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HIGH PERFORMANCE GEL PERMEATION CHROMATOGRAPHY OF POLYMERS

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Abstract - The determination of the molecular weight distribution by gel permeation chromatography requires the establishment of a molecular weight calibration and efficient columns in order to minimise chromatogram broadening. The separation process is described in terms of steric exclusion, retardation and partial exclusion mechanisms in order to show how the universal calibration method may be influenced by solute-gel interactions. Solute dispersion mechanisms are described to show how chromatogram broadening is determined by the particle size of the column packing, the eluent flow rate, and the solute diffusion coefficient. Consequently, extensive chromatogram broadening will occur for permeating high polymers at fast flow rates, the decrease in column efficiency with flow rate becoming more significant as solute size rises. Therefore, accurate molecular weight distributions may be determined by high performance gel permeation chromatography with microparticulate packings at low eluent flow rates. For small molecules efficient separations occur at fast flow rates, so that high speed and high resolution separations of prepolymers and low polymers may be performed by gel permeation chromatography.

INTRODUCTION

Although much effort has been directed to 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.

The description of gel permeation chromatography (GPC) by Moore (3) in 1964, and the subsequent availability of a commercial GPC instrument (4), attracted widespread interest. This technique is much more rapid (several hours) and convenient, giving reliable and reproducible chromatograms. Two types of process will determine the chromatographic resolution of molecules in a polymeric sample. The separation process in the stationary phase controls the differential migration of the molecules, and in our case includes all the mechanisms which may determine the permeation of molecules in the column packing. The dispersion process which generally consists of at least two mechanisms determines the chromatogram broadening of one type of molecule, and this broadening is influenced by molecular diffusion and the column packing. An understanding of the separation process is necessary because of the prevalent use of universal calibration methods which assume that GPC separations are controlled solely by an exclusion mechanism (5). An understanding of the dispersion process is also required because the experimental chromatogram will always correspond to a distribution of molecular weights which is too broad. Therefore, in order to calculate a molecular weight distribution from a gel permeation chromatogram, it is necessary to establish a calibration relation between molecular weight M and retention volume V_R and to have a correction procedure for chromatogram broadening (2).

It follows from theories for chromatogram broadening that column performance in liquid chromatography is markedly improved by reducing the particle diameter d_p of the column packing. In the past five years microparticulate packings for separations of large and small molecules by high performance gel permeation chromatography (HPGPC) have become available (6). Particular emphasis has been placed on the advantages of these small particles packed in short low capacity columns for high speed (<30 min) separations of polymers and for high resolution separations of small molecules and oligomers. For those workers involved in the characterization of high polymers, the precision of the molecular weight distribution calculated from the experimental chromatogram is just as important as the speed of separation.

However, careful attention to instrumentation and procedures is required in order to obtain accurate molecular weight data from chromatograms produced by HPGPC with short low capacity columns. If high efficiency columns are used in HPGPC, corrections for chromatogram broadening may be quite small, so that correction procedures may be omitted in the determination of the number average and weight average molecular weights, $\bar{M}_n$ and $\bar{M}_w$, from an experimental gel permeation chromatogram of a polydisperse polymer.

In this paper the basic principles of the separation and dispersion processes in GPC will be described. The contribution of non-exclusion mechanisms in some polymer separations will be discussed. Emphasis will be placed on the use of microspherical packings for high performance gel permeation chromatography (HPGPC), since HPGPC separations performed with carefully chosen conditions give low chromatogram broadening. In addition, HPGPC with short columns may be used for high speed separations and for high resolution separations of low polymers and prepolymers.

SEPARATION MECHANISMS

Thermodynamic Interpretation

Many theoretical models have been proposed for the size separation of polymers with a porous column packing in GPC. The theories are conveniently classified under two headings - equilibrium models and flow models. Whilst flow mechanisms are important in some experiments, there is abundant evidence indicating that most practical GPC separations are performed close to equilibrium conditions (7, 8). The retention behaviour of a polymer in a porous packing is given in terms of the distribution coefficient K_{GPC} by

$$V_R = V_o + K_{GPC} V_i \tag{1}$$

where V_o is the total volume of the mobile phase, i.e. interstitial or void volume, and V_i is the total volume of the stationary phase, i.e. solvent within the porous packing. The simplest situation to treat theoretically is a separation operating at equilibrium. The standard free energy change ΔG^o for the transfer of polymer molecules from the mobile phase to the stationary phase at constant temperature T is related to K_{GPC} by

$$\Delta G^o = - k T \ln K_{GPC} \tag{2}$$

where k is Boltzmann's constant.

