Calibration of GPC with Polydisperse Standards

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Synopsis

An iteration method has been developed to prepare a calibration curve for gel permeation chromatography (GPC). It requires a number of samples of the same polymer which may have broad molecular weight distributions (MWD) of which two molecular weight averages must be known previously. The method has been applied to dextran standards with known $\overline{M}_w$ and $\overline{M}_n$. Modifications involving the use of branched polymers are discussed.

INTRODUCTION

Measurements with GPC usually start with a calibration in order to relate the molecular weight M of a sample with its elution volume v. Thus, a range of molecular weights can be covered by preparing the calibration curve which is conveniently defined by the function

$$g(v) = \ln M \tag{1}$$

Once the function $g(v)$ is known, molecular weight averages can be calculated from the chromatogram according to

$$\overline{M}_x = \left[\int_{v_0}^{v_e} h(v) \exp\{xg(v)\}\, dv \right]^{1/x} \tag{2}$$

where $h(v)$ is the normalized chromatogram; v_0 and v_e are the elution volumes respectively at the beginning and the end of the chromatogram; and x may assume the values 1, -1, and the Mark-Houwink exponent a, corresponding respectively to $\overline{M}_w$, $\overline{M}_n$, and $\overline{M}_v$, the weight-average, the number-average, and viscosity-average molecular weights. The tedious procedure of calibration starts from the availability of polymer standards having very narrow distributions. However, such specimens are scarce due to their laborious preparation. Hence, many attempts have been undertaken to circumvent the problem of narrow distribution in calibrating GPC. Some of them are based on known molecular weight distributions[1–3] whereas others employ a known set of molecular weight averages.[4–6] In this paper we present a rapid iteration method belonging to the latter category and following a principle used by Bombaugh.[6] However, in two essential aspects we have modified his procedure. First, we have as much as possible avoided to employ peak elution volumes because they give rise to problems, especially where skew distributions are involved. Secondly, we have replaced Bombaugh's two-step calculation by a real iteration process with valid stopping criteria.

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THEORETICAL

Consider a polymer sample with a broad MWD and suppose that $\overline{M}_w$ and $\overline{M}_n$ are given. Let us further assume that as a first approximation the calibration curve is linear in the elution range of the chromatogram. Then we may write

$$Av + B = \ln M \tag{3}$$

where A and B represent constants. Let us carry out the transformations

$$v' = v - v_0 \qquad \text{and} \qquad h'(v') = h(v) \tag{4}$$

and let us substitute them combined with eq. (3) into eq. (2) applied to the cases $x = 1$ and $x = -1$. Then we find

$$\overline{M}_w = \exp(Av_0 + B) \int_0^{v_e - v_0} h'(v') \exp(Av')\, dv' \tag{5}$$

$$(\overline{M}_n)^{-1} = \exp\{-(Av_0 + B)\} \int_0^{v_e - v_0} h'(v') \exp(-Av')\, dv' \tag{6}$$

Evidently, the constants A and B can be solved from the eqs. (5) and (6). An appropriate way of performing this is to solve A first by applying the recursion formula

$$A_{k+1} = A_k + \frac{1}{v_e - v_0} \ln \frac{\overline{M}_n}{\overline{M}_w} \cdot \int_0^{v_e - v_0} h'(v') \times \exp(A_k v')\, dv' \cdot \int_0^{v_e - v_0} h'(v') \exp(-A_k v')\, dv' \tag{7}$$

in which k runs from zero to the number of the iteration wherein $|A_{k+1} - A_k|$ turns out to be sufficiently small. Note that the product of integrals appearing in eq. (7) represents the ratio $\overline{M}_w/\overline{M}_n$ as calculated from the chromatogram. If the starting value A_0 is chosen negative, it can be shown that the process reflected by eq. (7) is convergent and leads to the determination of A. Hereafter, B can be calculated directly from eqs. (5) or (6). Finally, elution volumes corresponding to $\overline{M}_w$ and $\overline{M}_n$ can be determined by substitution of $\overline{M}_w$ and $\overline{M}_n$ into eq. (3) in which A and B are known constants.

