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Isotachopheresis with two leading ions and migration behaviour in capillary zone electrophoresis

I. Isotachopheresis with two leading ions

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(First received December 1st, 1989; revised manuscript received January 22nd, 1990)

SUMMARY

A mathematical model for isotachopheresis with two leading ions is presented and, based on this model, computer programs are set up for the calculation of the parameters of the different zones. With this model it is possible to calculate at what level a second leading ion may be present, *e.g.*, carbonate ions in the separation of anions at a high pH, without disturbing the isotachopheretic separation.

Calculated values of the specific zone resistance and response values are compared with values experimentally obtained showing good agreement. Calculations with this model show that with the presence of a second leading ion the zone lengths elongate (higher values of the response factor) and at a certain concentration ratio of the two leading ions, also dependent on the effective mobilities of the ions, the isotachopheretic condition is lost and isotachopheresis changes into zone electrophoresis.

INTRODUCTION

Sometimes disturbances in isotachopheretic (ITP) separations can occur as a result of the presence of an (unwanted) second leading ion in the leading electrolyte (*e.g.*, carbonate ions in the separation of anions at high pH). Mikkers and Everaerts¹ mentioned earlier that ITP with two leading ions can be applied. In this paper, a previously described mathematical model for the steady state in ITP^{2,3} is extended for the use of leading electrolytes with two leading ions in order to establish the level at which a second leading ion can be present without disturbing the ITP separation. Calculations with this model show that at a certain concentration ratio of the two leading ions, also dependent on the effective mobilities of these ions, the ITP condition is lost. At that point, ITP changes into zone electrophoresis (ZE).

With this model, it can be understood why sometimes extremely high plate numbers can be obtained in capillary zone electrophoresis (CZE), as will be explained in Part II.

THEORY

Using a mathematical model for the description of the steady state in ITP, the reduced number of parameters is always four in all zones, viz., $[L]_t$ or $[A]_t$, $[B]_t$, E and pH. For all zones always four known parameters and/or equations are necessary, by means of which all parameters can be calculated. For the leading zone the known parameters are generally $[L]_t$ and $[B]_t$ and the equations are Ohm's law and the electroneutrality (EN). For all other zones the four available equations are the EN, Ohm's law, the buffer equation and the isotachophoretic condition (IC).

Considering a leading electrolyte with two leading ions (a so-called 2L system), the reduced number of parameters will be five for all zones. For the leading electrolyte the parameters are $[L1]_t$, $[L2]_t$, $[B]_t$, E and pH, where L1 is the leading ion with the highest and L2 that with the lowest effective mobility. For the sample zones the parameters will be $[A]_t$, $[L2]_t$, $[B]_t$, E and pH, under the assumption that L2 will remain behind and will form a mixed zone with the sample ions. Consequently, five known parameters and/or equations are needed for the calculations, viz., for the leading zone $[L1]_t$, $[L2]_t$, $[B]_t$, Ohm's law and the EN and for the sample zones the EN, Ohm's law, the buffer balance, the L2 balance and the IC.

For the use of the last equation, the crucial question is whether a component migrates in the ITP mode, because in principle it is not essential that a sample zone must follow the L1 zone owing to the presence of a second leading ion partially remaining behind in the preceding zone.

If the amount of L2 remaining behind L1 is sufficient to form a terminating L2 zone without any sample ionic species and if this terminating L2 zone has an E gradient smaller than that of a sample zone, the IC is no longer valid. In fact, the ITP mode changes into a ZE mode, whereby that sample zone migrates in a ZE way in the terminating L2 zone.

In our treatment, we assume that a sample ion migrates in the ITP mode if the calculated value of the specific zone resistance⁴ at 25°C (SZR_{25}) or the E are smaller than those of the terminating L2 zone. For the terminating L2 zone the reduced number of parameters is only four, viz., $[L2]_t$, $[B]_t$, E and pH. Only four equations are needed for the calculations, viz., the EN, Ohm's law, the buffer equation and the L2 balance.

The mathematical model is therefore extended in two ways, viz., for the calculation of parameters for a terminating L2 zone and for sample zones in a 2L system.

Mathematical model for the terminating L2 zone in a 2L system

At known pH, the concentrations of all ionic forms of a substance A with a charge of $z-i$ can be expressed as the total concentration of A by³

$$[A^{z-1}] = [A^z] \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i} = [A]_t \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i} \left(1 + \sum_{i=1}^n \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i} \right) \quad (1)$$

where K_j are the concentration equilibrium constants for the n protolysis reactions.

Tiselius⁵ 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

$$\bar{m} = \sum_{i=0}^n \alpha_i m_i = \sum_{i=0}^n [A^{z-i}] m_{z-i} / [A]_t \quad (2)$$

For simplicity, the effect of the ionic strength is not considered in this equation. In the computer programs, however, a correction is made for this effect using the Debye-Hückel-Onsager relation.

