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Relations between the separation coefficient, longitudinal displacement and peak broadening in size exclusion chromatography of macromolecules

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Abstract

The separation of a polymer by size exclusion chromatography is described as a series of interactions, i.e. consecutive establishments of equilibria between polymer fractions in the mobile and stationary phases followed by displacements of mobile phase containing the polymer. The elution curve is derived as the longitudinal concentration profile in the column observed in one position in space during the time of the analysis. The mean value of elution volume of a particular polymer species turns out to be the interstitial volume of the separation system divided by the mean fraction of polymer in the mobile phase. The number of the displacement–equilibrium steps can be estimated from the limiting values of the variance of the spreading function.

Keywords: Separation coefficient; Longitudinal displacement; Peak broadening; Polydispersity; Retention mechanisms; Macromolecules

1. Introduction

A combinatorial model was developed describing size exclusion chromatography (SEC) separation and elution as the longitudinal concentration profile in the separation column developing in time and being observed in one place of space, i.e. in the detector of concentration at the end of the column. The model describes the sequence in time of fractions of separated polymer occurring in a given place of the space. Each fraction appearing consecutively in the detector is a part of a different concentration profile. From the series of fractions, the concentration elution curve is constructed and its characteristics are derived.

SEC is an entropically controlled separation technique in which molecules in the mobile phase (MP) are separated by interaction with pores of the solid phase (SP) according to their size. Polymer molecules due to their thermal motion enter the pores of SP with a probability determined by their hydrodynamic volume and the accessible part of the inner pore volume [1–6]. Elution volume, V, of the polymer with a particular hydrodynamic volume and molecular mass, M, is a statistical average of the longitudinal migration of molecules with MP and interaction with SP.

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On the molecular level, the SEC separation is well understood [7–12]. On the contrary, our understanding and interpretation of the SEC experiments on the macroscopic level is hindered by the lack of a mathematical procedure permitting to observe in one place of space a function developing in time. Although a detailed description of the entire process, including peak broadening, on a molecular level is not possible, using a combinatoric approximation of this process, basic relations between V, separation coefficient and the extent of peak broadening can be obtained.

2. Theory

Migration of a polymer or another analyte of a given molecular mass along the chromatographic column will be described as a process independent of the presence of other analyte molecules, i.e. at concentration limiting to zero. The formation of the concentration profile along the separation column and the observation of the eluted polymer at the end of the chromatographic column will be considered separately.

2.1. Model of separation

The position of each polymer fraction along the separation system (column) will be described by a volume coordinate, related to the excluded volume V_0, i.e. volume between particles of SP [8–12]. Excluded volume is divided into m elements, in the following referred to as plates (not to be confused with an empirical quantity ''number of theoretical plates''), ΔV_k, of constant position, i.e. fixed with SP, of the size:

$$\Delta V = V_0/m \tag{1}$$

numbered from 0 to $m-1$. During the separation, V increases by steps of the size ΔV, numbered from 0 without any upper limit. By the increase of V by ΔV, a polymer fraction in ΔV_k of MP is transported into ΔV_{k+1}, but the position of a polymer in SP remains unchanged. The size of the plate ΔV_k, which can be defined as the domain where the local equilibrium between polymer fractions in MP, p, and in SP:

$$q = 1 - p \tag{2}$$

is formed, reflecting thus quality of separation. The ratio p/q is that of the excluded and the part of the pore volume accessible to a given polymer fraction. The equilibrium will be discussed in more detail below.

