Adsorption (2024) 30:337–349
https://doi.org/10.1007/s10450-024-00436-z

Estimating the parameters of competitive Langmuir adsorption isotherms from measured responses to rectangular pulse injections exploiting equilibrium theory

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Received: 1 August 2023 / Revised: 21 December 2023 / Accepted: 6 January 2024 / Published online: 4 March 2024

Abstract

Knowledge of adsorption isotherms is essential for the design and optimization of chromatographic separation processes. Since the experimental determination of these thermodynamic functions is a complicated and time consuming task, there is a need to develop methods which are fast and easy to apply. An attractive group of methods is based on neglecting in the analysis of measured dynamic elution profiles all kinetic effects. These methods assume the validity of an isotherm model equation and exploit the possibility to solve analytically the column mass balance equations of the equilibrium model. If just the dispersive part of an elution profile is, the method is known as "elution by characteristic point" (ECP). The ECP method has been applied successfully to analyse column effluent profiles of single component dissolved in a mobile phase. This work extends the ECP method to analyse just the shapes of elution profiles recorded after injecting samples that contain two key components to be separated. The extended ECP method requires recording only one overloaded elution profile for the two-component mixture and offers a fast and efficient way to estimate isotherm model parameters. The method is in particular attractive if there is limited access to the pure components, as for example in cases of enantiomers. The underlying theory is presented and applied for the case that the adsorption equilibria can be described satisfactorily by the classical competitive Langmuir model. Core of the theory are the available analytical equations describing the outlet concentration profiles of the two solutes for the equilibrium controlled case the. Considering a case study, it is shown that the extended ECP method can be applied successfully for columns characterized by 2500 or more theoretical plates. However, the method can be useful also for columns with lower efficiency. It provides then a rough estimation regarding the isotherm courses.

Keywords Adsorption isotherm determination · Equilibrium theory · Elution by characteristic point · Binary systems · Overloaded elution profiles · Langmuir isotherm model

1 Introduction

To design and optimise adsorption processes and preparative chromatography it is important to know the courses of the adsorption isotherms for the component that should be separated. A large number of experimental methods capable to determine the isotherms is available. A current overview related to quantifying adsorption from the gas phase is available (Shade et al. 2022). More specific studies devoted to carry out and analyse breakthrough (Wilkins et al. 2021) or zero column length experiments (Brandani and Mangano 2021) were also reported recently. The analysis of adsorption from solution considering the additional impact of specific solvents or solvent mixtures was summarized for example by Guiochon et al. (2006) and Schmidt-Traub et al. (2020).

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Choosing a suitable experimental method to determine adsorption isotherms is a challenging task. Each of the methods available has specific advantages and disadvantages (Seidel-Morgenstern 2004). Restrictions and difficulties arise when dealing with valuable substances, for example enantiomers of chiral compounds, since they are often not or only in limited amounts available as pure substances.

In this contribution, we will study theoretically and experimentally a dynamic method, which evaluates recorded responses to a single large pulse mixture injection. The key information exploited for isotherm estimation is the course of specific dispersed fronts characterizing the elution profiles. The concept is well known in the field of preparative liquid chromatography and designated as "Elution by characteristic point (ECP) method". This method is restricted so far to perform and analyse experiments with single solutes. The novel aspect of our work described here is an extension to mixtures.

The ECP method described below in this paper is based on an analytical solution exploiting the equilibrium theory of adsorption and chromatography. Profound overviews regarding this theory are given in the literature (Rhee et al. 1970, 1986; Helfferich and Klein 1970 and 1989; Golshan-Shirazi and Guiochon 1990 and 1994; Mazzotti and Rajendran 2013). Since the equilibrium model (designated also as the "ideal model of chromatography") assumes infinitely efficient columns, the results of the ECP method are effected by kinetic effects (Miyabe et al. 2000). However, the underlying simple algebraic equations exploit typical patterns of the development of the concentration profiles and provide useful approximations regarding the single component adsorption isotherms. Golshan-Shirazi and Guiochon (1994) and Guiochon et al. (2006) presented analytical expressions that describe equilibrium controlled elution profiles of binary mixtures in cases where the competitive Langmuir isotherm model is applicable. Considering larger injection sizes which do not lead to completely resolved elution profiles both the method of characteristics (Golshan-Shirazi and Guiochon 1989a, following Rhee et al. 1970) and the *h*-transform technique (Golshan-Shirazi and Guiochon 1989b, following Helfferich and Klein 1970) were applied. An in earlier publications missing explicit description of the shock of the first component was provided by Siitonen and Sainio (2011) solving the underlying quartic equation.

Based on earlier work of Glueckauf (1955) the ECP method was developed by Cremer and Huber (1961) assuming established adsorption equilibria and neglecting kinetic effects. Key feature of the method is the estimation of isotherm parameters from the diffusive part of a recorded overloaded elution profile. These parts are connected with the courses of local isotherm slopes. For Langmuir type isotherms the diffusive parts are always the rear (desorption) parts of the profiles. Originally considering only convex isotherms without inflection points (as the Langmuir-type isotherms), the method was adapted later to analyse also other isotherm shapes (Samuelsson et al. 2011). Attractive feature of the ECP method is the fact that it is sufficient to measure just the column response to a single injection. The injection size should be large enough to capture the nonlinear range of the adsorption isotherm. Due to its basic assumption, the method requires the usage of rather efficient columns. Recently, an extension of the method to columns with lower numbers of theoretical plates was reported (Hartig et al. 2015).

The ECP method offers a quick way to determine adsorption isotherms combined with the attractive feature that only small amounts of samples are required. These advantages make it attractive to extend the applicability of the method. Goal of this paper is to develop a ECP method for recording and analysing two-component elution profiles of systems that can be described with the binary competitive Langmuir isotherm model. We will first describe the method followed by providing for illustration simulation results. Finally, we will present results of an experimental validation study.

2 Theoretical basis of the method

A widely used column model for quantifying liquid chromatographic processes is the so-called equilibrium-dispersion model (EDM) (DeVault 1943; Ruthven 1984; Guiochon et al. 2006). The corresponding mass balance for a component *i* in a mixture of *n* components is:

$$\frac{\partial c_i}{\partial t} + F\frac{\partial q_i^*(c_1, c_2, \dots, c_n)}{\partial t} + u\frac{\partial c_i}{\partial x} = D_{app,i}\frac{\partial^2 c_i}{\partial x^2}, \; i = 1, n \quad (1)$$

where c_i are the liquid phase concentrations, q_i* are the corresponding equilibrium loadings of the stationary phase,$F = (1 - \varepsilon)/\varepsilon$ is the phase ratio connected to the total bed porosity ε, u is the linear velocity of the mobile phase, t is the time and x the space coordinate. The velocity of the liquid mobile phase is assumed to be constant. The $D_{app,i}$ are the apparent dispersion coefficients that describe as lumped parameters all band broadening effects. These coefficients are connected with the number of theoretical plates N_i and the length of the column L (Eq. 2):

$$D_{app,i} = \frac{uL}{2N_i}, \; i = 1, n \quad (2)$$

The number of theoretical plates can be estimated from recorded responses to small pulse injections assuming

Gaussian peak shapes (Guiochon et al. 2006, Schmidt-Traub et al., 2020):

$$N_i \approx 5.54\left(\frac{t_{R,i}}{w_{0.5,i}}\right)^2, \; i = 1, \mathrm{n} \tag{3}$$

In Eq. (2) the $t_{R,i}$ designate the mean retention times of the analysed peaks and the $w_{0.5,i}$ are the corresponding peak widths at half-height.

