Rapid estimation of overall mass-transfer coefficients in liquid chromatography

Ivett Bacskay and Attila Felinger*

Received 11th June 2010, Accepted 23rd September 2010
DOI: 10.1039/c0ay00372g

The stochastic or microscopic model of chromatography has been applied to determine overall mass-transfer coefficients from peak shapes recorded in reversed-phase liquid chromatography. The stochastic model of chromatography describes the separation process at the molecular level by probabilistic terms. This microscopic model is rather straightforward and it can furnish direct answers when one tries to understand the development of chromatographic peaks. In this study we demonstrate that this model can be rather simply used to estimate the fundamental mass-transfer characteristics of the separation process. The only information needed to obtain the mass-transfer coefficients is the first and the second moments of the chromatographic peak.

1 Introduction

Chromatographic processes are usually described with either equilibrium or kinetic macroscopic models. The modeling usually consists in formulating a proper differential mass balance equation that describes the physico-chemical processes of chromatography with a desired detail.[1,2] The mass balance models usually assume instantaneous equilibrium between the mobile and the stationary phases or use kinetic rate constants to characterize the resistance to mass transfer or adsorption–desorption.

A number of kinetic models of various degree of complexity have been introduced in chromatography. The most detailed kinetic model is the general rate model.[3] The solution of that model has been studied by several authors.[4,5] The moments calculated from the general rate model allow the derivation of a detailed plate height equation for both particulate and monolith columns.[6]

Lapidus and Amundson[7] introduced a simple nonequilibrium model, the lumped kinetic model, which has been used in various forms. The reaction-dispersive model attributes the nonequilibrium state to slow adsorption and desorption processes while the transport-dispersive model assumes that the adsorption–desorption is fast, but the mass-transfer kinetics is slow.

The lumped pore model[8] is the result of a different simplification of the general rate model. It seems to be more detailed than the lumped kinetic model because, similarly to the general rate model, it takes into consideration the difference between the mobile phase percolating through the interparticle space (or the through-pores of a monolithic column) and the mobile phase stagnant in the stationary phase pores. However, it ignores the radial concentration gradient within a stationary phase particle.

The microscopic – or stochastic – models depict the chromatographic processes at a molecular level *via* the random migration of the individual molecules.

The stochastic model of chromatography was introduced by Giddings and Eyring.[9] In their model, they assumed that while migrating along the column, a molecule performs a random number of adsorption and desorption steps characterized by a Poisson distribution. Furthermore, once a molecule is adsorbed on the stationary phase, the time spent until desorption – the sojourn time in the stationary phase – is a random variable, too. This latter random variable follows an exponential distribution. A significant effort has been devoted to the extension of the stochastic model to heterogeneous surfaces in the 1960s, but the handling of the problem in time domain resulted in rather complex expressions, inadequate for practical calculations.[10,11]

The characteristic function (CF) approach remarkably facilitates the use of the stochastic model of chromatography.[12,13] The use of CF facilitated the extension of the stochastic theory to two-site[14] or to generic multiple-site heterogeneous surfaces[15] that are very complex to handle otherwise.[9,16,17] Thus, the CF approach of the stochastic theory is able to model the band profile that is due to any unimodal or multimodal distribution of sorption energies. The stochastic model was further extended to describe the effect of mobile phase dispersion and size exclusion effects as well.[18–20]

By means of the CF method, closed form expressions are obtained in Fourier domain for the band profiles, thus the statistical moments can directly be calculated even if the transformation into time domain is possible numerically only.[21,22]

The dispersion in the mobile phase is very often neglected in the stochastic models. In some studies the contribution of the mobile phase dispersion was modeled simply with a Gaussian distribution[12,18,23] or by first-passage density calculated from the diffusion equation.[16,24]

There have been some attempts to compare the microscopic and the macroscopic models of chromatography. Usually this is restricted to the comparison of the first and the second moments. Cavazzini *et al.* showed that the number of theoretical plates calculated by either the microscopic or the macroscopic kinetic models of chromatography agree as long as the mobile phase dispersion is neglected.[15] Felinger *et al.* considered the mobile phase dispersion by a Gaussian peak and proved that the first and the second moments of the stochastic-dispersive and the lumped kinetic models are identical.[18] Later, Felinger *et al.*

Department of Analytical and Environmental Chemistry, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary. E-mail: felinger@ttk.pte.hu

showed that not only the first and the second moments but also the whole peak shape obtained with the stochastic-dispersive and with the lumped kinetic models are identical.[24]

In the present study, we will show a simple method to characterize the solute transfer between the mobile and stationary phases by using the stochastic model of liquid chromatography.

