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Review

Distribution isotherms in reversed-phase systems

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ABSTRACT

Experimental results on single-component isotherms and binary composite isotherms for reversed-phase liquid chromatography, for both sample and mobile phase components, and the modelling and molecular interpretation of these data are reviewed. The treatment of adsorption in terms of surface excess is discussed, mainly in relation to the determination of the void volume. The relevance of such activities for predicting elution curves in preparative LC, for understanding "system peaks" and for obtaining insight into the sorption process is discussed. Reasonably abundant data exist on single-component isotherms, but composite isotherms have rarely been measured in such systems. The success of various models in describing experimental isotherm data is also reviewed.

CONTENTS

1. INTRODUCTION

As the term indicates, a distribution isotherm describes the phase equilibrium between stationary and mobile phases at constant temperature. The latter condition corresponds to the belief that the phenomena in a liquid chromatographic column occur at constant temperature: neither the phase transition itself nor the propulsion of the mobile phase itself leads to heat effects large

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enough to make this notion invalid. Exceptions to this rule occur; *e.g.*, the effect of viscous heat dissipation on column transport has been studied [1] and the transport phenomena in an ("overloaded") chromatographic column under adiabatic conditions have been analysed mathematically [2]. However, in nearly all instances heat capacity and heat conduction in a liquid chromatographic column are sufficiently large that these effects can be neglected.

With a full knowledge of the isotherms, *i.e.*, of the distribution of all compounds involved, the chromatographic behaviour of a sample of any composition, *i.e.*, the column history after injection and (as part of that) the elution pattern, can be predicted [3,4]. This holds both for ideal chromatography (no diffusion or other dispersion at work, infinitely fast equilibrium) and for non-ideal chromatography (finite plate number), albeit that in the latter instance the extent of non-ideality has to be known, *e.g.*, via mass transfer coefficients or plate height equations, and the resulting mathematical complexity may strain the mind and the capacity of computers. The relationship between isotherms and chromatographic behaviour is therefore one of the cornerstones of comprehensive chromatographic theories and is indispensable for the understanding of many chromatographic effects. Also, the revolution in computer performance makes prediction on the basis of isotherms more and more useful in terms of both performance and accessibility.

The study of isotherms has also given an insight into the nature and cause of so-called system peaks and indirect detection schemes. Although these concepts have long been known [5], more recent publications have dealt with this subject and revived the general awareness of these relationships [6–8].

Another beneficial aspect of the study of isotherms is the possibility of interpreting the sorption process in terms of phenomena on the molecular scale. Depending on the certainty of the actual physical reality of the developed molecular picture, we may speak about a model, about hard experimental facts or even about a matter of first principles. The classical example, of course, is the Langmuir-type distribution. When, as is often the case, only molecules attached directly to the surface experience sufficient binding forces in order to keep them there at least temporarily, an adsorbent can accommodate only a limited number of molecules, the number of which can be found by dividing the total available surface area by the so-called "surface necessity" of each molecule. Alternatively, one assumes that each molecule occupies an adsorption site, leading to stoichiometric relationships. Such pictures can also explain in molecular terms the competition between adsorbates, *i.e.*, the fact that the presence of one adsorbate on the surface diminishes the adsorption of another adsorbate, a mechanism underlying the application of "moderators" in liquid chromatography.

In adsorption from the gas phase, one can actually observe the resulting monolayers of adsorbate molecules by various means, and this behaviour can be regarded as a hard experimental fact. However, the possibilities for such direct observations when adsorption takes place from the liquid phase are much more limited. With the the present state of knowledge the Langmuir description of the adsorption process, although it describes well the observed isotherms, is often not much more than a convenient model for the process or, if one wants, the corresponding equations can be regarded as correlations of experimental measurement results. It is important to make the distinction between this correlation and the often still elusive actual physical reality.

Non-linearity of isotherms, *i.e.*, curvature in the plot of stationary phase concentration *versus* mobile phase concentration, plays a central role in the understanding of peak shapes in chromatography at high concentrations. A brief summary of the theory in this respect is now given.

When the behaviour of a single solute, i, is studied, the slope of such plots is equivalent to the distribution constant, K_i. That is, with a linear plot (passing through the origin), the ratio of masses (or moles or even volumes) of component i in the two phases, equal to the capacity factor, k'_i, can be found from

$$k'_i = (V_s/V_m)K_i \quad (1)$$

where V_s and V_m are the volumes of the stationary phase and mobile phase in a column (segment), respectively. Linearity of the isotherm is usually preserved when the concentrations are kept low; in the language of the Langmuir "model", only a very small percentage (1–5%) of the surface is occupied at any position in the column by solute molecules.

It follows from chromatographic theory, due to, amongst others, DeVault [9], that when curvature in the isotherm does occur, the slope K_i that has to be substituted in eqn. 1 is the one corresponding to the prevailing mobile phase composition, *i.e.*, the derivative $dc_{i,s}/dc_{i,m}$. This means that each concentration c_i moves with its own migration velocity $u_i(c_{i,m})$:

$$u_i = \frac{1}{1 + k'_i(c_{i,m})} \cdot u_0 \quad (2)$$

where $k'_i(c_{i,m})$ is the differential capacity factor prevailing at concentration $c_{i,m}$, equal to $(V_s/V_m)(dc_{i,s}/dc_{i,m})$, and u_0 is the velocity of the mobile phase.

In the case of a Langmuir-type isotherm, at increasing concentrations the molecules find less and less place at the sorbent and the slope of the isotherm decreases. As a result, higher concentrations move faster than lower concentrations and the peak develops a tail at its rear. This transition from the higher concentration to the lower (zero) value is called a diffuse boundary.

The front of the peak cannot be described in this way, for obvious reasons: higher concentrations would precede lower concentrations, apparently not the situation at the peak front. Instead a shock develops, a sharp transition (discontinuity) from one (high) concentration to another (low) concentration. The position of the shock (in time or place) can be found by considering the total amount injected, which has to be equal to the area under the resulting curves. Alternatively, one can obtain the shock velocity by applying eqn. 2, while using the k' value corresponding to the chord connecting the operating point with the mobile phase composition tion point. For further details, publications by Guiochon and co-workers (*e.g.*, ref. 4) should be consulted.

In experimental work it is often more convenient to recast eqn. 2 in a form with retention times:

$$t_{R,i}(c_{i,m}) = t_{R0}[1 + k'_i(c_{i,m})] \quad (3)$$

indicating that each concentration elutes at its own retention time. When dispersion effects play a significant role (which is surprisingly seldom at high concentrations), corrections are necessary in these equations. The diffuse boundary is hardly affected and the shock position does not change much, but the transition is no longer infinitely sharp.

