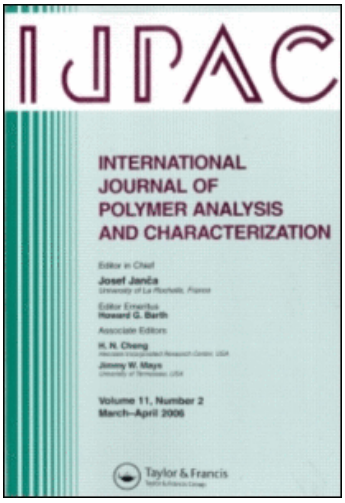

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Combining Detectors in Size Exclusion Chromatography: I. Interdetector Volume

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To cite this Article Thitiratsakul, R. , Balke, S. T. and Mourey, T. H.(1996) 'Combining Detectors in Size Exclusion Chromatography: I. Interdetector Volume', International Journal of Polymer Analysis and Characterization, 2: 4, 345 — 357
To link to this Article: DOI: 10.1080/10236669608033355
URL: http://dx.doi.org/10.1080/10236669608033355

Int. J. Polymer Analysis & Characterization
1996, Vol. 2, pp. 345–357
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Combining Detectors in Size Exclusion Chromatography: I. Interdetector Volume

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(Received September 28, 1995; in final form February 21, 1996)

Interdetector volume accounts for the time delay between detectors when more than one detector is used in size exclusion chromatography (SEC). In this work, interdetector volumes are determined from chromatograms for concentration detectors in series and in parallel, as well as for a parallel differential viscometer (DV)—differential refractometer (DRI) combination. The three methods examined for accomplishing this determination (peak apex, multipoint, and centroid methods) all provided equivalent results for truly monodisperse, low-molecular-weight compounds in all cases. For the DV-DRI combination, the interdetector volume obtained for the latter two methods increased with increasing molecular weight. This result was attributed to increasing peak skewness observed for DV chromatograms of narrow-molecular-weight standards as molecular weight increased. This occurred despite the fact that their normalized DRI chromatograms were all superimposable. In addition to helping to explain current widespread difficulties in determining interdetector volume, the observed effect also appears important in interpretation of narrow-molecular-weight distribution polymers. In Part II of this series we provide more evidence for the effect, examine its origins, and propose a solution.

KEY WORDS Size exclusion chromatography, interdetector volume, viscometer.

INTRODUCTION

When more than one detector is used on a size exclusion chromatography (SEC) the need to specify the volume between detectors, the "interdetector volume", becomes very apparent: chromatograms from each detector appear displaced from one another. Molecules require time to pass from one detector to another and data interpretation often requires combining information from two detectors at once. For example, for copolymer composition at each retention volume to be obtained from an ultraviolet (UV) detector and a differential refractometer (DRI) detector, the responses for the same molecules must be obtained from each detector. Also, when a DRI and a differential viscometer (DV) detec-

Presented at the International GPC Symposium, Lake Buena Vista, Florida, June 5–8, 1994

tor are used, calculation of intrinsic viscosity at each retention volume requires a concentration value from the DRI and a specific viscosity value from the DV, again for the same molecules. A similar situation exists when a DRI-light scattering (LS) combination is used. Thus, it is often the case that for multidetector SEC to provide property distributions, and not only overall average values, it is necessary to specify interdetector volume.

However, despite its importance and the apparent simplicity of the requirement, determination of interdetector volume has proven to be a significant obstacle to accurate SEC interpretation. A method of determining an effective interdetector volume which accounts for both interdetector volume and resolution correction simultaneously has been successfully used for a variety of broad-molecular-weight distribution polymers [1–6]. However, the method did not provide good interpretation of narrow-molecular-weight distribution polymers from DRI-DV or DRI-LS detector combinations. In this paper we investigate determination of interdetector volume for dual concentration detectors and for a parallel DRI-DV system.

THEORY

Interdetector Volume

There have been several publications on interdetector volume [1–13] including a recent review comparing several of these methods [5]. Many of the SEC variables fundamental to the problem can be readily identified. Different cell sizes in different detectors cause uncertainty in the definition of interdetector volume [7–8]. Interdetector band broadening, that is, axial mixing in tubing and detector cells also complicate matters [10]. For detectors connected in parallel, changes in the flowrate split during a run can change transport times of molecules between detectors [3]. The method of determining interdetector volume is probably the most significant variable of all. There are two major types of methods: those based upon directly evaluating the volume of tubing between detectors by measuring physical tubing dimensions, and those based upon chromatographic data. The former type has been found to be less likely to provide good quantitative results because of the uncertainties associated with rigorously defining the volume involved. The "effective" interdetector volumes necessary to provide good quantitative data are invariably different than those "geometrically determined" volumes [7]. Thus, in this study we focus upon methods which utilize chromatograms.

Some of the major chromatographic variables are: number of columns, mobile phase, polymer type, polydispersity of standards, injection concentration, injection volume, detector types, data acquisition rate, and finally, method of computation. Polydispersity has received special attention in the literature [10–15] because molecular weight variety in a standard can make it difficult to compare chromatograms from different types of detectors. The reason for this difficulty is that the response of a molecular-weight-sensitive detector is influenced by both concentration and molecular weight of the polymer.

