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System peaks and non-linearity in capillary electrophoresis and high-performance liquid chromatography

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Abstract

Non-linearity and systems peaks are closely related. In fact, the occurrence of system peaks at positions that are not so easy to predict is the consequence of non-linearity in the separation system. These things are observed in HPLC as well as in CE. In this presentation the theories and models developed in both fields are compared. An attempt is made to formulate a common framework for the description of these phenomena in the two techniques, including affinity capillary electrophoresis (ACE).

Keywords: System peaks; Non-linearity

1. Introduction

It is surprising how the theories in electrophoresis and chromatography, although both belonging in the realm of analytical separations, have developed independently. This holds especially for the aspect of non-linearity. The following few observations may partly explain this bifurcation.

Since the inception of gas chromatography, published dispersion theories have been founded partly on a chemical engineering approach to the transport equations (e.g., the 'van Deemter equation'), and partly been developed independently by early pioneers such as Golay and Giddings. A multitude of peak-broadening mechanisms was considered already in the early days, e.g., longitudinal diffusion, stream line effects ('eddy diffusion'), resistance to mass transfer in both phases, kinetic resistance to phase transfer. This multitude already led from the outset to rather complicated expressions. Theories were even further refined after the introduction of HPLC. Non-linearity was considered much less frequently, and came more in focus only after the introduction of preparative LC and displacement chromatography.

In electrophoresis there was not much attention for such elaborate dispersion theories. Indeed in that field there appeared not to be a great need for these; a simple migration/dispersion model, with dispersion mainly determined, at the low voltages used, by longitudinal molecular diffusion sufficed for most cases.

On the other hand, for non-linearity, the other aspect of zone shape, the situation was nearly the reverse. Among workers in electrophoresis there was great interest in non-linearity, especially also in the context of isotachophoresis. Most theoretical treatments went deep into the details of the effect of conductivity and the Kohlrausch regulating function (both associated with non-linearity), but if dispersion was taken into account at all, this was limited to simple longitudinal molecular diffusion. Indeed, that was justified, as the effects of non-linearity and

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mutual interaction on peak and boundary shapes were much more important and indeed more challenging than dispersion.

The analogy of isotachophoresis to displacement chromatography is obvious, but surprisingly this has not led to much interaction between the two groups of researchers.

In this work an attempt is made to bridge the gap between theoretical approaches in chromatography and electrophoresis, in particular HPLC and CZE, with special attention to non-linearity and mutual interaction of solutes.

2. Non-linear distribution, electromigration dispersion, moving boundary equations (MBE), concentration velocity and constituent velocity

In both techniques there is a 'carrier', a background electrolyte in CE, the mobile phase in HPLC. In the sequel the word carrier will be used for this 'initial composition', present before the injection has taken place and usually restored after the separation has performed.

In electrophoresis, it is common practice to consider ion velocities, u_i, being the product of the (effective) mobilities, μ_i, and the local electrical field strength, E:

$$u_i = E\mu_i \tag{1}$$

In the general case, both E and μ_i are functions of the entire composition of the solution. That is, E depends on the conductivity as current density should be constant over the length, and the u_i values, being effective values, may change due to pH shifts.

In order to describe the inherent non-linear effects, and the mutual interaction of solutes described by Eq. (1), two concepts have played an important role in electrophoresis: moving boundary equations (MBE) and Kohlrausch's regulating function (KRF).

The MBE are mass balances taken at a boundary between two regions, α and β, 'zones' in the solution, where β often is the carrier. The boundary generally moves, with a velocity indicated by U_B. If the boundary is to persist in time, as a plane separating α from β, the amounts per second per square meter transported to the plane should sum up to zero. The MBE are therefore of the form:

$$c_i^{\alpha}(u_i^{\alpha} - U_B) = = c_i^{\beta}(u_i^{\beta} - U_B) \text{ (each } i) \tag{2}$$

with a suitable dependence of the u_i values on the composition (or local conditions such as temperature) according to, e.g., Eq. (1).

The indication 'each i' in Eq. (2) requires some comment in the (nearly ubiquitous) case where fast reactions occur (for slow reactions the present treatment is inappropriate). One cannot apply the mass balances, Eq. (2), to such converting species, because they are produced and consumed by the reactions. Thus, the i values should refer to what we call 'constituent' concentrations, i.e., sums over whatever form a particular added compound occurs in (to some people known as analytical concentrations). For each mixture a exhaustive description in terms of such constituent concentrations is possible. Thus to describe a sodium acetate buffer only two numbers suffice; e.g., the total concentration of acetic acid plus acetate, and the concentration of sodium; the hydrogen ion concentration follows from this composition. Likewise, in an ethereal solution of benzoic acid dimers occurs, however, these should not be used as separate concentrations, as the partition between monomeric and dimeric form follows the mass action law at any moment, provided the equilibrium is fast.

It has to be noted that with a mixture of n constituents, of which the concentration can be chosen independently (e.g., using H^+ and OH^- for satisfying the electroneutrality condition), there are n MBE values, while with given c_i^{α} and c_i^{β} values there is only one unknown, U_B. It follows that the set of equations in general, with arbitrary choices of the concentrations, has no solution. Physically: only for certain combinations of the c_i^{α} values and c_i^{β} values there can be a stable boundary; the compositions ahead and astern of a boundary have to fulfil certain relations. This anticipates on the concept of coherence, introduced by Helfferich and Klein [1,2] for ion-exchange chromatography, to be discussed in the sequel.

The occurrence in Eq. (2) of two different types of velocities should be mentioned. A U_B (note the capital U) is the velocity of a zone or boundary,

named 'concentration velocity' by Helfferich and Klein [2], a quantity most readily experimentally accessible. The u_i values (note the lower case) are in fact velocities of molecules or ions (often not directly measurable), be it that they may be averages over various species, e.g., different degrees of ionization in electrophoresis. The u_i values are indicated as 'constituent velocities', reserving in this work the word species (as used in Ref. [1]), for ions, complexes, etc., in rapid equilibrium.

Another approach to understanding interdependence of compositions ahead and astern of a boundary is allowed by the KRF. This function of the concentrations, c, the (signed) nominal charges z and the (signed) mobilities μ, being,

$$\mathrm{KRF} = \sum_{\text{all ions } i} \frac{c_j z_j}{\mu_j} \tag{3}$$

is constant in time. It follows that KRF cannot change across a moving boundary; within a zone moving in the carrier the KRF should be the same as in the carrier. That comes down to the same thing: not every combination of concentrations before and after the boundary is allowed. Note, however, that in general case of more than three ions, the KRF equation (in combination with the electroneutrality) cannot replace the MBE; two equations are simply not sufficient to solve for the many unknowns. That is, two regions with the same KRF do not necessarily satisfy the MBE, only for a three-ion (constituent, not counting H^+, OH^-) system is this guaranteed.

In *chromatography* non-linearity is mainly caused by the non-linearity in the distribution between the mobile phase and the stationary phase. However, before considering the details, it must be mentioned that another cause was seen to be important in the early days of gas chromatography: the so-called sorption effect. It can be described physically as follows: When a solute has a non-zero concentration, the gas velocity cannot be uniform (even neglecting, for reasons of simplicity, the expansion of the carrier gas due to the pressure gradient).

