

# Comparison between adsorption isotherm determination techniques and overloaded band profiles on four batches of monolithic columns

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## Abstract

The adsorption isotherms of 4-*tert*-butyl phenol were measured on four different monolithic columns, using three different techniques, classical frontal analysis (FA), the perturbation on a plateau method (PP) and the recently introduced numerical procedure known as the inverse numerical method (IN). This last approach requires only the recording of a few overloaded profiles and has the potential advantage of affording a dramatic decrease of the amounts of compounds, solvent, and time needed to determine accurate estimates of the coefficients of the isotherm. The reproducibility of the adsorption data measured on the four columns is discussed with reference to the specific techniques used for obtaining these data and to the most suitable equation used for modeling them. The data obtained for the different columns were highly consistent. The inverse numerical approach was confirmed to provide a powerful, accurate, and economic method for measuring single component adsorption data.

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## 1. Introduction

Monolithic or rod columns represent the most interesting innovation in column technology since

the discovery of chromatography by Tswett, a century ago [1–6]. Today, various types of monolithic columns, made of different materials, are available. Their common characteristic is that they are constituted by one single piece of a porous material and that this piece fills the entire column.

Silica-based monolithic columns are characterized by a bimodal pore size distribution [1,7–10]. One of these modes is a macropore network with an average pore size of about 2  $\mu\text{m}$ , through which the stream of mobile phase can easily percolate. The permeability of the rod column is higher than that of columns packed with particles of a comparable size because

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the macropore channels in the rod are wider and less constricted than the channels between particles. The second mode is a mesopore structure with an average pore size of about 13–20 nm which gives to the column a sufficiently large surface area to afford enough retention for most solutes with mobile phase having a reasonable organic modifier concentration (hence a reasonable solubility). The bimodal pore size distribution explains the characteristics of the rod columns as separation media: a high efficiency and the possibility to operate at high flow rates. The bimodal porous structure allows both a low hydraulic resistance of the column and a fast mass transfer kinetics. Solute are quickly transported through the macropore network by convective flow and the time needed to diffuse through the small clumps of the mesopore structure is short [8]. Although rod columns have been extensively used and tested [1,2,11] under linear conditions for the separation of a wide variety of chemical compounds, their behavior under nonlinear conditions has been investigated far less systematically and only a few papers have been published regarding adsorption isotherms on these columns [12,9,13].

The empirical evaluation of adsorption isotherms represents the basis for the modeling and the optimization of separations in preparative or semi-preparative chromatography [14]. It is still a tedious, time consuming, and expensive task. This serious obstacle has limited the role of computer optimization in method development in preparative chromatography. Frontal analysis (FA) is probably the easiest and certainly the most accurate method for measuring isotherms. From FA data, the determination of the amount adsorbed as a function of the mobile phase concentration can easily be obtained [15]. The perturbation on a plateau (PP) technique is also frequently used for determining adsorption data [16,17]. By contrast with FA, PP does not give the amount of material adsorbed on the stationary phase as a function of the mobile phase concentration. In the single-component case, the slope of the isotherm at any mobile phase concentration can be obtained from the retention time of perturbation peaks measured on the column equilibrated with a mobile phase containing that mobile phase concentration [18]. The isotherm is derived from this set of data, through numerical integration. The most serious

drawbacks of these two techniques (FA and PP) are the large amount of material required to equilibrate the column at a given mobile phase concentration and the time required to make the systematic determinations needed in a wide enough concentration range. The cost can be prohibitive when expensive compounds (pure enantiomers, polypeptides, proteins) are investigated. Different empirical strategies have been established [16].

Single-component adsorption isotherms can also be evaluated from the diffuse rear boundary recorded in FA or from the rear part of overloaded band profiles. Techniques such as FA by characteristic point (FACP) and elution by characteristic point (ECP) utilize this approach. Their major advantages over FA and PP are the smaller amount of material needed and the faster determination of an isotherm. However, both FACP and ECP are only suitable for single-component systems and can be used only when the efficiency of the chromatographic system used is at least several thousand theoretical plates [14]. This is because both methods rely on the use of an equation of the ideal model, hence include a model error that becomes important at lower efficiencies.

