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COMPLEX-FORMING EQUILIBRIA IN ISOTACHOPHORESIS

IV. SIMULATION OF ISOTACHOPHORETIC EQUILIBRIA OF METAL IONS FORMING MULTI-COORDINATED COMPLEXES

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SUMMARY

A computational procedure is given for the qualitative analysis of isotachophoretic equilibria of metal ions forming labile multi-coordinated complexes with counter ions. For the zinc- α -hydroxyisobutyrate system, the qualitative index R_E of Zn^{2+} was simulated at pH = 4.4 by varying the coordination numbers (1-3) and the stability constants of the complexes and the concentration of the leading ion, C_L . Different dependences of the R_E values on C_L were obtained for the differently coordinated complexes, suggesting that the coordination numbers and the stability constants of complexes can be evaluated from the observed R_E values by the least squares method. The present method is applicable to the analysis of mixed complexes with different coordination numbers.

INTRODUCTION

In isotachophoresis, the interaction of sample ions with the counter ions to form kinetically labile complexes results in a decrease in the effective mobility of the sample and an increase in the observable qualitative index, R_E , in comparison with a non-complex-forming system. The increase in R_E is closely related to the stability constants of the formed complexes, therefore the stability constants can be evaluated by analysing the observed R_E values. In Part II¹ a computational procedure was applied to the equations² describing the isotachophoretic equilibria involved in the formation of one-coordinated complexes. However, it is well known that many metal ions form multi-coordinated complexes and it is of interest to determine how the R_E values of the metal ions are affected by the formation of these complexes.

In this study, the previously reported simulation procedure² was modified to deal with mixed complexes with different coordination numbers. The R_E values of complexes of Zn^{2+} with α -hydroxyisobutyrate ion were simulated for different coordination numbers and stability constants to estimate the effect of multi-coordinated complexes on the R_E values and to assess the utility of the simulation for the sim-

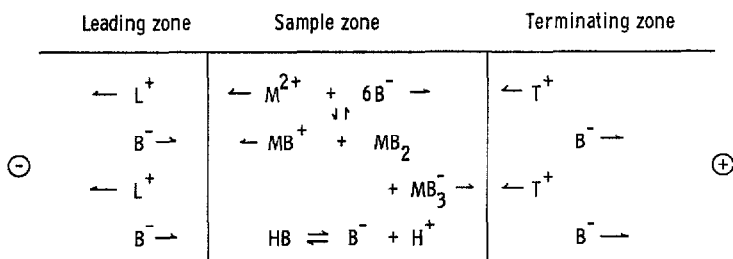


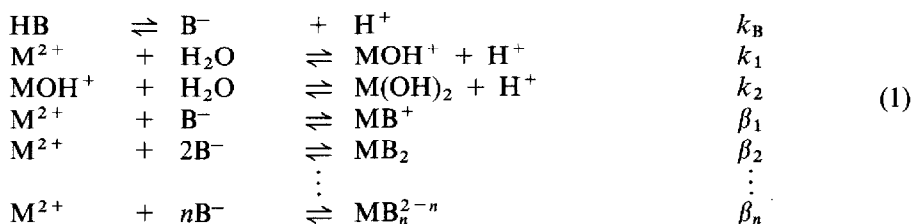
Fig. 1. Isotachophoretically separated zone of M²⁺ forming M-B complexes; the dissociation equilibria of the ligand ion, B⁻, are omitted in the leading (L) and terminating (T) zones.

ultaneous determination of the stability constants and the coordination numbers of the formed complexes.

COMPUTATIONAL PROCEDURE

Fig. 1 shows a schematic model of the isotachophoretically separated zones. The leading, sample and terminating ions are denoted by L⁺, M²⁺ and T⁺, respectively. A complexing agent with buffer action is denoted by HB, and B⁻ is the corresponding deprotonated anion. Although HB and B⁻ are present only in the leading zone at the initial stage of migration, at a steady state the original counter ions of M²⁺ are completely replaced by B⁻, forming kinetically labile complexes MB⁺, MB₂, etc., as shown in Fig. 1. The establishment of the equilibria may be rapid enough to enable a homogeneous migration of the formed complexes together with the non-complexed (free) M²⁺. 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. The pH_s, the concentrations of the zone constituents and their effective mobilities are regulated isotachophoretically under the particular leading electrolyte conditions.

