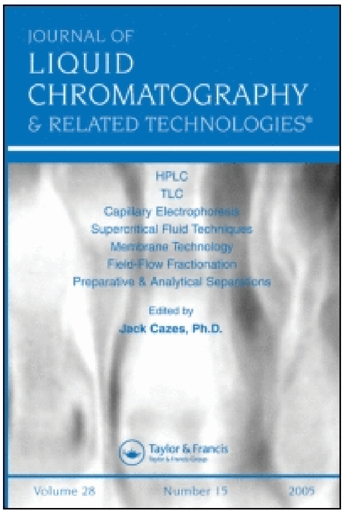

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Chiantore, Oscar and Guaita, Marino(1984) 'Concentration Dependence of Elution Volumes in Size Exclusion Chromatography of Polymer Molecules. 1. Effect of Viscosity and of Coil Contraction in Good Solvent', Journal of Liquid Chromatography & Related Technologies, 7: 9, 1867 — 1885

To link to this Article: DOI: 10.1080/01483918408068842

URL: http://dx.doi.org/10.1080/01483918408068842

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1867-1885 (1984)

CONCENTRATION DEPENDENCE OF ELUTION VOLUMES IN SIZE EXCLUSION CHROMATOGRAPHY OF POLYMER MOLECULES.

1. EFFECT OF VISCOSITY AND OF COIL CONTRACTION IN GOOD SOLVENT

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ABSTRACT

The contribution from viscosity phenomena and coil size contraction to the shift of polymer elution volumes with increasing concentration has been evaluated in size exclusion chromatography through a practical experimental procedure. It is shown that the viscosity effect is operative to different extents, depending on the different column systems. For most of the investigated polymer samples, however, macromolecular coil contraction seems to be the main contributing effect to the total concentration dependence of polymer elution volumes.

INTRODUCTION

It is well known that, in size exclusion chromatography (SEC) of polymer molecules, when the concentration of the injected sample is increased the peak maximum is shifted toward higher elution volumes. The change of elution volumes is particularly evident for narrow distribution polymers such as the standards normally used for calibration and, in the same chromatographic system, it increases with increasing both the sample molar mass and the thermodynamic quality of the solvent.

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This concentration dependence of elution volumes in SEC has been generally attributed either to an effect of contraction of the polymer coils with increasing concentration in the injected solution (1), or to a sum of contributing processes (2), namely, the mentioned coil contraction, the effect of the viscosity of the solutions in the interstitial volume of the columns, and the so-called secondary exclusion due to the reduction of the accessible pore volume of the packing when polymer molecules are already present in the pores. The reduction of the effective hydrodynamic volume of solvated polymer coils with increasing concentration can be accounted for by using the model developed by Rudin (1). Results from this model are in reasonable agreement with Yamakawa's theory relating concentration and hydrodynamic volumes of solvated polymers and were shown to describe SEC behaviour of several practical systems (3). The fact that the concentration dependence of elution volumes is lower in thermodynamically poor solvents (4-6) gives support to the hypothesis on which Rudin's theory is based. Quantitative relationships between concentration effects in SEC and thermodynamic quality of the solvents have been recently discussed (7).

The relative importance of the different contributing effects to the total concentration dependence of elution volumes was investigated by Janca (8-10). The viscosity of the injected polymer solutions was experimentally shown to drastically affect elution volumes and chromatogram widths of polymer standards which elute in the column interstitial volume only (8). Relationships for the quantitative description of this phenomenon and of the ratio of individual contributions to the overall concentration dependence were derived (9,10) and the application to experimental results led to an estimation of the viscosity effect as approximately 80% of the total concentration effect. The reported comparisons of experimental elution volumes with the predictions of Rudin's theory are therefore, according to Janca (11), incorrect because it is not considered that during the chromatographic process dilution along the column occurs; when this effect is not taken into account the polymer concentrations are obviously overestimated.

Attempt was also made of evaluating the contribution due to secondary exclusion (12): experimental results showed that, at least under stationary conditions, this process is probably operative to a very small extent. As a consequence it might be assumed that in SEC the increase of peak elution volumes with sample concentration is completely due, in real chromatographic systems as well, to the sum of the contributions from the macromolecular coil contraction and from the solution viscosity in the interstitial column volume.

In this paper we present results of an investigation intended to evaluate the relative amounts of the two contributing effects over the total concentration dependence of elution volumes under some practical experimental conditions. The study has been carried out in a good solvent, with narrow distribution polymer standards eluting either in the interstitial volume only, or in the permeation range of chromatographic columns with different pore sizes.

