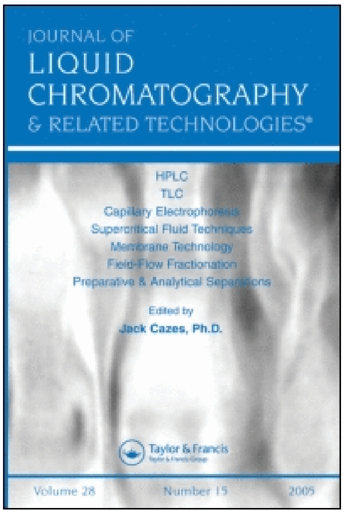

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Determination and Use of Cumulants of the Peak Broadening Function in Steric Exclusion Chromatography

Matthias Grüneberg[a][b]; Joachim Klein[a]
[a] Institut für Chemische Technologie, Technische Universität Braunschweig, Braunschweig, Germany [b] Institut für Anorganische Chemie und Kernchemie, Technische Hochschule Darmstadt, Darmstadt, Germany

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Determination and Use of Cumulants of the Peak Broadening Function in Steric Exclusion Chromatography

Matthias Grüneberg[x)], Joachim Klein
Institut für Chemische Technologie, Technische Universität
Braunschweig, Hans-Sommer-Str. 10, D - 3300 Braunschweig,
Germany

Summary

A procedure is developed which allows to deduce from recycle measurements the central moments and cumulants of the molecular mass distribution of polymers and the broadening function by the use of properties of statistical distributions. Results of an experimental application of the method are presented.

Equations for the calculation of true molecular mass averages from the elution curve are extended for the cases of nonlinear calibration curves and nonlinear broadening.

[x)] Present adress: Institut für Anorganische Chemie und Kernchemie,
Technische Hochschule Darmstadt, Hochschulstr.4,
D - 6100 Darmstadt, Germany

1. Introduction and Theory

In steric exclusion chromatography (SEC) dispersion effects result in broadening of the elution curve and thereby in a falsification of the calculated molecular mass averages. The experimental determination of the peak broadening contribution is therefore a prerequisit for any quantitative correction. Experiments on dispersion effects can also be used to examine theories of peak broadening.

While usually in chromatography the peak broadening function is identical with the elution curve, in the case of the SEC of synthetic polymers the determination of the broadening function turns out to be much more difficult. Here, no standards with a single molecular mass do exist, the broadening function is always convoluted with the molecular mass distribution of the sample under investigation. In the theory of SEC, the faltungstheorem is often named "Tung's equation" (1) :

$$F(V) = \int_{-\infty}^{+\infty} G(V-y)W(y)dy \qquad 1.$$

where F(V) represents the normalized curve, G(V-y) the broadening function and W(y) the corrected elution curve (i.e., the molecular mass distribution, expressed in elution volumina).

Three procedures have been proposed for the determination of parameters of the broadening function:

(1) T.Provder and E.M.Rosen (2) determined central moments of the broadening function by comparing the molecular mass averages calculated from the uncorrected elution curve with the "true"

molecular mass averages, which were determined by independent methods.

The ν. moment about the origin of a normalized distribution $\varphi(x)$ is defined by

$$\mu'_\nu = \int_{-\infty}^{+\infty} x^\nu \varphi(x)\,dx \qquad 2.$$

μ'_0 is the area under the curve (= 1) and μ'_1 the center of gravity.

Central moments are defined by

$$\mu_\nu = \int_{-\infty}^{+\infty} (x - \mu'_1)\,\varphi(x)\,dx \qquad 3.$$

where μ_1 is zero, μ_2 the variance and μ_3 is a measure of the skewness of the peak (strictly, the skewness is described by the parameter $\mu_3/\mu_2^{3/2}$). The higher even central moments are a measure of the symmetric deviation of the distribution from a gauss-function, the higher uneven central moments do refine the description of the skewness.

