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GENERATION OF OPERATIONAL ELECTROLYTES FOR ISOTACHOPHORESIS AND CAPILLARY ZONE ELECTROPHORESIS IN A THREE-POLE COLUMN

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SUMMARY

A new method has been developed for controlling the composition of the operational electrolytes directly in the separation capillary in isotachophoresis or capillary zone electrophoresis. The method is based on feeding the capillary with two different suitable ionic species from two separate electrode chambers by simultaneous electromigration. The composition and pH of the electrolyte in the separation capillary is thus controlled by setting the ratio of two electric currents. The theory has been developed and verified experimentally to predict both the electrolyte composition in the separation capillary and the time necessary to change this composition in the required way. Some of the possible ionic matrices realizable in the three-pole arrangement have been studied experimentally and used in isotachophoretic experiments. The technique described does not require moving parts in the instrumentation and provides the possibility to make very fine changes of pH in the capillary in a reproducible and easy way. The procedure itself is feasible for automation.

INTRODUCTION

In the analysis of real samples in capillary isotachophoresis (ITP) or zone electrophoresis (CZE) it is necessary to choose a convenient electrolyte[1]. There are two ways of doing this.

If the ionic mobilities and pK values of the substances to be separated are known, we can use a computing procedure[2]; if not, experiments must be done to create an ionic matrix in the separation column which ensures sufficient differences in the effective mobilities of the substances to be separated. All ions in the electrophoretic column move, and therefore the ionic matrix consists of coions and counter ions flowing between the electrode chambers through the separation column; the composition of this matrix depends on the composition of the electrolytes in these chambers. Commonly, only two electrode chambers are used and the ionic matrix can be changed only by altering the composition of the electrolyte in these chambers. In practice, when looking for a suitable electrolyte system, laborious and material-consuming wet chemistry procedures are performed, *e.g.*, preparation of new electrolytes, rinsing, filling until a successful separation is obtained or optimized.

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The essence of this paper is to describe a new procedure for the above mentioned purposes, where the required ionic matrix is created directly in the separation column by electrophoretic means. Here, each ionic species creating the ionic matrix is fed into the separation column by electromigration from its individual electrode (pole) chamber and the flow of each ionic species can be regulated electrically by controlling the magnitude of the current passing through each electrode chamber (each pole). This paper develops the necessary theory for calculation of the operational electrolyte composition, the description of suitable equipment for this procedure[3] and some model examples of its utilization.

THEORETICAL

The effective mobility of an ion is affected by all ions in its surroundings which may be called the ionic matrix. An important role in this ionic matrix is played by the solvolytic ions H^+ and OH^- which dramatically change the effective mobilities of weak acids and bases. In an electrophoretic column, when an electric field is applied, each ionic species migrates in the direction determined by its net charge and creates a flow of ions. These flows then create an ionic matrix around an individual ion, see Fig. 1. It is evident that the ionic matrix can be altered by changing these flows.

An electrode chamber filled with a working electrolyte represents a source of such a flow of ions. If an electrode of given polarity, *e.g.*, + is immersed in a working electrolyte containing a mixture of a common (G) and a solvolytic (S) ion of the same polarity, *e.g.*, Na^+ and H^+ the concentrations of which are c_G and c_S (see Fig. 2), then the respective electrode chamber produces flows of both ions, J_G and J_S. Their magnitude is proportional to the magnitude of the input electric current, I, passing through the electrode

$$J_i = Iu_i c_i/\kappa \qquad (\text{mol s}^{-1}) \tag{1}$$

where i = G or S, u_i is the electrophoretic mobility of ion i and κ is the conductivity of the electrolyte. For the ratio of both flows we may write:

$$\frac{J_S}{J_G} = \frac{c_S u_S}{c_G u_G} = \frac{c_S}{c_G} \cdot \text{constant} \tag{2}$$

It is obvious that in the commonly used two-pole arrangement (one cathode and one anode), the ratio of the flows of ions S and G in the ionic matrix can be changed only by an exchange of the working electrolyte in the respective electrode chamber, *i.e.*, by change of the ratio c_S/c_G.

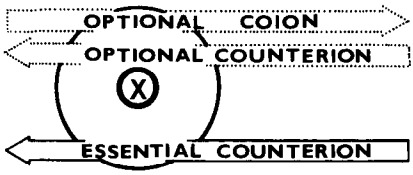

Fig. 1. Scheme of flows of ions forming the ionic matrix surrounding the analyzed ion X.

