HIGH PERFORMANCE GEL PERMEATION CHROMATOGRAPHY
WITH SILICA MICROSPHERES

by

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For Angela
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ABSTRACT

An instrument for high performance gel permeation chromatography (HPGPC) has been assembled with equipment designed for high performance liquid chromatography separations. The pump was a syringe-type giving uniform pulseless flow from 0.05 to 6.0 cm$^3$ min$^{-1}$ at pressures up to 3000 p.s.i.. Low dead-volume septum and septumless injection heads have been used for single columns and multiple column sets. Two ultraviolet detectors and a refractometer having low cell volumes were used to detect the low weights of solute which were separated.

Narrow distribution silica gels of varying particle size and porosity, as supplied by A.E.R.E. Harwell, have been packed into columns. Efficiencies of permeating and non-permeating polystyrenes determined with this HPGPC instrument gave an assessment of chromatogram broadening due to mass transfer as a function of eluent flow rate and polystyrene molecular weight. The results show that fast separations can be obtained in several minutes and that the most precise measurements of polydispersity are accomplished at very slow rates. A further assessment of chromatogram broadening was made by deactivating the surface of the silica particles with a hydrophilic bonded phase and examining the column efficiency and polydispersity of proteins in aqueous buffer.

Results show that even after surface modification the composition of the aqueous eluent used must be adjusted, e.g. by careful selection of pH and ionic strength, to minimize interactions. When interactions occur, they can be explained in terms of a thermodynamic representation of a mixed mechanism. A comparison of retention data for proteins, which do not participate in interaction effects with the stationary phase, dextrans, which are considered as "non-ionic" macromolecules, and polystyrenes suggest that solute diameter is a reasonable universal size parameter for the representation of macromolecules separating by a steric exclusion mechanism operating close to equilibrium conditions.
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CHAPTER 1  INTRODUCTION
Although much effort has been directed towards the determination of the molecular weight distributions of polymers by fractionation techniques, there are few accurate comparisons of experimental and theoretical distributions for polymers prepared under carefully controlled conditions. The limitations of the classical fractionation techniques relying on the molecular weight dependence of polymer solubility are well documented (1,2). These techniques are often tedious, time consuming and inefficient, so that in spite of considerable experimental effort spanning several days or weeks the molecular weight distribution is generally unreliable for quantitative use.

Gel permeation chromatography (GPC) is a form of liquid-liquid partition chromatography, which was first described in 1941 by Martin and Synge (3). 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 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; they stated that fast analysis by liquid chromatography would require small particles (microparticulate packings, particle diameter <30 μm), hence high pressure drops in order to achieve fast flow rates. The description of GPC by Moore (4) in 1964, and the subsequent availability of a commercial GPC instrument (5), attracted widespread interest. This technique is much more rapid (several hours) and convenient than other fractionation methods, giving reliable and reproducible chromatograms. Today, gel permeation chromatograms are widely used for routine polymer characterisation and for quality control measurements.

Although the experimental fractionation technique has greatly improved, the determination of molecular weight distribution is still
not entirely satisfactory. In order to calculate a distribution from a gel permeation chromatogram, it is necessary to establish a calibration relation between molecular weight and retention volume and to have a correction procedure for chromatogram broadening. Many reliable procedures for determining the calibration relation are available (6). However, correction for chromatogram broadening involves a long mathematical process, which was first proposed by Tung (7). Broadening 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 molecular weight distributions (MWDs) from gel permeation chromatograms. Much effort has been directed towards improving Tung's method as reviewed elsewhere (9-11) but many of these do not always produce reliable corrections. Consequently, broadening corrections are omitted from many publications in which distributions and average molecular weights have been calculated from gel permeation chromatograms.

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 eluent flow rate (12-19). Although the number of plates per minute was increased there was an overall decrease in resolution (19-21) owing to broadening. Investigations into the effect of particle size were carried out from the
beginning of GPC. Moore (4) described two particle sizes 50–75 and 75–100 μm, but their effect on column efficiency was not reported. Later Peaker and Tweedale (22,23) confirmed that a decrease in particle size gives progressive increase in efficiency over larger sizes using 15–45, 45–63, 63–90 and 90–150 μm particles. Narrower fractions of all these crosslinked polystyrene gels were prepared by Waters Associates (12,13), 10–15, 25–37 and 37–42 μm, but the increases in efficiency using small particles were not clearly shown. However, further work by Waters Associates (24) with equipment more suitable for small particles, 3–8, 8–12, 18–25 and 37–75 μm, identified the significant increase in efficiency obtainable.

During this period important advances in the theoretical understanding and in the experimental practice of conventional liquid chromatography indicated that high efficiency separations could be obtained with silica microspheres (25). A particularly significant publication in 1972 identified the performance which was possible with porous silica microspheres (<10 μm) prepared in narrow particle size ranges (26). These microspheres were packed into columns by slurry-packing techniques and plate heights below 100 μm were determined. This performance corresponded to about 1000 plates per min. and Kirkland (26) demonstrated excellent resolution of three polystyrene standards in less than 45 s at 1 cm³ min⁻¹ with a column (250 x 2.1 mm) packed with 5–6 μm silica. This performance is an order of magnitude better than the original GPC system (4,5) which generated 70 plates min⁻¹ at 1 cm³ min⁻¹, with banks of columns (500 cm x 7.5 mm) giving separation times of about 3 hours. During the 1970's GPC received considerable attention which was covered in the bibliography of three reviews (27–29). More recently a large amount of work has been done on GPC of water-soluble polymers (30).

The increase in the efficiency of polymer separations gained by the microparticulate packings has led to the name high performance GPC (HPGPC),
but the technique has been called high resolution, high speed, high pressure and even high price GPC. The prefix high performance is preferred because a polymer scientist will aim for both high resolution and high speed. The operating pressure may or may not be high depending on the regularity and particle size distribution of the packing and on column length. If HPGPC equipment is run to give analysis comparable to conventional GPC; the increase in resolution might be sufficient to dispense with the chromatogram broadening 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. Microparticulate packings with particle diameters in the range 8–20 μm having mean pore diameters in the range 8–120 nm were characterised and packed into columns using three packing techniques. These columns were used to study the broadening of chromatograms obtained for anionically prepared polystyrene standards of narrow MWD, with THF as eluent, and globular proteins, with aqueous buffer as eluent. Column efficiency results are described for permeating and non-permeating solutes as a function of flow rate, in order to assess the contributions to chromatogram broadening in HPGPC arising from solute dispersion in the mobile phase and during mass transfer. These results show how the mass transfer contribution increases for high polymers as the diffusion coefficient for solute in the stationary phase $D_s$ falls and estimates of $D_s$ are determined. Additionally, a procedure for including the contribution from the polydispersity of a polymer in theoretical plate height equations is described and using plate height data values for polydispersity are determined. The proposal that the extent of solute permeation in a steric exclusion mechanism is dependent on the mean external length or molecular projection for various types of molecules has been studied, and the occurrence of
secondary mechanisms arising from interactions between solute and stationary phase has been considered.
CHAPTER 2 THEORY
2.1 DISPERSION AND PERFORMANCE

Various theories of steric exclusion and restricted diffusion and theories 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 (31); however, it is generally agreed that the major mechanism of the GPC process is steric exclusion (32). This theory assumes that the separation of a solute of given size in solution is given by a distribution coefficient, $K_D$, which determines the volume of stationary phase solvent that is accessible within the porous gel particles to this solute. Using chromatographic terminology:

$$V_R = V_o + K_D V_i$$

where $V_R$ is the retention volume for the solute calculated from the point of injection onto the column to the appearance of the maximum height of the chromatogram.

$V_o$ is the interstitial volume (mobile phase volume).

$V_i$ is the stationary liquid volume (pore volume).

The dependence of $K_D$ on $V_R$ as a function of solute size and the size distribution of the pores within the gel particles can be calculated theoretically (33-36). When solute molecules are larger than the pore size, $K_D = 0$ and they are excluded from the gel. When the species are totally permeating $K_D = 1.0$. The slope of the calibration curve depends on the pore size distribution of the gel, the separation power being inversely proportional to the calibration slope, as shown in Figure 2.1.1.

Separations in which $V_R$ is higher than expected from a steric exclusion mechanism result from polymer-sorbent interactions which must be weak and reversible so that the polymer is not completely retained in the stationary phase. Dawkins and Hemming (37) suggest that the displacement of a calibration curve from that of a polymer separating solely by steric exclusion increases as polymer size decreases. Therefore,
Figure 2.1.1

Size separation and resolution in GPC.
the GPC mechanism can be considered as a network-limited separation, as proposed by Heitz and Kern (38,39). In liquid chromatography of small molecules (25) retention is expressed by the capacity factor, $k'$, defined by:

$$k' = \frac{K_{p}V_{i}}{V_{o}}$$ \hspace{1cm} (2.2)

where $K_{p}$ is the distribution coefficient for solute partition between the stationary and mobile phases. For polymers, the fraction of $V_{i}$ available will depend on solute size, therefore, Equation 2.2 becomes:

$$k' = \frac{K_{p}D_{i}V_{i}}{V_{o}}$$ \hspace{1cm} (2.3)

However, $k'$ is related to $V_{R}$ by

$$V_{R} = V_{o}(1 + k')$$ \hspace{1cm} (2.4)

From Equations 2.3 and 2.4 we obtain:

$$V_{R} = V_{o} + K_{p}D_{i}V_{i}$$ \hspace{1cm} (2.5)

For a steric exclusion separation with an inert stationary phase, Equations 2.1 and 2.5 are identical with $K_{p} = 1.0$. For separations operating close to equilibrium conditions at constant temperature $T$ the distribution coefficient is related to the free energy change for the transfer of solute molecules from the mobile phase to the stationary phase. The retention volume is determined by the entropy change $\Delta S$ and the enthalpy change $\Delta H$ is the Equation (40):

$$V_{R} = V_{o} + V_{i}\exp(-\Delta H/kT)\exp(\Delta S/k)$$ \hspace{1cm} (2.6)

where $k$ is Boltzmann's constant. Because steric exclusion is dominated by an entropy contribution (33 - 36) it was proposed that interaction effects as represented by $K_{p}$ are dominated by an enthalpy contribution (40). It follows from Equations 2.5 and 2.6 that $K_{p} > 1.0$ for an attractive interaction, such as partition and adsorption, and that $K_{p} < 1.0$ for a repulsive interaction. The early elution of some polymers has been interpreted in terms of $K_{p}$ below unity corresponding to a secondary mechanism of partial exclusion by polymer incompatibility with the stationary phase (41).
2.1.1 Resolution

In any chromatographic experiment the chromatogram for a monodisperse solute of a specific size does not appear as a narrow rectangle but has a Gaussian shape. The extent of this chromatogram broadening is characterised by the standard deviation $\sigma_v$, and is usually determined from the plate count or plate number $N$,

$$N = \left[ \frac{V_R}{\sigma_v} \right]^2$$

with a chromatogram for a monodisperse solute having a low molecular weight. For the Gaussian peak in Figure 2.1.2, $\sigma_v$ can be found from:

$$H_1 = \frac{1}{\sigma_v \sqrt{2\pi}} \exp \left[ \frac{-(V - V_R)^2}{2\sigma_v^2} \right]$$

where $H_1$ is the peak height at retention volume $V$. Using Equations 2.7 and 2.8, plate count is then given by:

$$N = 5.54 \left[ \frac{V_R}{w_1^{1/2}} \right]^2 = 16 \left[ \frac{V_R}{w} \right]^2$$

where $w_1^{1/2}$ is peak width at half peak height and $w$ the width at the base of the peak from tangents drawn at the points of inflection on the peak (Figure 2.1.2).

If a polymer contains several species of very different sizes, then peaks for each monodisperse species will be obtained when $w$ is minimised. The resolution, $R$, of a column is usually defined as the ability of a column to separate two Gaussian peaks of equal width $w$ by a distance $\Delta z$ where:

$$R = \frac{\Delta z}{w}$$

For the case of two monodisperse solutes 1 and 2 having different sizes, as shown in Figure 2.1.1:

$$R = \frac{\Delta z}{\frac{1}{2}(w_1 + w_2)} = \frac{2(V_2 - V_1)}{w_1 + w_2}$$
Figure 2.1.2

Relationship between peak height and peak width for a Gaussian-shaped peak.

\[ w_{\frac{1}{2}} = 2.354\sigma \]

\[ w = 4.000\sigma \]
To achieve good resolution, $R$ must be greater than unity, i.e. $(V_2 - V_1) > w$.

The restriction $0 < K_D < 1.0$ is unique to GPC as $K_D > 1$ is common in other forms of liquid chromatography where solute-gel interactions can occur. Therefore, the number $n$ of components in a sample which can be resolved by GPC is related to column efficiency as defined by plate count.

Giddings (42) suggested the following relation:

$$n \approx 1 + 0.2N_{t}^{1.2}$$

so that $n \sim 21$ for a column with 10000 plates.

The resolution of components in a sample also depends on pore size distribution which determines the separation power of a packing. Therefore, an increase in separation power raises the numerator in Equation 2.11.

A practical consequence of the importance of separation power is shown in Figure 2.1.1 for the separation of two polymers with different molecular weights $M_1$ and $M_2$. It is also evident from Figure 2.1.1 that raising $V_i$ also increases separation power. Resolution for the soft homogeneously cross-linked organic gels, originally developed for separations of biopolymers in aqueous media (43,44), is invariably higher than for semi-rigid gels, such as the gels developed by Moore (4), and for inorganic packings, such as silica (45) and porous glass (46,47), because soft gels have a greater total pore volume per gel particle. Altgelt (32) suggested that the maximum value for $R$ is given by:

$$R_{\text{max}} = \frac{N_{t}^{1.2}V_i}{4V_o}$$

Although Equation 2.11 is an acceptable definition of column performance, the definition of resolution can be extended to incorporate the linear semi-log calibrations in Figure 2.1.1 and the polydispersity of the two polymeric solutes. Since $\log M_1 - \log M_2$ is generally linearly proportional to $V_2 - V_1$, Bly (48) proposed a definition of specific resolution $R_s$:

$$R_s = \frac{2(V_2 - V_1)}{(w_1 + w_2)\log_{10}(M_1/M_2)}$$
Values for $R_s$ will be independent of the polymeric solutes provided the samples have narrow molecular weight distribution. Bly (48) proposed that weight average molecular weight $M_w$ values should be used for $M_1$ and $M_2$, which is substantiated by the work of Berger and Schultz (49) who observed that the molecular weight at the peak height maximum of a chromatogram is $M_w$ when the chromatogram corresponds to an exponential distribution function. Since the values of $w_1$ and $w_2$ will contain contributions from molecular weight distribution, Equation 2.14 must be extended for polydisperse samples; expressions are given elsewhere (48 - 51). In Figure 2.1.1 the slope of the calibration curve $D_2$ is given by $d(\log_{10} M/dV)$ and on substituting this in Equation 2.14 we obtain (50):

$$R_s = \frac{2dV}{(w_1 + w_2)d\log_{10} M} \quad 2.15$$

This is the inverse of the logarithm of the resolution index $R_I$ proposed by Smith and Feldman (52):

$$R_I = \frac{1}{\log_{10} M/dV} \quad 2.16$$

A simplified approach has been developed by Yau et al. (53) which assumes Gaussian peaks in a linear portion of the calibration curve. Equation 2.15 can be written as:

$$R_s = \frac{\Delta V}{w \Delta \log_{10} M} \quad 2.17$$

The linear calibration curves in Figure 2.1.1 can be expressed by (54):

$$M(V) = D_2 \exp(-D_2 V) \quad 2.18$$

where $D_2$ is given by:

$$V = \frac{\Delta \ln M}{D_2} \quad 2.19$$

substituting Equation 2.19 into 2.12 and using $w = 4\sigma_v$:

$$R_s = \frac{\Delta \ln M}{4D_2\sigma_v \Delta \log_{10} M} = 0.576 \frac{\Delta \ln M}{D_2\sigma_v} \quad 2.20$$
From Equation 2.15 resolution is only dependent on the slope of the calibration curve $D_2$ and the standard deviation of the chromatogram $\sigma_v$.

A similar relationship using a linear calibration has been derived by Hamielec and Ray (55) from Tung's axial dispersion Equation (7) in which the average molecular weights, $M_K(t)$, are compared to the experimental values $M_K(\infty)$.

$$\frac{M_K(t)}{M_K(\infty)} = \exp \left[ \frac{(3 - 2K)D_2}{4h} \right]$$

where $K = 1, 2, 3$ refers to the number-, weight-, and z-average molecular weights respectively and $h$ is a dispersion factor given by $1/2\sigma_v^2$ for a monodisperse solute. From Equation 2.16 a specific resolution factor can be defined (54):

$$R_s(K, M_i) = \frac{(-1)^K4h}{(2K - 3)D_2^2}$$

where subscripts $K$ and $M_i$ signify the average molecular weight and the molecular weight at $V$ respectively.

Meyerhoff (56) has used the polydispersity $d$ in a similar manner where subscripts $G$ and $T$ refer to the experimental and true molecular weight distributions:

$$R_s = \frac{d_G - 1}{d_T - 1}$$

Yau et al. (53,57) has developed this approach in order to calculate the relative errors in number- and weight-average molecular weights ($\overline{M}_n^*$ and $\overline{M}_w^*$) from the experimental ($G$) and true ($T$) values:

$$\overline{M}_n^* = \frac{(\overline{M}_n)_G - (\overline{M}_n)_T}{(\overline{M}_n)_T} = \exp \left[ -\frac{1}{2}(D_2\sigma_v)^2 \right] - 1$$

$$\overline{M}_w^* = \frac{(\overline{M}_w)_G - (\overline{M}_w)_T}{(\overline{M}_w)_T} = \exp \left[ \frac{1}{2}(D_2\sigma_v)^2 \right] - 1$$
Resolution can be increased by increasing the column length provided that the 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 are operated in a recycle mode (58). 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. Most of the above expressions can be extended to account for operating a GPC instrument in the recycle mode.

Hamielec (11) has extended Equation 2.23:

$$\frac{M_K(t)}{M_K(\infty)} = \exp \left[ \frac{1}{n} \cdot \frac{(3 - 2K)D_2^2}{4h} \right]$$

where $n$ is the number of recycle passes and $h$ and $D_2$ are found from a single pass.

2.1.2 Chromatogram Broadening

Theoretical interpretations (59) of column efficiency consider the dependence of the plate height $H$ on the mechanisms of solute dispersion in the mobile and stationary phases. The plate height may be thought of as the rate of change of peak (or solute zone) variance (in units of length) relative to the distance migrated, $L$. The variance is the square of the standard deviation $\sigma^2$, so that $H$ is defined by:

$$H = \frac{\sigma^2}{L}$$

If there are several solute dispersion mechanisms contributing to chromatogram broadening and if these mechanisms are independent of each other, it follows from the laws of statistics that the variance of the chromatogram will be the sum of the variances associated with the individual mechanisms, i.e.

$$H = \sum_{i} \frac{\sigma_i^2}{L}$$
The plate height for an experimental chromatogram is calculated from the expression:

\[ H = \frac{L}{N} \]  \hspace{1cm} \text{2.29} \]

having determined \( N \) with Equation 2.9.

The following expressions for \( H \), consisting of the summation of the terms for the solute dispersion mechanisms, follow from a random walk model developed by Giddings (59), but it should be remembered that Equations for \( H \) may be derived from a plate model, a non-equilibrium theory, and a mass balance model (59-62). In the random walk model the movements of the solute molecules are superimposed on the linear mobile phase velocity \( u \). In the flow of liquid between particles in a packed bed, four major dispersion mechanisms may be identified as follows:

(i) Dispersion arising from \textit{eddy diffusion} because some solute molecules are in mobile phase streamlines which move for some distance directly between particles, whereas other molecules are in streamlines whose path is obstructed by the particles and so the streamlines must go round the particles.

(ii) Dispersion arising from \textit{molecular diffusion} in the longitudinal direction in the mobile phase.

(iii) Dispersion due to resistance to \textit{mass transfer in the mobile phase} which consists of streamlines each with a different velocity, so that solute molecules in different streamlines will move with different relative velocities.

(iv) Dispersion due to resistance to \textit{mass transfer in the stationary phase} because at any instant a fraction of the molecules will be in the stationary phase and are left behind the remaining fraction which are in the mobile phase.
Expressions for $\sigma^2$ for all these mechanisms can be derived and, if it is assumed that the mechanisms are independent, the plate height is given by:

$$H = 2 \lambda d_p + \frac{2D_m}{u} + \frac{\omega d_P^2u}{D_m} + \frac{qrd^2u}{D_s}$$  \hspace{1cm} \text{(i) (ii) (iii) (iv)}$$  \hspace{1cm} 2.30$$

where $\lambda$ = a constant close to unity which depends upon the packing; $d_p$ = the particle diameter; $\phi$ = the tortuosity factor, which is the degree to which diffusion is restricted by the column packing ($<1$); $D_m$ = the diffusivity of the solute in the mobile phase; $\omega$ = column coefficient determined by packing structure, column diameter and column shape; $q$ = configuration factor, which depends on the shape of the stationary phase (uniform slab, rod, hemisphere, etc.); $r$ = a constant dependent upon the relative migration rate of solute and mobile phase; and $D_s$ = the diffusivity of solute in the stationary phase.

Equation 2.30 may be written in the more general form:

$$H = A + \frac{B}{u} + C_m u + C_s u$$  \hspace{1cm} 2.31$$

or in the form:

$$H = A + \frac{B}{u} + Cu$$  \hspace{1cm} 2.32$$

in which $A$, $B$, $C_m$, $C_s$ and $C$ are constants for the various dispersion processes. Equation 2.32, which has been widely used in gas chromatography, is known as van Deemter's equation which was derived from a mass balance model (63).

Although Equations 2.30 to 2.32 represent the qualitative dependence of $H$ on $u$, thorough studies of column efficiencies suggest that the representation of experimental GPC data at high $u$ is not always satisfactory. Giddings (59) recognised the inadequacies of Equation 2.30 which result from radial diffusion movements of a molecule. Consequently, there is a rapid interchange of solute molecules between streamlines, so
that the molecules will have a range of velocities and will move from an unobstructed streamline between particles to a streamline moving round a particle. It follows that mass transfer in the mobile phase, which is related to solute diffusivity, and eddy diffusion are interdependent. This is the basis of Giddings coupling theory \((59)\) in which the eddy diffusion term is coupled non-additively to a mass transfer term in the mobile phase according to the relation:

\[
H = \frac{B}{u} + C_s u + \sum \frac{1}{\left(\frac{1}{A}\right) + \left(\frac{1}{C_m u}\right)}
\]

where \(C_s\) and \(C_m\) are mass transfer constants in the stationary and mobile phases respectively. When applying Equation 2.33 specifically to GPC \((64)\) it can be shown that:

\[
H = \frac{2 \Omega d_m}{Ru} \frac{1}{30} R(1-R) \frac{d_p^2 u}{D_m} + \sum \frac{1}{\left(\frac{1}{2 \lambda d_p}\right) + \left(\frac{D_m}{\omega d_p^2 u}\right)}
\]

where \(R\) is the ratio of solute zone velocity to mobile phase velocity. The contribution of the third term in Equation 2.34 to \(H\) is smaller than the sum of the two terms (i) and (iii) in Equation 2.30.

An alternative expression to Equations 2.32 and 2.33 has been proposed by Billmeyer et al. \((60)\). Their treatment assumes that the solute dispersion mechanisms are independent of each other and utilizes Equation 2.32 in which \(C\) is a mass transfer constant for permeation into the stationary phase. An additional term arises from a non-uniform velocity profile effect owing to variations in packing density and particle segregation during packing, giving variable resistance to flow over the cross-section of the column. This is also enhanced by column wall effects where there is less resistance to flow because of a greater column void fraction \((8)\). Therefore, solute molecules may sample several velocity streamlines and flow paths. The expression of Billmeyer et al. \((60)\)
is:

\[ H = A + \frac{B}{u} + 2\psi R^2 \frac{u}{D_r} + Cu \]  \hspace{1cm} (v)  \hspace{1cm} 2.35

where \( R_c \) is the column radius, \( D_r \) is the average radial diffusivity, and \( \psi \) is a velocity profile constant. In the van Deemter equation, solute dispersion in the mobile phase in the longitudinal direction results from eddy diffusion and axial molecular diffusion. Radial diffusivity will therefore depend on eddy diffusion and molecular diffusion processes. Hence, the average radial diffusivity may be represented by:

\[ D_r = \phi D_m + \lambda d_p u \]  \hspace{1cm} 2.36

The application of Equations 2.35 and 2.36 to GPC separations of polymers has been thoroughly studied by Billmeyer and Kelley (8,55-57).

