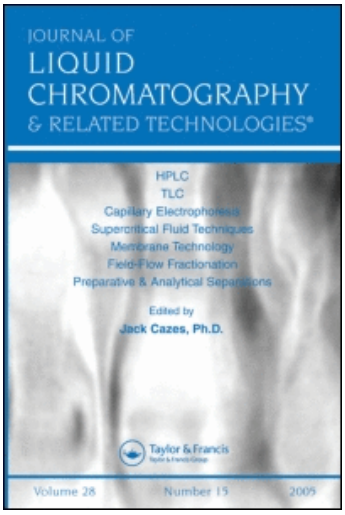

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Hydrodynamic and Size Exclusion Chromatography of Particle Suspensions-An Update

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HYDRODYNAMIC AND SIZE EXCLUSION CHROMATOGRAPHY OF PARTICLE SUSPENSIONS - AN UPDATE*

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ABSTRACT

The chromatographic separation of particle suspensions using packed beds has attracted considerable attention in recent years. It has the potential to provide accurate measurement of particle size and size distribution for spherical particles in the submicron range. There are two complementary approaches to the use of chromatography to separate particle suspensions according to size. Size exclusion chromatography (SEC) utilizes porous packing and relies mainly on steric exclusion from the pores of the packing. Hydrodynamic chromatography (HDC) utilizes non-porous packing and relies mainly on the velocity profile in the interstitial regions for size separation. In this paper, the developments in the understanding of these processes are critically examined and shortcomings of present theory are pointed out. Signal detection and chromatogram interpretation methods are reviewed.

INTRODUCTION

For over a decade, chromatographic methods using packed beds have been successfully used for the separation according to size of colloidal

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dispersions. Though vast strides have been made in the understanding of the separation process, some basic problems still remain, pertaining particularly to the holdup of colloid particles in the packed beds and the excessive broadening of colloid peaks (axial dispersion). While earlier research was mainly qualitative, adequate theory now exists to enable quantitative particle size measurement. A number of applications has been reported which demonstrates the considerable ease, rapidity and reliability of chromatographic techniques. These applications include detection of particle agglomeration [1,2] and swelling effects [2], measurement of particle growth kinetics [1,3,4,5], calculation of particle size distribution [6,7,8], etc.

In this paper, we critically review the theoretical and experimental developments concerning colloidal separations in packed columns. New insight is provided and new ideas are suggested for future research which will help to resolve present problems.

REVIEW OF EXPERIMENTAL INVESTIGATIONS

Chromatography has until recently been concerned exclusively with the separation of matter at the molecular level. One result of this restriction to the molecular domain was that, from a practical point of view, chromatographic methods invariably dealt with species in solution. Recently, however, chromatographic separations have been reported where the materials resolved were in suspension rather than in solution. Four major areas of particle chromatography have evolved; non-porous packed systems (HDC), porous packed systems (SEC), capillary chromatography [9] (CPC) and field-flow fractionation [10,11] (FFF). Here, we will be dealing with the first two.

Hydrodynamic chromatography (HDC) is a technique for separating small particles by flow through a packed bed of nonporous particles. This technique was invented by Small [12]. Similar developments using porous beds appeared through an independent study by Krebs and Wunderlich [13]. Studies on HDC have since been actively pursued by Stoisits et al [14], McHugh and co-workers [15,16,17], Nagy et al [18,19,20,21] and McGowan and Langhorst [6], whose major contribution is the development of a theory to explain colloid migration and then the application of this theoretical background in practical cases.

The object of using porous packing in size exclusion chromatography (SEC) has been to improve resolution over the non-porous HDC system by superimposing a steric exclusion effect on the flow separation. Particles smaller than the pore diameter can diffuse into the pores giving a second and more efficient mechanism of retardation and size separation.

Of course, there are relative advantages and disadvantages and the choice between HDC and SEC always depends on the physical system and the final objectives of the researcher. We now discuss briefly the experimental developments in HDC and SEC. To keep generality as much as possible, the principal results of these investigations are summarized at the end of the discussion.

A. Hydrodynamic Chromatography

When colloidal materials are carried in suspension through non-porous packed beds, it has been observed [1,2,22] that the rate of transport of the colloidal particles depends on such factors as the size of the colloid, the size of the particulate material that constitutes the packed column and the flowrate and ionic composition of the eluant.

The rate of migration (transport) of a colloid may be conveniently expressed by a dimensionless quantity, the R_F number, where:

$$R_F = \frac{\text{rate of transport of colloid through the bed}}{\text{rate of transport of the eluant}} \quad (1)$$

R_F gives the rate of migration of a colloid peak relative to a marker species.

In general, particle transport may be governed by one or a combination of the following effects: the hydrodynamic effect [1,2], the ionic effect [1,2] and the Van der Waals effect [1,2]. Figure 1 shows data [1] on the rate of transport of polystyrene latices through ion exchange beds of different diameter $\bar{D}$ (packing diameter). The fact that R_F clearly increases with increasing particle diameter of the latex, D, provides the basis for a chromatographic size separation. As the packing diameter is reduced, R_F increases. Furthermore, the slope of the R_F vs. D plot increases as the size of the packing is reduced, thereby resulting in improved resolution of different particle sizes. Most significantly, R_F is always greater than unity or in other words, the latex particles move more rapidly through the bed than either the carrier fluid or low molecular weight soluble species.

The dependence of R_F on the ionic strength of the eluant [1] is shown in Figure 2. Depending on the ionic strength, the colloidal forces can either enhance or hinder the average velocity of the particle. Decreasing the ionic strength, increases the volume of the relatively slow moving fluid in the interstitial regions from which particles are effectively excluded. Obviously, larger particles are excluded to a greater extent. Consequently, the mean velocity of the particle exceeds that of the fluid; the factor increases with the ratio of particle size to packing diameter. At high ionic strength, Van der

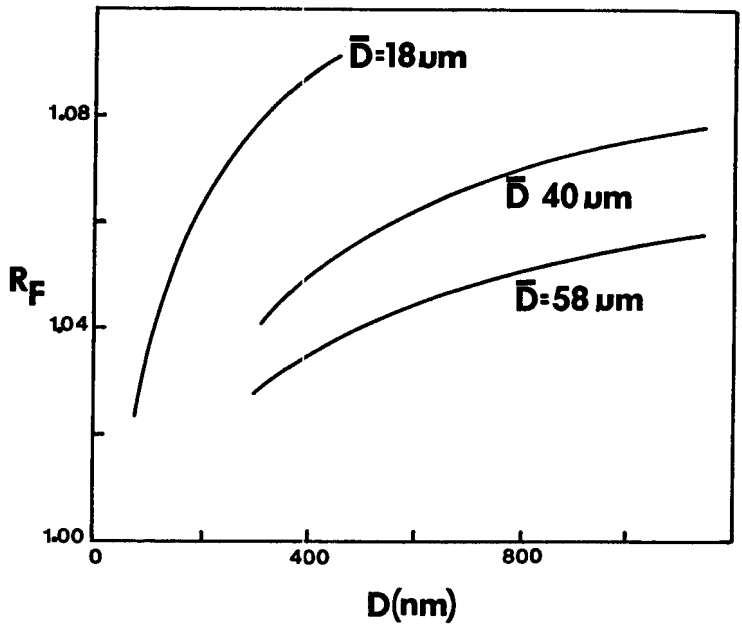

FIGURE 1: The dependence of R_F on latex particle diameter and packing diameter $\bar{D}$.

