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MODIFIED METHOD OF UNIVERSAL CALIBRATION FOR THE DETERMINATION OF MOLECULAR WEIGHT AND MOLECULAR-WEIGHT DISTRIBUTION

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SUMMARY

A rapid method is proposed for the determination of molecular weight and molecular-weight distribution, utilizing fully the gel permeation chromatographic data and the idea of universal calibration. The molecular weight so determined is very close to the weight-average molecular weight. The method applies regardless of whether or not the distribution is narrow.

INTRODUCTION

Gel permeation chromatography (GPC) has been used for the determination of molecular weight and molecular-weight distribution of polymers for more than 15 years. Although this method has been extensively used for so long, there are still problems to be solved. The most serious problem is that the molecular weights of polymers do not correlate linearly with retention volumes, because retention volume is more a function of effective volume than of molecular weight. One of the most reasonable proposals to solve this problem is to use the universal calibration[1], namely, the plot of hydrodynamic volume $[\eta]\ M$, *versus* retention volume, V_R, the symbol $[\eta]$ referring to intrinsic viscosity, and M to molecular weight. Unfortunately, there are two drawbacks to the universal calibration method: (1) The effective volume depends on the solvents, assuming that the temperature is kept constant (it is well known that the effective volume of a polymer molecule in a good solvent is not the same as that in a poor solvent[2]); (2) the GPC method has been reduced to be of secondary importance. Since intrinsic viscosity alone has been used for the determination of molecular weight, one wonders why one should bother with the GPC measurement.

This paper describes and assesses a rapid method for the determination of molecular weight and molecular-weight distribution, utilizing fully the GPC data and the idea of universal calibration. For well-known polymer systems, the viscosity constants K and a in the equation

$$[\eta] = KM^a \tag{1}$$

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are abundant in the literature, and one does not have to make any viscosity measurements. Only for a new system is a separate experiment on viscosity necessary to obtain the values of K and a.

EXPERIMENTAL

Materials

The two polymers investigated were polystyrene and poly(methyl methacrylate). Polystyrene standards were obtained from Pressure Chemical Co. (Pittsburgh, PA, U.S.A.). Four samples of poly(methyl methacrylate) were synthesized by polymeralization of methyl methacrylate in benzene at 37°C, using α,α′-azodiisobutyronitrile as initiator. Two other samples of poly(methyl methacrylate) were obtained from Aldrich (Milwaukee, WI, U.S.A.).

The eluent used was glass-distilled tetrahydrofuran (THF); "spectroquality" dioxane and purified benzene were also used for comparison.

GPC measurements

The GPC instrument was equipped with a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, a U6K injector, a variable-wavelength UV detector and a differential refractometer detector. Four μStyragel columns were used, with pore size of 10^3 Å, 10^4 Å, 10^5 Å and 10^6 Å. The GPC was run at 25°C and a flow-rate of 1.0 ml/min. The injection volume was 100 μl.

Viscosity measurement

Flow times were measured with Ubbelohde viscometer at 25°C. All the solutions as well as the solvents were filtered before measurements were made.

RESULTS

Table I shows the retention volumes of polystyrene standards at 25°C. The data on molecular weight were supplied by the manufacturer (determined with use of light-scattering and osmotic-pressure measurements.). In order to observe the effect

TABLE I
RETENTION VOLUME OF POLYSTYRENE STANDARDS IN VARIOUS SOLVENTS AT 25°C

Sample No	$M \times 10^{-4}$	*Retention volume (ml)*		
		THF	*Dioxane*	*Benzene*
1	0.220	40.5	41.0	40.9
2	0.400	40.0	40.8	40.4
3	1.50	36.6	—	—
4	3.50	33.8	—	—
5	11.0	31.1	31.4	31.3
6	47.0	27.8	—	—
7	90.0	26.2	26.4	26.3
8	180	24.3	24.6	24.6
9	270	24.0	24.5	24.2

of the solvent on the retention volume, GPC measurements were carried out with three different eluents: THF, benzene and dioxane. It can be seen that there is virtually no solvent effect on the retention volume. The solvents used are all good solvents (*i.e.*, solute–solvent interaction is low).

