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High-performance gel permeation chromatography

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HIGH PERFORMANCE GEL PERMEATION CHROMATOGRAPHY

by

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A Master's Thesis

Submitted in partial fulfilment of the
requirements for the award of Master of
Science of the Loughborough University
of Technology.

November 1977

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ORIGINALITY

All the work presented in this thesis has been carried out by the author except where otherwise acknowledged and has not previously been presented for a degree at this University or any other institution.

ABSTRACT

High performance GPC separations have been performed with columns containing microparticulate crosslinked polystyrene gels. Part of the work has involved individual columns containing gel with a single pore size ($\sim 80 \text{ \AA}$) and a narrow pore distribution, in order to compare column efficiencies for various solutes as a function of gel particle size ($16\text{--}37 \text{ }\mu\text{m}$) and eluent flow rate. Toluene, a totally permeating solute, was used to study the efficiency of the individual columns with respect to flow rate. A linear relationship between log height equivalent to a theoretical plate and log average particle size was demonstrated. The slope of this straight line was found to be similar to values obtained for silica gel in the same size range. Efficiency for non-permeating solutes on these gels was shown to be almost independent of flow rate and efficiencies were comparable to a permeating solute at low flow rates. This indicated that dispersion owing to velocity profile effects was minimal.

The remainder of the work has involved a set of four columns connected in series and containing gel (particle size $\sim 10 \text{ }\mu\text{m}$) of different porosities, in order to study column efficiency and chromatogram broadening as a function of solute diffusivity and eluent flow rate. Six narrow molecular weight distribution polystyrenes and a broad molecular weight distribution polystyrene were examined. High molecular weight polystyrenes gave high efficiencies at low flow rates while low polymers were less affected by flow rate. The slope of the log polydispersity versus flow rate plot for each polystyrene gave a linear relation when plotted against the reciprocal of the molecular diffusivity. It was proposed, therefore, that high resolution GPC at low flow

rate is of great importance when examining high molecular weight samples and high speed is of use when separating oligomers by GPC. A linear plot of log polydispersity versus flow rate for a broad distribution polystyrene was also constructed. It was demonstrated that this plot can be extrapolated to zero flow rate to give an intercept from which the true polydispersity of the polymer can be determined provided that the contribution owing to eddy diffusion was minimal. However, at the lowest flow rate examined it was demonstrated that broadening was still present.

The work shows that GPC is a very useful technique for measuring molecular weight distributions. These can be determined quickly and/or accurately depending on the molecular weight of the sample.

For My Parents

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CHAPTER 1 INTRODUCTION

Until the advent of gel permeation chromatography (GPC) the only methods of determining molecular weight distribution (MWD) were fractional precipitation, turbidimetric titrations, ultracentrifugation and various other column techniques (1). GPC is a form of liquid-liquid partition chromatography, which was first described in 1941 by Martin and Synge (2). Different molecular species are separated because they have unequal rates of migration owing to their different partition ratios between the mobile phase (solvent outside the particles) and stationary phase (solvent inside the porous particles) and are also sterically excluded from certain pores. Martin and Synge developed the plate height theory which is universally used as a specification of the efficiency of chromatography columns and stated that fast analysis by liquid chromatography (LC) would require small particles (microparticulate packings, particle diameter $< 30 \mu\text{m}$), hence high pressure drops, to achieve fast flow rates.

Since the development of GPC by Moore (3) there have been many improvements to the technique. Originally only crosslinked polystyrene gels were used (3) but the range of commercially available gels (4) now includes ones based on polyvinyl acetate, polyacrylamide, polydextrans, silica and glass. High temperature GPC (5) enables the analysis of polyolefins and other crystalline polymers. The latest GPC instruments were developed from high performance liquid chromatographs (6-7) and use small porous packings which operate at high pressures and flow rates. Primarily, these latest advances have decreased analysis times by an order of magnitude.

The efficiency of this separation process depends on the degree and nature of solute dispersion occurring in the column (8). Dispersion arises from each solute molecule taking a random path

through the column owing to molecular diffusion and collisions with the packing and other solute molecules, thus causing a distribution of solute molecules about the centre of a zone, which increases as the zone moves down the column. The particle size, type, packing method and instrument design all contribute to this dispersion resulting in the molecules of a single species being eluted over a volume larger than that injected even in the absence of a size separation. When a polymer containing a multitude of consecutive species is eluted the broadening of each species overlaps with neighbouring species, causing an inherent error when determining MWDs from GPC chromatograms. Correction for this broadening involves a long mathematical process which was first proposed by Tung (9). If the equipment for high speed GPC is run to give analysis times comparable to conventional GPC the increase in resolution might be sufficient to dispense with this correction without greatly decreasing the accuracy of the MWD.

In the present research it was first necessary to build a GPC instrument for high performance separations. This instrument is described in detail together with a review of the individual components available. The effect of plate height with decrease in particle diameter (d_p) was studied using narrow distribution crosslinked polystyrene gels of varying size ranges. A set of high performance columns containing small particles ($d_p \approx 10 \mu\text{m}$) of different porosities was used to study the broadening of chromatograms obtained for anionically prepared polystyrene standards of narrow MWD and for a radically prepared polystyrene of broad distribution.

CHAPTER 2 THEORY

2.1 HISTORY

Traditionally, GPC involves the use of semi-rigid macroporous crosslinked polystyrene gels first described by Moore (3). These are packed into columns, internal diameter (id) 7.6mm, which generate 60-100 plates per minute. This led to long analysis times (100-250 minutes) and high solvent consumption. Over the last eight years (10-27) much research has been undertaken to reduce this time by an order of magnitude generating 800-1000 plates per minute and allowing the technique to be applied to quality control areas where short analysis times are vital. Before the advent of small porous packings the columns used low efficiencies of separation and high permeabilities; hence initial attempts to reduce the analysis time, in most cases, involved increasing the flow rate (10-17). Although the number of plates per minute was increased there was an overall decrease in resolution (17-19) owing to broadening of the chromatogram.

Investigations into the effect of particle size were carried out from the beginning of GPC. Moore (3) described gels of two particle size ranges, 200-325 mesh (50-75 μm) and 100-200 mesh (75-100 μm), but their effect on column efficiencies was not reported. Later Peaker and Tweedale (20) confirmed that a decrease in particle size gives a progressive increase in efficiency over larger sizes (using 15-45 μm , 45-63 μm , 63-90 μm and 90-150 μm gel), obtaining 1300 plates per foot (ppf) at best (21). Narrower fractions of these crosslinked polystyrene gels were prepared by Waters Associates (10-11) under the trade-name Styragel (10-15 μm , 25-37 μm and 37-42 μm) but the increases in efficiency using small particles were not clearly shown. However, further work by Waters Associates (22) using equipment more suitable for small packings, 3-8 μm , 8-12 μm , 18-25 μm and 37-75 μm (Styragel), identified the

significant increase in efficiency obtainable. These packings ($d_p \approx 10 \mu m$) are now marketed as μ Styragel and are ideal for high speed GPC (22-24). Gels are also marketed by Toyo Soda Manufacturing Co. Ltd. (25-27) under the trade-name TSK-Gel and by Polymer Laboratories Ltd. (28) under the trade-name Micrel. From such columns efficiencies in the region 3000-12000 ppf are not uncommon. Microparticulate inorganic gels have also been developed (29-33) giving comparable efficiencies.

These packings can be used in short columns to give efficiencies comparable to conventional columns and as the volume is much less the analysis time is proportionally shorter for a given flow rate. However, very little work has been carried out to determine the optimum operating conditions for these columns for high resolution (25).

2.2 DISPERSION AND PERFORMANCE

Theories of steric exclusion, restricted diffusion and ones based on thermodynamics have been put forward to explain the physical process of GPC separation. Depending on the conditions one or more of these mechanisms is probably in operation (34). Using chromatographic terminology:

$$V_R = V_o + KV_i \quad 2.1$$

where V_R is the retention volume

V_o is the interstitial volume (volume between particles)

V_i is the stationary liquid volume (pore volume)

K is the distribution coefficient.

The distribution coefficient determines the fraction of pore volume accessible to a solute of a given size. When molecules are larger than the pore size $K=0$ and they are excluded from the gel. When the species are totally permeating $K=1$. The slope of the calibration curve depends on the pore size distribution of the gel. The gel will separate molecular weights M_1 to M_2 in Figure 2.2.1, the separation power being inversely proportional to the calibration slope. This is not often possible with a single gel, so combinations of gels are used covering different size ranges. If after careful selection of the gels poor separation is still obtained, such as in Figure 2.2.2(a), the broadness of the peak must be reduced (Figure 2.2.2(b)) to improve resolution.

FIGURE 2.2.1

Effect of Calibration Slope on Resolution

FIGURE 2.2.2

Effect of Peak Width on Resolution

2.2.1 Plate Height Theory

From a kinetic viewpoint the separation process can be considered to occur in a series of hypothetical steps or volume elements in which equilibrium is achieved between the mobile and stationary phases. Each step is termed a plate and a measure of the efficiency of a column is the height equivalent to a theoretical plate (HETP). There are many objections to the use of plate height since it depends on many variables and the assumptions made are oversimplified, viz. axial solute dispersion is confined to each plate or neglected, the flow of the mobile phase is discontinuous and the partition coefficient of the solute is the same in all plates and independent of concentrations. In spite of this the plate concept is very useful.

Basically there are two column dispersion processes which cause broadening:

- (i) dispersion mechanisms arising from liquid flow through a packed bed of particles (interstitial dispersion)
- (ii) diffusion in and out of the gel i.e. the permeation process itself (pore dispersion).

The main sources of interstitial dispersion are eddy diffusion, molecular diffusion and velocity profile effects. Eddy diffusion is due to the irregular packing of the bed. Flow occurs along interconnecting paths which differ in degree of constriction and tortuosity causing variations in the velocity of individual molecules from the mean. This and molecular diffusion usually cause symmetrical peak broadening. Flow variations caused by non-uniform packing and slow radial diffusion gives rise to velocity profile effects which causes asymmetrical peak broadening.

