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GENERAL MATHEMATICAL MODEL FOR THE STEADY STATE IN ISOTACHOPHORESIS

CALCULATION OF THE EFFECTIVE MOBILITY OF TERMINATING H^+ IONS AND TWO-BUFFER ELECTROLYTE SYSTEMS

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SUMMARY

An extension of the mathematical model for the steady state in isotachophoresis is given for the calculation of the effective mobility of terminating H^+ ions and two-buffer electrolyte systems. The model is verified using the specific zone resistance at 25°C (SZR_{25}) as an experimental parameter for several leading electrolytes with one and two buffering counter ionic species. The theoretically calculated SZR_{25} values show good agreement with the experimentally obtained values. The "enforced" migration of Al^{3+} in a two-buffer electrolyte system with acetic acid and α-hydroxyisobutyric acid as counter ionic species can be understood by comparing experimental and calculated data, using this model.

INTRODUCTION

In cationic isotachophoresis (ITP) at low pH, H^+ is often used as a terminator[1] and Bocek *et al.*[2] defined theoretically conditions for the ITP migration of cations with a controlled migration behaviour of H^+ and formulated the concept of the effective mobility of the terminating H^+ ions in cationic ITP[3]. Experimentally obtained values showed a good agreement with calculated data using their concept. However, the theory and its experimental verification were limited to the use of one monovalent counter ionic species.

In this paper, a general mathematical model for ITP is given that is useful for both the calculation of the effective mobility of terminating H^+ ions and the use of electrolyte systems with more than one buffering counter ionic species. It is an extension of the mathematical model for the steady state in ITP described previously[4,5].

The theoretical part is divided into three sections: (1) a recapitulation of the previously described mathematical model for the steady state in ITP, (2) an extension of the calculation of the effective mobility of terminating H^+ ions in cationic separations and (3) an extension of the use of electrolyte systems with more than one buffering counter ionic species.

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Computer programs based on these models were written and the results of calculations were verified partly experimentally and partly by comparing the results with those of other investigators.

THEORETICAL

The isotachophoretic model

For the description of a model useful for the calculation of the effective mobility of terminating H^+ ions and two-buffer electrolyte systems, we briefly repeat the ITP model published previously[4,5]. For this model, all substances will be regarded as amphiprotic polyvalent molecules.

For a molecule A (here only proton interactions are taken into account, for simplicity), the following equilibria can be set up:

$$A^{z} + H_2O \overset{pK_1}{\rightleftharpoons} A^{z-1} + H_3O^+ \tag{1}$$

$$A^{z-1} + H_2O \overset{pK_2}{\rightleftharpoons} A^{z-2} + H_3O^+ \tag{2}$$

$$A^{z-i+1} + H_2O \overset{pK_i}{\rightleftharpoons} A^{z-i} + H_3O^+ \tag{3}$$

where the superscript z refers to the highest charge of substance A. The general expression for the ith concentration equilibrium equation will be:

$$K_i = \frac{[A^{z-i}]\,[H_3O^+]}{[A^{z-i+1}]} \tag{4}$$

or

$$[A^{z-i}] = \frac{[A^{z-i+1}]\;K_i}{[H_3O^+]} \tag{5}$$

In the computations, all concentration equilibrium equations are calculated from the thermodynamic constants correcting for activities.

Replacing the ionic concentration on the right-hand side with the concentration of the higher charged forms, we find ultimately the relationship with the concentration of the highest charged ionic form, *viz.*,

$$[A^{z-i}] = \frac{[A^{z-i+1}]K_i}{[H_3O^+]} = [A^{z-i+2}]\cdot\frac{K_{i-1}K_i}{[H_3O^+]^2} = [A^{z}]\,\frac{\prod_{j=1}^{i} K_j}{[H_3O^+]^i} \tag{6}$$

In this way, all concentrations of the ionic forms can be expressed as the concentration of the ionic form with the highest charge by means of the equilibrium constants and the concentration of the hydrogen ions.

The total concentration of an ionic species is

$$[\mathrm{A}]_\mathrm{t} = [\mathrm{A}^{z}] + [\mathrm{A}^{z-1}] + [\mathrm{A}^{z-2}] + \ldots \tag{7}$$

Substitution of eqn. 6 gives

$$\begin{aligned}[\mathrm{A}]_\mathrm{t} &= [\mathrm{A}^{z}] + [\mathrm{A}^{z}] \cdot \frac{K_1}{[\mathrm{H_3O^+}]} + [\mathrm{A}^{z}] \cdot \frac{K_1 K_2}{[\mathrm{H_3O^+}]^2} + \cdots \\ &= [\mathrm{A}^{z}] \left(1 + \sum_{i=1}^{n} \frac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i} \right)\end{aligned} \tag{8}$$

if the number of pK values of substance A is n!

Combining eqns. 6 and 8, the ionic concentration with a charge of $z-i$ can be expressed as the total concentration of A by

$$[\mathrm{A}^{z-i}] = [\mathrm{A}^{z}] \cdot \frac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i} = [\mathrm{A}]_\mathrm{t} \cdot \frac{\dfrac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i}}{1 + \sum_{i=1}^{n} \dfrac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i}} \tag{9}$$

With these equations we can find an expression for the effective mobility of an ionic species.

Tiselius[6] pointed out that a substance that consists of several forms with different mobilities in equilibrium with each other will generally migrate as a uniform substance with an effective mobility given by

$$\bar{m} = \sum_{i=0}^{n} \alpha_i m_i = \sum_{i=0}^{n} [\mathrm{A}^{z-i}] m_{z-i} / [\mathrm{A}]_\mathrm{t} \tag{10}$$

For simplicity, the effect of the ionic strength is not considered in this equation. In the computer programs, however, this effect is corrected for using the Debye–Hückel–Onsager relationship.

Substituting eqns. 8 and 9 into eqn. 10, we can write for the effective mobility of an ionic species A

$$\bar{m} = \frac{\sum_{i=1}^{n} m_{z-i} \cdot \dfrac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i} + m_z}{1 + \sum_{i=1}^{n} \dfrac{\prod_{j=1}^{i} K_j}{[\mathrm{H_3O^+}]^i}} \tag{11}$$

Although in these general descriptions of equilibria and effective mobility of a substance no differences exist between the leading, sample, terminating and buffer ionic species, we shall distinguish between them using the symbols L, A, T and B, respectively.

In addition to the general descriptions of the equilibria and effective mobility of ionic species, we further need the mass balance of the buffer, the principle of electroneutrality, the modified Ohm's law and the isotachophoretic condition to describe the "steady state" in ITP.

