Monte Carlo Model of Nonlinear Chromatography: Correspondence between the Microscopic Stochastic Model and the Macroscopic Thomas Kinetic Model

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The Monte Carlo model of chromatography is a description of the chromatographic process from a molecular (microscopic) point of view and it is intrinsically based on the stochastic theory of chromatography originally proposed by Giddings and Eyring. The program was previously validated at infinite dilution (i.e., in linear conditions) by some of the authors of the present paper. In this work, it has been further validated under nonlinear conditions. The correspondence between the Monte Carlo model and the well-known Thomas kinetic model (macroscopic model), for which closed-form solutions are available, is demonstrated by comparing Monte Carlo simulations, performed at different loading factors, with the numerical solutions of the Thomas model calculated under the same conditions. In all the cases investigated, the agreement between Monte Carlo simulations and Thomas model results is very satisfactory. Additionally, the exact correspondence between the Thomas kinetic model and Giddings model, when near-infinite dilution conditions are approached, has been demonstrated by calculating the limit of the Thomas model when the loading factor goes to zero. The model was also validated under limit conditions, corresponding to cases of very slow adsorption–desorption kinetics or very short columns. Different hypotheses about the statistical distributions of the random variables “residence time spent by the molecule in mobile and stationary phase” are investigated with the aim to explain their effect on the peak shape and on the efficiency of the separation.

Chromatograms are the result of many very complex physicochemical processes. The processes involved are so complicated and numerous that is impossible, in almost all the real cases, to obtain an analytical function that describes the concentration profile at the exit of the column. The most important theories used to describe the chromatographic process are the mass balance and the statistical models.¹

Mass balance models (or macroscopic models) are the most popular ones. They can be divided in two major categories: equilibrium and kinetic. In mass balance models, the distribution of the solute between mobile and stationary phases is described by using one or more partial differential equations, whose integration—under proper initial and boundary conditions, and when an isotherm model or a kinetic expression are defined, gives the band profile.

On the other hand, statistical (or stochastic or microscopic) models approach the problem in a completely different fashion. They consider the behavior of a single molecule in the chromatographic column as a random sequence of “jumps” between mobile and stationary phase. Probability laws determine the frequency and the length of the jumps, and the chromatographic peak is obtained when a large number of molecules is collected at the exit of the column.

Two types of phenomena control the profiles of chromatographic bands: the nonlinearity of the adsorption isotherm and the finite rate of the mass-transfer kinetics (i.e., in general, the rate at which components move between the two phases of the chromatographic system).² The former, which is a pure thermodynamic phenomenon, explains why different concentrations migrate at different velocities in the column, resulting in the characteristic tailed profiles observed in nonlinear chromatography. It was fully explained by using the so-called ideal model of chromatography, where the efficiency of the column is assumed to be infinite.³ The latter includes all the nonthermodynamic contributions to band broadening, which are as follows: molecular diffusion, eddy diffusion, mass-transfer resistance, and finite rate of the kinetics of adsorption–desorption.¹⁻³ They dampen the concentration shock predicted by the ideal model. In many cases, especially with the modern chromatographic columns, mass-transfer phenomena are fast. This explains the wide success of the equilibrium dispersive model of chromatography in accounting for many real separations.¹ There are cases, however, where slow mass-transfer or retention mechanism kinetics cannot be neglected. For instance, this is often the case in the separation of biopolymers in bioaffinity chromatography⁴⁻⁶ and the separation

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of chiral compounds on protein stationary phases\(^7,8\) and on imprinted stationary phases.\(^9\) In all these cases, it is well known that the efficiency of the separation is poor. A number of kinetic models, able to account for the different kinetic aspects, have been accordingly introduced, in both linear and nonlinear chromatography.

The assumption of a linear isotherm function enormously reduces the mathematical complexity of the models. From a physical point of view, this means that no competition for the adsorption exists between molecules (near-infinite dilution conditions). Closed-form solutions in Laplace\(^{10-12}\) and Fourier\(^{13-17}\) domains were obtained for this case. The effect on the peak shape of slow adsorption–desorption kinetics, the adsorption on heterogeneous surfaces, and the outset of split peak phenomenon under specific conditions are now well-understood phenomena in linear conditions.\(^{15,16,18-23}\) Additionally, the equivalence between mass balance models and stochastic models was demonstrated when appropriate hypotheses were formulated.\(^{15}\)

In nonlinear chromatography, the situation is much more complex. The competition between molecules for the adsorption dramatically complicates the problem. No solution in closed form exists for mass balance models when the isotherm is nonlinear, except in some very simplified cases.\(^1\) However, numerical calculations allow the resolution of the system of the differential mass balance equations describing the model and, consequently, the achievement of the peak profile. Furthermore, no rigorous microscopic models of nonlinear chromatography are available.

