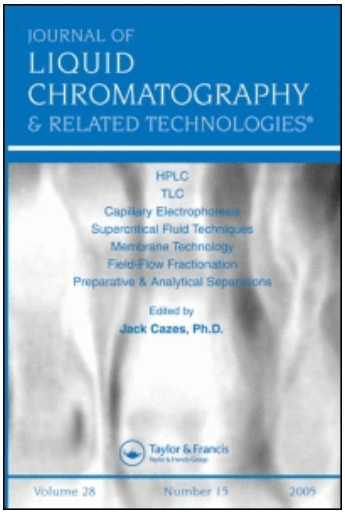

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A NEW METHOD FOR IDENTIFYING AND ESTIMATING THE PARAMETERS OF THE INSTRUMENTAL SPREADING FUNCTION IN SIZE EXCLUSION CHROMATOGRAPHY- APPLICATION TO PARTICLE SIZE ANALYSIS.

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ABSTRACT

Herein is reported a new method for identifying and estimating the instrumental spreading function in size exclusion chromatography. The method is based on the solution of the integral equation when the size distribution of the injected standards are known. A numerical method after Ishige et al.(1) to solve the integral equation for the corrected distribution is suitably modified to estimate instead the spreading function when the true and measured chromatograms are both known. The method is evaluated for synthesized chromatograms using the particle size distribution of Dow polystyrene latices. It is then applied to experimental chromatograms of the latices obtained by size exclusion chromatography. The resulting spreading functions were then analysed for variance, skewness and kurtosis.

INTRODUCTION

In size exclusion chromatography, the detector response F(v) is related to W(y), the chromatogram corrected for peak broadening by the integral equation,

$$F(v) = \int_{-\infty}^{\infty} W(y)\, G(v,y)\, dy \qquad (1)$$

where G(v,y) is the instrumental spreading function and both v and y denote retention volumes. To solve equation (1) for W(y) requires a knowledge of the spreading function, G(v,y). When W(y) is composed of essentially single sized species then the measured

chromatogram is in fact the spreading function for that species. The statistical properties of such a function are size or retention volume dependent and hence attempts to estimate the G(v,y) have involved the use of narrow distribution standards. When the standards are ultra-narrow then it is justified to assume that the measured chromatogram reflects the spreading characteristics of the chromatographic columns. Most available standards are however, not sufficiently monodisperse and the identification of the spreading function in general, requires a knowledge of the size distribution. One exception is the reverse flow technique, proposed by Tung, Moore and Knight (2); this allows an estimate of the spreading function independent of the size distribution function of injected standards.

The reverse flow technique is based on the assumption that when the flow is reversed the process of size separation is reversed also while instrumental spreading continues to broaden the peak. With this technique, a standard sample is allowed to flow through half of the column length; the direction of flow is then reversed. The resulting chromatogram reflects the spreading characteristics of that half of the column. The process is repeated for the other half. When a Gaussian spreading function is assumed, its variance σ^2 is related to σ_1^2 and σ_2^2, the variances of the measured chromatograms, by the following relationship,

$$\sigma^2 = (\sigma_1^2 + \sigma_2^2)/2 \qquad (2)$$

A less tedious procedure (3) involves fitting the leading edge of the chromatogram with a Gaussian function with variance, σ_c^2. If σ^2 (as determined by the reverse flow technique) and σ_c^2 are equal within experimental error, then it is inferred that the leading edge of the chromatogram is monodisperse. Otherwise the assumption of a Gaussian W(y) together with the calibration curve information leads to an estimation of the size distribution of the leading edge. Such information is then used for an unknown column to estimate its spreading characteristics. This procedure assumes that the leading edge of the chromatogram is composed of similar

sized species irrespective of the column's resolution. This clearly cannot be expected to hold in general.

A more direct approach and widely used, involves the use of moment equations derivable from equation (1). The parameters of the spreading function, either Gaussian or skewed are calculated from a knowledge of the averages of the size distribution, typically the number and the weight average. The spreading function generated from such estimates may not necessarily represent the actual spreading characteristic of the column and correlations of the spreading function parameters with retention volume must be regarded with caution.

Recently Berger (4) has suggested a calculation procedure based on the simultaneous solution of equation (1) and

$$C_n(v) = \int_{-\infty}^{\infty} \{W(y)\ G(v_n,y)\ \Delta y\}\ G(v,y)\ dy \qquad (3)$$

where $C_n(v)$ is the chromatogram of the re-injected fraction collected at retention volume v_n. No restriction was placed on the form of G(v,y) except that it is uniform.

In this paper we present a simple numerical procedure for estimating the spreading function from equation (1). It is assumed that W(y) is known. Such information for a latex sample can be obtained by electron microscopy. For a molecular weight sample, obtaining the molecular weight distribution is considerably more difficult. However, it is possible to synthesize polymers with known molecular weight distribution functions. When a sample has a narrow distribution, parameters of the calculated G(v,y) may be related to the mean size. However, when the sample is broad, the calculated G(v,y) is an effective spreading function which may be used to calculate W(y) for an unknown sample having a similar width.

Our procedure which is similar to that of Ishige et al.(1) for calculating W(y), is first assessed using synthesized chromatograms and then applied to chromatograms of Dow polystyrene latices obtained using size exclusion chromatography.

THEORY

Consider the chromatography of a latex sample. For a turbidity detector

$$W(y) \propto N(y)\, K(y)\, D^2(y) \tag{4}$$

where N and K are the number concentration and extinction coefficient respectively of a particle of size D having a mean retention volume y. For a linear calibration curve,

$$D(y) = D_1 \exp(-D_2 y) \tag{5}$$

$$dD(y) = -D_2\, D(y)\, dy \tag{6}$$

where D_1 and D_2 are the calibration constants. N(y) is related to the particle size distribution f[D(y)] as follows

$$f[D(y)]\, dD(y) = \frac{-N(y)\, dy}{\int_{-\infty}^{\infty} N(y)\, dy} \tag{7}$$

where f[D(y)] dD(y) is the fraction of particles in the size range D to D + dD. The negative sign in equation (7) is due to the negative slope of the calibration curve. It follows from the above equations that

$$W(y) \propto f[D(y)]\, K(y)\, D^3(y) \tag{8}$$

Substituting equation (8) in equation (1) yields

$$F(v) \propto \int_{-\infty}^{\infty} f[D(y)]\, K(y)\, D^3(y)\, G(v,y)\, dy \tag{9}$$

A discrete form of equation (9) is more suited when the function f[D(y)] is discontinuous and is given as

$$F(v) \propto \sum_{\text{over all D}} f[D(y)]\, K(y)\, D^2(y)\, G(v,y) \tag{10}$$

For a molecular weight analysis using a refractive index detector, corresponding to equation (9), one obtains

