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Peak-referenced integral method for size exclusion chromatography and its application to aromatic polyesters

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Abstract

A new peak-referenced integral method, PRIM, greatly improves the accuracy of single detector size exclusion chromatography, SEC, calibration and analysis using broad standards. PRIM combines advantages from the narrow standard peak position calibration method, which offers high precision, and the broad standard integral calibration method, which makes no assumption regarding the column's chromatographic behavior. PRIM calibration uses the calibrant's elution peak as a boundary condition to build the elution calibration. Separate cumulative integral matchings are made between the integrated signal area and the integrated molecular weight distribution on each side of the elution peak. SEC–PRIM is illustrated using well-characterized poly(trimethylene terephthalate) samples which follow the Flory most-probable molecular weight distribution. Calibrations are demonstrated which are independent of the sample molecular weight distribution. Valid calibrations can be made which are insensitive to the cyclic oligomer elution not being fully resolved from the linear polymer elution. A self-consistent comparison between the original integral method, Hamielec method and PRIM illustrates greatest accuracy from PRIM. The SEC–PRIM results for all molecular weight averages are accurate to within 5% of absolute, noncalibrated measurements considering all samples. The high accuracy is attributed to ensuring the calibrant peak molecular weight is assigned as accurately as possible.

Keywords: Peak-referenced integral method; Molecular mass distribution; Calibration; Size-exclusion chromatography; Polyester; Poly(propylene terephthalate); Poly(trimethylene terephthalate)

1. Introduction

Size exclusion chromatography, SEC, remains the most practiced analytical technique to measure molecular weight distribution, MWD, in polymeric materials. Classically, a liquid chromatographic separation monitored with a single detector is calibrated using either a series of narrow MWD standards or a single broad MWD standard [1–3]. More recently multiple detector methods have been developed which do not require standard MWD calibrants per se, e.g. see [4,5] and references cited therein. These multiple detector methods may soon become more widely and easily utilized, although these still require additional material property values be known for each sample and the properties can depend on molecular weight. Although the calibration methods for single detector SEC are facile and familiar, these methods typically provide very limited accuracy. This communication describes a new peak-referenced integral method, PRIM, which greatly im-

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proves the accuracy of single detector SEC calibration.

PRIM combines advantages from the peak position calibration [1–3] and integral calibration [1–3] methods. Peak position calibration using a series of narrow MWD standards precisely establishes a curve relating the peak retention time to molecular weight. The peak position calibration method has rather limited use in practice since the many required narrow standards may be prohibitively difficult or impossible to obtain for any given polymer. The integral method requires only one broad MWD standard. The presumed advantage of the integral method is that it makes no assumption regarding the column's chromatographic behavior and could accurately describe nonlinear calibration curves. However, this advantage often does not occur in practice. At the extreme ends of a calibration curve, the calibration points for the integral method are not very reliable because they can be greatly affected by: column dispersion, the choice of baseline and the choice of the integration limits. Hence even the molecular weight of the elution peak is not always assigned accurately. The increased accuracy of PRIM derives from its use of the most prominent, well-defined feature in the elution curve, the peak, as a boundary condition to build the elution calibration. Two separate cumulative integral matchings are made between the integrated signal area and the integrated molecular weight distribution. Each matching starts from the elution peak. One cumulative integral matching is done for molecular weights less than the peak and the other is done for molecular weights greater than the peak. The method does not assume or require that the column response be linear or ideal. PRIM calibration determines the actual column response. Like other broad standard approaches, PRIM–SEC offers a practical way of obtaining SEC calibration curves that are specific to polymer type. These methods require only one calibration standard of known broad MWD with the same structure as the unknown samples.

A widely-used broad standard method for single detector SEC was proposed by Hamielec [6], see also [1]. The Hamielec method requires only that molecular weight averages M_N and M_W be known for a calibration sample. The calibration consists of a search for an effective linear calibration making some account of instrumental peak broadening. When SEC is designed for linear column separations the original integral method is no more versatile or accurate than the Hamielec method [1]. The two major weaknesses of the Hamielec method are: there is no physical significance to the effective calibration line and the calculated molecular weight values are accurate only for samples having an MWD curve similar to that of the standard [1]. This communication provides evidence that PRIM can be substantially more accurate than the original integral method and the Hamielec method.

This communication characterizes the accuracy and reliability of the SEC–PRIM using simple, single detector liquid chromatography instrumentation. SEC–PRIM can be practiced with: a minimum number of calibration standards; no assumptions about column operation, i.e. linearity and dispersion; and self-consistency checks when something is known about the sample. Section 2 describes the main SEC instrumentation used as well as the synthesis of poly(trimethylene terephthalate), PTT, samples and their characterizations by viscometry, light scattering, NMR and SEC. Section 3 derives the key mathematical relationships used for PRIM calibrations. Section 4.1 illustrates that PRIM can establish a valid calibration which is independent of the sample's MWD. In Section 4.2 we compare the accuracy of SEC–PRIM relative to two absolute molecular weight measurement methods and two other broad-MWD calibrated methods.

2. Experimental

2.1. Chemicals and reagents

Four research-grade samples of poly(trimethylene terephthalate) were provided by DuPont (DuPont, Experimental Station, Route 141, Wilmington, Delaware 19880). These samples were synthesized by transesterification of dimethyl terephthalate and trimethylene glycol followed by rapid polycondensation in 100 lb. batch autoclave melt polymerizations. These four different batches were run to different polycondensation conversions under vacuum, extruded into ribbon and quenched in cold water. These samples were independently characterized by NMR,

light scattering, viscometry and multiple detector SEC as described in the next section.

The SEC carrier solvent is 1,1,1,3,3,3-hexafluoro-2-propanol, HFIP, with 10 m*M* sodium trifluoroacetate, NaTFA. The HFIP was supplied by DuPont at 99+% purity. The NaTFA was used as originally supplied by Aldrich Chemical Company at 98% grade.

