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Effect of the flow rate on the measurement of adsorption data by dynamic frontal analysis

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Abstract

The adsorption data of propyl benzoate were acquired by frontal analysis (FA) on a Symmetry-C_{18} column, using a mixture of methanol (65%, v/v) and water as the mobile phase, at three different flow rates, 0.5, 1.0 and 2.0 mL/min. The exact flow rates F_v were measured by collecting the mobile phase in volumetric glasses ($\delta F_v/F_v \leq 0.2\%$). The extra-column volumes and the column hold-up volume were accurately measured at each flow rate by tracer injections. The detailed effect of the flow rate on the value of the amount adsorbed was investigated. The best isotherm model accounting for the adsorption data was the same BET isotherm model at all three flow rates. Only slight differences (always less than 5%) were found between the three different sets of isotherm parameters (saturation capacity, q_S, equilibrium constant on the adsorbent, b_S and equilibrium constant on successive layers of propyl benzoate, b_L). The reproducibility of the same isotherm parameters measured by the inverse method (IM) is less satisfactory, leading to R.S.D.s of up to 10%. A flow rate increase is systematically accompanied by a slight increase of the amount adsorbed. This phenomenon is consistent with the influence of the pressure on the equilibrium constant of adsorption due to the difference between the partial molar volumes of the solute and the adsorbate. The larger average pressure along the column that is required to achieve a larger flow rate causes a larger amount of solute to be adsorbed on the column at equilibrium. This result comforts the high sensitivity and versatility of the FA method for isotherm determination under any kind of situation.

Keywords: Adsorption isotherm; Frontal analysis method; Calculation of elution profiles method; Flow rate; Liquid chromatography; Propylbenzoate; Column efficiency

1. Introduction

The prediction of individual band profiles that is required for the computer-assisted optimization of any mode of chromatography relies on the quantitative knowledge of the thermodynamic properties of the system considered [1]. This explains the importance given to improvements of the current methods of determination of equilibrium isotherm data on conventional chromatographic systems. Accurate adsorption data make easier the selection of the most appropriate isotherm model and to estimate the best values of the isotherm parameters. Various methods can be applied rapidly to measure adsorption data and to do so while using low amounts of the sample and of the mobile phase [1]. The most useful among such methods are the elution by a characteristic point (ECP) method [2,3] and the computation of elution profiles (CEP) method [4–6], also called the inverse method for isotherm determination (IM). Both ECP and CEP methods are based on the record of one or a few overloaded band profiles.

In the former method (ECP), the amount adsorbed at the concentration C_0 is calculated by applying the mass balance equation from the tail end of the band ($C = 0$) to the concentration C_0 recorded in the rear boundary of the band profile. This method is based on the ideal model and assumes that the column has an infinite efficiency, hence it includes a model error. The limits of the ECP method is that it can

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be applied conveniently only to convex upward isotherms. Although convex downward isotherms can be determined by applying the method to the front part of the elution profile, the result is usually less accurate. For S-shaped isotherms, however, the method is impractical at best.

In the latter method (CEP), a model of the adsorption isotherm is assumed, band profiles are calculated using a model of chromatography, these profiles are compared to experimental profiles, and the best parameters of the isotherm are estimated by minimizing the difference between the experimental and the calculated band profiles. The main difficulty in this method is the need for an initial guess of both the isotherm model and of the model of chromatography [1]. Because the column efficiency is finite, mass transfer resistances cannot be neglected, and the coefficients of the mass transfer kinetics cannot be conveniently assessed, the equilibrium-dispersive model is usually preferred to the lumped pore diffusion model and to the general rate model. This model assumes that the finite efficiency of the column results from an apparent diffusion coefficient that is independent of the solute concentration. This assumption introduces a model error, albeit one smaller than with the ECP method.

The only method of isotherm determination that gives data which do not depend on the column efficiency is the frontal analysis method (FA). The accuracy of the resulting adsorption data depends on the control of the column temperature, of the pressure, and of the mobile phase composition. It also depends on the accuracy of the determination of the column hold-up volume, the extra-column volumes (volumes of the connecting tubes upstream and downstream the column), and of the column dimensions. Since FA is a dynamic method, however, the parameter that must be most accurately known and controlled during the record of a series of breakthrough curves is the actual flow rate delivery. The mass adsorbed at equilibrium with a stream of solution at the concentration C_0 is derived from the integration of the breakthrough curves ($C = f(t)$) between two defined moments, the beginning of the injection of the sample solution at concentration C_0 and any time at which equilibrium has been reached in the column (i.e., when a stable plateau is detected at the column outlet). Whether the front is weakly or strongly dispersed, whether the breakthrough curve exhibits no, a slight, or a strong tailing, has no importance on the determination of the amount of solute adsorbed provided that the integration of the breakthrough curve is practically complete.

The effect of local temperature changes on the adsorption data has been studied recently [7]. It was shown that temperature fluctuations affect mostly the value of the adsorption constant, which follows Van't Hoff law. Although the effect of pressure on the amount adsorbed is usually small, it is significant with large molecules such as the buckminsterfullerenes [8] or for many bioanalytes [9,10]. The effect of the mobile phase composition and especially that of the organic modifier in aqueous solutions was already studied in RPLC [11,12]. The mobile phase composition affects both the surface heterogeneity of RPLC adsorbents [11,13] (typically the heterogeneity of the adsorbent surface is enhanced at low organic modifier concentrations) and the adsorption constants of solutes, which follow usually the linear strength solvent model (LSSM), at least in a certain range [11,7]. In principle and provided that it remains constant during the series of FA measurements, the flow rate used should not change the solute distribution between the adsorbent and the mobile phase. Provided that the equilibrium data are based on the integration of the breakthrough curve, they should be independent of the flow rate. However, changing the flow rate leads to a change in the average column pressure and this change may affect the way in which the molecules of the solute interact with the surface as well as the hold-up column volume. This issue is examined in this work. Investigations to assess the experimental errors that are made when adsorption data are measured at different flow rates by FA will be discussed.

2. Theory

2.1. Determination of single-component isotherms

Frontal analysis was used to measure the adsorption data of propyl benzoate on Symmetry-C_{18}. The step-wise replacement of the pure mobile phase with solute solutions of increasing concentrations was carried out. For each solute concentration, C, in the mobile phase in equilibrium with the adsorbent, the mass of propyl benzoate adsorbed per unit volume of adsorbent (q^*) was determined by applying the law of mass conservation of the solute between the times when the solution enters the column and when the plateau concentration is reached at the column outlet. This amount is best calculated by integrating the breakthrough curve (equal area method) [14]. The adsorbed concentration q^* is given by:

$$q^* = \frac{CF_v[t_{\text{eq.}} - (t_0 - t_{\text{e,a}}) - t_{\text{e,p}}]}{[V_c - F_v(t_0 - t_{\text{e,a}})]} \quad (1)$$

where F_v is the solution flow rate, $t_{\text{eq.}}$ the time of the equivalent area, calculated by integration of the breakthrough curve (injection of a large sample volume from the pump), t_0 the measured hold-up time (retention time of an infinitesimal volume of tracer injected from the auto-sampler), $t_{\text{e,a}}$ and $t_{\text{e,p}}$ are the extra- column times measured from the autosampler and from the pump, respectively, to the detector, and V_c is the geometrical volume of the column tube.

