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Calculation and Experimental Verification of Solute Retention in Liquid Chromatography Using Binary Eluents

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Abstract

The chromatographic transport problem is a kinematic wave problem that generally leads to a set of coupled, nonlinear partial differential equations. In the linear elution mode of chromatography an approximation can be made which allows linearization of the set of equations and hence solution by the matrix eigenvalue decomposition method. To make the solution explicit, an expression for a multicomponent distribution isotherm is required. Generally, Langmuir isotherms are used for this purpose. In this work a recently published multicomponent isotherm, that can be derived from first principles, is applied to the problem. For the case of binary eluents the capacity factors and peak compositions of both system peaks and analyte peaks are calculated using the elution mode approximation and the isotherm referred to above. Experimental results pertain to the ion-exchange separation of alkali ions. A good agreement between calculated and measured quantities is observed. An important consequence of interactions among sample and eluent constituents for the practice of chromatography lies in the field of indirect detection. The possibility of universal detection in LC is briefly discussed.

INTRODUCTION

In elution chromatography it is usually assumed that the various components of a mixture travel independently through the column, each component at its own "private" velocity. Thus, a sample consisting of N analytes is expected to yield an N-peak chromatogram, the retention times of the N peaks being determined by the respective distribution coefficients of the analytes. The distribution coefficient of a given component is assumed to be equal to the limiting slope at the origin of a plot of the stationary-phase concentration vs the mobile-phase concentration of that component. That is to say, retention is assumed to be determined by the individual distribution isotherms of the components (*1*, *2*).

In a qualitative sense, it has long been recognized that the assumption of independent motion of the sample components cannot be correct. There will be interferences among the different sample components on the one hand and among sample components and eluent components on the other. In other words, the process of separation is not governed by the individual isotherms of the sample constituents, but by coupled isotherms describing the composite behavior of all components in the system, analyte components as well as eluent components.

When using a multicomponent isotherm, one expects the stationary-phase concentration of a given component to be a complicated function of all concentrations prevailing locally at a given time, which seems to contradict the linear behavior observed in elution chromatography. However, in the linear elution mode the initial conditions for the separation are tailored in a very specific way. The amount injected is kept so small that injection of the sample represents only a minor disturbance of the column equilibrium. Under these conditions the multicomponent adsorption function can be expanded to first order in terms of the concentrations of all the components (*3–8*). Either mobile-phase, stationary-phase, or total concentrations can be used in this expansion. In this way, linearity is preserved without discarding the mutual interaction effects, as is done in the traditional description.

Poppe (*8*) has shown that such a linearized theory can be used successfully for quantitative predictions in capillary zone electrophoresis where one has Kohlrausch's regulating function taking the place of a multicomponent adsorption isotherm. In elution chromatography, however, the situation is less favorable. Here, one either has to rely on some questionable assumption, such as that Langmuir parameters do not change on going from a single component to a multicomponent mixture [discrepancies between theory and experiment have been attributed to the shortcomings of the Langmuir assumptions recently (*9*)], or one has to resort to actually measuring composite adsorption isotherms. The number of data needed to describe such a system naturally depends on the number of compositions at which one wishes to evaluate the adsorbed amounts. This number grows strongly with the number of components in the system. Due to the very large number of data needed, there are, to the best of our knowledge, no multicomponent adsorption data available pertaining to systems that contain more than two components. However, we have shown recently that for systems that exhibit enthalpy–entropy compensation, a multicomponent isotherm can be derived from first principles (*10–12*). Structural parameters appearing in the isotherm equation do not depend on the composition of the mixture or on the temperature. Using this isotherm therefore avoids the need to collect excessively large numbers of data points.

In this work we derive expressions for the capacity factors of all peaks appearing in the chromatogram as a function of the composition of a binary eluent. The consequences of the use of the new multicomponent isotherms for the calculation of peak retentions and compositions are discussed in the Theory Section. Our major goal is to compare calculations based on the linearized model and the new multicomponent isotherms with experiment. Experimental results pertain to the ion-exchange separation of alkali cations. The first reason for selecting this particular experimental system is that it has been the subject of previous studies so that values for the parameters appearing in the equations are well known. The second reason is that on using conductivity detection in (single-column) ion chromatography, all peaks, including the so-called system peaks that cannot be attributed to a particular solute, can be readily detected. The retention of system peaks can hence also be compared with model predictions.

THEORY

Formulation of the Transport Problem

Consider an isothermal column of length Z and constant void fraction ϵ. The mobile phase contains m different components, counting both sample and eluent constituents. The system is considered to be one-dimensional and uniform in the direction of flow and has constant interstitial velocity U. If c_i and n_i denote the molar concentrations of solute i in the mobile and the stationary phases, respectively, then by neglecting axial diffusion, the conservation of mass implies that

$$\epsilon U\frac{\partial c_i}{\partial z} + \epsilon\frac{\partial c_i}{\partial t} + (1 - \epsilon)\frac{\partial n_i}{\partial t} = 0 \tag{1}$$

where z measures the distance from the column inlet and t is the time, with $t = 0$ corresponding to the beginning of the injection.

By adding the Eqs. (1) corresponding to all components $i = 1, 2, ..., m$, it follows that

$$\epsilon U\frac{\partial C}{\partial z} + \epsilon\frac{\partial C}{\partial t} + (1 - \epsilon)\frac{\partial N}{\partial t} = 0 \tag{2}$$

where C is the total concentration in the mobile phase and N that in the stationary phase. We shall consider ion-exchange chromatography involving homovalent ions only. This implies that N is constant and hence by (2) that C is constant along the lines $dz/dt = U$. As clarified by Fig. 1, this

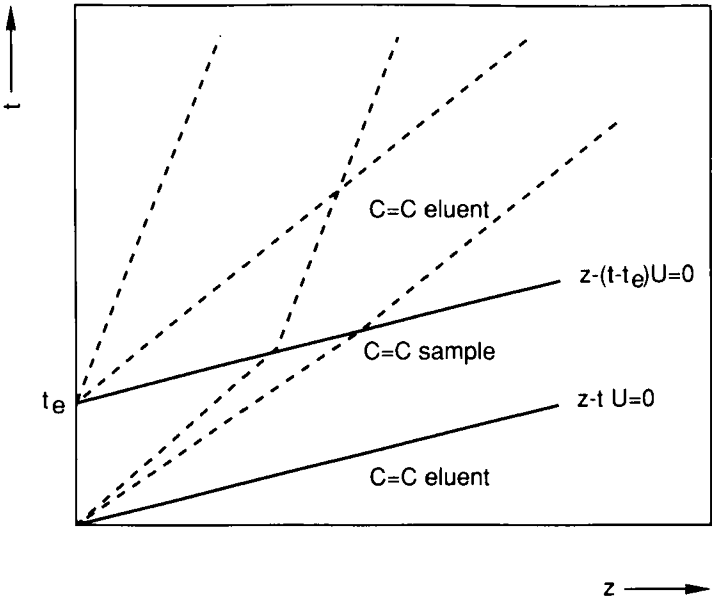

FIG. 1. Schematic portrait of the solution of Eq. (2) in the (z,t) plane showing zones of different total concentration.