The most widely used equation for predicting HETP values in

gas chromatography was developed by Van Deemter et al. (35) and is based on a mass balance approach. HETP (H) is given by the equation:

$$H = \frac{2 \cdot D}{U} + M_{tr} \quad 2.2$$

where U is the interstitial velocity (linear flow rate) and M_{tr} a mass transfer contribution. D is the overall mobile phase dispersion number given by:

$$D = \lambda U d_p + \phi D_m \quad 2.3$$

where λ is an eddy diffusion proportionality factor ($\approx 1/11$), d_p is the particle diameter, ϕ a tortuosity factor ($\approx \frac{2}{3}$) and D_m the diffusion coefficient of the solute. Combining Equations 2.2 and 2.3, then simplifying:

$$H = A + \frac{B}{U} + CU \quad 2.4$$

termI   termII   termIII

where A is an eddy diffusion constant

B is a molecular diffusion constant

C is a mass-transfer resistance constant

Equation 2.4 gives a curve as shown in Figure 2.2.3 which has an optimum flow rate with respect to efficiency. Various workers (10) have concluded that Van Deemter's Equation does not correlate well at high flow rates, because the solute molecules do not remain in the same flow paths but diffuse radially into other streamlines. Hence, the eddy diffusion term can be coupled non-additively to the resistance to mass-transfer in the mobile phase. This is the basis of Giddings Coupling Theory (36) and the equation can be written as :

$$H = \frac{B}{U} + C_s U + \sum \frac{1}{(1/A) + (1/C_m U)} \quad 2.5$$

FIGURE 2.2.3

Effect of Linear Flow Rate on HETP from Van
Deemter's Equation (Equation 2.4) and from
Giddings Coupled Approach

where C_s and C_m are mass-transfer effects in the stationary and mobile phases. When applying Equation 2.5 specifically to GPC (37) it can be shown that:

$$H = \frac{2\phi D_m}{RU} + \frac{1}{20} \frac{R(1-R)d_p^2 U}{D_m} + \sum \frac{1}{(1/2\lambda_i d_p) + (D_m/\omega_i d_p^2 U)} \quad 2.6$$

where R is the ratio of solute zone velocity to mobile phase velocity and λ_i and ω_i are geometric factors which for GPC depend on mobile phase velocity inequalities, trans column velocity differentials, trans channel diffusion, short and long range interchannel differentials.

A further approach has been to extend Van Deemter's Equation in order to explain the origin of coupling. This involves the addition of an extra term to the overall dispersion number. Variations in packing density and particle segregation during packing give a variable resistance to flow over the cross-section of the column which is enhanced by column wall effects where there is less resistance to flow because of a greater column void fraction (8). Equation 2.3 becomes:

$$D = \lambda U d_p + \phi D_m + \frac{h R_c^2 U}{\bar{D}_r} \quad 2.7$$

h is a velocity profile constant, R_c the column diameter which can be considered constant at aspect ratios >40 and $\bar{D}_r$ the average radial diffusivity given by:

$$\bar{D}_r = \phi D_m + \lambda d_p U \quad 2.8$$

Combination of Equations 2.2, 2.7 and 2.8 gives an equation for HETP where molecular diffusion is coupled to eddy diffusion as in Equations 2.5 and 2.6.

From the above equations (2.2 to 2.8) the amount of broadening should decrease with particle size as shown in Figure 2.2.4 for

FIGURE 2.2.4

Effect of Particle Size and Flow Rate on Efficiency
using Toluene in Tetrahydrofuran on μ Styragel columns.
Data Replotted from Limpert, Cotter and Dark (23).

cross-linked polystyrene gels (23). The optimum flow rate (minimum HETP) increases with decrease in particle size. Contributions to broadening from mass-transfer effects are minimal with small particles as its effect is proportional to d_p^2 and rapid mass-transfer occurs owing to the small penetration depth in the particles by the solute. Narrow particle sizes should also pack into columns more efficiently than those with a broad size distribution giving a uniformly packed column which minimizes velocity profile effects and has a relatively low pressure drop.

Some of these theoretical expressions and arguments have been used to develop empirical relationships. The main use of these are in quality control areas and for comparing one packing or system with another. The most commonly used expressions are discussed elsewhere (38).

The plate number (N) is calculated from the chromatogram by:

$$N = \left(\frac{V_R}{\sigma} \right)^2 \quad 2.9$$

where V_R is the retention volume at maximum peak height, σ is the standard deviation of the peak and for column length L HETP is given by:

$$H = \frac{L}{N} \quad 2.10$$

For a Gaussian shaped peak, as shown in Figure 2.2.5, σ can be found from:

$$H_i = \frac{1}{\sigma\sqrt{2\pi}} \cdot \exp \left[-\frac{(V_{Ri} - V_R)^2}{2\sigma^2} \right] \quad 2.11$$

where H_i is the peak height at retention volume V_{Ri} . Using Equation 2.9, plate height is then given by:

$$N = 5.54 \left(\frac{V_R}{W_{\frac{1}{2}}} \right)^2 \quad 2.12$$

or

$$N = 16 \left(\frac{V_R}{W_b} \right)^2 \quad 2.13$$

where $W_{\frac{1}{2}}$ is peak width at half peak height and W_b the width at the base of the peak from tangents drawn at the points of inflection on the peak (Figure 2.2.5).

FIGURE 2.2.5

Relationship between Peak Height and Peak Width for a Gaussian-shaped Peak.

2.2.2 Resolution

Since the advent of the first commercial GPC packing (3) and instrument (39), the theoretical plate number has been a guide for the efficiency of GPC columns. This is performed with a low molecular weight monodisperse solute and found using Equations 2.12, 2.13 and 2.10. The values of N (or HETP) obtained using low molecular weight solutes do not give a good indication of the ability of GPC columns to perform polymer separations (40). A better guide would also be a dimensionless number which can be given a physical interpretation.

Resolution (R_s) is usually defined as the ability of a column to separate two Gaussian peaks of equal width (W_b) by a distance Δz as in Figure 2.2.6 and Equation 2.14.

FIGURE 2.2.6

Separation of two Gaussian-shaped Peaks.

$$R_s = \frac{\Delta z}{W_b} \quad 2.14$$

and from Figure 2.2.6

$$\Delta z = V_{R2} - V_{R1} \quad 2.15$$

Equation 2.14 can be extended to allow for differing peak widths:

$$R_s = \frac{(V_{R2} - V_{R1})}{\frac{1}{2}(W_{b1} + W_{b2})} = \frac{2(V_{R2} - V_{R1})}{(W_{b1} + W_{b2})} \quad 2.16$$

Equation 2.16 can be normalized for the molecular weight of the sample generating each peak, this gives the specific resolution,

$$R_{sp}: \quad R_{sp} = \frac{2(V_{R2} - V_{R1})}{(W_{b1} + W_{b2}) \log_{10}(M_1/M_2)} \quad 2.17$$

Bly (40) further normalized Equation 2.17 to incorporate a factor to account for the polydispersity of each polymer.

The slope of the calibration curve is given by $d\log_{10} M/dV$ and on substituting this in Equations 2.14 to 2.17 gives (40):

$$R_{sp} = \frac{2dV}{(W_{b1} + W_{b2}) d\log_{10} M} \quad 2.18$$

This is the inverse of the logarithm of the resolution index (RI) proposed by Smith and Feldman (41):

$$RI = \text{antilog } W_b d(\log_{10} M/dV) \quad 2.19$$

A simplified approach has been recently developed by Yau et al. (42) which assumes Gaussian peaks in a linear portion of the calibration curve. Equation 2.18 can be written as:

$$R_{sp} = \frac{\Delta V_R}{W_b \Delta \log_{10} M} \quad 2.20$$

For a linear calibration curve (43):

$$M = D_1 \exp [-D_2 V_R] \quad 2.21$$

this reduces to

$$\Delta V_R = \frac{\Delta \ln M}{D_2} \quad 2.22$$

Substituting Equation 2.22 in 2.20 we obtain

$$R_{sp} = \frac{\Delta \ln M}{4 D_2^\sigma \Delta \log_{10} M} = \frac{0.576}{D_2^\sigma} \quad 2.23$$

From Equation 2.23 resolution is only dependent on the values of D_2 and σ as shown in Figures 2.2.1 and 2.2.2.

A similar relationship using a linear calibration has been derived by Hamielec and Ray (44) from Tung's axial dispersion equation (9) in which the average molecular weights, $M_K(t)$, are compared to the experimental values, $M_K(\infty)$.

$$\frac{M_K(t)}{M_K(\infty)} = \exp \frac{(3 - 2K) D_2}{4h} \quad 2.24$$

where $K=1,2,3$ refers to the number-, weight-, and z-average molecular weights and h is a dispersion factor given by Equation 2.25 for monodisperse polymers

$$h = \frac{1}{2\sigma^2} \quad 2.25$$

From Equation 2.24 a specific resolution factor $R_{sp}(K, M_o)$ can be defined (45)

$$R_{sp}(K, M_o) = \frac{(-1)^{K!} 4h}{(2K - 3) D_2^2} \quad 2.26$$

where subscripts K and M_o specify the average molecular weight and the molecular weight at V_R respectively.

Meyerhoff (46) has used the polydispersity d in a similar manner where subscripts G and T refer to the experimental and true

MWD.

$$R_{sp} = \frac{d_G - 1}{d_T - 1} \quad 2.27$$

Yau et.al. (42,47) have extended these approaches in order to calculate the relative errors in number- and weight-average molecular weights ($\bar{M}_n^*$ and $\bar{M}_w^*$) from the experimental (G) and true (T) values:

$$\bar{M}_n^* = \frac{(\bar{M}_n)_G - (\bar{M}_n)_T}{(\bar{M}_n)_T} = \exp -\frac{1}{2}(D_2\sigma)^2 - 1 \quad 2.28$$

$$\bar{M}_w^* = \frac{(\bar{M}_w)_G - (\bar{M}_w)_T}{(\bar{M}_w)_T} = \exp \frac{1}{2}(D_2\sigma)^2 - 1 \quad 2.29$$

Resolution can be increased by increasing the column length provided that other parameters, such as flow rate, remain constant. Eventually a point is reached where the pressure drop becomes a limiting factor and flow cannot be maintained. Therefore the columns can be operated in a recycle mode. Under these circumstances the limiting factor is the detector sensitivity. On repeated cycles the width of the chromatogram increases, hence, the solute concentration in the detector during peak analysis decreases. Hamielec has extended Equation 2.24 to account for the number of recycle passes n .

$$\frac{M_K(t)}{M_K(\infty)} = \exp \frac{1}{n} \frac{(3 - 2K) D_2^2}{4h} \quad 2.30$$

where h and D_2 are found from a single pass.

