Peak Distortion in Gel Permeation Chromatography at High Sample Concentration

In gel permeation chromatography (GPC), the sample concentration usually employed is not higher than 0.1–0.3 g/dl. However, in some cases high concentrations are used. In preparative separations, the material throughput is often increased by using high sample concentrations. Similarly, in the case of a low value of the refractive index increment, the sample concentration must be increased in order to obtain a sufficiently high detector signal. However, irregularly shaped chromatographic peaks might occur at high sample concentrations, as was detected also by others.[1–3] In this paper we examine the effect of high sample concentration on the reproducibility and separating power in GPC.

A GPC apparatus (column arrangement, 10^5, 10^4 and 3×10^3 Å Styragel columns) was used with tetrahydrofuran as a solvent at ambient temperature; the flow rate was maintained at 1 ml/min. Different polymers were used: polystyrene standards (Waters Ass.), poly(*n*-alkyl methacrylate) samples, and poly (styrene-*co*-*n*-alkyl methacrylate) copolymers of different molecular weight and molecular weight distribution (the characteristics of these latter materials were published elsewhere[4]). The volume of the injected sample solution was 1.67 ml in each case and three concentration levels (0.1, 0.5, and 1.0 g/dl) were examined (1 count = 1.67 ml).

In many cases of the high molecular weight samples, the chromatographic peaks were distorted, i.e., secondary maxima or shoulders occurred in the rear part of the chromatogram, if the sample concentration was higher than 0.1 g/dl. It was also found that this feature of the chromatogram is irreproducible as is seen in Figure 1(a), where three subsequent runs of the same solution of a Waters polystyrene standard of M_w 867,000 at a concentration level of 1 g/dl are visualized along with the chromatogram of the same sample obtained at a concentration of 0.1 g/dl.

Figure 1 also shows the results of an experiment that demonstrates the poor separation efficiency of GPC in this case. The sample was run three times, and the fractions were collected for further characterization. The cutting scheme of the fraction collecting is seen in Figure 1(b) on the average envelope of the curves in Figure 1(a). The fractions were isolated and rerun at a concentration of 0.1 g/dl; the chromatograms of the five fractions are seen in Figure 1(c). No significant difference between the chromatograms of the fractions can be detected, which means that practically no separation occurred in the case of distorted peaks.

As the phenomenon was reported in the literature in connection with viscous samples,[1–3] the occurrence of peak distortion was correlated with the sample specific viscosity, because this quantity reflects the relative difference of the viscosities across the interface of the moving chromatographic front. In order to visualize the effect of η_{sp} on peak distortion, we ascribed +1 to a chromatogram if a distinct peak distortion was observed, and −1 in the case of no peak distortion and good reproducibility of the peak. Finally, a chromatogram was denoted by 0 if no peak distortion was observed but irreproducible parameters (position and width) of the peak suggested the existence of anomalies. These values were plotted against sample specific viscosity in Figure 2. (The values of specific viscosity are plotted in a logarithmic scale in order to emphasize the most important region, $0.1 \leq \eta_{sp} \leq 10$.)

Figure 2 clearly shows a good correlation between the peak distortion and the sample specific viscosity. A limiting value of $\eta_{sp} = 0.8$ independent of sample type and concentration is clearly seen from the figure. Above this value the reproducibility of the chromatogram is impaired and artificial secondary peaks might occur.

In order to show that the peak distortion is not an exclusive property of the GPC set used, some additional measurements were performed on a Waters GPC-200 apparatus operated at 130°C with trichlorobenzene as solvent and using five Styragel columns (10^5, 3×10^4, 3×10^3, 10^3, and 250 Å). The polystyrene standard M_w 867,000 was run at three different concentrations. The chromatograms are shown in Figure 3, and the occurrence of the peak distortion is obviously also seen in this case at higher concentrations. We do not have sufficient experimental data for this instrument, therefore a reliable value of the limiting specific viscosity cannot be given, but the existence of such a limit could be confirmed.