leads to three regions in the (z,t) plane with different values for C. Solid lines in the figure refer to the transport of the total concentration changes, which have velocity U. Dashed lines refer to changes in composition. Hence, the reciprocals of their slopes are to be interpreted as chromatographic concentration velocities. Since in elution chromatography the injection time, t_e, is always very small, the composition peaks in the region $t > t_e + (z/U)$ are only of interest, and we shall therefore assume that C is equal to the eluent concentration everywhere:

$$C = C^E = \text{total concentration in the eluent} \qquad (3)$$

The conservation laws (1) can be made dimensionless in the following way. We define $x_i \equiv c_i/C^E$, $y_i \equiv n_i/N$, $\tau = (Ut - z)/Z$, and $\zeta = z/Z$. Equation (1) can then be written as

$$\frac{\partial y_i}{\partial \tau} + \frac{\epsilon}{1 - \epsilon} \frac{C}{N} \frac{\partial x_i}{\partial \zeta} = 0, \qquad i = 1, \ldots, m \qquad (4)$$

In the case of a constant mobile-phase concentration, the multicomponent isotherms that we study in this paper reduce to

$$x_i = \frac{y_i}{\sum_{j=1}^{m} \alpha_{ij}(y_1, \ldots, y_m)y_j}, \qquad i = 1, \ldots, m \tag{5}$$

where the separation factors $\alpha_{ij}(y_1, \ldots, y_m)$ are given by

$$\alpha_{ij}(y_1, \ldots, y_m) = \exp\left\{\frac{-(1 - T/T_c)}{RT} \sum_{k=1}^{m} (F_{ik} - F_{jk})y_k\right\} \tag{6}$$

where T is the absolute temperature and R is the gas constant, T_c is the compensation temperature of the class of components under consideration, and the F_{ij} are structural parameters describing the interaction between components i and j. The F_{ij} do not depend on the composition of the mixture nor on the temperature.

It has recently been shown in Refs. *10–12* that for systems exhibiting enthalpy–entropy compensation, (5) and (6) can be derived from first principles. Actually, Eqs. (5) and (6) are very similar to the regular-solution approach (*13*) [or, for adsorption, the Fowler–Guggenheim approach (*14*)] to multicomponent mixtures, except that in our case the interaction energies are temperature-dependent. This is a result of the fact that we have not used the random-mixing approximation in our derivation of the isotherm. It is also seen from Eq. (6) that selectivity is totally determined by the interactions among sorbed species.

We also remark that when the separation factors are chosen to be constants, an assumption usually made in the literature, the isotherms (5) reduce to the Langmuir type.

By substitution of (5) and (6) into (4), one obtains a coupled system of nonlinear equations involving only the y_i. Using the relation $\Sigma_{i=1}^{m} y_i = 1$, one of the y_i can be eliminated. By elimination of y_m using the relations $\alpha_{ij}\alpha_{jk} = 1$ and $\alpha_{ji} = 1/\alpha_{ij}$, we finally obtain

$$\frac{\partial y_i}{\partial \tau} + \frac{\partial}{\partial \zeta} f_i(y_1, \ldots, y_{m-1}) = 0, \qquad i = 1, \ldots, m - 1 \tag{7}$$

where

$$f_i(y_1, \ldots, y_{m-1}) = \frac{\epsilon}{(1 - \epsilon)} \frac{C}{N} \frac{\alpha_{mi}(\vec{y})y_i}{1 + \sum_{j=1}^{m-1} (\alpha_{mj}(\vec{y}) - 1)y_j} \tag{8}$$

with $\vec{y} \equiv (y_1, \ldots, y_{m-1})$ and $\alpha_{mj}(\vec{y})$ is given by (6) with $1 - \Sigma_{j=1}^{m-1} y_j$ substituted for y_m. Using the chain rule for differentiation, (7) can be written as

$$\frac{\partial \vec{y}}{\partial \tau} + \mathbf{A}(\vec{y})\frac{\partial \vec{y}}{\partial \zeta} = 0 \tag{9}$$

where $\mathbf{A}(\vec{y}) = (\partial f_i/\partial y_j\,(\vec{y}))$ is the Jacobian matrix of which the formulas are given in Appendix II.

In linear elution chromatography the injected amount is very small compared with the eluent concentrations $\vec{y}^E$, and therefore the equations are linearized around $\vec{y}^E$:

$$\frac{\partial \vec{y}}{\partial \tau} + \mathbf{A}^E\frac{\partial \vec{y}}{\partial \zeta} = 0 \tag{10}$$

where $\mathbf{A}^E = \mathbf{A}(\vec{y}^E)$.

Calculation of Peak Retention and Composition

The coupled linear system of Eq. (10) can be solved using the eigenvalue decomposition of the matrix $\mathbf{A}^E$. This method is well known (*3–8*, *15*) and we shall therefore give only a brief outline. We first assume that the matrix $\mathbf{A}^E$ has $(m = 1)$ real, distinct eigenvalues:

$$\lambda^1 < \lambda^2 < \cdots < \lambda^{m-1} \tag{11}$$

with eigenvectors $\vec{r}^k$, $k = 1, \ldots, m - 1$. Let Λ be the diagonal matrix of eigenvalues. Then

$$\mathbf{A}^E = \mathbf{B}\Lambda\mathbf{B}^{-1} \tag{12}$$

where $\mathbf{B}$ is the matrix of which the jth column is identical to the eigenvector $\vec{r}^j$. By substituting (12) into (10), it follows after multiplication by $\mathbf{B}^{-1}$ that

$$\frac{\partial \vec{w}}{\partial \tau} + \Lambda\frac{\partial \vec{w}}{\partial \zeta} = 0 \tag{13}$$

where

$$\vec{w} = \mathbf{B}^{-1}\vec{y} \tag{14}$$

Equation (13) implies that the ith component of the vector $\vec{w}$ is constant along the ith characteristic, $\zeta - \lambda_i\tau =$ constant, in the (ζ,τ) plane. Hence, the solution $\vec{w}$ can be readily constructed from the conditions at the inlet $\zeta = 0$.

For a system having two degrees of freedom ($m = 3$), the two characteristic directions corresponding to the two eigenvalues and local w_i values are shown in Fig. 2. The injection time, $\tau_e \equiv (Ut_e - z)/Z$, is exaggerated for reasons of clarity. In the limit $\tau_e \rightarrow 0$, two peaks occur by the overlapping of parallel characteristics at $\tau = 0$ and $\tau = \tau_e$.

For a system of m components, counting both sample and analyte constituents, there are $m - 1$ peaks. By analogy with the case $m = 3$, it follows that if $\vec{y}^F = (y_1^F, \ldots, y_{m-1}^F)$ and $\vec{y}^E = (y_1^E, \ldots, y_{m-1}^E)$ are the mole fractions of the first $m - 1$ components in the feed and the eluent, respectively, and if $\vec{w}^F = (w_1^F, \ldots, w_{m-1}^F)$ and $\vec{w}^E = (w_1^E, \ldots, w_{m-1}^E)$ are the corresponding $\vec{w}$ vectors, that the $\vec{w}$ vector in the jth peak is given by

$$\vec{w}^{P_j} = (w_1^E, \ldots, w_{j-1}^E, w_j^F, w_{j+1}^E, \ldots, w_{m-1}^E) \tag{15}$$

