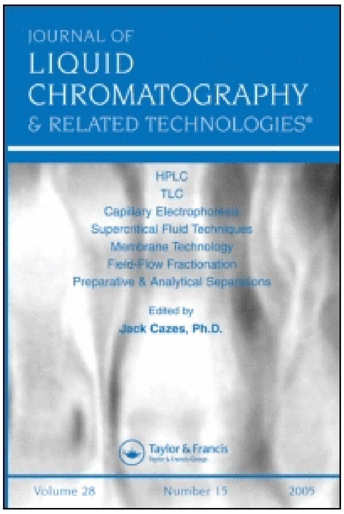

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T. H. Mourey[a]; S. M. Miller[a]
[a] Analytical Technology Division Research Laboratories Eastman Kodak Company, Rochester, New York

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MEASUREMENT OF INTERDETECTOR VOLUME IN SIZE EXCLUSION/LOW-ANGLE LASER LIGHT SCATTERING EXPERIMENTS

T. H. MOUREY AND S. M. MILLER
Analytical Technology Division
Research Laboratories
Eastman Kodak Company
Rochester, New York 14650-02136

ABSTRACT

The importance of an accurate value for the interdetector volume between a low-angle laser light scattering (LALLS) photometer and a concentration-sensitive detector on the calculation of local weight average molecular weights from size exclusion chromatography is discussed. Several methods of measuring interdetector volumes using narrow polymer standards are compared. Best results are obtained when peak onsets are used rather than peak maxima. An alternative approach that uses the LALLS as an absorption spectrophotometer is presented.

INTRODUCTION

Since its development (1-3) and commercialization in 1979, low-angle laser light scattering (LALLS) has become one of the more widely used detectors in size exclusion chromatography. One problem inherent to LALLS and any multidetector arrangement used to measure molecular weight distributions is determination of the interdetector volume between detectors. A configuration that we use frequently (Figure 1) illustrates the problem. Sample passes through molecular-weight-sensitive detectors (LALLS and differential viscometry (DV)) and a concentration-sensitive detector, such as a differential refractometer (DRI), at times t_1, t_2 and t_3, respectively. The interdetector volume for passage of sample between detectors is calculated from the lag time (Δt) and flow rate (Q_i) in each branch of the detector flow path.

The most important interdetector volumes are between the molecular-weight-sensitive and concentration detectors. Local molecular weight averages are calculated from the signals of the two detectors at each eluting slice. In the case of LALLS, local weight average molecular weights are calculated from the excess Rayleigh factor, $R_\theta(v)$, measured by the LALLS detector and c(v) measured by DRI:

$$\frac{1}{M_w} = \frac{K_\theta c(v)}{R_\theta} - 2A_2(v)c(v) \tag{1}$$

where K_θ is an optical constant and $A_2(v)$ is the second virial coefficient of polymer eluting at volume v.

The LALLS and DRI signals must be adjusted by an amount Δv_1 such that the same polymer species are compared in each detector.

Since our ultimate objective is to obtain accurate molecular weight distributions by SEC/LALLS, we define the "correct" or effective interdetector volume for calculation of molecular weight distributions as that which provides best agreement of local molecular weight averages with a narrow standard calibration curve of the same polymer. In Figure 2, interdetector volumes that are incorrectly high (shallow slopes) give molecular weight distributions that are falsely narrow. Low interdetector volumes (steep slopes in Figure 2) provide falsely broad molecular weight distributions.

It has been shown that the effective interdetector volume between detectors is usually not equivalent to the geometric volume because of the flow profiles in connecting tubing and mixing in the detector cells (4). For this reason, the effective interdetector volumes are measured by a flowing chromatographic experiment. The measurement of interdetector volumes is easier in the DV branch of Figure 1 or with any detector that is capable of responding to a small, monodisperse organic molecule. There is no complication, then, from size separation across the peak profile. In the case of LALLS, it is not possible to obtain detectable scattering intensities from solvent or small molecule peaks at reasonable concentrations. For this reason, several methods for measuring and calculating interdetector volumes using polymeric solutes are implemented:

Method 1. Measure the retention volumes of LALLS and concentration detector peak onsets or peak maxima of a narrow molecular weight standard eluted from a size-exclusion column.

