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Quantification of single solute and competitive adsorption isotherms using a closed-loop perturbation method

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Abstract

A modification of the classical method of perturbation chromatography for measuring isotherms of the adsorption of dissolved components is suggested. The general principle of the method consists in analyzing responses of the chromatographic system to small perturbations at different equilibrium concentrations. Essential advantages of the method are: (a) only retention times or volumes have to be measured and no detector calibration is required and (b) experiments with mixtures can be performed and analyzed efficiently. The modification suggested in this paper is the application of a closed-loop arrangement allowing the efficient exploitation of the sample. Experimental data for four different chromatographic systems are presented to illustrate the method. With the determined adsorption isotherms elution profiles could be predicted satisfactorily.

Keywords: Adsorption isotherms; Closed-loop perturbation

1. Introduction

The substantial progress that has been achieved in modeling preparative chromatography has been recently reviewed [1,2]. However, due to the lack of the required data, in industry the development of chromatographic separation processes for preparative purposes is still often based on trial and error methods. An empirical design and optimization of preparative chromatography may be more or less successful for classic elution. However, nowadays more sophisticated chromatographic technologies such as recycling, displacement, flow reversal schemes and the simulated moving bed concept are applied. To use these methods efficiently an a priori determination of the underlying thermodynamic functions is an important part of the design. In general, to model preparative chromatography adequately, the knowledge of the adsorption isotherms is the main prerequisite.

Despite the fact that there are several methods available the experimental determination of adsorption isotherms is still far away from being a routine job. The most frequently applied experimental methods have been compared and evaluated recently [3]. They can be divided in two groups. Static methods analyze only equilibrium data. Their application is usually connected with tedious laboratory work but no sophisticated mathematical tools are required for data processing. Dynamic methods exploit the information of concentration-time-curves. The mea-

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surements are often easier to perform since modern instrumentation can be used. However, the mathematical analysis requires the solution of differential equations and is thus more delicate.

The application of most dynamic methods requires columns filled with the adsorbent and equilibrated initially at a known concentration level. This equilibrium state is then perturbed in a defined way and the response is observed. Depending on the applied initial equilibrium state and the type and extent of the perturbation several methods can be distinguished. Most often rectangular injection profiles are introduced at the column inlet. If the size of this injection is large enough after a certain time a complete breakthrough of a new equilibrium state appears. The method exploiting such responses is called frontal analysis. For smaller sample sizes the shape of dispersed fronts can be used to estimate local slopes of the adsorption isotherms (Elution-by-characteristic point method [4]). Methods analyzing the position of the peak maxima for different injected amounts have be suggested [5] as well as methods based on matching the whole shape of an experimentally observed band profile to a theoretical profile by varying the parameters of an assumed isotherm model [6].

If the size of the perturbation is relatively small the difference between the initial equilibrium concentration and the detectable response concentrations is also small. The column remains essentially in equilibrium allowing one to apply the elegant tool of equilibrium theory [7–10] for analyzing the measured retention times. The principle of the method is well-known in the chromatographic and chemical engineering literature. It was applied successfully to measure isotherms of the adsorption of gases and dissolved components. Due to the broad spectrum of users the method was developed in different ways and is related to several names. Reilley et. al. [11] and Helfferich and Peterson [12] coined the term *Minor Disturbance Method* (MDM). In earlier studies of the thermodynamics of gas chromatography the method was called *Step and Pulse Method* [13–16]. Other names applied are *Concentration Pulse Chromatography* [17] and *Impulse Response Chromatography* [18]. Generalizing the two options of perturbing either by small concentration pulses or by pulses of detectable isotopes Hyun and Danner [19] called the concept *Perturbation Chromatography*. The latter name will be applied below.

The intention of this paper is to demonstrate the potential of the method of perturbation chromatography to quantify adsorption isotherms of different dissolved components. An important problem encountered during the design of a chromatographic process for the separation of value added solutes is addressed in particular. Frequently, in early development stages these substances are available only in limited quantities for parameter estimation studies. In this case a disadvantage of conventional perturbation chromatography is the fact that the column has to be saturated completely in different concentration ranges requiring relatively large sample amounts. Thus, the essential part of the paper consists of describing and testing a closed-loop arrangement allowing for an efficient exploitation of the sample material.

2. Theory and principles

2.1. General principle of perturbation chromatography

The general principle of perturbation chromatography is illustrated in Fig. 1 based on simulations for a single dissolved component. Initially the column was equilibrated with the solvent before a perturbation was triggered by injecting a small amount of sample. This is the typical situation of analytical chromatography. Thus, the first response time designates the retention time under analytical conditions. Then two subsequent concentration steps have been introduced to saturate the column at higher concentrations. The positions of the perturbations are marked by an arrow. The obvious decrease of the retention times for higher liquid phase concentrations is related to the nonlinear character of the assumed isotherm.

A similar presentation as in Fig. 1 for a single solute is given in Fig. 2a and b to illustrate the situation for two dissolved components. In Fig. 2a the individual concentration profiles of the two components are shown. For each equilibrium situation two characteristic response times result from the perturbations marked again by the arrows. In the

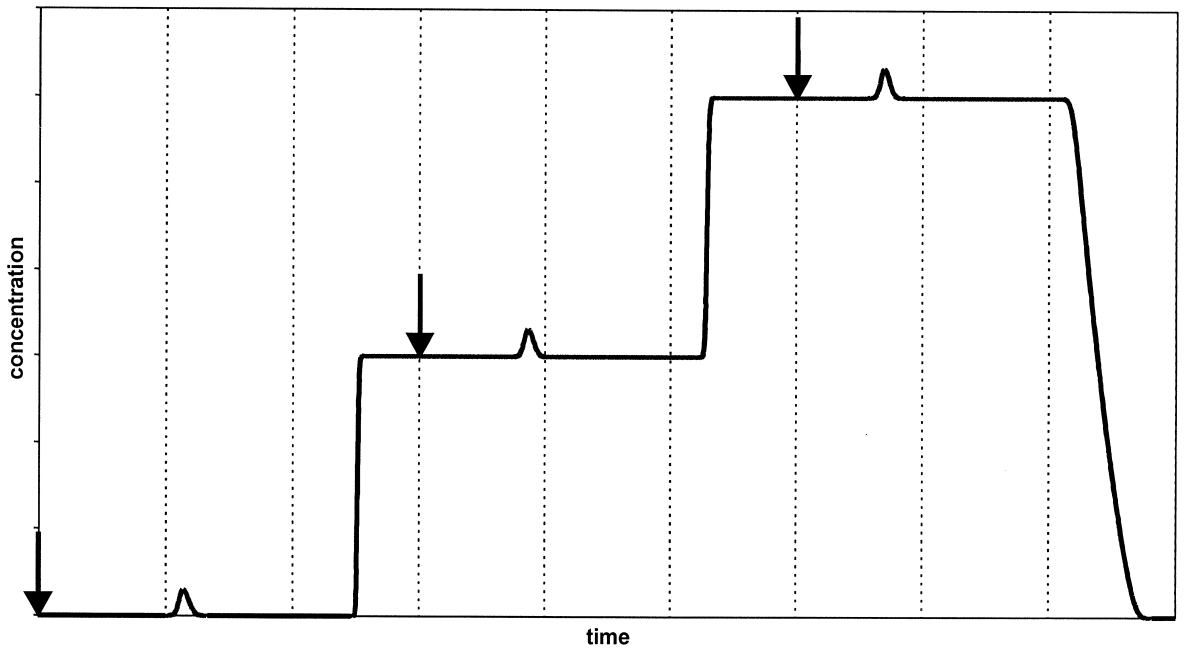

Fig. 1. Principle of perturbation chromatography. Single component case. Perturbations introduced at the column inlet for stepwise increased plateau concentrations.

case of preloaded columns the concentration profiles of each of the components show two response signals. The well-known coherence condition of equilibrium theory states that the characteristic retention times are the same for the two components [8]. As in the single component case a concentration dependence of the retention times can be observed. This dependence contains the information about the adsorption equilibrium. It should be further noted that positive as well as negative signals appear. In general selective detectors capable of following the individual concentration profiles are not available and usually nonspecific detectors are applied recording the course of total concentration at the column outlet. The course of the total concentrations corresponding to the individual band profiles shown in Fig. 2a is presented in Fig. 2b. From the figures it becomes apparent that some of the individual responses might be smaller than others and that there exists the possibility that positive and negative contributions of the individual components can compensate each other.

2.2. Analysis of perturbation chromatography

Here only a short summary of the relevant equations required for determining isotherm parameters from experimentally determined retention times is given. The analysis exploits the results of the classical equilibrium theory [7–10]. Further details can be found in [11–21]. In the frame of the equilibrium theory a chromatographic column is described by a pseudo-homogeneous model where the concentrations in the mobile and in the stationary phases are considered to be in permanent equilibrium. This appears to be a good assumption for the description of the migration of small perturbations.

