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A Valid Method of Calibration which Includes Correction for Dispersion in Gel Permeation Chromatography

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Synopsis

Two independently derived distribution function methods validate both the calibration curve and the dispersion correction of the "effective linear calibration" method used in gel permeation chromatography (GPC). Experimental conditions are specified for making the method more useful by permitting linear extrapolation of the calibration line,

$$V_R = C_1 + C_2 \log M,$$

and for using a minimum number of standards. The independent methods quantitatively relate known differential or integral distribution functions for standard samples to their respective chromatograms. As such, they are useful calibration methods also, but are limited in scope and range.

INTRODUCTION

Calibration is necessary in gel permeation chromatography to convert the raw data into molecular weight averages.[1-3] A variety of calibration methods have been proposed[4-16] which involve either narrow or broad molecular weight distribution standards and/or specific polymer types. Other methods have been concerned with the practical and theoretical basis for the "universal calibration" which is presently much debated.[17-25] Whatever calibration system is used, true molecular weight averages will not be obtained unless (1) the complete form of the calibration (equation) is known and used, and (2) correction is made for curve broadening caused by the chromatographic equipment, system, and process.[3,9,10,15,26-29]

Balke, Hamielec, LeClair, and Pearce[30] have proposed a practical method which incorporates conditions (1) and (2) above in a restricted way. Their method assumes an "effective linear calibration" line of the form

$$V_R = C_1 - C_2 \log M. \tag{1}$$

The molecular weight averages, $\bar{M}_n$ and $\bar{M}_w$, are computed from the GPC curve according to the method of Cazes[31] by means of eq. (1) for initial

approximations of C_1 and C_2. The computed $\bar{M}_n$ and $\bar{M}_w$ are compared to the true values obtained by osmometry and/or light scattering. New values of C_1 and C_2 are then assigned and the process repeated until an equation is obtained which yields minimum discrepancies from the true $\bar{M}_n$ and $\bar{M}_w$. This method of calibration corrects for curve broadening by means of the computation method. Data are taken across the curve for the summations which lead to the calculated averages. (The method can use one standard of known $\bar{M}_n$ and $\bar{M}_w$ or two standards of known $\bar{M}_n$ for each or $\bar{M}_w$ for each.)

The method suffers from the fact that the linear function, eq. (1), may not always represent the true form of the calibration curve. With unknown samples one is faced frequently with questionable extrapolation of this curve. Sufficient standards to define the proper form of the true calibration curve may not be readily available and are very costly to prepare and characterize.

In a different approach to calibration, considerable effort has been made to correct gel permeation chromatograms for curve broadening prior to calibrating. From a series of corrected standard chromatograms the retention volume V_R is related to a molecular weight parameter to define the calibration curve. Molecular weight parameters used have been $\bar{M}_n$, $\bar{M}_w$, $(M_n \cdot M_w)^{1/2}$, and distribution functions. For correction purposes Pickett, Cantow, and Johnson[32] have used a reshaping technique. Tung and coworkers have studied the Gaussian spreading function and also generalized shape functions.[33-38] The solution of the Gaussian spreading function may be obtained explicitly for given operating conditions (resolution), although a certain degree of mathematical sophistication and a computer are necessary. There must also be no mechanism bias caused by overloading (skewness), adsorption, viscosity drag, salting out, etc. Techniques for solving Tung's equation have been claimed by other workers.[39-41]

It is much more difficult to define properly a *general* spreading function and to obtain its accurate solution for a given set of GPC operating conditions. Provder and Rosen have studied this problem in detail,[42-44] as has Hamielec.[39] Our conclusions from these and other papers are that the Gaussian function is frequently inadequate, and it is usually impossible to define and solve the general function accurately.* Furthermore, the difficulty of acquiring and characterizing standards remains foremost. The "universal calibration" concept may solve this problem with standards,[17,22,23] but currently there are many areas of disagreement.

We have been intrigued with the simplicity and potential general utility of the "effective linear calibration" method,[30] eq. (1), and have sought to validate it and to reduce the number of restrictions. Of greatest concern to us were the questions: Does the method actually and properly correct

* Many useful concepts have resulted from the above studies including Provder's definitions of μ_2 and μ_3 for specifying column performance and Tung's finding that spreading depends on V_R, not M. The latter is important within the universal calibration concept but requires that no bias occur on the fractionation mechanism.

for curve broadening, and under what conditions are one or two standards sufficient for calibration? When are the linear function and linear extrapolations valid?

