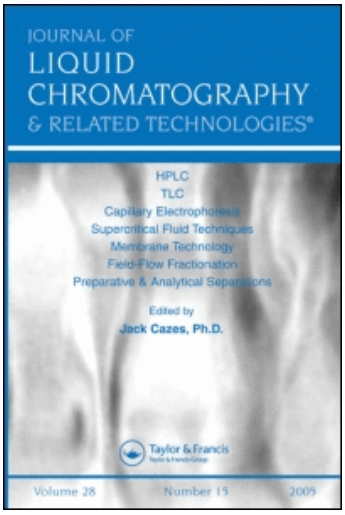

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THE ERRORS IN THE TREATMENT OF GEL PERMEATION CHROMATOGRAPHY DATA : ORIGINS AND CORRECTIONS

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ABSTRACT

This paper deals with the origins of errors in data interpretation when using modern GPC with dual detection (refractometer-viscometer) as a method of determination of average molecular weights of polymers. We describe here the different errors classified in two groups : typical chromatographic errors and data treatment errors and we show that they can lead to very miscalculated molecular weight values. For every case, we have tried to propose the best way to avoid or correct these errors so as to use modern GPC as a very accurate method of polymer characterization.

INTRODUCTION

Gel Permeation Chromatography (GPC) is a method of polymer characterization which is going to take the place of the traditional methods of average molecular weight determination. It can be used in two different ways: one can only consider recorded chromatograms as qualitative representations of molecular distribution and compare them to stress differences of polydispersity. But one can also achieve calculations from chromatographic peaks so as to obtain average molecular weight values. GPC may be then a very quantitative method of molecular weight determination and, accordingly, must withstand a critical analysis. For this reason, it is important to determine the main origins of errors in GPC data interpretation and their influence on the accuracy with which the average molecular weight values are obtained. We can thus number three kinds of errors :

- *errors arising from abnormal elutions*
- *typical chromatographic errors*
- *data treatment errors.*

Errors arising from abnormal elutions mainly occur when the stationary phase-mobile phase system is not suitable (1). Interactions between the packing and the solute may involve retention mechanisms such as adsorption or partition besides the steric exclusion process, increasing elution volumes and leading, thus to underestimated molecular weight values. We will not consider these non-exclusion effects and we will only analyze the origin of errors when the chromatographic system is well suited.

Typical chromatographic errors are directly related to instrumentation and chemicals (2). The main discrepancy is due to the calibration relationship between elution volumes and molecular weights, first because of the bad molecular weight accuracy of the standards used but also, the difficulty in determining precisely, flow rate stability and elution volumes. In addition, the sample dissolution and the accuracy on the injected solute amount may lead to very important errors.

Finally, data treatment must take into account experimental imperfections and include their corrections (3). We will examine the data treatment problems in the case of modern GPC using microgels with elution times of about 20 minutes.

EXPERIMENTAL

The experiments were run on a high performance liquid chromatograph composed with Waters Associates components : a M 6000 A solvent delivery system, a U6K injector, a R 401 differential refractometer, and a μ-styragel column set 10^3 Å, 10^4 Å, 10^5 Å, 10^6 Å. The GPC instrument was equipped with a continuous viscometer recently described (4) and a 9825 S Hewlett-Packard desk computer for data acquisition and treatment (16).

Standards used for calibration were polystyrene samples from Waters Associates. Solvent (T.H.F.) and solutions were membrane-

filtered via Millipore solvent and sample clarification kits. The ultrasonic bath used for sample dissolution was a Branson B 12 (80 watts) ultrasonic cleaner.

RESULTS

- *TYPICAL CHROMATOGRAPHIC ERRORS*

Measurement of elution volumes

In order to obtain a reliable relationship between elution volumes and molecular weights, it is essential to measure and check accurately, the mobile phase flow rate. A common technique is to use a siphon volume counter ; unfortunately, this device, which is very stable in a short period of time (better than 0.1 %), presents great variations in longer periods that can exceeds 1 %. These errors may be very important when, for example, the siphon is removed for cleaning and replaced without caution. Typically, Table 1 gives 3 series of measurements, achieved at different times for a same position of a 1ml siphon with a tetrahydrofuran (THF) flow rate of 2ml/mn.

