ELSEVIER

Journal of Chromatography A, 728 (1996) 15–23

JOURNAL OF CHROMATOGRAPHY A

Influence of the concentration dependence of the mass transfer properties on the chromatographic band profiles I. Apparent axial dispersion coefficient in frontal analysis

Peter Sajonz[a,b], Guoming Zhong[a,b], Georges Guiochon[a,b,*]

[a]*Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA*

[b]*Division of Analytical Chemistry, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120, USA*

Abstract

The influence of the concentration dependence of axial dispersion on the profiles of breakthrough curves was studied using the equilibrium–dispersive model. A simple linear relationship between the apparent axial dispersion coefficient and the concentration was assumed and calculations of band profiles were carried out for both increasing and decreasing values of the axial dispersion coefficient with increasing concentration. A comparison of the profiles with those obtained with a constant coefficient shows that the effect of a concentration dependence is significant even for moderate variations.

Keywords: Frontal analysis; Axial dispersion coefficient; Mass transfer; Brand profiles; Breakthrough curves; Thermodynamic parameters; Dispersion

1. Introduction

It has been established that molecular diffusion, axial dispersion and mass transfer kinetics all depend on the concentration [1–7]. However, the nature of the functional dependence has not been widely investigated, even though some results suggest that this dependence may be quite significant in cases of practical importance [6].

There is an increased interest in this problem among chromatographers and a number of investigations on the concentration dependence of mass transfer properties have been recently published. These studies are concerned with the dependence of the axial dispersion [1], the diffusion coefficient of proteins, and the effective diffusivity on the sorbate concentration [4]. In our group, the axial dispersion coefficient and the rate constant of mass transfer of some proteins (e.g., BSA) was found to be strongly dependent on the concentration [6]. While the diffusion coefficient of proteins has been found to vary linearly with their concentration [2,3], we have found that their mass transfer rate constant in chromatographic columns increases nearly linearly with increasing concentration [6]. The dependence of mass transfer properties on the concentration is of special concern for proteins because these are the compounds for which the development of separation techniques requires the most difficult compromises between retention, which should be reasonable, selectivity and efficiency, which should both be high, and cost, which should be low. Mass transfer of proteins tends to be sluggish in most cases and this prevents using high mobile-phase velocities, hence reduces the production rate which can be achieved.

*Corresponding author (University of Tennessee). Tel.: +1 (423) 974 0733; Fax: +1 (423) 974 2667.

0021-9673/96/$15.00
SSDI 0021-9673(95)01109-9

The systematic investigation of this phenomenon requires the availability of a calculation procedure and the investigation of the results of a number of computer experiments. These experiments permit considerable savings in time, money, and wasted chemicals. In this first report, we consider the simplest problem, a linear dependence of the axial dispersion coefficient on the concentration, and we use the simplest nonideal model, the equilibrium–dispersive model [8]. This linear dependence of the apparent dispersion coefficient represents the balance of the concentration dependence of molecular diffusion, eddy diffusion, and the mass transfer kinetics. The goal of this work is to illustrate the effect of the concentration dependence of the parameters which control the kinetics of equilibration in chromatographic columns on the profile of the breakthrough curves. This work should guide further investigations. More complicated relationships, the influence of this concentration dependence on the band profiles in overloaded elution chromatography, and the interpretation of various experimental results will be considered in the future.

2. Theoretical

We used in this work the equilibrium–dispersive model of chromatography [8]. Returning to the original formulation of the differential mass balance equation of chromatography gives the main equation of this new version of a classical model.

