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## USE OF NARROW AND BROAD MOLECULAR-WEIGHT DISTRIBUTION STANDARDS TO CALIBRATE THE GEL PERMEATION CHROMATOGRAPH

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### SUMMARY

A critical evaluation of common methods of column calibration in gel permeation chromatography is reported for a range of well characterised polymer standards. Of those methods based on the polystyrene standards, namely the  $Q$  factor method and the universal calibration method, the latter is shown to be more accurate over a wide range of molecular weights and polymer types. The universal calibration method, in which secondary standards with broad molecular-weight distributions are used to define the parameters of the universal calibration curve, is also shown to be superior to methods in which the calibration curve is attained directly from the secondary standards.

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### INTRODUCTION

The use of gel permeation chromatography (GPC) has increased dramatically over the last few years with the introduction of improved equipment<sup>1</sup> capable of rapid analysis and with high resolution. An analysis requiring, for example, 3 h five years ago may now be conducted in 30 min. The use of a captive mini-computer<sup>2</sup> or delayed processing techniques<sup>3</sup> ensures that calculation of the data is rapid, with an accuracy limited only by the particular needs of the user.

GPC does not give the molecular-weight distribution (MWD) directly. Instead, the chromatogram is the representation of the retention-volume distribution of the polymer sample, which is converted into the MWD via a relationship between molecular weight ( $M$ ) and retention volume ( $V$ ) of the polymer sample. This relationship (shown as eqn. 1) is termed the calibration curve and often takes the form

$$M = C_1 \exp(C_2 V) \quad (1)$$

where  $C_1$  and  $C_2$  are constants.

It has long been recognised, however, that the peak elution volume is not a function of the molecular weight of the solute species alone, but that molecular

structure plays a significant role. Thus, a calibration curve must be constructed for each polymer type. That such a simple process cannot be readily achieved in practice is due to the lack of suitable standards, *i.e.*, well characterised polymers with a narrow MWD, of polymers other than polystyrene. At present, a primary calibration curve is constructed (using polystyrene standards) which is then transformed by various techniques<sup>3</sup> so that it can be used for the whole range of polymeric materials.

The first of these methods is called the "*Q*" factor method<sup>5,6</sup>, where the *Q* factor is a relationship between molecular weight and molecular size of a particular polymer. The method has, however, many shortcomings (due mainly to the basic assumption that the molecular size of the polymer in solution can simply be represented by the product of the monomer contour length and the degree of polymerisation). Used with caution, however, and with certain polymers, it can provide a simple analysis with moderate accuracy, although in other instances the errors are considerable<sup>7</sup>.

The suggestion by Grubisic *et al.*<sup>8</sup> that the hydrodynamic volume of a polymer, as represented by the product of the intrinsic viscosity and the molecular weight  $[\eta]M$ , can be used as a measurement of the molecular size of any polymer in solution led to the introduction of the universal calibration curve.

It has been shown<sup>9</sup> that the molecular weight of a polymer sample eluting at volume  $V_R$  is related to the molecular weight of a polystyrene standard sample eluting at the same volume by the expression

$$M_2 = (K_1/K_2)^{1/a_2+1} \cdot M_1^{(a_1+1)/(a_2+1)} \quad (2)$$

where the subscripts 1 and 2 refer to the polymer standard and sample, respectively, and  $K$  and  $a$  are the respective Mark-Houwink parameters.

This method has been shown to have wide applicability<sup>10</sup>. The procedure does, however, require very accurate values of the Mark-Houwink constants for both the sample and the polymer standard in the same solvent at the same temperature and over the same molecular-weight range as the GPC analysis, a requirement not always readily achievable in practice.

Many of the calibration problems can be overcome by the use of secondary polymer standards, which are simply polymers whose average molecular weights have been determined by classical techniques. In most instances, these polymers have a broad MWD.

The methods in which these polydisperse polymers are used to determine their respective calibration parameters involve search techniques, combined with the actual chromatogram, to determine either the constants in the calibration equation (eqn. 1) or the Mark-Houwink parameters of the polymer sample.

Thus, Balke *et al.*<sup>11</sup> used a Rosenbrock computer-search procedure to determine the calibration constants  $C_1$  and  $C_2$  from the chromatogram of a secondary standard polymer. The objective function  $(\bar{M}_w - \bar{M}_w \text{EST})^2 + (\bar{M}_n - \bar{M}_n \text{EST})^2$  was minimised with the estimates of  $\bar{M}_w$  and  $\bar{M}_n$  (the weight- and number-average molecular weight of the polymer) being obtained from the chromatogram.

