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

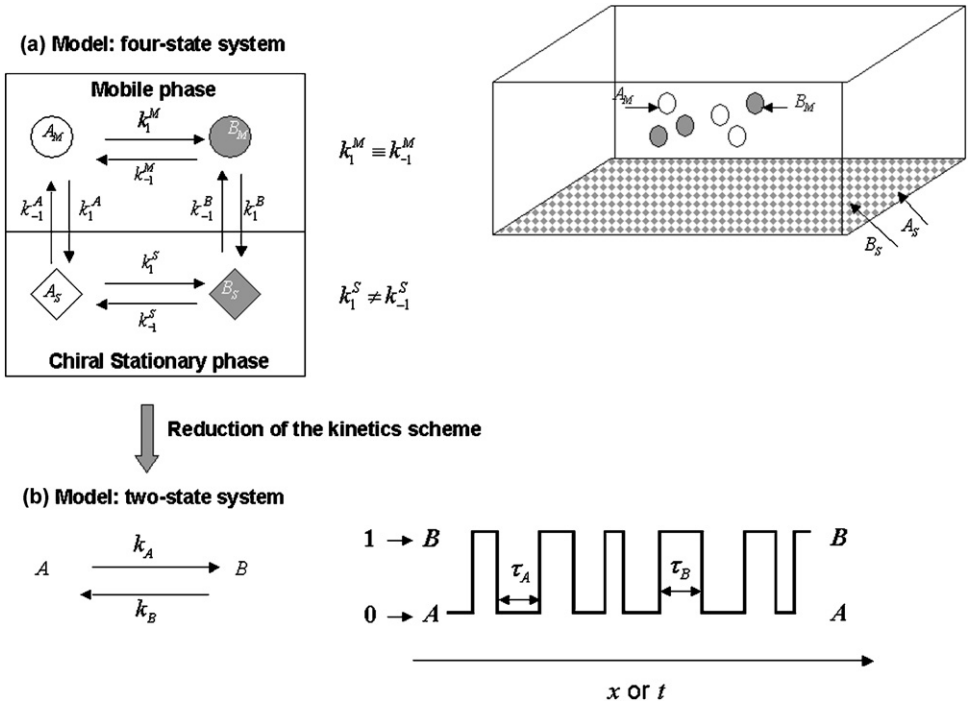

Fig. 1. (a) Kinetics scheme of a four-state system and (b) kinetics scheme of a two-state system.

containing, respectively, the mobile and stationary phase states of the enantiomers A and B:

$$\frac{dP_A}{dt} = k_B P_B - k_A P_A \tag{4a}$$

$$\frac{dP_B}{dt} = k_A P_A - k_B P_B \tag{4b}$$

where k_A and k_B are the two observed reaction rate constants and P_A and P_B are the molecular populations (i.e. the number of species) of the two states (enantiomers) A and B independently of their stay in the M or S phases (i.e. their sum).

The reduction of the four-state scheme to the two-state system in Fig. 1b can be achieved using different approaches, each implying different degrees of approximation [35–37]. Details of the approach adopted here are reported in Appendix C.

The apparent kinetic constants, already derived for a macroscopic system [31,38–40], in the case of the simplified two-states microscopic model are (see Appendix C):

$$k_B = \frac{(\bar{\tau}_{S,B} k^S_{-1} + \bar{\tau}_M k^M_{-1})}{\bar{\tau}_M + \bar{\tau}_{S,B}} = \frac{1}{1+k'_B} k^M_{+1} + \frac{k'_B}{1+k'_B} k^S_{-1} \tag{5a}$$

$$k_A = \frac{(\bar{\tau}_{S,A} k^S_{-1} + \bar{\tau}_M k^M_{-1})}{\bar{\tau}_M + \bar{\tau}_{S,A}} = \frac{1}{1+k'_A} k^M_1 + \frac{k'_A}{1+k'_A} k^S_1 \tag{5b}$$

Reaction dynamic chromatography experiments generally yield a peak cluster composed of two peaks of the unconverted enantiomers and a large central peak formed by the molecules involved in the interconversion reaction. Thus, it is conceptually useful to divide the chromatograms into two parts: one for the unconverted species, the other for the converted species.

2.4. Peak generated from reaction chromatography

In chromatographic experiments, retention times are the functions of numerous variables. Different instrumentation parameters such as flow velocity, column length, temperature can influence the eluted species peak profile as well as the contribution due to the chemical interaction between the analyzed species and both the stationary and mobile phases. In order to simplify the mathematical handling and unify the model description of these processes, changing the process time variable is convenient. Subsequently, a peak profile description for unreacted species is derived and finally a general expression for the profile of reacted species is obtained.

2.4.1. Change of the process variable

Let us define x as follows:

$$x = \frac{(t - t_{R,A})}{(t_{R,B} - t_{R,A})} = \frac{(t - t_{R,A})}{\Delta t_R} = \frac{(t' - t'_{R,A})}{(t'_{R,B} - t'_{R,A})} \tag{6}$$

where $t_{R,i}$ and $t'_{R,i}$ are the retention times and the corrected retention times, respectively referred to the species in the conformation state i with $i = A$ or B.

The x variable represents the normalized time of the interconversion reaction within the limited interval of the chromatographic experiment, defined as the difference in retention times of the two pure enanatiomers, Δt_R, here referred as "observational time", in analogy with single molecule photon transition experiments [26] (see in the following). We can thus consider a molecule of enantiomer A (less retained enantiomer) which is eluted at $t_{R,A}$ as a molecule that never visited a type B site and thus never remained in molecular state B, in the stationary phase. Note that the mobile phase is, in fact, assumed achiral and thus it is not possible to detect any interconversion process occurring therein. Conversely, a molecule of enantiomer B (most retained enantiomer) which is eluted at $t_{R,B}$ is a molecule that never visited site A and thus was never in the state A, in the stationary phase. Consequently, we can associate a molecule eluting at $t_{R,A}$ with a zero probability of transition from A to B. In fact, by substituting $t = t_{R,A}$ in Eq. (6) one has $x = 0$. Likewise, a molecule of enantiomer B, which is eluted at $t_{R,B}$, has a zero probability of transition from B to A.

