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Dependence of retention on the organic modifier concentration and multicomponent adsorption behavior in reversed-phase chromatography

FIROZ D. ANTIA and CSABA HORVÁTH*
Department of Chemical Engineering, Yale University, New Haven, CT 06520 (USA)

ABSTRACT

A three-parameter equation is derived to express the dependence of the logarithmic retention factor, κ, on the volume fraction of the retention modulator, φ, in a binary eluent (such as the organic modifier in the hydro-organic eluents used in reversed-phase chromatography). It is based on the competitive binary adsorption isotherm of the eluite and the modulator generated by employing the ideal adsorbed solution (IAS) method. The equation is found to describe adequately the trends in the κ–φ relationship experimentally observed in reversed-phase systems. Furthermore, the expression affords an estimation of the single-component adsorption isotherm of the eluite from the corresponding κ *versus* φ plot and thus provides a simple means to gather data of importance in the design of separations by non-linear chromatography. For instance, the method can be used to determine whether a pair of eluite isotherms cross one another, a situation that could lead to difficulties in preparative separations. The inherent limitations of the IAS approach may restrict the usefulness of the expression in specific cases. Nevertheless, the approach presented here establishes an explicit, thermodynamically consistent link between the eluite–modulator multicomponent isotherm and corresponding plots and allows a rational description of the generally observed retention behavior in reversed-phase chromatography. The results of this work also illustrate the limitations of the competitive Langmuir isotherm, which is most frequently used to treat competitive adsorption, in the study of the κ–φ relationship specifically and in investigating and modeling non-linear chromatography at large.

INTRODUCTION

Recent interest in industrial separations by preparative/process liquid chromatography has given renewed impetus to the study of adsorption from multicomponent liquid solutions. In such applications, feed components are usually present at concentrations high enough to render their adsorption behavior non-linear and competitive so that they interfere with one another as they traverse the column. The main features of the separation process are dictated by the non-linear multicomponent isotherm function, which describes the simultaneous adsorption of the different feed components. For this reason the process is called non-linear chromatography and it has been the subject of several recent reviews [1–3].

On the other hand, in linear elution chromatography, which is most commonly

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used in analytical work, the sample components are at such low concentrations that their adsorption isotherms are linear and the interference between them is insignificant. Nevertheless, in order to control retention of the eluites in linear elution chromatography, modifiers (retention modulators) are often added to the eluent at such high concentrations that their adsorption isotherms are non-linear and they compete with the eluites for binding sites. For this reason, multicomponent competitive adsorption can be important, not only in preparative applications, but also in analytical chromatography.

The relationship between the eluite retention factor and the modulator concentration is the key to the selection of the optimum eluent strength or gradient profile for a given separation. Since this relationship depends on the competitive adsorption of the modulator and eluite, it ought to be possible to describe it using an appropriate multicomponent isotherm. Yet, save in the case of electrostatic interaction chromatography [4,5], none of the current treatments of this important relationship make any explicit reference to multicomponent adsorption. In reversed-phase chromatography, where hydro-organic eluents are employed, empirical expressions [6,7] or theories of extra-thermodynamic origin [8] have been used to relate the logarithmic retention factor, κ, to the modulator volume fraction, φ. Treatments of chromatography with polar stationary phases have taken into account the adsorption isotherm of the modulator [9,10], but no connection has been made with either the multicomponent or the eluite isotherm.

The reasons for this lacuna become apparent when one considers that it is the multicomponent Langmuir equation that is almost exclusively employed to describe competitive adsorption behavior. Whereas this isotherm allows for great mathematical simplifications when modeling non-linear chromatography, it is thermodynamically inconsistent, except under special conditions, and more than often fails to reflect real behavior. For this reason, many of the simulations carried out with the multicomponent Langmuir isotherm formalism are likely to be of dubious value. The ideal adsorbed solution (IAS) method provides a thermodynamically consistent framework for generating multicomponent equilibrium relationships from arbitrary single component isotherms [11,12]. Due to its simplicity, the IAS method has serious limitations and is not expected to be universally applicable. Nevertheless, it represents a significant advance over the multicomponent Langmuir isotherm.

