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Simplified mathematical model of irreversible sample adsorption in capillary zone electrophoresis

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Abstract

In this paper, a very simple mathematical model of the irreversible adsorption of a sample onto the capillary wall in capillary zone electrophoresis is investigated analytically and numerically. With it, the influence of adsorption on the transport of sample is studied. The advantage of the model is that it contains only one parameter while other models usually contain many more. Mathematically, the problem is reduced to the construction of a solution of a partial differential equation coupled to an algebraic equation that imposes some restriction on the solution. Great attention is paid to the formulation of a correct mathematical model of the process. In diffusionless approximation, an analytical solution is obtained, while the influence of diffusion is studied by computer simulation. It is shown that in diffusionless approximation, the qualitative results regarding the behavior of the sample zone have a rather general character and do not depend on the specific mechanism of interaction between the substance and the wall.

Keywords: Adsorption; Mathematical models; Wall adsorption

1. I. Introduction

It is well known that sample sorption onto a capillary wall by means of ionic, hydrophobic and other mechanisms of interaction significantly reduces the efficiency of electrophoretic separation (see, e.g., Refs. [1–5]). In particular, the effect of sorption changes the sample's concentration profile, the velocity of zone motion and the amount of substance in the sample zone. Many works are devoted to the theoretical treatment of these phenomena, among which Refs. [5–12] should be mentioned. As a rule, almost all mathematical models are connected, to some extent, to those of chromatography (see, e.g., Refs. [13–17]). When describing the sorption effects, the chromatographic mechanism of interaction between the sample and the capillary wall, considered as a sorbent, is assumed. Such an interaction can be either linear [7–11], when the rate of sample adsorption is proportional to its concentration, or nonlinear [5,12,14–18], when the Langmuir adsorption isotherm is used. In the latter case, the concentration of the sorption sites on the capillary wall is also taken into account and thus the coefficient of proportionality also depends on the sample's concentration.

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Some of these works offer a rather detailed description of interaction mechanisms between the substance and the wall. As a result of such detail, these mathematical models contain a lot of parameters, which are often difficult to determine experimentally or to find in the literature. In contrast, when appropriately combined, practically any experiment can be fitted with reasonable qualitative and sometimes quantitative agreement. It is clear that the abundance of parameters in a model is not an advantage. Working on the models of non-linear adsorption in diffusionless approximation [12] and on that which takes into account the diffusion along a capillary as well as in a narrow sorption layer [5], we realized that similar effects can be observed using various combinations of parameters. Such parameters could be adsorption and desorption coefficients, the thickness of the sorption layer, its capacity, electrophoretic mobilities, diffusion coefficients, etc. (see, e.g., Refs. [5,8–12]). This means that the experimental determination of these parameters can be rather complicated, since some special experiments will be required to obtain isolated, "pure" effects in which an appropriate correlation "parameter effect" can be achieved.

Here the following question arises. What effects are observed by an experimenter working with capillary zone electrophoresis (CZE) in the presence of sample sorption effects? As a rule, he (she) has an electropherogram with a sample absorbance profile from which, after appropriate processing, the quantity of the sample in the zone and the velocity of zone motion can be derived. A visible attribute of the wall adsorption is the presence of a tail in the concentration profile, which often has a constant or almost constant height (the vast majority of electropherograms observed by the authors obeyed this rule [5]). Some parameters of process, such as electrophoretic mobilities, diffusion coefficients and the conductivity of the buffer solution, can be measured or obtained from reference books and they are usually known. Using one of the numerous CZE models available, it is also possible to determine the coefficient of interaction between the sample and the buffer solution (see, e.g., Refs. [19–22]) and thus to take into account electromigration dispersion, if present. On the contrary, there is a lack of information on parameters and mechanisms of adsorption processes. Even assuming that the sorption mechanism obeys, e.g., the Langmuir isotherm, it is impossible to find the adsorption and desorption coefficients, wall capacity, etc., in the literature. At best, only the orders of some values are known (see, e.g., Refs. [23]). Finally, the exact mechanism of adsorption is not known, even if it is described by Langmuir's isotherm in all models including our own [5,12].

In studying wall adsorption effects, it is reasonable to ask the following questions (the answers to some of them are rather obvious). The first one: How does sorption change the concentration maximum in the sample zone (peak height)? It is obvious that the peak height decreases, since the sample looses a part of its mass, being stuck to the capillary wall. The second question: Is there some deceleration in zone motion due to sorption effects and do all the points of the concentration profile move with the same velocity? The answer to this question is not so evident. It will determine whether the sample zone is deformed due to sorption or not. The third question is closely connected to the second: Do the sorption effects change the width of the zone (the distance between the zone's leading edge and the beginning of the "adsorption tail")? Finally, do the answers to these questions depend strongly on a particular mechanism of sample-wall interaction? For instance, does the qualitative behavior depend on the choice of formula describing the sorption process (Langmuir or non-Langmuir isotherm)? Is it possible to achieve quite good quantitative agreement between theory and experiment, based only on data from an electropherogram?

In this paper, we attempted to answer these questions by offering a very simple mathematical model of CZE with irreversible sample adsorption onto the wall. The sorption process in this model is characterized by only one parameter, which could either be determined from an electropherogram or be chosen empirically. In general, the model does not require a detailed description of the sorption mechanism. The advantage of the model is that it gives the solution in the form of algebraic formulas connecting all the parameters of the problem. Thus, the answers have a rather general character, while quantitative results can be obtained when necessary details of the transport mechanism are provided.

The general organization of the paper is as follows: In Section 2.1, a basic hypothesis and a mathematical model of the sample's transport are formulated. In Section 2.2, the diffusionless model is considered and its analytical solution is obtained. All principal conclusions about its qualitative behavior are formulated and proved. In Section 2.3, this model is extended to account for the diffusion effect and its influence on the sample zone's evolution. A specific model of the interaction between the sample and the wall (non-linear Langmuir isotherm) is considered in Appendix A. It is shown that, under certain assumptions concerning the parameters of the model, its solution is similar to that given by our model. Some mathematical details used in the text of the paper are given in Appendix B.

2. Theory

2.1. The simplest model of irreversible adsorption

As mentioned above, one of the most characteristic features by which sample adsorption manifests itself is the existence of an "adsorption tail" in the electropherogram. In many cases, the height of this tail or, in other words, the shift of the base line after the sample peak is constant with time. This height is actually a unique parameter of the sorption effect and is available directly from the electropherogram. Even in cases where the real mechanism of interaction is known or such a mechanism is supposed (see, e.g., Refs. [5–12]), the parameters of the sorption process, for instance, the adsorption and desorption coefficients for Langmuir's isotherm, are rarely available.

