



## Review

Experimental determination of single solute and  
competitive adsorption isothermsAndreas Seidel-Morgenstern <sup>a,b,\*</sup><sup>a</sup> *Institut für Verfahrenstechnik, Otto-von-Guericke-Universität, Magdeburg D-39106, Germany*<sup>b</sup> *Max-Planck-Institut für Dynamik komplexer technischer Systeme, Sandtorstraße 1, Magdeburg D-39106, Germany***Abstract**

In order to design and to optimise preparative liquid chromatography, the knowledge of the underlying thermodynamic functions, i.e. the adsorption isotherms, is of large importance. Usually these functions can not be predicted and various techniques have been suggested to determine them experimentally. In this paper, several important methods to measure adsorption equilibrium data are discussed and evaluated. The main focus is set on dynamic methods analysing concentration profiles that could be detected at the outlet of fixed-beds packed with the stationary phase of interest. The theoretical background of the different methods is explained using classical equilibrium theory and the equilibrium dispersion model. Each method is illustrated based on experimental data collected in our laboratory. Based on these personal experiences recommendations are given regarding the potential and the applicability of the methods discussed.

© 2003 Elsevier B.V. All rights reserved.

*Keywords:* Reviews; Adsorption isotherms; Mathematical modelling

**Contents**

1. Introduction .....	256
2. Adsorption isotherms and prediction of chromatograms .....	257
2.1. Equilibrium dispersion model .....	257
2.2. Equilibrium theory .....	258
2.2.1. Analysis of adsorption and desorption fronts (“very large” pulses) .....	259
2.2.2. Analysis of “very small” pulses .....	260
2.2.3. Analysis of “intermediate” pulses .....	261
3. Methods to measure adsorption isotherms .....	262
3.1. Static methods .....	262
3.1.1. Batch method .....	262
3.1.2. Adsorption–desorption method .....	263
3.2. Dynamic methods .....	263
3.2.1. Frontal analysis (responses to “very large” pulses) .....	263
3.2.2. Perturbation method (responses to “very small” pulses) .....	266
3.2.3. Elution by characteristic point (responses to “intermediate” pulses) .....	268
3.2.4. Exploiting characteristic peak features .....	268
3.2.5. Peak fitting method (or “inverse method”) .....	268
3.2.6. Influence of applied porosity $\epsilon$ .....	268
3.2.7. Other aspects .....	270
3.2.7.1. No pure substances available .....	270
3.2.7.2. Reducing the number of free parameters .....	270

\* Tel.: +49-39-1671-8644; fax: +49-39-1671-2028.

E-mail address: [anseidel@vst.uni-magdeburg.de](mailto:anseidel@vst.uni-magdeburg.de) (A. Seidel-Morgenstern).

3.2.7.3. More complex isotherm shapes .....	270
3.2.7.4. Gradients .....	270
3.2.7.5. Repeatability .....	270
3.2.7.6. Scale up .....	270
3.2.8. Summary .....	270
4. Modelling of adsorption isotherms .....	270
5. Conclusions .....	270
6. Nomenclature .....	271
Acknowledgements .....	271
References .....	271

## 1. Introduction

Preparative liquid chromatography becomes more and more an important separation process for the isolation and purification of pharmaceuticals, biomolecules and other value added products. Higher requirements on product purity, growing importance of enantioseparations and improved availability of highly selective stationary phases promote this trend. However, chromatographic techniques are expensive and require in an industrial scale a careful optimisation of the operating conditions with respect to production rates, recoveries and separation costs. The most common technique used in preparative chromatography is still isocratic batch elution, however more sophisticated concepts as recycling, gradient elution, displacement or the simulated moving bed (SMB) process are increasingly applied to enhance the productivity and yields [1]. In general, it is not an easy task to design and optimise these processes and to perform a quantitative comparison between rivaling concepts [2].

The substantial progress that has been achieved in modelling preparative chromatography was reviewed recently [3–6]. These works clearly emphasise, that an adequate simulation of a chromatographic separation process requires mainly reliable information how the components of a mixture to be separated are distributed under equilibrium conditions between the mobile and stationary phases. Depending on the specific combination of these two phases, nowadays several mechanisms are exploited to solve a concrete separation problem. Very often the specific enrichment of the dissolved feed components on the surface of the solid stationary phase is the main mechanism [7]. For this reason in this paper the focus is set on adsorption chromatography and thus on the analysis of adsorption equilibria. It should be noted that most of the methods described and the conclusions drawn below can be further generalised. This is due to the fact that there are many similarities between the general shapes of single solute and competitive distribution equilibria for other phase systems and/or other separation mechanisms.

The equilibrium of adsorption processes is usually presented using dependencies of the amounts adsorbed on the fluid phase composition for a constant temperature [8]. An attempt to classify possible shapes of single solute

adsorption isotherms was performed by Giles et al [9]. The broad spectrum of possibilities is illustrated in Fig. 1.

In general, adsorption isotherms can be determined only experimentally. Despite of the fact that there is currently a quite large number of experimental methods available, their appropriate and efficient application is still far away from being a routine job. In several papers, different experimental methods have been compared and evaluated (e.g. [10–14]). In 1996, already a review of the state of the art in the area of measuring adsorption isotherms was given [15]. In the meantime, the importance and the benefit of knowing these isotherms has been widely realised. This is also connected with the success of the SMB technology. To design and to optimise separations using this powerful concept the knowledge of the adsorption isotherms is mandatory [16–21]. In particular for this reason, in the last years many equilibrium studies have been performed.

It has to be realised that compared to the state described in [15] no new methods have been developed in the last years. No significant progress has been achieved regarding a direct measurement of the amounts adsorbed to substitute

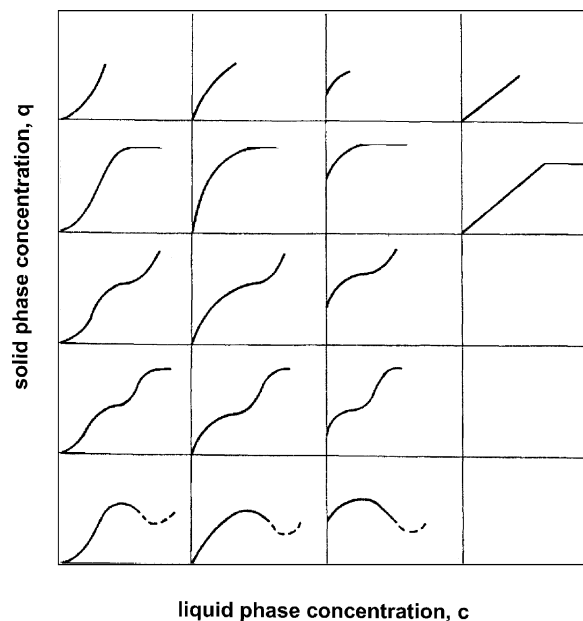


Fig. 1. Single component adsorption from solution. Classification of adsorption isotherms according to Giles et al. [9].

the usage of balance considerations in order to get access to these quantities. Still it appears to be the most difficult task to decide which method is appropriate to determine the adsorption isotherms for a specific separation problem. Often only the single solute isotherms are determined experimentally and the competitive isotherms are then predicted using isotherm models. However, frequently it was observed that the available theoretical concepts offer only limited accuracy and the experimental determination of at least a few multicomponent equilibrium data is recommended. Unfortunately, in this area measurements are more difficult and the number of reliable methods is smaller. An aspect to choose a suitable experimental method is often if and how much of the pure components are available to perform the measurements.

Before describing experimental methods capable to determine adsorption isotherms, the strong connection between these functions and the shapes of concentration profiles at the outlet of a chromatographic columns is explained. For this a simple model that is capable to simulate front propagation phenomena in fixed-beds is used. This equilibrium dispersion model can be easily reduced into the classical and instructive equilibrium (or ideal) model.

Then the most important methods to measure adsorption isotherms for single components as well as for mixtures are explained. To illustrate the individual methods, experimental data collected in the last years in our laboratory will be used. Based on these experiences it is attempted to derive some general conclusions concerning advantages and disadvantages of the methods.

Obviously, in the field of preparative chromatography the measurement of adsorption equilibrium data is only part of the work to be performed in order to simulate the separation process. Subsequently an appropriate mathematical model capable to describe the experimental data has to be found. This important and also not trivial aspect is not further elaborated. In this work, predominantly the well-known Langmuir equation is applied. Other useful adsorption isotherm equations are summarised, e.g. in [15,22,23].

## 2. Adsorption isotherms and prediction of chromatograms

In order to explain the relation between the course of the adsorption isotherms and the shape of concentration profiles in chromatographic columns it is expedient to introduce selected results of parametric calculations performed with simplified fixed-bed models. Since later essentially dynamic methods to measure adsorption isotherms are discussed, this chapter also supplies the theoretical basis for these methods.

### 2.1. Equilibrium dispersion model

A very successful and frequently applied model and frequently applied model to quantify chromatographic

processes under overloaded conditions is the equilibrium dispersion model (e.g. [4]). The mass balance of this model for solute  $i$  in a  $N$  component mixture and a volume element is:

$$\frac{\partial c_i}{\partial t} + \frac{1 - \varepsilon}{\varepsilon} \frac{\partial q_i(\bar{c})}{\partial t} + u \frac{\partial c_i}{\partial x} = D_{\text{ap}} \frac{\partial^2 c_i}{\partial x^2}, \quad (1)$$

$$i = 1, N \text{ with } \bar{c} = (c_1, c_2, \dots, c_N)$$

In this equation,  $c$  is the concentration in the fluid phase and  $q$  is the concentration in the solid phase. The dimension of both concentrations are identical (e.g. mol/l or g/l). The column porosity  $\varepsilon$  defines the fraction of the fluid phase in the column. Aspects of distinguishing more precisely between extraparticle and intraparticle porosities are discussed in [15]. Further,  $u$  stands for the linear velocity and  $t$  and  $x$  are the time and space coordinates, respectively. All contributions leading to band broadening (e.g. axial dispersion, finite rate of mass transfer processes) are lumped in a simplifying manner into an apparent dispersion coefficient,  $D_{\text{ap}}$ . In Eq. (1), it is assumed that the two phases are constantly in equilibrium expressed by the adsorption isotherms:

$$q_i = q_i(\bar{c}), \quad i = 1, N \text{ with } \bar{c} = (c_1, c_2, \dots, c_N) \quad (2)$$

Already for single solutes these functions can have very different courses (compare Fig. 1). A difficult task for mixture isotherm equations is to quantify properly the extent of competition effects. Typical effects occurring under overloaded conditions in nonlinear chromatography can be illustrated with the classical and well-known multi-Langmuir isotherm model which will be frequently used below to analyse experimental data:

$$q_i(\bar{c}) = \frac{a_i c_i}{1 + \sum_{j=1}^N b_j c_j}, \quad i = 1, N \quad (3)$$

In order to solve Eq. (1), there are additional initial and boundary conditions required. In most cases, the columns are uniformly preloaded prior to an experiment designed to measure adsorption isotherms:

$$c_i(t = 0, x) = c_i^{\text{Init}}, \quad i = 1, N \quad (4)$$

$$q_i(t = 0, x) = q_i^{\text{Init}}(c_i^{\text{Init}}), \quad i = 1, N \quad (5)$$

Often  $c_i^{\text{Init}}$  is zero. Appropriate boundary conditions for dispersion models have been formulated by Danckwerts [24]:

$$c_i(t, x = 0) = c_i^{\text{inj}}(t) + \frac{D_{\text{ap}}}{u} \frac{\partial c_i}{\partial x} \Big|_{t, x=0}, \quad i = 1, N \quad (6)$$

$$\frac{\partial c_i}{\partial x} \Big|_{t, x=L} = 0, \quad i = 1, N \quad (7)$$

Obviously, the injection profile  $c_i^{\text{inj}}(t)$  can be modulated during experiments. Often rectangular injection profiles of different width  $t^{\text{inj}}$  are applied:

$$c_i^{\text{inj}}(t) = \begin{cases} c_i^{\text{Feed}} & \text{for } t \leq t^{\text{inj}} \\ 0 & \text{for } t > t^{\text{inj}} \end{cases}, \quad i = 1, N \quad (8)$$

