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DETERMINATION OF MOLECULAR WEIGHT DATA IN SIZE-EXCLUSION CHROMATOGRAPHY BY USE OF BROAD-MOLECULAR-WEIGHT-DISTRIBUTED STANDARDS

H. GOETZ*, H. ELGASS and L. HUBER
Hewlett-Packard GmbH, D-7517 Waldbronn (F.R.G.)

SUMMARY

   The analysis of technical polycarbonates and nitrocelluloses by size-exclusion chromatography and the evaluation of chromatograms by conventional narrow-standard calibration and by a recently published broad-molecular-weight-distributed (MWD) standard calibration procedure [O. Chiantore and A. E. Hamielec, *J. Liq. Chromatogr.*, 7 (1984) 1753] are described. The broad-MWD standard calibration procedure calculates by an iterative computer search the sample calibration graph from a calibration graph obtained with narrow-MWD standards using one broad-MWD standard of the sample. The results from both procedures are evaluated with data obtained from absolute techniques (*e.g.*, light scattering, osmometry).

INTRODUCTION

   The evaluation of a size-exclusion chromatogram is based on a valid calibration graph for the sample polymer. Usually, however, narrow standards for a sample polymer are not available, and calibration has to be performed with narrow standards of a different chemical structure (*e.g.*, polystyrene for polymers soluble in organic solvents). The resulting molecular weight values are incorrect owing to structural differences between the sample and standards, but the chromatograms and results are comparable to each other.
   The objective of a broad-standard calibration procedure is to eliminate the problem of not having standards of the same chemical structure as the sample polymer. Various types of broad-standard calibration procedures have been described[1–4]. In this work, a method was used which employs one broad-molecular-weight-distributed (MWD) standard and the molecular weight calibration graph obtained with narrow standards, *e.g.*, polystyrene. The narrow-standard calibration graph is transformed into a calibration graph that is suitable for a sample polymer. This is done by using a chromatogram and known weight- (M_w) and number-average (M_n) molecular weights values for a broad standard with the same chemical structure as the sample polymer.

THEORY

A narrow-standard calibration can be performed with narrow polystyrene standards. The resulting calibration graph is described by the equation

$$M = C(V) \tag{1}$$

where M is the molecular weight, V is the elution volume and C is a constant. For a polymeric homologous series, the hydrodynamic volumes of the macromolecules are proportional to their molecular weights and, hence, the elution volumes are proportional to the molecular weights:

$$\text{Hydrodynamic volume} \approx [\eta]M \tag{2}$$

where $[\eta]$ is the intrinsic viscosity. Chemically different polymers which are eluted in the same volume have the same hydrodynamic volume[2]:

$$[\eta]_x M_x = [\eta]_{ps} M_{ps} \tag{3}$$

where x refers to polymer x and ps to polystyrene.
    Using the Mark–Houwink equation:

$$[\eta] = KM^h \tag{4}$$

where K and h are constants for a given polymer type, solvent and temperature, eqn. 3 can be transformed into

$$K_x M^{(h_x + 1)} = K_{ps} M_{ps}^{(h+1)} \tag{5}$$

Solving eqn. 5 for M_x and substituting for M_{ps} from eqn. 1 gives

$$M_x = \overbrace{\left(\frac{K_{ps}}{K_x}\right)^{\frac{1}{h_x + 1}}}^{\alpha} \cdot \overbrace{C_{ps}(V)^{\frac{h_{ps} + 1}{h_x + 1}}}^{\beta}$$

The multiplication constants are combined to give a new constant, α, and the exponential constants are combined to give β. Substituting for M_x from eqn. 1 gives

$$C_x(V) = \alpha C_{ps}(V)^\beta \tag{7}$$

Eqn. 7 allows a known narrow standard calibration graph [e.g., $C_{ps}(V)$ for polystyrene] to be transformed into a calibration graph for a different polymer [e.g., $C_x(V)$ for polymer x].
    To determine the constants α and β, a broad-distributed sample of polymer x is injected as broad standard. The M_w and M_n values for polymer x must have been determined by other absolute methods (e.g., light scattering and osmometry).

