Gel permeation chromatography of high molecular weight cellulose trinitrate

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Gel permeation chromatography measurements have been made upon cellulose trinitrate in ethyl acetate using samples which have already been well characterized by light scattering and osmometry. Columns were calibrated using polystyrene standards and it has been shown that the hydrodynamic volume calibration applies to cellulose trinitrate *provided that* all the data are extrapolated to zero concentration. It has also been shown that the resolution of the columns (as measured by the rate of change with count of logarithm of intrinsic viscosity), and the deviation from the true value of the apparent hydrodynamic volume at any given concentration depends only upon the relative viscosity of the sample. The validity of the hydrodynamic volume calibration does not necessarily mean that both polymers are fractionated solely by an exclusion process. It is possible that both react reversibly with the gel, and that there are compensatory hydrodynamic effects.

INTRODUCTION

Gel permeation chromatography (g.p.c.) is a type of gel filtration chromatography suitable for the separation of flexible polymer molecules according to size. It became possible with the development by J. C. Moore in 1964[1] of suitable column packing materials consisting of polystyrene and polystyrene–divinyl benzene gel particles, and was first applied to cellulose trinitrate by Meyerhoff in 1965[2], since when several investigations have been reported[3–11].

Separation according to size in gel permeation chromatography occurs because smaller macromolecules can diffuse in and out of gel particles more easily than larger ones, as the solvent and injected solution sample are pumped through the columns, so that a chromatogram is obtained with the larger macromolecules eluting first. In the early days of the technique the elution volume of a given molecular species was regarded as being entirely a function of chain length, and 'universal' calibration curves were obtained using narrow polystyrene fractions. Weight-average degrees of polymerization of cellulose trinitrate samples calculated from elution curves using this type of calibration have always been much higher than the intrinsic viscosities of the samples would suggest. However, in 1966 Benoit *et al.*[12] suggested that the relevant parameter for calibration purposes was the hydrodynamic volume measured as the product of intrinsic viscosity and molecular weight. The hydrodynamic volume calibration procedure has latterly been applied to cellulose trinitrate solutions[7]. Due to the fact that polystyrene is a much more flexible polymer than cellulose trinitrate the hydrodynamic volume calibration yields much lower values for degrees of polymerization than the chain length calibration, nearer to those found by other techniques. However, no extensive investigation has been made to establish its general validity as applied to cellulose trinitrate. In order to be a useful technique, g.p.c. must not only yield correct average degrees of polymerization but also distributions of the correct width and shape. Degrees of polydispersity found by g.p.c. have generally been higher than those found by fractional precipitation. In the case of cellulose trinitrate derived from undegraded cottom, unimodal distributions have been obtained by g.p.c.[3,5] but multimodal distribution by fractional precipitation[13].

G.p.c. is an attractive technique for the study of molecular weight distributions of native cellulose, as it is quick and easy and requires only small volumes of solution. However, it is necessary to establish the validity of the procedures used. This paper describes an extensive investigation of the g.p.c. characteristics of cellulose trinitrate using samples of low polydispersity which had already been well characterized by means of light scattering and osmometry[14].

EXPERIMENTAL

Cellulose trinitrate was obtained by directly nitrating cotton by the method of Alexander and Mitchell[15] for various periods and temperatures as previously described[14]. The characteristics of the samples relevant to the present work are summarized in *Table 1*.

The gel permeation chromatography apparatus consisted of parts supplied by Waters Associates comprising the pumping and injection system (2 ml sample), and one set of four Styragel columns in series. The eluate was collected in 5 ml fractions (counts), and refractive index differences between solution and solvent were subsequently measured with a Rayleigh differential refractometer using path lengths up to 40 mm. A flow rate of 1 ml/min was employed for all experiments with cellulose trinitrate. In every case the mass of solute eluted was found from the area under the chromatogram, and was compared with the mass of solute in the sample injected and found to agree to within ±10%. Each experiment was carried out at least three times and care was taken to avoid accidental contamination of the collection