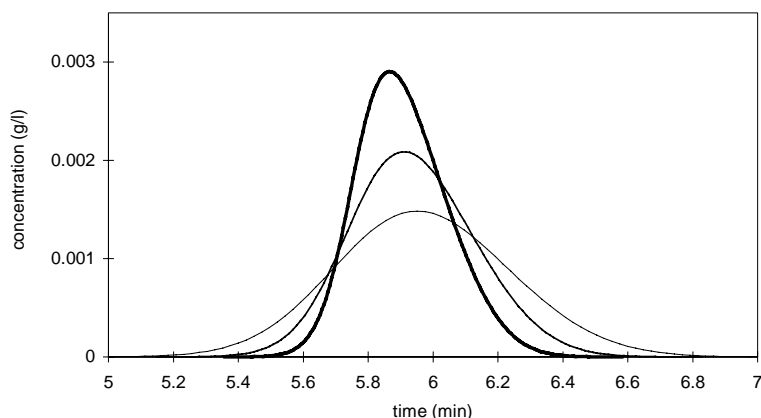


Fig. 2. Elution profiles under diluted conditions simulated with the equilibrium dispersion model (Eq. (1)). Parameters:  $a = 51/l$ ,  $b = 51/g$ ,  $\varepsilon = 0.5$ ,  $L = 20$  cm,  $V = 2$  ml,  $\dot{V} = 1$  ml/min,  $t^{\text{inj}} = 0.001$  min,  $c^{\text{Feed}} = 1$  g/l,  $N_p = 500$  (bold line)/1000/2000.

The selection of  $c_i^{\text{Feed}}$  and  $t^{\text{inj}}$  leads to different experimental methods which will be explained below.

For efficient columns of length  $L$  holds the well known relation between  $D_{\text{ap}}$  and the number of theoretical plates  $N_p$  [4]:

$$N_p = \frac{uL}{2D_{\text{ap}}} \quad (9)$$

Eq. (1) together with the given initial and boundary conditions can be solved numerically. This simple model was often found to be very accurate in describing elution profiles [4]. Here, it will be used to illustrate the fact that under overloaded conditions the adsorption isotherms have a significant influence on the propagation speed of concentration fronts in chromatographic columns and thus on specific retention times and shapes of effluent profiles.

Fig. 2 illustrates three simulated chromatograms for a single solute and small amounts injected, thus under diluted (analytical) conditions. The parameter varied is the plate number  $N_p$ . Fig. 3 reveals that, in contrast, under strongly overloaded conditions the effect of the plate number on the shape of the bands is much less important. The shape and position of the peaks is now essentially determined by the adsorption isotherm. The Langmuir isotherm equation used

in the simulations describes the frequently occurring reduction of retention times in combination with the formation of a sharpened front and a dispersed rear.

In Fig. 4 is given a comparison between the single solute elution profiles for two components under overloaded conditions and the corresponding profiles for the mixture. Again the peak deformation compared to analytical situations can be observed. In addition, the well-known consequences of competition are exemplified [4]. The extents of displacement of the first eluting component and tag along of the last eluting component depend strongly on the course of the competitive adsorption isotherms.

Effects of mass transfer and dispersion are often of minor importance in preparative chromatography. It is instructive to neglect them completely. This leads to the classical equilibrium (or ideal) model of chromatography which allows to study more directly the effect of the equilibrium functions and provides the basis for several experimental methods to measure adsorption isotherms.

## 2.2. Equilibrium theory

The basic equation of the equilibrium theory results from Eq. (1) if the apparent dispersion coefficient is set to zero

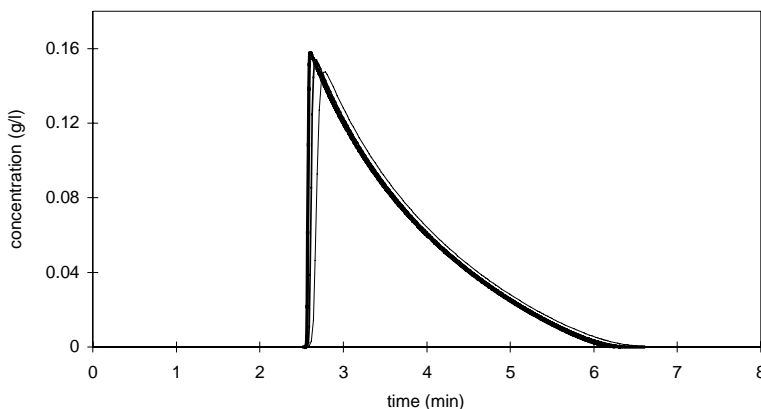


Fig. 3. Elution profiles under overloaded conditions simulated with the equilibrium dispersion model (Eq. (1)). Parameters as in Fig. 2, except  $c^{\text{Feed}} = 200$  g/l.

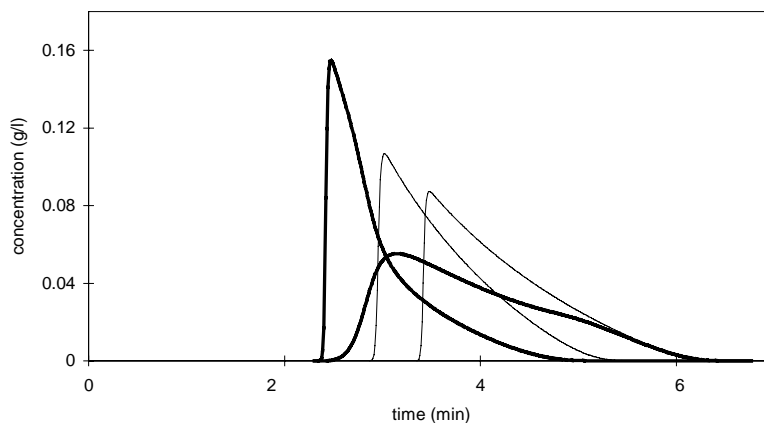


Fig. 4. Comparison between elution profiles for the two components of a binary mixture simulated with the equilibrium dispersion model (Eq. (1)). Thin lines: without competition (usage of single solute isotherms), Thick lines: with competition (isotherm Eq. (3)). Parameters:  $a_1 = 4 \text{ l/l}$ ,  $b_1 = 4 \text{ l/g}$ ,  $a_2 = 5 \text{ l/l}$ ,  $b_2 = 5 \text{ l/g}$ ,  $\varepsilon = 0.5$ ,  $L = 20 \text{ cm}$ ,  $V = 2 \text{ ml}$ ,  $\dot{V} = 1 \text{ ml/min}$ ,  $t^{\text{inj}} = 0.001 \text{ min}$ ,  $c_1^{\text{Feed}} = c_2^{\text{Feed}} = 100 \text{ g/l}$ ,  $N_p = 1000$ .

(i.e. if an infinite number of theoretical plates is assumed). Thus, the only transport mechanism considered is convection. Then results the following mass balance equation for a component  $i$ :

$$\frac{\partial c_i}{\partial t} + \frac{1 - \varepsilon}{\varepsilon} \frac{\partial q_i(\bar{c})}{\partial t} + u \frac{\partial c_i}{\partial x} = 0, \quad i = 1, N \quad (10)$$

The solution of this system of simplified partial differential equations requires the specification of only one boundary condition which typically is:

$$c_i(t, x = 0) = \begin{cases} c_i^{\text{Feed}} & \text{for } t \leq t^{\text{inj}} \\ 0 & \text{for } t > t^{\text{inj}} \end{cases}, \quad i = 1, N \quad (11)$$

Eqs. (10) and (11) have been studied intensively in the last decades for different adsorption isotherm models. The large number of instructive results is summarised in detail in excellent papers and monographs [25–28]. In particular, the method of characteristics has been applied successfully and analytical solutions have been achieved [27].

The shape of elution profiles and breakthrough curves predicted by the equilibrium theory is directly related to the course of the adsorption isotherms. This offers attractive possibilities to solve also the inverse problem consisting in the determination of the isotherms from measured elution profiles or breakthrough curves. Since three important methods capable to measure adsorption isotherms exploit solutions of the equilibrium theory, selected results provided by this theory will be summarised below. Separate attention is given to the three cases of (a) very large, (b) very small and (c) intermediate injection amounts.

### 2.2.1. Analysis of adsorption and desorption fronts (“very large” pulses)

If the injection time in Eq. (11) is large enough the whole fixed bed is completely transformed from a first equilibrium state corresponding to the initial condition  $(c^{\text{Init}}, q(c^{\text{Init}}))$  into a new equilibrium state corresponding to the feed concentration  $(c^{\text{Feed}}, q(c^{\text{Feed}}))$ . This is the classical and extensively

studied Riemann problem [27]. The shape of the resulting breakthrough curves depends on the direction of the process (adsorption for  $c^{\text{Init}} < c^{\text{Feed}}$ , desorption for  $c^{\text{Init}} > c^{\text{Feed}}$ ) and the curvature of the isotherm (Langmuir type or anti-Langmuir type). In each case, a compressed (shock) front or a dispersed front develop, respectively. The retention time of the shock front is related to the isotherm chord between  $c^{\text{Init}}$  and  $c^{\text{Feed}}$  and can be calculated according to:

$$t_{\text{R,shock}} = \frac{L}{u} \left[ 1 + \frac{1 - \varepsilon}{\varepsilon} \frac{q^{\text{Feed}}(c^{\text{Feed}}) - q^{\text{Init}}(c^{\text{Init}})}{c^{\text{Feed}} - c^{\text{Init}}} \right] \quad (12)$$

In a dispersed front the retention time for a certain concentration is related to the corresponding local slope of the isotherm and can be calculated as follows:

$$t_{\text{R,dispers}}(c) = \frac{L}{u} \left[ 1 + \frac{1 - \varepsilon}{\varepsilon} \left. \frac{dq}{dc} \right|_c \right] \quad (13)$$

It should be mentioned that the situation becomes more complex if the adsorption isotherms have one or more inflection points between  $c^{\text{Init}}$  and  $c^{\text{Feed}}$ .

Fig. 5 illustrates the discussed and important results of the equilibrium theory for two different adsorption isotherm curvatures and process directions.

There are also solutions of Eq. (10) available for mixtures. Using elegant coordinate transformations, there have been achieved instructive results, in particular for the multi-Langmuir isotherm equation (Eq. (3)) [27].

