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DETERMINATION OF COMPETITIVE ADSORPTION ISOTHERMS FOR MODELING LARGE-SCALE SEPARATIONS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Preparative and process-scale high-performance liquid chromatography has assumed an increasingly important role in the production of highly purified substances, such as proteins expressed by recombinant DNA technology for use as human pharmaceuticals. The theory for modeling chromatographic separations is well-developed, but requires data on the competitive adsorption behavior of all mixture components for accurate predictions and process design. This paper describes two new methods for determining competitive adsorption isotherms from multicomponent frontal chromatography experiments. The first new method reported here complements a method described previously that employed the theory of chromatography to estimate Langmuir isotherm parameters from the breakthrough volumes in frontal chromatography. The new method estimates Langmuir parameters from the experimentally determined compositions of the breakthrough zones, rather than from retention volumes, and so provides a check on the magnitudes of these parameters, but also yields values that may more accurately predict the concentrations of zones in a chromatogram than the Langmuir parameters estimated by other methods. The second method is based on both the mass balance and composition velocity approaches to analysis of the profile obtained in frontal chromatography. The method does not require measurement of concentrations in the profile, instead, concentrations are estimated according to the theory of chromatography from the breakthrough volumes of zones of different composition. The resulting method enables calculation of data points for mobile and stationary phase concentrations at equilibrium, yet it eliminates the need for a tandem rapid analytical high-performance liquid chromatographic unit to monitor effluent compositions.

INTRODUCTION

Preparative chromatography has long been an essential tool for purification of compounds at the laboratory and production scales. Recently, large-scale high-

performance liquid chromatography (HPLC) has become an important process purification step, and is used in the production of biosynthetic human insulin[1]. Such proteins, produced by recombinant DNA technology, require extremely high purity for their intended use as human pharmaceuticals[2]. HPLC is one of the most important analytical techniques for verifying purity[3] and for isolating contaminants or variants of a protein[4]. The separation by HPLC can be relatively easy to scale-up[5], and so has lately taken its place in the process stream as a purification step *par excellence*[6]. In order to support this extension of HPLC into large-scale applications, a growing body of theoretical work has been reported, to model the different modes of LC, to describe column behavior and to predict separation performance. Horváth has been a pioneer in this area, working on both theoretical and practical aspects of preparative HPLC[6–23], measuring adsorption isotherms[24,25] and demonstrating the importance of theory in understanding the workings of large-scale HPLC that differs in certain respects from the more familiar analytical modes[26–30].

Large-scale HPLC is most efficiently operated at relatively high concentrations, where manifestations of "column overloading" may be apparent[31]. These phenomena give rise to asymmetrical peak shapes[32], displacement effects[33] and selectivity reversals[19] that may impede simple strategies for scale-up from the analytical separation. Therefore, mathematical modeling of the process can provide valuable insight into the physical processes occurring within the column and aid in design of the HPLC system. Since HPLC is a relatively expensive process, and a very high resolution step, an adequate understanding of the dynamics of the chromatographic process is essential to an efficient and effective installation. While modeling is not a substitute for experimentation with a variety of mobile and stationary phase systems in the development process, it can be important to rapidly gaining insight into, and optimizing the HPLC step in use[34].

A key element in accurately modeling the chromatographic process is the data describing the equilibrium adsorption of each of the components in the system constituting the feed material and mobile phase components. No prediction of column performance can be made without first measuring or estimating adsorption isotherms for these components under the conditions of interest, and the accuracy of the prediction is strongly dependent on the accuracy of this data[27]. At elevated concentrations, such as employed in large-scale separations, interference[35–37] among components is important, and an adequate description of the way in which species mutually affect one another's adsorption properties also can improve the accuracy of the modeling effort. Little effort, however, has gone into determining multicomponent isotherms in chromatographic systems, until the work of Horváth cited above. This is due to the low efficiency of "classical" chromatographic columns, where the influence of such effects would be negligible compared to the large bandspreading forces, and to the *de facto* restriction of HPLC systems to low, analytical-scale concentrations, where interference effects are not manifest. Thus, the modern columns and equipment for large-scale HPLC need a higher level of sophistication in isotherm determination in order to represent column behavior that can differ substantially from that associated with separations in analytical modes of chromatography.

