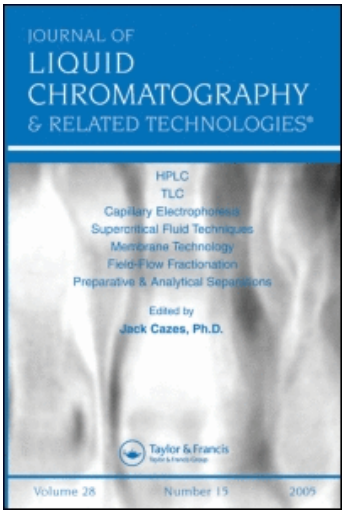

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Quantitative Size Exclusion Chromatography: Assessing New Developments

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QUANTITATIVE SIZE EXCLUSION CHROMATOGRAPHY: ASSESSING NEW DEVELOPMENTS

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ABSTRACT

Methods of assessing new technology are achieving increased importance because rapid technological changes cause obsolescence of evaluations before they are completed. Users now must often evaluate the specific version of the new technology that they receive. Four major areas are used here to provide examples of assessment methods: high resolution columns, coupled concentration-molecular weight-differential viscometer detectors, flow rate monitoring using a thermal pulse flowmeter and determination of Mark-Houwink constants from polydisperse standards. Specific methods of assessment examined centre about error analysis and sensitivity analysis. Several of the methods use the conventional calibration curve. The idea of correction priority (i.e. thoroughly examining the most fundamental significant corrections first) is emphasized.

INTRODUCTION

New column packings, instrumentation and computer implemented methods (chemometrics) offer great promise in the size exclusion chromatography (SEC) of industrial polymers. However, at the same time they are also dramatically increasing

uncertainty associated with quantitative results. Furthermore, advances are being made so rapidly in these areas that assessments are obsolescent before they are completed. Under these conditions, the methods of assessment gain new importance as we all must become assessors of the technology that we receive. New developments in SEC sometimes provide unusual problems and unexpected sensitivities which need to be revealed by an assessment. In this paper, evaluations now in progress of recent advances in four major areas are used to illustrate methods of assessment. The four areas are: high resolution columns, coupled molecular weight-intrinsic viscosity-concentration detectors, flow rate monitoring using a thermal pulse flow meter and determination of Mark-Houwink constants from polydisperse standards. Although some recent results are summarized, the emphasis in the paper is on the methods used rather than on the results obtained.

THEORY

High Resolution Columns: Calibration Curves and Plots of Residuals

Traditionally the emphasis in column evaluation has been the assessment of resolution. The problem of resolution (1) splits into two fundamental aspects: separation of the molecules (i.e. separation of the peaks of two truly monodisperse polymer standards) and band spreading. Height equivalent to a theoretical plate (hetp) determined by injection of small molecules (e.g. ortho dichlorobenzene) into an SEC measures only band spreading of small molecules. It does not provide information on how well polymer molecules of different molecular weights will separate or even how much band spreading each will incur. However, it is widely used in SEC because it provides a standard way of specifying the overall condition of

a column packing. A wide variety of quantities have been proposed to express both fundamental aspects of resolution of polymer molecules in one number. The primary difficulty that these methods address is the polydispersity of commercial "monodisperse" polymer standards.

Recent developments in SEC columns have focused upon obtaining linear calibration curves while maintaining high resolution. Therefore, it is the separation of the macromolecules which is now emphasized over band spreading reduction. Thus, at this time, once we are satisfied from hetp measurements that the columns are within manufacturers' band spreading specifications, our method of evaluating new columns should examine molecule separation. The conventional SEC calibration curve provides a comprehensive view of separation and is the natural basis for an assessment method. A difficulty with the calibration curve is that the logarithmic ordinate conceals the error represented by scatter of points around the fitted curve. One response to this situation is to define a new ordinate as:

$$\text{\% error in M} = \frac{100\,(M_{fit} - M_{std})}{M_{std}} \qquad (1)$$

where

M_{fit} is the molecular weight value obtained from the calibration curve fit to the data;

M_{std} is the manufacturers' published value of the peak molecular weight of the narrow polystyrene standards.

The choice of percent error in molecular weight is of direct interest to quantitative analysis. Also, it can be readily shown by an error propagation analysis (2) that the error in log M is proportional to the percent error in M.

A plot of % error in M versus retention volume should show a random scatter of points about zero and this scatter should reflect an error of the same order as the error in the known molecular weight values of the standards.

Coupled Concentration-Molecular Weight-Intrinsic Viscosity Detectors: Prioritizing Correction Methods and Devising Criteria

Multi-detector systems utilizing spectrophotometers set at different wavelengths or combining spectrophotometers with a differential refractometer have been known for many years in SEC (1). Combinations of concentration-molecular weight-intrinsic viscosity detectors are just beginning to appear (3,4). Furthermore, to minimize axial dispersion effects, flow from the SEC can be split so that only one, or at the most, two detectors will be in series (5,6). With the relatively large cell sizes in some of the detectors the second detector in a series will always receive sample that has been "premixed" in the cell of the previous detector.

Although experimental resolution from current SEC columns is now high, this combining of detectors and the fact that molecular weight and intrinsic viscosity

values often disagree with manufacturers' values for the standards even when less detectors are used, have caused much increased concern for resolution correction. Significant theoretical developments, particularly by Hamielec and co-workers (7), have provided the needed correction equations. However, a prime requirement in implementing resolution correction continues to be the determining the shape of the chromatogram of a truly monodisperse polymer. It is likely a function of concentration and molecular weight. Selection of correction factors to be used in the equations now appears as a very critical and uncertain issue. However, before resolution correction is used there are more fundamental "corrections" which need to be applied to the data: the time required for polymer molecules to move from one detector to another and mobile phase flow rate.

