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Eigenmobilities in background electrolytes for capillary zone electrophoresis

I. System eigenpeaks and resonance in systems with strong electrolytes

Milan Štědrý, Michal Jaroš, Bohuslav Gaš*

Faculty of Science, Charles University, Albertov 2030, CZ-128 40 Prague 2, Czech Republic

Abstract

A background electrolyte system for capillary zone electrophoresis which is composed of three strong univalent ionic constituents is investigated. The ion 1 is considered as a counter-ion and two ions, 2 and 3, are considered as co-ions in relation to the analyte ion 4. We investigate the linearized model of electromigration in such a system and calculate the eigenvalues of a corresponding matrix. The model is formulated in such a way that the eigenvalues of the system are certain mobilities, which we call eigenmobilities, which characterize specific features of the electrophoretic migration. One of the eigenmobilities is the system eigenmobility u_s causing the rise of the system peak, called here the system eigenpeak. A situation when the analyte has the same mobility as the system eigenmobility, $u_4 = u_s$, is analyzed in detail. We show that it leads to the resonance—the mutual jump in the concentration profile of both co-ions, 2 and 3, has a shape of the spatial derivation of the originally sampled analyte profile and, moreover, it grows linearly with time. After a sufficiently long time it can be “amplified” to any value. The resonance has then a great impact on signals of indirect detection methods, like indirect UV detection or conductivity detection. In the framework of the linearized model the relative velocity slope S_x , a measure of electromigration dispersion, is expressed as $S_x = F(u_1 + u_4)(u_2 - u_4)(u_3 - u_4)/[u_4(u_s - u_4)]$, where u_i is the mobility of the i th ion and F is the Faraday constant. As in practice the concentration of the analyte is not infinitely small and has a certain finite value, the analyte will be at the resonance severely dispersed to a much broader spatial interval. When a specific detector is used, the signal of such an analyte can apparently be missed without any notice. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

An important aspect of the movement of various ions in the electrolyte solution is that the movement cannot be regarded as independent. Due to strong

Coulombic forces the ions are forced to maintain a macroscopic electroneutrality, which means that in a sufficiently large volume of electrolyte the positive and negative charge of all ions is balanced. This is why the movement of every ion is dependent on the presence of other ions.

All considerations trying to describe the electromigration movement of ionic constituents in the electric field have to start from the continuity

*Corresponding author. Tel.: +420-2-2195-2437; fax: +420-2-2491-9752.

E-mail address: gas@natur.cuni.cz (B. Gaš).

equations (which are in fact the mass conservation laws) and Gulberg–Waag equations relating the species being in the acid–base equilibria. A complete description of a general situation in terms of such equations can easily be formulated [1]. What is much more difficult is solving the equations and revealing all the features of such general systems. Therefore, to analytically solve the equations there has to be adopted a series of simplifications omitting some aspects of the system but still enabling one to distinguish the features of interest.

With a specific capillary electrophoresis experimental setup, the equations can be linearized and solved analytically. The constituents forming a background electrolyte (BGE) are axially almost uniformly distributed along the column in concentrations C_i . On the other hand, analyte constituents are injected as a sample into a certain position in the column in a small concentration. It means that the concentrations of the analyte constituents are almost zero in all axial coordinates and only at a certain coordinate they attain a certain value, which is much smaller than C_i . Under such conditions linearization of continuity equations can be easily done while the solution of the linearized problem can still give important information about the behavior of the system in practice.

It was predominantly Poppe and co-workers [2–6], who analyzed the linearized model of electromigration. Moreover, they were able to explain various features of the system either as a property of the linearized system or as a result of the intrinsic nonlinearity [3]. When solving the linearized model there arises an eigenvalue problem for a matrix. They predicted that electrophoretic systems having N constituents generate N “eigenpeaks” in cases where the concentration of hydroxonium or hydroxide ions is small relating to the concentration of the other constituents. They also pointed out that the eigenpeaks have to be regarded especially in electrophoretic systems with indirect detection, where a constituent forming the background electrolyte is to be traced.

Poppe [2] also discussed an interesting phenomenon, noticed earlier in chromatographic transport by Crommen et al. [7] in connection with indirect detection: the response in indirect detection tends to reach \pm infinity, when the retention of an analyte

peak gradually moves across the position of the system eigenpeak. Poppe later showed [3] by means of numerical simulations that when during electromigration a solute mobility is close to that of the system eigenpeak, elements of the corresponding eigenvector become very large. This is manifested in reality as an unusually large response of the indirect detection signal.

Such “amplification” phenomena were also noticed by other authors. The system peaks in electrophoresis were observed by Beckers, who studied BGEs with two co-ions [8]. He found that a system peak has a mobility between the mobility of the two co-ions. He also noticed that the analyte ion in the vicinity of the system peak “interacts” with the system peak and both the sample and system peaks are enlarged and dispersed due to this interaction.

The system peaks in systems with multiple co-ions were further studied by Gebauer and Boček [9], Desiderio et al. [10], and Macka et al. [11]. The authors used the concept of vacancy electrophoresis for their explanation, in analogy to previously described vacancy chromatography. The system peaks and response in indirect UV detection were described in papers by Lu and Westerlund [12] and Bullock et al. [13].

The above authors also noticed the phenomena taking place when the velocity of an analyte peak is very close or matches the velocity of the system peak. First, they pointed out that a response of indirect detection at the site of the analyte has a tendency to reach both, highly positive and highly negative values and attains a characteristic zigzag shape. Second, the analyte undergoes unusually high dispersive forces causing its extensive spatial dispersion.

At this point it has to be stressed that electromigration dispersion, i.e., a kind of deformation and broadening of the analyte peak, is a consequence of the nonlinear nature of electromigration and cannot act in the linearized model. It can only take place for a finite analyte concentration, when the analyte constituent substantially influences the conductivity at the site where it is present. There can, however, be found a quantity that is regarded as a tendency to undergo electromigration dispersion, when the concentration of the analyte starts to grow from “limit-

ing zero". Gebauer and co-workers [14,15] and Horká and Šlais [16] previously introduced such a quantity called the relative velocity slope, which characterizes electromigration dispersion of an analyte in a given BGE. The relative velocity slope S_x is defined as:

$$S_x = \frac{\kappa}{v_x} \cdot \left(\frac{dv_x}{dc_x} \right)_{c_x \rightarrow 0} \quad (1)$$

where κ is the conductivity in the zone of the analyte, c_x and v_x are the concentration and electrophoretic velocity, respectively, of the analyte constituent X in the zone of the analyte. As the relative velocity slope S_x is defined as a limit for infinitely small analyte concentration, we will show how it can be analytically evaluated in the framework of the linearized model.