Neglecting all kinetic effects except convection, the mass balance equation of a component i in a N-component mixture can be expressed by the following partial differential equation

$$\frac{\partial c_i}{\partial t} + \frac{1-\epsilon}{\epsilon}\frac{\partial q_i(\bar{c})}{\partial t} + u\frac{\partial c_i}{\partial x} = 0$$
$$\text{with } \bar{c} = (c_1, c_2, \ldots, c_N) \quad i = 1, N \tag{1}$$

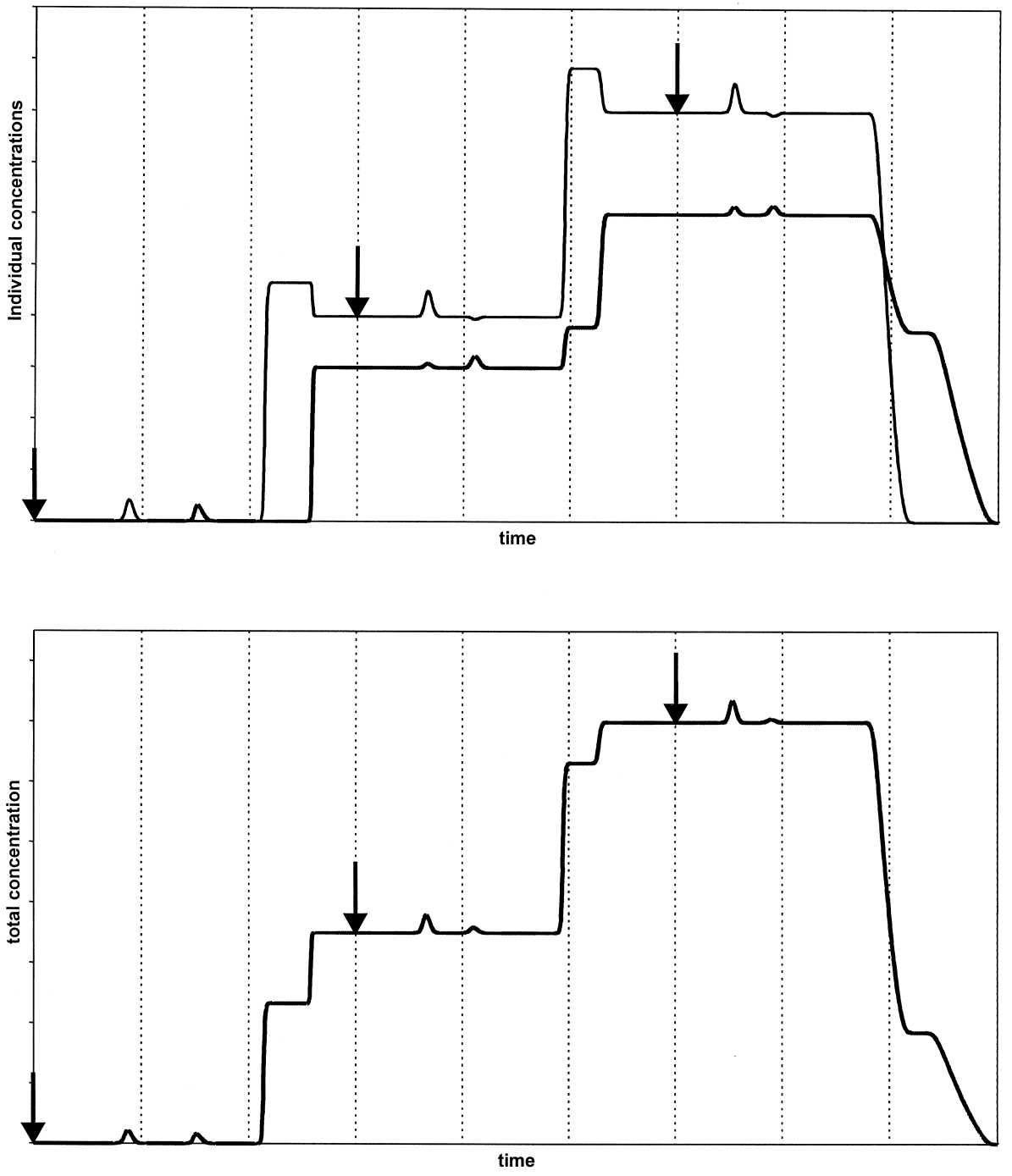

Fig. 2. (a) Principle of perturbation chromatography. Binary system. Individual concentration profiles of the two components. Perturbations introduced for stepwise increased plateau concentrations (ratio between components 4:3). (b) Principle of perturbation chromatography. Binary system. Total concentrations. Perturbations introduced for stepwise increased plateau concentrations (ratio between components 4:3).

In this study the linear velocity, u, is assumed to be constant. This assumption is reasonable only for the case of liquid chromatography where incompressible fluids are treated under nearly isothermal conditions and precise pumps are available. In the case of gas adsorption changes of the velocity have to incorporated in the analysis [18]. In Eq. (1) c stands for the concentrations in the liquid phase, q for the loadings and ϵ designates the total porosity.

From Eq. (1) characteristic retention times, $t_{R,i}(\bar{c})$, can be calculated for each component i and a given vector of equilibrium concentrations, $\bar{c}$, according to:

$$t_{R,i}(\bar{c}) = t_0\left(1 + \frac{1-\epsilon}{\epsilon}\left.\frac{dq_i}{dc_i}\right|_{\bar{c}}\right)$$

$$\text{with} \quad t_0 = \frac{\epsilon V_{col}}{\dot{V}} = \frac{L}{u} \quad i = 1, \ldots, N \tag{2}$$

The time t_0 designates the retention time of a non-retained component, L is the column length. To perform a more general analysis independent of the flow-rate it is more expedient to use retention volumes, V_R, instead of retention times, t_R. Multiplying Eq. (2) with $\dot{V}$ gives:

$$V_{R,i}(\bar{c}) = t_{R,i}(\bar{c})\dot{V} = V_0\left(1 + \frac{1-\epsilon}{\epsilon}\left.\frac{dq_i}{dc_i}\right|_{\bar{c}}\right)$$

$$\text{with} \quad V_0 = \epsilon V_{col} \quad i = 1, \ldots, N \tag{3}$$

Such retention volumes, measured for different equilibrium concentrations, $\bar{c}$, present the primary data of the method of perturbation chromatography. The isotherm information of interest is hidden in the total derivatives of the adsorption isotherms, $dq_i/dc_i|_{\bar{c}}$. For these derivatives holds:

$$\left.\frac{dq_i}{dc_i}\right|_{\bar{c}} = \sum_{j=1}^{N}\left.\frac{\partial q_i}{\partial c_j}\right|_{\bar{c}}\left.\frac{dc_j}{dc_i}\right|_{\bar{c}} \quad i = 1, \ldots, N \tag{4}$$

Thus, experimentally determined retention volumes essentially deliver information about the partial derivatives of the adsorption isotherms, $\partial q_i/\partial c_j$. By systematic collection of a sufficient amount of these derivatives the competitive isotherms, $q_i(\bar{c})$, can be determined by integration. Usually such isotherm data are subsequently used to determine free parameters of an isotherm model [18]. A simpler way is based on the immediate introduction of an isotherm model already on the level of the derivatives of the isotherm. Using this model theoretical partial derivatives, $\partial q_i/\partial c_j$, can be expressed analytically as a function of the not yet known isotherm parameters. A subsequent matching of the experimentally observed retention volumes and the predictions allows one to estimate the parameters of the isotherm model. This approach was used in this work and will be described below.

To predict retention volumes using Eqs. (3) and (4) the direction differentials dc_j/dc_i are needed. These differentials can be specified using the coherence condition [8] stating that after perturbing an equilibrium state in a N-component system for each component $N-1$ characteristic waves are triggered. The speed and thus the retention times or volumes of these waves are for all components synchronized (coherent). Thus for a given equilibrium composition, $\bar{c}$, holds for each wave k ($k=1, \ldots, N-1$):

$$V_{R,i,k} = V_{R,j,k} = V_{R,k} \quad i = 1, \ldots, N,\ j = 1, \ldots, N \tag{5}$$

In general only the concentrations of $N-1$ components are independent. The last concentration, c_N, is specified by the overall mass balance, i. e. $c_N = c_N(c_1, c_2, \ldots, c_{N-1})$. In the case of describing adsorption from solution the solvent is conveniently designated as component N. In preparative chromatography it is often assumed that the solvent is not adsorbed, i.e. $q_N = 0$ and thus $\partial q_N/\partial c_i = 0$. This allows to simplify Eq. (4):

$$\left.\frac{dq_i}{dc_i}\right|_{\bar{c}} = \sum_{j=1}^{N-1}\left.\frac{\partial q_i}{\partial c_j}\right|_{\bar{c}}\left.\frac{dc_j}{dc_i}\right|_{\bar{c}} \quad i = 1, \ldots, N-1 \tag{6}$$

For the example of two components 1 and 2 dissolved in a solvent ($N=3$) the prediction of the two ($N-1$) characteristic retention volumes requires the specification of two values for the direction differentials $dc_2/dc_1|_{\bar{c},k=1}$ and $dc_2/dc_1|_{\bar{c},k=2}$. For an assumed isotherm model, $q_1(c_1, c_2)$ and $q_2(c_1, c_2)$, these two values can be calculated from the two roots of the following quadratic equation that results from the coherence condition [8]:

$$(|dc_1/dc_2|_{\bar{c}})^2 + dc_1/dc_2|_{\bar{c}}\frac{\partial q_2/\partial c_2|_{\bar{c}} - \partial q_1/\partial c_1|_{\bar{c}}}{\partial q_2/\partial c_1|_{\bar{c}}} - \frac{\partial q_1/\partial c_2|_{\bar{c}}}{\partial q_2/\partial c_1|_{\bar{c}}} = 0 \tag{7}$$

With the two roots of Eq. (7) the two retention

volumes $V_{R,k=1} = V_{R,1,1} = V_{R,2,1}$ and $V_{R,k=2} = V_{R,12} = V_{R,22}$ can be predicted using Eqs. (3) and (6).

It should be further mentioned that the retention volumes do not depend on the sign of the difference between the concentrations in the injected simple and the equilibrium concentrations. ''Positive'' perturbations using more concentrated solutions or ''negative'' perturbations using e.g. solvent samples generate the same retention volumes and can be applied likewise.

To evaluate finally the mathematical aspect of the problem it can be stated that the prediction of theoretical retention volumes for a N component mixture essentially requires the determination of the roots of a polynomial of order $N-1$.