The data on molecular weight, intrinsic viscosity and retention volume for poly(methyl methacrylate) are given in Table II. The viscosity-average molecular weights were determined from the intrinsic viscosity data, by using[3] $K = 1.04 \cdot 10^{-4}$ dl/g and $a = 0.697$. Since there is a wide spread of GPC chromatograms for poly(methyl methacrylate), the viscosity-average molecular weight is expected to be much larger than the number-average and weight-average molecular weights.

TABLE II
INTRINSIC VISCOSITY AND RETENTION VOLUME OF POLY(METHYL METHACRYLATE) IN THF AT 25°C

Sample	$M_v \times 10^{-4}$	$[\eta]$ *(dl/g)*	V_R *(ml)*
C4	3.98	0.167	35.4
C3	4.65	0.186	34.9
C2	6.02	0.223	34.7
C1	6.51	0.236	34.1
Ald 03	8.48	0.283	33.1
Ald 10	46.4	0.926	28.4

ANALYSIS OF DATA

Determination of molecular weight for poly(methyl methacrylate) from GPC data and viscosity constants

The GPC calibration curve of polystyrene standards in the form of log M *versus* V_R is shown in Fig. 1; with the uncertainty indicated by the size of the circles, the data points are in general on a straight line. Given any value of V_R within the range 20 to 40 ml, the value of M can be read from the straight line.

According to the universal calibration theory, at a given elution volume, two polymers 1 and 2 have the same hydrodynamic volume, $[\eta]M$, that is

$$[\eta]_1 M_1 = [\eta]_2 M_2 \tag{2}$$

From eqns. 1 and 2, we obtain

$$\log M_2 = \frac{1}{1 + a_2} \cdot \log \frac{K_1}{K_2} + \frac{1 + a_1}{1 + a_2} \cdot \log M_1 \tag{3}$$

Let 1 represent polystyrene standard and 2 any sample [in our case, poly(methyl methacrylate)]. Since K_1 and a_1 are well known in the literature. and since K_2 and a_2 are either known in the literature or obtainable by carrying out a few measurements by intrinsic-viscosity and osmotic-pressure (or light-scattering) techniques, M_2 can be easily determined for any sample of the same species from GPC data V_r. From V_r one can read M_1 on the straight line of Fig. 1.

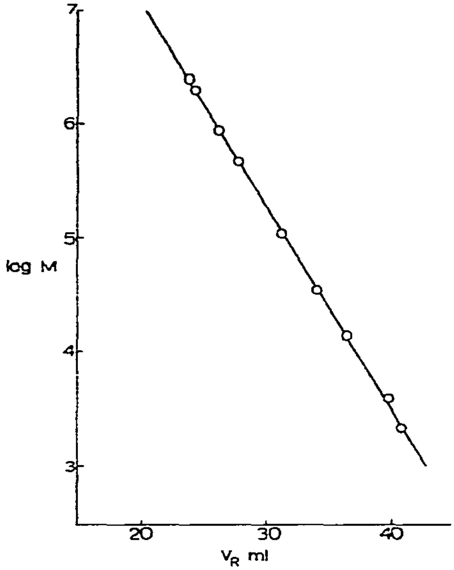

Fig. 1. GPC calibration plot: polystyrene standard in THF at 25°C

In Table III, the values of molecular weight of poly(methyl methacrylate) are listed (column 1); the values of viscosity constants used were $K_1 = 1.41 \cdot 10^{-4}$ dl/g; $a_1 = 0.70$.

