

An analytical correction for peak broadening in gel permeation chromatography with a molecular weight detector

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SUMMARY.

In this paper we present a general expression describing the peak broadening effect on molecular weight averages measured during the elution of a polymer in GPC. This expression is valid for nonlinear column calibration and nonuniform Gaussian spreading across the chromatogram. Comparison is made with analytical relationships derived by Yau, Marais, Hamielec and Netopilik. The methodology developed as an extension of the Pierce and Armonas method is applied to a model system and to elution data of dextrans.

INTRODUCTION.

It is well-known that peak broadening in GPC is adequately described by Tung's integral equation (1). Originally intended for chromatograms from a concentration detector, this equation may be generalized to chromatograms from a molecular weight detector. With the fast growing application of the LALLS (Low Angle Laser Light Scattering) and viscosimetric detection techniques several attempts have been made to solve analytically the generalized Tung's equation (2-7). In fact all these solutions represent relationships between the average molecular weight of the polymer species eluted at elution volume v , denoted here as $\bar{M}(v)$, and the column calibration molecular weight $M(v)$ (the subscript α specifies the kind of average). The function $M(v)$ is usually determined by measuring the peak positions of monodisperse standards. Contrary to $\bar{M}(v)$, the calibration function $M(v)$ is not affected by peak broadening.

The first reported relationship between $\bar{M}(v)$ and $M(v)$ is due to Yau et al. (2). Though restricted to linear column calibration and uniform Gaussian spreading across the chromatogram their expression is exact. Hamielec et al. (4-7) have generalized Yau's result. They apply the above restrictions only locally in the chromatogram i.e. in a very small elution range. As we shall see their expression is approximative. Other general relationships have been proposed by Marais et al. (3). However, their method has not come in practice.

On the same level of generality we deduce from the Pierce and Armonas expressions (8) a general relationship between $\bar{M}(v)$ and $M(v)$. The results are expressed in terms of the first and second derivatives of the chromatogram $F(v)$ and permit an explicit evaluation of the spreading factor $h(v)$. The latter is shown by a graphical procedure. Moreover Hamielec's analytical solution for the corrected chromatogram (5,7) is shown to be identical to an earlier version of this solution derived by Pierce and Armonas (8).

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THEORETICAL.

Let us first postulate the two integral equations describing the peak broadening effect on the different kind of chromatograms. The first equation, introduced by Tung (1), relates the experimental UV or RI chromatogram $F(v)$ to $W(y)$, the chromatogram corrected for broadening

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

Here $G(v-y)$ represents the spreading function of the polymer species eluted at elution volume v with elution volume variable y . The other integral equation, describing the chromatogram obtained from the molecular weight detector, reads

$$\bar{M}_\alpha(v)^\alpha F(v) = \int_{-\infty}^{+\infty} G(v-y) W(y) M(y)^\alpha dy \quad (2)$$

In eq.(2) $\bar{M}_\alpha(v)$ stands for the molecular weight average with α specifying the type of average

$$\bar{M}_1(v) \equiv \bar{M}_w(v) , \quad \bar{M}_{-1}(v) \equiv \bar{M}_n(v) , \quad \bar{M}_a(v) \equiv \bar{M}_{vis}(v)$$

(a' is the Mark-Houwink coefficient)

The l.h.s. of eq.(2) is proportional to the detector response, i.e. $\bar{M}_w(v) F(v)$ for the LALLS detector and $\bar{M}_{vis}(v)^{a'} F(v)$ for the connected viscosimeter. As $M(y)$ denotes the true molecular weight calibration function, $W(y)M(y)^\alpha$ is proportional to the height of the detector chromatogram corrected for peak broadening.

