

## Contribution of the polymer standards' polydispersity to the observed band broadening in size-exclusion chromatography

Y. Vander Heyden<sup>a,b</sup>, S.-T. Popovici<sup>a</sup>, B.B.P. Staal<sup>c</sup>, P.J. Schoenmakers<sup>a,\*</sup>

<sup>a</sup>*Polymer-Analysis Group, Department of Chemical Engineering, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands*

<sup>b</sup>*Pharmaceutical and Biomedical Analysis, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*

<sup>c</sup>*Chemical Technology, Technical University Eindhoven, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands*

Received 8 August 2002; received in revised form 25 November 2002; accepted 27 November 2002

### Abstract

The contribution of the polydispersity of polymer standards to the observed band broadening in size-exclusion chromatography was evaluated. Initially, theoretical predictions based on an equation by Knox et al. were found to overestimate this contribution, greatly due to the fact that the polydispersity values specified by the manufacturers are upper limits and therefore too high to be applied in this context. An improved estimate of the polydispersity values was obtained from the size-exclusion chromatography results and these new values were used to reassess the polydispersity contribution to band broadening. For two of three columns tested the best molar-mass-distribution parameters, i.e. those the least affected by extra-column and intra-column band broadening effects, can be obtained for polymers with a molar mass in the effective range of the given column and at rather low mobile-phase flow rates. At those conditions, for low-molar-mass polymers, the estimated polydispersity index values approach the theoretical ones derived from a Poisson distribution.

© 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Band broadening; Molecular mass distribution; Poisson distribution; Polydispersity; Size-exclusion chromatography; Polymers

### 1. Introduction

Size-exclusion chromatography (SEC) is a liquid chromatographic technique, introduced by Moore [1], in which the analytes are separated according to their hydrodynamic volumes. It is one of the most frequently used techniques to obtain molar-mass

distributions (MMDs) of both natural and synthetic polymers [2].

The main use of SEC is not to separate polymers, but rather to determine the MMD and the corresponding average molar-mass values, of which the most common are the number- ( $M_n$ ) and weight-average ( $M_w$ ) molar masses [2]. The ratio  $M_w/M_n$  between these two numbers, which is called the polydispersity,  $D$ , or the polydispersity index, PDI, provides an estimate of the width of the MMD. The larger the PDI, the broader is the distribution. The molar-mass data are used to characterise polymer

\*Corresponding author. Tel.: +31-20-525-6515; fax: +31-20-525-5604.

E-mail address: [pjschoen@science.uva.nl](mailto:pjschoen@science.uva.nl) (P.J. Schoenmakers).

samples or to control their quality [3–5]. This main application of SEC is at the same time the main challenge associated with the technique, as is discussed further.

While separation is based on the size of molecules in solution, the required information is usually molecular mass (or molar mass). The conversion from size data to molar-mass data is referred to as calibration in SEC [6]. For homopolymers the hydrodynamic volume is directly related to the molar mass, and a calibration curve is obtained by measuring the retention times of a number of well-defined (narrow) standards. If these standards have the same chemical composition as the polymer samples, this procedure allows obtaining correct (unbiased) MM information. Because the relationship between size in solution and molecular mass is different for different molecules, biased data will be obtained if the standards are chemically different from the sample. In that case the reported MMD and average molar-mass values are relative to the calibration standards used for the SEC calibration curve [7]. Several ways have been developed to obtain unbiased MM data without the need for specific standards for any given polymer. The principle of universal calibration [2] can be used to obtain a calibration curve for a particular type of polymer using standards from a different type.

Band broadening in SEC can result not only in distortion of the calculated MMD of the polymer *sample*, but also in errors in the average molar-mass values [8]. The three factors which contribute to the observed dispersion of a sample are the external band broadening, the column band broadening and the band broadening due to the polydispersity of the polymer standards. These contributions to band broadening can be expressed as:

$$\sigma_{\text{total}}^2 = \sigma_{\text{extra-column}}^2 + \sigma_{\text{column}}^2 + \sigma_{\text{PDI}}^2 \quad (1)$$

with  $\sigma_{\text{extra-column}}^2$  the variance of the peak caused by extra-column effects,  $\sigma_{\text{column}}^2$  by mixing phenomena in the column (longitudinal diffusion, eddy diffusion and mass transfer), and  $\sigma_{\text{PDI}}^2$  by the polydispersity of the polymer solute.

In SEC one wants the band dispersion due to extra- and intra-column effects to be minimised, while the band dispersion due to the sample PDI

(viz. the selectivity of the separation) should be maximised. To obtain good estimates of the MMD of polymers they should be measured under conditions at which the chromatographic peak width is completely determined by the polydispersity contribution and the other band broadening effects can be considered negligible.

When using relative calibration (e.g. relative to polystyrene) any eventual band broadening in the peaks obtained for the standards should have no effect on the calculated results for actual samples, given the nature of this calibration (the peak molar mass,  $M_p$ , vs. retention time or –volume). The band broadening of the sample, on the other hand, is obviously important as it will affect its estimated MMD parameters. However, this does not prevent us from using polystyrene standards as samples to evaluate under which conditions the column and external band broadenings are minimal, as is done in this paper.

In chromatography, dispersion is also commonly expressed in terms of plate height. From the plate theory of Martin and Synge [9], the total reduced plate height ( $h$ ) is given by:

$$h = \frac{H}{d_p} = \frac{L}{d_p} \frac{\sigma_t^2}{t_R^2} = \frac{L}{d_p} \frac{\sigma_v^2}{V_R^2} = \frac{L}{d_p} \frac{W_{0.5}^2}{5.54 \cdot t_R^2} \quad (2)$$

where  $H$  is the plate height,  $d_p$  the particle diameter,  $L$  the length of the separation column,  $t_R$  the retention time,  $V_R$  the retention volume, and  $W_{0.5}$  the peak width at half height in time units. Depending on whether retention time or volume is used,  $\sigma^2$  is expressed in time or volume units ( $\sigma_t$  or  $\sigma_v$ , respectively).

The total reduced (apparent) plate height is given by:

$$h = h_{\text{extra-column}} + h_{\text{column}} + h_{\text{PDI}} = h_{\text{kin}} + h_{\text{PDI}} \quad (3)$$

where  $h_{\text{kin}}$  and  $h_{\text{PDI}}$  are the kinetic (band-broadening) and polydispersity contributions to the total reduced plate height, respectively.

Knox et al. [10] proposed an equation to estimate the contribution of the polydispersity to the total (apparent) plate height:

$$h_{\text{PDI}} = \left(\frac{L}{d_p}\right) \cdot \left(\frac{S}{t_R}\right)^2 (\text{PDI} - 1)(\alpha + 1) \quad (4)$$

where,  $S$  is the negative inverse slope of the SEC calibration curve and  $\alpha$  a correction factor that depends on the polydispersity of the polymer, which was defined as:

$$\alpha = \frac{11}{4}(\text{PDI} - 1) + \frac{137}{12}(\text{PDI} - 1)^2 \quad (5)$$

Band broadening in SEC has been studied before. Busnel et al. [11] used very narrow polystyrene standards ( $\text{PDI} < 1.01$ ), but the contribution of polydispersity to the total band width was not evaluated. Possibly because the PDI contribution may be neglected for such standards according to Knox et al. [10].

In order to compute the PDI contribution to the (reduced) plate height, knowledge of the polydispersity of the narrow standards used is essential. The width of molar-mass distributions or the PDI has been directly estimated from size-exclusion chromatography with concentration and light-scattering detection [12] and also can be derived from MMDs obtained from mass-spectrometric measurements using soft ionization techniques [13,14]. In case of commercial standards, the manufacturer specifies a value. Usually, an upper limit is specified (e.g.  $\text{PDI} < 1.05$ ). Some researchers have suggested that the real PDI values are much smaller than those specified by the suppliers [15–17]. Stegeman et al. [15] claimed that  $h_{\text{PDI}}$  is overestimated, probably due to an overestimation of the PDI reported by the manufacturer of the standards. Also other authors [16,17] claim that the real PDI is considerably smaller than the nominal values reported, because SEC, which is used for their estimation, is significantly affected by band-broadening effects. Temperature-gradient interaction chromatography (TGIC) has been found to give much narrower peaks than SEC and thus leads to much lower PDI estimates [16,18]. For polymers (e.g. polystyrenes) made by anionic polymerisation the TGIC peaks observed approached a Poisson distribution and the estimated PDI values were close to those derived from the Poisson distribution.