For the case of a linear relation between the elution volume V_e and the logarithm of the molecular mass

$$\ln M = D_1 + D_2 V_e \qquad 4.$$

Provder and Rosen calculated a relation between the true k-th molecular mean $\overline{M}_k(t)$ and the uncorrected k-th molecular mean $\overline{M}_k(\infty)$, describing the broadening function by a Gram-Charlier-serie:

$$\frac{\bar{M}_k(t)}{\bar{M}_k(\infty)} = \exp\left[-(2k-3)D_2^{\,2}\mu_2/2\right] \frac{1 + \sum_{m=3}^{m=\infty} \left(\frac{A_m}{m!}\right) \left[(k-2)D_2\mu_2^{\,1/2}\right]^m}{1 + \sum_{m=3}^{m=\infty} \left(\frac{A_m}{m!}\right) \left[(k-1)D_2\mu_2^{\,1/2}\right]^m} \qquad 5.$$

$k = 1,2,3,$ and 4 correspond to the number-, the weight-, the Z- and the Z+1-average, $A_3 = \mu_3/(\mu_2^{3/2})$ ("skewness") and $A_4 = (\mu_4/\mu_2^{\,2}) - 3$ ("excess" of the broadening function). If the Gram-Charlier-serie is truncated to an Edgeworth serie, A_5 can be set zero, $A_6 = 10\,A_3^{\,2}$ and $A_m = 0$ for $m \geq 7$.

If the central moments of the order > 2 are neglected, the exponential term of eq. 5 remains. This equation for the case of gaussian peak broadening has been deduced by S.T. Balke and A.E. Hamiliec (3), too.

Eq. 5 can be used for the experimental determination of the central moments of the broadening function, but also - knowing these moments - for the correction of the molecular mass averages which have been calculated from the elution curve.

A drawback of this procedure lies in its sensitivity towards errors of the determined "true" molecular mass.

(2) L.H.Tung, J.C.Moore and G.W.Knight (4) proposed another procedure for the determination of parameters of the peak broadening. These authors cancel the chromatographic separation of the polydisperse sample by flow reversal after it has reached half of its elution volume. This way, at the column entrance

the pure broadening function is obtained. This procedure implies that the uneven central moments can be neglected. Besides this, it can not be used with columns which can only be operated with one direction of flow.

(3) J.L.Waters (5) proposed a third procedure, the recycle technique. In this approach the variances of the elution curves of polydisperse samples are measured as a function of the number of cycles, and the variance of the broadening function and of the molecular mass distribution (expressed in elution volumina) are calculated. The fact is used that the variance (μ_2) of the broadening function of the whole run is the sum of the variances of the broadening functions of the single runs, while for the polydispersity broadening the standard deviations ($\mu_2^{1/2}$) of the runs do add (this is the fact the chromatographic separation is based upon). The variance of the elution curve resulting after n runs (neglecting the extra-column broadening) is the sum of the variances of the broadening function and the polydispersity-broadening.

The variances of the peak broadening of one run, μ_{2d}, and the polydispersity, μ_{2p}, are obtained using the equation:

$$\mu_{2e}/n^2 = \mu_{2d}/n + \mu_{2p} \qquad 6.$$

The cumulant approach

The second central moment describes the whole broadening function only in the case of pure gaussian broadening, which is only a simplification of real chromatographic broadening (6).

In the present work the recycling approach shall be extended and generalized in order to get more informations about the peak broadening without limitation to gaussian broadening.

One possibility for getting the whole peak broadening function from recycle runs is the use of the characteristic functions of the elution curves. The characteristic function of the elution curve after n cycles is the product of the characteristic function of the n-fold extended molecular distribution function of one run and the n-th power of the characteristic function of the peak broadening of one run.

The numerical calculation of the characteristic functions and the retransformation of the defolded functions, however, is connected with great computational problems. Therefore in this work only the much easier determination of statistical parameters of the broadening- and molecular weight distribution function will be dealt with. These parameters, the cumulants, are deduced from properties of the characteristic function.

Cumulants of a distribution can be calculated from their central moments, which are easily determined. The cumulants of lower order are simply related to the moments as follows:

$$K_1 = \mu'_1 \qquad 7.$$

$$K_2 = \mu_2 \qquad 8.$$

$$K_3 = \mu_3 \qquad 9.$$

$$K_4 = \mu_4 - 3\mu_2^2 \qquad 10.$$

Cumulants behave additive with respect to the folding of two statistical independent distributions (7).

