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Calibration Methods for Field-Flow Fractionation Using Broad Standards. I. Thermal Field-Flow Fractionation

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ABSTRACT

Thermal field-flow fractionation (ThFFF) is used for the determination of molecular weight (MW) distributions of synthetic polymers. This is usually achieved utilizing a series of narrow MW polymer standards. A major drawback in this approach is the very limited number of polymers which have such standards available. This paper reports a method of calibration which utilizes one or more broad standards for which the average MW values are known. It was observed that 1) the use of number-average MW data tends to produce large variation in calibration constants and hence the MW generated, 2) the use of two polydisperse standards and their weight-average MW (M_w) provides reasonable calibration in comparison with the conventional method using narrow MW standards, and 3) even better calibration is obtained using multiple broad standards and their M_w values. The new method should expand the applicability of ThFFF to include a wider range of polymer types.

INTRODUCTION

Field-flow fractionation (FFF) is an emerging family of high resolution methods developed for separation and analytical characterization of macromolecules and particles. In FFF, separation is achieved by coupling a laminar carrier flow with an external field applied perpendicularly to the carrier flow. The field must be of a type that interacts with the species contained in the carrier fluid. The field forces the species toward one wall,

called the accumulation wall, of the channel. In the normal mode of FFF this process is balanced by the Brownian diffusion which drives the species away from the higher concentration region adjacent to the accumulation wall. Species with different characteristics interact differently with the field producing equilibrium clouds, each with a characteristic thickness. Consequently, species are carried downstream by fluid layers of different velocities within the laminar flow. Different types of external fields used in FFF create different subtechniques of FFF, which is useful for a specific range of sample types and sizes and may yield different information about the component particles or molecules (1).

The most common FFF techniques used for characterization of polymers are thermal FFF (ThFFF) and flow FFF (FlFFF). Although sedimentation FFF is used widely for sizing polymer latex beads, it is not so popular for polymer macromolecules because the sedimentation force is only efficient for rather big molecules (several million dalton). Although electrical FFF appears to have considerable potential, it is not well developed at present and is only applicable for charged species (2).

ThFFF is a subtechnique of FFF in which a temperature gradient dT/dx is employed as the external field. The temperature gradient is generated by heating one plate of the channel while cooling the other plate. Molecules migrate toward the cold plate which thus becomes the accumulation wall. The separation mechanism in ThFFF is based on a dynamic equilibrium between the thermal diffusion which draws species from the hotter region to the cooler region, and the normal diffusion which opposes the movement by driving species away from the region of higher concentration developed at the cold wall.

ThFFF is the preferred technique for characterizing synthetic polymers which can be dissolved in an organic solvent. Major applications of ThFFF include the determination of the molecular weight distribution (MWD), polydispersity, and thermal diffusion coefficient values (3–6).

However, a major drawback in ThFFF is that in order to determine the MWD of a polymer sample, calibration is generally carried out using narrow molecular weight standards. Such standards are only available for a limited number of polymer types.

We recently reported a method of calibration for ThFFF which utilizes either one broad standard having known M_n (number-average molecular weight) and M_w or two broad standards having known M_w or M_n (7).

The above method was then refined, first to include corrections to the ThFFF retention equations for variations in viscosity and thermal conduc-

tivity, and second to handle multiple broad standards for better estimation of the calibration constants. In this paper the theory of the calibration methods using multiple broad standards is discussed and tested with the polystyrene–tetrahydrofuran (PS–THF) combination.

THEORY DEVELOPMENT

Basic Theory of ThFFF

For constant field and normal mode FFF runs the retention ratio R is related to retention time t_r and retention volume V_r for a given component by

$$R = \frac{t^0}{t_r} = \frac{V^0}{V_r} \tag{1}$$

where V^0 and t^0 are the channel volume (or void volume) and retention time (or void time) for nonretained substances and is related to the retention parameter λ by (8)

$$R = 6\lambda \left\{ \coth\left(\frac{1}{2\lambda}\right) - 2\lambda \right\} \tag{2}$$

For high retention the term $\coth(1/2\lambda)$ approaches 1, and Eq. (2) reduces to

$$R = 6\lambda(1 - 2\lambda) \tag{3}$$

Equation (3) produces errors less than 5% for $\lambda \leq 0.23$ ($R \leq 0.78$), and errors less than 0.4% for $\lambda \leq 0.15$ (or $R \leq 0.63$). Under the condition of $\lambda \leq 0.15$, Eq. (3) can be rearranged to give

$$\lambda = \frac{3 - (9 - 12R)^{1/2}}{12} \tag{4}$$

For very high retention ($\lambda \rightarrow 0$),

$$R = 6\lambda \tag{5}$$

Equation (5) yields errors of $\leq 5\%$ for $R \leq 0.136$, although this equation was not used in this work.

In practice, Eq. (2) was corrected for the variations in viscosity and thermal conductivity across the channel according to Gunderson et al. (9) and Van Asten et al. (10).

The relationship between quantity λ and the thermal diffusion coefficient (D_T) in a dilute solution is described by (11)

$$\lambda = \frac{D}{D_T \left(\frac{dT}{dx}\right) w} \tag{6}$$

Utilizing the commonly obeyed expression for the Brownian diffusion coefficient D (12),

$$D = AM^{-b} \tag{7}$$

yields an equation in which λ is related to MW (M) as

$$\lambda = \frac{A}{D_T \left(\frac{dT}{dx}\right) wM^b} \tag{8}$$

where A and b are constants and w is the channel width. If we let

$$\Phi = A/D_T \tag{9}$$

then

$$\lambda = \frac{\Phi}{w \left(\frac{dT}{dx}\right) M^b} \tag{10}$$

It is generally assumed that D_T and hence Φ are independent of MW. However, it has been suggested recently that there may be a small dependence of D_T on MW (13), hence the experimentally determined calibration constants may not yield A and b exactly. To accommodate this discrepancy, we replace b by n which denotes the empirical calibration constant that will be used in MW determinations.