2 Theory

In the stochastic model of chromatography,[9] a distribution is determined for a given molecule describing the probability that the molecule can be found at location z within the column at time t. It is assumed that the k_a mass-transfer coefficient is a rate constant that represents the probability that a solute molecule will transfer from the mobile to the stationary phase. A similar k_d rate constant describes another mass-transfer coefficient – the solute transfer from the stationary to the mobile phase. The k_a constant represents the probability that a solute molecule will transfer to stationary phase during one second. The similar k_d rate constant describes the first-order process for the stationary-to-mobile phase transfer.[9]

In a similar manner, during a random walk of a molecule through the chromatographic bed, two mass-transfer time constants are defined. τ_m indicates the average time a molecule spends in the mobile phase between two visits to the stationary phase, and τ_s is the average time spent in the stationary phase during a single visit. These time constants can directly be related to the above introduced rate constants of the adsorption – or transfer from the mobile to the stationary phase – and desorption – or transfer from the stationary to the mobile phase – kinetics:

$$\tau_m = 1/k_a \tag{1}$$

$$\tau_s = 1/k_d \tag{2}$$

An unretained marker elutes from the column at time t_0, having stayed in the mobile phase only. Each retained molecule spends the same amount of time (t_0) in the mobile phase, and the adjusted retention time, t_R' indicates the time a retained molecule spends bound to the stationary phase.

Thus the void time and the adjusted retention time can be expressed by the average mobile and stationary phase sojourn times and by the average number of mass-transfer events (n)

$$t_0 = n\tau_m \tag{3}$$

$$t_R' = n\tau_s \tag{4}$$

Furthermore, the variance of the peak due to the stationary phase process can be given as:[13,15]

$$\sigma_s^2 = 2n\tau_s^2 \tag{5}$$

The stochastic model offers a rather simple way to evaluate the details of the separation process and to estimate the kinetics of the mass transfer. The only information needed to obtain the mass-transfer coefficients is the first and the second moments of the chromatographic peak.

3 Experiments

An Agilent 1100 liquid chromatograph with a dual solvent delivery system, an auto sampler, a column thermostat, a multi-wavelength UV detector and Chemstation software was used for all measurements. A Waters Symmetry C_{18} column was used (150 × 4.6 mm, average particle size 5 μm). Thiourea, toluene, ethylbenzene, propylbenzene, butylbenzene were purchased from Sigma-Aldrich; pentylbenzene was purchased from Fluka. Scharlau HPLC grade methanol and Baker water was used for all experiments.

Different flow rates were used, 0.25 mL min^{-1}, 0.50 mL min^{-1}, 1.00 mL min^{-1}, and 1.50 mL min^{-1}. The column thermostat was set at the following temperatures: 10 °C, 20 °C, 30 °C and 40 °C. Each measurement was executed with methanol–water (80 : 20, v/v) solution as mobile phase. The standard mixture contained 0.8 μg mL^{-1} of toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene and 1.6 μg mL^{-1} thiourea. The hold-up time of the column was determined from the elution time of thiourea. 20 μL of the standard mixture was injected in all measurements. The components were detected at 254 nm.

4 Calculations

In order to estimate the overall mass-transfer coefficients, the first and the second moments of the peaks must be determined. The first moment, or expectation of a transient signal is calculated as

$$\mu_1 = \frac{\int_{-\infty}^{\infty} t f(t) dt}{\int_{-\infty}^{\infty} f(t) dt} \tag{6}$$

The second central moment, or variance of a transient signal is calculated as

$$\mu_2' = \frac{\int_{-\infty}^{\infty} (t-\mu_1)^2 f(t) dt}{\int_{-\infty}^{\infty} f(t) dt} \tag{7}$$

Statistical moments are often difficult to determine with a good accuracy. The accuracy of the higher order moments is greatly affected by baseline drift and noise. One of the safest methods to calculate the moments is to fit a peak shape model to the experimental peaks, and then one can easily calculate the moments from the best-fit parameters.[1]

The exponentially modified Gaussian (EMG) function is one of the most frequently used asymmetrical models to describe peak profiles in chromatography. Although the peak shape parameters of the EMG model have no direct physical meaning, the moments of the recorded peak can be determined after the fitting procedure with sufficient accuracy. The following EMG model was fitted to the experimental peak profiles of thiourea and alkylbenzenes:

$$f(t) = \frac{A}{2\tau} \exp\left(\frac{\sigma^2}{2\tau^2} - \frac{t-t_R}{\tau}\right) \operatorname{erfc}\left(\frac{\sigma}{\sqrt{2}\tau} - \frac{t-t_R}{\sqrt{2}\sigma}\right) \tag{8}$$

where A is the peak area, t_R is the retention time of the Gaussian part, σ is the standard deviation of the Gaussian part and τ is the time constant of the exponential part of the EMG function.

