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COMPLEX-FORMING EQUILIBRIA IN ISOTACHOPHORESIS

I. COMPUTER SIMULATION OF THE CALCIUM TARTRATE SYSTEM IN THE STEADY STATE

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SUMMARY

A theory for kinetically labile complex equilibria in isotachophoresis has been described for the purposes of computer simulation. Simulated ratios of potential gradients, $R_E = E_S/E_L$, have been obtained for Ca^{2+} and tartrate ions coexisting with calcium tartrate complexes. The simulated values agreed well with the observed ones and the validity of the simulation has been confirmed. The simulation can be used for the determination of optimal separation conditions and also for the estimation of stability constants and/or mobilities of complexes.

INTRODUCTION

The theory of isotachophoresis in the steady state has been dealt with by several authors. The most general equations have been derived by Everaerts *et al.*¹, and these can be applied to many practical isotachophoretic systems. However, complex ion-pair-forming equilibria have not been taken into account.

The utility of complex-forming equilibria in isotachophoretic analysis has been proved for the separation of ions, which is difficult in normal electrolyte systems. Boček *et al.*² reported the separation of chloride, bromide and iodide ions in the complex-forming equilibria with Cd^{2+} , and the separation of several organic acids in the equilibria with Ca^{2+} , etc., has been reported by Kaniansky and Everaerts³. Recently, the technique has also been utilized for the separation of lanthanoids⁴.

The success of such separations depends on the kind of complexing agent used, its concentration, the pH of the leading electrolyte and the concentration of the leading ion. Therefore, the choice of optimum conditions may be often difficult without any theoretical estimation of isotachopherograms.

The object of this paper is to derive the detailed theoretical equations for the complex-forming equilibria in isotachophoresis, which would enable practical computer simulation at the steady state for a choice of the optimum separation conditions. To test the theory, simulations were carried out for both anionic and cationic

analysis in the calcium tartrate complex-forming system, and the simulated ratios of potential gradient of the sample zone to that of the leading zone, R_E , were compared with the observed values under several conditions.

THEORETICAL

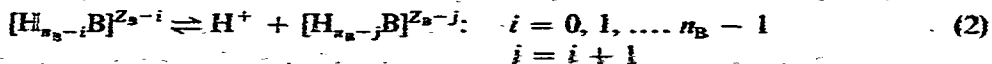
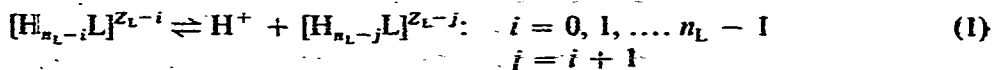
Electrolyte system

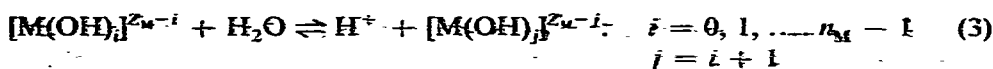
For the establishment of the steady complex-forming equilibria, the sample zone requires the continuous flow of a complexing agent from the leading zone into the succeeding zones. Therefore, the complexing agent should be contained in the leading electrolyte and should be of opposite charge to the sample ions. The complexing agent, in some instances, may have pH-buffering ability under the operational conditions, *i.e.*, if the pK_a of the complexing agent is approximately equal to the pH of the leading electrolyte, pH_L . However, if the desirable pH_L is different from the pK_a value of the complexing agent or cannot be achieved due to limitation of the optimal concentration of leading electrolyte, a pH-buffering agent must be added to the leading electrolyte. In the present case, the theory is described for anionic analysis, and it is assumed that the buffering ion has no complexing ability for the sample ions. On the other hand, in cationic analysis, an anionic counter ion with complex-forming and pH-buffering ability is assumed. Such an electrolyte system is useful when the formed complexes are very labile, as in the separation of Ba, Ca, Na, Zn, Cd and Cu by the use of a KOH leading electrolyte buffered by added acetic acid^{1,5}. By the appropriate combination of the derived equations, the complex-forming system with the counter ions of both the complex-forming reagent and buffering ions can be treated simultaneously.

Under steady isotachophoretic equilibria, the following conditions should be satisfied in the leading and the subsequent zones: (1) electroneutrality; (2) mass balance of counter ions (the complexing and the pH-buffering ions); (3) equality of migration velocity of the zones (For this it is desirable that the electric current be kept constant during the migration.); (4) the establishment of the complex-forming equilibria is rapid enough to enable electrophoretic migration of a homogeneous sample zone.

Leading zone

Although the leading ion, L, used is usually a monovalent ion such as K^+ or Cl^- , it was treated as multivalent ion to give a more general expression. The pH-buffering ion, B, and the added metal ion, M for anionic analysis, were also treated as polyvalent ions. It was assumed that complex formation does not occur in the leading zone. In practice, a decrease of the effective mobility of the leading ion is undesirable, since the increase in the step-height of the sample zone may sometimes be cancelled out. The dissociation equilibria in the leading zone can be expressed as





where n and Z are the number of dissociable hydrogens and the maximum charges of the L, B and M ions, respectively. For anionic species, the maximum charge is zero.

The electroneutrality relationship must be satisfied in every zone, and for the L zone it can be expressed as

$$C_{H,L} - C_{OH,L} + \sum_{i=0}^{n_L} (Z_L - i) C_{Z_L-i} + \sum_{i=0}^{n_B} (Z_B - i) C_{Z_B-i,L} + \sum_{i=0}^{n_M} (Z_M - i) C_{Z_M-i,L} = 0 \quad (4)$$

where C_{Z_L-i} is the concentration (mole/l) of the sub-species of the L ion with charge $Z_L - i$, $C_{Z_B-i,L}$ that of the B ion with charge $Z_B - i$ in the L zone and $C_{Z_M-i,L}$ that of the M ion with charge $Z_M - i$. $C_{H,L}$ and $C_{OH,L}$ are the concentration of H^+ and OH^- in the L zone: in the extreme pH region, i.e., $pH < 3$ or $pH > 11$, their contributions to the specific conductivity of the zone are very large and sometimes the isotachopheric equilibrium could not be established.