Method 2. Use a high molecular weight polymer that is totally excluded from the column packing. The interdetector volume may be estimated from peak onsets, peak maxima, or the point of zero slope in logM vs retention volume plots (5).

Method 3. Replace the SEC column with a piece of empty tubing and measure peak onsets or peak maxima at a reduced flow rate.

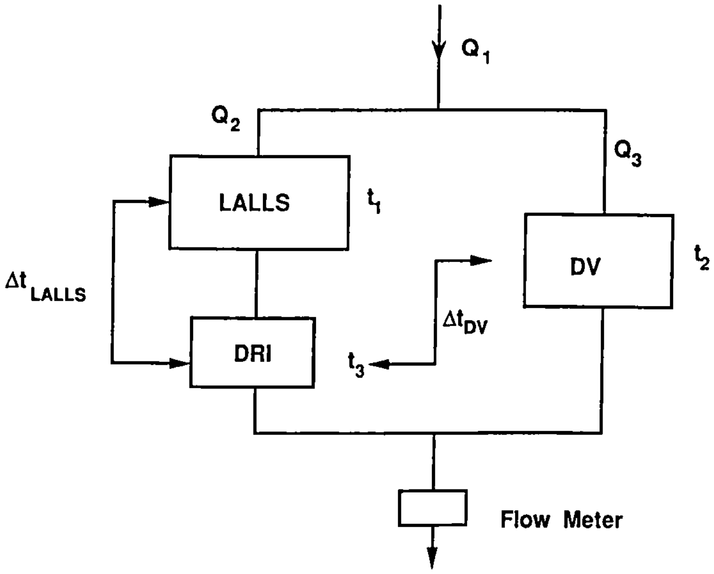

FIGURE 1. Multidetector schematic.

Method 4. Search for the interdetector volume which gives the best fit of LALLS local molecular weights of a broad standard to a narrow standard calibration curve (6). According to our results-oriented criteria, this is the best estimate of "effective" interdetector volume. This method assumes that axial dispersion does not significantly affect the local molecular weight averages. Theoretical and experimental evidence supports this assumption for broad standard chromatograms.

In evaluating the methods, we discount values of interdetector volumes that calculate irrational local molecular weights and whole polymer molecular weight averages. For example, an interdetector volume that calculates a molecular weight distribution of a standard polymer that is narrower than the true distribution must be erroneous because distributions are normally broadened by axial dispersion.

We have compared these methods according to how well they generate rational values of interdetector volume. Since each method has experimental complications, we also present an alternative procedure (Method 5) that uses the LALLS as an absorption spectrophotometer.

EXPERIMENTAL

The experimental setup depicted in Figure 1 is described in detail elsewhere (7). Tetrahydrofuran (THF, J. T. Baker HPLC-grade) was uninhibited and continuously sparged with helium. Flow rates were calculated from the time needed to collect 5 grams of eluent. Three 5 μm-particle-diameter 7.5 mm i.d. × 300 mm PLGel mixed bed columns (Polymer Laboratories, Amherst MA) were used to evaluate Method 1 at a nominal flow rate of 1.0 mL/min. A single 10 nm-pore-size, 5 μm-particle-diameter UltraStyragel column (Waters Associates, Milford, MA) was used at a nominal flow rate of 1.0 mL/min for Method 2. Polymer standards were injected in a volume of 100 μL at a concentration of 0.01% (wt/vol). Three 5 μm in-line stainless steel filters were placed in series with 1/16 in. o.d. × 0.01 in. i.d. stainless steel tubing between the autosampler and first detectors (Figure 1) for Methods 3 and 5. The filters provide turbulence in the flow stream. For use as an absorption spectrophotometer, the incident beam (P_0) of the KMX-6 LALLS (LDC Milton Roy, Riviera Beach, FL) was monitored with a 0.15 mm aperture. No attenuators were inserted and the coarse and fine gains were turned as low as possible. The baseline offset control was used to adjust the baseline to approximately 500 mV. Copper cyclohexanebutyrate (Eastman Kodak Company catalog number 10389) was dried at 100°C, and solutions, 2.0 mg/mL, were made in the eluent and injected in a volume of 100 μL. A Waters model 410 DRI was operated at a sensitivity of 4 for Method 5.