The corresponding $\vec{y}$ vector in the jth peak is given by

$$\vec{y}^{P_j} = \mathbf{B}\vec{w}^{P_j} \tag{16}$$

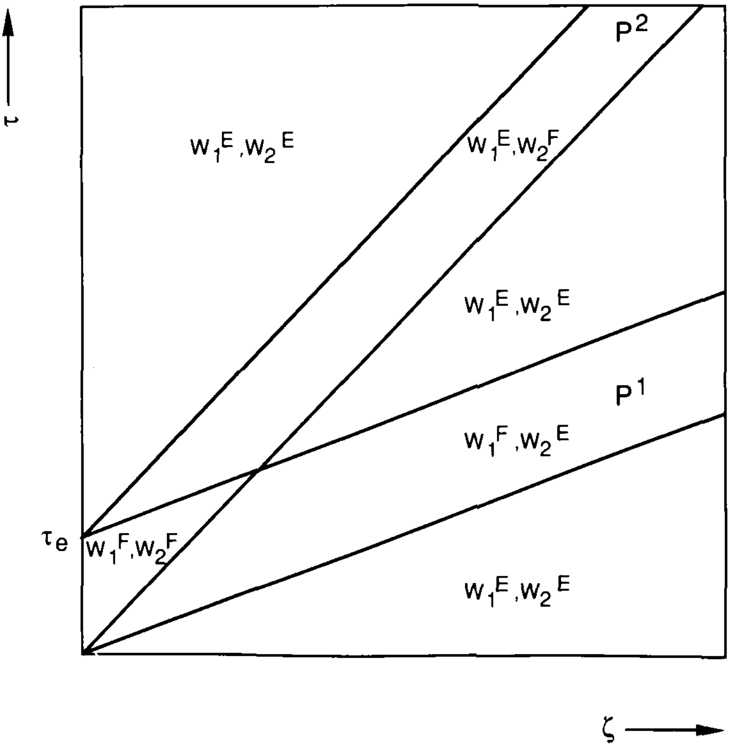

FIG. 2. Schematic portrait of the solution in the (ζ,τ) plane of the elution development of a single analyte in a binary eluent.

We now briefly consider the case that two or more eigenvalues of $\mathbf{A}^E$ coincide. This situation has occurred in one of the experiments discussed below. If the $(m - 1) \times (m - 1)$ matrix $\mathbf{A}^E$ has some coinciding eigenvalues, but still has $m - 1$ linearly independent eigenvectors, then the diagonalization (12) is still valid, with Λ now a diagonal matrix having a number of identical diagonal elements. However, when $\mathbf{A}^E$ does not have $m - 1$ linearly independent eigenvectors, the diagonalization of Eq. (13) does not exist, but instead there exists a so-called Jordan decomposition (*16*). Suppose that $\lambda^1 < \cdots < \lambda^r$ are the distinct eigenvalues of $\mathbf{A}^E$ and let s_k be the multiplicity of λ^k. Then there exists an invertible matrix $\mathbf{B}$ and a matrix $\mathbf{J}$ such that

$$\mathbf{A}^E = \mathbf{BJB}^{-1} \tag{17}$$

The matrix $\mathbf{J}$ is given by

$$\mathbf{J} = \begin{pmatrix} \mathbf{J}_1 & & & \mathbf{0} \\ & \mathbf{J}_2 & & \\ & & \ddots & \\ \mathbf{0} & & & \mathbf{J}_r \end{pmatrix} \tag{18}$$

where $\mathbf{J}_k$ is a $s_k \times s_k$ matrix of the form

$$\mathbf{J}_k = \begin{pmatrix} \boxed{\begin{matrix} \lambda^k & 1 & 0 & & \mathbf{0} \\ 0 & \lambda^k & 1 & 0 & \\ & \ddots & \ddots & \ddots & \\ \mathbf{0} & & 0 & \lambda^k & 1 \\ & & & 0 & \lambda^k \end{matrix}} & & \\ & \ddots & \\ & & \boxed{\begin{matrix} \lambda^k & 1 & 0 & & \mathbf{0} \\ 0 & \lambda^k & 1 & 0 & \\ & \ddots & \ddots & \ddots & \\ \mathbf{0} & & 0 & \lambda^k & 1 \\ & & & 0 & \lambda^k \end{matrix}} \end{pmatrix} \tag{19}$$

The number of submatrices of $\mathbf{J}_k$ as indicated in Eq. (19) is equal to the number of linearly independent eigenvectors corresponding to λ^k minus 1. For simplicity, we shall henceforth assume that every λ^k has only one eigenvector. The general case can be treated analogously.

As before, we define $\vec{w}$ by (13) and, analogously to (12), we now obtain

$$\frac{\partial \vec{w}}{\partial \tau} + \mathbf{J}\frac{\partial \vec{w}}{\partial \zeta} = 0 \tag{20}$$

Using the assumption that λ^1 has only one eigenvector, the first s_1 equations of (20) are

$$\frac{\partial w_i}{\partial \tau} + \lambda^1\frac{\partial w_i}{\partial \zeta} + \frac{\partial w_{i+1}}{\partial \zeta} = 0, \qquad 1 \le i \le s_1 - 1$$

$$\frac{\partial w_{s_1}}{\partial \tau} + \lambda^1\frac{\partial w_{s_1}}{\partial \zeta} = 0 \tag{21}$$

Using the conditions at the inlet $\zeta = 0$, it follows that for $1 \le i \le s_1$ we have

$$\begin{aligned} w_i &= w_i^E && \text{for } \lambda^1\tau < \zeta \text{ and for } \lambda^1\tau > \lambda^1\tau_e + \zeta \\ &= w_i^F && \text{for } \zeta < \lambda^1\tau < \lambda^1\tau_e + \zeta \end{aligned} \tag{22}$$

Analogously, one finds for $k = 2, \ldots, r$, that for all indices i with $\Sigma_{j=1}^{k-1} s_j + 1 \le i \le \Sigma_{j=1}^{k} s_j$ there holds

$$\begin{aligned} w_i &= w_i^E && \text{for } \lambda^k\tau < \zeta \text{ and for } \lambda^k\tau > \lambda^k\tau_e + \zeta \\ &= w_i^F && \text{for } \zeta < \lambda^k\tau < \lambda^k\tau_e + \zeta \end{aligned} \tag{23}$$

In the limit $\tau_e \rightarrow 0$, a number of r peaks P^k with $k = 1, \ldots, r$ arise. The $\vec{w}$ corresponding to the kth peak is given by

$$\begin{aligned} w_i^{P_k} &= w_i^F && \text{for } \sum_{j=1}^{k-1} s_j + 1 \le i \le \sum_{j=1}^{k} s_j \\ &= w_i^E && \text{for all other } i \end{aligned} \tag{24}$$

The corresponding $\vec{y}$ vectors are obtained from $\vec{y}^{P_k} = \mathbf{B}\vec{w}^{P_k}$.

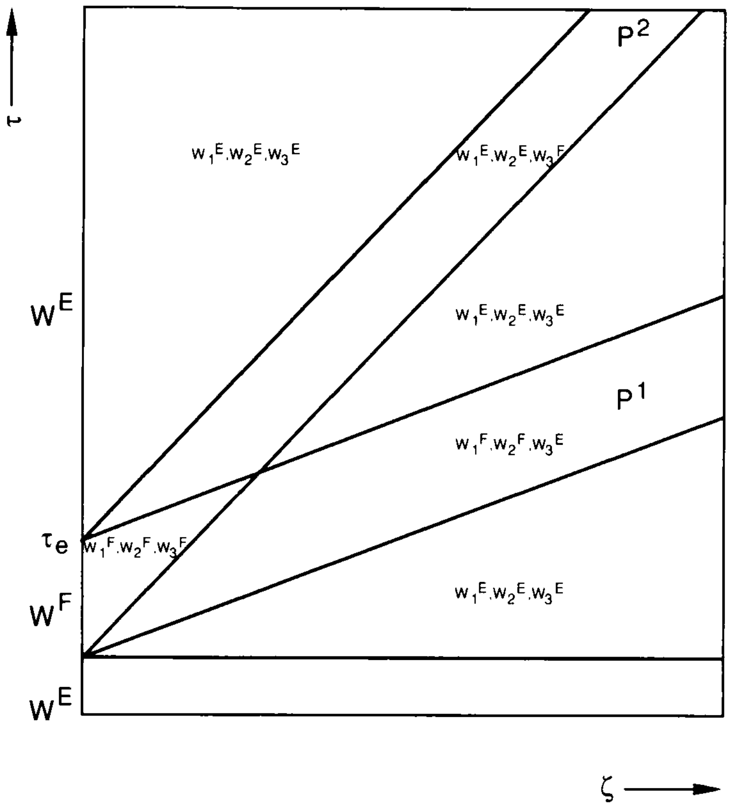

FIG. 3. Schematic portrait of the solution in the (ζ,τ) plane of the elution development of a single analyte in a ternary eluent for the case where there are two distinct eigenvalues.

