Journal of Chromatography A, 1217 (2010) 1000–1009

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Dynamic chromatography: A stochastic approach

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ARTICLE INFO

Article history:
Available online 17 October 2009

Keywords:
Dynamic chromatography
Reaction chromatography
Enantiomers separation
Chiral chromatography
Reaction rate

ABSTRACT

During the chromatographic separation process, analyte reactions are often observed leading to band broadening and/or elution of peak clusters. For many different chemical compounds the reaction can be reduced to a simple isomerisation kinetic scheme where elution is the result of adsorption–desorption on the surface stationary phase coupled with a flipping two-level reaction system. In this paper, the chromatographic peak shape for a reacting analyte is calculated in frequency domain when the reaction follows a simple reversible first order scheme. Both reaction and dynamic chromatographic systems have been considered. The derived solutions are expressed in closed form in the Fourier domain. Several limit solutions obtained under conditions of very slow and moderately fast kinetics are exploited. The effects of both kinetics rate constants and retention time on the chromatographic peak shape are singled out.

1. Introduction

Efficient, economical product or process design requires accurate fundamental data values such as transport properties, adsorption energy and kinetic rate. Likewise, the fundamental understanding of many processes requires the use of thermodynamics, mass transfer with chemical reaction in nonideal systems [1,2]. Moreover in product design, it is well known that the stability of certain chemicals is an important issue in chemical and pharmaceutical studies, since most biochemical processes or chemical properties are stereochemically controlled. Different methods can be adopted to evaluate these data and a comprehensive review of these can be found elsewhere [3].

One of these techniques is dynamic chromatography studied by Bürkle et al. [4], which offers several advantages over other methodologies. Indeed, chromatography makes it possible to obtain mass transfer, adsorption and kinetics data and can be employed in non-linear conditions [5]. However, a prerequisite for employing this technique is that reaction must take place within the separation timescale (on-column reaction chromatography) [6–12]. Different methods can be employed to extract kinetic parameters from the experimental chromatograms [3,4,13–16].

The focus of the present work is to represent the dynamic chromatography from a microscopic point of view in order to provide a new method for determining the kinetic constant by dynamic chromatography experiments. In particular, the on-column reaction chromatography process is described by using the stochastic model of chromatography [17]. Originally developed by Keller and Giddings [18], there has been renewed interest in this model since it makes it possible to correlate macroscopic classical chromatographic parameters with the behavior properties of individual molecules [19,20]. These arguments partially support the use of the stochastic approach even to study interconversion phenomena. The main reason for this is that, when coupled with the so-called characteristic function (*CF*) approach, the solution found using this model can be expressed in closed form in the frequency domain even when complex chromatographic cases are considered [19–25].

The theoretical dynamic chromatography model, here developed, follows the general formalism proposed for the photon statistics model of single molecule observation [26–28]. Since in this last case the model solution is available in terms of *CF*, the model of dynamic chromatography will be obtained by coupling the reaction kinetics description with the stochastic model of two sites chromatography [22]. By this way, the representation of the chromatography profile will be available in the Fourier domain, as a function of the process parameters: this makes it possible to estimate the kinetic constants of reaction–elution processes by fitting the whole experimental chromatographic profile in the Fourier domain [20]. Thus, the methodological approach followed here differs from both the original theoretical plate model of chromatography [4,24], and its extensions based on the introduction of stochastic terms describing the peak band broadening [29–31].

In the present approach to on-column reaction chromatography, the classical reduction of the kinetic scheme from the four states to two states will be followed [16,32,33]. This will imply a certain

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doi:10.1016/j.chroma.2009.10.036

degree of approximation. Moreover, the focus will be essentially on the band broadening coming from the phase exchange kinetics and reaction, neglecting other significant aspects such as the mobile phase diffusion and eddy diffusion. The aim of this study is, in fact, to explore the potential advantages of using a stochastic description of dynamic chromatography.

2. Theory

The theory section of the present paper starts by recalling some basics of the stochastic model of chromatography and ends with a treatment of the chromatographic elution of molecules undergoing first order reaction. Description of the model follows the general formalism for a four-states kinetics system [4,16,33] and then this kinetics scheme is reduced to obtain a two-states system. Finally, the general expression for the chromatographic peak in reaction chromatography is derived together with particular solutions valid for given reaction kinetics in the separation time considered (reaction time scale). The obtained solution does not account for important chromatographic phenomena, such as mobile phase and eddy diffusion, responsible for peak broadening. Details of the mathematical description of the process are reported in the Appendixes A–D given in Supporting Information.