The fact that the height of the "adsorption tail" is constant suggests that sample desorption is small compared to its adsorption during an electrophoretic run. Of course, this is not valid for all samples and, perhaps, is valid only for relatively short time periods compared with the time required for electrophoretic runs. In our model, we assume that the desorption is so minute that it can be neglected, i.e., the sorption has an irreversible character. Experiments with different sample concentrations [5] showed that approximately the same amount of sample was attached to the capillary wall irrespective of its initial concentration. At this point in our model, we suppose that the amount of substance attached to the wall does not depend on its concentration in the solution, provided that the latter exceeds some threshold value. One can suppose that the capillary wall has a certain capacity, due to a certain number of adsorption sites, and that when all of these sites are occupied the wall is saturated. This state of saturation corresponds to the amount of sample on the wall and, hence, to the height of the adsorption tail. At this point, it is necessary to assume that the process of adsorption is much faster than those of electrophoretic motion and diffusion and that saturation of the wall occurs almost instantaneously. The assumptions above allow us to describe the adsorption by introducing the concentration of wall saturation, which, in experiments, is observed as a tail. It should be noted that the same conclusion may be derived by analyzing a non-linear Langmuir isotherm (see Appendix A). However, we intentionally do not provide a detailed description of the interaction process since for the construction of a correct mathematical model, as will be shown, the detailed mechanism of interaction is not important.

Let us describe the transport of sample in CZE in one-dimensional approximations by means of the usual transport equation:

$$c_t + I_x(c) = \varepsilon c_{xx} \tag{1}$$

Here c is the molar concentration of the sample, $I(c)$ is the molar flux density under the impact of an external force, ϵ is the sample's diffusion coefficient. The subscripts x and t here and below denote partial derivatives of x and t, respectively. We will notice here that, speaking about an external force, migration under the action of an electric field is usually assumed; however, the flux density, $I(c)$, can also include terms connected with the motion of liquid by external pressure and by electroosmotic flow. We suppose that the chemical reactions in the solution and the interaction between the sample and the wall are instantaneous and, for this reason, Eq. (1) does not contain the source term that usually describes adsorption [5,12]. The flux density, $I(c)$, should be equal to zero when the concentration of the sample equals zero; it is quite natural that in the absence of a substance, its flux is absent too. In other words, the following relationship must be satisfied:

$$I(0) = 0, \text{ (if } c \neq 0 \text{ then } I(c) > 0) \tag{2}$$

To be more specific, let us suppose that sample motion occurs from left to right. The concentration of the sample attached to the wall is denoted as s. Actually, it is the height of the "adsorption tail" registered on the electropherogram, which, for Langmuir adsorption (see Appendix A), is the concentration of adsorption sites or the capacity of the wall. Since we assume that adsorption is irreversible, the substance stuck to the wall cannot go back to the solution and does not participate in the transport of sample by means of an external field. It means that if, in some moment, the sample with concentration $c(x, t) \geq s$ occupied the region $x \in \mathcal{M}$ (zone) and then, under the action of the electric field, it moved away, part of it, with concentration s, would remain attached to the wall. This requirement imposes the algebraic restriction $c(x, t) \geq s$ for the solution of Eq. (1) in any region, whenever occupied by the sample. It is clear that this restriction contradicts the relation shown in Eq. (2), as $I(c) \neq 0$ at $0 < c \leq s$. Hence, in order to account for the effect of sticking, it is necessary to redefine the expression for flux density.

The natural way to redefine the flux density while preserving the mathematical correctness of the problem is to subtract the portion describing the migration of the sample, which, in effect, is attached to the wall:

$$i(c) = \begin{cases} I(c) - I(s), & c \geq s \\ 0, & 0 < c \leq s \end{cases} \tag{3}$$

Here, $i(c)$ is the molar flux density accounting for the sorption effects. With such a definition, the part of the substance attached to the wall is excluded from the transport under the impact of an electric field.

At a first glance, such redefinition does not change Eq. (1), since $i_x(c) \equiv I_x(c)$ for $c \geq s$. However, in reality, the new flux density $i(c)$ is not a smooth function, as its derivative is discontinuous at $c = s$. This fact makes the properties of solution of Eq. (1) completely different. It will be shown for the diffusionless model considered in Section 2.2.

It is necessary to note that this is a unique way to correct the problem. For instance, it is impossible to do this by introducing the source term describing some chemical reactions, since the assumption of their instantaneous action sets this to zero. If not, there will be relaxation effects, bringing the system to a state of equilibrium, but this contradicts what has been specified above. Ways of redefining the flux density are discussed in more detail in Ref. [24], in which a strict mathematical treatment is given for the effect of a "chemical trap", as observed in electrophoresis [25]. The effect described in Refs. [24,25] is in many aspects similar to that examined in the present paper and, in fact, these works stimulated the development of the simplified model of adsorption, while the mathematical methods elaborated in Ref. [24] allowed us to obtain the solution in the simplest form.

Certainly, the problem of redefinition of flux density results from the absence of information about the mechanism of interaction between the sample and the wall. In particular, in Appendix A, a mathematical model is presented for which information about the mechanism of interaction that leads to the condition $I(s) = 0$ is shown. However, in most situations, the flux given by Eq. (3) seems to be more natural. At least, this way does not require any additional information and, hence, is more universal. Moreover, it will be shown that the results given by a general model and the model presented in Appendix A differ insignificantly from each other in cases where the concentration of sample is small. It is worth noting that it would be more logical to redefine a total flux density, including in it the diffusion flux as well. This will be discussed in detail later (in Section 2.3).

When accounting for the above considerations and adding the initial conditions typical for ZE problems, Eq. (1) can be rewritten as:

$$c_t + i_x(c) = \varepsilon c_{xx}, \; c \geq s \tag{4}$$

$$i(c) = \begin{cases} I(c) - I(s) \geq 0, & c \geq s \\ 0, & c \leq s \end{cases} \tag{5}$$

$$c|_{t=0} = \begin{cases} c_0, x \in [0, l_0], & c_0 = \text{const.} \\ 0, x \notin [0, l_0 \end{cases} \tag{6}$$

where c_0 is the initial sample concentration in zone region $[0, l_0]$ and l is the initial zone width.

2.2. Diffusionless model

It is well known that the diffusionless approximation is acceptable for studying the transport processes in electrophoresis for time intervals that

are considerably smaller than the characteristic time of diffusion (see, e.g., Refs. [21,22]). In particular, it works well when concentrated sample is considered and the electromigration dispersion is significant, since the shape of the concentration profile, the velocity of the zone's leading and trailing edges are basically determined by non-linear effects of interaction between the sample and the buffer solution. Diffusion leads to the decrease in the concentration maximum in the peak and to smoothing of concentration gradients in the profile, however, the peak shape remains essentially the same. Some advanced mathematical methods exist for diffusionless models that allow analytical solutions to be obtained, and these significantly simplify the analysis of the zone's evolution. This is why we first consider the problem described in Eqs. (4–6), in which the diffusion term is omitted, i.e., $\varepsilon = 0$, while electromigration dispersion is not negligible. Eqs. (4–6) are thus reduced to a quasi-linear hyperbolic equation, which could be solved by one of the methods described in Refs. [26–29]. A survey of such methods applied to chromatography is given in Ref. [18]. Some extension of these method was proposed by us [24], for a case with algebraic restrictions.