If thermal conductivity of the carrier solvent is assumed constant, then

$$dT/dx \approx \Delta T/w \tag{11}$$

where ΔT is the temperature difference across the channel. Therefore

$$\lambda = \Phi/\Delta TM^n \tag{12}$$

When the corrections due to the variations in thermal conductivity are taken into account, the dT/dx value at the cold wall temperature $(dT/dx)_c$ was used as an approximate value for dT/dx at the sample center of mass (4, 14).

$$\left(\frac{dT}{dx}\right)_c = \frac{1}{w}\left(\Delta T + \frac{1}{\kappa_c}\frac{d\kappa}{dT}\frac{(\Delta T)^2}{2}\right) = \frac{S}{w} \tag{13}$$

where S, the apparent field strength, is defined as (4)

$$S = \Delta T + \frac{1}{\kappa_c}\frac{d\kappa}{dT}\frac{(\Delta T)^2}{2} \tag{14}$$

Here $d\kappa/dT$ is calculated using the following expression for κ_T (14):

$$\kappa_T = \kappa_c + \frac{d\kappa}{dT}(T - T_c) \tag{15}$$

where κ_T and κ_c are the thermal conductivities at temperature T and the temperature at the cold wall T_c, and are obtained from thermodynamic data using the method described in Reid and Prausnitz (15).

Equation 12 can now be written as

$$\lambda = \Phi/SM^n \tag{16}$$

Calibration Using One or Two Broad MW Standards

The theory of the calibration method for ThFFF is based on the basic Eq. (12) or, more accurately, Eq. (16). The expressions for average MW in ThFFF were derived as (7)

$$M_w = \left(\frac{\Phi}{\Delta T}\right)^{1/n} \frac{\sum_{i=1}^{p} h_i \lambda_i^{(-1/n)}}{\sum_{i=1}^{p} h_i} \tag{17}$$

and

$$M_n = \left(\frac{\Phi}{\Delta T}\right)^{1/n} \frac{\sum_{i=1}^{p} h_i}{\sum_{i=1}^{p} h_i \lambda_i^{(1/n)}} \tag{18}$$

where for each of the broad MW samples h_i is the detector response at the ith point on the digitized fractogram.

Rearrangement of Eqs. (17) and (18) yields

$$M_w = I_t \frac{\sum_{i=1}^{p} h_i (\Delta T \lambda_i)^{-1/n}}{\sum_{i=1}^{p} h_i} \tag{19}$$

and

$$M_n = I_t \frac{\sum h_i}{\sum h_i(\Delta T\lambda_i)^{1/n}} \tag{20}$$

Here

$$I_t = \Phi^{1/n} \tag{21}$$

When correction for variation in viscosity is included, Eqs. (19) and (20) become

$$M_w = I_t \frac{\sum_{i=1}^{p} h_i(S\lambda_i)^{-1/n}}{\sum_{i=1}^{p} h_i} \tag{22}$$

and

$$M_n = I_t \frac{\sum_{i=1}^{p} h_i}{\sum_{i=1}^{p} h_i(S\lambda_i)^{1/n}} \tag{23}$$

According to Eqs. (22) and (23) (or 19 and 20), a broad sample can be used as a standard to obtain the calibration constants Φ and n if its average MW values M_w and M_n are available. Similarly, two broad standards can be used for calibration if each standard has one known MW value, either M_n or M_w. Here the combinations are two M_n, two M_w, or M_n and M_w (the use of both M_n and M_w for two broad standards is not included in this study). Some iteration method, such as Newton–Raphson, needs to be used to calculate the value of the constant n. Constant Φ will be obtained by substituting n back into either Eq. (22) or (23) as shown in Nguyen and Beckett (7).

Calibration with Multiple Broad Standards Using M_w Values

It will be seen later that the accuracy in the final values of Φ and n obtained using the above method is heavily dependent on the quality of the standard(s) used. If more broad standards are available, then a better estimate of the constants is expected to be obtained. The study was therefore expanded to investigate the use of multiple broad standards for calibration, and the theory is discussed in the following section. As will be

shown later, the value of M_n tends to have higher uncertainty than M_w and hence gives rise to larger errors in the calibration constant values. Consequently, calibration using multiple broad standards was developed for use with M_w data only.

Assuming m broad standards are available for calibration, the problem is to find the best fit values of Φ and n for this system of m equations of weight-average MWs. Such problems can be solved using some minimization approach such as Newton's method (16).

In general, let

$$\mathbf{f}_j = \mathbf{f}_j(I_t,n) = \left\{ (M_w)_j - \frac{I_t \sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n}}{\sum_{i=1}^{p_j} h_{ji}} \right\}^2 \tag{24}$$

where $j = 1, \ldots, m$ refers to the sample number assigned to each of the m broad MW standards and $(M_w)_j$ is the weight-average MW of the jth standard.

The function $\mathbf{f}_j$ here is the square of the difference of the nominal molecular weight value and its estimate generated when various values of Φ and n are used. The sum of these functions is the quantity to be minimized. Now let

$$\mathbf{F} = \mathbf{F}(I_t,n) = \sum_{j=1}^{m} \mathbf{f}_j \tag{25}$$

The values of I_t and n which need to be solved for here are those values which result in Eq. (25) passing a minimum. The conditions at the point where Eq. (25) goes through a local minimum are that its partial derivatives must both be equal to zero (17),

$$\frac{\partial \mathbf{F}}{\partial I_t} = \frac{\partial \mathbf{F}}{\partial n} = 0 \tag{26}$$

and its second-order derivatives satisfy the following conditions:

$$\left(\frac{\partial^2 \mathbf{F}}{\partial I_t^2}\right)\left(\frac{\partial^2 \mathbf{F}}{\partial n^2}\right) - \left(\frac{\partial^2 \mathbf{F}}{\partial I_t \partial n}\right)^2 > 0 \tag{27}$$

and

$$\frac{\partial^2 \mathbf{F}}{\partial I_t^2} > 0 \tag{28}$$

The first-order derivative of $\mathbf{f}_j$ with respect to I_t is

$$\frac{\partial \mathbf{f}_j}{\partial I_t} = 2I_t \left\{ \frac{\sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n}}{\sum_{i=1}^{p_j} h_{ji}} \right\}^2 - 2(M_w)_j \frac{\sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n}}{\sum_{i=1}^{p_j} h_{ji}} \tag{29}$$

and the first-order derivative of $\mathbf{f}_j$ with respect to n is

$$\frac{\partial \mathbf{f}_j}{\partial n} = \frac{2I_t^2}{n^2 \left\{ \sum_{i=1}^{p_j} h_{ji} \right\}^2} \sum_{n=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n} \sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n} \ln(S_j\lambda_{ji}) - \frac{2I_t(M_w)_j}{n^2 \sum_{i=1}^{p_j} h_{ji}} \sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n} \ln(S_j\lambda_{ji}) \tag{30}$$