To answer these questions, we developed two additional methods for calibration. The first utilizes knowledge of the full differential distribution for a sample, e.g., the most probable distribution function of a nylon, and the second a full integral distribution. It was expected that these new methods themselves would provide dispersion correction and a sufficient definition of the calibration curve shape over a wide range. While we were successful in these endeavors,† we observed that these additional distribution function methods were extremely sensitive to the tails of the GPC curve and to the tail broadening. To use these methods in calibration, it is required to know the distribution function accurately, to know that the standard samples follow the function very closely, and to know there is no mechanism bias. These restrictions become prohibitive for most analysis problems. However, the study of these methods provided the definition of the conditions to be employed for practical, general use of the "effective linear calibration" method.

DEVELOPMENT

Many polymers follow a predictable molecular weight distribution which depends on the type and conditions of polymerization. For example, anionic polymerizations often follow a Poisson distribution; certain vinyl polymerizations (termination via radical coupling) yield a Schulz-Zimm distribution; and condensation polymers give a Flory "most probable distribution" if prepared under equilibrium conditions.[46] We felt that it should be possible to apply these theoretical distribution functions to the measured GPC distributions in such a way that molecular weights could be assigned to the retention volume variable in the GPC experiment. Our methods follow.

Consider the most probable distribution function,

$$w_x = (1 - p)^2 \cdot x \cdot p^{x-1} \tag{2}$$

where w_x is the weight fraction of polymer with x repeat units and p is related to the probability or extent of reaction such that $p = (\bar{M}_n - M_0)/\bar{M}_n$. The area under the curve, eq. (2), is unity. For a condensation polymer made under equilibrium conditions with a known $\bar{M}_n$ and M_0 (repeat unit molecular weight), the Flory equation predicts the weight fraction as a function of x (degree of polymerization) only.

Now consider an experimental GPC curve (Fig. 1). The total area under the curve represents all the polymer. The fractional area (a) represents the weight fraction of polymer that falls between V_f and an arbitrarily chosen volume, V_i. The theoretical distribution plot is shown in

† During the course of our work a paper by L. Wild et al.[45] appeared describing a similar concept with regard to the integral distribution method.

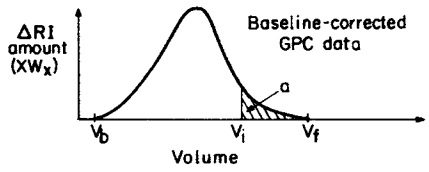

Fig. 1. GPC curve.

Figure 2. For the same area (a), there exists an x_i which defines the area boundary. The molecular weight at the boundary is x_iM_0 which is assigned to the V_i boundary of Figure 1. By making several such area comparisons over the entire curves, a series of volume-molecular weight pairs is generated, i.e., the calibration curve.

A relatively simple computer program determines the fractional area under the GPC data curve and also sums w_x for incremental x values until the sum $\sum_0^{x_i} w_x$ matches the fractional area. Consideration of the chromatogram's area is started at the highest volume end of the data (lowest molecular weight) since we are summing the theoretical function from $x = 0$.

Since $p < 1$, p^{x-1} gets exceedingly small for large values of x. Therefore, accurate calibration points for the highest molecular weights are unavailable. In practice, we sum w_x only to 99.98% of the theoretical area.

Note that the distribution function approach to calibration uses all the experimenal data. This method should "correct" for instrumental broadening of the sample because broadened "real" data are used to calculate the weight fractions of the sample. Skewing caused by nonuniform fractionation by the columns may also be corrected by this direct comparison approach. However, certain problems still remain. In reality, the polymer samples probably lack monomers, dimers, and trimers ($x = 1, 2, 3$) but we have arbitrarily chosen to sum from $x = 0$. Also, the volume chosen to begin and end the experimental GPC data is rather arbitrary. Many times the detector response (ΔRI) for low molecular weight species does not have the same proportionality to concentration as for the high molecular weight species. The baseline-corrected heights in both tails of the elution curve are subject to the greatest per cent error which automatically generates the greatest uncertainty in the calibration points at the extremes of the volume limits. Because of these uncertainties, extrapolation of the calibration curve is questionable.

