Calibration in gel permeation chromatography: Primary, universal and empirical methods

D. K. Gilding*, A. M. Reed* and I. N. Askill
Bioengineering Unit, University of Liverpool, PO Box 147, *Liverpool L*69 3*BX, UK*

(*Received* 16 *February* 1980; *revised* 29 *May* 1980)

This paper considers the need for g.p.c. to be used with many different solvent systems including dimethylacetamide/lithium bromide and HFIP in order to obtain estimates of absolute *MW* averages for industrial and medical polymers and copolymers. Several methods of calibration are discussed. The accuracy of the *MW* averages obtained by these methods compared with results obtained by independent methods is also given.

INTRODUCTION

Gel permeation chromatography (g.p.c.)[1], revolutionized polymer chemistry in that it allowed the complete molecular characterization, i.e. average molecular weight (*MW*), total range of *MW*s, and molecular weight distribution (*MWD*) to be obtained within 2–3 h. Subtle differences in *MWD*, which may endow a particular commercial grade of polymer with superior (or inferior) properties in a competitive market could be determined by the polymer scientist, whereas prior to g.p.c. they would have remained undetected.

The major limitation of g.p.c. has been that it is a secondary method of *MW* determination and requires calibration against a primary method such as osmometry or light scattering before it is possible to obtain absolute *MW* values. Once a particular set of g.p.c. columns has been calibrated for a particular polymer, under specified conditions of solvent, temperature and flow, then full molecular characterization of unknown samples of that polymer becomes a matter of routine. For many commercial applications, absolute values of *MW* averages are unnecessary and relative values are often sufficient.

The aim of this paper is to consider the various calibration methods used in g.p.c. and to examine some of their limits of extension, and the reproducibility of the data that these methods can potentially yield. Whereas g.p.c. is often used under constant conditions of solvent and temperature it is hoped to demonstrate that it is a relatively simple matter to design an instrument that allows solvent change-over within 4 h.

PRIMARY CALIBRATION

The primary calibration of a set of g.p.c. columns requires a series of narrow *MWD* standards to be available over a range of *MW*s. Such a series of anionically polymerized polystyrenes is available[2,3] over the range $600 < MW < 2\,000\,000$. These standards are injected sequentially onto the particular set of columns that require calibration, and the volume of solvent required to elute the sample to the peak of its *MWD* measures ($\bar{M}_w/\bar{M}_v \sim 1.1$) as quoted[2,3]. This volume of solvent, 'the elution volume', V_e, used to produce the primary calibration, is plotted as a function of log *MW* as shown in *Figure* 1 for polystyrene in 1:2:4 trichlorobenzene, (TCB), at 135°C at 1 ml min^{-1} flow through 10^7, 10^6, 10^5 and 10^3 Å Styragel^R columns[2].

The calibration is linear between 2×10^6 and 5×10^3 and the limit of resolution for this particular set of columns is 2×10^3. A typical broad *MWD* polymer can be described on the basis of total *MW* range covered, and by a series of average *MW*s defined below:

$$\bar{M}_{n-1} = \frac{\Sigma N_i}{\Sigma N_i M_i} = \frac{\Sigma h_i/M_i}{\Sigma h_i/M_i^2} \qquad (1)$$

$$\bar{M}_n = \frac{\Sigma N_i M_i}{\Sigma N_i} = \frac{\Sigma h_i}{\Sigma h_i/M_i} \qquad (2)$$

$$\bar{M}_v = \left[\frac{\Sigma N_i M_i^{1+a}}{\Sigma N_i M_i}\right]^{1/a} = \left[\frac{\Sigma h_i M_i^a}{\Sigma h_i}\right]^{1/a} \qquad (3)$$

$$\bar{M}_w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i} = \frac{\Sigma h_i M_i}{\Sigma h_i} \qquad (4)$$

$$\bar{M}_z = \frac{\Sigma N_i M_i^3}{\Sigma N_i M_i^2} = \frac{\Sigma h_i M_i^2}{\Sigma h_i M_i} \qquad (5)$$

$$\bar{M}_{z+1} = \frac{\Sigma N_i M_i^4}{\Sigma N_i M_i^3} = \frac{\Sigma h_i M_i^3}{\Sigma h_i M_i^2} \qquad (6)$$

$$[\eta] = K\bar{M}_v^a = K\frac{\Sigma h_i M_i^a}{\Sigma h_i} \qquad (7)$$

In equations 1–7 N_i is the number of polymer molecules of $MW = M_i$; h_i is the height of the chromatogram at M_i where $h_i \propto N_i M_i$, i.e. the weight fraction of species M_i. $\bar{M}_n$, $\bar{M}_v$, $\bar{M}_w$ and $\bar{M}_z$ are average *MW*s obtained from osmometry, solution viscosity, light scattering and sedimentation measurement respectively; whereas $\bar{M}_{n-1}$ and $\bar{M}_{z+1}$ are purely mathematical averages that are sensitive to low and high *MW* tails of the distribution respectively, but are particularly important in degradable[4] and impact resistant polymer[5] systems. $[\eta]$ is the intrinsic viscosity of the polymer in a particular solvent (not necessarily that

---

* Present address: Millipore Corporation, Ashby Road, Bedford, Mass 01730, USA.

0032-3861/81/040505-07$02.00

used in the g.p.c.) and '*K*' and '*a*' are the Mark Houwink parameters relating $[\eta]$ to the viscosity average molecular weight $\bar{M}_v$. *Table* 1 shows the reproducibility of a commercial polystyrene characterized over a period of two weeks in TCB/135°C.

The data shows an overall standard deviation of $\pm 8\%$ which is comparable with the reproducibility of both osmometry and light scattering measurements. Number average molecular weight, $\bar{M}_n$, is usually lower than the absolute value when measured by g.p.c., due to band spreading on the columns causing an apparent increase in the low *MW* tail of the *MWD*. On the other hand $\bar{M}_n$ by osmometry tends to be higher than the absolute value due to permeability of the membrane to low *MW* species. This is obviously more important for lower *MW* samples than higher ones. However, the correlation between all the *MW* averages obtained by g.p.c. and independent methods (*Table* 1) is well within the accuracy of the methods, which is an indication of how useful the much simpler g.p.c. technique can be in real situations.