2.2. Independent characterizations

Multiple detector SEC, MDSEC, was performed using a Waters (Waters Corporation, 34 Maple Street, Milford Massachusetts 01757) HPLC 150C with one Shodex® GPC HFIP800P and two Shodex® GPC HFIP80M columns in the same SEC solvent carrier as described in the previous section. The effluent was recorded by a Wyatt model DAWN F multiangle laser light scattering detector, a Viscotek 150R viscometer and a Waters 150C refractometer. The light scattering provides a direct measurement of the weight average molecular weight. Data collection and analysis was accomplished using VISCOTEK TriSEC v2.7 software.

The inherent viscosity, IV, is inferred by measuring the relative viscosity in a Viscotek (Viscotek Corporation, 15600 W. Hardy Road, Houston, TX 77060) forced-flow viscometry instrument model Y-500. The viscometer consists of differential transducers between two capillaries whose response signal is recorded and calibrated to infer viscosity. The polyester sample is dissolved 0.4 wt% and measured in the binary solvent system trifluoroacetic acid/methylene chloride 50/50 wt%. These results are correlated to the equivalent viscosity measured in phenol/tetrachloroethane 60/40 wt%. The latter solvent system is the more standard, common choice for lower molecular weight aromatic polyesters, but has limited use for high polymer. A correlation has been previously made by comparing the aforementioned IV with number-average molecular weight, M_N, determined directly and absolutely using H^1-NMR. Here the NMR spectra produce very accurate counting of the number of protons at both carboxylic acid and glycol ends. The number average molecular weight is simply determined knowing the ratio of the number of terephthaloyl units to the number of ends. The IV power law correlation, $3.901\times10^{-4}M_N^{0.7602}$, is based on over 20 melt polymerized samples spanning a molecular weight range which includes the samples mentioned in this communication. The correlation precision is 10% in number average molecular weight.

2.3. Chromatography instrumentation and methodology

The SEC carrier solvent is the aforementioned 10 m*M* NaTFA in HFIP. SEC samples were prepared by dissolving 3 mg polymer in 3 g of carrier solvent; passing the solution through virgin, sterilized 0.2 μm Teflon® filter syringes; and sealing the effluent in silicone capped glass vials. All samples were stored at 25°C and run through replicated SEC elutions within 24 h of sample preparation to avoid molecular weight loss via alcoholysis. Previous studies indicate molecular weight loss to be undetectable within this time frame.

SEC was performed using an HP1100 Series HPLC instrument (Hewlett Packard Company, 2850 Centerville Road, Wilmington, Delaware 19808). Solvent from the reservoir is degassed using a model G1322A on-line degasser and pumped through: one channel of a model G1312A binary pump, a model G1313A autosampler with a 6-port injection valve, a model G1316A thermostated column compartment, the separation columns, a model G1315A Diode Array Detector, DAD, then an HP1047A refractive index detector. The column compartment temperature is maintained at 45°C. The DAD spectral response is between 190 and 950 nm and has linear absorbance response up to 2 AU. The DAD was autobalanced before each sample to reference the spectrum to the carrier solvent. Two DAD signals were collected as PTT has absorption bands near 247 and 280 nm. The first sample signal is centered at 245 nm with a bandwidth of 5 nm and the second sample signal is centered at 280 nm with a bandwidth of 10 nm. Both signals are referenced to a baseline signal centered at 500 nm with a bandwidth of 10 nm. The response time is set at 2 s. Data collection rates for both the DAD and RI detectors were 1 Hz.

This study uses two PLGel® Mixed-B columns (Polymer Laboratories, 160 Old Farm Road, Amherst, MA 01002) with 10 μm particle sizes of

poly(styrene-co-divinyl benzene). Each column is 300 mm long and 7.5mm in diameter. These were obtained in tetrahydrofuran and transitioned slowly to the HFIP SEC carrier solvent. The theoretical number of plates was calculated from an elution of acetone in the carrier HFIP from the peak height, retention volume and peak area, see Yau et al. [1] p. 91. Two Mixed-B columns consistently provide greater than 25 000 plates. This system permits valid MWD analysis with negligible axial dispersion effects.

Basic instrument control and data collection was processed by a Hewlett Packard Vectra XM Series 4 with a 150 MHz Pentium® processor using HP-IB interfacing. The instrument control and data collection software was the Hewlett Packard ChemStation® for LC version A.04.02. The integral method calculations were performed using the Polymer Laboratories PL Caliber® software version 4.0. The new PRIM calculations were performed using the PL Caliber® software to define baselines and signal regions while calling a user-written program written in Microsoft Visual Basic® to perform the actual PRIM calibrations and analyses. A copy of the PRIM software is available on request.

3. Analysis

3.1. SEC elution

Before deriving the PRIM calibration it is necessary to understand how molecular weight, concentration and molecular weight distribution are related to an SEC signal. The description in this communication extends the analysis of Shortt [7] to derive the location of the peak molecular weight in the SEC elution. This Section also reviews the Flory distribution which is used to illustrate PRIM calibration examples in this communication.