METHODOLOGY

The following procedure has been used to evaluate the different contributions to the concentration effect in our systems. The overall concentration dependence of elution volumes is obtained from direct injections of several standards at different concentrations. In general, for values of concentrations not too high the measured elution volumes vary linearly with the injected concentration. Injection in the same column system of a totally excluded polymer gives the shift of elution volumes due to the pure viscosity effect taking place in the interstitial volume. It has been shown (8) that for such a polymer a linear relationship exists between the specific viscosity of the injected polymer solution and the elution volume, at least up to viscosity values where the solution behaviour is still newtonian. Owing to the fact that such a viscosity effect occurs in the interstitial volume, outside the gel pores, it might be assumed that a very same effect occurs for permeating polymers as well. In other words, for polymer solutions with the same specific viscosity, the same viscosity contribution should result, independently of the fact that the polymer molecules can diffuse into the gel pores. Therefore, such a viscosity contribution can be evaluated, for each injected sample, from the elution volume shift of the excluded polymer at the same specific viscosity. The specific viscosities of the polymer samples are calculated by the Huggins equation (13) at the different concentrations, and the amount of the viscosity effect can be subtracted from the total increase of the elution volumes.

EXPERIMENTAL

The chromatographic columns employed (25 cm length, 0.46 cm I.D.) were slurry packed with microparticulate spherical silica gels (average particle diameter 10 μm) supplied by E. Merck (Darmstadt, Germany). Mean pore sizes of the gels were 10 nm, 50 nm and 100 nm.

Two different column combinations were used in order to cover different molar mass ranges; their characteristics are reported in the next section.

Narrow distribution polystyrene (PS) standards (ArRo Laboratories, Joliet, Illinois, and Waters Associates, Milford, Massachusetts) were injected as tetrahydrofuran (THF) solutions at different concentrations; THF was used as eluent as well; injection volumes were 10 µl. An UV spectrophotometer (Zeiss PM2) at 260 nm wavelength was used as a detector. The chromatographic flow rate was approximately 0.5 cm^3/min.

All the measurements were done in triplicate and the results were averaged.

RESULTS AND DISCUSSION

The calibration curve of the first column system, two columns in series with 100 nm and 50 nm respectively as nominal pore size of the silicas, is shown in Fig. 1. The narrow distribution PS standards reported in Table 1 were injected at different concentrations (up to $2 \cdot 10^{-2}$ g/cm^3 for some of the samples) and the resulting peak elution volumes were measured.

As it appears from Fig. 1, the polymers with molar masses 17500, 111000, 200000, 390000 and 670000 are all eluting in the fractionation range of the columns, whereas the 10^7 standard is completely excluded from the pores. For the latter sample it has been checked by using Rudin's model (3) that even at the highest injected concentration, $2 \cdot 10^{-3}$ g/cm^3, his hydrodynamic volume is still large enough to make the molecules excluded.

With increasing polymer concentrations, not only peak elution volumes V_e and widths increased, but distorted chromatograms were obtained, especially for the high molar mass samples. Some examples are shown in Fig. 2 for two permeated polymers and for the excluded one. When the asymmetry and distortion of the chromatograms were severe, the average elution volumes of the polymer samples were obtained by calculating the first statistical moment of the peaks.

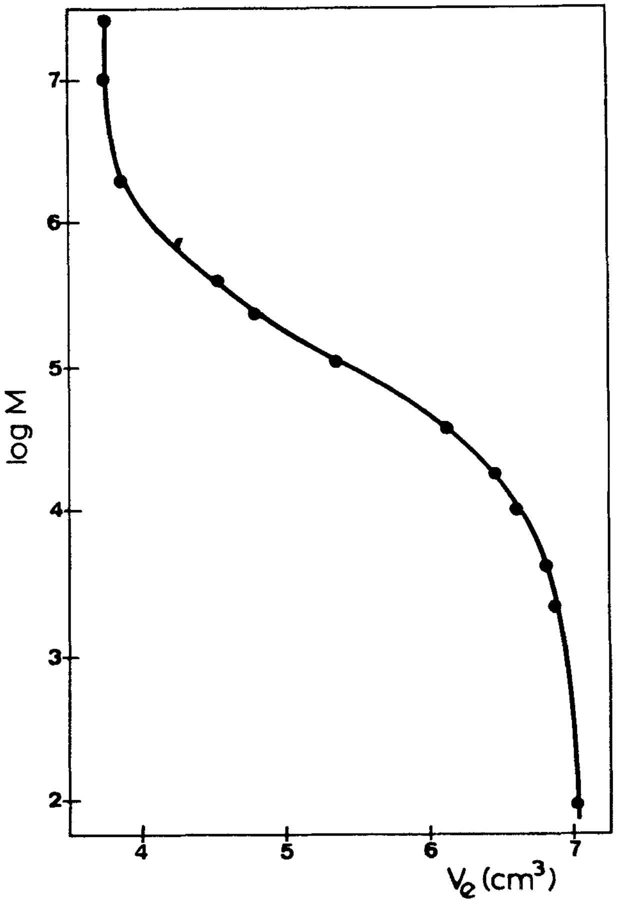

FIGURE 1. Calibration curve for the column system 100 nm + 50 nm.

