Size-Exclusion Chromatographic Determination of Polymer Molar Mass Averages Using a Fractal Calibration

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Abstract

The characterization of polymers by size-exclusion chromatography basically consists of the determination of the weight-average molar mass (M_w), number-average molar mass (M_n), and polydispersity index (I). An accurate estimation of these magnitudes requires the use of a reliable and trusted calibration curve. Three procedures for building up a calibration curve are analyzed in this work. The first is the classical universal calibration (UC), based on the elution of tetrahydrofuran–polystyrene in a system as reference. The second is based on the proper calibration curve made with standards of the sample under study. However, two main drawbacks arise when using these methodologies: the nonfulfilment of the UC when secondary mechanisms, other than pure size-exclusion, are present in the separation process; and the lack of a broad set of narrow standards of the sample under analysis in the second procedure. In order to circumvent these difficulties, a third, recently-proposed approach based on fractal considerations is applied. The accuracy and reliability of this method is proven through the calculation of the deviations observed in the estimation of the M_w values for polymer samples in different solvent–gel chromatographic systems. Whereas the classical UC shows a mean deviation of approximately 80% relative to the values given by the manufacturer, the fractal calibration yields a mean deviation of approximately 16%, similar to that obtained from the proper calibration. Moreover, the fractal procedure only needs one polymeric sample to generate the calibration curve.

Introduction

Size-exclusion chromatography (SEC) is a separation technique in which different analytes can be resolved based on their molecular size in a solution. It is widely used to determine molar masses and distributions of synthetic polymers (1–6) and biomacromolecules (7–9). The chromatographic profile is visualized through a variety of online detection systems such as refractometry (4,8–10), UV–vis spectroscopy (1,5), conductivity (7), Fourier-transform IR spectroscopy (FTIR) (11), viscometry (12), electrospray-ionization mass spectrometry (13), and multiangle laser light scattering (6,8,14,15). Also, combinations of these detection systems are used; two (2,3) or three (14,15) tandem detectors are a very common experimental setup.

In conventional SEC, calibration curves are commonly constructed by measuring the retention volumes (or retention times) of synthetic polymer standards with narrow molar mass distributions (3–5,10,16,17) and of monodisperse polymers in the case of biopolymers (15,16). The subsequent transformation of the chromatographic peak into a molar mass distribution (MMD) allows the determination of the characteristic parameters: the weight-average molar mass (M_w), number-average molar mass (M_n), and polydispersity index (I).

When separation of macromolecules is exclusively governed by size exclusion (ideal SEC), universal column calibration with polystyrene (PS) standards is normally used (1,18–22) and valid if enthalpic contributions during chromatographic separation are negligible (9). However, generally, the commercially available columns used in SEC separate different macromolecules not only according to their sizes but also by other mechanisms not exclusively related to size, such as adsorption or partition (or both) because of binary interactions between solvent, polymer solutes, and gel packing (23–28). Only the first mechanism is desirable when using SEC, especially for characterization purposes. The secondary mechanisms should be as insignificant as possible because, if the adsorption is important, the obtained MMD and calculated mass averages will be affected. Under certain conditions, interactions between the stationary phase and the analyte may additionally depend on the way the stationary phase [typically a copolymer of polystyrene–divinylbenzene (DVB)] is synthesized by the producer (25). In this regard, it is largely known (4,25,29–32) that when comparing columns with the same packing but from different commercial suppliers, they contain different auxiliary components (surfactants and protecting colloids) that remain in the columns, playing a significant role in the chromatographic resolution.

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Recently, for slightly interactive gels such as µ-styragel, TSK Gel H_{HR}, and TSK Gel H_{XL} where polymer-protecting colloid interactions are less important, it has been demonstrated that secondary mechanisms accompany the main, pure (ideal) SEC separation mechanism (32). In fact, deviations from the universal calibration curve, at a given temperature, are observed for different solvent–polymer systems in a given gel packing, or even for the same system when eluting in different commercial chromatographic supports (18,19,25,27,33–40). In all these situations, the universal calibration is not fulfilled for polymers of different types, and a proper calibration curve should be constructed for a given solvent–polymer–gel system, at constant temperature, based on standards of the same chemical nature as the polymeric sample under study. This fact implies that a set of well-characterized standards should be available for any putative polymeric sample. Obviously, this is not the real case, and the universal calibration with polystyrene standards is generally used as reference in spite of the inherent errors committed.