The first moment and the second central moment of the EMG model are:

$$\mu_1 = t_R + \tau \tag{9}$$

$$\mu_2' = \sigma^2 + \tau^2 \tag{10}$$

Both the first moment and the second central moment are built up by additive contributions. The stationary and mobile phase processes, and the extra-column contribution have to be identified separately. Their effect on peak position and width must be known when the shape chromatographic band is used for the estimation of any physical-chemical parameters.

At this point we have to emphasize that the EMG model is purely empirical and cannot be used to estimate kinetic or mass-transfer coefficients. Chromatographers often use this peak shape model, as it can mimic many experimental peaks. The τ parameter of the EMG model, however, has nothing to do with mass-transfer or adsorption–desorption kinetics. There are several reasons why one observes tailing peaks in HPLC. One of those reasons is indeed slow mass-transfer or sorption kinetics. However, kinetics must be extremely slow to have an effect on the peak asymmetry. In reversed-phase liquid chromatography, kinetics is fast enough and the number of mass-transfer events is high enough to observe symmetrical peaks. We only use the EMG model to assure accurate estimation of the moments.

5 Results and discussion

We applied the previously described theory to HPLC experiments, in order to determine overall mass-transfer coefficients. The reversed-phase separation of neutral compounds on a C_{18} silica-based stationary phase was studied. Samples of mixture of thiourea and alkylbenzenes were injected at flow rates between 0.25 and 1.5 mL min^{-1}, using aqueous solution of methanol with 80% methanol as mobile phase, under chromatographic conditions described in experimental section. Fig. 1 shows the chromatogram recorded at 10 °C and 0.25 mL min^{-1} flow rate.

The first step of the calculations is to eliminate the effect of mobile phase dispersion and extra-column broadening from the peaks using the moments calculated from the peak shape of an unretained marker. The peak shape and the moments of the unretained marker carry the influence of the extra-column and mobile phase processes, therefore they can be utilized for correction and to obtain moments characteristic for the stationary phase process. Thus $\Delta\mu_1 = \mu_1 - \mu_1^0$ and $\Delta\mu_2' = \mu_2' - \mu_2'^0$ are obtained, where μ_1^0 is the first moment and $\mu_2'^0$ is the second central moment of the thiourea peak.

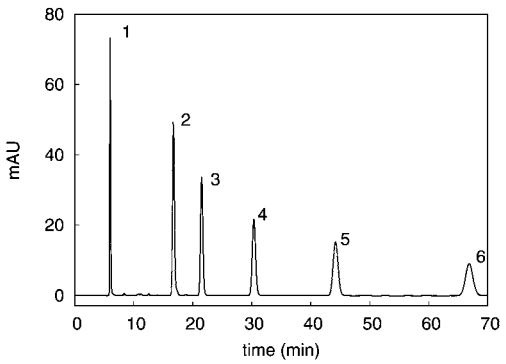

Fig. 1 Chromatogram of the standard mixture. Peaks: 1: thiourea, 2: toluene, 3: ethylbenzene, 4: propylbenzene, 5: butylbenzene, 6: pentylbenzene.

We used two sets of data to calculate the mass-transfer coefficients. The moments of the chromatographic peaks were calculated by two different ways: (i) using the moments reported by the Chemstation software and (ii) by fitting the exponentially modified Gaussian function, eqn (8). The Chemstation software calculates the moments by numerical integration, using a discretized form of eqn (6) and (7).

Tables 1 and 2 show a selected example from series of results for calculation of n, τ_s and τ_m. The average number of mass-transfer events (n) is calculated from the first moment and the second central moment corrected by the respective moments of thiourea using eqn (4) and (5):

$$n = \frac{2(\Delta\mu_1)^2}{\Delta\mu_2'} = \frac{2(t_R')^2}{\sigma_s^2} \tag{11}$$

Then the value of τ_s and τ_m can simply be determined as follows

$$\tau_s = \frac{\Delta\mu_1}{n} = \frac{t_R'}{n} \tag{12}$$

$$\tau_m = \frac{\mu_1^0}{n} = \frac{t_0}{n} \tag{13}$$

When the moments are calculated with numerical integration by the Chemstation software, the results are somewhat different from the more accurate peak fitting calculation. The average number of the mass-transfer events for ethylbenzene is, for instance $n = 16610$ and $n = 15363$ depending on the method of moment calculation.

