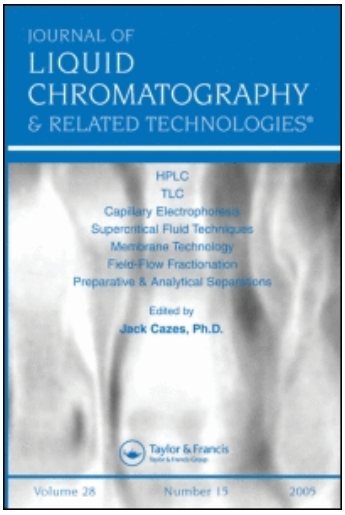

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Straightforward Procedure for Estimating the Spreading Factor in SEC

G. Glöckner[a]
[a] Department of Chemistry, Technical University of Dresden, Dresden, Mommsenstr

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STRAIGHTFORWARD PROCEDURE FOR ESTIMATING THE SPREADING FACTOR IN SEC

G. Glöckner
Technical University of Dresden
Department of Chemistry
DDR-8027 Dresden, Mommsenstr. 13

ABSTRACT

This contribution compiles SEC plate-height data obtained with various polymer standards and with a low-molecular probe. The latter value is easily measured and commonly given as a test of the apparatus used. Plate-height values h from the low and high-molecular range can be approximated by a straight line when plotted logarithmically:

$$\log h = A + B \log M$$

Knowledge of the slope factor B would enable plate-height data in the high-molecular range to be estimated on the basis of the reliable value from a low-molecular probe. The variance σ^2 and the spreading factor $1/(2\sigma^2)$ can easily be derived from the plate height.

INTRODUCTION

The spreading factor is a quantity which is needed for the evaluation of Tung's integral equation (1):

$$F(v) = \int_0^\infty W(y)\, G(v,y)\, dy \qquad (1)$$

F(v) is the uncorrected chromatogram, i. e. the detector response at elution volume v. W(y) is the chromatogram corrected

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for peak broadening. G(v,y) is the instrumental spreading function which contains the spreading factor. Graphically, G(v,y) is the detector response due to a single component with elution volume y. G(v,y) is usually assumed to be Gaussian:

$$G(v,y) = \frac{1}{\sigma\sqrt{2\pi}} \exp - \frac{(v - y)^2}{2\,\sigma^2} \qquad (2)$$

σ^2 is the variance of a Gaussian distribution. The quantity σ is the standard deviation. For a Gaussian curve, it is half the width at the inflection points, i. e. at 60.7 % maximum height of this curve.

Tung (1) called the quantity $1/(2\,\sigma^2)$ "spreading factor" but there are also papers which use this name for the expressions $1/(2\,\sigma^2)^{0.5}$ or $1/\sigma^2$. At any rate, the so called spreading factor is related to the reciprocal of the variance σ^2.

Eq. (1) reflects the fact that the chromatogram of a given sample is always broader than its component distribution. The band broadening is due to instrumental spreading. The higher the performance of a chromatographic apparatus, the less dramatically the bands will broaden - but band broadening remains a fundamental problem and especially influences the edges of a chromatogram. Here the uncorrected curve shows constituents which, in reality, are not present.

There are several numerical techniques for the solution of Eq. (1). (For survey, see Ref. 2, e.g.). The methods proposed

by Ishige et al. (3) or by Vozka and Kubin (4) proved to be very effective (5). An analytical solution of Eq. (1) has recently been given by Hamielec et al. (6).

All the correction techniques require precise knowledge of the standard deviation σ . With too small a value the correction will be insufficient, too high a value will yield overcorrection. In SEC of polymers, the quantity σ can be measured by several techniques: (i) by reverse-flow experiments (7), (ii) by chromatographic runs of polymers which are chromatographically monodisperse (8), (iii) by chromatographic runs of samples with precisely known molar mass distribution (MMD), (iv) using samples with exactly known values of average molar mass or statistical moments, or (v) by recycling.

Methods (i) and (v) need special equipment and are rather cumbersome, (ii) requires high effort in fractionating a synthetic polymer to the necessary degree of purity, (iii) and (iv) are strongly dependent on the precission to which the MMD or the average molar mass values of the standard polymers are known.

It is very difficult to obtain the precise data of $\sigma(v)$. In some papers dealing with correction of SEC data the value of σ is assumed to be independent of elution volume, but all experimental work shows a decrease of σ or σ^2 with increasing v. Some results reveal a maximum in the curve of σ^2 vs. v which is located in the vicinity of the exclusion limit of the column. This effect is due to the mass-transfer contribution and will be discussed later.