When more than one component is adsorbed, they will influence each other, *e.g.*, in the Langmuir case competition will occur. The elution patterns of such mixtures are complicated. When the load on the column is relatively large, and with two components, there will be three regions or zones in the elution pattern: in the first zone the least retained component is eluted in pure form, and this zone is followed by a mixed zone, which in turn is followed by a pure tail of the most retained component. The last zone in itself is complex, and consists of two separate zones with different properties [4].

A central theme in the discussion of such phenomena observed when components interact in the adsorption is the state of coherence, introduced with this wording by Helfferich and Klein [10] as early as 1970. A coherent transition from one composition to another (*e.g.*, front or tail of a chromatographic zone), a boundary, when it is coherent, moves in such a way that "a given concentration of one species then remains accompanied by the same set of concentrations of other species". Coherence is the ultimate, as it were natural, state to which the system tends to evolve, *i.e.*, non-coherent boundaries tend to split in coherent boundaries. These are the boundaries that are usually observed at the column outlet. The loci of the set of concentrations in a coherent boundary form a "path" in composition space, that is, a line in the $c_{1,m}$, $c_{2,m}$, $c_{3,m}$, . . . , $n-1$ dimensional plot. A diffuse boundary follows such a path.

When not considering "pathological" cases, that are degenerates in the mathematical sense, in each point in composition space there are as many paths as there are degrees of freedom in

the composition, *i.e.*, $n-1$. Minor disturbance peaks, including the so-called system peaks, are described by the $n-1$ paths, as has been discussed [5–7].

It goes without saying that the phenomena brought about by non-linearity are of utmost importance in preparative applications of LC. In these one seeks to increase the load, and hence the occupation of the surface (exploitation of the available surface for separation), as much as possible. At the resulting high concentrations one leaves the linear part of the isotherm(s). Often, then, the degree of separation is by no means determined any longer by the dispersion effect (insufficiently large plate number), but rather by the changes in k' values due to the non-linearity and component interaction. One speaks of thermodynamic broadening, as opposed to the kinetic broadening via dispersion (*e.g.*, ref. 3). The revived interest in preparative LC has lead to a wealth of publications on the determination and interpretation of (composite) isotherms.

When preparative chromatography is carried out in the displacement mode [11], the curvature in the isotherm and the competition effect are a prerequisite for operation. The front of the displacer has to move faster than any component of the mixture to be separated; on the other hand, the displacer, in order to do what its name indicates, must have a stronger affinity to the surface than the mixture components have. Hence its concentration should be fairly high, certainly beyond the range of linearity. Study of isotherms is therefore very important for the application of displacement chromatography.

As assumed above, the distribution equilibrium is usually described by giving the stationary phase composition as a function of mobile phase composition. The implementation of this abstract definition in practical, experimental terms is by no means without pitfalls. First there is the description of a "composition". For the mobile phase, and with a binary mixture, *e.g.*, one "solvent" and one "solute", one number, for which usually the concentration of the solute is taken, suffices to describe this, provided that in addition to the temperature the pressure is given. The latter effect does not generally lead to significant complications, as at HPLC pressures the compressibility of liquids is only of the order of a few percent, although interesting effects due to pressure pulses have been observed in chromatographic columns [12]. For one solute–one adsorption systems, simply plotting $c_{i,s}$ against $c_{i,m}$ gives complete characterization.

When more solute and/or more solvents are involved, the number of figures to describe the composition increases. For example, when using acetonitrile–water as the solvent, and studying the simultaneous distribution of phenol and nitrophenol as solutes, the mixture of four components can be fully described by three numbers. Often only two numbers, the concentrations of the solutes phenol and nitrophenol, are given; the tacit assumption then is that the concentration of acetonitrile, say 30% (v/v), is constant in the system. In principle this is incorrect; as the distribution of all components is coupled, the solute partitioning will bring about changes in the partitioning of the solvents also. As a result, the solvent concentrations in a chromatographic zone will not be equal to those in the incoming mobile phase. That is, each eluent component (except one, for which conveniently that with the largest percentage is chosen) is a potentially adsorbing component, of which the adsorption may be coupled to the adsorption of solutes. That it is indeed still possible to obtain reasonably consistent results when neglecting this is only the result of the fact that the acetonitrile (and water) concentration is often so large that the slight deviations are not visible in the experimental results. As soon as we turn to more active "moderator", such as butanol, in lower concentrations, such phenomena cannot be neglected any longer. Such effects have been studied in detail theoretically [13] and experimentally [14].

The equivalence of solutes and solvent components has another important consequence: classical studies of the influence of a moderator concentration are studies of composite isotherms, with one component (solute) at infinite dilution. Such studies often already reveal much about the applicability of a distribution model. This point is discussed in Section 3 and 4.

More tricky situations may arise when ionizing components are studied. The mobile phase often

contains a pH buffer, consisting of at least two constituents. For unambiguous definition of the phase composition one needs to state the concentration of solute and buffer ions, in addition to the counter ions.

The concentration in the stationary phase, for each component considered, is then a function of concentrations of all the components present. The amount of information needed to describe such composite isotherms increases dramatically with the number of components participating in the equilibrium. Most workers would agree that a plot of $c_{i,s}$ against $c_{i,m}$, for one component considered, would require at least about ten points, corresponding, *e.g.*, to ten different $c_{i,m}$ values, in order to cover a sufficiently large concentration range with a reasonable safeguard against missing particular details in the plot. With three components a comprehensive grid with ten points on each concentration axis would require 1000 points to be plotted, for each component giving the resulting stationary phase concentrations, leading to the need for the experimental determination of 3000 stationary phase concentrations. A less ambitious but perhaps still acceptable scheme still leads to a few hundred determinations. It is not surprising, therefore, that full experimental results on composite isotherms with three components are not available, and only a few cases with two components have been studied experimentally in detail.

Kováts and co-workers [15,16] have drawn attention to the fact that any thorough description of phase equilibria in LC has to start with an unambiguous, model-free, description of compositions. Mixing of concepts of stemming from molecular "mechanisms", such as ion exchange, ion pairing and competitive displacement, at this stage is very undesirable, as physical observation and interpretation conflict.

Stationary phase concentrations are usually not given in amounts per unit volume, but rather in amounts (moles, mass, volume) per gram or unit volume of adsorbent. When the specific surface area of the adsorbent is known, it is preferable to use amount per unit area, *e.g.*, μmol/m^2, often called surface concentrations. At first sight one would expect that there are n rather than $n-1$ meaningful concentrations in the adsorbed phase; however, these are not independent and one of them, or some sum of them, can be set to zero, as is discussed in Section 3.

