

CHROM. 13,732

CONCENTRATION EFFECTS IN GEL PERMEATION CHROMATOGRAPHY

VII. VISCOSITY PHENOMENA AND COLUMN GEOMETRY

J. JANČA*

Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, 611 42 Brno (Czechoslovakia)

S. POKORNÝ

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6 (Czechoslovakia)

and

L. Z. VILENCHIK and B. G. BELENKII

Institute of High-Molecular Compounds, Academy of Sciences of the U.S.S.R., Leningrad (U.S.S.R)

(First received December 9th, 1980; revised manuscript received February 10th, 1981)

SUMMARY

Viscosity phenomena were studied in columns with considerably diverse geometrical parameters. It was found that these phenomena, characterized quantitatively by statistical parameters and qualitatively by comparison of the shapes and changes in chromatograms, are identical for different geometrical arrangements. Hydrodynamic transport processes, which give rise to viscosity phenomena in chromatography of polymers, are thus general. The significance of the results ranges beyond the area of gel permeation chromatography of polymers since hydrodynamic transport processes are found every time polymer solutions pass through porous material, *e.g.*, in technological separation processes and the transport of macromolecules in biological media.

INTRODUCTION

As shown in our earlier papers^{1–11}, concentration effects in gel permeation chromatography (GPC), *i.e.*, changes in elution volumes which result from changes in the concentration or the total amount of the injected polymer solution, are caused by a number of contributing processes. Some of these processes are associated with the thermodynamic properties of and phenomena in the solute–solvent–gel system, and others with hydrodynamic transport phenomena in the mobile phase only. Hydrodynamic transport phenomena, which have been denoted as viscosity phenomena¹, are associated with the movement of the mobile phase and appreciable viscosity gradients and thus with the differences in mobilities of the pure solvent of the mobile phase and the solution of polymer in the zone.

Theoretical calculations and experimental results have shown that hydrodynamic phenomena account for 80–90% of the total change in elution volume with concentration^{4,7}. This paper considers in more detail of these hydrodynamic phenomena. It is possible to differentiate at least three effects associated with the dynamics of the passage of viscous zones through a porous medium, for example a chromatographic column.

The first effect is due to the formation of peripheral quasi-stationary zones in the channels of the mobile phase (see Fig. 1A). Having arrived in such a zone, the macromolecules can continue their motion along the column in this zone only. However, the main portion of the polymer solution passes outside the given zone and fresh solvent will dilute the solution and wash the solute out from the spot where it is retained. Consequently, we observe not only a shift of the peaks in a chromatogram towards higher elution volumes and an increase in their asymmetry, but also multimodality.

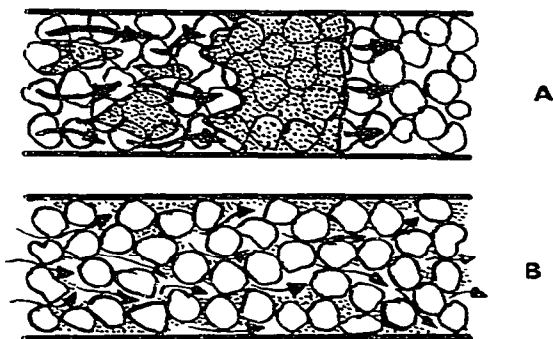


Fig. 1. Viscous fingering of the polymer solution flowing through the column packing.

The second effect is caused by the formation of quasi-stationary zones of the “plug” type in various parts of the column cross-section (see Fig. 1A and B). Under conditions of high viscosity, such a zone is formed due to non-homogeneity of the column packing in the transverse direction. At a cross-section containing a plug the motion of the solution is retarded or even stopped and the solvent, following the solution, “bombards” its rear boundary, deforming or sometimes even breaking it. This is sometimes called “viscous fingering”. The influence of this effect on the elution volumes is obviously analogous to that of the first effect. Additionally, the effect must increase with increasing density of the column packing and with decreasing size of the sorbent particles. It is more significant with narrow columns than with wide ones. The influence of this effect does not depend on the absolute viscosity of the solution in the chromatographic zone or that of the solvent, but only on viscosity gradients.

The third effect is associated with the dependence of the diffusion rate of the macromolecules on the viscosity of the solution. If the diffusion coefficients decrease substantially, this effect can give rise to a non-equilibrium chromatographic process. The consequence of this would be that the influence of the first two effects would be intensified, leading to significant asymmetry of the chromatographic peaks and to elongated tails.

