

Relation between the kinetic and equilibrium quantities in size-exclusion chromatography

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Abstract

Separation mechanisms in the adsorption and size-exclusion chromatography (SEC) modes are equivalent in the sense that both processes can be described in terms of a mean fraction of the analyte in mobile phase (MP) and the number of interactions of molecules of the analyte in MP and in pores of/on the solid phase (SP). This is derived by comparing a recent theory of SEC with the theory of Giddings and Eyring for the adsorption chromatography. The elution volume, i.e., the maximum of the elution curve of a species uniform in molecular weight and composition (i.e., the maximum of the spreading function) is the excluded volume divided by the mean fraction of the analyte in mobile phase. Relations between the probabilities of adsorption and desorption of the molecule and the fractions of the analyte in MP and SP were derived. This makes it possible to describe the SEC and adsorption separation mechanisms in the framework of a single unified theory, which is in accord with the Flodin model of separation.

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1. Introduction

The separation process in chromatography [1] was described by two different approaches related to the mechanism of interaction of analyte, dissolved in mobile phase (MP), with solid phase (SP). The mechanism can be based on adsorption [2] driven by van der Waals forces (adsorption chromatography), steric interaction (size-exclusion chromatography, SEC) [3] or it can be a combination of both mechanisms [4]. The mass exchange between MP and SP in general will be called ‘interaction’. The adsorption chromatography was described in terms of adsorption and desorption probabilities which are kinetic quantities [2]. The separation in SEC was described [3] by considering an equilibrium in the sense of non-equilibrium thermodynamics [5] between the analyte in MP and in pores of SP, formed by a porous gel [6,7]. This equilibrium results in the formation of a longitudinal concentration profile along the separation system (column) developing in time, observed at one place (mass detector) [3]. When the separation pro-

cess is governed by the molecular weight of the analyte, which is possible [4] both in the adsorption and in the SEC separation mode, it is quite unimportant for the statistics of polymer separation whether a molecule of the analyte is temporarily adsorbed on the surface or captured in a pore of SP. Hence, the mechanisms of adsorption chromatography and SEC are, except the mechanisms of the temporary anchoring of the analyte molecule on the surface or in the pores of SP virtually the same. The temporary anchoring in/on SP and liberation into MP will be, in the following, referred to as ‘adsorption’ and ‘desorption’ or each of them as ‘reaction’. Finding relations between the kinetic and equilibrium quantities enables ensuing description of chromatographic separation in a framework of a single unified theory. This is the goal of this paper.

2. Theory

The theory of chromatographic separation relates processes on molecular level, such as the interaction of the analyte with SP and its transport in MP, with macroscopically observed quantities, as elution volume, V , of a particular

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analyte, broadness of the band-broadening function, etc. The chemical heterogeneity brings about complex problems in chromatography [8] which are not the subject of the present paper and it will be assumed that the analyte is homogeneous from the point of chemical composition and molecular architecture (e.g., linear polymer chains or low-molecular weight compound), and, in case of linear polymers, its reaction with SP is governed by M . The elution curve, $F(V)$, of the analyte not uniform in M is composed of contributions of uniform species (fractions). This is expressed by the equation originally proposed by Giddings and Eyring [2] and frequently referred to as ‘Tung’ equation [9]:

$$F(V) = \int_{-\infty}^{\infty} W(y)G(V, y) dy \quad (1)$$

where $W(y)$ is called theoretical elution curve. When the separation is governed by M , the elution volume expressed by the variable y is related to M by equation, in first approximation linear, called ‘calibration dependence’:

$$\ln M = A + By \quad (2)$$

where A and B are constants. $G(V, y)$ is the spreading function, i.e., the elution curve with variable V of a fraction with molecular weight M related to elution volume according to Eq. (2). For a good separation system $G(V, y)$ is a narrow symmetrical peak and can be approximated by the normal distribution where the variables V and y are interchangeable.

The theories describing the elution curves of a uniform analyte will be now reviewed separately, and the relation between them will be discussed and demonstrated on experimental data in Section 4.

2.1. Adsorption chromatography

The probability per unit time that a molecule in MP adsorbs on SP is represented by a non-varying unimolecular rate constant, k , giving the fraction of molecules adsorbed per unit time, and, similarly, the probability of desorption of the adsorbed molecule is represented by k' [2]. In the following, the constants k and k' are considered to be unique characteristics of the interaction of the analyte of a given M with SP (derived by Giddings and Eyring as a “single-site” adsorption mechanism [2]). The probability of adsorbing a molecule in MP exactly r -times is given by the Poisson distribution [2]:

$$W(r) = \frac{(kt^\circ)^r}{r!} \exp(-kt^\circ) \quad (3)$$

where t° the time it takes the solvent to flow completely through the column [2], i.e., the time necessary to reach the value of excluded volume, V_0 , connected with t° by:

$$V_0 = t^\circ r_f \quad (4)$$

where r_f is flow-rate.

