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Fast size-exclusion chromatography—Theoretical and practical considerations

Simona T. Popovici [a], Peter J. Schoenmakers [a,b,*]

[a] *van't Hoff Institute for Molecular Sciencies (HIMS), University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands*
[b] *Dutch Polymer Institute, P.O. Box. 902, 5600 AX Eindhoven, The Netherlands*

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Abstract

Fast SEC is a very interesting modification of conventional SEC. The need for it emerges from combinatorial chemistry and high-throughput experimentation, where high-speed analyses are required. The different approaches to change the speed of analysis are extensively described in this paper. Special attention is paid to the trade-off between analysis time and resolution and to the selection of optimal column lengths and flow rates. Simulations are used to design and to understand experiments. Integrity plots are constructed to judge the quality of various SEC systems. Fast separations in size-exclusion chromatography are found to be more favorable than suggested by conventional theory. The results are based on experimental data obtained for polystyrene using THF as mobile phase.

Keywords: Size-exclusion chromatography; Fast-SEC; High-throughput screening; Band broadening; Polydispersity

1. Introduction

Size-exclusion chromatography (SEC) is a form of liquid chromatography (LC) that separates the analytes according to their hydrodynamic volume. SEC is the outstanding technique to measure molar-mass distributions (MMD) of natural and synthetical macromolecules. The analytes with the highest molar masses (M) are eluting first from the separation column, while the smaller molecules are eluting last, being retained for a longer time inside the column. Conventionally, SEC analyses require times up to several hours and not less than 10–15 min. One of the most important parameters in any kind of chromatography is the resolution. Good resolution is required to adequately characterize the sample. Because in case of polymers we invariable work above the optimal flow rate [1], the best resolution is usually obtained at the lowest flow rate, which is equivalent to the longest analysis time. However, there is a definite trend towards fast size-exclusion-chromatographic separations.

Fast SEC has been used by several polymer-science groups [2,3,21] and industrial laboratories [4–7] and dedicated columns for the purpose are available from several manufacturers [8,20]. The field is strongly application driven. Very little fundamental work has yet been devoted to the subject. The great interest in fast SEC separations arises from two sources. First, the emergence of combinatorial approaches to (industrial) research and development and the associated need for high-throughput experimentation [9]. Second, in polymer analysis two-dimensional techniques have been developing rapidly during the last years. This requires fast second-dimension separations. Other perceived advantages of fast SEC are the consumption of less eluent and the use of smaller columns. We believe neither of these latter two arguments to be truly relevant. Reduced consumption of solvents (and stationary phase) can much better be achieved by reducing the column diameter. The prevailing reason to pursue Fast SEC is speed.

Traditionally SEC of polymers has been performed on columns packed with relatively large particles (e.g. 10–20 μm). Columns with such large particles can provide high-resolution separations, but they do require long analysis times [29]. Large (high M) polymers are thought to require large particles to avoid shear degradation and recovery problems. To reduce the analysis times different approaches are possible (Table 1). The first one is to decrease the particle diameter of the stationary phase. The

* Corresponding author. Tel.: +31 20 5256642; fax: +31 20 525 5604.
E-mail address: pjschoen@science.uva.nl (P.J. Schoenmakers).

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Table 1
Different approaches to change the speed of analysis in SEC

Operating parameters			Anticipated results		
d_p (μm)	L (mm)	F (ml/min)	N[a]	t_R (min)	Δp (bar)
×2	×2	×2	(>) ×1/2	Cst.	Cst.
		Cst.	Cst.	×2	×1/2
		×1/2	(<) ×2	×4	×1/4
	Cst.	**×2**	(>) **×1/4**	**×1/2**	**×1/2**
		Cst.	×1/2	Cst.	×1/4
		×1/2	(<) Cst.	×2	×1/8
	×1/2	**×2**	(>) **×1/8**	**×1/4**	**×1/4**
		Cst.	**×1/4**	**×1/2**	**×1/8**
		×1/2	(<) ×1/2	Cst.	×1/16
Cst.	×2	×2	(>) Cst.	Cst.	×4
		Cst.	×2	×2	×2
		×1/2	(<) ×4	×4	Cst.
	Cst.	**×2**	(>) **×1/2**	**×1/2**	**×2**
		Cst.	Cst.	Cst.	Cst.
		×1/2	(<) ×2	×2	×1/2
	×1/2	**×2**	(>) **×1/4**	**×1/4**	**Cst.**
		Cst.	**×1/2**	**×1/2**	**×1/2**
		×1/2	(<) Cst.	Cst.	×1/4
×1/2	×2	×2	(>) ×2	Cst.	×16
		Cst.	×4	×2	×8
		×1/2	(<) ×8	×4	×4
	Cst.	**×2**	(>) **Cst.**	**×1/2**	**×8**
		Cst.	×2	Cst.	×4
		×1/2	(<) ×4	×2	×2
	×1/2	**×2**	(>) **×1/2**	**×1/4**	**×4**
		Cst.	**Cst.**	**×1/2**	**×2**
		×1/2	(<) ×2	Cst.	Cst.

d_p is the particle diameter, L the column length, F the flow rate, N the plate number, t_R the retention time and Δp is the pressure drop across the separation column. Potential approaches to fast SEC are printed in bold.

[a] The symbol ">" indicates "better than"; The symbol "<" indicates "worse than". Effects are moderated, because the plate height varies less than proportionally with the flow rate.

second possibility is to reduce the column length and the third to increase the flow rate.

The packing of SEC columns with smaller particles is the most attractive option for reducing the analysis times. When the length of the column is kept constant the efficiency will increase (Table 1). To reduce the separation time, the column length can be decreased or the flow rate increased. The efficiency can be (at least) maintained at the original level. The major disadvantage of this approach is the increased pressure drop across the separation column [10]. In order to overcome this mechanical limitation, Mc.Nair et al. [11,12] introduced ultra-high-pressure liquid chromatography (UHPLC) techniques. Small particles inherently produce very low column permeability and, therefore considerable heat is generated. Wu et al. [13,14] concluded that capillary columns (e.g. 30–150 μm internal diameter) are required to facilitate heat dissipation. Another solution to overcome the high pressure drop could be the use of monolithic columns. Due to their structure, silica-based monoliths can also offer an enhanced chromatographic performance [15–17]. They provide a unique combination of low pressure drop and high separation efficiency. Unfortunately, the selectivity offered by monolithic columns for SEC is still much inferior to that of typical columns packed with porous particles. So far, monoliths have a smaller volume of pores that contain stagnant mobile phase during analysis. Other alternatives to avoid working at high-pressure are size-exclusion electro-chromatography (SEEC) [18] and open-tubular or open-channel hydrodynamic-chromatography (HDC) [19]. Neither of these latter options is thought to be practical at this stage.

The third option for speeding up the SEC analysis is to increase the flow rate. This can be either be done using a conventional column, or while concomitantly using a shorter column (i.e. the second option). In the first case the efficiency and resolution decrease while the pressure drop will be higher. When increasing the flow rate in combination with a shorter column, the pressure drop may not increase, while the time of analysis will be shorter. However, the plate number will also decrease dramatically. Therefore, a compromise must be found between the gain in speed and the loss in efficiency (resolution).

