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# Determination of mobile phase effect on single-component adsorption isotherm by use of numerical estimation

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## Abstract

Numerical estimation was used to determine adsorption isotherm parameters of a single-component in a normal-phase system. The distribution isotherm of methyl deoxycholate was described between the mobile phase containing hexane, ethyl acetate, methanol with varied concentration and a silica gel adsorbent. The effect of the mobile phase composition on the isotherm parameters and the band profiles was investigated. The results obtained were used to simulate the overload gradient elution. The validity of the method proposed was verified by comparison of the computer simulations with the experimental band profiles. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Adsorption isotherms; Mobile phase composition; Band profiles; Overload gradient elution; Preparative chromatography

## 1. Introduction

Liquid chromatography is often performed with a multicomponent mixture of solvents as the mobile phase. The strongly adsorbed additives, or modifiers, enable suitable resolution between components in a reasonable time. The additives modulate the retention factors of the sample component in different ways. They interact with the stationary phase by bonding with active sites on adsorbent surface [1]. In this way the modifier competes with solutes and the concentration of each component in the adsorbed phase at equilibrium is a function of the local concentration of all the sample components and the modifier [2,3]. In some modes of chromatography, the modifier is assumed not to be adsorbed by the

stationary phase; it participates in the retention mechanism as a result of its influence on the chromatography distribution coefficient (capacity factor) of the components. The effect of adsorption of the modifier is usually neglected in reversed-phase chromatography if the columns are practically saturated by modifier at the initial concentration [4].

The composition of the mobile phase is used to control the chromatographic separation. The effect of the mobile phase composition on the isotherm should be quantitatively described. For this purpose, it is necessary to determine the isotherm parameters at different mobile phase compositions and then suitable equations for the dependencies of the isotherm parameters on the mobile phase composition. This allows numerical reproduction of the influence of modifier concentration on the retention and the shape of band profiles of the components separated. Moreover, it is useful for optimisation of the mobile phase

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composition to achieve maximum productivity from the separation process under overloaded isocratic [5] and gradient elution conditions [6–8].

The literature recommends the frontal analysis (FA) method [9] to determine adsorption isotherms. However, this method, especially for preparative chromatographic columns, requires a large amount of pure compounds. Other methods, the elution by characteristic points (ECP) method, the impulse method and the retention time method [9] require high-performance columns in which axial dispersion is negligible.

The alternative approach is the determination of isotherm parameters from chromatographic shapes. In this method, the initial isotherm parameters are used to calculate the band profiles and then are refined by minimisation of a sum of the squared differences between the experimental and theoretical concentration profiles.

This method was applied by Dose et al. [10] to the determination of the isotherm of *N*-benzoyl-L- and -D-alanine on immobilised bovine serum albumin. The implemented algorithm was a simplex optimisation one that worked successfully for a single component but failed for a two-component mixture. Dose estimated model parameters on the basis of all the experimental band profiles obtained. In Ref. [11], this estimation method was used for the determination of competitive isotherm parameters of the two main components of the post reaction mixture: benzophenone and diolone acetate. The numerical method applied was similar to that in Ref. [10], but for optimisation the Marquardt algorithm was implemented. The estimation of model parameters was performed for only one selected band profile. The good agreement between the experimental and simulated band profiles was obtained for different experimental conditions. Another approach, the steepest descend method algorithm has been proposed in Ref. [12] for the determination of the binary competitive isotherm from the individual chromatographic band profiles. The algorithm applied was based on the solution of the equilibrium model for ideal chromatography. The authors proved that if the isotherm model is satisfactory, the value of the isotherm coefficients derived from minimisation of a sum of the squared differences between experimental and theoretical data are in excellent agreement with those obtained from ECP or FA methods.

In this work, numerical estimation based on the Marquardt algorithm was used for determining isotherm parameters of methyl deoxycholate (derivative of deoxycholic acid — bile acid from ox bile) at various mobile phase compositions in a chromatographic system with finite efficiency. The simple (however, very common in liquid chromatography especially in normal-phase systems), Langmuir isotherm model was applied. For each mobile phase composition, the one overload chromatogram was selected for estimation procedure and the validity of the parameters obtained was confirmed by comparison of the simulation of the band profiles with experimental data under different feed conditions.

On the basis of the results obtained, the relationship between isotherm parameters and the modifier concentration was proposed. These dependencies were examined for overload gradient elution at different gradient programs.

## 2. Theoretical

### 2.1. Column model

The differential mass transport balance for each component is written as:

$$\frac{\partial C_i}{\partial t} + F \frac{\partial q_i}{\partial t} + w \frac{\partial C_i}{\partial x} = D_a \frac{\partial^2 C_i}{\partial x^2} \quad (1)$$

where:  $F = (1 - \varepsilon_i)/\varepsilon_i$  — phase ratio;  $w = u/\varepsilon_i$  — interstitial mobile phase velocity;  $D_a$  — the apparent dispersion coefficient;  $t$  and  $x$  are time and axial positions in the column, respectively;  $C$ ;  $q$  — the local concentrations in the mobile and stationary phases.

The initial conditions corresponding to a column saturated by modifier are given by:

$$C(t = 0, x) = 0 \quad (2)$$

for the sample component, and:

$$C_{\text{mod}}(t = 0, x) = C_{\text{mod}}^0 \quad (2a)$$

for the modifier.

