This is the post-print version of a paper published in Journal of Chromatography A

Citation for the original published paper (version of record): A Fast Estimation of Adsorption Isotherm Parameters in Gradient Elution Preparative LC I: The Single Component Case. D. Åsberg, M. Leśko, M. Enmark, J. Samuelsson, K. Kaczmarski, T. Fornstedt. Journal of Chromatography A (2013) 1299, 64-70

Access to the published version may require subscription.

doi:10.1016/j.chroma.2013.05.041

N.B. When citing this work, cite the original published paper.

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Fast Estimation of Adsorption Isotherm Parameters in Gradient Elution Preparative Liquid Chromatography I: The Single Component Case

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Abstract

The inverse method is a numeric method for fast estimation of adsorption isotherm parameters directly from overloaded elution profiles. However, it has previously only been used for isocratic experiments. Here we will extend the inverse method so it can be used for gradient elution too. This extended inverse method will make it possible to study the adsorption of substances whose retention factor vary strongly with the mobile-phase composition, like peptides and proteins, where the classic methods will fail. Our extended inverse method was verified using both simulations and real experiments. For simulated overloaded elution profiles, we were able to determine almost exact Langmuir adsorption isotherm parameters with the new approach. From real experimental data, bi-Langmuir adsorption parameters were estimated using both the perturbation peak method and the extended inverse method. The shape of the acquired adsorption isotherms did match over the considered concentration range; however, the adsorption isotherm parameters found with the two methods were not the same. This is probably due to the fact that adsorption isotherm estimated with the inverse method is only a good approximation up to the highest eluted concentration in the used chromatograms. But this is not a serious drawback from a process point of view where the main objective is to make accurate predictions of elution profiles. The bi-Langmuir adsorption isotherm obtained with both methods could accurately predict the shape of overloaded elution profiles.

Keywords

RPLC; Gradient elution; Inverse method; Adsorption isotherm determination

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1 Introduction

Gradient elution was introduced for liquid chromatography (LC) in the 50's by Alm et al. [1]. Since then it has been frequently used in analytical LC, but to a lesser extent in preparative LC [2]. Early work on gradient elution in preparative LC was done by Snyder and co-workers [3,4] and their focus was on tools for rough optimization of separation systems. Theoretical studies soon showed the potential of gradient elution in preparative LC [5,6] and the optimal operating conditions was calculated for theoretical [7,8] and experimental separation systems [9]. In recent years gradient elution has successfully been incorporated into SMB, e.g. [10-12], and other continuous chromatographic modes [13,14]. Nonlinear solvent gradients have also been investigated in preparative LC and the shape of the gradient has been optimized to maximize the yield and productivity [15].

A necessary step in numeric process optimization is estimation of the adsorption isotherm [2]. However, in cases where the retention factors of the solutes are very sensitive to the modifier concentration, it can be very difficult, or even impossible, to determine nonlinear adsorption isotherms in gradient elution mode through isocratic experiments [16]. The adsorption isotherms for the solutes are first estimated for several different fractions of modifier in the eluent, here the adsorption isotherm model is assumed to be independent of the fraction of modifier used and the modifier only affects the adsorption isotherm parameters [15,17-24]. Then a function which describes the adsorption isotherm parameters variation with the modifier must be determined. We refer to this approach as the "classical approach".

The aim of this study is to extend the inverse method for gradient elution and use it on overloaded elution profiles, obtained in gradient elution mode, to estimate the adsorption isotherm and the function describing the adsorption isotherm parameters variation with the modifier. The inverse method estimates the parameters in an adsorption isotherm model by fitting calculated elution profiles to experimental ones [25,26]. In the case of gradient elution, this has to our knowledge only been done on modifier plateaus, i.e., under isocratic conditions [15,21,24].

First we verified the extended inverse method using simulated data obtained from a known adsorption isotherm. Then we estimated adsorption isotherm parameters from gradient elution experimental data using both the classical approach [15,17–24] and the extended inverse method.