The factors listed in the plate-count column are based on the classical van-Deemter equation, which suggests that at high flow rates N is approximately proportional to $1/F$. However, in reality the situation is more favorable. When doubling F, N is expected to decrease by (much) less than a factor of two (see [1] and theory section below). This is indicated by the "<" and ">" signs in Table 1 for the situations in which the flow rate is altered.

Commercial fast SEC columns are much shorter than conventional SEC or LC columns (Table 2). Conventional LC columns have a length of 150–300 mm. The fast-SEC columns commercialized by Polymer Laboratories (PL) are 50 mm long and have an internal diameter (i.d.) of 4.6 or 7.5 mm, while those manufactured by Polymer Standard Service (PSS) have the same length, but a larger internal diameter of 20 mm.

Kilz et al. [20] have investigated options for fast-SEC analysis. They compared different approaches, such as increasing the flow rate on a conventional SEC column (8 mm × 300 mm),

Table 2a
Dimensions of Fast-SEC columns (d_c is column diameter, L is column length, d_p is particle diameter, V_{col} is column volume)

Column dimensions (mm)	Manufacturer	Column volume (ml)	Volume eluent for LC × SEC run (ml)[a]
4.6 × 50	PL	0.5	50
7.5 × 50	PL	1.3	130
20 × 50	PSS	10	1000

[a] Assuming 100 second-dimension runs.

Table 2b
Suggested flow rates and column diameters in LC × SEC

^{2}D column ($^2d_c \times {}^2L$) (mm)	^2F (ml/min)	^2t_R (min)	$^2V_{inj}$ (μl)	^1F (μl/min)	^1d_c (mm)
4.6 × 50	0.5	1	20	20	1
7.5 × 50	1.3	1	50	50	1.5 or 2
20 × 50	10	1	400	400	4.6

shortening the length to 50 mm, decreasing the i.d. to 4 mm, or increasing the column diameter to 20 mm. In a study by Pasch et al. [21] conventional styrene/divinylbenzene SEC columns (300 mm × 8 mm) and fast-SEC columns of 50 mm × 20 mm were compared. The accuracy and the precision of fast SEC columns were investigated using a broad reference standard. The analysis time was reduced from 10 to 2 min.

Applying the concept of integrity plots, which we introduced elsewhere [1], we are able to quantitatively investigate the influence of the particle size, pore-size distribution, flow rate, and column length on the quality of separation in fast SEC, based on a limited number of simple experiments. The aim of the present paper is to investigate whether fast SEC is a useful technique and, if so, for which types of analytes and under which conditions. Explicitly, we aim to establish the range of molar masses and polydispersities (PDI) for which an accurate MMD can be obtained. The MMD of polymers is characterized by their average molecular weights, such as number-average molecular weight (M_n) and weight-average molecular weight (M_w). The PDI of a polymer is defined as PDI = M_w/M_n.

The main fundamental obstacle to the fast and efficient chromatography of polymers is the slow diffusion of the analytes. As a consequence of this effect we have to deal with poor chromatographic efficiency and extra-column dispersion. The molecular diffusion of polymers is strongly dependent on their molar mass and on the mobile phase [22]. Typically, the diffusion coefficient decreases with increasing mobile-phase viscosity and it typically decreases with increasing M.

A SEC peak can be broadened due to the PDI of the sample and due to extra-column and column dispersion. Since in SEC we are aiming to measure the MMD of the sample, the broadening due to the PDI (i.e. the chromatographic selectivity) must be as high as possible, while the other two contributions to the total band broadening must be minimized.

The exact value of the polydispersity index (PDI) is very difficult to establish. The widths of molar-mass distributions (which are directly related to the PDI) have been directly estimated from SEC coupled with concentration and light-scattering detectors [23] and can also be derived from mass-spectrometric measurements using soft ionization techniques [24,25]. In the case of commercial standards the manufacturer specifies a value, which should be seen as an upper limit. Some researchers concluded that the real PDI values are somewhat smaller than the one specified by the supplier [26,27] others suggested that the true values are much smaller [28]. Strong evidence that the latter presumption is correct has been provided by Lee et al. [26] using temperature-gradient interaction chromatography (TGIC).

Two-dimensional chromatography progressed considerably in the last decade. This method involves the coupling of two different separation mechanisms on-line, exploiting the potential of both of them in order to obtain, so-called 'comprehensive' information. The first dimension is a slow separation while the second one is fast. The fractions of the sample eluting from the first dimension are collected by the modulation valve and re-injected in the second dimension. The faster the second dimension, the shorter can be the total analysis time (or the higher the overall resolution). The most common combination in comprehensive two-dimensional chromatography of polymers is interactive liquid chromatography with SEC. A second-dimension analysis time of 1 min implies an analysis time of several hours, if we are to maintain our first-dimension resolution. Thus, high-throughput experimentation requires moderately fast SEC; LC × SEC requires very fast SEC. In addition, LC × SEC has been gaining attention from a number of research groups and industries [31].

2. Theory

2.1. General aspects and overview

In chromatography, the degree of separation of two components i and j is given by the resolution R_s [29], which usually is defined as:

$$R_s = \frac{t_{R,j} - t_{R,i}}{(1/2)(w_i + w_j)} \quad (1)$$

where $t_{R,i}$ and $t_{R,j}$ represent the retention times and w_i and w_j are the peak widths (in time units) at the baseline of the second (j) and the first (i) analyte, respectively. Alternatively, retention volumes and peak widths in volume units may be used. In SEC a different resolution concept (R_{SEC}) is used, which is correlated to the calibration curve as follows:

$$R_{SEC} = \frac{\Delta V_R}{4\sigma} \frac{1}{\log(M_i/M_j)} \quad (2)$$

where $\Delta V_R = (V_{R,j} - V_{R,i})$, represents the difference in retention volumes between the second (j) and the first (i) analyte. σ is the peak standard deviation in volume units (proportional to the peak width), and M_j and M_i are the molar masses of the analytes where $M_j < M_i$, [30]. Note that R_{SEC} is equal to the conventional resolution (R_s) if we consider two peaks that differ in molar mass by an order of magnitude ($M_i = 10M_j$).

As described in the introduction of this paper, an important reason for our interest in fast SEC is its use as the second dimension in two-dimensional chromatography, in order to obtain comprehensive two-dimensional information. The most commonly employed coupling is liquid chromatography (LC) with size-exclusion chromatography (SEC), abbreviated to LC × SEC [33]. Reducing the column length of the second dimension and increasing the flow rate would greatly decrease the run time in SEC analysis (Table 1). As a result, the total analysis time in LC × SEC would become shorter. In Table 2 commercial short columns are listed. A smaller i.d. implies a smaller column volume. The PL columns have volumes of 0.5 and 1.3 ml while the PSS column has a volume of 10 ml. Typically, in LC × SEC a hundred fractions from the first dimension may be taken to characterize the sample. Because in SEC the volume of eluent required for one run is approximately equal to the column volume, the total volume required for a two-dimensional analysis assuming 100 fractions is 50 ml for the 4.6 mm i.d. column, 130 ml for the 7.5 mm i.d. column, and 1000 ml when the 20 mm i.d. column is used. The smallest column is most attractive from the points of view of toxic waste and cost of analysis. Also, the flow rate of 10 ml/min associated with the

20 mm i.d. column is not compatible with many common SEC detectors.