Numerical procedures can also be used for determining adsorption isotherms from overloaded profiles [19–22]. The so-called inverse problem of chromatography [23,24] consists of calculating the adsorption isotherm from the profiles of overloaded bands. Recently, Felinger et al. [25] set up an inverse numerical procedure (IN) that allows the rapid estimation of the best values of the isotherm parameters by minimizing the differences between experimental overloaded profiles of binary mixtures and the profiles calculated by solving the mass balance equation for the system under examination. Excellent results were obtained in the case of the competitive adsorption isotherms of enantiomers without the need of recording the corresponding single-component elution bands. The purpose of this work is to study the adsorption equilibrium of 4-*tert*-butylphenol on silica-based monolithic columns and to evaluate the reproducibility of these data for four monolithic columns [11] when the adsorption isotherm is measured in a wide range of concentrations. The adsorption data were measured on two columns of the same origin, using the classical

techniques, FA and PP. The isotherms were compared with those determined by the IN method from overloaded profiles recorded on these same columns. Once validated, the numerical method alone was used to predict overloaded profiles on the other two columns and calculated and experimental profiles on these columns were compared.

## 2. Theory

### 2.1. Equilibrium dispersive model

Several mathematical models can describe the chromatographic process [14,26,27]. Among them, the simplest is the equilibrium dispersive (ED) model. In spite of its simplicity, the ED model can be successfully applied when the mass transfer resistances are small and/or mainly controlled by diffusion in the mobile phase while the exchange of the elutes between the mobile and the stationary phases is fast. In this case, an apparent dispersion coefficient,  $D_L$ , is used to account for the non-equilibrium contributions (namely, axial and eddy diffusion and a finite mass transfer kinetics). The differential mass balance equation that accounts for the migration of the zone along the column is written:

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

where  $t$  and  $z$  are, respectively, the time elapsed from the injection and the distance traveled by the band along the column;  $u$ , the interstitial mobile phase velocity;  $C$ , the mobile phase concentration in equilibrium with the solid-phase concentration  $q$ ;  $F$ , the phase ratio, is defined by  $F = (1 - \epsilon)/\epsilon$ ,  $\epsilon$  being the total column porosity.  $D_L$  is evaluated under linear conditions, through the well-known equation:

$$D_L = \frac{HL}{2t_0} = \frac{Hu}{2} \quad (2)$$

where  $H$  is the height equivalent to a theoretical plate (HETP);  $L$ , the column length; and  $t_0$  is the column hold-up time ( $t_0 = L/u$ ). The ED model assumes that  $D_L$  does not depend on the solute concentration.

The following initial and boundary conditions are used to solve Eq. (1) [28,29].

– The initial condition corresponds to a column empty of solute:

$$C_i(0, z) = 0; \quad (3)$$

– The boundary condition at the column inlet is usually defined by the injection of a rectangular plug ( $t > 0$  and  $z = 0$ ):

$$C_i(t, 0) = C'_{f,i} \quad (4)$$

where  $C'_{f,i}$  is defined according to:

$$C'_{f,i} = \begin{cases} C_{f,i} & \text{if } 0 < t < t_p \\ 0 & \text{if } t_p < t \end{cases} \quad (5)$$

$t_p$  being the injection time and the subscript  $f$  indicating an “inlet value”. The more rigorous Danckwerts condition (where the effect of axial dispersion during the very injection is accounted for) was not used in this work, as is often the case when the column efficiency exceeds a few thousand theoretical plates [14]. It has been shown that, in such cases, the results obtained using the Danckwerts equation or Eqs. (4) and (5) are numerically equivalent. In practice, the real boundary condition often differs from Eq. (5) due to the dispersion of the sample that takes place between the injection port and the column inlet. This axial dispersion elongates the diffuse rear of the band profile when the isotherm is convex upward. Therefore, when the inverse method is used for isotherm determinations, it is necessary to measure the actual injection profile and to use it as the boundary condition.

– Boundary condition at the column outlet ( $t > 0$  and  $z = L$ ):

$$\frac{\partial C}{\partial z} = 0 \quad (6)$$

The differential mass balance equation (Eq. (1)) was integrated by using either the Rouchon (finite difference) algorithm [30,14] or a modified Rouchon algorithm [31].

### 2.2. Empirical determination of adsorption isotherms

The adsorption isotherm relates the equilibrium concentration of the studied component adsorbed in

the stationary phase to its concentration in the mobile phase.