2.3. Mathematical model

It is known that to correctly state the diffusionless problem, some additional relationship concerning discontinuities, known as the Rankine-Hugoniot relation, and also additional stability conditions for these discontinuities (see, e.g., Refs. [26–29], particularly in electrophoresis [12,19–22]), are required. The discontinuities in solution can be due to initial discontinuities in the concentration profile (pulse profile). Together with these relations, Eqs. (4–6) can be rewritten as:

$$c_t + i_x(c) = 0, c \geq s \tag{7}$$

$$i(c) = \begin{cases} I(c) - I(s), & c \geq s \\ 0, & c \leq s \end{cases} \tag{8}$$

$$V[c] = [i(c)], V = \frac{dx(t)}{dt}, \tag{9}$$

$$i'(c(x(t) - 0, t)) \geq V \geq i'(c(x(t) + 0, t)) \tag{10}$$

$$c|_{t=0} = \begin{cases} c_0, x \in [0, l_0], & c_0 = \text{const.}, c_0 \geq s \\ 0, x \notin [0, l_0] \end{cases} \tag{11}$$

Here, the symbol [...] denotes the jump of the function value across the line of discontinuity $x = x(t)$, i.e., $[f] = f(x(t) + O,t) - f(x(t) - 0,t)$; V is the velocity of the discontinuity propagation, and the flux derivative is calculated with respect to c. Eq. (9) is the so-called Rankine-Hugoniot relation and it corresponds to the mass conservation law written in terms of concentration on the line of discontinuity. The inequality (Eq. (10)) is a stability condition for discontinuity, which means that the group velocity of the substance (i.e., $i'(c)$) to the left of the line of discontinuity should be higher than the velocity of discontinuity propagation, which, in turn, should be higher than the velocity of the substance to the right of the discontinuity. This condition guarantees the existence of a discontinuity. When it is not satisfied, the initial discontinuity will transform into the so-called rarefaction wave. The Rankine-Hugoniot relation emphasizes a difference between the flux densities $I(c)$ and $i(c)$, as defined by Eq. (8). In particular, it is now possible to write the conditions on the boundary between regions in which in one the concentration is $c \geq s$, while in the other it is $c = 0$, correctly. The first region contains the sample that is able to stick to the wall, while the second region is free of sample and therefore there is no sample on the wall.

Let us introduce the following designations, which are required for the inequalities below to be valid:

$$v(c) = \begin{cases} i'(c) = I'(c), & c \geq s \\ 0, c < s, & c = 0 \end{cases} \tag{12}$$

$$v(c) > 0, v'(c) \equiv i''(c) > 0$$

Here $v(c)$ is a group velocity of the points on the concentration profile everywhere except for the discontinuity. It is this velocity that appears in the stability condition of discontinuity (Eq. (10)). Strictly speaking, it is possible not to define the velocity $v(c)$ for $c < s$, however, such a definition simplifies the algorithm of the solution. It is sufficient to define only $v(0)$. The value $v(0)$ is necessary for substitution into Eqs. (10,15).

The inequality, $v'(C) > 0$, limits our considerations to the case in which the sample profile is

fronting and its leading edge is sharp, i.e., discontinuous, while the trailing edge is diffuse, due to non-linear electromigration effects. It corresponds to the case encountered most often in electrophoresis in which the molar conductivity of the sample zone is less than that of the buffer solution (see, e.g., Refs. [19–22]). The case where $v'(c) < 0$ is solved similarly and does not cause any difficulties.

2.3.1. Evolution of the concentration profile

The theory of how to solve Eqs. (7–11) has been extensively treated in mathematical literature devoted to quasi-linear hyperbolic equations (see, e.g., Refs. [26–29]), and in works on electrophoresis (see, e.g., Refs. [19–22]). Some specific methods for problems with algebraic restrictions are developed in Refs. [24,25]. For the sake of simplicity, we present, later on, only the scheme of how the solution may be constructed and the figures illustrating the evolution of the concentration profile. Mathematical details of solution will be omitted. Qualitative features, such as the shape of the concentration profile, will be similar for an arbitrary equation for flux density, satisfying the conditions of Eqs. (8,12).

We determined the concentration profile in a moving zone by the equation:

$$v(c) = \frac{x}{t},\ c(x,t) = a\left(\frac{x}{t}\right),\ z = \frac{x}{t},\ s \le c \le c_0 \qquad (13)$$

Here, $a = a(z)$ is the so-called automodeling solution of Eqs. (7–12), corresponding to the rarefaction wave; z is the automodeling variable. The function $a(x/t)$ describes the concentration profile everywhere that it is non-constant. In practice, instead of the function $a = a(z)$, it is more convenient to use the inverse function, $z = z(a) \equiv v(a)$.

The solution of Eqs. (7–12), at $t = +0$, i.e., immediately after the application of an electric field, is shown in Fig. 1.1b.[3]* (in Fig. 1.1a the initial concentration profile, $t = 0$, is shown). The points α, β and γ of the concentration profile move according to the laws $x = x_\alpha(t)$, $x = x_\beta(t)$ and $x = x_\gamma(t)$.

[3] Fig. 1.1, 1.2 have a demonstrative character. The value $s = 0.75$ mM was chosen in order to show the presence of an "adsorption tail".

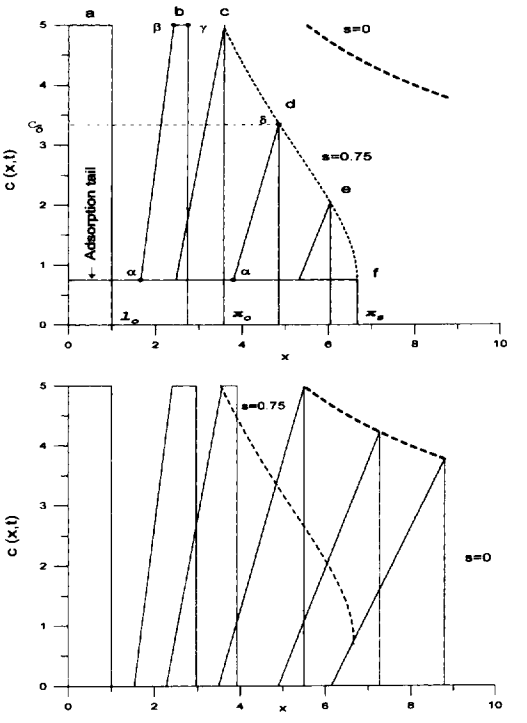

Fig. 1. Evolution of a sample concentration profile for different time moments: with (1.1) and without (1.2) adsorption. The thin dashed line shows the peak apex evolution in the case of adsorption ($s = 075$), thick dashed line shows the peak apex evolution in the absence of adsorption ($s = 0$). For further explanation, see text.

$$x_\alpha(t) = v(s)t,\ x_\beta(t) = v(c_0)t,$$
$$x_\gamma(t) = l_0 + V_0 t \qquad (14)$$

Here, V_0 is the velocity of the zone's leading edge (see Fig. 1.1b). The value of V_0 is determined by Eq. (9), on the line of the discontinuity $x = x_\gamma(t)$, where $c(x_\gamma(t) - 0,t) = c_0$ and $c(x_\gamma(t) + 0,t) = 0$. It is easy to check that the validity of inequalities (Eq. (10)) follows from Eq. (12) (see also Appendix B):

$$V_0 = \frac{i(c_0)}{c_0} = \frac{I(c_0) - I(s)}{c_0},\ v(c_0) > V_0 > v(0) = 0 \qquad (15)$$

The solution of Eqs. (7–12) for the interval $x \in [0,x_\alpha(t)]$ is a constant $c = s$, for the interval $x \in [x_\alpha(t),x_\beta(t)]$ it is $c = a(x/t)$ and for the interval $x \in$

$[x_\beta(t),x_\gamma(t)]$ it is $c=c_0$. The region $x\in[0,x_\alpha(t)]$ will be called an "adsorption tail".