2.1. Equations of the model

In the equilibrium–dispersive model, the equilibrium between the mobile and the stationary phase is assumed to be instantaneous and the contributions to the broadening effects of axial dispersion and the mass transfer resistances are lumped into an apparent axial dispersion coefficient, $D_a(C)$. The mass balance equation for one compound is then written:

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = \frac{\partial}{\partial z}\left[D_a(C)\frac{\partial C}{\partial z} \right] \quad (1)$$

where C and q are the concentrations in the mobile and the stationary phase, respectively, F is the phase ratio, and u is the mobile phase velocity. The simplest functional relationship, a linear dependence, is assumed for the apparent dispersion coefficient, $D_a(C)$, in this work:

$$D_a(C) = D_a^0 + D_a^1 C \quad (2)$$

where D_a^0 and D_a^1 are numerical coefficients.

The Langmuir isotherm is used for the adsorption equilibrium:

$$q = \frac{aC}{1 + bC} \quad (3)$$

The initial and boundary conditions used correspond to the frontal chromatography mode, with a column initially containing just pure mobile phase (no sample), and a step injection of a solution of concentration C_0 at the inlet of the column. Generally chromatography is carried out under such experimental conditions that apparent axial dispersion is relatively low, so no dispersion is necessary in the boundary condition. However, in this work we used the Danckwerts boundary condition because the Peclet numbers studied are very low and axial dispersion at the column inlet becomes significant [8]:

$$C(z,0) = 0,$$

$$uC(0,t) - D_a(C)\frac{\partial C}{\partial z}(0,t) = uC_0 \text{ for } t \geq 0,$$

$$\frac{\partial C}{\partial z}(L,t) = 0 \quad (4)$$

The system of Eqs. 1–4 states the equilibrium–dispersive model for a concentration-dependent apparent axial dispersion coefficient. There are no analytical solutions for this model.

2.2. Calculation of numerical solutions

Numerical calculations are easily carried out using the finite difference method with a Predictor–Corrector scheme [8,9]. A backward difference is used for the convection term in the Predictor step, a forward difference in the Corrector step. A central difference is always used for the diffusion term. The advantages of such a scheme are that it is stable and that the calculation error made is of second order.

The profiles of the breakthrough curves obtained are compared with those calculated with a constant

apparent axial dispersion coefficient, using the same calculation scheme. Because there is a large number of possible cases, it is more convenient to use the Peclet number, which is dimensionless, to classify them. The column Peclet number is given by:

$$Pe = 2N = \frac{uL}{D_a} \tag{5}$$

Note that a linear dependence of the dispersion coefficient on the concentration results in a hyperbolic relationship between both the Peclet number and the column efficiency on the one hand, and the concentration on the other and that Pe decreases with increasing axial dispersion coefficient. Combination of Eqs. 2 and 5 gives:

$$Pe = \frac{Pe^0}{1 + Pe^1 C} \tag{6}$$

with $Pe^0 = uL/D_a^0$ and $Pe^1 = D_a^1/D_a^0$.

3. Results and discussion

The results of the calculations are presented as plots of the concentration, C, versus the reduced time, t/t_0, where $t_0 = L/u$ is the hold-up time of the column. The following parameters have been kept constant throughout this study: the injection concentration, C_0=25 mg/ml, the phase ratio F=0.449, and the coefficients of the Langmuir isotherm, a=12 and b=0.024 ml/mg. Because the problem studied is important when mass transfer is slow (e.g., with most protein separations), relatively low values of the column plate number were selected in this work. To simplify the presentation of the results in spite of the complexity of the situation due to the large number of possible combinations, the results have been grouped and are discussed below. For the same reason, the Peclet number, Pe=2N, which is directly accessible from experimental data and well known in chromatography, is used as the main parameter in the whole discussion. In cases where it is useful to refer to D_a rather than to the Peclet number, we assume that uL = 1 cm^2/s, with e.g. L=10 cm and u=0.1 cm/s, corresponding to a value of t_0=100 s, a rather typical one.

In the following discussion the concentration dependence is expressed as Peclet number change from the initial value Pe (C=0) to the final value Pe (C=25).