TABLE III
MOLECULAR WEIGHT OF POLY(METHYL METHACRYLATE) DETERMINED FROM GPC DATA (AREA)

Sample	$M \times 10^{-4}$*	$\bar{M}_w \times 10^{-4}$	$\bar{M}_n \times 10^{-4}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_m$
C4	2 61	2 45	1.56	1.57	1 96
C3	3.14	2 88	1.97	1.46	2.39
C2	3.41	3.04	2.07	1.47	2.51
Cl	4.35	3.95	2.77	1.43	3.30
Ald 03	6.61	6.53 (6 10)**	2 61 (3.32)**	2 51	4.13
Ald 10	44.8	42 3	20.5	2 06	29.4

* Obtained by using a modified method of universal calibration.

** Manufacturer's value.

Calculation of molecular weight and molecular-weight distribution directly from GPC chromatograms

The chromatograms of four representative polystyrene standards are shown in Fig. 2 and those of poly(methyl methacrylate) in Fig. 3. The curves of polystyrene are strictly Gaussian with the exception of the one with the highest molecular weight ($2.7 \cdot 10^6$). The polydispersity is 1.26 for that particular sample, whereas that of each of the remaining three samples is 1.05. The curves of poly(methyl methacrylate) are widely spread, but, in general, they are symmetrical; for that reason, we can treat them as normal distribution.

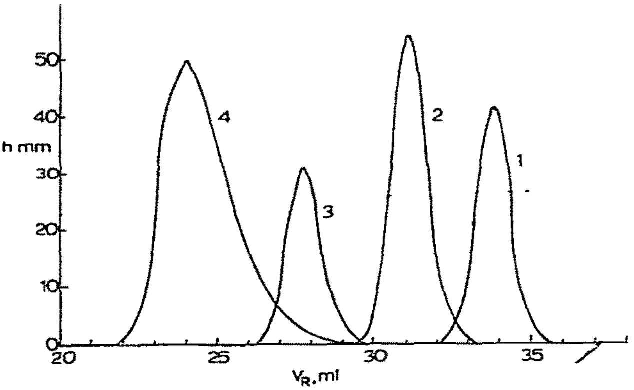

Fig 2. GPC chromatograms of polystyrene standard in THF at 25°C Molecular weight 1 = $3.5 \cdot 10^4$; 2 = $1.1 \cdot 10^5$; 3 = $4.7 \cdot 10^5$; 4 = $2.7 \cdot 10^6$.

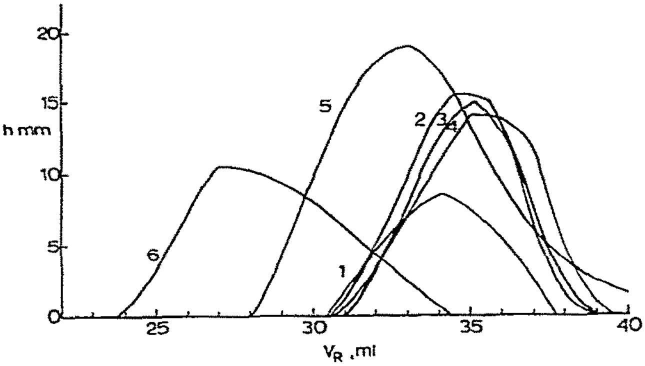

Fig. 3. GPC chromatograms of poly(methyl methacrylate) in THF at 25°C. Samples: 1 = C1; 2 = C2; 3 = C3; 4 = C4; 5 = Ald 03; 6 = Ald 10.

$\bar{M}_n$ and $\bar{M}_w$ are calculated from the following two equations:

$$\bar{M}_n = \frac{\Sigma\, h_i}{\sum \frac{h_i}{M_i}}$$

$$\bar{M}_w = \frac{\Sigma\, h_i M_i}{\Sigma\, h_i}$$

where h_i is the peak height and M_i is the molecular weight for compound i. As shown in Fig. 4(a), the values of h_i and V_{Ri} are read directly on the chromatogram. From the calibration curve in Fig. 1, the values of V_{Ri} are converted into M_i.