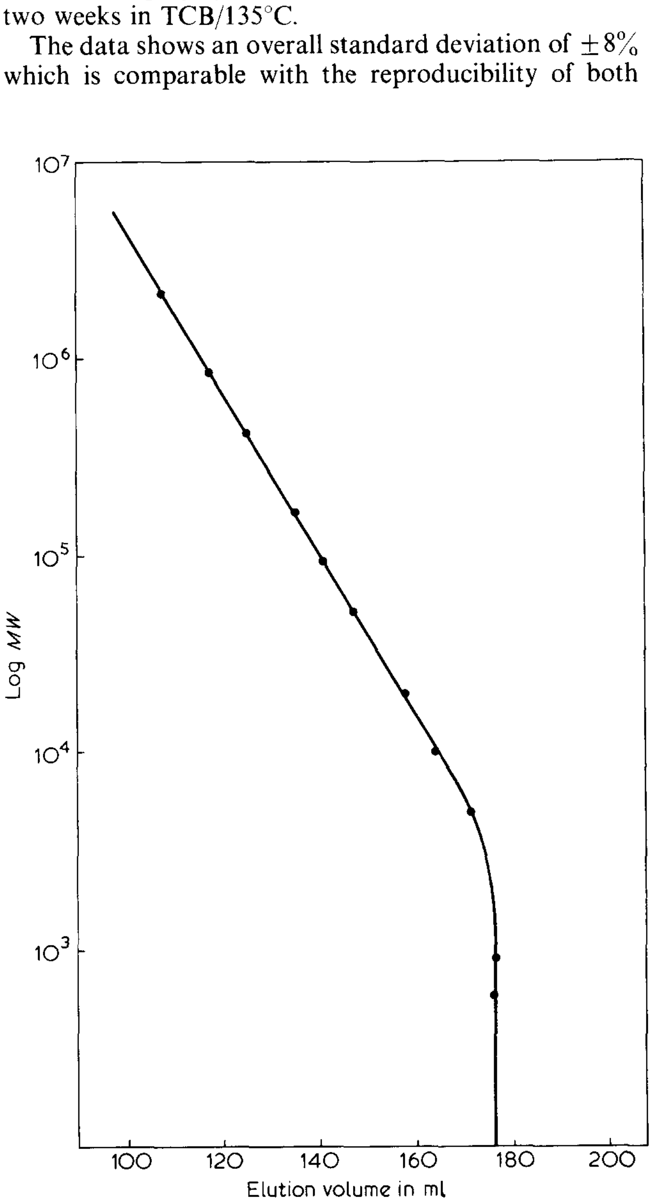

Figure 1 Primary g.p.c. calibration of polystyrene in 1,2,4-trichlorobenzene at 135°C

G.p.c. calibration becomes more difficult when other polymers besides polystyrene are considered, i.e. polymers for which narrow *MWD* standards are unavailable. In some instances *MW*s relative to polystyrene are quoted, which makes comparisons between laboratories difficult and in some cases impossible when different solvents and temperatures are used. In others, various methods of calibration are attempted e.g. the 'Universal' or theoretical calibration or empirical calibrations.

UNIVERSAL CALIBRATION

Attempts have been made to apply polymer solution theory[8] to convert the primary g.p.c. calibration for polystyrene, under a specific set of conditions, into a secondary calibration for a second polymer type. In order to do this it is assumed that (i) the g.p.c. separation takes place on the basis of molecular size; (ii) the polymer molecules are present in the form of simple swollen spheres in solution; and (iii) there is no adsorptive interaction between the polymers and the g.p.c. gel.

Radius of gyration[8,9], mean end to end distance[10-12] and unperturbed dimensions[13,14] have all been used as measures of hydrodynamic volume. Early attempts failed to achieve good correlations between polymers in good solvents and those in poorer solvents[10]. This was due to the assumption by Flory[15] that the segment density in a swollen polymer coil could be represented by a Gaussian distribution about the centre. When the Ptitsyn–Eizner theory[16] for non-Gaussian swelling was invoked[11] the validity for the Universal calibration approach was demonstrated for polystyrene, polyethylene, polypropylene and poly(α-methyl styrene) in TCB at 135°C [11] (see *Figure* 2).

Table 1 Reproducibility of g.p.c. data for a commercial polystyrene TCB/135°C

Sample Replicate No.	*MW* x 10^{-3}						$[\eta]^6_{\text{g.p.c.}}$ dl gm^{-1} Toluene 27°C
	$\bar{M}_{n-1}$	$\bar{M}_n$	$\bar{M}_v$	$\bar{M}_w$	$\bar{M}_z$	$\bar{M}_{z+1}$	
1	27.9	81.5	218	249	520	805	0.79
2	32.9	94.4	246	280	578	875	0.86
3	33.3	90.3	230	261	539	827	0.82
4	26.8	76.5	205	234	494	777	0.75
5	28.5	78.5	210	240	510	787	0.77
6	32.0	86.1	208	236	479	750	0.76
7	30.0	80.2	201	229	474	742	0.74
8	28.5	77.1	200	229	485	765	0.74
9	31.1	83.8	209	238	499	787	0.77
10	30.8	78.5	193	218	442	680	0.72
Mean	30.2	82.7	212	241	502	780	0.77
s.d.	2.2	6.0	15.7	17.9	37.7	52.2	0.04

Overall % error ± 8%, Light Scattering $\bar{M}_w$ = 290 000, Osmometry $\bar{M}_n$ = 102 000 Viscometry $\bar{M}_v$ = 225 000 $[\eta]$ Toluene 27°C ≈ 0.74

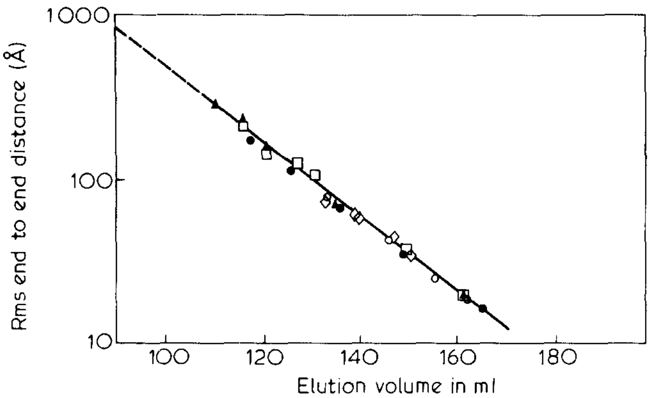

Figure 2 Universal calibration for polystyrene, polypropylene and poly(α-methyl styrene) in TCB at 135°C. ●, Narrow MWD polystyrenes; ○, narrow *MWD* poly(α-methyl styrene); ▲, polypropylene fractions M_w/M_n ~ 2; □, commercial polypropylenes M_w/M_n ~ 6; △, commercial polyethylenes M_w/M_n ~ 10

