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Influence of the concentration dependence of the mass transfer properties on chromatographic band profiles II. Accuracy of the determination of isotherm data by 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*

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Abstract

Using known relationships between the apparent axial dispersion coefficient and the concentration, the process of isotherm determination by frontal analysis is modeled by calculating numerical solutions of the equilibrium–dispersive model and processing these simulated experimental data using classical methods of frontal analysis. The results show that significant systematic errors can take place when the apparent axial dispersion coefficient depends strongly on the concentration in the range investigated.

Keywords: Adsorption isotherms; Frontal analysis; Equilibrium–dispersive model; Band profiles; Axial dispersion coefficient, apparent

1. Introduction

In a previous paper [1], we have shown that the profile of a breakthrough curve is not the same whether the apparent axial dispersion coefficient is constant or is a function of the concentration. When the dependence of this dispersion coefficient on the concentration is strong, the breakthrough curve becomes unsymmetrical and the retention volumes of its half-height point and of its inflection point depend on the extent of the concentration dependence. This effect is especially important when the dispersion coefficient is initially large (i.e., the column efficiency is poor) and decreases rapidly with increasing concentration. Obviously, this phenomenon could have an influence on the equilibrium isotherm data derived from frontal analysis because of the procedures commonly used to derive breakthrough volumes [1]. Since experimental results suggest that the concentration dependence of mass transfer coefficients (rate constant, axial dispersion coefficient, or apparent axial dispersion coefficient) depend significantly on the concentration [2–6], the accuracy of the common implementations of frontal analysis [7] should be investigated.

It is generally considered that the retention volume of the breakthrough curve is constant, independent of the coefficients of the mass transfer kinetics across

*Corresponding author. Address for correspondence: Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA.

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the column but depends only on the equilibrium isotherm [7,8]. This stems from the fact that, because of the self-sharpening effect arising from a non-linear isotherm, the profile of a breakthrough curve tends towards an asymptotic limit. Cooney and Lightfoot [9] have shown that this limit is a constant pattern, that it propagates at a constant velocity, without broadening and that this velocity is equal to that of the shock under ideal conditions. As a consequence, frontal analysis is considered as an accurate method of isotherm data determination. By contrast, elution by characteristic points (ECP) and frontal analysis by characteristic points (FACP) introduce a model error. ECP is based on the relationship between a concentration and its retention volume on the diffuse side of an overloaded elution band. FACP uses the same equation on a diffuse breakthrough curve (obtained with a concentration jump in the direction opposed to that used in FA). The model error originates from the fact that this relationship is based on the ideal model, a model which assumes the column to have an infinite efficiency [10]. This model error can be quite significant if the column efficiency does not exceed several thousand theoretical plates [11,12]. For this reason, frontal analysis is preferred to ECP or FACP for accurate determinations, especially when the column efficiency is poor [7,12]. However, for practical reasons reviewed below, experimentalists prefer to derive breakthrough volumes from the retention times of either the half-height or the inflection point of the breakthrough profiles. It is often not realized that the retention volumes of these features of the breakthrough curves are independent of the parameters of mass transfer kinetics only if these coefficients are large enough and independent of the concentration. The conclusion becomes invalid when they depend on the concentration.

The goal of this paper is to determine the extent of the potential error made, to investigate which experiment design could minimize it and to suggest procedures of data handling to reduce it. It is impossible to know in advance the extent of a systematic error when performing actual experiments if reference materials are not available. The immense interest of computer experiments is that we can calculate breakthrough profiles using any model of chromatography and whatever dependence of the parameters of these models on the concentration that we please. Then we can introduce these profiles in the software used to handle experimental data and derive, in the specific case discussed here, the best values of the isotherm data. The difference between the initial values and those derived from a best fit of the experimental profiles to the physical model gives an estimate of the bias introduced. In the present case, we calculate 'experimental data' using values of the apparent axial dispersion coefficient which are a function of the concentration and we use to evaluate these profiles the conventional procedures [7] which assume them to be constant.

2. Theoretical

2.1. Model used to generate the 'experimental profiles'

We use a modified equilibrium–dispersive model.

2.1.1. Mass balance equation

In the classical equilibrium–dispersive model, it is assumed that there is instantaneous equilibrium between the mobile and the stationary phase and that the contributions to the broadening effects due to axial dispersion and the mass transfer resistances across actual columns can be accounted for by lumping them into an apparent axial dispersion coefficient, $D_a(C)$. If we assume that this dispersion coefficient is a function of concentration, the mass balance equation for one compound is written [13]

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

where C and q are the concentrations in the mobile and the stationary phase, respectively, F is the phase ratio [$F = (1 - \varepsilon)/\varepsilon$, with ε total column porosity], and u is the mobile phase velocity.

2.1.2. Concentration dependence of the apparent axial dispersion coefficient

We assume a linear dependence of the apparent dispersion coefficient, $D_a(C)$, with

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

$$Pe(C) = \frac{uL}{D_a(C)} = \frac{Pe^0}{1 + Pe^1 C} \quad (2b)$$

where D_a^0, D_a^1, $Pe^0 = uL/D_a^0$, and $Pe^1 = D_a^1/D_a^0$ are numerical coefficients[1].

2.1.3. Isotherm model

The Langmuir isotherm is used for the adsorption equilibrium

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

Its numerical coefficients, a and b, are independent of the concentration.

2.1.4. Initial condition

The initial condition used corresponds to a column containing a constant concentration of solute. Two experimental procedures are classically used to implement FA. In the step series method (SSM), the column initially contains no sample, but is flushed with pure mobile phase and a step injection of a solution of concentration C_n is done at the inlet of the column. The column is flushed with pure mobile phase between two successive experiments, so for each breakthrough curve, the concentration increases from 0 to C_n. Thus the initial condition is

$$C(z, 0) = 0 \quad (4a)$$

In the staircase method (SCM), a series of steps is done and the column is not flushed between the injection of successive steps. Thus, a series of concentration steps C_{n-1} to C_n, to C_{n+1} are injected into the column and, as in a staircase, the height of each successive step is approximately constant. Then, the initial condition for the nth step is

$$C_n(z, 0) = C_{n-1} \quad (4b)$$

[1] Throughout this paper, the concentration dependence of Pe is expressed as $Pe(Pe_0 \rightarrow Pe_{25})$, where Pe_0 and Pe_{25} are the values of Pe for the concentrations $C = 0$ and $C = 25$ mg/ml, respectively.

2.1.5. Boundary conditions

The boundary conditions used are those of the frontal chromatography mode. Because chromatography is usually carried out under such experimental conditions that the apparent axial dispersion is relatively low, no dispersion is usually assumed in the boundary conditions [13]. However, in the present study, we use relatively high values of the apparent axial dispersion coefficient, in compliance with the requirement to simulate the conventional determinations of the isotherm data of biopolymers for which the column efficiency is rather poor. If the column efficiency is high, breakthrough profiles are nearly vertical and the determination of their retention volumes is accurate and precise. With high average values of $D_a(C)$, the approximation made in neglecting dispersion in the boundary condition is no longer valid. Therefore, we used the Danckwerts condition [13,15], which is an open–closed condition.

For the SSM method, with a concentration step from 0 to C_n, we have

$$uC(0, t) - D_a(C)\frac{\partial C}{\partial z}(0, t) = uC_n \text{ and}$$
$$\frac{\partial C}{\partial z}(L, t) = 0 \text{ for } t \geq 0 \quad (5a)$$

In the SCM method, the open–closed boundary conditions for the calculation of the nth breakthrough profile become

$$uC(0, t) - D_a(C)\frac{\partial C}{\partial z}(0, t) = u(C_n - C_{n-1}) \text{ and}$$
$$\frac{\partial C}{\partial z}(L, t) = 0 \text{ for } t \geq 0, \quad (5b)$$

The 'experimental data' consist of a set of breakthrough curves obtained with increasing values of C_n in a certain range. For the sake of an easy comparison between the two methods, we have taken, in both cases, the same series of values for C_n.

2.1.6. Equation system

The system of Eqs. 1–5 states the equilibrium–dispersive model for a concentration dependent apparent axial dispersion coefficient. There are no analytical solutions for this model. However, numerical solutions are easily calculated [1].

2.2. Model used to account for the 'experimental profiles'

'Experimental' breakthrough curves (BC) are calculated as explained in the previous section, each BC file containing 1000 data points. In this calculation, various relationships are used to account for the concentration dependence of the apparent axial dispersion coefficient, $D_a(C)$. The amount adsorbed in equilibrium with a concentration C_n is derived from the retention volume of the breakthrough curve, using the conventional methods of frontal analysis which assume D_a to be constant [7]. There are different methods available to determine this retention volume. It can be derived from the retention volume of the inflection point, of the point at half-height of the concentration wave, or it can be calculated from the area over the breakthrough curve, an area which is directly proportional to the amount adsorbed. We compare the results obtained with these three methods.

Once the isotherm data have been derived, the isotherm parameters are estimated by fitting these data to a Langmuir model. For this fitting procedure, a non-linear regression method (SAS) was used. The following function was minimized, using Marquard's method:

$$\sigma = \sqrt{\frac{1}{N_D - P} \sum_{i=1}^{N_D} \left(\frac{q_i^{ex} - q_i^{th}}{q_i^{ex}} \right)^2} \quad (6)$$

where P is the number of model parameters (2 for the Langmuir isotherm), N_D is the number of data points, q_i^{ex} and q_i^{th} are the 'experimental' data points and the values calculated from the model, respectively. The choice of Marquard's method is made in order to account more accurately for the initial slope of the isotherm, hence to improve the accuracy on the a parameter [7,14]. This approach is required to allow the calculation of band profiles and breakthrough curve profiles which have a retention time in close agreement with experimental results [6,7,10,14]. As a consequence and by compensation, the error made on the estimation of the b-term is larger.

Knowing the best value of the isotherm parameters, the average value of the apparent axial dispersion coefficient for each BC is derived by fitting the experimental data to breakthrough curves calculated with a constant dispersion coefficient and looking for the value of this coefficient which minimizes the distance between the two curves.

To limit the number of cases discussed and give a more concrete meaning to the values of the apparent axial dispersion coefficient used, it is practical to refer to the column Peclet number, $Pe = uL/D_a$, where L is the column length. The column efficiency is $N = Pe/2$. Note, however, that the concept of column efficiency is difficult to handle in non-linear chromatography when the plate number depends on the concentration.

3. Results and discussion

We discuss successively the errors made in the determination of the isotherm coefficients and in the derivation of the apparent axial dispersion coefficient.

3.1. Determination of the isotherm coefficients by frontal analysis

We need to select first a definition of the retention time of the BC profile among several possible ones. Then we will compare the parameters of the initial isotherm and those of the isotherms calculated from the simulated experimental data, using different strategies, namely frontal analysis with either a series of steps from $C = 0$ to increasingly large concentrations, C_n (SSM) or the staircase method (SCM). The parameters of the original Langmuir isotherm used for the calculations of all profiles were $a = 12$ and $b = 0.024$ ml/mg.

