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Numerical estimation of multicomponent adsorption isotherms in preparative chromatography: implications of experimental error

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Abstract

Since experimental methods for measuring multicomponent adsorption isotherms are extremely tedious, numerical approaches are an attractive alternative. Here, the variance in isotherm parameters as a function of experimental error in measured effluent concentrations is quantified. The number of experimental replicates needed to obtain isotherm parameters to a desired level of accuracy is calculated explicitly. After the covariance matrix of the parameters has been determined, Monte Carlo methods are found to be rapid and effective. The use of different kinds of experiments, the effect of resolution and loading, and the impact of the number of measured data points are described. © 2001 Published by Elsevier Science B.V.

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1. Introduction

Preparative chromatography is widely used in the isolation and purification of a variety of compounds from a range of feedstocks [1,2]. It has become clear that preparative chromatography is usually optimal with respect to production rate when the feed mass is high enough to cause nonlinear adsorption [2]. Thus, accurate knowledge of adsorption isotherms is an important prerequisite to optimization; clearly, these should be multicomponent isotherms, measured over the entire range of feeds and additive compositions that will be used in practical runs. In practice, however, multicomponent isotherms are rarely used to optimize a separation, principally because they require tedious and time-consuming experiments. Recently, the problem of estimating multicomponent isotherms numerically (preferably using a small amount of easily obtained experimental data) is receiving considerable attention in the literature [3–9]. The goal of this paper is to investigate the effect of experimental error on the precision of the numerically estimated isotherm parameters. To the best of our knowledge, this important problem of estimating the numerical back-propagation of error has not been addressed in the literature.

2. Theory

2.1. Brief review of literature

For the determination of single-component iso-

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therms, the most popular technique is probably elution on a characteristic point (ECP), since a single isocratic run gives many data points towards the isotherm. However, ECP has the drawback of not accounting for the contribution of nonequilibrium effects (pore diffusion, film mass-transfer) to the trailing edge of the chromatogram. The ensuing error in the extracted isotherm parameters has been characterized in the literature [9–15]. ECP cannot be used to measure multicomponent isotherms.

There are few experimental methods to measure multicomponent isotherms. Frontal analysis (FA) has been widely used to determine isotherms for binary systems [16–20], and recently this method has been applied to ternary systems [21,22]. Another experimental method that can measure multicomponent adsorption is elution on a plateau. One approach involves radio-labeling [23,24], which is tedious and inconvenient. The simpler approach to elution on a plateau, which is called the concentration pulse or the step-and-pulse method, is to use unlabeled pulses. This is easier to run, and has been more widely used in liquid–solid adsorption [25–27]. There are also methods based on theoretical calculations, such as the hodograph method [28–30]. Jacobson et al. [16] proposed a method based on the *h*-transform of Helfferich and Klein [31] that measures binary isotherms for approximately Langmuirian behavior; results from this method agreed with those from the classical frontal method for their system. Jacobson and Frenz [32] also combined these two approaches to formulate a hybrid mass balance method.

The alternative approach to determine the isotherm is to approximate it numerically. One disadvantage of this approach is that the isotherm functional form must be chosen at the outset. Then, by minimizing the difference between calculated and experimental profiles, the parameter values are adjusted until a best fit to the experimental data is found. In practice, several isotherm forms should be tried in order to estimate the most suitable model.

Dose et al. [3] applied the simplex optimization algorithm to determine single-component and binary isotherms numerically. Langmuir and bi-Langmuir model parameters were identified successfully. This method was also used by Guan et al. [9] to determine single-component isotherm using a simulated ‘‘experimental’’ profile, and found more accurate than ECP. Another optimization approach, the steepest descent algorithm, has been applied by James et al. [4] for determination of single- or binary-competitive isotherms. For the two binary systems studied (ketoprofen enantiomers in one case and benzyl alcohol and 2-phenylethanol in the other), numerically identified model parameters were in good agreement with those obtained from the ECP or FA methods. The authors suggested that one drawback of their method was that the computation was long and complex.

Choosing an isotherm model appropriate to a given experimental dataset is an important part of numerical estimation, and has been studied by Guiochon’s group. Experimental data on the competitive adsorption of 2-phenylethanol and 3-phenylpropanol in reversed-phase chromatography were fitted to a large number of different isotherm models [18,33–35]. Results from this particular case show that choosing model is a very complex and difficult task. Some of the models are implicit, which increases the run time dramatically. For this data set, the implicit models do not fit the data better than the explicit models, and are therefore unattractive. Among the explicit models, the 11-parameter quadratic model provides the most accurate fit to the binary data. Because no relation between the number of parameters in a model and the accuracy of that model can be derived from the results, simple models with fewer parameters would be preferable for the calculation of band profiles in practice.

Since mobile phase modulators (e.g., organic modifiers in reversed-phase and salts in hydrophobic and electrostatic interaction chromatography) are widely used in practice, it is important to know how they affect adsorption isotherms. Jandera and coworkers [36,37] investigated the effect of methanol concentration on single- and two-component solute isotherms in reversed-phase chromatography. Langmuir, Jovanovic, competitive Langmuir, competitive Jovanovic and quadratic isotherms were used to fit the experimental data. A quadratic dependence of the logarithm of the isotherm parameters on modulator (methanol) concentration was found to apply for the Langmuir isotherms in the range 0–40% methanol. By contrast, in the Jovanovic forms, the parameters themselves (and not their logarithms) were described well by a quadratic expression in the modulator

level. Jandera and co-workers [38,39] also investigated the mobile phase effects in normal-phase chromatography. Here, the competitive Langmuir model failed to describe the data and it was found necessary to take into account the simultaneous effects of a competitive adsorption and of a possible multi-layer association of the already adsorbed molecules.

Antos et al. implemented the Marquardt algorithm for optimization to determine numerically the competitive isotherms of diolone acetate and benzophenone from a real post-reaction mixture at one mobile phase composition in a reversed-phase system [5]. With a quadratic dependence of the logarithm of the isotherm parameters on modulator concentration, they were able to predict the separation for several different mobile phase compositions. Antos et al. also reported in a later paper [6] that using the same algorithm the numerical determination of the isotherm of methyl deoxycholate at various mobile phase compositions in a normal-phase system from isocratic elution profiles. The dependence of the isotherm parameters on the mobile phase composition was described by a three-parameter equation derived from the Snyder model [40]. The results were used to simulate overload gradient elution profiles. The predicted peak shapes are not in complete agreement with the experimental peak shapes. This is acceptable for single component runs, but for multicomponent runs, it may affect productivity or yield calculations appreciably.

2.2. Statement of problem

Many questions remain regarding the numerical estimation of isotherms. In analytical chromatography, the feed concentrations are so low that the various mass-transfer and diffusion parameters (such as the film mass-transfer coefficient, the pore diffusivity, the axial dispersion coefficient) are all independent of feed concentration. And, by definition, the large number of binding sites relative to the number of adsorbing molecules implies that the equilibrium binding constants are independent of the rate constants. Neither of these convenient assumptions holds in nonlinear chromatography. At high feed concentrations, rate parameters can depend on concentration [41,42]. Further, once competitive binding supervenes, the resulting mass balances become nonlinear, making the decoupling between the thermodynamic isotherm parameters and the kinetic rate parameters problematic. This nonlinearity also implies that not all lumped models are equivalent, leading to the question of which kind of lumped model is the best. In the worst case, all rate and equilibrium parameters may need to be determined simultaneously. Since we hope to use simple isocratic elution runs as the source of information for estimation, we must immediately be concerned with identifiability: can so many parameters in fact be uniquely and accurately extracted from a few simple runs? All these questions are beginning to be attacked in the literature as discussed earlier. In addition, such problems can be ‘‘ill-posed’’, i.e., the error in the results (the isotherm parameters) is extremely large even when the error in the input (the chromatogram) is very small. This question of ill posedness in chromatographic systems has been studied by James and co-workers [4,43].