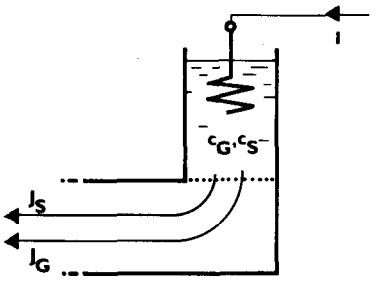

Fig. 2. Electrode chamber containing ions S and G of concentrations c_S and c_G, respectively, as a source of flow (J_S, J_G) of these ions.

The method suggested here allows the flows of ions of the same sign to be changed continuously and independently of those of the others. The electrode chamber serves again as the source of the flow of the ions. In this case, however, each electrode chamber contains only one cation and one anion, *i.e.*, there must be more than one electrode chamber for poles of the same sign. Each such chamber generates a flow of one kind of ions which is directly proportional to the magnitude of the electric current through the corresponding immersed electrode.

Assuming that the electrode chamber contains only one binary strong electrolyte, we may introduce the transference number, t_i, into eqn. 1 and write

$$J_i = I_i t_i / z_i F \tag{3}$$

where I_i is the electric current passing through the electrode in the chamber with ion i, z_i is the charge number of ion i and F is the Faraday constant. When neglecting the influence of ionic strength on t_i (within a limited concentration range), we may conclude from eqn. 3 that the flow of ions i is independent of their concentration in the electrode chamber.

A pair of electrode chambers of the same polarity which contain ions S and G and through which pass electric currents I_S and I_G produces ionic flows J_S and J_G (see Fig. 3) for which its holds that:

$$\frac{J_S}{J_G} = \frac{I_S \kappa_G c_S u_S}{I_G \kappa_S c_G u_G} = \frac{I_S}{I_G} \cdot k_1 \tag{4}$$

where k_1 in a proportionality constant. From this it follows that the ratio of the flows of the two ionic species S and G is directly proportional to the ratio of the respective electric currents.

In the case of the common ion G belonging to a uni-univalent weak electrolyte we must take into account that its solution contains also the solvolytic ion S of the same sign. The flow of this ion from electrode chamber G must be introduced into eqn. 4

$$\frac{J_S}{J_G} = \frac{I_S \kappa_G c_S u_S}{I_G \kappa_S c_G u_G} + \frac{c_{S,G} u_S}{c_G u_G} = \frac{I_S}{I_G} \cdot k_1 + k_2 \tag{5}$$

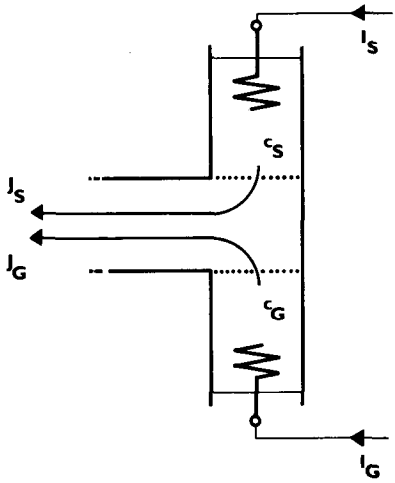

Fig. 3. Simultaneous control of the flow of two ions S and G by using two separate electrode chambers, each containing only one of these ions, by means of input electric currents I_S and I_G of the electrodes.

where $c_{S,G}$ is the concentration of ion S in chamber G and k_2 is an additive constant. The resulting ratio of flows depends on the ratio of the electric currents through both electrodes and its constant part depends also on the composition of the electrolyte in chamber G.

If a pair of electrode chambers of the described type (of the same polarity) are connected to a separation column filled with an electrolyte containing, *e.g.*, ion S, then we may call the electrode chamber which contains ion G the modifying electrode chamber. In comparison with the normal two-pole arrangement, the modifying chamber represents an addition which allows modification of the original electrolyte in the column. In the above example, *e.g.*, the original electrolyte containing ion S is modified to another one which contains a mixture of ions S and G. If, in the given analytical arrangement, the ions S and G are counter ions, then naturally the modifying electrode must be connected to the detection side of the separation column (see also Fig. 4). If the modification is performed by means of a coion, then the modifying electrode chamber is connected to the sampling side of the column. After switching on the electric current, a new zone of the modified electrolyte starts to be formed at the point of connection of the two electrode chambers to the column. This zone is displaced into the separation column and thus the modification of the original electrolyte proceeds. Let us call the original and modified electrolytes the primary and secondary ones, respectively.

In order to use the above arrangement for electrolyte modification successfully in practice, two things must be known, *viz.*, the composition of the resulting secondary electrolyte and the migration velocity of the boundary between the primary and the secondary electrolytes through the separation column.