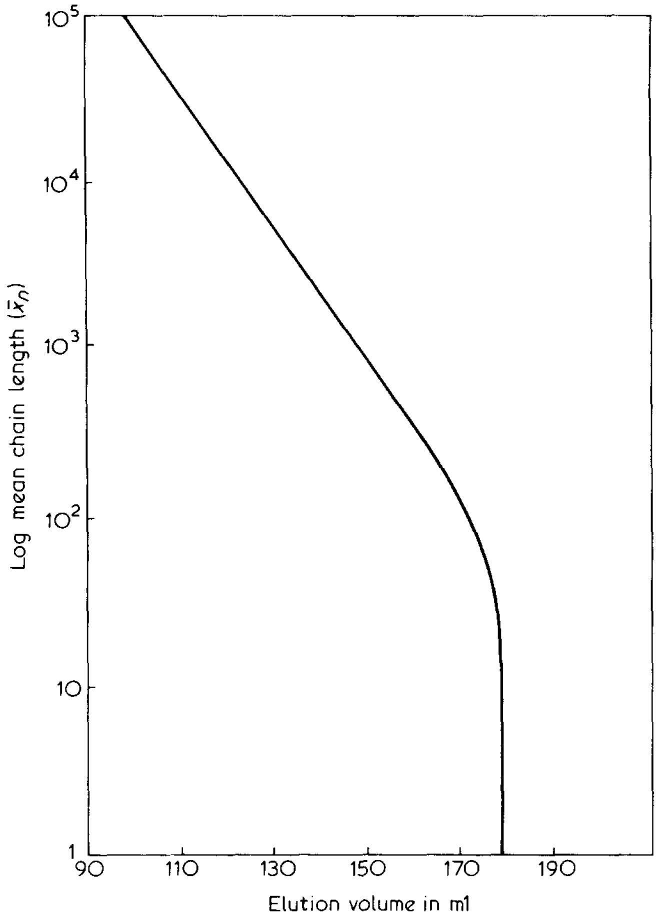

Figure 3 G.p.c. calibration for polyethylene and polypropylene in TCB at 135°C on the basis of $\bar{x}_n$

Application to copolymers

The data for polyethylene and polypropylene in *Figure 2* may be recast as functions of mean chain length, $\bar{x}_n$, (by dividing by the *MW* of the particular monomer) and elution volume. The result of such a treatment is shown in *Figure 3*, where a single function results. This is particularly important when copolymers (in particular random copolymers) of ethylene and propylene are considered. Absolute *MW* averages can in principle be obtained using the $\bar{x}_n$ calibration and applying the correct 'average' monomer *MW* applicable to the copolymer composition concerned.

This can be carried out providing a mass dependent detector is used e.g. flame ionization[34]. In the case of the differential refractometer for this to be strictly correct both monomers should be isorefractive. However, in the practical sense providing the reactivity ratios are not widely different, the range of compositions within the sample will be narrow[32], will not change significantly with *MW* across the distribution and hence any correction requested for this approach will be small.

The worst situation where this approach will be completely invalid is where two comonomers have widely differing refractive indices and reactivity ratios. In this case the refractive index chromatogram will bear no relationship to the true *MWD*.

Application to good and θ-solvents

The ultimate test of the 'Universal' calibration technique for non-polyelectrolytes is to select two polymers and a set of solvent conditions where one polymer is under θ-conditions, i.e. a truly random coil but on the verge of precipitation, and the other is under highly swollen conditions in the same solvent. Such a combination is polystyrene and polyisobutylene in benzene at 25°C [17,18], and cyclohexane at 34°C [17,19].

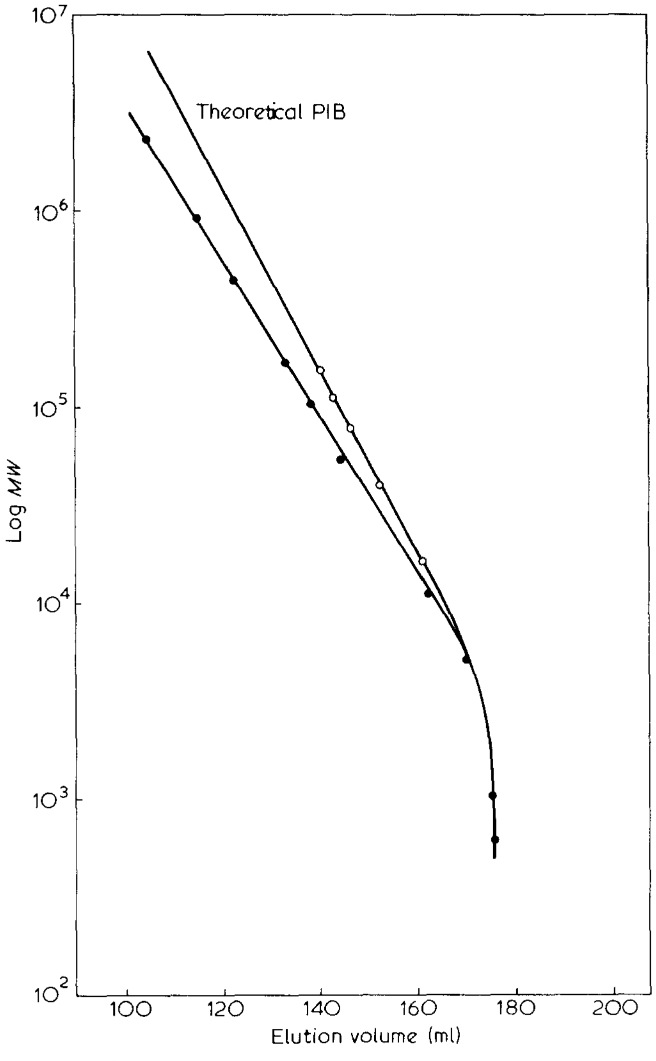

Figure 4 G.p.c. calibration for polystyrene and polyisobutylene in benzene at 25°C. ●, Experimental PS; ○, experimental PIB

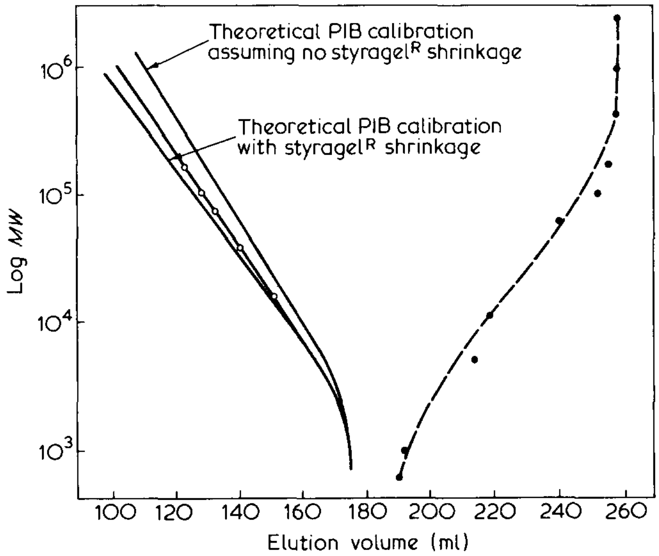

Figure 5 G.p.c. calibration for polystyrene and polyisobutylene in cyclohexane at 34°C on 10^7, 10^6, 10^5 and 10^3 Å styragel^R columns. Symbols as *Figure 4*

The study was carried out on a similar combination of Styragel^R columns as described previously, using the conventional narrow *MWD* polystyrenes[3], and some narrow *MWD* polyisobutylenes[20] kindly supplied by Dr. R. A. Cooper of Chevron Oil.