In recent years, simulation techniques have become increasingly more popular in the field of separation science. Modern PCs allow an elevated computational power at very low cost. Simulations are used not only for the optimization and scaling-up of productive processes\(^1\) but also to investigate the physicochemical phenomena that constitute the true essence of the chromatographic separation process: phase distribution and equilibria, flow hydrodynamics, and kinetics of phase transfer process.\(^{25-27}\)

Recently, Dondi et al.\(^28\) proposed a Monte Carlo (MC) model of nonlinear chromatography. The validation of the model was performed, under linear conditions, by comparing the results of the MC simulation with the results expected from the stochastic theory of chromatography, on which the model is intrinsically based. Some preliminary simulations, showing the onset of nonlinearity when the amount of the sample injected in the column increases, were also performed. The simulation program behaves as a virtual chromatograph, allowing the operator to choose the different experimental conditions: mobile-phase velocity, length of the column, number of molecules injected, frequency of data acquisition, etc. Potentially, it is able to face important aspects of chromatographic science, such as multsite adsorption, longitudinal diffusion, flow pattern effects, nonimpulsive injection profile, and structured heterogeneous stationary phases. Additionally, it appears to be a proper tool to investigate real-time dynamics of single-molecule separation chromatography, where just a few molecules (usually large biopolymers) are injected in the chromatographic column.\(^{29-32}\)

The purpose of this work is to further investigate the potentiality of the MC approach and to compare it with the correspondent macroscopic models, commonly accepted in nonlinear chromatography.

THEORY: MASS BALANCE AND STOCHASTIC MODELS

1. Mass Balance Approach. The complete derivation of the mass balance equation that describes the accumulation of materials due to convection and diffusion in a thin slice of the column can be found in many places.\(^{1,33}\)

\[
\frac{\partial C}{\partial t} + F \frac{\partial q}{\partial t} + u \frac{\partial C}{\partial z} = D_a \frac{\partial^2 C}{\partial z^2} \tag{1}
\]

In eq 1, \(C\) and \(q\) are respectively the mobile- and stationary-phase concentrations, \(t\) is the time, \(z\) is the column length, \(u\) is the mobile-phase velocity, \(D_a\) is the axial dispersion coefficient, and \(F\) is the phase ratio defined by \((1 - e)/e\), \(e\) being the total porosity of the column.

a. Equilibrium Dispersive Model. In the equilibrium dispersive model, eq 1 is used with an isotherm expression that relates \(q\) to \(C\). An apparent axial dispersion coefficient, evaluated under linear conditions, accounts for the finite rate of mass-transfer kinetics.\(^1\) It has been shown that the equilibrium dispersive model is correct if mass transfer in the chromatographic column is controlled only by molecular diffusion across the mobile phase flowing around the packing particles and if the exchange of eluates

(35) Giddings, J. C. Gas Chromatography; The Institute of Petroleum, 1965; p 3.
between the stationary and the mobile phases is very fast. From a practical point of view, several hundreds of theoretical plates are in many cases sufficient to successfully apply this model.1

b. Kinetic Model. Kinetic models use a kinetic expression (and, in some cases, even an isotherm model)1 together with the mass balance equation to describe the chromatographic separation. The kinetic equation relates the rate of variation of the concentration of each component in stationary phase with its concentration in mobile and stationary phases and with the rate constants of adsorption and desorption. The different kinetic models have been summarized by Ruthven34 and, more recently, by Guiochon.1

Two major categories of kinetic models can be defined: the first comprehends the so-called lumped kinetic models, the second one, the general rate model. In lumped kinetic models, all the contributions of the mass-transfer resistances are included in a single kinetic equation, using a “lumped” kinetic constant. For instance, the reaction dispersive models attribute nonequilibrium state to the slow adsorption—desorption process;2,3 while the transport dispersive models assume that the adsorption—desorption kinetic is fast but the mass-transfer phenomena are slow.3,39–41

The general rate theory of chromatography is the most complete. Obviously the complexity of the model increases markedly in this case, as well as the number of parameters used to describe the separation; some of them cannot be easily measured experimentally, and their physical meaning is sometimes a matter of opinion.42 The model independently accounts for all the kinetic phenomena affecting the separation: adsorption—desorption mechanism at the actual sites, axial dispersion, and all mass-transfer resistances, i.e., the external mass transfer of the solute molecules from the bulk phase to the external surface of the adsorbent particles and the diffusive transport through the pores of the adsorbent material.1,34,43

The conditions under which the different models hold and can be applied have been extensively studied.1,34,44

**Thomas Kinetic Model.** The combination of eq 1 and a second-order Langmuir kinetics, expressed by

\[ \frac{dq}{dt} = k'qC(q_s - q) - kdq \]  

(2)

constitutes the so-called Langmuir kinetic model.1,45 In eq 2, \( k' \) (dimensionally, \( (\text{time}^{-1} \cdot \text{concentration}^{-1}) \)) and \( kd \) (\( (\text{time}^{-1}) \)) are the adsorption and desorption rate constants, while \( q_s \) is the stationary-phase saturation capacity.1 At the equilibrium, the rate of adsorption and desorption are equal, \( \frac{dq}{dt} = 0 \), and eq 2 leads to the Langmuir model for the equilibrium isotherm.

