Data handling in g.p.c. for routine operations*

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The main points of importance in g.p.c. for routine operation are discussed. Concerning reproductibility, the major problem is the elution volume control, several methods are compared; the influence of number and precision of detector signal measurements are presented. For calibration, working with THF as the eluant, the use of narrow fraction polystyrene standards is a necessary primary step. It furnishes, by classical summations, number and weight average molecular weights in equivalent polystyrenes; these values are already useful in sample characterization. In estimating true molecular weights, the simplest method is use of the Benoit factor and some practical applications are given. A simple method for taking axial dispersion into account is proposed where the spreading function is not Gaussian.

Keywords Chromatographic analysis; gel permeation chromatography; calibration; characterization; polystyrene; Benoit factor; axial dispersion

INTRODUCTION

Most g.p.c. users work with a single detector apparatus, generally a differential refractometer. From the chromatogram, they wish to obtain number and weight average molecular weights of the analysed samples. With the increased use of minicomputers, calculations are simple and results are always printed. One question remains: 'Are these results reliable?' In our laboratory we are very interested in this problem: each year hundreds of g.p.c. analysis on polymer samples of various chemical compositions are made, and so, for us, g.p.c. is an analytical tool before being a fundamental research subject and we need to know the reliability of our routine results.

On this problem, three questions arise:

(1) What are the conditions for having an effective apparatus? In other words, a discussion on measurement reproducibility.

(2) How to determine the number and weight average molecular weights? In other words, what is the measurement accuracy?

(3) May the axial dispersion problem be treated simply?

On the first two points, we admit that the specific resolution of the apparatus is probably good enough to make axial dispersion negligible. With this assumption, average molecular weights (*Figure 1*) are obtained from the classical summations, so we need to measure detector signals at different time intervals, to fix for each measurement the elution volume value and to use a calibration curve.

CHROMATOGRAM REPRODUCIBILITY

The reproducibility for the same sample injected at the same concentration on the same apparatus depends on the measurement of detector signal and elution volume control.

Elution volume control

Elution volume control is a crucial point. For a typical apparatus where linear calibration is assumed from 1 000 up to 2.10^6 g.mol^{-1}, 0.2% error in the elution volume involves between 2% and 4% error in molecular weight. Several methods can be used to control this parameter.

The first precaution involves use of a high performance pumping system, generally a reciprocating pump with two or three heads which permit pulsating free flow. Suppliers claim long term accuracy of 0.2%, 0.3% if the pumping system is perfectly cleaned and eluant free from dust. Our experience permits us to say that these specifications are possible but, from time to time, there is inevitably failure in the pump performance, so an alarm system must be used. This consists of recording of the head pump pressure or the use of an internal standard of known elution volume. Any anomaly in the pressure recording and any increase in elution time means that there is lack of elution volume control.

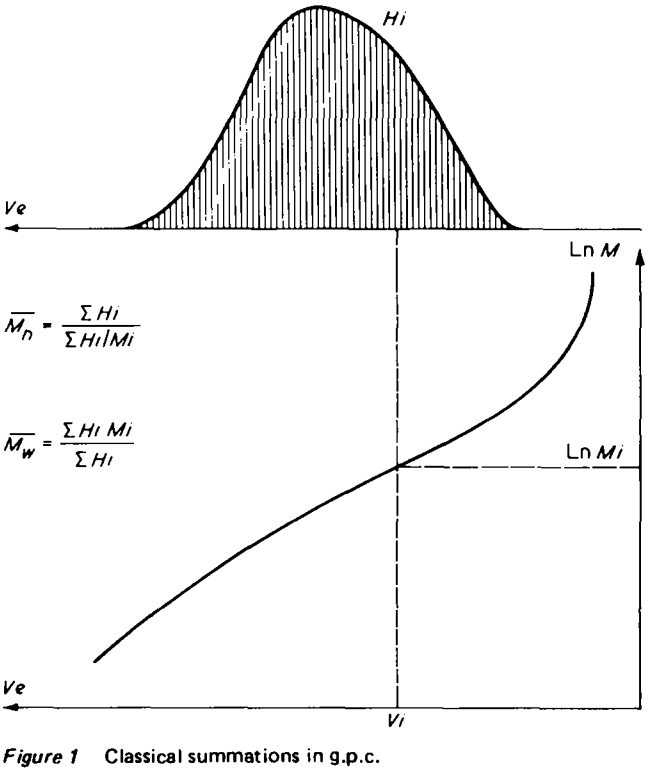

Figure 1 Classical summations in g.p.c.

