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Scale up in preparative chromatography

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Abstract

The subject of the paper is the application of a theoretical concept to scale up preparative chromatography. As an experimental example, the separation of the α- and β-isomers of a steroid compound using silica as the stationary phase is discussed. The applied concept is mainly based on measuring the adsorption isotherms in preliminary investigations using an analytical column. With these thermodynamic parameters it is attempted to simulate the separation behaviour in larger columns using the equilibrium dispersive model. To check the accuracy of the approach, experimental studies have been carried out using three other columns with increasing diameters (0.8, 5 and 30 cm). On the basis of the determined adsorption isotherms and a phase ratio adjustment, the main features of the separation process can be predicted for the larger columns. Thus, the presented concept can be effectively used in the process of optimizing preparative chromatography.

Keywords: Preparative chromatography; Adsorption isotherms; Thermodynamic parameters

1. Introduction

To fulfil the requirements of product purity in the pharmaceutical industry, in biotechnology and in the production of fine chemicals chromatographic methods are increasingly applied. This tendency is favoured by the considerable progress achieved recently in developing selective and stable stationary phases for a large variety of separation problems in an amount sufficient to pack preparative columns. Another important aspect that promotes chromatographic separation methods is the theoretical understanding of the main effects that control the process and related to this, the possibilities available to predict the process. The present state of the art was recently summarized by Guiochon et al. [1]. For different examples it was demonstrated, that the correct numerical solution of the column mass balance equation matches satisfactorily with experimental chromatograms if the required thermodynamic and kinetic data are available. To determine these data, measurements with smaller analytical columns are performed to save material and time. Hitherto, small scale comparisons between experimental and simulated chromatograms were performed to evaluate the model adequacy. The main subject of this paper is to check the possibility of using the parameters obtained from experiments with analytical columns to predict the behaviour of larger columns relevant for industrial separation processes. For this, the column diameter, starting from 0.4 cm,

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was increased in three steps by a factor of 2, 12.5 and 75.

2. Theoretical

In this section the equilibrium dispersive model applied to simulate chromatographic profiles is described. Details concerning the derivation of this model and the solution of the corresponding equations have been reviewed in the monograph by Guiochon et al. [1]. Adsorption isotherms describing the equilibrium distribution between the mobile phase and the stationary phase are the essential data for a simulation of the chromatographic separation process. The theoretical background of the method applied in this work to measure these functions for the pure components will be explained below. Knowing the single solute adsorption equilibrium, multicomponent isotherms can be estimated using thermodynamic concepts.

2.1. Equilibrium dispersive model

Assuming a local equilibrium between the concentrations in the mobile and stationary phases, C and q, and considering a backmixing described by an apparent dispersion coefficient, D_{ap}, the following mass balance equation holds for the column:

$$\frac{\partial C_i}{\partial t} + F\frac{\partial q_i}{\partial t} + u\frac{\partial C_i}{\partial x} = D_{ap}\frac{\partial^2 C_i}{\partial x^2}, \qquad i = 1,N \tag{1}$$

where q and C are related through the adsorption isotherm:

$$q_i = q_i(C_1,C_2,...,C_i,...,C_N), \qquad i = 1,N \tag{2}$$

In Eq. (1), u is the linear velocity of the liquid phase within the column and F is the phase ratio defined by:

$$F = \frac{1-\epsilon_t}{\epsilon_t} = \frac{V_s}{V_{l,t}} = \frac{V - V_{l,t}}{V_{l,t}} \tag{3}$$

This definition is based on the total porosity ϵ_t and considers the pores to be part of the liquid phase. Consequently the volumes V_s and $V_{l,t}$ are the volume of the stationary phase excluding the pores and the volume of the mobile phase including the pores, respectively. The coefficient D_{ap} should describe in Eq. (1) in a simple integral manner all possible kinetic effects causing band broadening. For low values this parameter is closely related to the number of theoretical plates N_p:

$$D_{ap} = \frac{uL}{2N_p} \tag{4}$$

The boundary condition at the column inlet for a rectangular injection profile is:

$$C_i(t,x=0) = \begin{cases} C_{inj,i} & \text{for } t \le t_{inj} \\ 0 & \text{for } t > t_{inj} \end{cases} \qquad i = 1,N \tag{5}$$

The most important information required to represent the chromatographic behaviour is the knowledge of the adsorption isotherm (Eq. (2)).

