Journal of Chromatography, 217 (1981) 209–224
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,851

MIGRATION BEHAVIOUR OF THE HYDROGEN ION AND ITS ROLE IN ISOTACHOPHORESIS OF CATIONS

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SUMMARY

A theoretical and experimental study has been made of the behaviour of hydrogen ions under the conditions of cationic isotachophoresis. It is shown that, for successful cationic isotachophoresis, the electrolyte conditions should be such that H^+ migrates in an enforced isotachophoretic manner as terminator and its front boundary shows self-sharpening.

A mathematical description of the migration of H^+ as the terminating zone is elaborated, giving the criteria decisive for the character of the terminating zone front boundary depending on the composition of the leading electrolyte and on the properties of the cationic substances separated (strong and weak bases). It has been found theoretically that, for a given set of cations to be separated and counter anion in the leading electrolyte, the concentration of the leading electrolyte must be higher than a certain critical value to obtain stable isotachophoretic migration.

Model experiments with different counter-anion systems (acetic acid–acetate and formic acid–formate) at various concentrations have given good agreement with the theory. Illustrative examples of cationic isotachophoresis of some amino acids and dipeptides are described.

The model described enables both the prediction of migration behaviour of cationic substances and the selection of suitable electrolyte systems in order to obtain stable isotachophoretic migration of these substances.

INTRODUCTION

When choosing a system of electrolytes for isotachophoretic separation of cations, attention must be paid not only to the leading and terminating cations but also to the suppression of hydrolysis of inorganic cations and to the provision of sufficient protonation of the organic bases to be separated. For these reasons, electrolyte systems of weak to medium acidity are generally used to separate cations by electrophoresis[1–19]. In isotachophoresis, free acids have sometimes been directly used as leading or terminating electrolyte, *i.e.*, hydrogen ion serving as the leading[1–6,12,20] or terminating[3,6–8,11] cation.

However, the application of such acidic electrolyte systems is not without problems. It was shown in both theoretical and experimental work[6] that in systems where the terminating electrolyte contains a large amount of H^+, or where hydrogen ions are produced at the terminating electrode by an electrode reaction, a strong flow of hydrogen ions occurs through the isotachophoretic system. In non-buffered systems (where an anion of a strong acid serves as counter ion), hydrogen ions from the terminating electrolyte pass freely through all zones and can modify substantially (or even disturb) the migration of zones of the cations to be separated. In cases where the contribution of H^+ to the conductivity of the migrating zones is not negligible, the H^+ serves as a background electrolyte and a moving boundary process occurs instead of isotachophoretic migration of zones.

In buffered systems, migration of H^+ through the zones of separated substances is affected by the presence of the buffering counter ion, *i.e.*, weak acid anion, migrating in the opposite direction. Migrating hydrogen ions recombine with this anion to an extent dependent on the dissociation equilibrium of the weak acid. The velocity of the migrating front of H^+ through the system is thus decreased (in effect, the effective mobility of H^+ is decreased).

It was demonstrated clearly in an experimental study[7] that the effective mobility of H^+ can be changed over a wide range and controlled by empirical selection of buffering counter ions. Under suitable conditions, hydrogen ion can serve as a terminating cation; its zone contains free weak acid corresponding to the anion applied. However, even in buffered systems, the above interfering effects caused by migration of H^+ may occur either over the whole system or in only some of its zones[6,7].

Some authors[11,16] have mentioned the necessity of choosing an appropriate concentration and pH of the terminating electrolyte, not too different from the corresponding values of the leading electrolyte; otherwise the results obtained may be difficult to interpret. If the pH of the terminator is too low, elution phenomena caused by migration of H^+ will occur and irreproducible results can be expected (*cf.*, refs. 6 and 20).

It is obvious that the migration behaviour of hydrogen ions is of fundamental significance for isotachophoresis of cations. The presence of H^+ in sufficient concentrations in the isotachophoretic zones is necessary for a successful separation of cations since the H^+ is the only direct source of positive charge for the bases. At the same time, however, the presence of H^+ may result in deterioration of the separation since it migrates in the same direction as the zones separated and its mobility in water is higher by an order of magnitude than that of any other cation. It is obvious that cations should be separated in acidic media, and that the migrating behaviour of H^+ in various zones must be properly controlled. Adequate attention has not yet been devoted to this problem.

The aim of the present work is to define theoretically, on the basis of a physicochemical model, conditions for isotachophoretic migration of cations with controlled migration behaviour of H^+ and to experimentally verify the theory. Further, we show, by using model examples, how the theoretical relationships derived can be used for selecting suitable electrolyte systems for the isotachophoretic separation of cations.

THEORETICAL

Based upon the knowledge summarized in the Introduction, the demands on the migration behaviour of hydrogen ions, or on an electrolyte system for cationic isotachophoresis, can be expressed as follows.

The concentrations of H^+ in the zones of separated compounds must be sufficient to prevent hydrolysis of inorganic cations and to protonate the organic bases. The counter anion used must buffer the concentrations of H^+ in all zones so that hydrogen ions from the terminator may no penetrate significantly the zone boundaries.

The first requirement can be simply and effectively satisfied by using a weak acid as the terminating electrolyte and hydrogen ion as terminating cation, as recommended by Vestermark[7]. The second requirement is equivalent to conditions for the steady-state moving boundary, which can be expressed in terms of a self-sharpening effect of the boundary as shown by Alberty[21] and Nichol[22]. In our case, this condition will be related to the boundary between the terminator and the preceding zone.

The aim of this section is to derive the relationship between the conditions for the steady-state moving boundary and the parameters of the applied system (concentration, dissociation constants). To denote the moving boundary systems, the notation introduced by Longsworth[23] (see also ref. 24) is adopted, in which a moving boundary is denoted by an arrow and a concentration boundary by a double colon. Phases or zones are denoted by Greek letters, α, β, etc., with λ and τ reserved for the leading and terminating electrolytes. The corresponding moving boundary is thus denoted by a pair of Greek letters denoting the neighbouring zones, *e.g.*, $\lambda\vartheta$ boundary.