To apply the model given by Eq. (1) the adsorption equilibrium functions need to be specified. Most often applied is the classical Langmuir isotherm model (Langmuir 1918), which describes the equilibrium loadings of a component i in a mixture by the following equation:

$$q_i^* = \frac{q_{0,i}b_ic_i}{1+\sum_{j=1}^{n} b_jc_j} = \frac{H_ic_i}{1+\sum_{j=1}^{n} b_jc_j}, \; i = 1, n \tag{4}$$

In this equation the $q_{0,\mathrm{i}}$ are component specific saturation capacities of the stationary phase, the b_i are isotherm parameters which depend on the adsorption energy and the H_i are Henry constants for which holds $H_i = q_{0,i}b_i$. To provide flexibility by offering more free parameters, we will not restrict Eq. (4) below to the thermodynamically consistent case that all saturation capacities are identical (Broughton 1948).

When all dispersion and further mass transfer effects can be neglected (i.e. $D_{app} = 0$ or $N \rightarrow \infty$), the EDM (Eq. 1) renders into the ideal model of chromatography, which is the basis for equilibrium theory. The following corresponding mass balance equations were studied extensively (Rhee et al., 1970; Helfferich and Klein 1970; Rhee et al. 1986 and 1989; Golshan-Shirazi and Guiochon 1994):

$$\frac{\partial c_i}{\partial t} + F\frac{\partial q_i^*(c_1, c_2, \ldots, c_n)}{\partial t} + u\frac{\partial c_i}{\partial x} = 0, \; i = 1, n \tag{5}$$

Even though Eq. (5) describes only an idealized process, they can serve as a powerful tool to elucidate important phenomena in chromatography. Thanks to their simplicity, it is possible to derive for specific isotherm models analytical solutions. Here we will focused on available solutions for narrow rectangular pulse injections. The term "narrow" designates here injections of volumes that not allow reaching in the recorded column outlet profiles the injection concentrations (Guiochon et al. 2006).

Solving Eq. (5) requires to respect initial and boundary conditions. Assuming an initially fully regenerated column and rectangular injection profiles holds:

$$c_i(x, t = 0) = 0, \; i = 1, n \tag{6}$$

$$\begin{aligned} &c_i(x = 0, t) = c_{i,inj} \text{ for } 0 < t \le t_{inj} \text{ and} \\ &c_i(x = 0, t) = 0 \text{ for } t > t_{inj}, \; i = 1, n \end{aligned} \tag{7}$$

In Eq. (7) t_{inj} stands for the injection time and $c_{i,inj}$ for the injection concentration of component i. The shapes of the elution profiles predicted by equilibrium theory depend on the courses of the adsorption isotherms. An overloaded chromatogram for a two-component mixture following the competitive Langmuir isotherm (Eq. 4) and characterized by incomplete breakthrough and limited resolution is illustrated in Fig. 1. The chromatogram consists of three zones (Z = I, II and III). Zone I contains only the first eluting component 1. Zone II represents a mixed zone and contains both components. This zone starts with the shock front of the second eluting component 2 and ends with the complete elution of component 1. Zone III contains only component 2. This zone is characterized in the beginning by a plateau concentration of component 2. It finishes with the completion elution of this component.

Analytical solutions which quantitatively describe the elution profiles shown in Fig. 1 are available (Guiochon et al. 2006; Golshan-Shirazi and Guiochon 1989a, b, 1994; Siitonen and Sainio 2011). They offer explicit equations for calculating the four characteristic retention times which define the borders of the three zones and the four continuous dispersive waves. We will designate the courses of these waves below as arcs with indices for the component and the zone (i.e.: arc$_{1,\mathrm{I}}$, arc$_{1,\mathrm{II}}$, arc$_{2,\mathrm{II}}$ and

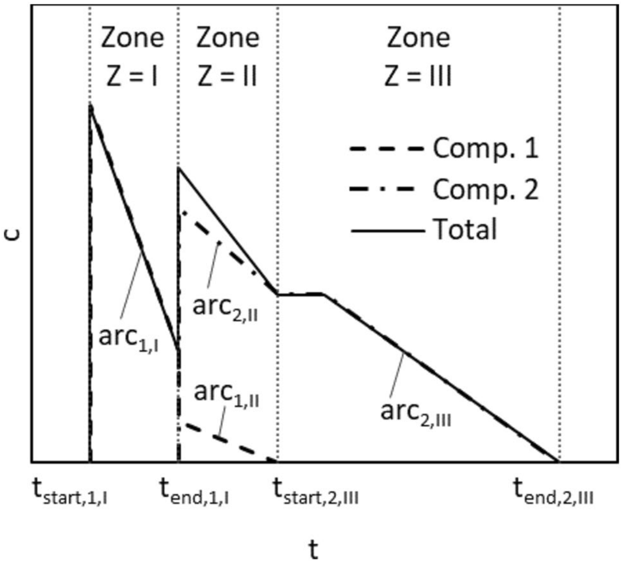

Fig. 1 Schematic representation of an ideal elution profile caused by a narrow rectangular pulse injection of a binary mixture characterized by competitive Langmuir isotherms [1]. The profile contains four characteristic arcs and can be divided into three zones Z framed by four by characteristic times

$\mathrm{arc}_{2,\mathrm{III}}$, see Fig. 1). To predict chromatograms with the equilibrium theory, besides the operating and geometric parameters, also the parameters of the adsorption isotherm model, $q_i^*\left(c_1, c_2, H_1, H_2, b_1, b_2\right), i = 1{,}2$ (Eq. 4), need to be provided. Identifying these four parameters from measured chromatograms is the objective of this paper.

2.1 Key equations of the method

The extended ECP method suggest here can be applied to analyse recorded chromatograms of binary mixtures in order to estimate adsorption isotherm parameters exploiting the analytical solutions for the chromatograms of both components summarized in the Appendix (Eqs. (13) to (26)). In particular easy to exploit are the courses of the arcs in Zone I and Zone III, in which only one component elutes, i.e. $\mathrm{arc}_{1,\mathrm{I}}$ and $\mathrm{arc}_{2,\mathrm{III}}$ (Fig. 1). For this reason we will concentrate below on the two equations that describe the retention times at which certain concentration elute at $x = L$. These key equations of the method are (see Eqs. (23) and (26)):

$$\text{For arc}_{1,\mathrm{I}}: \quad t_{R,1,\mathrm{I}}\left(c_1, x = L\right)$$
$$= t_{inj} + t_0\left(1 + H_1 F\left(\frac{1}{\left(1 + b_1 c_1\right)^2} - \frac{t_{inj}\, c_{2,inj}}{t_0\, F}\,\frac{\left(H_2 - H_1\right) b_2}{\left(H_2 - H_1 + H_2 b_1 c_1\right)^2}\right)\right)$$
$$\text{for } c_1 \text{ that lead to:} t_{start,1,I} < t_{R,1,\mathrm{I}}\left(c_1\right) < t_{end,1,I} \quad \text{(Zone I)} \tag{8}$$

$$\text{For arc}_{2,\mathrm{III}}: \quad t_{R,2,\mathrm{III}}\left(c_2, x = L\right) = t_{inj} + t_0\left(1 + \frac{H_2 F}{\left(1 + b_2 c_2\right)^2}\right) \tag{9}$$
$$\text{for } c_2 \text{ that lead to:} t_{start,2,III} < t_{R,2,\mathrm{III}}\left(c_2\right) < t_{end,2,III} \quad \text{(Zone III)}$$

It is worth noting that Eq. (9) contains only the single component isotherm parameters of component 2.

