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Peak tailing and mass transfer kinetics in linear chromatography

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Abstract

The origin of peak tailing under linear conditions, at very low analyte concentrations, is investigated using the equations of the transport–dispersive model of chromatography. It is shown that a general explanation can be obtained by assuming the existence of two different types of adsorption sites having different equilibrium isotherms and different rates of mass transfer kinetics. Even if the experimental conditions are such that both mechanisms operate linearly, tailing can be observed if the mass transfer kinetics is much slower on one type of sites than on the other. The most pronounced and typical peak tailings occur when the slow type of adsorption sites give a smaller contribution to the retention than the fast type and if the rate constant of mass transfer for the slow sites is between 20 and 2000 times smaller than that of the fast sites. Axial dispersion (caused by molecular and eddy diffusion) dampens the effects. Therefore, peak tailing of kinetic origin will be more important on highly efficient columns than on mediocre ones. An important case in point is that of enantiomeric separations. Chiral phases are designed as two-types-of-sites phases since it is impossible to eliminate the general non-selective interactions between analytes and bonded groups. The chiral selective sites involve strong interactions between the stationary phase and at least one of the enantiomers, conditions which slow down mass transfers.

Keywords: Peak tailing; Mass transfer; Kinetic studies; Band profiles; Adsorption isotherms; Thermodynamic parameters

1. Introduction

All models of linear chromatography predict a symmetrical Gaussian profile as long as the column efficiency exceeds about 100 theoretical plates [1]. Marked deviations from a Gaussian profile would take place only at very low efficiencies. Yet, it is rare to observe such profiles in practice. Depending on the experimental conditions, all bands tail to a smaller or greater extent. The origin of this tailing is puzzling. A number of explanations have been suggested [1]. Some involve the use of incorrect instrumental conditions, such as a faulty injection device. A large volume, empty injection loop does result in a strongly tailing injection function. A slow detector response when a fast column is used or the injection of a large sample causing column overload could also provide an explanation in some cases. These sources of extra-column band broadening and tailing are well-known nowadays however, and cannot provide a satisfactory explanation for a phenomenon which is as general as it is vexing.

Much effort has been devoted to the problem of modeling tailing peaks. An exponentially convoluted

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Gaussian profile has been suggested [2]. The only justification of this model is in the case of an injection profile with an exponential decay. Other models have been suggested on a purely empirical basis [3,4]. Chromatographers have been measuring and reporting peak asymmetry [5] for years without providing much of a satisfactory explanation of the origin of this asymmetry. Limited effort has been devoted to the fundamental issue of why symmetrical bands are so rarely observed since the initial work of Giddings [6,7], thirty years ago.

Eyring and Giddings [8] have already developed a stochastic theory of chromatography treating the chromatographic process as a Poisson distribution. This model neglects axial dispersion, assumes random adsorption–desorption processes, a single type of adsorption sites, and approximates the mass transfer kinetics in the mobile phase by a random walk model. Giddings [6] extended the model to a two-site surface by assuming that adsorption–desorption is very fast on one site, leading to an apparent dispersion which is easily calculated. "This is superimposed on the effect of the slow exchange process on the tail producing site", an effect which can be accounted for by the stochastic model. In other words, this model does not account straightforwardly for the band profile observed. This profile is calculated as the sum of two contributions. The complexity of this composite model results from the need to derive approximate analytical solutions to discuss such a problem at a time when powerful computers were not available. In spite of these important simplifying assumptions, the equation obtained for the band profiles was complex, involving Bessel functions. The calculations of actual solutions was too difficult to allow their systematic investigation. Only a few general conclusions could be derived from this work. A slow kinetics of adsorption–desorption on high adsorption energy sites is a plausible source of band tailing. Kinetic tailing is little affected by a decrease of the sample size in contrast to tailing of nonlinear origin. Kinetic tailing will be prominent at high velocities.

Later, Knox and Vasvari [9] studied the asymmetry of peaks obtained on a pellicular octadecyl silica. They found that the peak asymmetry, measured as the ratio of the trailing and leading "half widths", decreases with increasing velocity (although the asymmetry is constant for reduced velocities above 25 to 30). This phenomenon is in clear contradiction to the conclusions of Giddings [6]. It is explained by a nonlinear origin of the tailing. Pellicular adsorbents had a very low saturation capacity and detectors were less sensitive. The column was slightly overloaded at low velocity when the column efficiency was high. At high velocities, the column efficiency being lower, the band is more dilute and the nonlinear asymmetry reduced. This effect has no kinetic origin. Still we disagree with the comment by Knox and Vasvari [9] that "the kinetic contribution to peak width, which is symmetric, [..] decreases with fluid velocity". The kinetic contribution to peak width is symmetrical only when it is small enough to be included in an apparent axial dispersion coefficient.

Ohkuma and Hara [10] treated peak tailing as a kinetic effect, resulting from slow adsorption–desorption. They attempted to account for the experimental band profiles obtained using normal-phase chromatography on underivatized silica as the stationary phase, with an organic eluent. They fitted a Gaussian function to the leading part of the profiles of these experimental peaks while the excess portion, deviating from the Gaussian profile, was described by an empirically devised function as an "apparent tail portion". This is an original application of a purely empirical model designed by Chesler and Cram [11] to investigate the problems of accuracy and precision encountered in analog-to-digital (A/D) conversion, data acquisition and data handling of tailing peaks. Good agreement was obtained by Ohkuma and Hara between experimental and best calculated profiles, because of the flexibility of the fitting function, but the process is empirical, the model has no theoretical background, and the parameters derived have no physical meaning.

