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Factors influencing detector matching in multidetector SEC: solvent and concentration effects

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A study has been conducted on the origin of the molecular weight (MWT) dependence of the apparent interdetector-delay (IDD) in multidetector size exclusion chromatography (SEC). The IDD–MWT relationship proved to be independent of polymer concentration (for a given molecular weight). In contrast, the IDD–MWT relationship was found to be influenced by the polymer–solvent interaction, as shown by SEC experiments on polystyrene in a mixed tetrahydrofuran–water eluant. Confirmation of this discovery was provided by measured variations in the IDD–MWT relationship for polystyrene and poly(methyl methacrylate) in THF eluant. Possible reasons for the strong influence of the polymer–solvent interaction on the IDD are discussed in the paper. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: interdetector-delay; dead time; poly(styrene))

INTRODUCTION

Accurate molecular weight analysis of polymers by size exclusion chromatography (SEC) is possible provided that narrow polydispersity index (PDI) molecular weight standards of the analyte polymer are available. Unfortunately, only a limited variety of homopolymer standards are commercially available. In the absence of direct calibration standards, the universal calibration approach can be taken provided Mark–Houwink–Sakurada (MHS) or Stockmayer–Fixman constants are accessible. The quality of the reported MHS constants in the literature is poor, and rarely is it possible to assess the accuracy and precision of these constants. The preferred method for ensuring accurate molecular weight data for an unknown polymer sample is to use SEC with multiple detection methods. The use of on-line low-angle laser-light scattering (LALLS) or differential viscometry (DV) detectors coupled with a concentration sensitive detector such as a differential refractometer (DRI) will in theory yield the correct molecular weight distribution for any polymer of homogeneous composition.

The multiple detection option has been adopted by many laboratories as the SEC systems are versatile and relatively inexpensive. Numerous papers have been published on data reduction strategies for analysing the output from LALLS and DV detectors and several commercial packages are available to collect and transform the raw data. In a previous paper Zammit and Davis[1] expressed some concern over the reproducibility of molecular weight data obtained using a commercial software package and emphasized the problems associated with analytical procedures that require the operator to make subjective judgements (e.g. the selection of baseline and peak markers, integration ranges and data fitting procedures).

Another problem which can arise in interpreting SEC data is a deceptively simple issue relating to the assignment of the interdetector-delay (IDD) or dead time. Knowledge of the IDD is essential as the outputs from the DRI and DV detectors must be combined to derive the molecular weight distribution. The dead time between the detector systems must be taken into account so that the two signals can be overlaid, effectively attempting to match equivalent molecular weight slices on the two chromatograms. Suddaby *et al.*[2,3] derived expressions for multidetector SEC analyses, in which detector outputs are matched according to the hydrodynamic volume (HDV) of the eluant polymer in each detector. Having calibrated each detector independently, it is possible to interpolate an effective IDD for the SEC system. In cases where this has been done[1–3] it was found that the IDD is a function of molecular weight. Zammit and Davis[1] showed that ignoring the IDD–MWT relationship in an analysis can lead to serious errors in the calculation of MHS parameters using on-line DV detection. The work we report here is an attempt to understand the underlying cause of the variation in IDD with molecular weight.

EXPERIMENTAL

Materials

Anhydrous and unstabilized tetrahydrofuran (THF) (HiPersolv) was purchased from BDH and filtered through a 0.02 μm pore size filter (Whatman Anodisc 47, inorganic membrane) several times and stored in the dark until required. Triply distilled water was filtered several times and prepared when required.

Analysis by size exclusion chromatography (SEC)

SEC analyses were performed on a modular system comprising: a GBC Instruments LC1120 HPLC Pump

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operating at room temperature, an SCL-10A Shimadzu Autoinjector with 99 position sample rack and variable injection loop facility, a column set, which consisted of a PL 5.0 μm bead-size guard column (50 × 7.5 mm) followed by mixed bead Polymer Laboratories (PL) columns (300 × 7.5 mm, 10 μm mixed B, 2 × 5 μm mixed C and a 5 μm mixed D), an in-line filter (0.2 μm) and a DRI/DV detector set in series (Viscotek Model 250). As this column set is non-standard we were careful to ensure that the calibration curve contained no discontinuities and that all the calibration points fell on a best-fit curve represented by a third-order polynomial. The data were collected using PL Data Capture Units (DCUs) and analysed using PL Caliber version 6.0 GPC/SEC software[4]. The eluent was THF (or a THF/water mixture) at a flow rate of 1 ml min^{-1}. Polymer samples were dissolved in the particular SEC eluant and a 200 μl injection volume was used in all cases. Data were collected at two points per second.

The differential refractive index (DRI) and differential viscosity (DV) detectors were calibrated with a PSTY narrow PDI set consisting of 10 individual standards purchased from PL (1.25×10^3 to 2.95×10^6 MWT) along with five additional PSTY standards from Tosoh (1.8×10^4 to 2.89×10^6 MWT). A PMMA set purchased from PL (1.14×10^3 to 1.577×10^6 MWT) was also used. The supplier-determined peak maxima molecular weight (M_p) was used in the generation of all calibration curves (fitted with third-order polynomials). In all cases the concentration of standard calibration solutions was selected to optimize the detector response; thus the concentrations ranged from 0.3 to 2 mg ml^{-1} on going from high- to low-molecular-weight standards.

THEORY

A full description of the method of Suddaby *et al.* has been published earlier[1], consequently only a brief summary will be presented here to facilitate comprehension of the present study.