System 1

$$H^+, A^-, HA\ (\tau) :: H^+, A^-, HA\ (\vartheta) \rightarrow L^+, A^-\ (\lambda)$$

This is the most simple isotachophoretic system and is illustrated schematically in Fig. 1a. It is created by zone λ of the leading solution, containing cation L^+ (strong electrolyte) and anion A^- of weak acid HA, zone τ of the terminating electrolyte, containing a solution of acid HA, and boundary $\lambda\tau$. After the start of a constant d.c. current, cation L^+ migrates as the leading cation. Cation H^+ is in dissociation equilibrium with acid HA in zone τ. Showing higher ionic mobility, $u_H > u_L$, it tends to pass through boundary $\lambda\tau$ and penetrate to zone λ. At this boundary, however, the

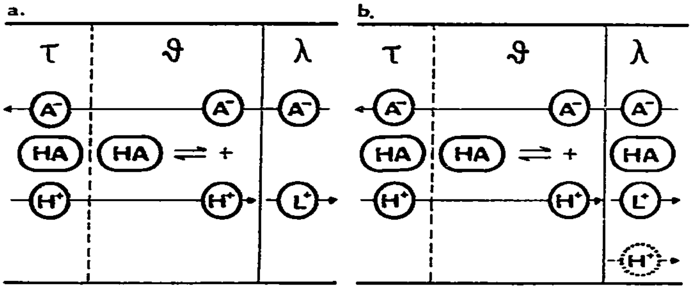

Fig. 1. Scheme for systems 1 (a) and 2 (b).

H^+ meets the counter ion A^- and reacts with it to reform acid HA, thus re-establishing the dissociation equilibrium characterized by the constant:

$$K_{HA} = [\mathrm{H}]\,[\mathrm{A}]/[\mathrm{HA}] \tag{1}$$

As long as certain conditions are maintained (see below) the above reaction causes the hydrogen ion to migrate in an enforced isotachophoretic[6] way behind the zone of ion L^+ of lower mobility and creates zone ϑ of acid HA (with a concentration adjusted to that of the leading electrolyte). Zone ϑ is separated from the initial zone τ by the concentration boundary $\vartheta\tau$.

As stated above, the condition of isotachophoretic migration of H^+ in system 1 is that $\lambda\vartheta$ is a steady-state moving boundary, *i.e.*, it has self-sharpening capability (*cf.*, ref. 21). This means that H^+ does not pass through boundary $\lambda\vartheta$ in the direction of the migration and the velocity of ion L^+ in zone ϑ is greater than in zone λ. Starting from the initial situation, see Fig. 1a ($\lambda\vartheta = \vartheta\tau$), then after a time d$t$

$$\mathrm{d}n_{\mathrm{H}} = [\mathrm{H}]_\tau u_{\mathrm{H}} E_\tau S \mathrm{d}t \tag{2}$$

moles of H^+ will pass through boundary $\vartheta\tau$, where $[\mathrm{H}]_\tau$ and E_τ are the concentration of H^+ and the potential gradient in zone τ, respectively, and S is the cross-section of the isotachophoretic column. During the same time

$$\mathrm{d}n_A = [\mathrm{A}]_\lambda (u_{\mathrm{A}} + u_L)\, E_\lambda S \mathrm{d}t \tag{3}$$

moles of counter ion A^- will pass through boundary $\lambda\vartheta$, and boundaries $\lambda\vartheta$ and $\vartheta\tau$ will designate the volume $\mathrm{d}V = u_L E_\lambda S \mathrm{d}t$ in the column after time dt. For the total (analytical) concentration of H^+ in zone ϑ, $c_{H,\vartheta} = [\mathrm{H}]_\vartheta + [\mathrm{HA}]_\vartheta$, the following equation holds:

$$c_{H,\vartheta} = \mathrm{d}n_{\mathrm{H}}/\mathrm{d}V = [\mathrm{H}]_\tau\, u_{\mathrm{H}} E_\tau / u_{\mathrm{L}} E_\lambda \tag{4}$$

By considering electroneutrality (the contribution of H^+ in zone λ being neglected), *i.e.*, $[\mathrm{H}]_\tau = [\mathrm{A}]_\tau$, $[\mathrm{H}]_\vartheta = [\mathrm{A}]_\vartheta$ and $[\mathrm{L}]_\lambda = [\mathrm{A}]_\lambda$, and the condition of constant electric current density.

$$[\mathrm{H}]_\tau\, E_\tau (u_{\mathrm{A}} + u_{\mathrm{H}}) = [\mathrm{H}]_\vartheta\, E_\vartheta (u_{\mathrm{A}} + u_{\mathrm{H}}) = [\mathrm{L}]_\lambda\, E_\lambda (u_{\mathrm{L}} + u_{\mathrm{A}}) \tag{5}$$

we obtain for the adjusted concentration of acid HA in zone ϑ, $c_{\mathrm{A},\lambda} = c_{\mathrm{H},\vartheta}$:

$$c_{\mathrm{H},\lambda} = [\mathrm{L}]_\lambda \cdot \frac{u_{\mathrm{H}}}{u_{\mathrm{A}} + u_{\mathrm{H}}} \cdot \frac{u_{\mathrm{A}} + u_{\mathrm{L}}}{u_{\mathrm{L}}} \tag{6}$$

The condition of a sharp boundary can be expressed simply in the form (see ref. 21)

$$E_\vartheta \bar{u}_{L,\vartheta} > E_\lambda \bar{u}_{L,\lambda} \tag{7}$$

where $\bar{u}_L$ is the effective mobility of ion L^+. Supposing ion L^+ is a cation of a strong base (*e.g.*, alkali metal cation), then $\bar{u}_{L,\vartheta} = \bar{u}_{L,\lambda} = u_L$ and expression 7 simplifies to:

$$E_\vartheta > E_\lambda \tag{8}$$

Combining relationships 8 and 5 we obtain:

$$[\mathrm{L}]_\lambda \cdot \frac{u_A + u_L}{u_A + u_H} > [\mathrm{H}]_\vartheta \tag{9}$$