In 1944, Thomas obtained an analytical solution for this model in the instances in which the axial dispersion was assumed to be negligibly small (i.e., \( D_z = 0 \) in eq 1) and for a step function input (frontal analysis chromatography).43 This model, called Thomas model, was later solved in closed form by Goldstein for the case of rectangular pulse injection46 and by Wade et al. for an infinitely narrow impulse injection (Dirac problem).45

Under this last hypothesis, the following equation was derived for the concentration profile at the column outlet:

\[ C_C(t) = \frac{1}{L_I \sqrt{t}} \left( 1 - \frac{1}{(1 - e^{-L_I N_{rea}})T(N_{rea}, N_{rea}t)} \right)^\frac{1}{2} \]  

(3)

where \( L_I \) is the first-order modified Bessel function and \( T(u, v) \) is a Bessel function integral.40 \( T(u, v) = e^{-\sqrt{u}} \int_0^1 e^{-\sqrt{v}y} dy \) \( (2\sqrt{vt})^{\frac{3}{2}} \) \( dt \), \( C_{eq,0} \) is the concentration of solute injected multiplied by the width of the injection pulse (as a fraction of column dead volume), \( L_I \) is the column loading factor, \( N_{rea} \) is the number of mass-transfer units, and \( t_r \), the “dimensionless time”, is defined by

\[ t_r = (t' - t_{h0}) / (t_k - t_{h0}) \]  

(4)

where \( t' \) is the time elapsed from the injection of the molecules in the column, \( t_{h0} \) the holdup time, and \( t_k \) the retention time at infinite dilution. The quantity \( (t_k - t_{h0}) \) represents the so-called corrected retention time (constant), while the numerator of eq 4 is, dimensionally, a time spent in stationary phase (independent variable). As expected by considering that the amount of material injected in the column has to be equal to the amount eluted, the integral of the RHS term of eq 3, evaluated from zero to infinity, is equal to 1. Equation 3 can be then considered to as a probability density function: the area under the peak—included between \( t_1 \) and \( t_2 \)—represents the fraction of the sample eluted between \( t_1 \) and \( t_2 \) (or the probability, \( \text{Prob}(t_1 \leq t \leq t_2) \)).

\[ \text{Prob}(t_1 \leq t \leq t_2) = \frac{1}{C_{eq,0}} \int_{t_1}^{t_2} f(t) \, dt \]  

(5)

where the symbol \( f(t) \)—to replace the original notation used by Thomas, \( C/C_{eq,0} \)—has been introduced for reasons that will be clear later on.

2. Stochastic Models. a. Linear Stochastic Models. Stochastic models are kinetic models. Since the pioneering works done by Giddings and Eyring in 1955,18 Giddings in 1957,49 and McQuarrie in 1963,12 significant improvements have been made in this field, with thanks also to more modern statistical tools.1,40,45 Stochastic models to describe adsorption on homogeneous and heterogeneous surfaces or size exclusion chromatography are available today.13–16,20,24 From a stochastic point of view, the
migration of the sample molecules in the column is described as a chain of ingress—egress random processes, i.e., state exchanges of the molecules between the mobile and the stationary phases. For the purposes of this paper, only a few remarks about the most important aspects and results of the stochastic model of chromatography will be emphasized, while for a full characterization and description of the stochastic theory, the reader is invited to refer to the specific references reported above.

The time spent by a molecule in the column is the sum of two independent and random contributions: the time spent in mobile phase and the time spent in stationary phase. The random nature of these macroscopic quantities (time spent in mobile and stationary phases) can be better understood when they are expressed in terms of the microscopic physical processes from which they are derived. The amount of time spent in the stationary phase is the sum—calculated on the number n of the times the molecule stops in the stationary phase—of the times, \( t_s \), of each adsorption:

\[
t_s = \sum_{i=1}^{n} t_{sj}
\]

In the same way, the time spent in the mobile phase (macroscopic quantity) is given, for each molecule, by the sum of all the microscopic contributions—random times, \( t_m \), spent by the molecule in the mobile phase between one adsorption and the following one—up to the elution of the molecule from the column. All the microscopic quantities \( t_s \), \( t_m \), and \( n \) are random variables. In principle, any kind of probabilistic law can be used as a distribution for these random variables, and their effect on the peak shape can be easily evaluated by using the stochastic theory.\(^{15,14,55}\)

A molecule adsorbed in the stationary phase continuously exchanges energy with the surface.\(^{56}\) It is released by the surface only when, after a random time, its desorption energy overcomes the adsorption energy. As discussed in specific literature (see, for instance, ref 57), a “waiting time” stochastic process—as is the adsorption of a molecule in the site—can be described, in first approximation, by an exponential distribution;\(^{33,57,58}\)

\[
f(t_s) = \frac{1}{\bar{t}_s} e^{-t_s/\bar{t}_s}
\]

where \( \bar{t}_s \) represents the average value of the distribution, related to the adsorption rate constant, \( k_s \), by

\[
\bar{t}_s = \frac{1}{k_s}
\]

In principle, any kind of probabilistic law can be used as a distribution for these random variables, and their effect on the peak shape can be easily evaluated by using the stochastic theory.\(^{15,14,55}\)

Although in many real cases this assumption is too simplified,\(^{59}\) it can be reasonably taken as a first approximation for linear chromatography.\(^{21,33,57}\)