2 Theory

The elution profiles was calculated using the equilibrium-dispersive (ED) model [2] because it has previously been shown to describe small molecules under gradient conditions well [21,24,27]. The differential mass transport balance for component i is,

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

where $F = (1 - \varepsilon_i)/\varepsilon_i$ is the phase ratio; ε_i is the total porosity; *w* is the interstitial mobile phase velocity; D_a the apparent dispersion coefficient; *t*, *x* are the time and axial positions in the column and *C*, *q* are the local mobile and stationary phase concentrations. The orthogonal collocation on finite elements (OCFE) method [28,29] was used to discretize the spatial derivatives of the ED model and the Adams-Moulton method implemented in the VODE procedure [30] was used to solve the system of ordinary differential equations. In all calculations the number of collocation points was equal to 3 in each element (subdomain) and the total number of elements was about one tenth of the number of theoretical plates.

At t = 0, the stationary phase was in equilibrium with pure mobile phase, i.e., the concentration of the solute studied was uniformly equal to zero. Danckwerts-type boundary conditions were used at the column inlet and outlet [31] and the inlet condition for the solute is described by the equation,

$$C_{\rm o}(t) = p_1 \left| 1 - \exp\left(-\frac{t - p_2}{p_3}\right) \right|^{p_4} \cdot \exp\left(-\frac{t - p_2}{p_5}\right),$$
 (2)

where p_1 , ..., p_5 are experimentally determined constants and C_0 is the mobile phase concentration at the column inlet. The area of the injection profile was corrected for the actual amount of solute injected into the column. When gradient elution was employed, the inlet condition for the modifier was given by,

$$\phi(t) = \begin{cases} \phi_{0} & 0 \le t < t_{p}, \\ \phi_{0} + \beta(t - t_{p}) & t_{p} \le t < t_{p} + t_{g}, \\ \phi_{0} + \Delta \phi & t_{p} + t_{g} \le t, \end{cases}$$
(3)

where ϕ_0 is the volume fraction of modifier in the mobile phase at the beginning of the gradient, t_p is the time when the gradient reaches the column inlet, β = $\Delta \phi / t_g$ is the slope of the gradient where $\Delta \phi$ is the change in modifier fraction between the beginning and the end of the gradient and t_g is the duration of the gradient. In the calculations, the modifier is treated as a second component, i.e. the system consists of the solute and the modifier. If the modifier is unretained then the gradient is simply translated along the column and the gradient outlet profile is given by substituting t_p with $t_p + t_0$ in Eq. (3). In this work the organic modifier was methanol and methanol gradients have been shown to not deform in RPLC [32]. Therefore, methanol is assumed to be unretained and this assumption was also made in a previous study on the same type of adsorbent [27].

In the inverse method the adsorption the isotherm is estimated by minimizing the sum of squared differences between experimental and calculated elution profiles. The minimization is here done by a modified least squares Marquardt method [33]. It is usually assumed that the adsorption isotherm model itself is not affected by the fraction of organic modifier in the mobile phase, only the adsorption isotherm parameters change [15,17–24]. When linear adsorption isotherm dependence on the modifier in reversed phase LC (RPLC) is considered, linear solvent strength (LSS) theory [34,35] provides the following relationship between the retention factor (*k*) and the volume fraction of organic modifier in the mobile phase, ϕ ,

$$k(\phi) = k_{o} \exp(-S\phi), \tag{4}$$

where k_0 and S are constants specific for the chromatographic system. The simplest nonlinear adsorption isotherm is the Langmuir model, which in modifier dependent form is,

$$q(C,\phi) = \frac{a_{\mathrm{I}} \exp\left(-S_{a,\mathrm{I}}\phi\right)C}{1 + b_{\mathrm{I}} \exp\left(-S_{b,\mathrm{I}}\phi\right)C},\tag{5}$$

where a_{I} is the Henry constant and b_{I} is the association equilibrium constant, which are both functions of the volume fraction of organic modifier. The exponential function describing the Henry constant is derived from LSS theory, see Eq. (4). Common practice is to use the same function for the association equilibrium constant b_{I} [15,17–21,24]. This version of the Langmuir model has been used extensively in gradient elution RPLC both for small molecules like 2-phenylethanol [17] and large molecules like peptides and proteins [19,24]. It is also used in this study, because of its simplicity, to generate simulated data.