Consider now a number of N polymer samples for which the chromatograms are eluted over different but, if possible, overlapping elution ranges. Let each sample again be characterized by values of $\overline{M}_w$ and $\overline{M}_n$ which may arise from a broad MWD. Then the same procedure as before can be applied to each separate sample. It yields a set of $2N$ elution volumes conjungated to $2N$ molecule weight averages used ($\ln M_j, v_j; j = 1,2 \cdots 2N$). The set of discrete points obtained so far reflects, though yet in the form of a table, the true calibration function. It can be approximated by a polynomial $g_0^n(v)$ of degree n such that a weighted sum of the square of the deviations is least.

Consider finally the function $g_0^n(v)$ as the first step (index zero) in an iteration process $g_k^n(v) k = 0,1,2 \cdots$ leading to the true calibration curve. An arbitrary loop of the iterations then looks as follows. Prepare by a polynomial least-squares fit the function $g_k^n(v)$ according to

$$g_k^n(v) = \sum_{i=0}^{n} (a_i)_k v^i \tag{8}$$

yielding values for the coefficients $(a_i)k$. Use these values for solving the elution volume v for each chromatogram from the equation

$$\sum_{i=0}^{n} (a_i)_k v^i = \ln \left[\int_{v_0}^{v_e} h(v) \exp \left\{ x \sum_{i=0}^{n} (a_i)_k v^i \right\} dv \right]^{1/x} \tag{9}$$

for $x = 1$ and $x = -1$. So for N chromatograms, $2N$ values of the elution volumes are obtained for which the corresponding molecular weights are given. Prepare from the tabulated set ($\ln M_j, v_j, j = 1,2 \cdots 2N$) the function $g^n_{k+1}(v)$ by the least-squares method according to

$$g^n_{k+1} = \sum_{i=0}^{n} (a_i)_{k+1} v^i \tag{10}$$

The process can be continued until $|(a_i)_{k+1} - (a_i)_k|$ turns out to be sufficiently small. Table I shows how the values of the coefficients $(a_i)_k$ converge to their final form. Once arrived at the true calibration curve, one can recalculate for each chromatogram molecular weight averages using eq. (2) for $x = 1$ and $x = -1$. These recalculated values of $\overline{M}_w$ and $\overline{M}_n$ ought to be consistent with the originally known values.

EXPERIMENTAL AND NUMERICAL OPERATIONS

Measurements were performed on a Waters Model 200 GPC equipped with a R.I detection and in which four columns had been installed with deactivated silica gel (Porasil, code AX, BX, CX, and DX Waters) with pore diameters in the range of 75–125 μ. The operational conditions were: solvents, water and DMSO; solute dextran standards (Pharmacia, for code see Table II); temperature of columns, 30°C; flow rate, 1 ml/min. Reverse flow experiments were performed with the system dextran–water. Hence, for this system the chromatograms were corrected for dispersion by a method presented earlier.[7]

The numerical calculations were performed by a computer (c.p.u. time 5 sec). For the polynomial fits, standard least-squares procedures were used. Equation (9) was solved for v by the method of Newton-Raphson using a starting value of v originating from the forgoing iteration step. Systematically, the degree of the polynomial was chosen equal to 3 ($n = 3$).

RESULTS AND DISCUSSION

Evaluation of Data

Calibration curves corresponding to three different cases are shown in Figure 1. Though the curves coincide partly, their extreme parts clearly diverge. It can be ascribed mainly to the effect of peak broadening or dispersion which, apparently is more pronounced for distributions in the low molecular weight range. In order to test the method, we have recalculated the molecular weight averages $\overline{M}_w$ and $\overline{M}_n$ and the ratio R ($=\overline{M}_w/\overline{M}_n$) using the three calibration curves.

The data have been compiled in Table II. Moreover, osmotic data[3] have been added in the last column for comparison. Within the limits of the accuracy of the method, agreement between the starting and recalculated molecular weights