The fractions of the analyte in the plate ΔV_k in MP and SP, respectively, will be denoted $f_{\mathrm{MP},k,n}$ and $f_{\mathrm{SP},k,n}$ where k is the number of the plate denoting its position in the column, starting with $k = 0$ (injection) and ending with $k = m - 1$ (elution) and n is the total number of displacement steps since the injection. Thus, the fractions of polymer at the beginning of observation in MP and SP (Fig. 1a) can be expressed by:

$$f_{\mathrm{MP},0,0} = p \tag{3}$$

and

$$f_{\mathrm{SP},0,0} = q \tag{4}$$

After the displacement of MP by ΔV (Fig. 1b) and formation of a new equilibrium (Fig. 1c), we have for the fractions in ΔV_0 and ΔV_1 in MP:

$$f_{\mathrm{MP},0,1} = pq \tag{5}$$

and

$$f_{\mathrm{MP},1,1} = p^2 \tag{6}$$

and for those in SP

$$f_{\mathrm{SP},0,1} = q^2 \tag{7}$$

and

$$f_{\mathrm{SP},1,1} = qp \tag{8}$$

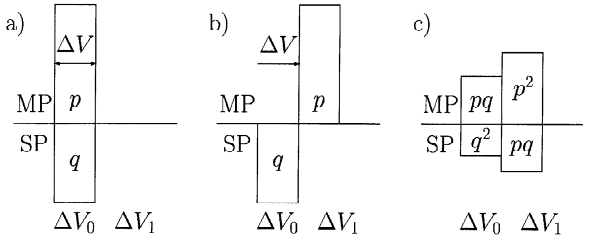

Fig. 1. The establishment of equilibrium between polymer fractions in SP and MP of ΔV_0 according to Eqs. (3) and (4) for $p = 0.6$ (a), displacement of MP by ΔV (b) and establishment of a new equilibrium (c) according to Eqs. (5)–(8).

This process can be repeated to any degree, say n. By comparing all fractions of two consecutive steps, one can see that the fraction of the polymer in the kth plate, ΔV_k, of MP and SP is given, respectively, by:

$$f_{\mathrm{MP},k,n} = \binom{n}{k} p^{k+1} q^{n-k} \tag{9}$$

and

$$f_{\mathrm{SP},k,n} = \binom{n}{k} p^{k} q^{n-k+1} \tag{10}$$

where $\binom{n}{k} = n!/(n-k)!k!$ is the binomial coefficient, where $k! = k(k-1)\ldots 1$ denotes the factorial. Eqs. (9) and (10) are binomial distributions, the former multiplied by p, the latter by q. They are not normalized because they describe distributions of the two fractions, p and q, in MP and SP. For $n \to \infty$, this result approaches the commonly accepted [11] Gaussian longitudinal concentration profile.

After the value of the excluded volume is reached, i.e. $V \geq V_0$, the analyte starts to be eluted from the column and registered by the mass detector but the analyte remaining in the column continues to be separated. Only the distribution of the analyte fractions with respect to volume of eluted MP, observed at the end of the separation column, is of interest because it is registered by the detector. This distribution will be now derived. The first eluted fraction is the most advanced one, given by Eq. (9) for:

$$k = m - 1 \tag{11}$$

and $n = m - 1$. In further steps, k, given by Eq. (11), is constant because it gives the position of the last plate of the column, where the polymer is detected, but the number of the elution volume steps, n, increases without any limit. A new counter, s, of elution volume is introduced which is a formal device making possible to start the counting fractions of polymer when they start to be eluted, i.e. after the condition $V \geq V_0$ is met. The order of the elution volume steps is then expressed by:

$$n = m - 1 + s \tag{12}$$

and the $(s+1)$th $(0 \leq s)$ eluted (subscript ''E'') and detected fraction is given by

$$f_{\mathrm{E},m,s} = f_{\mathrm{MP},m-1,m-1+s} \tag{13}$$

By introducing $f_{\mathrm{MP},n,k,}$ from Eq. (9) with k and n given by Eqs. (11) and (12) into Eq. (13), we have:

$$f_{\mathrm{E},m,s} = \binom{m-1+s}{m-1} p^{m} q^{s} \tag{14}$$

From the property $\binom{n}{k} = \binom{n}{n-k}$ of the binomial coefficients, it follows that Eq. (14) can be rewritten as:

$$f_{\mathrm{E},m,s} = \binom{m+s-1}{s} p^{m} q^{s} \tag{15}$$

which is the negative binomial distribution [13] of s in the range $0 \leq s < \infty$. Eq. (15) describes the distribution of polymer fraction, initially contained in ΔV_0, into m plates of MP, observed at the end of the column, during the elution of polymer (i.e. for $V > V_0$). The distribution is, unlike that given by Eq. (9), normalized which physically means that, after sufficient time, all polymer fractions are eluted from the separation system. For low m, the function given by Eq. (15) is not symmetrical and its plot (Fig. 2) resembles that of a function derived by Giddings and Eyering for adsorption chromatography [14]. However, the relation between the two functions is beyond the scope of this paper.