In addition to eqs.(1) and (2) we shall make two assumptions, which are valid to a high degree in a small range around v . First we linearize $\ln M(y)$ by writing

$$y = c_1(v) - c_2(v) \ln M(y) \quad (3)$$

Furthermore we restrict ourselves to Gaussian spreading

$$G(v-y) = \left\{ \frac{h(v)}{\pi} \right\}^{1/2} \exp \{-h(v)(v-y)^2\} \quad (4)$$

with a spreading factor $h(v)$. As a result eq.(1) becomes

$$F(v) = \left\{ \frac{h(v)}{\pi} \right\}^{1/2} \int_{-\infty}^{+\infty} W(y) \exp\{-h(v)(v-y)^2\} dy \quad (5)$$

Before rewriting also eq.(2) we transform eq.(3) to

$$M(y) = M(v) \exp\{-(y-v)/c_2(v)\} \quad (6)$$

Insertion of eqs.(4) and (6) into eq.(2) leads to

$$\bar{M}_\alpha(v)^\alpha F(v) = M(v)^\alpha \left\{ \frac{h(v)}{\pi} \right\}^{1/2} \exp \left\{ \frac{\alpha^2}{4h(v)c_2(v)^2} \right\} \int_{-\infty}^{+\infty} W(y) \exp \left\{ -h(v) \left(v - \frac{\alpha}{2h(v)c_2(v)} - y \right)^2 \right\} dy \quad (7)$$

$\alpha = 1, -1, a'$

Eq.(7) is a convenient starting point for deriving the various expressions found in literature as well as the expression obtained in this paper.

Let us assume that we are allowed to make the approximation $h(v - \alpha/(2h(v)c_2(v))) \approx h(v)$. In that case eq.(7) reduces with the use of eq.(5) to

$$\bar{M}_\alpha(v)^\alpha F(v) = M(v)^\alpha F\left(v - \frac{\alpha}{2h(v)c_2(v)}\right) \exp\left\{\frac{\alpha^2}{4h(v)c_2(v)^2}\right\} \quad (8)$$

$$\alpha = 1, -1, a'$$

This reduction has been shown by Yau et al.(2) assuming h and c_2 to be constant in the elution range of the chromatogram. Then eq.(8) is exact. The more general case, in which h and c_2 are assumed to be locally constant has been treated by Hamielec et al. (5,7) who arrive at eq.(8). However, it must be noted that eq.(8) remains approximative unless $\alpha/(2h(v)c_2(v))$ is a very small quantity.

Without loss of generality Marais et al. (3) solve the integral in eq.(7) by using a Taylor expansion followed by an integration of each term of the series. Their result is an expansion in powers of $1/(2h(v)c_2(v)^2)$. However, use of this open expression is restricted as the corrected chromatogram must be known beforehand.

In this treatment we solve eq.(7) by approximating $W(y)$ with the use of the method of Pierce and Armonas (8). Let us approximate $F(v)$ in a small range around v by the function $F(y,v)$ as follows

$$F(y,v) = \exp\{a(v) - b(v)(y-v) - c(v)(y-v)^2\} \quad (9)$$

with $a(v) = \ln F(v)$, $b(v) = -\{d \ln F(y)/dy\}_v$ and $c(v) = -\frac{1}{2}\{d^2 \ln F(y)/dy^2\}_v$. Then eq.(5) can be solved for $W(y,v)$ by means of the Fourier transform technique (8) resulting in

$$W(y,v) = \left\{\frac{h(v)}{h(v)-c(v)}\right\}^{1/2} F(v) \exp\left\{\frac{b^2(v)}{4c(v)}\right\} \exp\left\{-\frac{h(v)c(v)}{h(v)-c(v)}\left(y - v + \frac{b(v)}{2c(v)}\right)^2\right\} \quad (10)$$