TABLE I

Coefficients of the Third-Degree Polynomial of Calibration in the Successive Steps of Iteration[a]

k	$(a_0)_k$	$(a_1)_k$	$(a_2)_k$	$(a_3)_k$
0	54.22048116	−0.82824580	0.00555000	−0.0000130500
1	65.54156288	−1.10479740	0.00768761	−0.0000183535
2	72.22523196	−1.25986898	0.00885023	−0.0000211809
3	74.78620820	−1.32114418	0.00931920	−0.0000223376
4	76.41629826	−1.35910142	0.00960477	−0.0000230346
5	77.15008059	−1.37650317	0.00973729	−0.0000233606
6	77.59160312	−1.38682005	0.00981510	−0.0000235508
7	77.80810845	−1.39193160	0.00985392	−0.0000236461
8	77.93262153	−1.39484783	0.00987595	−0.0000237000
9	77.99647533	−1.39635196	0.00988736	−0.0000237280
10	78.03220476	−1.39718993	0.00989369	−0.0000237436
11	78.05096250	−1.38763126	0.00989704	−0.0000237518
12	78.06128963	−1.39787366	0.00989887	−0.0000237562
13	78.06678146	−1.39800279	0.00989985	−0.0000237598
14	78.06977837	−1.33807317	0.00990038	−0.0000237599
15	78.07138307	−1.39811088	0.00990067	−0.0000237606
16	78.07225448	−1.39813135	0.00990082	−0.0000237610
17	78.07272282	−1.39814236	0.00990091	−0.0000237612
18	78.07297646	−1.39814832	0.00990095	−0.0000237613
19	78.07311305	−1.39815153	0.00990097	−0.0000237614
20	78.07318691	−1.39815326	0.00990099	−0.0000237614
21	78.07322674	−1.39815420	0.00990100	−0.0000237615
22	78.07324825	−1.39815470	0.00390100	−0.0000237615
23	78.07325986	−1.39815497	0.00990100	−0.0000237615
24	78.07326613	−1.39815512	0.00990100	−0.0000237615
25	78.07326951	−1.39815520	0.00990100	−0.0000237615
26	78.07327134	−1.39815524	0.00990100	−0.0000237615
27	78.07327233	−1.39815527	0.00990100	−0.0000237615
28	78.07327286	−1.39815528	0.00990100	−0.0000237615
29	78.07327315	−1.39815529	0.00990100	−0.0000237615

30	78.07327330	−1.39815529	0.00990100	−0.0000237615
31	78.07327339	−1.39815529	0.00990100	−0.0000237615
32	78.07327343	−1.39815529	0.00990100	−0.0000237615
33	78.07327346	−1.39815529	0.00990100	−0.0000237615
34	78.07327347	−1.39815529	0.00990100	−0.0000237615
35	78.07327348	−1.39815529	0.00990100	−0.0000237615

[a] Arbitrary example; c.p.u. time, 5 sec.

TABLE II

Molecular Weight Averages. The Values of the Standards are Compared to the Recalculated Values Obtained from the Chromatograms

Code	Values supplied by manufacturer (Pharmacia) M_w/M_n	R	GPC, Solvent water: Corrected for dispersion M_w/M_n	R	GPC, solvent DMSO: Uncorrected for dispersion M_w/M_n	R	M_w/M_n	R	Osmose M_n
T-500	516,000/212,000	2.43	502,000/197,000	2.55	483,000/197,000	2.45	483,000/170,000	2.84	205,000
T-250	240,000/121,000	1.98	262,000/117,000	2.24	270,000/116,000	2.33	290,000/102,000	2.84	115,000
T-150	154,000/86,000	1.79	160,000/90,000	1.78	168,000/87,000	1.93	168,000/83,500	2.01	98,000
T-70	69,500/39,500	1.76	63,000/43,400	1.45	63,000/40,600	1.55	87,000/31,300	2.77	45,000
T-40	42,400/28,400	1.49	40,500/29,400	1.38	41,500/27,800	1.49	45,500/27,700	1.64	29,000
T-20	22,300/15,000	1.49	21,900/14,900	1.47	24,500/14,100	1.74	26,300/15,200	1.79	21,000
T-10	9,300/5,700	1.63	9,300/5,500	1.69	11,200/4,000	2.8			