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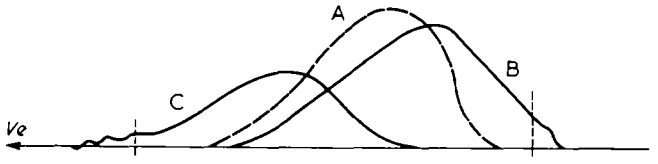

Figure 2 Typical chromatogram positions

Another method can be used, even with low cost pumps, which involves generating an elution volume scale independant of flow. The most popular system is the syphon counter. Syphon volumes as low as 1 cm^3 can be used which give 0.1% accuracy in elution volume if there is no syphon displacement and no large variation in the surface tension of the eluant. One can also use a gravimetric device which generates one peak for each fixed increment of eluted liquid. Such systems are very useful with aqueous eluants for which syphon counter is not convenient[1,2].

It should be noted that automated data handling does not always have sufficient protection against errors in elution volume control. Therefore each user needs to have a vigilant attitude towards this subject.

Detector signal measurement

Here we can look at the measurements of the detector signal. The number of data points is generally sufficient. For an unimodal chromatogram, a minimum of twenty points are required but a larger number of data points is useful since this is equivalent to reducing detector noise. In calculations of average molecular weights, terms Hi/Mi or $HiMi$ are important even for small relative values of Hi at the extremities of the chromatogram. Therefore we need a good signal/noise factor and an excellent baseline stability.

When working with a minimum number of data points, detector noise has to be less than 0.5% of the chromatogram maximum introducing less than 2% error in $\bar{M}_n$ and $\bar{M}_w$. Base line stability is more important because any uncontrolled drift introduces systematic errors. Only a very small linear drift (less than 1% full scale during a complete analysis) can be tolerated.

With the baseline, we have to note one major limitation in quantitative g.p.c. There will be baseline return behind calibration limits. These limits are not the classical total volume and interstitial volume but arbitrary chosen volumes defining the interval outside which there is no accurate calibration (*Figure 2*). So chromatogram A is convenient but chromatograms B and C are not, for having too high or too low molecular weight fractions. In these two latter cases, we can still make summations from the lower limit up to the upper limit. For sample B, $\bar{M}_n$ is quite exact and $\bar{M}_w$ is large in error, likewise for sample C, $\bar{M}_w$ is quite exact.

MOLECULAR WEIGHT MEASUREMENT ACCURACY

In this second section, we will discuss the analysis with THF eluant which is usually employed for synthetic polymers. The first task is to chromatograph narrow fraction polystyrene standard samples. These chromatograms furnish much information. Firstly, we can establish the polystyrene calibration curve, i.e. $\text{Ln}(M_s) = f(V_s)$ where M_s is the nominal molecular weight (or $[\bar{M}_w \cdot \bar{M}_n]^{1/2}$ and V_s is the elution volume at peak maximum. From this curve, we determine the practical upper and lower elution volume limits: a linear calibration inside these limits is a good simplification for subsequent calculations. By varying the concentration of standards, we can verify the stability of the elution volume. For molecular weights above 200 000 there is no major problem, on the condition that small concentrations are used. Up to this limit, generally there is a dependance of elution volume with concentration. Several authors have proposed methods taking this dependance into account[3,4]; the simplest is to make several injections with various concentrations and to extrapolate results at nil concentration.