If expression 9 is multiplied by K_{HA} and the result added to the square of 9, the following relationship is obtained

$$[\mathrm{L}]_\lambda^2 \cdot \left(\frac{u_A + u_L}{u_A + u_H}\right)^2 + [\mathrm{L}]_\lambda\, K_{HA} \cdot \frac{u_A + u_L}{u_A + u_H} > K_{HA} c_{H,\vartheta} \tag{10}$$

since, with respect to eqn. 1, it holds that $[\mathrm{H}]_\vartheta^2 + K_{HA}[H]_\vartheta = K_{HA} c_{H,\vartheta}$. Finally by combining 10 with eqn. 6

$$[\mathrm{L}]_\lambda > K_{HA} \cdot \frac{u_H - u_L}{u_L} \cdot \frac{u_A + u_H}{u_A + u_L} \tag{11}$$

which defines the range of concentrations of the leading cation for which the moving boundary $\lambda\vartheta$ remains sharp.

If relationship 11 holds true, from a comparison of eqns. 2 and 3 (with the use of eqn. 5) the following expression is obtained

$$\mathrm{d}n_H/\mathrm{d}t < \mathrm{d}n_A/\mathrm{d}t \tag{12}$$

which means that the flow of anion A^- through boundary $\lambda\vartheta$ is always larger than that of H^+ through boundary $\vartheta\tau$. Thus, for a sufficiently fast reaction $H^+ + A^- \rightarrow HA$, hydrogen ion cannot pass through boundary $\lambda\vartheta$ and disturb the migration of zones.

Expression 11 then represents for system 1 the limiting condition for enforced isotachophoretic migration of hydrogen ion as terminator. It gives an unambiguous dependence of the behaviour of the system on the concentration of the leading electrolyte. At a critical value, $[\mathrm{L}]_{\lambda,\mathrm{crit}}$, expression 11 becomes an equation, and, for $[\mathrm{L}]_\lambda < [\mathrm{L}]_{\lambda,\mathrm{crit}}$, the system does not create a sharp boundary. Assuming constant mobilities u_H, u_L and u_A, the dependence $[\mathrm{L}]_{\lambda,\mathrm{crit}}$ *vs.* K_{HA} yields a straight line (in logarithmic coordinates, see Fig. 2, line 1). In order to secure isotachophoretic migration, it is necessary in practice that, for a given K_{HA}, $[\mathrm{L}]_\lambda > [\mathrm{L}]_{\lambda,\mathrm{crit}}$, *i.e.*, $[\mathrm{L}]_\lambda$ lies above straight line 1 since the area above this line represents the range of validity of expression 11.

System 2

$$H^+, A^-, HA(\tau) :: H^+, A^-, HA(\vartheta) \rightarrow L^+, A^-, HA(\lambda)$$

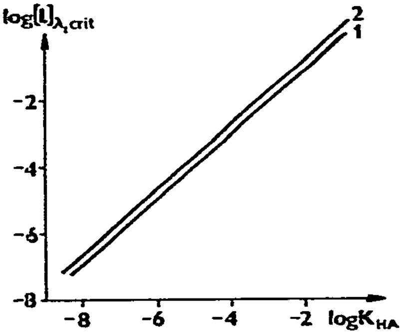

Fig. 2. Calculated dependence of log $[L]_{\lambda,crit}$ *vs.* log K_{HA} for systems 1 (line 1) and 2 (line 2), $[L]_\lambda/[HA]_\lambda = 1$. For the calculation, the following values of mobilities (25°C)[29] were used: $u_H = 362.6 \times 10^{-5}$ cm²/V·sec; $u_L = u_K = 76.18 \times 10^{-5}$ cm²/V·sec; u_A = constant $\approx 40 \times 10^{-5}$ cm²/V·sec.

In systems 2 and 2a (see Fig. 1b), ions L^+, A^- and free acid HA at a concentration $[HA]_\lambda$ are present in the leading electrolyte. Let us consider first the case when the presence of H^+ in zone λ can be neglected. Under this assumption, eqns. 2 and 3 hold for system 2, too; however, the adjusted concentration of acid HA in zone ϑ, $c_{H,\vartheta}$ (*cf.*, eqn. 6), is increased by the value $[HA]_\lambda = c_{H,\lambda}$:

$$c_{H,\vartheta} = [L]_\lambda \cdot \frac{u_H}{u_L} \cdot \frac{u_A + u_L}{u_A + u_H} + [HA]_\lambda \qquad (13)$$

Also in system 2 the condition of a sharp boundary, expressed as $E_\vartheta > E_\lambda$ (8), must be fulfilled. In further derivations, however, it is necessary to substitute in relationship 10 for $c_{H,\vartheta}$ from eqn. 13. After arrangement, we obtain

$$[L]_\lambda > K_{HA} \left[\frac{u_H - u_L}{u_L} \cdot \frac{u_A + u_H}{u_A + u_L} + \frac{[HA]_\lambda}{[L]_\lambda} \cdot \left(\frac{u_A + u_H}{u_A + u_L} \right)^2 \right] \qquad (14)$$

which expresses the condition of enforced isotachophoretic migration of H^+ in system 2. In comparison with relationship 11, an additional term appears, including the ratio of the acid and the salt concentrations in zone λ, which results in an increase of the value of $[L]_{\lambda,crit}$. The dependence of log $[L]_{\lambda,crit}$ *vs.* log K_{HA} is again a straight line (*cf.*, Fig. 2, straight line 2, for $[HA]_\lambda/[L]_\lambda = 1$, *i.e.*, for an equimolar mixture of the acid and its salt), but lying above the line corresponding to system 1.