The experimentally observed elution volumes at increasing concentrations, c, are reported in Fig. 3; the concentration dependence of V_e increases with the polymer molar mass as expected, and is linear either in the low concentration or in the whole range, depending on the different molar masses.

For each polymer concentration of Fig. 3 the specific viscosities were calculated by means of the Huggins equation

TABLE 1

Huggins Constants, k_H, and Contribution of Hydrodynamic Volume Contraction to the Concentration Effect for the PS Standards in the Column System 100 nm + 50 nm.

Polymer Molar Mass	k_H	$[d(\Delta V_s)/dc]/[d(\Delta V_t)/dc]$
17500	0.40	0.82
111000	0.33	
200000	0.31	0.77
390000	0.28	
670000	0.25	0.71
10^7	0.24	

$$\eta_{sp} = [\eta]\, c + k_H [\eta]^2 c^2 \qquad (1)$$

where the intrinsic viscosities $[\eta]$ were obtained from the equation (14)

$$[\eta] = 1.11\cdot 10^{-2}\ M^{0.723}. \qquad (2)$$

The values of the Huggins constant, k_H, for PS in the investigated molar mass range, in THF solution, were interpolated from the data by Spychaj et al.(15) and are reported in Table 1.

From Fig. 3 the elution volumes $V_{e,o}$ extrapolated at c=0 can be obtained. The total increment of elution volumes, $\Delta V_t = (V_{e,c} - V_{e,o})$, where $V_{e,c}$ is the elution volume of the polymer at concentration c, can be plotted against the polymer specific viscosities, as it is shown in Fig. 4. The increase of elution volumes for the

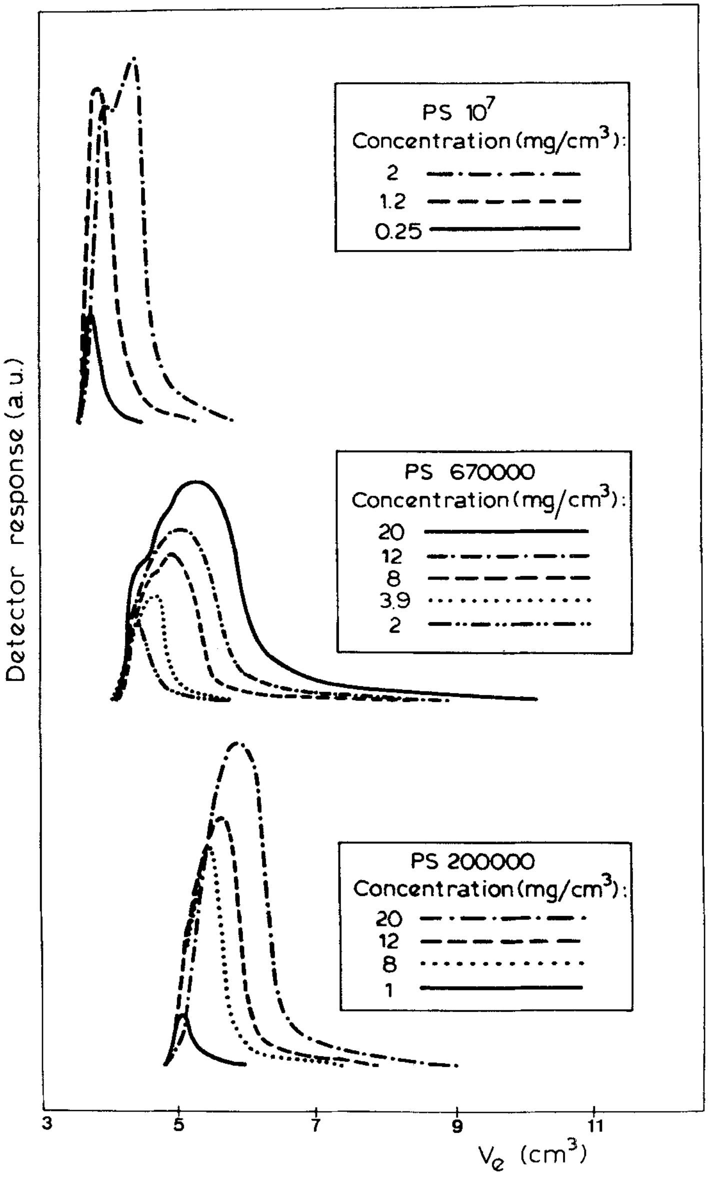

FIGURE 2. Chromatograms of permeated and excluded PS samples at different injected concentrations.

