Adsorption Isotherm Parameter Estimation in Nonlinear Liquid Chromatography

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Abstract

This thesis concerns the development and validation of methods for the industrially important area of adsorption isotherm parameter estimation in preparative, nonlinear high performance liquid chromatography (HPLC). Preparative chromatography is a powerful separation method to get pure compounds from more or less complex liquid mixtures, e.g., mixtures of mirror-image molecules. Computer simulations can be used to optimize preparative chromatography, but then competitive adsorption isotherm parameters are usually required. Here two methods to estimate adsorption isotherm parameters are treated: (i) the perturbation peak (PP) method and (ii) the inverse method (IM).

A new theory for the PP method was derived and led to a new injection technique which was validated experimentally. This injection technique solved the severe problem with vanishing peaks and enabled us to use the PP method to estimate binary competitive adsorption isotherms valid over a broad concentration range. Also, the injection technique made it possible to estimate competitive adsorption isotherms for a quaternary mixture for the first time. Finally, an interesting perturbation peak phenomenon, known as the “Helfferich Paradox”, was experimentally verified for the first time.

The IM is a relatively new method to determine adsorption isotherm parameters. It has the advantage of requiring very small samples, but also requires an advanced computer algorithm. An improved implementation of this computer algorithm was developed and tested experimentally. Also, a variant of the IM called “the inverse method on plateaus” was tested experimentally and the estimated adsorption isotherm parameters were shown to be valid over a broader concentration range than those estimated with the standard IM.

Keywords: High performance liquid chromatography (HPLC), Preparative chromatography, Competitive adsorption isotherm parameters, Perturbation peak (PP) method, Inverse method (IM), Computer simulation, Parameter estimation, Numerical method

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To my parents
This thesis is based on the following papers, which are referred to in the text by their Roman numerals I-VI.


**Papers not included in this thesis**
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Abbreviations

φ Boundary condition (injection profile)
Λ Eigenvalue matrix = diag(λi)
λ Eigenvalue of M
Ψ Perturbation peak area
μ Scalar used to determine Cinj in the PP method
Δt_{inj} Used for discrete boundary conditions
a Adsorption isotherm parameter vector
\tilde{a} Vector = \tilde{t}_{inj}(C_{inj} - C_0)
C Mobile phase concentration [M]
C_0 Initial concentration in mobile phase [M]
C_{inj} Sample concentration [M]
F Column phase ratio
HPLC High Performance Chromatography
I Unit matrix
IM Inverse Method
IMP Inverse Method on Plateaus
K_0 Jacobian of the adsorption isotherm model at C_0
L Column length [cm]
LC Liquid Chromatography
M Matrix = I + FK_0
N_{app} Apparent number of theoretical plates
N_c Number of components
N_{nj} Number of time increments corresponding to the injection time
N_t Number of time increments
N_s Number of space increments
P Eigenvector matrix M = \Psi \Psi^\dagger
PP Perturbation Peak (method)
q Stationary phase concentration [M]
t Time [s]
t_{inj} Injection time [s]
t_R Perturbation peak mean retention time
\text{t}_{stop} Total considered time range [s]
u Mobile phase linear flow velocity [cm/s]
\bar{u} Mean velocity of the components at infinite dilution [cm/s]
x Axial coordinate [cm]
1 Introduction

Liquid chromatography (LC) is a separation method of great importance to the chemical, pharmaceutical and biotechnological industry. It is based on the principle that a sample of molecules are injected into a column of an adsorbing porous material (stationary phase), and as a liquid (mobile phase) is pumped through the column, and the different kind of molecules are distributed differently between the two phases. Different types of solutes adsorb to different extents and therefore travel at different mean velocities through the column. Depending on the adsorption behaviors of the different types of molecules, they will emerge at different times from the column outlet, see Fig. 1. The technique was originally developed by the Russian botanist M.S. Tswett in 1903 [1, 2] and since then there has been an enormous development of this technique. The definite breakthrough for liquid chromatography of low molecular compounds was the introduction of chemically modified small diameter particles (3 to 10 µm) in the late 1960s [3]. The new technique became rapidly a powerful separation technique and is today called High Performance Liquid Chromatography (HPLC). Theoretical contributions to linear chromatography during this time period were summarized by Giddings [4].

