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Application of Triple-Detector Size Exclusion Chromatography (On-Line Differential Refractometer, Viscometer and Light Scattering Detectors) for the Characterization of Brominated Polystyrene

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A brominated polystyrene sample was used as a probe to explore the increased capabilities of size exclusion chromatography (SEC) with on-line light scattering and viscosity detectors—triple-detector SEC (TriSEC). Conventional SEC using only a concentration detector is not capable of providing accurate analysis for these types of polymers. Several flowrates and sample concentrations were used to study their effects on the precision of the various TriSEC-determined molecular parameters. Our study clearly shows the strength and weakness of each of the three detectors, and their interesting synergism in the TriSEC configuration. Overall, the molecular weight distribution results are better determined by light scattering compared to those determined by viscometer using the universal calibration approach. The viscometer, however, gives the important intrinsic viscosity distribution results. These intrinsic viscosity results can be considered for polymer quality control applications, because of their excellent precision and independence from SEC column calibration. The determination of the Mark-Houwink (intrinsic viscosity versus molecular weight) plot for any polymer conformational and structural studies is best done by using all detector signals from TriSEC.

KEY WORDS Size exclusion chromatography, molecular weight distribution, intrinsic viscosity distribution, viscosity detector, light scattering detector, brominated polystyrene

ABBREVIATIONS *BrPS* – brominated polystyrene; *CC* – conventional calibration; *IV* – intrinsic viscosity; *IVD* – intrinsic viscosity distribution; *LS* – light scattering; *MW* – molecular weight; *MWD* – molecular weight distribution; *PS* – polystyrene; *RALLS* – right angle laser light scattering; *RI* – refractometer; *SEC* – size exclusion chromatography; *THF* – tetrahydrofuran; *TriSEC* – triple detector size exclusion chromatography; *UC* – universal calibration; *Visc* – viscosity detector

INTRODUCTION

Size exclusion chromatography (SEC) separates polymer molecules by their size differences. Conventional SEC with only an on-line concentration detector is limited in its ability to determine the absolute molecular weight distribution (MWD) of polymers of differ-

ent chemical structures. The problem is that the relationship between polymer molecular size and polymer molecular weight (MW) is different from polymer to polymer depending on their chemical structures. Accurate SEC analysis requires calibration of the chromatographic retention volume against the MW standards of the same chemical structure as the unknown sample. Because of the lack of calibration standards, most conventional SEC results are reported in terms of the polystyrene-equivalent MW values, by using the commercially available polystyrene (PS) standards.

In a triple-detector SEC (TriSEC) technology, three on-line detectors are used together in a single SEC system [1]. In addition to a concentration detector, for example, a differential refractometer (RI), a viscosity detector (Visc) is included in TriSEC for the continuous monitoring of the polymer solution viscosity across the SEC chromatogram. A light scattering (LS) detector is the necessary third detector in TriSEC to monitor the polymer MW across the SEC chromatogram. With TriSEC, absolute MWD determination is possible for polymers that are very different in chemical composition or molecular conformation.

Brominated polystyrene (BrPS) is an interesting polymer to use for probing the TriSEC technology. One would expect that bromination of polystyrene would have a direct effect of adding MW to the polymer, but it would have only a minor effect on the hydrodynamic size of the molecule. Conventional SEC of only one concentration detector is not capable of providing accurate analysis for polymers of this kind. The presence of the heavy bromine atoms on the benzene ring affects the polymer MW-to-size relationship, which can no longer be represented accurately by a conventional polystyrene SEC calibration curve.

EXPERIMENTAL

Data were collected on a modular room temperature SEC system consisted of a Jordi 50cm \times 10 mm Gel DVB mixed-bed linear column (Jordi Associates, Bellingham, MA), Viscotek right-angle laser light scattering detector (RALLS), model 200 RI-Viscosity dual detector, and model 400 data manager (Viscotek Corp., Houston, TX). Laser wavelength was 670 nm. Chromatographic conditions: tetrahydrofuran (THF) solvent, 1.0 mL/min flowrate (unless otherwise specified), 100 μ L sample volume, 1% sample concentration (unless otherwise specified).

All polymer samples were obtained from American Polymer Standards Corp., Mentor, OH. The brominated PS sample (*BrPS*) has a reported bromination level of 66.3%, a reported M_z value of 1,300,000, M_w of 646,000, M_n of 300,000, and an intrinsic viscosity (IV or $[\eta]$) value of 0.284 dL/g. The broad MWD PS sample has a reported M_z value of 430,000, M_w of 250,000, M_n of 100,000, and IV value of 0.843 dL/g. Both conventional SEC calibration and universal calibration curves were established using a series of narrow PS standards ranging from 2,450 to 514,000 MW. Linear column calibration was assumed throughout the sample analyses [2]. All data processing and graphics were made using the Viscotek TriSEC software version 2.60. The dn/dc values for PS and BrPS in THF were determined, by the RI peak areas, to be 0.185 and 0.128 dL/g, respectively.

RESULTS AND DISCUSSION

Detector Volume Offset

Figure 1 shows the TriSEC chromatograms for a narrow polystyrene standard of 156,000 MW (Fig. 1A), and the broad brominated PS sample (Fig. 1B) at 2% sample concentration. The peak offset of the narrow standard detector responses is caused by the physical, geometric volume-offset of the interconnecting tubing between the three detectors. Correction for this tubing volume-offset is the necessary first step in a TriSEC data processing software. Because of its monodispersity, this volume-offset correction forces the perfect alignment of the three detector signals for the narrow standard. However, this same tubing-volume correction will still leave a significant amount of displacement of the three detector signals for the broad sample. These additional detector signal displacements that remained with the broad sample are caused mainly by the sample polydispersity.

A small portion of the remaining peak displacement seen with the broad sample is caused by SEC column axial dispersion and instrumental band broadening. In fact, it has been reported that an appropriate amount of extra intentional detector volume-offset can be used as a proper means to correct for the effect of symmetrical instrumental band-broadening in the TriSEC technique [3]. One can actually designate a legitimate quantitative "volume offset" term to quantify the symmetrical band broadening effect in TriSEC and correct for it in the software [4,5].

The accuracy of all TriSEC results is sensitive to the relative positioning of the three detector signals along the retention volume axis. Accurate TriSEC results can be obtained only if the detector responses are corrected for both the connecting tubing volume offset and this "instrument band broadening volume offset". This is likely to be the theoretical foundation behind the empirical approach to band-broadening correction using an adjustment factor, referred to by other authors as the "effective volume offset" [6,7].

For a broad sample (Fig. 1B), the sample MW polydispersity is closely related to the signal displacement of the LS and RI peaks. The following is the explanation. Let us focus on the two SEC slices halfway up on the either side of the RI peak. Equal RI signal means equal polymer concentration in these two SEC slices. However, the MW of the polymer in the two slices are different and they scatter light differently. The slice on the left elutes earlier, meaning higher MW and higher LS signal. The converse is true on the right side. The slice on the right contains lower MW polymer that gives a much lower LS signal. This net effect of increasing LS signal on the left, and decreasing on the right, causes the apparent shift of the LS peak to the earlier retention volume. In actuality, there is no actual volume shift. This result is merely the consequence of the natural manifestation of the different MW sensitivity among the two detectors.

For random-coil polymers, like BrPS, one expects the viscosity signal to lag slightly behind the light scattering signal. This is because of the viscosity detector has less MW sensitivity than the LS detector. The LS signal is proportional to MW to the first power, whereas the sensitivity of the viscometer has a MW dependency on the Mark-Houwink (M-H) exponent value, which has a value of about 0.7 for random-coil polymers.