Within the solute band the velocity of the gas is higher than outside it (see discussion by Jacob and Guiochon [3]), because at the tail of the band the solute leaves the stationary phase, and its large gas phase volume contributes to the overall flow. The gas passing through the front boundary of the band, on the other hand, loses part of its volume by sorption of the solute to the stationary phase; at that point the gas velocity decreases again. The correction for the gas velocity is readily found by considering the MBE values. With u_0 being the gas velocity outside the zone, u_{z0} that in the zone, u_X ($==U_{\text{zone}}$) that of the solute, k_i' the retention factor, X_i the mole fraction of the solute in its zone, the equations are:

$$\begin{aligned} &X_i u_{z0} - u_X X_i(1 + k_i') = = 0 \\ &\quad \text{for the solute,} \\ &(1 - X_i)(u_{z0} - u_X) = = 1(u_0 - u_X) \\ &\quad \text{for the carrier gas,} \end{aligned} \tag{4}$$

with the solution:

$$u_X = \frac{u_0}{(1 + k_i' - k_i' \cdot X_i)} \tag{5a}$$

$$u_{z0} = \frac{u_0}{1 - X_i k_i'/(1 + k_i')} \tag{5b}$$

This short excursion on a largely forgotten issue (with present day's detector sensitivity mole fractions >0.01 are rarely encountered) was made, as it shows that also in chromatography it is useful sometimes to consider the change in velocity from the outset.

However, as said, in the majority of cases there is non-linearity in the distribution; most often a result of too high concentrations in the stationary phase (in LC one could guess that also overloading in the mobile phase may occur under certain extreme conditions, since the mobile phase volume is not always very large compared to the stationary phase volume).

This change in distribution translates into a change in migration rate. Most popular to describe overloading is the Langmuir isotherm:

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

where the c are the concentrations, K is the adsorption equilibrium constant, and S measures the adsorbent saturation capacity for i; subscripts m and s refer to the mobile phase and stationary phase, respectively; i to the constituent.

From such isotherms the peak shape under conditions of ideal chromatography, i.e., in the absence of dispersion or slow equilibrium effects, can be derived, as has been shown, e.g., by Huber and Gerritse [4], or Knox and Pyper [5]. The result is often given as:

$$t_{R,i}(c_{i,m}) = L/u_0\left(1 + \frac{dc_{i,s}}{dc_{i,m}}\right) \quad (7)$$

Specialized for the above isotherm Eq. (2) it becomes:

$$t_{R,i}(c_{i,m}) = L/u_0\left(1 + q\,\frac{K_i}{(1 + 1/S_iK_ic_{i,m})^2}\right) \quad (8)$$

where q is the phase ratio, often m^2 adsorbent surface/liter mobile phase, and L is the column length.

Note that in such derivations, the velocity of the zones, and the velocities of the molecules themselves, nor the difference between the two, are mentioned explicitly.

When more constituents are involved, the 'composite' Langmuir expression is often taken to be:

$$c_{i,s} = \frac{K_ic_{i,m}}{1 + \sum_{\text{all constituents } j} 1/S_jK_ic_{j,m}} \quad (9)$$

Difficulties with thermodynamic consistency, especially occurring with differing S_j values, as have been discussed [6], will be ignored in the sequel, as the Langmuir relation is only used here as an illustration. The same holds for the observation that quite often experimental data do not fit such equations well. More sophisticated models have been developed in abundance; for the present general discussion these are too complicated.

The case of a mixture of two constituents is the most complicated one that lends itself to direct mathematical–analytical analysis [7], resulting in rather awkward expressions for the peak shapes or elution curves. For injection into a 'non-empty' carrier such analysis would become increasingly complex.

Eqs. (1)–(3) for electrophoresis on the one hand and Eqs. (7) and (8) for chromatography on the other hand do not seem to have much to do with each other. Nevertheless, the similarity of phenomena in electrophoresis and chromatography is striking. In both cases experiments with higher solute concentration leads to skewed peaks. Also, in both techniques, with more complicated carrier compositions, we encounter so-called system peaks, that without any doubt have to do with the non-linearity of the system, and with the mutual influence between carrier and sample constituents.

The idea of this contribution is to formulate electrophoresis and chromatography in a common formalism, so that results from the one field can be used in the other.

3. Constituent velocity as a function of composition

The proposal is to write for both techniques the constituent velocities, u_i, as a function of the entire composition:

$$u_i = u_i(\mathbf{c}) \quad (10)$$

where boldface **c** stands for the composition

For electrophoresis Eq. (10) is a direct equivalent to Eq. (1), as the field, E, follows from the conductivity, a composition function. However, for chromatography Eq. (10) is quite unconventional (and, we will see, unpractical for analytic evaluation). The u_i values are the velocities averaged over both phases, thus equal to:

$$u_i = u_0 \cdot \frac{c_{i,m}}{c_i} \quad (11)$$

where the elements c_i of **c** are defined as the 'total' concentrations (amount in both phases/volume mobile phase):

$$c_i = c_{i,m} + qc_{i,s} \quad (12)$$

For a linear distribution one simply has $u_i = u_0/(1 + k_i')$; in the non-linear case one has to evaluate Eq. (11) by using the isotherm such as Eq. (9): The result using Eq. (9) as an example is:

$$u_i = u_0 \cdot \frac{1}{1 + qK_ic_{i,m}/\sum_{j=1\ldots N} 1 + 1/S_jK_jc_{j,m}} \quad (13)$$

However, finding the $c_{i,m}$ values, needed in Eq. (13), when the c_i values are known is notably more

difficult than finding $c_{i,s}$ values and c_i values from a given set of $c_{j,m}$ values. The latter involves no more than insertion of the data in Eq. (9), the former necessitates the inversion of that equation, which even with one constituent leads to a quadratic-root expression, and for multi-constituent cases even cannot be given in a closed-form expression.

Nevertheless, we pursue this idea, because it gives valuable insight into the similar nature of system peaks in electrophoresis and chromatography. The necessary but intricate inversion of the isotherm turns to be no problem when numerical methods are used, something that is indicated anyhow, for other reasons, as will be seen below.

As will be discussed in the next section, the phenomena of slight overload, and of system peaks can be approached best by considering differential forms of the expressions for u_i and for the MBE.

4. Differential expressions

When the concentrations are still close to those in the carrier, it is useful to expand Eq. (10) in a Taylor series up to degree 1:

$$u_i(\mathbf{c}) = u_i(\mathbf{car}) + \frac{\mathrm{d}u_i}{\mathrm{d}c_1}\Delta c_1 + \frac{\mathrm{d}u_i}{\mathrm{d}c_2}\Delta c_2 \ldots + \frac{\mathrm{d}u_i}{\mathrm{d}c_N}\Delta c_N \tag{14}$$

or

$$u_i(\mathbf{c}) = u_i(\mathbf{car}) + \sum_{j=1\ldots N} A_{i,j}\,\Delta c_j \tag{15}$$

with i as well as j running from 1 to N. This leads to N^2 expressions for the elements of the square matrix, $|\mathbf{A}|$, being:

$$A_{i,j} = \frac{\mathrm{d}u_i}{\mathrm{d}c_j} \tag{16}$$

Each of these elements describes how a velocity of a constituent is influenced by its own concentration (the diagonal elements), or by that of another constituent (the off-diagonal elements). Thus, when all constituents are at such high dilution that they would not 'see' each other or themselves, and the velocities would not depend on the concentrations, the matrix $|\mathbf{A}|$ consist of zeros; any mutual interaction is reflected in non-zero elements in $|\mathbf{A}|$.

With that formalism the velocities, as a vector $\mathbf{u}$, can be written in vector notation:

$$\mathbf{u} = \mathbf{u}^{\mathrm{car}} + |\mathbf{A}|\boldsymbol{\Delta}\mathbf{c} \tag{17}$$

which is still equivalent to Eq. (15).