We consider a GPC separation consisting of two component mechanisms. The primary mechanism involves steric exclusion having a free energy change ΔG_D and the second mechanism, if present, will have a free energy change ΔG_P resulting from interaction of the polymer with the stationary phase. The total standard free energy change then is

$$\Delta G^o = \Delta G_D + \Delta G_P \tag{3}$$

Therefore, equations (1) - (3) give

$$V_R = V_o + V_i \exp(-\Delta G_D/kT) \exp(-\Delta G_P/kT) \tag{4}$$

Steric Exclusion

For an inert pore surface, the value of ΔG_P will be zero. The first theories of steric exclusion considered simple geometric models from which the fraction of pore volume accessible to a solute of given size may be calculated (9). This steric exclusion model is equivalent to statistical mechanical treatments of the loss in conformational entropy when a macromolecule approaches an inert surface. These thermodynamic theories (7, 10-12) calculate the accessible pore volume when a polymer molecule transfers from the mobile phase to a pore within the packing, showing how accessible pore volume depends on pore size for various models of pore shape and on solute size for both rigid and random coil polymers. The distribution coefficient K_D at equilibrium is defined as the ratio of accessible conformations for polymer within the porous packing to those in the mobile phase. It is assumed that the polymer is a random coil in a theta solvent, i.e. there is no change in the free energy of mixing when the solute transfers from one phase to the other, and that there is no polymer interaction with the inert porous packing, so that other enthalpy and entropy contributions are not considered. Consequently, ΔG_D is given by

$$\Delta G_D = - T\Delta S_D = - kT \ln K_D \tag{5}$$

where ΔS_D is the standard entropy change. Therefore, in the steric exclusion mechanism K_D becomes identical with K_{GPC}. For random coil and rigid polymers, the statistical mechanical interpretation of K_D shows that the separation is determined by the mean molecular projection independent of molecular geometry (8, 11, 12).

Universal Calibration

Experimental evidence that polymer size determines GPC separations was provided by Benoit and co-workers (13) who examined homopolymers and copolymers with crosslinked polystyrene gels and tetrahydrofuran as eluent. They showed that all their solutes fell on a single curve on a semi-logarithmic plot of the product $[\eta]$ and M versus V_R, where $[\eta]$ is the intrinsic viscosity (100 cm^3/g) of the polymer in the GPC eluent. It can be shown that $[\eta]M$ is proportional to the hydrodynamic volume of a polymer and to the size of a polymer with the Einstein and Flory-Fox equations respectively. Equations (4) and (5) suggest that K_D is independent of temperature, a characteristic of a mechanism controlled by entropy changes. Cooper and Bruzzone (14) have obtained an experimental calibration curve for polystyrene and polyisobutene in trichlorobenzene for porous glass columns at 25 and 150°C. Their separations were dependent solely on hydrodynamic size and were independent of polymer structure, polymer-solvent interaction and temperature. Experimental studies (see the papers cited in Refs.5 and 15) have confirmed the universal calibration plot of hydrodynamic volume for random coil polymers in eluents such as chloroform, o-dichlorobenzene, and trichlorobenzene. Furthermore, the exponent α in the Mark-Houwink equation is in the range 0.7-0.8 for polystyrene in these eluents, i.e. the eluent is a good solvent for polystyrene (15). Therefore, the eluent is very compatible with the crosslinked polystyrene gel, and in general solutes do not display preferential affinity for the mobile phase or the stationary phase. For polymers in good solvents, these results support the view that the separation is controlled by entropy changes and suggest that the free energy of mixing contribution is small compared with the conformational entropy change in the steric exclusion mechanism.

The equilibrium theories therefore predict that the behaviour of all polymers can be represented by a universal size parameter. It follows that a molecular weight calibration curve for one polymer may be calculated from a calibration curve established with well characterised polystyrene standards having narrow molecular weight distribution. The calibrations are related by the expression

$$\log M_p - \log M_{ps} = \log [\eta]_{ps}/[\eta]_p \qquad (6)$$

where p refers to the polymer and ps to an experimental study with polystyrene standards. Establishment of the M_p calibration with equation (6) is generally necessary because well characterised standards of the polymer requiring analysis are rarely available (5). Fractions with low polydispersity, i.e. $\bar{M}_w/\bar{M}_n < 1.1$, are ideal calibration standards, but it is only possible for polystyrene to establish a calibration curve over a wide range of V_R. Calibration methods employing fractions with broad molecular weight distributions are available, involving procedures such as representing the chromatogram by a theoretical distribution function and fitting the moments of a distribution by trial and error methods. It is generally necessary to assume a linear relation between ln M and V_R. When the right hand side of equation (6) has been established as a function of V_R, the M_p calibration may be calculated for a wide range of V_R. Anderson and Stoddart (16) observed that in the middle of the K_D range theoretical plots of K_D versus the logarithm of polymer size are essentially linear. Therefore, following their procedure and assuming that hydrodynamic volume is the universal size parameter determining a steric exclusion separation, we may write

$$K_D = - A \ln [\eta]M + B \qquad (7)$$

where A and B are constants.

Network-Limited Separation

Although steric exclusion dominates GPC separations, many experiments indicate that the assumption of an inert pore surface is not always valid. GPC separations with dimethylformamide (DMF) which is widely used as an eluent for polar synthetic polymers are influenced by interactions between polymer and stationary phase (17). In particular, the displacement of hydrodynamic volume calibration curves for polystyrene with respect to curves for other polymers in DMF has been observed with separations on crosslinked polystyrene gels (17, 18). DMF is a poor solvent for linear polystyrene with α values between 0.60 and 0.64. Consequently, deviations from the universal calibration plot of hydrodynamic volume for crosslinked polystyrene gels have been observed with organic eluents having α in the range 0.5-0.7 (17, 19, 20). In one of the first attempts to show that GPC separations with porous glass were size dependent (21), the plot of log polymer size versus V_R was influenced by eluent polarity. Subsequent studies with inorganic packings have suggested that deviations from the universal calibrarion plot of hydrodynamic volume are often observed (22).

