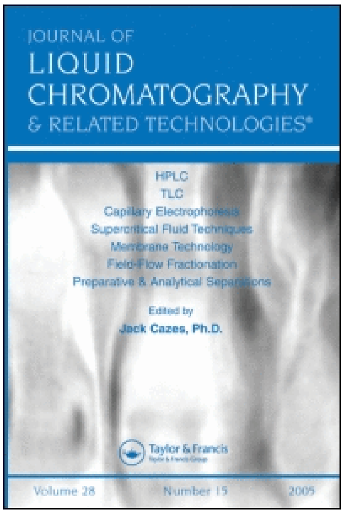

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Proposed Instrumental Spreading Shape Function (ISF) as Applied to Size Exclusion Chromatography (SEC)

S. N. E. Omorodion[a]; A. E. Hamielec[b]
[a] Department of Chemical Engineering, University of Benin, Benin City, Nigeria [b] McMaster Institute of Polymer Production Technology McMaster University Hamilton, Ontario, Canada

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PROPOSED INSTRUMENTAL SPREADING SHAPE FUNCTION (ISF) AS APPLIED TO SIZE EXCLUSION CHROMATOGRAPHY (SEC)

S.N.E. OMORODION*[1] AND A.E. HAMIELEC[2]
[1]*Department of Chemical Engineering*
University of Benin
Benin City, Nigeria
[2]*McMaster Institute of Polymer*
Production Technology
McMaster University
Hamilton, Ontario
Canada L8S 4L7

SYNOPSIS

Herein is proposed a new statistical ISF. This function which is gaussian and symmetric in shape, is an infinite series. The series is described by two parameters, instrument peak dispersion coefficient, σ^2, and a new coefficient, a polyplatykurtic coefficient A_k. The two parameters combine to form a peak broadening parameter, X. This new shape function seems to fall within the expectation of several authors in the past(1-11). This series was obtained during studies involving aqueous SEC with series of dextran standards. This paper is a continuation of a series of papers on these investigations (12,13)

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* Corresponding author

INTRODUCTION

Some twenty years ago, Tung and others(1,14-18), discussed the problem of peak dispersion correction in detail and gave answers on how to make adequate correction for dispersion effect. Tung, in particular, proposed a peak dispersion equation which relates an experimental chromatogram with the true molecular weight distribution (MWD) function, assuming that a monodisperse sample would give a Gaussian-shape chromatogram and the chromatogram of a polydisperse sample would be a composite of the Gaussian curves of all its constituents. During the overalapping process, the overall flattening process of the chromatogram which will depend on the polydispersity of the sample, has never been given adequate and proper considerations.

A great deal of work has been done to establish the major fundamental parameters in SEC with varying degrees of success. The importance of the slope (D2) of the calibration curve as a measure of peak separation has long been established. The existence and importance of peak dispersion phenomenon has also been accepted. It is well known to be an inherent phenomenon, but its relation to molecular weight (MW) or retention volume, or polydispersity or the nature of the system has not been fully well understood. $\sigma^2 = 0$ has always been a reflection of infinite resolution of SEC separation. From statistical considerations, it should be independent of the slope of the molecular weight calibration curve and the sample polydispersity(1,4). The Kurtosis phenomenon has not been given the attention it deserves. This coefficient is usually a measure of the excess flatness or thinness of the chromatogram peak compared to that of a Gaussian curve. It has been reported to be strongly related to peak broadening effects especially at very low flow-rates(19).

Skewing phenomenon has been extensively studied(18,20-26) in past years. Skewed chromatograms have been known to be produced under conditions where the experimental variables have been at extreme conditions of operation. These include high flow-rate or short residence times, increased viscosity either due to high molecular weight species, concentration effects or improper selection of mobile-phases or column overloading, poor resolution at the high or low MW ends of the calibration curve. Thus, unlike peak dispersion and Kurtosis phenomena which cannot be eliminated by control of the experimental variables, skewing can be eliminated under well controlled conditions. However, it still remains to be shown whether skewing of single chromatogram rather than the overall chromatogram of a polymer, is as fundamentally important as other parameters.

The newly proposed shape function was found to correlate excellently with the data based on the Two Broad Standard TBS(12,13,27) method of MW calibration. The five SEC multi-column systems employed are contained in Table 1. They are S4BR, S5CR, S5ER, S5FR and S6BR. These codings employed have been described before(13,27). They are all in series of 4, 5, 5, 5 and 6 columns respectively, arranged with the mobile-phase entering from the largest pore size column (R) as opposed to the conventional method of column arrangement. The order of column arrangement are shown in the table. The molecular weight data of the dextran standards employed are shown in Table 2. Experimental details of the operation of the SEC system have been described before(13,27,28).

