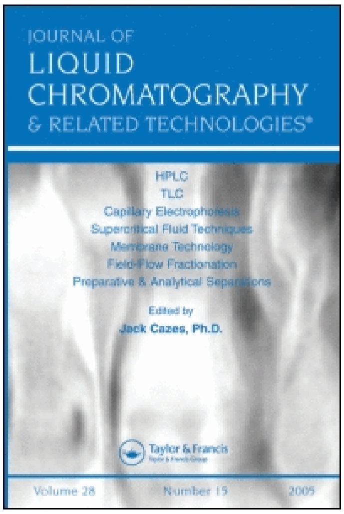

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Evaluation of Newly Proposed Instrumental Spreading Shape Function (ISF)

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EVALUATION OF NEWLY PROPOSED INSTRUMENTAL SPREADING SHAPE FUNCTION (ISF)

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SYNOPSIS

Herein is reported an evaluation of the newly proposed ISF[1]. Based on the shape function, it is now possible to estimate the peak dispersion coefficient of any sample given the chromatogram. To be able to apply the new concept to find sources of peak dispersion and provide a better understanding of other phenomena in Size Exclusion Chromatography (SEC), it is important to evaluate the concept in terms of reproducibility of estimated parameters. To this end, two dextran corning controlled porous glass (CPG-10) and one polyacrylamide/CPG-10 systems were chosen and the results are contained herein.

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INTRODUCTION

Evaluation of peak dispersion coefficients (σ^2) has been a very difficult task. Due to the difficulties involved and lack of adequate theories, it has either been usually neglected or assumed to be independent of molecular weight (MW) or retention volume, etc. during MW calibration/molecular weight averages calculation problems. Tung, Moore and Knight[2] were the first to develop an ingeneous technique for measuring peak dispersion coefficients - the "reverse-flow" technique. This method requires a special device which is not installed in usual GPC units. This method was developed with the use of monodispersed or very narrow MWD polymer standards. Therefore, for fairly narrow or broad MWD polymer standards, the method is not applicable. According to the physical interpretation of the newly proposed ISF, the flattening process which becomes very predominant as the polydispersity of the sample increases, cannot be eliminated whether the flow is reversed or not.

Due to the time-consuming and sometimes difficult procedures of the reverse-flow technique, several other methods have been proposed and used. Some of the older methods include curve fitting procedure[3], statistical consideration of variance[4,5), and Once-through technique of Hamielec and co-workers[6]. Of most of these methods, the most promising has been the Once-through technique, which makes use of the analytical solutions of Hamielec and Ray[7] of Tung's peak dispersion equation[8]. However, Tung and Runyon[3] and Kotaka[9] claimed that the method was not too accurate, due to the sensitiveness of σ^2 to the slope of the MW calibration curve. From statistical considerations, any adequate method of peak dispersion estimation should not depend on the slope of the MW calibration curve alone or on the true polydispersity of the sample.

In a recent work in the series[1], due to the symmetric form of the proposed ISF, a mathematical expression which relates peak dispersion coefficient to both the SEC polydispersity of a sample and the slope of a linear molecular weight calibration curve employed in obtaining the polydispersity was obtained. It is the objective of this study to evaluate the validity of this equation and reproducibility of the parameters σ^2 and A_k.

EXPERIMENTAL

The SEC equipment and procedures have been described[10]. For the present investigation, two multi-column systems were chosen. The descriptions of these systems are contained in Table 1. The MW averages of the dextran and polyacrylamide standards employed are shown in Tables 2 and 3 respectively. Though only the Weight Average MWs averages ($\bar{M}_{ws}$) were supplied for polyacrylamide standards, these values will not be required in the present investigation. The $\bar{M}_{ws}$ will only be employed in obtaining the $\bar{M}_w$ calibration curve rather than the true MW calibration curve.