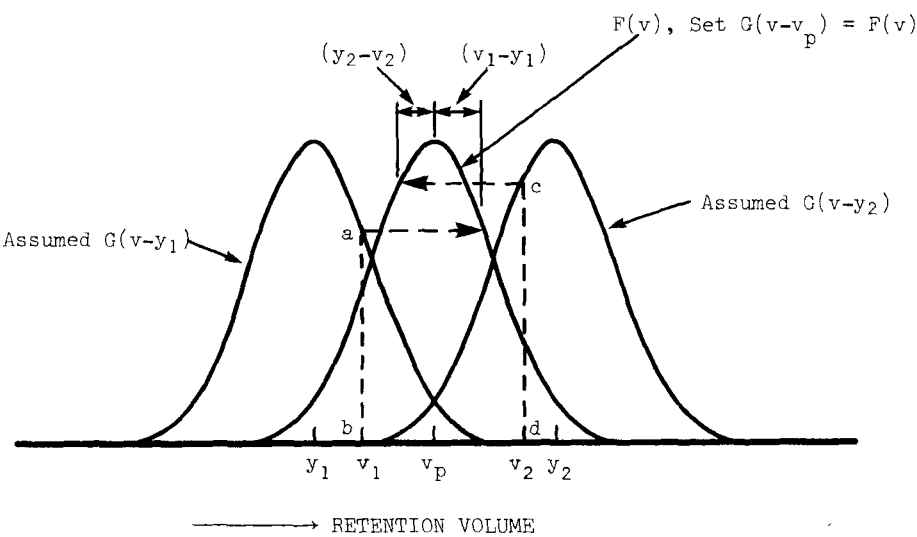
$$F(v) \propto \int_{-\infty}^{\infty} f[M(y)]\, M(y)\, G(v,y)\, dy \tag{11}$$

where M(y) is the molecular weight calibration curve. Extensions of equations (9) to (11) to a nonlinear calibration curve are straight-forward.

NUMERICAL PROCEDURE FOR CALCULATING G(v,y)

We now consider the numerical solution of equation (1) or any of its forms, equations (9), (10) or (11). The algorithm proposed is similar to that used by Ishige et al.(1) for calculating W(y). It is assumed that the spreading function is uniform, i.e. $G(v,y) = G(v-y)$. It is therefore necessary to solve only for one distribution function.

If one initially sets the spreading function, corresponding to $y = v_p$, equal to the measured chromatogram F(v), i.e.



Example:

$$G(v_1 - y_1) \equiv ab \equiv F(v_p + v_1 - y_1)$$

$$G(v_2 - y_2) \equiv cd \equiv F(v_p + v_2 - y_2)$$

Figure 1. The initial estimate for the spreading function

$$G_1(v - v_p) = F(v) \qquad (12)$$

then since the spreading function is assumed uniform, it follows that

$$G_1(v - y) = F(v_p + v - y) \quad \text{(see Fig.1)} \qquad (13)$$

v_p is the peak retention volume of the measured chromatogram. Equations (12) and (13) provide an excellent initial guess for the spreading function since the chromatograms of narrow standards largely reflect the spreading characteristics of the instrument.

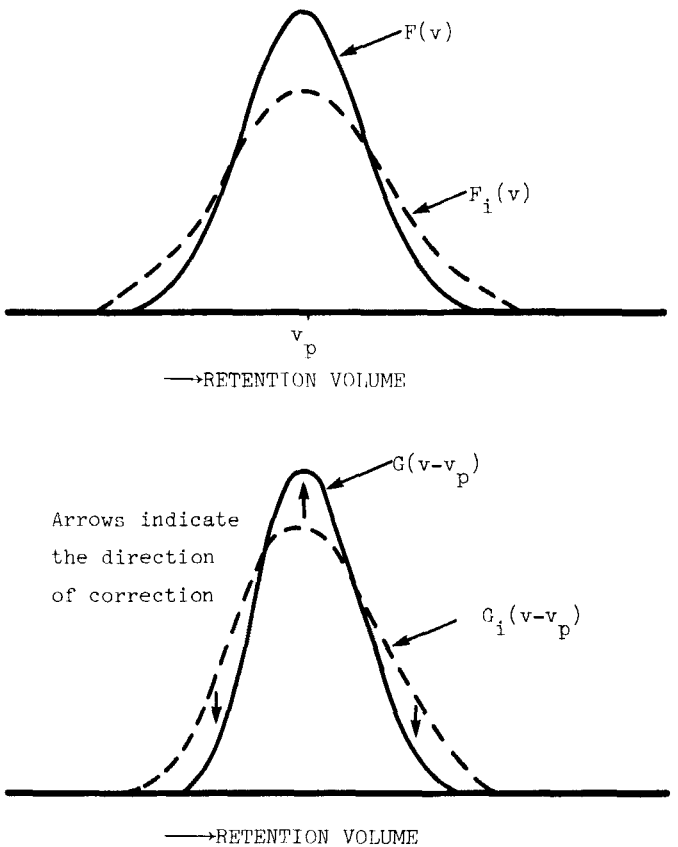


Figure 2. Graphical illustration of the algorithm (Equation 14).

Subsequent improved estimates of the spreading function are obtained as follows,

$$G_{i+1}(v - y) = \frac{F(v)}{F_i(v)} \quad G_i(v - y) \tag{14}$$

This is based on the fact that the initial estimate $G_1(v-y)$ which is broader than the actual $G(v-y)$ causes the calculated chromatogram $F_1(v)$ to be broader than the actual chromatogram $F(v)$. Hence for the calculated chromatogram to converge to the actual chromatogram, $G_1(v-y)$ requires to be sharpened. The algorithm is illustrated in Fig.2.

The procedure in equation (14) is repeated until convergence occurs. If

$$P = \int_{-\infty}^{\infty} | F(v) - F_i(v) | \, dv \tag{15}$$

is less than a given tolerance (a tolerance of 0.01 was set in our calculations) or if the value P for the i^{th} iteration exceeds the value at the previous iteration without the tolerance being satisfied, then the calculations are terminated. It is to be noted that each new estimate of the spreading function must be normalized.

EVALUATION OF THE NUMERICAL PROCEDURE

We evaluate the procedure stated in equation (14) by synthesizing $F(v)$ using an assumed spreading function and an assumed $W(y)$. The spreading function was either a Gaussian or a skewed function obtained by setting all coefficients except A_3 equal to zero in the statistical shape function proposed by Provder and Rosen (5) (see equation 16). To calculate $W(y)$, electron microscopy data of Dow polystyrene latices were used and the extinction coefficients were calculated using either Rayleigh or Mie theory. The results are presented in Figs.3A-H where the estimated $G(v-y)$ is compared with the actual function.