Let

$$\mathcal{L}_j = \frac{\sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n}}{\sum_{i=1}^{p_j} h_{ji}} \tag{31}$$

and

$$\mathcal{P}_j = \frac{\sum_{i=1}^{p_j} h_{ji}(S_j\lambda_{ji})^{-1/n} \ln(S_j\lambda_{ji})}{\sum_{i=1}^{p_j} h_{ji}} \tag{32}$$

Then Eq. (29) becomes

$$\frac{\partial \mathbf{f}_j}{\partial I_t} = 2I_t(\mathcal{L}_j)^2 - 2(M_w)_j\mathcal{L}_j \tag{33}$$

Eq. (30) becomes

$$\frac{\partial \mathbf{f}_j}{\partial n} = \frac{2I_t^2}{n^2}\mathcal{L}_j\mathcal{P}_j - \frac{2I_t(M_w)_j}{n^2}\mathcal{P}_j \tag{34}$$

Since $\mathbf{F} = \sum_{j=1}^{m} \mathbf{f}_j$, therefore

$$\frac{\partial \mathbf{F}}{\partial I_t} = \sum_{j=1}^{m} \frac{\partial \mathbf{f}_j}{\partial I_t} \tag{35}$$

and

$$\frac{\partial \mathbf{F}}{\partial n} = \sum_{j=1}^{m} \frac{\partial \mathbf{f}_j}{\partial n} \tag{36}$$

Expressions for the derivatives of function **F** are

$$\frac{\partial \mathbf{F}}{\partial I_t} = \sum_{j=1}^{m} \{2I_t(\mathscr{L}_j)^2 - 2(M_w)_j \mathscr{L}_j\} \tag{37}$$

or

$$\frac{\partial \mathbf{F}}{\partial I_t} = 2I_t \sum_{j=1}^{m} (\mathscr{L}_j)^2 - 2 \sum_{j=1}^{m} (M_w)_j \mathscr{L}_j \tag{38}$$

and

$$\frac{\partial \mathbf{F}}{\partial n} = \sum_{j=1}^{m} \left\{ \frac{2I_t^2}{n^2} \mathscr{L}_j \mathscr{P}_j - \frac{2I_t (M_w)_j}{n^2} \mathscr{P}_j \right\} \tag{39}$$

or

$$\frac{\partial \mathbf{F}}{\partial n} = \frac{2I_t^2}{n^2} \sum_{j=1}^{m} \mathscr{L}_j \mathscr{P}_j - \frac{2I_t}{n^2} \sum_{j=1}^{m} (M_w)_j \mathscr{P}_j \tag{40}$$

From Eqs. (26) and (38):

$$I_t^{(1)} = \frac{\sum_{j=1}^{m} (M_w)_j \mathscr{L}_j}{\sum_{j=1}^{m} (\mathscr{L}_j)^2} \tag{41}$$

and from Eqs. (26) and (40):

$$I_t^{(2)} = \frac{\sum_{j=1}^{m} (M_w)_j \mathscr{P}_j}{\sum_{j=1}^{m} \mathscr{L}_j \mathscr{P}_j} \tag{42}$$

In Eqs. (41) and (42), $\mathscr{L}_j$ and $\mathscr{P}_j$ are functions of n alone. To obtain n,

equate (41) and (42) and let

$$G = I_t^{(1)} - I_t^{(2)} \tag{43}$$

G is a function of n, and it is solved for n at $G = 0$ by some iteration method. A method which does not require the evaluation of the derivatives, such as the bisection method, is found useful here.

If term S in Eqs. (19) to (43) is replaced by ΔT, these approaches can be used for calibrations in which $\Delta T/w$ is used for the temperature gradient.

Note that this calibration method was developed based on the assumption that the sample average temperature is very close to the temperature at the cold wall ($T_{sample} \approx T_c$), hence it allows the use of (slightly) different ΔT values in calculations. However, to obtain more accurate results, ΔT should be the same for all samples. The use of different ΔT values may result in some errors due to the dependence of D_T on temperature (18).

A program in GWBASIC was written to carry out the computation. A graphic procedure was included in the program to plot the functions **F** and G to assist the choice of the initial values required by the bisection method, and to ensure the minimum value for **F**.

EXPERIMENTAL SECTION

ThFFF

Instrumentation

The ThFFF channel, model T100 from FFFractionation Inc., Salt Lake City, Utah, described in Nguyen and Beckett (7), was used in this study. The channel dimensions were tip-to-tip length 46.1 cm, width 1.60 cm, thickness 0.127 mm, giving a geometric void volume of 0.933 cm^3. The void volume was also determined experimentally using a nonretained solute injection at a low flow rate. A value of 0.840 cm^3 was first obtained (7). The volume was remeasured several months later for use in calculation of Φ and n in this research and this time gave a value of 0.750 cm^3. The shrinkage of the channel volume may be due to compression of the Mylar spacer, particularly when the temperature gradient is applied. The dead volume (i.e., the volume of tubing between the outlet of the channel and the inlet of the detector) was measured and had a value of 0.080 mL.

The pump used was an LDC Milton Roy Constametric III, and the UV detector was either a Waters model 480 or a Spectraphysics Spectra 100 set at a wavelength of 254 nm. A backpressure regulator was connected to the detector outlet to prevent the solvent boiling when using high ΔT values. The flow rates were measured by an in-house flowmeter comprised of an electronic balance (model FX300 from AND) which continously

monitors the mass of solvent from the outlet of the detector and a microprocessor unit for digital calculations and analogue data display. A chart recorder from ICI Instruments, model DP600, was used.

The thermal gradient field control and data acquisition were achieved using programs supplied by FFFractionation Inc., using an Epson PCe computer. The program FFF.EXE supplied by FFFractionation Inc. was used for adjusting baseline and removal of void peak. Fractograms were digitized when needed using a Hewlett-Packard plotter and the GRAPHPAD software package. Analysis of the fractogram data was achieved using in-house programs written in GWBASIC.

SEC-MALLS

SEC with a multiangle laser light-scattering detector (MALLS) on-line, and a differential refractive index detector (DRI) was employed to independently measure the average MW values for the broad standards used for calibration.