2.3 MOLECULAR WEIGHT DETERMINATION

GPC is not an absolute technique and a calibration must be constructed using a polymer or polymers of known MWD and/or known molecular weights. This is usually done by injecting a series of polystyrene standards (48) having a narrow MWD and measuring V_R at the maximum peak height. The calibration curve is made by a plot of $\log M$ versus V_R . Providing only polystyrenes are to be analysed a universal calibration is unnecessary. This method and other methods of calibration using broad MWD polymers have been fully reviewed (49-50)

It is not possible to convert the elution volume of an unknown polymer to the molecular weight by mere substitution because of the large errors involved (51-53). The MWD must be found and normalised. MWD may be defined by the differential weight distribution $w(M)$ or the cumulative weight distribution $I(M)$ as a function of the molecular weight (54)

$$w(M) = \frac{dI(M)}{dM} = \frac{dC(v)}{dv} \cdot \frac{dv}{d(\log M)} \cdot \frac{d(\log M)}{dM} \quad 2.31$$

where $C(v)$ is the weight fraction of polymer eluted between $V_R=0$ and v and $dC(v)/dv$ is the height of the chromatogram at $V_R=v$. As $d(\log M)/dM$ is $1/M$ the differential molecular weight distribution can be found using Equation 2.31. From this the average molecular weights can be found (49) from Equations 2.32 to 2.35

$$\bar{M}_n = \frac{1}{\int_m^\infty (1/M) w(M) dM} \quad 2.32$$

$$\bar{M}_v = \left[\int_m^\infty M^a w(M) dM \right]^{1/a} \quad 2.33$$

$$\bar{M}_w = \int_m^\infty M w(M) dM \quad 2.34$$

$$\bar{M}_p = (\bar{M}_n \cdot \bar{M}_w)^{\frac{1}{2}} \quad 2.35$$

$$d = \frac{\bar{M}_w}{\bar{M}_n} \quad 2.36$$

where $\bar{M}_n$, $\bar{M}_v$, $\bar{M}_w$ and $\bar{M}_p$ are the number-, viscosity-, weight- and peak-average molecular weights respectively, m is the molecular weight of the monomer, a is the Mark-Houwink exponent and d the polydispersity.

A computer program (55) based on that of Pickett et al. (52) was used to calculate these averages by this method.

CHAPTER 3 EXPERIMENTAL

3.1 EQUIPMENT DESIGN

Extra column broadening can be considered to be the sum of individual contributions due to injection, column fittings and detector (18,56-57). Therefore, the equipment used in high performance GPC, as in other modes of liquid chromatography, must be carefully designed to optimize performance. This involves reducing all waste volume areas or 'dead volumes' after the injection point. Mixing in these areas is diffusionally controlled giving rise to symmetrical peak broadening (58).

3.1.1 Packings

Various types of packing have been used in GPC (4) but not all are applicable to high performance systems. These gels can be classified into two categories:

- (i) porous or porous layer (pellicular)
- (ii) soft, semi-rigid or rigid gels

Pellicular packings are made from a non-porous bead ($d_p \approx 30 \mu\text{m}$) onto which a thin ($\sim 5 \mu\text{m}$) porous shell is bonded. These packings retain the advantages of large packings - low pressure drops, easily packed - and because of their effectively small pore depth they give high efficiencies due to rapid mass-transfer. However, they have not been used for routine GPC work (59) because they have low capacities requiring small sample sizes and highly sensitive detectors.

Microparticulate packings give high inlet pressures at normal GPC flow rates ($1-3 \text{ cm}^3 \text{ min}^{-1}$); hence, the gel type must be carefully chosen. The pore structure of all soft and some semi-rigid gels can collapse under high pressure and such gels are not used. The rigid gels (inorganics) have better mechanical and thermal properties than semi-rigid gels (e.g. crosslinked polystyrene) but their surface

is active which can cause severe tailing of polar solutes due to adsorption. To minimize this effect a polar solvent is used or the active sites are blocked. All commercially available rigid gels are either porous silica or glass and very little attention has been given to alumina despite encouraging reports in the literature (60-61).

Because of the various types and different methods of producing microparticulate packings, both spherical and irregular particles are encountered. If all GPC parameters are held constant, peak broadening in both types of particle is comparable. However, spherical particles are always preferred because:

- (i) the pressure drop over a packed column is smaller
- (ii) the efficiency of a column packed by a given method is more reproducible (62)
- (iii) sized fractions by an air classifier have a narrower particle size range.

The large number of microparticulate GPC packings now available has resulted in a search for the most efficient packing technique. The choice is governed by the gel type and its size and shape. Any packing technique which gives a uniform bed, packed as densely as possible without particle fracture, can be used.

3.1.2 Columns

Stainless steel columns are usually made from cold-drawn tubing which has a porous internal surface with longitudinal grooves. The internal diameter (id) of this tubing varies as a function of column length. Drilling the column removes wall defects, leaving radial grooves and burrs ~ 20 μm deep, and gives a constant id (62). This also improves packing reproducibility. Polishing the internal surface of drilled tubing gives no further improvement.

The size and bore can play an important part in the performance of a column. The pressure drop over a 7.6 mm id is thirteen times less than over a 2.1 mm id column for a given column length and volume flow rate. The sample size on the larger column can be sufficient for preparative GPC separations. Efficiency increases with increasing id for long columns because they are essentially of infinite diameter (63). If a sample is injected into the centre of a column, it can separate into solute zones which reach the column exit before the column wall. This allows the use of undrilled columns. However, the cost of packings is a limiting factor because a 7.6 mm id column holds thirteen times more packing than a 2.1 mm id column of equal length. It must also be noted that crosslinked polystyrene gels do not perform well in narrow bore columns (64).

Column end fittings should be designed with a minimum dead volume (65) and must form a tight seal with the frit. The frits used to retain the packings are usually made of porous stainless steel, or nickel, or a closely woven metal mesh. Their pores must be small enough to retain the packing and have similar sizes to prevent non-uniform flow.

In GPC particles of different porosity are packed into individual columns which are connected together using short lengths of low volume, narrow bore tubing. Under ideal conditions the decrease in efficiency is less than 5% (66). If two columns of different efficiencies are connected the result will not be an average column but one of poor efficiency. This effect could also happen if columns of different id or containing different gel types are connected. If coiled columns are packed, their eluent flow velocity profile will give extra broadening (67). If they are coiled after packing, broadening will also occur from particle crushing

and disruption of the packing geometry. Ideally a long column packed with a mixture of gels having different porosities, but the same particle size and distribution should be used, although difficulties in packing might be experienced.

3.1.3 Injection

The method of sample introduction greatly influences the efficiency of high performance columns. In an ideal system the sample is rapidly delivered to the top of the column and no mixing with the mobile phase occurs, i.e. the sample displaces the solvent at the head of the column. There are three main methods of introducing samples into the GPC instrument: stop-flow injection, sample-valve and on-column injection.

In the stop-flow injection the pump is stopped. When the column inlet pressure is atmospheric, the sample is injected onto the top of the column using a low pressure syringe. The pump is restarted and solvent flow continues. Pneumatic or small volume reciprocating pumps are used for this type of injection as they rapidly achieve the operating pressure of the system. Sample diffusion in the solvent is so slow that resolution is unaffected by the injection. The system is not ideal for high performance GPC because of the difficulties in measuring much smaller elution volumes (68).

Sample valve methods consist of a fixed volume loop filled with an aliquot of sample dissolved in the mobile phase. The solvent stream is diverted through the loop, flushing its contents to the column head. Sample sizes can be varied by changing the volume of the loop or, to a lesser extent, by controlling the time of flushing. This method of sample introduction has been commonly used in GPC instruments because of its reproducibility and convenience in

adapting it to automatic injection. Modern sample valves have been designed with a stream splitter (69) which increases efficiencies.

Because injection ports can only be used up to 500 psi, a special injection system is necessary for high resolution systems. The syringe must be designed to withstand the inlet pressures encountered so no sample is lost during injection. The weak point is the septum which must have sufficient strength to withstand many injections without rupture or extrusion through the needle inlet. A PTFE face on the septum can reduce these problems and help minimize solvent attack. The injected volume is less than for sample valve systems as the maximum operating pressure of a syringe normally decreases with increasing volume. If a large volume is injected rapidly, eddies may form round the needle tip and cause mixing, hence further broadening. However, on-column injection gives the highest efficiencies (69) because the sample is placed in the centre of the column directly onto the packing but must not disturb the packing otherwise broadening will occur.

The sample size injected can greatly effect the performance of a column causing poor resolution and a decrease in efficiency. For microparticulate columns the maximum injected volume is governed by the performance of the injection technique, sample complexity and viscosity effects. The latter is most important in respect to polymers, especially at high molecular weights, and results in an increase in V_R for the sample and broadening. Some quantitative work has been reported on the effect of high sample loads on resolution with 50 μm porous glass (70) and recently with $\mu\text{Styragel}$ (71).

3.1.4 Solvent Delivery

As high performance GPC necessitates the use of small packings, pumping systems which operate at high pressures are required. All these pumps were originally designed for liquid chromatography and most commercially available pumping systems have been reviewed (72-73). However, the criteria for GPC work is slightly different and a comparison is listed in Table 3.1

Reproducible flow is important in GPC as the much smaller elution volumes encountered in high performance systems cannot be readily broken up into many aliquots and measured accurately by syphon or drop counter. This has resulted in most workers assuming constant flow rate and measuring elution volumes as a time base along a recorder chart paper. However, small variations in flow rate can result in large errors when determining average molecular weights of polymers. Work at Du Pont (74-75) has shown that random fluctuations of 1 to 4% can be tolerated for a short time but flow rate repeatability must be better than 0.3% otherwise errors in $\bar{M}_n$ and $\bar{M}_w$ will be about 6%. These errors may be larger as flow rate fluctuation on separation efficiency and detector response are not considered.

Two different types of pump have been developed: pneumatic pumps which deliver the mobile phase at constant pressure and mechanical pumps which give constant flow, essentially independent of back pressure. Unfortunately, the cheaper, more reliable, pneumatic pumps are not applicable for high performance GPC as they cannot guarantee reproducible flow. Mechanical pumps are less sensitive to changes in viscosity caused by temperature rise due to liquid compression and changes in column permeability caused by swelling or settling of the packing.

Constant displacement pumps give accurately controlled flow

TABLE 3.1

Requirement for a modern analytical liquid chromatography pumping system	For GPC
Deliver mobile phase with consistancy, 1-2%.	0.3%
Pulse free to minimize detector noise.	Yes
Ease of solvent change.	unnecessary
Work at high pressures, 500-5000 psi.	300-3000 psi sufficient
Construction chemically resistant to mobile phase.	Yes
Compatibility with flow and solvent programming techniques.	unnecessary

by varying the voltage on a motor which moves a screw driven piston giving complete pulse-free flow independent of solvent properties. It can deliver at high pressures (3000 to 6000 psi) provided the seals on the piston are maintained. The pressure build up does not give immediate steady flow conditions (76-77) due to the compressibility of the mobile phase. Typically 15 to 60 minutes are required which consumes valuable time and depletes the limited solvent capacity (250-500 cm³). An improvement is to operate the pump at a higher flow rate until the operating pressure is achieved then reduce the flow rate to that required (77). The pumps disadvantages are its high cost and the pump must be stopped periodically for refilling. However, this type of pump is of great use in research work.