Obviously the ratio of the ionic flows from the two electrode chambers equals the ratio of the flows in the zone, Z formed. In analogy with eqn. 2 we may write:

$$\frac{J_S}{J_G} = \frac{J_{S,Z}}{J_{G,Z}} = \frac{c_{S,Z}u_S}{c_{G,Z}u_G} \tag{6}$$

By combination of eqns. 5 and 6 we obtain

$$\frac{J_{S,Z}}{J_{G,Z}} = \frac{c_{S,Z}}{c_{G,Z}} \cdot \frac{u_S}{u_G} = \frac{I_S}{I_G} \cdot k_1 + k_2 \tag{7}$$

which shows how the ratio of concentrations of both ions in the secondary electrolyte, $c_{S,Z}/c_{G,Z}$, depends on the ratio of the electric currents passing through both electrodes, I_S/I_G.

The velocity of generation of the secondary electrolyte in the separation column is an important parameter describing the velocity of displacement of the zone formed. For the calculation of this quantity, W_Z (expressed in $m^3\ C^{-1}$), the moving boundary equation[4] may be used, *e.g.*, for substance S (ion constituent S) in the form

$$W_Z = \left(\frac{c_{S,A} u_S}{\kappa_A} - \frac{c_{S,Z} u_S}{\kappa_Z} \right) \Big/ (\bar{c}_{S,A} - \bar{c}_{S,Z}) \tag{8}$$

where A and Z indicate the primary and secondary zones, respectively, and $\bar{c}_S$ is the total concentration of constituent S including, *e.g.*, for H^+ also the protonated base BH^+ and/or the neutral acid HA. In the case when the constituent S involves also such a charged form, *e.g.*, BH^+, an additional term must appear in the numerator in eqn. 8, $c_i u_i/\kappa$ with the corresponding subscript and sign depending on whether it is present in zone A and/or Z. In analogy, the corresponding balance for the counter constituent may be written:

$$W_R = \left(\frac{c_{R,Z} u_R}{\kappa_Z} - \frac{c_{R,A} u_R}{\kappa_A} \right) \Big/ (\bar{c}_{R,A} - \bar{c}_{R,Z}) \tag{9}$$

The unknown value of $c_{S,Z}$ may be obtained as follows. By combination of eqns. 5–9 with the electroneutrality condition and with the equation describing the present chemical (acid–base) equilibria, we may iterate for $c_{S,Z}$ as the parameter until:

$$W_Z = W_R \tag{10}$$

Then the value of $c_{S,Z}$ represents the solution of the system of equations and the corresponding W_Z is the true migration velocity. From these parameters, a complete description of the secondary zone Z may be obtained. For experimental purposes we may define the relative velocity as

$$v_r = \frac{W_Z}{W} = \frac{W_Z \kappa_A}{u_S} \tag{11}$$

where W is the reference frame selected so that it corresponds to the migration of a boundary between the primary and secondary zone where the secondary zone contains only G and no S. The value of v_r may easily be obtained by experiment from the time of passing of the boundary between the primary and secondary zones through the column (through the detector). From two experiments (one without S in zone Z), two passage times are obtained, their ratio being equal to v_r.

Usually it is convenient to select such experimental conditions that the boundary between the primary and secondary electrolytes (zones) is sharp. This is true as long as the modifying constituent has a lower effective mobility than that of the constituent in the primary zone (for a detailed treatment see ref. 5).

EXPERIMENTAL

The apparatus for the described method of regulation of the ionic matrix by either a modifying coion or a modifying counter ion must have at least three electrode chambers. An apparatus allowing simultaneous regulation of coions and counter ions must have four electrode chambers. Such four electrode equipment is suitable even in cases when the electrolyte generation is performed by the three-pole method since it facilitates the operation and manipulation of electrolytes.

Apparatus

The instrumentation for performing experiments with the three-pole arrangement is shown in Fig. 4. The apparatus consists of an electrolyte unit, a high-voltage power supply[6] and a device for controlling the electric current ratio.

The electrolyte unit consists of a PTFE separation capillary both ends of which are equipped with a potential-gradient detection cell. Both cells are connected to electrode blocks (Perspex), each equipped with a sampling device, input and output of electrolytes and two electrode chambers equipped with platinum electrodes and separated from the capillary by semipermeable membranes. The two electrode blocks are identical; however, they differ in the manner of connection to the power supply. The two electrode chambers of one block (M, see Fig. 4) are connected to the power supply and the ratio of the two input currents is controlled; one of these two electrode chambers contains the modifying electrolyte. From the second electrode block (S),