Thus x represents the fraction of the observational time Δt_R spent in the B state (in the stationary phase). It can be observed that x also represents the fraction of molecules which are in state B at any given time (i.e. $P_B/(P_A + P_B)$). One can thus set the two following

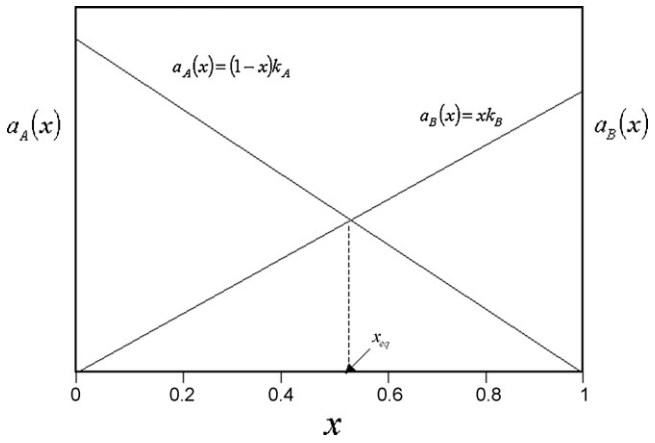

Fig. 2. Scheme of the propensity functions of the process.

expressions:

$$a_A(x) = (1-x)k_A \tag{7a}$$

$$a_B(x) = xk_B \tag{7b}$$

where $a_A(x)$ and $a_B(x)$ are the transition frequencies for the univariate processes $A \rightarrow B$ or $B \rightarrow A$, respectively. $a_A(x)$ and $a_B(x)$ are the so-called "propensity functions" characterizing, from a stochastic point of view, the reversible isomerisation reaction in Eq. (4a) as proposed by Gillespie [41] (see Appendix C). It is important to underline that the propensity functions here introduced do not include concentration of reacting species.

At the equilibrium (i.e. $x = x_{eq}$), the process becomes stationary and it has to satisfy the detailed balance condition [41]—that is, the frequency transitions are the same in each direction for every pair of states (see Fig. 2):

$$(1-x_{eq})k_A = x_{eq}k_B \tag{8a}$$

By rearranging Eq. (8a) one has:

$$x_{eq} = \frac{k_A}{k_A + k_B} \tag{8b}$$

which is the equilibrium condition of state B occupancy in the system.

The concentrations c_A and c_B can be obtained from the respective population P_A and P_B by dividing by the system volume. For $x = 1$ and $x = 0$ Eqs. (4a) and (4b) expressed in concentration units become:

$$\frac{dc_A}{dt} = -k_A c_A(t) \tag{9a}$$

$$\frac{dc_B}{dt} = -k_B c_B(t) \tag{9b}$$

The solution of the differential equations in the time domain are given, respectively, by

$$c_A(t_{R,A}) = c_A^0 \exp(-k_A t_{R,A}) \tag{10a}$$

$$c_B(t_{R,B}) = c_B^0 \exp(-k_B t_{R,B}) \tag{10b}$$

Eqs. (10a) and (10b), obtained in the framework of the kinetic microscopic description (see Appendixes B and C), correspond to those achieved by discontinuous equilibrium models based on the description of the chromatographic column as a sequence of theoretical plates [4,16]. However, a detailed comparison between the present approach and that presented in Refs. [29–31,38–40] would deserve a specific study, especially for that which concerns the introduced approximations, the limit conditions of the model and the applicability of limit solutions.

2.5. Case a: peaks generated from unconverted molecules

By combining Eqs. (10a) and (10b) with Eqs. A-6c, d and e, one obtains:

$$c_A(t_{R,A}) = c_A^0 \exp(-k_A t_{R,A}) = c_A^0 \exp(-k_1^S t'_{R,A} - k_{-1}^M t_M) \tag{11a}$$

$$c_B(t_{R,B}) = c_B^0 \exp(-k_B t_{R,B}) = c_B^0 \exp(-k_{-1}^S t'_{R,B} - k_1^M t_M) \tag{11b}$$

By assuming that the time spent in the mobile phase is constant for any eluted substance and that the forward and backward reaction rate constants in the mobile phase are equal (i.e. $k_{-1}^M = k_1^M$), it follows that:

$$\exp(-k_{-1}^M t_M) = \exp(-k_1^M t_M) = \text{const} \tag{11c}$$

The time spent in the mobile phase is constant for unreacting molecules. On the other hand, molecules involved in on-column reaction, are allowed to spend a variable amount of time in mobile phase (see Section 2.7 Eqs. (28a)–(28c)).

The above-defined quantities c_A and c_B are the concentration or number of species partitioned between the mobile and stationary phases in any given conformation (i.e. A or B, respectively). These molecules spent time only in one of the pairs of states related to a given conformation (i.e. A or B). In the stochastic description of chromatography, these molecules behave as though retained on a one-site chromatographic column since they only interact with one site of the stationary phase. This one-site model of chromatography (described in Appendix A) [19] can thus be applied to model the unconverted species and the functions describing the peak profile in frequency domain – scaled with respect to t_M – are:

$$\Phi(\omega)_{S,A}\big|_{unc} = c_A(t_{R,A}) \exp\left\{\bar{n}\left[\frac{1}{1 - i\omega\bar{\tau}_{S,A}} - 1\right]\right\} \tag{12a}$$

$$\Phi(\omega)_{S,B}\big|_{unc} = c_B(t_{R,B}) \exp\left\{\bar{n}\left[\frac{1}{1 - i\omega\bar{\tau}_{S,B}} - 1\right]\right\} \tag{12b}$$

$\Phi(\omega)_{S,A}\big|_{unc}$ and, $\Phi(\omega)_{S,B}\big|_{unc}$ are the *CFs* of the chromatographic process, over the sites A and B, respectively. Eqs. (12a) and (12b) do not account for any band broadening phenomena with the only exclusion of that coming from the randomness of the sorption–desorption process.

The chromatographic elution peaks for the unconverted species in the time domain can be obtained from the *CF* (Eqs. (12a) and (12b)) by a Fast Fourier Transform (*FFT*) algorithm as described elsewhere [19]. Alternatively, the parameters defining the peak (i.e. $\bar{n}$ and $\bar{\tau}_{S,A}$) can be estimated by fitting in the frequency domain [21]. The corresponding time domain solution is reported in Appendix A (see Eqs. A-5a and b).

2.6. Peaks generated from molecules that react during column migration

2.6.1. Case b: fast interconversion kinetic

Let us now consider molecules that react very fast vs. the separation process time scale. Such molecules can be present in both conformational states A and B in the stationary phase, and they interact with the corresponding sites of the stationary phase. During the time spent inside the column, those highly reacting molecules perform a high number of jumps (n) between the mobile and stationary phases and are in a stationary or equilibrium position, as above-described with reference to Eqs. (8a) and (8b). Consequently, by considering the equivalence between chromatographic site and molecular conformational state, the chromatographic process can be fully described by the two-sites stochastic model of chromatography [22].

