Elution and molecular weight parameters in exclusion chromatography

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The mode of the chromatogram is inadequate for characterizing experimental elution curves, which are generally dissymmetrical. It is also dependent on axial diffusion. When the molecular weight distribution is defined either by the generalized exponential function or by the log-normal function, simulation of elution at finite resolution shows that the calibration curve at infinite resolution, independent of the flow rate of the carrier fluid, is approximated validly by correlating the first moment of the chromatogram (MEV) with the geometrical compound average $(\bar{M}_n\bar{M}_w)^{1/2} = M_0$. The concentration effect seems to be controlled by a double extrapolation procedure defined by (1) treatment of the chromatograms using the calibration curve at zero concentration ($\ln M_0$ *versus* $\lim_{c\to o}$ MEV), and (2) linear extrapolation to infinite dilution of the calculated molecular weight averages.

INTRODUCTION

Exclusion chromatography is beyond doubt the most widespread technique for the analysis of molecular weight distribution. Its importance is demonstrated by the abundant literature which covers fundamental aspects of the technique and many of its practical applications[1].

Refinements are needed if more and more complex molecular structures are to be characterized by this method. However, one important problem is that exclusion chromatography is a *relative* method and relies on the validity of a calibration. Our knowledge of the molecular weight characteristics of the standard polymers used for this purpose, with few exceptions[2], is rather limited, and the lack of precision of the molecular weight averages given by manufacturers often exceeds 15%. This is a serious limitation of the method, at least for quantitative analysis. This factor is supposed to be lessened by statistical analysis of the calibration curve (smoothing)[3], based on mutual cancellation of presumably random uncertainties, provided that a broad range of 'standards' is used for establishing the reference function.

We are forced to accept an empirical, poorly defined terminology, despite the possibilities for more rigorous treatment of data by microprocessors. It is difficult to select from the literature a means to obtain quantitatively convincing results, either by impartial control or by objective neutralization of factors which confuse the separation process. In addition, several approximations, although heuristically useful, tend to propagate an ideal view which is no longer valid, prone to induce over-confidence in the potential of the method[4].

We will return to this point; but our main concern will be the calibration conditions of the instrument, which link molecular weight and elution parameters. These conditions have been discussed, mainly during the 1960s, on the basis of a crude model[5]. Obviously, they are related to the separation mechanism of exclusion chromatography, with necessary reference to the molecular weight characteristics of synthetic polymers.

Heuristic hypotheses and experimental reality

Any theoretical analysis of exclusion chromatography is usually based on the hypothesis of a *linear* calibration curve, i.e. the logarithmic version of the relation:

$$M = D_1 \times \exp(D_2 \times V) \qquad (1)$$

where M is the molecular weight, V is the elution volume and D_1 and D_2 are constants. Only the discontinuity of the slope at the two extremes of the calibration curve is eventually taken into consideration[6,7]. Yet despite appearances (see *Figure 2*), the reference function is essentially non-linear. *Figure 1* demonstrates this point for the experimental set-up described further on: the variation of the slope D_2 is apparent, and can be significant even in the restricted elution range of the standard polymers. The variance of the corresponding chromatograms is a function of the average molecular weight and ranges from 2 to 15 counts[2]. Perfect linearity of the calibration curve is one example of excessive idealization.

A second example concerns the elution curve more directly: the use of the term 'Gaussian' to qualify a chromatogram[8,9]. This adjective implies more than simply the notion of symmetry.

Gaussian analysis is inappropriate in practice, as shown by the positive (and significant) experimental values for skew ($\gamma_1 = \mu_3 \times \mu_2^{-3/2}$)* and excess ($\gamma_2 = \mu_4 \times \mu_2^{-2} - 3$) coefficients[10] which characterize the chromatograms of the standard polymers[3]. Abnormalities have also been observed in elution curves obtained under conditions of reversed flow[11]. The Gaussian function is not a general solution in chromatography[8,12] and actually constitutes an approximation of the Gamma function, which is intrinsically