When comparing GPC columns under different conditions of eluent flow rate, particle size and with different eluents, it is beneficial to be able to compare experimental data using common parameters. Giddings (59,68) has shown that the dimensionless parameters called reduced eluent flow rate \( \nu \) and reduced plate height \( h \) are suitable. These are defined by:

\[ \nu = \frac{ud_p}{D_m} \]  \hspace{1cm} 2.37

\[ h = \frac{H}{d_p} \]  \hspace{1cm} 2.38

the reduced velocity being the rate of flow compared to the rate of diffusion over a particle and the reduced plate height being the plate height scaled to the particle diameter. Equations 2.37 and 2.38 can be used to simplify Equations 2.30 to 2.36 leaving expressions independent of particle size and diffusion coefficient.
2.2 PRACTICAL CONSEQUENCES OF THEORY

Equations 2.30 to 2.36 describe the variables which influence chromatogram broadening. A graphical presentation of how the dependence of $H$ on $u$ is influenced by the separate solute dispersion mechanisms is shown in Figure 2.2.1. An alternative expression to Equation 2.39 has been proposed and investigated by Knox et al. (69, 70).

$$H = \frac{B}{u} + Cu + Au^m$$

where $B$ and $C$ are defined as in Equation 2.32. The third term is essentially empirical and represents solute dispersion in the mobile phase because of the complex flow of the eluent between particles. The exponent $m$ is between 0.25 and 0.35 and is generally assumed to be 0.33. Equation 2.39 can be written in the reduced form:

$$h = B' + C'v + A'v^m$$

and for many practical purposes the last two terms can be combined to give:

$$h = B' + D'v^m$$

If $v$ is above 30 then the first term in Equation 2.41 is very small compared to the second term and we obtain Snyder's Equation (71):

$$H = Du^{m'}$$

or $h = D'v^{m'}$  \hspace{1cm} 2.42

The constant $m'$ usually falls within the limits $0.3 \leq m' \leq 0.7$ but values outside this are known (61). For $v$ above 30, Snyder's Equation fits the data over a ten-fold range of $v$ but $m'$ increases gradually with $v$ above this; while Knox's Equation (Equation 2.39) fits over two orders of magnitude (61). When examining data using Equation 2.40 $A' \approx 1$ while $C' \leq 5 \times 10^{-2}$ and in principle $A'$ and $C'$ do not vary for columns equally well packed with the same materials, regardless of particle size.

Equations 2.39 and 2.42 are attractive because the empirical/theoretical constants $A$, $B$, $C$, $D$, $m$ and their derivatives can be determined.
Figure 2.2.1

Representation of the contributions of solute dispersion mechanisms to height equivalent to a theoretical plate (HETP) as a function of flow rate. Curve (1), Equation 2.32; Curve (2), Equations 2.33 to 2.35; Curve (v) is the term in Equation 2.35; Curves (i), (ii) and (iv) are the terms in Equation 2.30.
from experimental data. Whereas, the coupling terms in Equations 2.33 and 2.34 and the average radial diffusivity in Equations 2.35 and 2.36 are less easy to evaluate. Other approaches have been adopted by chromatographers to evaluate their systems, these include: column performance factor (72), the Huber Equation (73), modified Coupling Theory (74) and resolution or theoretical plates generated in a given time.

2.2.1 Particle Size

It is clear from Equation 2.30 that column efficiency increases with decrease in $d_p$ as shown in Figure 2.2.2 for cross-linked polystyrene gels (75). Small particles increase the rate of solute mass transfer between the shorter pore depths and the smaller interparticle channels, thus minimising the mass transfer contribution to $H$. At the minimum in Figure 2.2.1, eddy diffusion will be the major contributor to chromatogram broadening. For typical values of $d_p$, $u$, and $D_m$ of 10 μm, $10^{-1}$ cm s$^{-1}$, and $10^{-5}$ cm$^2$ s$^{-1}$ respectively, the eddy diffusion contribution to $H$ is predicted to be 0.1 mm (76). It can also be seen from the differential of $H$ in Equation 2.30, with respect to $u$, that as $d_p$ decreases the optimum flow rate (minimum $H$) increases. This is shown in Figure 2.2.2.

The dependence of column efficiency of a totally permeating solute on $d_p$ has been studied giving:

$$H \propto d_p^{-\delta}$$

where the exponent $\delta$ has been found to be 1.95 for cross-linked polystyrene gels (77), with mean particle diameters 16 – 34 μm, and 1.8 for silica gels (78), with mean particle diameters 5 – 40 μm. However, some data suggests that $\delta$ decreases as $d_p$ decreases (77, 79).

In order to reduce the eddy diffusion and velocity profile contributions to plate height, a uniform bed of regular monosized microspheres is required. The dependence of column efficiency of a non-permeating solute in the mobile phase on particle size distribution (80, 81) has
Effect of particle size and flow rate on efficiency using toluene in tetrahydrofuran on pStyragel columns; data replotted from Limpert, Cotter and Dark (75).

- , 20μm particles; ▲, 10μm particles; ■, 5μm particles.

Figure 2.2.2
been demonstrated and Ishida et al. (82) have also shown that narrow particle size distributions are preferred. Wide particle size distributions lead to multiple flow paths, thus raising the eddy diffusion contribution to $H$. Flow paths which differ in the degree of constriction and tortuosity cause variations of flow rate from the mean, thus generating a substantial non-uniform velocity profile effect. Small particles are not easy to pack satisfactorily and the best column efficiencies are obtained with slurry-packing techniques. If the particle size distribution is not narrow, the column packing procedure inevitably leads to particle segregation during packing, variations in packing density, and wall effects where there is less resistance to flow because of a greater column void fraction. A variable resistance to fluid flow across a column will increase the contribution to $H$ from a velocity profile effect (77).

2.2.2 Eluent Flow

In order to minimise chromatogram broadening, $H$ must be as low as possible. It is clear from Figure 2.2.1 that there is an optimum range of $u$. Very low flow rates are unattractive because the separations are slow and inefficient. Consequently, in most practical separations, longitudinal molecular diffusion has a rather small influence on chromatogram broadening. At the minimum in Figure 2.2.1 the separation will be performed with the best combination of speed, efficiency and pressure drop. The optimum value of $H$ at this flow rate will then depend on the type of particles and how well the column has been packed. Faster separations will be achieved at higher flow rates with higher pressure drops at the expense of lower efficiencies. From Giddings coupling theory (59) the increase in $H$ with $u$ is only gradual; hence little sacrifice in $H$ is expected. However, the mass transfer characteristics of a column packing may be poor, e.g. because of internal pore geometry, so that $H$ rises much faster than expected theoretically (8, 67).
Furthermore, because the mass transfer term contains the diffusivity, the magnitude of $H$ is solute size dependent. For typical values of $d_p$ and $u$ of $30 \, \mu m$ and $0.1 \, cm \, s^{-1}$ respectively, the mass transfer contribution of $H$ is predicted to be about $1 \, mm$ for polystyrene with a molecular weight of $10^4 (D_s \approx 2 \times 10^{-6} \, cm^2 \, s^{-1})$ and about $3 \, mm$ for polystyrene with a molecular weight of $10^5 (D_s \approx 5 \times 10^{-7} \, cm^2 \, s^{-1})$ (76).

2.2.3 Column Dimensions

If the length of a GPC column $L$ is increased, then the difference between $V_1$ and $V_2$ increases in Equations 2.11 and 2.14; hence, $D_2$ is proportional to the reciprocal of $L$. Additionally, Giddings (59) has shown that $\sigma_v$ is proportional $L^{1/2}$. Therefore, both $R_s$ and $R_s$ increase as $L$ increases and column packings of different types in various lengths can be compared using modifications of the resolution equations in Section 2.1.1. Specifically for Equation 2.20 Yau et al. (53) have shown that:

$$R_s = \frac{0.576}{D_2 \sigma_v L^{1/2}}$$  \hspace{1cm} 2.44

 Traditionally, GPC has been performed with columns of length $122 \, cm$, often with several columns linked together in series with short lengths of narrow-bore tubing. A comparison has been made between Styragel and $\mu$Styragel, trade names of Waters Associates (53) for typical high polymer separations. $\mu$Styragel ($d_p \approx 10 \, \mu m$) in a $122 \, cm$ long column set gives lower $H$ values than Styragel ($d_p \approx 50 \, \mu m$) in a $488 \, cm$ long column set, but resolution as defined by Equation 2.20 is similar for the two packings because of the large difference in $L$. In terms of performance criterion, the major gain is in the speed of analysis, a factor of 4 at the same flow rate. This factor may be increased by operating $\mu$Styragel columns at faster flow rates without raising $H$ excessively, provided the instrumentation will withstand the higher pressure drops.
Alternatively, if the objective is minimizing chromatogram broadening for a unimodal peak (optimising resolution for more than one peak), then shorter columns of microparticulate packing can be operated at lower flow rates. This gives the possibility of using high efficiency columns on "standard" GPC equipment with little sacrifice in analysis time.

If a very small volume of polymer solution is injected centrally into a column, the solute band will diverge as it progresses down the column. Because radial dispersion in columns typically used in liquid chromatography is slow, the molecules may never reach the column walls before leaving the column. Since substantial solute dispersion may occur at the column wall because of a non-uniform bed of particles and the greater void volume, the use of 'infinite diameter' columns is attractive. The criteria for obtaining this effect have been thoroughly studied by Knox et al. (70, 74, 83) who recommended the conditions:

\[
0.4 \frac{d_c}{d_p} > \frac{L}{d_c} \tag{2.45}
\]

\[(d_c - 60 d_p)^2 \frac{L d_p}{d_c} > 16 \left[ \frac{1.4 D_m}{u d_p} + 0.06 \right] \tag{2.46}
\]

where \(d_c\) is the diameter of the column. From Equation 2.45 we can calculate the limiting conditions for representative experimental parameters: \(L = 30\) cm, \(d_c = 0.75\) cm, \(d_p = 10\) \(\mu\)m, and \(u = 0.1\) cm s\(^{-1}\) (about 0.5 cm\(^3\) min\(^{-1}\)). The left-hand side of Equation 2.45 becomes 750 compared with 40 for the right-hand side. If the column length were extended to 120 cm, the column would still function in the infinite diameter mode. This suggests that future GPC experiments may be performed with single columns as in liquid chromatography. Instead of coupling together columns with packings having different average pore diameters, the packings will be contained in a single long column.

Equations 2.45 and 2.46 clearly demonstrate that very narrow bore columns should be avoided in GPC experiments. Knox et al. (83) have
demonstrated that the wall effect region may extend 30 particle diameters into the column, i.e. 0.3 mm for 10 μm particles. Peak asymmetry, such as the appearance of fronting on chromatograms, is explained by a substantial wall effect in short narrow-bore columns. The wall effect may become more important for small irregular particles with a wide particle size distribution which are not easy to pack into a uniform bed even by slurry techniques.
2.3 **EQUIPMENT DESIGN**

Extra column broadening can be considered to be the sum of individual contributions due to injection, column fittings and detector \((20, 66, 84)\). 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 \((85)\).

2.3.1 **Packings**

Various types of packing have been used in traditional GPC \((86)\) 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 \((d_p \approx 30 \mu m)\) onto which a thin \((\sim 5 \mu m)\) 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 \((87)\) 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 (81, 88).

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 (89)
(iii) sized fractions by an air classifier have a narrower particle size range.

The large number of microparticulate GPC packings now available (30, 90) 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.

2.3.2 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 $\sim 20 \mu m$ deep, and gives a constant id (89). 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. However, the cost of packings is often 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 cross-linked polystyrene gels do not perform well in narrow bore columns (91).

Column end fittings should be designed with a minimum dead volume (92) 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% (93). 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 (94). 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.

2.3.3 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 (95).

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 (96) which increases efficiencies.

On-column injection has widely been used in conventional LC to give high resolution separations. 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 (96) 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 affect the performance of a column causing poor resolution and a decrease in efficiency. For micro-particulate 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 $\mu$m porous glass (97) and with $\mu$Styrage (98).

2.3.4 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 (99,100). However, the criteria for GPC work is slightly different and a comparison is listed in Table 2.1.

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 (101) 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 number- and weight-average molecular weights 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
<table>
<thead>
<tr>
<th>Requirement for a modern analytical liquid chromatography pumping system</th>
<th>For GPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deliver mobile phase with consistency, 1 - 2%.</td>
<td>0.3%</td>
</tr>
<tr>
<td>Pulse free to minimize detector noise.</td>
<td>Yes</td>
</tr>
<tr>
<td>Ease of solvent change.</td>
<td>Unnecessary</td>
</tr>
<tr>
<td>Work at high pressures, 500 - 5000 psi.</td>
<td>300 - 3000 psi sufficient</td>
</tr>
<tr>
<td>Construction chemically resistant to mobile phase.</td>
<td>Yes</td>
</tr>
<tr>
<td>Compatibility with flow and solvent programming techniques.</td>
<td>Unnecessary</td>
</tr>
</tbody>
</table>
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 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 (102, 103) 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 (103). 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.

2.3.5 Detectors

Most commercial detectors have been reviewed (104) 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 (~3 μdm³) 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 (nD ≈ 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 (nD = 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 manufacturer cannot 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 μm³). 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 (105).

A low angle laser light scattering photometer having a small cell volume (106) has been coupled to a GPC instrument (107,108) in conjunction with a differential refractometer. 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.
2.4 **CALIBRATION AND DATA REDUCTION**

Molecular weights in GPC can be determined in two ways:

(i) Use of a molecular weight measuring system as a detector for the GPC eluent, such as that already described (107,108).

(ii) Use of a calibration curve.

The first system above will not be described in detail in this work.

2.4.1 **Calibration**

A calibration curve is usually constructed by injecting a series of polymer standards having a narrow or known molecular weight distribution (MWD) and recording the elution volume at various points on the resultant chromatogram, especially $V_R$ for narrow MWD standards. The calibration curve is obtained from a plot of log$M$ versus $V_R$. In general the calibration curve is dependent on polymer type and on the molecular weight average used i.e. number-, weight-, $z$-, viscosity- average molecular weights etc. (these are $M_n$, $M_w$, $M_z$ and $M_v$ respectively). The polymer standards should be of the specific polymer type as the samples to be analysed and should cover the whole molecular weight range of the calibration curve to be used because the calibration is never perfectly linear. Ideally, narrow MWD standards are used, where $M_w$ $\approx$ $M_v$ $\approx$ $M_n$, and the calibration utilizes $M_p$, the peak-average molecular weight. This method is ideal when the required standards can be readily prepared by living polymer techniques. Otherwise, the polymer can be separated into fractions with narrower MWD, by preparative GPC or partial fractionation, and determination of molecular weights made by conventional techniques to give the required standards. For practical reasons it is not always possible to do this and Frank et al. (109) and Weiss et al. (110) proposed special methods of obtaining the calibration curve from broad distribution polymers. These methods inevitably contain some assumptions such as MWD of the polymer and linearity of the calibration over areas of the molecular
weight range; therefore, the calibration contains basic errors.

The first method of using a universal calibration, where by one polymer type can be analysed using another for calibration, was the "Q" factor method (5, 111). This considered that the retention volume of a polymer depended on the length of the polymer molecule when fully extended as determined by the bond lengths and valence angles. This procedure is now known to be in error for many polymers because of the assumption of an incorrect model for the polymer molecule in solution.

A more useful universal calibration is obtained, when parameters relating to the true size and shape of polymer molecules in solution are chosen. Numerous publications have appeared on the subject of universal calibration and only a brief mention of them is made here. These relate $V_s$, the hydrodynamic volume as measured by various methods, to $V_R$ (112-116) using different polymer types, solvents and temperatures. The most commonly used method since the 1960's is that of a plot of $\log(\eta \cdot M)$ versus $V_R$ postulated by Benoit et al. (114), where $\eta$ is the intrinsic viscosity and $M$ is molecular weight. The justification for this is based on the Einstein Equation (117) and if the Mark-Houwink Equation (118) (Equation 2.48) is used it can be shown that (32):

$$2.5A_v V_s = [\eta] M = KM^{a+1}$$ (2.47)

where $[\eta] = KM^a$ (2.48)

$A_v$ is Avogadro's Constant and $K$ and $a$ are Mark-Houwink constants (118).

Therefore, at a given retention volume two polymers 1 and 2 are related by the Equation:

$$[\eta]_1 M_1 = [\eta]_2 M_2$$ (2.49)

From this it follows that:

$$K_1 M_1^{a_1 + 1} = K_2 M_2^{a_2 + 1}$$ (2.50)
Equation 2.51 is readily used because Mark-Houwink constants for many polymer/solvent systems are readily available \(\text{(119)}\). Since its first proposal much work has been carried out to test Benoit's theory. Boni et al. \(\text{(120)}\) have indicated that at molecular weights of 30000 to 50000 a gradual change in \(\alpha\) values occurs, because \(\alpha\) tends to decrease towards 0.5 for short chain polymers. This could give errors in Equation 2.51 when used in molecular weight determinations.

Whitehouse \(\text{(121)}\) has discussed how polydispersity effects \(K\) and \(\alpha\) values, and hence the intrinsic viscosity, proposing the use of Equation 2.52.

\[
[\eta] = KM_w^\alpha (1 - \Delta) \quad 2.52
\]

where \(\Delta\) is a function of polydispersity, typically when \(\alpha = 0.7\) and polydispersity \(d = 5\) then \(\Delta \approx 0.15\), or \(d = 2\) then \(\Delta \approx 0.07\).

2.4.2 Data Reduction

It is not possible to convert the elution volume of an unknown polymer to molecular weight by mere substitution because of the large errors involved \(\text{(122-124)}\). 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 \(\text{(125)}\).

\[
w(M) = \frac{dI(M)}{dM} = \frac{dC(v)}{dv} \cdot \frac{dv}{dM} \cdot \frac{d(logM)}{dM} \quad 2.53
\]

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 \(d(logM)/dM\) is \(1/M\) the differential MWD can be found using Equation 2.53.

From this the average molecular weights can be found \(\text{(6)}\) from Equation 2.54 to 2.57.

\[
M_2 = \left[ \frac{K_1}{K_2} \right]^{1/(\alpha_2 + 1)} \frac{(\alpha_1 + 1)}{(\alpha_2 + 1)} M_1 \quad 2.51
\]
\[ \overline{M}_n = \frac{1}{\int_m^\infty \frac{1}{M} w(M) dM} \]

\[ \overline{M}_v = \int_m^\infty M w(M) dM \]

\[ \overline{M}_w = \int_m^\infty M w(M) dM \]

\[ \overline{M}_p = (\overline{M}_n \overline{M}_w)^{\frac{1}{2}} \]

\[ d = \frac{\overline{M}_w}{\overline{M}_n} \]

where \( m \) is the molecular weight of the monomer and \( d \) the polydispersity, the other parameters having been defined in Section 2.3.1. \( \overline{M}_n \) is expressed as the mass concentration of the sample divided by the total number of moles in the sample. \( \overline{M}_w \) is defined as the mass fraction of the individual species multiplied by their molecular weights. \( \overline{M}_v \) is defined as the average molecular weight which would be found if the intrinsic viscosity of the polymer was measured. A computer program (126) based on that of Pickett et al. (123) was used to calculate these averages by this method.
2.5 CONTROL OF ADSORPTION WITH SILICA PACKINGS

At the present time silica gel is the most commonly used packing material for liquid chromatography. Porous silicas can be prepared by various routes (127). These include the hydrolysis of silicon compounds, such as silicon tetrachloride, in the liquid or vapour phase, by acid precipitation of silicates in solution, or by acid etching two colloidalially dispersed mutually interpenetrating glasses leaving one porous phase. Owing to these varied means of manufacture, commercial silicas have wide variations in properties such as pore and particle size and size distribution, surface area and surface pH.

The pH of the silica surface is critical for its use as a LC adsorbent. It is usually weakly acidic (pH 3 to 5); however, strong acidic surfaces are obtained if mineral acids, from preparation by gelation, contaminate the surface. Liquid-solid chromatography makes use of this acidity, separating basic solutes by their preferential affinity for the acidic surface. Two methods are generally used to modify the polar nature of the silica surface: use of a complexing agent and chemical modification of the surface.

2.5.1 Complexing Agents

Polyvalent metal ions can be adsorbed from an aqueous solution onto the surface of the silica providing the pH is not below the value at which the metal salt precipitates its hydroxide. The amount deposited depends on the concentration of the ion in solution; in general a 1 to 10% solution is used. Silver nitrate modified silica (128) has been used for the liquid-solid chromatographic separation of alkenes and alkynes from alkanes.

Organic compounds have been coated onto silica in a similar manner to the way in which gas chromatography packings are prepared (129). These compounds must contain polar atoms, e.g. oxygen or nitrogen, which are electrostatically attracted to the silanol groups on the silica surface.
The factors which influence the extent to which the organic compounds are adsorbed are listed in Table 2.2. The advantages of this method of coating are that packings are easy to prepare, the silica can be re-used with different coatings and the degree of coating is easily controlled. However, high linear flow rates tend to strip off the coating, temperature changes cause changes in the solubilities of the mobile and stationary phases, and stripping and salts in the carrier tend to interfere with the detection of the column eluent. These advantages/disadvantages are discussed in depth elsewhere (130).

The above problems can be remedied to a great extent by anchoring the coating, through a covalent bond, onto the surface of the silica's pores. This gives a "bristle" type of coating.

2.5.2 Chemical Modification

The extent to which any silica surface can be chemically modified is dependent upon the types of chemical groups present and the steric availability of these groups. Early workers (131,132) suggest the existence of three distinct groups:

\[ \text{silanol groups} \quad \text{siloxane groups} \quad \text{silanol + physically adsorbed water} \]

"bound water"  "dehydrated oxides"  

while others, such as Snyder (133,134), have postulated that the silica surface will contain two further groups:

\[ \text{geminal hydroxyl groups} \quad \text{bound and reactive hydroxyl groups} \]

the amount of each group being dependent on structural considerations.
TABLE 2.2

Factors influencing the adsorption of polar organic compounds on the surface silanol groups of silica

<table>
<thead>
<tr>
<th>Factors enhancing adsorption</th>
<th>Factors minimizing adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong interaction between polar organic groups and the silanol surface</td>
<td>Weakly polar groups.</td>
</tr>
<tr>
<td>(i) Basic groups acting as cations e.g. quaternary ammonium.</td>
<td></td>
</tr>
<tr>
<td>(ii) Electron-donor groups e.g. oxygen, nitrogen, amide, ether etc.</td>
<td></td>
</tr>
<tr>
<td>Soluble salts in eluent e.g. potassium chloride.</td>
<td>Alkali metal ions adsorbed on the surface.</td>
</tr>
<tr>
<td>Multiple polar groups providing multiple points of attachment to silanol surface.</td>
<td>Presence of organic solvents in aqueous eluent.</td>
</tr>
<tr>
<td>Increasing size and number of non-polar hydrocarbon groups in the molecule.</td>
<td>Minimizing size of non-polar hydrocarbon group e.g. methanol.</td>
</tr>
<tr>
<td>Low temperature.</td>
<td></td>
</tr>
<tr>
<td>High concentration of adsorbate.</td>
<td></td>
</tr>
</tbody>
</table>
Another approach is to assume that the surface is composed of a monolayer of silanol groups owing to the tendency of each silicon atom on the surface to maintain a tetrahedral co-ordination (135). The concentration of silanol groups is between 5 (136) and 8 (131) per nm$^2$, the latter value being calculated from the area covered by each hydroxyl group, based on crystal structure data. However, the actual number of groups available for chemical modification is dependent on the group being used, its reactivity, shape and size, and the size and shape of the pores.

Commercially available silicas have usually been heat treated to varying degrees during their manufacture, resulting in the formation of surface siloxane groups. These can be converted back to silanol groups by washing the silica with dilute hydrochloric acid and removing the water by controlled heating. Iler (131) claims that physically adsorbed water is evolved from silica below 150° with bound water being evolved from 150° to 600° without appreciable structural deformations; above 600° internal alterations occur together with a decrease in particle and pore size. However, later work (137,138) has shown that silanol concentration only decreases slightly up to 300° with a pronounced decrease attributable to condensation of bound or paired hydroxyl groups at 300 to 500°, condensation of free hydroxyl groups occurring with deformation above 600°.

Once the silica surface has been conditioned to produce the optimum number of silanol groups organic phases can be bonded to them by one of three types of reaction; esterification, reactions with an organochlorosilane, and chlorination followed by treatment with an organo-metallic. These methods produce three types of linkage:
(i) Si – O – C which is susceptible to hydrolysis by water and alcohols and, therefore, tends not to be used.

(ii) Si – O – Si – C which is more stable and normally used in the pH range 2 – 8 but can still be cleared by strong nucleophiles or electrophiles.

(iii) Si – C which is the most stable, but can be cleared by some electrophiles (139).

The concept of permanently modifying inorganic supports in order to control adsorption was first realised in 1950 (140) but the first paper reporting the modification and use of such a packing material appeared in 1968 (141). In this Steward and Perry (141) modified a diatomaceous earth support with octadecyltrichlorosilane giving a Si – O – Si – C linkage (142). Trichlorosilanes, being more reactive, are usually used, but this method is only suitable for certain organic phases, if a phase is used which contains an active hydrogen the chlorosilane part would react with it forming a condensation polymer. Hydrophilic phases are necessary for aqueous GPC otherwise the eluent will not wet the surface of the packing or enter its pores preventing separation by exclusion (143). Hydrophilic bonded phases are normally prepared using a trialkoxysilane in an organic or aqueous solvent (144,145).

