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SEC of Ultra-high Molar Mass Polymers: Optimization of Experimental Conditions to Avoid Molecular Degradation in the Case of Narrow Polystyrene Standards*

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Size exclusion chromatography (SEC) of commercially available polystyrene standards with narrow distribution of molar mass and weight average molar mass (M_w) up to 17,000 kg/mol was carried out in order to determine the influence of the concentration, injected volume of sample solutions, average size of the gel particles of the stationary phase within the columns, flow rate, as well as the system filters on the detected molar mass and its distribution. Low-angle laser light-scattering (LALLS) was used online to measure M_w and detect the drop in molar mass in the case of molecular degradation due to mechanical forces acting in the SEC system. For samples with $M_w < 5,000$ kg/mol, no molecular degradation could be observed at “standard conditions” (flow rate = 0.5 mL/min, concentration = 0.25 g/L, columns with 10 and 20 μ m diameter gel particles and exclusion limits varying from 10,000 to 100,000 kg/mol for polystyrene (PS), injection volumes from 100 to 300 μ L). Analysing ultra-high molar mass polystyrene with $M_w > 5,000$ kg/mol under these conditions, molecular degradation occurred. By systematic variation of the experimental conditions, the extent of molecular degradation could be minimized. These conditions were flow rate ≤ 0.2 mL/min, diameter of the gel particles of 20 μ m, injected volume of about 100 μ L and concentration of injected sample solution of ≤ 0.1 g/L.

Keywords: Size exclusion chromatography (SEC); Molecular degradation; Low-angle laser light scattering (LALLS); Polystyrene; Ultra-high molar mass

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INTRODUCTION

The fraction of high molar mass in molar mass distribution greatly influences the physical properties of polymers.^[1] Also, molecular degradation during processing changes the molar mass distribution mainly in the high molar mass range.^[2] Therefore, a precise determination of the high-molar-mass fraction as well as the whole molar mass distribution is of great interest for quality control of polymer production^[3, 4] and modification, *e.g.*, mastication of natural rubber.^[5]

The standard method to analyze, polymers with respect to their molar mass and molar mass distribution is size exclusion chromatography.^[6–8] When analyzing samples containing ultra-high molar mass, there is the danger of scission of the polymer chains by mechanical forces within the SEC system. During the last 25 years, this effect was preferably studied on narrow-distributed polystyrene samples. Slagowsky *et al.*^[9] observed molecular degradation of samples with $M_w > 10,000$ kg/mol at a flow rate of 0.6 mL/min. Kirkland^[10] found a flow rate dependency of the elution time of polystyrene with $M_p = 7,100$ kg/mol because of degradation in molar mass. Ye and Shi^[11] found degradation of high-molar-mass polystyrene with $M_w = 7,000$ kg/mol when they were using silica-packed columns but not with styrene-divinylbenzene columns. Barth and Carlin^[12] reviewed the work on polymer shear degradation in 1984 and came to the conclusion that there is serious molecular degradation of polystyrene with molar mass above 4,000 kg/mol. Nakamura *et al.*^[13] investigated the elution behaviour of polystyrene standards up to $M_w = 4,000$ kg/mol according to concentration and degradation effects, and observed the absence of degradation. In 1997, Zigon *et al.*^[14] compared the results from static light scattering to those from SEC/LALLS and found good agreement for polystyrene samples with $M_w \leq 5,800$ kg/mol at a flow rate of 0.6 mL/min; however, they observed molecular degradation at a flow rate of 1.0 mL/min.