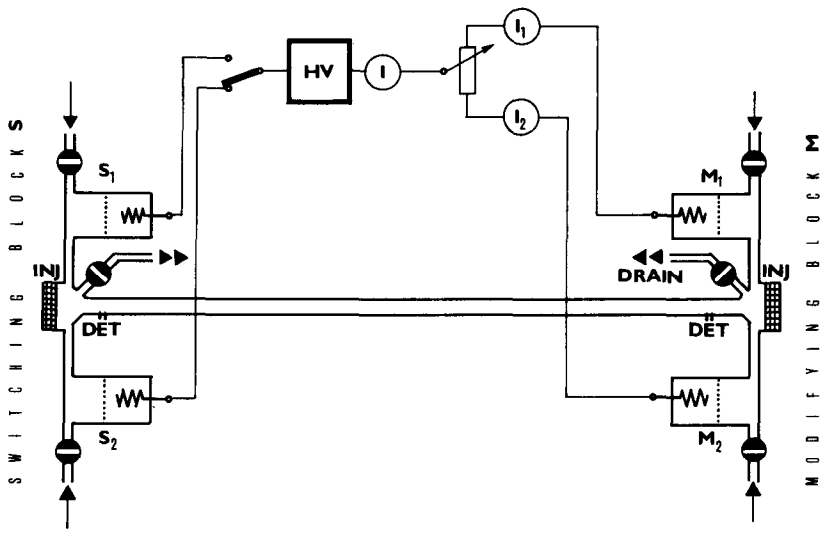

Fig. 4. Apparatus for the experiments with a three-pole column: INJ = injection port; DET = potential gradient detector; HV = high-voltage power supply; for explanation, see text.

only one electrode chamber is connected to the power supply and we may switch over from one chamber to the other.

Chemicals

All chemicals used were of analytical reagent grade (Lachema, Brno, Czechoslovakia).

Measurement of pH and v_r

For the verification of the theory, a simple apparatus was made equipped with a horizontal glass capillary (260 × 1.5 mm I.D.), three electrode chambers, sampling device and measuring scale. This apparatus was used for the determination of the two times necessary for the calculation of v_r (see Theoretical). The movement of the boundary was visualized by the sampling of a small amount of a suitable coloured indicator, *e.g.*, ferroin for cationic analyses. After the measurement of the migration velocity, the zone of the secondary electrolyte (volume approx. 150 μl) was purged from the capillary by a stream of air; its pH was measured by a capillary microelectrode (Radelkis, Budapest, Hungary).

Generation of ionic flows creating ionic matrices in a three-pole column

With a modifying counter ion. For zone electrophoresis the separation column and chambers M1 and S1 (see Fig. 4) were filled with the primary electrolyte, and chamber M2 was filled with the modifying electrolyte (differing from the primary one by containing another counter ion). After switching on the electric current, the zone of the secondary background electrolyte was formed in the separation column. After this zone had reached the detector in block S, the electric current was switched off, the sample introduced via the septum in block S and the analysis was performed.

For isotachophoresis the separation column and chambers M1 and S1 were filled with the primary electrolyte, chamber M2 was filled with the modifying electrolyte and chamber S2 with the terminating electrolyte. After switching on the electric current (so that it passes through chamber S1), the zone of the secondary (leading) electrolyte was formed in the separation column. After this zone had reached the detector in block S, the electric current was switched off, the sample introduced via the septum in block S and the electric current was again switched on (but now so that it passes through chamber S2) and the analysis was performed.

With a modifying coion. For zone electrophoresis the separation column and chambers M1 and S1 were filled with the primary electrolyte, chamber M2 with the modifying electrolyte, the sample was introduced via the septum in block M and the electric current was switched on for analysis.

For isotachophoresis the separation column and chamber S1 were filled with the primary electrolyte, chamber M1 with the modifying electrolyte, chamber M2 with the terminating electrolyte, the sample was introduced via the septum in block M and the electric current was switched on for analysis.

Note that the arrangement with the modifying coion is very advantageous since the analysis is performed simultaneously with the modification and there is no need to wait for the modification of the content of the whole separation column. The modifying constituent, however, must obviously have an higher effective mobility than those of the sample components the separation of which is to be controlled by the performed modification.

RESULTS AND DISCUSSION

For the verification of the theory and of the accuracy of setting of the pH, we compared the measured and calculated relative migration velocities, v_r, and the pH values in a buffered and a non-buffered electrolyte system.

In the case of the non-buffered system, 0.01 *M* HCl served as the primary electrolyte and 0.01 *M* tetrabutylammonium hydroxide (TBAOH) as the modifying electrolyte (modifying ion TBA^+). Table I shows the values of v_r and pH for various ratios I_H/I_{TBA}. The average relative difference between the measured and calculated values of v_r is 3.1% and that between the measured and calculated pH values is 0.03 units.

