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CONCEPT OF THE EFFECTIVE MOBILITY OF THE HYDROGEN ION AND ITS USE IN CATIONIC ISOTACHOPHORESIS

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SUMMARY

The concept of the effective mobility of the terminating H^+ ion for cationic isotachophoresis in an acidic medium is introduced and its application to the selection of the electrolyte system for a required separation is discussed. Based on the theoretical description of the migrating reaction boundary, a relationship is derived for the calculation of the effective mobility of the H^+ ion. Further, a rule is defined for the selection of a terminating cation such that the loss of the control of the migration of H^+ ions through the isotachophoretic system is avoided. The method for the calculation and the rule mentioned above have been verified experimentally for model systems.

INTRODUCTION

For isotachophoretic migration, it is necessary to have a state in which each zone contains virtually only one separated substance and a counter-ion system, and in which the contribution of other ions of the same sign as that of the substance being separated to the electrical conductivity in the given zone is negligible. This means that in an aqueous medium either H^+ or OH^- ions, for cationic or anionic isotachophoresis, respectively, must not contribute significantly to the conduction of electrical current.

In anionic isotachophoresis, this requirement can easily be met by performing the separation in the so-called "safe region"[1] at pH < 10. Here the concentration of OH^- ions is less than 10^{-5} *M* and is therefore negligible in comparison with the concentrations of the substances being separated in their zones (commonly 10^{-3}–10^{-2} *M*).

Analogously, for cationic analysis the "safe region" is represented by pH > 5. However, here a serious problem occurs in the separation of weak bases. It is necessary to shift the equilibrium $H^+ + B = BH^+$ towards BH^+ and, therefore, a pH range of 2–5 is required. Similarly, an acidic medium is required for suppressing hydrolysis in the electrophoresis of inorganic cations[2,3]. In the pH region mentioned, the concentration of H^+ ions cannot be neglected in comparison with other cations. Moreover, the H^+ ion here possesses the highest ionic mobility and unless the migra-

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tion of H^+ is controlled, these ions penetrate through all zones and, as a result, isotachophoretic migration is disturbed[4–6].

As shown earlier[4], uncontrolled migration of the front of H^+ ions occurs particularly in unbuffered systems, where the anion of a strong acid is used as a counter ion. In such a system the zone of a cation M^+ is always a mixed zone of M^+ and H^+, with more mobile H^+ ions penetrating easily through the boundaries of the M^+ zone. The result is that H^+ ions participate significantly in the flow of electrical current in the migrating zones and, instead of isotachophoretic migration, a moving boundary procedure takes place. This situation occurs particularly when an acidic solution is used as a terminator or when free H^+ ions are generated by electrode reactions on the terminating electrode.

In buffered cationic systems, free migration of H^+ ions is hindered by the buffering counter-ion system, *i.e.*, the anion of a weak acid accompanied by the undissociated free acid. Migrating H^+ ions recombine with the free anion, re-establishing the dissociation equilibrium of the applied acid and in the terminating electrolyte and a zone with an increased concentration of the acid is therefore created, which keeps increasing in the direction of the cationic migration. It can be said that the front of H^+ ions migrates at a certain speed through the given system. If this speed does not exceed the migration velocity of the zones of the separated substances, then H^+ ions do not disturb the separation, and H^+ can be applied successfully as a terminator for the given substances in the given system of electrolytes, which was verified experimentally[5,7,8]. If this speed is greater than the migration velocity of the zones, then H^+ ions penetrate through the zones and the isotachophoretic migration of these substances is disturbed.

As both theoretical and experimental work[9] has already shown, the front of H^+ ions does not interfere with the isotachophoretic analysis provided that the boundary between this front and the last zone of the sample has self-sharpening properties.

This paper presents another approach to the above problem, based on a comparison of effective mobilities, where the migrating front of H^+ ions is considered as a hydrogen-ion constituent[10–12] possessing a certain effective mobility. Based on theoretical considerations, a method is suggested for the calculation of the effective mobility of H^+ ions in cationic systems. The method has been verified experimentally and its application to the selection of suitable electrolyte systems and to the prediction of possible interfering effects in the given cationic system is considered. The theory and its experimental verification are limited to monovalent counter ions, which accords with practice and also makes it possible to express the effective mobility of H^+ ion in a simple explicit form.