3.1.1. Errors for different defined retention times

Table 1 gives the retention times of breakthrough curves obtained with a large concentration step, 0–25 mg/ml for different values of the column Peclet number, either constant or a function of the concentration. Some of these breakthrough curves have been previously published [1]. The retention times t_R are presented as reduced values t_R/t_0 where t_0 is the hold-up time of the column. Three definitions of the retention time of the breakthrough curve were considered, the retention time of the inflection point of

Table 1
Retention times and relative errors of some breakthrough curves with constant and concentration-dependent *Pe*

Pe	t_R/t_0 – Inflection point		t_R/t_0 – Half height	
	True retention time: $t_R/t_0 = 4.368$			
50	4.292	−1.74%	4.334	−0.78%
88.9	4.321	−1.08%	4.348	−0.46%
133.3	4.340	−0.64%	4.354	−0.32%
400	4.359	−0.21%	4.361	−0.16%
800	4.359	−0.21%	4.363	−0.11%
400→50	4.196	−3.94%	4.292	−1.74%
400→100	4.282	−1.97%	4.330	−0.87%
400→200	4.330	−0.87%	4.351	−0.39%
400→266.7	4.340	−0.64%	4.356	−0.27%
400→533.3	4.359	−0.21%	4.364	−0.09%
400→800	4.368	0.00%	4.367	−0.02%
50→400	4.483	2.63%	4.405	0.85%
100→400	4.407	0.89%	4.381	0.30%
200→400	4.368	0.00%	4.368	0.00%
266.7→400	4.368	0.00%	4.365	−0.07%
533.3→400	4.349	−0.43%	4.360	−0.18%
800→400	4.349	−0.43%	4.358	−0.23%

the profile, the retention time of the concentration $(C_{n+1} + C_n)/2$, at which the middle of the step is eluted, and the time given by an area based method. The bias is defined as $(t_R - t_{R,true})/t_{R,true}$, where $t_{R,true}$ is the theoretical retention time of the BC[2]. It depends on the definition selected for t_R and on the value of dispersion coefficient.

The area-based retention time is the only theoretically correct definition of the BC time[3]. Calculations confirmed in all cases that this definition gives results which are identical with the true retention time of the ideal model. Accordingly, this definition was not used in any further investigation. The agreement is expected in the present case since there is no noise in the simulated chromatograms and the integration limits could be chosen adequately. The agreement also states that the boundary conditions considered have been chosen correctly and that numerical errors for our calculations are small enough to be neglected. If the diffusionless boundary conditions are used, significant numerical errors do occur at low values of the Peclet number.

For a constant Peclet number, the bias decreases with increasing value of *Pe*. When the Peclet number is a function of the concentration, the bias depends also on the extent of the variation of *Pe* in the concentration range sampled by the breakthrough curve. For the inflection point method, the bias is −1.7% at the lowest constant value of *Pe* used. It decreases by nearly half whenever the Peclet number is doubled. It varies between ca. −3.9% and +2.6% when *Pe* depends on the concentration. The values of the retention time of the breakthrough curves measured by the half-height method are either smaller than those obtained from the inflection point or nearly equal.

Table 2 reports the relative values of the bias observed on the determination of the retention times for different values of the product bC_0 ($b = 0.024$ ml/mg) and of a Peclet number independent of the concentration. The concentration steps considered were from 0 to $C_0 = 26$, 14 and 2 mg/ml. It would be equivalent to change b instead of C for this study,

Table 2
Retention times and relative errors for constant *Pe* and different *bC*

Pe	t_R/t_0 – Inflection point		t_R/t_0 – Half height	
	True retention time: $t_R/t_0 = 4.318$, $bC = 0.624$			
50	4.244	−1.71%	4.284	−0.79%
88.9	4.273	−1.04%	4.298	−0.46%
266.7	4.301	−0.39%	4.310	−0.19%
533.3	4.311	−0.16%	4.313	−0.12%
1066.7	4.311	−0.16%	4.314	−0.09%
2133.3	4.321	0.07%	4.315	−0.07%
	True retention time: $t_R/t_0 = 5.033$, $bC = 0.336$			
50	4.924	−2.17%	4.986	−0.93%
88.9	4.972	−1.21%	5.008	−0.50%
266.7	5.010	−0.46%	5.023	−0.20%
533.3	5.020	−0.26%	5.027	−0.12%
1066.7	5.030	−0.06%	5.029	−0.08%
2133.3	5.030	−0.06%	5.031	−0.04%
	True retention time: $t_R/t_0 = 6.141$, $bC = 0.048$			
50	5.853	−4.69%	6.029	−1.82%
88.9	5.988	−2.49%	6.082	−0.96%
266.7	6.093	−0.78%	6.122	−0.31%
533.3	6.122	−0.31%	6.131	−0.16%
1066.7	6.131	−0.16%	6.135	−0.10%
2133.3	6.141	0.00%	6.138	−0.05%

[2] This retention time is given by the ideal model as $t_R/t_0 = 1 + Fa/(1 + bC)$ [10].

[3] This time is given by $t_R/t_0 = \int_0^\infty (C_{n-1} - C)\mathrm{d}(t/t_0)/(C_{n-1} - C_n)$.

if the breakthrough curves are normalized by reporting C/C_0. However, we have considered that the thermodynamical properties of the chromatographic system, hence the isotherm parameters, remain constant in the study. The experimental retention times obtained by either the inflection point or the half-height point methods are shorter than the true retention times and the differences increase with decreasing value of the product bC_0, i.e., with decreasing intensity of the non-linear behavior and decreasing influence of the self-sharpening effect of the convex upwards Langmuir isotherm.

Table 3 and Table 4 give the relative values of the bias observed for different values of the product bC_0 (as in Table 2) and for concentration dependent Peclet numbers. The change of the Peclet number in these tables refers to the actual concentration step performed and not to a step of 25 mg/ml as used throughout this paper. When the values of *Pe* increase with increasing *C*, the absolute value of the bias increases with decreasing values of bC_0, whether the determination of t_R is made by the inflection point or the half-height point method. The same is true when *Pe* decreases with increasing *C*. For low and moderate *Pe* number ranges we find that for an increasing *Pe* the bias is positive, for a decreasing *Pe* it is negative. For larger changes of the *Pe* number, this trend is less apparent and the bias is sometimes positive, sometimes negative.

Table 3
Retention times and relative errors for increasing *Pe* and different *bC*

Pe	t_R/t_0 – Inflection point		t_R/t_0 – Half height	
True retention time: $t_R/t_0 = 4.318$, $bC = 0.624$				
50→400	4.426	2.50%	4.353	0.81%
100→400	4.359	0.95%	4.330	0.28%
200→400	4.321	0.07%	4.318	0.00%
400→800	4.321	0.07%	4.317	−0.02%
800→1600	4.321	0.07%	4.316	−0.05%
1600→3200	4.321	0.07%	4.316	−0.05%
True retention time: $t_R/t_0 = 5.033$, $bC = 0.336$				
50→400	5.259	4.49%	5.099	1.31%
100→400	5.125	1.83%	5.063	0.60%
200→400	5.058	0.50%	5.039	0.12%
400→800	5.039	0.12%	5.035	0.04%
800→1600	5.039	0.12%	5.033	0.00%
1600→3200	5.039	0.12%	5.032	−0.02%
True retention time: $t_R/t_0 = 6.141$, $bC = 0.048$				
50→400	6.601	7.49%	6.239	1.60%
100→400	6.371	3.75%	6.198	0.93%
200→400	6.217	1.24%	6.159	0.29%
400→800	6.198	0.93%	6.157	0.26%
800→1600	6.189	0.78%	6.153	0.20%
1600→3200	6.170	0.47%	6.149	0.13%

Table 4
Retention times and relative errors for decreasing *Pe* and different *bC*

Pe	t_R/t_0 – Inflection point		t_R/t_0 – Half height	
True retention time: $t_R/t_0 = 4.318$, $bC = 0.624$				
400→50	4.148	−3.94%	4.244	−1.71%
400→100	4.234	−1.94%	4.282	−0.83%
400→200	4.282	−0.83%	4.302	−0.37%
800→400	4.301	−0.39%	4.309	−0.21%
1600→800	4.311	−0.16%	4.313	−0.12%
3200→1600	4.311	−0.16%	4.315	−0.07%
True retention time: $t_R/t_0 = 5.033$, $bC = 0.336$				
400→50	4.752	−5.58%	4.918	−2.28%
400→100	4.895	−2.74%	4.974	−1.17%
400→200	4.972	−1.21%	5.008	−0.50%
800→400	5.001	−0.64%	5.019	−0.28%
1600→800	5.020	-0.26%	5.025	−0.16%
3200→1600	5.030	−0.06%	5.029	−0.08%
True retention time: $t_R/t_0 = 6.141$, $bC = 0.048$				
400→50	5.518	−10.1%	5.928	−3.47%
400→50	5.786	−5.78%	6.021	−1.95%
400→200	5.978	−2.65%	6.084	−0.93%
800→400	6.045	−1.56%	6.105	−0.59%
1600→800	6.083	−0.94%	6.118	−0.37%
3200→1600	6.112	−0.47%	6.127	−0.23%

3.1.2. Errors made on the isotherm data in the SCM and SSM methods

When the retention time of the breakthrough curve, t_R, is measured as the retention time of the inflection or of the half-height point, the amount adsorbed in the staircase method is calculated as

$$q_{n+1} = q_n + \frac{C_{n-1} - C_n}{F} \frac{t_{R,n+1} - t_0}{t_0} \tag{7a}$$

In the SSM, $C_{n+1} = C_0$, $q_n = 0$, and $C_n = 0$, so Eq. 7a can be simplified to

$$q_{n+1} = \frac{C_0}{F} \frac{t_{R,n+1} - t_0}{t_0} \tag{7b}$$

Fig. 1a and b show the isotherms data points derived from the retention times of a series of 'experimental' breakthrough curves obtained in SSM, with $C_0 = 2, 6, 10, \ldots, 26$ mg/ml. The isotherm used for the calculation is also shown in the figures (solid line). The same values of the intermediate concentrations have been used in SCM, hence $C_n = 0, 2, 6, 10, \ldots, 22$ and $C_{n+1} = 2, 6, 10, \ldots, 26$ mg/ml. The isotherm data points obtained by SCM are compared with the initial isotherm in Fig. 2a and b. The insets in each show the variations of the difference, $q_{true} - q$ (where q_{true} is calculated from the isotherm parameters, $a = 12$ and $b = 0.024$ ml/mg) or absolute bias, with the concentration. In Fig. 1a and Fig. 2a, the isotherm data were derived from the retention time of the inflection points. In Fig. 1b and Fig. 2b, they were derived from the retention time of the half-points. Obviously, all these plots exhibit the same trends as reported earlier for the retention times. SCM gives larger deviations than SSM at high concentrations, because the errors made at each intermediate step accumulate and these errors are large for the first steps, for which bC is small. At low concentrations the deviations observed for SSM and SCM are comparable.

Several series of determinations were made in cases in which the column Peclet number is concentration dependent. For example, Fig. 3a, b and Fig. 4a, b show results obtained using SSM and SCM, respectively, when the Peclet numbers increases from 50 to 400 or decreases from 400 to 50, with a concentration step of 0 to 25 mg/ml[4]. Fig. 3a and Fig. 4a show isotherm data for the inflection point and Fig. 3b and Fig. 4b for the half-height method. A rather large systematic error is observed in these cases. When Pe increases with increasing C, the value derived by SSM from the retention time of the inflection point (Fig. 3a) for the amount adsorbed is lower than the true amount at low concentrations and larger at high concentrations (see also Fig. 5a where the difference $q_{true} - q$ is plotted vs. C). This effect results from the influence of the increase of Pe with increasing C on the breakthrough profile [1] and will cause a large error in the value of the b coefficient of the Langmuir isotherm, as we see later. In the other cases, the values measured for the amount adsorbed are always too small. The converse result is obtained when Pe decreases with increasing C. The amount adsorbed is much less underestimated at low than at high concentrations. This will also result in a large error made on the estimate of the b coefficient of the Langmuir isotherm. The retention time of the half height gives results similar to those derived from the retention time of the inflection point but with smaller systematic errors.