In the present article we continue in the way initiated by Poppe [2,3]. We consider the linearized model of electromigration and try to search for an analytical solution whenever we can. We will define a new term—the eigenmobility of the electrolyte system, which helps to understand the behavior of the system. Our aim is to reveal and explain all features of the solution that are interesting both for theoreticians and practical analysts. This will concern especially those aspects of capillary zone electrophoresis connected with indirect types of detection like photometric detection or conductivity detection.

We restrict all considerations to electrophoretic systems with exclusively strong electrolytes due to their relative simplicity as the equation for acid–base equilibria are not essential. Such systems, however, will be still able to reveal the behavior leading to occurrence of system eigenpeaks and their “interaction” or “resonance” with analytes. In the next article we will consider more complicated systems with weak electrolytes, where concentrations of hydroxonium or hydroxide ions are significant in relation to other background constituents.

2. Theory

2.1. Derivation of governing equations

Rather than taking into account the general case of n strong electrolytes, we shall investigate a very

simple system composed of three ions of BGE and possibly one ion of an analyte. The ion 1 is regarded as the counter-ion, and two ions, 2 and 3, are regarded as co-ions of the analyte ion 4. The respective concentrations of these ions are denoted by c_1 , c_2 , c_3 , and c_4 . All the ions will be called the constituents of the system. Such a system is able to demonstrate all the important features mentioned in the Introduction. We consider all ions as strong and univalent with the ionic mobility u_i . If we denote the conductivity $\kappa = F \sum_{i=1}^4 |z_i| u_i c_i$, where F stands for the Faraday constant and relative charges of ions are $z_1 = -1$ and $z_2 = z_3 = z_4 = 1$, the governing equation and initial conditions $c_i^{\text{in}}(x)$ for i th ion, $i = 1, 2, 3, 4$, are:

$$\begin{aligned} \frac{\partial c_i}{\partial t} &= -j \operatorname{sgn}(z_i) u_i \cdot \frac{\partial}{\partial x} \cdot \left(\frac{c_i}{\kappa} \right), \\ c_i(x, 0) &= c_i^{\text{in}}(x) \end{aligned} \quad (2)$$

Here x is the axial coordinate of the column, t is the time, j is the current density. If the cross-section of the column is constant along its length, the current density j is also constant along the x -axis. We will suppose that it is also constant in time, which can be easily achieved by an electronic circuitry. Further, the system has following approximating assumptions: the diffusion movement of ions caused by the gradient of chemical potential is neglected, ionic mobilities are constants, no thermal or sorption effects play a role, the radial distribution of all constituents in the column is uniform, there is no bulk flow like the electroosmotic flow of the electrolyte.

A specific capillary zone electrophoresis setup brings us to the study of such functions c_i , each of which remains close constant values C_i . Since the ion 4 is the analyte, it is natural to have $C_4 = 0$. This leads to the introduction of new (perturbation) variables \tilde{c}_i by $\tilde{c}_i(x, t) = c_i(x, t) - C_i$ and their initial ($t = 0$) values $\tilde{c}_i^{\text{in}}(x) = c_i^{\text{in}}(x) - C_i$.

Now we transform the set of governing Eq. (2) to the system with variables $\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4$ which — for simplicity — will be presented in the matrix form. This gives the whole problem a compact form, which needs only formal changes, when coming to a system with any number of constituents. For our case of four constituents we shall use the notation:

$$K = \sum_{i=1}^3 u_i C_i \quad \text{and} \quad k(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4) = \sum_{i=1}^4 u_i \tilde{c}_i$$

and the matrix \mathbf{M} of type 4×4 whose element at place (i, m) is:

$$\mathbf{M}_{im} = \frac{z_i K u_m}{(K + k)^2} \cdot [\delta_i^m (K + k) - u_i (C_i + \tilde{c}_i)]$$

where δ_i^m is the Kronecker symbol. Every element of the matrix \mathbf{M} depends on small quantities $\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4$, which is underlined by writing $\mathbf{M}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)$. The system of equations for \tilde{c}_i is:

$$\begin{aligned} \frac{\partial}{\partial t}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^T \\ = - \frac{j}{FK} \mathbf{M}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4) \frac{\partial}{\partial x}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^T \end{aligned} \quad (3)$$

where \mathbf{T} denotes transpose matrix, here applied to row vectors it gives column vectors. The initial conditions are:

$$\tilde{c}_i(x, 0) = \tilde{c}_i^{\text{in}}(x), \quad i = 1, 2, 3, 4 \quad (4)$$

Since the electroneutrality of the BGE and initial perturbations is supposed, i.e.:

$$C_1 = C_2 + C_3, \quad \tilde{c}_1^{\text{in}} = \tilde{c}_2^{\text{in}} + \tilde{c}_3^{\text{in}} + \tilde{c}_4^{\text{in}}$$

it is easy to get $\tilde{c}_1(x, t) = \tilde{c}_2(x, t) + \tilde{c}_3(x, t) + \tilde{c}_4(x, t)$ also for all positive t for which the solution exists.

For an initial value of $\tilde{c}_4^{\text{in}}(x)$ we take a small localized function (here by “localized” we mean a function which is different from zero only on a small interval of x , i.e., on the site of injection).

Linearization—a general approach that under some conditions enables us to get simple approximations to solution of nonlinear problems by treating them as linear ones—is in the case of this system carried by the idea that all \tilde{c}_i during time development do not go far away from zero values. We cancel all terms in the equations where at least two such small terms are multiplied, doing this we are left with the system:

$$\begin{aligned} \frac{\partial}{\partial t}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^T \\ = - \frac{j}{FK} \mathbf{M}(0, 0, 0, 0) \frac{\partial}{\partial x}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^T \end{aligned} \quad (5)$$

where the matrix $\mathbf{M}(0, 0, 0, 0)$, which for brevity will be shortened to $\mathbf{M}(0)$, has the form:

$$\begin{aligned} \mathbf{M}(0) \\ = \frac{1}{K} \begin{pmatrix} -u_1(K - u_1 C_1) & u_2 u_1 C_1 & u_3 u_1 C_1 & u_4 u_1 C_1 \\ -u_1 u_2 C_2 & u_2(K - u_2 C_2) & -u_3 u_2 C_2 & -u_4 u_2 C_2 \\ -u_1 u_3 C_3 & -u_2 u_3 C_3 & u_3(K - u_3 C_3) & -u_4 u_3 C_3 \\ 0 & 0 & 0 & u_4 K \end{pmatrix} \end{aligned}$$

The matrix $\mathbf{M}(0)$ has four eigenvalues:

$$\begin{aligned} \lambda_1 = \lambda_2 = 0 \\ \lambda_3 = \frac{u_2 u_3 C_1 + u_1 u_3 C_2 + u_1 u_2 C_3}{u_1 C_1 + u_2 C_2 + u_3 C_3} \equiv u_s \\ \lambda_4 = u_4 \end{aligned} \quad (6)$$

which all have the dimension of mobility and will be called the eigenmobilities of the system. We will show later that u_s is the mobility of a certain feature of the system—the system eigenpeak.