The Danckwerts-type boundary conditions were used:

$$uC_{fi} - uC_i = D_a \left. \frac{\partial C_i}{\partial x} \right|_{x=0} - \left. \frac{\partial C_i}{\partial x} \right|_{x=L} = 0 \quad (3)$$

where:

$$C_{fi}(t) = \begin{cases} C_{fi} & \text{for } t \in [0, t_p] \\ 0 & \text{for } t > t_p \end{cases} \quad (3a)$$

for the sample component, and:

$$C_{fmod} = \begin{cases} C_{fmod} & \text{for } t \in [0, t_p] \\ C_{fmod} + \beta(t - t_p) & \text{for } t > t_p \end{cases} \quad (3b)$$

for the modifier in gradient elution.

The set of Eqs. (1)–(3) was coupled with the Langmuir isotherm:

$$q_i^* = \frac{aC_i}{1 + bC_i} \quad (4)$$

## 2.2. The estimation of isotherm parameters

To solve the set of the Eqs. (1)–(4) the orthogonal collocation method on finite elements was used. The orthogonal collocation on finite elements (OCFE) method applied here is the same as described in Refs. [13–15]. The set of ordinal differential equations obtained after discretization due to OCFE was solved with the Adams–Moulton method implemented in VODE procedure [16] using the relative and absolute error equal  $10^{-6}$ . The VODE procedure automatically chooses the appropriate time increment to fulfil assumed error conditions. In each calculation the number of internal collocation points in subdomains (elements) was equal to 3. The number of subdomains was chosen in such a way as to have no visible oscillation in band profile simulation and was equal to 50 for calculations of chromatographic band profiles in isocratic and equal to 70 in gradient elution.

The estimation of the parameter  $\mathbf{p}$  value was performed through minimisation of a sum  $S$  of the squared differences between the experimental and the theoretical data:

$$S = \sum_{i=1}^n (C_{\text{exp},i} - C_{t,i}(\mathbf{p}))^2 \quad (5)$$

where:  $\mathbf{p}$  is the vector of model parameters;  $C_{\text{exp},i}$  are elements of the vector  $\mathbf{C}_{\text{exp}}$  containing the given experimental concentrations ( $n$  data points) and  $C_{t,i}$  are the corresponding theoretical values calculated by the model being studied.

The minimisation was performed with use of the

least-squares Marquardt method modified by Fletcher [17].

## 3. Experimental

### 3.1. Column and chemicals

A 25-cm long, 10-mm I.D. column packed with Lichrosphere 12  $\mu\text{m}$  (Merck, Darmstadt, Germany) was used. The column was operated with a mobile phase flow-rate of 5  $\text{cm}^3/\text{min}$ . Methyl deoxycholate with purity  $>99\%$  mass was isolated in the laboratory from a bile acids mixture of ox bile. This compound is substratum for progesterone hormones synthesis. The structure of analyte studied is given in Fig. 1.

The hexane, ethyl acetate and methanol were from Merck and were used as received; no attention was paid to their water content.

### 3.2. Instrumentation and methods

Experiments involving overloading elution in either the gradient or the isocratic modes were performed on a NovaPrep 200<sup>®</sup>-Preparative Scale High Performance Liquid Chromatography System (Merck, Darmstadt, Germany) with RI detector and a data station. The injector was a Rheodyne sampling valve with a 2.6  $\text{cm}^3$  loop. The solute was dissolved in the mobile phase. The mobile phase was hexane

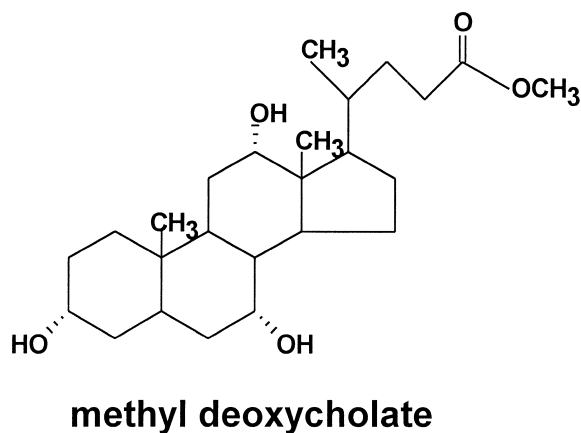


Fig. 1. Structure of methyl deoxycholate.

as a weak solvent 3.06 mol/dm<sup>3</sup> (40% v/v), ethyl acetate 6.14 mol/dm<sup>3</sup> (60% v/v) and methanol.

For isocratic elution, the methanol concentration was changed with successive 0.247 mol/dm<sup>3</sup> (1% v/v) upward steps going from 0.247 to 1.726 mol/dm<sup>3</sup> (1% to 7% v/v).

In all the gradient programs, the concentration of methanol was linearly changed from 0.493 to 1.726 mol/dm<sup>3</sup> (2% to 7% v/v). The solvent delivery system was programmed to deliver, at a set time interval, the concentration gradient of methanol.

Because of hold-up volumes in the line between the solvent delivery system and the column, the concentration gradient did not begin at the set time. The hold-up volumes of the delivery system were measured by injecting small amounts of hexane and connecting the injector directly to the detector, using the connection capillaries. A value of 25.06 cm<sup>3</sup> was obtained, including the extra column volume. In order to determine the extra column volume, the sample of hexane was injected through sampling valve and the volume 2.075 cm<sup>3</sup> was found. The experimental data were corrected by subtracting these values. The total column porosity determined by hexane injections and corrected for the extra column volume was  $\varepsilon_t = 0.77$ .

