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Adsorption and ion-exchange isotherms in preparative chromatography

Ajoy Velayudhan[☆] and Csaba Horváth*

Department of Chemical Engineering, Yale University, New Haven, CT 06520-8286 (USA)

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ABSTRACT

A systematic characterization of ion-exchange and adsorption isotherms is presented. This treatment serves to emphasize the singular nature of monovalent ion-exchange and classical Langmuir isotherms, and describes the more general homovalent and heterovalent isotherms that are applicable to both ion-exchange and adsorption. Such formalisms are applied to adsorptive systems for the first time, and could prove to be useful additions to the canon of available isotherms. The shielding effect of macromolecular adsorbates on the binding of other macromolecules is discussed, and its parallel to the characteristic charge of a macromolecule is brought out. The application of the general adsorptive formalism to reversed-phase chromatography yields a relationship between the retention factor and the modifier concentration that, in two limiting cases, reduces to two well-known results. The first, which is characteristic of small molecules, gives a linear relationship between the logarithmic retention factor and a linear or quadratic term in the modifier. The second, which is characteristic of large molecules, gives a linear relationship between the logarithmic retention factor and the logarithm of the modifier concentration. The use of appropriate concentration units is shown to resolve certain apparently non-standard (non-Tiselian) displacement patterns. The measurement of characteristic charges by displacement described above could be an interesting complement to the more common elution method, and could provide further insight into the underlying physico-chemical phenomena.

INTRODUCTION

Preparative chromatography is of ever-increasing importance in the downstream processing of biotechnological products. Non-linear chromatography, in which the feed components are at high enough mobile phase concentrations to lie within the non-linear regions of their respective adsorption isotherms and thus interfere both with themselves and with each other, is an attractive approach to preparative and process-scale separation, in that the stationary phase is more efficiently utilized and the throughput is higher than in linear elution chromatography [1]. One of the key pieces of information needed in the design of preparative chromatographic separations is multicomponent adsorption isotherms. This paper attempts to characterize the isotherm formalisms used in adsorption and ion exchange, and to resolve certain anomalies found in the literature related to isotherm formalisms.

ION-EXCHANGE ISOTHERM FORMALISMS

The most common isotherm used in ion-exchange chromatography is based on the monovalent form of the mass-action formalism [2,3]. This can be represented for two feed components A and B by

$$A + S^* \rightleftharpoons A^* + S \quad (1)$$

and similarly for B. Here, asterisks denote the corresponding adsorbed species, and S is the salt

* Corresponding author.
☆ Present address: Laboratory of Renewable Resources Engineering, 1295 Potter Center, Purdue University, West Lafayette, IN 47907-1295, USA.

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buffer counterion that maintains stationary phase electroneutrality and serves as the mobile phase modulator. For a species i, c_i is its mobile phase and q_i its stationary phase concentration. Electroneutrality then gives

$$q_S + q_A + q_B = \Lambda \tag{2}$$

where Λ is the total concentration of binding sites (fixed charges) on the ion exchanger. It can be seen that several assumptions have been made to arrive at this formalism. Activities have been replaced by concentrations; the binding process is assumed to be a stoichiometric exchange of counterions, *i.e.*, processes that involve the coions non-trivially, such as Donnan exclusion, are negligible. Further, the equilibrium constants, K_i, which are given by

$$K_i = \frac{q_i c_S}{c_i q_S}, \quad i = A, B \tag{3}$$

are assumed to be constant. Eq. 3 gives the familiar constancy of separation factors, S_{ij}:

$$S_{AB} = \frac{q_A c_B}{c_A q_B} = \frac{K_A}{K_B} \tag{4}$$

Eqns. 2 and 3 can be used to arrive at explicit expressions for the stationary phase concentrations:

$$q_i = \frac{\Lambda K_i c_i}{c_S + K_A c_A + K_B c_B}, \quad i = A, B \tag{5}$$

These results can be easily generalized to n feed components [4], for which

$$q_i = \frac{\Lambda K_i c_i}{c_S + \sum_j K_j c_j} \tag{6}$$

where the summation variable j runs over all the adsorbable components other than the buffer counterion (in this case, all the n feed components). The constancy of separation factors has the important consequence that the order of affinity of the components for the stationary phase, *i.e.*, the order of their selectivities, remains unchanged throughout the separation. Then column dynamics follow well-established trends [4,5]. As will be seen later, more complex isotherm formalisms can lead to non-constant selectivities, and thus the possibility of selectivity reversal in the composition range under consideration. This in turn leads to much more complex column dynamics, which have not yet been fully characterized.