When the adsorption energy distribution (AED) is bimodal, the bi-Langmuir adsorption isotherm can be used instead. If the exponential function derived from the LSS theory is used to describe the adsorption isotherm parameters dependence on the modifier we get that,

$$q(C,\phi) = \frac{a_{\mathrm{I}} \exp\left(-S_{a,\mathrm{I}}\phi\right)C}{1 + b_{\mathrm{I}} \exp\left(-S_{b,\mathrm{I}}\phi\right)C} + \frac{a_{\mathrm{II}} \exp\left(-S_{a,\mathrm{II}}\phi\right)C}{1 + b_{\mathrm{II}} \exp\left(-S_{b,\mathrm{II}}\phi\right)C},$$
(6)

where subscripts I and II refers to two adsorption sites with different adsorption energy. Eq. (6) is used in this study to model the adsorption of cycloheptanone on a C_{18} -column in gradient elution.

3 Experimental

3.1 Chemicals and Materials

The mobile phase consisted of varying compositions of HPLC grade methanol from Fisher Scientific (Loughborough, UK) and de-ionized water with conductivity 18.2 M Ω cm delivered from a Milli-Q Plus 185 water purification system from Millipore (Merck Millipore, MA, USA). After mixing, the mobile phases were degassed in an ultrasonic bath under low pressure. Cycloheptanone (99%), purchased from Sigma-Aldrich (Steinheim, Germany) was used as the solute.

A 150 × 4.6 mm i.d. Kromasil column (AkzoNobel Eka, Bohus, Sweden) packed with C_{18} -bonded, porous silica with an average particle diameter of 5 µm was used. The experiments were performed on an Agilent 1200 system (Palo Alto, CA, USA) consisting of a binary pump system, an auto-sampler with a 900 µL injection loop, a diode array UV detector and a column thermostat. The column temperature was held constant at 22°C with the thermostat and the flow rate was 1.0 mL/min for all experiments.

3.2 Simulated Data

A theoretical chromatographic system was used to investigate if the inverse problem can be solved for overloaded elution profiles obtained under gradient conditions. Here it was assumed that the column dimension is 150×4.6 mm, the flow rate is 1 ml/min, the column hold-up time is 1.5 min and the column efficiency is 1 000 theoretical plates. Samples, 400 µL with concentration 0.2 and 0.4 M, was introduced using rectangular injection profiles. We considered linear gradients with slopes 2 and 4 %/min that started 0.5 min after the injection started and the modifier fraction was varied from 30% to 60%. The Langmuir adsorption isotherm, see Eq. (5), with parameters presented in Table 1 was used. In total, four different elution profiles were used to estimate the adsorption isotherm parameters.

3.3 Experimental Data

At least two replicate measurements were performed for each injection. Five different mobilephase compositions, 35/65, 40/60, 46/54, 51/49 and 56/44 (v/v methanol-water), were investigated. For each mobile-phase composition a calibration curve for cycloheptanone was recorded at 280 nm and the column hold-up time was measured with methanol as marker. All calibration curves were found to be linear, but with slightly different slopes and the column holdup time decreased linearly with increasing methanol fraction. The column hold-up times were 88 s, 87 s, 86 s, 85 s and 84 s.

Perturbation pulses were created at 9 different solute concentration plateaus and the column was equilibrated for 30 min at each concentration plateau. The volume of the perturbation injections were 5 μ L and the concentration deficiency was about 10%. Below 0.4 M cycloheptanone was detected at 280 nm and for higher solute concentration plateaus the detection was made at 300 nm. These measurements were then repeated at the five different mobile-phase compositions. Hence, a total of 45 data points were recorded.