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Table 1

Sample	Time and temperature of nitration	DP_W light scattering	DP_N osmometry	$[\eta]$ (m^3/kg)	DP_W/DP_N absolute techniques	DP_W/DP_N by g.p.c. Hydrodynamic volume calibration	DP_W/DP_N by g.p.c. Intrinsic viscosity of fractions
1	1h at 20°C	5700[a]		4.4	>1.1[b]		
1a	1h at 0°C						
2	1h at −20°C	5000[a]					
3	24 h at −20°C	6500[a]		5.0		1.7[c] 1.2	1.3
4	2h at 20°C	3200	2600		1.2		
5	3h at 20°C			3.6		1.3	1.4
6	4h at 20°C	3300	2600		1.3		
7	6h at 20°C	2500	2100	2.6	1.2	1.4	1.4
8	25h at 20°C	2200	1600	2.1	1.4	1.3	1.5
9	54h at 20°C	600[a]	520	0.52	1.2	1.2	1.3

[a] The Zimm plots showed a downward curvature at low angles of scatter due to the presence of gel particles. The values shown are those obtained by ignoring the downward curvature. The gel present in sample 9 is thought to be different in origin from that present in samples 1 to 3. (For a detailed discussion of the nature of the Zimm plots see ref 14.) [b] Osmotic pressure measurements were not possible with the less degraded samples. The Figure shown is based on a fraction precipitation experiment (see ref 14). [c] See text

tubes. Although tetrahydrofuran has been used as carrier solvent in nearly all previous g.p.c. investigations it was found not suitable for cellulose trinitrate. The pumping pressure required to maintain a 1 ml/min flow rate increased continuously with successive experiments, a situation which could be temporarily rectified by removing some of the packing material near the inlet of the first column. The removed packing material, analysed by a semimicro Kjeldahl method, contained 0.3% nitrogen. Therefore, it is likely, that the increase in pumping pressure was due to blockage of the column with cellulose nitrate. For this reason ethyl acetate, which is a better solvent for cellulose trinitrate and less unpleasant to handle, was used as carrier solvent in most of the present work. Its use yielded more reproducible chromatograms than those obtained with tetrahydrofuran. All solutions were centrifuged at 30 000 g and decanted before being investigated.

The four columns used had nominal porosities of 70–200 nm, 500–1500 nm, 5000–15 000 nm, 70 000–50 000 nm, and were connected in that order. The figures refer to the range of pore sizes in each column, expressed in terms of the contour length of polystyrene molecules to which they are just accessible. Polystyrene standards having weight-average molecular weights 5.0, 51, 410, 870, 2150 kg/mol were used to obtain hydrodynamic volume calibration curves (*Figure 1*). The position of peaks in the elution curves could be determined correct to 0.2 counts after 3 experiments, and elution volumes were found to be independent of flow rate in the range 0.5 to 1.5 ml/min. Only the highest molecular weight sample exhibited any concentration dependence and this was slight (a decrease of 0.6 counts between 1.5 and 0.5 kg/mol). The performance of the apparatus was tested by investigating a mixture of the two standards with molecular weights 51 and 870 kg/mol. This gave the expected elution curve. The experiment was repeated five times so as to collect enough material from the centre of each peak to rerun them separately. These reruns yielded the original elution curves with no secondary peaks indicative of sample mixing. There were gradual changes in the elution volumes of the polystyrene standards during the course of the work and the columns were calibrated periodically. The elution volumes decreased, the magnitude of the decrease increasing with decreasing molecular weight. After about 2 years operation this variation became intolerable and the columns were

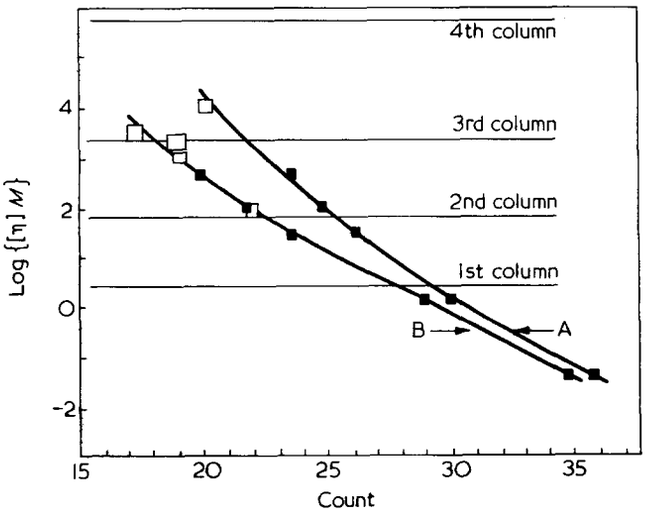

Figure 1 Gel permeation chromatograms hydrodynamic volume calibration for: A, the first set of columns; B, the second set of columns. ■, Polystyrene standards; □, cellulose trinitrate. The squares indicate the limits of error; the horizontal lines indicate the upper exclusion limits of the columns calculated by converting the contour lengths quoted by the manufacturers to hydrodynamic volumes of polystyrene

