

CHROM. 5121

PRACTICAL GEL PERMEATION CHROMATOGRAPHIC
COLUMN CALIBRATION FOR POLYMERS

M. ZINBO AND J. L. PARSONS

Scientific Research Staff, Ford Motor Company, Dearborn, Mich. 48121 (U.S.A.)

SUMMARY

A gel permeation chromatographic column calibration technique for polymers under a given set of operating conditions such as column, solvent, oven temperature, and elution rate is presented, using the exponent a of appropriate limiting viscosity number-molecular weight relationships of various polymers. The elution volumes V_e usually bear a linear, inverse relationship to the logarithms of molecular weight within a limited molecular weight range, permitting extrapolation to molecular weight unity as a measure of the apparent maximum elution volume V_m . A linear relationship between $\log V_m/V_e$ and $\log M$ over the linear range of $\log M$ versus V_e curves has been demonstrated for available polystyrene and polypropylene glycol standards for several given gel permeation chromatographic operating conditions. The indicated linear relationships for the polymer standards may be expressed by simple equations of the form $(V_m/V_e) = kM^{a'}$, where V_m and a' are constant for a given polymer under a given set of operating conditions and k is constant for a given operating condition. It seems likely that a' is a linear function of the exponent a of the limiting viscosity number-molecular weight relationship over the corresponding linear range of molecular weight. Thus, a given gel permeation chromatographic column system can be characterized for polymer samples by the column constant k and a conversion factor f between a and a' . The calibration technique is then of particular interest for polymers of different types than the available standards. An application of the technique to polyethylene oxide is presented.

INTRODUCTION

Calibration in gel permeation chromatography (GPC) has been one of the major problems for evaluating the chromatograms when the calibration curve for a given polymer sample in question is unknown. A functional relationship between the peak elution volume of a series of nearly monodisperse samples and their molecular weight is usually difficult to establish except for two series of commercially available polystyrenes (PS) and polypropylene glycols (PPG). It was apparent in the early stages of GPC development that the elution volume is not a function of molecular weight of the solute molecules alone, but that also interaction of GPC solvent with the polymer and chemical structure of the polymer play a role.

BENOIT *et al.*¹⁻³ have demonstrated that the logarithms of the products of molecular weight and intrinsic viscosity when plotted as a function of peak elution volumes for structurally different polymers yield a single curve. It is probably the most "universal calibration" to date which is independent of polymer type. The theoretical justification for using $M \cdot [\eta]$ or a similar quantity as a universal calibration function to transform the primary calibration curve into calibration curves for linear but structurally different polymers has been reported⁴. However, it should be noted that BENOIT's "universal calibration" procedure requires that intrinsic viscosities must be measured for each polymer sample, such as for a series of research-type polymers.

It was our primary objective to find a more practical way to calibrate a given solvent-column system for several different types of polymers, using available polymer standards and the reported values of the exponent α in the Mark-Houwink-Sakurada equation.

EXPERIMENTAL

Materials

The polystyrenes (PS) and polypropylene glycols (PPG) used were narrow-distribution samples supplied by Waters Associates (Framingham, Mass.). Carbowax 6000 and 20M were obtained from Applied Science Laboratories, Inc. and were characterized by NMR⁵, osmometry, and viscosity. The standards employed for calibration and study are listed in Table I together with their intrinsic viscosities in several solvents. The root-mean-square average molecular weights or molecular weights at peak for PPG standards have been estimated by the "comparative technique" described by BLY⁶.

Intrinsic viscosities

The instrument used was a modified Ubbelohde viscometer, originally described by CRAIG AND HENDERSON⁷, equipped with a Hewlett Packard Model 5901B