The silanol groups can be chlorinated using reagents such as thionyl chloride; this effectively makes the support a silylating agent. These groups can then be reacted with Grignard reagents to form Si – C bonds (137,146). This process is tedious and the metal from the Grignard reaction can be precipitated and block some of the pores in the support, and hence, they have not been made commercially available owing to the expense involved.

Many problems still exist concerning the use of bonded phases in LC and GPC. The erosion of the packing can easily occur which rapidly
renders an expensive column useless. The erosion is a function of time, buffer or eluent composition, temperature, solution pH, eluent flow rate, type of glass or silica used and surface area (147). In extreme cases erosion of the bonded phase can affect the detector response and alter the $V_o$ and $V_i$ characteristics of the column.

Various bonded phases prepared for use in aqueous GPC have been reported in the literature (144,148). The structure of some of these are listed in Table 2.3 (144), the most universally useful being the glycinamide phase (Structure 4) and the glycol phase (Structure 6) (144). However, only the glycol phase is commercially available (145), Corning Glycophase G, and hence, routinely used in GPC (149). This is supplied in a range of pore sizes from 4 to 150 nm with a 5 - 10 μm particle size (30).
### TABLE 2.3

Chemically bonded stationary phases of structure 

\( \text{Si-CH}_2-\text{CH}_2-\text{CH}_2-X \) for use in aqueous GPC (144)

<table>
<thead>
<tr>
<th>Bonded Phase (X)</th>
<th>Chemical Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) ( - \text{NH}_2 )</td>
<td>Amine</td>
</tr>
<tr>
<td>(2) ( - \text{NH} - \text{CO} - \text{CH}_3 )</td>
<td>Amide</td>
</tr>
<tr>
<td>(3) ( - \text{NH} - \text{CO} - \text{CF}_3 )</td>
<td>Trifluoroamide</td>
</tr>
<tr>
<td>(4) ( - \text{NH} - \text{CO} - \text{CH}_2 - \text{NH} - \text{CO} - \text{CH}_3 )</td>
<td>Glycinamide</td>
</tr>
<tr>
<td>(5) ( - \text{NH} - \text{SO}_2 - \text{CH}_3 )</td>
<td>Sulphonamide</td>
</tr>
<tr>
<td>(6) ( - \text{O} - \text{CH}_2 - \text{CHOH} - \text{CH}_2\text{OH} )</td>
<td>Glycol</td>
</tr>
</tbody>
</table>
CHAPTER 3 EXPERIMENTAL
3.1 MICROPARTICULATE PACKINGS

The macroporous silicas in this research were kindly provided by Dr. J.D.F. Ramsay and Dr. D.G. Sammon of A.E.R.E., Harwell. Spherisorb silica S.20.W was supplied by Phase Separations (Queensferry, Great Britain).

3.1.1 Surface Treatment

No detailed information on the preparative method of the silicas was available, but it is usual for silicas to be calcined at temperatures in excess of 600°C in order to impart strength (131). Therefore, it can be assumed that the surface was mainly composed of surface siloxane groups (137,138) which must be converted to silanol groups. This was achieved by refluxing the silica sample in 2.5M hydrochloric acid for 1.5 hours and then leaving the sample to cool for a further 2 hours. The silica was washed with distilled water, followed by AR acetone and then dried in vacuo (150°C, <0.1 torr) for 24 hours in order to remove physically bound water.

Bonded phase packings were prepared using Harwell S3 silicas pretreated in this way by reaction in aqueous media according to the method of Regnier and Noel (150). A silylating solution was prepared by the dropwise addition of 10 cm³ of γ-glycidoxypropyltrimethoxysilane (γ-G) (Union Carbide Ltd.) to deionized water (185 cm³) while maintaining the pH between 5.5 and 5.8 using 10⁻³M sodium hydroxide solution. The silylating solution was added to 10g of microparticulate silica packing in a 500 cm³ round bottomed flask fitted with a condenser. The contents were heated to 90°C ± 1°C for 30 minutes with shaking every 5 minutes. On cooling the packing was separated from the liquid using a Büchner funnel and was immediately washed with water to prevent any of the silylating agent from cross-linking within the pores of the packing and blocking them. The packing was further washed with 0.1M hydrochloric acid (50 cm³), water, then AR acetone and dried in vacuo (<0.1 torr).
The acid wash was needed to open the oxirane ring. The overall reaction is shown below:

\[
\text{Silica} + (\text{CH}_3\text{O})_3\text{Si(CH}_2)_3\text{OCH}_2\text{CH} - \text{CH}_2 \xrightarrow{\text{pH 5.5 to 5.8}} \text{HCl} \rightarrow \]

Elemental analysis was carried out at the Micro Analytical Laboratory, Department of Chemistry, The University of Manchester in order to measure the carbon content of the silica packing.

### 3.1.2 Scanning Electron Microscopy

Scanning electron microscopy was carried out in the Department of Chemical Engineering by Mr. M. Hayles. A dilute slurry of the micro-particulate packings in AR acetone was deposited onto a metal stub using a Pasteur pipette. The stub and packings were then coated in gold before being placed in the scanning electron microscope.

### 3.1.3 Particle Size

Particle size measurements were carried out in the Department of Chemical Engineering by Mr. D. Pinder using a Coulter Counter. This is a conductometric method of particle size analysis \((151)\). When particles, suspended in a dilute electrolyte, move through a capillary into a particle-free electrolyte solution, there is a sharp increase in the electrical resistance between the two solutions. The relationship which controls the magnitude of this electrical pulse, \(\Delta R\), at the moment a single particle passes through the capillary is given by \((152)\):

\[
\Delta R \approx \frac{V}{A^2}
\]

where \(A\) is the cross-sectional area of the capillary perpendicular to the direction of flow, \(V\) is the volume of the particle and \(\Delta R\) is the electrolyte resistance. The capillary is usually calibrated using spherical particles of known volume when Equation 3.1 becomes:
\[ AR \approx K_o V \quad 3.2 \]

where \( K_o \) is a constant for the apparatus. The resistance pulses thus obtained are then amplified before sorting and counting. This gives data about the particle size distribution of the particles with an overall precision of \( \sim 3\% \) (151).

The average particle diameters were calculated from the number fraction distribution with the relations:

\[
\begin{align*}
\bar{s}_n &= \frac{\sum n_i D_i}{\sum n_i} \quad 3.3 \\
\bar{s}_w &= \frac{\sum n_i D_i^3}{\sum n_i D_i^3} \quad 3.4
\end{align*}
\]

where \( s_n \) and \( s_w \) are the number-average and weight-average diameters and \( n_i \) is the number of particles with diameter \( D_i \) corresponding to the midpoint of interval \( i \). Equation 3.4 is used to calculate \( s_w \) rather than the incorrect Equation 3.5:

\[
\bar{s} = \frac{\sum n_i D_i^2}{\sum n_i D_i} \quad 3.5
\]

The justification for Equation 3.4 is that the weight of a particle is proportional to \( D_i^3 \) and, therefore, \( \sum n_i D_i^3 \) is proportional to the total weight of all the particles.

3.1.4 Transmission-Interferometry

A Jamin-Lebedeff system (153) was used to study the porosity of microparticulate silicas by lateral image separation transmission-interferometry. The microscope optics of the system are complex and therefore only a brief description is given.

Monochromatic light is first polarised and then split into dual beams by a 45\(^\circ\) prism which also orientates the beams at right-angles to each other. One beam passes through the specimen and the other through a reference point before recombination by a second prism, identical to
the first in nature and orientation. For a specimen with zero optical
path difference (OPD) with respect to the reference point, i.e. same
refractive index (RI), the beams remain at right-angles and on recombin-
ation the microscope image remains dark. A non-zero OPD will result in
elliptically polarised light and the microscope image will become brighter.

If a compensator is placed in one of the split beams, the plane of the
polarised light can be altered prior to recombination to give a dark
microscope image. The vernier marked compensator can be turned either
clockwise or anti-clockwise and an average reading taken for the OPD.

For a given monochromatic light the compensator can be calibrated to give
direct OPD values.

The above procedure was carried out using a microscope in the
Institute of Polymer Technology at Loughborough University of Technology.
The eye piece graticule had 27 divisions to 50 µm and green light was
used for measurements with a magnification of 625X. The OPD is given
by:

\[
\text{OPD} = T_n_e - [t n_p + (T - t)n_e]
\]

\[
= t(n_e - n_p)
\]

where \( t \) is sample thickness, given by \( d_p \) for spherical particles, \( T \) is
mounting medium thickness, \( n_p \) is RI of a porous silica particle and \( n_e \)
is RI of the mounting medium, which for air is \( n_a \). Equation 3.6 may be
written as:

\[
\frac{n_a - n_p}{d_p} = \frac{\text{OPD}}{d_p}
\]

If the silica microspheres are considered to consist of air and silica,
in fractions \( v_a \) and \( v_s \) respectively, and RI of silica is \( n_s \) then:

\[
\frac{n_p}{n_a} = v_a n_a - v_s n_s
\]

\[
v_s = \frac{n_p - n_a}{n_s - n_a}
\]
As \( v_a + v_s = 1 \), \( n_a = 1 \) and \( n_s = 1.459 \), then by combination of Equations 3.7 and 3.9 we obtain:

\[
v_s = \frac{OPD}{0.459d_p} \quad 3.10
\]

The volume of silica in a microparticulate packing is \( m/\rho_s \) where \( \rho_s \) is the density of silica and \( m \) is its weight in grams, and the pore volume is given by \( mP \) where \( P \) is pore volume/g. If the weight of the air within the packing is neglected, then the volume of the packing is \( m(P + 1/\rho_s) \).

Therefore, the fraction of silica in the packing, \( v_s \), is given by:

\[
v_s = \frac{m/\rho_s}{m(P + 1/\rho_s)} \quad 3.11
\]

\[
P = \frac{1 - v_s}{v_s \rho_s} \quad 3.12
\]

then, substituting Equation 3.10 in Equation 3.12 we obtain:

\[
P = \frac{0.459d_p - OPD}{2.19OPD} \quad 3.13
\]

where \( \rho_s = 2.19 \).

From an optical measurement of \( d_p \), using the microscope graticule and OPD, we can obtain values for the pore volume/g of the packings.
3.2 CHROMATOGRAPHIC EQUIPMENT AND ITS USE

In general, 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, schematically represented in Figure 3.2.1, was for research purposes only. It was chosen and designed firstly to give accurate, reproducible results under controlled conditions and secondly with economy in mind. Therefore, the system described is not necessarily ideal for routine polymer analysis.

3.2.1 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 cm$^3$ solvent capacity was considered adequate for running a series of columns for MW 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 cm$^3$, but this is still a large volume for high performance GPC systems which usually have a total permeating volume of $<$50 cm$^3$. The pump was capable of operating up to 3000 psi and at flow rates from 0.05 to 6.00 cm$^3$ min$^{-1}$. 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 system before opening the columns to the solvent again. This procedure prevented troublesome air-locks from forming in the detector cells and consumed $<$50 cm$^3$ of solvent.

In all cases the retention volume, $V_R$, was calculated by using an internal standard, as suggested by Patel (154) and used in HPGPC by Kohn and Ashcraft (155). The criteria used for an internal standard in
Figure 3.2.1

Schematic representation of the GPC equipment used in this study.
In general either a totally permeating or an excluded solute was used.

3.2.2 Injection

Three injection heads were used for introduction of sample into the columns. Two of these were used for on-column injection using single columns of \( \frac{1}{4} \) and \( \frac{3}{8} \) inch outside diameter (od). These were supplied by HETP, Macclesfield, Cheshire, part numbers 300 and 301 respectively, and are represented in Figure 3.2.2. In order to use this injection head the top of the column was prepared as shown in Figures 3.2.3a and 3.2.3b. The top 5 mm. of the packing was removed and a stainless steel mesh (2 or 5 \( \mu \)m) or, a nickel mesh (1 \( \mu \)m) and a tough stainless steel mesh (100 \( \mu \)m), were placed onto the top of the packing followed by Ballotini (\( \sim 150 \) \( \mu \)m). Finally, a porous PTFE plug (40 \( \mu \)m pores) was wedged into the top of the column to prevent displacement of the Ballotini if the column is moved. The injection procedure involved pushing a syringe needle through the porous PTFE plug and Ballotini until it touched the centre of the stainless steel mesh, which effectively allowed injection onto the top of the packing without disturbing it. If the mesh was punctured during injection, the top of the column was dismantled and the mesh replaced. With practice this injection procedure was reproducible and the 100 \( \mu \)m tough stainless steel mesh with 1 \( \mu \)m nickel mesh could be replaced by a less robust single 5 or 2 \( \mu \)m mesh as in Figure 3.2.3b. PTFE faced silicone septa were used and these were replaced every 10 - 25 injections, depending on the back pressure of the columns. These injection heads could be used for injections \( \leq 2000 \text{ psi} \). Two high pressure syringes were used with these heads; a 10 \( \mu \text{dm}^3 \) SGE high pressure syringe type B with needle guide (pressure limit \( \geq 8000 \text{ psi} \)) for injections of \( \leq 10 \mu \text{dm}^3 \) and a 100 \( \mu \text{dm}^3 \) Hamilton high pressure syringe No. 1710 (pressure limit \( \leq 1500 \text{ psi} \)) for injections of 10 - 100 \( \mu \text{dm}^3 \).

The third type of injection is shown in Figure 3.2.4 and was used...
Figure 3.2.2
Injection head for on-column injection
Figure 3.2.3
Methods used to prepare the heads of packed columns

(a) A - Porous PTFE plug (40µm pores)
   B - Ballotini
   C - 100µm tough stainless steel mesh

(b) A
   B
   E or F
   G

(c) t
   F
   G

D - 1µm nickel mesh
E - 5µm stainless steel mesh
F - 2µm stainless steel mesh
G - Packing material
Injection head for off-column injection.

(a) Teflon inner-ball seal
(b) Teflon wafer seal
(c) Zero dead-volume fitting to top of column
for off column injection when using multiple column sets or single columns for calibration work. This was supplied by Field Instrument Co. Ltd., Twickenham, Middlesex. An extra adaptor was constructed and attached, as in Figure 3.2.4, to allow injection to be made into the solvent flowing into the top of the column. A special syringe was supplied with this head which locked into the syringe guide while in the horizontal position (Figure 3.2.4a). When the guide and syringe were moved to the vertical (Figure 3.2.4b), the syringe could be pressed down and locked into the final injection position without loss of pressure or interruption of eluent flow rate. This allowed injection into the eluent passing into the top of the column. The above procedure was reversed for the removal of the syringe.

3.2.3 Columns and Column Packing

Three methods of column packing technique were used in this work. The dry tapping/tamping technique of Dawkins et al. (80,81) and two slurry techniques as described by Majors (156) and Bristow et al. (157).

(i) Tapping/Tamping Technique (80, 81)

This technique was used in packing Spherisorb S.20.W and Harwell Silicas, designated H2, H4 and H6. Silica was introduced into individual, seamless, 316 grade stainless steel columns (475 mm. x 3 mm. id, 1/4 in. od) terminated by a zero dead volume end fitting containing a 5 µm stainless steel mesh, using a Pasteur pipette. With the addition of each aliquot, enough to fill the column a further 15 mm., a 1.5 mm. diameter mild-steel rod having a 2.9 mm. diameter PTFE end-cap was placed into the column and tapped and tamped 20 times. (Some columns were packed without the tamping rod - see Section 4.2.1). This procedure was continued until the column was full. At the top of the column the silica packing was covered by a 2 µm stainless steel mesh above which was placed Ballotini, which in turn was covered by a porous PTFE (40 µm pores) plug as shown in Figure 3.2.3b. A syringe injection head was then attached
at the top of the column (Figure 3.2.2).

(ii) Balanced Density Slurry (156)

This technique was used in packing the same silicas as above. Prior to column packing all silicas were heat treated at 200°C for 3 to 5 hrs. to eliminate all surface moisture in order to help prevent agglomeration of the particles by mutual attraction owing to hydrogen bonding during dispersion in hydrophobic solvents. Column packing using this technique was carried out with the facilities at P.S.C.C., R.A.P.R.A., Shawbury, Nr. Shrewsbury where the apparatus schematically represented in Figure 3.2.5a was assembled. The pump was a single head Milton Roy reciprocating pump and the reservoir was constructed from a GPC column (1200 mm. x 7.6 mm. id, 3/8 in. od) which was connected to the column to be packed (200 mm. x 3 mm. id, 1/4 in. od) via a high-pressure ball-valve.

The components of the balanced density solvents, tetrabromoethane and tetrachloroethylene (SLR grade, BDH Chemicals Ltd.), were passed through individual activated 25 mesh silica gel columns to reduce the amount of halogen contaminants. The balanced density slurry was prepared by mixing a 60.6 : 39.4 ratio (w/w) of tetrabromoethane to tetrachloroethylene. This mixture (20 cm³) was placed in a 50 cm³ conical flask with the silica packing (2g) and the contents degassed in an ultrasonic bath for 2 minutes. This was then left to stand for 30 minutes to observe if any settling or floating of the silica occurred. If needed the solvent balance of the slurry was adjusted by the addition of the appropriate solvent followed by ultrasonic treatment.

The column (200 mm. x 3 mm. id, 1/4 in. od) to be packed, terminated as for the tapped/tamped columns, was filled with the balanced density solvent and the ball-valve was closed. The slurry was then carefully poured down the inside of the reservoir and the remaining volume of the reservoir was filled with toluene. The end-fitting was attached to the top of the reservoir ensuring that no air entered the system and the
pump set going at a slow rate pumping toluene to achieve the packing pressure fixed by a pressure relief valve. At the packing pressure the pump stroke was increased to maximum and the ball-valve opened forcing the slurry into the column. After 50 cm$^3$ of toluene had passed through the column the pump was turned off and the pressure in the system was allowed to relax before the ball-valve was closed and the column removed. The column head was then prepared as shown in Figure 3.2.2b.

(iii) Methanol Slurry (157)

This technique was used in packing the Harwell SG silicas, before and after surface treatment with $\gamma$-S, and S.7.W. Column packing by this method was carried out with the facilities at A.E.R.E., Harwell, Didcot, the apparatus being schematically represented in Figure 3.2.5b. This consisted of a Haskel, Model MCP.71 pneumatic amplifier pump working at 71 times the pressure of the nitrogen supply used, a high pressure needle-valve and a 75 cm$^3$ capacity packing bomb (HETP, Macclesfield, Part No. 361). The columns (300 mm. x 7.6 mm. id, $\frac{3}{8}$ in. od) were constructed of seamless, 316 stainless steel, terminated with zero dead-volume end-fittings containing a 2 $\mu$m stainless steel mesh. The silica materials were not heat treated to remove moisture prior to packing.

A slurry of silica material (11g) in methanol (70 cm$^3$, SLR grade, BDH Chemicals Ltd.) was prepared by vigorous shaking in a 150 cm$^3$ conical flask. This was poured into the packing reservoir, via the column connection point, and the column was quickly attached. Because a pneumatic amplifier pump instantly achieves its working pressure, the needle-valve was opened prior to activating the pump and the column packed at the required pressure. After 100 cm$^3$ of methanol had passed through the column the pump was turned off and the needle-valve closed. The inverted column was then quickly removed from the packing bomb to prevent the packing being disturbed owing to the effects of gravity. The head of the columns was then prepared in one of three ways as shown in Figures 3.2.3a
Figure 3.2.5a
Schematic representation of balanced density slurry packing apparatus

Figure 3.2.5b
Schematic representation of methanol slurry packing apparatus
3.2.4 Detection

Three detectors, two UV and one refractive index (RI), were used for monitoring the eluent from the column or column sets, but only one was operated for a given separation.

An ARL UV detector (254 nm, cell volume = 8 μdm$^3$, air reference) was modified. The tubing leading to the detector cell was shortened so that the column connected to the flow cell with a minimum of dead volume. This detector offered the maximum sensitivity available for polystyrene samples allowing a very small amount of sample to be injected, thus minimizing broadening due to viscous fingering and column overload. This detector was operated at maximum sensitivity at all times and always gave a steady base line on the chart recorder.

A Pye-Unicam LC-UV variable-wavelength detector (cell volume = 8 μdm$^3$, single beam) was used, as received, for the detection of proteins at 215 nm. Because the extinction coefficient of each protein varied at this wavelength, the detector sensitivity was adjusted during protein analysis to give peaks of equal height, for proteins of equal concentration, on the chart recorder.

RI detection was with a LDC Refracto Monitor Model 1107 (cell volume = 0.5 μdm$^3$), as received, for the detection of dextrans. The reference used was a static volume of eluent which had already passed through the chromatograph. This liquid was regularly changed in order to limit base-line drift on the chart recorder.

The chart recorder used for each detector was a Vitron Model UR.404 (Fisons Ltd.).

3.2.5 Errors in Chromatography

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

As described in Section 2.3.4, fluctuations in flow rate are
dependent on the pump system and errors of 1 - 4% can be tolerated for a short time. However, for MWD analysis the reproducibility of flow rate must be better than 0.3% otherwise errors in $\overline{M}_n$ and $\overline{M}_w$ will be about 6% (101). The retention volumes were calculated from the chart recorder speed and, where possible, a reference solute whose chromatogram for the column had been accurately characterised. This was usually a totally permeating or a totally excluded standard. 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 displayed by the recorder. Both these errors are small in comparison to the errors which result from flow rate.

Throughout the work no corrections were made for instrument dead-volume. It was considered that this was too small to measure accurately. In every case the injection time was negligible (<0.5s) compared to the time required for the peak to elute.

Operator errors arise from the measurement of peak height, peak width and peak retention volume. To calculate peak efficiencies the first part of Equation 2.9 was used as these results were more reproducible than from efficiencies measured by the last part of Equation 2.9. All results presented are the average of three consecutive determinations, under identical conditions, giving a maximum error in $N$ of ± 5%.
3.3 **MATERIALS**

This section contains information on the eluents and standards in this research, and where necessary their analysis. Owing to the varied nature of the experiments, specific information on the chromatographic conditions is given in Section 4.2.

3.3.1 Eluents

In conventional GPC eluents are normally degassed prior to their use. This is to prevent damage to polystyrene gel packings and reduce detection problems caused by air bubbles in the detector cell. Column efficiencies for the silica packings used did not change without degassing the solvent and detection problems did not arise. Therefore, the three types of eluent used in this research: tetrahydrofuran (THF), aqueous eluents and formamide, were not degassed.

THF (BDH Chemicals Ltd.) was destabilized, stored over calcium hydride for 4 hours, and then distilled from calcium hydride and copper (I) chloride before use. The THF residues were redistilled until the UV absorbance of the eluent was too large to allow adequate detector sensitivity. All water used as eluent, or used to prepare solutions of potassium chloride or phosphate buffer, was doubly distilled from glass. Formamide (SLR grade, BDH Chemicals Ltd.) was used as received.