The sub-species concentration of L, B and M ions can be expressed using the corresponding total concentrations, equilibrium constants and $C_{H,L}$, for example

$$C_{Z_M-i,L} = C_{M,L} \left(\frac{i}{H} K_{M,j} / C_{H,L} \right) / \left[1 + \sum_{j=1}^{n_M} \left(\frac{j}{H} K_{M,j} / C_{H,L} \right) \right] \quad (5)$$

where $K_{M,j}$ is the successive acidity constant of the M ion for the equilibrium in eqn. 3, and $C_{M,L}$ is the total concentration. Replacing the subscript M in eqn. 5 by L or B gives the corresponding equations for the L or B ion. Then, the electroneutrality (eqn. 4) can be expressed in terms of the total concentrations instead of the sub-species concentrations. In the practical case that C_L , $C_{M,L}$ and $C_{H,L}$ are known at the preparative stage of the leading electrolyte, the total concentration of buffering ion, $C_{B,L}$ can be estimated by

$$C_{B,L} \left\{ C_{OH,L} - C_{H,L} + C_L \left[Z_L + \sum_{i=1}^{n_L} (Z_L - i) \left(\frac{i}{H} K_{L,j} / C_{H,L} \right) \right] \right\} / \left[1 + \sum_{i=1}^{n_L} \left(\frac{i}{H} K_{L,j} / C_{H,L} \right) \right] + C_{M,L} \left[Z_M + \sum_{i=1}^{n_M} (Z_M - i) \left(\frac{i}{H} K_{M,j} / C_{H,L} \right) \right] / \left[1 + \sum_{i=1}^{n_M} \left(\frac{i}{H} K_{M,j} / C_{H,L} \right) \right] \left\{ \right\} / \left\{ \left[Z_B + \sum_{i=1}^{n_B} (Z_B - i) \left(\frac{i}{H} K_{B,j} / C_{H,L} \right) \right] / \left[1 + \sum_{i=1}^{n_B} \left(\frac{i}{H} K_{B,j} / C_{H,L} \right) \right] \right\} \quad (6)$$

where $K_{L,j}$ and $K_{B,j}$ are the successive acidity constants, and C_L the total concentration of the L ion. The concentration, $C_{B,L}$, is indispensable to estimate the total

concentration of the common buffering ion in the subsequent zones by the use of the mass balance equation¹. It is closely related to the pH of the subsequent zones.

The successive dissociation constants in eqns. 5 and 6 should be those at the finite ionic strength in the L zone. Thus, the thermodynamic constants are corrected by the use of activity coefficients of the constituents, which can be estimated by inserting the calculated ionic strength into the Debye-Hückel equations^{6,7}. For this correction, the sub-species concentrations of L, B and M ions are calculated. Iterative correction gives the constant concentrations of the leading constituents.

The effective mobilities, \bar{m} , of the leading, buffering and complexing ions are necessary for the evaluation of the concentrations of the constituents in the subsequent zones. For example

$$\bar{m}_L = \left[m_{Z_L} + \sum_{i=1}^{n_L} m_{Z_L-i} \left(\prod_{j=1}^i K_{L,j} / C_{H,L} \right) \right] / \left[1 + \sum_{i=1}^{n_L} \left(\prod_{j=1}^i K_{L,j} / C_{H,L} \right) \right] \quad (7)$$

where m_{Z_L} and m_{Z_L-i} are the corrected mobilities of the leading ions with charges Z_L and $Z_L - i$: The corrected mobility was obtained by inserting the absolute mobilities and the calculated ionic strength into the Onsager equation⁸, which can be applied to an isotachophoretical electrolyte system of concentration *ca.* 0.01 *M*. For anionic species, m_{Z_L} is zero. Replacing the subscript L in eqn. 7 by B or M gives the corresponding equation for B or M ions.

For the estimation of the potential gradient in the L zone, the specific conductivity, κ_L , is necessary and it is expressed as

$$\kappa_L = \left(C_{H,L} m_H + C_{OH,L} m_{OH} + \sum_{i=0}^{n_L} |Z_L - i| C_{Z_L-i} m_{Z_L-i} + \sum_{i=0}^{n_B} |Z_B - i| C_{Z_B-i,L} m_{Z_B-i} + \sum_{i=0}^{n_M} |Z_M - i| C_{Z_M-i,L} m_{Z_M-i} \right) F / 1000 \quad (8)$$

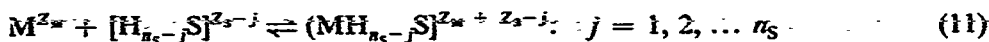
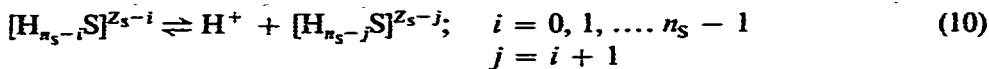
where m_H and m_{OH} are the corrected mobilities of H^+ and OH^- ions, and F is the Faraday constant. Then, the potential gradient of the leading ion, E_L , can be expressed as

$$E_L = 1000J / \kappa_L F \quad (9)$$

where J is the current density in the separation compartment.

Sample zone

Imagine a complex-forming equilibrium between anionic sample, S, and metal ion, M, as the counter ion, for example, tartaric acid and Ca^{2+} . When the M ion with maximum charge Z_M , *i.e.*, M^{Z_M} , can react with the sub-species of sample, the chemical equilibria in the S zone can be written as



where n_s is the number of dissociable hydrogens of the S ion and Z_s is the maximum charge, in this case, zero. In addition to the above equilibria, eqns. 2 and 3 must also be taken into account. The effective mobility of the S ion can be described using the mobility and concentration of the constituents, as follows

$$\bar{m}_s = \left(\sum_{i=0}^{n_s} m_{Z_s-i} C_{Z_s-i} + \sum_{i=1}^{n_s} m_{MS,Z_M+Z_s-i} C_{MS,Z_M+Z_s-i} \right) / (C_s^t + C_{MS}^t) \quad (12)$$

where C_{Z_s-i} is the sub-species concentration of non-complexing sample, C_{MS}^t that of complex, m the corrected mobilities and the superscript t indicates the total concentrations of the subscripted constituents. The mobilities of complexes, m_{MS} , are usually smaller than m_s , as expected from the decreased charges, therefore, the higher the concentration of MS, the lower is the effective mobility of the sample. The concentrations of complexes are uniquely defined by the conditions and the stability constants of the formed complexes. The concentrations of the constituents in the steady state, however, are not available at the initial stage of calculation. The correct pH in the S zone, and hence the concentration of the constituents, can be obtained after iterative calculation to fulfil the steady-state condition, as described later. Then, the other expression for \bar{m}_s using already known physico-chemical constants is necessary for the smooth convergence to self-consistent state in computer simulation, it follows that

$$\begin{aligned} \bar{m}_s = & \left[m_{Z_s} + \sum_{i=1}^{n_s} m_{Z_s-i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) + \right. \\ & \left. C_{Z_{M,S}} \sum_{i=1}^{n_s} m_{MS,Z_M+Z_s-i} K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \right] / \\ & \left[1 + \sum_{i=1}^{n_s} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) + C_{Z_{M,S}} \sum_{i=1}^{n_s} K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \right] \quad (13) \end{aligned}$$

where $K_{S,j}$ is the successive dissociation constant of sample S, $K_{MS,j}$ the stability constant of the formed complex MS, $C_{Z_{M,S}}$ the concentration of M ion with maximum charge Z_M , $C_{H,S}$ the proton concentration in the S zone and m is the corrected mobility. In eqn. 13, the concentration C_{Z_M} still remains and it can be expressed as:

$$C_{Z_{M,S}} = C_{M,S}^t \left[1 + \sum_{i=1}^{n_M} \left(\prod_{j=1}^i K_{M,j} / C_{H,S} \right) \right] \quad (14)$$