TABLE I

POLYMER STANDARDS AND DATA

| Standard No. | \bar{M}_{rms} | Intrinsic viscosity, $dl \cdot g^{-1}$ at 30.0° | | | |
|--------------|-----------------|--|-------------------|---------|---------|
| | | DMF | <i>p</i> -Dioxane | Toluene | Benzene |
| PS 1 | 4 800 | 0.0470 | 0.0578 | 0.0559 | 0.0587 |
| 2 | 10 000 | 0.0731 | 0.0888 | 0.0910 | — |
| 3 | 19 750 | 0.1110 | 0.1478 | 0.1487 | 0.1586 |
| 4 | 50 000 | 0.1971 | 0.2681 | 0.2800 | — |
| 5 | 97 200 | 0.2981 | 0.4237 | 0.4330 | 0.4748 |
| 6 | 171 000 | 0.4142 | — | 0.6610 | — |
| 7 | 402 000 | 0.6966 | — | 1.1069 | 1.2590 |
| 8 | 830 000 | — | — | 1.9040 | — |
| 9 | 1 987 000 | — | — | 3.5070 | — |
| PPG 1 | 830 | 0.0300 | 0.0307 | 0.0215 | — |
| 2 | 1 280 | 0.0382 | 0.0410 | 0.0315 | 0.0448 |
| 3 | 2 100 | 0.0525 | 0.0573 | 0.0520 | 0.0620 |
| 4 | 4 100 | 0.0778 | 0.0932 | 0.0941 | 0.1012 |

autoviscometer read-out. Measurements were carried out at four or five different concentrations within a range of 0.1 to 1.3 g·dl⁻¹. Reduced viscosities, η_{sp}/c , were plotted against c according to HUGGINS⁸.

Gel permeation chromatography

A Waters' Ana-Prep Gel Permeation Chromatograph used in this work was slightly modified to prevent solvent evaporation from the syphon⁹. Operating conditions varied and are listed in Table II.

TABLE II
GPC COLUMN SET AND OPERATING CONDITION

| GPC column set | Waters' column designation, (A) | Solvent | Oven temperature (°C) | Flow rate (ml/min) |
|----------------|---|-------------------|-----------------------|--------------------|
| A | 10 ⁴ + 10 ³ + 10 ² | DMF | 39 | 1.00 |
| B | 10 ² + 10 ³ + 10 ⁴ | <i>p</i> -Dioxane | 50 | 0.96 |
| C | 10 ⁴ + 10 ³ + 10 ² | <i>p</i> -Dioxane | 39 | 0.89 |
| D | 10 ⁴ + 10 ³ + 10 ⁵ | <i>p</i> -Dioxane | 50 | 0.83 |
| E | 10 ⁴ + 10 ³ + 10 ⁵ | <i>p</i> -Dioxane | 39 | 0.75 |
| F | 10 ⁵ + 10 ⁴ + 10 ³ | Toluene | 50 | 0.89 |
| G | 10 ² + 10 ³ + 10 ⁴ | Toluene | 50 | 0.96 |
| H | 10 ⁴ + 10 ³ + 10 ⁵ | Benzene | 36 | 0.86 |

RESULTS

Intrinsic viscosity–molecular weight relationships determined for PS and PPG standards in several solvents are summarized in Table III. There are some discrepancies of the exponent a with the values reported in the literature. The most likely source of error would be a difference in the polydispersity of samples for which the constants were measured.

Figs. 1(a), (b), (c) and (d) illustrate GPC calibration curves for PS and PPG

TABLE III
VISCOSITY–MOLECULAR WEIGHT RELATIONSHIPS, $[\eta] = K\bar{M}_{rms}^a$
All measurements were done at 30 ± 0.01°.

| Standard | Solvent | $K \times 10^4$ (dl/g) | a | Mol. wt. range $M \times 10^{-3}$ |
|----------------------|-------------------|------------------------|-------|-----------------------------------|
| Polystyrene | DMF | 2.606 | 0.612 | 5–400 |
| | <i>p</i> -Dioxane | 2.241 | 0.655 | 5–100 |
| | Toluene | 1.786 | 0.680 | 5–2000 |
| | Benzene | 1.623 | 0.695 | 5–400 |
| Polypropylene glycol | DMF | 5.284 | 0.598 | 0.8–4 |
| | <i>p</i> -Dioxane | 2.247 | 0.715 | 1–4 |
| | Toluene | 0.447 | 0.920 | 0.8–4 |
| | Benzene | 3.241 | 0.686 | 1–4 |

standards with column sets A, C, F, and H. Both the logarithms of molecular weight and of the products of molecular weight and intrinsic viscosity are plotted against elution volume.