Figure 3 shows the characteristic directions and the $\vec{w}$ vectors for the case that $m = 4$ and that $\mathbf{A}^E$ has two distinct eigenvalues $\lambda_1 < \lambda_2$ such that λ_1 has multiplicity 2 ($s_1 = 2$, $s_2 = 1$). Then two peaks occur with compositions as given in (22) and (23).

As remarked above, the $\vec{y}$ values are obtained from the $\vec{w}$ values using the transformation **B**. It is known from chromatographical practice that analyte components are present in a single peak. In fact, separation is possible for this very reason. However obvious it may seem to the chromatographer, the observation that an analyte can only be in one peak is not immediately clear from the analysis above. In fact, one can construct many functions $f_i(\vec{y})$ in (7) for which the corresponding linearized system (10) fails to have this property. Therefore, one could formulate a condition on any meaningful isotherm based on the property that every analyte should be present in only one peak. The isotherm of Eqs. (5) and (6) is shown to satisfy this condition. In fact, it can be proven that *every* isotherm of the form (5) with *arbitrary* functions $\alpha_{ij}(y_i, \ldots, y_{m-1})$ always has the desired property. The interested reader is referred to Appendix I for a proof.

EXPERIMENTAL

Apparatus and Materials

A PU4100 binary liquid chromatograph, equipped with a column-oven accessory (Philips Scientific, Cambridge, UK), was used throughout this work. The instrument was adapted to suit the requirements of ion-exchange chromatography of cations as described elsewhere (*11*). In all experiments an LDC Conductomonitor Mark III (LDC/Milton Roy, Riviera Beach, Florida, USA) conductivity detector was utilized. Fractions could be collected using a Model 201 fraction collector (Gilson, Villiers le Bel, France). A Philips PU6000 Integration System together with a P3202 computer were used for data capture and processing. The determination of sodium was carried out using a PU SP9 atomic absorption/emission spectrometer (Philips Scientific, Cambridge, UK).

All experiments were performed using a polystyrene-divinylbenzene-based strong cation exchanger (IC PAK C, Millipore/Waters, Milford, Massachusetts, USA), which was purchased prepacked in a 50 mm, 4.6 mm i.d. column. The analytical column was always used in conjunction with a guard column obtained from the same supplier. All salts used were nitrates of suprapur or a comparable quality (Merck, Darmstadt, FRG). Salts were dried overnight at 80°C and cooled to ambient in a desiccator before weighing. High-purity de-ionized water was used to make up the solution. Samples were made from 1000 ppm stock solutions stored in quartz flasks.

Procedures

The chromatographic system was characterized with respect to system dead volume, column void fraction, and the column exchange capacity. The system dead volume and the column void fraction were measured by injecting a sample containing Li at a concentration slightly different from the Li eluent, without and with the column installed, respectively. The system dead volume:volume in tubing was determined to be 0.216 ± 0.003 mL, the void fraction ϵ was 0.42 ± 0.01. The ion-exchange capacity was determined by frontal chromatography, measuring the net time needed to replace Li by Rb. The ion-exchange capacity of the column was 7.25 ± 0.05 μmol.

In the course of an experiment, both the analytical column and the guard column were first flushed for at least 1 h with a 0.1 *M* (total concentration) solution of a binary eluent having a preselected concentration ratio. The chromatographic system was allowed to equilibrate overnight with an eluent of the same concentration ratio, but now at a total concentration of 2 m*M*. It was found that long equilibration times (>1 h) were necessary to allow measurement at the most sensitive conductivity setting (0.1 μS, 10 mV output). Repeated injections were then made for several column temperatures. The total analyte concentration was always kept below 5% of the total eluent concentration to conform as closely to the elution mode approximation as was permitted by detectability. All capacity factors reported here are average values from at least three injections. The whole procedure was then repeated for a following eluent composition. Optionally, samples were collected to examine peak compositions using atomic spectrometry.

RESULTS AND DISCUSSION

In Fig. 4, two chromatograms are shown. The left-hand figure corresponds to the injection of a sodium sample in a 0.6 m*M* Li/1.4 m*M* Rb eluent; the right-hand figure corresponds to the injection of a potassium sample in the same eluent. In both chromatograms three peaks are discerned. In Fig. 5 the capacity ratios of these peaks have been plotted as a function of the Li/Rb ratio in the eluent. Drawn lines in the figures have been calculated using the elution-mode approximation as described in the theoretical section. From both figures it is evident that peak cross-overs occur on changing the eluent composition. For the case of potassium injection, the cross-over is very close to the origin of the mole fraction axis. For sodium there is a clear cross-over located at a Rb mole fraction of about 0.45.

It is the aim of this discussion to elucidate how the retention of all peaks in the chromatogram can be calculated, to calculate which peak(s) contain the injected analyte, and to compare the calculated result with experiment. Including the inert diluent water, we have four components. Water is not adsorbed. As stated in the Theory Section, there will always be a trivial system peak at $\tau = 0$, (i.e., $t = z/U$ in the (z,t) plane), representing the difference in total ionic concentration between the eluent and the injected sample. Among the remaining three components that is one relation, the electroneutrality condition, which leaves us with two degrees of freedom. This implies that we expect three peaks in the chromatogram: one at a position corresponding to the column hold-up time ($\tau = 0$) and two peaks showing retention. Referring to Fig. 4, this is indeed what is observed experimentally.

