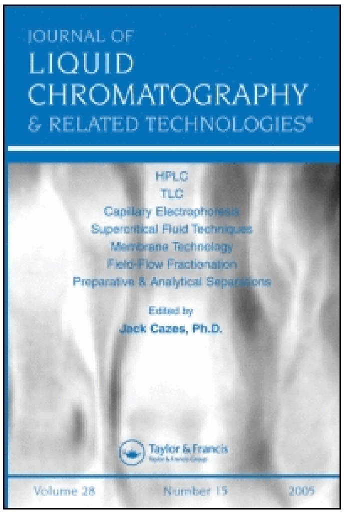

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Sommer, C. and Müller, G.(2001) 'RELATIONSHIPS BETWEEN SEPARATION POWER AND THE MW AVERAGES IN SEC MEASUREMENTS WITH MOLECULAR MASS SENSITIVE DETECTORS', Journal of Liquid Chromatography & Related Technologies, 24: 8, 1047 — 1060

To link to this Article: DOI: 10.1081/JLC-100103430

URL: http://dx.doi.org/10.1081/JLC-100103430

J. LIQ. CHROM. & REL. TECHNOL., 24(8), 1047–1060 (2001)

RELATIONSHIPS BETWEEN SEPARATION POWER AND THE MW AVERAGES IN SEC MEASUREMENTS WITH MOLECULAR MASS SENSITIVE DETECTORS

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ABSTRACT

Polystyrenes (standards with different molecular weights and broadly distributed polymers) and 1.4-cis polybutadienes had been characterized by online coupling of SEC columns with both light scattering (MALLS) and viscosity detectors. SEC columns with differences in separation power had been used.

Investigations were done on the dependence of the substance specific calibration curves and the exponent α the KMH-equation on the columns; the differences between the substance specific calibration curves for narrowly distributed standards and for polystyrenes with a broader distribution; the mutual influence of the components in polymer mixtures on separation and the effect on resulting molecular weight distribution.

The averages of the molecular weight, calculated from the measured distribution, depend on the resolution of the SEC

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columns. The reason for this is a mutual influence of the components in the polymer mixture. A consequence of this is a change in the distribution curve: the molecular weight distribution becomes broader despite the polydispersity decreasing (the M_n value is overestimated). The maximum of each peak is shifted in mixtures of standards related to single measurements. This fact does not appear in the use of high resolution columns.

The slope of the substance specific calibration curve also depends on the separation power of the columns in both the SEC light scattering and the SEC viscosity coupling. It can be neglected only for narrowly distributed polymers ($M_w/M_n < 1{,}2$). For higher polydispersties we have to reckon on a clear influence, and for those polymers we get wrong values for the number average molecular weight and for the exponent α in the KMH equation. The problem is that the molecular weight sensitive detectors always return weight averages of the measured values. In these cases, the measurement values depend on the broadness of the distribution in the examined slice. This means that the local dispersion of the slices in the elugram becomes important for the calculation of the desired value.

This effect is not only essential for determination of molecular weight distributions as described; it is also important for polymers with heterogeneity in the molecular architecture (LCB) or in copolymers with chemical composition distributions.

INTRODUCTION

The use of molecular mass sensitive detectors in SEC has become a common analytical technique, giving absolute values for the molecular mass distribution and its means, as well as for the frequently needed structure parameter ($\langle r^2 \rangle = f(M)$ from light scattering measurements) or Kuhn Mark Houwink (KMH) exponents from viscosity data. The instruments developed for this purpose are based on measurement principles suitable for flow technique. Static light-scattering and viscosity detectors[1] in various implementations are commercially available, in which the measurement procedures based on changes of solution viscosity require the validity of universal calibration.

The exclusion effect in chromatography, which had been confirmed as a theoretical model in SEC in recent studies by Liu,[2] is only used for separation of the macromolecules by size, respectively, by hydrodynamic volume and is no longer important for their identification. Further detectors working with dynamic light-scattering and membrane osmometric principles are still in the experimental stage.[3]

In spite of the highly sensitive light-scattering measurement technique, it was reported in the literature[4,5,6] that even for the shortest connections between separation columns and detectors there is an over-estimation of number average molecular masses (M_n) for polymers with broad distributions. On the other hand, the values for the weight averages (M_w) are found to be correct. The evaluation of the exponents of KMH equation using the commercial measurement techniques named above is also problematical. The values found by us often lie outside of the expected ranges.

