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COMPLEX-FORMING EQUILIBRIA IN ISOTACHOPHORESIS

II. EVALUATION OF STABILITY CONSTANTS OF TARTRATE AND CITRATE COMPLEXES

TAKESHI HIROKAWA* and YOSHIYUKI KISO

Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, Shitami, Saijo Higashihiroshima 724 (Japan)

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SUMMARY

A computational method is described for the evaluation of the thermodynamic stability constants of kinetically labile complexes using the qualitative indexes (R_E) observed by means of isotachophoresis. The constants obtained for Mg, Ca, Sr, Ba, Cd and Co–tartrate and –citrate complexes agreed well with previously reported values. The proposed method can be applied to mixed samples, and the constants obtained in anionic analysis could be also confirmed from cationic analysis in metal–citrate complex systems.

INTRODUCTION

In Part I¹, a computational procedure was described for the simulation of complex-forming equilibria in isotachophoresis. The observable qualitative index, R_E , which is the ratio of the potential gradient of the sample zone (E_S) to that of the leading zone (E_L : $R_E = E_S/E_L$), can be estimated for the sample zone with a steady-state of complex-forming equilibria, when the absolute mobilities, thermodynamic acid dissociation constants and stability constants of the sample and the leading, complex and pH-buffer ions are known. This suggests that the physico-chemical constants of the samples can be evaluated by analysing the variation in R_E observed under several different electrolyte conditions. In this instance, the analysis was treated by the least-squares method. A computational method for the evaluation of mobilities and acid dissociation constants has been already described for several amines and weak organic acids^{2,3}. The method has been proved to be a powerful technique especially for mixed samples. Similar applicability to the evaluation of stability constants is expected.

In this paper, experimental and computational methods for the evaluation of the stability constants of several metal(II) complexes are reported. The stability constants of many complexes reported previously have been measured in an electrolyte solution with a constant ionic strength; however, in isotachophoresis, the ionic

strengths of the separated zones including the leading zone are generally different. Therefore, thermodynamic stability constants are preferable to apparent values in the present experimental system. The stability constants were corrected for ionic strength using the activity coefficients estimated from Debye-Hückel equations^{4,5}. Hence, the obtained constants can conveniently be compared with each other.

The ligands used were tartrate and citrate ions. In isotachopheresis, these ions can be both a sample ion in anionic analysis and a counter ion in cationic analysis. The stability constants of metal(M)-tartrate complexes were evaluated using the cationic R_E indexes of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Cd^{2+} and Co^{2+} coexisting with tartrate ions as counter ions. The stability constants of Mg, Ca, Sr and Ba-citrate complexes were evaluated together with the absolute mobilities of the complex ions, using the anionic R_E values. The evaluated stability constants were compared with the previous values obtained by different methods. In order to reconfirm the constants for citrate complexes obtained by anionic analysis, the cationic R_E values were simulated using the values obtained in the anionic analysis in order to compare them with the observed values.

THEORETICAL

Tartrate complexes

Fig. 1 shows a schematic model of the separated zones in isotachopheresis. In cationic analysis, the leading, sample and terminating ions are K^+ , Ca^{2+} , and ϵ -aminocaproate, respectively. The tartrate ion acts as a pH buffer in the leading zone and also a complexing ligand in the cationic sample zone. The tartrate ion migrates in a counter direction to both the sample and terminating ions and interacts with Ca^{2+} to form a kinetically labile Ca-tartrate (CaTar) complex with an electrically zero charge. The complex itself cannot migrate electrophoretically, but the establishment of the equilibria may be rapid enough to permit homogeneous migration of a zone together with the non-complexing (free) M^{2+} .

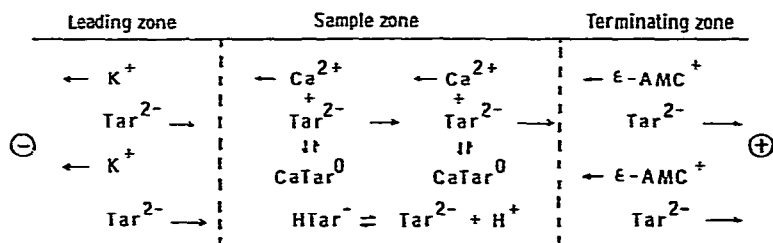
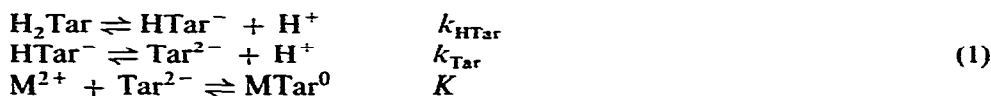


Fig. 1. Isotachophoretically migrating Ca^{2+} zone forming CaTar complex, the dissociation equilibria of the pH-buffering tartrate ion being omitted from the leading and terminating zones.

In the steady state, the complex-forming equilibria are achieved at a certain pH of the sample zone, pH_S , which is generally different from the pH of the leading electrolyte (pH_L). pH_S and the concentration and effective mobilities of the zone constituents are regulated isotachophoretically under the operational condition of the leading electrolyte. The complex-forming equilibria can be written as follows:



where k_{HTar} and k_{Tar} are the dissociation constants of tartaric acid and K is the stability constant of the CaTar complex. The other form of the complexes, CaHTar , was neglected as its abundance is negligibly small¹.

As has been discussed in Part I¹, the effective mobility of M^{2+} coexisting with tartrate ion can be expressed by the mobility and the dissociation constant of M^{2+} , the stability constant of the MTar complex and the concentration of non-complexing tartrate ion in the steady state. The observable R_E values for M^{2+} , $R_E(\text{M})$, can be expressed as follows:

$$\begin{aligned}
 R_E(\text{M}) &= \bar{m}_k / \bar{m}_{\text{M}} = \bar{m}_k([\text{MTar}] + [\text{M}^{2+}]) / (m_{\text{M}}[\text{M}^{2+}]) \\
 &= \bar{m}_k([\text{H}_2\text{Tar}] K k_{\text{HTar}} k_{\text{Tar}} / [\text{H}^+]^2 + 1) / m_{\text{M}}
 \end{aligned}
 \tag{2}$$

where \bar{m}_k is the effective mobility of K^+ , \bar{m}_{M} is that of M^{2+} coexisting with tartrate ion and m_{M} is the mobility of non-complexing M^{2+} , which are corrected for the finite ionic strength using Onsager's equation⁶. The hydroxyl complexes of the treated metal ions except Cd^{2+} hardly existed in the pH range used. In the isotachophoretic equilibrium, the concentration of the non-complexing tartaric acid in the sample zone, $[\text{H}_2\text{Tar}]$, can be calculated using the mass balance equation¹. This concentration is closely related to that in the leading electrolyte, *i.e.*, pH_L and the concentration of the leading ion. Fig. 2 shows the dependence of the stability constant of CaTar and pH_L on the simulated R_E values of Ca^{2+} coexisting with tartrate ion in an isotachophoretically steady state. The concentration of the leading ion was 10 mM and pH_L was varied by increasing the total concentration of tartaric acid. The thermodynamic stability constant of the CaTar complex ($\log K$) was varied in the range 2–3.4;

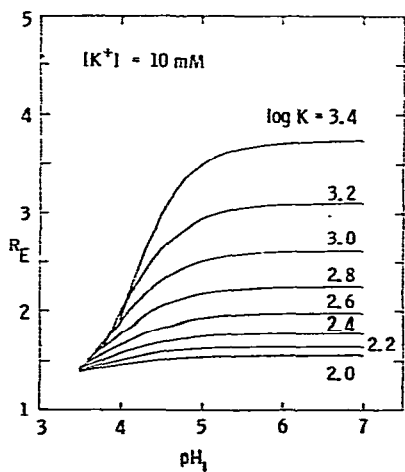


Fig. 2. Effect of the stability constant of CaTar complex ($\log K$) and the pH of leading zone (pH_L) on the R_E values of Ca^{2+} coexisting with tartrate ion. The concentration of the leading ion, K^+ , was 10 mM. The curves were plotted for the isotachophoretic steady state.

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

m_0 = Absolute mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) $\cdot 10^5$. pK_a = Thermodynamic acidity constants, assumed values being used for K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} and Cl^- .

Cation	m_0	pK_a	Anion	m_0	pK_a
K^+	75.72	13	Cl^-	79.08	-3
Mg^{2+}	55.0	12	HTar^-	32.6*	3.036
Ca^{2+}	61.7	12	Tar^{2-}	60.7*	4.366
Sr^{2+}	61.9	12	H_2Cit^-	28.7*	3.128
Ba^{2+}	66.0	12	HCit^{2-}	54.7*	4.761
Cd^{2+}	56.0	7.6	Cit^{3-}	74.4*	6.396
Co^{2+}	56.0	11.2			
Tris^-	29.5*	8.08			
$\epsilon\text{-AMC}^+$	28.8*	4.43			

* These absolute mobilities were obtained isotachophoretically and the other constants were taken from ref. 7.

the previously reported value⁷ was 2.8. The other physico-chemical constants used are listed in Table I⁸. It is apparent that in the higher pH range in Fig. 2 the R_E values are sensitive to the small difference in the stability constant due to the relatively high abundance of the CaTar complex. For the calculation of the stability constant, the R_E values for the least-squares method should be obtained under different electrolyte conditions, *i.e.*, different pH_L as shown in Fig. 2 and/or different concentrations of the leading electrolyte (Cl_L^-). Under such electrolyte conditions, $[\text{H}_2\text{Tar}]$ and/or $[\text{H}^+]$ in eqn. 2 are different, and therefore different R_E values may be obtained. Apparently, it is desirable for pH_L to be higher than the pK_a of tartrate ion (3.036 and 4.366) and to be in the pH range of full pH buffer capacity with tartrate ion. In the electrolyte systems used, a fixed pH_L of *ca.* 5 was selected and Cl_L^- was varied. Fig. 3 shows the dependence of the stability constant ($\log K$) and Cl_L^- on the R_E values at $\text{pH}_L = 5$. The value of $\log K$ was varied in the range 2-3 and Cl_L^- was varied in the range 5-20 mM. Fig. 3 implies that an accurate stability constant can be calculated by the least-squares method so as to reproduce the observed R_E values as well as possible, varying the objective constant.