Figs. 6 and 7 illustrate typical effluent profiles for a ternary system ( $N = 3$ ) as predicted by the equilibrium theory. Fig. 6 shows the adsorption on an initially not preloaded column ( $c_i^{\text{Feed}} > c_i^{\text{Init}} = 0$ ) and Fig. 7 shows the process of total regeneration of an initially preloaded column ( $0 = c_i^{\text{Feed}} < c_i^{\text{Init}}$ ). In both cases, the initial and feed states are divided by two ( $N - 1$ ) characteristic intermediate states (II and III). Due to the self-sharpening character of adsorption fronts for systems of the Langmuir type (compare Fig. 5a and b) the transitions between the four states occur in Fig. 6 at three ( $N$ ) characteristic retention times

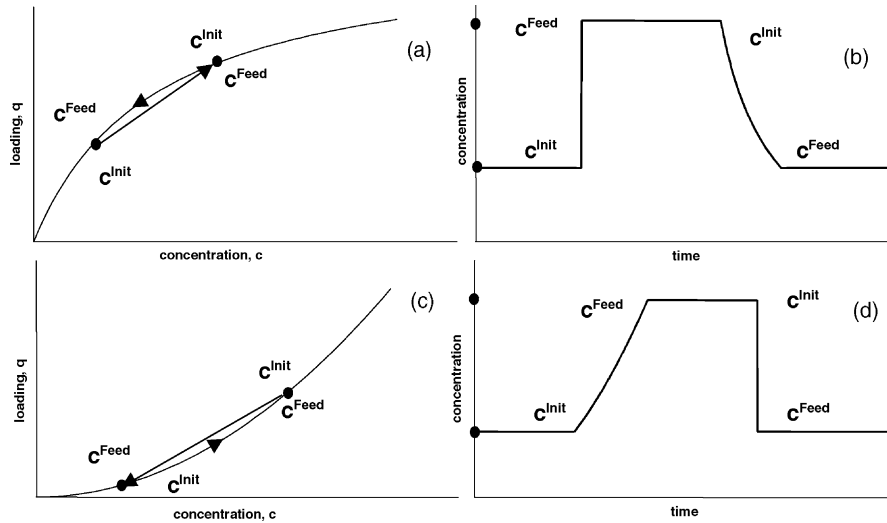


Fig. 5. Illustration of the relation between the curvature of the adsorption isotherms and the shape of adsorption and desorption fronts. (a) Langmuir isotherm, (b) corresponding sharpended adsorption front and dispersed desorption front, (c) anti-Langmuir isotherm, (d) corresponding dispersed adsorption front and sharpened desorption front.

( $t_R^1$ ,  $t_R^2$  and  $t_R^3$ ) as shocks. The displacement effect triggers the typical overshooting of some effluent concentrations. The corresponding transition for the desorption process happens in a smoother (dispersed) manner (Fig. 7). Equivalent averaged retention times of the simple waves can be calculated from mass balance considerations. Provided the adsorption isotherms are known, the equilibrium theory allows to calculate the characteristic intermediate plateau concentrations ( $c_i^{II}$ ,  $c_i^{III}$ , ...,  $c_i^{N-1}$ ), the retention times of the shocks ( $t_{R,S}^1$ ,  $t_{R,S}^2$ , ...,  $t_{R,S}^N$ ), the shape of the waves and their averaged retention times ( $t_{R,av}^1$ ,  $t_{R,av}^2$ , ...,  $t_{R,av}^N$ ). The latter mark the times where hypothetical shocks would fulfil the constraints set by the component mass balances. In both Figs. 6 and 7 are shown in addition also results of time consuming numerical calculations for the equilibrium dispersion model (Eq. (1),  $N_p = 500$ ). The close

agreement demonstrates the potential of the equilibrium theory.

2.2.2. Analysis of “very small” pulses

The equilibrium theory also allows to predict the responses to small perturbations introduced at the inlet of a column equilibrated at a known concentration level. A key result of the theory is the fact that a perturbation of an equilibrium state for a system with  $N$  solutes triggers for each solute  $N$  characteristic waves. According to the coherence condition the speeds and thus the retention times of these waves are synchronised for all components [26–28].

Analysing Eq. (10),  $N$  characteristic retention times of a component  $i$ ,  $t_{R,i}^k(\bar{c})$ ,  $k = 1, N$ , can be calculated for a given vector of equilibrium concentrations,  $\bar{c} = (c_1, c_2, \dots, c_N)$ ,

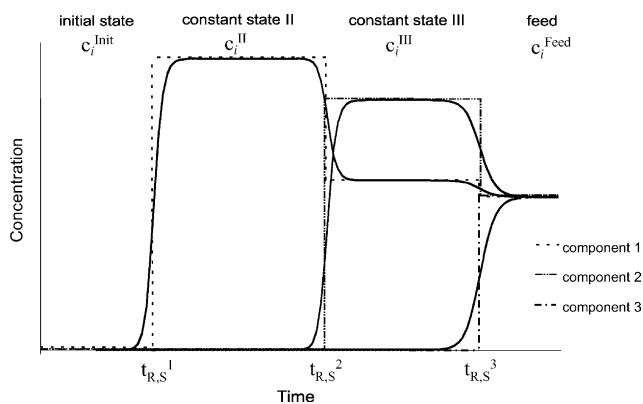


Fig. 6. Characteristic fronts for the adsorption on a not preloaded column ( $c_i^{Feed} > c_i^{Init} = 0$ ). Ternary mixture ( $i = 1-3$ ). Langmuir model (Eq. (3)). Dashed and dotted lines are predictions of equilibrium theory (Eq. (10)). Solid lines are the corresponding results of the equilibrium dispersion model (Eq. (1),  $D_{ap}$  corresponded to  $N_p = 500$ ) [33].

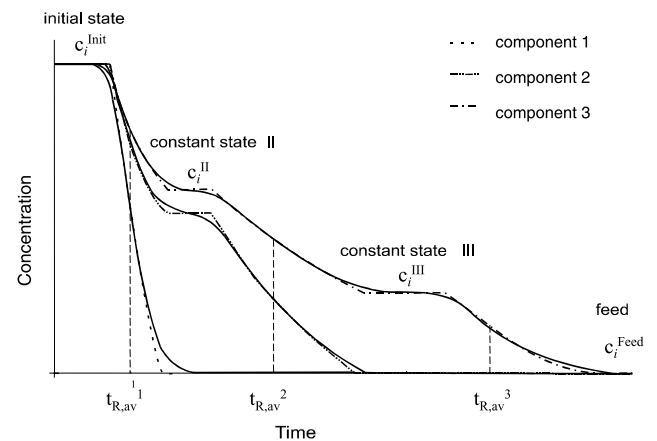


Fig. 7. Characteristic fronts for the desorption of a preloaded column ( $c_i^{Feed} = 0 < c_i^{Init}$ ). Ternary mixture ( $i = 1-3$ ). Langmuir model (Eq. (3)). Dashed and dotted lines are predictions of equilibrium theory (Eq. (10)). Solid lines are the corresponding results of the equilibrium dispersion model (Eq. (1),  $D_{ap}$  corresponded to  $N_p = 500$ ) [33].

according to:

$$t_{R,i}^k(\bar{c}) = \frac{L}{u} \left( 1 + \frac{1-\varepsilon}{\varepsilon} \frac{dq_i}{dc_i} \Big|_{\bar{c}} \right), \quad i = 1, N; k = 1, N \quad (14)$$

with

$$\frac{dq_i}{dc_i} \Big|_{\bar{c}} = \sum_{j=1}^N \frac{\partial q_i}{\partial c_j} \Big|_{\bar{c}} \frac{dc_j}{dc_i} \Big|_{\bar{c}}, \quad i = 1, N \quad (15)$$

To predict the retention times using Eqs. (14) and (15) the direction differentials  $dc_j/dc_i$  can be calculated using the coherence condition respecting that for each wave  $k$  holds:

$$t_{R,j}^k = t_{R,i}^k, \quad k = 1, N; i, j = 1, N \quad (16)$$

For the example of two dissolved components 1 and 2 the prediction of the two characteristic retention times is based on the specification of two values for the direction differentials, i.e.  $dc_2/dc_1|^{k=1}$  and  $dc_2/dc_1|^{k=2}$ . 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 roots of the following quadratic equation:

$$\left( \frac{dc_1}{dc_2} \Big|_{\bar{c}} \right)^2 + \frac{dc_1}{dc_2} \Big|_{\bar{c}} \frac{(\partial q_2/\partial c_2)|_{\bar{c}} - (\partial q_1/\partial c_1)|_{\bar{c}}}{\partial q_2/\partial c_1|_{\bar{c}}} - \frac{\partial q_1/\partial c_2|_{\bar{c}}}{\partial q_2/\partial c_1|_{\bar{c}}} = 0 \quad (17)$$

It should be mentioned that the obtained retention times do not depend on the direction in which the concentrations in the injected sample differ from the equilibrium concentrations. Small “positive” perturbations, using more concentrated sample solutions, or small “negative” perturbations, using, e.g. solvent samples, generate the same retention times.

Fig. 8 shows for isotherms of the Langmuir type the simulated dependence of the retention times on the equilibrium concentrations for single solutes and binary mixtures. For the binary mixtures besides the individual concentration courses are shown also the courses of the corresponding total concentrations [29].

### 2.2.3. Analysis of “intermediate” pulses

Also for the intermediate situation (no complete breakthrough into a new equilibrium state and no remaining at the initial equilibrium state) the equilibrium theory provides instructive analytical solutions. The analysis presented above for “very large” pulses (frontal chromatography) can be extended in order to calculate elution profile for smaller samples. Thus, in contrast to the case of the total breakthrough, each elution profile now simultaneously consists out of a compressed and a dispersed part. As discussed above the curvature of the isotherm will be decisive if the rear part of the peak (for anti-Langmuirian systems) or the front part (for Langmuirian systems) will be compressed. In the latter case,

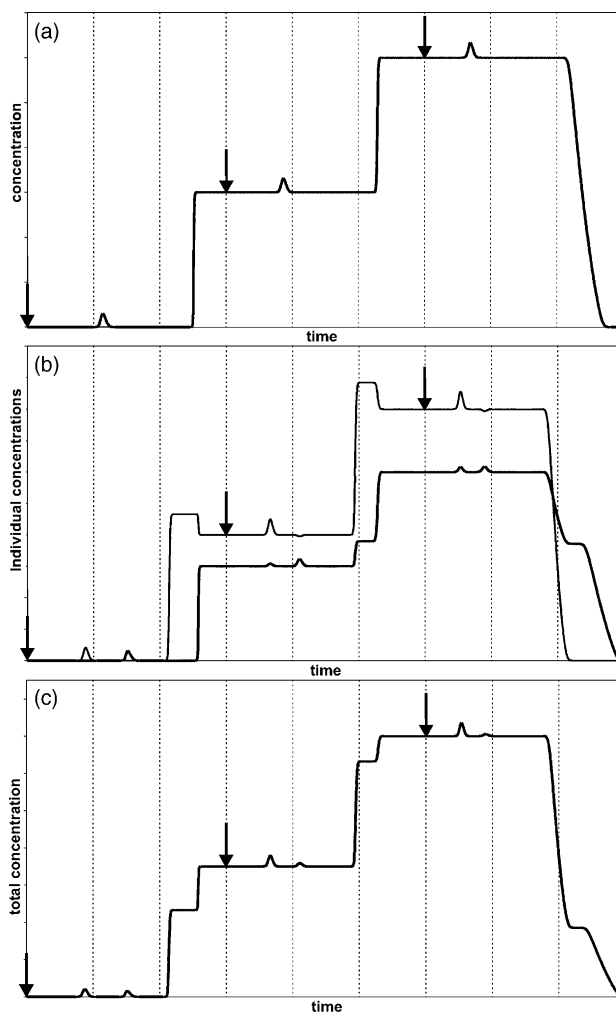


Fig. 8. Illustration of the concentration dependence of the retention times (pulse responses) [29]. (a) Retention times of single solute perturbations, three concentration plateaus. (b) Retention times for a binary mixture (individual concentrations). (c) Retention times for a binary mixture (total concentration).

the dispersed rear part of the peak can be calculated using Eq. (18) taking into account the duration of the injection:

$$t_R(c) = t^{\text{inj}} + \frac{L}{u} \left[ 1 + \frac{1-\varepsilon}{\varepsilon} \frac{dq}{dc} \Big|_c \right] \quad (18)$$

The position of the shock  $t_{R,\text{shock}}$  can be calculated from the following mass balance considering the amount injected:

$$\int_{t_{R,\text{shock}}}^{t^{\text{end}}} c(t_R) dt_R = t^{\text{inj}} c^{\text{Feed}} \quad (19)$$

with

$$t^{\text{end}} = t^{\text{inj}} + \frac{L}{u} \left[ 1 + \frac{1-\varepsilon}{\varepsilon} \frac{dq}{dc} \Big|_{c=0} \right] \quad (20)$$

Fig. 9 illustrates typical results for a single solute. For comparison in the figure are shown also results of predicting the elution profile with the equilibrium dispersion model (Eq. (1),  $N_p = 1000$ ).