Overloaded isocratic elution profiles were recorded at all mobile-phase compositions. Here 400 μ L of the sample was injected for each elution profile and four different sample concentrations were used: 0.1, 0.2, 0.3 and 0.4 M. Overloaded gradient elution profiles were recorded using the same injection volume and sample concentrations as for the isocratic experiments. Analytical peaks were also recorded at all gradient slopes. The gradients ran from 35% to 56% methanol and the rate of change of the methanol fraction, i.e. the slopes of the gradients, was 1, 2, 3 and 4 %/min. Before each gradient run, the column was equilibrated with the initial methanol fraction. The time it took for the gradient to reach the column inlet after the start of the injection was 86 s.

3.4 Estimation of Adsorption Isotherm Parameters

Here we used the extended inverse method to estimate bi-Langmuir adsorption isotherm parameters, see Eq. (6), directly from overloaded gradient elution profiles. For each profile, the calibration curve that best matched the methanol fraction in the eluent at the time the profile was recorded was used to convert the profiles to concentration. The total area under the peaks in the elution profiles was adjusted so it matched the injected amount of analyte. The total porosity used in the calculations was 0.56 which corresponded to the column hold-up time 84 s. The number of theoretical plates used in the calculations was 2 000, which was the average of the ones determined at the five mobilephase compositions.

The estimation of the parameters in Eq. (6) was done in three steps. The purpose of the first two steps was to obtain good initial values for the parameters. In the first step we assumed linear conditions, fixed $b_{\rm I}$ = $b_{\text{II}} = S_{b,\text{I}} = S_{b,\text{II}} = 0$ and used four experimental analytical peaks obtained with linear gradients. The parameter a_{I} , a_{II} , $S_{a,I}$ and $S_{a,II}$ was then estimated by minimizing the difference in retention time between calculated and experimental peaks. In the second step $b_{\rm I}$, $b_{\rm II}$, $S_{b,\rm I}$ and $S_{b,\rm II}$ were estimated using four overloaded elution profiles obtained with the highest sample concentration, 0.4 M, in gradient elution while the parameters estimated in the first step were kept fixed. In the final step, the same elution profiles as in step two were used but all eight parameters were estimated simultaneously.

4 Results and Discussion

The goal was to use, and verify, an extended inverse method to determine adsorption isotherm parameters in gradient elution mode by using only experimental elution profiles. This was done using four steps,

- (i) Validation using simulated elution profiles, section 4.1.
- (ii) Estimation of the bi-Langmuir adsorption isotherm parameters of cycloheptanone using the classical approach on modifier plateaus, section 4.2.
- (iii) Estimation of the adsorption isotherm parameters for the same system as in (ii) using the modified inverse method and elution profiles obtained in gradient elution, section 4.3.
- (iv) Comparison of the adsorption isotherm estimated with the classic approach in (ii) and the adsorption isotherm estimated with the inverse method in (iii), section 4.4.

4.1 Validation Using Simulated Data

Using the Langmuir adsorption isotherm, Eq. (5), with the exponential relationship derived from LSS

theory, we simulated four overloaded elution profiles according to section 3.2. These simulated overloaded elution profiles were then used to estimate the four adsorption isotherm parameters with our extended inverse method. The initial parameter values used in our extended inverse method were chosen arbitrarily to resemble a realistic starting point for the calculations. The average area of overlap [36], between the original and initial elution profiles, were 39% for these initial parameter values. The actual adsorption isotherm parameters were found with the extended inverse method, see Table 1, and an area overlap of 100% was obtained. From this we can conclude that our extended inverse method can estimate the adsorption isotherm parameters for the Langmuir model.