Our approach here is aimed at an even more fundamental question: if we have a full understanding of the appropriate model, and identifiability is guaranteed, how does error in the experimental data translate into error in the resulting isotherm parameters? In other words, given a bound on the typical error associated with any point in the experimental chromatogram, can suitable bounds be found on the estimated parameters? This is an essential question in any parameter estimation or inverse problem. Here, we present an argument to bound the error in the parameters, and thereby establish a relationship between the experimental error and the number of experimental replicates needed to obtain isotherm parameters with a specified standard error.

2.3. Methodology

We use data simulated by a numerical code, called the solver, to represent the experimental chromatogram in order to ensure that the *only* source of error is in the chromatogram; the isotherm model and the exact values of its parameters must therefore be known exactly. Since the latter assumption is never possible when using real data (even if a given isotherm model fits real data well, there will always

be some error involved), simulated chromatograms are the appropriate test bed for the present study. The rate parameters are also fixed, and the same numerical code that is used to generate the data is used in the estimation problem. Thus, error only arises from the chromatogram; we represent experimental error here by incorporating Gaussian noise. At each time, the exact chromatogram simulated by the solver is perturbed by adding a random number representing a certain relative error to produce an *experimental chromatogram*. We define relative error at a point in the chromatogram as the ratio of the error to the concentration at that point. The best-fit isotherm parameters are then generated iteratively using an optimizer that calls the solver repeatedly with changing isotherm parameter values as depicted schematically in Fig. 1. For a given set of isotherm parameter values, the solver generates a *simulated chromatogram*, which the optimizer compares with the experimental chromatogram. The error between these chromatograms is minimized by the optimizer, and the corresponding isotherm parameter values are the best-fit values. The expression to be minimized is given explicitly in Section 3.

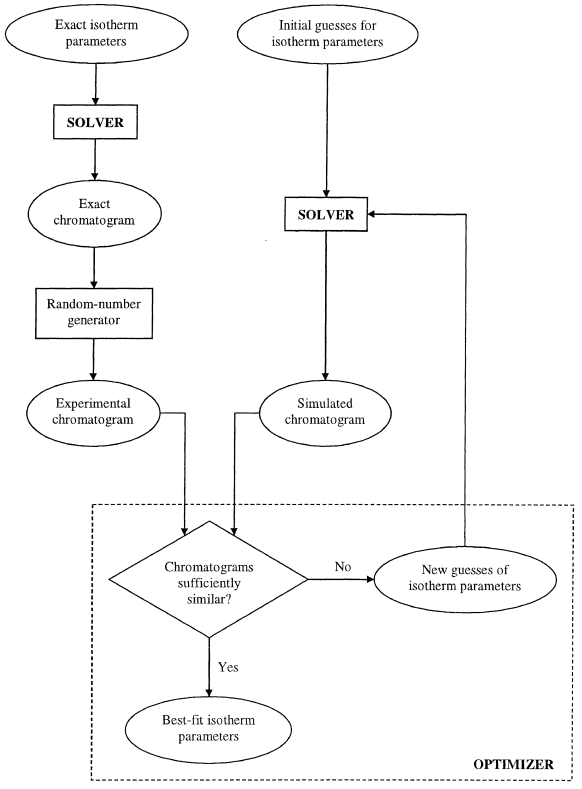

Fig. 1. Flowchart describing the process of estimating isotherm parameters numerically.

A variety of multicomponent isotherms has been used to capture chromatographic retention behavior [2,44,45]. Here, we restrict attention to the simplest of these, the multicomponent Langmuirian isotherm:

$$q_i = \frac{a_i c_i}{1 + \sum_{j=1}^{p} b_j c_j}, \quad i = 1, 2, 3, \ldots, p \tag{1}$$

where p is the total number of adsorbable components in the system. Each feed component contributes two parameters: a is the slope of the isotherm at the origin, and therefore represents the distribution coefficient at analytical concentrations (or Henry's-law constant); b is a measure of its affinity for the stationary phase; the ratio a/b is the corresponding saturation concentration. Although the Langmuirian isotherm is known to be thermodynamically inconsistent unless the saturation concentrations of all the species are identical [46,47], there are many situations in which it is a useful empirical description of realistic multicomponent adsorption. We use it here because it is a simple, explicit formalism, and allows us to concentrate on the question of estimation error. Ternary feeds are used in this work; consequently, six isotherm parameters must be obtained.

2.4. Statistical analysis

We first introduce some convenient notation. The chromatogram generated by the solver for the exact isotherm parameters is called the exact chromatogram. The variants generated by incorporating random errors into the exact chromatogram are called replicates, to distinguish them from real experimental runs, which will simply be called experiments. We will generate a large number of replicates for a given exact chromatogram, in order to be sure that a statistically representative distribution of the parameters is obtained. Later, we will see that a Monte Carlo distribution is found to be necessary for this purpose.

We have found that the variation in any estimated

parameter is well approximated by a Gaussian distribution. A representative result for two parameters from different exact data sets is shown in Fig. 2 (the corresponding isotherm parameter values and feed conditions are listed in Tables 1 and 2, respectively). However, correlation among the parameters must also be accounted for, and this is done in this section. The individual estimates for a_1 are a_{11}, $a_{12}, \ldots, a_{1n}$, where n is the number of replicate runs; similar notation applies for the other parameters. We scale the six average parameters before combining them into the parameter vector $\overline{\mathbf{X}}$; thus its first component, $\overline{\mathbf{X}}_1$, is $(\overline{a_1} - a_{1,\text{exact}})/a_{1,\text{exact}}$ where $a_{1,\text{exact}}$ is the exact value of a_1 and $\overline{a_1}$ is the averaged value of $a_{11}, a_{12}, \ldots, a_{1n}$; the second component, $\overline{\mathbf{X}}_2$, is $(\overline{b_1} - b_{1,\text{exact}})/b_{1,\text{exact}}$; and the sixth component, $\overline{\mathbf{X}}_6$, is $(\overline{b_3} - b_{3,\text{exact}})/b_{3,\text{exact}}$. This has the advantage of making the average value of each component unity. The error in the parameters obtained from the optimization process can be represented through the residual sum of squares (RSS), defined as:

$$\text{RSS} = \left[(\overline{\mathbf{X}}_1)^2 + (\overline{\mathbf{X}}_2)^2 + \ \ldots\ + (\overline{\mathbf{X}}_6)^2\right]^{1/2}$$

Since the standardized isotherm parameters were found to be Gaussian, we have:

$$\mathbf{X}_1 = \frac{a_{1,i} - a_{1,\text{exact}}}{a_{1,\text{exact}}} \sim N(0, \sigma_1^2)$$

where the N on the right-hand side represents a normal or Gaussian distribution with a mean of 0 and a standard deviation of σ_1; similar results hold for all the other parameters. Then the *averaged* parameters satisfy:

$$\overline{\mathbf{X}}_1 \sim N\left(0, \frac{\sigma_1^2}{n}\right) = \frac{\sigma_1}{\sqrt{n}} \cdot N(0,1)$$