The negative binomial distribution narrows with increasing m on a constant distance (Fig. 2) and can be replaced by the symmetrical Gaussian distribution [13]. The mean of the distribution is given by [13]

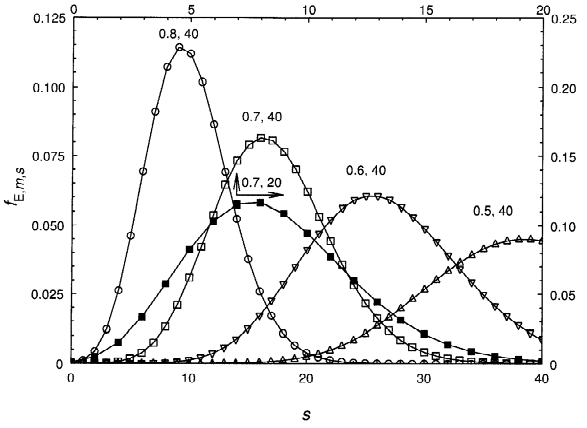

Fig. 2. A comparison of values of $f_{\mathrm{E},m,s}$ calculated according to Eq. (15) (negative binomial distribution) for $m = 40$ (open symbols) and 20 (■), right and upper scales (indicated by arrows), for p and m denoted, respectively, with the curves.

$$E_{NB}(s) = m\frac{1-p}{p} \tag{16}$$

The order of a step is given by Eq. (12) which means that m steps are to be added to obtain the mean of the distribution recorded from the injection; we have from Eq. (16):

$$E(s+m) = \frac{m}{p} \tag{17}$$

which is, according to Eq. (1) expressed in terms of elution volume:

$$y = V_0/p \tag{18}$$

The variance of the distribution given by Eq. (15) is [13]:

$$\mathrm{Var}(s) = m\frac{1-p}{p^2} \tag{19}$$

The variance of the distribution of s steps of the size ΔV, will be denoted σ^2_{sep}, where the subscript ''sep'' refers to the separation process and will be discussed later. The variance is found by considering the definition of variance, $\mathrm{Var}(x) = E(x^2) - [E(x)]^2$, and Eq. (1), as:

$$\sigma^2_{sep} = V_0\Delta V\frac{1-p}{p^2} \tag{20}$$

The function $(1-p)/p^2$ (Fig. 3), appearing in Eq. (20) is zero for $p = 1$ and with decreasing p it rises to a value about $(1-p)/p^2 \approx 2$ for $p \approx 0.5$, which is a value observed for a low-molecular-mass substance (see Results and discussion) and reflects the rising interaction of the analyte with SP.

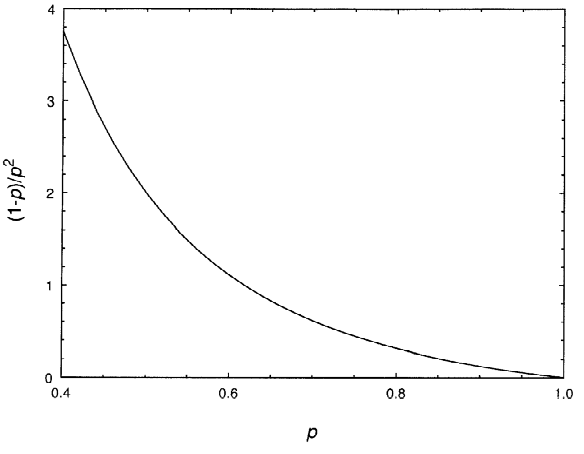

Fig. 3. The function $(1-p)/p^2$ appearing in Eq. (20).