These variations are too great to perform accurate measurements with a siphon volume counter and, instead, it seems better to use elution times for determining elution volumes, although this method requires a high quality pumping system with a well regulated flow rate. A difficulty appears when a leak or a pump malfunction occurs. The real flow rate is then smaller than the preset one

TABLE 1

Mean time in seconds	*Déviation*	*Elution volume of PS* 111,000*	*Peak molecular weight*	*Error*
25.62	*< . 1%*	*34.90 ml*	*122,000*	*10%*
25.43	*< . 1%*	*35.15 ml*	*111,000*	*0%*
25.26	*< . 1%*	*35.40 ml*	*102,000*	*-10%*

* *PS : polystyrene*

and this leads to underestimated molecular weights (typically 1 % flow variation corresponds to about 15 % error on molecular weight). However, it is possible to accurately check the flow rate by the use of a continuous viscometer, as we have shown in a previous paper (4).

Determination of molecular weight values

The second problem is to establish the relationship between elution volumes and molecular weights, since GPC is presently a non-absolute method and generally requires a calibration. Some "absolute" molecular weight detectors exist, such as the continuous viscometer (4) if the Mark-Houwink coefficients are known, or the low angle laser light scattering detector (5), but these detectors are rather used to provide information on molecular weight distributions. A calibration curve, obtained by injections of polystyrene standards, thus has to be used. Accordingly, the measurement accuracy is directly dependent upon the accuracy of these standards. It is important to select them carefully since all of them are not well-labeled and it is not rare to encounter errors greater than 20 %. Other standards, different from polystyrene, are now available (polytetrahydrofuran, polymethylmethacrylate, etc ...), but their accuracy is unknown since they are supplied in series of $\bar{M}w$ *= 300,000 ; 100,000 ; 30,000 ; 10,000 ; 3,000 ; 1,000 which are probably the expected values and not the real ones. In the best case, the supplier gives additional information as for the 100,000 polymethylmethacrylate standard whose label is :*

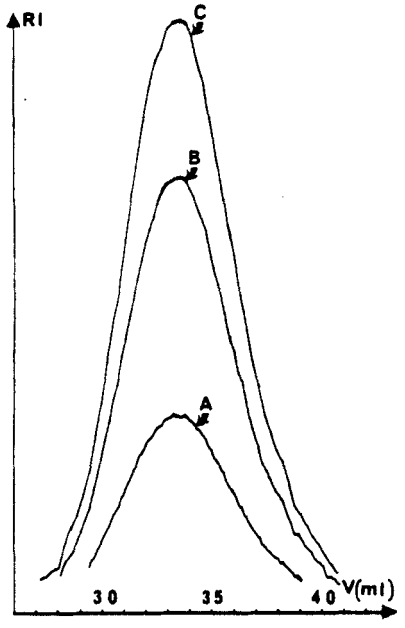
$\bar{M}w = 100{,}000 \; ; \; \bar{M}n = 77{,}000 \; ; \; \bar{M}w/\bar{M}n < 1.07$

After measurement, we found that the $\bar{M}w$ *value was obviously wrong.*

The reliability of polystyrene standards recently increased, and some of them can now be purchased with a certificate providing the different average molecular weights. However, we noticed some difficulties in the use of these values. For example, number average molecular weight can be greater than the corresponding viscometric average molecular weight. Anyway, the peak molecular weight obtained by GPC seems to be the most reliable value of these certificates.

Sample dissolution

In some cases, samples are not completely dissolved although the clarity of the solution before filtration is good. As a result, the chromatogram is obviously shifted towards low molecular weights. In addition, when using universal calibration ($[\eta].M$) with a viscometric detector, the intrinsic viscosity value of the whole sample is underestimated and, accordingly, its molecular weight is overestimated. The error on the injected sample amount is directly connected with the error on molecular weight. Figure 1 represents the chromatograms and the apparent molecular weights of a cellulose nitrate sample for different dissolution times. The THF/polymer mixture rapidly appears to be a clear solution.



solvent THF 30°

	dissolution	$\overline{M}_n$	$\overline{M}_w$
A	apparent	97,000	220,000
B	1 hour	65,000	130,000
C	3 hours	40,000	100,000

Figure 1

Variations of chromatograms and apparent molecular weights at different dissolution times for a cellulose nitrate sample in THF.

After membrane filtration, it is injected and nevertheless leads to molecular weight values that dramatically depend upon dissolution time. It is therefore important to avoid this phenomenon and to prepare polymer solutions very carefully. A good way, might appear to be the use of an ultrasonic bath, which would greatly reduce dissolution time. However, some authors report risks of degradation by chain breaking. We performed (2) GPC analysis of a polybutadiene sample solution ($\bar{M}w$ = 300,000) after different exposure times (10 minutes to 6 hours) in an ultrasonic bath (150W) and we did not notice any significant variation of the distribution curve.

