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GEL PERMEATION CHROMATOGRAPHIC CALIBRATION FOR POLYMERS MAKING USE OF THE UNIVERSAL CALIBRATION CURVE*

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SUMMARY

It is usually difficult to obtain narrow molecular weight distribution samples of a given polymer for calibrating the gel permeation chromatographic instrument in the conventional way. The literature reports several methods which have been used to obtain instrument calibrations with samples of broad distribution polymer. However, these methods generally must assume a linear calibration of the logarithm of molecular weight *versus* elution volume. This assumption may lead to significant errors in precise molecular weight distribution computations. It is possible to avoid this assumption by making use of the universal calibration curve proposed by BENOIT without the necessity of defining this curve by any mathematical function. A number of methods have been developed for combining the universal calibration curve with measurable parameters of the polymeric material (such as intrinsic viscosity, number average molecular weight and weight average molecular weight) to calculate molecular weight distributions.

Results will be reported on a careful evaluation of the accuracy and reproducibility of several of these methods.

INTRODUCTION

In order to provide quantitative data from gel permeation chromatographic (GPC) traces it is necessary to have an instrument calibration, *i.e.*, a relation between elution volume and molecular weight eluted. For a given GPC apparatus such a calibration will depend upon the chemical nature of the polymer. The most direct method of calibration consists of using narrow distribution samples of the same chemical nature as the experimental samples to be analyzed. The application of such a method is limited by lack of readily available narrow distribution samples of different chemical structures. An attempt to make allowances for differences in chemical structure was the Q factor approach[1, 2] which stated that the molecular species eluting at a given point is related to the size of the polymer chain. The size of the chain is assumed to be proportional to its molecular weight by a constant factor which depends on chain structure. Thus, having determined a calibration curve for one polymer type, say polystyrene using narrow distribution samples, the use of an appropriate Q factor allows a calibration to be calculated for another polymer type. However, this approach

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has not been found to be sufficiently accurate for most work[3]. However, an alternate and only slightly more complicated scheme for obtaining calibrations has been made possible by new developments in GPC theory. It was observed by BENOIT et al.[4] that for all polymers studied, a plot of molecular weight times intrinsic viscosity versus elution volume gave data that fell on a common curve. Thus, the calibration for any polymer may be defined by knowledge of the universal calibration for the column set involved along with the relationship between molecular weight and intrinsic viscosity for the particular polymer type involved. If the Mark-Houwink relationship is considered adequate the problem resolves itself into finding a way to establish the Mark-Houwink constants K and a from data on broad molecular weight distribution materials. An interesting method for accomplishing this has been reported[5], the mathematical basis for the method will be presented followed by an experimental test of the usefulness and validity of the technique.

MATHEMATICAL BASIS FOR CALIBRATION[5]

For the purpose of simplification and use of the universal calibration curve the parameter J is defined as the product of the intrinsic viscosity times the molecular weight:

$$J_i = [\eta]_i M_i \tag{1}$$

The function J with elution volume is considered to be independent of polymer type[4] and hence may be provided from a calibration curve with narrow distribution polystyrene samples. Making use of the Mark-Houwink relationship:

$$[\eta]_i = K M_i^a \tag{2}$$

and taking for the whole polymer:

$$[\eta] = \Sigma_i W_i [\eta]_i \tag{3}$$

where W_i $[\eta]_i$ and M_i are the weight fraction, viscosity and molecular weight respectively of the i-th species, then it follows directly that:

$$[\eta] = K^{1/(a+1)} \Sigma_i W_i J_i^{a/(a+1)} \tag{4}$$

and:

$$M_n = K^{-1/(a+1)} / \Sigma_i (W_i / J_i^{1/(a+1)}) \tag{5}$$

Use of intrinsic viscosities with GPC curves to obtain calibration

By taking two polymers of broad distribution and the same chemical nature but with different viscosities and measuring their intrinsic viscosities and GPC traces in the same solvent, then from eqn. 4:

$$\frac{[\eta]_1}{[\eta]_2} = \frac{\Sigma W_{i1} J_i^{a/(a+1)}}{\Sigma W_{i2} J_i^{a/(a+1)}} \tag{6}$$

In the equation W_i values are obtained from the ordinate of the GPC trace and hence a may be determined since it is the only unknown. The parameter K may then be determined through eqn. 4 using data from one of the polymers.