All probable interactions between solvent, polymer, and cross-linked gel can be simplified by considering the effect of the solute when it faces the gel as a rough surface. This effect can be characterized by means of the fractal dimension that measures the roughness of a porous surface. In this respect, a new fractal calibration (FC) that is accomplished by many solvent–polymer systems eluted in three different supports has been proposed (41). The present work demonstrates the usefulness of the FC by comparing the values of the molar masses estimated from this approach with those deduced from the proper calibration curves as well as from the THF–PS calibration curve as representative of the so-called universal calibration. Using TSK Gel H_{HR} and TSK Gel H_{XL} columns with similar size-exclusion range and different solvent–polymer systems, it is pointed out that important deviations are made when molar masses are evaluated using the THF–PS calibration curve. On the other hand, the deviations in molar mass determination obtained with the FC are very similar to those deduced with the proper calibration curves. Therefore, the use of the FC as a suitable procedure to characterize polymer samples by SEC in order to determine M_w, M_n, and I, especially when secondary effects (a very common event) are involved in the chromatographic separation process is proposed.

Theory

SEC is a widely used technique to characterize macromolecules in solution, that is, to determine the MMD, M_w, M_n, and I, which are defined as (42):

$$M_w = \sum_i w_i M_i \qquad \text{Eq. 1}$$

$$M_n = 1/\sum_i (w_i/M_i) \qquad \text{Eq. 2}$$

$$I = M_w/M_n \qquad \text{Eq. 3}$$

where w_i and M_i represent the weight fraction and the molar mass, respectively, of each sample fraction (i).

The main problem to deal with when characterizing an unknown polymeric sample by means of SEC consists of having a reliable and trusted calibration curve. For this reason, three procedures to build up this curve are presented.

Calibration curves in ideal SEC

Usually, SEC columns are calibrated by using a set of narrow PS standards of different molecular weights eluted in THF. The elution behavior of this system fulfills the so-called universal calibration (UC) relationship, provided that the separation mechanism is exclusively by size (or ideal SEC), which reads (43,44):

$$\log M_w[\eta] = a + bV_e \qquad \text{Eq. 4}$$

where $[\eta]$ is the intrinsic viscosity of M_w and V_e the sample elution volume. The product $M_w[\eta]$ represents a measure of the hydrodynamic volume (V_h) of the solute, and $[\eta]$ can be easily calculated through the well-known Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = KM^{\alpha} \qquad \text{Eq. 5}$$

because the MHS constants, K and α, are available in the literature at a given temperature.

From the classical UC curve and taking into account the MHS constants, the dependence of M on the elution volume can be derived as follows:

$$\log M_w = \frac{a - \log K}{1 + \alpha} + \frac{b}{1 + \alpha} V_e = A + BV_e \qquad \text{Eq. 6}$$

which is known as a mass or specific SEC calibration curve (16).

To characterize a polymer sample, different in chemical nature from PS in a given solvent, it is assumed that the UC of the THF–PS reference system is accomplished (i.e., that size is the only mechanism accounting for the separation process of the unknown sample). Therefore, the specific calibration curve for this case will read:

$$\log M'_w = \frac{a - \log K'}{1 + \alpha'} + \frac{b}{1 + \alpha'} V_e = A' + B'V_e \qquad \text{Eq. 7}$$

where a and b are the same coefficients as in equations 4 or 6; and K' and α' are the MHS constants of the solvent–polymer system being eluted. Therefore, the chromatogram of the sample can be deconvoluted by means of equation 7 into the corresponding MMD, from which the magnitudes given by equations 1–3 are determined.

Calibration curves in SEC with secondary mechanisms

The picture is completely different under real conditions, especially because of the existence of phenomena different from pure size-exclusion, mainly adsorption of solutes onto the gel packings as a consequence of enthalpic interactions between the components of the chromatographic system (solvent–polymer–gel). In these cases, deviations from the universal calibration curve appear, meaning that the THF–PS reference calibration is no longer valid (25,27,33–40). In fact, for the systems eluted by a mixture of main and secondary mechanisms, the appropriate universal calibration equation will be:

$$\log M_w[\eta] = a' + b'V_e \qquad \text{Eq. 8}$$

and the particular or specific dependence of M_w on V_e:

$$\log M''_w = \frac{a' - \log K'}{1 + \alpha'} + \frac{b'}{1 + \alpha'} V_e = A'' + B''V_e \qquad \text{Eq. 9}$$

Obviously, by using the presented solvent–polymer–gel calibration curve (equation 9) that takes into account all the interactions, the sample characterization will be more accurate than assuming the UC of the THF–PS reference system. However, this procedure has a main drawback because it is necessary to dispose of a set of narrow standards of the polymer under study in order to proceed with the calibration. This difficulty can be circumvented by using a recently proposed method that takes into account the fractal nature of the gel packing (41).