* μ_i is the ith moment around the mean elution volume

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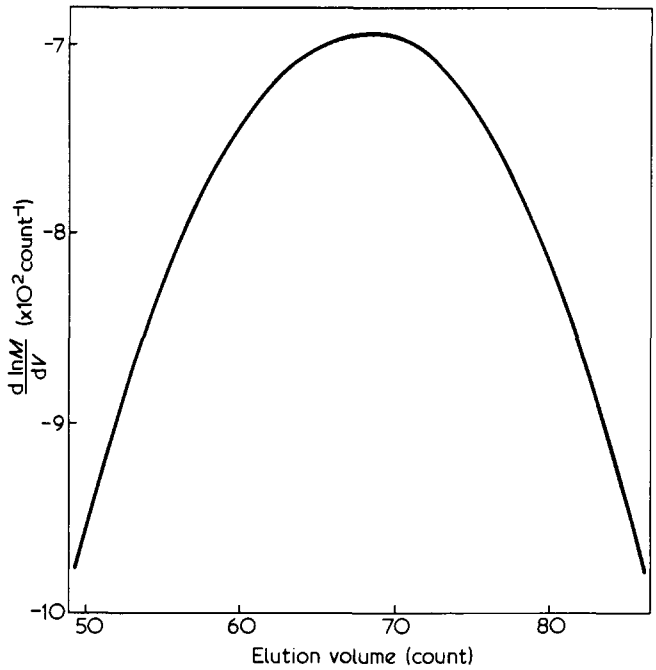

Figure 1 First derivative of the experimental exclusion calibration function which relates ln*M* to the mean elution volume at vanishing concentration of the injected solution

skewed to the right ($\gamma_1 > 0$) and leptokurtic ($\gamma_2 > 0$)[10].

Several works now contribute to a more realistic view of chromatography in general[6]. One essential element of this view concerns the consequences of the poor definition of the base line, due to uncertain limits of integration and to the signal:noise ratio[13]. The limitations of the method are becoming defined more clearly, particularly those limitations related to the use of moments for characterizing a chromatogram[14]. Thus, despite optimistic conclusions[4], a meaningful determination of higher order average molecular weights seems questionable (M_z), if not illusory ($M_{z+1}, \ldots$).

A thorough examination of the exclusion chromatography literature shows, with few exceptions[3,15,16], that reference is always made to the *mode* of the chromatogram as a parameter of central measure; yet, in practice, the peak elution volume (PEV) is clearly inadequate for characterizing even slightly asymmetrical elution curves. Its constant use is an unjustified generalization from a very specific study. Berger and Shultz[5] alluded to the association of molecular weight and elution parameters, and based their discussion mainly on the mode of the chromatogram and on the distance between inflexion points: with a linear calibration curve and *in the absence of axial dispersion,* the mode corresponds exactly to the weight-average molecular weight $\bar{M}_w$ if the molecular weight distribution is a generalized exponential function. It is related to the geometric compound average $(\bar{M}_n\bar{M}_w)^{1/2}$ if the molecular weight distribution is log-normal. In the latter case, the chromatogram is Gaussian *sensu stricto.*

A priori, the true molecular weight distribution of the standard polymers is unknown; but the two distributions considered here are much alike when polydispersity is low. 'Low', however, in exclusion chromatography, could be a polydispersity index $Q = (\bar{M}_w/\bar{M}_n) < 1.01$, because a higher value invalidates any attempt to determine the height of theoretical plate[17]. The usual approximation linking a Gaussian chromatogram to the elution of monodisperse species allows disregard of the asymmetry of a chromatogram which results, at infinite resolution, either from the exact analytical form of the molecular weight distribution or from the non-linearity of the calibration curve. It is therefore recommended[9,18], that the mode and the geometrical mean M_0 are correlated. This lends more weight to the log-normal distribution, although the generalized exponential distribution is much more representative in macromolecular chemistry[19].

Flow rate and concentration effects

Further analysis of the experimental conditions leads one to consider the influences of flow rate and the amount of injected polymer (concentration) on the elution volume (PEV). The effect of these factors is to shift the calibration curve in space (ln*M*-PEV), especially for molecular weights higher than 5×10^4. The resulting indetermination of the reference function in respect of the flow conditions of the carrier fluid is ignored generally. The flow is set once and for all at an optimal value which corresponds to a minimum height of theoretical plate, insensitive to small variations of flow around the chosen value[20].