The number of theoretical plates was determined for concentrations 1.233–1.726 mol/dm<sup>3</sup> of methanol from the half peak-width recorded in linear range of the apparent isotherm. The approximate value of 1000 theoretical plates was assumed. All the chromatograms recorded were transferred to the PC computer and converted into concentration profiles.

## 4. Results and discussion

### 4.1. Band profile in the isocratic elution mode

The modelling of the isotherm data is more complex when the mobile phase is a mixture of various solvents. It is necessary to choose which of the additives should be taken into account in the equilibrium isotherm and which ones can be neglected. If the components of the mobile phase are adsorbed, major distortions of band profiles can occur. However, when the initial slope of the ad-

sorption isotherm of an additive in the pure weak solvent is less than one-fifth that of the solute, the contribution of this additive is limited to its influence on the coefficient of the apparent isotherm [4,18,19]. Furthermore, the perturbation of equilibrium of the additive by solute can be neglected if the concentration of the additive is much higher than the maximum concentration of solute.

The mobile phase used in this study contained 6.1 mol/dm<sup>3</sup> ethyl acetate and methanol with a concentration in the range 0.247–1.726 mol/dm<sup>3</sup>. The maximum of the sample concentration was much lower and equal to 0.0615 mol/dm<sup>3</sup> for the lowest content of methanol (0.247 mol/dm<sup>3</sup>) and 0.0861 mol/dm<sup>3</sup> for the remaining compositions. Moreover, during the chromatography process the sample was diluted, thus, compared to the minimum of the methanol concentration, its concentration could be assumed as negligible.

#### 4.1.1. Parameter estimations

The band profiles recorded in isocratic elution after equilibrating the adsorbent with the mobile phase were used as the basis of the estimation procedure. The estimated parameters were coefficients  $a$  and  $b$  of the Langmuir isotherm. The coefficient  $a$  influences the retention time of a peak, the coefficient  $b$  determines overloading conditions. The initial, coarse values of  $a$  for estimation were chosen on the basis of time at which the elution of bands ends:

$$t_e = t_p + t_0 \cdot (1 + k'_0) \quad (6)$$

where:  $t_p$  — width of a rectangular injection pulse;  $t_0$  — hold-up time of the column;  $k'_0 = F \cdot a$  retention factor at infinite dilution.

The estimation was performed for the selected concentration of sample. Note that only one set of chromatographic band profiles is required for the determination of the isotherm parameters provided the solvent-pumping speed is accurate. In order to confirm the validity of the isotherm equation assumed and values of coefficients obtained, the computer simulations for different sample concentrations at the same mobile phase composition were performed and compared with experimental band profiles. The exemplary results were shown in Figs. 2 and 3. The estimation was performed at the feed

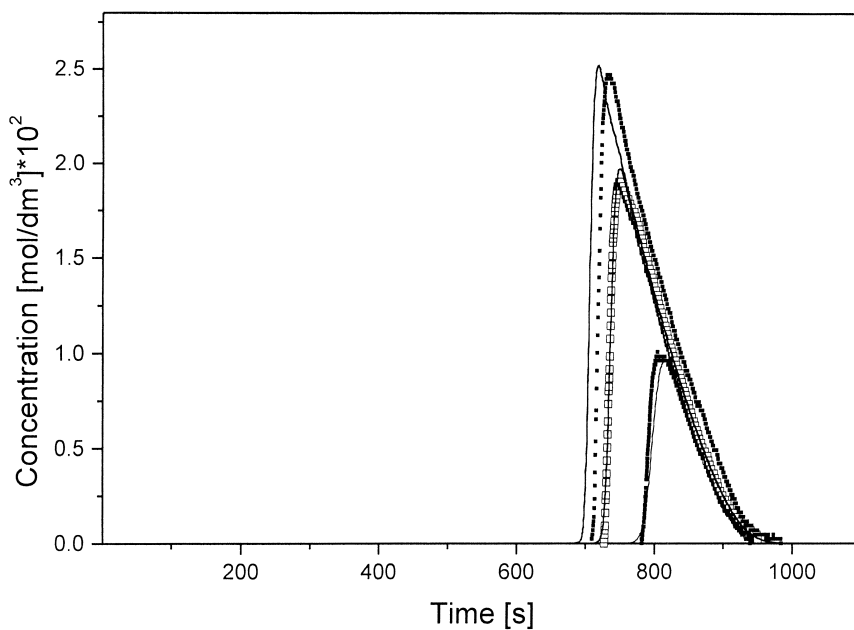


Fig. 2. Comparison of the computer simulations with experimental band profiles for the mobile phase containing  $0.740 \text{ mol/dm}^3$  methanol. The sample concentrations:  $0.0246$ ;  $0.0615$  (estimated);  $0.0861 \text{ mol/dm}^3$ . The square symbols are the experimental points; open squares are the experimental basis of the estimation; solid lines — the model predictions.