Then, if the total concentration of non-complexing M ion in the S zone, $C_{M,S}^t$, and $C_{H,S}$ are known, \bar{m}_s can be evaluated. The total concentration, $C_{M,S}^t$, is controlled by the following equation derived from the mass balance equation describing the complexing ion entering and leaving the S zone

$$\begin{aligned} C_{M,S} = & C_{M,L}^t [\bar{m}_s(\bar{m}_L + \bar{m}_{M,L})] / [\bar{m}_L(\bar{m}_S + \bar{m}_{M,S})] - C_{MS}^t = \\ & C_{M,L}^t [\bar{m}_s(\bar{m}_L + \bar{m}_{M,L})] / [\bar{m}_L(\bar{m}_S + \bar{m}_{M,S})] / \\ & \left\{ 1 + C_{Z_s} \left[\sum_{j=1}^{n_s} K_{MS,j} \left(\prod_{i=1}^j K_{S,i} / C_{H,S} \right) \right] / \left[1 + \sum_{p=1}^{n_M} \left(\prod_{i=1}^p K_{M,i} / C_{H,S} \right) \right] \right\} \quad (15) \end{aligned}$$

where $\bar{m}_{M,L}$ is the effective mobility of the complexing counter ion in the L zone, which can be evaluated by a similar equation to eqn. 7, and $\bar{m}_{M,S}$ is that in the S zone, which can be expressed by a similar equation to eqn. 13 considering the complex formation with sample ions as follows:

$$\bar{m}_{M,S} = \left[m_{Z_M} + \sum_{i=1}^{n_M} m_{Z_M-i} \left(\prod_{j=1}^i K_{M,j} / C_{H,S} \right) + C_{Z_S} \sum_{i=1}^{n_S} m_{MS,Z_M+Z_S-i} \times \right. \\ \left. K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \right] \left[1 + \sum_{i=1}^{n_S} \left(\prod_{j=1}^i K_{M,j} / C_{H,S} \right) + \right. \\ \left. C_{Z_S} \sum_{i=1}^{n_S} K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \right] \quad (16)$$

In eqn. 15, the effective mobility, \bar{m}_S , appears again. Combining eqns. 13, 14 and 15, a complicated quadratic expression for \bar{m}_S may be obtained, however, this is not necessary for the numerical calculation in the iterative procedure. Thus, if the initial values of the pH of the zone and $C_{Z_M,S}$ or $C_{M,S}^*$ are known one can obtain an initial value of \bar{m}_S by eqn. 13, and in the subsequent step of the iteration, $C_{Z_M,S}$ estimated by eqns. 14 and 15 can be employed for the evaluation of \bar{m}_S . Similarly, an initial value of C_{Z_S} or C_S^* is necessary for the evaluation of $\bar{m}_{M,S}$. If the quadratic equation for \bar{m}_S is used, a procedure for discriminating the physically incorrect solutions is necessary. Therefore, the present formulation is preferable for the computation of \bar{m}_S .

The total concentration of buffer, $C_{B,S}^*$, which is closely related with the pH of the sample zone, can be expressed by the following equation derived from the other mass balance equation

$$C_{B,S}^* = C_{B,L}^* [\bar{m}_S (\bar{m}_L + \bar{m}_{B,L})] / [\bar{m}_L (\bar{m}_S + \bar{m}_{B,S})] \quad (17)$$

where $\bar{m}_{B,S}$ is the effective mobility of the buffering ion in the S zone.

In order to obtain the total concentration of the non-complexing (free) sample ion, C_S^* , the electroneutrality relationship in the S zone is necessary, as follows:

$$C_{H,S} - C_{OH,S} + \sum_{i=0}^{n_B} (Z_B - i) C_{Z_B-i,S} + \sum_{i=0}^{n_M} (Z_M - i) C_{Z_M-i,S} + \\ \sum_{i=0}^{n_S} (Z_S - i) C_{Z_S-i} + \sum_{i=0}^{n_S} (Z_M + Z_S - i) C_{MS,Z_M+Z_S-i} = 0 \quad (18)$$

The concentration of complexes, C_{MS} , can be expressed using C_M^* (eqn. 15 and C_S^* as:

$$C_{MS,Z_M+Z_S-i} = C_{Z_M} C_{Z_S} K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) = C_S^* C_{M,S}^* / \\ \left[1 + \sum_{p=1}^{n_S} \left(\prod_{j=1}^p K_{S,j} / C_{H,S} \right) \right] \left[1 + \sum_{p=1}^{n_M} \left(\prod_{j=1}^p K_{M,j} / C_{H,S} \right) \right] \times \\ K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \quad (19)$$

The sub-species concentrations, C_B , C_M and C_S in eqn. 18, can be expressed by the corresponding total concentrations, then it follows that:

$$\begin{aligned}
 C_S^* = & \left\{ C_{OH,S} - C_{H,S} - C_{B,S} \sum_{i=0}^{n_B} (Z_B - i) \left(\prod_{j=1}^p K_{B,j} / C_{H,S} \right) \right\} \\
 & \left[1 + \sum_{p=1}^{n_B} \left(\prod_{j=1}^p K_{B,j} / C_{H,S} \right) \right] - C_{M,S} \sum_{i=0}^{n_M} (Z_M - i) \times \\
 & \left(\prod_{j=1}^i K_{M,j} / C_{H,S} \right) \left[1 + \sum_{p=1}^{n_M} \left(\prod_{j=1}^p K_{M,j} / C_{H,S} \right) \right] \left\{ \sum_{i=0}^{n_S} (Z_S - i) \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \right\} \\
 & \left[1 + \sum_{p=1}^{n_S} \left(\prod_{j=1}^p K_{S,j} / C_{H,S} \right) \right] + C_{M,S} \left[1 + \sum_{p=1}^{n_M} \left(\prod_{j=1}^p K_{S,j} / C_{H,S} \right) \right] \\
 & \left[1 + \sum_{p=1}^{n_M} \left(\prod_{j=1}^p K_{M,j} / C_{H,S} \right) \right] \times \sum_{i=1}^{n_S} (Z_M + Z_S - i) K_{MS,i} \left(\prod_{j=1}^i K_{S,j} / C_{H,S} \right) \} \quad (20)
 \end{aligned}$$

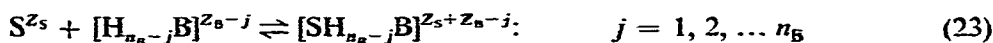
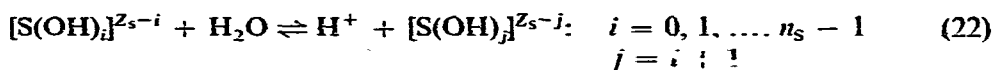
If the pH of sample zone is known, the sub-species concentrations of the zone constituents can be evaluated, and hence the ionic strength of the S zone.

