Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

The Overload Effect in Gel Permeation Chromatography

J. C. Moore[a]
[a] BASIC RESEARCH DEPARTMENT TEXAS DIVISION, THE DOW CHEMICAL COMPANY, FREEPORT, TEXAS

To cite this Article Moore, J. C.(1970) 'The Overload Effect in Gel Permeation Chromatography', Separation Science and Technology, 5: 6, 723 — 730

To link to this Article: DOI: 10.1080/00372367008055535

URL: http://dx.doi.org/10.1080/00372367008055535

SEPARATION SCIENCE, 5(6), pp. 723–730, December, 1970

The Overload Effect in Gel Permeation Chromatography*

J. C. MOORE
BASIC RESEARCH DEPARTMENT
TEXAS DIVISION
THE DOW CHEMICAL COMPANY
FREEPORT, TEXAS 77541

Summary

Overload phenomena in GPC are investigated with samples of narrow polystyrenes and short but efficient columns packed with Styragel. Viscous fingering is shown to be a leading cause of peak skewing and broadening. A correlation is proposed to define a safe operating range in terms of sample concentration, volume, and the average intrinsic viscosity of the solute polymer.

GPC presents an unusual problem in chromatography. The polymeric materials in the sample confer appreciable viscosity on their solutions, while a high resolution is demanded in a limited elution volume. Experience has shown that too much polymer in the sample causes not earlier but later elution of the peak. The curve starts up at the proper point but rises more slowly, and its return to baseline is delayed. There is a loss of resolution, particularly under the main part of the curve. This has been shown by collecting and rerunning the fractions (*1*). In such a case important details of the sample distribution may have been obscured. Attempts to extrapolate the mean values of the distribution (*2*, *3*) to a zero load condition may be useful with very regular distributions, but it would be desirable to have a measure of a safe sample load to avoid undue loss of resolution.

This study suggests that a relative measure of this sample load

---

* Presented at the ACS Symposium on Gel Permeation Chromatography, sponsored by the Division of Cellulose, Wood, and Fiber Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, February, 1970.

effect may be based on the sample volume V, its total concentration C, and its average intrinsic viscosity $[\eta]$. The latter value may usably be obtained from a test chromatogram's center of area and a calibration curve for elution volume versus $[\eta]$, or the integration $[\eta]_{av} = \Sigma W_i[\eta]_i/\Sigma W_i$ may be performed.

Sample load effects should most easily be seen when column efficiency is high and a narrow-distribution sample is used, especially if the column is short. In this study two columns were used, each 4 ft × 3/8 in. (7.8 mm i.d.) in size. Column 17I was packed with 10^6 Å permeability Styragel (Waters Associates, Inc.), a fraction was used in which 80% of the particles were within the range 13–47 μ in diameter. Column 18W was packed with 3×10^4 Å permeability Styragel of size range 29–53 μ diameter. The eluting solvent was tetrahydrofuran at 25°C; column efficiencies measured with small benzene samples were 2360 and 1900 theoretical plates per foot, respectively, for the two columns including the spreading effect of the connecting tubing (about 70 in., 0.020 in. i.d.) and the Waters R-4 refractometer whose cell volume was 0.010 ml. Samples used were the narrow anionic

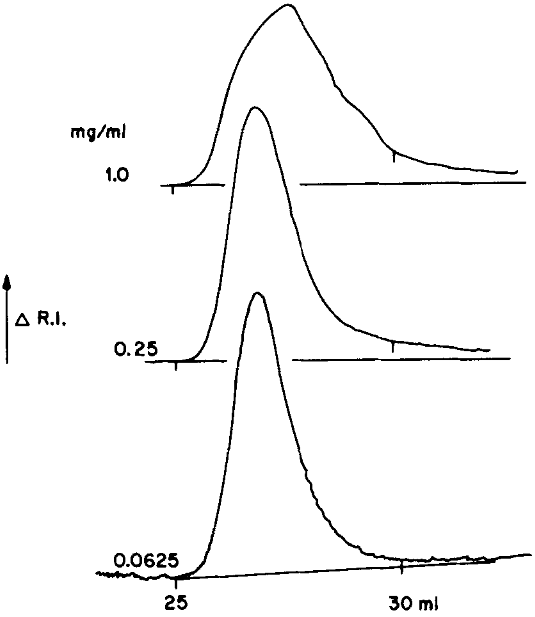

FIG. 1. Elution curves, Column 18w, 1 ml/min, 0.8 ml samples of polystyrene 14A in THF at three concentrations.