It is shown in Appendix A that Eq. (5) will be fairly simplified by the introduction of new dependent variables w_1, w_3, w_3, w_4 , which are some linear combinations of the original variables $\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4$. In the matrix notation:

$$(w_1, w_2, w_3, w_4)^T = \mathbf{V}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^T \quad (7)$$

where \mathbf{V} is a suitable regular matrix (the inverse \mathbf{V}^{-1} exists). Thus we can easily return from new variables w_i to original ones \tilde{c}_i when this relation is multiplied from the left side by the inverse matrix \mathbf{V}^{-1} .

2.2. System A—Electrophoretic system with analyte mobility u_4 different from the eigenmobility u_s

We start with the case $\lambda_3 \neq \lambda_4$, i.e., $u_4 \neq u_s$, in which only zero is a double eigenvalue. We can multiply Eq. (5) from the left by matrix \mathbf{V}_A of Appendix A and get the following very simple system in the variables w_1, w_2, w_3, w_4 :

$$\begin{aligned} \frac{\partial w_1}{\partial t} \equiv 0, \quad \frac{\partial w_2}{\partial t} = - \frac{4j u_s}{FK} \cdot \frac{\partial w_1}{\partial x}, \\ \frac{\partial w_3}{\partial t} = - \frac{j u_s}{FK} \cdot \frac{\partial w_3}{\partial x}, \quad \frac{\partial w_4}{\partial t} = - \frac{j u_4}{FK} \cdot \frac{\partial w_3}{\partial x} \end{aligned} \quad (8)$$

with the initial conditions:

$$w_1^{\text{in}} \equiv 0, \quad w_2^{\text{in}} = \sum_{i=1}^4 x_i \tilde{c}_i^{\text{in}}, \quad w_3^{\text{in}} = \sum_{i=1}^4 y_i \tilde{c}_i^{\text{in}},$$

$$w_4^{\text{in}} = \tilde{c}_4^{\text{in}} \quad (9)$$

Solving this system, we find that:

$$w_1(x, t) \equiv 0, \quad w_2(x, t) = w_2^{\text{in}}(x),$$

$$w_3(x, t) = w_3^{\text{in}} \left(x - \frac{j u_s}{FK} \cdot t \right),$$

$$w_4(x, t) = w_4^{\text{in}} \left(x - \frac{j u_4}{FK} \cdot t \right) \quad (10)$$

Using the second row of the matrix \mathbf{V}_A we immediately find that:

$$w_2 = 4 \left(\frac{\tilde{c}_1}{u_1} + \frac{\tilde{c}_2}{u_2} + \frac{\tilde{c}_3}{u_3} + \frac{\tilde{c}_4}{u_4} \right) \cdot u_s \quad (11)$$

Functions w_3 and w_4 are travelling waves which propagate along the x -axis with the velocity $j u_s / (FK)$ and $j u_4 / (FK)$, respectively. According to the structure of \mathbf{V}_A^{-1} (see Appendix A) we even find that:

$$w_4(x, t) = \tilde{c}_4(x, t) = \tilde{c}_4^{\text{in}} \left(x - \frac{j u_4}{FK} \cdot t \right)$$

This shows that—in the linear approximation—the analyte moves with the velocity, which does not bear any dependence on the analyte concentration and thus provides the velocity limit for the “zero” analyte concentration.

We now give a formula for $d\kappa/d\tilde{c}_4$ which is later used in deriving a value of S_x from Eq. (1). Since $u_4 \neq u_s$, the velocities of the waves represented by w_2 , w_3 , w_4 are different from each other and after some time w_4 representing the analyte is not influenced by either w_2 or w_3 . Then \tilde{c}_1 , \tilde{c}_2 , and \tilde{c}_3 are functions of only \tilde{c}_4 and calculating them at the side of the analyte only the elements of the fourth column of the matrix \mathbf{V}_A^{-1} are used. In Appendix A these are denoted by γ_i , thus $\tilde{c}_i = \gamma_i \tilde{c}_4$, $i = 1, 2, 3$, all under the assumption that \tilde{c}_i are very small. Thus:

$$\frac{d\kappa}{d\tilde{c}_4} = F \sum_{i=1}^4 u_i \frac{d\tilde{c}_i}{d\tilde{c}_4} = F \left(\sum_{i=1}^3 u_i \gamma_i + u_4 \right)$$

and some manipulations provide the formula:

$$\lim_{\tilde{c}_4 \rightarrow 0^+} \frac{d\kappa}{d\tilde{c}_4} = -F \frac{(u_1 + u_4)(u_2 - u_4)(u_3 - u_4)}{(u_s - u_4)u_4} \quad (12)$$

2.3. System B—Electrophoretic system without analyte 4

We check the case where there is no analyte in the BGE system consisting of three components. The linearized system of equations is:

$$\frac{\partial}{\partial t} (\tilde{c}_1, \tilde{c}_2, \tilde{c}_3)^T = -\frac{j}{FK} \mathbf{M}_3(0) \frac{\partial}{\partial x} (\tilde{c}_1, \tilde{c}_2, \tilde{c}_3)^T \quad (13)$$

where the matrix $\mathbf{M}_3(0)$ is of the form:

$$\mathbf{M}_3(0) = \frac{1}{K} \begin{pmatrix} -u_1(K - u_1 C_1) & u_2 u_1 C_1 & u_3 u_1 C_1 \\ -u_1 u_2 C_2 & u_2(K - u_2 C_2) & -u_3 u_2 C_2 \\ -u_1 u_3 C_3 & -u_2 u_3 C_3 & u_3(K - u_3 C_3) \end{pmatrix}$$

This matrix has three eigenvalues:

$$\lambda_1 = \lambda_2 = 0, \quad \lambda_3 = u_s \quad (14)$$

which are exactly the same as the first three of the preceding matrix $\mathbf{M}(0)$. To simplify the system, the matrix \mathbf{V}_B of Appendix A is applied.

2.4. System C—Electrophoretic system with analyte mobility u_4 equal to the eigenmobility u_s

In this part we shall solve linearized Eq. (5) with initial conditions Eq. (4) in the case of the additional double eigenvalue: $\lambda_3 = \lambda_4$, i.e., $u_4 = u_s$.