Having conducted a priori a standard pulse experiment with a non-retained component provides the column dead time and the phase ratio (t_0 and F). Having furthermore calibrated the detector applied with the pure components allows evaluating for known injection conditions (t_{inj}, $c_{1,inj}$, $c_{2,inj}$) a measured elution profile focusing on the two mentioned arcs, in which are present only the single components. Exploiting the above given theoretical solutions (Eqs. (8) and (9)) permits formulating an optimization problem for estimating the four isotherm parameters $H_1, H_2, b_1 \text{and} b_2$ minimizing a suitable objective function (OF), which evaluates for the two arcs the differences between the experimentally measured retention times as function of the concentration $t_{R,i}^{exp}\left(c_i^{exp}\right)$ and the theoretical retention times $t_{R,i}^{calc}\left(c_i^{exp}, parameters\right)$ calculated with Eqs. (8) and (9). An objective function can be constructed as the sum of the squared differences between the experimental and calculated values for arc specific j and k experimental combinations of retention times and concentrations. The number of experimental points analysed in these arcs is denoted by $n_{p,1,\mathrm{I}}$ (for component 1 in the arc of zone I) and by $n_{p,2,\mathrm{III}}$ (for component 2 in the arc of zone III). Evaluating for the sake of simplicity both branches with identical weight the overall objective function OF can be defined as the sum of two objective functions OF_1 and OF_2:

$$OF(parameters) = OF_1 + OF_2 \tag{10}$$

$$\text{with } OF_1 = \sum_{j=1}^{n_{p,1,I}}\left(\left(t_{R,1}^{exp}\left(c_1^{exp}\right)\right)_j - \left(t_{R,1}^{calc}\left(c_1^{exp}, parameters\right)\right)_j\right)^2$$
$$\text{for } c_1^{exp} \text{ such that}: t_{start,1,I} < t_{R,1}^{exp}\left(c_1^{exp}\right) < t_{end,1,I} \text{ (Zone I)} \tag{11}$$

$$\text{and } OF_2 = \sum_{k=1}^{n_{p,2,\mathrm{III}}}\left(\left(t_{R,2}^{exp}\left(c_2^{exp}\right)\right)_k - \left(t_{R,2}^{calc}\left(c_{i2}^{exp}, parameters\right)\right)_k\right)^2$$
$$\text{for } c_2^{exp} \text{ such that}: t_{start,2,III} < t_{R,2}^{exp}\left(c_1^{exp}\right) < t_{end,2,III} \text{ (Zone III)} \tag{12}$$

Minimizing OF with standard optimization software generates estimates for the four unknown parameter of the isotherm model.

Before starting the calculations it is important to is to specify the start and end positions between which the two characteristic arcs are exploitable for our purpose. These lengths depend on the specific kinetics of the chromatographic process investigated. All band broadening kinetic effects are represented in the mass balance Eq. (1) via the apparent dispersion coefficient or the corresponding plate number N. Without discussing other parameters used in solving numerically Eq. (1), Fig. 2 shows schematically for illustration two chromatograms generated for two different N values It can be seen that the parts of the characteristic arcs which are specified by the adsorption equilibrium move for reduced plate numbers away from the ideal start and end times for infinitely fast kinetics (Fig. 1) Thus, less information (indicated by the shaded ellipses) can be used for parameter estimation for lower plate numbers. This issue will be discussed based on a case study in more detail in Sect. 3.2.

3 Simulation study for method evaluation

At first the binary-mixture ECP method exploiting Eqs. (8) to (12) was tested processing chromatograms generated by solving numerically the equilibrium-dispersion model (Eq. 1) together with known parameters of the competitive Langmuir isotherm model (Eq. 4). The partial differential equations were solved using the explicit finite difference method according to the Rouchon algorithm (Rouchon et al. 1987), where the axial dispersion term is neglected and matched by a numerical dispersion term (Guiochon et al. 2006). This allowed, pre-specifying the numbers of

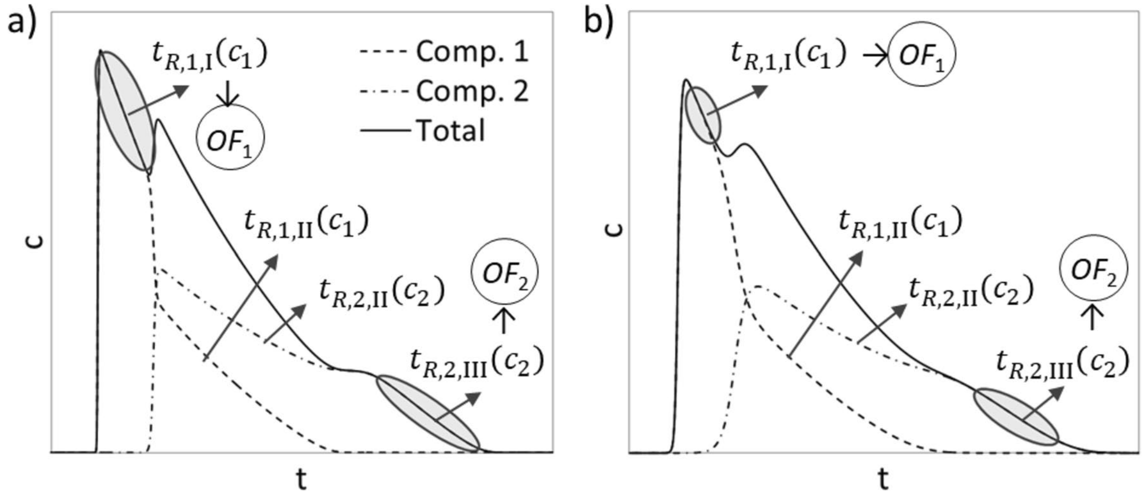

Fig. 2 Examples of binary-mixture elution profiles: Characteristic exploitable arcs are indicated by shaded ellipses (used in the objective functions OF, OF_1, OF_2, Eqs. (10)–(12), together with Eqs. (8) and (9). Two examples are shown: **a** For a highly efficient column ($N_1 = N_2 = 20{,}000$) offering long exploitable arcs (compare with the ideal case shown in Fig. 1); **b** For $N_1 = N_2 = 2000$, which provides shorter exploitable arcs

space and time increments in accordance with the number of theoretical plates. The simulations were performed in Matlab (MathWorks Inc, Natick, Massachusetts, USA, R2015b, 8.6.0.267246). The optimization needed for the estimation of the four isotherm parameters ($H_1, H_2, b_1 \text{and} b_2$) was performed by solving the described least-square problem (Eqs. (10) to (12) applying the Levenberg-Marquardt algorithm (Marquard 1963) available under Matlab.

3.1 Reference parameters

Table 1 lists the reference data used for preliminary simulations. The values of the isotherm parameters and the injection concentrations were chosen to match conventionally used HPLC column dimensions and typical operating conditions. The values for the isotherm parameters assumed are also representative for liquid chromatography studies reported in the literature (Seidel-Morgenstern 2004). The injection concentrations combined with the duration of the pulses injected are large enough to generate asymmetric overloaded chromatograms deviating from linear conditions.

Figure 3. shows two simulated chromatograms, namely a realistic one using the input parameters from Table 1 and another hypothetical one which can be considered as ideal. The latter was simulated with the same parameters, just the plate number was set very high ($N = 20{,}000$). The two elution profiles clearly follow a similar pattern. Smaller discrepancies can be recognized, in particular in the regions where the characteristic features of the bands change. The profile with the very high plate number is in excellent agreement with the predictions using the analytical solution given in the Appendix.