These uncertainties are caused not only by the evaluation methods (for instance the cutting problem in chromatogram flanks or the divergent extrapolations of the measured scattering intensities for LS detection with detectors of different types) but also directly by the combination of separation and evaluation methods.

While these effects are insignificant for the use of high resolution columns, for mixed bed columns with particle sizes of 10 μm and linear separation range across several decades (polystyrene in tetrahydrofuran) they clearly become important and have to be considered in evaluation of the measurements.[7] The problem of the influence of dispersion in SEC columns had been worked out in detail during the development of SEC as a routine method. After the introduction of high performance columns it had been considered of low importance and therefore neglectable. As the current paper shows, this is not always justified when molecular mass sensitive detectors are used.

It becomes more clear, especially when correlation between theoretically calculated molecular mass distributions based on a presumed kinetical scheme (for instance with frame program for multiple reactions PREDICI) and experimental data is needed,[8] and for some reason (for instance for very high molecular polymers) high resolution columns are not suitable.

EXPERIMENTAL

A huge number of polystyrene (PS) samples (polymer standards and polymers with a broader distribution) were investigated; the solvent was tetrahydrofuran (THF). Measurements for all sample sets were performed on a modular SEC apparatus. For separation, an universal separation column (Waters HMW6E) with a wide separation range (about $5{\cdot}10^3$ to $1{\cdot}10^7$ g/mol) but lower resolution was used, along with a combination of two high resolution columns (Waters HT5+HT4) with a linear separation range of $2{\cdot}10^4$ to $1{\cdot}10^6$ g/mol for comparison.[8]

Concentration detection was done with a refractive index detector (Wyatt Optilab DSP), for measurements of scattering intensity a multi angle light-scattering detector (Wyatt DAWN DSP-F), and for viscosity a flow capillary viscometer (Knauer 200) were used. All measurements were done at room temperature. The injection volume was 100 µL, the total concentration of the samples was about 1 mg/mL.

Calculations of the light-scattering data were performed with the software Astra 4.70, and for viscosity measurements we used WinGPC 4.02.

RESULTS

By investigation of narrow distributed PS standards with different molecular masses, and of technical products, as well as single substances as in mixtures, the degree of influence of SEC separation on resulting molecular mass distributions and their means, and on the exponents of KMH equation could be obtained.

First, we examined with light scattering position and tendency of the substance specific calibration curve (SSCC) using the universal column and the high resolution combination. The calibration curves were created with narrow and also with broader PS standards. As usual, the narrow distributed samples were evaluated by plotting the molecular mass at maximum of the elution curve via the elution volume.

Comparing the slopes of the linear part of the curves, they show clear differences for the HMW6E column. For the broadly distributed PS, the slope is significantly flatter. The curves cross at maximum of elution curve of the broader polymer (Figure 1). This effect does not depend on concentration and has been confirmed for a lot of other polymers (polystyrenes and polybutadienes). On the other hand, for the high resolution columns the slopes of SSCC are identical within the linear range of the curves ($2.5{\cdot}10^4 – 1{\cdot}10^6$ g/mol), regardless of the broadness of the sample distribution.

Furthermore, the SSCC of polystyrenes with comparable broadness of distribution show, on the universal column, a parallel shift in dependence of their weight average molecular masses. For polybutadienes, such a grading of the curves also was found; examples are also found in literature.[9] In Figure 2, this is shown for two polystyrenes with a polydispersity of $M_w/M_n = 2$.

The mutual influence of narrowly distributed polymers during separation was investigated using mixtures of PS standards. For a mixture of three polymers with close molecular masses (M_p: 97000, 201000, 404000 g/mol with a mass fraction of 1:2:1) with light scattering, we got M_n = 218000 g/mol and M_w = 242000 g/mol. For the named composition, the theoretically expected molecular mass was calculated from the LS data of the single standards to M_n = 221000 g/mol and M_w = 275000 g/mol. With the help of mathematical peak separation of the distribution of the mixture (software Peakfit 4.0, base of calculation: logarithmic normal distribution), the molecular masses and areas of the single components could be calculated (Table 1).