More recently, Marle et al [12] studied the influence of the mobile phase flow-rate on the efficiency and symmetry of the peaks of the enantiomers of several β-blockers[1] obtained in reversed-phase chromatography, using as stationary phase a protein (cellobiohydrolase I) immobilized on silica. These authors reported that the reduced plate height

[1]Important drugs against cardiac diseases, which are amino alcohols.

increased from 7 to 13 when the reduced velocity increases from 3 to 9, indicating a slow adsorption–desorption kinetics. Also, significant changes in the band profiles were observed. The peak asymmetry decreases with increasing velocity. At the lowest flow-rates the peaks were high and narrow but tailed strongly at the base. At high flow-rates, by contrast, the peaks become low and broad while the tailing observed at the base decreased. The authors concluded that the phenomenon was similar to the one reported by Knox and Vasvari [9]. At low flow-rates the column efficiency is high, the band narrow and high, and because of the limited capacity of the enantio-selective sites, the column is slightly overloaded, causing a peak tailing of thermodynamic origin. At high flow-rates, the band is more spread and dilute because of the reduced efficiency, so this tailing disappears. This explanation was not checked by comparing the band profiles obtained for different sample sizes. As noted by Giddings [6,7], a tailing of thermodynamic origin would disappear at low sample sizes. This leaves unsolved, however, the contradiction with the nature of the kinetic effect, which cannot be "symmetrical". As we show later, slow kinetics on the high adsorption energy enantio-selective sites is at the origin of the band asymmetry.

The lumped kinetic model of chromatography has been used earlier to study the influence of an homogenous mass transfer kinetics on the band profiles, in the linear [13] and the nonlinear case [13,14]. Its advantage over the stochastic model of Giddings and Eyring [8] is that it includes fewer restrictive assumptions [15]. The systematic calculation of numerical solutions of this model is now easy. Using this model, it has been demonstrated, in agreement with the early results of Giddings [6,7], that a slow kinetics cannot explain peak tailing in linear chromatography, except when this kinetics [1,15] is extremely slow. However, if we assume instead the existence of two different adsorption sites (the simplest model for an heterogeneous surface) and linear conditions concerning both types of sites, with different mass transfer kinetics on each type of site, the situation is entirely different as previously shown by Giddings [6,7] and later by Guiochon et al. [1].

The purpose of this work is a more detailed investigation of the properties of a two-site model, which has the potential of explaining most of the tailing effects reported so far. Systematic calculations of band profiles illustrate the nature and the possible importance of these effects and allow a definition of the conditions leading to the most severe peak tailing. Further work will show that these conditions are met in the chiral separations of β-blockers described above and that the model discussed explains quantitatively the peak tailing observed [16].

2. Theory

For the calculation of the chromatograms discussed in this work, we need (i) a proper chromatographic model, accounting for the propagation of concentration bands in the column; (ii) a relationship between the concentrations in the mobile and the stationary phase at equilibrium and (iii) a model for the mass transfer kinetics. The transport–dispersive model [13] was used for the systematic calculations of band profiles. In this lumped kinetic model, the mass transfer kinetics was represented by the equation of the solid film linear driving force model. Since we assume a heterogeneous surface with two types of sites, we need two such equations with different rate constants. Similarly, the adsorption isotherm on each site is normally accounted for by the Langmuir model. Thus, the global isotherm is given by the bilangmuir isotherm. However, we are interested here in the origin of band tailing at low concentrations, under linear conditions. Therefore, both b_1C and b_2C products are set to be much smaller than 1 throughout this work.

The thermodynamic equilibrium constant of linear chromatography is equal to the ratio of the adsorption and desorption rate constants. So, we cannot vary these two rate constants independently without changing the retention factor. This is consistent with the demonstration ([1]) that, in linear chromatography, the Langmuir kinetic model, which includes the adsorption and desorption rate constants, and the linear driving force models which include only one rate constant, are equivalent. The retention factor can be adjusted easily by changing the mobile phase composition, so it is sufficient to discuss the influence of the desorption rate constant at constant

value of the retention factor, i.e., to vary proportionally the adsorption and desorption rate constant. Alternately, the same result is obtained by varying the rate constant of a lumped kinetic model, which justifies the choice of this model.

2.1. Column model

A dispersive flow is assumed through the chromatographic column. Thus the mass balance equation is

$$u\frac{\partial C}{\partial z}+\frac{\partial C}{\partial t}+F\frac{\partial q}{\partial t}=D\frac{\partial^2 C}{\partial z^2} \quad (1)$$

where t and z are the time and axial positions in the column, respectively, C and q are the local concentrations in the mobile and stationary phase, respectively, u is the mobile phase velocity, F is the phase ratio, and D is the axial dispersion coefficient.

The initial condition characterizes the state of the column when the injection is performed. This corresponds to a column empty of sample and containing mobile and stationary phases in equilibrium

$$C(t=0,z)=0 \quad (2)$$

In most practical cases encountered in HPLC, the contribution to column efficiency due to axial-dispersion is quite small. Therefore, the dispersion effects can be neglected in the boundary conditions. The classical Danckwerts boundary conditions can be reduced to a rectangular injection pulse with a width t_p at the column inlet as follows

$$C(t,z=0)=C^0 \quad 0\le t\le t_p \quad (3a)$$

$$C(t,z=0)=0 \quad t<0 \vee t>t_p \quad (3b)$$

2.2. Equilibrium isotherms

The concentrations of a component in the mobile phase and in the stationary phase at constant temperature are related by an isotherm equation. In many cases in non-chiral chromatography the Langmuir isotherm equation accounts well for the adsorption of a single component on a homogeneous surface.

$$q^*=\frac{q_s bC}{1+bC} \quad (4)$$

where q^* is the stationary phase concentration at equilibrium with the mobile phase concentration C, q_s is the monolayer saturation capacity, bq_s is the initial slope of the isotherm, and b is a term related to the adsorption energy. In this work, we are only interested in the linear case, i.e., the product bC is neglected compared to 1.

In this work, we consider that the surface of the adsorbent is not homogeneous but is made of two kinds of homogeneous patches, with different adsorption energy, specific surface area and adsorption–desorption kinetics. This model applies particularly well to chiral stationary phases which contain both nonselective and enantio-selective sites. In this case, we will assume that the nonselective surface is homogeneous. We are of the opinion, however, that the surface of most adsorbents used in liquid chromatography (LC) are not homogeneous for at least a large number of analytes. The existence of so-called "active" sites has been postulated by many authors and this assumption seems to be supported by most experimental results, if only on a qualitative basis. Definite proofs are mainly encountered in gas chromatography investigations (e.g. [17]).