Table 1 Data used for the simulation study to evaluate the extended ECP method

Parameter	Value and unit
Column length (L) / inner diameter (d)	25 cm / 0.46 cm
Column hold-up time (t_0)	3.11 min
Total bed porosity (ε) / phase ratio (F)	0.75 / 0.333
Volumetric flowrate (Q) / linear velocity ($u = 4Q/(\varepsilon\pi\, d^2)$)	1 ml/min / 8.02 cm/min
Number of theoretical plates ($N_1 = N_2 = N$)	2500
Apparent dispersion coefficients ($D_{app,1} = D_{app,2} = D_{app}$)	0.00067 cm²/s
Injection time (t_{inj})[a]	1.2 min
Injection concentrations ($c_{inj,1} = c_{inj,2}$)	50 g/l
Parameters of competitive Langmuir isotherm (Eq. 4)[b]	$H_1 = 5.00$
	$b_1 = 0.0050$ l/g
	$H_2 = 7.00$
	$b_2 = 0.0060$ l/g

[a] Corresponds to injected volume (V_{inj}): $V_{inj} = Q\, t_{inj} = 1.2$ ml

[b] Provided for predicting chromatograms with Eq. (1), to be recalculated by the extended ECP method

3.1.1 Effect of arc lengths used for parameter estimation

The equilibrium controlled lengths of the two arcs in Zones I and II, which are exploitable for the purpose of this study, depend on the column efficiency (plate number). A rigorous analysis of this connection is beyond the scope of this

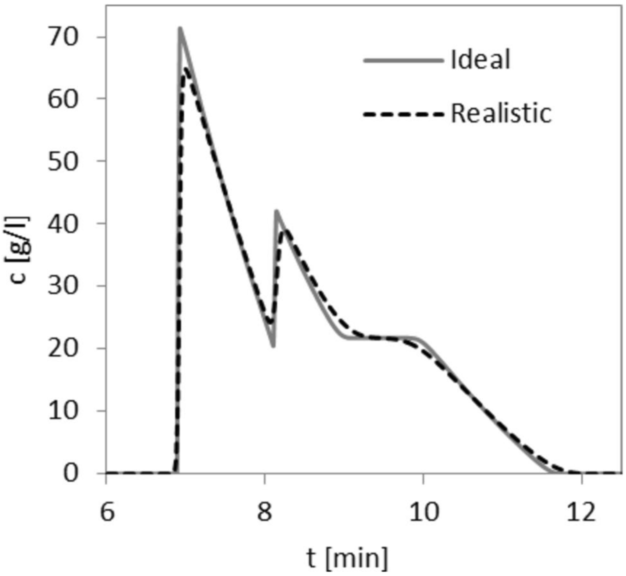

Fig. 3 Total concentrations of predicted elution profiles for a binary mixture (based on Eq. (1) and the parameters given in Table 1). The kinetically effected "realistic" case corresponds to $N_1 = N_2 = N$=2500 (Table 1), whereas the "ideal" (equilibrium controlled) case was approximated with a much larger plate number (N=20,000)

paper. Below we will just illustrate the effect for the specific parameter set of the reference study.

In Fig. 4 are shown two scenarios of analysing one and the same (predicted) chromatogram using the "whole ranges" of the two arcs and using "narrower ranges" of these arcs. In the "whole range scenario" arc$_{1,\mathrm{I}}$ starts at the peak maximum of the first component and ends at the first local minimum. The second arc$_{2,\mathrm{III}}$ is assumed to start shortly after the plateau of component 2 begins to deteriorate ends at the end of the profile (Fig. 4a). In the "narrow range scenario" the two arcs are shortened on both ends by reducing the number of points evaluated in the objective functions OF_1 and OF_2 (Eqs. (11) and (12) with smaller values $n_{p,1,I}$ and $n_{p,2,III}$). In this way the regions most strongly effected by kinetics are excluded from the analysis. In Fig. 4b shows the quite drastically performed reduction of the two characteristic arcs lengths.

Table 2 presents for the two scenarios of arc lengths the isotherm parameter values obtained using Eqs. (8) to (12) along with the original (pre-specified) isotherm parameters (Table 1). A general trend is that all four parameters are slightly higher than the pre-specified ones. Hereby the deviation is larger for the scenario evaluating the whole data range. In general the agreement with the original isotherm parameters is rather good. This is even more pronounced inspecting the courses of the re-calculated isotherms (Fig. 5a), which only slightly differ from the pre-specified ones. The comparison of the chromatograms (Fig. 5b) reveals that the simulation with both sets of re-calculated parameters predicts very well the exploited parts of the profile that belong to the two arcs in which the two components elute alone. This is seen as an important result, since in chromatographic separation processes these "extreme" arcs are typically of the largest interest, because they contain the components in pure form.

Summarizing the results of this parametric study, it is recommended not to use in the evaluation process the kinetically effected first and last sections of the two characteristic arcs. The reduction of data points can be done pragmatically a posteriori inspecting the shape of the chromatogram recorded.

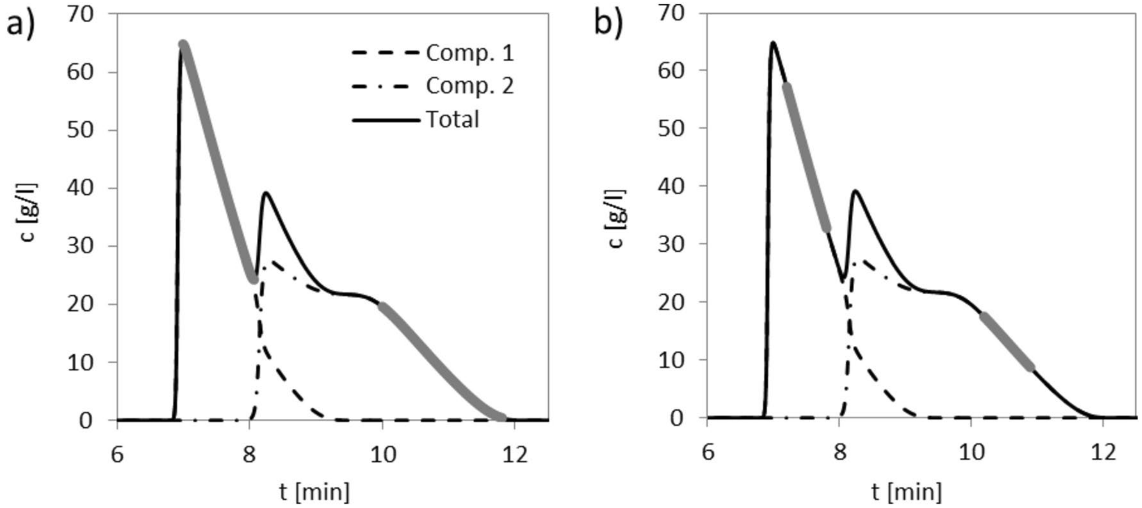

Fig. 4 Different lengths of the characteristic arcs used for isotherm estimation. The arcs exploited are indicated by thicker grey lines. **a** The arcs comprise the whole data range; **b** The arcs comprise significantly reduced (narrower) data ranges

Table 2 Competitive Langmuir adsorption isotherm parameters (H_i, b_i, i = 1, 2, Eq. (4)) as determined from chromatogram shown in Fig. 4 for two scenarios of exploited time intervals of the characteristic arcs