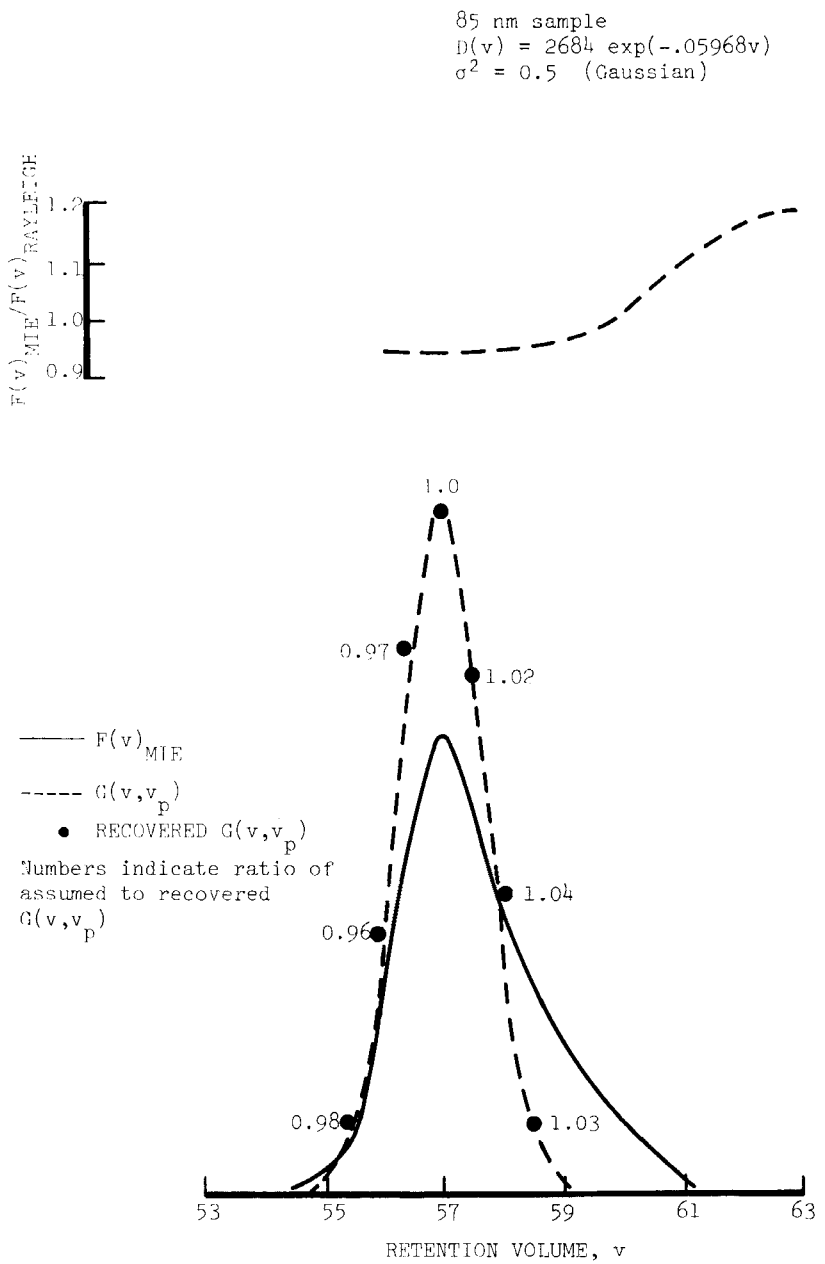
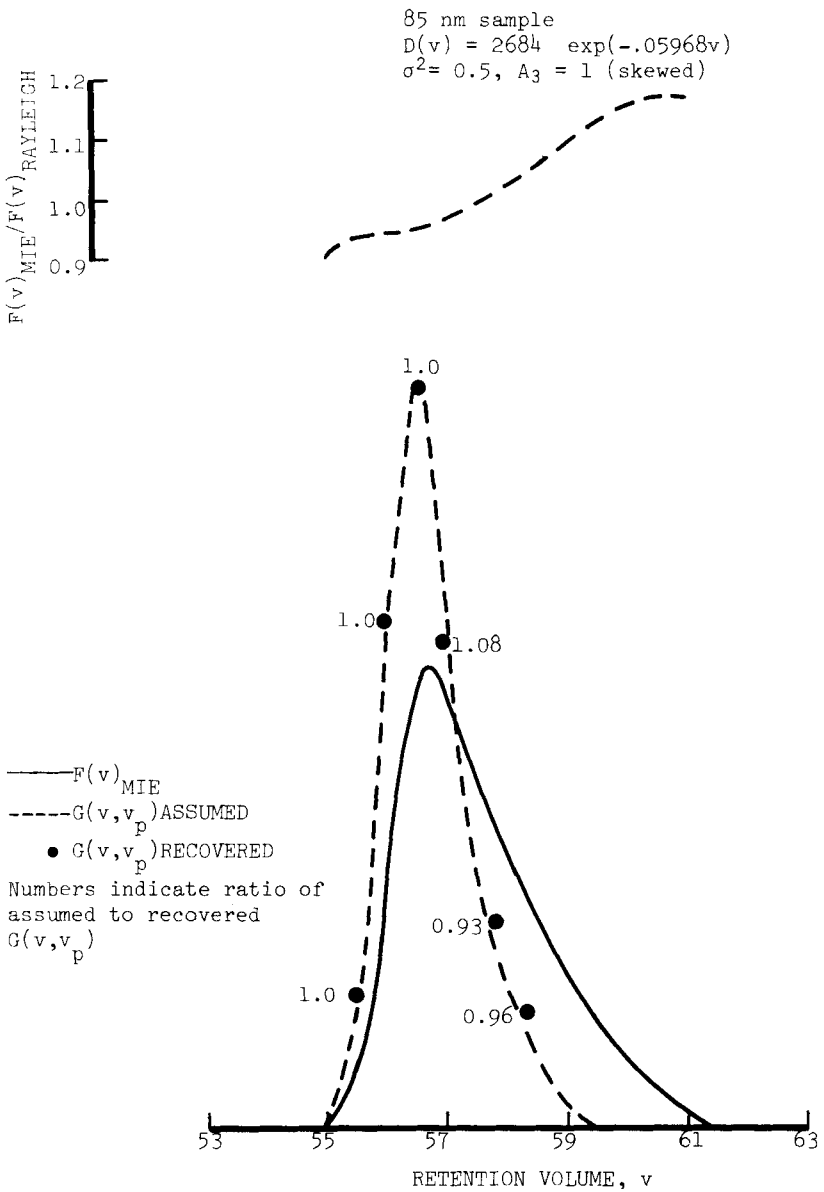


Figure 3. Comparison of assumed and recovered spreading functions.



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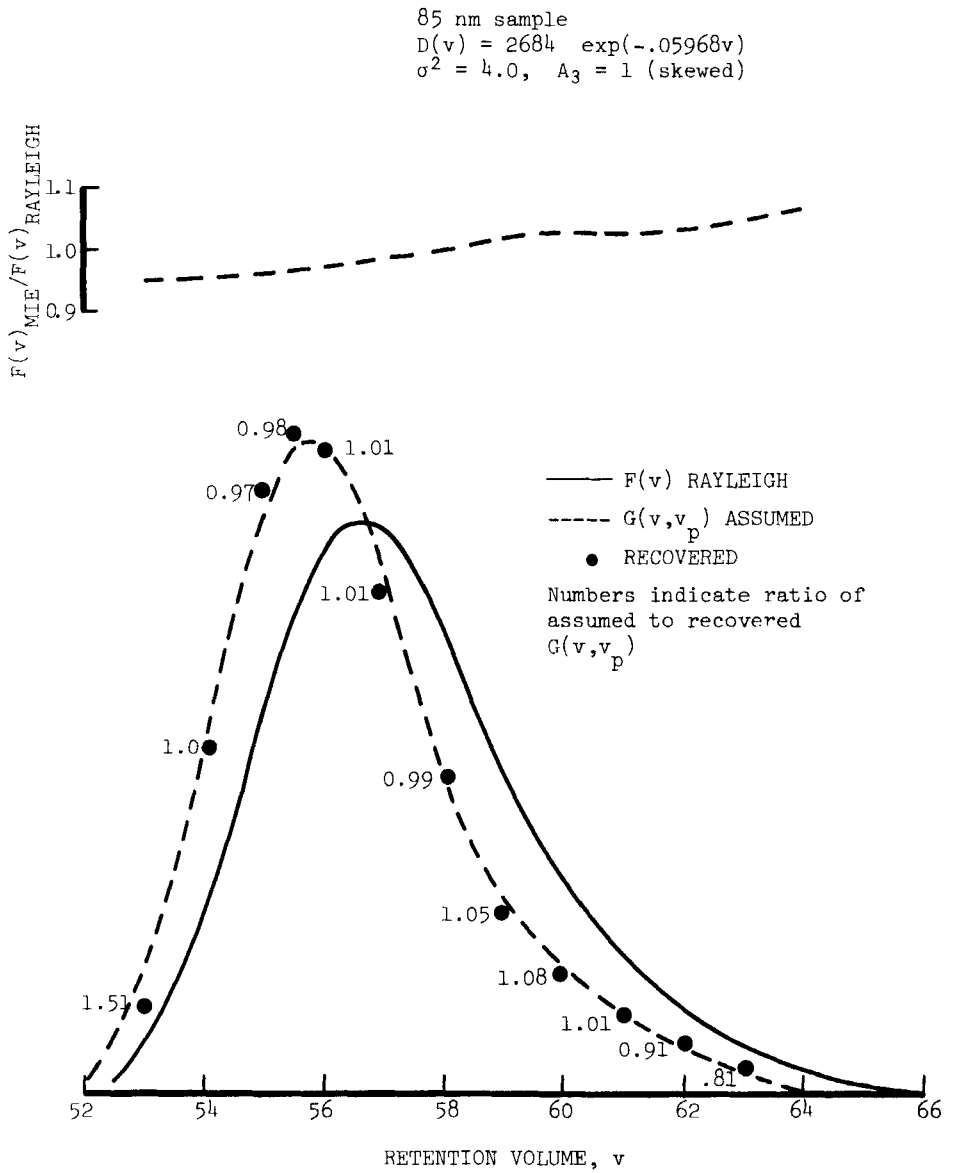

