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## COMPLEX FORMATION IN CATIONIC ISOTACHOPHORESIS: TERMINATION OF ACIDIC SYSTEMS

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### SUMMARY

The choice of suitable acidic electrolyte systems for isotachophoresis of metal cations forming weak complexes with the counter ion is considered. A method of calculation is suggested, making it possible to test whether a selected system of both leading electrolyte and separated ions can be terminated by the  $H^+$  ion, *i.e.*, whether the analysis may be performed without disturbing effects. The procedure described for the calculation of the critical concentration of the leading electrolyte (as well as the plotting of the effective mobility vs. concentration curves) provides more information, showing the range of suitable leading electrolyte concentrations. The mixed counter ion method is discussed, making it possible to work even with fairly acidic complexing counter ions. The theory is illustrated with experiments demonstrating the choice of electrolyte systems for practical use.

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### INTRODUCTION

The formation of kinetically labile complexes is a practically important procedure for selectively affecting the electrophoretic mobilities of ionic species in order to achieve their separation. The use of complex-forming agents in the zone electrophoresis of cations is a classical example<sup>1-3</sup>, where halide solutions<sup>4</sup>, organic acids (*e.g.*, glycollic acid<sup>5</sup>) or complexones (*e.g.*, EDTA<sup>6</sup>) have been used successfully. Complete separations of lanthanides with  $\alpha$ -hydroxyisobutyric acid ( $\alpha$ -HIBA)<sup>7</sup> or EDTA with a buffered ligand concentration<sup>8</sup> as background electrolytes belong to the most sophisticated applications. In isotachophoresis, complex-forming equilibria can be utilized in such a way that the complex-forming agent is a component of the leading electrolyte and, during the migration, it passes through the sample zones as a counter ion.

In the isotachophoresis of anions, complex formation has already been studied theoretically and experimentally<sup>9-11</sup> and applied practically<sup>12</sup>.

In the isotachophoresis of cations, the complex-forming interaction of the counter anion with the separated ions is a current phenomenon, but in some instances it can be observed only at high concentrations, *e.g.*, in the separation of an 18-

component mixture of cations with 5 *N* hydrochloric acid as a leading electrolyte<sup>13</sup>. With the use of organic acids as counter anions, complex-forming effects appear markedly at concentrations in the range 0.1–0.01 *M*<sup>14,15</sup>. The qualitative choice of the complexing agent is of great importance and has already been discussed. A recent paper<sup>16</sup> demonstrated clearly the potentialities of selective complex-forming effects by showing the complete separation of fourteen lanthanides with the use of  $\alpha$ -HIBA as the counter ion. However, little attention has been paid to the correct choice of the leading electrolyte concentration with regard to possible interferences due to uncontrolled migration of  $H^+$  ions through the isotachophoretic zones<sup>17,18</sup>. The authors<sup>16</sup> gave only an empirical observation that an increase in the concentration of the leading electrolyte leads to an improvement in its buffering capacity and thereby also to an increase in the pH of the terminator zone. Further, the same authors<sup>19</sup> showed that at higher pH values of a leading electrolyte consisting of acetate and cyclohexanediaminetetraacetic acid (CDTA), it is not possible to terminate zones of  $Ca^{2+}$  and  $Mg^{2+}$  with Tris as the pH in the terminating zone is too high.

Recent studies<sup>20,21</sup> on the isotachophoresis of simple cations and protonated bases have shown that the migration behaviour of  $H^+$  ions is the decisive factor for the stability of zones in cationic isotachophoresis. Also,  $H^+$  has been suggested as an ideal terminator for acidic systems.

The aim of this paper is to extend the previous theory to the case where the migration of the cation being separated is additionally affected by the formation of weak cationic or neutral complexes with the counter ion. Further, it is shown how the theory presented can be employed in practice for the correct choice of electrolyte systems.

## THEORETICAL

Let us consider isotachophoresis in which a complex-forming equilibrium between a cation  $M^{2+}$  and a univalent ligand  $Y^-$  takes place (cf., Fig. 1). An alkali metal cation  $L^+$  and an anion  $Y^-$  of a weak acid  $HY$  form the leading zone,  $\lambda$ . The acid  $HY$ , the dissociation of which provides the terminating  $H^+$  ion, is the terminator (zone  $\vartheta$ ). Between zones  $\lambda$  and  $\vartheta$  migrates a zone  $\mu$  of the cation  $M^{2+}$ , which forms labile complexes  $MY^+$  and  $MY_2$ , with anion  $Y^-$ .