Since the matrix $\mathbf{M}(0)$ has now two pairs of multiple eigenvalues, the matrix \mathbf{V}_C of Appendix A must be used. We come to the following system of equations for w_i :

$$\frac{\partial w_1}{\partial t} \equiv 0, \quad \frac{\partial w_2}{\partial t} = -\frac{4u_s j}{FK} \cdot \frac{\partial w_1}{\partial x},$$

$$\frac{\partial w_3}{\partial t} = -\frac{u_s j}{FK} \cdot \frac{\partial w_3}{\partial x},$$

$$\frac{\partial w_4}{\partial t} = -\frac{j}{FK} \left(u_s \frac{\partial w_4}{\partial x} + m_2 \frac{\partial w_3}{\partial x} \right) \quad (15)$$

with the initial conditions:

$$w_1^{\text{in}} \equiv 0, \quad w_2^{\text{in}} = \sum_{i=1}^4 x_i \tilde{c}_i^{\text{in}}, \quad w_3^{\text{in}} = \tilde{c}_4^{\text{in}},$$

$$w_4^{\text{in}} = \sum_{i=1}^4 y_i \tilde{c}_i^{\text{in}} \quad (16)$$

Having a small and localized analyte 4 in mind, we choose a small and smooth function φ and set $\tilde{c}_4^{\text{in}} = \varphi$. To obey the condition of electroneutrality, it

is possible to start with the following simple form of the initial conditions:

$$\tilde{c}_1^{\text{in}} = \varphi, \quad \tilde{c}_2^{\text{in}} = \tilde{c}_3^{\text{in}} \equiv 0, \quad \tilde{c}_4^{\text{in}} = \varphi \quad (17)$$

Solving this system, we find that:

$$\begin{aligned} w_1(x, t) &\equiv 0, \\ w_2(x, t) &= (x_1 + x_4)\varphi(x), \\ w_3(x, t) &= \varphi\left(x - \frac{j u_S}{FK} \cdot t\right), \\ w_4(x, t) &= (y_1 + y_4)\varphi\left(x - \frac{j u_S}{FK} \cdot t\right) \\ &\quad - \frac{j m_2}{FK} \cdot t \varphi'\left(x - \frac{j u_S}{FK} \cdot t\right) \end{aligned} \quad (18)$$

where φ' denotes the derivative of the function φ .

The presence of the variable t in the last term of w_4 means that, as long as the linear approximation is valid, the amplitude of w_4 grows linearly with time.

3. Discussion

In spite of its seeming simplicity, the background electrolyte system with exclusively strong electrolytes, having one counter-ion 1 of the opposite charge and two co-ions, 2 and 3, of the same charge in relation to the analyte 4, reveals many features that are important in electrophoretic practice.

We have formulated the problem in such a way that the eigenvalues of a matrix corresponding to the linearized system are certain mobilities, which we call eigenmobilities, and which characterize specific features of the electrophoretic migration. The number of eigenmobilities is the same as the number of constituents of the system.

3.1. System A—Electrophoretic system with analyte mobility u_4 different from the eigenmobility u_S

This particular setup with four ions (three ions compose the BGE, the fourth ion is the analyte) has four eigenmobilities, which are given in Eq. (6). Two of them equal zero, the third one is the system eigenmobility u_S and the fourth one equals the ionic mobility u_4 of the analyte. The analytical solutions

$\tilde{c}_i(x, t)$ of the linearized problem Eq. (5) are certain linear combinations of functions $w_i(x, t)$ which are given in Eq. (10) and are able to show features of the solution in a lucid way. Here we will discuss their physical meaning.

The function w_1 corresponds with the electro-neutrality condition. From the first row of the matrix \mathbf{V}_A it is obvious, that $w_1 = -\tilde{c}_1 + \tilde{c}_2 + \tilde{c}_3 + \tilde{c}_4$. Of course, we have then $w_1(x, t) \equiv 0$.

According to Eq. (11), the function w_2 has a very tight connection with the well known Kohlrausch regulating function, since:

$$\begin{aligned} \frac{w_2}{4u_S} + \sum_{i=1}^3 \frac{C_i}{u_i} &= \frac{\tilde{c}_1}{u_1} + \frac{\tilde{c}_2}{u_2} + \frac{\tilde{c}_3}{u_3} + \frac{\tilde{c}_4}{u_4} + \frac{C_1}{u_1} + \frac{C_2}{u_2} \\ &\quad + \frac{C_3}{u_3} = \frac{c_1}{u_1} + \frac{c_2}{u_2} + \frac{c_3}{u_3} + \frac{c_4}{u_4} \end{aligned} \quad (19)$$

Since:

$$\frac{\partial w_2}{\partial t} = -\frac{4j u_S}{FK} \cdot \frac{\partial w_1}{\partial x} \equiv 0$$

the $w_2(x, t) = w_2^{\text{in}}(x)$ is a function which is independent of time. It reveals that there is something like a “capillary memory”: some features of the concentration profiles “stay” at the same positions where they are at the beginning regardless of the pass of the electric current transporting all ions.

In the most common capillary electrophoresis setup a separation column is filled by axially uniform concentration of all ions of the BGE and the analyte is sampled at a certain “localized” site of injection. The injection will generally cause a perturbation in the originally uniform background electrolyte profile leading to a change in the w_2 . Such a perturbation is called the “water gap” or “water peak”. Its name comes from the fact that it can also be generated by the injection of water or diluted (or more concentrated) background electrolyte. Due to the feature of the w_2 function the perturbation—the water peak does not move and stays at the position of injection as its eigenmobility equals zero. If, possibly, there is a bulk flow of the electrolyte in the column, which is raised by the electroosmotic flow or by the laminar Poiseuille flow (which are of course different phenomena not governed by the presented equations), the water peak can serve for indication of their velocity when passing a detector site.

The third solution is $w_3(x, t) = w_3^{\text{in}}(x - ju_s t / FK)$ and belongs to the eigenmobility $\lambda_3 \equiv u_s$. It reveals that there is an additional feature in the solution, which moves with the mobility of u_s . As concentration of the counter-ion equals the sum of concentration of both co-ions, $C_1 = C_2 + C_3$, it can also be written:

$$u_s = \frac{u_3 C_2 (u_1 + u_2) + u_2 C_3 (u_1 + u_3)}{C_2 (u_1 + u_2) + C_3 (u_1 + u_3)} \quad (20)$$

If the injection of the analyte will cause a perturbation in the w_3 function, the perturbation is moved along the x -axis with the mobility of u_s in the same direction as both co-ions. Generally, $u_2 \neq u_3$ and $C_2 > 0$ and $C_3 > 0$, otherwise it is not a system with double co-ions. Without a loss of generality we suppose that $u_2 > u_3$, the eigenmobility u_s is then in the interval $u_2 > u_s > u_3$. The perturbation moving with mobility of u_s is sometimes called the system peak. Unfortunately, the term system peak is vague and has been so far used for designating various phenomena. We therefore propose to call such type of the peak as the system eigenpeak.