THEORY

Several shapes have been assumed to describe the instrumental spreading function. These include Gaussian and different types of

TABLE 1: DESCRIPTION AND OPERATING CONDITIONS FOR CASE STUDIES FOR DEXTRAN(a)

Case Study	Code No. (b)	Columns Combined in Series	Length of Column (ft)	Flow-rate (ml/min)	Conc. of Injection (wt.%)
1	S4BR(c)	729/700, 700/500/370, 240/120A°, 120/88A°	15.58	4.50	0.05
2	S5CR(c)	729/700, 700/500/370, 240/120, 120/88, 88A°	16.75	4.50	0.05
3	S5ER	729/700, 700/500/370, 370/327, 240/120, 88A°	18.08	4.50	0.05
4	S5FR(d)	729/700, 700/500/370, 370/327, 240/120, 125A°	18.41	4.50	0.05
5	S6BR	729, 700, 700/500/370, 370/327, 240/120, 120/88, 88A°	20.75	4.50	0.05

(a) Mobile-phase - 0.05MKF/0.02 wt.% NaN_3/1.0 gm/24 lit. Tergitol/1.0% CH_3OH (pH = 6.6).

(b) The coded form is used and will be preserved, since it may be used to illustrate other phenomena in SEC. The first letter S stands for series combination. The numbers next to S represent the number of columns combined in series. The letter next to the numbers, identifies the system in question and the last letter, R or C identifies the order of column arrangement. R is for reversed order. This begins with the largest pore-size, followed by the next pore-size in size in the decreasing order down to the smallest pore size, instead of the traditional order of column arrangement which begins with the smallest pore size - the conventional method C.

(c) These systems have MW gaps. The intermediate 370/327 A° pore size column which is present in the other systems is not used for these two systems.

(d) Irregular pore-size colum arrangement at the end of the multi-column combination (i.e. small pore-size end).

TABLE 2: BROAD MWD POLYDEXTRAN STANDARDS EMPLOYED WITH LINEAR CALIBRATION METHODS*

Sample	$\bar{M}_N \times 10^{-3}$	$\bar{M}_w \times 10^{-3}$	$\bar{M}_w/\bar{M}_N$	$\bar{M}_{rms} \times 10^{-3}$
T500	173.0	509.0	2.94	297.0
T250	112.5	231.0	2.05	161.2
T150	86.0	154.0	1.79	115.1
T110	76.0	106.0	1.39	89.8
T70	42.5	70.0	1.65	54.5
T40	28.9	44.4	1.54	35.8
T20	15.0	22.3	1.49	18.29
T10	5.7	9.30	1.63	7.28

* Supplied by Pharmacia Limited

non-symmetric shape-functions. Based on the fundamental principles of SEC, the proposed stasistical shape function of Provder and Rosen(17) is indeed important. The statistical shape function which accounts for deviation from the Gaussian shape has the form:

$$G(v,y) = G_o(v-y) + \sum_{n=3}^{\infty}(-1)^n \frac{A_n}{n!} \frac{G_o^{\,n}(v-y)}{(\sqrt{2h})^n} \quad ---- \quad [I-1]$$

where $G_o(v-y) = \frac{\sqrt{h}}{\pi} \exp\{-h(v-y)^2\}$ is the Gaussian part of the distribution and $G^n(v-y)$ denotes its nth order derivative, h is the resolution factor defined as $h = \frac{1}{2\sigma^2}$ and σ^2 is the variance of a single species chromatogram or the peak dispersion coefficient. The coefficients A_n are the function of μ_n, the n-th order moments about the mean retention volume μ_1 of the observed SEC chromatograms. Provder and Rosen, for practical purposes truncated the series at the third term neglecting A_5, A_7, A_8, ... and set A_6 equal to $10A_3^2$. This gave a model with three parameters σ^2, A_3 and A_4 as follows

$$G(v-y) = G_o(v-y) \{ 1 + \frac{A_3 \; H_3(v-y)}{6\sqrt{\sigma^2}} + \frac{A_4}{24} H_4 [\sqrt{\sigma^2}(v-y)] \} \quad \text{-----} \quad [I-2]$$

where $H_3[x] = x^3 - 3x$

$H_4[x] = x^4 - 6x^2 + 3$

The coefficients A_3 and A_4 are related to the moments as:

$$A_3 = \frac{\mu_3}{(\sigma^2)^{3/2}}, \quad A_4 = \frac{\mu_4}{(\sigma^2)^2} - 3 \quad \text{-------------------} \quad [I-3]$$

The coefficient A_3 or μ_3 provides a measure of skewness. A_3 can be zero, positive or negative. Finite value of A_4 gives a symmetrical distribution and provides a statistical measure of the flattening or Kurtosis of the chromatogram of the ideal monodisperse standard. The Kurtosis coefficient measures the excess flatness or thinness of the chromatogram peak compared to that of a Gaussian curve. A_4 can be zero (Gaussian), positive (leptokurtic) or negative (platykurtic).