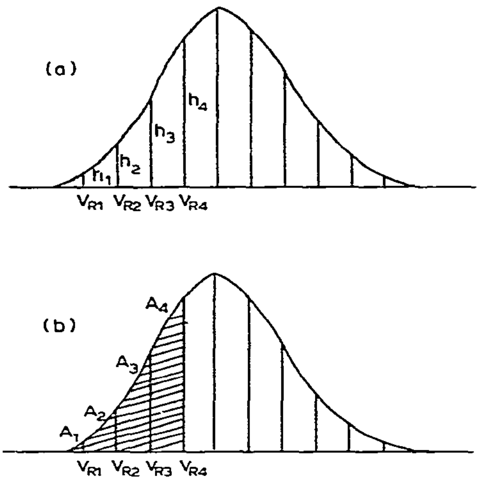

Fig. 4. Analysis of the chromatogram: (a) h_i vs V_{Ri}; (b) A_i vs V_{Ri}

We also tried to calculate the number-average molecular weight and the weight average molecular weight using slightly different equations, *viz.*:

$$\bar{M}_n = \frac{\Sigma A_i}{\sum_i \frac{A_i}{\bar{M}_i}}$$

$$\bar{M}_w = \frac{\Sigma A_i M_i}{\Sigma A_i}$$

where A_i is the peak area; Fig. 4(b) shows how the areas are evaluated. The values of $\bar{M}_n$ and $\bar{M}_w$ calculated from areas are listed in Table III, and those from heights in Table IV. The two methods seem to give the same values of $\bar{M}_n$ and $\bar{M}_w$. However, the area method is much more accurate and reliable for two reasons: (a) If the chromatogram is strictly Gaussian, the area method is just as good as the height method (actually, the two methods are identical in this case); (b) if the chromatogram is not

TABLE IV

MOLECULAR WEIGHT OF POLY(METHYL METHACRYLATE) DETERMINED FROM GPC DATA (HEIGHT)

Sample	$\bar{M}_w \times 10^{-4}$	$\bar{M}_n \times 10^{-4}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_m$
C4	2.75	1.75	1.57	2.19
C3	3.09	2.14	1.44	2.57
C2	3.38	2.32	1.46	2.80
C1	4.34	3.03	1.43	3.63
Ald 03	7.32	2.83	2.59	4.55
Ald 10	46.9	22.4	2.10	32.4

strictly Gaussian, the maximum height does not necessarily represent the medium (the area method would minimize the error).

Even for the determination of the retention volume, it is much better to use the area method (the center of mass) than the height method (the peak maximum)[3]. The values of V_R in Tables I and II were all determined with the area method.

Also included in Tables III and IV are two other parameters, the polydispersity, $\bar{M}_w/\bar{M}_n$, and the mean average molecular weight, $\bar{M}_m$, which is calculated with the equation:

$$\bar{M}_m = (\bar{M}_n \bar{M}_w)^{1/2}$$

Inspection of Table III reveals that the molecular weight determined by the modified universal calibration method is reasonably close to the weight-average molecular weight determined by analysis of the chromatogram alone; the difference is no more than 10%. For the samples with large molecular-weight distribution, the number-average molecular weight is always much smaller than weight-average molecular weight.

DISCUSSION

In regard to the determination of molecular weight of macromolecules, there seems to be a parallel between electrophoresis in biochemistry and GPC in polymer chemistry. Empirical correlation between the molecular weight and ion mobility has convinced biochemists for more than a decade that electrophoresis alone[4] can provide a reasonable estimation of the molecular weights of such biochemical species as enzymes and nucleic acids. An electrophoretic run is easy and fast; it has almost been a routine in any biochemistry research laboratory. For a very accurate determination of molecular weight, one has to use primary methods such as an analytical ultracentrifuge. The standard procedures for the ultracentrifuge are those proposed by Van Holde and Baldwin[5] and by Yphantis[6].

We have found that GPC measurements alone, with the aid of viscosity constants that are either available in the literature or can be obtained by separate measurement, can be used rapidly to determine the molecular weight of a polymer and that the molecular weight so obtained is equivalent to the weight-average molecular weight. The method is easy and rapid. For the determination of a more accurate value, one has to use such standard methods as osmotic-pressure and light-scattering measurements.

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