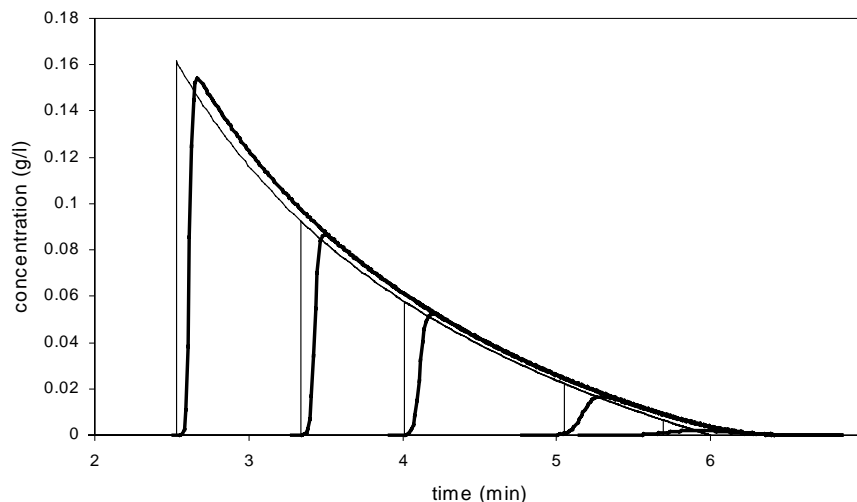


Fig. 9. Elution profiles simulated with the equilibrium model (thin line, Eq. (1)) and the equilibrium dispersion model (solid lines, Eq. (10),  $N_p = 1000$ ). Parameter:  $a = 5 \text{ l/l}$ ,  $b = 5 \text{ l/g}$ ,  $\varepsilon = 0.5$ ,  $L = 20 \text{ cm}$ ,  $V = 2 \text{ ml}$ ,  $\dot{V} = 1 \text{ ml/min}$ ,  $t^{\text{inj}} = 0.001 \text{ min}$ ,  $c^{\text{inj}} = 1/10/50/100/200 \text{ g/l}$ .

The prediction of single solute elution profiles for isotherms of the anti-Langmuir type can be performed in an analogous manner applying the concept summarised in Eqs. (18)–(20). More complicated is again the case when the isotherm has inflection points. Then composite waves form consisting out of compressed and dispersed parts.

The equilibrium theory can be also applied to predict elution profiles of mixtures not reaching the injection concentrations. However, the derivation of the solutions is much more difficult. Instructive analytical solutions for binary systems are given, e.g. in [4]. Fig. 10 shows predictions of elution profiles for a sample containing two components injected on a not preloaded column using these solutions. In the figure is again shown the good agreement of the results based on the equilibrium theory with independently obtained results corresponding to the equilibrium dispersion model.

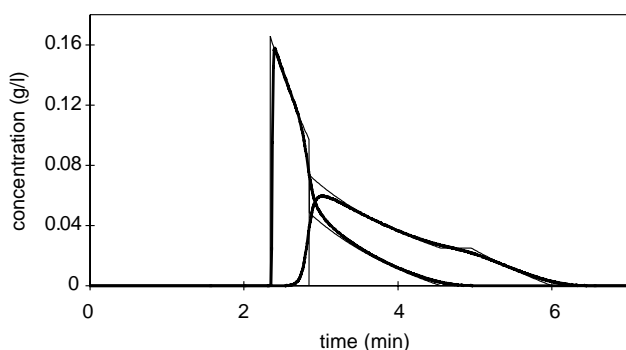


Fig. 10. Elution profiles simulated with the equilibrium model (thin lines, solution of Eq. (10) according to [4]) and the equilibrium dispersion model (solid lines, Eq. (1),  $N_p = 1000$ ). Parameter:  $a_1 = 4 \text{ l/l}$ ,  $b_1 = 4 \text{ l/g}$ ,  $a_2 = 5 \text{ l/l}$ ,  $b_2 = 5 \text{ l/g}$ ,  $\varepsilon = 0.5$ ,  $L = 20 \text{ cm}$ ,  $V = 2 \text{ ml}$ ,  $\dot{V} = 1 \text{ ml/min}$ ,  $t^{\text{inj}} = 0.001 \text{ min}$ ,  $c_1^{\text{inj}} = c_2^{\text{inj}} = 100 \text{ g/l}$ .

### 3. Methods to measure adsorption isotherms

In this chapter, various dynamic methods capable of measuring adsorption isotherms will be explained and illustrated. These methods are in close relation to the theoretical part given above. However, at first classical static methods will be shortly summarised.

#### 3.1. Static methods

Static methods of measuring adsorption isotherms do not analyse concentration time curves and use only the information of equilibrium states.

##### 3.1.1. Batch method

In the classical batch method known volumes or masses of adsorbent,  $V_{\text{ads}}$  or  $m_{\text{ads}}$ , are equilibrated in a closed vessel with a solution of volume  $V$  possessing known initial concentrations of the solutes,  $c_i^{\text{init}}$ . During the adsorption process the following mass balances have to be fulfilled:

$$\begin{aligned} Vc_i^{\text{init}} &= Vc_i(t) + V_{\text{ads}}q_i(t) \quad \text{or} \\ Vc_i^{\text{init}} &= Vc_i(t) + m_{\text{ads}}\bar{q}_i(t), \quad i = 1, N \end{aligned} \quad (21)$$

To determine the adsorption isotherms only the final equilibrium concentrations have to be measured, i.e.  $c_i^{\text{eq}} = c_i(t \rightarrow \infty)$ . The corresponding equilibrium amounts adsorbed per volume or mass of adsorbent,  $q_i(c_i^{\text{eq}})$  or  $\bar{q}_i(c_i^{\text{eq}})$  are calculated using Eq. (21).

To construct adsorption isotherms several experiments have to be performed. Free parameters that could be varied are the initial concentrations,  $c_i^{\text{init}}$ , and the adsorbent dosages,  $V_{\text{ads}}/V$  or  $m_{\text{ads}}/V$ . These parameters have to be chosen carefully to cover a broad region of the isotherms. The principle of the batch method is illustrated in Fig. 11a. An example of a single solute adsorption isotherm measured with the batch method is shown in Fig. 11b [30]. The data



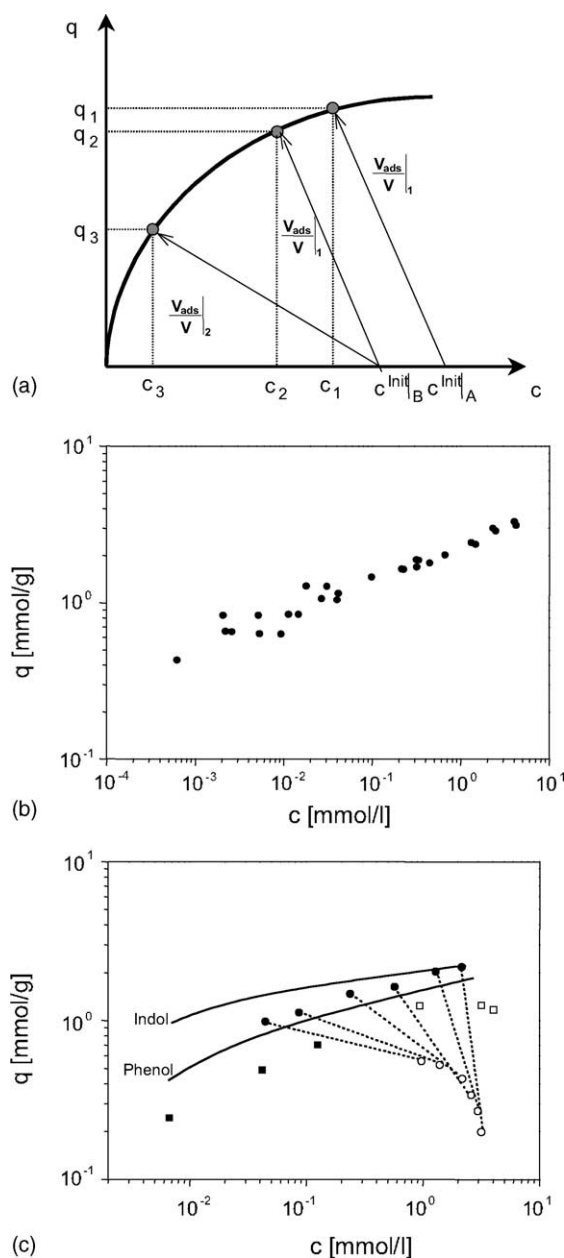


Fig. 11. Illustration of the batch method to measure adsorption isotherms. (a) Measurement of single solute adsorption isotherms. If one equilibrium point is determined (e.g.  $c_1$  and  $q_1$ ) corresponding to a certain initial concentration and adsorbent dosage ( $c^{\text{Init}}|_A$  and  $V_{\text{ads}}/V|_1$ ), other points can be measured varying the initial concentration and the adsorbent dosage. (b) Equilibrium data for the adsorption of phenol dissolved in water on the activated carbon Filtrasorb 400 measured with the batch method [30]. (c) Equilibrium data for the competitive adsorption of phenol and indol dissolved in water on the activated carbon Hydriffin 71 measured with the batch method [31]. The data sets belong to two different initial compositions (squares:  $c_{\text{Phenol}}^{\text{Init}} = 3.36$  mmol/l,  $c_{\text{Indol}}^{\text{Init}} = 4.27$  mmol/l; circles:  $c_{\text{Phenol}}^{\text{Init}} = 5.24$  mmol/l,  $c_{\text{Indol}}^{\text{Init}} = 0.86$  mmol/l) for which the adsorbent dosage was varied. The corresponding equilibrium points are connected for the first initial composition. The solid lines indicate the single solute isotherms.

cover a broad range of liquid phase concentrations. The relative large scattering is mainly due to the fact that each point corresponds to an individual experiment performed with a rather inhomogeneous adsorbent and to inaccuracies in the analysis of the liquid phase equilibrium concentrations. Fig. 11c gives results of measuring competitive adsorption equilibrium data for a binary system with the batch method. The results were obtained equilibrating two initial solutions with different amounts of adsorbent [31].

It can be stated that the batch method requires a significant amount of tedious labour work and is usually not very accurate. Difficulties are related to the required precise determination of the amount of adsorbent and the uncertainty concerning the time needed to reach equilibrium.

### 3.1.2. Adsorption–desorption method

Another static method is based on two steps. Initially a column is completely equilibrated with a feed solution of known concentrations,  $c_i^{\text{Feed}}$ . At equilibrium the quantity of component  $i$  in the column is:

$$n_i = \varepsilon V c_i^{\text{Feed}} + (1 - \varepsilon) V q_i(c_i^{\text{Feed}}) \quad (22)$$

In the second step, the column is completely regenerated with an eluent which is collected. This eluent is subsequently analysed and the  $n_i$  are determined. Now  $q_i(c_i^{\text{Feed}})$  can be calculated using Eq. (22).

The determination of the complete isotherm requires various labour intensive experiments varying the feed concentrations. The method was applied successfully, e.g. in [32].

### 3.2. Dynamic methods

The theoretical frame presented above gives some tools to determine adsorption isotherms from dynamic experiments. The methods are based on the mathematical analysis of the response curves corresponding to different well defined changes of the column inlet concentrations. Below examples for several systems analysed in our laboratory will be presented in order to illustrate different methods.