It is interesting to note that, whereas the total LS displacement in the corrected TriSEC chromatogram can give an indication of polymer polydispersity; the relative size of the

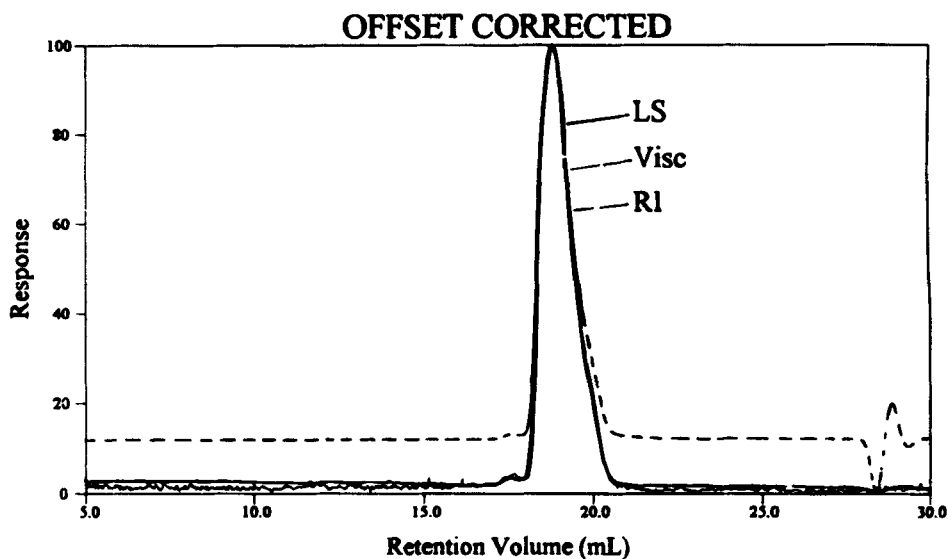
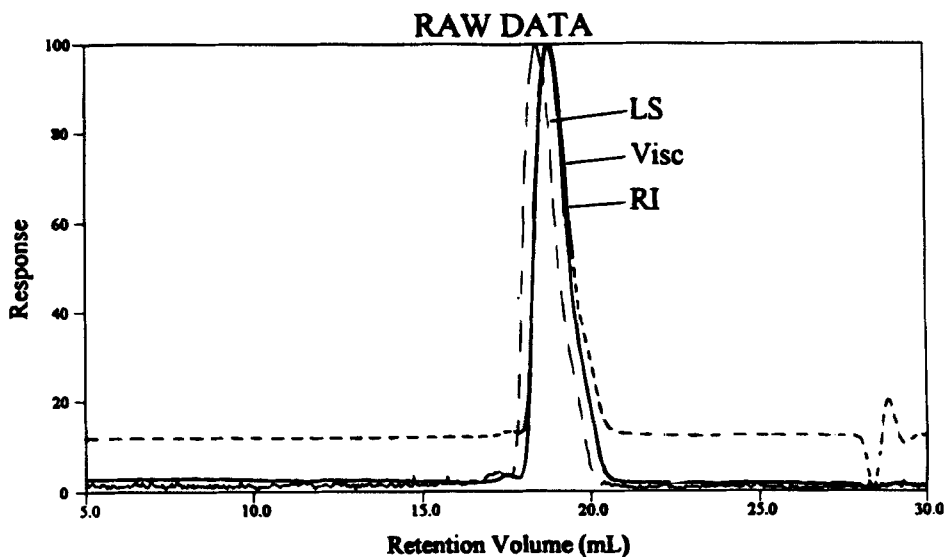


FIGURE 1A Volume offsets in triple-detector size exclusion chromatography using a narrow molecular weight distribution standard. Sample, polystyrene 156K MW, polydispersity ≈ 1.0 (Am. Polym. Std.); column, Jordi 50 cm, linear, solvent, THF; flowrate, 1.0 mL/min.

viscosity displacement against that of the LS can give an indication of polymer M-H exponent and polymer conformational differences. This is one of the attractive features of TriSEC. It allows the polymer structural features to be visualized directly from the display of the chromatograms [1].

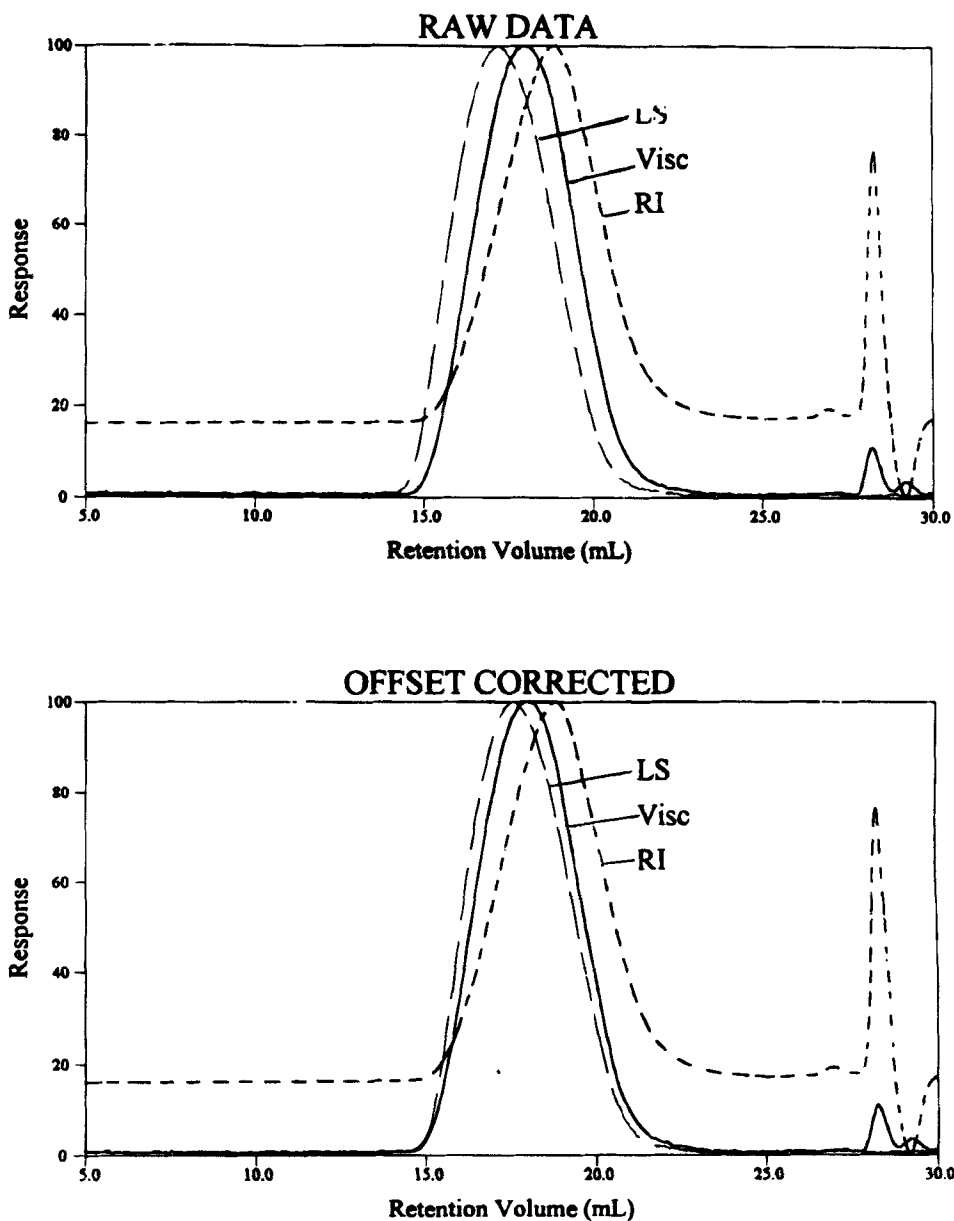


FIGURE 1B Volume offsets in triple-detector size exclusion chromatography using a broad molecular weight distribution sample. Sample, brominated polystyrene BrPS-646K, polydispersity ≈ 2.13 (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, THF; flowrate, 1.0 mL/min.