While Knox et al. [10] demonstrated the implication of Eq. (4) using simulations, in this study we wanted to evaluate the contribution of polydispersity to the total peak width in practical situations, on different SEC columns and applying different flow-rates. We also wanted to study more thoroughly the

statements in Refs. [15,16,18] that SEC overestimates the PDI. We wished to establish whether an overestimation of  $h_{\text{PDI}}$  indeed occurs, how large such an eventual overestimation may be, and what are the causes. Finally, we set out to evaluate under which SEC conditions and on which columns the MMD of a given polymer is best determined and whether under those conditions the estimated PDI values approach those corresponding to a Poisson distribution.

## 2. Experimental

### 2.1. Instrumentation and chemicals

PL-Gel Individual-Pore-Size GPC/SEC columns from Polymer Labs. (Church Stretton, UK) were used, with dimensions  $300 \times 6.8$  mm I.D. and packed with  $5\text{-}\mu\text{m}$  particles. Three columns with different pore sizes, (i)  $10^3$  Å (effective MM range: 500–60 000), (ii)  $10^4$  Å (effective MM range: 10 000–600 000), and (iii)  $10^5$  Å (effective MM range: 60 000–2 000 000) were applied. The measurements were performed on a Waters Alliance SEC system (Milford, MA, USA) equipped with a Waters 410 refractive-index (RI) detector. The temperature of the whole system was maintained at  $30^\circ\text{C}$ . Data were recorded using the Waters Millennium32 software. Calculations and data treatment on the chromatographic peaks were performed using laboratory written software on a Matlab 5.2 (MathWorks, Natick, MA) platform.

The eluent was non-stabilised tetrahydrofuran (THF) from Biosolve (Valkenswaard, The Netherlands). The standards used were polystyrenes (PS) from Polymer Labs. or Pressure Chemical (Pittsburgh, PA, USA). Their properties are shown in Table 1. The concentration of all standard solutions was 1 mg/ml in non-stabilised THF.

### 2.2. Procedure

The 14 polystyrene standards (Table 1) were injected on the three SEC columns. All polystyrene standards were injected at a series of different flow-rates, namely 0.1, 0.2, 0.3, 0.5, 0.7 and 1.5 ml/min, on each of the columns.

Table 1  
Molar mass, manufacturer and polydispersity of the polystyrene standards

Molar mass, $M_p$	Manufacturer	PDI
1700	Polymer Labs.	1.06
2450	Polymer Labs.	1.05
3250	Polymer Labs.	1.04
5050	Polymer Labs.	1.05
7000	Polymer Labs.	1.04
11600	Polymer Labs.	1.03
22000	Polymer Labs.	1.03
76600	Polymer Labs.	1.03
200000	Pressure Chemical	1.03
475000	Polymer Labs.	1.03
675000	Polymer Labs.	1.07
900000	Pressure Chemical	1.07
2000000	Pressure Chemical	1.03
2200000	Polymer Labs.	1.04

Values shown are those provided by the manufacturer.

### 3. Results and discussion

#### 3.1. Estimation of $h_{PDI}$ according to Knox' equation

For each standard,  $h$  (called  $h_{exp}$  further on) and  $h_{PDI}$  were calculated according to Eqs. (2) and (4), respectively. They were determined on all columns and at all flow-rates. The factor  $S$  in Eq. (4) was estimated in two different ways. A first value was derived from the calibration models best describing the entire range encompassed by all standards [6]. The second method employed the slope of local straight parts in the calibration curve. In the first situation, third-order polynomial models were used on the columns with pore sizes  $10^5$  Å and  $10^4$  Å, while for the  $10^3$  Å column a straight line (only spanning the effective range) was used. This was done based on the knowledge gained in Ref. [6]. In the second situation the locally straight line was determined by three standards, i.e. the one for which  $h_{PDI}$  is being estimated and the two situated around it. For the extreme (largest and smallest) standards, the two with the nearest MM values were used. The  $h_{PDI}$  estimated according to both approaches were, in general, similar as can be observed in Fig. 1. This figure shows  $h_{exp}$ ,  $h_{PDI}$  from modelling the entire range and  $h_{PDI}$  from the slope of local straight lines, each plotted as a function of the  $\ln(MM)$  of the

polystyrene standards and estimated on the three columns at a flow-rate of 0.5 ml/min. It was observed that on a given column the shape of the obtained plot was approximately independent of the applied flow-rate. In Fig. 2 the estimated  $h_{PDI}$  values are plotted as a function of the flow-rate. It can be seen that on a given column the value of  $h_{PDI}$  estimated for a standard is usually independent of the flow-rate. The most significant exceptions are some standards on the  $10^5$  Å column. The fraction of the total reduced peak height due to polydispersity, i.e.  $h_{PDI}/h_{exp}$ , was determined. As can be seen from Fig. 1, in a number of situations this fraction greatly exceeds 100% (Table 2, Knox approach). Values up to 450% were found for some conditions, indicating that the results exceeding 100% cannot only be due to experimental error. It also can be observed that the standards for which the 100% level is exceeded, are different on the different columns. For the large-pore-size columns these are rather high-MM standards, while for the small-pore-size column it are low-MM standards.

It is, of course, impossible that the true contribution from sample polydispersity exceeds  $h_{exp}$ . Therefore we wanted to find out the reason for overestimating  $h_{PDI}$ . Two possible phenomena are potentially responsible, (a) the equation proposed by Knox et al. [10] (Eq. (4)) is overestimating  $h_{PDI}$ , or (b) the PDI reported by the manufacturers, which is used in Eqs. (4) and (5), is overestimating the true values.

#### 3.2. Estimation of $h_{PDI}$ according to an alternative approach

We first wanted to evaluate whether or not Eq. (4) of Knox et al. [10], which in fact is an empirical expression, is overestimating  $h_{PDI}$ . Therefore an alternative approach to estimate  $h_{PDI}$ , independent of Knox' one, was used. Based on the retention time of a sample and on the calibration curve model [ $\ln(MM)$  as a function of retention time], the corresponding  $\ln(MM)$  or  $MM (=M_p)$  was predicted. From the equation for polydispersity (Ref. [10] and Eq. (6)), the width ( $\sigma_M$ ) of the polymer distribution was estimated:

$$\frac{\sigma_M^2}{M_n^2} = \frac{M_w}{M_n} - 1 = PDI - 1 \quad (6)$$

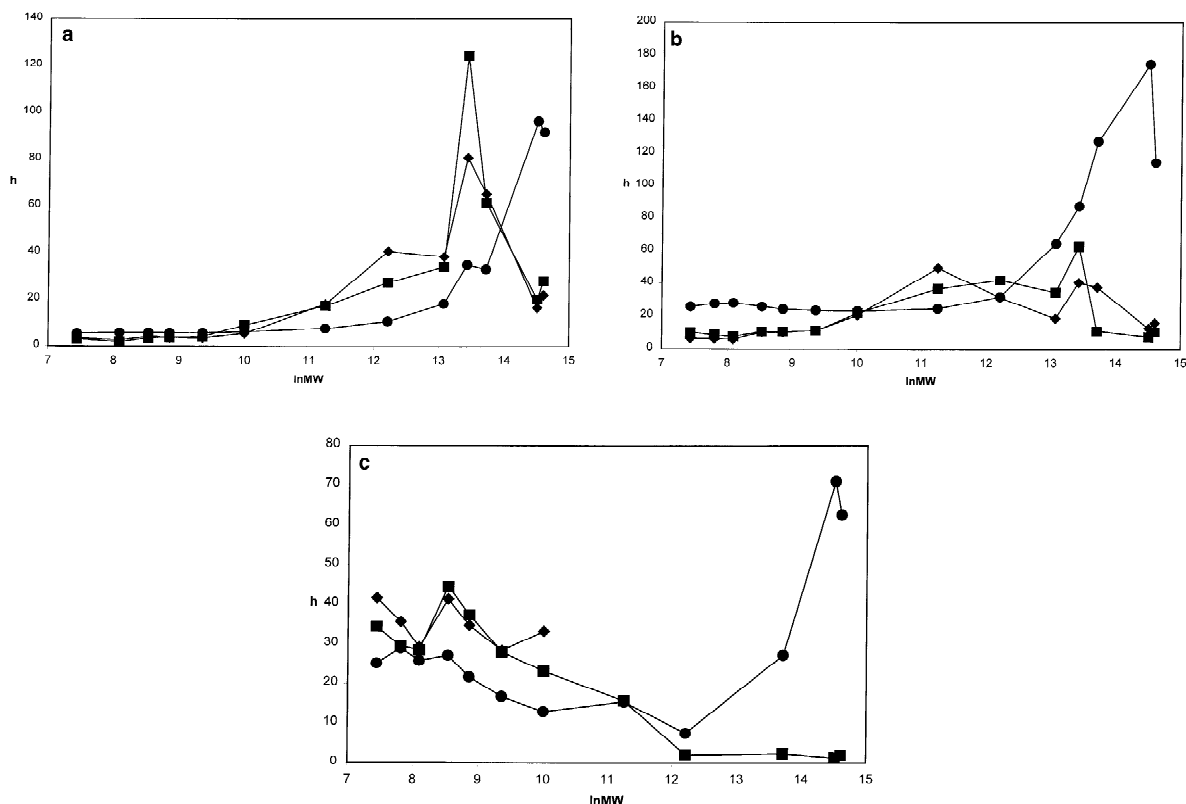


Fig. 1. Experimental reduced plate height,  $h_{\text{exp}}$  (●),  $h_{\text{PDI}}$  estimated from modelling across the entire range (◆) and  $h_{\text{PDI}}$  estimated from the slope of local straight lines (■) plotted as a function of the  $\ln(\text{MM})$  of the polystyrene standards. Flow rate: 0.5 ml/min; (a)  $10^5 \text{ \AA}$  column, (b)  $10^4 \text{ \AA}$  column, and (c)  $10^3 \text{ \AA}$  column.

In analogy with Ref. [10]  $M_p$  was used (instead of  $M_n$ ) in Eq. (6) to estimate  $\sigma_M$ . Using  $\sigma_M$ , the width of a normal (Gaussian) distribution around  $M_p$  at half height was estimated as:

$$\left[ M_p - \frac{\sqrt{5.54} \cdot \sigma_M}{2}, M_p + \frac{\sqrt{5.54} \cdot \sigma_M}{2} \right]$$

The real roots (the other are complex numbers) from the calibration equation for the natural logarithm of the interval extremes give an estimate of the width of the chromatographic peak at half height if it would have been entirely due to the polydispersity of a normally distributed polymer standard. As in the above section, this width was also estimated a second time using the root (linear interpolation) of locally straight calibration lines. It was found that both approaches lead to fairly similar results (Table 2).

The reduced plate height due to polydispersity  $h_{\text{PDI}}$  was then estimated applying Eq. (2) and its contribution to  $h_{\text{exp}}$  was determined. From Table 2 it can be seen that the results obtained with this alternative approach are comparable to those from the equation proposed by Knox et al. In general the former approach gave slightly lower values for  $h_{\text{PDI}}$  than the one by Knox. However, the %  $h_{\text{PDI}}/h_{\text{exp}}$  values still greatly exceeded 100%, indicating on the one hand that Eq. (4) proposed in Ref. [10] is appropriate and on the other that the PDI reported by the manufacturers—a value which was used in both approaches—seems to be overestimated as claimed in Refs. [15,16].

### 3.3. Upper limit of polydispersity

In a next step we wanted to evaluate how much

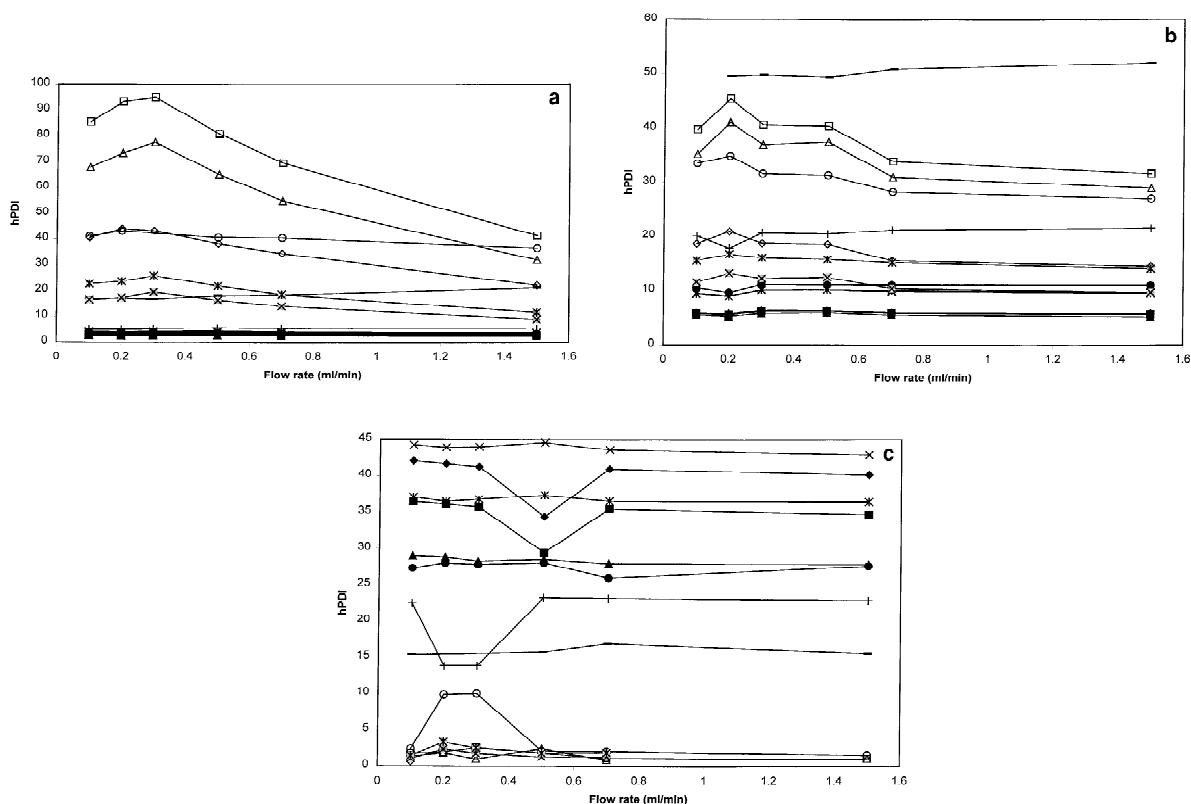


Fig. 2.  $h_{\text{PDI}}$  values for the different standards as a function of the flow-rate: (a)  $10^5$  Å column, (b)  $10^4$  Å column, and (c)  $10^3$  Å column. Legend for the standards: MM:  $\blacklozenge$ , 1700;  $\blacksquare$ , 2450;  $\blacktriangle$ , 3250;  $\times$ , 5500;  $*$ , 7000;  $\bullet$ , 11 600;  $+$ , 22 000;  $-$ , 76 600;  $\circ$ , 200 000;  $\diamond$ , 475 000;  $\square$ , 675000;  $\triangle$ , 900 000; shaded box with cross, 2 000 000; and shaded box with star, 2 200 000.

the PDI used in the above equations (Table 1) was overestimated. It will also be evaluated which of the experimental conditions applied, for instance which pore size or flow-rate, allow the best estimate of the real, but unknown, PDI. In order to estimate the upper limit for polydispersity, the situations in which  $\% h_{\text{PDI}}/h_{\text{exp}}$  exceed 100% were considered. It was estimated what could have been the maximal value for PDI to obtain an  $h_{\text{PDI}}$  value that does not exceed  $h_{\text{exp}}$ , i.e. considering the situation in which all contributions to the peak width, except the polydispersity of the polymer, are negligible. This maximal PDI ( $\text{PDI}_{\text{max}}$ ) was estimated for the situations where  $h_{\text{PDI}}$  estimated from Knox' and from the alternative approach exceeded 100%. Depending on the situation,  $\text{PDI}_{\text{max}}$  was estimated differently. If the initial  $h_{\text{PDI}}$  was estimated from Knox' equation (Eq. (4))

the following was done. Knox' equation can be rewritten as:

$$\frac{h_{\text{PDI}}}{L} \frac{d_p}{(S/t_R)^2} = \frac{137}{12} \text{PDI}^3 - \frac{387}{12} \text{PDI}^2 + \frac{357}{12} \text{PDI} - \frac{116}{12} \quad (7)$$

To estimate  $\text{PDI}_{\text{max}}$  when  $h_{\text{PDI}}$  exceeds  $h_{\text{exp}}$ ,  $h_{\text{PDI}}$  is replaced by  $h_{\text{exp}}$  in Eq. (7).  $\text{PDI}_{\text{max}}$  is then estimated as the real root from the third-order polynomial.