3.1. Constant initial Peclet number

In this case, we study the influence of D_a^1 at a constant value of D_a^0. Fig. 1 shows a series of breakthrough curves obtained for a constant initial value of the Peclet number equal to 400[1]. The values of D_a^1 considered are between $-5\cdot10^{-5}$ and $+7\cdot10^{-4}$ cm^5 s^{-1} mg^{-1}, corresponding to changes in the value of Pe ranging from an increase up to 800 to a decrease down to 50, respectively. The profiles obtained for these variable Peclet numbers can be compared with those obtained for a constant Peclet number (Pe=400, solid line). Experimental results [6] suggest that these values are typical.

Obviously because the column efficiency is proportional to the Peclet number, when the Peclet number decreases with increasing concentration the breakthrough curve becomes broader, beginning earlier and ending later than for a constant value of Pe. The effect is especially important for the largest decrease in Pe, from 400 to 50 (first curve). Further comparison requires the definition of the retention

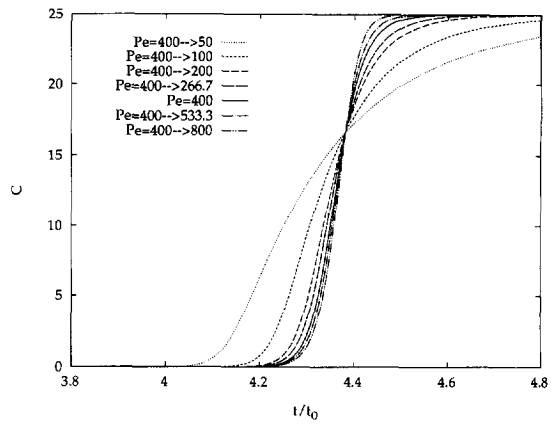

Fig. 1. Breakthrough curves at constant initial value of the Peclet number. The initial value is Pe=400. The final values are 50, 100, 200, 266.7, 400, 533.3, and 800, respectively.

[1] NB. This corresponds to D_a^0 = 0.0025 and to a column efficiency of 200 theoretical plates. Although low, this value is not unreasonable for proteins [6].

volume of the breakthrough curve. Although this is somewhat arbitrary [10], we consider in the following that this is the elution volume of the inflection point of the breakthrough curve. With this definition, the retention volume of the first breakthrough curve (*Pe* decreases from 400 to 50) is approximately 4% lower than that of the curve corresponding to $Pe=400$. This would cause the same relative error on the determination of isotherm data by frontal analysis if a constant Peclet number is assumed. A telling clue to the existence of such an effect would be the long time required to reach the equilibrium plateau.

Note that, because breakthrough profiles such as those shown here are constant patterns, the profile, hence the retention volume of the inflection point, depends on the whole set of Peclet numbers sampled by the concentration step. Higher concentrations have larger dispersion than lower ones, so they disperse faster than in the constant case. This faster dispersion spreads more sample molecules into the lower concentration zones and forces them forward. Thus, they elute from the column earlier. This explains why the retention time of the inflection point of the breakthrough curve varies with D_a^1, even though the Peclet number at $C=0$ is the same in all cases. A detailed study of the influence of the concentration dependence of the kinetic parameters of mass transfer on the retention volume of breakthrough curves and on the accuracy of the determination of isotherm data by frontal analysis will be published later [10].

Conversely, the breakthrough curve becomes sharper when *Pe* increases with increasing *C* (e.g., last curve in Fig. 1). Now, the column efficiency increases with increasing concentration, resulting in a top (i.e., end) part of the curve which is sharper than the bottom (i.e., beginning). The effect is not very important, however, because the profile at constant $Pe=400$ is already steep. It is noteworthy that all the breakthrough curves, whether corresponding to increasing or decreasing values of the Peclet number, intersect at the same point in the figure, located on the curve corresponding to the constant Peclet number $Pe=400$. Thus, the concentration corresponding to this point has a constant retention time, which can be obtained from the integration of breakthrough curves (area method) [7]. Except for the first two ones, the breakthrough curves have their inflection point close to the common intersect, so their retention volume is nearly independent of the value of *Pe*.

