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Theoretical evaluation of methods for extracting retention factors and kinetic rate constants in liquid chromatography

Xiaoping Li, Victoria L. McGuffin*

Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322 USA

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ABSTRACT

A three-dimensional stochastic simulation is used to provide a detailed understanding of mass transfer processes in liquid chromatography. In this simulation, the migration of individual molecules is established through diffusion and laminar convection within the mobile phase. The molecules interact with the stationary phase by a partition (absorption) mechanism. For these studies, the column length, linear velocity, stationary-phase diffusion coefficient, interfacial mass transfer coefficient, and equilibrium constant are varied in a system with a homogeneous surface. Heterogeneous surfaces are also investigated by having multiple types of interaction sites that are equally or unequally distributed. For each simulation, the molecular distribution is examined and characterized at specified times or column lengths. Five individual methods are then applied to extract the thermodynamic and kinetic information for transport between the mobile and stationary phases. In the first method, all of the molecules are initially distributed in the mobile phase and the fraction remaining is monitored as a function of time. These simulation data are fit to a single exponential decay by nonlinear regression to determine the "true" retention factors and rate constants. The other methods rely on evaluating the shape of the zone profiles along the column. The statistical moments of the profiles are used to calculate the mean and the variance, which are related to the retention factors and the rate constants, respectively. The profiles are also fit to the exponentially modified Gaussian equation, the Giddings equation, and the Thomas equation. The fitting parameters from these equations are then used to calculate the retention factors and rate constants. Comparisons of the accuracy relative to the "true" retention factors and "true" rate constants, as well as the advantages and limitations of each method are discussed.

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1. Introduction

The importance of kinetics in separation science has been recognized since the pioneering work of Giddings [1–5]. While thermodynamics governs the separation strength and selectivity, kinetics governs the extent of zone broadening and asymmetry, all of which affect the resolution. Thermodynamic processes have been studied for many years but, comparatively, kinetic processes have not been fully elucidated. A detailed understanding of kinetic behavior is essential to identify the rate-limiting processes so that separation speed may be increased without sacrificing resolution. For better understanding of kinetic behavior, accurate measurement of the kinetic rate constants is required.

There are several methods by which the kinetics of chromatographic interactions have been measured, including indirect and direct chromatographic methods [6]. The uptake/release method is an indirect chromatographic method, which has been performed in batch mode [7,8] and shallow-bed mode [9,10]. In these experiments, the concentration of solute in the solvent surrounding the particles is changed instantaneously, and the approach to equilibrium concentration is monitored. The adsorption/desorption rate curve is then fit to theoretical equations to extract the rate constants. This method is commonly used in engineering, biotechnology, and related fields. In the direct chromatographic approaches, elution chromatograms are obtained and analyzed by different methods. Among these, the plate height methods are most widely utilized to extract kinetic information [11–14]. In these methods, the zone profile is analyzed to determine the plate height, either from the width or the second statistical moment (variance), which is then related to the rate constants for mass transport. Guiochon et al. expanded the statistical moment method to study retention equilibrium and mass transfer kinetics in different kinds of chromatographic systems [14–17]. Information on axial dispersion, external mass transfer, intraparticle diffusion, and adsorption/desorption kinetics have been successfully derived. The exponentially modified Gaussian (EMG) model [18–20] has also

* Corresponding author. Tel.: +1 517 355 9715x244; fax: +1 517 353 1793.
E-mail address: jgshabus@aol.com (V.L. McGuffin).

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been extensively studied and applied in chromatographic analysis. McGuffin et al. [21–24] extracted the slow kinetics of mass transfer by fitting the zone profiles to the EMG function. The fitting parameters can be related to the rate constants for mass transport and, hence, the activation energy and activation volume. The models developed by Giddings [1] and by Thomas [25–27] are also popular to evaluate chromatographic zone profiles. These models have many applications in affinity chromatography [28] and preparative chromatography, where the fitting parameters can be directly related to the retention factor and adsorption/desorption rate constants. The Giddings model is utilized with single site adsorption in the linear isotherm range, whereas the Thomas model can be utilized with nonlinear isotherms.

To the best of our knowledge, there have been no studies dedicated to the validation and comparison of the experimental methods for extracting kinetic rate constants. For this purpose, stochastic simulation can be of great benefit [29–34]. Stochastic simulations are based on the migration of individual molecules by the sequential application of independently defined transport processes (Markov chain). The fundamental equations of motion used to describe these transport processes require few, if any, assumptions that limit their general applicability. Stochastic simulation models have shown excellent correspondence with macroscopic kinetic models [35,36]. All of the simulation parameters are specified, so the associated retention factors and kinetic rate constants can be easily calculated or extracted to serve as the "true" values. These true values can be used to compare the values from all other methods to validate the accuracy of the experimental methods.