THEORY

According to the form of the newly proposed ISF[1], the following equations were obtained:-

$$X = \frac{K_n}{D2^2} + \sigma^2 \qquad [I-1]$$

where σ^2 the peak dispersion coefficient of a sample's chromatogram was shown to be

TABLE 1: OPERATING CONDITIONS OF SYSTEMS EMPLOYED

#	Code No.	Columns combined in Series	Length of Column (ft.)	Flow-rate (ml/mm)	Conc. Inj. wt.%
1	S4CRA	C729, C500, C240B, C240/120 Polydextran*	13.18	3.00	0.05
2	S4CRB	C729, C500, C240B, C240/420 Polydextran*	13.18	1.90	0.05
3	S5AR	C3000, C3000, C2000, C1000, C729 Polyacrylamide**	19.00	2.50	0.05

* Mobile-phase: 0.00833MNa_2SO_4/1.0gm/24 lit.Tergitol (pH = 7.0)

** Mobile-phase: pH = 7.0, I = 0.25, 0.25 gm/L polyethlene oxide 1.5 gm/24 lit. Tergitol, 2.5% CH_3OH.

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

Sample	$\bar{M}_N x10^{-3}$	$\bar{M}_W x10^{-3}$	$\bar{M}_W/\bar{M}_N$	$\bar{M}_{rms} \times 10^{-3}$
T500	173.00	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

TABLE 3: CHARACTERISTICS OF POLYACRYLAMIDES*

Designation	Lot No.	$\bar{M}_N x10^{-3}$	$\bar{M}_w x10^{-3}$	$\bar{M}_{rms} x10^{-3}$	$\bar{M}_w/\bar{M}_N$
PAM 55	93-7	-	55.00	-	-
PAM 100	93-3	-	100	-	-
PAM 270	93-3	-	270.00	-	-
PAM 500	93-5	-	500.00	-	-
PAM 1000	95-6	-	1000.00	-	-
PAM 2000	95-4	-	2000.00	-	-
PAM 5000	94-3	-	5-6000.00	-	-

*Data supplied by Polysciences, Warrington, Pennsylvania

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

and K_n was given by

$$K_n = \frac{D2^4\sigma^4 A_k}{12} \qquad [I-3]$$

Here, D2 in equation [I-2] is the slope of any arbitrarily chosen linear MW calibration curve in which the true MW calibration may be one of them. P(app) is the apparent polydispersity of the sample obtained using the arbitrarily chosen linear MW calibration curve. D2 in equation [I-3] is the slope of the true MW calibration curve obtained using linear TBS method of calibration where both $\bar{M}_n$ and $\bar{M}_w$ are employed. K_n is the slope of the plot of X versus $1/D2^2$, which is also given by $K_n = -\ln P(t)$, P(t) being the true polydispersity of the sample. A_k is the polyplatykurtic or flattening coefficient which can only be less than zero for broad samples[1]. For Equation [I-1] to be used for obtaining peak dispersion coefficient, the absolute molecular weight averages ($\bar{M}_w$ and $\bar{M}_n$) of the sample must be known. The use of Equation [I-2] does not require the knowledge of the MW averages and the true MW calibration curve of the generating SEC/polymer system. Equation [I-3] is limited to the use of the true MW calibration curve alone, since K_n is a function of the true polydispersity of the sample. Therefore, for calibration/MW averages calculation problems, two or three calibration curves may subsequently be needed - the linear MW calibration curve, peak dispersion coefficient calibration curve (if desired) and that corresponding to A_k.

RESULTS AND DISCUSSIONS

Using TBS method, the true MW calibration curves for the dextran SEC systems (S4CRA and S4CRB) were obtained and these are shown in Fig. 1 in cubic centimeters (cm^3) rather than in counts, along with the $\bar{M}_w$ and root mean square average MW ($\bar{M}_{rms}$) calibration curves. At the two flow-rates employed, very little can be said about the influence of flow-rates on the molecular weight calibration curves. However, using X values obtained from the TBS method of calibration, plots of X versus $D2^{-2}$ were obtained. Fig. 2 shows the case of the system at lower flow-rate. From the plots, σ^2 and K_n for each sample were obtained. Using similar plot for the other system, σ^2 and K_n values were also obtained. These are listed in Table 4, along with the A_k values obtained using Equation [I-3]. Table 5 contains the molecular weight resolution correction factors with respect to σ^2 and A_k for these two dextran systems. Though peak dispersion correction is less at the lower-flowrate, it is prematurely early to draw any