The simplest isotherm model consistent with the surface model assumed in this work is the bilangmuir isotherm. This model has been used successfully to describe the adsorption equilibrium behavior of enantiomeric pairs on various chiral stationary phases [18–21]. The equation is

$$q^*=q_1^*+q_2^*=\frac{a_1 C}{1+b_1 C}+\frac{a_2 C}{1+b_2 C} \quad (5)$$

In this equation, the numerical coefficients a_1 and b_1 correspond to the first type of adsorption sites, those involving the general or non-selective interactions. In enantiomeric separations, these coefficients are the same for the two optical isomers. The coefficients a_2 and b_2 correspond to the second type of sites, those responsible for the high energy, the selective, or the enantio-selective interactions, depending on the nature of the separation studied. Usually, a_2 is much smaller than a_1 and b_2 much larger than b_1. In this work, we consider only the linear part of the isotherms and that the products b_1C and b_2C are negligible compared to unity.

At infinite dilution, i.e. under linear conditions, the

retention factor of the peak is directly proportional to the initial slope of the isotherm, which for a bilangmuir adsorption isotherm is given by

$$k' = F(a_1 + a_2) \quad (6)$$

Only the sum of these two terms can be measured in linear studies. Their independent determination requires the measurement of the isotherm in a sufficiently wide concentration range.

2.3. Kinetics of mass transfer

A solid film linear driving force model was used to account for the mass transfer kinetics on each of the two types of sites [15]. This leads to the following set of equations

$$\frac{\partial q_1}{\partial t} = k_1(q_1^* - q_1) \quad (7)$$

where q_1^* is the stationary phase concentration in equilibrium with the mobile phase concentration C_1 (i.e., $q_1^* = a_1 C_1$).

$$\frac{\partial q_2}{\partial t} = k_2(q_2^* - q_2) \quad (8)$$

$$q = q_1 + q_2 \quad (9)$$

The peak tailing is assumed to originate from a slow desorption rate of the selective type of sites, which, generally, has a higher adsorption energy, hence a longer average residence time than the nonselective site.

3. Results and discussion

Eqs. (1–3) and Eqs. (7–9) constitute a modified transport–dispersive model, with a bilangmuir isotherm and different kinetic coefficients for the two kinds of sites. This model is a more realistic and general representation of the surface of chromatographic adsorbents than the classical one. It assumes that there is only one mode for the high energy site distribution, which seems to be in agreement with experimental results [17]. Certainly, this model of heterogenous adsorption behavior is most suitable for the study of enantiomeric separations, particularly in the cases in which proteins are used as chiral selectors (under linear conditions). The system of equations can be solved numerically by using the backward–forward finite difference method [15].

The following study is mainly focused on the influence of a slow kinetics of adsorption–desorption on the band profiles under linear conditions. Hence, only the rate constants k_1 and k_2 are varied systematically. Different values have been used for the slope of the isotherm for the high adsorption energy sites, a_2, from 10.0 to 0.004. The other parameters are held constant, $a_1 = 10.0$, the column length (10 cm), the flow velocity (6.02 cm min^{-1}, i.e., 0.80 ml min^{-1} in a 4.6 mm I.D. column), the injection time (1.0×10^{-4} min) and the total packing porosity, ϵ (0.8), and thus the phase ratio, F (0.25). The study is divided in two parts. In an introductory section, the influence of an homogeneous kinetics is illustrated as a reference. In a second part, the changes caused by a heterogeneous kinetics are demonstrated and discussed. In both cases, we first neglect the axial dispersion due to molecular and eddy diffusion (i.e., by setting $D = 0$), in order to better illustrate the effect of the heterogeneous mass transfer kinetics on the two kinds of adsorption sites, an effect which is assumed to be the main reason of peak tailing in linear chromatography. In this case, the mass balance equation of the ideal model is used and the finite column efficiency arises only from the slow mass transfer kinetics. It is given by [15]

$$\frac{1}{N} = \frac{2k_0'}{(1 + k_0')^2} \frac{1}{St} \quad (10)$$

where k_0' is the retention factor and St is the Stanton number ($St = kL/u$), which compares the average residence time and the hold-up time. If the adsorption–desorption kinetics is very fast, we have a highly efficient column and then, the influence of axial dispersion may become important. Even for a slow kinetics, however, the influence of axial dispersion may be significant. It is also studied, by varying the value of D.

3.1. Homogenous mass transfer kinetics

We discuss successively two cases, one in which $a_1 = a_2$, which is identical to the single-site problem and provides a convenient starting point, and the more general case in which the retention factors of the two mechanisms are different.

3.1.1. The two isotherms have the same slope, $a_1 = a_2$

In this case, Eq. (5) reduces to Eq. (4) and Eqs. (7–9) reduce to one single equation, e.g., Eq. (7) with $q_1^* = q^*$ and $q_1 = q$. This is the single-site problem, previously studied [13]. Fig. 1 shows a series of band profiles calculated with $a = 10.0$ and $D = 0$, for different values of the rate constant, from 0.1 to 1×10^4 min^{-1}. The chromatograms are given as the concentration profiles (in arbitrary unit) versus the retention factor, $k' = (t - t_0)/t_0$. With the numerical values selected for the parameters, the peak should elute with a maximum at $k' = 5.0$ and an unretained tracer at $k' = 0$.

At the lowest value of the rate constant ($k = 0.1$ min^{-1}, peak 1) however, the compound behaves as if it were unretained. A narrow peak is eluted which exhibits no retention. When the rate constant increases, the initial, unretained peak decays and a long tail is formed ($k = 0.3$ min^{-1}, peak 2). This tail becomes a very broad band (1 min^{-1}, peak 3), then an actual, but very broad peak is formed ($k = 10$ min^{-1}, peak 4). For larger values of the rate constant, the peak becomes taller and narrower (peaks 5 to 7, $k = 100$, 1000, and 10 000 min^{-1}, respectively). Note that the retention time of peaks 5 to 7 are nearly equal. This is expressed by the corresponding Stanton number.