Equivalent polystyrene molecular weight

When a calibration curve is established, we can obtain for any linear polystyrene sample, number and weight average molecular weights $\bar{M}_n$ and $\bar{M}_w$. The accuracy of these results is limited by uncertainties concerning the standards' specifications and, after constructing a calibration curve with a tenth of standards, there remains typically an uncertainty of $\pm 10\%$.

When using the polystyrene calibration curve for another type of polymer sample, we obtain $\bar{M}_{nPS}$ and $\bar{M}_{wPS}$, number and weight average molecular weight of the sample in equivalent polystyrene, i.e. the $\bar{M}_n$ and $\bar{M}_w$ values of a polystyrene sample which have the same chromatogram. These results are useful for comparative tests or for qualitative discussion but most users want to know the true $\bar{M}_n$ and $\bar{M}_w$ of the sample.

Determination of true molecular weights

To solve this problem with a single detector apparatus, there are essentially two approaches; either using polymolecular standards with linear calibration approximation; or by using the concept of universal calibration in a simplified manner.

Linear calibration approximation. In a recent book[5] on practical aspects of g.p.c., great importance is given to this method. Let us note that some conditions must be fulfilled:

(i) the sample and the standard have to belong to the same homologous family (it is not advisable to apply this method to complex polymers as copolymers or branched ones);

(ii) the standard must be entirely chromatographed inside the linear calibration range of the apparatus.

Several calculation methods[6,7] have been proposed to determine the calibration parameters p and b in the expression:

$$\text{Ln } M = -pv + b$$

where M is the molecular weight of the species eluted at volume v. The starting point is always the same: only p has influence on $\bar{M}_w/\bar{M}_n$ calculated ratio. So, we can try values of p until the true $\bar{M}_w/\bar{M}_n$ value is obtained. Then, the exact value of b is immediately determined. To choose a good value of p for the first iteration, we can accept that for a log-normal molecular weight distribution:

$$\text{Ln } [\bar{M}_w/\bar{M}_n] = \sigma_c^2 \cdot p^2$$

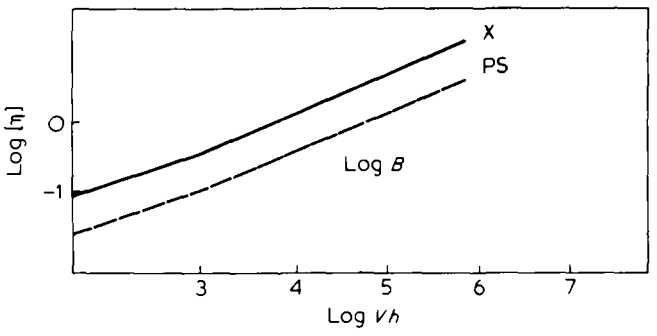

Figure 3 Conditions for having $M_X(v) = B.M_{PS}(v)$: Curves log [η] = $f(\log(Vh))$

so:

$$p = 4.\frac{\sqrt{\overline{\mathrm{Ln}[\bar{M}_w/\bar{M}_n]}}}{W} \quad \text{where } W = 4.\sigma_c \text{ is the peak width.}$$

This method is useful when working on one given family of polymers of similar molecular weight distribution but we consider it is rather limited for general use.

Simplified universal calibration use. To the contrary, the universal calibration concept[9] is now well established, especially when THF is used as eluant. So we can say that any macromolecule eluted at a given elution volume has the same 'hydrodynamic volume':

$$Vh(v) = [\eta]_x(v).Mx(v)$$

where $[\eta]_x(v)$ and $M_x(v)$ are the intrinsic viscosity and the molecular weight of the X species eluted at volume v.

A simple use of this concept is possible when the relationship below is verified[10,11].

$$\frac{[\eta]_{PS}(v)}{[\eta]_x(v)} = B$$

where the 'Benoit factor' B is a constant for the family X in a given eluant. In this case, for any point i of the chromatogram:

$$Mi_x = B.Mi_{PS} \quad \text{so } \bar{M}_{nx} = B.M_{nPS};\ \bar{M}_{wx} = B.M_{wPS}$$

We simply obtain true molecular weights $\bar{M}_{nx}$ and $\bar{M}_{wx}$ by multiplying equivalent polystyrene molecular weights $\bar{M}_{nPS}$ and $\bar{M}_{PS}$ by the Benoit factor B.