In this work we test whether the thermodynamically consistent multicomponent isotherm so generated provides a realistic description of the relationship between retention and modulator concentration (the κ–φ relationship). Concomitantly, we study the influence of the modulator on the isotherms of the eluites and examine whether estimates of the eluite adsorption isotherms can be made from κ–φ plots. The terminology of reversed-phase chromatography is employed and reference is made to data obtained in such systems. The general principles, however, are applicable to all non-electrolyte chromatographic systems.

THEORY

In general, the amounts of components adsorbed from liquid solutions cannot be quantitatively determined. It is therefore necessary to introduce the concept of excess adsorbed quantities and to adopt a convention for their definition [13–15]. In analytical liquid chromatography the concentrations of the feed components in the mobile phase are much lower than that of the solvent; thus no distinction has to be made between excess and absolute adsorbed amounts. The concentration of the mobile phase modulator, however, may vary in a wide range so that the treatment of its adsorption necessitates the choice of a suitable convention.

For this case, the most convenient is the "solvent-not-adsorbed" convention where the most weakly bound component is considered to be absent from the adsorbed phase [13,16]; the adsorbed amounts of other components are then represented by "relative excess" quantities. In the literature, the "molar reduced excess" is frequently used to express the adsorption of modulators [9,17–21], and for binary eluents this can be easily converted to the relative excess upon dividing by the mole fraction of the reference, or principal, solvent [13,14,19].

We will employ the "solvent-not-adsorbed" convention and consider only binary eluents containing a modulator and the principal solvent. The single component isotherms of the modulator, or of the individual feed components, determined in the presence of the principal solvent alone, are called "principal" isotherms.

Single component isotherms

For an eluite e, it is convenient to express the amount adsorbed per unit mass of sorbent, q_e^0, as a function of its molar concentration in the mobile phase, c_e^0, where the superscript 0 denotes that only a single component is being considered. For the modulator, m, q_m^0 represents the relative excess per unit sorbent mass, and mole fractions, x_m^0, rather than molar concentrations, are the appropriate units for the mobile phase composition. (For the rigorous derivation of adsorption isotherms in liquid systems, mole fractions are the required units [22]: molar concentrations are acceptable for eluites present at low concentrations, since, to a good approximation, they are linearly related to their mole fractions.) In this work, we still distinguish between feed components and modulator by employing molar concentrations for the former and mole fractions for the latter.

The single component, or principal, isotherms of feed components are most simply expressed by the Langmuir equation [23]

$$q_e^0 = \frac{\lambda_e^0 b_e^0 c_e^0}{1 + b_e^0 c_e^0} \tag{1}$$

where λ_e^0 and b_e^0 are the pertinent isotherm parameters for the eluite e; λ_e^0 represents the largest possible value for q_e^0 and is therefore called the saturation capacity of the sorbent for that eluite. Experimentally determined single-component isotherms of small and large molecular weight eluites in liquid chromatographic systems have been fit reasonably, with a few exceptions, to eqn. 1 [24–28].

Similarly, the simplest form for the relative excess isotherm of the modulator is given by (*cf.* ref. 22)

$$q_m^0 = \frac{\lambda_m^0 \beta_m^0 x_m^0}{1 + \beta_m^0 x_m^0} \tag{2}$$

where use of the symbol β_m^0 denotes that its units (dimensionless) are distinct from those of b_e^0 (molar concentration $^{-1}$) in eqn. 1. Even though q_m^0 is not equal to λ_m^0 at $x_m^0 = 1$, the parameter λ_m^0 does represent the saturation capacity of the sorbent for the modulator, as may be verified by applying Schay's method for determining the maximum adsorption capacity to eqn. 2 [29].

The molar reduced excess form of eqn. 2 —obtained by multiplying it by $(1 - x_m^0)$— yields an inverted U-shaped curve. This is the simplest of the several possible types of isotherms for binary miscible systems [19,30,31]. Molar reduced excess isotherms of modulators in reversed-phase systems have been experimentally determined [13,17,20,21,32], and often display a maximum and a minimum in the water-rich and organic-rich regions, respectively. For simplicity we assume that the modulator adsorption is well described by eqn. 2; later we will discuss briefly how other isotherm shapes can also be accommodated. With the parameters $\lambda_m^0 = 2.0$ mmol/g sorbent and $\beta_m^0 = 3.7$, eqn. 2 approximates the isotherm of methanol in water on μBondapak C_{18} [20], and these values are used in the calculations except where otherwise mentioned.