For the distribution, $P(t)$, of elution times t after the t° Giddings and Eyring derived a series expansion which can be approximated by its first term [2]:

$$P(t) = \frac{(kk't^\circ)^{1/4}}{2\sqrt{\pi}} \frac{1}{t^{3/4}} \exp[-(\sqrt{k't} - \sqrt{kt^\circ})^2] + \dots \quad (5)$$

For the estimation of the standard deviation (dispersion) of the function given by Eq. (5), the substitution $X = t/t^\circ$ is used and the dispersion is obtained as the difference between the its maximum value, $X_m = k/k'$ and its value in one half of the peak height, $X_{1/2}$,

$$X_{1/2} - X_m \approx \pm 2 \left(\frac{k \ln 2}{k^2 t^\circ} \right)^{1/2} \quad (6)$$

which is Eq. (20) in Ref. [2] and the variance is then estimated by:

$$\text{Var}(t) \approx ((X_{1/2} - X_m) \times t^\circ)^2 \quad (7)$$

2.2. Size-exclusion chromatography

The separation in SEC was described by a combinatorial model expressing the length of the separation system using a volume coordinate based on the excluded volume, V_0 , divided into n' plates of the size [3]:

$$\Delta V = \frac{V_0}{n'} \quad (8)$$

The spreading function starting at V_0 is expressed in terms of the negative binomial distribution of the $0 < s < \infty$ elements of elution volume, V , of the size ΔV being consecutively washed out from the last plate to the detector. In the following, the notation using expected value $E(x)$ and variance $\text{Var}(x)$ will be used in order to distinguish characteristics of the spreading function expressed in time and elution volume.

The mean of the negative binomial distribution is:

$$E_{\text{NB}}(s) = n' \frac{1-p}{p} \quad (9)$$

where

$$p = 1 - q \quad (10)$$

is the mean fraction of the analyte in MP and q is the fraction in/on SP, which, by adding n' steps necessary to reach V_0 gives [3]:

$$E(s + n') = \frac{n'}{p} \quad (11)$$

which for the average elution volume, taken as the maximum of the spreading function composed of n steps of the size expressed by Eq. (8), gives for the mean $E(V)$ of the elution volume:

$$E(V) = \frac{V_0}{p} \quad (12)$$

$E(V)$ is frequently denoted [3] y , because it is related to M by Eq. (2). The variance of the negative binomial distribution is given by [3].

$$\text{Var}(s) = n' \frac{1-p}{p^2} \quad (13)$$

The variance of the spreading function is given by expressing n' in Eq. (13) from Eq. (8) and, considering the definition of variance, $\text{Var}(x) = E(x^2) - [E(x)]^2$, by multiplying the result by $(\Delta V)^2$, as:

$$\text{Var}(V) = V_0 \Delta V \frac{1-p}{p^2} \quad (14)$$

The above discussed characteristics of the spreading function will be now expressed in terms of elution time:

$$t_e = t^\circ + t \quad (15)$$

related with elution volume by

$$t_e = \frac{V}{r_f} \quad (16)$$

The time needed for one equilibrium-displacement is

$$\Delta t' = \frac{t^\circ}{n'} \quad (17)$$

which, for the elution time defined as the maximum of the spreading function expressed in time, gives:

$$E(t_e) = \frac{t^\circ}{p} \quad (18)$$

and for the variance of the spreading function

$$\text{Var}(t_e) = t^\circ \Delta t' \frac{1-p}{p^2} \quad (19)$$

As the theory of adsorption chromatography [2] results in a complicated series whose terms are not integrable and therefore its statistical characteristics cannot be determined, the analytical proof of the consistency of the two theories does not seem to be easy. However, they can be compared in the limiting form for a large number of reactions and $p \rightarrow 1$ and numerically, showing the equivalence of the two theories.

2.3. Relation between the kinetic and equilibrium quantities

The theory of SEC describes the interaction of the analyte with SP by establishing the mean fraction in MP and in/on SP. The quality of separation is expressed as the number of the equilibrium-displacement steps per the passage of the analyte through the excluded volume, V_0 , of the separation system. On the other hand, the theory of adsorption chromatography by Giddings and Eyring [2] describes the interaction by postulating the unimolecular rate constants of adsorption and desorption, k and k' .