The most commonly used method of determining interdetector volume is simply to determine the difference in peak retention volumes of the consecutive chromatograms obtained:

$$\delta_{apex} = v_{2,\,peak} - v_{1,\,peak} \tag{1}$$

where δ_{apex} is the interdetector volume, $v_{2,peak}$ is the peak retention volume for detector 2, and $v_{1,peak}$ is the peak retention volume for detector 1. A prime concern with this method

when molecular-weight-sensitive detectors are used is that polydispersity in the fractions will result in the chromatograms obtained from a molecular-weight-sensitive detector (such as a DV) being shifted compared to that of the concentration detector. This shifting would be the result of the detector response being influenced by the variation in molecular weight across the chromatogram and would therefore cause an error in estimation of the interdetector volume by the peak apex method.

An alternative to the peak apex method is to use not just the retention volume at the peak of each chromatogram, but rather to use many retention times across both chromatograms. That is, many points on each chromatogram from different detectors would be superimposed in selection of the interdetector volume. The interdetector volume would be determined by finding the volume required to minimize the following function:

$$O(\delta_{multipt}) = \sum_{i=1}^{n} w_i (W_{N2,i} - W_{N1,i})^2 \tag{2}$$

where $O(\delta_{multipt})$ is the function to be minimized by finding the interdetector volume δ, n is the number of data points considered for each chromatogram, w_i is the weighting factor ($= 1/W_{N1,i}^2$), $W_{N2,i}$ is the normalized chromatogram height for retention volume i from detector 2, and $W_{N1,i}$ is the normalized chromatogram height for a corresponding retention volume i from detector 1. (By corresponding point is meant the point at the same retention volume in both chromatograms. Normalized heights are obtained by dividing each height of a chromatogram by the total area under the chromatogram).

This "multipoint" method has the advantage of not depending upon the precision and accuracy of only one point. However, if consecutive chromatogram shapes are not identical then the results obtained will depend upon the weighting factors used in Equation (2).

A third method has been proposed by Yau [16]. It relies on calculation of the first moment of the chromatogram:

$$\delta_{centroid} = \sum_{i=1}^{n_2} (W_{N2,i} v_{2,i}) - \sum_{i=1}^{n_1} (W_{N1,i} v_{1,i}) \tag{3}$$

This method uses the normalized heights of the chromatograms ($W_{N2,i}$ and $W_{N1,i}$ corresponding to normalized heights for detectors 2 and 1, respectively) and also would be expected to be sensitive to peak shape and to the precision of the tail heights [17]. However, the dependence on peak shape may be less than in the multipoint method.

Changes in shape of the chromatogram from one detector to another can strongly influence the determination of interdetector volume. It is well known that polydispersity of the fractions, column band broadening, and interdetector band broadening can cause such changes. Band broadening is normally the more dominated troublesome factor than the polydispesity. However, numerical correction for the effect is possible although the uncertain form of the spreading function is a source of error.

EXPERIMENTAL

Two SEC systems were used in this work: The first system was a Waters 150C (Waters Corp., Milford, MA) with 1,2,4-trichlorobenzene (TCB, BDH, Inc.) at 145 °C as the mobile phase. The instrument was equipped with a Waters differential refractometer and a

Viscotek differential viscometer Model 110 (Viscotek Corp., Houston, TX). Three PLgel 10-μm mixed-bed, 300 × 7.5-mm columns (Polymer Laboratories, Amherst, MA) were used.

The second system was a room temperature SEC with tetrahydrofuran (THF, BDH, Inc.) at 30 °C as the mobile phase. This instrument utilized in turn: a Waters Model 440 UV detector with twin cells (referred to below as two identical UV detectors when they were used together); a Waters Model 410 differential refractometer and a Viscotek differential viscometer Model 110. A total of 5.6 m of 0.228-mm I.D. tubing joined the twin UV cells. Some experiments were conducted with two Polymer Laboratories 10-μm mixed-bed columns and others with three such columns. The high temperature system was used only with detectors in a parallel arrangement. The room temperature system utilized both series and parallel arrangements.

Injection volume was 100 μL with the concentration of narrow-molecular-weight distribution polystyrene standards (Polymer Laboratories) between 3.2×10^6 MW and 3.2×10^3 MW ranging from 0.5 to 6.1 mg/mL, for high-to-low molecular weights, respectively.

With respect to computer implementation of the various methods, the trapezoidal rule was used for integrations. The centroid method was the method most sensitive to baseline noise: no height less than 5% of peak height were used to obtain the tabulated reproducibilities. For the multipoint methods, heights less than 2% of the peak height were necessarily omitted. Data acquisition rates varied from 2.1 to 3.6 s/point depending upon the detector.