2.2. Models of isotherm used

The isotherm model that best accounts for the adsorption data of propylbenzoate on Symmetry-C_{18} with a mixture of methanol (65%, v/v) and water as the mobile phase was the extended solid–liquid BET isotherm model [15]. The detailed derivation of this model is described in reference [15]. The

final equation is:

$$q^* = q_S \frac{b_S C}{(1 - b_L C)(1 - b_L C + b_S C)} \quad (2)$$

where q_S is the monolayer saturation capacity of the adsorbent, b_S the equilibrium constant for surface adsorption–desorption over the free surface of the adsorbent and b_L is the equilibrium constant for surface adsorption/desorption over a layer of adsorbate molecules.

2.3. Modeling of band profiles in HPLC

The overloaded band profiles of propyl benzoate were calculated using the equilibrium-dispersive model (ED) of chromatography [1,16,17]. The ED model assumes instantaneous equilibrium between the mobile and the stationary phases and a finite column efficiency originating from an apparent axial dispersion coefficient that accounts for the dispersive phenomena (molecular and eddy diffusion) and for the non-equilibrium effects (mass transfer kinetics) that take place in the chromatographic column. The axial dispersion coefficient is related to the column efficiency under linear conditions and is assumed to be independent of the sample concentration.

At $t = 0$, the stationary phase is in equilibrium with the pure mobile phase and the solute concentrations in both phases in the column are uniformly equal to zero. The boundary conditions used are the classical Danckwerts-type boundary conditions [18] at the inlet and outlet of the column.

The ED model was solved using the Rouchon program, based on a finite difference method [1,19–21].

3. Experimental

3.1. Chemicals

The mobile phase used in this work was a methanol–water (65:35, v/v) mixture. Both methanol and water (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The solute studied was propyl benzoate (99%) purchased from Aldrich (Milwaukee, WI, USA).

3.2. Columns

We used a 150 mm × 3.9 mm column, packed with Symmetry-C_{18} particles, given by the manufacturer (Waters, MA, USA). Its main characteristics are summarized in Table 1. This column was one of the 15 Symmetry-C_{18} columns previously used by Kele and Guiochon [22] for their study of the repeatability and reproducibility of retention and peak profile data under linear conditions. The column void volume was derived from the product of the average of the retention times of two consecutive thiourea injections ($t_0 - t_{e,a}$) and the measurement of the flow rate (F_v).

Table 1
Physico-chemical properties of the C_{18}-bonded packed Symmetry column (150 mm × 3.9 mm)

Particle shape	Spherical
Particle size (μm)	5
Pore size[a] (Å)	86
Pore volume[a] (mL/g)	0.90
Surface area[a] (m^2/g)	346
Total carbon (%)	19.6
Surface coverage (μmol/m^2)	3.18
Endcapping	Yes

[a] Data for the packings before derivatization.

3.3. Apparatus

All the breakthrough curves and overloaded band profiles were acquired using a Hewlett-Packard (now Agilent Technologies, Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a multi-solvent delivery system (volume of each tank, 1 L), an auto-sampler with a 250 μL sample loop, a diode-array UV detector, a column thermostat and a data station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operations of the pump, the auto-sampler, and the solvent sparging. The actual flow rate F_v delivered by the pump system was obtained from the measurement of the time t_{F_v} necessary to fill a volumetric glass of a given volume V_g (10, 25 and 50 mL volumetric glasses to measure accurately the flow rate around 0.5, 1.0 and 2.0 mL/min, respectively). The measured flow rate is given by

$$F_v = \frac{V_g}{t_{F_v}} \quad (3)$$

The accuracy on the flow rate measurements was ±0.2%.

The extra-column volumes from the auto-sampler ($V_{e,a}$) and from the pump system ($V_{e,p}$) were determined from the elution times of 10 μL pulses of a thiourea solution (injected with the autosampler, measured at the peak maximum, $t_{e,a}$) and of 5 mL pulses of the sample solution (injected with the pump, measured with the equivalent area method, $t_{e,p}$), respectively (see Fig. 1).

$$V_{e,a} = F_v t_{e,a} \quad (4)$$

$$V_{e,p} = F_v t_{e,p} \quad (5)$$

The values obtained are listed in Table 2. All the retention data were corrected for the one of these two contributions that applies. The flow-rate accuracy was determined by pumping the pure mobile phase at 23 °C and 1 mL/min during 50 min, from each pump head, successively, into a volumetric glass of 50 mL. The relative error was less than 0.4%, so that we can estimate the long-term accuracy of the flow-rate at 4 μL/min at flow rates around 1 mL/min. All measurements were carried out at a constant temperature of 23 °C, fixed by the laboratory air-conditioner. The daily variation of the ambient temperature

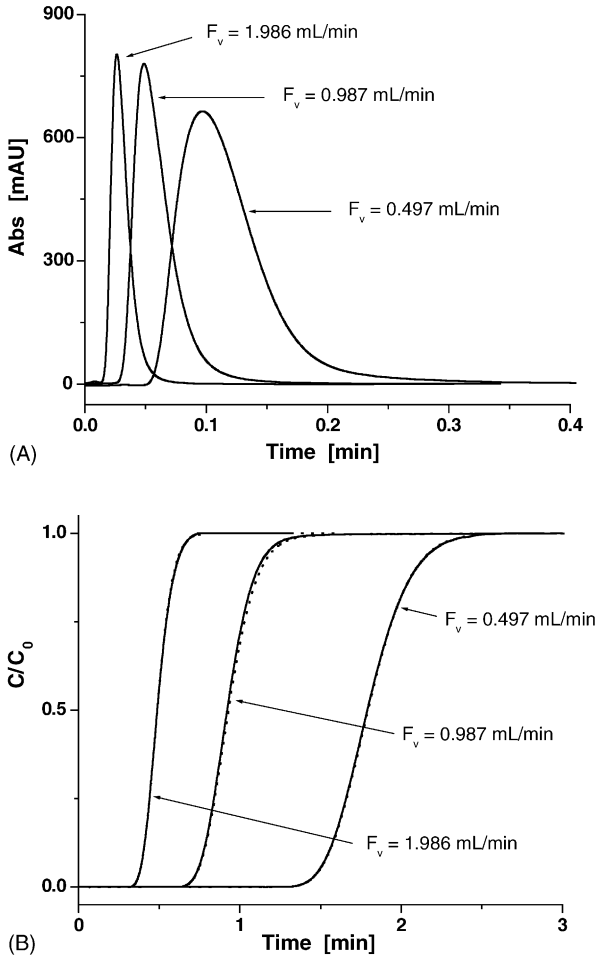

Fig. 1. Experimental determination of the extra-column volumes at room temperature. (A) Extra column volumes from the auto-sampler. Injection of 10 μL of a solution of 1 g/L of thiourea. Note the large peak broadening. (B) Extra column volumes from the pump delivery system. Injection of a solution of propylbenzoate (15 g/L, solid line; 7.5 g/L, dotted line) during 5 min. Note that the two normalized lines are almost undistinguishable.

never exceeded ±1 °C during the campaign of measurements.