Multicomponent isotherms

The simultaneous adsorption of more than one component, at a given temperature, is described by multicomponent isotherm functions. In general, the multicomponent function must be fit to equilibrium adsorption data obtained over the entire composition range of interest. However, only a few measurements have been made of multicomponent adsorption in liquid chromatographic systems [25,28]; for the most part, simple formalisms are employed to predict multicomponent adsorption using parameters obtained from single component data. We will outline here two formalisms for multicomponent isotherms when the principal isotherms of the eluite and modulator are well represented by eqns. 1 and 2: the competitive Langmuir isotherm and the multicomponent isotherm derived using the IAS theory. The results will then allow us to link the plots of the logarithmic retention factor of the eluites *versus* the modulator volume fraction to the principal isotherms of the eluites and the mobile phase modulator.

For brevity, the multicomponent isotherm equations will be written here in terms of concentrations; it is understood that for the modulator m, the term $b_i^0 c_i$ is replaced by $\beta_m^0 x_m$ and for the eluite e by $b_e^0 c_e$ —the absence of a superscript on the concentrations denotes that multicomponent systems are being considered. Furthermore, it is assumed that the mole fraction of the modulator is unaffected by the presence of the feed components.

The competitive Langmuir isotherm is given by [33]

$$q_i = \frac{\lambda_i^0 b_i^0 c_i}{1 + \sum_{j=1}^{N} b_j^0 c_j} \qquad i = 1,2,\ldots,N \tag{3}$$

where N is the total number of adsorbed components and the subscripts i and j refer to all the components including the eluites and the modulator. As mentioned before, a severe shortcoming of eqn. 3 is that it fails to satisfy the Gibbs' adsorption equation and is therefore thermodynamically inconsistent except in the rare case when [34,35]

$$\lambda_1^0 = \ldots = \lambda_i^0 = \ldots = \lambda_N^0 = \lambda \tag{4}$$

i.e., the saturation capacity of the system is the same for all components.

The Gibbs' adsorption equation relates adsorbed amounts to the spreading pressure, defined as the difference of the interfacial tensions between the multicomponent solution–sorbent and the pure principal solvent–sorbent systems. The ideal adsorbed solution (IAS) method provides a framework to generate from any set of single-component isotherms a multicomponent isotherm that satisfies Gibbs' equation [11,12]. We are interested in using the IAS approach to obtain such multicomponent isotherms from principal Langmuir isotherms. In this case the calculation proceeds in two steps [12,35]. First, the reduced spreading pressure, Π, is determined for the given set of Langmuir isotherm parameters and concentrations from the following implicit equation

$$\sum_{j=1}^{N} \frac{b_j^0 c_j}{\exp\{\Pi/\lambda_j^0\} - 1} = 1 \tag{5}$$

Next, for a known value of Π, the multicomponent isotherm is calculated from the following relationship

$$q_i = \frac{b_i^0 c_i/(\exp\{\Pi/\lambda_i^0\} - 1)}{\sum_{j=1}^{N} b_j^0 c_j \exp(\Pi/\lambda_j^0)/\{\lambda_j^0[\exp(\Pi/\lambda_j^0) - 1]^2\}} \qquad i = 1,2,\ldots,N \tag{6}$$

Eqn. 6 represents a multicomponent isotherm obtained from Langmuirian parent isotherms and we call it the IAS/L isotherm. In the case where eqn. 4 is satisfied, eqn. 6 reduces to the competitive Langmuir isotherm, eqn. 2, which is therefore a special case of the IAS/L model.

Relationship between logarithmic retention factor, κ, and modulator volume fraction, φ