Waals forces cause the larger particles to spend a greater fraction of their time in the sluggish interstitial regions, so that the R_F dependence on particle size may reverse.

Two approaches have been taken to model the role of the colloidal forces in HDC. The capillary model [1,14,15,16,23] considers the interstitial space as a system of interconnecting parallel capillaries of equal size. In the second approach [24], the speed of the chromatographic transients are calculated from the behaviour of a colloidal suspension in equilibrium in the vicinity of a plane interface. Expressed in this form, the theory is independent of the geometry of the particulate material that constitutes the HDC column. The capillary model approach tends to be more universally accepted.

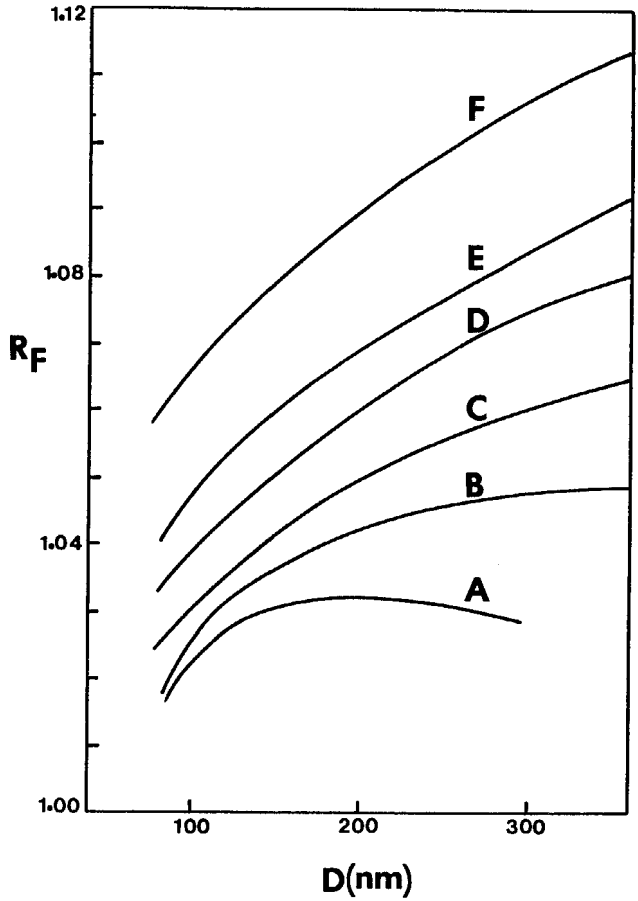

FIGURE 2: The effect of the ionic strength of the eluant on the R_F of polystyrene latices. Eluant concentration moles per liter of NaCl. A, 1.76×10^{-1}; B, $9. \times 10^{-2}$; C, 2.96×10^{-2}; D, 4.6×10^{-3}; E, 1.7×10^{-3}; F, 4.25×10^{-4}.

The experimental observations of Small [1] were subsequently confirmed and further extended by McHugh et al [15] and Nagy [18]. Recently, Nagy et al [19,20] reported a method for improving signal resolution in latex particle size analysis by HDC. Then, in a companion article [21], they presented a comparison of the column resolution characteristics of porous and non-porous packing systems. More recently, McGowan and Langhorst [6] reported an improved technique for the practice of HDC, which utilizes columns of higher efficiency and resolving power, thereby reducing the analysis time from 1.5 hr to 6 min. The integrated, computerized HDC they described, calculates the actual particle size distribution of the sample from molecular size to greater than 1 μm from the chromatogram in an additional 3-5 min. Several examples and experimental applications of their improved technique were discussed concerning HDC size distribution determination of a butyl acrylate/ butadiene latex and a polybutadiene latex reacted with methyl methacrylate.

B. Size Exclusion Chromatography

Krebs and Wunderlich [13] were the first to report a separation of polymethyl methacrylate and polystyrene latices using silica gel having very large pores (500 - 50,000 Å). This was followed by the work of Gaylor and James [25] who fractionated polymeric latices and inorganic colloidal silica, using columns packed with porous glass and water compatible polymeric porous gels. Coll et al [26] and Coll and Fague [27] experimenting with porous glass packing (CPG, 500 - 3,000 Å pore size), found it necessary to add electrolyte as well as surfactant to the aqueous eluant. In the absence of electrolyte, the colloids could not sample the pore volume. Peak broadening was observed to be more

extensive than in size exclusion chromatography of polymer molecules. They also observed that in SEC there is no limit as to how small the particle to be separated can be. The upper limit is a result of a greatly reduced diffusion coefficient for large particles and is probably about 4,000 Å. On the other hand, there appears to be a practical lower limit for HDC[28]. Singh and Hamielec [3], Hamielec and Singh [29] and Singh [30] presented the first comprehensive theoretical and experimental investigation of SEC. Using porous glass and silica packing (100 - 30,000 Å pore size), they established at low ionic strength the universality of the particle diameter-retention volume calibration curve. The slope of the calibration curve was essentially independent of the eluant flowrate; however, it became smaller (corresponding to a better resolution) with a reduction in packing size. The effects of the mobile phase flowrate and latex particle size on peak variance are shown in Table 1. Analytical expressions were derived to correct measured diameter averages for imperfect resolution. They concluded that SEC is sufficiently rapid for the off-line monitoring of latex particle growth in emulsion polymerization. With some modification it could be used in an on-line mode as a sensor for latex reactor control (8,31). However, the present state of SEC does require the development of a proper method for the complete extraction of a PSD from a chromatograph peak profile.

Nagy [18] and Nagy et al [21,32] investigated the chromatography of polystyrene latices using porous glass packing materials (CPG, 500 - 10,000 Å and Fractosil, 25,000 Å pore size). In a distinct departure from previous practice, only emulsifier (anionic) was added to the aqueous eluant, resulting in significantly reduced material loss within the packed bed.

TABLE 1

Peak Broadening Data for Polystyrene Latices Measured by Hamielec and Singh [29]

Eluant Flowrate (mℓ/min)	Chromatogram Variance ($m\ell^2$) PS 1000 Å	PS 2340 Å	PS 3120 Å
0.94	23.09	20.66	19.39
2.58	29.75	28.69	27.88
7.50	34.47	32.89	29.34

Johnston et al [33] reported the feasibility of chromatographing polystyrene latices using porous CPG columns (100 and 3,000 Å pore size). A reduction in packing size caused a small increase in R_F, while, significantly increasing sample loss. In general, the peak variance increased with particle size, attained a maximum and then started to decrease, analogous to the behaviour of polymer molecules (Table 1 gives this decreasing trend). An attempt was made to correlate the statistical properties of the chromatograms of narrow distribution latices with their mean retention volumes. Such an attempt is valid, provided the latices are sufficiently narrow to permit equating their spreading functions with the corresponding measured chromatograms. This condition is not fulfilled for the Dow latices used, as it was easily demonstrated theoretically [34].

Husain [34] and Husain et al [35] also examined the chromatography of polystyrene latices using porous CPG columns (1,000, 2,000 and 3,000 Å pore size). They advocated the merits of calibrating columns individually to weed out those with inadequate peak resolution and significant particle holdup. Common with previous observations, the

extent of skewing in the chromatograms of narrow distribution latices was observed to increase with particle size.

