

Molecular Movement in an HPLC Column: A Stochastic Analysis



The stochastic model of chromatography describes the separation process at the molecular level in probabilistic terms. Unfortunately, terms such as “stochastic” or “random” frighten chromatographers away from the use of this model. Nevertheless, this microscopic model is rather straightforward to comprehend, and it can furnish direct answers when one tries to understand the development of chromatographic peaks. In this study, we summarize briefly the basic concepts of the stochastic model and with an application to a simple reversed-phase high performance liquid chromatography (HPLC) separation, we show that it can be used easily to estimate the fundamental characteristics of the separation process.

The stochastic theory of chromatography is a microscopic model that probably provides the simplest picture of the chromatographic process. It describes the evolution of a chromatographic peak as the random migration of the molecules along the column combined with adsorption–desorption processes that also occur at random.

The stochastic theory of chromatography was introduced by Giddings and Eyring in 1955 (1) and then was developed further by Giddings (2). The random walk model can be used to understand several attributes of the separation process such as the contribution of different factors to zone broadening or the zone velocities in various areas of chromatography (3).

The stochastic theory of chromatography conveys very direct physical insights regarding the separation process, as it utilizes terms that are conceived easily (number of adsorption steps, average adsorption time, etc.). Accordingly, the stochastic theory can be used advantageously to understand or teach the fundamentals of the chromatographic processes.

The stochastic model of chromatography provides an attractive alternative to traditional macroscopic models, which formulate a differential mass balance equation to describe the physicochemical processes of chromatography with desired detail (4,5).

One major advantage of the stochastic model is that the heterogeneous adsorption

on the surface of the stationary phase can be considered rather easily when one works in Fourier domain via the characteristic functions (6,7). Size-exclusion chromatography also is modeled efficiently by the microscopic stochastic model (8,9).

The aim of the present study is to highlight the main features of the stochastic theory of chromatography, which deserves much more attention than it has been given. Fundamental characteristics of the separation process can be obtained with ease by some simple calculations with the stochastic model.

To enjoy the microscopic view of the chromatographic process, one must work through some probability theory; however, the vista offered is certainly worth the effort.

Theory

In a random walk of a molecule through the chromatographic bed, let τ_m designate the average time a molecule spends in the mobile phase between one desorption and the subsequent adsorption (fly-time). Let τ_s designate the average adsorption time, that is, the residence time of the molecule on the surface of the stationary phase. These time constants can be related directly to the rate constants of the adsorption (k_a) and desorption (k_d) kinetics: $\tau_m = 1/k_a$ and $\tau_s = 1/k_d$.

Figure 1 illustrates the random migration of the molecules. Once a molecule is adsorbed, it remains at the same position in the column as time passes. When it is des-

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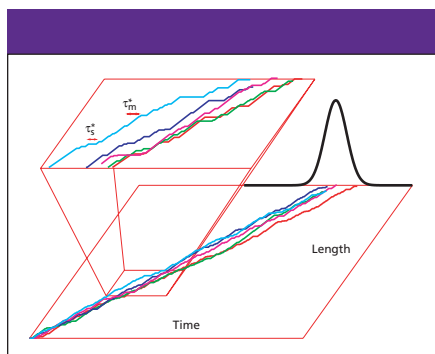


Figure 1: Chromatography as a stochastic process. The elution profile is the probability density function of the retention times of the individual molecules.

orbed, it swims with the mobile phase until it is adsorbed again on the stationary phase. As the individual values of the residence times in the stationary phase (τ_s^*) and in the mobile phase (τ_m^*) are random quantities (whose average values are τ_s and τ_m , respectively), each molecule follows a different trajectory and reaches the column outlet at a different time. Two identical molecules that entered the column at the same time soon will be found at different positions due to random influences. The elution profile is the probability density function of the retention times of the individual molecules.

During the elementary time dt , the probability of an adsorption is $\tau_m^{-1}dt$, and $\tau_s^{-1}dt$ is the probability of a desorption. In the stochastic model of Giddings and Eyring (1), each molecule spends a constant time t_0 in the mobile phase and a random time bound to the stationary phase. The probability $P_r dt$ that a molecule will undergo r adsorption–desorption steps during migration and that it will be eluted between t and $t + dt$ can be determined using the following three independent probabilities.