Quite different results are obtained with SCM. As a first approximation and provided that $C_{n+1} - C_n$ is small enough, each breakthrough curve of the staircase can be considered as corresponding to a constant Peclet number, independent of C and intermediate between the values corresponding to the low and high concentrations of this step. This average number, however, varies from one step to the next, according to the concentration dependence of Pe. This averaging process causes a systematic underestimate of the amount adsorbed, although much smaller than with SSM. Accordingly, significant errors on the b coefficient will still be made with this method.

Similar calculations have been made using different values for the coefficients Pe^0 and Pe^1 characterizing the numerical dependence of Pe on C. Our investigation includes cases in which the Peclet number (hence the column efficiency) is high and varies within a large range. The results are reported for SSM in Fig. 5a–5d for Pe increasing (Fig. 5a, b) and decreasing (Fig. 5c, d) with increasing concentration (Fig. 5a, c inflection point method, Fig. 5b, d half-height method) and for SCM in Fig. 6a–d. These figures confirm the trends identified in Fig. 3 and Fig. 4. There is a systematic underestimate of the amount adsorbed at low or moderate concentrations. This is apparent in all cases with low Pe number. As calculated from the retention time of the inflection point by SSM when Pe increases with increasing C (Fig. 5a), the absolute value of this bias is decreasing with increasing value of the initial Peclet number. In this case, an overestimate is observed at high concentrations. This overestimate disappears when Pe^0 exceeds 200. The systematic underestimate of the amount adsorbed derived from the retention time of the half-point (Fig. 5b) is smaller than the overestimate from the inflection point method. It also

[4] For $Pe(50 \rightarrow 400)$, $Pe^0 = 50$, $Pe^1 = -0.035$; and for $Pe(400 \rightarrow 50)$, $Pe^0 = 400$, $Pe^1 = 0.280$.

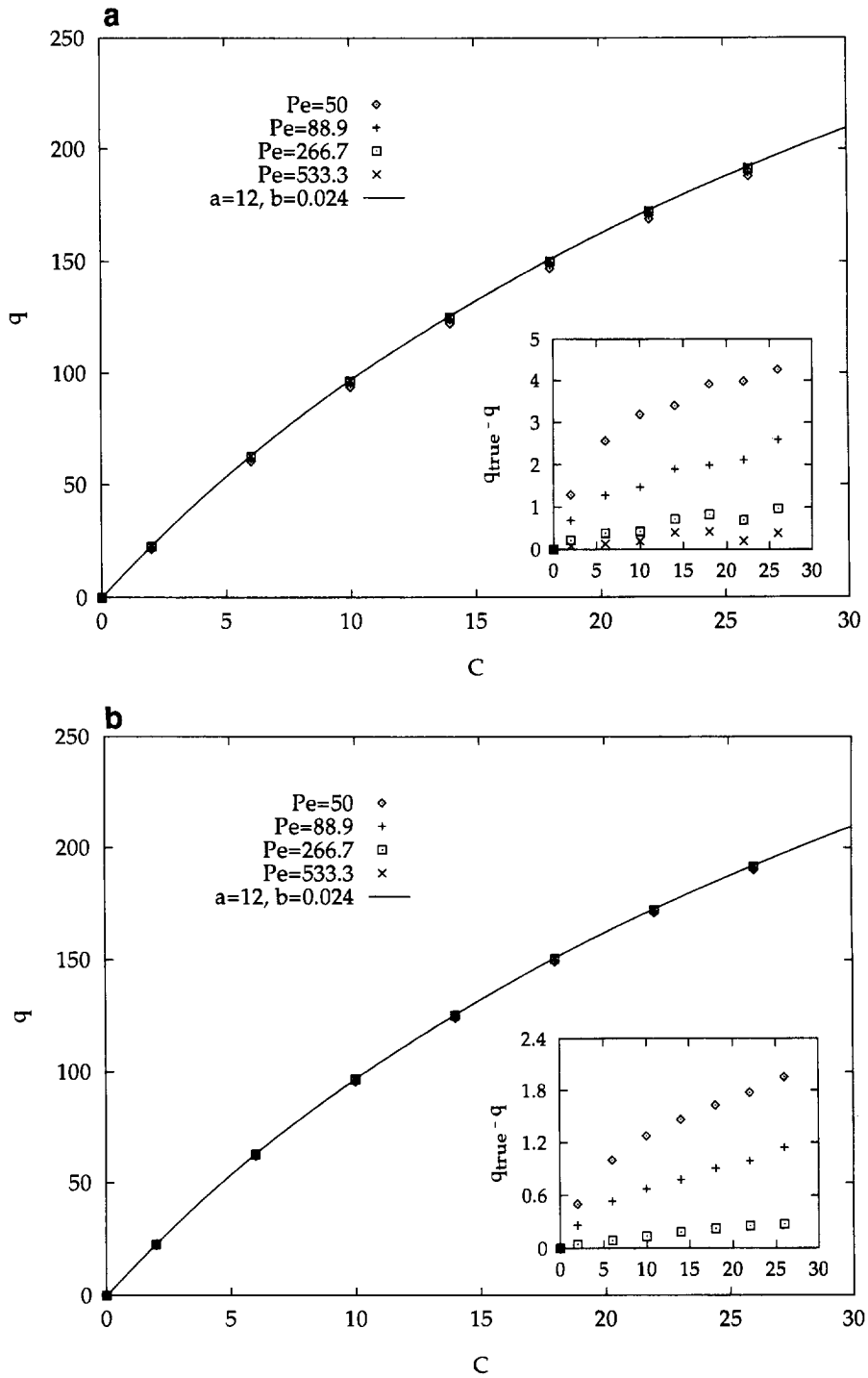

Fig. 1. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by step series method frontal analysis. 'Experimental' isotherms calculated from breakthrough curves with constant $Pe=50$, 88.9, 266.7, 533. (a) Inflection point method. (b) Half-height method.

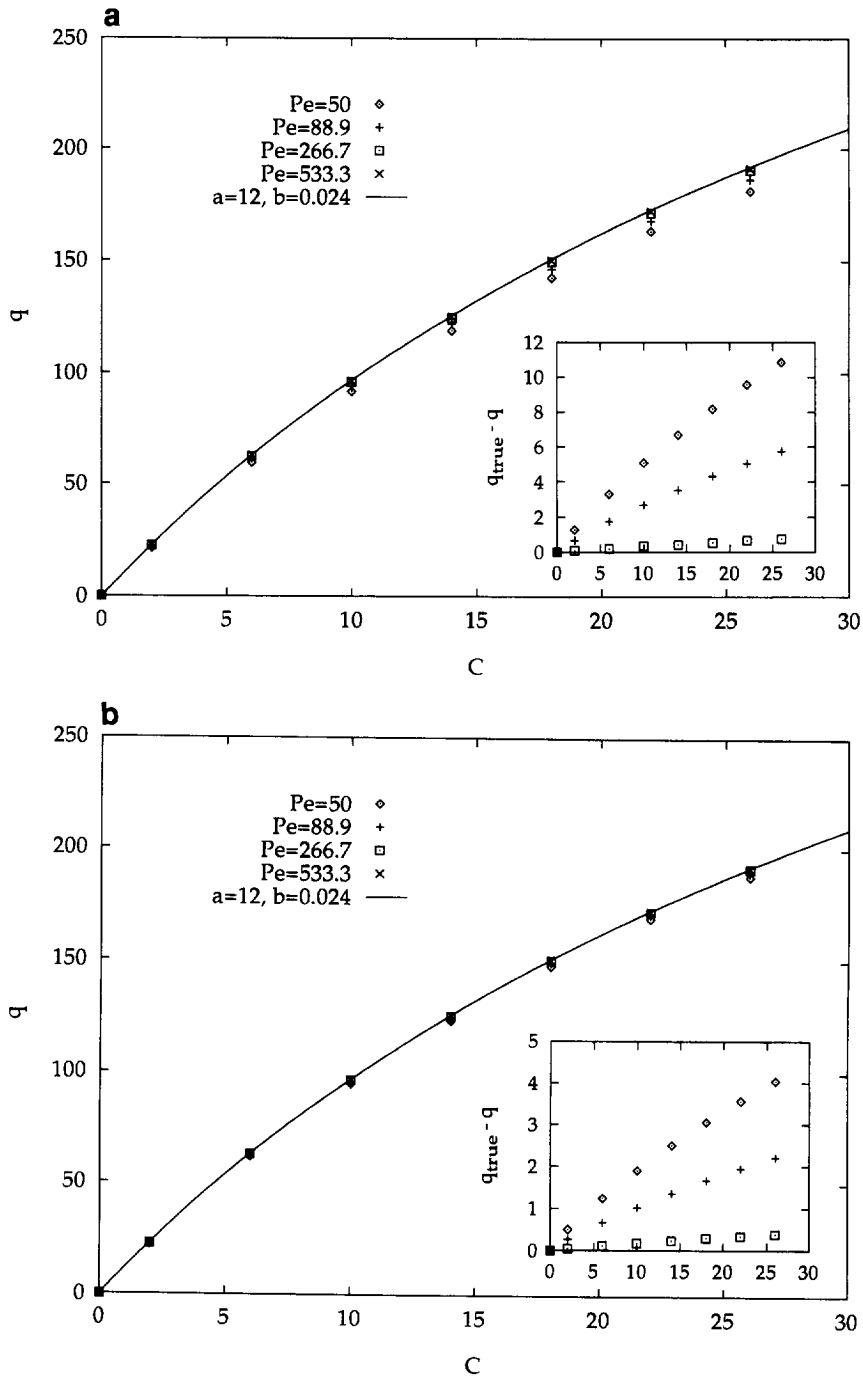

Fig. 2. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by frontal analysis in the staircase mode. 'Experimental' isotherms calculated from breakthrough curves with constant $Pe = 50$, 88.9, 266.7, 533. (a) Inflection point method. (b) Half-height method.

