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COMPLEX-FORMING EQUILIBRIA IN ISOTACHOPHORESIS

V. ESTIMATION OF COORDINATION NUMBERS AND STABILITY CONSTANTS OF METAL-OXYCARBOXYLIC ACID COMPLEXES BY MEANS OF COMPUTER SIMULATION

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SUMMARY

A computational method based on the least squares technique has been applied for the simultaneous estimation of the coordination numbers and stability constants of complexes formed between several metal ions and oxycarboxylic acids from the qualitative index R_E obtained by means of isotachophoresis. The metal ions were Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} , and the ligands were glycolate, lactate, β -hydroxybutyrate, α -hydroxybutyrate and α -hydroxyisobutyrate ions. The estimated coordination numbers and the stability constants agreed well with previously reported values. The proposed method can be applied even for mixed samples.

INTRODUCTION

In Part IV¹ a computational procedure was described to simulate isotachophoretic equilibria of a separated zone containing kinetically labile multi-coordinated complexes. It was suggested that the stability constants and coordination numbers of the complexes can be estimated by analysing the qualitative index, R_E , of the samples at different ligand concentrations. The least squares technique employed was applied repeatedly until satisfactory agreement was obtained between the observed R_E values and those simulated on the basis of a model. Since the isotachophoretic method for the evaluation of mobility and pK_a has proved to be a powerful technique especially for mixed samples², similar applicability to the simultaneous evaluation of stability constants and coordination numbers is expected.

In this study the proposed method is applied to evaluate the stability constants and coordination numbers of complexes formed by divalent metal cations, Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} , and monoanions of oxycarboxylic acids, glycolate, lactate, β -hydroxybutyrate, α -hydroxybutyrate and α -hydroxyisobutyrate ions respectively. The mobilities of the complex ions used were estimated

from a relationship between the formula weight and mobility of the monocarboxylate ions³. The estimated stability constants and coordination numbers are compared with previously published values evaluated by different methods.

EXPERIMENTAL

The R_E values of the metal ions were obtained using leading electrolytes of aqueous potassium hydroxide solution buffered by adding glycolic (Glyc), lactic (Lac), β -hydroxybutyric (β -HB), α -hydroxybutyric (α -HB) and α -hydroxyisobutyric acid (HIB), which are also the complexing agents in the sample zones. For each complexing agent, three to five kinds of leading electrolytes were prepared by varying the concentration of leading ion, $C_L^1 = 5.2$ – 26 mM. The pH of the leading electrolyte, pH_L , was adjusted to *ca.* 4.4, except for β -HB buffer ($pH_L = ca.$ 5). The surfactant Triton X-100 (0.2%) was added. The terminating electrolyte was 5 mM ϵ -aminocaproic acid (ϵ -AMC) and the pH was adjusted to *ca.* 4.5 by adding the buffers used for the leading electrolytes. The samples were chlorides or nitrates of the metal ions and all reagents used were commercial guaranteed grade. Table I summarizes the experimental conditions for the leading electrolytes used.

Isotachopherograms were obtained by the use of a Shimadzu IP-2A isotachopheretic analyzer equipped with a potential gradient detector Shimadzu PGD-2. A main separating tube (10 cm \times 0.5 mm I.D.) was connected to a pre-separating column (4 cm \times 1 mm I.D.). The separation compartment was thermostatted at 25°C and the driving currents applied were in the range of 50 (low C_L^1)–100 μ A (high C_L^1). For the precise measurements of R_E indices, internal standards were used such as Na^+ ($R_E = 1.48$ at $C_L^1 = 10$ mM) and Li^+ ($R_E = 1.94$ at $C_L^1 = 10$ mM) which hardly interact with the counter ions used. The use of an internal standard to correct

TABLE I

LEADING ELECTROLYTE CONDITIONS

pH_L = pH of leading electrolyte; C_L^1 = total concentration (mM) of leading ion, K^+ . Buffers: Glyc = glycolic; Lac = lactic; β -HB = β -hydroxybutyric; α -HB = α -hydroxybutyric; HIB = α -hydroxyisobutyric acids.

No.	pH_L	C_L^1	Buffer	No.	pH_L	C_L^1	Buffer
1	4.38	7.78	Glyc	13	4.35	5.19	α -HB
2	4.41	10.40	Glyc	14	4.38	10.37	α -HB
3	4.39	15.60	Glyc	15	4.37	15.56	α -HB
4	4.40	20.80	Glyc	16	4.37	20.74	α -HB
5	4.41	26.00	Glyc	17	4.38	25.93	α -HB
6	4.29	10.35	Lac	18	4.35	5.18	HIB
7	4.31	15.53	Lac	19	4.38	10.13	HIB
8	4.32	21.18	Lac	20	4.35	15.20	HIB
9	5.01	10.37	β -HB	21	4.36	20.26	HIB
10	5.02	15.56	β -HB	22	4.32	25.90	HIB
11	5.02	20.74	β -HB				
12	5.02	26.00	β -HB				

an asymmetric potential of a potential gradient detector has been detailed in ref. 2. Measurements of pH were made with a Horiba F-7ss expanded scale pH-meter.

Three examples of the observed isotachopherograms of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Li^+ and Zn^{2+} are shown in Fig. 1, and were obtained by varying C_L^+ (A, 10.37; B, 15.56; C, 25.93 mM) at $\text{pH}_L = \text{ca. } 4.4$ (α -HB buffer); Li^+ was the internal standard. An increase of the relative step heights of the sample zones with respect to that of Li^+ was observed with increasing C_L^+ , suggesting the occurrence of complex formation between the metal dications and α -hydroxybutyrate anion. Figs. 2-4 summarize the observed dependence on C_L^+ of R_E values using Glyc, Lac, β -HB, α -HB, and HIB as complexing agent. The R_E values shown are averages from three determinations. The curves are the best-fitted ones, which will be discussed in a later section. Apparently, the R_E values of each metal ion at similar C_L^+ differ significantly in the different electrolyte systems. Except in the β -HB system, the metal ions can be separated at appropriate C_L^+ values. Unless a mixed zone was formed, the samples were injected at once and the R_E values were measured.

RESULTS AND DISCUSSION

The evaluation of stability constants and data processing were made by the use of a SORD M223 Mark III microcomputer. The physico-chemical constants in Table II were used for simulation. They were taken from the literature⁴⁻⁸ except for the mobilities of α -HB and HIB which were determined by the isotachopheretic method².