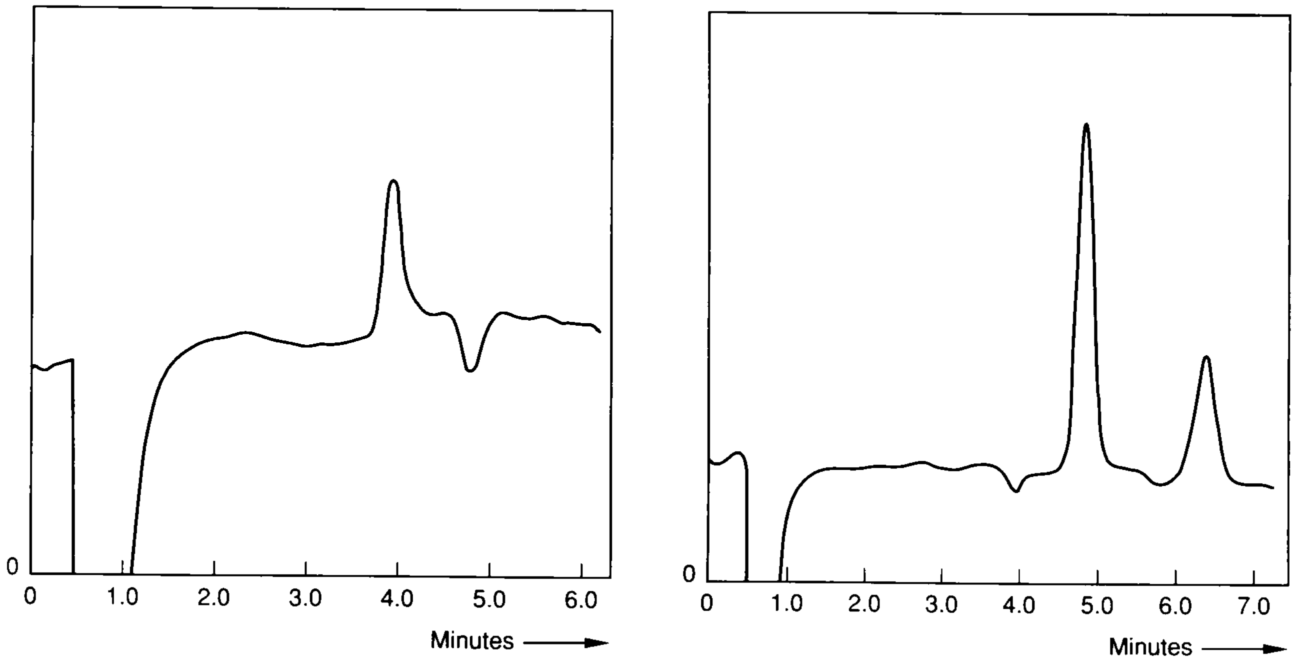

FIG. 4. Chromatograms resulting from the injection of Na (left-hand side) and K (right-hand side) samples in a 1.6 m*M* Li/0.4 m*M* Rb binary eluent. Column: Waters IC PAK C. Temperature: 295 K. Flow: 1.0 mL/min.

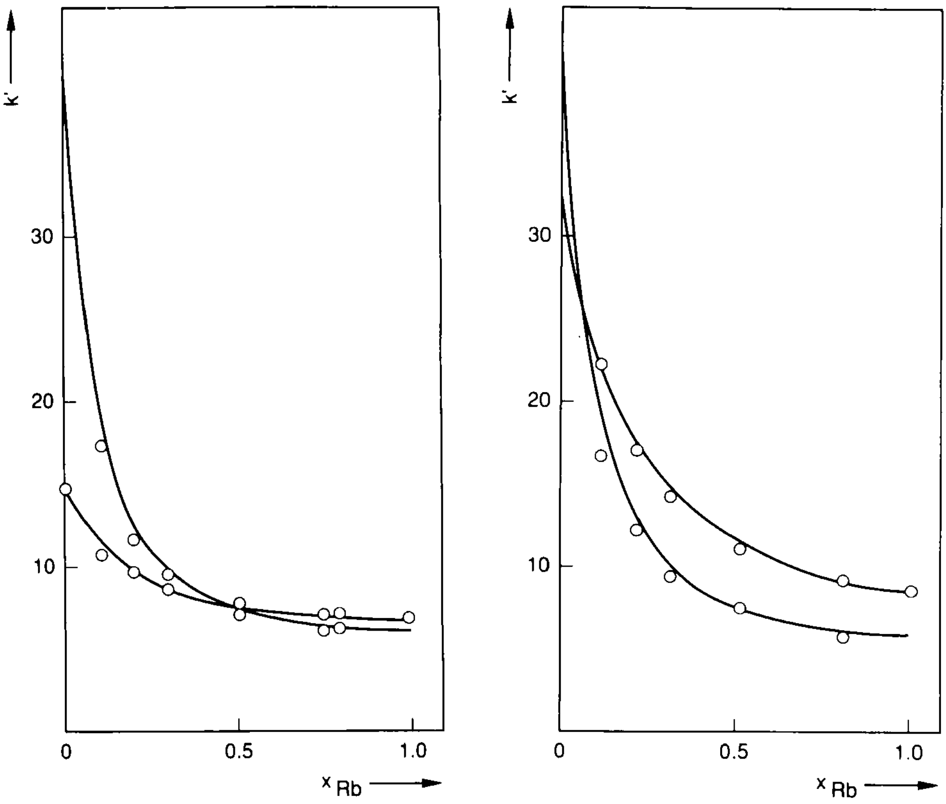

FIG. 5. Capacity factors of system and analyte peaks resulting from injection of Na (left-hand side) and K (right-hand side) as a function of the composition of a Li/Rb eluent. Total eluent concentration: 2.0 m*M*. Temperature: 295 K. Flow: 1.0 mL/min. Drawn lines in the figure have been calculated using the elution-mode approximation.

We now proceed to the calculation of the retention and the composition of the nontrivial peaks. Numerical values for the structural parameters needed in the calculations (the F_{ij} of Eq. 6 are taken from earlier work. For the sake of completeness, the set of f_{ij} values used is shown in Table 1. We are now equipped to solve Eq. (5). According to the discussion in the Theory Section, the following steps have to be taken to solve the problem:

(1) Calculate y_j by numerically solving Eq. (5)
(2) Set up matrix $\mathbf{A}^E$ by the appropriate substitution of y_j and f_{ij} values
(3) Solve the eigenvalue problem. For our simple 2 × 2 problem this is, of course, tantamount to solving a quadratic equation

TABLE 1
Values for the Structural Parameters f_{ij}
Used in the Calculations

p	q	$2a(f_{pq} - f_{\mathrm{Rb\ Rb}})$
Li	Li	12.11
Li	Na	9.95
Li	K	4.91
Li	Rb	3.36
Na	Na	6.33
Na	K	3.49
Na	Rb	2.75
K	K	1.70
K	Rb	1.18

At this point one is in a position to calculate capacity factors. In our formulation of the problem, the capacity factors are related to the eigenvalues according to

$$k_i' = \frac{1 + \lambda_i}{\lambda_i} - 1 \qquad (25)$$

To calculate peak compositions, one additionally has to:

(4) Calculate the Jordan decomposition $\mathbf{A}^E = \mathbf{BJB}^{-1}$
(5) Determine the $\vec{w}$ values of the different zones
(6) Calculate $\vec{y}$ values for the different zones from $\vec{y} = \mathbf{B}^{-1}\vec{w}$
(7) Calculate c values for the different zones by substitution in the multicomponent isotherm

In Fig. 6 the calculated compositions of the two nontrivial peaks obtained upon injection of sodium are plotted as a function of the Rb mole fraction in a Rb/Li eluent. It is seen that the injected analyte (thick solid line) is always present in only one peak. To a chromatographer this may seem self-evident as this is the situation he is familiar with from daily practice. Here, we wish to stress again that there is no *a priori* reason to discard the possible presence of an analyte component in several peaks. To investigate the predicted results, fractions have been collected in the case of sodium injection. These fractions were analyzed for their sodium content using flame emission spectrometry. The results are indicated in the figure. It is seen that the predicted reversal of system and analyte peaks is completely corroborated by the experimental results. To illustrate the temperature independence of the structural parameters, capacity factors referring to an elevated temperature are compared with experiment in Fig. 7.