System 2a

$$H^+, A^-, HA(\tau) :: H^+, A^-, HA(\vartheta) \rightarrow L^+, H^+, A^-, HA(\lambda)$$

Calculations for real systems (*cf.*, Results and Discussion) show that in general the presence of H^+ in zone λ cannot be neglected. Let us consider such a system. This system deviates from rigorous isotachophoresis; however, from a practical point of view the deviation is not substantial as long as the condition of a sharp boundary is satisfied.

The condition of constant current density in zones τ, λ can be expressed as follows

$$E_\tau[\mathrm{H}]_\tau(u_\mathrm{A} + u_\mathrm{H}) = E_\lambda \{[\mathrm{L}]_\lambda(u_\mathrm{A} + u_\mathrm{L}) + [\mathrm{H}]_\lambda(u_\mathrm{A} + u_\mathrm{H})\} \tag{15}$$

since $[\mathrm{A}]_\lambda = [\mathrm{L}]_\lambda + [\mathrm{H}]_\lambda$. For the adjusted concentration of acid HA, $c_{\mathrm{H},\vartheta}$, we obtain (*cf.*, eqns. 4 and 15)

$$\begin{aligned} c_{\mathrm{H},\vartheta} &= \frac{[\mathrm{H}]_\tau\, u_\mathrm{H} E_\tau}{u_\mathrm{L} E_\lambda} + [\mathrm{HA}]_\lambda \\ &= \frac{u_\mathrm{H}}{u_\mathrm{L}} \cdot \frac{(u_\mathrm{A} + u_\mathrm{L})\,[\mathrm{L}]_\lambda + (u_\mathrm{A} + u_\mathrm{H})\,[\mathrm{H}]_\lambda}{u_\mathrm{A} + u_\mathrm{H}} + [\mathrm{HA}]_\lambda \end{aligned} \tag{16}$$

and, since $c_{\mathrm{H},\lambda} = [\mathrm{H}]_\lambda + [\mathrm{HA}]_\lambda$,

$$c_{\mathrm{H},\vartheta} = [\mathrm{L}]_\lambda \cdot \frac{u_\mathrm{H}}{u_\mathrm{L}} \cdot \frac{u_\mathrm{A} + u_\mathrm{L}}{u_\mathrm{A} + u_\mathrm{H}} + [\mathrm{H}]_\lambda \cdot \frac{u_\mathrm{H} - u_\mathrm{L}}{u_\mathrm{L}} + c_{\mathrm{H},\lambda} \tag{17}$$

By combining relationships 8 and 15 (*cf.*, eqn. 5) it follows for the condition of a sharp boundary that:

$$[\mathrm{L}]_\lambda \cdot \frac{u_\mathrm{A} + u_\mathrm{L}}{u_\mathrm{A} + u_\mathrm{H}} > [\mathrm{H}]_\vartheta - [\mathrm{H}]_\lambda \tag{18}$$

By a procedure analogous to that for the derivation of relationship 10, substituting for $c_{\mathrm{H},\vartheta}$ from eqn. 17 and recombining with eqn. 1 in the form

$$[\mathrm{H}]_\lambda^2 + [\mathrm{H}]_\lambda([\mathrm{L}]_\lambda + K_\mathrm{HA}) = c_{\mathrm{H},\lambda} K_\mathrm{HA} \tag{19}$$

we obtain the condition for self sharpening of the boundary:

$$\begin{aligned} [\mathrm{L}]_\lambda > {} & K_\mathrm{HA} \cdot \frac{u_\mathrm{H} - u_\mathrm{L}}{u_\mathrm{L}} \cdot \frac{u_\mathrm{A} + u_\mathrm{H}}{u_\mathrm{A} + u_\mathrm{L}} + \\ & [\mathrm{H}]_\lambda \cdot \frac{u_\mathrm{A} + u_\mathrm{H}}{u_\mathrm{A} + u_\mathrm{L}} \cdot \left[\frac{u_\mathrm{A} + u_\mathrm{H}}{u_\mathrm{A} + u_\mathrm{L}} \cdot \left(\frac{K_\mathrm{HA}}{[\mathrm{L}]_\lambda} \cdot \frac{u_\mathrm{H} - u_\mathrm{L}}{u_\mathrm{L}} + 1 \right) - 2 \right] \end{aligned} \tag{20}$$

Instead of substituting for $[\mathrm{H}]_\lambda$ from eqn. 19 and expressing $[\mathrm{L}]_\lambda$ explicitly, relation 20 may be compared with the conditions for systems 1 and 2 (relationships 11 and 14). It can be shown that

$$[\mathrm{L}]_{\lambda,\mathrm{crit}} \text{ (system 2)} > [\mathrm{L}]_{\lambda,\mathrm{crit}} \text{ (system 2a)} > [\mathrm{L}]_{\lambda,\mathrm{crit}} \text{ (system 1)} \tag{21}$$

i.e., the critical concentration of system 2a lies between the values $[\mathrm{L}]_{\lambda,\mathrm{crit}}$ for systems 1 and 2. From a practical point of view, this means that (*cf.*, Fig. 2) if the concentration of the leading electrolyte is selected so as to satisfy condition 14, condition 20 is also satisfied.

System 3

$$H^+, A^-, HA(\tau) :: H^+, A^-, HA(\vartheta) \rightarrow BH^+, A^-, (HA, B)\ (\alpha) \rightarrow L^+, A^-\ (\lambda)$$

This system is illustrated in Fig. 3a and represents migration of zone α of a cation under analysis. In the general case, the cation can be a weak base, BH^+, with acid dissociation constant K_{BH}. The investigation of this system will be, again, limited to the determination of the necessary conditions for the boundary $\alpha\vartheta$ to be permanently sharp, *i.e.*, for isotachophoretic migration of zone α. The detailed composition of zone α is irrelevant for this purpose. Supposing that ions A^- and H^+ do not accumulate in zone α, then, analogously to system 1, eqn. 6 holds for the adjusted concentration of acid HA in zone ϑ.