Similar results can readily be written for the other parameters. Taking the square of $(\sqrt{n}/\sigma_1)\cdot\overline{\mathbf{X}}_1$ gives a χ^2 distribution with 1 degree of freedom [48]:

$$\frac{n}{\sigma_1^2}\cdot(\overline{\mathbf{X}}_1)^2 \sim \chi_1^2$$

If the isotherm parameters were uncorrelated (i.e., they were independent of each other), then the RSS would be a weighted χ^2 distribution with 6 degrees of freedom:

$$\begin{aligned}\text{RSS} &= \left[\frac{\sigma_1^2}{n}\chi^1 + \frac{\sigma_2^2}{n}\chi_1^2 + \ \ldots\ + \frac{\sigma_6^2}{n}\chi_1^2\right]^{1/2} \\ &= \frac{1}{\sqrt{n}}\cdot\left[\sigma_1^2\chi_1^2 + \sigma_2^2\chi_1^2 + \ \ldots\ + \sigma_6^2\chi_1^2\right]^{1/2}\end{aligned}$$

Note that the term in square brackets is independent of n. However, since the isotherm parameters

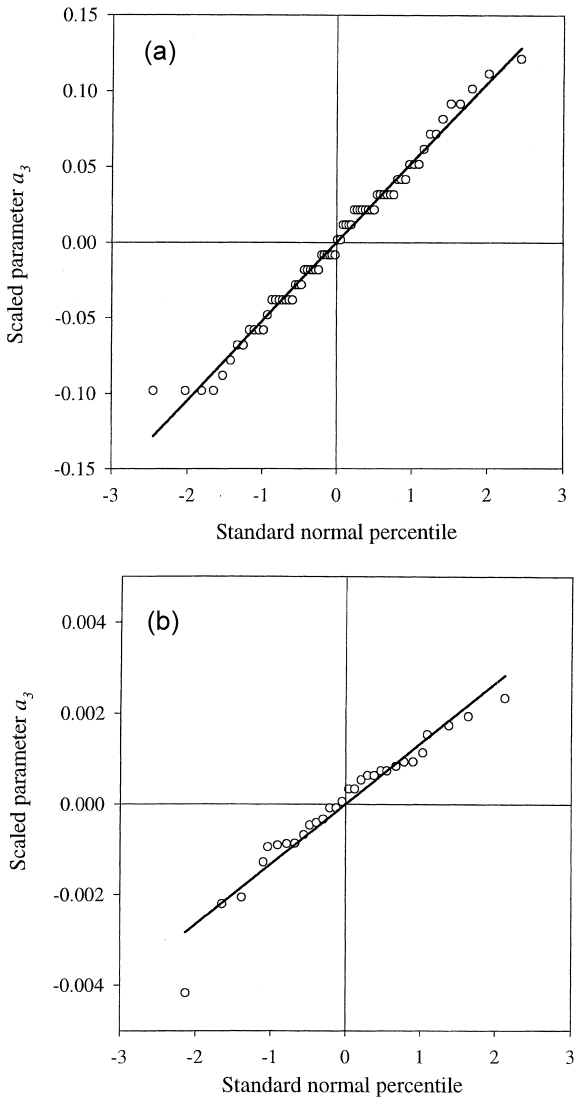

Fig. 2. Probability plot for estimated isotherm parameters. Part (a), estimated parameter a_3 from the exact data II with 10% relative error (70 replicates). Part (b), estimated parameter b_1 from the exact data I with 5% relative error (30 replicates).

Table 1
Isotherm parameter set

Set 1			Set 2		
Parameter	Exact value	Range	Parameter	Exact value	Range
a_1 (–)	3.00	2.4–3.6	a_1 (–)	2.50	2.25–2.75
b_1 (ml/mg)	0.100	10^{-3}–10	b_1 (ml/mg)	0.0714	10^{-4}–1
a_2 (–)	4.50	3.6–5.4	a_2 (–)	3.00	2.7–3.3
b_2 (ml/mg)	0.113	10^{-3}–10	b_2 (ml/mg)	0.075	10^{-4}–1
a_3 (–)	6.75	5.4–8.1	a_3 (–)	3.50	3.15–3.85
b_3 (ml/mg)	0.135	10^{-3}–10	b_3 (ml/mg)	0.078	10^{-4}–1

are correlated in an overloaded run, we must replace the previous expression by:

$$\mathrm{RSS} = \frac{1}{\sqrt{n}}\left[\overline{\chi_6^2}\right]^{1/2} \tag{2}$$

where the overbar on the chi-squared term indicates that the correlated structure of the parameters has been incorporated.

It is interesting to note that the mean and variance of RSS-squared can be calculated explicitly. The mean of RSS-squared is 6, which is exactly the mean of a chi-squared distribution with 6 degrees of freedom, χ_6^2. The variance of RSS-squared is given by:

$$\sigma^2_{\text{RSS-squared}} = 12 + 2\sum_{i=1}^{6}\sum_{j=1}^{6}\rho_{i,j}^2 \tag{3}$$

The first term on the right-hand-side of Eq. (3) is the variance of a chi-squared distribution with 6 degrees of freedom. The second term involves the sum of the correlatedness between the isotherm parameters taken pairwise, and is always positive. Therefore the correlatedness of the parameters always leads to an increase in the variance of RSS-squared. However, the mean and variance of RSS (which is what we are interested in) cannot be explicitly calculated from those of RSS-squared. We will therefore need to use simulations to determine the RSS distribution numerically.

Nevertheless, Eq. (3) is invaluable in pointing out that the variance of χ_6^2 is independent of the number of replicates, *n*. Since this is also true of the mean of χ_6^2, as pointed out above, we conclude that the mean and variance of χ_6^2 also do not depend on *n*. It therefore follows from Eq. (2) that the RSS will decrease as the square root of the number of replicates. We can determine how many replicates will be needed to make the RSS sufficiently small.

Usually, requiring the RSS to be 0.05 should be sufficient for most subsequent applications of the isotherm parameters, and this value has been used in all the calculations below. It can easily be seen from the definition of RSS that this choice (RSS = 0.05) guarantees that no estimated parameter is worse than 5% away from its exact value. Of course, RSS is also a random variable, with its own variance. So we will use the 95th quantile to be reasonably sure (with 95% confidence) that the calculated average value of RSS in a given optimization run lies below 0.05.

An important consequence of obtaining a sum of (correlated) χ^2 distributions is that the variance in RSS will be comparable to the mean. This implies that, in some cases, a large number of replicates may be needed in order to determine the isotherm param-

Table 2
Numerically simulated experimental data set

Exact data	Number of theoretical plates	Loading	Isotherm parameter set	Number of data points
I	1000	$c_{\text{feed}} = 2$ mg/ml, $V_{\text{feed}} = 0.3V_0$	Set 1	179
II	1000	$c_{\text{feed}} = 2$ mg/ml, $V_{\text{feed}} = 0.6V_0$	Set 1	199
III	1000	$c_{\text{feed}} = 5$ mg/ml, $V_{\text{feed}} = 0.4V_0$	Set 2	84
IV	1000	$c_{\text{feed}} = 2$ mg/ml, $V_{\text{feed}} = 0.6V_0$	Set 1	24
V	1000	$c_{\text{feed}} = 2$ mg/ml, $V_{\text{feed}} = V_0$	Set 1	27
VI	2500	$c_{\text{feed}} = 2$ mg/ml, $V_{\text{feed}} = 0.6V_0$	Set 1	188

eters to reasonable accuracy. We will see later that this is in fact the case (cf. Tables 10–12).

