Studies on Simultaneous Molecular Weight and Spreading Calibration in Steric Exclusion Liquid Chromatography

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STUDIES ON SIMULTANEOUS MOLECULAR WEIGHT AND SPREADING CALIBRATION IN STERIC EXCLUSION LIQUID CHROMATOGRAPHY

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ABSTRACT

Two improved methods of molecular weight cali-
bration are described where simultaneously parame-
ters of a symmetrical spreading function are obtained
through the use of polymolecular molecular weight
standards and of average retention volumes. In the
first method a linear molecular weight calibration
is assumed and the second method is based on a uni-
versal molecular weight calibration curve obtained
with narrow MWD polystyrene standards.
    The proposed methods have been tested using
two polymolecular polystyrene standards confirming
good convergence of the applied iteration procedures
and giving very promising results.

INTRODUCTION

Establishing of the molecular weight distribu-
tion /MWD/ curve of polymers by means of the size
exclusion liquid chromatography /GPC/ requires prior
calibration of the molecular weight, M, versus re-

tention volume, V, dependence /1-4/. In previous
publications /5-7/ it was demonstrated that intro-
ducing the concept of average retention volumes,
$\bar{V}$, one simplifies seriously the task of establis-
hing the molecular weight calibration in case of
standards of practically any polymolecularity. The
exponential or logarithmic nonlinearities of the
calibration dependences remain only in the defini-
tions of appropriate average retention volumes while
for the evaluation of the calibration parameters the
simple linear least squares method is adequate. In
this paper improvements of the above mentioned cali-
bration methods are described. Using two characteri-
zed polymolecular polymer standards the true molecu-
lar weight calibration may be established and, simul-
taneously, the spreading parameters of a Gaussian
spreading function determined. In the improved me-
thods either a linear model of the V - lnM dependen-
ce or the universal molecular weight calibration
curve of any shape are applied.

Below the theoretical basis of the proposed me-
thods is described. The performance of both methods
will be illustrated using two broad MWD polystyrene
standards with known values of the number-average,
$\bar{M}_n$, and the weight-average, $\bar{M}_w$, molecular weights.

<div align="center">THEORETICAL</div>

True average molecular weights, $\bar{M}_t$, of poly-
molecular polymer samples depend in the following
way on the experimental average molecular weights,
$\bar{M}_e$, calculated from a chromatogram according to
a true molecular weight calibration /1,8,9/

$$\bar{M}_t = X_{\bar{M}} \cdot \bar{M}_e \tag{/1/}$$

Factors $X_{\bar{M}}$ corresponding to $\bar{M}_n$ and $\bar{M}_w$ may be expressed as /8/

$$X_{\bar{M}_n} = X_n = \exp\left(\sigma^2/2C_2^2\right) \tag{/2/}$$

$$X_{\bar{M}_w} = X_w = \exp\left(-\sigma^2/2C_2^2\right) \tag{/3/}$$

where σ^2, the variance of a single species chromatogram, is the spreading parameter squared and C_2 is the slope of the molecular weight calibration curve at the peak position of the chromatogram or the slope of a linear calibration

$$V = C_1 - C_2 \cdot \ln M \tag{/4/}$$

Average retention volumes are expressed by average molecular weights using the actual model of the molecular weight calibration /5-7/. Using $\bar{M}_t$ the true average retention volume, $\bar{V}_t$, is defined, i.e. a quantity with eliminated spreading effects. One can thus evaluate $\bar{V}_t$ from the experimental chromatogram taking advantage of equation /1/.

Linear Molecular Weight and Spreading Calibration.
Equation /4/ may be written for the true quantities $\bar{V}_t$ and $\bar{M}_t$ as

$$\bar{V}_t = C_1 - C_2 \cdot \ln \bar{M}_t \tag{/5/}$$

Using equation /5/ and equations /1/ - /3/ one can get the following expressions defining $\bar{V}_t$ corresponding to either $\bar{M}_n$ or $\bar{M}_w$

$$\bar{V}_{\bar{M}_{n,t}} = \bar{V}_{n,t} = C_1 - C_2 \cdot \ln\bar{M}_{n,e} - C_2\ln X_n = \bar{V}_{n,e} - \mathfrak{S}^2/2C_2 \quad /6/$$

$$\bar{V}_{\bar{M}_{w,t}} = \bar{V}_{w,t} = C_1 - C_2 \cdot \ln\bar{M}_{w,e} - C_2\ln X_w = \bar{V}_{w,e} + \mathfrak{S}^2/2C_2 \quad /7/$$