When the initial  $h_{\text{PDI}}$  was estimated from the alternative approach,  $\text{PDI}_{\text{max}}$  was determined as follows. From the width at half height of the chromatographic peak, the corresponding time interval around the retention time ( $t_R$ ) was determined

Table 2  
 $h_{\text{PDI}}$  and  $\%h_{\text{PDI}}/h_{\text{exp}}$  for the different standards and on the different columns, estimated based on three approaches

MM	$h_{\text{PDI}}$			$\% h_{\text{PDI}}/h_{\text{exp}}$		
	Knox <sup>a</sup>	Curve <sup>b</sup>	Straight <sup>c</sup>	Knox <sup>a</sup>	Curve <sup>b</sup>	Straight <sup>c</sup>
$10^5 \text{ \AA}$						
1700	3.56	3.09	3.42	65	57	63
2450	(-)	(-)	(-)	(-)	(-)	(-)
3250	2.75	2.51	2.49	48	44	43
5050	4.21	3.74	3.74	73	65	65
7000	3.80	3.46	3.54	67	61	62
11600	3.57	3.34	3.44	63	59	61
22000	5.47	5.10	5.95	89	83	97
76600	18.00	16.69	15.72	235	218	205
200000	40.63	37.67	32.51	379	352	303
475000	38.28	36.54	37.42	211	201	206
675000	80.80	72.71	72.49	232	209	208
900000	65.17	58.70	59.18	198	178	180
2000000	16.36	15.66	19.69	17	16	20
2200000	21.84	20.54	27.29	24	22	30
$10^4 \text{ \AA}$						
1700	6.31	5.46	6.03	25	21	23
2450	6.21	5.51	5.54	23	20	20
3250	5.92	5.38	5.48	21	19	20
5050	10.16	8.99	8.96	39	35	35
7000	10.17	9.22	9.52	42	38	39
11600	11.05	10.28	10.73	47	44	46
22000	20.45	18.96	20.66	89	82	90
76600	49.43	46.20	36.79	202	188	150
200000	31.33	30.16	32.95	99	95	104
475000	18.58	17.79	20.29	29	28	31
675000	40.43	35.81	120.21	46	41	138
900000	37.45	33.10	33.65	29	26	26
2000000	12.47	11.87	11.96	7	7	7
2200000	15.76	14.71	17.00	14	13	15
$10^3 \text{ \AA}$						
1700	34.41	36.53	36.53	137	145	145
2450	29.47	32.04	32.04	101	110	110
3250	28.53	26.90	26.90	110	104	104
5050	44.58	37.22	37.22	164	137	137
7000	37.37	31.98	31.98	172	147	147
11600	28.06	26.69	26.69	167	159	159
22000	23.20	31.31	25.55	179	241	197
76600	15.72	#	12.71	102	#	82
200000	2.11	#	2.44	28	#	32
475000	(-)	(-)	(-)	(-)	(-)	(-)
675000	(-)	(-)	(-)	(-)	(-)	(-)
900000	2.43	#	2.38	9	#	9
2000000	1.31	#	1.55	2	#	2
2200000	1.84	#	1.39	3	#	2

Flow rate 0.5 ml/min. (-) not measured, # value cannot be determined.

<sup>a</sup> Knox approach.

<sup>b</sup> Alternative approach using the roots from the calibration curve.

<sup>c</sup> Alternative approach using the roots from local straight parts (cfr. text).

$$\left[ t_R - \frac{W_{0.5}}{2}, t_R + \frac{W_{0.5}}{2} \right]$$

$$\frac{W_M}{\sqrt{5.54}}$$

Using the calibration-curve equation the corresponding  $\ln(\text{MM})$  and  $\text{MM}$  values were predicted, as well as the half-height width of the molar-mass distribution ( $W_M$ ). From this width,  $\sigma_M$  was estimated as:

assuming a Gaussian distribution and  $\text{PDI}_{\text{max}}$  applying Eq. (6), in which for  $M_n$ , again  $M_p$  was used.

The  $\text{PDI}_{\text{max}}$  values obtained are shown in Table 3. For the  $10^5$  and  $10^3 \text{ \AA}$  columns they also are shown in Fig. 3. It can be observed that both approaches

Table 3

$\text{PDI}_{\text{max}}$  values obtained for the different standards, at different flow-rates, and on the different columns: (a) applying Knox' equation; and (b) from the alternative approach

MM	Column	Flow rate (ml/min)						Minimal $\text{PDI}_{\text{max}}$	Manufacturer specified PDI
		0.1	0.2	0.3	0.5	0.7	1.5		
<i>(a)</i>									
1700	$10^3$	1.045	1.045	1.046	1.046	1.046	1.048	<b>1.045</b>	1.06
2450	$10^3$	1.041	1.041	1.042	1.049	1.042	1.044	<b>1.041</b>	1.05
3250	$10^3$	1.036	1.036	1.037	1.037	1.038	1.039	<b>1.036</b>	1.04
5050	$10^3$	1.032	1.032	1.033	1.032	1.034	1.036	<b>1.032</b>	1.05
7000	$10^3$	1.024	1.024	1.025	1.024	1.026	1.027	<b>1.024</b>	1.04
11600	$10^3$	1.018	1.018	1.018	1.019	1.021	1.022	<b>1.018</b>	1.03
22000	$10^3$	1.016	1.027	1.027	1.017	1.019	1.023	<b>1.016</b>	1.03
22000	$10^4$	1.021	(-)	(-)	(-)	(-)	(-)	<i>1.021</i>	
76600	$10^3$	1.019	(-)	(-)	1.030	(-)	(-)	<i>1.019</i>	
76600	$10^4$	(-)	(-)	1.014	1.016	1.016	1.018	<i>1.014</i>	
76600	$10^5$	1.014	1.012	1.013	1.013	1.015	1.017	<b>1.012</b>	1.03
200000	$10^3$	(-)	1.024	1.025	(-)	(-)	(-)	<i>1.024</i>	
200000	$10^4$	1.018	1.022	1.027	(-)	(-)	(-)	<i>1.018</i>	
200000	$10^5$	1.009	1.007	(-)	1.008	1.010	1.014	<b>1.007</b>	1.03
475000	$10^5$	1.018	1.013	1.015	1.015	1.018	(-)	<b>1.013</b>	1.03
675000	$10^5$	1.039	1.032	1.030	1.034	1.042	(-)	<b>1.030</b>	1.07
900000	$10^5$	1.051	1.038	1.032	1.039	1.047	(-)	<b>1.032</b>	1.07
<i>(b)</i>									
1700	$10^3$	1.053	1.053	1.053	1.045	1.055	1.055	<b>1.045</b>	1.06
2450	$10^3$	1.047	1.047	1.048	1.049	1.049	1.050	<b>1.047</b>	1.05
3250	$10^3$	1.039	1.039	1.040	1.041	1.042	1.043	<b>1.039</b>	1.04
5050	$10^3 \text{ \AA}$	1.037	1.037	1.038	1.039	1.040	1.042	<b>1.037</b>	1.05
7000	$10^3$	1.027	1.027	1.028	1.029	1.030	1.032	<b>1.027</b>	1.04
11600	$10^3$	1.018	1.018	1.019	1.020	1.021	1.023	<b>1.018</b>	1.03
22000	$10^3$	1.011	1.012	1.012	1.013	1.014	1.017	<b>1.011</b>	1.03
22000	$10^4$	1.023	(-)	(-)	(-)	(-)	(-)	<i>1.023</i>	
76600	$10^3$	<i>1.004</i> <sup>#</sup>	(-)	(-)	(-)	(-)	(-)	<i>1.004</i> <sup>#</sup>	
76600	$10^4$	(-)	(-)	1.015	1.017	1.017	1.019	<i>1.015</i>	
76600	$10^5$	1.015	1.012	1.013	1.014	1.016	1.018	<b>1.012</b>	1.03
200000	$10^3$	(-)	<i>1.001</i> <sup>#</sup>	<i>1.001</i> <sup>#</sup>	(-)	(-)	(-)	<i>1.001</i> <sup>#</sup>	
200000	$10^4$	1.020	1.024	1.030	(-)	(-)	(-)	<i>1.020</i>	
200000	$10^5$	1.009	1.007	(-)	1.009	1.010	1.015	<b>1.007</b>	1.03
475000	$10^5$	1.019	1.014	1.016	1.016	1.020	(-)	<b>1.014</b>	1.03
675000	$10^5$	1.046	1.037	1.034	1.039	1.050	(-)	<b>1.034</b>	1.07
900000	$10^5$	1.064	1.044	1.037	1.046	1.058	(-)	<b>1.037</b>	1.07

(-) not determined, <sup>#</sup>PDI underestimated due to shape calibration curve (standards situated too close to total exclusion range).