The columns were initially calibrated using polystyrene in benzene at 25°C (see *Figure* 4). Using the two Mark Houwink equations:

$$[\eta]=9.52\times10^{-5}\ \bar{M}_v^{0.744} \qquad (8)$$

for polystyrene[17], and

$$[\eta]=107\times10^{-5}\ \bar{M}_v^{0.5} \qquad (9)$$

for polyisobutylene[15], it is possible to obtain the theoretical g.p.c. calibration for polyisobutylene in benzene at 25°C [11]. The mean end-to-end distance 'r' for a $MW=M$ is defined by:

$$(r^2)^{3/2}=\frac{[\eta]M}{\Phi_0 f(\varepsilon)} \qquad (10)$$

Φ_0 is the universal Flory constant[7] and (ε) the Ptitsyn–Eizner constant for non-Gaussian swelling[16], where:

$$\varepsilon=\frac{2a-1}{3} \qquad (11)$$

the function of ε in equation (10) is defined as

$$f(\varepsilon)=[1-2.63\varepsilon+2.86\varepsilon^2]=f(a) \qquad (12)$$

Hence the *MW* of a polyisobutylene with the same hydrodynamic volume as a polystyrene of a particular *MW* is calculated from the following equation:

$$\frac{K_{\mathrm{PIB}}M_{\mathrm{PIB}}^{1+a_{\mathrm{PIB}}}}{f(a_{\mathrm{PIB}})}=\frac{K_{\mathrm{PS}}M_{\mathrm{PS}}^{1+a_{\mathrm{PS}}}}{f(a_{\mathrm{PS}})} \qquad (13)$$

which when recast gives:

$$M_{\mathrm{PIB}}=\left(\frac{K_{\mathrm{PS}}}{K_{\mathrm{PIB}}}\cdot\frac{f(a_{\mathrm{PIB}})}{f(a_{\mathrm{PS}})}\cdot M_{\mathrm{PS}}^{1+a_{\mathrm{PS}}}\right)^{1/[1+a_{\mathrm{PIB}}]} \qquad (14)$$

If the values of the Mark Houwink parameters in equations (8) and (9) are inserted, equation (14) becomes:

$$M_{\mathrm{PIB}}=\left(\frac{9.52\times10^{-5}}{107\times10^{-5}}\cdot\frac{1}{0.652}\cdot M_{\mathrm{PS}}^{1.744}\right)^{0.667} \qquad (14a)$$

$$=(0.137M_{\mathrm{PS}}^{1.744})^{0.667} \qquad (14b)$$

The result is the theoretical line for PIB under θ-conditions in benzene at 25°C shown in *Figure* 4. The experimental points obtained using the narrow *MWD* polyisobutylenes are shown in *Figure 4*. They correlate perfectly with the theoretical curve indicating that this form of the Universal calibration is applicable to some polymers in good and θ-solvents.

If the same two polymers are dissolved in cyclohexane at 34°C, polystyrene is now under θ-conditions and polyisobutylene is highly swollen. The g.p.c. column train used above was set up in cyclohexane, the gels shrank, and additional swollen gel of such porosity was added to the columns. Flow was reversed and additions repeated until the columns were full when equilibrated. Reversal of flow assists in homogenizing the bed. When this work was originally carried out in 1968 Styragel^R was more prone to dimensional changes than contemporaty Styragel^R and greater care was necessary when changing solvents.

Figure 5 shows the theoretical polyisobutylene/cyclohexane/34°C calibration based on[19]:

$$[\eta]=27.5\times10^{-5}\ \bar{M}_v^{0.69} \qquad (15)$$

assuming no change in Styragel^R pore size or size distribution. Assuming the polystyrene will contract, then the calibration will be shifted to lower elution volumes, estimated using[17]:

$$[\eta]=82\times10^{-5}\ \bar{M}_v^{0.50} \qquad (16)$$

to be shown in *Figure* 4, prior to adding more gel. The shift to lower elution volumes occurs because fewer pores are available for the larger species as the pores shrink. The experimental calibration for polyisobutylene lies to the right of this 'corrected theoretical' calibration, indicating either that the added gel was providing additional resolution or that the polyisobutylene was interacting with Styragel^R. The results were not significantly different to those expected, indicating that providing the polymer is highly solvated it will not interact strongly with the stationary phase. This confirms the findings of Dawkins[13] and Grubisic[14].

Contrary to Dawkins[13], no size separation was found for polystyrene under θ-conditions with Styragel^R. All polystyrene standards appeared at higher elution volumes than that corresponding to total permeation, i.e. 170 ml. Standards up to 51 000 daltons appeared in reverse order between 190 and 260 ml. All chromatograms were broad with long trailing edges indicating adsorption. Since under θ-conditions polymer–polymer interactions are equally favoured to polymer–solvent interactions[7], and

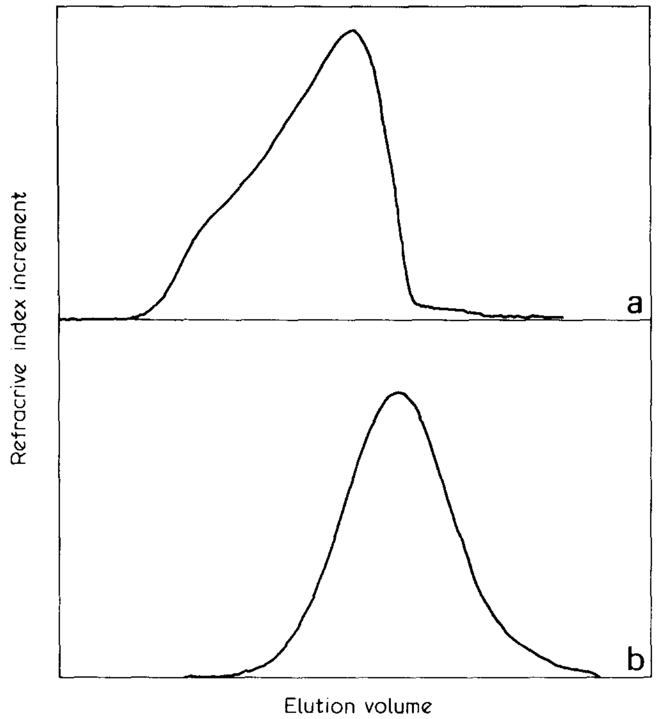

Figure 6 Gel permeation chromatograms of 80:20 polymethylmethacrylate/acrylic acid copolyelectrolyte. (a), Butanone; (b), DMAc̄/0.2%LiBr

partitioning might be expected either by adsorptive or liquid/liquid type mechanisms. Dawkins[13] and Grubisic[14] found under similar conditions that polystyrene standards appear in normal order. The calibration was displaced to lower *MW*s and was steeper, indicating that adsorption was present. Once interaction takes place between the polymer in transit and the gel, the result will depend on the degree of interaction. If mild, the result might be as described by Dawkins[13], if stronger, one would observe the effect described here.

