increase in the retention volume from an injection volume increase from 0.1 to 0.25 ml was 0.65 ml, whereas that from 0.25 to 0.5 ml was only 0.05 ml, suggesting that a precise or constant injection is required even if the injection volume is as small as 0.1 or 0.05 ml. In view of the significant effect of the injection volume on the retention volume, it is important to use the same injection volume for the sample under examination as that used when constructing the calibration curve. The use of a loop injector is essential, and the same injection volume must be employed for all sample solutions including calibration standards, regardless of their molecular-weight values. The increase in the injection volume results in a decrease in the number of theoretical plates, due to band broadening, which means that the calculated values of the molecular-weight averages and distribution deviate from the true values (Fig. 1).

The retention volume in SEC increases with increasing flow rate. This is attributed to non-equilibrium effects, because polymer diffusion between the intrapores and extrapores of gels is sufficiently slow that equilibrium cannot be attained at each point in the column. With a decreasing flow rate, the efficiency and the resolution are increased. Bimodal distribution of a PS standard (NBS706) with a narrow molecular weight distribution was clearly observed at the lower flow rate.

Separation of molecules in SEC is governed, mainly, by the entropy change of the molecules between the mobile phase and the stationary phase, and the temperature independence of peak retention can be predicted. However, an
An increase in retention volume with increasing column temperature is often observed. A temperature difference of 10°C results in a 1% increase in the retention volume, which corresponds to a 10–15% change in molecular weight. \[4\]

Two main factors that cause retention-volume variations with column temperature are assumed: an expansion or a contraction of the mobile phase in the column and the secondary effects of the solute to the stationary phase. When the column temperature is 10°C higher than room temperature, the mobile phase (temperature of the mobile phase is supposed to be the same as room temperature in this case) will expand about 1% from when it entered the columns, resulting in an increase in the real flow rate in the column due to the expansion of the mobile phase and the decrease in the retention volume. The magnitude of the retention-volume dependence on the solvent expansion is evaluated to be about one-half of the total change in the retention volume. The residual contribution to the change in retention volume is assumed to be that due to gel–solute interactions such as adsorption.

In order to obtain accurate and precise molecular-weight averages, the column temperature, as well as the difference of both temperatures, the solvent reservoir and the column oven, must be maintained.

Other factors affecting retention volume are the viscosity of the mobile phase, the sizes of gel pores, and the effective size of the solute molecules. Of these, the former two can be ignored, because they exhibit either no effect or only a small effect. The effective size of a solute molecule may also change with changing column temperature. The dependence of intrinsic viscosity on column temperature for PS in chloroform, tetrahydrofuran, and cyclohexane were tested. \[5\] The temperature dependence of intrinsic viscosity of PS solutions was observed over a range of temperatures. The intrinsic viscosity of PS in tetrahydrofuran is almost unchanged from 20°C up to 55°C, whereas the intrinsic viscosity in chloroform decreased from 30°C to 40°C. Cyclohexane is a theta solvent for PS at around 35°C and intrinsic viscosity in cyclohexane increased with increasing column temperature.

Because the hydrodynamic volume is proportional to the molecular size, the intrinsic viscosity can be used as a measure of the molecular size and optimum column temperatures and solvents must be those where no changes in intrinsic viscosity are observed.

**REFERENCES**