As long as *St* is not large compared to unity, the average residence time is of the order of the hold-up time and the compound molecule do not spend any significant amount of time in the stationary phase. By contrast, when *St* is large compared with unity, the retention time in linear chromatography is determined by the slope of the isotherm and the band width by the mass transfer kinetics [Eq. (10)]. Only for very small values of the rate constant, between 0.1 and 10 min^{-1}, can we observe band profiles which deviate from the Gaussian profile, are unsymmetrical and tail. But the corresponding efficiency is only a few theoretical plates [1].

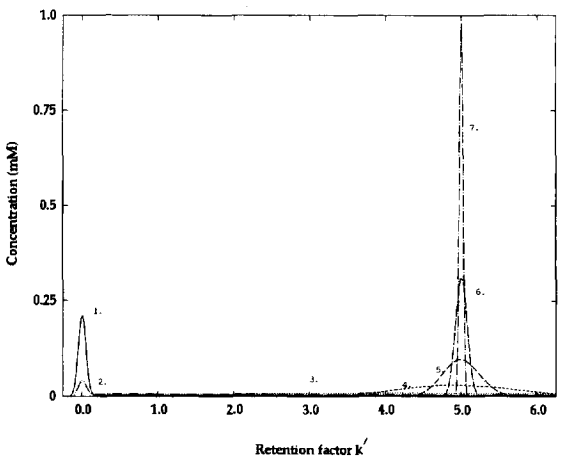

Fig. 1. Peak profiles calculated under linear conditions using the transport–dispersive model, assuming bilangmuir adsorption behavior with identical Langmuir terms and mass transfer coefficients. The column efficiency is infinite, thus N is ∞. The column length was 10 cm, the linear velocity was 6.02 cm min^{-1}, the injection time 0.1×10^{-4} min and the total packing porosity was 0.800. $a_1 = a_2 = 10.0$. In order to assure linearity, $b_1 = b_2 = 0$. The injected sample concentration was 1.0 *M*. The mass transfer coefficient corresponding to a_1 was k_1 and the one for a_2 was k_2. $k_1 = k_2$ and they were varied between 0.1 and 10 000 min^{-1}. The k values for the peaks 1–7 were (1) 0.1, (2) 0.3, (3) 1, (4) 10, (5) 100, (6) 1000 and (7) 10 000 min^{-1}.

3.1.2. The two isotherms have different slopes, $a_1 \neq a_2$

Fig. 2 shows the band profiles obtained under the same conditions as in Fig. 1, except for $a_1 = 10.0$ and $a_2 = 2.0$. The retention factor observed [Eq. (6)] is almost half as large as in the previous case. Otherwise, the band profiles are very similar to those in Fig. 1. There is no significant peak tailing, except for

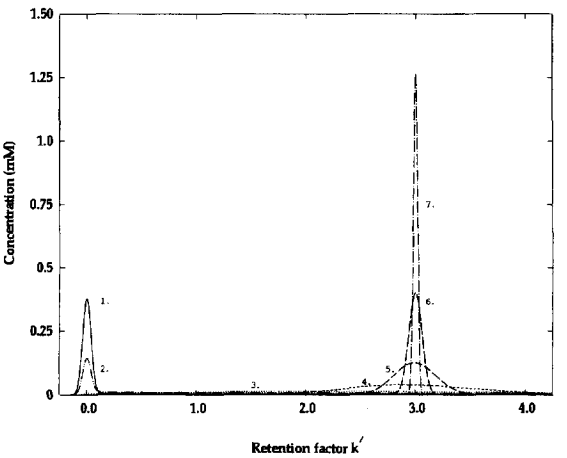

Fig. 2. As Fig. 1, but $a_2 = 2$.

very low values of the rate constant, between 0.1 and 10 min^{-1}, as in Fig. 1. These results seem to exclude the possibility of observing band asymmetry and tailing when the rate constant of the two retention mechanisms are similar. This will be demonstrated by a detailed study of the band profiles in the transition range.

3.1.3. Detailed study of the transition range

The results in Fig. 1 and Fig. 2 show that there is a transition range for values of the rate constant between 0.1 and approximately 10 min^{-1}. This transition requires further attention. Fig. 3 illustrates it further by showing profiles calculated under the same conditions as Fig. 1. It shows the progressive change of the band profile from an almost Gaussian profile when mass transfers are moderately fast (peak 6, $k=10$ min^{-1}) to the profile found at very low values of the rate constant, a small unretained peak followed by a very long tail (peak 1–3, $k=0.1$–0.5 min^{-1}). The intermediate profiles were obtained for values of k of 1.0, 2.5 and 10.0 min^{-1}. We note that the split peak phenomenon would be observed for values of the rate constant below 1 min^{-1}. The peak asymmetry factor (second or late part over first or early part of the peak width at 10% of the peak height) is given for the last three peaks in the figure.

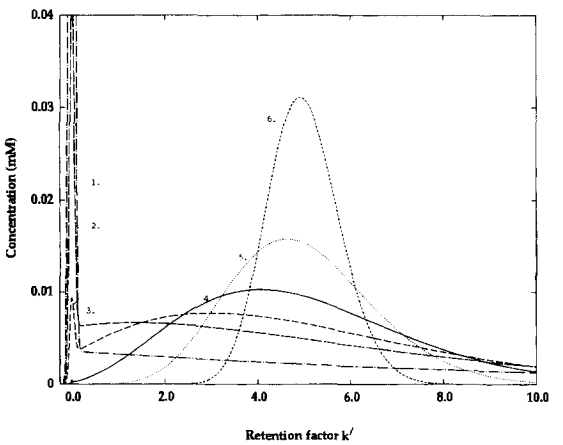

Fig. 3. As Fig. 1, but the variation of the mass transfer coefficients was focused on the intermediate region were the peaks are split and broaden, i.e. 1.0–100 min^{-1}. $k_1=k_2$ and their values for peaks 1–6 were: (1) 0.1; (2) 0.3; (3) 0.5; (4) 1.0; (5) 2.5 and (6) 10 min^{-1}. Peak asymmetry factors (at 10% of peak height): (4) 1.80; (5) 1.42 and (6) 1.19.

It shows a rapid decrease of the asymmetry with increasing value of the rate coefficient.

Tailing profiles, without splits, are observed only for values of the rate constant between 1.0 and 10 min^{-1}. This tailing is negligible compared to the one observed under conditions of heterogeneous kinetics, as will be shown later.