The specific conductivity of the S zone is as follows:

$$\begin{aligned}
 \kappa_S = & C_{H,S} + C_{OH,S} + \sum_{i=0}^{n_S} |Z_S - i| C_{Z_S-i} m_{Z_S-i} + \sum_{i=0}^{n_B} |Z_B - i| C_{Z_B-i} m_{Z_B-i} + \\
 & \sum_{i=0}^{n_M} |Z_M - i| C_{Z_M-i,S} m_{Z_M-i} + \sum_{i=1}^{n_S} |Z_M + Z_S - i| C_{MS,Z_M+Z_S-i} m_{MS,Z_M+Z_S-i} \quad (21)
 \end{aligned}$$

Then the potential gradient of the S zone can be evaluated using a similar equation to eqn. 9.

In the case that the buffer ion also acts as a complexing agent, the following equilibria in cationic analysis can be set up instead of eqns. 10 and 11



where S^{Z_S} is the sample ion with maximal charge Z_S . Eqn. 23 may represent the equilibria of a metal ion, S^{Z_S} , forming complexes with acids. The effective mobility, \bar{m}_S , is obtained by replacing subscripts M and MS in eqn. 16 by B and BS, respectively. Similarly, appropriate change of the subscripts in the preceding equations gives a set of equations for the electrolyte system. The total concentration of non-complexing buffer ion can be expressed as follows:

$$\begin{aligned}
 C_{B,S} = & (\text{Right hand of eqn. 17}) / \left\{ 1 + C_{Z_S} \left[\sum_{j=1}^{n_B} K_{BS,j} \left(\prod_{j=1}^j K_{B,j} / C_{H,S} \right) \right] \right\} \\
 & \left[1 + \sum_{j=1}^{n_B} \left(\prod_{p=1}^j K_{B,p} / C_{H,S} \right) \right] \quad (24)
 \end{aligned}$$

Determination of the pH of the sample zone at steady state

The pH of the S zone differs from that of the L zone, and only at a certain pH of the S zone are the isotachophoretical conditions fulfilled and the steady complex-forming equilibria allowed.

The correct pH of the S zone can be determined by the following procedure. Since the current density, J , is kept constant in the separation compartment during migration, and the density of the leading zone equals that of the sample zone, the following equation is valid:

$$1000J/F = E_L\kappa_L = E_S\kappa_S = Q \quad (25)$$

The amounts expressed by eqn. 25 for the L and S zones are denoted as Q_L and Q_S , hereafter. If these zones migrate with the same velocity, v , then the following relation is valid:

$$v = E_L\bar{m}_L = E_S\bar{m}_S \quad (26)$$

The electroneutrality of the zones and the mass balance equations for counter ions have already been taken into account when the specific conductivity was evaluated. Therefore, the establishment of the following relation¹ provides a measure of the steady state:

$$RFQ = Q_L/Q_S - 1 = \bar{m}_S\kappa_L/(\bar{m}_L\kappa_S) - 1 = 0 \quad (27)$$

In practice, the first stage of the iterative calculation was carried out using several assumptions: (1) the pH of the S zone is equal to that of the L zone; (2) the ionic strength of the S zone used for the correction of mobility and pK_a is equal to that of the L zone; (3) the total concentration of the complexing counter ion and sample ion is equal to those of the L zone. At this stage, the value of RFQ was never zero, and subsequently, the overall calculations for the S zone must be repeated, varying the pH appropriately without use of the above assumptions. In anionic analysis, the pH was increased, *vice versa* for cationic analysis. In the course of iteration, the absolute mobility, thermodynamic dissociation constants and stability constants were corrected to the values at finite ionic strength, and the effective mobilities and the concentrations of the constituents were evaluated. Also, if RFQ was zero at a certain pH, in practice $|RFQ| < 5 \cdot 10^{-5}$, the overall calculations were again carried out using the same pH to confirm self-consistency of the calculated results. If the relation $|RFQ| < 5 \cdot 10^{-5}$ was again satisfied, the iteration was terminated. This required the overall calculations to be repeated 15 to 30 times by our program.

Thus, the observable qualitative indexes, the ratios of the potential gradients, R_E , or specific conductivities of the separated zones in the steady state, can be expressed using the final values from the iteration, as follows:

$$R_E = E_S/E_L = \kappa_L/\kappa_S = \bar{m}_L/\bar{m}_S \quad (28)$$

The zone length of sample can easily be obtained using the obtained total concentration, the injected amount and the dimensions of the separation compartment. The time required for the sample zone to pass through the detector can be estimated using the zone length and the velocity of isotachophoretic migration.

EXPERIMENTAL

The validity of equations derived in the preceding sections can be confirmed, if the observed and simulated R_E values agree with each other for anionic and cationic samples respectively, coexisting with the common complexes.

The R_E values were measured for tartrate ion in the presence of Ca^{2+} (anionic analysis) and for Ca^{2+} in the presence of tartrate ion (cationic analysis). In anionic analysis, the leading ion was Cl^- (10 mM) and the concentration of Ca^{2+} added in the leading electrolyte was varied in from 0.5 to 3 mM. The pH of the leading electrolytes was adjusted to 6.0 by adding histidine. In cationic analysis, the leading ion was K^+ and its concentration was varied from 5.68 to 18.9 mM. The pH of the leading electrolyte was adjusted to 5.1 by adding tartaric acid. In this case, tartrate ion acts as both the complexing and pH-buffering ion. The terminating ions were 5 mM recrystallized N-(morpholino)ethanesulphonate for anionic analysis and 5 mM ϵ -aminocaproate for cationic analysis, respectively. All chemicals were commercial guaranteed reagents.