The purpose of this paper is the presentation of a straightforward procedure for estimating $\sigma^2(v)$. We intend to approach this aim via investigation of plate height as a function of molar mass, $h = f(M)$.

The plate height h (height equivalent to a theoretical plate, HETP) is:

$$h = L/N \tag{3}$$

The plate number N is related to elution volume v and variance σ^2:

$$N = v^2/\sigma^2 \tag{4}$$

Thus the plate height is:

$$h = L\,\sigma^2/v^2 \tag{5}$$

L is the length of column.

The plate height is a measure for the quality of column packing and influences the peak width. The peak width also increases with increasing column diameter and length. In order to get rid of these geometric effects and to approach a more general relation for peak broadening we shall investigate the behaviour of h instead of that of peak width.

According to Eq. (5) and the additivity rule of variances, the quantity h can be treated as the sum of contributions which are, e. g., due to polydispersity of sample (index: P), to diffusion and stream effects (D), and to resistance to mass transfer (index: MT):

$$h_{total} = h_P + h_D + h_{MT} \tag{6}$$

The sum of the second and third term at the right-hand side of Eq. (6) is the instrumental spreading. Only with monodisperse samples h_p is zero, with polydisperse samples the h_{total} should be corrected. This requires the precise knowledge of the distribution of species. If the calibration function of the SEC apparatus is linear,

$$\ln M = \ln D_1 - D_2 v \qquad (7)$$

and the sample distribution is logarithmic-normal, h_p can be calculated from the number and weight averages of molar mass, M_n and M_w, with the help of the equation:

$$\ln (M_w/M_n) = \sigma_p^2 D_2^2 \qquad (8)$$

EXPERIMENTAL

Apparatus: KNAUER Liquid Chromatograph LC/GPC 5050 with high-pressure pump FR 30 and differential refractometer 2025/50, with a home-made siphon of 1.289 cm^3 volume per count.

Column: L = 5 x 0.25 m, d_C = 4.6 mm, packed by supplier (KNAUER KG) with LiChrospher(R) Si 4000, Si 1000, Si 500 (2x) and Si 100, particle diameter d_p = 10 /um.

Solvent: Tetrahydrofuran (THF) "pro analysi", VEB LABORCHEMIE, Apolda, dried with KOH (24 hours), refluxed for 2 hours with Na wire, distilled under nitrogen using a VIGREUX column 0.30 m in length.

Samples: Polystyrene standards for SEC calibration, supplier: KNAUER KG, molar mass values given in column 1 of Table I.

Working conditions: concentration of sample solution c_o = 1.5 g/l, V_o = 538 µl, flow rate $\dot{v}$ = 1 cm^3/min.

RESULTS

The results obtained with this apparatus (9) are compiled in Table I. Column 3 of it shows the observed peak width, which is related to the standard deviation σ_{total} by the expression $W = 4\,\sigma_{total}$. The σ values listed in column 4 are calculated from the peak width after correction for injection volume (0.538 ml).

From the values of elution volume and molar mass (columns 1 and 2), the calibration function was calculated. It reads (for c_o = 1.5 g/l and $\dot{v}$ = 1 cm^3/min):

$$\log M_w = \sum_{i=0}^{7} a_i \, v^i \qquad (9)$$

with a_o = 449.977, a_1 = -144.251, a_2 = 18.0263, a_3 = -1.1768 a_4 = 6.49444-2, a_5 = -4.58180-3, a_6 = 2.25601-4, a_7=-4.3568-6.

The plate height data (column 5) plotted logarithmically vs. log M is shown in Fig. 1. The data are represented by a straight line:

$$\log h = A + B \log M \qquad (10)$$

Values of the slope factor B are compiled in Tab. II.

TABLE I

SEC Data Obtained with Toluene (M = 92) and Several Polystyrene Standards

M g/mol	v ml	W ml	σ ml	h /μm
92	17.04	0.951	0.103	46
600	16.78	1.075	0.134	80
4,000	16.20	1.245	0.177	149
20,400	14.89	1.280	0.186	194
33,000	14.20	1.280	0.186	213
51,000	13.82	1.411	0.218	312
110,000	12.84	1.316	0.195	287
173,000	12.30	1.266	0.182	274
200,000	12.19	1.256	0.180	271
390,000	11.38	1.204	0.167	268
670,000	10.93	1.235	0.174	318
867,000	10.82	1.319	0.195	407
2,000,000	10.27	1.655	0.279	924

DISCUSSION

The result shown in Fig. 1 fully corresponds to previous observation (2). In the course of the present work we used additional data from literature.