It will be shown that the two approaches are equivalent. The number of adsorptions and desorptions, N_{ads} and

N_{des} , respectively, will be related to time, t° , which a given molecule spends exactly in the liquid phase (MP) while it is in the chromatographic column [2]. In a situation close to equilibrium, the numbers of adsorbed and desorbed molecules can be, respectively, expressed:

$$N_{\text{ads}} = Npk t^\circ \quad (20)$$

and

$$N_{\text{des}} = Nqk' t^\circ \quad (21)$$

where N is the number of all molecules of the analyte. In equilibrium, the numbers are equal:

$$N_{\text{ads}} = N_{\text{des}} \quad (22)$$

which can be fulfilled only if their fractions are given by

$$p = \frac{k'}{k+k'} \quad (23)$$

and

$$q = \frac{k}{k+k'} \quad (24)$$

The total number of adsorptions per t° is $(N_{\text{ads}} + N_{\text{des}})$. Expressing terms in this sum from Eqs. (20) and (21) and dividing by the total number of molecules, N , the number of adsorptions and desorptions per one molecule:

$$n = \frac{N_{\text{ads}} + N_{\text{des}}}{N} \quad (25)$$

results as

$$n = t^\circ(k+k') \quad (26)$$

As for the formation of the equilibrium two kinds of interactions are necessary (adsorption and desorption), each equilibrium-displacement step is formed by two interactions which gives for the number of equilibrium-displacement steps:

$$n' = \frac{1}{2}n \quad (27)$$

and the time for one reaction, i.e., interval between reactions, is given by

$$\Delta t' = 2\Delta t \quad (28)$$

The fraction of molecules in/on SP can be expressed by combining Eqs. (24) and (26) as:

$$q = \frac{kt^\circ}{n} \quad (29)$$

or, with respect to Eq. (27), as

$$q = \frac{kt^\circ}{2n'} \quad (30)$$

By combination of Eqs. (17), (27) and (30), the probability, k , of adsorption of the desorbed molecule is given by:

$$k = \frac{2q}{\Delta t'} \quad (31)$$

and for the probability of desorption of a molecule in/on SP we have (see Appendix A):

$$k' = \frac{kp}{q} \quad (32)$$

When $\Delta t'$ is found from experimental data according to equation:

$$\Delta t' = \frac{\text{Var}(t)}{t^\circ} \frac{p^2}{1-p} \quad (33)$$

obtained from Eqs. (19), (31) and (32) can be used for the determination of kinetic from equilibrium quantities. This will be discussed in Section 4.

2.4. Limiting form of the binomial distribution

The treatment following in this section shows the correspondence of theory of chromatography [2] with the theory of gases [10]. It is valid only in the limit of $p \rightarrow 1$ and for a high number of interactions, $n \rightarrow \infty$. The distribution of probability of reacting s times, expressed by Eq. (3), with probabilities of adsorption and desorption given by k and k' , can be, in the limit of $n \rightarrow \infty$ and $p \rightarrow 1$, expressed as the binomial distribution:

$$W_n(r) = \binom{n}{r} q^r (1-q)^{n-r} \quad (34)$$

of n intervals of elution time of the size Δt defined by Eq. (28). The proof is analogous to that used in the theory of diffusion, i.e., that the Poisson distribution of density fluctuations results for $n \rightarrow \infty$ and $q \rightarrow 0$ in binomial distribution of occurrences [10] (see Appendix B). The mean of this distribution is:

$$E_B(r) = nq \quad (35)$$

These n steps of the size Δt (or $2n'$ steps of the size $\Delta t'/2$), according to Eqs. (17), (27) and (30), give $t^\circ q$ and for the mean we have, according to Eq. (15), the equation:

$$E(t_e) = t^\circ (1+q) \quad (36)$$

which, by considering $(1+q) \approx (1-q)^{-1}$ and Eq. (10), can be for $p \rightarrow 1$, i.e., for $q \rightarrow 0$, expressed as:

$$E(t_e) \approx \frac{t^\circ}{p} \quad (37)$$

which is an approximation of Eq. (18).

The variance of the binomial distribution given by

$$\text{Var}(r) = nq(1-q) \quad (38)$$

tends for $p \rightarrow 1$, i.e., for $q \rightarrow 0$, to $\text{Var}(n') \rightarrow np$, i.e., by considering Eqs. (27) and (28), approximately those given by Eq. (13) and therefore from Eq. (38) also follows the approximation of Eq. (19).

Eq. (34) is an approximation of the negative-binomial distribution of elution times, valid for $p \rightarrow 1$. However, the

recalculation of the kinetic to equilibrium quantities does not contain any approximation and therefore it can be expected that the predictions of the two theories differ only to the extent of approximations made in the theories themselves. This is the neglecting of higher terms in the expansion with the first term given by Eq. (5) in the theory of adsorption chromatography [2] and the postulation of a plate at which equilibrium occurs in the SEC theory [3]. In both cases the differences are expected to disappear for $n \rightarrow \infty$ and any p . This is demonstrated as follows. For the mean, approximated by maximum of function given by Eq. (5) we have:

$$E(t) = \frac{t^\circ k}{k'} \quad (39)$$

which gives according to Eq. (15) an equation

$$E(t_e) = t^\circ \frac{k+k'}{k'} \quad (40)$$

Expressing p from Eq. (23) and introducing it in Eq. (40) gives Eq. (18), i.e., the result obtained for SEC [3]. As Eqs. (12) and (18) are in accord [3] with the well-known Flodin model of separation [11], the same holds also for the theory of Giddings and Eyring.