In mobile phase, the situation is more complex. Here, the collisions between the solute molecule (Brownian motion) and the solvent combine with the effects of flow patterns, eddy diffusion, longitudinal diffusion, etc., determining the random nature of the “time-of-flight” process. The characterization and the description of the stochastic behavior of a molecule in mobile phase is a very difficult topic.\(^{40}\) Different degrees of simplification can be introduced;\(^{14,55}\) but in some cases,\(^{18}\) the random nature of the entry process (i.e., the probabilistic distribution used to describe \( n \)) “imposes” the distribution for \( t_m \). It is well known, for instance, that in the instances in which \( n \) is Poissonian, the distribution of the fly times is exponential,\(^{33,57}\) i.e.,

\[
f(t_m) = \frac{1}{t_m} e^{-t_m/t_m}
\]

where the average value, \( \bar{t}_m \), is related to the adsorption rate constant, \( k_s \), \( (k_s = k_s^\prime q_s = (\text{time})^{-1}), \) by

\[
\bar{t}_m = \frac{1}{k_s^\prime}
\]

The chromatographic peak in elution mode is obtained when a very high number of molecules, each of which behaves randomly, is collected at the exit of the column.\(^{34}\) It is usually characterized by means of the first and second statistical moments. From these, the other fundamental quantities used by chromatographers such as the retention factor and the column efficiency can be easily evaluated. The stochastic theory of chromatography gives a very clear and simple interpretation of these macroscopic quantities in terms of the above-described microscopic quantities \( t_s \), \( t_m \), and \( n \).\(^{21,32}\) The average time spent in stationary phase—i.e., the corrected retention time—can be expressed as the product between the mean number of adsorption—desorption steps performed by the molecules in the column, \( n \), and the meantime spent in each adsorption—desorption process, i.e.,

\[
\bar{t}_s = \bar{t}_s n
\]

The time spent in mobile phase, similarly, is expressed as

\[
\bar{t}_m = \bar{t}_m n
\]

Then the retention factor, \( k' \), is shown to be simply equal to the ratio between \( \bar{t}_s \) and \( \bar{t}_m \):

\[
k' = \frac{\bar{t}_s}{\bar{t}_m}
\]

and the efficiency of the column—expressed by means of the number of theoretical plates, \( N \)—is related to the mean number of molecules adsorbed in the column, \( n \), and the mean time per molecule spent in adsorption (i.e., the mean number of adsorption—desorption steps performed by each molecule)

\[
N = \frac{1}{k_s^\prime}
\]
of adsorption—desorption steps and to the retention factor (or to \( \tau_s \) and \( \tau_m \), through eq 13) by the following relationships: \(^{15}\)

\[
N = \frac{n'(k' + 1)^2}{2(k' - 1)^2} = \frac{n'(\tau_s + \tau_m)^2}{2(\tau_s - \tau_m)^2}
\] (14)

b. Simulation Approach. All the equations reported above (eqs 11–14) were obtained and hold only under the hypothesis of linear adsorption isotherm. On the other hand, the mathematical difficulty of handling nonindependent random variables is well known. This is the case for nonlinear chromatography where molecules compete for the adsorption and the retention time becomes a function of the concentration injected.

In this case, simulation techniques represent an important resource to model nonlinearity effects from a molecular point of view.\(^{25,28,61}\) A dynamic model of chromatography was recently proposed and validated under linear conditions.\(^{29}\) In the simulation program, a large number of molecules can be simultaneously injected into the column. This is depicted as a monodimensional grid of adsorption—desorption sites. For each molecule injected, the generation of a sequence of random “flight times” and random “stationary times”\(^{62,63}\) is required. The “stochastic” history of the molecule in the column is defined by the sequence of adsorption—desorption steps performed by the molecule before being eluted.

At the beginning of the simulation, two random numbers are associated with each molecule. One of them is used for the generation of the first random flight, while the second is used for the generation of the first stationary time. Then, the program generates by itself and associates with the molecule other random numbers each time the molecule stops in the stationary phase (for the generation of the actual stationary time), and when the molecule is ready to leave, after the random stationary time previously generated has elapsed, for the generation of the actual flight time.

The adsorption sites distributed along the column can allocate only a finite number of molecules, which can be modified in any simulation. As is usually done in nonlinear chromatography, the loading factor is the parameter used to quantify the degree of nonlinearity associated with a specific chromatographic run. In the case of the MC simulation, this is defined by\(^{28}\)

\[
L_f = N_{\text{mol}}/N_{\text{sites}}\text{CAP}
\] (15)

where \( N_{\text{mol}} \) indicates the number of molecules injected into the column, \( N_{\text{sites}} \) the number of sites in the column, and CAP the maximum number of molecules allocable in one site (capacity of the site). In this work, the CAP value was always assumed to be equal to 1 that means that a Langmuirian microscopic model of adsorption is assumed.\(^{56}\)

To simulate the very simplified case of Langmuir microscopic adsorption (i.e., the case in which an adsorption site can accept a molecule only if empty), a weighting factor was used to multiply the probability density function generating \( r_s \) (eq 7). Its value can be only equal to 1 (site empty) or to 0 (site full). In the former case, the generation of a random stationary time will be produced according to eq 7; in the latter, the molecule will not have the opportunity to access the site and it will be rejected back to the mobile phase.