3. Simulations

Several simulation methods have been used in our group for preparative chromatography [49,50]. Typically, the lumped solid-phase mass-transfer model is used, which can be represented as:

$$\frac{\partial c_i}{\partial t} + u\frac{\partial c_i}{\partial z} + \phi\frac{\partial q_i}{\partial t} = 0 \tag{4}$$

$$\frac{\partial q_i}{\partial t} = k_{\mathrm{M},i}(q_i^* - q_i) \tag{5}$$

Here, t and z are the independent variables time and distance into the column; the dependent variables are the mobile phase concentration c and the stationary phase concentration q for each adsorbable compound i. The mobile phase velocity is u, the volumetric phase ratio (the ratio of stationary phase to mobile phase volume) is ϕ, and the mass-transfer coefficient for the ith component is k_i. Note that q is not in equilibrium with c, but simply represents the stationary phase concentration. The stationary phase concentration in equilibrium with c is q^*, which is described through the multicomponent adsorption isotherm:

$$q_i^* = f_i(c_1, c_2, \ldots, c_p) \tag{6}$$

where p adsorbable components are present in the column. This represents the *solver*. As mentioned earlier, only Langmuirian isotherms for ternary feeds ($p=3$) will be used here. Both Craig plate models and rate models based on the method of characteristics are used to solve the lumped equations above. It was found for these data sets that the Craig plate simulations were significantly faster than the rate model; since the optimization process involves many calls to the solver, the Craig process was preferred, and was used for all the results shown here.

In this paper, 5 and 10% relative error are widely used. These values are reasonable for many overloaded runs, for which direct detection by, e.g., a UV detector is usually impossible because the high feed concentrations saturate the detector. Thus sample collection, dilution, and separate analysis are necessary. This increases the overall error associated with the reconstructed chromatogram. We expect that 5% relative error is fairly good for small molecules; proteins and other macromolecules often produce greater errors, and 10% might then be reasonable. The error is added into the exact chromatogram by using the random-number-generator FORTRAN code DRNOR, obtained from Netlib. This code generates normally distributed random iterates with mean 0 and variance 1; these are scaled to produce 5% relative error and added pointwise to the chromatogram to simulate experimental error. This is then used as the ‘‘experimental’’ chromatogram in the optimizer to find the best-fit isotherm parameters.

In order to scale the iterates, we must decide what fraction of the normal distribution lies within 5% relative error (since, with a Gaussian, there will always be some fraction of the values that lie outside of any specified bound). Here, we choose to specify that 90% of the iterates must lie within the specifications; thus, 10% of the distribution is allowed to lie outside of the ±5% limits. This choice was made in order to allow substantial likelihood for errant points (outliers). This implies that the estimate for the number of experiments obtained ultimately will be close to an upper bound; in other words, in most cases, fewer experiments may suffice. We therefore obtain a ‘‘worst-case’’ estimate with this choice.

The results from any run of the solver during the estimation process (as shown in Fig. 1) are then compared against the experimental chromatogram, and an estimate of how well the simulation fits the experimental data is constructed. Here, an output least-squares method [51] is used; thus the objective function is the sum of the squares of the differences between the experimental and simulated concentration values at various instants. For example, for a single-component run where the isotherm is to be fitted to the Langmuir isotherm, the objective function J would be given by:

$$J(c;a,b) = \sum_{m=1}^{M} \left[c_{\mathrm{sim}}(L,t_m;a,b) - c_{\mathrm{exp}}(L,t_m)\right]^2 \tag{7}$$

where M is the total number of data points at which the effluent history is known, c_{sim} represents the result of the simulation at the column exit (at $z=L$)

at those discrete times ($t=t_m$) for which experimental data is available. The isotherm parameters *a* and *b* are listed after the semicolon to emphasize that the simulated concentration depends on these parameters. The code that evaluates the objective function for given values of *a* and *b*, and on this basis chooses new values of *a* and *b*, is called the *optimizer*. The problem of estimating the ''best'' isotherm parameters, i.e., those that fit the experimental data best in the sense of minimizing the objective function *J*, is carried out iteratively. We start with a guess for *a* and *b*. The optimizer feeds these values to the solver, and thus obtains the chromatogram corresponding to these values. It then evaluates *J*, and thus decides on a new pair *a*, *b*. The process is repeated until the fit is acceptably good, i.e., the error lies below a specified tolerance. This description used a single-component isotherm for simplicity; as mentioned earlier, all the runs reported in this work are for ternary feeds. Such an iterative method is computationally convenient, allows for incorporation of regularization terms if the problem turns out to be sufficiently ill-posed to warrant it [52], and can be used for almost any kind of chromatogram. Methods that try to invert the problem directly (i.e., working directly from the chromatogram and attempting to calculate the resulting isotherm parameters) are often more ill-posed, and are always limited by the nature of the chromatogram. Thus, a completely resolved chromatogram cannot be used in the direct method to obtain isotherm parameters that depend solely on interaction among the feeds. However, an iterative method simulates the entire chromatographic process, and therefore captures competition among the feeds as they pass down the column subsequent to feed introduction. Such an iterative method is likely to capture pure interaction parameters even when given a chromatogram that is completely resolved.

There are many effective optimization methods available. We use here a public-domain code written by Professor Tits' group at the University of Maryland [53]. This state-of-the-art FORTRAN code solves nonlinear optimization problems with nonlinear and linear equality and inequality constraints, and simple bounds on the variables. The code is based on sequential quadratic programming (SQP) iterations [54,55], in which a sequence of quadratic sub-problems near the solution of the original problem are solved to generate directions of search. Along these directions, better approximations to the solution are determined.

In the Langmuirian isotherm, the *a* parameters are proportional to the linear (Henry's law) distribution coefficients, and can therefore be well estimated from separate analytical runs. The *b* parameters, which relate to the saturation concentration or equivalently the isotherm curvature, cannot be determined easily from independent experiments. In fact, it is the difficulty of determining these nonlinear parameters experimentally that leads us to try numerical estimation in the first place! The two sets of isotherm parameters used in this study are listed in Table 1. Our boundaries on the *a* parameters are fairly tight, at $\pm 20\%$ of the exact values for parameter set 1 and $\pm 10\%$ for parameter set 2. The bounds on the *b* parameters are much wider, to minimize the loss of *b*'s appropriate to the data but excluded because of unduly narrow bounds. (If we had found some optimization runs failing with values of *b*'s close to or at a boundary value, we would have enlarged these bounds suitably. This was not a factor in our runs, and so we are fairly confident that our bounds were appropriate.)

In order to ensure that we reached the global minimum, for each experimental chromatogram we ran six widely different sets of initial guesses. In all cases, all six initial guesses converged to a numerically unique solution. (Slight differences in the final results are sometimes obtained; however, the differences are less than 1%, and we concluded that these represent the same minimum perturbed by the different error in the chromatograms.) It is concluded that for this given bound on the parameters, convergence is guaranteed regardless the initial guesses.

4. Results and discussion

The first set of exact data used was produced by running the solver for the isotherm parameter set 1 and for a feed volume of $V_{\text{feed}}=0.3V_0$. All other conditions are listed in Table 2. The exact isotherm parameter values are: $a_1=3.00$; $b_1=0.100$ ml/mg; $a_2=4.50$; $b_2=0.113$ ml/mg; $a_3=6.75$; $b_3=0.135$ ml/mg. These could be viewed as representative of moderately retained feeds (perhaps amino acids or

small peptides) on a reversed-phase or hydrophobic interaction column under isocratic elution.