The proposed model of polymer separation will now be discussed in relation to two important concepts of the separation science: with the Flodin model of SEC separation and with the Tung equation.

2.2. Flodin model

The size exclusion separation is often described by a well-known equation [9,15]:

$$y = V_0 + K\left(\frac{V_{SA}}{V_S}\right)V_S \tag{21}$$

where V_S is the total pore volume of SP and V_{SA} is its fraction accessible to the molecules of the analyte. The quantity $K(V_{SA}/V_S)$ is called the volume separation coefficient [9]. (When the analyte concentration tends to zero the terms ''fractions'' have rather a meaning of probabilities of occurrence without any change to the present theory.) K is the solute distribution coefficient:

$$K = \exp(\Delta S/R) \tag{22}$$

where ΔS is the standard entropy difference between phases, due to the change in molecular conformation, of the analyte in the pores of SP, and R is the gas constant. When there is no interaction between the analytes and the surface of the pore walls, separation is a function of entropy. For low molecular-mass analytes, small enough to access all pore volumes, the elution occurs at total permeation volume, $\Delta S = 0$ (for the present paper, this would probably reflect the case of toluene), and $K = 1$ [11].

The average elution volume of the particular polymer, y, defined by Eq. (18), is related to M by the calibration dependence:

$$\ln M = A + By \tag{23}$$

where A and B are constants. The relation between Eqs. (18) and (21) is demonstrated as follows: the fraction p of polymer in MP can be expressed in terms of available volumes in MP and SP, V_0 and V_{SA}, respectively, as:

$$p = \frac{V_0}{V_0 + KV_{SA}} \quad (24)$$

Introducing p given by Eq. (24) into Eq. (18) gives:

$$y = V_0 + KV_{SA} \quad (25)$$

which is Eq. (21).

The driving force of motion on the molecular level is the difference in chemical potential in MP and in the pores of SP, pushing the system to equilibrium which is continuously being disturbed by the flow. The actual distribution of the analyte between SP and MP thus reflects not only the driving force to equilibrium properties (difference in chemical potential between phases) but also the resistance to flux on a molecular level expressed by diffusion coefficient. According to irreversible thermodynamics, sometimes called non-equilibrium thermodynamics [16], the fluxes are proportional to gradients or so-called ''generalized forces'' and the diffusional flux is proportional to the concentration gradient [16]. Thus, thermodynamic quantities describing the separation process, including entropy ΔS in Eq. (22), have to be understood in the sense of irreversible thermodynamics.

2.3. Tung equation

The non-ideality of separation, called peak or band broadening, is usually described by equation [17]:

$$F(V) = \int_{-\infty}^{\infty} W(y)G(V,y)\,dy \quad (26)$$

where $F(V)$ and $W(y)$ are, respectively, experimental and theoretical elution curves, expressed as functions of two elution volume variables, y and V. The former denotes the position of the maximum of eluted polymer, related to molecular mass by Eq. (23), the latter is the real elution volume at which the particular fraction is eluted. The spreading function, $G(V,y)$, usually approximated by the Gaussian distribution with variance σ^2, gives the contribution of a polymer fraction of molecular mass, related to the theoretical value of elution volume y by Eq. (23), to fractions *really* eluted at V. The variance σ^2 is usually divided into extra- and intra-column contributions [9,12]

$$\sigma^2 = \sigma^2_{ext} + \sigma^2_{int} \quad (27)$$

The intracolumn peak broadening can be divided into contributions from separation (''sep'') and non-separation (''nsep'') processes,

$$\sigma^2_{int} = \sigma^2_{nsep} + \sigma^2_{sep} \quad (28)$$

where σ^2_{nsep} has its origins in non-ideal flow between gel particles, and other parts of the separation system caused by viscosity effects due to the presence of a polymer, tortuosity of flow, diffusion etc. [9] and σ^2_{sep} defined by Eq. (20) is the variance due to the interaction of the analyte with SP in the absence of other non-separation processes, i.e. the variance of the elution curve of a unique species of the analyte, which can be expressed as:

$$G_{sep}(V,y) = f_{E,s,m}\Delta V \quad (29)$$

where the subscript ''sep'' refers to the fact that its variance refers only to ''separation'' and the real elution volume, according to Eq. (1), is given by:

$$V = (s + m)\Delta V \quad (30)$$

Individual contributions to peak broadening will be discussed in the Results and discussion.