1. The probability of r adsorptions during t_0 is given by the following Poisson distribution:

$$\frac{(t_0/\tau_m)^r e^{-t_0/\tau_m}}{r!} \quad [1]$$

As each molecule stays for time t_0 in the mobile phase, the average number of adsorption steps is $n = t_0/\tau_m$. Therefore, we can express equation 1 as

$$\frac{n^r e^{-n}}{r!} \quad [2]$$

2. The probability of $r - 1$ desorptions during t also is given by the following Poisson distribution:

$$\frac{(t/\tau_s)^{r-1} e^{-t/\tau_s}}{(r-1)!} \quad [3]$$

3. The probability that one desorption takes place between t and $t + dt$ is $\tau_s^{-1}dt$.

Therefore, for $P_r(n, t)$ we can write

$$P_r(n, t)dt = \frac{n^r e^{-n}}{r!} \frac{(t/\tau_s)^{r-1} e^{-t/\tau_s}}{(r-1)!} \tau_s^{-1} dt \quad [4]$$

We get the probability density function $P(n, t)$ of the retention times after a summation for all the possible values of r :

$$P(n, t) = \sum_{r=0}^{\infty} P_r(n, t) = \sqrt{\frac{n}{\pi t \tau_s}} e^{-n-t/\tau_s} I_1\left(\sqrt{\frac{4nt}{\tau_s}}\right) \quad [5]$$

where I_1 is a modified Bessel function of the first kind and first order.

Alternatively, we can utilize a slightly different derivation of the retention time distribution. When the random migration of the molecule is governed by a Poisson process, the time intervals between subsequent events are characterized by exponential distributions. Thus, it is reasonable to assume that when a molecule is adsorbed in the stationary phase, the residence time of a single adsorption step is given by an exponential distribution. Furthermore, the assumption of first-order adsorption–desorption kinetics also will result in exponentially distributed residence times. The calculation can be made in the Laplace domain as well, and this latter calculation yields much simpler results. In the following, we present a parallel derivation in the time and in the Laplace domains.

The exponential distribution and its Laplace transform are given by equation 6. After r adsorption–desorption steps, the residence time distribution is given by the gamma distribution, which is the r -fold convolution of r individual exponential decay functions (see equation 7, below). When we assume that a molecule performs on the average n adsorption–desorption steps while

migrating along the column, the probability of having exactly r sorption steps is given by the Poisson distribution in equation 2. The chromatographic band profile can be obtained as the sum of the residence times of the molecules multiplied by the fraction of the molecules going through r sorption steps as indicated in equation 8. The peak shape model for both the time- and the Laplace-domain representation is demonstrated by equation 9 (see equations 6–9, below).

Note that equation 5 and the time-domain expression in equation 9 are completely equivalent. Comparing the time- and the Laplace-domain expressions of equation 9, we can see that the solution in the Laplace domain is much simpler than the time-domain solution, and it allows a very simple determination of the position, the width, and the shape of the peak. The corrected retention time (that is, the time the molecules spend adsorbed on the stationary phase) is $t'_R = n\tau_s$, the width of the peak is $\sigma = \tau_s\sqrt{2n}$, while the skew that characterizes the peak asymmetry is $S = 3/\sqrt{2n}$. The details of the calculation of these parameters are given in a separate box.

Due to the remarkable simplicity of the solution in the Laplace domain, one can model with ease complicated separation processes with heterogeneous kinetics, mobile phase dispersion, and so forth by means of the characteristic function concept (6,7,10).