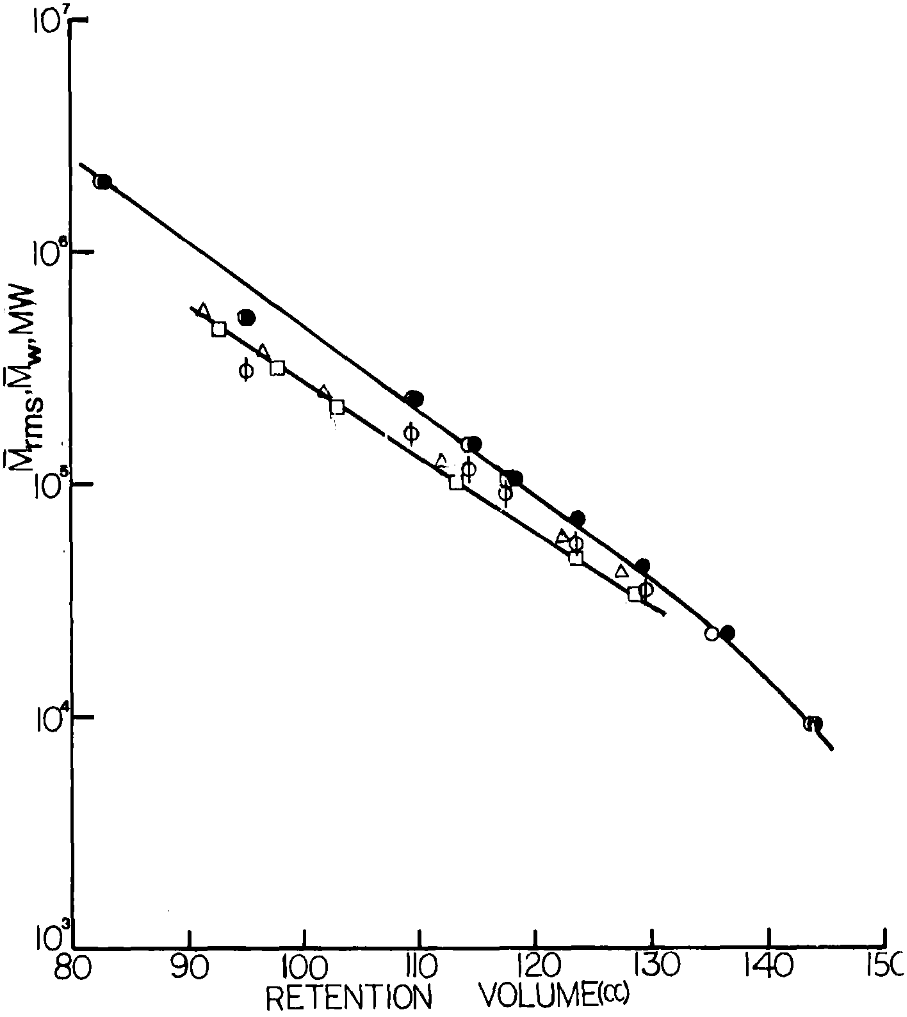

Figure 1. $\bar{M}_w$, $\bar{M}_{rms}$ and TBS method MW calibration curves for Dextran for case-studies # 1 and 2. O - $\bar{M}_w$, φ - $\bar{M}_{rms}$, □ - TBS method MW calibration curves ($M(v) = 0.356 \times 10^9$ EXP(-0.372V)) - for case study # 1 (flowrate = 3.0 ml/min); ● - $\bar{M}_w$, Δ - TBS method MW calibration curves ($M(v) = 0.370 \times 10^9$EXP(-0.364V)) - for case study # 2 (flowrate = 1.9 ml/min).