The Suddaby–Sanayei–O'Driscoll–Rudin (SSODR) method

The signal from a concentration detector for a given slice of the chromatogram is proportional to the concentration of the polymer eluting through the detector, and hence

$$S_{i,\mathrm{conc}} \propto c_i \tag{1}$$

Similarly, the signal from an on-line viscometer is proportional to the product of the concentration and intrinsic viscosity of the polymer eluting through the DV. Thus simulation of a DV signal can be achieved by making the following transformation:

$$S_{i,\mathrm{visc}} \propto c_i \cdot [\eta_i] \tag{2}$$

A simulated DV signal is shown in *Figure 1* for a narrow PDI calibrant. The crux of the SSODR procedure is that an independent calibration curve is generated for the DV by simulating each narrow PDI calibrant. In practice, the DV signal is simulated, using the DRI detector response and equation (2) above, and a new peak maximum molecular weight evaluated. This peak molecular weight is then attributed to the actual DV response, and so on for all the calibrants, thereby enabling a separate calibration curve to be derived for the DV detector.

Analysis of unknown polymers is performed by slice-matching hydrodynamic volumes (HDVs) on these separate calibration curves, as shown in *Figure 2*. The primary advantage gained in using this calibration procedure is that an estimation of the IDD is not required. However, it is possible to interpolate an IDD by comparing the chromatograms from the DRI and DV detectors.

Effect of polymer concentration on SEC

The universal calibration curve (UCC)[5] is widely used in SEC analyses because of its simplicity and wide applicability. The following expression is used to relate a standard (1) and test (2) polymer:

$$[\eta_1]M_1 = [\eta_2]M_2 \tag{3}$$

Rudin and co-workers[6–9] published extensively on deviations from the UCC which may be expected in practical usage as the transformation equations are only valid at infinite dilution. HDVs of solvated polymers are inversely related to concentration, and thus elution volumes (EVs) will be a function of concentration as well as the molecular

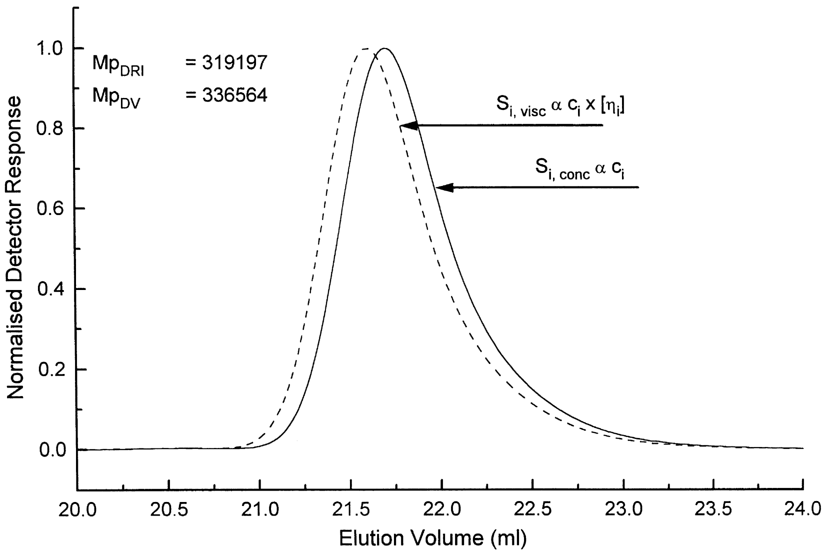

Figure 1 Comparison of responses from the concentration detector signal and the experimentally simulated viscometry response for a narrow PDI calibrant