It has been pointed out<sup>20</sup> that the condition of correct cationic isotachophoretic

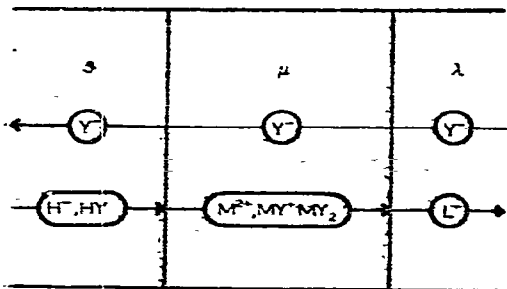


Fig. 1. Diagram of a migrating zone  $\mu$  of cation  $M^{2+}$  between zones  $\lambda$  of the leading ion  $L^+$  and  $\vartheta$  of the terminator  $H^+$ , with a complexing counter anion  $Y^-$ .

migration is equivalent to the state where  $H^+$  either forms the terminating zone or its use as a terminator is potentially possible, *i.e.*, in the case of simple cations its effective mobility<sup>21</sup> is the lowest of all. The self-sharpening ability of the terminating zone–last zone boundary is then the condition for the successful use of the  $H^+$  ion as a terminator. For the system described, it can be expressed in the following form:

$$E_{\lambda} \bar{u}_{M,\lambda} > E_{\lambda} \bar{u}_{H,\lambda} = E_{\mu} \bar{u}_{M,\mu} = E_{\lambda} u_L \quad (1)$$

where  $\bar{u}$  is the effective mobility,  $E$  is the potential gradient and the subscripts designate the corresponding ion and zone (ion charges are omitted). The equations on the right-hand side of the relationship express the condition of isotachophoretic migration of zones  $\lambda$ ,  $\mu$  and  $\lambda$ . From relationship 1, the criterion of the sharp boundary can be obtained in the simple form

$$\bar{u}_{M,\lambda} > \bar{u}_{H,\lambda} \quad (2)$$

which indicates that the effective mobility of  $M^{2+}$  in zone  $\lambda$  must be greater than the effective mobility of  $H^+$  in order to secure isotachophoretic migration (*i.e.*, control of  $H^+$  migration).

— Fulfilment of condition 1 or 2 does not exclude the case when  $\bar{u}_{M,\mu} < \bar{u}_{H,\lambda}$  and  $E_{\mu} > E_{\lambda}$ , representing enforced isotachophoretic migration. Hence, in principle, enforced isotachophoretic migration is possible not only in the cationic isotachopheresis of protonated bases<sup>20</sup> but also with simple inorganic cations, provided that a complex-forming ligand is used as a counter ion.

In order to determine the conditions under which expression 2 holds, the values of  $\bar{u}_{H,\lambda}$  and  $\bar{u}_{M,\lambda}$  must be determined for a given system. The effective mobility of  $H^+$  is defined by the relationship<sup>21</sup>

$$\bar{u}_{H,\lambda} = \mu_H \cdot \frac{[H]_{\lambda}}{c_{H,\lambda} - [HY]_{\lambda}} \quad (3)$$

where

$$[H]_{\lambda} = \frac{1}{2} \left[ \sqrt{K_{HY}^2 + 4 K_{HY} c_{H,\lambda}} - K_{HY} \right] \quad (4)$$

$$c_{H,\lambda} = [L]_{\lambda} \cdot \frac{\mu_H \cdot \mu_Y + \mu_L}{\mu_L \cdot \mu_Y + \mu_H} + [HY]_{\lambda} \quad (5)$$

Here,  $c_{H,\lambda} = c_{HY,\lambda}$  is the total analytical concentration of acid  $HY$  in zone  $\lambda$  and  $K_{HY} = [H][Y]/[HY]$  is the dissociation constant. The value  $[HY]_{\lambda}$  appears only with systems containing in the leading electrolyte additional free acid  $HY$  at a concentration  $[HY]_{\lambda}$ .