Table 1: Langmuir adsorption isotherm parameters, see Eq. (5), for the theoretical system and the result from parameter estimation using the inverse method with the given initial values.

using the inverse method with the given initial values.									
Parameter	True value	Initial value	Estimated value						
a_{I}	150.0	125.0	150.0						
$S_{a,{\scriptscriptstyle \mathrm{I}}}$	7.500	6.800	7.501						
$b_{\rm I} [{ m M}^{{\scriptscriptstyle -1}}]$	100.0	80.00	100.0						
$S_{b,{\scriptscriptstyle \mathrm{I}}}$	7.000	7.800	7.002						

4.2 The Classical Experimental Approach

The perturbation peak (PP) method, which is a wellknown and accurate method for acquiring adsorption data [2], was used to estimate adsorption isotherm parameters of cycloheptanone at different mobilephase compositions. The adsorption data were analyzed with a rigorous method prior to the model selection [36]. The raw slope data, obtained from the PP measurements, were integrated and Scatchard plots were constructed, Fig. 1a. The Scatchard plots were nonlinear and convex downward for all methanol fractions, which indicate adsorption isotherms with heterogeneous adsorption energy distributions (AEDs) like bi-Langmuir or Tóth [36].

From the Scatchard plots and the shape of the overloaded elution profiles, Fig. 1, we see that the adsorption can be described by type I adsorption isotherms [36]. From the raw slope data [37] obtained with the PP method the AEDs were calculated with the expectation-maximization (EM) method [38,39] which does not require any a priori assumptions about the global adsorption isotherm. The AEDs were found to be bimodal for all methanol fractions except 35% methanol, see Fig. 1b. The reason for this exception is probably that higher concentrations of cycloheptanone need to be investigated in order to resolve the second site at 35% methanol. Because the other four AEDs were bimodal and common practice is to assume that the adsorption model does not change, the bi-Langmuir model seemed to be a good choice.

The perturbation data were fitted to the bi-Langmuir model with nonlinear regression, Fig. 1c, and the fit was excellent for all cases, except for 35% methanol case. The relative error in the predicted retention factor was never larger than 1.5%. When the methanol dependence of the adsorption isotherm parameters was investigated it was found that the parameters for the 35% methanol fraction were outliers. Therefore, the bi-Langmuir model was refitted to the raw data for 35% methanol without incorporating the last data point (C = 0.6 M). The result was a better fit and a much better agreement with the adsorption parameters at the other methanol fractions. In the rest of the study, this was the adsorption isotherm used for the 35% methanol eluent.

The bi-Langmuir model was validated at each methanol plateau by comparing experimental elution

profiles with those calculated with the ED model. Four different sample concentrations were used for each methanol plateau, giving a total of 20 elution profiles. The average area overlap between the experimental and calculated profiles was 96% with a minimum overlap of 90%. Three elution profiles for the highest sample concentration are presented in Fig. 1d.



Fig. 1: Determination of adsorption isotherm with the perturbation peak method. In a) Scatchard plot for the adsorption of cycloheptanone on Kromasil C18 at five different methanol-water mobile phase compositions. In b) AEDs calculated with the EM method, the energy space was divided into 300 points, $b_{\min} = 10^{-1}$, $b_{\max} = 10^2$ and the number of iterations was 10⁷. In c) The bi-Langmuir model fitted with non-linear regression (solid lines) to the raw slope data (symbols). In d) Comparison between calculated and experimental elution profiles at mobile phase compositions 56%, 46% and 35% methanol. The injection volume was 400 µL and the concentration of cycloheptanone in the samples was 0.4 M.

To model the modifier dependence of the Henry constants, a, and association equilibrium constants, b, the exponential function derived from LSS theory, Eq. (4), was fitted with nonlinear regression to the values calculated at the methanol plateaus. In Fig. 2 the logarithm of the adsorption parameters are plotted against the methanol fraction in the mobile phase together with the best fit. The Henry constants, a, could be described by LSS theory with very good result. The methanol dependence of the association equilibrium constants, b, were also well described by an exponential equation. We therefore concluded that the modifier dependent bi-Langmuir adsorption isotherm, Eq. (6), with the adsorption isotherm parameters presented in Table 2 could describe the adsorption data very well. Note that this adsorption

isotherm is only valid in the investigated intervals of solute concentrations and modifier fractions.