RESULTS AND DISCUSSION

Concentration Detectors in Series and Parallel

Figure 1 shows normalized (unit area) chromatograms from the two identical UV detectors in series. In addition to an obvious displacement between the chromatograms, the chromatogram from the second detector appears broader than that of the first. This broadening was attributed to interdetector band broadening. Figure 2 shows the result of correcting the chromatogram from the second detector for interdetector band broadening effects and then shifting it to superimpose on the chromatogram from the first detector using Equation (2). The interdetector band broadening correction was accomplished by solving the Tung axial dispersion equation using the method of Ishige, et al. [18]. A Gaussian shape function was assumed with a standard deviation of 0.15. The standard deviation was found by dividing the chromatogram width at baseline by four. Figure 3 shows the interdetector volumes ($\delta_{multipt}$) obtained by injecting a series of polymer fractions with and without interdetector band broadening correction of the chromatograms. The estimates together with the error standard deviation (to define noise in the method) are shown in Table I. Also shown in this table are values obtained using the peak apex method (δ_{apex}) and the centroid method ($\delta_{centroid}$). In this case, all three methods provided essentially equivalent results and interdetector band broadening correction made no significant difference. Interdetector volume appears constant with molecular weight. Very similar results were obtained when identical UV detectors were used in parallel. Figure 4 and Table II show the interdetector volumes obtained.

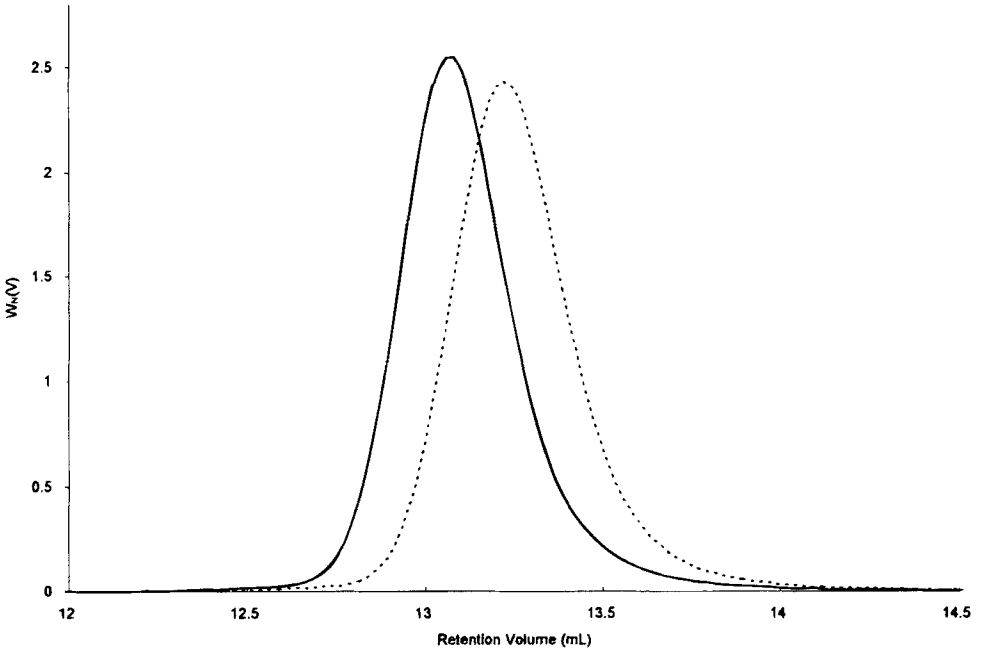

FIGURE 1 Normalized chromatograms from identical UV detectors in series: no interdetector band broadening correction.

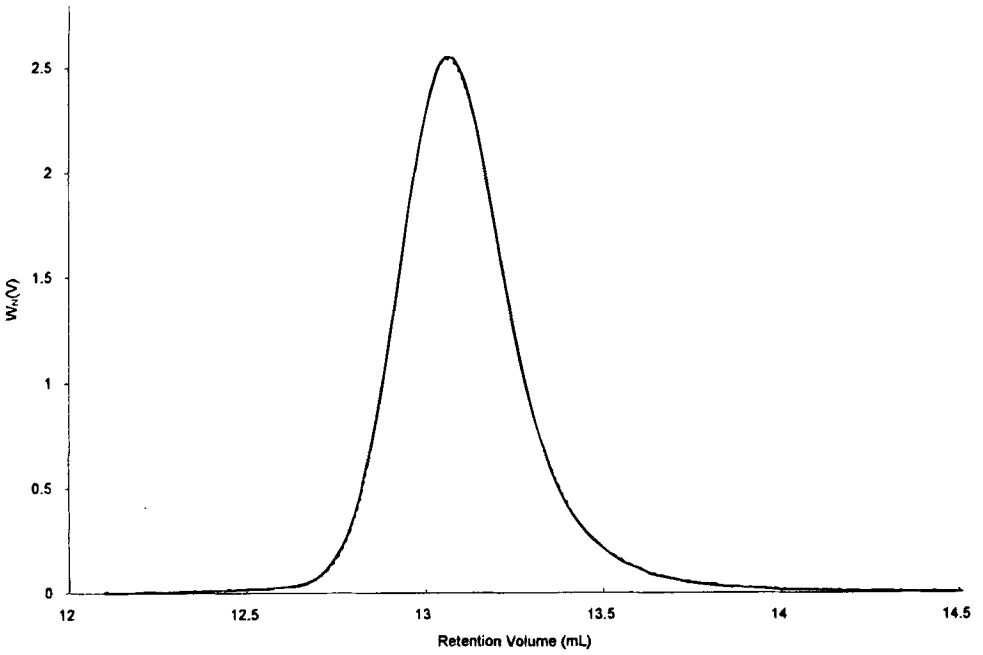

FIGURE 2 Normalized chromatograms from identical UV detectors in series: second chromatogram has been corrected for interdetector band broadening and shifted to superimpose on the first (using Equation (2) as a criterion).

