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INVERSE ELECTROLYTE SYSTEMS IN ISOTACHOPHORESIS

IMPACT OF THE TERMINATING ELECTROLYTE ON THE MIGRATING ZONES IN CATIONIC ANALYSIS

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SUMMARY

An insight is given into the nature of cationic isotachophoretic systems with H^+ as the leading constituent. It is shown both theoretically and experimentally that the parameters of all zones in such a system depend not only on the composition of the leading electrolyte, but also on the composition (pH) of the terminator. The theory presented enables one to predict the parameters of all zones involved, including the effective mobility of the leading hydrogen constituent, thus allowing the correct construction of such systems. Moreover, the theory predicts the impact of the terminating electrolyte on the quantitative analysis. The practical importance of the systems in question is exemplified showing the possibility of determining either very mobile ions (K^+ , NH_4^+) or very weak organic bases (with $pK_{XH} = 2-3$ or even lower).

INTRODUCTION

The fundamental role of a discontinuous electrolyte system consists in the creation of conditions under which the sample components show different effective mobilities and good separation. The leading electrolyte plays the key role here as it determines, according to the principle of zone adjustment, what qualitative and quantitative composition any arbitrary sample zone has and what the effective mobility of the corresponding substance is^{1,2}. In accordance with the importance of this role, one must pay great attention to the choice of a suitable leading electrolyte, which is usually selected in such a way that the resulting system is correct (*i.e.*, it provides sharp boundaries between all zones which are stable)². Simultaneously, the pH of the electrolytes is usually selected so that it lies in the so-called safe region³ where the disturbing effect of the migration of H^+ and OH^- ions can be neglected. We can say more exactly that the leading electrolyte is selected in such a way that the disturbing ion (H^+ or OH^-) forms the real or potential rear (terminating) zone of the system^{4,5}.

The actual terminator has first a practical role, fulfilling the following requirements: the effective mobility of the terminator in its adjusted zone should be lower than that of any sample substance so that a quantitative termination of all sample zones is ensured; and the effective mobility of the terminator and hence the

conductivity of the terminating zone should not be too low in order not to make the analysis time too long. Because in correct systems the composition of all adjusted zones is a function of the composition of the leading electrolyte, it is not necessary to pay excessive attention to the composition of the terminating electrolyte as the composition of the terminating zone is automatically adjusted to the parameters of the given leading electrolyte. We can say that in correct isotachophoretic systems which have been used and recommended so far, the composition of the terminator does not show any influence on the composition (properties) of the sample zones.

In isotachophoretic practice, however, there sometimes electrolyte systems are used that do not possess the above-mentioned properties; non-buffered electrolyte systems may serve as an example. Here the counter ion does not have any buffering properties, such as an alkali metal cation in anionic isotachopheresis or an anion of a strong acid (chloride, sulphate) in cationic isotachopheresis. As the leading ions H^+ and K^+ and/or OH^- and Cl^- are usually used. Even in the early days of isotachopheresis, the use of such systems was not recommended as a result of qualitative considerations on possible disturbing effects caused by uncontrolled migration of H^+ or OH^- ions from the terminating electrode chamber^{1,6,7}. A detailed analysis of the behaviour of H^+ and OH^- ions in isotachopheresis^{4,5,8} has also shown that for a correct isotachophoretic migration it is necessary to control the migration of one of these two ions by the counter ionic system in such a way that the respective ion (H^+ or OH^-) serves as the natural terminator of the system. Nevertheless, the practical use of non-buffered systems has grown as these systems allow one to analyse, *e.g.*, K^+ when using H^+ as the leader^{6,9} or Cl^- with OH^- as the leader⁹. Analytical practice, however, shows that for a reproducible analysis it is necessary to find empirically a suitable pH of the terminating solution and to perform each analysis with a fresh terminator in the terminating electrode chamber^{10,11}.

It is evident that a quantitative knowledge of the role of the terminator has not yet been established, especially the problem of the influence of the composition (pH) of the terminator on the zones of migrating substances. Further, the use of non-buffered systems has so far not overcome the stage of empirical searching and a theoretical idea of the conditions under which H^+ may be used as the leading ion in correct isotachopheresis is still lacking. This paper considers the above problems, establishes an adequate theory and gives answers to the related practical questions.

THEORETICAL

Description of the system

Let us consider a system (Fig. 1) in which the zone of a weak (or strong) acid HA (zone H) serves as the leading zone of cationic migration. The solution of this acid, HA, and of its salt with a weak (or strong) base, BH^+A^- , serves as the terminator (zone T). Let us assume that the composition of the terminator does not change with time. After switching on the electric current, the terminating zone B is created, its concentration being adjusted to the parameters of zone H.

The physico-chemical description of such a system starts from its understanding as a moving-boundary system, where not only the counter constituent but also the constituent " H^+ " are present in all zones. To describe such a type of system, the

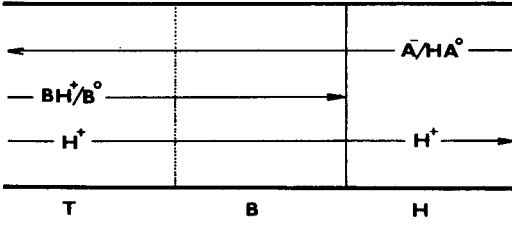


Fig. 1. Scheme of the formation of the adjusted terminating zone B between the leading zone H and the original terminating electrolyte T.

moving-boundary equations¹² may be advantageously adopted². In our case, the following equations may be written for the boundary B → H:

$$\frac{\bar{u}_{B,B}}{\kappa_B} = W_{B-H} \quad (1)$$

$$\frac{u_H c_{H,B} + u_{BH} c_{BH,B}}{\kappa_B} - \frac{u_H c_{H,H}}{\kappa_H} = W_{B-H} (\bar{c}_{H,B} - \bar{c}_{H,H}) \quad (2)$$

where u_i is the electrophoretic mobility of ion i , $\bar{u}_{i,j}$ and $\bar{c}_{i,j}$ are the effective mobility and the total (analytical) concentration, respectively, of constituent i in zone j , $c_{i,j}$ is the concentration of ion i in zone j , κ_j is the conductivity of zone j , and W_{B-H} is the volume swept by the boundary B–H per coulomb passed. Note that, *e.g.*,

$$\bar{c}_{H,B} = c_{H,B} + c_{BH,B} + c_{HA,B} \quad (3)$$

is the constituent concentration of H^+ in zone B. For the immobile concentration boundary T::B the following balances can be written:

$$\frac{u_{BH} c_{BH,T}}{\kappa_T} = \frac{u_{BH} c_{BH,B}}{\kappa_B} \quad (4)$$

$$\frac{u_H c_{H,T} + u_{BH} c_{BH,T}}{\kappa_T} = \frac{u_H c_{H,B} + u_{BH} c_{BH,B}}{\kappa_B} \quad (5)$$

from which

$$\frac{c_{H,T}}{\kappa_T} = \frac{c_{H,B}}{\kappa_B} \quad \text{and} \quad \frac{c_{BH,T}}{\kappa_T} = \frac{c_{H,T}}{c_{H,B}} \quad (6)$$