A further, and more persistent, difficulty in describing reversed-phase (RP) distribution equilibria is in the definition of the volume and composition of the stationary phase. Defining and characterizing the composition of the mobile phase presents no problem; it flows out of the columns and can be analysed by all available means. However, the stationary phase exists only within the column and its nature has been "modified" by the presence of the mobile phase. Taking the packing out and isolating it from the mobile phase will change its properties and composition. There is no way of studying the stationary phase in the absence of mobile phase (this is the reason why vacuum-based spectrometric surface analysis techniques such as SIMS, ESCA and mass spectrometry have been of so little use in the elucidation of distribution mechanisms in LC, and why Langmuir adsorption is often a model rather than a physical fact).

Kováts and co-workers [15,16] have put forward the only sound way to describe LC adsorption equilibria. The adsorbed amounts, *i.e.*, the composition of the stationary phase, is derived as the difference between the total (and experimentally observable) amount of each component in the column (column capacity) and the amount present in the mobile phase. This approach will be elaborated in Section 3.

2. MEASUREMENT OF ISOTHERMS

This aspect is considered only briefly, as there are some excellent papers that treat this subject in depth [17–23].

Some methods for composite isotherms, such as the *h*-root method [20], are valid only as long as the adsorption of the mixture adheres to the Langmuir model. The theory of coherence can then be worked out analytically (in the mathematical sense) [10], and the retention of boundaries and/or the experimentally observed composition paths can be interpreted in terms of Langmuir parameters. However, as soon as

there is a deviation from the Langmuir model, the resulting isotherm data are incorrect, in general to an unknown extent. Depending on the use of the isotherm data a larger or smaller risk of faulty predictions may be the outcome. For example, when the data from an *h*-root experiment are used for the prediction of a displacement experiment, one may obtain fairly good results, as the data themselves were obtained under similar conditions. Hence, and this holds in various situations, the data themselves may be incorrect, but may still be useful for optimization purposes. On the other hand, when aiming at a fundamental interpretation, or when a different sort of experiment (*e.g.*, elution *versus* displacement) is to be predicted, the effect of the errors can be significant.

Another comment is to reiterate what has been said above about mixed solvents. Many workers appear not to realize that an experiment with a mixed solvent of, say, n components with m solutes, should in principle be treated as an $(m + n - 1)$-dimensional system, with concurrent complications. In other words, an acetonitrile–water mixture is not equivalent to a hypothetical pure solvent of "acetonitrile–water". The preferential adsorption of components from such solvent mixtures have been demonstrated and studied in detail by various workers [24–28]. Discussion of the results will be given in Section 3. Also, the relevance of taking the full dimensionality of the composition into account has been demonstrated [13,14].

In choosing a method for the determination of (composite) isotherms, the following additional points should be kept in mind:

(i) Requirements for pure materials: some methods can give useful results when only (analysed) mixtures of the components are available. On the other hand, some methods (*e.g.*, frontal chromatography) require substantial amounts of pure materials.

(ii) With some methods, the mobile phase concentrations that ultimately are to be plotted are not imposed on the system, and therefore known via the preparation of solutions, but rather are generated by the chromatographic process. It follows that they have to be measured. Often this is not trivial, as virtually all available HPLC detectors are optimized for trace analysis and do not have a linear and reliable calibration graph at the high concentrations that occur in this type of work. A special warning should be given regarding the use of UV absorption at wavelengths where absorbance is low: minor impurities in solutes and solvents may cause drastic interference and erratic results.

(iii) A key point is often the amount of experimental work expected. Automation of the LC system is often mandatory. Also, obviously methods that produce data for a range of concentrations rather than for only one combination are preferable. However, the latter usually involve more assumptions, from the absence of non-idealities to full adherence to the (composite) Langmuir isotherm.

3. DEFINITION OF MOBILE PHASE VOLUME, V_m

The title of this section was chosen because this is the wording commonly encountered to indicate the problem. It is, however, somewhat too narrow; the definition of the stationary phase volume, V_s, is of course equally important.

Obviously, the total volume $V_m + V_s$ is easily accessible, *e.g.*, by weighing the column before and after flushing it with a liquid of known density. This, of course, is contingent on the assumption that the molar volumes of all components do not change on adsorption, which is an excellent approximation, except for ions. Removing this assumption leads to considerable complications in the derivations; therefore, we shall retain it throughout this paper.

One way to determine the mobile phase volume, V_m, is to inject a compound that is believed not to be adsorbed, that is, it does not enter the adsorbed layer, and observe its retention time; multiplication by the flow-rate then gives V_m. Unfortunately, this method does not give unambiguous results; it turns out that different neutral "V_m probes" of moderate molecular size yield slightly different V_m values, while deviations of the order of 50% occur when ions or polymers are used as probes. This uncertainty seems to corrupt the reliability of experimental data on distribution and its interpretation and therefore has led to extensive scientific efforts.

Many of these have been to propose alternative methods for the determination of V_m. For example, ^2H_2O or other isotopically labelled solvent components have been used as probes; it has been proposed to derive V_m from the linearity of the logarithm of the retention of solutes on the number of CH_2 units in the side-chain, while also disturbances in the solvent mixture composition have been injected as V_m markers. None of these methods, some of which are discussed below, have found general acceptance, and for good reasons. The problem is not solved by using alternative experimental methods; using them either means introducing additional uncertainties (*e.g.*, about the validity of the above-mentioned retention correlation with CH_2 number, which cannot be derived from any known physical law), or just obscures the problem. The problem is not in the experimental measurement, it is in the definition of the phase, *i.e.*, the region it occupies. As this problem arises every time one wants to measure or interpret isotherm data, it is imperative to discuss it here at some depth.

The foundation for this discussion has been formulated rigorously, although not in a very accessible manner, by Kováts and co-workers [15,16]. Based on Gibbs' treatment of adsorption, they argued that it is impossible to make a distinction between the mobile phase and stationary phase regions other than by adopting some arbitrary convention.

Following Knox and Kaliszan [24], we treat this concept here in molecular terms: moving from the bulk mobile phase, far from the surface, towards the surface, picometre by picometre, the average density of molecules (*i.e.*, counting their centres of gravity), *i.e.*, the average composition, will change. Eventually, when entering the inner matrix material of the reversed-phase packing, after passing through the "molecular fur" (Horváth) of C_n chains, all densities will drop to zero. Some densities will have gone through a maximum (which may or may not be within the fur), and these are generally the ones adsorbed, whereas others may decrease monotonically to zero. There is at present no way (see the remark above about non-applicability of spectrometric techniques) to observe how the changes occur. Under some conditions they may occur in an abrupt manner. However, we are fairly sure that the thermal random motion and orientation of molecules and C_n brushes in the liquid and (to a lesser extent) at the surface will always blur out the abruptness to some extent. We even do not know at what distance from the surface significant deviations from the mobile phase composition start to occur (with the exception of ions excluded under the influence of the electric charge of the surface, where this distance can be estimated as several times the Debÿe length). One could argue that rather than one stationary phase with a given composition, we have a continuity of stationary phases, each at a given distance from the surface and with its own composition. Hence, even if we had a "molecular razor blade" to separate the mobile and stationary phases, we would not know where the cut has to be made.

