Notes to the Editor

Some aspects of data treatment in the gel permeation chromatography of polymers

J. Janca and L. Mrkvicková
Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia
(Received 17 August 1978; revised 30 October 1978)

INTRODUCTION

Gel permeation chromatography (g.p.c.) is a method which makes possible the separation of molecules using differences in their effective sizes. If g.p.c. is performed by means of a detector directly monitoring a quantity proportional to the molecular weight of the polymer to be separated, e.g. an automatic viscometer[1] or a small-angle light-scattering detector[2], the molecular weight distribution (MWD) and the average molecular weights of the polymer can be determined. A more frequently used method involves the calibration of the separation system against a series of standard polymers. The plot thus obtained is frequently linear in its central part using the coordinates $\log M$ vs. V_e, where M is molecular weight and V_e is the elution volume of the given standard. However the calibration plot thus constructed is not generally valid for any polymer. If the product $[\eta] \times M$ proportional to the hydrodynamic volume of the polymer is used instead of M ($[\eta]$ being intrinsic viscosity), such calibration is universal[3]. For the evaluation of average molecular weights and MWD of a polymer different from that used in the calibration, it is sufficient to know the constant K and the exponent a of the Mark–Houwink equation $[\eta] = K \cdot M^a$ for this polymer in the solvent used and at a given temperature. The calculated MWD must also be corrected for longitudinal spreading[4]. Recently, microtechniques have been applied in g.p.c. which helped to accelerate the analyses and to reduce the quantity of the sample needed[5]. Under such conditions, it is difficult, if at all possible, to monitor molecular weights. For this reason among others, calibration methods have been reintroduced. It is important, at the same time, to make the treatment of data obtained in the measurements as simple as possible, utilizing on-line minicomputers, and also to make it at least as fast as the g.p.c. separation itself. In many cases, especially in fast g.p.c. as a check method of technology, polydispersity of the analysed polymer $I = M_w/M_n$ (M_w is the weight average of molecular weights and M_n is the number average of molecular weights) is the relevant result of the g.p.c. analysis. The reliability of I values calculated from g.p.c. data depends above all on correct absolute values of molecular weights of the calibration standards[6], on the method used in the construction of the calibration plot, on accuracy of the Mark–Houwink equation employed and on an adequate correction of longitudinal spreading.

RESULTS AND DISCUSSION

In most cases we are justified in using linear calibration in the central part of calibration plots, if the column system chosen by us is one in which the calibration plots of the individual columns are joined together smoothly. Deviations from the straight line, if any, should rather be attributed to incorrect molecular weights of polystyrene (PS) standards. The linear calibration plot possesses obvious advantages ensuing from the simpler treatment of g.p.c. data. The possibilities of a systematic preparation of separation system with linear calibration is studied by Belenkii[7].

Let us consider the way in which the effect of accuracy of the Mark–Houwink equation is reflected in the resulting value of I calculated from g.p.c. data, if the principle of universal calibration is employed. M_w and M_n are calculated from g.p.c. data using the expressions:

$$M_w = \frac{\Sigma M_i h_i}{\Sigma h_i}, \quad M_n = \frac{\Sigma h_i}{\Sigma \frac{h_i}{M_i}} \tag{1}$$

where h_i are heights of the chromatogram from the base-line in the i-th elution volume V_i, M_i are the respective molecular weights. From the universal calibration plot we read at V_i the value J_i proportional to the hydrodynamic volume:

$$J_i = [\eta]_i \times M_i \tag{2}$$

The respective M_i can be calculated, if K and a of the Mark–Houwink equation for the analysed polymer are known from the relationships:

$$[\eta]_i = K \cdot M_i^a \tag{3}$$

$$M_i = \left(\frac{J_i}{K}\right)^{\frac{1}{a+1}} \tag{4}$$

Table 1 Effect of the exponent *a* of the Mark–Houwink equation on polydispersity *I* calculated from GPC data for various absolute values of *I*

a	$I = M_w/_n$				
0.50	1.032	1.137	1.688	3.158	23.819
0.55	1.029	1.127	1.615	2.936	19.953
0.60	1.028	1.120	1.568	2.748	16.300
0.65	1.026	1.112	1.527	2.588	13.823
0.70	1.025	1.105	1.490	2.449	11.890
0.75	1.023	1.099	1.457	2.329	10.355
0.80	1.022	1.093	1.427	2.224	9.120
0.85	1.021	1.088	1.400	2.131	8.113
0.90	1.020	1.083	1.376	2.049	7.283
0.95	1.019	1.079	1.354	1.976	6.591
1.00	1.018	1.075	1.334	1.911	6.008

0032–3861/79/030388–02$02.00
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For the polydispersity index I it may hold, then:

$$I = \frac{\Sigma J_i^{\frac{1}{a+1}} \cdot h_i \Sigma J_i^{-\frac{1}{a+1}} \cdot h_i}{(\Sigma h_i)^2} \tag{5}$$

equation 5 shows that the accuracy of I may be affected by the accuracy of the exponent a or by its variation with molecular weight.

