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Theoretical study of the accuracy of the elution by characteristic points method for bi-Langmuir isotherms

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Abstract

The bi-Langmuir equation has recently been proven essential to describe chiral chromatographic surfaces and we therefore investigated the accuracy of the elution by characteristic points method (ECP) for estimation of bi-Langmuir isotherm parameters. The ECP calculations was done on elution profiles generated by the equilibrium-dispersive model of chromatography for five different sets of bi-Langmuir parameters. The ECP method generates two different errors; (i) the error of the ECP calculated isotherm and (ii) the model error of the fitting to the ECP isotherm. Both errors decreased with increasing column efficiency. Moreover, the model error was strongly affected by the weight of the bi-Langmuir function fitted. For some bi-Langmuir compositions the error of the ECP calculated isotherm is too large even at high column efficiencies. Guidelines will be given on surface types to be avoided and on column efficiencies and loading factors required for adequate parameter estimations with ECP. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Many substances of interest to the pharmaceutical industry exist in two different enantiomeric forms and the enantiomers may differ in pharmacological and/or toxicological effects and even in pharmacokinetic behaviour [1]. The US Food and Drug Administration (FDA) today recommend the pharmaceutical industry to investigate the pure enantiomers of all chiral candidate drugs already at an initial stage of drug development [2]. Since thousands to hundreds of thousands of candidate drugs are screened today the recommendations has created an urgent need in the industry for fast purification methods of milligram to gram amounts of pure

enantiomers. The production of enantiomers on the semi-preparative scale is time-consuming and tedious using traditional methods such as asymmetric synthesis methods. On the other hand, preparative chromatography has increased in importance today due to new chromatographic recycling techniques, such as closed loop recycling and simulated moving bed (SMB) [3–6]. Meanwhile, a large number of new and high capacity, chiral stationary phases has been made available [7–10].

The optimization of the more complex chromatographic methods are difficult using the empirical trial and error approach [5,6]. However, the development of computer simulation programs based on the equilibrium-dispersive model has during the last 10 years [11–13] provided an excellent tool for predictions of optimal separations [12,13]. The equilibrium-dispersive model of chromatography permits

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calculation of band profiles at the outlet of a column having a finite efficiency. The simulation requires mainly the knowledge of the thermodynamic equilibria between the stationary phase and the mobile phase in the column for the actual component; i.e., the adsorption isotherm. The most common isotherm used for describing a homogeneous surface is the Langmuir adsorption isotherm. For describing a heterogeneous surface with two types of sites, often the bi-Langmuir isotherm equation is used. Recently, the bi-Langmuir isotherm equation has been found essential in describing the chromatographic surfaces for chiral separations [14–21].

The main issue today is to find adequate and fast methods for isotherm determination. There are several methods for determination of the adsorption isotherm of a substance, however, most of them are time- and substance-consuming. Frontal analysis (FA) is the traditional method [12,22,23]. In FA the area from the dead time to the self-sharpened inflection point of the breakthrough curve gives the amount adsorbed; this technique is still dominating since it is the most accurate one so far. The drawback being that the method is time- and substance-consuming [12,24]. The pulse method (PM) is an alternative method, where an injection on a concentration plateau is detected [5,12,25,26]. The retention time of the peak corresponding to the excess solute eluted is dependent on the tangential slope of the isotherm. Another alternative is the elution by characteristic points (ECP) method where the isotherm is calculated from the rear diffusive part of an eluted and overloaded peak [12,27–29]. The method is based on the use of a simple equation giving the diffuse part of an overloaded elution band. However, the ideal model is assumed, which is equivalent to assuming infinite column efficiency. Therefore, ECP is used for derivation of single-component adsorption isotherms mainly on columns with high efficiencies. The advantages of the ECP method is its simplicity, rapid data acquisition and the small amount of component required [12,28,29].

There is a need to investigate the accuracy of the ECP method for chiral separation systems where the column surface often is heterogeneous and the column efficiency is relatively low. However, the only investigations made so far on the accuracy of the ECP method has been on pure Langmuir isotherms

describing homogeneous surfaces [28,29]. Among others, Guan et al. found that the error in the ECP method varied with both the mobile phase concentration of the compound studied and with the column efficiency [28]. The guideline was given that for a homogeneous Langmuirian surface the column efficiency of 2000 theoretical plates was required for determination of the Langmuir coefficients with a systematic error of less than 3% [28]. The aim of this study is to investigate the accuracy of the ECP method for estimating isotherms on a heterogeneous surface of the chiral-type, described by the bi-Langmuir isotherm equation.

2. Theory

2.1. Adsorption isotherms

The modeling of preparative chromatography requires an isotherm equation which relate the concentrations of a component in the mobile and the solid-phases at constant temperature [12,24]. Thermodynamic valid models are used successfully for predictions of new and similar separation conditions. The simplest isotherm model of physical relevance for the one component case is the Langmuir isotherm equation

$$q(C) = \frac{aC}{1 + bC} = \frac{bq_s C}{1 + bC} \quad (1)$$

where the numerical parameter a is the equilibrium constant, the term b is the binding constant per surface area, i.e., the interaction strength of the actual adsorption site, and the term q_s , equal to a/b , is the monolayer saturation capacity. The term a is also the initial slope of the isotherm and the term b its curvature while the value of q_s is the maximum level of the isotherm; i.e., the maximum stationary phase concentration $q(C)$ at infinite high mobile phase concentration, C . A slightly more complex isotherm is the bi-Langmuir isotherm equation

$$q(C) = \frac{a_I C}{1 + b_I C} + \frac{a_{II} C}{1 + b_{II} C} \quad (2)$$

consisting of two Langmuir terms on the right side of the equality sign, added to each other. The parameters in each Langmuir term gives the thermodynamic

parameters for the specific adsorption site: i.e., its equilibrium constant, interaction strength and monolayer capacity. The bi-Langmuir isotherm is used for adsorbents with heterogeneous surfaces and has recently been used successfully for describing the adsorption of enantiomers to enantio-selective columns where often two distinct types of adsorption sites are present; one non-selective site and one chiral-selective site [14–19]. In the actual study, the non-selective site is referred to as site I and the chiral-selective as site II. In the studies done so far the chiral site has a much stronger interaction than the non-chiral one, i.e., the term b_{II} is much larger than b_I [12,14–19]. The monolayer capacity of the chiral site is generally much smaller as compared to the non-chiral one, i.e., $q_{s,II}$ is much smaller than $q_{s,I}$.

2.2. Modeling of the elution profiles

Elution profiles are calculated with excellent accuracy by using the equilibrium dispersive model which describes the mass balance in the column, and allows us to take the finite column efficiency into account [12]. The method used for approximating the solution of the mass-balance equation is the widely used forward–backward difference scheme introduced by Rouchon et al. [30]. This method replaces the axial dispersion term of the mass balance equation by numerical dispersion. It has been proven to be sufficiently accurate and is very CPU efficient [11,12,31,32].

2.3. Single-component isotherms by the ECP method

Single-component isotherms can be derived by the ECP method from the rear part of an overloaded elution profile. When a large-sized sample is injected into a chromatographic column with a convex-upwards isotherm, the most common type, an unsymmetrical band is eluted with a steep front and a diffuse rear [12,33]. The method uses the ideal model of chromatography which is equivalent to assuming that the column efficiency is infinite and that the competitive effect of all other components can be neglected. The retention time (t_R) of the

diffuse rear part of an eluted profile at mobile phase concentration C is given by the equation:

$$t_R(C) = t_p + t_0 \cdot \left(1 + \frac{1 - \epsilon}{\epsilon} \cdot \frac{dq}{dC} \right) \quad (3)$$

where t_p is the width of the rectangular injected pulse, ϵ is the porosity, $q(C)$ is the equilibrium isotherm and C is the mobile phase concentration. Eq. (3) is solved for dq/dC , giving the relationship:

$$q(C) = \frac{1}{V_a} \cdot \sum_0^C (V - V_0) \delta_i C \quad (4)$$

where V_a is the volume of adsorbent in the column, V is the retention volume of the characteristic point of the diffuse profile at concentration C , V_0 is the hold-up volume, and $\delta_i C$ is the concentration increment so that $\sum \delta_i C = C$. As the efficiency of an actual column is finite, and Eq. (3) is the solution of the rear part of an elution profile of the ideal model, Eq. (4) gives an isotherm that includes an error. However, the size of this error decreases with an increased number of theoretical plates. In addition, the data points close to the top of the profile are more affected by the source of band broadening and should not be used in the determination of the isotherm. Unfortunately, it is not possible to reject the data points close to the baseline, since the integration of the profile has to be made from 0 to C . Thus, the systematic error due to the use of the inaccurate low-concentration data for the determination of the high-concentration points of the isotherm is a drawback of ECP [12,28]. This is the source of the error generated by the ECP calculation of the isotherm; the error (i) described below. The ECP method is, however, an attractive method both financially and environmentally for determining single component adsorption isotherms, since it is simple and fast, and it requires a low sample size compared to FA. Another benefit is that ECP will give many data points, thus giving a very good precision. Notice, however, that it is not equivalent to having a good accuracy [12,28,29]. FA is still the method preferred by careful experimentalists since it is highly accurate [12].

2.4. Loading factor

The loading factor, L_p , is defined for each com-

ponent as the ratio of its amount in the sample to the column saturation capacity for that component:

$$L_f = \frac{n}{(1 - \epsilon)SLq_s} \quad (5)$$

where n is the amount of the component injected, S and L are the column geometric cross-sectional area and length [12].