By combination of eqns. 1, 2 and 5, we obtain

$$\frac{u_H c_{H,T} + u_{BH} c_{BH,T}}{\kappa_T} - \frac{u_H c_{H,H}}{\kappa_H} = a = \frac{\bar{u}_{B,B}}{\kappa_B} (\bar{c}_{H,B} - \bar{c}_{H,H}) \quad (7)$$

When expressing the last term of eqn. 7 by using eqns. 3 and 6, the definition of effective mobility

$$\bar{u}_{B,B} = u_{BH}c_{H,B}/(c_{H,B} + K_{BH}) \quad (8)$$

and electroneutrality

$$c_{A,B} = c_{H,B} + c_{BH,B} \quad (9)$$

we obtain, after rearrangement an equation that is a function of only one unknown, $c_{H,B}$:

$$\frac{a}{c_{H,T}} \frac{\kappa_T}{\kappa_H} = \frac{u_{BH}}{c_{H,B} + K_{BH}} \left[c_{H,B} \left(1 + \frac{c_{H,B}}{K_{HA}} \right) \left(1 + \frac{c_{BH,T}}{c_{H,T}} \right) - \bar{c}_{H,H} \right] \quad (10)$$

where K_{HA} and K_{BH} are the dissociation constants of HA and BH^+ , respectively. The solution of eqn. 10 (and the use of eqns. 6 and 9) provides the complete description of the composition of zone B. From eqn. 10, one important conclusion can be drawn: the composition of the adjusted terminator (zone B) depends not only on the parameters of the leading zone (as is usual in normal isotachophoretic systems), but also on the composition of the terminating solution (zone T), especially on the ratio $c_{BH,T}/c_{H,T}$. Obviously, the above ratio can be expressed as a function of the total concentration $\bar{c}_{B,T}$: $c_{BH,T}/c_{H,T} = \bar{c}_{B,T}/(K_{BH} + c_{H,T})$.

In the treatment of systems as shown in Fig. 1, the question arises of whether the phenomenological description of isotachophoretic systems may be applied here. The understanding of the boundary $B \rightarrow H$ as an isotachophoretic boundary allows the application of the general isotachophoretic condition

$$W_{B-H} = \frac{\bar{u}_{B,B}}{\kappa_B} = \frac{\bar{u}_{H,H}}{\kappa_H} \quad (11)$$

where the concept of the effective mobility of hydrogen ion⁵, $\bar{u}_{H,H}$ (originally introduced for H^+ as the terminator), is generalized to any isotachophoretic zone containing H^+ as the only constituent (apart from the counter constituent). The combination of eqns. 7 and 11 gives, after rearrangement,

$$\bar{u}_{H,H} = \frac{c_{H,H}u_H}{\bar{c}_{H,H} - \bar{c}_{H,B} + c_{H,B} \left(\frac{u_H}{\bar{u}_{B,B}} \right) + c_{BH,B} \left(\frac{u_{BH}}{\bar{u}_{B,B}} \right)} \quad (12)$$

Comparison of this equation with eqn. 4 in ref. 5 shows additional terms expressing the contribution of B being a weak base; for $K_{BH} \rightarrow 0$ both of the compared equations become the same. It is clearly seen from eqn. 12 that $\bar{u}_{H,H}$ is a function of the parameters of both zones H and B (and thus T). The value of $\bar{u}_{H,H}$ is in direct proportion to W_{B-H} (*i.e.*, to the velocity of the isotachophoretic boundary) since κ_H is constant for a given leading electrolyte (*cf.*, eqn. 11).

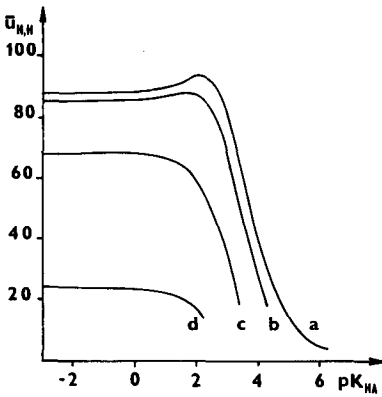


Fig. 2. Calculated dependence of the effective mobility of H⁺, $\bar{u}_{H,H}$ (in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) on $\text{p}K_{HA}$ of the counter constituent, for various $\text{p}K_{BH}$ of the terminating substance: (a) 20; (b) 5; (c) 4; and (d) 3. $u_A = -60 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{BH} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\bar{c}_{B,T}/\bar{c}_{H,T} = 0.5$.

Figs. 2–4 illustrate how the value of $\bar{u}_{H,H}$ depends on the parameters of the system. Fig. 2 shows the dependence of $\bar{u}_{H,H}$ on the value of $\text{p}K_{HA}$ of the counter constituent, for four selected values of $\text{p}K_{BH}$ of the terminating substance and for a constant ratio $\bar{c}_{B,T}/\bar{c}_{H,T} = 0.5$. It can be seen from Fig. 2 how the value of $\bar{u}_{H,H}$ depends on $\text{p}K_{BH}$ and that it decreases rapidly with increasing $\text{p}K_{HA} \geq 2$.

Fig. 3 shows the dependence of $\bar{u}_{H,H}$ on the content of free acid in the terminator expressed as $\bar{c}_{A,T} - \bar{c}_{B,T}$, for B being a strong base. For a strong acid (curve a, $\text{p}K_{HA}$

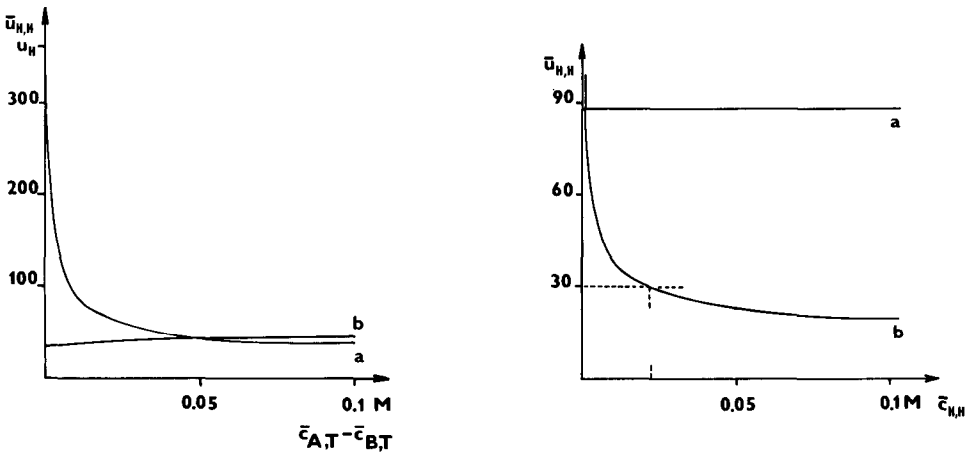


Fig. 3. Calculated dependence of $\bar{u}_{H,H}$ (in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) on the content of free acid in the terminator, $\bar{c}_{A,T} - \bar{c}_{B,T}$, for a strong (curve a, $\text{p}K_{HA} = -3$) and a weak (curve b, $\text{p}K_{HA} = 4$) acid as the counter constituent. $u_A = -60 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{BH} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\text{p}K_{BH} = 20$; $\bar{c}_{B,T} = 0.01 \text{ M}$.