3.2. Constant final Peclet number

The breakthrough curves obtained in the converse case of a constant initial Peclet number (Fig. 1) are illustrated in Fig. 2, where curves corresponding to a constant final value of the Peclet number, $Pe=400$, are plotted. For these breakthrough curves, the initial and final values selected for the curves in Fig. 1 have been reversed. The breakthrough curves corresponding to values of *Pe* increasing with increasing concentrations (hence starting with low values of *Pe*) are broader than the curve corresponding to a constant value, $Pe=400$. These curves appear more disperse in the first part than in the last part. By contrast, when *Pe* decreases with increasing concentration, steeper breakthrough curves are obtained but these curves are sharper in the beginning than at the end (Fig. 2).

As in the previous case (Fig. 1), the breakthrough curves have a common intersection point (Fig. 2). However, this point is at a lower concentration ($C=10.2$ mg/ml) than the one in Fig. 1 ($C=16.6$ mg/ml). Another important feature in this case is that the range of variation of the retention volume of the inflection point of the breakthrough curve with the value of D_a^1 is much narrower, hence the systematic error made in the measurement of isotherm data is reduced.

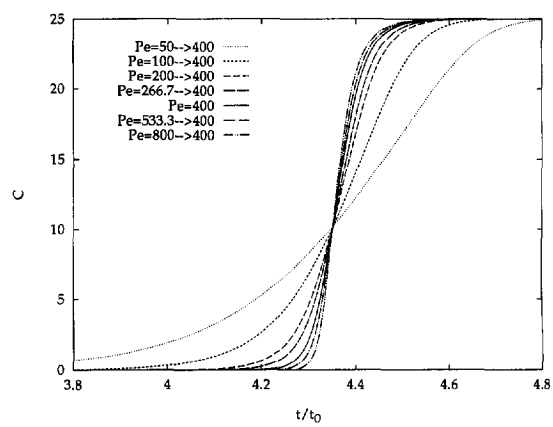

Fig. 2. Breakthrough curves at constant final value of the Peclet number. The final value is $Pe=400$. The initial values are 50, 100, 200, 266.7, 400, 533.3, 800, respectively.

3.3. Constant amplitude of the change of the value of the Peclet number

The effect of a constant absolute change of the Peclet number on the breakthrough curves is illustrated in Fig. 3. Two values of the change have been considered, $\Delta Pe = +250$ (Fig. 3a) and -250 (Fig. 3b). Other results (not shown) were obtained for changes of ± 50 and ± 150. In these figures, two sets of four curves are presented. The first set of curves was calculated with variable Peclet numbers. The other set was calculated with the constant Peclet number corresponding to the average value of the apparent axial dispersion coefficient (e.g., for *Pe* increasing from 50 to 300, D_a decreases from 0.02 to 0.003 $cm^2\ s^{-1}$; the average is 0.011667, corresponding to a Peclet number of 85.7). The inset shows a blow-up of the region around the inflection points of these curves.

By contrast with Fig. 1 and Fig. 2, in which all the curves intersect in the same point, the curves in Fig. 3 do not intersect at the same point (see insets). In the plots obtained for the lower values of the change, ΔPe, the various intersects look deceptively close, because these variations of ΔPe are not large enough to modify visibly the figure of the breakthrough curve compared to the one at constant *Pe* value. However, this behavior is clear in Fig. 3 in which a larger variation of ± 250 is used. The difference between a breakthrough curve with a variable Peclet number and the one corresponding to a constant average value of *Pe* is largest for the breakthrough curves with the lowest initial *Pe* in Fig. 3a and the lowest final *Pe* in Fig. 3b. This is due to the increasing amount of compound spread by dispersion.