To verify the effect of a upon I, we chose a model system, the linear calibration plot of which was determined by two points, namely, $M_1 = 10^6$, $V_1 = 90$ counts and $M_2 = 10^3$, $V_2 = 180$ counts. The universal calibration plot was also determined by means of a hypothetical Mark–Houwink equation, $[\eta] = 1 \times 10^{-4} M^{0.75}$. Hence the equation corresponding to the calibration straight line is:

$$\log J = 11.75 - 583\bar{3} \times 10^{-2} V_e \tag{6}$$

We also generated artificial chromatograms in the form of the Gaussian function (conclusions given below hold of course for any form of the chromatogram):

$$F(V) = \frac{1}{\sigma(2\pi)^{1/2}} \cdot exp\left(-\frac{1}{2}\frac{(V - V_{max})^2}{\sigma^2}\right) \tag{7}$$

where V_{max} is the elution volume of the maximum of the chromatogram. The standard deviation σ was varied so as to determine also the effect of width distribution, or in other words of the absolute value of I. Results of the calculations are summarized in *Table 1*. They show that for values up to $I \approx 1.1$ the choice of the exponent a is virtually without importance, because the change in I with a varying from 0.5 to unity lies within the limits of usual accuracy of g.p.c. (±5% relative). For the interval $1.4 < I < 2.5$, one can see that the error in the determination of the exponent $a \pm 0.05$ changes the resulting I again approximately within the limits of experimental error of g.p.c. Only at extreme values $6 < I < 23$ the error in the determination of I with the error $a \pm 0.05$ distinctly exceeds the limits of experimental error. In the case of linear calibration the values of I in *Table 1* do not depend on molecular weight, which may be proved by calculation after substitution of equation 6 into equation 5.

The correction of the polydispersity of I may be formulated e.g. by[8]:

$$I = e^{2D_2^2/h} \cdot I' \tag{8}$$

where I is the corrected value, I' is the uncorrected value, h is the correction factor and D_2 is one of the constants of the calibration function

$$M = D_1 e^{D_2 V_e}$$

defined as an inversion function to the linear function given above. For reasons of principle, the uncorrected value of I' calculated from g.p.c. data must be higher than the correct one. However, owing to the available accuracy of determination of the correct I by employing absolute methods, it seems useful to introduce correction only if the magnitude of this correction expressed as $(I - I')/I$ exceeds the experimental error of determination of I by absolute methods.

Thus, for instance, in the optimal case the error committed by us in the determination of M_w[9] in the range $M_w = 2.10^4$–2.10^6 is ±5%. M_n determined by membrane osmometry in the range $M_n = 2.14^4 - 3.10^5$ may in extreme regions be subjected to an error of ±10%. The minimum error of M_n values lies in the centre of this range, amounting to ±5%. M_n values up to 20 000 can be measured by vapour phase osmometry. Because of the concentration gradient in a drop of solution[10], the error of determination in the majority of solvents is approximately ±5% even at $M_n \approx 5.10^3$, while at the upper limit it amounts up to ±10%. Hence, I obtained from measurements employing absolute methods can in the best case be measured with an accuracy of ±10%.

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Acridine orange as a fluorescent probe for the study of polyelectrolyte complexes

R. B. Cundall, J. B. Lawton, D. Murray and D. P. Rowlands
Department of Chemistry and Applied Chemistry, University of Salford, Salford M4 4WT, UK
and G. O. Phillips
North East Wales Institute of Higher Education, Kelsterton College, Connah's Quay, Deeside, Clwyd, Wales, UK

(Received 2 August 1978; revised 6 November 1978)

INTRODUCTION

The acidic sites of several polyanions can be titrated spectrofluorimetrically with metachromatic dyes such as acridine orange[1]. A stoichiometric complex containing one dye cation to one acidic site is observed in most cases. The dye can readily be displaced from such a complex by a more strongly binding

0032–3861/79/030389–04$02.00
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