In the case of the buffered system, 0.01 *M* ammonium formate served as the primary electrolyte and 0.01 *M* formic acid as the modifying electrolyte (modifying ion H^+). The results for this system are given in Table II; here the average relative difference between the measured and calculated values of v_r is 1.4% and that between the measured and calculated pH values is 0.01 units.

From both Tables I and II it is seen that the experimental values are in good agreement with the theoretical ones and that the accuracy of the setting of pH is comparable with the accuracy of a common pH measurement.

For the change of pH in a real electrophoretic system, an example is the preparation of an extended buffer-free system[7] with a solution of HCl and KCl as the secondary electrolyte. For this, the separation column and the electrode chambers M1 and S1 were filled with 0.01 *M* HCl and the chamber M2 was filled with 0.01 *M* KCl. At $I_H/I_K = 1$, the secondary zone formed has the following composition: $c_{Cl} = 0.0067$ *M*, $c_H/c_K = 0.35$, pH 2.76. The calculated dependences of the pH, c_{Cl} and v_r of the secondary zone on I_H/I_K for this system are shown in Fig. 5. By varying the ratio I_H/I_K within the range 0–1 we may thus control the pH of the resulting secondary electrolyte within the range 7–2.7. In this way it is easy to prepare zones which would be otherwise

TABLE I

COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES OF pH AND v_r FOR THE NON-BUFFERED SYSTEM HCl–TBA

Exptl. = experimental value; S.D. = standard deviation; diff. = difference between calculated and experimental values; calc. = calculated values; R.S.D. = relative standard deviation; r.d. = relative difference between calculated and experimental values; the relative values (R.S.D. and r.d.) are given in %.

I_H/I_{TBA}	pH_Z				v_r			
	Exptl.	*S.D.*	*Calc.*	*Diff.*	*Exptl.*	*R.S.D.*	*Calc.*	*r.d.*
0.000	6.19	0.245	–	–	1.000	1.92	1.000	–
0.111	4.28	0.079	4.22	0.06	0.925	2.31	0.906	2.1
0.250	3.85	0.022	3.87	0.02	0.835	1.39	0.811	3.0
0.493	3.56	0.013	3.58	0.02	0.710	1.33	0.688	3.2
0.695	3.41	0.013	3.44	0.03	0.628	0.73	0.612	2.6
1.000	3.28	0.010	3.29	0.01	0.550	1.11	0.527	4.4
average difference				0.03	average difference			3.1%

TABLE II

COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES OF pH AND v_r FOR THE BUFFERED SYSTEM $HCOONH_4$–HCOOH

For abbreviations, see Table I.

I_{NH_4}/I_H	pH_Z				v_r			
	Exptl.	*S.D.*	*Calc.*	*Diff.*	*Exptl.*	*R.S.D.*	*Calc.*	*r.d.*
0.000	2.82	0.006	2.81	0.01	1.000	0.70	1.000	–
0.111	2.87	0.003	2.88	0.01	0.944	0.63	0.951	0.7
0.250	2.95	0.007	2.96	0.01	0.877	0.55	0.889	1.3
0.666	3.07	0.006	3.09	0.02	0.719	0.68	0.729	1.4
1.000	3.15	0.001	3.17	0.02	0.631	0.86	0.644	2.0
average difference				0.014	average difference			1.4%

difficult to obtain by classical means. As follows from the results, the time necessary for the formation of the secondary zone is approximately equal to the analysis time (when preparing the secondary electrolyte, we may use a higher electric current than for the analysis).

Analogously, we may prepare secondary electrolytes with a buffering modifier. The preparation time, however, increases to 2–4 times that of the analysis time, depending on the required pH. In this case the amount of the modifying ion in the secondary electrolyte depends also on the pH of the modifying electrolyte and it is convenient to select its pH so that it equals the pK_a of the buffering ion. An example of such a system is represented by 0.01 *M* HCl as the primary electrolyte and 0.01 *M* β-alanine + HCl (pH 3.6) as the modifying electrolyte. The calculated dependences

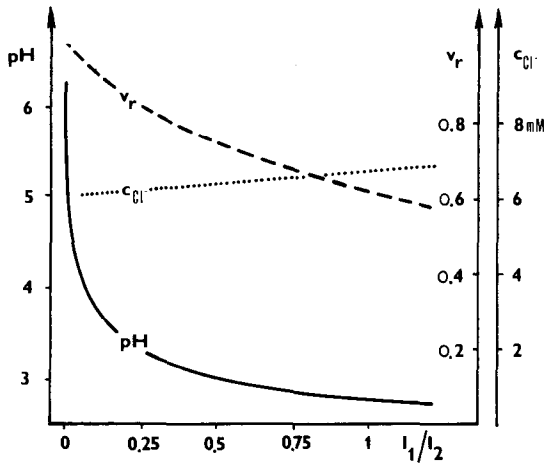

Fig. 5. Calculated dependences of pH, chloride concentration, c_{Cl}, and v_r of the secondary zone on the electric current ratio, $I_H/I_K = I_1/I_2$. The primary electrolyte was 0.01 *M* HCl and the modifying electrolyte was 0.01 *M* KCl.