If the cumulants of ν. order of the polydispersity function are named $K_{\nu p}$, the cumulants of the statistical broadening (dispersion) $K_{\nu d}$, and the cumulants of the resulting elution curve $K_{\nu e}$, the cumulants of the elution curve after n cycles are the sum of $K_{\nu d}$ and $K_{\nu p}$:

$$K_{\nu e,n} = K_{\nu p,n} + K_{\nu d,n} \qquad 11.$$

$K_{\nu d,n}$ is the folding of the statistical broadening functions of the single runs:

$$K_{\nu d,n} = n\, K_{\nu d,1} \qquad 12.$$

During n runs, the molecular mass distribution is extended by the factor n. The cumulants of the molecular mass distribution after n runs are:

$$K_{\nu p,n} = n^{\nu} K_{\nu p,1} \qquad 13.$$

From eqs. 11 to 13, one gets the relation between the cumulants of the elution curve after n runs and the cumulants of one run:

$$K_{\nu e,n} = n^{\nu} K_{\nu p,1} + n\, K_{\nu d,1} \qquad 14.$$

It is convenient to transform this equation into:

$$K_{\nu e,n}/n = n^{\nu -1} K_{\nu p,1} + K_{\nu d,1} \qquad 15.$$

Plotting $K_{\nu e,n}/n$ against $n^{\nu -1}$, one gets from the slope of the resulting linear relationship $K_{\nu p,1}$ and from its ordinate

$\kappa_{\nu d,1}$ is obtained. For the cumulants of the order 1 to 3, the moments about the origin resp. central moments can be set:

$$\mu'_{1e,n}/n = \mu'_{1p,1} \qquad 16.$$

$$\mu_{2e,n}/n = n\,\mu_{2p,1} + \mu_{2d,1} \qquad 17.$$

$$\mu_{3e,n}/n = n^2\mu_{3p,1} + \mu_{3d,1} \qquad 18.$$

Equation 16 is trivial; equation 17 corresponds to the relationship deduced by Waters (eq. 6).

Using eq. 15, the central moments of the broadening function can be calculated and used for the determination of the corrected molecular means (eq. 5).

From eq. 15, the cumulants and central moments of the molecular mass distribution (expressed in elution volumina) can be calculated too. Assuming a linear calibration curve in the range of the elution curve investigated, one gets the cumulants of the ln mass distribution (compare eq. 13):

$$\mu'_{1\ln M} = D_1 + D_2\,\mu'_{1\,p} \qquad 19.$$

$$\kappa_{\nu \ln M} = D_2^{\nu}\,\kappa_{\nu\,p} \qquad \nu > 1 \qquad 20.$$

With help of standards whose cumulants of the ln molecular mass distribution have been determined by the recycle technique, new columns can be calibrated. Using eq. 19 one gets a relation between the center of gravity of the elution curve and the corresponding molecular mass (the center of gravity of the ln mass distribution), a calibration procedure which is much easier than an iterative technique (8). The cumulants of the broadening function are the differences between the cumulants of the elution curve and the cumulants of polydispersity calculated by eq. 20.

One further application of the recycle equation 14 shall be mentioned: With the help of this equation and eq. 5, one gets a relationship showing the dependence of the correction factor $\bar{M}_k(t)/\bar{M}_k(\infty)$ on the number of cycles, n:

$$\frac{\bar{M}_k(t)}{\bar{M}_k(\infty)} = \exp\left[-(2k-3)\ D_2{}^2\mu_2/2n\right]\ \frac{1+\sum_{m=3}^{m=\infty}(n^{1-m})\left(\frac{A_m}{m!}\right)\left[(k-2)D_2\mu_2{}^{1/2}\right]^m}{1+\sum_{m=3}^{m=\infty}(n^{1-m})\left(\frac{A_m}{m!}\right)\left[(k-1)D_2\mu_2{}^{1/2}\right]^m} \qquad 21.$$

D_2 gives the slope of the calibration curve of a single run. If the peak broadening is approximated by a gauss function only the exponential term remains. This equation has already been given by A.E.Hamiliec (9)

2. Experimental Part

The applicability and the limits of the recycle technique for the determination of the cumulants and central moments of the polydispersity broadening and the dispersion shall be demonstrated by an experimental example.