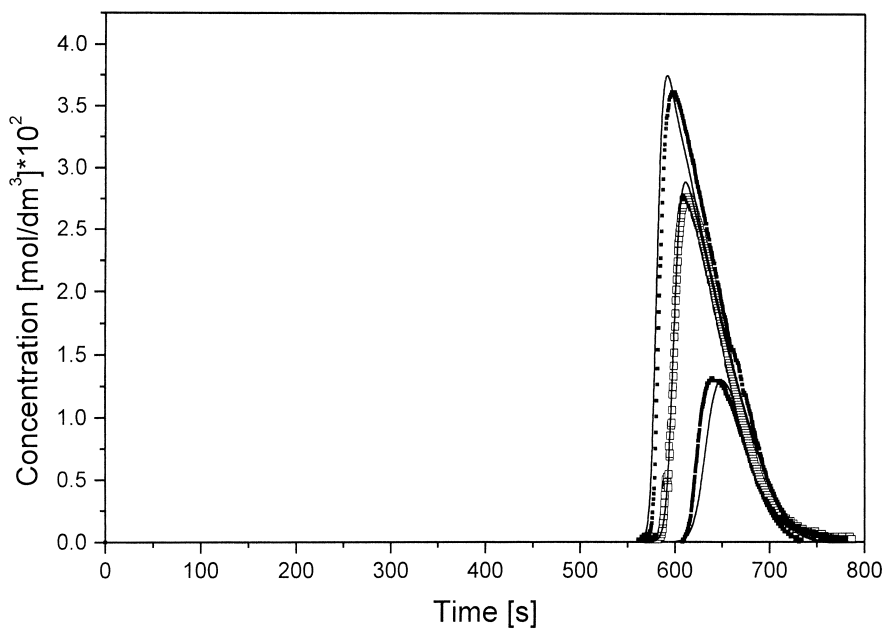


Fig. 3. The same as in Fig. 1 but  $0.986 \text{ mol/dm}^3$  methanol in mobile phase.

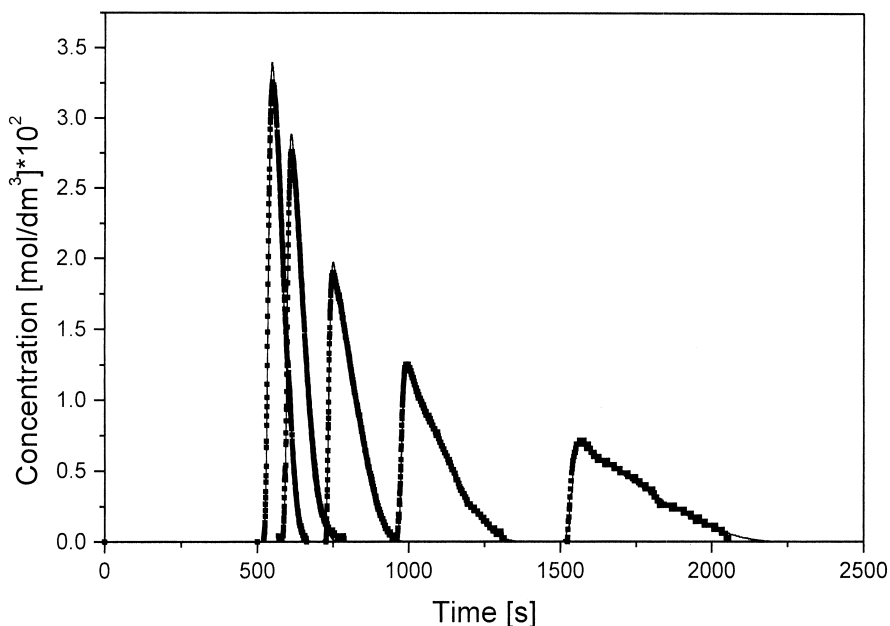


Fig. 4. Effect of the mobile phase composition on the band profiles. The square symbols from the right to left side are the experimental band profiles at 0.247–1.233 mol/dm<sup>3</sup> methanol in the mobile phase, respectively. The solid lines — results of the estimation. The sample concentration 0.0615 mol/dm<sup>3</sup>.

concentration 0.0615 mol/dm<sup>3</sup> and the coefficients obtained were used for simulations of chromatography process at the feed concentrations 0.0246 and 0.0861 mol/dm<sup>3</sup>. The same procedure was repeated for varied methanol concentration, going from 0.247 to 1.726 mol/dm<sup>3</sup>. The effectiveness of two-parameter estimation ( $a$  and  $b$ ) is limited to the conditions of concentration overloading. Such conditions were achieved for 0.247–1.233 mol/dm<sup>3</sup> of methanol in the mobile phase (see Fig. 4). Because of peak distortion that increases with the increasing concentration of methanol, at 1.479 and 1.726 mol/dm<sup>3</sup> of methanol concentration, the peaks with Gaussian shape were obtained. Thus, for these concentrations

only the coefficient  $a$  relating to retention factor  $k'_0$  was estimated.

Table 1 surveys estimated values of coefficients  $a$  and  $b$ . The values estimated lump all possible non-ideal behaviour as interactions between the adsorbed molecules of the solute and of polar solvents and between the adjacent adsorbed molecules of the polar solvents.

Moreover, the number of theoretical plates chosen as an approximate value affected the estimated values of isotherm parameters. However, under overload conditions, the sensitivity of the estimation results to changes of the number theoretical plates is minor.

Table 1  
The estimated isotherm parameters

$C_{\text{mod}}^0$ mol/dm <sup>3</sup>	0.247	0.493	0.740	0.986	1.233	1.479	1.726
$a$	33.93	19.22	12.73	8.986	7.463	5.567	4.804
[-]	±0.042	±0.020	±0.020	±0.0060	±0.010	±0.014	±0.0030
$b$	23.840	12.290	6.714	3.474	2.621	–	–
dm <sup>3</sup> /mol	±0.179	±0.068	±0.068	±0.016	±0.036		

Summarising, the estimated values should be treated as the lumped isotherm parameters rather than as the real values of the equilibrium constant and saturation capacity. The method is designed as a useful tool for process optimisation only and cannot support detailed information about all thermodynamic factors influencing the separation process.