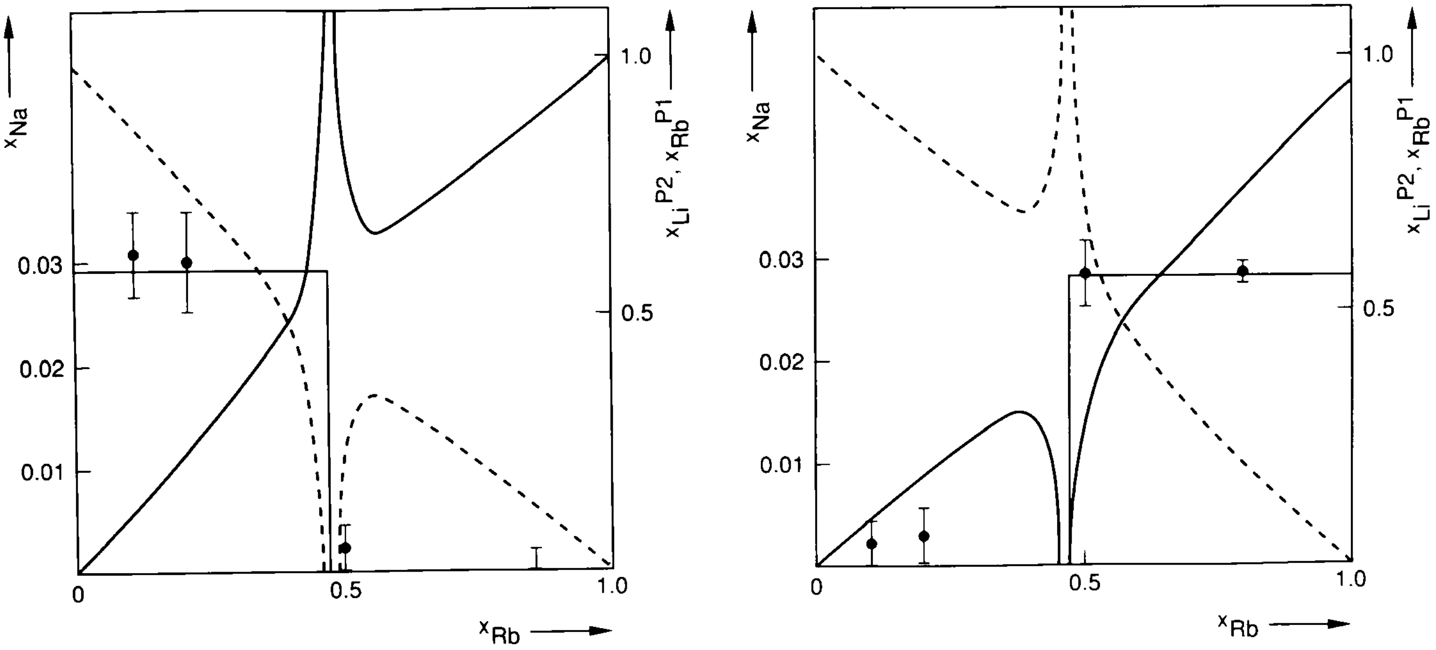

FIG. 6. Peak compositions as a function of the composition of the eluent. The injected sample was 2.7 ppm Na. Left-hand side: First eluting nontrivial peak. Right-hand side: Last eluting nontrivial peak. Dashed lines indicate the Li mole fraction in the peak, solid ones the Rb mole fraction. Thick solid lines refer to Na. Notice the different ordinate scales for sodium. Conditions: See Fig. 3.

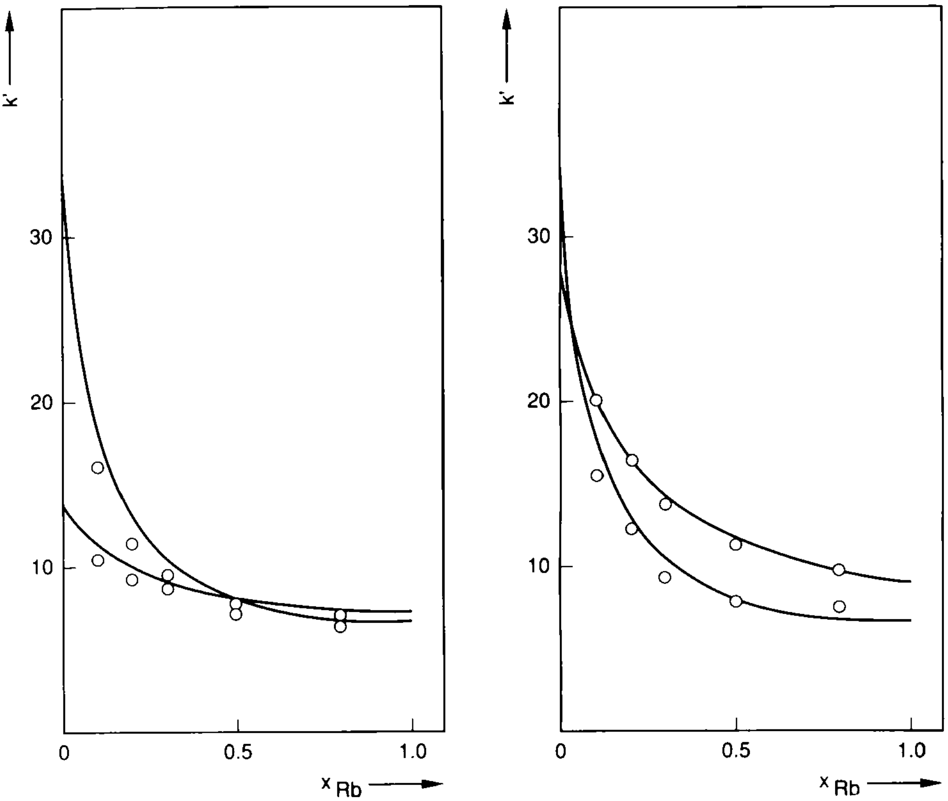

FIG. 7. Capacity factors of system and analyte peaks resulting from injection of Na (left-hand side) and K (right-hand side) as a function of the composition of a binary Li/Rb eluent. Temperature: 322 K. Other conditions: Same as Fig. 3.

The importance of knowing the composite adsorption behavior should not be underestimated. The close connection between mutual interferences and indirect detection has been pioneered in the work of Crommen, Schill, and coworker (*17*, *18*). The aim of indirect detection is to determine the concentration of an analyte that has no inherent detectability by measuring the change in concentration of a detectable eluent component. It follows from the theoretical section that this should always be possible when there are interferences between the analyte of interest and the eluent components. Understanding the details of response in indirect detection is not really possible without a thorough analysis, however. To illustrate this point let us refer to Fig. 6 for the case of a binary eluent. It is seen that the Li and Rb concentrations in the peak(s) rise and fall dramatically when the eluent composition approaches the value corresponding to the cross-over point. There seems to be a vertical assymptote for the mobile-phase concentrations of the eluent components.