Substituting eqn. 1 into eqn. 2, we can write for the effective mobility of an ionic species A

$$\bar{m} = \frac{\sum_{i=1}^n m_{z-i} \frac{\prod_{j=1}^i K_j}{[\text{H}_3\text{O}^+]^i} + m_z}{1 + \sum_{i=1}^n \frac{\prod_{j=1}^i K_j}{[\text{H}_3\text{O}^+]^i}} \quad (3)$$

Although in these general descriptions of the 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.

In addition to the general descriptions of the equilibria and effective mobilities of ionic species, we further need the principle of electroneutrality, the modified Ohm's law, the mass balance of the buffer and the mass balance of the leading ion L2 with the lowest effective mobility to describe the "steady-state" for the terminating L2 zone in ITP for two leading ions.

The principle of electroneutrality. In accordance with the principle of electroneutrality (EN), the arithmetic 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

$$[\text{H}_3\text{O}^+] - [\text{OH}^-] + \sum_{i=0}^{n_A} (z-i)[A^{z-i}] + \sum_{i=0}^{n_B} (z-i)[B^{z-i}] = 0 \quad (4)^a$$

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

$$E_L \sigma_L = E_T \sigma_T \quad (5)$$

^a Arbitrarily the contribution of a sample ion A and a buffer ion B are given. Of course, for the leading zone in an L2 system the contribution of L1, L2 and B and for the terminating L2 zone the contribution of L2 and B have to be taken into account.

or the function

$$RFQ = E_L \sigma_L / E_T \sigma_T - 1 \quad (6)$$

must be zero.

The overall electric conductivity σ of a zone is the sum of the values $c|\bar{m}z|F$ and consequently

$$E\{[\text{H}_3\text{O}^+][\bar{m}_\text{H}] + [\text{OH}^-][\bar{m}_\text{OH}] + \sum_{i=0}^{n_A} [\text{A}^{z-i}][\bar{m}_{z-i}(z-i)] + \sum_{i=0}^{n_B} [\text{B}^{z-i}][\bar{m}_{z-i}(z-i)]\} \quad (7)^a$$

in all zones is constant.

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 (8A) can be derived (see Fig. 1).

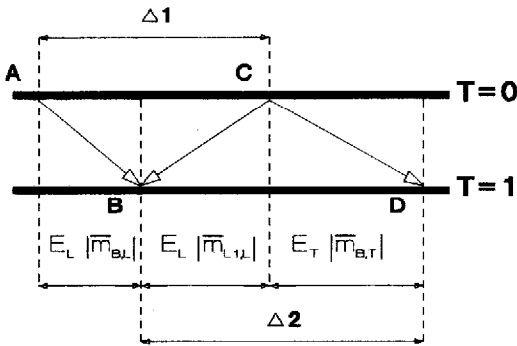


Fig. 1. Migration paths of the buffering counter ionic species over a zone boundary between a leading zone with two leading ions L1 and L2 and the terminating L2 zone.

The zone boundary between the leading electrolyte consisting of L1 and L2 and the terminating zone with L2, present at $t = 0$ at point C, moves in unit time to point B at $t = 1$ over a distance BC of $E_L|\bar{m}_{L1,L}|$. The buffer ionic species at time $t = 0$ present at the zone boundary C will reach point D at $t = 1$. The distance from C to D will then be $E_T|\bar{m}_{B,T}|$. The buffer ionic species at $t = 0$ present at point A will just reach the boundary in B at $t = 1$. The distance from A to B is then $E_L|\bar{m}_{B,L}|$.

This means that all buffer ionic particles present in the leading zone between A and C with a concentration $[\text{B}]_{t,L}$ at time $t = 0$ ($\Delta 1$) will be present in the terminating zone with a concentration $[\text{B}]_{t,T}$ between B and D at $t = 1$ ($\Delta 2$). Therefore, the buffer mass balance will be

$$[\text{B}]_{t,T}(E_T|\bar{m}_{B,T}| + E_L|\bar{m}_{L1,L}|) = [\text{B}]_{t,L}(E_L|\bar{m}_{B,L}| + E_L|\bar{m}_{L1,L}|) \quad (8A)$$

^a See footnote on p. 5.

or

$$[B]_{i,T}(E_T|\bar{m}_{B,T}|/E_L + |\bar{m}_{L1,L}|) = [B]_{i,L}(|\bar{m}_{B,L}| + |\bar{m}_{L1,L}|) \quad (8B)$$

Mass balance of L2. The zone boundary between the leading electrolyte consisting of L1 and L2 and the terminating zone with L2 present at $t = 0$ at point E (see Fig. 2) moves in unit time to point B at $t = 1$ over a distance EB of $E_L|\bar{m}_{L1,L}|$. The leading ionic species L2 (and terminating ionic species at the same time!) at time $t = 0$ present at point F will just reach C at $t = 1$. The distance from F to C will then be $E_T|\bar{m}_{L2,T}|$. The leading ionic species L2 at $t = 0$ present at point D will reach point A at $t = 1$. The distance from D to A is then $E_L|\bar{m}_{L2,L}|$.