The shape of the concentration profile shown in Fig. 1.1b will be changed in the moment t_0, when the point $x=x_\beta(t)$ reaches the point $x=x_\gamma(t)$.

$$t_0=\frac{l_0}{v(c_0)-V_0},\ x_0 = v(c_0)t_0,\ [x_0=x_\beta(t_0)=x_\gamma(t_0)] \quad (16)$$

Here, x_0 is the coordinate of the zone's leading edge in the moment of interaction t_0 (see Fig. 1.1c).

The further evolution of the concentration profile is shown in Fig. 1.1d–f. For comparison, the concentration profile in the absence of adsorption ($s=0$) is shown in Fig. 1.2. The dotted lines in Fig. 1.1 and Fig. 1.2 correspond to the maximum concentration for both $s=0$ and $s\neq0$. As previously stated, the solution on the interval $x\in[x_\alpha(t),x_\delta(t)]$ (see Fig. 1.1 d) is determined by the expression $c=a(x/t)$, however, unlike in Fig. 1.1b, the law of motion of the leading edge is different and it is determined by the differential equation (Eq. (9)) (see also Ref. [12]):

$$\frac{dx_\delta(t)}{dt}=\frac{i(c_\delta(t))}{c_\delta(t)},\ c_\delta(t)=a\left(\frac{x_\delta(t)}{t}\right) \quad (17)$$

with the initial condition:

$$x_\delta(t_0)=x_0 \quad (18)$$

Here c_δ is the sample concentration on the line of discontinuity, actually, it is a maximum of the sample's concentration in the zone. Fortunately, the Cauchy problem, Eqs. (17,18), can be solved using analytical methods (see, e.g., Ref. [24]). The solution may be written by using implicit functions:

$$v(c_\delta(t))c_\delta(t)-i(c_\delta(t))=\frac{l_0c_0}{t},\ x_\delta(t)=v(c_\delta(t))t \quad (19)$$

It is easy to prove that the formulas in Eq. (19) provide the solution to Eq. (17), which satisfies the condition (Eq. (18)). For this purpose, it is sufficient to find a derivative of Eq. (19) on t, taking Eq. (16) into account and the equality $c_\delta(t_0)=c_0$ (for details see Appendix B). As in the case of Eq. (13), in order to build a graph, for instance, $c_\delta=c_\delta(t)$, it is not necessary to solve Eq. (19). It is easier to build the inverse function $t=t(c_\delta)$ or $x=x(c_\delta)=v(c_\delta)t(c_\delta)$. Using this method, the inverse function for the maximum concentration, $c_\delta(t)$, with and without adsorption, was determined and is shown in Fig. 1.1 and Fig. 1.2 (dotted lines).

The evolution of the concentration profile (shown in Fig. 1.1c–e) occurs up to the moment t_s, when the trailing edge of the rarefaction wave [the line $x=x_\alpha(t)$ will reach its leading edge [the line $x=x_\delta(t)$]. Obviously, at this moment, the concentration is equal to $c_\delta(t_s)=s$. The value of t_s is derived by using Eq. (19) and taking into account that $i(s)=0$ (see Fig. 1.1f):

$$t_s=\frac{l_0c_0}{sv(s)},\ sx_s=c_0l_0 \quad (20)$$

Here, x_s is the point where the edges of the rarefaction wave will meet each other. Further evolution of the zone does not occur, since the sample is completely stuck to the wall and the equation $sx_s=c_0l_0$ is, in essence, the mass conservation law.

2.3.2. Analysis of the typical evolution law for the concentration profile

Despite the fact that the mechanism of interaction between the sample zone and the buffer solution is not known, i.e., the specific expression for the flux density is not defined and the mechanism of interaction between the sample and the wall is not specified, it is still possible to derive some general laws for the evolution of the concentration profile. Of course, more detailed and precise information can be obtained in the case when the formula for $I(c)$ is provided, in our case we took this to be:

$$I(c)=c(1+kc),\ k\geq0$$

Here, k is the coefficient of interaction between the sample and the buffer solution. Further on, for the values t_0, x_0, t_s, x_s, $c_\delta(t)$, $x_\delta(t)$ and V_0, we will use the notations $t_0(s)$, $x_0(s)$, $t_s(s)$, $x_s(s)$, $c_\delta(t,s)$, $x_\delta(t,s)$ and $V_0(s)$, emphasizing their dependence on s. All of the previous formulas are as valid at $s=0$, when adsorption is absent. Let us now pass from spatial coordinates to the temporal ones that represent the detector readings and let us denote by y the coordinate of the detector. The maximum sample concentration in the zone is denoted as θ. In our case, this is the concentration $c_\delta(t)$ at the point $x=y$ for an appropriate time moment. The detector should be

placed in the capillary so that it could register the motion of the zone, i.e., $l_0 \le y \le x_s(s)$. Where $y > x_s(s)$, the substance will never reach the detector, since it will be completely adsorbed before the point $x = x_s(s)$ (see Fig. 1.If). The trailing edge of the zone registered by the detector is obviously determined by the function $a(y/t)$ (see Eq. (13) Fig. 2). The simulation results presented in Fig. 2 correspond to the following parameters: initial sample plug length and its initial concentration are equal to unity, $k \approx 0.27$, $s = 0.01$, detector position $y = \tilde{y} = 37.3$.

Below we formulate some general statements valid for the evolution of the sample zone, regardless of the specific expression for the flux density $I(c)$.

(1) The part of the concentration profile described by the rarefaction, wave has the same form for all values of s. The velocity of the points for this part of the profile does not depend on s.

This evidently follows from Eqs. (12,23). As can be seen from Fig. 2, the part of the electropherogram that follows the leading edge ($t > t_{min}(s,y)$) is identical for electropherograms, independent of the concentration s. The difference between electropherograms will be in the moment at which the zone reaches the detector, i.e., in t_{min}. Fig. 2 shows two electropherograms corresponding to zero adsorption, $s = 0$ (thin solid line), and non-zero adsorption, $s = 0.01$ (thick solid line). The moment, t_{min}, when the zone's leading edge reaches the detector is determined by the formulas in Eq. (19), which, at $x = y$, have the form:

$$v(\theta)\theta - i(\theta) = \frac{l_0 c_0}{t_{min}},\ y = v(\theta)t_{min},\ t_{min} = t_{min}(s, y),\ \theta = \theta(s, y) \tag{21}$$

By excluding $t_{min}(s)$, it is possible to derive the function $\theta = \theta(y)$, giving the dependence of concentration maximum versus the detector position. Similarly, by excluding θ, the function $t_{min} = t_{min}(s,y)$ is derived. These functions are plotted in Fig. 3 for several values of s. The thick lines correspond to the function $\theta(s,y)$ at $s = 0$, 0.01, 0.02, 0.03, 0.04, 0.05 and the thin lines correspond to the function $t_{min}(s, y)$ at $s = 0$, 0.01, 0.02, 0.03, 0.04, 0.05. The following inequalities can be easily proven (see Appendix B):

$$\theta(s, y) < \theta(0, y),\ \frac{\partial \theta}{\partial s} < 0 \tag{22}$$