It was observed that the logarithm of τ/V_e is a linear function of the logarithm of M at peak position over the linear range of $\log M$ vs. V_e curves. Two examples are given in Fig. 2 (column set: A and B).

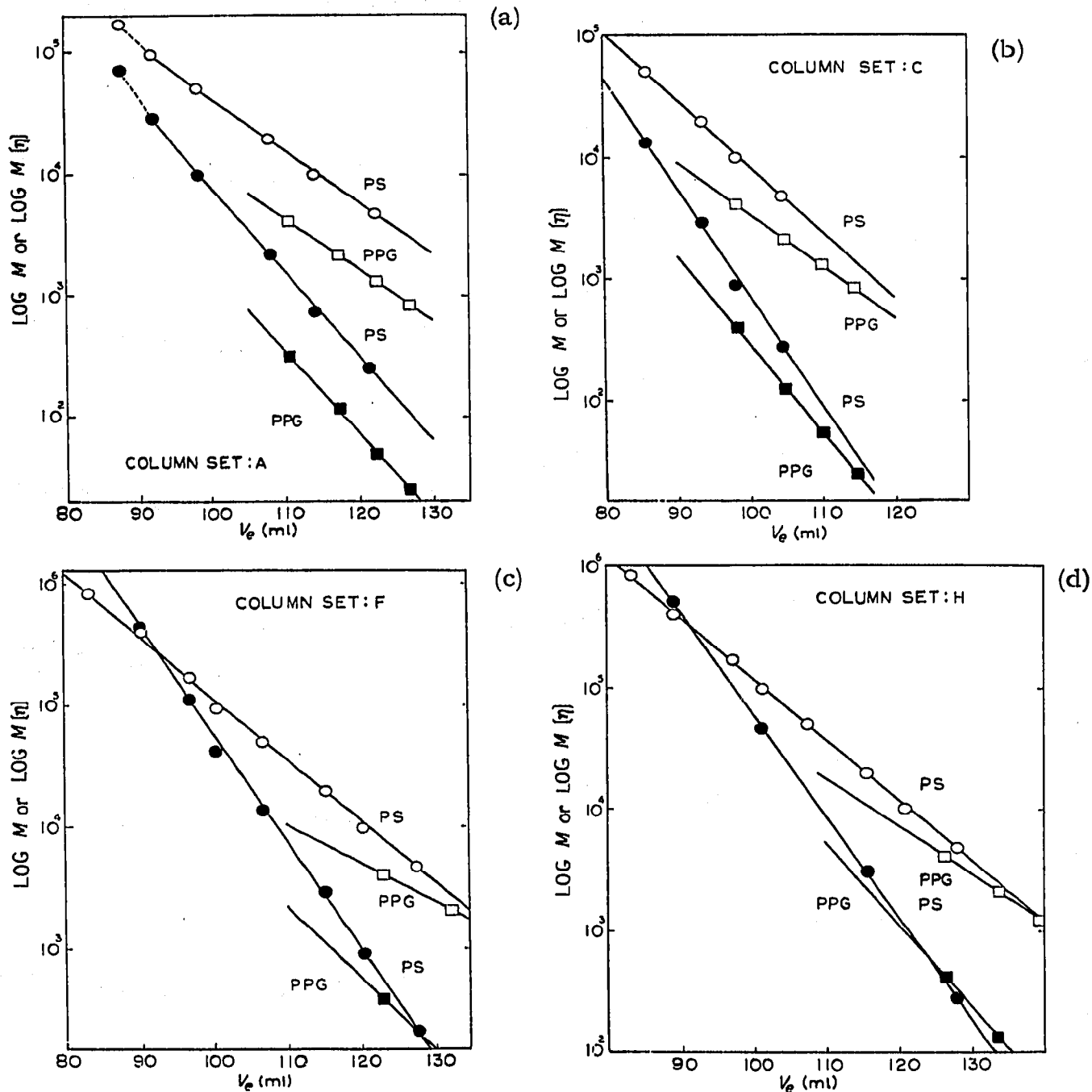


Fig. 1. (a) Calibration data for PS and PPG with column set A. (b) Calibration data for PS and PPG with column set C. (c) Calibration data for PS and PPG with column set F. (d) Calibration data for PS and PPG with column set H. \circ , $\log M$ vs. V_e for PS; \square , $\log M$ vs. V_e for PPG; \bullet , $\log M \cdot [\eta]$ vs. V_e for PS; \blacksquare , $\log M \cdot [\eta]$ vs. V_e for PPG.