The state of the system is continuously monitored and upgraded each time a molecule moves, and the collection of the molecules at the exit of the column generates the peak profile.

**RESULTS AND DISCUSSION**

1. Equivalence between Thomas Kinetic Model and Giddings–Eyring Stochastic Model in Linear Chromatography. As is well understood,\(^{1,33}\) the Thomas kinetic model\(^{45}\) and the original Giddings–Eyring stochastic model\(^{19}\) were derived from an identical set of assumptions: the same kinetic equation and hypothesis of negligible dispersion in the mobile phase. Expressed in terms of stochastic theory, the second assumption means that the time spent in mobile phase, \( t_M \) (eq 12), is a constant quantity for each molecule,\(^{14–16,55}\) The contribution of \( t_M \) to the total time spent in a chromatographic column of length \( L \) can be then simply accounted for by a shift of the position of the peak that accounts only for the time spent by the molecules in the stationary phase. The entity of the shift is\(^{35,28,32}\)

\[
t_M = \frac{L}{u} = \frac{\bar{n} \times \bar{\tau}_m}{\text{macroscopic model}}
\] (16)

As noted before, the dimensionless parameter \( \tau \) of Thomas model (eq 4) represents the ratio between the time spent in stationary phase

\[
t' - t_M = \frac{\sum_{1}^{n} \tau_{s,i}}{N_{\text{sites}} \times \bar{\tau}_s} = \frac{\tau_s}{\text{microscopic model}}
\] (17)

and the corrected retention time

\[
\frac{t_R - t_M}{t_M} = \frac{\sum_{1}^{n} \tau_{s,i}}{N_{\text{sites}} \times \bar{\tau}_s} = \frac{\tau_s}{\text{microscopic model}}
\] (18)

and can be then written as

\[
\tau = \frac{t' - t_M}{t_R - t_M} = \frac{\sum_{1}^{n} \tau_{s,i}}{N_{\text{sites}} \times \bar{\tau}_s} = \frac{\tau_s}{\frac{\bar{n}}{N_{\text{sites}}} \times \bar{\tau}_s}
\] (19)


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Additionally, the number of mass-transfer units, \(N_{rea}\), appearing in Thomas equation (eq 3), corresponds to the mean number of adsorption-desorption steps (\(n\) in the stochastic model) as previously demonstrated:\(^{15}\)

\[
N_{rea} = n
\]  

(20)

In light of these correspondences—and by keeping the unit area (see below)—eq 3 can be rewritten by using the proper language of stochastic theory as

\[
f(t_s, L_s) = \frac{(1 - e^{-L_s})e^{-(n + 1)/t_s}}{L_s\sqrt{n}} \ln(2n\sqrt{t_f/(n\sqrt{t_s})})
\]

\[(21)\]

where the dependence on the microscopic parameters \(n\) and \(t_s\) is now emphasized. \(f(t_s, L_s)\) represents the chromatographic peak expressed as a function of the time spent in the stationary phase: in the stochastic language, it is the probability density function of the time spent by the molecules in the stationary phase.\(^{18}\) One last consideration is needed for obtaining eq 21 from eq 3. It regards the area value of the functions (eq 3 and eq 21) when the change of the independent variables—in terms of which they are expressed (respectively, \(r\) and \(t_s\))—is considered. Because both eq 3 and eq 21 can be considered probability density functions, the change of the independent variable, when going from eq 3 to eq 21, requires the imposition of the following condition:\(^{65}\)

\[
f(t_s, L_s) = f'(\tau, L_s) \frac{dr}{dt_s} = f'(\tau, L_t) \frac{1}{n\tau_s}
\]

(22)

where the second equality is straightforwardly derived from eq 19. In other words, eq 3 has to be multiplied by the factor \(1/(n\tau_s)\) in order to keep the area of eq 21 unitary. Equation 21 represents another form of the analytical solution of the Thomas model, and it can be numerically solved as well (see below).

When the loading factor value tends to zero, the conditions of infinite dilutions are approached. Under these conditions, the convergence between Thomas model and the Giddings–Eyring model is expected. This can be easily demonstrated by using symbolic Mathematica packages\(^{64}\) when the limit of eq 21, for \(L_t \to 0\), is calculated:

\[
\lim_{L_t \to 0} f(t_s, L_s) = e^{-t_s + n}\sqrt{\frac{n}{t_s}}
\]

(23)

Equation 23 corresponds to the well-known Giddings–Eyring equation.\(^{18}\)

2. Correspondence between Thomas Kinetic Model and Monte Carlo Approach in Nonlinear Chromatography. The MC model described above is based on the stochastic description of the chromatographic process originally proposed by Giddings and Eyring. However, its ability to simultaneously account for a large number of molecules allows the study of nonlinearity effects.\(^{28}\) When the contribution of the dispersion in the mobile phase is negligible, it should then correspond to the Thomas model, while when the loading factor value goes to zero, it represents the Giddings–Eyring model of linear chromatography (eq 23).

Figure 1 shows the results of several MC simulations at different loading factors (\(L_t\) ranging from 2.5 to 25%). The number of molecules eluted from the column is reported as a function of the time spent in the stationary phase (expressed in \(\mu s\)).