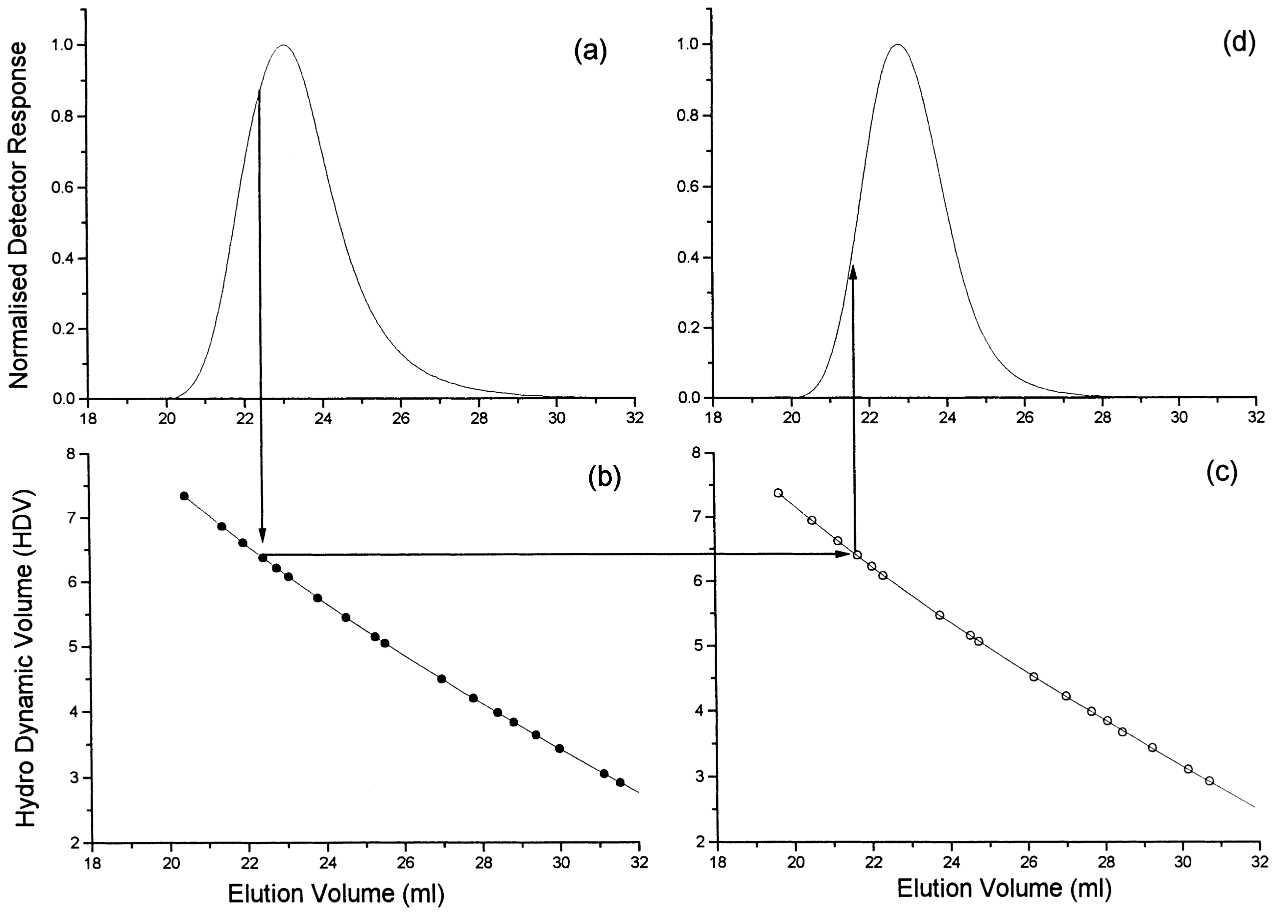

Figure 2 'Slice-matching' method of Suddaby *et al.* A slice is taken on the DRI response (a) and the corresponding HDV is found on the DRI calibration curve (b). The equivalent HDV is interpolated on the DV calibration curve (c) and then the equivalent 'slice' is matched on the DV response (d). (See text for further explanation)

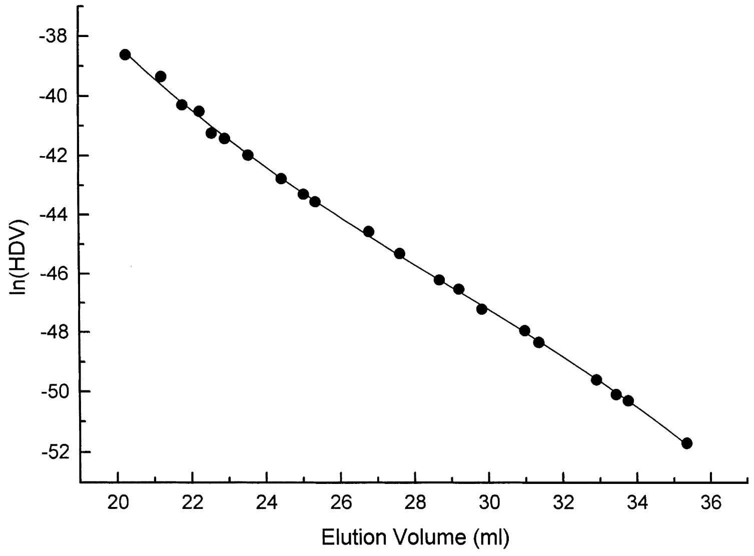

Figure 3 Universal calibration curve expressed as ln(hydrodynamic volume, HDV) *versus* elution volume for PMMA and PSTY in THF at 25°C

weight of the calibration samples. A semi-empirical model has been proposed by Rudin and co-workers[7–9] and has been applied to universal calibration in SEC to compensate for the reduction of the effective HDV of solvated polymer coils at high concentrations. In this model the calibration curve may be set up in terms of HDVs rather than molecular weights.

A general relation is:

$$\mathrm{HDV} = \frac{4\pi M[\eta]}{9.3 \times 10^{24} + 4\pi Nac([\eta] - [\eta]_\theta)} \tag{4}$$

where c is the concentration of a solution of narrow PDI calibration standard used to establish the calibration curve and Na is Avogadro's number. The quantity $[\eta]_\theta$ can be calculated from equation (5):

$$[\eta]_\theta = K_\theta \cdot M^{0.5} \quad (5)$$

For broad PDI polymers, the following expression is valid:

$$\mathrm{HDV} = \frac{4\pi M[\eta]}{9.3 \times 10^{24}} \quad (6)$$

These equations can be used to correct SEC for concentration effects. In most cases corrections only become necessary for high-molecular-weight polymers in good solvents. Using Rudin's model, the UCC can be expressed as ln(HDV) *versus* EV as shown in *Figure 3,* where PMMA and PSTY calibration standards were used to construct a calibration curve.

Polymer–solvent interactions[10]

One measure of the solvent–polymer interactions is the parameter β, the binary cluster integral. This represents the effective volume excluded to one segment of a polymer by the presence of another. In theta solvents β will have a value of 0. In poor solvents it will have a negative, zero, or small positive value. In good solvent systems, where there is preferential attraction between the chain segments and solvent molecules, it will take on large positive values.

The Stockmayer–Fixman equation is give below:

$$\frac{[\eta]}{M^{1/2}} = K_\theta + 0.51\Phi_0 \frac{\beta}{M_s^2} M^{1/2} \quad (7)$$

where $[\eta]$ is the intrinsic viscosity of the polymer, M is the molecular weight of the polymer, M_s is the segment molecular weight and all of the other terms are constant for a given polymer at a given temperature except for β.

Upon rearrangement, this equation can be expressed as

$$[\eta] = K_\theta M^{1/2} + K'M \quad (8)$$

where K' reduces to the product of β/M_s^2 and a constant. The parameter K' can thus be a measure of polymer–solvent interactions.