When n is in the order of the common adsorption–desorption steps observable in a modern chromatographic column, equation 5 can be approximated with the following Gaussian function

$$P(t) = \frac{1}{2\tau_s\sqrt{\pi n}} \exp\left[-\frac{(t/\tau_s - n)^2}{4n}\right] \quad [10]$$

Results and Discussion

We apply the previously summarized stochastic theory to a very simple HPLC separation, the reversed-phase separation of non-

Time domain

Exponential distribution:

$$f(t) = \frac{1}{\tau_s} e^{-t/\tau_s}$$

Convolution of r distributions:

$$f_r(t) = \frac{t^{r-1} e^{-t/\tau_s}}{\tau_s^r (r-1)!}$$

Sum over r

$$P(n, t) = \sum_{r=0}^{\infty} \frac{n^r e^{-n}}{r!} \frac{t^{r-1} e^{-t/\tau_s}}{\tau_s^r (r-1)!}$$

Peak shape model:

$$P(n, t) = \sqrt{\frac{n}{\pi t \tau_s}} e^{-n-t/\tau_s} I_1\left(\sqrt{\frac{4nt}{\tau_s}}\right)$$

Laplace domain

$$f(s) = \frac{1}{1+s\tau_s} \quad [6]$$

$$f_r(s) = \frac{1}{(1+s\tau_s)^r} \quad [7]$$

$$P(n, s) = \sum_{r=0}^{\infty} \frac{n^r e^{-n}}{r!} \frac{1}{(1+s\tau_s)^r} \quad [8]$$

$$P(n, s) = \exp\left(\frac{n}{(1+s\tau_s)} - n\right) \quad [9]$$

Laplace Transform

Laplace transform is applied often in the study of partial differential equations, signals, and other engineering problems. The Laplace transform of a function $f(t)$ is defined as

$$F(s) = L\{f(t)\} = \int_0^{\infty} f(t)e^{-st} dt$$

where s is a complex variable.

Several transform properties, such as linearity, scaling, shifting, convolution, differentiation, and integration, are defined for Laplace transform in a manner very similar to Fourier transform (11).

Here it is sufficient to recall the moment property that allows the simple calculation of the first statistical moments of $f(t)$ about the origin and the higher order statistical moments about the mean:

$$\mu_k = (-1)^k \lim_{s \rightarrow 0} \frac{d^k \ln F(s)}{ds^k}$$

When we write the Laplace transform of the peak shape model (equation 9) into the this equation, we have $\ln F(s) = -n\tau_s/(1 + s\tau_s)$ and

$$\frac{d^k \ln F(s)}{ds^k} = (-1)^k \frac{k! n \tau_s^k}{(1 + s\tau_s)^{k+1}}$$

Thus, we get a general expression for the moments as

$$\mu_k = k! n \tau_s^k$$

We obtain the corrected retention time as $t'_R = \mu_1 = n\tau_s$, the peak width as $\sigma = \sqrt{\mu_2} = \tau_s \sqrt{2n}$ and the asymmetry (skew) as $S = \mu_3/\mu_2^{3/2} = 3/\sqrt{2n}$.

The addition of independent random variables results in an often complex convolution process, which simplifies to multiplication in the Laplace or Fourier domain, offering an attractive alternative to time-domain calculations.

ionizable neutral compounds on a C18 silica-based stationary phase. Figure 2 shows the measured chromatogram. Because the model we have been discussing here concerns only the stationary phase process, we have to eliminate the effects of mobile-phase dispersion and extracolumn broadening from the peaks. To do so, we take the peak of the unretained marker — whose retention time, peak width, and shape are affected only by the mobile phase and extra-

Poisson Distribution

The Poisson distribution describes the probability distribution of the number of independent events found in a limited interval. The probability P_k of finding exactly k points during the time interval of length t is

$$P_k = e^{-\lambda t} \frac{(\lambda t)^k}{k!}$$

where λ determines the density of points along the time axis. λ should not be of an order of magnitude greater than 1; that is, the events should be rare in one interval unit. For instance, we assume that a rather small sample containing only 100 molecules was injected on a chromatographic column that contains a large enough number of adsorption sites so that the behavior of the molecules is independent. Let us take $\tau_s = 20$ ms for the average residence time in the adsorbed state. In this case, $\lambda = 100/20 = 5 \text{ ms}^{-1}$ (that is, on the average, five desorption events will occur during 1 ms). The probability that exactly three desorption events will occur during that time is

$$P_3 = e^{-5} \frac{5^3}{3!} \approx 0.14$$

The probability of no desorption occurring during one millisecond is $P_0 = e^{-\lambda t} = e^{-5} \approx 0.0067$.

column processes — and calculate the deconvolution of the whole chromatogram and the unretained peak (11). The resulting net chromatogram (plotted in Figure 2b) carries only the contribution of the stationary-phase process, so it can be used for our analysis.