In the least-squares method, the following quantity S was minimized by varying the stability constant:

$$S = \sum_{i=1}^n W_i [R_{E,i}(\text{obsd}) - R_{E,i}(\text{calcd})]^2 \quad (3)$$

where W_i is the weight of the observed R_E values, the inverses of the square of R_E being used in our program, and $R_E(\text{obsd})$ and $R_E(\text{calcd})$ are the observed and simulated R_E values, respectively. The "brute force" method was employed for the minimization of S . During the course of the minimization, the stability constants were decreased or increased by $0.1/2^n$ in $\log K$ units, where n was varied stepwise from 0 to 7. Thus, in the final step of the minimization procedure, $\log K$ was varied by $1/1024$.

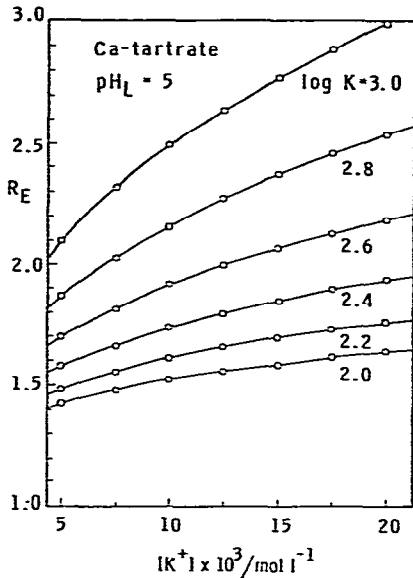


Fig. 3. Effect of the stability constant of CaTar complex ($\log K$) and the concentration of leading ion, $[K^-]$, on the R_E values of Ca^{2+} coexisting with tartrate ion. The pH of the leading zone, pH_L , was 5. The curves are for the isotachophoretic steady state.

The accuracy of the evaluated constants (the standard deviations, σ) can be estimated using the Jacobian matrix (J) as follows:

$$\sigma = [(\tilde{J}WJ)_{hh}^{-1} S/(q - p)]^{1/2} \tag{4}$$

where W is the weight matrix, q the number of the observed R_E values and p the number of constants determined. The subscript hh denotes the diagonal elements of the inverse matrix of $\tilde{J}WJ$. The elements of the Jacobian matrix may be approximated by $\Delta R_E / \Delta \log K$, and in practice the elements (J_j) can be calculated by the following:

$$J_{i,j} = [R_{E,i}(\log K_j + 0.01) - R_{E,i}(\log K_j - 0.01)]/0.02 \tag{5}$$

where $R_E(\log K \pm 0.01)$ denotes the calculated R_E values using the stability constants; the determined $\log K \pm 0.01$. Similarly, for the elements related to absolute mobilities the following approximation can be derived:

$$J_{i,k} = [R_{E,i}(m_{0,k} + 0.1) - R_{E,i}(m_{0,k} - 0.1)]/0.2 \tag{6}$$

Examples of the calculated Jacobian matrices will be shown later.

Citrate complexes

The known complex forms of citrate ion (Cit) with alkaline earths are MH_2Cit^+ , $MHCit^0$ and $MCit^-$. Fig. 4 shows a schematic model of the migrating citrate zone coexisting with Ca^{2+} . The chemical equilibria of complex formation can be written as follows:

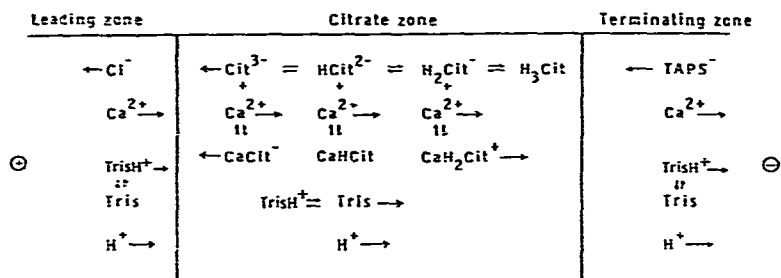
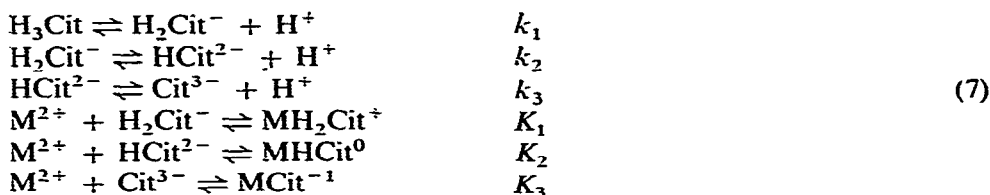


Fig. 4. Isotachophoretic equilibria of citrate ion coexisting with Ca²⁺. The pH buffer was Tris. No interaction was assumed between Tris and Ca²⁺.



where k_1 , k_2 and k_3 are the dissociation constants of citric acid and K_1 , K_2 and K_3 are the stability constants of the complexes formed. Typical values ($\log K$) reported for Ca complexes were 1.15, 3.05 and 4.90, respectively⁷. The formation of MHCit with zero charge and MCit⁻ with low mobility may strongly affect the effective mobility of citrate ion. The observable R_E , $R_E(\text{Cit})$, can be expressed as follows:

$$\begin{aligned}
 R_E(\text{Cit}) &= \bar{m}_{\text{Cl}}/\bar{m}_{\text{Cit}} = \bar{m}_{\text{Cl}}([\text{H}_2\text{Cit}^-] + [\text{HCit}^{2-}] + [\text{Cit}^{3-}] + \\
 &\quad [\text{MH}_2\text{Cit}^+] + [\text{MHCit}^0] + [\text{MCit}^{-1}]) / (m_1[\text{H}_2\text{Cit}^-] + \\
 &\quad m_2[\text{HCit}^{2-}] + m_3[\text{Cit}^{3-}] + m_4[\text{MH}_2\text{Cit}^+] + m_5[\text{MCit}^{-1}]) \\
 &= \bar{m}_{\text{Cl}} \{ 1 + k_1/[\text{H}^+] (1 + K_1[\text{M}^{2+}]) + k_1 k_2/[\text{H}^+]^2 (1 + K_2[\text{M}^{2+}]) + \\
 &\quad k_1 k_2 k_3/[\text{H}^+]^3 (1 + K_3[\text{M}^{2+}]) \} / \{ k_1/[\text{H}^+] (m_1 + m_4 K_1[\text{M}^{2+}]) + \\
 &\quad m_2 k_1 k_2/[\text{H}^+]^2 + k_1 k_2 k_3/[\text{H}^+]^3 (m_3 + m_5 K_3[\text{M}^{2+}]) \} \tag{8}
 \end{aligned}$$

where \bar{m}_{Cl} is the effective mobility of chloride ion and \bar{m}_{Cit} is that of citrate ion. The mobilities of H₂Cit⁻, HCit²⁻ and Cit³⁻ were abbreviated to m_1 , m_2 and m_3 , respectively, and m_4 and m_5 are the mobilities of MH₂Cit⁺ and MCit⁻, respectively. The mobility of the complex ions have not been reported. In this work, the stability constants and mobilities were obtained simultaneously, as shown below. However, it has become apparent, according to preliminary simulations using appropriate mobilities, that the precise evaluation of the stability constant and mobility of MH₂Cit was difficult owing to the relatively low abundance of the complex as for MHTar, even if pH_L was set at a low value. Then, the stability constant of MH₂Cit was fixed at the previously reported value⁷ of CaH₂Cit, i.e., $\log K_1 = 1.15$, and the mobility of MH₂Cit⁺ (m_4) was assumed to be equal to the value for MCit⁻ (m_5) throughout the subsequent calculations. The remaining three constants, $\log K_2$, $\log K_3$ and m_5 , were determined simultaneously in the present work.