Other polymers than polystyrene were studied concerning molecular degradation in molar mass in SEC by Huber and Lederer,^[15] who investigated the degradation behavior of polyisobutylene, and Chubarova and Nesterow^[16] who studied the degradation of polystyrene as well as poly (methyl methacrylate) with $M_w \sim 10,000$ kg/mol on silica gel columns. As it was shown in the past, degradation of

ultra-high molar mass polymers ($M_w > 5,000$ kg/mol) can be caused by various effects, such as shear and elongation stress in filters and inlet and outlet frits of the columns or in the pores of the gel particles. There are also nonexclusion effects, mainly depending on the volume and concentration of the injected sample solutions, which strongly influence the results of SEC of ultra-high molar mass polymers by broadening the peaks or by viscous fingering. Most of these effects were summarized in reviews by Giddings^[17] and Barth.^[18]

According to our own experience, SEC/LALLS-coupling^[19] is a powerful method for the detection of molecular degradation,^[15] for more precise measurement in the range of high molar mass^[20] and for calibration and correction of peak broadening.^[21] On the basis of this experience, this paper presents a systematic investigation on the influence of experimental conditions on molecular degradation in SEC and arrives at recommendations how to minimize molecular degradation in order to increase the molar mass range of SEC to higher molar mass. Furthermore, an estimate of the increase of peak broadening in SEC with ultra-high molar mass polymers was carried out.

EXPERIMENTAL

SEC/LALLS

The molecular weight and its distribution of the polystyrene samples were determined by size exclusion chromatography (SEC) coupled with low-angle laser light scattering (LALLS). All experiments were carried out at ambient temperature, 25 ± 0.5 °C. The SEC/LALLS apparatus consisted of a HPLC pump L-7100 (Merck, Darmstadt, Germany), a six-port injection valve 7010 (Rheodyne, Cotati, CA, USA) with sample loops of 103 and 305 μ L, respectively, a LALLS photometer KMX-6 (Chromatix, Sunnyvale, CA, USA), wavelength 633 nm, set to 4.33 degrees, with a 0.5 μ m metal-frit filter (Valco Instruments, Houston, Texas, USA) ahead of the measuring cell followed by a differential refractive index (DRI) detector L-7490 (Merck, Darmstadt, Germany).

The measurements were carried out in toluene "purest" (Merck, Darmstadt, Germany), which was purified by distillation over a

Vigreux distillation column (height of 120 cm) at 109°C, filtrated through 0.45- μm filters and degassed with helium prior to use. The elution accuracy was continuously monitored during the measurements with the aid of a flow meter LiquiFlow (Bronkhorst, Ruurlo, Netherlands) and was better than 99.8% for all selected flow rates.

Samples

All samples were narrow distributed polystyrene standards and were obtained from Polymer Laboratories (Church Stretton, United Kingdom). Table I shows the specification of the supplier and the results of our own measurements. M_w was obtained from the integrated LALLS signal^[22] $\int E(V) * dV$, M_n from the LALLS calibration curve $M_w(V)$ and the DRI detector signal, and M_p was approximated^[23] as $M_p = \sqrt{M_w * M_n}$.

Columns

All columns used were packed with cross-linked styrene-divinylbenzene (PS-DVB) (Waters, Milford, MA, USA). Their specification is shown in Table II.

Sample Preparation

The samples were dissolved in toluene and left over night, They were then rolled on a self-assembled apparatus at about 3 rounds/min for 3 h at room temperature and injected. This procedure homogenizes the samples with negligible thermal or mechanical stress.

TABLE I Specification of the polystyrene standards

Sample no.	M_p^* [g/mol]	M_w/M_n^*	M_p^\dagger [g/mol]	M_w/M_n^\dagger	M_w^\dagger [g/mol]
1	770,000	1.04	775,000	1.03	786,000
2	1,460,000	1.06	1,475,000	1.06	1,520,000
3	3,950,000	1.06	3,900,000	1.06	4,022,000
4	7,100,000	1.11	7,030,000	1.12	7,431,000
5	15,400,000	1.30	15,870,000	1.23	17,600,000

* Supplier's specification.

† SEC/LALLS.