2.2. Measurement of single solute adsorption isotherms

Several methods are available to determine adsorption isotherms [1,2]. An elegant and simple method to measure the isotherms for pure components is the so-called elution by characteristic point (ECP) suggested by Cremer and Huber [3]. This method evaluates chromatograms recorded after injecting samples of large size on a column filled with the stationary phase under consideration. As a basic requirement for the applicability of the ECP method the column has to be very efficient, i.e. the number of theoretical plates should be high (several thousands [4]). Under these conditions thermodynamics determine the shape of the chromatographic profiles and kinetic effects can be neglected. If a large sample size is injected on the column, usually the front of the obtained chromatogram is sharpened and the tail is dispersed. The concentration–time relation of the dispersed tail is completely defined by the course of the adsorption isotherm according to the following equation:

$$t(C) = \frac{L}{u}\left(1 + F\left.\frac{dq}{dC}\right|_C\right) \tag{6}$$

After rearrangement and integration follows:

$$q(C) = \int_0^C \frac{t(C) - (L/u)}{(L/u)F}dC \tag{7}$$

With Eq. (7) the isotherm can be determined from the dispersed tail of the peak, $t(C)$, approximately up to the maximum concentration of the band profile.

2.3. Modeling of single solute isotherms and prediction of competitive isotherms

Due to its well defined theoretical background and extensive experimental verification, the Langmuir isotherm equation is often used in preparative chromatography:

$$q_i = q_{s_i}\frac{b_i C_i}{1 + b_i C_i}; \qquad i = 1,N \tag{8}$$

However, if it is required to cover a broader concentration range the Langmuir equation was frequently been found to be insufficient. A large number of other isotherm equations with more than two free parameters is available [2]. The simple sum of two Langmuir terms has proven to be a very versatile and flexible equation:

$$q_i = q'_{s_i}\frac{b'_i C_i}{1 + b'_i C_i} + q''_{s_i}\frac{b''_i C_i}{1 + b''_i C_i}; \qquad i = 1,N \tag{9}$$

This so-called bi-Langmuir equation has four parameters and is able to describe the adsorption on a heterogeneous surface that consists of two different types of adsorption sites, I and II. Based on the knowledge of the parameters in Eq. (9) the adsorption isotherm for a component i in a mixture of N components can be calculated with the competitive bi-Langmuir equation:

$$q_i = q'_{s_i}\frac{b'_i C_i}{1 + \sum_{j=1}^{N} b'_j C_j} + q''_{s_i}\frac{b''_i C_i}{1 + \sum_{j=1}^{N} b''_j C_j}; \qquad i = 1,N \tag{10}$$

Eqs. (9,10) are only thermodynamically consistent if the q_{s_i}, q'_{s_i} and the q''_{s_i} are the same for all components i, respectively. Otherwise the ideal adsorbed solution theory (IAS) can be applied [5]. This theory allows the generation of consistent mixture isotherms using only the parameters of the single solute behaviour. The competitive isotherms can be calculated for any type of single solute isotherm equation. For a few special isotherm equations competitive isotherms can be derived explicitly, e.g. [6,7]. Generally applicable are numerical methods solving the system of IAS-equations in an iterative manner. More details concerning the application of the IAS theory are described elsewhere [8].

2.4. Scaling up preparative chromatography

For industrial applications and first approximations the effect of changing the column dimensions can be estimated by considering the ratios of the column cross section areas and column volumes. If one intends to reproduce a chromatogram that has been obtained using an analytical scale column 1 on a larger column 2, both the volumetric flow-rate and the amount injected should be modified according to the following formulas:

$$\dot{V}_2 = \dot{V}_1\frac{A_2}{A_1} = \dot{V}_1\frac{d_2^2}{d_1^2} \tag{11}$$

$$m_{\mathrm{inj}_2} = m_{\mathrm{inj}_1}\frac{V_2}{V_1} = m_{\mathrm{inj}_1}\frac{d_2^2 L_2}{d_1^2 L_1} \tag{12}$$

If length and efficiency (i.e. plate numbers, N_p, or heights equivalent to a theoretical plate, *HETP*, respectively) are the same for different columns, the obtained elution profiles should be identical. However, it is a well-known fact that differences in the packing states as well as in the flow-rates influence column efficiencies as a measure of band broadening. Thus, Eqs. (11,12) can be considered as a rough but useful guide for scaling up.

3. Experimental

In the experimental investigations two isomers of a steroid compound were separated using columns of different size. The problem under investigation represents an intermediate step for the production of an anti breast cancer drug. For the purpose of this study the formula of the compound is not relevant. To produce larger amounts of the α-isomer with a purity greater than 99% an optimization of the chromatographic method is important. The feed for the industrial process contains the α- and β-isomers with about 35% each. There are about 30% of

unspecified other compounds. Besides isolating the α-isomer it is also desirable to recover the β-isomer with high purity to recycle it in the frame of the whole production process. By comparing different stationary phases and eluent compositions the following chromatographic system was found to be suitable for the separation problem under investigation [9]:

Normal-phase silica (Kromasil, EKA Nobel, Bohus, Sweden), pore diameter 100 Å, particle size 10 μm) was used as the stationary phase.The mobile phase was hexane–methyl-*tert.*-butyl ether (MTBE) (70:30) (purity 99%, provided by several suppliers)

Under analytical conditions the separation factor for the separation of the two isomers was 1.5. Pure isomers of the steroid compound were available to measure the adsorption isotherms and to record chromatograms. They were isolated in preliminary runs from the feed mixture using preparative columns of different sizes.