Typical TriSEC Results

Figure 2 shows the results of the brominated PS sample. The M_w calibration curve in this figure is generated by taking the ratio of the LS signal divided by the RI signal, slice by slice, across the entire chromatogram. The differential MWD plot is calculated from the

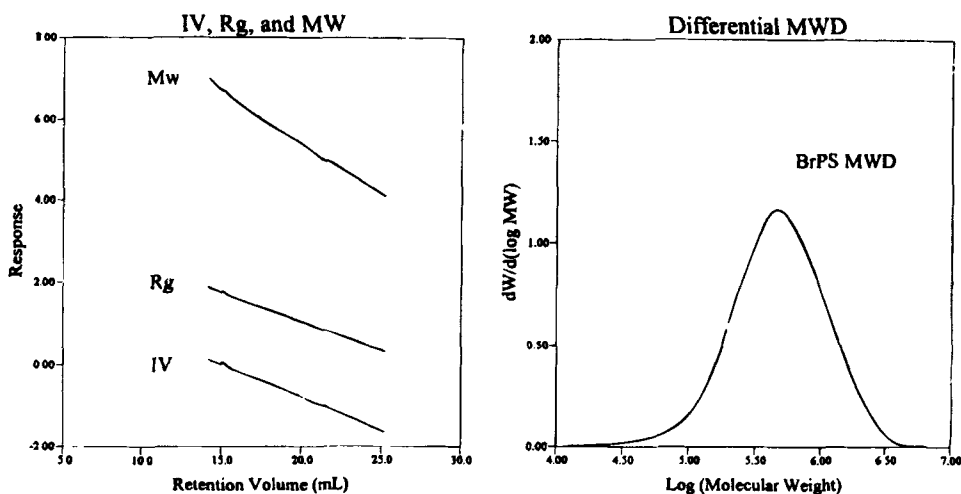


FIGURE 2 Triple-detector size exclusion chromatography results of brominated polystyrene: molecular weight distribution and M_w , R_g , and IV-calibration curves. Sample, brominated polystyrene BrPS-646K, polydispersity ≈ 2.13 (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min.

RI chromatogram using the LS- M_w calibration curve. The IV calibration is obtained similarly by the ratio of viscometer and RI signal. The radius of gyration R_g calibration is derived from the cubic root of the product between the M_w and IV values at every retention volume slice [8], using the Flory-Fox equation, assuming a linear flexible chain molecule [9]:

$$R_g = \frac{1}{\sqrt{6}} \cdot \left(\frac{IV \cdot M_w}{\Phi} \right)^{\frac{1}{3}} \quad (1)$$

where Φ is the Flory universal constant, equal to 2.86×10^{23} .

The slope of this M_w calibration is the direct result of the peak displacements existing between the LS and RI signals. Because the LS signal is situated to the left of the RI peak, the LS/RI ratio (or the M_w value) gives a high value on the left, the early eluting high MW side of the peak. Conversely, the LS/RI ratio is low on the right side of the peak and tilts the M_w calibration curve downward at the large retention volume region.

The same explanation can apply to the observed slope in the IV-calibration curve. Except that, the IV curve has a smaller slope because of the smaller displacement between the viscosity and the RI chromatograms. This relative difference in the slope of the M_w and the IV calibration curves determines the slope of the Mark-Houwink plot and the M-H exponent that is used frequently for polymer structural studies.

The above discussions reinforce the critical need for the proper volume-offset correction to account for both connecting tubing and band broadening effect in TriSEC. All key polymer structural information in TriSEC is highly sensitive to how well these volume-offset corrections are carried out in the software. In order to use TriSEC to its full potential, there is a real need to develop a better understanding and more effective algorithms of correcting band broadening.

Fortunately, several non-structural parameters calculated by the TriSEC technique are not so easily affected by the volume-offset correction of the chromatograms. In contrast to polymer structural parameters, (e.g., M-H exponent, and polymer long-chain branching index), the polymer MW averages and the MWD curves calculated from the LS/RI ratio are much less affected by volume-offset corrections. The same is true for the IV averages and intrinsic viscosity distribution curves obtained from the viscosity and RI chromatograms. Under usual circumstances, misalignment of detector signals would lead to errors of just a few percent in MWD and IVD information.

In Table I, the MW, IV and R_g values are reported in terms of the number, weight, z-average, and the weight-over-number polydispersity values (Pd). The results of this study are in good agreement with the vendor-reported values. Our value for the M-H exponent is 0.66, which is within the range one expects for a random coil polymer.

Differences in SEC-MWD Methods

With three detectors in one SEC system, there exist at least three different ways that the polymer MWD information can be calculated. An example of three such MWD calculations is given in this section using the TriSEC data in Figure 3 for the polystyrene and the brominated PS sample.

The conventional calibration (CC) method uses only the RI chromatogram. This particular CC calculation uses a PS calibration curve generated from the narrow PS standards. For any polymer except for polystyrene itself, this specific CC method is not capable of giving the true MWD information of the sample. What this CC method provides are only the "PS-equivalent MW and MWD results". One sees in Figure 3A that, because the PS and BrPS samples elute very closely, the PS-equivalent MW values and MWD plots are not very different for the two samples. However, in this case, the CC- M_w of 260,000 for the BrPS sample is much too low to compare with the true expected M_w value of 464,000 (Table II). Clearly, the CC method is not able to provide accurate MW information for this sample.

A better way to analyze the BrPS sample is to use the LS and RI combination in the LS-SEC method of calculation (Fig. 3B). In this LS approach, the M_w values of the sample across the chromatogram are directly determined by dividing the sample LS by RI signals at every retention volume. This M_w calculation in the Viscotek TriSEC software includes the RALLS dissymmetry correction that is discussed later. We see that with this LS-SEC approach, accurate MW averages and MWD plots are indeed obtained for both the PS and the BrPS samples. The MW calculations by LS-SEC require no SEC retention calibration.

TABLE I
TriSEC of BROMINATED POLYSTYRENE
(Am. Polym. Std. BrPS-646K*, 66.3%Br., IV = 0.284 dL/g., 2% sample concentration.)

MW	IV (dL/g)	R _g (nm)	M-H Constants
$M_n = 324,000$	$IV_n = 0.217$	$R_{g,n} = 14.50$	$N = 0.661$
$M_w = 659,000$	$IV_w = 0.291$	$R_{g,w} = 17.92$	$\text{Log}K = -4.346$
$M_z = 1,190,000$	$IV_z = 0.372$	$R_{g,z} = 21.56$	
$Pd(M_w/M_n) = 2.04$	$Pd(IV_w/IV_n) = 1.36$	$Pd(R_{g,w}/R_{g,n}) = 1.24$	
*Reported values (Am. Polym. Std.):			
$M_n = 300,000$	$M_w = 646,000$	$M_z = 1,300,000$	$IV = 0.284 \text{ dL/g}$