Another method, a little more complex introduces two parameters K and a, coefficient and exponent of the Mark Houwink viscosity law[12-14]. In effect, when knowing K and a, we can construct the calibration curve for any given X polymer: For each elution volume v, we have:

$$\left.\begin{aligned}[\eta]_X(v) &= KM_X^a(v)\\ [\eta]_X(v).M_X(v) &= Vh(v)\end{aligned}\right\} M_X(v) = \left[\frac{Vh(v)}{K}\right]^{1/a+1}$$

These two methods have been proposed under various forms by several authors. Here we want to specify the requirements needed to apply such methods.

The two-parameters method. Assuming a unique viscosity law, suppose that we analyse a simple type polymer of no variable branching degree and no compositional drift in copolymers.

A unique viscosity law also needs to be applied to a limited molecular weight range. It should be especially noted that generally there is[15] a break in the curve log $[\eta] = f(\log(M))$ from approximately $M = 10\,000$ g.mol^{-1} (*Figure 4*) so the two-parameters method is only suitable for sufficiently high molecular weight samples. It also needs a catalogue of viscosity laws in THF which is not still established. Progress must be made in the near future with the help of multidetection g.p.c. which, in principle, should be able to determine rapidly the viscosity law of any sample.

The one-parameter method. Figures 3 and *4* show precisely the requirements on which this method is based. The curve log $[\eta] = f(\log(Vh))$ for polymer X must be obtained from the same polystyrene curve by translation along the vertical axis.

When looking at the more familiar curve $\log[\eta] = f(\log(M))$ the polystyrene curve has a well established shape in THF: For low molecular weights the slope is 0.5 and near from 10 000 g.mol^{-1} the slope increases to 0.7.

For any polymer X, we ought to observe the same slopes but the two straight regions have to be translated along the vertical axis by quantities 1.5 log B and 1.7 log B.

Literature data does not give due consideration to show if these assumptions are verified exactly. Theoretical considerations help us no more, but results obtained by this method are quite satisfactory and this one-parameter method appears to be a good approximation for polymers for which THF is a good solvent.

There are various ways of determining practically the Benoit factor B for any family of polymers.

If we have a known standard:

$$B = \frac{\bar{M}_{nX}}{\bar{M}_{nPS}} = \frac{\bar{M}_{wX}}{\bar{M}_{wPS}}$$

(When base line return is not very good, it will be more accurate to use $\bar{M}_w$ ratio).

If we measure the intrinsiv viscosity $[\eta]_x$ of one sample X:

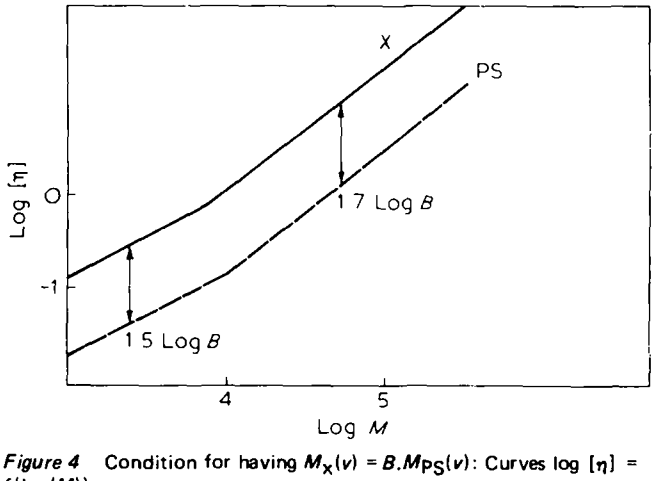

Figure 4 Condition for having $M_X(v) = B.M_{PS}(v)$: Curves log [η] = $f(\log(M))$