The same holds true with polystyrene samples whose molecular weights are smaller than 300,000. But, for higher molecular weight polystyrene samples,we observed a shifting down of molecular weight values ; the longer the exposure time in the ultrasonic bath, the more dramatic the effect. A chain degradation obviously occurs that prohibits the use of the ultrasonic bath for high molecular weight polymer dissolution.

This effect was observed with polystyrene standards, as shown in Table 2 in which we compare the elution volume values of solutions at a concentration of .125 %. A values were obtained via a classical dissolution and B values via an ultrasonic dissolution ; the higher the molecular weight, the stronger the effect.

Sample injection

As the use of a viscometer requires a precise knowledge of the injected sample amounts, the presence of non-soluble parts such as microgels in polymers presents a problem. Besides, when

TABLE 2

Molecular weights :	*650,000*	*1,200,000*	*2,700,000*	*3,800,000*
Elution volume A :	*29.15*	*27.50*	*26.45*	*26.20*
Elution volume B :	*29.35*	*30.25*	*30.30*	*31.55*

the injection is not achieved under perfect conditions, peak broadening with a skewed tail may occur. Figure 2 gives three consecutive injections of a mixture of two polystyrene standards and shows peak deformation leading to incorrect values, particularly for number-average molecular weights and polydispersities ; this occurs when an injector has gradually been plugged. The injection system must accordingly be very carefully checked to avoid these drawbacks.

Influence of solute concentration

The dependance of elution volumes on solute concentration is one of the most important sources of errors in modern GPC. This phenomenon, in connection with the effect of solution viscosity, is therefore dependent upon both polymer molecular weight and polymer concentration. It should then be more exact to refer to segment density, since the experimental observation is achieved by varying the amount of injected polymer and not the mole number. Increasing sample concentration leads to increasing elution volume (6) and distorted chromatographic peaks with skewed fronts. Moore (7) explained this distortion by overloading viscosity effects and Rudin (8) showed, theoretically, that hydrodynamic volume depends upon the solute concentration ; this is in good agreement with GPC experiments performed in theta solvents (9) where concentration effects are negligible. The influence of sample viscosity on the shift of chromatographic peaks was likewise studied by Janca (10-11).

This effect is shown in Figure 3, where chromatograms of a polystyrene standard ($\bar{M}w$ = 655,000) are obtained at four different concentrations (2). We can observe a peak shift towards high elution volumes when sample concentration increases and a peak distortion with a skewed front connected with non-equilibrium mass transfer. This results in drastic errors on measured molecular weights as shown in Table 3 for a polystyrene standard ($\bar{M}w$ = 830,000) and a calibration achieved with the same standard at a concentration of 0.0625 %.