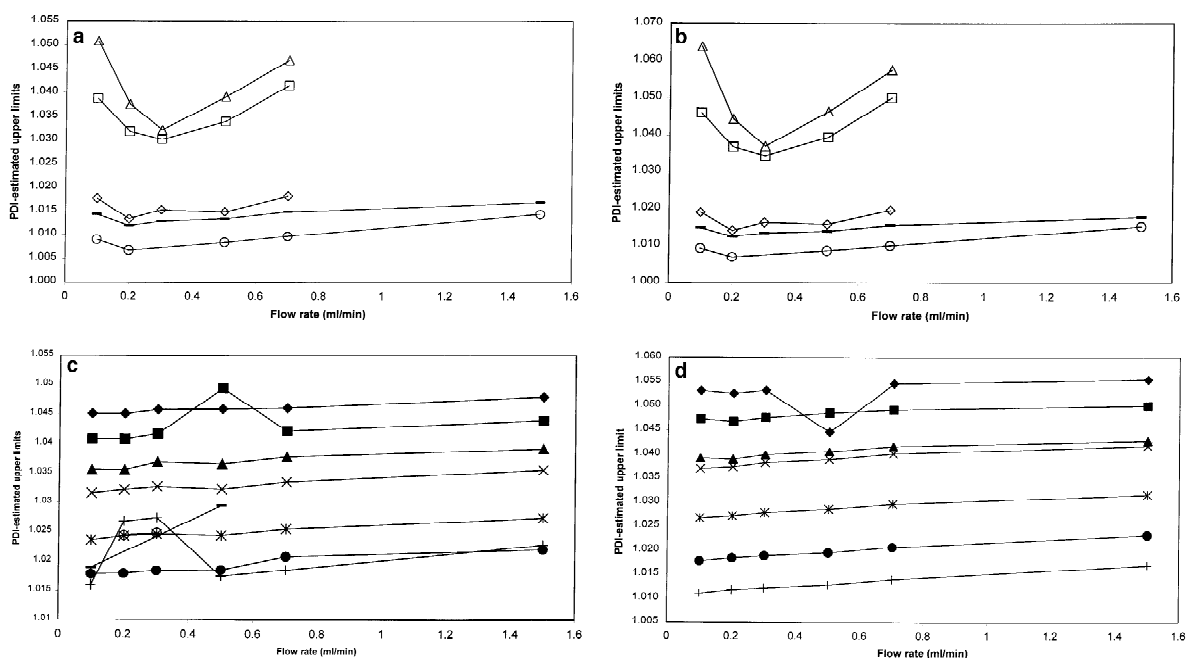


Fig. 3.  $PDI_{max}$  values for the different standards with  $\% h_{PDI}/h_{exp}$  exceeding 100%, as a function of the flow-rate: (a)  $10^5 \text{ \AA}$  column, Knox' approach; (b)  $10^5 \text{ \AA}$  column, alternative approach; (c)  $10^3 \text{ \AA}$  column, Knox' approach; and (d)  $10^3 \text{ \AA}$  column, alternative approach. Legend for the standards MM:  $\blacklozenge$ , 1700;  $\blacksquare$ , 2450;  $\blacktriangle$ , 3250;  $\times$ , 5500;  $*$ , 7000;  $\bullet$ , 11 600;  $+$ , 22 000;  $-$ , 76 600;  $\circ$ , 200 000;  $\diamond$ , 475 000;  $\square$ , 675 000;  $\triangle$ , 900 000.

lead to very similar estimates for  $PDI_{max}$ . The  $PDI_{max}$  values estimated from the alternative approach are, in general, somewhat higher than those from Knox' equation. From Fig. 3 it can be observed that, with the exception of some of the largest standards on the  $10^5 \text{ \AA}$  column, the estimated  $PDI_{max}$  values were approximately independent of the flow. This is in agreement with the results of Fig. 2 for which the estimated  $h_{PDI}$  values also were flow independent. It also can be seen from Table 3 that the minimal  $PDI_{max}$  values, i.e. those which should be the best possible estimates for the real PDI from this experimental set-up, are found on the  $10^3 \text{ \AA}$  column for the low-MM polymers (preferably at low flow-rate) and on the  $10^5 \text{ \AA}$  column for the higher molar mass polymers (preferably at intermediate flow-rate). At these conditions the contribution of the polydispersity of the polymer to the total peak shape is maximal. Therefore the MMD of polymers is best determined on a column for which it is situated in its

effective range and at relatively low mobile-phase flow-rates.

It also was observed that the minimal  $PDI_{max}$  values were *somewhat* smaller than those reported by the manufacturer, but not dramatically lower as claimed in Refs. [16,17].

### 3.4. Estimation of $PDI_{max}$ from other MMD distribution assumptions

In the above, the molar-mass distribution was always considered to be Gaussian. This corresponds to the most commonly observed chromatographic situation where peaks are skewed. However, in polymerisation theory and polymer analysis, other distributions are sometimes considered. In polymerisation, depending on the growth mechanism and when conducted under ideal circumstances, the MMD may follow a Poisson distribution [16,17,19,20]. In polymer analysis, for chromato-

graphic-elution curves that are rather Gaussian, the MMD can be approximated by a log-normal distribution [12,21]. We evaluated the influence that the occurrence of these distributions would have on the estimation of  $PDI_{\max}$  for the situations where earlier also a  $PDI_{\max}$  value was determined.

When for the MMD a log-normal distribution was assumed, the  $PDI_{\max}$  values were again calculated using Eq. (6). In this equation, the variance (Var) of the log-normal distribution (Eq. (8) and Ref. [22]) was used as  $\sigma_M^2$ , while for  $M_n$  either the mean of the log-normal distribution (Eq. (9) and Ref. [22]) or  $M_p$ , as in the previous estimates of PDI, was applied.

$$\text{Var} = (e^{\sigma^2} - 1)e^{2\mu + \sigma^2} \quad (8)$$

$$\text{Mean} = e^{\mu + \sigma/2} \quad (9)$$

with  $\mu$  the  $\ln(\text{MM}) = \ln(M_p)$  of a polymer, predicted through the calibration curve, from the retention time of its chromatographic peak, and  $\sigma$  the standard deviation of its  $\ln(\text{MM})$  distribution. An estimate for  $\sigma$  was obtained from the width at half height of the  $\ln(\text{MM})$  distribution. If MM is log-normally distributed then  $\ln(\text{MM})$  is normally distributed. This  $\ln(\text{MM})$  distribution width is predicted from the width of the chromatographic peak, applying an appropriate calibration-curve equation. The results obtained for  $PDI_{\max}$  are shown in Table 4. It can be observed that the results found when using the mean of the log-normal distribution (Table 4a) gave  $PDI_{\max}$  values that are, in general, very similar to those shown in Table 3 when Knox' equation was applied. The results found when  $M_p$  was applied (Table 4b) are close to those from the alternative approach in Table 3. The results of both approaches in Table 4 also are similar. This all indicates that the mean of the log-normal distribution and its top,  $M_p$ , are similar. It also means that if the MMD was log-normally distributed, this distribution was not very skewed and could without many problems be fitted by a normal distribution [22]. This can be confirmed by calculating the multiplicative standard deviations  $\sigma^* = e^\sigma$  of the distributions. The  $\sigma^*$  values obtained were below 1.28 for all situations considered in Table 4. For such small  $\sigma^*$  values, i.e. approaching 1.0, it is hard to distinguish a log-normal distribution from a normal one and usually the distribution will be considered normal [22].

A Poisson distribution is characterized by the fact that it only depends on one parameter,  $\mu$ , the mean of the distribution [23]. The standard deviation of this distribution is defined as  $\sigma = \sqrt{\mu}$ . In our situation,  $\mu$  represents the number of monomer units in a polymer. This leads to the  $PDI_{\max}$  values shown in Table 5. They were calculated using Eq. (6), which is valid for any form of MMD [10]. The obtained values are independent of the column or flow-rate used. It can be observed that they are smaller than the PDI values reported by the manufacturers. In Refs. [16,18] it was demonstrated that the PDI values estimated from a TGIC method approached those estimated from the theoretical Poisson distribution, which a polymer synthesized by anionic polymerization is expected to follow. The values derived from a SEC approach are reported to be considerably higher [16,18]. When evaluating our  $PDI_{\max}$  values from SEC (Tables 3 and 4) it can be seen that for polymers with a MM up to 200 000 values of a comparable order of magnitude as those found by TGIC [16,18] are obtained, which thus approach those of a Poisson distribution (Table 5). A possible explanation for the differences between the published values and our SEC estimates will be given below.