Mass balance of the buffer. With the mass balance of the buffer (Ohm's law and the principle of electroneutrality must also be obeyed) the leading zone determines the conditions of the proceeding zones. For the mass balance of the buffer, the following equation can be derived (see Fig. 1).

The zone boundary L/A moves in a unit of time over a distance $E_L \mid \bar{m}_{L,L} \mid$ or $E_A \mid \bar{m}_{A,A} \mid$. The buffer ionic species at time $t=0$ present at the zone boundary L/A will reach point D at $t=1$. The distance from L/A to D will then be $E_A \mid \bar{m}_{B,A} \mid$. The buffer ionic species at $t=0$ present at point C will just reach the boundary L/A at $t=1$. The distance from C to L/A is then $E_L \mid \bar{m}_{B,L} \mid$. This means that all buffer ionic particles present in the leading zone between L/A and C with a concentration of $[B]_{t,L}$ at time $t=0$ ($\Delta 1$) will be present in zone A with a concentration of $[B]_{t,A}$ between L/A and D at $t=1$ ($\Delta 2$). Therefore, the buffer mass balance will be

$$[B]_{t,A}(E_A|\bar{m}_{B,A}| + E_L|\bar{m}_{L,L}|) = [B]_{t,L}(E_L|\bar{m}_{B,L}| + E_L|\bar{m}_{L,L}|) \tag{12}$$

or

$$[B]_{t,A}(E_A|\bar{m}_{B,A}|/E_L + |\bar{m}_{L,L}|) = [B]_{t,L}(|\bar{m}_{B,L}| + |\bar{m}_{L,L}|) \tag{12a}$$

or, after applying the isotachophoretic condition (see eqn. 17),

$$[B]_{t,A}(|\bar{m}_{L,L}||\bar{m}_{B,A}|/|\bar{m}_{A,A}| + |\bar{m}_{L,L}|) = [B]_{t,L}(|\bar{m}_{B,L}| + |\bar{m}_{L,L}|) \tag{12b}$$

or

$$[B]_{t,A}(|\bar{m}_{B,A}|/|\bar{m}_{A,A}| + 1) = [B]_{t,L}(|\bar{m}_{B,L}|/|\bar{m}_{L,L}| + 1) \tag{12c}$$

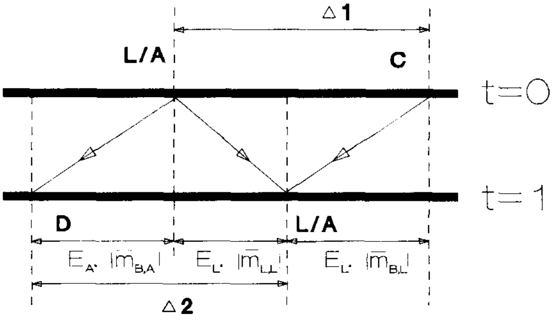

Fig. 1. Migration paths ofthe buffering counter ionic species over a zone boundary between the leading zone and a sample zone L/A. For further explanation, see text.

The principle of electroneutrality. In accordance with the principle of electroneutrality (EN), the arithmic sum of all products of the concentration of all forms for all ionic species and the corresponding valences, present in each zone, must be zero.

For the electroneutrality of a zone we can write

$$[H_3O^+] - [OH^-] + \sum_{i=0}^{n_A} z-i[A^{z-i}] + \sum_{i=0}^{n_B} z-i[B^{z-i}] = 0 \tag{13}$$

Modified Ohm's law. Working at a constant current density,

$$E_L\sigma_L = E_A\sigma_A \tag{14}$$

or the function

$$RFQ = E_L\sigma_L/E_A\sigma_A - 1 \tag{15}$$

must be zero. The overall electrical conductivity, σ, of a zone is the sum of the values $c|\bar{m}z|F$, and consequently

$$E\{[H_3O^+]|\bar{m}_H| + [OH^-]|\bar{m}_{OH}| + \sum_{i=0}^{n_A} [A^{z-i}]|\bar{m}_{z-i}(z-i)| + \sum_{i=0}^{n_B} [B^{z-i}]|\bar{m}_{z-i}(z-i)|\} \tag{16}$$

in all zones is constant.

Isotachophoretic condition. In the steady state, all zones move with a velocity equal to that of the leading zone, and therefore

$$E_L\bar{m}_{L,L} = E_A\bar{m}_{A,A} \tag{17}$$

Procedure of calculation. With the equilibrium constants, using eqn. 9, all ionic concentrations can be expressed as the total concentration for each type of ion. Further, $[OH^-]$ can be expressed as $[H_3O^+]$ using the pK_w. By this means, the reduced number of parameters is four for all ITP zones, *viz.*, E, pH, $[A]_t$ and $[B]_t$.

For all zones, four known parameters and/or equations, by means of which all parameters can be calculated, are always necessary. For the leading zone the known parameters are, *e.g.*, $[L]_t$ and $[B]_t$ and the equations are Ohm's law and the EN.

For all other zones, the four available equations are the EN, Ohm's law, the buffer equation and the isotachophoretic condition. In Figs. 2 and 3 the calculation procedure for the leading zone and a sample zone are shown schematically.

General model for hydrogen as terminator

If the effective mobility of hydrogen ions as the terminator in cationic ITP has to be calculated, the crux of the whole matter is whether *a steady state can be established whereby hydrogen ions migrate as the only positive ions in the terminating zone.* If such a steady state is possible, the reduced number of parameters for the terminating H^+ zone is only three, *viz.*, $[B]_{t,H}$, pH_H and E_H. Hence the EN, Ohm's law and the buffer mass balance are sufficient to calculate all parameters.

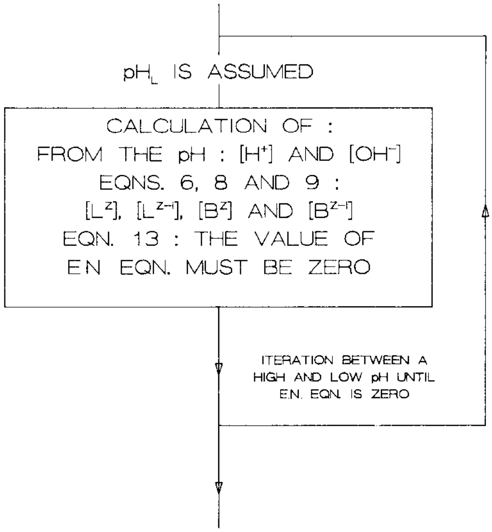

Fig. 2. Calculation procedure for the leading zone in ITP if the total concentrations of the leading and buffering counterionic species are known.