First it is necessary to define clearly our terms describing molecular weight distribution. A polymer chain's molecular weight, M, is equal to the chain length, x, times the molecular weight of the repeat unit, M_r. The cumulative weight fraction $W(M)$ is defined as the weight fraction of molecules in the sample having molecular weight less than M. The corresponding differential distribution, $w(M)$, for weight is defined by

$$w(M) = \frac{dW(M)}{dM} \tag{1}$$

such that the quantity $w(M)dM$ is the weight fraction of sample having a molecular weight between M and $M+dM$. It will also be useful to consider the cumulative weight fraction in terms of the integral, I_0^x, over chain lengths

$$I_0^x \equiv W(x) = \int_0^x w(x')\,dx' \tag{2}$$

At equilibrium all linear condensation homopolymers follow Flory's most probable distribution [8] and this work uses this distribution to illustrate the SEC–PRIM. This distribution is parametrically defined in terms of the polymerization conversion, p, which is zero for the equilibrium monomer and approaches unity for equilibrium, high molecular weight distributions. The weight fraction $w(x)$ of chains having length x for a given conversion is given by

$$w = x(1-p)p^{x-1} \tag{3}$$

.The cumulative weight fraction integral is therefore

$$I_0^x = 1 + p^x(x \ln p - 1). \tag{4}$$

In this communication *ln*() is the natural log, or logarithm with the base e. The number average molecular weight, M_N, weight–average molecular weight, M_W, and Z-average molecular weight, M_Z, can be expressed in terms of the repeat unit molecular weight and the polymerization conversion

$$M_N = M_r \frac{1}{1-p}, \tag{5}$$

$$M_W = M_r \frac{1+p}{1-p}, \tag{6}$$

$$M_Z = M_r \frac{p^2+4p+1}{(1-p)(1+p)}. \tag{7}$$

For calibrants having Flory's most-probable MWD, the PRIM software uses only M_r and M_W to define the entire molecular weight distribution. First the conversion, p, is computed from Eq. (6), then the

distribution integral in Eq. (4) is computed as required in the next Section.

Next, we relate the detector signal response to the eluted MWD. Let $S(t)$ be the concentration-sensitive detector response after subtraction of the baseline, where t is the elution time. If $S(t)$ is measured with a refractive index or UV/VIS spectrometer, we assume that $S(t)\mathrm{d}t$ is proportional to the mass of the sample in the detector between times t and $t+\mathrm{d}t$. Let $F(t)$ be the weight fraction of sample eluted up to retention time t. Then for each incremental change in time, the quantity $S(t)\mathrm{d}t$ is proportional to the incremental change in $F(t)$. If the detector signal were normalized by its integral over the entire elution, then we can write the equality

$$\frac{S(t)}{\int_{T_o}^{T_\infty} S(\tau)\,\mathrm{d}\tau} = \frac{\mathrm{d}F(t)}{\mathrm{d}t} \tag{8}$$

We use the convention that the highest molecular lengths elute first at time T_∞ and the monomers elute last at time T_o. Since the SEC elution times correspond to smaller molecular weights, the cumulative quantities $F(t)$ and $W(t)$ are related by

$$F(t) = 1 - W(t) \tag{9}$$

Thus Eqs. (1), (8) and (9) can be combined to yield

$$S(t) \propto w(t)\left(\frac{\mathrm{d}x(t)}{\mathrm{d}t}\right) \tag{10}$$

This equation can be used to determine the molecular length, x_p, at the peak elution time, T_p, if we know the molecular weight distribution $w(x)$. If the column response is not linear then x_p is determined implicitly from Eq. (10). In the most common practice of SEC, the column is designed for a ''linear'' response, i.e. the chain lengths are separated logarithmically with time, i.e.

$$-\mathrm{d}\ln(x) \propto \mathrm{d}t \tag{11}$$

Combining proportionalities (10) and (11), we see that the detector response is proportional to the molecular chain length times the weight fraction of that chain length eluting at the given time

$$S(t) \propto x(t)\,w(t) \tag{12}$$

under linear column chromatography. We can thus determine x_p by finding the maximum in

$$S[x(t)] \propto x(t)\,w[x(t)] \tag{13}$$

with respect to the molecular length, x. This result is general for any molecular weight distribution and SEC experiment when the peak elutes in the linear region of the column response.

Finally, let us consider the specific class of experiments in which we elute samples which have a Flory distribution of molecular weights. The weight fraction obeys Eq. (3) and the signal follows Eq. (13). Combining these results we find that the chain length, x_p, at the elution peak is given by

$$x_p = \frac{-2}{\ln p} = \frac{2}{\ln\left(\frac{M_W + M_r}{M_W - M_r}\right)} \tag{14}$$

as long as the peak elutes in the linear response of the SEC column and the sample has a Flory MWD.

3.2. PRIM calibration

The main advantage of the PRIM derives from its use of the most prominent, well-defined feature in the elution curve, the peak, as a boundary condition to build the elution calibration. We assume the molecular weight distribution is known for a broad MWD calibration standard. The peak elution time, T_p, of the sample is easily determined from the SEC experiment. Please note PRIM calibration neither requires nor assumes that the column response is either linear or ideal. PRIM calibration determines the actual column response. Calibration is facilitated if the column response is found to be linear in the region of the calibrant's elution peak since one can use Eq. (13) to determine M_p.

The PRIM calibration method is illustrated schematically in Fig. 1(a)–(c). Fig. 1(a) illustrates a typical aromatic polyester elution curve, depicting the DAD response as a function elution time. The middle vertical dashed line is drawn at the elution peak, defining the peak elution time, T_p, used for the calibration. Hence the first known point in the calibration curve in Fig. 1(c) is positioned at time T_p and molecular weight M_p. To complete a broad standard calibration, a user must define the begin-