2.1. Experimental Procedure

For the enforcement of the recycle measurements the method of "alternate pumping " was used, which has been developed by

I.Duvdevani, J.A.Biesenberger and M.Tan (10). It has the advantage that during recycling the probes do not flow through the pumpheads and the injection system and that therefore the extra-column broadening remains small.

The column set consisted of four high-resolution polystyrene gel columns from Toyo Soda Man. type TSK G3000H6, G4000H6, G5000H6 and G6000H6 which had 3/8 inch i.d. and 2 ft length each. The columns were switched in such way, that each half of the set had about the same distribution coefficient for each probe (6000,3000/5000,4000).

For column switching a six-way-valve was installed (Latek). As a pump the high-pressure piston pump 6000A was used, as the injection system the model U6K and as the detector the UV-photometer 440 (wave length 254 nm) (all instruments by Waters Ass.).

The column set was calibrated with 16 polystyrene standards of narrow distribution in the range of molecular mass from $2x10^3$ to $1.7x10^6$. Destilled THF was used as a solvent.

For the recycle measurement an anionic polymerized PS-standard from Waters Ass. was used. It had the batch number 25168 and the molecular mass averages given by Waters..Ass. were $\overline{M}_N$ = 20.000 and $\overline{M}_W$ = 20.800.

200 μl of a 600 ppm solution were injected.

The effective flow rate was determined using a 10 ml volumetric flask. For preventing systematical errors from evaporation, the opening of the flask was sealed with a plastic foil which was penetrated at the start of the measurement.

The flow rate determined this way was 1.02 ml/min.

2.2. Evaluation

A slight change of the amount of the amount of UV-visible traces in the solvent during the recycle run could not be prevented. This effect caused steps in the baseline after switching of the columns and a slight linear baseline drift. The steps had to be kept separate from the sample peak; the same holds for peaks caused by contaminations of the sample.

The measurement was evaluated only up to the fourth recycle run. Further runs resulted in superposition of the sample peak and baseline steps; besides, after four runs the dilution of the sample on the column was too high to give the stability of the baseline relative to peak height required.

The recorded elution curves were digitalized with the help of a pencil follower. The starting- and endpoints of the elution curve were considered to be points on a linear baseline. For the calculations the region of the elution curve was used in which the curve deviated visibly from the baseline (maximal baseline error: 0.5 percent of the maximal peak height). The ratio between the range of the elution curve used and the variance of the curve calculated was greater 20:1, so the range seems to be chosen broad enough.

The 2nd to 4th central moments and the centers of gravity of the peaks were calculated using a FORTRAN program. The cumulants calculated from the central moments were divided by the number of cycles n (table 1) and plotted against $n^{\nu-1}$ (fig.1-3). By linear regression the cumulants of the polydispersity and the dispersion broadening were calculated (table 2).

While the 2nd central moments measured deviate only slightly from the regression line, the higher cumulants scatter heavily; the regression lines calculated from these cumulants show a high degree of uncertainity.

Table 1 : 2nd to 4th cumulants of the recycle run

Run	$\kappa_{2e,n}/n$ ml^2	$\kappa_{3e,n}/n$ ml^3	$\kappa_{4e,n}/n$ ml^4
1	0.6220	0.2249	0.4876
2	0.8120	0.2864	0.1094
3	1.0671	0.5382	0.0336
4	1.2704	0.6261	-1.1580

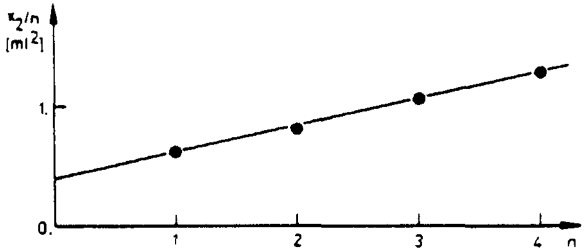

Figure 1. 2nd Central Moment.