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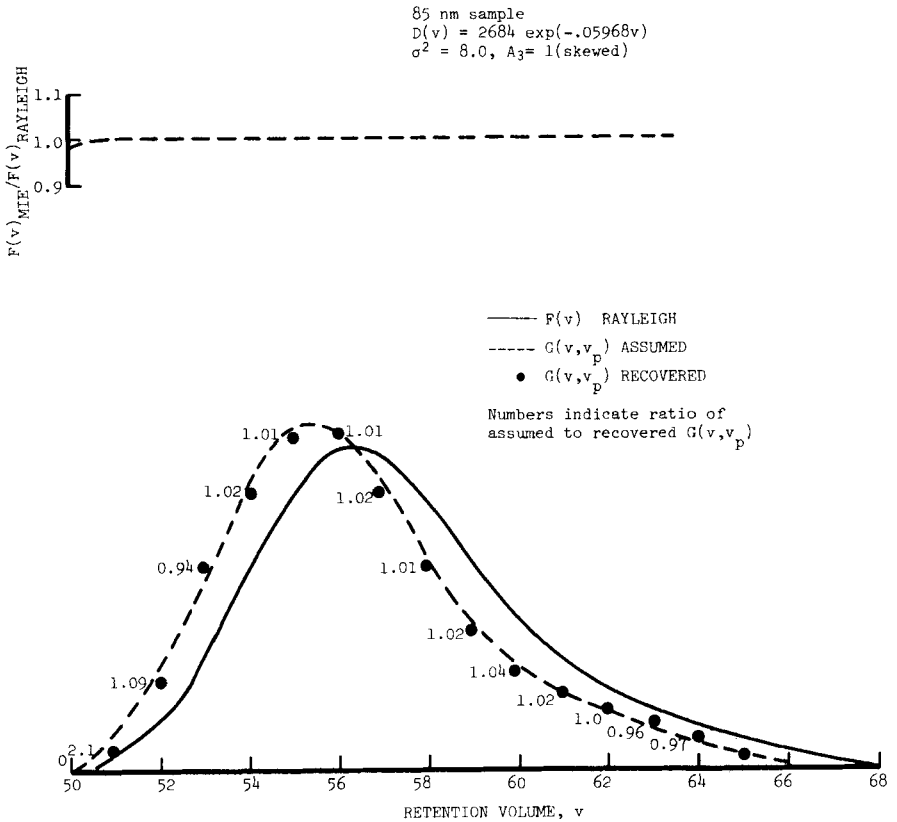

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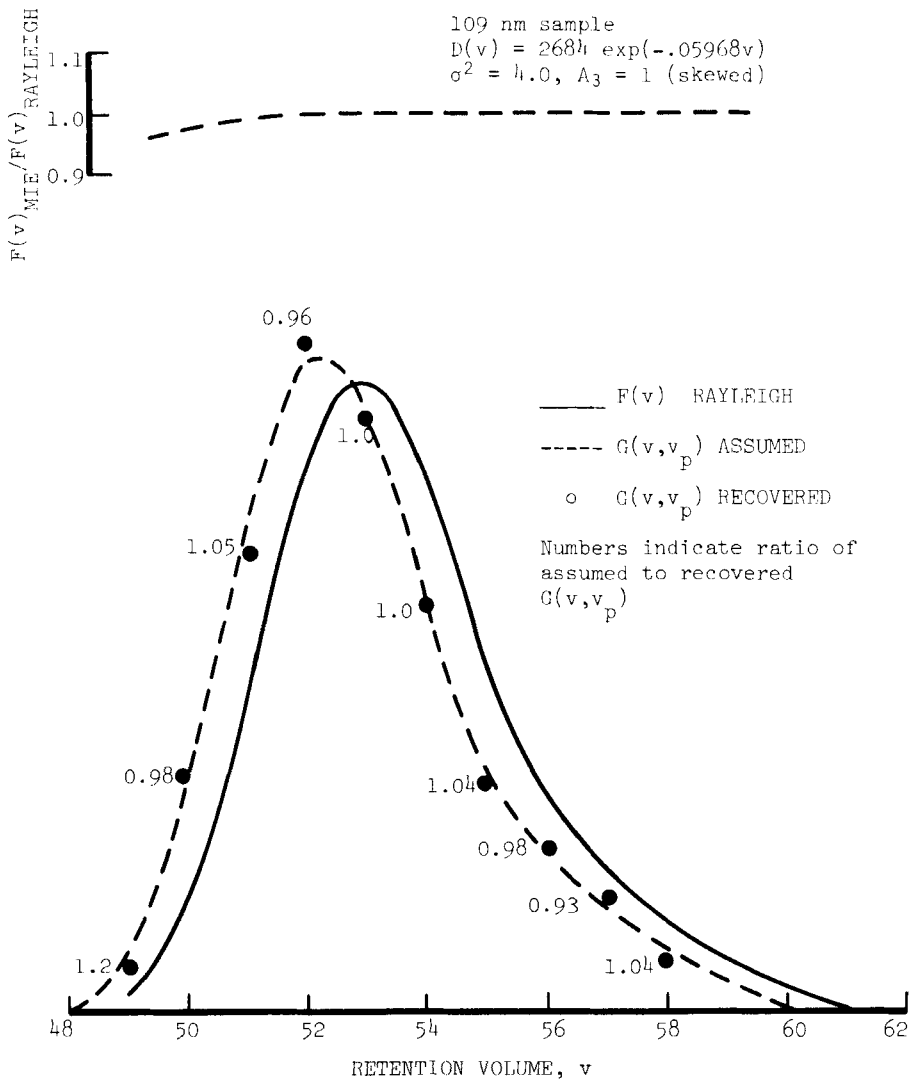