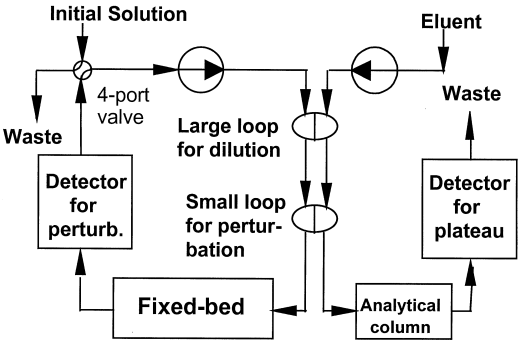

Fig. 3. Experimental set-up to implement the closed-loop perturbation method.

2.3. Perturbation chromatography in a closed-loop arrangement

Usually the concept of perturbation chromatography is applied as illustrated in Figs. 1 and 2. At the column inlet more or less concentrated solutions are continuously supplied to saturate the stationary phase at defined concentrations. Although it might be collected, the column effluent is more or less lost for the current experiment using this standard arrangement. To saturate the column completely at a sufficient number of concentration levels relatively large sample amounts are required. To reduce these amounts a closed-loop system is suggested below. The aspect of sample economy is especially relevant for pharmaceutical products where in early stages of process development only small amounts are available for experimental investigations. The main principle of the method and an appropriate experimental set-up are illustrated schematically in Fig. 3. Initially, a concentrated solution is prepared and fed continuously to equilibrate the fixed-bed. After reaching this state, indicated by a constant detector response, the loop (left circle) is closed using the 4-port valve. From now on no more feed solution is required to establish different equilibrium states and to record several retention volumes. A perturbation is triggered at the bed inlet by introducing with an injection loop a relatively small sample possessing a concentration different from the equilibrium concentration. The injection of small solvent pulses appears to be especially simple and attractive. The resulting retention times or volumes are recorded with a suitable detector. After that, the concentration in the closed volume is lowered by injecting a substantial quantity of solvent with a larger injection loop. The fixed bed is equilibrated now again by circulating the encapsulated liquid before another small perturbation is injected and the corresponding retention times or volumes are determined. This sequence of dilution, equilibration, perturbation and detection is repeated allowing the determination of several equilibrium data. Thus the whole experiment starts from a high concentration level and operates at stepwise decreased concentration levels. Obviously this procedure can be easily automated using modern HPLC equipment. An illustration of the signal at the outlet of the column is shown in Fig. 4. In this schematic representation it is not considered that the detector is usually reset before each new perturbation by flushing its reference cell. Thus, the optimal sensitivity is always available in order to detect the responses to small perturbations. In Fig. 4 the size of the signals is enlarged.

To exploit the measured retention volumes they have to be related to the corresponding equilibrium concentration for each plateau. There are in principle two ways possible to determine these concentrations. At first the concentrations can be measured for each plateau using, e.g., analytical HPLC (right part of Fig. 3). Since explicit concentration values are needed this method has to be calibrated. Alternatively the required concentrations can also be determined

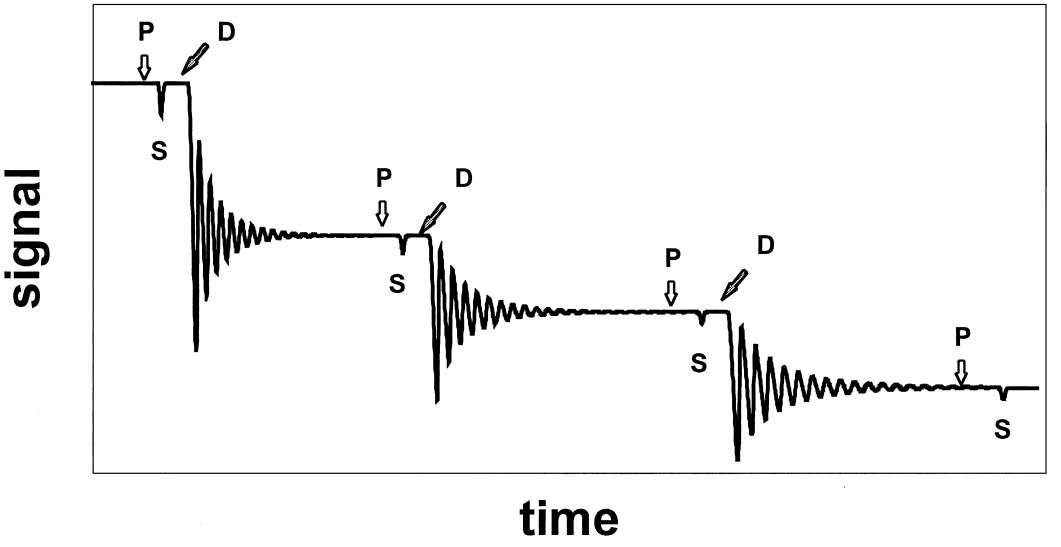

Fig. 4. Principle of the closed-loop perturbation method. Equilibrium states are perturbed (P) injecting small solvent samples. The corresponding response signals (S) are recorded. Equilibrium states are diluted (D) using large solvent samples. Local slopes of the isotherm can be determined from the retention times ($t_S - t_P$).

theoretically from mass balance considerations as shown in the next paragraph.

2.4. Parameter estimation and overall mass balance for the closed-loop arrangement

To match experimentally determined retention volumes, $V_{R,k,ex}$, and theoretical values, $V_{R,k,th}$, the latter have to be calculated with Eqs. (3), (5) and (6) for the corresponding equilibrium concentrations, $\bar{c}$. Measuring these equilibrium concentrations allows the use of the following objective function for parameter estimation.

$$OF_{\bar{V},\bar{c}} = \sum_{p=1}^{P} \sum_{k=1}^{N-1} \left(\left[V^p_{R,k,ex} - V^p_{R,k,th}(\bar{c}^p_{ex}) \right] / V^p_{R,k,ex} \right)^2 \tag{8}$$

The application of Eq. (8) is based on experimentally determined retention volumes, $\bar{V}$, and equilibrium concentrations, $\bar{c}$. The index p designates the number of the concentration plateau. To minimize $OF_{\bar{V},\bar{c}}$ a nonlinear regression routine is required, e.g. [22].

Of course the requirement of measuring the plateau concentrations causes further experimental work. To avoid these efforts mass balance considerations can be exploited to follow the concentration changes in the closed-loop caused by injecting larger solvent amounts for dilution and smaller pulses to trigger a perturbation.

The equilibrium concentrations of interest can be predicted provided all volumes in the system and the adsorption isotherms are known. Again, for the latter an isotherm model has to be assumed. The following equations allow one to track the concentrations in the loop. For an equilibrium state of a certain plateau $p-1$ the following mass balance holds for component i:

$$m_i^{p-1} = V_{\text{loop}} c_{i,th}^{p-1} + (1-\epsilon) V_{\text{col}} q_i^{p-1}(\bar{c}_{th}^{p-1}) \tag{9}$$

Eq. 9 states that the amount of i in the loop is distributed between the two phases. The concentrations in the liquid are $c_{i,th}^{p-1}$ and the corresponding loadings are q_i^{p-1}. The corresponding liquid phase volume, V_{loop}, contains a fraction in the column, ϵV_{col}, and an additional extracolumn volume, V_{ext}. For the first plateau the initial concentrations correspond to the known concentrations in the prepared feed solution, c_i^o.

Due to the dilution steps and to a smaller extent due to the perturbations, the amount of i is reduced from plateau $p-1$ to plateau p according to:

$$m_i^p = m_i^{p-1} - V_{\mathrm{dil}} c_{i,th}^{p-1} \tag{10}$$

The volume V_{dil} is related to the volumes of the two applied injection loops. After dilution the reduced amount in the closed-loop is again equilibrated and new equilibrium concentrations, $c_{i,th}^p$, can be calculated from:

$$m_i^p = V_{\mathrm{loop}} c_{i,th}^p + (1-\epsilon) V_{\mathrm{col}} q_i^p(\bar{c}_{th}^p) \tag{11}$$

Only for simple isotherm models the $c_{i,th}^p$ can be calculated analytically. Usually an iteration is required.

The concept of using this mass balance considerations in order to calculate theoretical retention volumes for each plateau and thus to estimate free parameters of an adsorption isotherm model can be summarized as follows:

1. Measure retention volumes for different equilibrium concentrations ($V_{R,k,ex}^p$, $k=1$, $N-1$, $p=1$, P).
2. Assume an isotherm model.
3. Estimate appropriate parameters of the isotherm model.
4. Use the actual isotherm model parameters to calculate the equilibrium concentrations for all plateaus, $\bar{c}_{th}^p$ (Eqs. (9)–(11), usually iterative solution).
5. Calculate for the calculated plateau concentrations, again with the actual isotherm model parameters, the corresponding theoretical retention volumes, $V_{R,k,th}^p(\bar{c}_{th}^p)$ (Eqs. (3), (5), (6))
6. Compare the retention volumes using the following objective function:

$$OF_{\bar{V}} = \sum_{p=1}^{P} \sum_{k=1}^{N-1} \left(\left(V_{R,k,ex}^p - V_{R,k,th}^p(\bar{c}_{th}^p) \right) / V_{R,k,ex}^p \right)^2 \tag{12}$$

7. Update the isotherm parameters using a nonlinear regression routine and repeat (4) until an appropriate termination criterion is reached.

Thus, Eqs. (8) and (12) offer two different objective functions that might be used to determine the free parameters of an isotherm model. Obviously, Eq. (12) requires less experimental work but more mathematical efforts. A third more complex objective function evaluating both differences between experimentally observed and predicted retention volumes and equilibrium concentrations [23] might be useful but will not considered here.

If no satisfying agreement between theoretical and experimental retention volumes is reached or if unrealistic model parameters are obtained the whole procedure should be repeated with other isotherm models. Based on comparing the minimum objective functions achieved for different isotherm models a model discrimination can be performed.