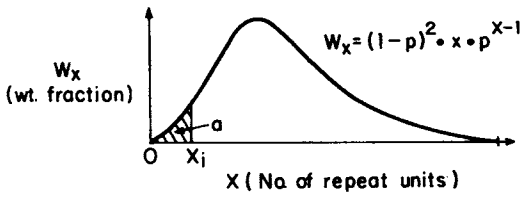

Fig. 2. Flory most probable distribution function.

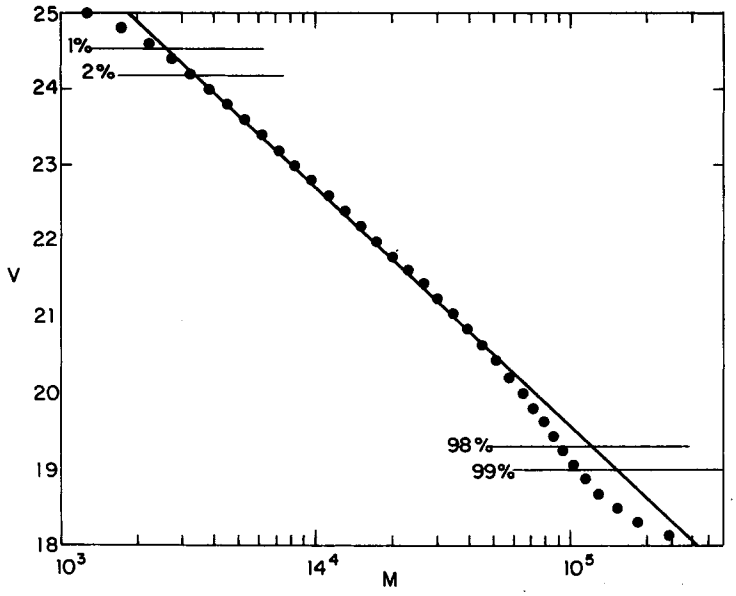

Fig. 3. Calibrations by most probable distribution method (● ● ●) and by Hamielec's linear method (——) for nylon 66 sample.

To prove this approach to calibration and to evaluate the "effective linear calibration" method, we have used a well-characterized nylon 66 standard. Calibration curves were generated via the most probable distribution function approach and compared to the calibration line obtained from the "effective linear calibration" method. As expected, the greatest deviations occur in the extremes of molecular weight (or volume). The slope and shapes of the calibration plots for the middle 98% of the sample were quite similar. The two calibration methods appear to be nearly equivalent and compatible (Fig. 3).

The second calibration procedure developed compares the cumulative or integral distribution data for a polymer obtained by independent means with the area under the GPC curve. The fractional area (a) under the baseline-corrected curve from the peak end (V_f) to any elution volume (V_i) is determined (Fig. 1). The molecular weight corresponding to the boundary of that fractional area (cumulative weight per cent) is the M_i associated with V_i (Fig. 4). Finding several of these (V_i, M_i) pairs over the entire distribution generates a calibration curve.

Again, all the real GPC data are used and any spreading or skewing should be compensated. However, the resulting calibration curve is only valid between V_f and V_b, and extrapolation may not be meaningful.

We have used this approach for the well-characterized polyethylene (NBS 1475) supplied by the National Bureau of Standards. A computer program determines areas under baseline-corrected curves and interpolates the data (cumulative wt-%, log MW) to generate the calibration curve at equal volume intervals. The resulting calibration curve was nearly coin-

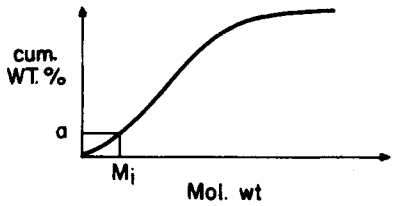

Fig. 4. Integral distribution curve.