The significance of the transport time between detectors originates from the need to superimpose the concentration values from the differential refractometer on the outputs of the molecular weight and intrinsic viscosity detectors. Selecting the wrong transport time results in a concentration error whose magnitude depends upon the shape of the chromatogram from the refractometer. Furthermore, for systems involving molecular weight detectors, use of retention volume rather than retention time is more appropriate because of the need to calculate concentration at each point. Then the "transport time" between detectors becomes the "volume of mobile phase" between detectors (the "inter-detector volume"). An error in determining the inter-detector volume can result in the wrong concentration being assigned to each point on the molecular weight or viscometer detector chromatogram.

Flow rate errors translate into errors in retention volume since the chromatograms are all recorded on a time axis which must be changed to retention volume by

multiplying by flow rate. Furthermore, as mentioned above, interpretation of both the molecular weight and the intrinsic viscosity detectors requires concentration. Concentration values depend upon accurate retention volume increments.

The errors in the final results caused by incorrectly specifying inter-detector volume and flow rate depend on many factors. The whole polymer weight average molecular weight from low angle laser light scattering (LALLS) and the whole polymer intrinsic viscosity from a differential viscometer detector (DV) will probably only be slightly affected. However, the local properties (weight average molecular weight and intrinsic viscosity at each retention volume) can be strongly affected (8). Conventional practice is to experimentally measure inter-detector volume and flow rate (1). For inter-detector volume this typically involves measurement of time between the peak retention times on each detector for narrow standards. Internal standards injected with the polymer are a common method of accounting for flow rate errors. Both of these methods have serious uncertainties. The inter-detector volume measurement depends upon flow-rate being constant and peak retention times being accurate. An auto-correlation method has recently been proposed by Lederer et al. (9) to circumvent the latter problem. A more fundamental uncertainty is that peak shapes can change because of mixing in detector cells (10). This may be the cause of some observations that the inter-detector volume varies from sample to sample (11). The flow rate measurement requires a suitable internal standard and cannot account for flow rate variations within a run (only between runs). The Thermal Pulse Flowmeter (12) is an instrument which is potentially capable of continuously monitoring flow rate for each sample and is discussed below. In this paper, we attempt to obtain the best possible inter-detector volume and flowrate for each sample injected by using only the data normally obtained in molecular property measurement.

The first step is selection of what calculated values of the data to examine (i.e. the identity of the "response variable") to assess whether or not the correct inter-detector volume and flow rate have been obtained. The requirement is a response that is sensitive to these quantities, independent of resolution correction and available from unknown samples. As previously mentioned, the LALLS value of M_w of polymer at each retention volume ($M_w(v)$) and the DV value of $[\eta]$ of polymer at each retention volume ($[\eta](v)$) are expected to have the required sensitivity and are not significantly affected by resolution correction over wide ranges of retention volume for broad molecular weight distribution polymers (13-15). Plots of these local values against retention volume should superimpose on the conventional calibration curve if inter-detector volume and flow rate have been correctly specified.

The strategy devised to assess the coupled detector system was as follows:

i. Examine the sensitivity of local values of M_w from LALLS and $[\eta]$ from the DV as a function of retention volume to changes in (a) inter-detector volume and (b) flow rate by changing the values in the computer program used to interpret the data and plotting the result;

ii. Using experimental estimates of inter-detector volume and flow rate, examine whether local values of M_w superimpose on the conventional calibration curve and whether local values of $[\eta]$ superimpose on the plot of $[\eta]$ against retention volume obtained from the conventional calibration curve.

iii. If the results in i show sensitivity and the results in ii show no superposition,

use a computer optimization method to search for the inter-detector volume and flow rate necessary to superimpose each set of curves.

This information provides the basis for determining whether lower priority corrections, such as resolution correction, are necessary.

The Thermal Pulse Flowmeter: Error Propagation Analysis

As mentioned above, the thermal pulse flowmeter (12) is a prime candidate for accomplishing flow rate correction of each SEC sample via direct experimental measurement of the flow rate. The principle of operation of the instrument is the measurement of the time required for a thermal pulse to be carried downstream a fixed distance. There have been several studies of this instrumentation (16,17). Precision has been stated at 0.1 % in pulse time. However, it is important to note that it is not pulse time, but rather flow rate which is needed to correct SEC chromatograms. Flow rate is related to pulse time according to (18):

$$t = A + \frac{B}{Q} + \frac{C}{Q^2} \qquad (2)$$

Error in Q is related to error in pulse time, t, according to:

$$s_Q^2 = \left[\frac{\partial Q}{\partial t}\right]^2 s_t^2 \qquad (3)$$

Therefore,

$$s_Q^2 = \left\{ \frac{-B}{2(t-A)^2} - \frac{(B^2 + 4(t-A)C)^{0.5}}{2(t-A)^2} + \frac{C}{(t-A)(B^2 + 4(t-A)C)^{0.5}} \right\}^2 s_t^2 \qquad (4)$$

The error in Q as a function of pulse time can be computed and compared to the SEC requirement. Usually percent error (100 s_Q/Q) is of interest. This is a simple illustration of the use of error propagation analysis in method assessment.

Mark-Houwink Constants from Polydisperse Standards: Error and Sensitivity Analysis

Calibration curve search methods to determine the Mark-Houwink constants, **K** and **a**, by utilizing the SEC universal calibration curve, are well known. Figure 1 shows a schematic which illustrate the methods. Essentially, **K** and **a** are guessed by a computer program until the molecular weight averages and/or the intrinsic viscosity calculated from the chromatograms of one or more polymers match the values of these properties known through independent measurements.