The number of molecules used in each simulation is listed in Table 1, first column.

![Figure 1](image1.png)

**Figure 1.** Validation of the MC microscopic model with the Thomas macroscopic kinetic model, at different loading factor values. Points: results of the MC simulations. Continuous lines: numerical solutions of the Thomas model, calculated using both the procedure described in ref 38 and Mathematica standard subroutines. The parameters used in the simulation are listed in Table 1, first column.

**Table 1. Parameters Used in the Monte Carlo Simulations**

<table>
<thead>
<tr>
<th>parameters</th>
<th>Figure 1</th>
<th>Figure 2</th>
<th>Figure 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L) ((\mu m))</td>
<td>2000</td>
<td>150</td>
<td>2000</td>
</tr>
<tr>
<td>(u) ((\mu m/\mu s))</td>
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<td>1.0</td>
<td>0.2</td>
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<tr>
<td>(N_{sites})</td>
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<td>CAP</td>
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<td>1</td>
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<tr>
<td>(t_s) ((\mu s))</td>
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<td>100</td>
<td>1</td>
</tr>
<tr>
<td>(T_m) ((\mu s))</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>


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The inset of Figure 1 shows the validation of the MC model in linear conditions (peak labeled “Giddings”). As expected, the peak simulated with the MC program (points) and the one calculated by numerically solving the Giddings–Eyring equation (continuous line) match perfectly. In this case, the number of molecules used in any MC simulation was only 20 (as before, a large number of simulations were performed, and consequently, a large number of molecules were collected in order to smooth out the bias of the experiments). Under these conditions, the $L_1$ value, calculated according to eq 15, is 0.5% The numerical solution of eq 23 was performed by using $t_0 = 10 \mu s$ and $n = 100$ (see Table 1). From eq 16, in fact, it is easy to see that $n$ can be calculated according to

$$ n = \frac{L}{u \bar{r}_m} \quad (24) $$

By increasing the loading factor values (while keeping constant all the other simulation parameters), the onset of nonlinearity appears. This is evident in Figure 1 when peaks calculated at different $L_1$ are compared. In the inset of the same figure, an enlarged representation of the cases corresponding to $L_1$ equal to 2.5–5% is represented. The peak profiles assume the characteristic shapes of nonlinear chromatography when a Langmuir-type microscopic isotherm is assumed, and they become continuously more asymmetrical when $L_1$ increases. Most importantly, the correspondence between the Thomas kinetic model and the MC model can be demonstrated. The results of the MC simulations are shown with points in Figure 1. The continuous lines are, instead, the results obtained by numerically solving eq 21 when the appropriate values for $L_1$, $t_0$ (from Table 1), and $n$ (calculated according to eq 24) are used. This is an important result because it represents the validation of a stochastic model in nonlinear chromatography. In a way, the Monte Carlo model could be defined as an applied solution of the Giddings–Eyring stochastic theory in the case of nonlinear chromatography (i.e., when the random variables upon which the model is based cannot anymore be assumed independent).

The numerical solution of kinetic models predicts the split peak phenomenon.$^{2,3,38,67}$ The split peak phenomenon is due to the fact that a fraction of the molecules is eluted from the column as an unretained peak (i.e., a peak with a retention time equal to the column holdup time). The split peak was even demonstrated by means of stochastic models, under linear conditions.$^{15,18,19,37,68}$ The split peak phenomenon can be explained if, for instance, the kinetics of adsorption–desorption are very slow or the saturation of the column is minimal. Additionally, in the instances in which the column is very short, the mobile-phase velocity is very high, or both, the split peak can appear. In all of these cases, the number of transfer units (macroscopic models) or the mean number of adsorption–desorption steps (in the stochastic models) is very small, as is the efficiency.

Figure 2 represents a MC study of the split peak effect, under near-linear and nonlinear conditions and its comparison with the results predicted by Thomas model. As before, the results of the MC simulation are represented by points and the numerical solution of Thomas equation by continuous lines. The chromatographic parameters used in the simulations for this case are listed in the third column of Table 1. The column used is very short. In all the simulations, the peak area was normalized to 1, and again, the time spent in the stationary phase ($\mu s$) is used as the independent variable. As evidenced by the figure, the peak profiles appear to be constituted by two parts: a spike of zero width eluted with the column holdup time (i.e., at $t_0 = 0$) which is represented in the figure by a boldface vertical narrow, and a very long tail. The shape of the peaks in the region included between 0 and 0.4 $\mu s$ does not have any physical meaning. It derives from the discretization of the time required by the simulation (discrete time simulation). The interval 0–0.4 simply represents the width of the classes (in particular of the first class) into which the time axis was divided in order to collect the molecules eluting from the column.

The peak split effect is studied at the different $L_1$ values listed in the figure. When $L_1$ is equal to 1%, the system is almost in linear conditions, as previously demonstrated (see above, the validation of the MC model with the Giddings model). In linear conditions, a significant result of the Giddings–Eyring model establishes that the fraction of molecules eluted without ever adsorbing in the stationary phase can be expressed as

$$ \text{unretained fraction} = e^{-n} \quad (25) $$

From the data reported in Table 1 and using eq 25, it is possible to estimate that approximatively one-fifth of the molecules are eluted without ever being adsorbed ($e^{-1.5} = 0.22$), under the condition chosen for the simulation. This means that, for the case corresponding to $L_1 = 1\%$ the part of the peak eluted at $t_0 = 0$, should be an infinitely narrow spike, whose total fractional area is, however, finite and equal to 0.22.