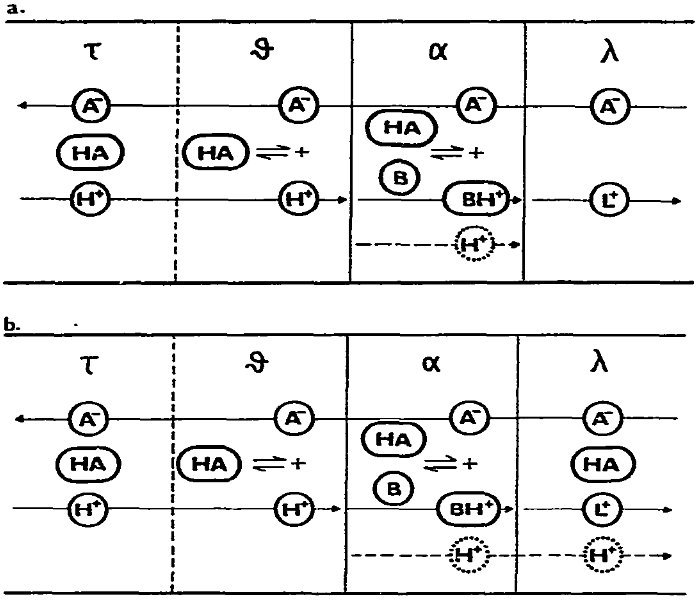

Fig. 3. Scheme for systems 3 (a) and 4 (b).

The condition for a sharp boundary $\alpha\vartheta$ in system 3 is of the form:

$$E_\vartheta \bar{u}_{BH,\vartheta} > E_\alpha \bar{u}_{BH,\alpha} = E_\lambda u_L \tag{22}$$

Applying eqn. 1 for the protonated base B, $K_{BH} = [H]\,[B]/[BH]$, the effective mobility of base B which penetrates into zone ϑ, $\bar{u}_{BH,\vartheta}$, can be expressed as:

$$\bar{u}_{BH,\vartheta} = u_{BH}\cdot\frac{[BH]_\vartheta}{[BH]_\vartheta + [B]_\vartheta} = u_{BH}\cdot\frac{[H]_\vartheta}{K_{BH} + [H]_\vartheta} \tag{23}$$

By combining eqns. 23 and 5 with condition 22, we obtain:

$$[L]_\lambda \cdot \frac{u_{BH}}{u_L} \cdot \frac{u_A + u_L}{u_A + u_H} > K_{BH} + [H]_\vartheta \tag{24}$$

Rearrangement as in the preceding cases (substitution from eqn. 6) yields the relationship:

$$[L]_\lambda^2 - [L]_\lambda \left(K_{HA} \cdot \frac{u_H - u_{BH}}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \cdot \frac{u_L}{u_{BH}} + \right.$$

$$\left. 2K_{BH} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \right) + (K_{BH}^2 - K_{HA}K_{BH}) \left(\frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \right)^2 > 0 \tag{25}$$

Since expression 25 is rather complicated, it will be discussed here from the point of view of two extreme cases.

(a) If $K_{BH} \ll K_{HA}$, expression 25 simplifies to:

$$[L]_\lambda > K_{HA} \cdot \frac{u_H - u_{BH}}{u_{BH}} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \tag{26}$$

This relation defines the condition for isotachophoretic migration of the zone of strong base BH^+, when the concentration of the leading ion, L^-, is $[L]_\lambda$, and hydrogen ion serves as the terminating cation, forming the weak acid HA as a terminating zone. The comparison of condition 26 with relationship 11 shows that they are identical for $BH^+ = L^+$. Condition 26 is also valid for the migration of a metal cation M^+ if u_{BH} is substituted with u_M. By plotting $[L]_{\lambda,\text{crit}}$ *vs.* K_{HA} a straight line is obtained (in logarithmic coordinates, Fig. 4, line 1).

(b) If $K_{BH} \gg K_{HA}$, expression 25 has the form:

$$[L]_\lambda > K_{BH} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \tag{27}$$

This corresponds to the migration of very weak bases (the protonated form BH^+ is a stronger acid than HA), and the critical concentration, $[L]_{\lambda,\text{crit}}$, is independent of the dissociation constant, K_{HA}. For a given system, $[L]_{\lambda,\text{crit}}$ is constant (*viz.*, the horizontal intercepts of curves 2–4 in Fig. 4). However, in this case, protonation of the base is minimal in both zones α and ϑ (if it were present there) and even though condition 27 for a sharp boundary is satisfied, a successful isotachophoretic run of base B is very difficult to achieve since the separation capacity of the column[25] approaches zero for the couple hydrogen ion–base B.

The practical significance of Fig. 4 is entirely analogous to that of Fig. 2: isotachophoretic migration of zone α is secured for values of $[L]_\lambda$ lying above the curve corresponding to the required value of K_{BH}. At the same time, bearing in mind the conclusions drawn in the preceding paragraph, it is not advisable to operate with a counter ion for which $pK_{HA} > pK_{BH} + 1$.

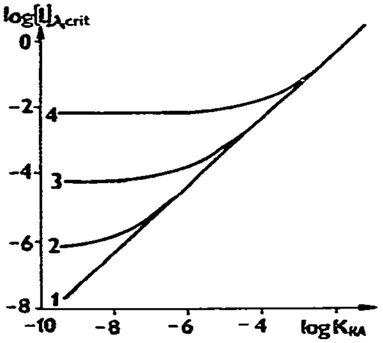

Fig. 4. Calculated dependence of log $[L]_{\lambda,\text{crit}}$ *vs.* log K_{HA} in system 3 for various values of K_{BH}: 1, $K_{BH} \ll K_{HA}$; 2, $K_{BH} = 10^{-7}$; 3, $K_{BH} = 10^{-5}$; 4, $K_{BH} = 10^{-3}$ mol·l^{-1}. For the calculation the data given in Fig. 2 were used, and $u_{BH} \approx 40 \times 10^{-5}$ cm^2/V sec.