Isotherm parameters	Original values	Selected times and re-calculated isotherm parameters (relative errors in brackets)	
		Larger range analysed $t_{1,\mathrm{I}}$= [6.99 min, 8.07 min] $t_{2,\mathrm{III}}$ = [10.0 min, 11.8 min]	Narrower range analysed $t_{1,\mathrm{I}}$ = [7.20 min, 7.81 min] $t_{2,\mathrm{III}}$ = [10.2 min, 10.9 min]
H_1 [-]	5.00	5.32 (6.5%)	5.27 (5.4%)
b_1 [l/g]	0.0050	0.0058 (16%)	0.0056 (12%)
H_2 [-]	7.00	7.17 (2.4%)	7.14 (2.1%)
b_2 [l/g]	0.0060	0.0071 (18%)	0.0069 (15%)

3.1.2 Effect of plate numbers N

To study the effect of finite process kinetics we generated based on Eq. (1) more chromatograms with the data given in Table 1 using different plate numbers (625, 1250, 2500 and 5000). In each case the lengths of the characteristic arcs were evaluated as described above exploiting a reduced narrower data range (Fig. 4b). The re-calculated Langmuir isotherm parameters for the four different plate numbers are summarized in Table 3. They are all larger than the original values. The results reveal that for increased column efficiencies, all parameter values become more accurate (i.e. closer to the pre-specified values). For the most efficient case considered ($N_1 = N_2 = N$=5000), the isotherm parameters are very close to the original ones. When N was 2500, also very good parameter values were estimated, while for the column with N = 1250, the results were still satisfactory and could be considered as useful. When the column efficiency was low (e.g. N = 625), the isotherm parameters obtained were not accurate anymore. The four sets of re-calculated isotherm parameters were used to simulate the chromatogram.

Figure 6 presents the elution profiles compared to the original one. The results of this parametric study that it is advisable for successful application of the extended ECP method to use columns that offer at least 2500 theoretical

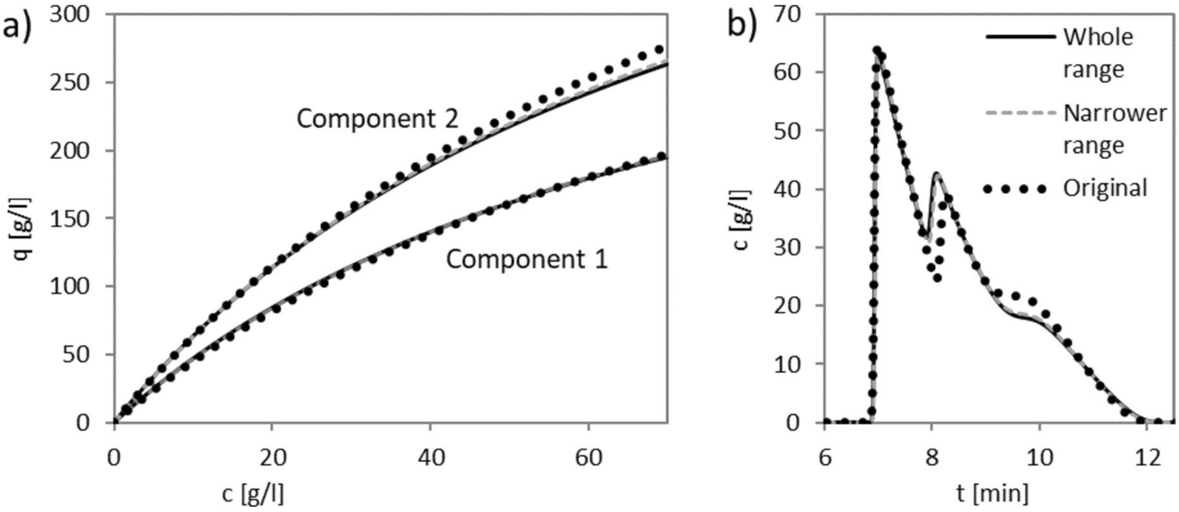

Fig. 5 Comparisons of the original isotherm based on the initially provided parameters (Table 1) and the corresponding elution profile (Fig. 4).
a Re re-calculated isotherms (Parameters in Table 2), the isotherms calculated from the whole and narrower range are almost overlapping, especially for component 1. **b** Elution profiles predicted with Eq. (1) for the two arc length scenarios using the re-calculated isotherm parameter sets

Table 3 Competitive Langmuir adsorption isotherm parameters (H_i, b_i, i = 1,2, Eq. (4)) determined using elution profiles simulated with different numbers of theoretical plates ($N1 = N2 = N$).

Isotherm parameters	Original values	Calculated values (relative errors in brackets)			
		N = 625	N = 1250	N = 2500	N = 5000
H_1 [-]	5.00	5.94 (19%)	5.48 (9.6%)	5.27 (5.4%)	5.14 (2.9%)
b_1 [l/g]	0.0050	0.0075 (50%)	0.0062 (24%)	0.0056 (12%)	0.0053 (6%)
H_2 [-]	7.00	7.41 (5.8%)	7.25 (3.6%)	7.14 (2.1%)	7.07 (1.1%)
b_2 [l/g]	0.0060	0.0086 (43%)	0.0076 (27%)	0.0069 (15%)	0.0065 (8.3%)

