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Fitting adsorption isotherms to the distribution data determined using packed micro-columns for high-performance liquid chromatography

Pavel Jandera[a,*], Simona Bunčeková[a], Kathleen Mihlbachler[b], Georges Guiochon[b], Veronika Bačkovská[a], Josef Planeta[c]

[a]*Department of Analytical Chemistry, University of Pardubice, Nám. Legií 565, 532 10 Pardubice, Czech Republic*
[b]*Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA*
[c]*Institute of Analytical Chemistry, Czech Academy of Sciences, Veveří, Brno, Czech Republic*

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Abstract

Knowing the adsorption isotherms of the components of a mixture on the chromatographic system used to separate them is necessary for a better understanding of the separation process and for the optimization of the production rate and costs in preparative high-performance liquid chromatography (HPLC). Currently, adsorption isotherms are usually measured by frontal analysis, using conventional analytical columns. Unfortunately, this approach requires relatively large quantities of pure compounds, and hence is expensive, especially in the case of pure enantiomers. In this work, we investigated the possible use of packed micro-bore and capillary HPLC columns for the determination of adsorption isotherms of benzophenone, *o*-cresol and phenol in reversed-phase systems and of the enantiomers of mandelic acid on a Teicoplanin chiral stationary phase. We found a reasonable agreement between the isotherm coefficients of the model compounds determined on micro-columns and on conventional analytical columns packed with the same material. Both frontal analysis and perturbation techniques could be used for this determination. The consumption of pure compounds needed to determine the isotherms decreases proportionally to the second power of the decrease in the column inner diameter, i.e. 10 times for a micro-bore column (1 mm I.D.) and 100 times for capillary columns (0.32 mm I.D.) with respect to 3.3 mm I.D. conventional columns.

Keywords: Adsorption isotherms; Benzophenone; *o*-Cresol; Phenol; Mandelic acid; Teicoplanin

1. Introduction

In preparative chromatography, mixtures are separated to prepare individual components at the required purity. The economy of this process dictates that the separation should be performed on overloaded columns, to maximize the production rate and minimize solvent consumption and labor costs. However, a compromise allowing a high recovery yield is often necessary. While analytical separations can be described by the theory of linear chromatography, separations on overloaded columns cannot. The more complex theory of non-linear chromatography is needed. Knowledge of the distribution isotherms is

*Corresponding author. Tel.: +420-40-603-7023; fax: +420-40-603-7068.
E-mail address: pavel.jandera@upce.cz (P. Jandera).

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required for its application, for an adequate description of the separation process and to optimize the separation conditions. Several models have been suggested to describe the distribution of one sample component between the stationary and the mobile phases. The most common and the most simple non-linear isotherms are the two-parameter Langmuir [1] isotherms:

$$Q = \frac{a \cdot c}{1 + b \cdot c} \quad (1)$$

Here, Q is the concentration of the sample compound in the stationary phase and c that in the mobile phases, and a and b are the coefficients of the isotherm ($a = k_0/\Phi$, where k_0 is the retention factor of the sample compound at infinite dilution, i.e. in analytical chromatography, and $b = a/Q_s$ is the ratio of the sorption and desorption rate constants, with Q_s the column saturation capacity).

Numerous and often complicated models have been suggested to describe the competitive equilibria involved between the components i, j of a binary sample mixture and the adsorbent, yielding various competitive isotherm equations [2]. The competitive Langmuir isotherm [3] is often used, but it is justified thermodynamically only if the column has the same saturation capacities for all sample components [1]:

$$Q_i = \frac{a_i \cdot c_i}{1 + b_i \cdot c_i + b_j \cdot c_j},$$

$$Q_j = \frac{a_j \cdot c_j}{1 + b_i \cdot c_i + b_j \cdot c_j} \quad (2a,b)$$

The parameters a_i, b_i, a_j and b_j are the Langmuir single-component coefficients for solutes i, j.

Single- and multi-component isotherms are now measured by dynamic methods. The most widespread of these is frontal analysis (FA) [4], but this technique is time-consuming and requires large amounts of pure compounds, which are often very expensive or difficult to obtain, for example in the case of pure enantiomers or proteins. Another popular method, elution by characteristic points (ECP) [5], derives the isotherm from the profile of the diffuse front of the band obtained in response to a single injection of a highly concentrated sample. This method is fast and needs only small amounts of sample, but it requires accurate calibration of the detector and an efficient column. Furthermore, it supplies only single-component isotherms. The extension of the method to the determination of multi-component (i.e., competitive) isotherms is most complex and not yet practical [6].