Table 1 Results of the calculation of τ_s and τ_m by the stochastic model of liquid chromatography, using moments calculated by Chemstation software, using chromatogram measured at 10 °C and 0.25 mL min^{-1}

	Thiourea	Toluene	Ethyl-benzene	Propyl-benzene	Butyl-benzene	Pentyl-benzene
μ_1 (s)	362.22	1004.6	1291.3	1823.2	2653.6	4014.7
μ_2' (s^2)	19.71	77.45	123.63	244.85	519.39	991.14
$\Delta\mu_1$ (s)		642.42	929.04	1461.0	2291.4	3652.4
$\Delta\mu_2'$ (s^2)		57.75	103.93	225.14	499.69	971.43
n		14293	16610	18962	21015	27465
τ_s (ms)		44.9	55.9	77.1	109.0	133.0
τ_m (ms)		25.3	21.8	19.1	17.2	13.2

Table 2 Results of the calculation of τ_s and τ_m by the stochastic model of liquid chromatography, using moments calculated by fitting the EMG model, using chromatogram measured at 10 °C and 0.25 mL min^{-1}

	Thiourea	Toluene	Ethyl-benzene	Propyl-benzene	Butyl-benzene	Pentyl-benzene
μ_1 (s)	362.3	1005.4	1291.4	1823.2	2653.3	4011.2
μ_2' (s^2)	22.39	96.07	134.76	267.98	569.3	1311.1
$\Delta\mu_1$ (s)		643.02	929.09	1460.9	2290.9	3648.8
$\Delta\mu_2'$ (s^2)		73.68	112.37	245.59	546.9	1288.7
n		11224	15363	17380	19192	20663
τ_s (ms)		57.3	60.5	84.1	119.4	176.6
τ_m (ms)		32.3	23.6	20.8	18.9	17.5

Fig. 2 shows the comparison of the experimental and theoretical profiles along the time axis for the calculation of the zeroth, first and the second central moments. The theoretical distribution was calculated from the fitting of the EMG function. The experimental data are the respective $f(t)$, $tf(t)$, and $(t - \mu_1)^2 f(t)$ series calculated from the chromatograms. Those terms are to be integrated when the moments are determined by numerical integration of the peaks in the chromatogram, as it is shown by eqn (6) and (7).

The experimental data and the theoretical data are in excellent agreement for the zeroth and first moments. The experimental data set for second central moment diverges from the theoretical data. Accordingly, it is difficult to make an accurate evaluation from the experimental data of the second central moment, since one cannot accurately determine where to start and stop the numerical integration.

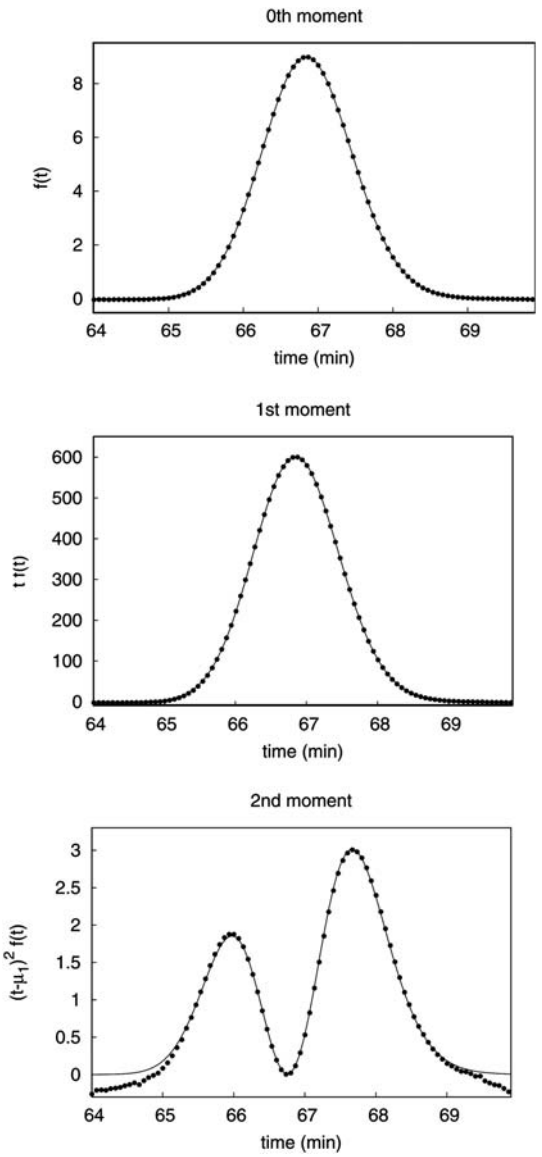

Fig. 2 Plots of the contributions of the signals to the moments *versus* the time. Symbols are the numerical integration data and the solid line is the theoretical data based on the EMG function, respectively.