Finally, Kirkland [36] investigated the properties of small porous silica microspheres (less than 10 μ compared with approximately 35 μ CPG packing size, pore size less than 75 nm) and superficially porous particles (solid core, porous crust, packing size less than 25 μ) for characterizing inorganic silica sols in the range 1 - 50 nm. Columns using both types of packing materials exhibited high resolution because of rapid equilibration of slowly diffusing colloids with the pores. The effect of flowrate on peak broadening and the role of ionic strength in colloid separation were observed to be similar to those in earlier studies.

C. Summary of Main Results

A brief summary of the main results of the above investigations is now given under three classifications, namely peak separation, peak broadening and material loss:

Peak Separation

1. The particle diameter-retention volume calibration curve, in general, is composed of two linear segments: a segment at low retention volumes, beyond the exclusion limit of the porous packing, corresponds to HDC size separation, while, a segment at high retention volumes corresponds to SEC size separation.
2. The calibration curve is insensitive to flowrate variations.
3. Increasing the ionic strength of the aqueous eluant causes a shift in the calibration curve to high retention volumes due to increased accessibility of the column voids. At low ionic strength, a universal calibration is obtained.

4. Reducing the packing size improves peak separation.
5. SEC is not limited by a minimum particle size. However, the effectiveness of separation in HDC decreases as the ratio of particle to capillary diameter approaches zero.

Peak Broadening

1. In general with SEC the peak variance increases with colloid size, reaches a maximum and then starts to decrease as the exclusion limit of the porous column is approached. In HDC, it decreases with increasing particle size.
2. An increase in flowrate causes increased peak broadening in SEC. In HDC, the effect is not known.
3. In SEC, dispersion increases at higher ionic strength due to increased pore permeability. A similar effect may be expected in HDC; however, no experimental data have been reported.
4. The chromatograms of narrow distribution particle standards are generally skewed.
5. A well designed packing can significantly reduce dispersion.

Material Loss

1. Increasing the electrolyte concentration of the eluant decreases sample recovery. However, if the ionic strength is adjusted by addition of an ionic emulsifier (within limits), material loss is reduced.
2. Sample loss increases with colloid size.
3. Reduction in the size of the packing, enhances material loss.

While factors governing peak separation are fairly well understood, those that affect peak broadening and particularly sample recovery are

not. A systematic study to determine the roles of colloid composition, packing type, pore size, operating temperature etc. is required to acquire a better understanding of these phenomena.

DETECTION OF COLLOIDAL PARTICLES

Light transmission has been a standard method for the measurement of size of colloidal spherical particles for many years. The fundamental theory was developed by Mie Heller and his coworkers [37,38,39] outlined the theory which gives size distribution curves in heterodisperse systems of nonabsorbing colloidal spheres from turbidity spectra. The assumption was made that the unknown PSD followed a log normal distribution, a distributional form commonly found in latex systems.

Two of the most commonly used modes of colloidal particle detection, namely turbidimetric and differential refractometry detection, are now briefly examined.

A. Turbidimetric Detection

The turbidity for very small particles which behave as Rayleigh scatterers is proportional to the sixth power of the particle diameter. For larger particles obeying Mie scattering theory, the corresponding dependence is lower. As a consequence of the above, the small particle signal is comparatively weak, though it can be augmented by using shorter wavelengths. However, for obtaining particle size distributions, the relative signal is of greater importance than the absolute signal. Calculations by Silebi and McHugh [17] indicate that a change of wavelength or refractive index has a small influence on the relative

signal for nonabsorbing particles. However, the relative signal is improved for absorbing particles due to a significant enhancement of the extinction coefficient of the smaller particles. These theoretical observations were confirmed by Nagy [18] and Nagy et al [32] who chromatographed mixtures of polystyrene latices at 220 and 254 nm (controversy exists as to whether particles absorb at 254 nm; at 220 nm, however, strong absorption occurs). One of Nagy's [18] results, shown in Figure 3, demonstrates the dramatic improvement in the 88 nm peak measured at a wavelength of 220 nm compared to that at 254 nm.

As Heller and Tabibian [40] indicated, appreciable error may result if instruments which are perfectly suitable for ordinary absorption measurements are used for turbidity measurements without proper modifications and precautions. There are three principle sources of error in turbidity measurements [40]: (1) interference of laterally scattered light (2) the corona effect and (3) the effect of the solid angle. While such errors were believed to be negligible by earlier workers [17], their existence was unequivocally demonstrated by Husain et al in a series of papers [34,41,42,43]. They compared the detector response to a suspension of polystyrene spheres with the response to a solution of sodium dichromate. Furthermore, they showed that impurities (such as residual styrene monomer in polystyrene particles) and additives (such as emulsifier) may cause the measured extinction coefficient to differ from theoretical calculations based on Mie theory. The discrepancy may theoretically be accounted for by employing an effective imaginary refractive index ratio (colloid to medium) [18,32].

Maron et al [44] applied turbidimetric techniques to measure the size distribution of polydisperse polybutadiene-styrene latices. Gledhill [45] described a method for constructing a graphical

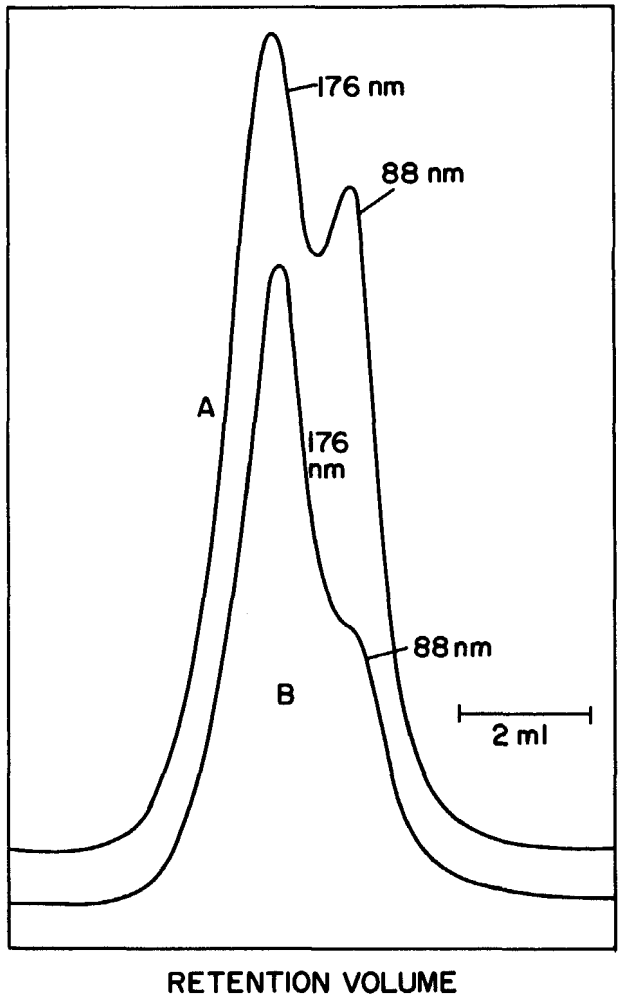

FIGURE 3: HCD separation of a bimodal mixture of 88 nm and 176 nm polystyrene latices. A, response at 200 nm; B, response at 254 nm.

calibration grid for a system of known optical constants and known distributional form, from which the weight mean diameter and standard deviation of the distribution corresponding to observed turbidity measurements could be read directly. However, as Maxim et al [46] indicated in their publication, the turbidity spectra analysis, though very attractive because of the simplicity of the experimental technique, should not be used alone for particle size analysis. This was further discussed in Kiparissides et al [5]. Recently, Nagy et al [20] reported a method for improving signal resolution in latex particle size analysis and data for the specific extinction coefficient for polystyrene indicates that improvement can be obtained for the small particle end of broad size distribution by using turbidity detection at wavelengths less than 254 nm.