THEORETICAL

If a zone λ of the leading electrolyte, containing a weak acid HA and its alkali metal salt L^+A^-, and a zone ϑ of the terminator, containing free weak acid HA, migrate isotachophoretically, then the migration velocity of boundary $\lambda\vartheta$ is equal to that of the leading cation:

$$v_{\lambda\vartheta} = E_\lambda u_L \tag{1}$$

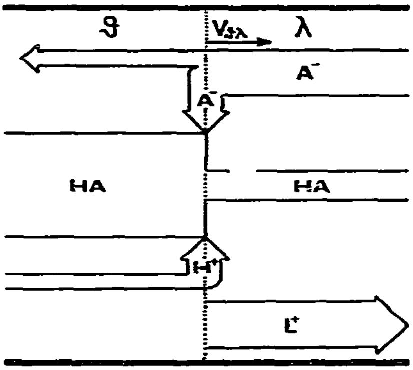

Fig. 1. Schematic diagram of the migrating reaction boundary.

where E_λ is the potential gradient in zone λ and u_L is the electrophoretic mobility of cation L^+. The H^+ ion is present virtually only on one side of the boundary $\lambda\vartheta$ (its concentration in zone λ is neglected) and thus in the sense of the authors'[10,11,13,14] definition of the effective mobility of weak electrolytes it holds that

$$\bar{u}_{H,\vartheta} = \frac{v_{\lambda\vartheta}}{E_\vartheta} \tag{2}$$

where $\bar{u}_{H,\vartheta}$ is the effective mobility of the H^+ ion in zone ϑ. The boundary $\lambda\vartheta$ is a migrating reaction boundary[14], at which the H^+ ion reacts with the counter ion A^- to form the slightly dissociated weak acid HA (see Fig. 1). The mass balance for the H^+ ion can be expressed as follows:

$$v_{\lambda\vartheta}\, c_{H,\vartheta} = E_\vartheta\, u_H\, [H]_\vartheta + v_{\lambda\vartheta}\, [HA]_\lambda \tag{3}$$

where c is the total concentration. By rearranging and combination with eqn. 2, a relationship is obtained for the effective mobility of the H^+ ion:

$$\bar{u}_{H,\vartheta} = u_H \cdot \frac{[H]_\vartheta}{c_{H,\vartheta} - [HA]_\lambda} \tag{4}$$

By expressing the condition of constant current density in the form

$$E_\vartheta\, [H]_\vartheta\, (u_A + u_H) = E_\lambda\, [L]_\lambda\, (u_A + u_L) \tag{5}$$

and by combination with eqns. 1 and 3, the adjusted concentration of acid HA in the terminating zone is given by

$$c_{H,\vartheta} = [L]_\lambda \cdot \frac{u_H}{u_L} \cdot \frac{u_A + u_L}{u_A + u_H} + [HA]_\lambda \tag{6}$$

By expressing the dissociation constant of acid HA, $K_{HA} = [H][A]/[HA]$, and by employing the condition of electroneutrality, $[\mathrm{H}]_\vartheta = [\mathrm{A}]_\vartheta$, the concentration $[\mathrm{H}]_\vartheta$ can be expressed as

$$[\mathrm{H}]_\vartheta = \frac{1}{2}\left[\sqrt{K_{\mathrm{HA}}^2 + 4\,K_{\mathrm{HA}}\,c_{\mathrm{H},\vartheta}} - K_{\mathrm{HA}}\right] \tag{7}$$

By combining eqns. 4, 6 and 7, the final relationship for the effective mobility of the H^+ ion in the terminator zone is obtained in the form

$$\bar{u}_{\mathrm{H},\vartheta} = u_{\mathrm{L}}\cdot\frac{u_{\mathrm{A}} + u_{\mathrm{H}}}{u_{\mathrm{A}} + u_{\mathrm{L}}}\cdot\frac{K_{\mathrm{HA}}}{2\,[\mathrm{L}]_\lambda}\cdot$$

$$\left[\sqrt{1 + \frac{4}{K_{\mathrm{HA}}}\left([L]_\lambda\cdot\frac{u_{\mathrm{H}}}{u_{\mathrm{L}}}\cdot\frac{u_{\mathrm{A}} + u_{\mathrm{L}}}{u_{\mathrm{A}} + u_{\mathrm{H}}} + [\mathrm{HA}]_\lambda\right)} - 1\right] \tag{8}$$

Eqn. 8 expresses the value of $\bar{u}_{\mathrm{H},\vartheta}$ explicitly as a function of the mobilities of the participating ions, of the concentrations of the leading ion and the free acid in zone λ, $[\mathrm{L}]_\lambda$ and $[\mathrm{HA}]_\lambda$, respectively, and of the dissociation constant of the acid, K_{HA}. This equation is suitable for numerical calculations of the value of $\bar{u}_{\mathrm{H},\vartheta}$.