$W(y,v)$ is an approximation of the corrected chromatogram around v . If $W(y)$ is replaced by $W(y,v)$ in eq.(7), the integration can be performed analytically. It yields

$$\bar{M}_\alpha(v)^\alpha = M(v)^\alpha \exp\left\{\frac{\alpha^2}{4h(v)c_2(v)^2}\right\} \exp\left\{\frac{b(v)\alpha}{2h(v)c_2(v)} - \frac{\alpha^2 c(v)}{4h(v)^2 c_2(v)^2}\right\} \quad (11a)$$

or in a more compact form

$$\bar{M}_\alpha(v) = M(v) \exp\left\{\frac{(h(v)-c(v))\alpha}{4h(v)^2 c_2(v)^2} + \frac{b(v)}{2h(v)c_2(v)}\right\} \quad (11b)$$

$$\alpha = 1, -1, a'$$

So we have a simple closed expression relating the detected molecular weight average to the true molecular weight. The required properties of the chromatogram are the local first and second derivatives. An additional remark on the derivation of eqs.(11) has to be made. Looking at eq.(2) it must be realized that the approximations for $W(y)$ and $M(y)$ are rather good in a small range around v , but loose their validity as y is farther from v . On the other hand the function $G(v-y)$ goes fast to zero for increasing

values of $(v-y)$. Thus the integral in eq.(2) is almost completely determined by the properties of $W(y)$ and $M(y)$ around v .

Of interest is that eq.(11) reduces to known results. At small values of $\alpha/(2h(v)c_2(v))$ we may substitute $y=v-\alpha/(2h(v)c_2(v))$ into eq.(9). Then we obtain the identity

$$\frac{F\left(v - \frac{\alpha}{2h(v)c_2(v)}\right)}{F(v)} = \exp\left\{\frac{b(v)\alpha}{2h(v)c_2(v)} - \frac{c(v)\alpha^2}{4h(v)^2c_2(v)^2}\right\} \quad (12)$$

We see that eq.(11a) becomes identical to eq.(8). Another case, where the chromatogram is Gaussian-shaped has been studied by Netopilik (9). In our terms it implies the equality $b(v) = 2c(v-y_0)$ where y_0 is the peak position and $2c$ the reciprocal variance of the Gaussian chromatogram. Under these specifications and with eq.(6) eq.(11b) can be rearranged to

$$\bar{M}_\alpha(v) = M(y_0) \exp\left\{\frac{(h(v)-c)\alpha}{4h(v)^2c_2(v)^2} + \frac{(h(v)-c)(v-y_0)}{h(v)c_2(v)}\right\} \quad (13)$$

$$\alpha = 1, -1, a'$$

If h and c_2 are considered to be independent of v in eq.(13) the previous analytical result (9) is found back easily.

Let us consider now two applications of eq.(11). Firstly, the resolution factor $h(v)$ can be solved if the other parameters are known. This point will be illustrated in the following sections. Secondly, eq.(11) will be useful for the calculation of the molecular weight averages of the polymer as a whole. For instance with the LALLS technique one measures $\bar{M}_w(v)$. For that situation eq.(11b) is conveniently rewritten as

$$\bar{M}_\alpha(v) = \bar{M}_w(v)^\alpha M(v)^{(1-\alpha)} \exp\left\{\frac{b(v)(1-\alpha)}{2h(v)c_2(v)}\right\} \quad (14)$$

$$\alpha = -1, a'$$

Thus expressions for $\bar{M}_w(v)$ and $\bar{M}_{vis}(v)$ follow from eq.(14) by taking respectively $\alpha=-1$ and $\alpha=a'$. The final step is the calculation of the molecular weight averages according to

$$\begin{aligned} \bar{M}_w &= \int_{-\infty}^{+\infty} \bar{M}_w(v) F(v) dv \\ \bar{M}_n &= \left[\int_{-\infty}^{+\infty} \frac{F(v)}{\bar{M}_n(v)} dv \right]^{-1} \\ \bar{M}_{vis} &= \left[\int_{-\infty}^{+\infty} \bar{M}_{vis}(v)^{a'} F(v) dv \right]^{1/a'} \end{aligned} \quad (15)$$

where the chromatogram has been normalized for the area under the curve.