The use of the intrinsic viscosity and number average molecular weight to obtain calibration
    By combining eqns. 4 and 5 we obtain:

$$[\eta]M_n = \Sigma_i W_i J_i^{a/(a+1)}/\Sigma(W_i/J_i^{1/(a+1)}) \tag{7}$$

The corresponding result published by WEISS AND COHN-GINSBERG[5] contains a typographical error.

By taking a single polymer sample of broad distribution and known number average molecular weight and intrinsic viscosity and obtaining a GPC trace, the application of eqn. 7 allows *a* to be calculated followed by *K* from eqn. 5.

It should be noted that in eqns. 6 and 7 there is not a simple analytical solution for *a*. In practice the right hand side of eqn. 6 or eqn. 7 is evaluated for several values of *a* and an interpolation is performed to find the value of *a* which yields the desired value of the function. The actual calculations are carried out using a computer program.

EXPERIMENTAL

Universal calibration with polystyrene
    The narrow molecular weight distribution polystyrene standards made by the Pressure Chemical Co and furnished to us by Waters Associates were used to establish the universal calibration curve. The viscosities of these standards were determined in THF at 30° using a Cannon dilution type micro-viscosimeter. All solutions and solvent were filtered through a glass frit before entering the viscosimeter. The original concentrations were adjusted so that the longest flow time was less than twice the solvent flow time. The results of the viscosity determinations are given in Table I. A plot of the logarithm of viscosity *versus* the logarithm of molecular weight is given as Fig. 1. Data points from the work of BENOIT *et al.*[4] are plotted as triangles. These are seen to be in good agreement with this work.
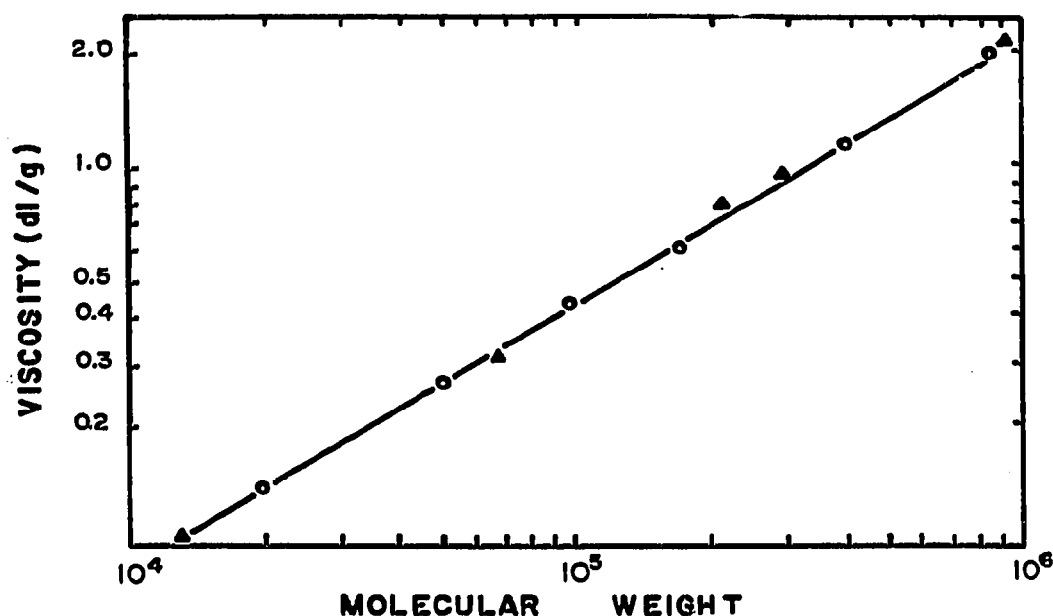

Fig. 1. The viscosity–molecular weight relationship for polystyrene in tetrahydrofuran at 30° from this work, ○ and from BENOIT[4], ▲.