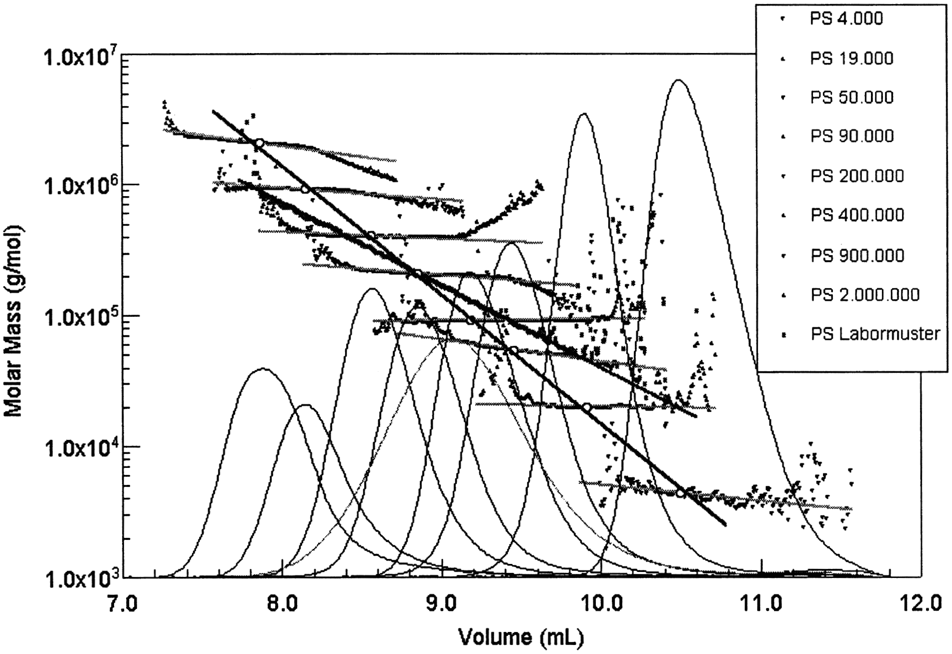


Figure 1. SSCC for PS standards (open circles = concentration maxima of elution curves) and a broadly distributed PS sample (universal column).

The molecular mass distribution of the mixture is, as expected, heavyly broadened compared with the individual standards. Formally viewed, it is found to be of nearly unimodal distribution. Of interest here, is that the signals of concentration profiles start and end at the same elution volume as for the single standards. However, the calculated molecular mass distribution shows parts in the high and low molecular ranges, which are physically impossible (Figure 3).

For the plot molecular mass vs. elution volume, there is a steady decrease of the curve without any steps across the entire concentration range. However, for each single standard the curve runs parallel to the X axis, corresponding to the theory. The mutual influence of both of the mixture parts is clearly visible. For elution volumes with higher molecular masses expected, small molecules are also detected and vice versa.

Table 1 shows that the molecular mass averages of the mixture is calculated correctly within the measurement error; but the area percents of the partial peaks obtained by peak separation differ significantly. The polymer standard with low M_w is shifted to higher molecular masses, the one with the highest M_w to lower molecular masses. The calculated peak areas clearly verify the compression of