In the SEC system, a series of two 10^3 Å, two 10^4 Å, and one 10^5 Å Ultrastyragel columns from Waters were used for the separation of the polymers in THF. A Waters 401 DRI detector was employed. The columns were calibrated using a series of polystyrene standards supplied by Waters. The MALLS instrument model DAWN F was from Wyatt Technology Corporation. ASTRA and EASI software packages from Wyatt Technology Corporation were used for analysis of light-scattering data. Data for conventional SEC with the DRI detector were analyzed with the BASELINE software package from Waters. HPLC-grade THF was used without any further treatment. The solvent was pumped at a flow rate of 1 mL/min.

ThFFF Run Conditions

A 20-μL sample was introduced onto the channel through a Rheodyne six-way loop injection valve. A constant ΔT value of 40 K was employed. The temperature at the cold wall, which varies somewhat with ΔT, was adjusted to 20 ± 1°C using a valve to control the cooling water flow rate through the cold wall copper block.

The sample was relaxed for 1 minute with the field strength applied but with the channel flow off. The run was then commenced with a flow rate between 0.130 and 0.180 mL/min. The detector response was recorded on a chart recorder and collected in digitized form on a PC computer for later processing to obtain MW information as outlined in the Theory Development Section using in-house programs.

TABLE 1
MW (M_p = peak maximum, M_w = weight average, M_n = number average) and Polydispersity (μ) Details for Six Narrowly Dispersed Polystyrene Standards as Supplied by the Manufacturer

Standard number	M_p (kdalton)	M_w (kdalton)	M_n (kdalton)	μ
N1	46	43	42	1.03
N2	92	85	80	1.05
N3	217	213	209	1.02
N4	440	427	417	1.03
N5	827	810	785	1.03
N6	1310	1260	1200	1.04

Materials

Narrow Molecular Weight Standards. For the ThFFF measurement, six narrow MW polystyrene standards were supplied by Polymer Standards Services (Mainz, Germany). MW information of these standards is indicated in Table 1.

Broad Molecular Weight Standards. Four broad MW polystyrene samples from different sources were used for the studies of the calibration method. Details of their nominal MW values given by the suppliers are indicated in Table 2.

All sample solutions were made up to 1–2 mg/mL in spectrophotometry-grade tetrahydrofuran (THF) which was also the carrier solvent. The same run conditions were applied for all narrow and broad standards, with the exception of the flow rates which were varied slightly in individual runs

TABLE 2
Details of M_n, M_w, and μ Values Given by the Suppliers (Dow and Pressure Chem. Co.) for Four Polydisperse Polystyrene Standards. The Flow Rates Employed in ThFFF Runs Are Also Given

Standard number	M_w (kdalton)	M_n (kdalton)	μ	Flow rate (mL/min)
B1	100	—		0.130
B2	250	100		0.138
B3	498	—	<1.2	0.180
B4	1000	—		0.130

in an attempt to maximize the separation between the sample and the void peak. The flow rate employed in narrow standard runs was 0.177 mL/min. For broad standard runs the flow rates used in each case are shown in Table 2.

RESULTS AND DISCUSSIONS

ThFFF fractograms of the six monodisperse polystyrene standards in Table 1 are shown in Fig. 1. Retention ratio (R) and the corresponding retention parameters λ data determined at the peak maximum of the fractograms are summarized in Table 3. For comparison, the retention parameter values uncorrected for variations in viscosity and in thermal conductivity are also calculated using Eq. (2) and included in Table 3. The corrected retention data were used to plot the calibration line in Fig. 2. The gradient of this line yields a value of the constant $n = 0.636$. The y-

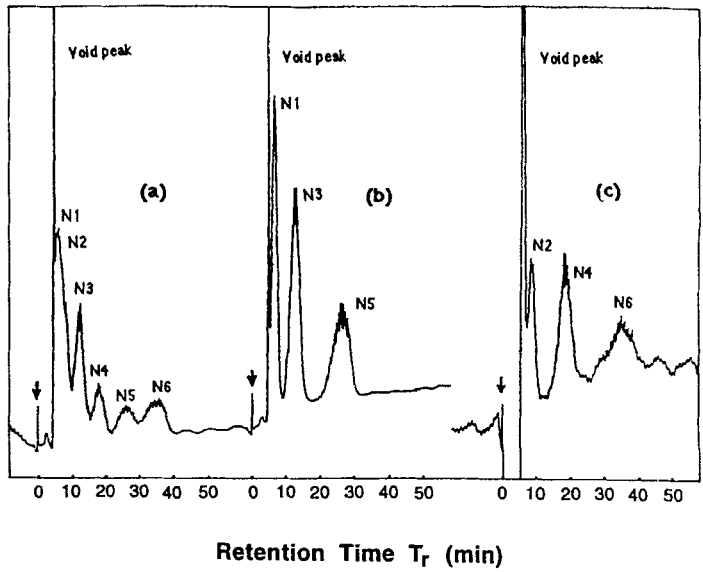

FIG. 1 ThFFF fractograms for six narrow polystyrene standards in three different mixtures recorded by chart recorder. Run conditions are identical in all three runs and are described in Table 3. The arrow indicates where the runs start. Mixture (a) contains all standards (N1–N6) with peaks for N1 and N2 not being resolved. Mixture (b) contains standards N1, N3, and N5. Mixture (c) contains standards N2, N4, and N6.

TABLE 3
ThFFF Data Obtained for Six Monodisperse Polystyrene Standards in THF. The MW at Peak Maximum (M_p) of SEC Separations Were Given by the Manufacturer. The Flow Rate Was 0.177 mL/min and ΔT was 40 K

Standard number	M_p (kdalton)	V_r (mL)	R	λ (uncorrected)	λ (corrected)
N1	46	1.07	0.701	0.1806	0.2037
N2	92	1.34	0.561	0.1245	0.1396
N3	217	2.08	0.361	0.0699	0.0787
N4	440	3.07	0.244	0.0447	0.0505
N5	827	4.52	0.166	0.0294	0.0333
N6	1310	6.03	0.124	0.0217	0.0247

intercept of the line is log Φ, which in this case gives a value for Φ of 7174.

Fractograms for the four polydisperse standards are shown in Fig. 3. Calibration constants n and Φ obtained from the calibration line (Fig. 2) were used to generate the MWD for these broad standards which are given in Fig. 4. The weight- and number-average MWs were calculated using Eqs. (22) and (23), respectively. MW averages of these broad standards were also independently determined by SEC-MALLS. These values are tabulated in Table 4 along with values specified by the supplier.