It has been pointed out that the **K,a** values obtained from different sets of data are often not equal and independent but rather are different and strongly correlated (19,20): high values of **K** are accompanied by low values of **a** and vice versa. This

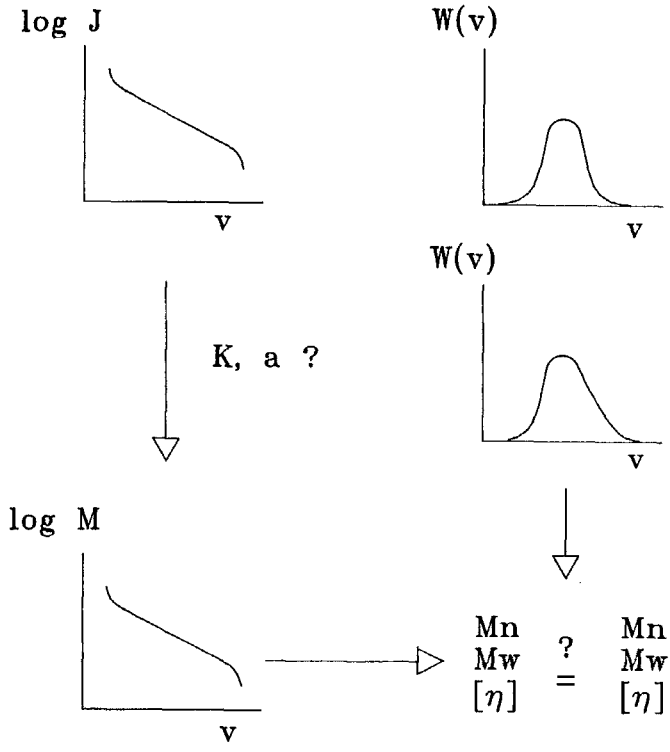

Figure 1. Schematic diagram of calibration curve search methods used to obtain Mark-Houwink constants from the SEC chromatograms of polydisperse standards.

is of most concern if the actual values of **K** and **a** are required. The resulting SEC molecular weight calibration curve obtained by using different **K** and **a** values may not be much affected since different **K,a** pairs may provide nearly the same value of intrinsic viscosity. Until very recently (21), there has been no attempt to quantitatively characterize this situation.

In this study, the worst results were obtained when a ratio of intrinsic viscosity values was being matched by the search. This was expected to be the best case. Using ratios of intrinsic viscosities is highly recommended by both Dobbin et al. (19) and by Kubin (20) for many good reasons: experimental intrinsic viscosity values are readily obtained; the search is simple numerically since ratioing intrinsic viscosity values for whole polymers eliminates the **K** values. [Thus the search is simply a single variable search for the correct **a** value. Once **a** is determined, **K** can also be determined by a single variable search]; axial dispersion effects on the results are minimized. A qualitative guideline from the literature is that the samples used should have widely differing molecular weights. Samay's (22) investigation of the variation of his objective function with the value of **a** is particulary notable.

A straightforward way to assess this method is to examine the effect on various viscosity ratios by systematically varying the value of **a** and plotting the value of the viscosity ratio obtained from the chromatograms versus each value of a. The next step is to superimpose the true value of the ratio on this "sensitivity analysis" plot showing the error present in the true value. The resulting intersection of the true value line and the SEC value line shows the "window" of **a** values on which the search would be expected to converge for that particular ratio. The location and width of the window can be used to determine why the search failed to provide reasonable values and to select samples.

EXPERIMENTAL

Column evaluations were done on two systems: System 1 consisted of a HP1050 Autosampler, a Waters Model 590 reciprocating piston pump and three five micron

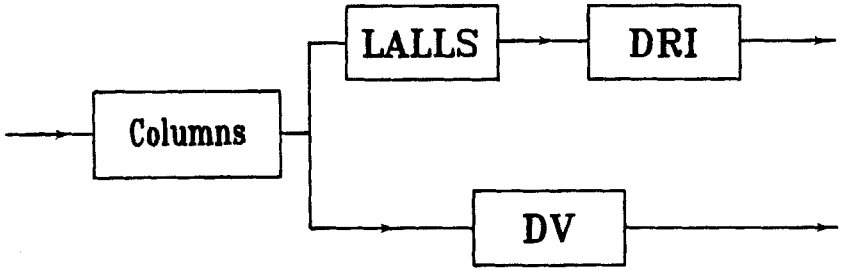

Figure 2. Schematic diagram of detector arrangement.
LALLS: low angle laser light scattering
DV: differential viscometer
DRI: differential refractometer

particle size mixed bed columns from Polymer Laboratories. The columns had been in use for approximately two years. Tetrahydrofuran at 30°C was the mobile phase. Three 5 micron particle size mixed bed columns from Polymer Laboratories were used. System 2 was a Waters 150C with 1,2,4-trichlorobenzene at 145°C as the mobile phase. It utilized three non-commercial prototype development columns from a different company than the five micron particle size columns.

For the work on evaluation of molecular weight detectors, System 1 was equipped with a low angle laser light scattering detector (Chromatix) and a differential viscometer (Haney) in addition to a Waters differential viscometer (see Figure 2). Inter-detector volume was determined experimentally by removing the columns from the instrument and injecting narrow standards at very low flow rate (0.1 cc/min). Flow rate was measured by collecting the eluent in a flask and weighing.
The thermal pulse flowmeter work was done using System 1 with acetone as the mobile phase using a Molytek Flowmeter.

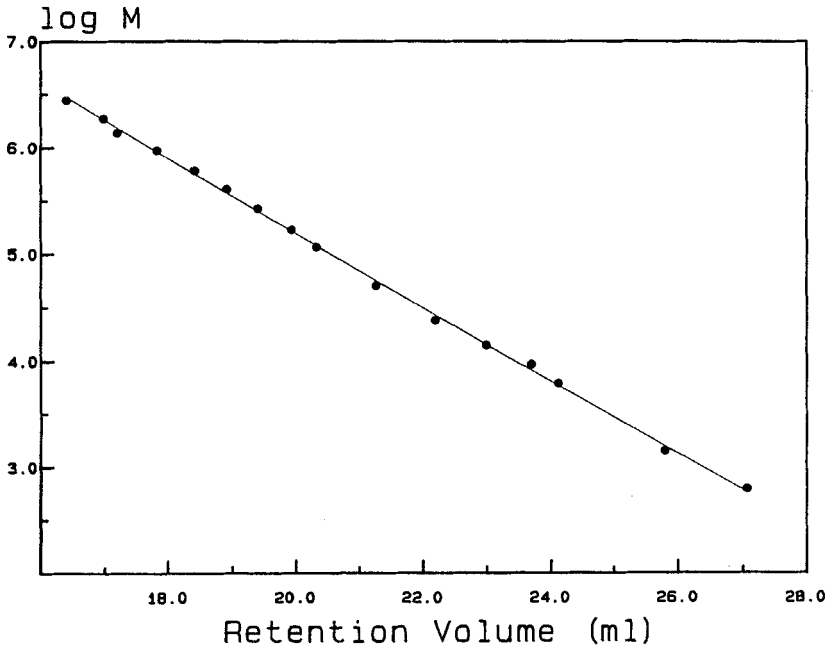

Figure 3. Conventional calibration curve obtained using narrow polystyrene standards and three 5 micron, mixed bed columns.