In linear elution chromatography the eluite is present in a trace amount so that its retention factor, k', is given by its mass distribution ratio in the stationary and mobile phases at vanishing eluite concentration as follows

$$k' = \phi\left(\lim_{c_e \to 0} \frac{q_e}{c_e}\right) \tag{7}$$

where ϕ is the phase ratio, which for simplicity is assumed here to be unity. In order to examine the dependence of the retention factor, or more conveniently, that of the logarithmic retention factor, κ, on the modulator concentration, we combine eqn. 7 with the IAS/L expression. In the limit $c_e \to 0$, eqn. 5 becomes explicit since there is no longer any need for the summation, and Π is found to be

$$\Pi = \lambda_m^0 \ln(1 + \beta_m^0 x_m) \tag{8}$$

If eqn. 8 is substituted into eqn. 6, applying eqn. 7 and taking logarithms yields the result:

$$\kappa = \kappa_L + \log\left[\frac{\Lambda\beta_m^0 x_m}{(1 + \beta_m^0 x_m)^\Lambda - 1}\right] \tag{9}$$

where Λ is the ratio λ_m^0/λ_e^0. The term κ_L is the logarithmic retention factor of the eluite when $\Lambda = 1$, *i.e.*, the IAS/L isotherm reduces to the competitive Langmuir isotherm, and is given by the expression

$$\kappa_L = \log a_e^0 - \log(1 + \beta_m^0 x_m) \tag{10}$$

where $a_e^0 = \lambda_e^0 b_e^0$ is the initial slope of the eluite isotherm in the absence of the modulator.

In the practice of reversed-phase chromatography the volume fraction of the organic modifier, φ, rather than its mole fraction, is most commonly used. For binary eluents, the modulator mole fraction, x_m is related to φ by the expression

$$x_m = \frac{\varphi}{\varphi + (1 - \varphi)\Omega} \tag{11}$$

Here $\Omega = \bar{v}_m/\bar{v}_s$, where $\bar{v}_m$ and $\bar{v}_s$ are the partial molar volumes of the modulator and the principal solvent, respectively. In eqn. 11 it is assumed that in hydro-organic eluents the ratio Ω is constant even though $\bar{v}_m$ and $\bar{v}_s$ usually depend on the composition [36]. Eqns. 9–11 together give the expression for κ *versus* φ. For a given value of Ω, eqns. 9–11 represent a relationship with three parameters: a_e^0, the initial slope of the eluite isotherm measured in the principal solvent proper; Λ, the ratio of the saturation capacities for the single component (parent) isotherms of the modulator and the eluite; and β_m^0.

RESULTS AND DISCUSSION

The most widely used branch of modern liquid chromatography is reversed-phase chromatography, carried out with aqueous eluents containing an organic modifier and it offers an eminently suitable means to examine the applicability of the derived equations for the dependence of κ on φ. Because of the importance of this chromatographic technique, the κ–φ relationships for eluites of widely different molecular weight and various modulators have been extensively documented; the plots are quasi linear and have negative slopes [37–45]. The negative slope of the κ *vs.* φ plot is termed the S value in the literature of gradient elution. The sign of the slope is due to the fact that the excess adsorbed amount of the modulator is positive [16]. Generally, in a given chromatographic system, it is found to increase with the molecular weight of eluites of similar chemical structure [42] and believed to be proportional to the area of contact between the bound eluite and sorbent [8,45]. The ordinate intercept of the plot, $\log a_e^0$, represents the κ value for the eluite in neat water; for sets of related molecules, it is also thought to increase with the area of contact [42,44].

As it shares a common physico-chemical basis with reversed-phase chromato-

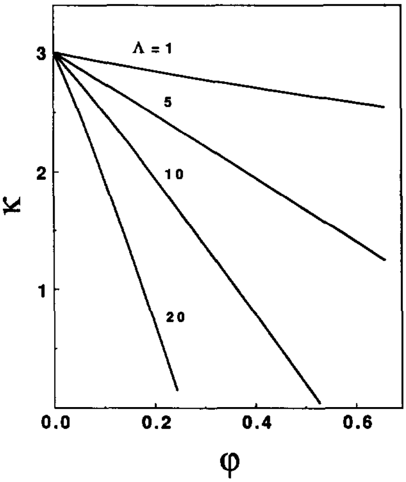

Fig. 1. Plots of the logarithmic retention factor, κ, *versus* modulator volume fraction, φ, for different values of the parameter Λ according to the IAS/L isotherm model. The data were calculated from eqn. 9 with fixed values of $a_e^0 = 1000$ and $\beta_m^0 = 3.68$ (dimensionless), $\phi = 1$ g sorbent/ml mobile phase.