In truly comprehensive two-dimensional liquid chromatography the maximum injection volume in the second dimension and the second dimension analysis time determine the maximum flow rate in the first dimension (Eq. (3)) [31].

$$ {}^1F_{\max} = \frac{{}^2V_{\mathrm{inj,max}}}{{}^2t_{\mathrm{R}}} \tag{3} $$

where, ${}^1F_{\max}$ is the maximum flow rate in the first dimension, expressed in μl/min, ${}^2V_{\mathrm{inj,max}}$ the maximum injection volume in the second dimension, expressed in μl, and ${}^2t_{\mathrm{R}}$ is the retention time in the second dimension, expressed in min.

The maximum flow rate (${}^1F_{\max}$) in turn suggests a suitable internal diameter for the first-dimension column (${}^1d_{\mathrm{c}}$). The advantage of using a 20 mm i.d. column in the second dimension is that a conventional column can be used in the first dimension. Choosing a 4.6 mm or a 7.5 mm i.d. second-dimension column necessitates the use of a narrow-bore column in the first dimension. Alternatively, the effluent can be split after the first column, but this may hinder quantitative applications of LC × SEC. The effluent viscosity varies greatly when polymeric samples are eluted and this would inevitably cause variations in the split ratio.

Optimizing the column configuration, flow rate and injection volume in conjunction with the mobile phase conditions can lead to an enhanced resolution. Ricker and Sandoval [32] have investigated the influence of the flow rate on the column efficiency and on the resolution using a Zorbax GF-250 column (250 mm × 9.4 mm). By decreasing the sample volume from 200 to 2 μL the resolution was improved from 0.7 to 1.3. Ricker et al. stressed that sample volume is the limiting factor in SEC and not sample concentration. However, above concentrations of roughly 5–10 mg/ml the sample becomes very viscous and then a loss in resolution can also be due to high concentrations.

The main objective in Fast SEC is to characterize the sample at the optimum experimental conditions representing the best compromise between speed and resolution.

The efficiency in chromatography is commonly expressed in terms of the plate height.

$$ h = \frac{H}{d_{\mathrm{p}}} \tag{5} $$

$$ \nu = \frac{u d_{\mathrm{p}}}{D_{\mathrm{m}}} \tag{6} $$

where h is the reduced plate height, ν is the reduced velocity, H is the plate height expressed in mm, d_{p} represents the particle diameter (mm), u is the interstitial velocity (mm/s) and D_{m} is the diffusion coefficient of the sample, expressed in mm^2/s.

Giddings [33] has introduced plots of the reduced plate height (h) versus the reduced velocity (ν). The reduced plate height reflects all dispersion terms in the conventional van-Deemter Eq. (4), i.e. Eddy diffusion (A term), molecular diffusion (B term), and resistance to mass transfer (C term).

$$ h = A + \frac{B}{\nu} + C\nu \tag{4} $$

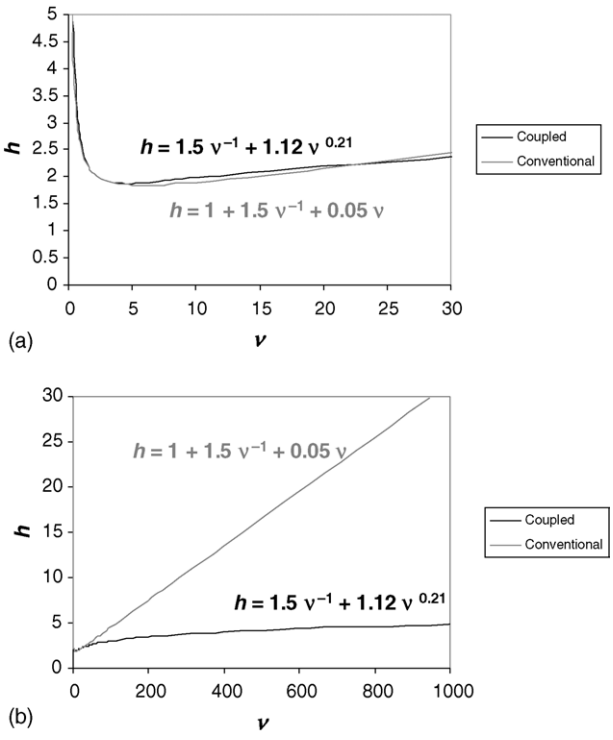

Fig. 1. Reduced plate height vs. reduced velocity plot according to conventional theory (Eq. (4)) and according to Eq. (4a).

Later Knox and Parcher [34] and Giddings have modified this by a coupling term (A_{C}), which represents the combined effects of the A and C terms ('coupling term').

$$ h = \frac{B}{\nu} + A_{\mathrm{C}}\nu^n \tag{4a} $$

The difference between Eqs. (4) and (4a) is especially relevant at high reduced velocities (see Fig. 1b). These are encountered most of all in the case of fast separations (high u) of large molecules (low D_{m}).

In Fig. 1 the conventional plate-height curve is compared with the plate-height curve obtained using Eq. (4). The values of the coefficients B and A_{C} are obtained from the best fit describing the experimental data (Fig. 2). The results were reported elsewhere [1]. At lower reduced velocities the two plate-height curves, conventional and coupled are almost identical (Fig. 1a). At higher reduced velocities (Fig. 1b) the plate-height curve obtained from Eq. (4a) shows much lower values than the conventional Eq. (4).

In SEC the samples of interest are synthetic and natural macromolecules. The diffusion coefficients of polymers depend on their molar mass (M) and on the solvent. For polystyrene in tetrahydrofuran (THF) at room temperature the linear diffusion coefficient is given by the following empirical equation [22]:

$$ D_{\mathrm{m}} = 0.0386(MM)^{-0.57} \tag{5} $$

where D_{m} is in mm^2/s and M is in g/mol.

The diffusion coefficient is thought to have a large effect on the chromatographic dispersion of polymers. The plate height for high-M polymers is thought to reach very large values, indi-

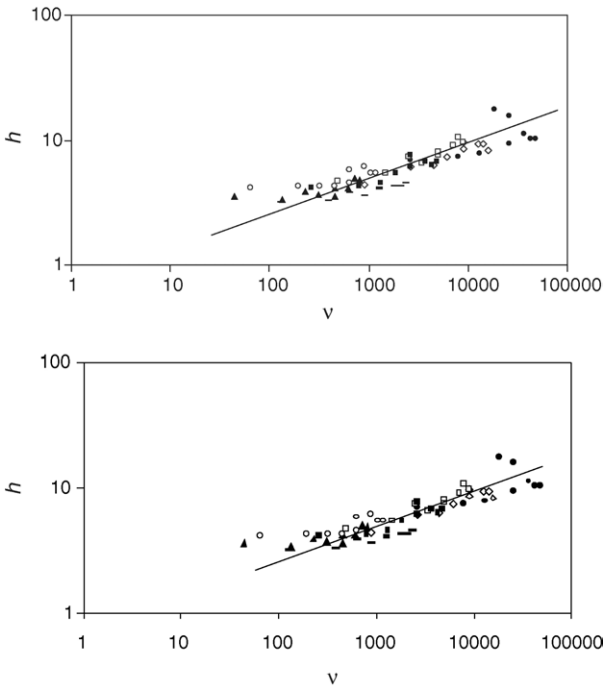

Fig. 2. Dimensionless plate-height (h) vs. reduced velocity (ν) plotted on a logarithmic scale for polystyrene standards (Table 3) on the PL-Gel-Mixed-C 50 mm × 7.5 mm i.d. column. See text for description of experimental data.

cating great losses in efficiency. Indeed, the main obstacle to fast polymer SEC is thought to be the slow diffusion of polymers. Another consequence of the slow diffusion is an increased risk of extra-column dispersion.