Using eqn. 7, α and β are fitted iteratively until the resulting polydispersity value of the injected broad standard approaches the polydispersity value calculated from the known M_w and M_n values. The iteration is terminated by the software when the difference between the two polydispersity values is less than 0.005.

EXPERIMENTAL

Chromatography was performed using a Hewlett-Packard HP1090 liquid chromatograph, equipped with a DR5 solvent-delivery system, a programmable variable-volume injector, an autosampler and a heated column compartment. Either a refractive index detector (HP1037A) or a programmable filter-photometric detector (HP1090 opt. 081) with a detection wavelength of 260 nm was connected to the column effluent.

The analogue output of the detector was connected to an HP3392 integrator, which transmitted the area slices via an RS232 interface to an HP85 computer. The HP85 was interfaced with the HP1090 liquid chromatograph and used to calculate the size-exclusion chromatographic (SEC) results and to generate the reports. The area slices were stored on an HP9121D disk drive. High-quality plots were obtained with an HP7470A plotter. Hewlett-Packard PLgel columns and polystyrene standards were used. The mobile phase was tetrahydrofuran (THF) of HPLC grade, supplied by E. Merck (Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

Polycarbonates

The chromatographic system was calibrated first with narrow-distributed polystyrene standards. Table I shows the molecular weights and the retention times of these standards and Fig. 1 is the calibration graph obtained using a third-order curve fit.

To transform the polystyrene calibration graph into the polycarbonate calibration graph, a broad-standard polycarbonate sample was chromatographed, and the area-slice data were stored on flexible disk. The broad-standard polycarbonate must fulfil the following requirements: (1) same chemical structure as the samples to be analysed; (2) polydispersity (D) greater than 1.5; and the M_n and M_w values must

TABLE I

RETENTION TIMES AND MOLECULAR WEIGHTS OF POLYSTYRENE STANDARDS

Retention time (min)	Molecular weight (daltons)
10.195	470 000
10.805	110 000
10.975	82 100
11.200	54 400
12.000	19 800
12.800	8500
13.690	3600

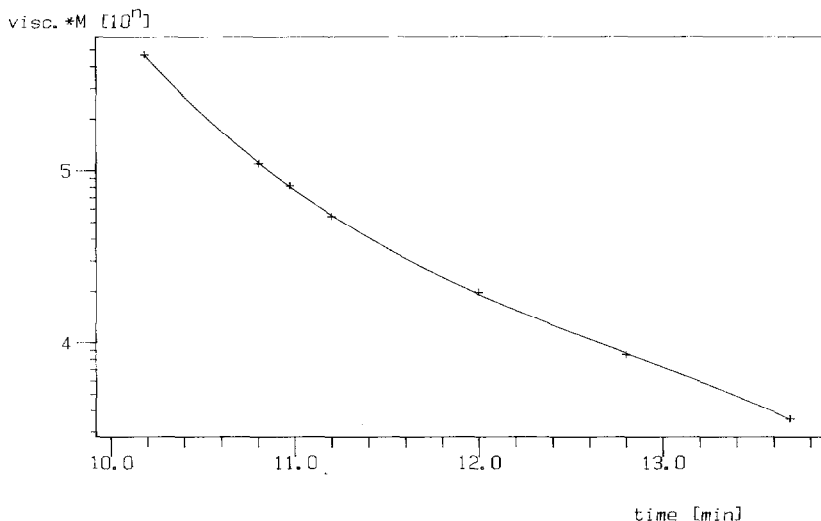visc. *M [10^n]

time [min]

Fig. 1. Calibration graph for polystyrene standards: $ax^3 + bx^2 + cx + d$; $a = -0.3657841167 \cdot 10^{-1}$, $b = 1.4070836103$; $c = -0.1844765079 \cdot 10^2$; $d = 86.242507686$; $\chi^2 = 0.00014$. Columns: HP79911GP-109, PLgel mixed, 10 μm, 300 × 7.7 mm I.D., in series with an HP79911GP-502, PLgel 500A, 5 μm, 300 × 7.7 mm I.D. Mobile phase: HPLC-grade THF. Flow-rate: 1 ml/min. Injection volume: 100 μl. Sample concentration: 0.1% in THF. Detection: wavelength 260 nm; bandwidth 10 nm.

be known from methods that give absolute data (*e.g.*, light scattering or osmometry).