At the equilibrium condition, the fraction of visits to B site is given by Eq. (8b). The corresponding two-sites chromatographic process can be described by:

$$\begin{cases} \Phi_S(\omega)\big|_{fast} = \exp\left[\bar{n}((1-x_{eq})\varphi_{S,A}(\omega) + x_{eq}\varphi_{S,B}(\omega)) - 1\right] \\ 1 - x_{eq} = \dfrac{k_B}{k} = 1 - p \\ x_{eq} = \dfrac{k_A}{k} = p \\ k = k_A + k_B \end{cases} \tag{13}$$

where $\varphi_{S,A}(\omega)$ and $\varphi_{S,B}(\omega)$ – given by Eqs. (12a) and (12b) with $c_A(t_{R,A}) = 1$ and $c_B(t_{R,B}) = 1$, respectively – are the *CFs* of the chromatographic process for a unit amount of species, over sites A and B, respectively (see Eq. (6b)). We observe that quantities x_{eq} and $1 - x_{eq}$ just correspond to the fraction of the two site types, p and $(1-p)$, in the two-sites stochastic model of chromatography [19,22].

Under the hypothesis that the number of jumps in the stationary phase is a Poissonian variable of average $\bar{n}$, in the case of a two-site model the probability density functions describing the number of jumps to sites A and B will have averages equal to $\bar{n}_A$ and $\bar{n}_B$, respectively. This hypothesis does not hold for slow reactions. In such cases, changes in the average number of jump of reacting species have to be accounted for (see Section 2.7). The retention time of the peak described by Eq. (13) can be expressed as:

$$t'_{R,eq} = \bar{n}\bar{\tau}_{S,eq} = \bar{n}\left(\frac{k_B}{k_A + k_B}\bar{\tau}_{S,A} + \frac{k_A}{k_A + k_B}\bar{\tau}_{S,B}\right) \tag{14a}$$

$t'_{R,eq}$ corresponds to the weighted retention time of a mixture of enantiomers, having weights equal to the equilibrium states population. The quantity $\bar{\tau}_{S,eq}$:

$$\bar{\tau}_{S,eq} = \left(\frac{k_B}{k_A + k_B}\bar{\tau}_{S,A} + \frac{k_A}{k_A + k_B}\bar{\tau}_{S,B}\right) \tag{14b}$$

is the corresponding "equilibrium" average sorption time of a single sorption step characteristic of the fast interchanging species.

Eq. (13) is the *CF* referred to a unit amount of species. The total amount of interconverting enantiomers c^* corresponds to the amount not eluted as pure enantiomers A or B at $t_{R,A}$ and $t_{R,B}$, respectively, and thus, from Eqs. (11a) and (11b), we get:

$$c^* = c_A^* + c_B^* = c_A^0(1 - \exp(-k_A^S t_{R,A})) + c_B^0(1 - \exp(-k_B^S t_{R,B})) \tag{15}$$

The searched solution of the peak profile in the Fourier domain, obtained from Eqs. (13) and (15) is

$$\Phi_S^*(\omega)\big|_{fast} = c^* \exp\left[\bar{n}((1-x_{eq})\varphi_{S,A}(\omega) + (x_{eq})\varphi_{S,B}(\omega)) - 1\right] \tag{16}$$

2.6.2. Case c: low interconversion kinetics

In the most general case, the reaction time scale can be similar to the separation time scale: each molecule spends part of the time in both conformations. Consequently, the average molecule migration velocity is expressed as a weighted sum of the migration velocity of the two pure enantiomers. The weights are related to the fraction of time spent in each of the two states. To determine the latter quantities, a two-states jump model is employed. Such a two-states jump process is characterized by a single relaxation rate constant $k = k_A + k_B$, and by $p = k_A/k$, the probability of finding the molecule in state B which is equal to x_{eq} (see Eq. (8)).

The concept of the "random trajectory" of a single molecule $\xi(t)$ (see Fig. 1b) constitutes the starting point for calculating the fraction of average time spent in each state. $\xi(t)$ is the stochastic occupation variable which is set to 0 when the molecule is in state A, and 1 when the molecule is in state B. The time spent in the state A or B is assumed to be exponentially distributed (corresponding to a first order kinetics). Consequently, the number of jumps between the two states follows Poisson statistics (see Eq. A-1, with $\mu = 1/k$). For a given trajectory lasting t, the time spent in state B is the integral of $\xi(t)$ over t. Consequently, the previously introduced quantity x (see Eq. (6)) can now be expressed as:

$$x = \frac{(t - t_{R,A})}{(t_{R,B} - t_{R,A})} = \frac{1}{\Delta t_R}\int_{t_{R_A}}^{t_{R_B}} \xi(t)dt = \frac{1}{T}\int_{t_{R_A}}^{t_{R_B}} \xi(t)dt \tag{17}$$

where $\Delta t_R = t_{R,B} - t_{R,A} \equiv T$ – sake of notation simplicity equal to T – and x is the fractional time spent in the state B and it is now a stochastic variable and is characterized by its distribution, since the "histories" of a molecule, $\xi(t)$, giving a value equal to $(t - t_{R,A})$ as the integral in Eq. (17), can be more or less probable.

The probability density associated with x and notated as $P(x|T)$ can be obtained as a sum of two contributions:

$$P(x|T) = ap(x|T, A) + bp(x|T, B) \tag{18}$$

where $p(x|T,A)$ and $p(x|T,B)$ are a molecule's probability density of spending xT time in state B for molecules initially in the states A and B, respectively ($x = 0$, with $t = t_{R_A}$, see Eq. (6)); a and b are the fractional populations of the states A and B at the initial condition ($a + b = 1$).

The solution of the problem (see Appendix D) can be obtained by following the method known as the Kubo and Anderson formalism [42,43], extensively employed in developing the line shape theory of interconverting chromophores [26–28,44,45]. The two-states enantiomeric interconversion and two-states dynamics processes of a chromophore molecule under stationary conditions [46] are, in fact, closely related from the stochastic–kinetic point of view because: (i) they follow the same first order kinetic scheme (see Figs. 1 and 2); (ii) they can be represented stochastically through two-states single molecule random trajectories (Eq. (17)); (iii) the statistics of these random trajectories are the same; and (iv) the probability density of trajectories at xT can be obtained as a sum of two contributions (Eq. (18)). As mentioned above in Section 2.2, the present approach can be applied to describe a macroscopic system composed of a large number of molecules or observed for a long period of time vs. the reaction time scale.

By applying the Fourier–Stiltjes transform to Eq. (18) one obtains:

$$\hat{P}(x|T) = a\hat{p}(x|T, A) + b\hat{p}(x|T, B) \tag{19}$$

where $\hat{P}(x|T)$, $\hat{p}(x|T, A)$ and $\hat{p}(x|T, B)$ are the *CFs* of $P(x|T)$, $p(x|T,A)$ and $p(x|T,B)$, respectively. $\hat{p}(x|T, A)$ and $\hat{p}(x|T, B)$ were solved in Ref. [47] (see Appendix D):

$$\hat{p}(\omega|T, A) == \exp\left(-\frac{kT - i\omega T}{2}\right)\left(\phi\cosh\frac{\phi T}{2} + (k + i\omega)\sinh\frac{\phi T}{2}\right)/\phi \tag{20a}$$

$$\hat{p}(\omega|T, B) = \exp\left(-\frac{kT - i\omega t}{2}\right)\left(\phi\cosh\frac{\phi T}{2} + (k - i\omega)\sinh\frac{\phi t}{2}\right)/\phi \tag{20b}$$

where

$$\phi = \sqrt{\left(\left(\frac{k}{2}\right)^2 - i\left(p - \frac{1}{2}\right)k\omega - \left(\frac{\omega}{2}\right)^2\right)} \tag{20c}$$

$\omega = 2\pi/T$ is the frequency. $t = T + t_{R,A}$ since the time windows in the considered chromatographic experiment is, as specified in Appendix D, $t_{R_A} \div t_{R_A} + T$.