3. Results and discussion

Two different errors are generated when the ECP method is used for estimating Langmuir parameters [12,28]: (i) the error of the ECP calculated isotherm as compared to the true one and (ii) the error of the fitted isotherm as compared to the ECP isotherm. The latter being the so-called model error. Since the two errors are the main sources for the inaccuracy in parameter estimation we focused on them in our study on the accuracy of the ECP method for estimating bi-Langmuir isotherm parameters. The simple Langmuir equation was used as a reference. Elution profiles were calculated using the true bi-Langmuir parameters as input data on a programming code based on the equilibrium dispersive model (see Theory) and developed in MATLAB 5.2 (MathWorks). The ECP method was applied to the elution profiles using a MATLAB code based on Eq. (4) for calculating the ECP generated isotherm. Thereafter, the ECP isotherm was fitted to the bi-Langmuir equation by nonlinear regression using the Levenberg–Marquardt method for least-squares minimization [34] as implemented in MATLAB 5.2.

3.1. Calculation of elution profiles

The column length was $L = 10.0$ cm and the inner diameter I.D. = 4.00 mm. The flow-rate was 1.00 ml/min and the hold-up volume, $V_0 = 1.00$ ml, corresponding to a porosity of $\epsilon = 0.796$. Six different column efficiencies were used ranging from $N = 200$ to 30 000. The true values used in the Langmuir reference isotherm were $a = 5.00$ and $b = 0.200$ mM^{-1} giving a monolayer capacity q_s of 25.0 mM (see Table 1). For all bi-Langmuir isotherms the parameters of site I, i.e., the high capacity site, had the same values as the Langmuir equation, i.e., $a_1 = 5.00$, and $b_1 = 0.200$ mM^{-1} (cf. Table 1). Five different values of the second bi-Langmuir term were investigated (cf. Table 1), chosen close to values earlier found for chiral stationary phases [14–21], as examples of Chiral AGP and an immobilized cellulase protein as stationary phase, CBH I [16–21].

The bi-Langmuir isotherms cases 1–3 had the following equilibrium constants: $a_{II} = 5.00$ and $b_{II} = 1.00, 10.0$ and 100 mM^{-1} (cf. Table 1). The corresponding monolayer capacities were $q_{s,II} = 5.00, 0.500$ and 0.0500 mM , respectively. In the case of the bi-Langmuir isotherms 4 and 5, the second site equilibrium constant was either lower or higher compared to the initial 3 cases, $a_{II} = 0.500$ and 50.0 respectively. The value of the term for the interaction energy was the same for the two last cases, i.e., $b_{II} = 10.0$ mM^{-1} . Thus, the monolayer capacities for cases 4 and 5 were $q_{s,II} = 0.0500$ and 5.00 mM , respectively.

Injections of two different sizes were made: 200 μl of 20.0 mM and 50.0 μl of 20.0 mM sample. The injections correspond to a loading factor of $L_f = 0.62$

Table 1

The true isotherm parameters used in the study. The homogeneous case is represented by the Langmuir parameters and five different compositions of heterogenous surfaces are represented by the five cases of bi-Langmuir parameters

Case	a_1	b_1 mM^{-1}	a_{II}	b_{II} mM^{-1}	$q_{s,I}$ mM	$q_{s,II}$ mM	Table
Langmuir	5.00	0.200			25.0		2
Bi-Langmuir 1	5.00	0.200	5.00	1.00	25.0	5.00	3
Bi-Langmuir 2	5.00	0.200	5.00	10.0	25.0	0.500	4
Bi-Langmuir 3	5.00	0.200	5.00	100	25.0	0.0500	5
Bi-Langmuir 4	5.00	0.200	0.500	10.0	25.0	0.0500	6
Bi-Langmuir 5	5.00	0.200	50.0	10.0	25.0	5.00	7

respectively, 0.16 when q_s is 25 mM. L_f for the low capacity site of the bi-Langmuir equation, site II, will be 5–500 times larger and will thus generally be $\gg 1$, i.e., the second site will always be more or less completely saturated. It is therefore irrelevant to calculate the loading factor for the low capacity site. Miyabe et al. [29] recently reported for the use of the dimensionless Langmuir isotherm that the loading factor will mainly influence the concentration range of the ECP isotherm; a larger size injection simply provides an ECP isotherm that reaches a higher concentration. This was confirmed in our study,

however, we also found that the loading factor will strongly influence the type of weighing desired for the regression of the bi-Langmuir function (see below).

Simulated elution profiles based on the Langmuir and the bi-Langmuir isotherm equations for different column efficiencies are shown in Fig. 1a and b. The bi-Langmuir equation used is case 2 in Table 1; thus $a_{II}=5.00$ and $b_{II}=10.0 \text{ mM}^{-1}$. The elution profiles when a heterogeneous surface is assumed differ markedly from the homogeneous case; the effect of the second site is a pronounced tail of the bi-

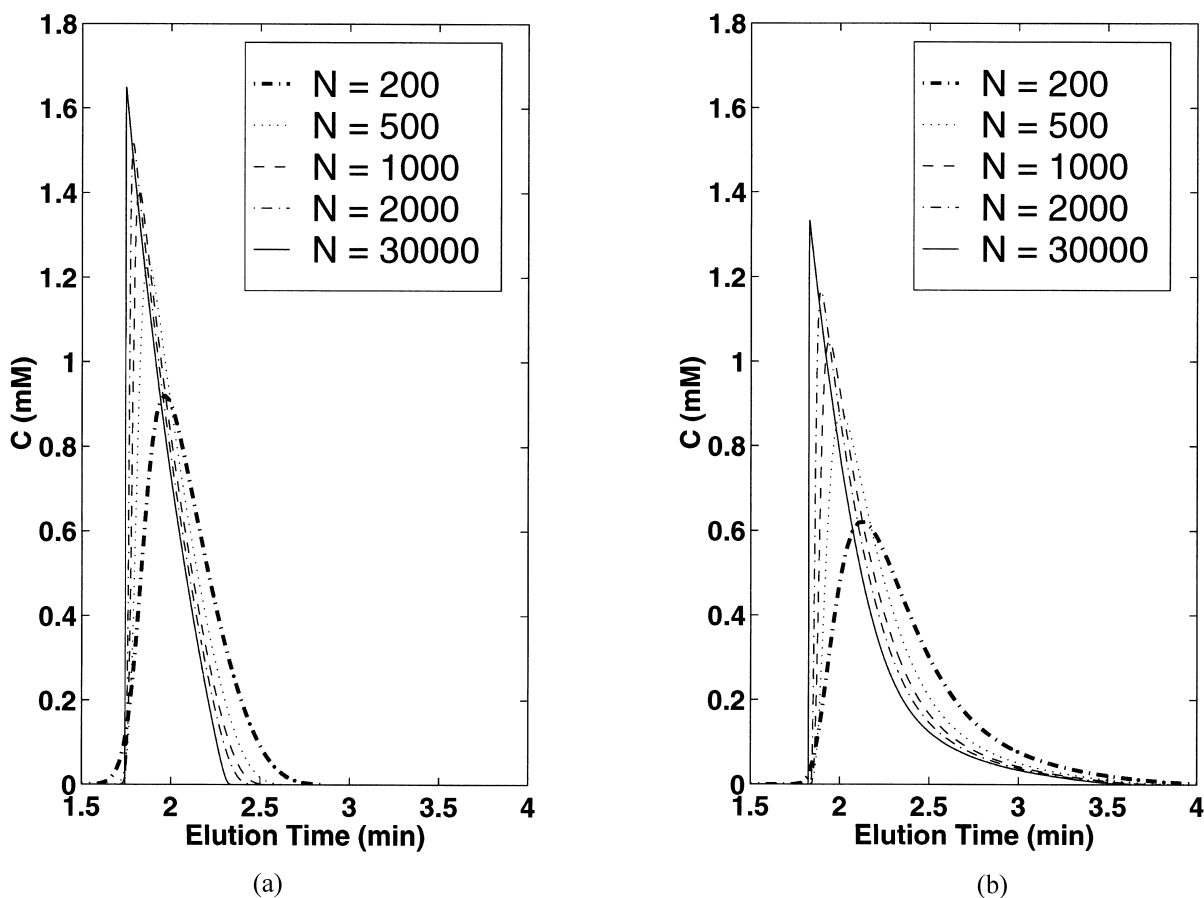


Fig. 1. Elution profiles of different column efficiencies as calculated with the equilibrium-dispersive model, demonstrating the difference between a Langmuir and a bi-Langmuir elution profile. Injection: 20.0 μl was injected of a 20.0 mM sample. Column length, $L = 10.0$ cm; column inner diameter, I.D. = 4.00 mm; flow-rate, 1.00 ml/min, hold-up volume, $V_0 = 1.00$ ml; porosity, $\epsilon = 0.796$. The Langmuir equation was used with $a = 5.00$, $b = 0.200 \text{ mM}^{-1}$. The bi-Langmuir equation was used with $a_I = 5.00$, $b_I = 0.200 \text{ mM}^{-1}$, $a_{II} = 5.00$ and $b_{II} = 10.0 \text{ mM}^{-1}$.

Langmuir elution profile (cf. Fig. 1b) [33]. The characteristic differences between the diffusive rear part of the Langmuir and bi-Langmuir elution profiles are not smoothed out by dispersion even at the lowest column efficiency investigated, i.e. $N=200$.