2.1. Dynamic microscopic description of the chromatographic process

In chromatographic separation, analyte molecules can be captured by the active surface sites and they can stay there for a random amount of time (τ_s); while they remain on the sites, the molecules are delayed vs. the main stream of the flowing mobile zone. After this time has elapsed they revert to the mobile phase until a new sorption event (n) occurs: the sojourn time undergoes to random jumps process. As a consequence, the analyte volume travels along the column at an average velocity, which is lower than that of the main stream. Moreover, the mobile phase volume occupied by the molecules is enlarged as a result of the random nature of the capturing and releasing processes (chromatographic band broadening).

The one site model of chromatography describes a chromatographic process involving solely a single kind of site (i.e. surface site homogeneity) (see Appendix A). In chiral chromatography at least two different active site types are present on the surface of the stationary phase and molecules could interact with both. The distribution of the time spent on each site is related to the molecule interaction energy on these two different sites. In chromatography literature the latter model is the so-called two sites model or biLangmuir model [5] and it is generally used to interpret enantiomeric separation. In enantiomeric chromatographic separation both enantiomers may be present in the mobile phase and they undergo different interactions with the two active stationary phase sites. In a simplified model, each enantiomer interacts selectively with just one site. If the two enantiomers can interconvert during the separation, then each molecule can be present in four different situations which correspond to the four states of the system (see Fig. 1a).

2.2. Model of first order dynamic chromatography

Let us consider an experimental interconversion batch involving enantiomers A and B partitioned between the M phase—a non-chiral homogenous phase and the S phase—the chiral phase (see Fig. 1a) The achiral and chiral phases are, respectively, the mobile and stationary phases in the chromatographic column corresponding to the batch system.

The considered system involves four different conformer types, which, in agreement with Refs. [4,29–31], are indicated as:

(1) A_M, lowest retained conformer in the mobile phase;
(2) B_M, highest retained conformer in the mobile phase;
(3) A_S, lowest retained conformer in the chiral stationary phase;
(4) B_S, highest retained conformer in the chiral stationary phase.

This model is a four-states system: at a given time t, a given molecule can be in one of the four above-described states. When the reaction rate constants are employed to describe the system, one should also consider the relationships existing between these constants. The principle of microscopic reversibility [4,29,34] states that, when a batch system is at equilibrium, the transition frequency is the same in both directions for each individual reaction step. Consequently, in any cyclic reaction the product of the rate constants going one way around the cycle is equal to the product of the kinetic constants describing the reverse reaction going the other way. With reference to Fig. 1 one has:

$$k^B_{-1}k^M_{-1}k^A_1k^S_1 = k^B_1k^M_1k^A_{-1}k^S_{-1} \quad (1)$$

where k^j_1, and k^j_{-1} with $j = M, S$ are the forward and backward reaction rate constants in the mobile (M) and stationary (S) phase, respectively; k^j_1, and k^j_{-1} with $j = A, B$ are the sorption and desorption rate constants of the species A and B, respectively.

The main difference between a batch system like the one described above and the corresponding chromatographic system is that, in the latter, it is important to evaluate the time evolution of a single molecule and its statistical properties instead of the molecular averaged chemical composition of the batch system at a given time. The batch results can be applied to the dynamic chromatographic process thanks to the ergodic hypothesis which states the averages obtained from a large molecular population and over a long observation time are equivalent.

In a macroscopic chromatographic system, under linear conditions, the microscopic reversibility principle holds true [4,16,34]. Under the hypothesis that $k^M_{-1} = k^M_1$, Eqs. (1) and A-6b show that this condition is verified in a chromatographic separation of enantiomers using an achiral mobile phase (see Fig. 1a) and, after rearrangement (see Appendixes B and C), one obtains:

$$K^S = \frac{k^S_1}{k^S_{-1}} = \frac{K_B}{K_A} = \frac{k'_B}{k'_A} = \frac{\bar{\tau}_{S,A}}{\bar{\tau}_{S,B}} \quad (2)$$

where $\bar{\tau}_{S,A}$ and $\bar{\tau}_{S,B}$ are the average time spent during one species A or B sojourn step in the stationary phase (S). Likewise, k'_A and k'_B are the capacity factors of the pure enantiomer A or B, respectively (see Eq. A-6a). K_B and K_A are the stationary-to-mobile phase partition coefficients for species B and A, respectively, and $K^S = B_S/A_S$.

Eq. (2) is obtained under the hypothesis that the mean time spent in the mobile phase (see Eq. A-2) is constant for all species involved. Moreover, Eq. (2) shows that, in the stationary phase, the interconversion rate is inversely proportional to a molecule's mean residence time in the corresponding conformational state in the stationary phase:

$$k^S_1 \propto \frac{1}{\bar{\tau}_{S,B}} \quad (3)$$

2.3. Reduction of the kinetic scheme

In a chromatographic experiment it is only possible to observe the history of the molecule inside the column as a whole—that is, the sum of the times spent in each of the four possible system states. The kinetic scheme can be simplified as a two states system: each