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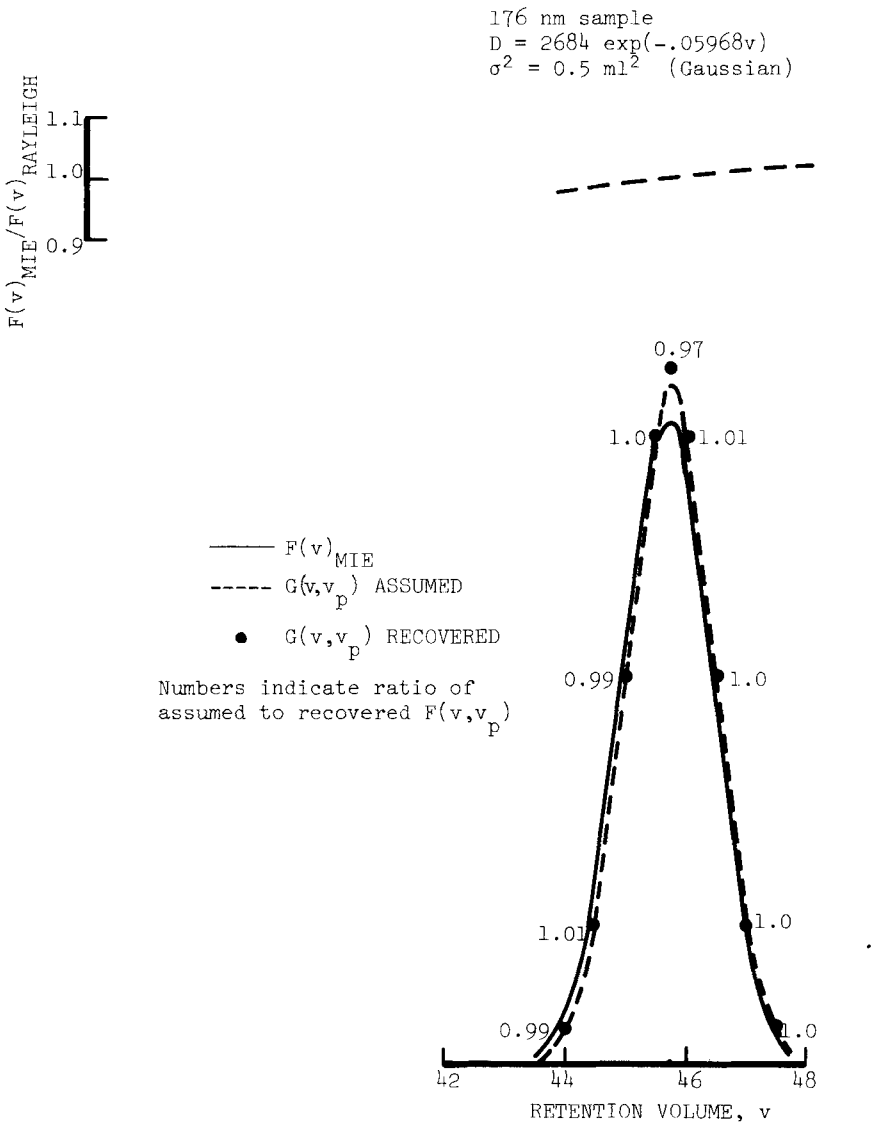

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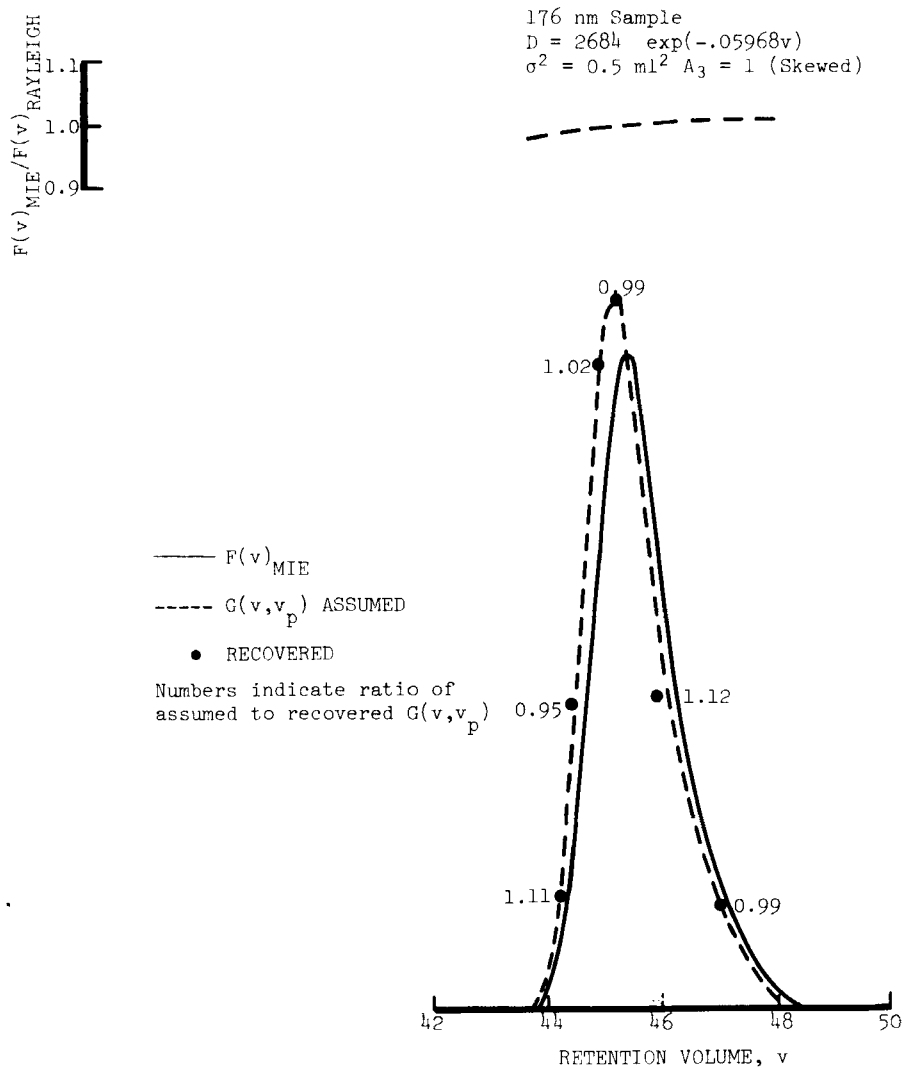