THF 25°C	$[\eta]_{PS} = 0.9\ 10^{-3}\ M^{0.5}$	$M < 10\,000$
[η] dl/g	$[\eta]_{PS} = 0.145\ 10^{-3}\ M^{0.7}$	$M > 10\,000$

Table 1 *B* factor values

	B	Method	Ref.
THF 25°C			
Polybutadiene 20% vinyl	0.55	I, II, III	14, 16
Polybutadiene 80% vinyl	0.60	I, III	10, 14, 16
Polyisoprene (1—4 *cis*)	0.67	I, III	16
PMMA	1.10	I, II, III	16
PVC	0.60	I, III	16, 17
Polyvinylacetate	0.96	I	16
Polycarbonate	0.58	I, III	16
Toluene 25°C			
PDMS	0.95	II, III	18
ODCB 138°C			
Polyethylene (linear)	0.47	II, III	19

Method I: In our laboratory, measurement of $[\eta]_X$ on line with g.p.c.
Method II: From standards
Method III: From Mark–Houwink relations

$$B=\frac{[\eta]_{PS}}{[\eta]_x}$$

where $[\eta]_{PS}$ is the intrinsic viscosity of the polystyrene samples which have the same chromatogram. This quantity $[\eta]_{PS}$ is easily computed from the chromatogram, PS calibration curve and PS viscosity law. Measurement of $[\eta]_X$ can be made with an automatic viscosimeter set after the refractometer, without any complicated calculations. So for any sample it will be possible with such a device to determine its proper *B* factor.

In using literature data, we can determine the *B* factor from the Mark-Houwink relations in THF when the exponent has the same value for PS and X family:

$$\left.\begin{aligned}[\eta]_X&=K_X.M_X^a\\ [\eta]_{PS}&=K_{PS}.M_{PS}^a\end{aligned}\right\}\frac{K_{PS}}{K_X}=B^{1-a}$$

Results obtained by these different methods are summarized in *Table 1*. In a few cases, the same method is usable in solvents other than THF. As against the *Q* factor[20] (based on extended chains length ratio), this one-parameter method would not be universal but only an approximation which can be applied to simple polymers for which THF is a good solvent. Inside these limitations this method is an effective improvement of the data interpretation of single detector g.p.c. apparatus. For the referenced families of polymers the uncertainty on the *B* factor lies around 10%.

AXIAL DISPERSION PROBLEM

Important literature has been published in this field, often concerning methods which needed extensive calculations. It was recently reviewed by Hamielec[21]. Here we only look at the simplest solutions available for routine operation. Two conditions are needed: linear calibration and axial dispersion independant from elution volume. When these two conditions are fulfilled, the problem is the same for any chromatogram position on elution volume scale, the problem is also the same for a family polymer X and for polystyrene, assuming we can apply the *B* factor method.

Let us recall some basic notations for characterizing apparatus performance:

$p=-\dfrac{\mathrm{d\,Ln}(M)}{\mathrm{d}v}$: calibration curve slope; σ: standard deviation of an isomolecular species peak; $\tau=\sigma.p$: efficiency parameter[22]; $Rs=\Delta v/4\sigma.\Delta(\log M)$: specific resolution.

If axial dispersion is independent of elution volume and in the case of linear calibration, all these parameters are constant for a given apparatus, eluant and flow stream.

In the *Table 2*, relations between these parameters are summarized. M_2/M_1 is the minimum molecular weight ratio needed for having a resolution better than unity.

When the peak of an isomolecular species is a Gaussian one, classical expression have been established for a long time:

$$\bar{M}_{nt}=\bar{M}_{nu}.\exp(\tau^2/2)\quad \bar{M}_{wt}=\bar{M}_{wu}.\exp(-\tau^2/2)$$

(subscripts t and u are used for true and uncorrected values).

The validity of these correction factors was first demonstrated for a log-normal molecular weight distribution[23] (Gaussian shape for the whole chromatogram) and then extended for any molecular weight distribution[24]. So, when single species give Gaussian peaks, axial dispersion is not a problem. The correction is generally small and moreover easy to make.