Small volume, dual reciprocating pumps have been developed operating 180° out of phase to reduce the amount of pulse-flow generated in conventional reciprocating pumps. If a specially shaped cam is used and a feed back system employed to control the pump motor speed, variations in flow can be reduced. This is achieved by a differential pressure transducer connected at the pump outlet which monitors the flow (not the system's back pressure) and compares it to the flow setting. A control circuit then adjusts the pump speed. The response time of the system is greater than the resultant pulsing caused by the dual pumping chambers giving almost constant flow without pulse dampers. The pump has no restrictions on reservoir size and is ideal for use in the recycle mode. A variation on this system uses two low volume positive displacement pumps at varying piston frequencies to give uniform pulse-free flow when their outputs are combined.

The final choice of pump depends on the GPC system used, versatility required and the cost.

3.1.5 Detectors

Most commercial detectors have been reviewed (78) and the choice will depend on the types of polymer samples to be analysed and the system conditions used. The main requirement of a detector is to give an accurate measurement of the concentration of the sample in the column eluent and produce an electronic signal proportional to it. Three methods of detection are available: monitor a property differential between the eluent and solvent, monitor a property unique to the sample injected and not the solvent, remove the mobile phase before attempting detection. Ideally, the detector used in GPC should be universal to all polymer samples and continuously monitor the eluent so the signal can be fed into a chart recorder giving a visual record. Almost any instrument capable of giving a response to a physical or chemical property of a polymer can be used as a detector. However, the technique must be adapted so that it fulfills specific requirements.

The amount of polymer in the eluent is present in a low concentration and only techniques which give a high signal-to-noise ratio are used. This can be significantly improved if a differential method is employed. The design of the flow cell is important; a compromise must be made between resolution and sensitivity. Sensitivity increases with increase in cell volume as more sample is available for detection, but the increase in cell volume allows greater mixing of the separated sample with the eluent, hence, a decrease in the overall resolution. Therefore, a detection method which is inherently sensitive is desirable. The signal output should also be linearly proportional to the solute concentration. Of the various types of commercially available detector for high performance systems, only a few of these

can be applied to polymer detection.

Differential refractometers are widely used in GPC instruments as they come nearest to being a universal detector. The refractive index of the eluent changes when a polymer sample is present unless the refractive index of the polymer is the same as that of the eluent. For most polymers refractive index is independent of molecular weight except for low molecular weights. The detector is based on two different designs. One utilizes Fresnel's Law of reflection. The intensity of the reflected light from incident light impinging at a liquid/glass interface, slightly less than the critical angle, changes inversely with the refractive index. The cell volume is very small ($\sim 3 \mu\text{dm}^3$) and does not limit the maximum flow rate. Variations due to temperature are minimized by differential measurement of the eluent and solvent. For efficient operation the cell window must be kept very clean. Unfortunately, this design has a limited linear response and two prisms are needed to cover a useful refractive index range ($n_D \approx 1.33-1.63$). The other refractometer design uses optical deflection of a light beam which passes through a triangular cell rather than light reflection from an interface. A single beam of light passes through the eluent and reference cells in series. The light is reflected from a mirror and passes through the dual cell again. When the concentration of solute in the sample cell changes the deflection of the light on each pass is additive and measured by photodetector. This method permits the use of one sample cell throughout the entire refractive index range ($n_D = 1.0-1.75$) and gives a wide range of linear response. The flow cell is not as cleanly swept or as small as the Fresnel type but its higher sensitivity is less affected by a build-up of dirt or air-bubbles in the cell. Refractometers are fairly reliable

but are extremely sensitive to changes in temperature. In practice, the sensitivity claimed by the manufactures can not be achieved with great precision due to poor thermal control.

The second most commonly used detector for GPC is a differential ultraviolet (UV) photometer. It has a high level of sensitivity to which other new detectors are often compared. UV radiation from a monochromatic source is utilized at a set wavelength, usually 254 or 280 nm. The amount of radiation absorbed is measured by a photocell which generates a current proportional to the intensity of the light. The flow cell used has an internal diameter of 1 mm and pathlength of 10 mm (cell volume $8 \mu\text{dm}^3$). Solvent or air can be used for the reference. Variable wavelength photometers (spectrophotometers) are now available at extra cost in which the wavelength is chosen to match a high extinction coefficient of the sample. UV detectors are less sensitive to changes in flow, temperature fluctuations and pulse-flow than refractometers, and most commercial instruments have a good linear response. Unfortunately, not all polymers have a significant UV absorbance at a convenient wavelength and the choice and purity of the solvent system must be considered.

Dual detectors have often been used in GPC analysis. The smallest amount of tubing between each flow cell must be used to decrease dead-volume, normally the detector with the smaller dead volume being placed first. A combination of selective detector (UV) with a universal detector (refractive index) using one flow cell has been developed (79).

Recently a low angle laser light scattering photometer having a small cell volume (80) has been coupled to a GPC instrument (81-82) in conjunction with a differential refract-

ometer. The light scattering data is a function of polymer concentration and also molecular weight. Therefore, this system can give molecular weights directly without the necessity of a GPC calibration and broadening correction, but is least accurate at low molecular weights.

3.2 APPARATUS

All equipment for high performance chromatography systems needs to be robust, long lasting, reliable and chemically inert. Stainless steel satisfies these requirements but because it needs to be machined to high tolerances the system chosen is always expensive. The equipment used in this study was for the purpose of examining single columns and column series with polystyrene samples. Therefore, this system is not necessarily ideal for routine polymer analysis.

3.2.1 Packing

In this study crosslinked polystyrene gels packed into 0.76 mm id stainless steel columns of varying lengths were used throughout. Gels produced by suspension polymerisation of styrene and divinylbenzene (21) were obtained in pre-packed columns from two sources:

(a) T. Stone Gel (83-84)

This gel was prepared, processed, classified, characterised and packed into columns at Loughborough University by the above worker.

The gel was prepared in one pore size range and separated into narrow particle size ranges using an Alpine Laboratory Zig-Zag centrifugal classifier. This process was repeated until gels with size ranges shown in Table 4.1 were produced. Optical micrographs (Figure 3.2.1) and scanning electron micrographs (Figure 3.2.2) of these fractions demonstrate the success of the size separation. The gel fractions were subjected to Coulter Counter analysis which counted the particles in 4 μm intervals for the size range 1-80 μm . The particle size distribution for

FIGURE 3.2.1

Optical Micrographs of T. Stone Gel Particles, by courtesy of
Mr. T. Stone, Loughborough University of Technology.

a, unclassified; b, $>28\text{ }\mu\text{m}$; c, $24\text{--}28\text{ }\mu\text{m}$; d, $20\text{--}24\text{ }\mu\text{m}$; e, $16\text{--}20\text{ }\mu\text{m}$;
f, $10\text{--}15\text{ }\mu\text{m}$

FIGURE 3.2.2

Scanning Electron Micrographs of T. Stone Gel Particles, by
courtesy of Mr. T. Stone, Loughborough University of Technology.

a, 24-28 μm ; b, 20-24 μm ; c, 16-20 μm ; d, 10-15 μm

each gel was found by constructing a curve through the weight fractions at the mid points of the $4\text{ }\mu\text{m}$ intervals. These are shown in Figure 3.2.3. The shape of the distribution for ARgel. (trade-name Applied Research of Laboratories Ltd., Luton, Beds.), grade 805, also shown in Figure 3.2.3 is because it was produced by wet sieving (83) even though its polydispersity from Table 4.1 is comparable to that of the air classified gels.

The column packing method used was similar to that of Peaker and Tweedale (21). Column end-fittings were kindly supplied by ARL and the column lengths were as shown in Table 4.1. From other investigations no significant changes in column efficiency (HETP) can be attributable to the differences in column length.

(b) μ Styragel

Four columns, each containing a gel of different pore size ($10^6\text{ }\text{\AA}$, $10^5\text{ }\text{\AA}$, $10^4\text{ }\text{\AA}$ and $10^3\text{ }\text{\AA}$), were purchased from Waters Associates Inc., Northwich, Cheshire. The particles have a $d_p \approx 10\text{ }\mu\text{m}$ and are packed into 30 cm columns. These can be connected in series, using 5 cm of connecting tubing 0.25 mm id, to give 120 cm of column in decreasing order of pore size.

3.2.2 Injection

The injection heads for sample introduction are shown in Figures 3.2.4 and 3.2.5. Because of the danger of exposing the polystyrene gel to the air, which causes gel shrinkage and irreversible channeling, it was decided not to place the injection head above the gel but directly above the column end-fitting. The sample could then be injected into the solvent flowing into the top of the column. The syringe needle was pushed through the

FIGURE 3.2.3

Weight Fraction Particle Size Distributions for T. Stone Gel,
courtesy of Mr. T. Stone, Loughborough University of Technology.

— · —, unclassified; — —, ARgeL 805; — —, $>28 \mu\text{m}$;
— —, $24-28 \mu\text{m}$; — — —, $20-24 \mu\text{m}$; ·····, $16-20 \mu\text{m}$;
————, $10-15 \mu\text{m}$

Figure 3.2.4 - Injector for T. Stone Gel Columns

Figure 3.2.5 - Injector for μ Styragel Column Set

A, PTFE backed silicone rubber septum; B, Mobile phase from pump;
C, PTFE plug (40 μ m pore size); D, 3 mm x 3 or 12 mm pocket; E,
woven stainless steel mesh (5 μ m pore size); F, 18 mm x 6.35 mm
od stainless steel tubing; G, 15 mm x 1.6 mm od stainless steel
tubing; H, nut for 1.6 mm od tubing; I, Spherisorb S.20.W.

FIGURE 3.2.4

FIGURE 3.2.5

porous PTFE disc (C) so that the needle would touch the centre of the stainless steel mesh (E). The porous PTFE prevented septum fragments from blocking the 0.25 mm id tubing (G) which connects the injector to the column inlet by the nut (H). To help minimize further blocking Spherisorb S.20.W (Phase Separations) was placed between two mesh discs, (Figure 3.2.5) to act as a further filter for MWD work on the μ Styragel column series. In all cases the PTFE faced silicone septa was replaced after 15-20 injections. These injection heads could be used for injections <2000 psi.

Two high pressure syringes were used throughout; a 10 μdm^3 SGE high pressure syringe type B with needle guide (pressure limit > 8000 psi) for injection of $\leq 10 \mu\text{dm}^3$ and a 100 μdm^3 Hamilton high pressure syringe No. 1710 (pressure limit ≤ 1500 psi) for injections of 10-100 μdm^3 .