3.3.2 Solutes

All solutes as received were freshly dissolved in the eluent used in order to prevent impurity peaks. Polystyrene standards (polydispersity $<1.3$) were obtained from three sources, as shown in Table 3.1. Because individual polystyrene oligomers are not readily available, three small molecules were used: tetraphenylethylene (TPE), 1,2-diphenylethylene (DPE) and toluene. Dextrans were obtained from two sources, as shown in Table 3.2. Although their polydispersities are $\sim1.5$ ($158$) they are supplied as calibrants for gel filtration work. Maltose was used as a totally permeating solute for studies with dextrans because it has a
**TABLE 3.1**

Polystyrene standards and low molecular weight molecules used as standards

<table>
<thead>
<tr>
<th>Name</th>
<th>Nominal Molecular weight(a)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-2,700K</td>
<td>2,700,000</td>
<td>Waters Associates Inc.</td>
</tr>
<tr>
<td>PS-1,987K</td>
<td>1,987,000</td>
<td></td>
</tr>
<tr>
<td>PS-955K</td>
<td>955,000</td>
<td>Polymer Laboratories Ltd.</td>
</tr>
<tr>
<td>PS-470K</td>
<td>470,000</td>
<td></td>
</tr>
<tr>
<td>PS-402K</td>
<td>402,000</td>
<td></td>
</tr>
<tr>
<td>PS-200K</td>
<td>200,000</td>
<td></td>
</tr>
<tr>
<td>PS-110K</td>
<td>110,000</td>
<td></td>
</tr>
<tr>
<td>PS-50K</td>
<td>50,000</td>
<td></td>
</tr>
<tr>
<td>PS-35K</td>
<td>35,000</td>
<td>Waters Associates Inc.</td>
</tr>
<tr>
<td>PS-20K</td>
<td>20,000</td>
<td></td>
</tr>
<tr>
<td>PS-9,800</td>
<td>9,800</td>
<td></td>
</tr>
<tr>
<td>PS-3,600</td>
<td>3,600</td>
<td></td>
</tr>
<tr>
<td>PS-2,350</td>
<td>2,350</td>
<td></td>
</tr>
<tr>
<td>PS-2,025</td>
<td>2,025</td>
<td></td>
</tr>
<tr>
<td>PS-600</td>
<td>600</td>
<td>Pressure Chemical Co.</td>
</tr>
<tr>
<td>TPE</td>
<td>332</td>
<td>Aldrich Chemical Co.</td>
</tr>
<tr>
<td>DPE</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>92</td>
<td>BDH Chemicals Ltd.</td>
</tr>
</tbody>
</table>

(a) Information supplied by the manufacturer.
<table>
<thead>
<tr>
<th>Name</th>
<th>Nominal Molecular Weight(a)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue dextran</td>
<td>2,000,000</td>
<td>Pharmacia Fine Chemicals Ltd.</td>
</tr>
<tr>
<td>D5251</td>
<td>500,000</td>
<td>Sigma Chemical Co. Ltd.</td>
</tr>
<tr>
<td>D5126</td>
<td>234,000</td>
<td></td>
</tr>
<tr>
<td>T70</td>
<td>70,000</td>
<td>Pharmacia Fine Chemicals Ltd.</td>
</tr>
<tr>
<td>T40</td>
<td>40,000</td>
<td></td>
</tr>
<tr>
<td>T20</td>
<td>20,000</td>
<td></td>
</tr>
<tr>
<td>T10</td>
<td>10,000</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>5,000</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>340</td>
<td>Sigma Chemical Co. Ltd.</td>
</tr>
</tbody>
</table>

(a) Information supplied by the manufacturer.
### TABLE 3.3

Proteins and Acetamide used as standards

<table>
<thead>
<tr>
<th>Name (a)</th>
<th>Molecular Weight (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>670,000</td>
</tr>
<tr>
<td>Catalase</td>
<td>250,000</td>
</tr>
<tr>
<td>Albumin</td>
<td>67,000</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>45,000</td>
</tr>
<tr>
<td>Pepsin</td>
<td>35,500</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17,800</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>13,900</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>12,400</td>
</tr>
<tr>
<td>Acetamide</td>
<td>59</td>
</tr>
</tbody>
</table>

(a) Supplied by Dr. J.N. Miller, Analytical Section, Department of Chemistry.

(b) Values taken from ref. (159) and (160).
similar structure and functionality. Proteins (Table 3.3) were obtained from various sources, their molecular weights being found in the literature (159, 160). Acetamide was used as a totally permeating solute for studies with proteins because of its similar functionality.

3.3.3 Characterisation of Dextrans by Viscometry

Solution viscosity (161) 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 flow time for the same volume of pure solvent, \( t_o \). The relative viscosity \( \eta_R \) and specific viscosity \( \eta_{sp} \) are given by Equations 3.12 and 3.13.

\[
\eta_R = \frac{t_c}{t_o}
\]

\[
\eta_{sp} = \frac{t_c}{t_o} - 1 = \eta_R - 1
\]

The limiting viscosity number (intrinsic viscosity), \([\eta]\), is given by Huggins' Equation (162):

\[
\frac{\eta_{sp}}{c} = [\eta] \left\{ 1 + k_{sp} [\eta] c + k'_{sp} ([\eta] c)^2 + \ldots \right\}
\]

and Kraemer's Equation (163):

\[
\frac{\ln \eta_R}{c} = [\eta] \left\{ 1 + k_{R} [\eta] c + k'_{R} ([\eta] c)^2 + \ldots \right\}
\]

where \( k_{sp}, k'_{sp}, k_{R}, k'_{R} \), etc. are constants. Equations 3.14 and 3.15 give linear plots of \( \eta_{sp}/c \) versus \( c \) and \( \ln \eta_R/c \) versus \( c \) at low concentrations of polymer because terms containing \( c^2 \) and higher powers of \( c \) have low values. If these plots are extrapolated to zero concentration then:

\[
[\eta] = \frac{L}{c \to 0} \frac{\ln \eta_R}{c} = \frac{L}{c \to 0} \frac{\eta_{sp}}{c}
\]

Solution viscosity at 25°C was found using a Ubbelohde suspended-level viscometer and four dilutions of dextran in water or potassium chloride solution. The time for the solvent was also noted. \([\eta]\) was obtained as the common intercept at \( c = 0 \) of plots \( \eta_{sp}/c \) versus \( c \) and \( \ln \eta_R/c \) versus \( c \). All runs were retimed until they agreed to within 0.1s.
CHAPTER 4  RESULTS
4.1 PACKINGS USED

This work used eight porous silica packings, some of which were surface modified. The details of these silicas as received, and their designations are shown in Table 4.1.

4.1.1 Surface Treatment

The surface of three of the macroporous silicas, SG30, SG60 and SG120, were modified using γ-G. Each silica, now designated SG30/G, SG60/G and SG120/G respectively, were analysed for carbon content, the results being shown in Table 4.2. Additionally the values for the theoretical carbon content for each silica is shown. This calculated carbon content is based on the following assumptions: 5 silanol groups per nm$^2$ (136), three being used for attaching each bonded phase molecule attached as γ-G is trifunctional and the surface area of each silica prior to modification for measurements supplied by A.E.R.E., Harwell (164).

It can be seen from Table 4.2 that the surfaces of SG30/G and SG60/G are effectively covered with the γ-G bonded phase while SG120/G is less than half as effectively covered.

4.1.2 Scanning Electron Microscopy

Scanning electron micrographs (SEMs) of all packings showed them to be spherical. Figure 4.1.1 demonstrates this for S.20.W and Harwell silicas H2, H4 and H6. It can also be seen that Harwell silicas tend to be less regular in shape and have a wider particle size distribution than S.20.W silica. Figure 4.1.2 shows a single S.20.W particle in which some of the larger pores can be seen. SEMs of SG30 silica before and after silylation are shown in Figure 4.1.3 indicating that neither particle breakdown nor fracture occurs during surface modification by the method of Regnier and Noel (150). Figure 4.1.3 also indicates that the SG30 material has a wider particle size distribution than H4 silica of similar pore size.
### TABLE 4.1

Details of Silicas as received from the suppliers

<table>
<thead>
<tr>
<th>Packing Name</th>
<th>Nominal Particle Size µm</th>
<th>Nominal Pore Size nm</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.20.W</td>
<td>20</td>
<td>8</td>
<td>Phase separations Queensferry.</td>
</tr>
<tr>
<td>H2</td>
<td>15–20</td>
<td>120</td>
<td>Experimental macro-porous silicas made at AERE, Harwell.</td>
</tr>
<tr>
<td>H4</td>
<td>15–20</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>15–20</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>SG.30</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>SG.60</td>
<td>10</td>
<td>60</td>
<td>AERE, Harwell.</td>
</tr>
<tr>
<td>SG.120</td>
<td>10</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>S.7.W</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 4.2

Surface Modification of Harwell SG Silicas

<table>
<thead>
<tr>
<th>Silica</th>
<th>Surface Area before Modification $\text{m}^2\text{g}^{-1}$</th>
<th>% Carbon Content</th>
<th>% Theoretical Carbon Content</th>
<th>% Efficiency of Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG30/G</td>
<td>204</td>
<td>3.0</td>
<td>3.7</td>
<td>81</td>
</tr>
<tr>
<td>SG60/G</td>
<td>80</td>
<td>1.2</td>
<td>1.5</td>
<td>82</td>
</tr>
<tr>
<td>SG120/G</td>
<td>58</td>
<td>0.4</td>
<td>1.1</td>
<td>38</td>
</tr>
</tbody>
</table>
Figure 4.1.1

Scanning electron micrographs of silicas:
(a) S.20.W; (b) H2; (c) H4; (d) H6.
Figure 4.1.2

Figure 4.1.3

Scanning electron micrographs of silicas:
(a) SG30; (b) SG30/G.
4.1.3 Particle Size

All silicas, as received, were subjected to Coulter Counter analysis. This method counted the particles in 4 μm intervals over the size range 1 - 60 μm for S.20.W, H2, H4 and H6 silicas and in 1 μm and 2 μm intervals over the size ranges 1 - 10 μm and 10 - 20 μm respectively for S.7.W, S30, S60 and S120 silicas. The particle size distribution for each silica was found by constructing a curve through the weight fractions at the mid-points of each interval. These are shown in Figures 4.1.4 and 4.1.5. A numerical representation of the Coulter Counter results can be made with Equations 3.3 to 3.5 as shown in Table 4.3.

Because Coulter Counters are calibrated with solid spherical particles, errors may occur when macroporous silicas are analysed owing to the electric field being able to flow through the particle (164). These errors will increase with increased pore size and pore volume and will possibly affect the measured values for S60 and probably affect values for S120 (164).

4.1.4 Transmission Interferometry

This work was only carried out on the macroporous silicas S30, S60 and S120. The results are shown in Table 4.4. Errors in measurement are larger when small particle sizes are used. Therefore, the smallest S30 particle might not have as large a pore volume as the results indicate. Under white light S120 appeared "textured" while S60 only exhibited a small amount of "texture" and S30 no "texture". In an attempt to understand this the compensator was used, under green light, to darken the centre of a S120 particle in order to measure the porosity of the "textured" area, as indicated in Table 4.4. This gave a lower OPD than when the whole of the particle was darkened using the compensator. The results, while not conclusive, infer that the centres of S120 particles are more porous than their outer layers.
TABLE 4.3

Number-average and weight-average particle diameters $s_n$ and $s_w$ (in $\mu$m) and polydispersity of silica particles as measured by Coulter Counter

<table>
<thead>
<tr>
<th>Silica</th>
<th>$s_n$</th>
<th>$s(a)$</th>
<th>$s_w$</th>
<th>$s/s_n$</th>
<th>$s_w/s_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.20.W</td>
<td>16.96</td>
<td>17.54</td>
<td>18.78</td>
<td>1.04</td>
<td>1.11</td>
</tr>
<tr>
<td>H2</td>
<td>13.92</td>
<td>15.00</td>
<td>16.98</td>
<td>1.08</td>
<td>1.22</td>
</tr>
<tr>
<td>H4</td>
<td>12.76</td>
<td>13.74</td>
<td>16.59</td>
<td>1.08</td>
<td>1.30</td>
</tr>
<tr>
<td>H6</td>
<td>8.50</td>
<td>10.11</td>
<td>14.17</td>
<td>1.19</td>
<td>1.67</td>
</tr>
<tr>
<td>SG30</td>
<td>5.62</td>
<td>6.22</td>
<td>8.12</td>
<td>1.11</td>
<td>1.44</td>
</tr>
<tr>
<td>SG60</td>
<td>7.43</td>
<td>7.89</td>
<td>8.78</td>
<td>1.06</td>
<td>1.18</td>
</tr>
<tr>
<td>SG120</td>
<td>6.43</td>
<td>6.94</td>
<td>7.96</td>
<td>1.08</td>
<td>1.24</td>
</tr>
<tr>
<td>S.7.W</td>
<td>6.91</td>
<td>7.05</td>
<td>7.30</td>
<td>1.02</td>
<td>1.06</td>
</tr>
</tbody>
</table>

(a) Defined as the ratio of the second and first moments, see Equation 3.5.
### TABLE 4.4

Results from transmission-interferometry

<table>
<thead>
<tr>
<th>Mean Reading</th>
<th>OPD nm</th>
<th>Particle Divisions</th>
<th>Diameter μm</th>
<th>OPD/d_p (n_a-n_p)</th>
<th>% Silica</th>
<th>Poor Volume cm^3 g^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SG30</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.59</td>
<td>1859</td>
<td>9.5</td>
<td>17.6</td>
<td>0.105</td>
<td>23.0</td>
<td>1.53</td>
</tr>
<tr>
<td>9.08</td>
<td>1668</td>
<td>8.0</td>
<td>14.8</td>
<td>0.113</td>
<td>24.5</td>
<td>1.40</td>
</tr>
<tr>
<td>5.56</td>
<td>627</td>
<td>4.5</td>
<td>8.3</td>
<td>0.075</td>
<td>16.4</td>
<td>2.33</td>
</tr>
<tr>
<td><strong>SG60</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.39</td>
<td>1107</td>
<td>7.8</td>
<td>14.4</td>
<td>0.077</td>
<td>16.7</td>
<td>2.28</td>
</tr>
<tr>
<td>6.52</td>
<td>862</td>
<td>6.7</td>
<td>12.4</td>
<td>0.070</td>
<td>15.1</td>
<td>2.56</td>
</tr>
<tr>
<td>5.08</td>
<td>523</td>
<td>3.7</td>
<td>6.9</td>
<td>0.076</td>
<td>16.6</td>
<td>2.29</td>
</tr>
<tr>
<td><strong>SG120</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.45</td>
<td>1805</td>
<td>9.3</td>
<td>17.2</td>
<td>0.105</td>
<td>22.8</td>
<td>1.54</td>
</tr>
<tr>
<td>6.98</td>
<td>988</td>
<td>5.0</td>
<td>9.3</td>
<td>0.108</td>
<td>23.8</td>
<td>1.51</td>
</tr>
<tr>
<td>6.46 (a)</td>
<td>845 (a)</td>
<td>6.1</td>
<td>11.3</td>
<td>0.075</td>
<td>16.3</td>
<td>2.34</td>
</tr>
<tr>
<td>7.34 (a)</td>
<td>1090 (a)</td>
<td></td>
<td></td>
<td>0.097</td>
<td>21.0</td>
<td>1.71</td>
</tr>
</tbody>
</table>

(a) the two values for OPD were obtained from darkening the whole particle and the "texture" region of the particle (lower OPD).
Figure 4.1.4

Weight fraction particle size distributions for H2, H4, H6 and S.20.W silicas determined by Coulter Counter analysis.

Figure 4.1.5

Weight fraction particle size distributions for SG30, SG60, SG120 and S.7.W silicas determined by Coulter Counter analysis.
Figure 4.14

Particulate Size Distribution

Weight % per 4 µm interval

Particle Diameter (µm)
Figure 4.15

![Graph showing particle diameter distribution with weight percent per 1um interval for different samples: S.7.W, SG30, SG60, SG120.](image)
The mean values for pore volume are shown in Table 4.5 together with literature values as measured by gas adsorption and mercury porosimetry (165). In all cases transmission interferometry gives larger values for the pore volume of Harwell SG silicas. This is expected because the technique measures the whole particle while gas adsorption and mercury porosimetry measure available pore volume, i.e. isolated pores are not measured. However, the pore volume data obtained by all methods show that SG60 is more porous than SG120 which is more porous than SG30. Errors in transmission interferometry are dependent on sample size which is small for Harwell silicas. This occurs because it is difficult to focus the microscope on the edge of particles and not their top, so that an accurate compensator reading can be obtained.
<table>
<thead>
<tr>
<th>Pore Volume $\text{cm}^3\text{g}^{-1}$ by</th>
<th>Transmission Interferometry</th>
<th>Gas Adsorption</th>
<th>Mercury Porosimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG30</td>
<td>1.75</td>
<td>1.54</td>
<td>-</td>
</tr>
<tr>
<td>SG60</td>
<td>2.38</td>
<td>1.77</td>
<td>1.25</td>
</tr>
<tr>
<td>SG120</td>
<td>1.59</td>
<td>1.43</td>
<td>0.90</td>
</tr>
</tbody>
</table>
4.2 **COLUMNS AND CHROMATOGRAPHY**

Because of the varied nature of the work, different packing techniques and availability of packing materials, the columns in this research had different dimensions. These are summarised in Table 4.6.

4.2.1 Tapped/Tamped Packed Columns

Four columns were packed by the tapping/tamping technique with S.20.W and Harwell silicas H2, H4 and H6. When THF was pumped through each separate column, it was not possible to achieve a 0.2 cm$^3$ min$^{-1}$ flow rate under 3500 psi pressure for any of the packed columns. Therefore, the packing technique was modified. This involved filling each column without using the tamping rod and tapping the sides of the column and the base of the column with a metal bar. The filled column was flushed with 150 cm$^3$ of THF at >4 cm$^3$ min$^{-1}$ and then the column was checked to see if any of the packing had settled. If settling had occurred the column was "topped-up" with more packing and was then ready for use. The packed columns containing S.20.W, H2, H4 and H6 silicas were designated S.20.W-T, H2-T and H6-T respectively (Table 4.6).

Calibration curves and toluene plate counts using THF as eluent were obtained for each column. This was carried out using the chromatographic conditions: 10 $\mu$m$^3$ injections of 0.12% w/v of solute in THF; 0.5 cm$^3$ min$^{-1}$ eluent flow rate; ARL detector at 254 nm. All results were reproducible but the chromatograms were very broad rendering them difficult to analyse for an accurate value of $V_R$ and $H$. The calibration curve for each packing is shown in Figure 4.2.1 and toluene efficiencies are listed in Table 4.7. Only the value for the S.20.W-T column ($H = 0.50$ mm) approaches an acceptable value for an ordinary GPC column, since the value anticipated for all these packings was $H \approx 100 \mu$m (75). It was assumed that this was caused by the packing technique and an alternative method was sought.
**TABLE 4.6**

Summary of HPGPC Columns in this Research

<table>
<thead>
<tr>
<th>Column Name</th>
<th>Packing Material</th>
<th>Packing Pressure psi (a)</th>
<th>Column id mm.</th>
<th>Column L mm.</th>
<th>Effective Column L mm.</th>
<th>Preparation at head of column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.20.W-T</td>
<td>S.20.W</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>H2-T</td>
<td>H2</td>
<td>-</td>
<td>3</td>
<td>475</td>
<td>470</td>
<td>a</td>
</tr>
<tr>
<td>H4-T</td>
<td>H4</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>H6-T</td>
<td>H6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>S.20.W-R</td>
<td>S.20.W</td>
<td>1800</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>H2-R</td>
<td>H2</td>
<td>500</td>
<td>3</td>
<td>200</td>
<td>195</td>
<td>a</td>
</tr>
<tr>
<td>H4-R</td>
<td>H4</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>H6-R</td>
<td>H6</td>
<td>750</td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>SG30-H1</td>
<td>SG30</td>
<td>850</td>
<td></td>
<td></td>
<td>245</td>
<td>b</td>
</tr>
<tr>
<td>SG30-H2</td>
<td>SG30</td>
<td>2130</td>
<td></td>
<td></td>
<td>250</td>
<td>c</td>
</tr>
<tr>
<td>SG60-H</td>
<td>SG60</td>
<td>2130</td>
<td>8</td>
<td>250</td>
<td>250</td>
<td>c</td>
</tr>
<tr>
<td>SG120-H</td>
<td>SG120</td>
<td>1420</td>
<td></td>
<td></td>
<td>250</td>
<td>c</td>
</tr>
<tr>
<td>S.7.W-H1</td>
<td>S.7.W</td>
<td>2130</td>
<td></td>
<td></td>
<td>245</td>
<td>b</td>
</tr>
<tr>
<td>S.7.W-H2</td>
<td>S.7.W</td>
<td>2130</td>
<td></td>
<td></td>
<td>250</td>
<td>c</td>
</tr>
<tr>
<td>SG30/G-H</td>
<td>SG30/G</td>
<td>850</td>
<td></td>
<td></td>
<td>245</td>
<td>b, a</td>
</tr>
<tr>
<td>SG60/G-H</td>
<td>SG60/G</td>
<td>2130</td>
<td>8</td>
<td>250</td>
<td>250</td>
<td>c</td>
</tr>
<tr>
<td>SG120/G-H</td>
<td>SG120/G</td>
<td>1420</td>
<td></td>
<td></td>
<td>250</td>
<td>c</td>
</tr>
</tbody>
</table>

(a) 1 psi = 6894.8 Nm⁻²

(b) a, b, c refer to preparation at head of column in Figure 3.2.3.

(c) -T refers to columns packed by tapping, -R refers to columns packed at RAPRA and -H refers to columns packed at Harwell.
TABLE 4.7

Toluene Efficiencies for Silicas packed by the Tapping Technique – Section 4.2.1

<table>
<thead>
<tr>
<th>Packing</th>
<th>Plates/m.</th>
<th>H mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.20.W</td>
<td>2040</td>
<td>0.49</td>
</tr>
<tr>
<td>H2</td>
<td>200</td>
<td>4.92</td>
</tr>
<tr>
<td>H4</td>
<td>200</td>
<td>5.00</td>
</tr>
<tr>
<td>H6</td>
<td>210</td>
<td>4.83</td>
</tr>
</tbody>
</table>
Figure 4.2.1

GPC calibration curves for polystyrene standards, TPE and toluene.

O, S.20.W-T; □, H2-T; Δ, H4-T; ●, H6-T.

Eluent: THF
Injection: 10 μdm$^3$ of 0.12% w/v solute
Detection: UV at 254 nm
4.2.2 Balanced Density Slurry Packed Columns

No problems were encountered when S.20.W, H2, H4 and H6 silicas were packed using this method to give columns designated S.20.W-R, H2-R, H4-R and H6-R respectively. The packing pressure used for each column is shown in Table 4.6. If the values for $s_w$ from Table 4.3 are used for $d_p$ in Equation 2.45 with $L = 195$ mm and $d_c = 3$ mm, then it can be seen that the above columns are close to functioning in the "infinite diameter" mode. The divergence of the solute bands down the column decreases for larger solutes at faster flow rates. The choice of column dimensions was mainly determined by the limited quantities of the silica samples.

Polystyrene calibration curves were obtained for each individual column, as shown in Figure 4.2.2. A study of the effect of flow rate on column efficiency was made for each column using totally permeating solutes (toluene and TPE), partly permeating polystyrene standards and totally excluded polystyrene standards, Figures 4.2.3 to 4.2.6.

4.2.3 Unmodified SG Silicas

Six unmodified silicas were packed by the methanol slurry technique into larger bore columns than those used for the balanced density slurry technique (see Table 4.6). The first column to be packed was a SG30 column, designated SG30-H1, for use with on-column syringe injection (Figure 3.2.2). A polystyrene calibration curve was obtained for this column, as shown in Figure 4.2.7, but severe tailing and mis-shaped peaks were noticed for peaks of polystyrene standards of molecular weight $>200,000$. When SG30-H1 was used for a study of the effect of flow rate on column efficiency for polystyrene standards (Figure 4.2.8) it was noted that the tailing of the excluded standards increased. This prevented the calculation of column efficiencies. In order to assess whether this was a viscosity or over-load effect a study of efficiency versus injected volume was made for various polystyrene standards (Figure 4.2.9). The efficiency decreased with increased injection volume, but no tailing or
Figure 4.2.2

GPC calibration curves for polystyrene standards, TPE and toluene.

O, S.20.W-R; □, H2-R; Δ, H4-R; ●, H6-R.

Eluent: THF

Injection: 2 μdm$^3$ of 0.2% w/v solute

Detection: UV at 254 nm
Figure 4.2.3

Dependence of plate height on flow rate for S.20.W-R:

◇, PS-470K and PS-110K; x, PS-2,350; ◆, TPE; ●, toluene.

Eluent: THF
Injection: 2 μdm$^3$ of 0.2% w/v solute
Detection: UV at 254 nm
Figure 4.2.4

Dependence of plate height on flow rate for H2-R:
•, PS-1,987K; △, PS-200K; □, PS-35K; ●, toluene.

Eluent: THF
Injection: 2 μl dm⁻³ of 0.2% w/v solute
Detection: UV at 254 nm
Figure 4.2.5

Dependence of plate height on flow rate for H₄-R:

●, PS-1,987K; □, PS-35K; ■, PS-9,800; ●, toluene.

Eluent: THF

Injection: 2 μdm³ of 0.2% w/v solute

Detection: UV at 254 nm
Figure 4.2.6

Dependence of plate height on flow rate for H6-R:

•, PS-1,987K; Δ, PS-200K; □, PS-35K; ●, toluene.

Eluent: THF

Injection: 2 μm³ of 0.2% w/v solute

Detection: UV at 254 nm
Figure 4.2.7

GPC calibration curves for polystyrene standards, TPE, DPE and toluene.

○, S.7.W-H1; □, SG30-H1; ■, SG30/G-H.

Eluent : THF

Injection: 10 μdm$^3$ of 0.2% w/v solute

Detection: UV at 254 nm
Figure 4.2.8

Dependence of plate height on flow rate for SG30-H1:

Δ, PS-200K; ▲, PS-110K; □, PS-35K; ■, PS-9,800;
+, PS-3,600; ×, PS-600; ○, TFE; ●, toluene.

Eluent : THF

Injection: 10 μdm$^3$ of 0.2% w/v solute

Detection: UV at 254 nm
Figure 4.2.9

Dependence of plate height on injection volume for SG30-H1.

Same symbols as Figure 4.2.8.

Eluent: THF

Flow rate: 1 cm³ min⁻¹

Injection: 0.5% w/v solute

Detection: UV at 254 nm
mis-shaped peaks occurred for PS-110,000 or PS-200,000 indicating that the problems were not caused by viscosity or over-load effects. A further possible cause could have been poor packing procedure. Therefore, a further column of SG30 silica designated SG30-H2 for use with off-column injection, Figure 3.2.4, was prepared with the packing pressure increased from 850 psi to 2130 psi. Tailing and mis-shaped peaks do not occur for excluded polystyrene standards with this column under test conditions identical to those for the SG30-H1 column. However, an efficiency versus flow rate study with the SG30-H2 column was not practical. This was because of its much lower column efficiencies for toluene, as shown in Table 4.8, compared to the SG30-H1 column owing to the injection procedure.