3.4. Constant relative change of the value of the Peclet number

In Fig. 1 to Fig. 3, the ratio of the final to initial values of *Pe* is different for the different curves. The influence of a constant relative change of the value of the Peclet number on the breakthrough curve is illustrated in Fig. 4. Curves are shown only for ratios of 3 (Fig. 4a) and 1/3 (Fig. 4b) and compared to those obtained for constant *Pe* (constant values corresponding to the average value of D_a in the first set of curves). All the curves in these figures intersect at the same point. However, the concentration of this intersection point varies with the value of the ratio selected. For example, these concentrations are 15.1, 14.0, 12.6, and 13.0 mg/ml for ratios of 1/3, 2/3, 3/2, and 3/1, respectively.

3.5. Comparison of breakthrough curves corresponding to symmetrical changes in the value of the Peclet number

Fig. 5a compares three breakthrough curves corresponding each to a constant *Pe* number and two corresponding to linear variations between the same limits, in the opposite directions. The variations of *Pe* considered are from 200 to 400 and from 400 to 200. The constant values of *Pe* used are the two limits, 200 and 400, and 266.7, the value corre-

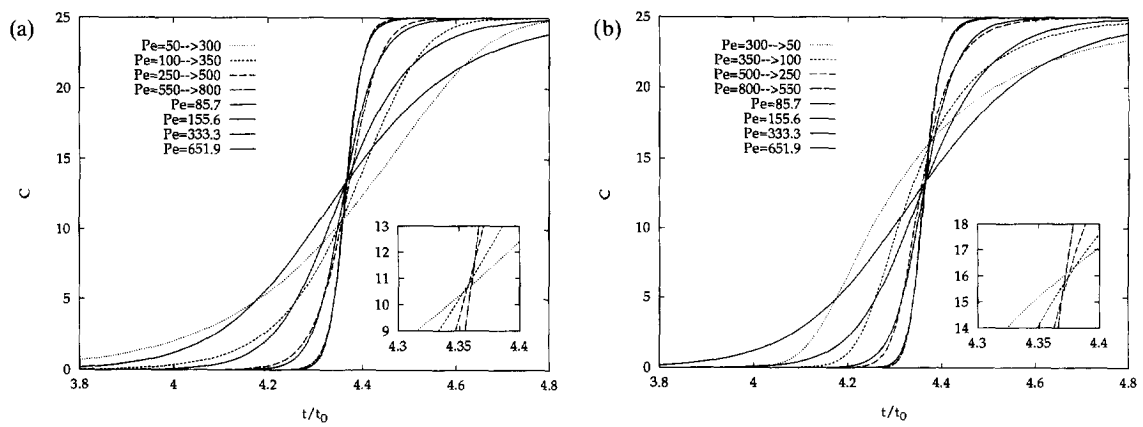

Fig. 3. Breakthrough curves at constant amplitude of the change of the Peclet number. (a) $\Delta Pe = +250$; (b) $\Delta Pe = -250$.