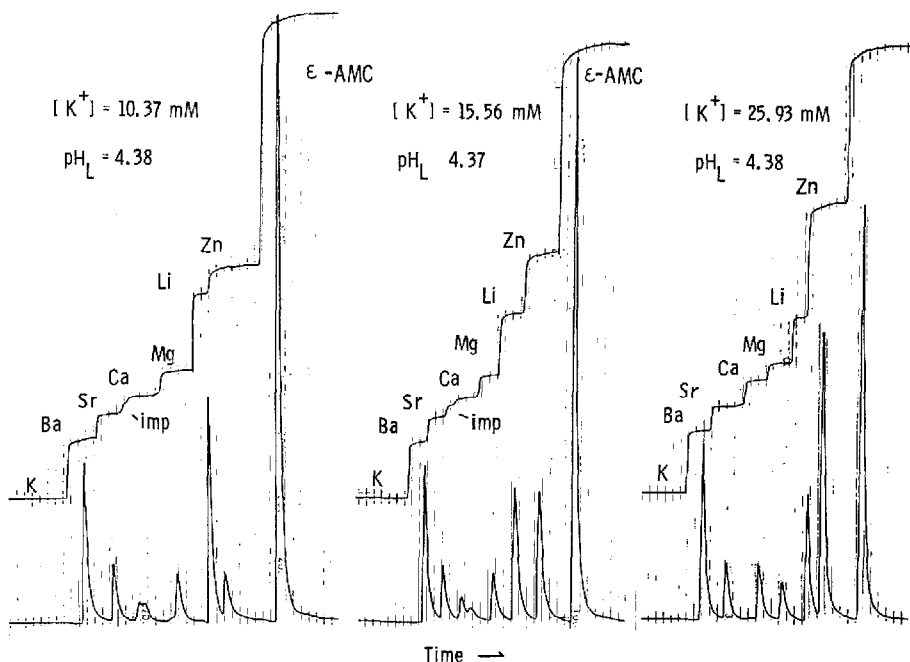


Fig. 1. Observed isotachopherograms (A, B and C, from left to right) of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Li^+ and Zn^{2+} at different C_L^+ values. The leading ion is K^+ and the terminating ion is ϵ -aminocaproate (ϵ -AMC). imp = Impurity in the electrolyte system.

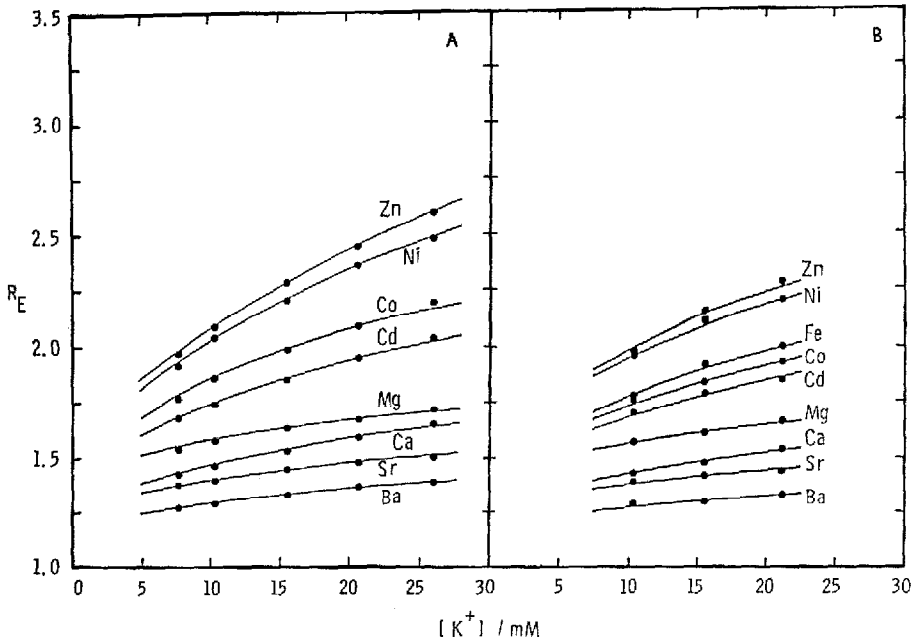


Fig. 2. Observed R_E values (●) of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} co-existing with glycolate (A) and lactate ions (B) at $\text{pH}_L = \text{ca. } 4.4$. The curves show the dependence on $[\text{K}^+]$ of simulated R_E values using best-fitted stability constants.

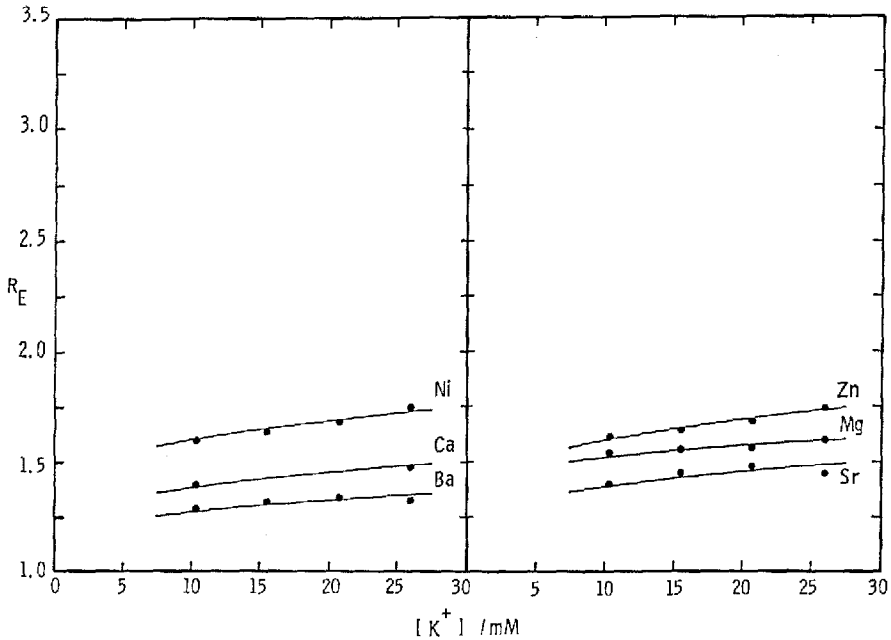


Fig. 3. Observed R_E values (●) of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Ni^{2+} and Zn^{2+} co-existing with β -hydroxybutyrate ions at $\text{pH}_L = \text{ca. } 5.0$. The curves show the dependence on $[\text{K}^+]$ of the simulated R_E values using best-fitted stability constants.