This means that all ionic particles L2 present between D and F (partially in the leading and partially in the terminating zone) at time $t = 0$ ($\Delta 1$) will be present between C and A at $t = 1$ ($\Delta 2$). The mass balance for ionic species L2 will therefore be

$$E_L|\bar{m}_{L1,L}|[L2]_{i,L} + E_T|\bar{m}_{L2,T}|[L2]_{i,T} = E_L|\bar{m}_{L2,L}|[L2]_{i,L} + E_L|\bar{m}_{L1,L}|[L2]_{i,T} \quad (9A)$$

or

$$|\bar{m}_{L1,L}|[L2]_{i,L} + E_T/E_L|\bar{m}_{L2,T}|[L2]_{i,T} = |\bar{m}_{L2,L}|[L2]_{i,L} + |\bar{m}_{L1,L}|[L2]_{i,T} \quad (9B)$$

It can be seen that if $[L2]_{i,L}$ is zero, this balance changes into the isotachophoretic condition

$$E_T/E_L|\bar{m}_{L2,T}|[L2]_{i,T} = |\bar{m}_{L1,L}|[L2]_{i,T} \quad (10A)$$

or

$$E_T|\bar{m}_{L2,T}| = E_L|\bar{m}_{L1,L}| \quad (10B)$$

Calculation procedure. If a pH_L is assumed, then $[H_3O^+]$ and $[OH^-]$ can be calculated. If $[L1]_{i,L}$, $[L2]_{i,L}$ and $[B]_{i,L}$ are known, all ionic concentrations of the ionic

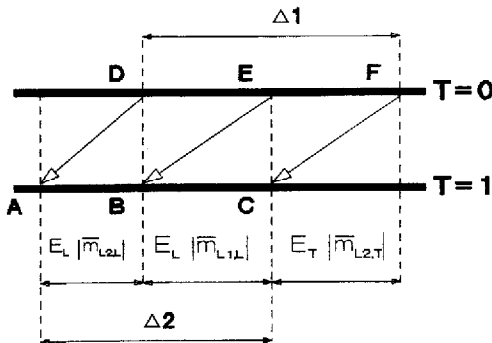


Fig. 2. Migration paths for the ionic species L2 over a zone boundary between a leading zone with two leading ions L1 and L2 and the terminating L2 zone.

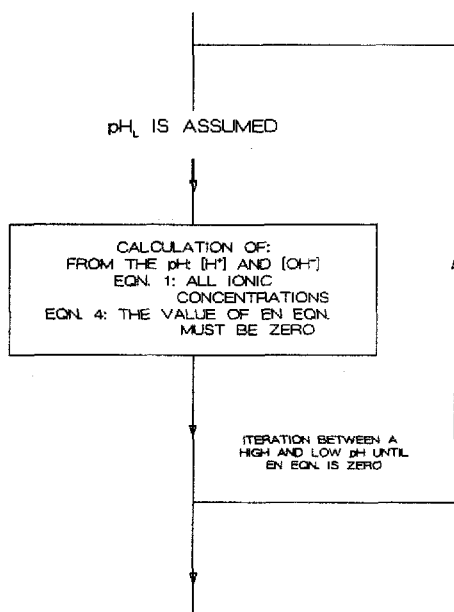


Fig. 3. Calculation procedure for the leading zone in ITP with two leading ions if the total concentrations of both the leading and buffering counter ionic species are known.

species can be calculated and by iterating between a low and high pH_L until the EN equation is met, the right pH_L can be obtained (see Fig. 3). For the calculation of the terminating L2 zone, first a pH_T is assumed (see Fig. 4), then the ratio E_T/E_L is assumed. From the buffer equation the buffer concentration and from the L2 balance the L2 concentration can be obtained. Using eqn. 1, all ionic concentrations can be obtained. Iterating, at the chosen pH_T , between a low and high E_T/E_L value the correct E_T/E_L value can be obtained using the EN. The correct pH_T can be found iterating between a low and high pH_T value using Ohm's law (see Fig. 4).

Mathematical model for the consecutive zones in a 2L system

The calculation of the parameters for the leading zone for a leading electrolyte with a known composition is similar to that for the foregoing model (see Fig. 3).

For the calculation of the sample zones in a 2L system, five equations are needed. The EN equation and Ohm's law are identical with those in the foregoing model, substituting the contribution of the ionic species L2 and A in these equations. The other three equations are the isotachophoretic condition and the mass balances of the buffer and L2 ions.