3.2. Heterogenous mass transfer kinetics

3.2.1. Overview over extreme conditions

We have shown in the previous section that when the rate constant is very low, 0.1 min^{-1}, the compound does not recognize the existence of the corresponding sites and is not retained on them. This is confirmed in the case of an heterogeneous, mixed retention mechanism, as shown in Fig. 4. In this figure four peak profiles are shown, corresponding to the four possible combinations of a rate constant equal to either 0.1 or 1×10^4 min^{-1} on either type of sites. The isotherm slopes for the two types of sites are $a_1=10$ and $a_2=2$. As expected, a rate constant equal to 0.1 min^{-1} is for all practical purposes equivalent to zero. When the rate constant is equal to

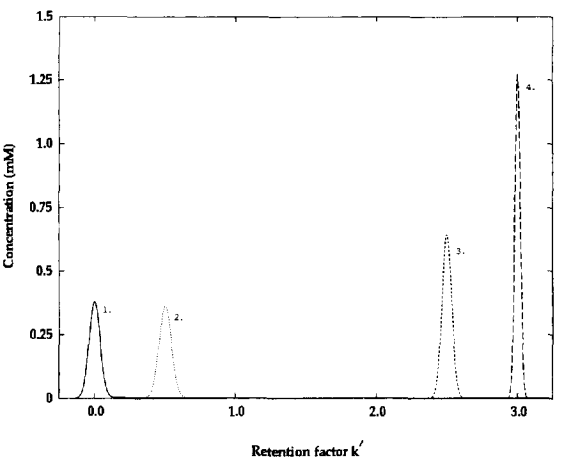

Fig. 4. Calculated profiles illustrating the different peak retention times possible at conditions where the mass transfer kinetics is either infinite fast at the one type of adsorption sites and infinite slow at the other one, or infinite fast or slow at both types of sites. $a_1=10$ and $a_2=2$. The values of the mass transfer coefficients k_1 and k_2 for peaks were: (1) $k_1=k_2=0.1$ min^{-1}; (2) $k_1=0.1$ and $k_2=10\,000$; (3) $k_1=10\,000$ and $k_2=0.1$ and (4) $k_1=k_2=10\,000$ min^{-1}.

10 000, near equilibrium is constantly achieved between the two phases. Thus, peak 1 ($k_1 = k_2 = 0.1$ min^{-1}) is not retained. For peak 2 ($k_1 = 0.1$, $k_2 = 10\,000$ min^{-1}) there is retention only on the second type of sites ($a_2 = 2.0$). Similarly, for peak 3 ($k_1 = 10\,000$ min^{-1}, $k_2 = 0.1$ min^{-1}) there is retention only on the first type of sites ($a_1 = 10.0$). Finally, for peak 4 ($k_1 = k_2 = 10\,000$ min^{-1}), there is retention on both types of sites and the eluted peak has a retention factor equal to $F(a_1 + a_2) = 3$.

3.2.2. Slow kinetics on one type of site and varied kinetics on the other one

We now study the changes in peak profile caused by a variation of the rate constant of adsorption–desorption on one type of sites while the rate constant for the other type of sites remains constant. Fig. 5A illustrates the effect for a value of the rate constant $k_2 = 10.0$ min^{-1}, while k_1 is increased from 0.1 (peak 1) to 10 000 min^{-1} (peak 6). Now, all peaks exhibit strong tailing effects, even the last one.