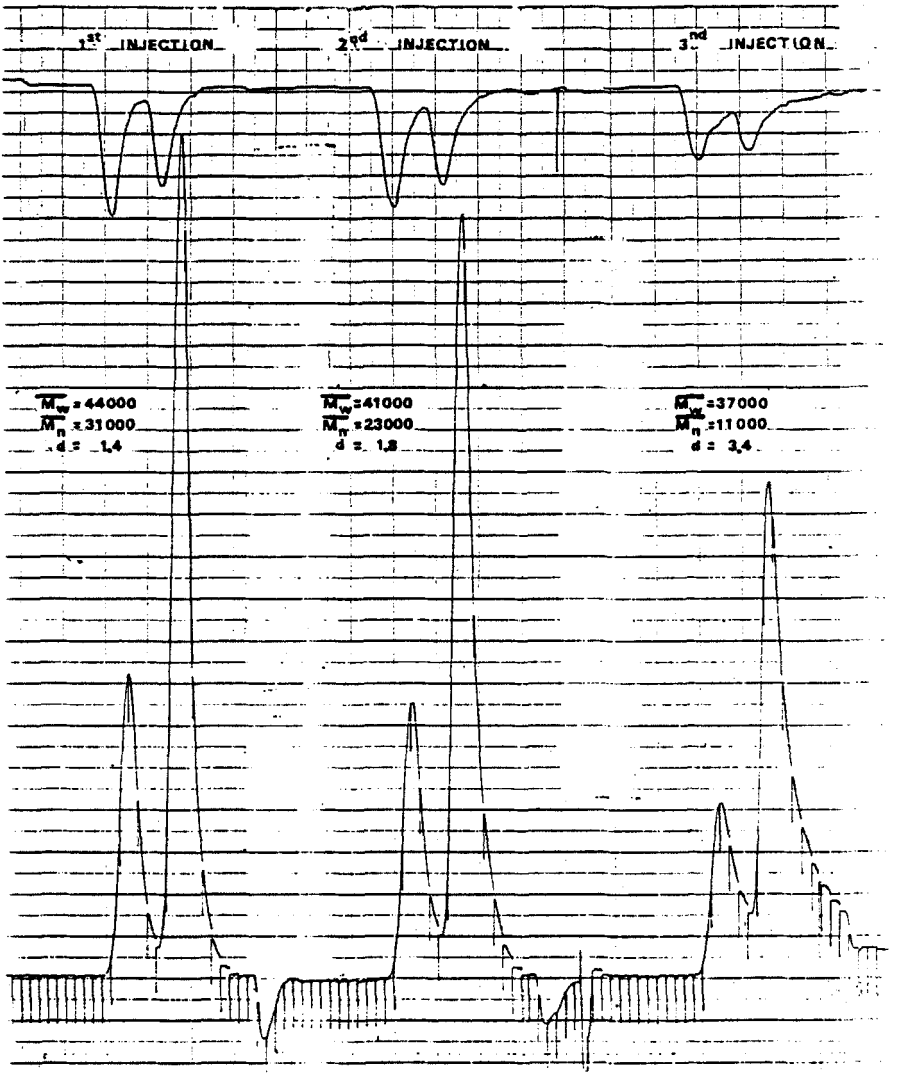


Figure 2

Influence of an injector malfunction on molecular weight values for a mixture of two polystyrene standards in THF.

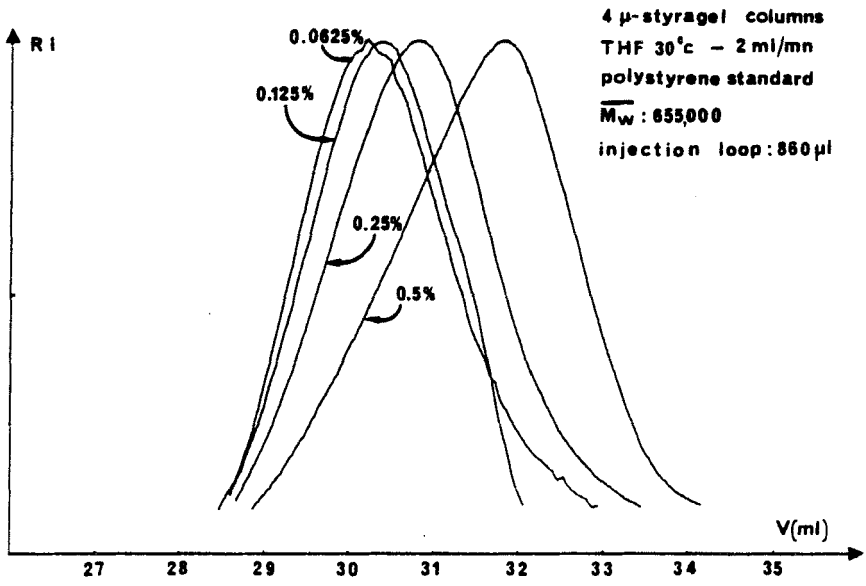


Figure 3

Influence of sample concentration on elution volume and peak shape. Sample : polystyrene standard 655,000. Injection volume 860 μl. Columns : μ-styragel 10^3Å, 10^4Å, 10^5Å and 10^6Å. THF : 2ml/mn.

TABLE 3

C (g/100ml) :	0.0625	0.125	0.25	0.5
$\bar{M}n$:	749,000	663,000	520,000	443,000
$\bar{M}w$:	839,000	742,000	676,000	513,000
$\bar{M}w/\bar{M}n$:	1.12	1.12	1.13	1.16

It is obvious that correct molecular weights are found for the same concentrations at which calibrations were performed, but errors can reach 50 % when concentrations used are different. With modern columns (particle size ≈ 10μ), this effect is negligible below $M \sim 10^4$ but affects measurements in the range of 10^5 and becomes dramatic above 10^6. It is impossible to avoid this problem completely and the only solution is to correct it as we will show later.

Peak broadening

The final typical chromatographic error is peak broadening due to axial dispersion, which occurs mainly in the columns and which is directly related to their efficiency. We will discuss its correction later ; since its decreases when the column efficiency increases, it is not unreasonable to think that this effect may become negligible in the future when more efficient columns are available.

A loop injector also produces peak broadening when the loop volume is too large but this effect can be corrected in the same time as axial dispersion in columns.