3.4. Measurements of the adsorption isotherm of propyl benzoate by FA

The adsorption isotherms of propyl benzoate were all measured with methanol–water (65:35, v/v). The maximum concentration of propyl benzoate applied in FA was fixed at 15 g/L to avoid any precipitation of the solute in the instrument. One single master solution was prepared at this concentration and seventeen solutions of lower concentrations (at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90 and 100% of 15 g/L) were generated. One pump of the HPLC instrument was used to deliver a stream of the pure mobile phase (1000 mL, methanol–water, 65:35, v/v) and the second pump a stream of the master solution (100 mL). The feed concentration in the FA stream is proportional to the concentration of the sample in the master solution and to the flow rate fractions delivered by the two pumps. The seventeen data points were acquired for concentrations between 0.75 and 15 g/L.

The breakthrough curves were recorded with a sufficiently long time delay between them (25 min) to allow for the complete reequilibration of the column with the pure mobile phase after the elution of each breakthrough curve. The injection time of the sample was fixed at 5 min for all FA steps, in order to reach a stable plateau at the column outlet, whatever the feed concentration used. The signal was detected at 293 nm. The calibration curve was determined from the concentration injected (flow rate fractions × 15 g/L) and the UV signal of the plateau of the breakthrough curves detected at the column outlet.

3.5. Measurements of overloaded band profiles

Low- and high-concentrations bands were injected with the pump. Pulses of 1 and 2 mL of solutions of 1.5 and 13.5 g/L of propyl benzoate were injected in the column and the band profiles were recorded at 293 nm.

4. Results and discussion

4.1. Extra-column volumes

According to Eq. (1), the mass of solute adsorbed per unit volume of adsorbent depends on the extra-column times, $t_{e,a}$ and $t_{e,p}$. These times permit also the determination of the exact hold-up column volume, independently of the length of the connecting tubes installed in the apparatus. However, these extra-column parameters are measured for a certain set value of the flow rate and it is important to assess their

Table 2
Measurement of the actual flow rate delivered by the pump (F_v) by using different volumetric glasses (10, 25 and 50 mL) and a stop watch (t_{F_v})

Volumetric glass (mL)	t_{F_v}	F_v (mL/min)	$t_{e,a}$ (min)[a]	$t_{e,a}$ (min)[b]	$t_{e,p}$ (min)	t_0 (min)	$V_{e,a}$ (mL)[a]	$V_{e,a}$ (mL)[b]	$V_{e,p}$ (mL)	V_0 (mL)
10	20′08″	0.497	0.121	0.097	1.804	2.207	0.060	0.048	0.897	1.049
25	25′17″	0.989	0.061	0.049	0.929	1.111	0.060	0.049	0.918	1.050
50	25′12″	1.986	0.031	0.026	0.491	0.564	0.061	0.052	0.975	1.068

Determination of the extra column volumes ($V_{e,a}$ and $V_{e,p}$) and the column hold-up volume (V_0) from the measurement of the corresponding extra column times ($V_{e,a}$ and $V_{e,p}$) and hold-up times (t_0), respectively.

[a] Calculated from the first moment.
[b] Calculated from the peak apex.

Table 3
Comparison between the expected (Th.) and experimental (Exp.) ratios of hold-up and extra column times (noted R) with respect to the measured flow rates ($R = t_{F_{v_i}}/t_{F_{v_j}}$)

F_{v_i}/F_{v_j}	Flow rate ratio	$R_{e,a}$ Th.	$R_{e,p}$ Th.	R_0 Th.	$R_{e,a}$ Exp.[a]	$R_{e,a}$ Exp.[b]	$R_{e,p}$ Exp.	R_0 Exp.
0.989/0.497	1.990	0.503	0.503	0.503	0.502	0.505	0.515	0.503
1.986/0.989	2.008	0.498	0.498	0.498	0.513	0.529	0.525	0.508
1.986/0.497	3.996	0.250	0.250	0.250	0.257	0.272	0.270	0.256

[a] Calculated from the first moment.
[b] Calculated from the peak apex.

reproducibility and the influence of the flow rate on the value obtained.

The former time ($t_{e,a}$) is the retention time of the maximum of the peak obtained upon the injection of a 10 μL pulse of a thiourea solution, with the auto-sampler and without column. This compound is not retained on reversed phases. Fig. 1A shows the experimental peaks obtained. These peaks are clearly unsymmetrical and exhibit a significant degree of tailing. As shown in Table 3, the retention times of the apex of the peaks are not inversely proportional to the flow rate but their first moments are. This result is explained by the peak asymmetry, itself the result of the combined influences of a very short retention ($\leq$0.2 min), a large peak width ($\geq$0.1 min), and a long tailing of the injection plug due to diffusion in the extra-column volume. The experimental ratios $t_{e,a}^{Fv_1}/t_{e,a}^{Fv_2}$ are systematically larger than those expected based on the flow rate ratios, so that when the flow rate is higher, the apex of the peak is displaced toward elution times that are shorter than the first moment of the peak. The reason is that the asymmetry of the peak increases with decreasing flow rate. The asymmetry measured at 5% of the peak height is 2.65 at 2 mL/min, 3.26 at 1 mL/min, and 3.01 at 0.5 mL/min. The asymmetry factor depends on the height of the peak at which it is measured and the expected trend could be found if a smaller peak height had been taken into account. However, the trend between the highest flow rate (2.0 mL/min) and the lowest ones (0.5 and 1.0 mL/min) is well respected. These results are consistent with the behavior of the connecting tubes as mixing reactors and the actual injection profile of the sample being described by an exponentially convoluted Gaussian profile [23].

The latter extra-column time ($t_{e,p}$) is measured from the integration of the breakthrough curve recorded without column (using the so-called equivalent area method), upon switching the stream percolating through the equipment from one of pure mobile phase to one of a solution of propyl benzoate, using the pump delivery system. Fig. 1B shows two breakthrough curves recorded at each of the flow rates applied, during a time sufficiently long for the outlet concentration to reach C_0. These two breakthrough curves correspond to streams of solutions of 7.5 and 15 g/L of propyl benzoate. When plotted in reduced coordinates, C/C_0, the two breakthrough curves are exactly overlaid. This shows that the extra-column volume does not depend on the concentration of the solution pumped into the instrument. The average extra-column times determined are listed in Table 2.

