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Secondary equilibria and their interaction with chromatographic transport

H. POPPE

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)

SUMMARY

The existence of "eigenpeaks" and the possibility of indirect detection in high-performance liquid chromatography and capillary zone electrophoresis can be understood with the same mathematical treatment. The coupled transport equations, when suitably linearized for small disturbances, can be treated as a linear eigenvalue problem. The solution predicts, in accordance with earlier results obtained by other workers, the existence of N eigenpeaks in a chromatographic or electrophoretic system, where N is the number of degrees of freedom in the description of the composition of the solution involved. Each eigenpeak corresponds to a capacity factor or mobility and is associated with an eigenvector that describes the relative intensities of the disturbances in all compounds that occur in the solution. An important experimental facility offered by these phenomena is indirect detection. A few general conclusions pertaining to this technique, invented before these phenomena were fully understood, are drawn.

INTRODUCTION

Usually in high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE), the distribution and transport of the various components in the column are considered separately; it is assumed that a solute can be transported without affecting the transport of another. This assumption is in sharp contrast with the basic facts about chromatographic and electrophoretic mechanisms. For instance, in adsorption chromatography (including reversed-phase chromatography), it is known that the adsorption of one component is associated with the desorption of another (displacement), the complete opposite of the above assumption! More incidental examples of mutual interactions are those brought about by so-called secondary equilibria, *e.g.*, acid–base reactions, ion-pair formation and van de Waals or covalent complexation.

Similarly, in free solution zone electrophoresis, the migration rate of an ion depends in general on the presence of other ions, because these determine the conductivity and with that the local electric field. In more particular cases, the effective

mobility of the ion considered depends on the various reactions (see above) that may alter its charge, size, etc. Therefore, here also causes for mutual interactions are abundant.

How could the theories based on such unrealistic assumptions be so successful in describing and predicting the multitude of phenomena, with respect to peak positions and peak widths, that occur in columns? The main reason is not that the sample constituents are eventually separated: first, one would still expect malfunctioning of the theory due to the interaction in the first part of the column; second, we know that independent behaviour is still a good description even if compounds coelute, *i.e.*, have been in each other's presence during their whole column history, the observed signal being simply the sum of that of the two components.

The success of the linear theory is rather due to the effective experimental techniques we use to make the behaviour of solutes adhere to idealized laws. Mostly, this can be described as buffering of conditions. Thus (a historically important example)[1], the activity of an adsorbent may be buffered by the addition of a moderator; when protolytic equilibria of the solutes occur, we intuitively choose a pH-buffered mobile phase; in CZE[2,3], the same is done, but the added salts also serve the purpose of buffering the electrical conductivity of the column.

Under such buffered conditions, the equations describing the transport of the solute species are linear in species concentration. This linearization can be described for chromatography as follows.

A general formulation of the transport equation is

$$\frac{\partial \tilde{c}_k}{\partial t} = \frac{\partial}{\partial z}\left(vR_k\tilde{c}_k + D_k \cdot \frac{\partial \tilde{c}_k}{\partial z}\right) \qquad \text{all } 1 \leqslant k \leqslant N \tag{1}$$

where

z = the coordinate in the length direction;
t = time;
v = cross-section-averaged velocity of the mobile phase;
$\tilde{c}_k$ = total concentration, averaged over the cross-section and both phases + possibly present support;
c_k = mobile phase concentration on the same volume basis;
D_k = a dispersion coefficient, describing, *e.g.*, lateral diffusion, non-equilibrium and flow irregularities (see below);
R_k = retardation factor, the fraction of solute in the mobile phase = $c_k/\tilde{c}_k$.

Some further comments should be made on eqn. 1. First, Riedo and Kováts[4] demonstrated that one cannot generally consider v as a constant, and that in an N-component mixture in LC there are only $N - 1$ degrees of freedom, and only $N - 1$ equations of the type of eqn. 1 should be considered. We work ourselves around this complication by assuming that v is constant, either because the $N - 1$ other volume fractions are small, or because all partial molar volumes are constants. Also, one component is considered as an inert diluent and left out from the calculations. Second, the D_k values are not equal to the commonly used dispersion coefficients, as a result of using $\tilde{c}$ rather than c. However, they may include diffusion in the stationary phase[5].

Third, it is assumed that diffusion, convective mixing and resistance to mass transfer can be lumped into one parameter D_k. This is asymptotically true for linear cases[6], and there is strong evidence[7] that it is also a good approximation for non-linear cases.

In the general case, R_k (and D_k and possibly v) depend on all the concentrations of $j = 1$ up to N. The resulting set of coupled, non-linear equations presents a virtually unsurmountable mathematical problem; even with the D_k terms cancelled and only one or two components, considerable effort is needed to obtain an insight into the system[8-10].