Applying the MBE (as vector form of Eq. (2)) to a boundary between a 'zone' of the carrier, 'car' and another zone 'α', that differs from the carrier only by small amounts Δc_i, for each i, the result is:

$$c_i^{\alpha}u_i^{\alpha} - c_i^{\mathrm{car}}u_i^{\mathrm{car}} = = (\Delta c_i)U_{\mathrm{B}}\ (\text{each } i) \tag{18}$$

On substitution of Eq. (15) in Eq. (18) it is found that many terms with c_i^{car} cancel. When then products of two Δ terms are neglected one obtains:

$$u_i^{\mathrm{car}}\,\Delta c_i + c_i^{\mathrm{car}}\sum A_{i,j}\,\Delta c_j = = U_{\mathrm{B}}\,\Delta c_i\ (\text{for each } i) \tag{19}$$

or

$$\sum_j H_{i,j}\,\Delta c_j = = U_{\mathrm{B}}\Delta c_i\ (\text{for each } i) \tag{20}$$

with

$$H_{i,j} = c_i^{\mathrm{car}}A_{i,i} \quad \text{If } (i=j) + u_i^{\mathrm{car}} \tag{21}$$

It is often convenient to write the elements of H as (in effect an equivalent definition):

$$H_{i,j} = \frac{\mathrm{d}(c_iu_i)}{\mathrm{d}c_j} = \frac{\mathrm{d}J_i}{\mathrm{d}c_j} \tag{22}$$

where the J_i are the fluxes of i. Eq. (20) can be given likewise in matrix notation:

$$|\mathbf{H}|\,\boldsymbol{\Delta}\mathbf{c} = = U_{\mathrm{B}}\boldsymbol{\Delta}\mathbf{c} \tag{23}$$

As discussed before [8–12], this is the classical eigenvalue/eigenvector equation. It can be solved only for particular values for the scalar U_{B}, the eigenvalues, yielding for each U_{B} a particular set of Δc_i values, forming a $\boldsymbol{\Delta}\mathbf{c}$ vector, indicated in the following by $\mathbf{e}$. As Eq. (23) is homogeneous in $\mathbf{c}$, only the direction of $\mathbf{e}$ is determined; when $\mathbf{e}$ is a solution (with associated U_{B}), multiplying $\mathbf{e}$ with a scalar gives another solution.

The physical meaning of this mathematics, in particular the fact that only particular values for U_{B} are allowed, is as follows: The MBE were derived

under the assumption that the boundary is stable; that is, ahead and astern of the boundary, and close to that boundary, the composition remains constant in time. That, apparently, can be the case only in combination with particular values of the boundary velocity, U_B, with associate values for the vectors **e**. The latter constitute another way to formulate the required relations between the concentrations in the two regions, that was noted already in Section 2.

Thus, the resulting U_B (eigen)values represent velocities with which stable boundaries can propagate (or remain stagnant, when $U_B = 0$). The associated vectors describe the (here, small) differences in the composition ahead and astern of the boundary.

A zone consists of a front boundary, possibly a 'flat' region of constant composition, and a rear boundary. As long as the step from carrier to zone is small, the two boundaries behave in the same way. Therefore, what holds for the migration of a boundary holds also for the migration of the zone (with a more complicated situation when dispersion is taken into account).

Before proceeding with the application of the above relations for particular cases, it is useful to summarize the properties of such eigensystems:

(a) Except for 'pathological' cases, with an N-constituent system there are N different values for U_B, indicated by $U_{B,k}$, each with an eigenvector $\mathbf{e}_k$.

(b) When a particular c_i^{car} value is zero, i.e., a constituent i does not occur in the carrier (the normal case in an analytical separation experiments for a sample constituent, normally not contained in the carrier), one eigenvalue $U_{B,k}$ equals u_i^{car}. Physically: The zone of the constituent in this case moves with a velocity equal to the velocity of the molecules. For the mathematically interested reader: This can be seen by considering that the i-th column in $|\mathbf{H}|$ contains an non-zero element only at the position (i, i); the rules of linear algebra say that for this case the (i, i) element is an eigenvalue.

(c) The extreme of this is when all constituents are entirely independent, i.e., all off-diagonal elements of $|\mathbf{H}|$ are zero. In that case the $U_{B,k}$ set is the same as the set of constituent velocities, u_i, being the diagonal elements of $|\mathbf{H}|$. Each zone corresponds to a particular i, the corresponding vector has only one non-zero element, that of i. Such a situation is impossible in CE, it is hardly imaginable in HPLC where mixed solvent are nearly always used, but it can be approached closely in GC (avoiding such high concentrations that the sorption effect can play a role).

(d) The case with a zero c_i^{car} is virtually the only one (except for shear accidental coincidence of values, and systems where there is no interaction between constituents at all) where an eigenvalues, $U_{B,k}$ is equal to one constituent velocity, u_i^{car}. In all other cases the $U_{B,k}$ values have differing values that can only be found by working out, e.g., Eq. (20). As mentioned, the U_B values are the concentration velocities as in introduced by Helfferich and Klein [2]. The associated eigenvectors contains non-zero elements for all constituents. Physically that means: all concentrations vary in a concerted manner within the zone.

(e) It must be stressed that under such conditions there is often no unambiguous identification of zones with constituents. It is impossible to state: this zone is that of constituent 'X'; it could be as well designated as being the zone of any other constituent, because all constituents vary in concentration. Only in the limit, when $c_X^{carr} \to 0$, there is only one zone where c_X varies (from zero upward) and this can then be identified as the zone of X.

5. Related approaches

It is important to mention the relation of this with earlier treatments. As indicated, Mangelsdorf [8] developed the same concept for gas chromatography. In about the same time Helfferich and Klein in a monumental piece of work [2], studying preparative operation of ion-exchange columns, introduced the concept of coherence. A coherent state is defined as one where a certain concentration of a constituent keeps being accompanied by a set of constant concentrations of the other constituents. That is, the concept is the same as that obtained when assuming a stable boundary. The eigenvector treatment discussed above is a specialization to small, 'infinitesimal', differences between carrier and zone, while coherence and the MBE can apply also to large concentration changes.

Later, Kohlrausch [13] arrived at an eigenvector treatment of band elution of minor disturbances in

liquid chromatography, a treatment that inspired us to earlier and to the present work. That work was formulated in terms highly specific for LC.

Interest in these phenomena used to be small in the field of analytical separations, except for some studies related to the determination of the mobile phase volume [14–18]. For straightforward operation of HPLC and GC there is indeed not much need to sort this all out: The carrier components are usually chosen to be non-responsive in the detector, so that any variations in the concentration of non-sample, carrier, constituents remain invisible; the sample constituent are the only ones generating any detector signals. The zones of the latter move with a velocity U_B, equal to u_i^{car} when the concentrations of sample constituent i are low (rule (b) given above). Thus, nothing related to the above discussion becomes manifest in such experiments. What one is usually aware of is the fact that the velocity u_i^{car} depends on the carrier composition ('moderators', 'modifiers', methanol content, pH (chiral) complexing agent, etc.), but in a once assembled carrier, the u_i and the corresponding zone velocities are virtually constant.

Much more interest in these aspects arose with the introduction of CE and with the introduction of indirect detection techniques in HPLC and CE, and partially because of the interest in isotherm studies.

In CE the concentrations of analytes is often not very small compared to those of the carrier constituent, a fact mainly brought about by the poor sensitivity of CE detectors, and the limitation on electrical conductivity of the carrier, limiting buffer concentrations to about 20 mmol/l. As a result, analyte velocities are not constant, the peaks are often asymmetrical, something often indicated as 'electromigration dispersion'. In order to assess this effect, i.e., how the velocity of a zone of an analyte depends on its concentration, it is necessary to know how the carrier composition within the zone varies with the analyte concentration, a task equivalent to solving the eigenvector problem stated above. As early as 1979, Mikkers et al. [19] performed this task, by quite other means, using the KRF.

Indirect detection in HPLC [10] and CE [20] is even more closely related to the above discussion. The variation of the concentration of one of the carrier constituent (the monitoring constituent), 'M', is followed with a suitable detector. This works only provided that the presence of analyte 'X' is accompanied by a change in the concentration of 'M'. That can be the case only when there is interaction between 'X' and 'M', i.e., the diagonal element in **A** corresponding to 'X' and 'M' must be non-zero.

Also, in the context of preparative LC, the interaction of the solutes among each other, and with constituents of a mixed mobile, can be very important, as has been shown by full numerical simulation to the chromatographic process [21]. However, by the nature of preparative purpose, the disturbances are always large, with the result that eigenvector approach can be used only with a thorough modification, discussed at the end of this article.