### 2.2.1. FA technique

From the breakthrough curves obtained in single-component FA experiments, the values of the stationary phase concentration,  $q$ , in equilibrium with the inlet concentration  $C^*$  can be obtained through the following equation [15]:

$$q = \frac{(V_R - V_D)C^*}{V_{\text{ads}}} \quad (7)$$

where  $V_R$  is the retention volume of the self-sharpening shock;  $V_D$ , the system holdup volume (including the column hold-up volume,  $V_0$ ), and  $V_{\text{ads}}$  is the volume of the adsorbent material filling the column. Eq. (7) assumes that the column was initially empty of solute and equilibrated with a stream of solution. A similar equation can be used if FA is carried out in the staircase mode.

### 2.2.2. PP technique

The concept of concentration velocity of a species is of critical importance in nonlinear chromatography [14]. It can be shown that a velocity is associated with each solute concentration and describes the rate of propagation of that concentration along the column. This concentration velocity gives describes the propagation of a perturbation or disturbance of this concentration. This velocity is different from the velocity at which the actual molecules move along the column [18,32,14]. The distinction is not commonly made in chromatography because both velocities are equal when the concentration is close to 0, i.e. under linear conditions. Neglecting all kinetic effects except the one due to convection (i.e. in ideal chromatography), Eq. (1) becomes:

$$\frac{\partial C}{\partial t} + F \frac{\partial q}{\partial t} + u \frac{\partial C}{\partial z} = 0 \quad (8)$$

This equation states that the velocity,  $u_{C'}$ , at which a given concentration  $C'$  travels through the column is [14]:

$$u_{C'} = \frac{1}{1 + F(\partial q / \partial C)|_{C=C'}} \quad (9)$$

where  $\partial q / \partial C|_{C=C'}$  is the slope of the isotherm at

$C=C'$ . Eq. (9) can be expressed in terms of the retention time of the concentration  $C'$ ,  $t_R(C')$ , as:

$$t_R(C') = t_0 \left( 1 + F \frac{\partial q}{\partial C} \Big|_{C=C'} \right) \quad (10)$$

where  $t_0$  is the hold-up time. By simply multiplying Eq. (10) and the mobile phase flow rate, we obtain the retention volume of concentration  $C'$ ,  $V_R(C')$ :

$$V_R(C') = V_0 \left( 1 + F \frac{\partial q}{\partial C} \Big|_{C=C'} \right) \quad (11)$$

Eqs. (9)–(11) constitute the basis for the method of isotherm determination by the perturbation method. The retention time of a small injection of a solute in a column equilibrated with the pure mobile phase (typical condition of analytical chromatography), gives the retention time under linear conditions (i.e. at infinite dilution), through the expression:

$$t_R = t_0(1 + aF) \quad (12)$$

where  $a$  is the initial slope of the isotherm. Similarly, the set of retention times of perturbation peaks measured in a column equilibrated with streams of the mobile phase of increasing concentrations allows the calculation of the slope of the isotherm at these different concentrations. This set of retention times constitutes the fundamental data set needed for determining the adsorption isotherm.

## 2.3. Numerical determination of adsorption isotherms

The inverse method of estimation of the best adsorption isotherm parameters is a promising alternative for measuring thermodynamic adsorption data. The algorithm recently proposed [25] is based on a nonlinear least-squares method. It calculates the best estimates of the isotherm parameters by minimizing the difference between one (or several) experimental overloaded profiles and the corresponding calculated profiles obtained by solving Eq. (1) under the set of initial and boundary conditions describing the experiment performed. The starting point is the choice of an isotherm model,  $q=f(C)$ . Although the examination of the shapes of experimental overloaded profiles (e.g. the fronting or tailing character of these profiles) does help in making this choice (e.g. upward or downward convex isotherm), this choice

requires great attention. A good optimization program will almost always give a set of best values of the parameters: only careful statistical tests can justify the choice of one isotherm model rather than any other one. Numerous isotherm models can describe a convex upward isotherm. The “best” model is usually chosen according to its ability to predict well the experimental overloaded profiles when used to solve Eq. (1)). This turns out to be a more important criterion than its capacity to fit to the original set of experimental isotherm data [29,14]. In this work, because we are more interested in comparing the ability of different techniques in evaluating the adsorption data than in comparing different adsorption models and because that model turned out to be an excellent approximation of the true isotherm in the case in point studied here, only the original Langmuir model was considered.