However, with a low c_k and other $\tilde{c}_i$s ($i \neq k$) buffered to almost constant values, R_k, v and D_k become virtually constant. The result is

$$\frac{\partial \tilde{c}_k}{\partial t} = vR_k \cdot \frac{\partial c_k}{\partial z} + D_k \cdot \frac{\partial^2 c_k}{\partial z^2} \tag{2}$$

an equation that can easily be solved. The well known result (see, *e.g.*, ref. 11) leads to the familiar Gaussian curve, when the input function is a sharp spike, of the shape

$$\tilde{c}_k = \frac{Q_k}{A\sqrt{2\pi\sigma_{z,k}}} \cdot \exp\left[-\tfrac{1}{2}\left(\frac{z - u_k t}{\sigma_{z,k}}\right)^2 \right] \tag{3}$$

where
  Q_k = amount injected;
  A = column cross-section;
  u_k = $R_k v$;
  $\sigma_{z,k}$ = $\sqrt{2D_k t}$.
while the response to a composite input can be obtained by convoluting eqn. 3 with that input[12].

In capillary electrophoresis, a similar situation exists. A general equation in that case is

$$\frac{\partial \tilde{c}_k}{\partial t} = -\frac{\partial J_k}{\partial z} = +\frac{\partial}{\partial z}\left(\frac{I}{\kappa} \cdot \mu_{\text{eff},k}\, c_k + D_k \cdot \frac{\partial^2 \tilde{c}_k}{\partial z^2} \right) \tag{4}$$

where
  I = current density, which is a constant when the tube diameter is uniform;
  κ = conductivity of the solution, equal to Faraday's constant times the sum
    $\sum_k \tilde{c}_k \mu_k z_k$ for all ionic species k;
  $\mu_{\text{eff},k}$ = effective mobility of species k, a signed quantity;
  z_k = charge of species k.

A few comments should be made on eqn. 4. First, spaces charges, which would invalidate the relationship for the local field, $E = I/\kappa$, are neglected. This is common in electrophoretic theories. Second, in that case, it is best to take N as one less than the number of independent ionic species, and the last concentration follows from the electroneutrality condition.

The values of $\mu_{\text{eff},k}$ may be variable, *e.g.*, owing to changes in ionic strength and reactions such as protolysis undergone by k. The value of κ is also variable in principle, because it is (to a good approximation) an additive function in all conductivities. As in chromatography, the usual experimental practice is to add such large concentrations of "indifferent" ions and reagents (such as H^+) that κ, $\mu_{\text{eff},k}$ and D_k are constants. Again, then, the equation is solved by a Gaussian function (or its convolution with any input function) and that is the usual peak shape we observe under such conditions.

It is interesting, and the main purpose of this paper, to investigate the disturbances in the mobile phase or carrier constituents (being at non-infinite dilution) brought about by the phase migration of solutes which are at high dilution. Thus, *e.g.*, in normal-phase adsorption chromatography with dichloromethane (DCM) as moderator in hexane as the mobile phase and ethyl acetate (EA) as the solute, with adsorption taking place at the front edge of the peak, EA adsorbs on the surface, inevitably displacing DCM. This means that the conditions at the very position of the peak are likely to be different from our set values. Similarly, when benzylamine (BA) is a solute in an electrophoretic experiment in an electrolyte buffered near to the pK_a value of BA, its transport is bound to disturb the buffer solution; only the protonated species migrate through the solution, but on entering an "empty" part of the liquid, however, it has to be partly deprotonated in order to re-establish the protolytic equilibrium. Thereby the pH value will be disturbed, again at the very position where we want it to be constant.

LINEARIZATION

The fully linear model is insufficiently detailed to investigate these effects. On the other hand, considering the general transport equations, which in principle is preferable, leads, as indicated, to a kind of mathematical complexity that precludes finding a solution in all but the simplest of cases, and puts very high demands on the mathematical ability of the investigator. However, one can go part way along this path: the dependence of the migration rate parameters on the concentrations can be simplified in such a way that the resulting set of equations is still linear, while preserving the mutual influence of the constituents.

We first demonstrate this for liquid chromatography. First, the diffusion terms in the set of eqn. 1 are dropped; they play no essential role in the problem and can be re-inserted afterwards. The R_k factors (equal to the R_F value in thin-layer chromatography) depend on all concentrations. However, close to the mobile phase composition for all reasonably behaving systems one can describe R_k as a linear function of the concentrations $\tilde{c}_j$ (j from 1 to N), as for all analytical experiments the deviation from the starting values will be small. Hence,

$$R_k = R_k^{\circ} + A_{k,1}' \Delta c_1 + A_{k,2}' \Delta c_2 + \ldots + A_{k,N}' \Delta c_N \qquad 1 \leqslant k \leqslant N \qquad (5)$$

where
R_k° is the R value for k at exactly the mobile phase composition;
$\Delta c_1 \ldots \Delta c_N$ are the deviations in the concentrations from that composition;
$A_{k,1}'$ are constants, to be found from an explicit expression $R_k = R_k(c_1 \ldots c_N)$ as

$$A'_{ki} = \left(\frac{\partial R_k}{\partial \tilde{c}_i}\right)_{c_{j\neq i}}$$