The isotachophoretic condition. In the steady state, all zones move with a velocity equal to that of the leading zone. This velocity is determined by the velocity of the L1 ion in the leading zone, and therefore

$$E_L \bar{m}_{L1,L} = E_A \bar{m}_{A,A} \quad (11)$$

Mass balance of the buffer. The mass balance of the buffer can be obtained by

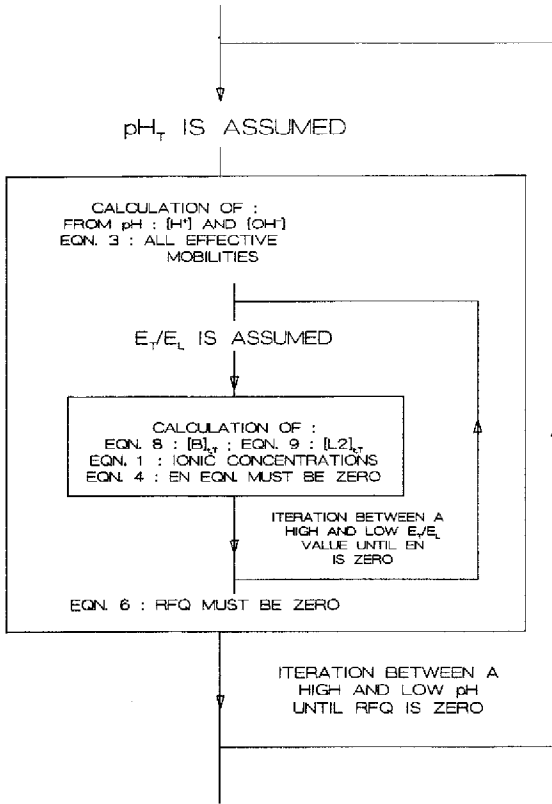


Fig. 4. Calculation procedure for the terminating L2 zone in ITP with two leading ions.

exchanging all parameters of the terminating zone by those of the A zone in eqn. 8. The mass balance will be

$$[B]_{i,A}(E_A|\bar{m}_{B,A}| + E_L|\bar{m}_{L1,L}|) = [B]_{i,L}(E_L|\bar{m}_{B,L}| + E_L|\bar{m}_{L1,L}|) \quad (12)$$

L2 balance. In a similar way to that described for the buffer balance, the L2 balance can be obtained by exchanging the T indices with A indices in eqns. 9. The L2 balance will be

$$E_L|\bar{m}_{L1,L}|[L2]_{i,L} + E_A|\bar{m}_{L2,A}|[L2]_{i,A} = E_L|\bar{m}_{L2,L}|[L2]_{i,L} + E_L|\bar{m}_{L1,L}|[L2]_{i,A} \quad (13A)$$

or

$$|\bar{m}_{L1,L}|[L2]_{i,L} + E_A/E_L|\bar{m}_{L2,A}|[L2]_{i,A} = |\bar{m}_{L2,L}|[L2]_{i,L} + |\bar{m}_{L1,L}|[L2]_{i,A} \quad (13B)$$

Calculation procedure. If a pH_A is assumed (see Fig. 5), then $[\text{H}^+]$ and $[\text{OH}^-]$ can be calculated and with eqn. 3 the effective mobilities can be calculated. With the IC (eqn. 11) the ratio E_A/E_L can be obtained. With the buffer balance the $[B]_{i,A}$ and with

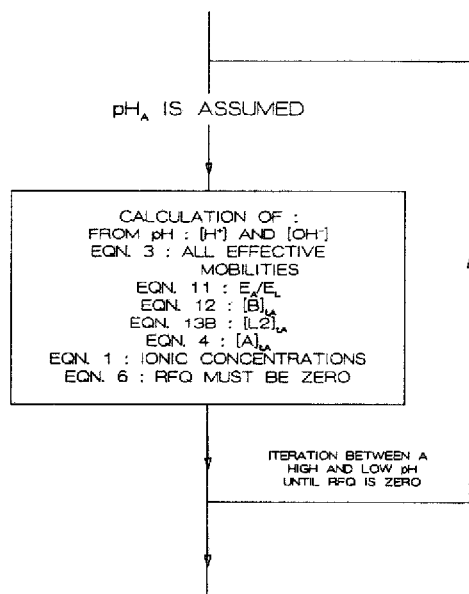


Fig. 5. Calculation procedure for the sample zones in ITP with two leading ions.

the L2 balance the $[L2]_{i,A}$ can be calculated. With the EN $[A]_{i,A}$ can be obtained. The correct pH_A can be found iterating between a low and high pH using Ohm's law.

EXPERIMENTAL

In the first instance, computer programs are set up for the calculation of parameters of the sample and terminating L2 zones with leading electrolytes consisting of two leading ions.