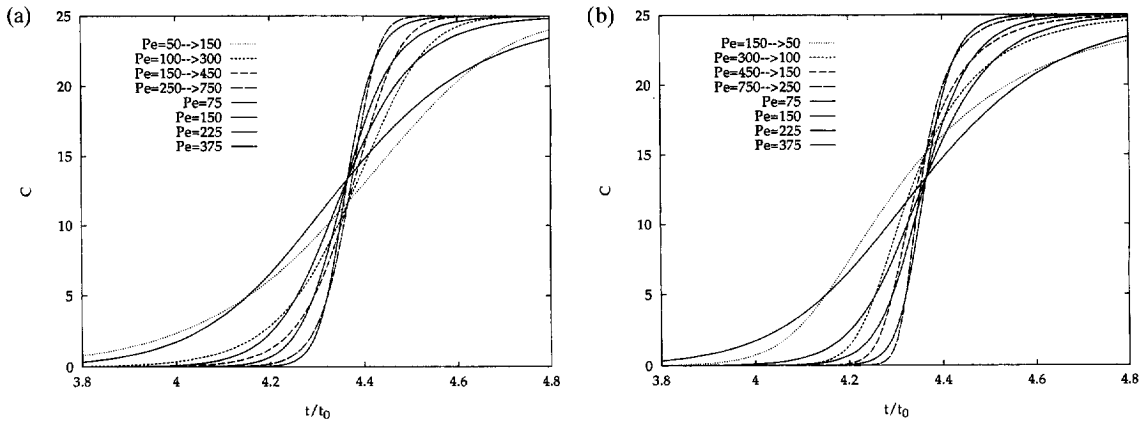

Fig. 4. Breakthrough curves at constant relative change of the value of the Peclet number. (a) The Peclet number increases threefold. (b) The Peclet number decreases threefold.

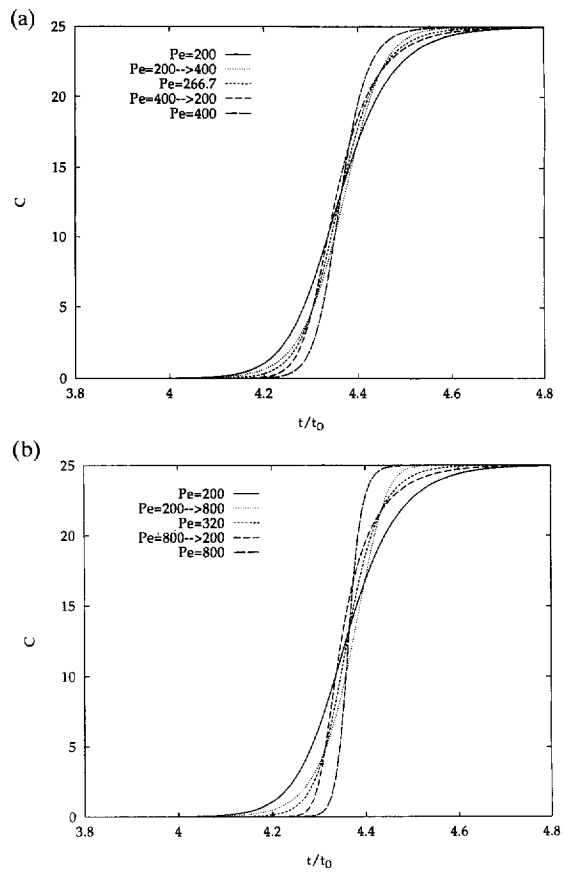

Fig. 5. Opposite increase and decrease of *Pe*. (Left) The Peclet number increases from 200 to 400 or decreases from 400 to 200. Breakthrough curves BC(200), BC(400), BC(266.7) (266.7 is the *Pe* corresponding to the average value of the dispersion coefficient), BC(200→400) and BC(400→200). (Right) The Peclet number increases from 200 to 800 or decreases from 800 to 200. Breakthrough curves BC(200), BC(800), BC(320) (320 is the *Pe* corresponding to the average value of the dispersion coefficient), BC(200→800), and BC(800→200).

sponding to the average dispersion coefficient. We call them BC(200), BC(400), and BC(266.7), respectively. These three curves intersect at the same point, almost in the middle of the concentration wave (13.4 versus 12.5 mg/ml for the average). The two curves corresponding to the opposite variations of the Peclet number are called BC(200→400) and BC(400→200). They intersect in two different points (concentrations, 4.7 and 21.7 mg/ml). The curve BC(266.7) goes through all these three intersection points (at 3.6, 15.5, and 22.25 mg/ml).

Compared with BC(266.7), curve BC(200→400) has a broader beginning and a sharper ending, while, conversely, curve BC(400→200) has a sharper beginning and a broader ending. This is because the axial dispersion is larger at the beginning of the former breakthrough curve and decreases with increasing concentrations, while the opposite is true for the latter. At moderate concentrations (below ca. 7 mg/ml), BC(200) and BC(200→400) are nearly parallel. At higher concentrations they come closer to each other and eventually intersect as BC(200→400) becomes steeper (Fig. 5a). The same is true for BC(400) and BC(400→200). The converse is true for the two couples BC(400) and BC(200→400), and BC(200) and BC(400→200) which intersect at low concentrations and become nearly parallel at high concentrations.