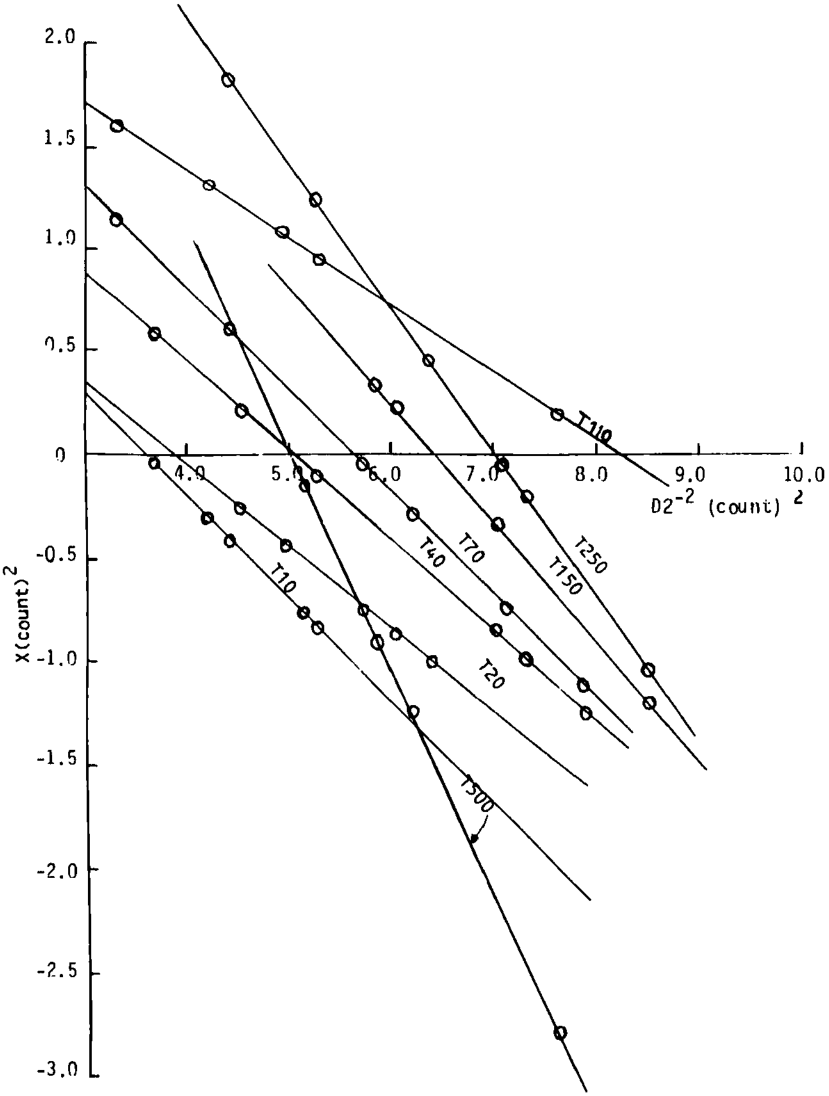

Figure 2. Overall Peak Broadening Parameter (X) versus D2^{-2} for case-study # 2 for Dextran.

TABLE 4: THE SLOPES AND INTERCEPTS OF THE PLOT OF X VERSUS $D2^{-2}$ FOR SYSTEMS # 1 AND 2

	3.0 ml/min.			1.9 ml/min.		
Sample	σ^{2*}	$-K_n$	$-A_k$	σ^2	$-K_n$	$-A_k$
T10	2.046	0.495	73.938	1.766	0.495	108.046
T20	1.883	0.401	70.728	1.529	0.401	116.041
T40	2.378	0.429	47.441	2.147	0.429	63.465
T70	2.969	0.498	35.337	2.787	0.498	43.935
T110	2.827	0.330	25.819	2.702	0.330	30.876
T150	4.018	0.580	22.466	2.737	0.580	28.501
T250	4.843	0.700	18.664	4.899	0.701	19.981
T500	5.403	1.052	22.496	5.272	1.056	26.020

$*\sigma^2$ in (count)^2

TABLE 5: PEAK DISPERSION RESOLUTION CORRECTION AND POLYPLATYKURTIC COEFFICIENT RESOLUTION CORRECTIONS FOR SYSTEMS # 1 AND 2