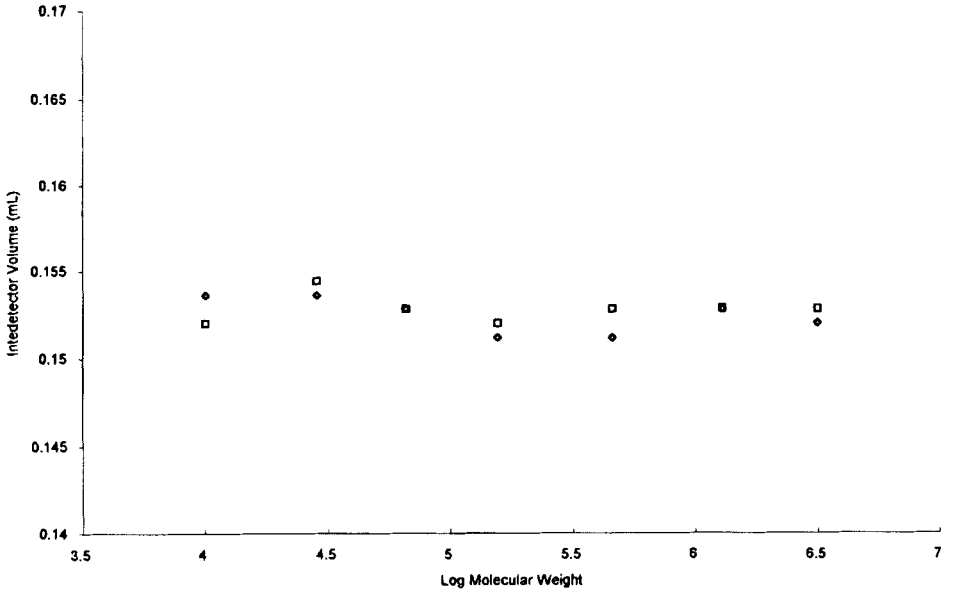

FIGURE 3 Interdetector volume ($\delta_{multipt}$) versus the logarithm of the peak molecular weight of narrow polystyrene fractions for two identical UV detectors in series: □ no interdetector band broadening correction to second chromatogram; ◇ with interdetector band broadening correction.

TABLE I

Interdetector volumes for identical UV detectors in series.

	average $\delta_{multipt} \pm \sigma$ (mL)	average $\delta_{apex} \pm \sigma$ (mL)	average $\delta_{centroid} \pm \sigma$ (mL)
No interdetector band broadening correction	0.1528 ± 0.0008	0.1521 ± 0.0010	0.1515 ± 0.0013
With interdetector band broadening correction	0.1524 ± 0.0010	0.1526 ± 0.0013	0.1513 ± 0.0011

Use of a UV-DRI detector combination in series posed no unusual problems. Figure 5 and Table III show that again all three methods were about equivalent and no variation of determined interdetector volume with molecular weight was evident.

DV-DRI Detectors in Parallel

Figure 6 shows normalized chromatograms from the DV and the DRI. Figure 7 shows a typical superposition obtained in determining $\delta_{multipt}$. Interdetector band broadening correction was done for the DV chromatogram. Superposition appears good but inferior to that obtained with the concentration detector systems. However, as indicated in Figure 8, the $\delta_{multipt}$ values show a definite trend with molecular weight. Figure 9 shows data from the room temperature SEC for all three methods of determining interdetector

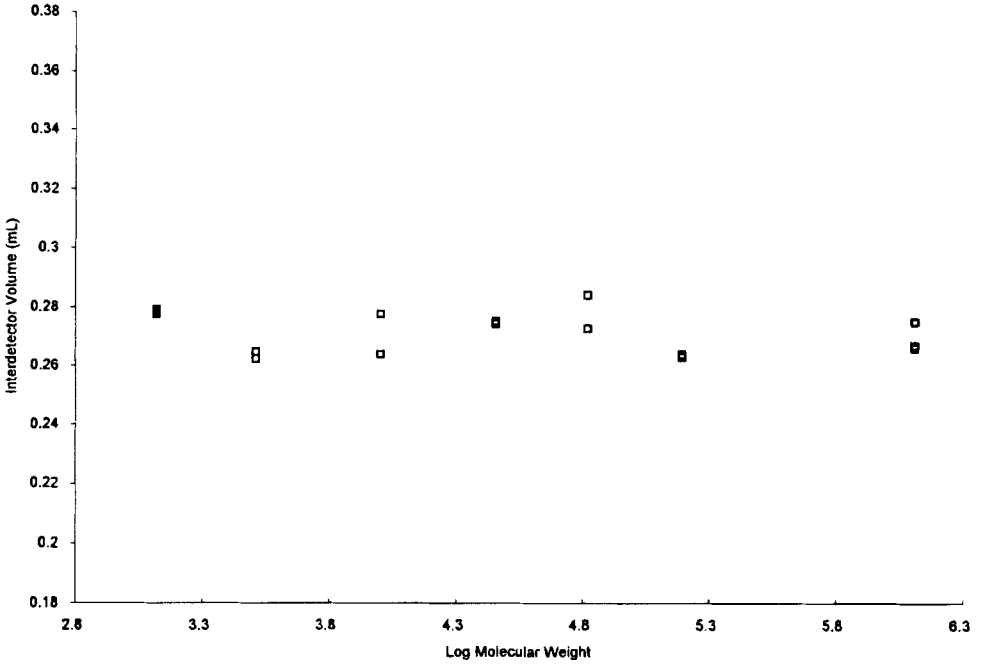

FIGURE 4 Interdetector volume ($\delta_{multipt}$) versus the logarithm of the peak molecular weight of narrow polystyrene fractions for two identical UV detectors in parallel (no interdetector band broadening correction).