TABLE I

MOLECULAR WEIGHTS AND INTRINSIC VISCOSITIES FOR POLYSTYRENE STANDARDS

Waters Standard No.	M_w	M_n	$[\eta]$
4 190 038	867 000	773 000	2.002
4 190 037	411 000	392 000	1.158
41 984	173 000	164 000	0.625
41 995	98 200	96 200	0.442
25 170	51 000	49 000	0.270
4 190 039	19 850	19 165	0.141

The line drawn represents the Mark-Houwink relation, eqn. 2, with $K = 1.25 \cdot 10^4$, and $a = 0.707$. The elution volume for each molecular weight was taken as the peak position of the GPC curve. Thus, the value of J versus elution volume was established. Fig. 2 shows the universal calibration curve for the four column set consisting of $1.5 \cdot 10^6$, 10^6, 10^5, and 10^4 Å columns. The unit for elution volume is counts (1 count = 5 cc). The concentrations used for GPC were about 0.1 % for the 411 000 and higher-molecular-weight standards and about 0.25 % for the lower molecular weight standards.
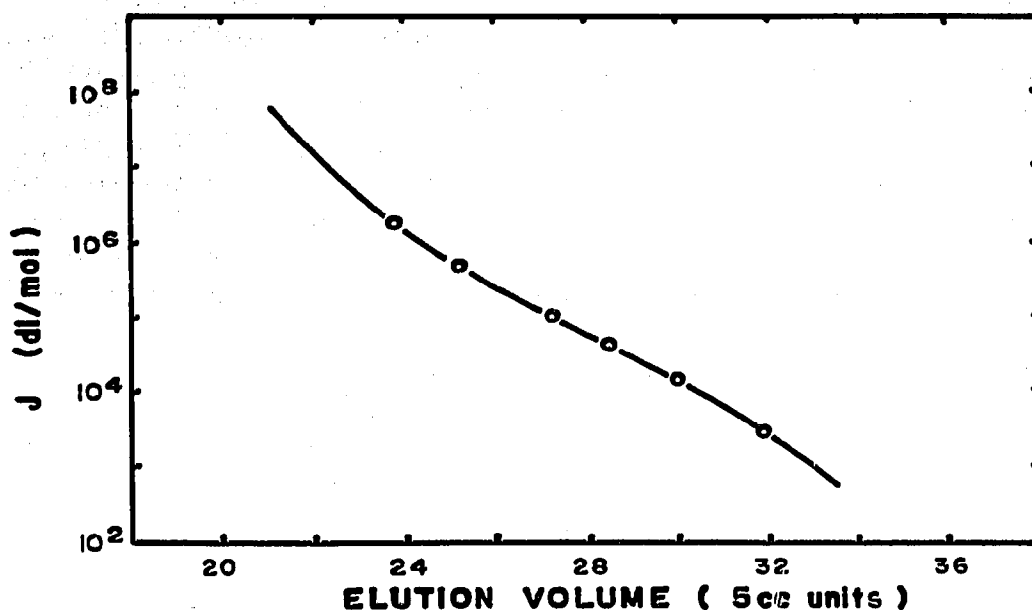

Fig. 2. The universal calibration curve for a styragel column set consisting of $1.5 \cdot 10^6$, 10^6, 10^5 and 10^4 Å columns.

Application to SBR polymers

In order to test these methods, intrinsic viscosities and number average molecular weights from osmometry were determined on two SBR polymers and GPC traces were obtained. The polymers were emulsion polymerized styrene–butadiene copolymers of about 23 % styrene.

The theory given allows the calculation of the molecular weight–intrinsic viscosity relation for a particular polymer and consequently its GPC calibration curve. Using various combinations of data Mark-Houwink parameters have been calculated and are shown in Table II. In addition, various molecular weight averages and in-