Recently, Loy<sup>12</sup> presented a simpler method in which the calibration constant  $C_2$  was obtained by minimising the function  $F_1$  as defined by

$$F_1 = \frac{\bar{M}_w}{\bar{M}_n} - (\sum W_i 10^{-V_i/C_2}) (\sum W_i 10^{V_i/C_2}) \quad (3)$$

by a suitable one-parameter search method. The term  $\Sigma W_i$  represents the weight fraction of polymer eluting at volume  $V_i$ .

The other constant,  $C_1$ , was determined from the relationship

$$C_1' = C_1 + C_2 \log_1 [M_w / \Sigma W_i \cdot 10^{(C_1 - V_i)/C_2}] \quad (4)$$

where  $C_1'$  is a better estimate of  $C_1$ .

In a similar approach, Mahabadi and O'Driscoll<sup>13</sup> determined the Mark-Houwink parameters of the polymer sample from the GPC of a standard polymer whose intrinsic viscosity  $[\eta]$  and  $\bar{M}_n$  were known. They used a search method to minimise the function shown in equation 5 to calculate an optimum value of  $a_2$ .

$$F_1 = \frac{[\eta] \bar{M}_n}{K_1} - [\Sigma (W_i M_i^{\beta a_2})] / [\Sigma (W_i / M_i^\beta)] \quad (5)$$

where  $K_1$  is the Mark-Houwink constant for polystyrene,  $\beta = (a_1 + 1)/(a_2 + 1)$ , and  $a_1$  and  $a_2$  are the Mark-Houwink exponents for polystyrene and the polymer sample, respectively.

The Mark-Houwink constant for the polymer sample,  $K_2$ , is then calculated from the equation

$$[\eta] = K_2 \bar{M}_{v2}^{\alpha_2}$$

$\bar{M}_{v2}$ , the viscosity-average molecular weight, being determined from the chromatogram. Once determined, these parameters are used in the construction of the universal calibration curve of the polymer sample.

This paper critically examines the shortcomings of each of these calibration methods in an attempt to recommend one as an accurate and reliable method.

## EXPERIMENTAL

The gel permeation chromatograms were obtained on equipment constructed from individual Waters Assoc. (Milford, Mass., U.S.A.) modules, namely, the 6000A pump, UK6 injector and R-400 differential refractive-index detector. Five columns with nominated exclusion limits of  $10^5$ ,  $10^4$ ,  $10^3$ , 500 and 100 Å were connected in series. The solvent tetrahydrofuran was distilled from potassium metal and filtered through a 0.45- $\mu$ m filter before use. It was kept under an atmosphere of nitrogen in the solvent reservoir.

The primary calibration curve was constructed by determining the peak-elution volume of polystyrene (PS) standards obtained from Waters Assoc. (Australia) (Sydney, Australia) and the National Bureau of Standards. The other characterised polymers used in this work, were polybutadiene (PB) (Phillips Chemicals, Kurnell, Australia), poly(vinyl chloride) (PVC) (ArRO Laboratories, Joliet, Ill., U.S.A., and ICI, Sydney, Australia), and polymethylmethacrylate (PMMA) (Rohm & Haas, Philadelphia, Pa., U.S.A., and Scientific Products, New York, N.Y., U.S.A.). The molecular-weight averages of these polymers were determined by the manufacturers.

The chromatograph was interfaced to a PDP11/40 digital computer for speed and accuracy of analysis<sup>3</sup>.

## RESULTS AND DISCUSSION

### The $Q$ factor

$\bar{M}_n$  and  $\bar{M}_w$ , the number- and weight-average molecular weights of a poly-disperse sample, are related to the molecular size of the polymer in solution by the relationship

$$\bar{M}_n = \bar{A}_n \cdot Q, \quad \bar{M}_w = \bar{A}_w \cdot Q \quad (6)$$

where  $\bar{A}_n$  and  $\bar{A}_w$  are, respectively, the number- and weight-average molecular sizes of the polymer. The reliability of this calibration method was examined by determining the  $Q$  factor from the known molecular-weight averages of a standard PVC.