However, when accepting a given convention, the position of the cut, the dividing plane, can be unambiguously established. We shall illustrate this with the case where acetonitrile–water is the mobile phase and phenol is the solute. One such convention is "acetonitrile is not adsorbed". That is, the dividing plane is situated at such a position that the total amount of acetonitrile in the column is accounted for by the product of mobile phase volume and mobile phase acetonitrile concentration. (Note that the total amount is an experimentally accessible quantity; if we cannot think of something better, we could blow out all the material in the column with a stream of inert gas under heating and determine acetonitrile in the effluent). In other words, with the dividing plane fixed in this way, it is by convention agreed that the region between this plane and the packing, *i.e.*, the "adsorbed layer", contains no acetonitrile.

Once such a convention is adopted, everything falls into place. The value of V_m, for example, can be determined as $Q_{ACN}/c_{ACN,m}$, where Q is the total amount in the column, c is concentration and ACN is acetonitrile. Water adsorption is found as the difference $Q_{H_2O} - V_m c_{H_2O,m}$; there is more (or less) water in the system than is accounted for by the mobile phase. Such a quantity is called (with suitable normalization on

the surface area) the surface excess, Γ_i (Γ_{H_2O} in this case). Riedo and Kováts [15] introduced the useful symbol $\Gamma_{H_2O,NAACN}$, meaning surface excess of water using the convention "acetonitrile is not adsorbed". If there is no preferential adsorption of water, the V_m value found in this way would coincide with the total volume of liquid in the column, accessible, etc., by filling the dry column with a one-component solvent and weighing. Naturally, one then finds that the surface excess of water is zero.

The surface excess of phenol is calculated in a similar way to that of water. However, for low concentrations it can be found experimentally in a straightforward manner by measuring the retention volume, finding the capacity factor, using the V_m value fixed, as discussed above, by the convention adopted, and finally finding Γ_i with the equation

$$k'_i = \Gamma_i(V_s/V_m) \quad (4)$$

where V_s is now the surface area of the adsorbent in the column; we retain the symbol V_s in order to retain the same form of the equations, irrespective of whether the amount of stationary phase is expressed in grams, square metres or even volume.

Other conventions are possible, each having their particular advantages and disadvantages. Thus, one could have "water not adsorbed" or "nothing is adsorbed" (that is, the total amount of material, adding up all components in either moles, mass or volume units, is accounted for by the mobile phase). For an inventory of the possible conventions, with their various complications, with the use of various composition units (moles, mass, volume), the reader is referred to ref. 14.

The approach is not without conceptual difficulties. Depending on the choice of the convention, the dividing plane may turn out to be outside the liquid, within the adsorbent matrix material, leaving us with a "negative" adsorption layer. Also, surface excesses may be negative, with the result that corresponding components are accelerated rather than retarded; *i.e.*, retentention volume smaller than V_m.

The convention "nothing is adsorbed" is tantamount to stating that all of the liquid volume belongs to the mobile phase; the stationary layer has no volume. This is the approach advocated by Knox and Kaliszan [24]. It leads to negative capacity factors when, as usual, there is preferential adsorption. Its sole advantage is that the definition is universal and one does not have to single out one component as being treated differently, *i.e.*, as the convention component.

An important drawback of the occurrence of negative k' values inherent in the "nothing is adsorbed" convention is that taking the logarithm of k' is not possible. Hence probably inaccurate but fairly useful retention correlations, *e.g.*, with the logarithm of the capacity factor found to be influenced in an additive way by substituents in the solute or the logarithm of capacity factor as a function of the volume percentage organic component in the mobile phase, become useless.

It might therefore be asked what has been gained by the introduction of surface excess. Again, with each set of data on retention it has to be stated what convention has been used; this is equivalent to stating the compound used as "unretained" in an experiment devised in a more elementary manner.

More importantly, from the point of view of interpretation, nothing has been improved. The rigour obtained is rather at the expense of the possibilities for molecular interpretation; useful concepts such as competition and displacement are very difficult to fit into this approach. This issue has been addressed recently by Foti *et al.* [25].

Notwithstanding, the surface excess approach has two important advantages. First, the connection between chromatographic knowledge and more general adsorption studies can be restored. Second, this treatment makes it clear from the outset that the uncertainty about the volumes of the phases (more correctly, amounts of phases) is not due to poor experimentation or a poor choice of marker compounds; it is intrinsic in macroscopic studies of adsorption, *i.e.*, studies in the absence of analytical methods on the molecular scale. Some sort of arbitrariness in the choice of the definition of a phase will always persist.

4. ISOTHERM MODELS

4.1. Basic equations

Most models used in chromatography are of the Langmuir type or derived from it. In other areas of science, *e.g.*, catalysis, soil adsorption and industrial separations, a wider variety of models are found to be useful. This is not surprising: adsorbents are useful in analytical chromatography only provided that the adsorption is linear at least in the lower part of the concentration range. In the development of packings and phase systems this is an important objective. For example, the Freundlich-type isotherm

$$c_{i,s} = A(c_{i,m})^n \tag{5}$$

where $n < 1$ does not have any linear portion at all, and adsorbents to which this expression applies will produce very poor analytical chromatograms in terms of resolution.

The most elementary form of the Langmuir adsorption isotherm is usually written for one component as

$$c_{i,s} = \frac{K_i c_{i,m}}{1 + b_i c_{i,m}} \tag{6}$$

where K_i (a_i is also often used) describes distribution of i at infinite dilution, while the saturation capacity of the sorbent for i is reflected in the value of b_i. Somewhat awkwardly, a high capacity corresponds to a small b_i; as can be seen from eqn. 6, by letting $c_{i,m}$ go to infinity, the saturation value of $c_{i,s}$, S_i, equals K_i/b_i. Another disadvantage of this traditional notation is that when the mobile phase conditions change, both K_i and b_i change, whereas their ratio S_i is often observed to be constant (as expected from the molecular picture). The author finds it more convenient to write eqn. 6 as

$$c_{i,s} = \frac{K_i c_{i,m}}{1 + K_i(c_{i,m}/S_i)} \tag{7}$$

In some treatments of column overload (*e.g.*, [30–32]), useful for moderate overload, where the k' values differ only slightly from the infinite dilution value, it is desired to express $c_{i,s}$ as a power series in $c_{i,m}$:

$$c_{i,s} = \alpha_i c_{i,m} + \beta_i c_{i,m}^2 \tag{8}$$

where a constant term is missing (zero $c_{i,m}$ means zero $c_{i,s}$) and the series is usually truncated after the second term. It holds that [3,30]

$$\alpha_i = K_i \quad \text{and} \quad \beta_i = -K_i b_i \tag{9}$$

and the value of the (differential) capacity factor is then given by

$$k_i' = K_i - 2K_i b_i = K_i - 2K_i^2/S_i \tag{10}$$

Inserting this in eqn. 3 leads to a triangular elution function as used in ref. 3. The steepness of the tail of the peak is smaller at large S_i, which is what one would expect with a large capacity.