To explain this, it should be noted that at the cross-over point the two eigenvalues λ^1 and λ^2 of $\mathbf{A}^E$ coincide and that $\mathbf{A}^E$ has only one eigenvector there. We shall write $\delta = \lambda^1 - \lambda^2$, then $\delta \rightarrow 0$ on approaching the cross-over point. The matrix **B** in Eq. (12) becomes singular in this limit; in fact, the elements of the matrix $\mathbf{B}^{-1}$ are of the order $1/\delta$ near the cross-over point:

$$\mathbf{B}_{ij}^{-1} = O\left(\frac{1}{\delta}\right) \tag{26}$$

If we write the feed state in $\vec{y}$-space as

$$\vec{y}^F = \vec{y}^E + \kappa\Delta\vec{y} \tag{27}$$

where $\kappa\Delta\vec{y}$ is the perturbation of the eluent and $\kappa > 0$ is a small parameter. Then we have

$$\vec{w}^F = \mathbf{B}^{-1}\vec{y}^F = \vec{w} + \kappa\mathbf{B}^{-1}\Delta\vec{y} \tag{28}$$

so that in particular: $w_1^F - w_1^E = O(\kappa/\delta)$. Since $\vec{w}^{P_1} = (w_1^F, w_2^E)$, it follows that

$$\begin{aligned}\vec{y}^{P_1} &= \mathbf{B}\vec{w}^{P_1} = \mathbf{B}\vec{w}^E + (w_1^F - w_1^E)\mathbf{B}\vec{e}^1\\ &= \vec{y}^E + (w_1^F - w_1^E)\vec{r}^1\\ &= \vec{y}^E + O\left(\frac{\kappa}{\delta}\right)\end{aligned} \tag{29}$$

Hence, if $\delta \rightarrow 0$ and κ is kept fixed, $|y_i^{P_1}| \rightarrow \infty$, which proves the existence of a vertical assymptote at the cross-over point. Physically, the mobile- and stationary-phase concentrations are, of course, bounded. It is to be understood that the restrictions on the magnitude of the first-order perturbation that can be allowed in a physically realistic calculation become more stringent as one gets nearer to the cross-over point. The large excursions of eluent component concentrations in the peaks as shown in Fig. 6, however, are in good agreement with the experimental results of Ref. *18*. We could now take the discussion on indirect detection a bit further. In LC a universal detector, such as the flame-ionization detector in GC, is much sought after. The main problem hampering the introduction of a universal LC detector is the presence of the liquid eluent that has to be removed before the detection. However, one could also consider the presence of the eluent as an advantage rather than as a drawback. The omnipresent interferences between eluent and sample species in LC systems offer an equally omnipresent opportunity for indirect detection. One could argue that the universal LC detector has been available from the outset. We just do not know how to exploit it properly through our lack of knowledge of the distribution isotherms that govern our separations.

To summarize, the retention times of all peaks and their compositions can be accurately calculated if and only if an isotherm describing the composite behavior of all species in the system is known.

APPENDIX I

A general observation in chromatography is that a given analyte is present in only one peak. This is true for every set of values of the concentrations in the eluent and, therefore, it seems that this experimental fact corresponds to a characteristic property that any meaningful distribution isotherm should have. In fact, as we shall show, the property that an analyte is present is only one peak can be derived from the conservation laws for *any* choice of the multicomponent adsorption isotherm when:

(1) The total concentration in the stationary phase is constant
(2) The conservation laws are linearized around the elution state

We shall first derive a necessary and sufficient condition on the isotherms in order that every analyte is always present in only one peak. Then we shall prove that when (1) and (2) hold, every isotherm satisfies this condition.

Let the analyte of interest be the component which is eliminated from the conservation laws using the relation $\Sigma_{i=1}^{m} y_i = 1$, that is, let the numbering of the components be such that y_m corresponds to the analyte. Because the observation that the analyte is present in one peak is independent of the number of peaks (mathematically speaking, independent of the number of distinct eigenvalues of the matrix $\mathbf{A}^E$), we shall consider the general case discussed in the theoretical section, which allows for multiple eigenvalues. Let $\lambda^1 < \cdots < \lambda^r$ be the distinct eigenvalues of $\mathbf{A}^E$ with multiplicities $s_1, \ldots, s_r$, respectively. Furthermore, put $\sigma_k \equiv \Sigma_{j=1}^{k} s_j$, $k = 1, \ldots, r$ and $\sigma_0 \equiv 1$. Then it follows from Eq. (24) that the composition of peak P^k in the $\vec{w}$-space can be concisely written as

$$\vec{w}^{P^k} = \vec{w}^E + \sum_{i=\sigma_{k-1}+1}^{\sigma_k} (w_i^F - w_i^E)\vec{e}^{\,i} \tag{AI.1}$$

where $\vec{e}^i = (0, \ldots, 0, 1, 0, \ldots, 0)$ denotes the ith unit vector. The corresponding $\vec{y}$-vector is given by

$$\vec{y}^{P^k} = \mathbf{B}\vec{w}^{P^k} = \vec{y}^E + \sum_{i=\sigma_{k-1}+1}^{\sigma_k} (w_i^F - w_i^E)\vec{b}^i \tag{AI.2}$$

where $\vec{b}^i = \mathbf{B}\vec{e}^i$. In the case that there are $m - 1$ distinct eigenvalues, we have $s_j = 1$ for all j, hence $\sigma_k = k$. Furthermore, in that case $\mathbf{B}\vec{e}^k = \vec{r}^k$, the eigenvector corresponding to λ^k, so that Eq. (AI.2) becomes

$$\vec{y}^{P^k} = \vec{y}^E + (w_k^F - w_k^E)\vec{r}^k \tag{AI.3}$$

In the general case of multiple eigenvalues however, only a subset of the vectors $\vec{b}^i$ are eigenvectors of $\mathbf{A}^E$. Since the analyte is not present in the eluent, one has

$$\sum_{j=1}^{m-1} y_j^E = 1 \tag{AI.4}$$

Furthermore, the analyte is *not* present in the kth peak if and only if

$$\sum_{j=1}^{m-1} y_j^{P^k} = 1 \tag{AI.5}$$

On combining Eqs. (AI.4) and (AI.5), it follows from Eq. (AI.2) that a given analyte is present in only one peak if and only if

$$\sum_{i=\sigma_{k-1}+1}^{\sigma_k} (w_i^F - w_i^E) \sum_{j=1}^{m-1} b_j^i = 0 \tag{AI.6}$$

for all but one $k \in \{1, \ldots, r\}$. Furthermore, this property holds independently of the concentrations in the eluent and the feed sample, provided Eq. (AI.6) not only holds for all but one k, but also for all values of $w_i^F - w_i^E$. From this we conclude that the analyte is always present in one peak if and only if one has

$$\sum_{j=1}^{m-1} b_j^i = 0 \qquad \text{for } \sigma_{k-1} + 1 \leq i \leq \sigma_k \tag{AI.7}$$

for all but one $k \in \{1, ..., r\}$. In particular, when $\mathbf{A}^E$ has $m - 1$ distinct eigenvalues, Eq. (AI.7) simply becomes

$$\sum_{j=1}^{m-1} b_j^k = \sum_{j=1}^{m-1} r_j^k = 0 \qquad \text{(AI.8)}$$

for all but one $k \in \{1, ..., r\}$. Hence, in this case, the vector (1, ..., 1) is orthogonal to all but one eigenvector, say $\overline{r}^l$. This is in turn equivalent to the statement that (1, ..., 1) is an eigenvector of the transpose $(\mathbf{A}^E)^T$ of $\mathbf{A}^E$ corresponding to the eigenvalue λ^l:

$$((\mathbf{A}^E)^T - \lambda^l)\begin{pmatrix} 1 \\ \cdot \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ \cdot \\ 0 \end{pmatrix} \qquad \text{(AI.9)}$$

In the general case of Eq. (AI.7), it follows that (1, ..., 1) is orthogonal to all linear spaces generated by $\{\overline{b}^i;\ \sigma_{k-1} \le i \le \sigma_k\}$ except for one, say for $k = l$. Since these linear spaces are identical to the kernel of the matrices $(\mathbf{A}^E - \lambda^k)^{s_k}$, and since the linear space which is orthogonal to the kernels of all $(\mathbf{A}^E - \lambda^k)^{s_k}$, $k \ne l$ is identical to the kernel of $((\mathbf{A}^E)^T - \lambda^l)^{s_l}$, we conclude that (1, ..., 1) is in the latter space, that is:

$$((\mathbf{A}^E)^T - \lambda^l)^{s_l}\begin{pmatrix} 1 \\ \cdot \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ \cdot \\ 0 \end{pmatrix} \qquad \text{(AI.10)}$$