2.5. Isotherm models

There is an abundance of adsorption isotherm equations that have been suggested and validated for different systems [1,3,24,25]. However, many of the systems relevant in the field of preparative liquid chromatography can be described successfully with the classical competitive Langmuir equation:

$$q_i = \frac{a_i c_i}{1 + \sum_{j=1}^{K} b_j c_j} \tag{13}$$

More difficult isotherm courses frequently can be modelled alternatively using the Bi-Langmuir model assuming two different types of adsorption sites (I and II)

$$q_i = \frac{a_i^{\mathrm{I}} c_i}{1 + \sum_{j=1}^{K} b_j^{\mathrm{I}} c_j} + \frac{a_i^{\mathrm{II}} c_i}{1 + \sum_{j=1}^{K} b_j^{\mathrm{II}} c_j} \tag{14}$$

In the above K is the number of adsorbable components. Eq. (13) has two and Eq. (14) has four free model parameters for each component that need to be specified. In principle all these parameters can be determined as described above from a set of experimentally determined retention volumes. However, to reduce the number of parameters it is expedient to take into account that the easily measurable retention volumes for a not preloaded column,

i.e. the analytical retention volumes $V_{R,i}^{anal}$, give direct access to the initial isotherm slopes, K_i according to:

$$K_i = \frac{V_{R,i}^{anal} - V_0}{V_0 \frac{1-\epsilon}{\epsilon}} \quad (15)$$

These adsorption equilibrium constants specify a_i in Eq. (13) and $a_i^{I} + a_i^{II}$ in Eq. (14) according to

$$a_i = K_i \quad \text{and} \quad a_i^{I} + a_i^{II} = K_i \quad (16)$$

3. Experimental

3.1. Chromatographic systems

The closed-loop perturbation method described above was applied for the determination of isotherm model parameters of the single solute and competitive adsorption isotherms of the following four systems:

1. System 1: solutes: cyclopentanone (C5)/cycloheptanone (C7) mobile phase: *n*-hexane/ethyl acetate (95:5) stationary phase: silica (Kromasil, Eka Nobel, mean particle diameter 10 μm)
2. System 2: solutes: dimethyl phthalate (DMP)/diethyl phthalate (DEP)/dibuthyl phthalate (DBP), mobile phase: methylene chloride, stationary phase: as for system 1
3. System 3: solutes: two isomers (α and β) of a steroid compound, mobile phase: *n*-hexane/methyl-*tert*.-butylether (70:30), stationary phase: as for system 1
4. System 4: solutes: the (+)- and (−)-enantiomers of a chiral hetrazipine called WEB2170 [26], mobile phase: methanol, stationary phase: cellulose triacetate (E. Merck, particle diameter: 15–25 μm).

The solvents and the solutes of systems 1 and 2 were purchased from different suppliers and used without further purification. Whereas systems 1 and 2 can be considered as model systems, the solutes of systems 3 and 4 are of more industrial relevance. For this reason no more details concerning their structures are given here. To perform the experiments analytical HPLC-columns of the following dimensions were used:

1. Columns I: silica, $L = 25$ cm, $d = 0.4$ cm, used for systems 1 and 3
2. Columns II: silica, $L = 10$ cm, $d = 0.4$ cm, used for system 2
3. Columns III: CTA, $L = 25$ cm, $d = 0.46$ cm, used for system 4.

Two of each of the columns I, II and III were available. Whereas the silica columns were packed by a supplier (Muder & Wochele, Berlin) the CTA columns were self packed as described in [27].

3.2. Set up

The set up was assembled as shown in Fig. 3. The essential parts are two conventional HPLC pumps, a refractive index detector to detect the responses after the perturbations and a UV-detector (all Knauer, Berlin). One of the columns was installed in the closed-loop to measure the retention volumes of the perturbations. The second column was installed to measure optionally the equilibrium concentrations of the plateaus and to record chromatograms under analytical or overloaded conditions. The detectors outlet signals were transferred to a PC for further processing. To maintain a high detector sensitivity of the RI-detector the reference side could be flushed with the solution in the loop prior to each perturbation. The small injection loops used to introduce the perturbations or to dilute the solution in the closed-loop were mounted on Rheodyne (7010) valves. According to the manufacturer they had internal volumes of 10 and 500 μl. Operating these injection loops always sent small (perturbation) or large (dilution) sample sizes to the first or second column. The responses of the UV-detector could be used to determine the initial slopes of the isotherms or to record elution profiles under overloaded conditions. The valve with the larger loop could be operated repeatedly between two equilibrium stages in order to impose larger concentration steps. In this case the valve was operated carefully in order to assure that only defined portions of concentrated solution and solvent were exchanged and thus the mass balance was kept under control. All experiments were carried out at ambient temperature. A flow-rate of 1 ml/min

was used in all experiments. More details can be found elsewhere [28].

3.3. Procedures

In the first stage of the investigation the standard chromatographic and geometric parameters of the applied experimental systems have been determined. The former are mainly the total porosities of the columns, ϵ, the adsorption equilibrium constants, K_i, and the number of theoretical plates, N_p. To determine these values conventional tracer experiments with not preloaded columns were performed. The latter parameters are mainly related to dead volumes in the closed-loop. In particular the total liquid phase volume outside the columns (V_{ext}) has to be determined carefully since it contributes significantly to the overall mass balance.

Using the described concept of perturbation chromatography for all four systems systematic measurements were performed using different initial solutions. These solutions contained the solutes as single solutes or as binary mixtures. Usually about three to six dilution steps have been performed for each initial solution. The measured retention volumes were used in the frame of the theory described above to determine free parameters of the assumed isotherm models.

As a side product, for each dilution step elution profiles under conditions where the second (analytical) column outside the loop was highly overloaded could be recorded with the UV-detector. Several of the obtained chromatograms were used to evaluate the accuracy of the determined isotherms. For this, theoretical chromatograms were simulated with the equilibrium dispersion model [1]. To allow for a quantitative comparison, the signals of the UV-detector were calibrated for all components at suitable wavelengths.

4. Results and discussion

4.1. Basic parameters and preliminary experiments

The fluid phase volumes in the column and thus the porosities have been determined from the retention times of solvent peaks. Adsorption equilibrium constants and column efficiencies have been estimated from the retention times and peak widths measured for the solutes of interest under analytical conditions. The obtained results have been found to be rather similar for the two columns of each type (I, II and III) used later simultaneously in the closed-loop and as the analytical column. Prior to calculating the adsorption equilibrium constants the extracolumn volume between injection position and detector was determined from tracer experiments with no column. This volume was later subtracted from the measured retention volumes. The obtained parameters (averaged for the two columns) are summarized in Table 1.

In further tracer experiments the total external liquid phase volume in the closed-loop was estimated to be $V_{ext} = 3.40$ ml. Finally, the correct sizes of the two injection loops were determined from peak areas and a calibration curve (areas vs. substance amounts) measured for smaller sample sizes which were injected with a syringe. The injection loop volumes were estimated to be 9.9 and 522.9 μl.

In another preliminary experiment it was confirmed that the retention volumes do not depend on the kind of perturbation imposed on the equilibrated system. In Fig. 5 measured response curves are shown for perturbing the equilibrium of a binary mixture (system 1) in four different ways. The concentrations of C5 and C7 in the solutions injected were (1) both smaller, (2) both larger, (3) larger and smaller or (4) smaller and larger than the equilibrium concentrations. As expected from the theory the

Table 1
Porosities and plate numbers of the three types of columns and the corresponding adsorption equilibrium constants of the solutes

Column	ϵ	N_p	K_i
I	0.756	4300–7000	$K_{C7} = 11.69$, $K_{C5} = 14.85$ (system 1) $K_\alpha = 6.87$, $K_\beta = 9.99$ (system 3)
II	0.826	1200–1400	$K_{DBP} = 21.51$, $K_{DEP} = 45.51$, $K_{DMP} = 52.16$ (system 2)
III	0.690	80–120	$K_{(+)\text{-WEB2170}} = 1.04$, $K_{(-)\text{-WEB2170}} = 2.58$ (system 4)

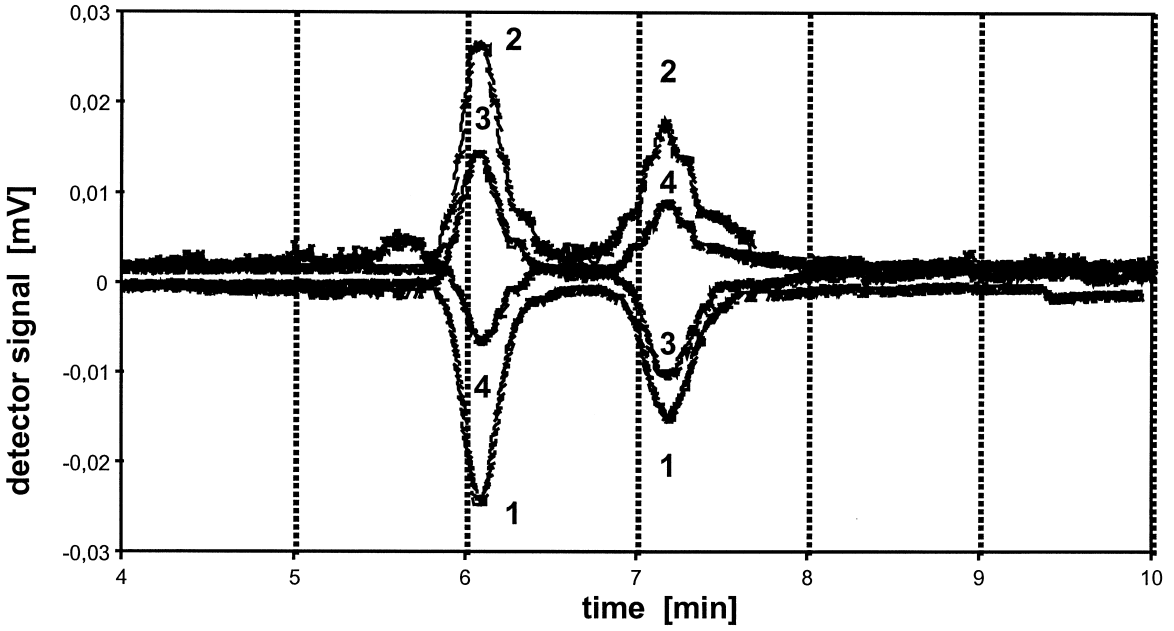

Fig. 5. Detector responses after four different types of perturbations (example for a binary C5–C7 mixture).

recorded retention times depend solely on the equilibrium state and not on the type of perturbation.