B. Differential Refractometry Detection

Zimm and Dandliker [47] derived a general refractive index expression based on the Mie theory. Their expression for the dispersion refractive index, n_s, is given by:

$$\frac{dn_s}{dc} = \frac{3n_m}{2a^3\rho_p} \; Re\left[\sum_{n=1}^{\infty} \frac{2n+1}{2n(n+1)} (\alpha_n - b_n) \right], \qquad (2)$$

where c is the weight concentration in gr/cm^3, ρ_p is the particle density, a is a dimensionless size parameter ($a = \pi D/\lambda$, where D and λ are respectively the particle diameter and the wavelength in the medium), n_m and n_s are the refractive indices of the medium and the dispersion, respectively, and α_n and b_n are functions of a and m (m is the refractive index ratio of particle to medium). The above equation (2) does not contain the restriction that a be small and allows calculation of the effect of light scattering on the refractive index of a

colloidal dispersion. In the limit, as a goes to zero, equation (2) reduces to:

$$\frac{dn_s}{dc} = \frac{3n_m}{2\rho_p} \frac{(m^2-1)}{(m^2+2)} \tag{3}$$

a result derivable from Heller's [48] equation. In accordance with equations (2) and (3), dn_s/dc is expected to be independent of c and at small values of a, independent of a as well.

Nakagaki and Heller [49] confirmed the validity of equation (2) for particle diameters as large as 500 nm. Measurements by Silebi and McHugh [17] show a surprising agreement of measured data with equation (3) for polystyrene latices as large as 350 nm. Both measurements were made with polystyrene at a wavelength of 546.1 nm. Subsequent data measured by Nagy [18] from the same laboratory indicates that dn_s/dc reverses in sign with increasing particle size; its implication, therefore, is that the signal is null for some intermediate particle size. Coll and Fague [27] observed that dn_s/dc was independent of c for a given latex, though its value increased linearly with particle diameter. Neither Nagy [18] nor Coll and Fague [27] were at the time able to explain their results satisfactorily. Interpretation of their data is complicated due to the use of a broad wavelength source.

Husain's [34] opinion is that the above seemingly conflicting data is in fact consistent with the Zimm and Dandliker [47] equation. Calculations [47,49] indicate that, depending on the values of m and a, dn_s/dc may either increase with particle size or decrease and eventually change sign.

Differential refractometry shows a less dramatic dependence on particle size (third order) than turbidimetry of nonabsorbing particles (sixth order). This advantage of differential refractometry is,

however, counterbalanced by the requirement of a higher sample concentration compared to the amount necessary for a photometric detection due to the limited sensitivity of available differential refractometers. Of course, with the advent of more sensitive detectors this drawback will likely be overcome.

THEORETICAL ANALYSIS OF PEAK SEPARATION

The passage of an injected sample through the columns and detector generates an output trace on the recorder, the chromatogram. For several reasons, a chromatogram can never fully represent the distribution of colloid sizes in the injected sample. Instrumental spreading (or axial dispersion) causes elution of a single species to occur over a range of retention volumes. The chromatogram of the sample is the superposition of these distributions. When the number of species is small, one might obtain a chromatogram involving many obvious but overlapping peaks. However, with a large number of species, the peaks of individual species are not evident; one usually obtains a unimodal chromatogram and sometimes a more complex one. Interpretation of a chromatogram must, therefore, account for this superposition and involve an evaluation of instrumental spreading and correction of the detector response to obtain the true concentrations of the component species.

While theory adequately predicts peak separation in HDC, a similar comprehensive treatment is lacking for SEC. Attempts [18] to predict peak separation in SEC have not been very successful. We now briefly examine the theories proposed to explain peak separation in HDC and SEC.

A. Hydrodynamic Chromatography

As it was mentioned earlier, two approaches have been taken to model HDC:

(a) The capillary model: A solute particle does not spend the same fraction of its total residence time at each radial position. If interactions between particles are negligible, the residence time distribution for the solute during a transient will be the same as in the case where the solute is continuously injected. From an analysis of the particle continuity equation in the presence of a radial force field, for the case of continuous injection, it can be shown that the radial concentration distribution is a Boltzmann one, given by:

$$C(r) \propto \exp\,[-\phi(r)/kT]\,, \tag{4}$$

where $\phi(r)$, the particle-wall total interaction energy is given by the superposition of the repulsive potentials arising from the double layer and Born repulsive forces and Van der Waals attractive potentials, as

$$\phi(r) = \phi_{DL} + \phi_B + \phi_{VW} \tag{5}$$

Then, the average particle velocity can be calculated by weighting the local particle velocity $V_{pz}(r)$ at a given radial position with the concentration at that position, to obtain

$$\bar{v}_p = \frac{\int_0^{R-\delta} v_{pz}(r)\,\exp\,[-\phi(r)/kT]rdr}{\int_0^{R-\delta} \exp\,[-\phi(r)/kT]\,rdr}\,, \tag{6}$$

where the upper integration limit accounts for the inability of a particle to approach the capillary wall closer than its radius, δ. $v_{pz}(r)$ is given by a modified Poiseuille equation which takes the wall effect into account.

For an ionic marker, ϕ_B and ϕ_{VW} are negligible and its average velocity, $\bar{v}_M$, is obtained by taking the limits of the above integrals in (6) as the particle radius δ tends to zero. R_F is then given by definition as:

$$R_F = \bar{v}_p / \bar{v}_M \tag{7}$$

(b) The equilibrium model: The corresponding expression for R_F is given by:

$$R_F = \frac{V}{A} \frac{1}{\int_0^\infty \exp\left[-\phi(h)/kT\right] dh} , \tag{8}$$

where V is the volume of the mobile phase and A is the surface area of the packing. The R_F dependence on packing diameter is manifested by the presence of A.

Both models adequately predict the variation of R_F with particle diameter over a wide range of ionic strength. Unlike the capillary model, Equation (8) predicts an increase in R_F with hydraulic radius V/A or packing diameter, contrary to the observed dependence. Therefore, the capillary model seems to be more powerful, as mentioned already.

B. Size Exclusion Chromatography

In addition to the factors governing the separation of colloids in HDC, the use of porous packing introduces the possibility of size separation due to steric exclusion from the pores. Due to the complex flow patterns in porous packed beds, the difficulty in predicting the migration of a colloid peak is obvious. Nagy [18] and Nagy et al [32] attempted to simplify this problem by using very large pores relative to the size of the colloids being separated (they used a porous column with a mean pore size of 2.5 μ). Their analysis, therefore, assumes that all particles enter the pores and accordingly, describes one extreme of SEC where permeation by all species occurs. No electrolyte was used and the ionic strength was varied by using surfactant alone, at concentrations below and above the critical micelle concentration. The qualitative

features of their data were similar to those observed in HDC, though, the R_F values were larger due to partial penetration of the pores by the particles.