Fig. 4. Calculated dependence of $\bar{u}_{H,H}$ (in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) on the total concentration of H⁺ in the leading zone, $\bar{c}_{H,H}$, for a strong (curve a, $\text{p}K_{HA} = -3$) and a weak (curve b, $\text{p}K_{HA} = 4$) acid as the counter constituent. The dashed line shows the $\bar{c}_{H,H}$ value corresponding to $\bar{u}_{H,H} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_A = -60 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{BH} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\text{p}K_{BH} = 20$; $\bar{c}_{B,T} = 0.01 \text{ M}$; $\bar{c}_{H,T} = 0.02 \text{ M}$.

$= -3$), the value of $\bar{u}_{H,H}$ increases with decreasing concentration of the free acid in zone T, reaching the limiting value $\bar{u}_{H,H} = u_H$ for values of $\bar{c}_{A,T} - \bar{c}_{B,T}$ near zero. Another type of dependence occurs for the case of a weak acid (curve b, $pK_{HA} = 4$), where the relatively low value of $\bar{u}_{H,H}$ increases only slightly with increasing $\bar{c}_{A,T} - \bar{c}_{B,T}$.

As follows from Fig. 4, the concentration in zone H, $\bar{c}_{H,H}$, has the opposite influence. For a strong acid ($pK_{HA} = -3$, curve a), $\bar{u}_{H,H}$ is almost independent of $\bar{c}_{H,H}$; in contrast, for a weak acid as the counter constituent ($pK_{HA} = 4$, curve b), the value of $\bar{u}_{H,H}$ increases rapidly with decreasing $\bar{c}_{H,H}$.

All the conclusions drawn from Figs. 2–4 are in accordance with eqn. 12 and illustrate some simple rules on how the value of $\bar{u}_{H,H}$ depends on the parameters of the electrolyte system in some special situations. For a strong acid HA and a strong base B, for example, it holds that $\bar{c}_{H,H} = c_{H,H}$ and $\bar{u}_{B,B} = u_{BH}$; eqn. 12 therefore becomes

$$\bar{u}_{H,H} = u_H \cdot \frac{1}{1 - \frac{c_{H,B}}{c_{H,H}} \left(1 + \frac{u_H}{u_{BH}}\right)} \quad (13)$$

This equation corresponds to curve a in Fig. 3 and explains both its course and the limiting value $\bar{u}_{H,H} = u_H$ (for $c_{H,B} = 0$).

As was shown above, the value of $\bar{u}_{H,H}$ depends on the parameters of the zones H and B in a defined way which is the same irrespective of whether B is the leader and H^+ the terminator or *vice versa*. The condition of correct migration⁸ of zones B and H can be formulated as follows:

(a) The system $H \rightarrow B$ is correct (the moving boundary $H \rightarrow B$ is sharp) if

$$\bar{u}_{B,H} > \bar{u}_{H,H} \quad (14)$$

(b) The system $B \rightarrow H$ is correct (the moving boundary $B \rightarrow H$ is sharp) if

$$\bar{u}_{B,H} < \bar{u}_{H,H} \quad (15)$$

In case (a), the electrolyte system in question is a normal cationic system with H^+ as the terminator. In case (b), the electrolyte system is that from Fig. 1 and condition 15 determines the region of correct migration. The only difference between the two situations is that in case (b) the behaviour of the system depends not only on the parameters of the leading zone but also on the parameters of the terminating zone, *viz.*, the ratio $c_{BH,T}/c_{H,T}$ (*cf.*, eqn. 10).

It is obvious that, when taking a series of systems with increasing $\bar{u}_{H,H}$, the transition between systems of type (a) and (b) can be documented. Let us, as an example, consider curve b in Fig. 4, depicting the dependence of $\bar{u}_{H,H}$ on the concentration in the zone H, $\bar{c}_{H,H}$, for a weak acid as the counter ion ($u_A = -60 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $pK_{HA} = 4$). A strong base B ($u_{BH} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) serves as the constituent of the zone B and the dashed line shows the transition corresponding to a concentration of $\bar{c}_{H,H} = 0.019 \text{ M}$ where $\bar{u}_{H,H} = u_B$. For concentrations higher than this (critical⁴) value, condition 14 holds true and the system shows a correct migration for the migration order $H \rightarrow B$. For concentrations lower than the transition value, a correct system is obtained if the inverse migration order is selected, *i.e.*, $B \rightarrow H$.

Correct migration of sample zones

Let us now describe the migration of the zone of a weak (or strong) base X in the type of systems under consideration (see Fig 5). The mass balance for the counter ion can be written in the usual way (note that u is a signed quantity):

$$\bar{c}_{A,H} \left(1 + \frac{|\bar{u}_{A,H}|}{\bar{u}_{H,H}} \right) = f_H = \bar{c}_{A,X} \left(1 + \frac{|\bar{u}_{A,X}|}{\bar{u}_{X,X}} \right) \quad (16)$$

The use of the electroneutrality condition, $c_{H,X} + c_{XH,X} = c_{A,X}$, and rearrangement lead to

$$(c_{H,X} + c_{XH,X}) \left(\frac{K_{HA} + c_{H,X}}{K_{HA}} + \frac{|u_A|}{u_{XH}} \cdot \frac{K_{XH} + c_{H,X}}{c_{H,X}} \right) = f_H \quad (17)$$

Simultaneously, the isotachophoretic condition

$$\frac{\bar{u}_{H,H}}{\kappa_H} = \frac{\bar{u}_{X,X}}{\kappa_X} \quad (18)$$

holds. The set of the two equations 17 and 18 can be solved for the two unknowns, $c_{H,X}$ and $c_{XH,X}$, by, *e.g.*, the usual RFQ method⁶.

Obviously, $\bar{u}_{H,H}$ in eqn. 18 is dependent on the composition of the terminating electrolyte, and hence the composition of zone X and the value of $\bar{u}_{X,X}$ are also dependent on the terminator.

Of greatest practical importance in any electrolyte system is the question of the correct migration of sample zones. In order to explain some new aspects which accompany the type of electrolyte systems described in this paper, we take advantage of the zone existence diagrams^{1,3}.