With the variance of the spreading function, the situation is more complicated because the series expansion with the first term given by Eq. (5) is not integrable and only the estimation by Eq. (6) is possible. For this reason, numerical calculations will be used for the comparison of the two theories.

3. Experimental

SEC measurements with two, light scattering and concentration, detectors were performed using following equipment: pump Deltachrom (Watrex International Inc., flow-rate 0.5 ml min^{-1}), autosampler Midas (Spark, Holland, injection-loop volume 0.1 ml), differential refractometer Shodex RI-71 operating at 30°C , light scattering photometer DAWN, measuring at 18 angles of observation (Wyatt Technology Corp.) placed between the columns and the refractometer as the first detector. The interdetector volume, 0.148 ml , was found by a procedure described elsewhere [12].

The separation system was formed by a precolumn of length 26 mm filled with PL gel of particle size of $5 \mu\text{m}$ and two columns PL gel MIXED-B LS (length 26 cm), particle size $10 \mu\text{m}$, separating in the range of molecular weights approximately $400\text{--}10^7 \text{ g mol}^{-1}$. The range was measured by the manufacturer with linear polystyrene standards in tetrahydrofuran at room temperature (mobile phase in our experiments).

Three arrangements of the separation system were used: in the first, 'one column' arrangement, one column was used as the separation system and the precolumn and the other column were placed between the pump and the autosampler, in order to maintain the same pressure drop on the system

Table 1

Results of SEC dual-detector analyses of polystyrene and poly(methyl methacrylate) standards denoted with weight-average molecular weight, \bar{M}_w (M and \bar{M}_w/\bar{M}_n given by the producers are in Section 3) and toluene

$\bar{M}_w \times 10^{-3}$	$(\bar{M}_w/\bar{M}_n)_c$	$(\bar{M}_w/\bar{M}_n)_d$	\bar{M}_w/\bar{M}_n	σ (ml)	σ_{MWD} (ml)	σ_{EC} (ml)	p	$\Delta t' \times 10^3$ (min)	k (min^{-1})	k' (min^{-1})
Polystyrene standards, one column ($t^\circ = 10.34$ min)										
1600	1.310	1.065	1.139	0.144	0.139	0.200	0.876	49.8	5.0	35.2
900	1.258	1.042	1.102	0.140	0.120	0.180	0.836	32.5	10.0	51.5
565	1.246	1.036	1.092	0.140	0.114	0.181	0.837	32.7	9.97	51.3
436	1.409	1.013	1.069	0.203	0.099	0.226	0.795	49.3	8.34	32.3
216	1.245	1.030	1.084	0.147	0.109	0.180	0.786	24.1	17.8	64.8
89.4	1.209	1.007	1.037	0.151	0.074	0.169	0.730	18.1	29.7	80.6
Toluene	–	–	–	0.115	–	–	0.517	2.84	340	364
Polystyrene standards, two columns ($t^\circ = 21.04$ min)										
1600	1.116	1.057	1.081	0.139	0.216	0.256	0.868	21.5	12.3	81.9
900	1.150	1.014	1.045	0.240	0.163	0.290	0.826	44.1	7.9	37.5
565	1.107	1.008	1.029	0.210	0.131	0.247	0.798	27.6	14.6	57.8
436	1.097	1.024	1.048	0.167	0.168	0.236	0.781	15.2	28.7	102
216	1.092	1.014	1.035	0.180	0.230	0.281	0.758	15.0	32.3	101
89.4	1.064	1.010	1.025	0.141	0.122	0.193	0.718	7.91	71.3	181
4.4	1.128	1.062	1.089	0.146	0.226	0.269	0.621	4.27	177	292
Toluene	–	–	–	0.155	–	–	0.521	2.65	362	393
Poly(methyl methacrylate) standards, two columns and precolumn ($t^\circ = 23.39$ min)										
1224	1.441	1.241	1.324	0.242	0.442	0.504	0.963	255	0.285	7.55
887	1.611	1.061	1.183	0.464	0.341	0.776	0.927	432	0.338	4.29
526	1.339	1.081	1.163	0.313	0.324	0.451	0.919	174	0.929	10.52
371	1.353	1.006	1.043	0.414	0.172	0.449	0.878	185	1.31	9.45
156	1.334	1.019	1.076	0.386	0.226	0.448	0.845	118	2.63	14.35