Finally we briefly discuss the approximative analytical solution of eq.(4) for the corrected chromatogram $W(v)$. Solutions of this kind have been reported by Pierce and Armonas (8) and by Hamielec et al. (5). The former solution is found by taking the limit $y \rightarrow v$ in eq.(10)

$$W(v) = \left\{ \frac{h(v)}{h(v)-c(v)} \right\}^{1/2} F(v) \exp\left\{-\frac{b(v)^2}{4(h(v)-c(v))}\right\} \quad (16)$$

Let us rewrite this expression in Hamielec's notation (5) with

$$\sigma(v)^2 = \frac{1}{2h(v)}$$

$$\bar{y}(v) = v - \sigma(v)^2 b(v)$$

$$\bar{\sigma}(v)^2 = \sigma(v)^2 - 2\sigma(v)^4 c(v)$$

It gives

$$W(v) = \frac{\sigma(v)}{\bar{\sigma}(v)} F(v) \exp \left\{ - \frac{(v - \bar{y}(v))^2}{2\bar{\sigma}(v)^2} \right\} \quad (17)$$

Eq.(17) has been derived by Hamielec along another route but is in fact the same as eq.(16).

RESULTS AND DISCUSSION.

Model calculation.

In order to check the properties of eq.(11) we shall choose the following test functions

$$W(v) = \frac{3}{4} \left(\frac{3}{20\pi} \right)^{1/2} \left\{ 1 + \frac{1}{10} (v-53)^2 + \frac{1}{50} (v-53)^3 \right\} \exp \left\{ - \frac{3}{20} (v-53)^2 \right\} \quad (18)$$

$$M(v) = 66171 \exp \left\{ - \frac{1}{4} (v-53) \right\} \quad (19)$$

Both functions are graphically shown in Figure 1. Notice that $W(v)$ is a skewed Gaussian chromatogram at infinite resolution ($h=\infty$), whereas for $M(v)$ linear column calibration is assumed ($c_2=4$ ml). For finite and constant h we find the corresponding chromatogram $F(v)$ by applying eq.(5)

$$F(v) = \frac{3}{4} \left\{ \frac{3h}{(3+20h)\pi} \right\}^{1/2} \left\{ 1 + \frac{1}{3+20h} + \frac{12}{(3+20h)^2} (v-53) \right. \\ \left. + \frac{1}{10} \left(\frac{20h}{3+20h} \right)^2 (v-53)^2 + \frac{1}{50} \left(\frac{20h}{3+20h} \right)^3 (v-53)^3 \right\} \\ \exp \left\{ - \left(\frac{3h}{3+20h} \right) (v-53)^2 \right\} \quad (20)$$

This chromatogram is represented in Fig.1 for $h=0.3$ ml⁻². Since h is constant across the chromatogram, eq.(8) provides an exact expression for the molecular weight average obtained by LALLS

$$\bar{M}_w(v) = M(v) \frac{F(v - \frac{1}{8}h)}{F(v)} \exp \left\{ \frac{1}{64h} \right\} \quad (21)$$

On the other hand this function is given by eq.(11a)

$$\bar{M}_w(v) = M(v) \exp \left\{ \frac{b(v)}{8h} - \frac{c(v)}{64h^2} \right\} \exp \left\{ \frac{1}{64h} \right\} \quad (22)$$

whereas analytical expressions for $b(v)$ and $c(v)$ are available from eq.(20). In Fig.1 the points corresponding to eq.(22) are seen to be in perfect agreement with the exact result according to eq.(21). Evidently the resolution factor $h(v)$ may be solved from eq.(11b). However, if experimental noise is generated a graphical representation is recommended. In Fig.2 a way of plotting has been chosen such that curves are obtained with a slope $1/h(v)$ according to eq.(11b). This curve may be useful if h is constant or at least locally constant. From Fig.2 we estimate $h=0.2998$ ml⁻² being nearly the exact value 0.3 ml⁻².