Table 3 gives the relative difference between the experimental and the calculated extra-column times obtained at the three different flow rates. The observations already made for $t_{e,a}$ remain valid for $t_{e,p}$. The experimental values of the extra-column times obtained by frontal analysis depend as much on the flow rate as those measured by elution. The time $t_{e,p}$ increases systematically with increasing flow rate, by 2.4% from 0.5 to 1 mL/min and by 5.4% from 1 to 2 mL/min. This phenomenon causes another source of inaccuracy in the determination of the amount of solute adsorbed at equilibrium.

The extra-column times are critical parameters in the accurate determination of the amount of solute adsorbed at equilibrium per unit volume of adsorbent. These times depend on the flow rate used, irrespective of the method used for their determination, whether from the retention time of the peak apex or by integration of the breakthrough curve. The differences observed are certainly related to the evolution of the band profile of the studied compound along the connecting tubes [23]. It is important to observe that, at a given flow rate, the values of the extra-column times derived from the two methods are very similar (Table 3). However, these values are not exact and systematic errors are made. Our experimental results give estimates of the extra-column volume measured from the autosampler and from the pump to the detector that are, respectively, between 48 and 52 μL (difference of 8.0%) and between 0.897 and 0.975 mL (difference of 8.3%). In principle, these values should be the same since they are estimate of the connecting tubes, the volumes of which are independent of the flow rate used in the measurements.

As indicated earlier, the results of measurements of the extra-column volumes vary with the mobile phase flow rate. The average values of the extra-column volumes measured from the autosampler and from the pump to the detector were 0.050 and 0.90 mL, respectively, and their range, for flow rates between 0.5 and 2 mL/min, was 10%. Since both parameters increase systematically with increasing flow rate (Table 2), we applied the same error factor to both volumes simultaneously. Fig. 2 shows the relative difference in the adsorption data that is calculated from Eq. (1) when errors of +5 and +10% are assumed to affect simultaneously both $t_{e,a}$ and $t_{e,p}$. The resulting error on the mass adsorbed is much lower, 1.1 and 2.2%, respectively. This is not surprising because the hold-up time, t_0, and the time of equivalent area, $t_{eq.}$, are at least 20 and six times larger than $t_{e,a}$ and $t_{e,p}$, respectively, which explains why the effects of a 10% error on the extra-column volumes on the numerator and the denominator of

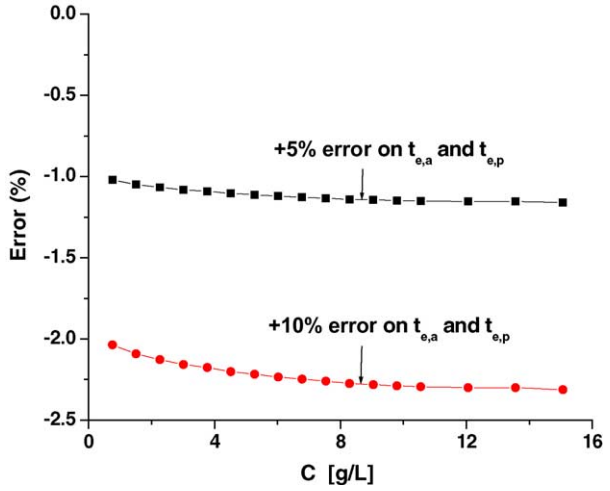

Fig. 2. Numerical errors made on the adsorption data of propylbenzoate on the Symmetry column from a methanol–water (65:35, v/v) mixture solution. (Eq. (1), $F_v = 1$ mL/min, $t_0 = 1.097$ mL) when +5 and +10% errors are made on the two extra-column volumes $t_{e,a}$ (0.048 mL) and $t_{e,p}$ (0.900 mL) simultaneously.

the right-hand side of Eq. (1) compensate to a large extent. This error disappears if the correction is made correctly, by using the first moments of the peaks, not the retention times of their apices.

Although the increase of the extra-column volume with increasing flow rate that we have observed is mostly due to the progressive increase in the tailing of the injection profile, it may also be in a small part due to the increase in the pressure and to the compressibility of the mobile phase (see next section). At 1 mL/min, the pressure drop through the instrument alone, with no column, is 23 bar. However, the elastic expansion of the injection and detection devices, which are made of relatively bulky metal parts, is probably much smaller than that of the column. These results illustrate the serious difficulties inherent to the acquisition of accurate adsorption data by dynamic methods. Although, at any given flow rate, the data are consistent, they may suffer from a systematic error.

4.2. Column hold-up volumes

The column hold-up volume is simply derived by subtracting the elution time of thiourea measured without column ($t_{e,a}$) from that measured with the column (t_0). The precedent section describes the dependence of $t_{e,a}$ on the flow rate applied. The same dependence is illustrated in Tables 2 and 3 for t_0. Note that the effect of the flow rate on t_0 is much smaller than on $t_{e,a}$ or $t_{e,p}$. The hold-up times t_0 measured at 0.5 and 1.0 mL/min are the same and variations of only +2 and +2.4% are observed when the flow rate is increased from 1 to 2 mL/min. This increase of the hold-up volume is now entirely explained by the consequence of the increase of the average column pressure which is, itself, caused by the flow rate increase. As shown in Fig. 3A, the hold-up time of the Symmetry-C_{18} column increases regularly from 1.018

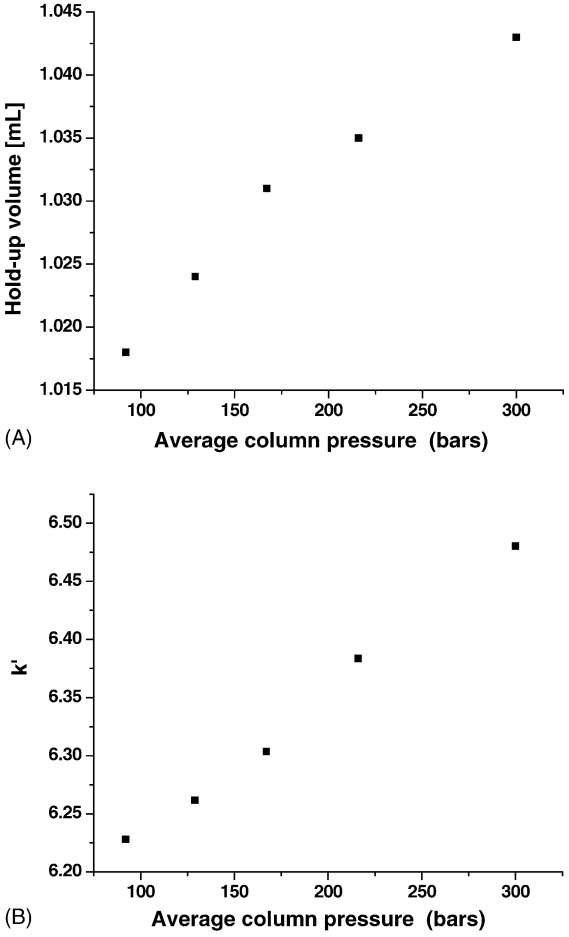

Fig. 3. Impact of the average column pressure on the hold-up time measured at atmospheric pressure on the Symmetry-C_{18} column (A) and the retention factor of propylbenzoate (B).

to 1.043 mL (+2.4%) when the average column pressure increases from 92 to 300 bar. This increase results from the compressibility of the methanol:water mixture (compressibility factor), the dilatation of the stainless steel column (Lamé factor) and the shrinkage of the compressed C_{18}-bonded silica particles [24].

