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# CHEMICAL KINETICS IN ISOTACHOPHORESIS

## EFFECTS OF NON-INSTANTANEOUSLY REVERSIBLE COMPLEXING EQUILIBRIA ON THE STABILITY OF ZONES

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SUMMARY

A theoretical description is given of kinetic effects in cationic isotachophoretic systems where anionic complexes are formed with the counter ion. The theory is based on a model of electrodiffusion across the isotachophoretic boundary. It shows, in agreement with experiment, that the slow dissociation of an anionic complex results (i) in the formation of a time-dependent tail where the loss of the cation is proportional to the square root of the electric field intensity and (ii) in zone bleeding (when the complex is kinetically inert), where the zone leaves behind a trace with a constant concentration of the complex.

## INTRODUCTION

Effects caused by slow kinetics of the chemical reaction or of the sorption equilibrium in chromatography and electrophoresis were described 20 years ago[1–5]. These effects are of key importance especially in the solution chemistry of metal ions, where very complicated systems of reversible and non-instantaneously reversible complexing equilibria take place, and such metal ions therefore do not yield one band, but a number of bands in chromatographic and/or electrophoretic systems[6].

In such cases, in principle, a substance undergoing chromatography or electrophoresis may exist in two different interconvertible forms A and B, each of them having a certain stability resulting in a slow conversion rate ($A \leftrightharpoons B$).

In the elution mode of these separation methods where A and B have different migration velocities, the slow interconversion $A \leftrightharpoons B$ leads to the formation of a multiple zone system[1]. Here, the system of the more or less separated forms A and B shows from one to three concentration maxima and the appropriate record shows a single, double and/or triple symmetrical or asymmetric zone system.

In isotachophoresis, the influence of slow kinetics has not yet been studied in detail and only experimental evidence of the presence of a slow complex formation reaction between the separated cations and the counter ion has been given[7]. As a result, analytically unstable sample zones occurred, where the self-sharpening isotachophoretic boundary was present between the unstable zone and its tail.

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Although, in general, one tends to work under conditions with fast reaction kinetics and with stable zones, this is sometimes impossible and, moreover, in a special case it may even be advantageous to use different zone stabilities as a tool for achieving selective separations. It is obvious that an extensive knowledge of the instabilities due to slow kinetics is necessary in order for them to be elucidated and considered properly in a given system. The selection of an optimal system with the absence of such effects is then much easier. From experiment[7] it follows that the nature of a migrating isotachophoretic system with slow kinetics of the complex formation reaction is not an analogue of the effects known from elution techniques. Therefore, it requires a separate theoretical treatment and discussion of its analytical aspects, which is the purpose of this paper.

As the use of complex formation reactions is a very effective tool for achieving very selective separations in isotachophoresis[8–12], especially for the separation of cations with a complexing counter anion (*e.g.*, the separation of lanthanides using complexation with α-hydroxyisobutyrate[12]), a model system for this study was selected from this field.

## THEORETICAL

Let us consider a cationic isotachophoretic system where, between the zones of the leading ion $L^+$ and $H^+$ as the terminator, there migrates the zone of a cation $M^+$ forming with the counter ion $Y^-$ the complex $MY^-$ (the signs of the charge have symbolic meaning only and do not express the number of charges on the ion); see Fig. 1. Certain concentrations of both free $M^+$ and complex $MY^-$ are present in zone M, determining the value of the effective mobility of the metal M. Taking the migration speed of the zones as the moving frame of reference, the processes in the zone may be described as follows. Cation $M^+$ migrates forwards in the zone and recombines on its front boundary in order to re-establish the equilibrium in the sense

$$M^+ + Y^- \underset{k_{MY}}{\overset{k'_M}{\rightleftharpoons}} MY^- \qquad (1)$$

The complex so formed migrates backwards in the zone; on the rear boundary the equilibrium is re-established by the decomposition of the complex.

If the decomposition of the complex is not fast enough to reach equilibrium

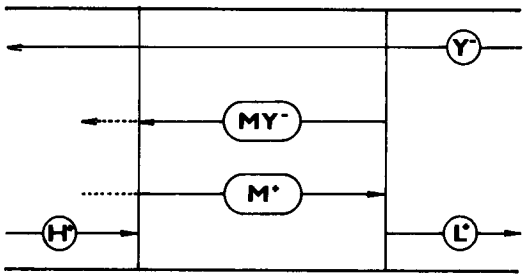


Fig. 1. Scheme of the cationic isotachophoretic system.

within the zone boundary, then complex $MY^-$ penetrates into the terminating zone and forms a tail containing metal M in the terminating zone. If the decomposition process of the complex in the acidic terminator is comparable to the rate of the electromigrational transport, then the behaviour of metal M in the terminating zone may be described as electrodiffusion[13–16] across the isotachophoretic boundary.