By considering Eq. (19) and given the linearity properties of the Fourier transform, by combining Eqs. (20a) and (20b) one obtains:

$$\hat{P}(x|T) = \exp\left(-\frac{kt - i\omega t}{2}\right) \times \left(\cosh\frac{\phi t}{2} + \frac{(k - i(p - (1/2))\omega)}{\phi}\sinh\frac{\phi t}{2}\right) \quad (21)$$

i.e. the *CF* total probability of being in the *B* state.

Eq. (21) was originally derived by Reilly and Skinner [47] to interpret chromophore transition of a two-level system. The probability density *CF* associated to *x* due solely to interconversion can be obtained from Eq. (21) by applying the following relationship:

$$\Phi_2(\omega|T) = \hat{P}(\omega|T) - \Phi_A^*(\omega) - \Phi_B^*(\omega) \quad (22)$$

where $\Phi_A^*(\omega)$ and $\Phi_B^*(\omega)$ are the *CFs* of the probability density functions of the fraction of unconverted molecules in *A* and *B*, respectively. The unconverted quantities in concentration units were discussed in paragraph 2.5 (Eqs. (12a) and (12b)). Thus the pertinent expressions – as fractional units and starting from equilibrium conditions in Fourier domain [48] – are:

$$\Phi_A(\omega) = (1 - p)\ \exp(-pkt) \quad (23a)$$

$$\Phi_B(\omega) = p\ \exp(-(1 - p)kt)\exp(-i\omega) \quad (23b)$$

Starting from Eq. (22), other useful relationships can be obtained, in particular:

2.6.3. Limit solutions

If $kt \gg 1$, which corresponds to moderately fast kinetics, Eq. (21) becomes:

$$\hat{P}(\omega|T) = \exp(i\omega pt)\ \exp\left(-\frac{p(1 - p)}{kt}\omega^2\right) \quad (24)$$

which is the *CF* of a Gaussian function [47] having a mean equal to *p* and variance σ:

$$\sigma = \frac{2p(1 - p)}{kt} \quad (25)$$

The limit for $kt \to \infty$, i.e. for very fast interconversion kinetics (see Section 2.6.1) one has:

$$\hat{p}(\omega|T) = p \quad (26a)$$

$$P(x|T) = \delta(x - p) \quad (26b)$$

Eq. (26a) is, in fact, the *CF* of a Dirac function (Eq. (26b)). The probability density $P(x|T)$ thus consists of a single spike located at $x = p$. In this case all the molecules rapidly interconvert vs. the separation time scale and the result here obtained confirms what was previously found in Section 2.6.1. The main difference between Eq. (26a) and Eq. (16) is that Eq. (26a) does not take into account the chromatographic band broadening contribution that will be considered later on.

The limit of Eq. (21) for $kt \to 0$ is [47]:

$$P(x|T) = (1 - p)\delta(x) + p\delta(1 - x) \quad (27)$$

Eq. (27) represents two spikes positioned at the extremes of the *x* domain and having abundances equal to the thermodynamic limit $(1 - p)$ and *p*, respectively. These functions correspond to the spikes of the non-reacting molecules handled in Section 2.5 and again do not include the contribution of the chromatographic sorption/desorption process.

2.7. From the probability distribution to the chromatographic peaks

The discussion developed in the previous paragraph (i.e. 2.6) was mainly concerned with the derivation of the *B* state population at a given time *xT*, resulting from the isomerisation reaction: $A_{M+S} \Leftrightarrow B_{M+S}$ (see Fig. 1b) under stationary conditions. The chromatographic process superimposes the reaction as a process of exchange between the mobile and stationary phases – $A_M \Leftrightarrow A_S$ and $B_M \Leftrightarrow B_S$ – and it contributes to broadening the elution band both because of the random nature of the stay of site *A* or *B* in the stationary phase and the random character of the interval between two subsequent adsorption processes from the mobile phase (see Section 2.2). Other band broadening processes, that can be significant and/or prevailing in chromatographic experiments [5], will be not handled in the present work. For each *x* (or *xT*) value, the number of jumps between the two states $n(x)$ is a Poisson variable (see Eq. A-1) which, when combined with the distribution of time spent in one state, gives the peak shape function in either the time domain, as developed by Giddings [18], Kramer [24] and Cremer [25], or in frequency domain (see Appendix A). This was essentially the result for fast reaction chromatography, when the equilibrium approximation $x = x_{eq}$ was adopted. Now, the approach must be extended to account for the distribution of *x* values (see Eq. (21)). The stochastic theory of dynamical chromatography includes this second stochastic variability thus far developed. The band broadening process in the interval $t_{R,B} \le t \le t_{R,B}$, i.e. in $0 \le x \le 1$ (see Eq. (6)) will be applied by rescaling this interval as number of jump units ($n(x)$). The chosen unit, as will be showed in the following, will be equal to $\bar{\tau}_{S,eq}$ since for it the chromatographic band broadening function is accessible in the Fourier domain (see Eq. (13) with $\bar{n} = 1$).

The fundamental quantity *x* defined in Eq. (17) can be redefined (see Eq. A-6d) as follows:

$$x = \frac{n(x)\bar{\tau}_{S,eq} - \bar{n}\bar{\tau}_{S,A}}{\bar{n}(\bar{\tau}_{S,B} - \bar{\tau}_{S,A})} \quad (28a)$$

$$t \equiv n(x)\bar{\tau}_{S,eq} \quad (28b)$$

In Eq. (28a), one can see that for $x = x_{eq}$ one has $n(x_{eq}) = \bar{n}$. By rearranging Eq. (28a) one obtains:

$$n(x) = \bar{n}\frac{(x(\bar{\tau}_{S,B} - \bar{\tau}_{S,A}) + \bar{\tau}_{S,A})}{\bar{\tau}_{S,eq}} \quad (28c)$$

According to this equation, $n(x)$ is rescaled with respect to *x* and, since in Eq. (28c) the averages terms ($\bar{\tau}_i$ and $\bar{n}$) are constants, $P(x)$ (or its *CF* $\hat{P}(x)$) can be directly transformed to a probability distribution of $n(x)$ (i.e. $P[n(x)]$) [48,49]. Note also that, according to Eq. (28c), $n(x)$ is a continuous variable like *x* or *t*.