#### 3.2.1. Frontal analysis (responses to “very large” pulses)

The determination of adsorption isotherms from frontal analysis is just the inverse problem compared to the prediction of breakthrough curves from known isotherms. Since the equilibrium theory provides a convenient tool to predict characteristic features of such curves (intermediate plateau concentrations and retention times of shocks) only these data have to be determined experimentally to solve this inverse problem. This concept is applied as a standard tool to determine single solute isotherms from the concentration dependence of the retention times of the breakthrough fronts. Less work was done up to now to exploit the concept to measure competitive isotherms. To determine whole isotherms (or isotherm branches) successive step changes at the column

inlet (increasing and/or decreasing concentrations) have to be performed and the resulting breakthrough curves have to be analysed to determine the following characteristic features:

- (i)  $N-1$  intermediate plateau concentrations:  $c_i^{\text{II}}, c_i^{\text{III}}, \dots, c_i^{N-1}$
- (ii)  $N$  retention times of shock fronts:  $t_{\text{R,S}}^1, t_{\text{R,S}}^2, \dots, t_{\text{R,S}}^N$  or
- (iib)  $N$  averaged retention times of dispersed fronts (hypothetical shocks)  $t_{\text{R,av}}^1, t_{\text{R,av}}^2, \dots, t_{\text{R,av}}^N$

In case (iib), a difficult and often inaccurate numerical integration of the individual breakthrough curves has to be performed. Thus the analysis of sharp fronts (case (iia)) leads to more reliable retention times and is preferable.

Provided the initial loadings ( $q_i^{\text{Init}}$ ), the column porosity  $\varepsilon$  and the linear velocity  $u$  (or the dead time  $t_0 = L/u$ ) are known, the unknown loadings  $q_i^{\text{Feed}}$  in equilibrium with all feed concentrations,  $\bar{c}^{\text{Feed}}$ , can then be determined using the following relations (e.g. [33]):

$$q_i^{\text{Feed}}(\bar{c}^{\text{Feed}}) = q_i(\bar{c}^{\text{Init}}) + \frac{(t_{\text{R},i}^* - (L/u))(c_i^{\text{Feed}} - c_i^{\text{Init}})}{((1 - \varepsilon)/\varepsilon)(L/u)} \quad (23)$$

with

$$t_{\text{R},i}^* = \frac{t_{\text{R}}^1(c_i^{\text{II}} - c_i^{\text{Init}}) + t_{\text{R}}^2(c_i^{\text{III}} - c_i^{\text{II}}) \cdots + \cdots + t_{\text{R}}^{k-1}(c_i^k - c_i^{k-1}) + t_{\text{R}}^k(c_i^{\text{Feed}} - c_i^k)}{c_i^{\text{Feed}} - c_i^{\text{Init}}}, \quad i = 1, N; k = 2, N \quad (24)$$

and

$$c_i^{k=1} = c_i^{\text{Init}} \quad (25)$$

The analysis based on Eq. (23) using retention times of shocks or averaged retention times of dispersed fronts and intermediate plateau concentrations is not sensitive to kinetic effects as long as the required intermediate plateau concentrations can still be identified (i.e. the plateaus are not eroded completely).

An example of measuring single solute isotherms with the frontal analysis method is given in Fig. 12 [33]. In Fig. 12a are shown 5 breakthrough curves for the adsorption of 2-phenylethanol on a reversed phase material using methanol-water (50:50) mixtures as the mobile phase. A significant reduction of the retention time of the sharp adsorption front with increasing concentration can be observed.

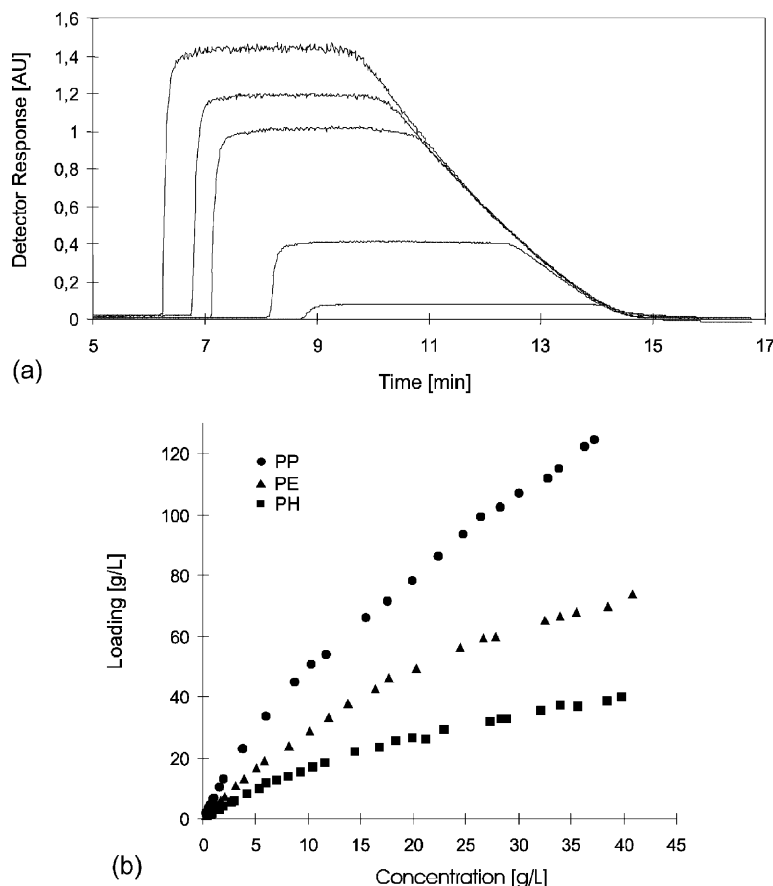


Fig. 12. Illustration of frontal analysis (single solutes) [33]. (a) Breakthrough curves of 2-phenylethanol (PE) on a reversed phase material using methanol-water (50:50).  $c_{\text{PE}}^{\text{Feed}} = 1.06, 5.87, 16.4, 20.3$  and  $30.3$  g/l (same injection volume). (b) Adsorption isotherms at ambient temperature of 2-phenylethanol (PE), phenol (PH) and 3-phenyl-1-propanol (PP) for the same chromatographic system.

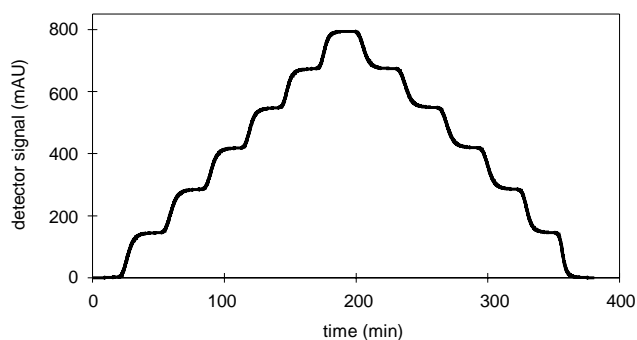


Fig. 13. Recorded profile ("staircase" frontal analysis) to determine the adsorption isotherm of the (+)-enantiomer of Tröger's base dissolved in ethanol on cellulose triacetate [34].

This indicates the presence of a nonlinear isotherm as shown in Fig. 12b. In this figure, more equilibrium data for 2-phenylethanol are shown together with data for the adsorption of phenol and 3-phenyl-1-propanol.

To obtain complete isotherms, always different breakthrough curves have to be analysed for different feed concentrations. It is useful to perform such experiments exploiting the mixing potential of modern HPLC gradient systems. This was done for example to measure the isotherms of the (+)-enantiomer of Tröger's base adsorbed from ethanol on cellulose triacetate (Fig. 13, [34]).

The possibility to determine competitive adsorption isotherms using frontal analysis is illustrated in Figs. 14 and 15 [33]. Fig. 14 shows the response curves for a ternary system detected at two different wavelengths and revealing the position of the shock fronts and the intermediate plateau concentrations required for the application of the methods. Equilibrium data determined from a series of such breakthrough experiments are given in Fig. 15. The effect of competition is obvious when the data are compared to the also depicted single solute isotherms.

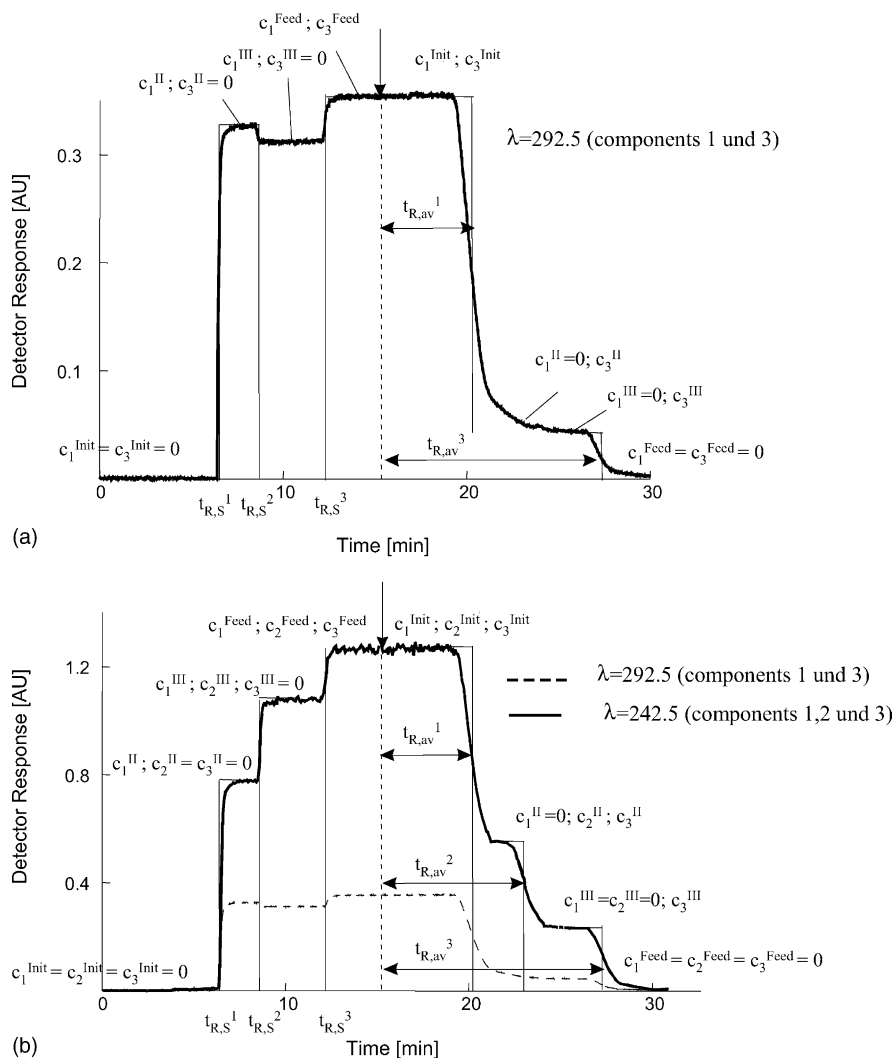


Fig. 14. Experimental breakthrough curves of a ternary mixture 2-phenylethanol, phenol and 3-phenyl-1-propanol (Kromasil C<sub>18</sub>, methanol-water (50:50),  $c_{\text{PH}}^{\text{Feed}} = c_{\text{PE}}^{\text{Feed}} = c_{\text{PP}}^{\text{Feed}} = 4.7 \text{ g/L}$ ,  $\dot{V} = 1 \text{ ml/min}$ ) [33]. (a) Detector response at  $\lambda = 292.5 \text{ nm}$ , where component 2 is not visible; (b) detector response at  $\lambda = 292.5 \text{ nm}$  (---) and at  $\lambda = 242.5 \text{ nm}$  (—).