The isotachophoretic analyzers used were a Shimadzu IP-1B for anionic analysis and IP-2A for cationic analysis. The former was equipped with a hand-made potential gradient detector⁹ and the latter with a Shimadzu PGD-2. The PTFE separation tube for the IP-1B was 0.5 mm I.D. and 20 cm long. The IP-2A was equipped with a pre-separation tube (4 cm \times 1 mm I.D.) joined to the main tube (10 cm \times 0.5 mm I.D.). A driving current, 50 or 75 μA , was applied to suppress the temperature increment in the separation tube. The temperature of the separation compartment was thermostatted at 25°C. The observed R_E values listed in Tables II and III are the averages from four or five determinations and the experimental deviations are within ± 0.03 . In order to cancel a slight drift of baseline or asymmetric potential, which could not be ignored for the precise R_E measurement, the internal standards ClO_3^- and Na^+ were used. The mobilities of these ions are well defined. The calculated R_E values were 1.19 for ClO_3^- ($L = 10 \text{ mM Cl}^-$, histidine buffer, $\text{pH}_L = 6.0$) and 1.48 for Na^+ ($L = 10 \text{ mM K}^+$, tartrate buffer, $\text{pH}_L = 5.1$), although these values varied slightly among the systems used, due to differences in ionic strength.

RESULTS AND DISCUSSION

All calculations described below were carried out using a SORD microcomputer, M223 Mark III.

The absolute mobility, $\text{p}K_a$ and stability constants used in the simulation are summarized in Table I. The absolute mobilities of tartrate ion have been obtained isotachophoretically by the computer analysis of the R_E values measured at different pH values, as described in ref. 10. The other constants were taken from the literature^{11,12}, but assumed $\text{p}K_a$ values were used for Cl^- , K^+ and Ca^{2+} . Exact values of them were not necessary, since the pH of the present system was 5–6. The stability constants of calcium tartrate shown in Table I were obtained by electromotive force measurements in 0.2 N KCl as electrolyte. The original values of 1.80 (CaTar) and 1.11 (CaHTar) were corrected to the thermodynamic values using activity coefficients. Other stability constants for calcium tartrate reported were 2.80 (conductivity method) and 2.98 (method not specified) at infinite dilution; however, the other value for CaHTar could not be found^{11,12}.

TABLE I
PHYSICO-CHEMICAL CONSTANTS USED IN SIMULATION (25°C)

m_0 = Absolute mobility $\times 10^5$ ($\text{cm}^2\text{V}^{-1}\text{sec}^{-1}$); pK_a = thermodynamic acidity constants, assumed values being used for Cl^- , K^+ and Ca^{2+} ; $\log K$ = thermodynamic stability constants.

Ion	m_0	pK_a	$\log K$
Cl^-	79.08	-3	
K^+	75.72	13	
Ca^{2+}	61.7	12	
	30.9	13	
HTar^-	32.6	3.036	
Tar^{2-}	60.7	4.366	
Histidine ⁺	29.6	6.04	
CaHTar^+	25	-	1.59
CaTar^0	0	-	2.83

The chemical equilibria in the tartrate zone can be written as



where tartrate is abbreviated as Tar, k_1 , k_2 are the acidity constants of HTar^- and Tar^{2-} and K_1 , K_2 the stability constants of CaHTar^+ and CaTar , respectively. In the anionic analysis, the dissociation equilibrium of histidine as the pH-buffering counter ion must also be taken into account.

The R_E values of tartrate ion can be expressed as

$$R_E(\text{Tar}) = \bar{m}_{\text{Cl}}/\bar{m}_{\text{Tar}} = \bar{m}_{\text{Cl}}([\text{H}_2\text{Tar}] + [\text{HTar}^-] + [\text{Tar}^{2-}] + [\text{CaHTar}^+] + [\text{CaTar}]) / (m_1[\text{HTar}^-] + m_2[\text{Tar}^{2-}] + m_3[\text{CaHTar}^+]) = \bar{m}_{\text{Cl}} \{1 + k_1/C_H(1 + K_1[\text{Ca}^{2+}] + k_1k_2/C_H^2(1 + K_2[\text{Ca}^{2+}]))\} / \{k_1/C_H(m_1 + m_3K_1[\text{Ca}^{2+}]) + m_2k_1k_2/C_H^2\} \quad (33)$$

where \bar{m}_{Cl} is the effective mobility of the chloride ion, and m_1 , m_2 and m_3 are the corrected mobilities of HTar^- , Tar^{2-} and CaHTar^+ , respectively. From eqn. 33, it is apparent that higher concentrations of complexes, i.e., a higher concentration of non-complexing (free) Ca^{2+} , results in higher R_E values. Eqn. 33 is useful for the estimation of rough R_E values. Fig. 1 shows the effect of the calcium concentration and the pH of the sample zone on R_E values of tartrate ion coexisting with Ca^{2+} . The curves were plotted by using eqn. 33. It should be noted that the curves are different from those of R_E vs. pH_L and R_E vs. pH_S at the steady state of isotachopheresis. The concentration of Ca^{2+} coexisting with tartrate was varied from 0 to 4 mM, and is actually controlled by the mass balance equation. The increase of the R_E values at low pH is essentially due to the lower effective mobility of tartrate ion, i.e., the lower

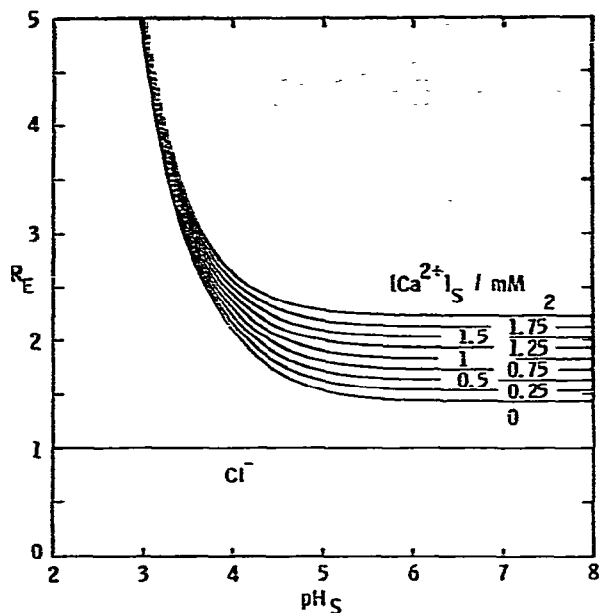


Fig. 1. Effect of calcium concentration and pH of sample zone, pH_S , on R_E values of tartrate ion coexisting with Ca^{2+} . The concentration of Ca^{2+} is that in the sample zone. The curves are not for the isotachophoretic steady state.

degree of dissociation. The similarity in R_E values results from the lack of complexation in this region. On the other hand, at pH values higher than 3.5, the effect of concentration on R_E increases. The limiting R_E values in this region can be estimated by use of eqn. 33, as follows:

$$R_E(\text{Tar})_{\text{lim.}} = \bar{m}_{\text{Cl}}(1 + K_2[\text{Ca}^{2+}])/m_2 \quad (34)$$

Table II lists the observed and the simulated R_E values for calcium concentrations from 0.5 to 3 mM in the leading zone, together with the concentrations and effective mobilities of the sample zone constituents. The concentration of CaHTar^+ complexes is not shown, since at most it was less than 0.04% of the total concentration of tartrate. It should be noted that the concentration of free Ca^{2+} in the tartrate zone is always smaller than that in the L zone. Thus, the R_E values estimated by eqn. 33 on the assumption that the calcium concentration in tartrate zone was equal to that in the L zone are always larger than those simulated at isotachophoretic equilibrium.