Two further macroporous silicas, SG60 and SG120, were packed by the methanol slurry technique, to give SG60-H and SG120-H columns respectively for use with off-column injection. Their polystyrene calibration curves are shown in Figure 4.2.10 together with SG30-H2 and their column efficiencies for toluene are shown in Table 4.8. Because of the low efficiency found for SG60-H, this column was not used for further work.

Two S.7.W columns were packed by the methanol slurry technique, to give S.7.W-H1 with on-column injection and S.7.W-H2 with off-column injection (Table 4.6). Their polystyrene calibration curves are shown in Figures 4.2.7 and 4.2.10 respectively and their column efficiencies for toluene in Table 4.8. The S.7.W-H1 column was used to study the efficiency of totally excluded polystyrenes versus flow rate (Figure 4.2.11).

Calibration curves for dextrins in water were also obtained for S.7.W-H2, SG30-H2 and SG120-H (Figure 4.2.12) and S.7.W-H2 + SG120-H and SG30-H2 + SG120-H column combinations (Figure 4.2.13). A combination of a high and low pore size column set, such as S.7.W-H2 + SG120-H has been promoted by Du Pont for polymer analysis in place of the conventional column set containing a larger number of different pore sizes (166). This they refer to as a bi-modal column set. The S.7.W-H2 + SG120-H
**TABLE 4.8**

Toluene efficiencies\(^{(a)}\) for silicas
packed by the methanol slurry technique

<table>
<thead>
<tr>
<th>Column Name (b)</th>
<th>Injection (c)</th>
<th>Plates/m</th>
<th>H (\mu)m</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG30-H1</td>
<td>A</td>
<td>52,050</td>
<td>19.2</td>
</tr>
<tr>
<td>SG30-H2</td>
<td>B</td>
<td>20,960</td>
<td>47.7</td>
</tr>
<tr>
<td>SG60-H</td>
<td>B</td>
<td>6,700</td>
<td>149.3</td>
</tr>
<tr>
<td>SG120-H</td>
<td>B</td>
<td>21,960</td>
<td>45.5</td>
</tr>
<tr>
<td>S.7.W-H1</td>
<td>A</td>
<td>74,650</td>
<td>13.4</td>
</tr>
<tr>
<td>S.7.W-H2</td>
<td>B</td>
<td>23,890</td>
<td>41.9</td>
</tr>
<tr>
<td>SG30/H2 + SG120-H</td>
<td>B</td>
<td>12,030</td>
<td>83.1</td>
</tr>
<tr>
<td>SG30/G-H1</td>
<td>A</td>
<td>50,290</td>
<td>19.9</td>
</tr>
<tr>
<td>SG30/G-H2</td>
<td>B</td>
<td>21,970</td>
<td>45.5</td>
</tr>
<tr>
<td>SG60-H</td>
<td>B</td>
<td>6,980</td>
<td>143.3</td>
</tr>
<tr>
<td>SG120-H</td>
<td>B</td>
<td>21,990</td>
<td>45.5</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Eluent : THF
Flow rate : 1 cm\(^3\) min\(^{-1}\)
Injection : 10 \(\mu\)dm\(^3\) of 0.2\% w/v toluene
Detection : UV at 254 nm

(b) See Table 4.6

(c) A, on-column injection Figure 3.2.2.
    B, off-column injection Figure 3.2.4.
Figure 4.2.10

GPC calibration curves for polystyrene standards, TPE, DPE and toluene.

O, S.7.W-H2; Δ, SG30-H2; ⋄, SG60-H; □, SG120-H.

Eluent : THF
Flow Rate: 1 cm³ min⁻¹
Injection: 10 µdm³ of 0.2% w/v solute
Detection: UV at 254 nm
Dependence of plate height on flow rate for S.7.W-H1:

- $\diamondsuit$, PS-1,987K;
- $\Phi$, PS-955K;
- $\lozenge$, PS-470K;
- $\Delta$, PS-200K;
- $\triangleleft$, PS-110K;
- $\bullet$, toluene.

Eluent: THF

Injection: 10 $\mu$m$^3$ of 0.01% w/v solute

Detection: UV at 254 nm
Plate Height (µm)

Flow Rate (cm³ min⁻¹)
Figure 4.2.12

GPC calibration curves for dextrans and maltose.

O, S.7.W-H2; □, SG30-H2; ●, SG120-H; ■, SG30/G-H.

Eluent: Water

Injection: 25 μm³ of 0.25% w/v solute

Detection: RI
Figure 4.2.13

GPC calibration curves for dextrans and maltose.


Eluent : Water

Injection: 50 μdm³ of 0.25% w/v solute

Detection: RI
calibration curve for dextrins was used to analyse T20, T40 and T70 dextrins for MWD. The chromatograms were analysed according to the method described in Section 2.2.3 and the results are shown in Table 4.9. Results for column efficiencies determined with maltose in water for all the columns described in the above dextran work are shown in Table 4.10. Dextran calibration curves were also obtained for the SG30-H1 column using water and formamide, but no difference in V_R for each dextran standard could be detected and there was no difference in column efficiencies for maltose.

Although information on MWD can be obtained for dextrins on the S7.5W-H + SG120-H column set (Table 4.9) the dextrins T20, T40 and T70 are better separated with the SG30-H2 + SG120-H column set (c.f. Figures 2.1.1 and 4.2.13). Table 4.11 shows the effect on V_R of adding potassium chloride to the eluent used for analysing the above dextrins on the SG30-H2 + SG120-H column set.

4.2.4 γ-G Modified SG Silicas

Three γ-G modified macroporous silicas, SG30/G, SG60/G and SG120/G, were packed by the methanol slurry technique to give SG30/G-H, SG60/G-H and SG120/G-H columns respectively (Table 4.6). The SG30/G-H column, for use with on-column injection, was packed at the same pressure as the SG30-H1 column. During its calibration with polystyrene standards, Figure 4.2.7, and a study of the effect of efficiency on flow rate, Figure 4.2.14, tailing and mis-shaped peaks occurred for polystyrene standards >200,000. However, with the off-column injection technique used for SG60-H and SG120-H for their polystyrene calibration curves, no tailing or mis-shaped peaks were obtained. The toluene efficiencies for the above columns (Table 4.8) again shows that the SG60/G-H column is much less efficient than other columns. As for the SG30-H1 column, there was no difference in the dextran calibration curves in water and formamide.
### Table of average molecular weights of dextrans by HPGPC (a)

<table>
<thead>
<tr>
<th>Dextran</th>
<th>$\overline{M}_n$</th>
<th>$\overline{M}_v$ (b)</th>
<th>$\overline{M}_w$</th>
<th>$\overline{M}_p$ (c)</th>
<th>$\overline{M}_p$(obs) (d)</th>
<th>$\overline{M}_w/\overline{M}_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20</td>
<td>19,190</td>
<td>26,320</td>
<td>28,440</td>
<td>23,360</td>
<td>28,900</td>
<td>1.482</td>
</tr>
<tr>
<td>T40</td>
<td>34,840</td>
<td>46,190</td>
<td>49,590</td>
<td>41,570</td>
<td>48,720</td>
<td>1.423</td>
</tr>
<tr>
<td>T70</td>
<td>52,500</td>
<td>66,747</td>
<td>71,290</td>
<td>61,180</td>
<td>70,800</td>
<td>1.358</td>
</tr>
</tbody>
</table>

(a) Column set : S.7.W-H2 + SG120-H  
Eluent : Water  
Flow rate : 1 cm$^3$ min$^{-1}$  
Injection : 75 pmol$^3$ of 0.5% w/v solute  
Detection : RI  

(b) $a = 0.50$ from ref. (167) used in Equation 2.55  
(c) from Equation 2.57  
(d) Observed $V_R$ for dextran.
TABLE 4.10

Maltose efficiencies\(^{(a)}\) for silicas packed by the methanol slurry technique

<table>
<thead>
<tr>
<th>Column Name(^{(b)})</th>
<th>Plates/m</th>
<th>H (\mu)m</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG30-H2</td>
<td>13,920</td>
<td>71.8</td>
</tr>
<tr>
<td>SG120-H</td>
<td>17,880</td>
<td>55.9</td>
</tr>
<tr>
<td>S.7.W-H2</td>
<td>22,730</td>
<td>44.0</td>
</tr>
<tr>
<td>SG30-H2 + SG120-H</td>
<td>7,890</td>
<td>126.7</td>
</tr>
<tr>
<td>S.7.W-H2 + SG120-H</td>
<td>7,110</td>
<td>140.6</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Eluent : Water  
Flow rate : 1 cm\(^3\) min\(^{-1}\)  
Injection : 20 \(\mu\)d\(^3\) of 0.3% w/v solute, off-column injection  
Detection : RI

\(^{(b)}\) See Table 4.6
TABLE 4.11

Retention volumes of dextrans using various eluents (a)

<table>
<thead>
<tr>
<th>Eluent</th>
<th>$V_R$ for T20 cm$^3$</th>
<th>$V_R$ for T40 cm$^3$</th>
<th>$V_R$ for T70 cm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>17.78</td>
<td>17.00</td>
<td>15.51</td>
</tr>
<tr>
<td>0.8M KCl</td>
<td>18.10</td>
<td>17.30</td>
<td>15.85</td>
</tr>
<tr>
<td>2.0M KCl</td>
<td>18.20</td>
<td>17.50</td>
<td>16.05</td>
</tr>
</tbody>
</table>

(a) Column set: SG30-H2 + SG120-H  
Flow rate: 1 cm$^3$ min$^{-1}$  
Injection: 75 µdm$^3$ of 0.5% w/v solute  
Detection: RI
Figure 4.2.14

Dependence of plate height on flow rate for SG30/G-H:

▲, PS-110K; □, PS-35K; ■, PS-9,800; +, PS-3,600;
×, PS-600; O, TPE; ●, toluene.

Eluent: THF

Injection: 10 μdm$^3$ of 0.2% w/v solute

Detection: UV at 254 nm
All the proteins in Table 3.3 were used to calibrate the SG30/G-H column, with an aqueous eluent of 0.1M phosphate buffer at pH 6.3, see Figure 4.2.15. Only lysozyme, of all solutes examined on all columns, eluted after the totally-permeating point, having $V_R = 14.6 \text{ cm}^3$. However, its peak was not distorted. Four solutes: albumin, ovalbumin, myoglobin and acetamide were used to examine the column efficiency of SG30/G-H at varying flow rates (Figure 4.2.16). During this work the nickel mesh at the head of the column, see Figure 3.2.3a, began to corrode and the column head was rebuilt using the method shown in Figure 3.2.3b. The SG60/G-H and SG120/G-H columns were not used for examining proteins because most proteins would perform as totally permeating solutes.

In all this work no more than two polymer solutes were used in a given injection. However, several standards can be incorporated into a single injection in order to obtain a rapid calibration or for demonstration purposes. Figure 4.2.17 pictorially demonstrates the decrease in column efficiency with increase in flow rate for six polystyrene standards in THF using the SG30/G-H column. As the flow rate increases the peaks begin to merge.
Figure 4.2.15

Molecular weight calibration curve for proteins on SG30/G-H.

Δ, thyroglobulin; ■, catalase; △, albumin; □, ovalbumin;
▼, pepsin; ○, myoglobin; ▼, cytochrome c.
Figure 4.2.16

Dependence of plate height on flow rate for SG30/G-H1:

Δ, albumin; □, ovalbumin; ○, myoglobin; ●, acetamide.

Eluent: 0.1M phosphate buffer, pH 6.3
Injection: 10 µdm³ of 0.1% w/v solute
Detection: UV at 215 nm
Effect of flow rate on the ability of SG30/G-H to separate a "cocktail" of polystyrene standards.

(a) PS-110K; (b) PS-35K; (c) PS-75K; (d) PS-9,800;
(e) PS-3,600; (f) PS-600; (g) impurity peak.

Eluent : THF
Injection: 10 pmol of 0.05% w/v of each solute (0.3% total)
Detection: UV at 254 nm

![Graph showing the effect of flow rate on separation](image-url)
4.3 VISCOSITY OF DEXTRANS

The solution viscosity of three dextrans, T20, T40 and T70, was measured in water, 0.8M and 2.0M potassium chloride solution. The plots of \( \eta_{sp}/c \) and \( \ln \eta_{R}/c \) versus \( c \) for water is shown in Figure 4.3.1 and their common intercepts at \( c = 0 \) (the intrinsic viscosity) for all solutions are listed in Table 4.12.
### TABLE 4.12

Intrinsic viscosities of dextrans in salt solutions at 25°C

<table>
<thead>
<tr>
<th>Dextran</th>
<th>$\bar{M}_v^{(a)}$</th>
<th>$[\eta]$ in water</th>
<th>$[\eta]$ in 0.8M KCl</th>
<th>$[\eta]$ in 2.0M KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20</td>
<td>17,700</td>
<td>0.126</td>
<td>0.120</td>
<td>0.129</td>
</tr>
<tr>
<td>T40</td>
<td>28,900</td>
<td>0.166</td>
<td>0.159</td>
<td>0.166</td>
</tr>
<tr>
<td>T70</td>
<td>50,300</td>
<td>0.219</td>
<td>0.230</td>
<td>0.228</td>
</tr>
</tbody>
</table>

(a) $\bar{M}_v$ in water at 25°C using K and a from ref. (143).
Figure 4.3.1

Solution viscosity of dextrans measured in water at 25°C.

Δ, T20 dextran; □, T40 dextran; O, T70 dextran.
CHAPTER 5 DISCUSSION
5.1 **DISPERSION MECHANISMS**

The theoretical treatments of chromatogram broadening, as described in Section 2.1.2, predict that column performance depends on flow mechanisms in the mobile phase and solute mass transfer between the stationary and mobile phases. Therefore, in order to interpret the experimental results given in Section 4.2 the individual contributions to chromatogram broadening must be separated and evaluated.

5.1.1 **Column S.7.W-H1**

Values of plate height $H$ versus flow rate for non-permeating polystyrene standards and toluene with S.7.W-H1 are shown in Figure 4.2.11. The efficiency decreases with increasing molecular weight for PS-110K, PS-200K PS-470K, PS-955K and PS-1,987K. But for a given polystyrene standard, Figure 4.2.11 shows that $H$ varies little with flow rate. Billmeyer and Kelley (64) reported for crosslinked polystyrene particles ($d_p \approx 40 \mu m$) that $H$ varies little with flow rate for a non-permeating polystyrene having a molecular weight of 160,000. The same polystyrene was also examined with a column containing non-porous glass particles ($d_p \approx 120 \mu m$) and again $H$ was independent of flow rate (65). The results of Billmeyer and Kelley (65) also suggest that $H$ remains constant as flow rate increases for polystyrenes with molecular weights 19,800 and 97,200 and that $H$ falls at lower flow rates for low molecular weight solutes ($\lesssim 10,300$). Giddings, Bowman and Myers (168) also observed that $H$ hardly changed for a non-permeating polystyrene (molecular weight 20,400) with a column of glass particles ($d_p \approx 55 \mu m$). Dawkins et al. have shown that non-permeating polystyrenes with molecular weights 111,000, 402,000 and 470,000 have $H$ values which exhibit little change with flow rate for silica particles ($d_p \approx 20, 44$ and 65 $\mu m$) (80,81), alumina particles ($d_p \approx 20 \mu m$) (81) and cross-linked polystyrene particles ($d_p \approx 20 \mu m$) (77). This has also been discussed in connection with µStyrageL columns ($d_p \approx 10 \mu m$) (69).

These results may be interpreted in terms of Equation 5.1 (same as Equation 2.30), van Deemter's equation (63):

---

64
where the variables are defined for Equation 2.30. Figure 2.2.1 shows the dependence of $H$ on flow rate using Equation 5.1. A minimum is observed because term (ii) is larger than terms (iii) and (iv) at low flow rates (seen at 2 cm$^3$ min$^{-1}$ for toluene in Figure 4.2.11). As the molecular weight of a polymer increases, the values of $D_m$ and $D_s$ decrease so that term (ii) decreases and terms (iii) and (iv) increase. Hence term (ii) becomes larger than terms (iii) and (iv) at much lower flow rates as solute size increases. Consequently, it is assumed that dispersion due to longitudinal molecular diffusion is unimportant for high polymers at practical flow rates. For non-permeating polymers term (iv) does not arise. From Figure 4.2.11 and the results of the above workers it is reasonable to assume that term (iii) is small and that term (i) may be regarded as independent of flow rate. However, this explanation is unsatisfactory because it implies that $H$ is also independent of molecular weight for non-permeating polymers which is not observed in Figure 4.2.11.

To see if the results for the S.7.W-H1 column are valid, the S.20.W silica was examined in columns having different dimensions and packed by a different technique.

5.1.2 Column S.20.W-R

Values of $H$ for toluene at a flow rate of 0.5 cm$^3$ min$^{-1}$, shown in Figure 4.2.3 for S.20.W-R, are a factor of ten lower than the values of $H$ for the S.20.W-T column, shown in Table 4.7, and the values reported in an earlier paper (80). This is because of the reliable slurry packing procedure and from the improved injection method (on-column syringe injection) over the one used in ref. (80). Additionally, the column dimensions used for the S.20.W-R work (see Table 4.6) and the Spherisorb S.20.W in ref. (80) do not allow the columns to function in the "infinite diameter" mode.
In Figure 4.2.3, the two non-permeating polystyrenes, PS-110K and PS-470K, give little variation of H with flow rate. At flow rates $>0.3 \text{ cm}^3 \text{ min}^{-1}$ the mean value for non-permeating polystyrenes is about $130 \mu m$ and at flow rates $<0.3 \text{ cm}^3 \text{ min}^{-1}$ the mean values is about $80 \mu m$. However, values of H for PS-470K were always greater than PS-110K which is in agreement with the results from S.7.W-H1. TPE which permeates slightly less pore volume than toluene (see Figure 4.2.2), gives a much more significant rise in H as flow rate increases than the non-permeating polystyrenes in Figure 4.2.3. Additionally, PS-2,350 permeates less pore volume than TPE (see Figure 4.2.2) and gives a greater rise in H as flow rate increases. The slope of the curve in Figure 4.2.3 is about eight times greater than the slope for TPE. Therefore, it is reasonable to assume that the value of term (iii) in Equation 5.1 is very small compared to term (iv) and the assumption that term (i) may be regarded as a constant independent of flow rate is valid for Figure 4.2.3 when considering permeating polymers. This work suggests that Equation 5.1 for high polymers, at practical flow rates, may be written as:

$$H = A + C_s u$$

where $A$ and $C_s$ are defined for Equation 2.31. Consequently, the polystyrene standard PS-2,350 in Figure 4.2.3 can be redrawn to give a linear relation between H and flow rate in terms of linear flow rate, as shown by Figure 5.1.1. Linear flow rate is calculated with use of $V_o$ determined with a non-permeating polystyrene standard. For toluene there is no increase in H as flow rate decreases, and term (ii) in Equation 5.1 can again be neglected. The dependence of H for toluene on flow rate, in Figure 4.2.3, suggests that terms (iii) and (iv) for mass transfer between the mobile and stationary phases is not very significant. This is explained by the high value of $D_m$ for toluene in Equation 5.1. Solute dispersion arising from a non-uniform flow velocity across a column, as proposed by Billmeyer and Kelley (8,65-67) and discussed in Section 2.1.2, can be used to
Figure 5.1.1

Dependence of plate height on flow rate for S.20.W-R.
A linear plot of Figure 4.2.3 with the same symbols.
interpret higher H values for a non-permeating polystyrene than for a totally permeating solute. In Figure 4.2.3, the H values for toluene and the two non-permeating polystyrenes are very similar, suggesting that the velocity profile effect is reduced considerably. A non-uniform flow velocity effect across a column is expected with particles having a wide size range, because of variable resistance to fluid flow across a column. It is evident in Figure 4.1.1 and Table 4.3 that S.20.W silica particles are very regular and have a narrow size distribution. Provided the column is packed carefully, a homogeneous bed of particles should result. The results for toluene and non-permeating polystyrenes with S.20.W-R are similar to H reported for cross-linked polystyrene gel particles (16-20 μm) having a spherical shape and a narrow particle size distribution, see Figure 6 in ref. (77). All these results suggest that optimum performance requires not only a low value of \( d_p \) but a narrow particle size distribution. Small differences between H values for silica and cross-linked polystyrene gels may arise from a contribution to H from solute adsorption onto the pore surface, and Giddings et al. (168) have identified a small adsorption contribution for solutes in dichloroethane separating on porous glass. This effect has not been considered in Equation 5.1.

Values of H for toluene, in Figure 4.2.3, for S.20.W-R are a factor of ten times higher than the values of H for the S.7.W-H1 column, shown in Figure 4.2.11. This is expected because of the smaller particle size of S.7.W. silica compared to S.20.W silica as shown in Table 4.3. For a better comparison of data between the two columns, at a given flow rate the linear flow rate must be used to account for differences in \( V_o \) caused mainly by the use of different column dimensions but also to a lesser extent by the different packing techniques and particle size distributions, Table 4.3. A plot of H versus linear flow rate for S.20.W-R and S.7.W-H1, redrawn from Figures 4.2.3 and 4.2.11, and with the use of \( V_o \) from Figures 4.2.2 4.2.7 respectively for calculating linear flow rate, are shown in Figure 5.1.2 for toluene and the excluded polystyrenes PS-1,987K, PS-955K,
PS-470K, PS-200K and PS-110K. For the S.20.W-R column a flow rate of $1 \text{ cm}^3 \text{min}^{-1} = 5.08 \text{ mm s}^{-1}$ while for the S.7.W-H1 column a flow rate of $1 \text{ cm}^3 \text{min}^{-1} = 0.08 \text{ mm s}^{-1}$. Hence, in Figure 5.1.2 the scale of the H axis has been expanded by a factor of about 20 compared to Figure 4.2.3 for the S.20.W-R column, while the scale of the flow rate axis remains unchanged. It can be clearly seen in Figure 5.1.2 that the comments made above concerning the linearity and zero slope of the toluene and non-permeating polystyrene plots in Figure 4.2.3 are only truly valid when comparing them to partly permeating polystyrenes, i.e. PS-2,350.

These conclusions are similar to those found by Knox et al. (69,70) who proposed Equation 2.39 for small molecules. At higher flow rates or for polymers at practical flow rates the molecular diffusion term is negligible and Equation 2.39 can be written as:

$$H = Au^m + Cu$$  \hspace{1cm} (5.3)

where the first term on the right side of Equation 5.3 is essentially empirical and arises from the non-additive coupling of eddy diffusion and mass transfer in the mobile phase, as shown in Equation 2.33. The exponent $m$ in Equation 5.3 is between 0.25 and 0.35 and is generally assumed to be 0.33. Equation 5.3 predicts that the plate height versus linear flow rate curve for PS-2,350 in Figure 5.1.1 should not be plotted linearly as at high flow rates the curve should bend away from linearity towards the flow rate axis. Therefore, if this equation is valid for permeating polymers then the second term dominates and the first term can be regarded as a constant; hence, Equation 5.3 becomes Equation 5.2. In order to investigate the applicability of empirical theories to the interpretation of chromatographic data, non-permeating polymers should be used. This will eliminate the dominant second term arising from mass transfer in the stationary phase in Equation 5.3 which then becomes the Snyder Equation (71) (Equation 2.42):

$$H = Au^m$$  \hspace{1cm} (5.4)

S.7.W-H1: same symbols as Figure 4.2.11.
All the curves for the non-permeating polystyrenes in Figure 5.1.2, except PS-955K and PS-1,987K on S.7.W-H1 are the same shape as those generated by Equation 5.4. The curve for toluene with S.20.W-R has a greater slope than the non-permeating polystyrenes because of the extra contribution from mass transfer in the stationary phase shown by Equation 5.3.