replaced. Although nominally of the same porosities as the first, the second set of columns had different characteristics, especially in the high molecular weight region (*Figure 1*). Intrinsic viscosities of polystyrene in ethyl acetate do not appear in the literature and these had to be determined in order to obtain hydrodynamic volume calibrations. The Mark–Houwink equation was found to be:

$$\{\eta\} = 2.58 \times 10^{-3} M^{0.57} \quad (1)$$

[$\{\eta\}$ in m^3/kg, M in kg/mol]. No shear-rate dependence was found with any of the samples and the Huggins constant was 0.28 in all cases.

As a matter of routine the intrinsic viscosity of each count was estimated for all the chromatograms obtained with cellulose nitrate. This was done by measuring the specific viscosity with an Ostwald viscometer at a mean rate of shear of approximately 200/sec. The intrinsic viscosity was then

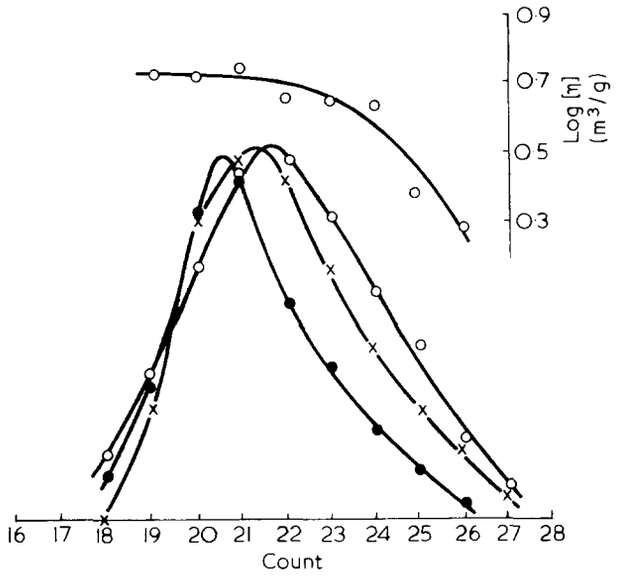

Figure 2 Gel permeation chromatograms for sample 3 (*Table 1*) and associated viscosity data: ○, 0.84 kg/m^3; x, 0.75 kg/m^3; ●, 0.36 kg/m^3

calculated assuming an effective Huggins constant of 0.3, a figure which was found from measurements on the whole samples. The counts were diluted as necessary so that the specific viscosity was always within 10% of the intrinsic viscosity.

RESULTS AND DISCUSSION

Preliminary experiments on a mildly nitrated sample using tetrahydrofuran as carrier solvent

G.p.c. experiments were carried out on sample 1a. Unfortunately sufficient material was not available after the g.p.c experiments had been carried out for light scattering experiments to be made but the conditions of nitration lie between those by which samples 1 and 2 were obtained. The experiments were carried out at a concentration of 0.1% and yielded chromatograms similar to the higher concentration curve of *Figure 2*. Under identical conditions elution volumes were approximately ½ count lower using ethyl acetate as solvent than with tetrahydrofuran. The results of viscosity measurements were also similar in that there was little variation in intrinsic viscosity in the leading edge of the chromatogram. 27 experiments were carried out in order to collect enough material to carry out experiments on individual fractions. The fractions were precipitated by pouring into a large excess of water. Some of the precipitate was redissolved in ethyl acetate and then allowed to evaporate so that the cellulose nitrate formed thin films suitable for infra-red measurements. Material from the leading edge exhibited strong hydrogen-bonded hydroxyl absorption in the region 2.8–3.0 μm. This fact, combined with the viscosity results and the fact that these fractions were only about 2/3 resoluble, indicates the presence of microgel in the leading edge of the chromatogram, and confirms the results of similar experiments on fractions obtained by fractional precipitation[14]. There was also some (much less) hydrogen-bonded hydroxyl absorption by material isolated from the centre of the chromatogram, supporting the authors' hypothesis that the microgel has a wide range of sizes[14]. The remainder of the fractions were redissolved in tetrahydrofuran at concentrations similar to the original sample and rerun separately. Chromatograms were narrow with fractions obtained from the trailing edge indicating good resolution of the columns and they appeared in the correct order but earlier than in the original chromatogram. Chromatograms obtained with fractions from the middle and leading edge, however, were comparable in width to the original chromatogram. It is clear that under the conditions described in this section, the fractionation of cellulose nitrate by gel permeation chromatography is a highly complex process. Several of the authors cited above have carried out their experiments under similar conditions with similar samples and there must be some doubt about the interpretation of their chromatograms.