Let us first consider the following model system with formate ($pK_{HA} = 3.75$) as the weak counter constituent. When taking $0.0053 M KCOOH + 0.002 M HCOOH$ as the leading electrolyte for cationic isotachopheresis, we have a normal system with H^+ as the terminator. Its effective mobility is $\bar{u}_{H,H} = 54.4 \cdot 10^{-9} m^2 V^{-1} s^{-1}$ and its adjusted concentration is $\bar{c}_{H,H} = 0.01 M$. The zone existence diagram of such a system is shown in Fig. 6 (the existence region of this system is K-A-H-D-K). The contours have the usual meaning^{1,3}; point K corresponds to the leading zone of K^+ and point H corresponds to the terminating zone of H^+ .

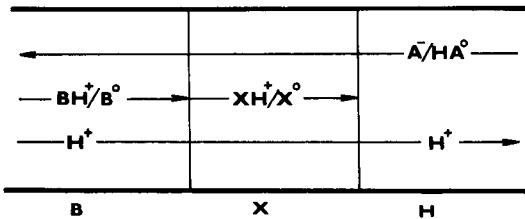


Fig. 5. Scheme of the migrating system of zones H, X and B.

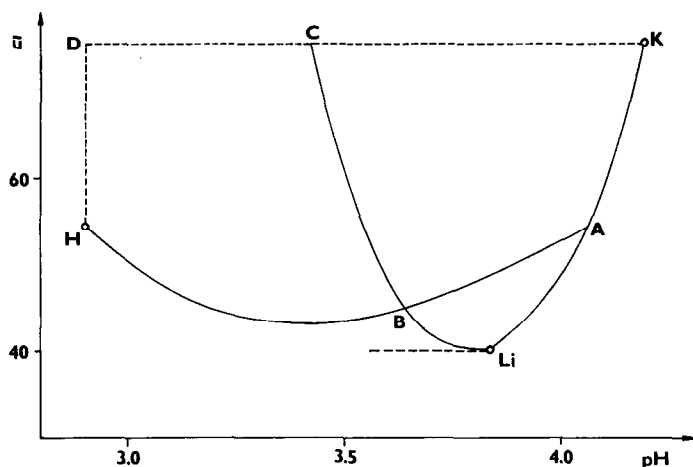


Fig. 6. Zone existence diagram, pH vs. \tilde{u} (in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$), for the cationic system with 0.0053 M potassium formate + 0.002 M formic acid as the leading electrolyte and H^+ as the terminator; for explanation, see text. $u_{\text{K}} = 76.2 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{For}} = -57.1 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{H}} = 362.5 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\text{p}K_{\text{HFor}} = 3.75$.

Let us now take an inverse system where 0.01 M HCOOH serves as the leading zone and the terminator is $0.00334 \text{ M LiCOOH} + 0.0029 \text{ M HCOOH}$. The terminator is selected so that its concentrations are adjusted to the leading H zone and, moreover, for the leading zone it holds again that $\tilde{u}_{\text{H,H}} = 54.4 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. When returning to Fig. 6, we can depict the leading zone H by the same point which depicts the terminating zone of the former system. The terminating zone of the present system is depicted by point Li which lies on the continuation of the curve KA. The existence region of the system involves points corresponding to bases X for which it simultaneously holds that $\tilde{u}_{\text{X,H}} < \tilde{u}_{\text{H,H}}$ and $\tilde{u}_{\text{X,Li}} > \tilde{u}_{\text{Li,Li}}$. The first condition holds true for points below curve AH in Fig. 6 (*i.e.*, below the lower existence contour of the former system). The second condition holds true for points above curve LiC (*i.e.*, above the upper sequence contour of zone Li). The resulting zone existence diagram of the present system has an unusual appearance; it is formed by the region Li–A–B–Li and by point H lying separately. All points lying on the diagram below curve LiC and above the lower sequence contour of zone Li (dashed line) do not give correct zones in the system as they are in the region of formation of steady-state mixed zones with the Li zone. Point H (zone H) represents here an apparent exception to this rule although, in fact, H^+ is present in all zones. We can say that point H represents the individual pure zone of the H^+ constituent, which is in steady-state coexistence with the terminating zone consisting of $\text{Li}^+ + \text{H}^+$.

Fig. 7 presents a schematic treatment of the various possibilities of constructing correct cationic electrolyte systems where 0.01 M HCOOH is involved as a separate zone. Fig. 7A shows the normal cationic system (A) where H^+ is the terminator; its concentration in the column adapts automatically to the value adjusted to the parameters of the leading electrolyte. In this system, substances from regions a and b (*cf.*, the diagram in Fig. 6) provide correct zones. Fig. 7B shows the novel inverse system (B) with the H^+ zone as the leader; here the terminating Li zone must have the

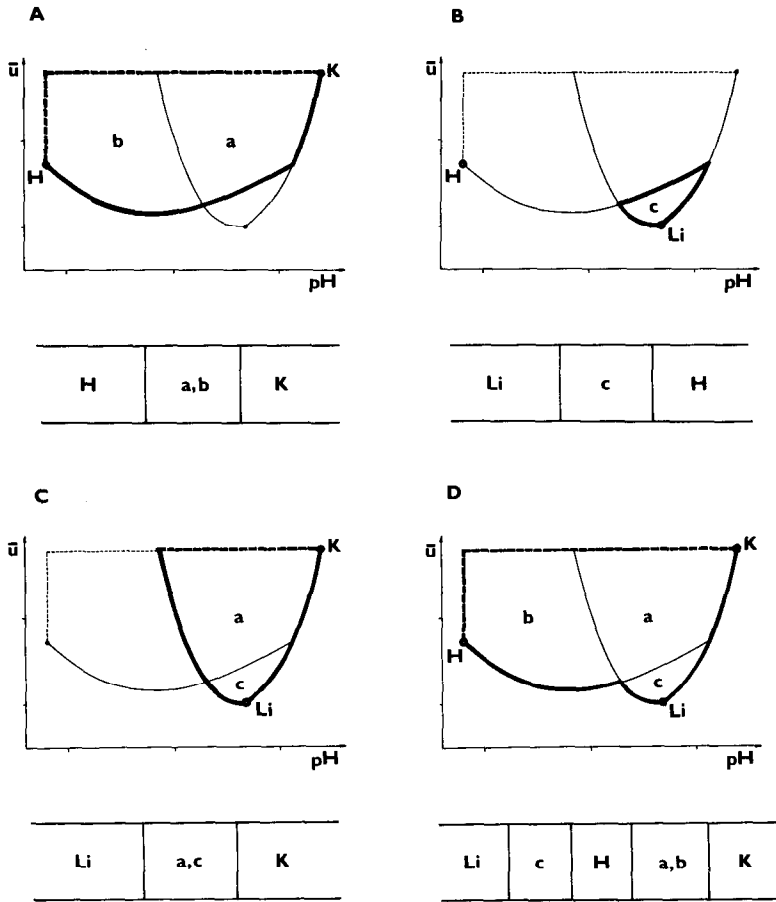


Fig. 7. Scheme of possible cationic isotachophoretic systems where 0.01 M formic acid is involved as an individual zone (the contours of the zone existence diagram are those from Fig. 6): (A) normal system; (B) inverse system; (C) combined system; (D) extended combined system. For explanation, see text.

concentrations of both LiCOOH and HCOOH selected so that $\bar{u}_{\text{H,H}}$ has the required value. In this system, substances from region c provide correct zones.