![Figure 1. A basic HPLC system. A sample with two components is injected into the mobile phase stream and exits the column at partially different times. An UV-detector is used to record when the components exits, i.e., the elution profile.](image)

In analytical or linear chromatography the aim of the separation is to obtain quantitative and qualitative information about the component(s) in a sample. For this purpose small volumes of diluted samples are injected into the column. In preparative or nonlinear chromatography the goal is to isolate as much as possible of the desired components in a complex sample mixture. Therefore, high sample concentrations and/or large volumes are
injected into the column. An example of elution profiles for analytic and preparative chromatography is shown in Fig. 2. Preparative chromatography is an important tool for the purification of fine chemicals and drug products. Theoretical contributions in the multi-component case were summarized by Helfferich 1970 [5]. During the 1990s Guiochon used the multi-component theory for computer simulations [6, 7] allowing systematic investigation of fundamental issues as well as computer-assisted optimization of practical problems.

![Figure 2](image_url)

**Figure 2.** Example of component elution profiles for a two component case (dotted and solid line). In a for an analytic (linear) case and in b for a preparative (non-linear) case.

The purpose of computer simulation is to optimize the separation of a desired component from the other components. The essential input data are the competitive adsorption isotherm parameters. Single component adsorption isotherms are easy to measure. However, in all chromatographic processes of practical importance, multi-component situations prevail and thus usually require measurement of competitive adsorption isotherms. Existing methods do not give much help and this has hampered the use of computer-assisted optimization. The issue of determining competitive adsorption isotherm parameters is therefore one of the most important research topics today.

One method to determine adsorption isotherm parameters is to use the perturbation peak (PP) method in the competitive mode. This method is examined in paper I-III and adsorption isotherm parameters for a quaternary mixture (four components) were, for the first time, acquired in paper III. An interesting phenomenon when dealing with perturbation peaks is the “Helfferich paradox”, suggested theoretically in 1964 [8]. For the first time we were able to verify it experimentally in paper IV.
Another relatively new method to determine adsorption isotherm parameters is the inverse method (IM). Compared to other methods this one requires little experimental work and only small samples. However, in order to get the adsorption isotherm parameters from the experiments an advanced computer algorithm is required [9]. An improved implementation of such an algorithm is presented in paper V, its main advantage being the considerably decreased run time. One problem with the IM is that the estimated isotherm parameters are only valid over a narrow concentration interval if one uses normal injection volumes, i.e., compared to the correct adsorption isotherm, the estimated one has an error less than some error limit over some narrow concentration interval $0$ to $\bar{C}$. In order to get isotherm parameters that are valid over a broader concentration range, i.e., $0$ to $\bar{C}$ where $\bar{C} > \bar{C}$, one could use large volume injections or the new variant “inverse method on plateaus” suggested by us in paper VI.
2 Theory for liquid chromatography

2.1 Continuous Mathematical Model

The equilibrium-dispersive (ED) model can be used to describe the migration of the molecules through a chromatography column, provided that the mass transfer kinetics and column efficiency are sufficiently high [6]. The migration of each component $i$ is described by a partial differential equation with initial and boundary conditions:

$$
\frac{\partial C_i(x,t)}{\partial t} + F \frac{\partial q_i(x,t)}{\partial t} + u \frac{\partial C_i(x,t)}{\partial x} = \frac{1}{N_{ap}} \frac{\partial^2 C_i(x,t)}{\partial x^2},
$$