By the use of the observed R_E values at pH_L = 8, $\log K_3$ and m_5 can be

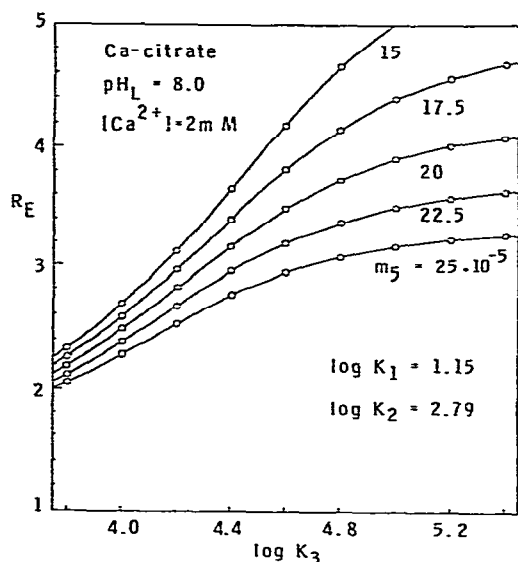


Fig. 5. Effect of the mobility of CaCit^- , m_5 ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) $\cdot 10^5$ and the stability constant, $\log K_3$, on the R_E values of citrate ion in the isotachophoretic steady state. The concentration of the leading ion, Cl^- , was 10 mM , the Ca ion concentration in the leading zone was 2 mM and the pH of the leading zone, pH_L , was 8 (Tris buffer). $\log K_1$ and $\log K_2$ (see text) were fixed at 1.15 and 2.79, respectively.

evaluated. It is apparent from the $\text{p}K_a$ values of citrate ions that the complexes MH_2Cit^+ and MHCit^0 can hardly exist under such pH conditions. The main constituents of the separated citrate zone may be the MCit^- complex, non-complexing M^{2+} and Cit^{3-} and the pH buffering counter ion. Fig. 5 shows the dependence of $\log K_3$, m_5 and the Ca^{2+} concentration of the leading electrolyte on the simulated R_E values of citrate ion. The mobility of the complex ion was varied in the range 15–25 ($\text{cm}^2/\text{V} \cdot \text{sec} \cdot 10^{-5}$) and $\log K_3$ was varied in the range of 3.8–5.4. The concentrations of the leading and the added Ca^{2+} were 10 and 2 mM , respectively. The buffer used was Tris and pH_L was set at 8. The values of K_1 , K_2 and m_4 were fixed appropriately. In the range of small $\log K_3$ in Fig. 5, the R_E value decreases, suggesting an increase in the abundance of the free citrate ion. On the other hand, in the range of large $\log K_3$, the upper limit of the curves corresponds to the R_E values of the CaCit^- complex. If the additive concentration increases, the gradients of the curves also increase and the plateau of the curves may be more apparent. Thus, the R_E values were strongly affected by the values of $\log K_3$ and m_5 , suggesting that the simultaneous evaluation of these constants may be possible by the best-fitting procedure for the observed R_E . If the number of observed R_E values is only one, an infinite number of combinations of different $\log K_3$ and m_5 are allowed, which can reproduce the observed R_E value exactly. Therefore, the R_E values should be obtained under different electrolyte conditions, in this instance, varying the additive concentration.

If the evaluation of $\log K_2$ is desired, another set of R_E values are necessary. pH_L should be lowered to keep the abundance of HCit^{2-} accordingly to that of MHCit^0 . However, the coexistence of MHCit^0 and MCit^- complexes is inevitable because the difference between $\text{p}K_2$ and $\text{p}K_3$ of citric acid is only 1.6 (see Table I).

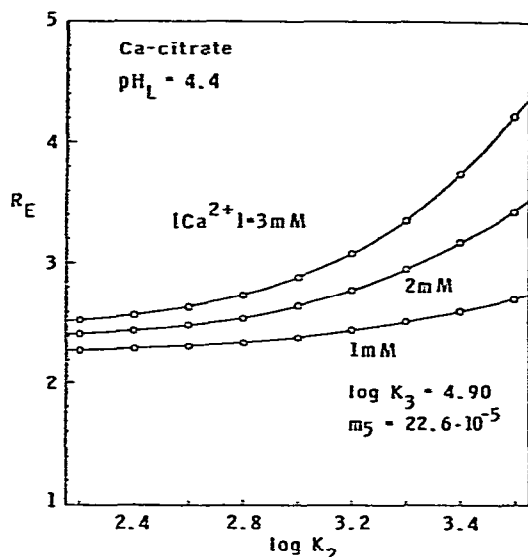


Fig. 6. Effect of the concentration (*mM*) of Ca²⁺ in the leading electrolyte and the stability constant of CaHCit, log *K*₂, on the *R*_E values of citrate zone in the isotachophoretic steady state. The pH of the leading electrolyte was 4.4 (*ε*-AMC buffer) and log *K*₃ and *m*₅ (see text) were fixed at 4.90 and 22.6, respectively.

Therefore, the exact values of log *K*₃ and *m*₅ are necessary for the evaluation of log *K*₂. If the values are not available, the best-fitting procedure should be applied for the *R*_E values observed at high and low pH_L.

Fig. 6 shows the dependence of log *K*₂ and the Ca²⁺ concentration in the leading electrolyte on the *R*_E values. pH_L was set at 4.4 and the pH buffer ion was *ε*-aminocaproate. The value of pH_L is smaller than p*K*₃ by *ca.* 2 and it is also smaller than p*K*₂ by *ca.* 0.4. Lower pH_L values may be inadequate for the decrease in the abundance of H*Cit*²⁻ and higher pH_L may also be inadequate for the increase in *Cit*³⁻ and the M*Cit*⁻ complex. The value of log *K*₂ was varied in the range 2.2–3.6 and the additive concentration was varied in the range 1–3 *mM*. The stability constant log *K*₃ and mobility *m*₅ used were fixed appropriately. The concentration of the leading ion was 10 *mM*. From Fig. 6, it is apparent that log *K*₂ can be evaluated by the use of the observed *R*_E values, varying the Ca concentration in the leading electrolyte. It should be noted that the curvatures of the lines in Fig. 6 are apparently different from those in Fig. 5. In Fig. 6, the simulated *R*_E values increase infinitely according to the increase in log *K*₂, *i.e.*, the increase in the abundance of MHCit⁰ with zero charge. The difference in the curvatures in Fig. 5 is due to the fact that M*Cit*⁻ has a finite mobility.

Thus, the simultaneous evaluation of log *K*₂, log *K*₃ and *m*₅ may be possible by the least-squares method for all of the observed *R*_E values at pH_L 8 and 4.4.

EXPERIMENTAL

Isotachopherograms were obtained by the use of Shimadzu IP-1B and IP-2A isotachophoretic analysers. The former was equipped with a hand-made potential

gradient detector⁹ and the latter with a Shimadzu PGD-2. For the IP-1B, the separating tube used was 20 cm × 0.5 mm I.D. For the IP-2A, a main separating tube, 10 cm × 0.5 mm I.D., was connected to a pre-separating column, 4 cm × 1 mm I.D. Although the separation compartment was thermostated at 25°C, the driving current was stabilized at 50 or 75 μA to suppress temperature increases. For the precise measurements of R_E values internal standards such as sodium ion ($R_E = 1.47$) and chlorate ion ($R_E = 1.18$) were used.

For the determination of the stability constants of tartrate complexes, the cationic R_E values were measured using the following electrolyte systems. The leading electrolyte was aqueous potassium hydroxide solution buffered by adding tartaric acid, which simultaneously acted as complexing agent in the sample zones. Six kinds of leading electrolytes were prepared by varying the concentration of leading ion (5.7–19 mM). The pH of all leading electrolytes was adjusted to 5.1. As the concentration of the tartrate ion increases as a function of that of the leading ion, the observable R_E values increases. The extent of the increase depends on the stability constants of the complexes formed, as discussed before.

The experimental conditions of the leading electrolytes used are summarized in Table II. Table II also shows the calculated concentrations of buffer ion and the effective mobilities of the leading and buffer ions, together with the ionic strengths of the leading electrolytes. The terminating electrolyte was 5 mM ϵ -aminocaproic acid (ϵ -AMC) and the pH was adjusted to 4.5 by adding tartaric acids. The samples were mixture of nitrates of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Cd^{2+} and Co^{2+} (0.01 M). They were injected simultaneously, unless some of them formed a mixed zone. Two examples of the isotachopherograms observed are shown in Fig. 7, and were obtained by varying the leading ion concentration (A, 5.675; B, 18.92 mM). The increase in the step heights of sample zones except for the standard (Na^+) zone was observed at higher concentrations of the leading ion, comparing Fig. 7A with Fig. 7B. In the isotachopherogram in Fig. 7B, the Co^{2+} zone could not be detected before the terminating zone owing to the extreme decrease in the effective mobility by the strong complexation.

TABLE II

EXPERIMENTAL CONDITIONS FOR THE EVALUATION OF STABILITY CONSTANTS OF Mg, Ca, Sr, Ba, Cd AND Co COMPLEXES WITH TARTRATE ION BY CATIONIC ANALYSIS, CALCULATED CONCENTRATIONS AND EFFECTIVE MOBILITIES OF LEADING ZONE CONSTITUENTS

pH_L = pH of leading electrolyte. C_L = Total concentration (mM) of leading ion. \bar{m}_L = Effective mobility ($cm^2 V^{-1} sec^{-1}$) of leading ion (K^+) · 10^5 . $C_{B,L}$ = Total concentration (mM) of buffer ion. $\bar{m}_{B,L}$ = Effective mobility ($cm^2 V^{-1} sec^{-1}$) of buffer ion · 10^5 . The counter direction is expressed by negative signs. I = Ionic strength · 10^3 .