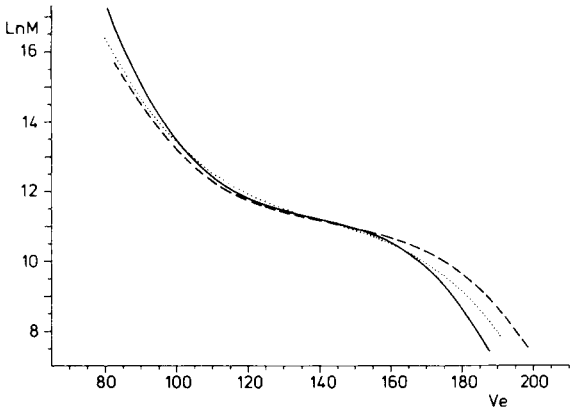

Fig. 1. Calibration of GPC with dextrans in different situations. Elution volume unit (count) is about 1 ml: (· · ·) uncorrected and (—) corrected for dispersion in waters; (- - -) uncorrected and in DMSO.

has been found. However, it may be noticed that the ratios R are significantly higher in the cases where dispersion has been neglected. This point emerges also strongly from Table III, in which the average and root-mean-square deviations of R with respect to the original values are shown. Relative low values found suggest a good reliability for the ratios R resulting from the corrected chromatograms.

In Figures 2 and 3, integral distributions calculated by the procedure above and obtained from the data sheets of Pharmacia are compared. Remaining discrepancies can be partly explained by the influence of dispersion. Finally, the data obtained from the uncorrected chromatograms (dextran/water) confirm results presented[3] but have been found by another route.

The Influence of Branching

The adoption of the function $g(v)$ in eq. (1) was based on the idea that the molecular mass is related to the elution volume in a unique way. This is always true if linear molecules are concerned. The presence of branched material, however, may give rise to disagreement with the concept above. Strazielle and Benoit[8] have given an illustrative example with a mixture of linear and star-shaped polystyrenes. They have found that the points belonging to branched molecules systematically fall outside the calibration curve drawn through the points belonging to linear molecules. It means that at the same elution volume, molecules of different molecular mass appear, whereas the branched molecules

TABLE III
Average and Root-Mean-Square Deviations of R with Respect to the Original Values

	Water	DMSO	
	Corrected for dispersion	Uncorrected for dispersion	
$\overline{\Delta R}$	0	0.25	0.49
$(\overline{\Delta R^2})^{1/2}$	0.17	0.48	0.59

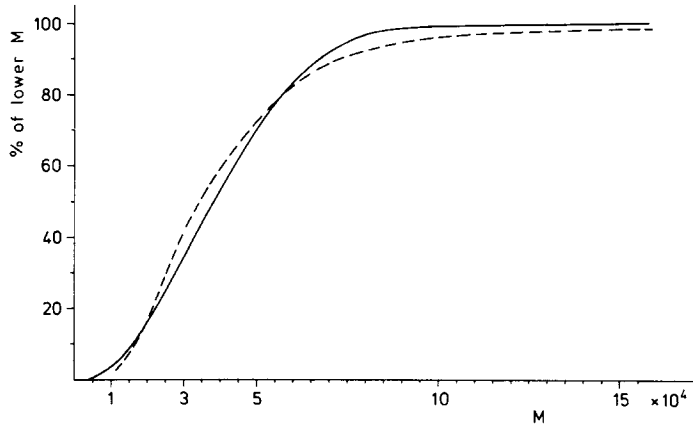

Fig. 2. Integral MWD of dextran T40, lot 9080. Integral distribution: (- - -) from data sheet Pharmacia (Sweden); (—) calculated.

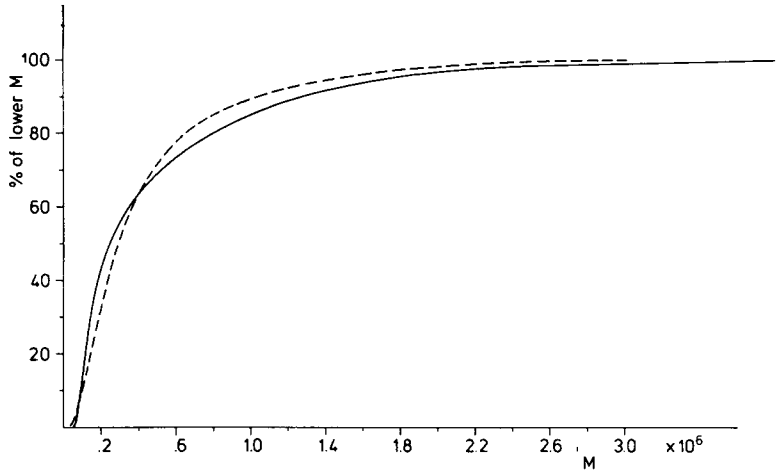

Fig. 3. Integral MWD of dextran T500, lot 3207. Integral distribution: (- - -) from data sheet Pharmacia (Sweden); (—) calculated.