4.2. Results for system 1

4.2.1. Single solutes

Perturbation experiments were performed at first with C5 and C7 as single solutes. Typical response curves for one run with C7 are shown in Fig. 6. Four different plateau concentrations have been perturbed and the responses have been recorded. The results are superimposed using the time for the perturbation as the origin. The first peak belongs to the prepared initial concentration (20 g/l). The next three peaks belong to the gradually lowered concentrations. Obviously there is a strong increase of retention volumes for decreasing equilibrium concentrations indicating a rather nonlinear isotherm.

Table 2 summarizes obtained retention volumes for four runs with C7 and different initial concentrations. For each initial concentration several plateaus were equilibrated after dilution and prior to the perturbation. The equilibrium concentrations have been measured using the second analytical column analyzing the 10 μl sample that was injected

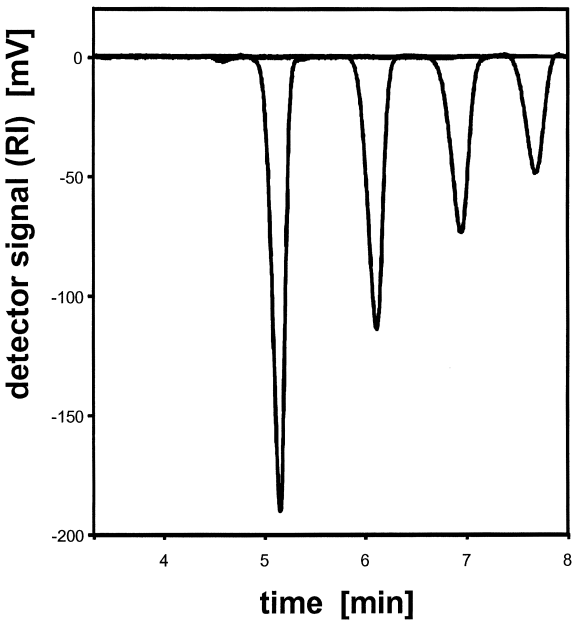

Fig. 6. Superimposed normalized detector responses after four perturbations (system 1, C7 as a single solute). The peaks elute in the order of decreasing equilibrium concentration. The concentration in the initial solution was 20.08 g/l.

Table 2
Retention volumes and equilibrium concentrations for four runs with C7

(Initial) and measured equilibrium concentrations [g/l]	Dilution step p	Retention volumes $V^{p}_{R,ex}$ [ml]
(100.03)	1	3.06
56.19	2	3.58
32.27	3	4.27
21.39	4	5.05
(20.08)	1	4.96
12.86	2	5.93
9.13	3	6.76
6.15	4	7.48
(10.13)	1	6.38
7.04	2	7.21
5.33	3	8.09
3.94	4	8.48
(2.02)	1	9.28
1.65	2	9.57

synchronously with perturbing the column in the closed-loop. All obtained retention volumes for C7 are given in Fig. 7a as a function of the measured equilibrium concentrations. A confirming overlap between the results for different initial concentrations can be noticed. Besides the experimental results, in Fig. 7a the best fit theoretical retention volumes are also given for the isotherm models defined in Eqs. (13) and (14). The free parameters (b_{C7}) or (a^{I}_{C7}, b^{I}_{C7}, b^{II}_{C7}) were obtained minimizing $OF_{\bar{V},\bar{c}}$ (Eq. (8)). It comes as no surprise that with the Bi-Langmuir model (Eq. (14)), offering three degrees of freedom, a much better fit can be achieved. A similar plot is shown in Fig. 7b for the local isotherm slopes of the more retained component C5.

In another series of calculations the free parameters of the two isotherm models have been fitted minimizing $OF_{\bar{V}}$ (Eq. (12)), thus without using the measured equilibrium concentrations. Both sets of obtained isotherm parameters are summarized in Table 3. To illustrate the differences between two Bi-Langmuir parameter sets, in Fig. 8 theoretical slopes of the isotherm for C5 are shown. Despite obvious differences there is agreement in the general course of the function indicating the applicability of the concept of using the overall mass balance to determine the isotherm parameters.

The determined single solute adsorption isotherms were subsequently used to predict chromatograms under overloaded conditions using the standard equilibrium dispersion model [1]. Besides the adsorption isotherms this model only needs apparent dispersion coefficients to describe band broadening effects. These coefficients can be estimated for efficient columns from the number of theoretical plates, N_p. For this, the numbers given in Table 1 were averaged. The system of partial differential equations forming the equilibrium dispersion model was solved numerically using an explicit finite difference scheme. In Fig. 9a a typical chromatogram is shown for C7 as a single solute. The two predictions given are based on the parameters of the Langmuir and the Bi-Langmuir isotherm models obtained from minimizing $OF_{\bar{V},\bar{c}}$ (Table 3). Both parameter sets describe the general shape of the measured band profile. A similar representation is given in Fig. 9b for a C5 profile. Here the parameters of the Bi-Langmuir equation belonging to the minimum of $OF_{\bar{V}}$ (Table 3) were taken for the prediction and again the agreement can be considered as satisfactory.

4.2.2. Binary mixtures

In further experiments retention volumes for different binary mixtures of C5 and C7 were determined. For each equilibrium composition two characteristic volumes were recorded. Typical experimental results with initial mixtures of different composition (C5:C7 = 1:1, 1:3 and 3:1) are summarized in Table 4. The obtained retention volumes were also analyzed using both isotherm models (Eqs. (13) and (14)) and both objective functions (Eqs. (8) and (12)). Here only the results for the most simple competitive Langmuir model (Eq. (13)) and for minimizing the objective function $OF_{\bar{V}}$ (Eq. (12)) are given. To further reduce the number of free model parameters it was assumed that the saturation capacities, $q_s = a_i/b_i$, are the same for the two components. With this assumption the b-values are linked according to:

$$b_j = b_i \frac{a_j}{a_i} \tag{17}$$

Thus, using too the available adsorption equilibrium constants K_i for a binary system, only one free parameter has to be specified. An analysis of all

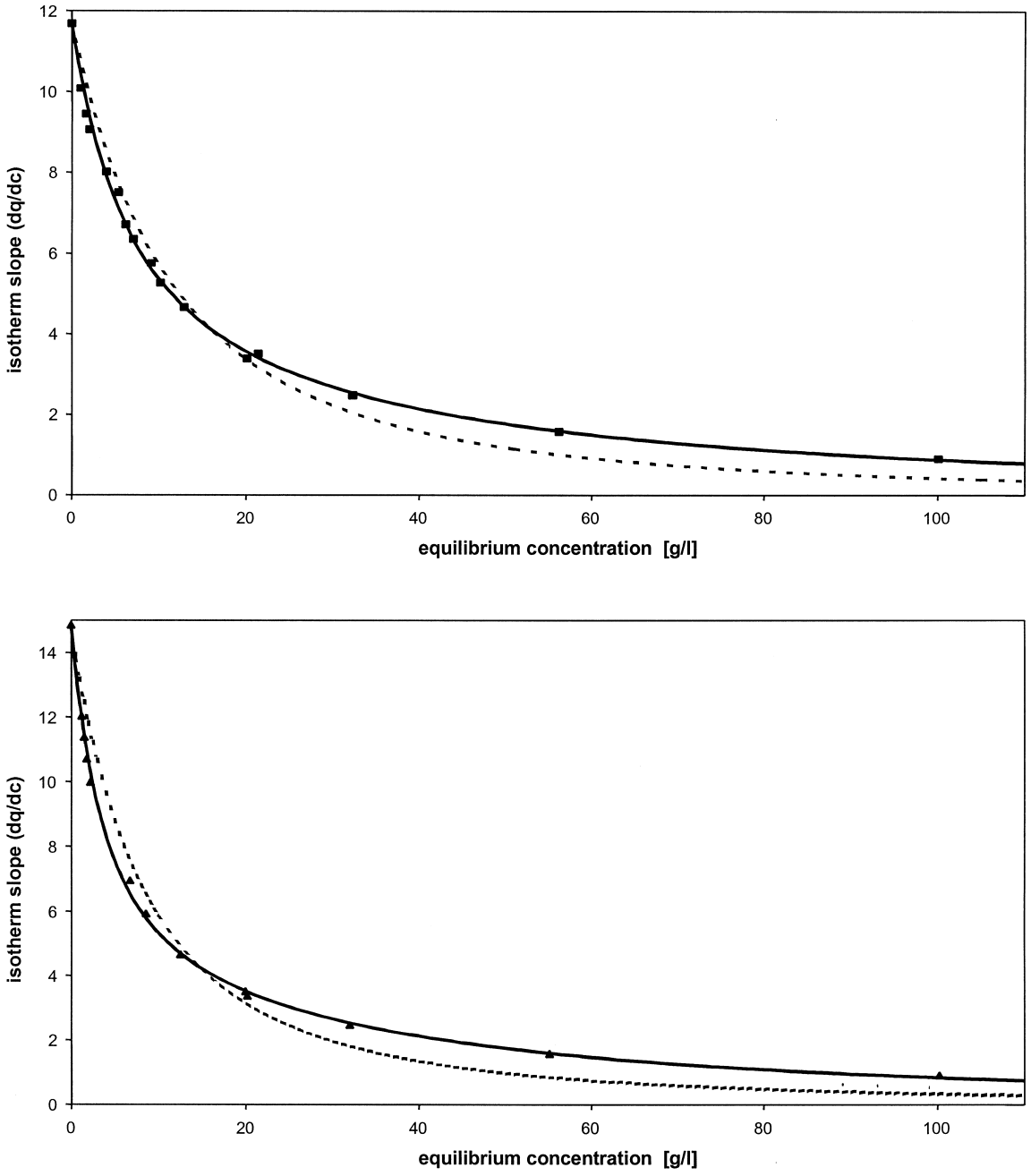

Fig. 7. (a) Local isotherm slope as a function of the equilibrium concentration for C7 as a single solute (system 1). ■: experimental data, dotted line: Langmuir isotherm (Eq. (13)), solid line: Bi-Langmuir isotherm (Eq. (14)). (b) Local isotherm slope as a function of the equilibrium concentration for C5 as a single solute (system 1). ▲ experimental data, dotted line: Langmuir isotherm (Eq. (13)), solid line: Bi-Langmuir isotherm (Eq. (14)).