Finally, we note that some workers use equations with the fraction of unoccupied surface, θ_f:

$$\theta_f = \frac{1}{1 + c_{i,m} b_i} = \frac{1}{1 + K_{i,m}(c_{i,m}/S_i)} \tag{11}$$

leading to

$$c_{i,s} = \theta_f K_i c_{i,m} \tag{12}$$

For multi-component systems these equations are easily generalized. The remaining "free surface fraction" θ_f now becomes

$$\theta_f = \frac{1}{1 + c_{1,m} b_1 + c_{2,m} b_2 + c_{3m} b_3 + \cdots} \tag{13}$$

and the individual expressions for the stationary phase concentrations become

$$c_{i,s} = \frac{K_i c_{i,m}}{1 + \sum_j b_j c_{j,m}} = \theta_f K_i c_{i,m} \tag{14}$$

where the second part is useful provided that it is realized that θ_f depends on all concentrations. One can also use the S_i values:

$$c_{i,s} = \frac{K_i c_{i,m}}{1 + \sum_j K_j(c_{j,m}/S_j)} \tag{15}$$

4.2. Differing saturation capacities

A number of papers (*e.g.*, refs. 33–36) have appeared on the effect of differing S_i values for the components in a mixture, both on the expected chromatogram when eqn. 7 holds and on

the effect on the validity of that equation. With respect to the first point it should be noted, as has been done earlier [37], that these do not offer new viewpoints regarding the prediction of elution curves: when eqn. 15 holds, identical profiles can be obtained by adjusting the amounts injected in simulations (taking $b_i c_i$ as a dimensionless variable). This follows from a transformation that leads to yet another representation of the Langmuir composite isotherm:

$$C_{i,m} \rightarrow c_{i,m}/S_i \quad \text{and} \quad C_{i,s} \rightarrow c_{i,s}/S_i = \theta_i$$

with the result

$$c_{i,s} = \frac{K_i C_{i,m}}{1 + \sum_j K_j C_{j,m}} = \theta_i \tag{16}$$

for which it can be noted that the $C_{i,s}$ values are the same as the fraction of surface occupied with i, θ_i, with $\theta_f + \Sigma\, \theta_i = 1$.

Using eqn. 16 rather than eqn. 15 is tantamount to expressing all amounts and concentrations in amounts corresponding to the saturation value for the surface for that component. All S values are unity. This allows one to account for variations in surface necessities by injecting different amounts in the simulation. We believe this remark is important, as it partly reduces the enormous number of different cases and conditions that have to be considered in a treatment of non-linear chromatography.

With respect to the physico-chemical interpretation, differing S_i values lead to serious difficulties, and it is doubtful whether under these conditions equations such as eqn. 15 can be of any theoretical and practical utility. A first problem is with the thermodynamic consistency. It has been derived [38,39] that the isotherm of eqn. 15 with differing S_i violates the Gibbs–Duhem relationship and therefore is thermodynamically inconsistent. From a practical viewpoint one could decide to ignore this and still use such an equation as a convenient "canning" of experimental results.

However, a second problem with eqn. 15, of immediate practical relevance, is that it contradicts a broad range of chromatographic experience [34–36]. It predicts that the selectivity factor $\alpha_{j,i}$,

$$\alpha_{j,i} = \frac{c_{j,s}}{c_{j,m}} \Big/ \frac{c_{i,s}}{c_{i,m}} \tag{17}$$

would not change with variation in concentration, as can be found by substituting eqn. 15 for i and j, respectively (the denominator of eqn. 15 cancels):

$$\alpha_{j,i} = \frac{K_j c_{j,m}}{c_{j,m}} \Big/ \frac{K_i c_{i,m}}{c_{i,m}} = \frac{K_j}{K_i} \tag{18}$$

Experiments do show a change in $\alpha_{j,i}$ with concentration, and even selectivity reversals have been seen. This observation has been treated [34–36] while still retaining eqn. 15 or similar as valid, as a direct consequence of the differing S_i values, on the basis of the equilibrium exchange reaction of i and j. This does not seem to be correct to the present author; it appears that as soon as selectivity is observed to change, eqn. 15 is no longer appropriate.

Another contradiction of eqn. 15 with differing S_i values is encountered when considering retention changes in analytical chromatography resulting from the addition of a modifier to the mobile phase. As noted above, this is equivalent to the study of competitive adsorption with one component at low concentration. Eqn. 15 predicts that the retention of i (solute) is inversely proportional to the first power of the moderator (j) concentration c_j, as soon as the surface is saturated with j (θ_f close to zero). However, probably more often than not a higher power of c_j is found in experiments. This has been explained [40] by considering the exchange reaction on the overcrowded surface:

$$I_m + nJ_s \rightleftharpoons I_s + nJ_m \tag{19}$$

where n is the exchange ratio, the number of moderator molecules displaced when one solute molecule is adsorbed. In the Langmuir model underlying these considerations, the exchange ratio is equal to

$$n = S_i/S_j \tag{20}$$

As the moderator molecule, i, is usually smaller than the solute molecule, j, and therefore occupies less surface on the adsorbent, one expects

S_i to be larger than S_j, on a molar basis (see also discussion of "footprints" of molecules in ref. 35 and their bearing on molar *versus* mass expressions), and $n > 1$. Working out the equilibrium "constant" for eqn. 19 one then expects that the retention of i is inversely proportional to the moderator concentration c_j to the nth power. Note in passing that this is in contradiction to eqn. 15. Recently, Velayudhan and Horváth [71] proposed a treatment based on an analogy to ion exchange, which appears to reconcile the conflict between eqns. 15 and 19.

In normal-phase liquid–solid chromatography such a dependence is usual. Also, in agreement with this theory, n is often constant. In RP chromatography, the situation is more complicated. One normally finds a linear plot of log k' *versus* φ, the percentage of organic modifier in the aqueous mixture. This means that an n-value observed in this way is not a constant, but increases with increasing concentration of the moderator.