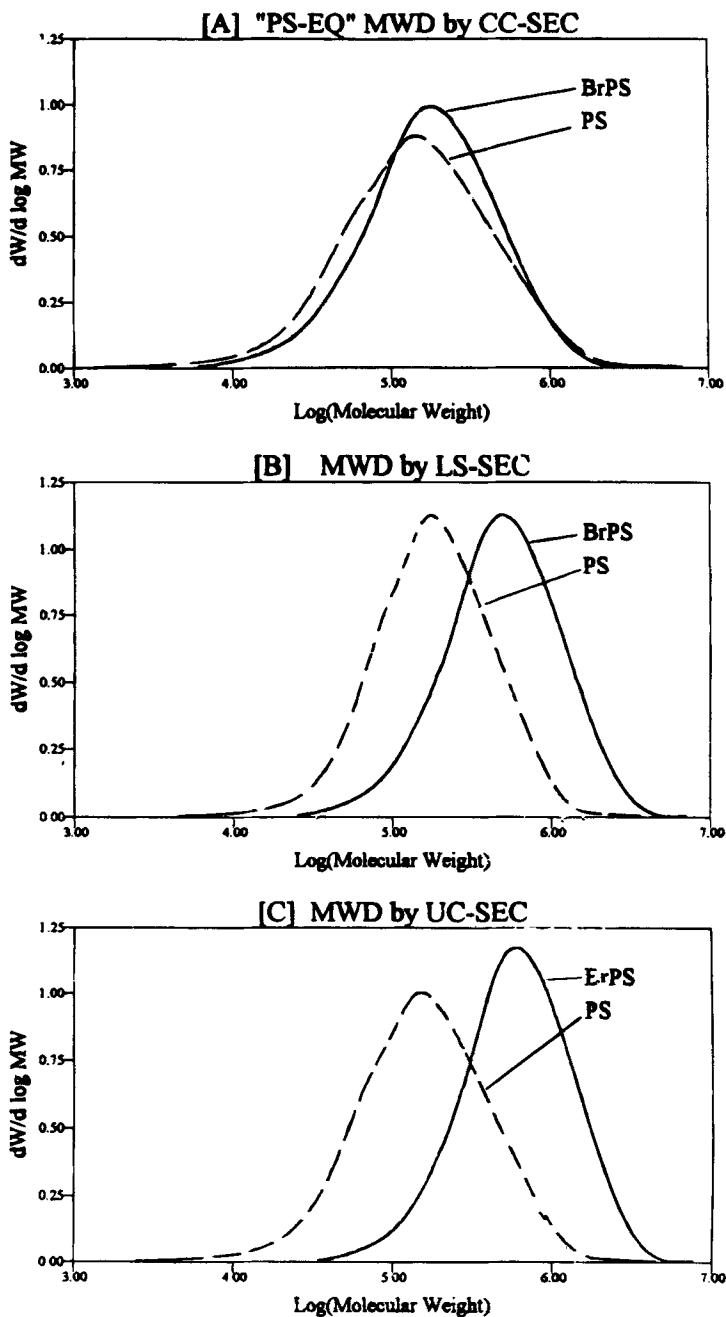


FIGURE 3 Different size exclusion chromatography-molecular weight distribution methods. (A) Conventional size exclusion chromatography using differential refractometer detector response alone and polystyrene-equivalent calibration curve; (B) SEC-light scattering method using both refractometer and light scattering detector responses; (C) Universal Calibration method using both refractometer and viscosity detector responses. Samples: brominated polystyrene BrPS-646K and broad molecular weight distribution PS-250K, polydispersity ≈ 2.5 (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min.

TABLE II
Difference in SEC-MWD Methods
(TriSEC Study of the Effect of Polystyrene Bromination)

BROMINATED PS (1% Sample Concentration)	
CC- M_w = 260,000	CC- M_n = 110,000
UC- M_w = 774,000	UC- M_n = 378,000
LS- M_w = 686,000	LS- M_n = 347,000
Reported* M_w = 646,000	Reported* M_n = 300,000
BROAD PS STD 250K MW	
CC- M_w = 251,000	CC- M_n = 77,200
UC- M_w = 242,000	UC- M_n = 96,200
LS- M_w = 250,000	LS- M_n = 116,000
Reported* M_w = 250,000	Reported* M_n = 100,000

*Taken from: "Polymer Standards Catalog", February, 1991, American Polymer Standards Corporation, Mentor, Ohio 44060.

This is the unique feature that gives this method its definite advantage in precision and accuracy compared to the CC method and the UC method discussed below. However, the overall precision of the LS method may or may not be better than the CC-method. The precision of the LS-SEC method can become very poor with low MW polymer samples, where the LS signal-to-noise level may no longer be acceptable.

One other alternative is the universal calibration (UC) method that uses the viscosity and the RI chromatograms [10,11]. In this method, the sample IV values at every retention-volume slice are obtained by dividing the viscometer signal by the corresponding RI signal. The UC method for this study uses a universal calibration curve established by plotting the $\log(\text{IV} \cdot \text{MW})$ value against the retention volume data of several narrow PS-standards. The MW calibration curve for the unknown polymer sample is then derived from the difference between the sample IV and this UC calibration curve in the logarithmic scale. We have:

$$\log(\text{IV} \cdot \text{MW}) = \log(\text{IV}) + \log(\text{MW}) \quad (2)$$

It is seen in Figure 3C that, the UC method is capable of giving the true MW values and MWD plots for the two samples. The UC method has a better low-MW capability than the light scattering method. This is because of the viscometer having a better low-MW sensitivity than the light scattering detector. The main drawback of the UC method is the fact that it is still a method relying on the SEC retention-volume calibration. Like the CC method, it also suffers many of the same precision uncertainties associated with many of the usual SEC retention and column resolution problems, such as flowrate fluctuations, sample overloading, and instrumental band broadening.

There are strengths and weaknesses for each of the three methods discussed above. The choice of one method versus the other may vary from sample to sample and depend also on the purpose of the analyses, the sample MW level, as well as the degree of structural and compositional complexity of a particular sample.

For routine MW and MWD analyses on a given polymer type, we recommend the following two-step process. The first step is to create a working SEC standard for the samples. This can be done by selecting a particular sample and determining its true MW val-

ues by using either the LS or the UC method that is available in a TriSEC system. The second step is to use this calibration standard to establish a conventional SEC calibration curve, and then proceed to analyze all samples by the CC approach using only the RI chromatogram.

Having established a true SEC-MW calibration curve, the conventional SEC method using a single RI detector does have one unique advantage over either the LS and the UC method. The LS and UC methods require the exact weight of polymer that is injected. The CC method does not require this polymer weight information; thus eliminates one source of MW error in the SEC results.

One point needs be made clear for our discussion up to this point. Our discussion on the options of different SEC methods presented above is limited to consideration of the polymer MW and MWD analyses. For polymer structural studies, and any attempts to determine polymer branching and conformational differences, the situation is quite different. In these latter cases, we need all the information we can obtain. All three detector chromatograms are needed in a TriSEC analysis when attempting to elucidate polymer structure. An example is given below for the BrPS sample.

Effect of Polystyrene Bromination

The substitution of hydrogen in PS by the heavier bromine atoms makes the BrPS molecules heavier and more densely packed than its polystyrene counterpart. This structural difference is clearly demonstrated in the next three figures, which give data from polymer solutions of the same weight concentration.

Conventional SEC may be considered as a one dimensional technique, where all the polymer MW information is derived from the sample elution profile along the horizontal retention volume axis alone. The addition of LS and viscometer is like adding new dimensions to the SEC technique. In TriSEC, the relative sizes of the LS, RI, and viscometer signals in the vertical axis are now becoming meaningful. They are indicative of the sample MW and IV anywhere across the chromatogram. The synergism existing among these three detectors has created a new dimension of using SEC in polymer characterization studies.

To view the relative size of the detector signals, the TriSEC data can be plotted on the absolute millivolt scale as that shown in Figure 4. We see in the RI overlay (Fig. 4A) that the PS and BrPS samples nearly co-elute in this SEC experiment, whereas conventional SEC would predict that both samples would have comparable MW values. Of course, we know this is not true. The fact that the BrPS sample has much higher MW can be seen only in the LS overlay plot (Fig. 4B). Here, we see the BrPS sample has a much larger LS peak, even though it has a lower dn/dc value (as seen by its smaller peak size in the RI overlay plot). The fact that the BrPS sample has a much smaller viscosity peak is also very interesting (Fig. 4C). It serves to elucidate the very densely packed structure of the BrPS molecules.

The discrimination of structural differences between PS and BrPS can also be visualized in the overlay plots in Figures 5A and 5B. In Figure 5A, we see the MWD curve of the BrPS sample is shifted to the right side of the PS sample, but its IVD curve is shifted to the opposite side. This gives the clear indication of the BrPS sample being a heavier, and more densely packed structure than PS. These observations are directly related to the vertical shifts seen in Figure 5B for the M_w and the IV calibration curves. The fact that, in the overlay plots, the R_g calibration and R_g distribution curves for these two co-eluting samples are

