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Accurate and rapid estimation of adsorption isotherms in liquid chromatography using the inverse method on plateaus

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Abstract

The inverse method (IM) is an attractive approach for estimating adsorption isotherm parameters in liquid chromatography (LC), mainly due to its experimental simplicity and low sample consumption. This article presents a new experimental approach, the inverse method on plateaus (IMP), which uses elution profiles on concentration plateaus together with IM. This approach enabled us to obtain very accurate adsorption isotherms that agreed well with those estimated by means of frontal analysis over the entire concentration range under consideration. IMP is recommended when accurate adsorption isotherm estimates are required, and standard IM is insufficient.

Keywords: Inverse method; Numerical method; LC; Competitive adsorption isotherms

1. Introduction

Liquid chromatography (LC) is often used for preparative purposes, for example, in the pharmaceutical industry. The yield of a multi-component separation is strongly dependent on the adsorption isotherms of all solutes in the system, because they dictate the column saturation capacities and separation factors. If the adsorption isotherms can be measured, numerical methods can be used for efficient process optimization and scale-up [1]. Numerical tools are especially important for large-scale separations due to the trend towards continuous and complex operational modes, such as recycling and simulated moving bed (SMB) [2]. The experimental method for adsorption isotherm determination needs to be not only accurate but also rapid, so that many stationary and mobile phases may be investigated in the search for optimal conditions. Furthermore, the solute consumption should be minimal.

Adsorption isotherm measurement is also an important analytical tool in the evaluation of new stationary phases. It gives a picture of the stationary phase selectivity and heterogeneity, helping us to understand what is happening inside the column. If proteins are immobilized on a stationary phase, then detailed drug–protein interaction studies may be performed. Important properties such as chiral selectivity, binding constants, drug–drug displacement, and multi-site interactions may be characterized by simply measuring the adsorption isotherms of the drug molecules to the protein stationary phase [3]. In this case, accuracy is of major importance.

Frontal analysis (FA) has been widely used for adsorption isotherm measurement [1]. The method is very accurate but suffers from several disadvantages: it is tedious, solute consumption is significant and multi-component adsorption isotherm measurement entails considerable difficulty. The perturbation method [4–6], which was recently validated for binary and quaternary systems, is more readily used for multi-component mixtures, but the solute consumption and time requirements are similar to those of FA.

The inverse method (IM) is an attractive alternative that has been developed in recent years [7–13]; it requires only a few injections with various sample concentrations, so the solute consumption and time requirements are very modest. The adsorption isotherm parameters are then estimated numerically by iteratively solving the chromatography mass balance equations, and tuning the adsorption isotherm parameters until optimal overlap (in a least squares sense) is obtained between the calculated and experimental elution profiles. The tuning requires a numerical optimization algorithm together with a partial differential equation solver.

One problem with IM is that an adsorption isotherm model must be chosen in advance. There are many such models of

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various degrees of complexity [14], and it takes considerable experience to judge from an elution profile which one applies. FA has sometimes been used at an initial stage to establish which model to use [13]; IM is then used to examine how the adsorption isotherm parameters change as salt and organic modifier levels vary.

The first use of the inverse method for determining a binary adsorption isotherm was reported in 1999 [8]. Fractionation of the eluate was performed in order to obtain the individual elution profiles, which were needed for the original version of the method. Later on, Felinger et al. modified the method, as to enable the binary adsorption isotherms of enantiomers to be determined directly from the elution profiles, without fractionation [11]. However, it was found that the obtained adsorption isotherms were satisfactory only for concentrations up to the highest eluted concentration, while deviations from the FA results increased significantly at higher concentrations. This is because the injected high-concentration pulse is very quickly diluted inside the column, so that the low-concentration range of the adsorption isotherm has a greater impact on the elution profile. There are several ways to compensate for the column dilution, for example, by using very short columns or by keeping the retention time low by other means. Extremely large sample volumes can be injected (typically 15% of the column volume) [8–12].

This work presents a straightforward alternative method that can be used to increase accuracy still further. By also using large perturbation peak profiles at a couple of concentration plateaus (see Fig. 1a) the high concentrations should be properly attributed. If the injection volume is moderate, the overloaded perturbation peaks will be separated, revealing further properties of the nonlinear adsorption isotherms. The peak shapes, retention time shifts, and degree of peak vanishing [4] are all unique attributes and should thus be valuable "identification tags" in the parameter estimation. This new approach can be considered a hybrid between the IM and the perturbation methods and will be called the inverse method on plateaus (IMP). Fig. 1b shows that even without the concentration plateaus, it is possible to achieve high elution concentrations by using a very large injection volume (11.8 times larger than that depicted in Fig. 1a). This large non-resolved elution profile does not provide the same distinct parameter "identification tags" as are observed when using the plateau approach. The simulation parameters used in Fig. 1 were kept constant, except for plateau concentrations and injection volumes.