These two studies have shown that the Universal calibration approach using the Ptitsyn/Eizner modification[11] may be valid for the widest range of hydrophobic polymers, providing the Styragel$^{\mathrm{R}}$ remains an inert sieve. Should the gel become adsorptive, as in the above case with polystyrene under θ-conditions, then the Universal calibration will fail.

Adsorptive effects are more pronounced with polar polymers. Meyerhoff[9] showed that polystyrene and cellulose trinitrate in THF do not obey the Universal calibration as the latter has a strong interaction with the gel. In cases where adsorption is present it can be reduced and even eliminated if a more polar solvent, or a small quantity (1.4%) of a highly polar liquid, e.g. acetic acid, is added to the mobile phase.

When polystyrene gels have been used for extended periods at high temperature, (this is particularly prevalent with *m*-cresol, *o*-chlorophenol or trichlorobenzene) these packings tend to oxidize and become adsorptive. Under these conditions all apparent *MW*averages, especially M_n are reduced, as the apparent *MWD* is broadened at the low end.

An experimental verification of adsorption can be obtained simply by comparing the chromatograms of benzoic acid and methyl benzoate, or stearic acid and methyl stearate. Under mild adsorptive conditions, both acid and ester will have the same elution volume but whereas the ester has an essentially symmetrical peak, the acid has an extended training edge. Under more severe adsorption, the acid will have a slightly higher elution volume[5].

Another class of polymers for which the Universal calibration fails in general terms is that of polyelectrolytes. Repulsion between like ionized groups or attraction between unlike ionized groups causes the chain either to be more extended in the former case, or pseudocrosslinked in the latter case. These effects can be circumvented in many cases by using anhydrous aprotic solvents to suppress the degree of ionization, and by using for example 0.2% LiBr or other similar salts in the mobile phase. Polyelectrolyte peaks with apparent bimodal distributions in polar or hydrated solvents become monomodal when chromatographed in for example dimethylacetamide (DMAc̄) or dimethylformamide (DMF) with 0.2% LiBr. An example of this is shown in *Figure* 6 for a copolymer of methyl methacrylate (80m%) and acrylic acid (20m%) chromatographed in Butanone and DMAc̄ with 0.2% LiBr.

EMPIRICAL CALIBRATIONS

(*i*) *Using* 3 *broad* MWD *polymers of known* $\bar{M}_w$ *and* $\bar{M}_n$

The empirical approach can be used when the molecular characterization of a polymer is required in a solvent for which the Mark–Houwink equation is unknown. This assumes that chromatographic conditions are such that the universal calibration approach is valid. The method involves the conversion of a primary polystyrene calibration using broad *MWD*s ($\bar{M}_w/\bar{M}_n > 2$) samples whose $\bar{M}_w$ and $\bar{M}_n$ are known. This method is particularly applicable to the type of standard polymers available from Cellomer Associates[21], particularly polyvinyl acetate (PVAc̄), PVC and the acrylics.

The initial example of this method is the use of polyvinyl acetate (PVAc̄) in THF at 25°C, for which there are 3 broad *MWD* standards available[21] with polydispersities $\bar{M}_w/\bar{M}_n \simeq 3$. A g.p.c. column configuration of 10^5 and 10^3Å equilibrated in THF was initially calibrated for polystyrene as described in the primary calibration section (*Figure 7*). The three broad *MWD* (PVAc̄) were chromatographed, and their weight and number average *MW*s determined relative to polystyrene.

The polystyrene calibration was converted to one for PVAc̄ by adjusting a particular $\bar{M}_w$ or $\bar{M}_n$ on the calibration to the absolute value obtained by light scattering or osmometry, i.e. keeping V_e constant. The resulting 6 point calibration, i.e. two *MW* averages for each of three samples is shown in *Figure* 7. The resulting *MW* averages, calculated on the basis of the PVAc̄ calibration, were within 5% of the absolute values.

(*ii*) *Using one broad* MWD *polymer for which* $\bar{M}_w$ *and* $\bar{M}_n$ *are known*

The above method (i) can be extended to work with a single broad *MWD* sample for which $\bar{M}_w$ and $\bar{M}_n$ are known. The example shown here demonstrates its limitation as we have chosen the case of a polymethyl methacrylate (PMMA)[21] with $\bar{M}_w/\bar{M}_n \simeq 2$. In this case the two points through which the calibration is adjusted are relatively close togehter, and iteration is required to produce the correct adjustment.

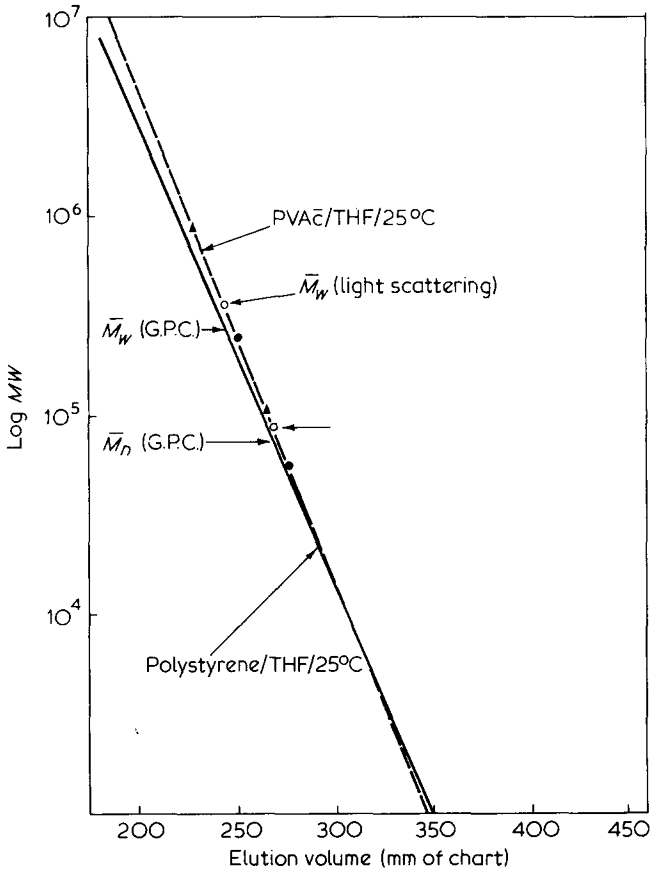

Figure 7 Polystyrene/polyvinyl acetate g.p.c. calibrations in tetrahydrofuran at 25°C. ●, Cellomer 024C–02; ○, cellomer 024C–01; ▲, cellomer 024C–03