Determination of Mark-Houwink Constants from Polydisperse Standards was done using System 1 with THF as the mobile phase and poly (methyl methacrylate) polydisperse standards.

RESULTS AND DISCUSSION

High Resolution Columns

Figure 3 shows a calibration curve for a set of three 5 micron particle size "mixed bed" columns. The curve was fit by a cubic polynomial. Figure 4 shows a plot of

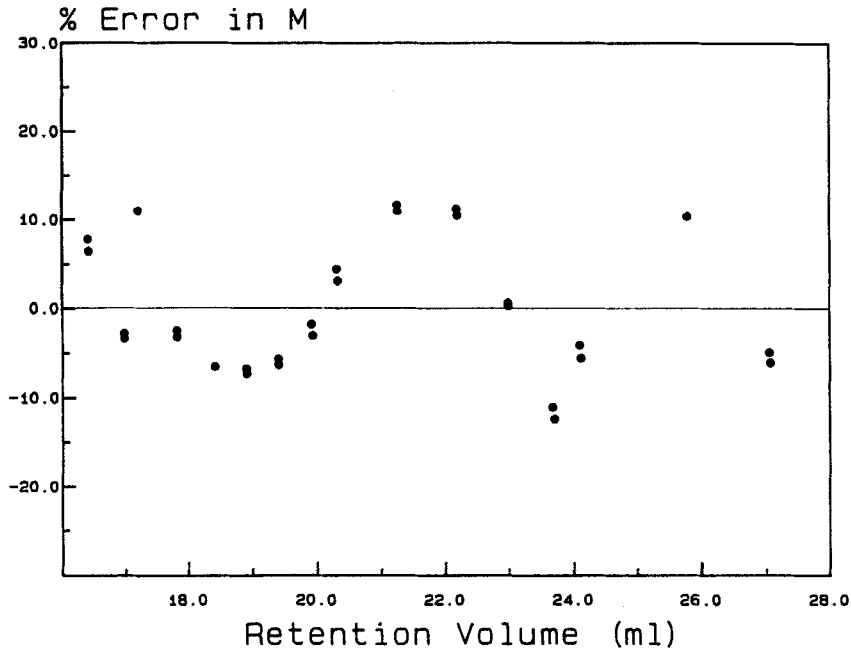

Figure 4. Plot of residuals calculated from Equation 1 versus retention volume for the calibration curve shown in Figure 3.

residuals (Equation (1) versus retention volume). Now a systematic variation of molecular weight with retention volume about the fitted curve is clearly evident. Such variations have been previously discerned by inspection of the calibration curve (23). However, the plot of residuals used here provides a magnified picture of the situation which facilitates observation of the trend. Furthermore, it also clearly shows that the maximum deviation on each side of the polynomial is 10%. If this magnitude of deviation is unacceptable, it must be determined whether or not the systematic variation reflects inaccuracy in the manufacturers' values of the molecular

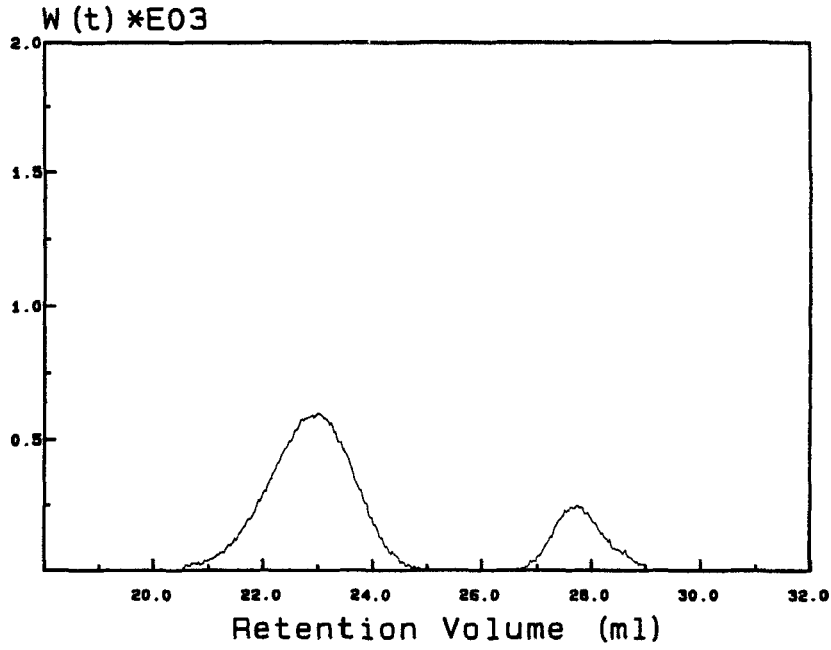

Figure 5. Chromatogram obtained by injection of a polydisperse polystyrene standard into the 10 micron, "prototype development" column set.

weights or is a property of the columns. If the latter is the case, then spline fits to different segments of the retention volume range can be considered.