Apparently, in RP systems, competition is always associated with activity changes in the mobile phase; in many discussions on RP retention the mobile phase activity effects are even held responsible for most of the changes in retention. In any event, in the dependence of retention on mobile phase composition in RP chromatography, other effects than those described by eqn. 15 are apparently of great importance. For competitive RP sorption of solutes these other effects may play a smaller role, one reason being that they adsorb at lower mobile phase concentrations. However, it is not likely that they diminish to insignificance, *e.g.*, when one changes the system from methanol–nitrophenol in water to phenol–nitrophenol in water. It is therefore unlikely that eqn. 15 would be very successful in RP chromatography.

4.3. Other expressions

Apart from the difficulties with differing S_i values, other possible explanations for deviating behaviour have led to alternative expressions, an extensively discussion is given in ref. 35. One possibility is to assume a heterogeneous surface. The resulting expression with, *e.g.*, two types of "sites", is the addition of two terms in eqn. 7. The difficulty with such complicated expressions is that it is hardly possible to ascertain that the parameters obtained after fitting the experimental data have some real physical significance, rather than being adjustments to the data at hand.

As mentioned, in the comparison of experimental data with Langmuir expressions, it has been found [18,41–43] that the representation is often adequate for single-component isotherms, whereas the representation of binary isotherms is usually poor [44,45]. An exception occurs with enantiomeric pairs, the composite adsorption of which is well described [46] by equations such as eqn. 7. This was rationalized [46] as a result of the similarities of the molecules and their identical S_i values. An alternative explanation can be given: the absolute values of S_i are small and at reasonable (experimentally manageable) k' values the mobile phase concentrations are also small under these conditions. In other words, in such systems, with small capacities, the affinity for the surface is large, and the system is similar to normal-phase adsorption in the sense that low mobile phase concentrations already lead to significant saturation of the sorbent. Under such conditions mobile phase non-idealities can be expected to be small compared with the surface saturation effects.

Poor fits of single-component data also occur. A recent study [47] of the non-aqueous RP distribution of cholesterol and related compounds provides an example. Attempts to improve the fit by modification with equations such as eqns. 14–16 by adding terms to the numerator and/or denominator were discussed. Also the Fowler equation, which takes interactions within the stationary layer into account (and cannot be formulated as an explicit expression for $c_{i,s}$), was tried. As expected, the fit improved when a larger number of adjustable parameters was available in the more complicated models. The physical reality of the underlying molecular pictures remains an open question; as stated [47], "a good fit alone is never a proof of the theoretical value of the model".

Surprisingly, it was found that an equation consisting of the sum of a basic Langmuir expression and a partition-type term:

$$c_{i,s} = \frac{K_i c_{i,m}}{1 + b_i c_{i,m}} + a' c_{i,m} \quad (21)$$

yields a very good fit to the experimental data. This equation was derived by assuming a kind of multi-layer adsorption: on top of an adsorbed molecule in the first layer, a second molecule can adsorb (with different affinity). This latter process can be repeated indefinitely, a third molecule adsorbing on the second (with the same affinity), etc.

As stated, when competitive adsorption is studied, the description by equations such as eqn. 15 nearly always fails. Attempts to obtain better fits are based on the same approaches as discussed above for the single-component case. In ref. 33, a mixed term $B_{1,2}c_{1,m}c_{2,m}$ was added to both the numerator and denominator of eqn. 14 for two components. In this and the next publication [41] it was argued that on the basis of statistical thermodynamics it can be derived that the combination of terms in the numerator and denominator cannot be chosen freely. Also, some isotherm models were discarded because of a lack of thermodynamic consistency. It seems doubtful whether such considerations are useful at this stage of our understanding of adsorption equilibria. First, in such derivations and checks ideality in both phases is assumed, and this is often not guaranteed. Second, the primary use of the isotherm expressions is, after all, to predict elution patterns, rather than to arrive at statements about the exact physical and/or chemical conditions at the surface. Under such conditions any mathematical functions will serve the purpose of correlating experimental results, and the one performed best would be preferable. Only if attempts were to be made to predict isotherms from first principles would one need to check equations for thermodynamic consistency.

In another publication [48], more terms were added to the numerator and denominator, with limited success in the accurate correlation of the adsorption of 2-phenylethanol and 3-phenylethanol in an RP system. These data correlated better with a generalized Fowler equation, which gives mobile phase concentrations as a function of surface concentrations. Only five adjustable parameters were needed in this correlation. However, the Fowler equation is awkward to handle in column simulation studies [48] (unless the whole simulation scheme were to be modified such that at one iteration only $c_{i,m}$ values have to be calculated from the $c_{i,s}$ set, which seems to be possible in principle).

It appears that a fundamental interpretation of adsorption isotherm data is at present beyond our capacity to describe adsorption from the liquid phase. One complicating factor, when compared with adsorption from the gas phase, is the effect of activity coefficients in the mobile phase. The free energy of components in the liquid phase, described by these, is generally a function of the concentration of all the components. As the free energy change on transfer to the adsorbed state ultimately describes the adsorption equilibrium, taking into account the changes in free energy in the liquid phase is a prerequisite for any fundamental interpretation of adsorption isotherm data. The activity effects in the liquid phases are in principle easily accessible, *e.g.*, by measuring the vapour pressure of the components. A similar approach has been successful in studying the adsorption process in normal-phase chromatography on silica [49]. Such measurements have been carried out [50,51] for this purpose, in RP-type mobile phases, for alkylbenzenes. For more typical RP solutes, attempts have been made in the author's laboratory that yielded erratic results, possibly owing to adsorption of the medium-polarity solute on various parts of the equipment.

4.4. Synergistic adsorption

All equations mentioned so far for (composite) isotherms apply to competitive adsorption: the adsorption of a given compound is decreased when increasing concentrations of all adsorbing compounds (including the one considered) are present. The opposite to this behaviour can be indicated as synergistic adsorption. It occurs less frequently in chromatography; however, a striking example is RP ion-pair adsorption [52]. Usually one considers the ion-pair-forming reagent as part of the mobility phase; however, as indicated above, in principle in the description of the phase system the role of solutes and mobile

phase components can be interchanged. Hence the observed increase in retention of charged solutes when an ion-pair-forming reagent (a salt with a relatively lipophilic ion with a charge opposite to that of the solute ion), is an extreme example of synergistic adsorption. Interesting overload experiments could by carried out if the reagent were to be co-injected with the (regular) solute ion, but such experiments do not seem to have been carried out.

Another example of synergistic adsorption is in the enhancement of retention and changes in selectivity observed by Daucik *et al.* [53] in experiments where a low concentration of a strongly adsorbing "surface modifier" such as hexanenitrile was found to increase the retention of a number of phenols, whereas the retention decreased on addition of small percentages of hexylamine.