Table 3
Isotherm parameters for C5 and C7 based on single solute measurements

Isotherm parameter	$OF_{\bar{V},\bar{c}}\rightarrow$MIN	$OF_{\bar{V}}\rightarrow$MIN
	C7	
a [−][a]	11.69	11.69
b [l/g]	0.0432	0.0394 (0.495)[b]
a^{I} [−]	3.79	5.13
b^{I} [l/g]	0.0121	0.0145
a^{II} [−][a]	7.90	6.56
b^{II} [l/g]	0.0840	0.0950
	C5	
a [−][a]	14.85	14.85
b [l/g]	0.0589	0.0509 (0.629)[b]
a^{I} [−]	4.71	6.43
b^{I} [l/g]	0.0144	0.0175
a^{II} [−][a]	10.14	8.42
b^{II} [l/g]	0.1432	0.1763

[a] Related to adsorption equilibrium constants K_i, Eq. (16).
[b] Based on mixture data.

available mixture retention volumes led to the following b-values: $b_{C7}=0.0495$ l/g. and $b_{C5}=0.0629$ l/g. These values obtained from experiments with mixtures are about 15 and 20% higher than the mean values obtained for the two objective functions from the single solute experiments (Table 3). A reason for this discrepancy might be errors related to the estimation of the equilibration concentrations using the overall mass balance. Nevertheless it was attempted to simulate with the parameters obtained from the mixture experiments a chromatogram under overloaded conditions. The comparison with the experiment shown in Fig. 10 indicates that the parameters are capable of predicting the retention times and the general shapes of the bands properly.

4.3. Results for system 2

For system 2 experiments with the single solutes and with all three binary mixtures were performed.

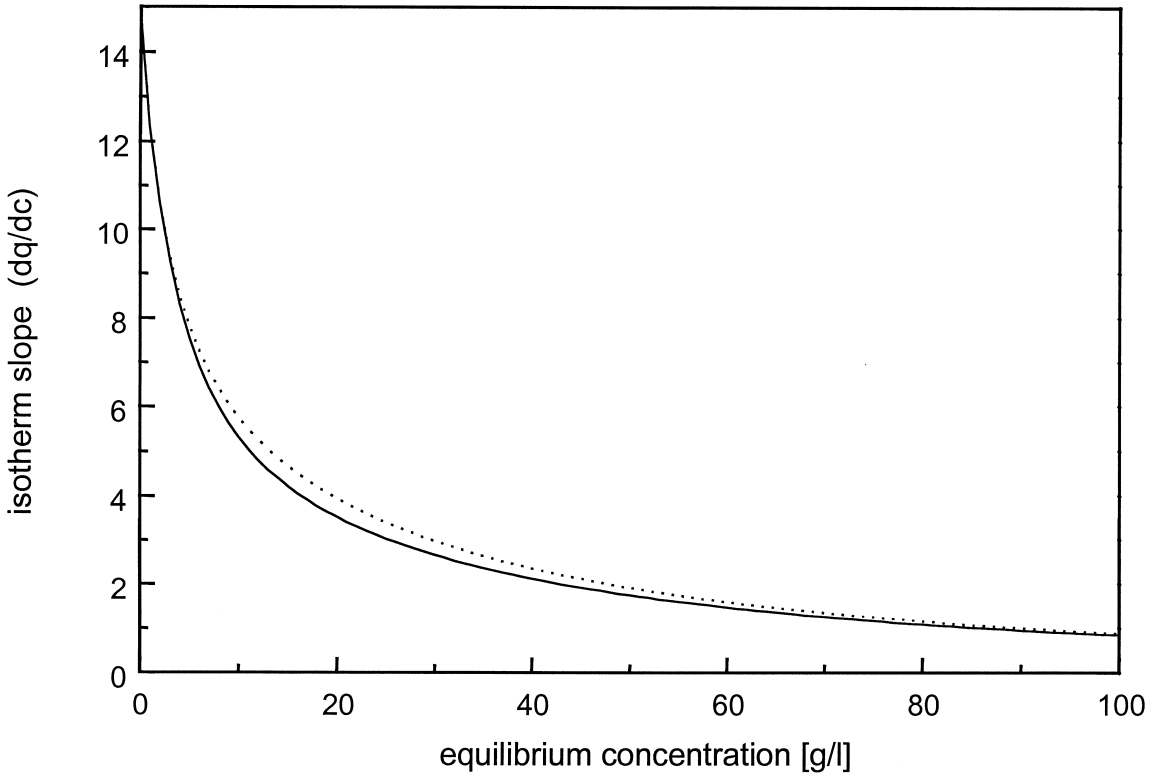

Fig. 8. Theoretical slopes of the adsorption isotherm of C5. Calculated with the parameters of the Bi-Langmuir equation obtained after minimizing $OF_{\bar{V},\bar{c}}$ (solid line) and $OF_{\bar{V}}$ (dotted line) (Table 3).

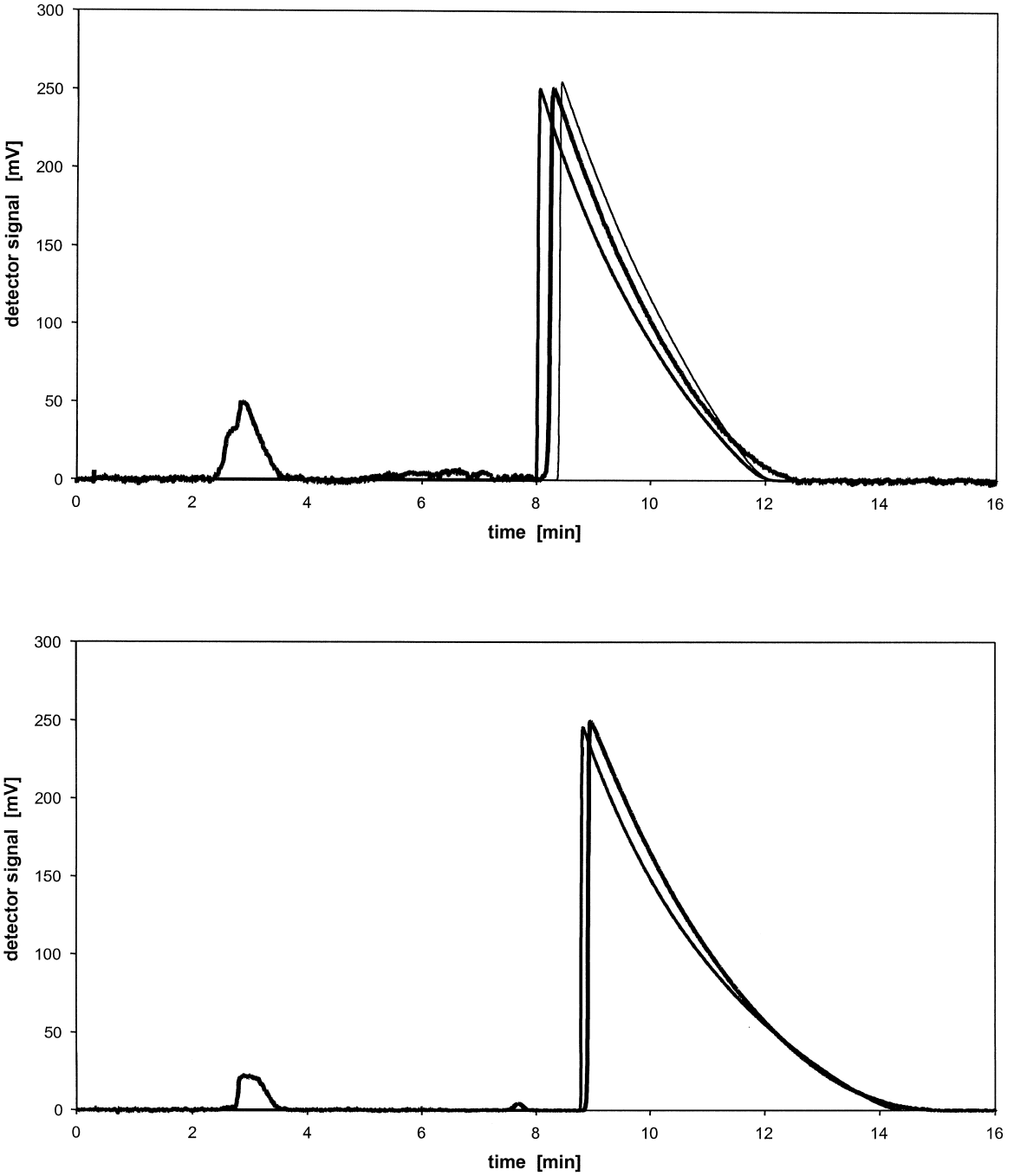

Fig. 9. (a) Comparison between an experimental elution profile (fat line) for C7 as a single solute ($c^{inj}=20{,}08$ g/l, $V^{inj}=523$ μl) with simulations using the Bi-Langmuir isotherm (Eq. (14), thin line) and the Langmuir isotherm (Eq. (13), thinnest line). Parameters as in Table 3 for minimizing $OF_{\bar{V},\bar{c}}$ (Eq. (8)). (b) Comparison between an experimental elution profile (fat line) for C5 as a single solute ($c^{inj}=20{,}17$ g/l, $V^{inj}=523$ μl) with simulation using the Bi-Langmuir isotherm (Eq. (14), thin line). Parameters as in Table 3 for minimizing $OF_{\bar{V}}$ (Eq. (12)).