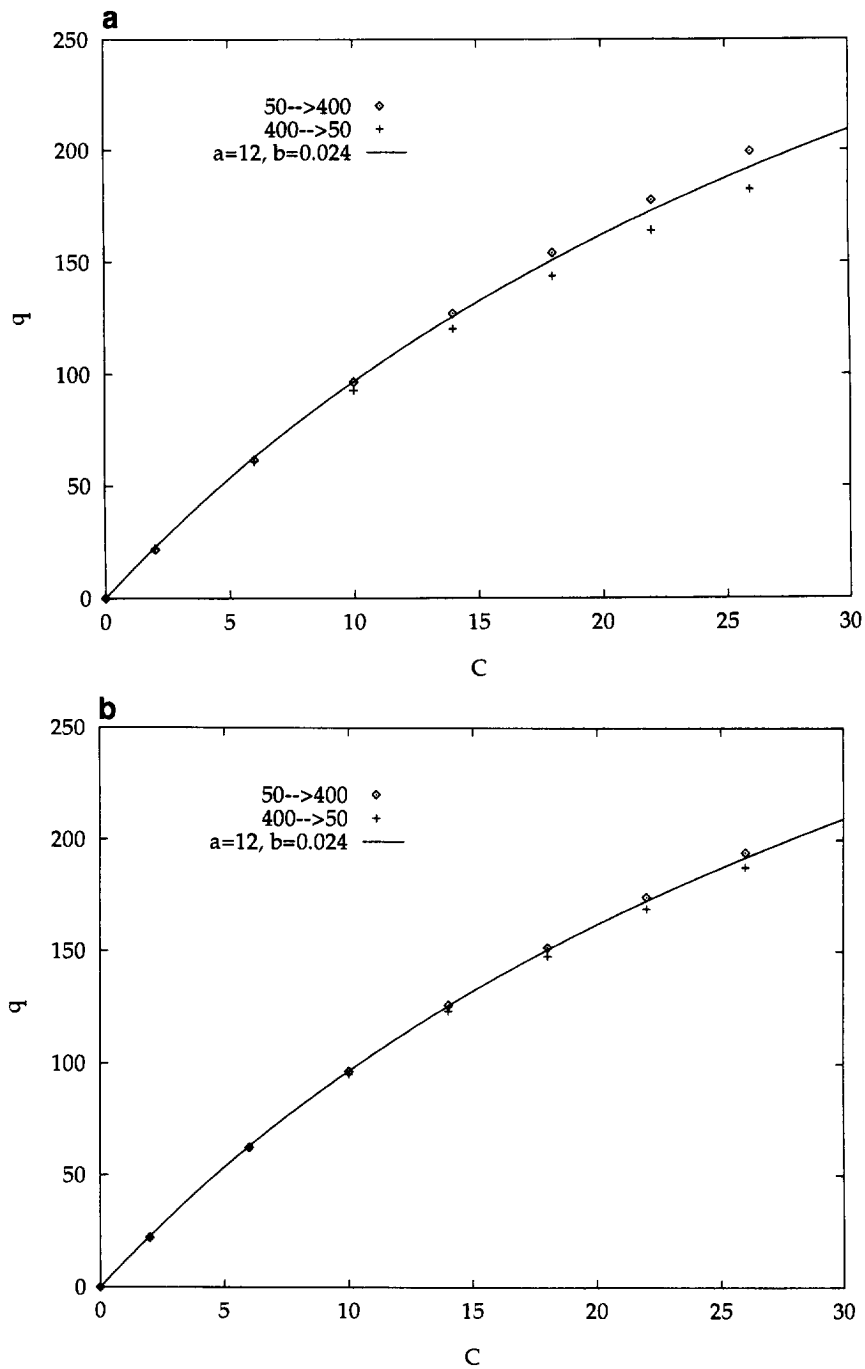

Fig. 3. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by step series method frontal analysis. 'Experimental' isotherms calculated from breakthrough curves with concentration dependent *Pe*. *Pe* increases from 50 to 400 or decreases from 400 to 50. (a) Inflection point method. (b) Half-height method.

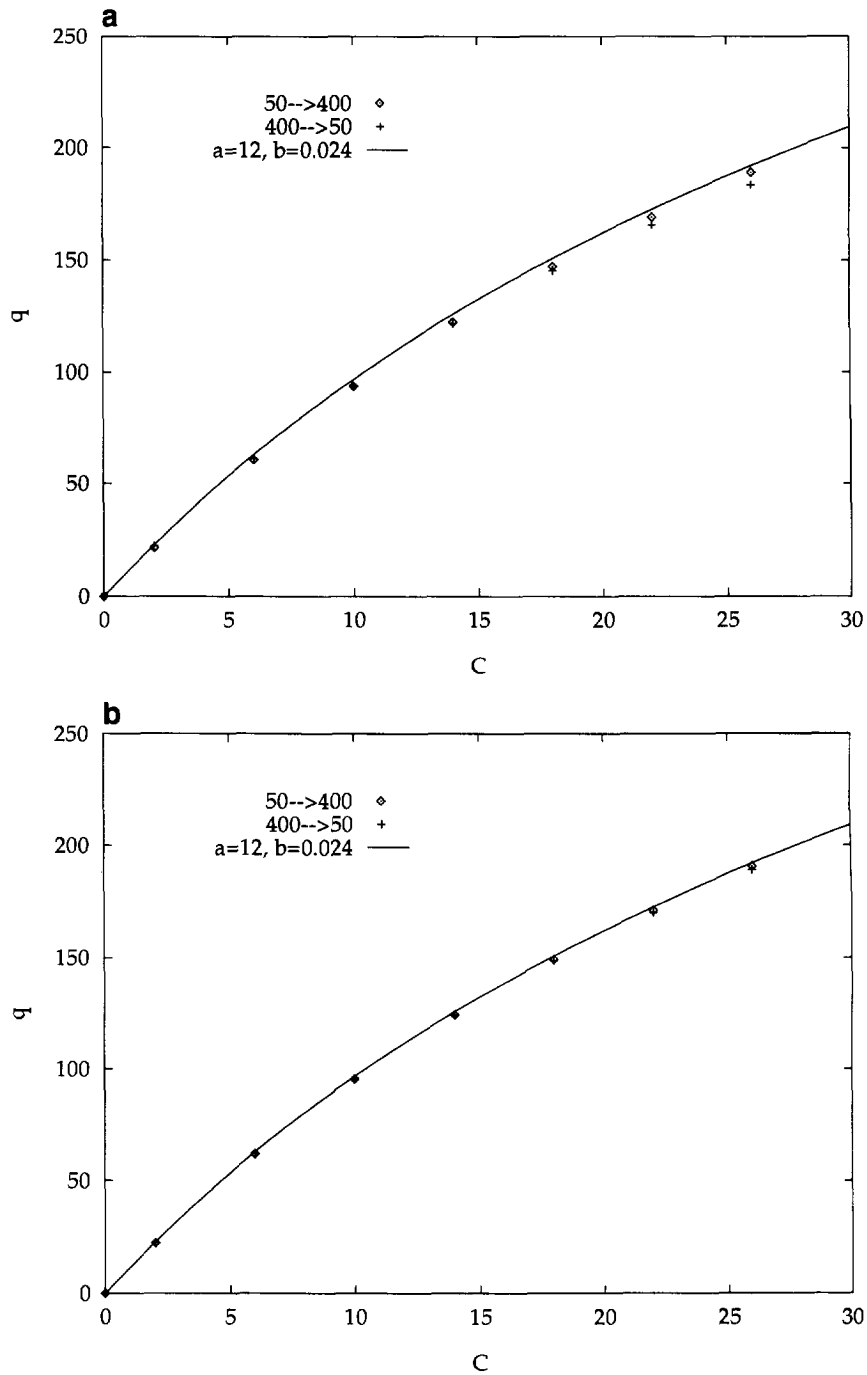

Fig. 4. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by frontal analysis in the staircase mode. 'Experimental' isotherms calculated from breakthrough curves with concentration dependent *Pe*. *Pe* increases from 50 to 400 or decreases from 400 to 50. (a) Inflection point method. (b) Half-height method.

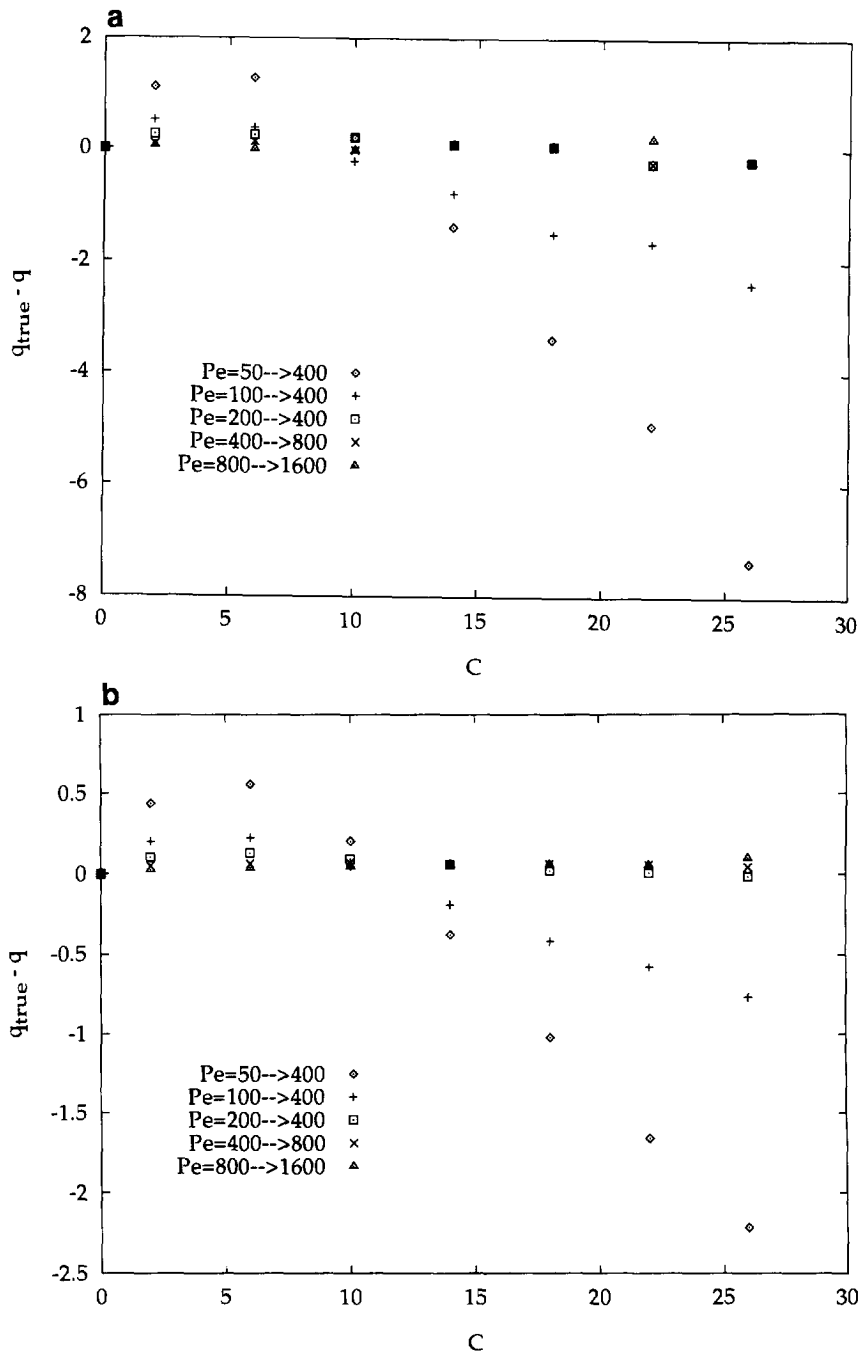

Fig. 5. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by step series method frontal analysis. Difference of true and 'experimental' isotherms calculated from breakthrough curves with concentration dependent *Pe*. (a) Inflection point method, increasing *Pe*. (b) Half-height method, increasing *Pe*. (c) Inflection point method, decreasing *Pe*. (d) Half-height method, decreasing *Pe*.