A more general description, which will be called the "homovalent" formalism, applies when all the feed counterions involved have the same valence α, while the buffer is still monovalent. If the buffer is of the same valence as the feed components, the problem reduces to that of monovalent exchange, treated above. All other assumptions being the same as for the monovalent case, the binding process for homovalent exchange with a monovalent counterion, which could therefore be called ideal homovalent exchange, is represented by

$$A + \alpha S^* \rightleftharpoons A^* + \alpha S \tag{7}$$

and similarly for B. The appropriate electroneutrality condition is

$$q_S + \alpha q_A + \alpha q_B = \Lambda \tag{8}$$

and the equilibrium constants are given by

$$K_i = \frac{q_i (c_S)^\alpha}{c_i (\Lambda - \alpha q_A - \alpha q_B)^\alpha}, \quad i = A, B \tag{9}$$

It can be seen from eqn. 9 that the separation factor is still constant and equal to the ratio of the equilibrium constants. However, general explicit expressions are no longer available for the stationary phase concentrations, which have to be found numerically from eqns. 8 and 9. The constancy of separation factor implies that selectivity reversal will not occur, and that the standard theories of column dynamics will still apply (*e.g.*, ref. 5).

However, the most common situation in practice involves several adsorbable components with different valences. When large molecules such as proteins and other biopolymers are involved, the number of charges on their surfaces that interacts with the stationary phase is smaller than their net charges, because their surface charges are in general too widely distributed for all of the charges to interact with the stationary phase [6]. In these cases, the valence is replaced by the characteristic charge [7,8], which must in general

be determined by experiment at the conditions under which the separation is run.

The ion-exchange process involving species of difference valences (or characteristic charges) is described by a heterovalent mass-action formalism. With all other assumptions as before, a system of two feed components, A with a characteristic charge of α and B with a characteristic charge of β, is considered. The binding processes are given by

$$A + \alpha S^* \rightleftharpoons A^* + \alpha S \quad (10)$$

$$B + \beta S^* \rightleftharpoons B^* + \beta S \quad (11)$$

The electroneutrality condition becomes

$$q_S + \alpha q_A + \beta q_B = \Lambda \quad (12)$$

The equilibrium constant for A is

$$K_A = \frac{q_A (c_S)^\alpha}{c_A (\Lambda - \alpha q_A - \beta q_B)^\alpha} \quad (13)$$

and that for B is

$$K_B = \frac{q_B (c_S)^\beta}{c_B (\Lambda - \alpha q_A - \beta q_B)^\beta} \quad (14)$$

From eqns. 13 and 14, it can be seen that the separation factor is no longer constant: it varies with the concentrations of all the adsorbing components. This allows for the possibility of concentration-dependent selectivity reversal, where the affinity order of the components for the stationary phase changes as the mobile-phase concentrations are varied. When selectivity reversal occurs during a chromatographic separation, unusual concentration profiles can result [9–12]. For example, it becomes possible in isocratic elution for one component to completely envelop another. Standard theories no longer account for column dynamics, and numerical simulations of the chromatographic process are needed to predict column effluent profiles.