The same conclusions can be drawn from similar figures obtained for different values of the change in *Pe* and different values of the Peclet number corresponding to the average axial dispersion coefficient. Another illustration is given in Fig. 5b which shows the modifications of the breakthrough curves which take place as a response to changes of *Pe* from 200 to 800.

3.6. Influence of the amplitude of the change in Peclet number

Fig. 6 shows three sets of curves similar to those shown in Fig. 5, with three different values of $|\Delta Pe|$, 100, 150 and 400, respectively. Each set has two intersection points as pointed out in Fig. 5a, one at a low, the other at a high concentration. The three upper intersection points and the three lower ones have the same concentration. This figure illustrates the symmetry of the breakthrough curves with respect to the exchange of the limits of the variation

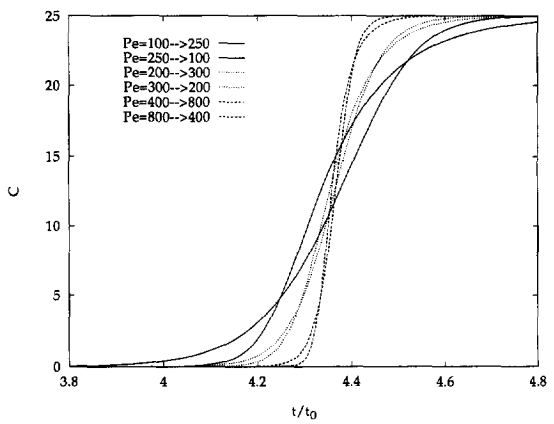

Fig. 6. Comparison between breakthrough curves corresponding to opposite step changes of *Pe*. The different curves correspond to increases and decreases of *Pe* by 100, 150 and 400.

of the Peclet number. As a consequence, this also underlines the fact that the breakthrough curve becomes unsymmetrical when the apparent axial dispersion coefficient is a function of the concentration. The stronger the concentration dependence, the more unsymmetrical the breakthrough curve. When *Pe* increases with increasing concentration the top or end part of the breakthrough curve becomes steeper than the bottom or initial part. The converse is true when *Pe* decreases with increasing concentration (Fig. 6).

3.7. Influence of the isotherm coefficients

A change in the first coefficient, *a*, of the isotherm has the trivial effect of shifting the retention time of the breakthrough curves. The value of the second coefficient, *b*, determines the saturation capacity of the stationary phase, hence the degree of nonlinear behavior experienced for a certain concentration. A change in *b* modifies the thickness of the shock layer (or mass transfer zone) [8]. However, a change in the value of *b* has the same effect as the same relative change in the height of the concentration step. The parameter *b* and the concentration should not be considered separately; the product *bC* is the important parameter (see Eq. 3). When this product increases, the breakthrough curves become steeper.

Fig. 7 shows the breakthrough curves calculated assuming a value of the second coefficient of the isotherm five times larger that in all previous figures

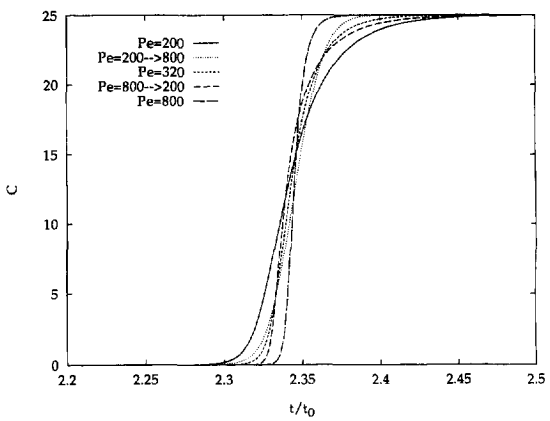

Fig. 7. Influence on the breakthrough curve of a change in the second coefficient of the isotherm. Same as in Fig. 5b, except the Langmuir parameter b=0.12 ml/mg (i.e., 5 times larger).