Fig. 2 shows the dependences on pH_L and calcium concentration of the simulated R_E values of tartrate ion at the isotachophoretically steady state. There is a distinct difference between Figs. 1 and 2 which can be attributed to the fact that the curves in Fig. 2 were plotted against pH_L and the calcium concentration is that of the leading zone. Fig. 3 shows the pH_L dependence of the effective mobility of Ca^{2+} and the pH-buffering histidine. Fig. 4 shows the dependences of pH_L on the concentrations of buffer, non-complexing sample, total sample and non-complexing Ca^{2+} for a typical electrolyte, together with R_E and pH_S . It should be noted that the histidine buffer was used in the pH region throughout the simulations.

TABLE II
OBSERVED AND SIMULATED R_f VALUES OF TARTRATE ION, AND EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)

pH_L = pH of leading electrolyte; C_{ML} = total concentration (mM) of Ca^{2+} in leading electrolyte; C_L = total concentration (mM) of leading ion; R_f = ratio of potential gradients, E_f/E_L ; n_f = effective mobility ($cm^2V^{-1}sec^{-1}$) of sample ion $\times 10^5$; pH_S = pH of sample zone; $C^* = C_f^* + C_{MS}^*$ = total concentration (mM) of sample; C_{MS} = concentration (mM) of CaTar complex; C_{MS}^* = total concentration (mM) of free Ca^{2+} ; n_{MS}^* = effective mobility ($cm^2V^{-1}sec^{-1}$) of $Ca^{2+} \times 10^5$; $C_{f,S}$ = total concentration (mM) of buffer ion; $n_{f,S}^*$ = effective mobility ($cm^2V^{-1}sec^{-1}$) of buffer ion $\times 10^5$.

pH_L	C_{ML}	C_L	R_f	n_f	pH_S	C^*	C_{MS}	C_{MS}^*	n_{MS}^*	$C_{f,S}$	$n_{f,S}^*$
6.00	0.50	10.16	1.52	1.55	6.063	4.616	0.307	0.262	24.50	15.57	13.31
6.00	0.75	10.15	1.57	1.60	6.067	4.617	0.448	0.392	24.86	14.60	13.26
6.01	1.00	10.14	1.62	1.65	6.081	4.616	0.581	0.521	25.22	13.81	13.06
6.01	1.25	10.14	1.70	1.70	6.085	4.616	0.707	0.649	25.57	12.87	13.01
6.01	1.50	10.13	1.74	1.75	6.090	4.608	0.824	0.776	25.94	11.94	12.95
6.01	1.75	10.12	1.80	1.80	6.094	4.598	0.934	0.901	26.31	11.02	12.90
6.00	2.00	10.11	1.86	1.85	6.088	4.585	1.036	1.026	26.68	9.988	12.99
6.00	2.50	10.09	1.95	1.95	6.097	4.549	1.220	1.272	27.42	8.220	12.88
6.00	3.00	10.07	2.08	2.05	6.105	4.503	1.378	1.512	28.17	6.496	12.77

Obs. Calc.

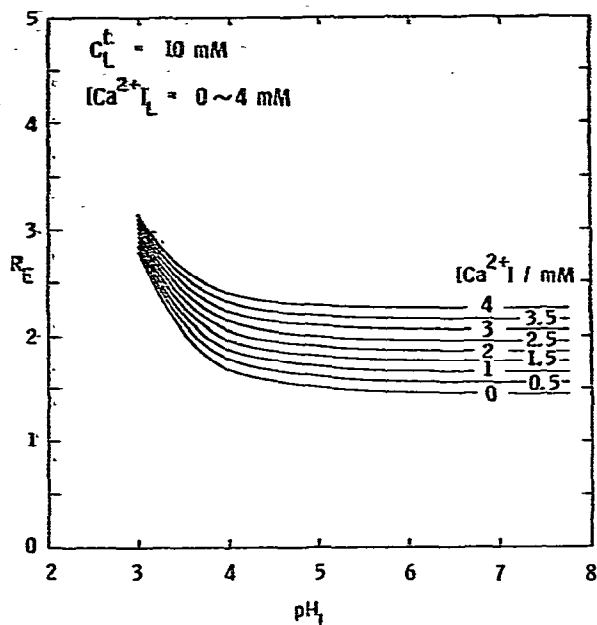


Fig. 2. Effect of calcium concentration and pH of leading zone, pH_L , on R_E values of tartrate ion coexisting with Ca^{2+} . The concentration of Ca^{2+} is that in the leading zone. The curves are for the isotachophoretic steady state. The leading ion is chloride (10 mM) and the pH-buffering ion is histidine.

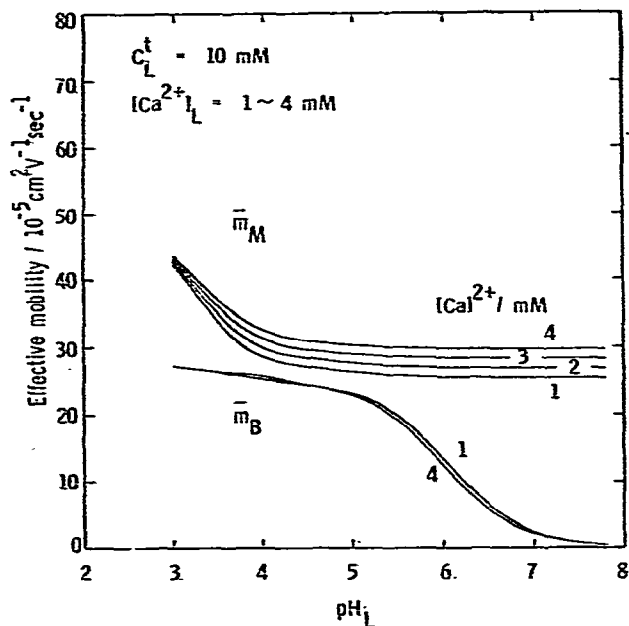


Fig. 3. Effect of calcium concentration and pH of leading electrolyte, pH_L , on the effective mobilities of counter ions (m_M , Ca^{2+} , m_B , histidine buffer) in the isotachophoretic steady state.

