Theoretical study of the accuracy and precision of the measurement of single-component isotherms by the elution by characteristic point method

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ABSTRACT

Using the equilibrium-dispersive model of chromatography, band profiles corresponding to a known Langmuir isotherm are calculated. A noise sequence is added to the calculated profile to simulate an actual chromatogram. The elution by characteristic point (ECP) method of isotherm calculation is then applied to this profile, and the derived isotherm is compared with the initial one. Significant differences between the "true" and the "measured" isotherms are observed at low or moderate column efficiencies. The direct method of determination of the isotherm from the band profile based on the numerical solution of the inverse mathematical problem gives more accurate results than ECP, especially at low column efficiency. It is recommended to use the ECP method only when the column efficiency exceeds markedly 2000 theoretical plates.

INTRODUCTION

The method of elution by characteristic points (ECP) is widely used for the determination of equilibrium isotherms. Arising from the work of Glueckauf [1,2], it was developed by Cremer and Huber over 30 years ago [3]. This method is based on the use of a simple equation that gives the profile of the diffuse part of an overloaded elution band. However, this equation is derived from the ideal model, and the ideal model assumes that the column has an infinite efficiency. As real columns have a finite efficiency, which broadens the diffuse parts of elution profiles, the ECP method contains a definite model error, and its use is recommended only with columns having a sufficient efficiency [3,4].

So far, no efficiency threshold has been defined, and there is no information available regarding the dependence of the model error on the column efficiency. In the past, several workers have attempted to make corrections to this method, but only limited success has been achieved.

Because of its simplicity, however, the ECP method is very attractive and has become widely popular for the determination of single-component adsorption isotherms [4,5]. It can be used with any conventional liquid chromatograph without requiring any modification of the hardware. The sample size required is low compared with the amounts needed in the frontal analysis (FA) and frontal analysis by characteristic point (FACP) methods. Although accurate detector calibration is required in the range of mobile phase composition studied, the possibility of deriving a large number of isotherm data points from a single run gives a good precision, which is sometimes mistakenly assumed to mean a high

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accuracy. Because of the model error and of our current lack of understanding of its actual importance, the accuracy of the ECP method remains in doubt and careful experimentalists rightly prefer frontal analysis, which is highly accurate as it is based on the measurement of the retention times of a series of self-sharpening fronts, results which are not affected by any model errors.

The major advantages of the ECP method, the rapidity of data acquisition and the small amount of component required, seem compelling to us. Because of increasing pressure from regulatory agencies to force waste reduction, we feel that the ECP method will be used more often in the future than it has been in the past. Further, our current interest is in the investigation of the relationships between (i) equilibrium isotherms in a diphasic system, (ii) band profiles in non-linear chromatography and (iii) the production rates and recovery yields of purified products achieved in preparative chromatography. The determination of adsorption isotherms is one of the critical problems that have to be solved to proceed successfully to any practical application [5–7]. Considering the fact that most relevant problems involve complex and expensive chemicals, the ECP method is especially attractive [6,7].

We have decided to re-examine the ECP method and its fundamental background. We know that the equilibrium-dispersive model of chromatography [5,8] permits the calculation of the profiles of bands eluted from columns having a finite efficiency, provided that the equilibrium isotherms and the column efficiency are known. The result obtained is accurate when the column efficiency exceeds 100 theoretical plates [8]. Therefore, we can use a computer to simulate an entire experiment. We first assume an isotherm, and calculate the band profile that would be recorded in the ECP method with a known column, for a known amount of that component. By adding an appropriate noise sequence, a chromatogram like that recorded by an actual detector is obtained. The ECP method can then be applied to this chromatogram. The comparison between the "true" (*i.e.*, assumed) and the "measured" (*i.e.*, ECP-calculated) isotherms

gives the model error. Repeating the same determination with different noise sequences gives the precision of the method. If needed, the effect of flow-rate or temperature fluctuations could also be included [9].

We report here on the results of this study. A forthcoming paper [10] will discuss experimental reproducibility.

THEORY

When a large-size sample is injected into a chromatographic column, an unsymmetrical band is eluted [5]. For a convex-upwards isotherm, by far the most frequent type, the band front is steep and the band rear is diffuse. The ideal model of chromatography, which assumes that the column efficiency is infinite, gives the equation of the diffuse part of the profile [11]:

$$t_R(C) = t_p + t_0 \left(1 + F \cdot \frac{dq}{dC}\right) \tag{1}$$

where $t_R(C)$ is the retention time of the mobile phase concentration C, t_p is the width of the injected pulse (assumed to have a rectangular profile), t_0 is the hold-up time ($t_0 = L/u$, $L =$ column length, $u =$ mobile phase velocity), F is the phase ratio $[V_s/V_m = (1 - \epsilon)/\epsilon$, V_s and V_m being the volumes of the stationary and mobile phase, respectively, and ϵ the packing porosity] and $q(C)$ is the equilibrium isotherm.

The direct use of eqn. 1 serves as the basis of the ECP method. This equation is solved for dq/dC, and the result is integrated as a function of C, hence the isotherm. In practice (Fig. 1), the isotherm is calculated from the elution profile of a large-size sample using the relationship

$$q = \frac{1}{V_a} \sum_{0}^{C} (V - V_0)\delta_i C \tag{2}$$

where q is the amount of the compound adsorbed on the stationary phase in equilibrium with the mobile phase at concentration C, V_a is the volume of adsorbent in the column, V_0 is the hold-up volume, V is the retention volume of the characteristic point of the diffuse profile at concentration C, and $\delta_i C$ is the concentration increment (with $\Sigma \delta_i C = C$).
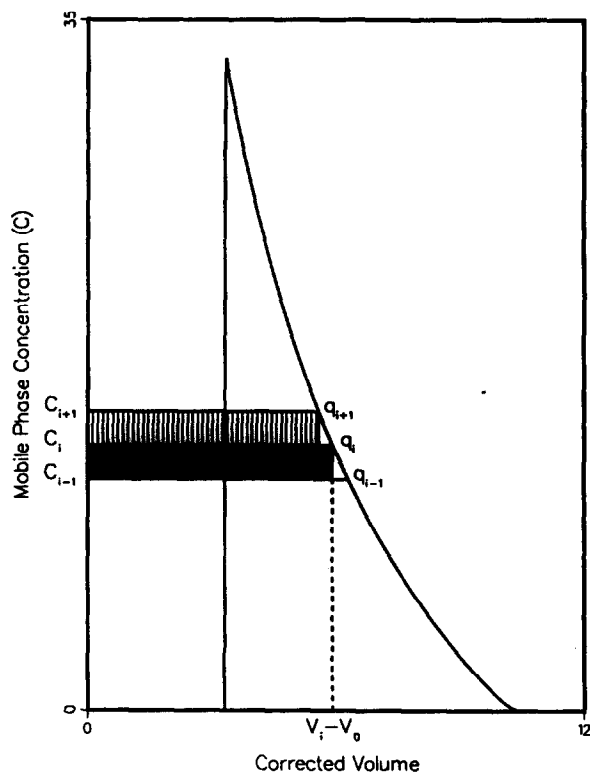
Fig. 1. Elution by characteristic point (ECP). Concentration in mg/ml; volume in ml.