It was necessary to calibrate the g.p.c. in dimethyl acetamide (DMAc̄) containing 0.2% LiBr with (PMMA) for a study of bioactive copolymers of MMA with various hydrophilic copolymers containing neutral, positive and negatively charged groups[22,23]. These polyelectrolytes, particularly the polyacids, had apparent multimodal *MWD*s especially in the presence of trace amounts of water. In the case of ter- and tetra-polymers[24] containing charged groups of opposite sign, these were insoluble in the presence of small amounts of water. 0.2% LiBr promotes dissolution in anhydrous DMAc̄, dissipates any polyelectrolyte effects and leads to the expected monomodal *MWD*, (see *Figure* 6).

As MMA was the more abundant monomer in all cases in this study[22], all *MW*data was reported on the basis of MMA. No compositional corrections were made as described above for ethylene/propylene copolymers. The expected magnitude of the corrections would not justify the labour involved in obtaining the fundamental data required to make them.

The primary polystyrene calibration and the three iterative stages to approach the final *PMMA* are shown in *Figure* 8, and the values of $\bar{M}_w$ and $\bar{M}_n$ from each stage of the iteration are given in *Table 2*. The results show that with a single sample of $\bar{M}_w/\bar{M}_n \simeq 2$ that a three stage iteration is required to obtain a satisfactory calibration extending over ~5 decades. The linear calibration can be drawn through two points covering half a decade and extended over 5 decades since the original polystyrene primary calibration is linear over the same range of elution volumes.

(iii) Using several 'broad' fractions of known $\bar{M}_n$

Biodegradable polymers pose experimental problems specific to their instability. Normal fractionation methods are inapplicable because hydrolysis always occurs in hydrophilic liquids (solvents or non-solvents) and exposure to elevated temperature accelerates this process.

A simple example of this is the case of the biodegradable copolymers of polyethylene oxide/polyethylene terephthalate (PEO/PET), these being hydrophilic elastomers soluble in chloroform[25,26]. Chloroform is an excellent solvent for g.p.c. in that it is hydrophobic, has good solvent power for many common polymers, and has an ideal boiling point so as to be stable at 25°C but easily evaporated to regain g.p.c. fractions for additional analysis.

The same column configuration described in (i) and (ii), i.e. 10^5 and 10^3 Å Styragel^R was used, this time with chloroform at 1 ml min^{-1} as the eluting solvent. The columns were calibrated for polystyrene as described in

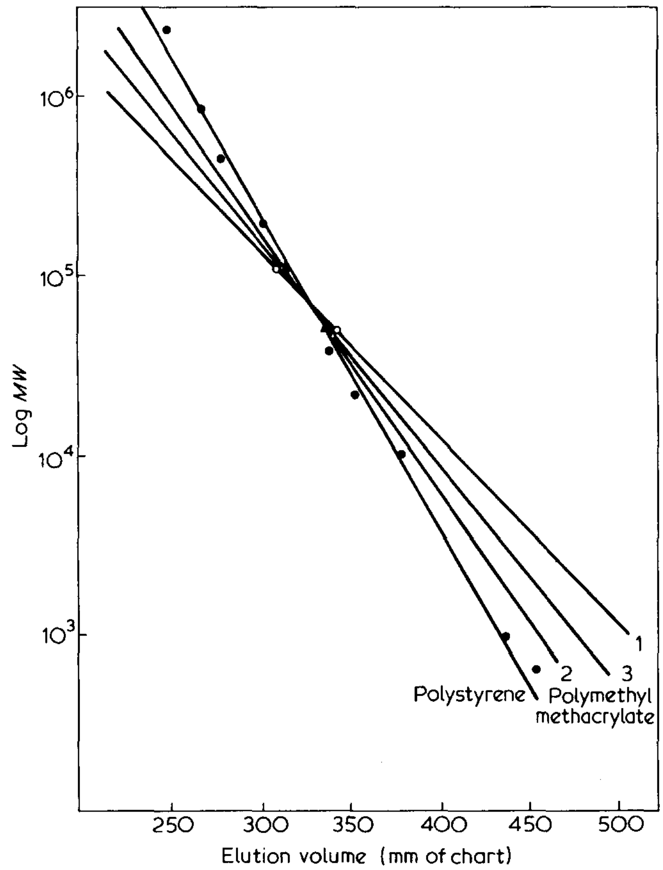

Figure 8 G.p.c. calibration for polymethylmethacrylate in DMAc̄ with 0.2%LiBr using iteration with a single broad *MWD* standard

Table 2 Polymethyl methacrylate *MW*s obtained by empirical calibration

	$MW \times 10^{-3}$				
	Light scattering or	g.p.c. calibration			
MW average	osmometry	Polystyrene	MMA1	MMA 2	MMA 3
$\bar{M}_w$	105	154	93	115	105
$\bar{M}_n$	48	38	57	46	53

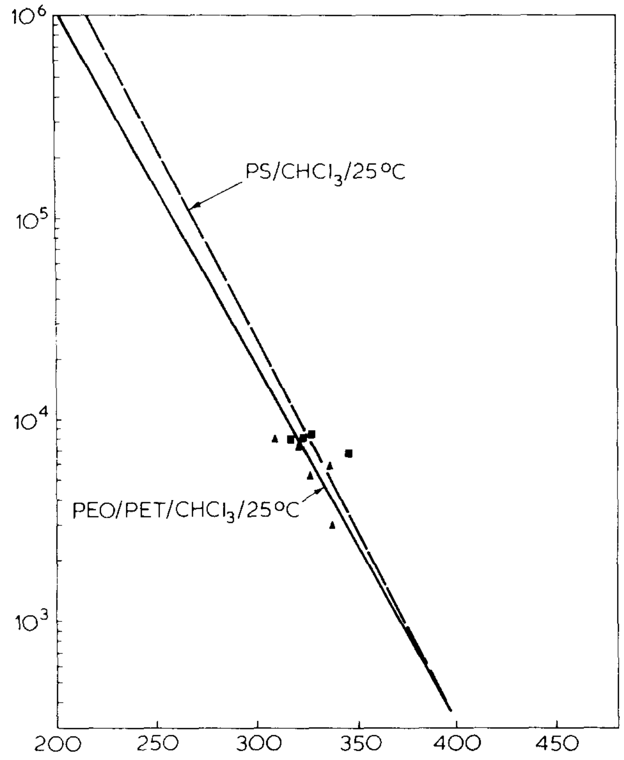

Figure 9 Comparison of $\bar{M}_n$ values for PEO/PET copolymers obtained by gel permeation chromatography and vapour phase osmometry. ■, $\bar{M}_n$ (v.p.o.) for 50/50 wt % PEO/PET; ▲, $\bar{M}_n$ (v.p.o.) for 70/30 wt % PEO/PET