Figs. 2 - 4 show results published by Dawkins and Yeadon in 1980 (10). These authors used columns 0.20 m in length and

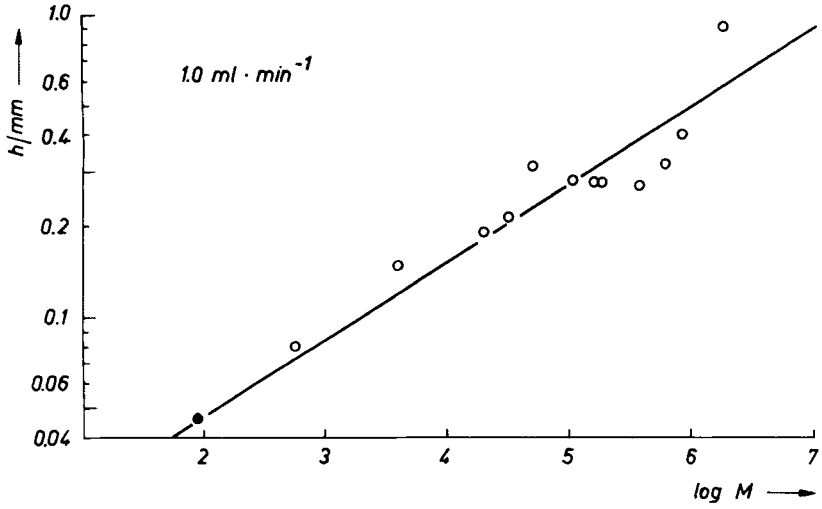

FIGURE 1

Plate height as a function of molar mass, log h vs. log M as measured with toluene (•) and polystyrene standards (o). Column: L = 1.25 m, 4.6 mm I.D., packed with silica microspheres. Eluent tetrahydrofuran, flow rate 1 ml/min, (redrawn from Ref. 9).

3 mm I.D. which had been slurry-packed with silica microspheres. The heterogeneity in particle diameter (weight to number average) was 1.22, 1.30, and 1.67, the number average values d_p = 13.9, 12.8, and 8.5 /um for the packing materials H 2, H 4, and H 6, respectively. The exclusion limits were $>10^6$, 5 x 10^5, and 10^5 g/mol (in the same sequence). The investigations were performed at various flow rates. The straight line for log h vs. log M, which was found at $\dot{v}$ = 1 ml/min, is repeated by a dashed line in the corresponding diagrams for higher (2.0) or lower values of flow rate (0.1 and 0.5 ml/min).

TABLE II

Values of B in Equation (10) as Calculated from Experimental Data by Least-Square Regression

Source	Fig.	Flow rate $\dot{v}$ ml/min	Linear velocity u mm/s	B
this work	1	1.5	2.51	0.24
Dawkins and Yeadon (10) H 2	2	0.1	0.54	0.15
		0.5	2.68	0.29
		1.0	5.36	0.34
		2.0	10.72	0.34
(10) H 4	3	0.1	0.54	0.24
		0.5	2.68	0.33
		1.0	5.36	0.38
		2.0	10.72	0.43
(10) H 6	4	0.1	0.54	0.19
		0.5	2.68	0.29
		1.0	5.36	0.36
		2.0	10.72	0.29
Kirkland (11)	5	0.88	0.76	0.17
		1.4	1.21	0.22
		2.5	2.16	0.26
		5.8	5.01	0.30
Cooper et al. (12)	6	0.055	0.05	0.13
		0.215	0.18	0.17
		1.040	0.90	0.27