Jacobson *et al.*[24,25] have presented methods for measurement of single- and multicomponent isotherms in HPLC by frontal methods that are accurate and economical of both time and sample. Two of the methods developed by these workers

and reported in the chemical engineering literature[25], describe techniques and instrumentation for rapidly measuring competitive adsorption isotherms of systems with, in principal, any number of components. These methods, described in the next section, differ in experimental complexity and in their restriction to Langmuir adsorption behavior. The first method utilizes a mass balance on the frontal chromatographic concentration profile, and so requires monitoring of the column effluent by a selective detector, such as the tandem HPLC analytical unit described in ref. 25. The second method did not require the selective detector, but was restricted to compounds whose adsorption behavior could individually be described by a Langmuir model. In this report, two additional means of determining competitive adsorption behavior are described and applied to the same data set as the earlier methods[25]. The first new method reported here employs the theory of chromatography[36] to obtain Langmuir adsorption parameters that best describe the concentration levels obtained within the frontal chromatogram. This complements the earlier method that was optimized to predict the breakthrough volumes of the steps in the chromatogram. The second new method employs a mass balance, but concentration values in the frontal chromatogram are not measured, rather they are estimated from the results of the compositions velocities method in ref. 25. This method has the advantage of yielding discrete surface concentration data points, without the need for an ancillary analytical HPLC. Incorporation of the mass balance approach in this method also relaxes the strict adherence to Langmuir adsorption behavior that is associated with the other simplified methods. This paper will also show how the two new methods, together with the earlier approaches, provide an arsenal of techniques for accurate data gathering for modeling column performance in large-scale HPLC.

THEORY

The Langmuir isotherm

The Langmuir adsorption isotherm[38] for a system of n components is commonly expressed as

$$q_i = \frac{a_i c_i}{1 + \sum_{j=1}^{n} b_j c_j} \tag{1}$$

where q_i is the surface concentration of component i in equilibrium with mobile phase concentration c_i, and a_i and b_i are parameters characteristic of species i. It has been shown[39] that eqn. 1 violates the Gibbs–Duhem isotherm, unless a_i/b_i is constant for all i, and so is thermodynamically inconsistent as an extension of single component isotherms. Alternative models have been proposed[40] but the resulting isotherms are more complex, require collection of more adsorption data in order to evaluate parameters, and do not satisfy the assumptions underlying the theory of chromatography[36], an analytic approach to solution of the equations for ideal multicomponent chromatographic behavior. Thus the Langmuir model has retained broad popularity as a means of expressing adsorption data. The aim of this and the previous report[25] is essentially to put forward methods for conveniently and accurately evaluating the

parameters in eqn. 1 for multicomponent systems in order to improve the reliability of the Langmuir formalism in predictions of chromatographic behavior.

The h-transformation

The theory of multicomponent chromatography[35–37] is a comprehensive treatment of ideal column performance that has been shown to accurately model high-performance displacement chromatography in a reversed-phase system[27]. This theory underlies one approach to determination of isotherms described earlier[25]. Only the results of the treatment are described here, and the reader is directed to the original references for a full description of the approach.

The *h*-transformation, as developed by Helfferich[35] and Helfferich and Klein[36], is a means for transforming the dependent variables, c_i, to natural variables, dubbed h_i, in order to simplify mathematical analysis of chromatographic performance. For compounds that obey the Langmuir isotherm, eqn. 1, the transformation is given by

$$\sum_{i=1}^{n} \frac{b_i c_i}{h(a_i/a_1) - 1} = 1 \tag{2}$$

Eqn. 2 is an *n*-th order polynomial with roots ordered as $h_1 > h_2 > \ldots > h_n$. The transformation simplifies calculation of breakthrough volumes, V_i, that are given in reduced terms as

$$v_i = \frac{V_i}{V_0 - V_i} = h_{ia} h_{ib} \frac{P_i}{a_1 \varphi} \tag{3}$$

where

$$P_i = \prod_{j=i+1}^{n} h_{ja} \prod_{j=1}^{i-1} h_{jb} \prod_{j=1}^{n} a_j/a_1 \tag{4}$$

and $\varphi = V_{sp}/V_0$ is the phase ratio in the column, V_{sp} is the volume of stationary phase in the column, V_0 is the column dead volume and v_i is defined as the reduced front velocity[36]. h_{ia} and h_{ib} are the values of h_i ahead of and behind the composition change, respectively.

The reverse of the *h*-transformation is given by

$$c_i = \frac{\prod\limits_{j=1}^{n} \dfrac{h_j a_i}{a_1} - 1}{b_i \left(\prod\limits_{\substack{j=1 \\ j \neq i}}^{n} \dfrac{a_i}{a_j} - 1 \right)} \tag{5}$$

from which concentrations within a zone can be calculated from known values of h_i.