Separations in which V_R is higher than expected from a steric exclusion mechanism are expected to arise from secondary partition (liquid-liquid) and adsorption (liquid-solid) mechanisms. Such interactions between polymer and stationary phase must be weak and reversible so that the polymer is not completely retained in the stationary phase. For some polymer-solvent-gel systems, K_D is greater than unity (9), which is inconsistent with a steric exclusion mechanism for which K_D must lie between zero and unity. Our observations for poor and theta solvents as eluents (17, 19, 20) suggest that the displacement of the hydrodynamic volume curve for polystyrene with respect to a curve for a polymer separating solely by

steric exclusion increases as polymer size decreases, i.e. the larger the volume of the total stationary phase volume accessible to a polymer, the greater the deviation of the polystyrene curve. Consequently, the GPC mechanism can be considered as a network-limited separation, as proposed by Heitz and Kern (23, 24). For such a mechanism we define a distribution coefficient K_P for the polymer-gel interaction, so that K_P is the ratio of solute concentration in the stationary phase to that in the mobile phase. The retention equation for a mixed mechanism involving steric exclusion and a secondary mechanism is derived to be (17)

$$V_R = V_o + K_D K_P V_i \tag{8}$$

which may be compared with equation (1). Equation (8) may describe both network-limited partition and adsorption mechanisms when $K_P > 1.0$.

Comparison of equations (4), (5) and (8) suggests that K_P is given by

$$K_P = \exp(-\Delta G_p/k\ T) \tag{9}$$

where K_P will be greater than unity when polymer is retarded in the stationary phase. In a somewhat simpler thermodynamic interpretation of a network-limited GPC separation (25), it was suggested that K_P is determined by an enthalpy contribution, i.e.

$$\Delta H_P = -\ k\ T\ \ln\ K_P \tag{10}$$

and the entropy change involved in interaction between polymer and stationary phase was neglected. Experimental universal calibrations are plots of log $[\eta]M$ versus V_R. In order to retain this presentation, equation (7) may be substituted into equation (8) to give

$$(V_R - V_o)/K_P = V_i(B - A \ln [\eta]M) \tag{11}$$

A plot of log $[\eta]M$ versus the left hand side of equation (11) will represent polymers separating by steric exclusion alone ($K_P = 1$), or polymers separating both by steric exclusion and interaction effects between polymer and stationary phase ($K_P \neq 1$). If the value of K_P has been evaluated, then the hydrodynamic volume universal calibration method is applicable provided the retention parameter is changed from V_R to $(V_R - V_o)/K_P$.

Retardation and Partial Exclusion

There is considerable experimental evidence for interaction between solute and stationary phase for separations with crosslinked polystyrene gels for polystyrene in eluents which are poor or theta solvents, and the results are summarised elsewhere (17, 19, 20). A plot of log hydrodynamic volume versus V_R for polystyrene is displaced to high V_R with respect to a plot for another polymer for which these same eluents are good solvents. Thus, cyclohexane is a good solvent for polyisoprene and poly(dimethyl siloxane) which follow the same plot of log $[\eta]M$ versus V_R, whereas the plot for polystyrene is displaced to much higher V_R values (17, 19). These results have been successfully represented by equation (11) as shown in Figure 1. Since K_P (polystyrene) = 1.45, it follows from equation (10) that ΔH_P is negative. This exothermic heat change is explained by polystyrene preferring the polystyrene-like gel environment rather than the mobile phase, because in a theta solvent polystyrene is close to its precipitation temperature. Interactions between polymer and solvent and between eluent and stationary phase are not favourable.

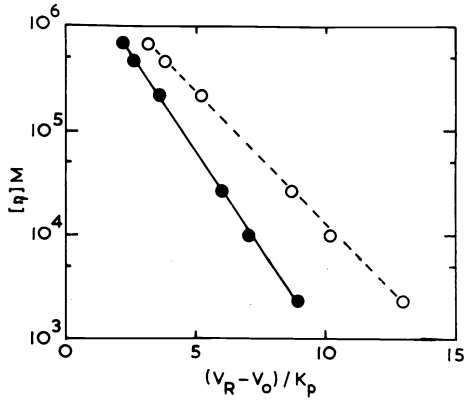

Fig. 1. Plot according to equation (11) for cyclohexane as eluent at 35°C (17); O polystyrene ($K_P = 1$); ● polystyrene ($K_P = 1.45$); (——) poly(dimethyl siloxane) ($K_P = 1$) and polyisoprene ($K_P = 1$).