3. Experimental

SEC measurements with dual light scattering/concentration detection were performed using the Watrex set (Pump Deltachrom, autosampler Midas-Spark Holland, two columns PL gel MIXED-B LS, particle size 10 μm, separating in the range of molecular masses ~400–10^7 g mol^{-1}, differential refractometer Shodex RI-71, measuring at a temperature of 30 °C). The injection loop volume was 0.1 ml. The DAWN DSP-F laser photometer, measuring at 18 angles of observation (Wyatt Technology) was connected between the columns and the refractometer as first detector. The data were accumulated and processed using the Wyatt Technology ASTRA 4.70.07 Software for Windows and some calculations were performed using laboratory-modified software. The system was calibrated using polystyrene reference standards. The dependences of molecular mass and root-mean-square radius, referred to, in the follow-

ing, as radius of gyration, r, on elution volume were fitted by straight lines according to equations [18]:

$$\log M = 11.825 - 0.436V \quad (31)$$

and

$$\log r = 5.18 - 0.260V \quad (32)$$

obtained from analyses of several polymer standards in a broad range of elution volume.

A polystyrene reference standard $M = 1.6 \times 10^6$ g mol^{-1} (Pressure Chemical), injected at a concentration $c_{inj} = 5.26 \times 10^{-4}$ g ml^{-1} was used for the experiments. The local calibration dependence was found from the local slope of the dependence of log r vs. V (Fig. 4), d log r/dV = -0.203 ml^{-1} by combining Eqs. (31) and (32) using a method described elsewhere [18,19], as d log M/dV = 0.335 ml^{-1}. The weight-to-number average molecular-mass ratio, $\overline{M}_w/\overline{M}_n = 1.05$, was found by combining [18,19] apparent non-uniformities obtained by use of dual light-scattering/concentration detection, $(\overline{M}_w/\overline{M}_n)_d = 1.046$, and the broad-range calibration dependence (Eq. (31)), $(\overline{M}_w/\overline{M}_n)_c = 1.07$. The solutions in tetrahydrofuran of this standard and of toluene (6×10^{-2} ml of toluene in 1 ml of solution) were analyzed at several flow-rates. The standard deviations of the elution curves were measured graphically (Fig. 5). The experiments were repeated three times and average values were calculated. The error of measurements was expressed as the standard deviation of the measurements.

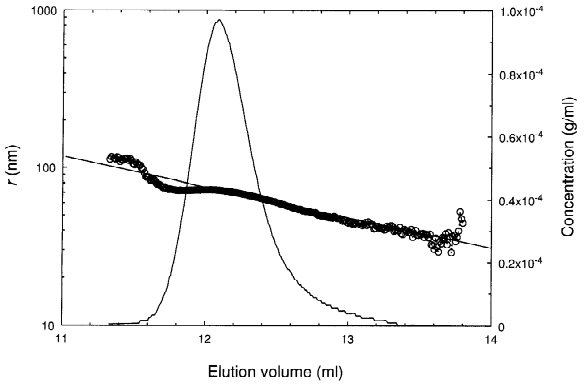

Fig. 4. A comparison of the logarithmic dependence of the radius of gyration, r, on the elution volume, determined for a polystyrene reference standard of molecular mass $M = 1.60 \times 10^6$ g mol^{-1} with the concentration elution curve.