When the leading zone does not contain free acid HA, the quantity $[\mathrm{HA}]_\lambda$ disappears from eqns. 4, 6 and 8. Eqn. 4 has then the form identical with the classical Tiselius definition of the effective mobility[15], which is commonly used in isotachophoretic practice[16]. Forms of the other equations are obvious.

EXPERIMENTAL

The isotachophoretic column used, the stabilized-current supply and the potential-gradient detector have been described earlier[17,18]. The effective mobilities were evaluated from the step heights in the potential-gradient record with the aid of the relationship[18] $\bar{u}_2 = \bar{u}_1\, h_1/h_2$, using tabulated (25°C)[19] values of mobilities of the leading cations. For extrapolating the values obtained to zero heating in the column, the procedure described earlier[20] was adopted. On measuring the model potassium–sodium boundary, the difference between the mobility u_{Na} obtained in the way and the tabulated value[19] was less than 2%.

Acetic acid, formic acid, their potassium salts, tris(hydroxymethyl)aminomethane (Tris) and the other chemicals used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia).

RESULTS AND DISCUSSION

The experimental verification of the method suggested for the calculation of the effective mobilities of the H^+ ion was performed for selected model systems (Table I). A 0.05 *M* solution of formic acid served as the terminator in all instances. Table I and Fig. 2 compare the experimental values of $\bar{u}_{\mathrm{H},\vartheta}$ with theoretical values calculated according to eqn. 8. Tabulated values of mobilities (at 25°C)[19] and dissociation constants[21] (the latter corrected for the ionic strengths of the solutions) were used for the calculation. It can be seen from Table I that, in accord with eqn. 8, $\bar{u}_{\mathrm{H},\vartheta}$

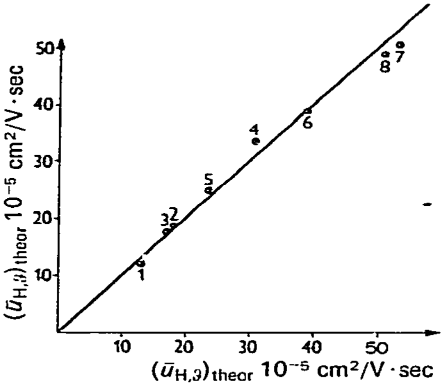

Fig. 2. Comparison of calculated and measured values of $\bar{u}_{H,\vartheta}$ for selected model systems (*cf.*, Table I).

decreases with increasing pK_{HA} of the counter anion [*cf.*, points 1, 5 and 6 in Fig. 2, when for 0.01 *M* solutions the effective mobility decreases in the series formate (pK_{HA} = 3.75), benzoate (pK_{HA} = 4.20) and acetate (pK_{HA} = 4.76)], for a given pK_{HA}, $\bar{u}_{H,\vartheta}$ increases with decreasing concentration of the leading electrolyte, $[L]_{\lambda}$ (*cf.*, points 1, 2 and 6, 7 in Fig. 2), and addition of free acid to the leading solution causes an increase in $\bar{u}_{H,\vartheta}$ (*cf.* points 1, 3 and 6, 8 in Fig. 2).

The agreement between the theoretical and experimental data is good and confirms the applicability of eqn. 8 to practical calculations. The method of calculation (*cf.*, eqn. 4) differs from the classical formulation of the effective mobility[15] according to which $\bar{u}_{H,\vartheta} = u_H[H]_\vartheta/c_{H,\vartheta}$. For the systems with a value of $[HA]_{\lambda}$ different from zero the classical relationship leads to wrong values of $\bar{u}_{H,\vartheta}$ (*e.g.*, for system 3, $\bar{u}_{H,\vartheta} = 10.0 \cdot 10^{-5}$ cm^2/V·sec is obtained, which is almost half the experimental value or the value calculated according to eqn. 4 or 8; *cf.*, Table I).