### 3.5. Estimation of PDI from matrix-assisted laser-desorption/ionisation time-of-flight mass spectrometry data

Given the fact that both the minimal  $PDI_{\max}$  values estimated from SEC and the PDI values from TGIC are slightly higher than those expected from a Poisson distribution, one could wonder whether our minimal  $PDI_{\max}$  values are realistic or whether they are still overestimated. The latter would be the case if the other band-broadening effects (column and extra-column) could not have been considered negligible under the most favourable conditions, as was done here. The former would occur when the MM distribution is deviating from a Poisson distribution. Therefore we tried to obtain a confirmation, for one or the other, from PDI estimates independent from the above-applied SEC approaches. Matrix-assisted laser-desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) offers this possibility for some polymers [13,14]. MALDI is a very soft ionisation technique, which yields large, non-frag-

Table 4

PDI<sub>max</sub> values obtained for the different standards, at different flow-rates, and on the different columns assuming a log-normal MMD: (a) applying the mean of the log-normal distribution; and (b) using  $M_p$

MM	Column	Flow rate (ml/min)						Minimal PDI <sub>max</sub>	Manufacturer specified PDI
		0.1	0.2	0.3	0.5	0.7	1.5		
<i>(a)</i>									
1700	10 <sup>3</sup>	1.045	1.044	1.045	1.038	1.046	1.046	<b>1.038</b>	1.06
2450	10 <sup>3</sup>	1.040	1.040	1.040	1.041	1.042	1.042	<b>1.040</b>	1.05
3250	10 <sup>3</sup>	1.034	1.033	1.034	1.035	1.036	1.036	<b>1.033</b>	1.04
5050	10 <sup>3</sup>	1.032	1.032	1.033	1.033	1.034	1.036	<b>1.032</b>	1.05
7000	10 <sup>3</sup>	1.023	1.024	1.024	1.025	1.026	1.027	<b>1.023</b>	1.04
11600	10 <sup>3</sup>	1.016	1.016	1.017	1.017	1.018	1.020	<b>1.016</b>	1.03
22000	10 <sup>3</sup>	1.010	1.011	1.011	1.012	1.013	1.015	<b>1.010</b>	1.03
22000	10 <sup>4</sup>	1.020	(–)	(–)	(–)	(–)	(–)	1.020	
76600	10 <sup>4</sup>	(–)	(–)	1.013	1.015	1.015	1.017	1.013	
76600	10 <sup>5</sup>	1.014	1.011	1.012	1.013	1.014	1.016	<b>1.011</b>	1.03
200000	10 <sup>4</sup>	1.017	1.020	1.026	(–)	(–)	(–)	1.017	
200000	10 <sup>5</sup>	1.009	1.007	(–)	1.008	1.009	1.014	<b>1.007</b>	1.03
475000	10 <sup>5</sup>	1.017	1.013	1.015	1.014	1.017	(–)	<b>1.013</b>	1.03
675000	10 <sup>5</sup>	1.038	1.031	1.029	1.033	1.041	(–)	<b>1.029</b>	1.07
900000	10 <sup>5</sup>	1.051	1.037	1.031	1.038	1.047	(–)	<b>1.031</b>	1.07
<i>(b)</i>									
1700	10 <sup>3</sup>	1.056	1.056	1.056	1.047	1.058	1.059	<b>1.047</b>	1.06
2450	10 <sup>3</sup>	1.050	1.049	1.050	1.051	1.052	1.053	<b>1.049</b>	1.05
3250	10 <sup>3</sup>	1.041	1.041	1.042	1.042	1.044	1.045	<b>1.041</b>	1.04
5050	10 <sup>3</sup>	1.038	1.039	1.040	1.041	1.042	1.044	<b>1.038</b>	1.05
7000	10 <sup>3</sup>	1.028	1.028	1.029	1.029	1.031	1.033	<b>1.028</b>	1.04
11600	10 <sup>3</sup>	1.018	1.019	1.019	1.020	1.021	1.024	<b>1.018</b>	1.03
22000	10 <sup>3</sup>	1.011	1.012	1.012	1.013	1.014	1.017	<b>1.011</b>	1.03
22000	10 <sup>4</sup>	1.024	(–)	(–)	(–)	(–)	(–)	1.024	
76600	10 <sup>4</sup>	(–)	(–)	1.015	1.017	1.018	1.020	1.015	
76600	10 <sup>5</sup>	1.015	1.013	1.014	1.014	1.016	1.018	<b>1.013</b>	1.03
200000	10 <sup>4</sup>	1.020	1.024	1.031	(–)	(–)	(–)	1.020	
200000	10 <sup>5</sup>	1.009	1.007	(–)	1.009	1.010	1.015	<b>1.007</b>	1.03
475000	10 <sup>5</sup>	1.019	1.014	1.017	1.016	1.020	(–)	<b>1.014</b>	1.03
675000	10 <sup>5</sup>	1.047	1.037	1.035	1.040	1.051	(–)	<b>1.035</b>	1.07
900000	10 <sup>5</sup>	1.066	1.045	1.038	1.047	1.059	(–)	<b>1.038</b>	1.07

(–) not determined.

mented ions. If the yield of the mass spectrometer (determined by the efficiency of ionisation, separation and detection) can be assumed independent of the molar mass across the range of molecules present in the sample, the MALDI-MS spectrum is directly indicative of the MMD of a polymer. From the spectrum,  $\sigma_M$  can be estimated, as well as  $M_p$ . Then PDI can be estimated with Eq. (6), using  $\sigma_M$  and either  $M_p$  (found from the MS spectrum) or the MM reported by the manufacturer.

This approach was applied to five polystyrene standards with MMs of 2100, 4700, 6770, 28 500

and 76 600, and with PDI values of 1.05, 1.05, 1.03, 1.03 and 1.03, respectively (values specified by the manufacturers). From the MS spectra (e.g. Fig. 4) PDI values of 1.039, 1.021, 1.020, 1.005 and 1.003, respectively, were estimated. The  $\sigma_M$  for these estimates were derived from the peak widths measured at half height of the observed distributions, assuming they were Gaussian. These estimates for PDI indicate that for the three smallest samples estimates were found that were comparable with those found with SEC and TGIC for similar polymers. For the largest polymer (MM 76 600) the MS

Table 5  
PDI<sub>max</sub> values obtained for the different standards when assuming a Poisson distribution

MM	Number of monomer units	PDI <sub>max</sub>	Manufacturer specified PDI
1700	16	1.06	1.06
2450	23	1.04	1.05
3250	31	1.03	1.04
5050	48	1.02	1.05
7000	67	1.01	1.04
11600	111	1.009	1.03
22000	211	1.005	1.03
76600	735	1.001	1.03
200000	1920	1.0005	1.03
475000	4561	1.0002	1.03
675000	6481	1.0002	1.07
900000	8641	1.0001	1.07
2000000	19202	1.00005	1.03
2200000	21123	1.00005	1.04

spectrum gives a PDI estimate that is even closer to the Poisson distribution (Table 5) than our SEC results (Tables 3 and 4) or the TGIC results [17]. This might indicate that for the larger polymers, both in TGIC and SEC, the kinetic band-broadening is still not negligible.

The PDI values estimated with MALDI can be considered as minimal values, PDI<sub>min</sub>. The reason for it is that the spectra, as one is shown in Fig. 4, have a lower sensitivity for the higher masses than for the lower ones, and secondly instead of numbers of molecules considered on the y-axis of the spectrum, one should consider masses, i.e. a distribution in weights instead of one in numbers would be more correct. Such a distribution tends to lead to higher PDI estimates as is explained below.

To account for that, two corrections were performed on the raw MS data. In a first instance the numbers were changed to weights by multiplying the intensity with the relevant MM. Secondly, a correction for differences in <sup>12</sup>C–<sup>13</sup>C isotopes composition between the different polymers within one standard also was introduced, because the measured intensity around a given *m/z* value is directly related to the number of molecules with the most frequently occurring <sup>12</sup>C–<sup>13</sup>C isotope composition. It can, for instance, be calculated from a binomial distribution that the highest intensity at *m/z* around 3500 (Fig. 4) is originating from the polymer containing three <sup>13</sup>C

and 269 <sup>12</sup>C carbon atoms and that it represents 22.5% (= *p*) of the polymer molecules with 272 carbons. The highest signal around *m/z* 6000 on the other hand is due to the polymer with 464 C atoms, of which five are <sup>13</sup>C. The highest signal represents 17.6% of this polymer. To account for these differences in isotope composition the signals were corrected in such a way that a prediction is made of the expected signals when a polymer would consist completely of the most frequent isotope composition (= intensity · 100/*p*).