always have the higher molecular mass. This difficulty has been overcome in a straightforward way by adopting instead of the molecular mass M the product of the molecular mass M and the intrinsic viscosity $[\eta]$ as describing the separation process.[8] Unique relationships between $M[\eta]$ and v have been observed in the case mentioned above and many others. Here, we shall indicate how the universal parameter $M[\eta]$, which we shall call μ, can be introduced in the calibration procedure. Consider N polymer samples previously characterized by values of $\overline{M}_n$ and $[\overline{\eta}]$; $[\overline{\eta}]$ refers to the sample as a whole and is in fact a weight-average quantity. Let the universal calibration function be represented by

$$G(v) = \ln \mu \tag{11}$$

Then, we have in one side

$$\overline{M}_n = \left[\int_{v_0}^{v_e} h(v)\, [\eta] \exp\{-G(v)\}\, dv \right]^{-1} \tag{12}$$

and

$$[\bar{\eta}] = \int_{v_0}^{v_e} h(v)\,[\eta]\,dv \tag{13}$$

On the other hand, we have for the number-averaged μ

$$\bar{\mu}_n = \frac{\int_{v_0}^{v_e} (c/M)\mu\,dv}{\int_{v_0}^{v_e} (c/M)\,dv} = \frac{\int_{v_0}^{v_e} h(v)\,[\eta]\,dv}{\int_{v_0}^{v_e} \{h(v)/M\}\,dv} = M_n[\bar{\eta}] \tag{14}$$

where c represents the weight concentration of the species with molecular weight M appearing at the elution volume v, and c/M consequently denotes the corresponding number of molecules.

The first sequence of the iteration is then as follows. Prepare a polynomial approximation using the set of N values of $\ln\{M_n[\bar{\eta}]\}$ and the corresponding peak elution volumes. It yields the starting function $G_0^n(v)$ reading

$$G_0^n = \sum_{i=0}^{n} (b_i)_0 v^i \tag{15}$$

Once the coefficients $(b_i)_0$ are known, solve for each chromatogram an elution volume v from

$$\sum_{i=0}^{n} (b_i)_0 v^i = \ln \frac{\int_{v_0}^{v_e} h(v)\,[\eta]\,dv}{\int_{v_0}^{v_e} h(v)\,[\eta]\,\exp\{-G(v)\}\,dv} \tag{16}$$

which equation follows immediately from eqs. (11)–(15). Use the N found values of v in combination with the N original values of $\ln\{M_n[\bar{\eta}]\}$ to prepare a new polynomial according to

$$G_1^n = \sum_{i=0}^{n} (b_i)_1 v^i \tag{17}$$

The process may be repeated then until the calibration function G_k^n remains constant. Upon inspection of eq. (16), it becomes clear that the procedure requires measurement not only of the chromatogram $h(v)$ but also of the intrinsic viscosity $[\eta]$ as a function of v. It is not problematic within the present-day GPC technique to measure the viscosity as a function of the elution volume.[3] However, the function $\{h(v)\cdot[\eta]\}$ does not allow easily to be corrected for dispersion.

Let us finally return to the dextrans investigated here. It is well known that they consist of branched molecules. This property would suggest treatment according to the universal calibration. However, due to the problematic application of the dispersion correction, it remains to be seen whether this approach is better. Furthermore, we may remark that when dealing with branched material, such particular cases as cited above will not always be met. Much will depend on the way in which the rate of branching has been distributed over the homologous series of polymer. In the particular case where the rate of branching is related uniquely to the molecular mass M, the latter quantity remains the

separating parameter. This may be the case in the dextran systems studied here.

CONCLUSIONS

The iteration described here can be handled as an efficient and rapid method for the calibration of GPC. Though the system investigated consisted of branched dextran molecules, the assumption that their molecular weights are uniquely related to the elution volumes leads to consistent results. The method was more successful when the chromatograms were corrected for dispersion. Complications resulting from the branching effect were discussed.

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