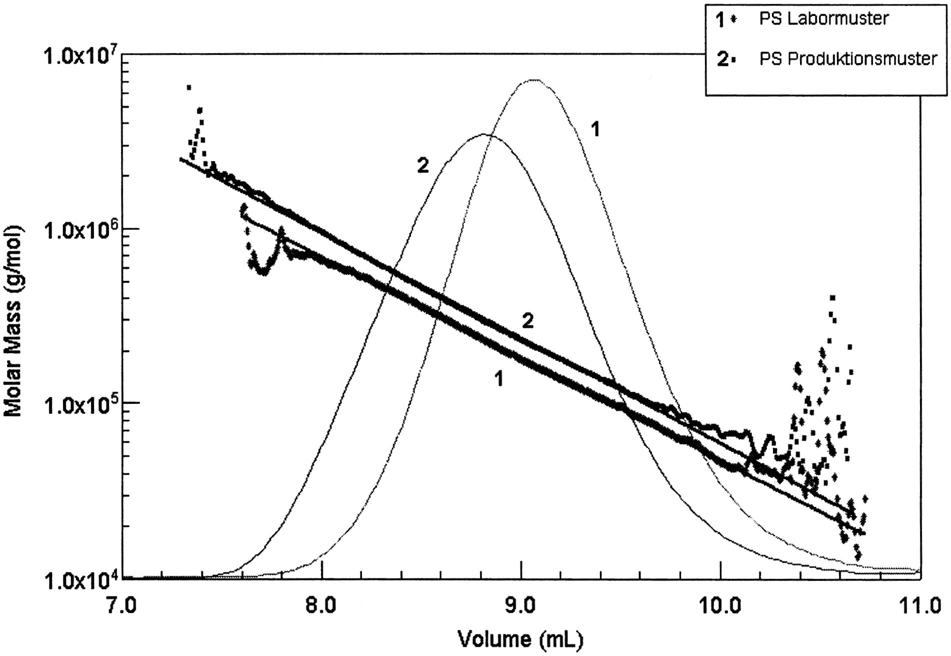


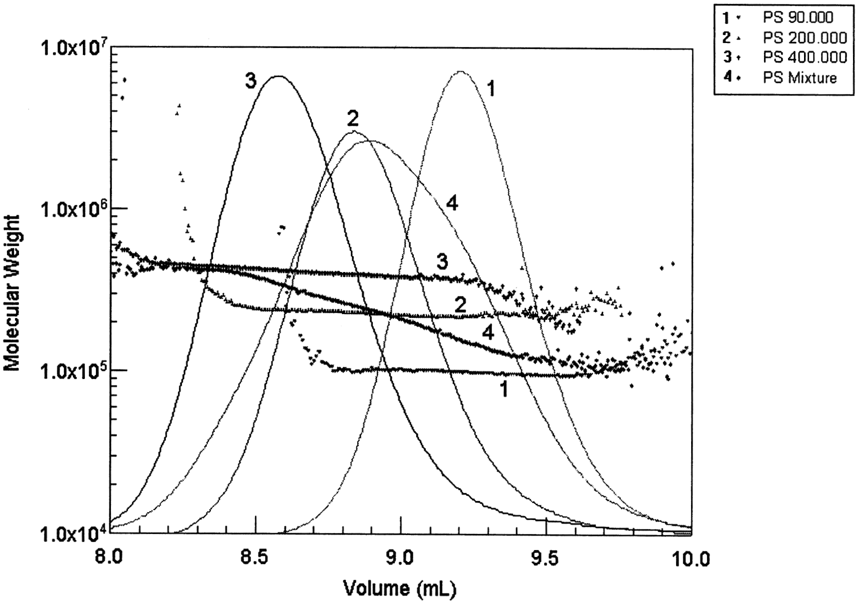
Figure 2. Shift of SSCC with molecular mass for two PS samples (universal column).

the distribution. Additionally, the three partial peaks are broadened in relation to the single measurements of the standards. M_w/M_n is on average 1.069 vs. 1.012 for the single standards.

With the universal column, the influence of uniformity of the samples on separation using mixtures of two PS standards has also been investigated. Polydispersity M_w/M_n has been varied by use of mixtures with different mass

Table 1. Averages of Molecular Masses and Area Percents of the Individual Components in a Mixture of Three PS Standards (Universal Column)

Sample	M_n (g/mol)	M_w (g/mol)	M_w/M_n	w (Calculated)	w (Mixed)
100000	140000	148500	1.061	17.5 %	23.9 %
200000	235000	256000	1.089	66.3 %	52.4 %
400000	377000	399000	1.058	14.9 %	23.7 %
Total	240000	278000	1.158	98.8 %	100 %



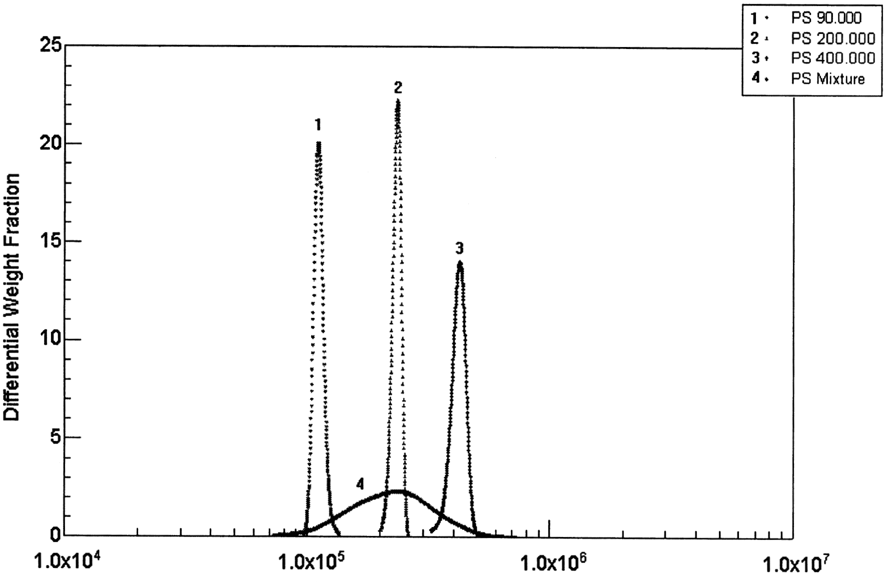


Figure 3. Above: SSCC for PS standards - comparison between individual measurements (1–3) and a mixture of standards (4); below: resulting molecular mass distributions (universal column).

fractions of two standards (molecular masses 55000 and 400000 g/mol). The broadening of the molecular mass distributions can plainly be seen (Figure 4), and the peak maxima are shifted to average values in relation to the individual standard measurements.