Nagy [18] modelled his data using a flow-through bank model as shown in Figure 4. The large tubes in a given bank represent the totality of interstitial regions at the same level in the column, while, the small tubes represent the totality of the pores within the packing at the same level. The spaces between the banks have zero volume and serve as a mixing region for altering particle trajectories. The probability of a particle entering a tube at the start of a bank is assumed equal to the ratio of flow through all such tubes to the total flowrate through all tubes in the bank. The principal result of their analysis predicts that:

$$\frac{1}{R_F} = \frac{(V_{pc}/V)}{R_{F,pc}} + \frac{(V_{ic}/V)}{R_{F,ic}} \tag{9}$$

where $R_{F,ic}$ and $R_{F,pc}$, the separation factors corresponding to the interstices and pores respectively, are calculated as before. V_{pc}, V_{ic} and V represent the pore volume, interstitial volume and total void volume, respectively.

Now, it is shown that the result in equation (9) may be obtained using a simpler model, which regards the column voids as a system of parallel capillaries of the interstitial and pore type (no mixing region is considered and capillaries are continuous across the length of the column). The peak retention volume of the colloid peak, V_p, is given by:

$$V_p = n_{pc}\, Q_{pc}\, \bar{t}_{pc} + n_{ic}\, Q_{ic}\, \bar{t}_{ic} \tag{10}$$

where n, Q and $\bar{t}$ denote tube number, flowrate and average residence time, respectively. It follows, therefore, that:

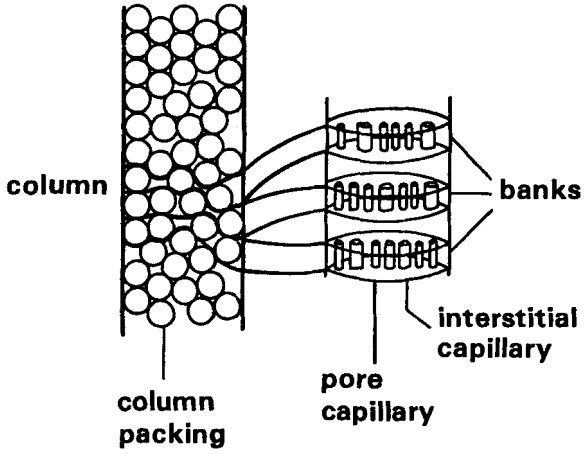

FIGURE 4: A bank model of a SEC column.

$$1/R_F = (n_{pc}\, Q_{pc}\, \bar{t}_{pc} + n_{ic}\, Q_{ic}\, \bar{t}_{ic})/V_m \tag{11}$$

where V_m, the retention volume of a marker peak, is equal to V. If the length of the column is L and the cross-sectional area of a capillary, A, then:

$$\begin{aligned} 1/R_F &= L/V_m\, [n_{pc}\, Q_{pc}/(\bar{v}_p)_{pc} + n_{ic}\, Q_{ic}/(\bar{v}_p)_{ic}] \\ &= L/V_m\, [n_{pc}\, A_{pc}\, (\bar{v}_m/\bar{v}_p)_{pc} + n_{ic}\, A_{ic}\, (\bar{v}_m/\bar{v}_p)_{ic}] \\ &= (n_{pc}\, A_{pc}\, L/V_m)/R_{F,pc} + (n_{ic}\, A_{ic}\, L/V_m)/R_{F,ic} \\ &= (V_{pc}/V)/R_{F,pc} + (V_{ic}/V)/R_{F,ic} \end{aligned} \tag{12}$$

The derived result is identical to equation (9) [18]. The apparent equivalence of the two models is a direct consequence of assigning, in the bank model, the probability that a particle travels through a given tube as equal to the ratio of flow through all such tubes to the total flowrate through all tubes. Therefore, it is not surprising that calculations based on equation (9) agree rather poorly with experimental data since, the equivalent model (which allows no fluid intermixing)

considered here, is hardly representative of the flow process in a packed column.

Nagy [18] cites several reasons, chief among which is the slow diffusion coefficient of colloids, to justify the use of a flow model as opposed to a diffusion model. As pointed out by Small [22], if a bank model is considered, separation by flow would seem unlikely, since very little fluid would flow through the extremely fine pores of the packing when the much less restricted pathway around the particles is available to it. It is beyond the scope of this paper to consider alternate models for SEC. It is, however, suggested [34] that, since the equilibrium theory for HDC is essentially independent of the complex flow geometry, it may be possible to extend the treatment to predict colloid behaviour in SEC. Further work in this direction would undoubtedly be facilitated by a critical review by Casassa [50] who has examined the various models proposed to explain peak migration in SEC.

CALCULATION OF PARTICLE SIZE DISTRIBUTIONS

As we have mentioned before, the axial dispersion phenomenon is a serious imperfection in the chromatography of particle suspensions. The input sample W(y) is distorted as a result, so that the diameter frequency distribution calculated based on the measured response F(v) may be significantly in error.

All rigorous methods of correcting detector response for peak broadening (or axial dispersion) use the following integral equation as the basis:

$$F(v) = \int_0^\infty W(y)\, G(v,y)\, dy \quad , \tag{13}$$

where F(v) is the detector response at retention volume v (i.e. it is related to the true chromatogram W(y) by equation (13)) and G(v,y) is the normalized detector response or spreading function for a particle of diameter D(y) or for a species with mean retention volume y. G(v,y) is often called the instrumental spreading function and is frequently considered to be uniform, i.e.

$$G(v,y) = G(v-y) \quad , \tag{14}$$

which considerably simplifies the mathematical treatment of equation (13). W(y)dy is the area under the detector response due to particles of diameter D(y). W(y) is called the detector response corrected for dispersion. Equation (13) is a Fredholme integral equation of the first kind and has been used extensively in various science and engineering applications. When detection is turbidimetric, both F(v) and W(y) represent turbidities while, in the case of differential refractometry, they represent refractive index increments.

The response for a general detector is given by:

$$F(v) = \int_0^\infty W(v,y)dy \quad , \tag{15}$$

where:

$$W(v,y) \propto N(v,y)\ D^{\gamma}(y) \tag{16}$$

for the Rayleigh scattering regime (γ=3 for refractive index and γ=6 for turbidity detector) and

$$W(v,y) \propto N(v,y)\ D^{2}(y)\ K(y) \tag{17}$$

for the Mie scattering regime, where K(y) is the extinction coefficient for particles of diameter D(y). In both (16) and (17), N(v,y) gives number of particles.

Comparing equations (13) and (15), it is clear that:

$$W(v,y) = W(y)\ G(v,y) \tag{18}$$

and

$$W(y) = \int_0^\infty W(v,y)dv \tag{19}$$

Equations (18) and (19) can be used to derive correction equations for dispersion in the detector cell itself.

Equation (13) may be solved both numerically and analytically. Numerically, it is solved either for $G(v-y)$[41], when $F(v)$ and $W(y)$ are known, or, as is usally the case, the integral equation is solved for $W(y)$, when $F(v)$ and $G(v-y)$ are known, which may then be converted into a particle size distribution. In contrast, analytical solutions enable the direct calculation of moments of the size distribution function; the PSD itself is not obtained. Both methods of solution will be discussed later in what follows.

Forms of the Spreading Function

(a) Uniform spreading function: In other words, its shape parameters are independent of retention volume, i.e. the shape parameters are the same for particles of different diameter. This limiting form should be valid for samples with relatively narrow particle size distributions. For this case, equation (13) becomes [51,52]:

$$F(v) = \frac{1}{\sqrt{2\pi\sigma^2}} \int_0^\infty W(y) \exp(-(v-y)^2/2\sigma^2)dy \tag{20}$$

where σ^2, the variance of the uniform Gaussian spreading function is independent of retention volume.