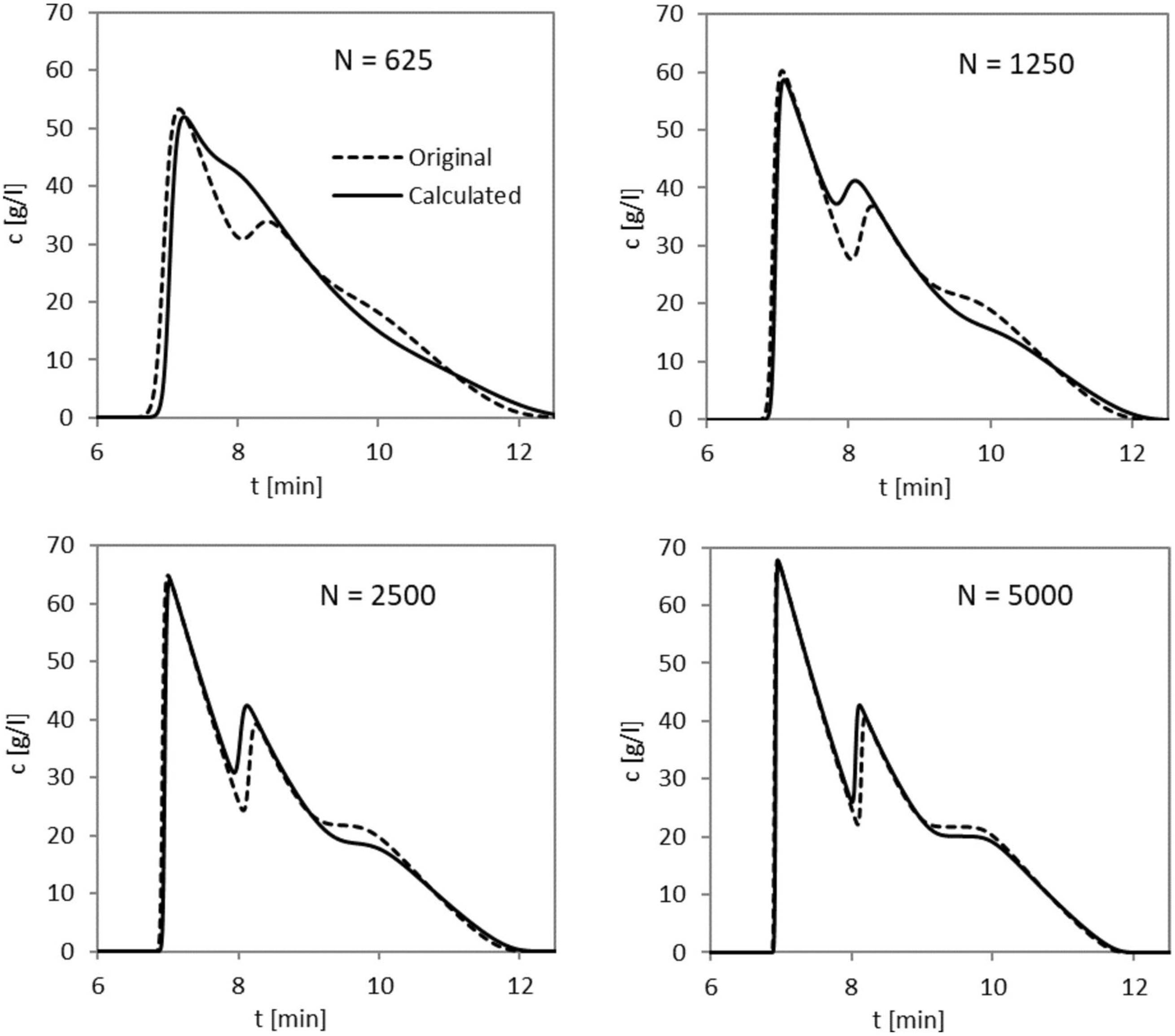

Fig. 6 Comparison of the original elution profiles (total concentrations) simulated with different numbers of theoretical plates (*N*) with predictions using the calculated isotherms

plates. For columns with *N* above 1000 the method can be still used for estimations of the band profile shapes. Even for lower efficiencies, the results can be still used to estimate the peak positions but not anymore for reliably predicting the shape of the elution profiles (as shown for 625 plates in Fig. 6a).

3.1.3 Effect of amounts injected

The analytical solutions used to estimate the isotherm model parameters with Eqs. (8) and (9) evaluate measured pairs of retention times and concentrations in Zones I and III in which pure components elute. The more the columns are overloaed the more the magnitude of these two zones shrink. This is due to the fact that the mixed Zone II expands.

Another simulation study was devoted to analyse to which extent the resolution and the lengths of the exploitable arcs affect the applicability of the method. To tests this, isotherms were re-calculated from profiles simulated using again the data in Table 1, but applying different injection times. The results for three characteristic examples are summarized in Fig. 7 and Table 4. The first example for $t_{inj} = 0.8$ min illustrates a rather high degree of separation connected with the longest exploitable arcs. The the second injection time ($t_{inj} = 1.2$) represents the reference case (Table 1) that was already analysed in the previous sections. The third example

Table 4 Competitive Langmuir adsorption isotherm parameters (H_i, b_i, $i = 1,2$, Eq. (4)) determined using simulated elution profiles for three different injection times (t_{inj})

Isotherm parameters	Original values	Calculated isotherm parameters (relative errors in brackets)		
		t_{inj} = 0.8 min	t_{inj}= 1.2 min	t_{inj}= 1.85 min
H_1[-]	5.00	5.17 (3.5%)	5.27 (5.4%)	6.34 (27%)
b_1 [l/g]	0.0050	0.0054 (8.0%)	0.0056 (12%)	0.0085 (70%)
H_2 [-]	7.00	7.14 (2.1%)	7.14 (2.1%)	7.17 (2.4%)
b_2 [l/g]	0.0060	0.0069 (15%)	0.0069 (15%)	0.0070 (17%)

corresponding to the largest injection imte (t_{inj} = 1.85 min) is connceted with a poor separation and shorter arcs.

The isotherm parameters re-calculated using the profile with best peak resolution (Fig. 7a) are more accurate than those calculated from the reference case (Fig. 7b). For the third case with almost no separation (Fig. 7c), the obtained isotherm parameter differ strongly and can be used only as rough estimates. The main cause for this limited accuracy are the short lengths of the characteristic arcs, which contain only a small number of data points and are significantly affected by kinetic effects.

4 Experimental demonstration of the method

4.1 Chromatographic system and materials used

To validate the suggested extended ECP method, experiments were carried out with a binary mixture of 2-phenyl-ethanol (PE, $i = 1$) and 3-phenyl-propanol (PP, $i = 2$). Preliminary published work by Katti et al. (1990) demonstrated that these two substances exhibit strong nonlinear Langmurian behaviour when adsorbed on octadecyl silica (C18) reversed phase stationary phases and using mixtures of water and methanol as the mobile phase.

For the experimental study we used a specific C18 column (Eclipse XDB, Agilent Technologies, USA) of dimensions $L = 10$ cm and $d = 0.46$ cm. The particle size was 5 µm. The mobile phase consisted of 60/40 (volume percentages) mixtures of water/methanol (Sigma-Aldrich, France) mixture. The investigated solutes PE and PP were purchased from Sigma-Aldrich (USA) and Alfa Aesar - Thermo Fisher GmbH (Germany), respectively.

The chromatographic experiments were realized using a standard HPLC system (Agilent Technologies, 1100 Series, Palo Alto, CA, USA) equipped with a high pressure four-channel pump, a sample injector, a column thermostat to keep the temperature at 25 °C, an auto-sampler and a diode array detector. The data were processed by the software of the Agilent 1100 ChemStation for LC 3D Systems.

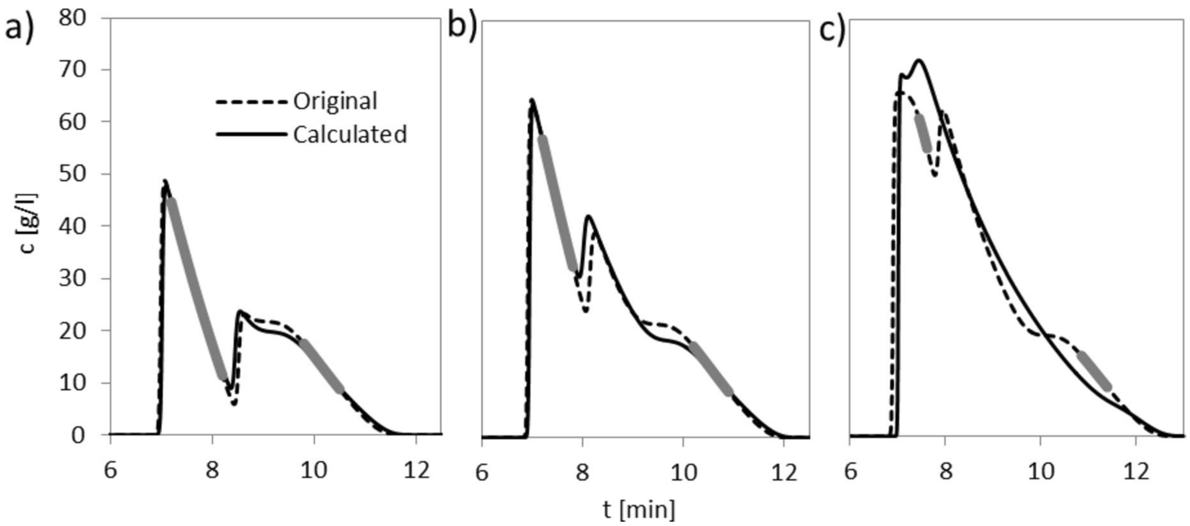

Fig. 7 Comparison of the three original elution profile (total concentrations) with simulated profiles for different amounts injected due to different injection times (t_{inj}). The simulations were generated with the corresponding re-calculated isotherm parameters (Table 4). **a** t_{inj} = 0.8 min; **b** t_{inj} = 1.2 min; **c** t_{inj} = 1.85 min. The specifically exploited characteristic arc lengths are indicated by thicker lines

4.2 Parameters and preliminary procedures

Preliminary tracer experiments were performed with small amounts of unretained thiourea (Merck, Germany) to determine the column total porosity (ε) and the phase ratio (F).