When the loading factor value increases, the fraction of molecules eluted without any adsorption–desorption step being performed, i.e., the amount of the split peak, increases as well.

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This is evidenced by the inset of Figure 2, where the magnitude of the phenomenon can be understood by looking at the amplitude of the peaks, at $t_0 = 0$, for the different $L_j$ values. The same result, which is completely unexpected for linear chromatography (see eq 25), was previously shown by other research groups under nonlinear conditions.\(^\text{36,38,67,69}\) The approach proposed to arrive at these conclusions is, however, quite original. Additionally, the stochastic approach allows a clear understanding of the different contributions to the split peak that come from a true kinetic effect (eq 25) and from the competition between molecules for the adsorption (see also below). The very satisfactory agreement between lines and points in Figure 2 represents the validation of the MC model also under limit conditions.

Figure 3 presents a more systematic and quantitative study of the split peak effect as a function of the loading factor. The fraction of molecules that are eluted without ever adsorbing on the surface is plotted against the $L_j$ value, when different $n$ values are assumed (the average number of adsorption–desorption steps is varied between one and four). These very small average numbers represent cases of separation that could be performed in very short columns or at very high flow rate, as well as situations where the kinetics of the adsorption–desorption process is extremely slow.

When the $L_j$ value is equal to zero, the unretained fraction amount corresponds to the one obtained with eq 25, as expected. When the loading factor increases, however, the curves clearly show that the unretained amount increases. If $n = 1$, an almost linear dependence between the unretained molecule fraction and $L_j$ can be observed. For higher $n$ values, the dependence is better described by a quadratic relationship.

It is interesting to observe that even for cases in which, under linear conditions, no significant split peak effect would be expected, the split peak phenomenon can become very important, when the $L_j$ value increases. For instance, if $n$ is equal to 4, less than 2% of the molecules would be eluted as split peak under linear conditions (see eq 25). However, this fraction becomes almost 10% of the peak when $L_j$ is $\sim 80%$.

On the basis of these observations, the open perspectives are important. The MC molecular model is able to deal with a large number of molecules by monitoring their positions in the chromatographic medium; it can show the onset of nonlinearity when increasing the number of molecules injected; it is able to study the interactions between molecules and, in theory, to investigate their behavior on different kinds of stationary phases. It seems, then, a very powerful tool to investigate situations in which phenomena of different origins overlap, as in the case of the split peak where a kinetic contribution and a nonlinear contribution are present (see above). There are, however, other important cases in which the overlapping of different phenomena determines the same macroscopic observable phenomenon. For instance, it is well known that when a surface is constituted by two or more different kinds of adsorption sites (heterogeneous surface), one of which is much more retentive than the other, but present in a very small amount (1–3%), very asymmetrical peaks can be observed even in linear conditions.\(^\text{15,37,70}\) Several practical separation cases match these conditions: for example, the adsorption of basic drugs on end-capped C-18 stationary phase, where a few free silanol groups can be present on the surface;\(^\text{71,72}\) the separation of enantiomeric compounds on new-design stationary phases;\(^\text{3,73}\) the separation of some compounds on monolithic silica-based columns.\(^\text{74}\) In all of these situations, it is not easy to recognize when the tailing originates as a result of kinetic reasons or, rather, as a result of nonlinear effects, although in principle performing different experiments at different flow rates could help.\(^\text{19}\) Furthermore, both phenomena could take place. On sites characterized by very slow kinetics, the molecules spend on the average a much longer time than on “regular” sites. This is the origin of the kinetic tailing effect.\(^\text{21}\) On these sites, on the other hand, the probability of interactions between molecules increases and nonlinear effects, also under near-infinite dilution condition, can appear.

The only adsorption–desorption mechanism considered up to now is derived from the model proposed by Giddings and Eyring in 1955.\(^\text{18}\) It assumes an exponential distribution for the random times spent in stationary phase and a Poissonian entry process. However, other kinds of dynamics could be hypothesized as well. The MC program implemented here, in fact, offers the opportunity to make different hypotheses about the random nature of $r_m$ and $r_s$ and to study their effect on the peak shape and, more generally, on the characteristics of the separation.

Figure 4 represents a very simple example of such an application, where two different distributions—uniform and exponential—are used for each possible combination of $r_m$ and $r_s$. Table 1, fourth column, reports the numerical values for the parameters used in these simulations. Although this could appear to be an unrealistic exercise, there is evidence that some specific classes of compounds show structured retention patterns.\(^\text{3,75}\) In the inset of Figure 4, the two probability density functions used, exponential and uniform, are represented. For an easier under-


\(^{\text{72}}\) Quiñones, I.; Cavazini, A.; Guiochon, G. J. Chromatogr., A 2000, 87, 1–11.