When using an equation to evaluate or interpret chromatographic data, the experimenter can use dimensionless or absolute kinetic variables. That is, the use of plate height H and linear velocity u or reduced plate height h, given in Equation 2.38, and reduced velocity v, given in Equation 2.37. Knox has, in a series of papers (61,69,70,170), advocated the use of the reduced parameters proposed by Giddings (59,68) because identical h versus v curves are obtained from columns which differ only in geometric scale and in the nature of eluent used. Knox (61) has replotted data of Majors (78), who examined the efficiency of azo dyes on six porous silicas covering the size ranges 6-45 µm, using the reduced parameters. His results showed that small particles performed better than larger ones. This he also confirmed by a comparison of data between 50 µm and 10 µm silica (61). Kelley and Billmeyer (8) have replotted their own data (67,171) against that of Le Page (45) with reduced parameters for permeating solutes on porous silica for dp ≈ 70, 90, 110, 140, 180 and 225 µm and found that reduced broadening occurred for high particle sizes. However, they attributed this to poor packing technique. The non-permeating polystyrenes in Figure 5.1.2 can be redrawn with reduced parameters as shown by Figure 5.1.3. Values of dp were taken as sw in Table 4.3, and values of Dm for all solutes in THF at 293K were estimated from the Wilke-Chang equation (172,173) and from the expression proposed by Rudin and Johnson (174). The average error in Dm using the Wilke-Chang equation is 10% (173). The data for the non-permeating polystyrenes in Figure 5.1.3 do not fit a single straight line as Equation 5.4 predicts. However, the spread in data corresponding to molecular weight in Figure 5.1.2
Figure 5.1.3

Dependence of reduced plate height on reduced velocity for S.20.W-R and S.7.W-H1 with same symbols as Figure 5.1.2.
Figure 5.1.4

Logarithmic plots of reduced plate height against reduced velocity for S.20.W-R and S.7.W-H1. Data from Figures 5.1.2 and 5.1.3 with the same symbols. Dotted curves show spread of data from ref. (170).
is not compensated for in Figure 5.1.3, but the relative positions of the curves are changed. The curves for PS-470K and PS-110K with S.20.W-R in Figure 5.1.3 have moved below the curve for PS-955K with S.7.W-H1. Values for toluene could not be included in Figure 5.1.3 because the high value of \( D_m \) for toluene, compared to \( D_m \) for the excluded polystyrene standards, gives a very short range of \( v \) over which to plot the data with the axis used in Figure 5.1.3. In order to present this type of data liquid chromatographers usually use a log h versus log \( v \) plot, allowing direct determination of the parameters \( D' \) and \( m' \) in the reduced form of the Snyder equation, Equation 2.42, although log/log plots are not considered mathematically sound. All the data in Figure 5.1.2 can be represented by this method, as shown in Figure 5.1.4. Again the spread in the curves of the non-permeating polystyrenes on S.7.W-H1 is evident in Figure 5.1.4.

Kelley and Billmeyer (70,171) found a single curve would pass through the efficiencies obtained from examining three polystyrene standards on one of three columns containing three non-porous glass particles (\( d_p \approx 70, 90 \) and \( 300 \mu m \)) when plotted against reduced flow rate. However, on close examination of their figures it is possible to see that there is an ordering of the data similar to the excluded polystyrenes in Figures 5.1.3 and 5.1.4 for the S.7.W-H1 column. From Figure 5.1.4 the results for FS-110K and FS-470K are closer together when examined with the S.20.W-R column than the S.7.W-H1 column. Thus, there appears to be a trend with particle size; where the visible spread of the curves for excluded polystyrenes on the S.7.W-R column (\( d_p = 7.3 \mu m \)) is larger than on the S.20.W-R column (\( d_p = 17.8 \mu m \)) which is hardly visible on the 70 \( \mu m \) non-porous glass particles of Kelley and Billmeyer (70,171). No data on the examination of a range of non-permeating polymers or a range of polymers with non-porous particles of similar \( d_p \) to S.7.W is available in the literature.

Figure 5.1.4 also shows data for the efficiency of toluene with S.20.W-R and S.7.W-H1, demonstrating the improved efficiency obtained by
decreasing particle size. No minima is observed in Figures 4.2.3, 5.1.2 and 5.1.4 for the efficiency of toluene versus flow rate for S.20.W-R, while such a minima is observed at 2 cm$^3$/min$^{-1}$ for toluene with S.7.W-H1 in Figures 4.2.11, 5.1.2 and 5.1.4. In Section 2.2.1 it was stated that from the differential of $H$ in Equation 2.30, with respect to flow rate $u$, that as $d_p$ decreases the optimum flow rate (minimum $H$) increases. Figure 5.1.2 can be used for a comparison of efficiency versus flow rate curves for toluene for S.20.W-R and S.7.W-H1. It is clear that the absence of a minima for toluene on S.20.W-R is due to insufficient low flow rate data as is also shown by the 20 pm cross-linked polystyrene gel packing in Figure 2.2.2 which is of similar particle size to S.20.W. A more direct comparison between Figure 2.2.2 and Figure 4.2.11 can be made because the columns used have similar internal diameters (75). In Figure 2.2.2 the minima occurs at 1 cm$^3$/min$^{-1}$ for 10 pm particles and at 2.5 cm$^3$/min$^{-1}$ for 5 pm particles which is in agreement with the 2 cm$^3$/min$^{-1}$ for the S.7.W-H1 column for 7.3 pm silica. Additionally, the higher efficiency at this minima for the 250 mm long S.7.W-H1 column versus the 5 pm packing in a 300 mm long column in ref. (75), 9 pm versus 25 pm, is mainly caused by the superior on-column injection procedure.

As the contribution due to molecular diffusion in the mobile phase is negligible for the toluene data in Figure 5.1.4 with S.20.W-R, the plot appears to be linear. This is in agreement with Snyder's equation (Equation 2.42) which holds for small molecules over a ten-fold range of $v$ above 30 (61) ($\log v$ values 1.5 to 2.5 in Figure 5.1.4). Therefore, values of $D'$ and $m'$ in Equation 2.42 can be calculated from Figure 5.1.4, giving $D' = 0.44$ and $m' = 0.25$ by least squares. A low value of $m'$ is desirable in liquid chromatography as higher flow rates may then be used to decrease separation times without a large sacrifice in column efficiency (78). However, porous packings give $m'$ values of about 0.6 (61,78) and lower values are associated with less well packed columns (61). This is in
agreement with Figure 5.1.4 where the reduced plot should theoretically compensate for changes in particle size between S.20.W-R and S.7.W-H1, but the column efficiency for toluene is much lower on the S.7.W-H1 column. Additionally if the toluene curve for S.7.W-H1 was extended to higher flow rates it would give a value of \( m' \approx 0.6 \). This is in agreement with the findings of other workers (170) using 5–10 \( \mu \)m silica for liquid chromatography separations, whose results are bounded by the dotted curves in Figure 5.1.4. From Figure 5.1.4, values of \( m' \) decrease with increasing molecular weight and are between 0.15 and 0.35 for PS-110K, PS-200K and PS-470K on both columns. Values of \( m' \) between 0.25 and 0.35 have been found for unretained solutes on porous glass and non-porous packings of \( d_p > 20 \mu m \) (69). A possible explanation of the variation in the results for excluded polymers with the small particle size S.7.W-H1 is that the eddy diffusion term is not a constant. Blackwell (175) has stated that mixing after stream splitting in liquid systems at low flow rates is diffusionally controlled. Therefore, it seems reasonable to have a set of values of \( \lambda \), defined in term (i) of Equation 5.1, dependent upon \( D_m \). Additionally, this effect would also be flow rate dependent, as suggested by the empirical relationships in Section 2.2 (61,69–71), Equations 5.2 to 5.4 and the eddy diffusion discussion in ref. (76). However, recent work by Rooney and Ver Strate (176) suggests that the variation in results for non-permeating polymers are likely to be caused by the practical difficulties normally associated with ultra-high molecular weight polymers. These are known to give additional chromatogram broadening owing to viscosity (177) and mechanochemical degradation effects (178) during GPC analysis. The original work carried out by Slagowski and Fetters (178) used conventional GPC instrumentation with cross-linked polystyrene gels (\( d_p > 30 \mu m \)) and found mechanochemical degradation to occur for polystyrenes of molecular weight >2,000,000. HPGPC instrumentation was used in the work of Rooney and Ver Strate (176), with cross-linked polystyrene
gels \( (d_p \approx 5 \, \mu m) \) and polyether coated, irregular porous silica \( (d_p = 5 \text{ to } 15 \, \mu m) \), who found that mechanochemical degradation occurred at lower molecular weights and was flow rate dependent giving increased degradation on increasing flow rate. All this work implies that such degradation is particle size and molecular weight dependent and great care should be taken when presenting chromatographic data for high molecular weight polymers determined with HPGPC instrumentation.

5.1.3 Columns H2-R, H4-R and H6-R

Curves demonstrating the dependence of H on flow rate are shown in Figures 4.2.4 to 4.2.6 for H2-R, H4-R and H6-R respectively. The values of H for toluene at a flow rate of 0.2 cm\(^3\) min\(^{-1}\) for all columns are a factor of forty lower than the values of H for the H2-T, H4-T and H6-T columns, shown in Table 4.7. This anomaly is again accounted for by the improved packing procedure. Spherisorb S.20.W differs from H2, H4 and H6 silicas by having: a narrower particle size distribution and more regular particles with a larger particle size, as shown by the SEMs in Figure 4.1.1 and the Coulter Counter analysis in Figure 4.1.4 and Table 4.3; a lower pore volume, as suggested by the calibration curves in Figure 4.2.2 and gas adsorption data \((164,165)\), hence a higher density. Therefore, S.20.W silica is better suited to packing by the tamping method \((80,81)\) than the macroporous silicas H2, H4 and H6 as demonstrated by the column efficiencies for toluene in Table 4.7.

It is observed that the non-permeating PS-1,987K and the totally permeating toluene gives little or no variation of H with flow rate, relative to partly permeating polystyrenes, and therefore the explanations discussed for S.20.W-R in connection with Equation 5.2 in Section 5.1.2, also hold for macroporous silicas. The values of H for PS-1,987K and toluene in Figures 4.2.4 to 4.2.6 are higher than those found with the S.20.W-R column in Figure 4.2.3. Therefore, it may be concluded from Equation 5.1 that the columns H2-R, H4-R and H6-R have higher mobile phase...
dispersions than the S.20.W-R column. This follows because terms (ii) and (iv) may be neglected for a non-permeating solute as discussed in Section 5.1.1. The increase in mobile phase dispersion for H2-R, H4-R and H6-R may be explained by the less regular particles, Figure 4.1.1, and the wider particle size distributions, Table 4.3, giving a heterogeneous bed of particles so that multiple non-uniform flow-paths are generated. The values of H for toluene and PS-1,987K with H6-R are somewhat higher than the values for H2-R and H4-R, as expected from the high value of s_w/s_n in Table 4.3. The increase in H, because of the wide particle size distribution of H6-R, will tend to be balanced by the decrease in H due to the lower value of d_p in Equation 5.1. Additionally the displacement of H values for PS-1,987K with respect to H for toluene in Figures 4.2.4 to 4.2.6 is larger than the displacement for toluene and PS-470K and PS-110K in Figure 4.2.3 for S.20.W-R. This is in agreement with the increased mechanochemical degradation expected for PS-1,987 over PS-470K and PS-110K discussed in Section 5.1.2.

In Figure 4.2.2 it appears that the H2-R and H6-R columns separate a similar range of molecular sizes, although the silica packings have different pore size distributions. (This similarity in calibration curves is also evident for the SG60-H and SG120-H columns in Figure 4.2.10 which have the same nominal pore sizes as H2 and H6 silicas, Table 4.1. The pore size distributions of SG60 and SG120 being different as shown by Figure 5.1.5 (165)). The dependence of H on flow rate for the permeating polystyrenes PS-35K and PS-200K is similar for H2-R and H6-R in Figures 4.2.4 and 4.2.6 suggesting that the mass transfer characteristics of the two columns are the same. The slopes of these curves at \( \leq 1.5 \text{ cm}^3\text{ min}^{-1} \) again show that H increases on permeation and that permeation dispersion increases for increasing molecular weight (decreasing diffusion coefficient). At the lowest flow rate of 0.05 cm\(^3\) min\(^{-1}\), in Figures 4.2.4 and 4.2.6, H for PS-35K is lower than H for PS-1,987K and not much larger than H for
toluene. This work further suggests that Equation 5.1 may be written as Equation 5.2. Therefore, Figures 4.2.4 to 4.2.6 can be redrawn to give a linear relation between H and flow rate in terms of linear flow rate for permeating polystyrenes, as shown by Figures 5.1.6 to 5.1.8 respectively.
Figure 5.1.5

Pore size distribution for S.7.W, SG30, SG60 and SG120 from ref. (165).
Figure 5.1.6

Dependence of plate height on flow rate for H2-R.
A linear plot of Figure 4.2.4 with the same symbols.
Figure 5.1.7

Dependence of plate height on flow rate for H4-R.
A linear plot of Figure 4.2.5 with the same symbols.
Figure 5.1.8

Dependence of plate height on flow rate for H6-R.
A linear plot of Figure 4.2.6 with the same symbols.
5.2 DIFFUSION COEFFICIENTS

5.2.1 Columns S.20.W-R, H2-R, H4-R and H6-R

The divergence of the curves for permeating solutes as flow rate rises, as shown in Figures 5.1.6 to 5.1.8 for polystyrenes in THF, suggest a dependence of permeation dispersion on solute diffusion coefficient. This is evident from Equation 5.2 which holds for partly permeating polymers at practical flow rate, e.g. >1 mm s\(^{-1}\). Equation 5.2 can be written out in full using the terms in Equations 2.34 and 5.1 giving:

\[
H = 2\lambda d_p + \frac{1}{30} \frac{R(1-R)}{D_m} \frac{d_p^2 u}{D_m}
\]

Equation 5.5 allows the determination of \(D_m\) for solutes in the stationary phase using Figures 5.1.1 and 5.1.6 to 5.1.8 for S.20.W-R, H2-R, H4-R and H6-R respectively. The slope of each linear plot \(D_3\) is given by:

\[
D_3 = \frac{1}{30} \frac{R(1-R)}{D_s} \frac{d_p^2}{D_s}
\]

where \(D_s\) is defined as the diffusion coefficient in the stationary phase. Estimates of \(D_s\) were obtained with Equation 5.5; where the retention ratio \(R\) is given by \(V_c/V_R\) from Figure 4.2.2 and values of \(d_p\) were taken as \(s_w\) from Table 4.3. The results are shown in Table 5.1 together with values of \(R\) and \(D_m\) from refs. (172-174).

5.2.2 Columns SG30-H1 and SG30/G-H

Because Equation 5.2 has been shown to be valid for the macroporous silica H4 it should be valid for the SG30-H1 and SG30/G-H columns whose packings have a similar pore size to H4 silica (as shown by the calibration curves in Figures 4.2.2 and 4.2.7). Figures 4.2.8 and 4.2.14 show the dependence of \(H\) on flow rate for permeating polystyrenes in THF with SG30-H1 and SG30/G-H respectively. A minimum is observed in the plots for toluene, TPE and PS-600 because term (ii) is larger than terms (iii) and (iv) in Equation 5.1 at low flow rates. The flow rate at which the minimum occurs decreases as molecular weight increases as predicted by
### TABLE 5.1

**Diffusion coefficients of polystyrene standards and proteins**

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>$D_m/10^{-7}$ cm$^2$ s$^{-1}$</th>
<th>Column Name (a)</th>
<th>R</th>
<th>$D_s/10^{-8}$ cm$^2$ s$^{-1}$</th>
<th>$D_s/D_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-2,350</td>
<td>37.2</td>
<td>S.20.W-R</td>
<td>0.77</td>
<td>21.4</td>
<td>0.058</td>
</tr>
<tr>
<td>PS-3,600</td>
<td>28.8</td>
<td>{ SG30-H1, SG30/G-H }</td>
<td>0.55</td>
<td>41.6</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
<td>30.6</td>
<td>0.106</td>
</tr>
<tr>
<td>PS-9,800</td>
<td>15.8</td>
<td>{ H4-R, SG30-H1, SG30/G-H }</td>
<td>0.58</td>
<td>30.1</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.60</td>
<td>20.7</td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.63</td>
<td>15.4</td>
<td>0.096</td>
</tr>
<tr>
<td>PS-35K</td>
<td>7.4</td>
<td>{ H2-R, H4-R, H6-R, SG30-H1, SG30/G-H }</td>
<td>0.60</td>
<td>11.3</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
<td>8.2</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
<td>11.7</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.73</td>
<td>6.0</td>
<td>0.082</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
<td>5.3</td>
<td>0.072</td>
</tr>
<tr>
<td>PS-200K</td>
<td>2.6</td>
<td>{ H2-R, H6-R }</td>
<td>0.73</td>
<td>4.3</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
<td>2.9</td>
<td>0.112</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>10.3</td>
<td>{ SG30/G-H }</td>
<td>0.53</td>
<td>7.8</td>
<td>0.076</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>7.8</td>
<td></td>
<td>0.60</td>
<td>7.5</td>
<td>0.096</td>
</tr>
<tr>
<td>Albumin</td>
<td>6.0</td>
<td></td>
<td>0.63</td>
<td>4.5</td>
<td>0.075</td>
</tr>
</tbody>
</table>

(a) see Table 4.6.
theory and described in Section 5.1. Therefore, the assumption that
dispersion due to longitudinal molecular diffusion is unimportant for
polystyrene of molecular weight \(>2,000\) at practical flow rates made in
Section 5.1 appears valid. Hence the data in Figures 4.2.8 and 4.2.14
for permeating polystyrenes can be redrawn to give a linear relation be-
tween \(H\) and flow rate in terms of linear flow rate, as shown by Figures
5.2.1 and 5.2.2 respectively. In Figure 5.2.1 the point for PS-3,600 at
0.25 cm\(^3\) min\(^{-1}\) and the point for PS-35K at 6 cm\(^3\) min\(^{-1}\) are omitted from
the linear plots and PS-200K points are omitted as its \(V_R\) is outside the
linear portion of the calibration curve in Figure 4.2.7. Similarly the
points at 4 and 6 cm\(^3\) min\(^{-1}\) for PS-110K in Figure 4.2.14 were omitted
from the linear plot in Figure 5.2.2.

Equation 5.2 may also be applied to the dependence of efficiency
on flow rate for the three proteins, albumin, ovalbumin and myoglobin,
in phosphate buffer at pH 6.3, shown in Figure 4.2.16. Hence the data in
Figure 4.2.16 can be redrawn to give a linear relation between \(H\) and flow
rate in terms of linear flow rate, as shown by Figure 5.2.3. All points
at 0.25 cm\(^3\) min\(^{-1}\) and at 6 cm\(^3\) min\(^{-1}\) for myoglobin omitted from the linear
plots. Estimates of \(D_s\) can again be obtained with Equation 5.5 and the
results are shown in Table 5.1 together with values of \(R\) and \(D_m\) from
refs. (172-174) for polystyrenes and from refs. (159,160) for proteins.

5.2.3 Diffusion in the Stationary Phase

The derived data for \(D_s\) in Table 5.1 are much less than the values
of \(D_m\), giving an arithmetic mean of \(D_s/D_m\) in Table 5.1 of about 0.1. This
is similar to the results of Giddings et al. (168) who studied the chromatogram broadening of low molecular weight polystyrenes \((\leq 4,000)\) separated
with porous glass particles having particle sizes in the range 44 - 74 \(\mu\)m
and found that \(D_s\) was about \(D_m/6\). Van Kreveld and van den Hoed (179)
evaluated the dispersion term for mass transfer in the stationary phase
for polystyrene standards (molecular weights 20,000 to 160,000) separating
Figure 5.2.1

Dependence of plate height on flow rate for SG30-H1. A linear plot of Figure 4.2.8 with the same symbols.
Figure 5.2.2

Dependence of plate height on flow rate for SG30/G-H.

A linear plot of Figure 4.2.14 with the same symbols.
Figure 5.2.3

Dependence of plate height on flow rate for SG30/G-H.
A linear plot of Figure 4.2.16 with the same symbols.
with porous silica particles \((d_p = 75 - 120 \, \mu m)\), finding \(D_s/D_m\) to decrease from 0.31 to 0.12 as molecular weight increased. Knox and McLennan (180) have also determined \(D_s\) values for polystyrene standards for a molecular weight range 2,000 to 33,000 separating with porous silica \((d_p = 7.5 \, \mu m)\) by evaluating the mass transfer term, quoting data for \(D_s/D_m\) 0.045 to 0.104 (or 0.059 to 0.167) depending on the method for calculating \(D_s\).

Large errors may result from the choice of particle size used because \(d_p^2\) appears in Equation 5.6. However, from Figures 4.2.1 and 4.2.3 to 4.2.5 and Table 4.3, the porous silica microspheres used have narrow particle size distributions. The calculation procedure used is simpler than that employed by Knox and McLennan (180) but the results are in reasonable agreement. All columns except H2-R show the decrease in \(D_s/D_m\) values as the molecular weight of the polystyrene standards increases over a narrower range of \(D_s/D_m\) values than found by van Kreveld and van den Hoed (179). These variations in \(D_s/D_m\) are caused by the variation in \(D_s\) values calculated by the above procedure and all \(D_s\) values in Table 5.1 decrease with increasing molecular weight.

The values of \(D_m\) for the polystyrene standards are assumed to be the diffusion coefficient in free solution at infinite dilution, but for lightly cross-linked gels of loose structure \(D_s/D_m\) was found to be 0.67 (181,182). This is in agreement with value found by Knox and McLaren (183) for gas chromatography. It is reasonable that in some gels, and particularly in rigid ones, there should be regions of restricted diffusion. Gel pores are not uniform varying in width as well as in general shape, where wide regions are connected to narrower ones. Very large molecules cannot diffuse through these constrictions, but smaller molecules permeate them freely. Intermediate size molecules should be able to diffuse through these regions, but only with difficulty. Renkin (184) has calculated the diffusion coefficient of spherical molecules of diameter \(V_h\) through pores of diameter \(d_s\). Figure 5.2.4 shows how the diffusion coefficient
in a pore $D_s$ decreases rapidly with increasing ratio $V_h/d_s$ (185). However, the pores should not be considered to be isolated channels but intimately connected regions which can be regarded as a microscopic version of the interstices between the particles (186). Therefore, restricted diffusion should increase with increasing molecular weight, hence, $D_s/D_m$ values decrease with increasing molecular weight as observed in Table 5.1. A consequence of this is that the largest solute molecules can diffuse only into a few of the large pores in the outer surface of the porous particles while smaller solute molecules can diffuse into the large pores and all the smaller pores also accessible to them. Haller (187) has demonstrated conclusively that the retention volume $V_R$ of a species is determined by a combination of restricted diffusion and steric exclusion, the contribution of each being determined by the ratio of solute size to pore size. Because $V_R$ can be altered by this ratio then the values of $R$ in Equations 5.4 and 5.5 are a function of diffusion coefficient. Dawkins and Yeadon (169) have shown that the slope of an efficiency versus flow rate plot, for polystyrene standards, versus $1/D_m$ is not greatly influenced by $R$; however, the values of $D_m$ used were literature values (172-174) and not influenced by restricted diffusion. In order to see if restricted diffusion is occurring, values of $R(1-R)$ calculated from $R$ in Table 5.1 are plotted against the calculated values of $1/D_s$, as shown in Figure 5.2.5. Although the values of $D_s$ and $D_s/D_m$ for SG30-H1, SG30/G-H and H4-R are varied they give a linear plot in Figure 5.2.5 for the polystyrene standards examined. The results for H2-R and H6-R in Figure 5.2.5 do not fit the same straight line. Because the calibration curve for H4 silica is similar to SG30 silica, Figures 4.2.2 and 4.2.7, the results in Figure 5.2.5 suggest that restricted diffusion is dependent upon pore size distribution and possibly pore geometry. Figure 5.2.5 also contains values of $R(1-R)$ and $1/D_s$ calculated from the data of Dawkins and Yeadon (169) assuming $d_p = 10 \mu m$ for the four column set of $\mu$StyrageL used. As their
Figure 5.2.4

Dependence of diffusion coefficient of a spherical molecule in a pore on the ratio of particle diameter $V_h$ to pore diameter $d_s$, from Ackers (185).
Figure 5.2.5

Dependence of $R(1 - R)$ on calculated values of $1/D_s$ from Table 5.1.

*, SG30-H1 data; O, SG30/G-H data; □, H2-R data; ■, H4-R data;
▲, H6-R data; Δ, data from ref. (169).
column set covers a wide molecular weight range the dependence of \(1/D_S\) on \(R\) is not as strong as for SG30-H1, SG30/G-H or H4-R.

The values for proteins do not fit the straight line for SG30/G-H in Figure 5.2.5. This is because the relationship between diffusion coefficient and molecular weight is different from proteins and polystyrenes and also does not hold for different proteins. However, the results in Table 5.1 demonstrate that restricted diffusion occurs not only for flexible chain molecules but also for rigid macromolecules such as the three globular proteins examined.