The concentration effect, however, is not controlled by an analogous procedure – choosing a constant value of concentration of the injected solution. This effect actually depends on both the molecular weight (or elution volume) and the molecular weight distribution of the sample under study, and consequently on the *relative abundance* of the different species. Neither the concentration entering the chromatograph (that of the injected solution) nor the concentration which emerges (given by the elution curve), are appropriate measures of the progressive dilution which occurs in the columns during elution. Dilution is a function of the porosimetric arrangement of the columns[21]. Corrections made on the basis of the chromatograms seem to improve the results[22], although the most objective approach, in our opinion, is to extrapolate the parameters obtained at finite concentration to infinite dilution of the injected solution[23,24].

Dispersion effects

Generally, an experiment gives quite satisfying semi-quantitative, but not strictly quantitative, conclusions. This is seldom accredited to the indetermination of the calibration curve. On the contrary, research was oriented towards the analysis of the consequences of dispersion effects[25], which deform the chromatogram at infinite resolution $W(V)$. This can be symbolized by the convolution equation:

$$F(V) = \int_{-\infty}^{+\infty} G(V - y) \times W(y) \times dy \qquad (2)$$

or, in matrical notation, replacing the integral by a sum:

$$f = G\omega \qquad (3)$$

where ω is the unknown function, f the observed function and G is the dispersion matrix, considered to be symmetrical at first approximation in a limited range of elution volume. The deconvolution:

$$\omega = G^{-1}f \qquad (4)$$

is operational after elaborate procedures designed to avoid oscillations of the function ω. The ambiguity of this solu-

Table 1 Mode (PEV), first moment (MEV) and corresponding molecular weight parameter for simulated exclusion chromatograms obtained from the combination of: (1) generalized exponential molecular weight distribution; (2) linear calibration curve (log M = 9 − 0.12 V); (3) Gaussian dispersion function of variance μ_{2D}

$\bar{M}_w/\bar{M}_n$	μ_{2D} count^2	PEV count →	M	$(\bar{M}_n\bar{M}_w)^{1/2}_{app}$	M	MEV ← count
1.06	0	35.006	63 000	61191	61122	35.109
	1	35.038	62436	61191	61122	35.109
	2	35.053	62178	61191	61122	35.109
	3	35.058	62093	61191	61122	35.109
1.10	0	35.006	63 000	60067	60167	35.172
	1	35.040	62402	60067	60167	35.172
	2	35.060	62058	60067	60167	35.172
	3	35.070	61887	60067	60167	35.172
1.15	0	35.006	63 000	58 747	58 949	35.246
	1	35.043	62351	58 747	58 949	35.246
	2	35.065	61973	58 748	58 949	35.246
	3	35.084	61648	58 748	58 949	35.246
1.20	0	35.006	63 000	57 510	57 836	35.315
	1	35.041	62385	57 512	57 836	35.315
	2	35.068	61921	57 516	57 836	35.315
	3	35.088	61580	57 525	57 836	35.315

tion is obviously related to the uncertainty attached to the kernal G, of which the inverse amplifies the experimental errors tied to f, and makes it necessary to smoothe the data[25]. One aspect, however, has never been discussed: how does concentration influence the deconvoluted solution?

We will attempt now to find the best possible approximation of the calibration curve at infinite resolution which will be independent of both dispersion and concentration effects. The latter will be eliminated by appropriate extrapolations to infinite dilution. The calibration, however, is inevitably carried out at *finite* resolution. Necessarily, it will be based on a judicious correlation between a molecular weight 'average' of the standard and the only parameter of central measure which truly represents the elution curve, the first moment of the chromatogram viz. the mean elution volume (MEV).

Molecular weight related to the MEV

Let us first reconsider the conclusions of the model of Berger and Shultz (linear calibration curve) with regard to dispersion effects. The convolution of a chromatogram at infinite resolution and of a symmetrical dispersion function (with a zero average and a constant variance in the range defined by the limits of elution of the sample under study) does not affect the value of the first moment of the elution curve. In the case of a log-normal molecular weight distribution, the central parameters remain identical (mode = median = mean) and correspond exactly with the geometrical average M_0. In the case of the generalized exponential function, however, it is difficult to relate analytically the first moment of the elution curve to a molecular weight parameter. The problem is avoided by numerical simulation.