Distribution isotherms can also be determined using the perturbation (PT) technique, originally developed for measuring gas-adsorbent equilibria [7–9]. The PT makes possible the determination of adsorption isotherms by measuring the retention times of small sample sizes injected onto a column equilibrated with sample solutions at different concentration levels. Isotherms measured using PT were recently utilized for the optimization of preparative HPLC separations of enantiomers [10]. The column used for the determination of the isotherm is first equilibrated with a solution containing either a single compound i (for the determination of single-component isotherms) or a mixture of compounds i and j (for the determination of two-component isotherms) dissolved in a non-adsorbable solvent. Then a small sample volume containing different (lower or higher) concentration(s) of the compound i (or of the compounds i and j) is injected onto the column. After the injection, the equilibrium condition is disturbed and perturbation waves arise which migrate along the column [11]. When such a wave reaches the column outlet, a negative or a positive peak is registered by the detector, depending on whether the concentrations of the sample compounds injected are higher or lower than their equilibrium concentrations at the start of the experiment. The total number of perturbation peaks is equal to the number of dissolved components i in equilibrium with the stationary phase and not to that of the compounds in the sample injected, as each component migrates in any perturbation traveling wave. Even though the same number of peaks as the number of sample compounds is observed, the peaks cannot be attributed to individual compounds as each peak represents a response to the perturbation of the concentrations of all sample compounds. The coefficients of an isotherm model can be determined directly from the experimental retention times.

In the case of a single-component equilibrium of a compound dissolved in a non-adsorbable solvent, one peak is observed and the distribution isotherm

depends only on the concentration of a single solute. Hence the experimental retention factors, k, of the peaks are directly proportional to the first derivation (slope) of the adsorption isotherm of the compound corresponding to its equilibrium concentration at the time of introduction of the disturbance, so that the experimental isotherm data can be obtained directly. These data measured at different equilibrium concentrations can be fitted to the selected model to obtain the best-fit isotherm coefficients. In the case of a single-component Langmuir isotherm (Eq. (1)), we have:

$$k = \frac{t_R}{t_0} - 1 = \Phi\frac{dQ}{dc} = \Phi\frac{a}{(1+bc)^2} \quad (3)$$

where t_R is the retention time of the solute, t_0 is the column hold-up time, i.e. the elution time of a non-retained compound, and $\Phi = V_S/V_M$ is the phase ratio in the column, i.e. the ratio of the volumes of the stationary (V_S) and of the mobile (V_M) phases in the column.

In contrast, the application of the PT technique to multi-component isotherms is less straightforward, however it is possible, as has been shown by Heuer et al. [10]. In the present work, we applied the solution for the two-component distribution equilibrium case. Here, two perturbation peaks are obtained, each of which consists of a contribution of both compounds i and j. The mathematical solution in this case employs the equality of the retention times corresponding to each perturbation peak for both solutes i and j, known as the coherence condition:

$$t^i_{R_1} = t^j_{R_1}, \quad t^i_{R_2} = t^j_{R_2} \quad (4a,b)$$

where $t^i_{R_1}$ is the retention time of the first perturbation peak with respect to compound i, $t^j_{R_1}$ is the retention time of the first perturbation peak with respect to compound j, $t^i_{R_2}$ is the retention time of the second perturbation peak with respect to compound i, and $t^j_{R_2}$ is the retention time of the second perturbation peak with respect to compound j. The retention times of the two perturbation peaks are related to the total derivatives of the isotherm at the equilibrium concentrations c_i and c_j at the time of injection, DQ_i/Dc_i and DQ_j/Dc_j (the subscripts 1 and 2 for the total derivatives relate to peaks 1 and 2, respectively). Using the coherence condition, we can write:

$$t^i_{R_1} = t_0\left(1 + \Phi\left(\frac{DQ_i}{Dc_i}\right)_1\right) = t^j_{R_1} = t_0\left(1 + \Phi\left(\frac{DQ_j}{Dc_j}\right)_1\right) \quad (5)$$

$$t^i_{R_2} = t_0\left(1 + \Phi\left(\frac{DQ_i}{Dc_i}\right)_2\right) = t^j_{R_2} = t_0\left(1 + \Phi\left(\frac{DQ_j}{Dc_j}\right)_2\right) \quad (6)$$

where t_0 is the column hold-up time. From Eqs. (5) and (6) it follows that the total derivatives of the adsorbed concentrations of i and j should be equal to each other at any retention time, t_{R_1} or t_{R_2} [10]:

$$\frac{DQ_i}{Dc_i} = \frac{DQ_j}{Dc_j} = \frac{\partial Q_i}{\partial c_i} + \frac{\partial Q_i}{\partial c_i}\cdot\frac{dc_j}{dc_i} = \frac{\partial Q_j}{\partial c_j} + \frac{\partial Q_j}{\partial c_i}\cdot\frac{dc_i}{dc_j} \quad (7)$$

By rearranging Eq. (7), a quadratic equation is obtained with respect to dc_i/dc_j:

$$\left(\frac{dc_i}{dc_j}\right)^2 + \left(\frac{(\partial Q_j/\partial c_j) - (\partial Q_j/\partial c_i)}{\partial Q_j/\partial c_i}\right)\frac{dc_i}{dc_j} - \frac{\partial Q_i/\partial c_j}{\partial Q_j/\partial c_i} = 0 \quad (8)$$