Of course for LC as well as for CE one can resort to full numerical simulation of the coupled transport of all the constituents, as has been done by the groups of Williams et al. [22] and earlier by Mosher et al. [23]. Although this may be the last possibility for some intricate cases, we believe that this procedure is too slow and of a too limited applicabilty to be useful as an aid in method development. Also, it does not provide the insight of coherence as a key in the understanding of the phenomena.

6. First example, two Langmuir distributed constituents in HPLC

According to Eq. (9), the distribution is described as (we have set the S_j to 1, and the phase ratio, q, equal to 1, which does not subtract anything from the essence of the argument):

$$c_{1,s} = \frac{K_1 c_{1,m}}{1 + K_1 c_{1,m} + K_2 c_{2,m}} \tag{24}$$

$$c_{2,s} = \frac{K_2 c_{2,m}}{1 + K_1 c_{1,m} + K_2 c_{2,m}} \tag{25}$$

For the calculation of $|\mathbf{H}|$ one needs (Eq. (22)) $d(c_i u_i)/dc_j$ values. Considering Eq. (24), these are equal to:

$$H_{i,j} = u_0 \frac{dc_{i,m}}{dc_j} \quad \text{If } (i = j) + u_i \tag{26}$$

We can define a matrix $|\mathbf{M\_from\_T}|$ ('Mobile from Total') having the derivatives in the RHS of Eq. (26)

as the elements. This matrix is the inverse of another matrix, |**T_from_M**| ('Total from Mobile') having elements:

$$\mathrm{T\_from\_M}_{i,j} = \frac{\mathrm{d}c_i}{\mathrm{d}c_{j,\mathrm{m}}} \tag{27}$$

Note that the derivatives in Eq. (26) are taken at constant $c_{z<>j,\mathrm{m}}$, whereas in Eq. (27) they are taken at constant $c_{z<>j}$. This leads exactly to the indicated matrix inversion.

Eq. (27) in turn can be written as:

$$\mathrm{T\_from\_M}_{i,j} = q\mathrm{S\_from\_M}_{i,j} \quad \text{If } (i=j)+1 \tag{28}$$

The +1 reflects the amount of a constituent present in the mobile phase.

The |**S_from_M**| matrix can be found straightforwardly by taking the derivatives of Eq. (25). Then, once numerical values have been found one can work back through Eqs. (28) and (27) to find |**H**|, using standard matrix routines.

The derivatives of Eq. (25) can also be given as analytic (although unwieldy) expressions, also with more than two constituents. These are not used here. A numerical method is more convenient and is inevitable anyway (because of the matrix inversion) for three or more constituents.

As an illustration, consider a case where $c_{1,\mathrm{m}}=0.0$, $c_{2,\mathrm{m}}=0.5$, $K_1=3$ and $K_2=4$, where for the sake of the argument one can take component 1 as the 'analyte' and component 2 as the 'moderator'. This leads to the following result (taking $q=1$ and $S=1$):

$$\begin{aligned}
|\mathrm{S_from_M}| &= 1 \quad 0\\
&\quad -0.667 \quad 0.444\\
|\mathrm{T_from_M}| &= 2 \quad 0\\
&\quad -0.667 \quad 1.444\\
|\mathrm{M_from_T}| &= 0.5 \quad 0\\
&\quad 0.231 \quad 0.692
\end{aligned} \tag{29}$$

|**S_from_M**| is the analogue of the distribution coefficient K_i used when only one constituent is considered. The element $|\mathbf{S\_from\_M}|_{1,1}$ describes the change of $c_{1,\mathrm{s}}$ with $c_{1,\mathrm{m}}$. It is considerably smaller than K_1 (=3), because most of the surface is covered by constituent 2. In fact, only a fraction $1/(1+c_2K_2)=0.3333$ is free to absorb, which explains the decrease of the retention factor from 3 to 1.

The zero in the upper row reflects the fact that with a zero $c_{1,\mathrm{m}}$, the $c_{2,\mathrm{m}}$ cannot exert an influence on the absorbed amount of 1.

The element −0.667 in |**S_from_M**| describes the 'modifying' effect of the carrier constituent 2; when $c_{2,\mathrm{m}}$ is increased, constituent 1 desorbs. Finally it must be noted that the value $|\mathbf{S\_from\_M}|_{2,2}$ (0.444) is much smaller than the K_2 value (4). This corresponds to the often observed fast migration of modifier disturbances; although their absorption may be high, there is relatively little change in absorption when the concentration varies by a small amount.

The conversion of |**S_from_M**| into |**T_from_M**| and |**M_from_T**| is straightforward. Note that the values in |**M_from_T**| are zero or positive: Adding more of whatever compound will increase all the $c_{i,\mathrm{m}}$, as less surface becomes available for all of them.

With a carrier velocity of $u_0=100$, as an example, we get for the matrix |**H**|, according to Eq. (26):

$$\begin{aligned}
|\mathrm{H}| &= 50 \quad 0\\
&\quad 23.1 \quad 69.2
\end{aligned} \tag{30}$$

The eigenvalues and eigenvectors found for this matrix are:

$$\begin{aligned}
&U_{b,1}=50.0 \quad e_{1,1}=0.640 \quad e_{1,2}=-0.768\\
&U_{b,2}=69.2 \quad e_{2,1}=0 \quad\quad e_{2,2}=1
\end{aligned} \tag{31}$$

Clearly, in this case one solution, the first, can be identified as 'that of the analyte'. It has a velocity 50, corresponding to a retention factor k' of 1, as was anticipated above. The eigenvector e_1 has two non-zero elements with opposite sign, indicating that at the position of the solute there is a decreased concentration of the moderator (which would allow indirect detection via constituent 2 as a monitoring substance). The second solution involves the 'moderator', 2, and describes the migration of a disturbance in the moderator concentration.

One of the pitfalls of considering such phenomena without insight into the concept of coherent transport is the following reasoning: by nature, the adsorptive interaction is competitive, when more of one compound is adsorbed, less of the other will be adsorbed. It would therefore lie at hand to expect that at the position of the solute always a positive disturbance in the modifier concentration would be present. In terms of indirect detection language: the response

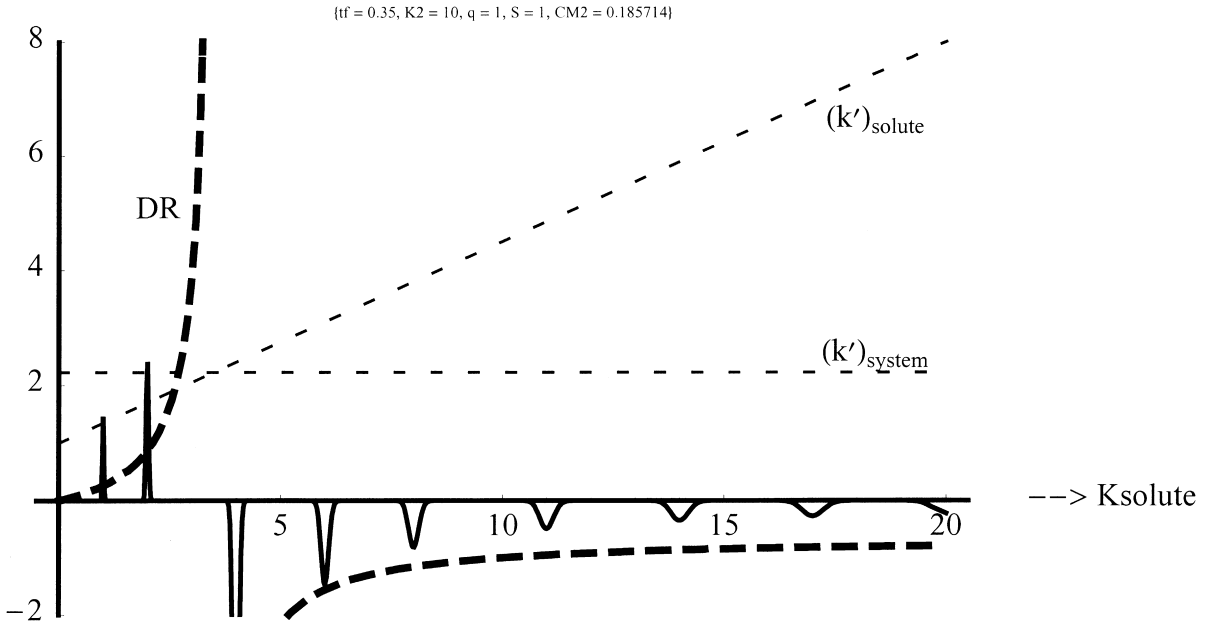

Fig. 1. Predicted chromatogram with indirect detection in a two-constituent system, with phase ratio, *q*, and saturation capacity, *S*, set to 1 for both constituents; distribution constants $K_2 = 10$; K_1 is abscissa, 'K_{solute}'. Concentration of constituent 2, moderator, chosen such that 65% of the adsorbent is covered. Inserted peaks were calculated on the basis of a plate number of 5000, with an intensity on the basis of the eigenvector ratio, assuming equal amounts injected.

