

A new approach to establishing universal calibration curves for size exclusion chromatography

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In size exclusion chromatography (s.e.c.) analysis both the universal calibration curve (UCC) and the secondary calibration curve (molecular weight calibration) can be constructed using the Mark-Houwink-Sakurada (MHS) relation, $[\eta] = KM^a$. Over a very wide range of molecular weights neither K nor a is constant; therefore, the interpretation of results using one set of K and a values may not be correct (i.e. in the case of commercial resins). An alternative model which relates molecular weight (MW) to $[\eta]$ over a very wide range of molecular weights is $[\eta] = K_0 \overline{M}_r^{1/2} + K' \overline{M}_w$ where K_0 and K' do not change. The intrinsic viscosities of polystyrene standards with MW s of 10^3 to 2×10^6 were measured and the results were used to establish a UCC for a multi-detector s.e.c. system. From the UCC two calibration curves were established using the MHS relation and the alternative model. Commercial polystyrene and polyethylene resins were analysed using the same analytical system. At the higher molecular weight end one observes a deviation between the results from absolute MW detectors and the calibration curve based on a particular set of K and a values. On the other hand, MW s obtained from the calibration curve using the alternative model were consistent with results from absolute molecular weight detectors. This indicates that the use of the alternative model will provide a more accurate calibration for s.e.c. analysis when the molecular weight distribution is relatively wide.

(Keywords: chromatography; calibration procedures; molecular weight)

INTRODUCTION

In size exclusion chromatography (s.e.c.) analyses the molecular weight distribution (MWD) of the polymer sample is determined either by direct measurement of the molecular weight of eluting species, using light scattering or continuous viscometer detectors at present, or by universal calibration. All methods require use of a separate concentration detector. The relative sensitivity of s.e.c. detectors has been discussed¹ and will not be addressed here. In this paper the focus is on the accuracy of s.e.c. calibration curves.

For a given polymer sample the distribution of molecular weights is only obtainable from s.e.c. analyses when macromolecules are separated according to their sizes. In practice, s.e.c. analyses provide only the size distribution of macromolecules with respect to elution volume. However, the MWD can be estimated by transformation of the polymer elution volume to its corresponding molecular weight (MW) by using a universal calibration curve (UCC). UCC relates elution volume to the hydrodynamic volume or size of solvated polymer, which, in turn, scales as intrinsic viscosity times molecular weight ($[\eta] \times MW$) (ref. 2).

Construction of a reliable UCC requires the determi-

nation of s.e.c. elution times for samples of known intrinsic viscosity and known MW . Commercial polymer standards with known molecular weight are available for this purpose. The following equation is most widely used to describe the molecular weight dependence of $[\eta]$:

$$[\eta] = KM^a \quad (1)$$

Equation (1) is known as the Mark-Houwink-Sakurada (MHS) relation³ and it can be applied to many different types of polymers. K and a in this relation are essentially empirical parameters. For a given polymer-solvent system different K and a values are reported depending on the range of molecular weights used in the estimation of these constants^{4,5}. The value of a is approximately 0.5 for $MW < 10\,000$ and is about 0.6–0.75 for $MW > 10\,000$ in a good solvent, as expected from the Flory-Fox treatment of polymers in solution⁶. In general, experimental values for K and a show an inverse correlation although the two constants are theoretically independent of each other⁷.

The MHS relationship is commonly used in s.e.c. data analysis to convert equivalent molecular weights to absolute molecular weights via equations such as:

$$\log M_2 = \frac{1}{1+a^2} \log \frac{K_1}{K_2} + \frac{1+a_1}{1+a_2} \log M_1 \quad (2)$$

when an on-line viscometer is not available. This equation

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assumes constant K and a values. The essential problem with equation (1) is that over a relatively wide range of molecular weights neither K nor a is actually constant^{8,9}. In practice, the K and a values which give an accurate $[\eta]$ along the mid-range of MW would be used. As MW falls below or exceeds this range the predictions of the MHS equation with fixed K and a become erroneous.

A recent modelling of $[\eta]/MW$ has led to a single adjustable parameter model¹⁰:

$$[\eta] = K_{\theta} \overline{M}_r^{1/2} + K' \overline{M}_w \quad (3)$$

where

$$\overline{M}_r \equiv \left[\sum w_i M_i^{1/2} \right]^2 \quad \text{and} \quad \overline{M}_w \equiv \sum w_i M_i$$

According to this model two polymer characteristics affect the intrinsic viscosity: (1) a newly defined radius average molecular weight, \overline{M}_r , which scales as the square of the unperturbed average radius of polymer coil; and (2) the coil expansion in a solvent, which is a function of \overline{M}_w . K_{θ} is constant and is known for many polymers, while K' reflects the extent of polymer-solvent interaction. The value of K' decreases as the solvent-polymer interaction decreases and becomes zero for a theta solvent.

