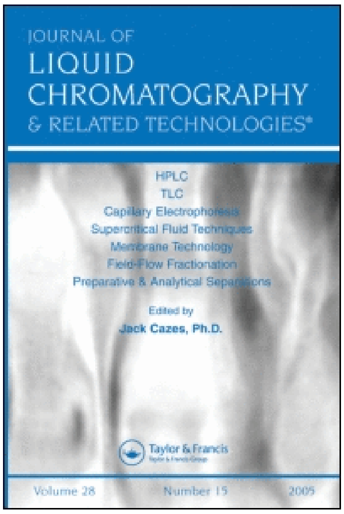

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Husain, A. , Vlachopoulos, J. and Hamielec, A. E.(1979) 'Chromatography of Suspensions, An Absolute Particle Size Detector Based on Turbidity-spectra Analysis - a Simulation Study', Journal of Liquid Chromatography & Related Technologies, 2: 4, 517 — 532

To link to this Article: DOI: 10.1080/01483917908060081

URL: http://dx.doi.org/10.1080/01483917908060081

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(4), 517-532 (1979)

CHROMATOGRAPHY OF SUSPENSIONS. AN ABSOLUTE PARTICLE SIZE DETECTOR BASED ON TURBIDITY-SPECTRA ANALYSIS - A SIMULATION STUDY

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ABSTRACT

In the chromatography of particle suspensions the use of a variable wavelength spectrophotometer can provide turbidity spectra continuously with retention volume. As suggested by Hamielec [1] this device could form the basis for an absolute particle size detector provided the size distribution in the detector cell could be represented by a known distribution function. In this work it is shown that the two parameter log-normal distribution function is an adequate representation of the detector cell contents. Further a computational scheme is outlined whereby the distribution parameters and the number of particles in the detector cell are calculated as a function of retention volume. It is then possible to obtain size distribution information of the original suspension.

INTRODUCTION

In the chromatography of submicron particle suspensions, axial dispersion phenomena is rather pronounced due in chief to the small diffusion coefficients of the particles. This requires very significant axial dispersion corrections to obtain absolute particle size distributions and particle diameter averages. To overcome this basic drawback, Hamielec[1] suggested the coupling of a chromatograph with an absolute detector such as one based on turbidity-spectra analysis. The latter requires a knowledge of the size distribution in the detector. It is reasonable to expect that this distribution would be narrow, unimodal and representable by a two parameter distribution function. Turbidity spectra analysis should then provide a measure of particle density in the cell along with the distribution parameters as a function of the retention volume, thus permitting the calculation of the particle size distribution of the original suspension.

In the present work, we describe a simulation study aimed at the identification of the aforementioned distribution function. We further show how the parameters of this distribution function may be evaluated and how this information leads to the size distribution of the sample.

THEORY

The instrument response F(v) to an input sample W(y) is given empirically by the integral equation:

$$F(v) = \int_{0}^{\infty} W(y)\, G(v,y)\, dy \qquad (1)$$

where G(v,y) is the instrumental spreading function which is frequently approximated by a Gaussian distribution:

$$G(v,y) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(- \frac{(v-y)^2}{2\sigma^2} \right) \qquad (2)$$

σ^2 is the variance of the instrumental spreading function which in general is a function of y.

As shown earlier[2] the frequency distribution of particle sizes in the cell of a turbidity detector at retention volume v, can be written as:

$$f(v,D)dD = -\frac{W(y)\ G(v,y)\ \{K(y)\ D^2(y)\}^{-1}\ dy}{\int_0^\infty W(y)\ G(v,y)\ \{K(y)\ D^2(y)\}^{-1} dy} \qquad (3)$$

where

f(v,D)dD is the fraction of the total number of particles at v in the size range D to D + dD.

K(y) is the extinction coefficient of particles eluting at retention volume y.

D(y) is the particle diameter-retention volume calibration curve.

For a turbidity detector, the instrument response F(v) at a wavelength λ in the suspending medium is also from light scattering theory given by:

$$F(v) = \frac{\pi}{4} N(v) \int_0^\infty K\ D^2\ f(v,D)\ dD \qquad (4)$$

where the extinction coefficient K for a given suspension is a function of $\frac{D}{\lambda}$. N(v) is the number of particles in the detector cell at retention volume v.