3.2.3 Detector

An ARL UV detector (254 nm, cell volume = 8 μdm^3 , air reference) was used for eluent detection. The tubing leading to the detector cell was shortened so that the column connected to the detector with a minimum of dead volume. The ultraviolet detector offered the maximum sensitivity available for polystyrene samples allowing a smaller amount of sample to be injected, thus minimizing broadening due to viscous fingering and column overload. Therefore, it was assumed that the effect of these latter two characteristics was unimportant. A steady base-line was obtained using the detector on maximum sensitivity at all times when coupled to a Vitron model UR404 chart recorder (Fisons Ltd.).

3.2.4 Solvent Delivery

A Perkin-Elmer Model 1220 positive displacement syringe pump was used because it gave a constant, reproducible, pulse-free flow rate which was essential for testing short columns. Its 500 cm³ solvent capacity was considered adequate for running a series of columns for MWD analysis of polymers. The downtime for refilling and allowing constant flow conditions to be achieved again was not a critical factor. At high flow rates the amount of solvent remaining when column testing can start is sometimes about 300 cm³, but this is still a large volume for high performance GPC systems which usually have a total permeating volume of <50 cm³. The pump operates up to 3000 psi and at flow rates 0.05-6.00 cm³min⁻¹ which exceeds the safe limits for crosslinked polystyrene gels (85). When the reservoir required refilling, the columns were isolated from the pump by a valve. Any air present in the system after refilling was purged out of an outlet in the pumping system before opening the columns to the solvent again. This procedure consumed <50 cm³.

In all cases the retention volume, V_R , was calculated from the chart paper travel because of the accuracy of the pump flow rate.

3.3 GPC CONDITIONS

The eluent was tetrahydrofuran (BDH Chemicals Ltd.) which was destabilized, stored over calcium hydride for 4 hours, then distilled from calcium hydride and degassed by stirring under vacuum before use. The residues from the high performance GPC were redistilled until too opaque to allow adequate detector sensitivity. The solutes were toluene (BDH Chemicals Ltd., AnalaR), tetraphenylethylene (TPE) (Aldrich Chemical Co.) and polystyrene standards (Waters Associates Inc.)

The concentration for all solutes are shown in Table 3.2.

TABLE 3.2

Solute	T. Stone Gel		μ Styragel	
	Injection	Concentration	Injection ^b	Concentration ^c
toluene	10 μ dm ³	0.6% w/v ^a	40 μ dm ³	0.2%
TPE	10	0.02	40	0.2
PS-600	10	0.6	40	0.2
PS-2350	10	0.6	40	0.2
PS-3600	-	-	40	0.2
PS-9800	-	-	40	0.2
PS-15000	-	-	40	0.2
PS-20000	10	0.6	40	0.2
PS-35000	-	-	40	0.2
PS-110000	-	-	40	0.15
PS-200000	-	-	40	0.13
PS-470000	10	0.6 ^a	40	0.1
PS-1987000	10	0.6	40	0.1
PStyGY2	-	-	80	0.2 ^d

(a) Reduced to 0.2% w/v for efficiency versus flow rate work.

(b) Reduced to 10 μ dm³ on calibration of ^{single} μ Styragel columns.

(c) All standard solutions contain 0.1% w/v TPE as internal standard except PS-600 which contains no TPE.

(d) Contains 0.005% w/v TPE.

3.4 BROAD MWD POLYSTYRENE

3.4.1 Preparation

Styrene (Fisons Ltd.), dried over calcium hydride for two weeks, was degassed by a series of freezing-thawing cycles under vacuum. It was then vacuum distilled into a calibrated tube already containing α, α' -azobisisobutyronitrile (AZBN) (BDH Chemicals Ltd.) which had been recrystallised twice from methanol. The styrene was degassed again and after refreezing the tube was sealed. On thawing and checking that the AZBN had dissolved the tube was placed into a thermostatted water bath at 60.0°C . The conditions are shown in Table 3.3.

After 1 hour the tube was withdrawn from the bath, broken open, and the contents tipped into 1 dm^3 of cold methanol. The tube was then washed by a small amount of toluene and the washings also tipped into the methanol. The bulk of the methanol was poured off and the precipitated polymer washed five times with 200 cm^3 methanol. Excess methanol was allowed to evaporate under nitrogen and the polystyrene was dried to constant weight at room temperature $<15\text{ mm}$ pressure. The polystyrene was then isolated by freeze-drying from benzene.

TABLE 3.3

Time	60 min
Temperature	60.0°C
AZBN	0.0896 g
Styrene	18.36 g
Wt. PStyGY2	1.03 g
Yield	5.1 %

Conditions for Bulk Polymerisation of Styrene to give PStyGY2

3.4.2. Characterisation of PStyGY2 by Osmometry

The theory of osmometry is described in detail elsewhere (86). An osmometer consists of two cells separated by a semi-permeable membrane, through which only solvent molecules can pass. If a polymer solution is placed on one side of the membrane and solvent on the other, the activity of the solvent is different in the two cells and solvent molecules diffuse through the membrane to achieve equilibrium. From the osmotic pressure π for a series of solutions having solute concentration c , the molecular weight is determined with Equation 3.1

$$\frac{\pi}{c} = \frac{RT}{\bar{M}_n} + B'c + C'c^2 + \dots \quad 3.1$$

where R is the gas constant, T is the temperature and B' and C' are virial coefficients.

Four dilute solutions of PStyGY2 (approximately 2, 4, 6 and 10 gdm⁻³) in toluene were examined in a Hewlett-Packard Model 502 high speed membrane osmometer at 17.5°C. In this osmometer there is no net solvent transfer through the membrane because of hydrostatic pressure applied to the solute side of the membrane which exactly balances the osmotic pressure at equilibrium. The readings of each solution were noted and between each measurement a reading was taken for pure solvent (toluene). Hence, the osmotic pressure of each solution was calculated. It was assumed that the concentrations used were sufficiently dilute to permit values of c^2 and higher exponents of c to be ignored. A plot of π/c versus c is shown in Figure 3.4.1 and is extrapolated to zero concentration. From the intercept on the π/c axis $\bar{M}_n$ was calculated as 75900.

FIGURE 3.4.1

3.4.3 Characterisation of PStyGY2 by Viscometry

Solution viscosity (86) is measured by comparing the time required, t_c , for a volume of solution having a polymer concentration c to flow through a capillary tube with the time for the same volume of pure solvent, t_o . The relative viscosity η_R and specific viscosity η_{sp} are given by Equations 3.2 and 3.3.

$$\eta_R = \frac{t_c}{t_o} \quad 3.2$$

$$\eta_{sp} = \frac{t_c}{t_o} - 1 = \eta_R - 1 \quad 3.3$$

The limiting viscosity number (intrinsic viscosity), $[\eta]$, is defined by Equations 3.4 and 3.5.

$$\frac{\eta_{sp}}{c} = [\eta](1 + k_1[\eta]c + k'_1([\eta]c)^2 + \dots) \quad 3.4$$

$$\frac{\ln \eta_R}{c} = [\eta](1 + k_2[\eta]c + k'_2([\eta]c)^2 - \dots) \quad 3.5$$

where k_1 , k'_1 , k_2 , k'_2 , etc. are constants. If the concentration c is small Equations 3.4 and 3.5 reduce to Equation 3.6.

$$[\eta] = \lim_{c \rightarrow 0} \frac{L}{c} \frac{\ln \eta_R}{c} = \lim_{c \rightarrow 0} \frac{L}{c} \frac{\eta_{sp}}{c} \quad 3.6$$

The intrinsic viscosity at 25°C was found using a Cannon Fenske viscometer and four solutions of PStyGY2 (approximately 0.3, 0.25, 0.2 and 0.15 g/100 cm³) in toluene. The time for pure solvent (toluene) was also noted. $[\eta]$ is obtained as the common intercept at $c=0$ of plots η_{sp}/c versus c and $\ln \eta_R/c$ versus c shown in Figure 3.4.2. All runs were retimed until

FIGURE 3.4.2

they agreed to within 0.2 s (time for toluene 420.8 s).

The viscosity average molecular weight was found using the Mark-Houwink Equation (Equation 3.7).

$$[\eta] = K \bar{M}_v^a \quad 3.7$$

where K and a are constants for a specific system. $K = 1.1 \times 10^{-4}$ and $a = 0.72$ (87) (same value used in Equation 2.33 for $\bar{M}_v$ by GPC) giving viscosity average molecular weight for PStyGY2 of 112000.

RESULTS
CHAPTER 4 AND
DISCUSSION

The results and discussion are divided into two major sections separated according to the type of gel used.

4.1 T. STONE GEL

4.1.1 Calibration

The calibration curves for each of the gel fractions are shown in Figure 4.1.1. In order to allow for differences in V_R of each standard owing to the non-uniform column length used, V_R divided by column length has been plotted as the abscissa. The error bars have taken account of variations in V_R for all of the standards on the individual columns and the calibration for gel in the size range 10-15 μm is plotted.

The curves are similar suggesting that the air classification procedure did not segregate particles having different porosities. The exclusion limit of the curve is equivalent to the polystyrene molecular weight of about 1500, the same as 60 Å and 100 Å Styragel and for ARgel, grade 805.

The values for PS-1987000 and PS-470000 are not plotted as their V_R values are identical to that of PS-20000. However, the PS-1987000 shows a tailing effect which increases with decrease in particle size. This is probably due to viscous fingering owing to the relatively high concentration of polymer used (85).

4.1.2 Column Efficiency

Toluene plate counts for these columns at a flow rate of 1.0 $\text{cm}^3 \text{min}^{-1}$ are shown in Table 4.1. The characteristic decrease in column efficiency as particle size falls is easily seen (22).

FIGURE 4.1.1

Polystyrene Calibration for T. Stone Gel Columns.

TABLE 4.1

Gel	$\bar{S}_n$ (μm)	$\bar{S}_w$ (μm)	$\bar{S}_w/\bar{S}_n$	N ppf	Column Length L cm
Unclassified	19.74	33.89	1.72	-	-
28 μm	26.34	35.36	1.34	1110	61.0
24-28 μm	27.24	30.60	1.12	1580	61.0
20-24 μm	22.41	25.42	1.13	2340	33.0
16-20 μm	19.31	23.07	1.19	3390	30.5
10-15 μm	13.57	16.17	1.19	4980	25.0
ARgeL 805	32.38	36.76	1.14	843	61.0

Average Particle Diameters and Efficiencies for T. Stone Gel.

However, before any theoretical interpretation can be attempted with these results, the particle size must be clearly defined.