Experiments with ethyl acetate as carrier solvent

Figure 2 shows chromatograms at various concentrations for sample 3, together with the associated viscosity data of the individual fractions for the highest concentration. Viscosity curves at lower concentrations were similar except that the region at low counts, over which there was little variation in intrinsic viscosity, became progressively shorter. Both the elution volume and the width of the chromatogram decreased with decreasing concentration. It was found that the elution volume of the peak could be extrapolated linearly to zero concentration. The extrapolated value is plotted against the hydrodynamic volume (calculated by multiplying the intrinsic viscosity by the weight-average molecular weight†) in *Figure 1*. The first set of columns was used to investigate this sample and it can be seen that the zero concentration data lie within experimental error on the hydrodynamic volume calibration curve obtained with the polystyrene fractions. Values of DP_W and DP_N calculated from the chromatograms using this calibration showed the apparent value of DP_W to be near the peak of the curve at all concentrations and that the apparent value of DP_W/DP_N decreased linearly with decreasing concentration from 2.8 at 8.4×10^{-1} kg/m^3 to an extrapolated value of 1.7 at zero concentration. However, by extrapolating the width at half-height, again linearly, to zero concentration and assuming a Schulz–Zimm distribution function (to which the chromatogram at the lowest concentration approximately corresponds) a value of $DP_W/DP_N = 1.2$ was obtained. It is likely that the discrepancy arises from the changing shape of the chromatogram which renders the validity of the extrapolation procedures doubtful. DP_W/DP_N calculated from the intrinsic viscosities of the individual fractions, and assuming each fraction to be monodisperse, increased with decreasing concentration from 1.1 to 1.3 at zero concentration. This increase is due to improving resolution. Taking the overall picture presented by the data shown in *Table 1*, it seems likely that 1.3 is near the true value as it is unlikely that sample 3

† According to Newman *et al.*[16] this procedure yields:

$$\phi[(\overline{r^2})^{3/2}]_N \frac{M_W}{M_N}$$

where the symbols have their usual meaning and M is assumed to be proportional to $\overline{r^2}$ over the range of the distribution. Within experimental error this is indistinguishable from the hydrodynamic volume corresponding to the weight-average molecular weight, $\phi[(\overline{r^2})_W]^{3/2}$. Assuming a Shulz–Zimm distribution, the ratio of the first expression to the second is:

$$\frac{\Gamma(a + 3/2)}{(a+1)^{1/2}\,\Gamma(a+1)} \quad \text{where} \quad \frac{M_W}{M_N} = \frac{a+1}{a}$$

For M_W/M_N = 1.33, 1.5, 2, 3, ∞, this expression yields 0.97, 0.96, 0.94, 0.92 and 0.89 respectively. There is some doubt, however, as to whether the expression given by Newman *et al.* applies to cellulose trinitrate in ethyl acetate (see main text).