$$
0 \leq x \leq L, t \geq 0, i = 1 \ldots N_c,
$$

$$
C_i(x,0) = C_{0,i},
$$

$$
\frac{\partial C_i(L,0)}{\partial x} = 0,
$$

$$
C_i(0,t) = \varphi(t).
$$

Here $C_i(x, t)$ and $q_i(x, t)$ are the mobile and stationary phase concentrations of each component $i$ at the time and space coordinates $t$ and $x$. $F$, $u$, $N_{ap}$ are constants, $F$ is the column phase ratio, $u$ is the linear flow velocity and $N_{ap}$ is the so called apparent number of theoretical plates, here assumed to be equal for all components. The boundary condition, or injection profile, $\varphi(t)$, can be measured experimentally. Otherwise it is usually assumed to be rectangular, i.e.:

$$
\varphi(t) = \begin{cases} 
C_{0,i}, & 0 \leq t \leq t_{inj}, \\
C_{inj,i}, & t > t_{inj}, 
\end{cases}
$$

where $C_{inj,i}$ is the sample concentration and $t_{inj}$ is the injection time. The initial condition $C_{0,i}$ describes the column state prior to injection, i.e., the initial concentration of the component in the mobile phase. In regular chromatography the initial concentrations of all components in the mobile phase are zero. But this is not the case when using so-called plateau methods, where the column is equilibrated with a mobile phase containing the components. The outlet condition, $\frac{\partial C_i(L,0)}{\partial x} = 0$, is needed to get a well-posed problem.
Here it also assumed that the stationary phase concentrations, \( q_i \), are given by differentiable functions of the mobile phase concentrations \( C_i \). These functions are called adsorption isotherm models and are assumed to be closed parameter expressions, i.e., \( q_i = q_i(a, C) \in C^a \) where \( a \) is a vector with the parameters and \( C \) is a vector with the component concentrations. The adsorption isotherm model is non-competitive if \( q_i \) is independent of concentrations \( C_j, j \neq i \) and is competitive otherwise. The simplest non-linear, competitive adsorption isotherm model is the Langmuir model,

\[
q_i([a,b],C) = \frac{a_i C_i}{1 + \sum_{j=1}^{N} b_j C_j}.
\]

Example of a single component and a two component isotherm model is shown in Fig. 3.

2.2 Discrete Mathematical Model

It is usually not possible to solve Eq. (1) analytically, i.e., to get \( C_i(x, t) \), using pen and paper. Instead one has to solve it numerically to estimate the solution \( C_i(x, t) \) at a number of discrete points. One could, for example, use the Rouchon finite difference scheme [10]. The apparent dispersion coefficient, \( 1/N_{ap} \), is here set to zero. The outlet condition can then be ignored and the following numerical scheme is used,
Here, \( N_c \) is the number of components, \( N_i = N_{ap} \) and \( N_t = t_{stop} / \Delta t \) where \( t_{stop} \) is the total considered time range. The space- and time increments \( \Delta x, \Delta t \) are set to \( \Delta x = L / N_{ap} \), \( \Delta t = 2L / \left( N_{ap} \bar{u} \right) \) where \( \bar{u} \) is the mean velocity of the components at infinite dilution calculated from,

\[
\bar{u} = \sum_{i=1}^{N_c} \frac{u}{N_c \left( 1 + F \cdot \partial q_i / \partial C_i \right)}.
\]  

With these choices of \( \Delta x, \Delta t \) it can be shown that the numerical dissipation in the calculations approximates the diffusion term \( 2F C_i(\partial^2 C_i(\partial x^2) \) in (1) [6]. The boundary condition \( q_i \) in (1) is approximated in (4), where \( N_{ij} = \left[ t_{ij} / \Delta t \right] \) and \( \Delta t_{ij} = t_{ij} / \Delta t - N_{ij} \). In the implementation the following update formula is used,

\[
C_{i,j+1}^{n+1} = C_{i,j}^{n+1} - \frac{\Delta x}{u\Delta t} \left( C_{i,j}^n - C_{i,j}^n + F \left( q_{i,j}^n - q_{i,j}^n \right) \right).
\]  

The numerical scheme above is first-order accurate and the work is proportional to \( N_{ap}^2 \). Higher order schemes have been proposed, but they demand considerably more work and are therefore, in general, slower [11].