In the absence of skewing, Equation [I-2] becomes:

$$G(v-y) = G_o(v-y) \, . \, (1 + \frac{A_4}{24} \sigma^4 \frac{[d^4 G_o(v-y)]}{dV^4})$$

$$= G_o(v-y) \, . \, (1 + x) \quad \text{-------------------------} \quad [I-4]$$

$$\text{where} \quad x = \frac{A_4 \sigma^4}{24} \frac{d^4 G_o(v-y)}{dV^4} \qquad -\infty < x < \infty \quad \text{-----------} \quad [I-5]$$

with corresponding analytical solutions from method of molecular weight averages given by:

$$\frac{\bar{M}_k(t)}{\bar{M}_k(app)} = \exp\left\{-\frac{(2k-3)D2^2\sigma^2}{2}\right\}\left[\frac{1+\frac{A_4}{24}[(k-2)^4 D2^4\ \sigma^4]}{1+\frac{A_4}{24}[(k-1)^4 D2^4\ \sigma^4]}\right] \text{ --- [I-6]}$$

where k = 1,2,3, correspond to number -, weight -, Z- average molecular weights. The subscript (t) refers to the instrumental spreading corrected or true molecular weight averages. The subscript (app) refers to the SEC or uncorrected MW averages. However, based on what is to be expected from chromatographic theories of SEC, Equation [I-4] is the first and second member of the following series equation, i.e. an approximate equation of equation [I-7].

$$G(v-y) = G_o(v-y) \,.\, \exp\left\{\frac{A_k}{24}\sigma^4 \frac{d^4G_o(v-y)}{dV^4}\right\}$$

$$= G_o(v-y) \,.\, \left[\sum_{n=1}^{\infty}\left(1+\frac{x^n}{n!}\right)\right] \text{ ------------------ [I-7]}$$

$$\text{with } x = \frac{A_k\ \sigma^4}{24}\ \frac{d^4G_o(v-y)}{dv^4} \qquad -\infty < x < 0 \text{ ------------ [I-8]}$$

where, A_k has replaced A_4 by virtue of the limitation imposed on x in equations [I-5] and [I-8] and the A_k is called the polyplatykurtic coefficient, since the newly proposed function (eqn. [I-7]) is an infinite series with only flattening of chromatogram. Unlike A_4, A_k can only be less than or equal to zero. Thus, when n = 1, equation [I-7] approximates to Provder and Rosen's proposed equation in the absence of skewing. Using the method of molecular weight averages, the analytical solutions of equation [I-7] for $\bar{M}_w$ and $\bar{M}_n$ are given by:

$$\frac{\bar{M}_w(t)}{\bar{M}_w(\mathrm{app})} = \exp\left\{\frac{-D2^2\,\sigma^2}{2}\right\} \cdot \exp\left\{\frac{-D2^4\,\sigma^4}{24}A_k\right\}$$

$$= \exp\left[\frac{-D2^2}{2}\left\{\sigma^2 + \frac{D2^2\,\sigma^4\,A_k}{12}\right\}\right] \qquad A_k \lesssim 0$$

$$= \exp\left\{\frac{-D2^2}{2}\,X\right\} = P_k \qquad \text{[I-9]}$$

where $X = \sigma^2 + \frac{D2^2\,\sigma^4}{12}A_k = X_{\bar{M}_w}$ $\qquad$ [I-10]

Similarly, $\frac{\bar{M}_n(t)}{\bar{M}_n(\mathrm{app})} = \exp\left\{\frac{D2^2\,\sigma^2}{2}\right\} \cdot \exp\left\{\frac{D2^4\,\sigma^4}{24}A_k\right\}$

$$= \exp\left\{\frac{D2^2}{2} \cdot X\right\} = P^{-1} \qquad \text{[I-11]}$$

where $X = \sigma^2 + \frac{D2^2\,\sigma^4}{12}A_k = X_{\bar{M}_N}$ $\qquad$ [I-12]

i.e. $X = X_{\bar{M}_n} = X_{\bar{M}_w}$ $\qquad$ [I-12a]

Equation [I-12a] is valid when both $\bar{M}_w$ and $\bar{M}_n$ of the standard samples are involved in obtaining the true MW calibration curve.

From Equations [I-9] and [I-11],

$$\frac{\bar{M}_w(t)}{\bar{M}_w(\mathrm{app})}\;\frac{\bar{M}_n(\mathrm{app})}{\bar{M}_n(t)} = \exp\{-D2^2\,X\}$$

or

$$\frac{P(t)}{P(\mathrm{app})} = \exp\{-\,D2^2\,X\} \qquad \text{[I-13]}$$

Where P(t) and P(app) are the true (or corrected) and apparent (or uncorrected) polydispersities respectively. Taking $\log_e$ of Equation [I-13], the following is obtained

$$X = -\frac{\ln(P(t))}{D2^2} + \frac{\ln P(\mathrm{app})}{D2^2} \qquad \text{[I-14]}$$

From equations [I-10] and [I-12]

$$X = \sigma^2 + \frac{D2^4\ \sigma^4\ A_k}{12\ D2^2}$$

$$= \sigma^2 + \frac{k_n}{D2^2} \quad \text{------------------------------} \quad [I-15]$$

where K_n is a negative dimensionless variable defined as

$$K_n = \frac{D2^4\sigma^4 A_k}{12} \quad \text{------------------------------} \quad [I-16]$$

k_n is a constant for a given polymer sample, when a true MW calibration curve is employed. According to equation [I-15],

(i) X is a function of two parameters σ^2 and A_k for a given SEC system.

(ii) For conditions of infinite or perfect resolution of the SEC separation, only X can be zero and not σ^2 since X is the instrument peak broadening parameter, except for monodisperse polymer sample, when $A_k = 0$. Under such conditions σ^2 for broad samples is given by

$$\sigma^2 = \frac{-12}{D2^2\ A_k} \qquad -\infty < A_k < 0 \quad \text{-------------} \quad [I-17]$$

(iii) Then when X is plotted against $D2^{-2}$ for any given sample one should expect a straightline with a slope of K_n and intercept of σ^2 to be obtained.