TABLE II

Interdetector volumes for identical UV detectors in parallel.

	average $\delta_{multipt} \pm \sigma$ (mL)	average $\delta_{apex} \pm \sigma$ (mL)	average $\delta_{centroid} \pm \sigma$ (mL)
No interdetector band broadening correction	0.2716 ± 0.0018	0.2728 ± 0.0016	0.2745 ± 0.0014

volume. A variation with molecular weight was evident for $\delta_{multipt}$ and for $\delta_{centroid}$. However, when the peak apex method was used to determine δ_{apex}, no variation was observed. Values of interdetector volume converge at lowest molecular weight. All methods yield the same values of interdetector volume for the lowest molecular weight. All methods yield the same values of interdetector volume for the lowest molecular weights. Thus, an obvious solution to the problem of determining interdetector volume for DV-DRI is to simply determine the interdetector volume from the peak apex of chromatograms from low molecular weight samples. When small molecules, such as toluene, are used then polydispersity is eliminated as a factor. Any inaccuracy in results may then be attributed to interdetector band broadening. The difficulty may then be overcome by either using this interdetector volume and accomplishing a separate interdetector band broadening correction or by determining a new "effective interdetector volume". This conclusion coincides very well with what is currently occurring in SEC interpretation development. However, it does not explain why considerable uncertainty

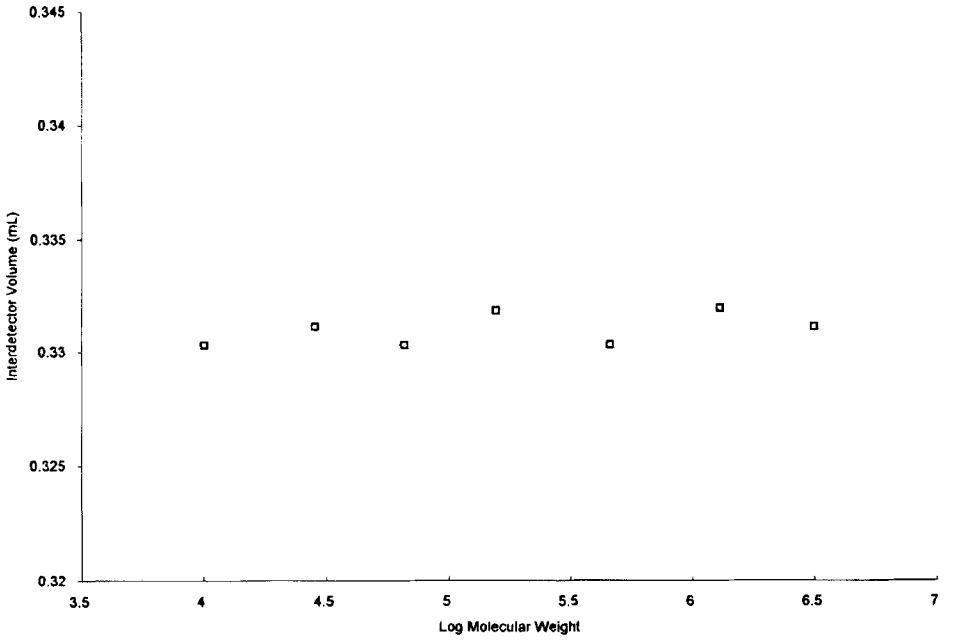

FIGURE 5 Interdetector volume ($\delta_{multipt}$) versus the logarithm of the peak molecular weight of narrow polystyrene fractions for UV-DRI detectors in series (no interdetector band broadening correction).

TABLE III

Interdetector volumes for UV-DRI detectors in series.

	average $\delta_{multipt} \pm \sigma$ (mL)	average $\delta_{apex} \pm \sigma$ (mL)	average $\delta_{centroid} \pm \sigma$ (mL)
No interdetector band broadening correction	0.3310 ± 0.0007	0.3313 ± 0.0018	0.3307 ± 0.0013

in DV-DRI analyses is generally associated with interpretation of narrow-molecular-weight distribution samples.

Figures 10a and 10b show the normalized DV and DRI chromatograms obtained in a series of runs on the room temperature SEC. For comparison purposes they have all been shifted so as to superimpose. The DRI chromatograms in Figure 10a show that all the samples have the same normalized concentration chromatogram shape. However, the shape of the corresponding DV chromatograms in Figure 10b appear markedly more broadened and skewed to higher retention volumes as the molecular weight of the sample increases. Table IV shows the areas under the DV chromatograms before normalization. This table shows that the mass injected was decreased so as to maintain these areas constant within about 5%. Replicated DV chromatograms for the same sample superimposed exceedingly well. Thus, the small differences in area observed between samples (which is of the same order as the within sample differences) is not responsible for the shape change with molecular weight.