As the efficiency of actual columns is finite, eqn. 1 does not give the true profile of an elution band. This profile can be calculated with excellent accuracy by using the equilibrium-dispersive model [8] which takes the finite column efficiency into account [5–8,11]. Unfortunately, this model has no analytical solutions. Numerical solutions are easy to calculate using one of the algorithms previously described [8]. Figs. 2 and 3 show the profiles calculated for columns of different efficiencies, using two different sample sizes. The isotherm used is the Langmuir model:

$$q = \frac{q_s bC}{1 + bC} \qquad (3)$$

where q_s is the saturation capacity of the adsorbent and b a coefficient related to the adsorption energy. This equation can be normalized by using a reduced concentration, $\Gamma = bC$. Instead of reporting the absolute sample size, it is more convenient and meaningful to report the loading factor, L_f, or ratio of the sample size to

the column saturation capacity (i.e., the amount of component needed to make a complete monolayer on the surface of the column packing).

RESULTS AND DISCUSSION

In the following, we refer to the concentration profiles derived from the equilibrium-dispersive model as calculated profiles and to the profiles obtained after addition of noise sequences as chromatograms. We refer to the initial Langmuir isotherm as the true isotherm, to the isotherms derived by ECP from the band profiles or the chromatograms as the ECP isotherms and to the isotherms fitted to the Langmuir equation with the coefficients derived from the ECP isotherm as the fitted isotherm. Correspondingly, there are two types of errors considered in this study: (1) the error between the true isotherm and that of ECP and (2) the error between the ECP isotherm and the fitted Langmuir model, which is the model error.

Derivation of the isotherm from calculated band profiles

The elution profiles of large-size samples were calculated on columns having 200, 500, 1000, 2000 and 5000 plates, using a true Langmuir isotherm with $b = 0.024$ ml/mg and $q_s = 500$ mg/ml. The profiles obtained for loading factors of 0.05 and 0.20 are shown in Figs. 2 and 3 respectively. The first value corresponds to a moderate degree of column overloading and the second to a strong overloading. The column dimensions were 25 cm × 4.6 mm I.D., the flow rate was 1 ml/min and the phase ratio was 0.25. The profiles obtained are typically Langmuirian. Their widths decrease with increasing column efficiency, the fronts become steeper and the tailing of their diffuse rear part becomes less important.

As a first step, the equilibrium isotherm was calculated by applying the ECP method described above to the elution band profiles shown in Figs. 2 and 3, before noise sequences were added. The ECP isotherms derived from the low-efficiency profiles in Fig. 3 ($L_f = 0.20$) are shown in Fig. 4. Similar results were obtained for a loading factor of 0.05. We see in Fig. 4 that
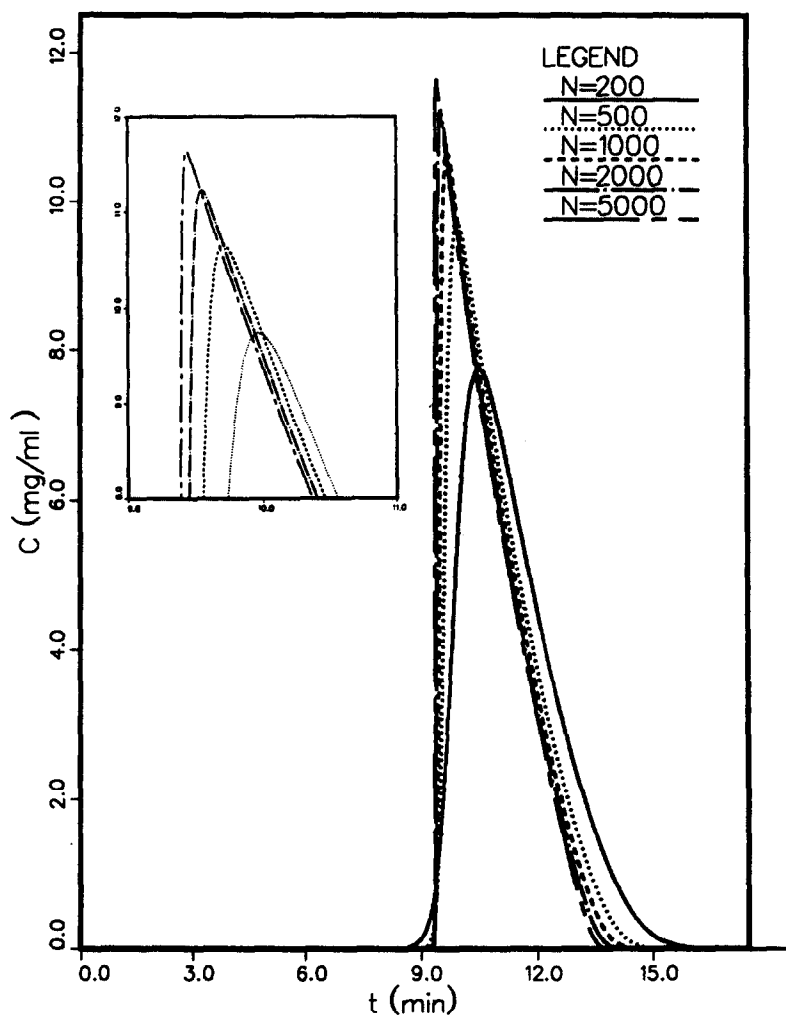
Fig. 2. Band profiles for $L_f = 0.05$ and $a/b = 500$. Inset: enlargement of the area around the top of the band profiles.

there are important, systematic differences between the "true" isotherm and the ECP isotherms derived from the profiles calculated with efficiencies of 200, 500 and 1000 theoretical plates. The ECP isotherm is always above the true isotherm.

Similar results were obtained for the profiles calculated with 2000 and 5000 theoretical plates. As the differences between ECP and true isotherms are now smaller, however, we show in Fig. 5 the ratio of the ECP to the true isotherm as a function of the sample concentration in the mobile phase. We see that, even at the highest efficiency considered (5000 theoretical plates), the ECP isotherm is nearly 5% higher than the

true one at very low concentrations, and still approximately 2% higher for $bC = 0.024$. Another striking feature of Fig. 5 is that the amount of solute adsorbed by the stationary phase at equilibrium is always overestimated, by approximately 1% for a column having as many as 5000 plates and by 3% for a column having 1000 plates.