The calculation of the effective mobility of the terminating H$^+$ ion is important

TABLE I

COMPARISON OF THE CALCULATED AND MEASURED EFFECTIVE MOBILITIES OF H$^+$, $(\bar{u}_{H,\vartheta})_{theor}$ AND $(\bar{u}_{H,\vartheta})_{exp}$, RESPECTIVELY, IN VARIOUS MODEL LEADING SYSTEMS

The average relative difference between the theoretical and experimental values, listed in the table, is 4.5 %.

No.	*Leading electrolyte*	$(\bar{u}_{H,\vartheta})_{theor}$ *(10^{-5} cm^2/V·sec)*	$(\bar{u}_{H,\vartheta})_{exp}$ *(10^{-5} cm^2/V·sec)*
1	0.01 *M* potassium acetate	13.0	12.3
2	0.005 *M* potassium acetate	18.1	18.6
3	0.01 *M* potassium acetate + + 0.01 *M* acetic acid	17.1	17.5
4	0.003 *M* potassium acetate + + 0.003 *M* acetic acid	30.6	33.7
5	0.01 *M* sodium benzoate	23.5	25.0
6	0.01 *M* potassium formate	38.8	38.8
7	0.005 *M* potassium formate	53.3	50.7
8	0.01 *M* potassium formate + + 0.01 *M* formic acid	51.0	49.1

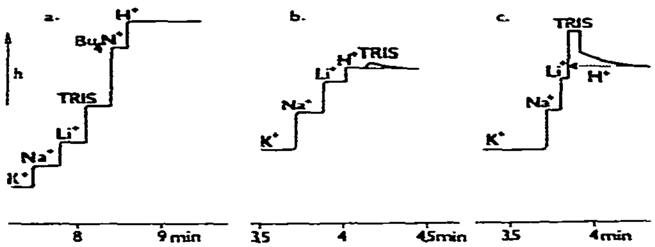

Fig. 3. Experimental results of analyses of a model mixture of cations. Leading electrolyte: (a) system 1, (b, c) system 4 (*cf.*, Table I). Terminator: 0.05 *M* formic acid. Sample: (a) 10 μl of 0.003 *M* $(C_4H_9)_4N^+Br^-$, 2 μl of 0.005 *M* Tris, 0.5 μl of 0.02 *M* LiCl, 0.7 μl of 0.015 *M* NaCl; (b) 1 μl of 0.002 *M* LiCl + 0.002 *M* NaCl + 0.00002 *M* Tris; (c(0.5 μl of LiCl + NaCl + ris (each 0.002 *M*). Driving current: (a) 100 μA, (b, c) 60 μA.

for the correct selection of electrolyte systems for practical separations. Fig. 3a shows the record of a cationic isotachophoretic analysis of a model mixture containing sodium, lithium, Tris and tetra-*n*-butylammonium in system 1 (*cf.*, Table I). Correct isotachophoretic migration of zones of all four components being separated can be seen, as the effective mobility of the terminator, $(\bar{u}_{H,S})_{theor} = 13.0 \cdot 10^{-5}$ cm^2/V · sec, is less than the mobility[19] of the slowest component being separated, $u_{Bu_4N} = 20.2 \cdot 10^{-5}$ cm^2/V · sec. Fig. 3b shows an analysis in system 4 (Table I), where the effective mobility of the terminating H^+ ion lies between the mobilities of Li^+ and Tris. In accord with the theory, isotachophoretic zones are formed by sodium and lithium only, while the slower Tris migrates in the terminator in a zone-electrophoretic manner[22,23]. When a large amount of Tris was injected (Fig. 3c), the H^+ front did not penetrate through the diffusion zone of Tris completely within the time of

