

Size-Exclusion Chromatography and Its Optimization for Material Science

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Abstract

A theoretical analysis of improving the separation power of size exclusion chromatography by decreasing flow-rate is conveyed. The variance of the elution curves is larger than expected from estimated dispersity in molecular weight, M_w/M_n . The shape of the elution curves depends strongly on experimental conditions. When the experimental conditions are approaching those to the ideal separation, judged by statistical properties of the curves, both skew and excess kurtosis of the elution curves increase near the exclusion limit in accord with theoretical prediction. In analyses of polystyrene standards by size exclusion chromatography for polymers of molecular weight about thirty thousand and higher, the longitudinal diffusion is not important even at low flow-rates.

Keywords: Separation mechanism; Size-exclusion chromatography; Band broadening function; Skew; Excess kurtosis

Introduction

The material science has ever been the driving force of the progress which has always advanced with the progress in analytical sciences. For polymer materials, the distribution of chain lengths and, therefore, molecular weight distribution (MWD) is of primary importance. MWD is characterized by its averages, M_n , M_w , M_z ,... and dispersities, M_w/M_n , M_z/M_w . Material properties depend on molecular weight of polymers in different ways and they are correlated with different averages. Thus M_n may be correlative with polymer colligative properties, e.g. freezing point depression, M_w with melt viscosity and M_z with toughness [1]. Several methods for the determination of the molecular weight averages were developed, e.g., membrane osmometry, cryoscopy and vapor pressure osmometry for the determination of M_n [2], static light scattering for the determination of M_w [2], sedimentation equilibrium for the determination of M_z [3] etc. However, all methods for the determination of individual molecular-weight averages have been dwarfed by size-exclusion chromatography (SEC) [4] which is a method widely used for the determination of MWD as well as of all averages and dispersities, M_w/M_n and M_z/M_w .

SEC with single concentration detector and results evaluated according to a calibration dependence, constructed from elution curves (maxima) of reference standards, frequently polystyrene or poly(methyl methacrylate), is a relative method and for polymers of different chemical composition the error may reach up to 200% [5]. Even if the analyte is chemically identical with the calibration reference standards, the correct value of M_w/M_n is not obtained because of the effect of the band-broadening [6]. This phenomenon follows from the principle of the method [7,8] and increases the value of M_w/M_n , influencing both M_w and M_n [6]. According to the theory, MWD of polystyrene prepared by anion polymerization (reference standards) is Poissonian type with dispersity decreasing with M [9]. This leads us to the conclusion that the dispersities of the reference standards given by the producers are systematically overestimated [8,10]. However, there is some evidence that efficiency of separation increases with decreasing flow rate, r_f [11,12], and there is evidence that the values of M_w/M_n of the reference standards are much lower than commonly believed [8,10,13,14]. The knowledge of the dispersity of the analyzed samples is extremely important for our understanding of the SEC separation of polymers, because of the above discussed dependences of the material properties on MWD and its averages. The experimental elution curve is related with broadening function (BBF) and theoretical elution curve, characterized by the sample dispersity, M_w/M_n , by the Tung equation [4,6].

In the theory of chromatographic separation and band broadening, BBF is called the elution curve of an analyte uniform in molecular weight and chemical composition [15]. For its description, several mathematical models of separation were developed. The kinetic model by Giddings and Eyring assumes that the capture of molecules of the analyte (analyzed substance) is described by two kinetic constants of ingress and egress [15].

The equilibrium model is based on the concept of theoretical plate on which the equilibrium is formed between molecules of the analyte moving together with MP and those anchored on the surface by enthalpic attractive forces or penetrated into the pores by entropic process basically of Brownian diffusion into pores of the stationary phase (SP). The plate model was first proposed by Martin and Synge [16]. The spacial distribution of the analyte with respect to the longitudinal axis of the separation system, developing in time, was expressed by the binomial distribution. However, further treatments of this physical situation were approximative. The exact solution to the problem gives the probability that an experiment with given probabilities of success and failure is successful, i.e., the molecule is eluted from the column in a volume of a plate after a given number of failures [17].