The retention profile for each *t* value is generated by the corresponding number of jumps, and the sorption time value given by $\bar{\tau}_{S,eq}$ is assumed to be common (see Eq. (14b)). The above assumption can be qualitatively explained by considering that the change in probably of state occupation due to system reaction can be viewed as a change in the number of jumps or, equivalently, in the corresponding change in permanence time, following the same approach proposed in the stochastic description of chemical kinetics processes [45]. In fact, in the chromatographic two-sites model (see paragraph 2.1), on one side the sorption time distributions do not change in time since the site adsorption properties are considered constant. Moreover, the number of type *A* and *B* sites in the stationary phase is also constant. Instead, the number of molecules which can specifically interact with sites *A* or *B* (see Fig. 1) evolves during the course of separation due to the interconversion reaction: the jump number is accordingly modulated (Eq. (28c)).

The assumed hypothesis that expresses the *t* quantity as the product of two sole random quantities, *n* and $\bar{\tau}_{S,eq}$, allows us to

obtain a solution for the coupled interconversion-phase exchange processes of dynamical chromatography by applying the mathematical formalism employed in handling the so-called stochastic dispersive model of chromatography [19]. In fact, in both cases, the band broadening due to reaction or to inhomogeneous flow velocity is translated into a probability distribution for the jump number. The jump process acts as a process that directs the sorption process.

The presented approach can be applied only to systems containing a large number of molecules. In terms of *CF*, the solution is expressed as follows:

$$\varphi_{tot}(\omega) = \varphi_{dis}\left(\frac{\log(\varphi_u(\omega))}{i}\right) \tag{29}$$

where φ_u is the *CF* of the chromatographic retention process referred to either to $\bar{n}$ or t_M unit values and φ_{dis} is the *CF* of the probability dispersion function of either $n(x)$ or $t_M(x)$ (equal to $= n(x)\bar{\tau}_M$, see Eq. (28b)).

In the present case, by assuming the initial condition corresponding to condition of equilibrium, under conditions of $n = 1$, Eq. (13) becomes:

$$\varphi_u(\omega) = \exp\left(\frac{1}{(k_A + k_B)}\left(\frac{k_B}{(1 - i\omega\bar{\tau}_{S,A})} + \frac{k_A}{(1 - i\omega\bar{\tau}_{S,B})} - (k_A + k_B)\right)\right) \tag{30}$$

The *CF* of the directing process (i.e. $\varphi_{dis}(\omega)$ in Eq. (29)) is obtained from Eq. (21) and by performing the variable change from x to $n(x)$ [46] one obtains:

$$\varphi_{dis}(\omega) = \hat{P}(x|T)\frac{p}{\bar{n}} = \exp\left(-\frac{kt - i\omega t}{2}\right)\left(\cosh\frac{\phi t}{2} + \frac{(k - i(p - (1/2))\omega)}{\phi}\sinh\frac{\phi t}{2}\right)\frac{p}{\bar{n}} \tag{31}$$

The final expression is obtained from the combinations of Eqs. (28)–(31). In particular the argument of φ_{dis} in Eq. (29) should be $\log(\varphi_u(\omega)/i)$.

The previously described coupling of the chromatographic band broadening process with the reaction process was based on the assumption that the unitary band broadening contribution was constant within the domain $t_{R,B} \le t \le t_{R,B}$, The consequent approximation degree is here estimated as follows: peak variance and the number of theoretical plates in standard stochastic theory of chromatography (e.g. Eq. (12a) or (12b)) [17,5] are given by

$$\sigma^2 = 2n\bar{\tau}_S \tag{32a}$$

$$N = \left(\frac{t_R}{\sigma}\right)^2 = 2n \tag{32b}$$

In the present case from Eq. (28c) one obtains , for x equal to 0, x_{eq} and 1, $n(0) = \bar{n} \times (\bar{\tau}_{S,A}/\bar{\tau}_{S,eq})$, $n(x_{eq}) = \bar{n}$ and $n(1) = \bar{n} \times (\bar{\tau}_{S,B}/\bar{\tau}_{S,eq})$, respectively. Consequently since $\bar{\tau} = \bar{\tau}_{S,eq}$, one has, by using Eq. (32a), $\sigma^2(0) = 2\bar{n}\bar{\tau}_{S,A}\bar{\tau}_{S,eq}$, $\sigma^2(x_{eq}) = 2\bar{n}\bar{\tau}^2_{S,eq}$ and $\sigma^2(1) = 2\bar{n}\bar{\tau}_{S,B}\bar{\tau}_{S,eq}$. By considering Eqs. (12a) and (12b), (13), (14b), and (32a)), the exact values should be $\sigma^2(0) = 2\bar{n}\bar{\tau}^2_{s,A}$, $\sigma^2(x_{eq}) = 2\bar{n}\bar{\tau}^2_{s,eq}$ and $\sigma^2(1) = 2\bar{n}\bar{\tau}^2_{s,B}$. Consequently one see that chromatographic band broadening calculated by using Eq. (29) is exact in the case of $x = x_{eq}$, and overestimated and underestimated in the domains $0 \le x < x_{eq}$ and $x_{eq} < x \le 1$, respectively. However one should remember that, beside the chromatographic band broadening there is, in addition, the contribution due to the reaction (see e.g. Eq. (25) in the case of fast kinetics) and they are additive in variance. In most cases the variance contribution due to reaction the most important one (see Section 4). Consequently the bias should be of minor relevance Alternatively, the whole process can be split into its two components labelled *A* and *B* (see Eqs. (19)–(21)) and the solution is then given as a sum of the respective contributions, i.e.:

$$\varphi_{tot}(\omega) = \varphi_{tot,A}(\omega) + \varphi_{tot,B}(\omega) \tag{33}$$

where the argument of $\varphi_{tot,A}$ and $\varphi_{tot,B}$ (see Eqs. (21) and (22) and D-9 a and b, respectively) are $\log(\varphi_{S,A}(\omega)/i)$ and $\log(\varphi_{S,B}(\omega)/i)$ with $\varphi_{S,A}(\omega)$ and $\varphi_{S,B}(\omega)$ given by Eqs. (12a) and (12b) when $c_A(t_{R,A})$ $c_B(t_{R,B})$ are both equal to one. The importance of Eq. (33) is that it represents the correct expression for the peak shape in Fourier domain. Its meaning will be thoroughly described in the discussion. The advantage of employing Eq. (33), expressed as separate components, instead of Eq. (31) is that it allows one to consider cases where the initial concentrations of the two reacting species differ from one another (i.e. on-column reaction chromatography).

Finally, the general solution is obtained by multiplying Eq. (31) by the total amount of the interconverting enantiomers c^* (see Eq. (15)).