As $R_k = c_k/\tilde{c}_k$, it follows that

$$A'_{ki} \ (i \neq k) = \frac{1}{\tilde{c}_k} \cdot \frac{\partial c_k}{\partial \tilde{c}_i}$$

$$A'_{kk} = \frac{1}{\tilde{c}_k} \cdot \frac{\partial c_k}{\partial \tilde{c}_k} - \frac{1}{\tilde{c}_k} \cdot R_k$$

Insertion into eqn. 5 leads to

$$\frac{\partial \tilde{c}_k}{\partial t} = -v\left(\sum_{i=1}^{N} A_{k,i} \cdot \frac{\partial \tilde{c}_i}{\partial z}\right) \qquad 1 \leqslant k \leqslant N \tag{6}$$

where $A_{k,i} = \partial c_k/\partial \tilde{c}_i$.

Two comments should be made. First, the use of the total concentration $\tilde{c}$ as the basic variables is unconventional, and leads to arithmic difficulties, e.g., when applying a Langmuir isotherm. For chromatography it can be avoided by starting from the equation

$$\frac{\partial \tilde{c}_k}{\partial t} = -v \cdot \frac{\partial c_k}{\partial z}$$

and expanding $\tilde{c}_k$ in terms of c_i rather than c_k (or R_k) in terms of $\tilde{c}_i$. However, the unconventional scheme was adopted here in order to demonstrate the analogy to electrophoresis. Second, eqn. 6 can be derived more directly by using

$$\frac{\partial \tilde{c}_k}{\partial t} = -v \cdot \frac{\partial c_k}{\partial z}$$

and expanding c_k in terms of $\tilde{c}_i$.

Returning to the set of eqns. 6, we note that it is linear. This leads to a drastic mathematical simplification. Most important are two facts: if a solution has been found, multiplication of this with a constant gives another solution; and any linear combination of solutions is also a solution.

The set of eqns. 6, as noted by numerous workers[4], constitutes an eigenvalue problem. There are N solutions E_p $(p = 1 \ldots N)$ of the form

$$c_{k,p} = e_{k,p}F(z - \lambda_p t) \tag{7}$$

where

λ_p is one of the set of N eigenvalues of the matrix $\| A_{k,i} \|$;
$e_{k,p}$ is the series of constants $k = 1 \ldots N$ that is indicated as the eigenvector $|e_p|$;
F is an arbitrary function; the dependence of F on $(z - \lambda_p t)$ indicates that a

translation along the z-axis with velocity λ_p occurs, while the shape of the disturbance is constant (*i.e.*, as long as the Ds are zero).

Insertion of eqn. 7 in the set of differential eqns. 6 reveals that in matrix notation, | | indicating a vector and ‖   ‖ a matrix:

$$\lambda_p \, |e_p| \; = \; - \, v \, \|A\| \cdot |e_p|$$

the standard eigenvalue problem.

It should be noted that, as a result of the chosen formalism, the eigenvalues emerge as velocities and, when v is divided out, as R_F values. Other formalisms, which are fully equivalent but do not have the close resemblance to the treatment of CZE, would lead to eigenvalues in terms of capacity factors, inverse velocities, etc. All these different treatments would be equivalent.

The same approach can be used for electrophoresis: expand $\mu_{\text{eff},k}/\kappa$ in terms of the c_is. It follows again that

$$\frac{\partial c_k}{\partial t} = - \, I \sum_{i=1}^{N-1} A_{k,i} \cdot \frac{\partial c_i}{\partial z} \qquad (k = 1 \ldots N - 1) \tag{8}$$

where

$$A_{k,i} = \frac{\partial \left(\dfrac{\mu_{\text{eff},k}}{\kappa} \right)}{\partial c_i}$$

The same mathematical treatment as given above for HPLC would then lead to the appearance of eigenvalues that are, in fact, the mobilities of "concerted" disturbances.

In zone electrophoresis there is one important favourable condition: with reasonable accuracy the differential quotients $A_{k,i}$ can be predicted, because κ is equal to

$$\kappa = F \sum_{\text{all ions}} c_i \mu_i z_i$$

where F is the Faraday, while the effective mobilities $\mu_{\text{eff},k}$ can be readily calculated when species mobilities and pK_a values are known. Therefore, all values A_{ki} can be found from first principles and physical constants that are tabulated for common ions. This is in sharp contrast to the situation in chromatography, where such calculations are normally impossible. Here one has to resort to some more or less arbitrary model, or to the experimental determination of the distribution isotherms. This is already a lot of work for a single component. When composite isotherms are to be determined, the work becomes very complex. The amount of data needed merely to describe such a system grows with $z^N(N)$, where z is the number of grid points in one concentration axis (*e.g.*, studying one component with 20 grid points leads to 20 data pairs, studying four components with mutual interactions leads to $20^4 \cdot 4 = 640\,000$ data). It is therefore not surprising that simultaneous isotherm data are scarce, and limited to two interrelated components.