The experimental average retention volumes $\bar{V}_{n,e}$
and $\bar{V}_{w,e}$ may be expressed as /7/

$$\bar{V}_{n,e} = C_2 \cdot \ln\left[\ \sum_i\ F(V_i)\ \exp\left(\ V_i/C_2\right)\right] \quad /8/$$

$$\bar{V}_{w,e} = -C_2 \cdot \ln\left[\ \sum_i\ F(V_i)\ \exp\left(-V_i/C_2\right)\right] \quad /9/$$

where $F(V_i)$ is the weight fraction of the experimental
chromatogram calculated for the retention volume V_i
and the summation includes all weight fractions.
As follows from equations /6/ - /9/ for the evalua-
tion of $\bar{V}_e$ one needs the experimental chromatogram,
$F(V)$, and the value of C_2 while for the evaluation
of $\bar{V}_t$ one requires additionally the value of $\mathfrak{S}$.
In order to develop expressions for the iterative
evaluation of parameters C_2 and C_1 equation /5/ may
be written for $\bar{M}_n$ and $\bar{M}_w$ of two samples with broad
MWD. After straightforward transformations of the
above mentioned 4 equations and using the relation

$$\bar{V}_{n,t} + \bar{V}_{w,t} = \bar{V}_{n,e} + \bar{V}_{w,e} \quad /10/$$

one gets the following solutions for C_2 and C_1

$$C_2 = \frac{\bar{V}_{n,e,1} + \bar{V}_{w,e,1} - \bar{V}_{n,e,2} - \bar{V}_{w,e,2}}{\ln\left(\bar{M}_{n,t,2} \cdot \bar{M}_{w,t,2}/\bar{M}_{n,t,1} \cdot \bar{M}_{w,t,1}\right)} \quad /11/$$

$$C_1 = \left[\bar{V}_{n,e,1} + \bar{V}_{n,e,2} + \bar{V}_{w,e,1} + \bar{V}_{w,e,2} + \right.$$
$$\left. + C_2 \cdot \ln\left(\bar{M}_{n,t,1}\bar{M}_{w,t,1}\bar{M}_{n,t,2}\bar{M}_{w,t,2}\right)\right]/4 \quad /12/$$

On RHS of eqs./11/ and /12/ indices 1 and 2 corres-
pond to samples 1 and 2, respectively.
Equation /11/ may serve as a simple expression for
the iterative evaluation of C_2. With a starting
value of C_2 one calculates $\bar{V}_{n,e}$ and $\bar{V}_{w,e}$ of both
samples using equations /8/ and /9/. A corrected C_2
is then calculated from equation /11/. The iteration
calculations are continued until the value of C_2 be-
comes constant at an assumed level of accuracy. In
order to speed up the convergence of the iteration
procedure, in every iteration step except the ini-
tial one, the temporary value of C_2 is taken as
equal to the arithmetic average of C_2 values of two
consecutive iterations /7/. In the last iteration
C_1 is calculated from equation /12/.
Knowing C_1 and C_2 the values of $\bar{M}_{n,e}$ and $\bar{M}_{w,e}$ may
be calculated using the expressions

$$\bar{M}_{n,e}= \exp(C_1/C_2)\cdot\left[\sum_i F(V_i)\exp(V_i/C_2)\right]^{-1} \quad /13/$$

$$\bar{M}_{w,e}= \exp(C_1/C_2)\cdot\left[\sum_i F(V_i)\exp(-V_i/C_2)\right] \quad /14/$$

From equations /1/ - /3/ follows that

$$\sigma_{\bar{M}_n} = \sigma_n = C_2\left[2\ln\left(\bar{M}_{n,t}/\bar{M}_{n,e}\right)\right]^{1/2} \quad /15/$$

$$\sigma_{\bar{M}_w} = \sigma_w = C_2\left[2\ln\left(\bar{M}_{w,e}/\bar{M}_{w,t}\right)\right]^{1/2} \quad /16/$$

Thus the described above simple iterative procedure
enables the evaluation of the linear molecular wei-
ght calibration parameters C_1 and C_2 as well as the
calculation of the spreading parameter σ using
experimental data of two characterized standards
with broad MWD.