If as suggested by Hamielec[1], f(v,D) could be represented by a unimodal two parameter distribution function, then measurement of turbidity as a function of wavelength should in principle allow one to solve equation (4) for N(v) and the two parameters. It would then be possible to calculate the particle size distribution of the original suspension. The identification of the distribution function f(v,D) is done by fitting a known functional form to f(v,D) calculated from equation (3). In the absence of experimental data, F(v) is synthesized from a known W(y) for a number of wavelengths. The details of the simulation are presented next.

SIMULATION

To establish the functional form of f(v,D), one must be able to compare the calculated frequency distribution and relevant particle diameter averages from two independent methods. Equations (3) and (4) furnish means of doing this. The number, weight, surface, specific surface, volume and turbidity average diameters of the particles eluting at v are defined respectively as:

$$D_n(v) = \{\int_0^\infty D\ f(v,D)dD\} \tag{5}$$

$$D_w(v) = \{\int_0^\infty D^4 f(v,D)dD\}/\{\int_0^\infty D^3 f(v,D)dD\} \tag{6}$$

$$D_s(v) = \{\int_0^\infty D^2 f(v,D)dD\}^{\frac{1}{2}} \tag{7}$$

$$D_{ss}(v) = \{\int_0^\infty D^3 f(v,D)dD\}/\{\int_0^\infty D^2 f(v,D)dD\} \tag{8}$$

$$D_v(v) = \{\int_0^\infty D^3 f(v,D)dD\}^{1/3} \tag{9}$$

$$D_T(v) = \{\int_0^\infty D^6 f(v,D)dD\}^{1/3}/\{\int_0^\infty D^3 f(v,D)dD\}^{1/3} \tag{10}$$

The corresponding diameter averages for the entire suspension are given by:

$$\bar{D}_n = \left\{\int_0^\infty D_n(v)\ N(v)\ dv\right\}/\left\{\int_0^\infty N(v)\ dv\right\} \tag{11}$$

$$\bar{D}_w = \left\{\int_0^\infty D_w^4(v)\ N(v)\ dv\right\}/\left\{\int_0^\infty D_w^3(v)\ N(v)\ dv\right\} \tag{12}$$

$$\bar{D}_s = \left\{\int_0^\infty D_s^2(v)\ N(v)\ dv\right\}^{\frac{1}{2}}/\left\{\int_0^\infty N(v)\ dv\right\}^{\frac{1}{2}} \tag{13}$$

$$\bar{D}_{ss} = \left\{\int_0^\infty D_{ss}^3(v)\ N(v)\ dv\right\}/\left\{\int_0^\infty D_{ss}^2(v)\ N(v)\ dv\right\} \tag{14}$$

$$\bar{D}_v = \left\{\int_0^\infty D_v^3(v)\ N(v)\ dv\right\}^{\frac{1}{3}}/\left\{\int_0^\infty N(v)\ dv\right\}^{\frac{1}{3}} \tag{15}$$

$$\bar{D}_T = \left\{\int_0^\infty D_T^6(v)\ N(v)\ dv\right\}^{\frac{1}{3}}/\left\{\int_0^\infty D_T^3(v)\ N(v)\ dv\right\}^{\frac{1}{3}} \tag{16}$$

When calculating diameter averages from equation (3), it is to be recognized that N(v) is given by the integral in the denominator of equation (3).

The simulation is done by first assuming W(y) at wavelength λ_1 to be Gaussian with mean $\bar{Y}$ and variance σ_0^2. For a known value of σ^2 and particle diameter-retention volume calibration curve, the frequency distribution was calculated from equation (3) as a function of retention volume. The particle diameter averages were then calculated from equations (5-16). Since

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

where N(y) represents the number of particles with mean retention volume y, it follows that,

$$W_{\lambda_i}(y) = K_{\lambda_i}(y)\ W_{\lambda_1}(y)/K_{\lambda_1}(y) \tag{18}$$