(i.e., b=0.12 ml/mg instead of 0.024 ml/mg), the same step concentration, the same values of the Peclet number and the same parameters of its concentration dependence as used in Fig. 5b. Comparing these two sets of figures shows that, due to the increased intensity of the nonlinear behavior, the breakthrough curves have become steeper (different scales in these two figures). The middle intersection point in Fig. 7 has a higher concentration than that in Fig. 5b, while the lower and upper intersection points have nearly the same concentrations.

4. Conclusion

The influence of the concentration dependence of the apparent axial dispersion coefficient on the profile of breakthrough curves may be very important. The extent of variation of Pe observed experimentally in the range of concentrations typically used in liquid chromatography can be as large as the widest ranges considered in the various examples given above. This phenomenon seems to be especially important for proteins [6].

A strong dependence of the shape of the breakthrough curve and the retention volume of its inflection point on the concentration has important consequences on the accuracy of measurements done by frontal analysis [7]. It is widely assumed that the retention volume of the breakthrough curve does not depend on the value of the rate coefficient of mass transfer or of the axial dispersion coefficient [8]. Although this assumption is true if the retention volume is derived from the integration of the breakthrough profile, its validity does not extend to retention times determined using alternative methods [7] which are more practical and much more widely used. In these cases, the validity of the assumption of breakthrough profiles independent from the rate coefficient of the mass transfer kinetics falters, as we have shown in this work, when the apparent axial dispersion coefficient depends on the concentration.

5. Glossary of symbols

a	First numerical parameter of the Langmuir isotherm
b	Second numerical parameter of the Langmuir isotherm
C	Liquid phase concentration of the component
C_0	Injection concentration of the component
D_a	Apparent dispersion coefficient
D_a^0	Constant term of the apparent dispersion coefficient
D_a^1	Coefficient of the concentration dependent term of the apparent dispersion coefficient
F	Phase ratio
L	Column length
N	Column efficiency
Pe	Column Peclet number ($Pe = uL/D_a$)
Pe^0	Value of the Peclet number at C=0 ($Pe^0 = uL/D_a^0$)
Pe^1	Coefficient of the concentration-dependent term of the Peclet number ($Pe^1 = D_a^1/D_a^0$)
q	Solid-phase concentration of the component
t	Time
t_0	Hold-up time of the column
u	Mobile-phase flow velocity
z	Position in the column

Acknowledgments

This work has been supported in part by Grant CHE-9201663 of the National Science Foundation

and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We acknowledge the support of our computational effort by the University of Tennessee Computing Center.

References

[1] K. Lederer, I. Amtmann, S. Vijaykumar and J. Billiani, J. Liq. Chromatogr., 13 (1990) 1849.
[2] W. Gallagher and C. Woodward, Biopolymers, 28 (1989) 2001.
[3] S. Gibbs, A. Chu, E. Lightfoot and T. Root, J. Phys. Chem., 93 (1991) 467.
[4] B. Al-Duri and G. McKay, J. Chem. Tech. Biotechnol., 55 (1992) 245.
[5] A. Seidel-Morgenstern, S.C. Jacobson and G. Guiochon, J. Chromatogr., 637 (1993) 19.
[6] H. Guan, P. Sajonz, G. Zhong and G. Guiochon, Biotechnol. Progr. (submitted).
[7] P. Sajonz, G. Zhong and G. Guiochon, J. Chromatogr. A, in press.
[8] G. Guiochon, S. Golshan-Shirazi and A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, NY, 1994.
[9] C. Hirsch, Numerical Computation of Internal and External Flow, John Wiley and Sons, Chichester, 1988, Vol. 2.
[10] P. Sajonz, G. Zhong and G. Guiochon, J. Chromatogr. (in preparation).