- *DATA TREATMENT ERRORS*

Data treatment errors arise when the calculation method does not take into account experimental peak distortions and does not include their corrections. The main corrections are connected with solute concentration, axial dispersion and hydrodynamic volume.

Solute concentration correction

As mentioned above, elution volumes depend upon solute concentration. Some corrections have been proposed to take this effect into account. The use of a theta solvent, suggested by Kato (12), is very difficult to apply practically. Extrapolation to zero requires several injections at different concentrations (13) and a calibration curve extrapolated to zero as well (14). A multiple calibration curve method was originally proposed by

Mori (15) who used a calibration curve set obtained at different concentrations.

We have proposed (2-3) a correction method which consists in giving an analytical form to the observed effect. At a given concentration, the calibration curve can be expressed by a third degree polynomial :

$$Log\ M = A_0 + A_1\ V + A_2\ V^2 + A_3\ V^3$$

where V is the elution volume. By varying the solute concentration, different calibration curves with their proper coefficients A_i can be obtained. But, it is possible to deduce the variation law of each coefficient under the form of a third degree polynomial :

$$A(c) = B_0 + B_1\ c + B_2\ c^2 + B_3\ c^3$$

where c is the solute concentration. We arrive at a general calibration equation :

$$Log\ M = A_o(c) + A_1(c)\ V + A_2(c)\ V^2 + A_3(c)\ V^3$$

This expression corresponds to an infinite number of calibration curves defined by a 16 coefficient matrix. Some of these curves are represented in Figure 4. For data treatment, the right calibration curve is determined for each point via the corresponding concentration given by the refractometer. This method obviously requires a significantly involved calculation, but provides excellent results (2,16).

Axial dispersion correction

Many methods have been proposed to correct axial dispersion whose variation (17 is generally assumed to be gaussian (18), but each of them : Fourier transform (19), polynomial methods (17,20) or minimization method (21), requires important computation treatments and does not provide very reliable results. A simple

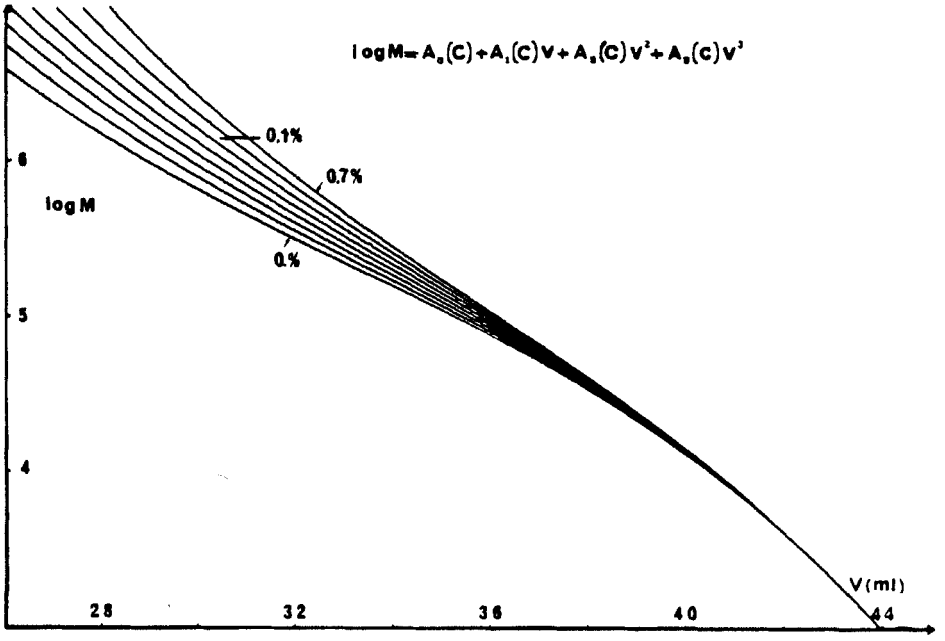


Figure 4

Calibration curves log M = f(V) for a μ-styragel column set (10^3Å, 10^4Å, 10^5Å and 10^6Å) with different solute concentrations. THF : 2ml/mn.

method, based upon a gaussian dispersion function, was recently published by Marais (22) who proposed a simple expression for the different average molecular weights of each fraction :

$$\bar{M}_{\beta i} = M_i \left(\exp \frac{\beta \tau^2}{2}\right) \cdot \left(1 + \tau^2 \frac{C'(\text{Log } M)}{C(\text{Log } M)}\right)$$