Illustration of the model error is provided in Fig. 6, where the model error is plotted *versus* the concentration, C, in the case of the profile calculated with 2000 theoretical plates. The model error is shown as the residual, or the difference between the value of the adsorbed amount at equilibrium obtained from the fitted
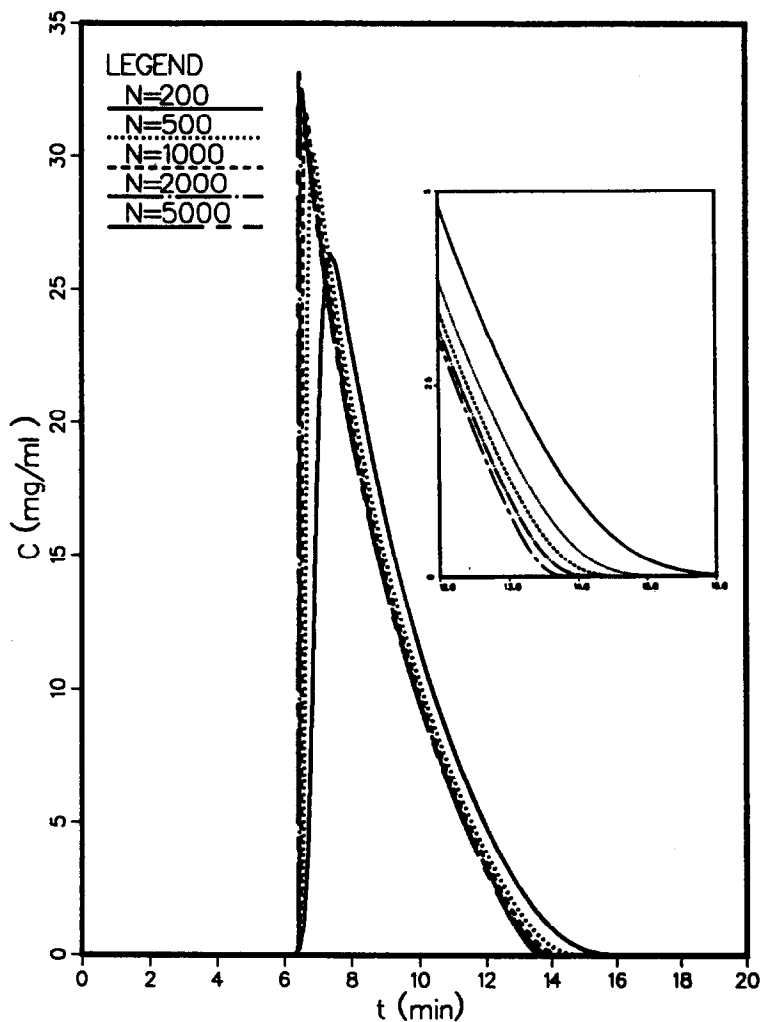
Fig. 3. Band profiles for $L_f = 0.20$ and $a/b = 500$. Inset: enlargement of the area around the tail of the band profiles.

Langmuir isotherm, and the value given by the ECP isotherm at the same concentration. The systematic error is important. Because of it, the ECP isotherm is not a Langmuir isotherm, and the fitting of the ECP isotherm data to the Langmuir equation will result in systematic errors on the coefficients q_s and b.

These errors could explain, at least in part, the difficulties encountered in previous studies with the accurate determination of adsorption isotherms of proteins in reversed-phase systems, and in the prediction of their elution band profiles in gradient elution [7]. The use of high-efficiency columns which have at least 5000

theoretical plates appears to be necessary in order to limit the systematic errors made to an extent compatible with the precision required in the prediction of band profiles for the optimization of the experimental conditions in preparative separations.

Derivation of the isotherm parameters from chromatograms

In the second step, noise sequences were added to the profiles shown in Figs. 2 and 3. These noise sequences were derived from a generator which gives random numbers, RN, between 0 and 1. A scale factor SF of 4σ was
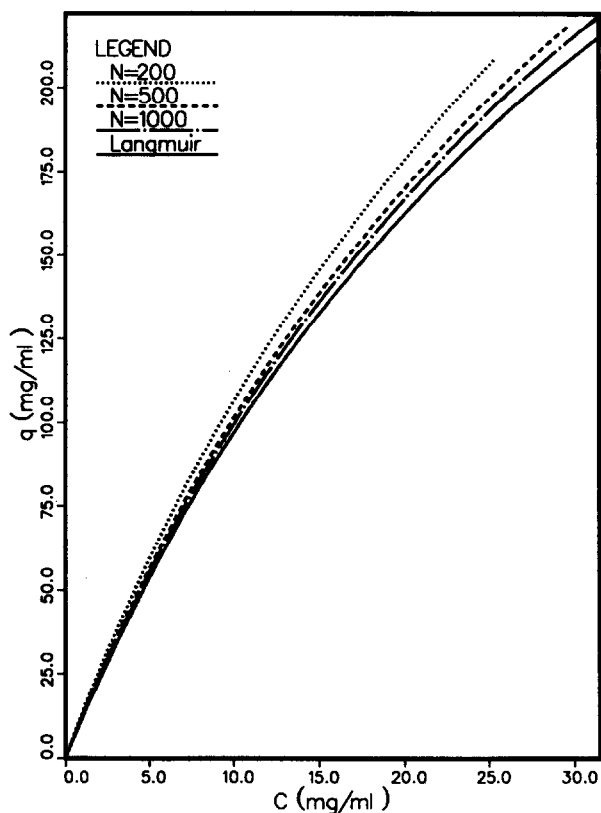
Fig. 4. Comparison between ECP isotherms and the true Langmuir isotherm for columns with low efficiencies ($L_f = 0.20$; $a/b = 500$).

Fig. 5. Comparison between ECP isotherms and the true Langmuir isotherm for columns with high efficiencies ($L_f = 0.20$; $a/b = 500$).