the section on primary calibration. Samples were recovered at different time periods from *in vitro* degradation experiments[26] and after compositional analysis by n.m.r. or i.r. spectroscopy to confirm the preservation of the original composition, the samples were characterized by g.p.c. and by vapour phase osmometry (v.p.o.) in chloroform at 30°C [27]. The calibration for polystyrene is shown in *Figure* 9. The number average molecular weights, $\bar{M}_n$, were obtained from each of the PEO/PET fraction's g.p.c. chromatogram, and their elution volumes used as the 'x' coordinate with the $\bar{M}_n$ value obtained from v.p.o. as the 'y' coordinate. The experimental points are shown for the various PEO/PET compositions, i.e. 50:50 and 70:30 wt % in *Figure* 9, together with the corrected calibration. The values of $\bar{M}_n$ obtained by the various methods are shown in *Table* 3.

The results in *Table* 3 show that this method can be used to produce an empirical calibration with a reasonable correlation with v.p.o. measurements. The limits of application are for $\bar{M}_n \leqslant 15\,000$ daltons because of the limitations of the v.p.o. method. In the region 3–10 000 daltons g.p.c. is fraught with difficulties arising from band spreading, and strictly speaking the refractive index measurements should be corrected for *MW* dependence up to 10^4 daltons[28]. This correction will have the effect of lowering $\bar{M}_n$ (g.p.c.) as there will be a relative increase in the lower *MW* species.

(*iii*) *Use of broad* MWD *samples to calibrate g.p.c. in non-solvents for polystyrene*

If a non-solvent for polystyrene is required in order to dissolve a polymer, is it possible to use this solvent for g.p.c. in order to obtain a molecular characterization of this sample? Aqueous non-solvents for polystyrene were investigated in the early 60s and proved unsuccessful even when the Syragel^R was sulphonated. The problem was probably one of surface tension and solvent exclusion from the pores of the gels.

A particularly good example of this situation is hexafluoroisopropanol (HFIP) which has low surface tension and is an excellent solvent for polyamides, polyamino-acids, polyesters and polyurethanes, at ambient temperatures and therefore circumvents problems due to degradation with high temperature g.p.c. in phenolic solvents. Over the past decade HFIP has gained added significance in that it is the only anhydrous solvent for polyglycolic acid (PGA), the first polymer specifically designed for biodegradable applications in medicine[29].

The feasibility of using this solvent in g.p.c. was demonstrated in 1970 [5], and its application perfected since that time[5,27]. HFIP must be used in the anhydrous state to obtain reproducibility in g.p.c. and requires thorough drying over a molecular sieve prior to distillation from the sieve before use. HFIP is expensive but 90%+ can be recovered from routine g.p.c. analyses[5,27].

The calibration technique for the use of HFIP requires one broad *MWD* sample that is soluble in both HFIP and a common g.p.c. solvent that is used with polystyrene, e.g. THF. Such a polymer is PVAc, and the initial step in the calibration procedure has been described in section (i). If one of the PVAc distributions is superimposed on the calibration, three points on the distribution are readily identifiable, the upper and lower *MW* limits of the *MWD* and the *MW* of the peak. If the same sample is run in a g.p.c. configuration, containing HFIP as the mobile phase, the same three *MWs* can be used to plot the calibration of PVAc in HFIP. This calibration provides the *MW* data shown in *Table* 4 for PVAc in THF and HFIP, and the validity of the method is proved.

Calibration for glycolic (GA) and lactic (LA) acid homo and copolymers in HFIP at 25°C

Once the g.p.c. has been calibrated for PVAc in HFIP at 25°C it is necessary to make several assumptions to

Table 3 $\bar{M}_n$ for PEO/PET copolymers by v.p.o. and g.p.c. methods in CHCl$_3$

	MW × 10^{-3}		
Copolymer composition	$\bar{M}_n$ (v.p.o.)	$\bar{M}_n$ g.p.c. (PS)	$\bar{M}_n$ g.p.c. (PEO/PET)
70/30	7.9(±10%)	15.1(±10%)	12.5(±10%)
	7.3(±5%)	9.1(±10%)	7.8(±10%)
	5.1(±5%)	7.1(±10%)	6.2(±10%)
	5.7(±3%)	4.7(±10%)	4.2(±10%)
	2.9(±10%)	4.4(±10%)	2.0(±10%)
50/50	7.7(±10%)	10.8(±10%)	9.2(±10%)
	8.0(±10%)	7.1(±10%)	6.2(±10%)
	7.9(±10%)	7.5(±10%)	5.7(±10%)
	6.8(±2%)	3.2(±10%)	3.0(±10%)

Table 4 Molecular characterization of PVAc in THF and HFIP

MW average	Quoted value	g.p.c./THF	g.p.c./HFIP
$\bar{M}_w$ × 10^{-3}	154	150	152
$\bar{M}_n$ × 10^{-3}	30.2	30.3	26

Table 5 Comparison of intrinsic viscosities for PGA in HFIP as measured directly by g.p.c.

PGA sample no.	$[\eta]$ viscosity	$[\eta]$ g.p.c.
1	0.68	0.69
2	0.83	0.85
3	1.24	1.30
4	1.29	1.33
5	1.47	1.49
6	1.54	1.58

convert this to a calibration for the glycolic/lactic acid family of polymers.

Firstly, PLA has one carbon atom less in the monomer than PVAc̄. PLA is a more compact chain and since the ester group is in rather than out of the chain, it will tend to be less solvated than the ester group of PVAc̄. Hence it would be expected that a PLA of higher mean chain length $\bar{x}_n$ would have an equivalent hydrodynamic volume to a PVAc̄ of given $\bar{x}_n$. Since the *MW* of vinyl acetate is ~18% greater than the *MW* of lactic acid, we have made the first assumption that the calibration for PVAc̄ is equivalent to that for PLA.