4.3 The Inverse Method

Using our extended inverse method we found that there were several parameter sets that gave retention times very close to the experimental ones when estimating the Henry constants, a, using linear conditions in step 1, section 3.4. This leads to difficulty finding the global minimum among several local ones. Some solutions for local minima yielded retention times which were equal to five digits. This difficulty might also lead to different sets of association equilibrium parameters, b, in the second step. The result of the inverse method is presented in Table 2 together with the result from the PP method for comparison.

Table 2: Bi-Langmuir adsorption isotherm parameters, see Eq. (6), estimated from experimental data using the perturbation peak (PP) method and the inverse method (IM).

Method	a_{I}	$S_{a,\mathrm{I}}$	<i>b</i> _I [М-1]	$\mathbf{S}_{b,\mathrm{I}}$	$a_{\scriptscriptstyle \mathrm{II}}$	$S_{a,\mathrm{II}}$	$b_{ m II}$ [M ⁻¹]	$S_{b,\mathrm{II}}$
РР	8.468	3.323	0.1715	-2.457	175.1	8.899	153.8	6.085
IM	4.166	1.064	61.73	3.474	111.4	8.243	173.5	10.16



Fig. 2: Methanol dependence of the bi-Langmuir adsorption parameters. The symbols are the parameter values found when fitting the bi-Langmuir model to the raw slope data, filled circles denote site I and open squares denote site II. Solid lines represent the best fit to Eq. (4).

4.4 Evaluation of Experimental Approaches

The estimated adsorption isotherm parameters were not the same for the two approaches in section 4.2 and 4.3, although some values were close. The raw PP data were also fitted directly to Eq. (6), i.e., without first fitting to the bi-Langmuir model on modifier plateaus. The result was slightly different parameter values than those presented for the PP method in Table 2, but they did not agree any better with the ones from the inverse method.

There are some reasons which could explain the difference between the PP method and the inverse method. The first reason is that in the PP method, solute mobile phase concentrations up to 0.6 M were used, but the highest inlet concentration in the inverse method was 0.4 M and the highest eluted concentration was 0.14 M. It is the eluted concentration which is important when using the inverse method because the maximum concentration decreases rapidly after the injection [25]. This difference in concentration affect the determination

of the adsorption parameters, especially the Henry constants, a [40,41]. When the maximum concentration used was lowered in the PP calculations the adsorption parameters calculated for modifier plateaus became closer to the ones estimated with the inverse method.

The second reason is that the PP method treats all modifier plateaus equally while in the inverse method the modifier compositions in the middle of the gradient are more important.

The third reason is that the exact shape of the gradient is not known and even if no adsorption of the modifier exists, some dispersion could be present due to the chromatographic system. The gradient shape was recorded without the column attached and the beginning and the end of the gradient was indeed somewhat smoothened.

The fourth reason is that the optimization algorithm may have stopped in a local minimum. When the theoretical data was used it was noted that the calculation accuracy needed to be high to find the global minima, but due to long calculation times it could not be increased further in the experimental case.



Fig. 3: Comparison between the bi-Langmuir adsorption isotherms with the parameters from the perturbation peak method (symbols and black lines) and the parameter values from the inverse method (red lines) at three different methanol-water mobile phase compositions.

The shape of the modifier dependent adsorption isotherm, with parameters from the two approaches, is plotted at three different modifier plateaus with the highest concentration equal to the highest one used in the inverse method, see Fig. 3. There is almost perfect agreement between the two adsorption isotherms at 46% methanol in the eluent; this was also the case for the 40% and 51% methanol compositions (not shown).



Fig. 4: Comparison between predicted (lines) and experimental (symbols) elution profiles for low and high concentration samples. The red lines are obtained with the estimated parameters from the inverse method while black lines are calculated with the parameters found with the perturbation peak method. The injection volume was 400 µL and the concentrations of cycloheptanone in the samples were 0.3 M and 0.1 M.