The complicated shapes of the elution curves obtained on injection of polymer solutions having high specific viscosities provide information on the heterogeneity of the porous interstitial structure of the column packing. It was considered necessary to investigate experimentally how columns having very diverse geometrical characteristics differ in the interstitial structure of the column packing which influences the shape of the elution curves. In the first place the ratio of the column diameter to its length, the way of injecting samples into the column and the ratio of the particle to the column diameter are understood by different geometrical characteristics of the chromatographic columns. The present paper is devoted to the study of the influence of these geometrical characteristics on viscosity phenomena.

EXPERIMENTAL

All the measurements were carried out in a Hewlett-Packard Model 1084 B liquid chromatograph equipped with a UV photometer operating at 260 nm. Two columns were used: 53×22 mm I.D., packed with Porasil B (Waters Assoc., Milford, MA, U.S.A.), particle size 63–71 μm ; 250×4.6 mm I.D., packed with Lichrosphere SI 100 silica gel (E. Merck, Darmstadt, G.F.R.), particle size 10 μm . Tetrahydrofuran, purified by distillation from cuprous chloride and potassium hydroxide, was used as the solvent. The columns were thermostated at 30°C.

A polystyrene standard (Knauer, Oberursel, G.F.R.) was applied with a narrow distribution of molecular weights, $\bar{M}_w/\bar{M}_n < 1.06$, where $\bar{M}_w = 694,000$. Solutions of the standard in tetrahydrofuran were prepared at various concentrations or specific viscosities. The specific viscosities were calculated using Mark-Houwink and Huggins equations valid for tetrahydrofuran, which were presented earlier¹.

RESULTS AND DISCUSSION

It was desired to determine the extent to which the viscosity phenomena which had been observed earlier under given experimental conditions would occur under different experimental conditions and thus to what extent they are general. Thus two columns were employed, differing substantially (more than twenty-fold) in the ratio of their column length to diameter, but with the ratio of the column diameter to the diameter of particles being almost constant. Furthermore, these two columns (denoted by B and C in Table I) differed in the way in which the injected solution of the polymer was introduced into the column. Column C, having the smaller diameter, was equipped with a stainless-steel frit the length of which was almost equal to the inside diameter of the column. This frit was used to secure more uniform injection of the sample onto the column packing throughout the column cross-section. For column B, having the larger diameter, the injected solution was introduced into the column via a capillary which projected into the column packing so that the sample was injected into the centre of the column. The geometrical characteristics of the column used earlier² and the experimental results obtained with it were used as reference data.

Table I presents relevant characteristics of the original column, denoted A. Compared with column B, the ratio of its length to diameter is *ca.* 15-fold higher and at the same average particle diameter the ratio of the column to particle diameter is

TABLE I

GEOMETRICAL CHARACTERISTICS OF THE CHROMATOGRAPHIC COLUMNS AND THE EXPERIMENTAL CONDITIONS USED

D = Column diameter; L = column length; \bar{d}_p = average particle diameter of column packing; V_I = volume of injected polymer solution; V_T = total volume of the column; q = flow-rate.

Column	D (mm)	L (mm)	\bar{d}_p (mm)	D/\bar{d}_p	L/D	V_I (ml)	V_T (ml)	$(V_I/V_T) \cdot 100$	q (ml/min)	$4q/\pi D^2$ (ml/min · cm ²)
A	8	300	0.067	119	37.5	0.080	15.08	0.53	0.334	0.664
B	22	53	0.067	328	2.41	0.100	20.15	0.50	2.070	0.545
C	4.6	250	0.010	460	54.3	0.020	4.15	0.48	0.525	3.159

almost three-fold lower. Compared with column C, the length-to-diameter ratio of column A is similar, the ratio of the column to the particle diameter is approximately four-fold lower and the particle diameter is approximately seven-fold larger. In order to eliminate other, undesirable factors, particularly the influence of the injected volume and of the flow-rate which could result in a non-Newtonian effect, these parameters were selected so that they should be approximately identical for all three columns. This was possible with an injected volume approximately 0.5% of the total column volume (see Table I). No difficulty was encountered in obtaining comparable flow-rates of the mobile phase through a cm² of the cross-sections (*i.e.*, comparable linear velocities) of columns A and B. With column C the measurements were performed at a minimum flow-rate of 0.5 ml/min; however, in comparison with column A, this corresponds to an almost five-fold higher linear velocity. On the other hand, the molecular weight of the polystyrene standard used in column C was almost four times lower than that in column A, thus the possibility of a non-Newtonian phenomenon, observed earlier², was slight, verified experimentally at higher-flow rates, up to *ca.* 3 ml/min in column C. Even in this range of flow-rates, no significant non-Newtonian phenomenon was observed with the polystyrene standard having $M_w = 694,000$. However, with the polystyrene standard having $M_w = 2,700,000$, a non-Newtonian phenomenon was observed.