	3.0 ml/min		1.9 ml/min	
Sample	$EXP(-D2^2\sigma^2/2)$	$EXP(-D2^4\sigma^4A_k/24)$	$EXP(-D2^2\sigma^2/2)$	$EXP(-D^4\sigma^4A_k/24)$
T10	0.867	1.279	0.889	1.280
T20	0.877	1.223	0.903	1.219
T40	0.848	1.237	0.867	1.238
T70	0.814	1.281	0.831	1.281
T110	0.822	1.179	0.836	1.179
T150	0.757	1.335	0.781	1.335
T250	0.715	1.416	0.723	1.418
T500	0.687	1.691	0.705	1.698

TABLE 6: REPRODUCIBILITY OF ESTIMATION OF σ^2 AND A_k VALUES

	T70			T250		
	X(count)^2	σ^2(count)^2	A_k	X(count)^2	σ^2(count)^2	A_k
1	-0.7013	2.8772	-37.6153	-0.2742	4.7435	-19.3136
2	-0.7125	2.8660	-37.9099	-0.1438	4.8739	-18.2939
3	-0.7469	2.8316	-38.8366	-0.1835	4.8342	-18.5956
4	-0.6807	2.8978	-37.0824	-0.1384	4.8793	-18.2535
5	-0.6440	2.9345	-36.1607	-0.1713	4.8464	-18.5021
6	-0.7041	2.8744	-37.6887	-0.2061	4.8116	-18.7707
7	-0.6542	2.9243	-36.4134	-0.2607	4.7570	-19.5312
8	-0.6281	2.9504	-35.7720	-0.2284	4.7893	-18.9459
9	-0.6974	2.8811	-37.5136	-0.2742	4.7435	-19.3136
10	-0.7018	2.8769	-37.6232	-0.1584	4.8597	-18.4010
11	-0.7009	2.8756	-37.6572	-0.1724	4.8453	-18.5105
12	-0.6282	2.9503	-35.7744	-0.2611	4.7566	-19.5345
13	-0.5868	2.9917	-34.7912	-0.1565	4.8612	-18.2897
14	-0.6955	2.8830	-37.4641	-0.1784	4.8393	-18.5565
15	-0.6457	2.9328	-36.2026	-0.2057	4.8120	-18.7676
	Avg. σ	2.9032 ±0.0422	-36.9670 ∓ 1.0660	Avg. σ	4.8169 ±0.0462	-18.7787 ∓ 0.4451

conclusion about the influence of flowrate on MW calibration/MW calculations.

For the system at higher flow-rate, two standards - T250 and T70 were injected fifteen times each over a period of one and half months. Using the true MW calibration curve for the system, their X values were obtained. These were used to estimate σ^2 and A_k values. These are listed in Table 6 which also contains the average values and their

TABLE 7: APPLICATION OF σ^2 EQUATION (EQN. [I-2]) FOR POLYACRYLAMIDE SAMPLES

For M(v) =	$0.617\times10^{10}\text{EXP}(-0.289v)$		$0.381\times10^{11}\text{EXP}(-0.333V)$		$0.150^{11}\text{EXP}(-0.309V)$	
Sample	P(app)	$\sigma^2(\text{count})^2$	P(app)	$\sigma^2(\text{count})^2$	P(app)	$\sigma^2(\text{count})^2$
PAM 55	2.397	10.47	3.189	10.46	2.713	10.46
PAM 270	2.302	9.98	2.985	9.87	2.579	9.92
PAM 500	2.811	12.37	3.868	12.21	3.231	12.28
PAM 1000	2.450	10.73	3.257	10.66	2.773	10.68
PAM 2000	1.664	6.10	1.963	6.09	1.788	6.08

standard deviations. With measured standard deviations of less than 1.5%, there is no doubt that σ^2 and A_k values are highly reproducible. There is also no doubt that the ISF of these very compact standards are in addition symmetric in shape.