#### 4.1.2. Variation of the isotherm parameters with the methanol concentration

In order to simulate band profiles and the optimisation of overloaded gradient elution, the functional dependency that describes the variation of the isotherm parameters with the mobile phase compositions must be found. Figs. 5 and 6 show the variation of the coefficients  $a$  and  $b$  with the concentration of methanol in the mobile phase. It is evident that  $a$  and  $b$  decrease with increasing concentration of methanol in the mobile phase.

Based on the original theoretical basis of the Snyder model, a three-parameter equation was derived [20] to describe the dependence of the retention factor of a sample compound on the concentration of

the stronger polar solvent. As the coefficient  $a$  is proportional to the retention factor the same form of equation describes its dependence on  $C_{\text{mod}}^0$ :

$$a = (p_1 + p_2 \cdot C_{\text{mod}}^0)^{-m} \quad (7)$$

where:  $p_1$ ,  $p_2$ ,  $m$  are experimental constants depending on the solute and on the chromatographic system.

Similar dependence on  $C_{\text{mod}}^0$  was assumed for parameter  $b$ .

## 4.2. Gradient elution

### 4.2.1. The adsorption of methanol by the stationary phase

The dependencies of isotherm parameters on the composition of the mobile phase obtained in isocratic chromatography were used for the simulations of gradient elution. Methanol, as a strong organic solvent, is adsorbed by the stationary phase. In order to calculate the local concentration of methanol in the column, its isotherm parameters should be determined. For the sake of simplicity, the methanol

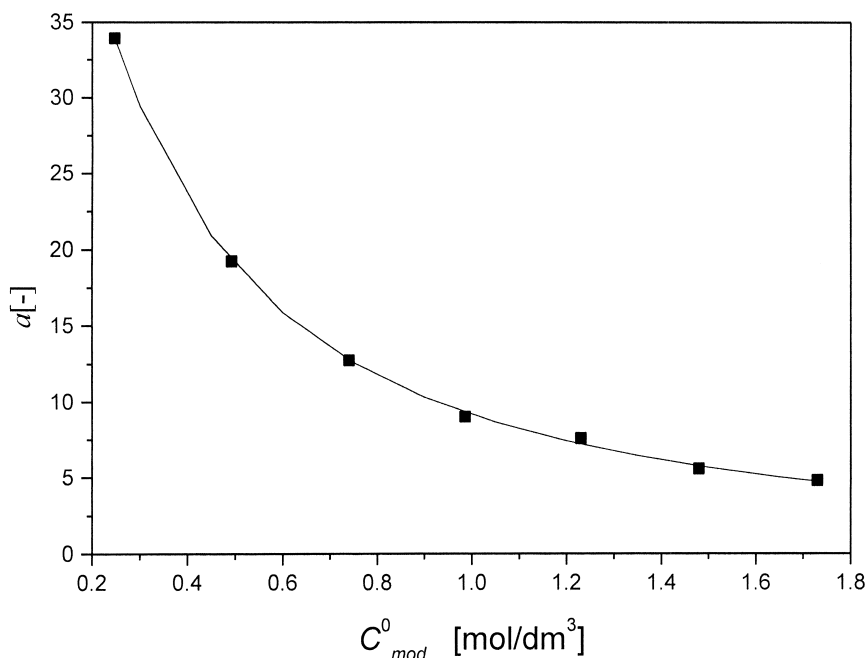


Fig. 5. The plot of the coefficient  $a$  of the Langmuir isotherm versus methanol concentration in the mobile phase. The values of the parameters of equation  $a = (p_1 + p_2 \cdot C_{\text{mod}}^0)^{-m}$ ;  $p_1 = 0.04407$ ;  $p_2 = 0.171$ ;  $m = 1.439$ .

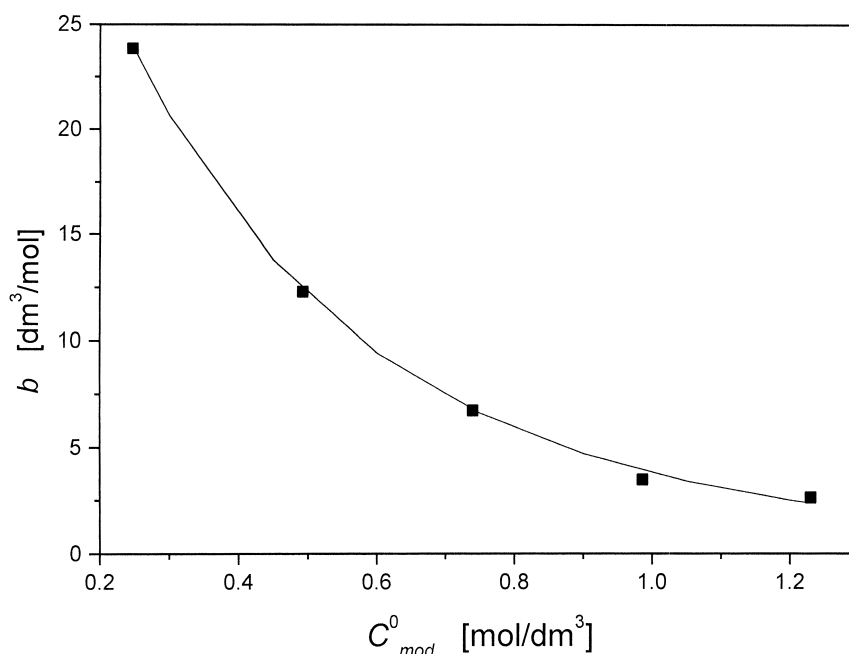


Fig. 6. The plot of the coefficient  $b$  of the Langmuir isotherm versus methanol concentration in the mobile phase. The values of the parameters of equation  $b = (p_1 + p_2 \cdot C_{mod}^0)^{-m}$ ;  $p_1 = 0.1805$ ;  $p_2 = 0.092$ ;  $m = 6.327$ .