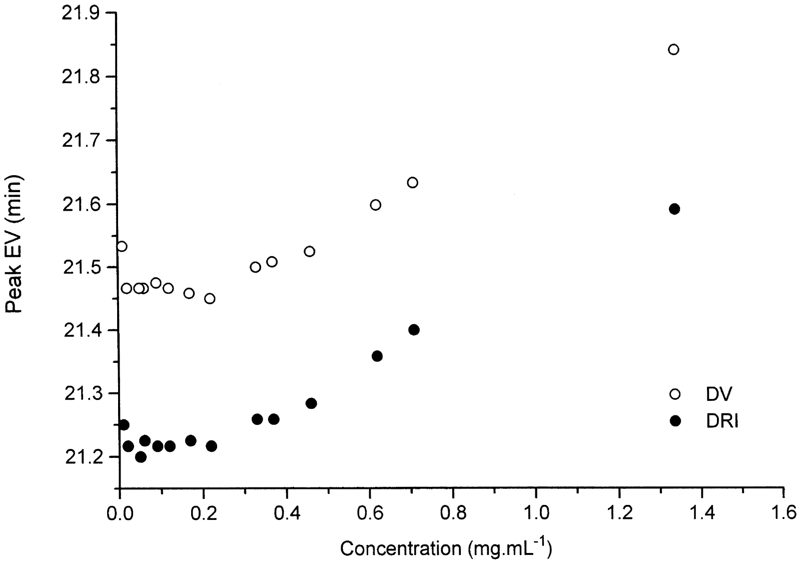

Figure 4 Peak maxima elution volume DRI and DV responses to a narrow PDI PSTY calibrant ($M_p = 2\,080\,625\ \mathrm{g\ mol^{-1}}$) injected over a range of concentrations

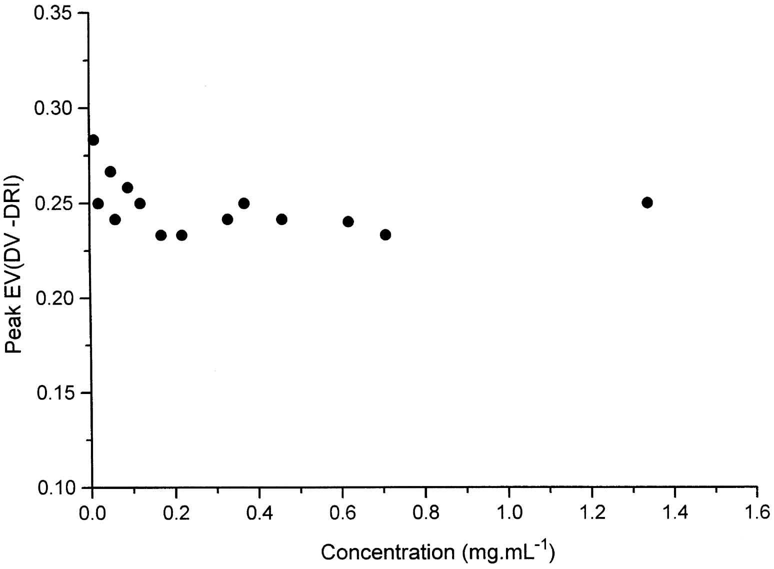

Figure 5 Subtraction of the peak maxima elution volume for each injection for the narrow PDI PSTY calibrant shown in *Figure 4*

RESULTS AND DISCUSSION

The problem of defining an accurate IDD has beset many workers in the SEC field. At face value the problem seems trivial and many commercial SEC analysis packages implement a simple measurement based on peak matching for a single broad or narrow PDI standard. Studies have shown that precise assignment of the IDD is essential for accurate molecular weight analyses, especially where the data are used to derive MHS coefficients. This area has been covered by previous studies[1,11–16]. As described earlier, Suddaby *et al.*[3] found that the IDD is a function of molecular weight and tentatively suggested that this may be due to complex flow profiles in the capillaries and cells of the detectors. A simple explanation for the behaviour is that the viscosity of the eluting polymer solution influences the flow through the capillaries giving rise to variation in the IDD. If this is the case then the IDD should also be sensitive to the polymer concentration.

Effect of polymer concentration on the IDD

An SEC experiment was conducted using THF as the eluant with two narrow PDI polystyrene standards (2 080 625 and 190 000 g mol^{-1}). These standards were injected at several concentrations (up to 1.5 mg ml^{-1}). In *Figure 4*, the detector responses for the high-molecular-weight standard are shown. The peak elution volume of the standard remains constant (within experimental error) up to 0.2 mg ml^{-1} injection concentration and thereafter increases linearly with concentration for both detectors. Shown in *Figure 5* is the subtraction of the DV and DRI peak maxima elution volumes shown in *Figure 4*. At each concentration the time difference between detectors results in a constant

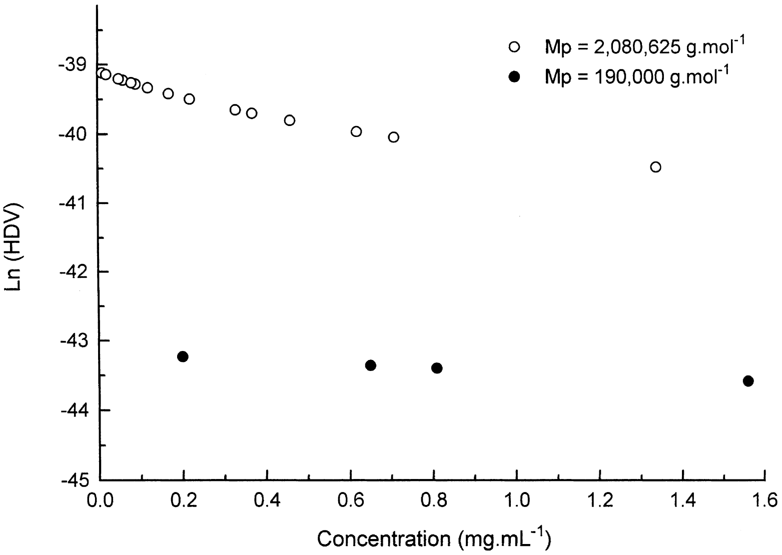

Figure 6 HDV variation of two narrow PDI PSTY calibrants ($M_p = 2\,080\,625$ and 190 000 g mol^{-1}) injected over a range of concentrations