We will apply both the IM and IMP approaches, and compare the results obtained with FA results. The competitive adsorption of the β-blockers alprenolol and propranolol on a Kromasil C18 stationary phase will be used as a model. Crucial analytical parameters, such as the highest injected concentration, injection volume, optimization algorithm, and number of profiles used in the numerical fitting will be kept the same to facilitate sound comparison. further optimization of the individual methods is beyond the scope of this initial work.

2. Theory

2.1. Column model

The equilibrium-dispersive (ED) model can be used to describe the migration of molecules through a chromatography column, provided the mass transfer kinetics and column efficiency are sufficiently high [1]. The migration of each component, i, is described by a partial differential equation containing both initial and boundary conditions, as follows:

$$\begin{cases} \dfrac{\partial C_i(x,t)}{\partial t} + F\dfrac{\partial q_i(x,t)}{\partial t} + u\dfrac{\partial C_i(x,t)}{\partial x} = D\dfrac{\partial^2 C_i(x,t)}{\partial x^2}, \\ \quad 0 \le x \le L, t \ge 0, i = 1 \ldots n, \\ C_i(x,0) = C_{0,i}, \\ C_i(0,t) = \varphi_i(t). \end{cases} \quad (1)$$

Here $C_i(x, t)$ and $q_i(x, t)$ are the mobile and stationary phase concentrations of each component, i, at time and space coordinates t and x, respectively; $F = (1 - \varepsilon_t)/\varepsilon_t$ is the column phase ratio (ε_t is the total porosity), u is the linear flow velocity, and

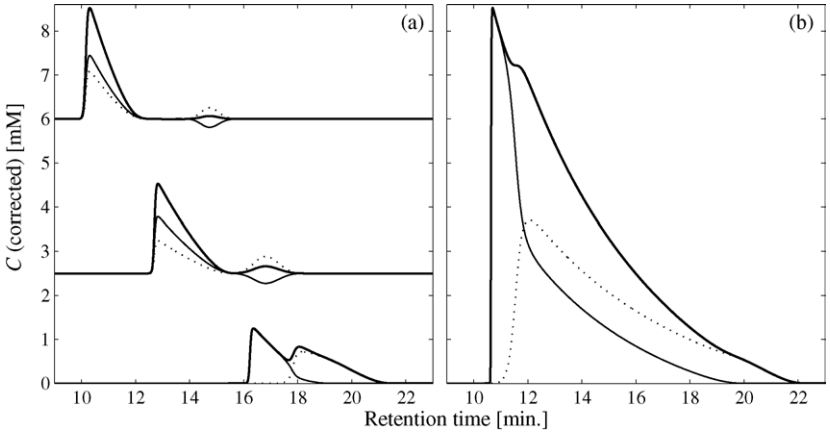

Fig. 1. Two-component simulated example of the type of elution profiles that can be used with (a) the inverse method on plateaus (IMP) and (b) the inverse method (IM). The thin lines are the individual components; the thick line the sum of both components. The sample concentration is the same in all simulations, whereas the sample volume is 11.8 larger in (b). Note how the retention times, shapes, relative compositions, and degree of peak vanishing of the large perturbation peaks are affected at the different plateaus in (a).

D is the apparent dispersion coefficient. The injection profile, φ_i, can be estimated experimentally by performing an injection directly into the detector cell [8,12]. Alternatively, the simpler but cruder rectangular pulse injection profile approximation [1] can be used, as follows:

$$\varphi_i(t) = \begin{cases} C_{\mathrm{inj},i}, & t \le t_{\mathrm{inj}}, \\ C_{0,i}, & t > t_{\mathrm{inj}}, \end{cases} \quad (2)$$

where $C_{\mathrm{inj},i}$ is the sample concentration and t_{inj} is the injection time. All results presented in this work were obtained using the experimentally measured injection profile as the boundary condition, unless stated otherwise. The initial condition, $C_{0,i}$, describes the column state prior to injection, i.e., the initial concentration of the component in the eluent. In regular elution chromatography, the initial concentrations of all components in the eluent are zero; however, this is not the case when using so-called plateau methods, in which the column is equilibrated with an eluent that does in fact contain the components.