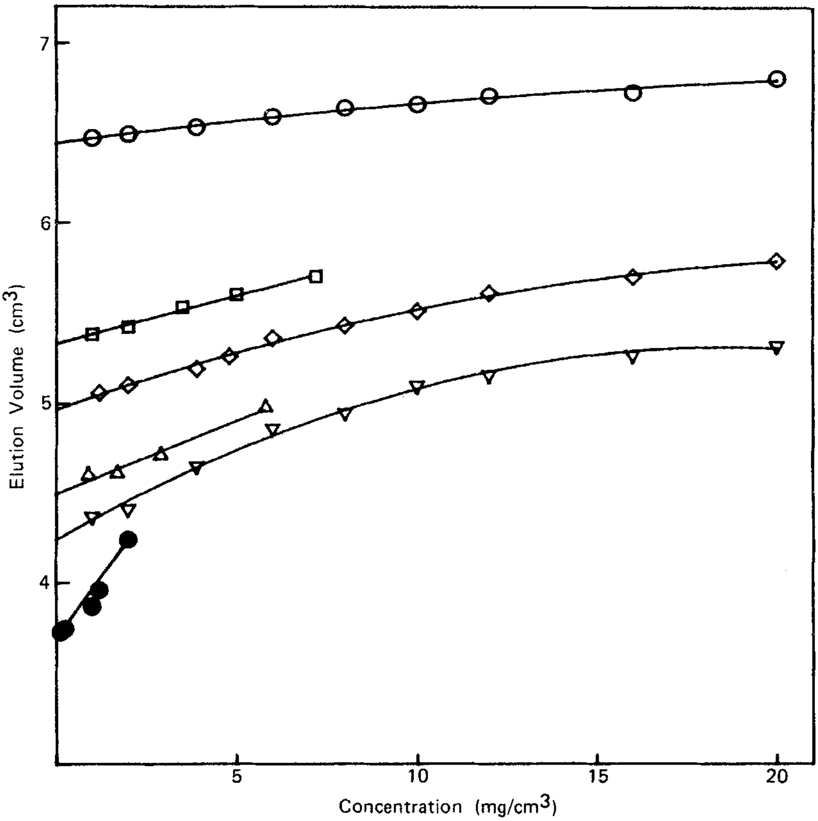

FIGURE 3. Elution volumes of PS standards at different concentrations; ○:17500; □ :111000; ◇:200000; △ :390000; ▽ :670000; ●:10^7.

excluded polymer, PS 10^7, is completely due to the viscosity effect in the interstitial volume. For all of the other samples, at each η_{sp} value, the ΔV_t values are higher than those of the excluded polymer, suggesting that in addition to viscosity, the effect of coil contraction is operative. The increase of ΔV_t is higher for the lower molar mass samples due to the fact that, for a same η_{sp} value, these polymer samples have a higher concentration.