Let us first consider the behaviour of M in the terminating zone; the direction of migration of cations will be taken as positive: $v_M > 0$, $u_M > 0$, $v_{MY} < 0$, $u_{MY} < 0$ ($v_i$ and $u_i$ are the migration velocity and electrophoretic mobility, respectively, of particles $i$). The following processes must be taken into consideration (see Fig. 2):

(a) the migration of particles $M^+$ with a speed $v_M = u_M E_H$ ($E_H$ is the electric field intensity in the terminating zone of $H^+$);

(b) the migration of particles $MY^-$ with a speed $v_{MY} = u_{MY} E_H$ in the opposite direction;

(c) the reaction $M^+ \rightarrow MY^-$ proceeding with pseudo-first-order kinetics with a rate constant $k_M = k'_M$ [Y] (the concentration of the counter ion $Y^-$ in the entire zone H is assumed to be constant with time);

(d) the reaction $MY^- \rightarrow M^+$ proceeding with first-order kinetics with a rate constant $k_{MY}$.

The mass balance for $M^+$ and $MY^-$ according to Fig. 2 in a volume element of the terminating zone (neglecting diffusion) is described by the following set of differential equations (*e.g.*, ref. 15):

$$\frac{\partial[MY]}{\partial t} = -E_H u'_{MY} \cdot \frac{\partial[MY]}{\partial x} - k_{MY}[MY] + k_M[M] \tag{2}$$

$$\frac{\partial[M]}{\partial t} = -E_H u'_M \cdot \frac{\partial[M]}{\partial x} + k_{MY}[MY] - k_M[M] \tag{3}$$

where $t$ is time, $x$ is the longitudinal coordinate ($x = 0$ at the boundary of zones H and M, $x > 0$ in zone H) and $u'_i$ means the ionic mobility corrected to the movement of the unidimensional coordinate system $x$ with a speed $v_{ref}$ as a frame of reference:

$$u'_i = u_i - v_{ref}/E_H \tag{4}$$

Mathematical difficulties in the solution of the set of eqns. 2 and 3 can be

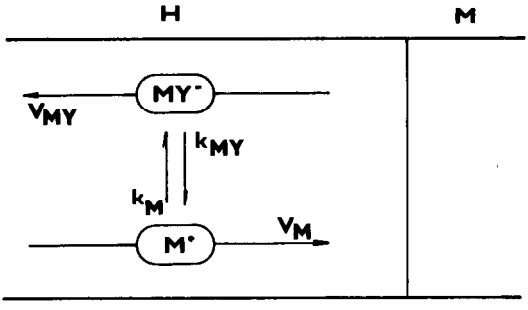


Fig. 2. Scheme of the processes that occur in the terminating zone.

overcome by using the random walk theory of electrodiffusion according to Scholten and Mysels[16], where the electrodiffusion coefficient $D_e$ is defined by analogy with the Einstein relation by

$$D_e = \langle \Delta^2 \rangle / 2 \langle \tau \rangle \tag{5}$$

where $\langle \Delta^2 \rangle$ is the mean square of distances $\Delta$ covered by unidimensional random independent flights during time intervals of average length $\langle \tau \rangle$.

By applying this theory to the situation in the terminating zone, we obtain

$$\langle \Delta^2 \rangle = 2 E_H^2 \left( \frac{u_M'^2}{k_M^2} + \frac{u_M' u_{MY}'}{k_M k_{MY}} + \frac{u_{MY}'^2}{k_{MY}^2} \right) \tag{6}$$