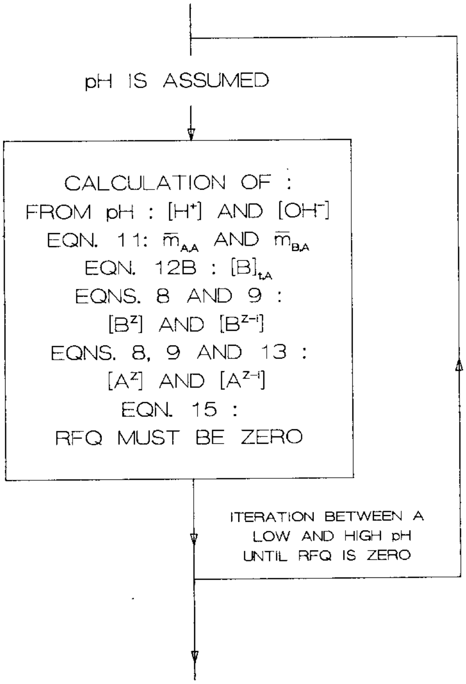

Fig. 3. Calculation procedure for the sample zones in ITP. In the calculation the EN (eqn. 13), the buffer equation (eqn. 12b), the isotachophoretic condition (included in eqn. 12b) and Ohm's law (eqn. 15) are used.

The isotachophoretic condition gives no further information, but can be used for the calculation of the effective mobility of H^+, conforming to the concept of Bocek *et al.*[3].

Procedure of calculation. All quantities of the leading zone can be calculated (see Fig. 2). If a pH for the terminating H^+ zone is assumed, the hydrogen and hydroxyl ion concentrations can be calculated. Further, all pH-dependent quantities such as the effective mobilities can be obtained. From the EN the concentration of the buffer ionic species in the terminating H^+ zone can be calculated and, using the mass balance of the buffer, the ratio E_H/E_L.

To find the correct value of pH_H, the pH can be iterated between a low and high value until Ohm's law is met (eqn. 15). Ultimately, the effective mobility of the terminating H^+ ions can be calculated with eqn. 17 (analogous calculations can be made for OH^- as terminator, if disturbances due *e.g.*, to the presence of carbonate can be suppressed).

Based on these equations, a computer program was set up. In Fig. 4 the calculation of the parameters of the terminating H^+ zone is shown schematically.

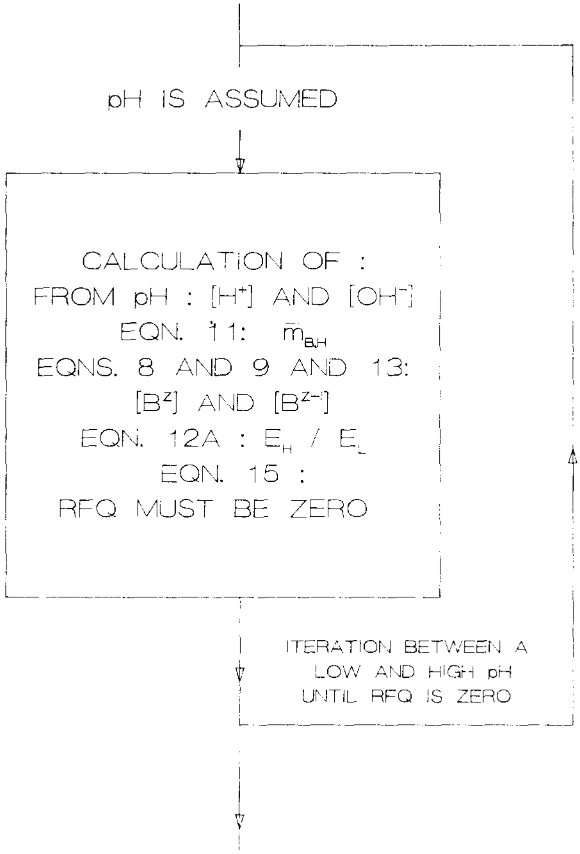

Fig. 4. Calculation procedure for the terminating H^+ zone. In the calculation only three equations are used, *viz.*, the EN (eqn. 13), the buffer equation (eqn. 12a) and Ohm's law (eqn. 15).

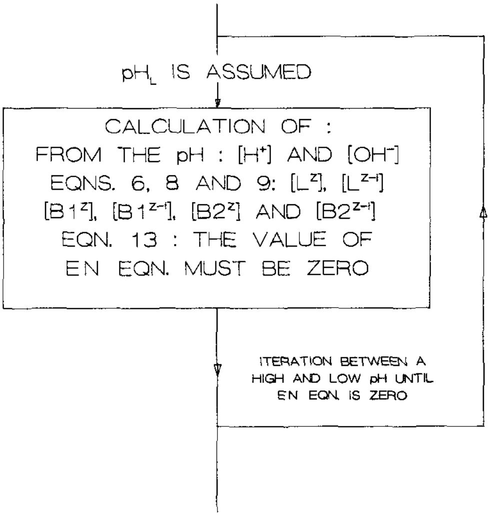

Fig. 5. Calculation procedure for the leading electrolyte in a two-buffer electrolyte, if the total concentrations of the leading and both buffering counter ionic species are known.

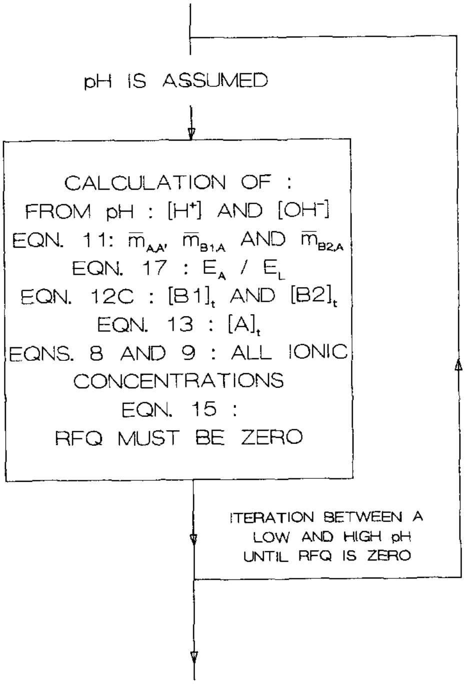

Fig. 6. Calculation of a sample zone in a two-buffer electrolyte. In the calculation five equations are used, *viz.*, the isotachophoretic condition (eqn. 17), two buffer balances (eqn. 12c), the EN (eqn. 13) and Ohm's law (eqn. 15).