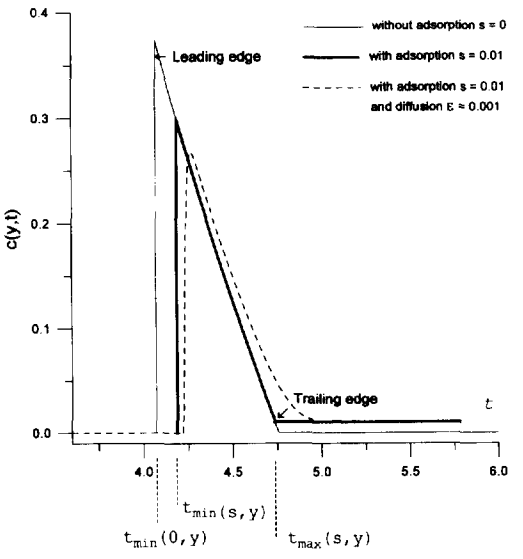

Fig. 2. Comparison of three different electropherograms without adsorption (thin solid line), with irreversible adsorption in the absence of diffusion (thick solid line) and with irreversible adsorption with diffusion (dashed line). For further explanation, see text.

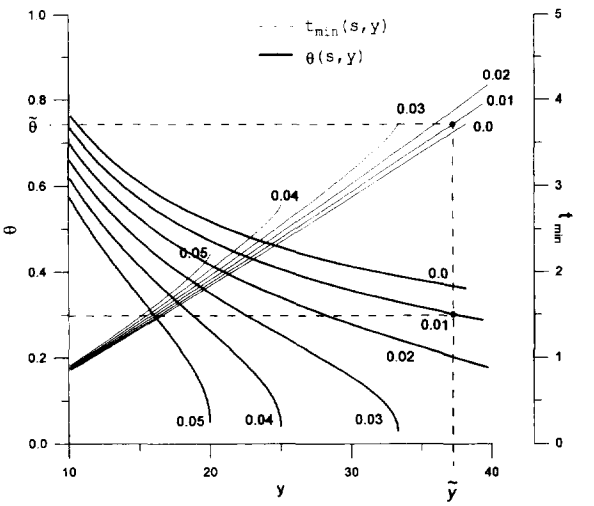

Fig. 3. The dependence of the concentration maximum θ (thick lines) and t_{min} (thin lines) on the effective length, y, of the capillary, for different values of wall capacity, s. For further explanation, also see text.

$$t_{\min}(s, y) > t_{\min}(0, y), \frac{\partial t_{\min}}{\partial s} > 0 \quad (23)$$

Two other statements obviously follow from these inequalities:

(2) The concentration maximum registered by the detector decreases with the increase in s.

(3) The velocity of the zone's leading edge decreases with the increase in s (t_{min} increases with increasing s).

As an example, the behavior of functions $t_{\min}(s,y)$ and $\theta(s, y)$ for $y=\tilde{y}$ is shown in Fig. 4. The statement 2° means that a part of the substance remains in the "adsorption tail" and it is rather obvious. The statements 1° and 3° mean that the adsorption retards the leading edge of the moving zone but it does not effect the motion of the rest of the zone behind it. In reality, the substance is not retarded but continuously removed from the leading edge. Let us denote as $t_{\max}$ the moment when the trailing edge of the zone reaches the detector. It is obvious that in the case of adsorption, this point is chosen as the coordinate of the point $x_\alpha(t_{\max}) = y$, where the sample concentration is $c = s$. Thus from Eq. (14), we have:

$$t_{\max} = \frac{y}{v(s)}, \frac{\partial t_{\max}}{\partial s} = \frac{-yv'(s)}{v_2(s)} < 0\, t_{\max} = t_{\max}(s, y) \quad (24)$$

(4) The velocity of the trailing edge increases with the increase of s (t_{max} decrease with increasing s).

The functions $t_{\max}(s,y)$ and $\theta(s, y)$ for $y=\tilde{y}$ are presented in Fig. 4. Since the function $v(c)$ is monotone, i.e., $v'(c) > 0$, then:

$$t_{\max}(s, y) < t_{\max}(0, y) \quad (25)$$

Let us define as $W(s)$ the width of the zone in the electropherogram in (c,t) coordinates by the formula:

$$W(s) = t_{\max}(s) - t_{\min}(s) = \frac{y}{v(s)} - \frac{y}{v(\theta)} \quad (26)$$

The "width" of the zone in the electropherogram is measured in units of time. The physical width of the zone is defined as $x_\gamma(t) - x_\alpha(t)$ at $t \geq t_0$.

The next statement is valid:

(5) The width of the zone in the presence of adsorption is less than the width of the zone without adsorption. The width of the zone decreases with increasing s.

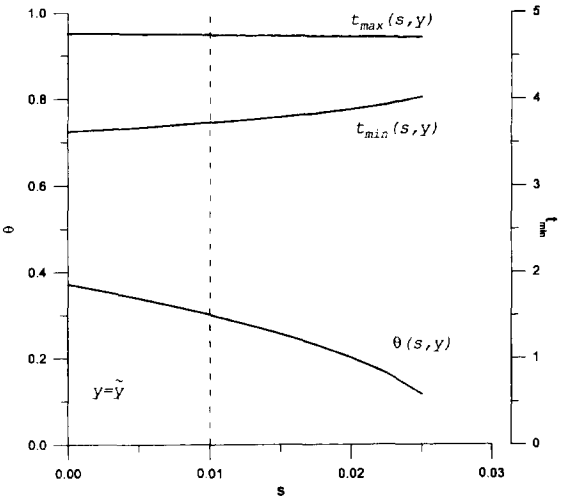

Fig. 4. The dependence of θ, $t_{\max}$ and $t_{\min}$ on the wall capacity, s, for a fixed capillary effective length of $y=37.3$. For further explanation, see text.

Indeed, we can determine from Eqs. (12,22,23,26) that $W(s) < W(0)$. The second statement, i.e., $W'(s) < 0$, is proved in Appendix B. Thus, adsorption reduces the zone width and, it might seem, improves the efficiency of the system. It can be seen clearly in Fig. 4 that the difference $t_{\max} - t_{\min}$ decreases with increasing s. The width of the zone becomes zero in a finite interval of time because the zone substance is pumped to the near-wall layer. In a normal separation system, the zone width never equals zero and, as a rule, it increases with time.