FC

The overall chromatographic distribution coefficient (K_D) is calculated from the elution volume by:

$$K_D = \frac{V_e - V_o}{V_p} \qquad \text{Eq. 10}$$

where V_o and V_p are the void or total exclusion volume and the pore volume of the SEC packing, respectively. This coefficient is related with the fractal properties of the chromatographic support by (45,46):

$$K_D = 1 - \left(\frac{R}{L}\right)^{3-D_f} \qquad \text{Eq. 11}$$

where L stands for the available pore size, D_f is the fractal dimension of the pore surface, and R represents the viscometric radius of the solvated macromolecular solute, which can be easily calculated (in Å) from the intrinsic viscosity (in mL/g) by using the Einstein equation:

$$R = \left(\frac{3 \times 10^{23} M[\eta]}{\pi N_A}\right)^{1/3} = 0.5412(M[\eta])^{1/3} \qquad \text{Eq. 12}$$

where N_A is the Avogadro's number.

On the other hand, it has recently been proposed that a linear relationship exists between K_D and D_f for a given solute size (i.e., $M[\eta] = 10^6$) given by (41):

$$D_f = 3.0697 - 0.8574K_D \qquad \text{Eq. 13}$$

This equation represents a FC because it is followed by many different solvent–polymer–gel systems, and serves as a tool to characterize an unknown sample. For this purpose, the proposed procedure requires the knowledge of the sample elution profile, the elution volume, the intrinsic viscosity, and the MHS constants (K' and α') for the system under study. With this information, the steps of the method are, briefly, the following: (*i*) the [η] value of the sample together with the MHS equation allow the transformation of:

$$M[\eta] = \left(\frac{[\eta]^{\alpha'+1}}{K'}\right)^{1/\alpha'} \qquad \text{Eq. 14}$$

which is necessary to assess that the hydrodynamic volume of the sample is 10^6; (*ii*) the maximum of the chromatogram gives the V_e value, which introduced into equation 10, provides the K_D value of the sample; (*iii*) from equation 13, the D_f value of the specific solvent–polymer–gel system under study is obtained; (*iv*) next, combination of Equations 11 and 12 provides the L value of the ternary chromatographic system; (*v*) once the fractal characteristics of the system (D_f and L) have been estimated with a unique sample, a relationship between V_e and $M[\eta]$ can be written:

$$V_e = V_o - V_p\left[1 - \left(\frac{0.5412(M[\eta])^{1/3}}{L}\right)^{3-D_f}\right] \qquad \text{Eq. 15}$$

and (*vi*) equation 15 allows the building up of the SEC calibration curve of the system (using only one sample) by giving values to $M[\eta]$ and obtaining the corresponding elution volumes. After that, by fitting the pair values ($\log M[\eta]$, V_e), a classical relationship will be generated:

$$\log M_w[\eta] = a'' + b''V_e \qquad \text{Eq. 16}$$

which is easily transformed into the specific calibration curve with the aid of the MHS constants:

$$\log M_w''' = \frac{a'' - \log K'}{1+\alpha'} + \frac{b''}{1+\alpha'}V_e = A''' + B'''V_e \qquad \text{Eq. 17}$$

Finally, the determination of M_w, M_n, and I is made from the deconvolution of chromatograms with equation 17, as will be explained later, in order to test the accuracy of the procedure.

Experimental

Chemicals

Four different types of polymers have been used in the present work: narrow standards of PS from Polymer Standard Service-USA Inc. (Silver Spring, MD) with M_w given by the supplier (in kD) of: 4.14, 6.87, 17.2, 30, 42, 90.1, 114, 207.9, 355, 400, 657, 1432, 2700, and 3800. Polybutadiene (PBD) was purchased from Polymer Source Inc. (Dorval, Canada) of M_w (in kD): 0.92, 6, 12.6, 34, 47, 67.3, 87, 94.3, 105.7, 268, 323, 360, and 1120. Poly(dimethylsiloxane) (PDMS) was purchased from Polymer Laboratories (Shropshire, U.K.) and Polymer Source Inc. (Dorval, Canada) of M_w (in kD): 1.14, 8.1, 33.5, 41.5, 76, 123, 188.4, and 681.6. Poly(methylmethacrylate) (PMMA) was purchased from Polymer Laboratories (Shropshire, U.K.) of M_w (in kD): 5.78, 26.9, 70.5, 160.5, 254.7, and 550. The ranges of I of the used standards were: PS (1.05–1.10), PBD (1.03–1.15), PDMS (1.06–1.23), and PMMA (1.03–1.15).

Tetrahydrofuran (THF), benzene (Bz), toluene (Tol), 1-4 dioxane (Diox), and cyclohexane (CHX) of chromatographic grade from Scharlau (Barcelona, Spain) were used as solvents or eluents.