Both the chromatogram in the absence of error, and a representative one for 5% error, are shown in Fig. 3a. The feed volume corresponds to bands that are just resolved at the column outlet. The number of theoretical plate is 1000, as listed in Table 2. We will see shortly that larger plate counts do not make much difference in these runs.

Since the estimation process is numerically intensive (recall that each simulated chromatogram was run for six initial guesses), we run a fair number of replicates (e.g., 30 or 40), and then try to extract the covariance matrix from these replicates using standard methods. A very large sample of replicates having this covariance is then generated by Monte Carlo simulations. The RSS distribution is then generated, and Eq. (2) is used to determine the number of experiments needed to obtain isotherm parameters to a certain level of accuracy (here, the 95th quantile is used). The examples below will clarify exactly how the process is carried out. The package S-PLUS is used for statistical calculations [56]. Table 3 shows the number of experiments needed to two significant digits (in practice, we will round this to the nearest integer) as a function of the number of replicates and the size of the Monte Carlo simulation for the results in Fig. 3a. In this case, we did not go further because the number of experiments needed is clearly going to be less than 1, implying that one experiment would be sufficient to identify the isotherm parameters in this case. Of course, in practice, we would always do at least two experiments.

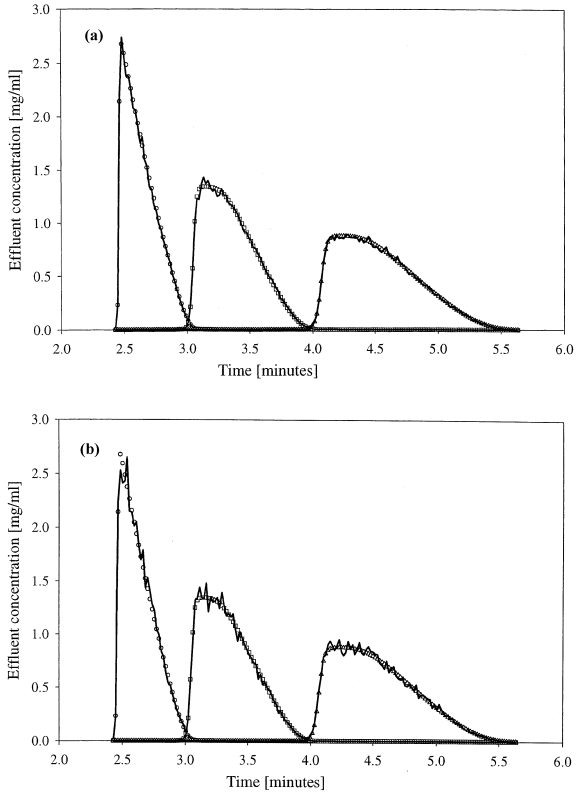

Fig. 3. Simulated chromatogram for exact data I ($V_{feed}=0.3V_0$). Part (a) compares the exact case, i.e., no error added (symbols) with a representative run involving 5% relative error (solid line). Part (b) compares the ''exact'' or ''no error'' case (same symbols as in part a) with a representative run involving 10% relative error (solid line). In all cases, concentrations are reported approximately every second.

Table 3
Number of experiments required for exact data I with 5% relative error

Size of Monte Carlo matrix	Number of replicates		
	10	20	30
1000	0.82	0.77	0.61
10 000	0.83	0.76	0.64
50 000	0.83	0.76	0.63

Table 4 shows the corresponding results for the case where the relative error in the chromatogram was increased to 10% (the feed volume remains at $V_{feed}=0.3V_0$). A representative chromatogram is shown in Fig. 3b. For 10 replicates (the first column of the matrix), it can be seen that the number of required experiments varies somewhat as the size of the Monte Carlo sample increases. This variation decreases as the number of replicates increases, as

Table 4
Number of experiments required for exact data I with 10% relative error

Size of Monte Carlo matrix	Number of replicates			
	10	20	30	40
1000	2.8	2.3	2.1	2.1
10 000	2.6	2.3	2.1	2.1
50 000	2.6	2.3	2.1	2.1

can be seen from the second and third columns of the matrix. Thus the numerical process involves running as many replicates and choosing as large a Monte Carlo sample as necessary to achieve numerical convergence. It is clear from Table 4 that 30 replicates of the numerical estimation process (corresponding to 30 versions of the exact chromatogram to which error is added randomly as described above) are needed to obtain two-digit accuracy in the result. On this basis, we conclude that two experiments should be done (a safe estimate would be three experiments). For each of these runs, the estimation process should be carried out to arrive at an estimate of the isotherm parameters. Taking the mean values of these parameters gives us the final result; the theory presented above indicates that the RSS associated with these mean isotherm parameters is below 0.05, with 95% confidence.

Next, a simulation (exact data II in Table 2) using a larger feed volume ($V_{feed} = 0.6V_0$) is used to generate the experimental chromatogram (see Fig. 4). Now the bands are mixed on emerging from the column. Since mixed bands represent greater interaction among the feed components, it is interesting to compare the results of this case to the previous one. It should be remembered, however, that the entire chromatographic process is being simulated in the solver. Thus, even if the bands are resolved at the column outlet (as in Fig. 3), the components were mixed over a considerable portion of the column, and interacted with each other over that length. Thus, feed–feed interactions may well be adequately described in this numerical approach even when the bands are resolved at the outlet. In this sense, this method is more general than methods based simply on the effluent chromatogram (such as all experimental methods). Again, we first generate 5% relative error using random iterates (a typical chromatogram is shown in Fig. 4a), and calculate the corresponding best-fit parameters. The results are shown in Table 5; now 40 replicates are needed for convergence to the RSS distribution, and we find two experiments should suffice to obtain isotherm parameters for which RSS = 0.05 with 95% confidence.

Table 6 shows the corresponding case where the relative error is increased to 10% as the feed volume remains at $V_{feed} = 0.6V_0$. A typical chromatogram is found in Fig. 4b. Considerably more variation is found than that in the previous runs; now 70 replicates are needed to provide convergence to two significant digits. Notice that the number of experiments needed has gone up significantly relative to that in Table 5. The RSS as a function of the number of experiments needed, *n*, is shown in Fig. 5.

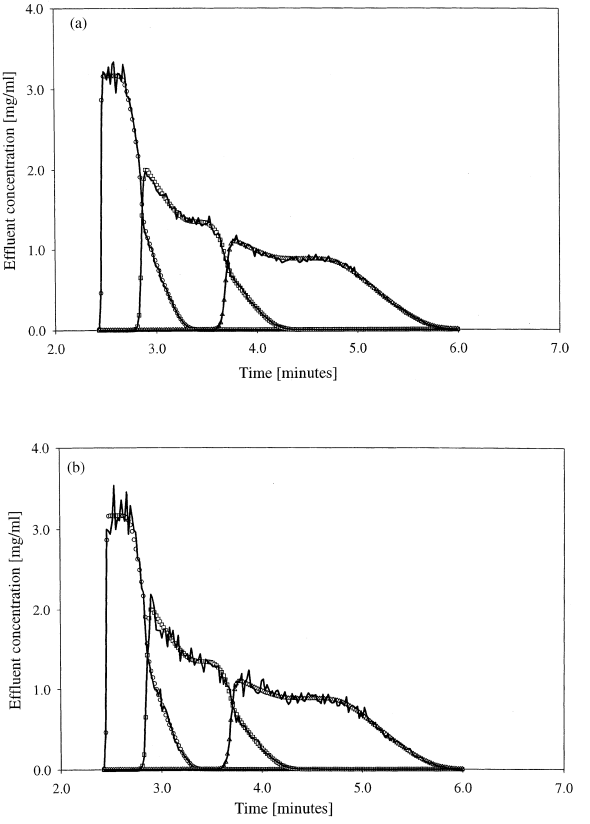

Fig. 4. Simulated chromatogram for exact data II ($V_{feed} = 0.6V_0$). Part (a) compares the exact case (symbols) with a representative run involving 5% relative error (solid line). Part (b) compares the exact case (same symbols as in part a) with a representative run involving 10% relative error (solid line). All other information as in Fig. 3.