2.2. Integrity plots

A quantitative tool to investigate the potential of fast SEC is provided by the concept of integrity plots [1]. Integrity plots portray the SEC resolving power as a function of the sample M and polydispersity. In SEC we are aiming to evaluate the MMD and to measure the PDI value of the sample. As in all chromatographic techniques, the contributions to the band broadening arising from the column and from the auxiliary flow path must be kept minimal. In SEC the PDI contribution should be dominant.

In our previous work [1] we defined the SEC integrity index, II_{SEC}, as:

$$II_{SEC} = \frac{\sigma}{\sqrt{\sigma^2_{PDI} + \sigma^2_{col} + \sigma^2_{extra\text{-}col}}} \quad (6)$$

where, σ^2_{PDI} is the contribution of the sample PDI to the variance of the peak, while σ^2_{col} and $\sigma^2_{extra\text{-}col}$ are the contributions of the column and extra-column dispersion to the variance of the peak, respectively. Two important cases can be recognized:

(1) If $\sigma^2_{PDI} > (\sigma^2_{col} + \sigma^2_{extra\text{-}col})$; $(0.8 < II_{SEC} \leq 1)$, the SEC elution profiles reflect the MMD and not the broadening due to the chromatographic dispersion. In ideal SEC, II_{SEC} is equal to unity.

(2) If $\sigma^2_{PDI} < (\sigma^2_{col} + \sigma^2_{extra\text{-}col})$; $(0 \leq II_{SEC} < 0.2)$, SEC cannot be used to measure the MMD. Only the location of the peak maximum, corresponding to the peak molar mass (M_p) is meaningful.

The SEC integrity index is defined such that it directly reflects variations in the width of the observed MMD. If $II_{SEC} = 1$ the observed chromatographic bandwidth can be converted without correction to the sample polydispersity. If $II_{SEC} = 0.9$ only 90% of the observed bandwidth is due to the polydispersity (and the calculated PDI will be approximately 20% higher than the true value) [1].

The manner used to calculate the SEC integrity index was described in detail previously [1]. The three terms affecting the SEC resolving power are the PDI contribution, column dispersion, and extra-column band broadening.

3. Experimental

3.1. Chemicals and procedures

Five separation columns from Polymer Laboratories (Church Stretton, Shropshire, UK) were used, all packed with 5 μm Mixed-C stationary phase. The reported data concern a 50 mm × 7.5 mm i.d. column, unless specified otherwise. Comparable results were obtained using a 50 mm × 4.6 mm i.d. column packed with the same material. The effects of column length and flow rate on resolving power in Fast SEC were studied by comparing the 50 mm × 4.6 mm i.d. column with 100 mm × 4.6 mm and 150 mm × 4.6 mm i.d. columns. The specified effective range of the Mixed-C stationary phase is from 200 to 2,000,000 Da. The Fast-SEC columns were compared with a PL-Gel column with dimensions 300 mm × 6.8 mm i.d. (5 μm particles; effective M range: 500–60,000 Da). The samples studied were polystyrene standards (PS) obtained from various manufacturers (Table 3). Standard solutions were prepared in tetrahydrofuran at concentrations of 1 mg/ml.

A Shimadzu (Kyoto, Japan) LC-10AD$_{VP}$ solvent-delivery module was used. The automated injection valve from VICI (Valco Instruments, Ontario, CA) was equipped with a 0.5 μL loop. The analytes were detected with a UV detector, model 200 from Linear Instruments (Reno, Nevada, USA). The detector-cell volume and band broadening were miniaturized by installing

Table 3
Polystyrene standards obtained from different manufacturers with their specified PDI values

Molecular weight (Da)	Manufacturer	Polydispersity index
1,700	PL	1.06
3,250	PL	1.04
10,900	PSS	1.03
39,200	PSS	1.03
117,000	PSS	1.03
325,000	PSS	1.03
1,260,000	PSS	1.05
2,200,000	MN	1.04
3,530,000	PSS	1.13

a fused-silica capillary (Polymicro Technologies, Phoenix, Arizona, USA) with an internal diameter of 250 μm. The UV detector was operated at a wavelength of 260 nm.

The applied flow rate was varied from 0.3 to 0.5, 0.7, 1.0, 1.2, 1.4, 1.6, and 1.8 ml/min, until the pressure limit of the column was reached.

3.2. Simulation program

The polymeric sample is assumed to feature a log normal MMD, the standard deviation of which is adjusted to match the specific PDI. This distribution is divided in one hundred equidistant fractions between $M_p - 3\sigma$ and $M_p + 3\sigma$. The concentrations of the fractions are calculated to follow the Gaussian profile. Each fraction is then considered as an individual sample to which conventional chromatographic theory applies. The retention time is obtained from an experimental SEC calibration curve and the peak width from the empirical Eq. (4a), obtained based on actual experiments. The efficiency of the 50 mm × 7.5 mm i.d. column is presented in Fig. 2. From the knowledge gained by Knox and Parcher [34] the reduced plate height versus reduced velocity was plotted on a logarithmic scale. In this way the reduced plate heights obtained at high-reduced velocities can be examined without extrapolation. A line can be fitted through all experimental data, and this was used to obtain the A_C and n coefficients that best describe the experimental results according to Eq. (4a). Thus, in this paper the following coefficients will be used: $B = 1.50$; $A_C = 1.12$ and $n = 0.21$

$$h = \frac{1.5}{\nu} + 1.12\nu^{0.21} \quad (7)$$

The 100 peak profiles are then added to obtain the SEC envelope profile. In general, the polymer distribution covers a much broader range than 100 members of the polymeric series (individual masses). Thus, the fractions used in the calculation are 'pseudo-oligomers' and not real oligomers. If any resolution between the separate fractions ('fingering') is observed in the SEC profile, then this is usually an artifact, which can be removed by increasing the number of pseudo-oligomers (e.g. from 100 to 500) at the expense of longer computations times.

4. Results and discussion

4.1. Influence of the polydispersity on the peak shape in SEC—results of simulations

The influence of the PDI on the peak shape in SEC was investigated using the simulation program described above.