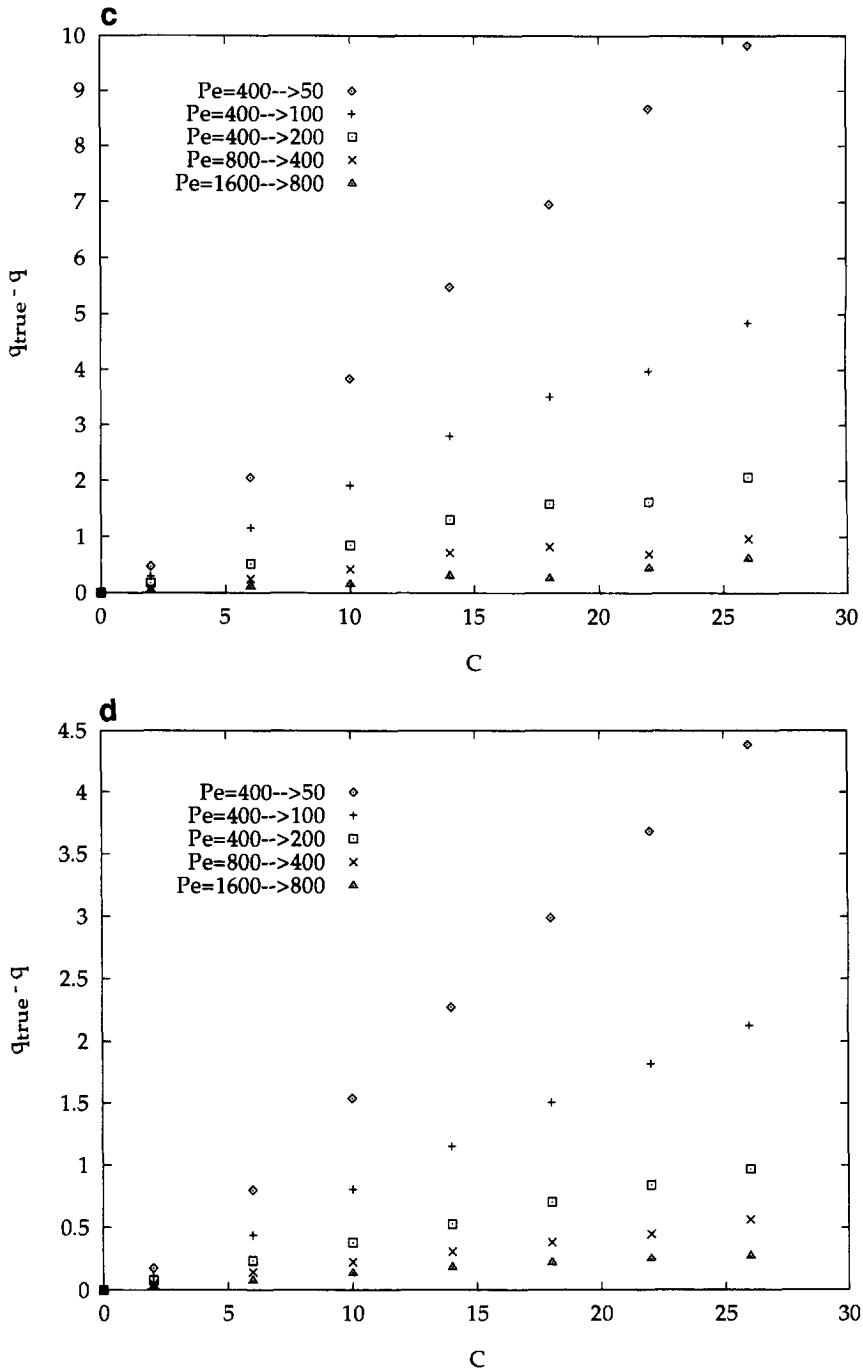

Fig. 5. *Continued.*

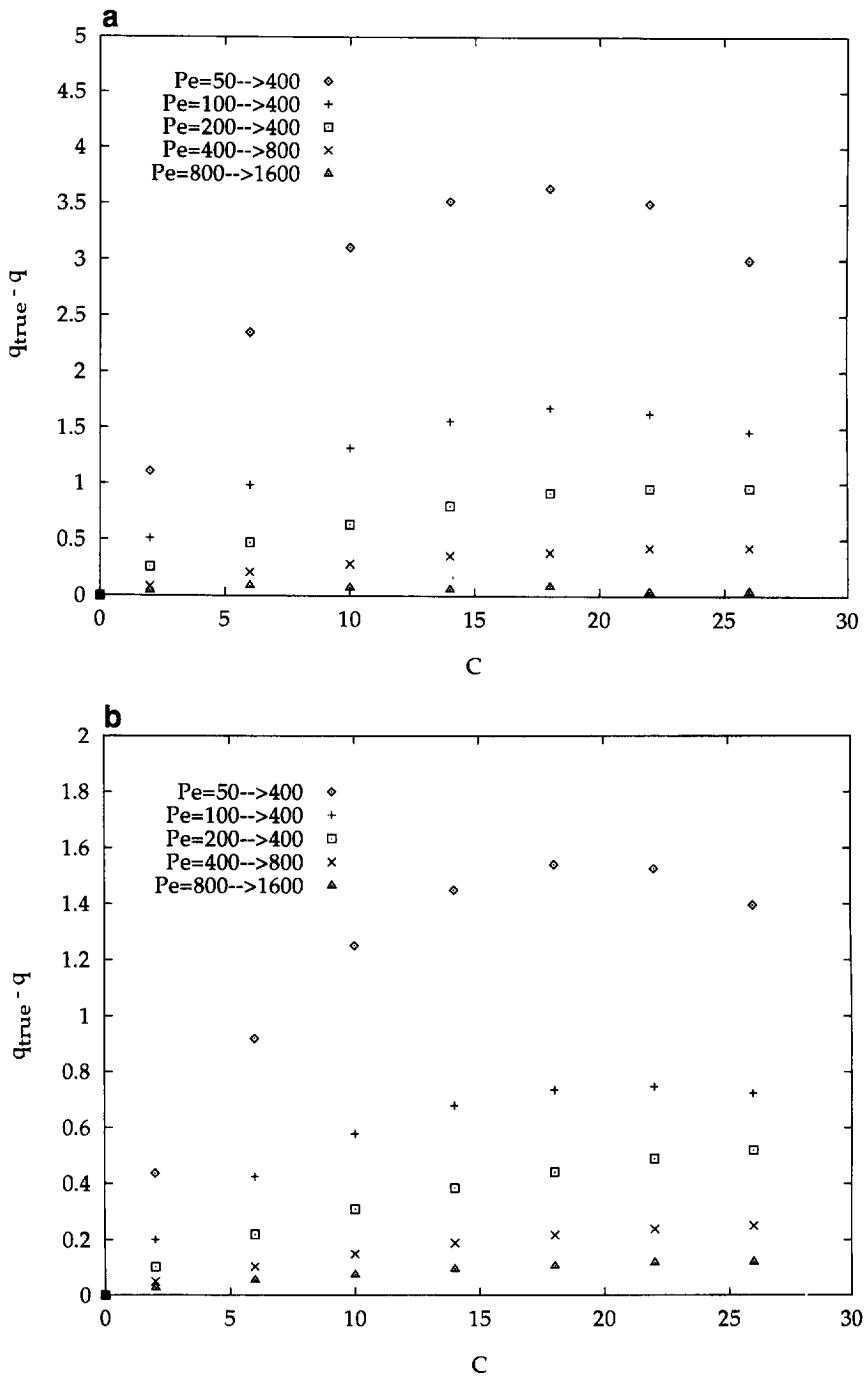

Fig. 6. Influence of the apparent axial dispersion coefficient in the determination of the isotherm by frontal analysis in the staircase mode. Difference of true and 'experimental' isotherms calculated from breakthrough curves with concentration dependent *Pe*. (a) Inflection point method, increasing *Pe*. (b) Half-height method, increasing *Pe*. (c) Inflection point method, decreasing *Pe*. (d) Half-height method, decreasing *Pe*.

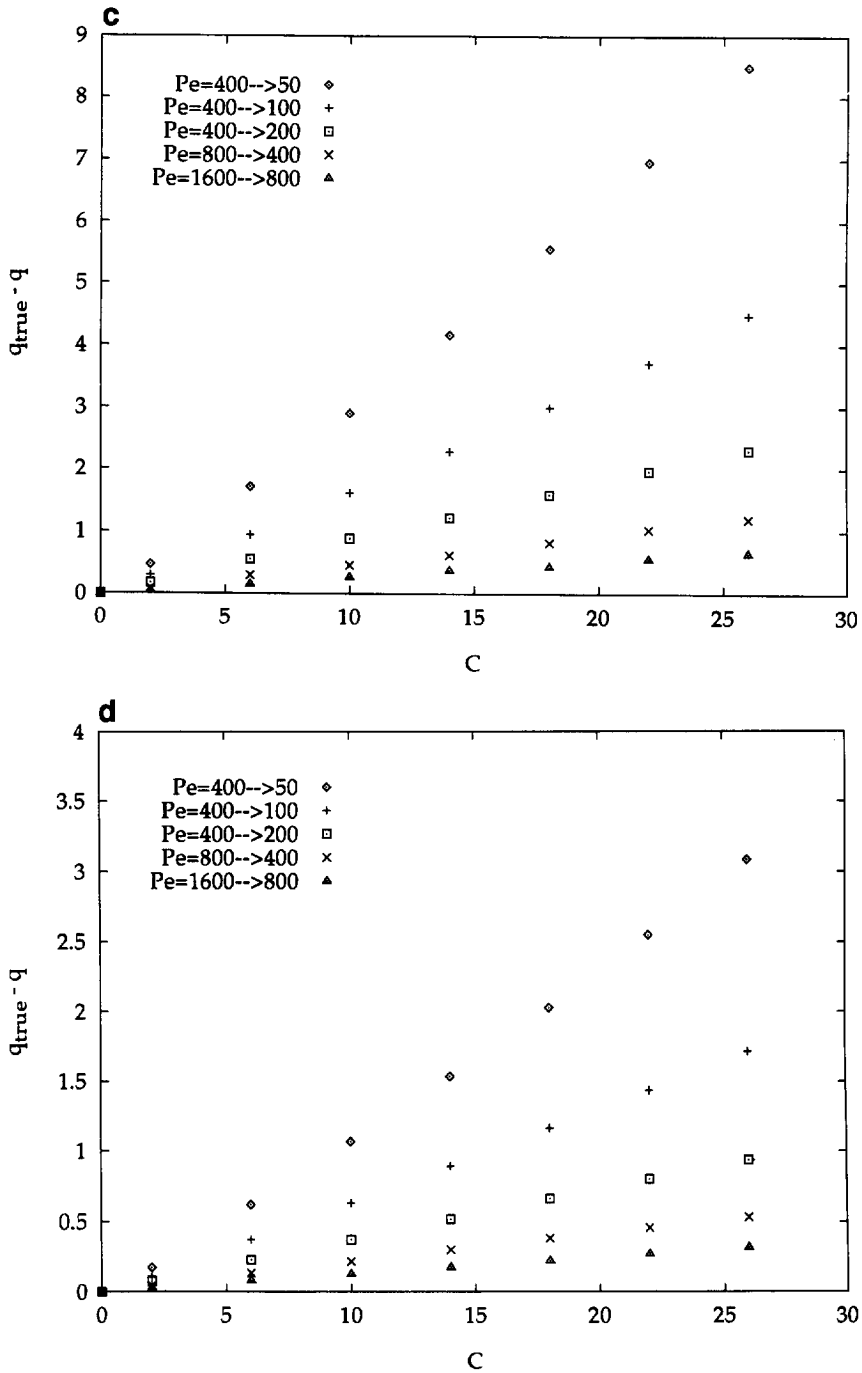

Fig. 6. *(Continued from p. 14).*

decreases with increasing column efficiency. Similar results were observed when *Pe* decreases with increasing concentration (Fig. 5c, d and Fig. 6c, d). However we found here only an underestimate of the amount adsorbed. Also, the bias decreases rapidly with increasing value of Pe^0, i.e., with increasing column efficiency.

3.1.3. Errors made in the derivation of the parameters of the Langmuir isotherm

The case of a Peclet number increasing from 50 to 400 when the concentration increases from 0 to 25 mg/ml is probably extreme. This represents an 8-fold increase from an unusually small value ($Pe=50$ corresponds to only 25 theoretical plates). Nevertheless, even in this case, the individual bias on any data point remains moderate. Thus, a superficial examination of the magnitude of the systematic error made on each successive data point would lead to the conclusion that the concentration dependence of the apparent axial dispersion coefficient does not cause any great systematic error in the measurement of isotherms by frontal analysis, even when this dependence is important and in spite of the fact that the retention volume of the inflection point is obviously not the same when the dispersion coefficient is constant or when it depends on the concentration [1].