multiplied to these random numbers to achieve the desired signal-to-noise ratio (SNR), which is defined here as the peak height obtained in the case while no noise signal was introduced divided by σ, the standard deviation of each point in the chromatogram. The number of data points, M, is neglected in this definition of SNR for reasons of clarity, because of its dependence on the number of theoretical plates as dictated by the algorithm and the arbitrary nature of SNR definitions. The number of points for each experiment are as follows: $N = 5000$, 2000, 1000, 500 and 200 plates correspond to $M = 1498$, 650, 356, 201 and 101 points (for $L_f = 0.20$) and $M = 953$, 439, 256, 156 and 88 points (for $L_f = 0.05$). However, the trends observed in this study are independent of M. The baseline estimate was taken as the numerical average of the above generated random numbers, R_{ave}. For each point of the band
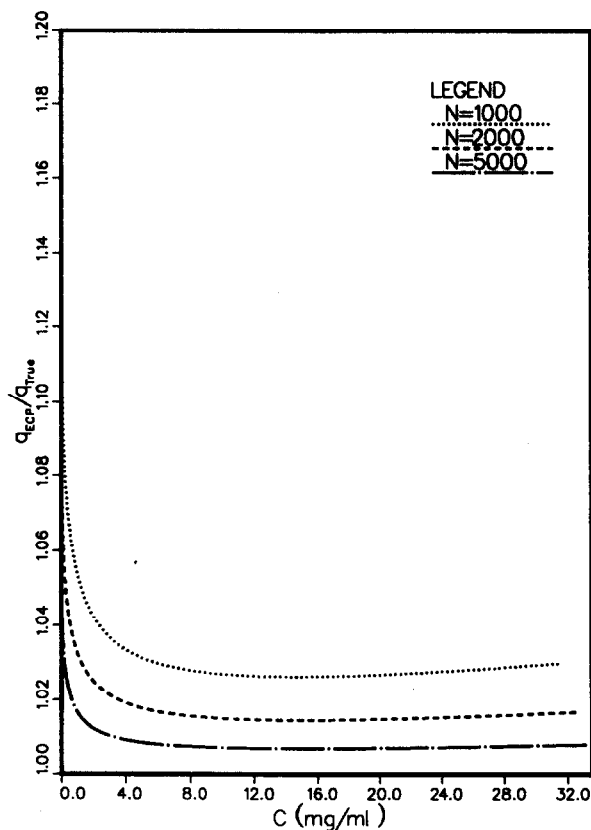
profile supplied by the equilibrium-dispersive model algorithm, a noise signal with the value $(RN \cdot SF - R_{ave})$, which is approximately $(RN - 0.5) \cdot h \cdot 4/SNR^a$, was added. The operation was repeated nine times, generating a total of ten chromatograms. For each chromatogram, the ECP isotherm was calculated, then the isotherm data points were fitted to the Langmuir model and the best values for the coefficients of the model were calculated. This provided a series of ten values for each coefficient and the final result was taken as the average of these ten values.

There are several ways to derive the best

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[a] Statistical study shows that the rectangular probability density function with width $4h/SNR$ gives a standard deviation of $4h/SNR\sqrt{12} \approx 1.15h/SNR$. Compared with the σ chosen in our paper, which is defined as h/SNR, there is no significant difference between these two.
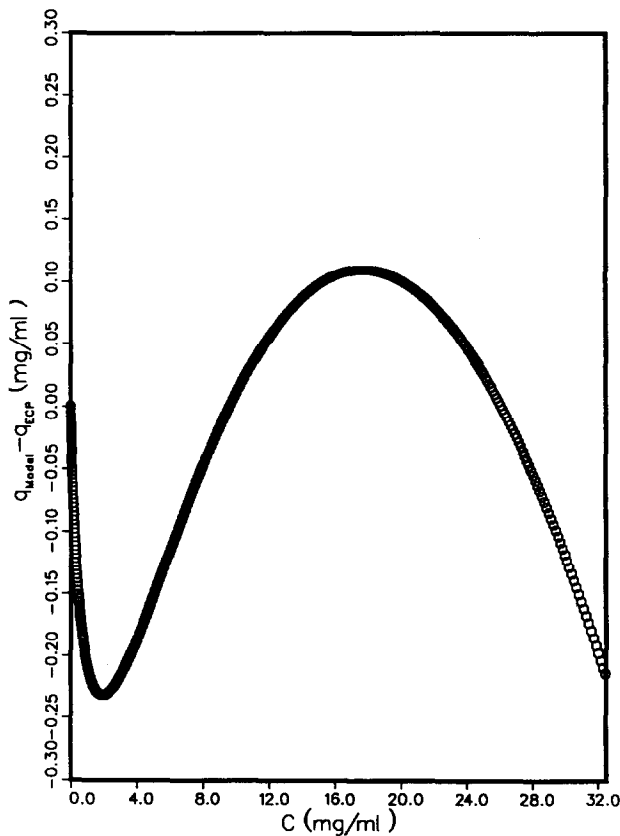
Fig. 6. Plot of the residual *versus* mobile phase concentration C. $L_f = 0.20$, $N = 2000$.

values of the model coefficients. First, the data points may or may not be smoothed prior to further calculations, and there are many smoothing algorithms. In our case, when the data were smoothed, a seven-point moving method, which takes the average of five points between the maximum and minimum, was used. Second, we can linearize eqn. 3 into several forms [12], the most popular being

$$q = aC - bqC \qquad (4)$$

with $a = bq_s$. By use of the conventional least-squares method, b and a (hence $q_s = a/b$) can be expressed by

$$a = \frac{\sum_i q_i C_i + b \sum_i q_i C_i^2}{\sum_i C_i^2} \qquad (5)$$

and

$$b = \frac{\sum_i q_i^2 C_i \sum_i C_i^2 - \sum_i q_i C_i \sum_i q_i C_i^2}{\left(\sum_i q_i C_i^2\right)^2 - \sum_i (q_i C_i)^2 \sum_i C_i^2} \qquad (6)$$

Equations may also be similarly derived to yield the standard deviation of the coefficients a and b and the standard deviation around the regression [13].

Alternatively, a non-linear regression that is directly based on the Langmuir model in eqn. 3 can be used. For this, the appropriate routine of the SAS software available at the University of Tennessee Computing Center was used. Our calculations were performed for five different signal-to-noise ratios: 200, 500, 1000, 2000 and 5000.

Table I summarizes all the calculations performed. For comparison, linear regression results derived for a and b from the band profiles without noise are given in Table II and non-linear regression results in Table III. The results obtained from the series of noisy chromatograms are reported in Figs. 7–13.

We find in Tables II and III that no matter what regression method is used, there is always a positive systematic error on the ECP yielded results for the coefficient a whereas for the coefficient b the error may be either positive or negative, depending on which regression method is used. For a moderate sample size ($L_f = 5\%$), the positive error for the coefficient a is 14% when the column has a poor efficiency (200 plates) and decreases to slightly more than 1% for an efficient 5000-plate column. The error is reduced by approximately one third when the larger sample size is used ($L_f = 20\%$). The b values derived from large size sample band profiles are accurate when the column efficiency is above 200 theoretical plates.

Influence of signal noise on measurement of the coefficient a. Figs. 7–10 illustrate the influence of the noise on the coefficient a. Each figure corresponds to a different procedure of analyzing the simulated experimental data (Table I), and contains five curves, one for each value of the column efficiency. In each figure, for each column efficiency, the numerical average of a de-

TABLE I

COMBINATIONS OF PARAMETERS CONSIDERED IN THE ECP SIMULATIONS

Column efficiency (number of theoretical plates) $N = 200$, 500, 1000, 2000, 5000. Signal-to-noise ratio $SNR = h/\sigma = 200$, 500, 1000, 2000, 5000.