Table 1 contains the results of a simulation (on the basis of a linear calibration curve) of the elution of four samples, each of which presents a Schulz molecular weight distribution. These products have identical weight-average molecular weight, but are distinguished by a polydispersity index $Q = (\bar{M}_w/\bar{M}_n)$ ranging from 1.06 to 1.20, in the interval which covers most values found in the standard polymers. The elution is or is not perturbed by dispersion which obeys a Gaussian function with a variance μ_{2D}. The order of magnitude of this spreading is typical of experimental values mentioned in the literature[11,27,28]. The M_0 averages are calculated using the true calibration curve for each chromatogram, spread or not. In the absence of dispersion (μ_{2D} = 0), one should notice that the calibration curve is validly approximated not only by associating the mode and $\bar{M}_w$ (exact correspondence), but also by correlating the MEV and the geometrical compound average M_0. In all cases, the difference with respect to the theoretical function is very small. It is also important to see the strong dependence of the mode on dispersion. Finally, commutativity is an essential property of convolution[29]: the test is therefore also representative of the spreading of a Gaussian chromatogram (log-normal molecular weight distribution) by a moderately asymmetrical dispersion function.

Is there some experimental evidence in favour of these conclusions?

EXPERIMENTAL

The chromatograph Model GPC 200 (Waters Associates) was operated at room temperature and was equipped with five four-foot columns of Styragel (Water Associates) placed in order of decreasing porosity (10^5, 10^5, 3×10^4, 10^4, 10^3). The solvent flow (toluene) was 1.0 ml min^{-1} and the thermostat-controlled syphon (23°C) had a capacity of 2.42 ml. The calibration was performed using 19 standard polystyrenes, the molecular weight parameters of which are represented in *Table 2*. They were either synthesized in this laboratory (L) or purchased from Waters Associates (W) or Pressure Chemical Company (PC). Each polymer was injected between four and eight times, at concentrations ranging from 0.02% to 0.2% (wt/wt).

The injection time was 120 s. An A/D converter and a serializer coupled to a chronoscope unit (Viscomatic, Fica) furnished the numerical data. These were treated using an HP9830 calculator (Hewlett-Packard). The molecular weight averages were determined by a calculation programme analogous to the one described by Pickett[30]. Prior application of the Fourier transform to the chromatograms enables smoothing of the data and simultaneous determination of the derivative and of the mode of the elution curve. The value obtained for the latter parameter is reproducible with a precision of 0.01 count (±0.02 ml) for successive injections of the same solution.

DISCUSSION

Table 3 lists, for each standard polymer, the values of the mode and of the mean elution volume, linearly extrapolated to infinite dilution of the injected solution.

Analysis of the correlation coefficients and of the slopes of the elution volume *versus* concentration diagrams confirms, on the whole, the excellent definition of the mode; the latter, incidentally, is notably more affected by the concentration than the MEV and both dependences increase with increasing molecular weight. A systematic, significant difference is obtained between the values of these two parameters throughout the elution range of the instrument, including those of low molecular weight samples which give the least dissymmetrical chromatograms.

Table 2 Molecular weight characteristics of the polystyrene samples

Symbol	Reference	Manufacturer's data ($M \times 10^{-3}$) $\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_v$	$\bar{M}_w$	Literature[a]
PS2200	W61970 PC14b	⩽1.30	1990	2340	2050 ± 4% 2610[f]	2400[32], 2840[33]
PS830	W25167		773		867	910[34]; Q = 1.24[34] Q = 1.006[35] → 1.003 [6]
PS655	PC13a	⩽1.10	640 ± 5%	678 ± 3%	670 ± 4%	702[33]; 598$_v$ → 619$_v$[37]
PS451	W25166		404		498	
PS373	PC3b	⩽1.10	355	388	392	377$_v$, 379$_v$[37]; 453 ± 14[38]; 392[33]; 462 ± 7[38]
PS300	L18F		300			
PS196	W41984 PCIc	⩽1.06	193 ± 4%	184 ± 4% 186 ± 4%	200 ± 5%	194[32]; 184[33]; 177$_v$ → 195$_v$[37]
PS170	LS50		170.8		172	
PS142	L22L		139.8		146	
PS111	W41995 PC4b	⩽1.06	111	111	111	107.8$_n$[39]; 123.6[39]; 116[33]; 113$_v$ → 117$_v$[37]
PS97	L23L		97			
PS67	L24L		66.6			
PS55	L20L		55.4		59.3	
PS34.5	W25170 PC7b	⩽1.06	36	38	33	33.7$_n$, 36.6[39]; 35.9[33] 37.8$_v$ → 37.9$_v$[37]
PS20.5	W25168 PC2b	⩽1.06	20.2 ± 0.6[c]	20.4 ± 0.6	20.8 ± 0.8	
PS9 8	W25171 PC8b		9.60		10	Q = 1.12[40]
PS3.55	W25169	⩽1.10	3.100		4	
PS2.025	W26971	⩽1.10	1.95		2.1	
PS0.6	PC16a	⩽1.10	0.524 ± 7% 0.585 ± 7% 0.55 ± 10%[k] 0.581 ± 5%[c]	0.578 ± 7%		