The TBS method could not be used with polyacrylamide since only the $\bar{M}_w$ of these standards were supplied. However, the peak dispersion coefficients of the standards were obtained using Equation [I-2]. This system was particularly chosen because of the very non-linear nature of the $\bar{M}_w$ calibration curve as shown in Figure 3. Three very different linear MW calibration curves were arbitrarily chosen and used with Equation [I-2] and the results are shown in Table 7. Despite the large differences in the apparent polydispersities of the same sample (P(app)), the σ^2 remains essentially the same varying only from one sample to the other. Thus, the chromatograms obtained in any SEC process contain a great deal of information about the performance of the SEC process itself, as well as the polymer sample in question.

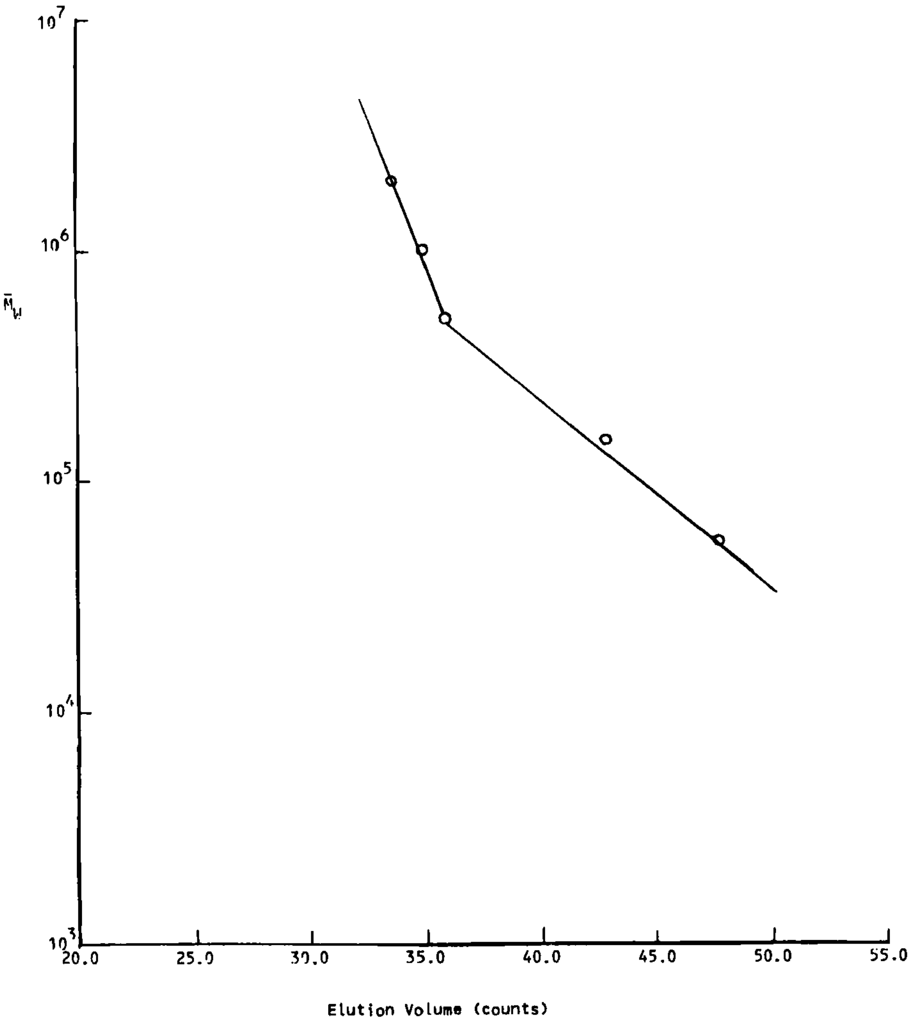

Figure 3. $\bar{M}_w$ calibration curve for Polyacrylamide (PAM) in case-study # 3 (flowrate = 2.5 ml/min); mobile-phase - pH = 7.0, Ionic strength (I) = 0.25 (See Table 1).

SUMMARY

In summary, based on the proposed ISF, peak dispersion coefficient can be easily obtained with great accuracy using any of the two methods described. There is no doubt that the proposed ISF should be of general application to polymers in general. Though, very narrow range of flow-rates were reported here, this will be a subject of further investigation in the series, since flow-rate studies is important in understanding the mechanism of separation in SEC.

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