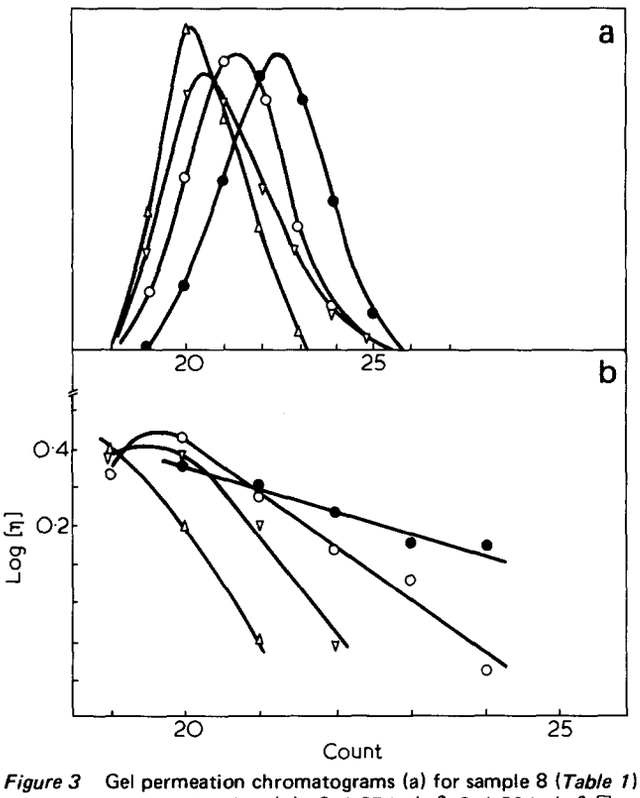

Figure 3 Gel permeation chromatograms (a) for sample 8 (*Table 1*) and associated viscosity data (b). ●, 1.85 kg/m^3; ○, 1.26 kg/m^3; ▽, 0.75 kg/m^3; △, 0.50 kg/m^3

is more polydisperse than the more degraded samples.

Similar experiments were carried out with samples 5, 7, 8 and 9 using the second set of columns and the results for sample 8 are shown in *Figure 3*. All samples showed a marked decrease in elution volume with decreasing concentration and a linear extrapolation to zero concentration (*Figure 4*) could be made. The extrapolated values are plotted against hydrodynamic volume in *Figure 1* and can be seen to lie within the experimental error on the hydrodynamic volume calibration curve. Values of DP_W/DP_N calculated using this calibration showed no systematic variation with concentration although it is clear from the viscosity data, e.g. *Figure 3b*, that this is fortuitous. It would appear that fraction-broadening and mixing effects tend to cancel out for these samples. Values of DP_W/DP_N calculated from the viscosity data increased with decreasing concentration and the extrapolated zero concentration values are shown in *Table 1*. Values of DP_W/DP_N calculated by both methods are in good agreement with values obtained by other techniques.

In general it may be concluded that the hydrodynamic volume calibration is applicable to high molecular weight cellulose trinitrate in ethyl acetate *provided that* extrapolation to zero concentration is made, and that at very low concentrations the chromatograms may be regarded as fair descriptions of the distributions of degree of polymerization. However, in practice, the usefulness of the hydrodynamic volume calibration is limited by the fact that polystyrene standards having comparable hydrodynamic volumes to high molecular weight cellulose trinitrate are not readily available, so that it is undesirable to rely solely on g.p.c. as the investigative technique. The validity of the contour length calibration has clearly been disproved by the present work, as on this basis all of the cellulose trinitrate samples should have eluted within the range of the polystyrene standards.

The fact that cellulose trinitrate and polystyrene each elute, at infinite dilution, according to their hydrodynamic volume, does not necessarily mean that fractionation takes place solely by an exclusion process, as in this case the hydrodynamic volume calibration would be expected to apply only in the case of molecules with similar geometry and flexibility[17]. There is some doubt as to whether the product of intrinsic viscosity and molecular weight represents the size of cellulose trinitrate molecules, as the near unity value of the exponent in the Mark–Houwink equation cannot be explained in terms of appropriate expansion factors[18,19]. Values of $\phi[(r^2)_W]^{3/2}$, calculated for the cellulose trinitrate samples by correcting the present authors' values of $(\bar{r^2})_z$ found from light scattering experiments for polydispersity[14], were found to be approximately twice the hydrodynamic volumes as plotted in *Figure 1*, except in the case of sample 3‡. This indicates that cellulose trinitrate molecules are eluted at higher volumes than polystyrene molecules having the same value of r^2. High elution volumes can result from reversible interactions between solute and gel and several authors have discussed the nature of these interactions[20–22]. The low exponent in the Mark–Houwink equation for polystyrene in ethyl acetate and the high polarity of cellulose trinitrate, would suggest that both react reversibly with the gel. It is possible that the effect is somewhat greater in the case of cellulose trinitrate. Thus the applicability of the hydrodynamic volume calibration may arise from the self-cancelling of several different effects.