It follows from equation (10) that K_P should decrease on raising the temperature because ΔH_P

is negative. In addition the magnitude of ΔH_p will change as the temperature of the eluent is raised above the theta temperature because of an increase in polymer-solvent interaction which contributes to the overall enthalpy change. As the eluent becomes a good solvent for the polymer, the solution tends towards an athermal mixture, i.e. a zero heat change. Then, the polystyrene molecules will not display preferential affinity for the gel or the eluent, so that the tendency for polymer retardation by interacting with the stationary phase is reduced considerably. This behaviour has been confirmed in experimental GPC separations with poly(dimethyl siloxane) having $K_p = 1$ (steric exclusion) and with polystyrene in trans-decalin which is a good solvent for poly(dimethyl siloxane) ($\alpha = 0.72$-0.76) and a poor solvent for polystyrene at 25°C (20). The displacement of the plot of log $[\eta]M$ versus V_R for polystyrene is shown to decrease as the temperature is raised (20). The results may be represented by equation (11), giving a decrease in K_p from 1.25 to 1.0 as the temperature is raised from 25 to 138°C. Therefore, the influence of temperature on the separation mechanism may be more important than has been previously considered. Even for tetrahydrofuran which is a good solvent with excellent compatibility with crosslinked polystyrene gels Mori and Suzuki (26) demonstrated that K_p changed significantly over the temperature range 10 to 45°C.

Kranz, Pohl and Baumann (27) showed that a plot of log $[\eta]M$ versus V_R for polystyrene in DMF ($\alpha = 0.60$-0.64) was displaced to high V_R with respect to a plot for polyacrylonitrile($K_p = 1$). Their results can be represented by equation (11), giving $K_p = 1.37$ for polystyrene (17). Results with DMF as eluent have suggested that the displacement of curves of log $[\eta]M$ versus V_R depends on polymer polarity when the eluent is not very compatible with the stationary phase. Thus, Dubin, Koontz and Wright (18) demonstrated that retardation was in the order polystyrene > poly(methyl acrylate) > poly(vinyl pyrrolidone) > poly(p-nitrostyrene), and they also demonstrated that retardation lessened on raising the temperature for both polystyrene and poly(ethylene oxide) in DMF, which is consistent with a negative enthalpy change for the interaction between polymer and the stationary phase.

Silica and porous glass packings have always appeared attractive for GPC fractionation because of their excellent thermal and mechanical stability and well-defined pore size distribution. However, because of the active surface sites, polymer retardation, and even irreversible polymer adsorption, is always possible (22). It is to be expected that both the polarity of the eluent and the degree of polymer-solvent interaction will have a considerable influence on the reversible and irreversible adsorption of a polymer at the surface of an inorganic oxide packing. This has been confirmed by Campos, Soria and Figueruelo (28) in an extensive study of the separation of polystyrene on porous silica with twenty one eluents. Their data may be represented by a network-limited separation mechanism, and a relation between the distribution coefficient K_p and solvent polarity may be demonstrated. Audebert (29) has commented that ΔG_p depends on the different interaction energies for solvent-gel and polymer-gel interactions. He demonstrated that a term representing these interaction energies is related to the product of the distribution coefficients K_D K_p, showing how the interaction energies determine whether the polymer separates by steric exclusion alone, by steric exclusion and a repulsive interaction, by steric exclusion and a slight attractive interaction, or by steric exclusion and strong adsorption.

The observations of the early elution of some polymers may be explained by polymer incompatibility with the gel (30, 31). A positive value of ΔH_p leads to a value of K_p below unity in equation (10). A polymer exhibiting repulsive interactions on transferring to the stationary phase will have a plot of log hydrodynamic volume versus V_R displaced to low V_R. Consequently, equation (11) should represent a separation involving steric exclusion and partial exclusion by incompatibility between polymer and the stationary phase. Data for poly(vinyl acetate) (30) have been interpreted in terms of equation (11), showing that values of K_p below unity decrease as molecular size increases as predicted from a thermodynamic interpretation of polymer incompatibility (32). Mori and Suzuki (26) have also shown that epoxy prepolymers having values of K_p below unity show a similar dependence of K_p on molecular size.

Recently, there has been considerable interest in microparticulate silica packings modified by silylation treatments for high speed and high resolution separations of water-soluble polymers generally, and biological macromolecules in particular. Several separations with porous silica and porous glass having a bonded phase resulting from reaction with γ-glycidoxypropyltrimethoxysilane (abbreviated here to γ-G) were first reported in 1976 (see Ref. 33). Some of our results for proteins in aqueous media with a γ-G silica (34) are displayed in Figure 2. The observations may be interpreted in terms of ionic effects as proposed by Schmidt *et al.*(35). These effects may be considered in terms of equations (10) and (11). Ovalbumin is assumed to separate by steric exclusion alone whereas catalase and pepsin separate by a mixed mechanism involving steric exclusion and interactions between protein and stationary phase. The modified silica will have a negative charge on the pore surface because of unmodified silanol groups which dissociate at pH 6.3. At pH 6.3 pepsin is negatively charged and will be subjected to partial exclusion by repulsive ionic interactions ($K_p < 1.0$). Lysozyme for which we were unable to obtain a chromatogram is positively charged at pH 6.3 and will be subjected to retardation by attractive ionic interactions ($K_p > 1.0$). Catalase is close to a neutral protein at pH 6.3 and the retardation of this protein

(K_p > 1.0) appears to arise, because of hydrophobic interactions with the bonded phase. It appears therefore that the preferred packing for aqueous separations should be non-ionic and should have a pore surface with the correct hydrophile-lipophile balance.