By analogy, the average value of the duration $\tau = t_M + t_{MY}$ of the composite flight may be obtained:

$$\langle \tau \rangle = \frac{1}{k_M} + \frac{1}{k_{MY}} \tag{7}$$

and, for the electrodifussion coefficient we have

$$D_e = E_H^2 \cdot \frac{k_M k_{MY}}{k_M + k_{MY}} \cdot \left( \frac{u_M'^2}{k_M^2} + \frac{u_M' u_{MY}'}{k_M k_{MY}} + \frac{u_{MY}'^2}{k_{MY}^2} \right) = E_H^2 K \tag{8}$$

where the abbreviation of the expression on the right-hand side of eqn. 8 is in agreement with the knowledge[13] that $D_e$ is proportional to the square of the electric field intensity. Eqn. 8 relates the electrodiffusion coefficient to any reference speed $v_{ref}$, which, in our case, should be taken as the migration velocity of the isotachophoretic zone boundary:

$$v_{ref} = u_L E_L = \bar{u}_{H,H} E_H \tag{9}$$

*i.e.*, $u_i' = u_i - \bar{u}_{H,H}$ ($\bar{u}_{H,H}$ is the effective mobility of $H^+$ in the terminating zone[17]).

Then, the formation of the tail may be described by analogy with the well known problem of the diffusion across a concentration boundary with a constant concentration on one side and zero starting concentration on the other side of the boundary. For the concentration profile of the substance in depending on the longitudinal coordinate $x$ and time $t$, we have

$$c(x, t) = c_0 \cdot \operatorname{erfc}(x/2\sqrt{D_e t}) \tag{10}$$

(where $c_0$ is the concentration at the point $x = 0$). The total amount of substance having diffused over the boundary after time $t$ is given by[18]

$$n_t = c_0 S \int_0^\infty \operatorname{erfc}(x/2\sqrt{D_e t})\, dx = 2 S c_0 \sqrt{D_e t/\pi} \tag{11}$$

where $S$ is the cross-section of the column.

For quantitative isotachophoretic analysis it is important to know the amount of metal retained in the tail at the moment when the quantitative recording of the zone in question has just finished, *i.e.*, to know the amount of metal that has passed the rear boundary of the zone within the time interval $t = t_a$ from the start of the analysis to the time of reaching the mentioned boundary at the detector. Neglecting the length of the sample zones in comparison with the length of the separation capillary, $l$, we can write $t_a = l/E_H\bar{u}_{H,H}$ and, by combination with eqns. 8 and 11, we obtain

$$n = 2\, c_0 S \sqrt{\frac{KE_H l}{\pi \bar{u}_{H,H}}} = \text{constant} \cdot \sqrt{E_H} \tag{12}$$

Eqn. 12 shows that the decomposition of the unstable zone of the tailing type is directly proportional to the square root of the electric potential gradient in the terminating zone. The decomposition is independent of the length of the zone M, *i.e.*, of the amount of sample, as long as the time interval of passing zone M through the detector is negligible in comparison with the analysis time. For the experimental evidence of a tail behind a decomposing zone in isotachophoresis, universal detectors may be used. If they are sensitive enough, they detect the tail as a deformation of the plateau corresponding to the following zone.

If the decomposition of the complex in the terminator is so slow that the complex can be considered as kinetically inert, then metal M (in the form of the complex $MY^-$) leaves the zone irreversibly. The description of this case can be based on the model of the bleeding zone[9] where the decomposition (bleeding) rate is constant and is given by

$$dn/dt = (\bar{u}_{H,H} - u_{MY})\, c_0 E_H S \tag{13}$$

For the total loss of the analysed substance at the end of the analysis, we then have

$$n = \frac{\bar{u}_{H,H} - u_{MY}}{\bar{u}_{H,H}} \cdot Slc_0 \tag{14}$$

## EXPERIMENTAL

The experiments were carried out in the isotachophoretic column described earlier[19], made of Perspex with a separation capillary of rectangular cross-section. The potential gradient detector and the high-voltage supply of stabilized current used have been described elsewhere[20].

The leading electrolyte contained 0.05 *M* ammonium formate, 0.05 *M* formic acid and various amounts of cyclohexane-1,2-diamine-N,N,N′,N′-tetraacetic acid (CDTA); 0.1 *M* acetic acid served as the terminating solution. Solutions of chlorides of sodium, calcium, lanthanum and holmium (0.01 *M*) were sampled using a 2-μl microsyringe (Hamilton, Bonaduz, Switzerland). All chemicals were of analytical-reagent grade (Lachema, Brno, Czechoslovakia).

The values of the total diffusion coefficient were determined from the zone electrophoretic experiments by means of the relationship $D_{tot} = \sigma^2/2t = l^2\sigma_t^2/2t^3$, where $\sigma_t$ is the time-based variance of the peak on the record; the effective length of the column was $l = 24$ cm.