Viscosity

[η] of each sample in a given solvent and at 25°C has been calculated according to the MHS equation ($[\eta] = KM^{\alpha}$). The values of the MHS constants for the THF–PS, traditionally used as reference system, are $K = 0.011$ mL/g and $\alpha = 0.725$ (47). The values for the remaining systems (named K' and α') are gathered in Table I. They were measured in our laboratory under the same experimental conditions (27), except for the THF–PMMA and Tol–PMMA systems that were taken from the literature (47).

Chromatography

A Waters set of equipment (pump 590, universal injector U6K and differential refractometer 410 (Milford, MA) has been used for SEC experiments. Two sets of columns (each one of 7.8- × 300-mm i.d.) based on a PS–DVB copolymer from Tosohaas, Tosoh Corp. (Tokyo, Japan) have been employed: (*i*) three TSK Gel H_{HR} columns (particle size 5 µm; effective M_w separation range between 200–4 million g/mol) with V_o = 16.4 mL and V_p = 16.8 mL; and (*ii*) three TSK Gel H_{XL} columns (particle size 5–9 µm; effective M_w separation range between 200–4 million g/mol) with V_o = 17.07 mL and V_p = 16.63 mL. In both cases, V_o and V_p were determined with a PS standard of high molar mass (M_w = 3,800,000) and with small molecules such as THF, Tol, or Bz, respectively.

All solvents used as eluents were previously degassed and filtered by passing them under vacuum through a 0.45-µm regenerated cellulose filter from Micro Filtration Systems (Dublin, CA). All chromatographic experiments were performed at 25ºC in a thermostated heater, and the columns were equilibrated overnight prior to starting any experiment. Chromatograms were obtained at a flow rate of 1.0 mL/min by injection of 100 µL of sample solution. To avoid concentration effects (25) on the elution volumes, all solute samples were injected at four concentrations and then extrapolated to zero concentration. The elution behavior (plotted as log $M[\eta]$ vs. V_e) of the THF–PS system was graphically fitted in the central linear region (according to Equation 4) by a line with intercept (a = 16.73 mL^{-1}) and slope (b = –0.470 mL^{-1}) in the TSK Gel H_{HR} packing and intercept (a = 18.24 mL^{-1}) and slope (b = –0.527 mL^{-1}) in the TSK Gel H_{XL}. For the remaining systems, the linear fit coefficients (named a' and b') are compiled in Table II. All fittings yield a correlation coefficient of r 0.998.

Table I. MHS Constants for Different Solvent–Polymer Systems at 25°C

System	K' (mL/g)	a'
THF–PBD	0.011	0.760
Bz–PBD	0.112	0.604
Diox–PBD	0.155	0.541
Bz–PDMS	0.058	0.572
Tol–PDMS	0.045	0.601
CHX–PDMS	0.159	0.534
THF–PMMA*	0.008	0.720
Tol–PMMA†	0.007	0.730
Diox–PMMA	0.011	0.714

* Data from (27).
† Data from (47).

Table II. Linear Fit Coefficients (Equation 8) of Different Systems Eluted in Two Gel Packings

	TSK Gel HHR		TSK Gel HXL	
System	a'	–b' (mL^{-1})	a'	–b' (mL^{-1})
THF–PBD	18.510	0.580	17.570	0.506
Bz–PBD	16.268	0.451	18.120	0.477
Diox–PBD	18.794	0.555	16.100	0.464
Bz–PDMS	16.770	0.469	17.160	0.465
Tol–PDMS	17.881	0.535	17.240	0.522
CHX–PDMS	16.852	0.456	16.230	0.462
THF–PMMA			18.116	0.529
Tol–PMMA			15.339	0.445
Diox–PMMA			18.642	0.602

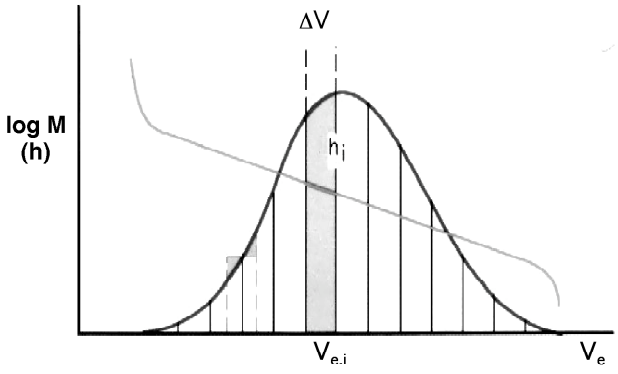

Figure 1. Typical elution profile and the corresponding transformation into a specific SEC calibration curve. (All experimental chromatograms were obtained by injecting 100 µL of 0.1 g/dL solution, and the height of the slices was measured every 2 mm.)