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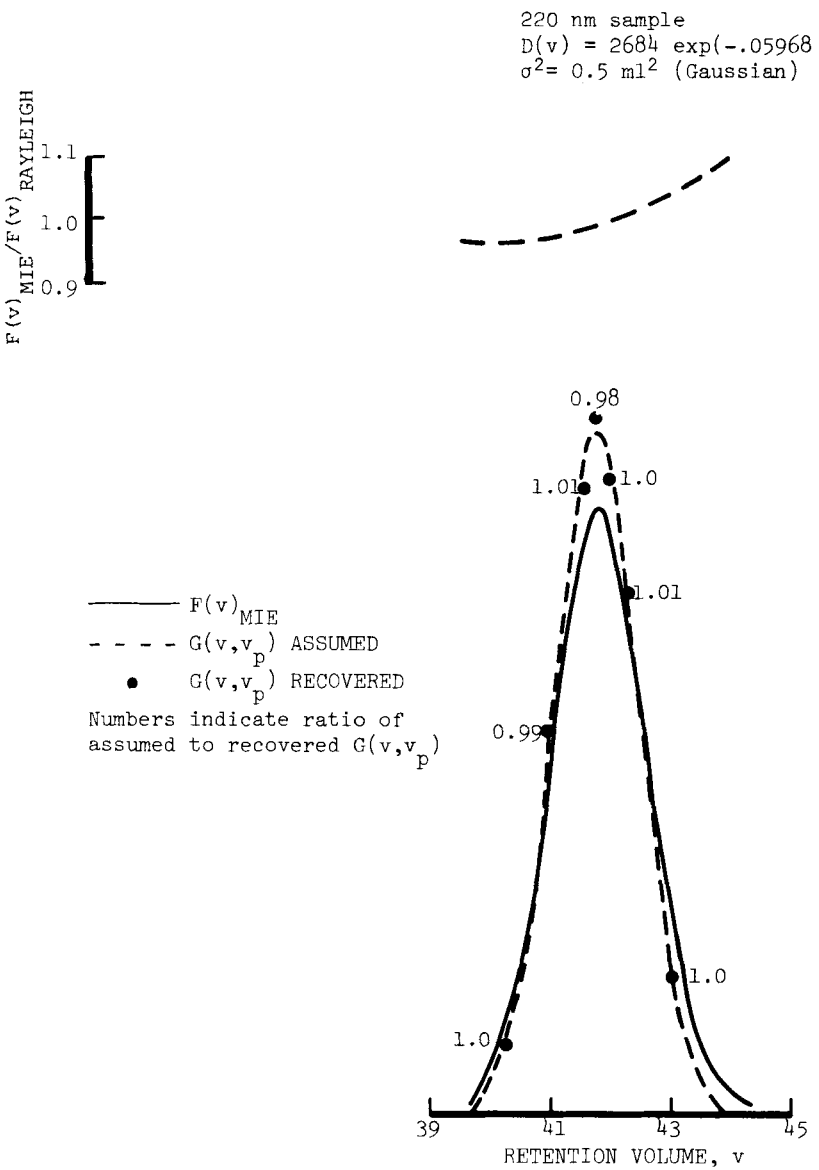

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The values of the parameter σ^2 used in the computations span a wide range with a value of 0.5 ml^2 being representative of columns used in hydrodynamic chromatography while larger values are encountered in size exclusion chromatography. Significant skewing is introduced by setting $A_3 = 1.0$. It is evident from the results that the calculated spreading functions compare very favourably with the assumed functions over the entire range of retention volumes.

Also shown in the plots are the ratios of the chromatogram heights calculated according to Mie theory to those according to Rayleigh theory. These indicate a rather low sensitivity of the calculated chromatograms to the light scattering theory applied.

ESTIMATION OF THE SPREADING FUNCTIONS FROM EXPERIMENTAL CHROMATOGRAMS

Having established the validity of the numerical procedure for estimating the spreading function, the method was applied to experimental chromatograms of a number of Dow polystyrene latices obtained by size exclusion chromatography. The data were measured at a wavelength of 254 nm. Mie theory was assumed valid. Errors resulting from this assumption (6) are likely to be insignificant due to the previously noted observation that the calculated F(v) are relatively insensitive to the light scattering theory applied.

The measured chromatograms and the corresponding estimated spreading functions are shown in Figs.4A-D. For the 220 nm sample the estimated spreading function did not differ markedly from the experimental chromatogram and hence this result is not graphed.

In size exclusion chromatography of polymer molecules, a statistical shape function proposed by Provder and Rosen (5) is frequently used to represent instrumental spreading. It is of interest to examine the fit of the spreading function data to this shape function. We first present a brief discussion of the

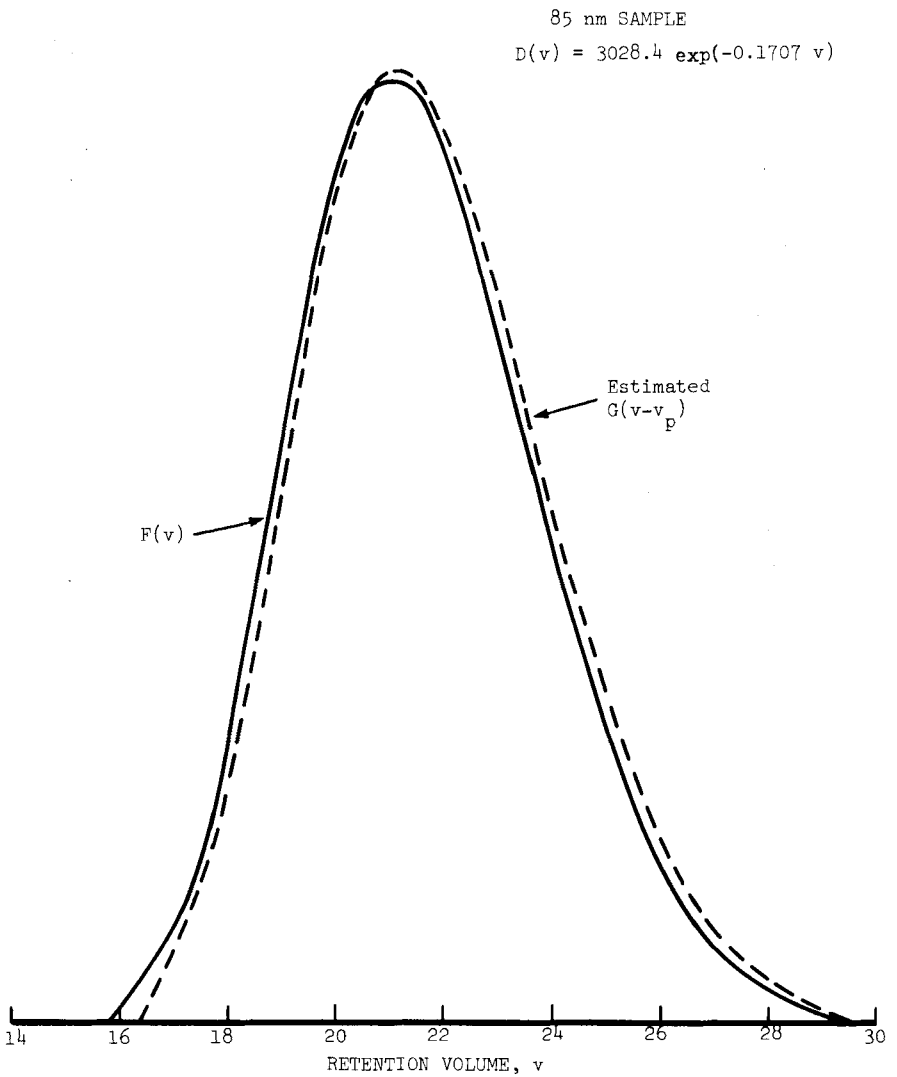
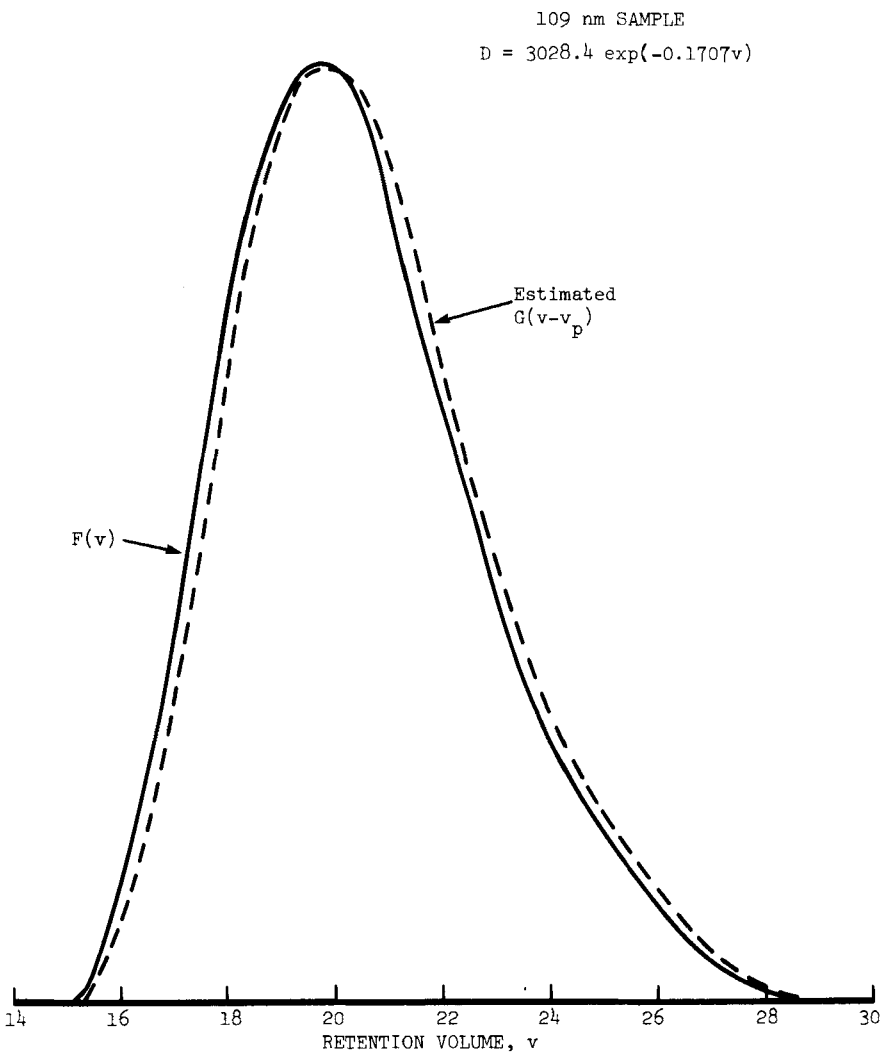