However, the bias varies systematically. So, when the isotherm data were fitted to the Langmuir equation, the best values obtained for the isotherm coefficients were somewhat different from those initially introduced in the calculation. The values obtained for these coefficients are reported in Table 5, Table 6 and Table 7. The relative errors are also shown in these tables.

First, we observe that the error on the coefficient *a* is generally very low. It is below 2% except when the Peclet number is small (either below 100 and constant or increasing from or decreasing to a low value of the order of 50). Even in the extreme case in which $Pe^0=50$, the error is still below 5%. The bias is generally negative (underestimate) except at high efficiencies, when the breakthrough curves are so narrow that the estimate of the retention time of the inflection point was not accurate for the limited number of data points. In such cases, the bias on *a* is of the order of 0.25%. Even then, the bias observed with the other method of processing the breakthrough curves (retention time of the half point) is negative and of a comparable magnitude (in absolute value).

Table 5
Isotherm coefficients with relative errors for constant *Pe*

Pe		Inflection point		Half height	
SSM					
50	a	11.465	−4.46%	11.798	−1.68%
	b	0.022507	−6.22%	0.023550	−1.88%
88.9	a	11.757	−2.03%	11.899	−0.84%
	b	0.023504	−2.07%	0.023826	−0.73%
266.7	a	11.925	−0.63%	11.968	−0.27%
	b	0.023903	−0.40%	0.023975	−0.10%
533.3	a	11.958	−0.35%	11.982	−0.15%
	b	0.023896	−0.43%	0.023996	−0.02%
1066.7	a	11.990	−0.08%	11.990	−0.08%
	b	0.024041	0.17%	0.024011	0.05%
2133.3	a	12.012	0.10%	11.995	−0.04%
	b	0.024062	0.26%	0.024022	0.09%
SCM					
50	a	11.414	−4.88%	11.777	−1.86%
	b	0.024479	2.00%	0.024149	0.62%
88.9	a	11.689	−2.59%	11.882	−0.98%
	b	0.024237	0.99%	0.024101	0.42%
266.7	a	11.908	−0.77%	11.963	−0.31%
	b	0.024029	0.12%	0.024041	0.17%
533.3	a	11.965	−0.29%	11.979	−0.18%
	b	0.024065	0.27%	0.024017	0.07%
1066.7	a	11.978	−0.18%	11.987	−0.11%
	b	0.023991	−0.04%	0.024006	0.03%
2133.3	a	11.998	−0.02%	11.994	−0.05%
	b	0.024056	0.23%	0.024007	0.03%

The bias on the estimate of the coefficient *b* made by SSM is quite large. It ranges between −20.1 and +7.3%. Such large errors are made, however, only in the extreme cases, when the initial or final Peclet number is low. For constant values of *Pe*, the bias is negative at low values of *Pe*, and sometimes positive (overestimate) at large values. Its absolute value decreases rapidly with increasing column efficiency. It is more difficult to derive general rules when *Pe* is a function of *C*. It still is possible to achieve a bias smaller than 1% at moderate column efficiencies.

The bias of the determinations of *b* made by SCM is usually larger than for those made by SSM, except when the range of variation of *Pe* is large. However the absolute errors are comparable. The bias of the determination of the coefficient *a* is some time larger, other times smaller. A general rule has not

Table 6
Isotherm coefficients with relative errors for increasing *Pe*

Pe		Inflection point		Half height	
SSM					
50→400	a	11.503	−4.14%	11.808	−1.60%
	b	0.019188	−20.1%	0.022259	−7.25%
100→400	a	11.883	−0.98%	11.935	−0.54%
	b	0.022603	−5.82%	0.023394	−2.53%
200→400	a	11.927	−0.61%	11.971	−0.24%
	b	0.023531	−1.95%	0.023838	−0.68%
400→800	a	11.964	−0.30%	11.984	−0.13%
	b	0.023739	−1.09%	0.023931	−0.29%
800→1600	a	11.983	−0.14%	11.993	−0.06%
	b	0.023905	−0.40%	0.023992	−0.03%
1600→3200	a	11.985	−0.13%	11.995	−0.04%
	b	0.023916	−0.35%	0.024001	0.00%
SCM					
50→400	a	11.409	−4.93%	11.773	−1.89%
	b	0.021906	−8.73%	0.023281	−3.00%
100→400	a	11.753	−2.06%	11.899	−0.84%
	b	0.023205	−3.31%	0.023714	−1.19%
200→400	a	11.893	−0.89%	11.954	−0.38%
	b	0.023760	−1.00%	0.023928	−0.30%
400→800	a	11.954	−0.38%	11.978	−0.18%
	b	0.023903	−0.40%	0.023966	−0.14%
800→1600	a	11.981	−0.16%	11.988	−0.10%
	b	0.023911	−0.37%	0.023979	−0.09%
1600→3200	a	12.003	0.03%	11.993	−0.06%
	b	0.023957	−0.18%	0.023983	−0.07%

been found. Accordingly, SCM is not necessarily better than SSM for the purpose of accuracy in isotherm determination.

It has to be pointed out here that for larger values of bC_0, i.e., in the case of a larger surface coverage, the error on b will be less. This result is expected, since the absolute error on the retention time will be less, as we saw before (Table 2, Table 3, Table 4). In order to get a more accurate value of b, frontal analysis experiments should be performed up to high concentrations or rather to high values of the product bC_0.

Finally, we must mention a limitation to the generality of the conclusions to be derived from this work. The range of concentrations within which the 'experimental' data were acquired is such that the largest value of the product bC is 0.61, corresponding to a surface coverage of 38%. Although non-linear effects are already most important in chromatography within that range, the determination of the coefficient b and the column saturation capacity requires a significant extrapolation, itself a cause of low accuracy. It is always advisable to acquire data up to the highest possible concentration. This is not always possible and in many cases, especially with low or moderate molecular mass compounds in reversed-phase chromatography, it is impossible, because the retention factor (i.e., a) is small, because of limited solubility or for other reasons. If it is possible, it is generally observed that the Langmuir model does not account well for the data, but this is another problem. The concentration dependence of the rate coefficient will remain a major concern in the evaluation of the experimental data.

3.2. Determination of the apparent axial dispersion coefficient

Obviously, any attempt at using the equilibrium–dispersive model with a constant dispersion coefficient (hence, *Pe*) for fitting the breakthrough curves

Table 7
Isotherm coefficients with relative errors for decreasing *Pe*

Pe		Inflection point		Half height	
SSM					
400→50	a	11.694	−2.55%	11.901	−0.83%
	b	0.025742	7.26%	0.024931	3.88%
400→100	a	11.817	−1.53%	11.937	−0.53%
	b	0.024618	2.58%	0.024370	1.54%
400→200	a	11.904	−0.80%	11.965	−0.29%
	b	0.024161	0.67%	0.024134	0.56%
800→400	a	11.942	−0.48%	11.979	−0.18%
	b	0.024000	0.00%	0.024066	0.28%
1600→800	a	11.994	−0.05%	11.984	−0.13%
	b	0.024153	0.64%	0.024007	0.03%
3200→1600	a	11.982	−0.15%	11.994	−0.05%
	b	0.023922	−0.32%	0.024021	0.09%
SCM					
400→50	a	11.817	−1.53%	11.930	−0.58%
	b	0.025841	7.67%	0.024633	2.64%
400→100	a	11.883	−0.98%	11.951	−0.41%
	b	0.024847	3.53%	0.024300	1.25%
400→200	a	11.924	−0.63%	11.966	−0.28%
	b	0.024351	1.46%	0.024125	0.52%
800→400	a	11.961	−0.33%	11.979	−0.18%
	b	0.024186	0.78%	0.024063	0.26%
1600→800	a	11.976	−0.20%	11.988	−0.10%
	b	0.024086	0.36%	0.024039	0.16%
3200→1600	a	11.998	−0.02%	11.994	−0.05%
	b	0.024056	0.23%	0.024031	0.13%

calculated with a concentration dependent dispersion coefficient will lead to large systematic errors in the case of SSM. In the case of SCM, this will lead to a variable average value of *Pe* but with biased estimates for the parameters of this dependence. In order to estimate these errors, we fitted the 'experimental data' to the breakthrough curves obtained with a constant value of the Peclet number and adjusted this number to obtain the best fit, i.e., the smallest sum of the squares of the distance between the points of the 'experimental' breakthrough curve and the points of a breakthrough curve calculated with a constant Peclet number [in this case, the objective function of the minimization problem is $OF = \sum(C^{ex} - C^{model})^2$]. This follows the procedure generally adopted to account for experimental data [6].

Fig. 7 shows an example of a plot of OF for SSM versus the estimate of *Pe* in the case of a Peclet number increasing from *Pe*=50 (*C* = 0) to *Pe*=400 (*C* = 25 mg/ml). The number for which OF is minimum gives the best estimate of *Pe*[5], hence of the apparent axial dispersion coefficient. Fig. 8a (*Pe*= 50→400) and b (*Pe*=400→50) show the plots of the differences between the concentration profiles of the 'experimental' and the best breakthrough curve obtained by SSM and calculated with the best estimates of the isotherm coefficients (previous section) and the apparent dispersion coefficient. Three cases were examined, the best fit using the true isotherm parameters (T), the isotherms parameters derived from the inflection point method (I) or the half-height method (H). The relative deviations observed in the cases of T and H increase with increasing step height. Finally, Fig. 9a and b compare the actual dependence of the apparent axial dispersion coefficient (solid line) with that measured

[5] In this work, the best value of each Peclet number is obtained with a precision of ±1 unit.

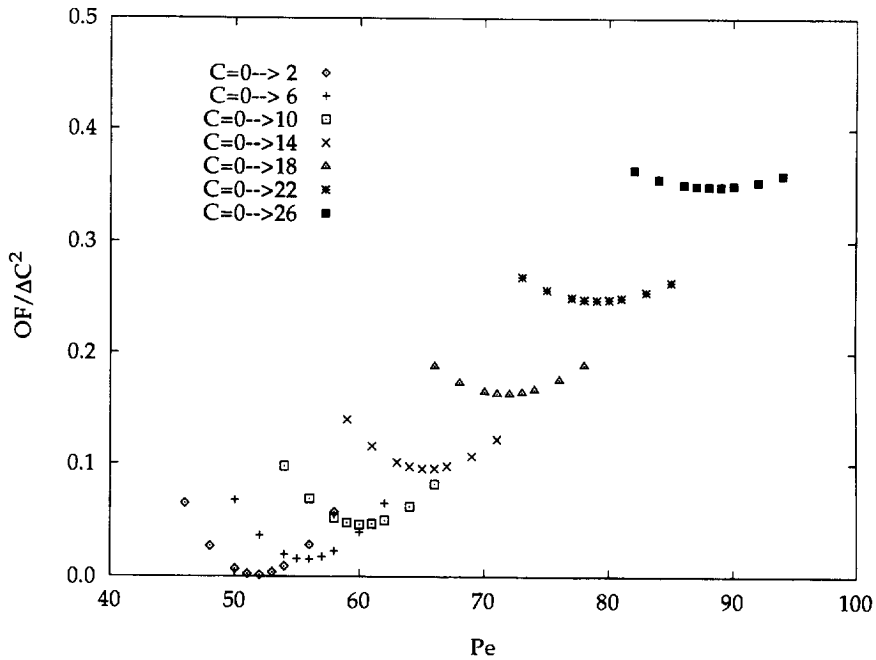

Fig. 7. Change of the objective function (OF) with average *Pe* number. Example of the fitting procedure for the SSM method with true isotherm parameters.

by SSM. The symbols show the results of the determination of the concentration dependence of these parameters using the different methods studied in this work (the symbols are reported to the average concentration of the step). The results obtained by SSM-T seem quite accurate while the bias observed with SSM-I becomes very important when the step height increases. Unfortunately, in actual practice, the experimentalist does not have access to the true isotherm parameters.