The dispersion of the band of a compound is much more important if this band is eluted along a packed column than if it is transported through an open tube. This dispersion slowly erodes away the contribution of the injection profile to the band profile. The estimated hold-up column volume varies between 1.049 and 1.068 mL when the flow rate increases from 0.5 to 2 mL/min (a +1.8% difference). This difference is accounted for by the increase of the average column pressure which increases from 47, to 92, and 183 bar for flow rates of 0.5, 1.0, and 2.0 mL/min, respectively and by the compressibility of the mobile phase. When the mobile phase is compressible, the NTP volume needed to sweep the column increases with increasing pressure drop. There is also a possibility that more mesopores become accessible at higher pressures (Washburn's law [25]) although the stationary phase of this phase system is completely wetted by the mobile phase.

4.3. Effect of the flow rate on the amount adsorbed measured by FA

Once the extra-column volumes and the hold-up column volume are known for a given flow rate, it is possible to derive the amount of a compound that is adsorbed at equilibrium from the elution time of the breakthrough curve given by the equal area method (see illustration in Fig. 4B). This is done for each breakthrough curve recorded (see Fig. 4A). In this work, we used a compound known to provide unusual S-shaped isotherms [26], the low molecular weight compound propyl benzoate. This choice offers several advantages. First, the isotherm is nearly linear, thus the systematic error made on $t_{eq.}$ will have nearly the same effect at low and at high concentrations. Therefore, the relative errors made on the masses adsorbed at different flow rates will be comparable at low and at high concentrations. Second, we had observed that, for this compound, the shapes of the elution band profiles and of the breakthrough curves normalized to the elution volume depend significantly on the flow rate (Fig. 5), through the flow rate dependence of the column efficiency. This makes this compound a good choice to validate the FA method. Finally, the mass transfer kinetics of propyl benzoate in the phase system used depends on the concentration [26,27]. The classical equilibrium-dispersive model of chromatography which assumes an homogeneous and constant dispersion parameters fails to predict accurately the overloaded band profiles of alkyl benzoates. More elaborate models (e.g., the lumped pore model, the general rate model) give better results [28]. Nevertheless, whatever the complexity of the mass transfer mechanism and the dependence of its kinetics on either the concentration or the mobile phase velocity, the use of the equivalent area method to determine the adsorption data ensures that the equilibrium isotherm remains the same, independently of the flow rate used to acquire the FA data.

The amounts of propyl benzoate adsorbed onto Symmetry-C_{18} from a methanol–water (65:35, v/v) mixture are plotted versus the concentration C of the solution in Fig. 6 for the three flow rates studied. There are some slight differences. The relative differences between the amount adsorbed at 1.0 and 2.0 mL/min, on the one hand, and at 0.5 mL/min, on the other hand, are plotted versus C in Fig. 7. Despite the complexity of the mass transfer mechanism and the different shapes in the breakthrough curves normalized to the same elution volume, the adsorbed amounts (Eq. (1)) remain very close. The amounts adsorbed at flow rates of 1.0 and 2.0 mL/min appear slightly larger (around +2%) than those measured at 0.5 mL/min.

This small positive difference is largely explained by the effect of the pressure on the equilibrium parameters, as previously observed on Chromolith-C_{18} with butyl benzoate as the solute and with a methanol–water (60:40, v/v) mixture as the solvent. The retention factor increased by about 2% when the average column pressure was increased from 100 to 275 bar at constant flow rate. In the same time, the column hold-up

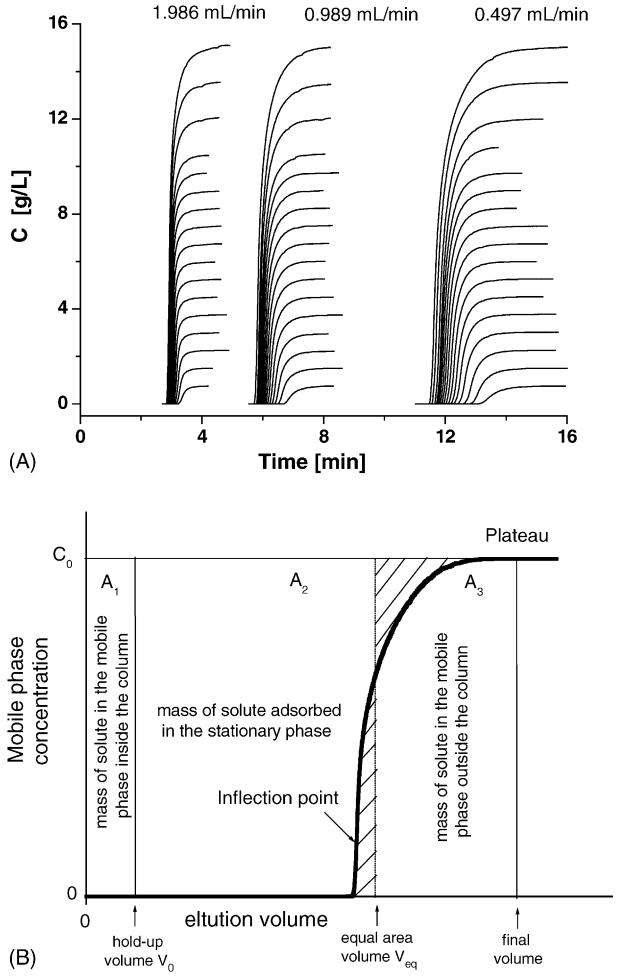

Fig. 4. Recording of the breakthrough curves and application of the equal area method for the isotherm determination. (A) Breakthrough curves of propylbenzoate injected in the Symmetry column with a methanol–water (65:35, v/v) mixture as the mobile phase. Note the effect of the flow rate on the position and shape of the frontal curves. (B) Description of the method used to derive the amount adsorbed from the breakthrough curves in part (A). Note that considering the inflection point is strongly erroneous.

volume of the Chromolith column increased by about 1.5%, a value consistent with the increase of the hold-up volume of the Symmetry-C_{18} column given in Table 2 (from 1.049 to 1.068 mL). To confirm the influence of the pressure on the retention, the retention factors of propylbenzoate were measured on the Symmetry-C_{18} column at five different average column pressures (92, 129, 167, 216 and 300 bar), by connecting polyether ether ketone (PEEK) capillaries of various lengths downstream the column. Fig. 3B shows a clear increase of the retention factor, by about 4%. This increase of the retention factor arises from the fact that the partial molar volume of the analyte changes when it is transferred from the liquid to the adsorbed phase.