In Table 2 the averages are collected. While the weight averages agree with the theoretically expected values within the measurement errors, for M_n there is a bigger discrepance. The values are calculated too high with increasing mass fractions of the high molecular component. A correlation with the distribution broadness of the mixture cannot be found; for the calculated molecular masses only the mass fractions of the polymers are decisive. In contrast to this, using the high resolution column combination there is only a little broadening of the distributions and no peak shift at all.

In cases of mixtures of broader distributed polymers rather than narrower standards, there are no considerable divergences in M_n and M_w from the theoretically expected values, in contrast to the above case. Both with the universal column and with the high resolution column the same results are found (Tables 3, 4).

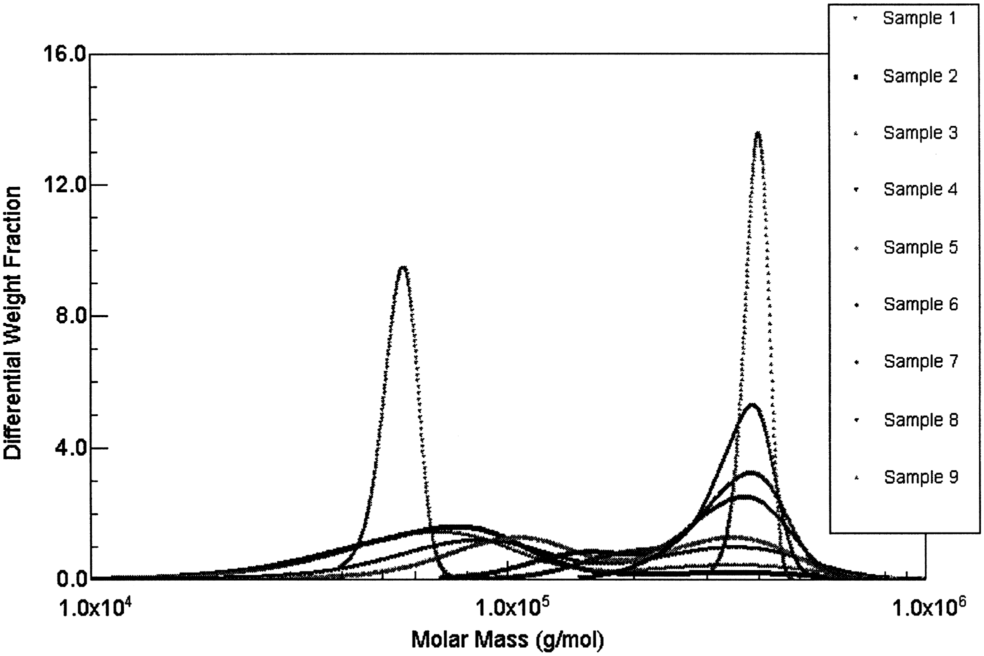


Figure 4. Molecular mass distributions for mixtures of PS standards 55000 (1) and 400000 (2) g/mol (variation of composition from 0 to 100 ma% (1), universal column).

Table 2. Average Molecular Masses, Theoretically Calculated and Experimentally Found from Mixtures of PS Standards (Universal Column)

Sample	w_2 (ma%)	M_n^{LS} (g/mol)	M_w^{LS} (g/mol)	M_w / M_n	M_n^{theor} (g/mol)	M_w^{theor} (g/mol)
PS 55000	0.0	54000	55000	1.019		
Mixture 1	10.9	57000	102000	1.789	60000	92000
Mixture 2	2.7	64000	136000	2.125	66000	125000
Mixture 3	44.5	102000	203000	1.990	88000	206000
Mixture 4	51.4	136000	222000	1.632	97000	229000
Mixture 5	77.7	247000	307000	1.243	164000	318000
Mixture 6	82.5	290000	332000	1.145	187000	335000
Mixture 7	91.5	337000	352000	1.045	256000	365000
PS 400000	100.0	392000	394000	1.005		

The result is understandable, because in this case the eluted parts of the measured polymers already show a distribution of the property, which is not intensified in mixtures. For narrowly distributed polymers (standards), however, the distribution of property is created during the separation process.