The electric current was measured with 2.5% accuracy with a moving coil ammeter.

## RESULTS AND DISCUSSION

Fig. 3 shows the calibration lines of calcium, lanthanum and holmium for cationic isotachophoretic analyses in acidic systems with formate and CDTA as a mixed counter ion. It can be seen that the graph for $La^{3+}$ and $Ho^{3+}$, although linear, intersects the abscissa at non-zero sample volumes, *viz.*, 0.2 and 2.3 μl of 0.01 *M* $La^{3+}$ and $Ho^{3+}$, respectively. This means that the zones of these cations are isotachophoretically unstable and decompose during their migration. The linear character of both dependences shows that (in accordance with theory) the amount of sample lost from the zone under the given conditions is constant and independent of the amount injected.

Fig. 4 shows potential gradient records of the zone electrophoretic migration of sodium and lanthanum when injected into the adjusted terminator behind the leading electrolyte containing 0 and 0.002 *M* CDTA, respectively. It can be seen that in the absence of CDTA $La^{3+}$ migrated faster than $Na^+$, both zones being asymmetric[21]. In the presence of CDTA, the $La^{3+}$ zone not only migrated more slowly than that of $Na^+$ but it also became very broad, thus demonstrating the presence of a selective dispersion effect. In this instance, electrodiffusion is responsible for the

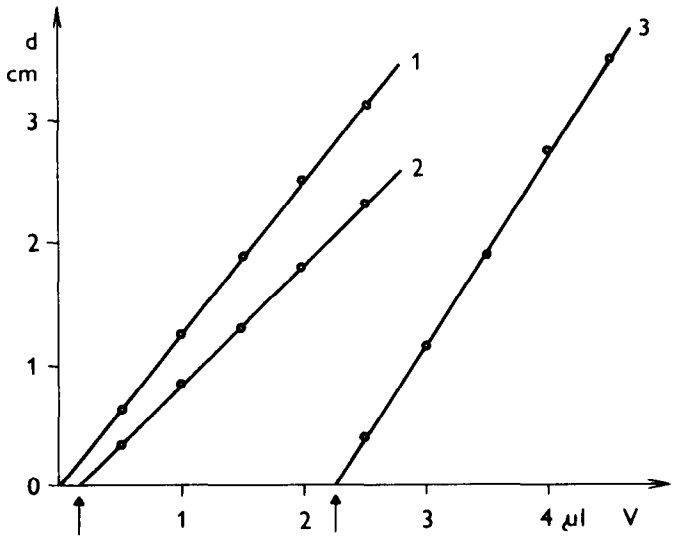


Fig. 3. Experimental dependence of the step length $d$ on the sampled volume $V$ (chart speed 12 cm/min); $I = 600$ μA. (1) $Ca^{2+}$, 0.0020 *M* CDTA in the leading electrolyte; (2) $La^{3+}$, 0.0020 *M* CDTA in the leading electrolyte; (3) $Ho^{3+}$, 0.0005 *M* CDTA in the leading electrolyte.

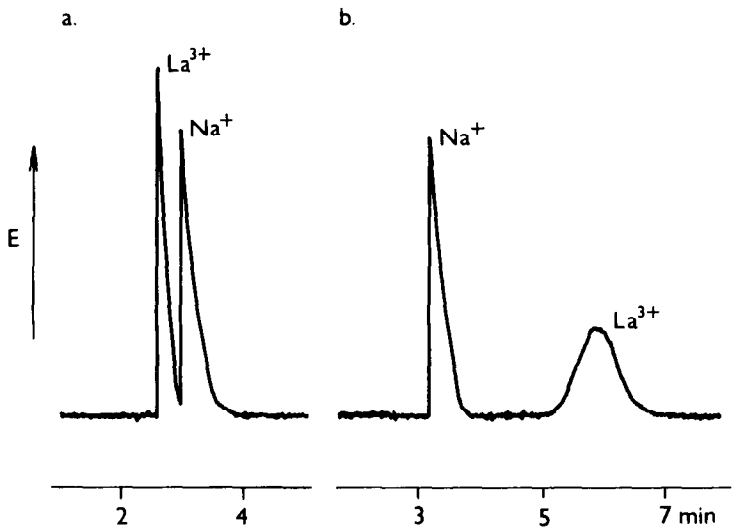


Fig. 4. Zone electrophoretic migration of sodium and lanthanum in the terminator ($H^+$) adjusted behind the leading electrolyte containing (a) no and (b) 0.0020 *M* CDTA. Sample: $Na^+ + La^{3+}$ (0.3 μl each); $I = 600$ μA.

zone broadening, its evidence in CDTA–lanthanide systems having been given earlier[22].