RESULTS AND DISCUSSION

Spectrophotometric Method

The incident beam of the LALLS detector is 632.8 nm. Any small molecule that absorbs radiation of this wavelength should attenuate the beam. We have chosen copper cyclohexanebutyrate because it is soluble in THF, has a characteristic green absorption (Figure 3) and is readily available. Unfortunately, the complex does not elute through polystyrene resin columns. Compounds with similar absorption characteristics should work equally well.

Typical LALLS and DRI signals for copper cyclohexanebutyrate are shown in Figure 4. Absorption of the incident LALLS beam by the copper complex produces a negative response. No LALLS signal above baseline noise is obtained with injection of pure solvent or high molecular weight polystyrene when the incident beam is examined, confirming that the LALLS response in Figure 5 results from absorption of the incident 632.8 nm radiation. Also note the absence of dust spikes characteristic of LALLS signals at low angles. Used as a spectrophotometer, the LALLS is insensitive to particulate scattering.

Occasionally, the DRI response is multimodal as in Figure 5. This results from slightly different amounts of water in the sample solvent and eluent. This effect is considerably more pronounced if the copper complex is not dried prior to dissolution. Peak distortions of this sort are to be expected without columns to separate water and dissolved gas peaks from the copper complex. For this reason, we use the peak onset of the LALLS and DRI detectors to calculate detector lag times. We have defined peak onset as the point at which the detector signal noticeably deviates from a longer draw to the baseline. From ten injections, we calculated an interdetector volume of 0.132 + /- 0.005 mL (Table 1).

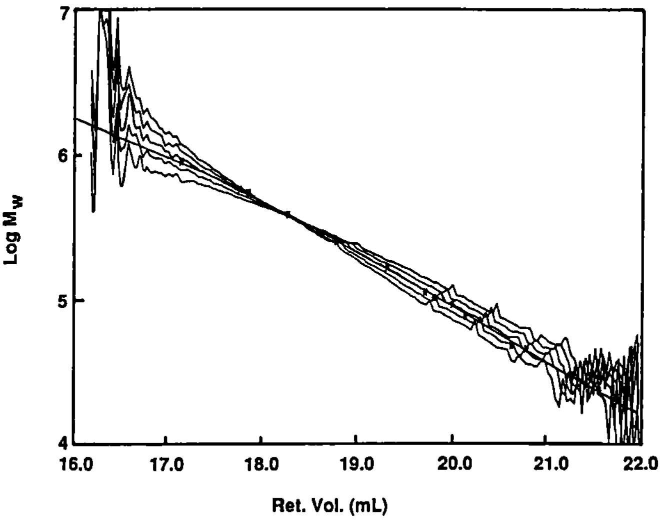

FIGURE 2. Effect of interdetector volume on local molecular weights of NBS 706 eluted from three 5 µm PLGel mixed bed columns. Symbols with smooth curve are the polystyrene narrow standard calibration. Interdetector volumes are 0.07 (steepest slope), 0.10, 0.13, 0.16, and 0.19 (shallowest slope) mL.