ADSORPTION ISOTHERM FORMALISMS

In the same way as was done above for ion exchange, isotherms for adsorption can be classified. The most common isotherm used in adsorption chromatography is the Langmuir isotherm, whose well-known multicomponent form is:

$$q_A = \frac{\Lambda_A K_A c_A}{(1 + K_A c_A + K_B c_B)} \quad (15)$$

The result above, for a binary feed mixture, is shown for comparison with the earlier ion-exchange results. This form assumes that each adsorbate molecule interacts with a single binding site on the stationary phase, and is thus in a sense equivalent to the monovalent ion-exchange process. In fact, Helfferich and Klein [4] have shown that the multicomponent Langmuir isotherms are in a sense equivalent to, and can be converted into, monovalent ion-exchange isotherms by the addition of a "dummy" component. As would be expected, the separation factor is constant, precluding selectivity reversal, and consequently detailed theories of column dynamics are available [4,5]. It is also well known that the multicomponent Langmuir form is thermodynamically consistent only when the saturation concentrations of all the adsorbates is equal [13,14]. The Langmuir description is in fact better suited to, and was originally derived [15] for, the description of gas adsorption, where fixed adsorption sites and a non-adsorbing carrier gas are more realistic. Nevertheless, multicomponent Langmuir adsorption is still the most widely used isotherm for non-linear liquid chromatography studies.

It is intriguing to view the binding sites in ion exchange as analogous to hydrophobic patches on the stationary phase available for binding in liquid adsorption; then the analogue to the characteristic charge in ion exchange is a "binding area" for each adsorbate in adsorption.

In the light of the above parallel between the monovalent ion-exchange isotherms and the Langmuir isotherms, we wish to examine whether analogues of the homovalent and heterovalent ion-exchange forms exist for adsorptive systems. Such generalizations can easily be formulated, as shown below.

The "adsorptive-homovalent," or "homosorptive," formalism would involve each feed component occupying α sites on binding; the adsorption equilibria would then take the form

$$A + \alpha I \rightleftharpoons A^* \quad (16)$$

and similarly for B, where I is a single binding site on the stationary phase. The equilibrium constants are given by

$$K_i = \frac{q_i}{c_i(\Lambda - \alpha q_A - \alpha q_B)^\alpha}, \quad i = A, B \quad (17)$$

where Λ is the total concentration of binding sites available. The similarity between eqns. 9 and 17 is striking, the only difference being the term involving the buffer counterion in the numerator of eqn. 9. Again, it can be seen that the separation factor is constant, as for the ion-exchange equations, and selectivity reversal will not occur. Similarly, no explicit expression is in general available for the stationary phase concentrations in the adsorptive-homovalent formalism.

The more general case is that of adsorbates with different binding areas or, in terms of the Langmuir adsorption formalism, that occupy different numbers of binding sites. This is analogous to the heterovalent version of the ion-exchange process. The formal representation of the "adsorptive-heterovalent," or "heterosorptive," binding equilibria are therefore

$$A + \alpha I \rightleftharpoons A^* \quad (18)$$

$$B + \beta I \rightleftharpoons B^* \quad (19)$$

The distribution coefficients are given by

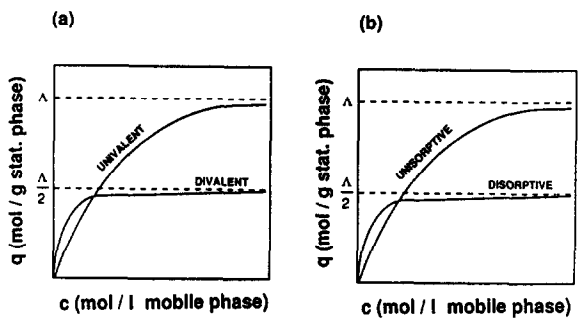

Fig. 1. The similarity between ion-exchange and adsorption isotherm formalisms. (a) Single-component isotherms for an univalent and a divalent adsorbate; (b) analogous case for heterosorptive single component isotherms, for species with binding areas of 1 and 2.

$$K_A = \frac{q_A}{c_A(\Lambda - \alpha q_A - \beta q_B)^\alpha} \quad (20)$$

and similarly for B; note the analogy to eqns. 13 and 14. Again, eqns. 13 and 20 are identical except for the modulator concentration term in the numerator of eqn. 13. The separation factors are not constant, and selectivity reversal becomes possible. As before, no explicit form for the stationary phase concentrations is available, and the single-component isotherms cross, as seen in Fig. 1.