An empirical constant V_m for both PS and PPG under a given set of operating conditions has been obtained by extrapolation of the linear range of $\log M$ vs. V_e calibration curves to molecular weight unity. Then another extrapolation to molecular weight unity of the plot of $\log V_m/V_e$ vs. $\log M$ for both PS and PPG standards

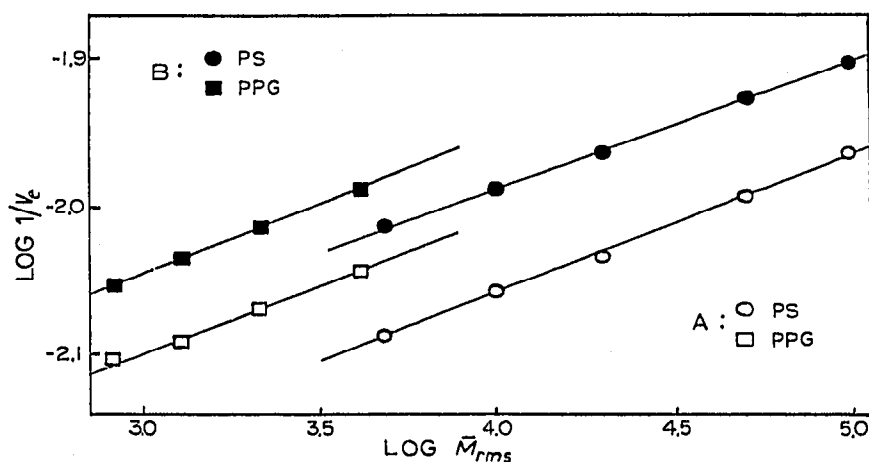


Fig. 2. Double logarithmic plots of M vs. $1/V_e$ in DMF and *p*-dioxane for PS and PPG. ○, PS with column set A; □, PPG with column set A; ●, PS with column set B; ■, PPG with column set B.

under the limited conditions described above comes to a point, giving another empirical constant, k . Figs. 3(a), (b), (c) and (d) illustrate the extrapolation described above for the column sets A, B, C, and G.

The indicated relationships for PS and PPG standards over the linear range of $\log M$ vs. V_e calibration curves may be expressed by simple equations of the form

$$(V_m/V_e) = k\bar{M}_{rms}^{a'}$$

where k and a' are constants determined, respectively, by the intercept and the slope of a plot of the type shown in Fig. 3 and V_m is a constant determined by extrapolation of the linear range of the log calibration curve to molecular weight unity. Empirical constants for the equation for both PS and PPG standards are summarized in Table IV together with the ratio between a and a' . The ratio f , which can be called a conversion factor, is assumed to be constant for any type of polymer within the following limitations:

- (1) molecular weight must be within a linear range of the log calibration curve,
- (2) intrinsic viscosity–molecular weight relationship should cover the linear range of the log calibration curve, and
- (3) a given set of GPC operating conditions must be used.

The equation indicates that having one known standard, two average molecular weights or one average molecular weight with polydispersity less than 1.10 is sufficient to find the effective linear calibration over the range of the linear log calibration of standards.

The present calibration procedure was applied to an intermediate mol. wt. polyethylene oxide–Carbowax 20M with column sets A and B. Carbowax 6000,