Meanwhile, in general, the analytical solutions to Equation [I-7] are:

$$\frac{\bar{M}_k(t)}{\bar{M}_k(\text{app})} = \exp\left\{\frac{-(2k-3)D2^2\sigma^2}{2}\right\} \exp\left\{\frac{-(2k-3)D2^4\sigma^4 A_k}{24}\right\} \quad \text{---} \quad [I-18]$$

and for any method of linear molecular weight calibration where one or both of $\bar{M}_n$ and $\bar{M}_w$ of at least two samples are employed, in general

$$X = \frac{X_{\bar{M}_w} + X_{\bar{M}_n}}{2} \qquad \text{[I-19]}$$

RESULTS AND DISCUSSIONS

The five Dextran/CPG-10 systems used here have been described before(13). The results of the application of the TBS and Effective Linear Calibration (ELC)(29) methods of calibration were presented therein. An ISF which was Gaussian in shape was found not to apply, since large negative values of σ^2 (which is now replaced by X) could not be tolerated. In evaluation of the methods of calibration, the ISF was found to be additionally symmetric in shape with no skewing(13). Tables 3 and 4 contain typical lists of X versus D2 values from the TBS methods of calibration for two of the five systems employed. Also, in this Table are X values from the ELC method of calibration. These ELC values were obtained using Equation [I-19]. For the case where a single broad MWD standard is used to obtain the MW calibration curve, X is meaningless. Where only the $\bar{M}_N$ of two standards are used $X_{\bar{M}_N} = 0$. Where only the $\bar{M}_w$ of two standards are used, $X_{\bar{M}_w} = 0$.

The plots of equation [I-15] are shown in Figures 1-5 for each of the five cases and for each polymer standard. The validity of the equation where all the plots are found to be perfectly and surprisingly linear is unquestionable. From these plots, the slopes (K_n) and intercepts (σ^2) for each sample were obtained and these are listed in Table 5. The σ^2 values which are now all positive are observed to be larger than expected and highly dependent on the system.

TABLE 3: Application of TBS and ELC methods to Case-study S5ER

Paired Samples	X Count)^2	D1 x 10^{-9}	D2 (count)$^{-1}$	P_x
T20 T250	-0.35 -1.43	0.538	0.289	1.015 1.062
T110 T500	0.42 -5.09	0.497	0.283	0.983 1.226
T150 T500	-0.33 -3.80	0.737	0.298	1.015 1.184
T70 T150	0.86 0.06	0.969	0.308	0.960 0.997
T20 T500	-0.14 -4.03	0.686	0.295	1.006 1.192
T40 T500	0.67 -4.57	0.579	0.289	0.972 1.210
T40 T250	0.24 -2.12	0.394	0.278	0.991 1.085
T70 T250	0.28 -1.25	0.587	0.292	0.988 1.055
T70 T500	0.69 -3.47	0.825	0.302	1.021 1.138
T20 T150	-0.17 0.51	0.655	0.294	1.083 1.121
T10 T500	-0.68 -2.00	1.451	0.324	1.036 1.111

ELC Method

	Double $\bar{M}_N$			Double $\bar{M}_W$			
Paried Sample	D2 (Count)$^{-2}$	X_{M_W}(count)^2	X (Count)^2	Paired Sample	D2 (Count)$^{-1}$	X_{M_N}(Count)^2	X(Count)$^{-2}$
T40 T70	0.233	4.18 6.02	2.09 3.01	T70 T150	0.323	2.69 1.24	1.35 0.62
T110 T250	0.190	9.22 25.18	4.61 12.59	T40 T250	0.293	1.64 1.64	0.82 0.82
T500 T150	0.236	22.05 8.52	11.03 4.26	T10 T500	0.329	-1.05 -3.34	0.53 1.67
T10 T20	0.516	-4.20 -5.80	-2.10 -2.90	T110 T250	0.288	1.10 -2.92	0.55 -1.46

TABLE 4: Application of TBS and ELC Method to Case-Study S6BR

TBS Method

Paired Samples	X (Count)^2	DI $\times 10^{-9}$	D2 (Count)$^{-1}$	P_X
T40	0.75	0.665	0.269	0.97
T250	-1.56			1.06
T20	-0.63	0.621	0.266	1.02
T150	-1.31			1.05
T40	0.80	0.690	0.270	0.97
T150	-1.12			1.04
T150	-1.38	0.598	0.265	1.05
T250	-1.82			1.07
T20	-0.64	0.616	0.266	1.02
T250	-1.75			1.06
T110	0.40	0.462	0.257	0.99
T250	-2.48			1.09
T10	-0.92	0.644	0.270	1.03
T70	0.40			0.99
T10	-0.54	0.902	0.278	1.02
T150	0.65			1.03
T150	0.61	2.141	0.305	0.97
T500	-2.24			1.11
T70	1.81	2.051	0.303	0.92
T500	-2.35			1.11