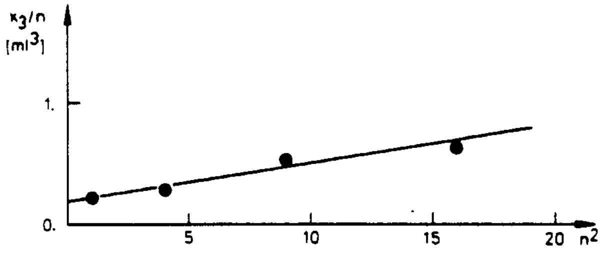

Figure 2. 3rd Central Moment.

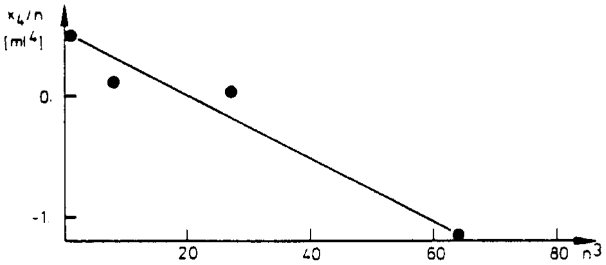

Figure 3. 4th Central Moment.

Table 2 : Cumulants of dispersion and polydispersity, determined with eq. 15

	κ_2 ml^2	κ_3 ml^3	κ_4 ml^4
dispersion	0.3928	0.2058	0.4817
polydispersity	0.2200	0.0284	-0.0254

The strong scattering of the higher cumulants results from the higher influence of experimental errors. It has been shown that the central moments calculated depend heavily on the exact measurement of the peak flanks (11,12), i.e. on the relative baseline error.

The variances of the curves were calculated from the tangents at the turning-points of the elution curves, too, assuming a gaussian distribution. The variances calculated this way deviate strongly from the value calculated numerically, the latter mostly being higher. This effect might be based on deviations from a gaussian curve, but the differences found (up to 20 percent) are considerably higher than expected.

The following measures can improve the determination of the polydispersity and the dispersion functions by the recycle technique:

- The absolute and relative constancy of the baseline should be improved. If theta-solvents are used, the concentration effects are minimized (13) and higher concentrations can be used.
- Even the molecular mass distribution of anionic polymerized standards may be improved considerably by preparative fractionation (14); this would result in a smaller range of the elution curve necessary for evaluation and in stronger flanks.
- A statistical distribution can be described by other parameters than by its moments resp. cumulants. Some alternative techniques like Laplace transform are less susceptible to baseline errors than the moment method (15) and might be better suited for an exact determination of the molecular mass.

Altogether the results of the recycle measurements quoted here do not yet satisfy high precision requirements, but the method as such can be developed further and seems promising.

The application of the theory of statistical distributions, as done in this work, gives a better understanding of the chromatographic separation of polydisperse samples during recycle runs. It allows the calibration of column sets for higher central moments of dispersion without the need for the determination of different molecular mass averages by independent methods.

2.3. Determination of the molecular mass averages and the cumulants of the logarithmic mass distribution

With the help of the calibration standards, the lnM-V_e-relationship of the column set was determined in four approach steps, following a graphical procedure which in principle has been described by W.Ring and W.Holtrup (8).

The molecular mass averages of the standard used for the recycle run were calculated under various assumptions. First, the digitalized calibration curve in the range of the elution curve was approximated by a polynom of fourth order (table 3). Because the molecular mass averages $\overline{M}_W$, $\overline{M}_Z$, and $\overline{M}_{Z+1}$ describe the higher molecular part of the molecular mass distribution, "$\overline{M}_{N-1}$"- and "$\overline{M}_{N-2}$"-mass averages may be used beside $\overline{M}_N$ to give a better description of the low molecular mass part (comp.(16)). They are defined by the equations

$$\overline{M}_{N-1} = \int (1/M(V))\ F(V)\ dV \Big/ \int (1/M(V)^2)\ F(V)\ dV \qquad 22.$$

$$\overline{M}_{N-2} = \int (1/M(V)^2)\ F(V)\ dV \Big/ \int (1/M(V)^3)\ F(V)\ dV \qquad 23.$$

The equation of Rosen and Provder for the correction of broadening (eq. 5) can be used with k = 0 resp. -1.