"Eigenpeaks"

The mathematics predict that N velocities occur, each with an associated eigenvector. This means that disturbances travel with fixed ratios of the intensities of the disturbances in the various concentrations. These disturbances can be indicated as "concerted"[13] or as "eigenpeaks", while the situation is also referred to as that of "coherence"[10].

Why should these disturbances be so rigidly interrelated? Let us return to the normal-phase chromatography of EA with DCM as the moderator. In a diagram such as Fig. 1 we can represent the mobile phase composition, each point corresponding to a composition. Point M represents the mobile phase. Eigenvectors are represented by arrows, emanating from M. There are two of them, e_1 and e_2. In this case (where $c_{EA} = 0$, for the eluent), the e_1 vector tells us that at the position of a positive deviation in EA (that is, in the commonly observed EA peak) there is a negative deviation in the DCM concentration, of a proportional intensity. Such a "concerted" disturbance travels with velocity λ_p. What if, by some peculiar history, the starting condition were to be different; let us assume that instead of a composition neatly in the direction of e_1 or e_2 one starts with a region of the column having composition "Q".
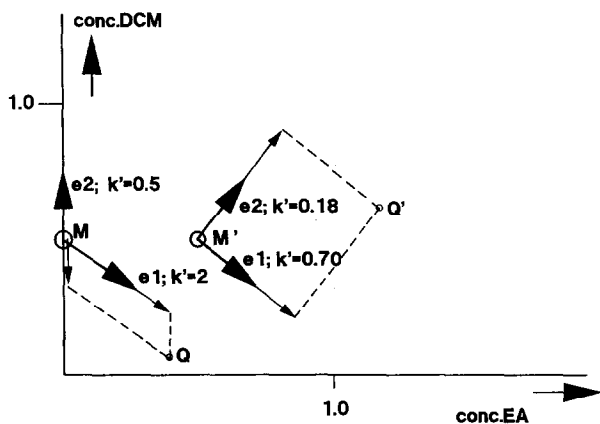

Fig. 1. Eigenvectors in a two-component system with dichloromethane (DCM) as the moderator and ethyl acetate (EA) as the solute (letters without primes) and with both components present in the mobile phase (primed letters). M, M', mobile phase compositions; Q, Q', compositions of injected solutions. The graph has been calculated assuming Langmuir adsorption with the expression $c_{i,s} = K_i c_{i,m}/(1 + K_{EA}c_{EA,m} + K_{DCM}c_{DCM,m})$ with $K_{EA} = 4$ and $K_{DCM} = 2$.

From Fig. 1, it is easy to see that the vector $\overline{MQ}$ can be described as the sum of vectors e_1 and e_2, each multiplied with a suitable constant. These would each start to travel with velocities λ_1 and λ_2, respectively, leading to a "separation". That means the vectors behave as if they were separate compounds in a classical chromatographic experiment.

This state of affairs becomes even more clear if we add some BA to the mobile phase, leading to mobile phase composition M' in Fig. 1, and study the profiles of BA and DCM after the application of some arbitrary injection, let us say of composition Q'. We obtain two peaks, with (in general) different velocities, but in each of the peaks

BA varies in addition to DCM. In this instance there is no "peak" identification", and one cannot say that one peak "belongs" to BA or DCM. What occurs would be described as the separation of mathematical vectors or, physically, only if the disturbances in DCM and EA are in the right proportions to each other can they move together; if not, the disturbance as a whole will be split into two sets of disturbances that do fulfil this requirement.

Indirect detection

An experimentally important aspect of the concerted disturbances is the possibility of indirect detection. This method has been studied by numerous workers. After the series of papers by Crommen and co-workers[13–15], the debate on the "mechanism" appears to have more or less terminated. In fact, it may be clear from their work and from this paper that there is no special physical mechanism involved; it is not necessary to assume special distribution phenomena such as two-site adsorption. Any transport mechanism with mutual interaction of constituents will lead to system peaks and the potential of indirect detection. This was well illustrated by the exploration of the method in capillary zone electrophoresis by two groups[16,17].

Indirect detection can be illustrated by the example given above. EA, if not detectable as such, can be detected via the disturbance in DCM, if that compound could be detected. The method is quantitatively reliable, because the nature of the phase system and the compounds and not, *e.g.*, the dispersion processes, govern the magnitude of the response.

The detailed calculation of Crommen and co-workers, *e.g.*, for the Langmuir isotherm case reveals that when $c_{EA} = 0$ the DCM response is proportional to $(k'_s/k'_{EA} - 1)^{-1}$, in which k'_{EA} and k'_s are the capacity factors for EA and the DCM system peak, respectively. This means first that the response changes sign when the k'_{EA} value passes that of the system peak, and second that when k'_{EA} approaches k'_s, the response (*e.g.*, effective molar absorptivity) tends to infinity. The responses may look as in Fig. 2, and these were observed experimentally even before the explanation could be given. Of course, the exact equality of both k values would lead to peak overlap and
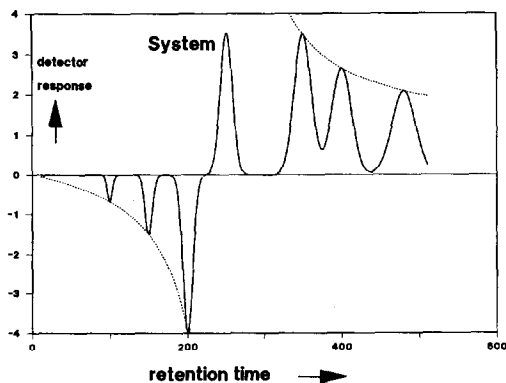