GENERAL FEATURES OF THE ISOTHERMS

The extension of the homovalent and heterovalent binding models from ion-exchange to adsorption processes can be viewed as a generalization of the classical Langmuir formalism. In this context, it could be interesting to examine the special case of a single component A. For both the homo- and heterosorptive cases, the equilibrium distribution can be expressed as:

$$K_A = \frac{q_A}{c_A(\Lambda - \alpha q_A)^\alpha} \quad (21)$$

This expression can be recast as

$$q_A = K_A c_A(\Lambda - \alpha q_A)^\alpha = K_A c_A \Lambda^\alpha \left(1 - \frac{\alpha q_A}{\Lambda}\right)^\alpha \quad (22)$$

For relatively small q_A, the last term in brackets in eqn. 22 can be expanded as a binomial series, since this term is always non-negative. Keeping only the linear term gives a Langmuirian form:

$$q_A = \frac{K_A \Lambda^\alpha c_A}{1 + K_A \Lambda^{\alpha-1} c_A} \quad (23)$$

Including the second-order term gives a quadratic algebraic equation for q_A. The result is

$$\frac{\alpha q_A}{\Lambda} = \frac{(\alpha\psi - 1) + \sqrt{(1 - \alpha\psi)^2 - 2\alpha(\alpha - 1)\psi^2}}{\alpha(\alpha - 1)\psi} \quad (24)$$

where

$$\psi = \alpha K \Lambda^{\alpha-1} c_A \quad (25)$$

A similar quadratic form can be obtained for the mass-action heterovalent ion-exchange formalism. In all cases, these approximate explicit forms will be monotonic and concave-down, like their parent isotherms.

The perturbative approximation above was only carried out for a single component, in order to avoid algebraic complexity. The generalization could be carried out to multicomponent systems with the aid of a symbolic calculation (or computer algebra) program such as Maple or Mathematica. The potential use for such approximations to the original isotherm is in computer simulations, which are widely used to model preparative chromatographic separations (*e.g.*, ref. 16). While it is easy enough to solve the original equations (a system of equations like eqn. 20) numerically on a computer, this step, of finding the stationary phase composition in equilibrium with a particular mobile phase composition, could occur several times in the calculation of the actual compositions at a given point in the column at a given time. When the number of discrete points in both space and time used in an accurate numerical program is considered, it is clear that the equilibrium calculation could prove to consume an appreciable fraction of the overall computing time. Thus explicit isotherm forms, such as those in eqns. 23 and 24, could prove valuable, in spite of their approximate nature.

However, one caveat must be emphasized: the approximate forms may not exhibit selectivity reversal in the same composition range as their original exact forms do. For instance, the approximation given by eqn. 22 is Langmuirian, even though the original isotherm was heterovalent. If the first-order approximations to the corresponding multicomponent isotherms were also Langmuirian, they could only be used in a composition range where the original exact isotherm did not exhibit selectivity reversal. Thus only those approximate explicit isotherms that exhibit similar selectivity-reversal behavior to the original exact isotherm in the composition range of interest can safely be used.

The characteristics of the various isotherms described above are summarized in Table I. The close analogy between the ion-exchange and adsorptive forms of the mono-, homo- and heterovalent formalisms is evident. All these isotherms are concave-down and monotonic, and their slopes obey the relations

$$\frac{\partial q_i}{\partial c_i} > 0\,; \quad \frac{\partial q_i}{\partial c_j} < 0\,, \quad i \neq j \tag{26}$$

These are the standard conditions for competitive binding [5], and imply that, if selectivity reversal does not occur in the range of compositions encountered during a separation, column effluent profiles that are qualitatively similar to those calculated for the monovalent ion-exchange and classical Langmuir formalisms [5,9] will result.