graphy, hydrophobic interaction chromatography (HIC) exhibits similar behavior for κ *versus* salt molality plots [46–49]. In HIC, however, the relative excess adsorption of the salt with respect to water is a negative quantity [50], and thus the slopes of the quasi-linear plots of κ *versus* salt molality are positive. Application of the Gibbs' adsorption equation shows that negative excess adsorption of salt in HIC implies an increase in the corresponding interfacial tension with salt concentration; the opposite is true for positively adsorbing organic modifiers in reversed-phase systems. This viewpoint is thus consistent with the solvophobic theory that is used to describe both HIC and reversed-phase chromatography [8,47]. Our treatment is therefore expected to be applicable to HIC *mutatis mutandis.*

As the Langmuir multicomponent equation has already been proved to be inadequate [51], the equation derived from the IAS/L isotherm will be used to interpret the κ–φ relationship in terms of the adsorption isotherms of the eluite and modulator. Although other thermodynamically consistent multicomponent isotherm models could conceivably found for the same purpose, here we consider only the IAS/L formalism. We have found no complete set of data that includes not only the isotherms of the modulator and eluites, but also the corresponding κ–φ plots. Therefore, we refrain from verifying the accuracy of eqn. 9. However, the general properties of the κ *versus* φ plots should allow us to evaluate the ability of eqn. 9 to describe, at least qualitatively, the experimentally observed behavior.

Our first concern is the linearity of the κ *versus* φ relationship. It is found that with β_m^0 values in the practical range, the equation gives rise to quasi-linear plots over the region $1 < \Lambda < 20$. Further investigations showed that the curvature depends on the ratio of molar volumes, Ω, and changes from slightly concave upward (positive second derivative) to slightly convex (negative second derivative) over $1 < \Omega < 3$, which covers the practical range for most modifiers. Despite the small changes in curvature, the dependence of the linearity on Ω is fairly weak in this region; all the plots can be fit reasonably with straight lines. In the calculations, we have used the value $\Omega = 2$, which is close to the value of 2.2 calculated for methanol–water systems from density data at 20°C [52].

As is evident from eqn. 10, the κ–φ relationship derived from the competitive Langmuir isotherm alone is unrealistic, in agreement with earlier observations [51]. The derivative of eqn. 10 with respect to x_m (or φ), and hence the slope of the corresponding κ–φ plot, depends solely on the parameter β_m^0, implying that for a given modulator, the κ–φ plots of all eluites, regardless of their isotherm parameters, would have the same slope. This absurdity reveals the limitations of one of the properties of the competitive Langmuir isotherm that makes it mathematically attractive, *viz*, constant separation factor (or selectivity, $\alpha_{21} = q_2c_1/q_1c_2 = a_2^0/a_0^1$, for any two components).

In contradistinction, according to the relationship derived from the IAS/L multicomponent isotherm, eqn. 9, the slopes of the plots, *i.e.* the S values, depend on the ratio of the saturation capacities of the modulator and eluite single component isotherms, Λ, and the parameter β_m^0. An approximate relationship for the dependence of S on these parameters is derived in the Appendix. Fig. 1 shows plots of κ *versus* φ for fixed values of β_m^0 and the intercept log a_e^0. The slope increases with increasing values of Λ. As the IAS/L isotherm correctly describes a wider range of behavior it offers a marked improvement in simulating dynamic adsorption behavior over the multicomponent Langmuir expression. In particular, in a given chromatographic system, the IAS/L model allows for eluites having different S values and thus separation factors that vary with changes in the modulator concentration. The slope also increases with increasing values of β_m^0 (not shown in Fig. 1), corresponding to the use of a more strongly sorbed modulator, other factors remaining constant.

The increase in the S values with the molecular contact area upon adsorption at

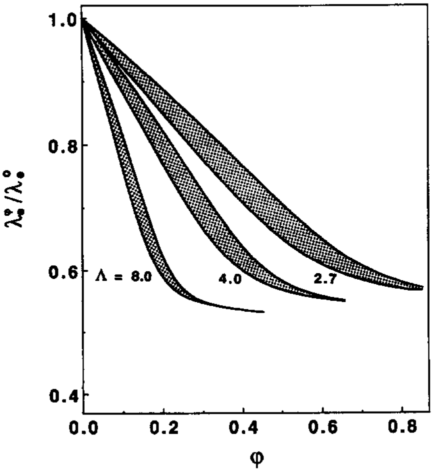

Fig. 2. Plots of the normalized apparent saturation capacity of eluite isotherms, $\lambda_e^\varphi/\lambda_e^0$, *versus* the modulator volume fraction φ, for different values of the parameter Λ. The curves representing the lower and upper envelopes of the shaded regions were calculated from eqn. 6 with $b_e^0 = 1000$ and 4000, respectively. Note that $\Lambda = \lambda_m^0/\lambda_e^0$ and $b_e^0 = a_e^0/\lambda_e^0$. Conditions: $\lambda_m^0 = 2$ mmol/g sorbent, β_m^0 and ϕ as in Fig. 1.