Details of the computational procedure have already been reported². Therefore in this paper the equations used to analyse the isotachophoretic equilibria are limited to those corresponding to a sample zone in which multi-coordinated complexes are formed. Divalent cations (M²⁺) are employed as samples and the monovalent anion B⁻ as the complexing agent. Assuming the coexistence of multi-coordinated complexes (coordination number 1 - n), the complex-forming equilibria in the sample zone can be written as



where k_{B} is the acid dissociation constant of HB, k_1 and k_2 are those of M and β_1

to β_n are the overall stability constants of complexes of MB^+ to MB_n^{2-n} . The effective mobility of M^{2+} , \bar{m}_M , coexisting with B^- at a steady state can be expressed using the mobilities, m , and the concentrations, C , of the metal species in the zone

$$\bar{m}_M = \left(\sum_{i=0}^2 m_{M^{2-i}} C_{M^{2-i}} + \sum_{j=1}^n m_{MB_j^{2-j}} C_{MB_j^{2-j}} \right) / (C_M^f + C_{MB}^f) \quad (2)$$

where C_M^f and C_{MB}^f are the total concentrations of free metal species, $C_{M^{2+}} + C_{MOH^+} + C_{M(OH)_2}$, and the complexes formed respectively. The C_{MB}^f can be expressed using the stability constants of the complexes and the concentrations of M^{2+} and the ligand B^- as follows:

$$C_{MB}^f = \sum_{j=1}^n \beta_j C_{M^{2+}} C_B^{j-} \quad (3)$$

Inserting eqn. 3 into eqn. 2, using the dissociation constants to express the concentrations of metal species and eliminating $C_{M^{2+}}$, gives the following equation for \bar{m}_M :

$$\bar{m}_M = \frac{m_{M^{2+}} + k_1/C_H m_{MOH^+} + \sum_{j=1}^n m_{MB_j^{2-j}} \beta_j C_B^{j-}}{1 + k_1/C_H + k_1 k_2 / C_H^2 + \sum_{j=1}^n \beta_j C_B^{j-}} \quad (4)$$

Similarly, the effective mobility of the ligand ion can be expressed as follows

$$\begin{aligned} m_B &= \left(m_{B^-} C_{B^-} - \sum_{j=1}^n m_{MB_j^{2-j}} C_{MB_j^{2-j}} \right) / (C_B^f + C_{MB}^f) \\ &= \frac{m_{B^-} - \sum_{j=1}^n m_{MB_j^{2-j}} \beta_j C_{M^{2+}} C_B^{j-1}}{1 + C_H/k_B + \sum_{j=1}^n \beta_j C_{M^{2+}} C_B^{j-1}} \end{aligned} \quad (5)$$

where C_B^f is the total concentration of free B, $C_{HB} + C_{B^-}$.

To calculate \bar{m}_M and \bar{m}_B using eqns. 4 and 5, C_H , C_{B^-} and $C_{M^{2+}}$ are necessary. The exact values of them at the steady state can be obtained after iterative calculation to satisfy isotachophoretic conditions^{2,3}, however, approximate initial values of \bar{m}_M and \bar{m}_B are necessary to advance the iteration. As a first step, C_{B^-} can be obtained using C_H and C_B^f of the leading zone:

$$C_{B^-} = (1 + C_H/k_B) C_B^f \quad (6)$$

Using this value of C_{B^-} and assuming that the value of $C_{M^{2+}}$ is half of the concentration of the leading ion, $C_L^i/2$, \bar{m}_M and \bar{m}_B can be evaluated. The total concentration of species B in the sample zone, namely the concentrations of free and complexed B, is regulated by the mass balance for the counter ion. The total concentration of species B can be expressed as:

$$\begin{aligned} C_{B^-}^i + C_{MB}^i &= C_B^i + \sum_{j=1}^n \beta_j C_{M^{2+}} [(1 + C_H/k_B) C_B^i]^j \\ &= C_B^i \left\{ 1 + \sum_{j=1}^n \beta_j C_{M^{2+}} [(1 + C_H/k_B) C_B^i]^{j-1} \right\} \end{aligned} \quad (7)$$