Frontal chromatography

Frontal chromatography can be operationally defined as that mode in which the

composition of the mobile phase flowing into the column is changed in a stepwise fashion and the breakthrough profile is monitored from the initial conditions until the final composition is reached. This technique can be used to very efficiently purify the least-retained component of a mixture[21]. Fig. 1 shows a general breakthrough profile obtained for a two-component mixture, in which the composition at the column inlet has been increased from c_{1a}, c_{2a} to c_{1b}, c_{2b}, where the subscripts 1 and 2 distinguish the two components of the feed, and a and b refer to the initial and final conditions, respectively. Fig. 1 shows that intercalated between the initial and final compositions is a band of intermediate composition, with concentrations c_{1m}, c_{2m}, where m indicates the middle, or "mezzanine" band. The breakthrough profile shown in Fig. 1 is typical of binary frontal chromatography, and in general, a frontal chromatogram of an n component mixture will contain $n-1$ zones of intermediate composition, each of them a mezzanine band[36]. The other characteristic feature of an n-component frontal chromatogram is that it contains n fronts between zones of changing composition, such as those labelled V_1 and V_2 in Fig. 1[36].

Methods for isotherm determination

The method of mass balance (MMB). One way to determine points on the competitive adsorption isotherm is by a mass balance on the profile shown in Fig. 1, as has been described earlier[25]. When the experiment is performed so that all concentrations increase from the initial to the final conditions, and sharp fronts are obtained as in Fig. 1, the increase in the amount of component i bound to the surface is

$$\Delta q_i = q_{ib} - q_{ia} = [(V_2 - V_D)(c_{ib} - c_{ia}) - (V_2 - V_1)(c_{im} - c_{ia})]/V_{sp} \qquad (6)$$

where V_D is the system dead volume, V_1 and V_2 are the breakthrough volumes of the fronts and V_{sp} is the volume of adsorbent in the column. Thus the complete adsorption
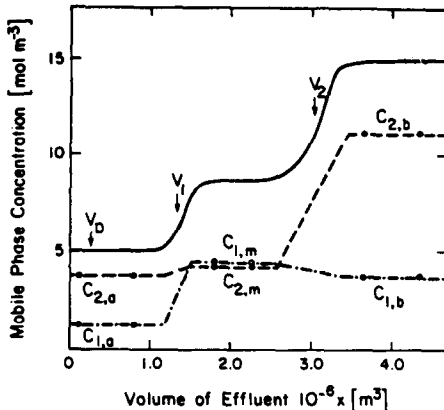

Fig. 1. Schematic of the profile obtained in a frontal chromatography experiment, in which the composition of the mobile phase pumped into the column is changed from c_{1a},c_{2a} to c_{1b},c_{2b}. The composition of the mezzanine zone in the column effluent is c_{1m},c_{2m}. V_1 and V_2 are the breakthrough volumes of the boundaries of the mezzanine zone and V_D is the dead volume of the system. Reprinted with permission of the American Chemical Society from ref. 25.

isotherm over the concentration range of interest can be determined by several frontal chromatography experiments with different combinations of initial and final conditions spanning the concentration range of interest. Jacobson et al.[25] have implemented this approach on a microbore column scale, and described an apparatus for determining the mezzanine concentrations, c_{1m}, c_{2m}, which must be measured along with the breakthrough volumes V_1 and V_2. One advantage of the mass balance method for isotherm determination is that it is not restricted to compounds that exhibit Langmuir adsorption behavior, but in principal can be applied to any system for which frontal chromatographic data can be obtained.

The method of composition velocities (MCV). An alternative means of estimating competitive adsorption isotherms of substances that approximate Langmuir behavior was also advanced in ref. 25. This method, based on the theory of chromatography, requires that the breakthrough volumes in the frontal chromatogram be measured, i.e, V_1 and V_2 in Fig. 1. These values can then be regressed to a model for chromatographic retention to determine isotherm parameters. The model used is that due to Helfferich and Klein[36], which uses the h-transformation, eqn. 2, in order to simplify the mathematical description of the chromatographic process. For two components eqn. 2 becomes a quadratic equation with roots given by

$$h_i = \frac{Q_1}{2} \pm \sqrt{\frac{Q_1^2 - 4Q_2}{4}} \tag{7}$$

where

$$Q_1 = \frac{a_1}{a_2} + 1 + b_1 c_1 + \frac{a_1}{a_2} b_2 c_2 \tag{8}$$

and

$$Q_2 = \frac{a_1}{a_2}(b_1 c_1 + b_2 c_2 + 1) \tag{9}$$

and the roots are indexed as $h_1 > h_2$.