We can express – using eqn (12) and (13) – the average stationary phase and mobile phase residence times for one mass-transfer event (τ_s and τ_m), respectively. The results summarized in Table 2 show that the stationary phase residence time ranges

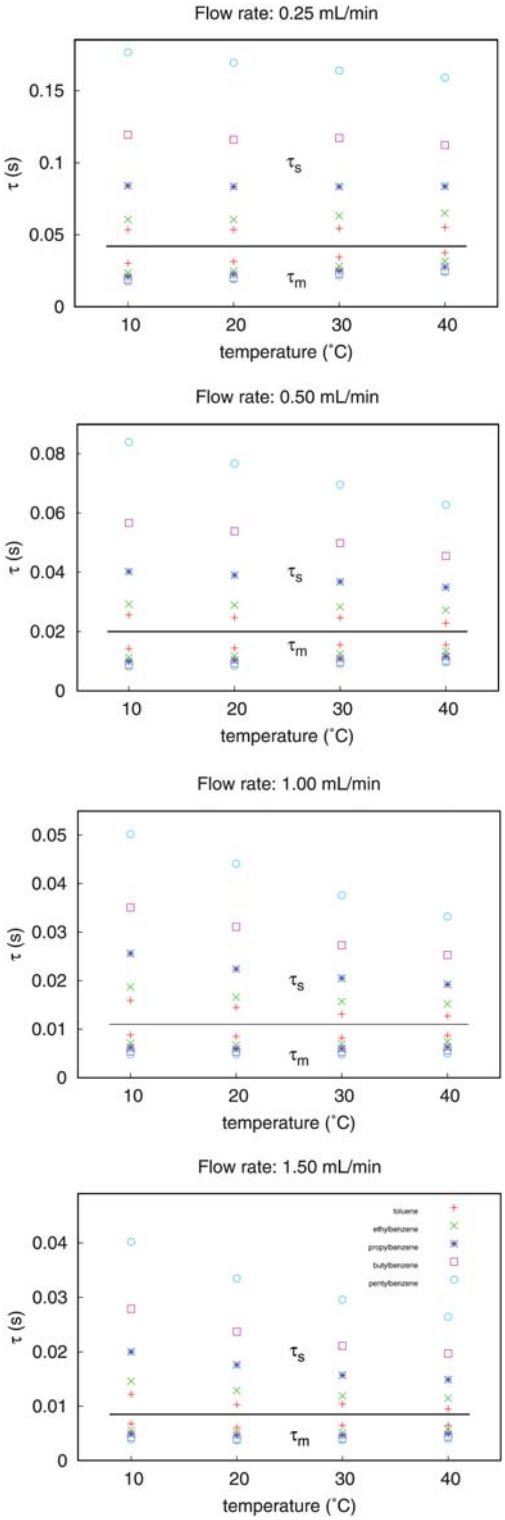

Fig. 3 Plot of the mass-transfer time constants *versus* temperature at different flow rates, calculated by stochastic model of liquid chromatography.

between $\tau_s = 57$–177 ms, and the mobile phase sojourn time is in the range of $\tau_m = 17$–33 ms for the given temperature and flow rate†. The average sojourn time in the stationary phase increases with the increase of the retention, and the average time a molecule spends in the mobile phase between leaving the stationary phase and the subsequent return there is decreasing.

Fig. 3 shows the significant decrease of the τ_s values with the increase of temperature and a slight decrease of the τ_m values with flow rate. The changes of sojourn times in the stationary phase are more significant for the strongly retained components than for the weakly retained ones. We can notice that when the flow rate is increased, the values of the average stationary phase sojourn times and the average time a molecule spends in the mobile phase are decreased.