5. ADSORPTION OF MOBILE PHASE COMPONENTS

5.1. Experimental techniques

As stated before, many workers have studied the ("selective" or "preferential", a not very meaningful term in view of the discussion in Section 3) adsorption of components from the typical mobile phases used in regular RP chromatography, *e.g.*, methanol–water, acetonitrile–water and tetrahydrofuran–water [15,16,24–28]. Some special techniques are used in the study of the preferential adsorption of solvent components, and these are now briefly discussed.

The systems can in principle be studied by injecting mobile phase mixtures, but with differing compositions. When the resulting disturbances are small ("minor disturbance", either small deviations in composition or small injection volumes), the retention of such disturbances is determined by the slope of the isotherm at the operating point [54,24,26,27]. Determination of the actual isotherm therefore requires an integration. With more than two components one obtains a number of peaks, each corresponding to an eigenvalue of the matrix of derivatives [15,24,55]. This leads to such complications that determination of isotherms with this technique becomes virtually impossible.

When using isotopically labelled solvent components it is possible to have more direct access to isotherm data. The retention of such labelled compounds is determined by the chord in the isotherm. In other words, the "column capacity" for a component, i, *i.e.*, the total amount of i present in it at a given mobile phase composition, can be found by injecting a sample containing a variety of i labelled with an isotope, i^*, and observing the elution volume or elution time of i^*. The product of concentration $c_{i,m}$ and the elution volume is then equal to the "capacity". This relationship, which can be derived from the mass balance in a very straightforward manner, is very general. It could only fail when part of i in the column is "inert", *i.e.*, it does not exchange with i^*. This may occur with material that is trapped in inaccessible pores of the packing matrix, but this is an unlikely assumption. Moreover, if this were to occur the corresponding amount of i would be of no interest in chromatography.

The labels on i to form i^* should not affect the distribution behaviour of i, just make i^* visible. Most often used is a deuterium-substituted version of i, such as C^2H_3CN in the case of acetonitrile, C^2H_3OH or CH_3O^2H in the case of methanol and ^2H_2O in the case of water, as such compounds are not too difficult to obtain and present no radiation hazards. However, the use of deuterated molecules is not without problems.

First, the deuterium substitution may have an "isotope effect" on the distribution constants, as witnessed by the many examples in the literature where components with various degrees of deuterium substitution have been separated by both GC and LC. Hence it is often necessary to study this isotope effect beforehand or, alternatively, to do the experiment in such a way (total substitution of i by i^* in a frontal mode) that the error does not occur. Second, when the deuterium is in OH groups, as in CH_3O^2H and ^2H_2O, the tags are labile and they exchange with other solvent components (hence using these components in a water–methanol experiment makes procedures very complicated) and also with silanol groups on the packing [27,56]. Hence one measures the "total mobile proton capacity" rather than the capacity for the com-

pound *i*. Proper evaluation of the measurements should take these two effects into account [29].

The deuterium exchange can also be taken advantage of, as it allows one to determine the silanol content of the column [56]. Such measurements have allowed the unequivocal determination of the degree of substitution of surface OH groups in modified silicas [57].

5.2. Results

The general picture that develops from the various experimental studies of distribution of solvent components is as follows:

(a) At low water concentrations, water is adsorbed to a slight extent. This is less pronounced to nearly invisible when methanol is the other solvent component.

(b) At higher water concentrations, starting at *ca.* 10–30% (v/v), the organic component is adsorbed. With the "nothing is adsorbed" convention, the surface excess concentrations are of the order of 0.5 μl/m^2 for acetonitrile adsorbed. However, in the case of methanol–water mixtures the adsorption is much smaller.

(c) It follows from the combination of (a) and (b) that there is an azeotropic point, where the total composition is equal to that of the mobile phase.

(d) When using other combinations of solvents it is generally found that the more lipophilic component is preferentially adsorbed from not too extreme compositions. However, some observations inconsistent with this have been reported [24].

(e) It appears that the more complete the coverage of the silica surface with alkyl chains, the less is the adsorption of water at the low water concentration end of the plot. This is consistent with the decreased accessibility of the residual OH groups, as inferred from the smaller retention of solutes such as ethers and amines observed on densely covered materials.

(f) An interesting fact that has been reported [29] is that adsorption from acetonitrile–water mixtures is virtually the same on two preparations of RP material that are claimed to have one of the highest coverages possible, one having an unbranched C_{14} and the other a branched C_6 (3,3-dimethylbutyl) moiety. This suggests that there is hardly any selective penetration of the less polar compound acetonitrile into the more voluminous C_{14} layer. This will be discussed in Section 6.3.

Adsorption of such solvent components has been modelled with a Langmuir isotherm expression [58,59]. This was done in order to allow calculation of the gradient deformation due to adsorption of the stronger component in gradient elution. Contrary to the case in normal-phase adsorption on silica, etc., it was not been done with the purpose of explaining the retention behaviour of solutes in dependence of the solvent composition.

Another area to be discussed here is ion-pair chromatography. The ion-pair reagents, usually ionic surfactants, *e.g.*, butylammonium, tetrabutylammonium, hexadecyltrimethylammonium, dodecyl sulphate or dodecyl sulphonate salts, are adsorbed on the RP packing, often also prior to the injection of solute ions of opposite charge, the more so the more lipophilic the reagent, including the counter ion, is. With very long chains the adsorption of the reagent is virtually irreversible; solute retention involves exchange with the counter ion of the reagent. This system has been termed "dynamic ion exchange" and indeed it behaves as an ion exchanger.

In this context determination of isotherms has been carried out by many workers, from the very beginning of the use of the technique in HPLC [60–63]. The data obtained have been fitted to Langmuir and Freundlich-type isotherms. However, it is now generally accepted that a Langmuir isotherm corrected for electrostatic effects [61–63] is the most appropriate.

6. ADSORPTION OF SOLUTES

6.1. General

These studies are obviously nearly always performed in the course of work on preparative uses of chromatography; analytically it may have significance to study isotherms only in rare cases, *e.g.*, in precolumn concentration procedures where matrix components may overload the

adsorbent. However, in these cases for good reasons a pragmatic experimental approach is generally preferred. Another analytical area where loadability and isotherms may be important is that of miniaturized chromatography, where concentration levels are often high because of detection limitations.

6.2. Langmuir behaviour and saturation values

A study of an RP system [17], with a typical solute such as phenol, revealed that the isotherms are linear over a fairly wide range in such systems, and this has been corroborated since then by many workers (*e.g.*, [18,41–43,48,59,64,65]). These later activities also made it clear that for most solutes of small molecular size (an exception [47] for non-aqueous RP chromatography has already been mentioned in section 4.3), the Langmuir expression gives a fairly good fit to the observed data. It follows that saturation surface concentrations can be derived and compared. Table 1 gives an overview of some values that could be extracted from the literature (they are rough, as often $c_{i,s}$ values are given per gram or millilitre of packing and calculation of the associated surface area is problematic).