Basic thermodynamic considerations [29,30] allow the derivation of the following relationship between the variation of the retention factor k and the local pressure in

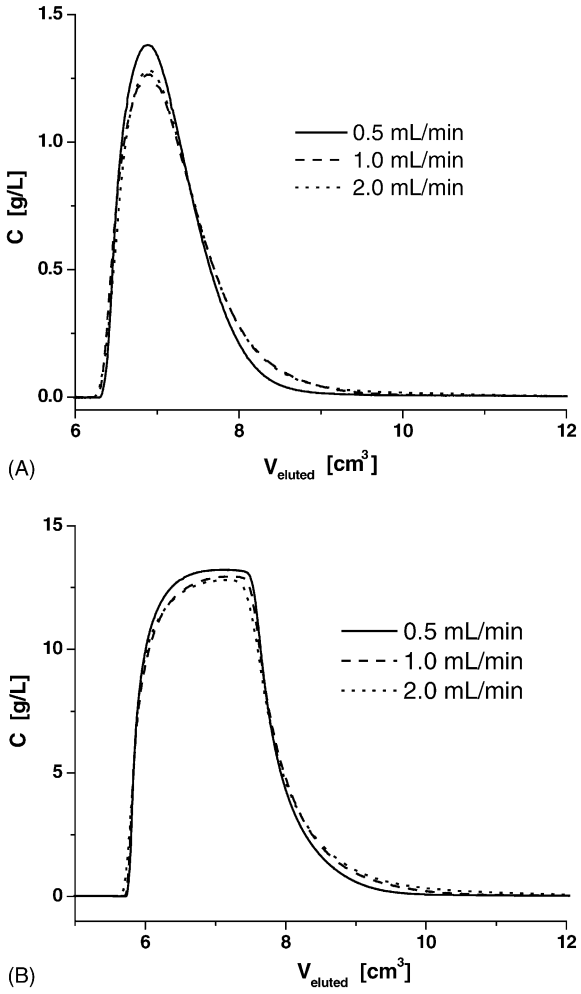

Fig. 5. Overloaded band profiles of propylbenzoate injected on the Symmetry-C_{18} column for three different flow rate. Same mobile phase conditions as in Fig. 2. Note the effect of the flow rate on the front and rear part of the band profiles. (A) Injection during 60 s of a 1.5 g/L solution of propylbenzoate. (B) Injection during 120 s of a 13.5 g/L solution of propylbenzoate.

the system

$$\left(\frac{\partial \ln k}{\partial P}\right)_T = -\frac{\Delta V}{RT} + \left(\frac{\partial \ln \phi}{\partial P}\right)_T \quad (6)$$

where ΔV is the change of the partial molar volume associated with the retention process, ϕ the column phase ratio and T the temperature. The change of partial molar volume associated with the transfer from the liquid to the adsorbed phase increases with increasing molecular weight. According to our data and Eq. (6), it is of 11.5 mL/mol for propyl benzoate. It is of 50–100 mL/mole for peptides or proteins (ΔV is 100 mL/mole for insulin [31]). The increase of the retention factor observed is consistent with many earlier results [31]. It is not due to an error of measurement but complicates the interpretation of experimental results since a correction is required for this effect.

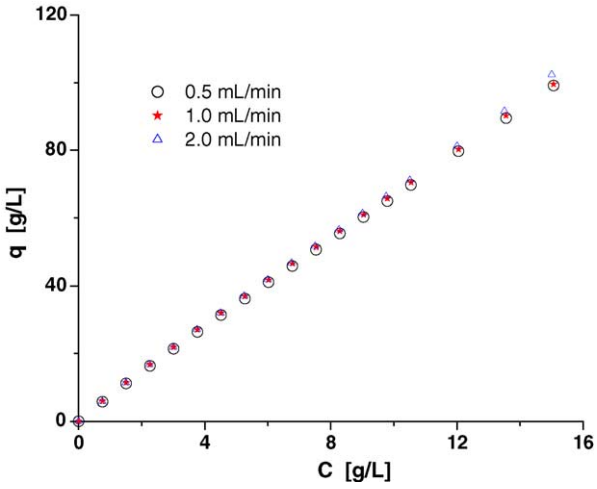

Fig. 6. Comparison between the adsorption data of propylbenzoate measured by FA with three different mobile phase velocities. Same experimental conditions as in Fig. 2. Note the very close adsorption data between the three data sets except for the flow rate of 2 mL/min.

The robustness of the frontal analysis method is illustrated by the data in Table 4 in which are listed the values of the best isotherm parameters of the BET isotherm model derived from the fitting to this model equation of the adsorption data obtained with the different mobile phase velocities. The saturation capacity q_S varies by only 3%, the adsorption constant on the adsorbent surface by 5%, and the equilibrium constant on layers of propyl benzoate by 4.5%. This shows that frontal analysis can be applied within a large range of mobile phase flow rate. As explained earlier, these variations are mostly due to the influence of the pressure on the equilibrium constants, not to experimental errors. Thus, the time needed to acquire the experimental data needed to determine an isotherm can be reduced several times without any significant loss in accuracy. The maximum inlet pres-

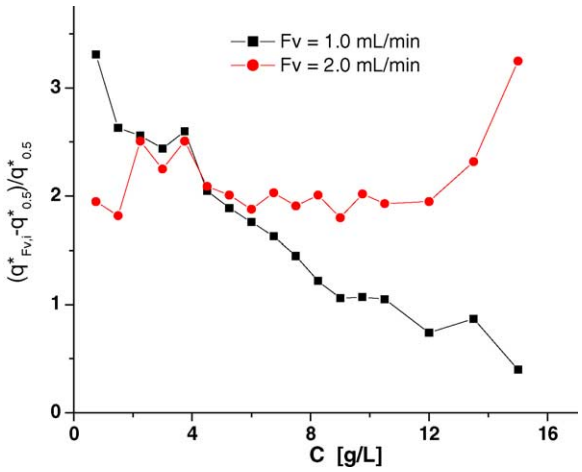

Fig. 7. Relative differences between the adsorption data measured at 1.0 and 2.0 mL/min with this measured at 0.5 mL/min. Note the increasing positive difference when the flow rate increases.

Table 4
Best BET isotherm coefficients derived from regression analysis of the adsorption data measured with three different mobile phase velocities (0.5, 1.0 and 2.0 mL/min)

Isotherm parameters	Frontal analysis (FA)			Calculation elution profiles (CEP)					
	0.497 mL/min	0.989 mL/min	1.986 mL/min	0.497 mL/min		0.989 mL/min		1.986 mL/min	
				Low	High	Low	High	Low	High
q_S (g/L)	102.1	100.3	99.2	99.0	98.4	99.1	89.5	102.0	102.0
b_S (L/g)	0.0761	0.0798	0.0799	0.0782	0.0788	0.0807	0.0890	0.0776	0.0777
b_L (L/g)	0.0233	0.0234	0.0244	0.0181	0.0240	0.0166	0.0259	0.0202	0.0236

Comparison with the best isotherm parameters determined by the CEP method for two different column loadings (low: 1.5 mg; high: 27 mg).