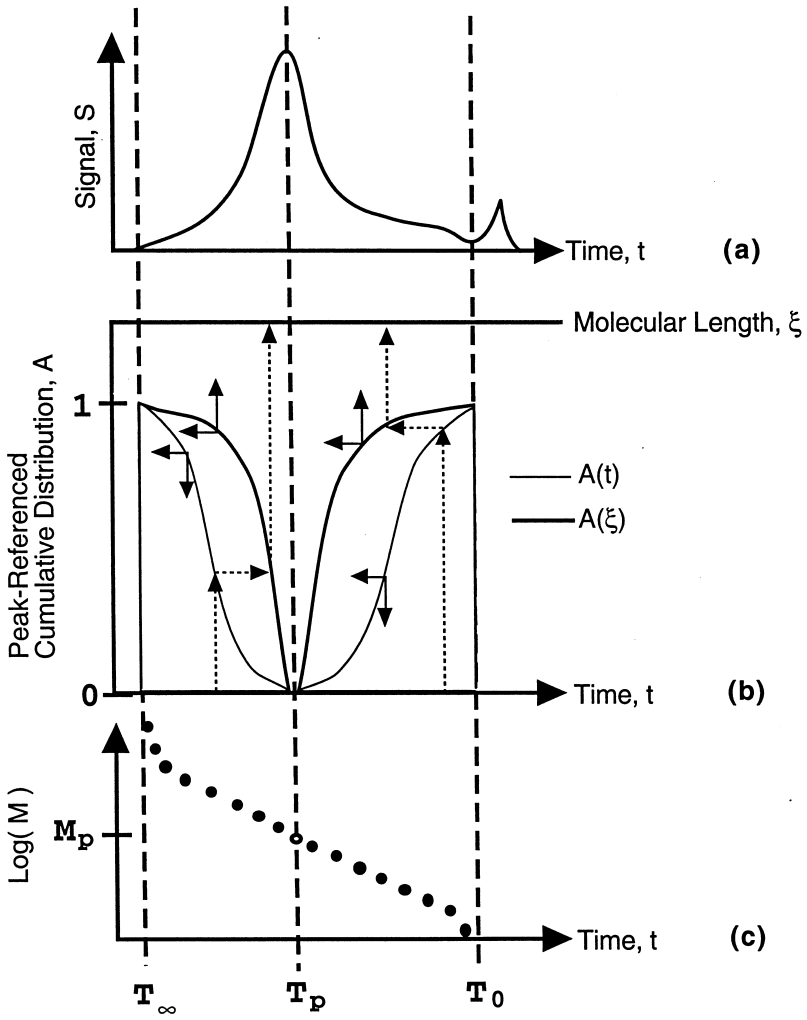

Fig. 1. Illustration of the Peak-Referenced Integral Method (PRIM). The sample elution (a), as typically observed by a UV/Vis spectrophotometric detector, is truncated between times T_∞ and T_0. The peak molecular weight, M_P, at the peak elution time, T_P, is the first point determined for the calibration in (c). The remaining calibration points in (c) are determined by two separate cumulative distribution matching sections illustrated in (b).

ning, time T_∞, and the end, time T_0, of the polymer calibrant elution signal, e.g. as shown by the first and third vertical dashed lines, respectively. In most cases there is no ambiguity setting the end of the polymer elution curve. In the case of condensation polymers, there will typically be a cyclic oligomer peak immediately following the polymer elution. Often the detector signal will not return to the baseline between the polymer and cyclic oligomer elution peaks, i.e. the low molecular weight linear oligomers might not be perfectly resolved from the cyclic oligomers. This is, in fact, the case with the aforementioned samples and columns. Here we choose to define the end of the polymer elution at the valley bottom between the polymer and cyclic oligomer peaks. Alternatively, one can fit the contribution due solely to the cyclic oligomer, subtract this contribution from the signal and analyze the remaining curve due soley from the polymer, e.g. see [9]. In this way there would be no ambiguity in setting a baseline and the appropriate fraction of the low molecular weight chains would be included in the

analysis. Otherwise, the method must simply be insensitive to the cyclic oligomer contribution.

The basis for the PRIM calibration is matching a cumulative detector signal area with a cumulative weight fraction of a known calibration MWD. Combining Eqs. (2), (8) and (9) we find

$$I_0^{x(t)} = 1 - \frac{\int_{T_\infty}^{t} S(\tau)\,\mathrm{d}\tau}{\int_{T_\infty}^{T_o} S(\tau)\,\mathrm{d}\tau} \tag{15}$$

where $T_0 \geq t \geq T_\infty$. This equation is valid for either linear or nonlinear column response and any calibrant MWD. The numerator is the cumulative area from the beginning of the elution to a given time, t, and the denominator is the cumulative area of the entire elution. The original Integral Method uses only Eq. (15) to calibrate a column set by matching a known molecular weight's cumulative weight fraction, e.g. Eq. (4) for a Flory MWD, to the elution time with the same cumulative signal area. The PRIM calibration uses two cumulative matchings instead.

We now divide the polymer elution curve into two sections: the high molecular weight section at times before T_p and the low molecular weight section at times after T_p. First, it is useful to compute the areas of each section relative to the total elution area. Let A_{low} designate the area fraction for the low molecular weight section and A_{high} designate the area fraction for the high molecular weight section. Comparison of these two area fractions can provide an internal consistency check for the validity of the calibration. The fraction A_{low} should equal the cumulative weight fraction integral up to the peak molecular weight, $I_0^{x_p}$. For the special case when the calibrant follows the Flory distribution, we have

$$I_0^{x_p} = 1 - \frac{3}{e^2} \approx 0.594$$

which is independent of polymerization conversion! For other distributions a similar comparison should be made between the fractional area of the elution curves and the known cumulative weight fraction up to the peak chain length, x_p. If the signal area for the low molecular weight fraction departs significantly from the theoretical value, then either the calibration sample is not well characterized and/or the detector signal is nonlinear with concentration.