isotherm was found by calculation of the outlet profile of gradient with simultaneous estimation of isotherm parameters. The Langmuir isotherm equation was assumed. The calculation procedure was the same as for the calculation of isocratic band profiles, as the experimental outlet gradient profile for estimation of the drift of baseline caused by the methanol gradient elution was used. Since the refractive signal of methyl deoxycholate and methanol was recorded simultaneously, the sample band profile had to be removed from the gradient chromatogram (see Fig. 7). For the estimation, the one less distorted by the signal of the sample chromatograms was selected (line 2 in Fig. 7).

Fig. 7 compares the calculated outlet profiles of methanol gradient with experimental data. The parameters estimated at a gradient program 3 min were used to simulate the outlet gradient profiles for the remaining time intervals (1; 6 and 12 min.). The very good agreement of the simulations with the experimental outlet profiles was achieved. It proves that the method proposed is sufficiently accurate to predict the local concentration of the modifier inside the column during gradient elution.

In the next step, the model parameters obtained were used to simulate gradient elution for different gradient steepness.

As mentioned above, the apparent isotherm of methyl deoxycholate does not take into account the competitive effect of the modifier. Thus, isotherm equations for both the sample and modifier were expressed by the simple single-component Langmuir isotherm (Eq. (4)).

Because adsorption of polar methanol cannot be neglected, the model consisting of two independent mass balance equations (Eq. (1)) — for the solute and modifier was built and solved by use of the orthogonal collocation method.

The simulations were performed for different gradient steepness. All gradient programs started with the same methanol concentration — 0.493 mol/dm<sup>3</sup>. For all gradient simulations CPU time was less than 1 min on a Pentium Pro 200 MHz computer.

In Figs. 8–11 the model predictions are compared with experimental elution profiles. The results obtained with all gradient programs are quite good.

Using strongly polar organic solvents such as methanol in the mobile phase in gradient elution



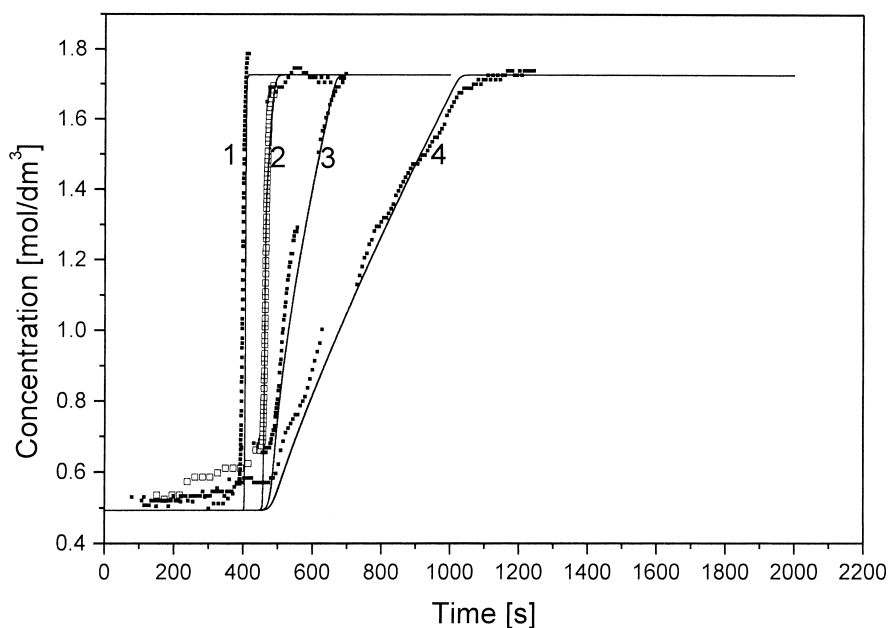


Fig. 7. Comparison between the experimental and calculated outlet gradient profiles. The band profiles of methyl deoxycholate are removed (lines 3, 4). Time intervals: (1) 1 min; (2) 3 min; (3) 6 min; (4) 12 min. The estimated isotherm coefficients of methanol:  $a = 8.993 \pm 0.092$  [-];  $b = 0.562 \pm 0.0086$  ( $\text{dm}^3/\text{mol}$ ). The lines as in Fig. 2.