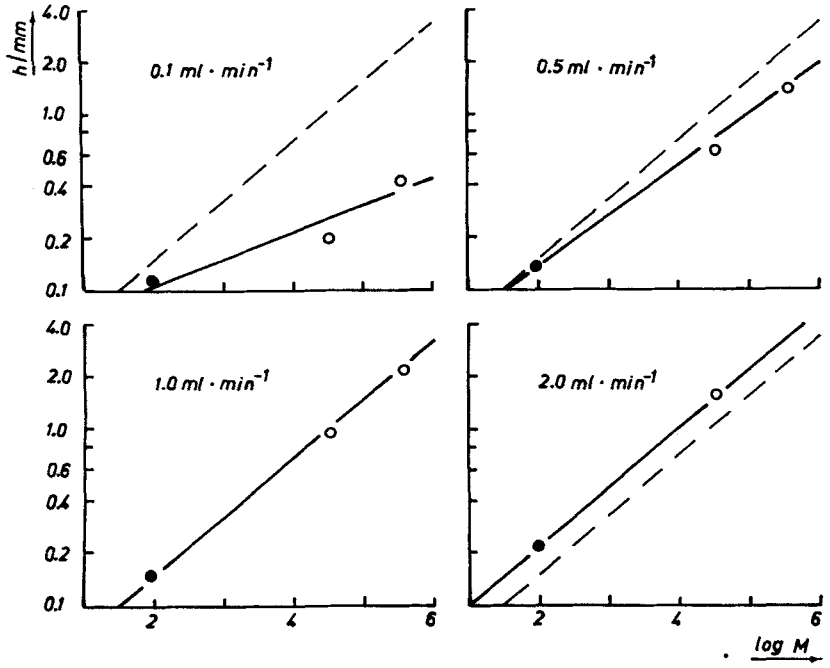

FIGURE 2

Plots of log h vs. log M for polystyrene standard samples (o) and toluene (•) at flow-rate values 0.1 ... 2.0 ml/min. Eluent THF. Column: L = 0.20 m, 3 mm I.D., slurry-packed with silica H 2 (exclusion limit >1,000,000 g/mol). (Data from Ref. 10).

Fig. 5 similarly presents results published by Kirkland in 1976 (11). Fig. 6 gives a corresponding view of data from Cooper et al. (12) which were used by this team again in 1983 (13). The value for a low-molecular probe was not given by the authors. The point indicated at log M = 2 has been estimated from the fact that a WATERS Styragel(R) column 10^5 Å was used which, according to the supplier's warranty, has at least 2100 plates per metre.

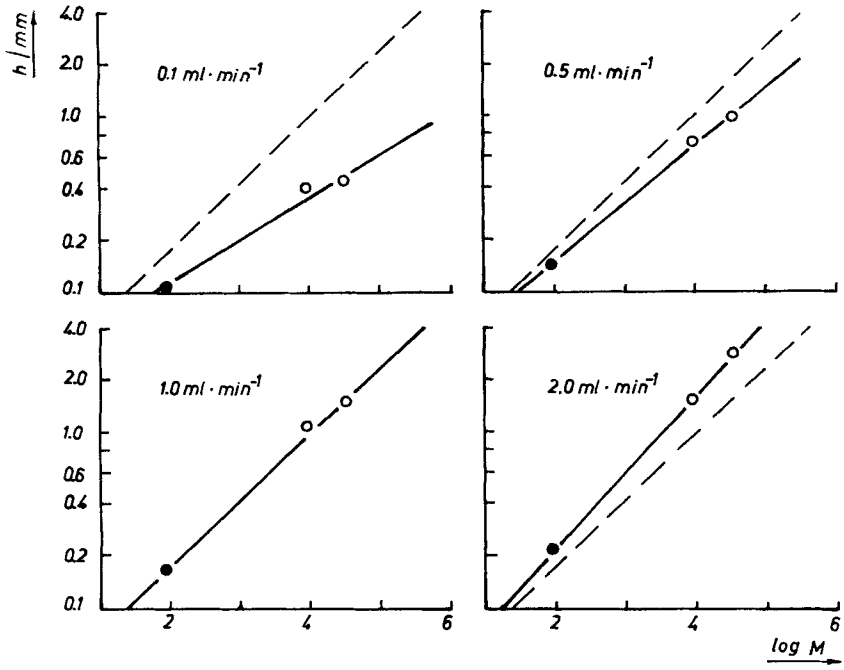

FIGURE 3

Same as Fig. 2, but column packed with silica H 4 (exclusion limit 500,000 g/mol). (Data from Ref. 10).

All the examples presented in Figs. 2 - 6 approximately support a linear relationship as given by Eq. (10). This linear dependence of log h from log M also includes the plate-height value of a low-molecular probe, which is easily measured. It is given as an additional bit of information in most papers. From this plain value and the knowledge of the slope B, the plate height valid for high-molecular samples can be estimated.