factor would be positive always. However, as has been clearly experimentally shown and explained in the work directed by Crommen and co-workers [10,24], the response often changes sign, depending on the relative affinity of analyte and monitoring substance. Fig. 1 illustrates this.

To end this section we note that the eigenproblem can be solved as well with the |**S_from_M**| and |**T_from_M**| matrices (yielding eigenvalues in terms of K and $(1+k')$, respectively); the translation into |**M_from_T**|, yielding ($u_0 R_f$ values) was only done here in order to fit the chromatographic case in the more general framework.

7. Second example: electrophoresis

The constituent velocity in a zone electrophoretic experiment depends on the local field and the effective mobility $\mu_{e,i}$, the latter being the average over all forms (e.g., acid–base forms, having differing charges) it occurs, according to Eq. (1). The field, E, however, is different from that in the undisturbed carrier. As the current in the capillary is uniform over its length it holds for zone α:

$$E^{\alpha}\kappa^{\alpha} = E^{\mathrm{car}}\kappa^{\mathrm{car}} \tag{32}$$

The conductivity, κ, in turn, depends on the composition of the electrolyte, via:

$$\kappa = \sum_{\text{all ions } m} s_m \mu_m z_m \tag{33}$$

where s_m is the concentration of a ionized form of the a constituent. For the calculation of κ it is needed to work in terms of the individual concentrations of ionized forms, and change the symbols for c to s and the index from j to m, as for multi-charged ions summing up over all *forms* is the only correct way to obtain the conductivity.

It follows from Eqs. (32) and (33) that in electrophoresis there is a very strong interaction. The analysis of such systems has been carried out

traditionally by using KRF; as early as 1979 Mikkers et al. [19] addressed this issue comprehensively for simple carrier solution containing strong ions. Some important results obtained by them are:

(a) When the mobility of the analyte equals that of the co-ion in the carrier, there is a 1:1 displacement of the co-ion by the analyte, while the concentration of the counter-ion remains unaffected (this has to be so in order to maintain electroneutrality). In that case the conductivity in the analyte zone is the same as in the carrier; the field, and with that the velocity does not depend on the concentration. As a result a symmetrical band develops, that is only broadened by diffusion and possibly other dispersion processes, but not by a non-linearity.

(b) For a mobility with a smaller mobility (later in the electropherogram) the displacement ratio is not 1:1, but smaller. Electroneutrality is maintained by a associated change in the counter-ion concentration. At high analyte concentration the conductivity is decreased. Qualitatively this can be understood when assuming—incorrectly—a 1:1 displacement: ions of small mobility are replacing ones with a high mobility (see Eq. (2)). As a result, the field is higher and the ion moves faster, when at higher concentrations. For a larger mobility of the analyte of course the reverse holds. This leads to the typical asymmetry observed in electropherogram of such simple systems: early peaks 'front', later peaks 'tail', at the position corresponding to the mobility of the co-ion peaks are narrow and symmetrical. The phenomenon is known as electromigration dispersion.

(c) A zone of zero mobility is present, consisting of the carrier in either a more diluted or a more concentrated form. This is the only zone (and this applies also for a system with many strong ions) in which the KRF differs from that of the carrier; for all the moving zones it is equal. Injection of a solution having not the same value for the KRF gives rise to such a stagnant zone.

The approach put forward in this work leads to exactly the same results. The eigenvector belonging to the analyte in the case of equal mobilities is $\{e_{1,X}=1,\ e_{1,C}=-1,\ e_{1,D}=0\}$, where subscripts X, C and D stand for analyte, co- and counter-ion. For other analyte mobilities explicit expressions in mobilities can be obtained (see Refs. [19,25], for a smaller value, e.g., a vector $\{e_{1,X}=1,\ e_{1,C}=-1.2,\ e_{1,D}=-0.2\}$ could be found. The transfer ratios, as introduced by Yeung are thus 1.0 and 1.2,, respectively, for these two cases.

The eigenvalue of the stagnant zone is zero. Using the index 'eo' for this zone, as it is often convenient to monitor electroosmotic flow by mean of it, its vector is $\{e_{eo,X}=c_X^{car},\ e_{eo,C}=c_C^{car},\ e_{1,D}=c_D^{car}\}$.

For more complicated carrier and acid–base reactions in both the carrier and the analyte, the derivation of such relations becomes increasingly complicated, while for polybasic constituent, as well as at high and low pH the analytic solution of the equations proves to be impossible. It is indicated in those cases to work numerically, as demonstrated above for multicomponent chromatography. The scheme then is as follows:

(1) With an analyte at concentration zero, for a given carrier, e.g., specified in terms of all constituent concentrations such as acetate, phosphate, sodium, the first step is to calculate pH, the distribution of each constituent over its ionization stages, as well the conductivity. The pH calculation usually requires an iterative solution of, e.g., the electroneutrality, the other steps can be explicit. Take as an example a phthalic acid/sodium phthalate ('Na'+'Ph') carrier with 'X' as a strong analyte ion. Its composition is specified by the total concentrations $c_{tot,Ph}$, c_{Na} and c_X. The fractions of 'Ph' with charges 0, −1 and −2 are indicated by $\alpha_{2,Ph}$, $\alpha_{1,Ph}$ and $\alpha_{0,Ph}$ (using the number of protons as index).

(2) Also the derivatives of the above quantities with respect to the concentrations of the constituents are needed. Therefore one has to find:

$$\frac{d(pH)}{dc_{tot,Ph}},\ \frac{d(pH)}{dc_{Na}},\ \frac{d(pH)}{dc_X} \tag{34}$$

$$\frac{d\kappa}{dc_{tot,Ph}},\ \frac{d\kappa}{dc_{Na}},\ \frac{d\kappa}{dc_X} \tag{35}$$

$$\frac{d(\alpha_{Ph,0})}{dc_{tot,Ph}},\ \frac{d(\alpha_{Ph,1})}{dc_{Na}},\ \frac{d(\alpha_{Ph,2})}{dc_X} \tag{36}$$

Fortunately, explicit expressions for all these can be derived.

With the Tiselius equation:

$$\mu_{i,\mathrm{eff}} = \sum_{\text{forms } m} \alpha_{i,\mathrm{m}}\,\mu_{i,\mathrm{m}} \qquad (37)$$

the velocities then can be found, as well as the derivatives of the velocities. That is, the matrices $|\mathbf{A}|$ and $|\mathbf{H}|$ are then available, and the velocities and the displacement ratios can be found as eigenvalues and eigenvectors.

This scheme reproduces the results obtained by using the KRF for all cases where the latter is appropriate, whereas it is easily applicable to rather complicated electrophoretic systems. [26–29].