TABLE I

CHARACTERISTIC FEATURES OF VARIOUS ISOTHERM FORMALISMS IN ION EXCHANGE (IEX) AND ADSORPTION (ADS)

AV = Always valid; NV = never valid; E = explicit; I = implicit, NP = not possible; P = possible.

Features	Monovalent		Homovalent		Heterovalent	
	IEX	ADS	IEX	ADS	IEX	ADS
Constancy of separation factors	AV	AV	AV	AV	NV	NV
Expression for stationary phase concentration	E	E	I	I	I	I
Selectivity reversal	NP	NP	NP	NP	P	P

In both the monovalent and the homovalent cases, the single-component isotherms will not cross, regardless of the units in which the concentrations are expressed (molarity, molality, equivalents, etc.) However, the heterovalent isotherms will, in general, cross, as has been shown [8]. We briefly repeat the argument here for completeness. Consider a monovalent adsorbate A and a divalent adsorbate B. If an adsorbent with Λ binding sites (in molar units, *e.g.*, moles of binding sites per unit area of stationary phase) is being used, the saturation concentration of A would be Λ M if it were the only adsorbable counterion. Under the same conditions, the saturation concentration of B would be $\Lambda/2$ M. Thus B would have a lower saturation concentration than A, but it would have the higher initial isotherm slope, reflecting its higher affinity for the adsorbent. The single-component isotherms of A and B would therefore cross, as seen in Fig. 1.

As mentioned earlier, Helfferich and Klein [4] have pointed out how the Langmuirian formalism (which we have called above monosorptive) can be converted into a monovalent ion exchange formalism by the inclusion of a dummy component, whose stationary phase concentration varied so as to keep the sum of all stationary phase concentrations constant, in analogy to an electroneutrality condition. The present work has described the forms of the homosorptive and heterosorptive generalizations of the Langmuir (monosorptive) isotherm. It remains an intriguing possibility to attempt to convert these formalisms into equivalent homo- and heterovalent ion-exchange (mass-action) formalisms by an analogous application of the method of Helfferich and Klein.

IMPLICATIONS FOR REVERSED-PHASE CHROMATOGRAPHY

Another interesting parallel between the ion exchange and adsorptive formalisms arises in the context of reversed-phase chromatography. It is well-known that, in ion exchange, the logarithmic retention factor varies linearly with the logarithm of the salt concentration in the mobile phase [2,7,8] when the adsorbate concentration is low enough to ensure that its adsorption is linear. Here we derive analogous expressions for the adsorption isotherm formalisms.

Consider an organic modifier S with unit adsorptive binding area, and an adsorbate A with binding area α. The equilibrium relations are

$$K_A = \frac{q_A}{c_A(\Lambda - \alpha q_A - q_S)^\alpha} \tag{27}$$

$$K_S = \frac{q_S}{c_S(\Lambda - \alpha q_A - q_S)} \tag{28}$$

from which follows

$$\left(\frac{q_A}{K_A c_A}\right)^{1/\alpha} = \left(\frac{q_S}{K_S c_S}\right) \tag{29}$$

Substituting eqn. 29 into eqn. 27 and neglecting αq_A with respect to Λ under the restriction of linear adsorption on A, we get

$$\left[\Lambda - K_S c_S \left(\frac{q_A}{K_A c_A}\right)^{1/\alpha}\right]^\alpha = \frac{q_A}{K_A c_A} \tag{30}$$

Let the distribution coefficient D_A represent q_A/c_A. Then

$$\Lambda - K_S c_S \left(\frac{D_A}{K_A}\right)^{1/\alpha} = \left(\frac{D_A}{K_A}\right)^{1/\alpha} \tag{31}$$

$$\frac{1}{\alpha}\ln D_A - \frac{1}{\alpha}\ln K_A = \ln \Lambda - \ln(1 + K_S c_S) \tag{32}$$

$$\ln D_A = \ln(K_A \Lambda^\alpha) - \alpha \ln(1 + K_S c_S) \tag{33}$$

When the product $K_S c_S$ is small with respect to unity, the last term in eqn. 33 can be simplified by using the usual approximation for the logarithm:

$$\ln(1 + x) \approx x - \frac{x^2}{2} \tag{34}$$

which gives

$$\ln D_A = \ln(K_A \Lambda^\alpha) - \alpha K_S c_S + \alpha \cdot \frac{K_S^2}{2} \cdot c_S^2$$

$$= K_1 - K_2 c_S + K_3 c_S^2 \tag{35}$$

where K_1, K_2, K_3 are constants. This is the usual expression for the dependence of adsorbate retention factor on organic modifier concentration in reversed-phase chromatography. It is

interesting that a formalism that is based solely on adsorbate interactions with the stationary phase can generate this result, since most reversed-phase retention theories are based on the interaction of adsorbates with the mobile phase [17–21]. However, it must be emphasized that the deviation above is limited to organic modifiers of unit binding area. (The binding area of the modulator can be regarded as the basic unit of interaction with the stationary phase, since it is unlikely that any feed component will be smaller than the modulator.) The calculation does not carry through in the general case, where the binding area of the modulator is arbitrary.

On the other hand, when the product $K_S c_S$ is large with respect to unity, the last term on the right-hand side of eqn. 33 can be simply replaced by the logarithm of $K_S c_S$; this leads to a linear relationship between the logarithmic retention factor and the logarithm of the modulator concentration:

$$\ln D_A = \ln(K_A \Lambda^\alpha K_S^\alpha) - \alpha \ln c_S \quad (36)$$

An analogous result has been reported by Geng and Regnier [22,23] based on a "displacement" model, in which the stationary phase is effectively considered to be saturated at all times, and adsorption of one species is necessarily accompanied by the desorption of another. Thus, Geng and Regnier's model is essentially the mass-action or ion-exchange formalism discussed earlier. Here we have shown that an adsorptive model, in which saturation of the stationary phase is not required, can also yield the usual log–log relationship as one limiting case, as seen in eqn. 36. Further, the other limiting case gives the "semilog" relation commonly used for small adsorbates (eqn. 35), and further provides a basis for the quadratic term in c_S that is frequently found necessary to describe experiments [19,24].

The two limiting cases described above can be physically motivated as follows. Prior to the introduction of feed components, the stationary phase can be regarded as saturated by the mobile phase components, *e.g.*, water and the modulator (here, an organic modifier). However, it cannot be concluded that the stationary phase is essentially covered by the modifier alone. In many cases of practical interest [25–27] the excess isotherm exhibits an adsorption azeotrope. If it is then assumed that the modifier alone adsorbs, the resultant individual isotherm for the modifier has a local maximum, which is physically unreasonable. It is therefore necessary to assume that the water also adsorbs. In fact, acetonitrile and water have been found to adsorb to comparable amounts over a wide range of mobile phase composition on octadecyl silica [25,26].

Given that both the modifier and the water adsorb, eqn. 35 can be regarded as corresponding to the region where the adsorption of a feed component is not necessarily accompanied by the desorption of the modifier. Instead, the feed can easily displace adsorbed water molecules. The other limiting condition corresponds to the region in which the only mobile phase component that is adsorbed to any appreciable extent is the modifier, and the adsorption of a feed component now results in the desorption of the modifier. The displacement model, eqn. 36, then results.

SHIELDING OF BINDING SITES BY MACROMOLECULAR ADSORBATES

All the isotherm formalisms described above are based on the assumption that all the adsorbates are able to access all the available binding sites on the stationary phase, *e.g.*, that a protein of characteristic charge α will adsorb on to an ion exchanger of binding site concentration Λ, and in the absence of other counterions, have a stationary phase (saturation) concentration of Λ/α. However, this excludes the possibility of shielding and exclusion, whereby the size of the protein makes a certain number of fixed charges on the stationary phase unavailable for binding by a molecule of comparable size. Thus, let the number of such excluded (occluded) binding sites associated with the protein A be ε_A; then the number of sites unavailable for binding after a single molecule of A has adsorbed is $(\alpha + \varepsilon_A)$, and the saturation concentration is decreased to $\Lambda/(\alpha + \varepsilon_A)$. Of course, electroneutrality requires that all the sites on the stationary phase be