The solution of Eq. (8) for dc_i/dc_j represents two roots, corresponding to the two retention times, t_{R_1} and t_{R_2}. Introducing corresponding expressions for the total derivatives into Eqs. (5) and (6) we obtain Eqs. (9a,b) which allow us to calculate the expected elution times of the two disturbances, $t_{R_1(calc)}$ and $t_{R_2(calc)}$:

$$t_{R_1(calc)} = t_0\cdot\left[1 + \Phi\cdot\left(\frac{\partial Q_i}{\partial c_i} + \frac{\partial Q_i}{\partial c_j}\cdot\left(\frac{dc_j}{dc_i}\right)_1\right)\right],$$

$$t_{R_2(calc)} = t_0\cdot\left[1 + \Phi\cdot\left(\frac{\partial Q_i}{\partial c_i} + \frac{\partial Q_i}{\partial c_j}\cdot\left(\frac{dc_j}{dc_i}\right)_2\right)\right] \quad (9a,b)$$

Analogous equations are obtained with respect to the concentrations of the compound j. To solve Eqs. (9a,b), we have to introduce the two roots of Eq. (7)

for $(dc_j/dc_i)_1$ and $(dc_j/dc_i)_2$ and the appropriate expressions for the partial derivatives, which can be derived from the isotherm model. For the two-component competitive Langmuir isotherm (Eqs. (2a,b)), Eqs. (10)–(13) are obtained:

$$\frac{\partial Q_i}{\partial c_i} = \frac{a_i(1 + b_j c_j)}{(1 + b_i c_i + b_j c_j)^2} \tag{10}$$

$$\frac{\partial Q_j}{\partial c_j} = \frac{a_j(1 + b_i c_i)}{(1 + b_i c_i + b_j c_j)^2} \tag{11}$$

$$\frac{\partial Q_i}{\partial c_j} = \frac{-a_i(b_j c_i)}{(1 + b_i c_i + b_j c_j)^2} \tag{12}$$

$$\frac{\partial Q_j}{\partial c_i} = \frac{-a_j(b_i c_j)}{(1 + b_i c_i + b_j c_j)^2} \tag{13}$$

After this substitution, Eqs. (9a,b) enable the calculation of the expected retention times of perturbations using the estimated coefficients of the two-component Langmuir isotherm, a_i, a_j, b_i and b_j. The retention times calculated in this way, $t_{R_1(calc)}$ and $t_{R_2(calc)}$, are compared with the experimental values of the retention times for all perturbation experiments at different combinations of concentrations c_j and c_i. As there are more experimental retention times measured by perturbation injections of a mixed sample than unknown variables, numerical solution is employed to determine the isotherm coefficients yielding minimum squares of the differences between the calculated and the experimental retention times, $t_{R_1(calc)} - t_{R_1(exp)}$ and $t_{R_2(calc)} - t_{R_2(exp)}$, at various combinations of c_i and c_j. To this aim, the initial estimates of the isotherm coefficients are subsequently corrected in repeated iterative calculation steps using the Marquardt method of minimization of the objective function *OF*:

$$OF = \sum_{p=1}^{P} (t_{R_1(calc)} - t_{R_1(exp)})^2 + \sum_{p=1}^{P} (t_{R_2(calc)} - t_{R_2(exp)})^2 \tag{14}$$

where P is the number of all perturbation experiments. The values of the best-fit isotherm coefficients corresponding to the minimum *OF* represent the desired solution for the competitive Langmuir isotherm.

The initial estimated values of the isotherm coefficients for calculations of $t_{R_1(calc)}$ and $t_{R_2(calc)}$ can be set equal to the single-component Langmuir or linear isotherm coefficients determined in earlier experiments. In the present work, a program written in Pascal was used for the numerical solution of Eq. (14). More details on the calculation procedure can be found elsewhere [10,11].

The main advantage of the perturbation method consists in using a simpler instrumentation for the acquisition of the experimental data than in the frontal analysis method: the determination of the concentrations of the individual compounds at the intermediate plateaus of the frontal analysis curves is no longer needed [12,13]. Furthermore, if the separation selectivity of sample compounds is not large, the intermediate plateaus are often poorly developed, which makes the accurate evaluation of the breakthrough volumes difficult or even impossible, whereas the perturbation volumes can still be precisely determined.

At a constant retention factor, the retention volume of a compound is directly proportional to the second power of the column inner diameter. Thus, the consumption of compounds necessary for the determination of isotherms can be very significantly decreased by using columns with smaller inner diameters. For example, using a 0.32 mm I.D. capillary tube packed with the same material as a conventional analytical column of the same length but 10 times greater (3.2 mm) I.D., should reduce by 100 times the retention volumes and the amounts of sample compounds needed. This holds true also for the mobile phase flow-rate, which — at the same usual linear flow velocity — would be 5–10 μL/min with the capillary column instead of 0.5–1.0 mL/min with the conventional 3.2 mm I.D. column. Accordingly, special instrumentation is necessary.

The isotherm coefficients are thermodynamic quantities that are independent of the column dimensions and their values should theoretically be the same, regardless of whether they have been determined on a conventional or on a capillary column. However, the packing density of a conventional column may differ from the density of a packed capillary column and the phase ratio may differ in the two column types. Further, the contribution of the extra-column volumes is much more difficult to

determine accurately with capillary columns, but recently we have worked out an approach allowing accurate determination of this quantity [14].

The effects of the extra-column contributions to band broadening and of possible flow-rate fluctuations on the accuracy of the results may be important with packed capillary columns, so that these effects must be investigated.