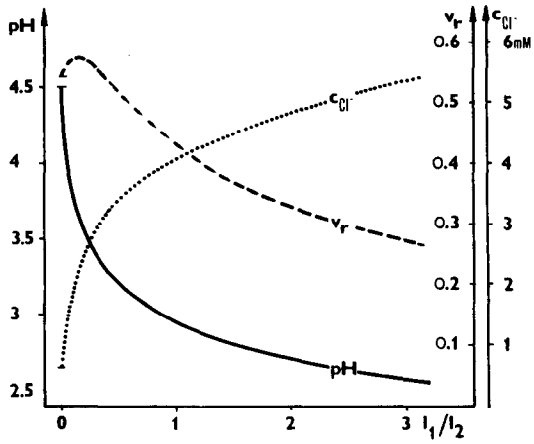

Fig. 6. Calculated dependences of pH, c_{Cl} and v_r of the secondary zone on the electric current ratio, I_1/I_2. The primary electrolyte was 0.01 *M* HCl and the modifying electrolyte was 0.01 *M* β-alanine + HCl (pH 3.6).

of pH, c_{Cl} and v_r on I_1/I_2 are shown in Fig. 6. It is seen that, by changing the electric current ratio, the whole buffering range of β-alanine can be covered.

The possibility to use the method described for an easy change of the ionic matrix in ITP is illustrated by the following example of anionic separation of citrate, lactate and succinate. The primary electrolyte was 0.01 *M* HCl, the modifying electrolyte was 0.01 *M* KCl (modifying ion K^+) and the terminator was 0.01 *M* caproic acid. At the ratio $I_1/I_2 = 0$ (the secondary electrolyte contains only K^+), the migration order of the three acids is citrate, succinate, lactate (see Fig. 7a). A current ratio, $I_1/I_2 = 0.5$ results in the order citrate, lactate, succinate (Fig. 7b).

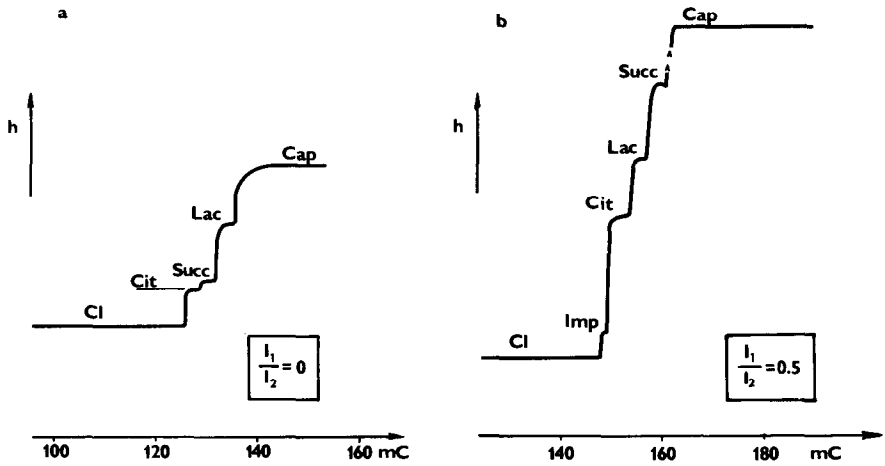

Fig. 7. Potential gradient records of the analysis of citrate (Cit), succinate (Succ) and lactate (Lac) in the system with 0.01 *M* HCl (primary electrolyte), 0.01 *M* KCl (modifying electrolyte), Cl^- (leading ion), 0.01 *M* caproic acid (Cap, terminator) for $I_1/I_2 = 0$ (a) or 0.5 (b). (a) $I = 150$ μA (modification and separation), 100 μA (detection); (b) $I = 150$ μA (modification and separation), 60 μA (detection). Sample amounts (nmol): (a) 3.75, Cit; 7.5, Succ and 18.8, Lac; (b) 7.15, Cit; 14.3, Lac and 28.6, Succ; Imp = impurity.

Another example shows the cationic ITP separation of methylamine and tetrabutylammonium (TBA), where 0.01 *M* $Ca(OH)_2$ served as the primary electrolyte and 0.01 *M* KCl was the modifier. At the ratio $I_{OH}/I_{Cl} = 0.05$, methylamine migrates in front of TBA (TBA is the terminator), see Fig. 8a. At $I_{OH}/I_{Cl} = 1.7$ methylamine migrates behind TBA (Fig. 8b) and NH_4^+ serves as the terminator; at $I_{Cl} = 0$, the effective mobility of methylamine was further changed (Fig. 8c).