For both corrections the change is larger for higher molar masses, which theoretically would tend to increase the skewness of the distributions. However, in practice these corrections lead to distributions which give rise to about equal PDI values as those obtained from the intensities. For instance, for the standard with MM=6770, PDI values of 1.020, 1.018 and 1.018 were found for the measured, the MM corrected and the isotope corrected spectrum, respectively.

Therefore, taking into account the above, we can assume that the minimal PDI<sub>max</sub> values estimated in the SEC measurements (Table 3) approach the real (theoretical) PDI value reasonably well for low-MM polymers and that at the conditions they were estimated the peak widths are almost entirely due to the polydispersity of the measured polymer. For larger polymers the SEC measurements still seems to overestimate the real PDI values.

### 3.6. Re-assessment of the $h_{PDI}$ contribution to $h_{exp}$

Based on the above observations we reconsidered the  $h_{PDI}$  contribution to  $h_{exp}$ . The minimal PDI<sub>max</sub> values obtained in Table 3a were used to estimate  $h_{PDI}$  according to Eq. (4). The percent fraction of the total reduced plate height due to polydispersity, i.e.  $100 \cdot h_{PDI}/h_{exp}$ , was determined. It is plotted as a function of the flow-rate in Fig. 5. One can see in Fig. 5a that on the  $10^5$  Å column (effective MM range: 60 000–2 000 000), for the polymers with MM 76 600, 200 000, 475 000, 675 000 and 900 000 the contribution of  $h_{PDI}$  to  $h_{exp}$  is above 99% at either 0.2 or 0.3 ml/min. However, taking into account the MS observations the  $h_{PDI}$  contribution of these standards still might be somewhat overestimated. Anyway, these conditions should allow on

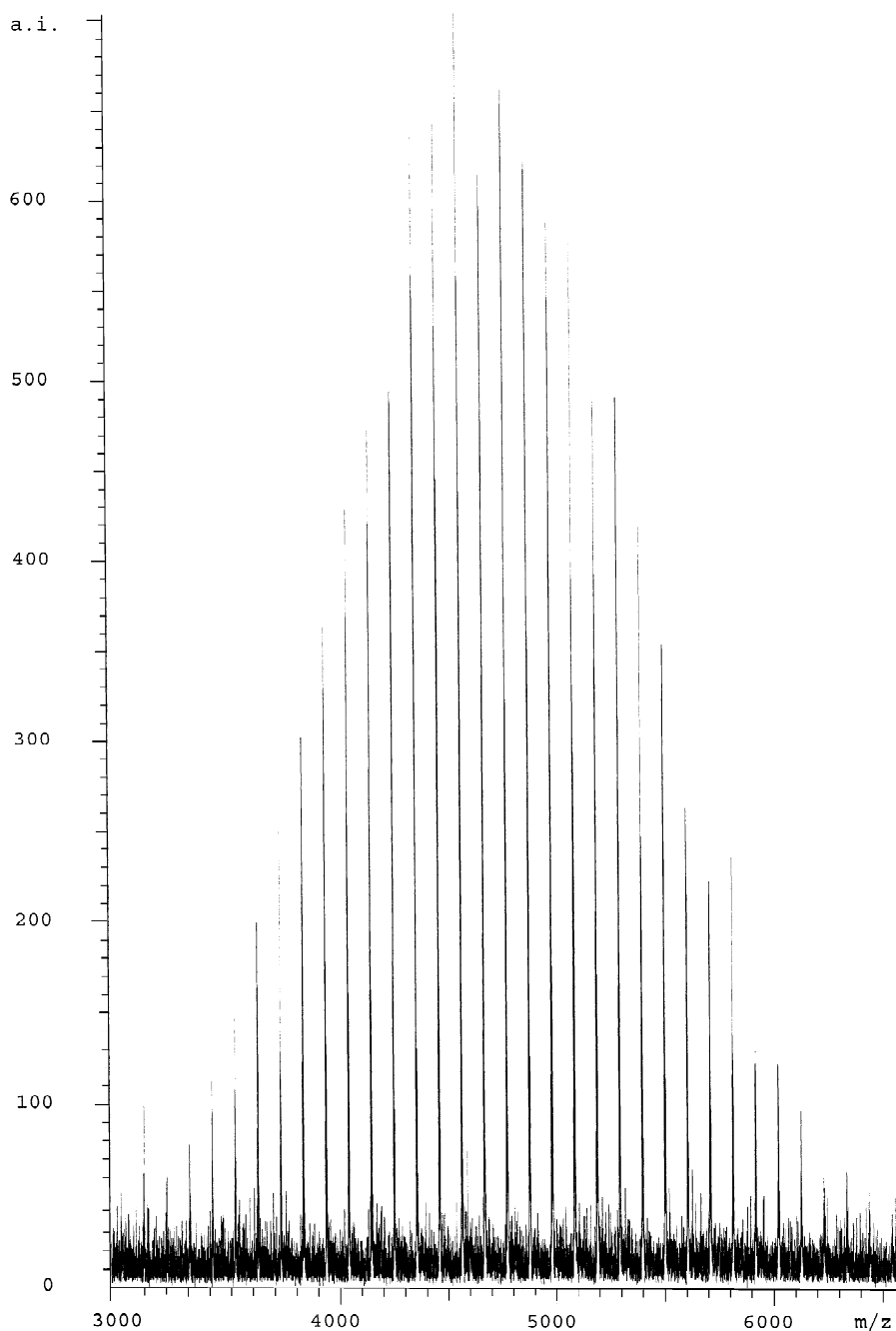


Fig. 4. MALDI-TOF-MS spectrum for a polystyrene standard with reported values, by the manufacturer, of 4700 for MM and of 1.05 for PDI.

this column the best MMD parameter estimates for polymer samples with similar molecular masses. It is also seen that the contribution of  $h_{\text{PDI}}$  to  $h_{\text{exp}}$  is flow

dependent for these standards. Lower and higher flow-rates lead to increased contributions of  $h_{\text{kin}}$  to  $h_{\text{exp}}$ . For the other standards, both smaller and larger