System	pH_L	C_L	\bar{m}_L	$C_{B,L}$	$\bar{m}_{B,L}$	I
1	5.10	5.675	71.74	3.028	-50.64	8.34
2	5.06	7.567	71.20	4.049	-49.64	11.10
3	5.08	9.459	70.73	5.038	-49.11	13.90
4	5.09	11.351	70.32	6.027	-48.59	16.69
5	5.09	14.189	69.79	7.519	-47.83	20.88
6	5.09	18.918	69.03	9.998	-46.75	27.86

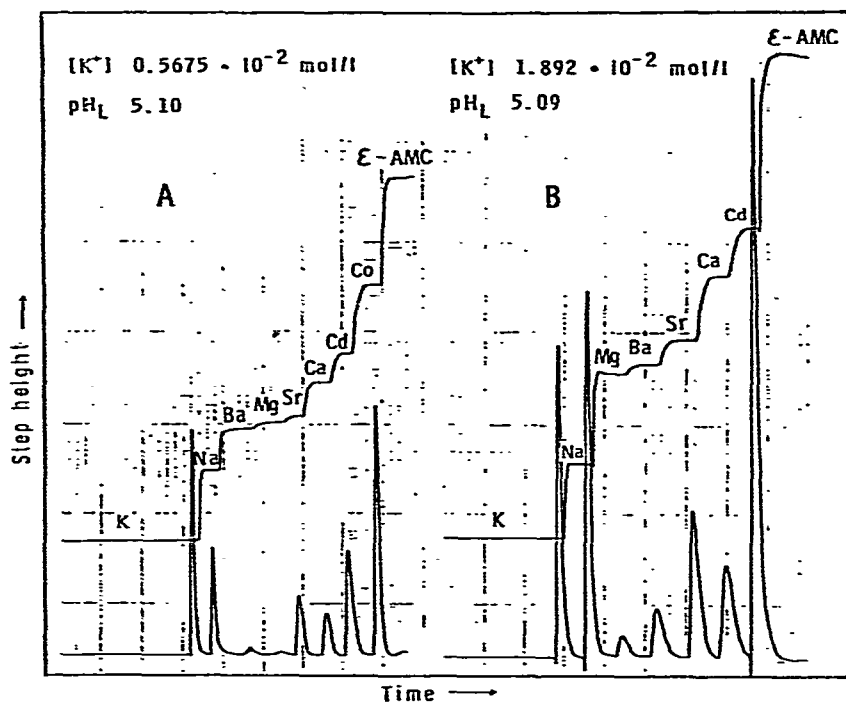


Fig. 7. Observed isotachopherograms of Na^+ , Ba^{2+} , Mg^{2+} , Sr^{2+} , Ca^{2+} , Cd^{2+} and Co^{2+} . The concentration of the leading ion, K^+ , was (A) 5.675 and (B) 18.92 mM. The pH of the leading electrolyte was adjusted to (A) 5.10 and (B) 5.09 by adding tartaric acid.

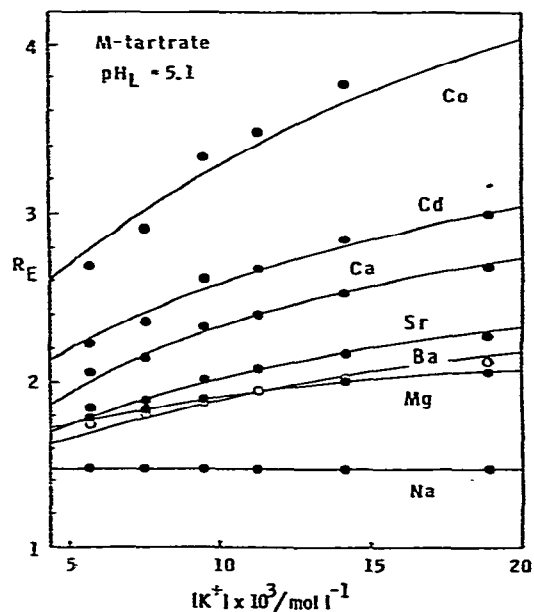


Fig. 8. Observed R_E values of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Cd^{2+} and Co^{2+} coexisting with tartrate ion. The concentration of the leading ion was varied in the range 5.7–18.9 mM. Na^+ was the internal standard. The best-fitted curves were also plotted.

TABLE III

EXPERIMENTAL CONDITIONS FOR THE EVALUATION OF STABILITY CONSTANTS OF Mg, Ca, Sr AND Ba COMPLEXES WITH CITRATE ION BY ANIONIC ANALYSIS, CALCULATED CONCENTRATIONS AND EFFECTIVE MOBILITIES OF LEADING ZONE CONSTITUENTS

$C_{M,L}$ = Total concentration (mM) of added ion. $\bar{m}_{M,L}$ = Effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) of added ion $\cdot 10^5$. The counter direction is expressed by negative signs. For other notations, see Table II.

Metal	System	pH _L	C _L	C _{M,L}	\bar{m}_L	C _{B,L}	$\bar{m}_{B,L}$	$\bar{m}_{M,L}$	I
Mg	1	8.00	10.09	1.00	74.78	14.13	-14.87	-48.00	11.09
	2	8.00	10.05	1.50	74.70	12.30	-14.85	-47.89	11.55
	3	8.00	10.00	2.00	74.63	10.46	-14.82	-47.78	12.00
	4	8.00	9.96	2.50	74.56	8.64	-14.80	-47.68	12.46
	5	8.04	9.92	3.00	74.48	7.11	-14.19	-47.58	12.92
	6	4.40	10.12	1.00	74.77	14.85	-13.76	-48.00	11.12
	7	4.40	10.09	1.50	74.70	12.94	-13.74	-47.88	11.59
	8	4.40	10.06	2.00	74.62	11.04	-13.71	-47.77	12.06
	9	4.40	10.04	2.50	74.54	9.16	-13.69	-47.66	12.54
	10	4.40	10.01	3.00	74.47	7.27	-13.67	-47.56	13.01
Ca	1	8.03	10.42	1.00	74.70	15.15	-14.41	-53.94	11.42
	2	8.03	10.37	1.50	74.63	13.25	-14.39	-53.82	11.87
	3	8.03	10.32	2.00	74.55	11.35	-14.37	-53.70	12.32
	4	8.03	10.27	2.50	74.48	9.46	-14.34	-53.58	12.77
	5	8.03	10.21	3.00	74.41	7.55	-14.33	-53.47	13.21
	6	4.40	10.48	0.50	74.78	17.35	-13.77	-54.07	10.98
	7	4.40	10.42	1.00	74.70	15.39	-13.74	-53.95	11.42
	8	4.40	10.37	1.50	74.63	13.45	-13.72	-53.82	11.87
	9	4.40	10.32	2.00	74.55	11.51	-13.70	-53.70	12.32
	10	4.40	10.27	2.50	74.48	9.58	-13.68	-53.58	12.77
Sr	1	8.00	10.12	1.00	74.75	14.18	-14.87	-53.99	11.12
	2	8.00	10.09	1.50	74.67	12.37	-14.84	-53.85	11.59
	3	8.00	10.06	2.00	74.60	10.57	-14.82	-53.72	12.06
	4	8.00	10.04	2.50	74.52	8.78	-14.80	-53.58	12.54
	5	8.00	10.01	3.00	74.45	6.98	-14.77	-53.45	13.01
	6	4.40	10.15	0.50	74.83	16.75	-13.78	-54.14	10.65
	7	4.40	10.14	1.00	74.75	14.88	-13.76	-53.99	11.14
	8	4.40	10.12	1.50	74.67	13.00	-13.73	-53.85	11.62
	9	4.43	10.10	2.00	74.59	11.48	-13.28	-53.71	12.10
	10	4.40	10.09	2.50	74.51	9.53	-13.69	-53.57	12.59
	11	4.40	10.07	3.00	74.44	7.38	-13.67	-53.44	13.07
Ba	1	7.99	10.24	1.00	74.72	14.25	-15.01	-57.89	11.24
	2	7.99	10.21	1.50	74.64	12.46	-14.98	-57.74	11.71
	3	7.98	10.18	2.00	74.57	10.57	-15.10	-57.61	12.18
	4	8.03	10.15	2.50	74.49	9.24	-14.35	-57.47	12.65
	5	7.99	10.12	3.00	74.42	7.10	-14.91	-57.34	13.12
	6	4.40	10.15	0.50	74.82	16.75	-13.78	-58.07	10.65
	7	4.40	10.13	1.00	74.74	14.86	-13.76	-57.92	11.13
	8	4.40	10.11	1.50	74.66	12.98	-13.73	-57.78	11.61
	9	4.40	10.09	2.00	74.58	11.10	-13.71	-57.63	12.09
	10	4.40	10.07	2.50	74.50	9.22	-13.69	-57.50	12.57
	11	4.40	10.06	3.00	74.42	7.36	-13.67	-57.36	13.06

The observed R_E values are summarized in Fig. 8. The R_E values are the averages of four determinations, which were corrected with the step-height of the standard ion, non-complexing Na^+ . The curves in Fig. 8 were the best-fitted ones, which will be discussed in the next section.

The stability constants of citrate complexes were determined using the following electrolyte systems for anionic analysis. The leading electrolytes were dilute hydrochloric acid buffered by adding tris(hydroxymethyl)aminomethane (Tris) and ϵ -AMC. The pH_L value of the former electrolyte was adjusted to 8.0 and the latter to 4.4. The chlorides of Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} were added individually to the leading electrolyte (1–3 mM). The total concentration of the leading ion, Cl^- , was adjusted to *ca.* 10 mM for all electrolyte systems used. Five or six kinds of leading electrolytes of a similar pH were prepared by varying the concentration of the added alkaline earths. The terminating electrolytes were 10 mM N-tris(hydroxymethyl)-methyl-3-aminopropanesulphonic acid (TAPS) and ϵ -AMC. The former electrolyte was used in combination with the leading electrolyte of pH 8 and the pH was also adjusted to 8.0 by adding Tris. The latter was used together with the leading electrolyte of pH 4.4 and the pH was adjusted to 4.4 by adding ϵ -AMC. The sample was 0.01 M citric acid.