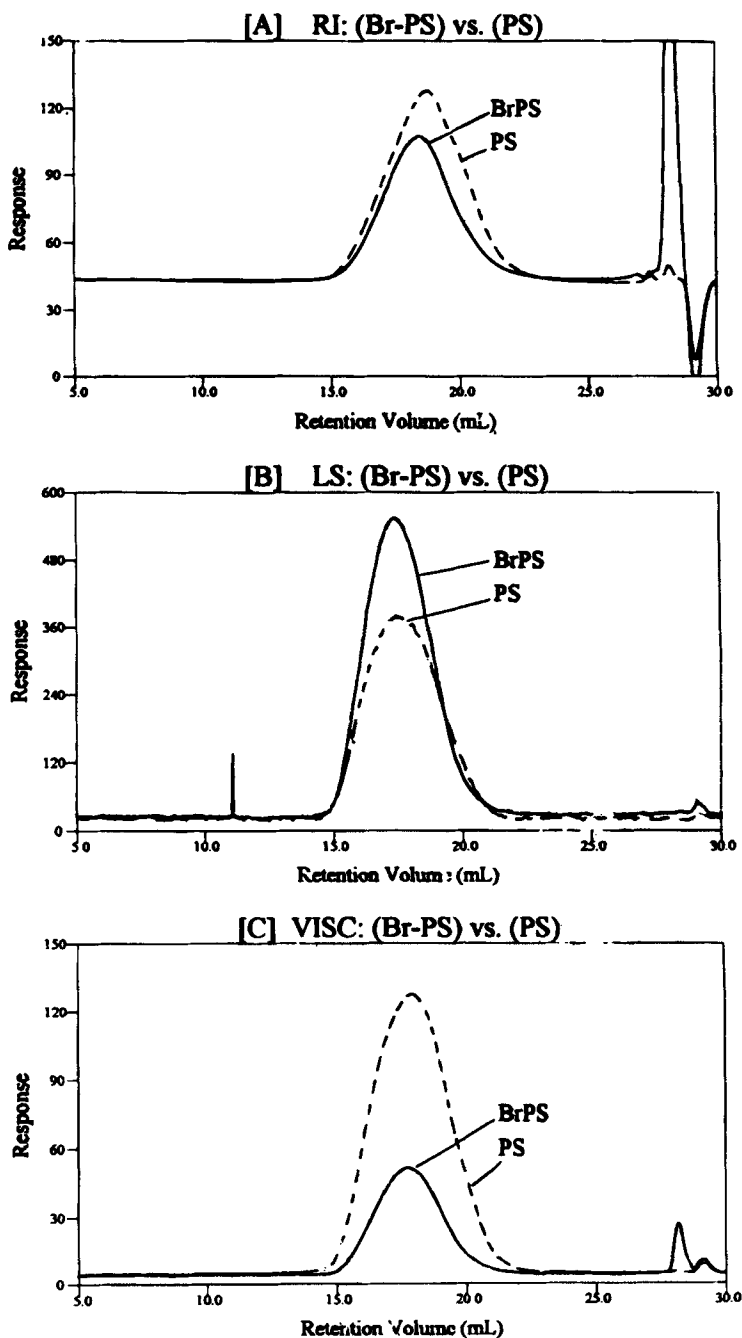


FIGURE 4 Effect of polystyrene bromination on triple-detector size exclusion chromatography (TriSEC) chromatograms. (A) Overlay of differential refractometer-size exclusion chromatography chromatograms of nearly co-eluting brominated-polystyrene and polystyrene peaks; (B) Larger BrPS peak area in light scattering-size exclusion chromatography chromatogram; (C) Smaller BrPS peak area in Visc-SEC chromatogram. Samples: brominated polystyrene BrPS-646K and broad MWD PS-250K (Am. Polym. Std.); column, Jordi 50cm linear; solvent, THF; flowrate, 1.0 mL/min; sample concentration, 1.0% for both the BrPS and the PS sample.

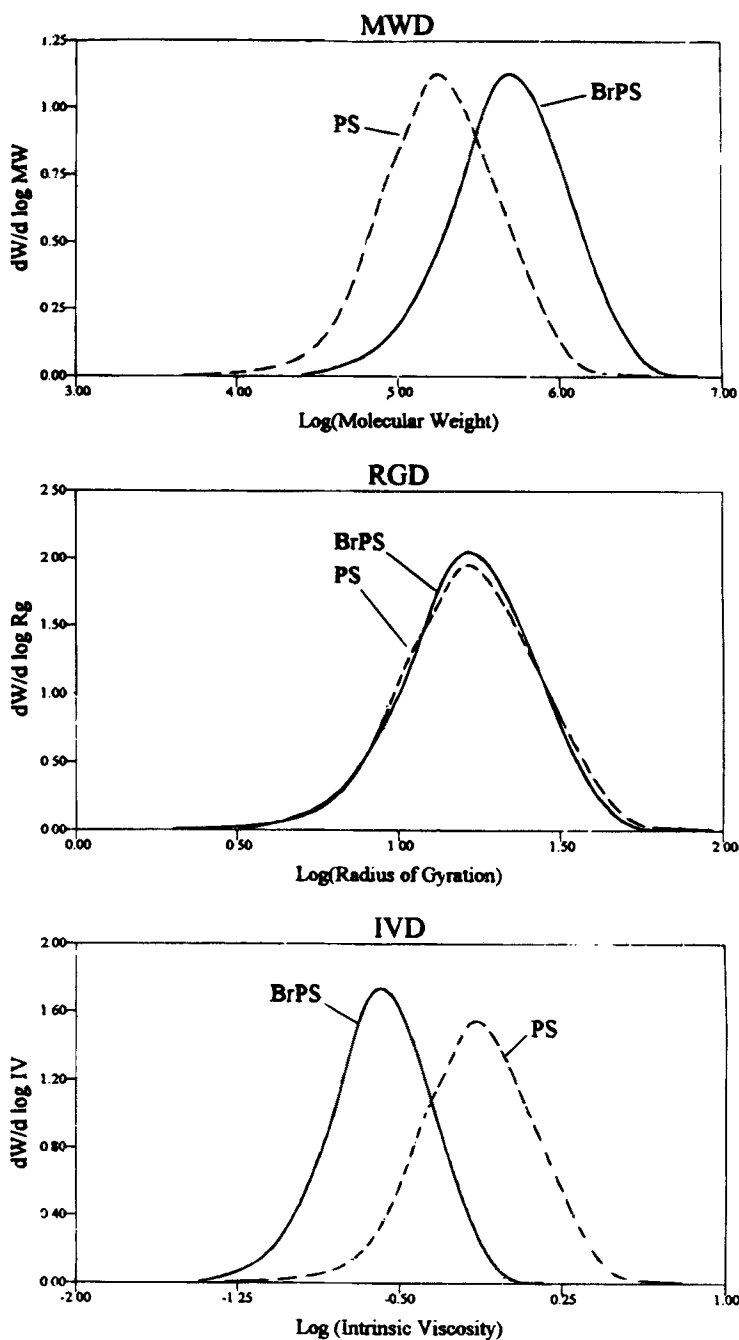


FIGURE 5A Effect of polystyrene bromination on triple-detector size exclusion chromatography results. Compared to the polystyrene peak, the brominated polystyrene peak shifts toward higher molecular weight in the molecular weight distribution plot, stays at nearly same location in $R_g D$ plot, and shifts to lower intrinsic viscosity in the intrinsic viscosity distribution plot. Samples: brominated polystyrene BrPS-646K and broad molecular weight distribution PS-250K (Am. Polym. Std.); column, Jordi 50cm, linear, solvent, tetrahydrofuran; flowrate, 1.0 mL/min.