3.1. Equipment

Using the above combination of stationary and mobile phases different chromatographic set-ups have been used in the experiments. Four columns were applied. Two smaller analytical columns (25×0.4 and 25×0.8 cm) were packed by a supplier (Muder and Wochele, Berlin, Germany). Two axial dynamic compression columns (Prochrom, Champigneulles, France) were packed by us (31×5 and 27×30 cm) using a slurry technique. A mixture of toluene and 5% 2-propanol was applied as pushing solvent at a pressure of 60 bar. The compression times were 30 s (31×5 cm) and 120 s (27×30 cm), respectively. The same batch of silica was used to pack all four columns.

The measurements with the two smaller columns were carried out using a high-pressure pump (Knauer, Berlin, Germany, Pump 64) and a continuous-flow diode array detector (LKB, Bromma, Sweden, 2140) with the accompanying data acquisition system (LKB, Wavescan 1.02). The stability of the volumetric flow-rate was measured with a digital flow-meter (Phase Separations, Deeside, UK). The columns were thermostated at 21°C.

The separations on the larger columns were performed using pumps of the following suppliers: 31×5 cm column: Lewa, Bad Leonhard, Germany, maximum flow-rate 16 l/h, maximum back pressure 100 bar; 27×30 cm column: Milton Roy, Rouen, France, maximum flow-rate 400 l/h, maximum back pressure 70 bar. The volumetric flow-rates were controlled manually and with a mass flow meter (Rosemount, Boulder, USA, Micromotion 9729), respectively. The chromatograms have been recorded by a UV detector (Knauer, Berlin, Germany, variable-wavelength monitor) equipped with a super prep cell. All of the experiments on the larger columns were processed at ambient temperature (approximately 21°C).

4. Results and discussion

4.1. Adsorption isotherms

In the first stage of the investigation the detectors were calibrated. At a wavelength of 300 nm, linear response curves for the two isomers were observed with both detectors used. Subsequently the ECP method as described above was used to determine experimentally the single solute adsorption isotherms of both isomers. For this, a series of elution profiles using the smallest column (25×0.4 cm) was recorded. The obtained isotherms were analysed and an appropriate model equation was chosen to fit the data. To avoid time consuming and complicated measurements the competitive isotherms have been predicted using the theoretical concepts presented above.

In Fig. 1 two elution profiles of the pure α-isomer obtained with the 25×0.4 cm column are superimposed. Theoretically, the rear parts of the profiles should coincide for different amounts injected if the column efficiency is large. In fact, there are only small differences noticeable in Fig. 1. This justifies in combination with the relatively large plate number (see below) the application of the ECP method for determining the adsorption isotherm. The arrow further designates the retention time of an analytical size injection. The perturbation caused by small differences between the solvent composition for the sample solution and the mobile phase can be also recognized. A similar presentation is shown in Fig. 2 for the longer retained β-isomer.

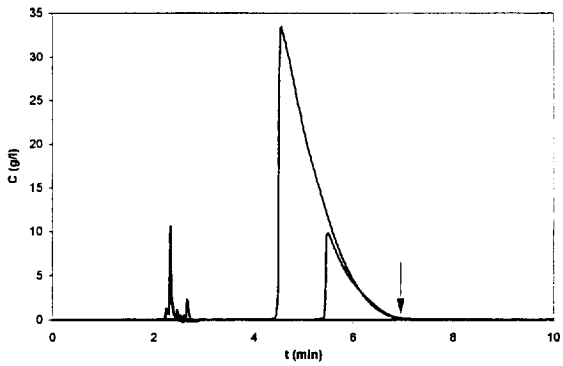

Fig. 1. Experimental elution profiles for two injections of the α-isomer. The retention time of an analytical peak is designated on the time axis. Column, 25×0.4 cm, packed with 10 μm silica, pore size = 100 Å; mobile phase, hexane–MTBE (70:30); flow-rate = 1 ml/min; detection, UV at 300 nm; C_{inj} = 50 and 250 g/l, respectively; volume injected = 0.1 ml.

Using the rear parts of the peaks measured for the larger sample size the adsorption isotherms of both isomers were calculated according to Eq. (7). To model these curves both the Langmuir equation, Eq. (8) and the bi-Langmuir equation, Eq. (9), were used. The parameters of these equations obtained with Marquard's method [10] minimizing the relative standard deviation between the theoretical and experimental loadings σ are presented in Table 1. The theoretical Henry constants $K_{th,ref}$ given in Table 1 for both components correspond to the initial slope of the adsorption isotherm according to Eq. (8) and Eq. (9), respectively:

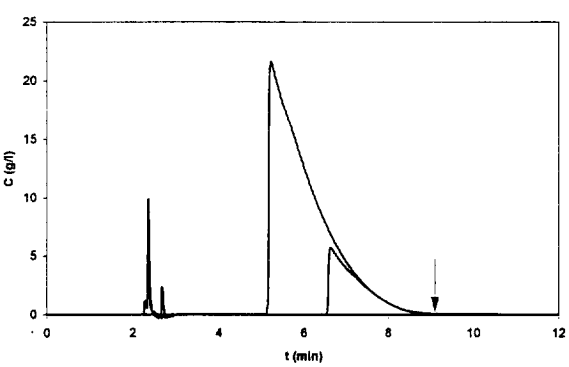

Fig. 2. Experimental elution profiles for two injections of the β-isomer. The retention time of an analytical peak is designated on the time axis. Experimental conditions as in Fig. 1.