The transformation is based on the concept of the hydrodynamic volume[5], which states that calibration graphs of different polymers merge into a single plot when log $M[\eta]$ (see eqn. 2) instead of log M is plotted against the retention time. Fig. 2 shows that this is fulfilled for the chosen polystyrene standards and the polycarbonates.
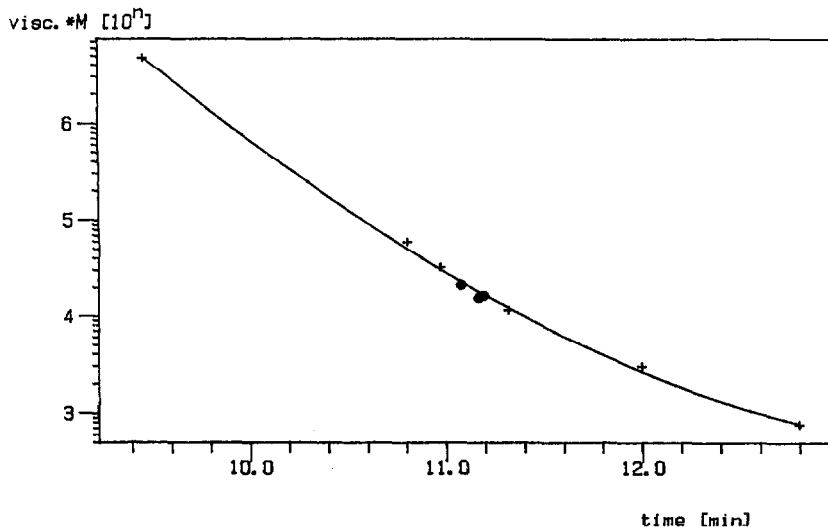
visc. *M [10^n]

time [min]

Fig. 2. Plot of log $M[\eta]$ against retention time for polystyrene standards (+) and polycarbonates (●). Conditions as in Fig. 1.
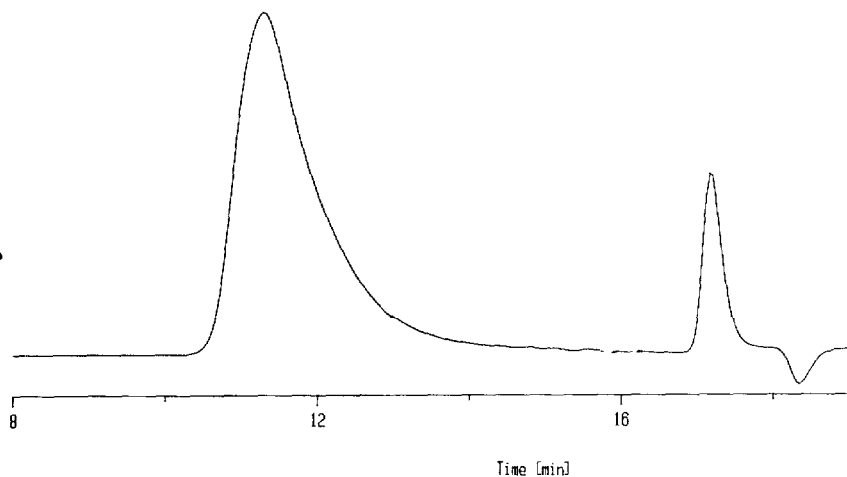
Time [min]

Fig. 3. Chromatogram of broad-standard polycarbonate. Conditions as in Fig. 1.

Fig. 3 shows the chromatogram of the broad-standard polycarbonate. For this polycarbonate, M_n was 12 400 ± 5% when determined by membrane osmometry and M_w was 23 800 ± 5% when determined by light scattering.