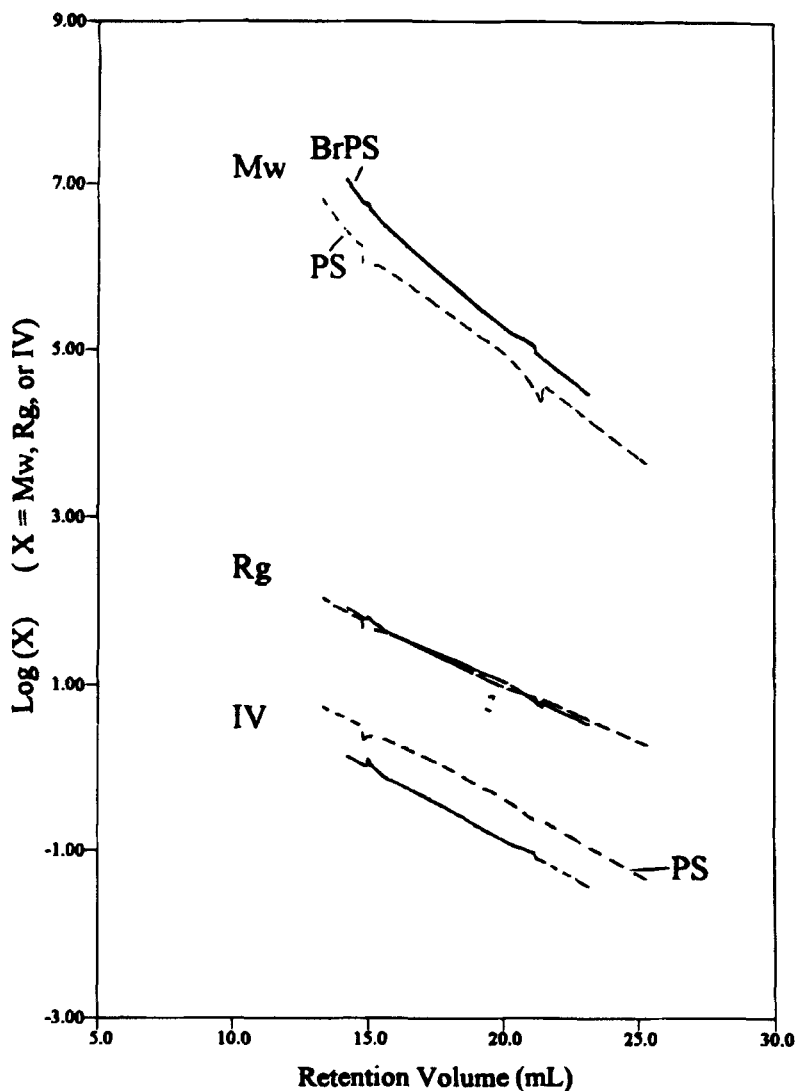


FIGURE 5B Effect of polystyrene bromination on "size exclusion chromatography Calibration" curves. Compared to polystyrene, the effect of bromination causes the brominated polystyrene calibration to shift toward higher molecular weight and lower intrinsic viscosity, but causes no shift of R_g calibration curve. Samples: brominated polystyrene BrPS-646K and broad molecular weight distribution PS-250K (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min.

so closely matched is also interesting. It provides confirmation of the universal calibration principle and the true size exclusion mechanism that is present in the SEC separation.

The Mark-Houwink plot in Figure 6 gives us yet another visualization of the structural differences between the two samples. There seems to have a slight decrease in the M-H exponent for the BrPS sample as compared to PS. The reason for this slope difference is not clear. The data may suggest a possible heterogeneity in the degree of bromination,

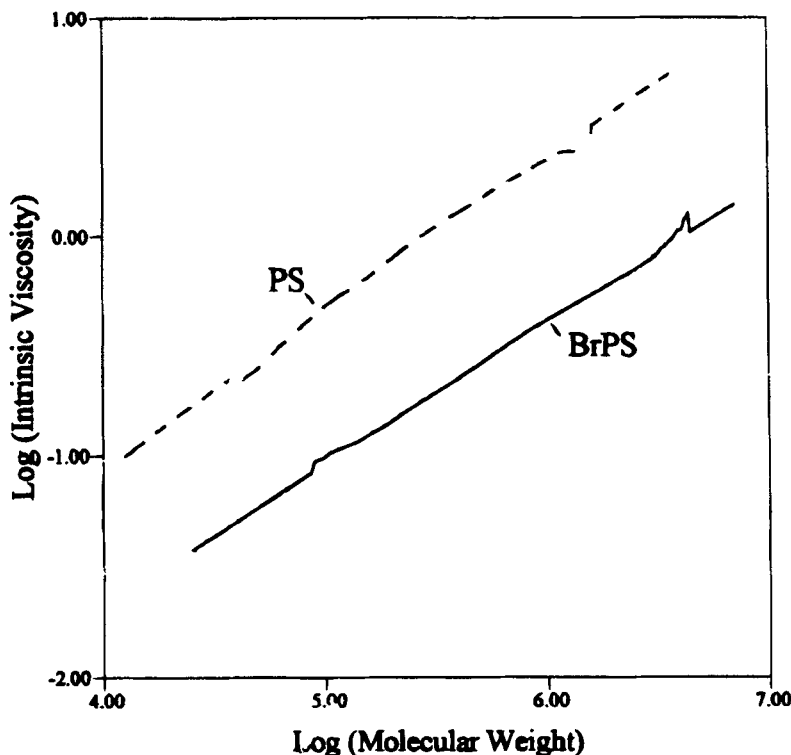


FIGURE 6 Effect of polystyrene bromination on the Mark-Houwink plot. Samples: brominated polystyrene BrPS-646K and broad molecular weight distribution PS-250K (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min.

favoring the high MW population of the sample. Nevertheless, as shown in Table III, M-H exponent values for both samples fall well within the window that is expected of the random-coil polymer conformation. The more significant result in this M-H plot is the large vertical displacement between the two curves, which resulted in a factor two change in the K value. This again confirms the very densely packed structure of the BrPS molecules. We see that even though the two samples co-elute, the BrPS sample has a MW value that is more than two times higher, but its IV value is that much lower, indicating a compact structure having a smaller molecular volume per unit mass.

LS Dissymmetry Correction

There have been concerns of possible angular dissymmetry errors in using the RALLS instrument for the TriSEC application; however, the errors in most cases are quite minimal [8,12]. We have found that such errors are generally negligible, especially after a correction for this effect is made in the TriSEC software. For our BrPS sample, we have found that this LS dissymmetry correction is a relatively unimportant factor in interpreting the BrPS results. Nevertheless, to assure accuracy of the results, all M_w and R_g calculations in this study have included RALLS dissymmetry correction.

TABLE III
Mark-Houwink Parameters of Polystyrene and Brominated Polystyrene
[η] = $K \cdot M^\alpha$

	α	$K (10^{-4})$	[η] (dL/g)	M_w by LS	M_w by CC
Polystyrene	0.725	1.098	0.839	250,000	251,000
Brominated PS	0.650	0.512	0.291	686,000	260,000*

*Note: Conventional calibration (CC) of using polystyrene standards grossly underestimated the true M_w of the brominated polystyrene sample. This "apparent" PS-equivalent M_w value is close to that of the polystyrene sample, because the two samples nearly co-elute from the SEC column.

For this work, the LS dissymmetry correction that is based on the random-coil molecular model is used. This correction of 90° RALLS dissymmetry is made with the Viscotek's TriSEC software. This software uses an iterative search algorithm to determine R_g (Equation (1), the Flory-Fox equation) and the following particle scattering function $P(\theta)$ derived by Debye for flexible-coil molecules [13]:

$$P(\theta) = \frac{2}{x^2} \cdot (e^{-x} + x - 1) \quad (3)$$

with,

$$\sqrt{x} = \left(\frac{4\pi n_0}{\lambda_0} \right) \cdot R_g \cdot \sin \theta \quad (4)$$

where n_0 is solvent refractive index, λ_0 is the wavelength of the incident light, and θ is the scattering angle [8,12,13].

Figure 7 shows the MWD and RgD curves of the BrPS sample, with and without dissymmetry correction. The Rg values are calculated using Equation (1) and the corresponding M_w values, with or without dissymmetry correction. Obviously, as shown in Table IV, the software correction factor in this case is rather small, and amounts to only a few percent. From this observation, we can conclude that any small errors in the dissymmetry-correction algorithm would lead to an even smaller, negligible error in the calculated molecular weight. It is interesting to note in Table IV the correction factor is larger for the higher order statistical averages of the MW and Rg values. This is understandable because these higher order averages are favoring molecules of larger sizes, which require larger correction factors [12]. The same observation is made in Figure 7, which shows no detectable effect of dissymmetry correction in the low-molecular-weight portion of the MWD and RgD curves. The correction factor becomes only noticeable when molecular weight values exceed much beyond 100,000 g/mol.