When the polymer sample is monodisperse equation (3) simply reduces to:

$$[\eta] = K_{\theta} M^{1/2} + K' M \quad (4)$$

Intrinsic viscosities of different polymers in different solvents over a very wide range of molecular weights (from 10^3 up to 7×10^6) are well represented by this alternative model¹⁰.

The UCC converts the elution volume of a given polymer sample to $[\eta] \times M$. When K_{θ} and K' are known the molecular weight of each slice can be obtained from:

$$[\eta] \times M = K_{\theta} M^{3/2} + K' M^2 \quad (5)$$

The left-hand side of the above equation is known; thus, by help of an iterative (i.e. Newton-Raphson) method, the molecular weight of each slice can be estimated.

In this paper we compare the molecular weights obtained from universal calibration curves using equations (1) and (4) with molecular weights measured by an absolute molecular weight detector.

EXPERIMENTAL

The s.e.c. system used in this study consisted of a high-temperature g.p.c., a differential refractive index detector (d.r.i.), a low angle laser light scattering detector (LALLS), an on-line differential viscometer (CV) detector, an on-line degasser, a thermopulse flowmeter, and a mixed bed column (Jordi Linear). The experiments were run at a flow-rate of 0.7 ml/min at 145°C.

The mobile phase was filtered through an on-line 0.5 μm filter prior to entering the LALLS cell. After passing through the LALLS the eluant was split 50:50 between the d.r.i. and CV detectors. The instantaneous flow-rate was monitored after passing through the d.r.i.

Details of the setup of the existing system have been previously reported¹. Polymer concentration in the eluant was monitored using the d.r.i. detector. Scattering intensity data were collected with the 6-7° annulus and 6328 Å wavelength, He-Ne laser. The values of (dn/dc)

for the polyethylenes were determined independently with a differential refractometer. The analog signals from all three detectors were collected and digitized through an interface. The data were subsequently processed using a microcomputer with a software package developed in our laboratory.

Interpretation of results from a multi-detector s.e.c. system requires correct matching of the responses from the d.r.i. detector with corresponding responses from LALLS and CV detectors. In doing so, a broad distribution polystyrene sample was used to determine the detector offset¹². When the detector offset was estimated correctly, the absolute molecular weights of each slice from d.r.i., LALLS and CV analysis over the whole elution volume profile were successfully superimposable. In this approach, the inherent assumption is that the interdetector volume remains constant throughout the whole molecular weight distribution. Note that this assumption may not necessarily be valid in all cases.

Filtered 1,2,4-trichloro-benzene (TCB) containing antioxidant (0.1% Irganox-1010) was the s.e.c. eluant. Polymer solutions were prepared by dissolving a known amount of the polyethylene (PE) in a known volume of filtered TCB. Dissolution of PE samples was achieved by rotating the samples at 160°C for 16-24 h. To prevent oxidative degradation of sample, 0.1 w/v% of a phenolic antioxidant (Irganox 1010) was added. The mixed bed column was calibrated using 19 polystyrene (PS) standard samples with molecular weights ranging from 950 to 1900 000.

RESULTS AND DISCUSSION

The intrinsic viscosity of standard polymers (monodisperse) can be determined from s.e.c. results using CV chromatograms alone. Table 1 presents $[\eta]$ values of 19 PS standards with MW s ranging from 950 to 1900 000 in TCB at 145°C.

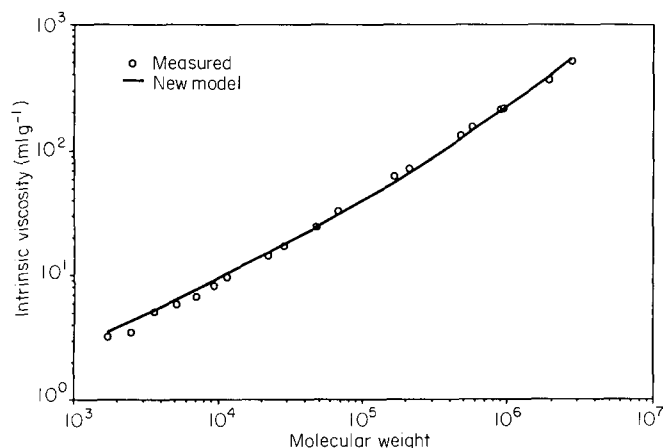
Although equation (4) is linear with respect to parameters K_{θ} and K' , an ordinary least squares procedure may result in inaccurate parameter estimation when the error variance is not constant (i.e. when the molecular weight range is wide). The error variance in the intrinsic viscosity estimation depends on the magnitude of the $[\eta]$ being measured; therefore, the error variance is heteroscedastic (the error variance is correlated with independent variable). In order to overcome this problem a logarithmic transformation has been adopted since it is a custom to plot $[\eta]$ versus M on logarithmic coordinates. The logarithmic