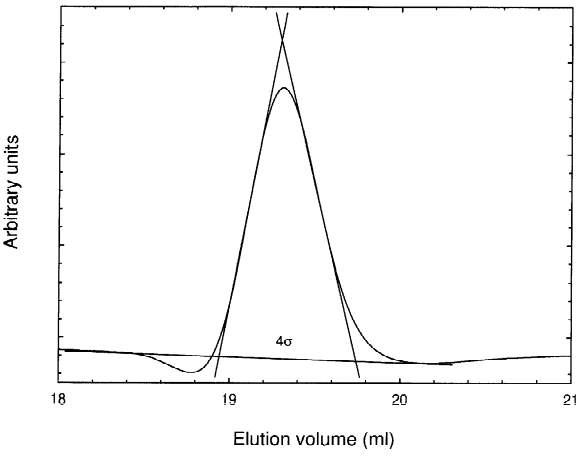

Fig. 5. Determination of the standard deviation, σ, from the concentration elution curve of toluene ($r_f = 0.108$ ml min^{-1}).

The TSK polystyrene reference standard, $M = 2.06 \times 10^7$ g mol^{-1} (Toyo Soda Manufacturing, $M = 1.98 \times 10^7$ g mol^{-1} according to our measurements), injected at concentration $c_{inj} \approx 7.5 \times 10^{-4}$ g ml^{-1} was used for the determination of the exclusion limit.

4. Results and discussion

In a real separation system, the number of separation–equilibrium steps is high and the contribution of separation to peak broadening, expressed by σ^2_{sep} in Eq. (28), is low. This makes possible only a rough estimation of its limits from the fraction of analytes in MP and SP and limiting values of σ^2.

4.1. Fractions of analytes in MP and SP

The exclusion limit of the separation system was found from the position of the maximum of the light scattering (90 degree) elution curve of the TSK standard at 10.26 ml (denoted by an arrow in Fig. 6). The elution curve is asymmetrical which could be due to the elution at the exclusion limit (cf. curves in Fig. 2). However, the sample molecular-mass distribution (MWD) as well the $\overline{M}_w/\overline{M}_n$ ratio are not

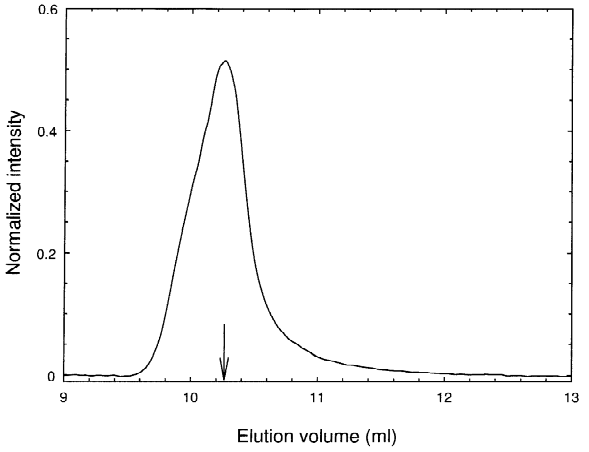

Fig. 6. Determination of the exclusion limit, 10.26 ml (denoted by the arrow), from the light scattering (90 degree) elution curve of the reference standard of $M = 2.06 \times 10^7$ g mol^{-1}.

known with sufficient precision to solve this question.

From the exclusion limit, and elution volume maxima, 19.5 and 12.6 ml, the fractions of toluene and the $M = 1.6 \times 10^6$ mol g^{-1} polystyrene standard in MP, respectively, $p = 0.52$ and 0.80, were found according to Eq. (18).

4.2. Contribution of separation to peak broadening

For toluene, uniform in M, the elution curve is identical with the spreading function. Although a small negative peak due to the presence of moisture is present at the beginning of the curve, its standard deviation, σ, can be directly found [9,10] from the intersection of the tangents through the inflection points of its elution curve with the baseline (Fig. 5).