For two components, eqns. 3 and 4 become

$$v_1 = \frac{V_1}{V_0 - V_1} = \frac{h_{1a} h_{1b}}{a_1 \varphi} h_{2a} \frac{a_2}{a_1} \tag{10}$$

and

$$v_2 = \frac{V_2}{V_0 - V_2} = \frac{h_{2a} h_{2b}}{a_1 \varphi} h_{1b} \frac{a_2}{a_1} \tag{11}$$

A non-linear regression can be performed, as described previously, using eqns. 7–11 and values of V_i measured in several frontal chromatography experiments to obtain Langmuir adsorption parameters, a_i and b_i, for each of the components of the mixture.

The isotherms so obtained closely approximate the adsorption behavior as determined by the more involved mass balance approach[25], at least for the system examined.

The method of mezzanine concentrations (MMC). The two new isotherm determination methods reported here are complements to the approaches developed earlier, *viz.*, MMB and MCV. The first, termed the method of mezzanine concentrations (MMC), employs measured compositions of the mezzanine bands and correlation with the "reversed *h*-transformation", given for two components by

$$c_{1m} = \frac{h_{1b}h_{2a} - 1}{b_1\left(\dfrac{a_1}{a_2} - 1\right)} \tag{12}$$

and

$$c_{2m} = \frac{h_{1b}h_{2a}(a_2/a_1)^2 - 1}{b_2\left(\dfrac{a_2}{a_1} - 1\right)} \tag{13}$$

Together with eqns. 7–9, eqns. 12 and 13 express mezzanine concentrations in terms of the Langmuir parameters a_i and b_i and the initial and final concentrations of the step change in concentration at the inlet of the column. This model therefore provides an alternative set of equations to which measured c_{im} values can be regressed in order to obtain values of a_i and b_i that are optimized for prediction of concentration values in the reversed *h*-transformation.

The hybrid method of mass balance (HMMB). The second method advanced here is a modification of the mass balance approach, in which the values of c_{im} in eqn. 6 are not measured, but estimated by eqns. 12 and 13 using the Langmuir parameters given by the regression of MCV. Thus in this method, only breakthrough volumes, V_i, are determined exprimentally, obviating the need for the tandem HPLC experimental set-up employed in the rigorous mass balance approach[25]. This modification of the mass balance, which is a hybrid of MCV and MMB, is thus termed the hybrid method of mass balance (HMMB). While HMMB employs the mobile phase concentration values estimated by MCV, it yields discrete data points on the adsorption isotherm, like MMB, and the restriction to Langmuir adsorption behavior is relaxed, compared to MCV and MMC.

RESULTS AND DISCUSSION

Comparison of the methods

The two methods proposed here, together with those reported earlier[25] provide four distinct approaches for the determination of competitive adsorption isotherms from frontal chromatographic experiments. The approaches are all suited to measurements with columns and equipment developed for HPLC, and rapidly provide data of high precision with relatively small sample amounts. The four methods differ in certain important respects that are summarized in Table I. The mass balance method (MMB) requires measurement of both V_i and c_{im} for isotherm determination, while the

TABLE I

COMPARISON OF FOUR METHODS FOR DETERMINING COMPETITIVE ADSORPTION ISOTHERMS

Y = Yes, N = no.

	Method name abbreviation			
	MMB	MCV	MMC	HMMB
Data required by each method				
Measured V_i	Y	Y	N	Y
Measured c_{im}	Y	N	Y	N
Estimated c_{im}[a]	N	N	N	Y
Values determined by each method				
Isotherm data points[b]	Y	N	N	Y
Langmuir parameters	N[c]	Y	Y	N[c]
Restricted to Langmuir behavior	N	Y	Y	N[d]

    [a] c_{im} estimated by Langmuir parameters from MCV and eqns. 12 and 13.
    [b] Y indicates that discrete surface concentrations are determined for each mobile phase composition.
    [c] Langmuir parameters can be determined by regression of isotherm data points to eqn. 1 in a subsequent analysis.
    [d] Mezzanine concentrations are estimated by assuming Langmuir behavior, but the calculated isotherm can show departures from the Langmuir form.

methods of composition velocities (MCV) and hybrid mass balance (HMMB) require measurement only of V_i. The method of mezzanine compositions (MMC) employs only the measured mobile phase concentrations, c_{im}. In HMMB, c_{im} values are employed in the calculations, but these values are estimated from the results of the MCV technique. The four methods also differ in the values that are produced by the data manipulations. Both MMB and HMMB yield discrete isotherm data points, *i.e.*, values of surface concentrations, q_i, for each experimental value of mobile phase concentration, c_i. The MCV and MMC methods, on the other hand, yield parameters of the Langmuir equation, a_i and b_i, since these methods are regressions of measured data to models derived from the theory of chromatography that assumes Langmuir behavior. In the discussion below the results of the isotherm determinations by each new model are described and compared with the other methods.