General model for a two-buffer electrolyte system

For a two-buffer electrolyte system (and this can easily be extended to more buffer ionic species), the reduced number of parameters will be five in all ITP zones, *viz.*, E, pH, $[A]_t$, $[B1]_t$ and $[B2]_t$. For the calculation of all parameters, five known parameters and/or equations are necessary. In the leading zone the known parameters are $[L]_t$, $[B1]_t$ and $[B2]_t$ and with Ohm's law and the EN all parameters can be calculated (see Fig. 5). For all other zones the five available equations are the EN, Ohm's law, the isotachophoretic condition and two buffer equations (see Fig. 6).

Procedure of calculation. All quantities of the leading zone can be calculated (see Fig. 5). If a pH in a sample zone is assumed, the hydrogen and hydroxyl concentrations and all pH-dependent quantities such as the effective mobilities can be calculated. With the isotachophoretic condition, the ratio E_A/E_L can be found and with the buffer equations $[B1]_t$ and $[B2]_t$. From the EN the $[A]_t$ can be obtained. Iterating between a low and a high pH, the correct value of the pH can be found using Ohm's law (see Fig. 6). A computer program for this procedure was written.

Analogously to the procedure described above, the mobility of terminating H^+ ions can also be calculated in a two-buffer electrolyte system. In this instance, the reduced number of parameters in the terminating H^+ zone is only four, *viz.*, $[B1]_t$, $[B2]_t$, pH and E. The available equations are two buffer balances, Ohm's law and the EN.

In fact, more ways of iterating are possible. We use the following procedure for the calculation of parameters of the terminating H^+ zone in a two-buffer electrolyte system. A pH is assumed and from this the hydrogen and hydroxyl concentrations and the pH-dependent parameters can be calculated. Then a ratio $E_H/E_L$is assumed. Using the buffer equations the $[B1]_t$ and $[B2]_t$ can be obtained. Iterating, at the chosen pH, between a low and a high E_H/E_L value, the correct E_H/E_L value can be obtained using the EN. The correct pH can be found iterating between a low and a high pH value using Ohm's law (see Fig. 7).

EXPERIMENTAL

In order to check the validity of the extended steady-state model for ITP, useful for calculations of the mobility of terminating H^+ ions and two-buffer electrolyte systems, on the one hand the results of calculations based on this model were compared with those of Bocek *et al.*'s model and on the other further experiments were carried out.

As the experimental parameter we used the specific zone resistance at 25°C (SZR_{25})[7]. For not too large electric currents and ionic species with not too small mobilities, a linear relationship between the step heights and SZR_{25} values is obtained[7]. By this means the SZR_{25} of a substance can be obtained using two standard substances for which the SZR_{25} values can be calculated (based on the mathematical model for the steady state in ITP). From the step heights of these two standard substances, a linear relationship between step height and SZR_{25} can be set up and from this relationship and the step height of an ionic species its SZR_{25} can be calculated.

The SZR_{25} values obtained in this way are used as experimental parameters for the check of our extended mathematical model. As standard substances both the leading ions, terminating ions and other ionic species can be used. For substances with very low mobilities, standards can be chosen with mobilities close to that of the sample component.

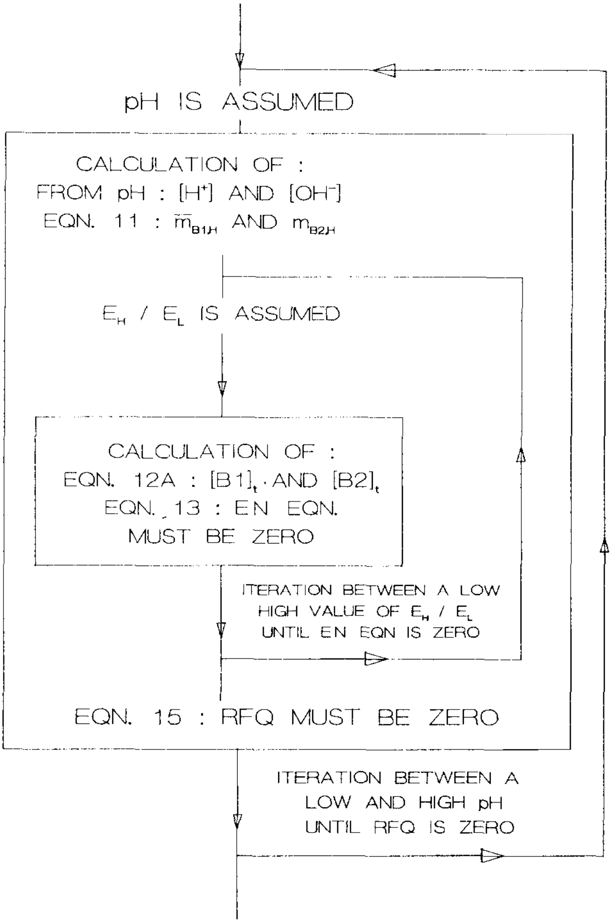

Fig. 7. Calculation of the terminating H^+ zone in a two-buffer electrolyte. Four equations are used, *viz.*, two buffer balances (eqn. 12a), the EN (eqn. 13) and Ohm's law (eqn. 15).

TABLE I

p*K* VALUES AND ABSOLUTE IONIC MOBILITIES FOR THE IONIC SPECIES USED IN THE CALCULATIONS

Ionic species	*Absolute ionic mobility (10^{-5} cm^2/V · s)*	*pK_a*
Acetic acid	42.4	4.756
Benzoic acid	33.6	4.203
Formic acid	56.6	3.75
Hydrochloric acid	79.1	−2.0
α-Hydroxybutyric acid	33.5	3.971
Histidine	29.6	6.04
Lithium	40.1	>14
Potassium	76.2	>14
Sodium	51.9	>14

In all calculations, corrections for the activities are made and for the concentration effects on the mobilities using the Debye–Hückel–Onsager equation. All absolute ionic mobilities (at infinite dilution) and pK values of the leading, counter and sample ionic species, used in the calculations, are given in Table I.

Model for hydrogen as terminator

As a first check we compared the results of calculations with our model for the calculation of the effective mobilities of hydrogen as terminator with those of Bocek *et al.*[3].

As we did not know how Bocek *et al.* carried out the corrections for the influence of temperature and ionic strength, we give in Table II the values of the effective mobility of terminating H^+ ions, (1) experimentally determined by Bocek *et al.*, (2) the values calculated by Bocek *et al.* (3) the values calculated using his model (eqns. 7 and 8 in ref. 3) without corrections and calculated values using our model, (4) without and (5) with corrections.

The calculated values of Bocek *et al.*'s model and those of our model are comparable, although our value for the last one (at low pH!) is significantly lower. To check if the models are satisfactory at lower pHs, we carried out some experiments at low pH. As experimental parameter we used the SZR_{25} as described before, using always the lading ion K^+ and Na^+ as standards.