In order to construct the PRIM calibration, we must match peak-referenced cumulative curves between the signal area and MWD in both the low and high molecular weight elution sections. The matching operations are illustrated in Fig. 1(b). The peak-referenced cumulative signal area curve, $A(t \geq T_p)$, for the low molecular weight section describes the normalized signal area starting from the elution peak until the low molecular weight limit of the elution curve, T_0.

$$A(t \geq T_p) \equiv \frac{\int_{T_p}^{t} S(\tau)\,\mathrm{d}\tau}{\int_{T_p}^{T_0} S(\tau)\,\mathrm{d}\tau} \tag{16}$$

The $A(t)$ functions are represented by the narrow curves in Fig. 1(b). The peak-referenced cumulative molecular length distribution, $A(\xi)$, for the low molecular weight section is defined by

$$A(\xi \leq x_p) \equiv \frac{I_0^{x_p} - I_0^{\xi}}{I_0^{x_p}} \tag{17}$$

The $A(\xi)$ functions are represented by the thick curves in Fig. 1(b). In building our calibration, each elution time, t, is paired with a molecular length value ξ such that $A(t) = A(\xi)$. In this way we obtain the calibration points in Fig. 1(c) between times T_p and T_0. This matching procedure can be done with any broad MWD, whether the MWD I_0^{ξ} integrals are determined by either a numerical table or an analytical expression. In the special case of a Flory most-probable distribution, we have an analytical expression for the peak-referenced cumulative molecular weight distribution for the low molecular weight elution section.

$$A(\xi \leq x_p) = \frac{p^{x_p}(x_p \ln p - 1) - p^{\xi}(\xi \ln p - 1)}{p^{x_p}(x_p \ln p - 1) + 1} \tag{18}$$

Likewise, the peak-referenced cumulative signal area curve, $A(t \leq T_p)$ for the high molecular weight sec-

tion describes the normalized signal area starting from the elution peak until the high molecular weight limit of the elution curve at time T_∞.

$$A(t \leq T_p) \equiv \frac{\int_{T_p}^{t} S(\tau)\,d\tau}{\int_{T_p}^{T_\infty} S(\tau)\,d\tau} \quad (19)$$

The peak-referenced cumulative molecular length distribution, $A(\xi)$, for the high molecular weight section is defined by

$$A(\xi \geq x_p) \equiv \frac{I_0^{\xi} - I_0^{x_p}}{I_0^{\infty} - I_0^{x_p}} \quad (20)$$

In building our calibration, each elution time, t, is paired with a molecular length value ξ such that $A(t) = A(\xi)$. In this way we obtain the calibration points in Fig. 1(c) between times T_p and T_∞. This matching procedure can be done with any broad MWD, whether the MWD I_0^{ξ} integrals are determined by either a numerical table or an analytical expression. In the special case of a Flory most-probable distribution, we have an analytical expression for the peak-referenced cumulative molecular weight distribution for the high molecular weight elution section.

$$A(\xi \geq x_p) = 1 - \frac{p^{\xi}(\xi \ln p - 1)}{p^{x_p}(x_p \ln p - 1)} \quad (21)$$

Therefore, two separate cumulative integral matchings are made between the integrated signal area and the integrated molecular weight distribution. Each matching starts from the elution peak. Fig. 1(b) and (c) illustrate that one cumulative integral matching is done for molecular weights less than the peak and the other is done for molecular weights greater than the peak. The method using Eqs. (17) and (20) does not assume or require that the column response be linear, or that an analytical expression for the MWD be known. The method utilizing Eqs. (18) and (21) is appropriate for a Flory most probable MWD where the peak elutes in the linear response regime.

Ideally one selects a broad standard for PRIM calibration whose elution spans the chromatographic column's total exclusion and permeation limits. The polymer standards utilized in this work have MWDs which follow Flory's most probable distribution in which the polydispersity index is two. The highest molecular weight standard contains detectable macromolecules which approach the column total exclusion limit. This type of broad distribution has been used previously to demonstrate the original integral method [10].

4. Results and Discussion

4.1. Experimental method analysis

An SEC experiment represents a choice among several method variables such as carrier solvent, column configuration, elution flow rate, injection volume, detector signal and baseline corrections. A method is considered valid if samples of different MWD elute reproducibly and according to the same relationship expressing molecular weight eluted as a function of time, such as the calibration illustrated in Fig. 1(c). For this communication we have four PTT samples spanning a wide range of MWDs each carefully characterized by independent techniques. We wish to demonstrate briefly that the aforementioned method variables influence the PRIM to establish a calibration which is independent of the sample's MWD.

Consideration of experimental variables will be limited. The solvent carrier and temperatures are identical for all elutions. A few examples are presented to illustrate the DAD at two different wavelengths and two choices for defining the elution baselines. A ''peak'' baseline refers to defining the baseline as the line defined by the signal at the immediate ends of the polymer elution peak, beginning at T_∞ and ending at T_0. A ''signal'' baseline within the elution peak is defined by interpolating the observed flat, horizontal signal between times very much earlier and later than the elution peak. The signal baseline method truncates the elution signal at T_0 with a vertical line. Since the elution of the linear and cyclic oligomers are almost completely resolved, there can be a small, but noticeable difference between the two baseline choices. The peak intensity changes slightly and the low molecular weight signal

Table 1
Normalized signal area fractions, A_{low}, between the elution peak at T_p and the low-molecular weight tail ending at T_0. The total areas for all elution curves are normalized to unity. For each of the four PTT samples, results are tabulated using either: the refractive index, RI, detector or DAD methods; signal or peak baseline definitions; and injection amounts. DAD signals are analyzed at spectral peaks at either 245 nm or 280 nm. All elutions are from identical sample concentrations and two PLGel® Mixed-B columns. The standard deviation for the area fraction from repeated injections of the same sample is ca. ±0.003. The theoretical value for the normalized area fraction between the elution peak and low-molecular weight tail in all cases is 0.594

Sample	RI detector signal base 5 μl inject	RI detector peak base 5 μl inject	DAD 245 nm signal base 5 μl inject	DAD 245 nm peak base 5 μl inject	DAD 280 nm signal base 5 μl inject	DAD 280 nm peak base 5 μl inject	DAD 280 nm signal base 50 μl inject	DAD 280 nm peak base 50 μl inject
PTT−1	0.598	0.597	0.593	0.588	0.592	0.589	0.586	0.579
PTT−2	0.621	0.605	0.610	0.599	0.606	0.599	0.604	0.597
PTT−3	0.599	0.589	0.604	0.596	0.605	0.596	0.598	0.592
PTT−4	0.548	0.573	0.566	0.563	0.565	0.558	0.563	0.555

area fraction A_{low} is consistently larger for the signal baseline choice.