the chromatographic surface in reversed-phase chromatography can also be inferred from the IAS/L isotherm. Eluite molecules having larger contact areas when bound to the stationary phase would be expected to occupy larger area at the surface and consequently to have smaller values for the molar saturation capacity, λ. The larger Λ values then result in greater S values as illustrated in Fig. 1. A similar conclusion can be drawn for the dependence of S on the molecular weight of the eluites.

Influence of the modulator on the eluite adsorption isotherm

So far we have considered only the principal isotherm of the eluite, *i.e.* the single-component eluite isotherm measured in principal solvent in the absence of the modulator. Here we turn our attention to the effect of the modulator concentration measured by its volume fraction, φ, on the isotherm of the eluite measured at a fixed value of φ in the eluent. Since the dependence on φ of the initial slope of the resulting isotherm is described by the κ–φ relationship discussed before, our concern here is the effect of the modulator on the observed saturation capacity, λ_e^φ, that differs from the saturation capacity of the eluite in neat principal solvent, λ_e^0. As shown in the Appendix, λ_e^φ can be found as a function of φ from eqn. 6. In Fig. 2, the normalized observed saturation capacity, $\lambda_e^\varphi/\lambda_e^0$, is plotted against the modulator volume fraction, φ, for different values of the parameters Λ and b_e^0. As seen in Fig. 2 the observed saturation level decreases with increasing φ in agreement with experimental findings [24]. The rate of the decrease, or suppression rate, increases with Λ, but is only a weak function of b_e. Since the slopes of κ–φ plots also increase with Λ, both the initial slope and the saturation capacity of the isotherm are suppressed more strongly with increasing values of Λ. This conclusion is in agreement with the observed sharp drops in the retention factor and in the saturation capacity of large molecules with small changes in modulator concentration [53].

Estimation of the eluite isotherm from the κ–φ plot

In the design of process scale separations by chromatography, there is a significant need to estimate the isotherms of the components to be separated in the chromatographic system under consideration. Since κ–φ plots can be conveniently determined from a few gradient runs [45,54], a method using such data for even a crude estimate of the parameters of the principal eluite isotherm could be of importance because of the considerable saving in time with respect to the tedious conventional methods. Unfortunately, as mentioned before, the expression derived from the competitive Langmuir isotherm, eqn. 10, contains only the initial slope and therefore does not provide sufficient information to estimate the two isotherm parameters. On the other hand, the dependence of κ on φ, eqn. 9, derived from the IAS/L multicomponent isotherm entails both parameters of the principal eluite isotherm and provides an opportunity to estimate them from a κ–φ plot. If the parameters of the modulator isotherm, λ_m^0 and β_m^0, and Ω are known independently, $\lambda_e^0(=\lambda_m^0/\Lambda)$ can be determined from the S value (*vide* eqn. A2) and a_e^0 from the intercept. Knowing λ_e and a_e^0, b_e^0 $(=a_e^0/\lambda_e^0)$ is readily obtained. If the modulator isotherm is not Langmuirian, the IAS method can still be used to generate multicomponent isotherms, as discussed briefly later.