With the chromatogram in Fig. 3 and the known M_n and M_w values, the polystyrene calibration graph was transformed into the polycarbonate calibration graph using the method described above. Fig. 4 shows an overlay of the resulting polycarbonate graph and the original polystyrene calibration points. A significant difference, especially in the high-molecular-weight region, can be seen.
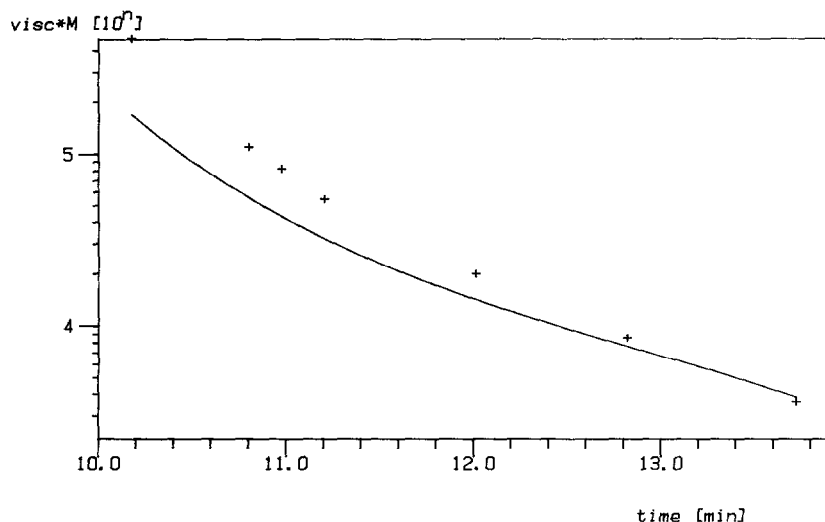

time [min]

Fig. 4. Overlay of polystyrene (+) and polycarbonate (−) calibration graphs: $ax^3 + bx^2 + cx + d$; $a = 0.3657 \cdot 10^{-1}$, $b = 1.4071$, $c = -0.1844 \cdot 10^2$, $d = 86.243$; $\chi^2 = 0.00014$, log $\alpha = 8.1927 \cdot 10^{-1}$, $\beta = 7.7734 \cdot 10^{-1}$.
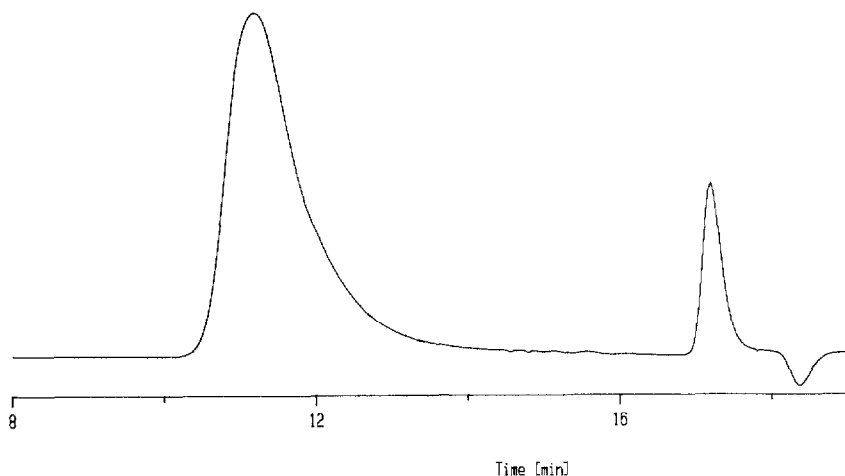
Fig. 5. Chromatogram of polycarbonate No. 2. Conditions as in Fig. 1.

The validity of the new calibration can be tested by injecting polycarbonates of known M_n and M_w.

Fig. 5 shows the chromatogram for polycarbonate No. 2. Comparison of the results obtained by the absolute techniques and SEC (Table II) shows that the M_w and polydispersity data, calculated from the narrow polystyrene calibration graph, differ considerably from the data obtained by light scattering and membrane osmometry. However, the M_n value is in reassonably good agreement with the osmometric value, because M_n is influenced more by the low-molecular-weight fractions for which the calibration graphs are similar for both the polycarbonate and the polystyrenes (Fig. 4). When the polycarbonate calibration graph, obtained by broadstandard calibration, was used for the evaluation of polycarbonate No. 2, close agreement between the SEC data and the absolute data was obtained.

The molecular-weight distribution is also influenced by the calibration graph. Fig. 6a shows that 60% of the polymer consisted of molecular weights of less than 25 800 daltons, but by conventional narrow-standard calibration we would have calculated that 60% of the polymer consisted of molecular weights of less than 41 900 daltons (Fig. 6b).