(b) Non-uniform Gaussian spreading function: The integral equation (13) now takes the form:

$$F(v) = \int_0^\infty W(y) \frac{1}{\sqrt{2\pi\sigma^2(y)}} \exp(-(v-y)^2/2\sigma^2(y))dy \tag{21}$$

(c) General spreading function: Provder and Rosen [52[have proposed the use of a general statistical shape function to account for deviations of the spreading fucntion from the Gaussian shape. It has the form:

$$G(x) = \phi(x) + \sum_{n=3}^{\infty} (-1)^n A_n \phi^n(x)/n! \qquad (22)$$

where

$$\phi(x) = \frac{1}{\sqrt{2\pi}} \exp(-x^2/2) \qquad (23)$$

$$x = \frac{v - y}{\sigma}$$

and $\phi^n(x)$ denotes n^{th}-order derivative. The coefficients A_n are functions of μ_n, the n^{th}-order moments about the mean retention volume, μ, of the normalized detector response for a single species.

Numerical Solution of the Integral Equation

Several numerical methods have been reported for the solution of the integral equation. These have been reviewed by Friis and Hamielec [53] and evaluated by Silebi and McHugh [17] for their application to particle chromatography. They conclude that the method of Ishige et al [54] performs better than other available methods. A noteworthy undesirable feature of the method, however, is its tendency to overestimate the number of small particles in a polydispersed sample. Modifications of Ishige's algorithm fail to overcome this defect [53]. Unless a more effective numerical method is developed for solving for the corrected detector response, W(y), it is recommended that analytical methods be used to calculate particle diameter averages.

Analytical Solution of the Integral Equation

Three analytical methods for solving the integral equation have been reported [29,42,55]. Their main features are compared in Table 2.

TABLE 2

A Comparison of the Analytical Methods for Solving the Integral Equation

Attributes	Method* 1 Hamielec and Singh[29]	2 Hussain et al.[42]	3 Hussain et al.[55]
1. Calibration curve	Linear	Nonlinear	Nonlinear
2. Spreading function	Eqn.(25) or Provder and Rosen's[52] shape function	Eqn.(25)	Eqn.(25) or Provder and Rosen's[52] shape function
3. Light scattering theory which may by applied	Rayleigh	Mie	Mie
4. Chemical absorption may be present in tubidimetric detection.	No	Yes	Yes
5. Diameter averages are calculated as a function of retention volume	No	Yes	No

* The refractive index detector (dn_s/dc = constant) can be treated using all the above methods. However, Method 3 is most general.

It is important to realize that, the solution derived for a Gaussian spreading fucntion $G_o(v-y)$ is equally applicable to a whole family of functions of the form:

$$G(v,y) = G_o(v-y)\ \Psi(y) \tag{25}$$

where $\Psi(y)$ is an unspecified function of y. This considerably extends [55] the applicability of the solution for a Gaussian spreading function to an infinite set of non-Gaussian, non-uniform functions.

The first solutions of this kind were based on the use of bilateral Laplace transformations and uniform instrumental spreading functions

51,52] and they were applied to the SEC of polymer molecules. The first application to the chromatography of spherical suspensions was made by Hamielec and Singh [29] and Husain et al [55]. Yau et al [56] obtained similar solutions for the case of a uniform spreading function and a linear molecular weight calibration curve (equivalent to a linear particle diameter-retention volume calibration curve in this context). Yau et al [56] focussed on dispersion in the detector cell as did Hamielec [57] and Hamielec et al [58] in accounting for a non-uniform Gaussian spreading function and a nonlinear calibration curve.

The case of a non-uniform spreading function and a nonlinear particle diameter-retention volume calibration curve has been treated by Husain et al [35,42]. A novel method for identifying and estimating the parameters of the instrumental spreading function for column chromatography has been developed and applied to the SEC of particle suspensions [41]. This has revealed that for SEC, the spreading function of polystyrene latex standards in the size range 85-312 nm is skewed towards longer retention volumes. The Provder and Rosen [52] general spreading function gives reasonable fit to experimentally measured spreading functions for particles in the size range 85-220 nm. This is clearly demonstrated in Figures 5, 6 and 7. In these three figures [34], F(v) represents the experimental chromatogram and $G(v-v_p)$ the estimated spreading function. Figure 8 gives the change of the variance σ^2 with particle diameter, and Figure 9 shows a plot of the coefficient A_3 in equation (22) versus particle diameter, as they were used in fitting the general spreading function given by equation (22) to experimentally measured spreading functions. As it is easily understood from Figures 8 and 9, the change of σ^2 and A_3 with particle diameter is consistent with experimental data[34], which showed an increase in

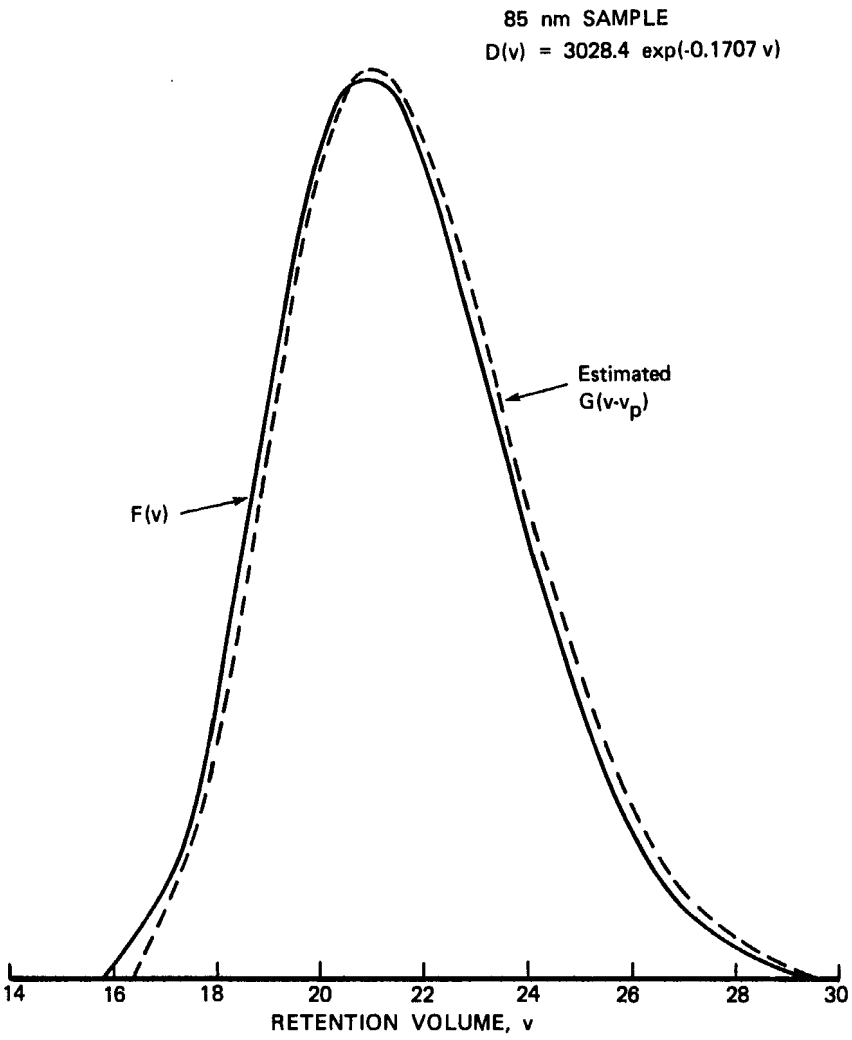

FIGURE 5: Estimation of the spreading function from experimental chromatogram.