In Table III the pH values of the systems, the calculated effective mobilities of the terminating H^+ ions (1) with and (2) without corrections using our model and (3) those of the model of Bocek *et al.* without corrections are given. Further, the (4) calculated and (5) measured SZR_{25} values with corrections are given.

TABLE II

COMPARISON OF (1) MEASURED AND CALCULATED VALUES OF THE EFFECTIVE MOBILITIES FOR TERMINATING H^+ IONS USING (2) BOCEK *et al.*'s VALUES[3], (3) USING BOCEK *et al.*'s MODEL WITHOUT CORRECTIONS, AND USING OUR MODEL, (4) WITHOUT AND (5) WITH CORRECTIONS

System	*Effective mobilities for terminating H^+ ions (in 10^{-5} $cm^2/V \cdot s$)*				
	Measured	*Calculated*			
	(1)	*(2)*	*(3)*	*(4)*	*(5)*
0.01 *M* Potassium acetate	12.3	13.0	12.63	12.62	12.88
0.005 *M* Potassium acetate	18.6	18.1	17.73	17.72	18.00
0.01 *M* Potassium acetate–0.01 *M* acetic acid	17.5	17.1	16.63	16.49	16.53
0.003 *M* Potassium acetate–0.003 *M* acetic acid	33.7	30.6	30.02	29.37	29.43
0.01 *M* Sodium benzoate	25.0	23.5	22.63	23.00	23.19
0.01 *M* Potassium formate	38.8	38.8	37.29	37.19	38.15
0.005 *M* Potassium formate	50.7	53.3	51.57	51.43	52.49
0.01 *M* Potassium formate–0.01 *M* formic	49.1	51.0	48.69	46.36	46.42

TABLE III

CALCULATED EFFECTIVE MOBILITIES OF TERMINATING H^+ IONS USING OUR MODEL, (1) WITH AND (2) WITHOUT CORRECTIONS, AND (3) USING BOCEK *et al.*'s MODEL WITHOUT CORRECTIONS, AND (4) CALCULATED AND (5) MEASURED SZR_{25} VALUES, USING OUR MODEL WITH CORRECTIONS

System	*pH*	*Effective mobilities for terminating H^+ ions (10^{-5} $cm^2/V \cdot s$): calculated*			*SZR_{25} (Ω m)*	
					Calc.	*Exptl.*
		(1)	*(2)*	*(3)*	*(4)*	*(5)*
0.01 *M* Potassium acetate–acetic acid	4.91	15.56	15.48	15.55	43.10	41.43
	4.78	16.39	16.35	16.45	40.86	39.15
	4.51	18.86	18.94	19.15	35.31	35.27
	4.30	21.69	21.90	22.29	30.48	30.70
0.01 *M* Potassium benzoate–benzoic acid	4.40	29.51	29.54	29.98	24.38	21.70
	4.38	29.71	29.75	30.22	24.19	23.22
	4.30	30.59	30.68	31.25	23.42	21.90
	4.17	32.30	32.48	33.29	22.02	20.67
	3.97	35.76	36.12	37.54	19.58	18.75
0.01 *M* Potassium formate–formic acid	4.12	42.09	41.54	42.56	13.84	12.69
	3.90	44.33	44.03	45.81	12.92	12.44
	3.56	49.85	50.16	54.55	10.93	10.81
	3.35	54.65	55.52	63.40	9.44	9.36

From Table III it can be concluded that the results from the two models correlate fairly well, although our results at the lowest pHs seem to fit the experimental values better. It must be noted that, although Bocek *et al.*'s model is rather simplified, it fits remarkably over a wide range of pH.

Model of H^+ as terminator in two-buffer systems

To check the model for the calculation of the effective mobility of terminating H^+ ions in the use of two-buffer electrolyte systems, we calculated the SZR_{25} for the terminating H^+ zone and compared those values with the experimentally obtained SZR_{25} values, using the Na^+ and K^+ zones as standards. This was done for several leading electrolytes consisting of 0.01 *M* potassium hydroxide and a specific concentration of α-hydroxyisobutyric acid (HIBA), adding acetic acid to a pH_L of 4.5 and 4.0, respectively, and formic acid to a pH_L of 3.75.

In Table IV, the concentration of HIBA in the leading electrolyte, the calculated effective mobility of the terminating H^+ ions, the calculated $HIBA^-$ concentration, the calculated SZR_{25} and the measured SZR_{25} values are given for the terminating H^+ zone. It can be concluded that the experimentally obtained values fit the calculated values, although the former are slightly too high for low-mobility H^+ zones. Because in these instances a large difference exists between the SZR_{25} values of the standards and terminating H^+ zone, we repeated the experiments for some electrolyte systems using Li^+ instead of Na^+ as a standard. The results (in parentheses) are better, showing that more accurate measurements are obtained if the SZR_{25} values of the standards are close to that of the sample.

TABLE IV

CALCULATED EFFECTIVE MOBILITIES OF TERMINATING H^+ IONS, CALCULATED CONCENTRATION OF $HIBA^-$, CALCULATED AND MEASURED SZR_{25} VALUES FOR TERMINATING H^+ ZONES IN SEVERAL TWO-BUFFER SYSTEMS, WITH DIFFERENT CONCENTRATIONS OF HIBA IN THE LEADING ELECTROLYTE AT DIFFERENT pH VALUES

Sustem	*Concentration of HIBA in the leading zone (M)*	*Effective mobility of H^+ (10^{-5} $cm^2/V \cdot s$)*	*[$HIBA^-$] (10^{-4} M)*	*SZR_{25} (Ωm)*	
				Calculated	*Measured*
0.01 *M* Potassium acetate–acetic acid at pH_L = 4.50	0	19.47	0.00	34.19	37.73 (35.06)
	0.0002	19.29	0.29	34.56	37.46 (35.13)
	0.0008	20.03	1.12	33.40	35.22 (33.20)
	0.001	20.29	1.40	33.02	35.00 (34.24)
	0.002	21.54	2.71	31.29	33.01
	0.003	22.83	3.93	29.71	31.23
	0.004	24.11	5.09	28.31	29.31
	0.005	25.40	6.17	27.05	27.96
	0.006	26.68	7.20	25.91	26.49
	0.007	27.96	8.17	24.89	25.05
	0.008	29.22	9.09	23.96	24.59
	0.009	30.49	9.97	23.12	23.47
	0.010	31.75	10.81	22.35	22.23
0.01 *M* Potassium acetate–acetic acid at pH_L=4.00	0	27.65	0.00	23.46	23.95
	0.001	28.29	1.04	23.02	23.27
	0.002	28.94	2.02	23.60	23.64
	0.003	29.61	2.99	22.18	23.35
	0.004	30.27	3.93	21.79	23.24
	0.005	30.94	4.85	21.41	23.15
	0.006	31.62	5.74	21.04	22.70
	0.007	32.30	6.61	20.68	22.11
	0.008	32.98	7.45	20.35	21.75
	0.009	33.67	8.28	20.01	21.12
	0.010	34.36	9.08	19.69	20.94
0.01 *M* Potassium formate–formic acid at pH_L=3.75	0	46.69		12.05	12.22
	0.001	46.66		12.14	12.20
	0.002	46.63		12.23	12.44
	0.003	46.60		12.33	12.58
	0.004	46.57		12.42	13.05
	0.005	46.54		12.52	12.84