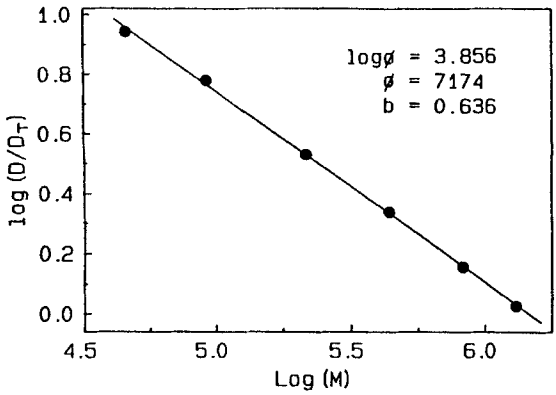

FIG. 2 Calibration line for polystyrene dissolved in THF obtained using data for monodisperse standards given in Table 3. The retention parameter used was corrected for variations in viscosity and thermal conductivity.

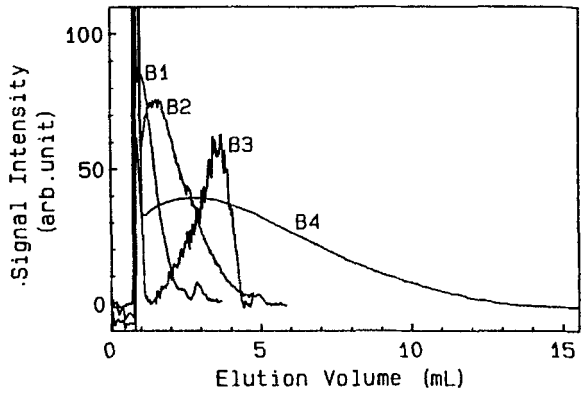

FIG. 3 ThFFF fractograms for four polydisperse polystyrene samples dissolved in THF. For all samples, ΔT was 40 K. Flow rates were measured in each case as shown in Table 2.

The digitized fractograms in Fig. 3 were used to estimate the calibration constants n and Φ utilizing the average MW values as outlined in the Theory Development Section. Two groups of MW average values used in these estimations were 1) generated from the ThFFF calibration line in Fig. 2, and 2) determined by SEC-MALLS.

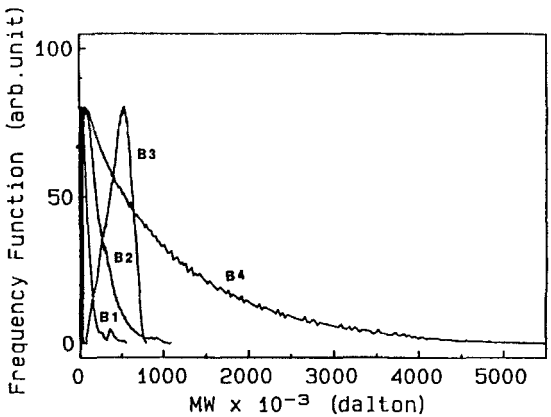

FIG. 4 MWD curves for four polydisperse polystyrene samples calculated using the fractograms in Fig. 3 and the calibration constants b = 0.636 and Φ = 7174 obtained from ThFFF using monodisperse standards (Fig. 2).

TABLE 4

M_n and M_w for Four Broad Polystyrene Standards as Given by the Manufacturer, Obtained from ThFFF Using Conventional Calibration with Narrow Standards, and Obtained from SEC-MALLS. The Values in Parentheses Indicate the Deviation from the Manufacturers' Value

Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	ThFFF (kdalton)
M_n:			
B1	—	64	63
B2	100	137	125
		(+37%)	(+25%)
B3	—	446	403
B4	—	857	358
M_w:			
B1	100	97	103
		(−3.0%)	(+3.0%)
B2	250	240	226
		(−4.0%)	(−9.5%)
B3	498	467	466
		(−6.3%)	(−6.5%)
B4	1000	1039	1082
		(+3.9%)	(+8.2%)

When the average MW values generated from the narrow standard calibration line were used in the broad sample calibration method, identical values of n and Φ to those evaluated directly from conventional calibration were obtained in each case. This is to be expected as the MW averages for the broad standards were calculated using these same constants. However, it does demonstrate that the computer programs written for the broad standard calibration method are valid.

When average MW values determined by SEC-MALLS were used, deviations in the values of n and Φ were observed. The values of n and Φ obtained in these cases were then used for backcalculation of number and weight MW averages (M_n and M_w) for the broad standards and the MW at peak maximum of the ThFFF fractogram (M_p) for the narrow standards. Values of n and Φ and the generated MW averages obtained using calibration with one or two broad standards are summarized from Tables 5 to 13.

From these tables it can be seen that the results obtained from these calibration methods depend on the choice of standards and the particular

TABLE 5
Comparison of Calibration Constants Φ and n Obtained by ThFFF Calibration with Broad Standards for PS–THF Using Both M_n and M_w Values for One Broad Standard. The ThFFF Data in Each Case Were Corrected for Variations in Viscosity and Thermal Conductivity. The MW Values Were Determined by SEC-MALLS

Standard number	M_n (kdalton)	M_w (kdalton)	Φ	n
B1	64	97	13,810	0.695
B2	137	240	9,491	0.655
B3	446	467	4.53×10^6	1.129
B4	857	1039	2.40×10^9	1.545

calibration procedure used. When one standard was used, only 2 out of 4 sets of constants (Φ = 13800, n = 0.695 and Φ = 9490, n = 0.655) gave results which were close to the manufacturers values as illustrated in Tables 8 and 9. Constants resulting from calibration using two M_n values tend to produce large errors in calculated MW values. Unrealistic constants and MWs were sometimes observed as shown in Tables 6, 10, and 11. Calibration using two M_w values generally gave reasonable MW results as shown in Tables 7, 12, and 13. However, errors produced by this cali-

TABLE 6
Comparison of Calibration Constants Φ and n Obtained by ThFFF Calibration with Broad Standards for PS–THF Using M_n Values for Two Broad Standards in Various Combinations. The ThFFF Data in Each Case Were Corrected for Variations in Viscosity and Thermal Conductivity. The M_n Values Were Determined by SEC-MALLS

Standard number	M_n1 (kdalton)	M_n2 (kdalton)	Φ	n
B1 and B2	64	137	3326	0.564
B1 and B3	64	446	5500	0.610
B1 and B4	64	857	292	0.337
B2 and B3	137	446	7262	0.632
B2 and B4	137	857	49.4	0.179
B3 and B4	446	857	[a]	[a]

[a] The Combination of Standards Having M_n of 446 and 857 kdalton did not convert.