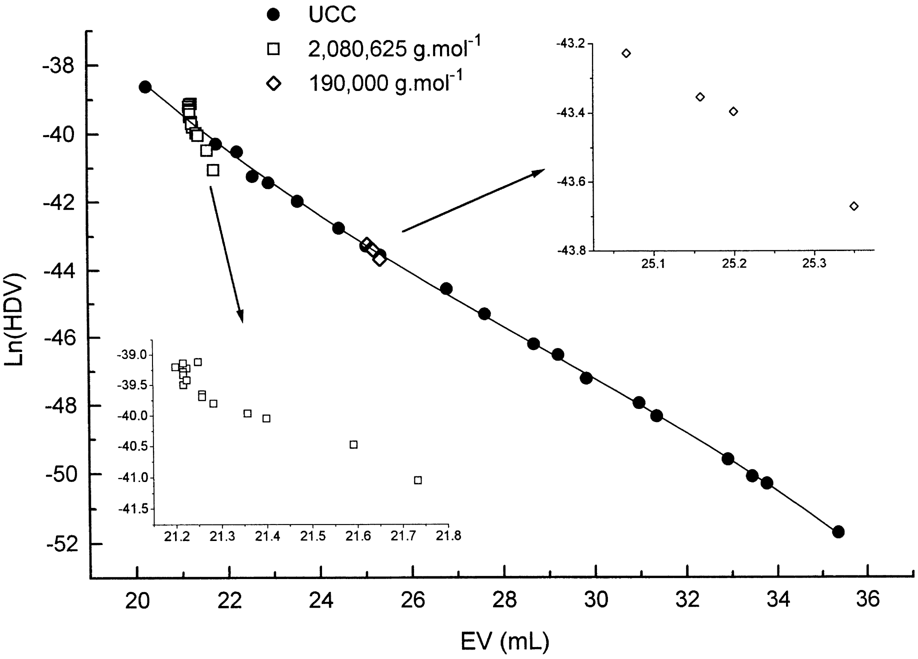

Figure 7 HDV *versus* elution volume figures for the 190 and 2080 K narrow PDI PSTY calibrants superimposed onto the universal calibration curve

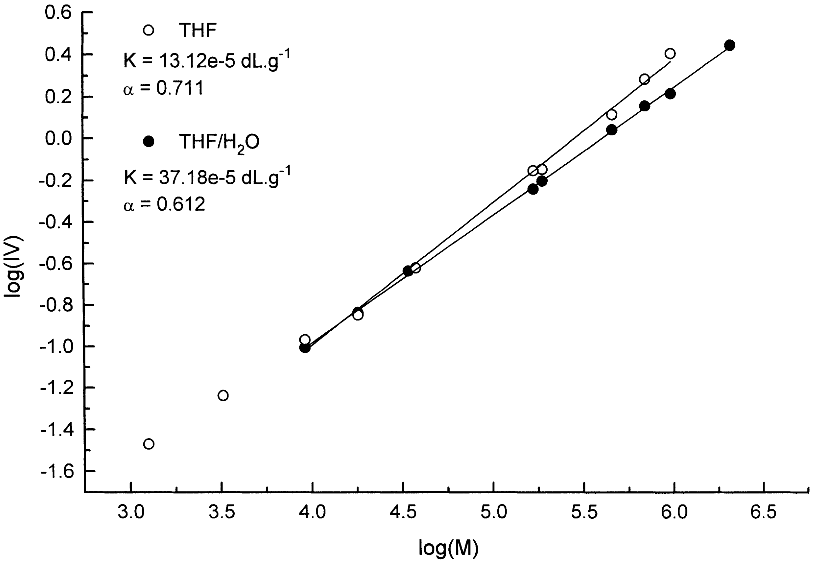

Figure 8 Mark–Houwink–Sakurada plots of narrow PDI PSTY calibrants in THF and THF/water eluents

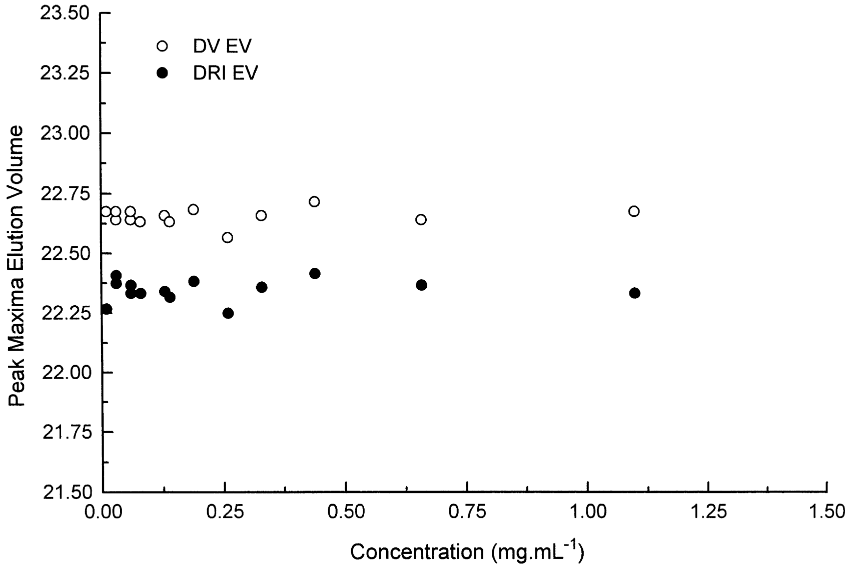

Figure 9 Peak maxima elution volume DRI and DV responses to a narrow PDI PSTY calibrant ($M_p = 2\,080\,625\ \mathrm{g\,mol^{-1}}$) injected over several concentrations

IDD of 0.25 min. A similar analysis was performed with a medium-molecular-weight (190 000 g mol^{-1}) standard and equivalent results were obtained with a measured IDD of 0.23 min.