ELC Method

Paired Sample	Double $\bar{M}_W$			Double $\bar{M}_N$			
	D2 (Count)$^{-1}$	$X_{\bar{M}_N}$	X (Count)^2	Paired Sample	D2 (Count)$^{-1}$	$X_{\bar{M}_W}$	X (Count)^2
T10	0.301	1.15	0.58	T10		1.13	0.57
T20		0.80	0.40	T20	0.306	1.41	0.71
T40	0.237	-1.90	-0.95	T40	0.214	5.32	2.66
T70		-3.30	-1.65	T70		7.28	3.64
T110	0.304	3.66	1.83	T110	0.137	24.59	12.30
T150		1.15	0.58	T150		48.24	24.12
T250		7.43	3.72	T250		-.6.82	-3.41
T500	0.399	4.84	2.42	T500	0.247	-16.54	-8.27

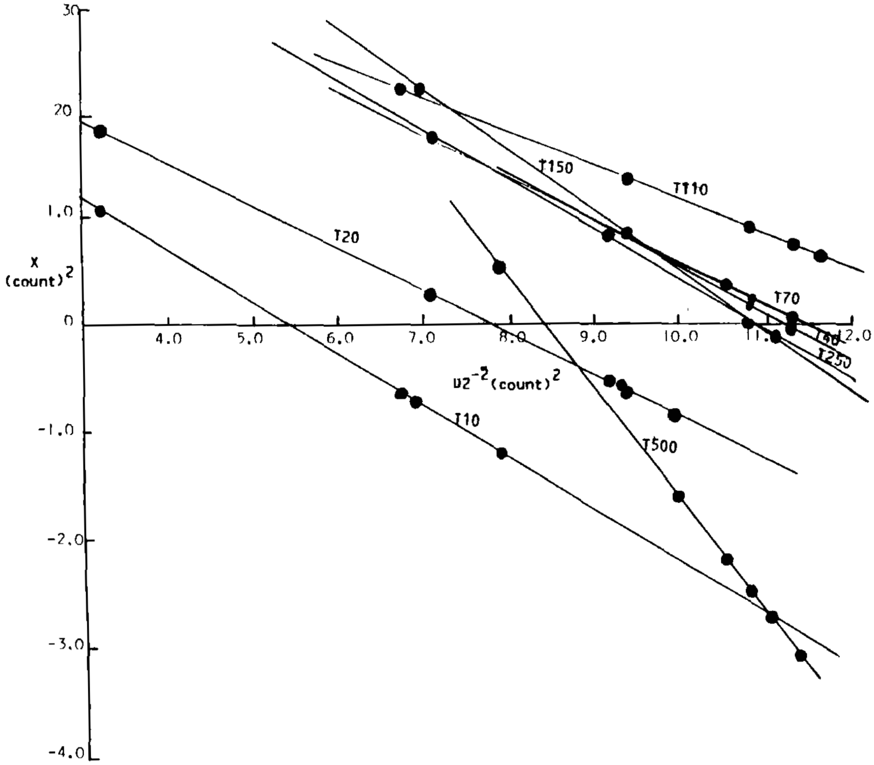

Figure 1. Overall peak broadening parameter (X) versus D2^{-2} for Dextran standards for case study # 1.

It is interesting to note that the K_n values are the same for all the SEC systems for each sample and as shown in Table 6, these values (with the exception of the high MW sample within the non-linear region of the linear MW calibration) are the $\log_e$ of the true polydispersities of the samples as supplied by the manufacturers. Therefore,