The use of a modifying coion is illustrated by the cationic isotachophoretic analysis of a mixture of K^+, Na^+, tetrapropylammonium (TPA) and aniline. The primary electrolyte was 0.01 *M* HCl, as was the modifying electrolyte (modifying coion H^+) and the terminating electrolyte was 0.01 *M* TBACl. At $I_2 = 0$ ($I_H = 0$), aniline did

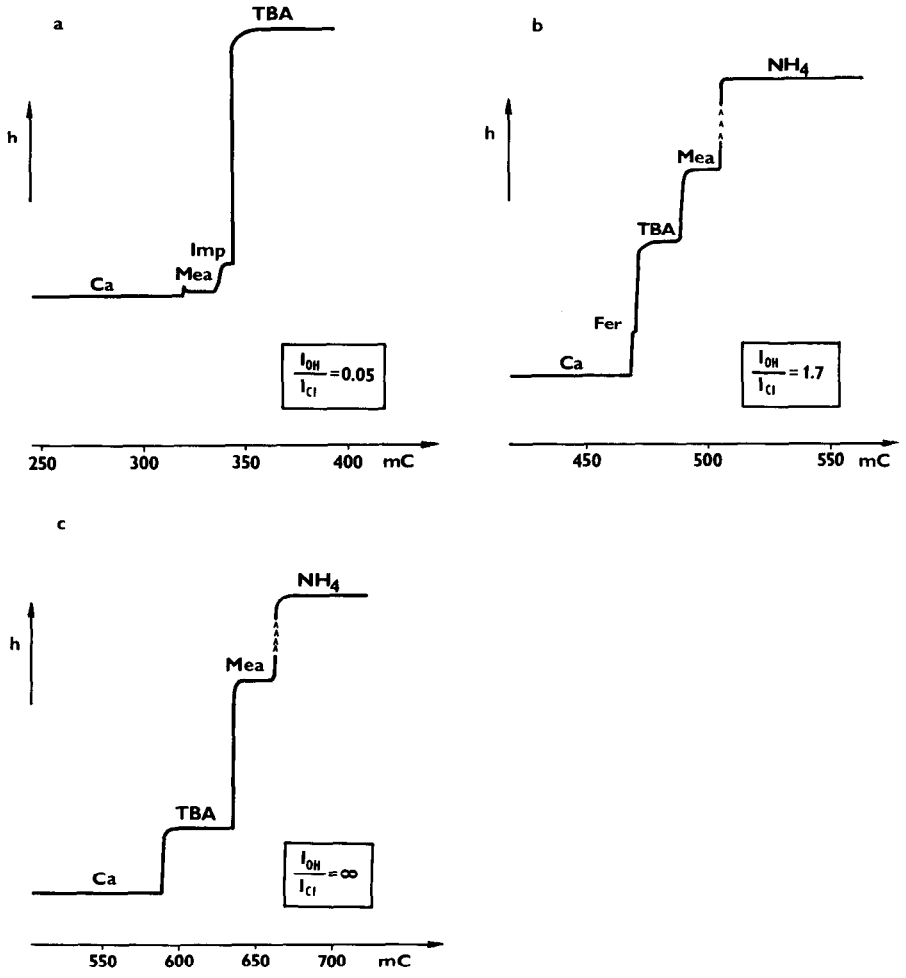

Fig. 8. Potential gradient records of the analysis of methylamine (Mea) and tetrabutylammonium (TBA) in the system with 0.01 *M* $Ca(OH)_2$ (primary electrolyte), 0.01 *M* KCl (modifying electrolyte), Ca^{2+} (leading ion), 0.01 *M* TBAOH (a) or 0.01 *M* NH_4OH (b, c) (terminator), for (*a*) $I_{OH}/I_{Cl} = 0.05$, (b) $I_{OH}/I_{Cl} = 1.7$ and (c) $I_{Cl} = 0$; $I = 140$ μA (modification and separation), 70 μA (detection). Sample amounts: (nmol) (a) 50, MeaCl; (b) 26, MeaCl and 13, TBACl; (c) 67, MeaCl and 33, TBACl; Imp = impurity, Fer = ferroin.