Equation (18) can be used along with equation (1) to calculate $F_{\lambda_i}(v)$ at desired wavelengths. From equation (4) it follows that,

$$\frac{F_{\lambda_i}(v)}{F_{\lambda_1}(v)} = \frac{\int_0^\infty K_{\lambda_i} D^2 f(v,D)dD}{\int_0^\infty K_{\lambda_1} D^2 f(v,D)dD} \tag{19}$$

If f(v,D) is assumed to be log-normal (this assumption was found valid in several case studies made as shown later) given by,

$$f(v,D) = \frac{1}{\sqrt{2\pi\bar{\sigma}^2(v)D^2}} \exp\left\{-\frac{(\ln D - \mu(v))^2}{2\bar{\sigma}^2(v)}\right\} \tag{20}$$ [1]

where $\mu(v)$ and $\bar{\sigma}^2(v)$ are retention volume dependent parameters, then equation (19) can be written as,

$$\frac{F_{\lambda_i}(v)}{F_{\lambda_1}(v)} = \frac{\int_0^\infty K_{\lambda_i} D \exp\left\{-\frac{(\ln D - \mu(v))^2}{2\bar{\sigma}^2(v)}\right\}dD}{\int_0^\infty K_{\lambda_1} D \exp\left\{-\frac{(\ln D - \mu(v))^2}{2\bar{\sigma}^2(v)}\right\}dD} = f_i'(v) \tag{21}$$

If K_{λ_j} is represented by a polynomial in D, the integrals can be evaluated analytically leading to considerable simplification. However this cannot always be done without compromising accuracy. The parameters $\mu(v)$ and $\bar{\sigma}^2(v)$ are obtained from equation (21) by minimizing the following criteria:

$$\phi = \sum_{i=2}^{I} \left| \frac{F_{\lambda_i}(v)}{F_{\lambda_1}(v)} - f_i'(v) \right| \tag{22}$$

---

[1] The nth moment of the log-normal distribution function is given by $(n\mu + \frac{n^2\bar{\sigma}^2}{2})$.

where I is the total number of wavelengths scanned. Due to the negative slope of the calibration curve, the search is constrained by the requirement that

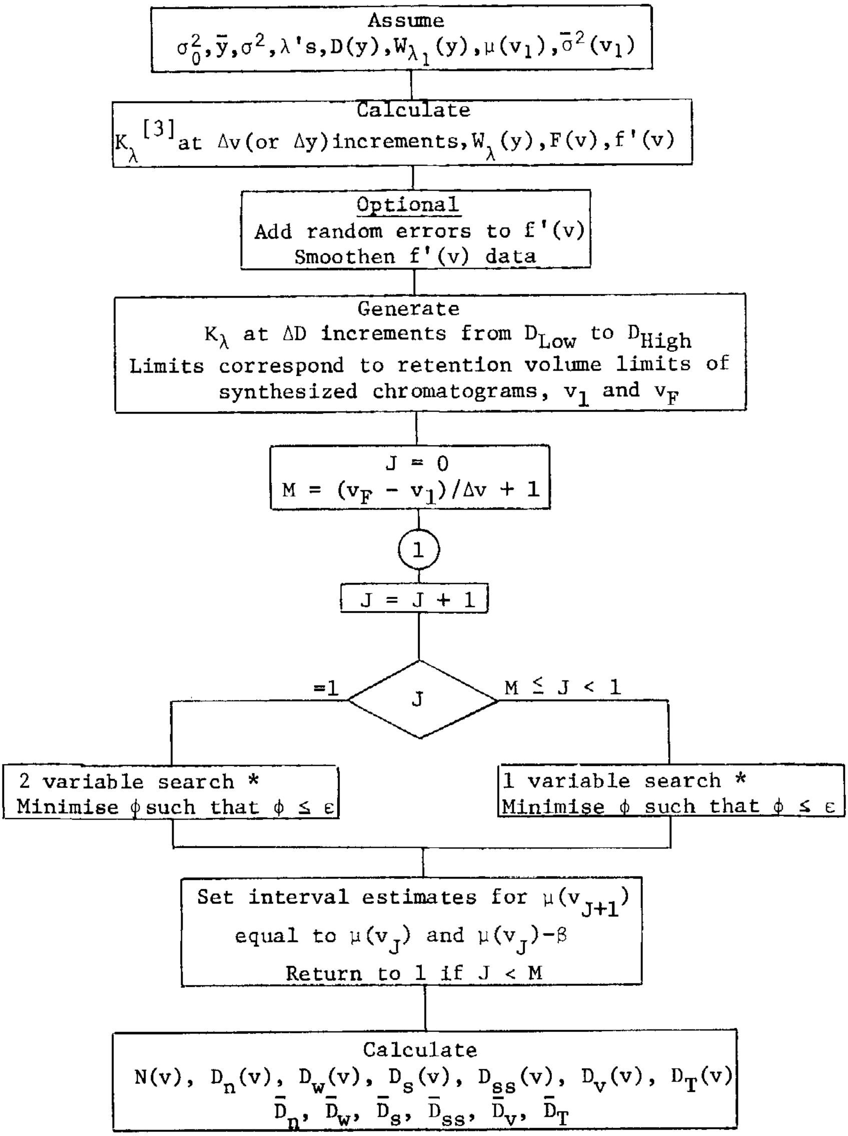
$$\mu(v_{i+1}) < \mu(v_i) \qquad \text{for } v_{i+1} > v_i \qquad . \tag{23}$$