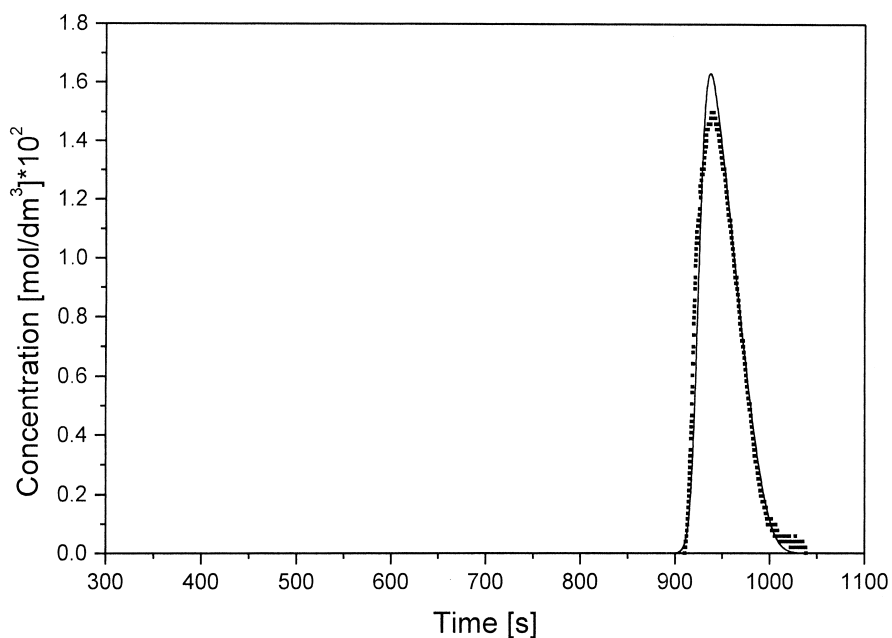


Fig. 8. Comparison between the experimental and calculated band profiles of methyl deoxycholate with gradient program 0.493–1.726  $\text{mol}/\text{dm}^3$  in 12 min. The sample concentration  $C_j = 0.0246$   $\text{mol}/\text{dm}^3$ . The experimental data were corrected with the drift of baseline.

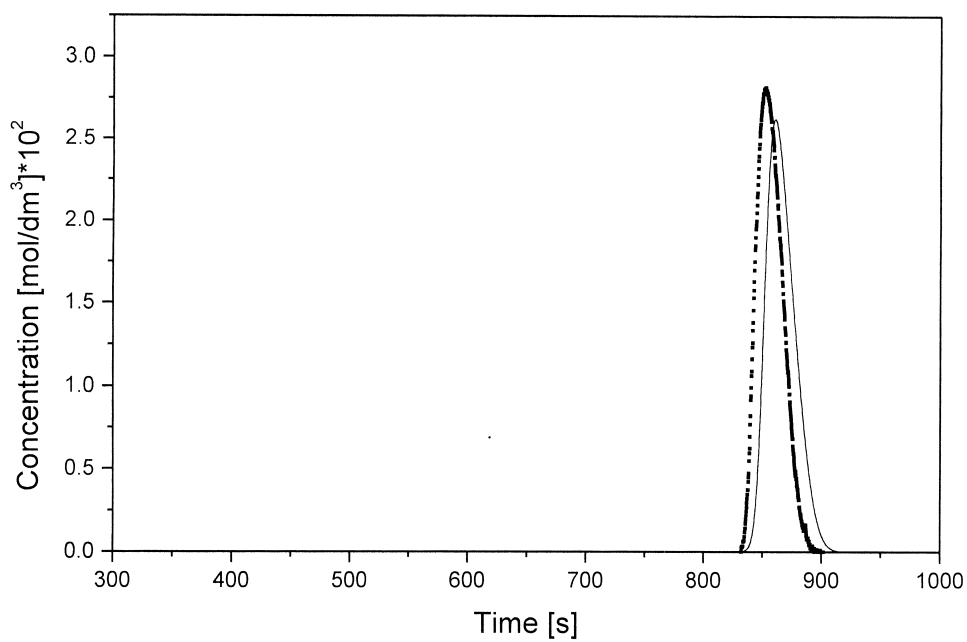


Fig. 9. The same as in Fig. 8, but with gradient program 0.493–1.726 mol/dm<sup>3</sup> in 6 min.

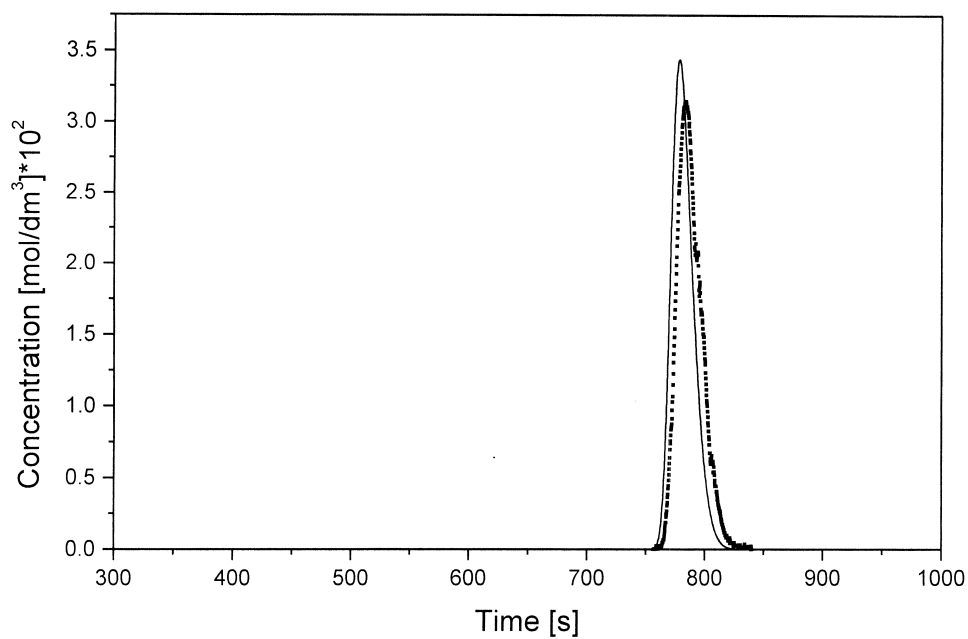


Fig. 10. The same as in Fig. 8, but with gradient program 0.493–1.726 mol/dm<sup>3</sup> in 3 min.