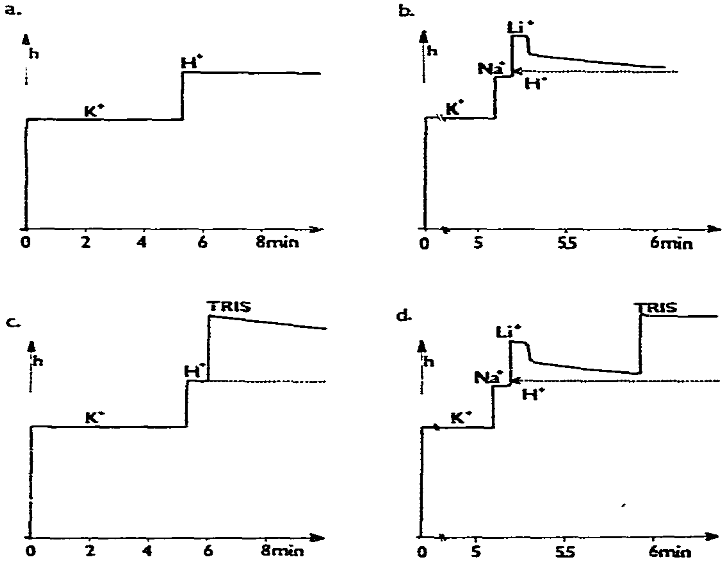

Fig. 4. Experimental results of analyses in system 8 (*cf.*, Table I). Terminator: (a, b) 0.05 *M* formic acid; (c, d) 0.1 *M* Tris. Sample (b, d): 3 μl of 0.002 *M* LiCl + 0.002 *M* NaCl. Driving current: 200 μA.

analysis. It can be seen that the front part of the Tris zone is still homogeneous, whereas its rear shows a diffusion tail. The slope and the length of the diffusion part of the zone depend on u_{Tris} and on the analysis time[22].

A knowledge of the effective mobility of the H$^+$ ion is important even in "classically" terminated cationic isotachophoresis, because here also the control of the migration behaviour of this ion must be ensured. In system 8 (Table I) the H$^+$ ion possesses a relatively high effective mobility, $(\bar{u}_{\text{H},\vartheta})_{\text{theor}}/u_{\text{K}} = 0.669$ (*cf.*, Fig. 4a). When running the same model mixture as in Fig. 3a, it is only sodium that provides an isotachophoretic zone (*cf.*, Fig. 4b, for an analysis of a mixture of Na$^+$ and Li$^+$). Here the isotachophoretic zone of sodium is followed by a mixed zone of lithium with H$^+$ ion, analogous to the migration of Tris in Fig. 3c. If a 0.1 *M* solution of Tris is used as terminator for system 8 (Fig. 4c), with respect to the sequence of the mobilities $\bar{u}_{\text{H},\vartheta} > u_{\text{Tris}}$, Tris migrates in a frontal manner inside the zone of H$^+$ ion, which is the real terminator. The analysis of a mixture of Na$^+$ and Li$^+$ under the given conditions provides an obscure record of the analysis (Fig. 4d). Based on the above comments on Fig. 4a–c, and with respect to the sequence of mobilities $u_{\text{Na}} > \bar{u}_{\text{H},\vartheta} > u_{\text{Li}} > u_{\text{Tris}}$, Fig. 4d can clearly be interpreted. It is only sodium that provides a correct isotachophoretic zone. Lithium and Tris provide mixed zones with H$^+$ ion and migrate in a zonal or frontal manner, respectively. Thus, H$^+$ ion serves as background electrolyte for the Li$^+$ and Tris zones. Analogously, earlier described[4] cases of non-isotachophoretic zone behaviour can be similarly interpreted on the basis of a knowledge of the effective mobility of the H$^+$ ion.

CONCLUSION

The migration behaviour of the H$^+$ ion in cationic isotachophoresis in an acidic medium can be described by means of the effective mobility, $\bar{u}_{\text{H},\vartheta}$, defined by eqn. 4 or 8. In order to obtain correct isotachophoretic migration it is necessary that the front of the H$^+$ ion does not pass through the boundary between the terminator and the last zone, *i.e.*, that its effective mobility is lower than the mobility of the slowest cation being separated. If for any cation M$^+$ $\bar{u}_{\text{H},\vartheta} > u_{\text{M}}$, then H$^+$ ion penetrates through its zone and causes the isotachophoretic character of the migration to deteriorate; M$^+$ migrates in a zonal or frontal way on the background of the H$^+$ ion.

According to eqn. 8, $\bar{u}_{\text{H},\vartheta}$ is dependent only on the parameters of the leading electrolyte. The method proposed here enables one to predict theoretically $\bar{u}_{\text{H},\vartheta}$ for the given leading system and to compare the value thus obtained with the tabulated values of the mobilities of the substances being separated. It is thus possible to evaluate the suitability of a given system for a required separation. As a rule it can be analogously formulated for the selection of the terminator that it is not advisable to terminate a given leading electrolyte (which determines the value of $\bar{u}_{\text{H},\vartheta}$) with a cation M$^+$ for which $u_{\text{M}} < \bar{u}_{\text{H},\vartheta}$.

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