2.2. Adsorption isotherm model

The retention time and elution profile of a component are governed by its ability to adsorb to the stationary phase surface and by how it competes with other components. Adsorption isotherm models describe the distribution between the mobile and stationary phases. In this work, the competitive bi-Langmuir model was primarily used [15], a model in which the stationary phase is assumed to consist of two types of binding sites, to which each component can adsorb, as follows:

$$q_i(C_1, C_2, \ldots, C_n) = \frac{a_{\mathrm{I},i} C_i}{1 + \sum_{j=1}^{n} b_{\mathrm{I},j} C_j} + \frac{a_{\mathrm{II},i} C_i}{1 + \sum_{j=1}^{n} b_{\mathrm{II},j} C_j}. \quad (3)$$

Here $a_{k,i}$ and $b_{k,i}$ are the parameters describing the adsorption of component i to site k (I or II). The a terms are related to the chromatographic retention factor and dictate adsorption under linear conditions. The b terms are the thermodynamic association constants for the respective binding sites. By taking the quotient, the adsorption capacity, q_s, can be calculated for each site by means of $q_{s,k,i} = a_{k,i}/b_{k,i}$.

2.3. Calculation of elution profiles

The system of mass balance equations, Eq. (1), was solved numerically using the Rouchon finite difference scheme [16]. The apparent dispersion coefficient, D, was set to zero and the time and space steps were chosen so that the numerical dispersion approximates the observed apparent dispersion. The algorithm was modified so that constant sections in the (x, t) plane were not considered in the calculations, and this decreased the calculation time drastically. The output elution profiles were converted from concentration to detector response using an empirical calibration curve. The algorithm is described in detail in Forssén et al. [17].

2.4. The inverse method (IM)

The IM estimates adsorption isotherm parameters by fitting simulated elution profiles to experimental ones. In our algorithm, the difference between the simulated and experimental elution profiles was minimized using a subspace trust region method based on the interior-reflective Newton method [18,19]. The fitting procedure requires the Jacobian of the simulated elution profile with respect to the adsorption isotherm parameters. This is calculated by means of a finite difference approximation in which the parameters are perturbed, one at a time, by a small imaginary part. A procedure was also introduced that enables quick scanning of various adsorption models [17].

3. Experimental

An Agilent 1100 chromatography system (Agilent Technologies, Palo Alto, CA, USA) was used, consisting of binary pump, auto injector, and diode array UV detector modules. A Lauda pump thermostat (Lauda, Königshofen, Germany) controlled the column temperature by circulating water through a plastic jacket in which the column was placed. PEEK capillaries (i.d. 0.13 mm) were used. A C18 column (Kromasil, Bohus, Sweden), length 150 mm, i.d. 4.6 mm, particle size 3.5 μm, pore size 100 Å, was used. The column efficiency was 17,000 plates.

Alprenolol hydrochloride and propranolol hydrochloride (>99% purity), acetonitrile (HPLC grade), and phosphoric acid and sodium hydroxide (analytical grade) were all purchased from Sigma–Aldrich. Water was of Millipore quality. The HPLC eluent consisted of acetonitrile:buffer 28:72 (v/v), using filtered (0.22 μm) sodium phosphate as the buffer (pH 2.53, ionic strength 0.10 M). Eluent reservoirs were carefully sealed to prevent acetonitrile evaporation and were degassed by ultra sonication prior to use.

Experiments were performed at $25 \pm 0.1\,^{\circ}\mathrm{C}$ and $0.70\,\mathrm{ml\,min^{-1}}$ flow. The injection volume was 50 μl (i.e., 2% of the column volume) and the sample concentrations were 0–15 mM. The column void volume was measured by means of thiourea injections. UV absorbance was measured at two different wavelengths, 250 and 330 nm.

The injection profile was estimated by displacing the column and injecting directly into the detector. We tried to maintain the same system operating conditions, even though the column was removed. Long PEEK capillaries (i.d. 0.13 mm) were connected before the injector and after the detector for this purpose.

Binary samples containing both alprenolol and propranolol were first injected at zero plateau concentration, i.e., using pure eluent. Then samples were injected onto a 0.75 mM binary plateau (eluent containing alprenolol and propranolol, both 0.75 mM). Finally samples were injected onto a 5 mM binary plateau.