In the present work, we compare the performance of packed capillary, micro-bore and conventional analytical HPLC columns for the determination of adsorption isotherms using simple model compounds and reversed-phase chromatographic systems to explore the possible problems encountered when attempting to use narrow-bore columns for the determination of distribution isotherms when only small quantities of samples are available. The main objective was to check whether the errors associated with the instrumental differences between the conventional analytical columns and with capillary columns packed with the same material are small enough so that the results with the capillary column can still be meaningful.

2. Experimental

2.1. Chemicals

Acetic acid, phenol, *o*-cresol and ethanol (96%), all analytical grade, were obtained from Lachema (Brno, Czech Republic). Before use, phenol was purified by distillation and *o*-cresol by crystallization from methanol. Benzophenone (99%, GC), triethylamine D-, L- and L,D-mandelic acid (all 99%+) were used as obtained from Sigma–Aldrich (Prague, Czech Republic). Methanol, HPLC grade, was obtained from Merck (Darmstadt, Germany). De-ionized water was doubly distilled in a glass vessel, with addition of potassium permanganate. The solvents were filtered on a Millipore 0.45-μm filter. The mobile phases were prepared by mixing the components in the required ratios and degassed by ultrasonication before use. The sample solutions used for the determination of the distribution data were prepared by weighing the required amounts of solutes and dissolving them in the mobile phases.

2.2. Instrumentation

A 1090M liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a 3 DR solvent delivery system, solvent reservoirs continuously stripped with helium and a temperature-controlled column compartment, a diode-array UV detector and a data workstation, was used for the acquisition of the data needed for the adsorption isotherms on conventional analytical columns: (1) a 150×3.2 mm I.D. glass cartridge, packed with Biospher C_{18}, 7 μm, purchased from Bio Lab (Prague, Czech Republic) and (2) a 150×4.6 mm I.D. stainless steel column packed with Chirobiotic T, 5 μm, obtained from Astec (Whippany, NJ, USA).

For measurements on the micro-bore column, 290×1 mm I.D., stainless steel, packed in the laboratory with Chirobiotic T, 5 μm (Astec), the instrument was modified by using an external single-wavelength (254 nm) UV detector (M 440, Waters, Milford, MA, USA) equipped with a flow-through micro-cell and an injection valve with a fixed-volume 500 nL sample loop.

The apparatus for HPLC on a packed capillary micro-column was assembled using an LC-10AD pump (Shimadzu, Kyoto, Japan), an ACI4W.06 high pressure sample valve equipped with a 60 nL internal sample loop switched by a DVI pneumatic actuator (all from Valco, Houston, TX, USA) with helium as the pressure gas, an SP 100 UV–Vis detector (Spectra Physics, San Jose, CA, USA), and an analog–digital converter (760 Interface, Hewlett-Packard) was used to process the detector data by a 1090 chromatograph data station. The capillary column was connected to the injector valve by a piece of silica capillary tubing of 75 μm I.D. The connection tubing to the detector was also made of fused-silica capillary, 75 μm I.D., and served as a detector cell with windows burned in the polyimide coating. All connections were made as short as possible to reduce the extra-column contributions to the band broadening. The column was placed in a thermostated compartment. The preparation and characterization of the packed capillary HPLC columns and the instrumentation used were as described earlier [15]. A capillary HPLC column, 143×0.32 mm I.D., was prepared by packing the Biospher C_{18}, 5 μm, material into an empty fused-silica capillary (Chrom-

pack, Prague, Czech Republic) with porous ceramic frits prepared within the column by polymerizing solutions containing potassium silicate in situ according to Cortes et al. [16]. The column was packed at the Institute of Analytical Chemistry, Brno, using a supercritical-fluid slurry technique [17].

2.3. Procedures

On the Biospher C_{18} columns, single-component isotherms of benzophenone (in the concentration range 0.0025–0.025 mol/L) were measured in 70:30 (v/v) methanol–water, whereas single-component isotherms of phenol and *o*-cresol (each in the concentration range 0.01–0.1 mol/L) and a two-component isotherm of a mixture of phenol and *o*-cresol (at a constant concentration ratio, from 0.01:0.01 to 0.1:0.1 mol/L) were measured in 50:50 (v/v) methanol–water, at 40°C. On Teicoplanin columns, single-component isotherms of the L- and D-enantiomers and a two-component isotherm of the racemate of mandelic acid (in the concentration range 0.025–0.25 mol/L) were measured in ethanol–water–triethylamine–acetic acid (17.5:70.3:8.7:3.5, v/v/v/v) as the mobile phase, at 35°C. UV detection was used to record the frontal analysis and the perturbation technique signals, at a wavelength of 352 nm for benzophenone, 289 nm for phenol and *o*-cresol and 254 nm for mandelic acid. The flow-rate of the mobile phase was set to 1.0 mL/min with both the C_{18} and Teicoplanin conventional analytical columns, to 0.005 mL/min with the packed capillary C_{18} column and to 0.05 mL/min with the micro-bore Teicoplanin column.