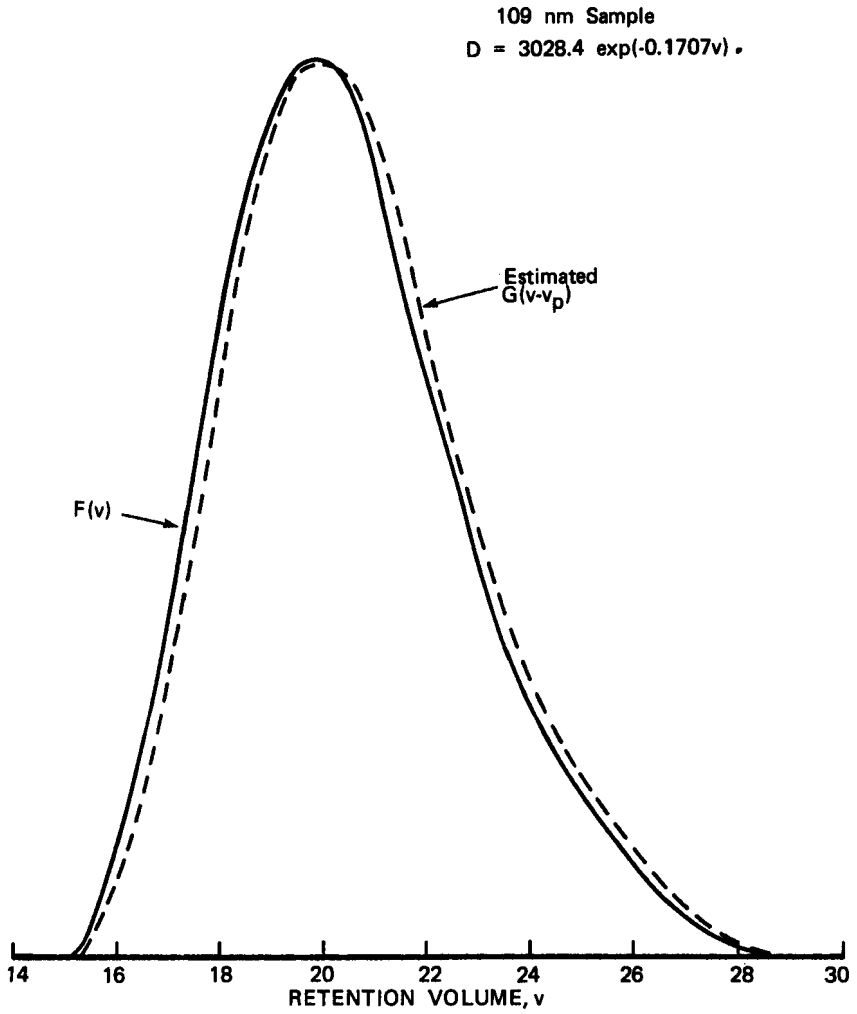

FIGURE 6: Estimation of the spreading function from experimental chromatogram.

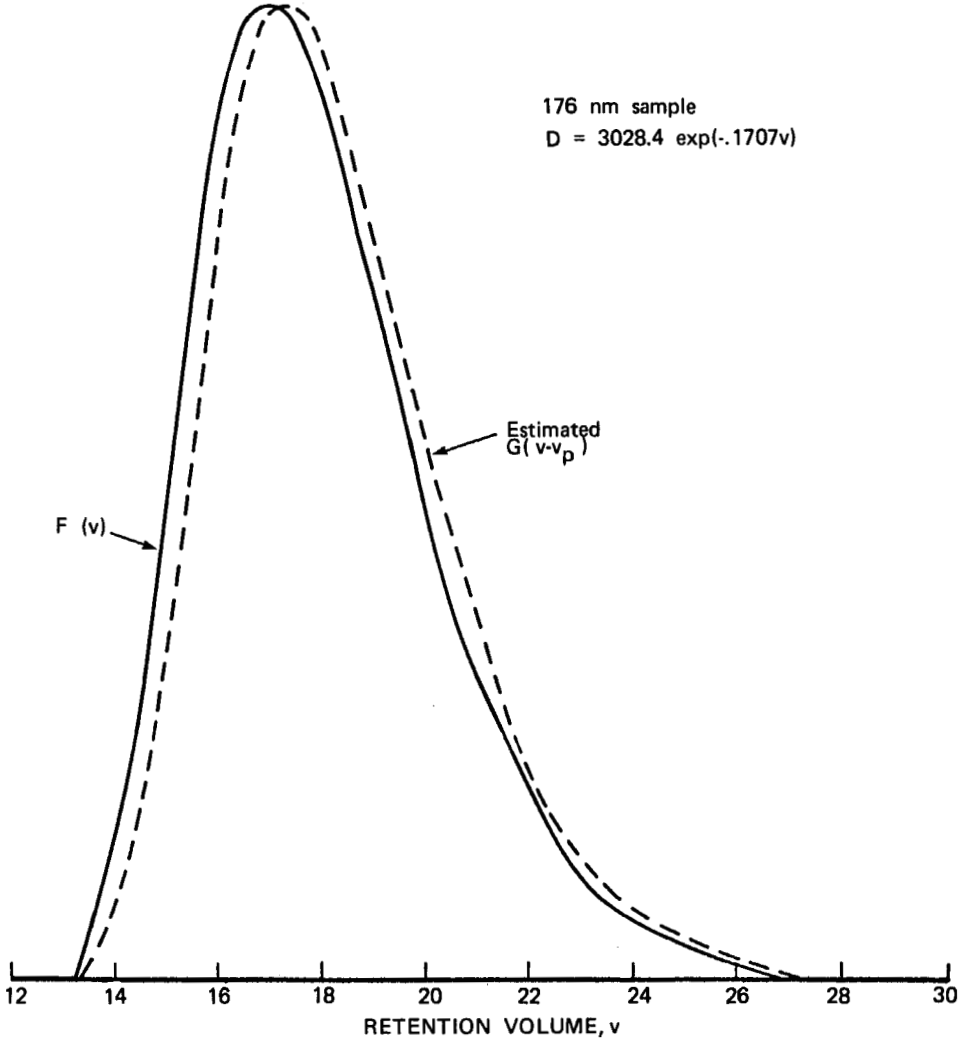

FIGURE 7: Estimation of the spreading function from experimental chromatogram.