Fig. 2. Responses in indirect detection as a function of capacity factor of the solute, with analyte peaks and system peak, showing the increase in response when k'_A approaches k'_s and the sign reversal in the response when the analyte peak moves across the position of the system peak. The dashed line describes the hyperbolic curve, with a dependence like $k'_i/(k'_i - k'_s)$ on the capacity factor of the solute k_i.

the impossibility of quantification. An example may serve to illustrate the profound difficulty in the understanding of these phenomena. Experimentalists such as chromatographers are used to considering cause–effect relationships, *e.g.*, the fact that a displacement mechanism is at work, suggests at first (but false) sight that a positive EA peak would be accompanied by a positive DCM peak, because the addition of EA to a phase system with DCM would always release DCM from the stationary phase, no matter what the ratio of the k'_s values is. More detailed treatments show this view to be entirely wrong; the slot machine of mathematics delivers another result, that described above, in which the sign changes with change in capacity factor.

There seems to be no way to explain the behaviour of such systems, even in quantitative terms, without using complex mathematics. We simply have to accept as facts that only concerted disturbances can move as identities through the column and that the quantitative relationships, such as peak positions (and relative magnitudes of disturbances) can only be found as eigenvalues and eigenvectors, respectively. However, the perseverence of eigenpeaks in the column may become more familiar with the following reasoning. In every column slice, the change in the total concentration of each component is determined by the local gradient in the mobile concentration. When these changes for all components are exactly in proportion to the deviations already present, these new deviations will still have the same ratios. In this way the relative rate of change and hence the speed is the same for every component. The changes are determined by the prevailing concentrations and the transport matrix. That explains that only particular combinations of deviations can travel without being fragmented (in fact resolved in the chromatographic sense) into disturbances moving with different velocities.

Relationship with theories from preparative chromatography

It is important to stress that the close relationship between the phenomenon of system peaks, indirect detection, etc., and those observed in heavily overloaded columns, as studied by the pioneers of chromatography in the 1940s, reviewed by Helfferich and Klein[10] and more recently by Rouchon *et al.*[9], and in chemical engineering by Rhee and Amundson[7]. In these discussions the concept of "paths"[10], Γ[7] or hodograph[9] is developed. These are lines in the composition space formed by using the *N* concentrations as coordinate axes. A disturbance following such a path has the same character as the concerted set of minor disturbances described above: all concentration variations are rigidly interrelated. Also, the transport through the column of a disturbance following a path does not split and is described by relatively simple equations: a translation with constant velocity λ [a function $F(z - \lambda t)$] or a dilatation [a function $F(z/t)$].

The linear eigenvectors described above are the tangents to the *N* paths in composition space (Fig. 3).

EIGENPEAKS IN CZE

It has been demonstrated above that the same phenomena have to occur in electrophoretic experiments. Eqn. 9, for the simpler case of simple ions with no reaction such as protolysis, can be written as
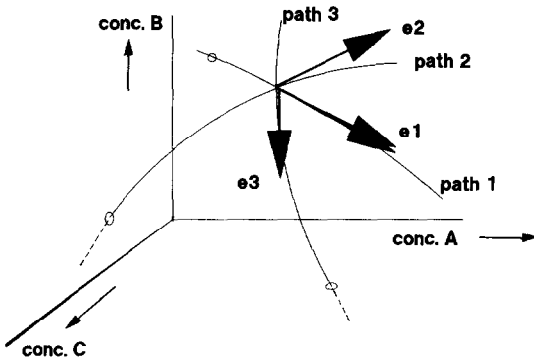
Fig. 3. The "paths" according to Helfferich and Klein[10] in coherent boundaries and the eigenvectors for minor disturbances. The latter are the tangents to the former.

$$\frac{\partial c_k}{\partial t} = \mu_k I \cdot \frac{\partial}{\partial z}\left(\frac{c_i}{\kappa}\right) \qquad \text{all } 1 < i < N \tag{10}$$

where $\kappa = \sum_i \mu_i c_i z_i F$

where F is Faraday's constant and the z values are the ionic charges. Thus:

$$\frac{\partial c_k}{\partial t} = \frac{I}{F} \cdot \frac{\partial}{\partial z}\left(\frac{c_k}{\sum_j \mu_j c_j z_j}\right)$$