3.2. Calculation of ECP isotherms from the elution profiles

ECP isotherms were calculated from the elution profiles in Fig. 1a and b and the generated isotherms are shown in Fig. 2a for the Langmuir case and in Fig. 2b for the bi-Langmuir case, respectively. In each Figure there is an inset at the right hand bottom corner showing the low concentration range of the respective isotherm. The difference in appearance in the low concentration region of the elution profiles are reflected at the low concentration region of the ECP isotherms. Thus, the Langmuir isotherms are more or less linear in the low concentration range (cf. Fig. 2a) whereas the bi-Langmuir ECP isotherms are nonlinear at the same low concentration range (cf. Fig. 2b).

As mentioned, the ECP calculation introduces an error in the ECP generated isotherm as compared to the true ones. The isotherms calculated from elution profiles of column efficiencies of $N=30\,000$ deviates negligible from the true isotherms and can therefore be considered as true ones in the Fig. 2a and b. As can be seen from Fig. 2a and b, a decreased number of theoretical plates will increase the differences between the ECP generated isotherms and the true isotherm. In both the Langmuir and the bi-Langmuir cases the ECP method overestimates the concentration in the solid-phase. At the lowest column efficiencies the maximum mobile phase concentration of the isotherms declines. The reason being that the elution profiles are less concentrated due to a larger degree of dispersion and, thus, the ECP calculation must end at a lower concentration level.

3.3. Calculation of the ECP generated error

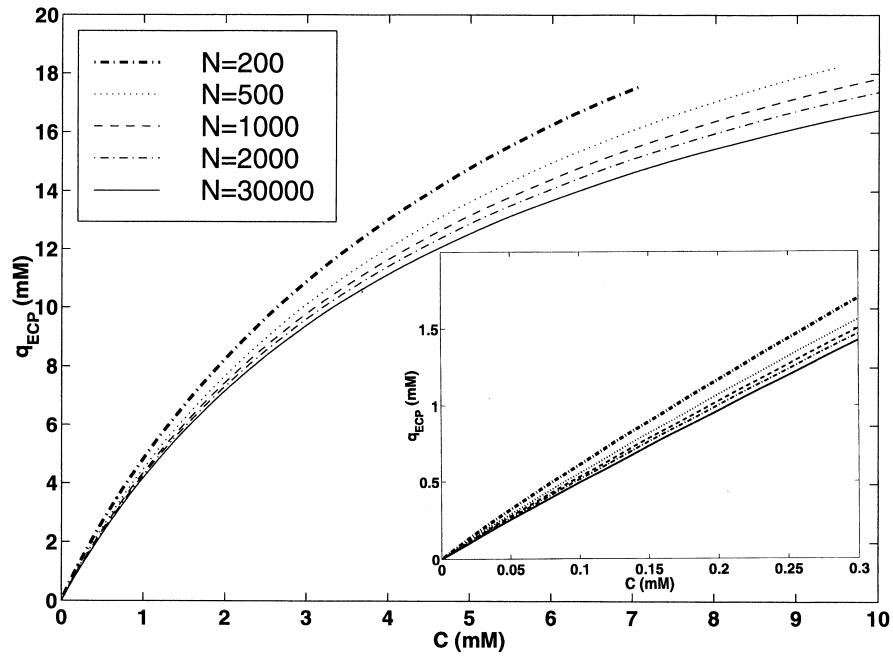
To illustrate the error of the ECP isotherm the ratio of the ECP isotherms over the true isotherms, as a function of the mobile phase concentration of the component was calculated [28]. In Fig. 3 $q_{\text{ECP}}/q_{\text{true}}$ is plotted versus the mobile phase concentration

C in the case of the Langmuir equation. The parameters are the same as in Fig. 2a but a run with $N=5000$ is presented instead of the $N=30\,000$ result. Fig. 3 describes that the ECP generated error is smaller the higher the efficiency and has a maximum at low respective high mobile phase concentrations. Thus, there is a minimum of error at moderate low mobile phase concentrations; the minimum error is easier to recognize at lower column efficiencies (cf. Fig. 3). The mobile phase concentration, C , where the minimum error takes place is shifted to slightly higher values at higher column efficiencies. At $N=200$, 2000 and 5000 the minimum errors are 15, 2.0 and 1.0%, and appear at C equal to 1.57, 1.72 and 1.75 mM, respectively. This is close to previous reported errors for the Langmuir case [28].

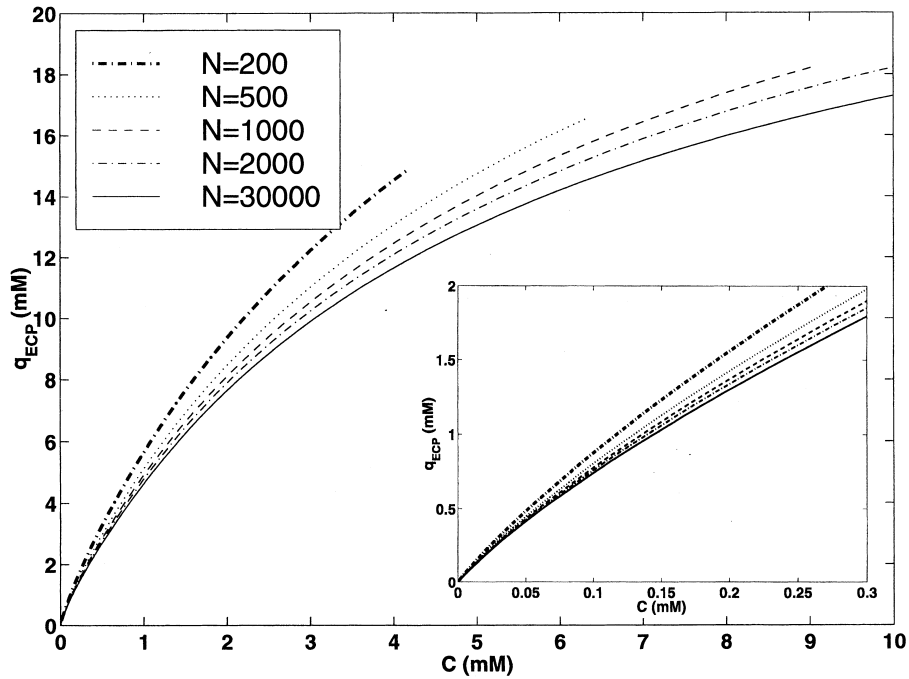
In Fig. 4 the same type of plot is made for the bi-Langmuir isotherm case 1 which is a mildly heterogenous surface. The interaction strength, the b term of the low capacity site is only 5 times stronger and its capacity the q_s term is only five times smaller as compared to the corresponding terms of the high capacity site (cf. Table 1). This bi-Langmuir case 1 follows the same general trend as the Langmuir case but the dip with the minimum error is more pronounced and is generally also shifted towards lower mobile phase concentrations, C . At $N=200$, 2000 and 5000 the minimum errors are 13, 1.8, and 0.8%, respectively, and takes place at $C=0.53$, 0.61 and 0.62 mM, respectively. Another slight difference from the Langmuir case is that the maximum errors at high concentrations are somewhat larger in the bi-Langmuir case at the lower column efficiencies.

Note, that in Figs. 3 and 4 the plots for two different loading factors, $L_f=0.62$ and 0.16 are shown. We reach a higher concentration range if the larger loading factor is used. In fact, the plots quota $q_{\text{ECP}}/q_{\text{true}}$ versus C for the two loading factors coincides in the Langmuir case (cf. Fig. 3) as well as in the bi-Langmuir case (cf. Fig. 4). Therefore, only the larger loading factor was printed in Figs. 5b–8b.

Fig. 5a shows the same type of plots but for the bi-Langmuir case 2 (cf. Table 1.) with a strongly heterogenous surface. The term $b_{\text{II}}=10.0\text{ mM}^{-1}$, a 50 times stronger interaction as compared to the non-chiral site, and $q_{s,\text{II}}=0.500\text{ mM}$, which is a 50 times lower capacity as compared to the high



(a)



(b)

Fig. 2. Isotherms calculated with the ECP method on the (a) Langmuir and (b) bi-Langmuir elution profiles. The solid line isotherm is calculated from an elution profile of $N=30\,000$ theoretical plates and is close to the true isotherm. Inset shows the low concentration range. $200\ \mu\text{l}$ was injected, other conditions as in Fig. 1.

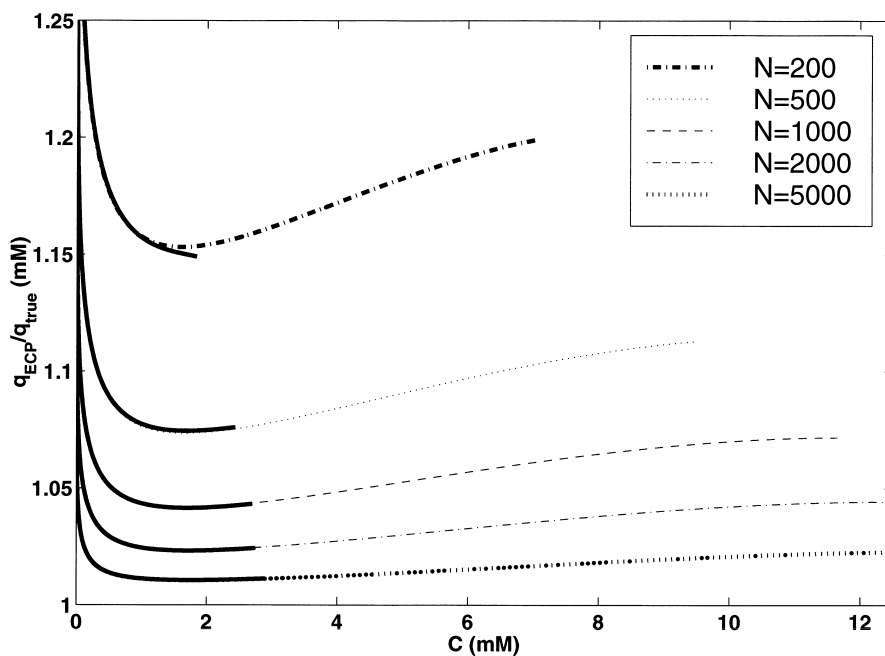


Fig. 3. The plot shows $q_{\text{ECP}}/q_{\text{true}}$ as a function of the mobile phase concentration for a homogeneous surface. $L_f=0.62$ except for the solid lines when $L_f=0.16$. Other conditions as in Fig. 2a, however, $N=30\,000$ is replaced by $N=5000$.