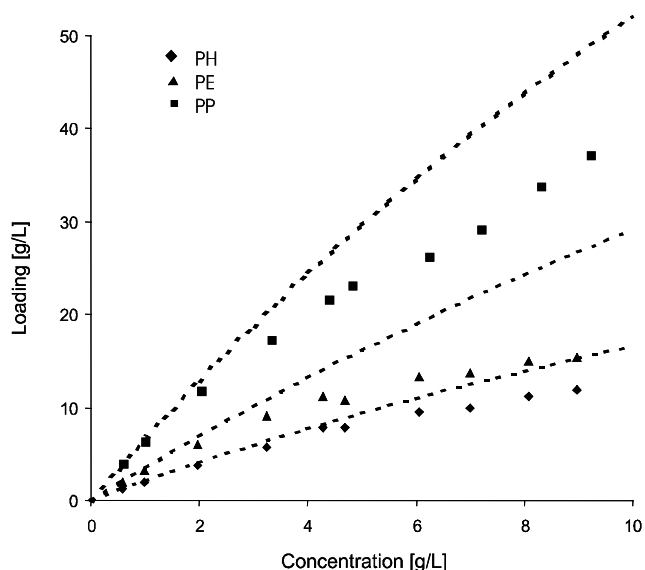


Fig. 15. Adsorption equilibrium data (symbols) for ternary mixtures of 2-phenylethanol (PE), phenol (PH) and 3-phenyl-1-propanol (PP) (Kromasil C<sub>18</sub>, methanol-water (50:50)) determined from frontal analysis. Dashed lines: single solute isotherms calculated with the Langmuir model [33].

Another successful application of the frontal analysis method to measure competitive adsorption isotherms was recently published [35].

### 3.2.2. Perturbation method (responses to “very small” pulses)

One requirement for a successful application of the perturbation method is that the injections performed are small enough to leave the column in equilibrium. If this is the case the resulting retention times of the responses will not depend on the type of perturbation. This is shown in Fig. 16a, where four different perturbations have been introduced in a column which was equilibrated before with a binary mixture. If larger perturbations are required due to detection limits, the retention times of injections with concentrations higher and smaller than the plateau values can be averaged before the analysis is started. Fig. 16b depicts four superimposed response curves for a column equilibrated before the perturbations were introduced with a single solute at different concentration levels. The decrease of retention time with increasing plateau concentrations indicates a Langmuir behaviour of the system.

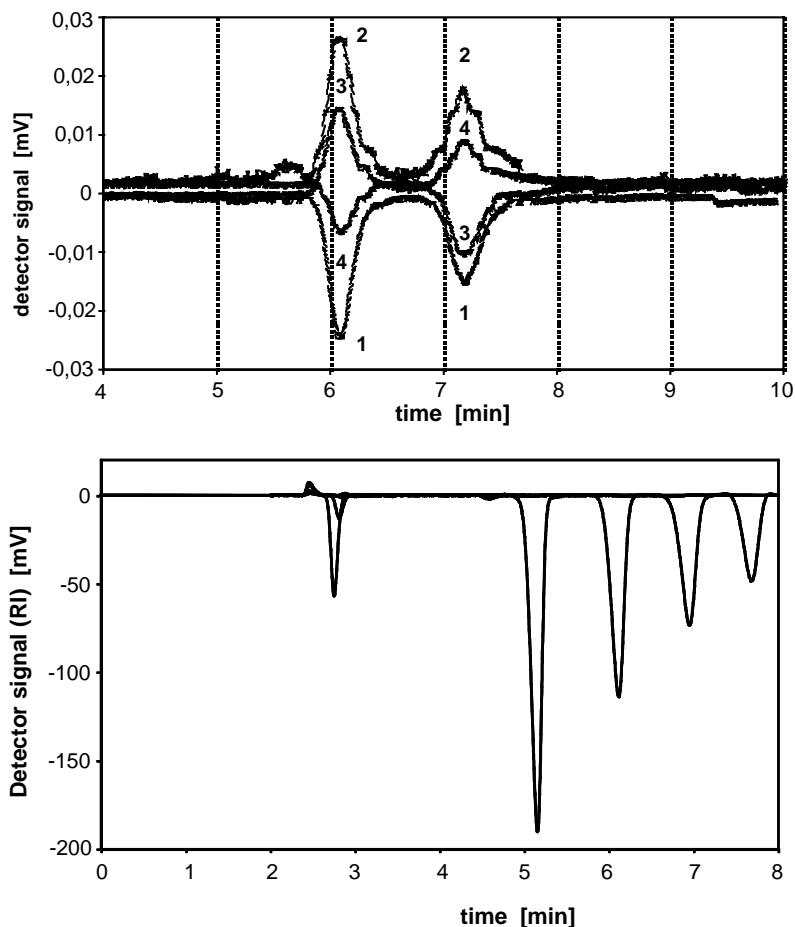


Fig. 16. Illustration of the perturbation method [29]. Top: Detector responses after four different types of perturbations. Example: binary mixture of cyclopentanone and cycloheptanone (1:1), silica, *n*-hexane–ethyl acetate (95:5). Bottom: Superimposed normalized detector responses after four perturbations (cyclopentanone as a single solute). The largest retention time belongs to the lowest plateau concentration.

The experimentally determined retention times deliver essentially information about the local total derivatives of the adsorption isotherms,  $dq_i/dc_i$ . By systematic collection of a sufficient amount of these derivatives,  $q_i(\bar{c})$  can be determined by integration. Usually such isotherm data are subsequently used to determine free parameters of a selected isotherm model. A simpler way to obtain these parameters 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  $\partial q_i/\partial c_j$  and thus total derivatives  $dq_i/dc_i$  (Eq. (15)) can be expressed analytically as a function of not yet known isotherm parameters. A matching of the experimentally observed retention times or volumes and the predictions allows to estimate the

parameters of the isotherm model. This approach was, e.g. used in [29,36]. Fig. 17 illustrates a typical migration behaviour of the retention times as a function of the plateau concentrations for a binary system. The corresponding parameters of the Langmuir model describing these retention times are given in [36].

Further details concerning the application of the method and successful applications are reported in [37,38]. An example where the method was used to detect inflection points in the course of an isotherm is given in [22]. The aspect of a possible vanishing of peaks at certain plateau concentrations is analysed in [39].

A general and significant advantage of the perturbation method is that no detector calibration is required. Pointing

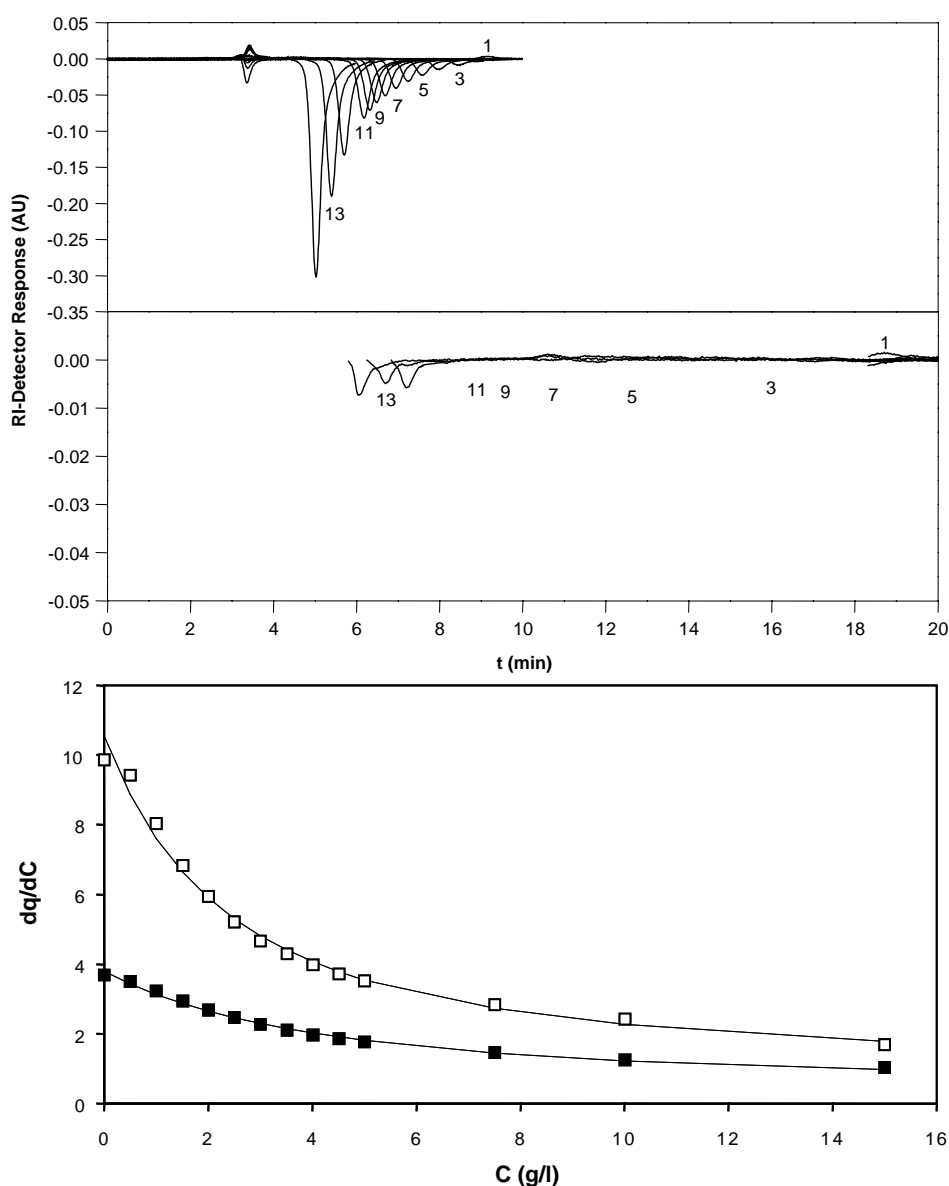


Fig. 17. Illustration of the perturbation method [36]. Top: Detector responses after equilibrating the column with 1:1-mixtures of the two enantiomers of 1-phenoxy-2-propanol (Chiralcel OD, *n*-hexane–isopropanol (90:10)). For each of the 14 plateaus the smaller retention times are illustrated in the upper part and the corresponding larger retention times in the lower part. Bottom: Total isotherm derivatives obtained from the determined retention times [36].

on a mathematical aspect of the problem, it should be finally stated that the prediction of theoretical retention times for  $N$  solutes requires essentially the determination of the roots of a polynomial of order  $N$ .

### 3.2.3. Elution by characteristic point (responses to “intermediate” pulses)

The theoretical frame of the equilibrium theory allows to analyse dispersed fronts in overloaded chromatograms in order to determine adsorption isotherms [4,10]. This method can be used only if the shape of the fronts are predominantly determined by the isotherms. This condition is fulfilled for very efficient columns allowing for fast mass transfer.

If a dispersive front is measured in an efficient column, the knowledge of the course of the retention time  $t_R(c)$  allows to determine the course of the slope of the single solute adsorption isotherm  $dq/dc|_c$  according to Eq. (18). This equation is valid if the desorption front of the peak is dispersed, which is true for Langmuirian systems. In case of anti-Langmuirian systems, the adsorption front is dispersed and the injection time must be neglected in Eq. (18). The more sample is injected the longer is the arc of the isotherm that can be determined from a single chromatogram. The method is frequently called “elution by characteristic point (ECP)”, or “frontal analysis by characteristic point (FACP)” [4].

From a theoretical point of view, the method is accurate and a single elution profile allows the determination of a complete arc of the isotherm. As already mentioned, the applicability is in practise restricted to columns possessing a high plate number. The analysis of dispersive fronts has hitherto been applied only for the determination of single solute isotherms. An extension to mixtures is probably too complicated for practical applications.