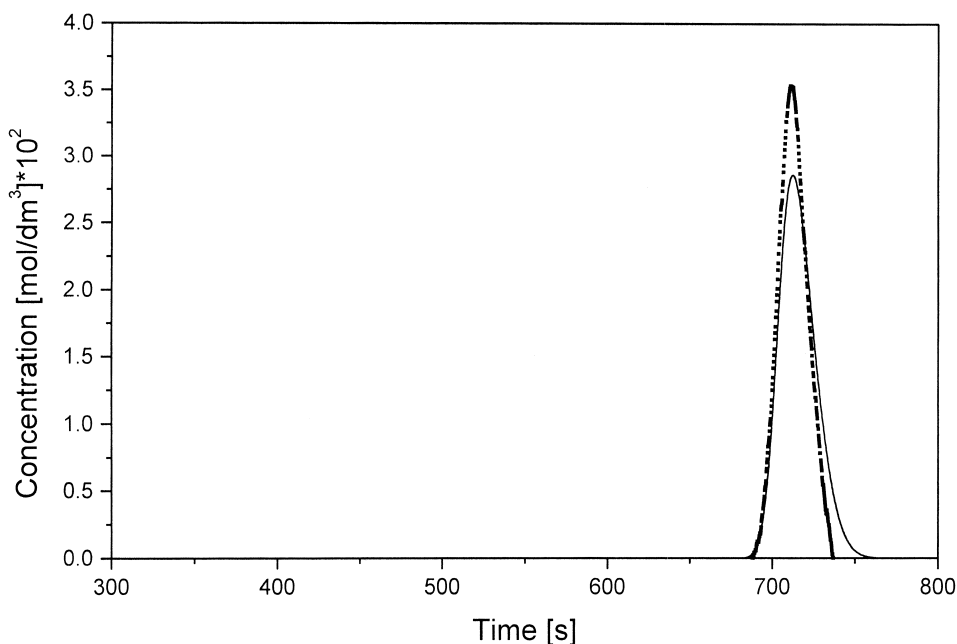


Fig. 11. The same as in Fig. 8, but with gradient program 0.493–1.726 mol/dm<sup>3</sup> in 1 min.

'buffers' the effect of trace concentrations of water present in the solvents. Furthermore, polar adsorbents fill up the micropores diminishing heterogeneity of the adsorbent surface [21]. It reduces excess band broadening resulting from surface heterogeneity. Furthermore, initial saturation of the column by the polar solvents: ethyl acetate 6.14 mol/dm<sup>3</sup> and strongly polar methanol 0.493 mol/dm<sup>3</sup> allows a shortened equilibration time. The relative change of polarity of mobile phase during gradient elution was moderate. It resulted in good reproducibility of gradient elution that is declared satisfactory mainly for solvents of low and moderate polarity [22]. The good agreement of the model predictions with experimental band profiles was achieved for all the gradient programs examined. The three-parameter dependencies determined in the isocratic condition were found to be still valid in gradient elution with continuous changes of the mobile phase composition.

For the steepest gradient program (time interval 1 min) the very sharp front of adsorbed methanol is formed. In this case the displacement effect involved by a sudden change in solvent strength leads to a narrowing of the band profile (so called 'demixing effect' [23]) and the experimental band profile is

higher than that predicted (Fig. 11). Nevertheless, as the adsorbent is initially saturated with the polar mobile phase, this effect is weak and experimental band profiles are still well reproduced to that calculated.

## 5. Conclusions

The isocratic and gradient elution of methyl deoxycholate in a normal-phase system was investigated. In order for fast determination of the adsorption isotherm parameters and to determine the effect of mobile phase composition on band profiles, numerical estimation was used. For the fitting procedure, one set of experimental data was selected at each mobile phase composition. The results obtained in isocratic elution were used to simulate overload gradient elution and satisfactory agreement with the experimental band profiles was achieved. This confirmed the validity of the proposed method of modelling. The effectiveness of the estimation method was examined for the Langmuir isotherm.

The algorithm proposed can be recommended for preparative and industrial scale optimisation of the

chromatographic process. The method allows problems with scaling-up to be avoided; the experiments may be repeated in any process scale without consumption of large amounts of the pure component.

## 6. Abbreviations

$a, b$	Langmuir isotherm coefficients
$C$	local concentrations in the mobile phase
$C_{\text{mod}}$	modifier concentration
$D_a$	apparent axial dispersion coefficient
$F = \frac{1 - \epsilon_t}{\epsilon_t}$	phase ratio
$k'_0$	retention factor at infinite dilution
$L$	column length
$u$	superficial mobile velocity
$w = \frac{u}{\epsilon_t}$	interstitial mobile phase velocity
$t$	time positions in the column
$t_p$	width of a rectangular injection pulse
$t_0$	hold-up time of the column
$x$	axial positions in the column

### Greek letters

$\beta$	gradient steepness
$q$	local concentrations in stationary phase
$\epsilon_t$	total porosity

### Subscript

$i$	component index 1,...,NC
$f$	inlet value

### Superscript

$o$	initial value
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