Detector signals for PE/PP mixtures were recorded at a wavelength of 275 nm, at which for both components linear responses were observed in the concentration range covered. Thus, the concentrations of the two eluting components could be calculated from the measured single component responses exploiting the component specific calibration factors.

Preliminary recording of the column response to a very small amount injected were used to estimate a mean number of theoretical plates (Eq. (3)) and to pre-inspect whether equilibrium controlled band profiles can be expected.

Small amounts of pure samples of PE and PP were needed only to carry out the calibration procedure.

The experimental details including the measured column characteristics are summarized in Table 5.

4.3 Measured chromatogram for estimating the isotherm parameters

To produce for the flowrate selected an overloaded chromatogram characterized by providing arcs in zones I and III combined with partial peak resolution a concentrated 1:1 feed mixture (total concentration (150 g/l) was prepared and an injection time of 6 s (far above analytical conditions) was considered (Table 6).

Figure 8 shows the total concentrations (i.e. the sum of both concentrations) obtained from processing the recorded UV traces at the two wavelengths together with the calibration matrix. For the conditions selected the characteristic zones I and III in which the pure components elute and also zone II can be well identified. If this would not have been observed in the first trial a repetition of the measurement with a new injection time (larger or smaller) is straightforward (respecting the trends described in Sect. 3.4).

To analyse the chromatogram, as in the simulation study presented above, two different alternative lengths of the two characteristic arcs were selected as marked in Fig. 8.

Table 5 Experimental data. Separation of 2-phenyl-ethanol (PE, component 1) and 3-phenyl-propanol (PP, component 2)

Parameter	Value
Column length (L) / inner diameter (d)	10 cm / 0.46 cm
Total bed porosity (ε) / phase ratio (F)	0.54 / 0.85
Temperature (T)	25 °C
Mean number of theoretical plates (N, Eq. (15))	3459
Mean apparent dispersion coefficient (D_{app}, Eq. (14))	0.00027 cm^2/s
Volumetric flowrate (Q) / linear velocity ($u = 4Q/(\varepsilon\pi d^2)$)	1 ml/min / 11.4 cm/min
Column hold-up time (t_0)	0.90 min

Table 6 Injection conditions used to generate an overloaded experimental chromatogram as the input information

Parameter	Value
Injection time (t_{inj})	0.1 min
Corresponds to injected volume ($V_{inj} = Q\, t_{inj}$)	100 µl
Feed concentration of each component ($c_{1,inj} = c_{2,inj}$)	75 g/l

4.4 Isotherm parameters based on the ECP method and re-calculated elution profile

Following the same procedure as for the simulation study described above, the four isotherm parameters of Eq. (4) were calculated minimizing for the two types of arc lengths the objective functions Eqs. (10)–(12). The results obtained are summarized in Table 7. It can be seen that the usage of different arc lengths does not produce very large differences in the isotherm parameters estimated.

The isotherm parameters determined were finally used for simulating the whole elution profile by solving numerically Eq. (1) order to compare the predictions with the

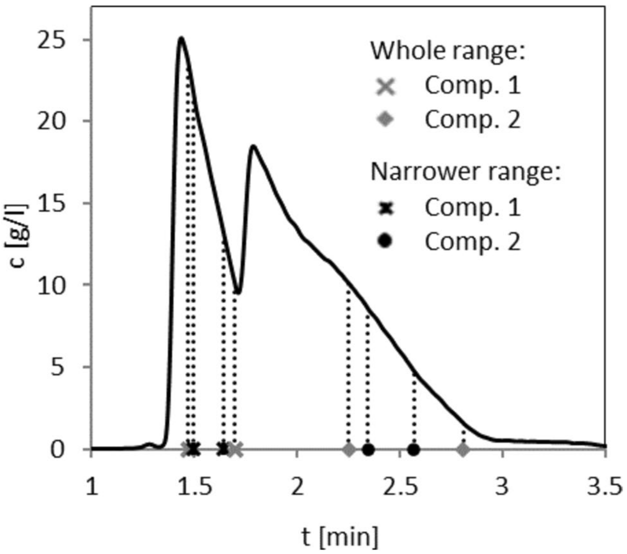

Fig. 8 Experimentally determined profile of the total concentration ($c_1 + c_2$) for an injection of a 1:1 mixture of PE and PP for the conditions given in Tables 5 and 6. Symbols and thin dotted lines represent the start and end points of the characteristic arcs selected (a wider and a narrower range)

Table 7 Competitive Langmuir adsorption isotherm parameters (H_i, b_i, $i = 1,2$, Eq. (4)) determined at 25 °C from the experimental chromatogram shown in Fig. 8. The time intervals ($t_{arc,1,I}$ and $t_{arc,2,III}$) represent two different scenarios regarding the exploited lengths of the characteristic component specific arcs

Isotherm parameters	Whole range analysed $t_{arc,1,I}$= [1.47 min, 1.70 min] $t_{arc,2,III}$= [2.25 min, 2.81 min]	Narrower range analysed $t_{arc,1,I}$= [1.50 min, 1.64 min] $t_{arc,2,III}$= [2.34 min, 2.57 min]
H_1[-]	1.63	1.68
b_1 [l/g]	0.0227	0.0241
H_2 [-]	2.55	2.54
b_2 [l/g]	0.0236	0.0232

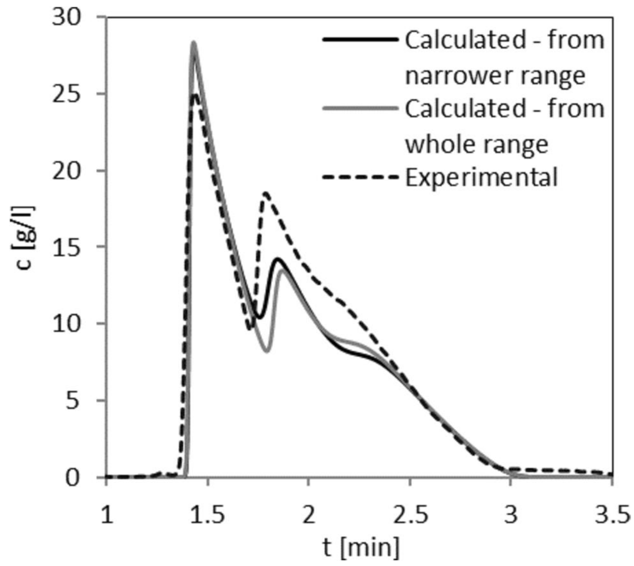

Fig. 9 Comparison of the experimental elution profile and the profiles simulated with the calculated isotherm parameters obtained by exploiting the whole and a narrower range of the characteristic arcs

experimental chromatogram. This can be considered as a validation of the extended ECP method suggested here.

In Fig. 9 are shown the relevant total effluent concentration profiles. The general position of the experimentally observed chromatogram is well described by the predictions. For both isotherm parameter sets there is an excellent matching with the experimental profile in the exploited pure component regions. However, the mixed band between the pure components could not be predicted as accurately (i.e. zone II). Hereby, the agreement is slightly better for the narrower range exploited.

5 Conclusions

A method derived from equilibrium theory and based on the analysis of dispersive fronts of chromatograms (Elution by characteristic point method) is extended to treat binary mixture elution profiles of components which follow the Langmurian adsorption isotherm model. The idea is to exploit only one measured elution profile which shows only partial peak resolution by analysing the two characteristic dispersive fronts, in which the components are pure. From these data one can calculate the isotherm parameters in a fast and easy way based on two explicit equations (Eqs. (8) and (9)). This extended ECP method allows obtaining the isotherm parameters with low amounts of sample applying well established standard experiments.