Table 1
Adsorption isotherm parameters determined from chromatograms on the reference column (25×0.4 cm)

Parameter	Langmuir equation		bi-Langmuir equation	
	α-isomer	β-isomer	α-isomer	β-isomer
q_s'(g/l)	381:5	308.8	453.0	376.8
b'(l/g)	0.013	0.023	0.010	0.017
q_s''(g/l	–	–	2.470	3.248
b''(l/g)	–	–	0.377	0.492
$K_{th,ref}$	4.96	7.10	5.46	8.00
σ (%)	2.8	3.3	0.25	0.31

$$K_{th,ref_i} = q_{s_i} b_i$$

and

$$K_{th,ref_i} = q'_{s_i} b'_i + q''_{s_i} b''_i \quad (13)$$

The theoretical isotherms are shown in Fig. 3. In this plot the differences between the two isotherm models appear to be relatively small. However, the application of the bi-Langmuir equation led to significantly lower standard deviations (Table 1).

The capability of simulating elution profiles is a severe test of the quality of an isotherm model. The accuracy of the isotherm must be sufficient to allow a reliable prediction of the first derivative of this function [1]. In Fig. 4 predictions of the equilibrium dispersive model are compared to an experimental profile obtained for the α-isomer. Obviously, the results using the Langmuir equation (Eq. (8)) are

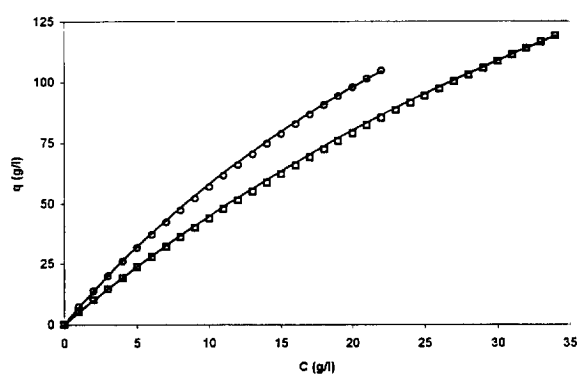

Fig. 3. Adsorption isotherms of the α-isomer (□) and β-isomer (○) on silica from hexane–MTBE (70:30) at 21°C according to the Langmuir (solid line) and the bi-Langmuir (symbols at equidistant concentrations) equation, respectively.

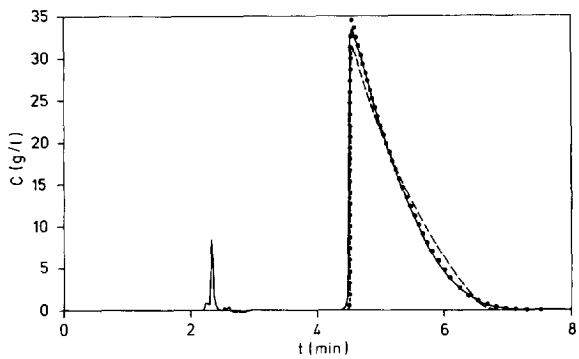

Fig. 4. Comparison between experimental (solid) and calculated chromatograms using the Langmuir (dashed) and the bi-Langmuir (dotted) equation for an overloaded sample of the α-isomer. Experimental conditions as in Fig. 1, except $C_{inj}=250$ g/l.

worse than the results with the bi-Langmuir equation (Eq. (9)). The improvement of using the isotherm Eq. (9) is noticeable especially in the regions of lower concentrations. The general agreement between simulations using the bi-Langmuir equation and measurements shown in Fig. 4 could be confirmed for other sample sizes and for the β-isomer.

4.2. Measurements in the analytical range and phase ratio adjustment

The dynamic behaviour of each column has been evaluated by analysing chromatograms in the analytical range generated by injections of small sample sizes. The size of these samples has been chosen approximately in accordance with Eq. (12). Further, the retention time of a non-retained component, t_0, was measured using a small sample of hexane, which is one of the components of the mobile phase. The basic chromatographic data that characterize the chromatographic system investigated and the columns applied are summarized in Table 2.

The volumetric flow-rates $\dot{V}$ for the three larger columns given in Table 2 are based on the value for the smallest column. Slightly different $\dot{V}$ were actually used than predicted by Eq. (11) due to imprecisions of the pumps. The numbers of theoretical plates N_p were extracted from the retention times and peak widths at half height of the α-isomer. The values of the β-isomer were found to be quite similar.