TABLE II

COMPARISON OF RESULTS FOR POLYCARBONATE NO. 2

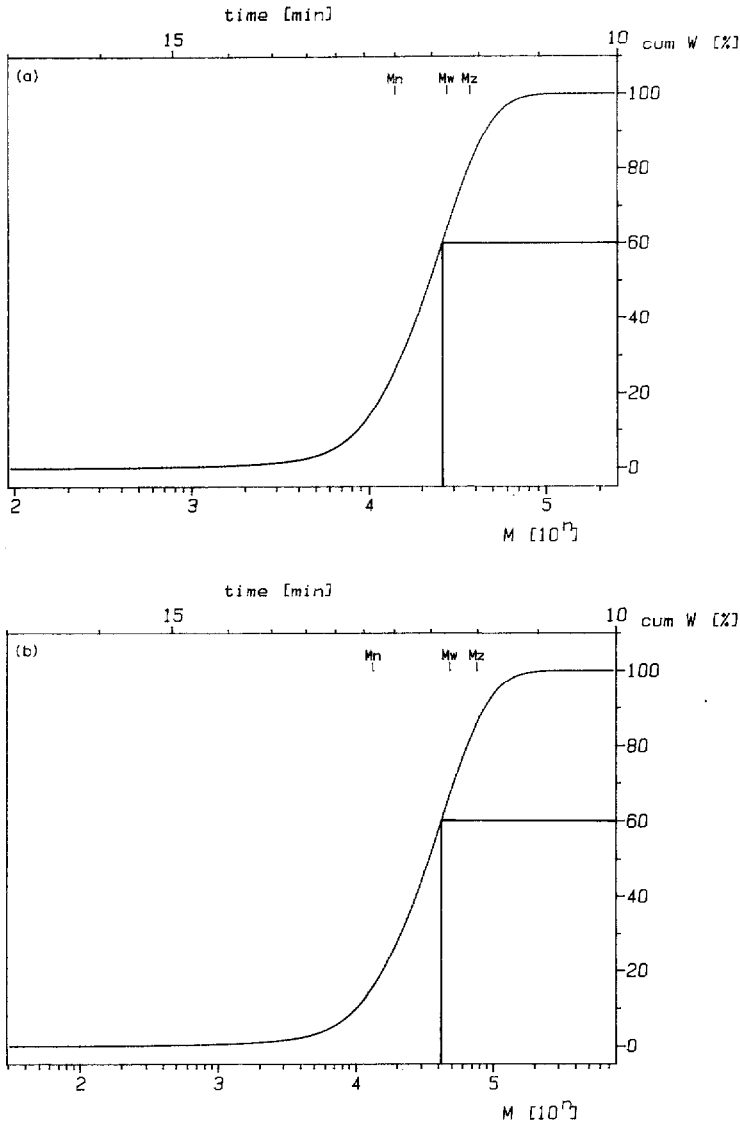| Parameter	Light scattering/ osmometry	Narrow-standard calibration (polystyrene standards)	Error (%)	Broad-standard calibration	Error (%)
M_w	27 800	48 510	75	27 960	0.57
M_n	14 200	13 390	6	14 030	0.12
D	1.96	3.62	85	1.99	1.53

Fig. 6. Cumulative molecular-weight distributions for polycarbonate No. 2. (a) Obtained with broad-standard calibration; M_n = 14 120, M_w = 27 650, M_z = 37 240, polydispersity = 1.957. (b) Obtained with narrow-standard calibration; M_n = 13 390, M_w = 48 510, M_z = 76 470, polydispersity = 3.62.