The general relationship<sup>22</sup> holds true for the effective mobility of metal ion,

$$\bar{u}_M = \frac{u_M + u_{MY} \beta_1 [Y]}{1 + \beta_1 [Y] + \beta_2 [Y]^2} \quad (6a)$$

which, with regard to the condition of electroneutrality  $[Y]_3 = [H]_3$ , is valid in zone 3 in the following form:

$$\bar{u}_{M,3} = \frac{\mu_M + u_{MY}\beta_1[H]_3}{1 + \beta_1[H]_3 + \beta_2[H]_3^2} \quad (6b)$$

where

$$\beta_1 = \frac{[MY]}{[M][Y]} \quad (7)$$

and

$$\beta_2 = \frac{[MY_2]}{[M][Y]^2} \quad (8)$$

are the total stability constants of complexes  $MY^+$  and  $MY_2$ .

The comparison of the calculated values of  $\bar{u}_{M,3}$  (eqns. 6b, 4 and 5) and  $\bar{u}_{H,3}$  (eqns. 3, 4 and 5) in the sense of relationship 2 then decides whether zone  $\mu$  of the given cation  $M^{2+}$  can be terminated by hydrogen ion with the leading electrolyte chosen, *i.e.*, whether correct isotachophoretic migration of  $M^{2+}$  can be achieved in the given system. For testing the validity of relationship 2, a graphical treatment is useful, plotting the dependences  $\bar{u}_{H,3} = f([L]_\lambda)$  and  $\bar{u}_{M,3} = f([L]_\lambda)$ .

As shown earlier<sup>20,21</sup>, the value of  $\bar{u}_{H,3}$  depends strongly on the concentration of the leading electrolyte. Generally, with decreasing concentration of the leading ion  $L^+$ ,  $\bar{u}_{H,3}$  increases rapidly (non-linearly), for  $[L]_\lambda \rightarrow 0$  being equal to the ionic mobility,  $u_H$ , the greatest of all of the cations. It is obvious that for a given acid  $HY$ , leading ion  $L^+$  and cation  $M^{2+}$ , a certain extreme, critical<sup>20</sup>, concentration exists that defines the range of the validity of condition 2. At concentrations lower than the critical concentration condition 2 is not fulfilled and the migration is not isotachophoretic. Hence, the determination of the critical concentration for a given system definitely establishes the range of concentrations suitable for the isotachophoretic separation.

The calculation of the critical concentration includes the condition of constant current density and electroneutrality,  $[H]_3 = [Y]_3$ ,  $[L]_\lambda = [Y]_i$  ( $[H]_\lambda$  is neglected), by combination of which the following relationship is obtained:

$$E_\lambda [H]_3 (u_Y + u_H) = E_\mu (2[M]_\mu u_M + [MY]_\mu u_{MY} + [Y]_\mu u_Y) = E_\lambda [L]_\lambda (u_Y + u_L) \quad (9)$$

Using eqn. 9, condition 1 is obtained in the form

$$[L]_\lambda > [H]_3 \cdot \frac{u_L}{\bar{u}_{M,3}} \cdot \frac{u_Y + u_H}{u_Y + u_L} \quad (10)$$

with the values of  $\bar{u}_{M,3}$  and  $[H]_3$  being determined by relationships 6b and 4. Expression 10 gives the range of the leading ion concentrations, within which the migration of  $M^{2+}$  is isotachophoretic.

If in relationship 10 equality is considered, the value of the critical concentra-

tion,  $[L]_{\lambda, \text{crit}}$ , is obtained. The explicit expression of the critical concentration in the form  $[L]_{\lambda, \text{crit}} = f(u_Y, u_L, u_H, u_M, u_{MY}, K_{HY}, \beta_1, \beta_2)$  leads, by substituting eqns. 4-6b into eqn. 10, to very complicated expressions and, for an actual case, it is therefore more expedient to determine the value of  $[L]_{\lambda, \text{crit}}$  by an iterative calculation with the aid of eqns. 5, 4, 6b and 10.

In cases where species involved in either the first or the second step of the consecutive complex formation predominate in a zone, certain simplifications can be performed:

(a) The formation of  $MY_2$  is negligible. Then the first equilibrium (eqn. 7) only may be taken into account. This case corresponds, e.g., to acetate complexes of alkaline earth metals<sup>15</sup>. Then the term involving  $[H]_3^2$  is eliminated from eqn. 6b; substituting this equation into eqn. 10 and by combination with eqns. 4 and 5 ( $[HY]_{\lambda} = 0$ ), the following expression (squared with regard to  $[L]_{\lambda}$ ) is obtained:

$$\left\{ [L]_{\lambda}^2 \beta_1^2 - [L]_{\lambda} \cdot \frac{u_Y + u_H}{u_Y + u_L} \cdot \frac{u_L}{u_{MY}} \left[ \frac{u_M^2}{u_H u_{MY}} \cdot \frac{1}{K_{HY}} + \beta_1 \left( 2 - \frac{u_M}{u_H} - \frac{2 u_M}{u_{MY}} \right) + \beta_1^2 \cdot \frac{u_H - u_{MY}}{u_{MY}} \cdot K_{HY} \right] + \frac{u_H - u_M}{u_H} \left( \frac{u_Y + u_H}{u_Y + u_L} \cdot \frac{u_L}{u_{MY}} \right)^2 (1 - \beta_1 K_{HY}) \right\} \begin{matrix} > \\ < \end{matrix} 0 \quad (11)$$

where the sign  $>$  is valid for  $\beta_1 K_{HY} - u_M/u_H > 0$  and the sign  $<$  for the opposite case. For the limiting cases  $\beta_1 \rightarrow 0$  (only M exists in the solution) and  $\beta_1 \rightarrow \infty$  (only MY exists in the solution), condition 11 will be simplified to the form derived earlier<sup>20</sup>, valid for isotachophoretic migration of zones of simple  $M^{2+}$ , and/or  $MY^+$ , respectively, terminated by  $H^+$ :

$$[L]_{\lambda} > K_{HY} \cdot \frac{u_H - u_i}{u_i} \cdot \frac{u_Y + u_H}{u_Y + u_L} \cdot \frac{u_L}{u_i} \quad (12)$$

( $i = M$  or  $MY$ , respectively).

Fig. 2a shows how the value of  $[L]_{\lambda, \text{crit}}$  depends, for a given  $\beta_1$ , on the dissociation constant of the acid used,  $K_{HY}$ . It can be seen that the dependence consists of two straight-line sections, corresponding to eqns. 12, with the point of inflection in the transition part being at  $K_{HY} = u_M/u_H \beta_1$ . The range of permitted values of  $[L]_{\lambda}$  lies above the curve in Fig. 2a.

(b) The concentration of free  $M^{2+}$  is negligible. This simplified case of assuming the second equilibrium (eqn. 8) only illustrates how the value of the critical concentration is affected by the formation of neutral complex particles. The first terms in both the nominator and the denominator are eliminated in eqn. 6b; by combining this equation with relationships 10, 4 and 5 ( $[HY]_{\lambda} = 0$ ) and eliminating  $[H]_3$  and  $c_{H,3}$ , condition 1 is obtained in the following form:

$$[L]_{\lambda} > K_{HY} \cdot \frac{u_H - u_{MY}}{u_{MY}} \cdot \frac{u_L}{u_{MY}} \cdot \frac{u_Y + u_H}{u_Y + u_L} \cdot \frac{1 - (\beta_2/\beta_1) K_{HY}}{\left( 1 - K_{HY} \cdot \frac{\beta_2}{\beta_1} \cdot \frac{u_H}{u_{MY}} \right)^2} \quad (13)$$