As it is expected, the mobile phase sojourn times are independent of the solute. For one set of experimental conditions, the solute molecules show identical mobile phase sojourn times, with a very small temperature dependence. On the other hand, the stationary phase time constants strongly depend on temperature and on the retention of the solute. Since selectivity arises from the stationary phase processes, therefore this is the expected behavior. In a similar manner, the weaker retention observed at higher temperatures is due to the decrease of the τ_s time constant that characterizes the stationary phase sojourn time.

6 Conclusions

The stochastic model of chromatography offers a rather interesting view of the chromatographic process. The determination of the stationary phase sojourn times and the number of mass-transfer events gives a practical view of the molecular process of separation. From the first moment and the second central moment, the overall mass-transfer coefficients can easily be determined. All the information needed to calculate the mass-transfer coefficients is the first and second moments of the peaks. That information is easily conveyed by the data handling software of an HPLC instrument.

The analysis of a reversed phase HPLC separation shows that the typical time constants are the stationary phase residence time ranges between $\tau_s = 10$–180 ms, and the mobile phase sojourn time is in the range of $\tau_m = 5$–20 ms, depending on solute, temperature, and flow rate.

The typical numbers of mass-transfer events is in the range of $n = 15000$–25000, *i.e.* on the average, every molecule enters and leaves the stationary phase that many times during the migration along the chromatographic column. The enormous separation power of chromatography arises from the differences in the stationary phase sojourn time combined with the large number of mass-transfer events.

† The retention of toluene is the weakest, so the width of the toluene peak is mostly affected by mobile phase dispersion and not stationary phase processes. Therefore the time constants obtained for toluene are prone to error.

Acknowledgements

This work was supported by grants GVOP-3.2.1-0168, RET 008/2005, OTKA 75717, and OTKA-NKTH 68863.

References

1 G. Guiochon, A. Felinger, D. G. Shirazi and A. M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography*, Academic Press, Amsterdam, 2nd edn, 2006.
2 G. Guiochon and B. Lin, *Modeling for Preparative Chromatography*, Elsevier, Amsterdam, 2003.
3 E. Kučera, *J. Chromatogr., A*, 1965, **19**, 237–248.
4 M. Suzuki and J. M. Smith, *Adv. Chromatogr.*, 1975, **13**, 213–263.
5 A. M. Lenhoff, *J. Chromatogr., A*, 1987, **384**, 285–2990.
6 K. Miyabe and G. Guiochon, *J. Phys. Chem. B*, 2002, **106**, 8898–8909.
7 L. Lapidus and N. R. Amundson, *J. Phys. Chem.*, 1952, **56**, 984–988.
8 M. Morbidelli, A. Servida, G. Storti and S. Carrà, *Ind. Eng. Chem. Fundam.*, 1982, **21**, 123–131.
9 J. C. Giddings and H. Eyring, *J. Phys. Chem.*, 1955, **59**, 416–421.
10 J. C. Giddings, *Anal. Chem.*, 1963, **35**, 1999–2002.
11 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
12 D. A. McQuarrie, *J. Chem. Phys.*, 1963, **38**, 437–445.
13 F. Dondi and M. Remelli, *J. Phys. Chem.*, 1986, **90**, 1885–1891.
14 A. Cavazzini, M. Remelli and F. Dondi, *J. Microcolumn Sep.*, 1997, **9**, 295–302.
15 A. Cavazzini, M. Remelli, F. Dondi and A. Felinger, *Anal. Chem.*, 1999, **71**, 3453–3462.
16 G. H. Weiss, *Sep. Sci. Technol.*, 1970, **5**, 51–62.
17 G. H. Weiss, *Sep. Sci. Technol.*, 1982, **17**, 1609–1622.
18 A. Felinger, A. Cavazzini, M. Remelli and F. Dondi, *Anal. Chem.*, 1999, **71**, 4472–4479.
19 F. Dondi, A. Cavazzini, M. Remelli, A. Felinger and M. Martin, *J. Chromatogr., A*, 2002, **943**, 185–207.
20 L. Pasti, F. Dondi, M. Van Hulst, P. J. Schoenmakers, M. Martin and A. Felinger, *Chromatographia*, 2003, **57**, S171–S186.
21 A. Felinger, *Data Analysis and Signal Processing in Chromatography*, Elsevier, Amsterdam, 1998.
22 A. Felinger, *J. Chromatogr., A*, 2008, **1184**, 20–41.
23 J. H. Beynon, S. Clough, D. A. Crooks and G. R. Lester, *Trans. Faraday Soc.*, 1958, **54**, 705–714.
24 A. Felinger, A. Cavazzini and F. Dondi, *J. Chromatogr., A*, 2004, **1043**, 149.