To investigate how much of the broadening of the peak is due to the chromatographic dispersion and how much to the sample polydispersity, several profiles were simulated, i.e. a monodisperse PS standard (PDI = 1) and polydisperse standards (PDI > 1). The differences between the various profiles indicate to what extend the observed peak profile is due to the MMD of the sample. Peaks were simulated for a conventional (300 mm × 7.8 mm i.d.; Fig. 3) and a short (50 mm × 7.5 mm i.d.; Fig. 4) SEC column, both packed with Mixed-C material. The flow rate is 1 ml/min and the sample of interest is 117,000 Da. The dashed profiles in Figs. 3 and 4 represent the corresponding monodisperse polymer (PDI = 1). This signal reflects the chromatographic band broadening. Only in case of Fig. 4a the observed band broadening seems to be equal to the

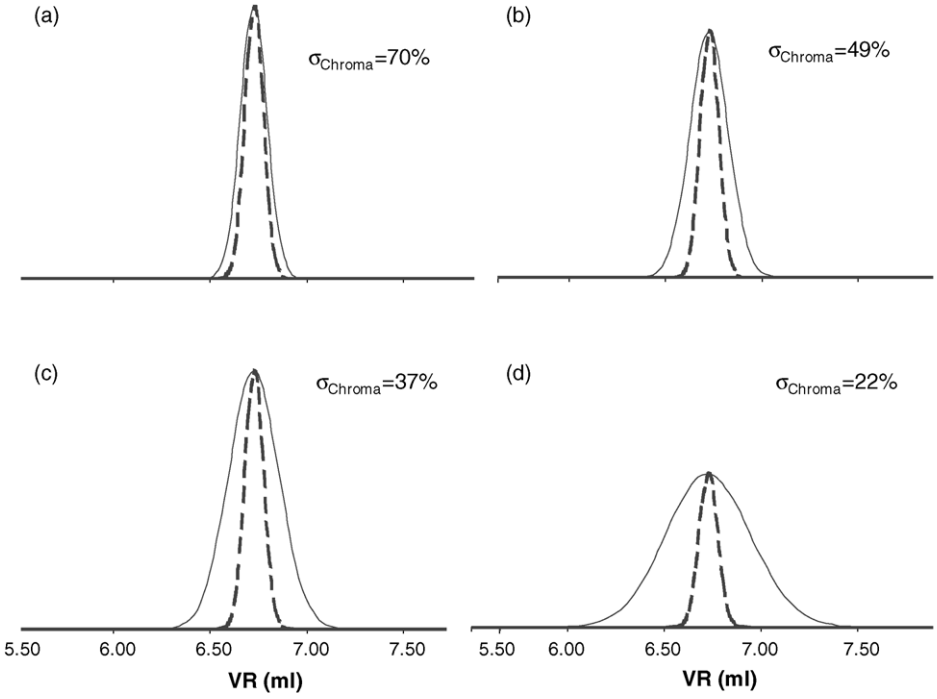

Fig. 3. Simulated chromatogram for a PS standard of 117,000 Da on a 300 mm × 7.8 mm i.d. column, flow rate 1.0 ml/min, comparison between PDI = 1.00 (- - -) and (a) PDI = 1.01, (b) PDI = 1.03, (c) PDI = 1.06 and PDI = 1.2.

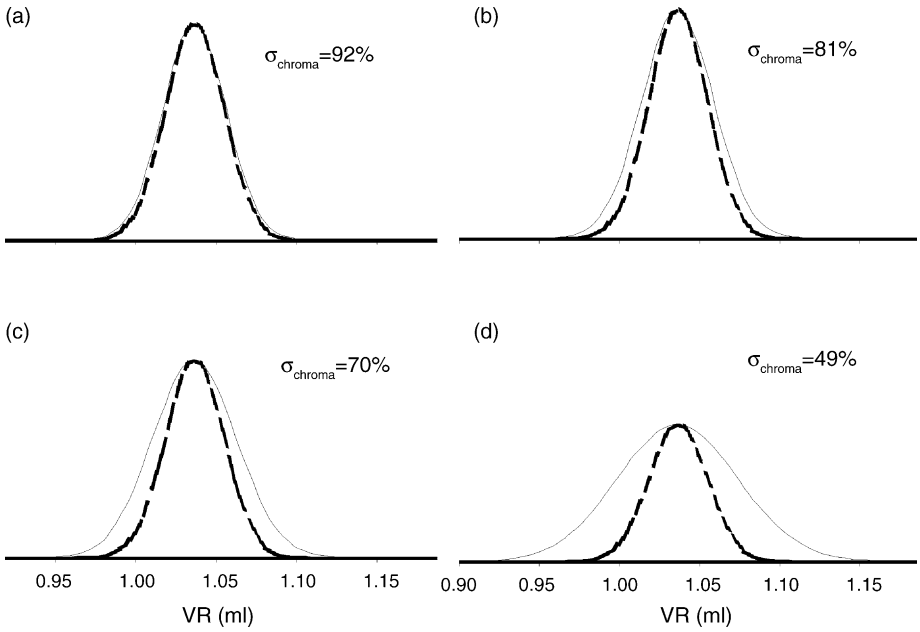

Fig. 4. Simulated chromatogram for a PS standard 117,000 Da on a 50 mm × 7.5 mm i.d. column, flow rate 1.0 ml/min, comparison between PDI = 1.00 (- - -) and (a) PDI = 1.01, (b) PDI = 1.03, (c) PDI = 1.06 and PDI = 1.2.

chromatographic band broadening. This is also approximately the case for Figs. 3a and 4b. Thus, for very narrow (PDI = 1.01) or narrow standards (PDI = 1.03), which are analysed on short (50 mm long) or conventional (300 mm long) SEC columns, only on the short column the chromatographic band broadening truly dominates. In other cases the band broadening due to separation selectivity (given by the actual PDI) plays a significant role. In broad samples (PDI ≥ 1.2; Figs. 3d and 4d) the polydispersity-contribution is dominant and SEC is an excellent tool for measuring MMDs.

In Fig. 5 the simulated chromatogram is compared with the experimental results for the same mixture. Now the PDI values

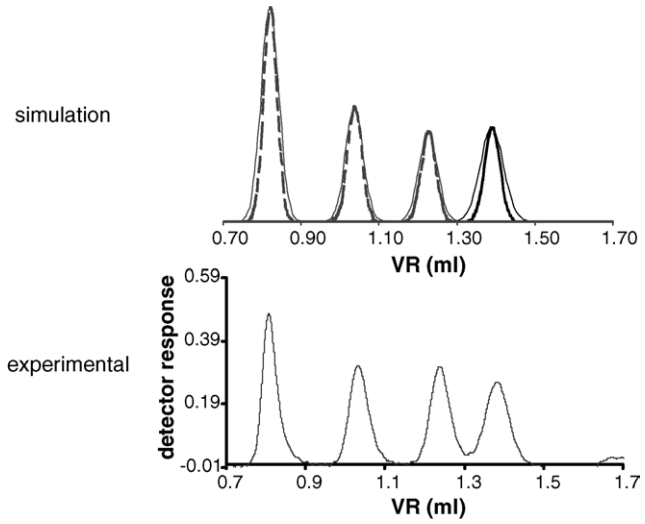

Fig. 5. Comparison between simulation and experimental chromatograms (- - -) PDI = 1 and (—) PDI values as specified by the manufacturer (see Table 3). Experimental conditions: column 50 mm × 7.5 mm i.d. at 1 ml/min and polystyrene *M*: 1700; 10,900; 117,000; and 2,200,000 Da.

(as specified by the manufacturers; see Table 3) are used in the simulation. In this experiment, a flow rate of 0.3 ml/min was used. The similarity between the experimental and simulated figures demonstrates the accuracy of the simulation program. From the knowledge gained in Figs. 3 and 4, we can conclude that the loss in resolution between the *M* 1700 Da (PDI = 1.06) and *M* 10,900 (PDI = 1.03) peaks is due to sample polydispersity and thus reflects the properties of the sample.