Briefly, the inverse method involves the following steps [25].

- Choice of the isotherm model and initial guesses for the values of its parameters. For instance, in the case of the Langmuir isotherm:

$$q = \frac{aC}{1 + bC} = \frac{q_s b C}{1 + bC} \quad (13)$$

where  $a = q_s b$  is the Henry constant;  $b$ , the equilibrium adsorption constant; and  $q_s$  is the saturation capacity. The retention factor measured for a chromatographic injection made under linear conditions allows the estimation of the parameter  $a$  (through Eq. (12)). At this point, the least-squares estimation of  $b$ —starting from an initial guess—does not present any difficulty.

- An overloaded profile is calculated by integrating Eq. (1) and using the initial guesses for the isotherm parameters.
- The sum of the squares of the differences between the empirical and the calculated band profiles (objective function) is calculated:

$$\min \sum_i r_i^2 = \min \sum_i (C_i^{\text{sim}} - C_i^{\text{meas}})^2 \quad (14)$$

where  $C_i^{\text{sim}}$  and  $C_i^{\text{meas}}$  are the calculated and the measured concentrations at point  $i$  and  $r_i$  is their difference.

- The isotherm parameters are changed to minimize the squares of the differences between calculated

and experimental profiles. Two nonlinear least-squares algorithms were used for this purpose: the super-modified downhill simplex search [33] and the Levenberg–Marquardt steepest descent method [34].

### 3. Experimental

#### 3.1. Equipment

An HP 1090 liquid chromatograph (Hewlett-Packard, now Agilent, Palo Alto, CA, USA) was used for all the experimental determinations. This instrument was equipped with a multi-solvent delivery system, an automatic sample injector with a 25  $\mu\text{l}$  loop, a diode array detector, and a computer data station.

#### 3.2. Materials

##### 3.2.1. Mobile phase and chemicals

The mobile phase used for the determination of the adsorption data was a methanol–water solution (60:40, v/v). Both methanol and water were HPLC grade solvents, purchased from Fisher Scientific (Fair Lawn, NJ, USA). Uracil and 4-*tert*-butylphenol were purchased from Aldrich (Milwaukee, WI, USA). The solutions used for the FA experiments were filtered before use (Gelman Sci. filter 0.2  $\mu\text{m}$ , Gelman Science, Ann Arbor, MI, USA).

##### 3.2.2. Columns

Four Chromolith Performance 100 $\times$ 4.6 mm columns (Ref. #19, 20, 21, 22, Merck, Darmstadt, Germany, EU) were used. They belonged to the same set as was used in a previous study [11]. The hold-up times were measured using uracil as an unretained compound. The observed hold-up time values were (average of seven repeated injections): 1.38 $\pm$ 0.01 (col. #19); 1.42 $\pm$ 0.01 (col. #20); 1.39 $\pm$ 0.01 (col. #21); 1.47 $\pm$ 0.01 (col. #22). The retention times of 4-*tert*-butylphenol were (average of many repeated injections, performed in different days and relative standard deviation): 5.21 $\pm$ 0.12 (col. #19); 5.31 $\pm$ 0.09 (col. #20); 5.33 $\pm$ 0.09 (col. #21); 5.42 $\pm$ 0.10 (col. #22).

### 3.3. Isotherm determination

The isotherm determination was achieved in three different ways. The FA technique [14] was used for the determination of the adsorption equilibrium data for the columns #19 and 21. In this case, one pump of the HPLC instrument was used for delivering the sample solution to the mixing chamber, the other pump for delivering the pure mobile phase. The desired sample concentration was achieved by selecting the concentration of the primary sample solution and the ratio of the flow rates delivered by the two pumps. Twenty data points were measured for each column (ten in the range 0–1 g/l and ten in the range 1–13 g/l). After each injection, the column was re-equilibrated with the pure mobile phase. The acquisition of these data series were repeated three times.