When taking the leading zone K from system A and the terminating zone Li from system B, a composed system (C) as depicted in Fig. 7C is the result. In this system, substances from region a and c provide correct zones. An interesting possibility of how to extend the system C should be mentioned here. By introducing the zone H^+ as a sample, a system (D) arises representing the coupled systems A and B (Fig. 7D). When comparing systems C and D, we can see that in system D also the substances from region b provide correct zones, their correct migration being enforced by the H^+ zone migrating behind them. It should be stressed here that both systems C and D are very sensitive to any change in the composition of either the leading or terminating electrolyte. A change in the composition of the terminator (Li zone) leading to a decrease in the corresponding $\bar{u}_{\text{H,H}}$ value, for example, results in the virtual existence

of two different $\bar{u}_{H,H}$ values in the system; one of them is defined with respect to the terminating zone of Li^+ , $\bar{u}_{H,H}(\text{Li})$, and the other with respect to the leading zone of K^+ , $\bar{u}_{H,H}(\text{K})$. In our example $\bar{u}_{H,H}(\text{Li}) < \bar{u}_{H,H}(\text{K})$, the boundaries $\text{Li} \rightarrow \text{H}$ and $\text{H} \rightarrow \text{K}$ migrate with different velocities (here $v_{\text{Li-H}} < v_{\text{H-K}}$) and consequently, a growing H^+ zone will appear between the leading (K) and terminating (Li) zones. The (originally) single isotachophoretic system splits in this way into two isotachophoretic systems moving with two different velocities, bound by the common zone of the H constituent which had a double role, being simultaneously terminator for one system and leader for the other.

For systems with a strong acid as the counter constituent, the zone existence diagram shows a very narrow existence region which can be approximated by only a single line. Fig. 8, for example, shows the diagram for the system with 0.05 M HCl as the leading zone and 0.02 M hydroxyproline + 0.06 M HCl as the terminating solution. In this instance the pH of zones of strong bases decreases with increasing u_{XH} (in contrast to the former formate system). It is obvious that the above-mentioned operational electrolyte system offers the possibility of analysing even very weak bases in a correct way (see Results and Discussion, Fig. 15).

Quantitative aspects

As mentioned above, the composition of the terminating electrolyte strongly influences the qualitative parameters of the sample zones in the systems studied here. This fact has a strong impact on quantitative analysis.

The basic principle of quantitation in isotachopheresis is expressed by the known equation²

$$N_i = k_i Q_i \quad (19)$$

which expresses the proportionality between the amount N_i of a substance i and its

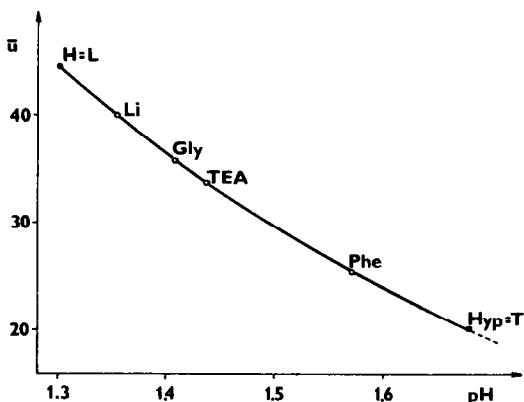


Fig. 8. Zone existence diagram, pH vs. \bar{u} (in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$), for the cationic system with 0.05 M HCl as the leading electrolyte and 0.02 M hydroxyproline + 0.06 M HCl as the terminator. Gly = glycine; TEA = tetraethylammonium; Phe = phenylalanine; Hyp = hydroxyproline. $u_{\text{HypH}} = 32 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{PheH}} = 28 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{TEA}} = 32.9 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{GlyH}} = 40 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{Cl}} = -79.1 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\text{p}K_{\text{HypH}} = 1.92$; $\text{p}K_{\text{PheH}} = 2.58$; $\text{p}K_{\text{GlyH}} = 2.35$.

zone-passage charge Q_i . The proportionality (calibration) constant for the given electrolyte system may be expressed by

$$k_i = \bar{c}_{i,t} \frac{|\bar{u}_{i,i}|}{\kappa_i} \quad (20)$$

When considering an acidic cationic system where a constant flow of H^+ passes through the sample zones, it is obvious from eqn. 19 that the higher the flow of H^+ , the lower is k_i . An explicit description of the effect of H^+ may be given here by considering the simple example of the system in Fig. 5 with the assumption that all of the constituents involved are strong acids and bases. Then we may write the counter ion balance for zones B and X as

$$c_{A,B} \left(1 + \frac{|u_A|}{u_{BH}}\right) = c_{A,X} \left(1 + \frac{|u_A|}{u_{XH}}\right) \quad (21)$$

and the isotachophoretic condition for these zones is

$$\frac{u_{BH}}{\kappa_B} = \frac{u_{XH}}{\kappa_X} \quad (22)$$

By using the condition of electroneutrality and after rearrangement, eqns. 21 and 22 provide the following expression:

$$k_X = \frac{u_{XH} c_{X,X}}{\kappa_X} = \frac{1}{F} \cdot \frac{u_{XH}}{u_{XH} + |u_A|} \left(1 - \frac{u_H - u_{BH}}{u_H - u_{XH}} \cdot \frac{1}{1 + \frac{c_{BH,B}}{c_{H,B}} \cdot \frac{u_{BH} + |u_A|}{u_H + |u_A|}}\right) \quad (23)$$

showing that k_X is a function of $c_{H,B}/c_{BH,B} = c_{H,T}/c_{BH,T}$ (*cf.*, eqn. 6), *i.e.*, of the ratio of H^+ and BH^+ in the terminating zone. For $c_{H,T} = 0$ we obtain the constant $k_X = u_{XH}/F(u_{XH} + |u_A|)$. With increasing $c_{H,T}/c_{BH,T}$ the value of k_X decreases and becomes zero at $c_{H,T}/c_{BH,T} = (u_{BH} + |u_A|)(u_H - u_{XH})/(u_{XH} - u_{BH})(u_H + |u_A|)$.

Fig. 9 shows the calculated dependence of $1/k_X F$ on the pH of zone B for the system 0.01 M HCl (leading electrolyte), 0.01 M Tris + HCl (terminator). Curve a shows the dependence for a strong base ($u_{XH} = 55 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) and curve b an analogous dependence for a weak base ($u_{XH} = 35 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $pK_{XH} = 6$); the end of the latter curve towards the alkaline region indicates the end of correct migration owing to insufficient protonation of base X.