The variation of HDV with concentration can be analysed in terms of Rudin's model as shown in *Figure 6* for the two narrow PDI standards. The ordinate in *Figure 6* (concentration) can be exchanged for peak elution volume (see insets *Figure 7*), and superimposed on the UCC (shown earlier as *Figure 3*). *Figure 7* shows the universal calibration curve (via Rudin's equation) with overlays of the 190 and 2080 K PSTY standards injected over several concentrations. The insets demonstrate that the dependence of HDV on concentration is exacerbated at high molecular weights. Note that Rudin's model obviously fails to accurately describe the effect on HDV of injected concentration; however, our data show qualitative agreement with this model, i.e. at higher concentrations coil shrinkage causes standards to have longer elution times.

The retention time–concentration data show two important points. The first is that the retention times of the narrow PDI standards are observed to vary with concentration, as expected. The second is that the IDD (between DV and DRI) is found to be independent of the injection concentration over a wide range of injection concentrations. While the variation of the IDD with molecular weight could originate in fluid mechanics, this latter observation suggests that this is not the case. Thus solvent–polymer interactions were examined.

Effect of solvent type on the IDD

It is well known that in a theta solvent the concentration dependence of HDV is eliminated[9]. A theta cosolvent (at 25°C) for polystyrene is a water/THF mixture[17] (7.7 vol% water, $K = 110\ \mathrm{dl\,g^{-1}}$, $\alpha = 0.500$). To avoid possible problems with the columns and to reduce the build-up of back-pressure in the SEC the experiments were conducted using a slightly lower water concentration (6.85 vol%

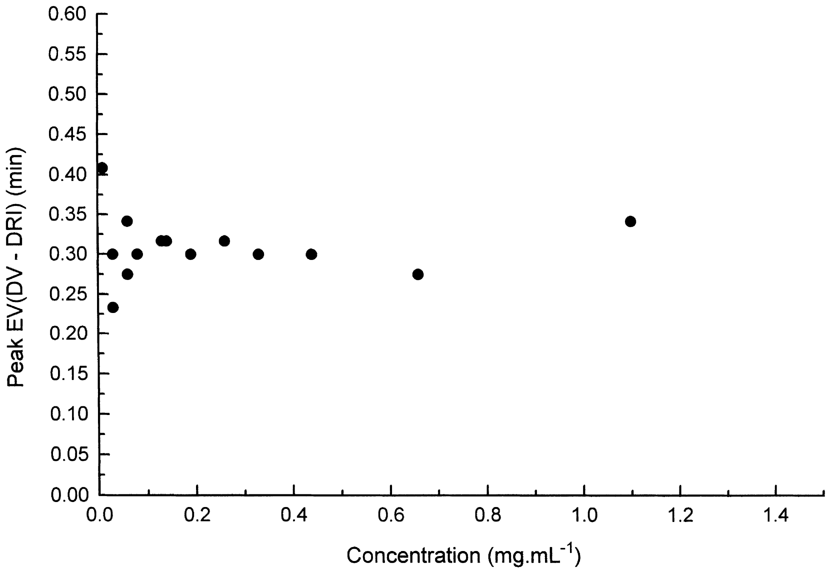

Figure 10 Subtraction of the peak elution volumes at each concentration from the data shown in *Figure 9*

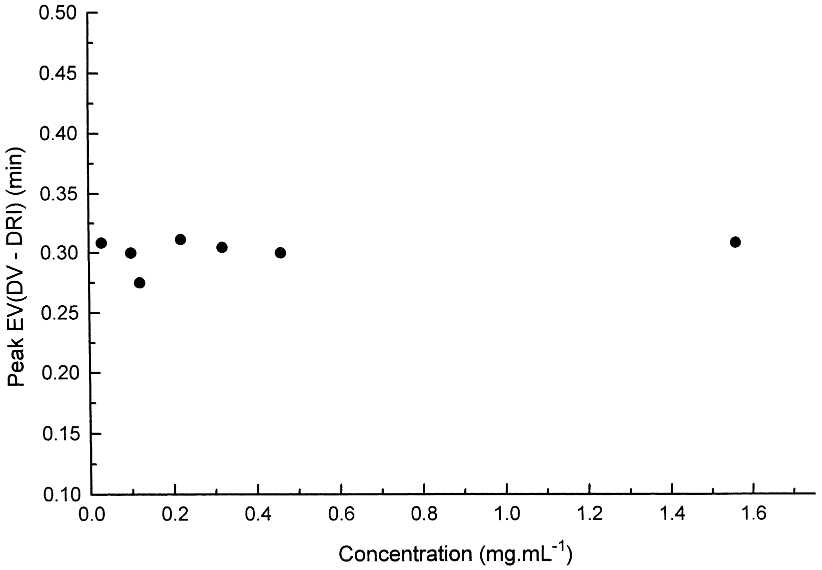

Figure 11 The subtraction of peak maxima elution volume DRI and DV responses to a narrow PDI PSTY calibrant ($M_p = 190\,000$ g mol^{-1}) injected over several concentrations

water) than those for theta conditions. An MHS plot was constructed for narrow PDI polystyrene standards yielding values for K and α of 37.18×10^{-5} dl g^{-1} and 0.612 respectively. These values can be contrasted with the literature values of PSTY in THF of 14.10×10^{-5} dl g^{-1} and 0.700[18] for K and α respectively. These values compare well with our values of 13.12×10^{-5} dl g^{-1} and 0.711 for K and α respectively (obtained in pure THF). The MHS plots are shown in *Figure 8*.