4.2. Experimental results and discussion

4.2.1. Analysis time versus resolution

For this experimental study two different mixtures were prepared. Because the results were equivalent, only one mixture is discussed in this paper. The polymer-standard mixture was injected on the fast-SEC column at different flow rates varying from 0.3 to 1.8 ml/min (Fig. 6). 1.0 ml/min was found to be the apparent optimum flow rate, in agreement with the specification of the manufacturer of this column. At the lower flow rates (0.3 and 0.5 ml/min) the chromatograms show a very good resolution. When increasing the flow rate, the analysis time becomes shorter, but resolution starts to be diminished. At a flow rate of 0.3 ml/min (Fig. 6) the total analysis time is 3.5 min, decreasing to 3.1 min (at 0.5 ml/min), 2.1 min (at 1.0 ml/min), 1.3 min (at 1.2 ml/min), and finally 0.8 min (at 1.8 ml/min). At flow rates higher than 1.0 ml/min the resolution starts to decrease more quickly. At 1.8 ml/min the gain in analysis time no longer seems to outweigh the loss in resolution.

4.2.2. Calibration curves

To create integrity plots, calibration curves must be measured. We have found small, but systematic effects of the flow rate on the calibration curve, especially in the region of high *M* (Fig. 7a). In this range (Fig. 7b) the slope of the calibration

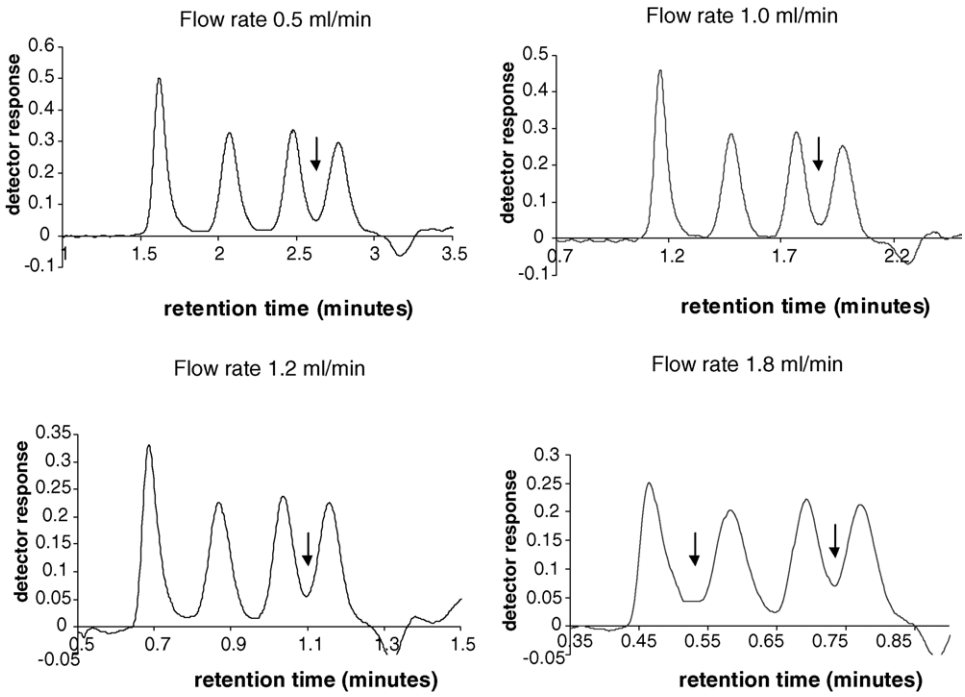

Fig. 6. Separation of a mixture of 1700, 10,900, 117,000, and 2,200,000 Da polystyrene standards at different flow rates (conditions see text).

curve decreases systematically with increasing flow rate. Therefore, the selectivity of the separation is decreasing somewhat at higher flow rates. As a consequence, the PDI contribution to the total peak width is also decreasing at higher flow rates. Tentatively, the largest molecules, around the exclusion limit may experience shear deformation or degradation. As a result, these analytes may be slightly retained (less excluded). It has been noted before [26,35] that close to the exclusion limit of the column not only the SEC mechanism prevails, but also hydrodynamic-chromatography effects play a role.

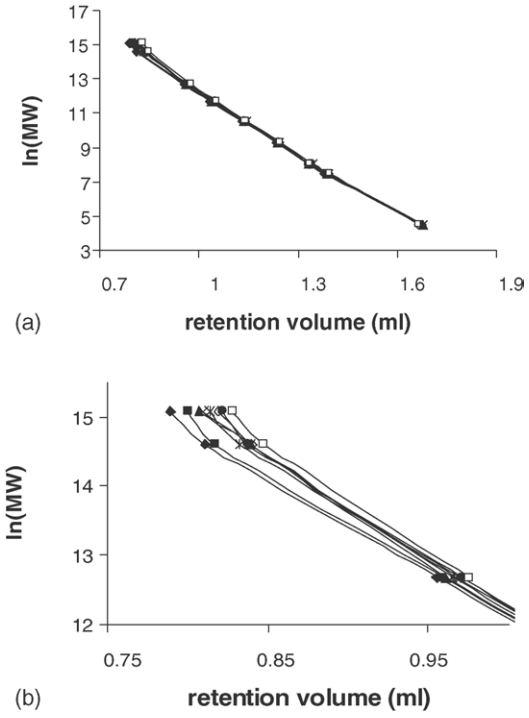

Fig. 7. (a) Calibration curves at different flow rates (0.3, 0.5, 0.7, 1.0, 1.2, 1.4, 1.6, and 1.8 ml/min) on the 7.5 mm × 50 mm i.d. column (b) Enlarged region of part (a) (flow rates increase from left to right).

4.2.3. Integrity plots

The Integrity Plots are illustrating the quality of SEC information obtained with a certain column at a chosen flow rate. They display the SEC integrity index, II_{SEC} (Eq. (8)), as a function of the *M* and the PDI of the sample. Using integrity plots we can decide in which range of *M* and PDI a given column, at given conditions, can provide reliable information on the MMD. First, the influence of the flow rate on the performance of the Fast-SEC column is evaluated (Fig. 8). Then we will show examples to illustrate the effects of the length of the column and the pore-size distribution of the packing material.

4.2.3.1. Influence of the flow rate on the integrity plot. Fig. 8a shows the integrity plot of the 50 mm × 7.5 mm i.d. fast-SEC column obtained at 0.5 ml/min. At 0.5 ml/min this particular column can be used to characterize the MMD of broad polymers (light zone on the right-hand side). If the sample of interest has a narrow distribution (PDI < 1.1), then another (longer) column must be used. For (ionizable) narrowly distributed polymers MALDI-MS offers a viable alternative [36]. The sample *M* has little effect on these conclusions across the range from 10,000 to 1,000,000 Da. The column truly behaves as a 'linear column'.