Effect of Flowrate Variation

Figure 8 and Table V show the result of a flowrate study in which the SEC solvent flowrate was purposely varied in the experiment to study the precision of the TriSEC method versus the conventional and universal calibration methods. It is obvious from these results that, without the use of a flowrate marker, a 5% error in flowrate can cause huge MW errors in both the conventional and the universal calibration methods. This is

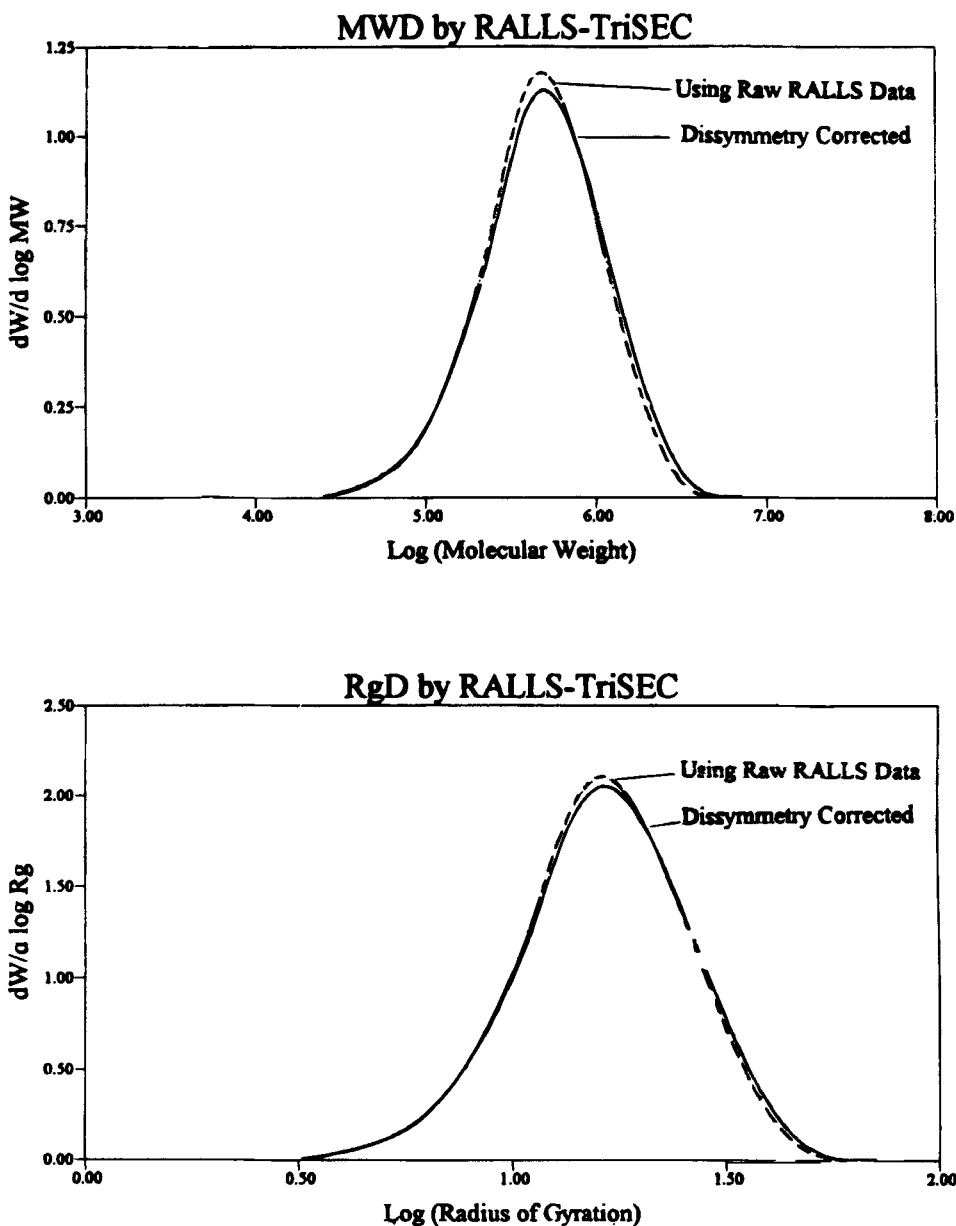


FIGURE 7 Effect of right-angle laser light scattering dissymmetry correction on molecular weight distribution and RgD curves. Sample, brominated polystyrene BrPS-646K, polydispersity ≈ 2.13 (Am. Polym. Std.); column, Jordi 50cm linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min; right-angle laser light scattering detector (Viscotek Corp.).

because these methods are retention-volume-based methods and thus sensitive to any sample retention errors. Large errors are also observed in the Rg and the M-H exponent values determined by the universal calibration method using the on-line viscometer.

TABLE IV
Correction of 90° Dissymmetry in TriSec Using Ralls

Molecular Parameter	Raw RALLS Data	Dissymmetry Correction	% Correction
M_z	1,046,000	1,226,000	+14.7
M_w	632,000	686,000	+7.8
M_n	339,000	347,000	+2.2
IV_z	0.372	0.372	no effect
IV_w	0.291	0.291	—
IV_n	0.219	0.219	—
$MH-\alpha$	0.678	0.650	-4.2
$R_{g,z}$	21.12	21.89	+3.5
$R_{g,w}$	17.76	18.14	+2.1
$R_{g,n}$	14.61	14.76	+1.0

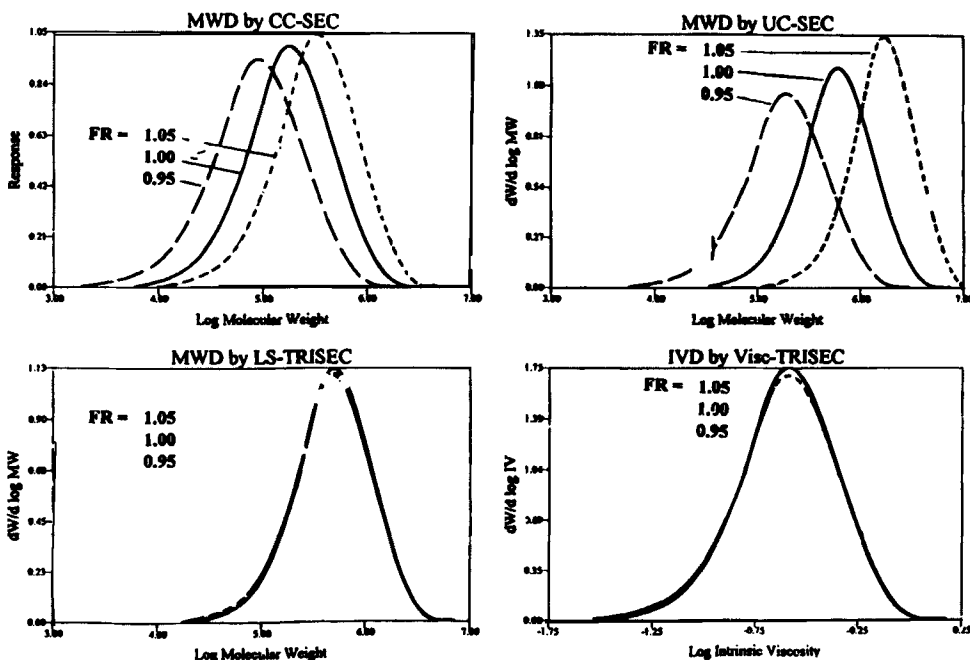


FIGURE 8 Effect of flowrate changes on TriSEC results. Strong flowrate dependency of the molecular weight distribution results obtained from conventional size exclusion chromatography or universal calibration method; flowrate insensitivity of the molecular weight distribution results obtained by light scattering-size exclusion chromatography or the intrinsic viscosity distribution results by Visc-SEC. Sample, brominated polystyrene BrPS-646K, polydispersity ≈ 2.13 (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrates, 0.95, 1.00, and 1.05 mL/min.