System 4

$$\mathrm{H^+, A^-, HA}(\tau) :: \mathrm{H^+, A^-, HA}(\vartheta) \rightarrow \mathrm{BH^+, A^-, HA\,(B)}\,(\alpha) \rightarrow \mathrm{L^+, A^-, HA}\,(\lambda)$$

This represents the migration of zone α of a base BH^+ behind the leading zone λ containing a mixture of free acid HA and its salt L^+A^- (*cf.*, system 2). For this system, a most of the conclusions derived for systems 2 and 3 are valid, *i.e.*, eqn. 13 and conditions 22 and 24. By combining relationships 13 and 24, the condition for a sharp boundary $\alpha\vartheta$ is:

$$[L]_\lambda^2 - [L]_\lambda \left(K_{HA} \cdot \frac{u_H - u_{BH}}{u_{BH}} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} + 2K_{BH} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \right) +$$

$$(K_{BH}^2 - K_{HA}K_{BH} - K_{HA}[HA]_\lambda) \left(\frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \right)^2 > 0 \qquad (28)$$

By comparing expressions 28 and 25, it is seen that an additional term is present in 28 involving the concentration of free acid in zone λ, $[HA]_\lambda$. Also in this case, simplifications can be made, yielding the forms:

(a) for $K_{BH} \ll K_{HA}$

$$[L]_\lambda > K_{HA} \left[\frac{u_H - u_{BH}}{u_{BH}} \cdot \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} + \frac{[HA]_\lambda}{[L]_\lambda} \left(\frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \right)^2 \right] \qquad (29)$$

(b) for $K_{BH} \gg K_{HA}$

$$[L]_\lambda > (K_{BH} + \sqrt{K_{HA}\,[HA]_\lambda})\, \frac{u_L}{u_{BH}} \cdot \frac{u_A + u_H}{u_A + u_L} \qquad (30)$$

The character of the relationships derived is best seen from a comparison of relationship 29 with 14 and 26, and of relationship 30 with 27. Thus, it is possible to estimate the course of the dependence of log $[L]_{\lambda,crit}$ *vs.* log K_{HA} for system 4. The straight line section of this dependence passes above straight line 1 in Fig. 4 (parallel with it); the curved section follows the analogous curve of system 3 (for given K_{BH}) and coincides with it for $K_{HA} \rightarrow 0$.

In analogy with the conclusions drawn for systems 2 and 2a, in practice it is sufficient to apply relationship 28 even in cases where the presence of H^+ in zone λ cannot be neglected.

EXPERIMENTAL

The isotachophoretic column consisted of a block of Perspex containing electrode chambers, injection port, control valves and sensing electrodes of the potential gradient detector. The separations were performed in a capillary of rectangular cross-section (1.0 × 0.2 mm), 200 mm long. created by a groove in the organic glass block and covered with PTFE foil pressed on the block with a thermostated metal plate. A high-voltage constant-current supply controllable up to 400 μA at a maximum of 16 kV was used. Potential gradients in zones were detected by a device consisting of a voltmeter with a high input resistance and recorded by means of a Servogor RE 571 line recorder (Goertz, Vienna, Austria). A detailed description of the isotachophoretic column, power supply and detection system can be found elsewhere[26,27].

The potential gradient values measured at different driving currents were extrapolated to the thermostatting temperature (22°C) by a procedure analogous to ref. 28.

Formic acid, acetic acid and their alkali metal salts were of analytical grade (Lachema, Brno, Czechoslovakia). The other chemicals used, histidine (Reanal, Budapest, Hungary). β-alanine (Loba-Chemie, Vienna, Austria), aniline, glycine, Tris and chloroacetic acid (Lachema) were of reagent grade.

RESULTS AND DISCUSSION

To verify the theoretical description of the enforced isotachophoretic migration of hydrogen ion as terminator in systems 1, 2 and 2a, the ratio of E_λ/E_3, available both theoretically and experimentally, was utilized, *cf.*, relationships 7 and 8. For a given weak acid HA, this ratio is dependent on the concentration of the leading solution. For $[L]_\lambda > [L]_{\lambda,crit}$, $E_\lambda/E_3 < 1$ and the boundary $H^+ \rightarrow L^+$ is sharp. For $[L]_\lambda \leqslant [L]_{\lambda,crit}$, $E_\lambda/E_3 \geqslant 1$ and the condition of a sharp boundary does not hold.

The electrolyte system acetic acid :: potassium acetate, which has already proved to be very useful[11,13,14] in cationic isotachophoresis, served as a model of system 1. Table I lists the values of E_λ/E_3 calculated and measured for various concentrations of the leading electrolyte. Theoretical values were calculated by using eqns. 5, 6 and 1. The required mobility values were obtained by extrapolating tabulated[29] values to the working temperature (22°C): $u_H = 344.7 \times 10^{-5}$ cm^2/V·sec; $u_K = 70.87 \times 10^{-5}$ cm^2/V·sec; $u_{Ac} = 39.33 \times 10^{-5}$ cm^2/V·sec. The dissociation constant of acetic acid ($pK_{HA} = 4.7561$) (see ref. 30) was corrected for the ionic strength of the solutions. The agreement between the theoretical and experimental values was satisfactory.