Single-component and binary frontal analyses were performed for reference purposes. The analyses were performed in the staircase mode [1], using step gradients of one- or two-component eluents, respectively. Three stairs, each containing 10 steps, per analysis were constructed in this way. The low stair ranged from 3.75 to 37.5 μM, the mid stair from 75 to

750 μM and the high stair from 1.5 to 15 mM. The stairs were also used to construct non-linear concentration–response curves for alprenolol and propranolol. These curves were used to convert the simulated elution profiles from concentration to UV detector response [17].

4. Results and discussion

4.1. Frontal analysis

Binary frontal analysis was performed to obtain a reference measurement of the competitive adsorption isotherm parameters. A binary step gradient was introduced into the column and the retention times of the primary and secondary fronts and intermediate plateau compositions were measured in each step [1]. The two fronts eluted closely so the intermediate plateau and secondary front disappeared even at quite low concentrations. Consequently, no reliable adsorption isotherm parameters could be obtained using binary frontal analysis. However, single-component frontal analysis could be performed, so these results were compared with the ones obtained by the inverse methods.

4.2. IM and IMP

In this work we investigated whether IMP could be used for competitive adsorption isotherm determination and whether it can produce more accurate results than standard IM does. The methods are very similar and the same software can be used for both. In both cases, the adsorption isotherm parameters are estimated by fitting to experimental elution profiles; the difference between the methods lies in the kind of elution profiles used.

The IMP injections are performed not only on a zero plateau, but also on one or several non-zero plateaus, i.e., the eluent contains a non-zero concentration of the studied components, besides buffer and organic modifier. The eluted profiles consist of large perturbation peaks covering a concentration range determined by the chosen plateau and sample concentrations [20]. Consequently, the high-concentration region of the adsorption isotherm should have a much greater impact on the plateau elution profiles than on standard pure-eluent elution profiles.

Several injections were performed in this study, both with and without the use of alprenolol and propranolol in the eluent.

Table 1
Plateau and injection concentrations for the elution profiles used for adsorption parameter estimation with IM and IMP

IM		IMP	
C_0	C_{inj}	C_0	C_{inj}
0	0.75	0	0.75
0	5	0	15
0	10	0.75	15
0	15	5	0

Table 2
Adsorption isotherm parameters obtained using IM, IMP and FA

	Propranolol				Alprenolol			
	a_I	b_I	a_{II}	b_{II}	a_I	b_I	a_{II}	b_{II}
IM	1.53	0	3.37	515	1.66	0	3.74	514
IMP	1.37	0.43	3.51	430	1.54	3.84	3.79	473
FA	1.35	0.06	3.55	402	1.45	2.53	3.91	422

The b parameters are given in M^{-1}.

The injected sample volume was 50 μl throughout. Two sets of four elution profiles were measured and used separately to test the new method (see Table 1). The IM set corresponds to the standard inverse method with four injections onto a zero-plateau. At the lowest concentration the eluted peaks were completely resolved; at higher concentrations the propranolol and alprenolol co-eluted, and competition was pronounced. The IMP set was used in the IMP with two non-zero plateaus. It consisted of two injections on a zero plateau, one injection onto a 0.75 mM binary plateau and one injection onto a 5 mM binary plateau. UV-responses at both 250 and 330 nm were recorded and used, except for the 5 mM binary plateau where only the response at 330 nm was recorded and used.

Adsorption isotherm parameters for various models were estimated for each set of elution profiles using either IM or IMP. We found that the bi-Langmuir adsorption isotherm model, Eq. (3), gave the best results statistically; the same conclusion could be drawn from the FA experiments. The estimated parameters derived from FA, IM and IMP is presented in Table 2. Some of the experimental elution profiles used in the parameter estimations