Table 1 Intrinsic viscosity of PS in TCB at 145°C

MW	$[\eta]$ (ml/g)	MW	$[\eta]$ (ml/g)
950	3.299	28 500	17.251
1 700	3.207	47 500	24.489
2 450	3.467	68 000	32.628
3 600	5.087	165 000	63.850
5 050	5.878	207 700	72.244
7 000	6.790	475 000	132.025
9 200	8.274	575 000	155.310
11 600	9.627	900 000	213.565
22 000	14.333	950 000	216.556
		1 900 000	363.778

Table 2 Estimates of K_θ and K' with 95% confidence interval for PS and PE at 145°C

Polymer	$K_\theta \cdot 10^2$	$K' \cdot 10^4$
PS (std)	7.597 ± 0.636	1.549 ± 0.210
PS (DOW 1683)	7.622 ± 0.213	1.528 ± 0.049
LLDPE (DOW 2056)	32.868 ± 0.539	3.946 ± 0.254


Figure 1 The fit of equation (2) to the $[\eta]$ - MW data of PS standards in TCB at 145°C

transformation of equation (4) is:

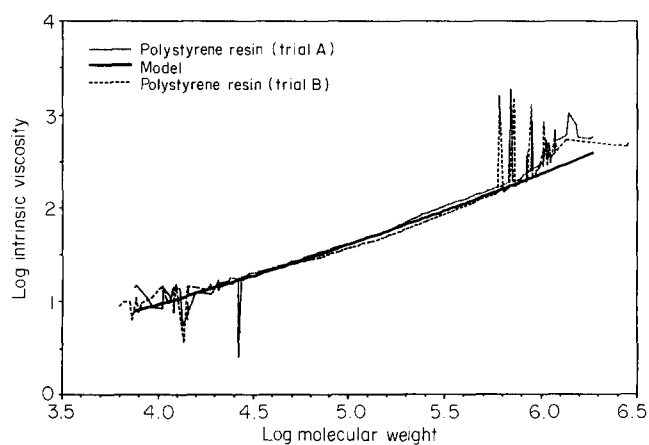
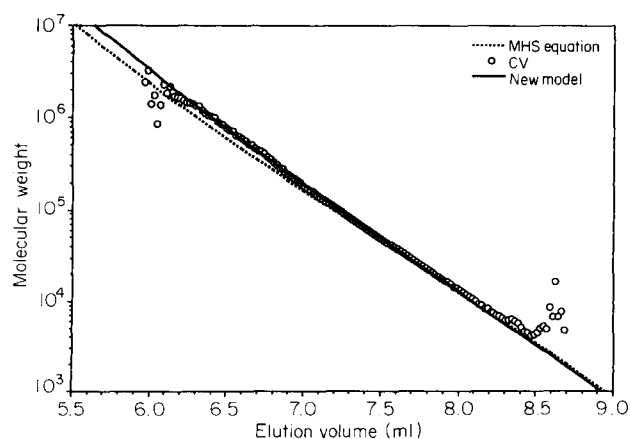
$$\log[\eta] = \log(K_\theta M^{1/2} + K'M) \quad (6)$$

The intrinsic viscosities in Table 1 were fitted to equation (6) using a non-linear regression analysis (a modified Gauss Newton algorithm) to estimate K_θ and K' . Inspection of the results in Table 2 indicates that the estimate of K_θ is in excellent agreement with the reported value of 7.6 ± 0.2 in the literature¹¹. Figure 1 shows the fit of equation (3) for the PS standards.

A UCC was established, using these 19 PS standards. A cubic polynomial represents $[\eta] \times M$ as function of elution volume for these standards. In order to ensure that this UCC is accurate, a commercial PS resin (Dow 1683) with $M_n = 100\,000$, $M_w/M_n = 2.5$ was analysed on the same s.e.c. system. The intrinsic viscosities of the eluting PS species in TCB were estimated using CV data in conjunction with d.r.i. data. Two runs were performed on this sample. In order to reduce the effect of random noise appearing at the tails of MWD due to the relatively low polymer concentrations in the eluants, results of both trials were considered in the regression analysis. In this analysis MWs of 12 000–800 000 were included, where the signal-to-noise ratio was acceptable. Figure 2 displays the measured $[\eta]$ versus MW (dotted lines) plus the fit of equation (3) through these data (solid line). In this plot, the experimental results are displayed without data smoothing. The cut-offs at the low and high molecular weight ends of the distribution are dictated by the relatively high noise-to-signal ratio from the d.r.i. detector¹. The estimates of K_θ and K' for PS resins are given in Table 2 and are found to be statistically identical with the estimates of the same parameters from PS standards.