In Table I all ionic mobilities and pK values used in the calculations are given. In Table II some characteristic calculated parameters for the leading, sample and terminating L2 zone are given using these computer programs, *viz.*, the concentrations

TABLE I

pK VALUES AND IONIC MOBILITIES (m) FOR THE IONIC SPECIES USED IN THE CALCULATIONS

<i>Ionic species</i>	$m \times 10^5$ ($cm^2/V \cdot s$)	pK_A
Acetic acid	-42.4	4.76
Formic acid	-56.6	3.75
Histidine	+29.7	6.03
Hydrochloric acid	-79.1	-3.00
MES	-28.0	6.10
Propionic acid	-37.1	4.87

TABLE II

CALCULATED VALUES OF THE TOTAL CONCENTRATIONS OF THE SAMPLE IONS ($[A]_t$) AND L2 ($[L2]_t$), pH, SPECIFIC ZONE RESISTANCE AT 25°C (SZR_{25}), R_E AND RF FOR THE LEADING ZONE, SAMPLE ZONES AND TERMINATING L2 ZONE FOR SEVERAL COMPOSITIONS OF THE LEADING ELECTROLYTE AT A pH OF 6

Zone	$[A]_t$ (mol/l)	$[L2]_t$ (mol/l)	pH	SZR_{25} (Ω m)	R_E	RF (10^5 C/mol)
<i>Leading: 0.01 M Cl⁻ + 0 M MES at pH 6:</i>						
Leading L1/L2	—	0	6.0	10.33	1.00	1.30
70/3	0.0097	0	6.02	11.73	1.14	1.35
60/3	0.0092	0	6.04	13.76	1.33	1.42
50/3	0.0086	0	6.06	16.63	1.61	1.52
40/3	0.0078	0	6.10	21.01	2.03	1.67
30/3	0.0068	0	6.16	28.48	2.76	1.92
20/3	0.0053	0	6.27	43.93	4.26	2.46
Terminating L2	—	0.0065	6.42	43.21	4.18	—
<i>Leading: 0.01 M Cl⁻ + 0.005 M MES at pH 6:</i>						
Leading L1/L2	—	0.0050	6.0	9.33	1.00	1.45
70/3	0.0094	0.0052	6.02	10.60	1.14	1.54
60/3	0.0086	0.0054	6.04	12.43	1.33	1.69
50/3	0.0075	0.0058	6.08	15.04	1.61	1.94
40/3	0.0058	0.0067	6.13	19.02	2.04	2.51
30/3	0.0020	0.0096	6.24	25.74	2.76	7.43
20/3	No real values					
Terminating L2	—	0.0115	6.29	27.76	2.98	—
<i>Leading: 0.01 M Cl⁻ + 0.01 M MES at pH 6:</i>						
Leading L1/L2	—	0.0100	6.00	8.51	1.00	1.60
70/3	0.0092	0.0103	6.02	9.68	1.14	1.74
60/3	0.0080	0.0108	6.04	11.36	1.33	1.99
50/3	0.0064	0.0117	6.08	13.75	1.62	2.52
40/3	0.0036	0.0135	6.14	17.39	2.04	4.50
30/3	No real values					
20/3	No real values					
Terminating L2	—	0.017	6.22	20.90	2.45	—
<i>Leading: 0.01 M Cl⁻ + 0.015 M MES at pH 6:</i>						
Leading L1/L2	—	0.0150	6.00	7.84	1.00	1.75
70/3	0.0090	0.0155	6.02	8.92	1.14	1.95
60/3	0.0074	0.0162	6.05	10.48	1.34	2.35
50/3	0.0052	0.0176	6.09	12.69	1.62	3.37
40/3	0.0013	0.0205	6.16	16.06	2.05	14.02
30/3	No real values					
Terminating L2	—	0.0215	6.18	16.93	2.16	—
<i>Leading: 0.01 M Cl⁻ + 0.02 M MES at pH 6:</i>						
Leading L1/L2	—	0.0200	6.00	7.28	1.00	1.90
70/3	0.0087	0.0206	6.02	8.27	1.14	2.17
60/3	0.0069	0.0216	6.05	9.72	1.34	2.77
50/3	0.0040	0.0234	6.09	11.78	1.62	4.72
40/3	No real values					
30/3	No real values					
Terminating L2	—	0.0265	6.15	14.30	1.96	—

of the sample ions A and L2, the pH of the zones, the specific zone resistances at 25°C (SZR_{25}), the R_E values and the RF values⁴. The leading electrolytes consisted of 0.01 M Cl^- (L1) and different concentrations of MES (L2), at a pH_L of 6 by adding the buffer histidine. For the sample ionic species a pK of 3 was assumed and ionic mobilities varying from -70 to $-20 \cdot 10^{-5} \text{ cm}^2/\text{V} \cdot \text{s}$. A sample ionic species denoted by 30/3 has a pK value of 3 and an anionic mobility of $30 \cdot 10^{-5} \text{ cm}^2/\text{V} \cdot \text{s}$.