Figure 5 shows a the result of injecting a broad molecular weight distribution standard into the 10 micron column set. The chromatogram was bimodal rather than unimodal as expected. Figure 6 shows the calibration curve. The curve was non-linear over a wide range of retention volumes. However, there appeared a distinct gap between 100,000 and 150,000 molecular weight. Figure 7 shows the raw chromatograms of the injected monodisperse polystyrene standards. Standards in the

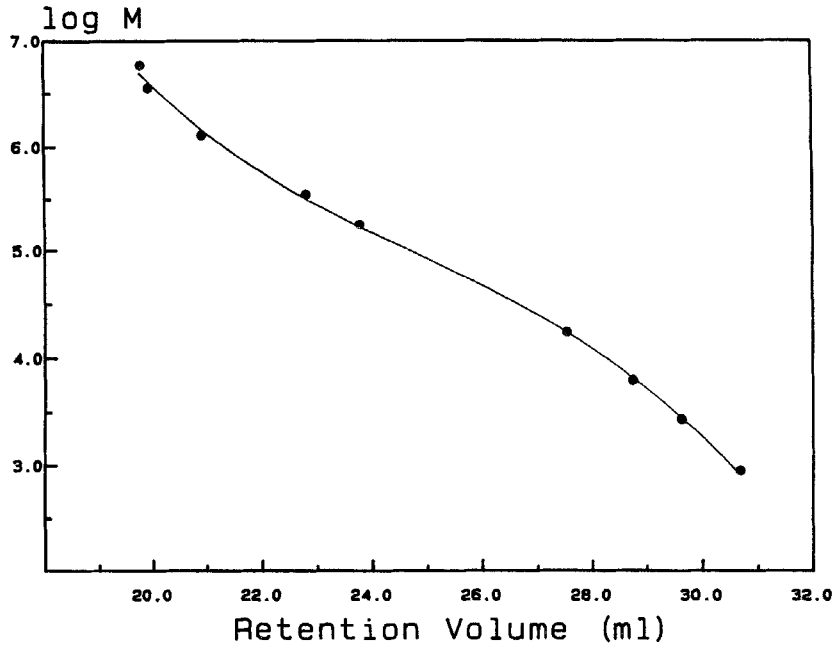

Figure 6. Conventional calibration curve obtained using narrow polystyrene standards and three 10 micron, non-commercial "prototype development" columns.

100,000 to 150,000 range did not exit the columns. The standard at the upper end of the range exited at a much reduced concentration. This range corresponds to the space between the two peaks of Figure 5. Samples were injected in triplicate over several days. Although this effect has yet to be satisfactorily explained, very recently it has been observed by the manufacturer using these same columns.

Coupled Concentration-Molecular Weight-Intrinsic Viscosity Detectors

Figure 8 shows the conventional calibration curve and the calibration curve obtained by plotting the local values of M_w from the LALLS versus retention volume for

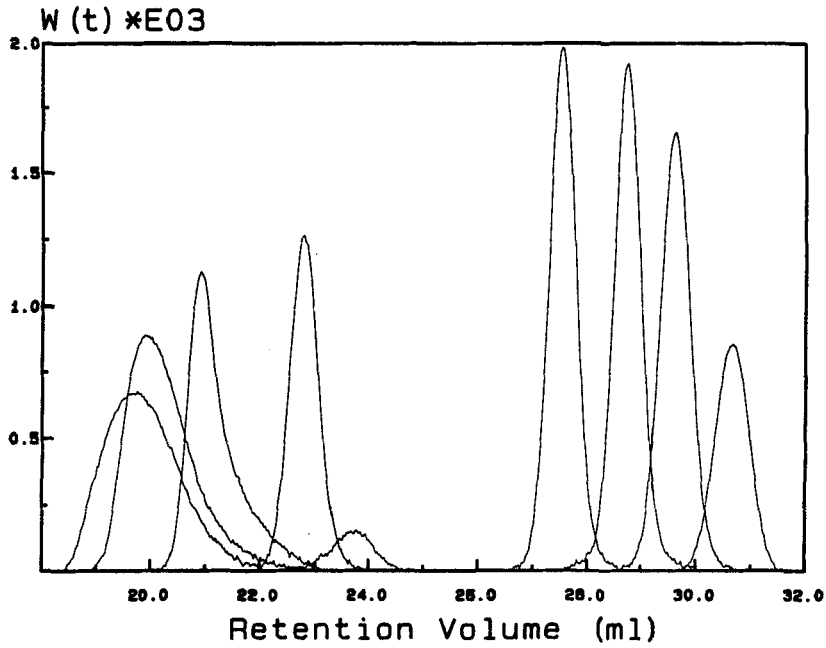

Figure 7. Chromatograms of the narrow polystyrene standards which were each separately injected into the 10 micron, "prototype development" column set.

different inter-detector volumes. Figure 9 shows a similar plot for different flow rates. In each case, the experimentally determined values of flow rate and inter-detector volume (0.980 cc/min and 0.270 cc respectively) did not provide superposition on the conventional calibration curve. Also, both flow rate and inter-detector volume have very significant effects on the local values. Similar results were obtained with the DV detector. Figure 10 shows the result of a search for the correct flow rate and inter-detector volume using a single broad standard (0.967 cc/min and 0.356 cc respectively). It is evident that we can superimpose the local