To get an estimate of the number of sorption steps and the residence times in the mobile and stationary phases, we fit equation 10 to the peaks in the net chromatogram. The results of the fitting are tabulated in Table I. As analytes 1 and 2 are retained weakly, they spend less time in the stationary phase than in the mobile phase. After deconvolution, their peaks cannot be used for precise parameter estimation, so only data for analytes 3–8 are tabulated.

It is interesting to note that neither the number of sorption steps nor the fly-time in the mobile phase are affected strongly by the retention of the analyte. The typical value of the number of sorption steps is between

13,000 and 20,000, whereas the fly-times vary roughly from 3 to 5 ms. The residence time in the stationary phase, however, strongly correlates with the retention of the analytes, so selectivity of the separation and retention is due primarily to the variation of τ_s .

The HPLC column ($L = 15$ cm and $d = 0.39$ cm) used in the experiment is packed with $d_p = 5 \mu\text{m}$ spherical particles. Thus, the total column volume is 1.8 mL and the volume of a single particle is $V_p = 6.5 \times 10^{-11}$ mL. When we pack a column with spherical particles, it is reasonable to assume that the external porosity is in the range of 0.35–0.4 (that is, the spheres fill about 60–65% of the volume available). Therefore, the number of particles packed in the column is about 1.8×10^{10} .

When this column is packed with a porous C18 stationary phase and the flow rate is 1.0 mL/min, the unretained marker is eluted at $t_0 = 62.3$ s. This indicates the linear mobile-phase velocity $u = 0.24$ cm/s.

We can express the average fly-time of the molecules between a desorption and the subsequent adsorption as the ratio of the void time and the number of sorption steps: $\tau_m = t_0/n$. Results summarized in Table I show that $\tau_m = 3$ –5 ms. During that fly-time, the mobile phase travels $l = u\tau_m = 7.2$ –12 μm , a distance that is 1.5–2.3 times that of the particle diameter.

At the current linear velocity, the mobile phase travels the distance of d_p in $\tau_{m,d} = 2.1$ ms. Because fly-times follow an exponential distribution, with a simple integration of

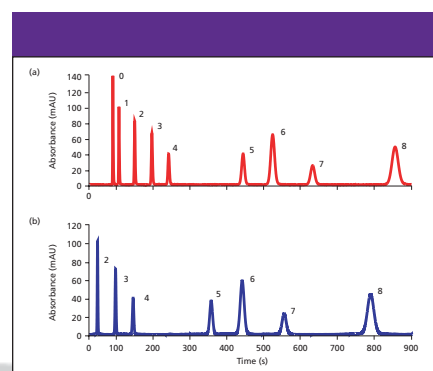


Figure 2: Chromatograms obtained from (a) reversed-phase liquid chromatography separation of neutral compounds and (b) after the deconvolution of the original chromatogram with the peak of the unretained marker (peak 0). Column: 150 mm \times 3.9 mm, 5- μm d_p Symmetry C18; mobile phase: 80:20 (v/v) methanol-water; flow rate: 1 mL/min; temperature: 25.0 $^{\circ}\text{C}$; detection: UV absorbance at 254 nm. Peaks: 0 = thiourea, 1 = phenol, 2 = 1-chloro-4-nitrobenzene, 3 = toluene, 4 = ethylbenzene, 5 = butylbenzene, 6 = *o*-terphenyl, 7 = amylbenzene, 8 = triphenylene.