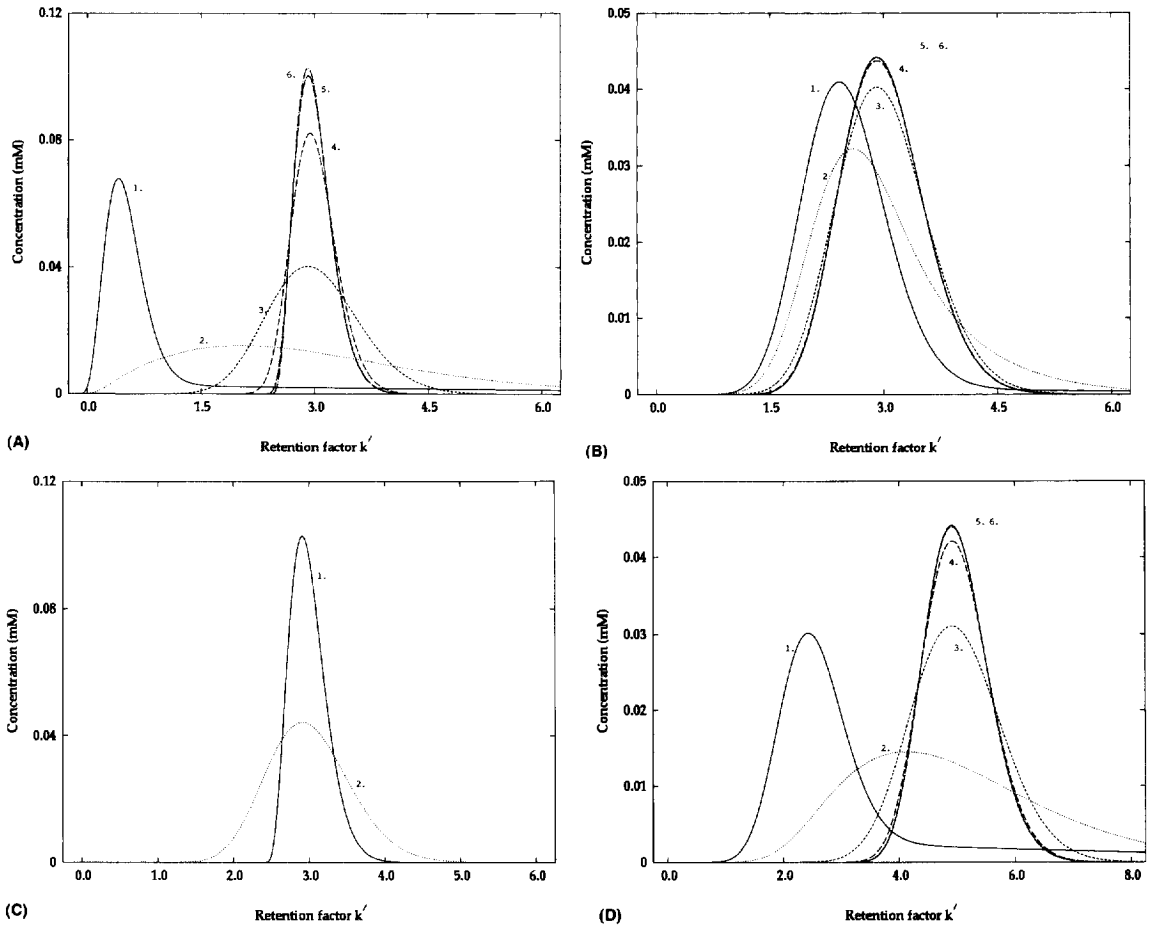

Fig. 5. As Fig. 2 ($a_1 = 10$ and $a_2 = 2$) but with unchanged, slow mass transfer kinetics on one of the types of adsorption site while the mass transfer kinetics were varied in a broad range for the other type of adsorption site. (A) k_2 was held constant at 10.0 min^{-1} and k_1 was varied between 0.1–10 000 min^{-1}. Peak asymmetry factor: (3) 1.24; (4) 1.36; (5) 1.71 and (6) 1.81. (B) k_1 was held constant at 10.0 min^{-1} and k_2 was varied between 0.1–10 000 min^{-1}. Peak asymmetry factors: (3) 1.25; (4) 1.34; (5) 1.27 and (6) 1.28. (C) Peak 1, k_1 is 10 000 min^{-1} and k_2 is 10, peak 2, k_1 is 10 and k_2 is 10 000 min^{-1}. Peak asymmetry factor: (1) 1.81 and (2) 1.28. (D) as (a) but $a_1 = a_2 = 10$. Peak asymmetry factor: (3) 1.19; (4) 1.24; (5) 1.27 and (6) 1.27.

As a matter of fact, the effect is especially important for peaks 5 and 6, for which adsorption–desorption is very fast on the sites of type 1 but slow on the type 2 sites. This tailing effect takes place in spite of the fact that the type 1 sites contribute most of the retention ($a_1 = 10$, $a_2 = 2$, $a_1/a_2 = 5$), and that exchanges are very fast on these sites. The slow type of specific site gives only a minor contribution to the total retention factor, still its influence on the peak shape at slow mass transfer kinetics is dramatic. Note that the peak asymmetry factor (asf 10%) increases with increasing value of the rate coefficient. This tailing is due to the low value of k_2 and the decreasing contribution of the mass transfer resistance on the type 1 sites to the band broadening.

Fig. 5B shows the converse case. The rate constant for the first type of sites was kept constant ($k_1 = 10.0$ min^{-1}) and k_2 was increased progressively from 0.1 (peak 1) to 10 000 (peak 6) min^{-1}. So, in this case, the retention mechanism which contributes most to the retention is also the one with the slow kinetics. All the bands obtained are unsymmetrical, very broad and their tailing is severe. They are much broader than the peaks in Fig. 5A, corresponding to a much lower efficiency. The reason is that the tailing originates from the part of the surface which contributes most to the retention. In this case, the profiles are close to those observed with a slow homogeneous kinetics (compare peaks 4 and 5 in Fig. 3 with the peaks in Fig. 5B). In the case of heterogenous mass transfer kinetics, a significant efficiency can be observed only if the slow type of sites make a small contribution to the retention. Because they are few in proportion, these sites are difficult to eliminate in practice and this is why tailing can be so hard to reduce. In this case also, the peak asymmetry factor increases with increasing value of k_2 but the effect is nearly negligible. This is because the retention is now controlled by the slow sites.

A comparison between the two situations discussed above is provided in Fig. 5C which shows the two complementary profiles obtained with a high value of one rate constant and a low value of the other rate constant. For the first peak, $k_1 = 10\,000$ min^{-1} and $k_2 = 10$ min^{-1}. For the second peak, $k_1 = 10$ and $k_2 = 10\,000$ min^{-1}. Although not very efficient, the first peak is closely related to the tailing peaks observed in linear chromatography, whether in enantio-selective separations or during the elution of strongly polar (e.g., basic) solutes in reversed-phase liquid chromatography. We also illustrate in Fig. 5D the situation when the contribution of the "active" sites to the retention factor is comparable to that of the general, nonselective interactions. In Fig. 5D $a_1 = a_2$ and all the other parameters are as in Fig. 5A. The peak tailing is again important, but the bands are broad and have a low efficiency. The profiles are intermediate between those shown in Fig. 5A and Fig. 5B because the contribution of the second type of sites to the retention factor is too large.

The above result shows that when the contribution of the slow sites to the retention factor becomes large and dominates over the contribution of the fast sites to the retention, the profile of the whole peak becomes more similar to that obtained in cases of slow homogenous mass transfer kinetics (cf. Fig. 3). In contrast, when the contribution of the slow sites to the retention becomes small or even negligible, the band becomes narrow, its upper part tries to advertise a high column efficiency, but significant peak tailing appears close to the baseline. This tailing is due to the heterogenous mass transfer kinetics. The limits of this situation were investigated. In Fig. 6A and Fig. 6B, the value of a_2 is very small and k_2 is either (A) 10 or (B) 50 min^{-1}. The values of a_2 were 0.1, 0.04, 0.01 and 0.004 for the peaks 1, 2, 3, and 4, respectively. In other words, the ratios a_1/a_2 and the relative contributions of the two retention mechanisms to the retention factor were 100, 250, 1000 and 2500, respectively. The figures illustrate the L-shaped nature of the profiles and the importance of the tailing at the peak base in all cases. The inserts of the two figures show a considerable enlargement of the rear profile close to the baseline. A definitive albeit small degree of tailing appears even for a value of a_2 which is more than 1000 times smaller than a_1. The smaller the contribution to the retention of the slow type of sites, the less important the area of the tail. The duration of this tail is more weakly affected and the L-shaped character of the peak tailing is enhanced. The same trend can be seen in Fig. 6B for which the kinetics on the slow sites is 5 times faster than for Fig. 6A. In this case, however, the L-shaped character of the peak flattens out and the tail decays faster when the kinetics of adsorption–desorption on the slow sites become faster.