TABLE II Specification of SEC columns given by the supplier (HT = "high temperature", HMW = "high molecular weight")

<i>Supplier</i>	<i>Type</i>	<i>Column dimensions, diam × length [mm]</i>	<i>Gel particle size [μm]</i>	<i>Gel pore size [Å]</i>	<i>Exclusion limit for PS [kg/mol]</i>
Waters	Styragel HT 6	7.8 × 300	10	1×10^6	10,000
Waters	Styragel HMW 7	7.8 × 300	20	1×10^7	100,000

Calibration of the DRI Detector

To ensure that there is no adsorption of the polymer in the columns, the concentration detector (DRI) had to be calibrated by measurements with sample solutions of various concentrations. The signal $S(V)$ at a certain elution volume is proportional to the respective concentration $c(V)$ of the sample solution

$$S(V) = kc(V), \quad (1a)$$

and therefore

$$\int S(V)dV = k \int c(V)dV = km_{el} \quad (1b)$$

In absence of adsorption in the column, the total eluted mass m_{el} is equal to the total injected mass m_{inj} .

For the determination of the calibration constant k (*cf.* Eqs. (1a) and (1b)), a series of SEC runs with varying injected mass m_{inj} was carried out with polystyrene standard $M_p = 7,100$ kg/mol using a silica column (LiChrosorb SI 60, $7\mu\text{m}$, 250×4 mm, Merck, Darmstadt, Germany) to completely separate the polymer peak from the solvent peak. As can be seen from Figure 1, a strictly linear relationship

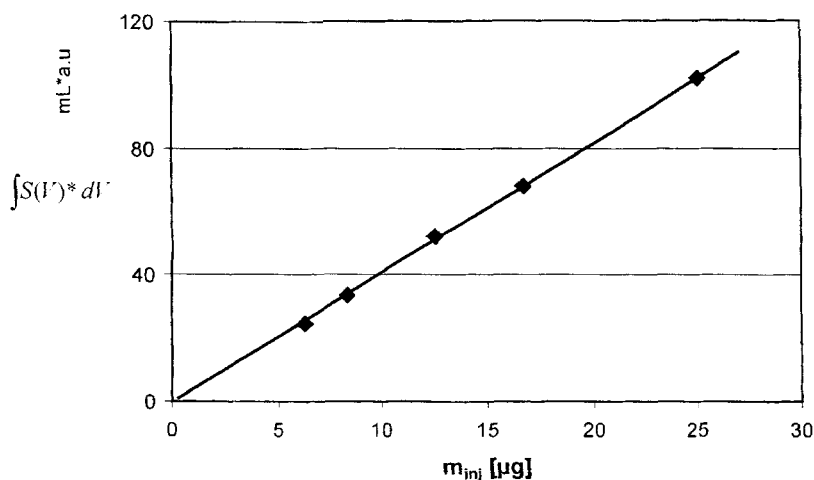


FIGURE 1 Calibration of the DRI detector (see text). Column: normal-phase silica gel; flow rate: 0.5 mL/min; injected volume: 103 μL ; a.u.: arbitrary units.

between $\int S(V)dV$ and m_{inj} was obtained. The SEC runs with PS standards with the PS-DVB columns were carried out with defined injected mass gave values of $\int S(V)dV$ lying on this calibration curve. As it is very improbable that the extent of adsorption would be identical on these chemically very different columns, adsorption on these columns can be excluded.

With the calibration constant k and thus $c(V)$, correct evaluation of the light scattering signal can be carried out according to Eq. (2).^[24]

$$M_w(V) = \frac{1}{(Kc(V)/R_\theta(V)) - 2A_2c(V)} \quad (2)$$

where K is the optical constant, R_θ is the Rayleigh factor, A_2 is the 2nd virial coefficient. For the system polystyrene/toluene, $K = 2.91 \times 10^{-9}$ mol cm²/g² and A_2 was assumed to be 2×10^{-4} mL mol/g², independent of molar mass. The angular dependence in the range from 0 to 4.33° could be neglected in all cases.

With the precondition that no adsorption occurs in the SEC system, the weight-average molar mass of the total eluted polymer sample M_w is calculated by

$$M_w = \int E(V)dV \quad (3)$$

where

$$E(V) = e(V)M_w(V) = \frac{1}{((K/R_\theta) - 2A_2)m_{el}} \quad (4)$$

with

$$e(V) = \left(c(V) / \int c(V)dV \right) \text{ (normalized concentration signal)} \quad (5)$$

and $m_{el} = m_{inj}$ (no adsorption).