$L_f{}^a = 0.05$				$L_f{}^a = 0.20$			
SD[b]		NSD[c]		SD[b]		NSD[c]	
LR[d]	NLR[e]	LR[d]	NLR[e]	LR[d]	NLR[e]	LR[d]	NLR[e]

[a] Loading factor.
[b] Data smoothed first.
[c] Data non-smoothed.
[d] Linear regression.
[e] Non-linear regression.

TABLE II

PARAMETERS DERIVED BY ECP FROM CALCULATED BAND PROFILES (SNR INFINITE) (LINEAR REGRESSION)

True values: $a = 12.00$; $b = 0.024$; $q_s = a/b = 500$.

L_f	Parameter[a]	$N = 200$	$N = 500$	$N = 1000$	$N = 2000$	$N = 5000$
0.05	a	13.737	12.826	12.479	12.271	12.132
	B	32.519	27.533	25.984	25.112	24.531
0.20	a	13.277	12.566	12.319	12.178	12.084
	B	24.508	23.947	23.920	23.955	23.968

[a] $B = b \cdot 100$.

rived from a series of ten chromatograms is plotted *versus* the logarithm of the signal-to-noise ratio. For the high and low N values, error bars corresponding to the standard deviation based on the ten simulated a values are given, to indicate the influence of the noise on the precision of the derived coefficients. The higher is SNR, and as for the data in Table II the higher

TABLE III

PARAMETERS DERIVED BY ECP FROM CALCULATED BAND PROFILES (SNR INFINITE) (NON-LINEAR REGRESSION)

True values: $a = 12.00$; $b = 0.024$; $q_s = a/b = 500$.

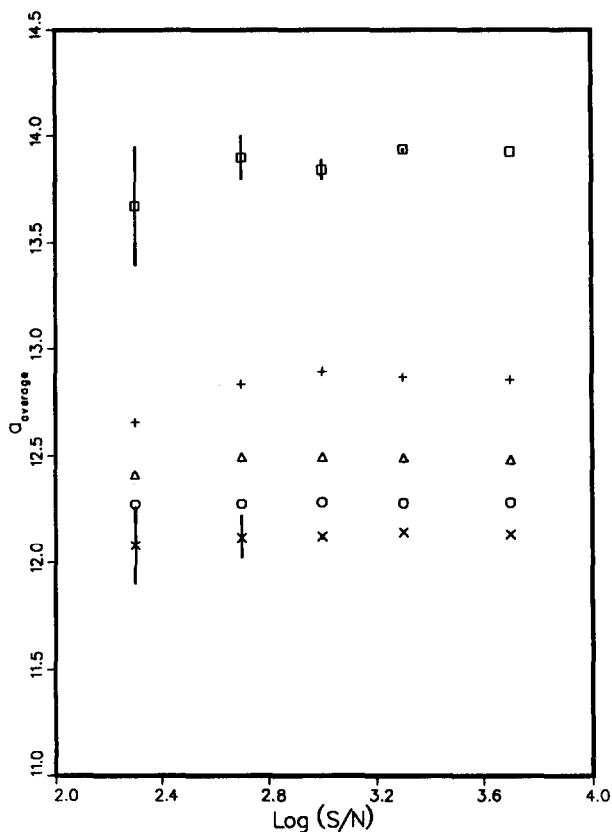| L_f	Parameter[a]	$N = 200$	$N = 500$	$N = 1000$	$N = 2000$	$N = 5000$
0.05	a	13.755	12.838	12.487	12.276	12.135
	B	32.807	27.705	26.091	25.176	24.565
0.20	a	13.318	12.594	12.337	12.189	12.090
	B	24.784	24.121	24.030	24.021	24.003

[a] $B = b \cdot 100$.

Fig. 7. Parameter a yielded by linear regression for smoothed data *versus* the logarithm of the signal-to-noise ratio (L_t = 0.05). N = (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

Fig. 8. Parameter a yielded by linear regression for smoothed data *versus* the logarithm of the signal-to-noise ratio (L_t = 0.20). N = (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

the column efficiency, the more accurate are the results. However, the reproducibility at high efficiency tends to be slightly less than at low efficiency.

Figs. 7 and 8 show the results obtained by linear regression using eqn. 4 for two different loading factors, 0.05 and 0.20, respectively. Compared with the results shown in Table II, we see that the accuracy is better for the larger loading factor, when a chromatogram or a profile without noise was used. The improvement is more significant at very low efficiency than at high efficiency. On the other hand, the precision is poorer for the larger loading factor because the peak is now higher, hence the baseline noise is higher for a given SNR. This causes wider fluctuations of the end time of the integration.

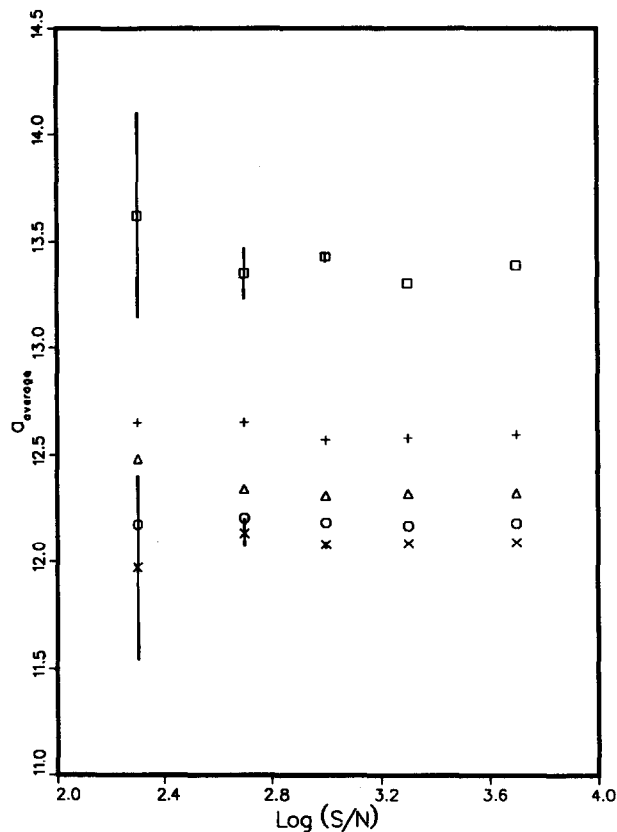Figs. 8 and 9 show the results obtained with

the same series of data when using linear (Fig. 8) and non-linear (Fig. 9) regression methods. There is virtually no difference, which shows that in this case there does not seem to be any particular advantage in using the slightly more complex approach of non-linear regression. It must be noted, however, that the results from linear and non-linear regression are different at the 95% confidence level.