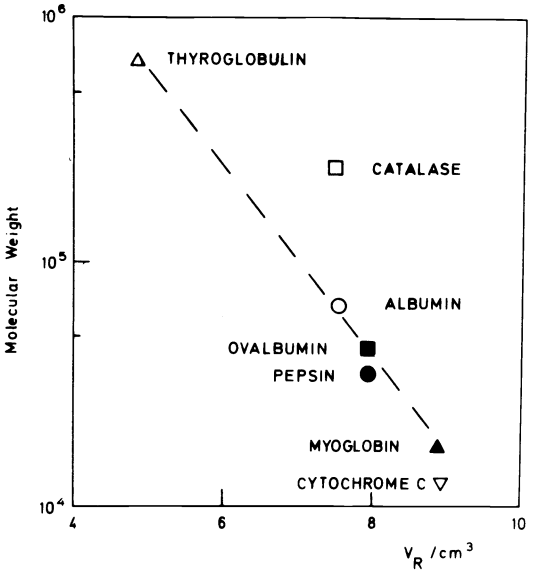

Fig. 2. Molecular weight calibration for proteins eluted from γ-G SG30 silica (34); Δ, thyroglobulin; □, catalase; O, albumin; ●, pepsin; ▲, myoglobin, ∇, cytochrome c; ■, ovalbumin.

DISPERSION MECHANISMS

A measure of the efficiency of a chromatography column is the height equivalent to a theoretical plate or plate height H(36). The plate height for an experimental chromatogram is calculated from the expression

$$H = L/N \tag{12}$$

where L is the column length and N is the plate number which may be determined from

$$N = 5.54\ (V_R/w_{0.5})^2 \tag{13}$$

where V_R is retention volume and $w_{0.5}$ is the width of the chromatogram at half its height. Equation (13) assumes a symmetrical chromatogram corresponding to a normal error (or Gaussian) function.

Experimental data for plate height H for a solute having constant retention volume V_R over the range of linear flow rate u of the eluent may be interpreted in terms of the dispersion mechanisms occurring in the mobile and stationary phases. In the first detailed theoretical treatment of chromatogram broadening in GPC, Giddings and Mallik (37) proposed an expression for H which for a monodisperse polymer is

$$H = (B/u) + C_s u + \Sigma\ 1/[(1/A) + (1/C_m u)] \tag{14}$$

in which A, B, C_s and C_m are coefficients depending on several parameters (see later) where the first term results from dispersion owing to molecular diffusion in the longitudinal direction in the mobile phase, the second term results from solute dispersion owing to mass transfer in the stationary phase, and the third term containing contributions from eddy diffusion (A) and mass transfer ($C_m u$) results from solute dispersion in the mobile phase. There is abundant experimental evidence, as reviewed elsewhere (38), indicating that the first term in equation (14) may be neglected for high polymers at practical flow rates, e.g. u > 1 mm s^{-1}. Experimental plate height data plotted as a function of u in Figure 3 do not display the minimum required by the first term in equation (14). The polystyrene standard PS-1987000 in Figure 3 may be regarded as a non-permeating solute, see the GPC calibration curve for H4 silica reported in an earlier study (39). The plate height data for this excluded solute shown in Figure 3 suggest that for high polymers chromatogram broadening

due to solute dispersion in the mobile phase exhibits little or no change with the eluent flow rate u, in agreement with experimental data for non-permeating polymers reviewed elsewhere (38). Theoretical calculations suggest that the term (1/A) is considerably larger than the term ($1/C_m u$) for high polymers (40). Consequently, it is concluded that the eddy diffusion term dominates mobile phase dispersion. It follows that only two dispersion terms, namely eddy diffusion in the mobile phase and mass transfer in the stationary phase, have to be considered in the expression for H for a monodisperse high polymer.

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 (36). The variance is the square of the standard deviation σ^2, so that H is defined by

$$H = \sigma^2/L \tag{15}$$

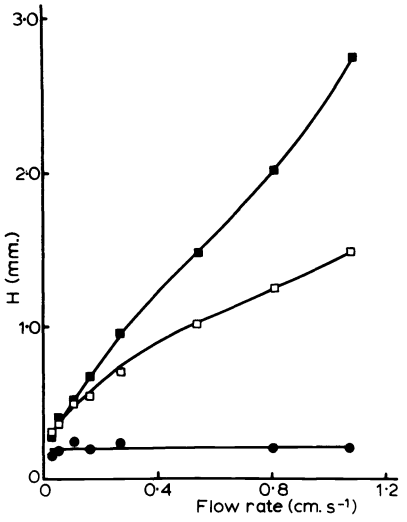

Fig. 3. Dependence of plate height on flow rate for polystyrene standards with H4 silica (39); □, polystyrene (M = 9800); ■, polystyrene (M = 35000); ●, polystyrene (M = 1987000).