Using the viscosity detector for evaluation of the KMH equation, analoguos results are found. If the column HMW6E is used, the SSCC (intrinsic viscosity [η] vs. Elution volume V_e) for broadly distributed samples runs much flatter than the curve obtained for calibration with narrow standards ([η] at concentration maximum plotted against elution volume). This leads to a much too small exponent, α, in KMH equation (Table 3). Additionally, there is a significant parallel shift between the sample curves, exactly as that found in light scattering.

Table 3. Theoretically Calculated and Experimentally Found Molecular Masses and Exponent α of KMH Equation for Various Mixtures of Broadly Distributed PS Standards (Universal Column)

Sample	w_2 (ma%)	M_n^{LS} (g/mol)	M_w^{LS} (g/mol)	M_w / M_n	M_n^{theor} (g/mol)	M_w^{theor} (g/mol)	α_{KMH}
Labormuster	0.0	104000	181000	1.740			0.293
Mixture 1	21.7	116000	216000	1.896	115000	218000	0.297
Mixture 2	32.0	120000	231000	1.934	122000	236000	0.300
Mixture 3	59.9	144000	289000	1.979	143000	283000	0.310
Mixture 4	81.9	166000	319000	1.934	166000	321000	0.311
Produktionsmuster	100.0	192000	352000	1.833			0.302

Table 4. Theoretically Calculated and Experimentally Found Molecular Masses and Exponent α of KMH Equation for Various Mixtures of Broadly Distributed PS Standards (High Resolution Columns)

Sample	w_2 (ma%)	M_n^{LS} (g/mol)	M_w^{LS} (g/mol)	M_w / M_n	M_n^{theor} (g/mol)	M_w^{theor} (g/mol)	α_{KMH}
Labormuster	0.0	93000	188000	2.022			0.636
Mixture 1	30.8	116000	238000	2.243	107000	240000	0.668
Mixture 2	42.9	109000	261000	2.289	114000	261000	0.723
Mixture 3	56.2	130000	279000	2.309	123000	284000	0.729
Mixture 4	74.8	137000	317000	2.283	138000	315000	0.729
Produktionsmuster	100.0	165000	358000	2.170			0.663

However, for separation with high resolution columns, only a slightly parallel shift of the curves occurs. The slopes are nearly equal (Table 4) and fluctuate statistically around the value for PS in THF, as reported in the literature (α = 0.71).

In comparison of the constants of KMH equation obtained from narrow PS standards (single measurements, evaluation: [η] and M at maximum of elution curve) between universal column and high resolution combination, the values agree (Table 5). This was expected, because the molecular mass sensitive property at maximum of the elution curve is independent of the quality of separation (Figure 1), and therefore, a correlation between separation power and average of property doesn't exist.

DISCUSSION

With light-scattering, as well as with viscosity detection, comparable results are found. The slopes in plots M_w vs. V_e and [η] vs. V_e depend on separation power of the column and on distribution broadness of the characterized poly-

Table 5. Relation Between [η] and M for PS Standards; Molecular Mass and [η] at Maximum of Elution Curve (Intrinsic Viscosity in dl/g)

	(M < 15.000)		(M ≥ 15.000)	
Column	α	K	α	K
HT5+HT4	0.553	$5.3 \cdot 10^{-4}$	0.713	$1.1 \cdot 10^{-4}$
HMW6E	0.521	$6.7 \cdot 10^{-4}$	0.708	$1.2 \cdot 10^{-4}$

mer. If separation power is high, the effects shown above are small and can be neglected. Otherwise, they have to be considered in interpretation of the measurement results. The reason for this is that the molecular weight sensitive detectors respond to averages. As for the light-scattering result, it is always a weight average molecular mass $M_{w,i}$ even for very small elugram slices. In the same way, the calculated intrinsic viscosity is only an average value.

If the selected column cannot separate the macromolecules in a sufficient amount of monodisperse fractions, then for summation of the product of concentation and property value an error results, which becomes larger as the separation power becomes smaller. This means that if the individual slices in elugram show a distribution (local dispersion), the M_i used for calculation of average molecular masses are really $(M_w)_i$ values. Calculating the averages gives true M_w, but M_n is only roughly correct for narrow polymers. For broad samples this value will be overestimated.