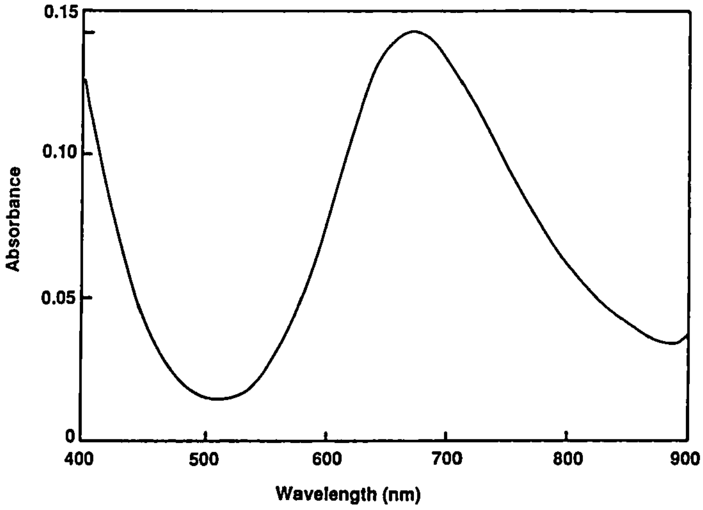

FIGURE 3. Absorption spectrum of copper cyclohexanebutyrate, approximately 4×10^{-4} moles/L in THF, 1 cm pathlength.

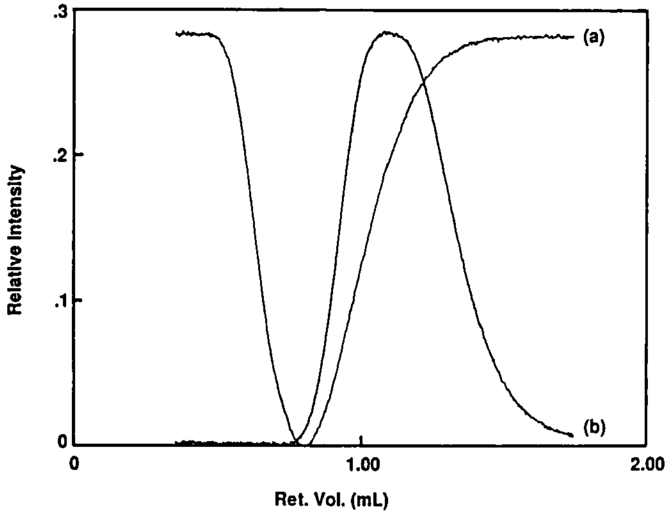

FIGURE 4. Spectrophotometric LALLS (a) and DRI (b) responses for dried copper cyclohexanebutyrate.

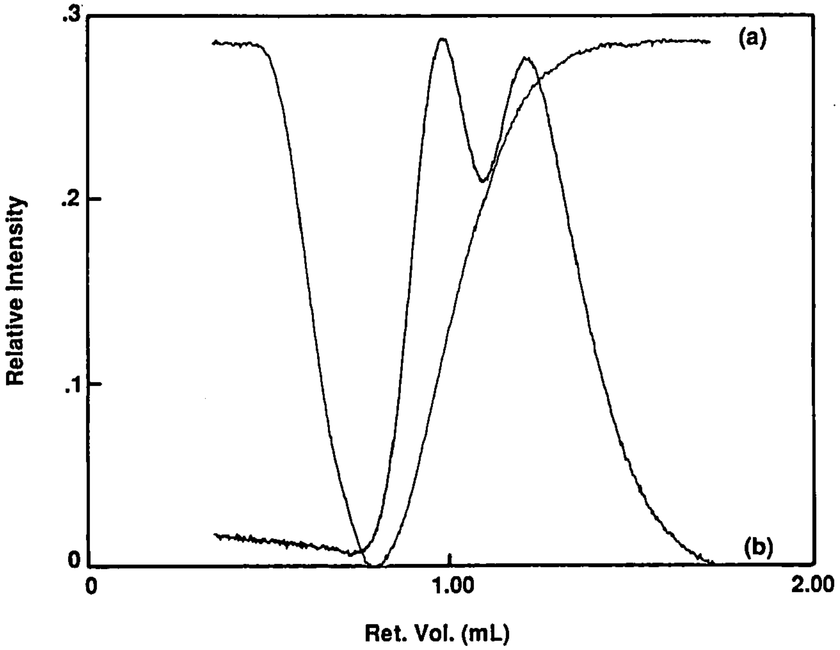

FIGURE 5. Spectrophotometric LALLS (a) and DRI (b) responses for undried copper cyclohexanebutyrate.