TABLE 7
Comparison of Calibration Constants Φ and n Obtained by ThFFF Calibration with Broad Standards for PS–THF Using M_w Values for Two Broad Standards in Various Combinations. The ThFFF Data in Each Case Were Corrected for Variations in Viscosity and Thermal Conductivity. The M_w Values Were Determined by SEC-MALLS

Standard number	M_w1 (kdalton)	M_w2 (kdalton)	Φ	n
B1 and B2	97	240	2,413	0.547
B1 and B3	97	467	4,829	0.606
B1 and B4	97	1039	6,470	0.631
B2 and B3	240	467	19,500	0.712
B2 and B4	240	1039	12,420	0.676
B3 and B4	467	1039	9,876	0.660

TABLE 8
Comparison of M_p Values (kdalton) for the Six Monodisperse Polystyrene Standards Given by the Manufacturer with Those Obtained from ThFFF Using Calibration with Both M_n and M_w Values for One Broad Standard. The Calibration Constants Used in Each Case Are Indicated in Table 5. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values; the Deviation Is Not Shown Where It Is Greater than 100%

Standard number	Manufacturer (kdalton)	B1 Φ = 13,800 n = 0.695	B2 Φ = 9,490 n = 0.655	B3 $\Phi = 45.3 \times 10^6$ n = 1.129	B4 $\Phi = 2.40 \times 10^9$ n = 1.545
N1	46	50 (+7.8%)	54 (+17%)	132	320
N2	92	85.3 (−7.6%)	96.2 (+4.2%)	184	408
N3	217	195 (−10%)	231 (+6.5%)	307 (+41%)	591
N4	440	368 (−16%)	453 (+3.0%)	454 (+3.1%)	788 (+79%)
N5	827	672 (−19%)	858 (+3.7%)	657 (−21%)	1030 (+25%)
N6	1310	1030 (−21%)	1354 (+3.3%)	856 (−35%)	1250 (−4.4%)

TABLE 9

Comparison of M_n and M_w Values (kdalton) for the Four Polydisperse Polystyrene Samples Determined by SEC-MALLS with Those Obtained from ThFFF Using Calibration with Both M_n and M_w Values for One Broad Standard. The Calibration Constants Used in Each Case Are Indicated in Table 5. The Numbers in Parentheses Indicate the Percentage Deviation from the MW Values Determined by SEC-MALLS; the Deviation Is Not Shown Where It Is Greater Than 100%

Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	B1 Φ = 13,800 n = 0.695	B2 Φ = 9,490 n = 0.655	B3 Φ = 45.3 × 10^6 n = 1.129	B4 Φ = 2.40 × 10^9 n = 1.545
M_n:						
B1	—	64	63.9 (−0.16%)	69.9 (+9.2%)	161	373
B2	100	137	121 (−12%)	137 (0%)	243 (+77%)	507
B3	—	446	348 (−22%)	425 (−4.7%)	446 (0%)	778 (+74%)
B4	—	857	326 (−62%)	385 (−55%)	481 (−44%)	852 (−0.58%)
M_w:						
B1	100	97	97 (0%)	112 (+15%)	189 (+95%)	406
B2	250	240	199 (−17%)	240 (0%)	295 (+23%)	562
B3	498	467	392 (−16%)	487 (+4.3%)	467 (0%)	797 (+71%)
B4	1000	1039	828 (−20%)	1090 (+4.9%)	689 (−34%)	1030 (−0.87%)

bration method vary with the particular combination of standards used. Some combinations did result in quite large errors, especially at the extreme ends of the MW range and when the samples were outside the ranges covered by the broad MW standards used for that particular calibration.

In general, the use of M_n resulted in values of n and Φ which were widely scattered when different broad standards were used. This in turn causes large deviations in the MW values generated using these constants when compared to the corresponding nominal values. This can possibly be explained by the fact estimates of M_n determined by various methods are often inaccurate because it can be strongly influenced by the presence of small amounts of low MW impurities in the sample. In addition, low

TABLE 10

Comparison of M_p Values (kdalton) for the Six Monodisperse Polystyrene Standards Given by the Manufacturer with Those Obtained from ThFFF Using Calibration with Two M_n Values for Two Broad Standards. The Calibration Constants Used in Each Case Are Indicated in Table 6. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values; the Deviation Is Not Shown Where It Is Greater than 100%

Standard number	Manufacturer (kdalton)	B1 and B2 Φ = 3326 n = 0.564	B1 and B3 Φ = 5500 n = 0.610	B1 and B4 Φ = 292 n = 0.337	B2 and B3 Φ = 7262 n = 0.632	B2 and B4 Φ = 49.4 n = 0.179
N1	46	49 (+6.5%)	49 (+6.5%)	53 (+14%)	53 (+15%)	37 (−20%)
N2	92	95.7 (+3.7%)	91.2 (−1.2%)	161 (+75%)	96 (+4.0%)	305
N3	217	265 (+22%)	233 (+7.7%)	887	238 (+9.7%)	7530
N4	440	580 (+32%)	482 (+9.5%)	3,300	479 (+8.8%)	8.92×10^4
N5	827	1216 (+47%)	955 (+15%)	11,400	928 (+12%)	9.23×10^5
N6	1310	2070 (+58%)	1560 (+19%)	27,700	1490 (+14%)	4.90×10^6

MW components are sometimes not completely resolved from the void peak with ThFFF separations.

The use of M_w data alone gave a smaller range of scatter in both n and Φ. Consequently, MW values generated using these calibration constants have smaller deviations from the corresponding nominal values. It can be concluded that the method of calibration using broad standards is more reliable when M_ws are used. Therefore, when the method was expanded to handle multiple broad standards, only weight-average MW values were included in the computations.

For calibration using multiple broad standards, digitized fractograms of all four broad standards given in Fig. 3 were used to estimate the values of n and Φ as described in the Theory Development Section. These constants were then used to generate MW values for narrow and broad standards. Calibration constants from these calculations and resultant MWs are summarized in Tables 14 to 17. Results obtained using multible broad standards have been compared with corresponding values calculated using narrow standards.