Peak Broadening

In the case of a Gaussian chromatogram and Gaussian peak broadening, the following equation holds:

$$\sigma_{mes}^2 = \sigma_{sample}^2 + \sigma_0^2 \quad (6)$$

where σ_{mes}^2 is the variance of the detected Gaussian peak, σ_0^2 is the variance of the Gaussian peak broadening and σ_{sample}^2 is the variance of the Gaussian distribution of the sample, which can be calculated in the case of a linear calibration curve, $\ln M(V)$, with

$$\sigma_{\text{sample}}^2 = \frac{\ln(M_w/M_n)}{B^2} \quad (7)$$

where B is the slope of the linear calibration curve.

The peak broadening parameter σ_0^2 can be considered as a sum of the constant factor σ_{ex}^2 , which contains all the extracolumn peak broadening effects within the system, and a elution volume dependent parameter σ_{SEC}^2 which represents the peak broadening due to the size exclusion effect within the column.

$$\sigma_0^2 = \sigma_{\text{ex}}^2 + \sigma_{\text{SEC}}^2 \quad (8)$$

According to Cheng *et al.* [25] the dependence of the peak broadening parameter σ_0^2 on the elution volume V can be expressed by

$$\sigma_0^2 = \sigma_{\text{ex}}^2 + a(V_i - V_0) \exp(-bV) \quad (9)$$

where

$$a = (c^{\text{§}}/V_i) \exp(\varepsilon A)$$

and

$$b = \varepsilon B$$

where $c^{\text{§}}$ is a constant, V_i is the total pore volume, ε is a constant nearly equal to one, A is the intercept of the linear part of the calibration curve, $\ln M(V) = A - BV$, B is the slope of the linear part of the calibration curve, and V_0 is the interstitial volume.

Equation (9) relates $\sigma_0^2(V)$ to the characteristics of the column and the molecular weight of the sample. $\sigma_0^2(V)$ shows a maximum at $V = V(\sigma_{0\text{max}}^2)$, with

$$V(\sigma_{0\text{max}}^2) = V_0 + \frac{1}{b} \quad (10)$$

Experimental Conditions

Injection volumes of sample solutions were 103 or 305 μL . The concentration varied from 0.075 to 0.25 g/L, the injected mass from about 8 to 60 μg . Flow rates from 0.1 to 0.5 mL/min were used to optimize the system with respect to analysis time and avoidance of molecular degradation.

RESULTS AND DISCUSSION

To investigate the effect of the concentration of the injected sample solution on the detected molar mass, different concentrations of the same sample were analyzed under identical conditions. The flow rate was set to 0.2 mL/min; the injected sample volume was 103 μL . Figure 2 shows, that the weight-average molar mass of the eluted polymer M_w (cf. Eq. (4)) is not significantly influenced by the sample concentration in the concentration range from 0.08 to 0.250 g/L. All investigated concentrations were below the overlapping concentration,