Behaviour of Al^{3+} in two-buffer electrolytes

In cationic separations, H^+ is often used as a terminator applying two-buffer electrolyte systems, whereby one of the buffering counter ionic species is used because of its complexing properties with the cations. As a complexing agent HIBA often used, whereby several cations migrate in an "enforced" way. A typical example of this phenomenon is the migration of Al^{3+}, where, the concentrations of the HIBA and pH of the leading zone can be critical, resulting in inaccurate and irreproducible quantitative determinations[8].

The mechanism of the "enforced" migration can be easily understood. In the

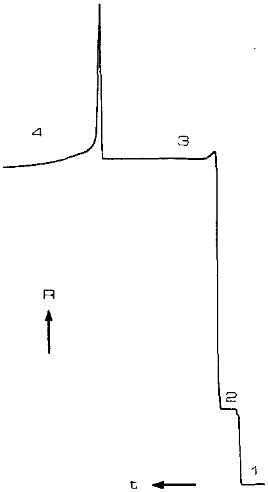

Fig. 8. Isotachopherogram for the separation of Na^+ and Al^{3+} using H^+ as terminator. The leading electrolyte consisted of 0.01 *M* K^+, 0.0034 *M* HIBA and acetic acid at a pH_L of 4.5. (1) K^+; (2) Na^+; (3) Al^{3+}; (4) H^+.

aluminium zone[a], the $HIBA^-$ concentration is fairly high (depending on the concentration of the leading HIBA concentration and pH) pH) and Al^{3+} will form Al–HIBA compleses with a low mobility. If the Al–HIBA complex remains in the terminating H^+ zone, with a lower pH and $HIBA^-$ concentration, the complex will decompose and the Al^{3+} (with its higher mobility) will move forwards, will pass the front of the terminating H^+ zone, will reach its own zone with a higher $HIBA^-$ concentration and will form the less mobile Al–HIBA complexes again. In this way a stationary situation is created whereby Al–HIBA complexes with low effective mobilities migrate in front of the more mobile terminating H^+ zone, because the $HIBA^-$ concentration in the terminating H^+ zone is much lower.

In Fig. 8, a typical isotachopherogram is shown of a sample consisting of Na^+ and Al^{3+} with the terminator H^+. The leading electrolyte was 0.01 *M* KOH with 0.0034 *M* HIBA and acetic acid to a pH_L of 4.5. In Figs. 9 and 10, the istachopherograms of the same mixture in the same system are given with HIBA concentrations of 0.009 and 0.011 *M*. In the latter instance the Al^{3+} does not migrate in the isotachophoretic mode but migrates in a zone electrophoretic mode in the terminating H^+ zone, owing to the higher $HIBA^-$ concentration in the terminator zone.

Using computer programs based on the mathematical models for H^+ as terminator and the use of two-buffer electrolyte systems, were explain this effect quantitatively.

The question is, at what HIBA concentration in the leading electroplyte does Al^{3+} not migrate in the ITP mode? It may be assumed that Al^{3+}will remain in the terminating H^+ zone if the concentration of $HIBA^-$ in this zone is such that an

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[a] It must be borne in mind that when speaking about an aluminium zone, a zone consisting of different ionic forms of aluminium and/or aluminium complexeses is always meant.

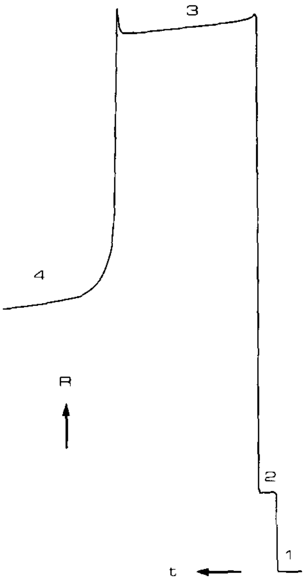

Fig. 9. As Fig. 8, with 0.009 *M* HIBA.

Al–HIBA complex is formed with an effective mobility smaller than that of the H^+ ions. Therefore, it is important to know the relationship between the effective mobilities of Al–HIBA complexes and the $HIBA^-$ concentration in a zone. A principal problem here is that the $HIBA^-$ concentration cannot be changed alone. Measuring these effective mobilities, the varying condition in the zones with a specific $HIBA^-$ concentration must be taken into consideration: a different pH, different concentrations of the complexes and of acetic acid, different activity coefficients etc. We even do

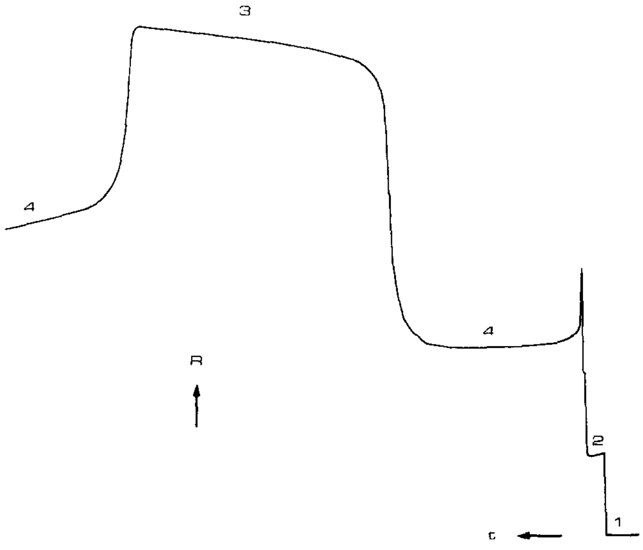

Fig. 10. As Fig. 8, with 0.011 *M* HIBA.

not know the charge and type of the complexes, because in water Al^{3+} shows pH-dependent protolysis reactions in addition to the complexation with $HIBA^-$.