At 35% methanol the curves diverge marginally at the highest concentrations and at 56% the PP curve is somewhat below the inverse method curve, but overall the agreement is good at these plateaus too. It is logical that differences between the two methods can be seen at the highest and lowest modifier plateaus. The highest methanol fraction is only reached when the solute is close to the column outlet, and only for the two steepest gradient slopes, while the lowest methanol fraction only exists during the injection of the sample.

Unless the goal is a rigorous adsorption study, it is not a major drawback if the parameter values do not match if the shape of the adsorption isotherms are the same, i.e., if the shape of $q(C,\varphi)$ is preserved, the actual functions describing them are less important [25]. From a mathematical point of view, there often exist multiple sets of adsorption isotherm parameters which give almost the same functional values of the adsorption isotherm, at least over a limited range. In process chromatography the goal is to use the adsorption isotherm to predict the elution profiles at different conditions, i.e. the physical meaning of the adsorption isotherm parameters are irrelevant.

In process optimization especially, the most important thing is the models ability to make predictions which agree with experiments. To compare the ability to predict elution profiles, elution profiles were simulated using adsorption isotherm parameters from the two methods and these simulations were compared to experimental elution profiles for samples with concentration 0.1 M, 0.2 M and 0.3 M at four gradient slopes. In Fig. 4 the results for 0.1 M and 0.3 M are presented. The agreement is excellent for both approaches with an average area overlap of 97% for the PP method and 95% for the inverse method. Because only the 0.4 M elution profiles were used in the final step in the inverse method, its ability to predict low concentration profiles is not as good as that of the PP method.

5 Conclusions

In this study we showed that our extended inverse method could be used to determine single component, nonlinear adsorption isotherms directly from overloaded elution profiles obtained in gradient elution. The main advantage of this approach, compared to the classical methods, is that it makes it possible to determine adsorption isotherms in cases where isocratic experiments are not feasible, i.e. when the retention factor vary sharply with the modifier fraction. The amount of experimental work is also less because no tedious/impractical isocratic experiments are needed which saves time/money.

The new approach was first validated with a set of simulated data obtained with the Langmuir adsorption isotherm, where the modifier dependence of the parameters was described by LSS theory. We showed that the actual values of the parameters in the Langmuir adsorption isotherm could be found with our extended inverse method when using simulated overloaded elution profiles.

Then the modifier dependent adsorption isotherm of cycloheptanone was estimated using the perturbation peak method. We found that the bi-Langmuir model described the adsorption behavior well; this data was used as reference data to validate how well the new approach can estimate experimental adsorption isotherms.

Finally, the modifier dependent adsorption isotherm of cycloheptanone was estimated using the new approach. The parameters for the bi-Langmuir model estimated using the perturbation peak method did not match the ones estimated by the inverse method; however the shape of the adsorption isotherm did match. That the parameters for the bi-Langmuir model estimated using the perturbation peak method did not match the ones estimated by the inverse method is probably due to the fact that adsorption isotherm estimated with the inverse method is only a good approximation up to the highest eluted concentration in the used chromatograms. But this is not a serious drawback in process chromatography, were the goal is to make accurate predictions of the elution profiles. The ability to predict elution profiles in gradient elution was excellent for both methods, with very good area overlap between experiments and predictions.

In part II of this series we will extend the approach presented here to a binary mixture, i.e., an actual separation problem.

6 Acknowledgement

This work was supported by the Swedish Research Council (VR) in the project grant "Fundamental interactions studies on molecular aimed at preparative separations and biospecific measurements". The work was also supported by (i) the Swedish Knowledge Foundation for the KK HÖG 2011 project "Improved Purification Processes to Satisfy Modern Drug Quality Assurance and Environmental Criteria" and by (ii) the Research Council for Environment, Agricultural Sciences and Spatial Planning for the project "High-Value Compounds from Agricultural and Forestry Waste by Sustainable Methods, -an Interdisciplinary Approach for Bioresource Utilization".

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