It has already been pointed out that there is not a general value of the quantity B. Some of the results presented in Ref.

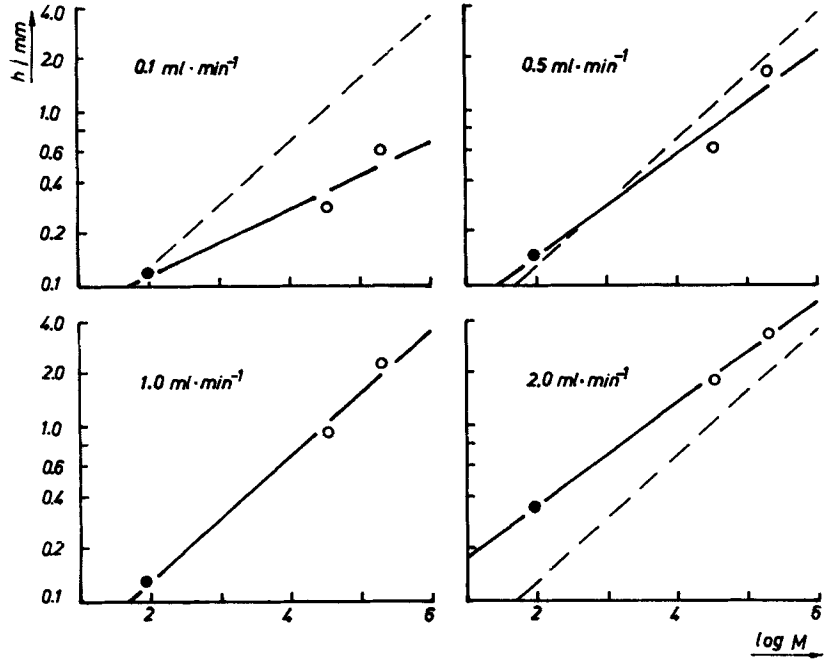

FIGURE 4

Same as Fig. 2, but column packed with silica H 6 (exclusion limit 100,000 g/mol). (Data from Ref. 10).

(2) yielded B = 0.3, but three of the six sets of data investigated led to a smaller value[+].

The B data compiled in Tab. II of this paper obviously show the influence of flow rate. Fig. 7 is a synoptic representation of data measured at different flow rates. In the range of a linear velocity u = 0.05 - 10 mm/s the data given

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[+] Equation (16-34) in Ref. (2) should read:

$$h_M = h_{Bzn}(M/M_{Bzn})^{0.3}$$

Unfortunately, the M_{Bzn} was omitted.

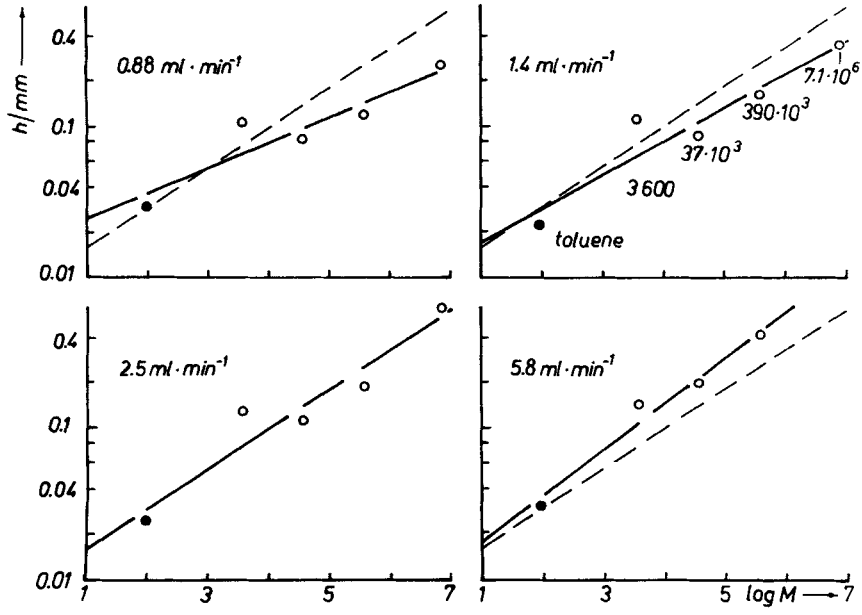

FIGURE 5

Plots of log h vs. log M for polystyrene standards (o) and toluene (•) at flow-rate values 0.88 ... 5.8 ml/min. Eluent THF. Column: L = 0.60 m (concatenation of 2 x 0.15 and 3 x 0.10 m tubes), 7.8 mm I.D., individually packed with 5 species of silanized silica microspheres. (Data from Ref. 11).

by Cooper et al. (12), by Kirkland (11), and by Dawkins et al. (10) yield an almost linear decrease of (d log h / d log M) with log u. The slope of this decrease is about 0.13 and indicated by the thick line in Fig. 7.