2.4. Influence of diffusion effect on the concentration profile

In Section 2.3.2, results were obtained for a wide class of flux functions, $I=I(c)$, for which $v(c) > 0$, $v'(c) \equiv i''(c) > 0$, assuming that diffusion is absent. Qualitative results for a particular sample substance and buffer composition can be easily calculated, when the formula for $I(c)$ is provided. In our case, the following expression was used:

$$I(c) = c(1 + kc), k > 0 \quad (27)$$

In this section, the influence of diffusion effect on the evolution of the concentration profile is analyzed and no conditions are imposed on the k value. Eqs. (4–6), presented in Section 2.1 are solved by using numerical methods. This model requires some specification. As already mentioned, it would be more natural (and more correct) not to redefine the flux density $I(c)$, but rather the full flux density, including the diffusion term, that is:

$$I_{\text{diff}} = -\varepsilon c_x + I(c)$$

In the region with a concentration of $c > s$, it is necessary to keep the expression in the form:

$$I_{\text{diff}} = -\varepsilon c_x + i(c), c \geq s$$

while in the region $c < s$, it is necessary that the flux be equal to zero:

$$I_{\text{diff}} = 0, c < s$$

For this purpose, it is sufficient to only redefine the derivative c_{xx} using the following formula:

$$c_{xx} = \begin{cases} c_{xx}, & c \geq s \\ 0, & c < s \end{cases}$$

However, such redefinition is rather dangerous, as the diffusion term εc_{xx} becomes a non-smooth function. Generally speaking, it contradicts the purpose with which the diffusion term was introduced into equations. It is this term that should provide the high smoothness of the solution and eliminate the discontinuities.

The results of simulations for the diffusion coefficient $\epsilon = 2 \cdot 10^{-4}$ and different k values are presented in Fig. 5. Unlike in Section 2.3.2, here all three typical situations were simulated, i.e., when the sample peak is tailing $k = 0.3$, fronting $k = -0.3$ and Gaussian $k = 0.0$. Initially the sample profile had a pulse shape. Solid lines represent sample concentration profiles along the capillary column, for which the wall capacity factor was assumed to be $s = 0.03$. For comparison, corresponding profiles are also drawn (dashed lines) when wall adsorption is absent. Evidently, the principal conclusion formulated in the previous section are equally valid in the case with diffusion. It is seen that wall adsorption retards the propagation of the sample's front edge compared to that in the adsorptionless case. At the same time, the

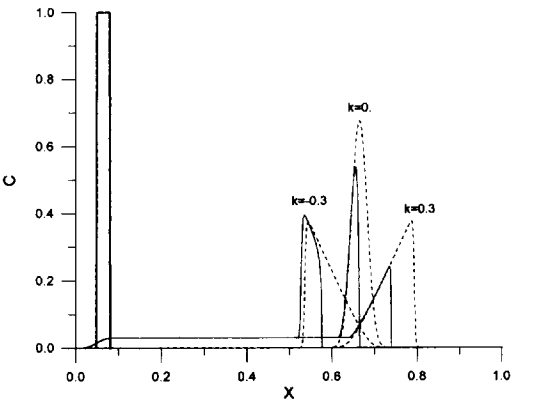

Fig. 5. Concentration profiles along the capillary column with (solid lines) and without (dashed lines) adsorption for different parameters, k. Diffusion coefficient, $\varepsilon = 2 \cdot 10^{-4}$, wall capacity, $s = 0.03$, time $t = 0.6$. Initial concentration profile was a rectangular pulse (on the left). For further explanation, see text.

rear edge remains practically the same. Consequently, the width of the sample zone is always narrower with adsorption. It is worth noting that Statement 2 is not valid for $k < 0$, since in this case, Eq. (12) is not true. It can be seen from Fig. 5 that the peak maximum for $k = -0.3$ remains the same in the presence and absence of adsorption. The other interesting feature is that in all cases the peak's leading edge becomes sharp.

The effect of diffusion itself leads to a little lagging of the sample peak compared to that observed in the diffusionless case (see Fig. 2, dashed line, in comparison with the solid thick line). A more pronounced difference can be seen in the trailing edge, where diffusion results in much stronger tailing. The sample peak is lower but it preserves its shape. As can be seen from the graphs, the redefinition of the flux did not produce significant changes in the case of diffusion. This means that the time considered is much shorter than the characteristic time of diffusion. On the other hand, the non-linear effects leading to a characteristic triangular peak shape dominate the diffusion.

As a concluding remark one can specify that the problem of sample adsorption onto the capillary wall is complex and has not been resolved yet. Different methods for controlling the state of the capillary wall were proposed, among them, buffer changes and additives; the use of organic solvents; adsorption of

neutral and/or charged macromolecules (including surfactants) to the wall; chemically bonded phases, etc. An extensive review of these methods may be found in Ref. [30]. None of them completely eliminates the sample's adsorption, hence, a further study of this phenomenon is necessary. The simple mathematical model of sample adsorption offered above may be useful in understanding some general features of sample behavior, since it is applicable to a wide class of interaction mechanisms and does not require detailed information.

3. Experimental and results

3.1. Materials and methods

All chemicals were of analytical-reagent grade; acetic and formic acids, potassium hydroxide and cytochrome *c* were purchased from Merck (Darmstadt, Germany) and poly-L-lysine was from Sigma (St. Louis, MO, USA). Samples were prepared by dissolving them in the running buffer.

Experiments were performed using a Beckman P/ACE System 5000 capillary electrophoresis unit (Palo Alto, CA, USA), running under GOLD Software (Beckman). An untreated fused-silica capillary (27.0 cm (20.1 cm effective length)×75 μm I.D.; Polymicro Technologies, Phoenix, AZ, USA) was used. Electropherograms were collected using a UV detector set at 214 nm. A voltage-stabilized regime was used in all experiments. The temperature of the liquid-cooled capillary was maintained at 25°C. Between runs, the capillary was washed with 0.1 *M* NaOH for 2 or 5 min and then with buffer for 2 min. Injection of sample was by means of pressure excess.

3.2. Results

In order to evaluate how the theoretical predictions correlate with experimental data, a series of experiments were performed. It should be noted that, in general, the reproducibility of results depends strongly on the capillary treatment performed between the runs and the environment to which the capillary surface was subjected previously.

Unlike theoretical studies, it is very difficult, if indeed possible, to perform two experiments under the same conditions so that in the first the sample–wall interaction is zero while in the second it exists. In other words, if the sample adsorption is initially negligible, one should change the pH value of the buffer or its ionic strength or something else to increase the adsorption, but this will inevitably change other characteristics of the sample's migration. This is why a series of experiments was performed in which the only parameter changed was the injection time. Experimental electropherograms with injection times of 1, 2 and 3 s are plotted in Fig. 6. Sample peaks demonstrate strong tailing due to adsorption. As expected, shorter injection times gave lower peaks, as a bigger portion of the substance was attached to the wall before the peak arrived at the detector. Due to adsorption, sample peaks with shorter injection times are retarded, thus, their front boundary arrives at the detection point later. However, unlike theoretical predictions, the part described by the rarefaction wave (to the right of the peak apex) is not the same for all three runs. We assume that the reason for this could be the finite rate of adsorption–desorption kinetics (the model assumes that the adsorption is infinitely fast, while the desorption rate is zero). In fact, one should bear in mind that first the sample is adsorbed from the liquid volume near the capillary wall and then some time is