$$K_n = - \ln P(t) \qquad \text{------------------------} \qquad [I\text{-}20]$$

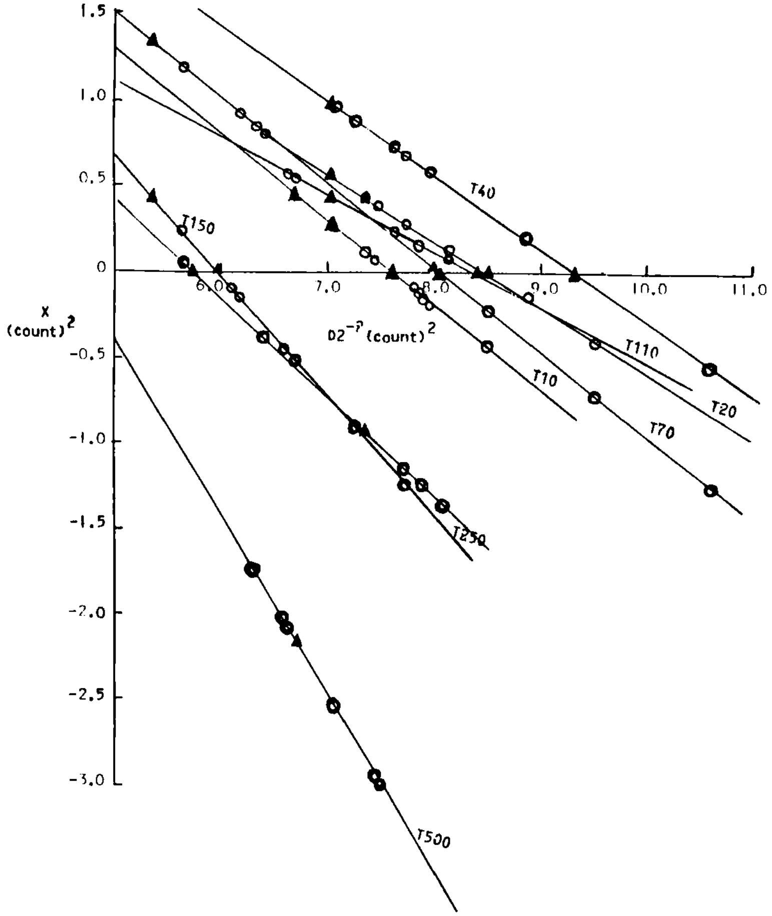

Figure 2. Overall peak broadening parameter (X) versus D2^{-2} for Dextran standards for case-study # 2. ▲ - based on ELC method; ● - based on TBS method.

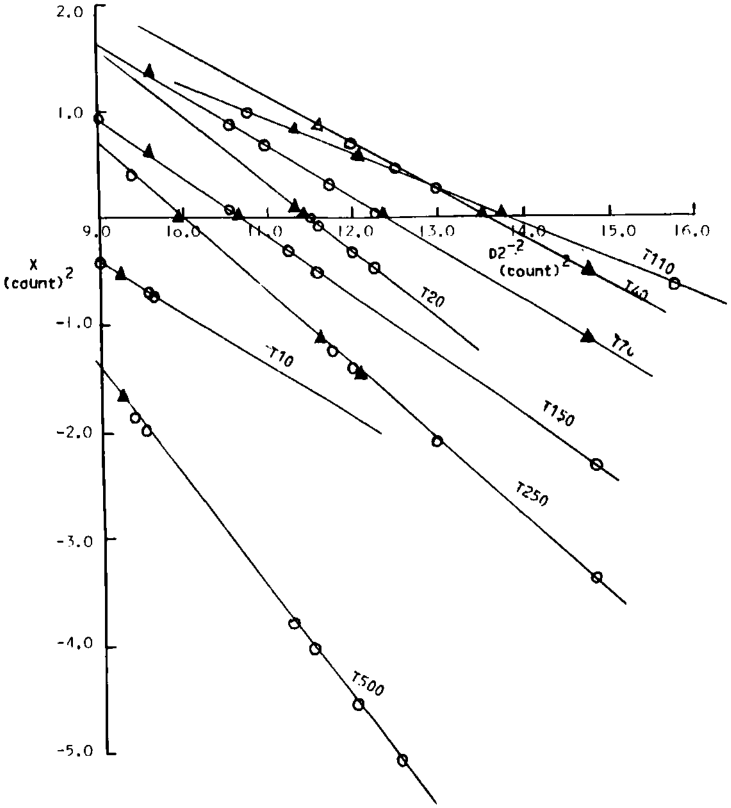

Figure 3. Overall peak broadening parameter (X) versus D2^{-2} for Dextran standards for case-study # 3. ▲ - based on ELC method; 0 - based on TBS method.