bound, and thus ε_A molecules of salt (buffer or modulator) must adsorb to the ε_A binding sites occluded by molecule of A. The adsorption of large molecules on, say, reversed-phase sorbents, can be similarly generalized from the adsorptive formalisms presented earlier. Again, an excluded binding area, which we will also represent by ε_A, is associated with the binding of a protein A with binding area α. Such a shielding or screening effect was proposed by Velayudhan [28], and has been expanded upon by Cramer and co-workers [29,30].

One important consequence of this generalized formalism is that it is no longer necessary for single-component isotherms to cross. Consider a protein A with characteristic charge 6 and occluded charge 3, which is found to be more retentive in analytical runs than another protein B with characteristic charge 5 and occluded charge 5. The effective saturation concentration of A is $\Lambda/9$, while that of B is $\Lambda/10$. In this example, the single-component isotherms need not cross.

Implications for displacement chromatography

As we have pointed out in the previous section, all the isotherms discussed above should give "normal" separation profiles as long as selectivity reversal (for the heterovalent formalisms only) does not occur during the separation. Thus, in displacement, a final pattern of contiguous rectangular bands of pure feed components should result on using a sufficiently long column. This is the classical Tiselian description of displacement [31], and is based upon monotonic, concave-down, non-crossing single-component isotherms for all the feed components as well as the displacer. Since these isotherms do not cross, there is a natural retention order of the feed components; by excluding selectivity reversal, this order is maintained throughout the separation. The displacer's single-component isotherm must overlie all of the others, so that it is the most-strongly retained component, and its front can displace the bands of all the other components, eventually forming a displacement train (final pattern). The concentrations of the bands in the final pattern increase monotonically in increasing order of retention, with the displacer's concentration being the highest.

However, it has sometimes been found that displacement trains are formed even though the single-component isotherms cross, and that the concentrations in the final pattern do not form a monotonic sequence. We seek to clarify these results based on the previous discussion on isotherms. This is clearly a heterovalent case, since the single-component isotherms cross. However, if the concentrations were replaced by equivalents —this would involve scaling by the valence or characteristic charge in ion exchange, and by the binding area in adsorption— the single-component isotherms would no longer cross, since they would all have the same saturation concentrations. In effect, we have converted heterovalent isotherms into monovalent isotherms by using equivalents. The same conversion is obviously also applicable to homovalent systems.

In the example given above, the saturation levels of both A and B would be Λ equivalents, and the single-component isotherms no longer cross. Further, consider a displacement of A and B in which a final pattern of fully resolved bands of A and B are formed, but the concentration of A —which emerges first— is higher than that of the later-eluting band of B. If the concentrations in the displacement train were replaced by equivalents, the equivalents of B would be twice its concentration, while the numerical value of A in equivalents would be identical to that in concentration units. Thus the non-monotonicity of concentrations in the final pattern could well be removed. Fig. 2 shows a schematic of such a displacement, using a trivalent displacer. This situation, in which heterovalent ion exchange in the absence of selectivity reversal gives rise to apparently non-Tiselian behavior, is apparently quite widespread. The remedy of using equivalents, while standard in classical ion-exchange batch studies [3], does not seem to have been widely used, to the best of our knowledge, for heterovalent systems in column chromatography.