Unlike the water peak, the system eigenpeak moving with the eigenmobility u_s is invoked by a perturbation in w_3 function, which is zero before sampling. As noted in the Appendix, the initial value of function $w_3 \equiv 0$, when three initial functions \tilde{c}_1^{in} , \tilde{c}_2^{in} , \tilde{c}_3^{in} satisfy:

$$\frac{\tilde{c}_1^{\text{in}}(x)}{C_1} = \frac{\tilde{c}_2^{\text{in}}(x)}{C_2} = \frac{\tilde{c}_3^{\text{in}}(x)}{C_3}$$

in all coordinates x . It means that the system eigenpeak cannot be generated by the injection of water or a diluted (or more concentrated) background electrolyte. To rise the system eigenpeak, the injected electrolyte must contain a different ratio of both co-ions than is in the original background electrolyte. Of course, in practice the system eigenpeak is the most often generated by the introduction of a sample containing analyte constituents, which, in general, causes a perturbation in w_3 function.

The fourth function $w_4(x, t) = \tilde{c}_4^{\text{in}}(x - ju_4 t / FK)$ belonging to the eigenmobility $\lambda_4 = u_4$ simply tells that the analyte peak having infinitely small concentration is moved along x -axis by its own mobility u_4 . A very interesting question is, how the electro-

phoretic velocity v_4 of the analyte depends on its own concentration \tilde{c}_4 . Such dependence can be hardly found by application of the presented linear model. What we, however, can calculate, is a slope of such dependence for infinitely small concentration of the analyte. This quantity, which is called the relative velocity slope [16,17]:

$$S_x = \lim_{\tilde{c}_4 \rightarrow 0^+} \frac{\kappa}{v_4} \cdot \frac{dv_4}{d\tilde{c}_4}$$

was introduced to characterize a tendency of the sample to undergo the electromigration dispersion. Since the analyte velocity v_4 is given by $v_4 = ju_4 / \kappa$, it follows after a simple computation that:

$$\frac{\kappa}{v_4} \cdot \frac{dv_4}{d\tilde{c}_4} = - \frac{d\kappa}{d\tilde{c}_4} \quad (21)$$

From this and from Eq. (12), the relative velocity slope S_x is in our model:

$$S_x = F \frac{(u_1 + u_4)(u_2 - u_4)(u_3 - u_4)}{(u_s - u_4)u_4} \quad (22)$$

It is obvious that when the mobility u_4 of the analyte peak is close to the eigenmobility of the system eigenpeak, u_s , the relative velocity slope has a tendency to reach \pm infinity. It consequently means that the analyte will undergo the electromigration dispersion to an infinitely large extent.

Fig. 1 shows an example of a dependence of S_x on the sample mobility u_4 to get an impression about its shape. This is a hypothetical electrophoretic system, where the counter-ion 1 is in concentration $C_1 = 10$ mM and both co-ions 2 and 3 are in concentrations $C_2 = C_3 = 5$ mM. Their ionic mobilities are $u_1 = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $u_2 = 80 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $u_3 = 40 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and the Faraday constant $F = 96487 \text{ C mol}^{-1}$. The system eigenmobility u_s is according to Eq. (6) $u_s = 55.56 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$.

The quantity $\lim_{\tilde{c}_4 \rightarrow 0^+} d\kappa/d\tilde{c}_4$ is in fact the molar conductivity detection response b_x , which is a measure of the sensitivity of conductivity detection [18]. According to Eq. (21) it has the same value but opposite sign as the relative velocity slope, $S_x = -b_x$. This is valid for the systems with strong electrolytes, as is the case in the consideration. We

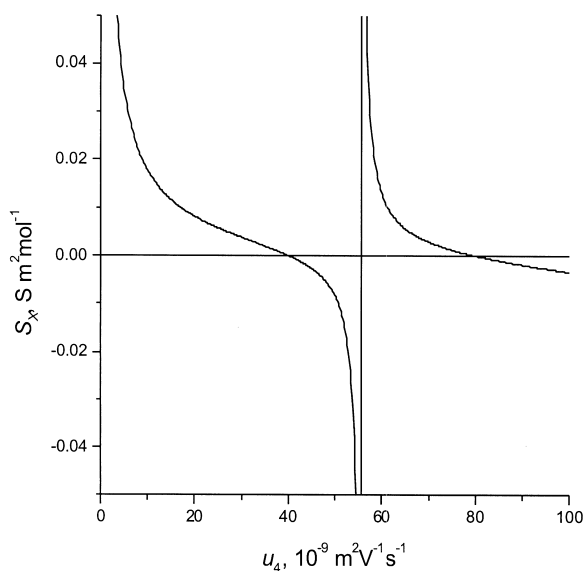


Fig. 1. Dependence of S_x on sample mobility u_4 according to Eq. (22). Ionic mobility of counter-ion, $u_1 = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. Mobility of co-ions, $u_2 = 80 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $u_3 = 40 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. The system eigenmobility $u_s = 55.56 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, according to Eq. (6).

will show in the next paper that weak electrolytes behave differently and that it can be found BGE systems where analytes undergo very low electromigration dispersion, $S_x \rightarrow 0$, and still are able to give a good conductivity signal, $b_x \neq 0$.

3.2. System B—Electrophoretic system without analyte 4

It is worth noting the fact that both the water peak and system eigenpeak can be invoked also in the background electrolyte with double co-ions even without presence of any analyte injected, as such a system has the eigenmobilities responsible for them, $\lambda_2 = 0$ and $\lambda_3 = u_s$ (see Eq. (14)). As was discussed in the previous part, to arise the water peak the diluted (more concentrated) background electrolyte must be injected, while for arising the system eigenpeak the injected electrolyte must contain a different ratio of both co-ions than is in the original background electrolyte.

3.3. System C—Electrophoretic system with analyte mobility u_4 equal to the eigenmobility u_s

Here we shall discuss a situation when the analyte has the same mobility as the mobility of the system eigenpeak, $u_4 = u_s$. This case, when two nonzero eigenmobilities of the system are the same, $\lambda_3 = \lambda_4$, is the general situation, called the resonance in mechanical or electrical systems, where the frequency of the forcing term equals the frequency of free oscillations. We are therefore tempted to call this case the resonance, too. Such a situation can happen in practice by chance but quite often, especially in cases of complex samples. After the injection of the analyte its movement coincides with the movement of the system eigenpeak, so they are constantly “in touch”. The analytical solution is given by functions w_i , see Eq. (18).