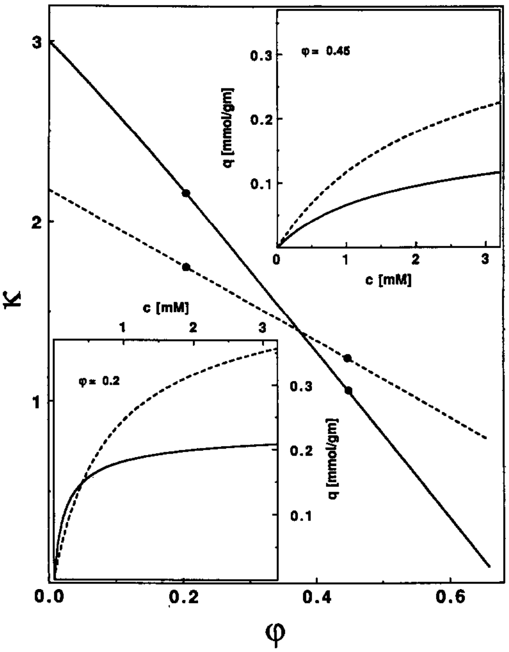

Fig. 3. Intersecting κ *versus* φ plots calculated from eqn. 9. The corresponding eluite isotherms were calculated from eqn. 6 with $\varphi = 0.2$ and $\varphi = 0.45$ and are shown in the lower and upper insets respectively. (— — —) $\lambda_e^0 = 0.5$ mmol/g sorbent, $\Lambda = 4$, $b_e^0 = 300\ M^{-1}$; (———) $\lambda_e^0 = 0.25$ mmol/g sorbent, $\Lambda = 8$, $b_e^0 = 4000\ M^{-1}$. Other conditions as in Fig. 2.

Convergent κ–φ plots and crossing isotherms

Consider a pair of eluites whose κ–φ plots converge and intersect at modulator concentrations of practical interest, as shown in Fig. 3. According to eqn. 9, the eluite with the steeper slope, *i.e.* the greater S value, must have the higher value of Λ, and hence a lower saturation capacity, λ_e^0. On the other hand this eluite also has a higher intercept, and consequently a greater value for the initial slope of its principal isotherm, a_e^0. As a result, the principal isotherms of the two eluites, *i.e.* their single-component isotherms measured in the absence of the modulator, must cross at a certain concentration when viewed in the same plane. Several recent reports have stressed the adverse effects of such isotherm crossing in non-linear chromatography [55–59], and therefore a simple method for diagnosing such behavior can be helpful in the design of a preparative separation.

If the principal isotherms of both the modulator and the eluites are known, the isotherms of the eluites in solutions containing various concentrations of the modulator can be calculated from eqn. 6. Such isotherms, determined separately for each of the two eluites under consideration at two different values of φ are plotted in the insets of Fig. 3. Recent advances in this area of research [60] allow us to generalize the results: for values of φ less than that corresponding to the intersection of the κ–φ plots, the isotherms cross, whereas at φ values beyond this point, the isotherms no longer intersect. Since the isotherm of the eluite with the greater Λ value is more strongly suppressed by increasing modulator concentration than the other, it lies completely below the latter isotherm at sufficiently high values of φ. It is seen that the initial slopes and apparent saturation capacities of both isotherms are lower at the higher φ values; thus addition of a modulator uncrosses the offending isotherms at the cost of lowering the retention of the eluites and adsorption capacity of the system. The practical benefit of adding a modulator to uncross the isotherms depends on the κ value of the intersection point, κ^*. If it is sufficiently large, the uncrossing can be accomplished with modest reduction of the retention and saturation capacity of the components. If, on the other hand, κ^* is too low, the retention and adsorption capacity are likely to be suppressed to unacceptable levels, and other means to uncross isotherms, such as changing the modifier, pH, temperature, or even the stationary phase itself, might be necessary.

In what circumstances might isotherms of eluites be expected to cross? Let us consider, as an example, a series of homologous compounds; each successive homologue binds more tightly to the stationary phase, therefore having a higher initial isotherm slope and thus a greater value for the intercept of its respective κ–φ plot. Since higher homologues are likely to occupy a larger area on the surface of the adsorbent, they would have smaller values, on a molar basis, for the saturation capacity, λ_e. Consequently the single-component isotherms of homologues are expected to cross. Since, in a given system, lower λ_e values correspond to greater A and consequently larger S values, the κ–φ plots of the homologues would intersect as well. Experimental observations indeed bear out this prediction for the κ–φ plots [42–44].