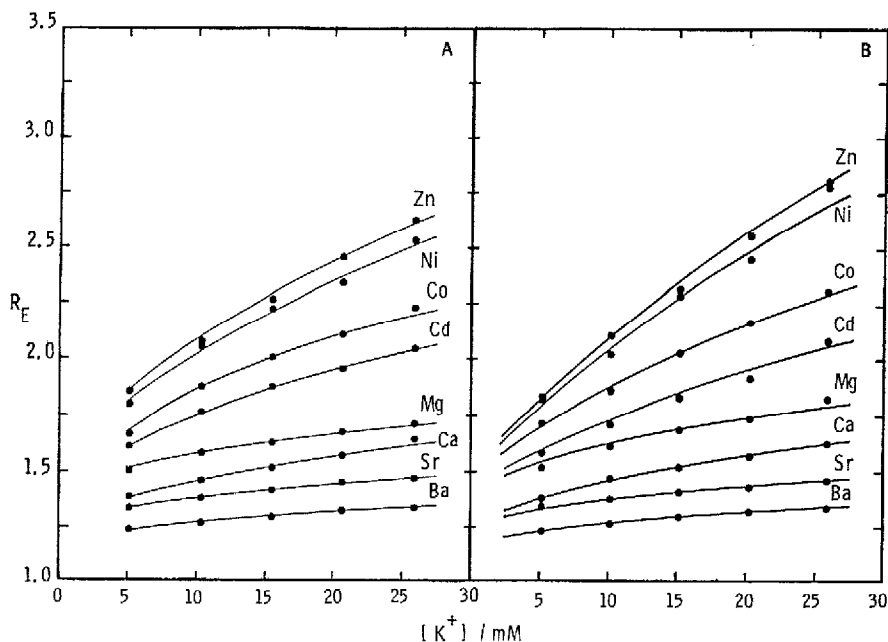


Fig. 4. Observed R_E values (●) of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} co-existing with α -hydroxybutyrate (A) and α -hydroxyisobutyrate ions (B) at $pH_L = ca. 4.4$. The curves show the dependence on C_1 of the simulated R_E values using best-fitted stability constants.

TABLE II

PHYSICO-CHEMICAL CONSTANTS USED IN SIMULATIONS (25°C)

m_0 = Absolute mobility ($cm^2 V^{-1} sec^{-1}$) $\times 10^5$; pK_a = thermodynamic acid dissociation constants; \hat{a} = mean ionic diameter (cm) $\times 10^8$ (ref. 10). For metal ions the mobilities and pK_a s of the monoanions are assumed to be $m_0/2$ and $pK_a + 1$ respectively. Exact values of them are not necessary in simulation.

Cation	m_0	pK_a	\hat{a}	Anion	m_0	pK_a	\hat{a}
Ba^{2+}	66.0	13.2	5	Glycolate	42.4*	3.886	4
Sr^{2+}	61.6	13.2	5	Lactate	36.5	3.858	4
Ca^{2+}	61.7	12.6	6	β -Hydroxybutyrate	33.3	4.519	4
Mg^{2+}	55.0	11.4	8	α -Hydroxybutyrate	33.5*	3.979	4
Cd^{2+}	56.0	7.6	5	α -Hydroxyisobutyrate	33.5*	3.971	4
Co^{2+}	57.0	11.2	6				
Fe^{2+}	56.0	8.3	6				
Zn^{2+}	54.7	9	6				
Ni^{2+}	53.9	9.3	6				
Na^+	51.9	13.7	4				
Li^+	40.1	13.8	4				
K^+	76.2	—	4				
ϵ -Amino-caproic acid	28.8*	4.373	4				

* Isotachophoretically evaluated.

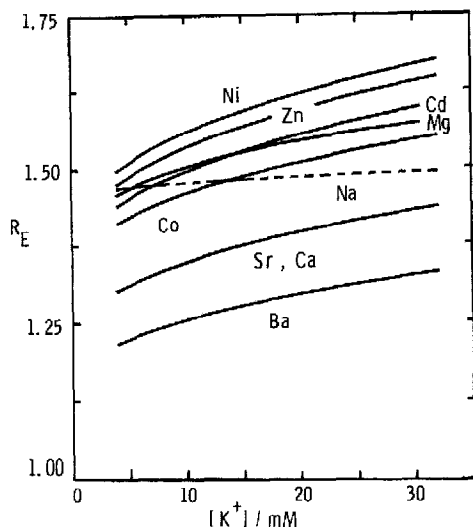


Fig. 5. Dependence on C_L^- of the simulated R_E values of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Co^{2+} , Cd^{2+} , Zn^{2+} and Ni^{2+} on the assumption that no interaction takes place between the metal and α -hydroxybutyrate ions. $pH_L = 4.4$.

Fig. 5 shows the dependence on C_L^- of the R_E values of the metal dications in the range C_L^- 4–32 mM on the assumption that the samples do not interact with the counter ions (α -HB, $pH_L = 4.4$). At the pH_L all of the metal ions are in their fully charged states, therefore the increase in R_E values of the dications with increasing C_L^- is attributed to the decrease in effective mobility accompanied by the increase in ionic strength. The broken line represents the internal standard Na^+ . Apparently, the divalent cations having R_E values in the range of *ca.* 1.4–1.7, namely Co, Cd, Mg, Zn and Ni, may form a mixed zone if no interaction takes place between them and the counter ion. In practice, however, they can be separated at an appropriate C_L^- value by using α -HB buffer, as shown in Fig. 1. Therefore the separation between the samples and the increase of R_E with increasing C_L^- are caused by complex formation. The order of increasing R_E values in Fig. 5 is different from that in Fig. 4 suggesting that the stability constants and/or the coordination numbers may be different amongst the complexes formed.

To analyse the complex-forming systems we require the absolute mobilities of the complexes formed. A relationship between the formula weights, FW, and absolute mobilities of the monocarboxylate ions was used to estimate the mobilities of the complex ions:

$$m_0 = (329.9/\sqrt{FW} - 1.1) \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1} \quad (1)$$

The values of the stability constants are dependent on the estimated mobilities and the validity of the latter may be assessed by comparing the evaluated stability constants with reported values.

Two examples will be given to clarify the procedure for the assessment of

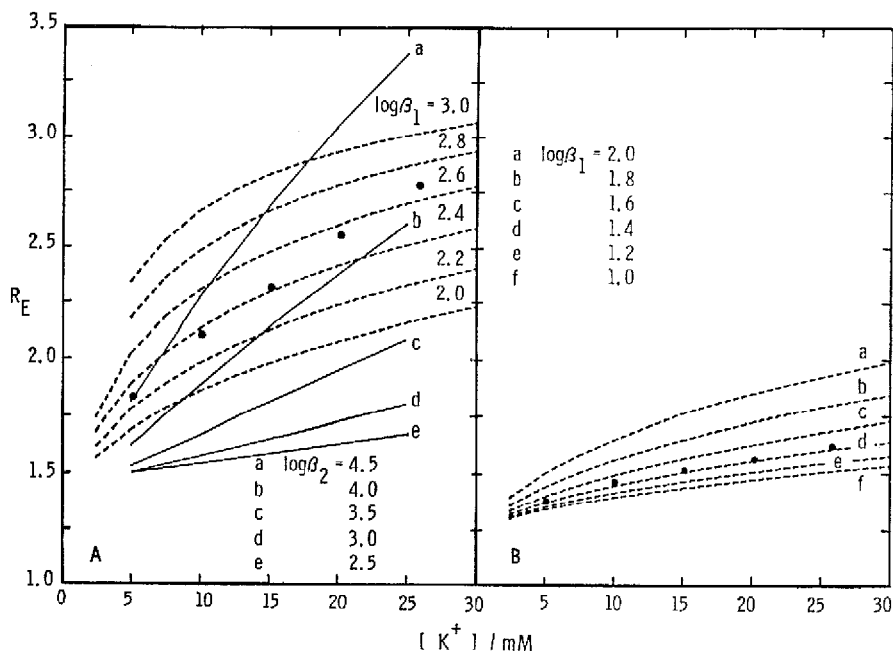


Fig. 6. Dependence on Cl^- of the observed (\bullet) and simulated R_E values of Zn^{2+} (A) and Ca^{2+} (B) co-existing with α -hydroxyisobutyrate ion. The broken curves represent the one-coordination model and the solid curves are for the two-coordination model.