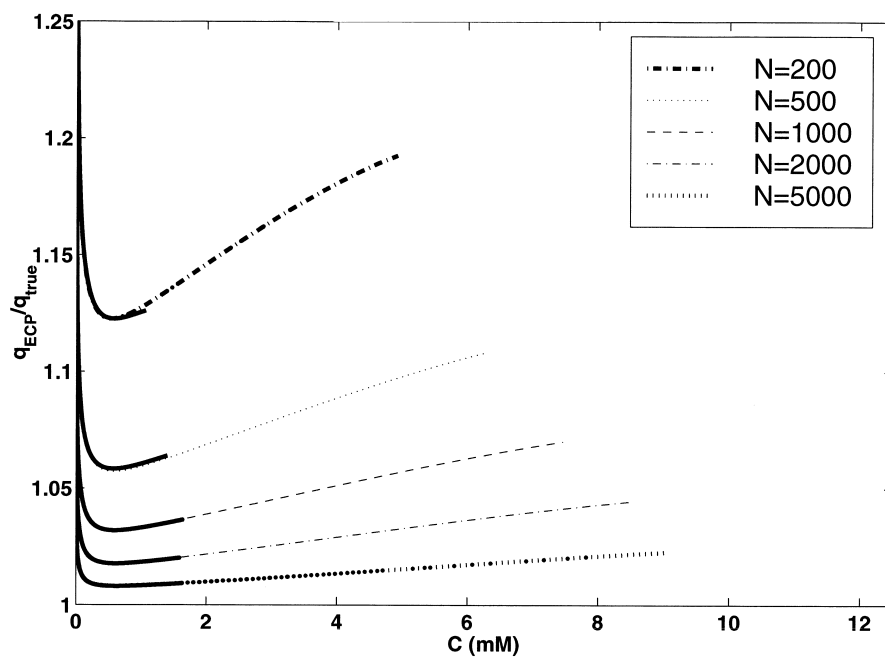


Fig. 4. The plot shows $q_{\text{ECP}}/q_{\text{true}}$ as a function of the mobile phase concentration for a heterogeneous surface. Parameters from the bi-Langmuir equation case 1 in Table 3. $L_f=0.62$ in all cases except for solid lines where $L_f=0.16$. Other conditions as in Fig. 2b, however, $N=30\,000$ is replaced by $N=5000$.

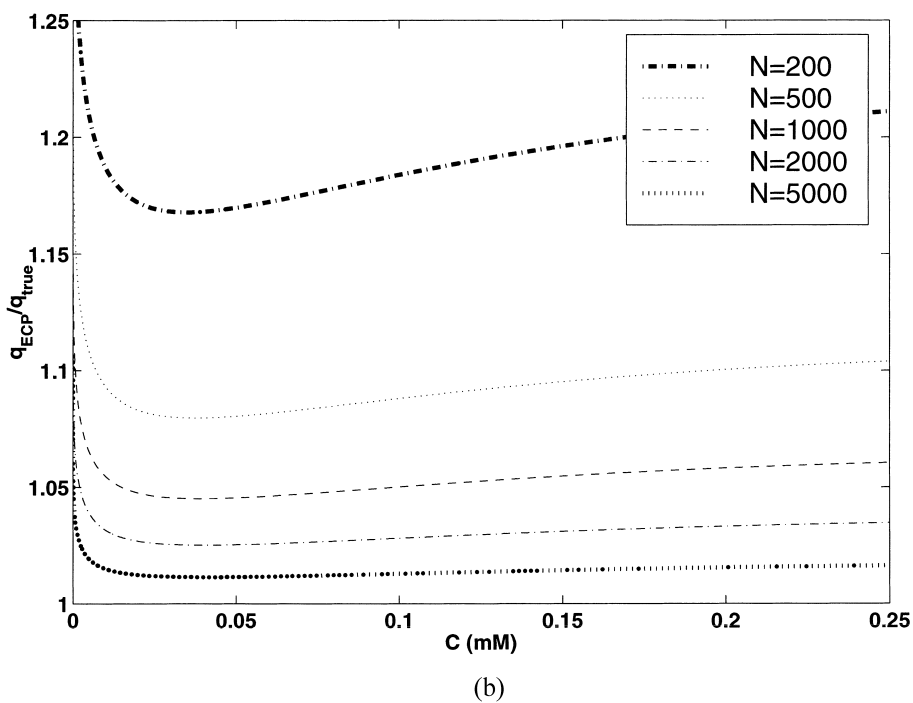
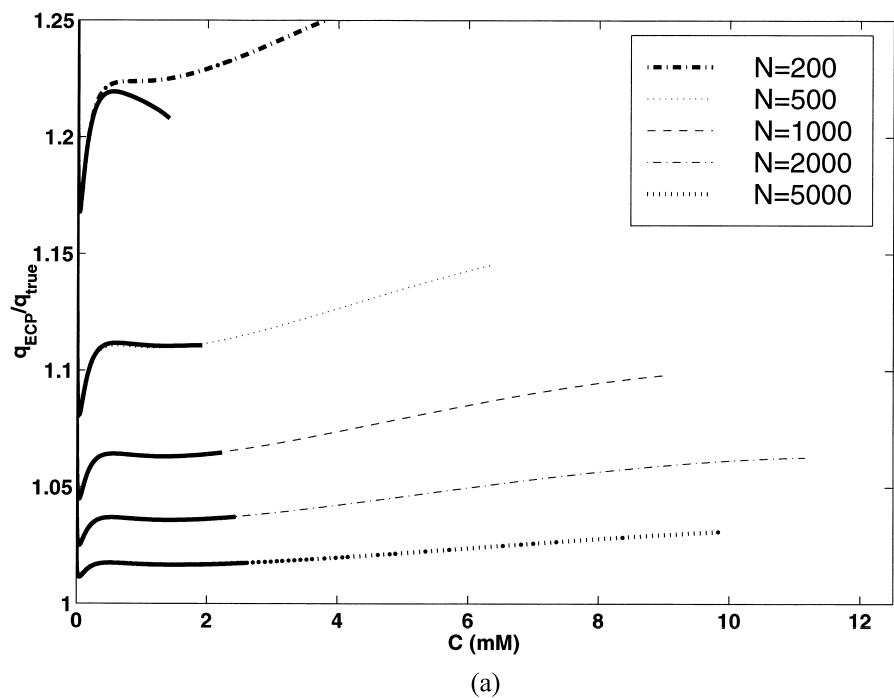


Fig. 5. As in Fig. 4 but bi-Langmuir parameters from the case 2 in Table 4. $L_f=0.62$ in all cases except for solid lines where $L_f=0.16$. (a) The plot shows the full concentration range, up to 12 mM. (b) The plot shows a low concentration range, up to 0.25 mM.