[f], data from fractionation; [c], cryoscopy; [k], kinetic; [a], weight-average molecular weight except: $n = \bar{M}_n$; $v = \bar{M}_v$; $Q = \bar{M}_w/\bar{M}_n$

Table 3 Linear concentration dependences of the mode (PEV) and of the first moment (MEV) of polystyrene standards chromatograms

	Extrapolation ($c \rightarrow o$) (count)		Slope (count/%weight)		Correlation	
Sample	PEV	MEV	PEV	MEV	PEV	MEV
PS2200	47.68	50.51	7.30	4.88	0.995	0.932
PS830	54.18	55.04	4.50	3.17	0.999	0.964
PS655	55.47	56.63	3.14	2.81	0.969	0.985
PS451	57.11	57.99	1.80	1.76	0.963	0.618
PS370	58.92	59.52	2.32	1.55	0.965	0.864
PS300	60.36	61.02	2.19	1.39	0.986	0.948
PS196	63.66	64.04	1.83	0.73	0.999	0.769
PS170	64.28	64.44	1.41	1.60	0.924	0.990
PS142	65.45	65.64	1.74	0.96	0.983	0.891
PS111	66.58	66.90	1.34	0.50	0.996	0.693
PS97	67.70	68.05	1.23	0.62	0.970	0.885
PS67	70.28	70.33	1.19	0.56	0.997	0.984
PS55	71.72	71.79[a]	0.90	–	0.931	–
PS34.5	73.94	74.12[a]	0.74	–	0.977	–
PS20.5	77.54	77.70[a]	0.37	–	0.969	–
PS9.8	81.25	81.29[a]	0.40	–	0.783	–
PS3.55	86.09	86.26[a]	0.25	–	0.850	–
PS2.1	88.25	88.49[a]	0.30	–	0.814	–
PS0.6	92.60[a]	92.60[a]	–	–	–	–

[a] mean value; non-significant slope

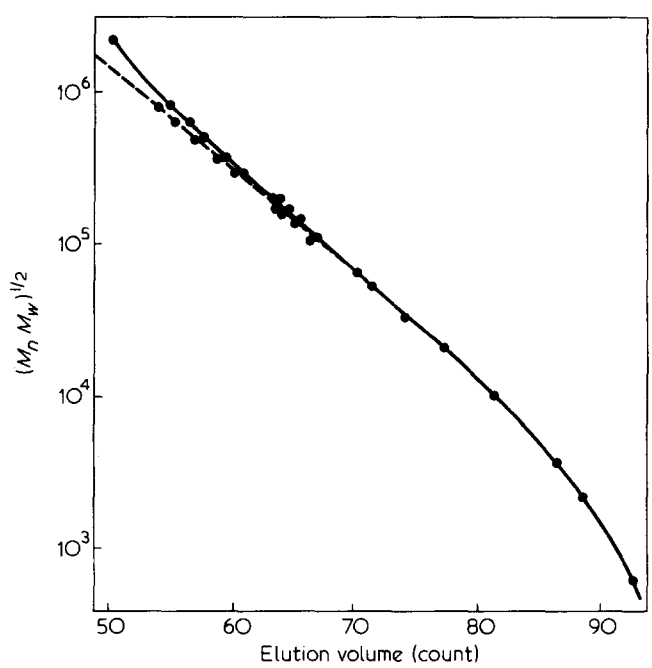

Figure 2 Conventional (– – – –, PEV) and first moment (——, MEV) exclusion calibration curves at vanishing concentration of the injected solution

Our results are comparable to those obtained by Provder and Rosen[3] under similar experimental conditions, if care is taken to normalize the unitary elution volumes (or counts) in view of the different capacities of the syphons used in the two experimental set-ups.