Table 4
Retention volumes and equilibrium concentrations for runs with C5–C7 mixtures

Dilution step p	(Initial) and measured equilibrium concentration C5 [g/l]	(Initial) and measured equilibrium concentration C7 [g/l]	First retention volume $V^{p}_{R,1,ex}$ [ml]	Second retention volume $V^{p}_{R,2,ex}$ [ml]
1	(75.12)	(25.06)	3.15	3.92
2	40.56	14.81	3.58	4.86
3	22.72	8.82	4.33	5.90
4	15.49	6.26	5.02	6.09
5	10.05	3.74	5.76	6.93
1	(50.00)	(50.03)	3.06	3.71
2	28.03	30.13	3.58	4.55
3	15.49	18.28	4.25	5.50
4	11.26	13.09	5.00	6.08
1	(5.02)	(5.03)	6.32	7.85
2	3.43	3.47	7.16	8.73
3	2.84	2.98	7.88	9.45
4	1.85	1.91	8.40	9.90
5	1.39	1.65	8.94	10.37
1	(25.21)	(75.37)	3.06	3.56
2	12.94	46.55	3.58	4.33
3	7.07	29.64	4.24	5.21
4	4.82	15.90	5.02	6.11
1	(2.55)	(7.56)	6.35	7.42
2	1.79	5.72	7.23	8.27
3	1.49	4.33	7.85	8.89
4	0.92	3.16	8.34	9.33

In Fig. 11 a typical course of the local isotherm slopes is shown for DBP as a single solute. This dependence can be described well by the Langmuir model. Only a slightly better agreement can be achieved using the Bi-Langmuir equation. The isotherm parameters obtained from minimizing the objective function $OF_{\bar{V},\bar{c}}$ for the single solute retention volumes are summarized in Table 5 for all three phthalates. In further experiments with binary mixtures, three different initial compositions were prepared and all equilibrium concentrations were measured. The fitted parameters of the Langmuir isotherm are given in Table 6. For comparison the values calculated from the single solute experiments are repeated. Obviously there is a relatively good agreement between the corresponding values for the different experiments with binary mixtures and the single solute experiments.

Using the Bi-Langmuir parameters given in Table 5 it was attempted to predict a chromatogram for a ternary mixture of all three phthalates. The comparison with the experimentally determined elution profile is shown in Fig. 12 (top). For this comparison the simulated concentration profiles shown in Fig. 12 (bottom) have been transferred into theoretical signals using calibration factors for all components. The general agreement between the measurements and the predictions is relatively satisfactorily. Similar results have been found for other feed compositions [28]. These results indicate again the applicability of the performed isotherm parameter estimation using perturbation chromatography.

4.4. Results for system 3

Due to the success of applying only experiments with mixtures for studying systems 1 and 2 this strategy was exclusively performed for system 3. Five solutions of the two isomers (α and β) were prepared. For each of them the initial concentrations

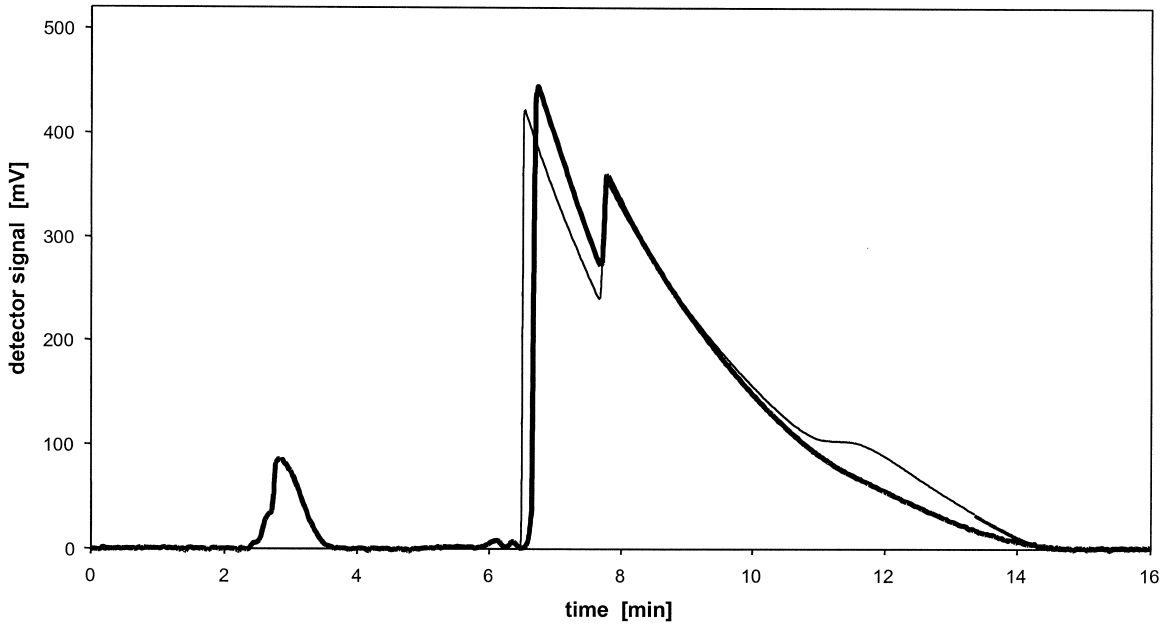

Fig. 10. Experimental elution profile (fat line) of a binary mixture of C5 and C7 ($c_{C5}^{inj}=14.8$ g/l, $c_{C7}^{inj}=40.6$ g/l, $V^{inj}=523$ μl). Simulation (thin line) using the Langmuir model (Eq. (13)) and the parameters obtained from minimizing $OF_{\bar{V}}$ (Eq. (12)) using exclusively retention volumes from experiments with mixtures.

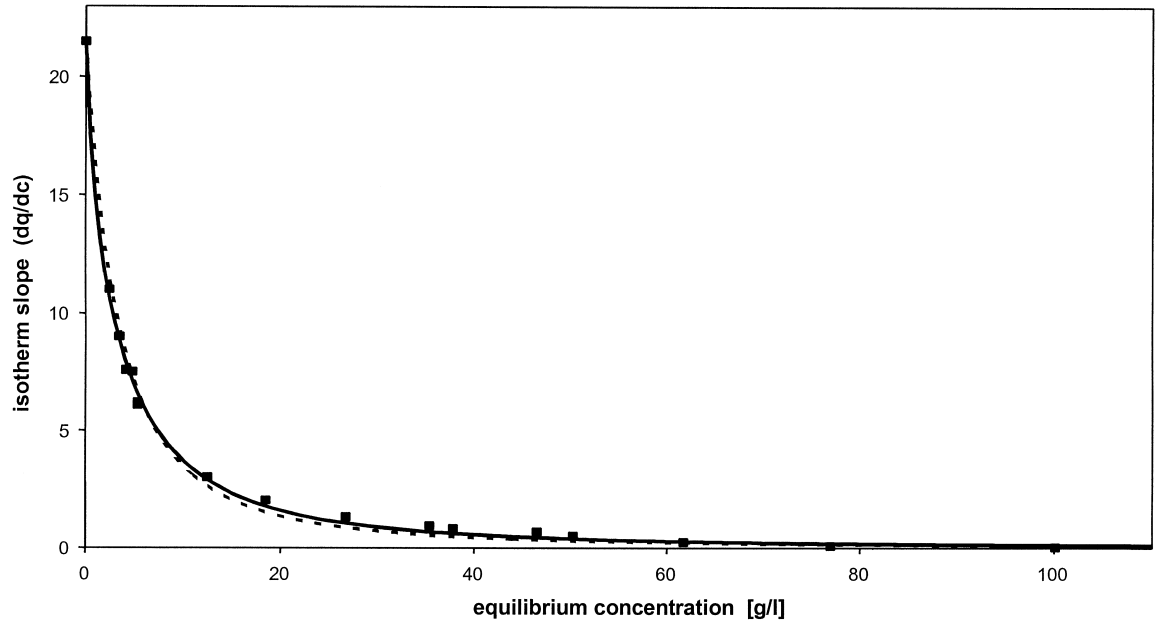

Fig. 11. Local isotherm slope as a function of the equilibrium concentration for DBP as a single solute (system 2). ■: experimental data, dotted line: Langmuir isotherm (Eq. (13)), solid line: Bi-Langmuir isotherm (Eq. (14)).

Table 5
Parameters of the Langmuir and the Bi-Langmuir equation for the single solutes of system 2 (based on single solute experiments, $OF_{\bar{V},\bar{c}} \rightarrow$MIN)

Isotherm parameter	DBP	DEP	DMP
a [−][a]	21.51	45.51	52.16
b [l/g]	0.148	0.337	0.280
a^{I} [−]	15.10	24.67	43.16
b^{I} [l/g]	0.105	0.138	0.517
a^{II} [−][a]	6.41	20.84	9.00
b^{II} [l/g]	0.567	1.745	0.049

[a] Related to adsorption equilibrium constants K_i, Eq. (16).

and three less concentrated plateau concentrations were perturbed. Thus 40 relevant retention volumes were recorded. It should be mentioned that due to impurities in the feed material other additional retention volumes have been detected. Since these signals were completely resolved from the peaks of interest they were not included in the analysis. Attempts to fit the measured retention volumes with the simple Langmuir isotherm were of limited success. This is in agreement with the results of an independent study performed recently [29]. In [29] the ECP-method was applied alternatively to study the thermodynamics of the same system and the Langmuir model failed to represent the experimental data satisfactorily. To match the measured retention volumes using the Bi-Langmuir model and exploiting the available initial isotherm slopes (Table 1) there are six free parameters that need to be determined (for each isomer 3). To reduce this number to four the following relation between the energetic parameters b was assumed to be valid for each of the two sites I and II:

$$b_j^{\text{site}} = b_i^{\text{site}} \frac{K_j}{K_i} \quad \text{site} = \text{I, II} \tag{18}$$

The four free parameters were determined minimizing both $OF_{\bar{V},\bar{c}}$ and $OF_{\bar{V}}$. The results are summarized in Table 7. There is a remarkable agreement between the two parameter sets. This result supports again the applicability of the experimentally more simple method of minimizing $OF_{\bar{V}}$.