To simulate chromatograms with Eq. (1) the values for D_{ap}, u and F have to be determined in addition to the adsorption isotherm. D_{ap} can be calculated from Eq. (4) if the number of theoretical plates and the linear velocity, u, are known. This velocity is given by:

$$u = \frac{\dot{V}(F+1)}{A} \tag{14}$$

Consequently, all necessary data can be calculated from the column geometry and the flow-rate, if the phase ratio F is known. This parameter is very important for quantitative predictions in chromatography and many authors have studied it theoretically and experimentally [11–13]. The most convenient and frequently applied way to determine F is to measure the retention time of a non-retained component, t_0 . From this time F can be calculated according to the following equation:

$$F_{ex} = \frac{V - t_0\dot{V}}{t_0\dot{V}} \tag{15}$$

Alternatively, the possibility exists to determine the phase ratio from the retention time of an analytical

Table 2
Summary of relevant parameters for the four columns

Parameter	Column I	Column II	Column III	Column IV
Column length (cm)	25	25	31	27
Column diameter (cm)	0.4	0.8	5	30
N_p	4700	6500	2700	2000
$\dot{V}$ (ml/min)	1	4	169	5667
t_0 (min)	2.34	2.44	2.80	2.51
$t_{R,\alpha}$ (min)	6.94	7.87	9.02	5.78
$t_{R,\beta}$ (min)	9.21	10.49	12.27	6.94

Particle size: 10 μm, pore diameter: 100 Å.

injection for a retained component, t_R. In this case the adsorption equilibrium or Henry constant, K, has to be known. This parameter can be measured by any independent method [1,2]. A suitable way is to use the value obtained from experiments with a reference column, $K_{th,ref}$. The subsequent calculation of the phase ratio for another column, F_{th}, is based on the usual mass balance:

$$t_R = \frac{V_{l,t}}{\dot{V}}(1 + F_{th}K_{th,ref}) = \frac{V}{\dot{V}(1 + F_{th})}(1 + F_{th}K_{th,ref}) \quad (16)$$

This leads to the following equation to calculate F_{th} from t_R and $K_{th,ref}$:

$$F_{th} = \frac{V - t_R\dot{V}}{t_R\dot{V} - K_{th,ref}V} \quad (17)$$

Table 3 presents for each of the investigated columns the F_{ex} obtained using Eq. (15) and the F_{th} calculated from retention times of the α- and β-isomers using Eq. (17).

In principal, the obtained phase ratios F_{ex} given in Table 3 are very similar for the different columns. This generates the impression that despite the differences in column size and packing technique the quality of the beds does not differ. Corresponding to the theory F_{ex} and F_{th} are nearly the same for the reference column I. The small discrepancies are mainly caused by inaccuracies of the theoretical isotherms in the low concentration range (i.e. of $K_{th,ref}$). Much more pronounced are the differences between the F_{ex}- and the F_{th}-values for the larger columns. Considering the similarity of the phase ratios F_{ex}, these differences in F_{th} are astonishing. Since all columns are packed with the same stationary phase and possess (according to the F_{ex}-values) similar packing densities, also the phase ratios F_{th} should be theoretically similar for all columns. However, from the results can be concluded that the quality of the packings is not as equal as suggested by the similarity of the phase ratios F_{ex} determined as described. In a recent NMR imaging study it was proven that chromatographic columns are usually not homogeneously packed [14].

Table 3
Phase ratios based on the retention times of a non-retained component (F_{ex}, Eq. (15)) and retained components (F_{th}, Eq. (17))

Phase ratio	Column I	Column II	Column III	Column IV
F_{ex}	0.34	0.29	0.29	0.34
$F_{th,\alpha}$	0.36	0.49	0.49	0.19
$F_{th,\beta}$	0.39	0.51	0.53	0.18

For simulating overloaded chromatograms on columns of different size, the isotherm as a universal thermodynamic function should be applied in all simulations identically. The phase ratio F_{th} determined from the retention time of an analytical peak on the column of interest and the Henry-constant of the reference column (Eq. (17)) might be able to position predicted chromatograms properly on the time axis. This concept appears to be capable to balance the observed differences in the packings. Also in an industrial scale it is possible to determine the required analytical retention times. Below, results of simulating different chromatograms for the larger columns using both F_{ex} and F_{th} will be reported. In all studies performed the columns have been overloaded corresponding to a loading factor of about 0.15. Thus about 15% of the available adsorption capacity of the column was injected [1].

4.3. Comparison between measured and simulated elution profiles on the 25×0.4 cm column

First, a few chromatograms were measured on the smallest column to verify the applicability of the equilibrium dispersive model and the accuracy of the determined adsorption isotherms. Defined synthetic mixtures prepared from both pure isomers were injected. For different flow-rates and feed compositions simulations have been compared with experimental profiles.