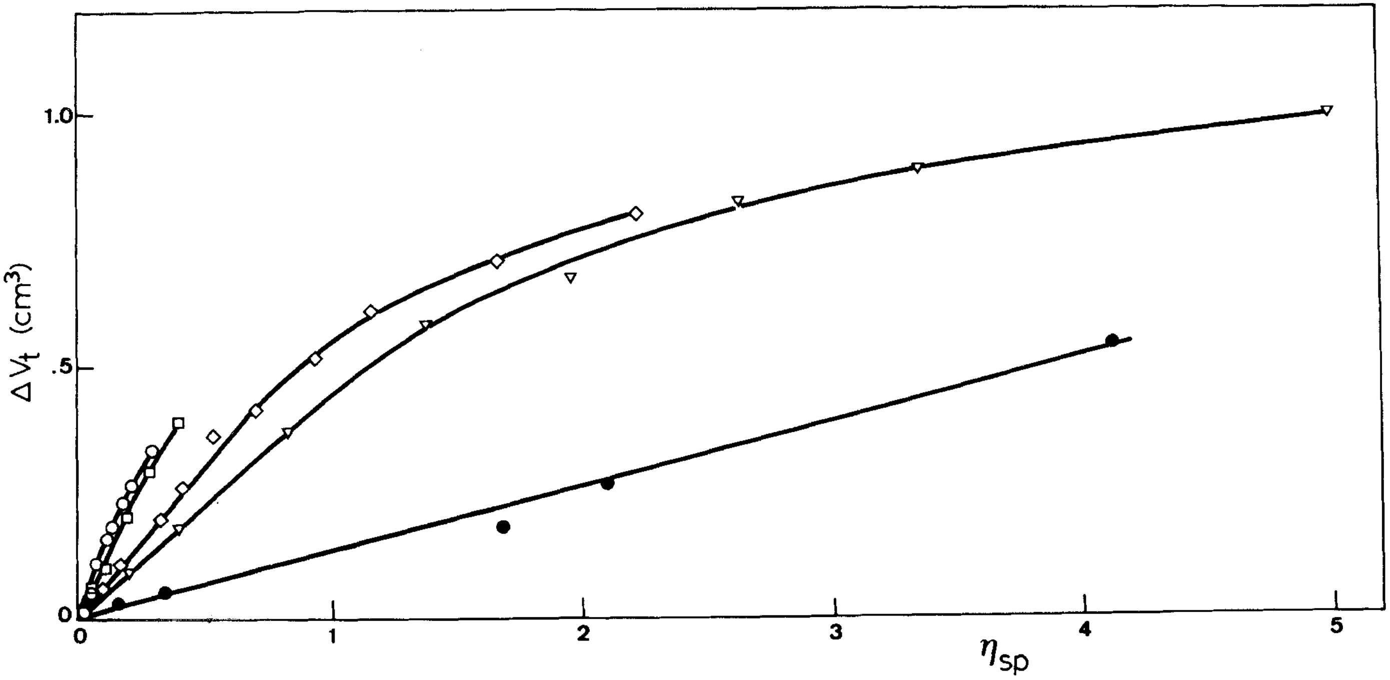

FIGURE 4. Changes of elution volumes, ΔV_t, with specific viscosities. Columns 100 nm + 50 nm. Symbols as in Fig. 3.

The contribution to the total concentration effect on elution volumes from viscosity phenomena is given, under the experimental conditions adopted and the assumptions already made, for each of the permeated polymer samples by the ΔV_t of the 10^7 PS sample at the same η_{sp} value;this contribution can be subtracted from the total change of elution volumes ΔV_t at the appropriate concentration and the resulting increment of elution volumes, ΔV_s, is due to the macromolecular coil contraction only.

In Fig. 5 the change of ΔV_t with injected polymer concentration is shown for three different molar mass samples, eluting in three different parts of the column permeation range, i.e. at the beginning (PS 670000), in the middle (PS 200000) and at the end (PS 17500) of the practically linear part of the calibration curve (see Fig. 1). After subtraction of the viscosity contributions, the resulting increment of elution volumes, ΔV_s, at different concentrations are plotted in Fig. 6 for the three PS standards. Both in Figs. 5 and 6 an initial linear dependence of the incremental elution volumes ΔV_t and ΔV_s on concentration is evident. For each polymer sample, the ratio of the slopes $d(\Delta V_s)/dc/d(\Delta V_t)/dc$ will give an estimate of the fractional amount of the contribution due to coil size contraction over the total concentration dependence of elution volumes. This ratios are reported in the last column of Table 1 for the samples considered. One can see that the contribution of coil size contraction is dominant, in our experimental system, in determining the increasing of elution volumes. The viscosity effect seems to be responsible for 20-30% only of the total elution volume change.

Similar experiments were also performed with a different column set (2 columns packed with 10 nm pore size silica gel) covering the molar mass range 10^3-10^5, as it is shown by the calibration curve

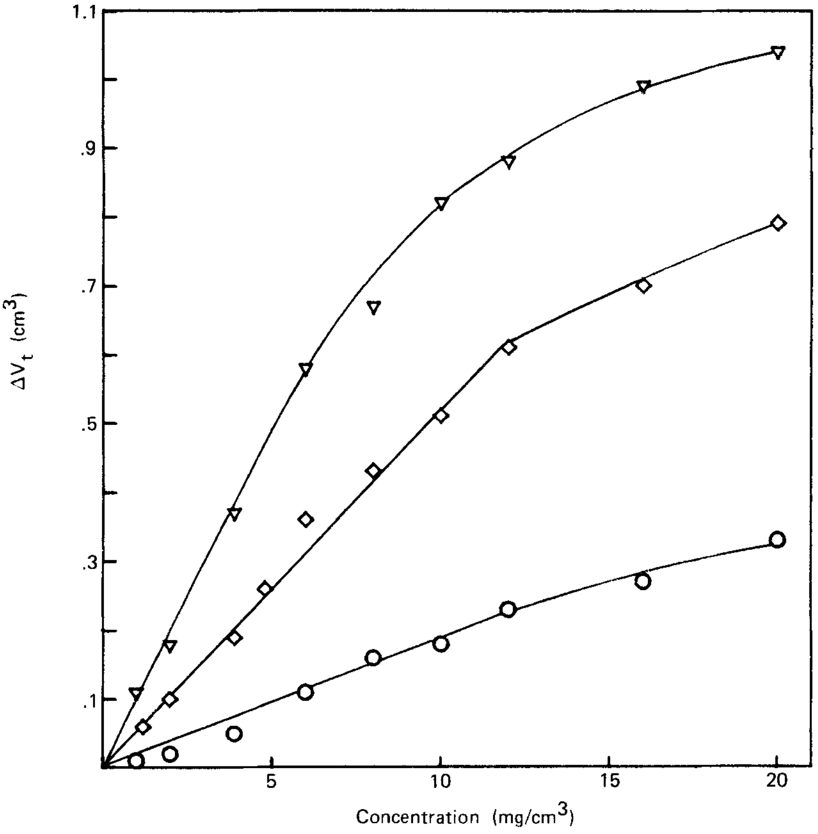

FIGURE 5. Changes of elution volumes, ΔV_t, with injected polymer concentration. Symbols as in Fig. 3.

in Fig. 7. The PS standards listed in Table 2 were injected at increasing concentrations. The results are shown in Fig. 8.