It can be concluded from Table II that with increasing concentration of L2 in the leading zone for all sample zones the SZR_{25} values decrease (increasing conductivity), the R_E values are nearly constant (according to the IC) but the R_E value of the terminating L2 zone decreases rapidly to low values, implying that a growing number of sample ions with low effective mobility will be passed by the terminator and consequently will not migrate in the ITP mode. In Fig. 6 the relationship between the calculated R_E values and the anionic mobility is shown. The arrows indicate the R_E value of the terminating L2 zone for a specific concentration of L2 in the leading electrolyte.

Ionic species with R_E values smaller than those of the terminating L2 zone will migrate in a proper ITP mode and ionic species with higher R_E values will migrate in a ZE mode. Further, the RF values of the sample ions increase at the same time because the sample ion concentrations are decreasing.

As a check on these mathematical models, we measured the RF values for formate, acetate and propionate in leading 2L systems, consisting of 0.01 M Cl^- and different concentrations of MES at a pH_L of 6 by adding the counter ionic species histidine. In Table III all calculated and experimentally determined RF and SZR_{25}

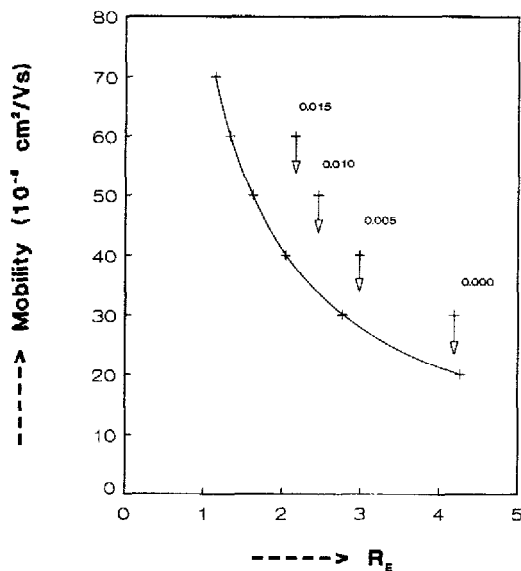


Fig. 6. Relationship between calculated R_E values and anionic mobility of ionic species for ITP systems with 0.01 M Cl^- and various concentrations of MES as leading ions at a pH_L of 6. The arrows indicate the calculated R_E values of the terminating MES zone. The numbers represent the concentration of MES (in M) in the leading electrolyte.

TABLE III

CALCULATED AND EXPERIMENTALLY DETERMINED SZR_{25} VALUES (Ω m) AND RF VALUES (10^5 C/mol) FOR THE ZONES IN A 2L SYSTEM WITH 0.01 M Cl^- AND DIFFERENT MES CONCENTRATIONS AS LEADING IONS AT A pH_L OF 6

Value	Leading [MES] (M)	Calc.	Exptl.	Formate		Acetate		Propionate		Terminating	
				Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.
				Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.
SZR_{25}	0.000	10.33	10.33	14.67	14.61	20.50	20.49	23.78	23.76	43.21	39.55
	0.005	9.33	9.51	13.27	13.34	18.52	18.70	21.45	21.39	27.76	26.29
	0.010	8.51	8.45	12.12	11.91	16.94	16.61	19.60	19.02	20.90	19.76
				Formate		Acetate		Propionate			
				Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.		
RF	0.000	1.45	1.41	1.62	1.61	1.72	1.71				
	0.005	1.76	1.80	2.41	2.71	3.15	3.68				
	0.010	2.13	2.25	4.07	5.00	10.81	12.20				

values are given. It can be concluded that the experimentally determined SZR_{25} and RF values fit the calculated values, although for high concentrations of MES in the leading electrolyte the experimentally determined RF values are too high. Possibly the steady state was not reached because very long zones with small differences in SZR_{25} values were the result.

In addition to the strong shift in RF values, another interesting point was the decreasing effect of the R_E values of the terminating L2 zone. For systems with 0.01 M Cl^- and MES concentrations varying from 0 to 0.02 M in the leading electrolyte, the R_E values of the terminating L2 zone changed from 4.18 to 1.96. It can be assumed, however, that at relative higher concentrations of L2 in the leading zones this effect will be even greater.

In Table IV the results of the calculations are given for leading electrolytes with 0.01 M MES and Cl^- concentrations varying from 0 to 0.01 M for the sample ionic species formate, acetate and propionate. The column mode indicates whether the ionic species really migrate in the ITP or CZE mode during experiments.