3. Computation

Computations were done by using Mathematica version 4.1 or Matlab version 5.0 routines. The numerical FFT inversion procure was described elsewhere [17].

4. Results and discussion

Eqs. (12a) and (12b) represent the chromatographic peak profile in Fourier domain of the pure enantiomers *A* and *B*, and Eqs. (33) the corresponding peaks due to first order reaction chromatography. By summing the contributions due to only one enantiomer *A* (or *B*) it is thus possible to obtain the equations describing the chromatographic response of a single species. The peak profile in the time domain can be obtained as numerical inversion of the abovementioned equations. This set of equations (i.e. Eqs. (12a) and (12b) and (33)Eqs. (12) and (33)) represent thus a solution of the on-column reaction chromatography model, however, they appear mathematically cumbersome to be applied to experimental chromatograms. For this reason several approximate or limit solutions were in addition obtained. In particular, a simplified solution that describes the effect of fast or moderately fast kinetics (i.e. $kt \gg 1$, see Eq. (24)) under linear elution condition, can be approximated by a Gaussian-like central peak. With respect to the previously reported handlings, the present stochastic approach was able to obtain general and specific solutions in closed form under frequency domain, fully describing the dynamical chromatography (Eqs. (29) and (33)). In the following basic features of dynamical chromatography and its dependence on interconversion kinetics and operative variables such as column length are exploited. The retention time data and column efficiency values of the selected examples were chosen in order to mimic real cases.

In Fig. 3 the time domain solution for the peak profile of two separated reacting species is plotted. Each profile represents the chromatographic peak corresponding to the elution of an injected solution containing only one component which reacts during the chromatographic run. They were obtained by numerical inversion of the function generated by summing the chromatographic peak profile in the Fourier domain for the pure enantiomers *A* and *B* (Eqs. (12a) and (12b)) with the corresponding peaks resulting from first order reaction chromatography (Eqs. (20a) and (20b)), i.e. the description of reaction chromatography, where only one of the species is present at the beginning of the separation process. One can see that the first enantiomer A appears as a peak at $t_R \cong 23$ min (which is product obtained by multiplying n(=9045) by the sum of $\bar{\tau}_M + \bar{\tau}_A$, according to Eqs. (7a) and (7b)Eq. (7e)). A decaying tail,

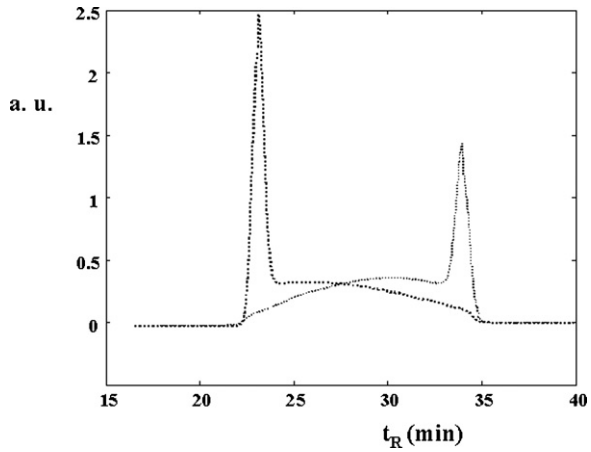

Fig. 3. Simulated peak: (a) $n = 9045$, $\tau_{S,A} = 0.1331$ s, $\tau_{S,B} = 0.2032$ s, $k_A = 0.0252\ \text{min}^{-1}$, $T = 33.66$ min; (b) $k_B = 0.0222\ \text{min}^{-1}$ (all other parameters have been kept constant and equal to (a)).

corresponding to the transformation, follows this peak $A \rightarrow B$. In the same figure, the B peak appears with an increasing front followed by the peak located at t_B (≅34 min). The reported example refers to a slow interconverting enantiomeric mixture ($k_A = 0.0252\ \text{min}^{-1}$, $k_B = 0.0222\ \text{min}^{-1}$). If the injected solution contains both the species A and B, the resulting peak profile can be obtained by summing the contribution of each.

In dynamic chromatography experiments the observation time T plays a key role in determining the shape of the resulting chromatographic peak for a given reactive substance. In fact, the quantity kT characterizes the reaction kinetic time scale (k, see Eq. (13)) vs. separation time scale (T). Fig. 4 provides an example of simulated chromatograms corresponding to increasing k values from a to f while keeping T constant. In this series of chromatograms, the retention time for the pure enantiomers is kept constant by fixing the values of $T\ (=t_{R,B} - t_{R,A})$, $\bar{n}$, $\bar{\tau}_B$ and $\bar{\tau}_A$ for all simulations. In Fig. 4a no interconversion takes place (see paragraph 2.5); in Fig. 4b–d interconversion rates are steadily increasing and all the components due to pure enantiomers and reacting species are present. Finally, in Fig. 4e and f, only one central peak is present. These last two cases correspond to the moderately fast kinetics discussed in Section 2.6.3. By comparing the peak shapes seen in Fig. 4e and f, one can see that peak width decreases as kinetics constant values increase and this is in agreement with what has been predicted from theory.

Increasing the observation time of moderately fast reactions should lead to a decrease in peak variance; this follows from the inverse proportionality relationship between kinetics constant and observation time. In chromatographic practice, a species' residence time in a column is related to column length or mobile phase velocity, two related variables (see Appendix A). Fig. 5 reports simulated chromatographic peaks obtained for different separation times, thus providing an example of the influence of this feature. It can be observed that peak band broadening increases with time. Two factors have to be considered in evaluating the band broadening in reaction chromatography, namely: (1) chromatographic (i.e. sorption/desorption) band broadening and (2) the global process separation time. From stochastic theory of chromatography one knows that peak variance for a one-site column is given by Eq. (32a). Therefore band broadening increases with n. Figs. 4 and 5a reports the peak corresponding at the highest n value. In this case, the calculated chromatographic band broadening was found to be $\sigma^2 = 0.188\ \text{min}^2$ (for $n = 10{,}000$, tau = $0.1838\ \text{s}^{-1}$), a small value when compared to the total peak variance $\sigma^2 = 2.25\ \text{min}^2$. It follows