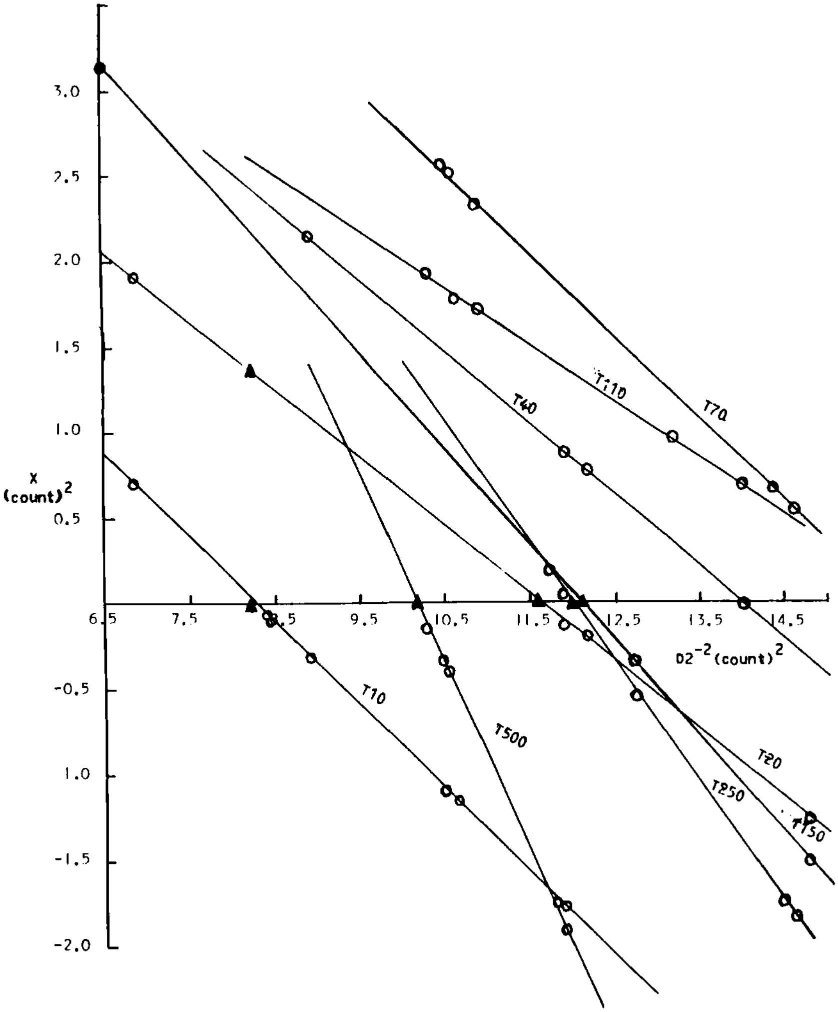

Figure 4. Overall peak broadening parameter (X) versus D2^{-2} for Dextran standards for case-study # 4. ▲ - based on ELC method; 0 - based on TBS method.

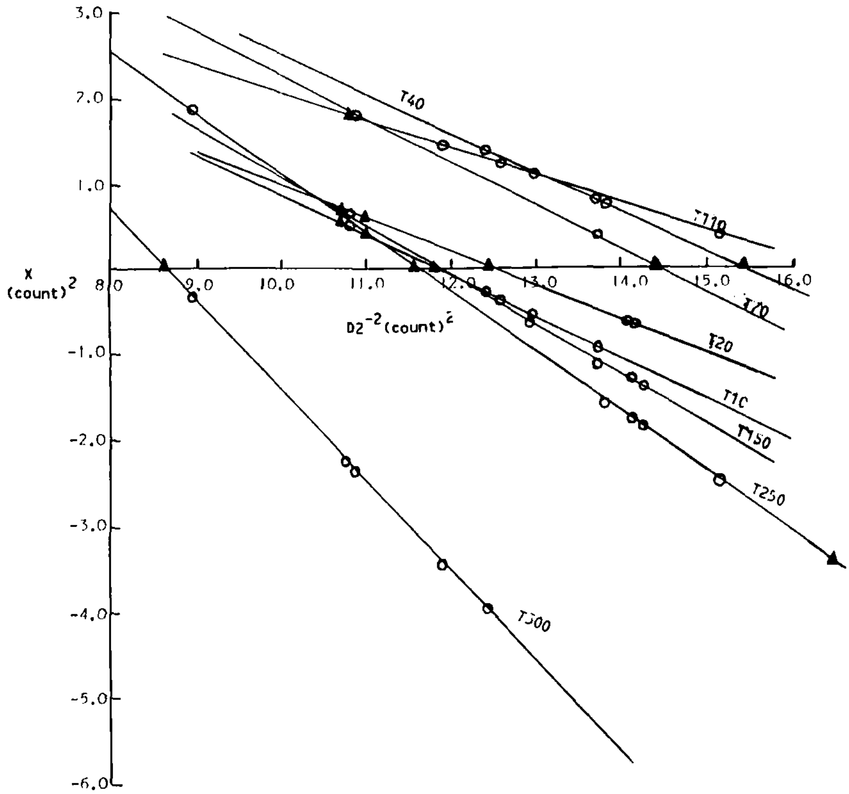

Figure 5. Overall peak broadening parameter (X) versus D2^{-2} for Dextran standards for case-study # 5. ▲ - based on ELC; O - based on TBS method.

Using equation [I-16], the A_k value for each sample was calculated and these are listed in Table 7 for all the systems. Just like the σ^2 values, nothing significant can be said now about the A_k values. However, Table 8 lists the molecular weight resolution correction with respect to peak dispersion, while those corresponding to polyplatykurtosis are listed in Table 9. In this table, the

TABLE 5: CALCULATED σ^2 (count)^2 AND K_n VALUES FOR EACH SYSTEM (FROM THE PLOTS)

System	S4BR		S5CR		S5ER		S5FR		S6BR	
Sample	σ^2	$-K_n$	σ^2	$-K_n$	σ^2	$-K_n$	σ^2	$-K_n$	σ^2	$-K_n$
T10	2.64	0.486	3.72	0.488	3.89	0.480	4.16	0.500	5.74	0.485
T20	3.08	0.395	3.34	0.393	4.43	0.399	4.66	0.395	4.99	0.392
T40	4.78	0.426	3.99	0.427	5.82	0.430	5.89	0.421	6.62	0.422
T70	5.58	0.490	3.98	0.494	6.03	0.490	7.74	0.493	7.23	0.498
T110	4.46	0.330	2.76	0.330	4.55	0.331	5.33	0.330	5.39	0.330
T150	6.21	0.573	3.35	0.582	6.15	0.575	6.74	0.570	6.97	0.589
T250	7.55	0.701	4.25	0.710	6.95	0.700	8.47	0.707	8.21	0.695
T500	8.93	1.060	4.98	1.061	8.15	1.060	10.80	1.067	9.27	1.065
*D2 (count)$^{-1}$	0.300		0.353		0.292		0.286		0.267	

*D2 is the slope of the true MW calibration curve obtained by averaging D2s in the linear region of the MW calibration curve.