TABLE 1

	interdetector volume (mL)
Method 1 (narrow standard)	
peak onset	0.140 +/-0.008
peak maxima	0.155 +/-0.010
Method 2 (excluded narrow standard)	
peak onset	0.134 +/-0.004
peak maxima	0.161 +/-0.004
zero slope	0.160 +/-0.010
Method 3 (no column)	0.100 +/-0.020
Method 4 (numerical optimization)	0.130 +/-0.005
Method 5 (spectrophotometric)	0.132 +/-0.005

Comparison of Methods

The interdetector volume which provides the best fit of LALLS local weight average molecular weights of NBS 706 polystyrene with a narrow standard calibration curve is 0.13 mL (Method 4 in Table 1). Comparison of results obtained by the five methods (Table 1) reveals the following:

Method 1. The interdetector volume calculated from the peak maxima of a narrow standard eluted from a mixed bed SEC column (Method 1) is high. This interdetector volume calculates molecular weight distributions that are narrower (shallow slopes in Figure 2) than the correct distribution of NBS 706. Measurement of peak onsets provides better agreement, but is also slightly high. This may be attributed to some separation by size of the narrow standard.

Method 2. Interdetector volumes measured by peak maxima, as well as the method of zero slope, are erroneously high. One complication with the zero slope method is shown in Figure 6. Linear plots are not obtained and the slopes must be approximated. Measurements that rely on peak maxima measurement are likely to be in error because of distorted peak shapes. Figure 7 shows that the outputs of the two detectors are not superimposable if aligned at peak onsets. We presume this distortion to be a result of mixing in the LALLS cell. As with Method 1, peak onset measurements agree with the best fit.

Method 3. The interdetector volume calculated from DRI and LALLS peak onsets of a high molecular weight polymer, without columns, are smaller than the effective interdetector volume. This value calculates molecular weight distributions that are too

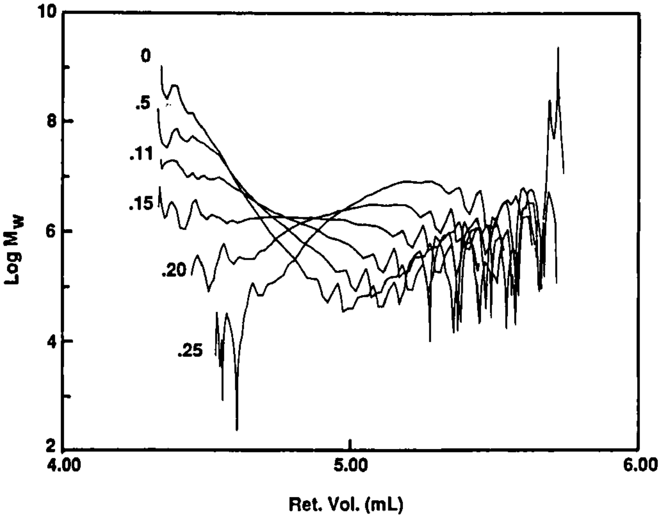

FIGURE 6. Zero slope method of reference 5. Interdetector volumes are given on the figure.

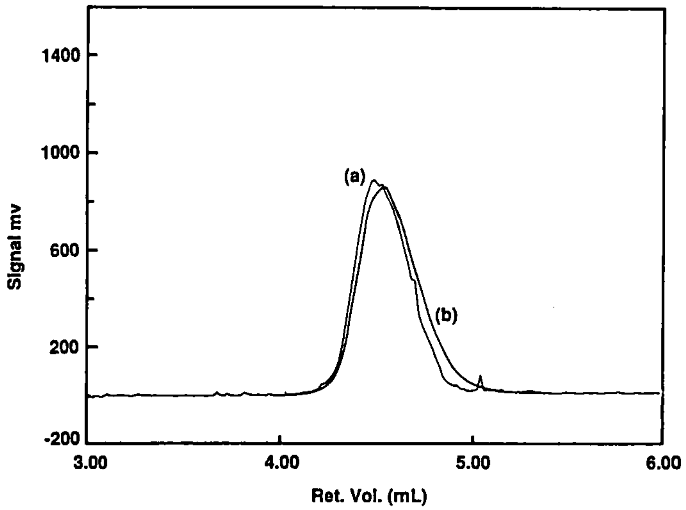

FIGURE 7. Polystyrene 1,900,000 eluted from one 10-nm pore-diameter column. LALLS (a) and DRI (b) peaks are aligned at onsets to illustrate peak distortion.