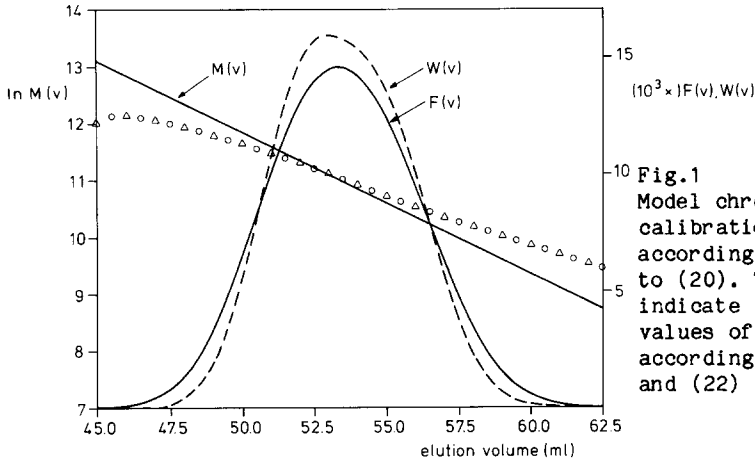


Fig.1
Model chromatograms and calibration curve according to eqs.(18) to (20). The points indicate calculated values of $\bar{M}_W(v_i)$ according to eqs.(21) (Δ) and (22) (○).

In Table 1 various values of \bar{M}_W and \bar{M}_n of the model sample are compared. In the first column are given the exact values calculated with eqs.(18) and (19). The estimates in the second column of Table 1 have been made by applying eqs.(14) and (15). They turn out to be accurate. In the third column of Table 1 small errors in the values occur. These figures have been found by using eqs.(16) and (19). Thus a small inaccuracy has been introduced by the analytical approximation of $W(v)$.

Table 1

	exact	eq.(15)	approximative
\bar{M}_W	67,450	67,450	66,580
\bar{M}_n	50,280	50,290	50,760

Number-average and weight-average molecular weights of the model sample.

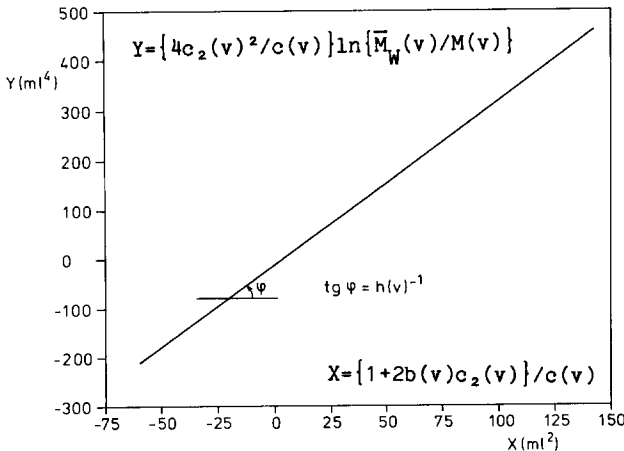


Fig.2
Graphical evaluation of $h(v)$ for the model according to eqs.(18) to (21).

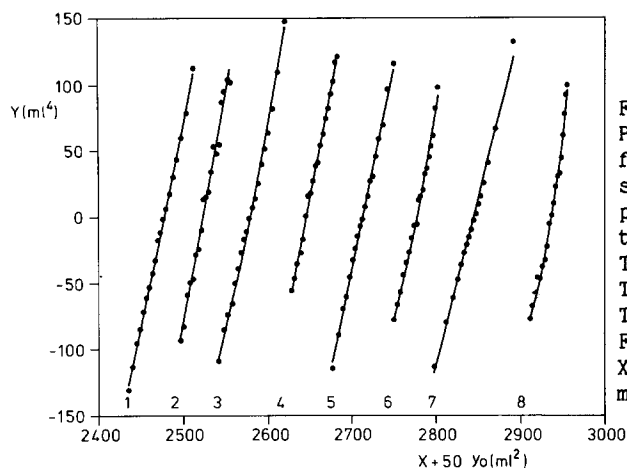


Fig.3
Plot of Y versus $X+50y_0$
for eight different dextran
samples (y_0 being the
peak elution volume). From
the left to the right:
T70-II-1(1); T150-921(2);
T70-II-4(3); T70-1730(4);
T70-II-7(5); T40-1600(6);
FDR-7314(7); T10-9846(8).
 X and Y have the same
meaning as in figure 2.