In order to visualize the effect of the pH and HIBA concentration of the leading electrolyte on the charge and migration behaviour of Al^{3+}, we use the fact that the relationships between calculated response factor (RF) and calculated R_E values for mono- and divalent cationic species are different. The RF value[7] is the slope of a calibration graph (in C/mol) of the product lI (zone length l in s and applied electric current I in A) plotted against the amount of the sample injected (in mol). The R_E value[9] is the ratio E_{Al}/E_L.

In fact, these relationships are different for different electrolyte systems. Calculating the relationships for several leading electrolyte systems consisting of 0.01 M KOH with varying concentrations of HIBA between 0 and 0.01 M, at pH_Ls between 3.6 and 4.9 by adding acetic acid, the maximum differences between the values were about 8%. Using the average values of the calculated RF and R_E values, the maximum differences with all electrolyte systems are 4%. In Fig. 11 the average calculated RF values are plotted against the R_E values.

For several systems, with different pHs and different HIBA concentrations in the leading electrolyte, we measured the RF and R_E values for the aluminium zone. In Table V the experimentally determined RF and R_E values are given and are plotted in Fig. 11. Although this procedure is an estimation (remember that for all systems, parameters such as ionic strength and pH in the aluminium zone are slightly different), we can observe some interesting points. Line I shows the shift in the RF value as a function of increasing pH between 3.6 and 4.9. At a low pH of about 3.6 aluminium

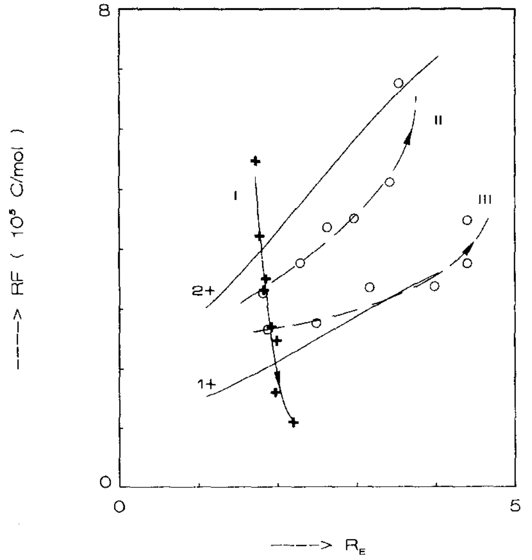

Fig. 11. Relationship between average calculated RF and R_E values for (1+) strong monovalent and (2+) strong divalent cations and between measured RF and R_E values for Al^{3+} for several leading electrolytes, (I) without HIBA at a varying pH_L of 3.6–4.9, (II) with varying $[HIBA]_L$ at a pH_L of 4.0 and (III) with varying $[HIBA]_L$ at a pH_L of 4.5. The arrows indicate increasing pH_L and $[HIBA]_L$ respectively. For further explanation, see text.

TABLE V

EXPERIMENTALLY OBTAINED RESPONSE FACTORS (*RF*) AND R_E VALUES OF ALUMINIUM ZONES IN SEVERAL LEADING ELECTROLYTE SYSTEMS BASED ON 0.01 *M* POTASSIUM ACETATE–ACETIC ACID

For further explanation, see text.

pH_L	*Concentration of HIBA in the leading zone (M)*	*RF (10^5 C/mol)*	R_E
4.5	0.0000	2.66	1.87
	0.0002	2.76	2.49
	0.0034	3.35	3.16
	0.0050	3.37	3.98
	0.0070	3.76	4.40
	0.0090	4.48	4.40
4.0	0.0000	3.26	1.81
	0.0002	3.77	2.29
	0.0030	4.36	2.63
	0.0040	4.51	2.97
	0.0060	5.12	3.42
	0.0080	6.76	3.53
3.6	0.0000	5.47	1.72
3.8	0.0000	4.22	1.77
4.0	0.0000	3.50	1.85
4.1	0.0000	3.31	1.82
4.3	0.0000	2.70	1.92
4.5	0.0000	2.47	1.99
4.8	0.0000	1.61	1.97
4.9	0.0000	1.10	2.20

seems to behave as a trivalent cation whereas at increasing pH its effective charge decreases. The fact that at pH 4.9 its *RF* value strongly decreases means that the aluminium zone is not stable and that the substance remains in the terminator zone, as can also be concluded from the negative axis-intercept of the calibrationgraphs. At higher pH (between 4.5 and 4.9) the aluminium zone is often not a single step. These effects may be connected with the slow protolysis equilibria of Al^{3+}.

Lines II and III show the changes in the *RF* values due to the effect of complex formation with $HIBA^-$ at a pH_L of 4.0 (line II) and 4.5 (line III). The average charge at a pH_L of 4.0 is about 2+ and at a pH_L of 4.5 the charge is 1+ (larger effect of the protolysis of Al^{3+}). In order to determine the effective mobility of the Al–HIBA complex as a function of the $HIBA^-$ concentration in a zone, we measured in several leading electrolytes at a pH_L of 4.5 and 4.0 with different HIBA concentrations the SZR_{25} values of the Al–HIBA zone and calculated the effective mobility of the Al–HIBA complex zone using the relationship

$$\bar{m}_{\text{Al-HIBA}} = \bar{m}_{\text{L}} SZR_{25,\text{L}}/SZR_{25,\text{Al-HIBA}}$$

From the effective mobilities the absolute mobilities can be calculated assuming the

TABLE VI

ABSOLUTE MOBILITIES AND HIBA⁻ CONCENTRATIONS IN THE ALUMINIUM ZONES, CALCULATED FROM EXPERIMENTAL DETERMINED STEP HEIGHTS FOR ELECTROLYTE SYSTEMS AT A pH_L OF 4.0 AND 4.5, FOR SEVERAL HIBA CONCENTRATIONS IN THE LEADING ELECTROLYTE

For further explanation, see text.

Concentration of HIBA in the leading zone (M)	*0.01 M potassium acetate–acetic acid*			
	pH_L = 4.50		*pH_L = 4.00*	
	[$HIBA^-$] (10^{-4} M)	*m_{Al} (10^{-5} $cm^2/V \cdot s$)*	*[$HIBA^-$] (10^{-4} M)*	*m_{Al} (10^{-5} $cm^2/V \cdot s$)*
0.000	0.00	39.42	0.00	42.26
0.001	5.53	33.92	3.85	37.24
0.002	10.37	30.47	7.15	33.68
0.003	14.16	26.38	9.82	29.89
0.004	16.98	22.79	11.72	26.59
0.005	19.31	20.30	13.35	24.71

charge of the complex. As no large differences are obtained using a charge of 1+ or 2+, the calculated absolute mobilities, arbitrarily assuming a charge of 1+, are given in Table VI. For the determination of the SZR_{25} values we used K^+ and Na^+ as standards.