The expression in parentheses can be expanded as a linear function of the c values. In so doing, as indicated, one should consider $N - 1$ ions, leaving the concentration of the Nth ion to be determined afterwards from the electroneutrality condition. Rather than going through this exercise in general equations, we shall do it for a particular example, which demonstrates the essential features. Suppose a Li^+ ion is electrophoresed at low concentration in a carrier of KBr. Take Br^- as the indifferent ion, and set I/F to unity, as this is just a constant. We then have (with a negative μ_{Br} value)

$$\kappa = c_{Li}(\mu_{Li} - \mu_{Br}) + c_k(\mu_k - \mu_{Br})$$

$$\frac{\partial c_{Li}}{\partial t} = \frac{\partial}{\partial z}\left[\frac{\mu_{Li}c_{Li}}{c_{Li}(\mu_{Li} - \mu_{Br}) + c_K(\mu_K - \mu_{Br})}\right]$$

$$\frac{\partial c_K}{\partial t} = \frac{\partial}{\partial z}\left[\frac{\mu_K c_K}{c_K(\mu_K - \mu_{Br}) + c_K(\mu_K - \mu_{Br})}\right]$$

Expansion in Δc values of the factors in parentheses gives

$$\frac{\partial \Delta c_{Li}}{\partial t} = A_{Li,Li} \cdot \frac{\partial \Delta c_{Li}}{\partial z} + A_{Li,K} \cdot \frac{\partial \Delta c_K}{\partial z}$$

$$\frac{\partial \Delta c_K}{\partial t} = A_{K,Li} \cdot \frac{\partial \Delta c_{Li}}{\partial z} + A_{K,K} \cdot \frac{\partial \Delta c_K}{\partial z}$$

with

$$A_{Li,Li} = \frac{\mu_{Li}}{\kappa} - \frac{\mu_{Li} c_{Li}(\mu_{Li} - \mu_{Br})}{\kappa^2} = \frac{\mu_{Li}{}^a}{\kappa}$$

$$A_{Li,K} = -\frac{\mu_{Li} c_{Li}(\mu_K - \mu_{Br})}{\kappa^2} = 0^a$$

$$A_{K,Li} = -\frac{\mu_K c_K(\mu_{Li} - \mu_{Br})}{\kappa^2} = \frac{\mu_K c_K(\mu_{Li} - \mu_{Br})}{\kappa^2}$$

$$A_{K,K} = \frac{\mu_K}{\kappa} - \frac{\mu_K c_K(\mu_K - \mu_{Br})}{\kappa^2} = 0^a$$

The eigenvector problem is therefore

$$\left\| \begin{matrix} \dfrac{\mu_{Li}}{\kappa} & 0 \\ -\dfrac{\mu_K c_K(\mu_{Li} - \mu_{Br})}{\kappa^2} & 0 \end{matrix} \right\| \left\| \begin{matrix} e_{Li} \\ e_K \end{matrix} \right\| = \left\| \begin{matrix} \lambda e_{Li} \\ \lambda e_K \end{matrix} \right\|$$

In this case, it is easy to find the two solutions:

$$\lambda_1 = \mu_{Li}/\kappa, \quad e_K{:}e_{Li} = -\frac{\mu_K c_K(\mu_{Li} - \mu_{Br})}{\kappa \mu_{Li}}$$

$$= -\frac{\mu_K c_K(\mu_{Li} - \mu_{Br})}{c_K(\mu_K - \mu_{Br})\mu_{Li}}$$

$$= -\frac{\mu_K}{\mu_{Li}} \cdot \frac{(\mu_{Li} - \mu_{Br})}{(\mu_K - \mu_{Br})}$$

$$\lambda_2 = 0, \quad e_{Li}{:}e_K = 0$$

The first solution corresponds to the normal electrophoresis of Li$^+$ (the factor $1/\kappa$ in λ_1 occurs because I/κ was set to 1). The K disturbance accompanying Li$^+$ is related to this by the factor $\mu_K(\mu_{Li} - \mu_{Br})/[\mu_{Li}(\mu_K - \mu_{Br})]$. The disturbance in Br$^-$ can be calculated from the electroneutrality, $e_{Br} = e_{Li} + e_K$:

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^{a} Equal to these terms as $c_{Li} \approx 0$ and $\kappa \approx c_K(\mu_K - \mu_{Br})$.

$$e_{Br}:e_{Li} = 1 - \frac{\mu_K(\mu_{Li} - \mu_{Br})}{\mu_{Li}(\mu_K - \mu_{Br})} = - \frac{\mu_{Br}(\mu_{Li} - \mu_K)}{\mu_{Li}(\mu_K - \mu_{Br})}$$

We note in passing that the expressions for e_{Br} and e_K can be obtained from each other by interchanging the indices K and Br, which indicates that the same solution would be found if K had been chosen as the Nth ion.

The above shows that (a) The indirect detection of K cannot be described as a direct consequence of electroneutrality. This would give $e_K = - e_{Li}$, with $e_{Br} = 0$. Thus, as in Langmuir chromatography, the direct application of intuitive notions leads to an erroneous result. (b) K^+ as well as Br^-, $i.e.$, ions of both charges, could be used as a marker ion. (c) Only when $\mu_{Li} = \mu_K$, a situation realized, $e.g.$, when using isotopically marked K^+ as the solute, is the intuitive result $e_K = - e_{Li}, e_{Br} = 0$ correct. This is what is to be expected physically; in that case, the Cl^- ions are "decoupled" from the transport, as they would not "see" the substitution of K^+ by Li^+.