coordination numbers and the evaluation of stability constants. Fig. 6 shows the dependence on Cl^- of the observed and simulated R_E values of Zn^{2+} and Ca^{2+} co-existing with HIB. The broken curves show the dependence on Cl^- of the stability constants and the simulated R_E values on the assumption that only a one-coordinated complex is formed in the sample zone (one-coordination model). For the Zn-HIB system the stability constant was varied in the range of $\log \beta_1 = 2-3$ and for the Ca-HIB system in the range 1-2. For the calcium system the observed R_E values can be explained in terms of the one-coordination model and $\log \beta_1 = ca. 1.4$ from Fig. 6, which can be refined by the least squares method using this model. On the other hand, the R_E values of the Zn-HIB system apparently cannot be explained by such a model. The solid curves represent the simulated values of Zn^{2+} on the assumption that only a two-coordinated complex is formed (two-coordination model). The increase in the simulated R_E values does not agree with that of the observed values, even when the best-fitting procedure is applied. From Part IV¹ it is apparent that the three-coordination model and other multi-coordination model also cannot explain the observed R_E values, suggesting the co-existence of differently coordinated complexes. The increase in the observed R_E values with increasing Cl^- was intermediate between those for one- and two-coordination models, therefore it is very probable that one- and two-coordinated complexes may co-exist in the Zn-HIB system.

Fig. 7A shows the dependence on Cl^- of the simulated R_E values of Zn^{2+} using the best-fitted stability constants obtained by the least squares method on the basis of three different models of complex formation. One broken curve (- · -) shows the

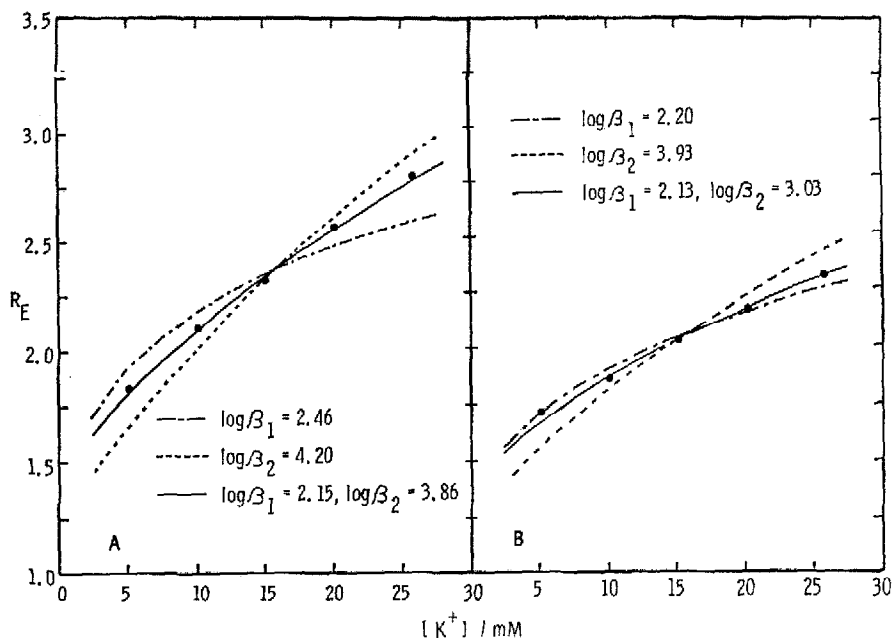


Fig. 7. Dependence on Cl^- of the observed (\bullet) and simulated R_E values of Zn^{2+} (A) and Co^{2+} (B) co-existing with α -hydroxyisobutyrate ion. - · - ·, The best-fitted dependence using the one-coordination model; ---, the dependence using the two-coordination model; —, the dependence using the co-existence model.

best-fitted dependence using the one-coordination model ($\log \beta_1 = 2.46$). The agreement was not satisfactory, and the mean error between the observed and simulated R_E values was 4.2%. The other broken curve (---) shows the corresponding dependence for the two-coordination model ($\log \beta_2 = 4.20$). The best-fitted R_E values at high Cl^- were overestimated and those at low Cl^- were underestimated. The mean error was again high (4.9%). On the other hand, the solid curve shows the dependence evaluated on the assumption that one- and two-coordinated complexes co-exist (co-existence model: $\log \beta_1 = 2.15, \log \beta_2 = 3.86$). The mean error was 0.52%, supporting this model of the Zn-HIB system.

The coordination numbers and stability constants were similarly estimated for the metal-HIB systems. It is concluded that the observed R_E values of Ca^{2+} , Sr^{2+} and Ba^{2+} can be explained by the one-coordination model with mean errors of 0.33%, 0.53% and 0.16%, respectively. For Mg^{2+} , Cd^{2+} and Co^{2+} almost all of the complexes formed may be one-coordinate, however small amounts of the two-coordinated complexes might also occur. The dependence on Cl^- of the R_E values of Co^{2+} is shown in Fig. 7B. The difference in the simulated R_E values between the one-coordination and the co-existence models is small in comparison with Zn-HIB system. When the one-coordination model was applied, $\log \beta_1$ was 2.20 and the mean error was 1.34%, for the co-existence model, $\log \beta_1$ and $\log \beta_2$ were 2.13 and 3.03 with a mean error of 0.96%. Owing to its lower mean error, the coexistence model is marginally preferred. For Mg^{2+} and Cd^{2+} , the mean errors for the one-coordination model were 1.05 and 1.58% and those for the coexistence model were 0.43 and 0.70%.

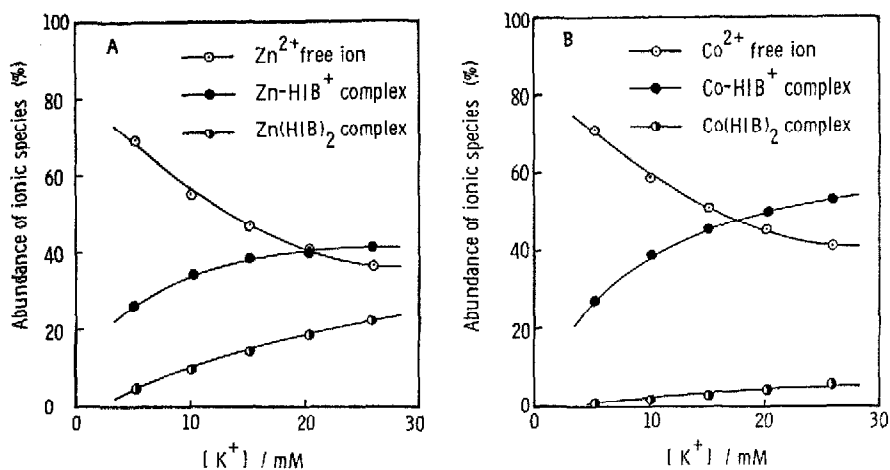


Fig. 8. Dependence on Cl^- of the abundance of one-coordinated (●) and two-coordinated complexes (○) and non-complexing ions (○) of Zn^{2+} (A) and Co^{2+} (B) co-existing with α -hydroxyisobutyric acid (HIB). The co-existence model was used for the simulation.