The obtaining of the separation parameters and the description of elution curves by the Giddings-Eyring equation was shown for low-molecular weight substances separated in the adsorption mode by liquid chromatography (LC). This was possible because the LC separation is effective, the retention (capacity) factor [18] high and the baseline-separation of the sample-components is in many cases achieved. For polymers, separated by SEC such phenomena were not observed. In SEC compared to LC, the situation concerning BBF is much less favourable. The capacity factor [18] in SEC is low and the analyzed polymer samples are almost always disperse in molecular weight. The elution curve of such sample is then the convolution of BBF with theoretical elution curve corresponding with the sample MWD

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[6]. On the other hand, the elution curves of narrow-MWD are much broader than expected from their MWD [19] and therefore we expect that their shape reflects properties of BBF rather than those of narrow-MWD sample MWD. It has been known soon that the concentration detector signal gives unacceptably broad MWD and the evidence suggests that MWD of polymer standards are much narrower than believed [13,14], which supports the idea of the peak-shape formation by the BBF mechanism. According to the theory, its skew and excess kurtosis (referred to sometimes as 'excess' only [7,20]) increase and variance decreases near the exclusion limit of the separation system [7].

From the plate-height mechanism [7,16] it follows strictly that the polymer occurring in MP only is not separated at all and is eluted in the volume approaching that of one plate. In this case, the variance of its BBF approaches zero. For the separation of low-molecular weight substances, characterized by high capacity factor [18], the expectation of the decrease in variance has been verified [7]. With high-molecular weight polymers, the situation is more complicated. Just below the exclusion limit the capture of macromolecules in the pores causes hindered diffusion and the egress process is expected to be slow and the BBF variance to increase. This effect has been frequently observed in times when the injected concentration was usually independent of molecular weight [21,22] and it may have been the consequence of concentration effects as discussed [7]. With concentration decreasing with molecular weight the BBF variance does not change considerably [7]. Tung and Runyon [23] report increasing variance with decreasing elution volume. This is followed by a steep decrease in the vicinity of the exclusion limit. An ample decrease throughout the whole span of elution volume cannot be expected. Such effects do not follow any consistent pattern and can hardly form a part of a theory describing the separation. Polymers eluted far after the exclusion limit show almost entirely Gaussian peaks (and therefore BBF). This can be expected from the random character.

The polymers which elute near the exclusion limit in a given separation system are those of the highest molecular weight. It is therefore necessary to increase the separation power of the system. As recognized by Giddings, two kinetic processes, viz., tortuous and obstructed flow of eluent through and around the particles of packing and the flow profile in the mobile phase whose dispersive effect is checked by transversal diffusion, may combine to reduce and control band broadening. To avoid band broadening, the individual molecule has to sample the complete range of linear flow velocities in a random way as it moves along the column; this is achieved by transverse diffusion [24]. Compared to low-molecular weight substances studied by Giddings by the gas chromatography, the rate of diffusion of polymers in solutions separated by SEC is low [25]. Therefore, by decreasing the flow-rate, an increase in separation power of polymers elution close to the elution limit is expected just by letting time enough to molecules just to compensate for the low rate of transversal diffusion as well as to reach the equilibrium of the polymer between volumes inside and outside the pores of SP in accord with experimental observation [11,12].

The aim of this paper is the demonstration of the improvement of efficiency of separation in SEC aimed at the material research with decreasing flow-rate on the basis of expected statistical parameters of BBF, derived on theoretical model [26].

Materials and Methods

The SEC analyzes were performed using a Pump Deltachrom (Watrex Ltd.) with computer-controlled piston movement, autosampler MIDAS (Spark Ltd.), one column PL gel mixed D

(Polymer Laboratories), separating according to the producer in the range approximately $10^2 \leq M \leq 4-5 \times 10^5$, particle size 5 μm . UV/VIS DeltaChrom UVD 200 detector (Watrex) with flow-cell volume of 8 μl , operating at wavelength $\lambda=264$ nm, the light-scattering photometer DAWN Heleos II, measuring at 18 angles of observation and Optilab T-rEX differential refractometer (both Wyatt Technology Corp.) were the detectors in the order of flow. The data were collected into the Astra 6.1 (Wyatt Technology Corp.) and Clarity (DataApex Ltd.) softwares, communicating with detectors using U-PAD2 USB acquisition device.