With the determined isotherm parameters several chromatograms were predicted and found to be in close agreement with experimental observations [28].

4.5. Results for system 4

System 4 differs from the previous ones in two respects. There is at first a much larger separation factor for the two enantiomers available than for the components in the other three systems (Table 1). Another important feature is the fact that cellulose triacetate usually offers only very limited efficiency. In the particular case the plate numbers were only around 100. Similar results for the same system have been reported in [26]. In comparison to our results given in Table 3 in [26] the following values were reported: $K_{(+)} = 1.2$ and $K_{(-)} = 2.12$.

The low column efficiency caused some difficulties in detecting the retention volumes of small sample sizes. However, even for this system the method of perturbation chromatography was found to be applicable. The measurements were performed using the racemic mixture to prepare different initial solutions. Only the retention volumes have been determined and no plateau concentrations have been measured. For parameter estimation $OF_{\bar{V}}$ was minimized. The Langmuir model was found to represent the data satisfactorily. The obtained parameters are presented in Table 8. With these parameters the retention times and the general shape of elution profiles could be predicted [28].

Table 6
Comparison of b-parameters of the Langmuir equation for the single solutes of system 2 (based on experiments with single solutes and mixtures, $OF_{\bar{V},\bar{c}} \rightarrow$MIN)

Isotherm parameter	From single solute experiments	From mixture experiments with DBP and DEP	From mixture experiments with DBP and DMP	From mixture experiments with DEP and DMP
b_{DBP} [l/g]	0.148	0.165	0.144	–
b_{DEP} [l/g]	0.337	0.349	–	0.289
b_{DMP} [l/g]	0.280	–	0.350	0.331

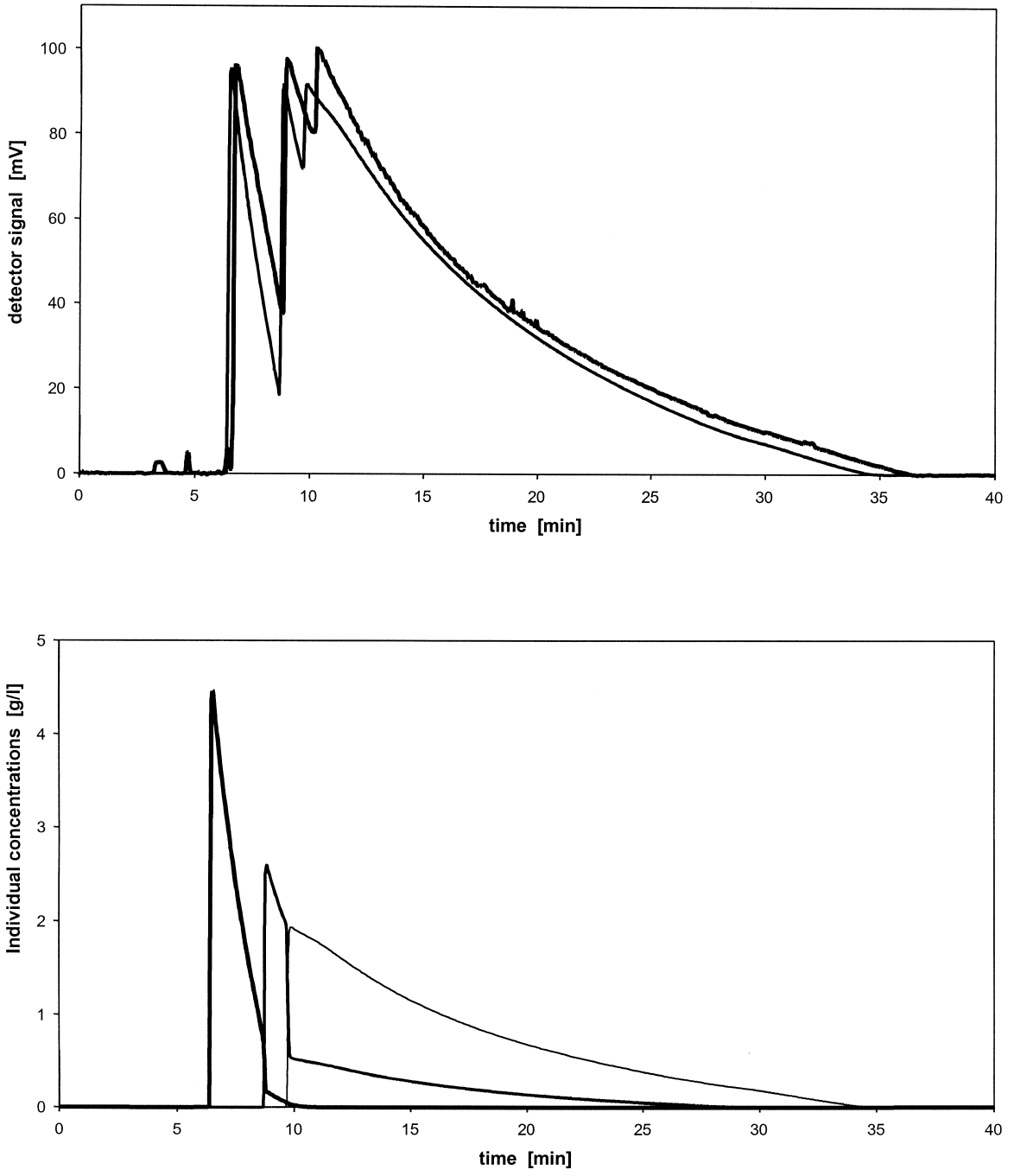

Fig. 12. (top) Experimental elution profile (fat line) of a ternary mixture of DBP, DEP and DMP (system 2, $c^{inj}_{DBP}=10.1$ g/l, $c^{inj}_{DEP}=10.5$, $c^{inj}_{DMP}=30.0$, $V^{inj}=523$ μl, $\dot{V}=0.926$ ml/min). Simulation (thin line) using the Bi-Langmuir model (Eq. (13)) an the parameters in Table 5. (bottom) Simulated individual concentration profiles corresponding to the total signal shown in (top).

Table 7
Parameter of the Bi-Langmuir equation (system 3, from experiments with mixtures)

Isotherm parameter	$OF_{\bar{V},\bar{c}}\rightarrow$MIN	$OF_{\bar{V}}\rightarrow$MIN
	α-isomer	
a^{I} [−]	4.66	4.69
b^{I} [l/g]	0.0083	0.0083
a^{II} [−][a]	2.21	2.18
b^{II} [l/g]	0.2902	0.3038
	β-isomer	
a^{I} [−]	6.19	6.19
b^{I} [l/g]	0.0121	0.0121
a^{II} [−][a]	3.80	3.80
b^{II} [l/g]	0.4219	0.4418

[a] Related to adsorption equilibrium constants K_i, Eq. (16).

5. Conclusions

Perturbation chromatography was applied to determine the free parameters of isotherm models for four different chromatographic systems. In particular a closed-loop arrangement was developed and tested. Essentially only retention volumes had to be determined. In addition the equilibrium concentrations in the closed-loop were measured. Two possibilities to analyze the primary retention data using different objective functions were presented. No detector calibration is required if the proposed overall mass balance concept is used. The closed-loop method appears to be especially attractive in terms of sample economy. It can be easily automated. Another interesting feature of the method is that experiments with mixtures can be exploited to determine the parameters of an isotherm model.

Table 8
Parameter of the Langmuir equation (system 4)

Isotherm parameter	$OF_{\bar{V}}\rightarrow$MIN (experiments with the racemate)
$a_{(+)\text{-WEB2170}}$ [a] [−]	1.04
$b_{(+)\text{-WEB2170}}$ [l/g]	0.0716
$a_{(-)\text{-WEB2170}}$ [a] [−]	2.58
$b_{(-)\text{-WEB2170}}$ [l/g]	0.1777

[a] Related to adsorption equilibrium constants K_i, Eq. (16).

6. Symbols

a [−]	parameter of the Langmuir equation (Eq. (13))
b [l/g]	parameter of the Langmuir equation (Eq. (13))
c [g/l]	concentration of the liquid phase
K [−]	adsorption equilibrium constant
L [m]	column length
N [−]	number of components in a mixture
N_P [−]	number of theoretical plates
q [g/l]	solid-phase concentration
t [s]	time
t_0 [s]	retention time of a nonretained component
t_R [s]	retention time
u [m/s]	linear velocity
$\dot{V}$ [l/s]	volumetric flow-rate
V_0 [m^3]	retention volume of a nonretained component
V_{loop} [m^3]	liquid phase volume in the closed loop
V_{col} [m^3]	column volume
V_{ext} [m^3]	liquid phase volume in the closed loop outside of the column
V_R [l]	retention volume
V_{dil} [l]	dilution volume
x [m]	axial coordinate in the column

6.1. Greek letters

ϵ [−]	total porosity

6.2. Superscripts

anal	for analytical (diluted) conditions
I	first adsorption site, Bi-Langmuir equation (Eq. (14))
II	second adsorption site, Bi-Langmuir equation (Eq. (14))
p	plateau number

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