Non-Langmuirian isotherms

In principle, the IAS method can be employed with all types of single-component isotherms: therefore, κ–φ plots can be calculated, combining eqn. 7 with the guidelines given in the Appendix of ref. 12, for any type of modulator and eluite principal isotherms, even those that are fitted to experimental data by a polynomial or other suitable function. If isotherm parameters of eluites are to be estimated from the κ–φ plots, an *a priori* postulate for the form of the principal eluite isotherm is required; the Langmuir form is usually adequate, but again, in principle, any appropriate function may be employed. The practicability of using the approach, however, may be limited because small errors in the experimental data may give inaccurate results due to the sensitivity of the complicated calculations. Indeed, unless great care is taken in the measurement and appropriate fitting of the data to suitable functions representing all the isotherms involved, substantial errors may be introduced [61].

Eqn. 2 is expected to describe adsorption of the modulator only in the water rich region *i.e.* up to about $\varphi = 0.7$. At higher φ values, the reduced molar excess of modifier is often observed to be negative [9,17–21]. Under such circumstances, the IAS theory would predict positive slopes for κ–φ plots. This is consistent with an increase in retention with increasing modifier concentration in the eluent as observed sometimes with organic-rich mobile phases in reversed-phase chromatography [62,63].

CONCLUSIONS

On the basis of its ability to describe qualitatively the observed trends in the relationship between logarithmic retention factor and modulator concentration in reversed-phase chromatography, the IAS/L isotherm should be viewed as a distinct improvement over the more restrictive, and more than often thermodynamically inconsistent, competitive Langmuir isotherm. Indeed, the competitive Langmuir isotherm, which is seen to be a special case of the IAS/L equation, fails to describe all but the most rudimentary cases; in this light its indiscriminate use in the past to model the dynamics of non-linear chromatography calls for criticism.

The IAS/L multicomponent isotherm itself, however, is far from perfect and for this reason the literature is replete with elaborate schemes to incorporate liquid and adsorbed-phase activity coefficients into the fundamental IAS formalism [12,15,22, 64–66]. As mentioned above it is also sensitive to small errors in the measurement of single-component isotherms. Nevertheless, for sets of related compounds having commensurate activity coefficients, it is likely that the trends predicted by the IAS/L isotherm provide a reasonable picture of reality. Indeed the simple expression, eqn. 9, derived from the IAS/L multicomponent isotherm to describe the κ–φ relationship, embodies much of the experimentally observed behavior. In particular, it not only provides the missing link between κ–φ plots and the eluite isotherms, but also offers a rapid means to diagnose isotherm crossing, a source of potential difficulties in preparative separations. In general the approach presented here offers a method to treat the effect of non-linear adsorption in linear elution chromatography on the basis of thermodynamically sound multicomponent isotherms.

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APPENDIX

Dependence of the S value on isotherm parameters

For a given value of the ratio Ω ($= \bar{v}_m/\bar{v}_s$) the negative slope, S, of the nearly linear κ–φ plots can be determined from

$$S \approx (\kappa_{\varphi=0} - \kappa_{\varphi=0.5})/0.5 \tag{A1}$$

where $\kappa_{\varphi=0}$ (given by $\log a_e^0$) and $\kappa_{\varphi=0.5}$ are κ values evaluated at $\varphi = 0$ and $\varphi = 0.5$ respectively. Since at $\varphi = 0.5$, $x_m = 1/(1 + \Omega)$ (*vide* eqn. 11), substitution of eqn. 9 into eqn. A1 yields

$$S \approx S_L + 2\log\left[\left(1 + \frac{\beta_m^0}{1+\Omega}\right)^A - 1\right] - 2\log\left[\frac{A\beta_m^0}{(1+\Omega)}\right] \tag{A2}$$

where S_L is the slope obtained with the multicomponent Langmuir isotherm, given by

$$S_L \approx 2\log\left(1 + \frac{\beta_m^0}{1+\Omega}\right) \tag{A3}$$

which is a function only of β_m^0. The value of S reduces to S_L when $\Lambda = 1$ which is a condition unlikely to have practical significance.

Calculation of the apparent saturation capacity of the eluite isotherm at fixed φ

For the dependence of the apparent saturation capacity of the eluite isotherm on φ (or x_m) no explicit relationship has been found. Therefore we fitted the isotherms generated using eqn. 6 at fixed values of φ to a single component Langmuir expression (*vide* eqn. 1) using a Scatchard diagram, *i.e.* a plot of q_e/c_e *vs.* q_e. The intercept of the straight line thus obtained with the q_e axis yields the apparent saturation capacity, λ_e^φ, plotted in Fig. 2.

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