Fig. 8 shows the dependence on Cl^- of the simulated abundances of the non-complexing ions, and of the one- and two-coordinated complexes of Co^{2+} and Zn^{2+} co-existing with HIB. The abundance of $Co(HIB)_2$ is at most 5%. On the other hand the abundance of $Zn(HIB)_2$ is 22% at $Cl^- = 25.9$ mM. For Mg^{2+} and Cd^{2+} the abundances of the one-coordinated complexes are similar to that in the Co-HIB system.

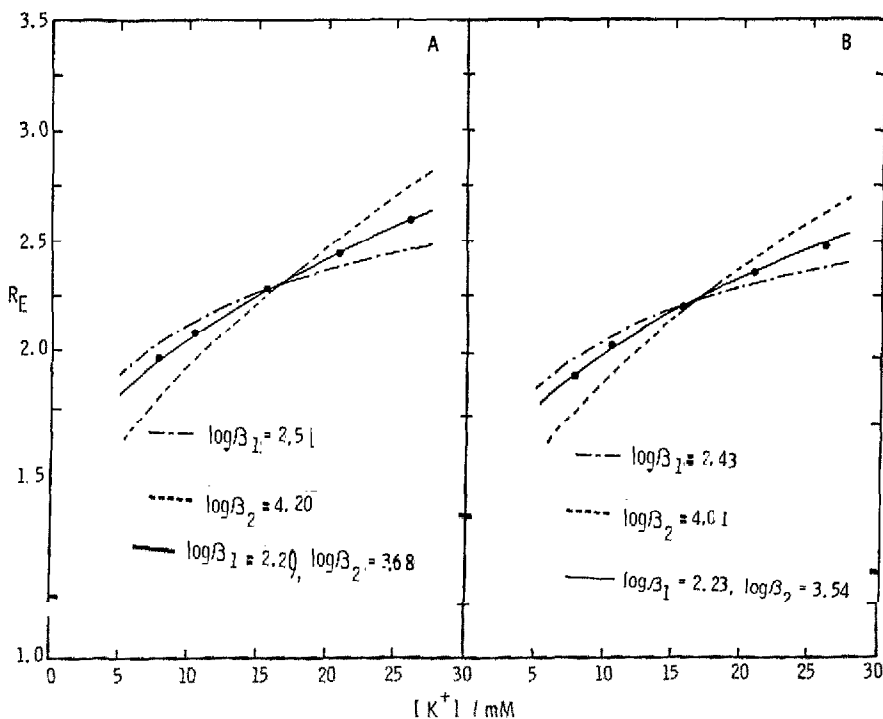


Fig. 9. Dependence on Cl^- of the observed (●) and simulated R_E values of Zn^{2+} (A) and Ni^{2+} (B) co-existing with glycolate ion. Key to curves as in Fig. 7.

TABLE III

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

R_E = Ratio of potential gradients, E_S/E_L ; \bar{m}_S = effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) $\times 10^5$ of sample ion; pH_S = pH of sample zone; C^i = total concentration (mM) of metal ions including complexes; P_1 = abundance (%) of 1:1 complex; P_2 = abundance (%) of 1:2 complex; $C_{B,S}^1$ = total concentration (mM) of non-complexing buffer ion; $\bar{m}_{B,S}$ = effective mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) $\times 10^5$ of buffer ion; I = ionic strength $\times 10^3$.

Metal ion	System	R_E		Dev. (%)	\bar{m}_S	pH_S	C^i	P_1	P_2	$C_{B,S}^1$	$\bar{m}_{B,S}$	I
		Obs.	Calc.									
<i>Buffer: glycolic acid</i>												
Ca	1	1.43	1.435	-0.31	50.05	4.271	3.53	15.4	-	9.02	24.8	9.56
	2	1.47	1.474	-0.22	48.36	4.302	4.75	18.7	-	11.6	24.3	12.5
	3	1.53	1.538	-0.50	45.79	4.277	7.18	23.9	-	17.2	22.6	18.2
	4	1.59	1.591	-0.04	43.83	4.283	9.63	28.0	-	22.3	21.6	23.5
	5	1.65	1.637	0.83	42.26	4.288	12.1	31.4	-	27.3	20.8	28.7
Co	1	1.77	1.787	-0.96	40.19	4.178	3.50	39.6	-	8.31	19.1	7.79
	2	1.86	1.867	-0.40	38.17	4.212	4.76	45.2	-	10.6	18.2	10.1
	3	1.98	1.990	-0.49	35.39	4.191	7.29	52.9	-	15.5	16.2	14.2
	4	2.09	2.084	0.29	33.47	4.199	9.87	58.3	-	20.0	15.1	18.2
	5	2.19	2.160	1.37	32.02	4.207	12.5	62.2	-	24.4	14.2	22.0
Zn	1	1.97	1.961	0.48	34.25	4.091	3.39	33.9	4.36	8.18	17.5	7.10
	2	2.08	2.081	-0.03	34.25	4.106	4.63	44.2	6.12	10.4	16.5	9.02
	3	2.28	2.278	0.10	30.91	4.053	7.08	49.2	9.30	15.2	14.3	12.4
	4	2.44	2.445	-0.19	28.52	4.029	9.57	51.7	12.1	19.5	13.0	15.4
	5	2.59	2.591	-0.04	26.69	4.006	12.1	53.3	14.7	23.8	12.0	18.2
<i>Buffer: lactic acid</i>												
Ca	6	1.43	1.434	-0.10	49.71	4.222	4.77	12.2	-	12.5	21.3	13.2
	7	1.48	1.486	-0.63	47.37	4.229	7.16	16.0	-	18.2	20.4	19.3
	8	1.54	1.535	0.56	45.40	4.237	9.78	19.3	-	24.1	19.6	25.6
Co	6	1.78	1.790	-0.85	39.81	4.168	4.78	33.9	-	11.4	16.8	11.0
	7	1.92	1.904	0.88	36.98	4.169	7.26	43.2	-	16.4	15.3	15.6
	8	2.00	2.002	-0.17	34.80	4.173	10.0	48.9	-	21.6	14.1	20.3
Zn	6	1.98	2.010	-1.26	35.46	4.140	4.82	47.8	-	10.9	14.6	9.94
	7	2.15	2.148	0.24	32.77	4.141	7.36	55.4	-	15.7	13.1	11.6
	8	2.28	2.263	0.93	30.78	4.146	10.2	61.1	-	20.6	11.6	18.2
<i>Buffer: β-hydroxybutyric acid</i>												
Mg	9	1.54	1.523	1.20	46.84	4.937	4.53	1.79	-	12.1	21.6	13.5
	10	1.55	1.549	0.05	45.45	4.946	6.79	2.47	-	17.8	21.2	20.0
	11	1.56	1.571	-0.71	44.38	4.944	9.04	3.09	-	23.5	20.7	26.6
	12	1.59	1.590	0.00	43.48	4.942	11.3	3.67	-	29.3	20.3	33.1
Ni	9	1.60	1.604	-0.27	44.42	4.924	4.50	5.78	-	11.8	20.9	13.0
	10	1.64	1.652	-0.72	42.63	4.930	6.73	7.78	-	17.3	20.2	19.2
	11	1.68	1.692	-0.68	41.22	4.926	8.96	9.50	-	22.8	19.6	25.2
	12	1.75	1.727	1.33	40.04	4.923	11.2	11.0	-	28.3	19.1	31.2
Zn	9	1.61	1.596	0.87	44.65	4.924	4.53	7.58	-	11.8	20.6	12.9
	10	1.64	1.648	-0.51	42.74	4.929	6.79	10.1	-	17.3	19.9	19.0
	11	1.68	1.692	-0.73	41.20	4.924	9.04	12.3	-	22.7	19.2	24.9
	12	1.74	1.731	0.50	39.93	4.920	11.3	14.2	-	28.1	18.6	30.8