TABLE 11

Comparison of M_n and M_w Values (kdalton) for the Four Polydisperse Polystyrene Samples Determined by SEC-MALLS with Those Obtained from ThFFF Using Calibration with Two M_n Values for Two Broad Standards. The Calibration Constants Used in Each Case Are Indicated in Table 6. The Numbers in Parentheses Indicate the Percentage Deviation from the MW Values Determined by SEC-MALLS; the Deviation Is Not Shown Where It Is Greater than 100%

Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	B1 and B2 $\Phi = 3326$ $n = 0.564$	B1 and B3 $\Phi = 5500$ $n = 0.610$	B1 and B4 $\Phi = 292$ $n = 0.337$	B2 and B3 $\Phi = 7262$ $n = 0.632$	B2 and B4 $\Phi = 49.4$ $n = 0.179$
M_n:							
B1	—	64	64 (0%)	64 (0%)	64 (0%)	69 (+7.0%)	26 (−60%)
B2	100	137	137 (0%)	131 (−4.4%)	199 (+45%)	137 (0%)	137 (0%)
B3	—	446	528 (+18%)	446 (0%)	2,400	446 (0%)	2.21×10^4
B4	—	857	427 (−50%)	385 (−55%)	857 (0%)	395 (−54%)	857 (0%)
M_w:							
B1	100	97	120 (+24%)	110 (+13%)	354	114 (+17%)	4960
B2	250	240	290 (+21%)	248 (+3.3%)	1,430	250 (+4.2%)	5.00×10^4
B3	498	467	635 (+36%)	522 (+12%)	4,110	516 (+10%)	1.65×10^5
B4	1000	1039	1720 (+65%)	1270 (+22%)	30,700	1210 (+16%)	1.76×10^7

TABLE 12

Comparison of M_p Values (kdalton) for the Six Monodisperse Polystyrene Standards Given by the Manufacturer with Those Obtained from ThFFF Using Calibration with Two M_w Values for Two Broad Standards. The Calibration Constants Used in Each Case Are Indicated in Table 7. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values

Standard number	Manufacturer (kdalton)	B1 and B2 $\Phi = 2413$ $n = 0.547$	B1 and B3 $\Phi = 4829$ $n = 0.606$	B1 and B4 $\Phi = 6470$ $n = 0.631$	B2 and B3 $\Phi = 19,500$ $n = 0.712$	B2 and B4 $\Phi = 12,420$ $n = 0.676$	B3 and B4 $\Phi = 9876$ $n = 0.660$
N1	46	38 (−17%)	43 (−6.3%)	45 (−2.2%)	62 (+35%)	57 (+24%)	53 (+15%)
N2	92	75.9 (−18%)	80 (−13%)	82 (−11%)	105 (+14%)	100 (+8.0%)	93 (+1.2%)
N3	217	216 (−0.1%)	207 (−4.3%)	204 (−6.0%)	236 (+8.8%)	233 (+7.4%)	223 (+2.7%)
N4	440	486 (+10%)	430 (−2.2%)	411 (−6.7%)	439 (−0.3%)	448 (+1.7%)	435 (−1.1%)
N5	827	1040 (+26%)	858 (+3.7%)	797 (3.7%)	789 (−4.6%)	830 (+0.4%)	819 (−0.9%)
N6	1310	1800 (+37%)	1400 (+7.2%)	1280 (−2.3%)	1200 (−8.4%)	1290 (−1.4%)	1290 (−1.7%)

TABLE 13

Comparison of M_n and M_w Values (kdalton) for the Four Polydisperse Polystyrene Samples Determined by SEC-MALLS with Those Obtained from ThFFF Using Calibration with Two M_w Values for Two Broad Standards. The Calibration Constants Used in Each Case Are Indicated in Table 7. The Numbers in Parentheses Indicate the Percentage Deviation from the MW Values Determined by SEC-MALLS

Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	B1 and B2 $\Phi = 2413$ $n = 0.547$	B1 and B3 $\Phi = 4829$ $n = 0.606$	B1 and B4 $\Phi = 6470$ $n = 0.631$	B2 and B3 $\Phi = 19{,}500$ $n = 0.712$	B2 and B4 $\Phi = 12{,}420$ $n = 0.676$	B3 and B4 $\Phi = 9876$ $n = 0.660$
M_n:								
B1	—	64	50 (−22%)	56 (−12%)	58 (−8.7%)	80 (+24%)	74 (+15%)	68 (+6.6%)
B2	100	137	109 (−20%)	115 (−16%)	117 (−15%)	149 (+8.8%)	142 (+3.6%)	133 (−2.9%)
B3	—	446	439 (−1.6%)	397 (−11%)	382 (−14%)	416 (−6.7%)	421 (−5.6%)	408 (−8.5%)
B4	—	857	345 (−60%)	341 (−60%)	338 (−61%)	395 (−54%)	390 (−54%)	372 (−57%)
M_w:								
B1	100	97	97 (0%)	97 (0%)	97 (0%)	119 (+23%)	115 (+18%)	108 (+11%)
B2	250	240	240 (0%)	221 (−7.9%)	214 (−11%)	240 (0%)	240 (0%)	231 (−3.7%)
B3	498	467	535 (+15%)	467 (0%)	443 (−5.1%)	467 (0%)	479 (+2.0%)	467 (0%)
B4	1000	1039	1510 (+45%)	1150 (+11%)	1040 (0%)	961 (−7.5%)	1040 (0%)	1040 (0%)

TABLE 14

Calibration Constants Φ and n Obtained Using Narrow and Multiple Broad Standards. M_w Values in Each Case Were From SEC-MALLS. Corrected Values Refer to Calculations in Which the ThFFF λ Values Have Been Corrected for Variations in Viscosity and Thermal Conductivity across the Channel

	Φ		n	
Calibration method	Uncorrected	Corrected	Uncorrected	Corrected
Narrow standards (see Fig. 2)	7,169	7,174	0.639	0.636
Broad standards (M_w from SEC-MALLS)	10,300	9,968	0.667	0.661

TABLE 15
Comparison of M_p Values (kdalton) for the Six Monodisperse Polystyrene Standards Given by the Manufacturer with Those Obtained from ThFFF at the Fractogram Peak Maximum Using Either Narrow or Broad Standards. The Calibration Constants Used in Each Case Were Obtained as Indicated in Table 14. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values. Data for Both Uncorrected and Those Corrected for Viscosity and Thermal Conductivity Are Given and Explained in the Text