Figure 4 shows that, in the case of cellulose trinitrate, the concentration dependence of elution volume increases with molecular weight. The *apparent* proportional change in hydrodynamic volume relative to that at infinite dilution can in fact be shown to depend, within experimental error, only on the relative viscosity of the sample (*Figure 5*) although this applies only for a given set of columns. *Figure 6* shows

‡ The value calculated for sample 3 was in agreement with the hydrodynamic volume. However values of $(r^2)_z$ found from light scattering measurements on cellulose trinitrate samples obtained by mild nitration procedures, are thought to be too low, due to the influence of microgel on the slopes of Zimm plots. It is the contention of the present authors that cellulose trinitrate is a less flexible molecule than hitherto believed[14].

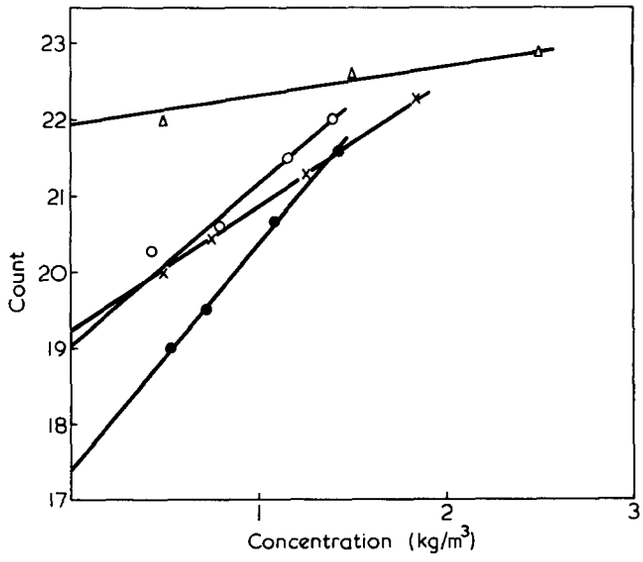

Figure 4 Concentration dependence of chromatogram peaks. ●, Sample 5; ○, sample 7; x, sample 8; △, sample 9 (*Table 1*)

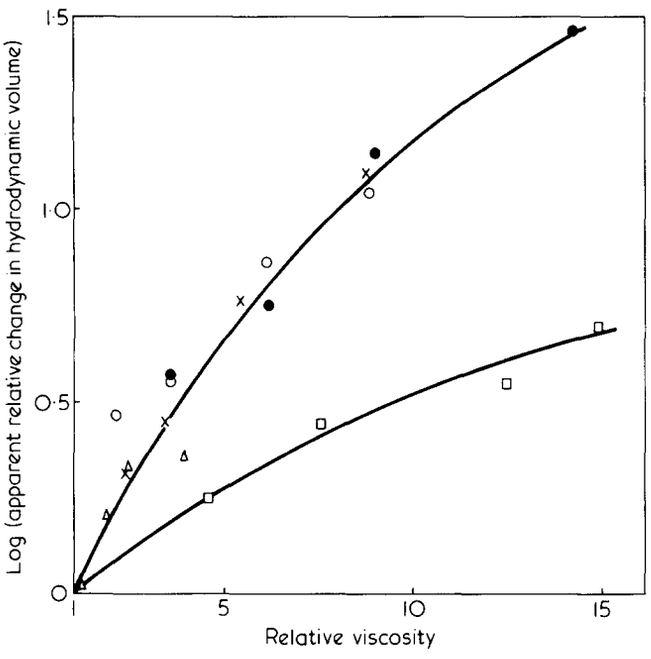

Figure 5 Shift in chromatogram peak relative to the extrapolated zero concentration value, as a function of relative viscosity of sample (see text). □, Sample 3; ●, sample 5; ○, sample 7; x, sample 8; △, sample 9 (*Table 1*)

that a correlation can also be made between the resolution of the columns (as measured by the rate of change of intrinsic viscosity with count) and the relative viscosity of the sample. The fact that the relative viscosity is the dominant factor in determining the concentration dependence of both elution volume and resolution, explains the results of the preliminary experiments on sample 1a described in the previous section. Material isolated from the trailing edge would have a lower relative viscosity at a given concentration than that of the whole sample, and hence if rerun separately would be expected to elute earlier and with improved column resolution. A similar result was obtained by running fractions of sample 1 obtained by fractional precipitation[14]. Ethyl acetate was used as carrier solvent in this experiment and it was found that the chromatogram obtained by combining those of the fractions had a leading edge in the same position as the chromatogram obtained with the whole sample, but that the trailing edge had a lower elution volume.