Combining eqn. 7 and a mass balance equation^{2,3}, we obtain the following equation

$$C_{B^-}^i = \frac{C_{B,L}^i [\bar{m}_M (\bar{m}_L + \bar{m}_{B,L})] / [\bar{m}_L (\bar{m}_M + \bar{m}_B)]}{1 + \sum_{j=1}^n \beta_j C_{M^{2+}} [(1 + C_H/k_B) C_B^i]^{j-1}} \quad (8)$$

where $C_{B,L}^i$ is the total concentration of B in the leading zone, \bar{m}_L and $\bar{m}_{B,L}$ the effective mobilities of leading and B ions in the leading zone. In the leading zone, B is merely a pH buffer. Eqn. 8 includes C_B^i in the right hand side. Mathematically, C_B^i is one solution of an n th order equation.

To evaluate physically meaningful values of C_B^i the iterative calculations are made using the C_B^i value obtained in the earlier stage of iteration. As a first step, using $C_{B,L}^i$ as C_B^i , the value of C_B^i in the sample zone can be obtained for the next iteration step; $C_{B,L}^i$, $\bar{m}_{B,L}$ and \bar{m}_L were evaluated beforehand. The C_B^i value obtained by eqn. 8 is different from initially used $C_{B,L}^i$ and the advanced highly approximated C_{B^-} can be evaluated from C_B^i using eqn. 6. This value is then used to calculate the concentrations of metal species on the basis of the following electroneutrality relationship:

$$C_H - C_{OH} - C_{B^-} + \sum_{i=0}^2 (2-i) C_{M^{2+i}} + \sum_{j=1}^n (2-j) C_{MB_j^{2-j}} = 0 \quad (9)$$

Replacing the partial concentrations of the zone constituents in eqn. 9 by the total concentrations, dissociation constants and stability constants, the total concentration of free metal species can be expressed as:

$$\begin{aligned} C_M^i &= [C_{OH} - C_H + C_B^i k_B / (k_B + C_H)] / [1 + k_1 / C_H + k_1 k_2 / C_H^2] / \\ &\left\{ 2 + k_B / C_H + \sum_{j=1}^n (2-j) \beta_j [C_B^i k_B / (k_B + C_H)]^j \right\} \end{aligned} \quad (10)$$

The concentrations of the free metal constituents, $C_{M^{2+}}$, C_{MOH^+} and $C_{M(OH)_2}$, can be

calculated using C_M^i . The value of C_M^{2+} so obtained is different from the initially used value of $C_L^i/2$. Using this value the concentrations of the complexes can be obtained.

The specific conductivity of the sample zone, κ_M , can be obtained using the evaluated concentrations of the zone constituents

$$\kappa_M = \left[C_H m_H + C_{OH} m_{OH} + \sum_{i=0}^1 (2-i) C_M^{2-i} m_M^{2-i} + C_B^- m_B^- + \sum_{j=1}^n |2-j| C_{MB_j}^{2-j} m_{MB_j}^{2-j} \right] \times F/1000 \quad (11)$$

where F is the Faraday constant. At the isotachophoretically steady state, the values of the current density, J , for the leading and the sample zone, and of the velocity, should be equal

$$J = E_L \kappa_L = E_M \kappa_M \quad (12)$$

$$v = E_L \bar{m}_L = E_M \bar{m}_M \quad (13)$$

where E_L and E_M denote the potential gradients of the leading and sample zones respectively and κ_L is the specific conductivity of the leading zone. Combining eqns. 12 and 13, RFQ^{2,3} can be obtained,

$$\text{RFQ} = J_L/J_M - 1 = \bar{m}_M \kappa_L / (\bar{m}_L \kappa_M) - 1 \quad (14)$$

which can be used for the assessment of the isotachophoretically steady state^{2,3}. When the isotachophoretic conditions are fulfilled, the value of RFQ is zero. In the first iteration stage the value of RFQ is never zero since several assumptions are included as discussed above. To simulate the steady state at which the zone constituents are equilibrated, the pH value of the sample zone, pH_S , should be changed and the above calculations should be repeated until $\text{RFQ} < \text{ca. } 10^{-4}$. Usually, in cationic analysis, the simulated pH_S is lower than pH_L . It should be noted that the establishment of isotachophoretic equilibria is limited to the range pH_L ca. 3-11. In cationic analysis this establishment is sometimes critical below ca. $\text{pH}_L = 4$. At the steady state, the qualitative index, R_E , can be obtained from:

$$R_E = E_M/E_L = \kappa_L/\kappa_M = \bar{m}_L/\bar{m}_M \quad (15)$$

SIMULATION OF ISOTACHOPHORETIC EQUILIBRIA

Using the above computational procedure, the effective mobilities, R_E values, concentrations and pH shifts of pH_S from pH_L were simulated for Zn^{2+} coexisting with α -hydroxyisobutyric acid (HIB). The physico-chemical constants used in the simulations are listed in Table I. For the simulation a SORD microcomputer M223

TABLE I

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

The values were taken from refs. 5 and 6. m_0 = Absolute mobility ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) $\times 10^5$; pK_a = thermodynamic acid dissociation constant.

Cation	m_0	pK_a	Anion	m_0	pK_a
K^+	75.8	—	α -Hydroxyisobutyrate	33.5*	3.971
Zn^{2+}	54.7	9			

* Isotachophoretically evaluated.

mk III was used and a Watanabe x-y plotter Model WX4671 was used for plotting figures. The simulation conditions were as follows. The leading ion was K^+ and a pH_L value of 4.4 was selected since when pH_L is below 4, isotachophoretic conditions are not always fulfilled in cationic analysis. The pH_L value may be the upper limit for the buffering capacity of HIB ($pK_a = 3.971$). At pH_L 4.4 the hydroxy complexes of Zn^{2+} may be negligible, namely all the free zinc ions ($pK_a = ca. 9$) are in the fully charged state of Zn^{2+} . To assess the effect of the complex-forming equilibria established by different chemical species of Zn^{2+} , HIB, HIB^- and Zn-HIB complexes with different coordination numbers (1-3), the isotachophoretic equilibria were simulated by varying the concentration of ligand (HIB^-) in the leading electrolyte. At constant

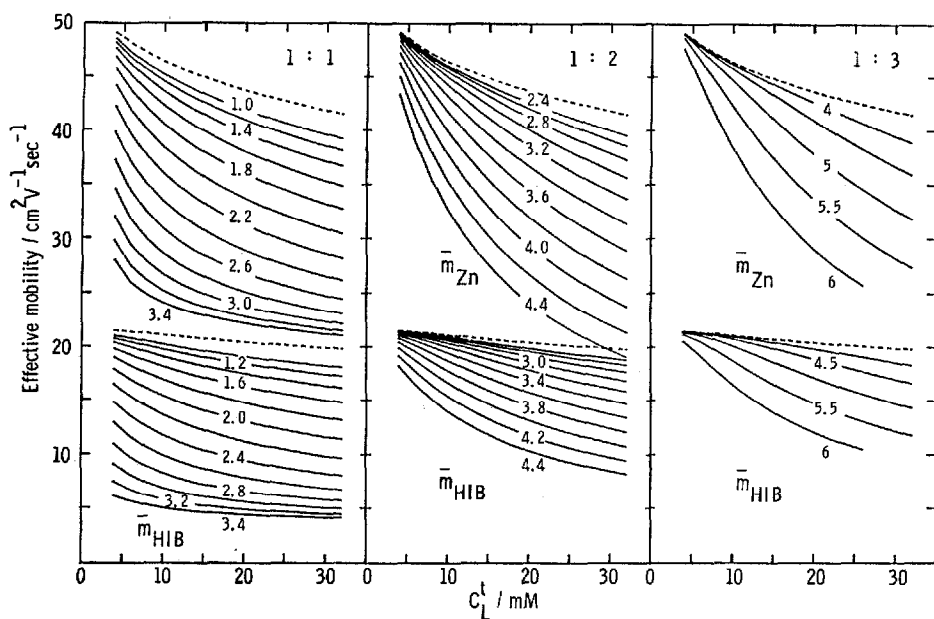


Fig. 2. Dependence on C_L of the effective mobilities of zinc and α -hydroxyisobutyrate ions (HIB) forming one- (1:1), two- (1:2) and three-coordinated complexes (1:3) at the isotachophoretically steady state. The broken curves represent the effective mobilities of Zn without any interaction with HIB. The numbers on the curves denote the overall stability constants, $\log \beta$.

pH_L this was achieved by varying the total concentration of leading ion, K^+ (C_L^t), in the range of 4–32 mM. In this range the total concentration of HIB was in the range from 5.4 to 42 mM. Since the C_L^t value in the actual leading electrolyte is known, several characteristics of the sample zone are discussed in terms of this value.