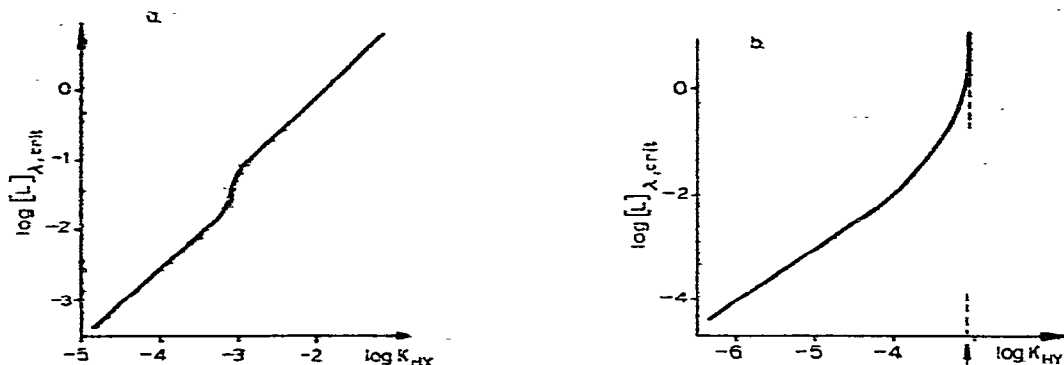


Fig. 2. Calculated dependence of  $\log [L]_{\lambda, \text{crit}}$  vs.  $\log K_{\text{HY}}$  (a) for the equilibrium  $M^{2+} + Y^{-} = MY^{-}$  and the value of  $\beta_1 = 10^2$ ; (b) for the equilibrium  $MY^{+} + Y^{-} = MY_2$  and the value of  $\beta_2/\beta_1 = 10^2$ . For the calculation, tabulated<sup>23</sup> values of  $u_{\text{H}^+}$ ,  $u_{\text{L}} = u_{\text{K}}$  and  $u_{\text{Y}}$  (acetate) were used. The values of  $u_{\text{M}} \approx 55 \cdot 10^{-5} \text{ cm}^2/\text{V} \cdot \text{sec}$  and  $u_{\text{MY}} \approx 30 \cdot 10^{-5} \text{ cm}^2/\text{V} \cdot \text{sec}$  used are estimates.

For  $\beta_2/\beta_1 \rightarrow 0$ , relationship 13 becomes identical with relationship 12 (where  $i = \text{MY}$ ).

By analogy with case (a), Fig. 2b shows the dependence of  $\log [L]_{\lambda, \text{crit}}$  vs.  $\log K_{\text{HY}}$  for a chosen value of  $\beta_2/\beta_1$ . The straight-line dependence (in accord with eqn. 12) here becomes a curve at greater values of  $K_{\text{HY}}$ , with the value of  $[L]_{\lambda, \text{crit}}$  approaching infinity for  $K_{\text{HY}} = \beta_1 u_{\text{MY}}/\beta_2 u_{\text{H}^+}$ . For still greater values of  $K_{\text{HY}}$ , a zone of the migrating complex (with the given  $\beta_2/\beta_1$ ) cannot be terminated successfully.

In practice, the selected complex-forming counter anion need not be suitable for the use of  $\text{H}^+$  as the terminator, owing to either too high or too low values of  $\bar{u}_{\text{H}^+, \text{g}}$ . Frequently, the requirement of a sufficiently low effective mobility of  $\text{H}^+$  is not fulfilled (see above), owing to the low  $\text{p}K_{\text{HY}}$  of the counter ion. This problem can be solved by using a leading electrolyte with a mixed counter ion<sup>16,19</sup>, which contains an

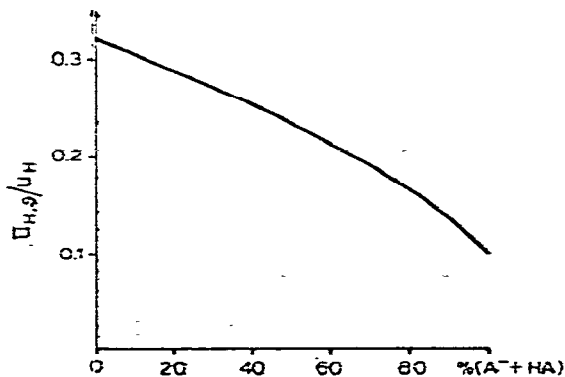


Fig. 3. Calculated dependence of the relative effective mobility of  $\text{H}^+$ ,  $\bar{u}_{\text{H}^+, \text{g}}/u_{\text{H}^+}$ , on the percentage of  $\text{A}^{-} + \text{HA}$  in the leading solution containing  $0.01 \text{ M}$  ( $\text{Y}^{-} + \text{A}^{-}$ ) and  $0.01 \text{ M}$  ( $\text{HY} + \text{HA}$ ). The values used for the calculation:  $K_{\text{BY}} = 10^{-3}$ ,  $K_{\text{HA}} = 10^{-4}$ ;  $u_{\text{H}^+} = 362.55 \cdot 10^{-5}$ ,  $u_{\text{K}} = 76.18 \cdot 10^{-5}$ ,  $u_{\text{Y}} = u_{\text{A}} \approx 40 \cdot 10^{-5} \text{ cm}^2/\text{V} \cdot \text{sec}$ .

anion  $Y^-$  of a strong acid forming complexes of the required stability and an anion  $A^-$  of a weak acid providing a sufficiently low value of  $\bar{u}_{H, \vartheta}$ .