TABLE II

APPLICATION OF UNIVERSAL CALIBRATION METHODS TO SBR

Calibration method	Standard sample and computed results	SBR	Calculated from GPC curve		$[\eta]$
			M_n	M_w	
Using eqn. 7 with $[\eta]$ and M_n one sample one GPC curve	Case A SBR 1808 $K = 0.2984 \cdot 10^{-3}$	1808	114 800[a]	453 500	2.47[a]
	$a = 0.7043$	1507	88 360	308 700	1.89
	Case B SBR 1507 $K = 0.5463 \cdot 10^{-3}$	1808	111 100	475 700	2.38
	$a = 0.6545$	1507	84 900[a]	320 000	1.85[a]
Using eqn. 6 two $[\eta]$ two GPC curves	Case C SBR 1808 SBR 1507 $K = 0.8066 \cdot 10^{-4}$	1808	129 700	445 600	2.47[a]
	$a = 0.8021$	1507	101 100	310 000	1.85[a]

[a] Indicates directly measured value used in establishing calibration.

trinsic viscosities have been calculated from the GPC curves and are listed in Table II. All number average molecular weights are seen to fall within a total range of about 20 %. The worst case is case C of Table II where the calibration curve was obtained from Eqn. 6 without reference to a molecular weight measurement on an SBR sample.

Weight average molecular weights fall within a range of 10 %. While the Mark-Houwink parameters K and a shown in Table II vary in the three cases, low values of a are compensated by high values of K so that the mid-range of the calibration checks out very well. The calibration curves are tabulated in Table III for comparison.

Application to butyl rubber

It was desirable to take several well characterized rubber samples and obtain replicate GPC traces on each in order to better establish the reproducibility and accuracy of the molecular weight averages. For this reason, five replicate GPC curves were obtained for each of three samples of butyl rubber, one control and two unknowns.

Using the calibration procedures demonstrated earlier and using eqn. 7 with

TABLE III

COMPARISON OF CALIBRATIONS FOR SBR SAMPLES

Elution volume (counts)	Molecular weight		
	Case A	Case B	Case C
16	35 100 000	41 100 000	28 300 000
20	1 772 000	1 894 000	1 677 000
24	145 400	144 200	157 700
28	30 150	28 520	35 600
32	5 369	4 822	6 962
36	645	544	939

TABLE IV

BUTYL CALIBRATIONS FROM CONTROL REPLICATES

Replicate	No. 1	No. 2	No. 3	No. 4	No. 5
$K \times 10^4$	0.8540	1.597	0.7789	1.287	0.8580
a	0.746	0.700	0.755	0.716	0.747
Elution volume (count)	Molecular weight corresponding to elution volume				
19	37.9×10^6	42.0×10^6	36.6×10^6	40.7×10^6	37.7×10^6
23	1.40×10^6	1.41×10^6	1.37×10^6	1.42×10^6	1.39×10^6
27	178 900	171 400	177 400	174 600	177 800
31	31 200	28 500	31 200	29 500	31 000
35	4 046	3 501	4 090	3 693	4 025
M_w	526 000	513 000	504 000	534 000	519 000
M_z	1 890 000	1 610 000	1 630 000	2 340 000	1 880 000

$[\eta] = 1.42$ and M_n 172 600 for the control polymer, five sets of K and a values were obtained. They are listed in Table IV. Also listed are molecular weight values at specific elution volumes which result from use of the K and a values with the universal calibration curve. As with the SBR polymers, while the K and a values vary considerably, a high value of K is compensated by a low value for a so that the molecular weight values in the mid-range of the calibration are very simular.

In Table V, typical number average molecular weight results are given for calculating each of the sample replicates against the various control replicates. Thus, going across the table horizontally for a given sample, the standard deviation over different controls is about 4100 or 2.2 % of the average value. Comparing the values vertically, the standard deviation for different butyl No. 1 replicates over the same control runs about 1.2 %. Since 95 % confidence limits run about twice the standard deviation, we find confidence limits of about ±5 % for variation in M_n due to the uncertainty of controls and about 2.5 % due to the uncertainty in the sample trace.