For the polystyrene standard, not uniform in M, the procedure is more complicated. The determination of $\overline{M}_w/\overline{M}_n$ was described in the Experimental section. The values of σ^2 were calculated from those of σ_E^2, found from elution curves (subscript ''E'') in the same way as σ^2 for toluene, according to a formula [20–22]

$$\sigma^2 = \sigma_E^2 - \frac{\ln^2 \overline{M}_w/\overline{M}_n}{B^2} \quad (33)$$

where B is the slope in Eq. (23) (cf. Eq. (31)), taking

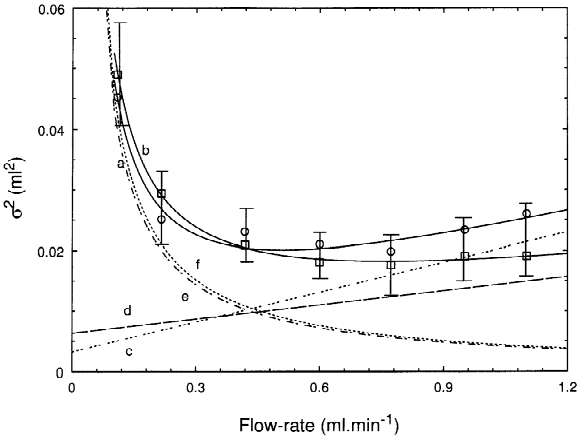

Fig. 7. A comparison of the dependences of the variance of the spreading function, σ^2, calculated from the concentration elution curves (data points, curves ''a'' and ''b''), with straight lines with intersections and slopes given by a and c (lines ''c'' and ''d'') in Eq. (34) and reciprocal terms b/r_f (curves ''e'' and ''f'') found for toluene (○), dependences ''a'', ''e'' and ''c'', and the polystyrene standard of $M = 1.6 \times 10^6$ g mol^{-1} (□), dependences ''b'', ''c'', and ''f''. The error bars express the standard deviations of the average values obtained from three experiments.

thus the part of peak broadness due to non-uniformity of the sample into consideration.

The dependences (Fig. 7) of σ^2 on flow-rate, r_f, are described by an equation frequently referred to as the van Deemter [11,23] equation:

$$\sigma^2 = a + b/r_f + cr_f \quad (34)$$

where the constants a, b and c are associated, in the first approximation, with eddy diffusion, longitudinal diffusion and mass transfer, respectively. The values of the parameters $a = 3.226 \times 10^{-3}$, $b = 1.650 \times 10^{-2}$ and $c = 4.314 \times 10^{-3}$ were found by linear regression for toluene and $a = 6.320 \times 10^{-3}$, $b = 7.824 \times 10^{-3}$ and $c = 4.458 \times 10^{-3}$ for the polystyrene standard.

For comparison with other separation systems, for the minimum of the curve for toluene, $\sigma^2 \approx 0.02$ ml^2 at $r_f \approx 0.5$ ml min^{-1}, the number of theoretical plates [11] was found according to the equation:

$$N = (y/\sigma)^2 \quad (35)$$

where $y = 19.8$ ml is the elution volume in the maximum of the curve, as $N = 2.00 \times 10^4$.

The constant term, a, in Eq. (34), summarizing

under the term ''*a*-term dispersion'' a large number of processes such as viscosity effects, tortuous flow, transverse diffusion etc. [24], can be understood as the limiting value of σ^2 close to equilibrium conditions, i.e. at $r_f \to 0$ in the absence of longitudinal diffusion [25] (expressed by the reciprocal term b/r_f).

With increasing flow-rate, the resistance to the mass transfer causes σ^2 to rise as expressed by the linear cr_f term (lines ''c'' and ''d'' in Fig. 7) in Eq. (34). On the other hand, with $r_f \to 0$, σ^2 rises as expressed by the reciprocal term b/r_f (hyperbolic curves ''e'' and ''f'' in Fig. 7).

The ranges of σ^2_{sep} and ΔV and their dependence on conditions of analysis will be estimated from an equation:

$$\Delta V = \frac{\sigma^2_{sep}}{V_0} \frac{p^2}{1-p} \tag{36}$$

obtained from Eq. (20). The minimum of both curves for toluene and the polystyrene standard is about $\sigma^2 \approx 0.02$ ml^2 which gives, according to Eq. (36), $\Delta V \approx 1.12 \times 10^{-3}$ ml for toluene and $\Delta V \approx 6.35 \times 10^{-3}$ ml for the polystyrene standard.