The theory of restricted diffusion predicts that for high molecular polymers the retention volume changes with increasing flow rate and leads to pronounced tailing (32,184,185). Neither of these were seen in this study but changes in \(V_R\) with flow rate have been reported by Mori (98). Therefore, in this study a molecule must have had sufficient time to diffuse into and out of a pore, even if restricted diffusion was occurring, before the eluent flow rate moved the molecule past the pore. Errors may result in the above procedure for determining values of \(D_S\) if the eddy diffusion term in Equation 5.4 contains a contribution for molecular diffusion, as discussed in Section 5.1.2. However, any such contribution would be minimized for permeating polymers because of their lower molecular weight. Similarly, contributions from mass transfer in the mobile phase are reduced for permeating polymers. Figures 4.2.3 to 4.2.6 show that with the high molecular weight excluded polymers, where mass transfer in the mobile phase is increased, the effect is minimal.
5.3 POLYDISPERSITY CONTRIBUTIONS

In order to interpret further the experimental results for plate height on the solute dispersion mechanisms contributing to chromatogram broadening, a contribution from polydispersity must be included. Hendrickson (20) expressed the width of a chromatogram in terms of solvable variables including the MWD of the polymer and the chromatogram broadening arising in the column from solute dispersion mechanisms. It follows that according to Equation 2.28 the standard deviation \( \sigma \) from an experimental chromatogram may be expressed by:

\[
\sigma^2 = \sigma^2_I + \sigma^2_{II} + \sigma^2_{III} + \sigma^2_M
\]  \hspace{1cm} 5.7

where \( \sigma_I, \sigma_{II} \) and \( \sigma_{III} \) follow from the solute dispersion terms in Equation 2.32 and \( \sigma_M \) in units of length is the standard deviation for the true MWD of the polymer. Therefore, Equation 2.32 can be extended to give:

\[
H = A + \frac{B}{u} + Cu + \frac{\sigma^2_M}{L}
\]  \hspace{1cm} 5.8

By analogy with definitions for \( H \) given in Section 2.1.2, \( \sigma_M \) (in units of length) may be defined in terms of \( \sigma_V \) (in units of \( V_R \)) with:

\[
\frac{\sigma^2_M}{L} = \frac{L \cdot \sigma^2_V}{V_R^2}
\]  \hspace{1cm} 5.9

where \( \sigma_V \) represents a contribution to the experimental chromatogram.

If it is assumed that the true MWD of a polymer standard may be represented by a logarithmic normal distribution, then for a permeating polymer the true polydispersity, written as \( [\bar{M}_w/\bar{M}_n]_T \), may be calculated from:

\[
\ln [\bar{M}_w/\bar{M}_n]_T = \sigma^2_D
\]  \hspace{1cm} 5.10

where \( \sigma_D \) is the standard deviation in terms of \( \ln \) (molecular weight) (188). Because the experimental chromatograms for polymer standards are almost symmetrical (see Figure 4.2.17) and because the GPC separation gives an almost linear calibration plot of \( \ln \) (molecular weight) versus \( V_R \) over the
permeation range, the polydispersity may be calculated from the standard deviation $\sigma_v$ in volume units with the relation:

$$\ln\left[\frac{\bar{W}}{\bar{N}}_n\right]_T = \sigma_{v_2}^2$$

where $D_2$ is defined in Equation 2.18. Equation 5.9 and 5.11 can be used with Equation 5.8 to give:

$$H = A + \frac{B}{u} + Cu + \frac{L \ln[\bar{W}/\bar{N}]_T}{D_v^2/2R}$$

5.3.1 Column S.20.W-R

The vertical displacement of the curve for PS-2,350 with respect to the curves for toluene and TFE results from the polydispersity term in Equation 5.12. This term may be evaluated approximately by considering that at very low flow rates mobile phase dispersion is the major contributor to chromatogram broadening. The results suggest that term (ii) in Equation 5.1 is unimportant in this study, as described in Section 5.1.1 and that the efficiency versus flow rate curves for toluene and non-permeating polystyrene standards are close together, compared to the permeating solute PS-2,350. Therefore, it is proposed that the plate height for toluene $H_t$ could represent the mobile phase dispersion in Equation 5.12 at the lowest practical flow rate, this is at 0.05 cm$^3$ min$^{-1}$ in Figure 4.2.3. Consequently, the plate height for a permeating polystyrene standard $H_{PS}$ at the same flow rate can be assumed to be given approximately by:

$$H_{PS} = H_t + \frac{L \ln[\bar{W}/\bar{N}]_T}{D_v^2/2R}$$

in which the second term on the right-hand side of Equation 5.13 is for the permeating polystyrene standard. The results in Figure 4.2.3 and discussion in Section 5.1.2 suggest that terms (iii) and (iv) in Equation 5.1 are very small for toluene compared to a permeating polystyrene.

Therefore, provided mass transfer dispersion is less important than mobile
phase dispersion at very low flow rates, the use of toluene data for $H_t$ will not be too unreasonable. From the difference between the $H_{PS}$ and $H_t$ values at the lowest flow rate of 0.05 cm$^3$ min$^{-1}$ in Figure 4.2.3 a value of $[\tilde{M}_w/\tilde{M}_n]_T = 1.15$ was calculated for PS-2,350 and is given in Table 5.2. Values of $D_2$ and $V_R$ in this procedure were obtained from Figure 4.2.2. The calculated true polydispersity is not unreasonable considering the assumptions involved. For a polystyrene prepared by "living" anionic polymerisation, the theoretical value of $[\tilde{M}_w/\tilde{M}_n]_T$ for a sample of molecular weight 2,350 is 1.04 (188) and a somewhat higher practical value might be expected because of the rigorous conditions required in the experimental polymerisation technique.

5.3.2 Columns H2-R, H4-R and H6-R

The displacement of $H$ for PS-1,987K with respect to $H$ for toluene in Figures 4.2.4 to 4.2.6 suggests that the mobile phase dispersion is polymer size dependent. But the differences are probably caused by the difficulties associated with the chromatography of high molecular weight polymers as discussed in Section 5.1.2. Since the permeating polystyrenes in Figures 4.2.4 to 4.2.6 have molecular weights similar to or below the molecular weights of the non-permeating polystyrenes in Figure 4.2.3, where mobile phase contribution to $H$ is assumed to be approximately that of toluene, it appears reasonable to use the $H$ value for toluene from H2-R, H4-R and H6-R in Equation 5.12.

A similar calculation may be made for the polydispersity of PS-9,800, PS-35K and PS-200K polystyrene standards on the H2-R, H4-R and H6-R columns. The results are shown in Table 5.2 together with the theoretical value of $[\tilde{M}_w/\tilde{M}_n]_T$ for each polystyrene standard (188). Figure 4.2.4 was preferred for PS-200K since the calibration curves in Figure 4.2.2 suggest that H2-R gives a better resolution of the high molecular weight chains, and therefore, a more reliable chromatogram. The average of the values for PS-35K in Table 5.2 may be regarded as acceptable, as the $H_{PS}$
### TABLE 5.2

Polydispersities of polystyrene standards obtained from plate height data\(^{(a)}\) on balanced density slurry packed columns

<table>
<thead>
<tr>
<th>Column Name(^{(b)})</th>
<th>Polystyrene Standard</th>
<th>(H_{ps}) (mm)</th>
<th>(H_t) (mm)</th>
<th>(\left[\frac{\overline{M_w}}{\overline{M_n}}\right]_T)</th>
<th>Theoretical Polydispersity(^{(c)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.20.W-R</td>
<td>PS-2,350</td>
<td>0.52</td>
<td>0.062</td>
<td>1.16</td>
<td>1.044</td>
</tr>
<tr>
<td>H4-R</td>
<td>PS-9,800</td>
<td>0.31</td>
<td>0.070</td>
<td>1.08</td>
<td>1.011</td>
</tr>
<tr>
<td></td>
<td>PS-35K</td>
<td>0.28</td>
<td>0.070</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>H6-R</td>
<td>PS-35K</td>
<td>0.24</td>
<td>0.110</td>
<td>1.02</td>
<td>1.003</td>
</tr>
<tr>
<td>H2-R</td>
<td>PS-35K</td>
<td>0.12</td>
<td>0.110</td>
<td>1.00</td>
<td>1.001</td>
</tr>
<tr>
<td></td>
<td>PS-200K</td>
<td>0.31</td>
<td>0.110</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

\(^{(a)}\) Eluent: THF  
Flow rate: 0.5 cm\(^3\) min\(^{-1}\)  
Injection: 2 µdm\(^3\) of 0.2% w/v solute  
Detection: UV at 254 nm

\(^{(b)}\) See Table 4.6

\(^{(c)}\) From ref. (188).
values are similar at the flow rate used. However, the slope of the \( H \) versus flow rate plots in Figures 4.2.4 and 4.2.6 for PS-35K is lower than for the H4-R column in Figure 4.2.5. This is explained in Section 5.2 as the value of \( D_s \) is proportional to the slope and \( D_s \) for PS-35K with H4-R is lower than for H2-R and H6-R because of the differences in pore size and possibly pore geometry between the packings.

5.3.3 Column SG30-H1

In Sections 5.3.1 and 5.3.2 the mobile phase dispersion was assumed to be the major contributor to mobile phase dispersion at low flow rates because of the similarities between the efficiency versus flow rate curves for a totally permeating and an excluded solute. When totally excluded or partly excluded polystyrene standards, molecular weight 470,000, were examined with SG30-H1 (and SG30/G-H) mis-shaped chromatograms having shouldered or multiple peaks were obtained. In order to check if this was caused by viscosity effects or column overload, SG30-H1 was examined using polystyrene standards at higher concentrations, increased from 0.2% w/v to 0.5% w/v, at flow rate 1 cm\(^3\) min\(^{-1}\), by varying the injected volume as shown in Figure 4.2.9. The slope of the curves in Figure 4.2.9 increase with increasing molecular weight owing to the increase in viscosity with molecular weight; with only slight change in plate height for toluene and PS-3,600. No tailing or mis-shaped peaks were observed for PS-110K or PS-200K indicating that the problems with the higher molecular weight standards were probably not caused by viscosity or overload effects. Figure 4.2.9 suggests that if these high polymers were analysed a discrepancy, between the values obtained and the values at zero concentration or zero injection volume, would result and this may be important in the analysis of the data for S.7.W-H1. However, the weight of the sample injected at the lowest injection volume used (2 µdm\(^3\)) in Figure 4.2.9 is 10 µg and the weight injected in the S.7.W-H1 study with non-permeating polystyrenes is 1 µg. The difference between efficiencies of the
extrapolated data for PS-200K at 1 μg and 0 μg column loadings is <1 μm, therefore it is assumed that the results obtained for S.7.W-H1 in Section 5.1.1 are valid. An approximate guide line for the concentration of the injected sample is that the relative viscosity of the sample as compared to the mobile phase should be less than 2 (189,190). Samay and Kubin (191) have found that overloading effects occur at a relative viscosity of 1.8 with polystyrenes in THF with Styragel columns while an extensive study by Jancic and Pokorny (192-198) into concentration effects in GPC has been made; for polystyrenes in THF with a silica packing they have shown that overloading appears at a relative viscosity of 1.1 with severe peak distortion at 2.1. From the results in Figure 4.2.9 the probable cause of the mis-shaped peaks is either poor packing because of the relatively low packing pressure used, as suggested in Section 4.2.3 or mechanochemical degradation, as suggested for the non-permeating polystyrenes with S.7.W-H1.

In Equation 5.1 the contributions owing to mass transfer in the mobile phase, term (iii), mass transfer in the stationary phase, term (iv), and eddy diffusion, term (i), can be approximately evaluated. For a flexible chain polymer of molecular weight 20,000, having $D_m$ of about $10^{-5}$ cm$^2$ s$^{-1}$ (171), and with the parameters $\lambda$ and $\omega$ of order unity then term (iii), at $u = 2.5$ mm s$^{-1}$ and with $d_p = 8$ μm, will be about $1/2$ of term (i). Therefore, for SG30-H1 the eddy diffusion term dominates the mobile phase dispersion, as discussed in Section 5.1, hence Equations 5.1, 5.2, 5.12 and 5.13 can be combined to give:

$$H_{PS} = H_t + \frac{1}{30} R(1-R) \frac{D^2}{D_s} \frac{u}{d_p} + \frac{L \ln[M_w/M_n]}{D_m^2 V R}$$

In Section 5.2.4 the mass transfer in the stationary phase, the second term on the right-hand side of Equation 5.14, was evaluated. This can then be used in Equation 5.14 to calculate $[M_w/M_n]_T$ for PS-3,600, PS-9,800 and PS-35K at flow rates 2, 4 and 6 cm$^3$ min$^{-1}$ and the results are shown in
Table 5.3. Data for PS-110K have not been calculated because this polystyrene standard has $V_R$ close to the exclusion limit (see Figure 4.2.7), so that mis-shaped peaks, particularly at high flow rates, may occur because part of the solute is excluded while the remainder permeates the pores. The value of $[\bar{M}_w/\bar{M}_n]_T$ for PS-35K at a flow rate of 6 cm$^3$ min$^{-1}$ is expected to be over estimated because the value of $H$ for this polystyrene standard is not on the linear portion of the $H$ versus flow plot (see Figure 5.2.1). Apart from PS-3,600, the data for polydispersity are reasonable, being somewhat higher than the theoretical values of 1.011 for PS-9,800 and 1.003 for PS-35K calculated for polystyrenes prepared by "living" anionic polymerisation (188). The theoretical value of $[\bar{M}_w/\bar{M}_n]_T$ for PS-3,600 is 1.029, indicating that the values in Table 5.3 are too low. This may arise because the calibration curve in Figure 4.2.7 suggests the PS-3,600 is separating close to the total permeation volume. The value of $D_2$ assumed from the plateau region of Figure 4.2.7 when calculating $[\bar{M}_w/\bar{M}_n]_T$ may not be valid, as in practice the slope of the calibration curve will increase as the total permeation volume is approached. This will effectively give higher values of $[\bar{M}_w/\bar{M}_n]_T$ for PS-3,600 with Equation 5.14 than shown in Table 5.3. The low values of $[\bar{M}_w/\bar{M}_n]_T$ may also result from incomplete fractionation of chains in a polymer eluting close to the total permeation volume.

### 5.3.4 Column SG30/G-H

The procedure outlined in Section 5.1.3 can also be applied to SG30/G-H column using polystyrenes in THF from Figures 4.2.7, 4.2.14 and 5.2.2, and three proteins in phosphate buffer from Figures 4.2.15, 4.2.16 and 5.2.3. The $[\bar{M}_w/\bar{M}_n]_T$ values are shown in Table 5.4. Apart from PS-3,600, the data for the polydispersities of the polystyrene standards are reasonable and comparable to the data in Table 5.3 for the SG30-H1 column. The values of $[\bar{M}_w/\bar{M}_n]_T$ for PS-3,600 in Table 5.4 are lower than those in Table 5.3 which are below the theoretical value of 1.029. In
**TABLE 5.3**

Polydispersities of polystyrene standards obtained from plate height data\(^{(a)}\) on SG30-H1 at various flow rates

<table>
<thead>
<tr>
<th>Polystyrene Standard</th>
<th>Theoretical Polydispersity(^{(b)})</th>
<th>(\frac{\overline{M}_w/\overline{M}_n}{T}) at 2 cm(^3) min(^{-1})</th>
<th>(\frac{\overline{M}_w/\overline{M}_n}{T}) at 4 cm(^3) min(^{-1})</th>
<th>(\frac{\overline{M}_w/\overline{M}_n}{T}) at 6 cm(^3) min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-3,600</td>
<td>1.029</td>
<td>1.017</td>
<td>1.017</td>
<td>1.013</td>
</tr>
<tr>
<td>PS-9,800</td>
<td>1.011</td>
<td>1.033</td>
<td>1.029</td>
<td>1.031</td>
</tr>
<tr>
<td>PS-35K</td>
<td>1.003</td>
<td>1.010</td>
<td>1.010</td>
<td>1.018</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Eluent: THF
Injection: 10 \(\mu\text{dm}^3\) at 0.2% w/v solute
Detection: UV at 254 nm

\(^{(b)}\) From ref. (188).
TABLE 5.4

Polydispersities of polystyrene standards and proteins obtained from plate height data\(^{(a)}\) on SG30/G-H at various flow rates

<table>
<thead>
<tr>
<th>Macro-molecule</th>
<th>Theoretical Polydispersity(^{(b)})</th>
<th>(\frac{M_w}{M_n} ) at 2 cm(^3) min(^{-1})</th>
<th>(\frac{M_w}{M_n} ) at 4 cm(^3) min(^{-1})</th>
<th>(\frac{M_w}{M_n} ) at 6 cm(^3) min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-3,600</td>
<td>1.029</td>
<td>1.012</td>
<td>1.010</td>
<td></td>
</tr>
<tr>
<td>PS-9,800</td>
<td>1.011</td>
<td>1.035</td>
<td>1.034</td>
<td></td>
</tr>
<tr>
<td>PS-35K</td>
<td>1.003</td>
<td>1.011</td>
<td>1.013</td>
<td>1.009</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1.000</td>
<td>-</td>
<td>1.011</td>
<td>1.0(^{(c)})</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>1.000</td>
<td>1.004</td>
<td>-</td>
<td>1.008</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Same conditions as in Table 5.2 for polystyrene standards in THF.

For proteins:

- **Eluent**: 0.1M phosphate buffer, pH 6.3
- **Injection**: 5 \(\mu\)dm\(^3\) of 0.1% w/v solute
- **Detection**: UV at 215 nm

\(^{(b)}\) From ref. (188)

\(^{(c)}\) Plate height contribution from polydispersity was negative.
Figure 4.2.7 the PS-3,600 standard on SG30/G-H is separating closer to the total permeation volume than on SG30-H1. This implies that the explanation of the low $\bar{M}_w/\bar{M}_n$ values for PS-3,600 given in Section 5.3.3 for the SG30-H1 column is valid.

Before the plate height data for proteins can be analysed by this method, using values of $H$ for acetamide, it is necessary to confirm that no adsorption or retardation of the solutes in Figure 4.2.16 is present under the conditions used. Schmidt et al. (201) have shown that ovalbumin separating in aqueous media at pH 5.0 with a $\gamma$-G coated silica shows no change in $V_R$ as the ionic strength of the eluent is changed. Regnier and Noel (148,150) demonstrated by dynamic recovery studies that no adsorption of ovalbumin occurred with $\gamma$-G coated porous glass. The results in Figure 4.2.15 when converted to solute diameter (see Figure 5.4.2 in Section 5.4.2) suggest that the separation of albumin is determined mainly by steric exclusion, and satisfactory separations of albumin with $\gamma$-G coated porous glass and silica have been reported by other workers (145,148,149,199,200). A high recovery of albumin from $\gamma$-G coated porous glass was reported by Regnier and Noel (148,150). However, values of $H$ for albumin in Figure 4.2.16 were much higher than for ovalbumin and myoglobin and tailing was observed on the chromatograms. This, together with displacement of retention volumes to higher values of $V_R$ in Figure 4.2.15, as the flow rate is increased, indicates an interaction between albumin and the stationary phase. Therefore, the data on albumin were not analysed further. From Figure 4.2.15, myoglobin is almost a totally permeating solute, which suggests that its separation is mainly determined by steric exclusion. Values of $V_R$ for totally excluded macromolecules (i.e. $V_o$) on SG30/G-H, PS-1,987K in Figure 4.2.7 and thyroglobulin in Figure 4.2.15, are in good agreement, but elution of toluene ($V_R = 9.55 \text{ cm}^3$) and acetamide ($V_R = 10.05 \text{ cm}^3$) suggest different accessible pore volumes. An alternative explanation is that interactions between solute and
stationary phase, which are more prevalent for small molecules than for macromolecules (202), may be occurring and may differ from acetamide and toluene.

From the above information it will be assumed that ovalbumin, myoglobin and acetamide are separated solely by steric exclusion with no adsorption or retardation occurring. The values of $\frac{\bar{M}_w}{\bar{M}_n}$ for ovalbumin in Table 5.4 determined by using plate height data for acetamide in Figure 4.2.16 and Equation 5.13 are much lower than the data for polystyrene standards, confirming that this protein may be regarded as monodisperse. The values of $\frac{\bar{M}_w}{\bar{M}_n}$ for myoglobin also indicate a narrow distribution, although the agreement between the two values is poor.

5.3.5 True Polydispersity

In summary, this simple method gives reasonable estimates of polydispersity; a more involved procedure having been proposed by Knox and McLennan (180, 203). The values of the true polydispersity in Tables 5.2 to 5.4, calculated using Equations 5.13 and 5.14 involves several assumptions and the determination of experimental values of $H$ involves the standard deviation $\sigma$ of the chromatogram. A much more reliable evaluation of polydispersity results from the use of all the chromatogram, as in the computer programme discussed in Section 2.4.2 (123, 126). This experimental polydispersity $\frac{\bar{M}_w}{\bar{M}_n}$ may be related to $\frac{\bar{M}_w}{\bar{M}_n}$ if it is assumed that the chromatogram and the molecular weight distribution are represented approximately a logarithmic normal function. The experimental value of $H$ is given by $\sigma^2/L$ (59). It follows from Equations 5.1 and 5.9 to 5.11 that Equation 5.12 may be transformed to:

$$\ln \left[ \frac{\bar{M}_w}{\bar{M}_n} \right] = \frac{D^2}{2vR} \frac{2\lambda d_p}{u} + \frac{2D_m}{u} + \frac{qR(1-R)}{d_p^2} + \ln \left[ \frac{\bar{M}_w}{\bar{M}_n} \right]_T$$

Equation 5.15

It follows from Equation 5.14 that the chromatogram broadening terms in Equation 5.15 may be evaluated from experimental $H$ data for toluene at low flow rates. Consequently, $\frac{\bar{M}_w}{\bar{M}_n}$ may be calculated from $\frac{\bar{M}_w}{\bar{M}_n}$
determined experimentally from the chromatogram of a permeating polymer at low flow rates. The drawback of this procedure is that even at low flow rates the mass transfer term for a permeating polymer will be somewhat higher than for a totally permeating solute. Therefore, estimates of $[\bar{M}_w / \bar{M}_n]_T$ are likely to be high.
5.4 CALIBRATION AND COLUMN PERFORMANCE

Results in Section 5.2 show that the diffusion coefficient of polymer molecules in the pores of the silica particles is reduced compared to their diffusion coefficient in free solution. However, the predicted change in retention volume for a high molecular weight solute separating by a restricted diffusion mechanism was not observed. For every solute retention volume was calculated as the maximum height of a chromatogram above its base-line.

5.4.1 Calibration Curves for Uncoated Silicas

The shape of all the calibration curves in this study, Figures 4.2.1, 4.2.2, 4.2.7, 4.2.10, 4.2.12 and 4.2.15, suggest that the silicas used have very narrow pore size distributions compared to commercially available cross-linked polystyrene gels (169,204,205). This is confirmed by the pore size distribution data for S.7.W, SG30, SG60 and SG120 silicas in Figure 5.1.5 from nitrogen adsorption isotherms measured at 77K (164,165,206). The calibration curves also suggest that the total pore volumes of H2, H4, H6, SG30, SG60 and SG120 silicas are about twice the total pore volume of S.20.W and S.7.W; this is in agreement with Figure 5.1.5. A measure of pore volume can be made from the difference between the retention volumes of non-permeating and totally permeating solutes. From Figure 4.2.10 the ratio of this value for SG30 : SG60 : SG120 silicas is 1.00 : 1.03 : 0.93 while results from transmission interferometry and gas adsorption in Table 4.5 give 1.00 : 1.36 : 0.91 and 1.00 : 1.15 : 0.93 respectively. This shows very good agreement between values for SG30 and SG120 for all methods but results for SG60 are not consistent. This is probably caused by the poor packing of the SG60 silica as shown by the column efficiencies for toluene in Table 4.8. The difference in the above results from Table 4.5 for pore volumes by transmission interferometry and gas adsorption for SG60 are due to transmission interferometry measuring the total pore volume and gas adsorption measuring available
pore volume. If the total pore volume of SG60 is very high, as these results suggest, then the porous particles will be prone to fracture during packing which would result in the low efficiencies seen in Table 4.8.

The calibration curves, in Figure 4.2.10, suggest that the pore-size distribution of S.7.W, SG30, SG60 and SG120 silicas are almost identical to that of S.20.W, H4, H2 and H6 silicas respectively. Therefore, the methods used to manufacture the smaller size silicas do not change the separation power of the silica when changing particle size. The broad, low efficiency peaks obtained with the silicas packed by the tamping method were used to construct a calibration curve, Figure 4.2.1. Although some difficulty was encountered in measuring $V_R$ because of the width of the peaks, the calibration curves appear identical to those of the more efficient columns packed by the balanced density slurry method, Figure 4.2.2. Therefore, the calibration curve appears to be independent of column efficiency and packing procedure.

The calibration curves and column efficiencies for column sets will be discussed with the results for dextrans on uncoated silicas in Section 5.4.3.

5.4.2 γ-G Modified SG Silicas

Although many chemically modified silicas are available for liquid chromatography (207), several of which have been investigated for use in GPC (144,145,148), many details concerning the physical properties of the packings are not known. Aue (208) has used scanning electron microscopy to examine dimethyl silane and octadecyl silane groups bonded to chromosorb supports. He found that the majority of the bonded phase covered the outside of the particles rather than the interior of the pores. Heat treating the packing did not visibly alter the distribution of the bonded phase. This indicates that a large proportion of the surface area is not covered with bonded phase during modification. The 3.0%
carbon content for modified SG30 silica in Table 4.2 compares well with values of 2.63% and 2.41% obtained for Corning CPG packings having a 25 nm pore size (145,148), but much less than the 5.48% obtained for Merck SI-100 silica (144).