It is possible that the observed effects of relative viscosity on elution volume and resolution result from flow non-uniformities within the void volume. These could enable solvent following part of the injected sample through the less constricted pathways to overtake the rest of the sample moving through the more constricted pathways. Also lower molecular weight material fractionated within the less constricted pathways could overtake higher molecular weight material fractionated in the more constricted pathways. The magnitudes of these effects would depend upon the relative viscosity of the injected sample and would manifest themselves as a retardation of the chromatogram peak and a mixing of fractions within the chromatogram. On the basis of this explanation the leading edge of the chromatogram would be expected to be less concentration dependent than the trailing edge and *Figures 2* and *3* show this to be the case. Viscosity effects would be expected to be less for a higher void volume. *Figure 5* shows that the effect of viscosity was less with the first set of columns than with the second and *Figure 1* indicates that the void volume within the first set of columns was higher.

Clearly there are qualitative differences in the elution characteristics of the samples obtained by a mild nitration procedure (samples 1a and 3) and the more degraded samples. To a large extent, the non-linearity of plots of the logarithm of intrinsic viscosity against count, obtained with samples prepared by mild nitration procedures, is due to the presence of microgel in the leading edge of the chromatogram as stated above – there being much less microgel present in samples obtained by more extensive nitration[14]. This does not explain the difference in the concentration dependence of the apparent poly-dispersity. It is possible that the degree to which fraction broadening and mixing effects cancel out depends upon where, in the sequence of columns, fractionation takes place. *Figure 1* shows that, assuming the validity of the hydrodynamic volume calibration, fractionation of sample 3 took place entirely within the last column; this is not the case for the other samples. Experiments in which the order of the columns were changed would be useful, although lengthy and expensive on account of the finite lifetime of individual columns.

Concentration effects have been observed with g.p.c. by several other authors[23–27]. It is not suggested that the above explanation applies in all cases. Examination of *Figure 5* shows that the small concentration dependence of the elution volume of polystyrene observed in the present work for instance, cannot be so explained, because the relative viscosities at the concentrations used were not high enough. However, the authors believe that viscosity effects dominate all others in the case of very long polymer chains with low flexibility.

CONCLUSION

In practice, the hydrodynamic volume calibration is applicable to gel permeation chromatography of high molecular weight cellulose trinitrate in ethyl acetate, provided that extrapolation to zero concentration is made. The resolution of the columns and the deviation of the apparent hydrodynamic volume at any given concentration from the true value, depends only upon the relative viscosity of the sample.

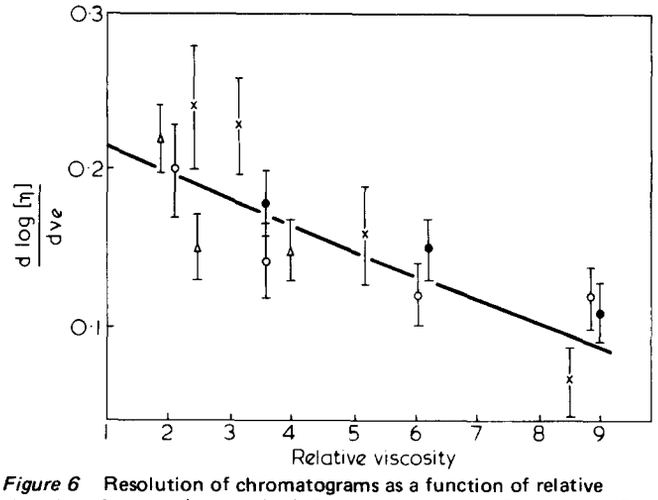

Figure 6 Resolution of chromatograms as a function of relative viscosity of sample (see text). ●, Sample 5; ○, sample 7; x, sample 8; △, sample 9 (*Table 1*)

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