It is seen that (AI.10) reduces to (AI.9) when $s_l = 1$, i.e., when $\mathbf{A}^E$ has $m - 1$ distinct eigenvalues. To summarize, we have:

If the $(m - 1) \times (m - 1)$ matrix $\mathbf{A}^E$ obtained after eliminating the analyte of interest has r distinct eigenvalues, then there are r peaks. Let $\lambda^1 < \cdots < \lambda^r$ be the distinct eigenvalues of $\mathbf{A}^E$ and let $s_1, ..., s_r$ be their respective multiplicities. Then the given analyte is always present in one peak for all concentrations in the eluent and in the feed, if and only if there is $l \in \{1, ..., r\}$ such that Eq. (AI.10) holds. In that case the analyte is present in the lth *peak, corresponding to the eigenvalue λ^l.*

Let the isotherms be written as

$$\frac{y_i}{x_i} = \alpha_{ij}(y_i, ..., y_m)\frac{y_j}{x_j}, \qquad 1 \le i, j \le m \qquad \text{(AI.11)}$$

where $x_i = c_i/C$ and $y_i = n_i/N$ are the mole fractions of the ith component in the mobile and the stationary phase, respectively. Compatability requires that the separation factors satisfy

$$\alpha_{ii} = 1, \qquad \alpha_{ij}\alpha_{jk} = \alpha_{ik} \tag{AI.12}$$

Apart from these compatibility conditions, the separation functions are arbitrary functions of $y_1, \ldots, y_m$ and the following derivations are therefore valid for any isotherm. Equation (AI.11) implies

$$\frac{x_i}{x_m} = \alpha_{mi}(y_i, \ldots, y_m)\frac{y_i}{y_m} \tag{AI.13}$$

If all sites are always occupied, we have

$$\sum_{i=1}^{m} x_i = \sum_{i=1}^{m} y_i = 1 \tag{AI.14}$$

By adding all the Eqs. (AI.13), using (AI.14) one obtains

$$\frac{y_m}{x_m} = \sum_{k=1}^{m} \alpha_{mk}(\vec{y})y_k = 1 + \sum_{k=1}^{m-1} [\alpha_{mk}(\vec{y}) - 1]y_k \tag{AI.15}$$

where $\vec{y} = (y_1, \ldots, y_{m-1})$ and $\alpha_{mk}(\vec{y})$ is obtained from $\alpha_{mk}(y_1, \ldots, y_m)$ by substitution of $y_m = 1 - \Sigma_{k=1}^{m-1} y_k$. Since $x_i = c_i/C$, it follows by comparing Eqs. (4), (11) and (AI.15) that

$$f_i(\vec{y}) = \frac{C}{\nu N}\frac{\alpha_{mi}(\vec{y})y_i}{1 + \sum_{k=1}^{m-1} [\alpha_{mk}(\vec{y}) - 1]y_k} \tag{AI.16}$$

where $\nu = (1 - \epsilon)/\epsilon$. We write

$$V \equiv 1 + \sum_{k=1}^{m-1} [\alpha_{mk} - 1]y_k \tag{AI.17}$$

Then Eq. (AI.16) implies

$$\sum_{i=1}^{m-1} f_i(\vec{y}) = \frac{C}{\nu N}\left\{1 - \frac{1 - \sum_{k=1}^{m-1} y_k}{V(\vec{y})}\right\} \tag{AI.18}$$

Hence for $1 \leq j \leq m - 1$:

$$\sum_{i=1}^{m-1} \frac{\partial f_i}{\partial y_j}(\bar{y}) = \frac{C}{\nu N V(\bar{y})^2} \left\{ V(\bar{y}) + \left[1 - \sum_{k=1}^{m-1} y_k \right] \frac{\partial V}{\partial y_j}(\bar{y}) \right\} \qquad \text{(AI.19)}$$

Now, if y_m corresponds to an analyte, then the eluent state $\bar{y}^E$ satisfies

$$\sum_{i=1}^{m-1} y_i^E = 0 \qquad \text{(AI.20)}$$

Hence, Eq. (AI.19) implies for $1 \leq j \leq m - 1$:

$$\sum_{i=1}^{m-1} \frac{\partial f_i}{\partial y_j}(\bar{y}^E) = \frac{C}{\nu N V(\bar{y}^E)} \qquad \text{(AI.21)}$$

Since the right-hand side of Eq. (AI.21) is independent of j, it follows that Eq. (AI.21) is equivalent to the statement that the vector (1, ..., 1) is an eigenvector of the matrix $(\mathbf{A}^E)^T$, where $\mathbf{A}^E \equiv (\partial f_i / \partial y_j\ (\bar{y}^E))$. The eigenvalue is given by the right-hand side of Eq. (AI.21):

$$\lambda = \frac{C}{\nu N} \frac{1}{\sum_{k=1}^{m-1} \alpha_{mk}(\bar{y}^E) y_k^E} \qquad \text{(AI.22)}$$

We therefore conclude that a given analyte is always present in only one peak irrespective of the choice of the mathematical form of the multicomponent isotherm provided that the conservation laws are linearized around the elution state and all adsorption sites are permanently occupied.

APPENDIX II

In this Appendix, expressions for the elements of the matrix $\mathbf{A}_{ij}(\bar{y}) = \partial f_i / \partial y_j\ (\bar{y})$ are given. For the isotherm considered in this paper, the separation factors α_{ij} are functions of all y_j (cf. Eq. 6):

$$\alpha_{ij}(\bar{y}) = \exp \left\{ \frac{-(1 - T/T_c)}{RT} \times \left[F_{im} - F_{jm} + \sum_{k=1}^{m-1} (F_{ik} - F_{jk} + F_{jm} - F_{im}) y_k \right] \right\} \qquad \text{(AII.1)}$$

where y_m has again been eliminated. Using

$$f_i(\bar{y}) = \frac{C}{\nu N} \frac{\alpha_{mi}(\bar{y}) y_i}{1 + \sum_{k=1}^{m-1} [\alpha_{mk}(\bar{y}) - 1] y_k} \tag{AII.2}$$

we obtain for the partial derivatives:

$$\frac{\partial f_i}{\partial y_j}(\bar{y}) = \frac{C}{\nu N} \left\{ \frac{V \alpha_{mi} \delta_{ij} - (\alpha_{mj} - 1) \alpha_{mi} y_i}{V^2} - \frac{1 - T/T_c}{RT} \right.$$
$$\times \frac{(F_{mj} - F_{ij} + F_{im} - F_{mm}) \left(1 - \sum_{k=1}^{m-1} y_k \right) \alpha_{mi} y_i}{V^2}$$
$$\left. - \frac{1 - T/T_c}{RT} \frac{\sum_{k=1}^{m-1} (F_{im} - F_{km} + F_{kj} - F_{ij}) \alpha_{mk} y_k \alpha_{mi} y_i}{V^2} \right\} \tag{AII.3}$$

where V has been defined in Eq. (AI.17). Note that the two terms of (AII.3) with factor $(1 - T/T_c)/RT$ are due to the $\bar{y}$-dependence of the α_{ij}. These terms are absent when ideal (Langmuir) adsorption behavior is assumed.

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