The first three eigenvalues λ_1 , λ_2 , λ_3 and functions w_1 , w_2 , w_3 are the same as in previous systems A and B. This is obvious as the maximum concentration of the analyte 4 even in the small localized area—the injection site—is supposed to be very small. We denote such initial axial distribution of the analyte as $\varphi(x)$. We can have in mind $\varphi(x)$ like a profile which reminds a Gaussian peak, the width of which is much less than the total length of the column. The second term on the right-hand side in the formula for w_4 :

$$w_4(x, t) = (y_1 + y_4)\varphi\left(x - \frac{ju_s}{FK} \cdot t\right) - \frac{jm_2}{FK} \cdot t\varphi'\left(x - \frac{ju_s}{FK} \cdot t\right)$$

reveals that there is an additional type of feature moving with the mobility $u_s = u_4$. It has a shape of the spatial derivation φ' of the original analyte profile $\varphi(x)$ and, moreover, as it is multiplied by t , it grows linearly with time. After sufficiently long time the amplitude of w_4 can be “amplified” to any value (in the framework of the linearized model). It is important to realize that the grow in w_4 is reflected by the amplification of the “mutual jump” in the concentration profile of both co-ions, 2 and 3. On the other hand, the axial concentration profile of the sample, w_3 , is not affected by the amplification phenomenon and still has a shape of the original peak $\varphi(x)$ moving with the mobility of u_s :

$$w_3(x, t) = \varphi\left(x - \frac{ju_s}{FK} \cdot t\right)$$

All these aspects are demonstrated in Fig. 2. It shows results of the simulation program, which was described elsewhere [19,20] and which is able to solve numerically the system of continuity equations, Eq. (2). The hypothetical electrolyte system is the same as used in Fig. 1—the column is uniformly filled with solution of counter-ion 1 in concentration $C_1=10$ mM and both co-ions 2 and 3 in concentrations $C_2=C_3=5$ mM. The analyte is introduced at a position $\text{Injsite}=0.002$ m as a small Gaussian peak, $\varphi(x) = c_{4\text{max}} \exp\left[-(x - \text{Injsite})^2 / (2\sigma^2)\right]$, with concentration at the maximum of peak $c_{4\text{max}}=0.002$ mM and variance $\sigma^2=4 \cdot 10^{-8}$ m². The mobilities u_1 , u_2 and u_3 are the same as in Fig. 1,

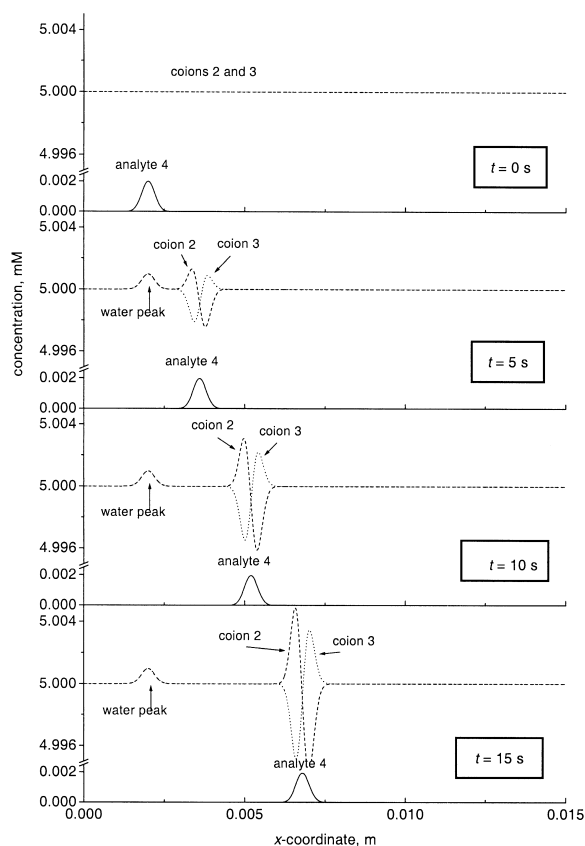


Fig. 2. Computed axial distributions of concentrations of the co-ions c_2 (dashed curve), c_3 (dotted curve) and of the analyte c_4 (solid curve) at various time t . For other conditions see text.

i.e., 30, 80 and $40 \cdot 10^{-9}$ m² V⁻¹ s⁻¹, respectively. The mobility u_4 of the analyte 4 is here the same as the system eigenmobility, $u_4 = u_s = 55.56 \cdot 10^{-9}$ m² V⁻¹ s⁻¹ and the current density $j=500$ A m⁻². Fig. 2 shows the output of the Simul program [20]—distributions of concentrations of both co-ions, c_2 and c_3 , and of the analyte, c_4 , at various times. (The Simul program had to be adapted to compute Eq. (2) which are without diffusion terms). As the original perturbation φ had a Gaussian shape, the moving features appearing in concentrations of co-ions have a shape of its derivative, the amplitude of which increases during its movement. This is a result straightly explaining why in indirect detection the signal attains the zigzag shape under such situations, as noticed previously (see e.g., Ref. [8]). It also elucidates the amplification phenomenon earlier simulated numerically by Poppe [3], who calculated that elements of the corresponding eigenvector become very large.

The coincidence of the system eigenmobility and the mobility of the analyte, $u_4 = u_s$, can have an additional aspect in electrophoretic practice. As derived in Eq. (22) and shown in Fig. 1, the relative velocity slope has a tendency to reach \pm infinity in such a case. The presented model is based on the assumption of no diffusion and a very small concentration of the analyte compared to the concentration of BGE. Nevertheless, in practice the maximum concentration of the analyte is not infinitely small. Under such conditions the velocities of the analyte at points with a higher concentration will be significantly different from the velocity at points where the concentration is small. If there were no diffusion, the crest of the peak would override the parts of the peak where the concentration is low, which would result in a physically unrealizable situation. This case cannot occur in reality as the diffusion is always present and leads to a strong dispersion of the peak to a much broader spatial interval.

The maximum analyte concentration will be decreasing. The phenomenon is called schizoprenic [14] or anomalous [21] dispersion. If a specific detector is used, i.e., the detector recognizing the analyte presence directly, the signal of such an analyte can apparently be missing without any notice due to its severe spatial dispersion [17]. In spite of

the decreasing concentration of the analyte, the indirect detector signal is still unusually high.