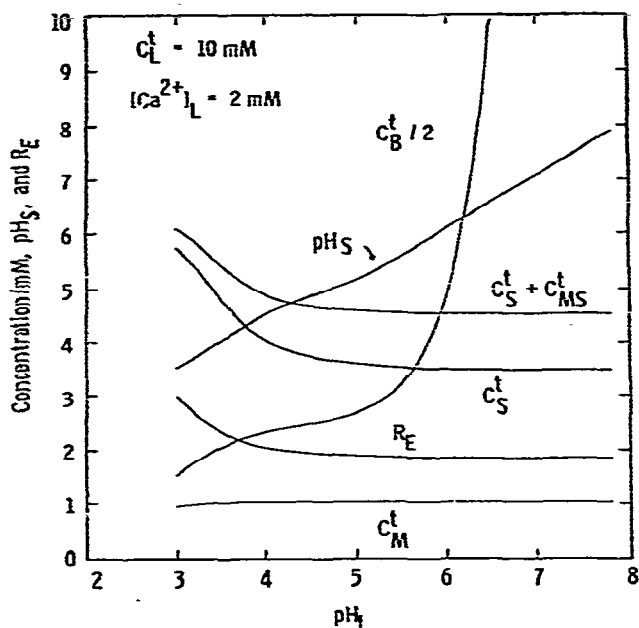


Fig. 4. Effect of pH of leading electrolyte, pH_L , on the concentration of the zone constituents, pH_S and R_E in the isotachopheric steady state. The calcium concentration in the leading zone is 2 mM. C_B^t = Total concentration of buffer; C_S^t = non-complexing tartrate; C_{MS}^t = calcium tartrate complex and C_M^t = non-complexing Ca^{2+} .

As shown in Table II, the observed and simulated R_E values agreed well with each other, and the differences were less than the accuracy of the experiments.

In cationic analysis, the R_E value of Ca^{2+} in the presence of tartrate ion can be expressed as

$$R_E(\text{Ca}) = \bar{m}_K / \bar{m}_{\text{Ca}} = \bar{m}_K \{ [\text{CaHTar}^+] + [\text{CaTar}] + [\text{Ca}^{2+}] \} / (m_3 [\text{CaHTar}^+] + m_4 [\text{Ca}^{2+}]) = \bar{m}_K \{ [\text{H}_2\text{Tar}] (K_1 k_1 / C_H + K_2 k_1 k_2 / C_H^2) + 1 \} / (m_3 K_1 [\text{H}_2\text{Tar}] k_1 / C_H + m_4) \quad (35)$$

where \bar{m}_K is the effective mobility of K^+ ion and m_4 is the corrected mobility of Ca^{2+} . Fig. 5 shows the dependences on pH and tartrate concentration of the R_E values of Ca^{2+} coexisting with CaTar . The curves were calculated by the use of eqn. 35, varying the concentration of coexisting tartrate from 0 to 4 mM. The point of inflection may correspond to the $\text{p}K_2$ value of the tartrate ion. At lower pH, the R_E value converges to the value of free Ca^{2+} , whereas at higher pH the CaTar complex is formed. The limiting value in the latter region can be expressed, ignoring the formation of hydroxyl complexes of Ca^{2+} , as follows:

$$R_E(\text{Ca})_{\text{lim.}} = \bar{m}_K (1 + K_2 [\text{Tar}^{2-}]) / m_4 \quad (36)$$

Eqns. 35 and 36 can be used for the estimation of rough R_E values; however, the strict simulation gives different R_E values, as shown in Fig. 6. This difference can be attri-

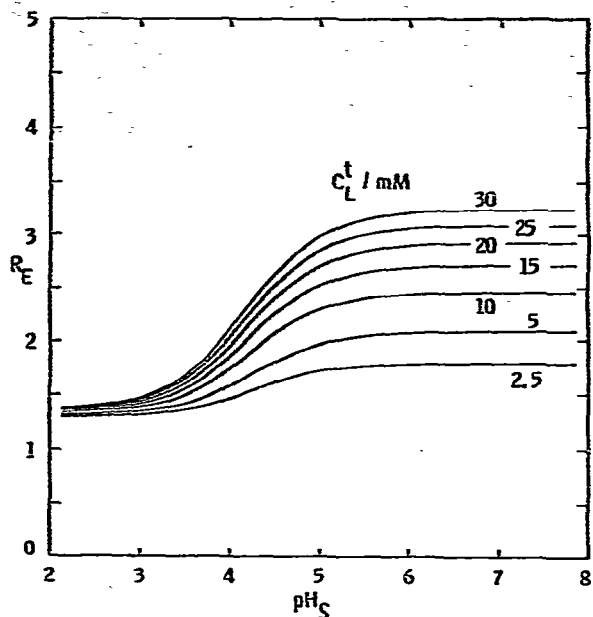


Fig. 5. Effect of total concentration of leading ion, C_L^t , and pH of sample zone, pH_S , on the R_E values of Ca^{2+} co-existing with tartrate ion. The curves are not for the isotachophoretic steady state.

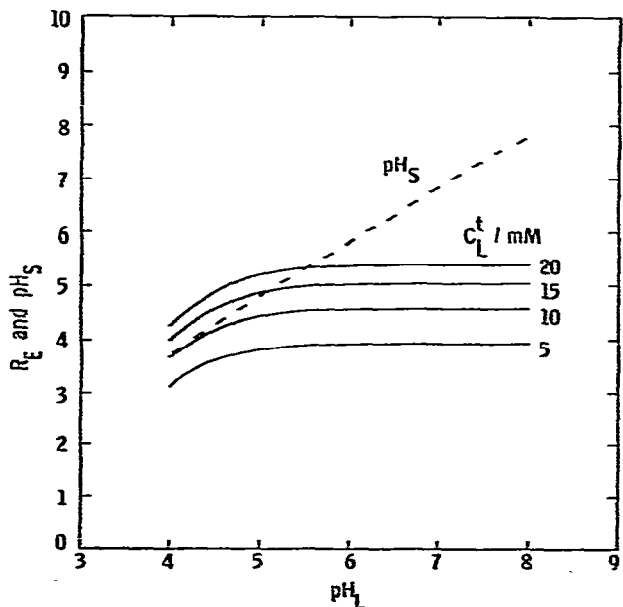


Fig. 6. Effect of total concentration of leading ion, C_L^t , and pH of leading electrolyte, pH_L , on the pH of the sample zone, pH_S , and the R_E values of Ca^{2+} co-existing with tartrate ion.