The method of mezzanine concentrations (MMC)

The data employed in the regression of the mezzanine concentration values to eqns. 12 and 13, taken from refs. 25 and 41, are given in Table II. Each line in Table II represents one frontal chromatography experiment, in which the column equilibrated with the solution of composition c_{1a}, c_{2a} was switched to c_{1b}, c_{2b} and the effluent monitored with a fast analytical HPLC unit to determine the mezzanine composition c_{1m}, c_{2m}. The data were collected over three regimes of compositions: the 3:1 regime in which initial and final concentrations of *p*-cresol and phenol were in a 3:1 molar ratio, the 1:1 or equimolar regime, and the 1:3 regime, in which the *p*-cresol to phenol molar ratios were 1:3. To determine the Langmuir parameters of the two components these data were regressed to the model for c_{im} given by eqns. 12 and 13 as outlined above.

TABLE II

CONCENTRATION DATA EMPLOYED IN REGRESSION FOR DETERMINATION OF LANGMUIR ISOTHERM PARAMETERS BY THE METHOD OF MEZZANINE CONCENTRATIONS

Concentrations in mol/l, component 1 = p-cresol, component 2 = phenol, a = ahead of the composition change, m = mezzanine composition, b = behind the composition change.

Initial		Mezzanine		Final	
c_{1a}	c_{2a}	c_{1m}	c_{2m}	c_{1b}	c_{2b}
0.0000	0.0000	0.0000	0.0015	0.0038	0.0013
0.0038	0.0013	0.0042	0.0046	0.0113	0.0038
0.0113	0.0038	0.0122	0.0073	0.0188	0.0063
0.0188	0.0063	0.0205	0.0102	0.0263	0.0088
0.0263	0.0088	0.0293	0.0148	0.0375	0.0125
0.0375	0.0125	0.0403	0.0188	0.0488	0.0163
0.0488	0.0163	0.0515	0.0223	0.0600	0.0200
0.0600	0.0200	0.0649	0.0272	0.0750	0.0250
0.0000	0.0000	0.0000	0.0021	0.0025	0.0025
0.0025	0.0025	0.0026	0.0080	0.0075	0.0075
0.0075	0.0075	0.0082	0.0133	0.0125	0.0125
0.0125	0.0125	0.0133	0.0185	0.0175	0.0175
0.0175	0.0175	0.0191	0.0265	0.0250	0.0250
0.0250	0.0250	0.0263	0.0342	0.0325	0.0325
0.0325	0.0325	0.0332	0.0404	0.0400	0.0400
0.0400	0.0400	0.0416	0.0489	0.0500	0.0500
0.0000	0.0000	0.0000	0.0037	0.0013	0.0038
0.0013	0.0038	0.0016	0.0111	0.0038	0.0113
0.0038	0.0113	0.0042	0.0186	0.0063	0.0188
0.0063	0.0188	0.0071	0.0268	0.0088	0.0263
0.0088	0.0263	0.0100	0.0389	0.0125	0.0375
0.0125	0.0375	0.0138	0.0493	0.0163	0.0488
0.0163	0.0488	0.0172	0.0583	0.0200	0.0600
0.0200	0.0600	0.0223	0.0713	0.0250	0.0750

The Langmuir isotherm parameters for p-cresol (component 1) and phenol (component 2) resulting from this regression (MMC) are listed in Table III, along with the corresponding parameters reported earlier for the mass balance (MMB) and composition velocity (MCV) methods. The three methods yield differing values of the