The second solution has velocity zero, a stagnant disturbance, with identical variation in c_K and c_{Br}, while Li is not involved. This means that a disturbance in the carrier electrolyte concentration does not move (relative to the liquid; of course, the osmotic flow will make it move). This is a well known fact in electrophoresis; it can be derived directly from the requirement that Kohlrausch's regulating function (KRF) is constant in time:

$$KRF = \sum \frac{c_i z_i}{\mu_i}$$

where z_i is the charge of the ion i. As $\Delta c_{Li} = 0$, it follows, from electroneutrality, that

$$\Delta KRF = \frac{\Delta c_K}{\mu_K} + \frac{\Delta c_{Br}}{\mu_{Br}} = \Delta c_K \left(\frac{1}{\mu_K} - \frac{1}{\mu_{Br}} \right)$$

which indicates that Δc_K does not change with time, $i.e.$, the disturbance does not move.

It should be noted that the K^+ and Br^- disturbances travelling with Li^+ can also be derived directly from KRF; two equations are needed to relate e_K and e_{Br} to e_{Li}: one is the constancy of KRF, the other the electroneutrality. However, for more complicated systems one needs the eigenvector treatment.

Effect of a given injection composition

The "composition" of peaks, $i.e.$, the relative intensities in the various component disturbances, are now described in the $|e_p|$ vectors. However, another problem is to describe the intensities of the various peaks that result from a given injection. In Fig. 1 it was already indicated how the vector V_{inj} of the injection disturbance has to be decomposed into the vectors, e_p, corresponding to the various "eigenvelocities". However, the graphical tool will not be of much help when three or more components are relevant (see Fig. 4); in that case, one needs a numerical treatment.

The decomposition of $|c_{inj}|$ into the $|e_p|$ is a straightforward example of linear
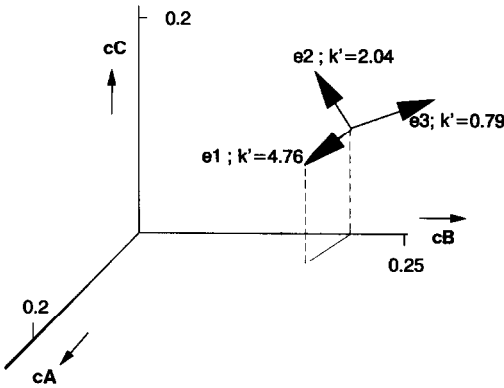
Fig. 4. Eigenvectors in a three-component system, containing A, B and C in the mobile phase. Vectors $e_2 b$ and e_3 are in the back-plane ($c_A = 0$); e_1 protrudes out of this plane and corresponds to the elution of compound A that does not occur in the mobile phase. The graph has been calculated assuming Langmuir adsorption with an expression $c_{i,s} = K_i c_{i,m}/(1 + K_A c_{A,m} + K_B c_{B,m} + K_C c_C)$ with $K_A = 10$, $K_B = 3.0$ and $K_C = 5.0$.

algebra. The $e_{p,i}$ matrix (all eigenvectors assembled to form a square matrix), indicated by $\|E\|$, is bound to have an inverse, because the e_ps are independent. The result $\|R\| = \|E\|^{-1}$ is also equal to the set of left-eigenvectors of the transport matrix $\|A\|$ (left and right eigenvectors have the same set of eigenvalues). Once $\|R\|$ is known, the intensities of the various peaks, denoted by P_p, are given by $|P| = \|R\| \cdot |c_{inj}|$. The electrophoretic example with Li$^+$ in KBr may illustrate this:

$$\|E\| = \left\| \begin{matrix} 1 & 0 \\ -\alpha & 1 \end{matrix} \right\|$$

where $\alpha = + \mu_K(\mu_{Li} - \mu_{Br})/\mu_{Li}(\mu_K - \mu_{Br})$. It follows that

$$\|R\| = \left\| \begin{matrix} 1 & 0 \\ \alpha & 1 \end{matrix} \right\|$$

  Case 1. Injection solution deviates from carrier by 0.0001 M Li$^+$ and 0.0001 M K$^+$ (*i.e.*, the Cl$^-$ concentration is the same).

$P_1 = 1 \cdot 0.0001 \, M + 0 \cdot 0.0001 \, M = 0.0001 \, M$
$P_2 = \alpha \cdot 0.0001 \, M + 1 \cdot 0.0001 \, M$

  The first peak has intensity 0.0001 M in Li and $-\alpha$ times this value in K$^+$. The second peak (stagnant) has intensity 0.0001 $M(1 + \alpha)$ in K$^+$. Note that the K$^+$ sum over both peaks (and that of Li$^+$) equals the injected concentration.