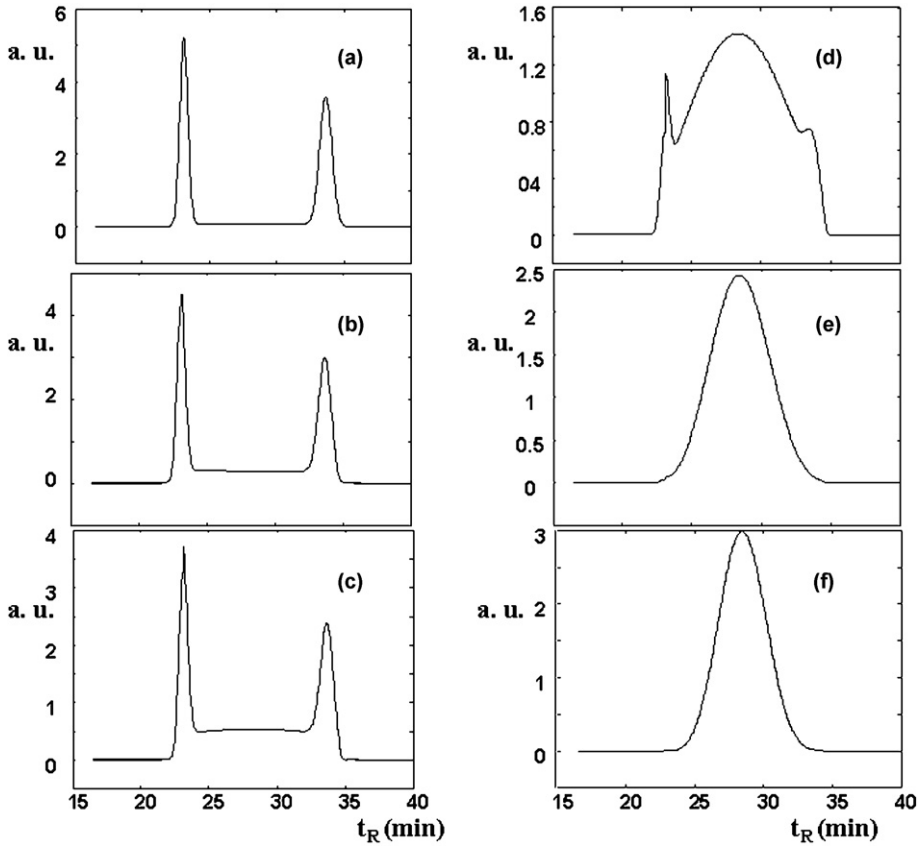

Fig. 4. Simulated chromatograms. Effect of increasing k value: $n = 9045$, $\tau_{S,A} = 0.1331$ s, $\tau_{S,B} = 0.2032$ s, $p = 0.53$ (a) $kT = 0.16$, (b) $kT = 0.8$, (c) $kT = 1.6$, (d) $kT = 8$, (e) $kT = 16$, and (f) $kT = 24$.

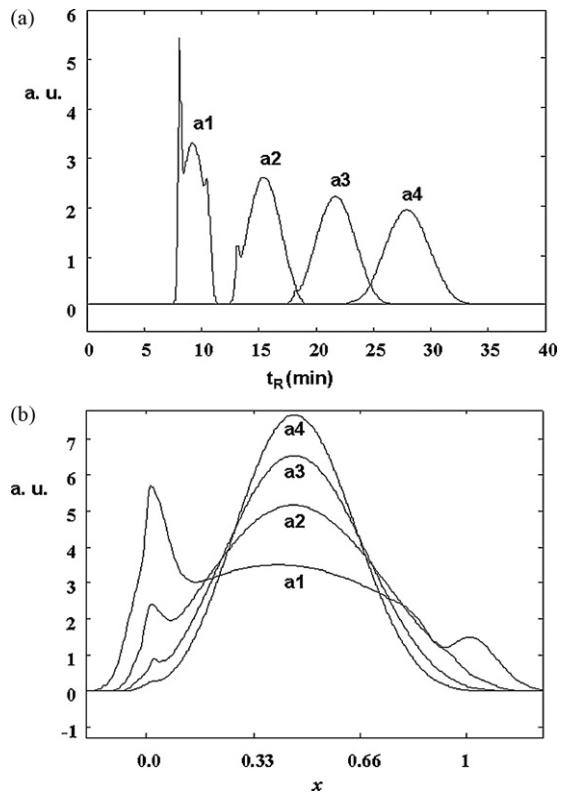

Fig. 5. a) Simulated chromatograms. Effect of increasing process time: $\tau_{S,A} = 0.1204$ s, $\tau_{S,B} = 0.1838$ s, $p = 0.47$, $k = 0.474\,\text{min}^{-1}$, (a1) $\bar{n} = 2500$ (solid line), (a2) $\bar{n} = 5000$ (dashed line), (a3) $\bar{n} = 7500$ (dotted line) and (a4) $\bar{n} = 10000$ (dash-dot line). (b) Simulated chromatograms of (a) scaled by the process time.

that, for the reported cases, kinetics makes the main contribution to peak width. In fact, by plotting the simulated chromatograms in the normalized x-axis (see Eq. (6).) one obtains Fig. 5b.

The simulated chromatograms do not account for axial and eddy diffusion contributions to the overall to peak band broadening which, in experimental chromatograms, can be relevant. When the above-mentioned additional band broadening contributions are negligible, the proposed procedure makes it possible to obtain the kinetic parameters by non-linear fitting of the acquired chromatogram. This procedure has to be applied in the Fourier domain where the solution is available in closed form. The topic lies beyond the aim of the present study since it requires a specific extended numerical handling. Nonetheless some simple computations in order to allows one to obtain preliminary kinetic data is selected cases is presented in the following.

For moderately fast reactions vs. elution time scale, once the retention times of the two pure enantiomers are available, it is possible to obtain an approximated value of the kinetic constant (see paragraph 2.6.3). Experimental chromatograms satisfying such requirements have been already reported in the literature [50,51]. In such cases the following procedure can be applied:

(a) Normalization of the retention times:

$$x_A = \frac{t_{R,eq} - t_{R,A}}{T} = \frac{(\bar{\tau}_{S,eq} - \bar{\tau}_{S,A})}{\bar{\tau}_{S,B} - \bar{\tau}_{S,A}} \tag{34a}$$

$$x_B = 1 - x_A = \frac{t_{R,B} - t_{R,eq}}{T} = \frac{(\bar{\tau}_{S,B} - \bar{\tau}_{S,eq})}{\bar{\tau}_{S,B} - \bar{\tau}_{S,A}} \tag{34b}$$

where $t_{R,eq}$ is the retention time of the Gaussian peak of the two interconverted species.

Form Eq. (13) one has:

$$x_A = 1 - p = \frac{k_B}{k} \tag{35a}$$

$$x_B = p = \frac{k_A}{k} \tag{35b}$$

(b) Evaluation of normalized standard deviation.

When limit condition 2.6.3 is applicable and the band broadening of the reaction is the most important contribution to the peak variance, after subtraction of the chromatographic band brading contribution (see Eq. (32a)) from the peak variance one has:

$$\sigma(x) = \frac{2p(1-p)}{kt} = 2\frac{x_A x_B}{kt} \tag{36}$$

By substituting Eqs. (35a) and (35b) in Eq. (36) one has:

$$k_A = \frac{2x_A x_B^2}{\sigma(x)t} \tag{37a}$$

$$k_B = \frac{2x_A^2 x_B}{\sigma(x)t} \tag{37b}$$

The outlined theoretical model can be thus the basis for setting up and validating a numerical procedure based on least square fitting in the Fourier domain for determining the kinetic constants of the reaction.