This shape change is apparently what is influencing many of the interdetector volume determinations. If this effect is generally observed on such detector systems, in addition to

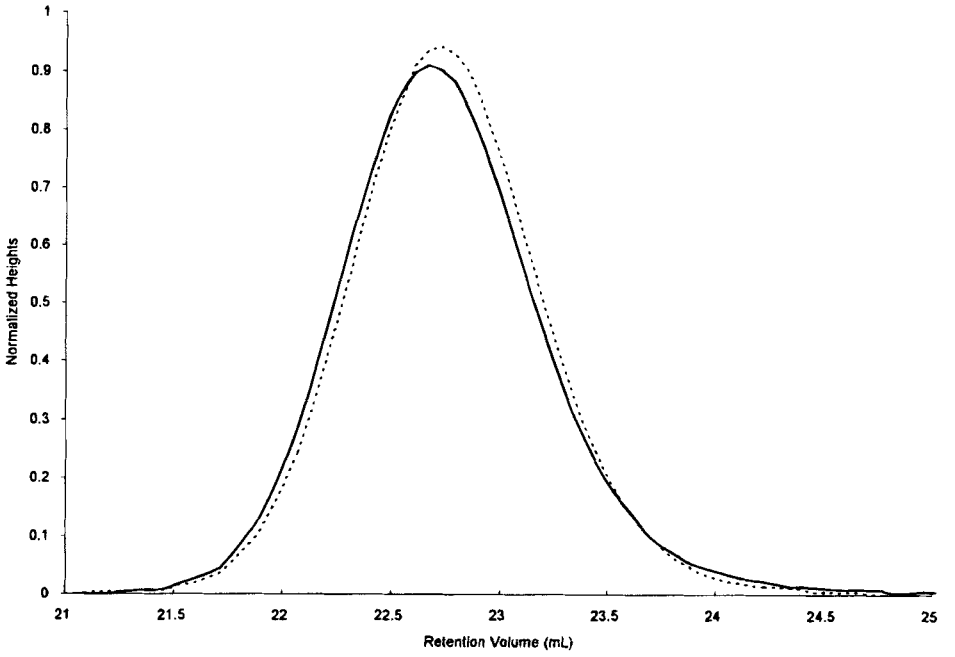

FIGURE 6 Normalized chromatograms from DV-DRI detectors in parallel (high temperature SEC system): no interdetector band broadening correction.

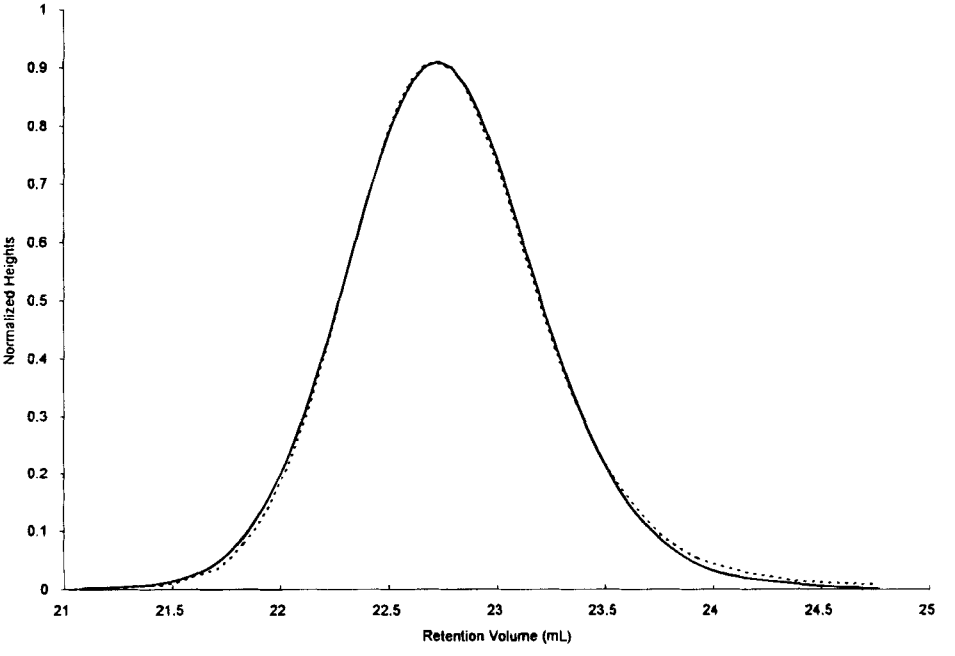

FIGURE 7 Normalized chromatograms from DV-DRI detectors in parallel (high temperature SEC system): DV chromatogram has been corrected for interdetector band broadening and shifted to superimpose on the DRI chromatogram (using Equation (2) as a criterion).