All the chromatograms were regular in shape, with only slight distortion for the peaks of the excluded polymer, PS 470000, shown in Fig. 9. For these latter samples the elution volumes were obtained from the first moment of the peaks.

It can be seen from Fig. 8 that the changes of elution volumes with concentration are small for the low molar mass permeating sam-

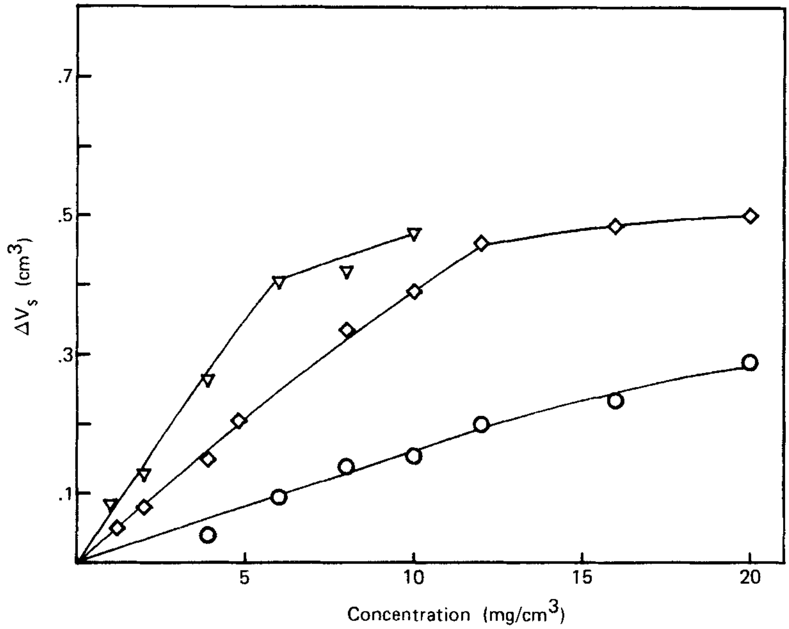

FIGURE 6. Increment of elution volumes for macromolecular coil contraction. Symbols as in Fig. 3.

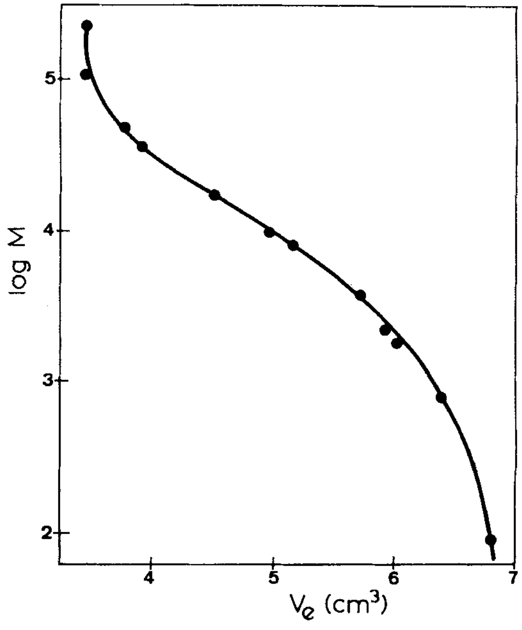

FIGURE 7. Calibration curve for the column system 2 x 10 nm.

TABLE 2

Huggins Constants, k_H, and Contribution of Hydrodynamic Volume Contraction to the Concentration Effect for the PS Standards in the Column System 2 x 10 nm.

Polymer Molar Mass	k_H	$[d(\Delta V_s)/dc]/[d(\Delta V_t)/dc]$
1800	0.50	0.65
8500	0.50	0.63
17500	0.40	0.47
50000	0.35	0.36
470000	0.27	

ples. The changes for PS 17500 are also slightly smaller than those observed for the same polymer in the first column system investigated. The specific viscosities of the injected samples were calculated at the different concentrations by using the Huggins constant values reported in Table 2. The $[\eta]$ values were obtained from Eq. (2) for all the standards, with the exception of PS 1800 and PS 8500, for which the viscometric equation (16)

$$[\eta] = 1.0\cdot 10^{-1} \; M^{0.50} \qquad (3)$$

recommended for low molar mass samples in good solvents, was employed. The k_H value for these two latter polymers were taken from reference data relative to low molar mass polystyrene samples in different solvents (16).