The same two standards (2080 and 190 K) of narrow PDI polystyrene were injected onto the columns with THF/water mixture as eluant. The effect of concentration on the elution volume of 2080 K is shown in *Figure 9* and the influence of concentration on the IDD is shown in *Figure 10*. As expected, the influence of concentration on HDV has diminished and once again the IDD (0.3 min) is independent of polymer concentration. The same procedure was repeated with the 190 K standard with similar results, as shown *Figure 11*. However, in this case the IDD for the medium-molecular-weight standard is identical to that obtained for the high-molecular-weight standard (0.3 min) indicating that the IDD is constant regardless of injection concentration and molecular weight (for the two molecular weights shown here).

Effect of the polymer–solvent interaction on the IDD

The independent calibration curves for the DRI and DV detectors were generated using the SSODR method for the entire series of PSTY calibrants under both solvent conditions; the ‘good’ solvent (THF) and the ‘poor’ solvent system (THF/water). The relationship between IDD and molecular weight can be interpolated from the individual calibration curves. The results for an IDD–MWT analysis can be seen in *Figure 12* overlaid onto the IDD *versus* molecular weight data shown previous. It is apparent that as the ‘goodness’ of the solvent diminishes, the IDD becomes

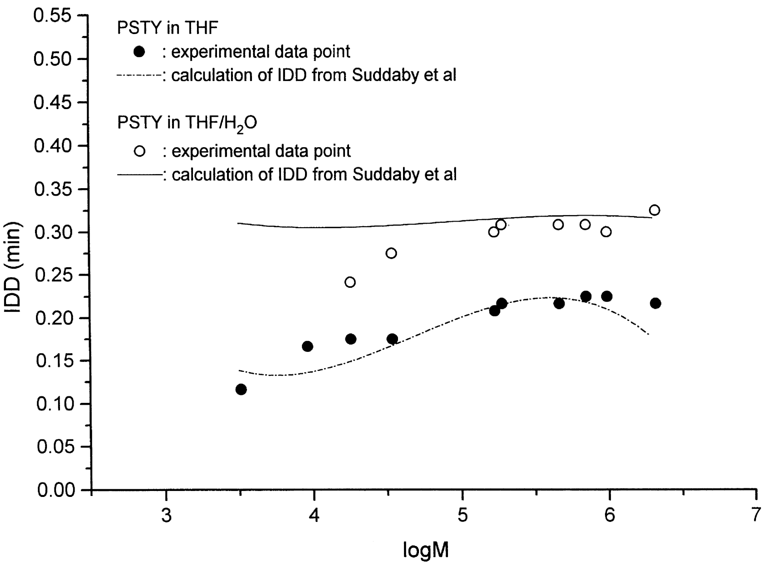

Figure 12 Variation of the interdetector-delay between DRI and DV detectors as a function of molecular weight for PSTY in the solvent systems THF and THF/water. The continuous lines are determined from the SSODR method and the individual data points are experimental values

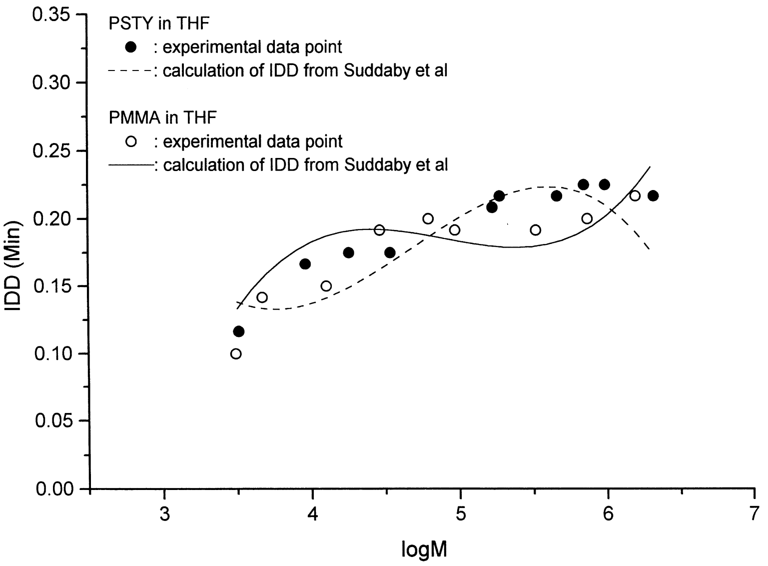

Figure 13 Variation of the interdetector-delay between DRI and DV detectors as a function of molecular weight for PSTY and PMMA in the solvent THF. The continuous lines are determined from the SSODR method and the individual data points are experimental values

less dependent on molecular weight, tending to a maximum value (approx. 0.325 min).

Initially, this influence of solvent quality may seem to indicate an influence of polymer coil dimensions on the IDD. However, this would contradict earlier findings showing no effect of concentration on IDD. Furthermore, the coil dimension of the low-molecular-weight standards (less than say 5 K) should be the same regardless of solvent quality, yet these standards are the ones showing the largest change on going to a poorer solvent. In contrast, the high-molecular-weight samples which show the most significant change in coil size show the least effect. It appears that the influence of molecular weight on IDD is related to solvent–polymer interactions. Further evidence for this can be found by comparing Suddaby–Sanayei-type analyses for PSTY and PMMA narrow PDI standards in the same SEC system. The IDD–MWT relationships obtained for these two standards in neat THF are plotted in *Figure 13*. Clearly, the two polymers show differing behaviour.

The parameter K', as defined previously, has been determined for both PMMA and PSTY in neat THF[19]. Values of 17.42×10^{-5} and 11.2×10^{-5} ml g^{-1} were reported for PSTY and PMMA respectively. Since the segment molecular weights (M_s) are essentially the same for these two polymers, the values of K' can be used as a comparison of β. The observed difference in behaviour can be attributed to this difference in solvent quality.