The frontal analysis approach used for the determination of the distribution data on conventional analytical columns was described earlier [18]. The ratio of the flow-rates of the mobile phase and of the solution of a sample compound was adjusted from 0 to 100% in successive 10% steps using the gradient programming capability of the 1090 M instrument. Time was allowed for the stabilization of the detector signal after each concentration change. The solute concentration in the stationary phase was determined from the integral mass balance equation using the experimental concentrations of the sample compounds at the plateaus of the frontal analysis curve and the retention (breakthrough) volumes corresponding to the inflection points on the breakthrough curve, corrected for the volume of the tubing between the mixing point of the liquids pumped in each channel and the column top (0.35 mL). The steps on the frontal analysis curve were steep and the inflection points were close enough to the half-heights of the steps so that possible errors in the breakthrough volumes were less than 0.01 mL, which was within the limits of the accuracy of the instrumental determination of the elution volumes. The details of the technique are described elsewhere [19].

The elution volume of non-retained uracil was determined experimentally. This is comprised of the volume of the mobile phase in the column, V_M (the column hold-up volume), and of the extra-column volume of the system, the relative contribution of which is much larger with the packed capillary column than with the conventional analytical and micro-bore columns and may cause significant errors if the experimental data are not corrected for its contribution. Hence, the contributions of the real column hold-up volume (8 μL) and the extra-column volume of the system (7.5 μL) to the elution volume of non-retained compound were determined from the retention volumes of homologous *n*-alkylbenzenes as described earlier [15]. The volume of the stationary phase, V_S, necessary for the calculation of the phase ratio, Φ, and for the evaluation of the frontal analysis results using the mass-balance equation, was calculated as the difference between the inner volume of the empty column and V_M. The phase volumes for the individual columns are listed in Table 1.

For the perturbation measurements of the distribution data on the conventional analytical columns, the same instrumentation was employed as for

Table 1
Dimensions of the empty column volume, V_C, the volume of the mobile, V_M, and of the stationary, V_S, phases of the conventional analytical Biospher C_{18} (1) and Chirobiotic T (2) columns, of the micro-bore column packed with Teicoplanin (3) and of the capillary column packed with Biospher C_{18} (4)

Column	Length (mm)	I.D. (mm)	V_C (cm^3)	V_M (cm^3)	V_S (cm^3)
1	150	3.3	1.28	0.86	0.42
2	150	4.6	2.49	1.68	0.81
3	290	1	0.228	0.16	0.068
4	143	0.32	0.0115	0.008	0.0035

the frontal analysis. Each isotherm data point was measured in 11 subsequent steps after equilibration of the column with a solution containing a sample compound (0, 0.0025, 0.005, 0.0075, 0.01, 0.0125, 0.015, 0.0175, 0.02, 0.0225 and 0.025 mol/L benzophenone or 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mol/L phenol or *o*-cresol or 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2, 0.225 and 0.25 mol/L of the enantiomers and of the racemate of mandelic acid) until a stable detector response was obtained. Small volumes (5 μL) of water and of the most concentrated sample were subsequently injected onto the column. Each experiment was repeated three times. The differences between the elution times of perturbations caused by injection of the sample and the times of perturbations caused by injection of water were in the range 0.01–0.02 min, which is within the limits of the experimental error. No significant differences were observed between the elution times of the concentration waves caused by the injection of water and of the concentrated sample into the equilibrated system.

The same approach was used with the packed capillary column (injection volume 60 nL) and with the micro-bore column (injection volume 500 nL).

The coefficients of the single-component Langmuir isotherm (Eq. (1)) were evaluated from single disturbance peaks by fitting Eq. (3) to the experimental retention times using non-linear regression. For the evaluation of the coefficients of the two-component Langmuir isotherm (Eqs. (2a,b)), the iterative Marquardt approach was used to fit the best isotherm coefficients' values, as shown in the Introduction.

3. Results and discussion

The single-component distribution data of benzophenone between a conventional and a packed capillary C_{18} column and an aqueous solution of methanol were measured by the frontal analysis (FA) method. The Langmuir isotherm model was found to describe adequately the experimental data on the two columns. Although obtained with two columns of different diameters, the isotherms are practically identical (Fig. 1). The best values of the constants of Eq. (1) obtained by non-linear regression for the two

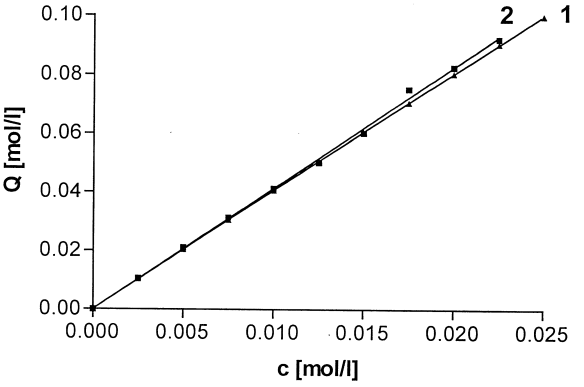

Fig. 1. Adsorption isotherms of benzophenone on a capillary column, 143×0.32 mm I.D. (1), and on a conventional analytical column, 150×3.2 mm I.D. (2), both packed with Biospher C_{18}, 7 μm, in methanol–water (70:30) mobile phase. The distribution data were determined using the frontal analysis method. *c*, *Q*, concentrations of benzophenone in the mobile and in the stationary phases, respectively, in mol/L.

columns are very similar to each other (Table 2). The isotherms obtained for benzophenone were almost linear up to a concentration of 0.025 mol/L. Because of the limited solubility of this compound in the mobile phase, it was not possible to carry out measurements at higher concentrations.