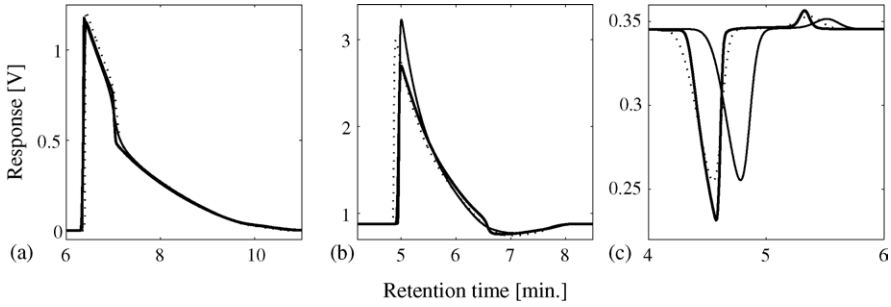

Fig. 2. Experimental and simulated binary elution profiles. The thick solid line represents the experimental profiles; the other lines are simulated profiles using the adsorption isotherm parameters presented in Table 2, the thin solid line using the IM parameters and the dotted line using the IMP parameters. In (a) the initial concentration of the components in the eluent was 0 and a binary mixture of 15 mM propranolol and alprenolol was injected. In (b) the initial concentration of both components in the eluent was 0.75 mM and a binary mixture of 15 mM propranolol and alprenolol was injected. Finally in (c) the initial concentration of both components in the eluent was 5 mM and a binary mixture containing no components was injected. For other experimental conditions, see Section 3.

are shown in Fig. 2 together with the corresponding profiles simulated using IM and IMP parameters. Due to the successful adsorption isotherm parameter estimation, most profiles overlap to a great extent. However, it must be mentioned that, since the IM parameters were not based on the plateau elution profiles the corresponding simulated profiles in Fig. 2b and c deviate considerably.

The two binding sites described by the bi-Langmuir model turned out to be very different from each other. The first site has a very low association constant, b_I, for both components, but especially for propranolol; in other words, its binding strength is weak and its capacity is very high. The second site is much stronger, in terms of binding strength ($b_{II} \gg b_I$), though its capacity is much lower.

The low binding strength of site I complicated the parameter estimation, because of the small adsorption isotherm curvature. In this case, the capacity greatly exceeds the solubility, so the estimation must be based on lower concentrations, and this introduces uncertainty. The adsorption isotherm shape in the studied concentration range may therefore be relatively insensitive to variations in the value of b_I. The b_I parameters in Table 2 are close to zero and vary considerably, in relative terms, when the different methods are compared. It is clear that the parameters obtained using IM method deviate more from the FA results than the corresponding IMP parameters do.

The single-component adsorption isotherms of propranolol and alprenolol obtained using the different methods are plotted in Fig. 3. The adsorption isotherms are remarkably linear in the high-concentration region. Standard IM obviously overestimates the adsorption, a deviation that becomes very pronounced at high concentrations, especially for alprenolol. IMP, however, gives adsorption isotherms that are very similar to the FA results. For both alprenolol and propranolol, IMP using two non-zero plateaus produces adsorption isotherms that are almost indistinguishable from those of FA across the entire range of studied concentrations (i.e., 0–15 mM), an agreement that prevails even at higher concentrations. It was calculated that when using IMP the adsorption isotherm exceeds 5% relative error at 172.5 mM for propranolol and at 106.5 mM for alprenolol. With IM the corresponding concentrations are 19.3 mM for propranolol and 10.2 mM for alprenolol. Interestingly both methods gave isotherm estimates that are valid at remarkably high concentrations. It is generally accepted that the accuracy of the inverse method is limited by the maximum eluted concentration [11,12]. Here, the error is less than 5% up to approximately 15 times the maximum eluted concentration with IM and 25 times with IMP. It should be noted, however, that the validity of the FA isotherms cannot be guaranteed at such high concentrations.

To investigate how well the binary competitive adsorption isotherms, estimated by both the IM and the IMP, agree with the FA results, we calculated the L2-error over the studied concentration range, i.e., 0–15 mM of propranolol and alprenolol. The L2-error is a measure of the distance between two adsorption isotherm surfaces; it is here defined as:

$$\frac{\sqrt{\int_\Omega (q_{i,\text{method}} - q_{i,\text{FA}})^2 \, \mathrm{d}C}}{\int_\Omega 1 \, \mathrm{d}C}, \quad \Omega = [0, 15] \times [0, 15], \qquad (4)$$

where *method* is IM or IMP; i.e., q_i is calculated using the corresponding adsorption isotherm parameters presented in Table 2. The denominator ensures that the size of the L2-error is independent of the size of the studied concentration range. The IM method gave the following L2-errors: 76.2 mM for the propranolol and 88.6 mM for the alprenolol binary adsorption isotherm. With IMP the corresponding errors were 8.6 and 27.9 mM, respectively. Using IMP the error was reduced several times compared to when no concentration plateau was used (IM). But, as mentioned earlier, the accuracy of the inverse method is generally limited by the maximum eluted concentration [10,11]. Under the present experimental conditions, maximum elution concentrations are higher in the IMP case. A fair L2-error analysis should therefore be based on concentrations ranging from zero to the maximum eluted concentrations for both the IM and IMP experiments. We call this concentration region