Finally, a comparison between Figs. 8 and 10 illustrates the influence of smoothing data before undergoing the regression calculation. As expected, when no smoothing was involved, there appears to be a larger scatter of a values (Fig. 10). Actually, the standard deviation for a values when SNR = 200 (ca. 11.90, not shown) now becomes comparable to the true a value (12.00). This scatter is such that, at low SNR, the

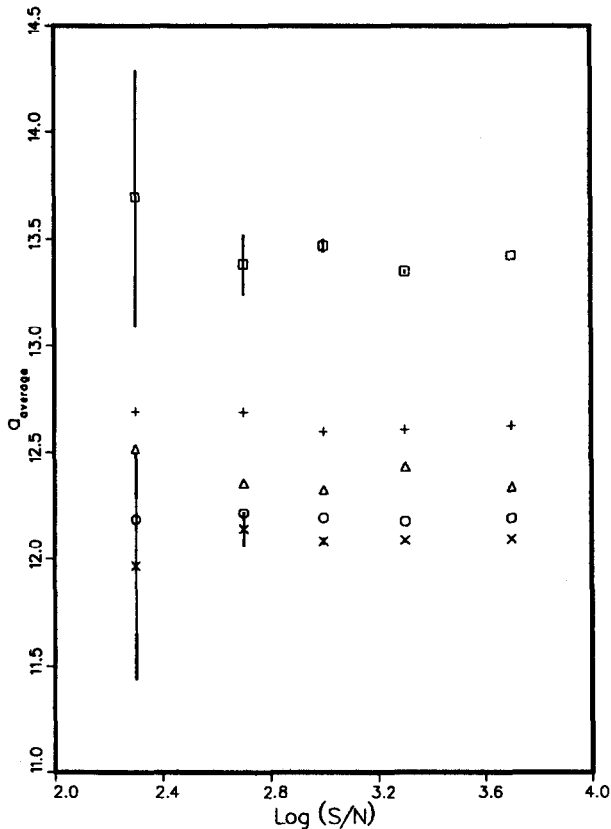Fig. 9. Parameter a yielded by non-linear regression for smoothed data *versus* the logarithm of the signal-to-noise ratio ($L_f = 0.20$). $N =$ (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

Fig. 10. Parameter a yielded by linear regression for non-smoothed data *versus* the logarithm of the signal-to-noise ratio ($L_f = 0.20$). $N =$ (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

systematic error which appears on the derivation of a does not necessarily decrease with increasing column efficiency. Inversion of the order takes place between *SNR* of 200 and 500. Similar results were obtained also for the loading factor of 0.05.

Influence of signal noise on measurement of the coefficient b. Figs. 11–13 show similar results for the coefficient b of the Langmuir model. These figures demonstrate that even when *SNR* is small, the noise signal does not seem to affect the average b value estimate to a large extent. The calculated standard deviations for each *SNR* based on ten simulated b values are less than 10^{-4}. Also, all the b values derived for columns having 500 theoretical plates or higher are identical within the limits of error. Similar results
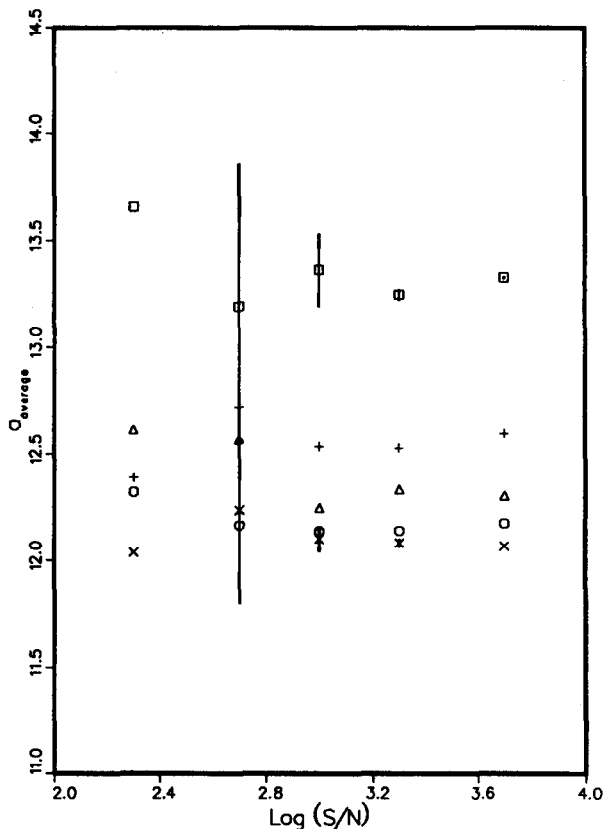
regarding both the accuracy and the precision are obtained no matter whether the data are smoothed (Fig. 12) or not (Fig. 13) in advance. Data obtained from non-linear regression (not shown) lead to the same conclusions as before. Once again, we can see that the precision of the results derived from non-smoothed data is lower than that from smoothed data.

Influence of integration path in the ECP method

In all the above studies, the ECP integration, (eqn. 2) was evaluated directly from the chromatograms. However, numerical integration is typically performed with the independent (or "known") variable as the integration variable, and the dependent (or "measured") variable is placed in the integrand. In eqn. 2, the indepen-
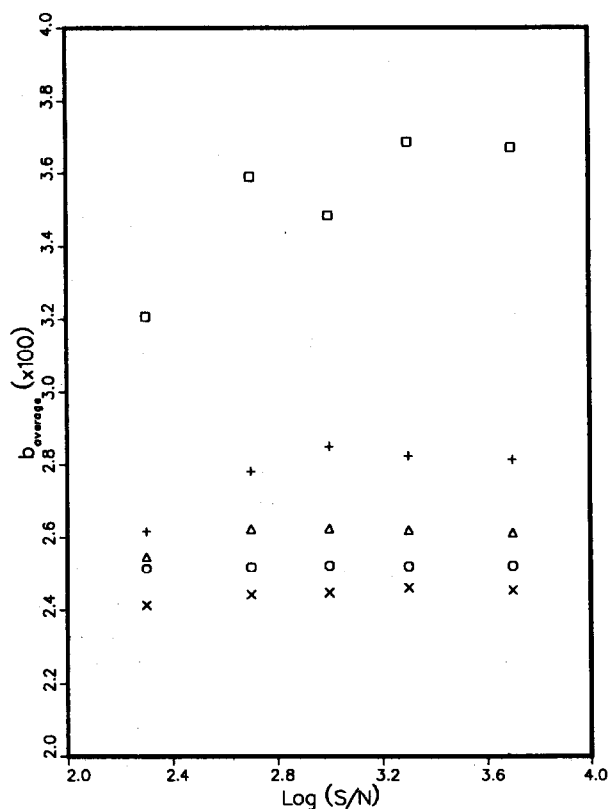
Fig. 11. Parameter *b* yielded by linear regression for smoothed data *versus* the logarithm of the signal-to-noise ratio ($L_f = 0.05$). $N =$ (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

Fig. 12. Parameter *b* yielded by linear regression for smoothed data *versus* the logarithm of the signal-to-noise ratio ($L_f = 0.20$). $N =$ (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

dent variable (concentration) is in fact measured. Hence a noisy step length, $\delta_i C$, is used in the integration. An alternative way of evaluating eqn. 2 is to order the entire data set in terms of increasing concentration instead of increasing time. This is easily performed with vector ordering algorithms commonly supplied in many software packages. This procedure effectively transfers the experimental noise from the concentration variable to the time variable, so that the integration can proceed with a noise-free step length. Further, as negative concentrations do not make any sense, the tail of the data may be cut at the first occurrence of a negative concentration at the beginning of the algorithm, immediately after baseline correction.