Any suitable multivariable search method may be used. The search procedure used in this study was based on a Hook and Jeeves direct search method. Without any loss of accuracy, it was possible to assume in all cases that $\bar{\sigma}^2(v)$ is constant across the chromatogram. This significantly reduces computational effort requiring a two variable search only at the initial retention volume followed by a single variable search at all other retention volumes. The computational scheme is given in Figure 1.

RESULTS

Without any loss of generality, the particles were assumed to be nonabsorbing in the wavelength range scanned, 2540-4500 Å. The suspension was of polystyrene particles in aqueous media. Up to ten turbidity ratios were used in the minimization step. Cases analyzed were of particle suspensions in the range of application of liquid exclusion chromatography and capillary chromatography. Both linear and non linear calibration curves were treated. σ^2, the variance of the instrumental spreading function was varied across the chromatograms.

The results for Case 1 and all relevant simulation data are given in Table 1. When a two variable search for $\mu(v)$ and $\bar{\sigma}^2(v)$ was performed at each count across the chromatogram, $\bar{\sigma}^2(v)$ was



* When calculating the integrals in equation (21), the integration is done between appropriate cut-off diameters corresponding to current values of search variables.

Figure 1 Computer flow-sheet for calculating particle diameter averages.

TABLE 1. Simulation Results for Case 1 (Linear Calibration Curve)

$\sigma_0^2 = 1.25\ \text{count}^2$ $\bar{y} = 11.5\ \text{count}$ $\sigma^2 = 0.75\ \text{count}^2$ $\bar{\sigma}^2(v) = 0.0405$ $D = 36584.0 \exp(-0.295\ y)$

		$D_n(v)$		$D_w(v)$		$D_s(v)$		$D_{ss}(v)$		$D_v(v)$		$D_T(v)$	
v	$\mu(v)$	Eqn(3)	Eqn(22)	Eqn(3)	Eqn(22)	Eqn(3)	Eqn(22)	Eqn(3)	Eqn(22)	Eqn(3)	Eqn(22)	Eqn(3)	Eqn(22)
7	7.74	2448	2336	2772	2638	2500	2384	2661	2533	2552	2433	2884	2747
8	7.61	2022	2059	2296	2325	2066	2101	2201	2233	2110	2144	2395	2421
9	7.42	1668	1704	1896	1924	1704	1739	1817	1848	1741	1775	1978	2004
10	7.25	1378	1438	1564	1623	1407	1467	1499	1559	1437	1497	1632	1691
11	7.10	1142	1238	1293	1398	1166	1264	1241	1343	1190	1290	1348	1456
12	6.88	945	997	1072	1126	965	1017	1028	1081	986	1038	1118	1172
13	6.65	777	791	885	894	795	808	848	858	812	824	924	930
14	6.48	639	668	728	754	653	681	697	724	667	695	760	785
15	6.30	528	554	598	626	539	565	574	601	550	577	625	651
16	6.04	449	429	498	485	456	438	480	465	464	447	518	505