Results and Discussion

The dependence of a physical property P on the particular average of molecular weight M_x can often be described by

$$P = P_\infty (1 + k/M_x)^{-1} \quad (1)$$

where P_∞ is the value of the property at infinite molecular weight and k is a constant. As an example, Figure 1 shows the dependence of the melting point on the chain length expressed as the number of monomer units published by Flory and Vrij [27]. The dependence (Figure 1) can be described by eqn. (1) with $M_x = M_n$, $P_\infty = 418.8$ and $k = 7.33$. Hence, the determination of the correct chain-length and therefore molecular weight is of highest importance in macromolecular science.

The improvement of the separation can be shown on elution curves for the standard $M_p = 4 \times 10^5$. With decreasing r_f the curves are becoming narrower and in the near the exclusion limit they become moreover non-symmetric (Figure 2). The Figure suggests that flow-rate $r_f = 0.5$ ml·min⁻¹ as a very good compromise between separation power and the time of analysis. On the other hand, in the region $0.01 \leq r_f$ ml·min⁻¹ ≤ 0.035 the shape of the curves changes showing the expected increase in skew, indicating thus increasingly efficient separation. The change in variance of elution curves with r_f can be seen in the van Deemter plot [28]

$$\sigma^2 = a + b/r_f + cr_f \quad (2)$$

where the constants a , b and c are associated, in the first approximation, with eddy diffusion (the contribution of MWD is in this case neglected), longitudinal diffusion and mass transfer [4], respectively, in logarithmic scale on the r_f axis (Figure 3). Only the curve for toluene rises continuously which is apparently due to the effect of longitudinal diffusion of low molecular weight substance, expressed by the term b/r_f in eqn. (2). For the standards of higher M minima appear at $r_f = 0.02$ ml min⁻¹.

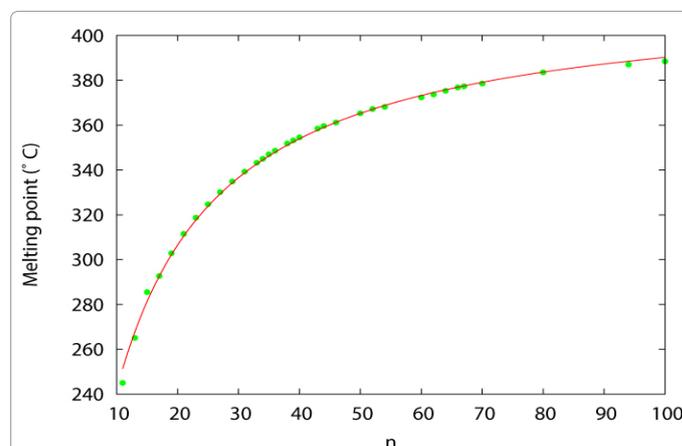


Figure 1: The dependence of the melting point temperature on the number of monomeric units in the molecule [27].

The decrease in broadness of the elution curves can be seen also in values of the apparent dispersity in molecular weight, M_w/M_n , obtained according to calibration dependence constructed at each r_f (Table 1). The values of M_w/M_n for the majority of them significantly lower than those given by the producer were obtained at very low r_f . This is in contrast with relatively high values obtained at widely used $r_f \approx 1$ ml·min⁻¹ (Table 1).

The variability of the shape with the change of experimental conditions (r_f) and low dispersity justify the approximation of BBF for the studies of its statistical properties by elution curve. The values of the skew are highly scattered (Figure 4), but the lowest values are always those for the lowest flow-rate $r_f=0.01$ ml·min⁻¹ and are close to the theoretical line [8] calculated for parameters of the standard of $M_p=4 \times 10^5$ (see below). The order of points from the left to the right is the same as the order of standards in Table 1. Near the elution limit, estimated as 4.64 ml, the curve as well as the experimental value rises abruptly together with the theoretical line and for the standard of $M_p=4 \times 10^5$ at $r_f=0.01$ ml·min⁻¹ the value of σ^2 is the highest and is close to the rising part of the theoretical curve.

The large scatter of points at lower r_f suggests that operational conditions and changes of the character of the flow, characterized by irregular change in shape of elution curves with r_f with r_p discussed Figure 2, rather than instrumental defects (e.g., voids in the separation system) are the cause of the skew.