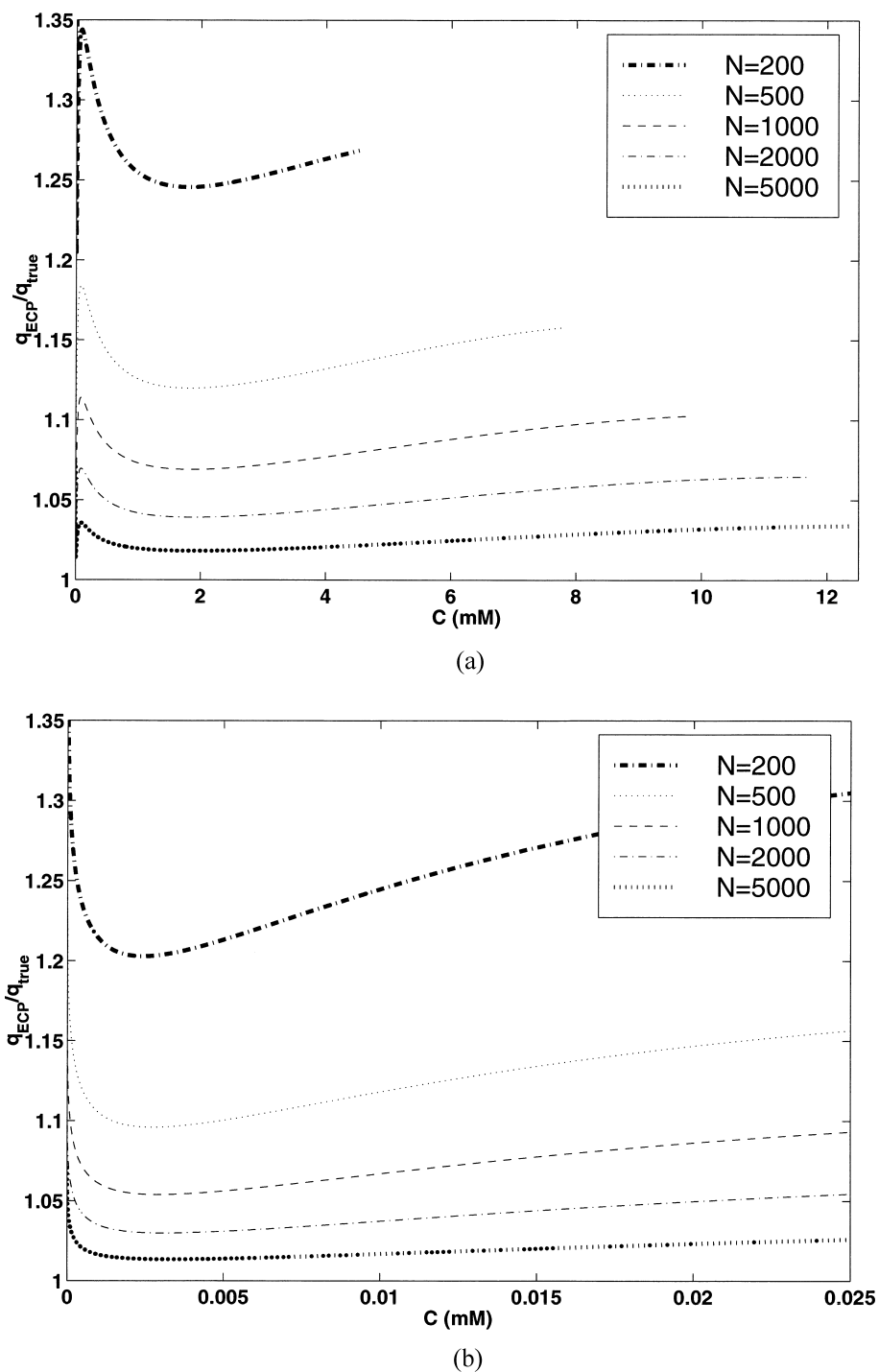


Fig. 6. As in Fig. 4 but bi-Langmuir parameters from case 3 in Table 5. $L_f=0.62$. (a) The plot shows the full concentration range, up to 12 mM. (b) The plot shows a low concentration range, up to 0.025 mM.

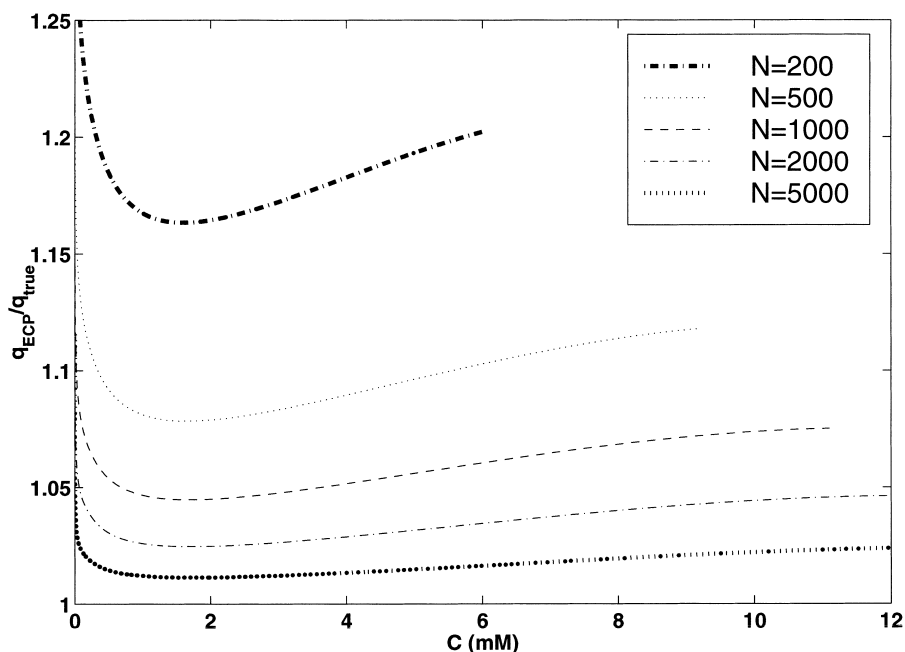


Fig. 7. As in Fig. 4 but bi-Langmuir parameters from case 4 in Table 6. $L_j=0.62$.

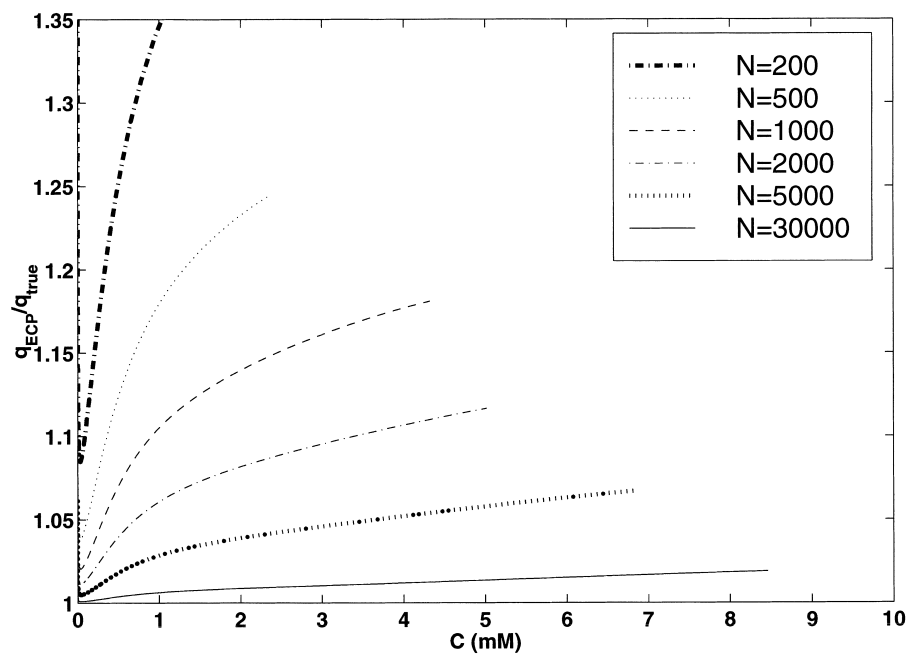
capacity site. The pattern looks quite different; the minimum error is a sharp negative dip and has been strongly shifted towards the low mobile phase concentration area. On the other hand, the enlargement, Fig. 5b, shows close resemblance to the Langmuir case. In the enlargement, we can see that at $N=200$, 2000 and 5000, the minimum errors are 17, 2.5, and 1.1%, respectively, and takes place at C equal to 0.035, 0.041 and 0.042 mM, respectively.

Fig. 6a is the same type of plot but for the bi-Langmuir case 3 (cf. Table 1.) and Fig. 6b its enlargement. In case 3, the difference in interaction strength of the site I and II b terms, is extraordinarily large. The term $b_{II}=10.0 \text{ mM}^{-1}$ is 500 times larger as compared to site I and $q_{s,II}=0.05 \text{ mM}$ which is a 500 times lower capacity as compared to that of site I. The situation is unusual in chiral separations but a 150 times ratio has been found for the *R*-metoprolol adsorbed on a cellulase protein CBH I as selector [19]. It can be observed that the minimum error has been shifted even more strongly towards the low concentration area; the minimum errors can only be recognized in the enlargement (cf. Fig. 6b). In this very low concentration range, at $N=200$, 2000 and 5000 the minimum errors are 21, 3.0, and 1.3%, and

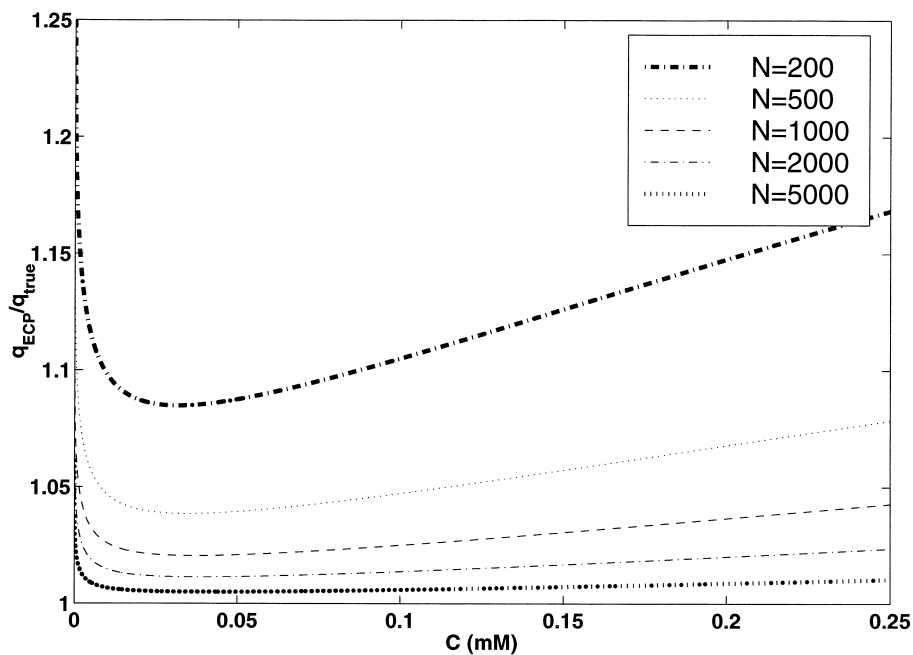
takes place at C equal to 2.4, 3.1 and 3.3 μM respectively. In addition, as can be seen from Fig. 6 a new minimum error is introduced at the high concentration region. At $N=200$, 2000 and 5000 the new minimum errors are 25, 3.9, and 1.8%, and takes place at $C=1.78$, 1.88 and 1.90 mM respectively. Those minimum errors are similar to but larger than the Langmuir case in Fig. 3.

