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CONCENTRATION EFFECTS IN GEL PERMEATION CHROMATOGRAPHY

V. DIFFERENTIAL ELUTION AND VISCOSITY PHENOMENA

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SUMMARY

The viscosity phenomena that take place in the interstitial volume of a chromatographic column and are connected with changes in the concentration and specific viscosity of an injected polymer solution have been studied. These phenomena were also studied under conditions of differential elution when, instead of a pure solvent, solutions of the same polymer at concentrations both higher and lower than those of the injected sample were used as the mobile phase. Linear relationships were found between the elution volume and specific viscosity and between the efficiency and specific viscosity. It was also ascertained that these dependences hold under conditions of differential elution; accordingly, the absolute viscosity of the mobile phase is of minor importance for the phenomena investigated.

INTRODUCTION

The complexity of processes that lead to changes in the elution volume, V_e , and in the peak width with changes in the concentration of an injected polymer solution has been elucidated in a systematic study of concentration effects in the gel permeation chromatography (GPC) of polymers under model conditions^{1–4}. A hypothesis that viscosity phenomena in the interstitial volume manifest themselves to an extent that is proportional to the specific viscosity of the polymer solution, η_{spec} , was proposed and formulated mathematically in Part I¹. In Part II², this hypothesis was confirmed experimentally and it was proposed to evaluate the data by means of statistical parameters of elution curves; this enabled us to correlate the experimental results when the shape of the elution curves was very complex. It clearly follows from the experimental study³ and from the theoretical analysis⁴ that viscosity plays a very important role in the complex phenomena that contribute to the overall concentration effect. This paper is devoted to a more detailed experimental study of viscosity phenomena.

Measurements² with polystyrene standards with molecular weights above the exclusion limit of the packing have been complemented by differential elution ex-

periments, in which solutions containing a known amount of a polymer with a molecular weight above the exclusion limit served as the mobile phase; solutions of the same polymer (with concentrations either higher or lower than that of the mobile phase) were injected. In this manner, the effect of the viscosity of the mobile phase on the phenomena brought about by the difference in the viscosities of the mobile phase and the injected sample solutions could be evaluated. Thus, the correlation of experimental results from various measurements enabled us to generalize to a certain extent our knowledge of viscosity phenomena in the interstitial volume.

A similar method of differential elution was used by Chuang and Johnson⁵ for discerning small differences in the shapes of molecular weight distributions. Bartick and Johnson⁶ outlined a possible utilization of differential GPC for studying the concentration effects from a point of view somewhat different from ours. Finally, Bakoš *et al.*⁷ used differential GPC in a study of polymer incompatibility and the connected concentration effects. This paper is intended to contribute to the elucidation of some aspects of viscosity effects under conditions of differential GPC.

EXPERIMENTAL

Gel permeation chromatography

GPC measurements were performed on the instrument, designed in our laboratory, which was described in detail in Part II². Samples were injected by means of a six-port valve (Waters Assoc., Milford, Mass., U.S.A.) using a loop with a volume of 80 μ l. The column (30 \times 0.8 cm) was packed with Porasil B (Waters Assoc., particle size 63–71 μ m) and maintained thermostatically at $25 \pm 0.01^\circ$. The exclusion limit of this packing for polystyrene (PS) corresponds to a molecular weight of *ca.* 100,000. A Model 2025 differential refractometer (Knauer, Oberursel/Taunus, G.F.R.) was employed as a detector; the cell volume of this instrument was 8 μ l and the dead volume between the column end and the refractometer cell was less than 10 μ l (a capillary tube of diameter 0.2 mm). Tetrahydrofuran (THF), employed as the solvent, was distilled from copper(I) chloride and potassium hydroxide. The other experimental details were described in Part II².

In differential elution experiments, a solution of a PS standard at a given concentration (with a given η_{spec} with respect to pure THF) was used as the mobile phase. Solutions of the same standard PS in THF but of a different concentration (both higher and lower) were injected.

Polymer sample

A PS standard (Knauer) with a molecular weight of 670,000 ($M_w/M_n < 1.1$) was used throughout. The specific viscosities were calculated from the Huggins equation with Huggins constant $k_H = 0.362$; the constants of the Mark–Houwink equation (valid for linear PS in THF at 25°) employed were the same as in Part I¹. $[\eta] = 1.17 \cdot 10^{-2} M^{0.717}$ (ml/g), where $[\eta]$ is the intrinsic viscosity and M is the molecular weight.