Table III summarizes the experimental conditions of the leading electrolytes used, the calculated concentrations of the buffer and added (M) ions and the effective mobilities of the leading, buffer and added ions. Figs. 9 and 10 show the ob-

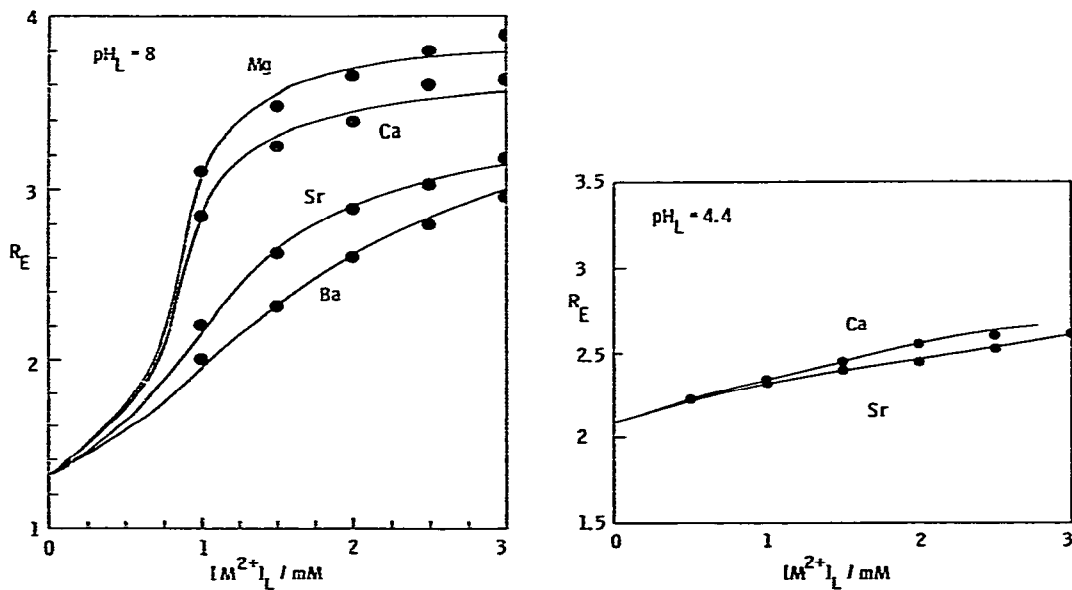


Fig. 9. Observed R_E values of citrate ion coexisting with Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} . The concentration of the leading ion was 10 mM and the pH of the leading electrolyte ion was 8 (Tris buffer). The concentration of the added metal ion was varied in the range 1–3 mM. The best-fitted curves were also plotted. The lower limit of the R_E value was that of free citrate ion.

Fig. 10. Observed R_E values of citrate ion coexisting with Ca^{2+} and Sr^{2+} . The concentration of the leading ion was 10 mM. The pH of the leading electrolyte (pH_L) was 4.4. The concentration of the added metal ion was varied in the range of 0.5–3 mM. The best-fitted curves were also plotted.

served R_E versus the added metal ion concentration in the leading electrolyte at pH_L 8 and 4.4, respectively. At pH_L 8, a rapid increase in the R_E of citrate ions was observed in the range of M^{2+} concentrations 0.5–1 mM, especially with the addition of Mg^{2+} and Ca^{2+} . The accuracy of the observed R_E values in this range was low, and therefore the obtained R_E values are not shown in Fig. 9 and were not used in the least-squares method. On the other hand, the R_E values at pH_L 4.4 showed a moderate increase owing to the lower complexibility of citrate ion (see Fig. 10). For added Mg^{2+} and Ba^{2+} , the observed R_E values were similar to those with Ca^{2+} and Sr^{2+} , respectively. The observed R_E values were averages of three determinations.

The leading electrolyte used contained a surfactant, 0.2% Triton X-100. All of the chemicals used were commercial guaranteed reagents. Measurements of pH were made with a Horiba F-7ss expanded-scale pH meter.

RESULTS AND DISCUSSION

The evaluation of the stability constants and data processing were carried out with a SORD M223 Mark III microcomputer.

For M-tartrate systems, Table IV summarizes the observed and the best-fitted R_E values, the calculated effective mobilities and concentrations of the samples, M^{2+} , and the common complexing ion, tartrate. The mean errors of the calculated R_E values were Mg^{2+} 1.01, Ca^{2+} 0.76, Sr^{2+} 0.95, Ba^{2+} 1.24, Cd^{2+} 1.37 and Co^{2+} 3.12%. As the existence of CoTar_2 and CoTar_3 complexes has been reported¹⁰, the relatively large error with Co^{2+} may be attributed to the fact that the present model treated only the monoligand complex. If the other two complexes are taken into account, the gradient of the best-fitted curve of Co^{2+} in Fig. 8 will increase and the calculated discrepancy will decrease. Such cases will be treated in detail in later papers.

The stability constants obtained are summarized in Table V, together with their standard deviations and the previously reported values obtained by different methods. Good agreement was obtained between the present and the reported values for Mg, Ca, Sr and Ba-tartrate complexes. For the Cd complex, the stability constant ($\log K$) of CdTar_2 in the literature⁷ is 4.49 (medium not specified). However, all six values of the observed R_E agreed with the estimated values by means of the present monoligand model. Therefore, at least under the present experimental conditions, almost all of the complexes formed may be of the CdTar type. It may be a further use of the proposed method that the number of ligands in a complex can be determined by R_E measurement and the best-fitting procedure. In principle, only one highly accurate R_E value can be used for the calculation of $\log K$; however, this is allowed only when the model of a complex used is confirmed by a different method, in addition to the statistical problem of the evaluation of $\log K$ (see eqn. 4; the dispersion is infinite in this instance). The small mean errors between the observed and best-fitted R_E values except for Co^{2+} are meaningful, suggesting that the accuracy of the evaluated stability constants is high and the present monoligand models are valid for Mg, Ca, Sr, Ba and Cd-tartrate complexes.

In order to confirm the obtained values of the stability constants, an isotachopherogram was simulated using these values, as shown in Fig. 11, which corresponds to Fig. 7B. The concentration of the leading ion (C_L) was 18.9 mM and pH_L was 5.09. The agreement between the observed and the simulated isotachophero-

TABLE IV

OBSERVED AND SIMULATED R_E VALUES OF Mg, Ca, Sr, Ba, Cd AND Co IONS, EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)

R_E = Ratio of potential gradients, $E_S/E_L \cdot \bar{m}_S$ = Effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) of sample ion $\cdot 10^5$ $\cdot \text{pH}_S$ = pH of sample zone. C = Total concentration (mM) of sample. C_{MB} = Concentration (mM) of M-tartrate complex. $C_{B,S}$ = Total concentration (mM) of non-complexing tartrate ion. $\bar{m}_{B,S}$ = Effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) of tartrate ion $\cdot 10^5 \cdot I$ = Ionic strength $\cdot 10^3$.

Metal	System	R_E		\bar{m}_S	pH_S	C	C_{MB}	$C_{B,S}$	$\bar{m}_{B,S}$	I
		Obs.	Calc.							
Mg	1	1.79	1.76	40.68	4.981	2.318	0.385	2.104	-43.73	0.758
	2	1.84	1.85	38.92	4.940	3.076	0.587	2.721	-41.94	0.974
	3	1.90	1.89	37.46	4.960	3.827	0.814	3.276	-40.71	1.181
	4	1.95	1.94	36.24	4.969	4.574	1.055	3.813	-39.64	1.381
	5	2.00	2.01	34.76	4.967	5.688	1.438	4.596	-38.24	1.668
	6	2.06	2.10	32.84	4.965	7.526	2.122	5.830	-36.41	2.121
Ca	1	2.06	2.04	35.14	4.893	2.466	0.905	1.732	-33.67	0.609
	2	2.15	2.17	32.77	4.851	3.275	1.320	2.184	-31.50	0.762
	3	2.33	2.29	30.86	4.871	4.079	1.765	2.568	-29.93	0.903
	4	2.40	2.40	29.35	4.879	4.877	2.224	2.934	-28.64	1.036
	5	2.53	2.53	27.59	4.878	6.069	2.933	3.460	-27.09	1.225
	6	2.69	2.72	25.42	4.876	8.036	4.156	4.271	-25.17	1.516
Sr	1	1.85	1.81	39.54	4.941	2.466	0.700	1.939	-37.86	0.691
	2	1.89	1.91	37.25	4.898	3.272	1.036	2.469	-35.78	0.874
	3	2.02	2.00	35.38	4.915	4.072	1.399	2.934	-34.30	1.045
	4	2.08	2.08	33.88	4.924	4.868	1.779	3.379	-33.08	1.209
	5	2.17	2.17	32.10	4.920	6.050	2.367	4.022	-31.56	1.442
	6	2.28	2.31	29.87	4.918	8.005	3.393	5.024	-29.65	1.806
Ba	1	1.75	1.70	42.14	4.947	2.550	0.731	1.994	-37.71	0.712
	2	1.81	1.79	39.71	4.902	3.385	1.080	2.542	-35.63	0.901
	3	1.88	1.88	37.72	4.918	4.213	1.458	3.021	-34.15	1.078
	4	1.95	1.95	36.14	4.926	5.037	1.853	3.480	-32.92	1.246
	5	2.02	2.04	34.25	4.922	6.264	2.466	4.145	-31.40	1.487
	6	2.12	2.16	31.89	4.918	8.290	3.533	5.181	-29.49	1.863
Cd	1	2.23	2.26	31.72	4.879	2.335	0.854	1.649	-33.74	0.577
	2	2.36	2.41	29.55	4.841	3.100	1.245	2.077	-31.59	0.722
	3	2.61	2.54	27.80	4.863	3.858	1.663	2.440	-30.03	0.855
	4	2.66	2.66	26.43	4.874	4.612	2.096	2.785	-28.76	0.981
	5	2.85	2.81	24.82	4.875	5.735	2.762	3.282	-27.22	1.160
	6	3.00	3.02	22.82	4.875	7.582	3.907	4.047	-25.31	1.435
Co	1	2.69	2.80	25.62	4.775	2.334	1.155	1.352	-27.14	0.457
	2	2.92	3.03	23.52	4.744	3.106	1.652	1.676	-25.06	0.563
	3	3.36	3.24	21.84	4.772	3.871	2.174	1.936	-23.50	0.658
	4	3.48	3.42	20.55	4.787	4.632	2.708	2.183	-22.27	0.747
	5	3.76	3.66	19.08	4.793	5.767	3.522	2.536	-20.83	0.872
	6	-	3.99	17.31	4.801	7.638	4.907	3.071	-19.07	1.062

grams was good. The details of the M-tartrate system in an isotachophoretic steady state have been reported in Part I¹.