($\bar{M}_{\beta i} = \bar{M}_{ni}$ for $\beta = -1$, $\bar{M}_{vi}$ for $\beta = \alpha$, $\bar{M}_{wi}$ for $\beta = 1$) where $\tau = \sigma/a$, with σ = standard deviation of the gaussian dispersion function, a = slope of the calibration curve, C(Log M) = real polydispersity curve and C'(Log M), its derivative. As the ratio C'(Log M)/C(Log M) is unknown, it can be approximately expressed through H'(V)/H(V) =

ratio of the derivative of the experimental chromatogram to the chromatogram itself. Polymer average molecular weights are then obtained by summation of the different averages $\bar{M}_{\beta i}$. *Parameter* τ *has just ot be determined throughout the chromatogram by Waters' recycle method (23) (see Figure 5), which leads also to a precise determination of the standard polydispersity (24). In the case of high efficiency columns, wa have noticed that the first point (see Figure 5) is not located as the others on the same straight line (2-3), since solute does not pass through the pump in the first cycle. This distinction allowed us to determine both the standard deviations of the broadenings in columns and in the pump. In Figure 6, variations of* σ *and* τ^2 *coefficients are plotted as functions of elution volume for a* μ*-Styragel column set.*

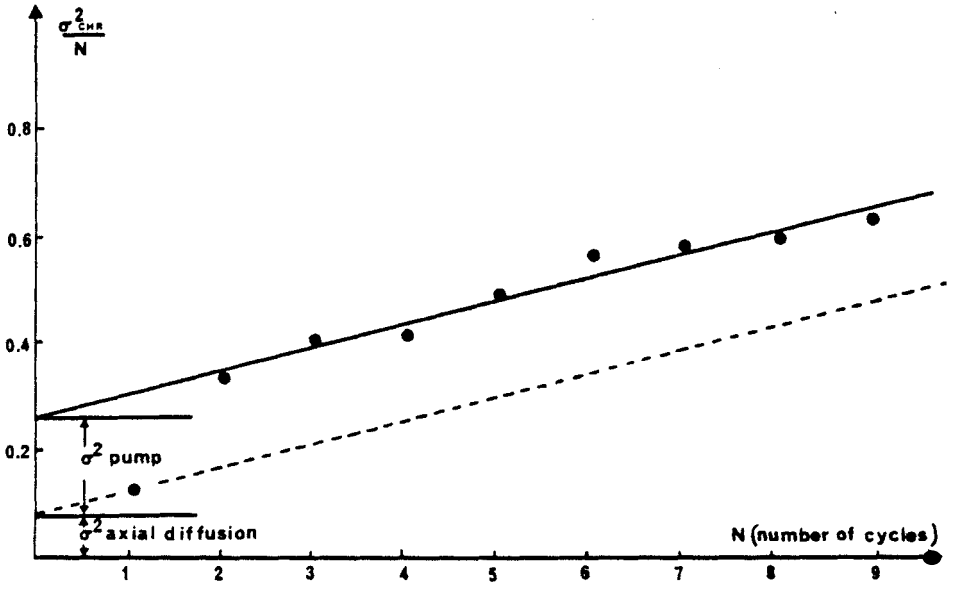


Figure 5

Recycling method of column axial dispersion calibration and absolute polydispersity determination.

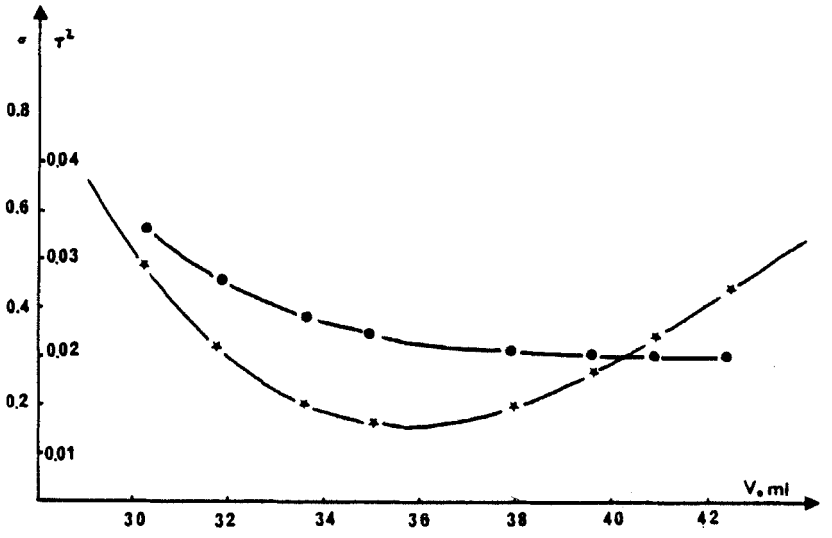


Figure 6

Variations of axial dispersion parameters as a function of elution volume : σ : ● ; $\tau^2 = \frac{\sigma^2}{2}$: ★
μ-styragel 10^3Å, 10^4Å, 10^5Å and 10^6Å. THF : 2ml/mn.