It must be emphasized that the suggestion offered here is purely formal: it does not in any way affect the dynamics of separation. For instance, if selectivity reversal is observed ex-

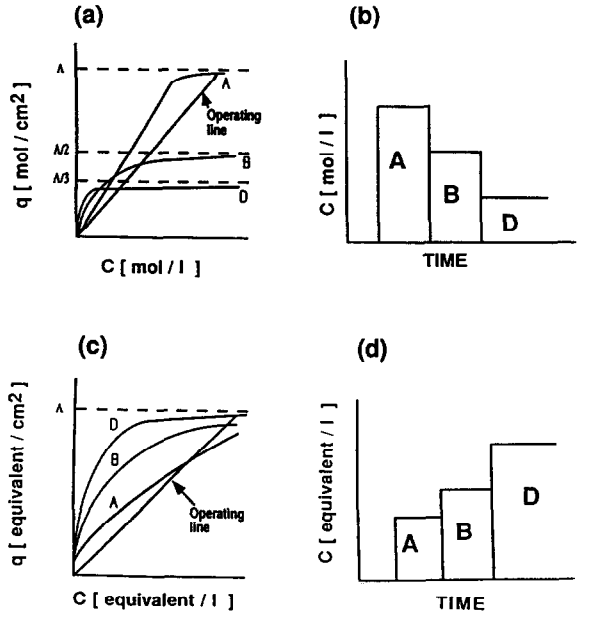

Fig. 2. Representation of single-component isotherms and consequences for final patterns in displacement. (a) Crossing single-component isotherms for two feed components and the displacer; here shielding effects are not considered; (b) the resulting displacement chromatogram; (c) the isotherms in (a) redrawn in terms of equivalents; now the single-component isotherms no longer cross, since they all have the same saturation level in equivalents; (d) the final pattern in (b) redrawn in terms of equivalents. The normal Tiselian final pattern is shown. C = mobile phase concentration; q = stationary phase concentration.

perimentally, changing concentrations into equivalents will not alter the non-Tiselian behavior found in practice. The present suggestion is merely intended to add a modicum of conceptual clarity to the understanding of systems that might be regarded theoretically as non-Tiselian, but do in fact give fully resolved displacement trains (as opposed to systems where selectivity reversal is encountered during the separation, and fully resolved component bands are not in general formed). The result might be summarized thus: in the absence of selectivity reversal, ion-exchange and adsorptive systems exhibiting crossing single-component isotherms can give fully resolved displacement trains. In these situations, using equivalents rather than concentrations will clarify why such results are obtained, and reduce them to classical Tiselian patterns.

CONCLUSIONS

The preceding discussion assumes that the heterovalent ion-exchange (ideal mass-action) formalism is applicable, *i.e.*, that stationary and mobile phase activities can be replaced by their respective concentrations, and that mechanisms other than pure mass-action, such as Donnan equilibrium, can be neglected. While certainly being far from the truth [32], the ideal mass-action formalism is still widely used to approximate chromatographic behavior in the ion-exchange mode for a large class of realistic operating conditions.

The use of valences is essential to converting concentrations to equivalents. However, the equivalent for proteins and other macromolecules, the "characteristic charge," [7,32] is by no means as well-defined a concept. In fact, the characteristic charge is unlikely to be solely a function of the adsorbate, even when the environmental conditions such as temperature and pH are fixed: it could well vary with the adsorbent. For example, the characteristic charge of a protein is likely to be low on an adsorbent with a very low density of fixed charges, since there are not too many fixed charges available within a protein diameter. If another adsorbent with a higher density of fixed charges were used, all other conditions remaining the same, it is likely that the measured characteristic charge of the protein would increase. The characteristic charge could also vary with loading. In addition, the charge carried by the ionogenic groups of the protein can depend on the dielectric of the local microenvironment as well as on the electrostatic field generated by the fixed charges. These points merely emphasize the need for more fundamental studies on protein adsorption. Nevertheless, the use of characteristic charges measured at the conditions under which the displacement separation is to be run should prove adequate to remove the kind of anomaly described above.

In turn, fully resolved displacement trains could be used to measure characteristic charges in the absence of selectivity reversal, as long as the characteristic charge or valence for one component is known. It would be interesting to

see how the characteristic charges measured from displacement runs compare with those measured in the standard fashion from analytical elution experiments (as the slope of a log–log plot of retention factor *versus* the salt concentration in the mobile phase [7], or from a plot of the logarithmic retention factor against the reciprocal square root of the ionic strength [32]).

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