In the online detection of solution viscosity, the evaluation algorithm is different but leads to the same results. The intrinsic viscosity values calculated for every point of the elugram are averages in the case of the existence of a noticeable local dispersion, and differ numerically from those for monodisperse fractions. The bigger the polydispersity, the smaller the value for $[\eta]$ becomes (supposing equal molecular mass). Each $[\eta]$ value refers to a molecualar mass by universal calibration. Since the evaluation is based on the idea of constant products of intrinsic viscosity and molecular mass $([\eta]\cdot M)_{Ve}$ at given elution volumes, intrinsic viscosity values found to be too low result in the calculation of molecular masses which are too high because of local dispersion. This leads to consequences for KMH plot. The slopes in the double logarithmic plot decrease with increasing dispersion.

A molecular mass M_v obtained in this way can be described for each slice with the inequation $M_n < M_v < M_w$. Summation of $(c\cdot M_v)_i$ then leads to a difference between the averages M_n and M_w describing the analyzed polymer and the averages detected by light-scattering. This becomes especially noticeable for the number average value in agreement with the assumptions of average calculation in viscosity and LS detection. When M_n^{Visc} is plotted against M_n^{LS}, this correlation is also clearly visible in the dependence of polymer broadness (Figure 5).

With the help of a simple model consideration, it can be estimated how large the influence of local dispersion on the molecular mass averages is. The starting point is a model distribution of Gaussian type and polydispersity of $M_w/M_n = 1.6$. Each slice of the distribution is assumed not to be unimodal with molecular mass M_i (ideal separation), but rather a Gaussian distribution with an average molecular mass $\underline{M}_i$ and standard deviation $\sigma_{M,i}$. σ increases with molecular mass though the uniformity of all slices is constant.

That means, that like with real separation, the molecules with M_i will be eluated not only in slice i (at elution volume $V_{e,i}$), but also partially in slices i-n, ... ,

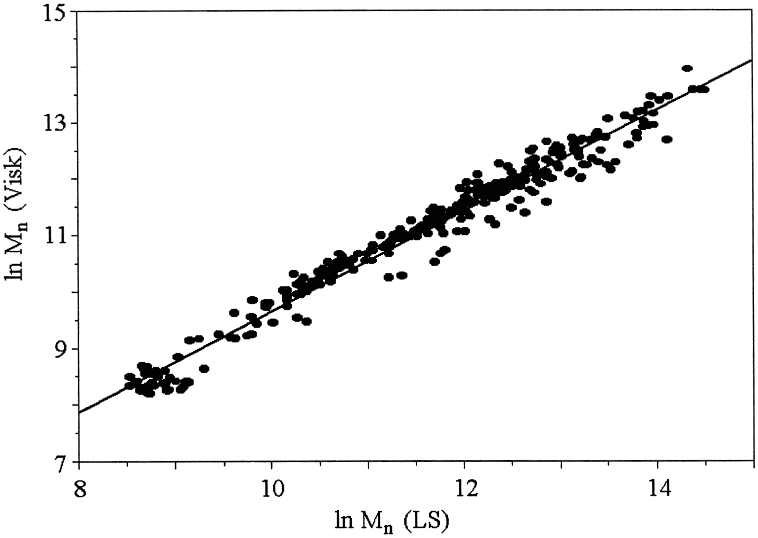


Figure 5. Plot of M_n (light scattering) vs. M_n (viscosity) for 1.4-cis PB samples with M_w/M_n of 1.3..10 (universal column).

i−1, i+1, ... , i+n. At the same time additional molecules with M_{i-n} ... M_{i+n} appear in slice i.