$(\bar{M}_w/\bar{M}_n)_c$ is the value of \bar{M}_w/\bar{M}_n obtained using the calibration dependence (41), $(\bar{M}_w/\bar{M}_n)_d$ that obtained using the dual detection and \bar{M}_w/\bar{M}_n is the value obtained using the procedure described in Ref. [15] together with standard deviation of spreading function, σ . (For toluene, σ was obtained using graphical procedure shown in Fig. 3a and b.) The values σ_{MWD} and σ_{EC} were calculated according to Eq. (43) using \bar{M}_w/\bar{M}_n and $(\bar{M}_w/\bar{M}_n)_c$, (see text for details). The mean fraction, p , of the analyte in MP was calculated according to Eq. (12), the equilibrium-displacement step, $\Delta t'$, according to Eq. (33) and the rate constants k and k' according to Eqs. (31) and (32)).

as in the second, ‘two column’ arrangement where both columns were used as the separation system (and the precolumn was between the pump and the autosampler) and in the third, ‘two columns and precolumn’ (the precolumn and the two columns were the separation system), arrangements.

The system was calibrated using several Pressure Chemical polystyrene and Polymers Standards Service poly(methyl methacrylate) reference standards. Some of the polystyrene standards with nominal $M \times 10^{-5} = 16, 9, 4, 2.07, 0.9$, and 0.04 (\bar{M}_w/\bar{M}_n : $\leq 1.12, \leq 1.10, \leq 1.06, \leq 1.05, \leq 1.04$ and ≤ 1.06 , respectively, given by the producer) and poly(methyl methacrylate) ones with nominal $\bar{M}_w \times 10^{-5} = 12, 7.3, 5.7, 3.15$ and 1.45 (\bar{M}_w/\bar{M}_n : $1.26, 1.12, 1.14, 1.032$ and 1.07 , respectively, given by the producer) were used for experiments.

The dependences of molecular weight on elution volume were fitted by equation:

$$\log_{10} M = A' + B'V \quad (41)$$

The constants of Eq. (41) were for polystyrene $A = 12.90$ and $B = -1.127 \text{ ml}^{-1}$ in the ‘one column’ arrangement, $A = 12.93$ and $B = -0.560 \text{ ml}^{-1}$ in the ‘two columns’ arrangement and for poly(methyl methacrylate) $A = 12.441$ and $B = -0.521 \text{ ml}^{-1}$ in the ‘two columns and precolumn’ arrangement.

The values of excluded volume and time, t° (Table 1, cf. Eq. (16), were found from the maxima of elution curves of high-molecular weight polystyrene reference standard (TSK polystyrene standard, $M = 2.06 \times 10^7 \text{ g mol}^{-1}$, Toyo Soda Manufacturing Co. Ltd., $M = 1.98 \times 10^7 \text{ g mol}^{-1}$ according to our measurement, injected at concentration $c_{\text{inj}} \approx 7.5 \times 10^{-4} \text{ g ml}^{-1}$) from the position of the maximum of the elution curve).

Toluene (A.C.S. reagent, 99.5%) at concentration $c_{\text{inj}} \approx 6 \times 10^{-3} \text{ g/ml}$ was injected as a low molecular-weight analyte.

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.

Correction of the light-scattering data for finite concentration was made by the Astra Software using the values of the second virial coefficient, A_2 in mol ml/g^{-1} , calculated according to equation:

$$A_2 = K \times M^\alpha \quad (42)$$

with $K = 10^{-2}$ and $\alpha = -0.25$ [13] for polystyrene and $K = 8.56 \times 10^{-3}$, $\alpha = -0.25$ calculated from published data [14] for poly(methyl methacrylate).

4. Results and discussion

4.1. Numerical calculations

The values (Table 2) of the variance of the function given by Eq. (5) calculated numerically for several values of k and k' are in agreement with those calculated from Eq. (19). The error due to numerical calculation for low values of k and k' (broad spreading function) is negligible and for higher values not observable. The values calculated from an approximate formula (Eq. (7)) are consistently somewhat higher. The corresponding elution curves (Fig. 1) calculated according to Eq. (3) are somewhat asymmetric but with increasing n the asymmetry decreases.

4.2. Comparison with experiment

The results of analyses of several polystyrene and poly(methyl methacrylate) standards (the nominal values of M and \bar{M}_w/\bar{M}_n are in Section 3) and toluene as a low molecular-weight analyte are in Table 1.