TABLE III (continued)

Metal ion	System	R_E		Dev. (%)	\bar{m}_S	pH_S	C'	P_1	P_2	$C'_{B,S}$	$\bar{m}_{B,S}$	I
		Obs.	Calc.									
<i>Buffer: α-hydroxybutyric acid</i>												
Ca	13	1.39	1.379	0.81	52.54	4.256	2.35	8.76	—	6.76	19.3	6.70
	14	1.46	1.458	0.15	48.87	4.289	4.76	14.4	—	12.8	18.4	13.0
	15	1.52	1.519	0.06	46.33	4.274	7.17	18.7	—	18.8	17.3	18.9
	16	1.57	1.571	-0.04	44.37	4.270	9.59	22.2	—	24.6	16.4	24.6
	17	1.61	1.616	-0.36	42.77	4.275	12.0	25.2	—	30.1	15.9	30.1
Co	13	1.66	1.683	-1.35	43.05	4.168	2.28	28.4	—	6.36	15.5	5.61
	14	1.87	1.870	0.04	38.11	4.205	4.74	40.8	—	11.7	13.7	10.4
	15	2.00	2.004	-0.15	35.13	4.190	7.24	48.4	—	17.0	12.2	14.8
	16	2.10	2.109	-0.41	33.05	4.186	9.77	53.8	—	22.1	11.2	18.9
	17	2.23	2.197	1.51	31.46	4.192	12.3	57.8	—	26.9	10.5	22.8
Zn	13	1.85	1.818	1.75	39.85	4.111	2.21	30.3	2.23	6.28	14.4	5.22
	14	2.07	2.081	-0.52	34.24	4.122	4.62	41.0	5.37	11.4	12.4	9.41
	15	2.26	2.286	-1.15	30.79	4.081	7.06	46.3	8.29	16.4	10.8	13.0
	16	2.46	2.461	-0.01	28.33	4.052	9.52	49.3	10.9	21.6	9.74	16.2
	17	2.63	2.615	0.57	26.43	4.033	12.0	51.1	13.3	26.2	8.98	19.1
<i>Buffer: α-hydroxyisobutyric acid</i>												
Ca	18	1.38	1.383	-0.19	52.39	4.254	2.35	9.25	—	6.70	19.3	6.67
	19	1.47	1.461	0.61	48.80	4.287	4.65	14.9	—	12.4	18.4	12.6
	20	1.52	1.523	-0.23	46.24	4.253	7.01	19.3	—	18.5	17.0	18.4
	21	1.57	1.576	-0.39	44.26	4.258	9.38	22.9	—	24.1	16.3	23.9
	22	1.63	1.626	0.22	42.49	4.213	12.0	26.2	—	31.1	15.1	29.8
Co	18	1.72	1.692	1.62	42.81	4.157	2.27	27.9	0.75	6.31	15.4	5.56
	19	1.86	1.890	-1.59	37.73	4.183	4.44	39.2	1.86	11.3	13.6	10.0
	20	2.03	2.042	-0.58	34.50	4.138	7.02	45.8	3.03	16.8	11.9	14.1
	21	2.17	2.169	0.07	32.17	4.131	9.48	50.2	4.17	21.6	10.9	17.8
	22	2.31	2.288	0.95	30.20	4.077	12.2	53.6	5.37	27.9	9.79	21.6
Zn	18	1.83	1.817	0.71	39.86	4.092	2.18	26.4	4.35	6.26	14.6	5.19
	19	2.11	2.104	0.28	33.88	4.079	4.44	34.8	9.76	11.2	12.6	9.01
	20	2.32	2.346	-1.09	30.03	4.003	6.74	38.5	14.5	16.5	10.7	12.2
	21	2.56	2.559	0.06	27.26	3.968	9.07	40.4	18.5	21.2	9.59	14.9
	22	2.78	2.768	0.45	24.97	3.894	11.6	41.4	22.3	27.4	8.40	17.6

Fig. 9 shows the dependence on C'_L of the R_E values for the zinc- and nickel-glycolate systems. In the zinc system the stability constants for the one- to four-coordinated complexes have been reported ($\log \beta_1 = 2.32$, $\log \beta_2 = 3.52$, $\log \beta_3 = 4.06$ and $\log \beta_4 = 3.39$; the successive stability constants obtained by polarography⁶ were converted into overall stability constants). For the nickel system values of $\log \beta_1 = 2.02$, $\log \beta_2 = 3.30$ and $\log \beta_3 = 3.65$ were obtained by spectrophotometry⁶. In the present method however the existence of three- and four-coordinated complexes in these systems could not be confirmed, since the observed R_E values could be explained in terms of the coexistence model as shown in Fig. 9. The mean errors were 0.17 and 0.27% respectively. The coordination numbers and stability constants were estimated for the other complexes in a similar manner.

TABLE IV

ESTIMATED MOBILITIES AND STABILITY CONSTANTS OF METAL-GLYCOLATE, -LACTATE, - β -HYDROXYBUTYRATE, - α -HYDROXYBUTYRATE AND - α -HYDROXYISOBUTYRATE COMPLEXES (25°C)

$\log \beta$ (IP) = Evaluated thermodynamic stability constant; σ = dispersion; $\log \beta$ (corr.) = corrected thermodynamic stability constant ($I = 0$). In the "method" column the following abbreviations have been used: H = electromotive force (emf) measurement with electrode of H_2 , sol = solubility, cond = conductivity, pol = polarography, qh = quinhydrone electrode, sp = spectrophotometry, ix = ion exchange, gl = glass electrode, oth = other methods.