Initially two competitive isotherm models were used. Fig. 5 gives a comparison of the simulations with Eq. (10) and with the IAS theory, based on parameters of the bi-Langmuir model for the single solute isotherms. Especially in the region where both components are not resolved the IAS theory delivers results that are in better agreement with the experimental chromatograms. The competitive bi-Langmuir equation underestimates the degree of competition between both isomers. Similar differ-

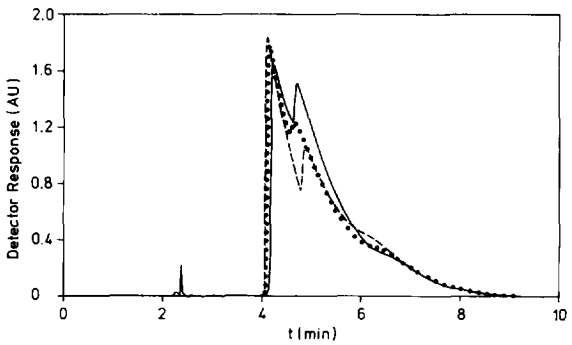

Fig. 5. Experimental (solid) and calculated chromatograms for a synthetic 1:1 mixture of the α- and β-isomers. The dotted line was calculated using the competitive isotherms predicted by the IAS theory with the bi-Langmuir model. The dashed line was calculated using the competitive bi-Langmuir model, Eq. (10). Experimental conditions as in Fig. 1, except C_{inj}(total) = 500 g/l.

ences have been observed for other conditions as well. Consequently, only the IAS theory was used in further simulations.

The results shown in Fig. 6 were obtained simulating the separation for an injection where the α-isomer was three times more concentrated than the β-isomer. The good agreement between theory and experiment shows that the applied model is applicable to simulate chromatograms for various operating conditions.

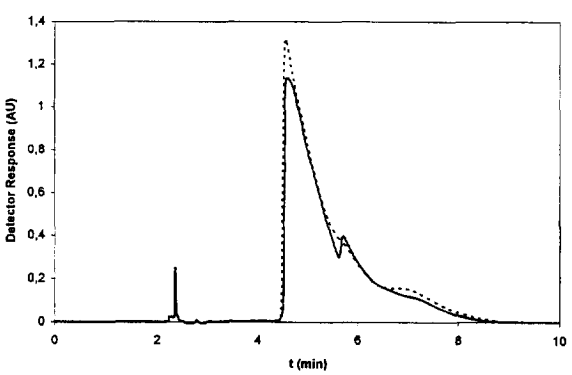

Fig. 6. Experimental (solid) and calculated (dotted) chromatograms for a synthetic 3:1 mixture of the α- and β-isomers. The prediction was calculated using the IAS theory with the bi-Langmuir model. Experimental conditions as in Fig. 5, except feed composition and C_{inj}(total) = 300 g/l.

4.4. Predictions for the 25×0.8 cm column

Based on the thermodynamic parameters obtained from the measurements using the smallest column, chromatographic profiles for the three larger columns were predicted. To check the quality of these predictions several chromatograms were recorded using these columns.

Fig. 7 presents an experimental chromatogram for the 25×0.8 cm column in comparison to simulations using (a) the phase ratio calculated from the retention time of a non-retained component (F_{ex} = 0.29, Eq. (15)) and (b) the adjusted phase ratio based on the retention time of the retained components (F_{th} = 0.50, Eq. (17)). The flow-rate and the amount injected have been determined from the data given in Fig. 4 using the scale up formulas, Eqs. (11,12). Whereas F_{ex} delivers theoretical profiles that elute too early, a good agreement between experimental and simulated chromatograms is observed if the larger adjusted phase ratio F_{th} is used in combination with the parameters of the bi-Langmuir equation given in Table 1. The agreement shown in Fig. 7 could be confirmed for several other conditions. Another example is given in Fig. 8 for the separation of a 1:1 mixture of both isomers using a smaller flow-rate. Based on the results for the 25×0.8 cm column, the same methodology, based on the calculation of the

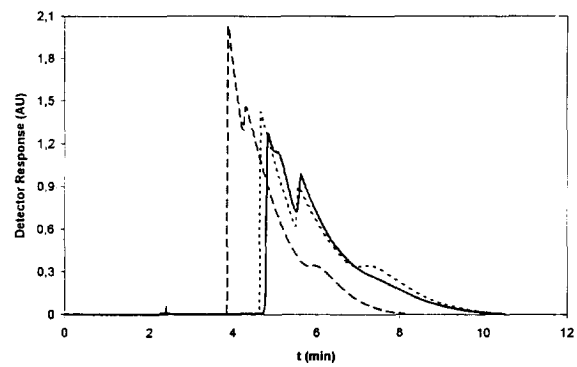

Fig. 7. Experimental (solid) chromatogram for a synthetic 1:1 mixture of the α- and β-isomers on the 25×0.8 cm column. Stationary and mobile phase as in Fig. 1. flow-rate = 4 ml/min; detection, UV at 300 nm; C_{inj}(total) = 400 g/l; volume injected, 0.5 ml. Dashed line based on F_{ex} Eq. (15), dotted line based on F_{th} Eq. (17).