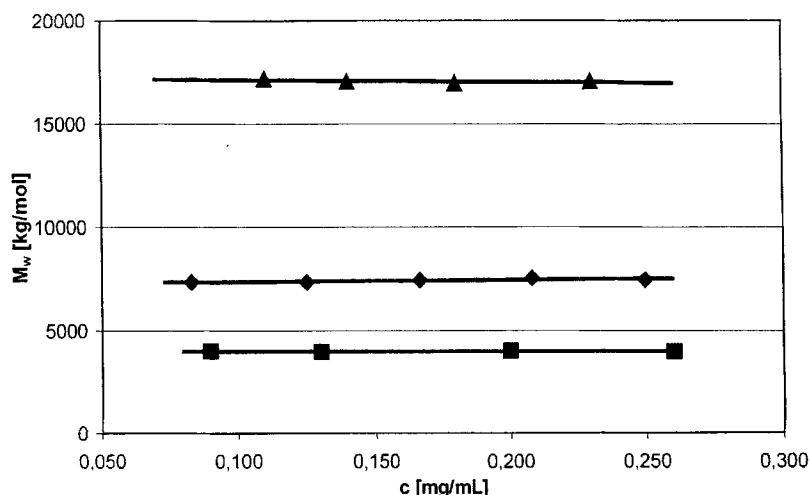


FIGURE 2 Weight-average molar mass M_w of the eluted sample obtained as a function of the injected polymer concentration c of various polystyrene standards (■: $M_p = 3,950$ kg/mol, ◆: $M_p = 7,100$ kg/mol, ▲: $M_p = 15,400$ kg/mol). column: Waters HMW7, flow rate: 0.2 mL/min; injected volume: 103 μL .

which was estimated as the reciprocal viscosity number of the investigated sample. For the highest molar mass standard ($M_p \sim 15,500$ kg/mol) the overlapping concentration equals 0.6 g/L. The same results were obtained when using both types of columns (*cf.* Tab. II).

The scission of the polymer chains leading to a decrease in molar mass is caused by high flow rates. This leads to high mechanical forces within filters, at the inlet and outlet frits of the columns, and between the gel particles within the columns. The molecular degradation in the space between the gel particles (interstitial volume) strongly depends on the average particle size. This effect was investigated by comparing the high molar mass part of the calibration curves obtained at two different flow rates (0.2 and 0.5 mL/min) using the DRI detector only. With the 20- μm packing material (Waters HMW7), the high molar mass regions of the calibration curves show no difference in the retention volume at flow rates of 0.5 mL/min and 0.2 mL/min, whereas with the 10- μm packing material (Waters HT6), the two highest molar mass samples elute later at 0.5 mL/min than they do at 0.2 mL/min (*cf.* Fig. 3). This indicates degradation within the column and/or at the inlet frit. Scission of the polymer chains after separation within the

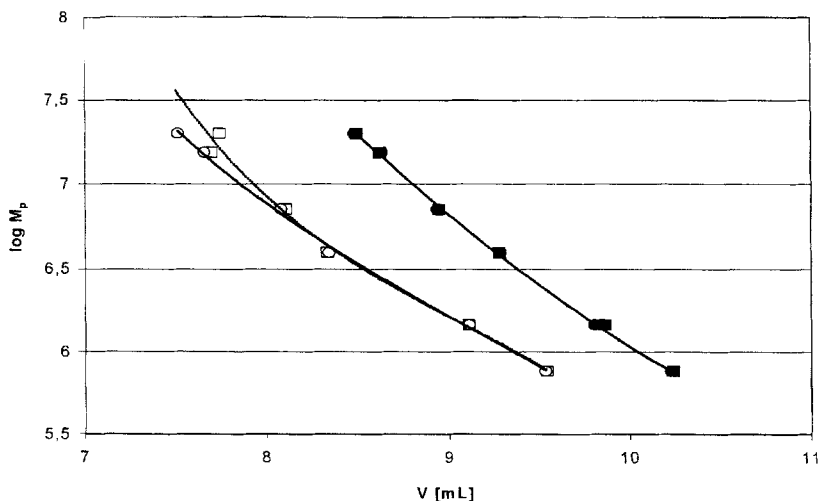


FIGURE 3 Plot of the calibration curves in the high-molar-mass range of the columns Waters HT6 (open symbols: average gel particle diameter: 10 μm) and Water HMW7 (filled symbols: average gel particle diameter: 20 μm) with different flow rates V . □, ■: $V = 0.5$ mL/min, ○, ●: $V = 0.2$ mL/min. Injected volume: 103 μL .

columns would not have affected the retention volume of the samples and would therefore not be detectable with the DRI detector. For the samples with $M_p < 5,000$ kg/mol, no such indication for molecular degradation within the column and/or its inlet frit could be observed, even in the case of the 10 μm packing material.