8. Example 3, complexation in CZE, affinity CZE

Affinity electrophoresis for studying complex formation in solution has become very popular in recent years, because the method offers many advantages, especially for the study of interactions of biochemical interest [30–33]. Briefly, the most popular method, in which information is gained from the migration rates, works as follows:

Studying the interaction between a compound 'M' and a ligand 'L' (e.g., a protein-binding drug and a protein), the migration rate of one constituent, say 'M', is studied as a function of the concentration of 'L' in the carrier, c_{L}. When the complexation is fast, and when results are reported in terms of observed mobilities, $\langle\mu\rangle$, the data plotted as a function of c_{L} vary between the mobility of the parent compound 'M', μ_{M}, and that of the fully formed complex ML_n, at very high c_{L} values. The degree of complexation can thus be derived—for simple cases by linear interpolation—from the observed mobility change. From these, in turn, the stability constant, e.g., K_{ML} can be estimated.

This method looks sound when the occurring concentrations of M (which can be either the drug or the protein) can be kept low. One difficulty is often that the limiting value u_{ML} cannot be found directly, as the corresponding high c_{L} concentrations cannot be accessed. In this case a two-parameter estimation for both K_{ML} and u_{ML} has to be performed.

There are several reasons why it is interesting to consider cases where the concentrations c_{M} are not very much smaller than L_{t}. In the first place the question arises whether the consumption of L by the formation of ML decreases the actual L concentration. In the second place, the c_{M} values often cannot be chosen very low, as detection is not very sensitive; frequently lower limits are in the range of 10^{-6} mol/l. The ligand concentration in the buffer, L_{t}, on the other hand, has to be chosen not too far from $1/K_{\mathrm{ML}}$, as otherwise either no or complete complexation takes place. Thus, with K_{ML} values larger than 10^5 l/mol it will often prove impossible to maintain a large $c_{\mathrm{L}}/c_{\mathrm{M}}$ ratio.

Further, the two-parameter estimation often used puts stricter requirement on the requires a reliability of the model. This it is important to investigate whether the use of a straightforward equilibrium equation, taking $c_{\mathrm{L}}^{\mathrm{car}}$ for $c_{\mathrm{L}}^{\mathrm{a}}$ introduces errors.

A last reason is in a recent proposal [34] to do affinity CZE (vacancy affinity electrophoresis) at non-zero concentration of both reaction partners, injecting minor disturbances on this carrier composition.

The methods explained above allow to obtain insight into such situations. As an illustration, the case of a 1:1 complexation will be treated below. It is assumed that the normal non-idealities of CE such as conductivity changes and pH changes are absent; the field E is constant and the velocities can be replaced by the mobilities:

The MBE (variation on Eq. (2)) for a zone, α, of an equilibrium mixture M/ML in a carrier, 'carr', containing only L are then:

$$\text{for M: } c_{\mathrm{M}}^{\alpha}\mu_{\mathrm{M}} + c_{\mathrm{ML}}^{\alpha}\mu_{\mathrm{ML}} - (c_{\mathrm{M}}^{\alpha} + c_{\mathrm{ML}}^{\alpha})U_{\mathrm{B}} = = c_{\mathrm{M}}^{\mathrm{car}}\mu_{\mathrm{M}} + c_{\mathrm{ML}}^{\mathrm{car}}\mu_{\mathrm{ML}} - (c_{\mathrm{M}}^{\mathrm{car}} + c_{\mathrm{ML}}^{\mathrm{car}})U_{\mathrm{B}} \qquad (38)$$

$$\text{for L: } c_{\mathrm{L}}^{\alpha}\mu_{\mathrm{L}} + c_{\mathrm{ML}}^{\alpha}\mu_{\mathrm{ML}} - (c_{\mathrm{L}}^{\alpha} + c_{\mathrm{ML}}^{\alpha})U_{\mathrm{B}} = = c_{\mathrm{L}}^{\mathrm{car}}\mu_{\mathrm{L}} + c_{\mathrm{ML}}^{\mathrm{car}}\mu_{\mathrm{ML}} - (c_{\mathrm{L}}^{\mathrm{car}} + c_{\mathrm{ML}}^{\mathrm{car}})U_{\mathrm{B}} \qquad (39)$$

8.1. Zone M/ML in carrier containing only L

For that case the RHS of Eqs. (38) and (39) is zero, and it follows from this equation:

$$\mu_B = \frac{c_{\mathrm{M}}^{\alpha}\mu_{\mathrm{M}} + c_{\mathrm{ML}}^{\alpha}\mu_{\mathrm{ML}}}{(c_{\mathrm{M}}^{\alpha} + c_{\mathrm{ML}}^{\alpha})} = \mu_{\mathrm{M}} + p(\mu_{\mathrm{ML}} - \mu_{\mathrm{M}}) \qquad (40)$$

where p is the fraction complexed; indeed the weighted averaged mobility as was indicated above, and which would be independent of the concentration level of M. However, although the measurement of p is correct, it is still uncertain whether c_{ML} represents the equilibrium concentration of c_M^{α} and c_L^{car}, as one assumes in the data handling. That is, it holds:

$$c_{ML}^{\alpha} = K_{ML} c_L^{\alpha} c_L^{\alpha}, \text{ NOT: } c_{ML}^{\alpha} = K_{ML} c_L^{car} c_L^{car} \tag{41}$$

The unknown ligand concentration in the zone, c_L^{α}, can only be estimated (normally not be measured) by analyzing the transport equations. Eqs. (38) and (39) can be solved directly for that purpose. However, it is preferred here to again resort to the case of minor disturbances, where an eigenvector solution can be found. A convenient approach to that is similar to the one used above in chromatography, consisting in first expressing the fluxes in terms of the free concentrations changes (an easy, explicit relation). This lead to a matrix $|\mathbf{J\_from\_F}|$ (derivatives of Fluxes **J** with respect to the **F**ree Concentrations, in the sequel for clarity indicated as M_f and L_f), These are easily found as the derivatives of, e.g., $u_M M_f + u_{ML} K_{ML} M_f L_f$. Next the variations in the free concentrations as a function of the disturbances in the total concentrations, M_t and L_t, are found, leading to a matrix $|\mathbf{F\_from\_T}|$. This is done by inversion of $|\mathbf{T\_from\_F}|$; for the latter again an easy explicit form is available (e.g., the derivatives of $M_t = M + K_{ML} M_f L_f$). The matrix product $|\mathbf{J\_from\_F}| \cdot |\mathbf{F\_from\_T}|$ gives the desired **J_from_T** matrix.

For the particular case of 1:1 complexation of M and L the $|\mathbf{J\_from\_T}|$ matrix is found to be:

$$\begin{matrix} \dfrac{u_M + K_{ML} M_t u_M + K_{ML} L_f u_{ML}}{1 + K_{ML} L_f + K_{ML} M_f} & \dfrac{K_{ML} M_f (-u_M + u_{ML})}{1 + K_{ML} L_f + K_{ML} M_f} \\ \dfrac{K_{ML} L_f (u_L + u_{ML})}{1 + K_{ML} L_f + K_{ML} M_f} & \dfrac{u_L + K_{ML} L_f u_L + K_{ML} M_f u_{ML}}{1 + K_{ML} L_f + K_{ML} M_f} \end{matrix} \tag{42}$$

Explicit expressions for the eigenvalues/vectors can be found. Those for arbitrary M_t and L_t are, however, too complicated to print here and to inspect visually. For the case where $M_t = 0$, that is, the usual experimental condition in ACE where one constituent does not occur in the carrier, the expressions are illustrative: The first eigenvalue has the trivial value described by Eq. (40), i.e., the expression used universally in this field. The second eigenvector is less trivial:

$$\left\{ 1, \frac{K_{ML} L_f (u_L - u_{ML})}{u_L + K_{ML} L_f u_L - u_M - K_{ML} L_f u_{ML}} \right\} \tag{43}$$

describing the 'displacement effect' (cf indirect detection), i.e., what change in the L_f concentration accompanies the presence of M in the zone. As can be seen, the (total!) concentration in L is disturbed, when the mobility of the complex u_{ML} differs from that of the ligand u_L, $u_{ML} <> u_L$. This potential source of error has been noticed before [32]. The absence of this deviation when $u_{ML} = u_L$ can be understood by an elementary consideration: In that case the total transport of L is not affected at all by the complexation, so L behaves in that case as if M is not present. This situation may be less unlikely than it seems: e.g., when L is a protein and M is a drug of which the binding is studied, the mobility of the complex may not be much different from that of the parent protein.