The values of $(\bar{M}_w/\bar{M}_n)_c$, the weight-to-number average molecular-weight ratio obtained by evaluation according to the ('conventional') broad-range calibration dependence (Eq. (41)) are larger than $(\bar{M}_w/\bar{M}_n)_d$, obtained in the dual

Table 2

A comparison of the number of reactions calculated from Eq. (26) for $t^\circ = 10$ with the variances of the spreading function calculated numerically from values calculated from Eqs. (5), (19) and (7) for several values of probabilities of adsorption and desorption, k and k'

k	k'	$\log n$	Var(t) calculated from		
			Eq. (5)	Eq. (19)	Eq. (7)
0.2	0.8	1	399.6	400	554
2	8	2	40	40	55.4
20	80	3	4	4	5.5
200	800	4	0.4	0.4	0.5

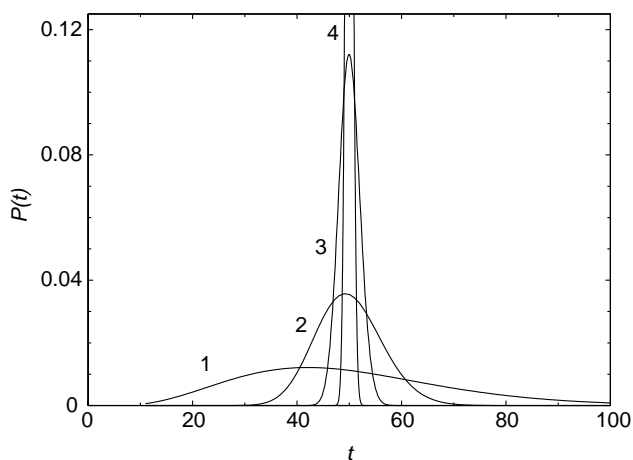


Fig. 1. Comparison of the theoretical elution curves calculated for $t^\circ = 10$ and different numbers of interaction, n . The curves are denoted with $\log_{10} n$; the values of k and k' are, together with n , given in Table 2.

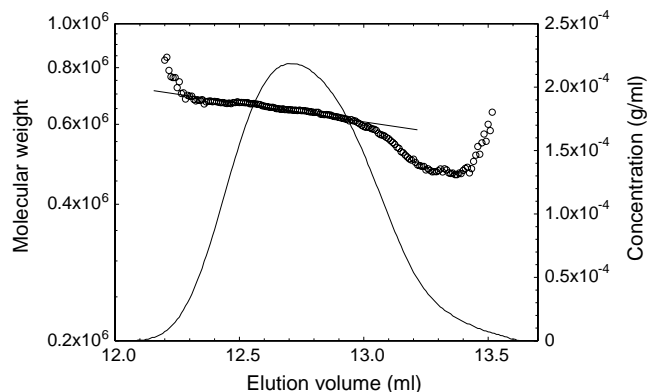


Fig. 2. Elution curve of a polystyrene reference standard of nominal $M = 5.75 \times 10^5$ (pressure chemicals) together with the local calibration.

analyses. A typical example of the results of a dual analysis (standard of $M = 5.65 \times 10^5$) is in Fig. 2. The values of \bar{M}_w/\bar{M}_n , obtained by a band-broadening correction procedure [15] are between them in accord with the theory [16,17]. The values of the variance of band-broadening function, σ , are somewhat larger for the 'two column arrangement' but the differences are small in accord with the finding that the extracolumn band-broadening contributions, especially in the refractometer, are considerable [18]. It is also interesting to compare with σ the contribution of broadness due to molecular-weight distribution (MWD), calculated as [17,19,20]:

$$\sigma_{\text{MWD}}^2 = \frac{\ln \bar{M}_w/\bar{M}_n}{B^2} \quad (43)$$

to the broadness of the entire elution curve, expressed as σ_{EC} , calculated also according to Eq. (43) but with \bar{M}_w/\bar{M}_n expressed by $(\bar{M}_w/\bar{M}_n)_c$. In accord with findings of other authors [18], the contribution of band-broadening to the elution-curve broadness of narrow-MWD sample is considerable.

The values of p , calculated from Eqs. (16) and (18) as

$$p = \frac{t^\circ}{E(t_e)} \quad (44)$$

decrease with decreasing M of the analyte to a value of $\approx 1/2$.

With decreasing M of the analytes, the values (Table 1) of equilibrium-displacement step calculated according to Eq. (33) decrease and the values of k and k' , calculated according to Eqs. (31) and (32), increase reflecting thus increasing mass transfer between MP and SP. The ratio k' to k , decreases, reflecting thus decreasing ratio of the analyte in MP to that in/on SP.

The values of both k and k' are a little higher (and those of Δt lower) for the 'two column' arrangement, reflecting lower contribution of the extracolumn band-broadening but the differences are negligible compared with the experimental error. The observed values of k and k' are decreased by band-broadening. Therefore their actual values expressing real frequencies of interactions with SP are probably much higher.