More accurate results are expected from the staircase frontal analysis method when the dispersion coefficient is concentration dependent. Fig. 10a (Pe= 50→400) and b (Pe=400→50) show plots of the relative differences between the concentration profiles of the 'experimental' and the 'best-fit' breakthrough curves for the 4 different isotherm sets, versus time. The values of the dispersion coefficients obtained by this method are those plotted in Fig. 11a and b versus the concentration at the center of the breakthrough curve [i.e., $(C_{n-1} + C_n)/2$]. The results obtained demonstrate a significant improvement over the results afforded by SSM. Because the step height is small in SCM, the concentration dependence of Pe is negligible over the concentration range sampled in the step. Then, the concentration dependence determined for the apparent axial dispersion coefficient is in better agreement with the actual one, the one which was used for the calculations.

This latter procedure was used in a recent publication [6] to investigate the concentration dependence of the column efficiency ($N=Pe/2$) for the two enantiomers of Tröger's Base in ethanol on microcrystalline cellulose triacetate. In this work, the isotherm was determined by SCM. A linear increase of the apparent plate number with increasing concentration was reported for one of the enantiomers, a parabolic variation for the other one. Since the column efficiency was very low (ca. 120 theoretical plates), it seems probable that the values measured for q were underestimated and that the concentration dependence obtained was biased accordingly.

The data points reported in Fig. 9 and Fig. 11 were fitted to straight lines, using a linear regression. The correlation coefficients R^2 were, in almost all cases, close to 1, indicating that the dependence observed is

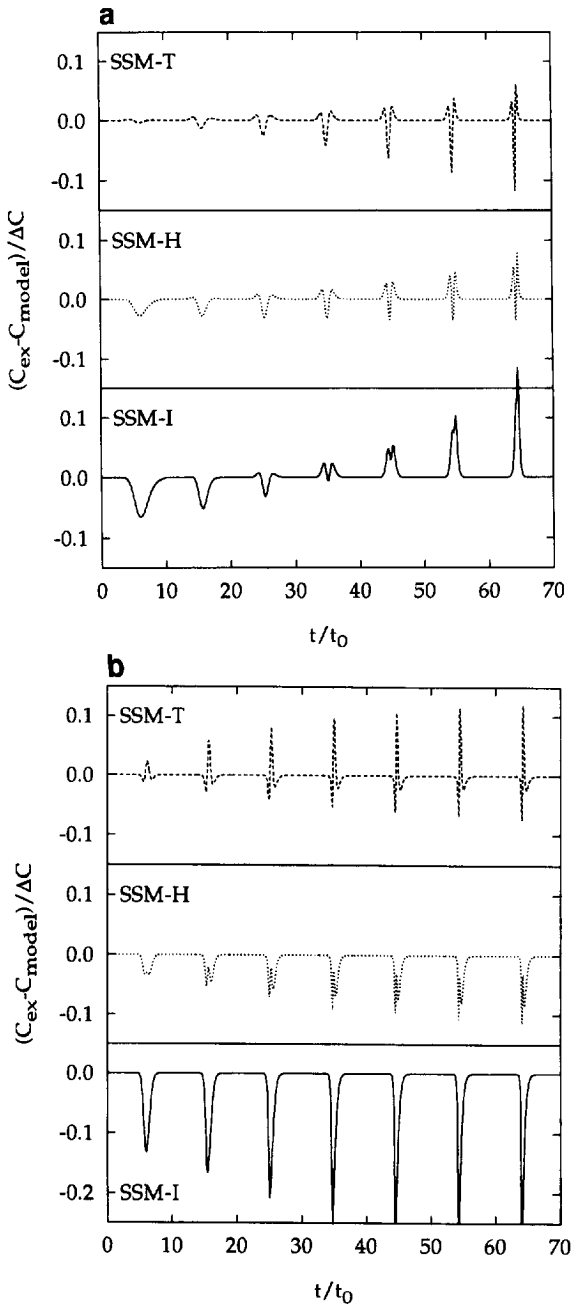

Fig. 8. Differences (for SSM) between the concentration profiles and the best-fit profiles obtained by the model with constant *Pe*. Time differences of 0, 10, ..., 60 have been added to the breakthrough curves with increasing plateau concentrations. SSM-I, inflection point method; SSM-H, half-height method; SSM-T, true isotherm method. (a) $Pe=50\rightarrow400$. (b) $Pe=400\rightarrow50$.

practically linear. The values obtained for the coefficients Pe^0 and Pe^1 are listed in Table 8. The coefficients determined by SSM have a relatively large bias. On the other hand, the values obtained by SCM agree very well with the initial values. There is another advantage of SCM, visible in Fig. 9a and b. The data points are reported to the concentration corresponding to the middle of the step, i.e., to $C_0/2$ in SSM. Therefore, the concentration range accessible in our measurements is 1–24 mg/ml for SCM, but only 1–13 mg/ml for SSM. This explains, in part, the large differences between the values calculated by SSM and SCM for the range of variation of *Pe* when the concentration increases from 0 to 25 mg/ml. Thus, SCM permits a more accurate determination of the coefficients of the linear dependence than SSM.

4. Conclusion

In actual experimental practice, the determination of the retention volume of a breakthrough curve by the integration procedure is less precise and less convenient than alternative methods. This explains why the only procedure which is theoretically sound is generally ignored. Peak area integrators and software developed for quantitative analyses are inconvenient. Dedicated software is easy to write but still requires computer acquisition of the breakthrough profiles. However, the major difficulty and source of error is found in the determination of the end point of the integration. The signal noise is the less important contribution to this error. Much more significant is the observation that, in most cases of applications of FA to biochemicals, the breakthrough curves do not end smoothly, as predicted by simple chromatographic models. A variety of more or less reproducible accidents on these curves suggest that the kinetics is more complex than assumed in chromatographic models [16]. For these reasons, experimentalists prefer 'quick and dirty' procedures.

Two procedures are commonly used. The retention time of the point at half-height of the concentration step can be measured with a better precision than the retention time of the inflection point, which is difficult to locate in the presence of signal noise. Thus, the former method is preferred in nearly all

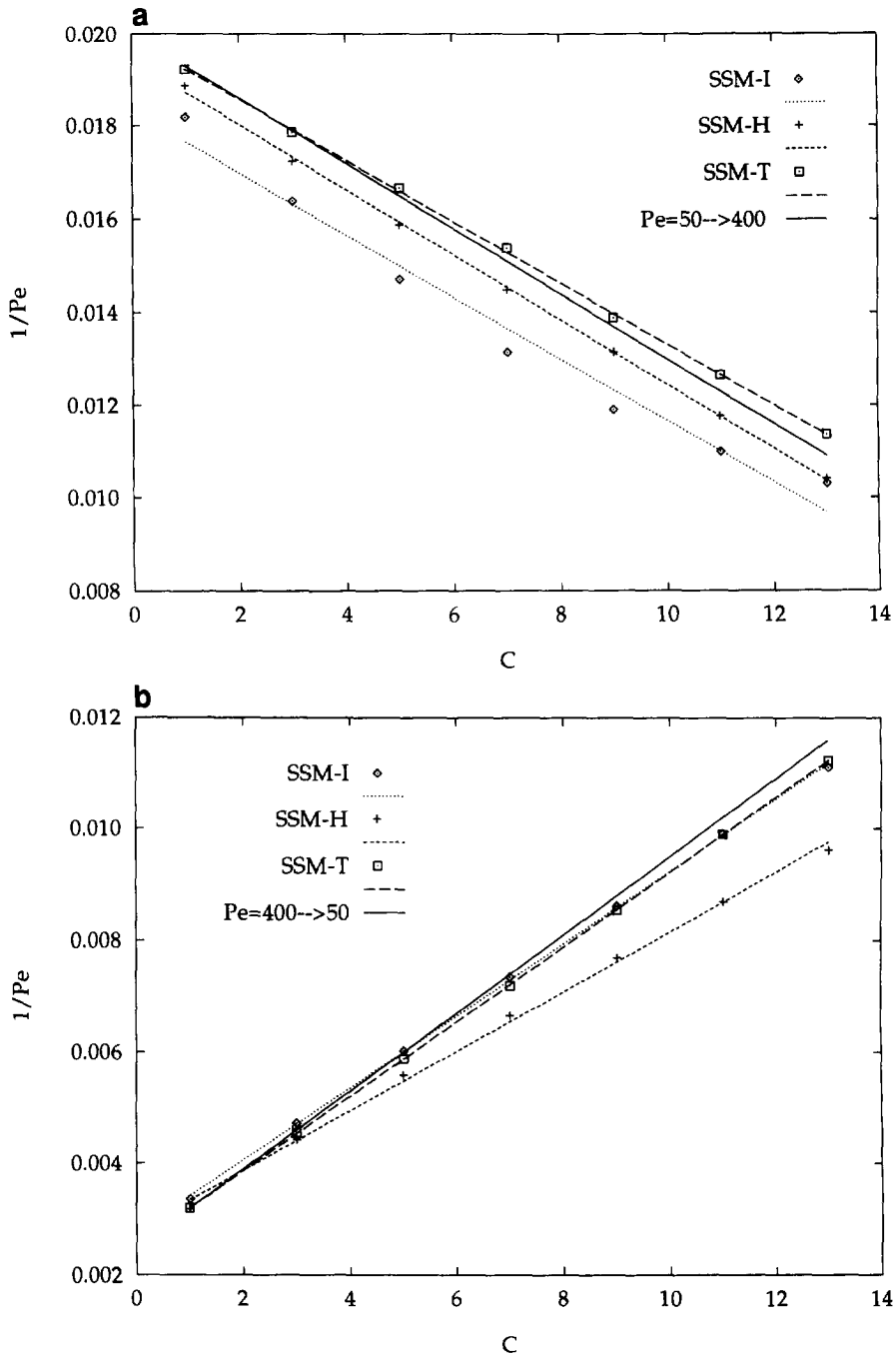

Fig. 9. Original concentration dependence of Pe and concentration dependence obtained by frontal analysis in the step series mode. (a) Pe=50→400. (b) Pe=400→50.