The chromatogram for polycarbonate No. 3 is shown in Fig. 7. Table III summarizes the results obtained for polycarbonate No. 3. Again, a significant difference was found between the data obtained using the narrow-standard calibration graph and the data obtained using absolute techniques. In contrast, the molecular-weight averages and the polydispersity, obtained by broad-standard calibration, agreed with those obtained by light scattering and membrane osmometry.
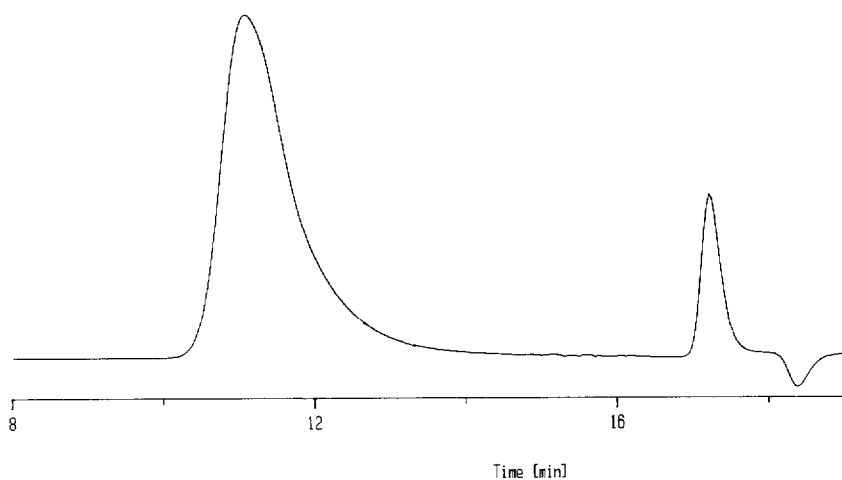
Time [min]

Fig. 7. Chromatogram of polycarbonate No. 3. Conditions as in Fig. 1.

TABLE III

COMPARISON OF RESULTS FOR POLYCARBONATE NO. 3

Parameter	Light scattering/ osmometry	Narrow-standard calibration (polystyrene)	Error (%)	Broad-standard calibration	Error (%)
M_w	33 700	56 500	68	31 200	7.4
M_n	17 400	19 200	10	17 600	1.2
D	1.94	2.94	51	1.77	8.8

Nitrocelluloses

For the analyses of nitrocelluloses, a different set of PLgel columns was used, which were first calibrated with polystyrene standards. Fig. 8 shows the chromatogram of the broad-standard nitrocellulose No. 1 and Table IV the M_n and M_w data obtained by viscosimetry and osmometry. With nitrocellulose the broad standard and sample should have the same nitrogen content. It is known[6] that the viscosity depends on the nitrogen content, which can be explained by a difference in hydrogen bonding. From Fig. 9, it can be seen that the iteratively derived nitrocellulose calibration graph is different from the original polystyrene calibration graph, especially in the higher molecular weight region.

Fig. 10 shows the chromatograms of a nitrocellulose sample and Table V compares the results obtained by the absolute and SEC techniques. Table V shows that the error is large when narrow-standard calibration is used but that results agree well with those of the absolute techniques when broad-standard calibration is used.

The chromatogram for nitrocellulose No. 2 is shown in Fig. 11. Because of the different nitrogen content (12.6%) of that sample, the original polystyrene calibration
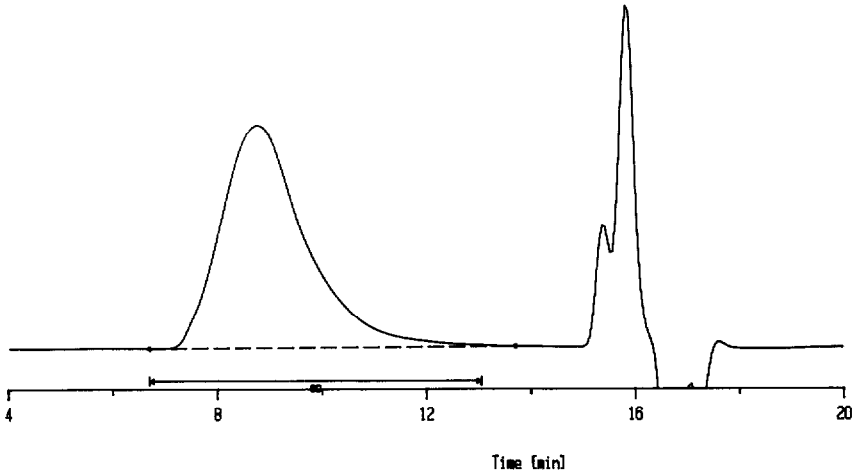
Time [min]

Fig. 8. Chromatogram of broad-standard nitrocellulose No. 1. Mobile phase: HPLC-grade THF. Flow-rate: 1 ml/min. Columns: HP79911GP-502 (PLgel 5 × 10^2 A, 5 μm) in series with HP79911GP-504 (PLgel 10^4 A, 5 μm). Sample concentration: 0.1% in THF. Injection volume: 100 μl.