Figure 4. Estimation of the spreading functions from experimental chromatograms.



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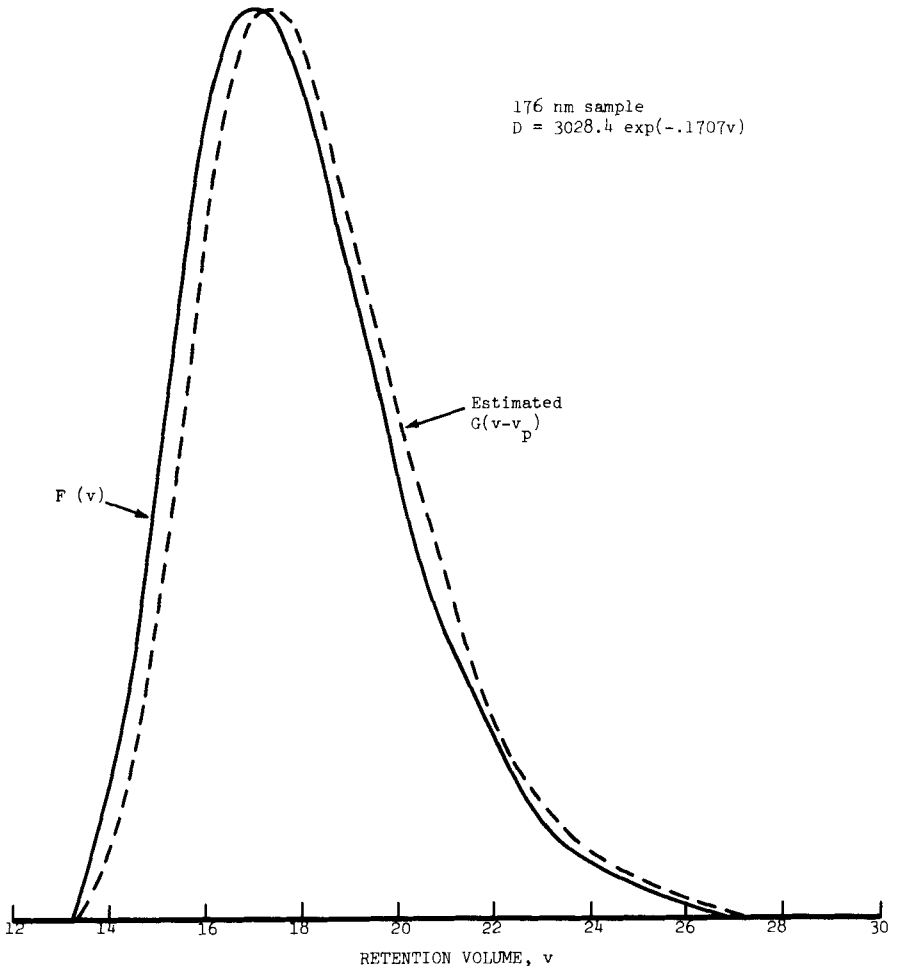

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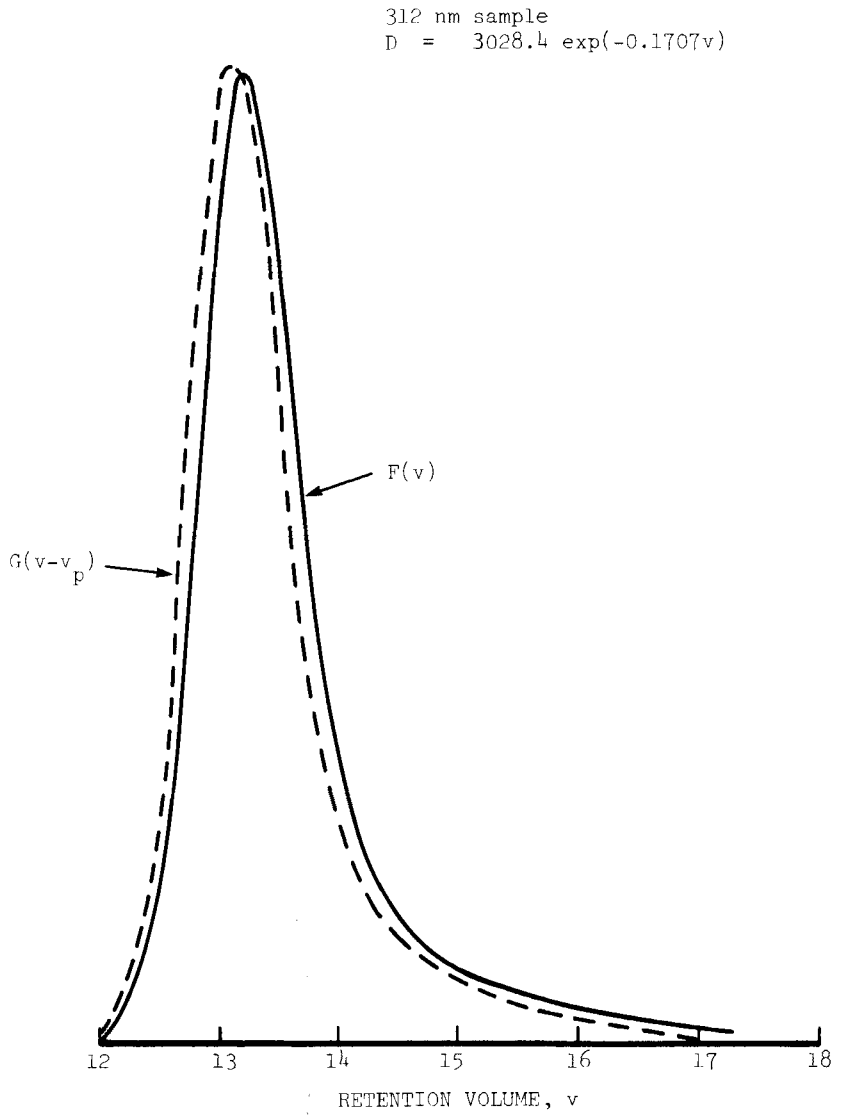