Table 5
Number of experiments required for exact data II with 5% relative error

Size of Monte Carlo matrix	Number of replicates			
	10	20	30	40
1000	2.5	1.8	1.9	1.9
10 000	2.5	1.7	1.8	1.8
50 000	2.5	1.7	1.8	1.8

Table 6
Number of experiments required for exact data II with 10% relative error

Size of Monte Carlo matrix	Number of replicates						
	10	20	30	40	50	60	70
1000	11	7.2	7.3	7.2	6.3	6.3	6.4
10 000	10	6.7	7.1	7.4	6.4	6.3	6.3
50 000	11	6.8	7.1	7.4	6.4	6.3	6.3

Table 7
Number of experiments required for combination of exact data I and II both with 5% relative error

Size of Monte Carlo matrix	Number of replicates		
	10	20	30
1000	0.58	0.41	0.36
25 000	0.53	0.42	0.38
50 000	0.53	0.42	0.38

Consideration of the results in Tables 3–6 indicates that the chromatogram representing complete separation (Fig. 3) needed fewer experiments for the same level of error than did the chromatogram with overlap (Fig. 4). This is an interesting and somewhat unexpected result. This may be because of the iterative nature of the optimization method used here. As mentioned earlier if the bands are fully separated at the column outlet, the solver simulates the entire chromatographic process, including the period at the beginning of the column over which the bands underwent strong mutual interference. So even fully separated bands may be efficiently estimated by this method.

If another isotherm formalism were used that contained interaction terms, i.e., terms that vanished when only one component was present, then it is clear that mixed data of the kind shown in Fig. 4 would be essential to identify the parameters contained in these interaction terms. Isotherms with such interaction terms have been proposed in the literature [18,33–35].

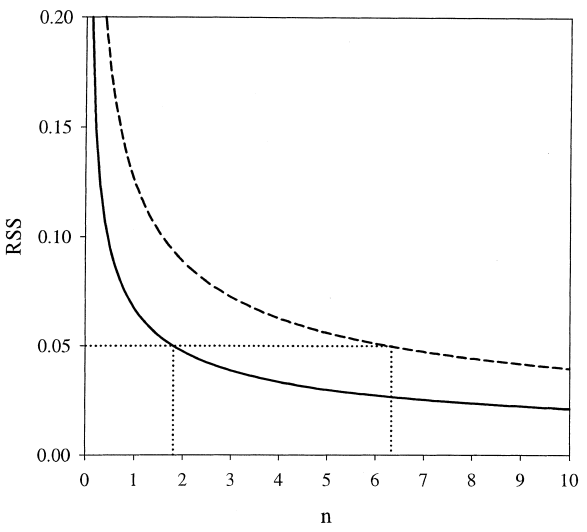

Fig. 5. RSS as a function of *n*, the number of experiments needed for results listed in Table 3 (solid line) and Table 4 (dashed line).

We now examine the possibility of using *both* datasets above simultaneously in the optimizer, i.e., will fitting both small and large feed volume chromatograms at the same time provides better isotherm information? The results for 5% relative error in both datasets are shown in Table 7, and for 10% error in Table 8. The results seem better than those for either the small or large feeds individually, since the number of experiments needed is smaller. But it should be kept in mind that a ''single'' experiment in Tables 7 and 8 corresponds to doing one small-feed *and* one large-feed run. Thus, for 10% error, using the small-feed run alone requires two runs, from Table 4; using the large run alone requires six (or seven) runs, from Table 6; using them both together requires two runs of each kind. However, this kind of combined run is often useful in that it is likely to be not much worse than the best case, and much better than the worst case. Since we do not know beforehand (for an arbitrary isotherm) which case is the best, the combined run may be quite attractive in practice.

As another way of examining the variation in the parameters, the individual results of the various

Table 8
Number of experiments required for combination of exact data I and II both with 10% relative error

Size of Monte Carlo matrix	Number of replicates				
	10	20	30	40	50
1000	2.1	1.8	1.7	1.5	1.6
25 000	2.3	1.8	1.6	1.5	1.5
50 000	2.3	1.8	1.6	1.5	1.5

replicates for the calculations above are shown in Fig. 6. It is clear that the relative variation in the *a* parameters is far less than in the *b* parameters. This is to be expected in part because of the much tighter bounds on the former.

The runs above were based on simulated chromatograms for a ternary system with moderate selectivity (around 1.5 for each adjacent pair of components). While this is realistic for preparative

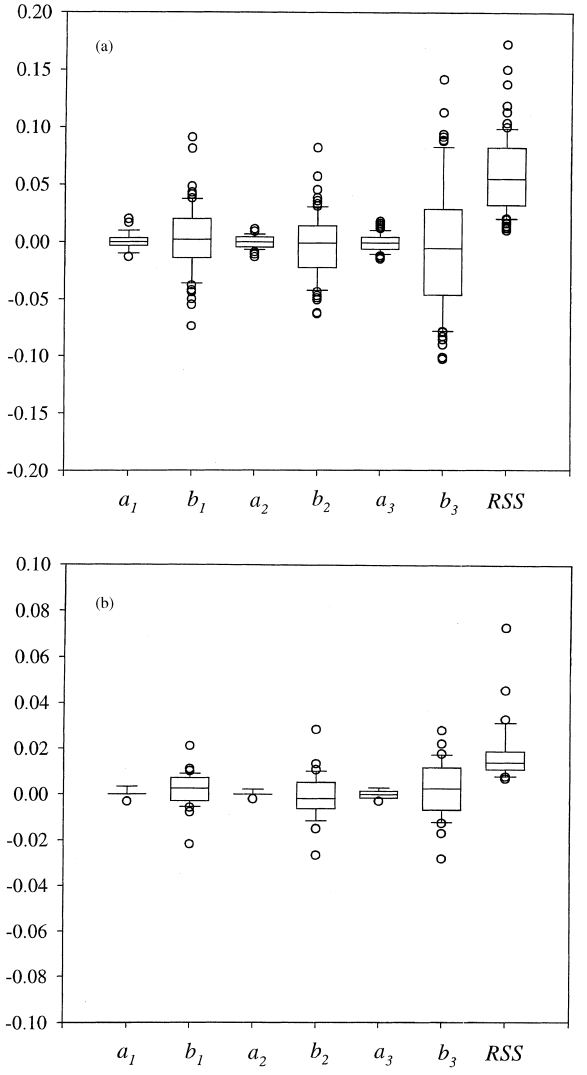

Fig. 6. Variations in simulated parameters (scaled with respect to the exact parameters) and RSS. Part (a), exact data II with 10% relative (70 replicates). Part (b), combination of two exact data I and II both with 5% relative error (30 replicates).