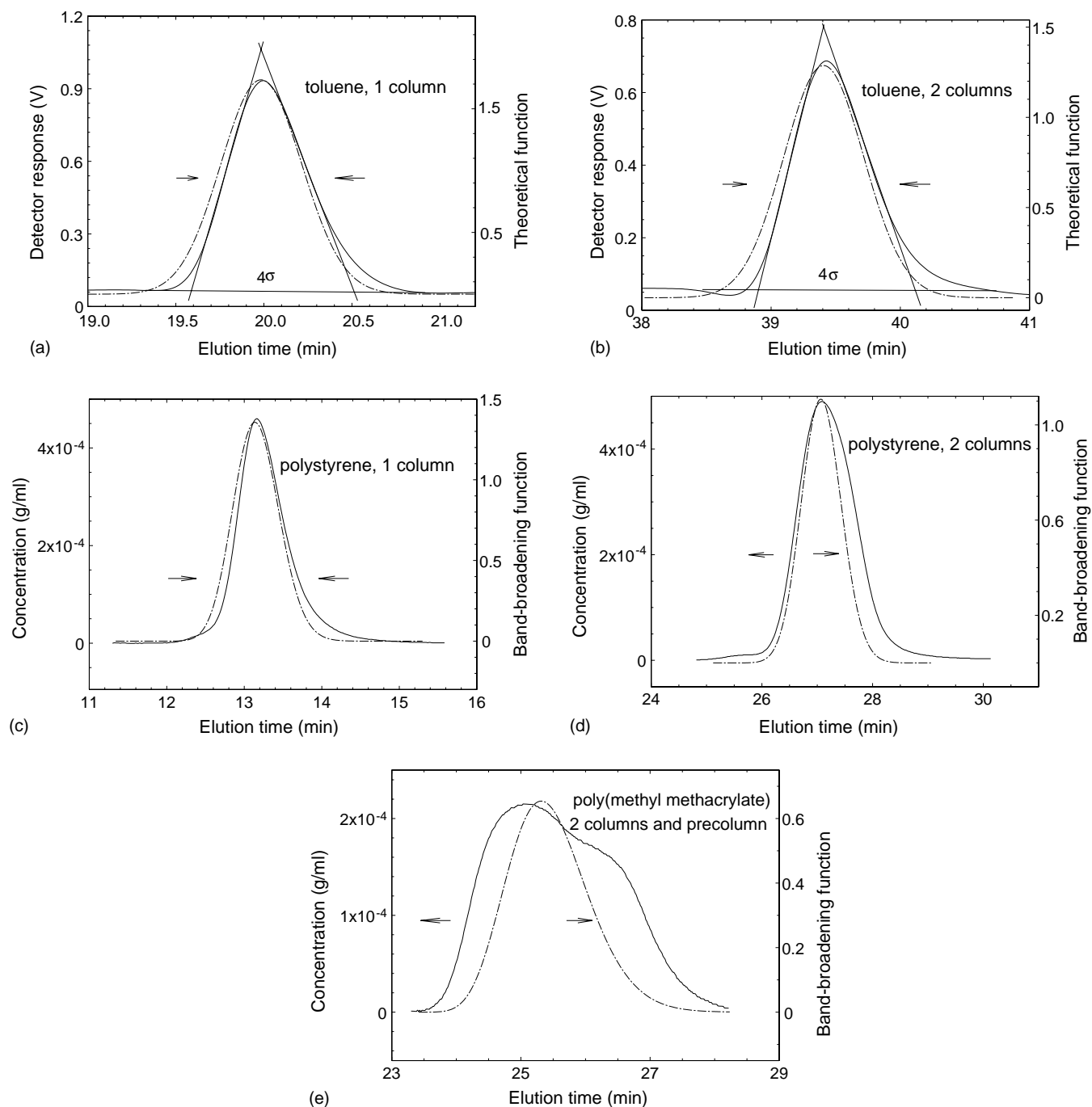


Fig. 3. Comparison of the concentration elution curves (solid lines, left scales, denoted by arrows near the curves) of toluene (a and b), polystyrene reference standard of nominal $M = 2.07 \times 10^5$ (c and d) and poly(methyl methacrylate) reference standard of nominal $M = 6.66 \times 10^5$ (e) with the theoretical functions for toluene and band-broadening functions for polymer analytes (dashed lines, right scales, denoted by arrows) calculated according to Eq. (5) using parameters t° , k and k' given in Table 1. Experimental data were obtained with the separation system formed by one column (a and c), two columns (b and d) and two columns and a precolumn (e). (See Section 3 for details.)

The values of k and k' for poly(methyl methacrylate) are somewhat lower (and those of σ^2 and Δt higher) which may reflect the fact that samples with broader MWD (cf. values of \bar{M}_w/\bar{M}_n in Section 3 and Table 1) show larger band-broadening (higher σ) compared with those with narrower MWD [15].