Figure 4 (Continued).

latter. The shape function is given by,

$$G(v-y) = G_0(v-y)\left[1 + \sum_{n=3}^{\infty} \frac{A_n}{n!} H_n(x)\right] \quad (16)$$

where

$$G_0(v-y) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-\frac{(v-y)^2}{2\sigma^2}\right] \quad (17)$$

and

$$x = \frac{(v-y)}{\sigma} \quad (18)$$

$H_n(x)$ are the Hermite polynomials, σ^2 is the second moment of the spreading function about the mean retention volume y and the coefficients A_n are functions of the n^{th} order moments, μ_n of the spreading function about the retention volume y. The first two coefficients are of direct statistical significance and are given as,

$$A_3 = \frac{\mu_3}{\mu_2^{3/2}} \quad (19)$$

$$A_4 = \frac{\mu_4}{\mu_2^2} - 3 \quad (20)$$

The coefficient A_3 provides an absolute statistical measure of skewness while A_4 is a measure of the flattening or kurtosis of the spreading function.

A special case of equation (16) is called the Edgeworth series and is obtained when $A_6 = 10A_3^2$ and $A_n = 0$ for $n \geq 7$. In a subsequent paper, we develop an analytical solution to equation (1) (as applied to particle chromatography) using the Edgeworth series. Therefore we examine the fit of the spreading function data to this series rather than considering additional terms in equation (16).

Figure 5 illustrates the fit obtained for the 85 nm sample. The central portion is adequately represented while the low and high retention volume ends are respectively underestimated and

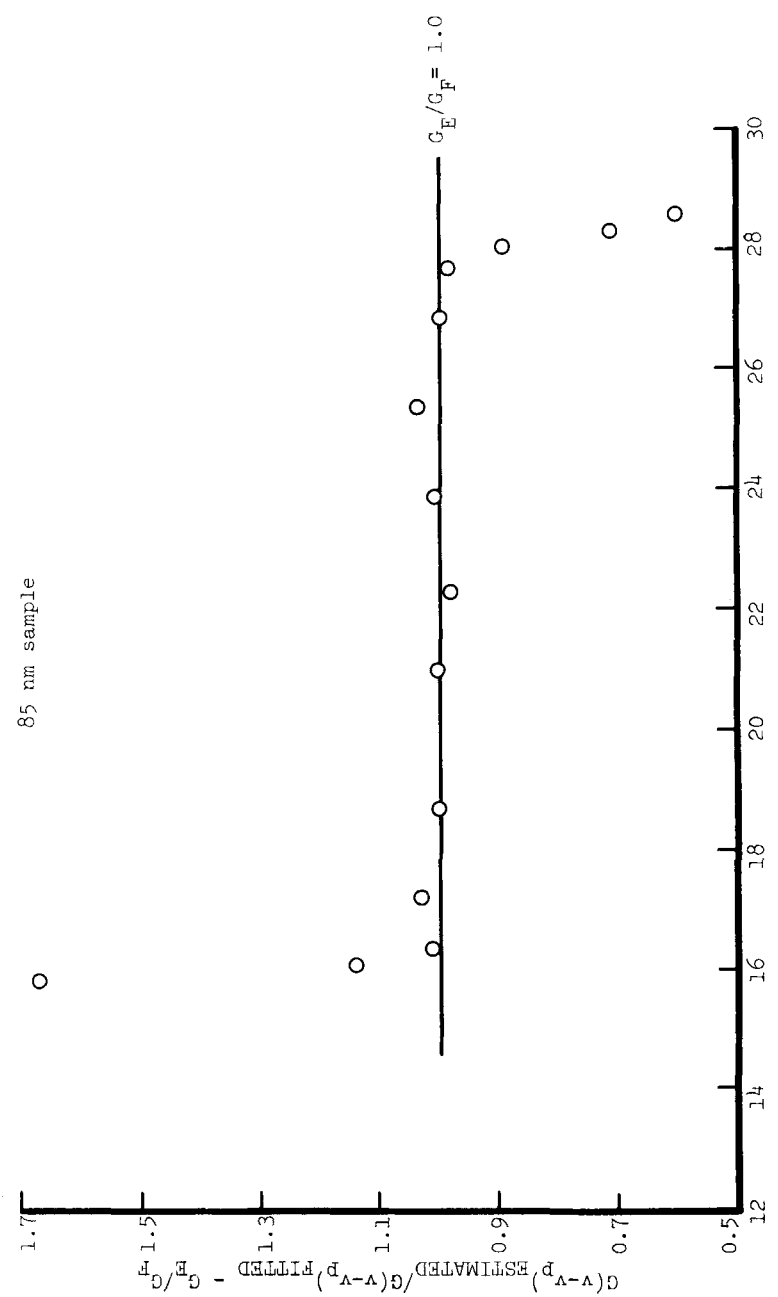

Figure 5. Fit of the estimated instrumental spreading to Provder and Rosen's shape function.

TABLE 1

Fit of the Spreading Function Data by the Edgworth Series. (G_E/G_F denotes the ratio of the estimated G(v-y) to the fitted G(v-y))

Sample	Span of the lower retention volume end where $G_E/G_F > 1.1$ (%)	Span of the central portion of the spreading function where $0.9<G_E/G_F<1.1$ (%)	Span of the high retention volume end where $G_E/G_F < 0.9$
85	4.1	79.6	16.3
109	6.2	73.0	21.8
176	12.4	66.2	21.4
220	10.3	70.0	19.7

overestimated. The fits for 109, 176 and 220 nm samples were similar, while that for the 312 nm sample was rather poor. Table 1 summarises the results of the fitting.

The values of σ^2, A_3 and A_4 for the spreading functions are given in Table 2. A similar trend is observed for all three entities, i.e. they indicate an optimum with retention volume. The trend indicated may be fortuitous since the data is rather limited. However, the method suggested in this paper can form the basis of a systematic investigation of the variation of these parameters with retention volume under a wide range of operating conditions.

TABLE 2

Parameters of the Statistical Shape Function

Sample	Peak Retention Volume	Variance σ^2	Skewness A_3	Kurtosis A_4
85	21.02	5.13	0.396	-0.0992
109	19.70	5.62	0.509	-0.0137
176	17.00	5.34	0.737	0.4830
220	15.35	3.58	0.716	0.0930

It should be stressed that the σ^2, A_3 and A_4 values calculated in the manner shown represent the actual second moment, skewness and kurtosis of the spreading function rather than manipulated parameters such as are obtained when searching for their values with the help of moment equations.

CONCLUSIONS

A numerical method has been developed and evaluated to estimate the instrumental spreading function in size exclusion chromatography. It requires the size distribution information of the injected standards. Such information can be obtained for particle standards by electron-microscopy. The numerical technique performs satisfactorily and is simple to apply. No restriction is placed on the form of the spreading function except that it be uniform.

A Gaussian function or the statistical shape function are frequently used to represent instrumental spreading. The calculated spreading function data allows an independent assessment of the adequacy of functions assumed to describe them. Also it enables their parameters to be estimated unambiguously.

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