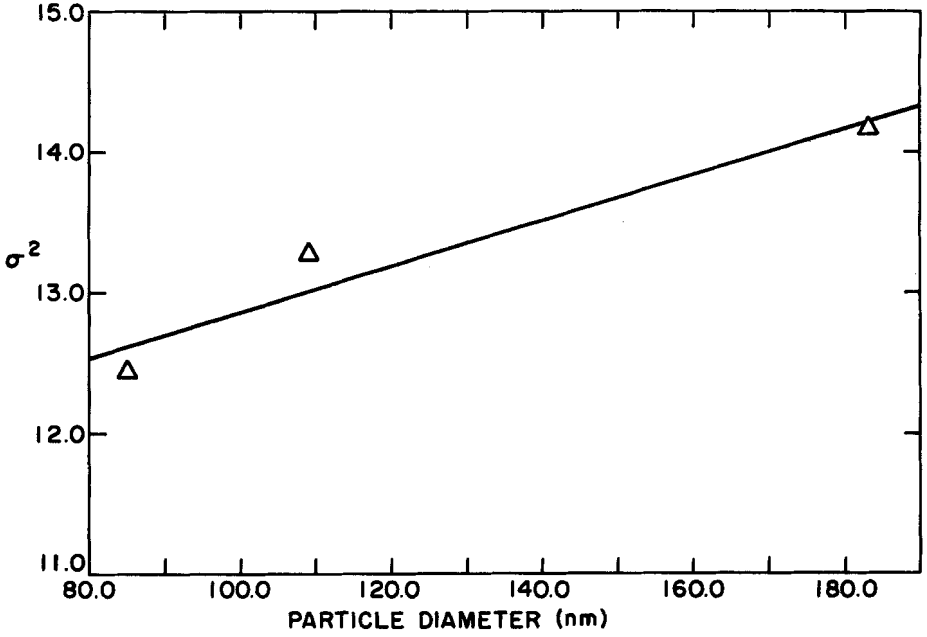

FIGURE 8: Variance versus particle diameter.

skewness of the chromatograms with an increase in particle diameter of the sample. For the 312 nm standard, the fit was poor. It would be of interest to compare experimental G(v,y) with the spreading function predicted by the plug flow dispersion model. It appears that for the HDC or SEC of particles, a skewed instrumental spreading function should be used to properly account for dispersion.

Instrumental Correction for Dispersion

An HDC or SEC operating with normal resolution should provide unimodal and relatively narrow frequency distributions of particle size in the detector cell across the chromatogram of a whole sample. Therefore, a detector system which can provide, say, two moments of the frequency distribution and the particle concentration of the detector

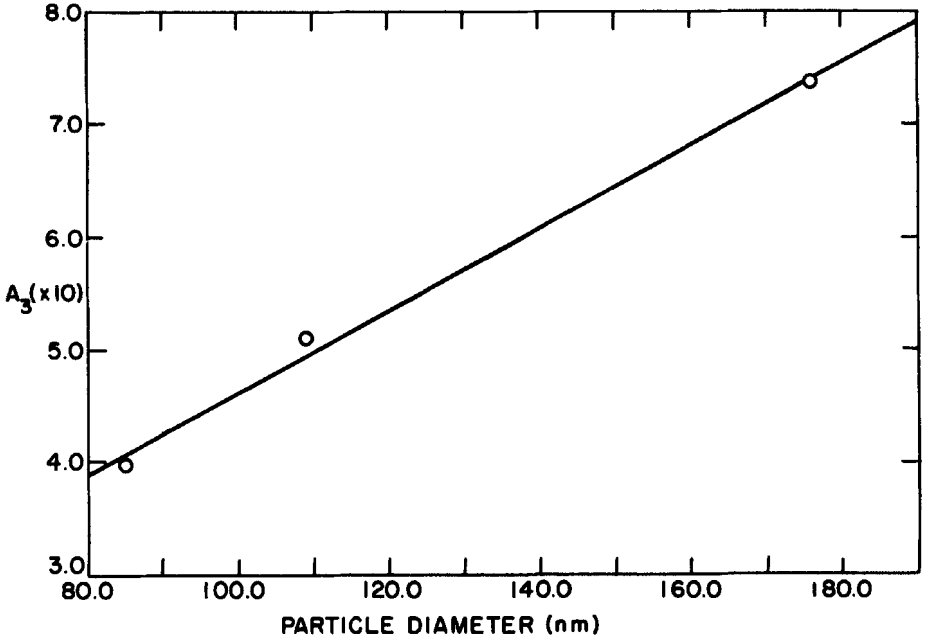

FIGURE 9: Coefficient A_3 in equation (22) versus particle diameter.

cell contents should in principle provide a measure of the frequency distribution of the whole sample and this measure should be largely independent of the resolution of the chromatograph.

To date, the use of a detector system to this end has not been reported. There are, however, at least two detector systems based on turbidity-spectra 43,59] and quasi-elastic light scattering (photon correlation spectroscopy [60,61,62,63,64]) which seem to have the potential for this task.

Fitting the Plug-Flow Dispersion Model

Discussing previously the different forms of the spreading function G(v,y), we have seen that a general statistical shape function

can account for deviations from the Gaussian shape [52]. If we consider equation (22) again and especially the coefficients denoted by A_n, we can see that the first two coefficients are of direct statistical significance and also represent the most useful terms in the infinite series for applications in chromatography.

$$A_3 = \mu_3/\mu_2^{3/2} \tag{26}$$

$$A_4 = (\mu_4/\mu_2^2 - 3) \tag{27}$$

μ_2 is the variance and is equivalent to σ^2. The coefficient A_3 provides an absolute statistical measure of skewness (when μ_3=0, the spreading function is symmetrical about the mean retention volume μ_1 or y. When $\mu_3 > 0$, skewing is towards longer retention volumes). The coefficient A_4 provides a statistical measure of flattening or kurtosis. When $A_4 > 0$, the shape function is taller and slimmer than a Gaussian and so forth. Tung and Runyon [66] used a simpler form to fit skewed detector responses in the SEC of polymer molecules. Silebi [67] has recently shown that skewed instrumental spreading functions derived from the plug-flow dispersion model [68] adequately fit data for particle separations by HDC. This spreading function has the form:

$$G(v,y) = \frac{1}{2\sqrt{\pi Pe^{-1}(v/y)}} \exp\left(-(v-y)^2/4\, Pe^{-1}(v\, y)\right) \tag{28}$$

where Pe = (uL/D) is the Peclet number, u is the superficial velocity in the column, L is the length of the packed bed and D is a dispersion coefficient. The plug-flow dispersion model predicts symmetrical broadening in the packed-bed; however, when dispersion is large, the detector gives a response which is skewed towards larger retention volumes. For small dispersion, Pe > 100 and G(v,y) reduces to a Gaussian shape.

Efforts by the authors to use equation (28) to fit data for particle separations by SEC were proven unsuccessful. It seems that

equation (28) should be used with HDC data and it is the intention of the authors to check the validity of the plug-flow dispersion model in the near future using vinyl acetate latices from their continuous emulsion reactors.

RECOMMENDATIONS

Some recommendations for future work with HDC/SEC can now be made:

(1) An extensive evaluation of various packing materials with differing pore geometry is required. This is necessary to minimize dispersion and particle loss in the columns. The optimum packing particle is probably one with a solid core and superficial surface pores.

(2) Factors affecting particle loss such as (a) ionic strength of eluant, (b) use of ionic surfactant alone as opposed to a mixture of surfactant and electrolyte, (c) effect of glass transition temperature of particles, (d) effect of packing type, (e) effect of column temperature, etc., need to be better understood.

(3) A multiple wavelength UV/Visible light turbidity detector has been theoretically evaluated and found to have reasonable potential as an analytical tool for particle size measurement [43]. Likewise, IR detection has been shown to have some useful features [18]. An experimental investigation of both these detectors is desirable. Also, more sensitive refractometers with a monochromatic light source should be evaluated.

(4) The theory of HDC should be extended to investigate the possibility of predicting the chromatogram shape. The corresponding development of a theory of SEC, to adequately predice peak separation as well as peak shape, may be facilitated by the use of model porous spheres.

(5) The numerical treatment of chromatographic data is rather inadequate. It is desirable to develop new improved methods for recovery of W(y).

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