For a correction of the molecular mass averages using eq. 5 it is necessary to calculate the molecular mass averages under the assumption of a linear calibration curve. As will be discussed later, calculating the linear regression it is useful to give special weight to the range of the calibration curve which gives a high contribution to the molecular mass averages Therefore, the calibration curve was weighted with the height of the elution curve.

Two corrections of the molecular mass averages were carried out, both using eq. 5. The first one assumed a gaussian broadening; for the second correction the 3rd and 4th cumulants of the broadening function were used too (table 3).

Although the probe under investigation elutes in the low molecular mass, nonlinear range of the column set, it is seen from table 3 that the nonlinearity does not affect the calculated molecular mass averages to a greater extent. This is due to the narrow distribution of the standard.

The correction of the molecular mass averages for dispersion, however, has a stronger effect, especially on $\overline{M}_W/\overline{M}_N - 1$. With the high-resolution columns used, the influence of cumulants of higher order on the calculated molecular mass averages seems to negligible. Their influence will be higher for probes of higher molecular weight and under the conditions of fast SEC.

Table 3 : Molecular mass averages calculated by different approaches

molecular means	nonlinear calibration	linear calibration		
	not corrected		corrected for K_{2d}	corrected for K_{2d} to K_{4d}
$\overline{M}_{N-2}$	17, 122	17,137	18,059	18,272
$\overline{M}_{N-1}$	17, 927	17,919	18,491	18,561
$\overline{M}_{N}$	18, 607	18,602	18,798	18,807
$\overline{M}_{W}$	19, 217	19,230	19,030	19,037
$\overline{M}_{Z}$	19, 794	19,830	19,216	19,256
$\overline{M}_{Z+1}$	20, 359	20,419	19,377	19,460
$\overline{M}_{W}/\overline{M}_{N}$	1.033	1.034	1.012	1.012

The values of $\overline{M}_W$ and $\overline{M}_N$ calculated deviate rather strongly from the values given by the manufactorer. This fact might be attributed to an inaccurate construction of the calibration curve at its nonlinear edge. ~~However~~, some standards measured in the linear range show such deviations, too. So, the reason for the discrepancy might be an inexact measurement of the "true" molecular mass averages (comp. (17)).

The high resolution of the column set used makes a determination of the broadening, which is based on the comparison of "true" $\overline{M}_W/\overline{M}_N$ and the values calculated from the SEC measurements quite inexact. In fact, in the present case, $\overline{M}_W(t)/\overline{M}_N(t) > \overline{M}_W(\infty)/\overline{M}_N(\infty)$ so that a calculation based on eq. 5 gives a negative value for the dispersion.

Finally, the cumulants of the ln-molecular mass distribution shall be calculated with the help of eq. 19 and 20. The ordinate and the slope of the weighted regression line of the calibration curve in the range of the elution curve are $D_1 = 24.52$ and $D_2 = -0.2310\ \mathrm{ml}^{-1}$, respectively; the center of gravity is at the elution volume of 63.51 ml. The cumulants calculated are listed in table 4.

Table 4 : Cumulants of the ln-molecular mass distribution

$\kappa_{1\ln M}$: 9.852
$\kappa_{2\ln M}$: 1.174×10^{-2}
$\kappa_{3\ln M}$: -3.501×10^{-4}
$\kappa_{4\ln M}$: -7.229×10^{-5}

3. Refinements of the correction equation

Equation 5, given by Rosen and Provder, allows a simple correction of the molecular mass averages calculated from the elution curve. This equation, however, is based upon some simplifications. The extension on some more realistic cases will be discussed here. The first simplification lies in the assumption of a linear calibration curve (eq. 4). Especially with samples having a broad molecular mass distribution, this approach may lead to greater deviations from the real molecular mass average than the uncorrected average.

A.E.Hamiliec (9) has extended the calculations of Rosen and Provder for the case of a nonlinear calibration curve. The resulting equation, however, seems not to be solvable analytically One therefore has to be contented with an iterative approach.

A refinement of the linear approach has been used in the experimental part: the points of the digitalized calibration curve were weighted with the corresponding height of the elution curve and then a linear regression in the elution range was applied.