If there are several solute dispersion mechanisms contributing to chromatogram broadening, as represented by equation (14), 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.

$$H = \Sigma\, \sigma^2/L \tag{16}$$

For a polydisperse polymer, Hendrickson (41) expressed the width of an experimental chromatogram for a permeating polymer in terms of several variables, including the molecular weight distribution of the polymer and chromatogram broadening arising in the column (or columns) from solute dispersion mechanisms. It follows that according to equation (16) the standard deviation σ_o from an experimental chromatogram may be expressed by

$$\sigma_o^2 = \sigma_I^2 + \sigma_{II}^2 + \sigma_M^2 \tag{17}$$

where σ_I^2 and σ_{II}^2 are the variances for eddy diffusion and mass transfer respectively and σ_M (in units of length) is the standard deviation for the true molecular weight distribution of the polymer. It follows that the experimental plate height for a polydisperse solute is given by

$$H = A + C_s u + (\sigma_M^2/L) \tag{18}$$

By analogy with definitions for plate height (6), we may define σ_M (in units of length) in terms of σ_V (in units of retention volume) with

$$(\sigma_M^2/L) = (L\, \sigma_V^2/V_R^2) \tag{19}$$

where σ_V represents a contribution to the experimental chromatogram. A procedure allowing for the polydispersity in the expression for plate height has been described by Knox and McLennan (42, 43). We shall assume that the true molecular weight distribution of the polystyrene standards may be represented by a logarithmic normal distribution, which is reasonable for polymers with narrow molecular weight distributions (5, 15). For a permeating polymer

the true polydispersity defined as the ratio of the weight average and number average molecular weights $[\bar{M}_w/\bar{M}_n]_T$, where $\bar{M}_w$ and $\bar{M}_n$ are the weight average and number average molecular weights, may be calculated from

$$\ln[\bar{M}_w/\bar{M}_n]_T = \sigma_D^2 \qquad (20)$$

where σ_D is the standard deviation in terms of ln molecular weight. Because the experimental chromatograms for polystyrene standards are almost symmetrical and because the GPC separation gives an almost linear calibration plot of log molecular weight versus V_R over the permeation range, the polydispersity may be calculated from σ_V with the relation

$$\ln[\bar{M}_w/\bar{M}_n]_T = \sigma_V^2 D_2^2 \qquad (21)$$

where D_2 is the slope of the GPC calibration relation between ln molecular weight M and V_R. Substitution of equations (19) and (21) into equation (18) for H for a permeating polydisperse high polymer gives

$$H = 2\lambda d_p + [R(1-R)\ u d_p^2/30 D_s] + (L \ln [\bar{M}_w/\bar{M}_n]_T/D_2^2 V_R^2) \qquad (22)$$

where the first and second terms for eddy diffusion and mass transfer respectively follow from the treatment of Giddings and Mallik (37), λ (close to unity) is a constant characteristic of the packing, R is the retention ratio defined by V_o/V_R, D_s is the diffusion coefficient of the solute in the stationary phase, and D_2 is the slope of the linear calibration curve of ln molecular weight against V_R in the partial permeation range.

Although equation (22) may be used as a basis for the interpretation of experimental chromatograms for polymers having narrow molecular weight distributions, the practical polymer scientist will generally be concerned with polydisperse samples which will be evaluated in terms of average molecular weights. The experimental polydispersity $[\bar{M}_w/\bar{M}_n]$ may be related to $[\bar{M}_w/\bar{M}_n]_T$ if it is assumed that the chromatogram and the molecular weight distribution are represented approximately by a logarithmic normal function. The experimental value of H is given by σ_o^2/L in equation (17) and σ_o may be related to $[\bar{M}_w/\bar{M}_n]$ in the same way that σ_M was related to $[\bar{M}_w/\bar{M}_n]_T$ in equations (19), (20), and (21). It follows that equation (22) may be transformed to

$$\ln [\bar{M}_w/\bar{M}_n] = \ln [\bar{M}_w/\bar{M}_n]_T + (D_2^2 V_R^2/L) \left(2 \lambda d_p + [R (1-R)\ u\ d_p^2/30\ D_s]\right) \qquad (23)$$

EXPERIMENTAL TECHNIQUES

Column Packings

It follows from equation (22) that chromatogram broadening in gel permeation chromatography is markedly reduced by lowering the particle diameter of the column packing, as shown by results for microspheres having well-defined particle size distributions (44). Regular spherical particles with a narrow size distribution should be preferred. From a practical view-point, such a distribution of microspheres minimises the resistance to fluid flow, so that fast separations at low pressures may be performed. It has been demonstrated that plate height increases for columns having a heterogeneous bed of particles because of the wider particle size distributions (39). A range of microparticulate packings is now available for both organic and aqueous separations of polymers and small molecules by HPGPC (45), and a listing of commercially available rigid packings is given in Table 1.

The crosslinked polystyrene gels are compatible with a wide range of organic eluents. It is preferable to use an eluent with a similar polarity to that of polystyrene, i.e. similar solubility parameter, when adsorption and partition effects are generally absent. Possible disadvantages of rigid organic gels are a susceptibility to thermal degradation and a decrease in mechanical stability at the elevated temperatures which are required in separations of polyolefins and some condensation polymers. In order to obtain compatibility with aqueous eluents, some manufacturers have modified the surfaces of crosslinked polystyrene gels, e.g. by sulphonation.