The total increments of elution volumes are plotted in Fig. 10 against the η_{sp} values: one can see that the viscosity effect, as

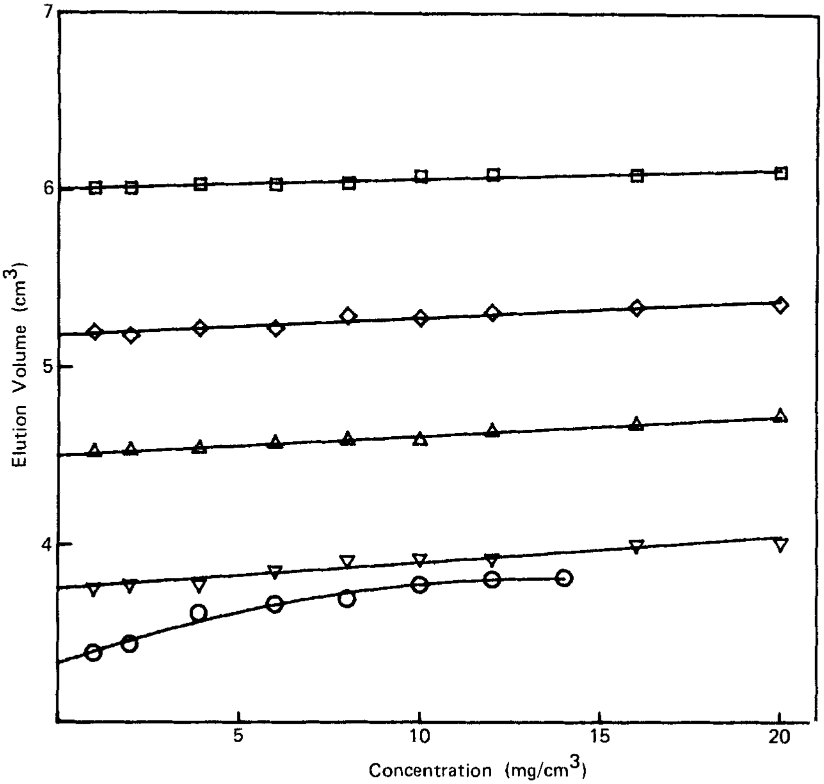

FIGURE 8. Elution volumes of PS standards in the column system 2 x 10 nm. □ :1800; ◇ :8500; △ :17500; ▽ :50000; ○ :470000.

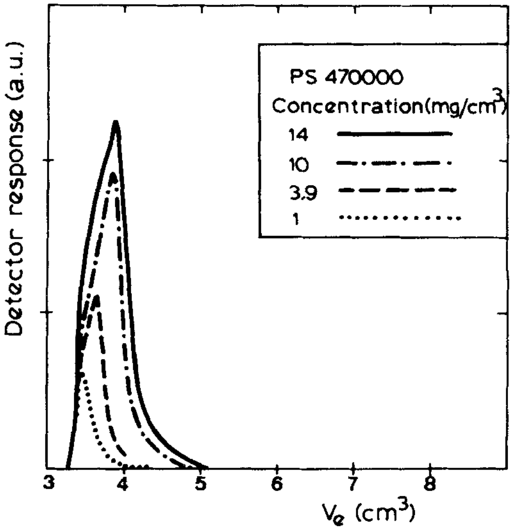

FIGURE 9. Chromatograms of excluded PS 470000 standard at different concentrations.