Hydrodynamic volume correction

A current method to apply the Benoit hydrodynamic volume concept is to use the "universal" calibration curve by means of the [η].M product (25). This calibration was widely accepted and used but we have noticed that it introduces, in spite of its great utility a systematic error, as shown in Table 4, where we compare the polydispersity values of a polystyrene standard (M = 110,000) obtained with and without axial dispersion correction and calculated either via a classical polystyrene calibration or via a universal calibration.

A systematic difference appears between values obtained with classical and universal calibrations. This discrepancy can be explained from the axial dispersion effect on the dual refractometer-viscometer detection (2-3). We have represented, in Figure 7, the

TABLE 4

Axial dispersion Correction	*Polystyrene Calibration*	*Universal Calibration*
NO	$d = 1.07$	$d = 1.20$
YES	$d = 1.03$	$d = 1.07$

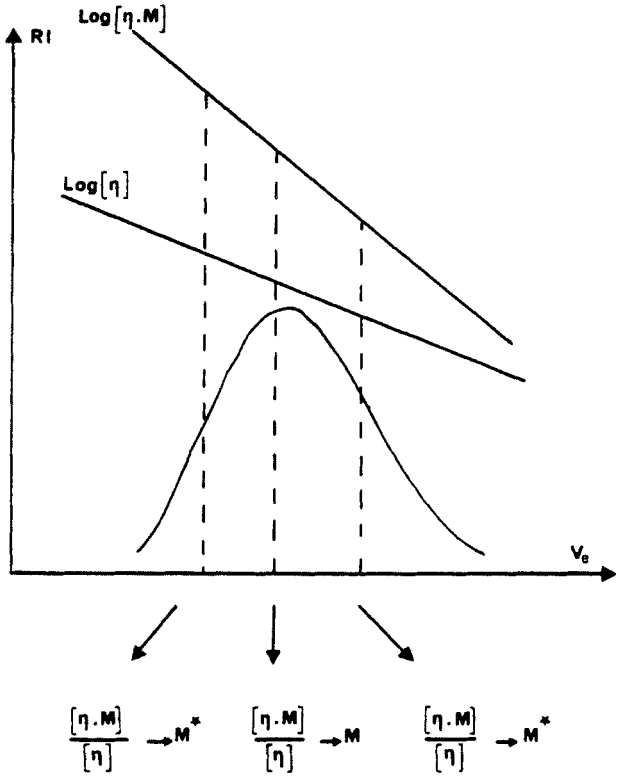


Figure 7

Calculation of molecular weight through universal calibration curve.

universal calibration curve $Log[\eta].M = f(V_e)$ *and a polymer/solvent viscosity law from the viscometer* $Log[\eta] = g(V_e)$. *At each point, M value is obtained by dividing* $[\eta].M$ *by the corresponding* $[\eta]$. *The calculation is most valid at the peak apex but introduces an error at the peak extremities where* $[\eta].M$ *values given by the universal calibration curve are wrong on account of axial dispersion ; viscometry provides correct values of* $[\eta]$.*The calculated M is therefore overestimated on the high molecular weight side and underestimated on the low molecular weight side and, consequently, the sample polydispersity is overestimated, as shown in Table 4. The same holds true when an axial dispersion correction is applied to molecular weights (2-3).*

If $[\eta]'_i.M'_i$ *is the experimental hydrodynamic volume and* $[\eta]_i.M_i$ *the correct value :*