EXPERIMENTAL

For all calculations, a PMD 85-2 microcomputer (Tesla, Piešťany, Czechoslovakia) was used.

Experiments were carried out using a CS isotachophoretic analyser (URVJT, Spišská Nová Ves, Czechoslovakia) equipped with one capillary (170 mm \times 0.3 mm I.D.) and conductivity detection. Some experiments were carried out on a Shimadzu

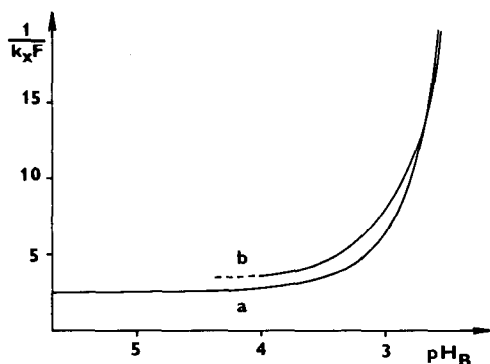


Fig. 9. Calculated dependence of $1/k_x F$ (reciprocal calibration constant) on pH_B of the adjusted terminating zone for a strong (curve a, $u_{\text{XH}} = 55 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $\text{p}K_{\text{XH}} = 20$) and a weak (curve b, $u_{\text{XH}} = 35 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $\text{p}K_{\text{XH}} = 6$) base in the system with 0.01 M HCl as the leading electrolyte and 0.01 M Tris [tris(hydroxymethyl)aminomethane] + HCl as the terminator. $u_{\text{TrisH}} = 30 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $u_{\text{Cl}} = -79.1 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\text{p}K_{\text{TrisH}} = 8$.

IP-3A isotachophoretic analyser (Shimadzu, Kyoto, Japan) equipped with a separation (0.7 mm I.D.) and a detection (0.2 mm I.D.) tube, with potential gradient and UV-absorption (254 nm) detection. When performing experiments only in the detection capillary, the sample was pumped into the separation capillary and electric current was applied for some time. After stopping this electromigration sampling, the sample solution in the separation capillary was replaced with the terminating solution and the analysis was carried out.

An OP-208 Precision Digital pH meter (Radelkis, Budapest, Hungary) with glass and calomel electrodes served for pH measurements.

All chemicals were of analytical-reagent grade (Lachema, Brno, Czechoslovakia) and were dissolved in distilled water deionized with a mixed-bed ion exchanger.

RESULTS AND DISCUSSION

As stated under Theoretical, in systems with H^+ as the leading constituent, the composition of the terminating solution shows a strong influence on the migration behaviour and on all substantial parameters of zones migrating in front of it. For practical users of these systems, the impact of this fact on quantitative analysis is very important. Related to the *Quantitative aspects* section, Fig. 10A illustrates the influence of the pH of the terminator on the step lengths of Na^+ and K^+ . The system used was 0.01 M HCl as the leading electrolyte and 0.01 M (0.1 M) Tris + HCl as the terminator. The results show good coincidence with the theory: with decreasing the pH of the terminator, the step lengths increase (*cf.*, Fig. 9, curve a). Fig. 10B shows a similar experimental dependence for a weak base (ϵ -aminocaproic acid) in the system with 0.005 M formic acid as the leading electrolyte and 0.005 M tetrabutylammonium hydroxide + formic acid as the terminator. Also here we can make a good comparison with Fig. 9 (curve b); it is seen that weak bases can be analysed only under conditions of sufficient protonation (at a sufficiently low pH value of the terminator). The point marked with an arrow in Fig. 10B indicates incorrect migration of the sample zone.

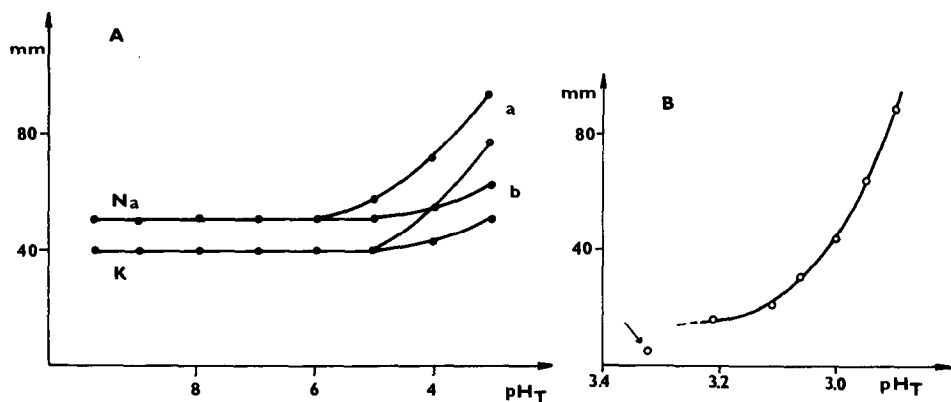


Fig. 10. (A) Experimental dependence of the step lengths of the zones of K^+ and Na^+ ($25 \mu\text{l}$ of $0.0019 M$ KCl + $0.0017 M$ $NaCl$) on the pH_T of the terminator in the system with $0.01 M$ HCl as the leading electrolyte and (a) $0.01 M$ and (b) $0.1 M$ $Tris + HCl$ as the terminator. (B) The same dependence for ϵ -aminocaproic acid ($25 \mu\text{l}$ of $30 \mu M$ solution) in the system with $0.005 M$ formic acid as the leading electrolyte and $0.005 M$ tetrabutylammonium hydroxide + formic acid as the terminator. Both experiments were performed in the CS isotachophoretic analyser with a detection current I of (a) $250 \mu A$ and (b) $25 \mu A$ and a chart speed of 1 mm/s .