In Table VI the averages and standard deviations are summarized for both butyl unknowns. For number average molecular weights, the results for the second butyl rubber shows slightly more deviation over different sample traces. The variations

TABLE V

NUMBER AVERAGE MOLECULAR WEIGHTS FROM GPC FOR BUTYL NO. 1

Sample	Control replicate						
	1	2	3	4	5	Average	S.D.
1	192 400	181 500	191 300	186 000	191 200	188 480	4137
2	191 900	181 100	190 800	185 500	190 700	188 000	4104
3	187 600	176 700	186 500	181 100	186 400	183 660	4150
4	187 500	176 700	186 400	181 100	186 400	183 620	4117
5	191 900	181 100	190 800	185 500	190 800	188 020	4117
Average	190 260	179 420	189 160	183 840	189 100	Overall 186 356	
S.D.	2 220	2 226	2 220	2 245	2 211		

TABLE VI

STATISTICAL RESULTS

Sample replicate	Vary calibration, fixed sample							
	Butyl No. 1				Butyl No. 2			
	$\bar{M}_n$	S.D.	$\bar{M}_w$	S.D.	$\bar{M}_n$	S.D.	$\bar{M}_w$	S.D.
1	188 500	4137	552 000	4455	209 500	4198	558 300	4484
2	188 000	4104	542 200	4258	214 200	4172	556 000	4310
3	183 700	4150	557 600	4620	204 600	4211	551 200	4253
4	183 600	4117	545 700	4420	212 400	4061	529 600	3803
5	188 000	4117	543 500	4258	199 400	4156	541 100	4141
	~2.2%		~0.8%		~2.0%		~0.8%	

Cali-bration	Fix calibration, vary sample replicate							
	Butyl No. 1				Butyl No. 2			
	$\bar{M}_n$	S.D.	$\bar{M}_w$	S.D.	$\bar{M}_n$	S.D.	$\bar{M}_w$	S.D.
1	190 300	2220	549 900	5749	211 600	5063	546 500	11 205
2	179 400	2226	551 200	6004	200 500	5042	547 100	11 686
3	189 200	2220	540 600	5610	210 200	4989	537 400	10 930
4	183 800	2245	553 100	5934	205 100	5087	549 200	11 577
5	189 100	2211	546 200	5680	210 300	5014	542 900	11 138
	~1.2%		~1.0%		~2.5%		~2.1%	

Overall: $\bar{M}_n = 186360$; $\bar{M}_w = 548200$; osmotic $M_n = 186000$.

Overall: $\bar{M}_n = 207540$; $\bar{M}_w = 544600$; osmotic $M_n = 212000$.

due to calibration are almost identical for the two unknowns as would be expected. In terms of accuracy the average M_n values are in excellent agreement with osmotically determined values.

The standard deviations for the M_w values given in Table VI are seen to be smaller than those for M_n. Estimated 95 % confidence limits are ± 2 % due to calibration and ± 4 % due to sample trace variation.

The values for the Z average molecular weight cover a range of about ± 10 % the average values. The M_z values found for the two rubbers were not significantly different having average values of 1707000 and 1604000, respectively. The larger deviations for M_n and M_z reflect their greater reliance upon the extremes of the calibration curves where the calibrations are least accurate and reproducible.

CONCLUSION

Intrinsic viscosities in THF at 30° were determined on six of the standard polystyrene samples furnished by Waters Associates. The viscosity values made it possible to set up the universal calibration curve which was successfully applied in obtaining specific polymer calibrations.

The results shown with SBR served to check different cases of the theory. Satisfactory agreement of calculated M_n values with the osmotic pressure values was found for SBR. The work with butyls served as an independent test of the theory and

also as an examination of the reproducibility to be expected. Again the calculated M_n values were in good agreement with osmotically determined values. Reproducibility was determined to be very good and it was estimated that one can expect to be able to differentiate between samples having M_n values 5 % or more apart when the GPC traces are run against the same calibration.

However, certain assumptions are made in the derivation which results in the following restrictions. The Mark-Houwink viscosity relation must apply to the polymer. Use of the ordinate of the GPC curve as the measure of concentration means that all the material involved should be of the same refractive index. Therefore the method would not be expected to apply to mixtures of polymers or non-random copolymers and grafts.

The methods presented are especially attractive for routine use with GPC work since intrinsic viscosities and number average molecular weights are relatively easily obtainable. Also a distinct advantage comes about through the use of Mark-Houwink parameters K and a. It should be possible to apply the K and a values to obtain specific polymer calibrations for other similar column sets. This may be expected to facilitate comparison of results from different instruments or to correct for calibration drift of one instrument. Work is presently proceeding along these lines.

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