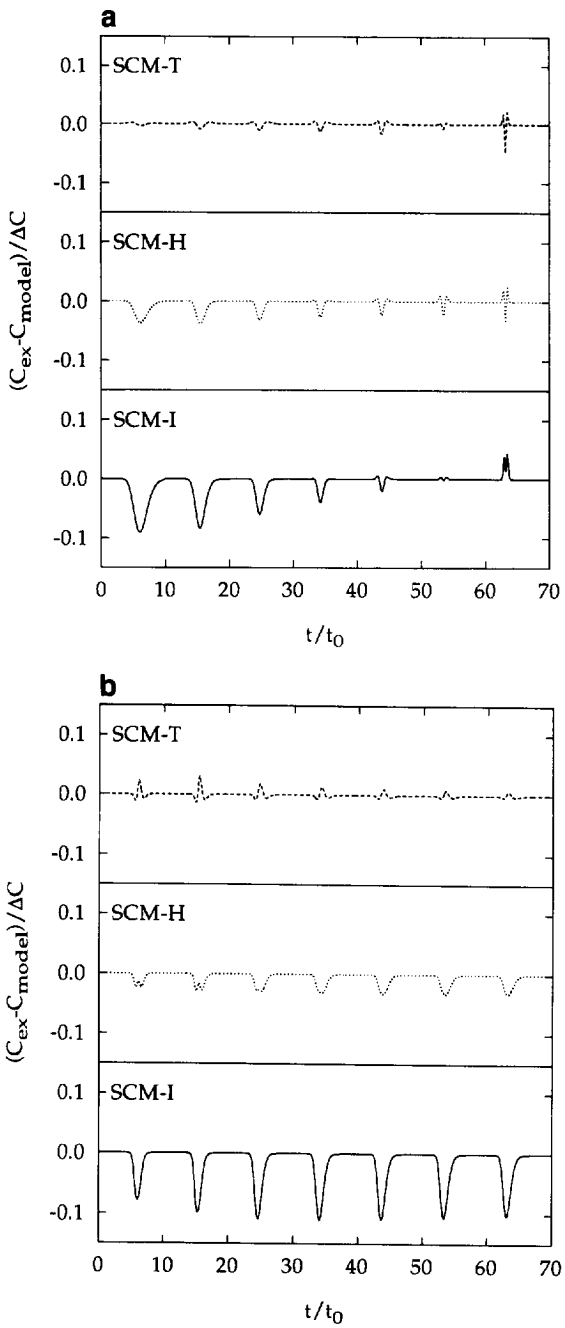

Fig. 10. Differences (for SCM) between the concentration profiles and the best-fit profiles obtained by the model with constant *Pe*. Time differences of 0, 10, ..., 60 have been added to the breakthrough curves with increasing plateau concentrations. SCM-I, inflection point method; SCM-H, half-height method; SCM-T, true isotherm method. (a) $Pe = 50 \rightarrow 400$. (b) $Pe = 400 \rightarrow 50$.

cases. It is obviously precise and convenient. It has always been known to be quick. We have shown above that it is also dirty.

When the coefficients characterizing the mass transfer kinetics in a chromatographic column (axial dispersion, mass transfer resistances, apparent axial dispersion) depend on the concentration, considerable systematic errors can be made when determining by frontal analysis the equilibrium isotherms of compounds under conditions when the column efficiency is low or moderate. This phenomenon is important in the case of proteins or on certain phases such as cellulose derivatives [6]. Important systematic errors, which in some cases may exceed 10 to 25%, can be made on the second coefficient of a Langmuir isotherm, hence on the saturation capacity of the stationary phase. In the same time, the residual in the non-linear regression of the data to the Langmuir model (or any other model) increases, which could cast unfounded doubts on the validity of the adsorption model used.

The use of staircase frontal analysis with steps of nearly constant (and moderate) height does not markedly reduce the importance of these errors compared to the use of steps from $C = 0$ to C_n, having an increasing height. Compared to ECP or FACP, where an accurate determination of the isotherm coefficients requires large values of *Pe*, typically above 5000, the method of frontal analysis is much more accurate. If *Pe* exceeds 300 to 500, the bias becomes practically negligible.

When they depend on the concentration, which might be more often than generally recognized, the values of the apparent axial dispersion coefficient determined by staircase frontal analysis are much more accurate than those given by the method using a series of steps of increasing height. Whenever possible, the former method should be preferred.

An alternative procedure to obtain both the isotherm and the apparent axial dispersion coefficient D_a is to make small, analytical size injections at different flow velocities on the plateaus achieved for different concentration steps. In this case D_a would be obtained directly as a function of the concentration. Breakthrough curves could be fitted to those obtained by a model considering this concentration dependence in order to obtain a best-fit which gives the isotherm coefficients. The isotherm coefficients

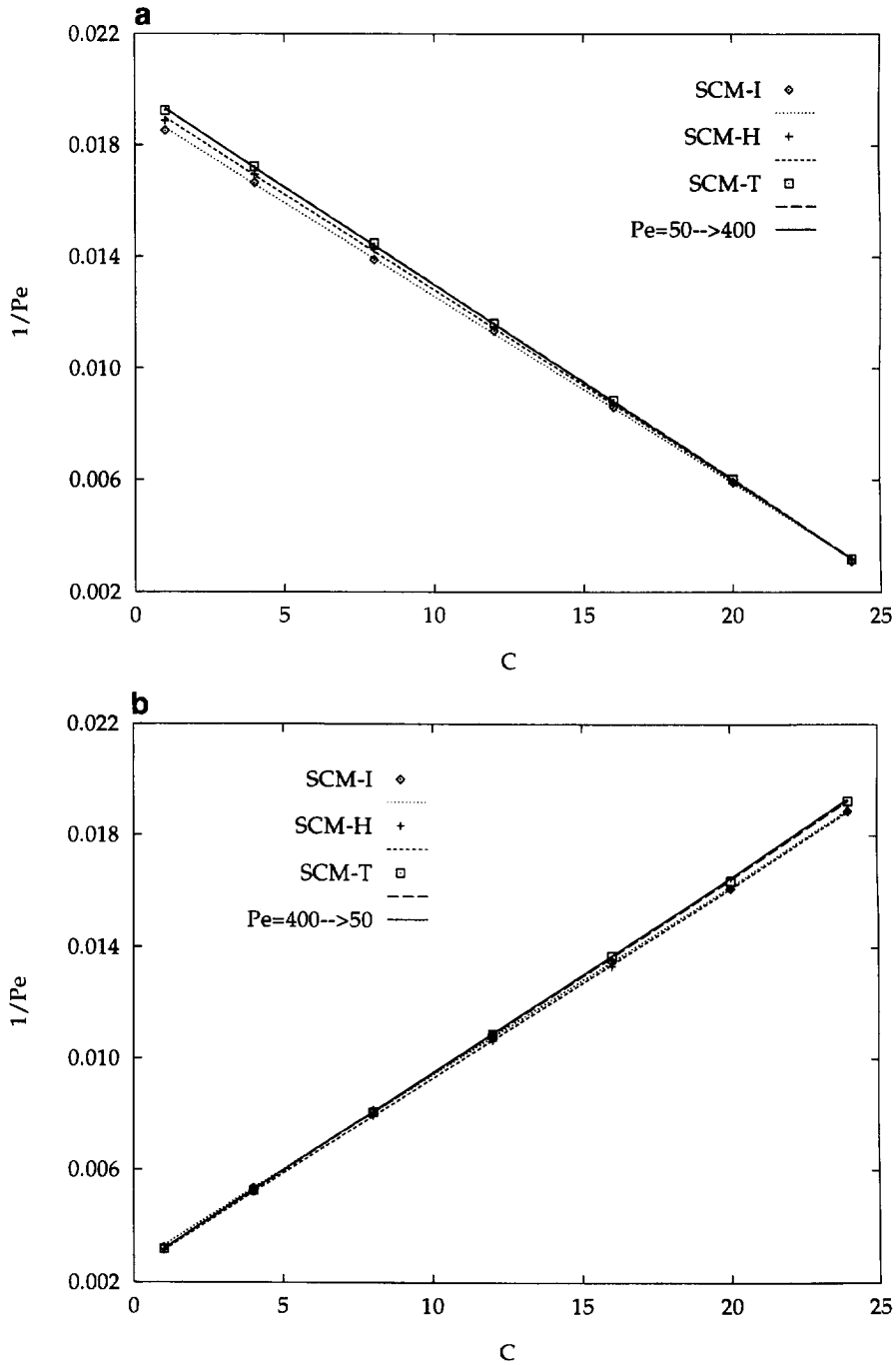

Fig. 11. Original concentration dependence of Pe and concentration dependence obtained by frontal analysis in the staircase mode. (a) $Pe = 50 \rightarrow 400$. (b) $Pe = 400 \rightarrow 50$.

Table 8
Recalculated values Pe_0 and Pe_1.

Method		$Pe_0 = Pe(C=0)$		Pe_1		$Pe(C=25)$	R^2
Case I: $Pe = 50 \rightarrow 400$							
SSM	I	54.60	9.2%	−0.03629	3.7%	589.23	0.979125
	H	51.49	3.0%	−0.03588	2.5%	499.49	0.999296
	T	50.28	0.6%	−0.03302	−5.7%	288.05	0.999496
SCM	I	51.85	3.7%	−0.03472	−0.8%	392.95	0.999788
	H	50.84	1.7%	−0.03481	−0.5%	392.13	0.999832
	T	49.97	−0.1%	−0.03493	−0.2%	394.61	0.999928
Case II: $Pe = 400 \rightarrow 50$							
SSM	I	361.19	−9.7%	0.23368	−16.5%	52.79	0.999725
	H	356.55	−10.9%	0.19082	−31.9%	61.79	0.997552
	T	395.72	−1.1%	0.26498	−5.4%	51.90	0.999987
SCM	I	377.80	−5.6%	0.25586	−8.6%	51.08	0.999861
	H	404.68	1.2%	0.27604	−1.4%	51.22	0.999979
	T	401.24	0.3%	0.27978	−0.1%	50.19	0.999976

can also be obtained from the retention time of the injections made on the plateau, which gives the possibility of an independent check. This would probably be the most accurate way of measuring isotherm coefficients for columns with very low efficiency.

5. Glossary of symbols

a First numerical coefficient for Langmuir isotherm
b Second numerical coefficient of Langmuir isotherm
C Liquid phase concentration of the component
C_0 Injection concentration of the component
D_a Apparent axial dispersion coefficient
D_a^0 Constant term of the apparent axial dispersion coefficient
D_a^1 Coefficient of the concentration dependent term of the apparent axial dispersion coefficient
F Phase ratio
L Column length
N Column efficiency
N_D Number of data points
OF Objective function
P Number of model parameters
Pe Column Peclet number
Pe^0 Value of the Peclet number at $C=0$
Pe^1 Coefficient of the concentration dependent term of the Peclet number
q Solid-phase concentration of component
t Time
t_R Retention time
t_0 Hold-up time of the column
u Mobile phase velocity
z Position in column

Greek letters

ε Total porosity of the column
σ Standard deviation defined in Eq. 6

Superscripts

ex 'Experimental' value
model Value obtained from a model
th Theoretical value

Subscripts

n Index indicating successive concentration steps
true True value

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References

[1] P. Sajonz, G. Zhong and G. Guiochon, J. Chromatogr. A, 728 (1996) 15.
[2] K. Lederer, I. Amtmann, S.V. Vijayakumar and J. Billiani, J. Liq. Chromatogr., 13 (1990) 1849.
[3] W. Gallagher and C. Woodward, Biopolymers, 28 (1989) 2001.
[4] S. Gibbs, A. Chu, E. Lightfoot and T. Root, J. Phys. Chem., 93 (1991) 467.
[5] B. AlDuri and G. McKay, J. Chem. Biotechnol., 55 (1992) 245.
[6] A. Seidel-Morgenstern, S.C. Jacobson and G. Guiochon, J. Chromatogr., 637 (1993) 19.
[7] G. Guiochon, S. Golshan-Shirazi and A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, NY, 1994, Ch. III.
[8] G. Guiochon, S. Golshan-Shirazi and A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, NY, 1994, Ch. XIV.
[9] D.O. Cooney and E.N. Lightfoot, Ind. Eng. Chem. Fundam., 4 (1965) 233.
[10] G. Guiochon, S. Golshan-Shirazi and A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, NY, 1994, Ch. X.
[11] J.F.K. Huber and R.G. Gerritse, J. Chromatogr., 58 (1971) 137.
[12] H. Guan, B.J. Stanley and G. Guiochon, J. Chromatogr. A, 659 (1994) 27.
[13] G. Guiochon, S. Golshan-Shirazi and A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, NY, 1994, Ch. II.
[14] S. Jacobsen, S. Golshan-Shirazi and G. Guiochon, AIChE J., 37 (1991) 836.
[15] P.W. Danckwerts, Chem. Eng. Sci., 2 (1953) 1.
[16] H. Guan-Sajonz, P. Sajonz, G. Zhong and G. Guiochon, Biotechnol. Progr., submitted.