However, even when $u_{ML} = u_L$, the problem indicated above below Eq. (41) still persists, as it is the free concentration, not the total, that has to be inserted in the equilibrium expression. In alternative cases, i.e., when $u_L <> u_{ML}$ the uncertainty about the ligand concentration in the zone is of course exacerbated, the deviation being caused not only by stoichiometric consumption, but also by differing migration rates.

For the case $M_t = 0$ the other eigenvalue, equal to u_L, corresponds to the migration of a disturbance in the L concentration, L_t, a one-dimensional phenomenon, the eigenvector naturally being {0, 1}.

When M_t is not zero, often the case for reasons of detectability, different values are found. Fig. 2 illustrates, as a function of the concentration of the injected constituent M, the behaviour for three velocities for M: the constituent velocity, the concentration velocity, as predicted here, and the velocity calculated assuming undisturbed c_L concentration, with stoichiometric consumption of L within the zone. The assumed u values were $u_M = 10$, $u_{ML} = 40$ and $u_L = 60$, and the ligand concentration was

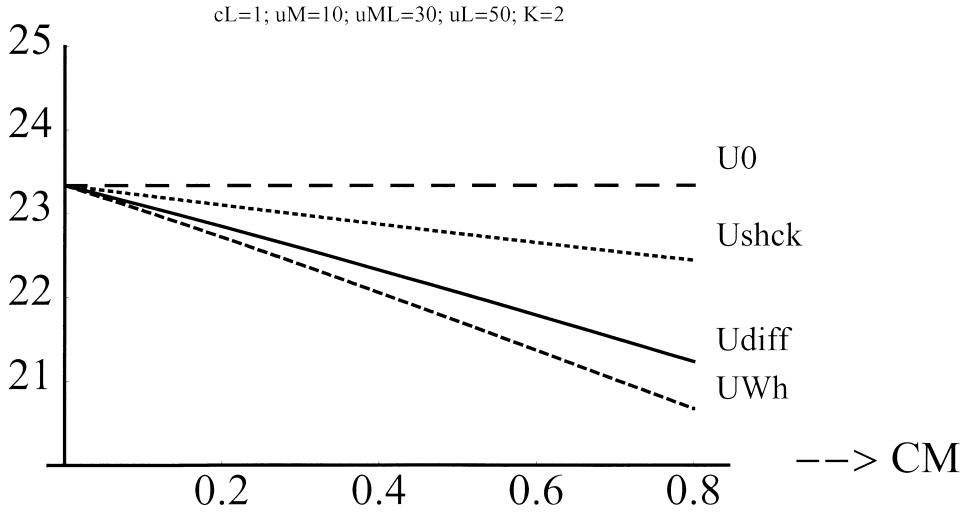

Fig. 2. Plot of three velocities in a hypothetical affinity capillary electrophoresis experiment, for the 1:1 complexation of M to L. Assumed mobilities $\mu_M = 10$, $\mu_{ML} = 40$, $\mu_L = 60$. Concentration L in carrier $c_L = 1$, formation constant $K_{ML} = 2$. Lines: U_{diff} (full), mobility of diffuse boundary (or of minor disturbance at that concentration level); U_{shck} (dotted), mobility of M in zone, also equal to the velocity of the steep boundary; U_{Wh} (dashed), calculated with Eq. (40), with allowance for consumption of L in formation of ML; U_0 (long dashed), with Eq. (40) without such allowance.

$c_L = 1$, $K_{ML} = 2$. As can be seen, the deviations appear not to be very serious in this case, considering the expanded scale of the figure (full range of complete complexation corresponds to μ values from 10 to 40) and the large ratios c_M/c_L up to 0.4. However, with some combinations of mobility values, especially when the u_L is in between u_M and u_{ML}, rather drastic deviations may occur. Also, for unknown u_{ML} and multiple-step reactions, the effects of such deviations on the accuracy of the reclaimed data are quite unpredictable. The consequences for ACE and VACE are presently under study.

9. Intensity of system peaks in electrophoresis

In the above it was indicated how the velocities of zones and with that their position after a time lapse or their elution functions can be predicted from known properties of the system at hand. How intense these zones are, depends of course on the way they are 'excited', i.e., normally on the injection process. As briefly indicated before for CE [11], the vector formulation allows to derive the necessary equations in a quick and clear manner. It is discussed here in terms of position distribution after a time lapse ('column maps'), but with suitable modification it can be used also for elution function or detector traces.

Define the intensity function $IF_k(z)$ of a zone k, migrating with velocity $U_{B,k}$ with eigenvector $e_{k,i}$ such that:

$$c_i(z) = c_i^{car} + e_{k,i}\, IF_k(z) \tag{44}$$

which should be possible as all variations in the c_i within the zone k are in proportion to the elements $e_{k,i}$. That is, $IF_k(z)$ behaves as a zone distribution function, as if it were one of a constituent. The concentration distribution function $(c_i(z) - c^{car})$ follows by multiplication with $e_{k,i}$.

As 'EigenAreas', $Area_{i,k}$, we define the integrals of the $IF_k(z)$ values:

$$EigenArea_k = \int IF_k(z)\, dz \tag{45}$$

These values indicate the intensity of the total

propagating zone, analogous to a peak area. The total integral of the deviations in concentration, over the whole system, i.e., 'catching' all zones, is

$$\int (c_i(z) - c_i^{\mathrm{car}})\,\mathrm{d}z = \sum_k e_{k,i}\,\mathrm{EigenArea}_k \tag{46}$$

As 'Injections', Inj_i, are defined:

$$\mathrm{Inj}_i = \Delta z_{\mathrm{inj}}(c_i^{\mathrm{inj}} - c_i^{\mathrm{car}}) \tag{47}$$

where is the length of the injection plug, i.e., the part of the capillary where the carrier is thought to be replaced by the injection.

Clearly, the task is to find the relation between $|\mathbf{EigenArea}|$ on the one hand, which allows the find the concentration excursions of each i on the one hand, and the known **Inj** on the other hand.

After a time lapse, but before any zone has left the column (which can be conveniently be assumed to be infinitely long at both sides, so that there is no problem with the latter condition), the integral mass balance for each constituent must still hold.

That is, the expressions in Eqs. (46) and (47) must be equal:

$$\mathrm{Inj}_i = \sum_k \mathrm{EigenArea}_k e_{k,i} \tag{48}$$

In vector notation:

$$\mathbf{Inj} = \mathbf{EigenArea}|\mathbf{e}| \tag{49}$$

Thus, the **EigenArea** can be found by:

$$\mathbf{EigenArea} = |\mathbf{e}|^{-1}\,\mathbf{Inj} \tag{50}$$

10. Non-linearity in coherent zones

The above was all derived under the assumption that the disturbances are infinitesimally small, so that the MBE (or any other formulation of the mass balance) can be linearized into a set of equations such as Eqs. (20) and (23). However, once the numerical machinery for finding eigenvectors/values is available, there is an elegant way to describe the non-linearity effects when disturbances are not so small. In this way, shifts in peak positions and the formation of non-symmetrical zones can be readily described. In principle, provided sufficient numerical brute force is applied, the method is also suitable for heavily overloaded systems.

The principle has apparently been used for the first time by Helfferich [35], in the context the chromatography-related processes occurring in oil recovery. For HPLC and CE is was applied [11,12]; after reinvention.