TABLE III

LANGMUIR PARAMETERS FOR p-CRESOL AND PHENOL DETERMINED BY THREE MULTICOMPONENT FRONTAL CHROMATOGRAPHIC METHODS

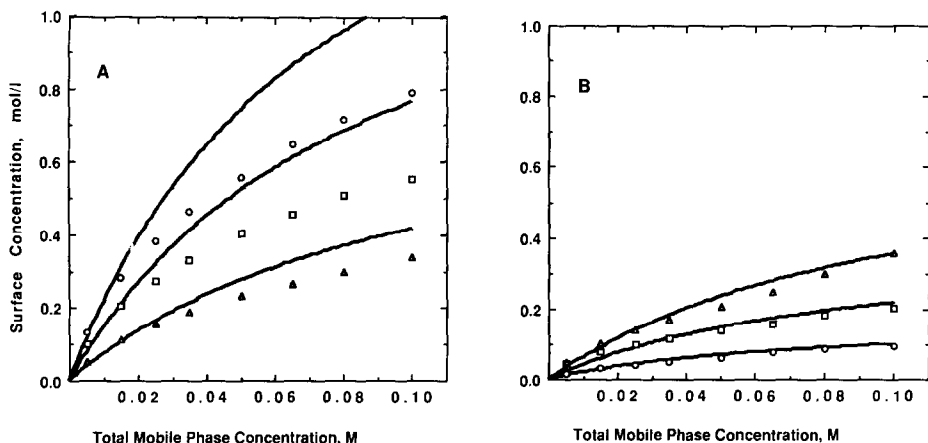| Method	p-Cresol		Phenol	
	a	b (M^{-1})	a	b (M^{-1})
MMB	32.0	24.2	11.0	11.6
MCV	23.1	13.2	10.3	10.8
MMC	33.0	14.9	9.34	7.97

Fig. 2. Comparison of competitive adsorption isotherm of (A) p-cresol and (B) phenol determined by two different methods. The data points, from ref. 25, were obtained by the method of mass balance with p-cresol to phenol molar ratios of 3:1 ($\bigcirc$), 1:1 ($\square$) and 1:3 ($\triangle$). The solid lines are the corresponding isotherms calculated by eqn. 1 using the MMC parameters from Table III.

four parameters. These differences demonstrate that even the relatively simple system examined here does not rigorously follow Langmuir adsorption behavior, since an ideal Langmuirian system would yield identical parameters by all three methods. The Langmuir model has been shown to be thermodynamically inconsistent[39], so this result is unsurprising. As an empirical tool, however, the Langmuir isotherm provides an adequate representation of the adsorption behavior of many systems[41] and is the most convenient competitive isotherm that satisfies the assumptions underlying the theory of ideal multicomponent chromatography. Since the HPLC system does not rigorously adhere to the assumptions underlying the Langmuir model, selection of the appropriate method of determination of Langmuir parameters provides a means of optimizing the parameters to most accurately model the system of interest. Thus, the availability of several different means of determining isotherm parameters also enhances the utility of the Langmuir model.

The adsorption isotherms predicted by MMC deviate significantly from the adsorption data obtained by MMB, as seen in Fig. 2. The data obtained for each of the three regimes of concentration are shown together in Fig. 2. In all three regimes the MMC parameters greatly overestimate the amount of p-cresol adsorbed to the surface, while surface-bound phenol is accurately estimated. Examination of the Langmuir parameters in Table III shows that b_1 for p-cresol is lower when determined by the MMC method than by MMB, while the a_1 values are similar. Since b_1 appears in the denominator of the compound Langmuir isotherm, eqn. 1, the result is an overestimation of the amount of p-cresol bound. The large deviation in Fig. 2A indicates that this approach is not an accurate method for determining the adsorption isotherm of individual species, at least for the system examined here. An analogous result has been obtained for modeling displacement chromatography in a reversed-phase system[27]. The Langmuir parameters obtained by frontal chromatography in that study yielded results that matched retention times well, but were less successful at predicting compositions of individual bands. Thus, a single set of parameters is inadequate to

describe all features of the chromatographic system. These results reiterate the finding that Langmuir models have limited scope in modeling the adsorption behavior even of simple systems such as p-cresol and phenol in reversed-phase chromatography, so the parameters must be optimized for the intended use.

MMC poorly predicts the adsorption isotherms of these compounds but, since it is a regression of a model for c_{im} data, it would be expected to accurately predict mobile phase concentration values. The Langmuir isotherm is a convenient means of empirically representing adsorption data so a judicious choice of the protocol for regression of the data can improve the accuracy of the predictions of a model based on this isotherm. Fig. 3A compares the mezzanine concentrations predicted by the Langmuir parameters of the MMB approach, with the experimentally measured mobile phase concentrations. The calculated concentrations in the ordinate were obtained with the MMB parameters of Table III and using eqns. 7–9, 12 and 13. The results of the same comparison using the MMC parameters are shown in Fig. 3B, and demonstrate that the MMC parameters, which are optimized for this calculation, perform slightly better for prediction of mezzanine concentrations, particularly at high concentrations. In calculations involving estimates of the concentrations in a band, therefore, the MMC approach may be the best choice for representing Langmuir parameters. In this way the choice of the approach to determining Langmuir parameters may compensate for part of the inaccuracy of this means of data representation in particular applications.