An example for the application of the ECP method is given in Fig. 18 [40]. A good indication that the method can be applied is the fact that the dispersed fronts belonging to different samples sizes nearly coincide. The isotherm shown in the lower part of the figure was determined analysing the peak belonging to the largest sample size.

### 3.2.4. Exploiting characteristic peak features

The possibility to exploit certain characteristic features in order to determine adsorption isotherms should be also mentioned. For efficient columns it is possible to extract from the retention times of peak maxima or shock fronts local isotherm slopes or chords. Performing this analysis for several peaks gives access to determine whole isotherms. Such approach was found to be somewhat less sensitive to deviations from the infinite plate number assumption, compared to the ECP method which analyses the whole dispersive front of one chromatogram [41].

### 3.2.5. Peak fitting method (or “inverse method”)

Provided that models for the simulation of the band profiles (for instance the equilibrium dispersion model, Eq. (1))

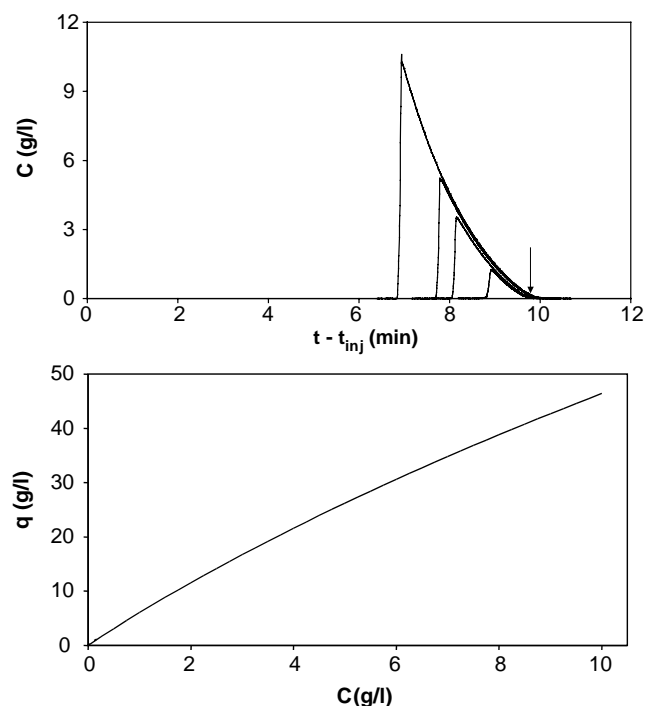


Fig. 18. Top: Experimental elution profiles of anilin on silica from hexane/methyl *tert*-butyl ether (60:40) at 21 °C for four sample sizes. The retention time of an analytical peak is designated on the time axis by an arrow. Bottom: Adsorption isotherm of anilin determined with the ECP method (analysing the largest peak) [40].

and for the description of the isotherms (for instance the Langmuir equation, Eq. (3)) are available, one can optimise the free parameters by minimising the discrepancies between an experimental chromatogram determined under overloaded conditions and the model predictions. This method becomes more and more attractive and feasible because a large number of band profiles could be nowadays generated rapidly within efficient optimisation routines. Examples and contributions to develop this method for single solutes and mixtures are given in [42–47]. An example is shown in Fig. 19 [48].

Usually several isotherm models should be tested alternatively. If the injection can not be introduced as a perfect rectangular profile it can be important to include the real shape in the boundary condition (Eq. (6)). The accuracy of the method depends obviously to a large extend on the accuracy of the applied column model. It is recommended to test critically the validity of this model.

### 3.2.6. Influence of applied porosity $\varepsilon$

This paragraph should point on an important problem connected with the application of the dynamic methods described above. All methods require to specify the porosity  $\varepsilon$  (or the linear velocity  $u$ ). Thus, the determined equilibrium data and finally the free parameters of an adsorption isotherm model will depend on this specification. Usually, the retention time of a non retained component is used to determine  $\varepsilon$  experimentally. If this value does not represent

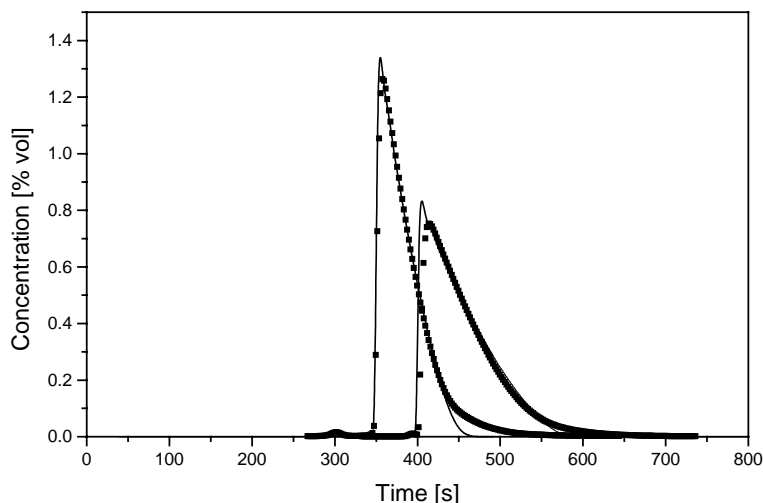


Fig. 19. Results of the peak fitting method. Simulated (lines, equilibrium dispersion model, Eq. (10), using the determined parameters of the Langmuir isotherm model) and measured (symbols) elution profiles for the single solutes cycloheptanone (first peak) and cyclopentanone (silica, *n*-hexane–ethyl acetate (85:15)),  $c_1^{\text{Feed}} = c_2^{\text{Feed}} = 10\%$  vol,  $t^{\text{inj}} = 6$  s,  $\dot{V} = 20$  ml/min [48].

the correct porosity (or phase ratio  $F$ , with  $F = (1 - \varepsilon)/\varepsilon$ ), then also the isotherm can not be a real thermodynamic function characterising the equilibrium of the two phases in the column. However, erroneous isotherms, in combination with the incorrect porosity, are nevertheless often able to describe quite accurate the propagation of concentration fronts in the column. This is due to the fact that in situations close to equilibrium only the product of the phase ratios and the isotherm slopes defines the retention times (see Eqs. (12)–(14), (18) and (20)).

The effect discussed is illustrated in Fig. 20. In the analysis performed at first for five different feed concentrations single component peaks were generated using the equilibrium dispersion model. The same parameters as already applied in Fig. 9 were used, in particular  $\varepsilon = 0.5$  and the Langmuir isotherm parameters  $a = 5$  and  $b = 5$  l/g. The peaks were considered as quasi-experimental chromatograms to be analysed with the ECP method. This analysis was performed with three different porosities:  $\varepsilon = 0.5$  (the assumed “correct” value),  $\varepsilon = 0.4$  and  $\varepsilon = 0.6$ . Specifying that the

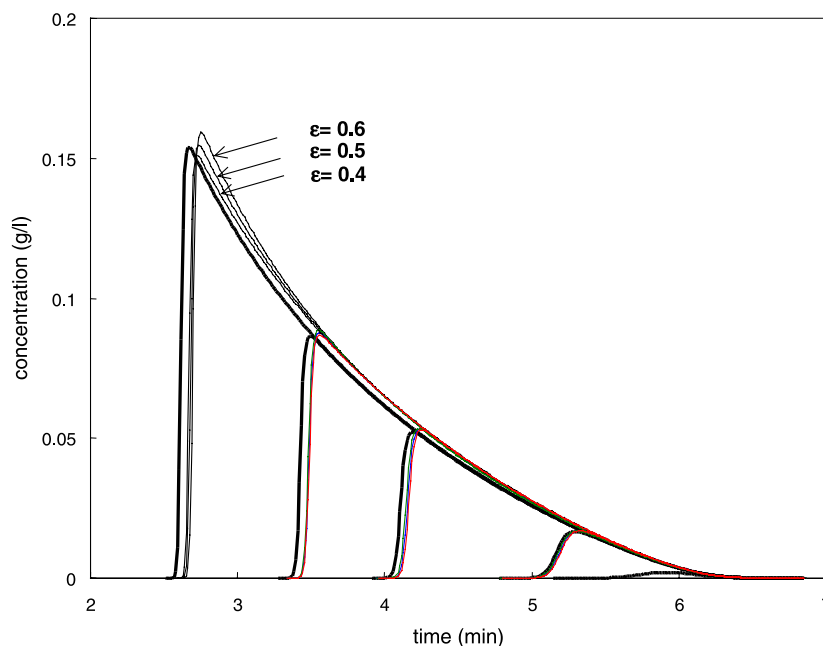


Fig. 20. Elution profiles simulated with the equilibrium dispersion model (Eq. (10)). Parameters for solid lines (as in Fig. 9):  $a = 5$  l/l,  $b = 5$  l/g,  $\varepsilon = 0.5$ ,  $L = 20$  cm,  $V = 2$  ml,  $\dot{V} = 1$  ml/min,  $t^{\text{inj}} = 0.001$  min,  $N_p = 1000c^{\text{inj}} = 1/10/50/100/200$  g/l. Varied parameters for thin lines: (a)  $\varepsilon = 0.5$ ,  $a = 5$  l/l,  $b = 4.730$  l/g (recalculated after analysing solid lines with ECP method), (b)  $\varepsilon = 0.4$ ,  $a = 4.333$  l/l,  $b = 4.442$  l/g, (c)  $\varepsilon = 0.6$ ,  $a = 6.0$  l/l,  $b = 5.057$  l/g.

analytical retention time is in all cases the same (6 min) the following  $a$ -values result: 5.0 (correct), 4.333 and 6.0. Applying the ECP method to the peak for the largest injection concentration allows to determine the following three corresponding  $b$ -values: 4.730 (instead of 5.0), 4.442 and 5.057. Obviously the three isotherm parameter sets are rather different. However, the back calculations of the original peak are quite satisfactory as shown in Fig. 20. This demonstrates that there can be less severe restrictions to specify the correct column porosity than often assumed. Of course, the applied porosity value must be applied consistently in the determination of the isotherms and in subsequent chromatogram simulations.

### 3.2.7. Other aspects

**3.2.7.1. No pure substances available.** A difficult problem, in particular in the field of enantioseparation, is the fact, that often not enough pure substance is available in order to measure the single solute isotherms. Then only measurements with mixtures can be performed. If thermodynamically consistent competitive isotherm models are applied the obtained parameters should give also access to describe the single solute behaviour. Recently successful descriptions of band profiles of mixtures using isotherm data determined from experiments with racemates have been reported [45,49].

**3.2.7.2. Reducing the number of free parameters.** It is always recommended to determine the retention time of a small (analytical) sample size on a non preloaded column. This simple experiment delivers the initial slopes of the single solute isotherms (i.e. the parameters  $a_i$ ) which should be introduced in the analysis of other experiments.

**3.2.7.3. More complex isotherm shapes.** Often the adsorption isotherm have more complex shapes and possess inflection points. Most of the methods can be adapted to handle such situations. A more detailed analysis is outside the scope of this paper. Examples of such cases were studied, e.g. in [22,50].

**3.2.7.4. Gradients.** It should be also mentioned that chromatographic processes are often performed exploiting gradients in the mobile phase composition. To simulate such processes the adsorption isotherms have to be known as a function of the solvent composition. The determination of this dependency increases considerably the experimental efforts. Examples demonstrating the effect of changes in the solvent composition on the courses of the adsorption isotherms are given, e.g. in [50–52].

**3.2.7.5. Repeatability.** Several studies have been performed to check the repeatability and reproducibility of

adsorption isotherms. A detailed and instructive analysis for reversed-phase liquid chromatography using C18 columns was recently published [53].

**3.2.7.6. Scale up.** Due to the rather uniform and small particles sizes of chromatographic media and the achieved experiences in packing columns rather homogeneously the scale up in preparative chromatography is usually quite successful. Thus, if the isotherms are measured properly using a small laboratory column, they can be directly used for scale up calculations as demonstrated in [54].