The theoretical line in Figure 4 was calculated from parameters of the sample of $M_p=4 \times 10^5$, which is eluted close to the exclusion limit,

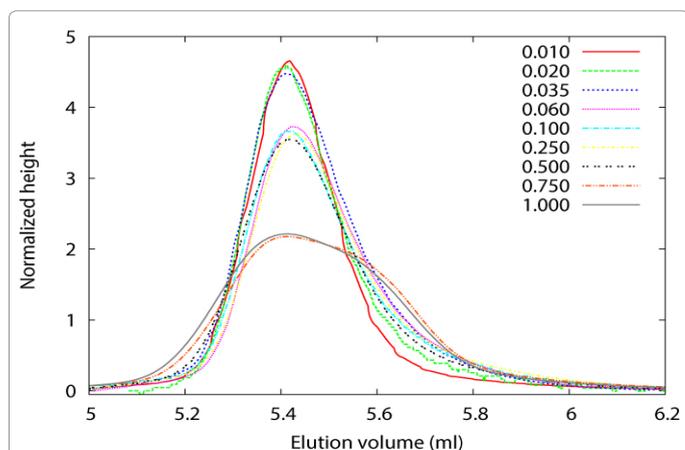


Figure 2: Elution curves of standards of nominal $M_p=4 \times 10^5$ at different r_f /ml·min⁻¹, denoted with the curves. The legends are ordered according to heights of curves in maximum.

shows a curvature. The points at $r_f=0.01$ ml·min⁻¹ are for polymers of $M_p < 4 \times 10^5$ the lowest and close to the theoretical dependence which was calculated for data of sample $M_p=4 \times 10^5$. However, for this sample the value of γ_s is the highest of all, in accord with the theoretical curve. The points for the standard of $M_p=9 \times 10^5$, which are in front of V_0 , are scattered according no rule, indicating imperfect separation, the point at $r_f=0.01$ ml·min⁻¹ being just in the middle.

The excess kurtosis, γ_E calculated shows also a strong increase of values obtained at the lowest flow-rate, $r_f=0.01$ ml·min⁻¹, near the exclusion limit (Figure 5). Theoretical curve obtained by the same procedure [8] as that of γ_s , shows again a strong increase near the exclusion limit and the curve calculated for the standard of $M_p=4 \times 10^5$ fits the experimental data for this standard best. The points at $r_f=0.01$ ml·min⁻¹ for polymers of $M_p < 4 \times 10^5$ are the lowest and close to the theoretical dependence. For data of sample $M_p=4 \times 10^5$ the value of γ_E is the highest of all in accord with the theoretical curve. The points for the standard of $M_p=9 \times 10^5$ in front of V^* do not also obey any rule.

Conclusions

Due to the transversal diffusion and increases the time necessary for a molecule to sample the complete range of flow velocities which is a condition for a good separation. This is achieved in the region of high M at extreme low r_f in accord with the idea of transversal diffusion as a factor decreasing band broadening, as recognized by Giddings [24].

For polymers with approximately $M \geq 3 \times 10^4$ the longitudinal

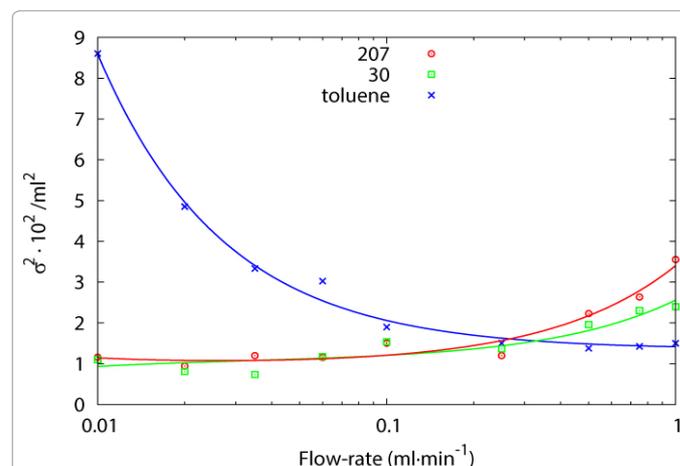


Figure 3: Van Deemter plot (eqn. (2)) for several polystyrene standards with M_p denoted with the curves and toluene.