The hybrid method of mass balance (HMMB)

In the hybrid mass balance method of competitive isotherm determination, the mezzanine concentrations are estimated by the MCV approach, and used *in lieu* of measured concentrations in eqn. 6. This method is thus a hybrid of the MCV and MMB methods, since it is experimentally as simple as the MCV, yet it retains some of the stringency of the mass balance approach, which is not restricted to a particular isotherm model. Fig. 4 shows the mezzanine concentrations estimated using eqns. 7–9,
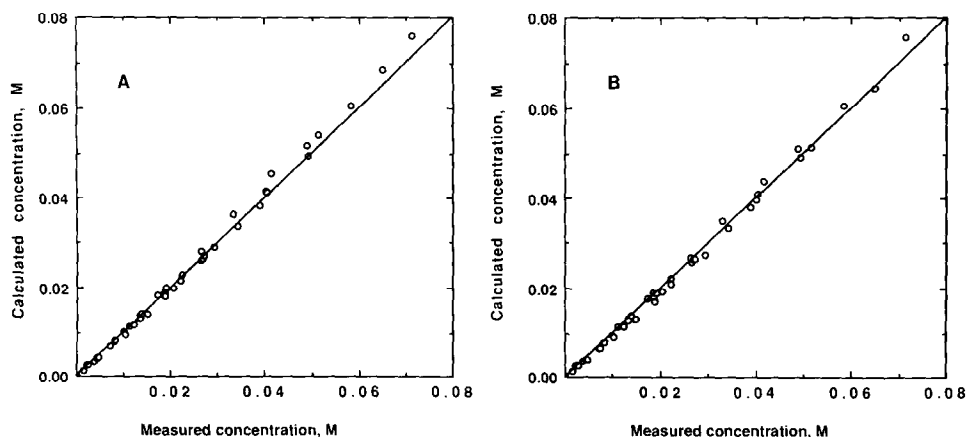

Fig. 3. Comparison of mobile phase concentrations in the mezzanine band, c_{1m} and c_{2m}, calculated by eqns. 7–9, 12 and 13 with the Langmuir isotherms parameters determined by (A) MMB and (B) MMC. The parameters were taken from Table III.
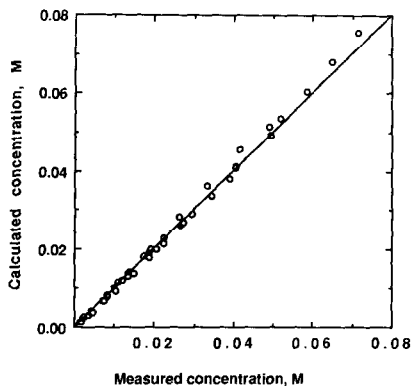
Fig. 4. Comparison of mezzanine concentrations calculated by eqns. 7–9, 12 and 13 with the Langmuir isotherm parameters determined by MCV. The parameters were taken from Table III.

12 and 13 with the MCV Langmuir parameters from Table III. The calculated HMMB and measured MMB concentrations using this approach agree well. When these estimated concentrations are used in Eqn. 1 along with the measured V_1 and V_2 breakthrough volumes given in Table I of ref. 25, a good fit to the measured isotherm points results, as shown in Fig. 5. At the upper limit of the range of concentrations for which the isotherms were determined the deviation between the two isotherms is greatest. According to eqn. 6, errors in determination of c_{im} in this range will result in a proportional error in Δq_i. Nevertheless, over most of the concentration range studied the fit to data points measured by MMB is excellent. Since it has been shown above that this chromatographic system deviates from the Langmuir model, an approach such as HMMB may be the most convenient for gathering accurate competitive adsorption data.

The hybrid approach employs MCV only to estimate mezzanine concentrations, so can be expected to more accurately estimate adsorption isotherms than MCV proper, which is forced to obey the Langmuir model. Fig. 6A shows the excellent
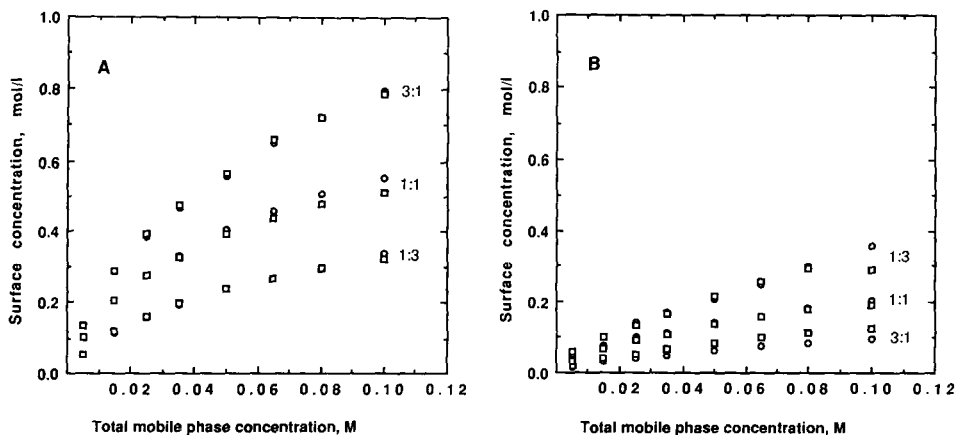