The reciprocal of the step, $(\Delta V)^{-1}$, gives some idea about the number of interactions (the equilibrium establishment and displacement) of the analyte with SP per unit of elution volume necessary for the separation. As the separation process is superimposed by the deleterious effect of other processes discussed above, it is clear that the number of interactions is higher than estimated from ΔV obtained from the minimum of the curves in Fig. 7. This number can be estimated from the approximation of σ^2 at $r_f \to 0$, i.e. in the absence of the longitudinal diffusion, i.e. as the *a* term. As the error in estimation of the term *a* in Eq. (7) is large, as can be seen from the error bars in Fig. 7, only rough estimation is possible, $\Delta V \approx 1.81 \times 10^{-4}$ ml for toluene and $\Delta V \approx 2.02 \times 10^{-3}$ ml for the polystyrene standard. This means that the number of interactions of the analyte between SP and MP can be estimated as of the order of 10^4 for toluene and 10^3 for the standard per 1 ml of elution volume.

As was demonstrated, the number of interactions of the analyte with SP is very high. This can explain the question why the molecules of the analyte in a densely packed system appear concentrated in a narrow flow-zone although one could expect infinite dispersion of the analyte because of interactions with SP [24]: the analyte *is* really infinitely dispersed. However, with increasing number of interactions of the analyte in MP and SP, i.e. with decreasing ΔV, the probability of exclusion is concentrated into a small zone with a maximum given by Eq. (18) and variance due to separation given by Eq. (20). Other, non-separation processes superimposed on the analysis, act in the opposite sense, increasing σ^2 by σ^2_{nsep} as expressed by Eq. (33).

As viscosities of toluene and tetrahydrofuran are almost the same, respectively, 0.59 and 0.55 mPa s at 20 °C [26], the influence of viscosity effects on dispersion is eliminated. With increasing molecular mass of the analyte, and according to the conditions of analysis, the decreasing interaction of polymer with SP with increasing *M* is partially or fully compensated by the viscosity, kinetic and other non-separation effects. This can explain why no observable dependence of σ^2 on *M* [18] or even an abrupt increase in the range of very high *M* [20,27–29] is observed. Thus, the exact determination of σ^2_{sep} is complicated by σ^2_{nsep} which makes the separation process more complicated than envisaged by the proposed simplified model.

5. Conclusions

1. The position of the fractions of the analyte in MP as well as in pores of SP of the SEC separation system is described by the volume coordinate, related to the excluded volume, divided into plates of size characterizing the efficiency of the separation process.
2. The separation process is described as a series of interactions of the analyte with SP, i.e. of equilibrium establishments between the fractions in MP and accessible part of the pores of SP on each particular plate, followed by a displacement by a step of the size of the plate and a formation of a new equilibrium.
3. The elution curve of an analyte uniform in *M* (''spreading function'') is obtained as a series of consecutive concentration profiles developing in time and space observed in one point of the space.

4. The elution volume of a particular polymer species, i.e. the volume at which the maximum of analyte is eluted, is the excluded volume divided by the fraction of the analyte in MP from the total amount of polymer in MP and in the accessible part of the pores of SP, i.e. by the volume separation coefficient.
5. The interaction of the analyte with SP is a source of peak broadening. The distribution of the analyte uniform in M with respect to V is described by a function which is, in principle, asymmetrical but with increasing number of interactions of the analyte with SP (displacement–separation steps) narrows and tends quickly to symmetrical normal distribution, justifying thus the use of normal distribution as the approximation of the spreading function. As a consequence, other sources of peak broadening prevail: at low flow-rates the longitudinal diffusion and/or tortuous flow and at high flow-rates the resistance to mass transfer. The size of the equilibrium–displacement step as well as number of interactions can be thus only roughly estimated.

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