Average particle diameters given in Table 4.1 were calculated from the number fraction distribution given by the Coulter Counter using Equations 4.1 and 4.2

$$\text{number average diameter } \bar{S}_n = \frac{\sum n_i D_i^2}{\sum n_i} \quad 4.1$$

$$\text{weight average diameter } \bar{S}_w = \frac{\sum n_i D_i^4}{\sum n_i D_i^3} \quad 4.2$$

where n_i is the number of particles having diameter D_i corresponding to the mid-point of interval i (these being plotted in Figure 3.2.3).

The size range settings on the air classifier were found from calibration curves for particles having a density of $2.5 \times 10^3 \text{ kg m}^{-3}$, by assuming a density of $1.0 \times 10^3 \text{ kg m}^{-3}$ for a T. Stone gel particle. The values of $\bar{S}_n$ in Table 4.1 fall within these ranges except gel fraction $> 28 \mu\text{m}$ which was not reclassified to decrease the tail at low particle sizes. Hence, its $\bar{S}_n$ value is less than the lower limit of the particle size range. Values of $\bar{S}_w$ approach the maximum peak values of the weight fraction size distribution in Figure 3.2.3. This diagram also shows that a substantial weight of the gel fractions is outside the 4 or 5 μm air classifier size range settings. Hence, there is an overlap among the fractions.

Limpert, Cotter and Dark suggest (23) that high efficiency columns packed with particles having $d_p < 20 \mu\text{m}$ can not be packed if $\bar{S}_w / \bar{S}_n > 1.1$. For $\mu\text{Styragel}$ ($d_p \approx 10 \mu\text{m}$) Waters Associates guarantee a minimum plate count of 4000 ppf for a

100 Å column. In Table 4.1 the plate count for the 10-15 μm gel fraction exceeds this guarantee and the value of $\bar{S}_w/\bar{S}_n$ is in excess of 1.1. Table 4.1 suggests that the upper limit is at least 1.20. This anomaly is probably due to the method of calculating the average particle size.

Limpert, Cotter and Dark (23) have stated that $\bar{S}_n$ and $\bar{S}_w$ are calculated the same way as $\bar{M}_n$ and $\bar{M}_w$ which are given by Equations 4.3 and 4.4

$$\bar{M}_n = \frac{\sum N_i M_i}{\sum N_i} \quad 4.3$$

$$\bar{M}_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \quad 4.4$$

where N_i is the number of molecules having molecular weight M_i . Equations 4.3 and 4.4 are derived from analysing the integrals 2.32 and 2.34 by Simpson's Method. From this reasoning $\bar{S}_w$ should be given by Equation 4.5

$$\bar{S}_w = \frac{\sum n_i D_i^2}{\sum n_i D_i} \quad 4.5$$

Therefore, if $\bar{S}_w/\bar{S}_n$ is calculated using Equations 4.1 and 4.5 its value is smaller than if Equations 4.1 and 4.2 are used. However, the weight of a particle is proportional to D_i^3 , therefore, the total weight of all the particles is proportional to $\sum n_i D_i^3$. A log-log plot of HETP versus weight average particle diameter is shown in Figure 4.1.2. The straight line is a least squares plot through the filled symbols. The open symbol for ARgeL 805 was omitted from this plot because it was sized by sieving and had a different shaped distribution (Figure 3.2.3). This straight line obeys the relation:

FIGURE 4.1.2

Dependence of Efficiency on Weight Average Particle Diameter.

●, T. Stone Gel; ○, ARGel 805

$$H \propto \bar{S}_w^{1.95}$$

4.6

The exponent in Equation 4.6 differs from the value 1.8 found for liquid chromatography separations using silica gels (88) having mean particle diameters 5-40 μm . Unfortunately, not all workers give either their method of calculating or their definition of particle size. Therefore, the value 1.95 is probably acceptable. The points in Figure 4.1.1 can be used to construct a curve where the exponent of $\bar{S}_w$ in Equation 4.6 decreases with particle size as in Equation 4.7.

$$H \propto \bar{S}_w^{f(\bar{S}_w)}$$

4.7

where $f(\bar{S}_w)$ is a function of $\bar{S}_w$. A similar effect has been observed in liquid chromatography by several workers (89). If the data is extrapolated as:

- (i) a straight line, then HETP for $\bar{S}_w = 10 \mu\text{m}$ is about 0.02 mm (16000 ppf)
- (ii) a curve, then HETP for $\bar{S}_w = 10 \mu\text{m}$ is 0.033 mm (9000 ppf).

The value for a curve is a good approximation to observations made for $\mu\text{Styragel}$ ($d_p \approx 10 \mu\text{m}$) under identical conditions during this research (see Section 4.2.1).

Work has shown that column efficiency is dependent on eluent flow rate (23). A minimum in the HETP versus flow rate plot (Figure 2.2.3) is generally observed. This has been studied using a column packed with the 16-20 μm T. Stone gel. Results for a completely permeating solute, toluene (molecular weight = 92), for a partly permeating solute, TPE (molecular weight = 332), and a non-permeating solute, PS-470000, are shown in Figure 4.1.3. These solutes were chosen because of the calibration curve, Figure 4.1.1. The minimum is not present

FIGURE 4.1.3

Dependence of Plate Height on Eluent Flow Rate using T. Stone Gel.

●, toluene; ▲, tetraphenylethylene; ■, PS-470000

for the non-permeating solute which is not subjected to solute mass transfer between the mobile and stationary phases. From Figure 4.1.2 the best column performance is obtained at a flow rate of $0.5 \text{ cm}^3 \text{ min}^{-1}$ when the plate count is 4230 ppf, 840 ppf greater than at $1.0 \text{ cm}^3 \text{ min}^{-1}$.

This work confirms that HETP for a non-permeating solute having a low diffusivity hardly changes as a function of flow rate which was previously shown by Kelley and Billmeyer using porous glass and also Styragel (8,56,90-91). They also suggest that the major contribution to peak broadening for Styragel arises from a non-uniform flow velocity profile across the column (8,56,91). The higher values of HETP for a non-permeating solute are ascribed to a velocity profile effect (8). From Figure 4.1.2, the HETP values for toluene and polystyrene are similar at flow rates below 1.0 min^{-1} , which indicates that the velocity profile effect is much reduced.

For particles having a wide size range care must be taken during packing to prevent them from segregating. This would produce variations in packing density, hence variations in eluent flow across the column. By using regular particles having a narrow size distribution, such as T. Stone gel (Figures 3.2.2 and 3.2.3), packing should result in a homogenous bed of particles. Therefore, peak broadening due to velocity profile effects and eddy diffusion are kept to a minimum in the columns. Column permeability increases as the particle size distribution becomes narrower. For the columns in Table 4.1, pressure drops even at a flow rate of $4.0 \text{ cm}^3 \text{ min}^{-1}$ were not recorded on the pump pressure gauge which only registers pressures in excess of 400 psi.

4.2 μSTYRAGEL COLUMN SET

4.2.1 Calibration

The calibration curves for each of the columns at a flow rate of $1.0 \text{ cm}^3 \text{ min}^{-1}$ are shown in Figure 4.2.1. The values used by Waters Associates for pore size are only approximations as mean pore size may differ from batch to batch owing to chemical factors during gel preparation. The claimed exclusion limits for the gel type and experimental values obtained from the individual calibration curves, Table 4.2, suggest that the $10^6 \text{ \AA}$ column is better classified as a $10^5 \text{ \AA}$ column. This is not an isolated case as a previous $10^6 \text{ \AA}$ μ Styragel column showed the same characteristics and other workers have reported similar observations (92). No detailed work was conducted using the individual columns, except for calibrations. However, it was noted that the column efficiencies were between 6000-9500 ppf using a totally permeating solute (i.e. toluene).

The calibration curve for the four columns connected in series in decreasing pore size ($10^6 \text{ \AA}$ first, $10^3 \text{ \AA}$ last) is shown in Figure 4.2.2. In order to increase the reliability of the work, because measurement of retention volumes by syphon or drop counter was not considered to have sufficient accuracy, an internal standard (TPE) was used, as suggested by Patel (93). The retention volume of TPE was assigned as 100% and the polystyrene standards measured as a ratio to this. TPE was not completely satisfactory as an internal standard for PS-600 because the tails of the two peaks overlapped. To check the assignment of the retention volume for PS-600 in terms of 100 % TPE, independent chromatograms for TPE and PS-600 were obtained from two separate injections. Hence, by resolving the chromatogram of the mixture into two components

FIGURE 4.2.1

Polystyrene Calibration Curves for Individual uStyragel Columns.

■ , $10^6 \text{ \AA}$; ● , $10^5 \text{ \AA}$; Δ , $10^4 \text{ \AA}$; ○ , $10^3 \text{ \AA}$

TABLE 4.2

Gel Type λ	Polystyrene Exclusion Limit		Agreement
	Manufacturer's	From Fig. 4.2.1	
10^6	5×10^7	5×10^6	V. Poor
10^5	2×10^6	5×10^6	Good
10^4	7×10^5	7×10^5	Good
10^3	5×10^4	5×10^4	Good

Table of Exclusion Limits for μ Styragel - Manufacturer's
from Dark W.A. + Limpert R.J. (4) and Values from Fig. 4.2.1

FIGURE 4.2.2

Polystyrene Calibration Curve for four μ Styragel Columns
in Series (10^6 Å, 10^5 Å, 10^4 Å and 10^3 Å)

according to the independent peaks, the retention volumes of TPE (100 %) and PS-600 (in terms of TPE) in the mixture could be assigned. The method of determining elution volumes with reference to 100 % TPE was used for each chromatogram in the determination of peak heights for the calculation of average molecular weights.

From Figures 4.1.1, 4.2.1 and 4.2.2 it is clear that none of the calibration curves are linear according to Equation 2.21. This does not invalidate the equations shown in Section 2.2.2 which can be used to express resolution over a small linear range of the calibration curve.

4.2.2 Polystyrene Standards

A series of polystyrene standards was analysed using the μ Styragel set of four columns at flow rates of $0.1-2.0 \text{ cm}^3 \text{ min}^{-1}$. The chromatograms were analysed using Equations 2.10-2.12 and 2.23 and the results are shown in Tables 4.3-4.8. The position of the calibration curve was hardly altered by flow rate and D_2 was assumed independent of flow rate over the range examined. D_2 was calculated by subjecting the points obtained from all the polystyrene standards used, with molecular weight ≤ 470000 , to leastsquares analysis.