Fig. 3 demonstrates the calculated dependence of  $\bar{u}_{H, \vartheta}$  on the composition of the leading electrolyte composed of an equimolar mixture of  $HY + HA$  and  $KY + KA$  for the chosen values of  $pK_{HY} = 3$  and  $pK_{HA} = 4$ . The calculation was performed according to eqn. 3 with the value of  $[H]_{\vartheta}$  being given by a cubic equation (*cf.*, eqn. 4). It can be seen from Fig. 3 that the replacement of  $HY$  by  $HA$  at a constant total concentration leads to a considerable decrease in the value of  $\bar{u}_{H, \vartheta}$ . The complexing characteristic of the system, given by the concentration of  $Y^-$ , remains nearly constant up to a value of 50%  $HA + A^-$ , as anion  $A^-$  is almost entirely bonded in the undissociated acid  $HA$ .

## EXPERIMENTAL

Tabulated values (at 25°C) of mobilities<sup>23</sup>, dissociation constants<sup>24</sup> and stability constants of complexes<sup>24,25</sup> were used for calculating actual systems. The values of  $\mu_{MY}$  (calculated according to Joki's equation<sup>26</sup> with parameters<sup>15</sup>  $a = 244.2$ ,  $b = 4.32$  [ $10^{-5}$  cm<sup>2</sup>/V · sec]) and  $\beta_{Cu(HCOO)_2} = 2 \cdot 10^3$  (estimate) used are approximate. The values of  $\beta$  and  $K$  were corrected approximately for the ionic strengths of the solutions.

The experiments were carried out in an isotachophoretic column made of Perspex with a separation capillary of rectangular cross-section<sup>27</sup>. The potential-gradient detector and the high-voltage supply of stabilized current (maximum parameters 460  $\mu A$  and 16 kV) were described earlier<sup>28</sup>.

The chemicals, *i.e.*, acetic, formic and lactic acids, their potassium salts, and chlorides or nitrates of separated metal cations, were of analytical-reagent grade (Lachema, Brno, Czechoslovakia).

## RESULTS AND DISCUSSION

To verify the theory, the simple model system  $Cu^{2+}$ -formate was selected. For an equimolar mixture of potassium formate and formic acid in the leading electrolyte,  $[L]_{\lambda} = [HY]_{\lambda}$ ,  $[L]_{\lambda, crit}$  was calculated according to eqn. 10. The result,  $[L]_{\lambda, crit} = 0.010$ , represents the lowest limiting concentration of the leading electrolyte for correctly terminating the zone of  $Cu^{2+}$  by hydrogen ion. Fig. 4 illustrates a more detailed calculation, representing the dependence of the effective mobilities of  $Cu^{2+}$  and  $H^+$  on the concentration of the leading ion,  $[L]_{\lambda}$ . Eqns. 3, 4 and 5 served to calculate the effective mobility of hydrogen ion,  $\bar{u}_{H, \vartheta}$ ; the calculation of  $\bar{u}_{M, \vartheta}$  and  $\bar{u}_{M, \mu}$  was performed with the aid of relationships 6b, 4 and 5, and 7, 6a, 9 and 1 (and the condition of electroneutrality in zone  $\mu$ ,  $2[M]_{\mu} + [MY]_{\mu} = [Y]_{\mu}$ ), respectively. With respect to the fact that the migration behaviour of  $H^+$  is expressed in Fig. 4 directly by its effective mobility, the range of isotachophoretic migration of the  $Cu^{2+}$  zone can be selected by direct comparison of the values of  $\bar{u}_{H, \vartheta}$  and  $\bar{u}_{M, \mu}$ .

As pointed out earlier<sup>21</sup>, the effective mobility of  $H^+$  decreases rapidly with increasing  $[L]_{\lambda}$  (*cf.*, Fig. 4). From the starting point  $\bar{u}_{M, \mu} = \mu_{M, \mu}$  (for  $[L]_{\lambda} = 0$ ), the mobility curves of  $Cu^{2+}$  in zones  $\vartheta$  and  $\mu$  also show a decreasing tendency. The curves intersect with the  $H^+$  curve at points dividing the concentration axis into three sec-