For the M-citrate systems, the least-squares method was applied to the observed R_E values at pH_L 8 and 4.4 simultaneously. Table VI summarizes the observed

TABLE V

EVALUATED STABILITY CONSTANTS OF METAL-TARTRATE COMPLEXES AND THEIR DISPERSIONS (25°C)

Log K = Thermodynamic stability constant of M-tartrate. σ = Standard deviation. Lit. = Literature values; H = hydrogen electrode, con = conductivity. * = the method has not been specified, ix = ion exchange and E = electromotive force method.

Metal	Present method		Other method (lit.): log K (method)
	log K	σ	
Mg	2.349	0.017	2.304 (H)
Ca	2.895	0.007	2.80 (con), 2.98 (*), 2.809 (H), 2.727 (ix)
Sr	2.690	0.011	2.904 (*), 2.700 (H), 2.574 (ix)
Ba	2.686	0.015	2.540 (con), 2.904 (E), 2.664 (ix), 2.54 (ix), 2.670 (H)
Cd	2.913	0.010	—
Co	3.225	0.020	—

and the best-fitted R_E values, the calculated concentrations and the effective mobilities of the sample zone constituents. Good agreement was obtained between the observed and the calculated R_E values. The mean error was less than 1.5%. The evaluated stability constants and mobilities are listed in Table VII together with their standard deviations and the literature values⁷. The dispersions of log K_2 were fairly large, especially for MgHCit, because of a lack of a sufficient number of effective R_E values and the small abundance of M-HCit under the present conditions. However,

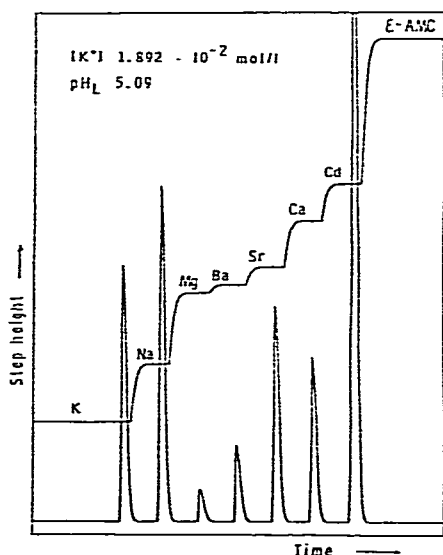


Fig. 11. Simulated isotachopherogram of an equimolar mixture of Na⁺, Mg²⁺, Ba²⁺, Sr²⁺, Ca²⁺ and Cd²⁺ under the experimental condition of Fig. 7B, the evaluated stability constants being used.

TABLE VI
OBSERVED AND SIMULATED R_{it} VALUES OF CITRATE ION, EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)
 P_2 = Abundance (%) of non-complexing HCit^{2-} ion, P_3 = Abundance (%) of non-complexing Cit^{3-} ion, P_5 = Abundance (%) of M-HCit⁰ complex, P_6 = Abundance (%) of M-HCit⁻ complex, $C_{M,S}$ = Total concentration (mM) of non-complexing metal ion, $n_{M,S}$ = Effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) of complexing metal ion $\cdot 10^4$. For other notations, see Table III.

Metal	System	R_g	n_{iS}	pH_S	C_N^*	P_1	P_2	P_3	P_5	P_6	$C_{M,S}$	$n_{M,S}$	I		
		Obs.	Calc.												
Mg	1	3.10	3.08	8.185	4.480	0.14	13.1	0.00	0.00	86.7	11.2	-12.3	0.229	14.6	7.66
	2	3.48	3.57	8.219	4.803	0.05	5.35	0.00	0.00	94.6	9.43	-11.8	0.577	10.9	6.67
	3	3.65	3.70	8.227	4.799	0.04	3.69	0.00	0.00	96.3	7.94	-11.7	0.843	8.07	6.53
	4	3.80	3.76	8.232	4.741	0.03	2.88	0.00	0.00	97.1	6.53	-11.6	1.090	5.55	6.52
	5	3.90	3.80	8.275	4.659	0.02	2.38	0.00	0.00	97.6	5.42	-11.0	1.329	3.20	6.54
	6	2.34	2.29	4.572	4.963	39.0	0.95	0.45	0.45	13.2	13.1	-11.3	0.529	11.4	9.49
	7	2.38	2.36	4.566	4.923	36.2	0.87	0.64	0.64	18.3	11.3	-11.4	0.800	12.9	9.39
	8	2.43	2.43	4.560	4.870	33.9	0.80	0.80	0.80	22.6	9.51	-11.5	1.072	14.4	9.31
	9	2.49	2.49	4.554	4.813	31.9	0.74	0.95	0.95	26.4	7.82	-11.6	1.344	15.8	9.27
	10	2.51	2.54	4.548	4.744	30.1	0.69	1.08	1.08	29.6	6.15	-11.7	1.617	17.1	9.24
Ca	1	2.84	2.82	8.196	4.619	0.16	16.2	0.01	0.01	83.6	12.4	-12.1	0.224	15.3	8.60
	2	3.25	3.32	8.232	5.023	0.06	6.54	0.01	0.01	93.4	10.4	-11.6	0.572	11.4	7.25
	3	3.39	3.46	8.241	5.036	0.04	4.47	0.01	0.01	95.5	8.84	-11.5	0.843	8.44	7.01
	4	3.60	3.52	8.245	4.973	0.03	3.47	0.01	0.01	96.5	7.33	-11.4	1.094	5.72	6.93
	5	3.63	3.57	8.248	4.874	0.03	2.85	0.01	0.01	97.1	5.83	-11.3	1.336	3.14	6.91

Sr	6	2.23	2.23	33.5	4.584	5.160	42.3	1.07	2.67	5.27	15.4	-11.1	0.229	11.3	9.81
	7	2.36	2.34	31.9	4.581	5.127	38.9	0.97	5.02	9.81	13.5	-11.2	0.463	12.7	9.48
	8	2.45	2.45	30.5	4.578	5.080	35.9	0.88	7.07	13.7	11.6	-11.2	0.698	14.0	9.21
	9	2.58	2.55	29.3	4.577	5.015	33.4	0.82	8.87	17.2	9.84	-11.3	0.935	15.3	8.98
	10	2.60	2.64	28.2	4.576	4.937	31.2	0.76	10.5	20.2	8.10	-11.3	1.171	16.6	8.78
	1	2.20	2.15	34.7	8.114	3.956	0.40	35.2	0.03	64.4	12.3	-13.2	0.253	13.3	11.2
	2	2.62	2.65	28.2	8.152	4.383	0.20	18.6	0.03	81.2	10.2	-12.7	0.540	10.4	9.01
	3	2.88	2.90	25.7	8.171	4.560	0.13	12.4	0.03	87.5	8.53	-12.5	0.836	7.39	8.23
	4	3.02	3.05	24.5	8.181	4.612	0.10	9.41	0.03	90.5	7.00	-12.3	1.111	4.59	7.91
	5	3.18	3.14	23.7	8.188	4.598	0.08	7.68	0.03	92.2	5.53	-12.2	1.379	2.00	7.75
	6	2.23	2.20	33.9	4.590	4.972	43.8	1.12	3.22	2.19	15.0	-11.1	0.250	21.9	9.61
	7	2.31	2.29	32.6	4.593	4.943	41.5	1.06	6.11	4.18	13.2	-11.0	0.497	22.6	9.43
	8	2.40	2.38	31.4	4.596	4.896	39.5	1.01	8.74	6.02	11.4	-11.0	0.742	23.4	9.25
	9	2.43	2.45	30.5	4.624	4.822	38.5	1.05	11.3	8.28	10.0	-10.6	0.982	23.5	9.15
	10	2.53	2.54	29.3	4.606	4.777	36.1	0.94	13.3	9.35	8.00	-10.9	1.222	24.9	8.96
	11	2.61	2.62	28.4	4.613	4.700	34.7	0.92	15.3	10.9	6.34	-10.8	1.458	25.6	8.84
Bi	1	2.00	1.93	38.7	8.087	3.730	0.55	46.9	0.03	52.5	12.6	-13.5	0.304	8.29	12.8
	2	2.31	2.30	32.4	8.117	3.972	0.35	31.0	0.04	68.6	10.6	-13.2	0.544	5.98	10.7
	3	2.60	2.61	28.6	8.129	4.121	0.25	22.2	0.04	77.6	8.72	-13.0	0.810	3.29	9.51
	4	2.79	2.83	26.4	8.194	4.183	0.17	17.2	0.04	82.6	7.57	-12.1	1.075	0.61	8.86
	5	2.96	2.99	24.9	8.164	4.189	0.15	14.1	0.05	85.7	5.69	-12.5	1.332	-1.97	8.47
	6	2.23	2.18	34.3	4.592	4.953	44.6	1.14	2.60	1.31	15.0	-11.0	0.268	30.9	9.71
	7	2.27	2.25	33.2	4.596	4.901	43.0	1.11	4.99	2.54	13.2	-11.0	0.531	31.5	9.59
	8	2.33	2.31	32.3	4.601	4.841	41.6	1.09	7.21	3.71	11.5	-10.9	0.790	32.1	9.49
	9	2.39	2.37	31.4	4.607	4.773	40.3	1.06	9.27	4.83	9.77	-10.8	1.044	32.7	9.40
	10	2.43	2.44	30.6	4.614	4.696	39.2	1.05	11.2	5.94	8.08	-10.7	1.295	33.2	9.31
	11	2.46	2.50	29.8	4.624	4.617	38.2	1.04	13.0	7.05	6.43	-10.6	1.541	33.7	9.24