### 3.2.8. Summary

Dynamic methods usually provide a larger information density. Typically less experiments and less time are required compared to static methods. However, to perform dynamic experiments in a reliable manner more advanced equipment is required. In particular, the accuracy of the flowrate must be assured. As mentioned above there are differences in the amounts of substance needed to perform the experiments. The ECP method requires the smallest amount of substance since usually from one overloaded injection a significant part of a single solute isotherm can be estimated. Quantitative comparisons of isotherms obtained with different methods have been performed several times. A very recent study [55] confirms again that similar results are achievable.

## 4. Modelling of adsorption isotherms

It is not the intention of this paper to review the theoretical concepts developed to describe single solute and competitive adsorption isotherms. The interested reader should consult here in particular classical textbooks and papers (e.g. [4,8,56–60]). Recent developments were described, e.g. in [22,23].

## 5. Conclusions

There are several reliable methods available to measure adsorption isotherms in order to design and optimise preparative liquid chromatographic separations. Correctly applied, all these methods should lead to parameters that could be used to predict the performance of chromatographic columns. Important features of the methods are summarised in Table 1. Obviously, all methods are most reliable for single solutes. The determination of competitive isotherms for mixtures possessing more than two components is possible but difficult. Important aspects to consider for a decision which of the available methods discussed in this paper is most suitable are: (a) the required accuracy, (b) the equipment available, (c) the nature of the separation problem and (d) the costs and availability of the components under consideration.



Table 1  
Features of different methods capable to measure adsorption isotherms (according to [15])

Method and characterization	Special favourable feature	Special unfavourable feature	Applicable for one solute	Applicable for two solutes	Applicable for more than two solutes
Batch (static)		Tedious, not accurate	Yes	Yes	Yes
Adsorption–desorption (static)	Accurate	Tedious	Yes	Yes	Yes
Frontal analysis (dynamic, large samples)	Easy automation		Yes	Yes	Yes
Perturbation (dynamic, small samples)	No detector calibration required	Isotherm model required	Yes	Yes	Difficult
Dispersed front analysis (ECP) (dynamic, intermediate samples)	Low sample amount, small number of experiments	High column efficiency required	Yes	no	No
Chromatogram fitting (dynamic)	Low sample amount, small number of experiments	Models for the isotherms and to simulate the chromatogram required	Yes	Yes	Difficult

## 6. Nomenclature

$a$	parameter of Langmuir equation, adsorption equilibrium constant, Eq. (3)
$A$	column area, $A = (\pi/4)d^2$
$b$	parameter of Langmuir equation, Eq. (3)
$c$	concentration (in mol/l or g/l)
$d$	column diameter
$D_{\text{ap}}$	apparent dispersion coefficient
$L$	column length
$m$	mass
$n$	number of moles
$N$	number of components
$N_{\text{p}}$	number of theoretical plates
$q$	loading of the solid phase (in mol/l or g/l)
$\bar{q}$	loading of the solid phase (in mol/g or g/g)
$t$	time $\dot{V}$
$u$	linear velocity, $u = \dot{V}A\varepsilon$
$V$	column volume, $V = AL$
$V_{\text{ads}}$	volume of adsorbens
$\dot{V}$	volumetric flowrate
$x$	space coordinate
$\varepsilon$	porosity

### Subscripts

ads	adsorbens
R	retention

### Superscripts

eq	equilibrium state
Feed	feed state
Init	initial state
II, III	constant intermediate states

## Acknowledgements

The author is in particular grateful to Drs. Dorota Antos, Oksana Lisec, Clemens Blümel, Christian Heuer and Malte Kaspereit for their contributions to this paper and for

the splendid cooperation. The financial support of Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie, Schering AG (Berlin) and Knauer (Berlin) is also gratefully acknowledged.

## References

- [1] G. Ganetsos, P.E. Barker (Eds.), Preparative and production scale chromatography, Marcel Dekker, New York, 1993.
- [2] C. Heuer, H. Kniep, T. Falk, A. Seidel-Morgenstern, Chem. Eng. Technol. 21 (1998) 469.
- [3] F.G. Helfferich, P.W. Carr, J. Chromatogr. 629 (1993) 97.
- [4] G. Guiochon, S. Golshan-Shirazi, A. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, New York, 1994.
- [5] G. Guiochon, J. Chromatogr. A 965 (2002) 129.
- [6] G. Guiochon, B. Lin, Modelling for Preparative Chromatography, Academic Press, Amsterdam, 2003.
- [7] L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968.
- [8] D.M. Ruthven, Principles of Adsorption and Adsorption Processes, Wiley, New York, 1984.
- [9] C.H. Giles, T.H. Mac Ewan, S.N. Nakhwa, D. Smith, J. Am. Chem. Soc. (1960) 3973.
- [10] J.F.K. Huber, R.G. Gerritse, J. Chromatogr. 58 (1971) 137.
- [11] A.W.J. De Jong, J.C. Kraak, H. Poppe, F. Nooitgedacht, J. Chromatogr. 193 (1980) 181.
- [12] J.M. Jacobson, J. Frenz, Cs. Horváth, Ind. Eng. Chem. Res. 26 (1987) 43.
- [13] J.M. Jacobson, J. Frenz, J. Chromatogr. 499 (1990) 5.
- [14] S. Golshan-Shirazi, S. Godbane, G. Guiochon, Anal. Chem. 60 (1988) 2630.
- [15] R.M. Nicoud, A. Seidel-Morgenstern, Isolat. Purif. 2 (1996) 165.
- [16] D.B. Broughton, C.G. Gerhold, US Patent 2,985,589 (1961).
- [17] D.M. Ruthven, C.B. Ching, Chem. Eng. Sci. 44 (1989) 1011.
- [18] M. Mazzotti, G. Storti, M. Morbidelli, J. Chromatogr. A 769 (1997) 3.
- [19] C. Migliorini, M. Mazzotti, M. Morbidelli, J. Chromatogr. A 827 (1998) 161.
- [20] H. Kniep, G. Mann, C. Vogel, A. Seidel-Morgenstern, Chem. Eng. Technol. 23 (2000) 853.
- [21] M. Kapereit, P. Jandera, M. Škavrada, A. Seidel-Morgenstern, J. Chromatogr. A. 944 (2002) 249.
- [22] K. Mühlbacher, K. Kaczmarek, A. Seidel-Morgenstern, G. Guiochon, J. Chromatogr. A 955 (2002) 35.

- [23] F. Gritti, G. Guiochon, *J. Colloid Interf. Sci.* 264 (2003) 43.
- [24] P.V. Danckwerts, *Chem. Eng. Sci.* 2 (1953) 1.
- [25] H.-K. Rhee, R. Aris, N.R. Amundson, *Proc. Roy. Soc. London, Philos. Trans.* 267 (1970) 419.
- [26] G. Helfferich, G. Klein, *Multicomponent Chromatography*, Marcel Dekker, New York, 1970.
- [27] H.K. Rhee, R. Aris, N.R. Amundson, *First-Order Partial Differential Equations*, Prentice-Hall, Englewood Cliffs, NJ, vol. I, 1986, and vol. II, 1989.
- [28] G. Helfferich, P.W.J. Carr, *J. Chromatogr.* 629 (1993) 95.
- [29] C. Blümel, P. Hugo, A. Seidel-Morgenstern, *J. Chromatogr. A* 865 (1999) 51.
- [30] A. Seidel, E. Tzscheuschler, K.-H. Radeke, D. Gelbin, *Chem. Eng. Sci.* 40 (1985) 215.
- [31] A. Seidel, G. Reschke, S. Friedrich, D. Gelbin, *Adsorpt. Sci. Technol.* 3 (1986) 189.
- [32] R.M. Nicoud. A. Seidel-Morgenstern, in: R.M. Nicoud (Ed.), *Simulated moving beds, basics and application*, Institut National Polytechnique de Lorraine, Nancy, 1993.
- [33] O. Liseac, P. Hugo, A. Seidel-Morgenstern, *J. Chromatogr. A* 908 (2001) 19.
- [34] A. Seidel-Morgenstern, G. Guiochon, *J. Chromatogr.* 631 (1993) 37.
- [35] D. Zhou, X. Liu, K. Kaczmarski, G. Guiochon, *Biotechnol. Progr.* 19 (2003) 945.
- [36] C. Heuer, E. Küsters, T. Plattner, A. Seidel-Morgenstern, *J. Chromatogr. A* 827 (1998) 175.
- [37] C.B. Ching, K.H. Chu, D.M. Ruthven, *AIChE J.* 36 (1990) 275.
- [38] D. Tondeur, H. Kabir, L. A. Luo, J. Granger, *Chem. Eng. Sci.* 51 (1996) 3781.
- [39] P. Forssen, J. Lindholm, T. Fornstedt, *J. Chromatogr. A* 991 (2003) 31.
- [40] C. Heuer, P. Hugo, A. Seidel-Morgenstern, *Sep. Sci. Technol.* 34/2 (1999) 173.
- [41] J.A. Jönsson, P. Lövkvist, *J. Chromatogr.* 408 (1987) 1.
- [42] E.V. Dose, S. Jacobson, G. Guiochon, *Anal. Chem.* 63 (1991) 833.
- [43] F. James, M. Sepulveda, F. Charton, I. Quinones, G. Guiochon, *Chem. Eng. Sci.* 54 (1999) 1677.
- [44] L. Zhang, J. Selker, A. Qu, A. Velayudhan, *J. Chromatogr. A* 934 (2001) 13.
- [45] A. Felinger, A. Cavazzini, G. Guiochon, *J. Chromatogr. A* 986 (2003) 207.
- [46] A. Felinger, D. Zhou, G. Guiochon, *J. Chromatogr. A* 1005 (2003) 35.
- [47] A. Cavazzini, F. Gritti, G. Guiochon, K. Muhlbachler, Determination and modeling of equilibrium isotherm data, Workshop at the 16th International Symposium PREP 2003, San Francisco, 29 June–2 July, 2003.
- [48] D. Antos, A. Seidel-Morgenstern, *Sep. Sci. Technol.* 37 (2002) 1469.
- [49] D. Zhou, D. E. Cherrak, A. Cavazzini, K. Kaczmarski, G. Guiochon, *Chem. Eng. Sci.* (2003) 3257.
- [50] A. Seidel-Morgenstern, G. Guiochon, *Chem. Eng. Sci.* 48 (1993) 2787.
- [51] L. Gueorguieva, D. Beltscheva, D. Antos, A. Seidel-Morgenstern, Adsorption from solvent mixtures with varying composition, in: *Fundamentals of Adsorption*, Japanese Adsorption Society, 1070, 2002.
- [52] F. Gritti, G. Guiochon, *J. Chromatogr. A* 995 (2003) 37.
- [53] F. Gritti, G. Guiochon, *J. Chromatogr. A* 1003 (2003) 43.
- [54] C. Heuer, P. Hugo, G. Mann, A. Seidel-Morgenstern, *J. Chromatogr. A* 752 (1996) 19.
- [55] A. Cavazzini, A. Felinger, G. Guiochon, *J. Chromatogr. A* (2004) in press.
- [56] T.L. Hill, *Introduction to Statistical Thermodynamics*, Addison-Wesley, Reading, 1960.
- [57] A.L. Myers, J.M. Prausnitz, *AIChE J.* 11 (1965) 121.
- [58] C.J. Radke, J.M. Prausnitz, *AIChE J.* 18 (1972) 761.
- [59] A.L. Myers, *AIChE J.* 29 (1983) 691.
- [60] G. Gamba, R. Rota, G. Storti, S. Carra, M. Morbidelli, *AIChE J.* 35 (1989) 959.