TABLE 1

Pressure Chemicals batch	$\bar{M}_w{}^a$	$\bar{M}_w/\bar{M}_n{}^a$	$[\eta]_{\mathrm{THF}}$, 25°C
14A	1.8×10^6	1.2	3.8
6A	8.6×10^5	1.15	2.0
3A	4.1×10^5	1.06	1.2
1A	1.6×10^5	1.06	0.65

[a] Pressure Chemicals' property sheets.

polystyrenes obtained from Pressure Chemicals Inc., as shown in Table 1.

Elution curves from three concentrations of Sample 14A on Column 18W, in which the main part of the sample has little or no penetration of the gel, show in Fig. 1 that severe overloading has occurred in the highest concentration. The pattern of erratic delay is indicative of viscous fingering (*4*), an interstitial phenomenon. The drop in viscosity at the rear boundary of the sample has made the radial velocity profile

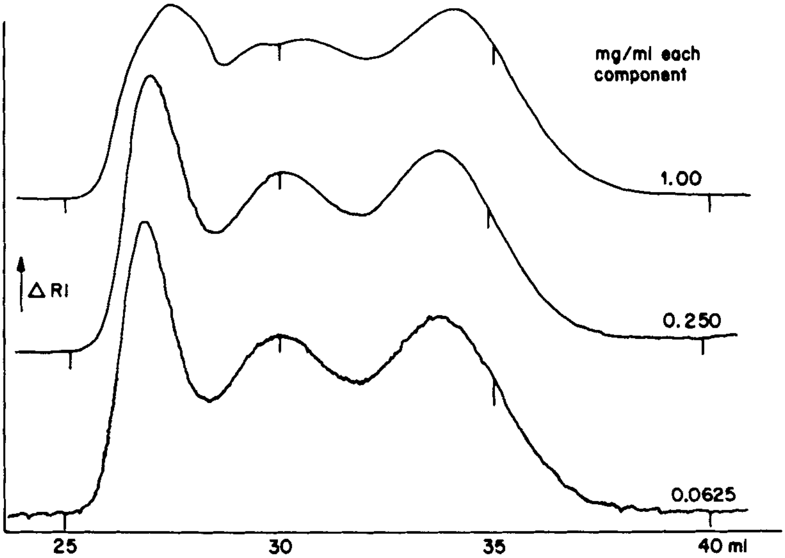

FIG. 2. Elution curves, Column 18w, 1 ml/min, 0.8 ml samples, polystyrenes 14A, 3A, and 1A in equal mixture, three concentrations.

unstable. In this zone it is easier for solvent to push through a slightly wider passage between grains than to maintain an even plug-type flow. Fingers of solvent may punch ahead while fingers of sample are temporarily delayed. As the sample becomes spread out, farther down the column the effect diminishes so that later eluting components may reflect the distortion effect in a lesser degree.

Elution curves from the same column showing the same samples with two lower molecular weights components added are shown in Fig. 2. Here it is evident that the distortion pattern is now largely super-imposed on the curve at the same elution volumes. If the distribution had been smoother, there might have been little evidence of the loss of resolution. It would be desirable to detect this in its early stages even with a broad distribution.

Since we observe a delay of material, it is interesting to test the intensity function that has been proposed as a detector of stagnancy in a residence time distribution (*5*, *6*). The intensity function $L(V)$ measures the probability that a molecule still in the system may escape in the next volume increment.

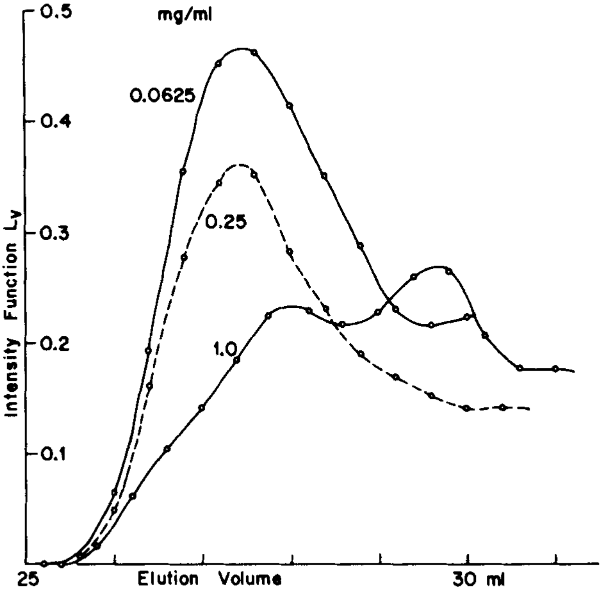

FIG. 3. Intensity function for elution curves in Fig. 1: Column 18w, 0.8 ml samples of polystyrene 14A at three concentrations.