The PP technique was used for measuring the isotherm data for column #19. Perturbations on the plateau can be empirically made in different ways, through positive or negative peaks [14]. In the former case, a solution slightly more concentrated than the one equilibrating the column is injected; in the latter, it is a solution slightly more dilute (or a pulse of the pure mobile phase). The retention times of the two perturbation peaks (positive and negative) should coincide [14,16]. In this work, we first checked that a negative pulse achieved by injecting 3  $\mu$ l of the pure mobile phase led to the same pulse retention times as those given by a positive pulse of similar size in the whole concentration range. Then, all the perturbations were achieved by injecting the negative pulse of the pure mobile phase just described. Seventeen perturbation peaks were recorded, in the concentration range 0.1–13 g/l.

For columns #20 and 22 only overloaded profiles were measured. From them, we determined the best isotherm parameters using the IN method previously described (see earlier) [25]. Some overloaded profiles were also recorded for column #21.

All the measurements were carried out at room temperature (22.5–24.5 °C) and at a flow rate of 1 ml/min. The wavelength used to record the overloaded profiles and the FA signals was 296 nm. A calibration curve was measured each time that an overloaded profile was recorded. The repeatability of these different calibration curves was not better than

approximately 5%. However, the mass balance (amount eluted from the column, calculated by the integration of the peak profile vs. amount injected) was satisfied within an error of approximately 3% for all the chromatograms recorded. All the sample solutions used for FA and PP were filtered before use. Otherwise, a systematic 2–3-fold increase of the initial back pressure of the column was observed. The same trend had also been noticed in the past [9]. In such cases, the obvious advantage of being able to operate the monolithic columns at high flow rates is lost.

## 4. Results and discussion

The Langmuir isotherm (Eq. (13)) is the most frequently used model to represent adsorption data in liquid–liquid or liquid–solid chromatography. Although the basic theoretical assumptions made in deriving this isotherm model are not verified in most experimental cases, it is often found that experimental adsorption data fit reasonable well to the Langmuir equation in a rather wide range of concentrations [14,26].

In Fig. 1 the experimental isotherm data measured using the FA technique for the columns #19 and 21 are shown (symbols). The solid line represents the Langmuir isotherm whose best parameters were

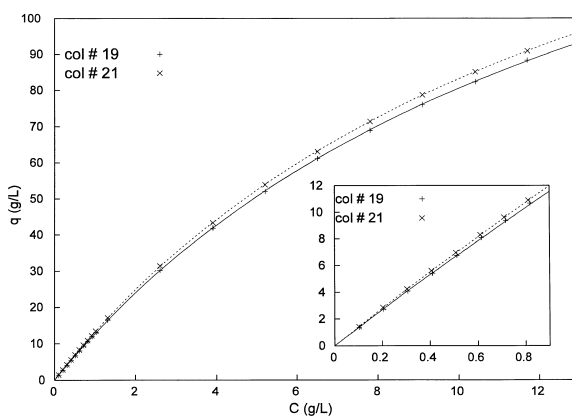


Fig. 1. FA technique: comparison between empirical adsorption data and Langmuir model.  $q$ , stationary phase concentration;  $C$ , mobile phase concentration. Points, empirical adsorption isotherms. Lines, best Langmuir isotherms (cols. #19 and 21).

Table 1  
Best isotherm parameters

Column reference	Technique	$a$ (g/l)	$b$	$q_s$ , (g/l)	FSSR
19	FA	13.636±0.009	0.0697±0.0004	195.7±0.7	0.237
21	FA	14.151±0.006	0.0706±0.0003	200.4±0.6	0.156
19	PP	13.26±0.08	0.068±0.001	195±3	0.495

FA, frontal analysis; PP, perturbation on the plateau.

obtained through the nonlinear least-squares fitting of the data for column #19 to Eq. (13). The values of these coefficients are listed in Table 1. The last column of Table 1 reports the final sum of the squares of the residuals (FSSR). The smaller the FSSR value, the better the “goodness” of the fit [35]. Three series of data were acquired for each column. The data reported are the average values of these three sets of data. The repeatability of these data series was excellent, with an error smaller than 2%.