The prolongation of the zones at low pH of the terminator may apparently offer a means of increasing the sensitivity of the analysis; however, this is accompanied by an increase in the analysis time. Another important feature for practical applications is the impact of the terminating electrolyte on the reproducibility of the analysis. This can be ensured only if a constant composition of the terminator is provided during all analyses. If Cl^- is used as the counter ion, the reproducibility of analyses can be considerably decreased owing to the change in the composition of the terminator by the production of H^+ at the anode. Fig. 11 shows the variation of the zone lengths of Na^+ and K^+ in five consecutive analyses when the content of the terminating electrode chamber was not replaced with fresh electrolyte after each analysis. The prolongation of the zones caused by the increasing concentration of H^+ in the terminator is clearly

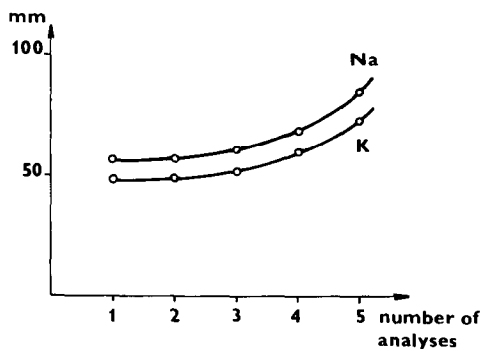


Fig. 11. Experimental dependence of the step lengths of the zones of K^+ and Na^+ ($25 \mu\text{l}$ of $0.0019 M$ KCl and $0.0017 M$ $NaCl$) on the number of analyses (without refreshing the content of the terminating electrode chamber) in the system with $0.01 M$ HCl as the leading electrolyte and $0.01 M$ $Tris + HCl$ ($pH_T = 7$) as the terminator. CS isotachophoretic analyser, $I = 250 \mu A$, chart speed 1 mm/s .

seen, indicating that the refilling of the terminating electrode chamber after each analysis is necessary. The original pH of the terminator, $\text{pH}_T = 7$, decreased to 3.8 after the fifth analysis, which caused a prolongation of the Na^+ zone by 46% (see Fig. 11). In a similar experiment an original value of $\text{pH}_T = 3.2$ decreased to 3.05 after the third analysis, resulting in a prolongation of the Na^+ zone by 35%.

A possible means of eliminating the negative effects of H^+ production at the anode is the use of a carboxylic acid instead of HCl in the terminating solution. Fig. 12 shows the record of an analysis of a relatively complex mixture of cations with 0.01 M HCl as the leading electrolyte and 0.01 M tetrabutylammonium hydroxide + acetic acid as the terminator at $\text{pH}_T = 4.9$. The system showed excellent reproducibility (repeatability) without the need to refill the terminating electrode chamber after each analysis as acetate did not produce any H^+ at the anode.

Under *Description of the system*, the effects of the concentration of the leading zone on the migration behaviour were described and discussed (*cf.*, Fig. 4). The experimental evidence is given in Fig. 13, where the possibility of how we can change the role of the H^+ constituent from the leader to the terminator by only a change in concentration is exemplified. Fig. 13a shows the record of an analysis in a system with 0.015 M sodium formate as the leading electrolyte and 0.01 M formic acid as the terminator. In this system, the calculated value of the effective mobility of H^+ is $\bar{u}_{\text{H,H}} = 28.1 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and H^+ thus acts as the terminator, also for Li^+ migrating as a sample zone. Fig. 13b shows the case where the concentrations were decreased by an order of magnitude. Here 0.0018 M formic acid serves as the leading zone as the calculated effective mobility of H^+ is $\bar{u}_{\text{H,H}} = 97.1 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. In this system sodium is the terminator and K^+ can be determined here without problems.

Under *Correct migration of sample zones*, the coexistence of normal and inverse systems of zones was predicted, resulting in a combined system with the potential to

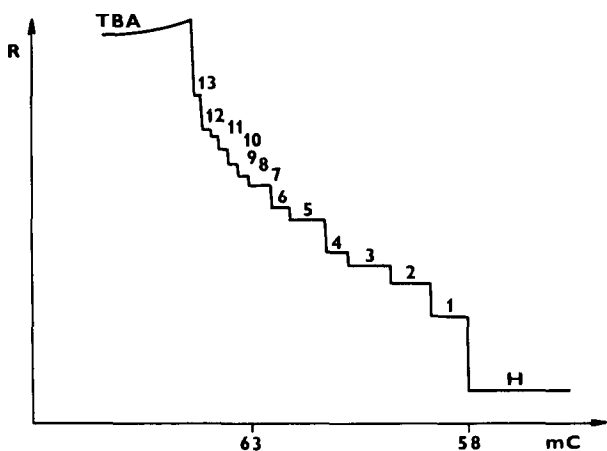


Fig. 12. Experimental record of an analysis in the system with 0.01 M HCl as the leading electrolyte and 0.01 M TBAOH + acetic acid ($\text{pH}_T = 4.9$) as the terminator. The sample was $1 \mu\text{l}$ of a solution containing *ca.* 0.5 mM of the following bases: (1) K^+ , (2) Ca^{2+} , (3) Na^+ , (4) pyridine, (5) piperidine, (6) lutidine, (7) ammediol, (7) collidine, (8) Tris, (9) ornitine, (9) histidine, (10) lysine, (10) citrulline, (11) ephedrine, (12) arginine and (13) procaine; steps 7, 9 and 10 on the record correspond to mixed zones. TBA = tetrabutylammonium; CS isotachophoretic analyser, $I = 50 \mu\text{A}$, R = conductivity detection signal.

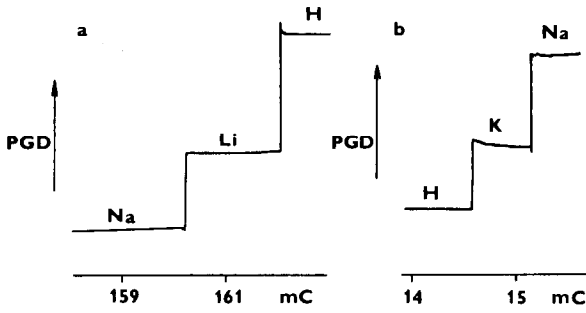


Fig. 13. (a) Isotachopherogram of 1 μ l of 0.01 M LiNO₃ in the system with 0.015 M sodium formate as the leading electrolyte and 0.01 M formic acid as the terminator. Shimadzu IP-3A instrument, $I = 300 \mu$ A (separation), 40 μ A (detection). (b) Isotachopherogram of 0.1 μ l of 0.015 M KCl in the system with 0.0018 M formic acid as the leading electrolyte and 0.0015 M sodium formate as the terminator. Shimadzu IP-3A instrument, $I = 50 \mu$ A (separation), 5 μ A (detection), PGD = potential gradient detection signal.

show the correct isotachopheretic migration and analysis of the zone of the H⁺ constituent. Fig. 14 confirms this theoretical assumption by experiment; the leading electrolyte was 0.005 M ammonium formate + 0.0024 M formic acid and the terminating electrolyte was 0.0036 M lithium formate + 0.0024 M formic acid. Fig.14a shows a blank run; the small zone of H⁺ on the record indicates that the

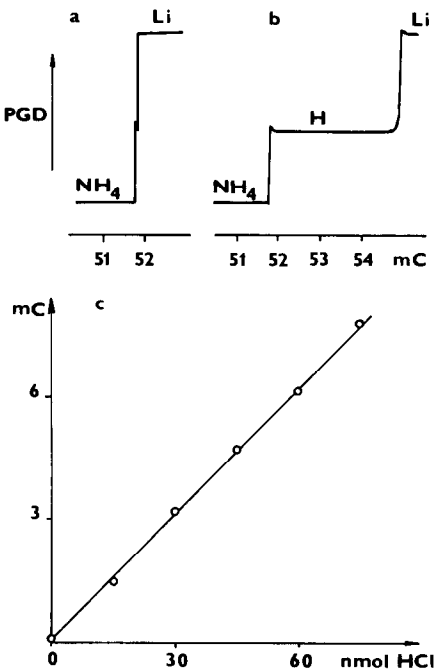


Fig. 14. (a, b) Isotachopherograms in the system with 0.005 M ammonium formate + 0.0024 M formic acid as the leading electrolyte and 0.0036 M lithium formate + 0.0024 M formic acid as the terminator. (a) Blank run; (b) 2 μ l of 0.015 M HCl sampled (zone H). Shimadzu IP-3A instrument, $I = 100 \mu$ A (separation), 25 μ A (detection). (c) Calibration line for the above system and 0.015 M HCl as the sample in the range 0–5 μ l.