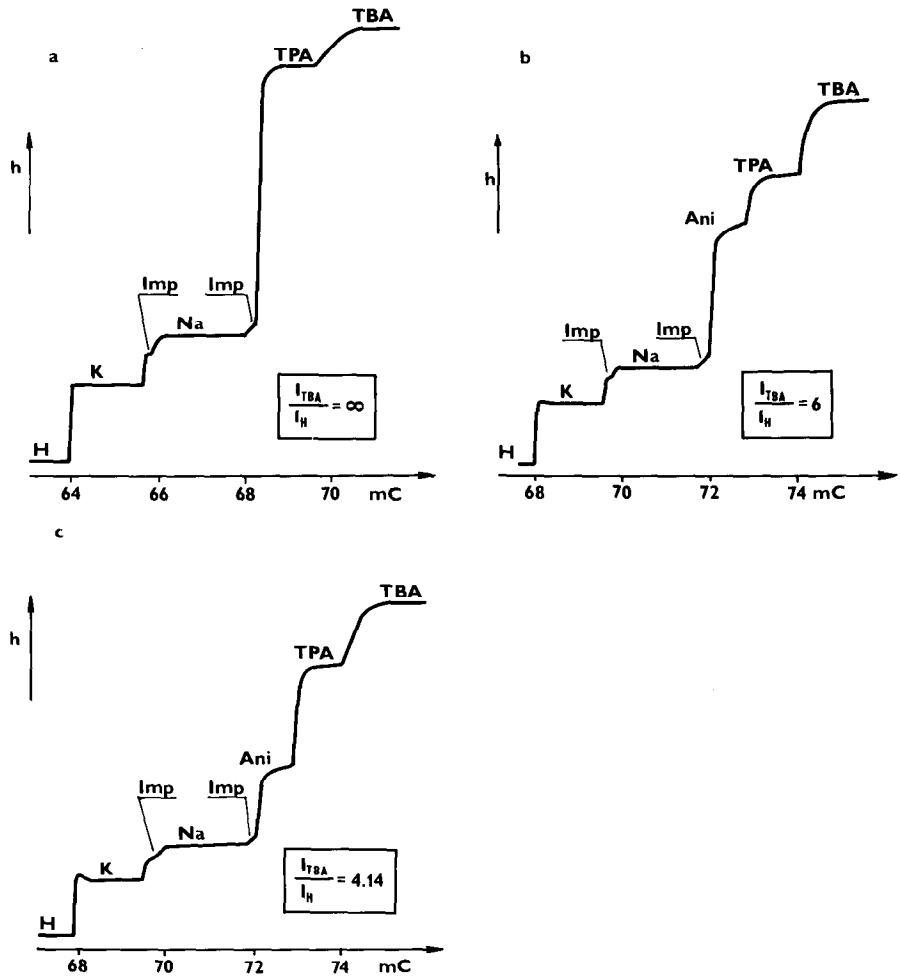

Fig. 9. Potential gradient records of the analysis of KCl, NaCl, tetrapropylammonium (TPA) iodide and aniline (Ani) (each 2.5 nmol) in the system with 0.01 *M* HCl (primary electrolyte and modifying electrolyte), H^+ (leading ion), 0.01 *M* TBACl (terminator) for (a) $I_2 = I_H = 0$, (b) $I_{TBA}/I_H = 6$ and (c) $I_{TBA}/I_H = 4.14$. (a) $I = 315\ \mu A$ (separation), 105 μA (detection); (b) $I = 200\ \mu A$ (separation), 100 μA (detection); (c) $I = 270\ \mu A$ (separation), 50 μA (detection); Imp = impurity.

not migrate (see Fig. 9a); at the ratios $I_{TBA}/I_H = 6$ and 4.14, aniline migrated and changed its effective mobility (Fig. 9b and c).

CONCLUSIONS

The composition of the ionic matrices in isotachophoresis and zone electrophoresis can be changed directly in the separation capillary by electromigration with two different ions of the same charge from two separate electrode chambers. The flows of these ions from the electrode chambers can be regulated electrically by the

magnitude of the input current to each electrode, and thus the ionic matrix can effectively be changed.

The composition of the ionic matrix and the time necessary for its change can be calculated from the ratio of the input currents on the basis of moving boundary equations, which was verified experimentally. The calculated and experimental values of pH and v_r for a non-buffered system (primary electrolyte HCl, modifying electrolyte TBACl) and a buffered system (primary electrolyte $HCOONH_4$, modifying electrolyte HCOOH) were compared. The values of pH agreed very well and did not differ on average by more than 0.03 units for the non-buffered and 0.013 units for the buffered system. The values of v_r for the non-buffered and buffered systems differed by about 3 and 1.4%, respectively.

The procedure described enables one to make very fine changes in the composition of the ionic matrix directly in the separation column in an easy and reproducible way, which is useful especially for experimental determination of suitable separation conditions in electrophoretic techniques.

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