At flow rates above 0.2 mL/min, another effect takes place which strongly influences the results obtained by light scattering: the high molar mass fractions are degraded when passing through the filter ahead of the LALLS detector. This filter has an average pore diameter of 0.45 μm which is smaller than the hydrodynamic radius of the high molar mass fractions of polystyrene with $M_p > 7,000$ kg/mol. Calculation of the hydrodynamic volume of polystyrene in toluene on the basis of the Staudinger-Mark-Houwink coefficients^[22] $K = 0.01069$ mL/g and $a = 0.724$ suggests that the molecular diameter of polystyrene with a molar mass of about 16,000 kg/mol is in the order of magnitude of the average pore size of this filter. The two highest molar mass standards ($M_p = 7,100$ kg/mol and $M_p = 15,400$ kg/mol) contain fractions with molar mass higher than 16,000 kg/mol and are therefore affected most.

Molecular degradation at this point does not affect the retention time and the signal of the concentration detector because it takes place after separation within the columns, different from the case which is demonstrated by the dashed line Figure 3. To avoid molecular degradation within the column and/or the inlet frit, these experiments were all carried out in columns with a diameter of 20 μm of the gel particles. Only the use of the LALLS detector can show this kind of post-separation degradation (*cf.* Fig. 4). Samples with $M_w < 3,000$ kg/mol are not affected. However, Figure 4 clearly reveals that it is possible to analyse polystyrene samples with M_w up to 17,000 kg/mol without any degradation.

As is shown in Figure 5, increasing the concentration leads to an increase in peak broadening going along with broadening of the apparent molar mass distribution due to overloading effects. This influences mainly the ends of the molar mass distribution, leading to an erroneously large fraction of ultra-high molar mass. Therefore concentrations below 0.15 g/L are recommended.

Besides concentration, the injected sample volume plays an important role in broadening of the peaks. To study its influence,

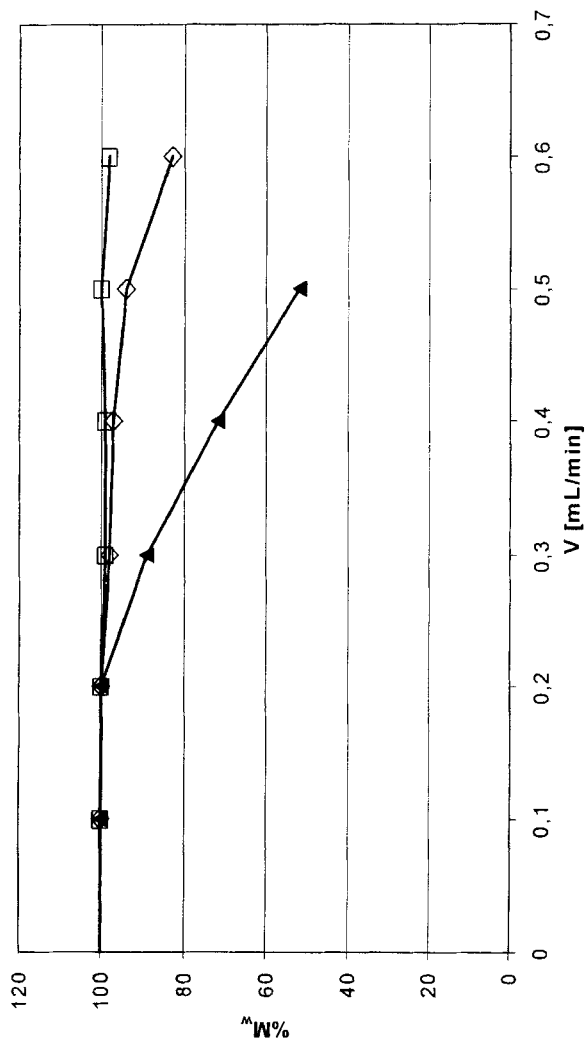


FIGURE 4 Molecular degradation of various polystyrene samples as a function of the flow rate V (see text). (\square : $M_p = 15,400$ kg/mol, \blacktriangle : $M_p = 3,950$ kg/mol, \diamond : $M_p = 7,100$ kg/mol).