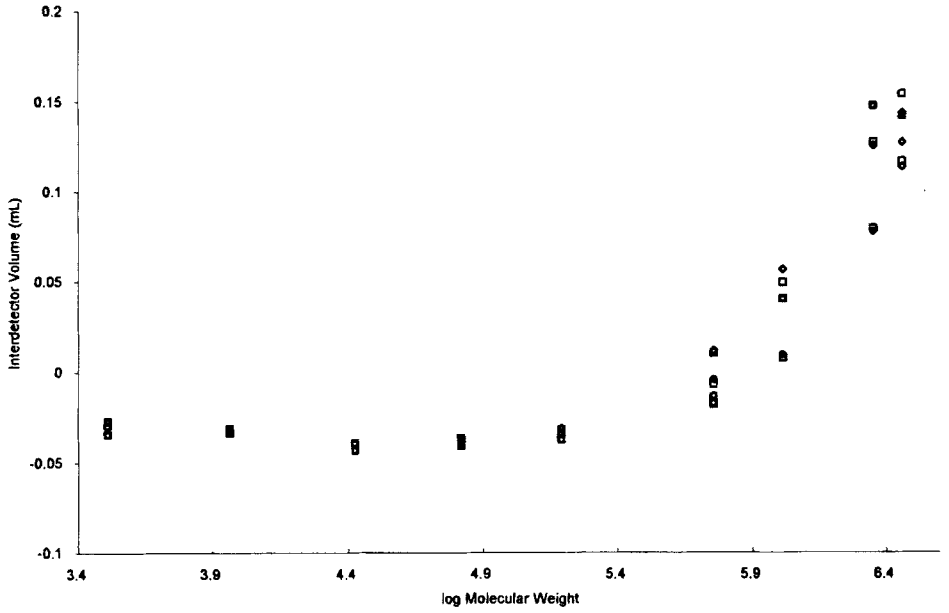

FIGURE 8 Interdetector volume ($\delta_{multipi}$) versus the logarithm of the peak molecular weight of narrow polystyrene fractions for DV-DRI detectors in parallel (high temperature SEC system): □ no interdetector band broadening correction; ◇ with interdetector band broadening correction.

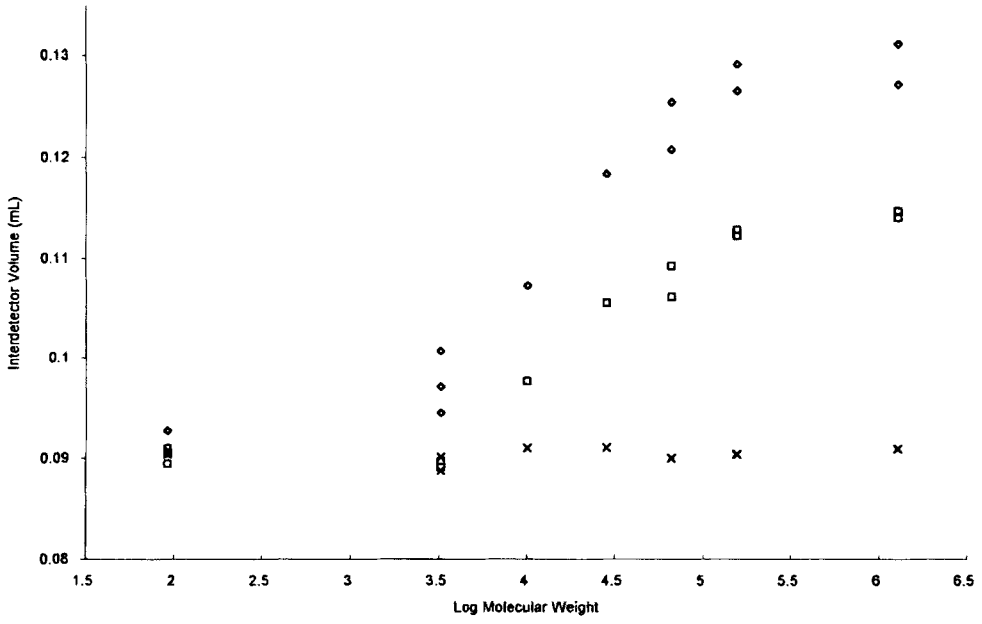

FIGURE 9 Interdetector volume versus the logarithm of the peak molecular weight of narrow polystyrene standards for DV-DRI detectors in parallel (room temperature SEC system): ×, δ_{apex}; □, $\delta_{multipi}$; ◇, $\delta_{centroid}$.