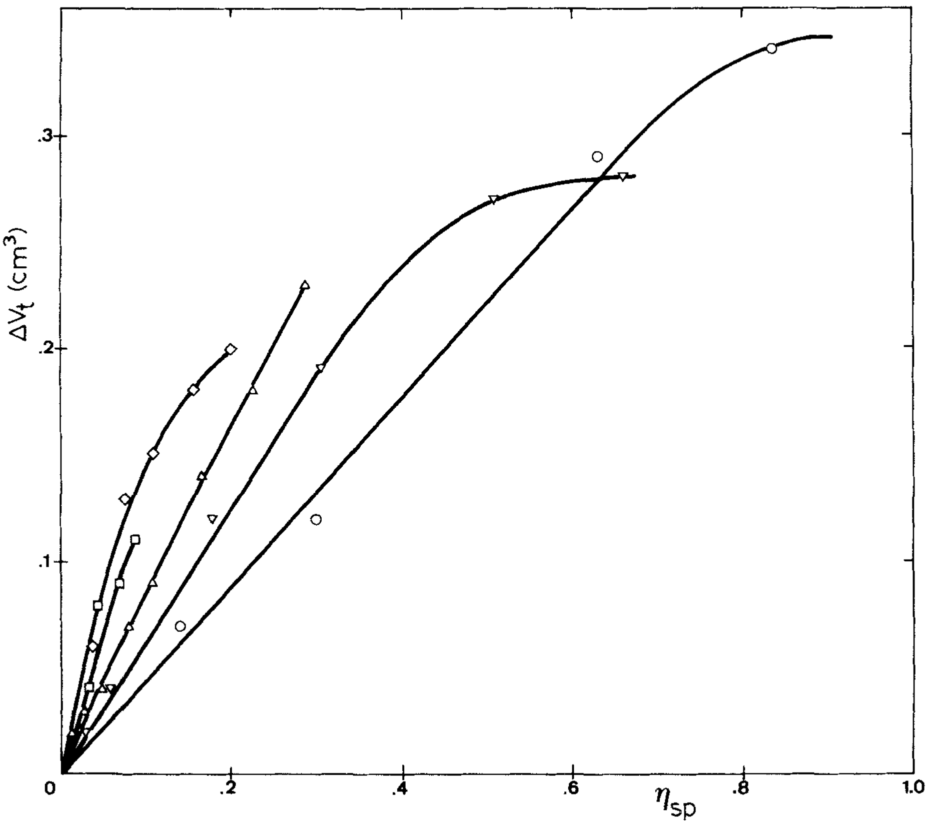

FIGURE 10. Changes of elution volumes, ΔV_t, with specific viscosities. Columns 2 x 10 nm. Symbols as in Fig. 8.

represented by ΔV_t changes of the excluded PS 470000 sample, is in this column system higher than in the previous case.

After obtaining at the different concentrations the viscosity contributions to ΔV_t with the procedure described above, the values of ΔV_s could be calculated, and these are plotted in Fig. 11 vs injected polymer concentrations for three of the standards. The relative amount of the concentration dependence of elution volumes due to the hydrodynamic volume reduction was again estimated

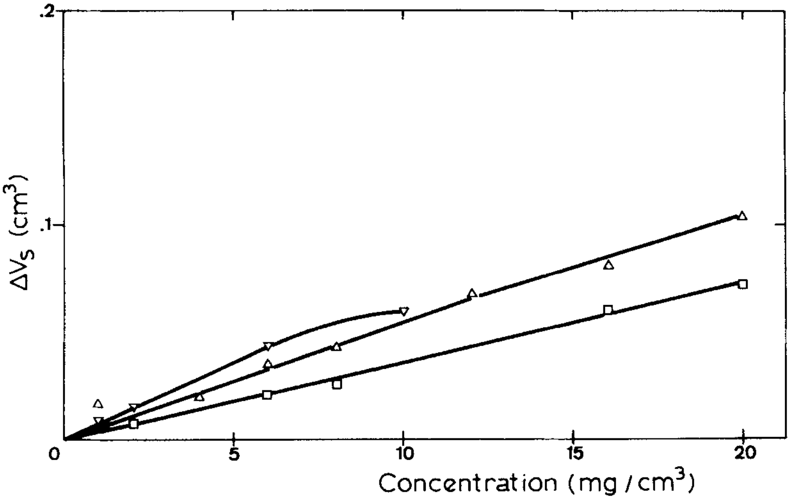

FIGURE 11. Increment of elution volumes for macromolecular coil contraction. Columns 2 x 10 nm. Symbols as in Fig. 8.

from the ratio $d(\Delta V_s)/dc/d(\Delta V_t)/dc$, for the different standards, and the results are shown in the last column of Table 2.

The effect of macromolecular coil contraction turns out to be reduced in respect of the results of Table1;particularly, for the samples PS 17500 and PS 50000 this contribution is acting either at the same level than the viscosity effect, or with a lower relative importance. In practice, in this column system, the increased viscosity contribution overcomes the one from the higher pore permeation of the macromolecules. The results relative to the lower molar mass samples, PS 1800 and PS 8500, cannot be given too much confidence, because it is questionable wether, for such short polymer chains, the same concentration dependence of molecular sizes and of solution viscosities used for random coil molecules can be still employed. On the other hand, elution volume changes occurring for these samples are quite low, and large differences in the calculated contributions can result from small errors in measurements.

CONCLUSIONS

A practical experimental procedure for evaluating the ratio of the viscosity effect to the total change in elution volumes in SEC of polymers at different concentrations has been applied to real chromatographic systems. It is assumed that all the concentration effect comes from two main contributions: the frictional forces acting in the interstitial volume during sample elution in the columns (viscosity effect) and the higher pore volume permeated because of the macromolecular size contraction with increasing concentrations.

The calculated relative amount of these contributions show that the viscosity effect can be operative to different extents, depending on the column system; under the experimental conditions investigated, however, the macromolecular coil contraction seems to account for 50-80% of the total elution volume changes for most of the investigated samples.

In the analysis of data herewith shown use has been made of the injected nominal concentrations of the polymer solutions. It is known that during the chromatographic elution a dilution process occurs, and this should be taken into account when looking for quantitative relationships (11). The employed methodology, however, of plotting elution volume changes against nominal concentrations and against the correspondent specific viscosities is not incorrect if it is assumed that the dilution of the sample equally affects the concentration and viscosity dependence of elution volumes. To check that this assumption is reasonable the experimental elution volumes were also correlated to the average effective concentration of the samples estimated by using the procedure suggested by Janca (11) and the different contributions to the changes of elution volumes were evaluated. The results so obtained were only slightly different from those reported here, showing in general an even bigger effect of the hydrodynamic volume contraction.

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