The values of t° , k and k' (Table 1) can be used for the construction of theoretical elution curves, for the analyte non-uniform in M identical with the band-broadening function with a maximum identical with the elution curve.

Fig. 3 shows the comparison of theoretical curves calculated according to Eq. (5) with experimental ones of

polystyrene standard ($M = 5.65 \times 10^5$) and toluene in the first two arrangements. For polystyrene standards, the theoretical elution curves are narrower than the experimental ones because they do not reflect the contribution σ_{MWD} to the broadness of elution curve, expressed by σ_{EC} , as expressed by equation [6]:

$$\sigma_{\text{EC}}^2 = \sigma^2 + \sigma_{\text{MWD}}^2 \quad (45)$$

The difference, however, is not very large, in accord with the values of σ and σ_{MWD} (Table 1). For all curves, the maximum of theoretical elution curves agrees well with the experiment, the differences are given only by the precision of the graphical determination of $E(t_e)$.

The theoretical elution curves show asymmetry below any limit of observation in accord with the finding that the equilibrium chromatographic separation leads to a symmetrical band-broadening function [18]. The experimental elution curves, on the other hand, show some tailing. This is prominent especially on elution curves of poly(methyl methacrylate) standards (Fig. 3e).

A possible explanation of the asymmetry of elution curves could be sought in the influence of pore-size distribution, flow in the junction zones [18], etc., and, for samples non-uniform in M and differing in chemical composition, also in the shape of their MWD, differences in diffusion coefficient of the molecules of the analyte as well as in viscosity properties of their solutions including hydrodynamical interaction [21] of the molecules, etc. The asymmetry of elution curves requires further examination.

5. Conclusions

1. The theories of adsorption and SEC predict the elution volume $E(V)$ (or time $E(T)$) as excluded volume V_0 (or time t°) divided by the mean fraction of the analyte in solution, p . The theories therefore comply [3] with the Flodin model [11] of separation.
2. The mean fraction of the analyte in solution is related to the probabilities of transition of a molecule in MP to SP and of a transition of a molecule in/on SP into MP.
3. The number of interactions of a molecule is related to the broadness (variance) of the spreading function and equals twice the number of equilibrium-displacement steps.
4. For a particular analyte, the parameters of the spreading function determined in terms of the SEC theory [3] can be expressed in terms of the theory of chromatography by Giddings and Eyring [2]. From them, the spreading function can be calculated. The calculated theoretical elution curves and band-broadening functions are in agreement with the experiment.

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Appendix A

From Eq. (24) we have

$$qk' + qk = k \quad (A.1)$$

or

$$qk - k = -qk' \quad (A.2)$$

which by using Eq. (10) is

$$kp = k'q \quad (A.3)$$

which gives Eq. (32).

Appendix B

The distribution of fluctuations of the number of adsorbed molecules, given by Eq. (3), will be expressed in the limit $n \rightarrow \infty$ and $p \rightarrow 1$ as distribution of occurrences on SP, i.e., of elution times starting at t° . Eq. (34) can be rewritten:

$$W_n(r) = \frac{n!}{r!(n-r)!} \left(\frac{v}{n}\right)^r \left(1 - \frac{v}{n}\right)^{n-r} \quad (B.1)$$

where

$$v = kt^\circ \quad (B.2)$$

which is

$$\begin{aligned} W_n(r) &= \frac{1}{r!} n(n-1)(n-2) \cdots (n-r+1) \left(\frac{v}{n}\right)^r \left(1 - \frac{v}{n}\right)^{n-r} \\ &= \frac{v^r}{n!} \left(1 - \frac{1}{n}\right) \left(1 - \frac{2}{n}\right) \cdots \left(1 - \frac{n-1}{n}\right) \left(1 - \frac{v}{n}\right)^{n-r} \end{aligned} \quad (B.3)$$

For $n \rightarrow \infty$,

$$\begin{aligned} W(n) &= \lim_{n \rightarrow \infty} W_n(r) \\ &= \frac{v^r}{r!} \lim_{n \rightarrow \infty} \left\{ \left(1 - \frac{1}{n}\right) \left(1 - \frac{2}{n}\right) \cdots \right. \\ &\quad \left. \times \left(1 - \frac{r-1}{n}\right) \left(1 - \frac{v}{n}\right)^{n-r} \right\} \end{aligned} \quad (B.4)$$

which is

$$= \frac{v^r}{r!} \lim_{n \rightarrow \infty} \left(1 - \frac{v}{n}\right)^n \quad (B.5)$$

which is

$$W(r) = \frac{v^r \exp(-v)}{r!} \quad (B.6)$$

which is Eq. (3).

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