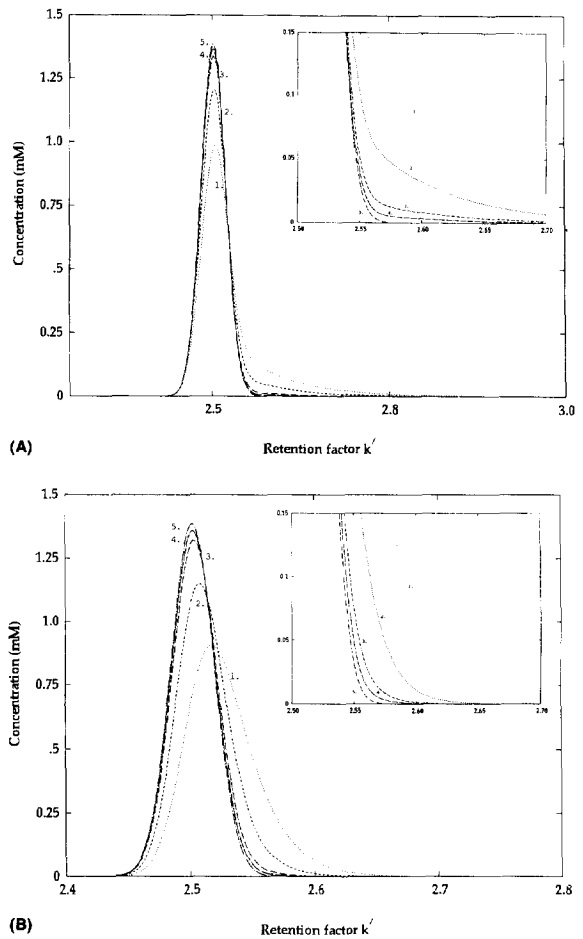

Fig. 6. As Fig. 1 but $a_1 = 10$, and a_2 has different and small values, k_1 is 10 000 and k_2 is either (A) 10 or (B) 50 min^{-1}. The a_2 values for the peaks 1–4 were: (1) 0.1; (2) 0.04; (3) 0.01 and (4) 0.004. Peak 5 was used as a reference since it lacked any kind of peak tailing since $a_2 = k_2 = 0$. (A) Peak asymmetry factor: (1) 1.86 and all other peaks 1.0. (B) Peak asymmetry factor: (1) 1.50; (2) 1.19 and all other peaks 1.0.

Note that the peak asymmetry factor at 10% of the peak height is not a good measure of the peak asymmetry in Fig. 6A and Fig. 6B. When a_2 becomes small, the tail affects only the low part of the profile and only a peak asymmetry factor at a few % of the peak height could illustrate it. Unfortunately, such a peak asymmetry factor would be of impractical use, being too sensitive to baseline noise or drift.

3.2.3. Very fast kinetics on the general type of sites and slow kinetics on the selective type of sites

This is an important case in actual practice. Adsorbents used in HPLC often contain, wilfully or not, active sites which contribute to some extent to the retention. They may be nefarious active sites which cannot possibly be entirely eliminated. They may be special groups bonded to create or enhance some selectivity (e.g., in enantio-selective separations or in complexation separations). Fig. 7 shows profiles calculated with $a_1 = 10$ and $a_2 = 2$ (as in Fig. 5A and Fig. 5B) and $k_1 = 10\,000$ min^{-1}. k_2 was varied between 1.0 and 100 min^{-1}. Starting from $k_2 = 1.0$ min^{-1} gives a peak which is eluted with a retention factor equal to Fa_1 and has a strong tail (peak 1). This is due to the slow kinetics of adsorption–desorption on the second type of site which prevents most molecules of the compound from interacting with these sites. Increasing the value of k_2 causes the peak height to decrease at first. Then the peak maximum moves progressively toward the retention factor $F(a_1 + a_2)$ and its height starts to increase again when the kinetics of exchange on the type 2 sites becomes fast enough. In this case, the

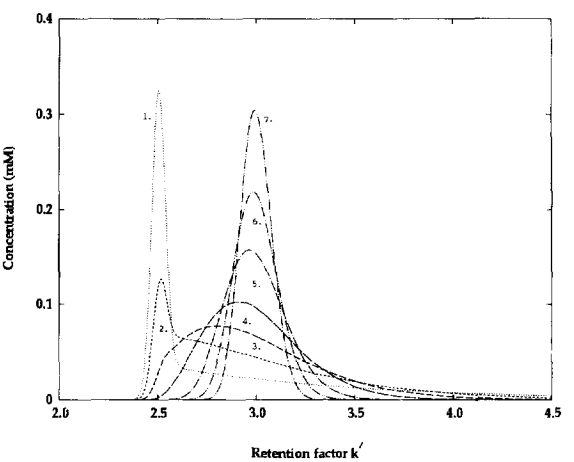

Fig. 7. As Fig. 2 ($a_1 = 10$ and $a_2 = 2$) but with k_1 held constant at 10 000 min^{-1} and k_2 varied between 1.0 $min-1$ and 100 min^{-1} illustrating the tailing profile region. The k_2 values for the peaks 1–7 are: (1) 1.0; (2) 2.5; (3) 5; (4) 10; (5) 25; (6) 50 and (7) 100 min^{-1}. Peak asymmetry factor:(4) 1.81; (5) 1.39; (6) 1.25 and (7) 1.19.

transition appears to be for k_2 between 1.0 and 25 min^{-1}. Finally, we should note the important difference between the shape of the band profiles calculated with the transport model and shown in Figs. 1–7 and those derived from the stochastic model of Giddings and Eyring [8]. A detailed comparison between the profiles predicted by these two models warrants further investigations.

In all cases, the peaks exhibit some degree of tailing. This tailing is strong for low values of the mass transfer kinetics but its intensity decreases with increasing value of the rate constant. When the rate constant of the mass transfer kinetics becomes relatively high and the type 2 sites fully contribute to retention, i.e. for k_2 larger than ca. 50.0 min^{-1} (peaks 6 and 7 in Fig. 7), the tailing decreases as shown by the values of the peak asymmetry factor (see caption). There is a slight tailing left when $k_2 = 100$ min^{-1} (peak 7 in Fig. 7, peak asymmetry factor at 10% equal to 1.19).