Results and Discussion

The estimation of molar masses has been made by deconvoluting the elution profiles or chromatograms and by transforming them, in the classical way, through the SEC calibration curves given by Equations 7, 9, and 17. To visualize the procedure, Figure 1 depicts how the raw data collected by the refractive index detector (h_i) are divided in equidistant slices with a corresponding elution volume ($V_{e,i}$). The height of each slice is proportional to the concentration of the eluting species, c_i, and the product ($\Delta V \times c_i$) is the mass of the eluting fraction (m_i). Therefore, as ΔV is constant, the corresponding weight fraction can be calculated from

$$w_i = \frac{m_i}{\sum_i m_i} = \frac{h_i}{\sum_i h_i} \qquad \text{Eq. 18}$$

and the elution profile transformed into a MMD (w_i vs. $V_{e,i}$). Finally, the molar mass of each slice (M_i) is calculated from the corresponding calibration curve. Note that, it has been designed by M_w', M_w'' and M_w''' to the weight-average molar masses calculated with Equations 7, 9, and 17, respectively, previously (Theory section). In all the calculations, both concentration and axial dispersion effects have not been taken into account. The former effect is not considered because the M_w values of the samples analyzed were less than 125,000 Da. On the other hand, the influence of the dispersion effects is negligible given the column dimensions, flow rate, and monodispersity of the samples.

In order to test the validity of the proposed FC, firstly the differences between the real or true value of M_w (given by the supplier) and those calculated according the three types of

calibration curves must be calculated. These differences are defined, respectively, as:

$$d'_{M_w} = \frac{\left| M'_w - M_w \right|}{M_w} \times 100 \qquad \text{Eq. 19a}$$

$$d''_{M_w} = \frac{\left| M''_w - M_w \right|}{M_w} \times 100 \qquad \text{Eq. 19b}$$

$$d'''_{M_w} = \frac{\left| M'''_w - M_w \right|}{M_w} \times 100 \qquad \text{Eq. 19c}$$

in a similar way as recently describe by other authors (5,8).

Next, the results obtained were analyzed, in terms of their deviations, with the three procedures of M_w calculation (in the same order previously described in the Theory section) to test the reliability and accuracy of each one.

First, using the THF–PS system as reference, the particular calibration curve of a different solvent–polymer system (at the same temperature and set of columns) can be constructed according to equation 7. Table III compiles the values of the A' and B' coefficients, calculated from K' and α' (in Table I) and the corresponding intercept (a) and slope (b) of the reference system previously (Experimental section) for all the systems assayed in both TSK Gel H_{HR} and H_{XL} columns. Moreover, the values of M_w' obtained from the chromatographic profile, as explained preciously, are included in Table III, together with those given by the vendors for comparison purposes. As can be seen, the M_w' values estimated by the procedure of assuming the THF–PS universal behavior as reference differ considerably from the true values given by the suppliers as denoted by the enormous deviations calculated in Equation 19a (see last column in Table III). In fact, an overall mean deviation of about 80% in the molar mass has been estimated, which indicates that (in practice) the classical universal calibration procedure is poorly accomplished by any solvent–polymer system when other separation mechanisms are present, as experimentally evidenced in the literature (26–28,41).

Second, in order to establish the degree of confidence in the determination of molar masses for a given solvent–polymer system, it is necessary to assume a new calibration curve made-up with standards of the same chemical nature as the polymeric sample under characterization, which is usually different enough to the THF–PS one at the same temperature (41). In this case, the proper specific calibration is given in equation 9. The coefficients A" and B" are gathered in Table IV for all the systems studied and were recalculated from K' and α' (in Table I) together with the intercepts and slopes (a' and b' in Table II) of the experimental calibration curves (41). The values of M_w' evaluated by transforming the chromatograms according to equation 9 (i.e., with the new calibration curve as reference) are also included in Table IV, together with the nominal values given by the producer. Overall, for any solvent–polymer system, the deviations dM_w'' (expressed in percentage) estimated with equation 19b were considerably lower than those obtained with the preceding methodology in both sets of TSK Gel columns. The analysis of the results in Table IV allow the estimation of a mean deviation value of approximately 10%. These findings provide quantitative evidence for the importance of using a proper calibration curve for each polymeric system studied (built-up with standards of the same chemical composition as the polymer under study) because the use of the universal calibration to characterize M_w, introduces important errors. However, from a practical point of view, it can be very difficult (if not impossible) to obtain a broad set of narrow standards (tailor-made) for a given polymer sample. This fact provides a possible explanation for the wide use of PS standards in THF and other solvents, and the so-called universal calibration curve for polymer characterization.