Fig. 7 also shows data from Chuang et al. (13) who have recently measured SEC efficiency at very small flow rate. They used two polymer samples with molar mass values within the limits of the separation range of the column. Results for low-molecular probes have not been given. In view of this restric-

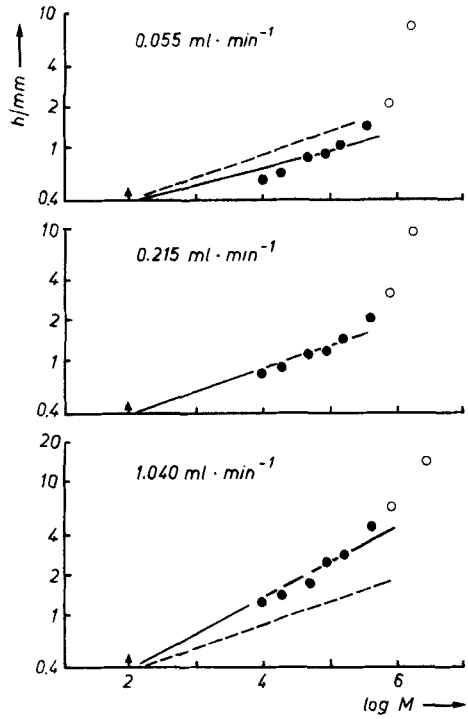

FIGURE 6

Plots of log h vs. log M for polystyrene standards (o, •) at flow-rate values 0.055 ... 1.040 ml/min. Eluent THF. Column: L = 1.22 m, packed with polystyrene gel of nominal porosity of 100,000 Å. (Data from Ref. 12). (The value indicated at M = 100 (▲) is estimated from supplier's column warranty. In calculating the position of the straight line, the open circles were not taken into account.)

tion, the data can only provide approximate information. Nevertheless, they are included in Fig. 7 in order to stress the fact that the thick line must not be extrapolated beyond the range of experimental evidence. Within this range, the data measured by Chuang et al. also support the location of this line.

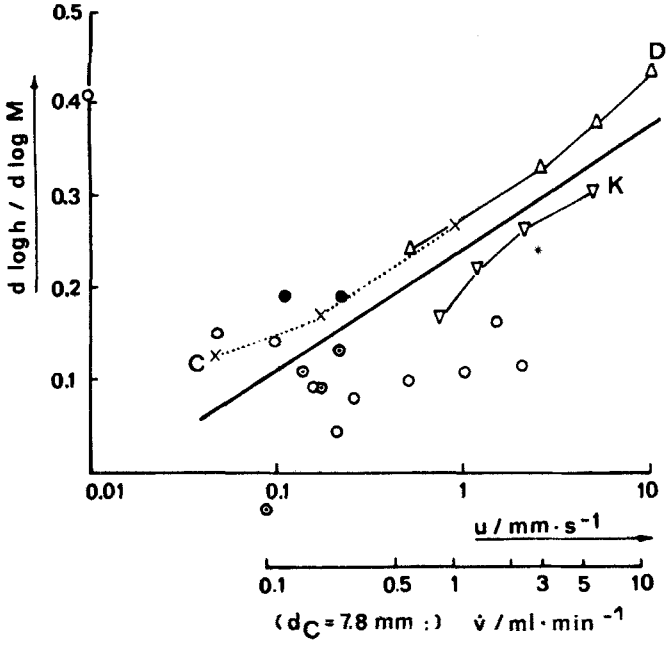

FIGURE 7

Synoptic representation of the flow-rate dependence of dlog h / dlog M. The thick line corresponds to

$$\text{dlog h /dlog M (= B)} = 0.239 + 0.130 \log u$$

and gives a good approximation of experimental data.