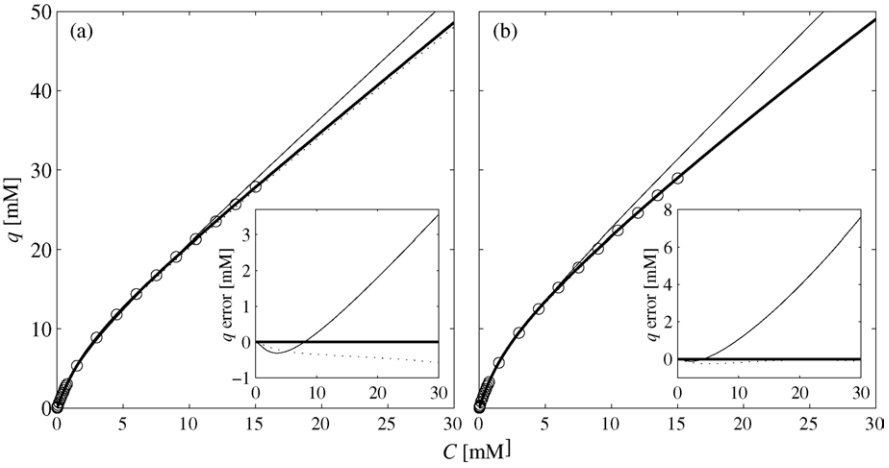

Fig. 3. Bi-Langmuir adsorption isotherm curves, in (a) for propranolol and in (b) for alprenolol. The thick solid line is the single component FA adsorption isotherm and the other are calculated using the parameters in Table 2: thin solid line is the IM adsorption isotherm, the dotted line the IMP adsorption isotherm. The differences between the FA adsorption isotherm and the other adsorption isotherms are presented in the insets.

Table 3
Calculated L2-error for the adsorption isotherms over different concentration ranges

i	L2-error (mM)			
	IM		IMP	
	Alprenolol	Propranolol	Alprenolol	Propranolol
1	35	41	19	41
2	32	36	9	31
3	23	28	9	27

$\Omega_1 = [0,C_1] \times [0,C_2]$. Such L2-errors, calculated according to,

$$\frac{\sqrt{\int_{\Omega_i} (q_{i,\text{method}} - q_{i,\text{FA}})^2 \, \mathrm{d}C}}{\int_{\Omega_i} 1 \, \mathrm{d}C}, \quad \Omega_i = [0, i \cdot C_1] \times [0, i \cdot C_2], \tag{5}$$

are presented in Table 3. In the IM experiments, the maximum eluted concentrations were 1.30 mM for propranolol and 0.62 mM for alprenolol, such that $\Omega_1 = [0,1.30] \times [0,0.62]$ mM. In the IMP experiments, the maximum eluted concentrations were 5.14 mM for propranolol and 5.00 mM for alprenolol, such that $\Omega_1 = [0,5.00] \times [0,5.14]$ mM. By studying the L2-error for different values of i, one can compare the methods accuracies at different multitudes of Ω_1. For $i = 1$, the L2-error of the alprenolol adsorption isotherm is 46% lower with IMP while no significant difference is seen in the propranolol case. For alprenolol, the isotherms obtained by IMP have much lower L2-errors even for higher i values ($i = 2$ and 3) as compared to IM. In the propranolol case, the L2-error obtained by IMP is only slightly lower than with IM ($i = 2$ and 3).

4.3. Binary FA simulation

Even though binary frontal analysis could not be used to acquire adsorption isotherm parameters in this study, the experimental curves contain valuable information that can be used in the evaluation. The binary stairs capture the competitive adsorption over a wide range of concentrations. By simulating the stairs using the adsorption isotherm parameters obtained with IM and IMP and then comparing them to the experimental binary FA response curves, the validity under competitive conditions can be examined in yet another way. The adsorption isotherm parameters (presented in Table 2) were used to simulate three binary stairs, and then the retention times of the primary fronts in the simulated and experimental binary FA response curves were measured. A high-concentration binary FA breakthrough curve is presented in Fig. 4. The average differences in retention time between the simulated and experimental fronts are shown in Table 4. The IM parameters show slightly better agreement with binary frontal analysis at the low concentrations (3.75–37.5 μM) whereas at mid (75–750 μM) and high (1.5–15 mM) concentrations the IMP parameters show significantly better agreement. Thus, the IMP parameters describe the competitive binding with high accuracy throughout the entire studied concentration range.