The data in Table 1 show that the reproducibility of the FA results for the two columns (#19 and 21) is also very satisfactory. The difference between the estimated values of the  $b$  term is about 1%, resulting in a difference of approximately 2.5% in the value of the saturation capacity. Recently, it was shown that the specific saturation capacity of silica-based monolithic columns—i.e. the saturation capacity referred to the unit mass of packing material—is about 50% larger than the saturation capacity of traditional silica C-18 packed columns [13]. These authors obtained a saturation capacity value roughly equal to 141 g/l in the case of a C-18 packed column against a value of about 218 g/l for the monolith ([13], Table 2, 4-*tert.*-butylphenol—Langmuir model). All the other physico-chemical properties characterizing the two silica surfaces (for instance, the binding constants of the compounds investigated) were the same within the limits of experimental errors. Using a monolithic column belonging to the same initial set (Chromolith

Performance, ref. #24) [11,13] and considering that the amount of material contained in a column is less for the monolithic than for the packed column, these authors concluded that the alkyl chains bonded to the monolith expand more than those bonded to the particles of the packed column or that the mobile phase does not have access to the whole surface area of the particles [13] (for a more detailed discussion about the reasons of the different behavior of these two columns, the reader is addressed to [13]). These results agree with the theoretical prediction of Liapis et al. [10], who showed that the porous structure of the monolith—characterized by relatively large through-pores and a high through-pore connectivity [10,8]—is responsible for an increased saturation capacity.

As indicated earlier, the value obtained for  $q_s$  (4-*tert.*-butylphenol, Langmuir model) in that earlier study was about 218 g/l (vs. a value of about 141 g/l for 4-*tert.*-butylphenol on the C-18 packed column) which is about 10% larger than the values measured in this work. Nevertheless, the present results substantially confirm the former observations of a markedly larger saturation capacity for the monolithic columns [13,10]. This result makes this type of column attractive for some applications.

Fig. 2 shows the plot of the differentials of the isotherm,  $\partial q/\partial C$ , against the mobile phase concentration (symbols). These differentials were derived from the retention data of the perturbation peaks through Eq. (10). The point at  $C=0$  was extrapolated by using Eq. (12). The solid line represents the best fitting of the experimental differentials to the Langmuir equation:

$$\frac{\partial q}{\partial C} = \frac{a}{(1 + bC)^2} \quad (15)$$

The best isotherm parameters afforded by the PP

Table 2  
Best isotherm parameters through inverse numerical approach

Column reference#	$a$ (g/l)	$b$	$q_s$ (g/l)	FSSR
20	14.77	0.0882	167.6	0.661
21	14.24	0.0825	172.6	0.742
22	14.49	0.0843	171.9	0.598

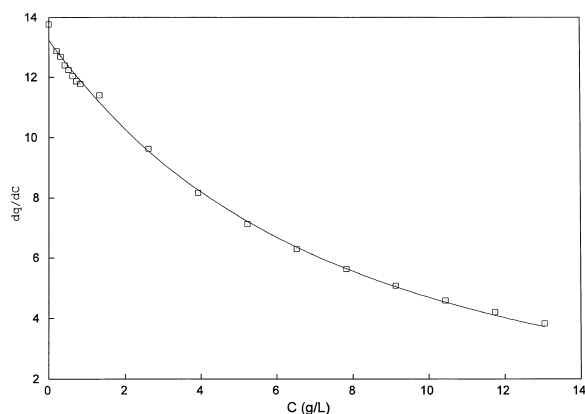


Fig. 2. PP technique: plot of the derivatives,  $\partial q/\partial C$ , against the mobile phase concentration,  $C$ . Points, experimental data. Line, best fitting of derivative of the Langmuir isotherm (col. #19).

method for column #19 are also reported in Table 1. The FSSR value in this case is larger than the one obtained for the FA data. This is not surprising and can be explained by considering that, at high concentrations, the response of the UV detector is nonlinear and the signal/noise ratio decreases rapidly with increasing concentration. Then, the correct evaluation of the peak moments becomes difficult and less accurate. This problem is more serious for the second moment [37] but the loss of precision is significant even for the first moment. Yet, because of the importance of the signal noise, calculating the peak first moment is the most precise method of determining the average of a Gaussian peak. Additionally, the measurement of the slope of the isotherm (PP technique) is more strongly affected by small random errors (for instance in the sample preparation) than the measurement of the slope of the chord of the isotherm (FA technique). As shown by the data reported in Table 1, the agreement between the best isotherm parameters obtained by the FA and PP methods for column #19 is very satisfactory. The relative error made on the isotherm parameters is of the order of 1%.