<u>Universal Molecular Weight Calibration</u>. The universal molecular weight calibration curve may be generally expressed by a polynomial, i.e.

$$V = \sum_{i=0}^{n} a_i \left(\ln [\eta] M \right)^i = f \left(\ln [\eta] M \right) \qquad /17/$$

and

$$\ln [\eta] M = \sum_{i=0}^{m} b_i V^i = g(V) \qquad /18/$$

where a_i and b_i are parameters of the polynomials f and g, respectively, while n and m are their degrees. Values of a_i and b_i may be evaluated using a series of polystyrene standards with narrow MWD. Before applying the equations /17/ and /18/ for another polymer one should evaluate the α and K parameters of the Mark-Houwink relation

$$[\eta] = K \cdot M^{\alpha} \qquad /19/$$

It follows from equations /18/ and /19/ that

$$\ln K + \left(\alpha + 1 \right) \ln M = g(V) \qquad /20/$$

For polymolecular samples equation /20/ may be expressed as

$$\ln K + \left(\alpha + 1 \right) \ln \bar{M}_t = g(\bar{V}_t) \qquad /21/$$

where $\bar{M}_t$ and $\bar{V}_t$ have their former meanings. Writing equation /21/ for $\bar{M}_{n,t}$ and $\bar{M}_{w,t}$ of one polymolecular standard a set of two equations is obtained which may be solved for α and K giving the following expressions .

$$\alpha = \left[g\left(\bar{V}_{w,t}\right) - g\left(\bar{V}_{n,t}\right)\right]/\ln\left(\bar{M}_{w,t}/\bar{M}_{n,t}\right) - 1 \qquad /22/$$

$$\ln K = \left[g\left(\bar{V}_{n,t}\right) + g\left(\bar{V}_{w,t}\right) - (\alpha + 1)\ln\left(\bar{M}_{n,t}\cdot\bar{M}_{w,t}\right)\right]/2 \quad /23/$$

Equation /22/ may serve as a simple expression for the iterative evaluation of α. Values of $\bar{V}_{n,t}$ and $\bar{V}_{w,t}$ may be calculated according to equations /6/ and /7/, respectively, while $\bar{V}_{n,e}$ and $\bar{V}_{w,e}$ are to be calculated from the following equations /7/

$$\bar{V}_{n,e} = f\left[-(\alpha + 1)\ln\left(\sum_i F(V_i)\exp\left(-g(V_i)/(\alpha+1)\right)\right)\right]/24/$$

$$\bar{V}_{w,e} = f\left[(\alpha + 1)\ln\left(\sum_i F(V_i)\exp\left(g(V_i)/(\alpha+1)\right)\right)\right] \quad /25/$$

The procedure of iterative evaluation of α according to equation /22/ is identical to the procedure of evaluation of C_2 based on equation /11/. In order to calculate values of σ from either equation /15/ or /16/ the $\bar{M}_{n,e}$ and $\bar{M}_{w,e}$ values should be evaluated using the following relations /7/

$$\bar{M}_{n,e} = K^{-1/(1+\alpha)}\left[\sum_i F(V_i)\exp\left(-g(V_i)/(\alpha+1)\right)\right]^{-1} \quad /26/$$

$$\bar{M}_{w,e} = K^{-1/(1+\alpha)} \sum_i F(V_i)\exp\left(g(V_i)/(\alpha+1)\right) \qquad /27/$$

Thus the described simple iterative procedure enables the evaluation of the parameters α and K of the universal molecular weight calibration curve using experimental data of one characterized standard with broad MWD. Preliminary evaluations of the slope C_2 of the linear V-lnM relation and of the spreading parameter σ are needed prior to the above iterative calculations.

EXPERIMENTAL

All experiments were carried out at ambient temperature using the Waters ALC/GPC model 301 instrument equipped with a set of 3 linear columns of 91.5 cm length and 0.78 cm inner diameter filled with styragel of 10^4 nm nominal porosity. Tetrahydrofuran /THF/ was used as the mobile phase and differential refractometer model R-401 was the detector. The model linear molecular weight calibration was measured with a series of 8 narrow MWD polystyrene standards using the peak retention volumes, V_p, of the chromatograms and the corresponding to them peak molecular weights, M_p, supplied by Waters. In order to obtain a non-linear universal molecular weight calibration curve of the applied set of columns two additional standards with low molecular weights were used. The molecular weight and GPC data of all the PS standards and of the styrene monomer are given in Table 1.