Table 4
Difference between experimental and simulated primary front retention times in binary FA

	Average frontal deviation (s)		
	Low	Mid	High
IM	−2.2 (±1.3)	−4.1 (±1.7)	15.4 (±9.3)
IMP	−3.2 (±1.7)	−0.9 (±2.9)	−1.6 (±4.1)

Average frontal retention times in the low (3.75–37.5 μM), mid (75–750 μM) and high (1.5–15 mM) concentration ranges were studied. Simulations were based on the adsorption isotherm parameter sets presented in Table 2.

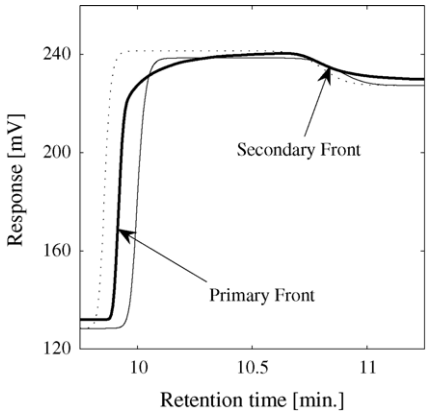

Fig. 4. Experimental and simulated binary fronts. The thick solid line is the experimental result and the other lines are simulated fronts determined using the adsorption isotherm parameters presented in Table 2: the thin solid line using the IM parameters, the dotted line the IMP parameters. The initial concentration of propranolol and alprenolol is 1.5 mM which is increased to 3 mM in the front step. The average difference in retention time between the experimental and simulated primary fronts is presented in Table 4. For other experimental conditions, see Section 3.

4.4. Prediction of elution profiles

We have seen that IMP better estimates the adsorption isotherm than standard IM does. This is important for column characterization and in drug–protein interaction studies, in which the binding constants and capacities must be accurately estimated. The same accuracy may not be required for the simulation and numerical optimization of chromatographic separations. As long as the input adsorption isotherm parameters produce a good approximation of the low- and mid-concentration regions of the true adsorption isotherm, the simulations may be sufficiently accurate. In Fig. 2, we see that both sets of parameters produce simulated elution profiles that overlap nicely with the experimental peaks. The parameters were optimized with respect to these elution profiles, so the results are not unexpected. However, it should be possible to predict other elution profiles as well, using the same parameters.

A number of binary injections with various sample concentrations were performed. The adsorption isotherm parameters obtained with standard IM and IMP with two plateaus were used as input data to simulate the elution profiles (cf. Table 2). One of the binary elution profiles is shown in Fig. 5, overlaid with the corresponding simulated profile: (a) is the sum elution profile, and (b and c) the individual elution profiles for propranolol and alprenolol, respectively. The last two profiles

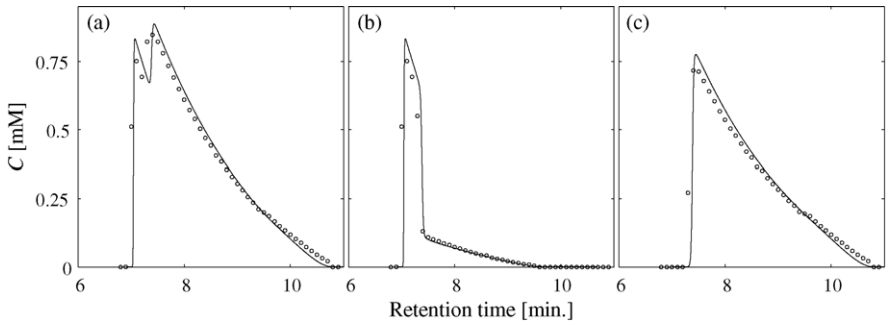

Fig. 5. Experimental and simulated binary elution profiles showing (a) the sum profile, (b) the individual profile for propranolol and (c) the individual profile for alprenolol. A sample of 5 mM propranolol and 15 mM alprenolol was injected on a zero-plateau. The symbols represent experimental data; the thin lines represent simulated profiles using the IMP adsorption isotherm parameters presented in Table 2. For other experimental conditions, see Section 3.