Analogous measurements for system 2 were performed in the arrangement acetic acid :: potassium acetate + acetic acid (Table I). Theoretical values of E_λ/E_3

TABLE I

CALCULATED AND MEASURED VALUES OF E_λ/E_3 FOR MODEL ACETATE SYSTEMS

Zone composition	$(E_\lambda/E_3)_{calc.}$	*System*	$(E_\lambda/E_3)_{exptl.}$
0.005 *M* CH_3COOK	0.243	1	0.244
0.01 *M* CH_3COOK	0.174	1	0.161
0.02 *M* CH_3COOK	0.124	1	0.128
0.05 *M* CH_3COOK	0.079	1	0.090
0.01 *M* CH_3COOK +	0.229	2	0.230
+ 0.01 *M* CH_3COOH	0.228	2a	
0.0005 *M* CH_3COOK +	0.962	2	*
+ 0.0005 *M* CH_3COOH	0.885	2a	

* The high-voltage and d.c. current requirements were outside the operating regime of the power supply used.

were calculated by using eqns. 5, 13 and 1 (system 2) or 15, 17, 19 and 1 (system 2a). Experimental results for 0.01 *M* solution of potassium acetate and 0.01 *M* acetic acid are in good agreement with the theoretical values. Evidently, theoretical values of E_λ/E_3 for systems 2 and 2a differ only slightly from one another in the range of higher concentrations (0.01 *M*). However, at concentrations down to $[L]_{\lambda,crit}$ the values of E_λ/E_3 calculated for systems 2 and 2a differed substantially (*cf.*, Table I). The approximate value obtained by applying the more simple model 2 is higher than the correct value (2a), *i.e.*, for practical selection of electrolyte systems the more simple calculation (system 2) is sufficient.

Bearing in mind that the critical concentration for acetate systems under study, $[L]_{\lambda,crit} = 5 \times 10^{-4}$ *M*, arises out of the working range of the apparatus used, the extinction of the stable isotachophoretic boundary was studied for a sodium formate–formic acid system. Fig. 5 shows experimental records of potential gradient detection for various concentrations of the leading electrolyte (equimolar solution of HCOOH and HCOONa). It follows that on decreasing the concentration to the critical value (for system 2a, theoretical $[L]_{\lambda,crit} = 0.0066$ *M*), the ratio E_λ/E_3 approaches 1. If $[L]_\lambda$

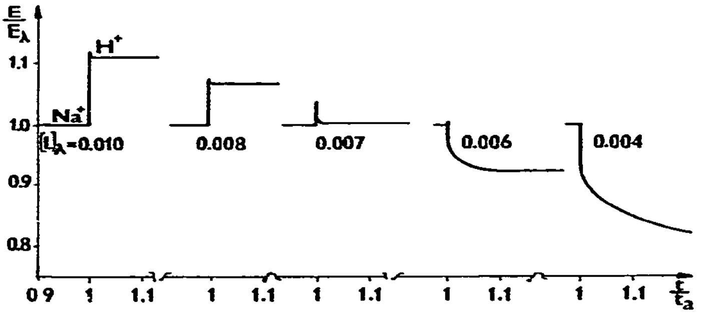

Fig. 5. Experimental records of the potential gradient detection of the moving boundary in the system HCOOH :: HCOOH + HCOONa (1:1) for various concentrations of the leading electrolyte. The relative time of analysis, t/t_a ($t = t_a$ on passage of the boundary through the detector), is plotted on the x axis.

$< [L]_{\lambda,\mathrm{crit}}$, a diffusion boundary is created followed by a decrease in potential gradient.

The effect of the dissociation constant, K_{HA}, on the character of the migrating boundary is illustrated in Fig. 6, which shows records of the migration of a boundary created by free acid as terminator and by a solution of the acid (0.007 *M*) and its sodium salt (0.007 *M*) as leading electrolyte. It is seen that, while the acetate system creates a permanently sharp boundary, the formate system, at the same concentration, is at the limit of the steady-state (*cf.*, Fig. 5) and the chloroacetate system creates a wide diffusion boundary.

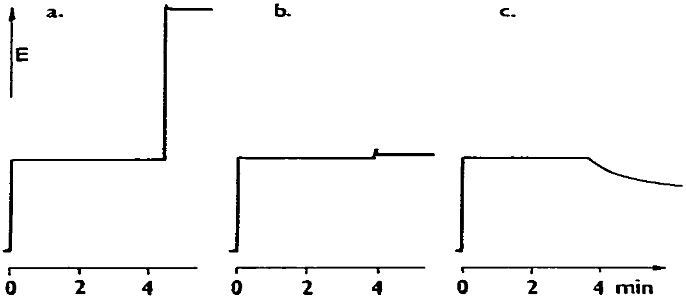

Fig. 6. Experimental records of the potential gradient detection of the moving boundary in the systems HA :: 0.007 *M* HA + 0.007 *M* NaA, for the following acids and their salts: a, acetic acid (pK_{HA} = 4.76); b, formic acid (pK_{HA} = 3.75); c, chloroacetic acid (pK_{HA} = 2.86). Driving currents: 140 μA (a); 190 μA (b); 250 μA (c).

To verify experimentally the isotachophoretic migration of selected bases, in respect of the theoretical condition 25, the bases were run under model conditions and the presence and/or absence of sharp zones (or steps in the record) served as a diagnostic criterion. In this case, the ratio of E_x/E_ϑ with the critical value 1, *viz.*, expression 8, cannot be used as the criterion of a sharp boundary since, in view of relationship 22, the critical value is given by the ratio $\bar{u}_{BH,y}/\bar{u}_{BH,x}$. Due to a lower pH in zone ϑ ($[H]_\vartheta > [H]_x$), $\bar{u}_{BH,\vartheta} > \bar{u}_{BH,x}$ ($\bar{u}_{BH,\vartheta}/\bar{u}_{BH,x} > 1$). In practice, it enables isotachophoretic migration of the zone of the terminating hydrogen ion with lower potential gradient behind the zone of the base with a higher potential gradient. Fig. 7 shows records of the migration of selected bases in the system acetic acid :: 0.005 *M* potassium acetate. It is seen that the migration behaviour of the bases is significantly dependent on the value of their dissociation constant, K_{BH}. While for zones of tris(hydroxymethyl)aminomethane (Tris) and histidine (His), $E_x/E_\vartheta < 1$ (Fig. 7b, c), for migrating aniline (Ani) and β-alanine (β-Ala), $1 < E_x/E_\vartheta < \bar{u}_{BH,\vartheta}/\bar{u}_{BH,x}$ (Fig. 7d, e). Condition 22 or 25 is not satisfied for glycine in the given system (theoretical $[L]_{\lambda,\mathrm{crit}}$ = 0.036 *M*, *i.e.*, $[L]_\lambda < [L]_{\lambda,\mathrm{crit}}$) and that is why the glycine zone is absent from the record (Fig. 7f). If the concentration of the leading electrolyte is decreased to 0.002 *M*, it becomes less than the critical concentration even for β-alanine (theoretical $[L]_{\lambda,\mathrm{crit}}$ = 0.0037 *M*). Fig. 8 presents records of the analyses of a model mixture containing histidine, aniline and β-alanine for 0.005 and 0.002 *M* leading electrolyte. As expected, the zone of β-alanine is absent at the lower concentration.