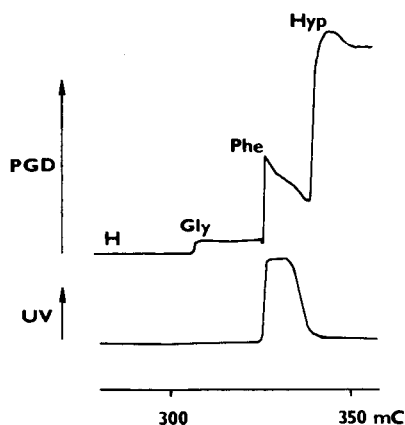


Fig. 15. Isotachopherogram of phenylalanine (Phe) and glycine (Gly) in the system with 0.05 *M* HCl as the leading electrolyte and 0.02 *M* hydroxyproline (Hyp) + 0.06 *M* HCl as the terminator. Shimadzu IP-3A instrument; the sample (0.01 *M* glycine, 0.02 *M* phenylalanine, 0.04 *M* HCl) was introduced electrically (20 μ A for 1 min) into the detection capillary; $I = 100 \mu$ A.

balance of the velocities of the $\text{H}^+ \rightarrow \text{NH}_4^+$ and $\text{Li}^+ \rightarrow \text{H}^+$ boundaries is nearly perfect. Fig. 14b shows the record of the analysis of 2 μ l of 0.015 *M* HCl; the calibration line in Fig. 14c confirms the possibility of isotachophoretic determination of H^+ .

An important practical aspect of the theory presented here is the possibility of constructing correct isotachophoretic systems working at very low pH (see last part of the section *Correct migration of sample zones* and Fig. 8). This opens the way to the protonation and analysis of even very weak bases. Fig. 15 shows the results for glycine ($\text{p}K_{\text{XH}} = 2.35$) and phenylalanine ($\text{p}K_{\text{XH}} = 2.58$) in the system with 0.05 *M* HCl as the leading electrolyte and 0.02 *M* hydroxyproline + 0.06 *M* HCl as the terminating solution. In accordance with theory (*cf.*, Fig. 8), correct migration of both sample zones is seen.

CONCLUSIONS

The fundamental feature of a correct isotachophoretic system is that the migration of H^+ (in cationic isotachopheresis) and/or OH^- (in anionic isotachopheresis) is fully controlled. This is achieved, from the classical point of view, by the selection of a suitable leading electrolyte with a suitable buffering counter ionic system which controls fully the effective mobility of H^+ and/or OH^- so that it forms the real or potential terminator of the whole system. In such a system the parameters of the terminating zone are also unambiguously controlled by the leading zone and the parameters of the terminating electrolyte itself (especially its pH) do not show any influence on the behaviour (parameters) of the sample zones.

It has been shown in this paper both theoretically and experimentally that inverse systems where H^+ (in cationic isotachopheresis) and OH^- (in anionic isotachopheresis) serve as the leading ions may show, under certain conditions, correct behaviour. In cationic isotachopheresis, *e.g.*, H^+ serves as the leading cation and the terminator is formed by a suitable cation (*e.g.*, organic base, B) the zone of which

contains also some amount of H^+ . Thus, the velocity of the $T \rightarrow L$ boundary is a function of the composition of both the leading and terminating zones. As H^+ forms here its individual zone, the concepts of the H^+ constituent and of its effective mobility may be introduced; the actual value of the latter depends on the parameters of both the leading and terminating zones (*cf.*, eqn. 12).

By calculation of the effective mobility of the leading H^+ constituent and of the terminating constituent B, and also the pH values of their zones, one may decide for a given substance X whether it provides a correct isotachophoretic zone in the $B \rightarrow H$ system.

From a more detailed treatment of the theory of cationic systems with a weak acid as the counter ionic substance, it follows that from each combination of two arbitrary zones of "acid" (constituent H^+) and "base + acid" (constituent B), one can construct a correct isotachophoretic system. There remains only the question of which is the correct order of these two zones (*i.e.*, B as the terminator and H^+ as the leader or *vice versa*). This order can be established by the calculation of the parameters of both zones (as indicated under Theoretical), especially of the effective mobilities $\bar{u}_{H,H}$ and $\bar{u}_{B,H}$, and by their comparison according to conditions 14 and 15. The concentration level of the electrolyte system here plays an important role. The detailed study of both classical and inverse systems has revealed that a combined system may exist where H^+ can migrate and be determined as an individual zone constituent, *e.g.*, between the acidic zones of ammonium formate (leader) and lithium formate (terminator).

From the quantitative point of view, the systems in question show prolongation of the sample zones. This prolongation is the more pronounced the more acidic the terminator is. To achieve a good reproducibility of the analyses, the composition (pH) of the terminating solution should remain constant. This could be ensured by using fresh terminating electrolyte in the terminating electrode chamber after each analysis. To avoid the disturbing electrode reactions producing H^+ at the anode it is advisable to use acetate or formate instead of chloride for the preparation of the terminating solution.

The practical aspects of acidic systems with H^+ as the leading constituent consist not only in the possibility of determining very mobile ions (such as K^+ or NH_4^+) but, especially, in the potential to determine also very weak bases with pK_{XH} even lower than 2.

SYMBOLS

$c_{i,j}$	concentration of ion i in zone j ;
$\bar{c}_{i,j}$	concentration of constituent i in zone j ;
F	Faraday constant;
k_i	calibration constant of constituent i ;
K_{HA}	dissociation constant of acid HA;
K_{BH}	dissociation constant of protonated base B;
L	leading zone, leading constituent;
N_i	amount of constituent i ;
Q_i	zone passage charge of constituent i ;
T	terminating zone, terminating constituent;
u_i	electrophoretic mobility of ion i ;

$\bar{u}_{i,j}$	effective electrophoretic mobility of constituent i in zone j ;
$W_{B \rightarrow H}$	rate of movement of boundary $B \rightarrow H$ (volume per charge);
κ_j	conductivity of zone j ;
::	stationary boundary;
\rightarrow	moving boundary with indicated direction of movement.

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