Table 1: Results of fitting equation 10 to the peaks of the net chromatogram

peak	n	τ_s (ms)	τ_m (ms)	$u\tau_m/d_p$
3	12,965	8.42	4.81	2.31
4	14,335	10.88	4.34	2.08
5	18,328	19.88	3.40	1.63
6	14,606	30.61	4.26	2.05
7	19,864	28.15	3.13	1.50
8	16,710	47.27	3.73	1.79

the density function, we can calculate the fraction of fly-times that are shorter than needed to travel a distance longer than a particle diameter.

$$P(l < d_p) = \int_0^{\tau_{m,d}} \frac{1}{\tau_m} e^{-t/\tau_m} dt = 1 - e^{-\tau_{m,d}/\tau_m} \quad [11]$$

If τ_m is 3 ms, the previous integration yields 0.5. Accordingly, in about half of the adsorption steps, the molecule returns to the particle just visited, and the total number of particles visited by one molecule is roughly one half of the sorption steps.

This simple model does not consider that there are pores with stagnant mobile phase, and that once a molecule is trapped in such a pore, it will adsorb and desorb several times before leaving that pore. This phenomenon will further reduce the number of particles visited by a single molecule.

If $d_p = 5 \mu\text{m}$ particles were put along a line, one would need 30,000 particles to fill the 15-cm length of the column. Notice that this number is just in the order of magnitude of the number of adsorption steps and surely is greater than the number of particles visited by a molecule.

The specific surface area of the stationary phase is $344 \text{ m}^2/\text{g}$, and the surface coverage is $3.2 \mu\text{mol}/\text{m}^2$. The total porosity is 65%, and the skeleton density of the stationary phase is $1.82 \text{ g}/\text{cm}^3$ (12). These numbers indicate that the amount of C18 ligands in the column is 1.26 mmol; that is, there are 7.6×10^{20} ligands on the surface. Considering the number of particles, this gives 4.2×10^{10} ligands per particle.

If we compare the number of sorption steps and the number of particles in the column, we see that one molecule visits only a tiny fraction of particles.

In the previous example, only one out of every two million particles is visited by a single molecule. An injected sample might contain a few nanomoles of analyte; that is, the number of molecules injected is in the order of 10^{15} , a number that is roughly a million times smaller than the total number

of ligands in the column and surely larger than the number of particles.

Conclusions

The stochastic model provides an attractive means to evaluate the details of the separation process. With rather simple calculations, one can assess the kinetics of the adsorption-desorption phenomena and get a view of the chromatographic process at the molecular level.

For the sake of simplicity, only homogeneous adsorption kinetics are discussed here. The model, however, is capable of explaining peak tailing often observed due to heterogeneous kinetics, such as in the case of reversed-phase liquid chromatography separation of basic analytes or chiral separation of enantiomers (7,13).

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References

- (1) J.C. Giddings, and H.J. Eyring, *Phys. Chem.* **59**, 416–421 (1955).
- (2) J.C. Giddings, *Dynamics of Chromatography* (Marcel Dekker, New York, 1965).
- (3) J.C. Giddings, *Unified Separation Science* (John Wiley & Sons, Hoboken, New Jersey, 1991).
- (4) L. Lapidus and N.R. Amundson, *J. Phys. Chem.* **56**, 984–988 (1952).
- (5) G. Guiochon, S.G. Shirazi, and A.M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography* (Academic Press, Boston, Massachusetts, 1994).
- (6) A. Cavazzini, M. Remelli, F. Dondi, and A. Felinger, *Anal. Chem.* **71**, 3453–3462 (1999).
- (7) A. Felinger, A. Cavazzini, M. Remelli, and F. Dondi, *Anal. Chem.* **71**, 4472–4479 (1999).
- (8) F. Dondi, A. Cavazzini, M. Remelli, A. Felinger, and M. Martin, *J. Chromatogr., A* **943**, 185–207 (2002).
- (9) L. Pasti, F. Dondi, M. Van Hulst, P. Schoenmakers, M. Martin, and A. Felinger, *Chromatographia* **57**, S171–S186 (2003).
- (10) F. Dondi and M.J. Remelli, *Phys. Chem.* **90**, 1885–1891 (1986).
- (11) A. Felinger, *Data Analysis and Signal Processing in Chromatography* (Elsevier: Amsterdam, The Netherlands, 1998).
- (12) M. Kele and G. Guiochon, *J. Chromatogr., A* **830**, 41–54 (1999).
- (13) P. Jandera, V. Bačková, and A. Felinger, *J. Chromatogr., A* **919**, 67–77 (2001). ■