Table 1 summarizes the observed low molecular weight signal area fraction for several of the varied methods. The theoretical value of 0.594 pertains to all methods and samples. Although systematic deviations are observed, none of these area fractions deviate from theory by more than 8% in absolute value. Deviations from theory using the DAD are limited between +2.7% to −6.6% for all methods. The method with the lowest deviation range, +1.8% to −3.5%, uses the refractive index detector and peak baseline. If the original Integral Method is used to formulate a calibration with deviations of this magnitude, the peak molecular weight will not be assigned accurately and significant errors will propagate in analyzing unknown samples, see Section 4.2.

In the remainder of this Section, each of the PTT samples will be example PRIM calibration standards. Each sample calibration will be identified based on its M_W value as determined by NMR, which is underlined in Table 2. Further discussion of the results in Table 2 is left for the next section. In Figs. 2–4 each calibration curve illustrates molecular weight elution as a function of time for each sample as computed by PRIM calibration. Each sample is assumed to contain a Flory MWD. Since each sample elution consists of nearly one thousand data points, the calibrations are drawn using lines between data points with only six filled markers at data points to distinguish between samples. In Figs. 2–4 the peaks in each sample elution are depicted explicitly by open circles at the peak data point. The best exponential function fitting the four peaks is obtained by linear regression and illustrated by the wider gray line in each figure. This regression line is equivalent to an independent SEC calibration using the samples like separate peak position standards! This calibration serves as a linear response reference when comparing the following experimental methods.

Peak baseline methods provide valid MWD-independent PRIM calibrations. Figs. 2 and 3 illustrate calibrations using the DAD data at 245 nm with signal and peak baseline definitions, respectively. The signal baseline method in Fig. 2 exhibits MWD-dependent molecular weight assignments for molecular weights less than 10^4. Note the highest MW samples are eluted with greater resolution from the cyclic oligomer peak than the lower MW samples. Hence, the vertical termination of the elution in a signal baseline method does not well approximate the true polymer elution signal shape. This effect is less severe for the low molecular weight calibrations. There is greater overlap between the cyclic and linear oligomers so the low molecular weight side of the signal is more similar to a vertical. Since Fig. 2 indicates an apparent MWD-dependent calibration at the low molecular weights, the signal baseline method does not provide a valid calibration. In contrast, the peak baseline method better preserves the appropriate shape of the polymer elution curve at the ends. Fig. 3 illustrates the peak baseline method curves are sensibly independent of MWD, so this method provides a valid calibration. Furthermore, the molecular weight assignment curves more closely superpose to the overall linear response for molecular

Table 2
Self-consistent comparisons of molecular weight averages for four samples of poly(trimethylene terephthalate), PTT. IV–NMR indicates a correlation between NMR determination of M_N and IV. MDSEC is Multiple-Detector SEC. The Hamielec Method, SEC–HM, and original Integral Method, SEC–IM, were implemented using Polymer Laboratories PLCaliber® software. Results reported for SEC–HM, SEC–IM and SEC–PRIM all use the same elution curve for each sample

Sample	IV (l/kg)	IV–NMR[a] M_N M_W M_Z	MDSEC M_N M_W M_Z	SEC–HM[b] M_N M_W M_Z	SEC–IM[b] M_N M_W M_Z	SEC–PRIM[b] M_N M_W M_Z
PTT-1	24.4	4 800	4 300	6 000	5 700	4 700
		9 300	7 800	10 300	9 900	8 900
		14 000	10 900	15 600	15 100	13 700
PTT-2	33.4	7 100	7 200	8 700	8 300	7 100
		14 200	12 100	16 100	15 600	14 000
		21 300	16 700	24 800	24 100	21 900
PTT-3	67.8	18 300	14 000	22 000	20 700	18 200
		36 500	36 000	41 000	40 000	36 300
		55 000	48 500	60 500	59 300	54 600
PTT-4	87.8	25 500	17 800	26 100	24 900	25 700
		51 100	49 100	50 200	49 000	51 400
		77 000	73 700	76 000	73 300	76 900

[a] M_N computed from IV–NMR correlation; M_W, M_Z computed from M_N assuming Flory distribution.
[b] Sample PTT-4 taken as standard assuming $M_w = 51\,100$ and Flory distribution, signal DAD at 280 nm, elutions through two Mixed-B columns, peak baselines used for analysis.

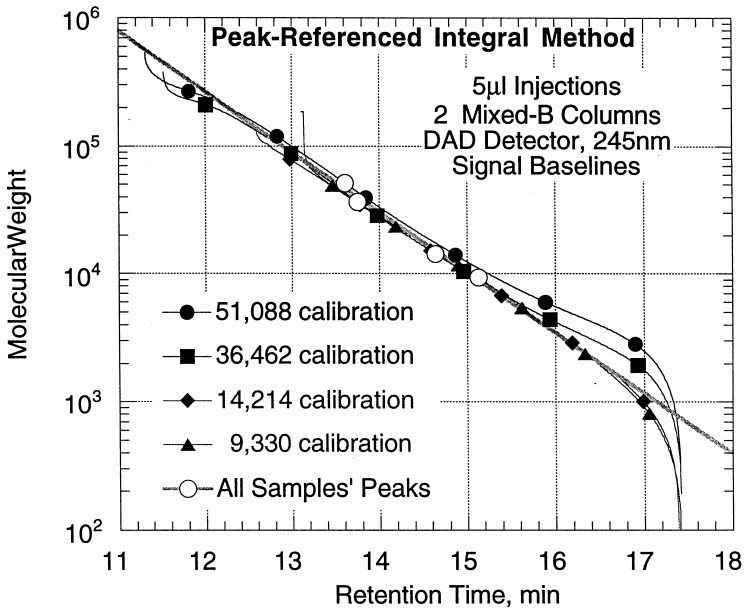

Fig. 2. PRIM calibrations for the conditions indicated assuming each sample contains a Flory distribution with a weight average molecular weight inferred from NMR measurement of M_N. Calibrations are drawn using lines between data points with only six filled markers at points to distinguish samples. The peaks for each sample are depicted explicitly by open circles. The best exponential function fitting the four peaks is obtained by linear regression and depicted by the wider gray line.