The extended ECP method was first tested in a theoretical simulation study and then validated experimentally analysing chromatograms of binary 1:1 mixtures. The results of simulations demonstrate that the method can be successfully applied for efficient columns with more than 2500 theoretical plates. To increase the precision the two characteristic arcs should not be exploited close to the edges, where kinetic effects have an increased influence. Also for columns with lower efficiency the method then still provides good estimates for the isotherm courses and the positions of the chromatograms. Since the method does not require recording single component profiles, it appears to be especially suitable for determining adsorption isotherms of enantiomers from injections of racemic mixtures. However, the applicability is not restricted to 1:1 feed mixtures.

Further work will be devoted to extend the ECP method by analysing more characteristic regions of the elution profiles for mixtures that contain more than two solutes and to apply it to systems that follow the Bi-Langmuir model or more complex isotherm models. In the latter case the courses of the characteristic arcs must be predicted using numerical methods.

Appendix

The complete analytical solution of the ideal model of chromatography (equilibrium theory) for a narrow rectangular pulse injection in case of binary Langmuir isotherm is available in the literature (Guiochon et al. 2006; Golshan-Shirazi and Guiochon 1989a, b, 1994; Siitonen and Sainio 2011). Below the equations most relevant for the ECP method described and valid for the column outlet ($x = L$) are summarized.

The first characteristic arc (arc$_{1,\mathrm{I}}$) corresponds to the part of the elution profile of the component 1 in the zone I (defined in Fig. 1). The analytical solution is expressed as dependence of the retention time $t_{R,1}(c_1)$ on the concentration of the first component (c_1):

$$t_{R,1}\left(c_1\right) = t_{inj} + t_0 + \left(t^0_{R,1} - t_0\right) \left[\frac{1}{\left(1 + b_1 c_1\right)^2} - L_{f,2}\frac{\alpha_{2,1} - 1}{\alpha_{2,1}} \cdot \frac{1}{\left(\frac{\alpha_{2,1}-1}{\alpha_{2,1}} + b_1 c_1\right)^2}\right] \tag{13}$$

In this equation $\alpha_{2,1}$ represents the selectivity under linear conditions:

$$\alpha_{2,1} = \frac{H_2}{H_1} \tag{14}$$

The column hold-up time t_0 is defined as:

$$t_0 = \frac{L}{u} = \frac{\epsilon V}{Q} \tag{15}$$

where Q is the volumetric flowrate.

The times t^0_{Ri} are the retention time under linear conditions for component i ($i = 1{,}2$):

$$t^0_{R,i} = t_0\left(1 + H_i F\right)\ i = 1{,}2 \tag{16}$$

$L_{f,2}$ denotes the loading factor for the single second component:

$$L_{f,2} = \frac{b_2 c_{2,inj} t_{inj}}{t^0_{R,2} - t_0} \tag{17}$$

The second arc of component 1 (arc$_{1,\mathrm{II}}$), i.e. the part of the elution profile of component 1 in zone II, is expressed as:

$$c_1(t) = \frac{1}{b_1 + \frac{b_2}{\left(\alpha_{2,1} r_1\right)}}\left(\sqrt{\frac{\gamma}{\alpha_{2,1}} \cdot \frac{t^0_{R,1} - t_0}{t - t_{inj} - t_0}} - 1\right) \tag{18}$$

The parameter r_1 is the positive root of the following quadratic equation:

$$\alpha_{2,1} b_1 c_{2,inj} r^2_{1,2} - \left(\alpha_{2,1} - 1 + \alpha_{2,1} b_1 c_{1,inj} - b_2 c_{2,inj}\right) r_{1,2} - b_2 c_{1,inj} = 0 \tag{19}$$

The variable γ is expressed as a function of the isotherm parameters and r_1:

$$\gamma = \frac{\alpha_{2,1} b_1 r_1 + b_2}{b_1 r_1 + b_2} \tag{20}$$

The first and the second arc of component 2 (arc$_{2,\mathrm{II}}$ and arc$_{1,\mathrm{III}}$), i.e. the parts of the elution profiles for component 2 in zones II and III, are described with Eqs. (21) and (22), respectively:

$$c_2(t) = \frac{1}{b_2 + \alpha_{2,1} b_1 r_1}\left(\sqrt{\gamma \frac{t^0_{R,2} - t_0}{t - t_{inj} - t_0}} - 1\right) \tag{21}$$

$$c_2(t) = \frac{1}{b_2}\left(\sqrt{\frac{t^0_{R,2} - t_0}{t - t_{inj} - t_0}} - 1\right) \tag{22}$$

All equations that describe the component and zone specific arcs of the elution profiles can be expressed uniformly in the form of functional dependencies $t_R = f(c)$ (as already used for the first arc of component 1, Eq. (13)). The corresponding final expressions for the four arcs are:

$$t_{R,1,\mathrm{I}}\left(c_1\right) = t_{inj} + t_0\left(1 + H_1 F\left(\frac{1}{\left(1 + b_1 c_1\right)^2} - \frac{t_{inj} c_{2,inj}}{t_0 F}\frac{\left(H_2 - H_1\right) b_2}{\left(H_2 - H_1 + H_2 b_1 c_1\right)^2}\right)\right) \tag{23}$$

$$\begin{aligned} t_{R,1,\mathrm{II}}\left(c_1\right) &= t_{inj} + t_0 + \frac{t^0_{R,1} - t_0}{\frac{\alpha_{2,1}}{\gamma}\left[1 + c_1\left(b_1 + \frac{b_2}{\alpha_{2,1} r_1}\right)\right]^2} \\ &= t_{inj} + t_0 + \frac{t_0 H_1 F\left(b_1 r_1 + \frac{H_1 b_2}{H_2}\right)}{\left(b_1 r_1 + b_2\right)\left(1 + b_1 c_1 + \frac{H_1 b_2 c_1}{r_1 H_2}\right)^2} \end{aligned} \tag{24}$$

$$\begin{aligned} t_{R,2,\mathrm{II}}\left(c_2\right) &= t_{inj} + t_0 + \frac{\gamma\left(t^0_{R,2} - t_0\right)}{\left(1 + b_2 c_2 + \alpha_{2,1} b_1 c_2 r_1\right)^2} \\ &= t_{inj} + t_0 + \frac{t_0 H_2 F\left(\frac{b_1 r_1 H_2}{H_1} + b_2\right)}{\left(b_1 r_1 + b_2\right)\left(1 + b_2 c_2 + \frac{H_2 b_1 c_2 r_1}{H_1}\right)^2} \end{aligned} \tag{25}$$

$$\begin{aligned} t_{R,2,\mathrm{III}}\left(c_2\right) &= t_{inj} + t_0 + \frac{t^0_{R,2} - t_0}{\left(1 + b_2 c_2\right)^2} \\ &= t_{inj} + t_0 + \frac{t_0 H_2 F}{\left(1 + b_2 c_2\right)^2} \end{aligned} \tag{26}$$

The equations for the four times which define the start and end times of the three zones are provided in the mentioned references.

Eqs. (23) and (26) are identical to Eqs. (8) and (9), which were used in Sect. 2 for describing the extended ECP method.

Acknowledgements The authors are grateful to Luise Blach for technical support and to Jacqueline Kaufmann for help regarding the experimental investigations.

Author contributions ASM designed the project. IM carried out the experiments, did the calculations and prepared the figures. IM and ASM wrote the manuscript text. Both authors reviewed the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. The project was funded by the Max Planck Society. No other support was received.

Data availability The datasets used can be received upon request from the authors.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Not applicable.

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