$$[\eta]'_i.M'_i = [\eta]_i.M_i + d([\eta]_i.M_i)$$

the calculated value of molecular weight M''_i *is therefore :*

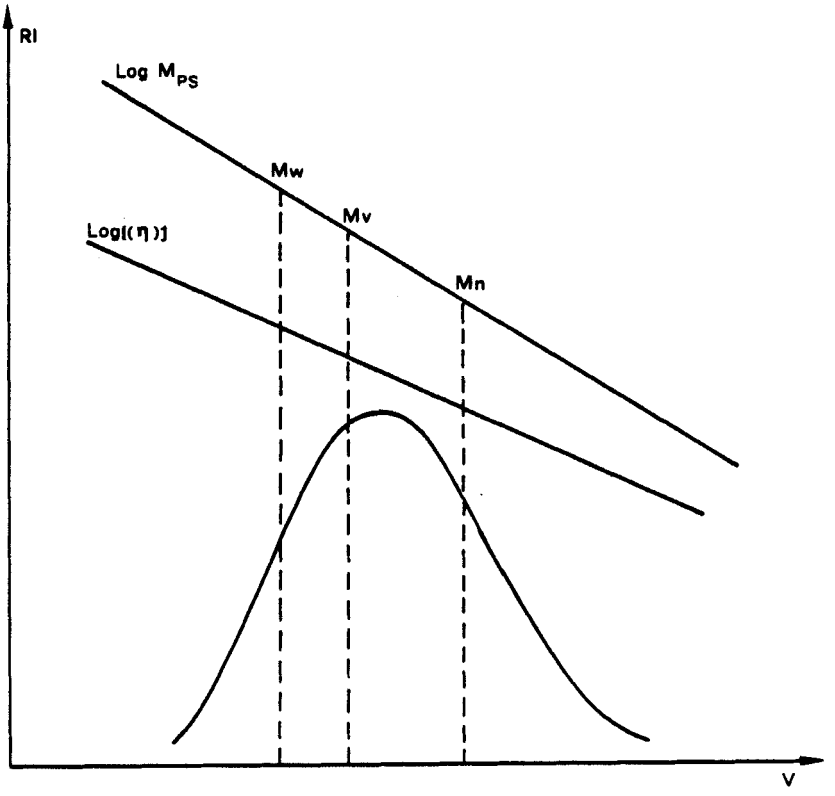
$$M''_i = \frac{[\eta]'_i.M'_i}{[\eta]_i} = M_i + dM_i + M_i \frac{d[\eta]_i}{[\eta]_i}$$

and using the Mark-Houwink relationship : $[\eta] = KM^{\alpha}$:

$$M''_i = M_i + (1 + \alpha)\, dM_i$$

As the axial dispersion correction dM_i *is actually applied to molecular weights (but not to hydrodynamic volumes), this correction is complete with a polystyrene calibration* ($M''_i = M_i + dM_i$), *but only partial with the universal calibration, for the* αdM_i *term is not taken into account.*

To avoid this drawback in the use of the hydrodynamic volume concept, we have proposed adoption of the following method. In a first step, average molecular weights are calculated with a polystyrene calibration including axial dispersion correction and are accordingly expressed in polystyrene units ($\bar{M}_{nps}$, $\bar{M}_{vps}$, $\bar{M}_{wps}$). *In a second step, hydrodynamic volume correction is performed by writing the equalities of the sample and polystyrene hydrodynamic volumes at* $\bar{M}_n$, $\bar{M}_v$ *and* $\bar{M}_w$ *values, as shown in Figure 8 :*



$$\overline{Mn} = \frac{\overline{Mn}_{PS} \times \eta_{PS(\overline{Mn})}}{\eta(\overline{Mn})}$$

$$\overline{Mv} = \frac{\overline{Mv}_{PS} \times \eta_{PS(\overline{Mv})}}{\eta(\overline{Mv})}$$

$$\overline{Mw} = \frac{\overline{Mw}_{PS} \times \eta_{PS(\overline{Mw})}}{\eta(\overline{Mw})}$$

Figure 8

Principle of average molecular weight calculation through polystyrene calibration and sample viscosity law.

$$\bar{M}_n \cdot [\eta]_{\bar{M}_n} = \bar{M}_{nps} \cdot [\eta_{ps}]_{\bar{M}_n}$$

$$\bar{M}_v \cdot [\eta]_{\bar{M}_v} = \bar{M}_{vps} \cdot [\eta_{ps}]_{\bar{M}_v}$$

$$\bar{M}_w \cdot [\eta]_{\bar{M}_w} = \bar{M}_{wps} \cdot [\eta_{ps}]_{\bar{M}_w}$$

This sample method only requires a knowledge of the viscosity law for polystyrene in THF and the measurement of the sample viscosity law that respectively allows the determination of $[\eta_{ps}]_{\bar{M}_n}$ *and* $[\eta]_{\bar{M}_n}$ *values at the elution volume corresponding to* $\bar{M}_n$ *and the same for the other molecular weights. Excellent results have been obtained with this method (2) and will be shortly published in this Journal (16).*

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