The  $Q$  factor was determined by calculating  $\bar{A}_n$  and  $\bar{A}_w$  from the chromatogram, after which an average value of  $Q$  was determined from eqn. 6,  $\bar{M}_n$  and  $\bar{M}_w$  representing the average molecular weights of the polymer sample determined by classical methods of osmometry and light-scattering. This value was then used to calculate the molecular-weight averages of other PVC polymers; the results are collected in Table I. As can be seen from these results, the agreement between the tabulated and calculated molecular weights varies widely over the molecular-weight range of polymers studied, the closest agreement being achieved with polymers of similar molecular weight. The values of  $Q$  in the sixth column were calculated for the respective polymers from their actual molecular-weight averages. No meaningful result could be recorded for the final sample, as the weight-average and number-average molecular weights yielded widely different values of  $Q$ .

These results suggest that the simple relationship suggested by eqn. 6 does not adequately represent the relationship between molecular size and molecular weight and that the  $Q$  factor should in fact be some function of the molecular weight.

TABLE I  
GPC DATA OF PVC STANDARDS

Manufacturer's data		$Q$	From GPC — $Q$ factor		$Q^*$	From GPC — Loy calibration	
$\bar{M}_w$	$\bar{M}_n$		$\bar{M}_w$	$\bar{M}_n$		$\bar{M}_w$	$\bar{M}_n$
118,000**	41,000	23.9	117,200	40,750	23.9	117,600	41,500***
68,600**	25,500	23.9	84,100	31,100	19.5	57,800	22,300
132,000**	54,000	23.9	134,600	57,800	25.6	138,000	57,200
100,000 <sup>†</sup>	45,500	23.9	95,600	43,450	25.1	115,000	52,000
140,000 <sup>†</sup>	55,000	23.9	152,000	62,100	21.8	127,000	61,000
200,000 <sup>†</sup>	64,000	23.9	252,600	52,100	—	145,000	56,000

\* Values calculated from manufacturer's data.

\*\* Standards from ArRo Labs., Joliet, Ill., U.S.A.

\*\*\*  $C_1 = 10.8$ ,  $C_2 = -0.17$  calculated from these data.

<sup>†</sup> Samples supplied by ICI (Australia).

### Calibrations based on polydisperse standards

The technique of Loy<sup>12</sup> was used in preference to that of Balke *et al.*<sup>11</sup> to determine the constants  $C_1$  and  $C_2$  in a linear calibration curve as the method, in trial tests, used much less computer time. The PVC standard with  $\bar{M}_w = 118,000$  was used to determine the calibration constants  $C_1$  and  $C_2$ , and these constants were then used in calculating the molecular weights as shown in Table I. The agreement was reasonable except for the two PVC samples of highest molecular weight. An examination of the polystyrene calibration curve in Fig. 1 reveals that this curve is linear only over the range of molecular weights to about 700,000, deviating upwards from this point. Thus, a linear calibration curve based on a lower-molecular-weight polymer underestimates the molecular weights, as shown by the dashed line in Fig. 1. Such an under-estimation of molecular weight will lead to a significant lowering of  $\bar{M}_w$ . The number-average molecular weight is only slightly affected for the polymers studied, although a more noticeable effect would have been observed for polymers of higher molecular weight.

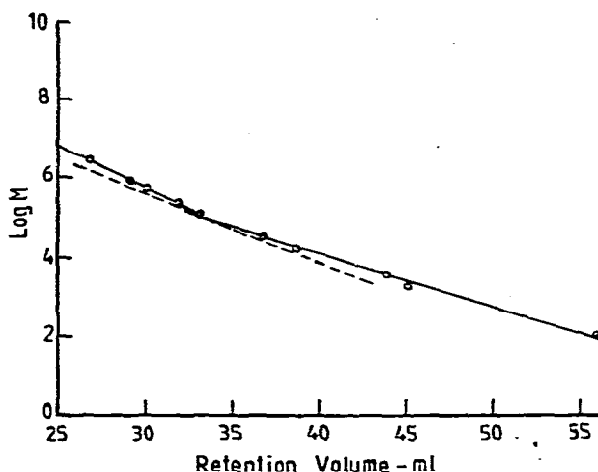


Fig. 1. GPC calibration curve experimentally determined for polystyrene standards (O). The dashed line represents the calibration line determined by the procedure of Loy<sup>12</sup>.