  Case 2. Injection of Li, 0.0001 M, "on top of" the base composition KBr, *i.e.*, $V_{inj,Br}$ is also 0.0001 M. This yields

$$P_1 = 1 \cdot 0.0001 \, M + 0 \cdot 0 = 0.0001$$
$$P_2 = \alpha \cdot 0.0001 \, M + 1 \cdot 0 = \alpha \cdot 0.0001$$

The first peak again has intensity 0.0001 M in Li^+, of course, and $-\alpha \cdot 0.0001$ M in K^+. The second, stagnant, peak has $\alpha \cdot 0.0001 \, M$ in K^+. The sum of the K^+ responses is zero, as it should be.

Case 3. Only by exactly "tuning" the injection of K^+ to *e.g.*, 0.0001 M Li and $-\alpha \cdot 0.0001 \, M \, K^+$ can one avoid the "excitation" of the second peak:

$$P_2 = \alpha \cdot 0.0001 \, M + 1(-\alpha \cdot 0.0001 \, M) = 0.$$

Similar calculations are possible for liquid chromatography although, instead of using first principles to describe the non-linearities, one has to either use experimental composite isotherms (which are scarce), or rely on a model for the distribution behaviour such as the Langmuir composite isotherms. When suitably programmed, the arithmetic does not cause any problems.

DYNAMIC RANGE OF INDIRECT DETECTION

In both separation techniques the buffering of conditions is not perfect; it can be exhausted when high concentrations of solutes are present. When increasing these concentrations or (which amounts to the same) increasing the mass load while not changing the injection volume, inevitably at some point the non-linear behaviour will be visible again. Usually peaks then start to develop a triangular shape. This is the result of the fact that high concentrations travel [*i.e.*, the velocity "of a concentration" is $(dz/dt)_{ci}$] at a different velocity than do low concentrations. Such effects have been described in HPLC[18,19] as "mass overload", "concentration overload" or "thermodynamic broadening", which are good descriptors. Unfortunately, in CZE the term "electromigration dispersion" has found acceptance. In fact, there is no dispersion at work or anything that can be described formally as such. This is clear from the fact that the same effects can lead to zone narrowing, *e.g.*, occurring in isotachophoresis. It is proposed here to retain a more neutral term, such as "concentration overload", in order to indicate this type of extra zone broadening.

Differences in the velocities of high and low concentrations will become visible in the electropherogram or chromatogram as soon as they are larger than the "natural" uncertainty in the velocity connected with peak dispersion. When the latter is expressed as standard deviation and taking relative to the peak position, it equals $1/\sqrt{N}$. It follows that relative variations in migration rates should exceed this value; this is more critical for CZE with 100 000–300 000 plates than for HPLC where plate counts often do not exceed 10 000. How strongly the migration rate varies with the concentration of i depends very much, of course, on the particular type of solute and system. However, when indirect detection is used, for detecting a solute i with a marker M, the marker concentration c_M is necessarily coupled to c_i, otherwise no response would be obtained. If the marker M is involved in the migration of i, it can be assumed that the migration of i is also influenced by the concentration c_M. Indeed, carrying out various numerical experiments, *e.g.*, with Langmuirian adsorption in HPLC, or for

CZE systems, shows that the marker concentration always, under conditions where indirect detection works, has a strong influence on the migration rate of i, 1% in c_M leading to variations of the order of 1%.

As the migration rate has to be kept constant within a factor of $1 \pm 1/\sqrt{N}$, as a general rule of thumb it can be stated that the marker concentration is to be held constant within the same factor. Thus, only a fraction of $1/\sqrt{N}$ of the detection range offered by the detected marker at its given concentration can be exploited; when a 1:1 response is obtained, the solute concentration has to be kept below $c_M/\sqrt{N}$, in order to avoid peak deformations. As, on the other hand, c_M cannot be chosen to be indefinitely high (e.g., a UV detector would not work at absorbances of 3 or higher), the indirect detection principle can in general be expected to have a negative effect on the loadability and dynamic range of the system. The problem may turn out to be more severe in CZE than in HPLC, as the plate numbers are larger and the detectors work under less favourable conditions.

This reasoning, crude as it is, obviously is strongly connected with the necessity, most explicitly formulated by Kuhr and Yeung[16], to use instrumentation with a "high dynamic reserve", i.e., a capability of detecting small relative changes in the concentration of the marker.

CONCLUSION

It has been shown that system peaks and indirect detection can be treated mathematically from one viewpoint. The quantitative relationships follow readily from straightforward mathematics and can be obtained, once the distribution or transport behaviour is known, by programs that are straightforward but may sometimes be fairly complicated.

Unfortunately, it remains difficult to develop an intuitive direct understanding of the phenomena. It has been shown here that it is even risky to rely on such intuitive notions, as they tend to lead to wrong conclusions.

The common basis of indirect detection in HPLC and CZE has been elucidated. These possibilities do not depend on particular physical conditions, such as displacement in the distribution process or the concept of electroneutrality, but are always present when for some reason or another (displacement, mutual reactions, coupled protolysis) compounds influence each other in their transport behaviour.

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