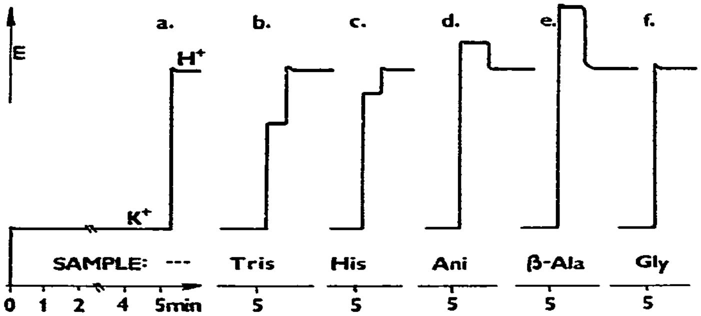

Fig. 7. Experimental isotachophoregrams of analyses in the system acetic acid :: 0.005 *M* potassium acetate. a, Boundary; b, injection of 2 μl of 0.005 *M* Tris (pK_{BH} = 8.07); c, 2 μl of 0.005 *M* histidine (pK_{BH} = 6.04); d, 2 μl of 0.005 *M* aniline (pK_{BH} = 4.80); e, 1 μl of 0.005 *M* β-alanine (pK_{BH} = 3.55); f, 1 μl of 0.005 *M* glycine (pK_{BH} = 2.35). *I* = 80 μA.

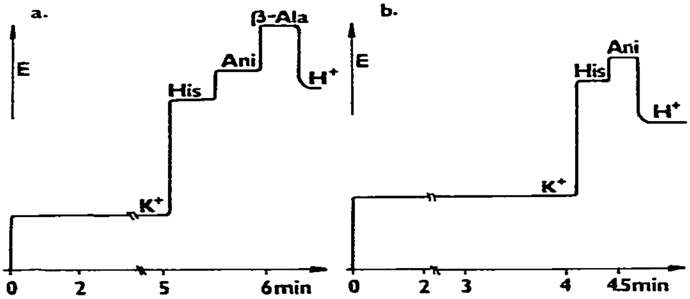

Fig. 8. Experimental isotachophoregrams of analyses in acetic acid :: potassium acetate systems. Sample: 0.005 *M* β-alanine + 0.01 *M* aniline + 0.02 *M* histidine. a, Leading electrolyte 0.005 *M* potassium acetate, 0.5 μl of sample injected. *I* = 80μA; b, leading electrolyte 0.002 *M* potassium acetate. 0.2 μl of sample injected. *I* = 40 μA.

Fig. 9 shows an example of practical application[13,14] of the systems described; a record of the analysis of the artificial sweetener Aspartam (L-aspartylphenylalanine methyl ester hydrochloride, MEAP). Because of the low value of pK_{BH} = 3.18 of the

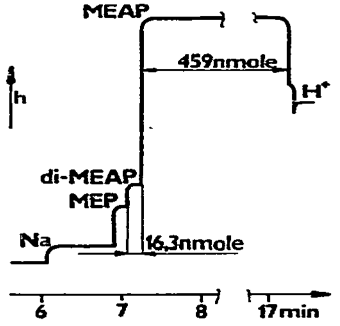

Fig. 9. Cationic analysis of a sample of the artificial sweetener Aspartam. Leading electrolyte 0.05 *M* acetic acid + 0.05 *M* ammonium acetate, terminating electrolyte 0.1 *M* acetic acid. *I* = 400 μA. Injection of 10 μl of a solution of the sweetener (97 mg per 5 ml water). Besides the main component, MEAP, the following admixtures were separated: sodium; methyl ester of phenylalanine (MEP) and dimethyl ester of aspartylphenylalanine (di-MEAP).

main component, a strongly acidic leading electrolyte (0.05 *M* acetic acid + 0.05 *M* ammonium acetate) was used to ensure enforced isotachophoretic migration of the sweetener (MEAP).

CONCLUSIONS

The presence of hydrogen ions and their migration behaviour play a decisive part in cationic isotachophoresis. For steady-state isotachophoretic migration, the electrolyte systems should be selected so that the H^+ may migrate as terminating cation by enforced isotachophoresis with self-sharpening of the front boundary (*cf.*, condition 7), or that its migration be possible in principle.

The nature of the migration of H^+ with a given selected counter anion is dependent on the concentration of the leading electrolyte. For isotachophoretic migration of H^+, the concentration of the leading ion must be higher than a certain value, in accordance with relationship 11 or 14.

For isotachophoretic migration of a protonated base in the above system, analogous conditions hold, where the requirements for the concentration of the leading electrolyte are expressed by relationship 25 or 28.

The condition of a self-sharpening boundary for weak protonated bases can be expressed in the form 22. Bearing in mind the dependence of the effective mobility of the given base on pH, this condition can be satisfied even if $E_3 < E_\alpha$. Enforced isotachophoretic migration of the base (*cf.*, *e.g.*, migration of β-alanine, Fig. 7e) then results.

The criteria derived permit the selection of a suitable counter ion and a suitable concentration of the leading electrolyte for isotachophoretic separation.

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