Table 2.

Code	\bar{M}_w $\times 10^{-3}$ (REFERENCE)	\bar{M}_n $\times 10^{-3}$	\bar{M}_w $\times 10^{-3}$ eq.(15)	\bar{M}_n $\times 10^{-3}$ eq.(15), $h=\infty$	h ml^{-2}	\bar{M}_n $\times 10^{-3}$ (corrected)
T10-3205	9.3	5.7	10.4	7.9	0.367	3.9
T10-9846	10.5	6.4	10.1	8.6	0.394	6.0
T20-7968	22.3	15.0	20.8	19.0	0.348	13.9
T20-5382	21.6	16.7	22.2	20.6	0.348	12.1
T40-9086	42.4	28.4	42.1	34.1	0.358	20.1
T40-2540	44.4	28.9	44.7	38.1	0.319	26.6
T40-1600	35.9	24.1	38.3	29.9	0.300	21.3
T70-693	69.5	39.5	74.2	54.7	0.248	39.7
T70-1730	70.0	42.5	76.4	62.1	0.314	42.8
T110-5404	101	62.0	96.5	79.1	0.272	52.9
T110-9071	106	76.0	104	90.4	0.299	77.3
T150-921	154	86	157	121	0.285	85.8
T250-8374	240	121	245	159	0.343	101
T500-21917	465	192	462	223	0.256	171
T70-II-4	98	84	98.4	94.7	0.305	86.7
T70-II-7	50.8	43.6	48.1	45.0	0.315	41.1
T70-II-1	158	135	152	148	0.327	155
FDR-7314	21.3	18.9	21.0	20.5	0.327	16.5

Molecular weight data of dextrans in 0.1 M KNO_3 aqueous solution.

Real System.

Experimental data were obtained from a recent study on the elution of dextrans in aqueous GPC with a LALLS detector (10). Sufficient salt (0,1 M KNO_3) was added to enhance a pure size exclusion mechanism. The functions $b(v)$ and $c(v)$ defined by eq.(9) were determined as proposed by Pierce and Armonas (8). An estimation of $c_2(v)$ was made by approximating $\bar{M}_w(v) \approx M(v)$ at the peak of each RI chromatogram and by plotting $\ln \bar{M}_w(v)^w$ vs v^p for the different dextran samples. This approximation follows from a reduction of eq.(11b) with $\alpha=1$, $b(v)=0$ and sufficiently small values of the first term between braces. In this case the latter was about 0.03, making an iterative approach unnecessary. In Fig.3 plots are shown similar to that in Fig.2, however, restricted to a small range around the top of the chromatogram. They were obtained by a quadratic least squares approximation. The slopes of the curves vary only marginally for the different samples reflecting the weak dependence of h on v . Estimates of h are found in Table 2. They agree reasonably with estimates recently obtained (10) from a numerical solution of eqs.(1) and (2). Further inspection of Table 2 reveals that the values of \bar{M}_w calculated according to eq.(15) are in agreement with the reference values. Notice that no prior knowledge of h is required for the calculation since $\bar{M}_w(v)$ is available from the measurement. In column 5, Table 2, values of \bar{M}_n are mentioned which have been calculated according to eq.(15) but taking $\bar{M}_n(v) = \bar{M}_w(v)$ or $h = \infty$. These values turn out to be too large. Corrected values of \bar{M}_n obtained for finite h are found in the last column of Table 2.

The correction implies an improvement apart from a few exceptions. In the latter cases the solution for $\bar{M}_n(v)$ was not stable. Obviously it can be circumvented by taking the other calculation pathway via $W(v)$ and $M(v)$ as shown in the model calculation.

CONCLUSIONS.

With eq.(11) we have the disposal of a simple closed expression accounting for imperfect resolution in GPC involving a molecular weight detector. Compared to other similar analytical relationships in literature it has the advantage of combining a general validity to an easy determination of the resolution function $h(v)$.

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