A plot of column efficiency (HETP from Tables 4.3-4.8) versus flow rate for these standards, Figure 4.2.3, shows that at flow rates above $0.1 \text{ cm}^3 \text{ min}^{-1}$ no minimum is observed, except for toluene whose plot is also shown. However, from Figures 4.2.1 and 4.2.2 it can be assumed that toluene is a totally permeating solute. For the polystyrene standards the slope of the efficiency (HETP) versus flow rate curves at any point (D_3) is greater the higher the molecular weight of the standard. This is not as evident from plotting specific resolution (R_{sp} from Tables 4.3-4.8)

FIGURE 4.2.3

Dependence of Plate Height on Flow Rate using μ Styragel

□ , PS-470000; Δ , PS-200000; ▲ , PS-110000; ● , PS-35000;
○ , PS-9800; ■ , PS-3600; x , toluene

<u>TABLE 4.3</u>	-	PS-3600
<u>TABLE 4.4</u>	-	PS-9800
<u>TABLE 4.5</u>	-	PS-35000
<u>TABLE 4.6</u>	-	PS-110000
<u>TABLE 4.7</u>	-	PS-200000
<u>TABLE 4.8</u>	-	PS-470000

Tables Containing Values for Polystyrene Standards on
 μ Styragel Columns

TABLE 4.3

Flow Rate	N ppf	HETP mm	R_{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 $\text{cm}^3 \text{min}^{-1}$	4 500	0.271	2.42	1.115	0.109
0.25	4 350	0.280	2.38	1.113	0.107
0.5	3 900	0.312	2.26	1.111	0.105
1.0	3 620	0.337	2.17	1.116	0.110
2.0	3 460	0.352	2.13	1.135	0.127

TABLE 4.4

Flow Rate	N ppf	HETP mm	R_{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 $\text{cm}^3 \text{min}^{-1}$	2 920	0.417	2.09	1.092	0.088
0.25	2 810	0.433	2.05	-	-
0.5	-	-	-	1.099	0.094
1.0	2 440	0.500	1.91	1.108	0.103
2.0	2 140	0.570	1.78	1.121	0.114

TABLE 4.5

Flow Rate	N ppf	HETP mm	R_{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 $\text{cm}^3 \text{min}^{-1}$	8 110	0.150	3.80	1.005	0.005
0.25	6 530	0.187	3.41	-	-
0.5	5 120	0.238	3.02	1.015	0.014
1.0	3 940	0.310	2.65	1.030	0.030
2.0	2 830	0.431	2.24	1.039	0.038

TABLE 4.6

Flow Rate	N ppf	HETP mm	R _{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 cm ³ min ⁻¹	10 170	0.120	4.64	1.023	0.023
0.25	7 750	0.157	4.05	1.029	0.029
0.5	5 190	0.235	3.32	1.036	0.035
1.0	3 240	0.377	2.62	1.048	0.047
2.0	2 310	0.529	2.21	1.063	0.061

TABLE 4.7

Flow Rate	N ppf	HETP mm	R _{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 cm ³ min ⁻¹	9 120	0.134	4.61	1.024	0.024
0.25	6 380	0.191	3.86	-	-
0.5	4 760	0.256	3.33	1.038	0.037
1.0	2 890	0.423	2.60	1.051	0.050
2.0	1 770	0.689	2.03	1.078	0.075

TABLE 4.8

Flow Rate	N ppf	HETP mm	R _{sp}	$\bar{M}_w / \bar{M}_n$	$\ln(\bar{M}_w / \bar{M}_n)$
0.1 cm ³ min ⁻¹	5 350	0.228	3.81	1.046	0.045
0.25	4 070	0.299	3.32	1.057	0.055
0.5	3 070	0.398	2.88	1.068	0.066
1.0	1 910	0.639	2.27	1.083	0.080
2.0	1 280	0.954	1.86	1.136	0.127

against flow rate, Figure 4.2.4, even though from Equations 2.9-2.10 and 2.23 it follows that:

$$R_{sp} \propto \frac{1}{\sqrt{H}} \quad 4.8$$

providing that the slope of the calibration curve D_2 is constant. However, variations in D_2 mask the slope/molecular weight effect in Figure 4.2.4 and variations in polydispersity among the polystyrene standards will provide different polydispersity contributions to R_{sp} . Comparison of two columns requires R_{sp} to be higher in the molecular weight range where maximum resolution is required. In Figure 4.2.4, the values of R_{sp} at $2.0 \text{ cm}^3 \text{ min}^{-1}$ are higher than the value of 1.14 for a polystyrene standard (molecular weight = 97200) quoted by Yau and co-workers (42) for an equivalent length of $\mu\text{Styragel}$ column.

Values of $\ln(\bar{M}_w/\bar{M}_n)$ calculated from the chromatograms of the standards (shown in Tables 4.3-4.8) may be plotted versus flow rate, Figure 4.2.5, to give a similar plot to that of efficiency (HETP) as a function of flow rate. The dependence of D_3 on molecular weight is again clearly evident as the plots in Figure 4.2.5 are linear at flow rates $\leq 1.0 \text{ cm}^3 \text{ min}^{-1}$. The peak width contains a polydispersity contribution from the MWD, which is not truly monodisperse, as well as a broadening contribution. Hendrickson (18) suggested that the standard deviation is a measure of peak width. The overall standard deviation of a molecular weight distribution σ_o is given by:

$$\sigma_o^2 = \sigma_d^2 + \sigma_I^2 + \sigma_{II}^2 + \sigma_{III}^2 \quad 4.9$$

where σ_d is due to the polydispersity contribution of the polymer and σ_I , σ_{II} and σ_{III} follow from the dispersion terms in Van Deemter's Equation (Equation 2.4). The polydispersity for a

FIGURE 4.2.4

Dependence of Specific Resolution (from Equation 2.23) on Flow Rate for Narrow Molecular Weight Distribution Polystyrenes.

■, PS-3600; ○, PS-9800; ●, PS-35000; ▲, PS-110000;
 △, PS-200000; □, PS-470000

FIGURE 4.2.5

Dependence of Log Polydispersity on Flow Rate for Narrow Molecular Weight Distribution Polystyrenes.

■, PS-3600; ○, PS-9800; ●, PS-35000; ▲, PS-110000;
 △, PS-200000; □, PS-470000

logarithmic normal molecular weight distribution is related to σ_m by (49):

$$\frac{\bar{M}_w}{\bar{M}_n} = \exp \left[\frac{\sigma_M^2}{2} \right] \quad 4.10$$

which can be rewritten as:

$$\ln \left[\frac{\bar{M}_w}{\bar{M}_n} \right] \propto \sigma_M^2 \quad 4.11$$

It follows from Equations 2.4, 2.9-2.10, 4.9 and 4.11 that an expression for the overall polydispersity $\bar{M}_w/\bar{M}_n$ in terms of true polydispersity $(\bar{M}_w/\bar{M}_n)_T$ and flow rate U may then be written in the form:

$$\ln \left[\frac{\bar{M}_w}{\bar{M}_n} \right] = \underbrace{A_I}_{\text{termI}} + \underbrace{\frac{B_{II} \cdot D_m}{U}}_{\text{termII}} + \underbrace{\frac{C_{III} \cdot U}{D_m}}_{\text{termIII}} + \underbrace{\ln \left[\frac{\bar{M}_w}{\bar{M}_n} \right]_T}_{\text{termIV}} \quad 4.12$$

where A_I , B_{II} and C_{III} are constants. TermI results from eddy diffusion in the mobile phase. It has been shown in Figure 4.1.2 that the column efficiency for a non-permeating solute hardly changes as a function of flow rate. Consequently, termI in Equation 4.12 may be regarded as constant and independent of flow rate. Furthermore, for one standard, $(\bar{M}_w/\bar{M}_n)_T$ will be constant. As the molecular weight of a polymer increases, the diffusion coefficient D_m of a polymer molecule decreases. The minimum in the efficiency versus flow rate plot occurs because termII in Equation 4.12 is larger than termIII at low flow rates. As D_m decreases termII decreases and termIII increases; therefore, termII becomes larger than termIII at much lower flow rates as solute size increases. In Figure 4.2.5 it is evident that a minimum occurs at a flow rate of $0.5 \text{ cm}^3 \text{ min}^{-1}$ for PS-3600 and that a minimum is not

observed at experimental flow rates below $0.5 \text{ cm}^3 \text{ min}^{-1}$ for PS-9800. Since $\ln(\bar{M}_w/\bar{M}_n)$ and HETP are a measure of chromatogram broadening, it is clear that the slope D_3 is proportional to C_{III}/D_m in Equation 4.12 as shown in Figure 4.2.6. The slope of the linear portion of the curves in Figure 4.2.5 (at flow rates $\leq 1.0 \text{ cm}^3 \text{ min}^{-1}$) has been plotted against $1/D_m$. D_m is estimated for the polystyrene standards in tetrahydrofuran at 20° C using the Wilke-Chang Equation (94-95). Error bars are shown for values of $1/D_m$ as the average error in D_m using the Wilke-Chang Equation is 10 % (95). The value for PS-3600 was omitted from Figure 4.2.6 as PS-3600 shows a minimum in Figure 4.2.5; hence, D_3 for PS-3600 is not proportional to term III in Equation 4.12 at experimental flow rates $< 1.0 \text{ cm}^3 \text{ min}^{-1}$.

From these results it is clear that high polymers can give high efficiencies at low flow rates, while low polymers are less affected by flow rate. Therefore, high speed GPC is of use when separating low polymers, and high resolution at low flow rates is more important when separating high polymers.

4.2.3 Broad MWD Polystyrene

A free radical bulk polymerisation of styrene initiated by the thermal decomposition of AZBN, such as in PStyGY2, is not susceptible to chain transfer reactions at low conversion. It is suggested that the termination mechanism is by bimolecular combination of radicals (96). This gives a polymer of polydispersity 1.50 (97). The high performance GPC described in Section 3.2 using μ Styragel columns was used to analyse PStyGY2 at various flow rates. The values for average molecular weights are shown in Table 4.9 and $\ln(\bar{M}_w/\bar{M}_n)$ versus flow rate is plotted in Figure 4.2.7. An independent determination of average molecular

FIGURE 4.2.6

Dependence of the Slope of Log Polydispersity Versus Flow
Rate on Reciprocal of Diffusivity for Polystyrene Standards

TABLE 4.9

Flow Rate	$\bar{M}_n$	$\bar{M}_v$	$\bar{M}_w$	$\bar{M}_w / \bar{M}_n$
-	75 900	112 000	-	-
0.1 cm ³ min ⁻¹	79 700	121 000	128 500	1.613
0.25	79 450	120 400	128 800	1.622
0.5	79 250	120 400	127 750	1.625
1.0	78 250	123 000	130 050	1.665
2.0	76 050	125 100	129 200	1.699
1.0 (RAPRA)	66 750	115 200	126 500	1.847

Table of average molecular weights from computer analysis (55) of the GPC chromatograms and values of $\bar{M}_n$ from osmometry and $\bar{M}_v$ from viscometry.