Secondly, utilizing the observation discussed above that polymers of ethylene and propylene of the same $\bar{x}_n$ elute at the same position, we are able to use the same analogy in this case as glycolic and lactic acids are the direct ester analogues of the ethylene and propylene olefins. Hence if as a first approximation, the calibration for PLA is assumed to be identical to that for PVAc̄, the calibration for PGA can be obtained from:

$$M_{\mathrm{PGA}} = \tfrac{58}{72} M_{\mathrm{PLA}} \qquad (17)$$

i.e. homo or copolymers of the GA/LA family of the same $\bar{x}_n$ have identical elution volumes.

Since PGA is so hydrolytically unstable[30], it is impossible to obtain narrow *MWD* fractions without severe degradation. Light scattering and osmometry in HFIP are impractical. In order to obtain an independent estimation of the validity of the two assumptions above it was necessary to estimate the probable values of the Mark–Houwink parameters '*K*' and '*a*'. For PGA in HFIP at 25°C. HFIP is a good solvent for PGA, therefore '*a*' should lie within the range 0.75–0.80. Since PGA is less flexible than the normal carbon–carbon backbone, '*a*' will probably be at the higher end of this range.

'*K*' and '*a*' are interdependent[31] and for polymers with small pendant groups in good solvents, i.e. $a > 0.75$, it is found that '*K*' is typically 10^{-4} dl gm^{-1}. Hence a reasonable estimate of the Mark–Houwink equation is:

$$[\eta] = 10^{-4} \cdot \bar{M}_v^{0.8} \qquad (18)$$

If $[\eta]$ for PGA in HFIP is caluclated from g.p.c. chromatography using equation 18 (via the route shown in equations 3 and 7) the values are found to be 2–3% larger than those obtained from direct viscometric measurements of $[\eta]$ over the range 0.6–1.6 dl gm^{-1} (see *Table* 5).

Taking these factors into consideration we feel that the calibration approach for PGA and PLA proposed above is valid to within ±10% and that the two assumptions made are reasonable. The calibrations for the copolymers of these two materials will lie between the calibrations for the two homopolymers, the actual positions being dependent on copolymer composition.

Besides obtaining intrinsic viscosity data for PGA, g.p.c. allows details of the *MWD* to be elucidated; which are of particular importance when studying degradation phenomena.

CONCLUSIONS

In conclusion, we have found g.p.c. a versatile method for providing total molecular characterization of homo and copolymers[23,32]. Unlike many groups where fixed g.p.c. operating conditions tend to dictate workload, we have used g.p.c. under the optimum conditions required to solve the particular problem at hand. Providing the solvent system is designed to be only as complex as necessary, then it is possible to have a complete change over from one system to another within 4 h. The methods of calibration we have described are by no means meant to be exhaustive, but more as examples of how basic data available within the polymer literature[33] can be used with simple experiments to obtain more meaningful molecular characterizations of commercial polymer systems.

REFERENCES

1 Moore, J. C. *J. Polym. Sci.* 1964, **A2**, 835

2 Waters Associates Inc., Milford, Ma., USA

3 Pressure Chemical Co. Pittsburg, Pa., USA

4 Gilding, D. K. and Reed, A. M. *Polymer* 1979, **20**, 1459

5 Gilding, D. K., unpublished results

6 Bawn, C. E. H., Freeman, C. and Kamaliddin, A. *Trans. Faraday Soc.* 1950, **46**, 862

7 Flory, P. J. 'Principles of Polymer Chemistry', Cornell U. Press, 1953

8 Cantow, M. J. R., Porter, R. S. and Johnson, J. *J. Polym. Sci.* 1967, **A1(5)**, 987

9 Meyerhoff, G. *Makromol. Chem.* 1965, **89**, 282

10 Benoit, H. *et al.*, 3rd International Seminar on g.p.c., Geneva, 1966

11 Coll, H. and Gilding, D. K. *J. Polym. Sci.* 1970, **A2**, 8, 89

12 Boni, K., Sleimers, F. A. and Stickney, P. B. *J. Polym. Sci.* 1967, **A2(6)**, 1567

13 Dawkins, J. V. *Makromol. Sci. Phys.* 1968, **B2**, 623; Dawkins, J. V. and Hemming, M. *Die Makromol. Chemie* 1975, **176**, 1777; Dawkins, J. V. and Hemming, M. *ibid.* 1975, **176**, 1795; Dawkins, J. V. and Hemming, M. *ibid.* 1975, **176**, 1815

14 Mencer, H. J. and Grubisic, Z. *J. Liquid Chromatogr.* 1979, **2(5)**, 649

15 Flory, P. J. and Krigbawn, W. R. *J. Chem. Phys.* 1950, **18**, 1986

16 Ptitsyn, O. B. and Eizner, Y. E. *Zhur. Fiz. Chim.* 1958, **32**, 2464

17 Krigbawn, W. R. and Flory, P. J. *J. Polym. Sci.* 1953, **11**, 37

18 Fox, T. G. and Flory, P. J. *J. Am. Chem. Soc.* 1951, **73**, 1909

19 Krigbawm, W. R. and Flory, P. J. *J. Am. Chem. Soc.* 1953, **75**, 1775

20 Cantow, M. J. R., Porter, R. S. and Johnson, J. F. *J. Polym. Sci.* 1963, **C(1)**, 187; Genert, J. F., Cantow, M. J. R., Porter, R. S. and Johnson, J. F. *ibid.* 1963, 195

21 Cellomer Associates Inc., *Characterized Polymers* 202, PO Box 311, Webster, New York 14580

22 Gilding, D. K., Wilson, J. and Pigott, G. H. *J. Biomed. Mat. Res.* 1981 in press

23 Gilding, D. K. and Askill, I. N. *Ibid* in press

24 Gilding, D. K., Askill, I. N. and Wilson, J. *Ibid* in press

25 Gilding, D. K. and Reed, A. M. *Polymer*, 1979, **20**, 1454

26 Reed, A. M. and Gilding, D. K. *Polymer* 1981, **22**, 342

27 Reed, A. M., Ph.D. Thesis, University of Liverpool, 1978

28 Barrall, E. M., Cantow, M. J. R. and Johnson, J. F. *J. Appl. Polym. Sci.* 1968, **12**, 1373

29 Frazza, E. and Schmitt, E. E. *J. Biomed. Mater. Res. Symp. 1*, 1971, **43**

30 Reed, A. M. and Gilding, D. K. *Polymer* 1981, **22**, 494

31 Gilding, D. K. Ph.D. Thesis, University of Birmingham, 1965

32 Askill, I. N. and Gilding, D. K. *Polymer* 1981, **22**, 499

33 Brandrup and Immergut, 'Polymer Handbook', 2nd Edn, Wiley, 1975