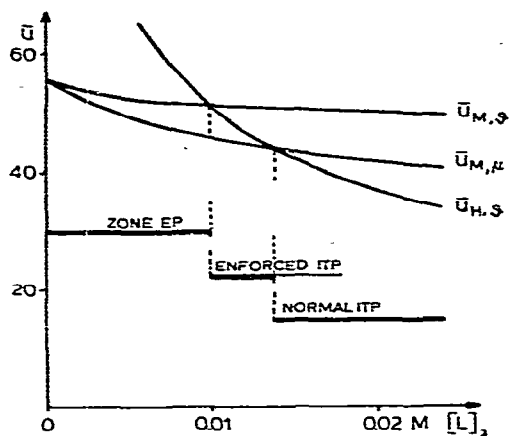


Fig. 4. Calculated dependence of the effective mobilities  $\bar{u}$  (in  $10^{-5} \text{ cm}^2/\text{V} \cdot \text{sec}$ ) on the concentration of the leading electrolyte,  $[\text{L}]_\lambda$ , for the model system. Leading electrolyte (zone  $\lambda$ ), potassium formate–formic acid (1:1); sample (zone  $\mu$ ),  $\text{Cu}^{2+}$ ; terminator (zone  $\vartheta$ ), formic acid. For explanation, see text.

tions, the  $[\text{L}]_\lambda$  values of which correspond to different migration behaviour of the zone of  $\text{Cu}^{2+}$ :

(a) In the range of higher concentrations of  $\text{L}^+$ ,  $\bar{u}_{\text{M},\vartheta} > \bar{u}_{\text{M},\mu} > \bar{u}_{\text{H},\vartheta}$  and the zone migrates in a normal isotachophoretic mode.

(b) In the intermediate range of  $[\text{L}]_\lambda$  values,  $\bar{u}_{\text{M},\vartheta} > \bar{u}_{\text{M},\mu}$ . Thus condition 2 of the sharp boundary  $\mu\vartheta$  is still valid. However, with regard to the inverse sequence of mobilities,  $\bar{u}_{\text{H},\vartheta} > \bar{u}_{\text{M},\mu}$ , zone  $\mu$  migrates in an enforced isotachophoretic mode.

(c) In the range of concentrations below the value of  $[\text{L}]_{\lambda,\text{crit}}$  the condition of the sharp boundary is not valid. Then  $\bar{u}_{\text{M},\vartheta} < \bar{u}_{\text{H},\vartheta}$  and  $\text{Cu}^{2+}$  migrates in the terminating zone of  $\text{H}^+$  in a zone electrophoretic mode.

The three modes of migration described above are illustrated in Fig. 5 by experimental records of the migration of the  $\text{Cu}^{2+}$  zone in the given model system at various concentrations of the leading electrolyte. Whereas at a concentration of 0.03 M (Fig. 5a) the  $\text{Cu}^{2+}$  zone shows normal isotachophoretic migration, at a value of  $[\text{L}]_\lambda = 0.012$  (Fig. 5b) this zone migrates in an enforced isotachophoretic mode. At the

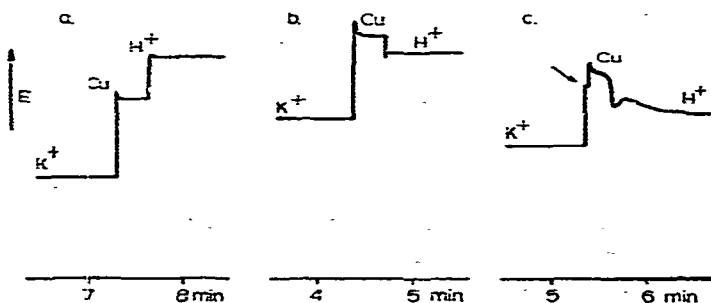


Fig. 5. Experimental records of the isotachophoretic migration of the  $\text{Cu}^{2+}$  zone. Leading electrolyte: potassium formate–formic acid (1:1): (a) 0.03 M; (b) 0.012 M; (c) 0.006 M. Terminator: 0.1 M formic acid. Sample: 0.01 M  $\text{CuCl}_2$ : (a) 2  $\mu\text{l}$ ; (b) 0.6  $\mu\text{l}$ ; (c) 0.3  $\mu\text{l}$ . Current: (a) 400  $\mu\text{A}$ ; (b) 240  $\mu\text{A}$ ; (c) 120  $\mu\text{A}$ .