In conclusion, it can be supposed that a limiting value of sample specific viscosity exists probably for each GPC set. Above this value the results of the GPC measurements are not reliable. As this

Journal of Applied Polymer Science, Vol. 23, 1879–1881 (1979)

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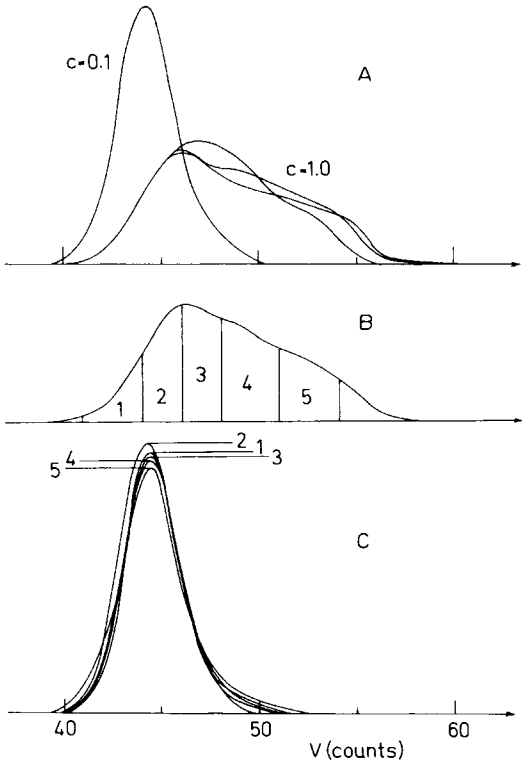

Fig. 1. Loss of reproducibility and separation efficiency of the GPC set at higher concentration of the sample: (a) chromatograms obtained in three subsequent runs of the same sample solution of a polystyrene standard (M_w 867,000) at c = 1 g/dl and 0.1 g/dl, respectively; (b) scheme of fraction collecting; (c) chromatograms of the fractions obtained, measured at sample concentration of 0.1 g/dl.

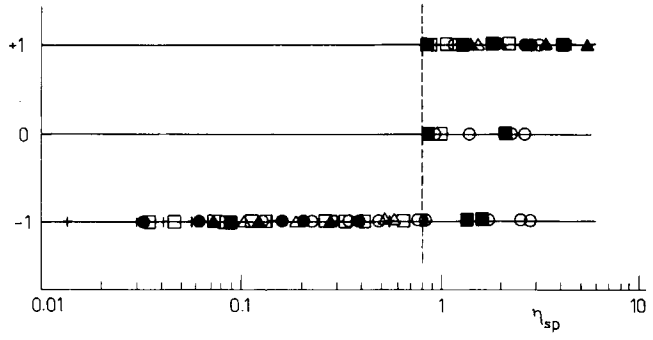

Fig. 2. Occurrence of peak distortion in dependence on sample specific viscosity (see text for explanation): (+) polystyrene; (○) poly(ethyl methacrylate); (□) poly(butyl methacrylate); (△) poly(*n*-octyl methacrylate); (●) poly(styrene–*co*–ethyl methacrylate); (■) poly(styrene–*co*–butyl methacrylate); (▲) poly(styrene–*co*–*n*-octyl methacrylate).

limiting value might depend on the experimental arrangement, it is recommended that this limit be found experimentally and that the specific viscosity of the injected samples be estimated before GPC runs at higher sample concentrations are attempted.

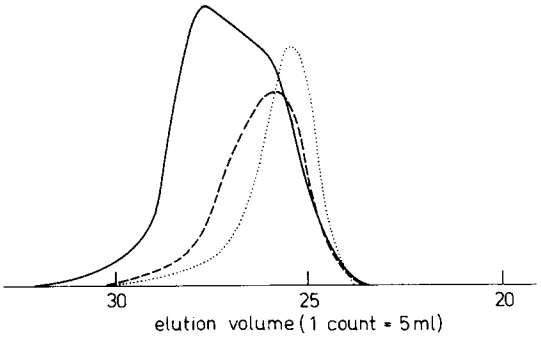

Fig. 3. GPC peaks of a polystyrene standard (M_w 867,000) at different concentrations in trichlorobenzene at 130°C: (—) c = 1 g/dl; (- - -) c = 0.5 g/dl; (. . . . . .) c = 0.1 g/dl.

References

1. P. Flodin, *J. Chromatogr.*, **5**, 103 (1961).
2. J. C. Moore, *Separ. Sci.*, **5**, 723 (1970).
3. K. P. Goetze, R. S. Porter, and J. F. Johnson, *J. Polym. Sci. A-2*, **9**, 2255 (1971).
4. J. Podešva, M. Bohdanecký, P. Kratochvíl, and G. Samay, *J. Polym. Sci., Phys. Ed.*, **15**, 1521 (1977).

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Received September 15, 1978