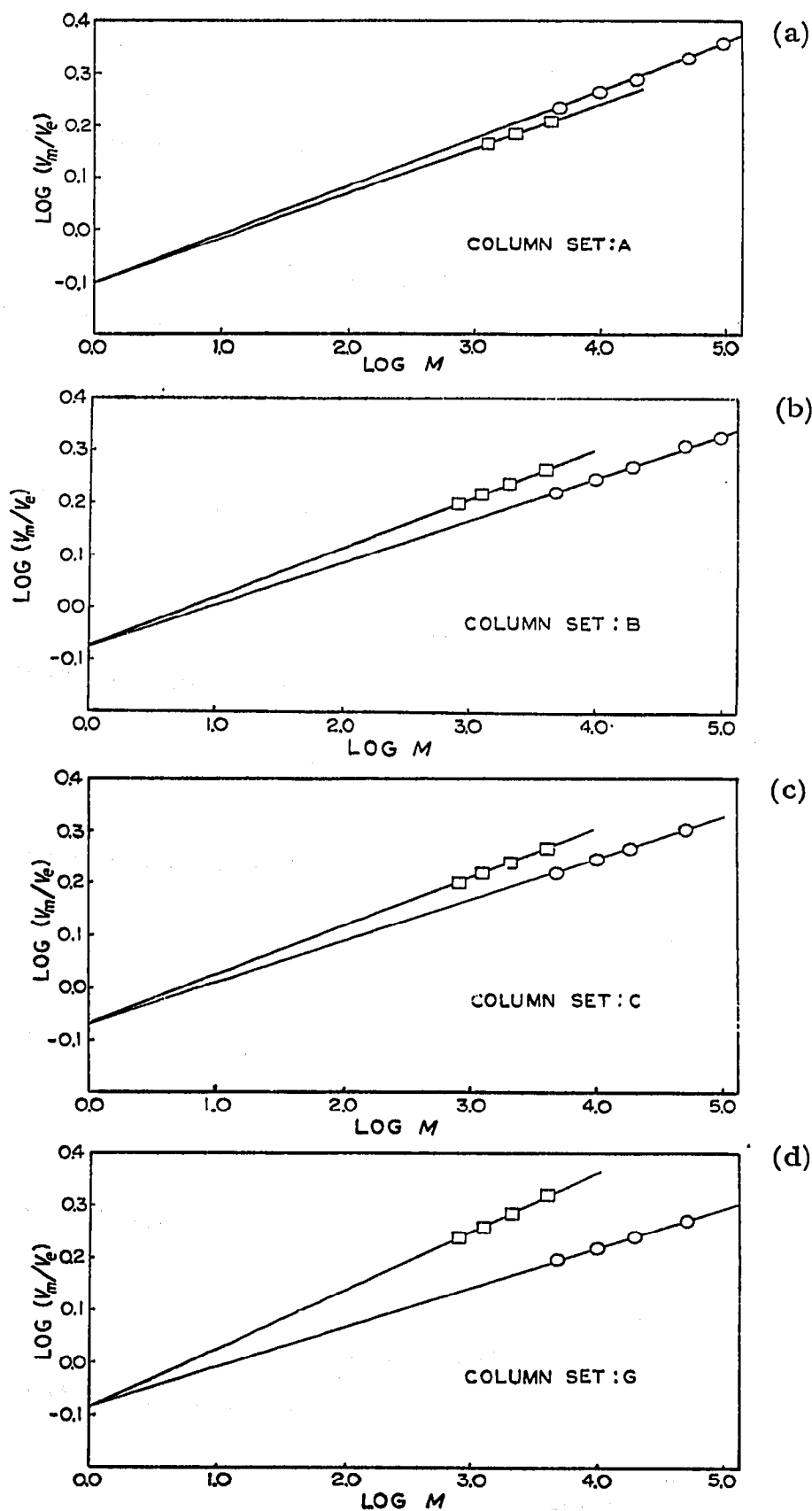


Fig. 3. (a) Double logarithmic plots of M vs. V_m/V_e for PS and PPG with column set A. (b) Double logarithmic plots of M vs. V_m/V_e for PS and PPG with column set B. (c) Double logarithmic plots of M vs. V_m/V_e for PS and PPG with column set C. (d) Double logarithmic plots of M vs. V_m/V_e for PS and PPG with column set G. \circ , PS; \square , PPG.