Collins and Haller (209) have shown that an elution coefficient for a protein–sodium dodecyl sulphate complex is proportional to the logarithm of the pore diameter for CPG packings. Regnier and Noel (148,150) used this method to obtain elution coefficients on a series of uncoated porous glass packing for chymotrypsin and compared their data with values obtained before and after modifying a 50.7 nm pore size CPG with γ-G. They concluded that the pore diameter reduced to 47.0 nm, and therefore, the bonded phase gives a 1.8 to 1.9 nm coating on the surface of the pores. This is larger than theoretical value of 1.4 nm for a fully extended γ-G chain. Giddings et al. (36) proposed that separations of rigid molecules are determined by the mean external length or molecular projection $\bar{L}$ which is equal to the diameter $V_h$ of a spherical solute. As $V_h$ is proportional to molecular weight and $V_h$ governs how much a molecule permeates a pore because of steric exclusion, then Equation 2.18 explains why $\log$(pore size) enters the above calculation. Casassa (35) has proposed that $\bar{L}$ should also be regarded as an acceptable size parameter for flexible chain polymers. If it is assumed that polystyrenes and dextrans may be represented by a model of an equivalent hydrodynamic sphere (210), then the diameter $V_h$ (cm) of this sphere is given by:

$$V_h^3 = \frac{2.4K^1+\alpha}{A_v}$$  \hspace{1cm} 5.16

where $A_v$ is Avogadro's number and $K$ and $\alpha$ are the Mark–Houwink constants in Equation 2.48. The permeation range of polystyrenes with SG30–H1 and SG30/G–H can be calculated from the calibration curve in Figure 4.2.7, giving a molecular weight of 3,600 to 108,000 for SG30–H1 and 2,600 to 104,000 for SG30/G–H. The γ-G bonded phase reducing the permeation range
at both high and low molecular weights, and hence, reducing pore volume. A calculation of the change in size of molecules which just permeate and are just excluded from the pores, before and after coating with γ-G, will give the depth of coating. Equation 5.16 can be used to determine $V_h$ (cm) for the polystyrenes using values of $K = 1.164 \times 10^{-2} \text{ cm}^3 \text{ g}^{-1}$ and $a = 0.73$ at 25°C (211). This gives the reduction in pore size of 19.6 to 19.2 nm at the exclusion limit and 2.7 to 2.3 nm at the totally permeating point corresponding to coating depths of 0.2 nm. Within experimental error these results indicate hardly any change in pore diameter which is in agreement with the findings of Aue (208). However, these results are almost an order of magnitude less than those found by Regnier and Noel (148,150). The results above assume that the γ-G coating and the uncoated silica exhibit the same or no interaction with polystyrene in THF under the conditions used. The value for the depth of coating is expected to be less than the theoretical 1.4 nm as the groups in γ-G will hydrogen bond to any surface silanol groups present. If the depth of coating was 1.8 nm then the exclusion limit of SG30/γ-H would be about 76,000 for polystyrenes. A possible explanation of the variance of these results is that the wider pores of the packing used by Regnier and Noel (148,150) allows the γ-G groups to enter the pores, while with SG30 the bonding reaction is sterically hindered. Regnier et al. (212) have noted that supposedly identical bonded phases behave differently. They concluded that this was probably due to the technique of applying the bonded phase and also the history of the bonded phase.

The protein calibration curve in Figure 4.2.15 can be interpreted with the aid of Equation 2.5. Values of $V_R$ for pepsin ($K_p < 1.0$), catalase ($K_p > 1.0$) and lysozyme ($K_p >> 1.0$) which had $V_R$ greater than the totally permeating volume, may be considered in terms of ionic effects as suggested by Schmidt et al. (201). If unreacted silanol groups are present on the surface or in the pores of the γ-G modified SG30, as suggested by
the results of Regnier and Noel (148,150), they might dissociate in aqueous media at pH 6.3 giving a nett negative charge on the silica surface (200,201). The ionic strength of the eluent used lies in the range investigated by Schmidt et al. (201) and so interactions between charged proteins and the silica surface may not be completely neutralized. The behaviour of lysozyme in particular is very dependent on the ionic strength of the eluent (212). Regnier et al. (213) have shown that the distribution coefficient $K_d$ of lysozyme, when analysed on a commercial $\gamma$-G modified support as a function of pH in 0.1M ionic strength eluent, changed with pH. Values of $K_d > 1.0$ were obtained at pH $> 5.0$; $K_d$ is given by the product of $K_p$ and $K_p$ in Equation 2.5. At pH 6.3, lysozyme is positively charged and pepsin is negatively charged (201,214), so that these two proteins will be subjected to attractive and repulsive interactions respectively. This ionic explanation is less satisfactory for catalase which is close to a neutral protein at pH 6.3 (214).

However, of the 16 proteins subjected by Regnier and Noel (148,150) to dynamic recovery studies, catalase exhibited the worst recovery with $<50\%$ of the protein eluting from the column containing $\gamma$-G modified porous glass. An increased interaction ($K_p > 1.0$) at high ionic strength giving higher values of $V_R$ has been demonstrated for several proteins, which is interpreted in terms of hydrophobic interactions (201,215).

The molecular weight calibration curves for polystyrenes in THF (Figure 4.2.7) and dextrans (Figure 4.2.12) and proteins (Figure 4.2.15) in aqueous media do not give a single curve as shown in Figure 5.4.1. The polydispersity of a calibration standard will determine the accuracy of a calibration curve. For polystyrene standards and proteins, there should be little error in assigning the molecular weight at $V_R$. The dextran standards have polydispersities in the range 1.35 to 1.50 (216) and data for $M_w$ were used because they have an exponential molecular weight distribution (217) and for these $M_p \propto M_w$ (49). Experimental
evidence that these assumptions are valid is given in Section 5.4.3 for dextran.

In order to demonstrate that the macromolecules shown in Figure 5.4.1 are separating predominantly by steric exclusion, although data in Section 5.2 suggest steric exclusion is occurring, retention data must be interpreted in terms of solute size rather than molecular weight. This can be done using Equation 5.16 for polystyrenes and dextran, with $K = 0.1 \text{ cm}^3 \text{ g}^{-1}$ and $a = 0.5$ for dextran in water (167). For proteins $V_h$ has been evaluated from tabulated data for the Stokes' radius (218). Values of $K_D$ for each solute were determined from $V_R$ data with Equation 2.1 in which $V_o$ was assumed to be $4.9 \text{ cm}^3$ for excluded solutes ($K_D = 0$) in Figure 5.4.1 and $V_i$ was calculated from values of $V_R$ for acetamide and toluene ($K_D = 1$). A semi-logarithmic plot of $V_h$ against $K_D$ is shown in Figure 5.4.2. The results for proteins and dextran in aqueous media lie almost on the same curve, suggesting that the hydrodynamic diameter $V_h$ of the equivalent sphere is a reasonable choice of size parameter for a flexible coil polymer and that these separations operate by steric exclusion without perturbations from interaction effects. Good agreement is also observed between the aqueous separation data and the results for polystyrene in tetrahydrofuran in the low range of $K_D$, the range which is less easy to work out theoretically (34 - 36). The slight deviation between the curves for the aqueous and tetrahydrofuran separations at high $K_D$, in particular at $K_D \approx 0.8$, may arise from differences between $V_i$ for the two eluents and/or interaction effects between small molecules (toluene and acetamide) and the stationary phase which are dependent on small molecule and eluent.

5.4.3 GPC of Dextran

Column efficiencies for maltose in water on three uncoated silica columns and two column sets are shown in Table 4.10. The plate heights are slightly higher than those obtained with the same columns using
Figure 5.4.1

Molecular weight calibration curves for proteins, dextran and polystyrenes on SG30/G-H. Data replotted from Figures 4.2.7, 4.2.12 and 4.2.15.

△, thyroglobulin; △, albumin; □, ovalbumin; ○, myoglobin; ▽, cytochrome c; ◇, dextran; ●, polystyrene standards; +, acetamide; ×, toluene.
Universal calibration curve of solute diameter against the distribution coefficient for steric exclusion on SG30/G-H, with the same symbols as Figure 5.4.1.
toluene in THF as shown in Table 4.8. The efficiencies of the two column sets S.7.W-H2 + SG120-H and SG30-H2 + SG120-H are much lower than the sum of the individual column efficiencies in both Tables 4.8 and 4.10. A detailed study by Barker et al. (166,218) has shown that column efficiencies for glucose with various uncoated silicas, of comparable efficiencies to the individual columns in Table 4.10, decrease with time. This they ascribe to dissolution of silica leading to dead-volumes forming at the top of their columns when the packings settle. They also demonstrate (166) that the addition of salts to the eluent increases dissolution of the silica with increasing salt concentration. This decrease can be partly recovered by removing the heads from the columns and topping up with extra packing material; the lower efficiencies being caused by shoulders on the glucose peak which did not markedly affect the results from the analysis of dextrans. Therefore, it is assumed that the lower efficiencies of the column sets, which were measured after the individual columns had been used with salt solutions and their efficiencies determined, were caused by dead-volume areas.

The S.7.W-H2 + SG120-H column set was used to analyse three dextrans, T20, T40 and T70 with water as eluent. The MWD of the dextrans in Table 3.2 were assumed to be exponential and data for $M_w$ from the manufacturers was used to construct a calibration curve (202), as shown in Figure 4.2.13. This was used to produce the results listed in Table 4.9. The values found for $M_w$ of each sample are in good agreement with the values used for the calibration and these, with the values for polydispersity, are similar to other workers (216). For a Schulz-Zimm exponential distribution function (125,202) the polydispersity indices are given by:

$$\frac{(\beta + 1)}{M_n} = \frac{(\beta + 2)}{M_w} = \frac{(\beta + 3)}{M_z}$$

where $\beta$ is a parameter varying inversely with the breadth of the distribution (125). For a logarithmic normal distribution, the polydispersity
indices are identical (225):

\[
\frac{\bar{M}_w}{\bar{M}_n} = \frac{\bar{M}_z}{\bar{M}_w}
\]

and Equation 2.57 is valid. Equations 5.17 and 5.18 are plotted graphically, with the MWD results for T20, T40 and T70, in Figure 5.4.3 (values of \(\frac{\bar{M}_z}{\bar{M}_w}\) are not shown in Table 4.9). The results for the dextrans follow Equation 5.17 confirming that they have an exponential distribution which was assumed for the calibration curves in Section 5.4.2. However, all the results have slightly high \(\frac{\bar{M}_w}{\bar{M}_n}\) values compared to \(\frac{\bar{M}_z}{\bar{M}_w}\) in Figure 5.4.3. This is probably caused by a low molecular weight shoulder on the peaks which increases with decreasing molecular weight, arising from diffusonally controlled dispersion in dead-volume areas, which then gives low estimates of \(\bar{M}_n\).

The values for \(\bar{M}_w\) for T20, T40 and T70 can be determined in water by viscometry. Polymers having a fairly broad but well-known distribution fit Equation 2.52 where \(V_h\) is a constant and is incorporated into the value for \(K\). Pekic et al. (219) have determined values for \(K = 0.258 \, \text{cm}^3 \, \text{g}^{-1}\) and \(\alpha = 0.604\) for several dextrans which give \(\bar{M}_w\) values of 28,300, 44,700 and 70,800 for T20, T40 and T70 dextrans respectively from the data in Table 4.12. These values compare well with \(\bar{M}_w\) values determined by GPC and shown in Table 4.9. However, the values determined for \(\bar{M}_v\) for intrinsic viscosity, are much lower than those determined by GPC in Table 4.9. This is possibly caused by using the incorrect values for \(K\) and \(\alpha\) (167).

Kiciak (220) has shown that \(K\) and \(\alpha\) values vary with molecular weight of dextrans. The coefficient \(\alpha\) decreases with increasing molecular weight from 0.496 (for \(\bar{M}_v = 13,000\)) to 0.390 (for \(\bar{M}_v = 145,000\)), while \(K\) values increase from \(1.04 \times 10^{-3}\) to \(2.95 \times 10^{-3} \, \text{cm}^3 \, \text{g}^{-1}\) over the same molecular weight range. This can account for errors in \(\bar{M}_v\) of over 10% but these are not sufficient to account for the differences in \(\bar{M}_v\) between Tables 4.9 and 4.12. The values of \(\alpha < 0.5\) and variations in \(K\) and \(\alpha\) values are caused by the high degree of branching in dextrans (220).
Figure 5.4.3

Polydispersity indices for dextrans. Curve A - exponential distribution according to Equation 5.17; Curve B - logarithmic normal distribution according to Equation 5.18.

Δ, T20 dextran; □, T40 dextran; ○, T70 dextran.
When uncoated silica is used with water as eluent the silanol groups can act as weak cation-exchange groups (220, 221). Therefore, it is advisable to work at low pH in order to suppress this ionization (187) or by the addition of an electrolyte to the eluent to compete for any ionic sites (222, 223) to prevent a high molecular weight shoulder on the chromatogram from ion exclusion. A 0.05 to 0.1M solution is normally used (189) but strengths of 0.2M may be required to eliminate this effect for proteins (201). Barker et al. (166) suggest that 0.001M potassium phthalate or potassium dihydrogen phosphate are satisfactory at suppressing ion exclusion of dextrans without accelerating silica dissolution. But this was found to be unnecessary in the determination of MWD of dextrans with water as eluent in this study. The SG30-H2 + SG120-H column set was used to examine T20, T40 and T70 dextrans with water and with 0.8M and 2.0M potassium chloride solutions. An increase in $V_R$ occurred with increase in salt concentration for each dextran as shown by Table 4.11 and Figure 5.4.4. Spatorico and Beyer (224) showed that by increasing the concentration of sodium sulphate in the aqueous mobile phase from 0.2M to 0.8M the retention volume of dextrans, analysed on porous glass, decreased. This they attributed to an increase in molecular size which they supported with intrinsic viscosity data (224); however, no explanation for these observations was offered. Cooper and Matzinger (225) examined a dextran of molecular weight 40,000 in 0.01, 0.1 and 1.0M phosphate buffer at pH 7 with porous glass particles and concluded that their retention volumes were "essentially independent" of salt concentration; but their data suggests that $V_R$ increases with increase in salt concentration. In order to clarify these observations, intrinsic viscosity measurements were carried out on T20, T40 and T70 dextrans using water, 0.8M and 2.0M potassium chloride solutions. The flow times for the salt solutions were less than for water because the presence of salt reduced the amount of hydrogen bonding in the water, and hence,
Figure 5.4.4

GPC calibration curves for dextran on SG30-H2 + SG120-H.

Eluent: ○, Water; □, 0.8 M KCl; △, 2.0 M KCl.

Flow Rate: 1 cm$^3$ min$^{-1}$

Injection: 50 µdm$^3$ of 0.25% w/v solute

Detection: RI
decreased its viscosity. Within experimental error, no change in intrinsic viscosity was observed, as shown by Table 4.12. Steward and Tate (226) used ~2M salt solution in order to suppress ion exclusion for the analysis of inorganic and organic phosphates. At high ionic strengths hydrophobic interactions may occur (227) and/or alteration in the hydrogen bonding between the dextran and the bonded phase (228,229). Both these effects could explain the change in $V_R$ for dextrans with high salt concentrations. These changes in $V_R$ with salt concentration can be interpreted in terms of Equation 2.5; when ion exclusion is occurring $K_p < 1.0$ while hydrophobic interactions can be interpreted as $K_p > 1.0$. This indicates that for a pure size exclusion mechanism to operate in GPC a specific solvent system must be used for a specific column packing. The results in Section 5.4.2 for dextrans in pure water, proteins in 0.1M phosphate buffer and polystyrenes in THF on γ-G coated silica represent ideal conditions where the separation mechanism is predominantly size exclusion.

Additionally this work indicates that if coated or uncoated silica packings are to be used with aqueous eluents, then the columns are likely to have a finite life. With care, the life of a column can be greatly extended, especially by storing under the correct conditions when not in use. Two methods suggested for extending column life (166) are: flushing and storing the column in acetone; using a 10:90 mixture of methanol to water containing two parts glycerol as eluent.
CHAPTER 6 CONCLUSIONS
In the present work the ability of porous silica microspheres to separate different molecular species has been examined. Plate height has been used throughout to monitor the effect of flow rate on narrow molecular weight distribution polymers in an effort to run GPC columns in a high performance mode.

The similarity of plate height values for non-permeating polystyrenes and toluene compared to partly permeating polystyrenes suggests that the mobile phase dispersion is the major cause of chromatogram broadening for small molecules. Because mass transfer dispersion is quite low for small molecules, high resolution-GPC separations of oligomers and low polymers may be performed at fast flow rates. High performance will not, however, be obtained from microparticulate packing if the particles are not regular and do not have a narrow size distribution. Mass transfer dispersion becomes much more important for high polymers and increases as polymer diffusion coefficient decreases, and therefore as molecular size increases. Consequently, extensive chromatogram broadening will occur for permeating high polymers at fast flow rates. Efficient separations of high polymers, giving plate heights similar to those for small molecules, are only obtained at extremely low flow rates, e.g. below 1.0 mm s\(^{-1}\). Mass transfer dispersion is then much reduced. The variation in plate height data for non-permeating polystyrenes on microparticulate packings (d\(_p\) ≤ 8 μm) indicates that mechanochemical degradation could be occurring. This would also limit the use of high flow rates in the analysis of high polymers.

The general expression proposed for the plate height of small molecules may be simplified for high polymers which have a mobile phase contribution to plate height from the eddy diffusion dispersion mechanism. Consequently, the contribution to plate height from mass transfer in the stationary phase may be evaluated from experimental data for plate height. The diffusion coefficients of globular proteins and flexible coil
polystyrenes during mass transfer in the stationary phase are low, and from this work the data for polystyrene are in fair agreement with the results reported by other workers. A trend in these low values is also noted where the ratio between calculated diffusion coefficient and diffusion coefficient in free solution decreases with increasing molecular weight which is related to increased restricted diffusion effects and pore geometry.

The simplified expression for plate height may be extended to include a polydispersity term. Provided the mobile phase contribution to plate height from eddy diffusion can be estimated, polydispersity may be determined from experimental data for plate height. Values of the polydispersity of polystyrene standards were in reasonable agreement with theoretical expectation. While values for proteins, which can be regarded as monodisperse when separating solely by steric exclusion, gave an assessment of the accuracy of the method. By decreasing particle size the resolution of the packing increased and polydispersities for polystyrenes were closer to the theoretical values.

The modification of silica using γ-G has been successfully carried out to give a packing suitable for the analysis of charged macromolecules. However, the composition of the aqueous eluent must be adjusted, e.g. by careful selection of pH and ionic strength, to minimize interactions between the solute and stationary phase. If secondary interaction effects, e.g. adsorption or partial exclusion owing to ionic repulsion (ion exclusion) do occur, the retention data may be interpreted in terms of a thermodynamic representation of a mixed mechanism. A comparison of retention data for proteins, e.g. ovalbumin and myoglobin, which do not participate in interaction effects with stationary phase, dextrans, which are considered as "non-ionic" macromolecules, and polystyrenes suggest that solute diameter is a reasonable universal size parameter for the representation of macromolecules separating by a steric exclusion mechanism operating
close to equilibrium conditions. Although dextrans are considered non-ionic macromolecules, retention data obtained using water and salt solutions suggest that mechanisms other than steric exclusion are operating when using uncoated silica packings. Viscosity data suggests that no change in hydrodynamic volume occurs; therefore, variations are probably caused by hydrophobic interactions which can also be interpreted in terms of a thermodynamic mixed mechanism.

A bimodal column set containing uncoated silica can be used to obtain molecular weight distribution data for dextrans in water in 18 minutes at a flow rate of $1 \text{ cm}^3\text{ min}^{-1}$. However, by lowering the flow rate to $0.2 \text{ cm}^3\text{ min}^{-1}$ polymer analysis with improved resolution should be obtained in about $1\frac{1}{2}$ hours which is not unreasonable compared to traditional GPC separations. The main practical disadvantage is the determination of retention volume for short columns, but the precission may be optimised with an accurately controlled constant flow pump. This work shows that separations for a single column could be performed in under two minutes and for a bimodal set in about 3 minutes but the chromatogram broadening for high polymers would be extensive giving inaccurate values for molecular weight distribution.
CHAPTER 7  RECOMMENDATIONS
Further information on mass transfer in the mobile phase could be obtained using non-porous microparticulate packings. Small molecules, oligomers and low molecular weight polymers could be analysed and any effect of solute diffusion coefficient on mass transfer could be observed without the risk of inference owing to mechanochemical degradation of the solute. These non-porous microparticulate packings are not readily available, and therefore, may have to be prepared by high temperature calcination of the silica in an inert atmosphere; this should shrink the pore size and particle size of a silica packing (230). Alternatively, the work with S.7.W-H1 could be repeated with high molecular weight polymers at various concentrations and then plate height data of each solute used after extrapolation to zero concentration and injection volume. However, this solution will not be as satisfactory as the use of non-porous packings.

Observations of the decrease in diffusion coefficient in the stationary were made with polystyrene in THF, which behaves as a random coil, and globular proteins in aqueous buffer, which are rigid molecules. Further work should be carried out to examine this effect with other types of solute, such as rigid rods. This work could then be extended to the calculation of polydispersities for rigid rods.

Plate height data on column sets has shown that a marked decrease in column efficiency can occur when columns are connected together or when an off-column injection procedure is used. Because column packing procedure by low viscosity slurry techniques is relatively simple packings, made by mixing silicas having different pore sizes, could be packed into a single 500 mm long column of 8 mm internal diameter, fitted with on-column injection. This type of column should give high resolution separations provided it performs in the "infinite diameter" mode and that the individual silicas give a packing having a narrow pore size distribution.
REFERENCES
5. Maley L.E. - J. Poly. Sci. (C) 8 253 (1965)
10. Smith W.V. - Rubb. Chem. & Tech. 45 667 (1972)
17. Telepohak M.J. - J. Chromatogr. 83 125 (1973)


27. Atomic and molecular data series, ASTM D-20.70.04 - "Bibliography on liquid exclusion chromatography (gel permeation chromatography)", ASTM, Philadelphia (1975)


35. Casassa E.F. - Macromol. 2 182 (1976)


37. Dawkins J.V. + Hemming M. - Makromol. Chem. 176 1777 (1975); ibid. p1795; ibid. p1815


41. Dawkins J.V. - Polymer 19 705 (1978)

42. Giddings J.C. - Anal. Chem. 39 1027 (1967)


59. Giddings J.C. - "Dynamics of chromatography, Part 1, Principles and theory", Dekker, New York (1965)
77. Dawkins J.V. + Stone T. + Yeadon G. - Polymer 18 1179 (1977)
79. Rajcsanyi P.M. + Rajcsanyi E. - "High speed liquid chromatography", Marcel Dekker, New York (1975) Ch1
82. Ishida Y. + Kawai K. + Ueda S. + Takeda T. - Kobunshi Kagaku 30 34 (1973)
86. Dark W.A. + Limpert R.J. - J. Chromatogr. Sci. 11 114 (1973)
88. Baba N. + Sato S. - Japan Anal. 20 208 (1971)
92. Rabel F.M. - Int. Lab. July/August 35 (1975)
94. Heitz W. - J. Chromatogr. 83 223 (1973)
102. Martin M. + Blu G. + Eon C. + Guiochon G. - J. Chromatogr. 112 399 (1975)
103. Martin M. + Blu G. + Eon C. + Guiochon G. - J. Chromatogr. 130 458 (1977)
105. KG Dr.-Ing. Herbert Knauer & Co. GmbH.
111. Harmon D.J. - J. Poly. Sci. (C) 8 243 (1965)
115. Drott E. - 4th Int. GPC Seminar, Miami Beach (1967)
121. Whitehouse B.A. - Macromol. 4 463 (1971)
135. Carmon P.C. - Tran. Faraday Soc. 36 964 (1940)
141. Stewart H.N.M. + Perry S.G. - J. Chromatogr. 37 97 (1968)
142. Locke D.C. - J. Chromatogr. Sci. 11 120 (1973)
143. Biran R. - Loughborough University of Technology, personal communication
148. Noel R.J. - PhD Thesis; Purdue University, Lafayette (1977)
153. Hemsley D.A. - Institute of Polymer Technology, Loughborough University of Technology, personal communication
156. Majors R.E. - Anal. Chem. 44 1722 (1972)
162. Huggins M.L. - J. Am. Chem. Soc. 84 2716 (1942)
164. Ramsay J.D.F. - A.E.R.E., Harwell, personal communication
169. Dawkins J.V. + Yeadon G. - Polymer 20 981 (1979)
179. van Kreveld M.E. + van den Hoed N. - J. Chromatogr. 149 71 (1978)
185. Ackers G.K. - Biochem. 3 723 (1964)
187. Haller W. - J. Chromatogr. 32 676 (1968)
199. Becker N. + Unger K.K. - Chromatographia 12 539 (1979)
211. Spychaj T. + Lath D. + Berek D. - Polymer 20 437 (1979)


227. Hjersten S. - J. Chromatogr. 87 325 (1973)


230. Bristow P.A. - I.C.I., Macclesfield, personal communication