Fig. 8a–e show the integrity plots at 0.5, 1.0, 1.2, 1.4, and 1.6 ml/min. In general the conclusions for 1.0 ml/min are identical to those drawn for 0.5 ml/min. Raising the flow rate to 1.0 ml/min does not significantly affect the performance of the

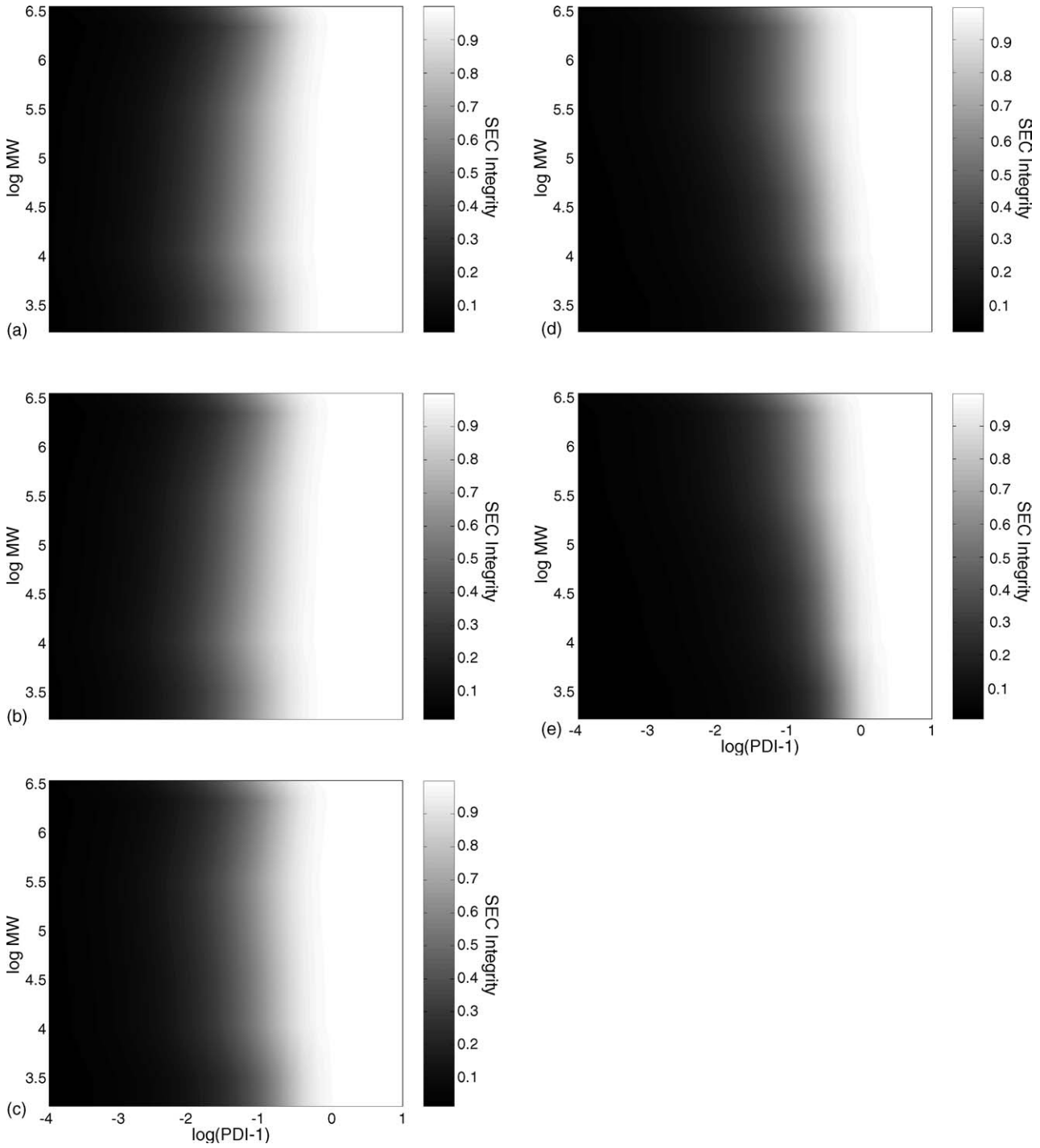

Fig. 8. Integrity plots for the fast-SEC column 7.5 mm × 50 mm i.d. at different flow rates (a) 0.5 ml/min, (b) 1.0 ml/min (c) 1.2 ml/min, (d) 1.4 ml/min, and (e) 1.6 ml/min.

SEC system. If the samples of interest have a PDI of about 1.1, then the highest flow rate at which reliable information on the MMD can be obtained is around 1 ml/min. This flow rate seems to offer the best compromise between speed of analysis and resolution. When the flow rate reaches 1.2 ml/min, a significant loss in resolution starts to be observed (Fig. 8c). This continues further at higher flow rates (Fig. 8d and e). Samples of high M may deform at such flow rates and different separation mechanisms may start to play a role. The calibration curves around the exclusion limit are shifting to the right, so that selectivity is lost (Fig. 7a and b). The integrity plots in Fig. 8c–e illustrate this effect. At the highest flow rates, the MMD of samples of relatively low M can only be characterized on this particular column if their PDI is very large (e.g. at 1.6 ml/min PDI > 1.5 for $M < 10^4$, see Fig. 8e). At higher M the situation is a bit more favorable (at 1.6 ml/min PDI > 1.3 for $M \approx 10^5$ and PDI > 1.2 for $M \approx 10^6$). The flow rate affects the separation of samples with an M lower than 40,000 Da most strongly.

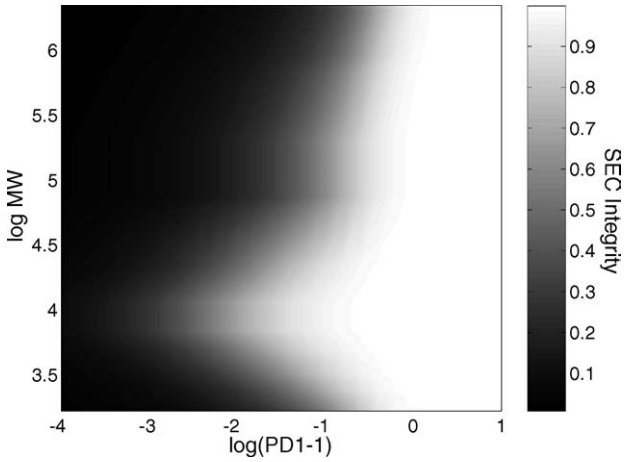

Fig. 9. Integrity plot for the Plgel 10^3 Å column at 0.7 ml/min flow rate.

4.2.3.2. Effect of the pore-size distribution. In Fig. 9 the SEC integrity index of a PLgel 10^3 Å at 0.7 ml/min is plotted. A different trend in the curvature of the integrity plots is observed in comparison with the plots obtained for the 'mixed-bed' columns. The shape of the curve is dependent on the pore-size distribution [21]. The calibration curve of this column shows an inflexion point around an M of 10,000. Around this M (and at this flow rate), we can use this column to characterize very narrowly distributed polymers, possibly down to PDI = 1.0. It is well known that to measure reliable MMDs for low-PDI samples long columns with a narrow pore-size distribution are recommended.