The application of FA with the conventional analytical column was straightforward. It was more difficult with the packed capillary column. No low-

Table 2
Coefficients *a* and *b* of single-component (SC) and a_1, b_1, a_2 and b_2 of two-component (TC) Langmuir isotherms of benzophenone (B), phenol (P) and *o*-cresol (C) determined on a conventional analytical (CA) and on a packed capillary (PC) HPLC column (Biospher C_{18}, 7 μm) in 50% (P and C) and in 70% (B) methanol in water, using the frontal analysis (FA) and the perturbation (PT) methods (values±standard deviation, *a* is dimensionless)

Coefficients of Langmuir isotherm	CA	PC (SC)	PC (TC)
B (FA) *a*	4.12±0.10 (SC)	4.20±0.02	
B (FA) *b* (L mol^{-1})	1.1±0.30 (SC)	1.05±0.19	
P (FA) *a*	3.56±0.02 (TC)		
P (FA) *b* (L mol^{-1})	0.43±0.06 (TC)		
P (PT) a_1		3.74±0.02	3.87±0.02
P (PT) b_1 (L mol^{-1})		0.51±0.06	0.49±0.05
C (FA) *a*	5.10±0.04 (TC)		
C (FA) *b* (L mol^{-1})	1.24±0.03 (TC)		
C (PT) a_2		5.38±0.04	5.52±0.07
C (PT) b_2 (L mol^{-1})		2.21±0.07	2.05±0.11

volume gradient-elution instrument is available to perform FA the way it is done with conventional columns, by running a step-gradient program and pumping two solutions of different compositions at a constant flow-rate [4]. A suitable procedure was developed, consisting of first filling the 250 μL sample loop of the sampling valve with an adequate solution of the sample in the mobile phase. The FA experiment was then started by switching the valve and keeping it in the switched-on position during the whole elution of the breakthrough curve. The experimental difficulty arises from the need to pressurize the liquid contained in the sample loop at the beginning of this step. The stability of the flow-rate was sensitive to the way the sample switch was actuated.

For this reason, the perturbation technique (PT) seemed more suitable for the determination of the adsorption isotherms on the packed micro-columns and we used only the PT technique for the determination of the experimental distribution data of phenol and *o*-cresol on the Biospher C_{18} capillary HPLC column. The isotherms of these compounds in reversed-phase systems were measured in earlier studies, but either in pure water [12,13], or in mobile phases with lower concentrations of organic modifiers [18,19]. In these earlier works, Langmuir or quadratic isotherms were found to describe adequately the experimental distribution data, but the isotherm parameters cannot be compared directly with our new data because of the different column type and higher methanol concentration used in the present work. Generally, the saturation capacity available for the adsorption of sample compounds is found to decrease with increasing concentration of the organic modifier in reversed-phase systems [18,19].

In the following, we compare in Fig. 2 the single-component isotherms of phenol (P) and of *o*-cresol (C) on Biospher C_{18} columns. The distribution data were measured using the PT technique on the packed capillary column (dashed lines) and by the FA technique on the conventional analytical column (full lines). In both cases, the differences between the isotherms determined on the two columns are barely significant for *o*-cresol and slightly more important for phenol.

As a further test of the validity of the data on the

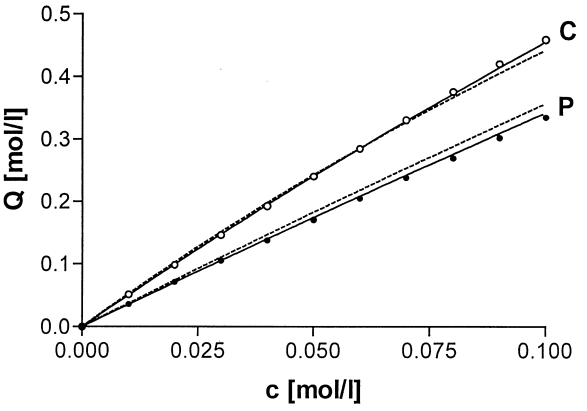

Fig. 2. Single-component adsorption isotherms of phenol (P) and *o*-cresol (C) on a conventional analytical column, 150×3.2 mm I.D. (experimental values — symbols; calculated using the Langmuir coefficients determined by frontal analysis — full lines), and on a capillary column, 143×0.32 mm I.D. (calculated using the Langmuir coefficients determined by the perturbation technique — dashed lines), packed with Biospher C_{18}, 7 μm, in methanol–water (50:50) mobile phase (Table 2). *c*, *Q*, concentrations of phenolic compounds in the mobile and in the stationary phases, respectively, in mol/L.

packed capillary column, we compare in Fig. 3 the two-component competitive isotherms of a 50:50 mixture of phenol (P) and *o*-cresol (C) measured experimentally by the FA method on a conventional analytical Biosphere C_{18} column (symbols) and those calculated from the equations of the competitive Langmuir isotherms (Eqs. (2a,b)) using the coefficients a_i and b_i determined from the two-component data by the PT technique on a packed capillary column (solid lines); see Table 2 for the values of the coefficients. The competitive isotherms in Fig. 3 are more significantly curved than the single-component isotherms in Fig. 2, which indicates the importance of the competitive effect in the system studied. The calculated competitive isotherm of phenol is in good agreement with the experimental FA data. The two-component Langmuir isotherm calculated from the PT data determined on the capillary column predicts higher adsorbed concentrations of *o*-cresol than the experimental FA data on the conventional analytical column, which results from higher isotherm coefficients *a* and *b* determined by the PT data (Table 2) with respect to the FA coefficients on the conventional analytical column.