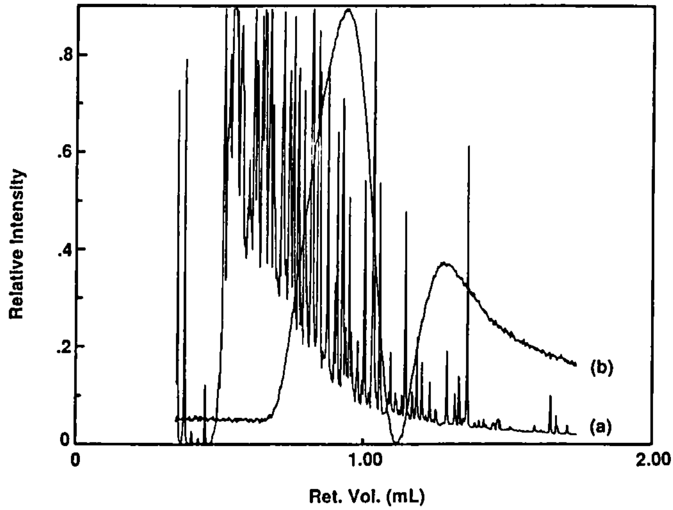

FIGURE 8. Polystyrene 600,000 eluted through empty tubing. LALLS (a) scattering at 6-7° and DRI (b) response.

broad (refer to Figure 2). Peak maxima cannot be accurately measured because of the dust spikes shown in Figure 8, and in general this method is difficult to reproduce.

Method 4. Numerical optimization of the interdetector volume by the method of reference 6 generates the best effective value according to our criteria, provided a broad polymer standard is examined.

Method 5. The spectrophotometric method measures interdetector volumes that agree closely with the best effective interdetector volume.

CONCLUSIONS

Five different methods were examined in this work:

· Methods 1, 2, and 3 which use peak maxima calculate erroneously high interdetector volumes. The molecular weight distributions calculated from these values are narrower than the true values. Narrower values are considered infeasible because distributions are normally broader than true due to axial dispersion effects. Interdetector volumes that give molecular weight distributions narrower than the true distribution should not be used.

· Peak onsets of LALLS and DRI responses for polymer eluting through an empty tube give low values of interdetector volume. The resulting molecular weight distribution is substantially broader than the true distribution.

Peak onsets provide best agreement with the best fit obtained by numerical optimization (Method 4). A totally excluded polymer (Method 2) or the spectrophotometric method (Method 5) provides the greatest precision and accuracy. The new spectrophotometric method is easy to implement because, unlike scattering, it is insensitive to dust particles.

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REFERENCES

(1) Ouano, A. C. and Kaye, W, *J. Polym. Sci., Polym. Chem. Ed.*, **12**, 1151, 1974.

(2) Kaye, W. and Havlik, A. J., *Appl. Opt.*, **12**, 54 1973.

(3) Kaye, W. and McDaniel, J. B., *Appl. Opt.*, **13**, 1934 1974.

(4) Bruessau, R, in "Liquid Chromatography of Polymers and Related Materials—II," J. Cazes, Ed., Marcel Dekker, Inc., New York, 1980, pp. 73-93.

(5) Lecacheux, D. and Lesec, J., *J. Liq. Chromatogr.*, **5**, 2227, 1982.

(6) Balke, S. T., Cheung, R., Lew, R. and Mourey, T. H., Submitted to *J. Liq. Chromatogr.*

(7) Mourey, T. H., Miller, S. M., Balke, S. T., Submitted to *J. Liq. Chromatogr.*