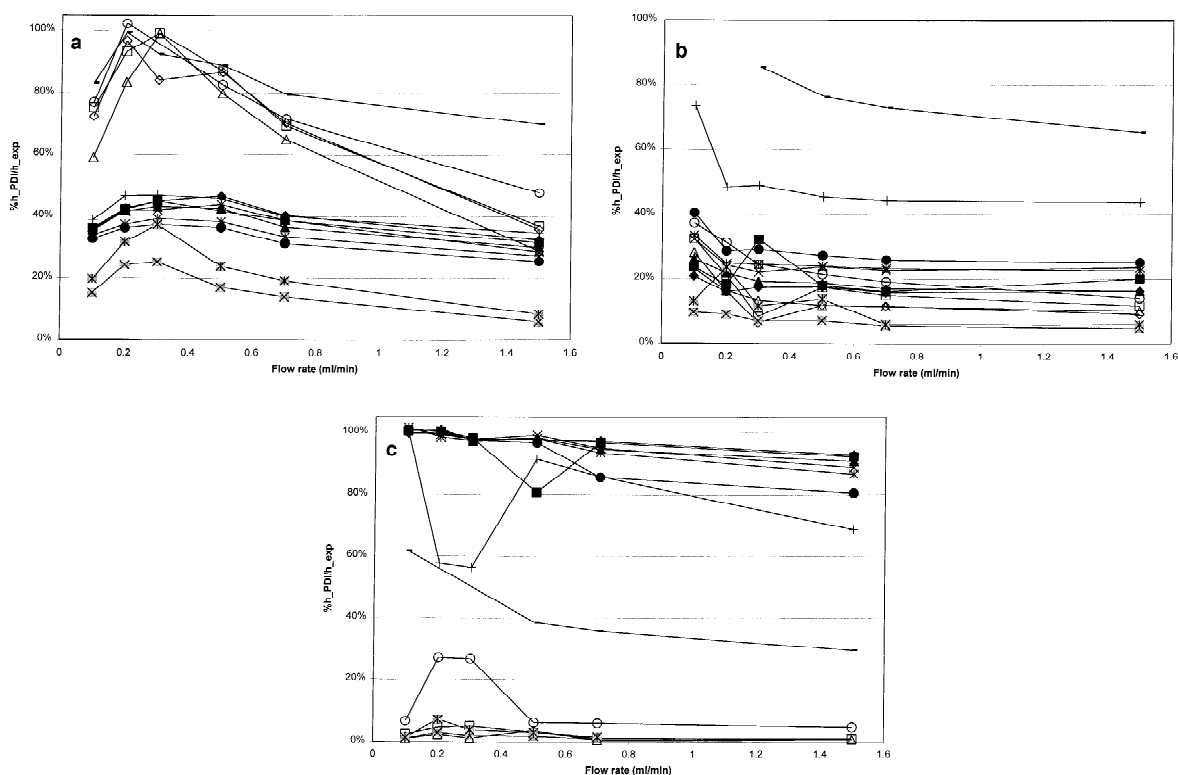


Fig. 5. The percent fraction of the total reduced plate height due to polydispersity, i.e.  $100 \cdot h_{\text{PDI}}/h_{\text{exp}}$ , plotted as a function of the flow-rate: (a)  $10^5$  Å column; (b)  $10^4$  Å column; and (c)  $10^3$  Å column. Legend for the standards MM:  $\blacklozenge$ , 1700;  $\blacksquare$ , 2450;  $\blacktriangle$ , 3250;  $\times$ , 5500;  $*$ , 7000;  $\bullet$ , 11 600;  $+$ , 22 000;  $-$ , 76 600;  $\circ$ , 200 000;  $\diamond$ , 475 000;  $\square$ , 675 000;  $\triangle$ , 900 000; shaded box with cross, 2 000 000; and shaded box with star, 2 200 000.

ones, the contributions of  $h_{\text{PDI}}$  to  $h_{\text{exp}}$  were below 50%, meaning that one should be careful when estimating MMD parameters for samples with such low or high molar masses on this column.

The results on the  $10^3$  Å column (effective MM range: 500–60 000) demonstrate that for the polymers with MM 1700, 2450, 3250, 5050, 7000 and 11 600 the  $h_{\text{exp}}$  is completely determined by  $h_{\text{PDI}}$  at low flow-rates, i.e. at 0.1 or 0.2 ml/min (Fig. 5c). At higher flow-rates the influence of  $h_{\text{kin}}$  to  $h_{\text{exp}}$  seems to increase. However, the influence of the flow-rate for the polymers in the effective range of this column is less than for those in the effective range of the  $10^5$  Å column. The  $h_{\text{PDI}}$  contribution of the polymer with MM 22 000 seems to show a large variability (Fig. 5c). This is due to the fact that the polymer is eluting close to the exclusion limit of the column used, which makes it not always evident to estimate  $S$  in

Eq. (4) correctly from the calibration curve measured [6]. For the polymers situated outside the effective range of this column large contributions of  $h_{\text{kin}}$  to  $h_{\text{exp}}$  were observed. When the polymer is fully excluded,  $h_{\text{PDI}}$  approaches zero, and thus the contribution of  $h_{\text{kin}}$  100%.

Finally, on the  $10^4$  Å column (effective MM range: 10 000–600 000), for most polymers, also those situated in the effective range, a large contribution of  $h_{\text{kin}}$  to  $h_{\text{exp}}$  was seen (Fig. 5b). It usually exceeded 50%, which makes this column less suitable to estimate MMD parameters in any range. Note that this conclusion pertains to the present column and not necessarily to all columns with the same denomination.

The above observations might give us an explanation why the PDI estimates from SEC measurements can be considerably higher than those found with the

TGIC [16,18]. If the SEC experiments are performed at suboptimal conditions, i.e. at a too high flow-rate and/or on a less appropriate column (situations in which there is still a considerable  $h_{kin}$  contribution to  $h_{exp}$ ) the SEC results overestimate the real PDI values.

#### 4. Conclusions

The above study indicates that the true PDI values of narrow standards are below the upper limits specified by the suppliers. Assuming the specified values as correct, leads to largely overestimated contributions of the PDI to the observed peak width or to the reduced plate height,  $h_{PDI}$ , for narrow standards in SEC. The estimation of  $h_{PDI}$  itself seems to be correct since different approaches lead to comparable results.

It was also found that if the molar masses of the examined polymers are in reality log-normally distributed, the distribution is of such a nature that it can be approximated by a normal one.

It was demonstrated that for low-MM polymers the PDI estimates from SEC can approach the theoretical ones derived from a Poisson distribution reasonably well when the SEC measurements are performed at optimal flow-rate and on a column with an appropriate pore-size distribution.

The estimations of PDI from mass spectra seem to indicate that the  $PDI_{max}$  values for low-MM polymers derived from the SEC experiments are reasonably correct and certainly not much too high. For higher-MM polymers they indicate that the best SEC measurements are still overestimating the real PDI.

To estimate MMDs of polymers it was confirmed that the best estimates, i.e. those least affected by extra-column and column band-broadening effects, should be obtained when measuring a polymer in the effective range of a given column and at a rather low flow-rate. An exception to the above was the column with the intermediate pore size on which all tested polymer peaks were greatly affected by these other band-broadening effects.

#### Acknowledgements

Y.V.H. is a postdoctoral fellow of the Fund for

Scientific Research (FWO), Vlaanderen, Belgium. The authors would like to thank S. Eeltink and F. Fitzpatrick for providing useful data, Paul le Comte for technical assistance, and N. Bacchini for logistic support.

#### References

- [1] J.C. Moore, J. Polym. Sci. A 2 (1964) 835.
- [2] W.W. Yau, J.J. Kirkland, D.D. Bly, Modern Size-Exclusion Liquid Chromatography, Wiley, New York, 1979.
- [3] B. Porsch, M. Andersson, B. Wittgren, K.-G. Wahlund, J. Chromatogr. A 946 (2002) 69.
- [4] S.X. Ci, T.H. Huynh, L.W. Louie, A. Yang, B.J. Beals, N. Ron, W.-G. Tsang, P. Soon-Shiong, N.P. Desai, J. Chromatogr. A 864 (1999) 199.
- [5] E.M. Fujinari, J.D. Manes, J. Chromatogr. A 763 (1997) 323.
- [6] Y. Vander Heyden, S.T. Popovici, P.J. Schoenmakers, J. Chromatogr. A 957 (2002) 127.
- [7] E.G. Malawer, Handbook of Size-exclusion Chromatography, Chromatographic Science Series, Vol. 69, Marcel Dekker, New York, 1995, pp. 1–24.
- [8] S.T. Balke, T.H. Mourey, C.A. Harrison, J. Appl. Polym. Sci. 51 (1994) 2087.
- [9] A.J.P. Martin, R.L.M. Synge, Biochem. J. 35 (1941) 1358.
- [10] J.H. Knox, F. McLennan, Chromatographia 10 (1977) 75.
- [11] J.P. Busnel, F. Foucault, L. Denis, W. Lee, T. Chang, J. Chromatogr. A 930 (2001) 61.
- [12] M. Netopilik, S. Podzimek, P. Kratochvíl, J. Chromatogr. A 922 (2001) 25.
- [13] E. Scamporrino, D. Vitalini, in: R.A. Pethrick, J.V. Dawkins (Eds.), Modern Techniques for Polymer Characterization, Wiley, Chichester, 1999.
- [14] C.A. Jackson, W.J. Simonsick Jr., Curr. Opin. Solid State Mater. Sci. 2 (1997) 661.
- [15] G. Stegeman, R. Oostervink, J.C. Kraak, H. Poppe, K.K. Unger, J. Chromatogr. 506 (1990) 547.
- [16] W. Lee, H. Lee K, J. Cha, T. Chang, K.J. Hanley, T.P. Lodge, Macromolecules 33 (2000) 5111.
- [17] D.W. Shortt, J. Chromatogr. A 686 (1994) 11.
- [18] H.C. Lee, T. Chang, Polymer 37 (1996) 5747.
- [19] G. Odian, Principles of Polymerization, 3rd ed, Wiley, New York, 1991.
- [20] H. Höcker, J. Molec. Catal. 65 (1991) 95.
- [21] M. Netopilik, J. Chromatogr. A 915 (2001) 15.
- [22] E. Limpert, W.A. Stahel, M. Abbt, BioScience 51 (2001) 341.
- [23] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Part A, Elsevier, Amsterdam, 1997.