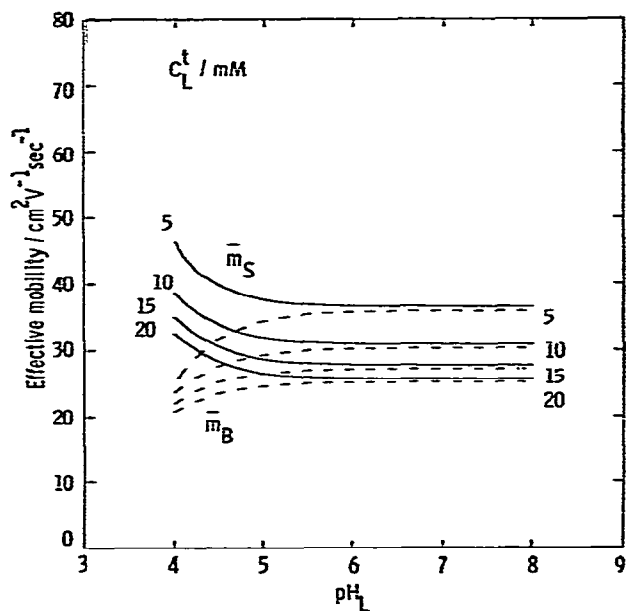


Fig. 7. Effect of total concentration of leading ion, C_L^t , and pH of leading electrolyte on the effective mobilities of Ca^{2+} , \bar{m}_S , and tartrate ion, \bar{m}_B .

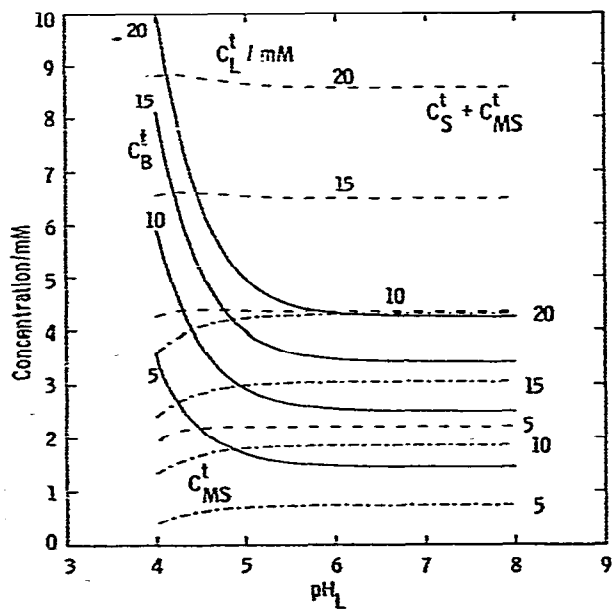


Fig. 8. Effect of total concentration of leading ion, C_L^t , and pH of leading electrolyte, pH_L , on the concentration of the sample zone constituents; for definitions of C_B^t , C_S^t and C_{MS}^t , see Fig. 4.

buted to the fact that Fig. 5 was plotted using corrected mobilities and the total concentration of buffer at certain pH values. Fig. 7 shows the dependences on pH_L and C_L of the effective mobilities of Ca^{2+} (sample) and tartrate ion (buffering and complexing agent), and Fig. 8 the concentrations of the zone constituents.

Table III gives the observed and simulated R_E values together with the concentrations and effective mobilities of the zone constituents. The good agreement between the simulated and observed R_E values was obtained also in cationic analysis.

TABLE III

OBSERVED AND SIMULATED R_E VALUES OF Ca^{2+} , AND EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)

$C = C_S + C_{BS}$ = Total concentration (mM) of sample; C_{BS} = concentration (mM) of CaTar complex; for other definitions see Table II.

pH_L	C_L	R_E		\bar{m}_S	pH_S	C	C_{BS}	C_B	\bar{m}_B
		Obs.	Calc.						
5.10	5.675	2.06	1.97	36.37	4.918	2.510	0.854	1.821	33.50
5.06	7.567	2.15	2.09	34.04	4.876	3.341	1.253	2.307	31.23
5.08	9.459	2.33	2.20	32.12	4.894	4.168	1.683	2.727	29.61
5.09	11.35	2.40	2.30	30.61	4.903	4.991	2.129	3.127	28.28
5.09	14.19	2.53	2.42	28.84	4.900	6.223	2.822	3.706	26.67
5.09	18.92	2.68	2.59	26.65	4.897	8.263	4.021	4.605	24.65

Thus, the present theory for the isotachophoretic steady state in complex-forming equilibria could be confirmed. The slight differences between the observed and calculated R_E values in cationic analysis might be meaningful, suggesting the existence of a small amount of a different type of complexes or ion pairs which were not considered in the present equilibria.

In this paper the simulation was carried out only for a simple case. However, it is apparent that the present simulation can be applied not only for the optimization of practical separations but also for the estimation of the stability constants and/or mobilities of complexes. Examples will be reported in later papers.

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REFERENCES

- 1 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, 1976.
- 2 P. Boček, I. Miedziak, M. Deml and J. Janák, *J. Chromatogr.*, 137 (1977) 83.
- 3 D. Kaniánsky and F. M. Everaerts, *J. Chromatogr.*, 148 (1978) 441.
- 4 I. Nukatsuka, M. Taga and H. Yoshida, *J. Chromatogr.*, 205 (1981) 95.
- 5 Y. Kiso and T. Hirokawa, *Chem. Lett.*, (1980) 745.
- 6 P. Debye and E. Hückel, *Physik. Z.*, 24 (1923) 185.
- 7 P. Debye and E. Hückel, *Physik. Z.*, 24 (1923) 305.
- 8 L. Onsager, *Physik. Z.*, 28 (1927) 277.
- 9 F. Nishiyama, T. Hirokawa and Y. Kiso, *Bull. Chem. Soc., Jap.*, 54 (1981) 933.
- 10 Y. Kiso and T. Hirokawa, *Chem. Lett.*, (1979) 891.
- 11 *Landsolt-Börnstein, Zahlenwerte und Funktionen*, Vol. II, Part 7, Springer, Berlin, 6th ed., 1960.
- 12 L. G. Sillen and A. E. Martell (Editors), *Stability Constants of Metal-Ion Complexes*, Special Publication No. 17, Chemical Society, London, 1964.