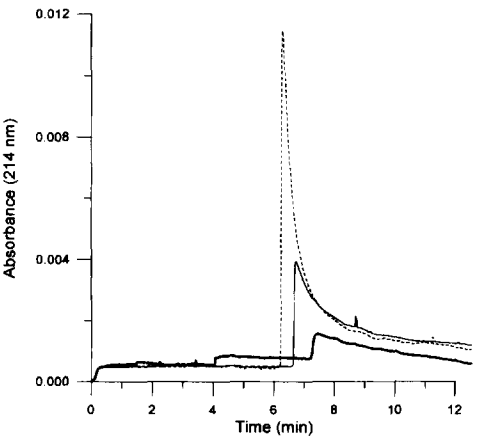

Fig. 6. Effect of different injection times on the absorbance profiles of the sample (0.5 mg/ml of cytochrome *c*): 1 s, solid bold line; 2 s, solid thin line, 3 s, dashed line. The buffer solution was 100 m*M* formic acid titrated to pH 3.5 with NaOH. The running voltage was 5 kV and the capillary was washed for 5 min with 0.1 *M* NaOH in-between runs.

necessary for the substance to diffuse from the region close the capillary axis towards the wall, in order to be adsorbed. This time can be roughly estimated as a characteristic diffusion time $t_{diff} = r^2/D$, where r is the radius of the capillary and D is a diffusion coefficient. For the typical values, $2r = 75$ μm and $D = 1 \cdot 10^{-10}$ m^2/s, it is $t_{diff} = 14.1$ s that is not negligible.

The above experiments demonstrated a rather strong sample interaction with the wall. We performed some experiments in which this interaction seemed to be weaker. A series of runs with poly-L-lysine was performed in acetate buffer. Initially the interaction was very weak and the baseline on the electropherogram returned to its initial level after the peak passage (Fig. 7, solid line). After several washings with a 0.1 *M* NaOH solution, we got evident signs of the sample wall interaction, i.e., baseline shift and a decrease in the sample peak area (Fig. 7, dashed line). Peak integration showed that, due to interaction, about 72% of its mass was lost, assuming that the first peak (solid line) did not loose it. If we suppose that the capillary wall is uniformly covered with a layer of substance from the inlet to the detection point (approx. 20 cm) and that the amount of substance on the wall is such that it corresponds to the shift in the baseline, we determined that the loss of the sample mass should be ca. 11%. Such a big difference in these two estimations may be attributed to the fact that the sample is adsorbed to the wall non-uniformly, perhaps its larger part was attached to the capillary wall close to the inlet end.

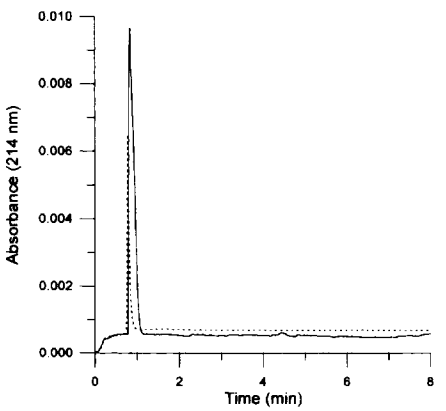

Fig. 7. Poly-L-lysine (0.5 mg/ml) absorbance profiles with zero sample loss due to the sample–wall interaction (solid line) and when a part of it was lost on the wall (dashed line). The running buffer was 100 m*M* acetic acid titrated to pH 4.0 with NaOH; the voltage applied was −10 kV, the injection time was 1 s and the capillary was washed for 5 min with 0.1 *M* NaOH in-between runs.

4. Conclusions

A simple one-parameter mathematical model of sample sorption onto a capillary wall is proposed in this paper. The model is based on the assumption that sample adsorption is irreversible and the unique parameter characterizing this process is the number of sorption sites or the sorption capacity of the wall. This parameter can be calculated directly from the electropherogram from the baseline shift before and after the sample peak. Mathematically, the model is described by a partial differential equation with an algebraic condition, which is eliminated by redefining the sample flux term. An advantage of the model is that it allows some preliminary conclusions to be drawn about the sample's evolution, without having detailed information about its transport mechanism. Another advantage is that the solution can be obtained in the form of algebraic formulas connecting all the parameters of the problem. It is shown that in a diffusionless approximation, sample adsorption reduces the peak height and the velocity of the zone's leading edge (when the peak is tailing). However, the rear part of the concentration profile is not effected by adsorption. The diffusion effect slightly retards the sample zone, simultaneously giving a more tailing profile. It is shown that in the limiting case of irreversible adsorption, Langmuir adsorption practically coincides with the described model.

Acknowledgments

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Appendix A

Model of non-linear Langmuir adsorption

In this section, we compare the model proposed in the paper with a usual model of non-linear adsorption, showing that, for small concentrations, the difference between the models is insignificant.

Let us consider a model of adsorption of a sample onto a capillary wall, corresponding to Langmuir's adsorption (see, e.g., electrophoresis models [5,12] and chromatographic models [13–18]):

$$(u+q)_t + I_x(u) = 0, I(0) = 0 \quad \text{(A.1)}$$

$$q_t = k_a(s-q)u - k_d q \quad \text{(A.2)}$$

Here u is the concentration of sample in the liquid phase; q is the concentration of sample attached to the wall (solid phase); s is the amount of adsorption sites per unit area (capacity of the wall); k_a and k_d are adsorption and desorption coefficients; $(u+q) = c$ is the total concentration of sample. We assume that the process of adsorption has an irreversible character and that desorption is absent, i.e., $k_d = 0$. In addition, we consider that the time scale of adsorption is very small compared to that of the processes associated with external forces; e.g., electrophoretic migration, i.e., $k_a \to +\infty$.

Then from Eqs. (A.2) we have:

$$q = s,\ u = c - q = c - s,\ \left(q = \frac{k_a s u}{k_a u + k_d}\right)$$

Substituting the obtained expression in Eqs. (A.1), we derive:

$$c_t + I_x(c-s) = 0 \quad \text{(A.3)}$$

Note that $I(c-s)|_{c=s} = 0$, i.e., the requirement for the absence of sample transport by means of an electric field is automatically satisfied when $c = s$. For small concentrations of c and s, by performing a Taylor series expansion and keeping only the terms up to the second order of magnitude, we have [only if $I(0)=0$]:

$$I(c-s) \approx I(c) - I(s),$$

since

$$I(c-s) = I(0) + I'(0)(c-s) + \ldots = I'(0)(c-s) + \ldots$$

$$I(c) - I(s) = I(0) + I'(0)c + \ldots - I(0) - I'(0)s - \ldots$$
$$= I'(0)(c-s) + \ldots$$

Thus, the model described in the paper does not differ much from the model expressed in Eqs. (A.1), (A.2) for small concentrations. However, as already stated, it has an advantage, since it does not require specific information about the mechanism of sample–wall interaction given, for instance, by Eqs. (A.2). Of course, in cases in which the mechanism of adsorption is known (e.g., Eqs. (A.2)) and the values of k_a, k_d and s are also known, it is necessary to use the model in Eqs. (A.1), (A.2). In contrast, in the absence of such information, the model shown in Eqs. (7–12) is preferable, since it still offers some qualitative results. It may seem that the model described in this paper is a special case of Eqs. (A.1), (A.2), when $k_d = 0$. However, this is wrong, since the direct passage to the limit when $k_d \to 0$ is incorrect, because

$$\lim_{u\to 0}\lim_{k_d\to 0} q(u) = s \neq \lim_{k_d\to 0}\lim_{u\to 0} q(u) = 0$$

Appendix B

Mathematical details

The proof of inequalities in Eqs. (22,23) is based on the following inequalities:

$$ci'(c) - i(c) > 0,\ (cv(c) - i(s) > 0),\ \forall c \geq s \quad \text{(B.1)}$$

$$l_0 c_0 < \theta y \quad \text{(B.2)}$$

In order to prove Eqs. (B.1), we consider the function $\varphi(c) = ci'(c) - i(c)$. Differentiating c and accounting for Eq. (12), we obtain $\varphi'(c) = cv'(c) > 0$. This means that the function $\varphi(c)$ monotonically increases and $\varphi(\theta) > \varphi(s) = sv(s) > 0$, i.e., $\theta > s$. In particular, from the inequality in Eqs. (B.1), the validity of the left part of Eq. (15) follows. The right

part of Eq. (15) follows from Eq. (12), since $v(0) = 0$.