Figs. 2 and 3 show the results obtained on the viscosity phenomena in columns B and C. The course of the dependence of the average elution volume, V_{av} ,

$$V_{av} = \Sigma V_i h_i / \Sigma h_i \quad (1)$$

where h_i is the height of the chromatogram from the baseline at the corresponding elution volume V_i , on the specific viscosity, η_{spec} , of the injected polymer solution is in qualitative agreement with that obtained in the previous study with column A under the same experimental conditions (see Fig. 5, ref. 2). Despite the fact that a straight (dashed) line is drawn through all the experimental points in Figs. 2 and 3, with regard to the previous studies a non-linear course cannot be ruled out, particularly at higher values of η_{spec} . It is therefore better to draw the straight line through only the experimental points at lower values of η_{spec} (up to $\eta_{spec} \approx 3$), which is with regard to the earlier results, the approximation still valid. The slopes of these straight lines are

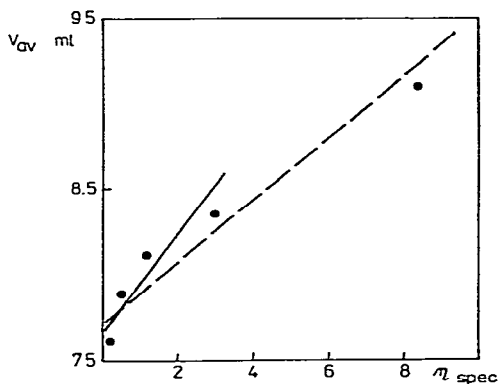


Fig. 2. Dependence of the average elution volume on the specific viscosity of the injected solution of the polystyrene standard in column B. ---, The solution of highest viscosity was considered; —, the solution of highest viscosity was not considered.

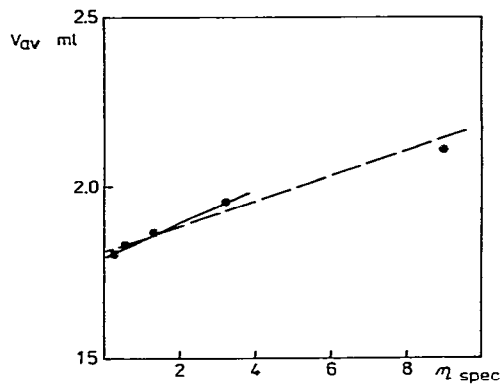


Fig. 3. Dependence of the average elution volume on the specific viscosity of the injected solution of the polystyrene standard in column C. Other details as in Fig. 2.

now expressed in a way that enables columns B and C, with different total volumes, to be compared:

$$S = \Delta V_{av} / (V_{av}^0 \cdot \Delta \eta_{spec}) \quad (2)$$

Here ΔV_{av} is the difference in the average elution volumes corresponding to the difference in the specific viscosities, $\Delta \eta_{spec}$, and V_{av}^0 is the average elution volume extrapolated to zero concentration. The values of S , calculated as above, are 3.2 and 2.7 for columns B and C, respectively. It is seen that, despite the considerably diverse geometries of columns B and C, the parameters of the viscosity phenomena are very similar. The value $S = 6.0$, calculated from the earlier results² for column A, is thus twice as high; however, this difference can be explained by the higher efficiency of column A, whose height equivalent to a theoretical plate (HETP) is 0.42 mm for the excluded polystyrene standard at the lowest of the measured concentrations, while for columns B and C, $HETP = 1.04$ mm under identical conditions. The more pronounced dependence of V_{av} on η_{spec} at higher column efficiencies is in accord with both theoretical assumptions^{1,4,7} and experimental results³.

Figs. 4 and 5 show chromatograms obtained from measurements at various concentrations in columns B and C. The overall appearance of these chromatograms and their behaviour at increasing concentration are similar for columns B and C. They are also similar to the chromatograms obtained on column A (see Fig. 2, ref. 2). In the rather chromatograms the fine structure is more complicated, which is again associated with the higher efficiency of column A. With columns B and C, this fine structure is suppressed owing to greater broadening.