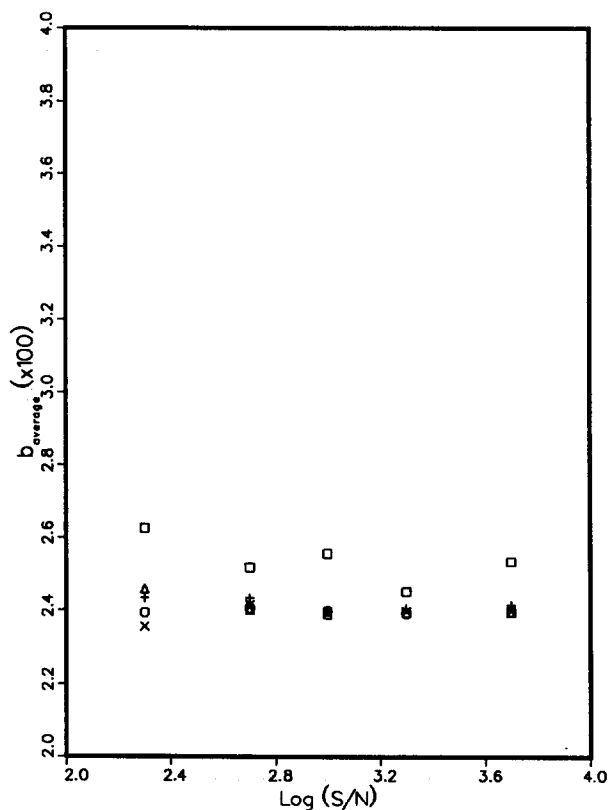The effect of cutting and integrating the data in this manner greatly increases the precision of the computed isotherm, in effect better preserving the low-pass filtering characteristic of integration. In Table IV the ECP results are shown for $L_f = 0.20$, $N = 2000$ and $SNR = 500$ and 1000, where the parameters were determined by simplex optimization. These results clearly show that transferring the noise to the time variable increases the precision of the results. Further, the accuracy of *b* is retained at lower SNR. The two orders of magnitude increase in precision is due to the effect of a smoothly varying integration variable, which subsequently filters the isotherm more effectively. Integrating in this manner eliminates the need for data smoothing and the performance of several sample trials in order to reduce the effect of random noise. The accuracy for any one trial, on average, will always be greater than if noise is allowed on the integration variable.
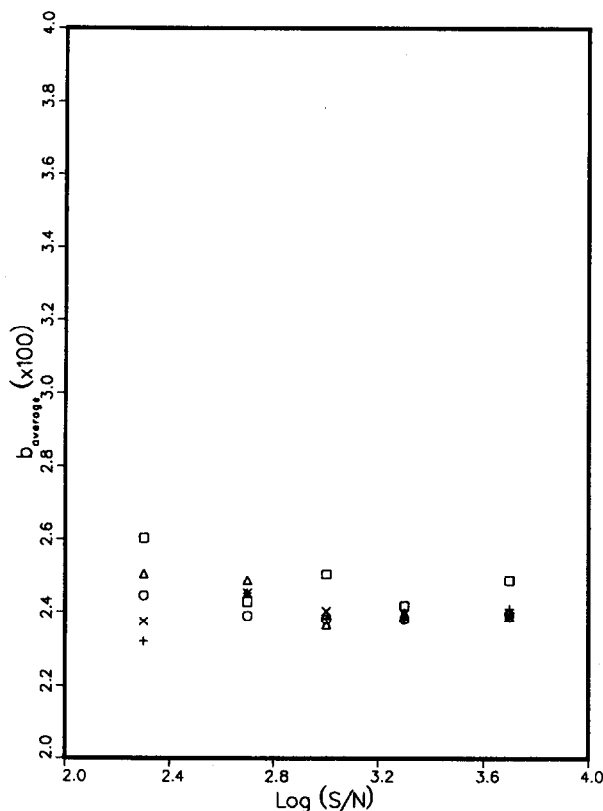
Fig. 13. Parameter b yielded by linear regression for non-smoothed data versus the logarithm of the signal-to-noise ratio ($L_f = 0.20$). N = (□) 200; (+) 500; (△) 1000; (○) 2000; (×) 5000.

Effect of determining a independently from the retention time at infinite dilution

With an infinite dilution experiment, an accurate measure of the retention factor, k', can be obtained. The accuracy and precision of this measure depends on SNR, the data density and the fluctuation of the experimental parameters (i.e., pressure drop, temperature, etc.). With a value of the phase ratio, F, the initial slope of the true isotherm, $a = k'/F$, can be determined more accurately than that yielded by the ECP method for columns of less than 5000 plates (often to within 0.05%) [14]. The effect of fixing this measure of a and then determining b from the ECP isotherm is to place the systematic error arising from the finite efficiency on the b coefficient.

This effect is illustrated for $N = 1000$ plates, $SNR = 1000$ and $L_f = 0.20$ in the residual plot of Fig. 14. In this figure, the fitted Langmuir isotherms (obtained via simplex optimization of the coefficients with respect to the ECP isotherm) are compared with the true isotherm and the ECP isotherm in terms of their relative error, $r = [q_{fit}(C) - q(C)]/q(C)$, where $q_{fit}(C)$ is the fitted isotherm. The solid line represents the true isotherm prediction error when both a and b are optimized. The upper dashed line represents the true isotherm prediction error when a is fixed to the infinite dilution value (assumed as the correct

TABLE IV

PARAMETERS DERIVED FROM ECP METHOD WITH DIFFERENT CONCENTRATION INTEGRATION PATHS

$L_f = 0.20$, $N = 2000$. True values: $a = 12.00$; $b = 0.024$; $q_s = a/b = 500$.