4. Nomenclature

x, t	Axial coordinate of the column, time
F, j	Faraday constant, current density
u_i, v_i, z_i	Mobility, velocity and relative charge of i th ion
$c_i(x, t)$	Concentration of i th ion
$c_i^{\text{in}}(x) = c_i(x, 0)$	Initial condition for $t = 0$
C_i	Constant for which $c_i(x, t) - C_i$ is small
$\tilde{c}_i(x, t) = c_i(x, t) - C_i$	Perturbation of concentration of i th ion
$\tilde{c}_i^{\text{in}}(x) = \tilde{c}_i(x, 0)$	Initial condition for $t = 0$
$C = C_1 + C_2 + C_3$	
κ	Conductivity
S_x	Relative velocity slope
$K = \sum_{i=1}^3 u_i C_i, k(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4) = \sum_{i=1}^4 u_i \tilde{c}_i$	
$\mathbf{M}, \mathbf{M}(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)$	Matrix of elements M_{im}
λ_i	Eigenmobilities, i.e., eigenvalues of $\mathbf{M}(0) \equiv \mathbf{M}(0, 0, 0, 0)$
$u_s \equiv \lambda_3$	Eigenmobility characterizing the movement of the eigenpeak
$\mathbf{V}, \mathbf{V}_A, \mathbf{V}_B, \mathbf{V}_C; \mathbf{V}^{-1}$	Transformation matrices; inverse of transformation matrix
w_i	Variables to which \tilde{c}_i are transformed using matrices $\mathbf{V}, \mathbf{V}_A, \mathbf{V}_B, \mathbf{V}_C$
x_i, y_i	Elements of matrices $\mathbf{V}_A, \mathbf{V}_B, \mathbf{V}_C$
$\alpha_i, \beta_i, \gamma_i$	Elements of the inverse matrix \mathbf{V}_A
m_i	Constants in a vector relation involving eigenvalues λ_i and eigenvectors
$\varphi = \tilde{c}_1^{\text{in}} = \tilde{c}_4^{\text{in}}$	Initial condition for analyte

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Appendix A

We explain how the eigenvalue and eigenvectors arise when solving Eq. (5). Here a system of four equations is dealt with, the procedure is quite the same in a general case of n variables. For any left (i.e., row) eigenvector $v = (\xi_1, \xi_2, \xi_3, \xi_4)$ of the matrix $\mathbf{M}(0)$ which corresponds to an eigenvalue λ , i.e., $v\mathbf{M}(0) = \lambda v$, we multiply the i th equation of the system (5) by ξ_i , then sum up for $i = 1, 2, 3, 4$, to get for a function: $w(x, t) = \sum_{i=1}^4 \xi_i \tilde{c}_i(x, t)$ an equation $\partial w / \partial t = -(j\lambda / FK) \cdot (\partial w / \partial x)$. The initial condition for this function is $w(x, 0) = w^{\text{in}}(x)$, where $w^{\text{in}}(x) = \sum_{i=1}^4 \xi_i \tilde{c}_i^{\text{in}}(x)$, and thus one immediately gets:

$$w(x, t) = w^{\text{in}}\left(x - \frac{j\lambda}{FK} \cdot t\right)$$

a travelling wave which propagates with the velocity $j\lambda / (FK)$.

If there are four eigenvectors v_1, v_2, v_3, v_4 , which, respectively, correspond to the eigenvalues $\lambda_1, \lambda_2, \lambda_3, \lambda_4$ of the matrix $\mathbf{M}(0)$, we get four new functions $w_1(x, t), w_2(x, t), w_3(x, t), w_4(x, t)$, of which each satisfies a simple equation of the above type and the appropriate initial condition. In matrix notation, if \mathbf{V} is a matrix whose rows are the left eigenvectors v_i , then the new functions and their initial conditions are:

$$(w_1, w_2, w_3, w_4)^{\text{T}} = V(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^{\text{T}},$$

$$(w_1^{\text{in}}, w_2^{\text{in}}, w_3^{\text{in}}, w_4^{\text{in}})^{\text{T}} = V(\tilde{c}_1^{\text{in}}, \tilde{c}_2^{\text{in}}, \tilde{c}_3^{\text{in}}, \tilde{c}_4^{\text{in}})^{\text{T}}$$

As above, the functions w_i are given by:

$$w_i(x, t) = w_i^{\text{in}}\left(x - \frac{j\lambda_i}{FK} \cdot t\right)$$

If, as a last assumption, \mathbf{V} is a nonsingular matrix, the functions \tilde{c}_i can be recovered with the help of the inverse matrix \mathbf{V}^{-1} through the matrix relation:

$$(\tilde{c}_1, \tilde{c}_2, \tilde{c}_3, \tilde{c}_4)^{\text{T}} = \mathbf{V}^{-1}(w_1, w_2, w_3, w_4)^{\text{T}}$$

If the matrix \mathbf{V}^{-1} exists, then $\mathbf{VM}(0)\mathbf{V}^{-1}$ is a diagonal matrix with eigenvalues $\lambda_1, \lambda_2, \lambda_3, \lambda_4$ on the diagonal. A simple sufficient condition for this is that every two eigenvalues of $\mathbf{M}(0)$ are different, i.e., there are no multiple eigenvalues.

Unfortunately, for $\mathbf{M}(0)$ we have $\lambda_1 = \lambda_2 = 0$. All other eigenvalues are different from zero, but further multiple eigenvalues may appear—as in System C. Generally, it is possible to find a matrix \mathbf{V} such that $\mathbf{VM}(0)\mathbf{V}^{-1}$ is a Jordan matrix—a matrix which is not far from a diagonal one. This Jordan matrix has eigenvalues on the diagonal and all other elements are zero with a possible exception of elements standing just under the diagonal. It is the Jordan form of a matrix we shall use.

If $\lambda = \lambda_i = \lambda_{i+1}$, a double eigenvalue, we proceed as follows. An eigenvector v_i is found for λ , i.e., $v_i\mathbf{M}(0) = \lambda v_i$. If, for this eigenvalue λ , it is not possible to find another eigenvector v_{i+1} linearly independent of v_i , one must accept as v_{i+1} a vector satisfying a relation $v_{i+1}\mathbf{M}(0) = \lambda v_{i+1} + m v_i$ in which m is a suitable constant. The presence of such vectors v_{i+1} in the matrix \mathbf{V} leads to the above mentioned nonzero off-diagonal terms in the Jordan matrix.