M_p				
Standard number	Manufacturer (kdalton)	Calculation type	Φ and n from narrow standards	Φ and n from 4 broad standards using M_w obtained from SEC-MALLS
N1	46	Corrected	48 (+5.1%)	53 (+15%)
		Uncorrected	49 (+5.8%)	54 (+17%)
N2	92	Corrected	88 (−5.2%)	94 (+1.5%)
		Uncorrected	87 (−5.7%)	94 (+1.8%)
N3	217	Corrected	216 (−0.5%)	223 (+2.9%)
		Uncorrected	215 (−0.9%)	224 (+3.2%)
N4	440	Corrected	432 (−1.8%)	436 (−0.9%)
		Uncorrected	432 (−1.8%)	437 (−0.7%)
N5	827	Corrected	834 (+0.8%)	820 (−0.9%)
		Uncorrected	833 (+0.7%)	821 (−0.8%)
N6	1310	Corrected	1330 (+1.8%)	1290 (−1.5%)
		Uncorrected	1340 (+2.2%)	1290 (−1.5%)

Comparisons of results obtained from calibration using multiple broad standards (summarized in Tables 14 to 17) and those obtained from calibration using only two broad standards (summarized in Tables 12 and 13) showed that similar to conventional calibration with narrow standards, an increase in the number of standards will improve the accuracy of the

TABLE 16

Comparison of M_n Values (kdalton) for the Four Polydisperse Polystyrene Samples Determined by SEC-MALLS with Those Obtained from ThFFF Using Either Narrow or Broad Standards. The Calibration Constants Used in Each Case Were Obtained as Indicated in Table 14. The Numbers in Parentheses Indicate the Percentage Deviation from the MW Values Determined by SEC-MALLS. Data for Both Uncorrected and Those Corrected for Viscosity and Thermal Conductivity Are Given and Explained in the Text

M_n					
Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	Calculation type	Φ and n from narrow standards	Φ and n from 4 broad standards using M_w obtained from SEC-MALLS
B1	—	64	Corrected	63 (−1.6%)	68 (+6.9%)
			Uncorrected	59 (−7.8%)	66 (+2.8%)
B2	100	137	Corrected	125 (−8.8%)	134 (−2.2%)
			Uncorrected	125 (−8.8%)	135 (−1.5%)
B3	—	446	Corrected	403 (−9.6%)	408 (−8.5%)
			Uncorrected	402 (−9.9%)	409 (−8.3%)
B4	—	857	Corrected	358 (−58%)	372 (−57%)
			Uncorrected	358 (−58%)	374 (−56%)

calibration constants and hence molecular weight values determined by the calibration method. This is reflected by the fact that the deviations between the calculated average MWs and the SEC-MALLS values are generally less in the case when four broad standards are used compared to when only two are utilized.

Errors stemming from the assumption of parabolic velocity profiles for the laminar flow and the use of the $\Delta T/w$ as an approximation for the temperature gradient across the channel were also investigated. In this investigation the uncorrected retention parameters were calculated using either Eq. (2) or Eq. (4). The results for n and Φ (Table 14) and MW values (Tables 15, 16, and 17) obtained using both the corrected and uncorrected retention parameters have been compared. It can be seen that the differences in corresponding values between results based on corrected and uncorrected data are small, which is consistent with recent work reported

TABLE 17
Comparison of M_w Values (kdalton) for the Four Polydisperse Polystyrene Samples Determined by SEC-MALLS with Those Obtained from ThFFF Using Either Narrow or Broad Standards. The Calibration Constants Used in Each Case Were Obtained as Indicated in Table 14. The Numbers in Parentheses Indicate the Percentage Deviation from the MW Values Determined by SEC-MALLS. Data for Both Uncorrected and Those Corrected for Viscosity and Thermal Conductivity Are Given and Explained in the Text

M_w				Φ and n from narrow standards	Φ and n from 4 broad standards using M_w obtained from SEC-MALLS
Standard number	Manufacturer (kdalton)	SEC-MALLS (kdalton)	Calculation type		
B1	100	97	Corrected	103 (+6.2%)	109 (+12%)
			Uncorrected	98 (+1.0%)	104 (+7.2%)
B2	250	240	Corrected	226 (−5.8%)	231 (−3.7%)
			Uncorrected	226 (−5.8%)	232 (−3.3%)
B3	498	467	Corrected	466 (−0.2%)	467 (0%)
			Uncorrected	466 (−0.2%)	469 (+0.43%)
B4	1000	1039	Corrected	1082 (+4.1%)	1039 (0%)
			Uncorrected	1083 (+4.2%)	1039 (0%)

by Van Asten et al. (10). Since the values of the uncorrected λ may differ by up to 10% from the corrected values (see Table 3), it would seem that some cancellation of errors occurs in the computation of Φ and n and the resultant MW.

Corrections for band-broadening effects were not applied in this work. They are least likely to be significant for broad MW samples and were considered to be negligible by Schimpf et al. (4).

CONCLUSION

ThFFF is a promising new method for characterizing synthetic polymers. However, it requires calibration in order to derive absolute MWDs. Calibration can be readily achieved using narrow molecular weight standards, but the availability of these is restricted to a few specific polymers.

The aim of this research was to develop a calibration method which can use broad standards of a given polymer type. The theory was tested using samples of the polystyrene–THF system.

Six narrow polystyrene standards dissolved in THF were used to construct a conventional calibration line in order to provide reference data for later comparisons. The ThFFF fractograms of four broad polystyrene samples also dissolved in THF were generated, and the average MW values were calculated using the calibration constants obtained from the conventional approach. The average MW values were also measured independently by a SEC-MALLS system. These broad samples were then used as secondary standards to investigate the proposed methods utilizing each of the following calibration procedures.

1. Calibration with one broad standard using both number and weight MW averages.
2. Calibration with two broad standards using number MW averages.
3. Calibration with two broad standards using weight MW averages.
4. Calibration with multiple broad standards using weight MW averages.

Calculations of calibration constants were based on average MW data for each secondary broad standard either 1) generated by the conventional calibration line using narrow standards, or 2) obtained from the SEC-MALLS system.

It has been shown that the use of weight-average MWs is more reliable than the use of number-average MWs, and the former method yields results that are similar to those obtained with narrow standards. Increasing the number of broad standards employed for calibration improves the accuracy of the calibration constants obtained.

Errors created from the assumption of parabolic velocity profiles and the approximation of $\Delta T/w$ for the temperature gradient across the channel were also investigated. It was shown that for the polystyrene–THF combination, ignoring these assumptions produces only small errors in the final results when compared with the corresponding values obtained using the more accurate equations.

In conclusion, calibration methods for ThFFF using broad MW standards have been developed and generally validated. This approach should greatly expand the range of applicability of ThFFF, allowing absolute MWDs of different classes of polymers to be determined.

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