TABLE VII

EVALUATED STABILITY CONSTANTS AND MOBILITIES OF ALKALINE EARTH-CITRATE COMPLEXES AND THEIR DISPERSIONS

Log K_2 = Thermodynamic stability constants of M-HCit (25°C). Log K_3 = Thermodynamic stability constants of M-Cit (25°C). m_3 = Absolute mobilities ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) of $\text{MCit}^- \cdot 10^5 \cdot \sigma$ = Standard deviation. Lit. = Literature values; pol = polarography. For other methods, see Table V. Log K_1 was fixed at 1.15.

M^{2+}	Log K_2	σ	Lit.	Log K_3	σ	Lit.	m_3	σ
Mg	1.672	0.601	—	4.917	0.022	4.65 (pol) 4.60 (—) 4.44 (pol)	21.1	0.242
Ca	2.786	0.066	3.05 (sol) 3.09 (ix) 3.29 (gl)	4.850	0.023	4.90 (sol) 4.68 (ix) 4.59 (E)	22.3	0.258
Sr	2.815	0.028	—	4.410	0.024	4.53 (ix) 4.32 (ix) 4.17 (con)	23.3	0.397
Ba	2.686	0.036	—	4.150	0.021	4.43 (E) 4.13 (ix) 4.01 (ix) 3.79 (ix)	22.0	0.617

the accuracies of the other constants were satisfactory. This can be easily understood from the calculated Jacobian matrices. For example, the matrices for Mg-citrate and Ba-citrate are as follows:

$$J(\text{Mg}) = \begin{pmatrix} m_3 & m_5 & \log K_1 & \log K_2 & \log K_3 \\ 0.000 & -0.027 & 0.000 & 0.000 & 2.661 \\ 0.000 & -0.139 & 0.000 & 0.000 & 1.037 \\ 0.000 & -0.158 & 0.000 & 0.000 & 0.713 \\ 0.000 & -0.168 & 0.000 & 0.000 & 0.561 \\ 0.000 & -0.174 & 0.000 & 0.000 & 0.468 \\ 0.000 & -0.007 & 0.013 & 0.018 & 0.264 \\ 0.000 & -0.011 & 0.020 & 0.025 & 0.363 \\ 0.000 & -0.014 & 0.028 & 0.036 & 0.442 \\ 0.000 & -0.018 & 0.037 & 0.043 & 0.507 \\ 0.000 & -0.020 & 0.045 & 0.051 & 0.556 \end{pmatrix} \begin{matrix} R_{E,1} \\ R_{E,2} \\ R_{E,3} \\ R_{E,4} \\ R_{E,5} \\ R_{E,6} \\ R_{E,7} \\ R_{E,8} \\ R_{E,9} \\ R_{E,10} \end{matrix}$$

$$J(\text{Ba}) = \begin{pmatrix} m_3 & m_5 & \log K_1 & \log K_2 & \log K_3 \\ 0.000 & -0.011 & 0.000 & 0.000 & 0.763 \\ 0.000 & -0.029 & 0.000 & 0.001 & 1.325 \\ 0.000 & -0.050 & 0.000 & 0.002 & 1.528 \\ 0.000 & -0.068 & 0.000 & 0.002 & 1.514 \\ 0.000 & -0.082 & 0.000 & 0.003 & 1.435 \\ 0.000 & 0.000 & 0.006 & 0.100 & 0.027 \\ 0.000 & -0.001 & 0.012 & 0.200 & 0.054 \\ 0.000 & -0.002 & 0.019 & 0.300 & 0.078 \\ 0.000 & -0.003 & 0.025 & 0.399 & 0.101 \\ 0.000 & -0.004 & 0.030 & 0.497 & 0.123 \\ 0.000 & -0.005 & 0.036 & 0.595 & 0.144 \end{pmatrix} \begin{matrix} R_{E,1} \\ R_{E,2} \\ R_{E,3} \\ R_{E,4} \\ R_{E,5} \\ R_{E,6} \\ R_{E,7} \\ R_{E,8} \\ R_{E,9} \\ R_{E,10} \\ R_{E,11} \end{matrix}$$

where the subscripts 1–11 on R_E correspond to the number of the electrolyte systems listed in Table IV. The negative signs mean that R_E decreases with increase in the constants, *vice versa* for positive values. The elements of $J(\text{Mg})$ related to $\log K_2$ are apparently smaller than those of $J(\text{Ba})$, suggesting that the exact determination of the constant is difficult. However, the accuracies of the other constants were satisfactory.

It should be noted that the effective mobilities of M^{2+} in the sample zones at pH 8 are of the same sign for citrate ion as the sample, except for the present Ba–citrate systems, as shown in Table VI. One might doubt the validity of the mass balance relationship for M^{2+} in the complex-forming equilibria, as the effective mobility of the usual counter ion, the pH buffer, is of opposite sign to the leading and sample ions. The M^{2+} in the leading zone does not form any complex and it is actually the counter ion that migrates to the sample and terminating zones. On the other hand, in the sample zone, owing to the complex formation with citrate ions, the apparent proceeding direction of the complexing M^{2+} is the same as the sample. Such behaviour was not found for tartrate as the counter ion in the M–tartrate systems (see Table IV).

The effective mobility of M^{2+} coexisting with citrate ion can be expressed as

$$\begin{aligned} \bar{m}_M &= (m_M[\text{M}^{2+}] + m_3[\text{MH}_2\text{Cit}^+] + m_5[\text{MCit}^-]) / \\ &\quad ([\text{M}^{2+}] + [\text{MH}_2\text{Cit}^+] + [\text{MHCit}^0] + [\text{MCit}^-]) \\ &= (m_M + m_3k_1/[\text{H}^+] K_1[\text{H}_3\text{Cit}^0] + m_5k_1k_2k_3/[\text{H}^+]^3 K_3[\text{H}_3\text{Cit}^0]) / \\ &\quad (1 + k_1/[\text{H}^+] K_1[\text{H}_3\text{Cit}^0] + k_1k_2/[\text{H}^+]^2 K_2[\text{H}_3\text{Cit}^0] + \\ &\quad + k_1k_2k_3/[\text{H}^+]^3 K_3[\text{H}_3\text{Cit}^0]) \quad (9) \end{aligned}$$

where the signs of the mobility of m_M and m_3 are positive and that of m_5 is negative. Therefore, in the high pH range, a change in the sign of m_M may occur in the citrate zone according to the decrease in the abundance of M^{2+} and the increase in MCit^- . Even if the sign changes, the effective mobility of M^{2+} in the citrate zone is smaller than that of citrate ion (see Table VI), *i.e.*, the migration velocity of M^{2+} is smaller than the isotachophoretic velocity and M^{2+} is forced to flow out. This process meets the simple requirement that the amount of M^{2+} coexisting with the sample is restricted by the amount of sample in the equilibrium. Thus, the mass balance relationship for the counter ion¹ is fulfilled for M^{2+} in the M–citrate systems, together with the pH buffer ion. For the Ca–citrate system, the pH dependence on the effective mobility of Ca^{2+} coexisting with citrate ion is shown in Fig. 12. The pH range, 4–8.5, was buffered by ϵ -AMC, histidine and Tris. The concentration of added Ca^{2+} in the leading electrolyte was varied in the range 0.5–4 mM. The concentration of the leading ion, Cl^- , was fixed at 10 mM. The evaluated constants were used for the simulation. Fig. 12 shows that the sign of the effective mobility of Ca^{2+} changes in the pH range 4.8–5.3. In this pH range, the abundance of MCit^- increases rapidly. On the other hand, the concentration of free Ca^{2+} in the citrate zone did not vary significantly, even if pH_L was varied, as shown in Fig. 13. Electroneutrality in the sample zone is maintained by the pH buffer. The effect of pH_L and Ca^{2+} concentration on the total concentration of the buffer in the sample zone is shown in Fig. 14.