Columns containing porous silica and glass particles can be used at high flow rates and high pressures. Inorganic packings are suitable both for aqueous and organic eluents and are particularly suitable for separations at high temperature, e.g. polyolefins, because of the excellent mechanical and thermal stability of the gel particles. The main deficiency of inorganic packings is the presence of surface sites which may facilitate retardation because of adsorption of some polymers onto the packing. Adsorption may be irreversible, i.e. total retention of the polymer in the particles, or reversible when V_R increases and chromatograms are broadened. For polar polymers in both organic and aqueous phases, it is advantageous to coat inorganic packings with a surface bonded phase in order to minimise solute-gel interaction effects. Packings produced by reaction of porous silica or porous glass with γ-glycidoxypropyltrimethoxysilane have given size exclusion separations for a range of water-

TABLE 1 Rigid microparticulate packings

TYPE	NAME	SUPPLIER
Crosslinked polystyrene	PLgel	Polymer Laboratories
	μ-Styragel	Waters Associates
	Shodex A	Showa Denko
	HSG	Shimadzu
	TSK Type H	Toyo Soda
Porous silica	Zorbax PSM	Du Pont
	LiChrospher	Merck
	LiChrospher Diol	Merck
	Synchropak GPC	SynChrom
	Chromegapore	Beckman
	Spherosil	Rhone Poulenc
	μ-Bondagel E	Waters Associates
	Protein Column	Waters Associates
	TSK Type SW	Toyo Soda
Porous glass	CPG	Corning
	CPG Glycophase G	Corning
Hydrophilic polymer gel	TSK Type PW	Toyo Soda
	Ionpak	Showa Denko
	OHpak	Showa Denko

-soluble polymers (33, 46). However, silanisation may not be completely effective for all polymer-eluent pairs. Hydrophobic polymer-surface interactions may occur with very polar eluents and have been proposed to explain polymer retention behaviour both with DMF (18) and aqueous salt solutions (46) as eluents. A further method of reducing polymer retardation and irreversible polymer adsorption is to optimise the composition of the eluent which must be a good solvent for the polymer and must be more polar than the polymer. If the eluent is not very polar because of polymer solubility considerations, small quantities of an adsorption-active substance may be added to the eluent in order to suppress adsorption.

Instrumentation

The accurate determination of V_R is an essential part of the procedure in the evaluation of molecular weight distributions and average molecular weights of polymers by HPGPC. Short columns require precise measurements of the low retention volumes. A typical GPC system consists of four columns connected in series with short lengths of low volume narrow bore tubing, with each column having L∿30 cm with an internal diameter 0.7-0.8 cm. For L = 120 cm the value of V_i is about 20 cm^3. A constant flow pump which is reproducible and essentially independent of back pressure should be preferred. Then, a pulse-free liquid flow giving minimum detector noise will be generated, and if the eluent flow is truly constant, V_R may be measured as a time base along a recorder paper, thus avoiding experimental errors in the determination of V_R by siphon or by drop counter. It must be stressed that small variations in flow rate can cause large errors in this procedure for determining molecular weights from the chromatogram of a polymer (47, 48). A useful procedure is to monitor each HPGPC separation in terms of the retention volume of an internal standard (49).

Injection of polymer solutions in HPGPC is commonly performed with valve-loop injectors. Low injection volumes are necessary and the polymer concentration should be chosen to minimise overloading and viscosity effects. An examination of the dependence of chromatogram broadening on injection conditions has been performed by Mori (50).

The common detectors for monitoring polymer concentration in the eluent are based on the measurement of ultraviolet absorbance, differential refractive index and infrared absorbance. For calibration of V_R in terms of ln M, it may be necessary to have an automatic viscometer detector for determining $[\eta]_D$ in equation (6). The recent development of the low angle laser light scattering technique for the direct determination of M of the polymer in the eluent may simplify the determination of the molecular weight distribution.

RESULTS

High Speed Separations

It is clear from equations (22) and (23) that the flow rate and the solute diffusion coefficient influence chromatogram broadening, so that the chromatographic conditions will have to be chosen by considering the type of separation being performed. In Figure 3 it is

observed that the partially permeating polymeric solutes exhibit a much more significant rise in H as u increases than the non-permeating solute. The same trend again for a silica packing is shown in Figure 4 where the slope of the curves increases as the molecular weight of polystyrene increases. A detailed study confirming the molecular weight dependence of the plot of H against u for a wide range of permeating polystyrene standards with crosslinked polystyrene gels is reported elsewhere (38). The divergence of the curves in Figure 4 as u rises may be interpreted by the dependence of the mass transfer term on D_s. Consequently, larger molecules which have lower values of D_s will have higher mass transfer dispersion. Therefore, the mass transfer term will be the major contributor to plate height for permeating high polymers which must have greatly increased chromatogram broadening at fast eluent flow rates.

Particular emphasis has been placed on the advantages of microparticulate packings in short low capacity columns for high speed separations. The results in Figures 3 and 4 suggest that for low polymers the mass transfer term will not be too significant because D_s is high so that fast separations may be performed with little loss in efficiency. However, the pronounced rise in H for high polymers at fast eluent flow rates shows that high speed separations will operate with a considerable fall in column efficiency.