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	$\bar{D}_T$
Equation (3)	767	1086	812	966	860	1222
Equation (22)	792	1131	838	1001	889	1275
% Deviation	3.3	4.1	3.2	3.6	3.4	4.3

found to oscillate about a mean value. Therefore it was decided to treat $\bar{\sigma}^2(v)$ as a constant across the chromatogram the value of which was determined by a two variable search at the starting count. All cases dealt with in this paper were similarly analysed. As shown in Table 1 there is a consistent decrease in the value of $\mu(v)$ from 7.74 at count 7 to 6.04 at count 16. This corresponds to the negative slope of the calibration curve which in this case is linear. The assumption of log-normal distribution in the detector cell appears to be extremely well validated as shown by good agreement between diameter averages (at each count and overall) calculated with this assumption and those computed from equation (3). This is further illustrated in Figure 2 where the diameter distribution is plotted.

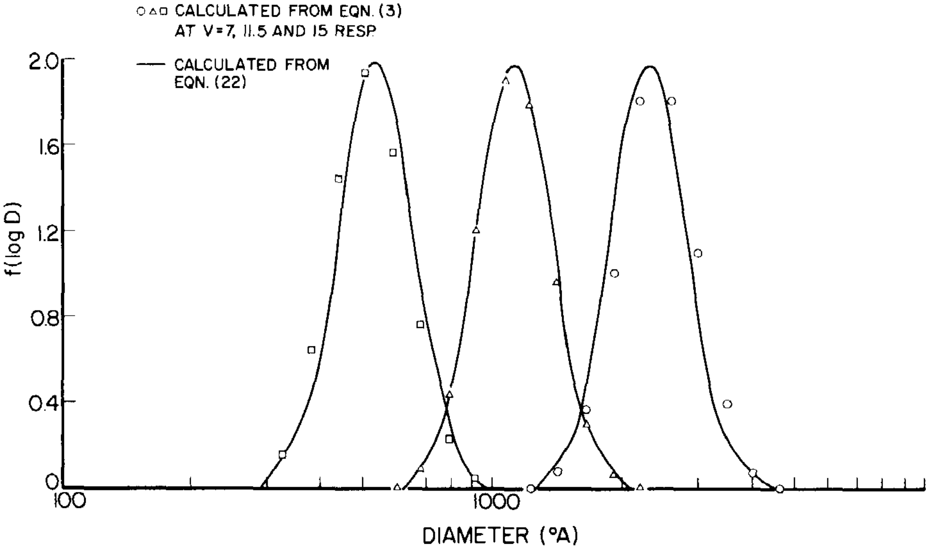


Figure 2 Size distribution calculated as a function of retention volume.

It would seriously limit the applicability of this method if the search procedure was overly sensitive to initial parameter estimates. To this end computations were done with varying initial parameter guesses and this fortunately does not seem to be the case. Poor guesses can however lead to a completely different solution and the defect cannot be remedied by increasing the number of wavelengths scanned. One intuitively would expect that for a given instrument calibration and a given G(v,y), $\bar{\sigma}^2(v)$ would be uniquely determined. Unfortunately this is not true with $\bar{\sigma}^2(v)$ being dependent on the input sample.

In Figure 3 are shown some calculated individual species chromatograms. (Only portions of the chromatograms are drawn,