Fig. 5 shows the result of the measurement of the electrodiffusion coefficient of lanthanum in the adjusted terminator for case (b) from Fig. 4 using the method described in ref. 22. The dependence of the total diffusion coefficient on the square of the electric current (and thus on the square of the electric potential gradient) can be clearly seen. The equation[13] $D_{tot} = D_e + D$ is valid and the plot intersects the

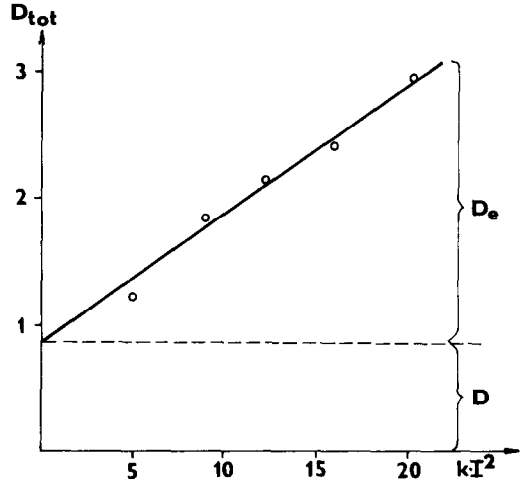


Fig. 5. Experimental dependence of the total diffusion coefficient, $D_{tot}$ ($10^{-3}$ $cm^2$/sec), on the square of the electric current. Sample: 0.5 μl of 0.01 *M* $La^{3+}$. $I = 200, 300, 350, 400, 450$ μA.

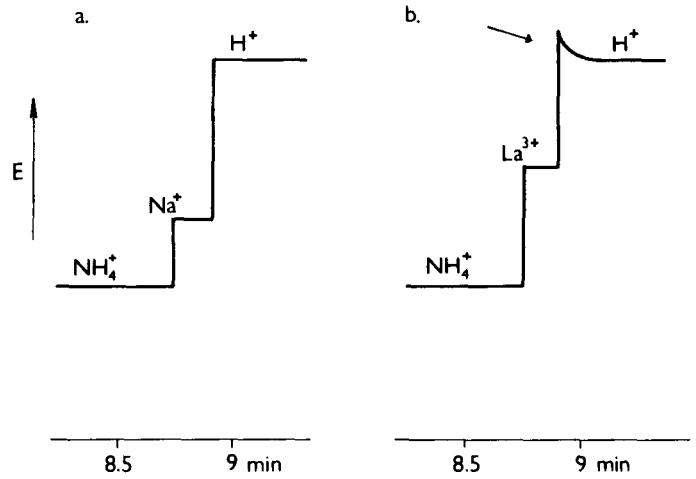


Fig. 6. Experimental isotachophoregrams in the system with 0.002 *M* CDTA in the leading electrolyte. Sample: (a) $Na^+$, (b) $La^{3+}$ (2.0 $\mu$l each). $I$ = 600 $\mu$A.

ordinate at the value of the effective diffusion coefficient $D$ covering all other (non-electrodiffusional) dispersion effects in the separation column, for the given case being of the order $10^{-3}$ $cm^2/sec$.

Fig. 6 shows isotachophoregrams of sodium and lanthanum in the system with CDTA. As expected, sodium gave the step-like potential gradient record, and the lanthanum zone was followed by an adjacent potential gradient peak in the terminator, indicating the migrating tail. It should be mentioned that although electrodiffusion takes place in this system, the condition of the sharp boundary between zone M and terminator H is still valid, *i.e.*, the effective mobility of lanthanum in the terminator is greater than the effective mobility of the terminating $H^+$ ion[23]. The amount of lanthanum retained in the tail is given by the point of intersection of the calibration graph with the abscissa (see Fig. 3). Fig. 7 shows the experimental de-