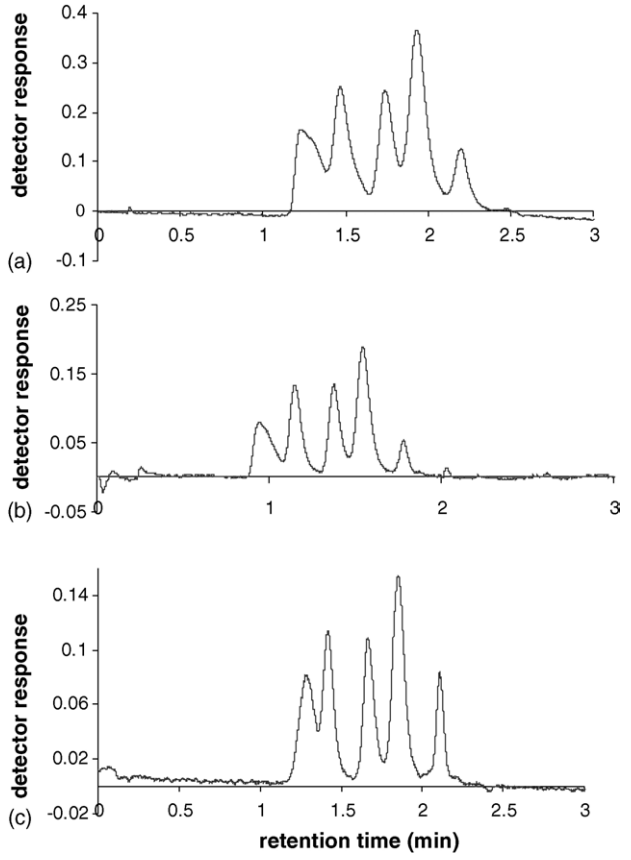

Fig. 10. Effect of concomitant changes in the column length and flow rate on the resolving power of Fast-SEC columns (a) 4.6 mm × 50 mm i.d. at 0.3 ml/min; (b) 4.6 mm × 100 mm i.d. at 0.6 ml/min; (c) 4.6 mm × 150 mm i.d. at 0.9 ml/min.

4.2.4. Effect of column length and flow rate

When increasing the flow rate the analysis times become shorter, while the resolution diminishes. Earlier in this paper the approach of using short columns at high flow rates was studied. In Table 1 several approaches for increasing the separation speed are highlighted. The way to increase the resolution, while realizing fast separations is to double the length of the column

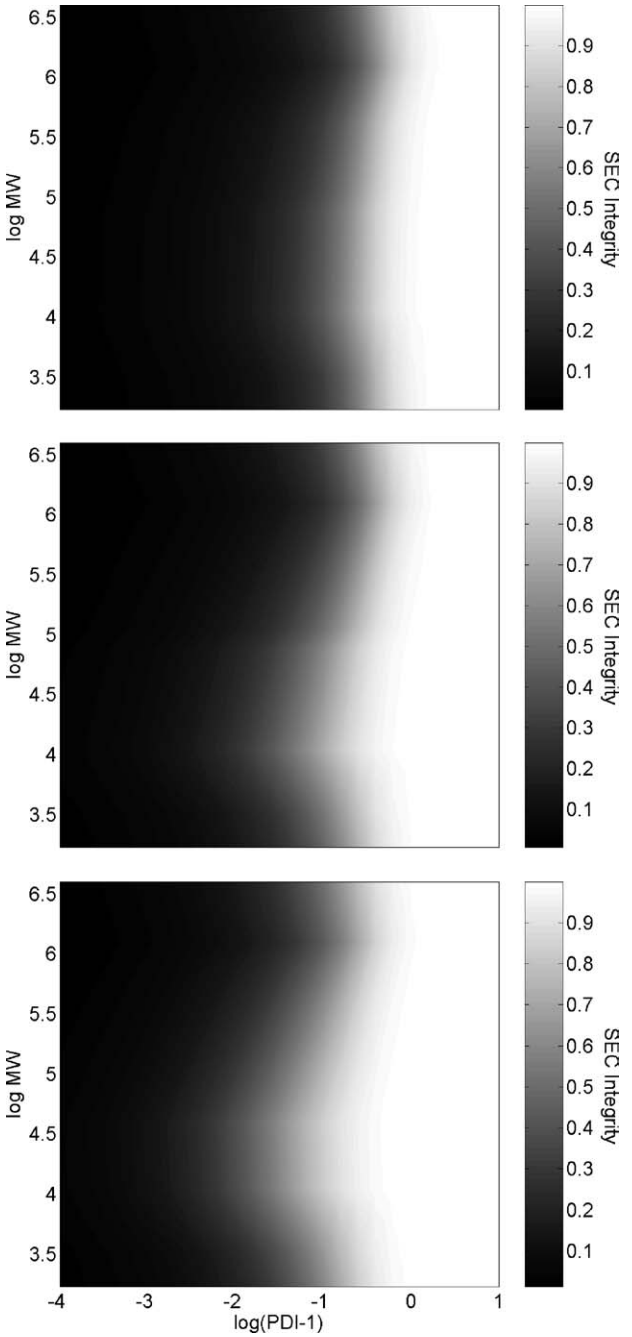

Fig. 11. Integrity plots of 4.6 mm × 50 mm i.d. (at 0.3 ml/min) compared with 4.6 mm × 100 mm i.d. at 0.9 ml/min and 4.6 mm × 150 mm i.d. at 0.9 ml/min flow rate.

and the flow rate. This is expected to result in an increased efficiency at constant analysis time.

Three separation columns were studied 50 mm × 4.6 mm i.d., 100 mm × 4.6 mm i.d., and 150 mm × 4.6 mm i.d. The same mixture as before (1700; 10,900, 117,000, and 1,260,000 Da) was injected on all columns at the corresponding flow rate (Table 1), viz. 0.3, 0.6, and 0.9 ml/min. The results are shown in Fig. 10. From this result we can conclude that higher flow rates and longer columns result in better resolution. Similar results are obtained at 0.5 ml/min (4.6 mm × 50 mm i.d.) compared with 1.0 ml/min (4.6 mm × 100 mm i.d.) and 1.5 ml/min (4.6 mm × 150 mm i.d.).

The integrity plots of the 4.6 mm × 50 mm i.d. column (at 0.5 ml/min), compared to 4.6 mm × 100 mm i.d. (at 1.0 ml/min) and 4.6 mm × 150 mm i.d. (at 1.5 ml/min) show (as described in Table 1) that the longer the column the more efficient the separations are (Fig. 11), despite the increase in flow rate to keep the analysis time constant. Thus, the concomitant change of length and flow rate will enhance the usefulness of the column towards characterizing MMDs of narrower samples.

5. Conclusions

In this paper we have confirmed that fast size-exclusion chromatography can be performed in practice for the analysis of synthetic polymers. The main limitation remains the loss in resolution. A compromise must be struck between the loss in resolution and the gain in speed.

Fast SEC is a very interesting modification of conventional SEC, due to its advantages in terms of the speed (analysis time) and production of toxic waste. Fast SEC has emerged in combination with combinatorial chemistry and high-throughput experimentation, as well as in comprehensive two-dimensional liquid chromatography. Several approaches to change the speed of analysis have been presented, highlighting the ones corresponding to the interest of this study.

Fast SEC can only be used to measure the MMD of broadly distributed polymers. An increase in the length of the column at higher flow rates will improve the resolution. The main limitation becomes the pressure drop across the column.

Based on the observed peak widths for a series of standards and the SEC calibration curves, SEC integrity plots can easily be constructed for any kind of column or column configuration. The influence of the pore-size distribution, flow rate and column length have been discussed. These plots are providing clear and objective information for the selection of suitable SEC columns and they can provide guidelines for a study of various types of SEC columns (e.g. miniaturized SEC, Fast SEC).

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