The intrinsic viscosities of the ethylene–octene copolymer (linear low-density polyethylene, LLDPE) species in TCB were calculated from combined CV and DRI chromatograms. K_θ and K' were estimated from $[\eta]/MW$ data for LLDPE. These results are shown in Table 2. The reported value of K_θ for linear PE in the literature¹³ is $(31 \pm 1.0) \times 10^{-2}$ (g/ml), and agrees well with our estimate. This is expected, since the LLDPE contains no long branches and the short (C_6) branch density is low. Note that the MWs of slices were obtained from the absolute MW detector (LALLS).

As a comparison, two calibration curves were established; one using equation (4) with the determined K_θ and K' values, and the other using equation (1) with K and a equal to 0.0596 and 0.69, respectively¹⁴. Figure 3 shows MWs obtained from CV data with respect to elution volume for a different LLDPE resin and also molecular weights calculated from calibration curves using the MHS equation and the new model. Figure 4 shows the results from LALLS analysis in comparison with these calibration curves. Results in Figures 3 and 4 indicate that the calibration curve using equation (4) is more accurate than the calibration curve using equation (1). This is particularly true at higher MWs . In Figures 3 and 4 the measured MWs at very high and low MW regions are scattered due to very low polymer concentrations. In Table 3 the estimates of MWs for PE from either of the calibration curves at different elution


Figure 2 The fit of equation (2) to the $[\eta]$ of slices of a polydisperse PS in TCB at 145°C

Figure 3 Molecular weight versus elution time for LLDPE from viscometer detector

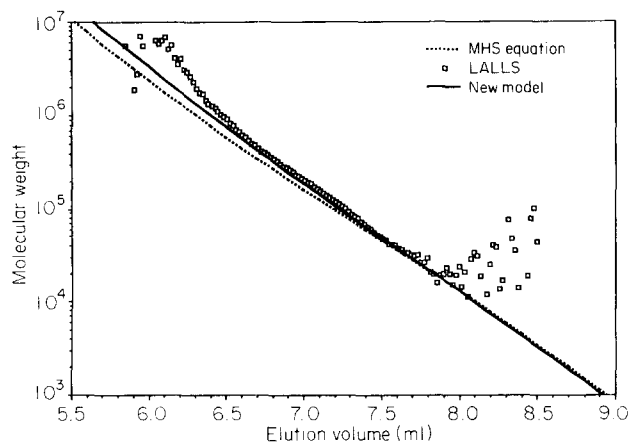


Figure 4 Molecular weight versus elution time for LLDPE from the LALLS detector

Table 3 Estimates of MW s from calibration curves (MHS, the new model) and LALLS detector

Elution volume (ml)	MW (MHS)	MW (new model)	MW (LALLS)
6.4	777 000	1 060 000	1 230 000
6.6	455 000	573 000	679 000
6.8	269 000	326 000	382 000
7.4	58 000	62 800	77 200
7.6	35 100	36 600	37 100

volumes are compared with those obtained from the LALLS detector. It can be seen that the UCC with measured MHS constants seriously underestimates the molecular weight in the high molecular weight region. The remaining discrepancies between the revised UCC and the LALLS detector are due to the very low concentration of polymer at the tail ends of distribution.

CONCLUSION

The results from s.e.c. analysis on PS and PE resins using two different calibration curves were compared with results from absolute MW detectors. At the higher molecular weight end a deviation is apparent between MW s obtained from absolute molecular weight detector (LALLS) and MW s estimated using a universal calibration curve based on the MHS relationship with a

particular set of K and a values. Therefore, over a relatively wide range of molecular weights the interpretation of results may not be correct when one set of K and a is being used.

The estimates of MW s from the calibration curve using the alternative model with a single parameter were consistent with results from absolute molecular weight detectors. This indicates that accurate calibration of the s.e.c. system could be achieved over a wide range of MW s when the appropriate functional relation is used to establish the universal calibration curve.

Another advantage of the new method is apparent in the low molecular weight region of the chromatogram, where the oligomeric species (with degree of polymerization less than 100) are known to exhibit theta solution behaviour ($a=0.5$). Therefore, use of a single set of MHS constants that is more suitable for the mid-range of MWD is inadequate for the low molecular weight region. The alternative model with a single system-dependent parameter provides accurate $MW-[\eta]$ values for these small polymeric species.

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