* :slope factor from Fig. 1 (PS in THF, silica)
△ —— △ :data from Ref.(10), (PS in THF, silica H4)
▽ —— ▽ :data from Ref.(11), (PS in THF, silica)
x.....x :data from Ref.(12), (PS in THF, Styragel(R) 10^5 Å)
o, ⊙, ● :data from Ref.(13), (PS in trichloromethane, Bio-Glass packings, three different columns)

The presentation of log h vs. log M used here and in Ref. (2) is by no means the only effort to correlate peak broadening and molar mass or SEC elution volume. Eq. (10) obviously works well in most cases, but one should be aware of the fact that the pore-size distribution of the packing mate-

rial might influence the applicability of this equation. We have some experience of this kind with CPG packings.

Bly plotted plate number N as $\sqrt{N}$ vs. elution volume v and found a linear correlation in the high molecular range (14) but the plate number determined with acetone was far aside. Cooper et al. plotted N vs. log M and found correspondence in the high molecular range (12). (Low-molecular values were not given.) Kirkland presented a straight-line correlation between ó and log M which met the value obtained with toluene but was rather badly obeyed by polymers of intermediate molecular weight. This mode of plotting has repeatedly been employed. McCrackin and Wagner (15) found good correlation in the range of 9,000 - 300,000 g/mol. The value for a low-molecular probe was not given, but the extrapolation of the straight line towards M = 100 g/mol would lead to a negative σ which has no physical meaning.

Elution volume and standard deviation are dependent on column diameter and length. Plotting of h vs. M overcomes the shortcomings of other evaluation procedures and enables columns of different size to be compared.

The relationship given by Eq. (10) is in accord with conclusions from general knowledge about polymer solutions and liquid chromatography. The plate height depends on the coefficient of diffusion by:

$$h/d_p = \text{const}(u\, d_p / D')^n \qquad (11)$$

The expression given in brackets on the right-hand side is the reduced velocity ν. For $\nu > 10$, the exponent n in Eq. (11) approximately becomes invariable, $n = 0.4$.

The coefficient of diffusion D' is related to the molar volume V of solute by:

$$D' = 0.00014 / (V^{0.6} \eta') \qquad (12)$$

The viscosity of solvent is indicated by η'.

The combination of Eqs. (11) and (12) yields (for a given solvent and a given velocity)

$$h = \text{const } V^{0.6n} \qquad (13)$$

or

$$\log h = \log \text{const} + 0.6n \log V \qquad (14)$$

If the volume of the solute is proportional to molar mass one obtains Eq. (10) with $B = 0.24$, if it is proportional to unperturbed coil volume one obtains Eq. (10) with $B = 0.36$. Of course, these values are rough approximations only. In pores, the coefficient of diffusion is strongly influenced by the ratio of molecular size to pore diameter.

CONCLUSIONS

The accuracy of plate-height values calculated through the approximation given by Eq. (10) is not less than the precision of most experimental data in the high-molecular range. The advantage of Eq. (10) is the inclusion of the reliable and easily measured value for a low-molecular sample as a base for the estimation of values in the high-molecular range.

On base of this perception, the following procedure for correcting SEC chromatograms can be recommended:

(a) Evaluation of the plate height with a low-molecular probe, e.g. with toluene.

(b) Estimation of another plate-height value using a polymer with a molar-mass value sufficiently smaller than the exclusion limit of the column. This condition is essential because the contribution h_{MT} in Eq. (6) diminishes with excluded samples. Consequently, plate heights measured with excluded samples are smaller than those with penetrating polymers (10, 16). In the vincinity of the exclusion limit, a plot of h (or σ^2) vs. v will show a maximum. Corresponding to this, a plot of $1/(\sigma\sqrt{2})$ will have a minimum. This was demonstrated by Tung and Runyon as early as in 1969 (17).

The distribution of the sample polymer must be either narrow or precisely known. Under favourable circumstances, the contribution of sample heterogeneity can be calculated via Eqs. (5, 6, and 8).

Repetition of this step with another suitable polymer would provide information whether the system really follows the dependence indicated by Eq. (10).

(c) Estimation of the constants A and B in Eq. (10) with the help of the values measured in steps (a) and (b).

(d) Calculation of the M value corresponding to a certain value of elution volume v in the uncorrected chromatogram.

(e) Estimation of the plate-height value at this molar mass via Eq. (10) and calculation of σ^2 or the spreading factor $1/(2\sigma^2)$ via Eq.(5).

(f) Performing the correction of the chromatogram with the help of a suitable algorithm.

(g) Repetition from (d) to (g) for the next value of v.

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