RESULTS AND DISCUSSION

We found in a previous paper² that the average elution volume, V_{av} , of totally excluded PS standards was proportional to the specific viscosity, η_{spec} , of the injected

solution, regardless of the sample molecular weight; moreover, within certain ranges of η_{spec} and of the flow-rate, this dependence was linear. We also found that the dependence of V_{av} on the standard deviation, σ , of a peak calculated as the square root of the second central moment (variance), was also linear. Accordingly, one can correlate the experimental data at different values of η_{spec} and different flow-rates either on the basis of V_{av} or by means of the height equivalent to a theoretical plate, HETP:

$$\text{HETP} = (1/L) (\sigma/V_{\text{av}})^2$$

where L is the column length. Table I gives the calculated values of η_{spec} for the injected samples (solutions of the PS standard or pure THF) with respect to the pertinent mobile phase (THF or a solution of the same PS standard with a different concentration); these values were calculated from the Mark-Houwink equation and from the

TABLE I
CALCULATED SPECIFIC VISCOSITIES OF INJECTED SOLUTIONS OF A POLYSTYRENE STANDARD

C^* (% w/v)	η_{spec}^{**}						
	0	1.883	0.941	0.471	0.235	0.118	0.059
3.766		1.839	5.446				
2.824		0.800	3.086				
2.353		0.370					
2.118		0.177					
2.000		0.087					
1.941		0.043					
1.883	7.287		1.270	2.990	4.614	5.776	6.486
1.824		0.044					
1.765		0.116					
1.647		0.194					
1.412	4.720	0.449	0.567	1.754	2.875		
1.177			0.267				
1.059			0.129				
1.000			0.064				
0.941	2.650	1.270		0.757	1.473	1.984	2.297
0.882			0.065				
0.824			0.137				
0.706			0.303	0.349			
0.588				0.167			
0.529				0.081			
0.471	1.077		0.757		0.407	0.698	0.876
0.412				0.085			
0.353				0.179	0.193	0.440	
0.294					0.094		0.459
0.235	0.476		1.473	0.407		0.207	
0.176					0.097	0.101	0.216
0.118	0.223				0.207		0.105
0.059	0.107					0.105	
0		7.287	2.650	1.077	0.476	0.223	0.107

* Concentration of the injected solution.

** Specific viscosity with respect to the mobile phase of concentration C (% w/v).

Huggins equation with the given k_H value. Experimentally determined η_{spec} values for PS solutions of different concentrations were identical with calculated data, within the limits of experimental error of viscometry.

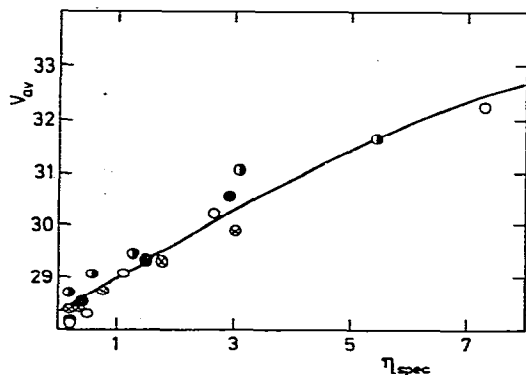


Fig. 1. Dependence of the average elution volume, V_{av} (counts), on specific viscosity (η_{spec}) of the injected polymer solution for different (lower) concentrations of the same polymer in the mobile phase. Flow-rate, 0.334 ml/min. Polymer concentration in the mobile phase (% w/v): \circ , 0.0; \bullet , 0.235; \otimes , 0.471; \odot , 0.941.

A plot of V_{av} against η_{spec} is given in Fig. 1 for cases when the viscosity (concentration) of the injected solution was higher than that of the mobile phase. Although there is a certain scatter of points in Fig. 1, it can be stated that the relationship between η_{spec} and V_{av} , which is linear up to $\eta_{spec} \approx 4$ under the given experimental conditions (when the viscosity or concentration of injected solutions was higher than that of the mobile phase), remains generally valid also for differential elution. It can therefore be concluded that, to a good approximation, the absolute viscosity of the mobile phase in this instance is unimportant so far as viscosity effects in GPC are concerned.

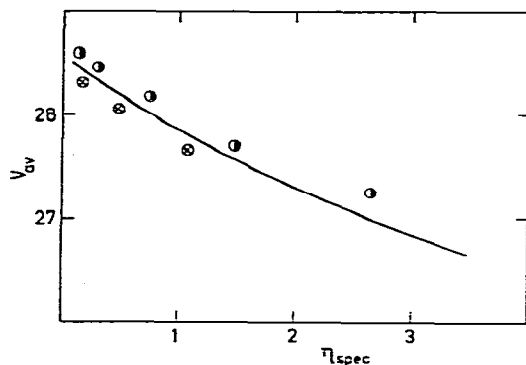


Fig. 2. Dependence of the average elution volume, V_{av} (counts), on specific viscosity (η_{spec}) of injected polymer solutions at different (higher) concentrations of the same polymer in the mobile phase. Flow-rate, 0.038 ml/min. Symbols as in Fig. 1.