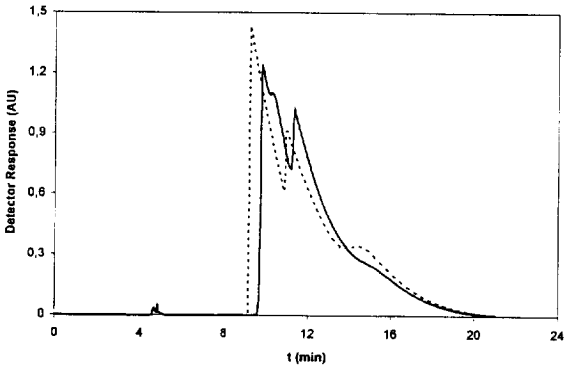

Fig. 8. Experimental (solid) and calculated (dotted) chromatograms for a synthetic 1:1 mixture of the α- and β-isomers. Experimental conditions as in Fig. 7, except flow-rate = 2 ml/min.

phase ratio according to Eq. (17), was applied for the analysis of chromatograms on the larger industrial scale columns.

4.5. Predictions for the 31×5 cm column

In Fig. 9 an experimental and a simulated chromatogram are compared for the 31×5 cm column. The feed was again a 1:1 mixture prepared from the pure isomers. The flow-rate and the injected amount have been scaled up using Eqs. (11,12) from the corresponding values of the smallest column. The agreement between theory and experiment is of the same quality as for the 25×0.8 cm column (Figs. 7, 8). The applied phase ratio based on the retention times of the α- and β-isomers was according to Eq. (17) $F_{th} = 0.51$. With this value again significantly better results have been achieved than with $F_{ex} = 0.29$.

Hitherto only mixtures prepared from the pure products have been injected. In further experiments the applicability of the model was tested for injections with the industrial crude product. This feed contained, besides the α- and β-isomer of the steroid compound (each approximately 35%), several other unspecified components eluting between and after the isomers of interest. Although these additional components are neglected in the calculations, Fig. 10 demonstrates that the main features of the separation process are represented by the model.

4.6. Predictions for the 27×30 cm column

A comparison between a predicted and an experimental chromatogram for the largest industrial scale column is given in Fig. 11. A synthetic 1:1 mixture of the pure isomers was injected. The position on the time axis and the general shape of the experimental elution profile is also predicted for the other columns. A phase ratio $F_{th} = 0.185$ according to Eq. (17) was used in the calculation (for comparison F_{ex} was 0.34).

Finally, Fig. 12 compares the experimental chromatograms of the four columns for operating con-

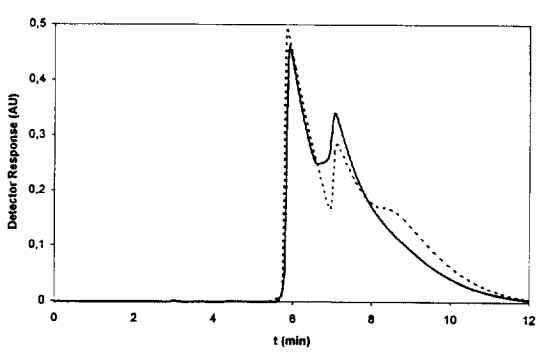

Fig. 9. Experimental (solid) and calculated (dotted) chromatograms for a synthetic 1:1 mixture of the α- and β-isomers on the 31×5 cm column. Flow-rate = 169 ml/min; detection, UV at 300 nm; C_{inj}(total) = 400 g/l; volume injected = 20 ml.

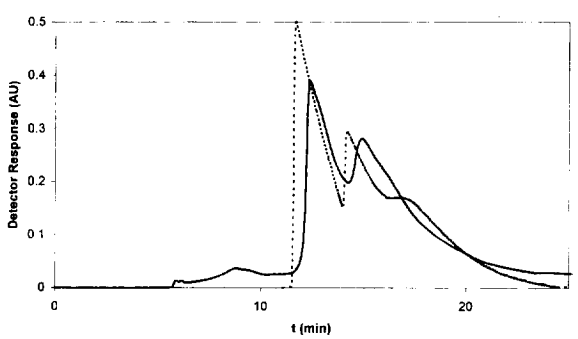

Fig. 10. Experimental (solid) chromatogram of the crude product containing the α- and β-isomers (1:1) and 30% impurities (31×5 cm column). The simulation (dotted) was calculated neglecting the impurities. Experimental conditions as in Fig. 9, except flow-rate = 84 ml/min and C_{inj}(total) = 520 g/l.