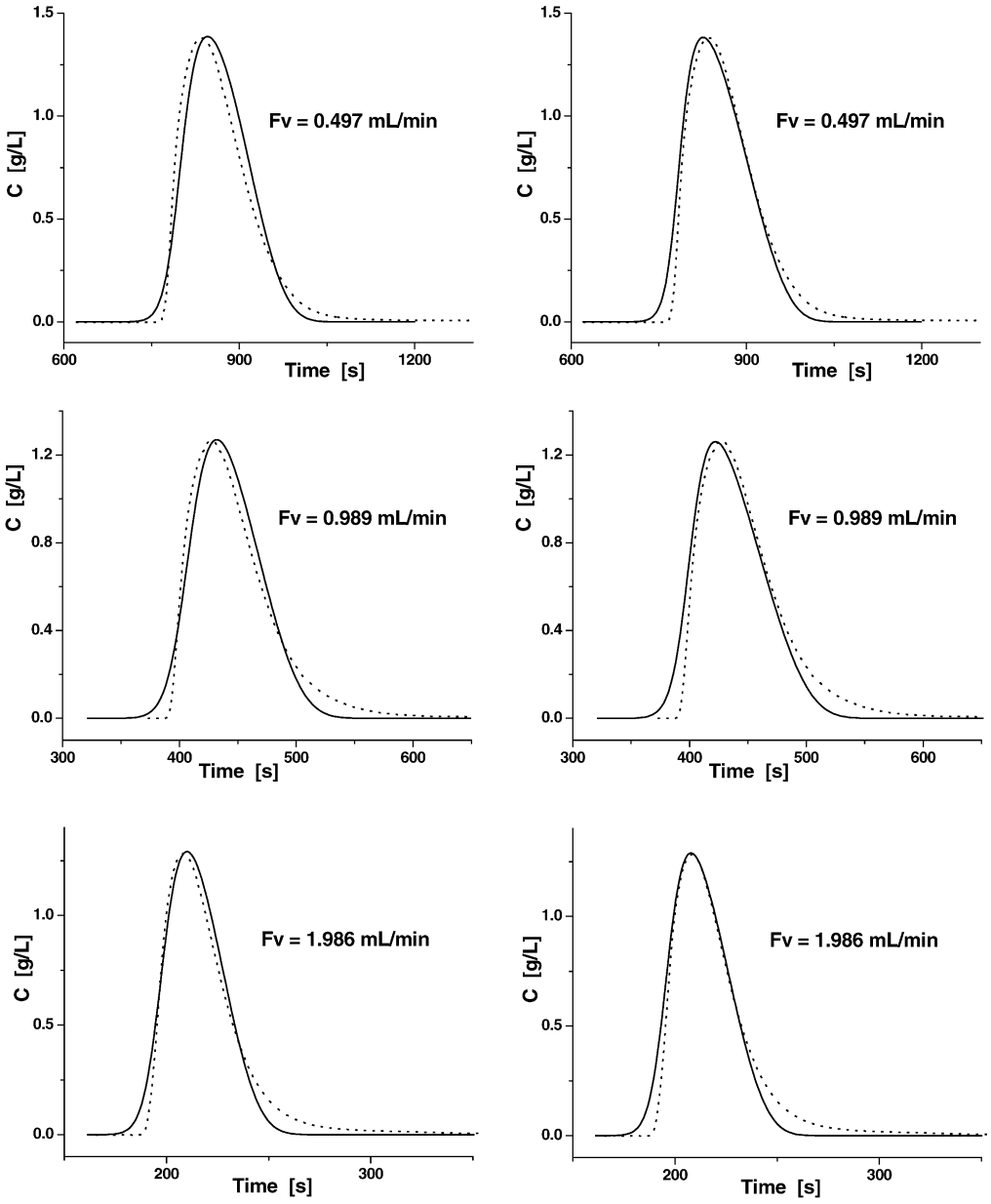

Fig. 8. Comparison between calculated (solid lines, ED model of chromatography) and experimental (dotted lines) overloaded band profiles for three different flow rates. Injection during 60 s of a 1.5 g/L solution of propylbenzoate. Left: Calculation using the isotherm parameters derived from the FA data. Right: Best calculated band profiles by using the CEP method assuming the BET isotherm model. Note that the ED model cannot describe perfectly the experimental band profile.

sure allowed, hence the viscosity of the mobile phase and the column length, are the main constraints limiting the flow rate at which FA data may be acquired. In the particular case of propyl benzoate discussed here, the major advantage of the frontal analysis method is that its results are not influenced by the mass transfer kinetics. If differences exceeding 5% between the adsorption data measured by FA at different flow rates are observed, it is likely that either this is due to errors made in the determination of the extra-column volumes and the column hold-up volume or that it is caused by a larger than usual influence of the pressure on the equilibrium data, in which case the compound studied must have a large molecular weight or follow an unusual retention mechanism.

4.4. Comparison of the accuracy of isotherms determined by FA and IM at different flow rates

In the precedent section, we showed that, provided that the extra-column volumes and the hold-up column volume are carefully and accurately measured, frontal analysis gives isotherm parameters at different flow rates that are in excellent agreement, provided that proper correction is made to account for the systematic influence of the pressure. If this influence is neglected, the relative standard deviations (R.S.D.) of the data, σ_{n-1} ($n = 3$) on the series of values obtained are still only 1.5, 2.8 and 2.6% for q_S, b_S and b_L, respectively (but this value would depend on the compound selected and on its partial molar volume of retention). This result confirms