TABLE 1

Narrow MWD PS Standards and Styrene Employed for Evaluating of Linear Molecular Weight and Universal Calibrations

Standard	M_p	$V_p(cm^3/5cm^3)$
1	830 000	15.08
2	451 000	15.94
3	196 000	17.45
4	111 000	18.11
5	35 000	19.73
6	20 500	20.68
7	9 800	21.68
8	3 550	22.98
9	2 025	23.61
10	104	26.9

For the first 8 PS standards the following linear molecular weight calibration was obtained

$$V = 35.077 - 1.462 \ln M, \quad cm^3/5cm^3 \qquad /28/$$

The universal molecular weight calibration curves were obtained using the polynomial regression for 10 pairs of values of V_p and $\ln K + (\alpha + 1) \ln M_p$ where for polystyrene in THF at ambient temperature the following often applied Mark-Houwink constants were used /9/ : $K = 1.60 . 10^{-2}$ cm^3/g, $\alpha = 0.706$. According to statistical criteria /10/ polynomial of second degree was the optimum model of the $V-\ln[\eta]M$ relation - equation /17/, and the a_i coefficients of the polynomial f have the following values

$$(a_0, a_1, a_2) = (28.76, -0.5042, -0.01229) \quad /29/$$

For the $\ln[\eta]M - V$ relation - equation /18/, polynomial of the third degree fitted the experimental data best and the b_i coefficients of the polynomial g are as follows

$$(b_0, b_1, b_2, b_3) = (38.82, 0.05751, -1.901, -0.001331)$$
$$/30/$$

In order to evaluate the proposed in this work two new calibration methods, 2 well characterized PS standards with broad MWD were applied. With polystyrene as the testing polymer comparisons of corresponding results are straightforward. The molecular weight data for the two PS standards are given in Table 2 where also are values of $\bar{M}_n$ and $\bar{M}_w$ calculated with the calibration equation /28/ from the

TABLE 2

Broad MWD PS Standards Employed with Molecular
Weight and Universal Calibration Methods

Stan-dard	$\bar{M}_n \cdot 10^{-3}$		$\delta \bar{M}_n$ %	$\bar{M}_w \cdot 10^{-3}$			$\delta \bar{M}_w$ %
	Osm.	GPC		l.sc.[1]	sed.[2]	GPC	
1.Lus-trex	70	68	-2.9	230[1]		234	1.7
2.NBS 706 a/	136.5	120.3	-11.9	257.8[1]		290.3	12.6
b/					283.1[2]		

uncorrected chromatograms of the samples together
with their percentage deviations from the true va-
lues, i.e. from the values of $\bar{M}_n$ and $\bar{M}_w$ measured by
the osmometric /osm./ and the light scattering
/l.sc./ or the sedimentation /sed./ methods.

RESULTS

Linear Molecular Weight and Spreading Calibrations.
The linear molecular weight calibration was establi-
shed with the data of two broad MWD PS standards gi-
ven in Table 2 and their experimental chromatograms
using equations /11/ and /12/. The calculations were
made for pairs of $\bar{M}_n$ and $\bar{M}_w$ values of the standards.
Two variants of the performed calculations correspond
to two different values of $\bar{M}_w$ of NBS 706. The calcu-
lations were started with an initial value of $C_2=2$
and continued until C_2 became constant with a rela-
tive accuracy of 10^{-4} what corresponds to the per-

centage deviations of average molecular weights
being less than 1%. Number of the iteration steps
did not exceed 15. Finally, values of the spreading
parameter σ were obtained from either equation /15/
or /16/. Results of the calibration calculations are
given in Table 3.

Universal Molecular Weight Calibration. Using equa-
tions /22/ and /23/ the Mark-Houwink coefficients
were calculated separately for each of the standards
characterized in Table 2. The average retention vo-
lumes $\bar{V}_{n,t}$ and $\bar{V}_{w,\tau}$ were corrected for spreading
with equations /6/ and /7/, respectively, and values
of the coefficients C_2 and σ were taken from
Table 3 since the preceding linear molecular weight
calibration based on the same PS standards could
serve as the necessary linear approximation of the
true calibration curve. The iteration calculations
were started with α =0.706 and in all 4 variants of
possible C_2-σ combinations taken from Table 3 no

TABLE 3

Results of Linear Molecular Weight and Spreading
Calibration with 2 Broad MWD PS Standards with
Known $\bar{M}_n$ and $\bar{M}_w$