At this time, it is predicted that errors in the estimation of molar masses will decrease when using the recently described FC approach (41). In this regard and according to the procedure indicated previously (FC section), a specific calibration curve can be generated from equation 17. The corresponding A''' and B''' values (calculated with the MHS constants in Table I and the coefficients a" and b" from equation 16) for each system in the two gel packings are gathered in Table V. Also, the values of M_w''' estimated from the chromatographic profile and equation 17 are compiled in Table V. In general, it can be observed from the data that, for a given solvent–polymer–gel system, the deviations in M_w''' relative to the values from the manufacturer and referred to as dM_w''', are noticeably lower than those estimated from the universal calibration (see dM_w' in Table III). In fact, with the FC procedure, the mean percentage deviation is around 16%, slightly higher than that

Table III. Linear Fit Coefficients and Molar Mass (M_w') Calculated with Equation 7, and the Difference (dM_w') Estimated with Equation 19a, as Percentage, for Two Gel Packings.

Packing	System	A'	–B' (mL^{-1})	M_w*	M_w'	dM_w' (%)
TSK Gel HHR	THF–PBD	10.622	0.267	47,000	114,400	143.4
				67,300	140,600	108.9
	Bz–PBD	11.024	0.293	47,000	43,500	7.4
	Diox–PBD	11.383	0.305	47,000	37,800	19.6
				67,300	50,700	24.7
	Bz–PDMS	11.431	0.299	8,100	8,100	0.0
				41,470	39,800	4.0
	Tol–PDMS	11.294	0.293	8,100	16,300	101.2
				41,470	65,000	56.7
	CHX–PDMS	11.428	0.306	41,470	27,300	34.2
				76,035	45,300	40.4
TSK Gel HXL	THF–PBD	11.480	0.300	34,000	48,200	41.8
	Bz–PBD	11.966	0.329	12,600	2,600	79.4
				34,000	6,700	80.3
	Bz–PDMS	12.391	0.335	33,500	19,100	43.0
				123,000	89,000	27.6
	Tol–PDMS	12.237	0.329	8,100	31,900	293.8
				33,500	166,200	396.1
	THF–PMMA	11.841	0.307	70,500	75,400	6.9

* Molar mass given by the supplier.

estimated from the proper calibration curve (dM_w' 10%) but greatly lower than that from the THF–PS curve (dM_w" 80%). Moreover, it should be noted that an analysis of the deviations in each type of columns shows the same trend, and in general, the values are higher in TSK Gel H_{XL} packing than in H_{HR} one. This finding could be in agreement with the fact that systems eluted in H_{XL} gel exhibit higher polymer-gel interactions, as it was shown through the values of the adsorption distribution coefficients, K_p (32,41).

Table IV. Linear Fit Coefficients and Molar Mass (M_w") Calculated with Equation 9, and the Difference (dM_w") Estimated with Equation 19b, as Percentage, for Two Gel Packings.

Packing	System	A"	–B" (mL^{-1})	M_w*	M_w'"	dM_w" (%)
TSK Gel HHR	THF–PBD	11.632	0.329	47,000	57,900	23.2
				67,300	74,200	10.2
	Bz–PBD	10.735	0.281	47,000	39,500	15.9
	Diox–PBD	12.721	0.360	47,000	48,800	3.8
				67,300	68,500	1.8
	Bz–PDMS	11.453	0.298	8,100	8,700	7.4
				41,470	43,000	3.7
	Tol–PDMS	12.012	0.334	8,100	8,700	7.4
				41,470	42,500	2.5
	CHX–PDMS	11.506	0.297	41,470	52,600	26.8
				76,035	86,100	13.2
TSK Gel HXL	THF–PBD	11.099	0.287	34,000	38,000	11.8
	Bz–PBD	11.888	0.297	12,600	14,400	14.3
				34,000	33,300	2.1
	Bz–PDMS	11.704	0.296	33,500	35,300	5.4
				123,000	135,200	9.9
	Tol–PDMS	11.611	0.326	8,100	8,900	9.9
				33,500	45,700	36.4
	THF–PMMA	11.768	0.308	70,500	65,000	7.8

* Molar mass given by the supplier.

Table V. Linear Fit Coefficients and Molar Mass (M'''_w) Calculated with Equation 17, and the Difference (dM_w'") Estimated with Equation 19c, as Percentage, for Two Gel Packings.