Once the eigenvectors have been found at a particular composition, as described above, one can find a new composition, which can migrate in a coherent manner, by adding a small amount of one of the vectors to the composition. In the new point the eigenvalues/vectors can be found again, normally yielding only one new eigenvalue that deviates only slightly from the starting eigenvalue, whereas the vector direction may also slightly deviate. For the new composition the process can be repeated, so that a 'path' is formed by the subsequent vector segments, describing the compositions of a persistent (coherent) boundary. Along the path, a said, the eigenvalue, i.e., the boundary velocity, will vary; that is the velocity is found as a function of the concentration change along the coherent path. In this way one of the boundaries of the zone (the diffuse, not the shock-type, sharp one) can be constructed, by translating velocities into positions or residence times.

For small overload, for 'reasonable' system where the 'paths' are fairly straight and the change of velocity behaves linearly, one such an iterative step suffices; it yields the velocity at the 'origin', the eigenvector, and the change of the velocity with the intensity of the disturbance. Linear extrapolation than allows to construct the full diffuse boundary. The approach is quite analogous to the one presented by Beckers [36], where the MBE in its original form is iteratively solved for the diffuse boundary of a zone. The eigenvector scheme has the advantage of being much more generally applicable.

The latter scheme [11] has been used for predicting CE elution patterns [37], with applications in the CE of peptides [29], as well as for the quantitative evaluation of pH-mediated electromigration dispersion [26]. In such situations the effect of dispersion is nearly always important. This can be accounted for by modifying the triangles or trapezium shapes obtained by means of the Houghton/Haarhof and

van der Linde expressions [27], inserting a suitable dispersion coefficient.

In preparative LC, where overloading is more severe, and the occurrence of not-yet-coherent boundaries is much more likely, the approach appears [12] to be less successful.

11. Conclusion

A more or less general approach for analyzing the effect of mutual interaction in differential migration separation systems has been formulated. It allows to predict system peaks and indirect detection phenomena, quantitatively, for minor disturbances, in principle for any assumed shape of isotherms, mobility function, complexation degree, etc. The prediction include position and intensity of concentration disturbances. For larger disturbances an extension of the method can be used, allowing the prediction of peak asymmetry. For some simple cases the method can yield (with the help of a symbolic manipulator such as Mathematica) explicit expressions.

It is hoped that this formalism will contribute to the understanding of system peaks, so that incorrect direct interpretation of observed 'displacement effects' in terms of chemical phenomena such as competition and complexation can be avoided in the future.

12. Glossary

$\lvert\mathbf{A}\rvert$	$N\times N$ matrix with elements $A_{i,j}$
$A_{i,j}$	matrix element, $\mathrm{d}u_i/\mathrm{d}c_j$
c	composition vector of length N, i.e., set of concentrations c_i that exhaustively describe composition
c_i^{α}	concentration of i in zone α
car	composition vector when there is no solute
c_i	concentration of component i
c_i	in section of chromatography: total concentration of i, i.e., $c_{i,\mathrm{m}}+qc_{i,\mathrm{s}}$
$c_{i,\mathrm{m}}$, $c_{i,\mathrm{s}}$	concentrations of i in mobile and stationary phase
E	electric field strength
E^{ω}	electric field strength in zone ω
$\lvert\mathbf{e}\rvert$	matrix consisting of all eigenvectors $\mathbf{e}_k$
$\mathbf{e}$, $\mathbf{e}_k$	eigenvector with elements e_i or $e_{k,i}$, respectively
e_i, $e_{k,i}$	eigenvector element, describing the change of i in a zone (in a zone k)
$\mathrm{EigenArea}_k$	integral of $\mathrm{IF}_k(z)$ with respect to z
eo	as a subscript refers to the stagnant zone (moving with the electro-osmotic velocity)
$\lvert\mathbf{F\_from\_T}\rvert$	$N\times N$ matrix with elements $F\_\mathrm{from}\_T_{i,j}$
$\lvert\mathbf{H}\rvert$	$N\times N$ matrix with elements $H_{i,j}$
$H_{i,j}$	matrix element equal to $A_{i,j}$ except when $i=j$, when it is $A_{i,j}+u_{\mathrm{i}}^{\mathrm{car}}$
$\mathrm{IF}_k(z)$	function of the position in the column z, describing the shape of a zone k
Inj_i	amount injected per unit cross-section area, taking the carrier as the zero point, i.e., $\Delta z_{\mathrm{inj}}(c_{\mathrm{i}}^{\mathrm{inj}}-c_{\mathrm{i}}^{\mathrm{car}})$
J_i	flux of i
$\lvert\mathbf{J\_from\_F}\rvert$	$N\times N$ matrix with elements $J\_\mathrm{from}\_F_{i,j}$, the derivative of flux of i with respect to free concentration of j
$\lvert\mathbf{J\_from\_T}\rvert$	$N\times N$ matrix with elements $J\_\mathrm{from}\_T_{i,j}$
k_i'	retention factor
K_i	equilibrium constant of component i in Langmuir isotherms
K_{ML}	equilibrium formation constant of complex ML from M and L
KRF	Kohlrausch' regulating function $=\Sigma_{\text{all ions } i}c_jz_j/\mu_j$
L	ligand
L	column length
L_{f}, L_{t}	free and total concentration of L, respectively
M	compound to react with ligand L in ACE experiment
$\lvert\mathbf{M\_from\_T}\rvert$	$N\times N$ matrix with elements $M\_\mathrm{from}\_T_{i,j}$
$M\_\mathrm{from}\_T_{i,j}$	matrix element, $\mathrm{d}c_{i,\mathrm{m}}/\mathrm{d}c_j$
M_{f}, M_{t}	free and total concentration of M, respectively

N	number of independent components
p	fraction of M converted to ML
q	phase ratio, amount of stationary over amount of mobile phase
$\|\mathbf{S\_from\_M}\|$	$N\times N$ matrix with elements $S\_\text{from}\_M_{i,j}$
$S\_\text{from}\_M_{i,j}$	matrix element, $\mathrm{d}c_{i,\mathrm{s}}/\mathrm{d}c_{j,\mathrm{m}}$
s_i	concentration of ionized form i of some component
S_i	saturation value of $c_{i,\mathrm{s}}$ in Langmuir isotherm
$\|\mathbf{T\_from\_F}\|$	$N\times N$ matrix with elements $T\_\text{from}\_F_{i,j}$
$T\_\text{from}\_F_{i,j}$	matrix element, derivative of total concentration of i with respect of free concentration of j
$\|\mathbf{T\_from\_M}\|$	$N\times N$ matrix with elements $T\_\text{from}\_M_{i,j}$
$T\_\text{from}\_M_{i,j}$	matrix element, $\mathrm{d}(c_{i,\mathrm{m}}+qc_{i,\mathrm{s}})/\mathrm{d}c_{j,\mathrm{m}}$
$t_{\mathrm{R},i}$, $t_{\mathrm{R},i}(c_{i,\mathrm{m}})$	retention time, retention time of concentration $c_{i,\mathrm{m}}$
U, U_B, U_k	velocity of boundary, of boundary B or k
u_i^{α}	velocity of component i in zone α.
u_0	velocity of mobile phase (c.q. outside solute zone)
u_i	velocity of component i
$u_{\mathrm{z}0}$	gas velocity in gas chromatography inside solute zone
X_i	mole fraction of i in gas phase
z_i	nominal charge of ion i

12.1. Greek symbols

$\alpha_{i,\mathrm{m}}$	fraction of component i in form with m protons attached
$\mathbf{\Delta c}$	vector consisting of Δc_i values
Δz_{inj}	length of injection plug
Δc_i	(small) deviation in concentration of i, in zone, relative to carrier
κ^{ω}	conductivity in zone ω
μ_B	velocity of zone expressed as mobility, i.e., u_B/E^{car}
μ_i	electrophoretic mobility of ion i
$\mu_{i,\mathrm{eff}}$	effective (averaged over various forms) mobility of component i
$\mu_{i,\mathrm{m}}$	mobility of form m of component i

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