The discrepancies between the two-component

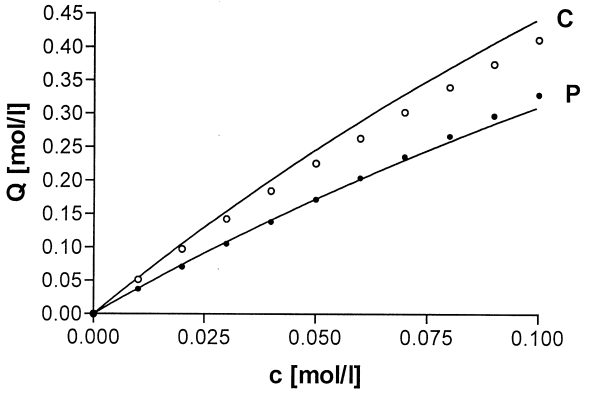

Fig. 3. Competitive adsorption isotherms of phenol (P) and *o*-cresol (C) on Biospher C_{18} columns in methanol–water (50:50) mobile phase. Symbols — experimental two-component distribution data for a 1:1 mixture of phenol and *o*-cresol determined using frontal analysis on a conventional analytical column; solid lines — competitive Langmuir isotherms calculated using Eqs. (2a,b) with the coefficients a_i, b_i, a_j and b_j determined using the perturbation method from the two-component distribution data on the capillary column (Table 2). *c*, *Q*, concentrations of the individual phenolic compounds in the mobile and in the stationary phases, respectively, in mol/L.

isotherms of *o*-cresol can possibly be attributed either to experimental errors or to the imperfect suitability of the competitive Langmuir model to describe the two-component distribution data. However, the agreement between the other single- and two-component isotherms determined on the conventional and on the packed capillary columns shown in Figs. 1–3 is acceptable. This suggests that the distribution data measured using small sample quantities on a packed capillary column may be useful for the determination of the isotherms of expensive compounds, such as of enantiomers on chiral stationary phases.

To test the possibilities for the determination of the isotherms of chiral compounds on a micro-column, we selected the L- and D-enantiomers of mandelic acid on a Teicoplanin macrocyclic chiral stationary phase, which has been reported to show excellent enantioselectivity for underivatized amino acids, hydroxy carboxylic acids and other chiral acids [20,21]. Recently, we studied the effects of the mobile phase composition on the separation of mandelic acid enantiomers [22]. The enantiomers can be separated up to concentrations of 0.25 mol/L in a buffer containing ethanol–water–triethylamine (TEA)–acetic acid (AA) 17.5:70.3:8.7:3.5 (v/v/v/v) as the mobile phase at pH 6.5. In the present work, we used this mobile phase for a comparison of the distribution isotherms on a commercial analytical column (150×4.6 mm I.D.) and on a micro-column, both packed with Chirobiotic T. The commercial column is packed with 5 μm particles, however only the 10 μm particle bulk material is available commercially and we did not succeed in preparing sufficiently efficient capillary columns of 0.32 mm I.D. packed with these particles. Hence, we filled this material into a 1 mm I.D. stainless-steel micro-bore column using a slurry technique, the efficiency of which was suitable for measuring single-component distribution data using the perturbation (PT) technique.

The single-component distribution data of L- and D-mandelic acid on Chirobiotic T columns on the two columns tested could be described by the Langmuir isotherm (Eq. (1)). The coefficients of the Langmuir isotherm determined on the micro-bore column by the PT technique are in good agreement with the data determined by frontal analysis (FA) on a commercial Chirobiotic T column (Table 3), hence the micro-bore column is potentially useful for isotherm determination.

The intermediate plateaus on the FA breakthrough curve for the racemate were poorly developed, hence we could not measure the two-component distribution data using this technique. We determined the two-component Langmuir coefficients (Eqs. (2a,b))

Table 3
Coefficients *a* and *b* of single-component (SC) and a_1, b_1, a_2 and b_2 of two-component (TC) Langmuir isotherms of the L- and D-enantiomers of mandelic acid determined on a conventional analytical (CA, commercial Chirobiotic T, 5 μm, 150×4.6 mm I.D.) and on a micro-bore (MB, packed in the laboratory, 10 μm, 290×1 mm I.D.) Teicoplanin column in ethanol–water–triethylamine–acetic acid (17.5:70.3:8.7:3.5, v/v/v/v), using the frontal analysis (FA) and the perturbation (PT) methods (values±standard deviation, *a* is dimensionless)

Coefficients of Langmuir isotherm	CA (FA, SC)	CA (PT, TC)	MB (PT, SC)
L-Mandelic, *a*	0.69±0.01	0.57±0.01	0.71±0.02
L-Mandelic, *b* (L mol^{-1})	1.42±0.01	0.96±0.07	1.48±0.19
D-Mandelic, *a*	1.15±0.03	1.02±0.02	1.23±0.04
D-Mandelic, *b* (L mol^{-1})	3.60±0.20	4.43±0.22	3.90±0.29

on the commercial analytical Chirobiotic T column by the PT technique (Table 3), but the efficiency of the micro-bore column was too low for sufficient resolution of the enantiomers at higher mandelic acid concentrations.