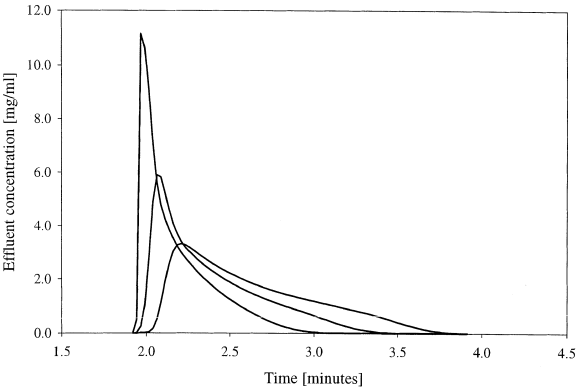

Fig. 7. Simulated chromatogram for exact data III ($V_{feed} = 0.4V_0$). Different isotherm parameters from those used in the earlier figures; this figure represents a more difficult separation.

runs, it is of interest to see how the results would change if the selectivity were decreased. Fig. 7 is the exact chromatogram (exact data III in Table 2) for a ternary system in which the binary selectivities are 1.1, with $V_{feed} = 0.4V_0$. The exact isotherm parameter values are listed in Table 1. Considerably more mixing is found than that in the earlier runs with comparable feed volumes for the system with higher selectivity (cf. Figs. 3 and 4). When 5% relative error was added in the usual way, the results for up to 60 replicates are shown in Table 9. Now seven experiments are needed, compared to one (Table 3) or two (Table 5). When 10% relative error is used, the number of experiments needed increases to 36 (Table 10). This is an indication that highly overloaded systems may have too much mixing among the feed bands to obtain the isotherm parameters effectively. Although this is a single result, we might cautiously suggest that there is a range of loading for which experimental estimation is facilitated, and that

Table 9
Number of experiments required for exact data III with 5% relative error

Size of Monte Carlo matrix	Number of replicates				
	20	30	40	50	60
1000	5.7	6.0	6.7	6.5	6.4
10 000	5.9	5.7	6.6	6.2	6.3
50 000	5.8	5.8	6.6	6.5	6.2
100 000	5.8	5.8	6.6	6.5	6.2

Table 10
Number of experiments required for exact data III with 10% relative error

Size of Monte Carlo matrix	Number of replicates				
	20	30	40	50	60
1000	51	38	41	32	36
10 000	46	39	39	36	35
50 000	47	38	39	36	36
100 000	51	40	40	36	36

very low or very high loading complicates the estimation process.

Another factor that may affect the results is the number of data points in the experimental chromatogram. In the earlier runs (Figs. 3 and 4), data points were generated approximately every second. While this is realistic for detectors in current use (in fact, typical detectors collect several points per second), in many cases the feed concentrations in preparative runs are high enough to saturate the detector. Then fractions must be collected, diluted if necessary, and re-analyzed in order to reconstruct the preparative chromatogram. In this case, the number of points in the chromatogram will be dramatically reduced. Fig. 8 (exact data IV in Table 2) shows the same chromatogram as in Fig. 4 (for $V_{feed}=0.6V_0$), but with data points being generated every 10 s. The usual process of estimating the parameters was done, and the result for 5% relative error is shown in Table 11. It can be seen that the number of experiments

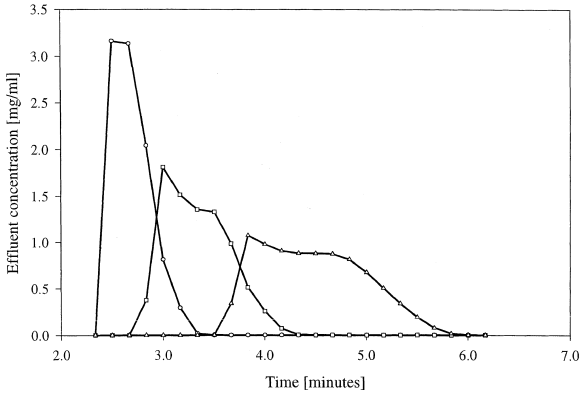

Fig. 8. Simulated chromatogram for exact data IV ($V_{feed}=0.6V_0$). The same exact case as in Fig. 4, but here the concentrations are reported approximately every 10 s (21 nonzero points).

Table 11
Number of experiments required for exact data IV with 5% relative error and less data points than that of Table 5

Size of Monte Carlo matrix	Number of replicates			
	10	20	30	40
1000	8.2	12	11	12
10 000	8.9	12	11	11
50 000	8.8	12	11	11

required has increased significantly, to 11. This indicates that the large number of points in the earlier runs (exact numbers are listed in Table 2) are in fact useful in minimizing the variance of the converged result.

One way to increase the number of data points when collecting fractions is to increase the loading; for isocratic elution, this should result in a wider band. The feed volume was increased to $V_{feed}=V_0$ in Fig. 9 (exact data V in Table 2), and the corresponding results shown in Table 12. It can be seen that the number of experimental data points did not

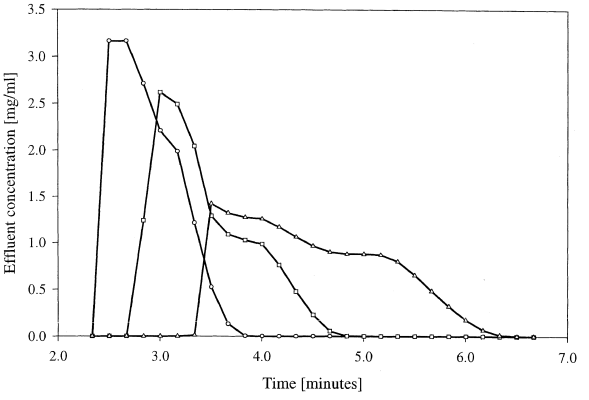

Fig. 9. Simulated chromatogram for exact data V ($V_{feed}=V_0$) with 24 nonzero points.

Table 12
Number of experiments required for exact data V with 5% relative error

Size of Monte Carlo matrix	Number of replicates		
	10	20	30
1000	7.9	9.3	10
10 000	8.5	10	10
50 000	8.8	10	10

Table 13
Number of experiments required for exact data VI with 5% relative error

Size of Monte Carlo matrix	Number of replicates		
	10	20	30
1000	1.7	1.4	1.4
10 000	1.6	1.4	1.4
50 000	1.6	1.4	1.4

increase appreciably in Fig. 9, and the required number of experiments went to 10, which is a very small change from 11. Notice that the pure portion of the middle component is already quite narrow in Fig. 8, and does not exist in Fig. 9. Increasing the loading much further would in effect produce a case analogous to that of Fig. 7, where the bands are highly mixed; the number of experiments required would therefore increase again. The issue of increasing the number of experimental data points by using an appropriate combination of sampling (fraction collection) and detection is therefore one of great practical importance.