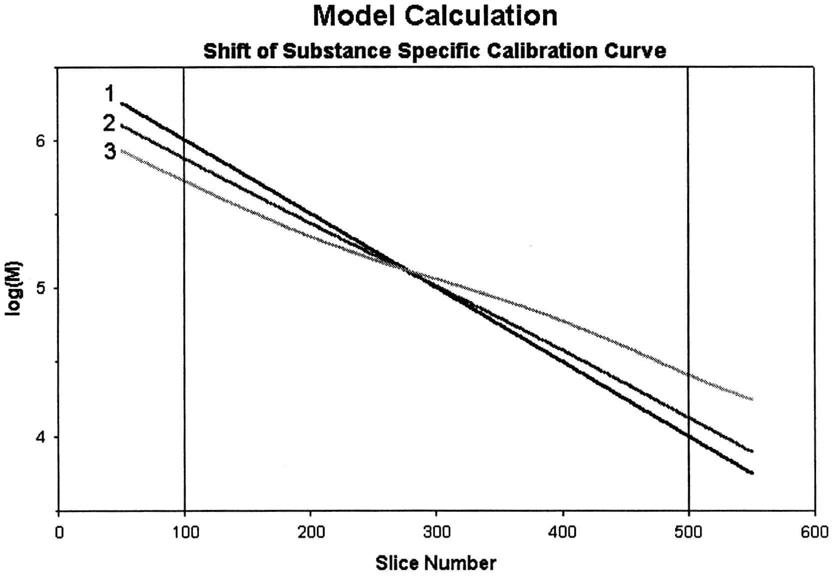
Based on the molecular mass distribution of the ideal separation, we can now calculate a corrected mass fraction w’ and a corrected (average) molecular mass M’ for each slice by consideration of n neighbour slices:

$$w'_{(\mathrm{Slice})} = f_{-n} \cdot w_{-n} + f_{-n+1} \cdot w_{-n+1} + \ldots + f_{n-1} \cdot w_{n-1} + f_n \cdot w_n$$

$$M'_{(\mathrm{Slice})} = (f_{-n} \cdot w_{-n} \cdot M_{-n} + f_{-n+1} \cdot w_{-n+1} \cdot M_{-n+1} + \ldots + f_{n-1} \cdot w_{n-1} \cdot M_{n-1} + f_n \cdot w_n \cdot M_n) / w'_{(\mathrm{Slice})}$$

with f = factor of the nth neighbour from the assumed Gaussian dispersion function.

As a result, the SSCC M-V_e changes its shape with local dispersion. The bigger the dispersion (consideration of more neighbours) becomes, the lower the slope of the curve associated with an increasing S-shape bending will be. This corresponds to the experimental results reported above. Simultanously, the elugram, and therefore, the molecular mass distribution broadens because of the dispersion of edge slices (Figure 6). By cutting the elugram at the former peak limits and because of the increasing asymmetry of M-V_e curve, an (apparent) compression of the elugram appears.



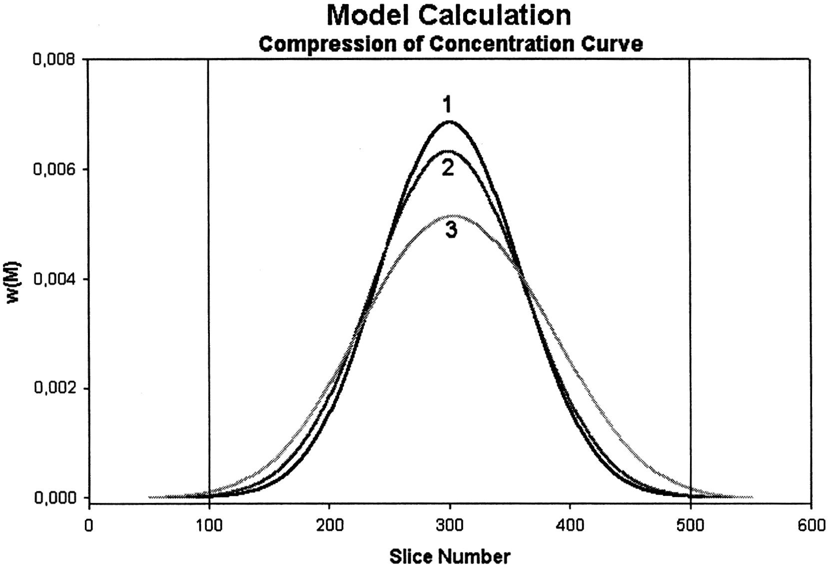


Figure 6. Model calculation - above: change of SSCC with local dispersion, below: change of elugram with local dispersion (1 = without dispersion, 2 = slightly dispersion, 3 = strong dispersion).

Table 6. Calculated Number and Weight Average Molecular Masses of a Model Distribution with Consideration of n Interfering Neighbour Slices

n	M_n (g/mol)	M_w (g/mol)
0	79800	125300
50	85200	125200
100	97700	125000

The weight average molecular mass remains constant, but the number average increases visibly (Table 6). This is in good agreement with the measured average values.

ACKNOWLEDGMENTS

We have to thank the Bundesministerium für Bildung und Forschung and the Fond für Chemische Industrie for financial support.

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Received October 5, 2000
Accepted October 19, 2000
Manuscript 5408