The *a* coefficients of the competitive Langmuir isotherms (Eq. (2)) of the two enantiomers of mandelic acid determined from the two-component distribution data on the commercial Chirobiotic T column are slightly lower than the single-component Langmuir coefficients, but the two-component isotherm coefficient *b* of the L-enantiomer is lower, and the two-component coefficient *b* of the D-enantiomer is higher, than the single-component Langmuir coefficients on both columns (Table 3). However, the differences between the profiles of the two-component isotherms determined on the conventional Chirobiotic T column by the PT technique (full lines) and the isotherms calculated from the single-component perturbation data on the micro-bore column (dashed lines) are not very significant (Fig. 4).

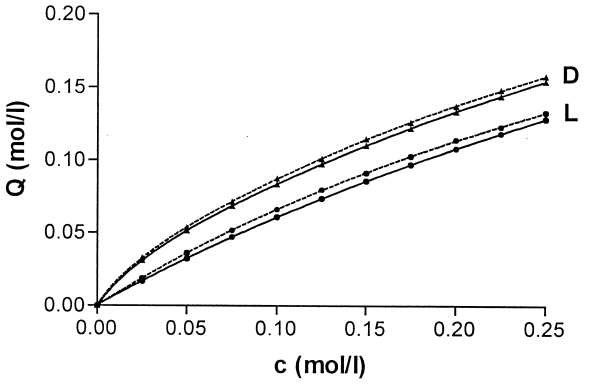

Fig. 4. Competitive adsorption isotherms of L-mandelic (L) and D-mandelic (D) acid on Teicoplanin columns in ethanol–water–triethylamine–acetic acid (17.5:70.3:8.7:3.5, v/v/v/v) mobile phase. Full lines — two-component Langmuir isotherms determined on a conventional Chirobiotic T column (150×4.6 mm I.D.) using the perturbation method for the two-component data for mandelic acid racemate; solid lines — competitive Langmuir isotherms calculated with the coefficients a_i, b_i, a_j and b_j determined using the perturbation method from the single-component distribution data (Table 3) measured on a micro-bore column (290×1 mm I.D.). *c*, *Q*, concentrations of the individual mandelic acid enantiomers in the mobile and in the stationary phases, respectively, in mol/L.

4. Conclusions

We found reasonable agreement between the isotherm coefficients of benzophenone, phenol and *o*-cresol measured under reversed-phase conditions on a packed HPLC capillary column and on a conventional analytical column packed with the same C_{18}-silica material. The perturbation technique is more reliable than the FA method because of the better stability of the flow-rate during the experiments. Also, the isotherms of the enantiomers of mandelic acid determined on a Teicoplanin chiral stationary phase determined on a commercial analytical and on a micro-bore column did not differ significantly from each other.

Our results indicate that micro-bore or packed capillary columns can provide realistic values of the isotherm coefficients comparable to the data which can be obtained on conventional analytical HPLC columns. The discrepancies between the single-component and competitive isotherm data found in this work can probably be attributed to the limited suitability of the simple Langmuir competitive model, assuming equal saturation capacities for the adsorbed compounds, to describe experimental two-component distribution data. Deviations from the Langmuir model caused by association of the molecules in the adsorbed layer in reversed-phase systems have recently been reported [23]. For enantiomers, two different adsorption centers are assumed, one for non-specific (achiral) adsorption and the other for specific (enantioselective) adsorption on chiral stationary phases. This behavior can be described by a bi-Langmuir model [24]. However, more complex isotherm equations taking into account these effects require more complex programs for numerical calculations of the results of perturbation analysis, and these are not yet available. Further work is in progress in this direction.

The amount of compound necessary for the determination of adsorption isotherms decreases proportionally to the amount of the adsorbent (stationary phase) employed for the measurement. In dynamic determination methods, this amount is directly proportional to the column inner volume. Using a micro-bore Teicoplanin column, 290 mm long, 1 mm I.D., the consumption of material necessary for the determination of the isotherms was decreased 10

times with respect to a commercial column, 150 cm long, 4.6 mm I.D. The material savings that can be obtained using packed capillary HPLC columns are even greater; for example, using a packed capillary column with an inner diameter of 0.32 mm, it is possible to reduce the consumption of the pure compounds necessary for the determination of the isotherm by 100 times with respect to a 3.2 mm inner diameter conventional column. This approach may be especially attractive for the determination of the isotherm data for optimization of the preparative separations of expensive compounds such as pure enantiomers.

Acknowledgements

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