In order to prove Eqs. (B.2), we substitute the expression for y obtained from Eq. (21) by excluding t_{min}:

$$l_0c_0 < \frac{\theta i'(\theta) l_0c_0}{\theta i'(\theta) - i(\theta)} = \theta y$$

By dividing by l_0c_0 and accounting for Eqs. (B.1), we get an obvious inequality $i(\theta) > 0$.

In order to prove Eq. (22), we differentiate Eq. (21) on s and obtain:

$$\frac{\partial \theta}{\partial s}\left(\theta - \frac{l_0c_0}{y}\right)v'(\theta) = -v(s)$$

When calculating di/ds we have to keep in mind that the flux density i depends on $\theta(s)$ and s. Hence, the following formula is valid:

$$\frac{\mathrm{d}i}{\mathrm{d}s} = \frac{\partial I}{\partial \theta}\frac{\partial \theta}{\partial s} - \frac{\partial I}{\partial s} = v(\theta)\frac{\partial \theta}{\partial s} - v(s)$$

Together with Eqs. (B.2) and Eq. (12), we obtain $\partial\theta/\partial s < 0$. Differentiating t_{min} on s, one obtains:

$$\frac{\partial t_{min}}{\partial s} = \frac{-yv'(\theta)}{v^2(\theta)} \cdot \left(\frac{d\theta}{ds}\right) > 0$$

Finally, differentiating Eq. (26) on s, we have:

$$\frac{\mathrm{d}W}{\mathrm{d}s} = y\left\{-\frac{v'(s)}{v^2(s)} + \frac{v'(\theta)}{v^2(\theta)}\frac{\partial \theta}{\partial s}\right\} < 0$$

Let us demonstrate the analytical method for solving Eqs. (17,18). Eq. (17), accounting for Eq. (13), has the form:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{i(c)}{c},\ x = i'(c)t,\ (i'(c) \equiv v(c))$$

Excluding x, we obtain:

$$\frac{ci''\,dc}{ci'(c) - i(c)} = -\frac{\mathrm{d}t}{t};$$

then integrating it, we derive Eq. (19):

$$cv(c) - i(c) = \frac{K}{t},\ K = l_0c_0$$

where K is the integration constant. The value of K is derived from Eqs. (15,16,18) and the fact that $c(x_0,t_0) = c_0$.

References

[1] S. Hjertén, Chromatogr. Rev., 9 (1967) 122–219.
[2] S. Hjertén, J. Chromatogr., 347 (1985) 191–198.
[3] J.K. Towns and F.E. Regnier, Anal. Chem., 64 (1992) 2473–2478.
[4] F.E. Regnier and D. Wu, in N.A. Guzman (Editor), Capillary Electrophoresis Technology, Marcel Dekker, New York, NY, 1993, pp. 287–309.
[5] S.V. Ermakov, M.Yu. Zhukov, L. Capelli and P.G. Righetti, J. Chromatogr. A, 699 (1995) 297–313.
[6] S. Hjertén, Electrophoresis, 11 (1990) 665–690.
[7] E. Grushka, in F. Dondi and G. Guiochon (Editors), Theoretical Advancement in Chromatography and Related Separation Techniques, Kluwer, Dordrecht, 1992, pp. 607–632.
[8] M.R. Schure and A.M. Lenhoff, Anal. Chem., 65 (1993) 3024–3037.
[9] M. Minárik, B. Gaš, A. Rizzi and E. Kenndler, J. Cap. Elec., 2 (1995) 89–96.
[10] B. Gaš, E. Kenndler, A. Rizzi and M. Štědrý, Electrophoresis, 16 (1995) 958–967.
[11] M. Štědrý, B. Gaš and E. Kenndler, Electrophoresis, 16 (1995) 2027–2033.
[12] M.S. Bello, M.Yu. Zhukov and P.G. Righetti, J. Chromatogr. A, 693 (1995) 113–130.
[13] E. Grushka, in N. Catsimpoolas (Editor), Methods of Protein Separation, Vol. I, Plenum Press, New York, NY, 1975, Ch. 6, pp. 161–192.
[14] J.L. Wade, A.F. Bergold and P.W. Carr, Anal. Chem., 59 (1987) 1286–1295.
[15] G. Guiochon, S. Golshan-Shirazi and A. Jaulmes, Anal. Chem., 60 (1988) 1856–1866.
[16] S. Golshan-Shirazi and G. Guiochon, J. Chromatogr., 506 (1990) 495–545.
[17] T. Gu, Mathematical Modelling and Scale-up of Liquid Chromatography, Springer, Berlin, 1995.
[18] F.G. Helffrich and P.W. Carr, J. Chromatogr., 168 (1993) 97–122.
[19] F.E.P. Mikkers, F.M. Everaerts and J.A.F. Peek, J. Chromatogr., 168 (1979) 293–315.
[20] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169 (1979) 1–10.
[21] V.G. Babskii, M.Yu. Zhukov and V.I. Yudovich, Mathematical Theory of Electrophoresis, Consultants Bureau, New York, NY, 1989.
[22] R.A. Mosher, D.A. Saville and W. Thormann, The Dynamics of Electrophoresis, VCH, Weinheim, 1992.
[23] K.K. Unger, Packing and Stationary Phases in Chromatographic Techniques, Marcel Dekker, New York, NY, 1979.
[24] M.Yu. Zhukov, S.V. Ermakov and P.G. Righetti, SIAM J. Appl. Math. (submitted).
[25] S.V. Ermakov, M.Yu. Zhukov, L. Capelli and P.G. Righetti, Anal. Chem., 66 (1994) 4034–4042.
[26] P.D. Lax, Comm. Pure Appl. Math., 10 (1957) 537–566.
[27] P.D. Lax, Hyperbolic Systems of Conservation Laws and Mathematical Theory Shock Waves, Society for Industrial and Applied Mathematics, Philadelphia, PA, 1972.

[28] G.B. Withem, Linear and Non-Linear Waves, Wiley, New York, NY, 1974.
[29] B.L. Rozdestvenskii and N.N. Yanenko, The Systems of Quasilinear Equation, Nauka, Moscow, 1978 (in Russian).
[30] M. Chiari, M. Nesi and P.G. Righetti, in P.G. Righetti (Editor), Capillary Elecetrophoresis: An Analytical Tool in Biotechnology, CRC Press, Boca Raton, FL, 1996, Ch. 1, p. 1–36.