Complex	m	$\log \beta$ (IP)	σ	$\log \beta$ (lit.) (method ^b)	I	$\log \beta$ (corr.)
Ba(Glyc) ⁺	21.6	0.935	0.017	1.00 (H)	0	
				1.04 (sol)	0	
				0.66 (H)	0.2	1.21
Sr(Glyc) ⁺	24.8	1.079	0.012	—		
Ca(Glyc) ⁺	29.7	1.612	0.011	1.59 (H)	0	
				1.59 (sol)	0	
				1.11 (H)	0.2	1.66
Mg(Glyc) ⁺	32.0	1.441	0.021	—		
Cd(Glyc) ⁺	23.0	1.841	0.085	1.866 (cond)	0	
				1.41 (pol)	2.0	1.80
				1.51 (qh)	2.0	1.91
				1.84 (qh)	2.0	2.44
Cd(Glyc) ₂	0			2.11 (pol)	2.0	2.69
				1.975 (cond)	0	
				1.48 (qh)	2.0	1.88
Co(Glyc) ⁺	27.4	2.216	0.012	1.30 (sp)	2.0	1.70
				1.30 (sp)	2.0	1.70
				2.29 (qh)	2.0	2.89
Co(Glyc) ₂	0			2.08 (sp)	2.0	2.68
				2.08 (sp)	2.0	2.68
				2.08 (sp)	2.0	2.68
Ni(Glyc) ⁺	27.5	2.430*	0.044	—		
Ni(Glyc) ⁺	27.5	2.230	0.016	1.62 (sp)	2.0	2.02
				1.69 (qh)	2.0	2.09
				2.70 (sp,qh)	2.0	3.30
Ni(Glyc) ₂	0	3.544	0.026	—		
Zn(Glyc) ⁺	26.8	2.456*	0.062	—		
Zn(Glyc) ⁺	26.8	2.286	0.012	1.93 (pol)	2.0	2.32
				1.72 (qh)	2.0	2.12
				2.94 (pol)	2.0	3.52
Zn(Glyc) ₂	0	3.684	0.018	2.88 (qh)	2.0	3.48
				2.88 (qh)	2.0	3.48
				2.88 (qh)	2.0	3.48
Ba(Lac) ⁺	20.9	0.540	0.091	0.77 (sol)	0	
				0.64 (H)	0	
				0.55 (H)	0.2	1.10
Sr(Lac) ⁺	23.7	0.777	0.091	0.98 (H)	0	
				0.70 (H)	0.2	1.23
				0.50 (ix)	0.16	1.21
Ca(Lac) ⁺	28.0	1.381	0.021	1.07 (H)	0.2	1.62
				0.8 (ix)	0.16	1.32
				0.82 (emf)	0.15	1.33
Mg(Lac) ⁺	29.9	1.255	0.025	1.370 (H)	0	
				0.93 (H)	0.2	1.48
Cd(Lac) ⁺	22.2	1.662	0.015	1.695 (con)	0	
Co(Lac) ⁺	26.1	2.027	0.014	1.896 (con)	0	
				1.28 (sp)	2.0	1.68
				1.38 (oth)	2.0	1.77
				1.39 (gl)	2.0	1.78

TABLE IV (continued)

Complex	<i>m</i>	$\log \beta$ (IP)	σ	$\log \beta$ (lit.) (method ⁶)	<i>I</i>	$\log \beta$ (corr.)
Co(Lac) ₂	0			2.53 (sp)	2.0	3.12
				2.30 (oth)	2.0	2.88
				2.36 (gl)	2.0	2.94
Fe(Lac) ⁺	26.3	1.877	0.012			
Ni(Lac) ⁺	26.1	2.191	0.011	2.216 (con)	0	
				1.57 (sp)	2.0	1.97
Ni(Lac) ₂	0			2.94 (sp)	2.0	3.54
Zn(Lac) ⁺	25.5	2.262	0.017	2.239 (H)	0	
				1.860 (H)	0.2	2.407
Ba(β-HB) ⁺	20.2	0.512	0.193	0.43 (H)	0.2	0.98
Sr(β-HB) ⁺	22.8	0.819	0.158	0.47 (H)	0.2	1.02
Ca(β-HB) ⁺	26.5	1.235	0.122	0.82 (sol)	0	
				0.60 (H)	0.2	1.15
Mg(β-HB) ⁺	28.2	0.491	0.143	0.60 (H)	0.2	1.15
Ni(β-HB) ⁺	24.9	1.038	0.053	—		
Zn(β-HB) ⁺	24.3	1.163	0.032	1.06 (H)	0.2	1.61
				0.99 (qh)	2.0	1.39
Zn(β-HB) ₂	0			1.71 (qh)	2.0	2.31
Ba(α-HB) ⁺	20.2	0.357	0.058	—		
Sr(α-HB) ⁺	22.8	0.723	0.023	—		
Ca(α-HB) ⁺	26.5	1.471	0.010	—		
Mg(α-HB) ⁺	28.2	1.297	0.019	—		
Cd(α-HB) ⁺	21.4	1.800	0.042	1.27 (gl)	0.1	1.73
				1.23 (pol)	2.0	1.63
Cd(α-HB) ₂	0			2.13 (pol)	2.0	2.73
Co(α-HB) ⁺	24.8	2.131	0.013	1.95 (gl)	0.1	2.41
Ni(α-HB) ⁺	24.9	2.155	0.040	1.72 (sp)	2.0	2.12
Ni(α-HB) ₂	0	3.426	0.093	2.91 (sp)	2.0	3.51
Zn(α-HB) ⁺	24.3	2.383*	0.035	2.05 (gl)	0.1	2.51
Zn(α-HB) ⁺	24.3	2.209	0.048	1.72 (qh)	2.0	2.12
				3.02 (qh)	2.0	3.62
Zn(α-HB) ₂	0	3.572	0.096	—		
Ba(HIB) ⁺	20.2	0.414	0.310	—		
Sr(HIB) ⁺	22.8	0.666	0.072	—		
Ca(HIB) ⁺	26.5	1.495	0.089	—		
Mg(HIB) ⁺	28.2	1.556*	0.035	—		
Mg(HIB) ⁺	28.2	1.350	0.078	—		
Mg(HIB) ₂	0	2.733	0.124	—		
Cd(HIB) ⁻	21.4	1.776*	0.031	—		
Cd(HIB) ⁻	21.4	1.581	0.089	—		
Cd(HIB) ₂	0	3.110	0.158	—		
Co(HIB) ⁻	24.8	2.198*	0.022	—		
Co(HIB) ⁺	24.8	2.130	0.051	—		
Co(HIB) ₂	0	3.033	0.287	—		
Ni(HIB) ⁺	24.9	2.008	0.163	—		
Ni(HIB) ₂	0	3.865	0.122	—		
Zn(HIB) ⁺	24.3	2.145	0.037	—		
Zn(HIB) ₂	0	3.862	0.038	—		

* Evaluated on the basis of the mono-coordination model.