In Fig. 7 all electropherograms with the sample ionic species formate (F), acetate (A) and propionate (P) are shown, obtained using different leading electrolytes at pH_L 6 by adding the buffer histidine. The terminating zones are indicated with L2, the starting point of the analyses with an arrow and the leading electrolyte with two numbers indicating the concentrations of Cl^- and MES of the two leading ions. The experiments were carried out in a laboratory-made ITP apparatus with a conductivity detector (I.D. 0.4 mm)². The electric current was 70 μ A and the paper speed was 10 mm/min for all experiments. In order to see the ITP zones, large amounts of the sample have to be introduced. The sample consists of a mixture of 0.01 M formate, 0.01 M acetate and 0.01 M propionate. In this instance the zone electrophoretic zones are very broad and small because the amplification of the signals was the same as in the ITP experiments. Further, the rear sides of the zone electrophoretic peaks are sharp because the effective mobilities of the sample anions are higher than that of the anion

TABLE IV

CALCULATED VALUES OF THE CONCENTRATIONS OF SAMPLE IONS ($[A]_i$) AND L2 ($[L2]_i$), THE MIGRATION MODE, THE SPECIFIC ZONE RESISTANCE AT 25°C (SZR_{25}), R_E AND RF FOR THE LEADING ZONE, SAMPLE ZONE AND TERMINATING L2 ZONE FOR SEVERAL COMPOSITIONS OF THE LEADING ZONE AT A pH_L OF 6

Zone	$[A]_i$ (mol/l)	$[L2]_i$ (mol/l)	Mode	SZR_{25} (Ω m)	R_E	RF (10^5 C/mol)
<i>Leading: 0 M Cl^- + 0.01 M MES:</i>						
Leading L1/L2	—	0.01	—	42.35	1.00	—
Formate	No real values		Zone			
Acetate	No real values		Zone			
Propionate	No real values		Zone			
<i>Leading: 0.002 M Cl^- + 0.01 M MES:</i>						
Leading L1/L2	—	0.01	—	23.26	1.00	—
Formate	0.00029	0.011	ITP?	32.94	1.42	19.7
Acetate	No real values		Zone			
Propionate	No real values		Zone			
Terminating L2	No real values					
<i>Leading: 0.004 M Cl^- + 0.01 M MES:</i>						
Leading L1/L2	—	0.01	—	16.12	1.00	—
Formate	0.0021	0.0111	ITP	22.87	1.42	3.95
Acetate	No real values		Zone			
Propionate	No real values		Zone			
Terminating L2	—	0.0126	ITP	29.34	1.82	—
<i>Leading: 0.007 M Cl^- + 0.01 M MES:</i>						
Leading L1/L2	—	0.01	—	11.11	1.00	—
Formate	0.00481	0.0111	ITP	15.79	1.42	2.53
Acetate	0.00152	0.0133	ITP	22.01	1.98	8.00
Propionate	No real values		Zone			
Terminating L2	—	0.0146	ITP	24.34	2.19	—
<i>Leading: 0.01 M Cl^- + 0.01 M MES:</i>						
Leading L1/L2	—	0.01	—	8.51	1.00	—
Formate	0.00752	0.0111	ITP	12.11	1.42	2.13
Acetate	0.00394	0.0133	ITP	16.94	1.99	4.07
Propionate	0.00148	0.0152	ITP	19.6	2.30	10.81
Terminating L2	—	0.0165	ITP	20.90	2.45	—

of the background electrolyte (self-correcting boundary) where the fronts are diffuse.

However, some interesting conclusions can be drawn (see also Table IV). In the ITP experiment with the leading ion Cl^- at a concentration of 0.01 M (0.01/0, injected sample volume 4 μ l), all sample ions migrate in the ITP mode. Using the leading ions 0.01 M Cl^- and 0.01 M MES (injected sample volume 3 μ l), the sample ions also migrate in the ITP mode, although a relatively strong elongation of the propionate can be observed. In the system 0.007/0.01, formate and acetate migrate in the ITP mode (injected sample volume 2 μ l; note the elongation of the acetate zone), whereas propionate migrates in the ZE mode. In the system 0.004/0.01 (injected sample volume 2 μ l) only formate migrates in a proper ITP mode. In the system 0.002/0.01 (injected sample volume 2 μ l), formate migrates just (or just not?) in the ITP mode and in the