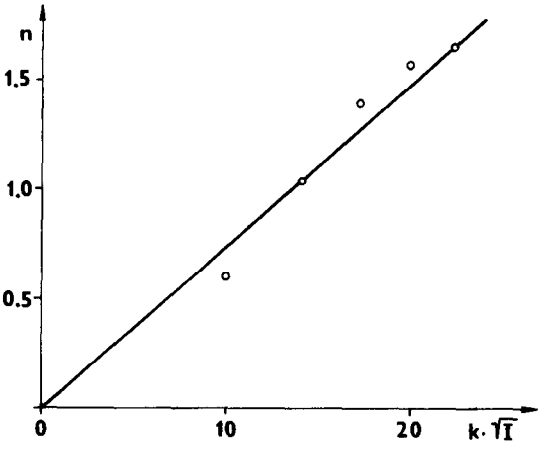


Fig. 7. Experimental dependence of the loss of the lanthanum zone, $n$ (nmol), on the square root of the electric current in the system in Fig. 6. $I$ = 100–500 $\mu$A.

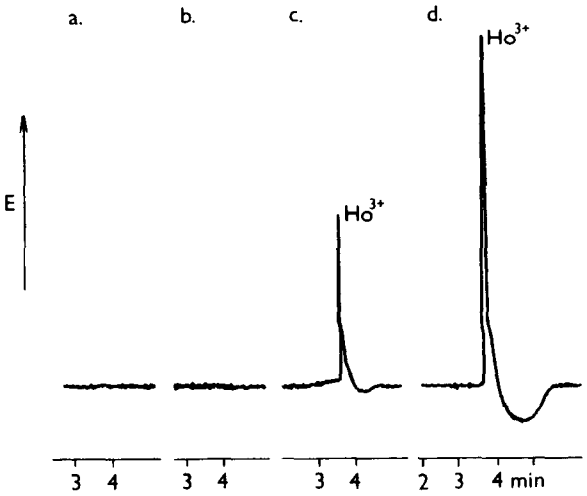


Fig. 8. Zone electrophoretic migration of $Ho^{3+}$ in the terminator ($H^+$) adjusted behind the leading electrolyte containing 0.0005 *M* CDTA. Sample: (a) 1.0; (b) 2.0; (c) 2.2; (d) 2.5 $\mu$l. $I$ = 600 $\mu$A.

pendence of this amount on the square root of the electric current (and thus on $\sqrt{E}$). The course of the dependence is in agreement with the conclusion drawn from eqn. 12 that the amount of the metal retained in the tail is proportional to the square root of the electric potential gradient in the terminating zone.

For the description of the type of instability caused by very slow kinetics, the zone electrophoresis of holmium in the adjusted terminator may serve (see Fig. 8). On sampling 2.0 $\mu$l or less of 0.01 *M* $Ho^{3+}$, no zones could be detected (Fig. 8a and b). For sample volumes of 2.2 $\mu$l and more, sharp zones of holmium were observed (Fig. 8c and d). This demonstrated the very slow kinetics of the dissociation of the complex, far out of the electrodiffusion region where the migrating zone leaves behind a trace of the virtually undissociated anionic complex and, for small amounts of sample the zone decomposes completely before it reaches the detector.

## CONCLUSIONS

The kinetics of chemical reactions and the arrangement of electrolyte systems are important factors that influence considerably the analytical stability of isotachophoretic zones, especially in the isotachophoresis of complexes or when complex formation reactions are involved.

If the mentioned chemical reactions are slow, zone tailing results, where the migrating zone is followed by a time-dependent tail of the analysed substance. The calibration graph for this substance does not pass through the origin.

In isotachophoretic analyses with universal detection, tailing zones manifest themselves as deformed plateaux of the following zones on the record. Moreover, the presence of tailing zones may be shown by zone electrophoretic experiments, where an additional selective zone broadening occurs.

Tailing of zones may be described as electrodiffusion across the isotachophoretic boundary, where the electrodiffusion coefficient is proportional to the square of

the electric field intensity in the following zone. In analytical practice this means that the loss of the substance from the zone is proportional to the square root of the electric field intensity in the terminator (eqn. 12), it is therefore dependent on the working conditions, *i.e.*, on the driving current used.

If the decomposition of the complex formed is very slow, then the zone bleeds, *i.e.*, it loses the analysed substance in the form of the virtually non-dissociated complex. The rate of bleeding and the concentration of the complex in the trace behind the zone are constant and independent of time. Zone bleeding may be evidenced from the shape of the calibration graph, but it neither has an adverse effect on the stepwise signal from a universal detector nor shows electrodiffusion broadening of bands in zone electrophoretic experiments.

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