A.1. System A

We start by describing the construction of the dimensionless matrix \mathbf{V}_A in the case $u_4 \neq u_S$. As the left eigenvector corresponding to $\lambda_1 = 0$ it is possible to take $(-1, 1, 1, 1)$, i.e., $(-1, 1, 1, 1)\mathbf{M}(0) = \lambda_1(-1, 1, 1, 1)$. (On the right side, due to $\lambda_1 = 0$, there is a null vector). If we define:

$$\begin{aligned}x_1 &= \left(\frac{3}{u_1} + \frac{1}{u_2} + \frac{1}{u_3} + \frac{1}{u_4} \right) u_S, \\x_2 &= \left(\frac{1}{u_1} + \frac{3}{u_2} - \frac{1}{u_3} - \frac{1}{u_4} \right) u_S, \\x_3 &= \left(\frac{1}{u_1} - \frac{1}{u_2} + \frac{3}{u_3} - \frac{1}{u_4} \right) u_S, \\x_4 &= \left(\frac{1}{u_1} - \frac{1}{u_2} - \frac{1}{u_3} + \frac{3}{u_4} \right) u_S\end{aligned}$$

the vector (x_1, x_2, x_3, x_4) satisfies

$$\begin{aligned}(x_1, x_2, x_3, x_4)\mathbf{M}(0) &= \lambda_2(x_1, x_2, x_3, x_4) \\ &+ m_1(-1, 1, 1, 1)\end{aligned}$$

where $m_1 = 4u_S$ [on the right hand side, due to $\lambda_2 = 0$, there is only a m_1 multiple of the preceding eigenvector $(-1, 1, 1, 1)$].

When we use the sum of all constant concentrations $C = C_1 + C_2 + C_3$, a vector (y_1, y_2, y_3, y_4) is the left eigenvector corresponding to $\lambda_3 = u_S$, i.e.,

$$(y_1, y_2, y_3, y_4)\mathbf{M}(0) = \lambda_3(y_1, y_2, y_3, y_4)$$

if we set:

$$\begin{aligned}y_1 &= \left(\frac{1}{u_3} - \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_1}, \\y_2 &= - \left(\frac{1}{u_1} + \frac{1}{u_3} \right) u_S \cdot \frac{C}{C_2}, \\y_3 &= \left(\frac{1}{u_1} + \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_3}, \\y_4 &= \frac{(u_1 + u_2)(u_1 + u_3)(u_2 - u_3)u_4 u_S C}{u_1 u_2 u_3 [(u_2 u_3 - u_1 u_4)C_1 + (u_1 u_3 - u_2 u_4)C_2 + (u_1 u_2 - u_3 u_4)C_3]}\end{aligned}$$

It is easy to see that the denominator of the last relation y_4 equals zero only if $u_4 = u_S$.

Finally, the fourth vector $(0, 0, 0, 1)$ is the left eigenvector corresponding to the eigenvalue λ_4 , i.e., $(0, 0, 0, 1)\mathbf{M}(0) = \lambda_4(0, 0, 0, 1)$. Now, we are able to define a dimensionless matrix:

$$\mathbf{V}_A = \begin{pmatrix} -1 & 1 & 1 & 1 \\ x_1 & x_2 & x_3 & x_4 \\ y_1 & y_2 & y_3 & y_4 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

which reduces Eq. (5) with initial conditions Eq. (4) to system (8) and initial conditions (9) for functions w_i .

The inverse matrix \mathbf{V}_A^{-1} has the form:

$$\mathbf{V}_A^{-1} = \begin{pmatrix} * & \alpha_1 & \beta_1 & \gamma_1 \\ * & \alpha_2 & \beta_2 & \gamma_2 \\ * & \alpha_3 & \beta_3 & \gamma_3 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

where the elements in places of * are of no importance for us, since $w_1 \equiv 0$, and α_i, β_i , and γ_i can be

expressed through u_i and C_i which we omit to present here.

A.2. System B

In the case of three variables c_1, c_2, c_3 , the matrix \mathbf{V}_B can be given the form:

$$\mathbf{V}_B = \begin{pmatrix} -1 & 1 & 1 \\ x_1 & x_2 & x_3 \\ y_1 & y_2 & y_3 \end{pmatrix}$$

where:

$$x_1 = \left(\frac{2}{u_1} + \frac{1}{u_2} + \frac{1}{u_3} \right) u_S,$$

$$x_2 = \left(\frac{1}{u_1} + \frac{2}{u_2} - \frac{1}{u_3} \right) u_S,$$

$$x_3 = \left(\frac{1}{u_1} - \frac{1}{u_2} + \frac{2}{u_3} \right) u_S$$

and:

$$y_1 = \left(\frac{1}{u_3} - \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_1},$$

$$y_2 = - \left(\frac{1}{u_1} + \frac{1}{u_3} \right) u_S \cdot \frac{C}{C_2},$$

$$y_3 = \left(\frac{1}{u_1} + \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_3}$$

The vector (x_1, x_2, x_3) satisfies $(x_1, x_2, x_3)\mathbf{M}(0) = 3u_S(-1, 1, 1)$.

The question may arise whether there are initial conditions for which no eigenpeak appears, i.e., the function $w_3 \equiv 0$. This is really possible when three initial functions $\tilde{c}_1^{\text{in}}, \tilde{c}_2^{\text{in}}$, and \tilde{c}_3^{in} satisfy:

$$\frac{\tilde{c}_1^{\text{in}}(x)}{C_1} = \frac{\tilde{c}_2^{\text{in}}(x)}{C_2} = \frac{\tilde{c}_3^{\text{in}}(x)}{C_3}$$

in all points x .

A.3. System C

We finish by dealing with the case $u_4 = u_S$. The first two rows of the matrix \mathbf{V}_C are identical with those of matrix \mathbf{V}_A . The eigenvalues $\lambda_3 = \lambda_4 = u_S = u_4$. The vector on the third row is $(0, 0, 0, 1)$. The fourth row is formed by (y_1, y_2, y_3, y_4) , for which the relation:

$$(y_1, y_2, y_3, y_4)\mathbf{M}(0) = \lambda_4(y_1, y_2, y_3, y_4) + m_2(0, 0, 0, 1)$$

is satisfied with an appropriate constant m_2 . This may be achieved by taking:

$$y_1 = \left(\frac{1}{u_3} - \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_1},$$

$$y_2 = - \left(\frac{1}{u_1} + \frac{1}{u_3} \right) u_S \cdot \frac{C}{C_2},$$

$$y_3 = \left(\frac{1}{u_1} + \frac{1}{u_2} \right) u_S \cdot \frac{C}{C_3},$$

$$y_4 = 0,$$

$$m_2 = \left(\frac{1}{u_1} + \frac{1}{u_2} \right) \left(\frac{1}{u_1} + \frac{1}{u_3} \right) \left(\frac{1}{u_3} - \frac{1}{u_2} \right) \times \frac{u_1 u_2 u_3 u_4 u_S C}{K}$$

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