Figure 4. Effect of different hypotheses made on the random nature of the processes “flight time” and “time spent in the site” (\(t_m\) and \(t_s\), respectively) on the observed peak shape. The parameters used in the simulations are listed in Table 1, third column.

standing by the readers, the uniform probability density function is defined as:

\[
f(t) = \begin{cases} 
  1/(b-a) & \text{if } a < t < b \\
  0 & \text{otherwise}
\end{cases} \quad (26)
\]

For the sake of simplicity, the same average value equal to 1 was assumed for both the distributions in this figure. In Figure 4, four peaks are plotted against the time spent in stationary phase (\(\mu s\)). The area of the peaks is normalized. The average time spent in the stationary phase is the same for all the peaks, as expected from eq 11 and from the data in Table 1. The simulations were performed under near-linear conditions (\(L_f \approx 0\)), because only under these conditions is the concept of column efficiency meaningful.\(^1\) As expected, all the peaks seem to have Gaussian profiles. However, the efficiencies are markedly different, as can be qualitatively seen by comparing the widths of the peaks (evaluated at half of their height) for the different cases. The Giddings model, for which both \(t_m\) and \(t_s\) are exponential, is characterized by the lowest efficiency value. On the other hand, the sharpest peak is observed when both \(t_m\) and \(t_s\) are uniformly distributed. “Mixed mechanisms” (uniform \(t_m\) vs exponential \(t_s\) or uniform \(t_s\) vs exponential \(t_m\)) result in almost indistinguishable peaks of intermediate efficiency.

Peak efficiency is the result of the combination of several independent (in linear chromatography) effects.\(^20\) When attention is paid only to the adsorption–desorption kinetics, there are two independent contributions that broaden the peak: the dispersion resulting from the entry process of the molecules in the site and the dispersion deriving from the stationary-phase process.\(^55\) It is important to note that the former is not related to any kind of mobile-phase dispersion, as commonly understood in macroscopic models: this effect is explained by considering that each molecule behaves randomly in the chromatographic column.\(^13,55\) Assumed an average value, \(n\), for the mean number of adsorption–desorption steps, any other molecular history (characterized by a greater or inferior number of stops) is still possible, though less probable. The phenomenon is then characterized by a certain amount of dispersion. In any case, \(t_m\) is a constant value for each molecule.

The second effect that must be considered is related to the permanence of the molecules in the adsorption sites, which is also characterized by some statistical fluctuations. A short description of the random nature of this process was given above.\(^56\)

The efficiency of the system is shown to be proportional to\(^55\)

\[
H \propto (\sigma_n/n)^2 + (\sigma_{\bar{t}}/\bar{t}_s)^2 \quad (27)
\]

where the first RHS term is the square of the relative dispersion related to the entry process and the second RHS term is the square of the relative dispersion related to the actual residence in the site. \(H\), the height equivalent to a theoretical plate (HETP), is related to \(N\) by the well-known relationship

\[
H = L/N \quad (28)
\]

The estimation of \(H\), performed by means of eqs 27 and 28, refers to the so-called C term of the van Deemter equation, which accounts only for the adsorption–desorption process.

The first and second RHS of eq 27 assume well-defined values depending on the probability density functions of the corresponding random variables. For an “exponential” distribution, the average and the standard deviation are equal, and the corresponding relative dispersion value is 1. For the “uniform” distribution, instead, the relative dispersion value is only 1/3. These considerations explain why peaks obtained under different hypotheses on the stochastic nature of the microscopic processes controlling the adsorption process have very different shapes. The Giddings case corresponds to least efficient peak because, under the hypotheses it was derived, both contributions in eq 27 hold one; the sharpest profile is obtained when \(t_m\) and \(t_s\) are uniform, because in this case both RHS terms of eq 27 are only 1/3. Intermediate cases cannot be recognized: it is the sum of the two effects that appears in the expression for the peak efficiency value (eq 27).

CONCLUSIONS

The M C model of chromatography has been validated under nonlinear conditions, by comparison with the Thomas macroscopic kinetic model. The M C model is, by nature, a kinetic model. It is based on the original stochastic theory of chromatography proposed by Giddings and Eyring and attention is paid to the dynamics of single molecules. The Thomas model is a macroscopic kinetic model, but is one in which only the contributions to the broadening coming from slow adsorption–desorption kinetics are considered.

The correspondence between the Giddings–Eyring model and Thomas kinetic model, when the loading factor goes to zero, is expected. It was demonstrated by analytically calculating the limit (for \(L_f \rightarrow 0\)) of the Thomas equation expressed as a function of the microscopic quantities typically used in the stochastic description. When the loading factor increases and the assumption of

near-linear condition cannot be assumed, the Giddings equation does not hold anymore. However, MC simulations have shown that the Giddings–Eyring model still corresponds to the Thomas model. The correspondence between the two approaches was demonstrated under different chromatographic conditions and also in the extreme case of very slow adsorption–desorption kinetics, very short columns, or both.

The MC model represents the first stochastic model of nonlinear chromatography. It opens important perspectives for investigating cases that cannot be modeled by macroscopic models: for instance, it will be possible to study the behavior of columns in which the stationary phase is characterized by ordered patches of sites and to investigate the effect of different arrangements on the separation performance.

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