Table III shows the observed and best-fitted R_E values of Ca^{2+} , Co^{2+} and Zn^{2+} , and the effective mobilities and concentrations of the zone constituents evaluated by the least-squares method.

Table IV summarizes the evaluated and previously reported stability constants and coordination numbers together with the estimated mobilities of the complex ions. The stability constants of the metal ion-HIB systems have not been reported previously. In the present method the evaluated constants are the thermodynamic values corrected to ionic strength $I = 0$ M. However, almost all of the previously reported values have been obtained using electrolytes with high ionic strengths, for example $I = 2$ M. To enable comparison between the reported and the evaluated stability constants, the former values were corrected to $I = 0$ using the Davies equation⁹. The stability constants listed are restricted to $\log \beta_1$ and $\log \beta_2$. For several metal ions $\log \beta_3$ and $\log \beta_4$ have been reported but they are not cited here; as discussed for the Zn-Glyc and Ni-Glyc systems, they are not large enough to affect the R_E values under the present electrolyte conditions.

From Table IV, the agreement between the evaluated and the reported values of the stability constants and coordination numbers is satisfactory. Values of $\log \beta_2$ have been reported for several systems, although they could not be determined by the present method. In most cases this can be attributed to the fact that the values are not large enough to have a significant effect on the R_E values: for the metal-Lac systems all of the observed R_E values can be explained by the one-coordination model, although for Co^{2+} and Ni^{2+} the stability constants of two-coordinated complexes have been reported. The complex $\text{Co}(\text{Lac})_2$ may be overlooked by the present method since the reported $\log \beta_2$ is small (2.9–3.1). However, the reported $\log \beta_2 = 3.54$ for $\text{Ni}(\text{Lac})_2$ is sufficiently large to enable the complex to be identified by the present method, as shown for the nickel- and zinc-glycolate systems. However, the actual value of $\log \beta_2$ may be smaller than that reported. In the metal β -HB systems the stability constants were obtained for Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Ni^{2+} and Zn^{2+} . The coordination numbers of the complexes formed under the present electrolyte conditions were one except for zinc. The stability constants of the metal- β -HB systems were the smallest of the metal-oxycarboxylic acid systems examined, as expected. In the metal- α -HB system, two-coordinated complexes have been reported for Cd^{2+} , Ni^{2+} and Zn^{2+} , and we have confirmed this for Ni^{2+} and Zn^{2+} . The others were all one-coordinate as found previously.

Fig. 10 shows the observed and the simulated isotachopherograms of the metal ions at $C_L = 20.74$ mM and $\text{pH}_L = 4.4$ (HIB buffer). The agreement is satisfactory. Once the physico-chemical constants are evaluated, such a simulation can be made for any electrolyte condition and utilized to assess the separation conditions and to assay objective samples.

Thus it seems that this simulation of isotachophoretic equilibria can be applied for the estimation of the stability constants and the coordination numbers of kinetically labile complexes formed between sample ions and counter ions. The minimum evaluable $\log \beta_1$ by the present system is above *ca.* 0.5–1 and $\log \beta_2$ is above *ca.* 3–3.5. For the precise measurement of stability constants the abundance of the complex should be greater than *ca.* 5%. It should again be stressed that the evaluated stability constants are dependent on the values employed for the mobilities of the complex ions. We used values estimated from the formula weights (eqn. 1); this estimation is

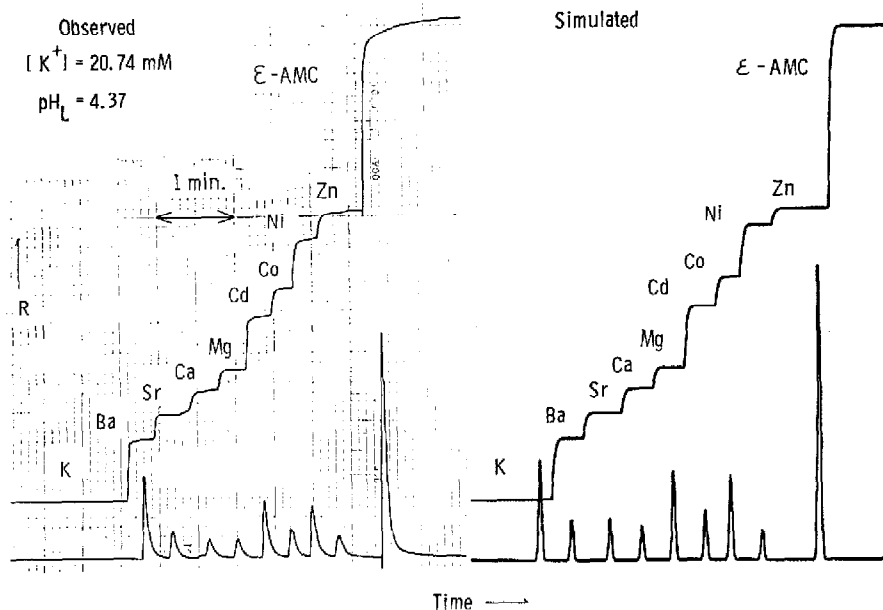


Fig. 10. Observed and simulated isotachopherograms of Ba^{2+} , Sr^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} . The complexing counter ion is α -hydroxybutyrate, the leading ion is K^+ and the terminator is ϵ -aminocaproic acid (ϵ -AMC).

valid for the present purpose since the evaluated stability constants agreed satisfactorily with reported values. For barium complexes however the evaluated stability constants were always smaller than the reported ones suggesting the value of the mobility used might have been an underestimate.

REFERENCES

- 1 T. Hirokawa, H. Takemi and Y. Kiso, *J. Chromatogr.*, submitted for publication.
- 2 T. Hirokawa, M. Nishino and Y. Kiso, *J. Chromatogr.*, 252 (1982) 49.
- 3 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 257 (1983) 197.
- 4 R. A. Robinson and R. H. Stokes, *Electrolyte Solutions*, Butterworths, London, 2nd ed., 1959.
- 5 Landolt-Bornstein, *Zahlenwerte und Funktionen*, Vol. II, Part 7, Springer, Berlin, 6th ed., 1960.
- 6 L. G. Sillen and A. E. Martell (Editors), *Stability Constants of Metal-Ion Complexes*, Special publication No. 17, The Chemical Society, London, 1964.
- 7 D. D. Perrin, *Stability Constants of Metal-Ion Complexes, Part B, Organic Ligands*, IUPAC Chemical Data Series No. 22, Pergamon, Oxford, 1979.
- 8 *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*, Vol. VI, McGraw-Hill, New York, London, 1933.
- 9 C. W. Davies, *J. Chem. Soc., London*, (1938) 2093.
- 0 J. Kielland, *J. Amer. Chem. Soc.*, 59 (1937) 1675.