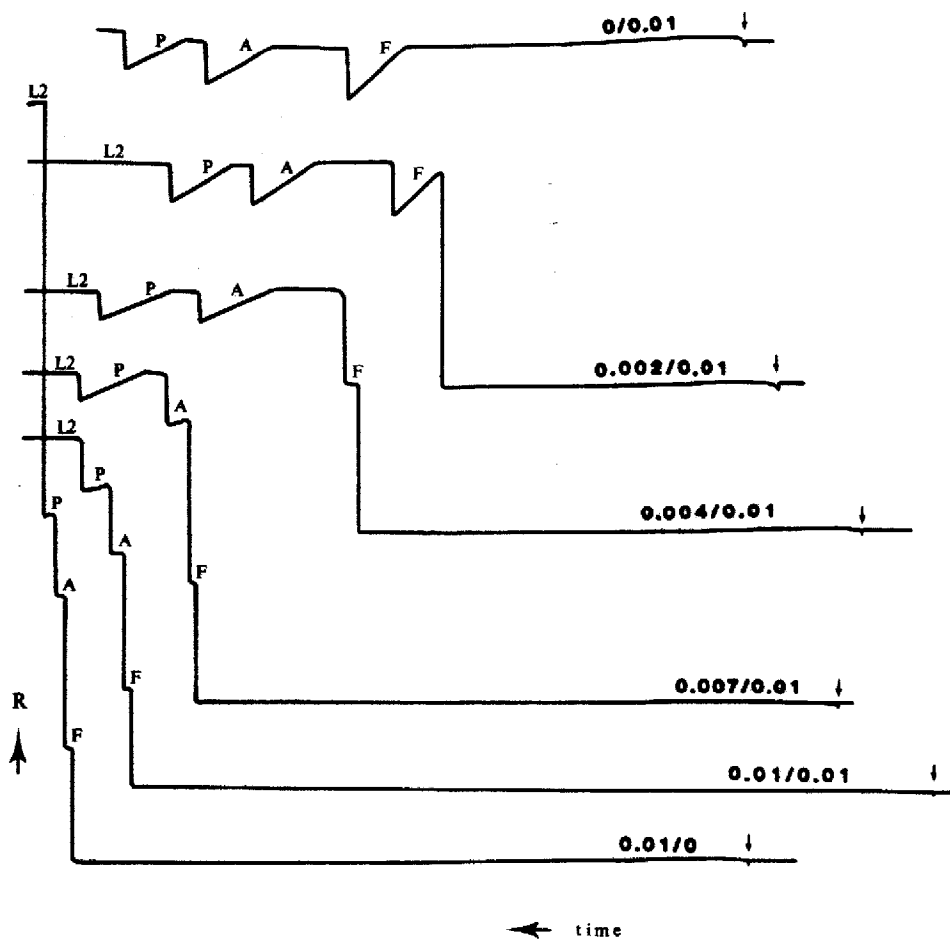


Fig. 7. Electropherograms for the separation of formate (F), acetate (A) and propionate (P) in different electrolyte systems. The leading electrolytes contain two leading ions, Cl^- (L1) and MES (L2), at a pH_L of 6 adjusted by adding the buffer histidine. The numbers refer to the leading ion concentrations L1 and L2, e.g., 0.002/0.01 means that the concentrations of Cl^- and MES are 0.002 and 0.01 M, respectively. The terminator was 0.01 M MES. It can clearly be seen that ITP changes into ZE at lower Cl^- concentrations. All experiments were carried out at an electric current of $70 \mu\text{A}$ and a paper speed of 10 mm/min with an ITP apparatus with a capillary tube of 0.4 mm I.D. and a conductivity detector.

system 0/0.01 (injected sample volume $2 \mu\text{l}$) all sample ions migrate clearly in the ZE mode.

It is clear that it is possible to obtain in one electropherogram several different modes¹, e.g., first sample ions migrating in the ZE mode (if their effective mobilities are larger than that of the leading ion), followed by ionic species migrating in the ITP mode, followed by sample ions again migrating in the ZE mode (if their mobilities are smaller than that of the terminating ion).

CONCLUSION

The mathematical model for the steady state in ITP has been extended for the use of leading electrolytes with two leading ionic species. Experimentally obtained values for R_E and RF confirm the validity of this model. The interesting point in the use of 2L systems is that RF values increase in the presence of a second leading ion L2, with a low effective mobility, through which the detection limit can be lowered. This may be the reason for too high RF values in anionic systems at higher pH_L values, owing to the presence of unwanted (hydrogen)carbonate in the leading electrolyte. Further, this model shows that using a second leading ion L2, the R_E value of the terminating L2 zone decreases strongly and the ITP mode changes into a ZE mode for many sample ions.

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
L1	leading ionic species with the highest mobility in a 2L system
L2	leading ionic species with the lowest mobility in a 2L system
m	mobility at infinite dilution ($m^2/V \cdot s$)
\bar{m}	effective mobility ($m^2/V \cdot s$)
n	number of protolysis steps
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 zone of substance A, B, T and L
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Superscripts

z	maximum charge of an ionic species
i	to the i th degree

Examples

$[B]_{L,A}$	total concentration of substance B in zone A
$ \bar{m}_{B,A} $	absolute value of the effective mobility of substance B in the zone of substance A

ABBREVIATIONS

2L	system with two leading ions
CZE	capillary zone electrophoresis
EN	electroneutrality condition
IC	isotachophoretic condition
ITP	isotachophoresis
MES	2-(N-morpholino)ethanesulphonic acid
R_E	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)

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