It is generally believed that the surface concentrations of OH groups on the SiO_2 matrix, when properly hydrated, is about 8 μmol/m^2, and that about half of these groups can be derivatized in a silylation reaction as used in the preparation of RP adsorbents, 4 μmol/m^2 [57]. For a monofunctional reagent this should then be the surface concentration of the (densely packed) alkyl chains. The values in Table 1 are of this order of magnitude, suggesting that the maximum adsorbed surface concentration is limited in the same steric manner as the modification of the silica. However, on the basis of these results, we cannot decide where the adsorbed solute molecules actually sit.

Further conclusions for the data are difficult to draw; there appear to be no clear trends visible between the erratic set of values. It seems that larger molecules "occupy more surface", in agreement with a molecular picture. Thus, the saturation capacity for angiotensin on a molar

TABLE 1

ROUGH ESTIMATES OF OBSERVED SATURATION CAPACITIES, S_i, IN REVERSED-PHASE SYSTEMS

Packing	Eluent	Solute	S_i (approx.) (μmol/m^2)	Ref.
YMC ODS	ACN–H_2O (10:90)	2-Phenylethanol	7	59
LiChrosorb RP-2	MeOH–H_2O (25:75)	Phenol	2	17
LiChrosorb RP-2	MeOH–H_2O (25:75)	4-*tert.*-Octylphenol	3	17
LiChrosorb RP-18	MeOH–H_2O (25:75)	Phenol	1	17
LiChrosorb RP-18	MeOH–H_2O (25:75)	4-*tert.*-Octylphenol	0.6	17
Spherisorb ODS-2	Aqueous buffer (pH 7)	L-Phenylalanine	2	65
Spherisorb ODS-2	Aqueous buffer (pH 7)	L-Phen-L-Ala	1	65
Spherisorb ODS-2	H_2O	Phenol	3	18
Spherisorb ODS-2	H_2O	Nitrobenzoic acid	0.5	18
Vydac Spher. ODS	MeOH–H_2O (50:50)	2-Phenylethanol	6	59
Vydac Spher. ODS	MeOH–H_2O (50:50)	3-Phenylpropanol	5	59
Zorbax ODS	ACN–H_2O (15:85)	Angiotensin II	0.35[a]	35
Zorbax ODS	MeOH–H_2O (20:80)	Benzyl alcohol	3.5	35
Zorbax ODS	MeOH–H_2O (40–60)	Benzyl alcohol	3.6	35
Porous carbon	ACN	Phenyldodecane	0.6[b]	69

[a] Lower affinity term in a two-site model.

[b] From Langmuir-type term in composite Langmuir–quadratic fit.

basis is about one tenth of that of benzylalcohol, under the same conditions.

6.3. Influence of chain length, adsorption vs. partition

In the data in Table 1 there is no trend visible that longer alkyl chains lead to higher capacity. This, together with similar results discussed in section 5, suggests that there is no partitioning into the brush layer of alkyls, but rather an enrichment of solutes in a narrow region; the brush layer hardly acts as a liquid phase. In line with this is the observation by Eble *et al.* [35] that the volume fraction of moderator has no influence on the saturation capacities.

Retention studies usually lead to the same conclusion. Although in one study [25] it was found that solutes (at infinite dilution) do have larger retentions at the C_{14} surface and thus apparently do penetrate this layer, other indications [66,67] are that the retention levels off when the chain length is increased beyond a limit, depending on the type of solute. Related is a study [68] where sorption into a alkane liquid was compared with that on to an ODS packing, also leading to the conclusion that a partition type of distribution is very unlikely for RP systems.

Accepting that enrichment in a confined region exists, no matter whether the enrichment occurs at the silica surface or in the region where the alkyl brush meets the solvent, one should use the term adsorption. However, it would probably be incorrect to associate adsorption too closely with competition, *e.g.*, by taking eqn. 7 to be valid, or by considering an equilibrium constant for the exchange reaction between a solute and n strong solvent molecules as governing the influence of one component on the other. Two strong experimental facts weigh against this: the linear log k' *versus* φ plots (changing n) and the occurrence of synergistic adsorption (formal n negative).

6.4. Solute size

Larger molecules adsorbing on RP packings have also been studied. Huang and Horváth [65] studied, among others, the 2′,3′-cyclic monophosphates of adenosine and guanosine, adenosine monophosphate, benzyltrimethylammonium bromide and the polypeptide α-MSH. Sigmoid isotherms were obtained that were attributed to "solvent-mediated molecular associations or conformational changes". The latter group of phenomena represent non-idealities in the mobile phase, the activity no longer being proportional to concentration. In fact, this means that the mobile phase is overloaded before or at the same time as the stationary phase. This may be a general problem in the RP chromatography of large molecules; in the terms of Horváth: the solvophobic effect that is the deriving force in these separations may for large molecules lead to non-ideality by self-aggregation and precipations in the eluent with increase in sample concentrations.

On the other hand, in view of our poor understanding of the adsorbed phase itself, it could well be that such deviations should be attributed to special effects, *e.g.*, related to competition, that occur with these large molecules. A treatment of this kind was given by Eble *et al.* [35].

7. COMPOSITE ISOTHERMS OF SOLUTES

The most striking general result of these studies is that the Langmuir expression, which was reasonably successful for the description of one-component data, often fails altogether when the composite form is applied to binary mixtures. That is, the K_i and b_i (or S_i) data for each component, that would (conveniently) allow one according to eqn. 6 to predict the isotherms over the full range of mixed composition, are often useless in this respect. Adapting these values such that they represent all of the data better of course improves the fit slightly, but in general the resulting representation of the data (and especially the single-component lines in composition space) is unacceptable poor. Such cases have been reported by several workers [33,69,70], whereas others have reported reasonable success in this respect [19].

For several reasons it is very important to find mathematical models that do fit well to such

composite isotherms. First, the availability of a (preferably explicit [48]) mathematical expression for the equilibrium is a prerequisite for carrying out simulation studies; attempts to use the smoothed isotherms data directly are hardly successful [70]. Second, the development of a model of some general validity would possibly point the way to a better fundamental understanding of these equilibria and, with that, to better predictability for not yet measured combinations. Even predictability from measured single-component data would be an important asset. Needless to say, this is a key point for arriving at reasonable development times for preparative separations.

Other mathematical isotherm shapes have indeed been tried in order to obtain a better representation, and the Fowler equation was found in one instance to give better results [48]. Nevertheless, it was concluded that the problem (predictability on the basis of single-component data), even for their particular case studied, "remains unsolved".

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