The effect of column efficiency on parameter estimation was investigated by repeating the runs above with higher plate counts. An exact data with 2500 plates was made (exact data VI in Table 2) with all other conditions the same as exact data II. Table 13 shows the number of experiments needed for this data set with 5% relative error. Convergence is achieved by 30 replicates, and two experiments should suffice. The results corresponding to 10% relative error are shown in Table 14, where six experiments are seen to be necessary. Comparing the chromatogram due to 2500 plates to that due to 1000 plates (Fig. 10), a very slight increase in resolution is seen, due to the sharper peaks at $N=2500$. Slight differences can also be observed at the top and

Table 14
Number of experiments required for exact data VI with 10% relative error

Size of Monte Carlo matrix	Number of replicates		
	10	20	30
1000	6.5	5.7	5.7
10 000	6.7	5.2	5.5
50 000	6.6	5.5	5.5

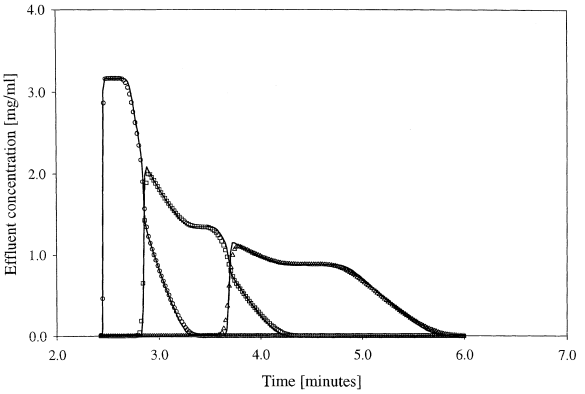

Fig. 10. Simulated chromatograms for exact data II and VI ($V_{feed}=0.6V_0$). Solid line for exact dataset VI (2500 plates) and symbols for exact data II (1000 plates).

plateau portion of the second and third band. The effect on the estimation process is also small: the number of experiments needed decreased by 1 for 10% error, and remained the same for 5% error. Increasing the column efficiency further would make even less difference to the exact chromatogram, and it is likely that the number of experiments will be similarly insensitive.

Finally, we compare the distributions generated by the relatively small number of replicates and those produced by the large Monte Carlo simulations. Fig. 11 shows the histogram from the replicates and the density distribution generated by the Monte Carlo simulations for the case described in Table 6. It is clear that the original replicates would have provided a poor estimate of the 95th quantile, from which we calculate the number of experiments required to obtain 5% precision in the final isotherm parameters. The density distribution is seen to be asymmetric, with a significant tail. It should be noted that the number of experiments calculated from the Monte Carlo estimate of the 95th quantile is itself a variable with some error. The process of generating Monte Carlo distributions is therefore repeated several times (typically 20) for the bottom right entry in each of the tables, in order to determine a standard deviation for the reported number of experiments. In all cases we have studied, these relative standard deviations are less than 1% of the mean, which indicates that the results of the tables can be regarded with confidence.

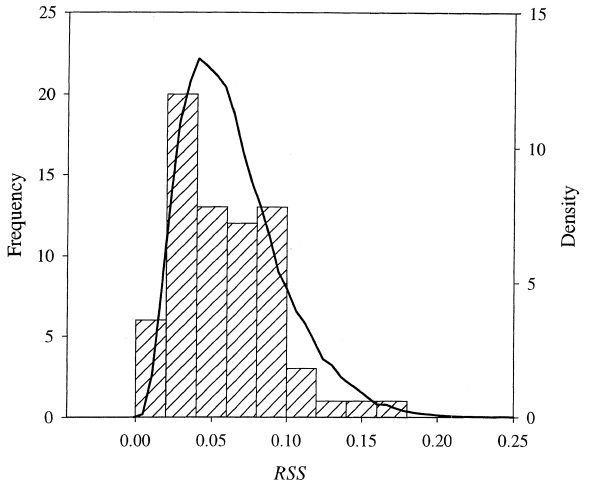

Fig. 11. Plot of the histogram obtained from the 70 replicates used in Table 6 (exact data II with 10% relative error), and the corresponding density of the Monte Carlo distribution with the same covariance with 50 000 replicates (solid line).

Fig. 12 shows the same comparison of the histogram from the replicates with the density generated from the Monte Carlo simulations for the case described in Table 7. Again, a tailing density distribution is found. The single entry in the histogram with an RSS between 0.07 and 0.08 is an event of extremely low probability, since the corresponding Monte Carlo simulation with 50 000 replicates does not have a significant density value in this range.

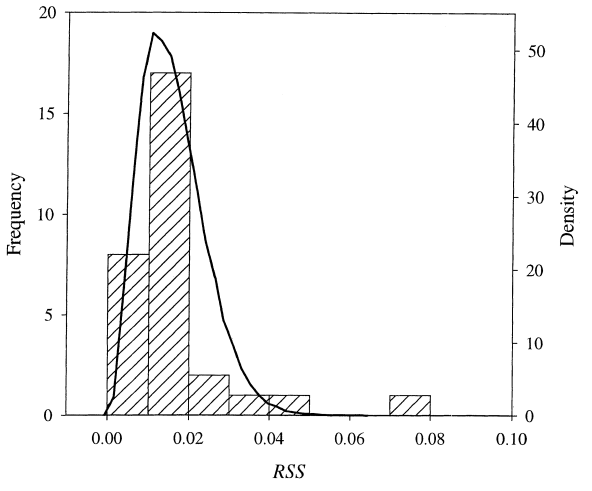

Fig. 12. Plot of the histogram obtained from the 30 replicates used in Table 7 (combination of exact data I and II both with 5% relative error), and the corresponding density of the Monte Carlo distribution with the same covariance with 50 000 replicates (solid line).

The work presented here could be expanded in several ways. Firstly, other perturbations on the experimental data could be considered. For example, slight changes in flow-rate or mobile phase composition or gradient timing and slope would result in changes in the times at which the bands emerged from the column. Since we have so far only considered perturbations in the effluent concentrations, but kept the times at which they emerge fixed to their exact values, this would provide an additional source of data regarding error propagation in the estimation of isotherms. Secondly, and more importantly, this work must be tested on real experimental data, for which the ''exact'' isotherm model is unknown; then the combined effect of model error *and* experimental error on the required number of experiments must be assessed. While this is a vital problem, it can only be studied once the role of each kind of error has been estimated. The current work provides an explicit basis for quantifying the effect of experimental error on isotherm parameter estimation.

5. Conclusions

Numerical estimation of isotherm parameters is rapidly becoming an attractive and viable method for determining multicomponent adsorption data. In this paper, the influence of experimental error on the variance of the resulting parameters is addressed quantitatively. It is shown that the variation in the RSS, which is a measure of the goodness of fit, varies inversely as the square root of the number of replicates. From this result, we have shown how many experiments of a certain kind will be needed to identify parameters in a ternary mixture to 5% accuracy. Using experimental data of different kinds is discussed; in many practical separations, this may be a simple way to explore a larger fraction of the parameter space effectively. An attractive feature of the numerical approach used here is its effectiveness even when the effluent chromatogram consists of fully resolved peaks. The extent of loading, resolution and the number of data points in the experimental chromatogram are shown to be significant

parameters in the estimation process; however, estimation is relatively insensitive to plate count for N larger than 1000. Comparison of the discrete histograms with the Monte Carlo density distributions shows that the process is statistically sound.

An important feature of the results is the wide variation in the number of experimental replicates needed to obtain tightly bounded isotherm parameters. In some cases (e.g., Table 10), the estimation method described here would require an unrealistically large number of experimental replicates. As against that, the marked decrease in the number of experimental replicates required when more than one kind of experiment was run (e.g., Table 8) is very promising, and may provide a way to achieve good estimates from relatively few experimental runs.

In the present work, we have focused on experimental errors that changed the effluent concentration values. As mentioned in the text, other sources of error can lead to changes in the effluent times. We hope to present results incorporating these additional sources of error in a future publication.

Acknowledgements

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