### 2.3 Theory for Perturbation Peaks

If one makes a small injection on a column equilibrated with a mobile phase containing the injected components the resulting elution profile will consist of a number of small peaks, so called perturbation peaks. The number of detected perturbation peaks will be equal to, or less than, the number of components in the sample/mobile phase (not counting the main solvent).
Figure 4. Perturbation peak elution profiles on a non-linear plateau for a two component case. In a using the traditional blank injection and in b using the new injection technique proposed by us in paper I. The thick solid line is the total response assuming that the two components have equal response, the thin solid and dotted line is the component elution profiles.

The previous theory for perturbation peaks in liquid chromatography only considers the retention times of the peaks, but our new theory also considers their areas. The theory for the binary case is presented papers I and II and is a special case of the general theory presented here.

Assuming that Eq. (1) holds and the injection is small one can derive expressions for both the perturbation peak retention times and areas. Here we will only show the expressions and not how they are derived but they are basically derived in the same manner as the ones presented in [12] for gas chromatography. Let

$$\mathbf{M} = \mathbf{I} + FK_0,$$

where

$$K_0 = \begin{pmatrix}
\frac{\partial q_1}{\partial C_1} & \cdots & \frac{\partial q_1}{\partial C_N}, \\
\vdots & \ddots & \vdots \\
\frac{\partial q_N}{\partial C_1} & \cdots & \frac{\partial q_N}{\partial C_N}
\end{pmatrix}_{C_0}. \tag{7}$$

Here $\mathbf{I}$ is the unit matrix and $K_0$ is the Jacobian of the adsorption isotherm models evaluated at the initial concentrations $C_{0,1}, \ldots, C_{0,N}$. Now diagonalize $\mathbf{M}$, i.e., $\mathbf{M} = \mathbf{P}\Lambda \mathbf{P}^{-1}$, where $\mathbf{P}$ is a matrix with the eigenvectors of $\mathbf{M}$ as columns and $\Lambda = \text{diag}\left(\lambda_i\right)$ is a diagonal matrix with the eigenvalues of $\mathbf{M}$ as diagonal elements. Without loss of generality, we will assume that the diagonal elements of $\Lambda$ and the columns of $\mathbf{P}$ are ordered so that

$$\lambda_1 \leq \lambda_2 \leq \ldots \leq \lambda_N.$$
One can show that the elution profile of each component will consist of \( N_c \) perturbation peaks and that the mean retention time of peak \( j \) will be equal to \( L \lambda_j / u \). Let \( \tilde{a} = t_{ij} (C_{ij} - C_0) \), then one can show that perturbation peak \( j \) in the elution profile for component \( i \) will have an area \( \psi_{ij} \) equal to
\[
\psi_{ij} = p_{ij} \sum_{k=1}^{N_c} p_{jk} \tilde{a}_k, \tag{8}
\]
where \( p_{ij} \) and \( p_{jk}^{-1} \) are elements of \( \mathbf{P} \) and \( \mathbf{P}^{-1} \). Notice that the area \( \psi_{ij} \) can be positive or negative. In practice it is not possible, in general, to detect the individual peaks of the separate components, but only the sum of all peaks of all components. The total area, \( \psi_j \), and the mean retention time, \( t_{R,j} \), of peak number \( j \) is then,
\[
\psi_j = \sum_{i=1}^{N_c} p_{ij} \cdot \sum_{k=1}^{N_c} p_{jk}^{-1} \tilde{a}_k, \tag{9}
\]
\[
t_{R,j} = L \lambda_j / u .
\]

Although we see that the number of perturbation peaks will be the same as the number of components in the sample, it is not possible to attribute any peak to an individual component, see paper II and III. This is because a peak represents the response to the perturbation of the concentrations of all sample components.
3 Summary of Papers

3.1 Paper I

Adsorption isotherm parameters determined from single-component experiments cannot always predict multi-component elution profiles satisfactorily [13, 14]. Therefore, adsorption isotherm parameters determined from multi-component experiments are desirable. Also multi-component experiments are often required when dealing with racemic mixtures, i.e., mixtures of mirror-image molecules, because the pure components (enantiomers) are not always available in large quantities. There exist only a small number of reports on the determination of multi-component isotherm parameters. Frontal analysis (FA) can be used to determine binary adsorption isotherm data, but it is time-consuming. The PP method is an alternative method to determine adsorption isotherm parameters from binary mixtures. It has been reported that the PP method works well up to weakly non-linear conditions [15, 16].