3.2.4. *Influence of axial dispersion*

In all the band profile calculations discussed so far, the influence of axial dispersion was neglected. The axial dispersion coefficient was set to 0 in Eq. (1). It is important however, to understand what influence axial dispersion may have on the band profiles. Fig. 8 illustrates band profiles exhibiting the combined effects of a slow mass transfer kinetics and finite axial dispersion. Except for the value of the axial dispersion coefficient, these profiles were calculated under the same conditions as the last three profiles in Fig. 7. The values of the axial dispersion coefficient used corresponded to column efficiencies of ∞, 1000 and 300 theoretical plates, respectively.

Fig. 8A shows the influence of axial dispersion on the peak profile obtained for $a_1 = 10$, $a_2 = 2$ and $k_2 = 10$ min^{-1}. The three profiles correspond to values of the plate number of ∞, 1000 and 300, respectively. Fig. 8B shows the same result for $k_2 = 25$ min^{-1}, and Fig. 8C for $k_2 = 50$ min^{-1}. These figures demonstrate that the slower the mass transfer kinetics on the selective type of sites and the higher the column efficiency due to molecular diffusion and eddy diffusion, the more pronounced the tailing effect is. The influence of dispersion on the rear of

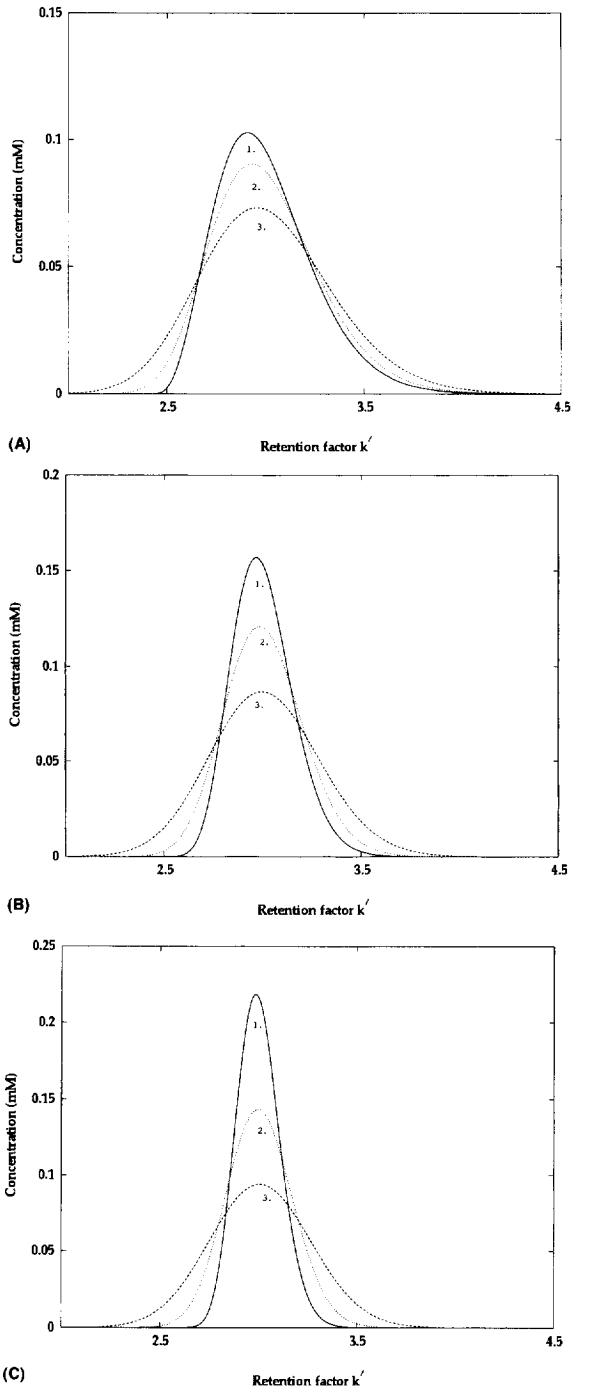

Fig. 8. As Fig. 7 but the effects of the axial dispersion was taken into consideration and the number of plates, N, for the peaks were: (1) ∞; (2) 1000 and (3) 300. The value of the k_2 was: (A) 10; (B) 25 and (C) 50 min^{-1}.

the profile decreases with decreasing value of the rate constant. It is nearly negligible in Fig. 8A, moderate in Fig. 8B, significant in Fig. 8C. This is explained by the considerable dispersion of the rear diffuse boundary of the profile at slow mass transfer kinetics. On the other hand, the front of the band becomes more disperse at low column efficiency as expected. When the rate constant is sufficiently high, the peak becomes symmetrical. This does not happen when the rate constant is low.

4. Conclusions

The excessive peak tailing often encountered in analytical separations performed by chromatography under linear concentrations can be explained by a mixed retention mechanism. This mechanism combines a classical adsorption on a nonselective surface having a moderate adsorption energy and a fast kinetics of adsorption–desorption and strong, selective interactions with a small number of active sites and a high interaction energy. The selective interactions contribute moderately to the total retention but have a slow kinetics which can cause, under the proper circumstances, serious peak tailing while keeping relatively high the apparent column efficiency.

This phenomenon is particularly important in the case of the strongly heterogenous biopolymeric surfaces which are often used for enantio-selective separations. Tailing is particularly intense when the ratio between the contributions to the retention factor of the general mechanism and the selective one exceeds 5 (i.e., $a_1/a_2>5$). The tailing becomes more L-shaped at the base when this ratio increases. It is important even when $a_1/a_2>1000$. Also, for tailing to occur, the ratio between the corresponding mass transfer coefficients should be between 20 and 2000. When the latter ratio exceeds 10 000 there are no tailing effects.

Experimental results illustrating these effects in the case of the separation of the enantiomers of propranolol on a protein, cellobiohydrolase I, chemically bonded to silica will be discussed in the near future [16].

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