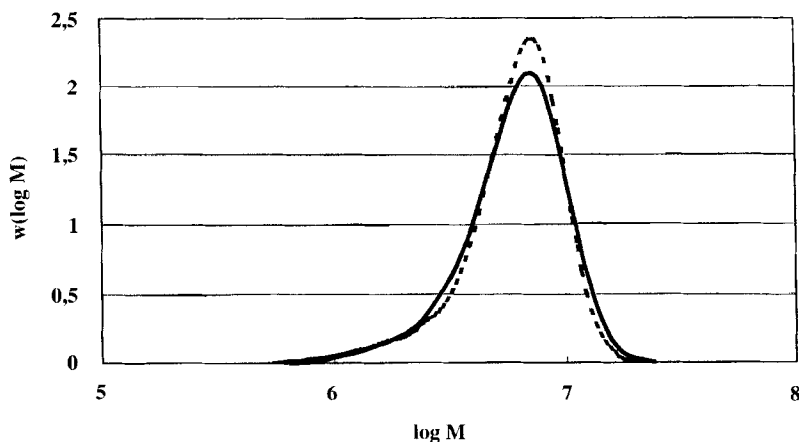


FIGURE 5 Apparent molar mass distribution of a polystyrene standard ($M_p = 7,100$ kg/mol) measured with two different concentrations of injected sample solutions (—: 0.200 g/L, - - - - -: 0.080 g/L). column: Waters HMW 7; flow rate: 0.2 mL/min; injected volume: 103 μ L.

certain sample masses with dilution by 103 or 305 μ L of solvent were injected and the peak width σ_{mes} was estimated (*cf.* Eq. (5)) by taking the difference of the extremes of the first derivative function of a peak as $2 \sigma_{mes}$. This showed that only with the small sample volume of 103 μ L the peak width could be reduced by lowering the concentration (*cf.* Fig. 6). At the higher sample volume of 305 μ L no decrease of the peak broadening with lower sample concentration could be observed. Apparently, the increase of peak broadening due to the larger injection volume predominates the concentration effect.

The peak broadening parameter σ_0^2 estimated according to Eq. (9) increases with increasing molar mass (*cf.* Fig. 7). The fitted curve goes through a maximum at $V(\sigma_{0,max}^2) = 7.57$ mL (*cf.* Eq. (10)). At higher molar mass σ_0^2 is expected to decrease and finally reach the value of σ_{ex}^2 at the exclusion limit, where it is only influenced by extra-column broadening effects.

As can be seen from Figure 7, the peak broadening parameter σ_0^2 increases by one order of magnitude in the molar mass range from $M_p = 66$ to 15,400 kg/mol. In spite of this dramatic drop of column performance at ultra-high molar mass, there is no interference, which influences the elution behaviour, between higher and lower molar mass

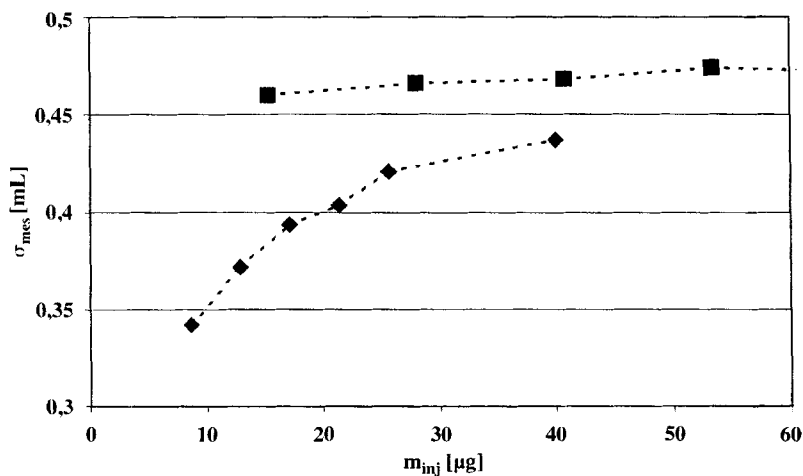


FIGURE 6 Peak width σ_{mes} as a function of the injected sample mass m_{inj} with two sample loops of different volume (\blacklozenge : 103 μL , \blacksquare : 305 μL).