TABLE IV

EMPIRICAL CONSTANTS OF THE EQUATION $(V_m/V_e) = k\bar{M}_{rms}^{a'}$

| GPC column set | Standard | V_m (ml) | a' | k | $f = a/a'$ |
|----------------|----------|------------|-------|-------|------------|
| A | PS | 209.50 | 0.094 | 0.791 | 6.51 |
| | PPG | 179.50 | 0.092 | | 6.50 |
| B | PS | 172.00 | 0.087 | 0.841 | 7.53 |
| | PPG | 178.25 | 0.095 | | 7.53 |
| C | PS | 172.25 | 0.084 | 0.849 | 7.80 |
| | PPG | 181.75 | 0.092 | | 7.77 |
| G | PS | 157.50 | 0.076 | 0.822 | 8.94 |
| | PPG | 199.20 | 0.112 | | 8.21 |
| H | PS | 203.00 | 0.087 | 0.832 | 7.99 |
| | PPG | 210.20 | 0.086 | | 7.98 |

$\bar{M}_w/\bar{M}_n = 7500/7300$, which had been characterized by NMR near end-group assay¹⁰, vapor phase osmometry, and viscosity, was used as a single standard for the procedure. The viscosity-molecular weight relationships used for the exponent a were:

$$[\eta] = 0.02 + 24 \times 10^{-5} \cdot \bar{M}_w^{0.73}$$

$$(1.0 \times 10^3 < \bar{M}_w < 3.0 \times 10^4; \text{DMF; at } 25^\circ) \text{ (see ref. 11)}$$

and

$$[\eta] = 0.0075 + 35 \times 10^{-5} \cdot \bar{M}_n^{0.71}$$

$$(6.0 \times 10 < \bar{M}_n < 1.9 \times 10^4; p\text{-dioxane; at } 20^\circ) \text{ (see ref. 12).}$$

The results are given in Figs. 4 (column set A) and 5 (column set B).

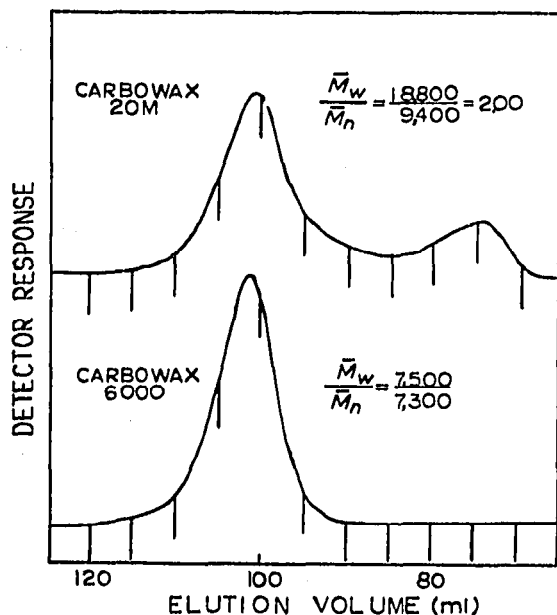


Fig. 4. GPC chromatograms of Carbowax 6000 and 20M. Column set A, $k = 0.791$, $f = 6.50$, $a' = 0.112$, and $V_m = 216.5$ ml.

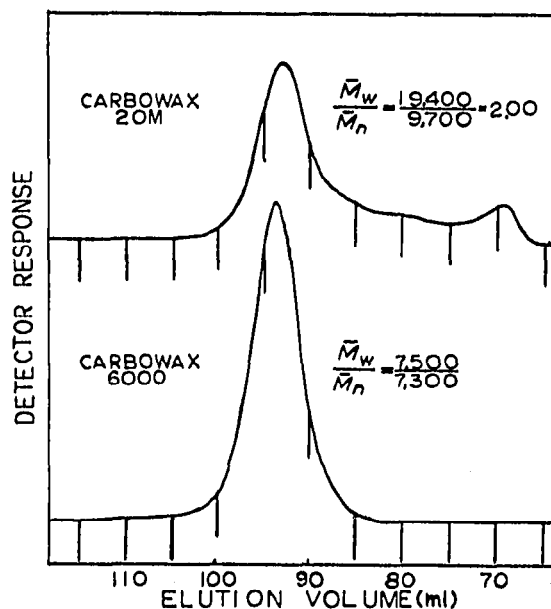


Fig. 5. GPC chromatograms of Carbowax 6000 and 20M. Column set B, $k = 0.841$, $a' = 0.094$, and $V_m = 181.5$ ml.

DISCUSSION

It has been reported¹³ that available PS and PPG standards below 5000 mol. wt. do not give calibration curves consistent with the Benoit "universal calibration". The same deviations have been observed by us in several given sets of GPC operating conditions (Fig. 1).