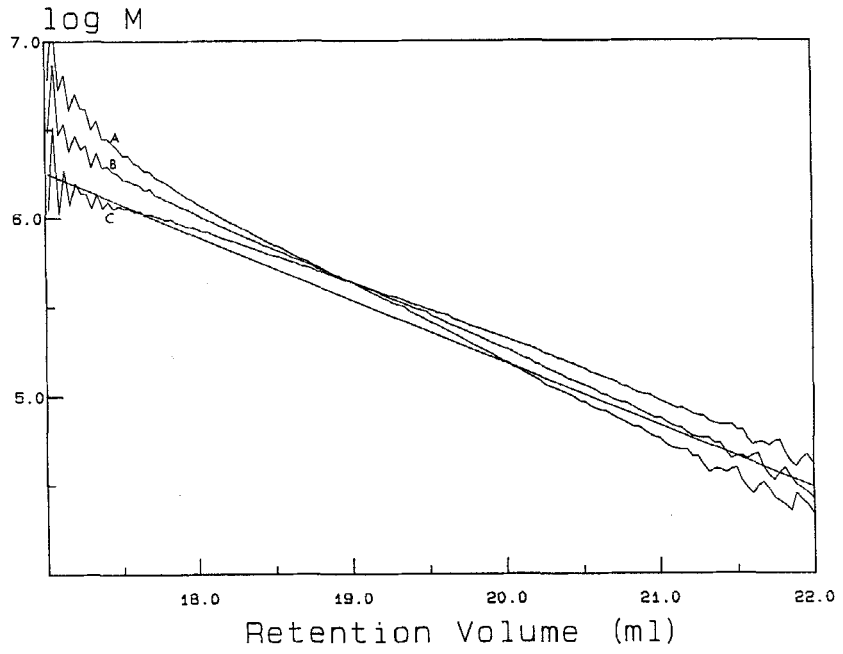

Figure 8. Conventional calibration curve (smooth unlabelled curve) from narrow polystyrene standards and calibration curves obtained from injecting a single polydisperse polystyrene standard and monitoring using the LALLS for different inter-detector volumes between the LALLS and the DRI using a flow rate of 0.98 cc/min. in the computations. Inter-detector volumes used were: 0.164 cc for curve A, 0.270 cc for curve B and 0.376 cc for curve C.

values of M_w and the conventional calibration curve over the central range of retention volumes of the standard by changes in the assumed flow rate and inter-detector volume which are probably within the reproducibility of experimentally determined values of these two parameters. Values of local M_w at each end of the range appear noisy and are not superimposed. It is possible that resolution correction could correct this remaining discrepancy. Another possibility is the use of some

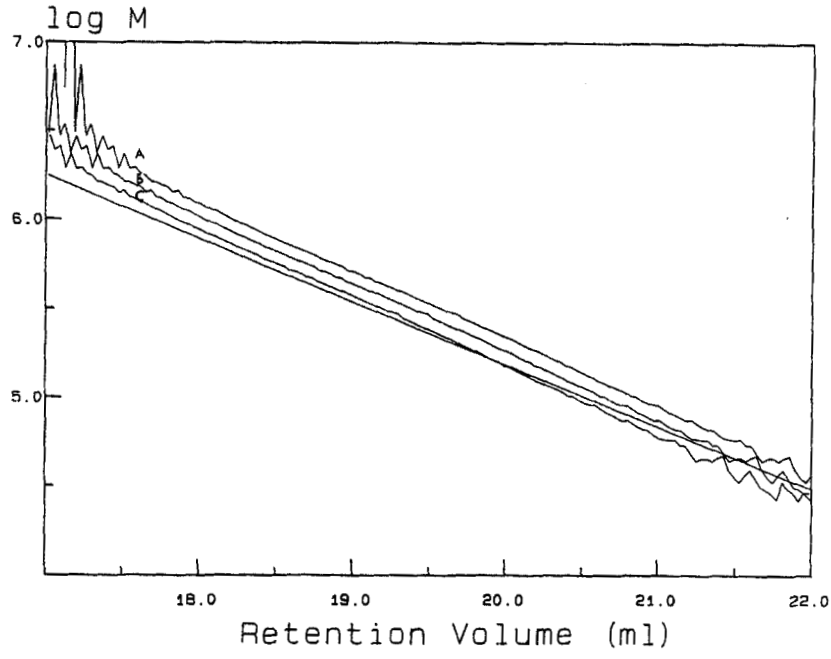

Figure 9. Same as Figure 8 except a inter-detector volume of 0.270 cc was used in the computations for flow rates of: 0.990 cc/min for curve A, 0.980 cc/min for curve B and 0.970 cc/min for curve C.

method for allowing for the different sensitivities of each detector at very high and very low molecular weight instead of resolution correction.

The Thermal Pulse Flowmeter

Figure 11 shows a calibration curve obtained from the thermal pulse flowmeter. Figure 12 shows how flow rate varies with the pulse time to emphasize the fact that

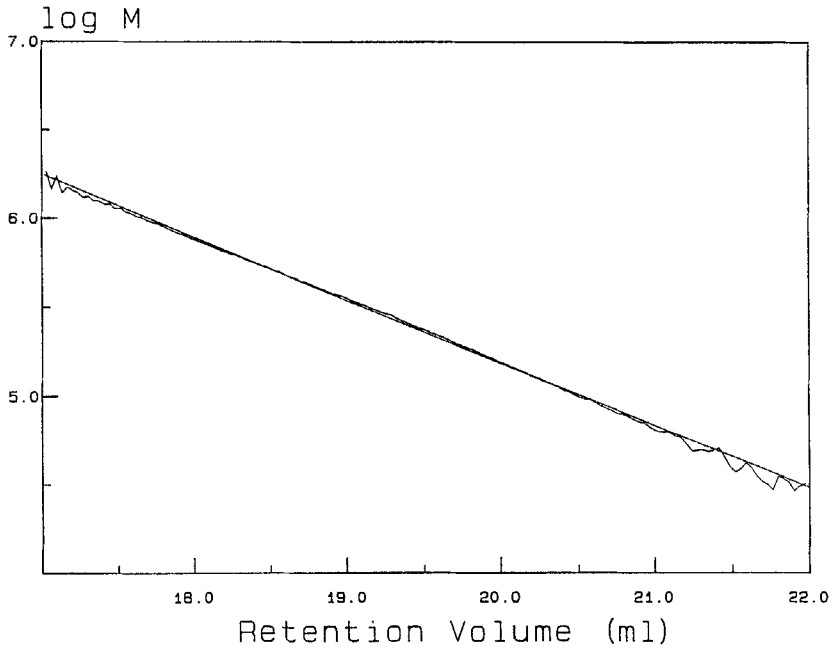

Figure 10. Same as Figure 8 except a inter-detector volume of 0.356 cc and a flow rate of 0.967 cc/min was used to compute the single LALLS curve.