square root of the true polydispersities (that supplied by the manufacturers) of the samples are also included for the purpose of comparison. From Table 8, it is apparent that peak dispersion phenomenon is indeed inherent, in view of the fact that the corrections are of the same magnitude for these dextran samples, varying slightly according to the quality of the system. Though the σ^2 for each system and sample seem to differ quite widely (Table 5), this is not reflected in Table 8, where peak dispersion corrections seem not to vary much from one system to the other. However, careful observation shows that peak dispersion corrections may be

TABLE 6: COMPARING CALCULATED K_o WITH THOSE SUPPLIED BY THE MANUFACTURES

Supplied by Manufacturers			Calculated
Sample	P(t)	ln P(t)	$-\text{Avg } K_n$
T10	1.63	0.489	0.488
T20	1.49	0.399	0.396
T40	1.54	0.432	0.425
T70	1.65	0.501	0.495
T110	1.33	0.329	0.330
T150	1.79	0.582	0.580
T250	2.05	0.718	0.703
T500	2.95	1.078	1.063

TABLE 7: POLYPLATYKURTIC COEFFICIENT VALUES OF DEXTRAN FOR THE DIFFERENT SYSTEMS

$-A_k$ Values					
Sample	S4BR	S5CR	S5ER	S5FR	S6BR
T10	103.86	27.29	53.30	50.64	35.02
T20	62.28	27.63	35.54	33.80	37.82
T40	28.00	20.96	21.04	22.32	23.27
T70	24.34	24.43	22.73	14.99	22.62
T110	22.34	30.44	23.92	18.94	24.38
T150	22.36	40.08	25.40	22.98	28.29
T250	18.22	30.38	23.92	17.68	24.35
T500	19.09	33.06	26.34	16.40	29.26

TABLE 8: PEAK DISPERSION RESOLUTION CORRECTION FOR EACH SYSTEM AND SAMPLE

$EXP(-D2^2\sigma^2/2)$					
Sample	S4BR	S5CR	S5ER	S5FR	S6BR
T10	0.888	0.793	0.847	0.844	0.815
T20	0.871	0.812	0.828	0.829	0.837
T40	0.807	0.780	0.780	0.786	0.790
T70	0.778	0.780	0.773	0.729	0.773
T110	0.818	0.842	0.824	0.804	0.825
T150	0.756	0.812	0.769	0.759	0.780
T250	0.712	0.767	0.744	0.707	0.746
T500	0.669	0.733	0.707	0.643	0.719

TABLE 9: POLYPLATYKURTIC MW RESOLUTION CORRECTIONS FOR EACH SYSTEMS AND SAMPLE

$EXP(-D2^4\sigma^4\ A_k/24)$						
Sample	S4BR	S5CR	S5ER	S5FR	S6BR	$\sqrt{P(t)}$
T10	1.277	1.277	1.276	1.277	1.278	1.277
T20	1.221	1.220	1.221	1.221	1.220	1.221
T40	1.242	1.241	1.240	1.241	1.241	1.241
T70	1.278	1.285	1.285	1.286	1.284	1.285
T110	1.180	1.179	1.180	1.179	1.179	1.179
T150	1.338	1.338	1.338	1.337	1.340	1.338
T250	1.420	1.413	1.426	1.420	1.423	1.432
T500	1.699	1.701	1.705	1.706	1.708	1.432

dependent on the SEC MWD of the polymer as well as the quality of the system.

In table 9, the corrections due to polyplatykurtosis are observed to be exactly the same for all the systems, varying only from one sample to another, unlike the A_k values. The excellent agreement between these values and $\sqrt{P(t)}$ for each sample clearly indicates the validity of the newly proposed ISF as far as SEC separation according to size is concerned. This implies that

$$\sqrt{P(t)} = \mathrm{EXP}(-D2^4\sigma^4 A_k/24) \quad \text{------------------------} \quad [I-21]$$

and on the basis of equation [I-20], equation [I-15] becomes

$$X = \sigma^2 - \frac{\ln P(t)}{D2^2} \quad \text{------------------------------} \quad [I-22]$$

When equation [I-22) is now compared with equation [I-14], σ^2 which is a peak dispersion coefficient (rather than axial dispersion coefficient) is given by:

$$\sigma^2 = \frac{\ln P(app)}{D2^2} \quad \text{-------------------------------} \quad [I-23]$$

Thus, σ^2 is virtually constant for any linear MW calibration curve of the form M(v) = D1 exp(-D2 V), for any sample's chromatogram.

SUMMARY

In summary, it has been shown that the ISF of chromatographed broad MWD dextran standards are symmetric as well as gaussian in shape. The solutions of TBS linear methods of theoretical MW calibration were found to fit the analytical solutions of the form of Equation [I-18] very perfectly. According to this equation, σ^2 can never be zero, unless under conditions of perfect resolution for a monodisperse molecular species. From the proposed ISF, it

was found that the conditions of infinite resolution for SEC separation is defined by the peak broadening parameter, X = 0, rather than $\sigma^2 = 0$, since σ^2 has been shown to be a strong function of the width of the experimental chromatogram for at least three different polymer standards, dextran(27), polyacrylamide(27,30) and sodium polystyrene sulfonate(27,31).
Equation [I-23] which has been derived from analysis of experimental data using the newly proposed ISF will be the subject of further investigations.

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