The bi-Langmuir cases 4 and 5 are special cases of heterogenous surfaces. Case 4 has a smaller contribution of the second site, since its equilibrium constant $a_{II}=0.5$ is 10 times smaller than site I (cf. Table 1). The capacity of site II is as low as in the previous case, i.e., $q_{s,II}=0.05 \text{ mM}$. In the first 3 cases the a terms of the first and second sites has been equal, thus the contribution to retention from site II has been 50%. In case 4 the chiral contribution to retention is only 9%. The plots of the ratios q_{ECP}/q_{true} versus C for various column efficiencies for case 4 are shown in Fig. 7. Interestingly, the pattern and the values of the minimum errors are similar to the reference Langmuir case in Fig. 3.

In opposition to the bi-Langmuir case 4, the contribution to retention from site II in case 5 is high, i.e., 91%. This is because the equilibrium



(a)



(b)

Fig. 8. As in Fig. 4 but bi-Langmuir parameters from case 5 in Table 7. $L_r=0.62$. (a) The plot shows the full concentration range, up to 10 mM. (b) The plot shows a low concentration range, up to 0.25 mM.

constant of site II is 10 times that of site I, i.e., $a_{II}=50$. The capacity of site II is $q_{s,II}=5$ mM which is five times lower than site I. The plot q_{ECP}/q_{true} versus the mobile phase concentration C in case 5 is shown in Fig. 8a and it is obvious that the effect of a 10 times larger equilibrium constant of site II than site I is dramatic. The error is tremendously high at moderate and high mobile phase concentrations. However, there is a minimum of error at low mobile phase concentrations as recognized in Fig. 8b providing an enlargement. In Fig. 8b we can see that at $N=200$, 2000 and 5000 the minimum errors are 8.5, 1.1, and 0.5%, and take place at C equal to 32, 38 and 40, μM respectively, which is similar but lower than case 2 (cf. Fig. 5b). But the errors are extremely high at increased mobile phase concentrations, even at high column efficiencies. At $C=5$ mM and $N=1000$ and 2000 respectively, the error is 18 and 10%, respectively.

To summarize, the ECP generated isotherm error is strongly dependent on the type of surface. For the heterogenous surface, the error versus the mobile phase concentration has in most cases a typical pattern in contrast to a homogeneous surface. For heterogenous surfaces, there is a relation between the C at the minimum error and the value of the site II capacity. When the latter is decreased the minimum error is shifted towards lower mobile phase concentrations. As an example, in the bi-Langmuir cases 1–3 the capacity of site II, $q_{s,II}$, is decreased 10 times for each higher number of case, i.e., from 5.00 mM (case 1) to 0.500 mM (case 2) to 0.0500 mM (case 3). As a consequence, the concentration of the minimum error is decreased by about the same magnitude: from 0.61 mM to 41 and 3.1 μM , as calculated when $N=2000$. For all cases the minimum error takes place at a concentration which is between 10 and 20 times lower than the site II capacity.

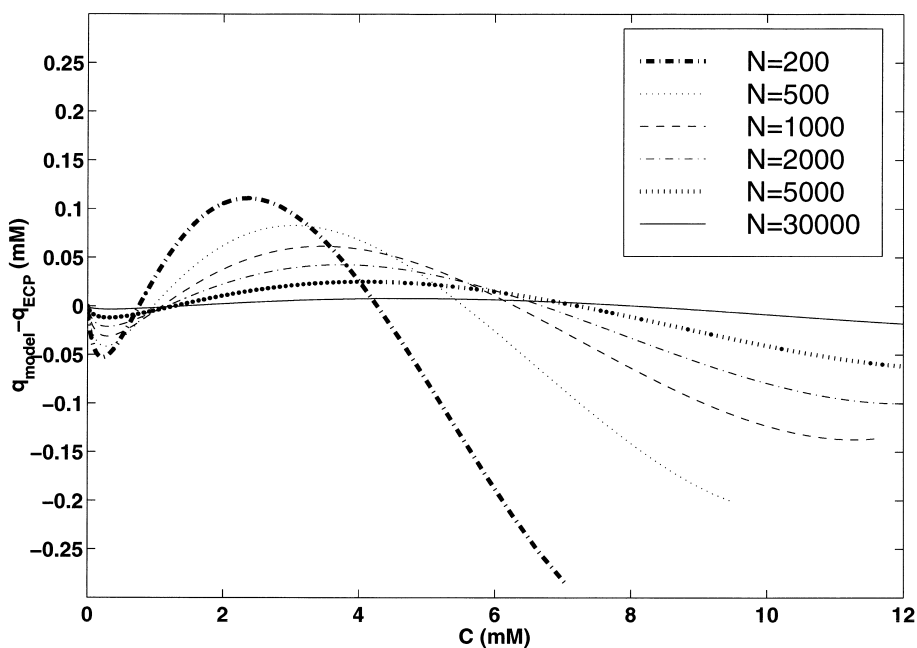
In the cases described above, the retention contribution of the chiral site and the non-chiral site is equal, i.e., $a_I=a_{II}$. However, as the second equilibrium constant goes to extreme values, as an example when a_{II} is 10 times smaller (cf. Fig. 7) or 10 times larger (cf. Fig. 8a and b) as compared to site I the pattern is quite different. In the first case, the retention contribution of the second site to the global retention will be less than 10% causing the typical

bi-Langmuir error pattern to almost disappear and to turn almost identical to the Langmuir case (cf. Fig. 3). In the latter case, the pattern will still be as described in general for the bi-Langmuir case but the error will be too high in moderate and high mobile phase concentration regions (cf. Fig. 8a and b). Although this is a situation close to real conditions [19] it is obvious that bi-Langmuir parameters can not be derived using the ECP method on such surfaces, especially since the efficiency of chiral separations often is low.

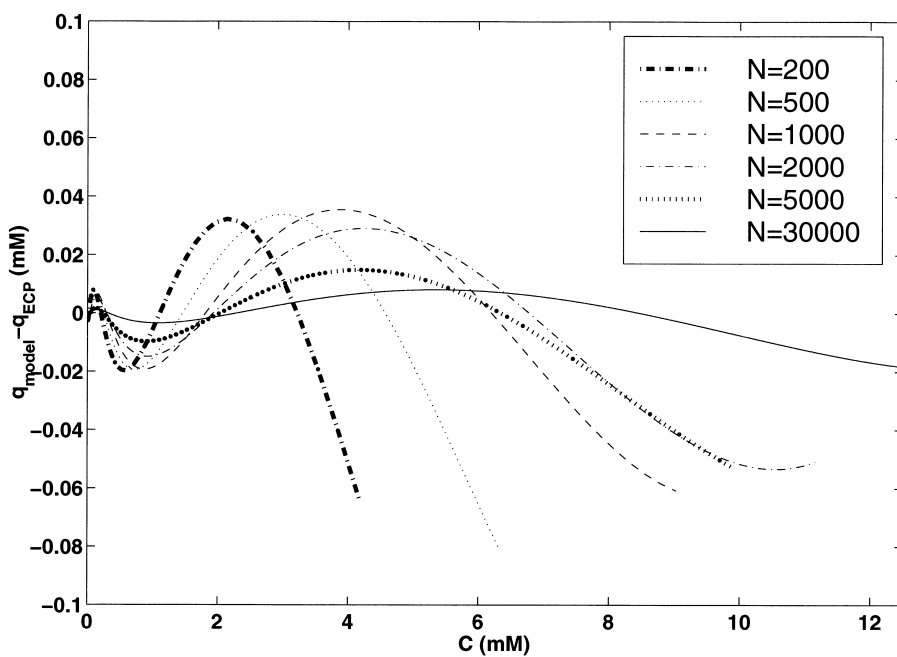
3.4. Estimation of bi-Langmuir parameters from ECP isotherms

In the next step, the ECP generated isotherms are fitted by proper isotherm equations: this is the step introducing the model error. The model error is best visualized by plotting the difference between the value of the adsorbed amount at equilibrium obtained from the fitted isotherms and the value given by the ECP isotherm at the same concentration. This was done previously for the Langmuir case with the efficiency $N=2000$ [28]. Fig. 9a and b show the residual $q_{model}-q_{true}$ versus the mobile phase concentration C for the Langmuir case and bi-Langmuir case 2. The pattern is similar to the previous homogeneous case [28] except that we did the plot with six different column efficiencies from $N=200$ to $N=30\,000$. The error has the shape of a wave-curve and is dependent on the column efficiency; the error decreases when the number of theoretical plates increases (cf. Fig. 9a and b). As the column efficiency is increased (i) the wavelength increases and (ii) the amplitudes decrease. In the case of the bi-Langmuir equation, the plot of the model error versus C is similar to the Langmuir one but contains more waves at the lower mobile phase concentrations (cf. Fig. 9b).

The issue of weighting the nonlinear regression has not been discussed in previous studies probably since the concept is not as important for a Langmuir isotherm equation as for a bi-Langmuir equation. In the latter case, we have both one high and one low capacity site and it is necessary to put more emphasis on the data points at the lower concentration ranges in order to determine the low capacity site properly. Fig. 9c shows the model error of the bi-Langmuir



(a)



(b)

Fig. 9. Residual plots, $q_{\text{model}} - q_{\text{ECP}}$ in the area of concentration examined for the respective model. $L_f = 0.62$. Weighting factor is $1/F$ if no other weighting is stated. Other conditions as in Fig. 2. (a) Parameters are the homogeneous case Langmuir in Table 1, as in Fig. 2. (b) Parameters are the heterogeneous case bi-Langmuir 2 in Table 1, as in Fig. 3. (c) As in (b) but the weighting factor was varied.