a constant error at any pulse time results in an error in the flow rate which depends upon the magnitude of the flowrate. Figure 13 shows that in this particular case, even a constant percent error in pulse time results in a variable percent error in flowrate. It shows a plot of the error in the flow rate for a 0.1% error in the pulse time. The error in flow rate is observed to vary from 0.15% at 0.290 ml/min to 0.25% at 2.26 ml/min. This illustrates the fact that the error in computed values depends upon the error in the experimental data and the form of the equation used to

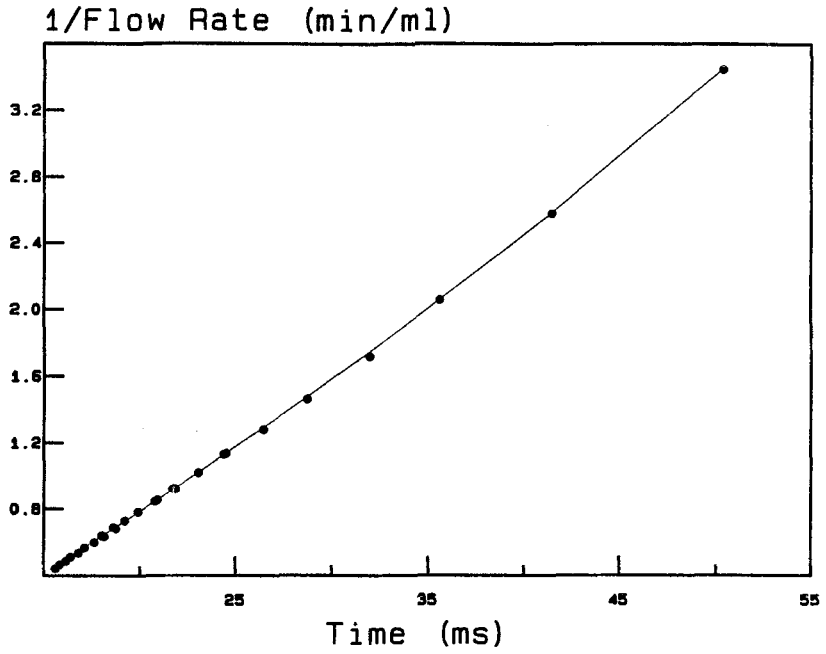

Figure 11. Calibration curve for the thermal pulse flow meter: reciprocal of mobile phase flow rate versus time for pulse to be transported to detecting thermistor.

calculate the computed value. An extensive evaluation of this instrument is in progress (18).

Mark-Houwink Constants from Polydisperse Standards

Four standards were available for the determination of the Mark-Houwink constants. They were numbered 1 through 4 in order of increasing molecular weight (21). Five

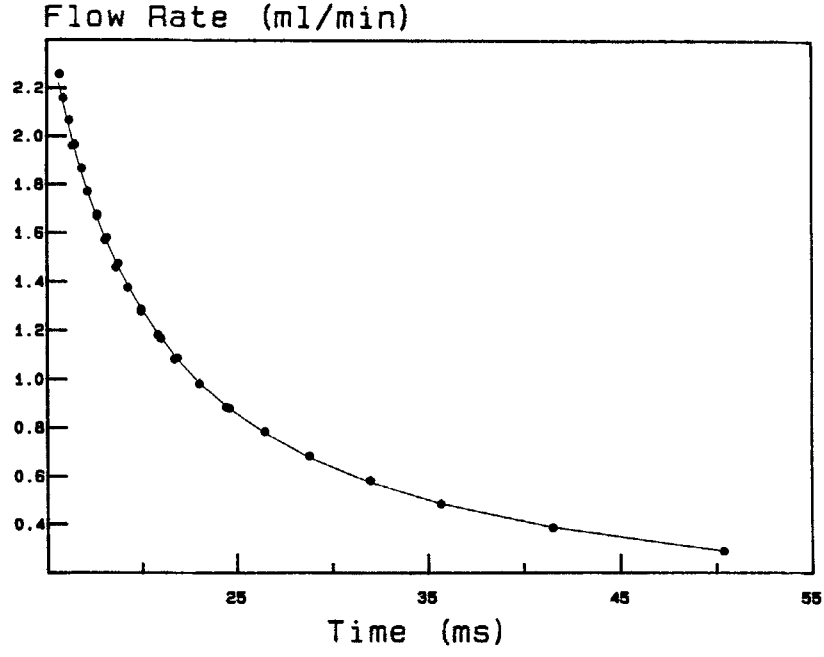

Figure 12. Plot of flow rate versus pulse transport time for thermal pulse flowmeter.

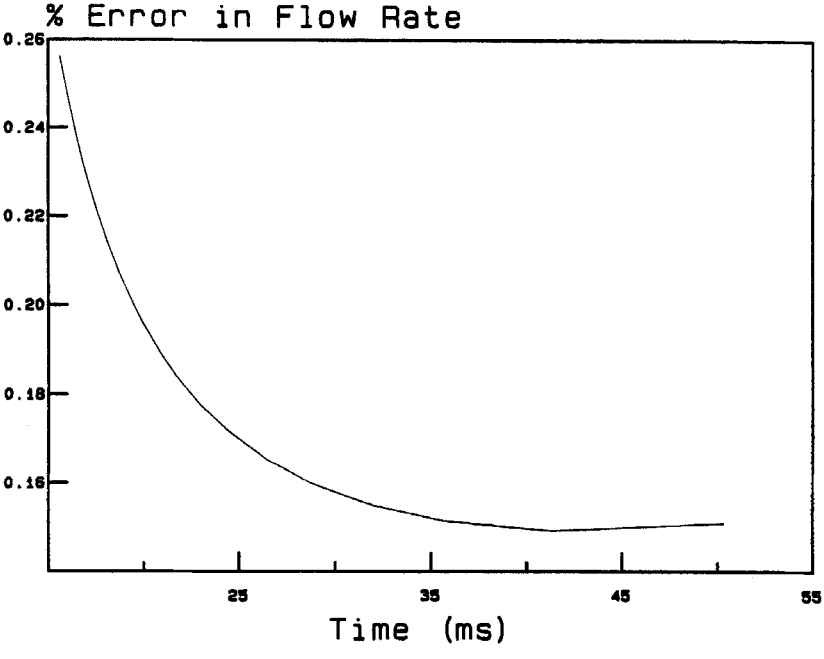

Figure 13. Percent error in flow rate for a 0.1 % error in pulse transport time for the thermal pulse flowmeter.