However, we see from the TriSEC results in Table V that, one can obtain flowrate-independent MWD and IVD curves by using the LS-RI and the Visc-RI combination, respectively. The MW and MWD determination in TriSEC is made by taking the ratio of the LS signal over the RI signal, whenever the polymer molecules happen to emerging from the SEC columns and reaching the two detectors. Therefore, the same MW value is measured

TABLE V
Effect of Flowrate on SEC-MWD
Brominated Polystyrene (BrPS-646K, 0.284 dL/g IV).
CC and UC calibration: PS standard at 1.00 mL/min without flowrate marker

	1.05 mL/min	1.00 mL/min	0.95 mL/min	% Std. Dev.
CC-SEC (PS-equivalent MW values)				
M_z	811,000	496,000	273,000	41.9
M_w	456,000	260,000	134,000	46.7
M_n	203,000	110,000	49,000	52.8
UC-SEC				
M_z	2,911,000	1,241,000	463,000	66.4
M_w	2,027,000	774,000	261,000	72.7
M_n	1,103,000	378,000	102,000	80.0
IV_z	0.395	0.373	0.368	3.1
IV_w	0.288	0.292	0.287	0.7
IV_n	0.211	0.222	0.215	2.0
MH- α	<u>0.781</u>	<u>0.669</u>	<u>0.594</u>	11.2
$R_{g,z}$	31.9	23.7	16.8	25.7
$R_{g,w}$	25.8	18.7	12.9	27.5
$R_{g,n}$	20.0	14.3	9.4	29.6
TriSEC				
M_z	1,391,000	1,255,000	1,293,000	4.4
M_w	679,000	686,000	671,000	1.0
M_n	316,000	347,000	315,000	4.5
IV_z	0.379	0.372	0.367	1.3
IV_w	0.288	0.291	0.287	0.6
IV_n	0.206	0.219	0.213	2.4
MH- α	<u>0.659</u>	<u>0.650</u>	<u>0.632</u>	1.7
$R_{g,z}$	22.0	21.9	21.8	0.5
$R_{g,w}$	17.9	18.1	17.8	0.7
$R_{g,n}$	14.2	14.8	14.3	1.7

for a molecule, regardless of whether the molecule elutes from SEC earlier or later due to flowrate differences. The MW values determined in this manner is not sensitive to flowrate variations. The same reason can be used to explain the insensitivity of the IV and IVD determination by Visc-SEC, using the ratio of the viscosity over the RI signal. TriSEC is also able to provide repeatable R_g values for the BrPS sample, regardless of the large flowrate changes. More importantly, the TriSEC approach is able to make significant improvement in the precision of determining the M-H exponent, as compared to the UC method (or the multi-angle laser light scattering SEC approach [3,12,14]).

Effect of Concentration Overloading

Sample concentration overloading in SEC is caused by a molecular crowding effect. When this occurs, the sample peak tends to skew towards increased retention time, giving rise to a distorted SEC elution curve favoring a lower MW appearance [2]. This is exactly what we see in the MW results from the conventional and the universal calibration methods shown in Figure 9 and Table VI.

Because of its round-about way of calculating the MW values, the UC method is found to be even more sensitive to SEC retention errors than the conventional SEC method. The

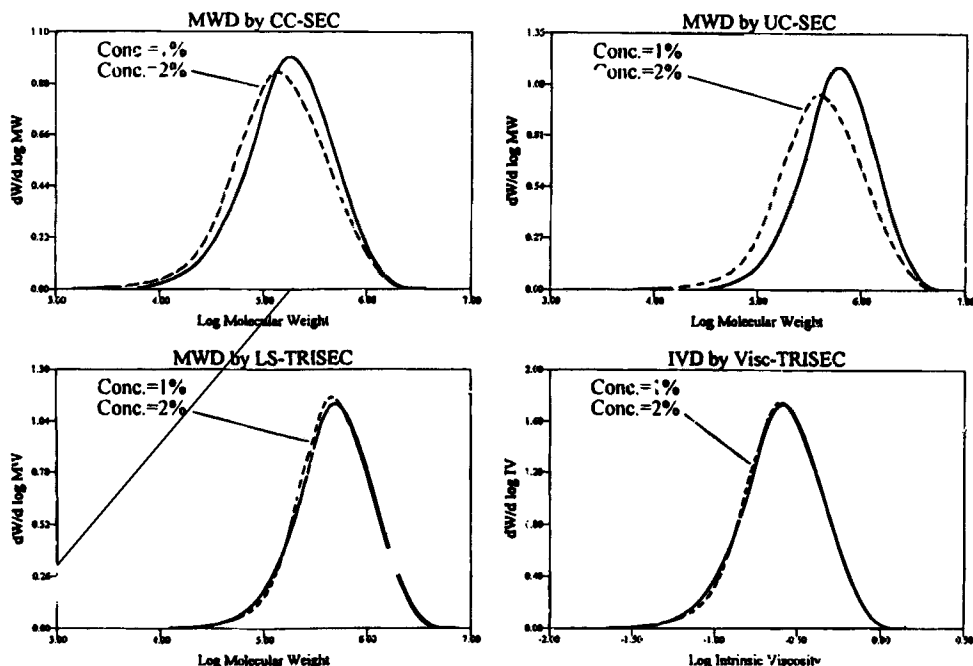


FIGURE 9 Effect of sample overloading on TriSEC results. Strong effect of sample-overloading on the molecular weight distribution results obtained from conventional size exclusion chromatography or universal calibration method; much less sample-overloading effect in the molecular weight distribution results obtained by light scattering-size exclusion chromatography or the intrinsic viscosity distribution results by Visc-SEC. Sample, brominated polystyrene BrPS-646K, polydispersity ≈ 2.13 (Am. Polym. Std.); column, Jordi 50cm, linear; solvent, tetrahydrofuran; flowrate, 1.0 mL/min; sample concentration, 1.0% and 2.0%; injection volume, 100 μ L.

UC results in Table VI show this method has poor precision performance against the effect of sample overloading. Only IV values (Table VI) from UC method using the viscometer-RI combination gave highly reliable results. This is only because the IV determination requires no SEC retention calibration. However, the results clearly show that the TriSEC method is highly insensitive to sample overloading effects.

From the flowrate (Table V) and the concentration study (Table VI), we see that the TriSEC method can determine the polymer M-H exponent much more precisely than the UC method. This makes TriSEC the method of choice for studying polymer branching and conformational differences. The ability to determine the M-H exponent is the very foundation to any polymer structural studies using SEC.

CONCLUSIONS

In summary, we have seen in this study how a TriSEC technique can be used to reveal the structure of a brominated polystyrene sample. With respect to general application of TriSEC, we have seen the strong insensitivity of the TriSEC results to the effects of

TABLE VI
Effect of Concentration Overloading on SEC-MWD
(Using brominated polystyrene BrPS-646K, 0.284 dL/g IV, 100 μ L injection)

	1% Concentration	2% Concentration	% Difference
CC-SEC (PS-equivalent MW values)			
M_z	496,000	472,000	-4.9
M_w	260,000	224,000	-13.8
M_n	110,000	79,000	-27.8
UC-SEC			
M_z	1,241,000	1,132,000	-8.8
M_w	774,000	598,000	-22.7
M_n	378,000	234,000	-38.1
IV_z	0.373	0.372	-0.2
IV_w	0.292	0.292	-0.1
IV_n	0.222	0.219	-1.3
MH- α	<u>0.669</u>	<u>0.582</u>	-13.0
$R_{g,z}$	<u>23.0</u>	<u>22.3</u>	-2.9
$R_{g,w}$	18.2	17.0	-6.3
$R_{g,n}$	13.8	12.4	-10.6
TriSEC			
M_z	1,255,000	1,190,000	-5.2
M_w	686,000	659,000	-3.9
M_n	347,000	324,000	-6.6
IV_z	0.372	0.372	-0.1
IV_w	0.291	0.291	-0.0
IV_n	0.219	0.217	-1.0
MH- α	<u>0.650</u>	<u>0.661</u>	+1.7
$R_{g,z}$	<u>21.9</u>	<u>21.6</u>	-1.5
$R_{g,w}$	18.1	17.9	-1.2
$R_{g,n}$	14.8	14.5	-1.8

flowrate fluctuations and sample overloading. In contrast, we have seen that the UC method is highly susceptible to SEC retention disturbances.

Instrumental band broadening is a problem in TriSEC that is closely related to the detector signal displacement. How well can TriSEC determine polymer branching and conformational differences is largely dependent on the ability of computer software to correct for instrumental band broadening. Software development will continue to play an important role in improving the TriSEC technology. For routine polymer MW analyses, TriSEC may or may not be the method of choice over the CC method.

It is noteworthy to point out that the most consistent results in our flowrate and overloading study are the IV and the IVD data. One can also expect the IVD results to be highly insensitive to many other chromatographic variabilities as well, such as column deterioration, instrumental band broadening, non-SEC retention effects, and so forth. We believe the IVD determination has the right attributes for becoming an important polymer quality control measurement. High precision IVD determination requires no SEC column calibration. Also, intentional sample overloading could be used to improve detector S/N and further enhance the IVD precision.

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