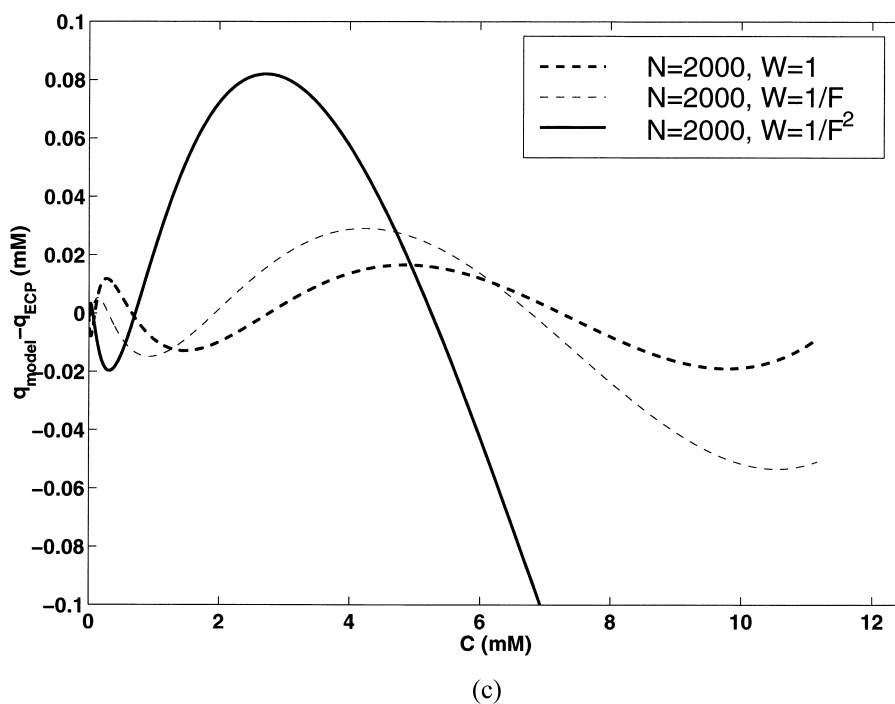


Fig. 9. (continued)

case 2 as plotted versus C using the three investigated weights (W) of the nonlinear regression, i.e., $W=1$, $W=1/F$ and $W=1/F^2$, where F is the function that is fitted to the data. It is obvious that the issue of weighting is extremely important for the estimation of the parameters in ECP of bi-Langmuir equations. We can see in Fig. 9c that when $W=1/F^2$ the regression does not work at all for the medium and high concentration ranges. On the other hand, at very low concentrations the weight $W=1/F$ seems to be the best choice (cf. Fig. 9c). In the case in Fig. 9c, the ratio of the q_s terms is 50. If the ratio of the q_s terms of site I over site II is 500, the weight $1/F^2$ might be the best choice for a good determination of the constants of site II, since this put a lot of emphasis on the data points very close to 0. However, in this context it is important to remember that the first ECP error is large at concentrations very close to zero (cf. Figs. 3–8) where a minimizing of the model error does not necessarily yield better final parameter estimations. In addition, in real experimental situations the presence of noise makes estimations at low concentrations an even more

difficult task. The need for weighting of the isotherm is also affected by the loading factor L_f . In unpublished results we have seen that a too large loading factor puts too much emphasis on the high concentration area; an effect which can be counteracted by weighting. On the other hand, it is important when the bi-Langmuir equation is fitted to the ECP isotherm that both sites reach nonlinearity, so the loading factor should not be too small either.

In the bi-Langmuir cases investigated the weight $1/F$ was in general most successful to determine both site I and site II parameters. The weight $1/F$ was therefore used in the following parameter estimations (Tables 2–7).

3.5. Accuracy of estimated ECP parameters

ECP parameters were calculated for ECP isotherms derived from two injection sizes corresponding to the loading factors $L_f=0.62$ and 0.16 respectively. We already showed that the type of heterogenous surface, i.e., the set of true bi-Langmuir parameters, strongly affects both the ECP

Table 2

Estimated ECP parameters at two different loading factors and at different column efficiencies. The ECP calculations were made on elution profiles calculated from the true Langmuir parameters in Table 1: $a=5.00$ and $b=0.200 \text{ mM}^{-1}$. Injection volumes were $200 \mu\text{l}$, (giving $L_f=0.62$) and $50.0 \mu\text{l}$ (giving $L_f=0.16$)

<i>N</i>	$L_f=0.62$		$L_f=0.16$	
	<i>a</i>	<i>b</i> (mM^{-1})	<i>a</i>	<i>b</i> (mM^{-1})
200	5.79	0.194	6.08	0.252
500	5.34	0.191	5.52	0.221
1 000	5.18	0.192	5.29	0.212
2 000	5.09	0.195	5.17	0.207
5 000	5.05	0.197	5.08	0.203
30 000	5.01	0.199	5.02	0.201
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>0.200</i>

generated error and the the model error. Thus, the accuracy of the estimated bi-Langmuir parameters is dependent on both type of errors. The final estimates of the bi-Langmuir isotherm parameters in Table 1 calculated with ECP on the elution profiles are

Table 3

As in Table 2 except the ECP calculations were made on elution profiles calculated from the true parameter case bi-Langmuir 1 in Table 1: $a_I=5.00$, $b_I=0.200 \text{ mM}^{-1}$, $a_{II}=5.00$ and $b_{II}=1.00 \text{ mM}^{-1}$

<i>N</i>	$L_f=0.62$				$L_f=0.16$			
	a_I	b_I (mM^{-1})	a_{II}	b_{II} (mM^{-1})	a_I	b_I (mM^{-1})	a_{II}	b_{II} (mM^{-1})
200	7.08	0.201	4.81	2.00	10.1	0.389	2.43	1.08
500	5.84	0.187	5.05	1.37	9.04	0.347	2.22	5.05
1 000	5.31	0.184	5.19	1.17	8.21	0.314	2.50	2.85
2 000	5.04	0.185	5.24	1.07	7.75	0.303	2.66	2.15
5 000	4.94	0.190	5.19	1.02	6.85	0.270	3.34	1.51
30 000	4.95	0.196	5.07	1.00	5.51	0.220	4.53	1.10
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>1.00</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>1.00</i>

Table 4

As in Table 2 except the ECP calculations were made on elution profiles calculated from the true parameter case bi-Langmuir 2 in Table 1: $a_I=5.00$, $b_I=0.200 \text{ mM}^{-1}$, $a_{II}=5.00$ and $b_{II}=10.0 \text{ mM}^{-1}$

<i>N</i>	$L_f=0.62$				$L_f=0.16$			
	a_I	b_I (mM^{-1})	a_{II}	b_{II} (mM^{-1})	a_I	b_I (mM^{-1})	a_{II}	b_{II} (mM^{-1})
200	6.04	0.185	4.51	8.88	6.63	0.265	5.28	12.9
500	5.39	0.181	5.23	8.43	5.74	0.219	5.14	10.9
1 000	5.14	0.182	5.11	8.34	5.41	0.208	5.07	10.4
2 000	5.05	0.187	5.05	8.72	5.23	0.204	5.03	10.2
5 000	5.02	0.193	5.02	9.29	5.11	0.202	5.01	10.1
30 000	4.99	0.198	5.00	9.72	5.02	0.200	5.00	10.0
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>10.0</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>10.0</i>

discussed below. The estimated parameters can be seen in Table 2 for the Langmuir case and in Tables 3–7 for the five bi-Langmuir cases. The weight $W=1/F$ was used.

In the case of the Langmuir equation, the a parameter will be overestimated at low column efficiencies at both loading factors (cf. Table 2). The overestimation decreases with increased column efficiency and is less for the larger injection size. Using the large loading factor at the column efficiencies $N=200$, 2000 and 5000 the error is +16, +1.8 and +0.8%, respectively. The b term will be more or less correct using the large loading factor and overestimated for the small loading factor. For the large loading factor and column efficiencies $N=200$, 2000 and 5000 the error is -3.0, -2.7 and -1.6%, respectively. Thus, in the case of a Langmuir isotherm we should use a large loading factor.

In the mildly heterogenous case 1, the bi-Langmuir estimates are shown in Table 3. The a_I term

Table 5

As in Table 2 except the ECP calculations were made on elution profiles calculated from the true parameter case bi-Langmuir 3 in Table 1: $a_1=5.00$, $b_1=0.200 \text{ mM}^{-1}$, $a_{II}=5.00$ and $b_{II}=100 \text{ mM}^{-1}$

N	$L_f=0.62$				$L_f=0.16$			
	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$
200	6.08	0.188	4.23	23.5	6.54	0.265	4.64	53.0
500	5.43	0.183	4.03	28.4	5.75	0.225	4.51	59.0
1 000	5.23	0.187	4.14	39.0	5.42	0.212	4.55	66.4
2 000	5.12	0.191	4.33	52.5	5.24	0.207	4.66	75.9
5 000	5.05	0.195	4.66	71.0	5.12	0.204	4.80	86.7
30 000	5.01	0.198	4.88	91.1	5.03	0.201	4.94	96.3
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>100</i>	<i>5.00</i>	<i>0.200</i>	<i>5.00</i>	<i>100</i>

Table 6

As in Table 2 except the ECP calculations were made on elution profiles calculated from the true parameter case bi-Langmuir 4 in Table 1: $a_1=5.00$, $b_1=0.200 \text{ mM}^{-1}$, $a_{II}=0.500$ and $b_{II}=10.0 \text{ mM}^{-1}$