Packing	System	A'''	–B''' (mL^{-1})	M_w'*	M_w'''	dM_w''' (%)
TSK gel HHR	THF–PBD	13.754	0.436	47,000	43,200	8.1
				67,300	62,800	6.7
	Bz–PBD	12.331	0.358	47,000	33,600	28.5
	Diox–PBD	12.378	0.348	47,000	33,100	29.6
				67,300	57,300	14.9
	Bz–PDMS	10.423	0.254	8,100	10,700	32.1
				41,470	41,300	0.4
	Tol–PDMS	10.891	0.282	8,100	11,900	46.9
				41,470	44,800	8.0
	CHX–PDMS	10.726	0.267	41,470	42,800	3.2
				76,035	67,000	11.9
TSK gel HXL	THF–PBD	10.588	0.269	34,000	31,100	8.5
	Bz–PBD	10.404	0.241	12,600	13,900	10.3
				34,000	27,100	20.3
	Bz–PDMS	10.075	0.229	33,500	33,300	0.6
				123,000	93,000	24.4
	Tol–PDMS	12.573	0.375	8,100	5,900	27.2
				33,500	38,500	14.9
	THF–PMMA	11.159	0.280	70,500	62,700	11.1

* Molar mass given by the supplier.

Figure 2 shows, as an example, the overlays of the MMD (as w_i vs. M_i) obtained with the three calibration approaches compared herein, equations 7, 9, and 17. Figure 2A corresponds to PBD of M_w = 12,600 Da eluted with Bz in TSK Gel H_{XL}; Figure 2B is for PBD of M_w = 47,000 Da and Figure 2C for PBD of M_w = 67,300 Da, both eluted with THF in TSK Gel H_{HR}. As can be seen, the MMDs obtained with the FC approach (equation 17) are nearly overlapping the real MMD of the samples (equation 9), whereas those obtained with the classical THF–PS universal calibration (equation 7) are further apart. Moreover, it is important to note that not a fundamental difference in the shape of the MMDs is observed given that the three equations used to transform the elution profiles into a MMD have the same mathematical functionality. However, substantial shifts along the molar mass values are observed, which leads to important errors (as shown in Tables III–V) when determining the molar mass of a sample.

Figure 3 depicts, as an example, specific calibration plots (as log M_w vs. V_e) obtained at 25ºC for the THF–PBD system eluted in TSK Gel H_{HR} columns (Figure 3A) and Bz–PBD system in TSK Gel H_{XL} columns (Figure 3B), in order to graphically visualize and compare the three calibration methods in the complete M_w range assayed. Again, and in both sets of columns, the FC curves are near to the proper calibration curves, whereas the universal calibration dependence is shifted apart, denoting that parallel to size-exclusion, enthalpic mechanisms also govern the chromatographic separation. As recently stated (9), the validity of the UC curve should be confirmed prior to its use, for each particular chromatographic system, in order to avoid unacceptable errors. However, the alternative new calibration can be a tedious and time-consuming task and even impracticable in the absence of proper standards. Consequently, the FC emerges as a valuable tool to determine M_w values with a similar accuracy. Obviously, from the values of the M_w, the corresponding M_n, and I can be derived in order to complete the polymer characterization by SEC. Moreover, another advantage of the proposed approach is that it is not expensive from a methodological and practical point of view because a simple refractive index detector is needed. In contrast, actual absolute calibration methods are based on the combination of concentration-sensitive detectors (e.g., refractive index or UV–vis) with molar mass-sensitive

detectors, such as light scattering or viscometer (or both) (2,3,8,48) and combined SEC matrix assisted laser desorption time-of-flight mass spectrometry (1,49). Finally, it should also be emphasized that when using the FC, the $M[\eta]$ value of the particular sample is not a drawback because a FC equation (similar to Equation 13) can be generated for any $M[\eta]$ value. In this sense, work is in progress in our lab to extend the formalism to any hydrodynamic size.

Conclusion

The precise M_w of an unknown polymer sample is not easy to obtain by SEC because a proper calibration curve in the given solvent–polymer–gel system is needed. In other words, it is necessary to have several narrow standards of different molecular weight but with identical chemical structure as the sample under study to proceed with the calibration. For this reason, much work is done in polymer characterization based on the universal calibration of the THF–PS system as reference. However, this method originates important quantitative errors, especially when secondary mechanisms, other than ideal size-exclusion, are involved. Experimental evidenced shows (using polymers such as PBD, PDMS, and PMMA in different solvents such as THF, Bz, Diox, CHX, and Tol, eluted in two TSK gels) that a mean deviation of about 80% in the estimation of M_w was committed when using the classical UC; whereas a 10% was estimated from the proper calibration curves.