This approach can be refined further, if one includes the fact, that calculating the moments about the origin of the molecular mass distribution, whose ratios comprise the molecular means (eq. 24), different parts of the elution curve (and therefore of the calibration curve too) contribute to the result to a different extent. This fact can be considered in an iterative way by calculating the moments about the origin of the (uncorrected) molecular mass distribution using respective specifically weighted linear regressions of the calibration curve (eq.24).

$$\overline{M}_k = \mu'_{k-1}/\mu'_{k-2} \qquad 24.$$

One gets the weighting factors by taking the molecular weight which is attached to a point of the calibration curve to the power of the respective moments about the origin of the molecular mass distribution.

The resulting slopes of the linear regression are used for the correction of the molecular means, too. If $D_{2,k-1}$ is the slope of the regression line which is attached to the higher moments about the origin, and $D_{2,k-2}$ the corresponding slope attached to the moments about the origin of the lower order, one gets from the calculations of Rosen and Provden the following extension of equation 5 :

$$\frac{\overline{M}_k(t)}{\overline{M}_k(\infty)} = \frac{\exp[(k-2)^2\mu_2 D^2_{2,k-2}/2][1 - \sum_{m=3}^{m=\infty} (\frac{A_m}{m!}) [(k-2)D_{2,k-2}\mu_2^{1/2}]^m]}{\exp[(k-1)^2\mu_2 D^2_{2,k-1}/2][1 + \sum_{m=3}^{m=\infty} (\frac{A_m}{m!}) [(k-1)D_{2,k-1}\mu_2^{1/2}]^m]} \qquad 2$$

A second simplification of equation 5 lies in the assumption, that the respective cumulants of the broadening function are constant in the range of the elution curve. Especially the higher cumulants of the broadening function, however, depend rather strongly on the elution volume (6). This nonconstancy of the cumulants of dispersion, too, has its greatest influence in the case of broad molecular mass distributions.

Mean central moments of dispersion can be calculated with the help of equations given by Rosen and Provder (13):

$$\bar{\mu}_{2d} = \int_{-\infty}^{+\infty} \mu_{2d}(y)W(y)dy \qquad 26.$$

$$\bar{\mu}_{3d} = \int_{-\infty}^{+\infty} \mu_{3d}(y)W(y)dy + 3\int_{-\infty}^{+\infty} \mu_{2d}(y)yW(y)dy \qquad 27.$$

$$- 3\mu'_{1e}\int_{-\infty}^{+\infty} \mu_{2d}(y)W(y)dy$$

$$\bar{\mu}_{4d} = \int_{-\infty}^{+\infty} \mu_{4d}(y)W(y)dy + 6\int_{-\infty}^{+\infty} \mu_{2d}(y)y^2W(y)dy \qquad 28.$$

$$- 12\mu'_{1e}\int_{-\infty}^{+\infty} \mu_{2d}(y)yW(y)dy - 6\mu'^{2}_{1e}\int_{-\infty}^{+\infty} \mu_{2d}(y)W(y)dy$$

The problem of these equations lies in the fact, that W(y) is the true molecular mass distribution (expressed in elution volumina), which normally is not known. The mean central moments can be calculated in an iterative way: In the first step, the elution curve is taken as the true molecular mass distribution and $\kappa_p(y)$ in the elution range is approximated by polynom series in such way, that from the integrals of eq. 26. - 27. sums of moments about the origin of the elution curve are obtained.

The moments about the origin are easily calculated from the central moments of the curve (7).

The calculated mean central moments are used for the calculation of new moments about the origin of the approximated molecular mass distribution (in elution volumina) and the iteration can be carried on.

Conclusions

It is thus possible to get the cumulants of the peak broadening function with the help of the recycle technique and to calculate the corrected molecular mass averages also in case that the elution volume / molecular mass relationship is nonlinear or that the dependence of the cumulants on the elution volume can not be neglected.

The numerical correction of the whole elution curve, using cumulants of order higher 2 and taking into consideration non-constant broadening, poses much higher difficulties. Even for the simpler case of a gaussian constant broadening a numerical calculation of the true molecular mass distribution shows considerable practical problems (19).

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