Pair of Standards	C_1 C_2 $cm^3/5cm^3$		No. of Standard	σ_n σ_w $cm^3/5cm^3$	
1 + 2a	35.437	1.492	1	0.1784	0.1782
			2	0.6853	0.6852
1 + 2b	33.735	1.545	1	0.7227	0.7227
			2	0.7579	0.7579

TABLE 4

Results of Universal Molecular Weight Calibration
with Single Broad MWD PS Standard with Known $\bar{M}_n$
and $\bar{M}_w$

Standard C_2, σ	α	$K \cdot 10^2$ cm^3/g	σ_n	σ_w	$(\sigma_n + \sigma_w)/2$
				$cm^3/5cm^3$	
1 - a 1.492, 0.1783	0.7127	0.96	0.0582	0.2880	0.1731
1 - b 1.345, 0.7227	0.5489	6.35	0.7079	0.7392	0.7236
2 - a 1.492, 0.6853	0.6896	1.29	0.6461	0.7097	0.6779
2 - b 1.345, 0.7579	0.5261	8.53	0.7263	0.7733	0.7498

more than 8 iteration steps were needed in order to
establish α with an absolute accuracy of 10^{-4}.
With the   obtained values of α and K the $\bar{M}_{n,e}$
and $\bar{M}_{w,e}$ average molecular weights were calculated
with equations /26/ and /27/ and, finally, values
of σ_n and σ_w were recalculated according to equa-
tions /15/ and /16/, respectively. Results of the
calibration calculations are given in Table 4.

DISCUSSION

The described above improved methods of molecu-
lar weight calibration are based on the assumption
that the instrumental spreading is Gaussian. For the
case of linear molecular weight calibration equation
/28/ obtained with a set of narrow MWD PS standards

may be considered as the true calibration relation. Thus the percentage deviations $\delta\bar{M}$ of average molecular weights of the 2 polymolecular PS standards which are given in Table 2, columns 4 and 7, characterize the instrumental spreading. Inspection of the above mentioned $\delta\bar{M}$ values leads to the conclusion that in the case of the standard NBS 706 either the value of $\bar{M}_w$ measured by light scattering is the correct one or the instrumental spreading is not symmetrical. On the other hand values of $\delta\bar{M}$ obtained for the standard Lustrex support the assumption of Gaussian spreading. It is most probable that in the case of NBS 706 the value of $\bar{M}_w$ measured by the sedimentation method is less reliable than that obtained by light scattering.

The applied experimental data allowed thus testing of the proposed calibration methods in the case of fulfilled assumptions of the symmetrical spreading /light scattering $\bar{M}_w$ values corresponding to variants a/ and in the case of either non-Gaussian spreading or, what seems to be more probable, of less accurate value of $\bar{M}_w$ of one of the PS standards /sedimentation value of $\bar{M}_w$ for NBS 706 corresponding to variants b/.

Results of the tests in the first case are good both for the linear as well as for the universal molecular weight calibrations. The evaluated values of the parameters C_1 and C_2 are very close to the true ones in equation /28/ /compare results obtained for the variant 1+2a, Table 3/. Similarly values of the parameters α and K obtained by the improved universal molecular weight calibration method for variants 1-a

and 2-a /compare Table 4/ are close to the true va-
lues estimated with a set of narrow MWD PS standar-
ds, i.e. to values α =0.706 and K=1.60.10^{-2} cm^3/g.
Obviously, results obtained with the new calibra-
tion methods are less satisfactory when either the
measured average molecular weights of the broad MWD
standards are erroneous or the spreading deviates
from a symmetrical one. This is illustrated by the
results obtained for the parameters C_1 and C_2
/Table 3/ as well as for α and K /Table 4/ which
correspond to variants b.

Values of the spreading parameter σ of the two
broad MWD PS standards obtained for variants a and
given in Table 3 are consistent with the correspon-
ding $\sigma \bar{M}$ deviations given in Table 2. The differen-
ces between values of σ_n and of σ_w given in Table
4 are due to the more accurate modelling of the ca-
libration relation in the region of low molecular
weights what was achieved by the addition of two
standards with low M while establishing the univer-
sal molecular weight calibration with narrow MWD PS
standards /compare Table 1, rows 9 and 10/.

It may be concluded that the proposed in this
work two improved methods of linear molecular weig-
ht and spreading calibration and of universal mole-
cular weight calibration using broad MWD standards
are simple in application. The performed experimen-
tal evaluations indicate that both methods give very
promising results and the convergence of the cor-
responding iteration procedures is good.

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