The viscosity of a polymer solution depends in the first place on the factors which determine the volume occupied by the dissolved macromolecule in solution (molecular weight, interaction of the solvent with the polymer, chemical structure of the polymer) and on the concentration of the solution. Thus, the intrinsic viscosity of a polymer solution itself is a measure of the capacity of a polymer molecule to enhance the viscosity, which depends on the size and the shape of the molecule as well as molecular weight. The intrinsic viscosity is widely used in polymer studies, as it provides a simple and easy method of molecular weight determination of polymers for which the dependence of intrinsic viscosity on molecular weight has been established experimentally. Intrinsic viscosity-molecular weight relationships for various polymers have been compiled¹⁴.

It is generally assumed that both K and a in the viscosity-molecular weight relationship become insensitive to the temperature when a exceeds about 0.70, and that they may be used in a ten-degree range on either side of the temperature at which the constants were determined. Note should be made that the exponent a does not vary with the kind of average molecular weight when a series of polymer samples used for viscosity measurements possess the same degree of polydispersity, but the constant K does vary with the particular average molecular weight. Furthermore, the exponent a is not expected to vary with molecular weight for a linear polymer in a good solvent at a given temperature except at rather low molecular weight, where the long-range interactions are reduced to zero¹⁵.

If we exclude specific interactions between the solute molecules and the Styragel in the GPC process, the factors related to the viscous properties of polymer solutions are also the major factors involved in size separation by the GPC process. The fingering effect¹⁶ or other concentration effects can be minimized by appropriate choice of experimental conditions.

It is generally easy to choose the column combination⁶ so that there is a linear $\log M$ vs. V_e region which covers the interested range of molecular weights. The linear log regions for polymers, which are tedious to obtain a series of well characterized samples, can be safely estimated by the commercially available PS and PPG standards for a given set of operating conditions. As shown in Table IV, the exponent a seems to be a linear function of the constant a' in the equation obtained for PS and PPG standards; however, the relation should be further verified with several additional series of well characterized polymer samples. Even though the exponent a determined in ordinary solvents is valid only within a rather limited range of molecular weights, the value can be useful to find the constant a' for the corresponding molecular weight range of a linear $\log M$ vs. V_e curve. Thus, a and a' can be related for the corresponding molecular weight-solvent-temperature system as follows:

$$a = \frac{d \log [\eta]}{d \log M} = f \cdot a' = \frac{d \log (V_m/V_e)}{d \log M} \cdot f$$

The column constant k can be obtained graphically as shown in Fig. 3; however, it is recommended that the method of least squares be used for a series of polymer standards.

Very recently the use of the apparent maximum elution volume, V_m , as a measure of the apparent effective internal solvent volume has been reported¹⁷ for the reverse gel permeation process. The constant V_m for a polymer in a given set of GPC operating conditions would be defined as the apparent elution volume of molecular weight unity.

Polyethylene oxide has been chosen for the examination of the calibration method with the column sets of A and B. The results are shown in Figs. 4 and 5. Number-average molecular weight of Carbowax 20M, obtained by the NMR near-end group analysis¹⁰, was found to be 10500. Most likely sources of error would be the instrumental spreading and the temperature difference between viscosity measurements and GPC operation. However, it seems that the agreement between number-average molecular weights obtained by the present method and by NMR is satisfactory.

DUERKSEN AND HAMIELEC¹⁸ have reported the increasing importance of the resolution correction with increasing molecular weight and decreasing residence times. For the linear region of a given linear log calibration ($\log M$ vs. V_e), provided that efficient columns are used, resolution varies linearly with $\log M$ ¹⁹. When the variation of resolution with molecular weight is known, the calculation of molecular weight parameters from the chromatogram *via* the calibration of a given polymer become valid. According to HAMIELEC²⁰, the specific resolution R_s derived by BLY¹⁹ is consistent with the analytical solution of Tung's integral dispersion equation for symmetrical axial dispersion. Considering the present results and the specific resolutions derived by BLY and HAMIELEC, it is clearly indicated that different types of polymers have different specific resolutions at a given elution volume for a given set of GPC operating conditions. Thus we feel that the logarithm of molecular weight *versus* elution volume calibration for polymers should be used to evaluate chromatograms with valid molecular weight parameters, such as for resolution correction and for "analytical solution"²⁰.