To enable the calibration curve to be more fully covered, it was decided to use the method of Balke *et al.*<sup>11</sup> extended to solve a quadratic calibration equation of the form

$$M = C_1 \exp(C_2 V + C_3 V^2) \quad (7)$$

The objective function  $(\bar{M}_w - \bar{M}_w \text{EST})^2 + (\bar{M}_n - \bar{M}_n \text{EST})^2$  was minimized in this technique. However, when tested with PS standards for which the calibration equation could be accurately determined, this method invariably arrived at a local minimum for the objective function, which yielded calibration constants considerably removed from their actual values. Changes to the objective function had little over-all effect. The results for the PS standard (NBS-706) are collated in Table II and show that, unless a very accurate initial guess of the calibration constants is obtained, little progress is made in arriving at the correct solution.

TABLE II

## ROSENBRÖCK THREE-PARAMETER SEARCH: RESULTS FOR POLYSTYRENE STANDARD NBS-706

For this material,  $\bar{M}_w = 257,000$  and  $\bar{M}_n = 136,000$ , and the calculated calibration constants are  $C_1 = 28.3$ ,  $C_2 = -1.31$ ,  $C_3 = 0.0163$ .

Constant	Value of constant		Objective function value		Calculated value	
	Initial	Final	Initial	Final	$\bar{M}_w$	$\bar{M}_n$
$C_1$	26	26.25				
$C_2$	- 1.2	- 1.20	$7.9 \cdot 10^7$	$3.46 \cdot 10^6$	292,200	157,000
$C_3$	0.015	- 0.15				
$C_1$	27	28				
$C_2$	- 1.3	- 1.29	$3.6 \cdot 10^8$	$1.62 \cdot 10^6$	218,100	148,200
$C_3$	0.016	-				
$C_1$	29	28				
$C_2$	- 1.4	- 1.29	$1.8 \cdot 10^8$	$2.81 \cdot 10^6$	205,700	150,400
$C_3$	0.016	0.016				
$C_1$	21	21.65	$1.53 \cdot 10^7$	$3.9 \cdot 10^6$	415,000	252,000
$C_2$	- 0.84	- 0.843				
$C_3$	0.08	0.081				

*Universal calibration method*

The applicability of the technique of Mahabadi and O'Driscoll<sup>13</sup> was examined by obtaining gel permeation chromatograms of the available range of PVC, PB and PMMA secondary standards. The Mark-Houwink parameters were calculated from the GPC of one of the standard polymers of each type. These parameters were then applied to the calculation of the molecular-weight averages of the other polymers; the results are collected in Table III.

TABLE III

## MOLECULAR-WEIGHT DATA CALCULATED ACCORDING TO METHOD OF MAHABADI AND O'DRISCOLL

Polymer standard	Manufacturer's data		From GPC		Source
	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	
Polybutadiene	17,000	16,000	15,500	14,100	} Phillips Chemicals
	170,000	135,000	168,000	110,000	
	272,000	206,000	269,000	208,000*	
	332,000	226,000	382,000	241,000	
Poly(methylmethacrylate)	60,000	33,200	60,450	33,000**	} Scientific Products
	50,000	20,800	30,500	17,200	
	270,000	123,000	165,000	86,000	
Poly(vinylchloride)	68,600	25,500	70,200	25,900***	} ArRO Laboratories
	118,000	41,000	104,000	46,000	
	132,000	54,000	134,000	55,000	
	83,500	37,400	76,000	36,000	

\*  $K = 1.84 \cdot 10^{-3}$ ;  $\alpha = 0.58$  calculated for polybutadiene from the GPC of this polymer.

\*\*  $K = 7.87 \cdot 10^{-5}$ ;  $\alpha = 0.75$  calculated for poly(methyl methacrylate) from the GPC of this polymer.

\*\*\*  $K = 4.76 \cdot 10^{-4}$ ;  $\alpha = 0.673$  calculated for poly(vinyl chloride) from the GPC of this polymer.

Agreement is excellent over the range of polymer types and molecular weights studied, with the exception of the high-molecular-weight PMMA and PB samples. This discrepancy may arise from the fact that the Mark-Houwink parameters are slightly molecular-weight dependent. Hence, when used for calculations of molecular weights well removed from the calibration standard, errors may arise. It should not be forgotten, however, that the manufacturers' data can often be in considerable error<sup>14</sup>. Nevertheless, the method appears to be more reliable over the whole range of polymers and molecular weights than any of the others. This is possibly because the molecular-weight parameters of the standards utilised, namely  $\bar{M}_n$  and  $[\eta]$  can be determined with considerably more accuracy and reliability than can  $\bar{M}_w$ , which is one of the parameters used in the other methods.

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