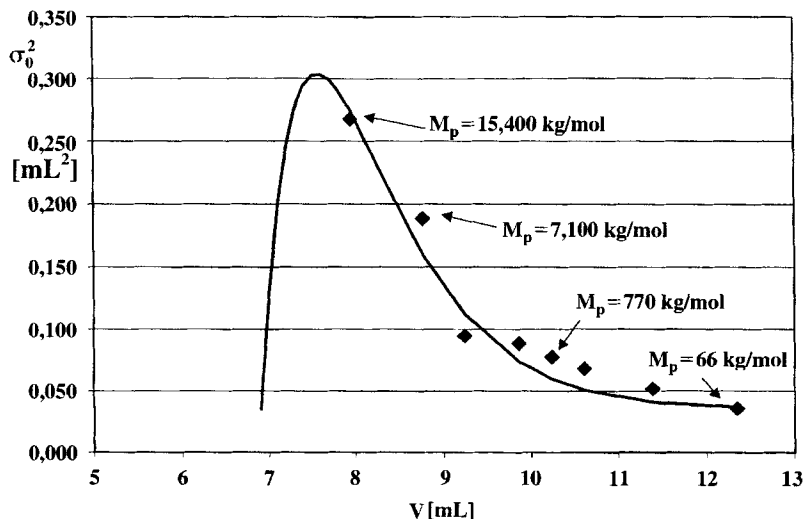


FIGURE 7 Dependency of the peak broadening parameter σ_0^2 on the elution volume V . —: fit according to Cheng *et al.* [25] \blacksquare : experimental data. column: Waters HMW 7; flow rate: 0.2 mL/min; injected volume: 103 μL .

fractions as was proved by measurement of mixtures of polystyrene standards with $M_p = 15,400$ and 3,950 kg/mol in a test of validity of superposition (*cf.* Fig. 8).

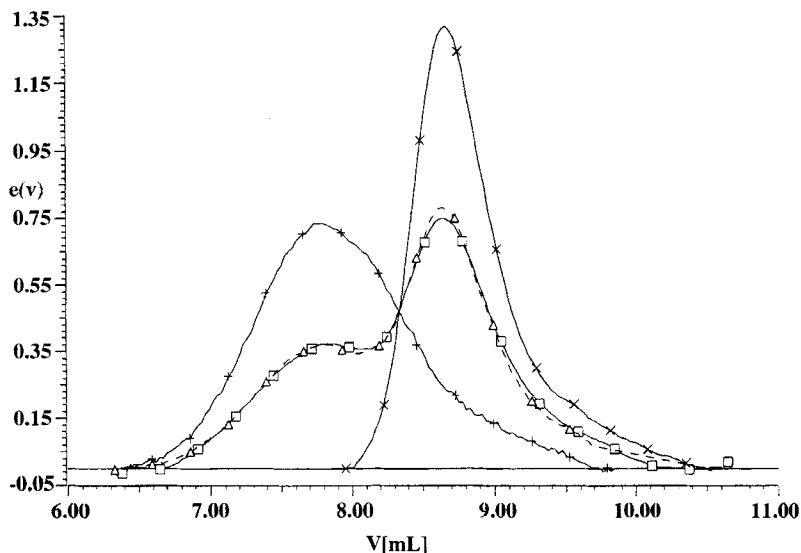


FIGURE 8 SEC of polystyrene standards (\times : $M_p = 3,950$ kg/mol, and $+$: $M_p = 15,400$ kg/mol); column: Waters HMW7; flow rate: 0.2 mL/min; injected volume: 103 μ L; concentration: 0.1 g/L each; test of validity of superposition under these conditions: Δ : experimental eluogram of 1/1 mixture of both polystyrene standards, \square : eluogram calculated for the 1/1 mixture from separate measurement of both PS standards.

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