$$L(V) = H(V)/[A - C(V)]$$

where H is the curve height and C is the cumulative area under the curve at the elution volume V, and A is the total area under the curve. This is the unnormalized form of Eq. (20) in Ref. *6*. A decrease in this function would indicate stagnancy if the polymer sample were effectively mondisperse. In Fig. 3 values are shown as calculated from the three elution curves of Fig. 1. The sharp decrease shown is not related to viscous fingering since it is most evident at the lowest concentration. More likely, it may reflect the low-molecular weight tail of this sample which was capable of penetrating some of the gel pores. The sharply lower initial slope of the overloaded curve may be significant, but with these narrow samples the increased width of the curve is also sensitive. With the broader distributions in Fig. 2, the same relative pattern was followed by their calculated intensity functions. This may be more reliable than a direct measure of the initial slope of the chromatogram relative to total area.

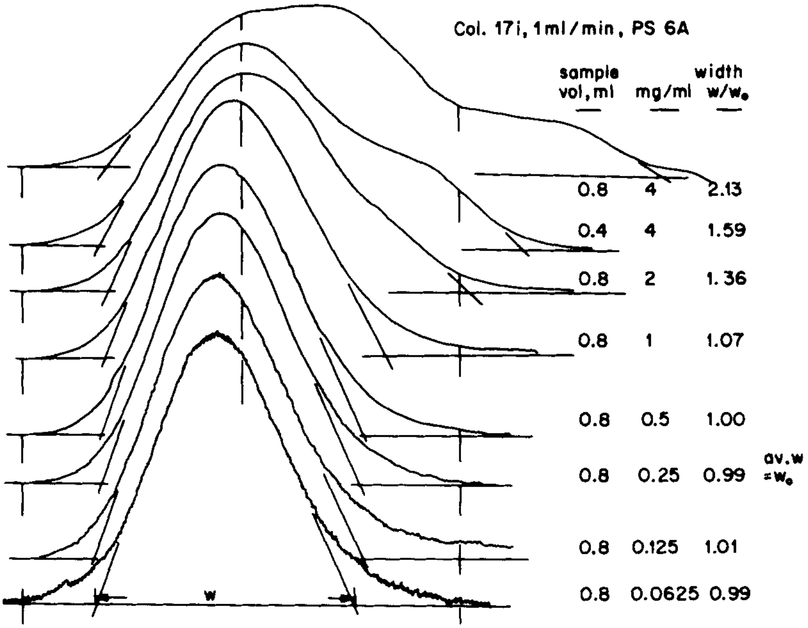

FIG. 4. Elution curves, Column 17I, 1 ml/min, 0.8 and 0.4 ml samples of polystyrene 6A at seven concentrations, relative baseline width between tangent lines as shown.

TABLE 2

Peak Width Measurements under Varying Sample Loads

Col. No.	Sample Pressure Chemicals No.	Flow (ml/min)	Ml Baseline width between first and last tangent intercepts, at concentration (mg/ml)										
			0.0625	0.125	0.25	0.5	1.0	2.0	4.0	4.0	5.0	10.0	10.0
17i	14A	1.0	8.32	8.40	8.52	8.42	9.68	11.90	—	—	—	—	—
17i	14A	0.2	—	—	6.53	7.12	8.13	11.18	—	—	—	—	—
18w	14A	1.0	2.56	—	2.78	—	4.68	—	—	—	—	—	—
17i	6A	1.0	5.92	6.07	5.96	6.00	6.40	8.26	9.45[a]	12.70	—	—	—
17i	6A	0.2	—	—	—	4.46	5.11	6.96	—	10.61	—	—	—
18w	6A	1.0	3.88	—	3.80	—	4.30	4.84	5.35[a]	7.75	—	—	—
17i	1A	1.0	—	—	5.28	—	5.21	5.13	—	—	5.25	5.55[a]	8.20

[a] 0.4 ml sample, otherwise 0.8 ml.