Fig. 5. Comparison of competitive adsorption isotherms of surface concentrations of (A) *p*-cresol and (B) phenol determined by MMB (○) and HMMB (□) for the three concentration regimes described in Fig. 2.
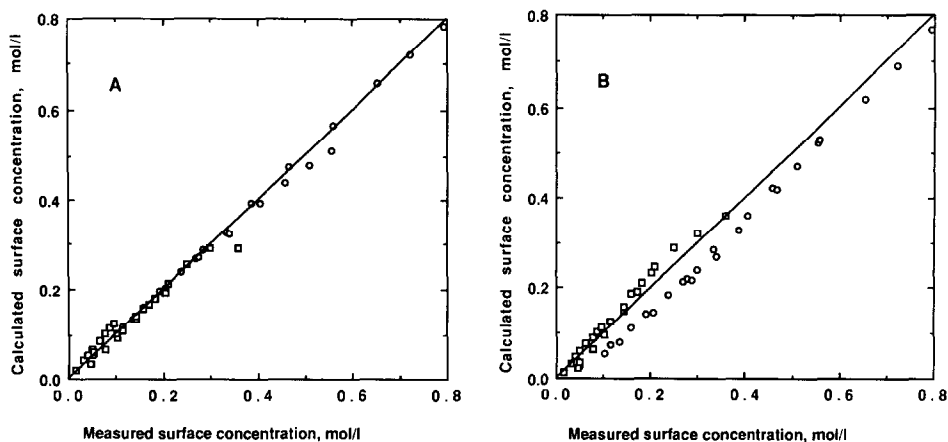
Fig. 6. Comparison of measured surface adsorption isotherm data from MMB with that calculated by (A) the hybrid mass balance method and (B) using eqn. 1 and the Langmuir parameters obtained by MCV. The measured surface concentrations are those determined by MMB for p-cresol ($\bigcirc$) and phenol ($\square$) and shown in Fig. 2.

match between surface concentrations determined by MMB and calculated by HMMB. Fig. 6B shows the comparison between surface concentrations measured by MMB with those calculated using the MCV parameters from Table III and the Langmuir isotherm model, eqn. 1. Comparison of the two figures illustrates the significant improvement in representation of adsorption data afforded by the hybrid approach. Since the reversed-phase system employed here does not stringently adhere to the assumptions underlying the Langmuir model, the added flexibility provided by the hybrid mass balance approach improves the accuracy of isotherm determination.

CONCLUSIONS

As noted previously, the Langmuir isotherm is not an exact representation of the adsorption behavior of this reversed-phase system, as evidenced by the deviation of adsorption parameters obtained by the four different approaches applied to the same set of data. One way to improve accuracy in the face of this deviation is by regression of measured data to a model for the variable of interest. This is the approach employed in the method of mezzanine concentrations advanced here. The Langmuir parameters then are optimized so that the best fit of calculated to measured concentration values in the frontal chromatogram are obtained. This method is shown here to improve the calculated concentration values, compared to other means of determining Langmuir parameters. The parameters obtained by MMC are tailored for this calculation, and poorly estimate other adsorption behavior, such as the amount of a component adsorbed to the surface. Nevertheless, this method is one way to improve the utility of the Langmuir model in predictions of column performance.

The second new method described here is an improvement on the method of concentration velocities (MCV) previously described. The new method, termed the hybrid mass balance method, requires the same experimental data as the MCV, but is more accurate for systems, such as that examined here, that deviate from strict

adherence to Langmuir adsorption. The method is a hybrid of the mass balance and concentration velocity procedures reported earlier, with nearly the accuracy of the former and the experimental simplicity of the latter approach.

With the increasing emphasis on large-scale HPLC, interest has grown in modeling of column performance, so that techniques for assessing adsorption behavior of systems of interest are required. The two new methods described here, building on the approaches reported earlier, provide the tools for such an assessment. Analysis of frontal chromatographic data with these methods can reveal the nature of competitive adsorption, the degree of adherence to Langmuir isotherm behavior, and provide optimal parameters to facilitate use of the Langmuir model in prediction of HPLC system performance.

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