For column #21 the isotherm data were measured using the IN method described earlier. In this case, it suffices to record a few band profiles under overloaded conditions. The best parameters obtained through the IN method are listed in Table 2. Fig. 3 compares the experimental profile recorded on this

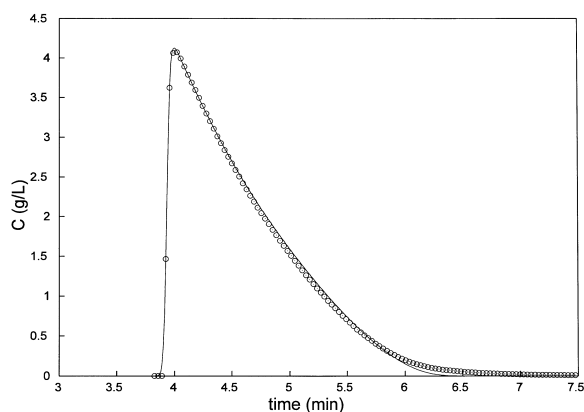


Fig. 3. Comparison between an empirical profile (points) and that simulated (line) by solving the mass balance equation (Eq. (1)) with the Langmuir model whose best parameters were estimated through IN (col. #21). Experimental conditions, pulse 0.3 min in length; concentration injected 12.4 g/l.

column (concentration injected: 12.8 g/l; injection time: 0.3 min; symbols) and a calculated profile obtained through IN (solid line). The calculated peak was obtained by solving Eq. (1) and using the isotherm parameters listed in Eq. (2) (col. #21). These parameters were obtained by minimizing the differences between the experimental profile and the profile calculated with the “intermediate” set of isotherm parameters. The agreement between the calculated and the experimental peaks is excellent. A very small difference can be observed only at the end of the peak tail. As already noted [11,9], this difference may be due to some degree of energetic heterogeneity of the surface of the stationary phase. By contrast, the prediction of the retention time of the shock is most accurate. This result confirms that for the modeling of the adsorption of a small molecule—such as 4-*tert.*-butylphenol—the simple ED model is more than adequate. The differences between the isotherm parameters obtained with the IN and the FA method on the same column (col. #21) are more significant than the differences between the parameters estimated on the same column, using either FA or PP (see earlier, results for col. #21 and 19). A difference as large as 15% can be observed for both  $b$  and  $q_s$ . By contrast, the values obtained for  $a$  are practically the same, with a difference of less than 1%. The reason for this can be



understood by considering the effective range of concentrations on which the inverse numerical minimization is carried out. Fig. 3 shows that the maximum concentration recorded at the column outlet is about 4.2 g/l. This means that only the concentration range between 0 and 4.2 g/l is considered in the inverse numerical estimation of the isotherm parameters [25]. Admittedly, a wider concentration range is involved in the profile calculation (between 0 and the sample concentration at injection) but the residuals are defined only within the concentration range of the eluted profile. Under such conditions, an excellent agreement between the data given by IN and by FA/PP can be expected at relatively low concentrations while, at high concentrations, the ability of IN correctly to estimate the isotherm parameters decreases. This is illustrated in Fig. 4, where the isotherms calculated by the FA and IN methods are compared. The main part of the figure refers to the actual interval 0–4.2 g/l of the peak concentration in Fig. 3. In this range, the discrepancies between the isotherms provided by the two approaches are less than 5%. The inset of the figure reports the FA data (symbols) obtained in the entire concentration range that was investigated and compares them to the best IN isotherm (solid line). It shows that the range 0–4.2 g/l is too small to allow the accurate extrapolation and the calculation of  $q_s$ . The value of  $bC$  for  $C=4.2$  g/l is only approximate-

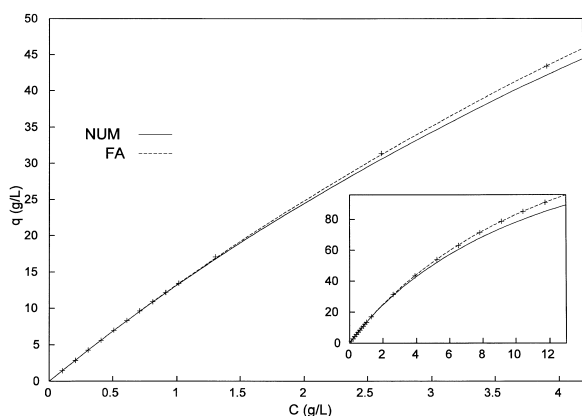


Fig. 4. Comparison between adsorption data obtained through FA and IN.  $q$ , stationary phase concentration;  $C$ , mobile phase concentration. Points, empirical adsorption isotherms. Lines, best Langmuir isotherms (col. #21).