However, with the increased speed of analysis, isomolecular species peaks are often non Gaussian and non symmetrical. This problem was first treated with some approximations by Hamielec[19] and recently reviewed[21]. When the axial dispersion is constant but not Gaussian, the corrections still involve two different factors of $\bar{M}_n$ and $\bar{M}_w$:

$$\bar{M}_{nt}=K_n\bar{M}_{nu}\qquad \bar{M}_{wt}=K_w\bar{M}_{wu}$$

For any chromatogram constituted by one isomolecular species j of molecular weight M_j, these relations are obvious. When making summations for a general sample comprising a set of j species (M_j molecular weight and W_j weight fraction) we can establish that the same relations remain exact[25]. Similar results expressed by a less simple form are used in a recent ASTM method on g.p.c.[26].

So K_n and K_w are constants for a given apparatus, eluant and flow, usable for any molecular weight distribution.

The values of these two parameters are determined from the chromatogram of narrow fraction polystyrene standards. To prevent including errors coming from the calibration curve construction or from the elution volume control it is better to take:

Table 2 Efficiency representations

τ	R_s	M_1/M_2
0.046	12.6	1.2
0.10	5.7	1.5
0.17	3.3	2
0.27	2.1	3
0.40	1.4	5

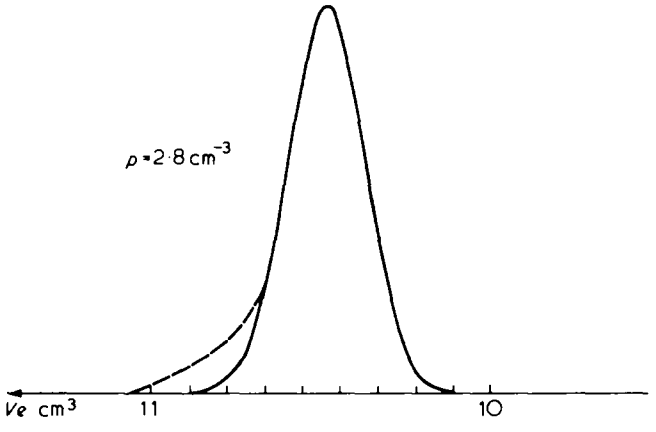

Figure 5 Influence of peak tailing: Gaussian peak $K_n = 1.04$; $K_w = 0.96$; Skewed peak $K_n = 1.20$; $K_w = 1.03$

$$K_n = \frac{M_S}{\bar{M}_{nu}} \qquad K_w = \frac{M_S}{\bar{M}_{wu}}$$

where M_S is the molecular weight at peak maximum (and not the nominal molecular molecular weight of the standard).

This axial dispersion treatment permits us to calculate the influence of a peak tailing. In *Figure 5*, with a Gaussian peak for isomolecular species we have $+4\%$ correction factor for $\bar{M}_n$ and -4% correction factor for $\bar{M}_w$. With the single difference of peak tailing (classical peak width is not modified), correction factors become $+20\%$ on $\bar{M}_n$ and $+3\%$ on $\bar{M}_w$. These corrections are totally different and we can see that strong errors can be made if we do not take into account the exact shape of the isomolecular species peak.

CONCLUSION

In conclusion, we give below, some points which could be of importance when using g.p.c. for routine operations.

(1) The necessity for a precise elution volume control. Failure on this point is the source of the greatest errors in g.p.c.

(2) The importance of a good calibration curve construction with a sufficient number of narrow fraction polystyrene standards.

(3) We believe, when using THF as eluant, that the B factor method is a good way to obtain molecular weights of simple polymers. When coupled with an automatic viscometer, to simply measure intrinsic viscosity of the whole sample, the proper B factor for each chromatographed sample can be computed and results are more certain.

(4) Finally, to improve the quality of results, much care has to be taken in choosing column sets with wide linear calibration franges and by taking into account the exact peak shape of single species, for correcting axial dispersion.

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