It seemed desirable to study the onset of the delay phenomenon in more detail. A series of samples of three polystyrenes in the two columns were run at two flow rates, 1.0 and 0.2 ml/min, with two sample sizes, 0.8 and 0.4 ml, and a wide range of concentrations, as summarized in Table 2. Figure 4 illustrates the range of patterns encountered. At the lowest concentrations baseline drift and instrument noise limit the resolution. Then for a range the chromatogram is independent of sample concentration, with detector-recorder sensitivity adjusted to keep curve area constant. Above this range, gross distortion sets in and rapidly becomes severe. At the highest concentration overloading was sharply reduced by halving the sample volume, but it was reduced a little more by halving the concentration instead.

At lower molecular weights the safe and usable range is broader; at some higher molecular weight it would seem to disappear. For most samples the extremely high molecular weight components are minor in quantity but their accurate analysis is important.

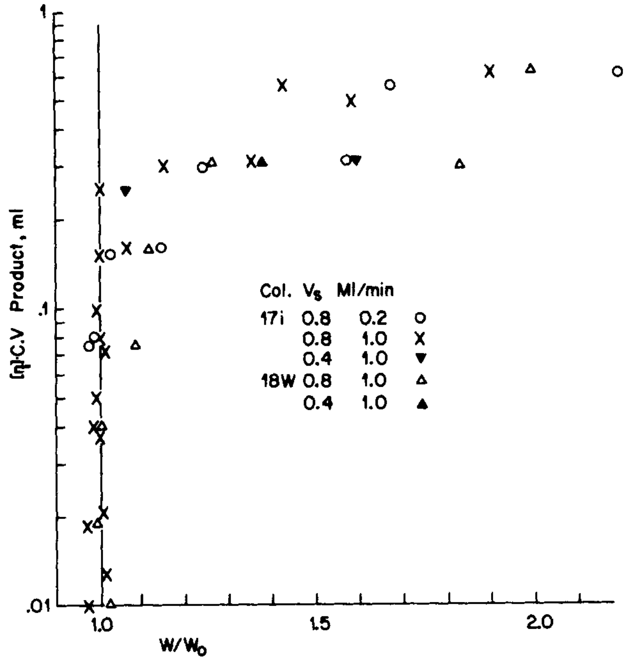

FIG. 5. Correlation of Table 2 data, relative peak broadening vs. solvated molecular volume of sample load.

Since intrinsic viscosity measures the ratio of molecular volume at infinite dilution to molecular weight, the product of sample concentration and intrinsic viscosity should give a ratio of solvated molecular volume to sample volume. Then multiplying this by sample volume gives a measure of load which implies that a given column can safely handle some total volume of solvated polymer molecules. Figure 5 shows Table 2 data treated in this way. With consistent units (dl/g $\times$ 0.1 $\times$ mg/ml $\times$ ml = ml) the safe limit for these columns appears to lie between product values of 0.05 and 0.10 ml.

It appears that much GPC work has been done with samples in the overload range, while long columns, broad distributions, and lower column efficiencies obscured the evidence. With improvements in column-packing techniques and in corrections for the system's spreading effects, it becomes possible to ask for faster and more accurate analyses, and these column load effects become more important. While comparison of a rerun cut with its expected distribution (*7*) is still considered the most revealing and fundamental technique, it seems desirable to direct attention to the causes of the gross distortions seen in this study, and if possible to find a simple expression to describe a safe operating range with a variety of samples as usually encountered.

REFERENCES

1. H. E. Adams, K. Farhat, and B. L. Johnson, *Ind. Eng. Chem., Prod. Res. Develop.*, **5**, 126 (1966).
2. M. J. R. Cantow, R. S. Porter, and J. F. Johnson, *J. Polym. Sci., Part B,* **4**, 707 (1966).
3. A. Lambert, *Polymer,* **10**, 213 (1969).
4. R. E. Collins, *Flow of Fluids Through Porous Materials,* Reinhold, New York, 1961, pp. 196–200.
5. P. V. Dankwerts, *Chem. Eng. Sci.,* **2**, 1 (1953).
6. P. Naor and R. Shinnar, *Ind. Eng. Chem., Fundam.,* **2**, 278 (1963).
7. L. H. Tung, J. C. Moore, and G. W. Knight, *J. Appl. Polym. Sci.,* **10**, 1261 (1966).

Received by editor February 2, 1970