The dependence of pH_L on the effective mobility of citrate ion is shown in Fig. 15. Fig. 16 shows the dependence of pH_L on the observable R_E values in the isotachophoretically steady state. The observed large gradient in R_E versus Ca^{2+} concen-

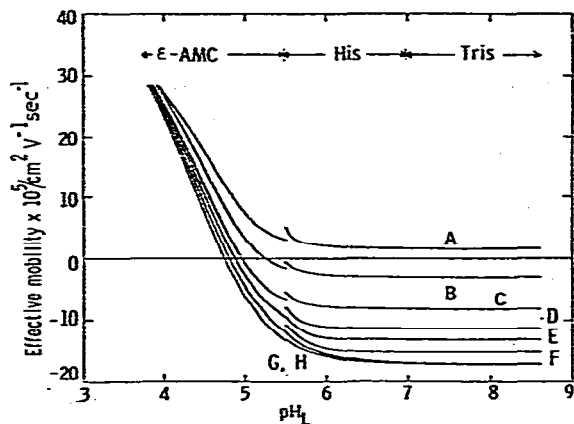


Fig. 12. Effect of the Ca^{2+} concentration in the leading electrolyte and the pH of leading zone (pH_L) on the effective mobility of Ca^{2+} coexisting with citrate ion in the isotachophoretic steady state. The evaluated stability constants and mobility were used. The pH-buffering agents used were ϵ -aminocaproic acid (pH_L 4–5.5), histidine (5.5–7) and tris(hydroxymethyl)aminomethane (7–8.6). Ca^{2+} concentration (mM); A, 4; B, 3; C, 2; D, 1.5; E, 1.25; F, 1; G, 0.75; H, 0.5. In the pH range 4–5.5 the curves for E and G were omitted.

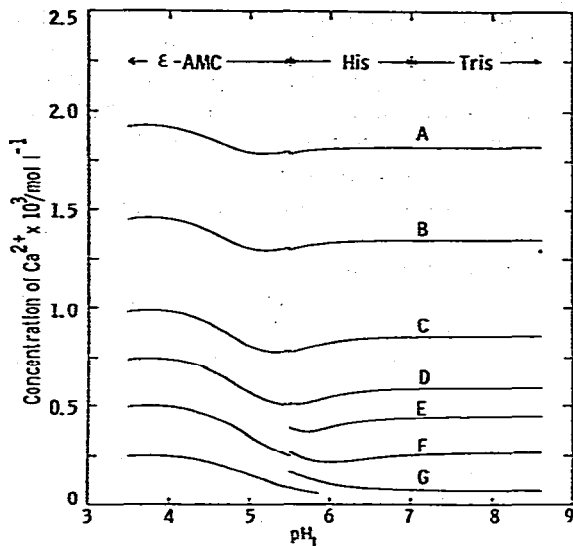


Fig. 13. Effect of the Ca^{2+} concentration in the leading electrolyte and the pH of the leading zone (pH_L) on the concentration of Ca^{2+} in the citrate zone. Ca^{2+} concentration (mM): A, 4; B, 3; C, 2; D, 1.5; E, 1.25; F, 1; G, 0.75; H, 0.5. In the pH range 3.5–5.5 the curves for E and G were omitted. For buffers, see caption of Fig. 12.

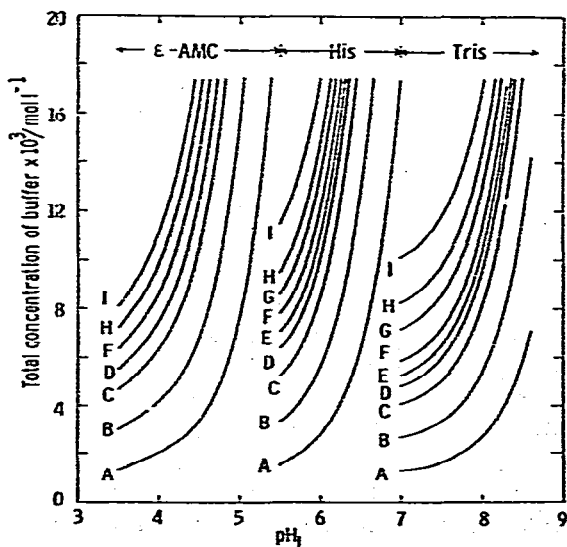


Fig. 14. Effect of the Ca^{2+} concentration in the leading electrolyte and the pH of leading zone (pH_L) on the concentration of pH-buffering agents in the citrate zone. Ca^{2+} concentration (mM): A, 4; B, 3; C, 2; D, 1.5; E, 1.25; F, 1; G, 0.75; H, 0.5.

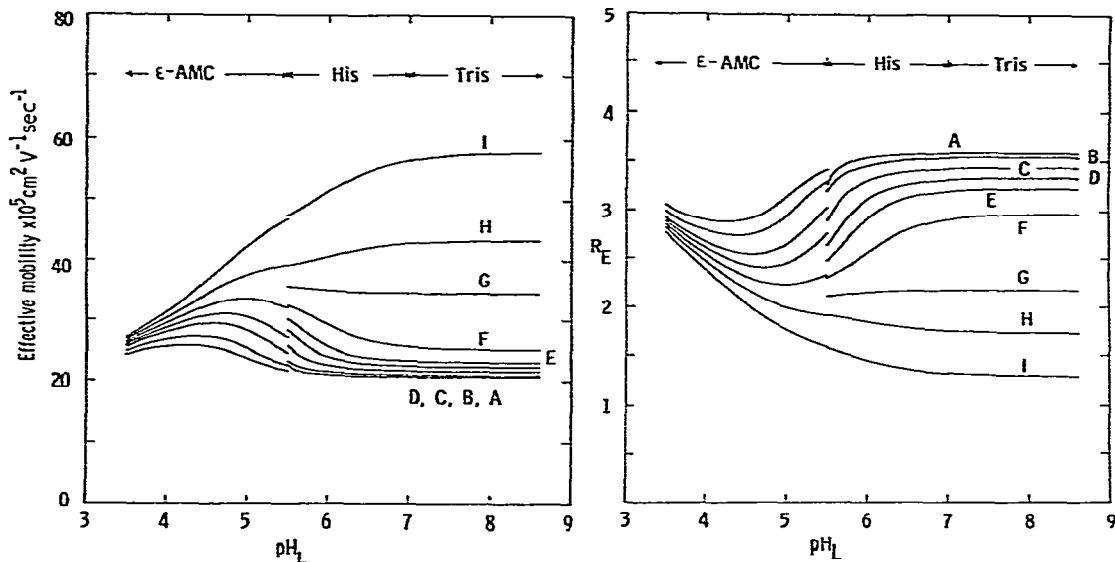


Fig. 15. Effect of the Ca^{2+} concentration in leading zone and the pH of the leading electrolyte (pH_L) on the effective mobility of citrate ion. Ca^{2+} concentration (mM): A, 4; B, 3; C, 2; D, 1.5; E, 1.25; F, 1; G, 0.75; H, 0.5; I, 0. In the pH range 3.5–5.5 the curves for E and G were omitted.

Fig. 16. Effect of the Ca^{2+} concentration in the leading zone and the pH of the leading electrolyte (pH_L) on the R_E values of citrate ion. Ca^{2+} concentration (mM): A, 4; B, 3; C, 2; D, 1.5; E, 1.25; F, 1; G, 0.75; H, 0.5; I, 0. In the pH range 3.5–5.5 the curves for E and G were omitted.

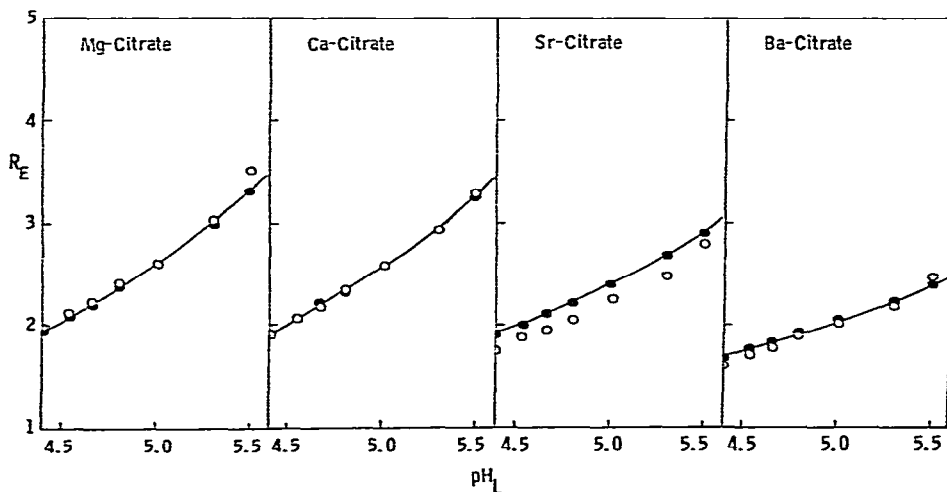


Fig. 17. Observed and simulated R_E values of Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} coexisting with citrate ion. The concentration of the leading ion, K^+ , was 9.845 mM. The pH of the leading electrolyte was varied in the range 4.4–5.5 by adding citric acid. O, observed; ●, calculated.

tration curves at pH_L 8 shown in Fig. 9 and the small gradient at pH_L 4.4 in Fig. 10 can be easily understood from the comparison of the R_E variations at the corresponding pH_L values in Fig. 16.

Finally, in order to confirm the obtained stability constants, the R_E values obtained in cationic analysis were compared with the simulated R_E values using the constants obtained in anionic analysis. The leading electrolyte was 9.845 mM potassium hydroxide solution buffered by adding citric acid. Fig. 17 shows the comparison between the observed and the simulated R_E values. The agreement was satisfactory except for the Sr-citrate system.

Thus, the stability constant obtained by this method was in good agreement with the previously reported value. It can be concluded that the present method should be useful for the evaluation of complex stability constants and absolute mobilities. The accuracy of the constants obtained depends mainly on the precise measurement of R_E values. The accuracy may be improved by the use of a more stable detector with greater linearity between the step height and potential gradient or conductivity of the zones. The high-frequency contactless conductivity detector¹¹ may meet the requirements, although the reported model lacks linearity as yet.

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