Because for trivalent, divalent and monovalent ionic species the relationships between the SZR_{25} values and the [$HIBA^-$] were nearly identical, we could also calculate the [$HIBA^-$] in these Al–HIBA zones, irrespective of the charge.

In Fig. 12 the absolute mobilities of the Al–HIBA zones and the effective mobilities of the terminating H^+ zones for the systems at pH_L of 4.5 and 4.0 are plotted against the actual zone [$HIBA^-$]. Although the different points were obtained from different leading electrolyte systems, *i.e.*, the actual pHs, activity coefficients, etc., are slightly different, it can be concluded, as an estimation, that at an [$HIBA^-$] of $7.4 \cdot 10^{-4}$ *M* for the pH_L of 4.0 and at 10^{-3} *M* for the pH_L of 4.5 the absolute mobility of the Al–HIBA complex is lower than that of the terminating H^+ ions and Al^{3+} will not migrate in an enforced system at this [$HIBA^-$]. In fact, we ought to calculate the effective mobility from the absolute mobility of the complex, but the difference will be only a few percent because the ionic strength in the terinator zone is low.

In Fig. 13, the [$HIBA^-$] in the terminating H^+ zone is given as a function of the leading $[HIBA]_L$ (for data see Table IV). It can be concluded that [$HIBA^-$] values of $7.4 \cdot 10^{-4}$ and 10^{-3} *M* correlate with $[HIBA]_L$ values of about 0.008 and 0.009 *M*, respectively.

To find experimentally the $[HIBA]_L$ at which Al^{3+} does not migrate in the ITP mode, we carried out separations of a mixture of Na^+ and Al^{3+} in leading electrolyte systems with different $[HIBA]_L$ and found experimentally values of about 0.008 and 0.009 *M*.

It must be noted that in practice these $[HIBA]_L$ values depend both on the amount of the sample and length of the capillary tube. In isotachophoretic equipment

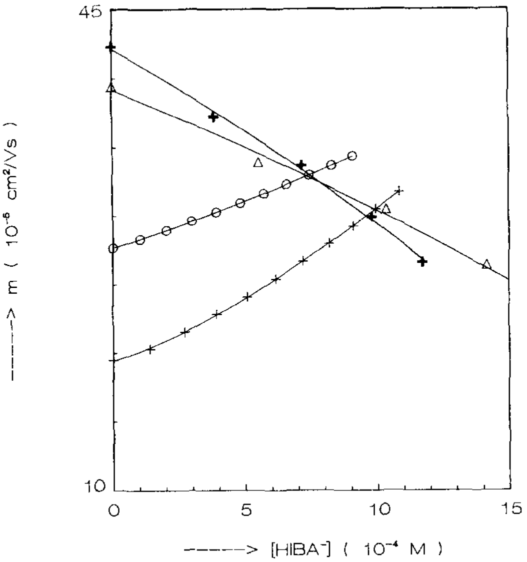

Fig. 12. Relationship between calculated mobilities and calculated [HIBA$^-$] for terminating H$^+$ ions at a pH$_L$ of (+) 4.5 and (○) 4.0 and between the absolute mobility and calculated [HIBA$^-$] for Al–HIBA complexes at a pH$_L$ of (△) 4.5 and (+) 4.0. For further explanation, see text.

with a very sort capillary tube, higher critical [HIBA]$_L$ values were obtained, whereas in longer capillary tubes these [HIBA]$_L$ values were smaller, indicating that in these instances overruling of the Al–HIBA zone by the terminating H$^+$ ions occurs, which is time and concentration dependent.

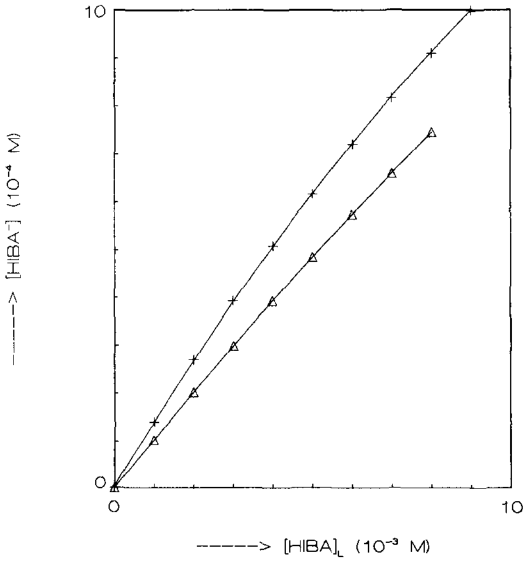

Fig. 13. Relationship between calculated [HIBA$^-$] in the terminating H$^+$ zone and [HIBA]$_L$ in the leading zone for a pH$_L$ of (+) 4.5 and (△) 4.0. For further explanation, see text.

CONCLUSION

Comparison of calculated and experimentally obtained values shows that the mathematical model presented is useful for the calculation of the effective mobility of terminating H^+ ions and also for two-buffer electrolyte systems. The relationship between RF and R_E values can be used to obtain information about the charge of complexes. With these tools the "enforced" migration behaviour of aluminium in two-buffer electrolytes can be understood. Measurements of the RF values show that the quantitative determination of aluminium is strongly affected by the pH and [HIBA] of the leading electrolyte. Similar problems can be expected in analyses of cations such as Fe^{3+}, Cr^{3+} and Zr^{2+}/Z^{4+}.

SYMBOLS

A	Sample ionic species A
B	Buffering counter ionic species B
E	Electric field strength (V/m)
F	Faraday constant (C/equiv.)
K	Concentration equilibrium constant
L	Leading ionic species L
m	Mobility at infinite dilution ($m^2/V \cdot s$)
$\bar{m}$	Effective mobility ($m^2/V \cdot s$)
n	Number of protolysis steps
R_E	The electric field strength in a zone divided by the electric field strength of the leading zone
RF	Response factor (C/mol)
SZR_{25}	Specific zone resistance at 25°C (Ω m)
T	Terminating ionic species
z	Charge of an ionic species (equiv./mol)
α	Degree of dissociation
σ	Zone conductivity ($\Omega^{-1}m^{-1}$)

First subscripts

A, B, T and L	According to substance A, B, T and L
t	Total

Second subscripts

A, B, T and L	In the zon of substance A, B, T and L
H	In terminating H^+ zone

Supercripts

z	Maximum charge of an ionic species
$( )^i$	To the ith power

Examples

$[B]_{t,A}$	Total concentration of substance B in zone A
$\bar{m}_{B,A}$	Effective mobility of substance B in the zone of substance A

Abbreviations

HIBA	α-Hydroxyisobutyric acid
$HIBA^-$	α-Hydroxyisobutyrate

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