Association of the geometrical average M_0 with the elution parameters PEV and MEV leads us to define two distinct calibration curves at zero concentration. These are represented in *Figure 2*. Analysis of the calibration chromatograms using these functions yields two independent series of molecular weight averages, each of which is a function of the concentration of the injected solution. The values of these averages, linearly extrapolated to infinite dilution, are in *Table 4*. The two calibration curves give different results.

Table 4 Molecular weight averages of the polystyrene standards computed by the double extrapolation procedure

	($M \times 10^{-3}$)					
	PEV			MEV		
Sample	$\bar{M}_n$	$\bar{M}_w$	M_0	$\bar{M}_n$	$\bar{M}_w$	M_0
PS830	(640)	(730)	(684)	(744)	(930)	(832)
PS655	475	570	520	545	700	618
PS451	409	459	433	456	536	494
PS370	329	359	344	350	400	374
PS300	258	292	274	272	318	294
PS196	170	183	176	175	188	181
PS170	160	172	166	163	177	170
PS142	134	143	139	136	146	141
PS111	111	118	114	112	120	116
PS97	92.9	101	97.0	93.5	102	97.8
PS67	64.4	70.3	67.3	65.0	71.0	67.8
PS55	51.3	56.0	53.6	52.0	56.0	54.2
PS34.5	34.1	36.4	35.2	34.9	37.2	36.0
PS20.5	18.4	19.8	19.1	19.2	20.5	19.8
PS9.8	8.89	9.91	9.39	9.42	10.5	9.95
PS3.55	3.01	3.37	3.18	3.18	3.58	3.37
PS2.025	1.69	1.97	1.82	1.77	2.08	2.11
PS0.6	0.567	0.674	0.614	0.561	0.670	0.613

Which values are more probable?

Comparison with the 'absolute' data of *Table 2* reveals differences between the two groups of calculated average values (PEV and MEV bases) and the values given by the manufacturers. The uncertainty attached to the latter can be estimated, for only a few samples, by taking into consideration the last column of *Table 2*, which lists complementary data obtained from the literature. On the other hand, the calculated molecular weight characteristics are independent of the concentration, but might not ignore dispersion effects. Assuming the dispersion to be symmetrical, distinct values can be predicted for the calculated averages and for the absolute (•) averages. The former 'contain' the latter[28]:

$$\bar{M}_n < \bar{M}_n^{\bullet} < \bar{M}_w^{\bullet} < \bar{M}_w$$

The symmetry of the observed differences is emphasized by examination of *Table 1*, which shows that dispersion has little effect on the apparent geometrical average M_0 resulting from the analysis of the simulated spread chromatograms.

Let us examine the content of *Table 4*, taking into consideration both the absolute values and the effects of dispersion. It appears that the calibration curve at zero concentration based on the MEV gives, on the whole, more plausible values. The difference between the two groups of calculated values becomes extremely noticeable for molecular weights above 10^5. The averages corresponding with lower molecular weight samples are also more reliable (MEV basis), despite the obvious similarity (*Figure 2*) between the two reference functions at high elution volumes.

CONCLUSION

The experimental chromatograms show the dissymmetry which results, among others, from the independent or combined effects of three factors: the exact analytical form of the molecular weight distribution; dispersion; and the nonlinearity of the calibration curve. The mode of the elution curve is without fundamental meaning with respect to elution, and depends on kinetic transfer[8,15,31] and the phenomena responsible for the spreading of the chromatogram. The first moment, on the other hand, is strictly dependent on the equilibrium properties. It is insensitive to the flow rate of the carrier fluid[15] and to axial diffusion, provided the variance of the dispersion function does not change significantly with the elution volume.

When the molecular weight distribution of the samples used is either log-normal or a generalized exponential function, the geometrical compound average M_0 can be correlated with the mean elution volume of the chromatogram at finite or infinite resolution.

Linear extrapolation of the mean elution volume to infinite dilution defines the calibration curve at zero concentration ($\ln M_0$ *versus* $\lim_{c \to 0}$ MEV), which constitutes a valid approximation of the ideal calibration curve at infinite resolution. After extrapolation and as predicted from analysis at infinite resolution, the average molecular weights resulting from use of the reference function to treat the chromatogram yield values which contain the absolute averages and within the limits of uncertainty attached to the latter if $M < 5 \times 10^5$.

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