FIGURE 4.2.7

Dependence of Log Polydispersity on Flow Rate for PStyGY2.

weights was carried out by the SRC sponsored Polymer Supply and Characterisation Centre (PSCC) (RAPRA, Shawbury, Shrewsbury) using a conventional GPC having 16 ft of Styragel columns (gel porosities 700-2000 Å, 1.5×10^4 - 5×10^4 Å, 7×10^5 - 5×10^6 and 5×10^6 - 10^7 Å each packed into 4 ft long columns, 7.6 mm id (98)). The PSCC chromatogram and calibration curve were processed by the same computer program (55) as that used for the results quoted from the μ Styragel experiments in Table 4.9.

From the interpretation of Figure 4.2.5 in terms of Equation 4.12, the slope of a plot of $\ln(\bar{M}_w/\bar{M}_n)$ versus U is directly proportional to term III. Therefore, it follows from Figure 2.2.3 that extrapolation of such a plot to zero flow rate will give a value of $\bar{M}_w/\bar{M}_n$ containing contributions from chromatogram broadening owing to eddy diffusion and from polydispersity. If $(\bar{M}_w/\bar{M}_n)_T$ is the major contribution, then the value of $\bar{M}_w/\bar{M}_n$ for PStyGY2 of 1.609 obtained by extrapolating the data in Figure 4.2.7 can be compared with polydispersity expected from the polymerisation mechanism. $(\bar{M}_w/\bar{M}_n)_T$ is larger than the value 1.50 predicted by the polymerisation and can be justified if part of the polymerisation termination process is by a disproportionation mechanism (96). For termination entirely by disproportionation $\bar{M}_w/\bar{M}_n$ would be 2.00 (97).

Berger (99-100), has attempted to measure the amounts of termination by combination and disproportionation over a range of temperatures. From the work of Berger (100), the fraction of combination at 60°C is approximately 75%. An equation can be derived (101-102) involving the weight fraction of combination w_2 and $(\bar{M}_w/\bar{M}_n)_T$

$$2 \left[\frac{\bar{M}_w}{\bar{M}_n} \right]_T = 4 - w_2^2 \quad 4.13$$

with the extrapolated value of 1.609 for $\bar{M}_w/\bar{M}_n$ from Figure 4.2.7 the fraction of combination is approximately 90%. If a minimum appears in $\ln(\bar{M}_w/\bar{M}_n)$ versus flow rate plot for PStyGY2 as argued in Section 4.2.2, the fraction of combination would be nearer the value found by Berger.

A comparison between experimental and theoretical values for the differential weight distribution $w(M)$ of PStyGY2 at a flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$ is shown in Figure 4.2.8. The experimental values were calculated by a computer program (55) and the theoretical values are calculated from Equations 4.14 for termination entirely by combination and 4.15 for termination by both combination and disproportionation (97,102).

$$w(M) = \frac{4M^2}{\bar{M}_n^3} \exp\left[-\frac{2M}{\bar{M}_n}\right] \quad 4.14$$

$$w(M) = (1 - w_2) \cdot \frac{M}{\bar{M}_{n1}^2} \exp\left[-\frac{M}{\bar{M}_{n1}}\right] + w_2 \frac{4M^2}{\bar{M}_{n2}^2} \exp\left[-\frac{2M}{\bar{M}_{n2}}\right] \quad 4.15$$

where M is molecular weight and $\bar{M}_{n1}$ and $\bar{M}_{n2}$ are the number average molecular weights of the fractions resulting from disproportionation and combination respectively. w_2 is found from Equation 4.13 using the value of $(\bar{M}_w/\bar{M}_n)_T = 1.613$ for PStyGY2 at $0.1 \text{ cm}^3 \text{ min}^{-1}$ and $\bar{M}_{n1}$ and $\bar{M}_{n2}$ are calculated from Equation 4.16 (97,102).

$$\bar{M}_{n1} = \frac{\bar{M}_{n2}}{2} = \bar{M}_n \left(1 - \frac{w_2}{2}\right) \quad 4.16$$

The value $\bar{M}_n = 75900$ from osmometry was used. It is clear from Figure 4.28 that neither of the theoretical distributions fit the experimental curve. Both the theoretical values peak at higher molecular weights and their high molecular weight tail is too short. These results suggest that broadening owing to eddy diffusion is still significant at a flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$.

FIGURE 4.2.8

Comparison between Experimental and Theoretical Differential Weight Distributions for PStyGY2 on μ Styragel at $0.1 \text{ cm}^3 \text{ min}^{-1}$.

Curve for Experimental Values; O, from Equation 4.14; ●, from 4.15

The values of $\bar{M}_n$ and $\bar{M}_w$ obtained by high performance GPC all compare well with the values found by osmometry and viscometry. At worst the value for $\bar{M}_n$ at $0.1 \text{ cm}^3 \text{ min}^{-1}$ is 5% greater than that of osmometry. Even this compares well with the independently determined value (RAPRA) which is 12% lower. The analysis time for RAPRA was approximately 160 minutes while analysis by high performance GPC at 2.0 cm^3 was 22 minutes, therefore, faster, more accurate GPC measurement can be made using smaller particle sizes.

Figures 4.2.5 and 4.2.6 show that not all molecular species are broadened to the same degree. Therefore as flow rate is reduced the front of the chromatogram for a polymer having a broad MWD is affected more than the tail. Hence, the shape of the chromatogram will be dependent on flow rate. However, this doesnot appear to be significant for PStyGY2.

4.3 ERRORS

The errors involved can be divided into two categories, machine error and operator error.

The main source of machine errors is centred around the pump. Fluctuations in flow rate are dependent on the pump system and errors of 1-4% can be tolerated for a short time, but flow rate repeatability must be better than 0.3% otherwise errors in $\bar{M}_n$ and $\bar{M}_w$ will be ~6% (74-75). For the syringe pump in this work adequate time must be allowed for the system to reach equilibrium otherwise errors ~ 0.3% will result (76-77). Both these considerations are important as retention volumes were calculated from the chart recorder speed used. The effect of variations in flow rate can be magnified by variations in chart speed. Using TPE as an internal standard should help reduce flow rate errors. The response time of the detector to give a signal to the chart recorder is also important, as is the time for this signal to be recorded by the chart recorder. Both these errors are small in comparison to errors which result from flow rate. Throughout the work no corrections were made for machine dead volume. It was considered that this was too small to measure accurately. In every case the injection time was negligible (< 0.5 s) compared to the time required for the peak to elute.

Operator errors arise from the measurement of peaks on the chromatograms. This involves measurement of peak height, peak width and peak retention volume. To calculate peak efficiencies Equation 2.12 was used as these results were more constant than from efficiencies measured by Equation 2.13. Variations in peak for PStyGY2 gives rise to further errors when calculating average molecular weights, but these errors are minimized by using the internal standard method based on TPE. If the peak position is

moved the variation in average molecular weights is not proportional to the peak movement as the calibration curve, Figure 4.2.2, is not linear.

All results presented are the average of three or more determinations under identical conditions.

CHAPTER 5

CONCLUSIONS
AND
RECOMMENDATIONS

In the present work the ability of crosslinked polystyrene gels to separate different molecular species has been examined. Resolution and efficiency have been used to monitor the effect of flow rate on narrow and broad molecular weight distribution polystyrenes in an effort to run GPC columns in a high performance mode.

Toluene, a totally permeating solute, was used to study with respect to flow rate the efficiency of individual columns, containing gel with a single pore size ($\sim 80 \text{ \AA}$) and a narrow pore size distribution. The empirical relationship $H \propto \bar{S}_w^\beta$ was demonstrated for these gels (particle size 16-37 μm) where the value of $\beta = 1.95$ is similar to values obtained for silica gel in the same size range (89). It was suggested that this relationship is not truly linear when plotted on a log-log scale, and β is a function of weight average particle size $\bar{S}_w$. Efficiency for a non-permeating solute on these gels was shown to be almost independent of flow rate as found for larger porous glass and Styragel particles (8, 56, 90-91). At low flow rates the efficiency of the non-permeating solute was similar to the totally permeating solute, indicating that dispersion owing to velocity profile effects was minimal.

The remainder of the work has involved a set of four μ Styragel columns connected in series and containing gel (particle size $\sim 10 \mu\text{m}$) of different porosities ($10^6 \text{ \AA}$, $10^5 \text{ \AA}$, $10^4 \text{ \AA}$ and $10^3 \text{ \AA}$). Six narrow molecular weight distribution polystyrenes and a broad molecular weight distribution polystyrene were examined in order to study column efficiency and chromatogram broadening as a function of flow rate. High molecular weight polystyrenes gave high efficiencies at low flow rates while low polymers were less affected by flow rate. The slope of the log polydispersity versus flow rate plot for each polystyrene gave a linear relation when

plotted against the reciprocal of the molecular diffusivity. Therefore, high resolution GPC at low flow rates is of great importance when examining high molecular weight samples and high speed is of use when separating oligomers by GPC. Hence, the shape of the chromatogram of a very broad polymer will change with flow rate as the high molecular weight tail is more susceptible than low molecular weight species to such flow rate changes. A similar log polydispersity versus flow rate plot was also constructed for a broad distribution polystyrene. This plot was extrapolated to zero flow rate to give an intercept from which the true polydispersity of the polymer could be determined. However, it was demonstrated that this was only valid when the contribution owing to eddy diffusion was minimal. A comparison between theoretical and experimental differential weight distributions for the broad distribution polystyrene at the lowest flow rate examined showed that broadening was still present.

A better insight into the use of high performance GPC would be gained if detailed knowledge of the particle size distribution of the packings in the μ Styragel column set was known. It is believed that μ Styragel (23) has a wider particle size distribution than the gel particles in the columns in Table 4.1 (84). Particle size, particle size distribution and choice of pore sizes could be varied to observe any change in slope of the log polydispersity versus flow rate plot for all the polystyrenes examined on the column set. A very narrow particle size distribution should help minimize eddy diffusion, hence, allow more accurate estimation of true polydispersity. Although a particle size of 5 μ m would lower the eddy diffusion contribution, narrow particle size distributions for very small polystyrene particles are difficult to produce experimentally. Consequently, for particles with sizes below

10 μm , silica packings with narrow particle size distributions may be preferable. It would also be advantageous to examine a series of polymers having a range of known polydispersities so an assessment of the eddy diffusion contribution to broadening can be made.

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