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 form 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, $M_w/M_n$, $M_w/M_z$, $M_n/M_z$. Material properties depend on molecular weight of polymers in different ways and they are correlated with different averages. Thus $M_n$ may be correlatable 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 $M_w$ [2], static light scattering for the determination of $M_z$ [2], sedimentation equilibrium for the determination of $M_n$ [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 $M_w/M_z$.

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 different chemical composition the error may reach up to 200% [5]. Poly(methyl methacrylate), is a relative method and for polymers of molecular weight distribution (MWD) is of primary importance. MWD for polymer materials, the distribution of chain lengths and, therefore, material properties depend on molecular weight of polymers in different ways and they are correlated with different averages. Thus $M_n$ may be correlatable 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 $M_w$ [2], static light scattering for the determination of $M_z$ [2], sedimentation equilibrium for the determination of $M_n$ [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 $M_w/M_z$.

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Conclusion
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.
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 10^5 ≤ M ≤ 4−5 × 10^6, particle size 5 μm. UV/VIS DeltaChrom UVD 200 detector (Watrex) with flow-cell volume of 8 μl, operating at wavelength λ=264 nm, the light-scattering photometer DAWN Helios 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.

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, \( M_p/M_n \), obtained according to calibration dependence constructed at each \( r_f \) (Table 1). The values of \( M_p/M_n \) 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 \( r_f = 1 \) 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 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 \( \sigma^2 \) 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 \( r_f \), 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, shows a curvature. The points at \( r_f = 0.01 \) ml·min\(^{-1} \) are for polymers of \( M_p < 4 \times 10^4 \) 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 \( \gamma_S \) 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 \( V_p \), 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, \( \gamma_E \), 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 \( \gamma_S \), 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 \( M_p < 4 \times 10^4 \) are the lowest and close to the theoretical dependence. For data of sample \( M_p = 4 \times 10^5 \) the value of \( \gamma_E \) 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 \( V_p \) 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

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**Table 1:** The molecular weights \( M \) of polystyrene standards given by the producer and obtained by the on-line light scattering (LS), dispersity \( M_p/M_n \) 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).

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

**Figure 2:** Elution curves of standards of nominal \( M_p = 4 \times 10^5 \) at different \( r_f/ml \cdot min^{-1} \), denoted with the curves. The legends are ordered according to heights of curves in maximum.

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