Statistic	Parameter	SNR = 500		SNR = 1000	
		c-Noise[a]	t-Noise[a]	c-Noise[a]	t-Noise[a]
Mean	a	12.0	12.175	12.2	12.176
	b	0.023	0.02400	0.024	0.024003
Standard deviation	σ_a	0.3	0.005	0.2	0.001
	σ_b	0.001	0.00002	0.001	0.000005
Error (%)	a	0	+1.46	+1	+1.46
	b	−3	0	0	+0.01

[a] c-Noise refers to evaluating eqn. 2 as is, allowing the noise to reside on the concentration data. t-Noise refers to transferring the concentration errors to the time or volume variable by ordering the concentration data monotonically from low to high.
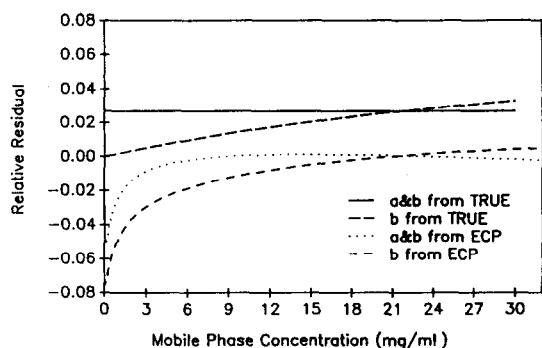
Fig. 14. Relative residual error of the predicted isotherms. Solid and upper dashed lines correspond to the predicted error with respect to the true isotherm ($a = 12.0$, $b = 0.024$); solid line corresponds to conventional ECP analysis, where both a and b are fitted to the ECP isotherm; upper dashed line corresponds to accurately determining a independently and fitting b to the ECP isotherm. Dotted and lower dashed lines correspond to the respective residuals of estimation of the ECP analyses (with respect to the fit ECP isotherm).
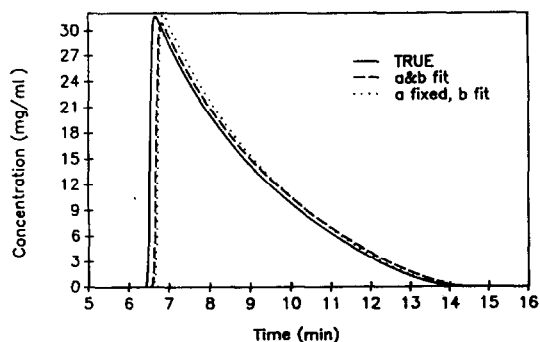

Fig. 15. Band profiles corresponding to the predicted isotherms derived by the different methods in this study. Solid line represents the "true" chromatogram. Dashed line originates from the isotherm determined by fitting both a and b in ECP analysis. Dotted line originates from the isotherm determined by accurately measuring a independently and fitting b to the ECP isotherm.

value here), and only b is fitted to the ECP isotherm. The dotted and lower dashed lines represent the corresponding residual errors from the optimizations (with respect to the ECP isotherm). The a and b fit isotherm is shifted above the true isotherm by an approximately constant amount, whereas the b fit isotherm converges to the true isotherm at low C. The statistical results of these determinations are given in Table V. The effect of these results on the predicted band profiles are shown in Fig. 15.

From these results, it is apparent that at low efficiencies most of the positive systematic error

is placed on the a coefficient when conventional regression on ECP isotherms is performed (for high loading factors). If a is determined accurately beforehand, and b is fitted to the ECP isotherm, a negative systematic error is placed on b. Although the former method fits the data better (ECP isotherm), it does not cumulatively reflect the true isotherm any better until large mobile phase concentrations are encountered (>21.5 mg/ml, which corresponds to a loading factor of approximately 12% in the above simulation). However, as the error in the b term inherently increases as the loading factor decreases, the advantage of determining a separately is not great. This is illustrated by the

TABLE V

COMPARISON OF DIFFERENT METHODS OF OPTIMIZATION OF THE LANGMUIR PARAMETERS

$L_f = 0.20$, $N = 1000$, $SNR = 1000$. True values: $a = 12.00$; $b = 0.024$; $q_s = a/b = 500$.

Result	Parameter	a and b fitted	a fixed, b fitted b fitted	Fit to band profile
Coefficients	a	12.32	12.00	11.85
	b	0.02399	0.02216	0.02530
Error (%)	a	+2.7	0.0	−1.2
	b	−0.04	−7.7	+5.4
σ_{fit} [a]		0.01598	0.02942	0.01231

[a] Defined as the relative root-mean-squared (rms) value of fit. Defined for the predicted and ECP isotherms for the first two columns and for the predicted and observed chromatograms for the last column.

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similarity of the aggregate error in the predicted chromatograms shown in Fig. 15.

Analysis by fit of the band profile

Dose et al. [15] showed that the isotherm parameters can be determined from the band profile data by simulating the band profile with a known efficiency and optimizing the isotherm coefficients with a careful simplex routine. If this algorithm is applied to the above data $L_f = 0.20$, $SNR = 1000$ and $N = 1000$, the results in Table V and Fig. 15 ("fit to band profile") are obtained. The predicted chromatogram is overlaid with the true chromatogram on the scale of Fig. 15. However, the error in the Langmuir coefficients remains significant. The effects of underestimating a and overestimating b cancel each other. This false minimum is obtained because of the systematic direction of convergence of the simplex and the shallow nature of the chi-squared surface. The initial estimates for this algorithm were obtained from the a and b fit of the ECP isotherm.

This method of analysis exhibits high precision, but the gain in accuracy over the ECP analysis in terms of the isotherm parameters decreases as the column efficiency increases. The accuracy with respect to the observed band profile, however, is always good. Therefore, this is a good practical method for predicting nonlinear chromatograms, although it bears the stigma of a circular argument, and for this reason must be used cautiously. As the convergence of this algorithm can be prohibitively slow, and little advantage exists for the high-efficiency cases, the use of this program is advised for low efficiency columns only (<2000 plates).

CONCLUSIONS

The experimental requirements to achieve the precision and accuracy which are needed in the determination of isotherms by ECP are more stringent than generally believed. The column efficiency should exceed 2000 and preferably be close to 5000 theoretical plates. The signal-to-noise ratio (maximum height of the band divided by the standard deviation of the noise) should exceed 500. A large size sample should be injected, corresponding to a loading factor of about 0.20, to achieve a better estimate of the coefficient a. Smoothing the raw data in advance can reduce the large scatter of the results to a certain extent, but integrating the data with the error placed on the time variable eliminates the scatter more effectively. Finally, the detector response should be linear in the range of concentrations sampled, or it should be accurately calibrated.

The independent determination of the retention factor at infinite dilution can be performed by the injection of a sample small enough to be eluted under linear conditions. This procedure provides often a value of the initial slope of the isotherm that is more accurate than the value derived from the ECP method for $N < 5000$ plates. There are cases, however, where these values are difficult to reconcile, for example because of the existence of a low density of active sites on the surface [16].

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