In paper I we first used the traditional PP method, i.e., small injections of pure mobile phase were made at different binary plateau concentrations. At low plateau concentrations the two perturbation peaks could be detected, but at higher plateau concentrations the second peak vanished and made it impossible to measure the required retention time. The problem with vanishing peaks is common when one have components with identical detector response, for example, UV-detection of mirror-image molecules.

Because of this problem a new injection technique, based on theory, was tested. In this technique the injections should be made with the same excess of one of the component equal to the deficiency of the other component, i.e., \( C_{inj,1} = C_{0,1} - \mu \) and \( C_{inj,2} = C_{0,2} + \mu \) for some number \( \mu \) chosen so that the concentrations always are equal to or greater than zero. It was proven that this injection will always yield two perturbation peaks with the same area but with opposite signs, i.e., one positive and the other negative. This holds regardless of the adsorption isotherm model used. By this technique the retentions can be measured for both peaks also in the high concentration range, thereby allowing the PP method to be used to determine adsorption isotherm parameters for strongly non-linear systems.
3.2 Paper II

The most practical and economical approach is to perform the PP method using only one mixture. However, most often the PP method has been performed using several mixtures of various relative compositions to obtain high accuracy, the drawback being that this approach requires pure chemicals. No comparison has been made between this method and adsorption isotherm parameters determined using only one mixture. This was done in paper II, where the new injection technique developed in paper I was used.

The adsorption isotherm parameters obtained were validated by using them to simulate elution profiles. The simulated elution profiles were compared with experimental ones. The overlap between the simulated and experimental profiles was great using only adsorption isotherm parameters determined from racemic mixtures, and this validates the accuracy of the parameters. In fact, the overlap was almost as high as when adsorption isotherm parameters determined using several plateau ratios were used. This is an important result, allowing major savings in time and money when determining competitive adsorption isotherm parameters. We also investigated how the disturbance degree and the injected volume affect the accuracy of the adsorption isotherm parameters.

3.3 Paper III

There are, so far, only two reports on the determination of adsorption isotherm data from mixtures containing more than two components. In both cases, the FA method was used for ternary mixtures (three components) [17, 18]. There are no reports on the experimental determination of isotherms for quaternary mixtures (four components) using any chromatographic method. The competitive quaternary adsorption isotherm parameters could be very valuable in separations of the four isomers of a compound with two chiral centers, i.e., four different geometric configurations of the same compound, or for the preparative separation of two components in the presence of one or two impurities.

The purpose of this paper was twofold: (i) to investigate how to detect all four perturbation peaks on a quaternary concentration plateau and to (ii) validate the accuracy of the adsorption isotherm parameters obtained directly from quaternary mixtures of 1/1/1/1 compositions. The injection technique developed and validated for the binary case in papers I and II was extended to the multi-component case, i.e., $C_{i,j}^\text{ inj} = C_{0,j} - (-1)^i \mu$, where $i$ is the component number and $\mu$ is a number chosen so that the injected concentrations always are equal to or greater than zero. This injection technique made all
perturbation peaks clearly detectable, although their areas were not the same. The accuracy of the determined adsorption isotherm parameters was validated by comparing simulated multi-component elution profiles with experimental ones.

To conclude: accurate adsorption isotherm parameters were determined without the need of pure components in large quantities. With our injection technique only about 0.3 mg of two of the components has to be available in their pure form. This should be compared with the roughly 80 mg of each pure component required if single-component adsorption isotherm parameters are to be determined. This is an important result, allowing major savings in time and money in the determination of the competitive isotherm parameters.