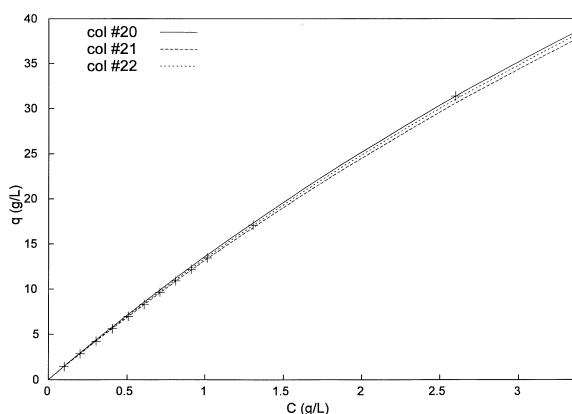


Fig. 5. Comparison between adsorption data obtained through IN for columns #20, 21 and 22.  $q$ , stationary phase concentration;  $C$ , mobile phase concentration. Points, empirical adsorption isotherms. Lines, best Langmuir isotherms.

ly 0.3, which is not sufficient for the accurate estimate of  $q_s$  [14].

The satisfactory results of the comparison between the data obtained with FA, PP and IN show that IN can be used as a precise, time- and material-saving approach for isotherm determination. This method was applied to columns #20 and 22. For these measurements, only a few pulse experiments, made with large sample sizes (pulses of 0.3 min duration, of a solution with a concentration of about 12.4 g/l) were performed and the profiles acquired and treated with the IN technique. Table 2 lists the best values of the parameters of the Langmuir isotherm obtained. Fig. 5 shows the isotherms estimated by IN for the three columns. The column-to-column reproducibility of these results is excellent. The three isotherms cannot be distinguished (as shown in Fig. 5, where the isotherms nearly overlaid). In all three cases, the agreement between the experimental and calculated profiles was also excellent (figures not reported here).

## 5. Conclusions

The adsorption data obtained with the two most popular methods of measurement available (FA and PP) were compared with those given by a new numerical procedure (IN). The validation of this

inverse numerical method (IN) was done: (1) by comparing the isotherm parameters obtained with the FA and PP methods and those given by IN; (2) by comparing the experimental profiles with those calculated as solution of the mass balance equation with the best isotherm parameters estimated through the IN method. The results obtained showed that, if the best values of the isotherm parameters are derived only from the data within the concentration range of the largest peak used in the IN method, the differences observed between the parameters obtained with the three methods are less than a few percent. However, the results obtained with the IN method in the range between the sample concentration and the maximum concentration of the largest peak used in the regression have an accuracy that decreases with increasing concentration. Hence, the overloaded peaks used in the IN method must be as large as possible.

The results obtained with a series of four identical monolithic columns show that the column-to-column reproducibility of the parameters of the adsorption isotherms is better than a few percent. This result is highly satisfactory because these columns had been extensively used in the past 2 years, under very different experimental conditions and for different purposes [11,9,36,13]. Yet, except for a downward drift in permeability they do not seem to have aged. The main advantage of the numerical approach lies in the fact that the amount of material and time required for the determination of the adsorption isotherm parameters is dramatically reduced compared with those needed with the FA and PP methods. Furthermore, the extension of this method to the case of competitive isotherms is straightforward. Accordingly, this method seems particularly suitable for the study of the adsorption of expensive compounds and/or of compounds that are available only in small amount (e.g. polypeptides, proteins, pure enantiomers, etc.). Finally, the saturation capacity values obtained in this study are in substantial agreement with those derived in a different study. They are significantly larger than those previously obtained for the same compound on a packed C-18 column, confirming that the monolithic structure allows a better access of the mobile phase to the stationary phase than the one encountered in the traditional C-18 packed materials.

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