N	$L_f=0.62$				$L_f=0.16$			
	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$
200	5.37	0.168	1.69	4.53	5.78	0.209	1.55	11.3
500	5.03	0.174	1.24	3.82	5.31	0.195	1.18	9.01
1 000	4.96	0.180	0.965	3.91	5.16	0.196	0.961	8.94
2 000	4.94	0.186	0.800	4.32	5.08	0.197	0.804	9.17
5 000	4.96	0.192	0.648	5.38	5.03	0.198	0.667	9.62
30 000	4.98	0.198	0.532	7.89	5.01	0.200	0.547	10.0
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>0.500</i>	<i>10.0</i>	<i>5.00</i>	<i>0.200</i>	<i>0.500</i>	<i>10.0</i>

will be overestimated to a large extent at low column efficiencies. Using the large loading factor at $N=200$, 2000 and 5000 the error is +42, +0.8 and -1.2%. With the small loading factor the overestimation is even larger (cf. Table 3). The b_1 term is more or less correct using the large loading factor and quite much overestimated using the small load-

ing factor; in the latter case the error is +94.5% at $N=200$. The a_{II} term will be slightly overestimated at almost all column efficiencies for the large loading factor and quite underestimated using the small loading factor; at $N=200$ the error is -51%. The b_{II} term is overestimated using the large loading factor at $N=200$, 2000 and 5000 the error is +100, +6.9

Table 7

As in Table 2 except the ECP calculations were made on elution profiles calculated from the true parameter case bi-Langmuir 5 in Table 1: $a_1=5.00$, $b_1=0.200 \text{ mM}^{-1}$, $a_{II}=50.0$ and $b_{II}=10.0 \text{ mM}^{-1}$

N	$L_f=0.62$				$L_f=0.16$			
	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$	a_1	$b_1 (\text{mM}^{-1})$	a_{II}	$b_{II} (\text{mM}^{-1})$
200	10.1	0.295	50.5	11.8	17.4	1.47	44.1	14.8
500	7.40	0.222	50.2	10.9	9.31	0.604	48.6	11.7
1 000	6.26	0.194	50.1	10.4	7.27	0.389	49.4	10.9
2 000	5.69	0.191	50.1	10.2	6.16	0.285	49.8	10.5
5 000	5.28	0.190	50.0	10.1	5.50	0.231	49.9	10.2
30 000	5.05	0.196	50.0	10.0	5.10	0.204	50.0	10.0
<i>True</i>	<i>5.00</i>	<i>0.200</i>	<i>50.0</i>	<i>10.0</i>	<i>5.00</i>	<i>0.200</i>	<i>50.0</i>	<i>10.0</i>

and +2.0%, respectively. At small loading factors the overestimation is much worse; as an example at around $N=200$ the error is 980%. Thus, even in case 1, a large loading factor should be used. However, even if the large loading factor is used the error is considerable on the a_I term and especially on the b_{II} term at limited column efficiencies.

Also in the more strongly heterogenous case, bi-Langmuir case 2, the large loading factor gave generally the best estimations (cf. Table 4) except for the term b_{II} . Using this loading factor the site I parameters, a_I and b_I , were decided with a similar accuracy as compared to case 1 when the injection size is large. However, using the large loading factor at $N=200$, 2000 and 5000 the error of the term b_{II} is -11.3 , -12.9 and -7.0% , respectively. Using the small loading factors the corresponding errors are $+29.2$, $+1.54$ and $+0.6\%$, respectively.

The bi-Langmuir case 3 represents a surface with a very small amount of site II interactions, but however, the interactions are also very strong. The capacity of site II is $q_{s,II}=0.0500$ mM which is 10 times lower as compared to case 2. As showed above the ECP generated error versus C is observed in a broad concentration range between two minima; a range which is important for estimation of site II parameters (cf. Figs. 6 a and b). The accuracy of the estimated parameters are shown in Table 5. The large loading factor gave the best estimations for the site I parameters and the small loading factor gave best estimates of site II terms. The reason being that less emphasis will be put on the data at the low concentration range as the total range will be broader at the higher regions. This will be evident in case 3 since the site II capacity is very small as compared to site I. We found that the large loading factor gave better estimates if the weight was changed to $W=1/F^2$ which again put more importance to the low concentration area. However, the large loading factor is necessary to use because otherwise the errors of the site I terms will become too large. Using the large loading factor at $N=200$, 2000 and 5000 the error of the term a_{II} is -15.4 , -13.4 and -8.0% , respectively, and that of the b_{II} term is -76.5 , -47.5 and -29.0% , respectively. Thus, this surface can not be decided with the ECP method.

The heterogenous surface case 4 represents a case when there is only a 10% contribution to the total

retention from site II and also when site II has a very low capacity, i.e., 500 times lower than site I. We showed the plot of the ECP generated error versus C has an almost identical error pattern as the Langmuir case (cf. Figs. 7 and 3). Thus the minimum error has the same position as in the Langmuir case, i.e., at C around 1.6–1.7 mM (cf. Fig. 7) and the error turns to extremely large values at the lower C values where the estimates should be done at the site II terms. The error using the best loading factor (the smallest) of the a_{II} term at $N=5000$ was $+33.4\%$. Thus ECP should be avoided for such surfaces.

In bi-Langmuir case 5 we have the opposite situation to case 4; the retention contribution of the second site to the total retention is 91%. The plot q_{ECP}/q_{true} versus C showed that the ECP generated errors are tremendously high at the high concentration ranges where site I terms are estimated but not at low C (cf. Fig. 8). This pattern is also the opposite to case 4. Consequently the site II terms are estimated with good accuracy but not the site I terms (cf. Table 7). The errors using the large loading factor of the a_I and the b_I terms at $N=5000$ are $+5.6$ and -5.0% and for the small loading factor they are $+10$ and $+15.6\%$, respectively. Thus, the ECP method is not recommended for this type of heterogenous surface.

4. Conclusion

The accuracy of the ECP method for calculating bi-Langmuir isotherm parameters on heterogenous surfaces was investigated. The background is that it has recently been found that chiral stationary phases most often are described by the bi-Langmuir equation [12,14–21]. Site I is considered to be the non-selective site, and site II the chiral-selective one.

The ECP method gives rise to two different types of errors; (i) the error of the ECP calculated isotherm compared to the true one, and (ii) the error of the fitted isotherm relative to the ECP isotherm. Both errors are dependent on the mobile phase concentration and decreases with increasing column efficiency. When the first type of error is plotted versus the mobile phase concentration there will be a minimum at low mobile phase concentrations. This concentration depends on the capacity of the low

capacity term, i.e., on $q_{s,II}$. The second type of error, the model error, is also dependent on the weight made during nonlinear regression and on the loading factor. The weight used in this work is $1/F$, where F is the value of the fitted function. Accurate estimation of the low capacity term requires a proper weight so that adequate emphasis is put on low concentration data. The loading factor should be large enough to reach nonlinearity of both sites I and II. On the other hand, extremely high loading factors will lead to a less accurate estimation of the low capacity terms, especially for ratios of $q_{s,I}/q_{s,II}$ 50 or larger. However, a too high loading factor, can be counteracted by a changed weight giving more emphasis to the low mobile phase concentrations.

Because of the first type of error, accurate bi-Langmuir parameters estimations can not be done for all types of heterogenous surfaces. Those are the cases when the second equilibrium constant, a_{II} , is too big or too small in comparison to the first one, i.e., when the ratio a_{II}/a_I is less than 0.1 or larger than 10. In the latter case it is the non-chiral high capacity sites that can not be determined accurate enough. In addition, the ratio of the site I capacity over that of site II, i.e., $q_{s,I}/q_{s,II}$, must be between 5 and 500. For other heterogenous surface compositions accurate estimations of bi-Langmuir parameters might done if the column efficiency is high enough. However, a higher column efficiency is required as compared to the Langmuir case where $N=2000$ (less than 3% error) is a minimum and $N=5000$ (less than 2% error) is recommended [28]. For the bi-Langmuir case N higher than 5000 is required for an error less than 5% for all estimated bi-Langmuir parameters. Thus, the ECP method is limited for use only on a few number of high efficiency chiral stationary phases.

5. Nomenclature

a	Constant of Langmuir equation, slope of the isotherm at infinite dilution.
a_I	Constant of bi-Langmuir equation, a term of the bi-Langmuir site I with higher q_s value.
a_{II}	Constant of bi-Langmuir equation, a

	term of the bi-Langmuir site II with lower q_s value.
b	Constant of Langmuir equation (mM^{-1}) control the curvature of the isotherm.
b_I	Constant of the bi-Langmuir equation (mM^{-1}), b term of the bi-Langmuir site I.
b_{II}	Constant of the bi-Langmuir equation (mM^{-1}), b term of the bi-Langmuir site II.
C	Mobile phase concentration (mM).
L	Column length (cm).
L_f	Loading factor.
N	Number of theoretical plates.
$q(C)$	Equilibrium isotherm (mM).
q_s	Monolayer capacity (mM), equal to a/b .
$q_{s,I}$	Monolayer capacity (mM) of site I.
$q_{s,II}$	Monolayer capacity (mM) of site II.
S	Column geometric cross-sectional area (cm^2).
t_0	Hold-up time (min) (equal to L/u).
t_p	Width of injected pulse (s), assumed to be rectangular.
$t_R(C)$	Retention time (min) of diffuse profile of an overloaded elution profile.
V	Retention volume (cm^3) of the characteristic point of the diffuse profile at concentration C .
V_0	Hold-up time volume (cm^3).
V_a	Volume of adsorbent (cm^3).
V_m	Volume of the mobile phase in the column (cm^3).
V_s	Volume of the solid-phase (cm^3).
ϵ	Porosity of the adsorbent.

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