5. Conclusions

To summarize, the developed model allows for both general and specific solutions in frequency domain, fully describing the dynamic chromatography process. The general equation can be simplified in the limit cases: (a) for slow reaction rate (compared to the adsorption kinetics) the chromatographic response is a sum of two separated peaks; (b) very fast reaction rates generate just one peak having a retention related to the kinetic constants of the reaction (p); (c) for intermediate rates, a peak cluster is generated. The model was able to describe both interconversion and reaction during separation. Some basic features of the process – such as interconversion kinetics constants – and/or operative variables – such as column length (or flow velocity) – and their influence on the chromatographic output have been exploited.

Acknowledgments

This work has been supported by the Italian University and Scientific Research Ministry (CHEM-PROFARMA-NET, RBPR05NWWC_008) and by PRRIITT (ER) misura 4 azione 2.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.10.036.

References

[1] D. Do Duong, Adsorption Analysis: Equilibria and Kinetics, Imperial College Press, 1998.
[2] I.M. Richard, Principles of Adsorption and Reaction on Solid Surfaces, Wiley–IEEE, 1996.
[3] O. Trapp, G. Schoetz, V. Schurig, Chirality 13 (2001) 403.
[4] W. Bürkle, H. Karfunkel, V. Schurig, J. Chromatogr. 288 (1984) 1.
[5] G. Guiochon, A. Felinger, A.M. Katti, D. Shirazi, Fundamentals of Preparative and Nonlinear Chromatography, 2nd ed., Elsevier, The Netherlands, 2006.
[6] R. Thede, Encyclopedia of Chromatography: Rate Constants: Determination from On-Column Chemical Reactions, 2nd ed., Taylor & Francis, 2006.
[7] C. Wolf, Dynamic Stereochemistry of Chiral Compounds—Principles and Applications, RSC Publishing, Cambridge, 2008.
[8] C. Wolf, Chem. Soc. Rev. 34 (2005) 595.

[9] O. Trapp, S.K. Weber, S. Bauch, W. Hofstadt, Angew. Chem. Int. Ed. 46 (2007) 7307.
[10] O. Trapp, J. Chromatogr. A 1184 (2008) 160.
[11] O. Trapp, S.K. Weber, S. Bauch, T. Baecker, W. Hofstadt, B. Spliethoff, Chem. Eur. J. 14 (2008) 4657.
[12] O. Trapp, Chem. Today 26 (2008) 26.
[13] J. Krupcik, P. Oswald, P. Màjek, P. Sandra, D.W. Armstrong, J. Chromatogr. A 1000 (2003) 779.
[14] B. Lina, F. Songa, G. Guiochon, J. Chromatogr. A 1003 (2003) 91.
[15] J. Veciana, M.I. Crespo, Angew. Chem. Int. Ed. 30 (1991) 74.
[16] O. Trapp, V. Schurig, Comput. Chem. 25 (2001) 187.
[17] N.G. Van Kampen, Stochastic Processes in Physics and Chemistry, North Holland, Amsterdam, 1981.
[18] R.A. Keller, J.C. Giddings, J. Chromatogr. 3 (1960) 205.
[19] L. Pasti, A. Cavazzini, A. Felinger, M. Martin, F. Dondi, Anal. Chem. 77 (2005) 2524.
[20] A. Felinger, L. Pasti, F. Dondi, M. van Hulst, P.J. Schoenmakers, M. Martin, Anal. Chem. 77 (2005) 3138.
[21] A. Felinger, A. Cavazzini, M. Remelli, F. Dondi, Anal. Chem. 71 (1999) 4472.
[22] A. Cavazzini, M. Remelli, F. Dondi, A. Felinger, Anal. Chem. 71 (1999) 3453.
[23] F. Dondi, A. Cavazzini, L. Pasti, J. Chromatogr. A 1126 (2006) 257.
[24] R. Kramer, J. Chromatogr. 107 (1975) 241.
[25] E. Cremer, R. Kramer, J. Chromatogr. 107 (1975) 253.
[26] A.M. Berezhkovskii, A. Szabo, G.H. Weiss, J. Chem. Phys. 110 (1999) 9145.
[27] I. Gopich, A. Szabo, J. Chem. Phys. 122 (2005) 014707.
[28] E. Barkai, Y. Jung, R.J. Silbey, Phys. Rev. Lett. 87 (2001) 207403.
[29] O. Trapp, V. Schurig, J. Chromatogr. A 911 (2001) 167.
[30] O. Trapp, V. Schurig, Chirality 14 (2002) 465.
[31] O. Trapp, Anal. Chem. 78 (2006) 189.
[32] K. Cabrera, M. Jung, M. Fluck, V. Schurig, J. Chromatogr. A 731 (1996) 315.
[33] M. Jung, V. Schurig, J. Am. Chem. Soc. 114 (1992) 529.
[34] R.C. Tolman, Proc. N.A.S. 11 (1925) 436.
[35] M. Scappin, P. Canu, Chem. Eng. Sci. 56 (2001) 5157.
[36] G. Zumofen, J. Klafter, Chem. Phys. Lett. 219 (1994) 303.
[37] J. Cao, Chem. Phys. Lett. 327 (2000) 38.
[38] O. Trapp, Electrophoresis 27 (2006) 2999.
[39] O. Trapp, Electrophoresis 27 (2006) 534.
[40] O. Trapp, Chirality 18 (2006) 489.
[41] D.T. Gillespie, J. Chem. Phys. 113 (2000) 297.
[42] R. Kubo, J. Phys. Soc. Jpn. 9 (1954) 935.
[43] P.W. Anderson, J. Phys. Soc. Jpn. 9 (1954) 316.
[44] M. Boguñá, J. Masoliver, G.H. Weiss, Physica A 289 (2001) 307.
[45] I. Gopich, A. Szabo, J. Chem. Phys. 124 (2006) 154712.
[46] Y. Jung, E. Barkai, R.J. Silbey, Chem. Phys. 284 (2002) 181.
[47] P.D. Reilly, J.L. Skinner, J. Chem. Phys. 101 (2) (1994) 959.
[48] R.N. Bracewell, The Fourier Transform and Its Applications, 2nd ed., McGraw-Hill, NY, 1978.
[49] W.C. Hamilton, Statistics in Physical Science Estimation, Hypothesis Testing, and Least Squares, The Ronal Press Company, NY, USA, 1964.
[50] D.H. Hochmuth, W.A. Konig, Lieb. Ann. 6 (1996) 947.
[51] R. Cirilli, R. Costi, R. Di Santo, F. La Torre, M. Pierini, G. Siani, Anal. Chem. 81 (2009) 3560.