The quantitative analysis of the same ternary systems based on the FC approach allows the mean deviations of about 16% with respect to the M_w nominal values given by the supplier to be obtained. This error is only slightly higher than that determined with the proper calibration curves but considerably lower than that estimated with the classical UC. Moreover, in the FC method, the A''' and B''' coefficients of Equation 17 can be obtained only with one polymer sample in a given solvent–gel system and are valid for any other sample of the same chemical nature in the solvent–gel system considered, that is, it allows the building up of a calibration curve. This fact alleviates the necessity of having a broad number of standards for polymer characterization, and provides a reliable determination of M_w, M_n, and I.

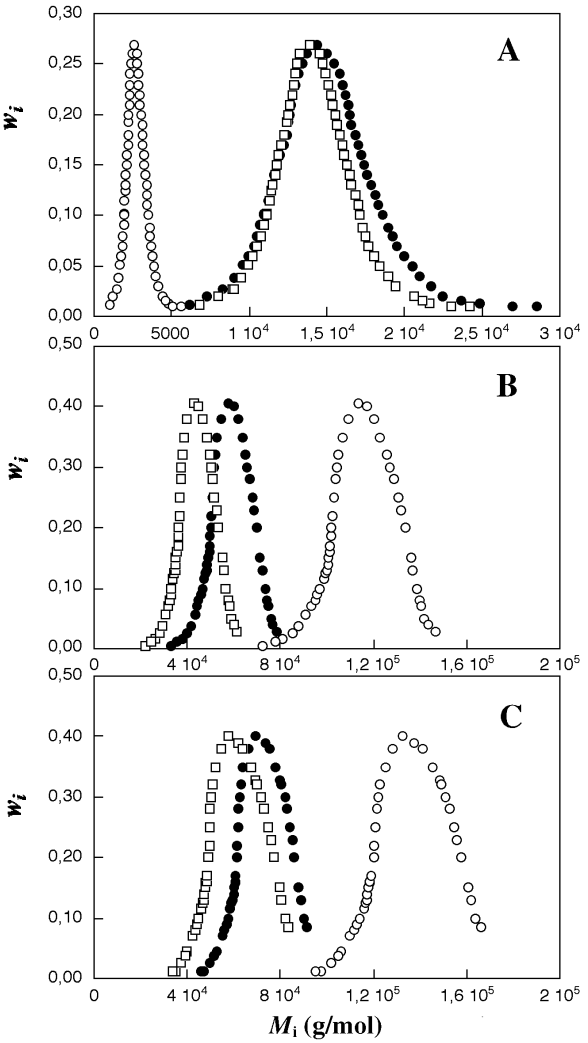

Figure 2. Molar mass distributions obtained from the deconvolution with Equation 7 (○), equation 9 (●), and equation 17 (□) of the elution profiles of: PBD (M_w = 12,600) in Bz–TSK gel HXL (A); PBD (M_w = 47,000) in THF–TSK gel HHR (B); and PBD (M_w = 67,300) in THF–TSK gel HHR (C).

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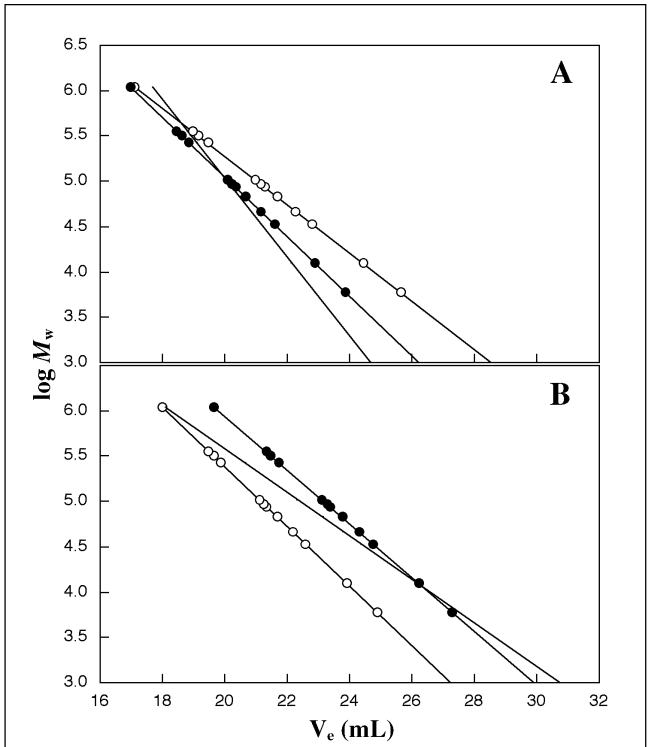

Figure 3. Calibration curves at 25°C fitted according to equation 7 (○), equation, 9 (●), and generated from equation 17 (—) for the systems: THF–PBD–TSK gel HHR (A) and Bz–PBD–TSK gel HXL (B).

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