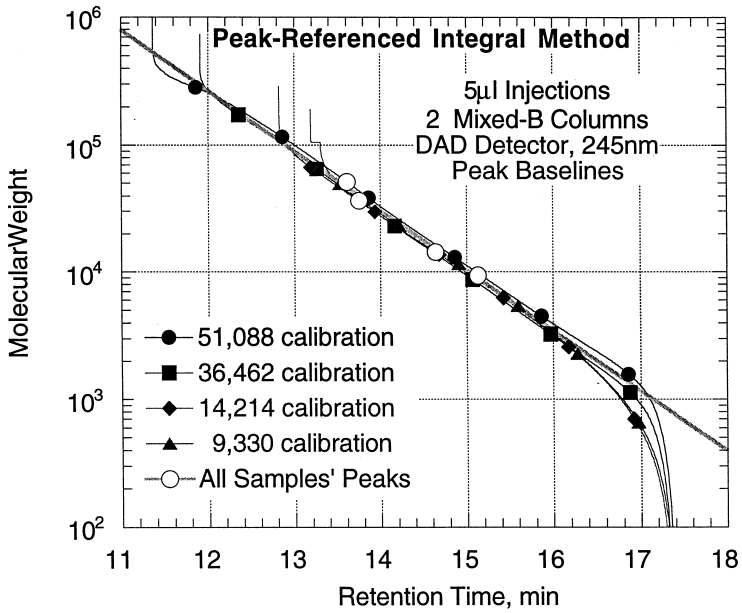

Fig. 3. PRIM calibrations for the conditions indicated assuming each sample contains a Flory distribution with a weight average molecular weight inferred from NMR measurement of M_N. Calibrations are drawn using lines between data points with only six filled markers at points to distinguish samples. The peaks for each sample are depicted explicitly by open circles. The best exponential function fitting the four peaks is obtained by linear regression and depicted by the wider gray line.

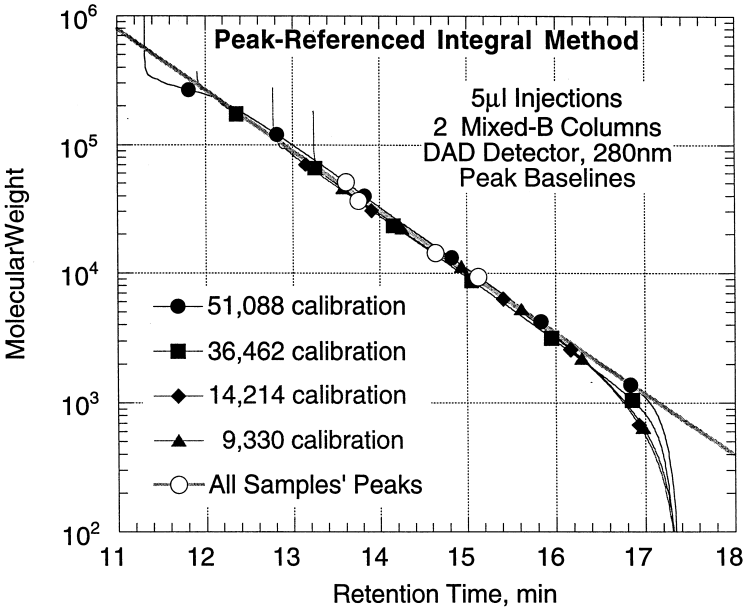

Fig. 4. PRIM calibrations for the conditions indicated assuming each sample contains a Flory distribution with a weight average molecular weight inferred from NMR measurement of M_N. Calibrations are drawn using lines between data points with only six filled markers at points to distinguish samples. The peaks for each sample are depicted explicitly by open circles. The best exponential function fitting the four peaks is obtained by linear regression and depicted by the wider gray line.

weights over 2×10^3, which is the reported total permeation threshold for this column type. Table 1 indicates that the peak baseline method does not significantly better reproduce the theoretical signal area for the low molecular weight fraction. Similar curves are obtained for the DAD data at 280 nm comparing signal and peak baseline methods. Fig. 4 illustrates that PRIM calibrations using the DAD at 280 nm and peak baselines also provides a valid calibration method. Similar valid calibration is also obtained with the refractive index signal data using the peak baseline method.

The methods illustrated in Figs. 2–4 exhibit some similar features. In all cases the peaks elute in a linear response regime of the method. All calibration curves superpose each other within the linear response regime. Furthermore, the linear response regime is described identically by both the fitted peak position line and each of the sample's PRIM calibration. These observations are independent results from SEC–PRIM, not assumed a priori. Some of the following methods exhibit less deviation from linear response than others. The two highest M_W calibration curves exhibit slight exclusion limitation for the longest chain lengths in the MWDs, as evident by an increasing slope deviating from the linear response line. This is in accord with the column manufacturer's data. The two lowest M_W calibration curves also exhibit an increasing slope deviation for their longest chain lengths, but this is not due to a size exclusion in the columns. This apparent slope deviation is actually at only the couple end data points which are the last data points to be matched using Eqs. (19) and (21). The last data point must reconcile the entire remaining cumulative signal area with the infinite MWD limit and suffers from signal noise or detection threshold error. Therefore a positive deviation from the linear response is observed. Negative deviations from the linear response at the lowest molecular weights and longest elution times are related to column performance and is typically observed for PTT at molecular weights near 10^3 or chain lengths much less than ten. Here all the smallest chains permeate all the smallest pores present in the column packings. Smaller pore sizes would be required to maintain the linear response. Differences between calibrants at the end of their elutions are more significantly influenced by the relative overlap between the linear and cyclic oligomers. Cyclic oligomer content is known to be a function of polymerization conversion [8], but this effect is small for the polymer conversions considered herein.