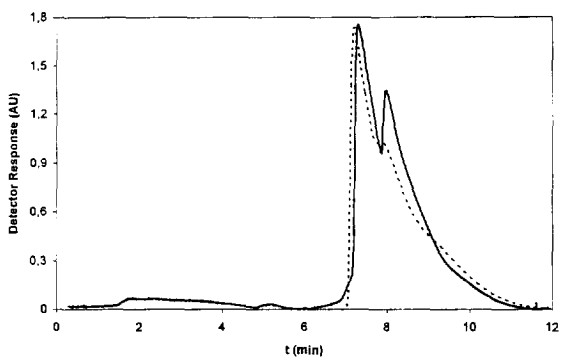

Fig. 11. Experimental (solid) and calculated (dotted) chromatograms for a synthetic 1:1 mixture of the α- and β-isomers on the 27×30 cm column. Stationary and mobile phase as in Fig. 1. flow-rate = 3667 ml/min; detection, UV at 300 nm; C_{inj}(total) = 152.5 g/l; volume injected = 1000 ml.

ditions similar according to the scale up formulas. The peak area of the elution profile for the 31×5 cm column is considerably smaller than the other ones due to the fact that the amount injected was calculated for an estimated column length of 25 cm. After subsequent opening of the column the real bed length was found to be 31 cm.

The fact, that the chromatograms have different retention with respect to the normalized time scale used in Fig. 12, t/t_0, again demonstrates that a simple consideration of t_0 is not sufficient to compensate the differences in the separation behaviour of the columns. Consequently the attempt to use F_{ex} in scaling up has proven to be less successful compared to the suggested application of a phase ratio F_{th} based on the retention times of the components to be separated.

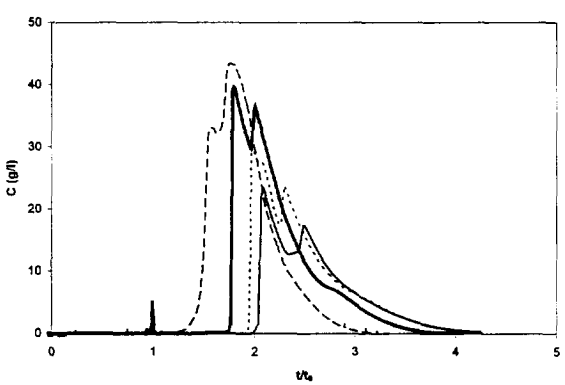

Fig. 12. Experimental normalized chromatograms for a synthetic 1:1 mixture of the α- and β-isomers obtained under comparable conditions on different columns: 25×0.4 cm (solid fat), 25×0.8 cm (dotted), 31×5 cm (solid thin) and 27×30 cm (dashed). Experimental conditions as in Fig. 5, except parameters depending on increasing column size: flow-rate = 1, 4, 169 and 5667 ml/min; C_{inj} = 500, 400, 400 and 305 g/l; volume injected = 0.1, 0.5, 20 and 1000 ml, respectively.

5. Conclusions

The chromatographic separation of two isomers of a steroid compound was compared on columns of four different sizes. On a 25×0.4 cm column the adsorption isotherms of both isomers were determined experimentally. Based on this information and on a simple phase ratio adjustment analysing the retention times of analytical peaks, chromatograms for larger columns could be qualitatively predicted. Despite inaccuracies the results demonstrate the applicability of the equilibrium dispersive model and the developed methodology as a tool to design and optimize preparative chromatography. Further progress requires mainly a more detailed incorporation of the heterogeneity of chromatographic beds into modeling.

6. List of symbols

A	Column cross section area, $A = (\pi/4)d^2$
b	Parameter in isotherm equations, Eqs. (8–10)
C	Liquid phase concentration
C_{inj}	Concentration in the feed
F	Dimensionless phase ratio, based on the solid-phase concentration without pores and the liquid phase concentration with pores, Eq. (3)
F_{ex}	phase ratio as F, based on the determination of the retention time of a non-retained component, Eq. (15)
F_{th}	phase ratio as F, based on the determination of the retention time of a retained component and the Henry constant, Eq. (17)
d	Column diameter
D_{ap}	Apparent dispersion coefficient
K	Henry constant
L	Column length

m_{inj} Mass injected
N Number of components
N_p Number of theoretical plates
q Stationary phase concentration
q_s Saturation capacity
t Time
t_{inj} Time of injection
t_R Retention time
t_0 Retention time of a non-retained component, $t_0 = V_{lt}/\dot{V}$
u Linear velocity
V Column volume, $V = LA$
$\dot{V}$ Volumetric flow-rate
V_s Volume of the solid phase in the column excluding the pores
$V_{l,t}$ Total volume of the liquid phase in the column including the pores of the solid
x Axial coordinate of column

5.2. Subscripts

ex Value determined experimentally
i,j Component
ref Value determined for the reference column
th Value used in simulation
α Value determined for the α-isomer
β Value determined for the β-isomer

5.3. Greek

ϵ_t Total porosity, $\epsilon_t = V_{l,t}/V$
σ Standard deviation,

$$\sigma = 100\% \sqrt{\frac{1}{N_D - P} \sum_{i=1}^{N_D} \left(\frac{q_{i,ex} - q_{i,th}}{q_{i,ex}}\right)^2}$$

with N_D and P being the numbers of data and model parameters, respectively.

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