From the results presented, it is seen that viscosity phenomena in the interstitial volume, resulting from the different mobility of the polymer solution in the chromatographed zone in comparison with the pure solvent of the mobile phase, and constituting the dominant contribution to the concentration effects, were observed in columns having considerably diverse geometry. A quantitative comparison of the results obtained on the various columns showed that the viscosity phenomena appear to be general, regardless of the geometrical arrangement of the experiment, and to occur to similar extents. This is confirmed not only by statistical parameters charac-

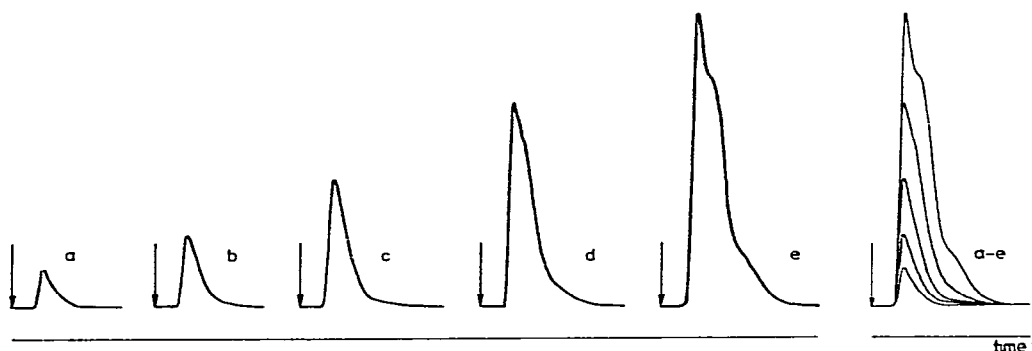


Fig. 4. Chromatograms of the polystyrene standard at various concentrations in column B. Specific viscosities of the injected polystyrene solutions: 0.244 (a); 0.525 (b); 1.197 (c); 2.984 (d); 8.327 (e).

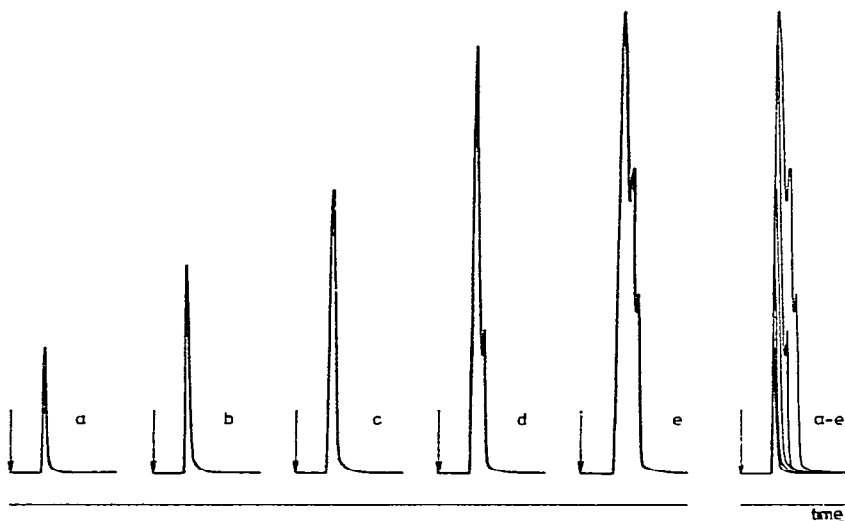


Fig. 5. Chromatograms of the polystyrene standard at various concentrations in column C. Specific viscosities of the injected polystyrene solutions: 0.256 (a); 0.553 (b); 1.268 (c); 3.182 (d); 8.950 (e).

terizing chromatograms at various concentrations or at various specific viscosities of the injected polymer solutions, but also by qualitative comparison of the character and the overall shape of chromatograms. Thus the hydrodynamic transport processes in the chromatographic columns are entirely general. Further theoretical and experimental studies of these phenomena are in progress.

REFERENCES

- 1 J. Janča, *J. Chromatogr.*, 134 (1977) 263.
- 2 J. Janča and S. Pokorný, *J. Chromatogr.*, 148 (1978) 31.
- 3 J. Janča and S. Pokorný, *J. Chromatogr.*, 156 (1978) 27.
- 4 J. Janča, *J. Chromatogr.*, 170 (1979) 309.
- 5 J. Janča and S. Pokorný, *J. Chromatogr.*, 170 (1979) 319.
- 6 J. Janča, *J. Chromatogr.*, 187 (1980) 21.
- 7 J. Janča, *Anal. Chem.*, 51 (1979) 637.
- 8 J. Janča, *Polym. J.*, 12 (1980) 405.
- 9 J. Janča, S. Pokorný, M. Bleha and O. Chiantore, *J. Liquid Chromatogr.*, 3 (1980) 953.
- 10 J. Janča, *J. Liquid Chromatogr.*, 4 (1981) 181.
- 11 L. Z. Vilenchik and B. G. Belenkii, *Vysokomol. Soedin., Ser. A*, 14 (1972) 1874.