TABLE IV

M_n AND M_w DATA OF BROAD-STANDARD NITROCELLULOSE NO. 1

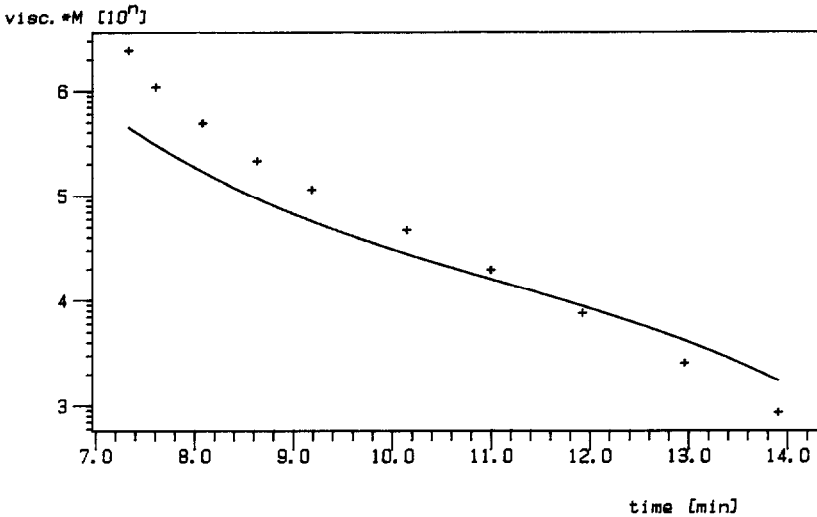| M_w (viscosimetry)	M_n (osmometry)	D (M_w/M_n)
90 300 ± 10%	51 500 ± 10%	1.75

time [min]

Fig. 9. Nitrocellulose calibration graph (————) obtained by broad-standard iteration: $ax^3 + bx^2 + cx + d$; $a = -0.1134069263 \cdot 10^{-1}$, $b = 3.7925534982 \cdot 10^{-1}$, $c = -4.614371258$, $d = 24.233240826$; $\chi^2 = 0.00255$, $\log \alpha = 1.1841$, $\beta = 7.0703 \cdot 10^{-1}$. Polystyrene calibration graph (+). Conditions as in Fig. 8.
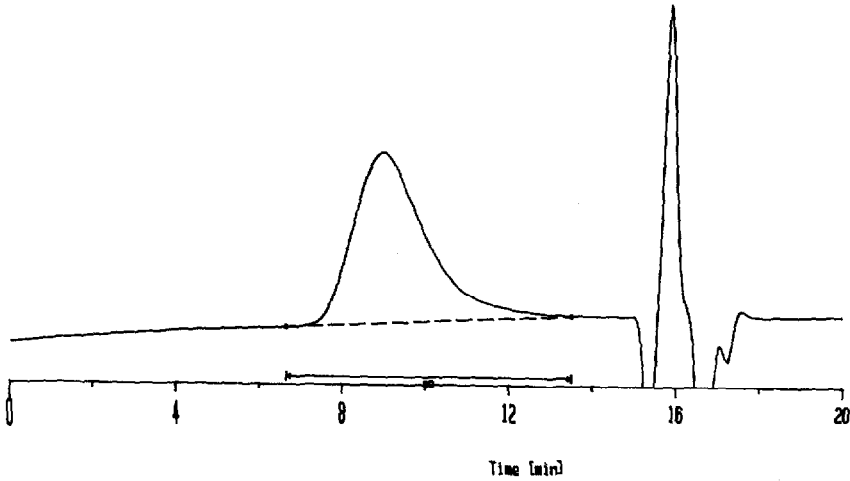
Fig. 10. Chromatogram of nitrocellulose No. 1. Conditions as in Fig. 8.