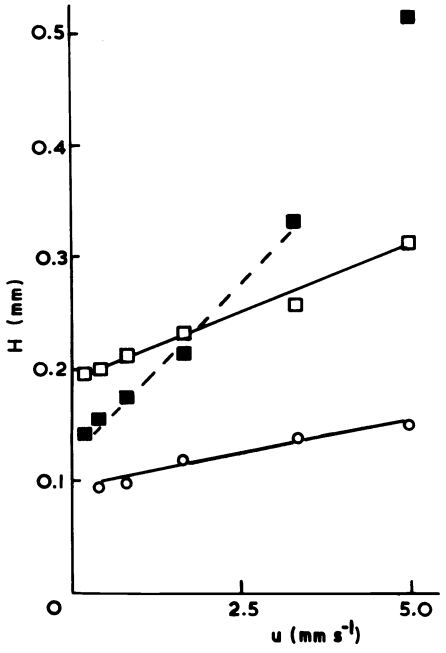

Fig. 4. Dependence of plate height on flow rate for polystyrene standards with SG30 silica (40): O, polystyrene (M = 3600); □, polystyrene (M = 9800); ■, polystyrene (M = 35000).

Molecular Weight Distributions of High Polymers

For workers involved in the characterisation of high polymers, the precision of the molecular weight distribution calculated from the experimental chromatogram will be important. It follows that the most accurate molecular weight data for high polymers will be obtained at low eluent flow rates with columns having high efficiencies, i.e. well packed columns containing the smallest particles. Furthermore, careful attention to instrumentation and experimental procedures is necessary in order to realise the theoretical advantages of microparticulate packings in practice (6, 38). Dawkins and Yeadon (38) have examined the polydispersity of a broad distribution polystyrene PSGY2, ($\bar{M}_n$ = 76000) with μ-Styragel columns (L = 120 cm) as a function of flow rate. Typical HPGPC results are shown in Table 2. The fall in $[\bar{M}_w/\bar{M}_n]$ as u is decreased which is predicted by equation (23) is confirmed. Polystyrene PSGY2 was prepared under controlled conditions so that the true polydispersity $[\bar{M}_w/\bar{M}_n]_T$ is 1.5 because the dominant termination mechanism in the polymerisation of styrene is radical combination. Even for chromatograms obtained at the lowest practical value of u, the experimental molecular weight distribution determined by HPGPC is always broader than the theoretical distribution predicted from the polymerisation mechanism (38). Equation (23) suggests that a plot $\ln [\bar{M}_w/\bar{M}_n]$ against u is a straight line yielding an intercept containing contributions from $[\bar{M}_w/\bar{M}_n]_T$ and a broadening effect owing to eddy diffusion. Procedures for estimating mobile phase dispersion with equations (22) and (23) have been discussed

TABLE 2 Dependence of polydispersity on eluent flow rate for polystyrene PSGY2 by HPGPC

u ($cm^3\ min^{-1}$)	$\bar{M}_w/\bar{M}_n$
0.1	1.61
0.25	1.62
0.5	1.63
1.0	1.67
2.0	1.70

elsewhere (34, 40), so that it is possible in principle to calculate $[\bar{M}_w/\bar{M}_n]_T$ from the experimental dependence of H, or $[\bar{M}_w/\bar{M}_n]$, on u. Values of $[\bar{M}_w/\bar{M}_n]_T$ calculated from the results for polystyrene standards in Figure 4 are shown in Table 3. For comparison, a value of $[\bar{M}_w/\bar{M}_n]_T$ calculated from data of H against u by the same method is shown for ovalbumin in aqueous media separating on a Y-G modified silica. The polystyrene results are in reasonable agreement with theoretical values predicted for polystyrene from a "living"anionic polymerisation (40). The value of polydispersity for ovalbumin is much lower confirming that this protein may be regarded as monodisperse.

TABLE 3 Polydispersity for polystyrene standards and ovalbumin determined from equation (22)

Polymer	$[\bar{M}_w/\bar{M}_n]_T$
Polystyrene-3600	1.017
Polystyrene-9800	1.033
Polystyrene-35000	1.010
Ovalbumin	1.004

High Resolution Separations of Low Polymers

In a GPC separation which functions by steric exclusion with K_{GPC} always between 0 and 1.0, all the solutes are eluted within the total solvent volume in the column. The limited range of distribution coefficients is unique to GPC, since values exceeding unity are common in other forms of liquid chromatography. Because of this restriction, the number n of components in a sample which can be resolved by GPC is related to column efficiency as defined by plate count N by a relation proposed by Giddings (51)

$$n \sim 1 + 0.2\ N^{0.5} \qquad (24)$$

so that $n \sim 21$ for a GPC column with 10000 plates. The consequence of the restriction of K_{GPC} to values ≤ 1.0 is that n for GPC separations is considerably less than for conventional liquid chromatography (about 3 times larger) and for gas chromatography (about 5 times larger).

Separation of individual components in a prepolymer or low polymer by HPGPC therefore requires very efficient columns. Several examples demonstrating fast high resolution separations of prepolymers by HPGPC are given elsewhere (6). As long as the molecular species have different sizes and the number of species is not excessive, excellent separations may be achieved by HPGPC. The separation of small molecules may be influenced by secondary mechanisms, but for some mixtures these mechanisms may provide greater peak resolution. The examination of monodisperse biopolymers is an excellent way of assessing GPC resolution of macromolecules, and resolved peaks for a mixture of proteins reported by Regnier and Gooding (33) illustrate the fine performance which may be obtained with rigid packings for separations with aqueous eluents. There is considerable interest in new microparticulate aerogel packings for aqueous HPGPC, because it is anticipated that much of the analytical gel filtration work with soft xerogels could be performed by HPGPC. Fast routine analytical separations of many biological macromolecules will then be possible.

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