4.2. Assessment of SEC–PRIM advantages

In this Section we compare the accuracy of SEC–PRIM relative to two absolute molecular weight measurement methods and two calibrated methods for the PTT samples. The data required for this comparison are collected in Table 2. The absolute methods, NMR and MDSEC, are described in Section 2.2. NMR directly determines the number of ends, thus providing M_N. Values for M_W and M_Z are then inferred assuming each sample contains a Flory MWD using Eqs. (5)–(7). MDSEC infers these MWD averages directly, the values reported do not depend on an assumption that each sample contains a Flory MWD. The calibrated methods considered are the original integral method, SEC–IM, the Hamielec method, SEC–HM, and the peak-referenced integral method, SEC–PRIM. The SEC–IM uses only a single cumulative matching between a calibrant's MWD and elution signal based on Eq. (15). No assumption that the column operates with a linear response is made in an SEC–IM calibration. The SEC–HM assumes that the column operation can be described with an effective linear response and makes some account of instrumental peak broadening. Both the SEC–IM and SEC–HM are described in standard texts, see [1–3].

The molecular weight averages based on the absolute methods agree remarkably well. The average molecular weights inferred by NMR and MDSEC agree within 16% for all samples. NMR is believed to be more accurate for the lowest molecular weight samples as the relative number of ends is easily quantified in the spectrum while some parameters, such as the change in refractive index with concentration, required for MDSEC are not concentration invariant for low MWDs. The number average molecular weights inferred from NMR are considered upper bounds since the number of acid ends are not as accurately quantified as glycol ends, but this issue is mainly relevant for high MWD samples. Results from MDSEC are noticeably affect-

ed by baseline selection method and the relative overlap between linear and cyclic oligomers. This is most problematic for the number average molecular weight precision, and lesser problematic for the *Z*-average molecular weight. With these considerations in mind, we have chosen the NMR values underlined in Table 2 as the targets for the calibrated SEC methods. The reader should note that if we had chosen the NMR M_W values for PTT-1 and PTT-2, but the MDSEC M_W values for PTT-3 and PTT-4, then the PRIM calibrations in Figs. 2–4 would collapse more closely together within the linear response regimes. We note that there are currently no commerically available standards for PTT and universal calibration for PTT in buffered HFIP has not yet been proven.

The SEC–IM, SEC–HM and SEC–PRIM comparison is self-consistent because the same elution data are used for all methods. Since these methods require a single calibration standard, we have assigned the highest MWD sample PTT-4 as having the number average molecular weight as inferred by NMR, computed the corresponding Flory MWD using Eqs. (4)–(6), and used this sample's elution data to build calibration curves according to the recipe for each method. The remaining samples are then treated as unknowns and we compare how closely the three calibrated SEC methods compare to each other as well as the absolute methods. Deviations in M_W for PTT-4 is due to the precision of the numerical methodology.

Using PTT-4 as the single, broad-MWD calibration standard, the SEC–PRIM most accurately and consistently reproduces the molecular weight averages than SEC–HM and SEC–IM. Each of these calibrated methods reproduce the averages for PTT-4 within 5%. Errors in M_W are consistently smaller than the errors in M_N and M_Z for SEC–IM and SEC–HM for the remaining samples, but these errors are significantly larger than for SEC–PRIM. Errors for SEC–HM and SEC–IM values in M_N and M_Z increase rapidly with decreasing MWD, i.e. the results for PTT-1 are far less accurate than those for PTT-3. For SEC–HM the maximum error in: M_N is 16%, M_W is 14% and M_Z is 16%. For SEC–IM the maximum error in: M_N is 19%, M_W is 10% and M_Z is 13%. These errors are attributed to the aforementioned and inevitable discrepancies between the theoretical and experimental signal area fractions on each side of the peak. Effectively, not even the peak molecular weight is assigned correctly in the calibration and this error is propagated in the analysis of the unknowns. The SEC–PRIM results for all molecular weight averages are accurate to within 5% considering all samples. The PRIM's observed high-accuracy is attributed to this calibration's ensuring that the elution peak molecular weight, which is very close to the sample's weight average molecular weight, is assigned as accurately as possible.

5. Conclusions

In summary, this communication provides rigorous derivation of PRIM and illustrates some important features to the PRIM calibration technique. First, methods have been identified which produce valid SEC calibration curves which are independent of MWD. In these cases SEC–PRIM can be used reliably to analyze unknown samples of the same polymer as the calibrant. Second, the two cumulative matchings between the signal area and calibrant MWD have demonstrated a significant linear response regime for the SEC method. The peak elutions are observed in the linear response regime, thus Eq. (14) is self-consistently valid for the samples with Flory MWDs. In addition, the slope of the calibration curve at the peak elution time is continuous. The slope resulting from the high MW cumulative matching independently equals the slope resulting from the low MW cumulative matching. Although we observed that the signal area fraction, A_{low}, for the low MWD section deviated up to 8% from theory, deviations of this magnitude are numerically insignificant to the overall calibration. Furthermore, valid calibrations can be made which are sensibly insensitive to the cyclic oligomer elution not being fully resolved from the linear MWD elution. Finally, we are able to use SEC–PRIM to assess the efficiency and operating limits of the columns and overall method.

6. Supporting software

The Visual Basic® software written to implement

both PRIM calibration from standards and MWD analysis of unknowns can be made available via electronic mail. Please remit inquiries to the author at Steve.R.Lustig@usa.dupont.com.

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