were extracted by a linear combination of the 250- and 330 nm concentration–response curves. The elution profile was also fractionated (circles) to confirm that the detector non-linearity introduced no serious errors. The simulated elution profiles in Fig. 5 are based on IMP parameters. Almost identical profiles were obtained using IM simulations, so those profiles are excluded for clarity. The overlap between experimental and simulated profiles is defined as follows:

$$1 - \frac{\int_0^\infty |C_{\mathrm{sim}}(t) - C_{\mathrm{exp}}(t)|\,\mathrm{d}t}{2\int_0^\infty |C_{\mathrm{exp}}(t) - C_0|\,\mathrm{d}t}, \tag{6}$$

where C_{sim} is the simulated sum elution profile, C_{exp} the measured sum elution profile and C_0 is the total initial concentration of the components in the eluent. When the profiles completely coincide the overlap is 100% and when they are totally separated the overlap is 0%. The degree of overlap was 95.8% (±1.9%) with IM and somewhat lower, 93.3% (±1.9%) with IMP; the accuracy is given here as standard deviation. If the parameter estimates are to be used only for process optimization, the more economical IM is preferable.

4.5. Impact of the boundary condition

Both rectangular and experimentally measured injection profiles were used as boundary conditions. We initially believed that the asymmetry of the injection profile had a negligible effect on the elution profiles for such small injection volumes, so that a rectangular boundary condition could be assumed. However, we found that the adsorption isotherm parameters obtained when assuming the rectangular injection profile gave somewhat worse binary elution profile predictions. Using this boundary condition in predictions and simulations, the obtained overlap decreased to 94.5% (±2.6%) with IM and 92.1% (±2.7%) with IMP, the accuracy being given here as standard deviation. It should be noted, however, that this overlap decrease is not significant at the 5% level.

The injection profile asymmetry may vary considerably from instrument to instrument so it is probably sensible always to use the measured injection profile to minimize potential systematic errors.

5. Conclusion

A hybrid between the inverse method (IM) and the perturbation method was suggested and investigated in this study. The hybrid method, the inverse method on plateaus (IMP), is very similar to IM in that adsorption isotherm parameters are obtained by numerical fitting to experimental elution profiles. The difference is that IMP uses concentration plateaus and large perturbation elution profiles. By means of this perturbation approach, adsorption nonlinearity is better attributed, possibly because the distinct and separated perturbation peaks add useful system information. The retention times, shapes, and the degree of peak vanishing are strongly dictated by the adsorption parameters. IMP represents an attempt to combine the desirable properties of both the perturbation method and IM, while excluding the undesirable properties to obtain a rapid, accurate, economical method for multi-component adsorption isotherm determination.

Standard IM as well as IMP were used to measure the competitive adsorption isotherms of alprenolol and propranolol on a Kromasil C18 column. We found that the adsorption isotherms obtained using the IMP approach agreed better with the frontal analysis (FA) results. This was especially evident in the high-concentration region, where the adsorption isotherms obtained using IM diverged clearly from those of the FA reference. Adsorption isotherms obtained using the inverse method are considered valid only up to the maximum eluted concentrations. In this study, these concentrations were found to differ for IM and IMP. We therefore performed an error analysis that took this difference into account. Also, this analysis showed that IMP gave better results than IM did, especially when considering the alprenolol isotherm. These results indicate that the new experimental approach should be more accurate than standard IM, even if the maximum eluted concentration is increased by larger injection volumes something that should be investigated experimentally.

It was shown that the error in the high concentration range has very little impact on the elution profile. Consequently, IM is suitable for use for measurements to be used in process optimization. However, in situations where higher accuracy is required, such as for column characterization and drug–protein interaction studies, where the actual isotherm parameters are of interest, the

use of IMP is worthwhile. The solute consumption is considerably higher with IMP than with IM, since stable concentration plateaus must be obtained; however, it is still approximately 10 times less than that used in frontal analysis and 15 times less than that required with the perturbation method, where 30 such plateaus are typically introduced.

The addition of concentration plateaus results in higher accuracy. Increasing the number of plateaus to three or four, or even 10 would most likely increase the accuracy even further. However, then the advantages of IM, such as rapid analysis and low sample consumption, would then be lost. A balance must thus be found between experimental complexity and accuracy. We think that IMP with one, or possibly two, concentration plateaus is sufficiently accurate, economical, and efficient for most purposes.

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