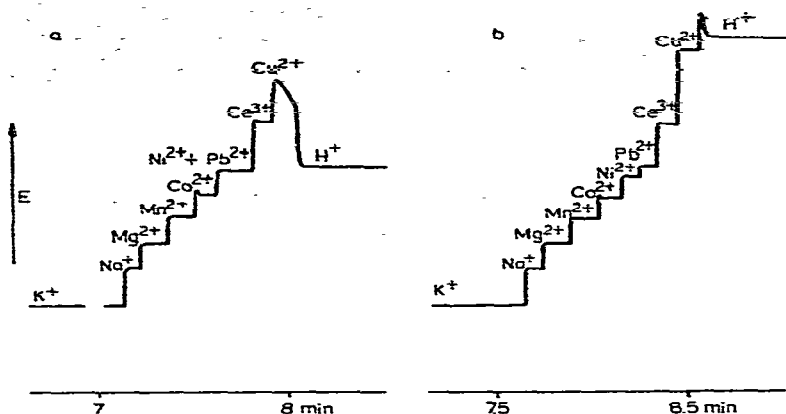


Fig. 6. Experimental records of the analyses of a model mixture of cations. Leading electrolyte: (a) 0.05 *M* potassium lactate–0.05 *M* lactic acid; (b) 0.05 *M* potassium lactate–0.05 *M* acetic acid. Terminator: 0.1 *M* acetic acid. Sample: Na<sup>+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Ce<sup>3+</sup>, Cu<sup>2+</sup>; 10 nmole (1  $\mu$ l of 0.01 *M* solution) of each.  $I = 460 \mu\text{A}$ .

concentration  $[L]_z = 0.006 < [L]_{z,\text{crit}}$  (Fig. 5c) it can be seen that Cu<sup>2+</sup> migrates in a zone electrophoretic mode. The front of H<sup>+</sup> ions penetrates through the front boundary of the zone (see arrow), whereas the lack of sharpness of the rear boundary is indicated by a non-constant course of the potential gradient record.

Fig. 6 illustrates the possibility of suppressing the effective mobility of H<sup>+</sup> by the selection of a mixed counter ion. In analysing an eight-component model mixture of cations with a leading electrolyte containing 0.05 *M* potassium lactate and 0.05 *M* lactic acid (Fig. 6a), the effective mobility of H<sup>+</sup> is higher than the effective mobilities of Ce<sup>3+</sup> and Cu<sup>2+</sup>. Whereas Ce<sup>3+</sup> migrates in an enforced isotachophoretic mode, Cu<sup>2+</sup> provides a diffuse mixed zone with H<sup>+</sup>. Replacement of free lactic acid in the leading solution by acetic acid results in a substantial decrease in the value of  $\bar{u}_{H,3}$  (Fig. 6b) and all components of the mixture migrate in a normal isotachophoretic mode. Comparison of the two records shows that the modification of the leading electrolyte performed did not change the complexing properties of the system markedly. Only a slight decrease in the effective mobility of Pb<sup>2+</sup> occurred owing to formation of relatively stable acetate complexes, making the separation of the model mixture in Fig. 6b complete.

## CONCLUSION

A sufficiently low value of the effective mobility of H<sup>+</sup> is a necessary condition for stable isotachophoretic migration of cations in acidic electrolyte systems where the counter anion used forms complexes with the cations being separated.

This condition corresponds to the use of H<sup>+</sup> as a terminating ion and can be expressed in a simple form,  $\bar{u}_{H,3} < \bar{u}_{M,3}$  (eqn. 2), *i.e.*, the effective mobility of cations under separation in the terminating zone must be greater than the effective mobility of the terminating H<sup>+</sup> itself, where both  $\bar{u}_{H,3}$  and  $\bar{u}_{M,3}$  are strongly dependent on the concentration of the leading electrolyte. Their values can be calculated by using eqns. 3 and 6b, thus enabling one, for an actual system and the given concentration of the

leading electrolyte, to determine whether the resulting migration is or is not isotachophoretic. More detailed information on the range of suitable concentrations of the leading electrolyte may be provided either by calculation of the critical concentration of the leading electrolyte (eqn. 10), or by plotting  $\bar{u}_{H,3}$  and  $\bar{u}_{M,3}$  vs.  $[L]_2$  (cf., Fig. 4). Generally, the critical concentration increases with increasing values of the dissociation constant of the counter ion acid and the stability constants of the complexes formed. The analysis made for simplified cases of single consecutive complex-forming equilibria confirms this rule (cf., eqns. 11 and 13 and Fig. 2).

If the required complex-forming counter anion is too strong an acid which does not permit to use  $H^+$  as terminator, then it is possible to use a mixed counter anion (cf., Fig. 6). Here a mixture of the complexing anion with a suitable weak acid is employed, thus ensuring both a sufficiently low  $\bar{u}_{H,3}$  and stable complexes.

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