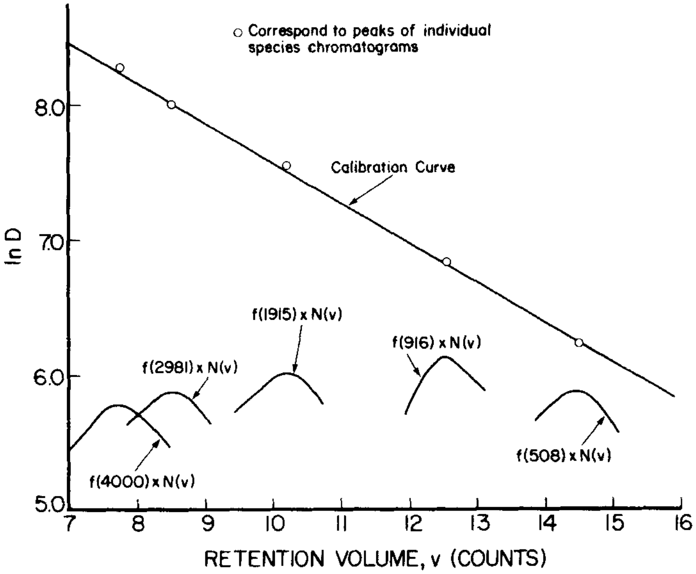


Figure 3 Plot showing agreement of calculated peak retention volumes with assumed calibration curve.

sufficient to identify the peak retention count. Curves are not drawn to scale). A point on the chromatograms is calculated from the product f(v,D) × N(v) for a known D. When the peak retention volumes are plotted against corresponding diameters, the points lie close to the assumed calibration curve. It is suggested that this procedure be used to ascertain the correctness of the solution.

Table 2 summarises the results for Cases 2 and 3. These differ from Case 1 in the magnitude of the particle diameters. The data for Case 3 is typical of capillary chromatography, the retention volume abscissa being replaced by time for convenience. It is interesting to note from Case 2 that a non-uniform σ^2 has

TABLE 2 Simulation Results for Cases 2 and 3
(Linear Calibration Curve)

Case 2 $\sigma_0^2 = 1.25$ count^2 $\bar{y} = 11.5$ count $\sigma^2 = 0.75$ count^{2*}

$D \approx 8 \times 10^4 \exp(-0.295y)$

* Bracketted number obtained when σ^2 was varied linearly from 0.75 (v = 7) to 1.25 (v = 16) count^2.

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	$\bar{D}_T$
Equation 3	1814	2587	1921	2291	2037	2929
	(1822)	(2593)	(1928)	(2297)	(2043)	(2935)
Equation 22	1789	2669	1911	2332	2042	3053
	(1760)	(2707)	(1888)	(2340)	(2028)	(3132)
% Deviation	1.4	3.2	0.5	1.8	0.2	4.2
	(3.4)	(4.4)	(2.1)	(1.9)	(0.7)	(6.7)

Case 3 $\sigma_0^2 = 1.25$ sec^2 $\bar{y} = 905$ sec σ^2 varied linearly from 30 (t=855) to 45 (t=955)sec^2 $D = 2.56\times10^{13}\exp(-0.0248\ t)$

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	$\bar{D}_T$
Equation 3	3579	4579	3728	4216	3884	4975
Equation 22	3616	4645	3769	4271	3929	5056
% Deviation	1.0	1.4	1.1	1.3	1.2	1.6

little effect on calculated diameter averages. Evident from the table is the excellent agreement between diameter averages computed from equations (3) and (22).

Cases 4 and 5 involving nonlinear calibration curves are given in Table 3. In Case 4 nonlinearity in the calibration curve is restricted to the higher diameter end while it occurs at both ends in Case 5. Imperfect resolution can lead to bimodal distribution in the detector cell even when the input sample is unimodal. This was observed at higher retention volumes for Case 5 which explains the larger discrepancy between calculated $\bar{D}_n$, $\bar{D}_s$ and $\bar{D}_v$ from equations (3) and (22). These averages are more sensitive to the smaller diameter particles and hence suffer due to the inadequate

TABLE 3 Simulation Results for Cases 4 and 5 (Nonlinear Calibration Curve)

Case 4 $\sigma_0^2 = 1.25$ count^2 $\bar{y} = 11.5$ count $\sigma^2 = 0.75$ count^2

$$D = 36584 \exp(-0.295y) + 3 \times 10^{12} \exp(-3y)$$

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	D_T
Equation 3	767	1086	812	966	860	1223
Equation 22	763	1134	814	991	869	1295
% Deviation	0.5	4.4	0.3	2.6	1.0	5.9

Case 5 $\sigma_0^2 = 1.25$ count^2 $\bar{y} = 11.5$ count $\sigma^2 = 0.75$ count^2

$$D = 1.75 \times 10^6 \exp(-0.8543y) + 3.51 \times 10^3 \exp(-0.0531y) - 1.521 \exp(0.4138y)$$

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	$\bar{D}_T$
Equation 3	1537	1773	1587	1706	1629	1844
Equation 22	1357	1743	1413	1601	1473	1900
% Deviation	11.7	1.7	11.0	6.2	9.6	3.0

quate representation of the particle distribution function by a log-normal distribution function at the lower diameter end. This was not observed with Case 4 where agreement is better and similar to cases analysed earlier.