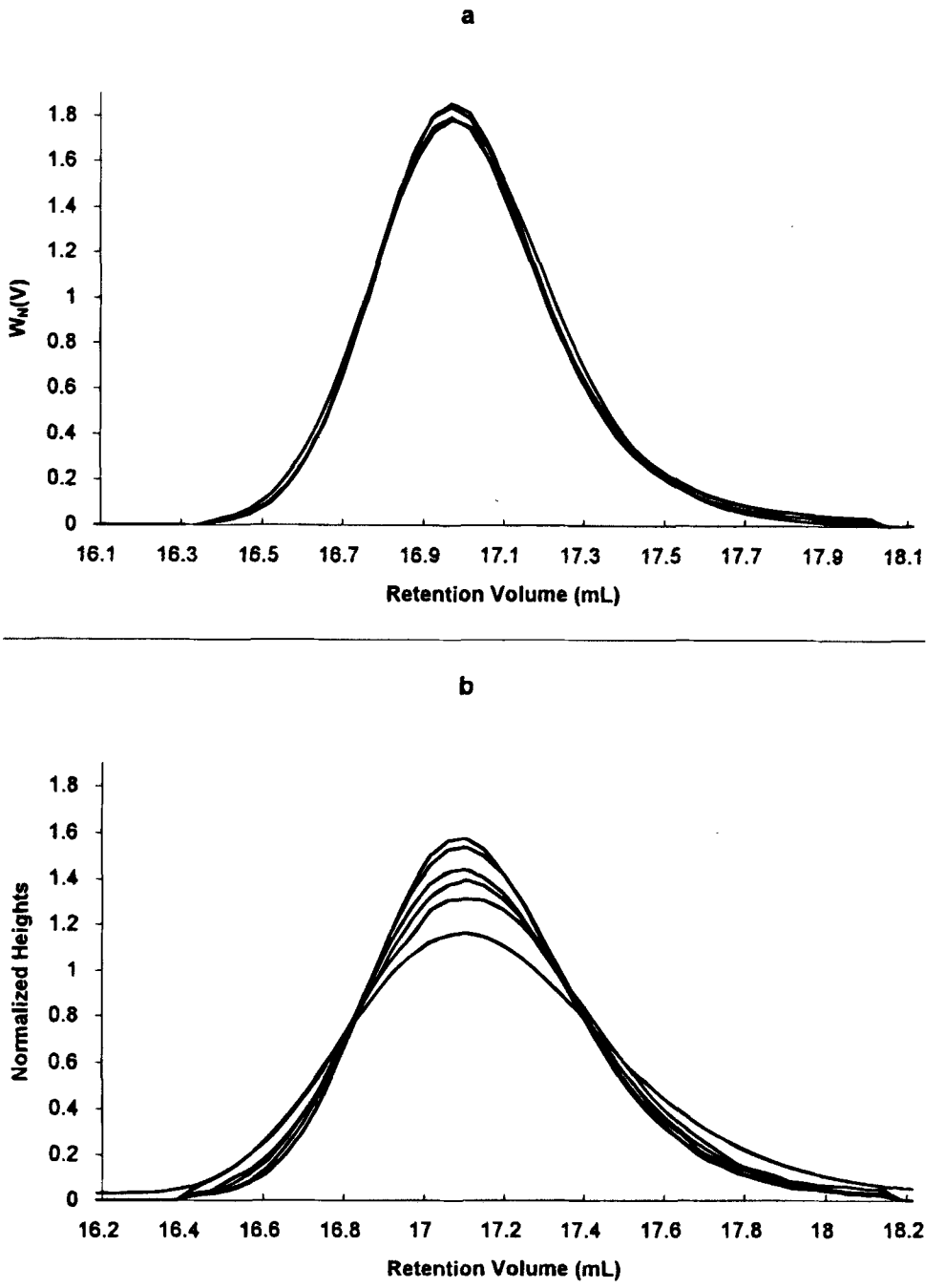

FIGURE 10 a. Normalized DRI chromatograms of narrow-molecular-weight distribution polystyrene standards (different lines correspond to standards with peak molecular weights from 28,500 to 1,290,000 g/mol) superimposed to show absence of shape change with molecular weight.

b. Normalized DV chromatograms superimposed as for Figure 10a. The shorter the chromatogram the higher the molecular weight of the standard.

TABLE IV

Areas under DV chromatograms before normalization.

Molecular Weight (g/mol)	Concentration (mg/mL)	Area (mL)
1,290,000	0.50	0.014
	0.49	0.014
460,000	0.99	0.014
	0.99	0.014
156,000	1.98	0.0139
	1.96	0.0133
66,000	4.05	0.0137
	4.06	0.0138
28,500	6.11	0.0135

helping to explain current widespread difficulties in determining interdetector volume, the observed effect would also then be important in interpretation of narrow-molecular-weight distribution polymers. Possible origins for this effect include the polydispersity of the narrow-molecular-weight distribution standards, the assumption of constant viscosity in the viscometer bridge at any time, axial dispersion, flowrate fluctuations during the run originating from the parallel detector arrangement.

CONCLUSIONS

For concentration detectors in series or in parallel, all three methods of determining interdetector volume (peak apex, multipoint or centroid methods) appear to work satisfactorily. No trend with molecular weight for the determined interdetector volumes was observed and interdetector band broadening correction did not appear to significantly affect results. A simple and conservative recommendation would be to determine interdetector volumes using the peak apex method and a low molecular weight truly monodisperse standard such as toluene.

For the DRI-DV parallel combination, all three methods work satisfactorily only for the lowest molecular weights. The peak apex method appeared least sensitive to these aspects for the commercial fractions examined. If a "true" interdetector value is required, our recommendation here is to determine it for this detector combination by using a truly monodisperse low molecular weight standard (e.g., toluene) and the peak apex method. Use of this value with interdetector band broadening correction is possible for broad-molecular-weight distribution polymers. However, the observation that significant normalized chromatogram shape changes occur for the DV detector as molecular weight is increased while the corresponding DRI chromatogram shapes are invariant indicates that more than band broadening effects may be influencing the chromatograms. It is possible that "effective" interdetector volumes widely used to interpret broad-molecular-weight distribution polymers are correcting for several effects.

In Part II of this series we provide more evidence for the observed peak shape changes, examine their origins, and propose a solution [19].

Acknowledgment

We wish to thank the Eastman Kodak Company (Rochester, NY) and the Natural Sciences and Engineering Research Council of Canada for funding this research. Also, we are grateful to the Canadian International Development Agency for awarding a scholarship to R. Thitiratsakul.

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