$M \times 10^{-3}$		Producer	M_w/M_n			SEC with LS
Producer	LS		r_f /ml min ⁻¹			
900	1084	1.1	0.01	0.5	1	1.026
400	381	1.06	1.027	1.087	1.196	1.028
207	206	1.05	1.029	1.092	1.019	1.021
90	84	1.04	1.033	1.066	1.068	1.014
30	31	1.06	1.024	1.068	1.064	1.012
4	4.5	1.06	1.023	1.072	1.071	1.012
Toluene			1.106	1.113	1.116	1.026

Table 1: The molecular weights M of polystyrene standards given by the producer and obtained by the on-line light scattering (LS), dispersity M_w/M_n given by the producer and obtained by the calibration dependence constructed at a given flow-rate F and obtained from the dual light-scattering concentration detection corrected for band broadening ("SEC with LS").

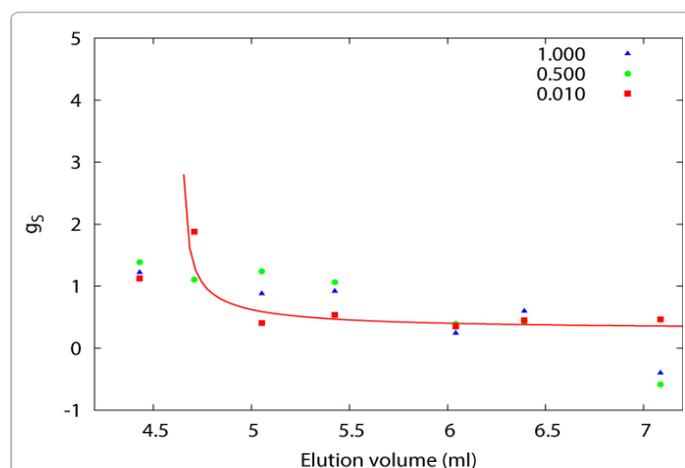


Figure 4: Skew of elution curves calculated as a function of elution volume V at $r_f=0.2 \text{ ml}\cdot\text{min}^{-1}$. The flow-rate, $r_f/\text{ml}\cdot\text{min}^{-1}$, denoted with the points and M_p in thousands with curves. The order of analytes from left to right is the same as in Table 1 from top to bottom.

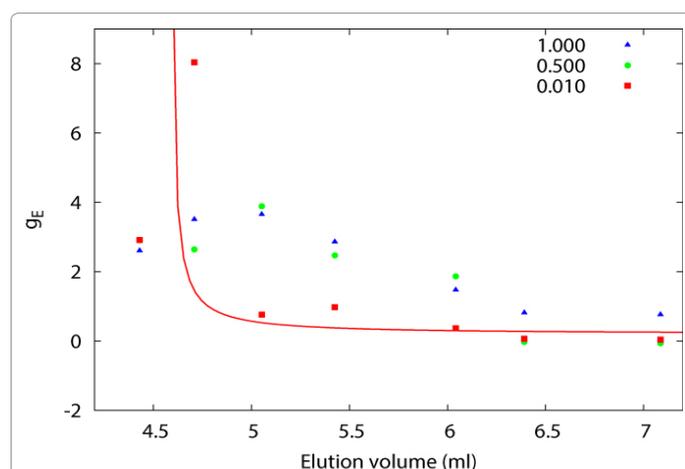


Figure 5: Excess kurtosis of elution curves as a function of elution volume. For the details of the points and lines calculated see legend to Figure 4 and text.

diffusion ceases to play an important role in the SEC separation process. With decreasing r_f the efficiency of separation increases and the separation mechanism appears, using γ_s and γ_E as a criterion, to approach the mechanism of equilibrium on theoretical plate combined with longitudinal shift, proposed by Martin and Synge [16] and solved exactly by Netopilík [26].

For polystyrene in THF for $9 \times 10^5 \leq M \leq 4 \times 10^3$, the flow-rate $r_f=0.5 \text{ ml}\cdot\text{min}^{-1}$ appears, rather than usual $r_f=1 \text{ ml}\cdot\text{min}^{-1}$, a good compromise between efficiency of separation and the time of analysis. Further decrease in r_f has no observable effect down to $r_f \approx 0.06 \text{ ml}\cdot\text{min}^{-1}$, then a new region of increase in separation power is for some standards of higher M observed.

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