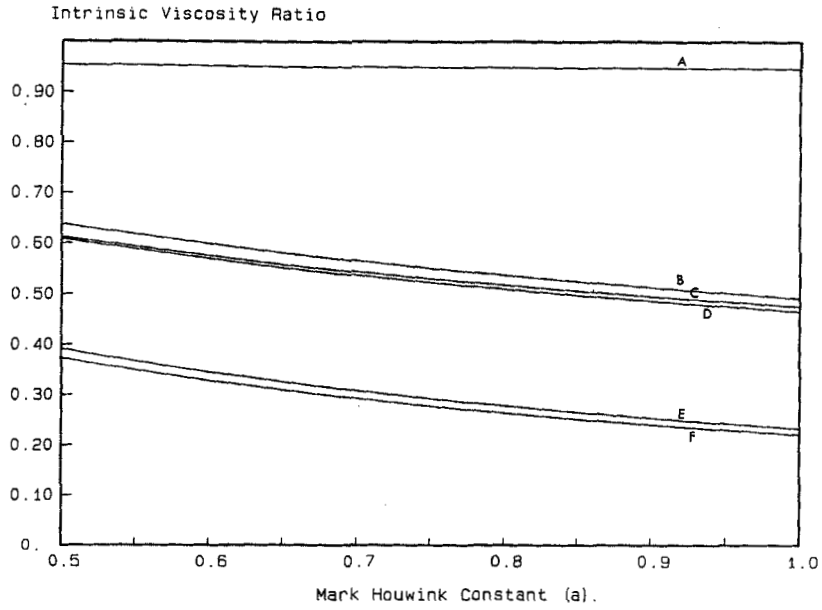

Figure 14. Variation of intrinsic viscosity ratios for six different pairs of standards with the value of **a** used to calculate the intrinsic viscosity ratios from the respective chromatograms of the standards. Ratios shown are: A: std. 3/std. 4; B: std. 2/std. 3; C: std. 1/std. 2; D: std. 2/std. 4; E: std. 1/std. 3; F: std. 1/std. 4.

independent ratios of intrinsic viscosity were therefore possible: 1:2, 1:3, 1:4, 2:3, 2:4, and 3:4. The variation of these ratios with the Mark-Houwink constant **a** is shown in Figure 14. This sensitivity analysis shows that some of the ratios vary much less than others as **a** is varied. The ratio 3:4 is particularly invariant. Now, Figure 15 shows the results of an error analysis superimposed on the sensitivity analyses for 2:3. The dashed lines represent a ±2% error in the experimental ("true") value of the ratio. Figure 15 shows that a reasonable value of **a** can be obtained at which the true value matches the value calculated from the SEC chromatogram.

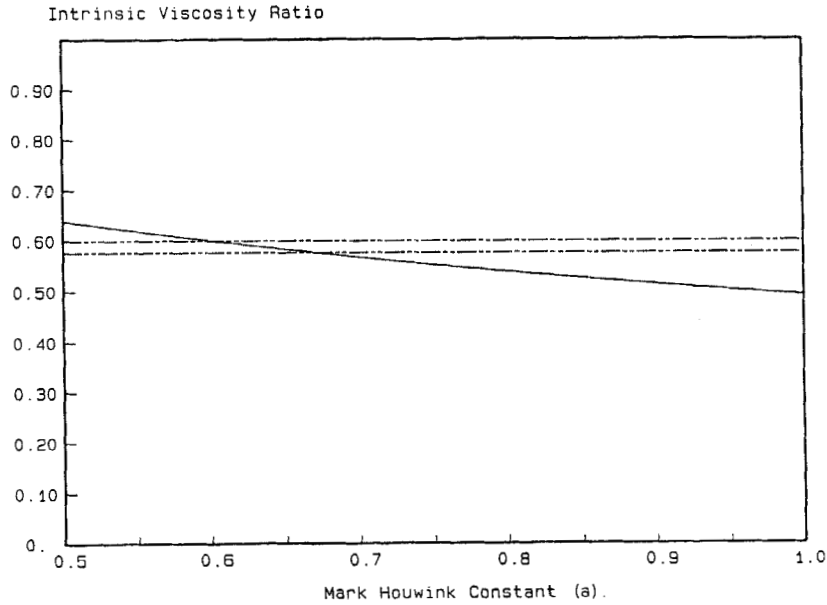

Figure 15. Superposition of the "true value" of intrinsic viscosity ratio of two standards (as a "band" of values with ±2% error defined by the two dashed lines) on the viscosity ratio calculated from chromatograms of std. 2/ std. 3 for different values of **a**.

Several other ratios did not show acceptable matches. Thus, this method allowed selection of standards for the search. It also showed that there is a high level of subjectivity in application of the method.

CONCLUSIONS

- Methods of assessment are increasingly important because the rapid development of new technology often results in obsolescent evaluations in publications.

- Specific methods of assessment examined centered about error analysis and sensitivity analysis. The conventional calibration curve maintains a high level of importance in assessing new technologies.

- Particularly with coupled detector systems where a variety of correction methods are available for data, prioritizing corrections is recommended. This means fully investigating the most fundamental corrections first. In the example shown, finding best values of flow rate and transport inter-detector volume between detectors considerably improved results and so lessened the correction burden on resolution correction.

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