3.4 Paper IV

In this article a “paradox” related to perturbation peaks in non-linear chromatography was experimentally verified for the first time. This “paradox” was first suggested 40 years ago by Helfferich and Peterson in an article published in Science [8]. They theoretically predicted that when an excess of sample molecules is injected into a chromatographic column, equilibrated with a constant stream of identical molecules, the observed peak will not contain the injected molecules. Instead, the observed peak will only contain molecules from the stream, whereas the injected molecules will exit the column in a slower, “invisible” peak. It was considered paradoxical that a single injection into a single component system could cause the successive elution of two peaks.

Two different experimental strategies were employed to verify the “paradox”: (i) a radiochemical approach and (ii) a method based on the use of two mirror-image molecules (enantiomers) in a non-chiral separation system, i.e., a system that does not differentiate between them. In particular, it was verified that the mean retention time of the observed perturbation peak depends on the tangential slope of the adsorption isotherm and that the mean retention time of the injected molecules in the “invisible” perturbation peak depends on the slope of the adsorption isotherm chord.

3.5 Paper V

In this paper an improved computer algorithm for the IM is presented and tested. The IM is an attractive approach to estimate adsorption isotherm parameters in liquid chromatography; this is mainly due to its experimental
simplicity and low sample consumption. The IM is based on “tuning” the adsorption isotherm parameters so that experimental and simulated elution profiles coincide.

Because the IM often requires thousands of simulations of elution profiles it is essential that these can be calculated efficiently. By using a coarse pre-solver we were able to cut away portions in the time-space calculation domain that are constant, thereby improving the calculation speed significantly.

In order to “tune” the adsorption isotherm parameters one needs to calculate the Jacobian of the elution profiles with respect to those parameters. By using complex numbers to do the differentiation, the calculation of the Jacobian could be done accurately and with only minor modifications to the existing simulation algorithm.

We also suggested a way to deal with the fact that one nearly always measures “total detector response”, i.e., some, often non-linear, function of all the component concentrations. Instead of using experiments to get component concentrations in the experimental elution profiles, we proposed using measured response functions to convert concentration in the simulated elution profiles to “total detector response”.

By using a statistical test based on the Fisher’s test we could also determine which adsorption isotherm models that best fits the experimental profiles.

3.6 Paper VI

Unfortunately the adsorption isotherms estimated using the IM are usually only valid over a narrow concentration interval when using normal injection volumes. This is because the estimated adsorption isotherms are only valid up to the highest eluted concentration in the used elution profiles. One way to deal with this problem is by using elution profiles with extremely large injection volumes [9, 19-22].

In this paper we describe a new, alternative experimental approach, the inverse method on plateaus (IMP), which consists of using elution profiles on concentration plateaus together with the IM. The algorithm presented in paper V was used and enabled us to obtain adsorption isotherms that agreed well with those estimated by FA over the entire concentration range under consideration.
4 Future Work

The IM is a relatively new method and some of the issues that remains are:

- The run time for the algorithm is still quite long, typically several hours but this should be possible to reduce it.

- The algorithm does not guarantee convergence to a global optimum, i.e., the determined adsorption isotherm parameters might not be correct. This needs to be studied further.

- How many and what type of elution profiles should be used to get accurate results? Too many profiles mean unnecessarily long run times, but too few give inaccurate results. The type of elution profiles used is also important, as shown in paper VI. Plateau data might be very advantageous.
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6 Swedish Summary


IM är en relativt ny metod för att uppskatta adsorptionsisoterm-parametrar. Fördelen med den är att det behövs mycket små provmängder, men den kräver också en avancerad datoralgoritm. Vi utvecklade och implementerade en förbättrad variant av denna datoralgoritm vilken vi sedan testade experimentellt. Dessutom utvecklade vi en variant av IM som kallas ”inversa metoden på platåer” (IMP), den visade sig göra det möjligt att uppskatta adsorptionsisoterm-parametrar som var giltiga över ett större koncentrationsområde än de som uppskattades med vanlig IM.
7 References

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