Verifications and checks

In SEC studies it is necessary to verify the absence of significant peak distortion and/or axial dispersion. Thus in this work, several consistency checks were made. First, we verified that the peak shapes were invariant to solvent type or injection volume concentration. In *Figure 14* we show four typical chromatograms that indicate the elution peaks are not distorted under the experimental conditions described.

The influence of axial broadening was investigated using two approaches. In *Figure 15* we show the actual viscometer

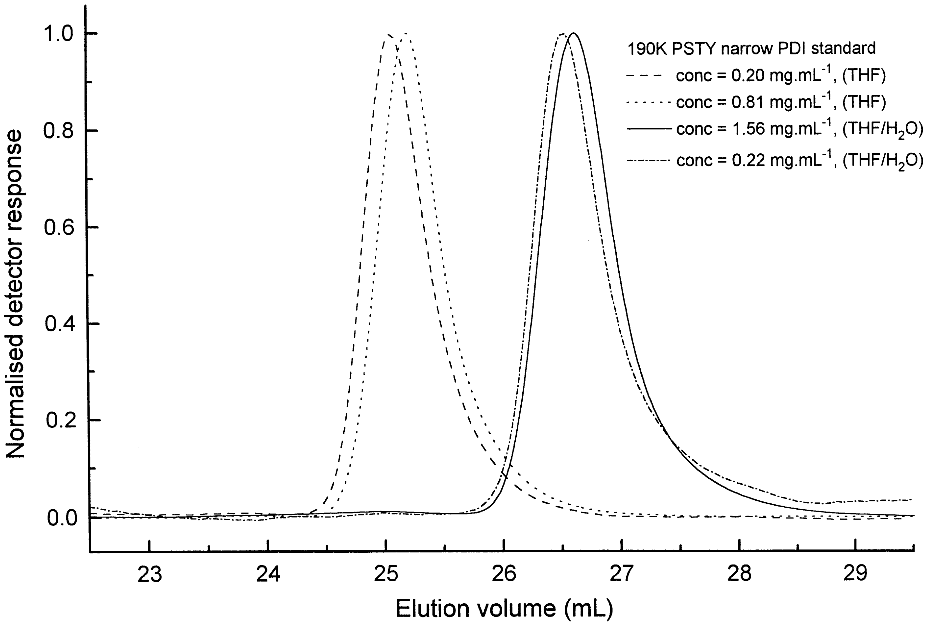

Figure 14 DRI response to 190 K PSTY standard at several concentrations and in both eluent systems

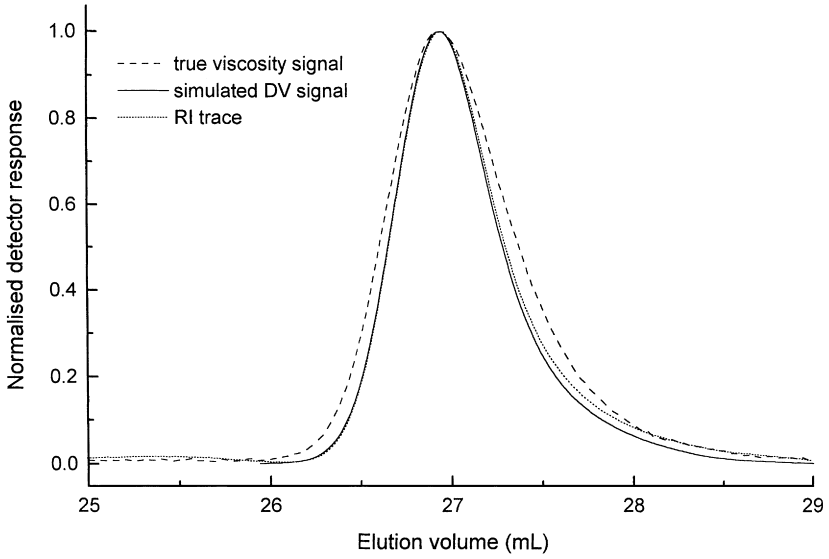

Figure 15 Viscosity response to a 190 K PSTY standard overlayed with the DRI response and the simulated viscosity signal all matched at their peak maxima

Table 1 Comparison of experimentally determined PDIs *versus* supplier-determined values for PSTY in THF and THF/H$_2$O SEC eluents

Molecular weight (g mol^{-1})	Supplier	Supplier quoted PDI	PDI, THF system	PDI, THF/H$_2$O system
2 890 000	Tosoh	1.09	1.304	1.295
2 100 000	PL	1.06	1.135	1.052
980 000	PL	1.07	1.061	1.062
706 000	Tosoh	1.05	1.055	1.053
465 000	PL	1.04	1.047	1.045
190 000	Tosoh	1.04	1.035	1.037
170 000	PL	1.03	1.04	1.042
34 500	PL	1.04	1.035	1.03
18 100	Tosoh	1.01	1.027	1.026
9200	PL	1.03	1.034	1.033
3250	PL	1.05	1.057	1.053
1250	PL	1.08	1.109	1.111

response overlaid with the DRI response and the simulated viscosity signal aligned by their peak maxima (190 K PSTY standard, injection concentration $= 0.31$ mg ml^{-1} in THF/H_2O eluent). The responses are congruous and the actual DV trace is only slightly broader indicating that axial dispersion plays only a small role. In order to quantify the influence of axial dispersion in our experimental set-up we report the values obtained for polydispersity for the narrow standards and compare these with the supplier-determined values as shown in *Table 1*. The results from this comparison indicate minimal axial dispersion and also that the SSODR procedure yields accurate and precise polydispersity indices, except for the highest-molecular-weight sample where some degradation may have occurred under the given analysis conditions.

CONCLUSIONS

The fact that the IDD is independent of sample concentration, despite changes in elution volume, combined with the observed influence of solvent quality on IDD indicates that polymer–solvent interactions influence the IDD. This has important implications for polymer characterization by SEC.

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