TABLE V

COMPARISON OF RESULTS FOR NITROCELLULOSE NO. 1 OBTAINED BY THE ABSOLUTE AND SEC TECHNIQUES

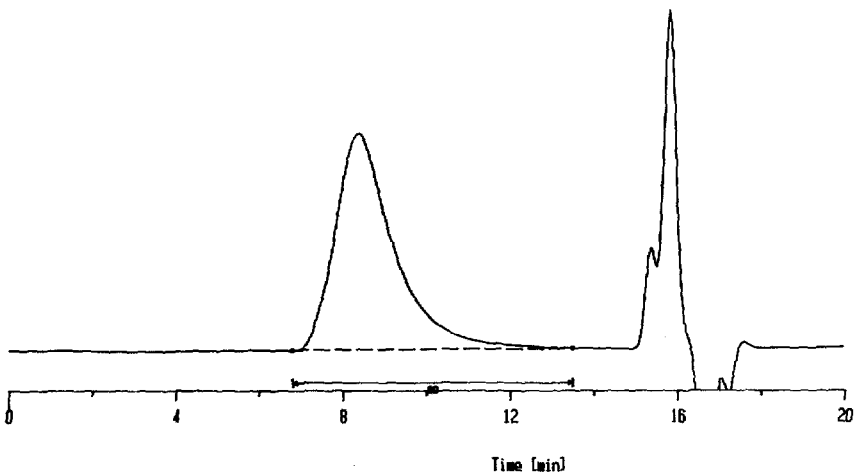| Parameter	Viscosimetry/ osmometry	Narrow-standard (polystyrene)	Error (%)	Broad-standard	Error (%)
M_w	68 400	185 600	171	72 520	6.0
M_n	37 000	55 780	51	35 250	4.7
D	1.85	3.33	80	2.06	11.2

Fig. 11. Chromatogram of nitrocellulose No. 2. Conditions as in Fig. 8.

TABLE VI

COMPARISON OF RESULTS FOR NITROCELLULOSE NO. 2 OBTAINED BY THE ABSOLUTE AND SEC TECHNIQUES

Parameter	Viscosimetry/ osmometry	Narrow–standard (polystyrene)	Error (%)	Broad–standard	Error (%)
M_w	178 000	379 300	113	188 400	5.8
M_n	106 000	107 300	1.2	103 800	2.1
D	1.68	3.53	110	1.81	8.0

was transformed with a broad-standard nitrocellulose with the same nitrogen content. Table VI shows a comparison of the results obtained by absolute and SEC techniques. The superiority of the broad standard calibration procedure is furthermore demonstrated. In Fig. 12 the data obtained for the two nitrocelluloses by absolute techniques and SEC are compared. The agreement is very good if the described broad-standard calibration procedure is used for the calibration of the SEC system.
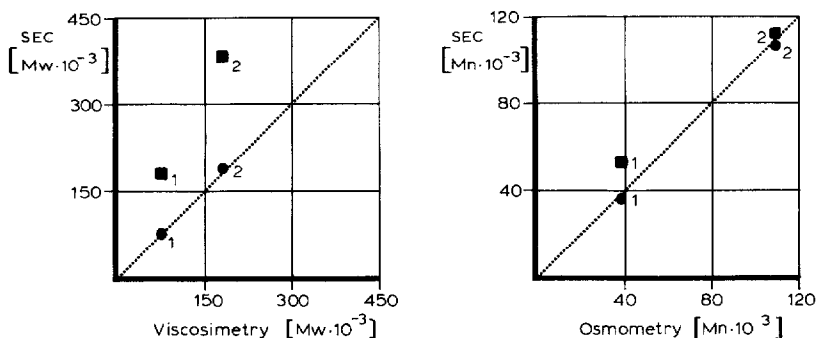

Fig. 12. Comparison of results for nitrocelluloses Nos. 1 and 2. ●, Broad standard; ■, narrow standard.

CONCLUSION

It has been shown that polycarbonate and nitrocellulose molecular-weight data obtained by SEC can be directly compared with data obtained by absolute techniques when using the discussed broad-standard calibration procedure. The agreement between the data from the absolute techniques and SEC is significantly improved if the original polystyrene calibration graph is transformed by an iterative computer search into the polycarbonate or nitrocellulose calibration graph. Similar results are expected for other types of polymers.

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