The goals of this research are as follows: (1) to perform stochastic simulations under conditions representative of liquid chromatography systems with homogeneous or heterogeneous stationary phases, (2) to compare the generated zone profiles under different conditions, (3) to extract retention factors and kinetic rate constants by using different theoretical models, and (4) to compare the different methods with respect to their accuracy, advantages, and limitations.

2. Computer simulation method

A three-dimensional stochastic simulation was written in the FORTRAN 90 programming language and has been described in detail elsewhere [37–40]. This program incorporates algorithms for the processes of diffusion, laminar convection, and retention. The molecules can interact with a homogeneous or heterogeneous stationary phase by a partition (absorption) mechanism. These processes are repeated for each molecule at each time increment until the total simulation time is reached.

The input parameters required for the simulation are divided into three categories. The system parameters describe properties of the mobile and stationary phases, as well as the spatial dimensions of the system to be simulated. These include the radius of the mobile phase (R_m), depth of the stationary phase (d_s), column length (L), linear velocity (u_0), etc. The molecular parameters describe attributes of the molecules, such as the diffusion coefficients in the mobile (D_m) and stationary (D_s) phases, and the distribution coefficient between the two phases (K). The computational parameters describe certain constraints that are required for the simulation, such as the number of molecules (N), time increment (t), and total simulation time (T). To initialize the simulation, the molecules are distributed in a statistically random manner entirely in either the mobile or stationary phase.

The results of the simulation are output as the time distribution of molecules at specific column lengths or, correspondingly, as the distance distribution of molecules at specific times. The statistical moments of the molecular distribution are calculated in time units for simulations at specified column lengths (or in length units at specified times). In addition to these numerical output parameters, the molecular population is summed in discrete segments of time (or length) and then connected in a continuous zone profile for graphical display or for zone profile fitting by the different models.

3. Data analysis methods

3.1. "True" method

In the partition process, the solute molecules transfer between the mobile and stationary phases. The rate of transfer between these two phases can be treated as a pseudo-first-order reaction [41]. Under these conditions, the process can be described by a simple kinetic model of reversible reactions. To determine the rate constants, the molecules are initially distributed in a uniform manner in the mobile phase. The number of molecules in each phase is then recorded as a function of simulation time. The mass transfer rate constants from the mobile to stationary phase (k_{sm}) and from the stationary to mobile phase (k_{ms}) are determined by fitting the data to the kinetic equation for a first-order reversible reaction

$$\frac{N_m}{N} = \frac{[k_{ms} + k_{sm}\ \exp(-(k_{sm} + k_{ms})T)]}{k_{sm} + k_{ms}} \tag{1}$$

where N_m is the number of molecules in the mobile phase, N is the total number of molecules, and T is the time elapsed in the simulation. The rate constants determined in this manner will be called the "true" kinetic rate constants. Furthermore, the ratio of the kinetic rate constants defines the retention factor (k)

$$k = \frac{k_{sm}}{k_{ms}} = \frac{\overline{N_s}}{\overline{N_m}} \tag{2}$$

which is equal to the ratio of the number of molecules in the stationary and mobile phases at equilibrium ($\overline{N_s}$ and $\overline{N_m}$, respectively). The retention factor determined in this manner will be called the "true" retention factor. Three repetitive simulations with 10,000 molecules are performed for each set of simulation conditions. Rate constants are then determined by nonlinear regression of the data to Eq. (1). The three values are then averaged to obtain the rate constants and their standard deviations. Similarly, the retention factor and its standard deviation are determined by Eq. (2).

3.2. Statistical moment method

In the statistical moment method, all of the moments are directly obtained from the molecular zone profiles. These statistical moments are calculated in the time domain as

$$M_1 = N^{-1}\sum_{i=1}^{N} T_i \tag{3}$$

$$M_2 = N^{-1}\sum_{i=1}^{N} (T_i - M_1)^2 \tag{4}$$

$$M_3 = N^{-1}\sum_{i=1}^{N} (T_i - M_1)^3 \tag{5}$$

where T_i is the arrival time of an individual molecule (i) at the specified column length and N is the total number of molecules. The first moment (M_1) is the mean retention time. It can be related to the retention factor by

$$k = \frac{M_1 - t_0}{t_0} \tag{6}$$

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