All data in Fig. 1 pertain to a flow-rate of 0.334 ml/min; however, similar results were obtained at 0.038 ml/min. On the other hand, values of V_{av} in Fig. 2 were obtained in experiments in which the concentration (and the viscosity) of the injected solution was lower than that of the mobile phase; at a flow-rate of 0.038 ml/min V_{av} decreases with increasing η_{spec} , so that the zone of the injected sample moves faster under these conditions than in the previous instance. However, within the limits of acceptable experimental scatter, the absolute viscosity of the mobile phase again does not influence the shape of the dependence of V_{av} on η_{spec} to any marked extent.

Plotting the values of σ against V_{av} for the latter experimental arrangement, a single correlation curve was obtained (Fig. 3). One can say that this dependence is approximately identical with the curve obtained in experiments in which the sample viscosity was higher than that of the mobile phase. Similar conclusions can be drawn also from experiments performed at a flow-rate of 0.334 ml/min.

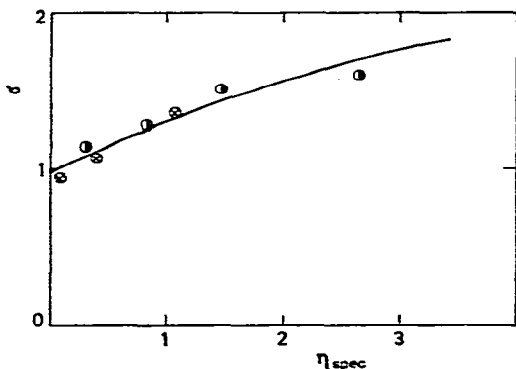


Fig. 3. Dependence of the standard deviation, σ (counts), of the elution curve on specific viscosity (η_{spec}) of the injected polymer solution at different (higher) polymer concentrations in the mobile phase. Flow-rate, 0.038 ml/min. Symbols as in Fig. 1.

We also employed some previous experimental data² in order to find the dependence of the efficiency, characterized by the HETP, on η_{spec} and the solvent flow-rate for different values of η_{spec} of injected PS solutions. Fig. 4 shows the HETP as a

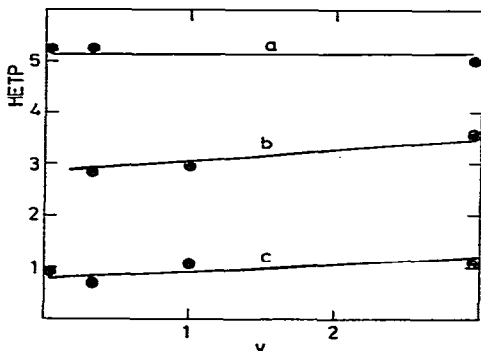


Fig. 4. Dependence of the plate height, HETP (mm), on the flow-rate (ml/min) at different values of specific viscosity (η_{spec}) of the polymer solutions injected into THF. η_{spec} : (a) 7.286; (b) 1.077; (c) 0.476.

function of the flow-rate for different η_{spec} of injected PS solutions. It can be seen that under the experimental conditions used, the HETP of the excluded polymer sample remains virtually constant at flow-rates in the range 0.038–3.000 ml/min. This result agrees in principle with the findings of Giddings *et al.*⁸.

The dependence of HETP on η_{spec} of the injected solutions at a flow-rate of 0.334 ml/min is linear within the investigated range of specific viscosity, as shown in Fig. 5. To a good approximation the single curve in Fig. 5 is also valid for the differential elution in all instances when the concentration (and viscosity) of injected solutions was higher than that of the mobile phase. For other investigated flow-rates, the plots of HETP against η_{spec} were virtually identical (the slightly different absolute values of HETP in Fig. 5 in comparison with Fig. 4 are due to a change in the properties of the column).

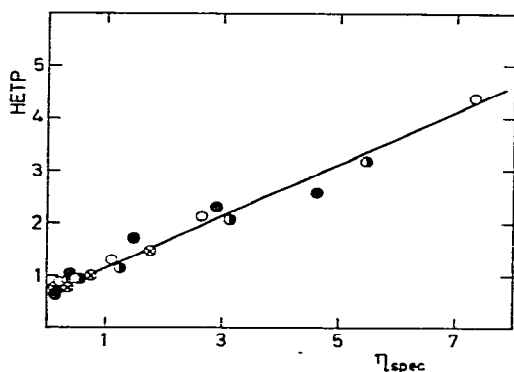


Fig. 5. Dependence of the plate height, HETP (mm), on the specific viscosity (η_{spec}) of polymer solutions injected into a mobile phase of different concentrations. Symbols as in Fig. 1.

The results show clearly how the changes in the elution volume and the efficiency with changes in the sample concentration are connected with viscosity phenomena in the interstitial volume. In view of the fact that these phenomena play a very important, if not decisive, role in the complex contributions to the overall concentration effect (as was shown in previous parts in this series), the importance of their study for the elucidation of the detailed mechanisms of processes that take place in a chromatographic column is evident.

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