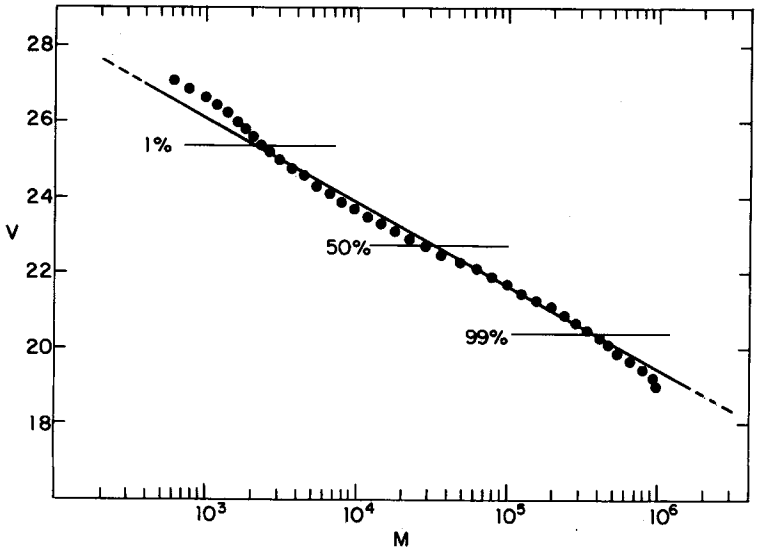

Fig. 5. Calibrations by integral distribution method (● ● ●) and by Hamielec's linear method (——) for NBS 1475.

cident with that obtained from the "effective linear calibration" method using $\bar{M}_n$ = 18,400 and $\bar{M}_w$ = 55,400. The greatest deviations were again seen in the extreme tails of the chromatogram. The middle 98% of the sample coincides nicely (Fig. 5).

Although this integral distribution method may be useful for those cases where accurate fractionation data are available, we feel that its greatest utility was to show that the "effective linear calibration" method is valid over a significant range of the sample. The problem of uncertain extrapolation is lessened with a linear calibration line.

EXPERIMENTAL

The nylon 66 sample used in this work was a Du Pont commercial resin prepared under conditions where the Flory distribution is expected. This sample was characterized in our laboratory by endgroup analysis, osmometry, and light scattering to give $\bar{M}_n$ and $\bar{M}_w$ of 15,800 and 32,500, respectively. The linear polyethylene is the standard reference material 1475 certified and supplied by the National Bureau of Standards.

GPC data were obtained from a modified Waters Associates Model 200 instrument. The nylon sample was chromatographed at 100°C in *m*-

cresol (0.5% w/v) through a 10^7, 10^5, 10^3 Å column series. The polyethylene sample was chromatographed at 138°C in 1,2,4-trichlorobenzene (0.25% w/v with 1 mg/cc Tenamine-3 antioxidant) through a 10^7, 10^5, 10^3 Å column series.

Digital data were taken via an A/D teletype coupler at the same time as the analog curve was made. The data conversion and reduction were accomplished by a computer program, which incorporates the features of the Hamielec[30] and Pickett et al.[41] methods into one master program. The data were also analyzed by in-house computer programs which accomplish the area calculations and comparisons mentioned in the body of this paper.

CONCLUSIONS

The interactive method of finding the best (effective) straight line calibration in the form of $V = C_1 - C_2 \log M$ has been shown to be valid. It corrects for peak broadening and skewing with the minimum of assumptions and it generates a calibration line that can easily be extended over a wide, useful range. To ensure even greater utility, standard samples should have a broad molecular weight distribution and "linear columns" should be used. (By "linear columns" we mean column packings that factionate the polymer so that log MW varies linearly with V for all molecular weights.) If these two conditions are met, only *one* well-characterized standard (known $\bar{M}_w$ and $\bar{M}_n$) is needed for each polymer type to be analyzed. Until the various problems associated with the "universal calibration" method are solved, the "effective linear calibration" scheme appears to be the method of choice.

We cannot overemphasize the utility of "linear columns." The calibration method gives valid molecular weight values for volumes encompassing the standard curve. Outside of this range the calibration curve must be extrapolated. If curve broadening and/or skewing is not excessive and the fractionation is linear with log M, then rather extensive extrapolation of the linear calibration curve is *permissible*.

"Linear columns" can be made in several ways. One involves use of "single pore size" columns in series and has been discussed by Bly.[16] Another is to add together approximately equal *numbers* of all pore sizes. To do this, one simply mixes 7 parts 10^7 Å + 6 parts 10^6 Å + 5 parts 10^5 Å, etc., gel. Our experience shows it is preferable to weight somewhat the amount of larger pore sizes, for example, by adding 12 parts gel.

Contribution No. 1920.

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