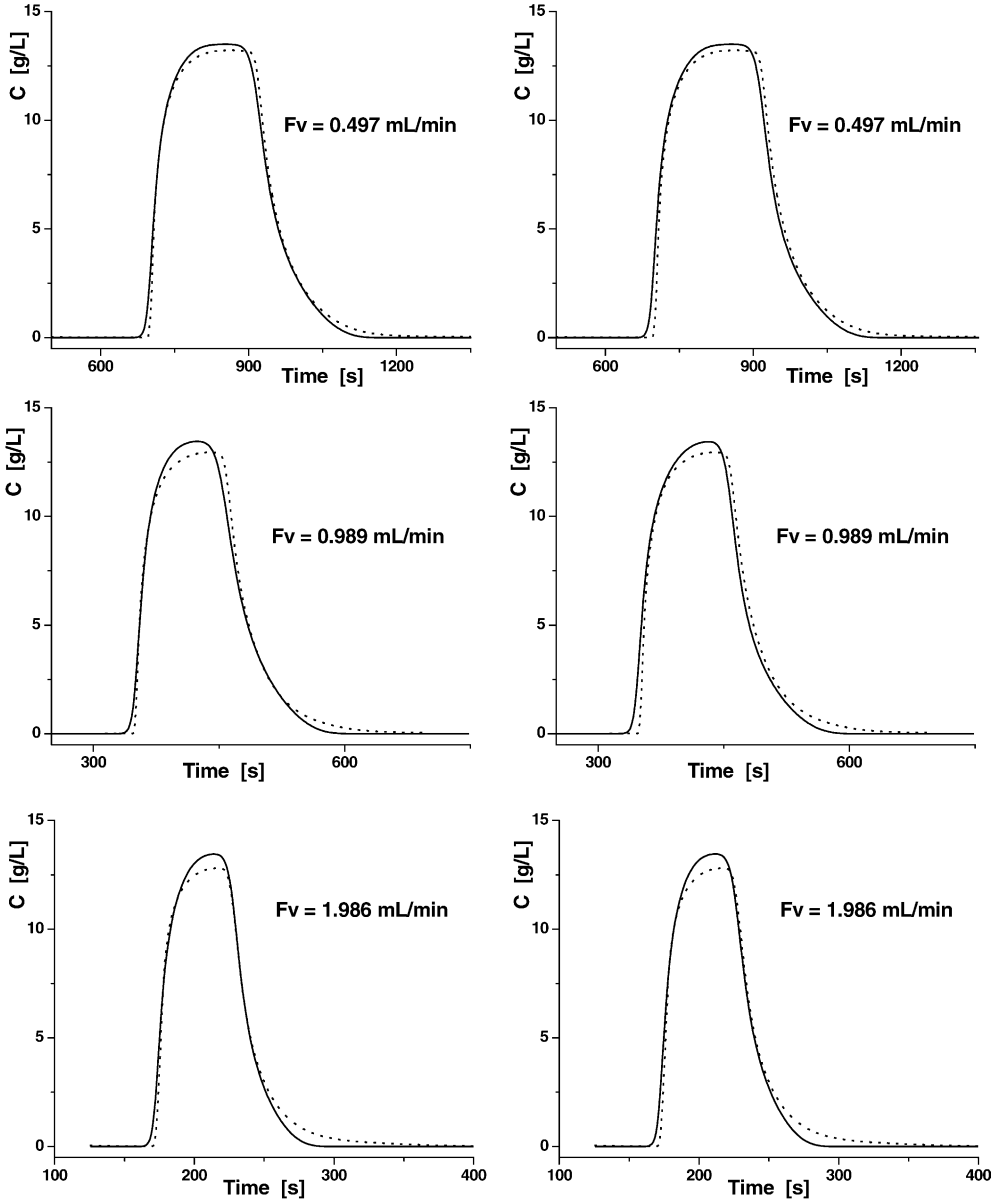

Fig. 9. Same as in Fig. 8, except the injection of a 13.5 g/L solution.

that frontal analysis gives results that are independent of the column efficiency in a rather broad range of flow rates and pressures.

To compare the performance of different methods of isotherm determination with that of FA, we investigated the influence of the flow rate on the isotherm parameters derived from the IM method, a method that may be, at least in principle, strongly dependent on the column efficiency. The isotherm model used with IM was the BET model, because it is supported by the FA data.

The best values of the parameters afforded by the IM method are also given in Table 4. At low column loadings, the apparent R.S.D.s calculated for the three flow rates are 1.7, 2.1 and 9.9% for q_S, b_S and b_L, respectively. The reproducibility of the first two parameters is the same as that of FA. At high column loadings, the R.S.D.s always exceeds 5% (6.6, 7.6 and 5.0%, respectively). The influence of the flow rate is more important on the IM than on the FA data because the band profiles contain kinetics information that the equilibrium dispersive model of chromatography cannot take into account properly and the mass transfer parameters of propyl benzoate are complex functions of the concentration [26,27].

Figs. 8 and 9 show that there is an excellent agreement between the calculated and the experimental band profiles at low (Fig. 8, 1.5 mg injected) and high (Fig. 9, 27 mg injected) column loading, at the three different flow rates. On the right hand side the calculated profiles are those provided by the IM method. On the left hand side, the calculated profiles are obtained with the parameters derived from the FA data. The agreement is very good with the FA parameters and it is excellent with the IM parameters. The algorithm of the IM method tries to account for the whole band profile and, since it contains a model error (it assumes that the mass transfer kinetics is independent of the concentration, which is incorrect in this case), it has to change the isotherm parameters to account for the changes in band profiles due to the concentration effect.

5. Conclusion

This work confirms that dynamic frontal analysis is the most accurate method of acquisition of isotherm data and that it remains accurate even when the mass transfer kinetics and the adsorption–desorption mechanism are concentration dependent and the column efficiency is as low as it is in the case of propyl benzoate [26,27]. When the influence of the pressure on the adsorption equilibrium is neglected, the R.S.D.s of the isotherm parameters derived at three different flow rates with a ratio of one to four are below 3% while, under the same conditions, the R.S.D.s of the same parameters derived with the IM method are as high as 10%. In certain cases, the estimate of the isotherm parameters made with IM from a simple overloaded band profile may deliver inaccurate values of the coefficients because the model of chromatography used in the calculations does not describe correctly the mass transfer kinetics.

The FA method gives very accurate adsorption data when the breakthrough curves are recorded in series, during a single experimental sequence to minimize fluctuations of the experimental conditions, and when the same method is used to treat the breakthrough curves in the whole concentration range. This method can be the direct determination of the retention time of the inflection point in favorable cases (high column efficiency, simple and fast kinetics). It should be the equivalent area method in cases of compounds having a complex or slow kinetics of phase equilibrium. However, the separation scientist should be aware that the derivation of an accurate and precise isotherm and its further use requires that a few more steps be followed carefully.

First, the accurate determinations of the extra-column volumes and of the hold-up column volume are important. The measurements of the extra-column volumes may very well give results that depend on the flow rate used because the injection profile is always strongly asymmetrical. The results depend then on whether the measurement of the elution time of the tracer is based on the retention time of the peak apex or on its first moment. Corrections for this effect are needed, both at the measurement stage and in the calculation of band profiles under nonlinear conditions. The first moment method is strongly recommended. Systematic errors on this volume may lead to significant differences in the calculated amount adsorbed at different flow rates.

Second, the accuracy that is expected for isotherm data and that modern equipment make easy to deliver requires that the influence of the column pressure on the equilibrium data be taken into account. Otherwise, the calculation of overloaded band profiles of a compound on a given adsorbent, using data acquired with different HPLC instruments, using columns of different dimensions (e.g., analytical or preparative size) may lead to significant differences. This may explain the disappointing results recently reported by several engineers who have tried and used the simplistic approaches that are suggested by different manufacturers and are implemented in the canned programs that they supply [32].

Acknowledgements

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