CONCLUSION

It has been assumed that the conversion factor f and the empirical constant k for a given set of operating conditions are constant for any type of polymer. They are the key parameters for the present calibration technique. It has been felt that the constancy of k and f should be confirmed further with some different series of well characterized polymer samples. However, it seems that by using a series of well characterized polymer samples, a given set of operating conditions can be characterized by a column constant k and a conversion factor f over the corresponding linear range of molecular weight and temperature. Thus, by using a well characterized polymer sample with a known value of the exponent a from an appropriate viscosity-molecular weight relationship, the $\log M$ vs. V_e calibration can be obtained for the polymer by using the equation.

The application of this column calibration procedure to a specific polymer is straightforward and average molecular weights are simple to estimate when there is

a viscosity-molecular weight relationship and a well characterized polymer sample available. Such a polymer sample can be collected from the analytical column and characterized by classical methods.

Column calibration for a polymer for which the viscosity-molecular weight relationship is not available, will be reported in the near future.

ACKNOWLEDGEMENT

We wish to express our thanks to Dr. J. F. KINSTLE for valuable discussions of this work. We also wish to acknowledge the help of Mr. W. K. OKAMOTO and Miss A. W. TAUB in calculating the GPC data.

REFERENCES

- 1 H. BENOIT, Z. GRUBISIC, P. REMPP, D. DECKER, AND J. ZILLIOX, *J. Chim. Physiol.*, 63 (1966) 1507.
- 2 Z. GRUBISIC, P. REMPP AND H. BENOIT, *Polym. Lett.*, 5 (1967) 753.
- 3 H. BENOIT AND Z. GRUBISIC, *Proc. 5th Intern. Seminar Gel Permeation Chromatography, London, 1968*.
- 4 H. COLL AND D. K. GILDING, *J. Polym. Sci.*, A2, (1970) 89.
- 5 K. LIU, *Makromol. Chem.*, 116 (1968) 146.
- 6 D. D. BLY, *Proc. 6th Intern. Seminar Gel Permeation Chromatography, Florida, 1968*.
- 7 A. W. CRAIG AND D. A. HENDERSON, *J. Polym. Sci.*, 19 (1956) 215.
- 8 M. L. HUGGINS, *J. Amer. Chem. Soc.*, 64 (1942) 2716.
- 9 W. W. YAU, H. L. SUCHAN, C. P. MALONE AND S. W. FLEMING, *Proc. 5th Intern. Seminar Gel Permeation Chromatography, London, 1968*.
- 10 J. E. TANNER, private communication.
- 11 T. A. RITSCHER AND H. G. ELIAS, *Makromol. Chem.*, 30 (1959) 48.
- 12 C. SADRON AND P. REMPP, *J. Polym. Sci.*, 29 (1958) 127.
- 13 J. R. RUNYON, *159th ACS National Meeting Abstract, Cell-26, Houston, 1970*.
- 14 M. KURATA, M. IWAMA AND K. KAMADA, in J. BRANDRUP AND E. H. IMMERGUT (Editors), *Polymer Handbook*, ch. IV, Interscience, New York, 1967, pp. 7-46.
- 15 C. ROSSI, U. BIANCHI AND E. BIANCHI, *Makromol. Chem.*, 41 (1960) 31.
- 16 J. C. MOORE, *159th ACS National Meeting Abstract, Cell-21, Houston, 1970*.
- 17 L. F. MARTIN, F. A. BLOUIN AND S. P. ROWLAND, *159th ACS National Meeting Abstract, Cell-16, Houston, 1970*.
- 18 J. H. DUERKSEN AND A. E. HAMIELEC, *J. Polym. Sci.*, C, 21 (1968) 83.
- 19 D. D. BLY, *J. Polym. Sci.*, C, 21 (1968) 13.
- 20 A. E. HAMIELEC, in J. C. MOOR (Editor), *Gel Permeation Chromatography*, Appendix 1, St. Louis, 1969.

J. Chromatog., 55 (1971) 55-64