To evaluate the robustness of the search procedure when the data have experimental error, random normal errors with zero mean and standard deviations σ_e were added to the turbidity ratio data of Case 1. The data was subsequently smoothened using a data smoothing routine. The data is shown in Figure 4 for two values of σ_e. Tabulated results in 4 indicate substantially the same values of diameter averages as for Case 1.

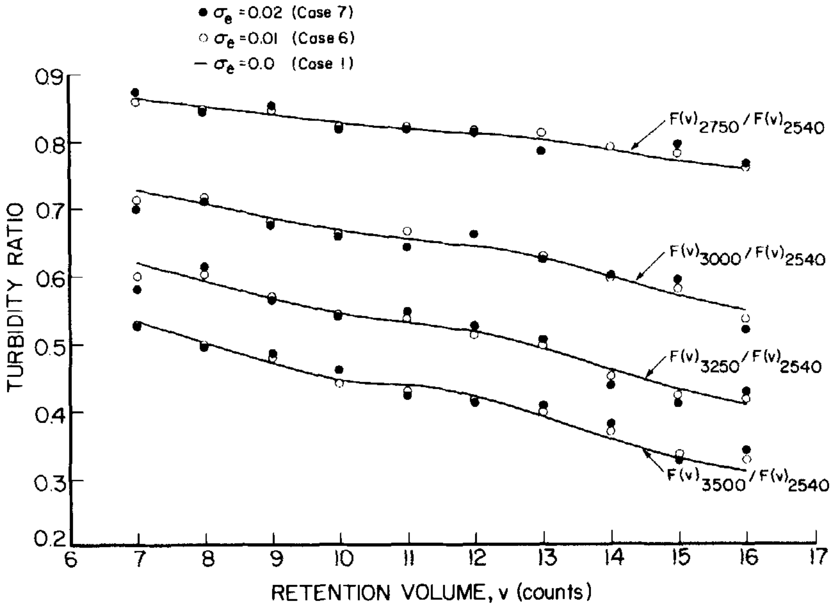


Figure 4 Turbidity ratio data for Cases 1, 6 and 7.

TABLE 4 Effect of Random Error in Turbidity Data on Diameter Averages

	$\bar{D}_n$	$\bar{D}_w$	$\bar{D}_s$	$\bar{D}_{ss}$	$\bar{D}_v$	$\bar{D}_T$
Case 1 ($\sigma_e = 0$)	792	1131	838	1001	889	1275
Case 6 ($\sigma_e = 0.01$)	791	1110	835	988	883	1250
Case 7 ($\sigma_e = 0.02$)	798	1088	838	977	882	1215

DISCUSSION

In all the simulation cases, chromatograms at any one wavelength were derived from a Gaussian W(y) at $\lambda = 2540$ Å. The synthesized chromatograms do not retain the Gaussian form and at higher wavelengths become increasingly skewed towards the low diameter tail. Therefore the validity of the log-normal representation of the detector contents must not be linked with any specific form of a chromatogram and appears to be general. These comments apply to unimodal chromatograms when the resolution of the instrument is adequate. Caution must be excercised when the calibration curve is nonlinear.

Results for the cases discussed earlier do not differ significantly from those evaluated using a two variable search at each retention count. This is due to the relatively large magnitude of the parameter $\mu(v)$ compared to $\bar{\sigma}^2(v)$ as indicated for Case 1 so that a small discrepancy in the value of $\bar{\sigma}^2(v)$ has little effect. This is indeed a useful approximation and results in considerable savings of computation time. Typical computing time (CDC 6400) for the reported cases was in the order of 300-400 secs.

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