The simulation was carried out for a zinc zone containing three complexes with coordination numbers of 1, 2 and 3. As a first approximation, three different complex-forming models were used, in which one-, two- and three-coordinated complexes respectively are formed. For the simulation the absolute mobilities of $ZnHIB^+$ and $Zn(HIB)_3^-$ are necessary. Their values are not available in the literature, therefore we used $24.3 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ for $ZnHIB^+$ estimated from the relationship between the absolute mobility and the formula weight of monocarboxylate ions⁴. The overall stability constants of $ZnHIB^+$, $Zn(HIB)_2$ and $Zn(HIB)_3^-$ were varied in the ranges of $\log \beta_1 = 1.0\text{--}3.4$, $\log \beta_2 = 2.4\text{--}4.4$ and $\log \beta_3 = 4\text{--}6$ respectively. Fig. 2 shows the dependence on C_L^t of the effective mobility of Zn, \bar{m}_{Zn} , and HIB ions, \bar{m}_{HIB} , equilibrated in a zone. The broken curves show the corresponding dependences of \bar{m}_{Zn} and \bar{m}_{HIB} assuming no interaction between Zn^{2+} and HIB^- ; the decrease in the effective mobilities is therefore due to the increase in ionic strength, since the Debye-Hückel correction was made in the computation². The solid curves are for the complex-forming system. As the stability constants and C_L^t increase, both \bar{m}_{Zn} and \bar{m}_{HIB} decrease. The difference between the behaviours of \bar{m}_{Zn} and \bar{m}_{HIB} among the models used is significant especially at low C_L^t values and is large for the multi-coordination models.

The R_E value increases as the mobility of Zn decreases. Fig. 3 shows the dependence on C_L^t of the R_E values of one-, two- and three-coordinated zinc complexes

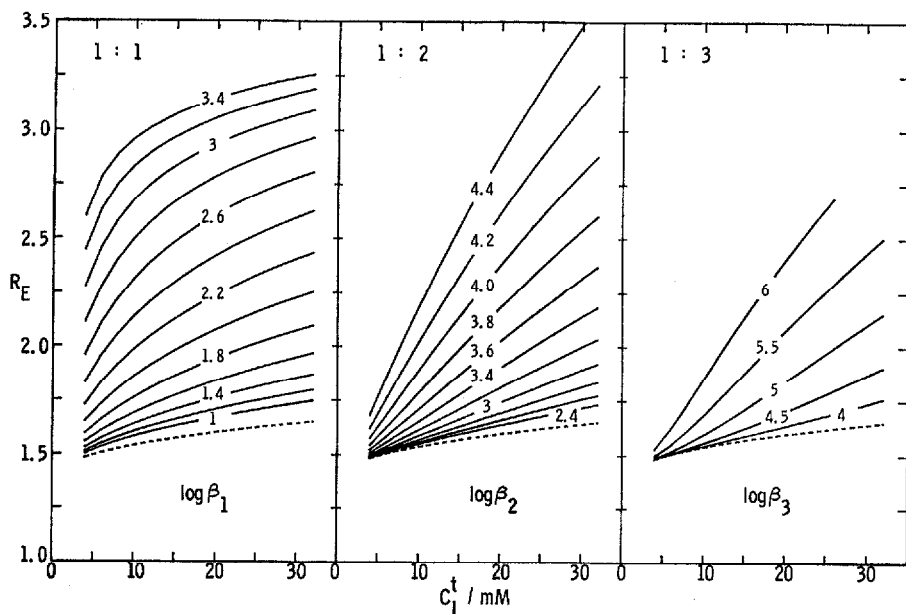


Fig. 3. Dependence on C_L^t of the R_E values of Zn^{2+} co-existing with α -hydroxyisobutyrate ion forming one- (1:1), two- (1:2) and three-coordinated complexes (1:3). The broken curves represent the R_E values of Zn^{2+} without any interaction with HIB.

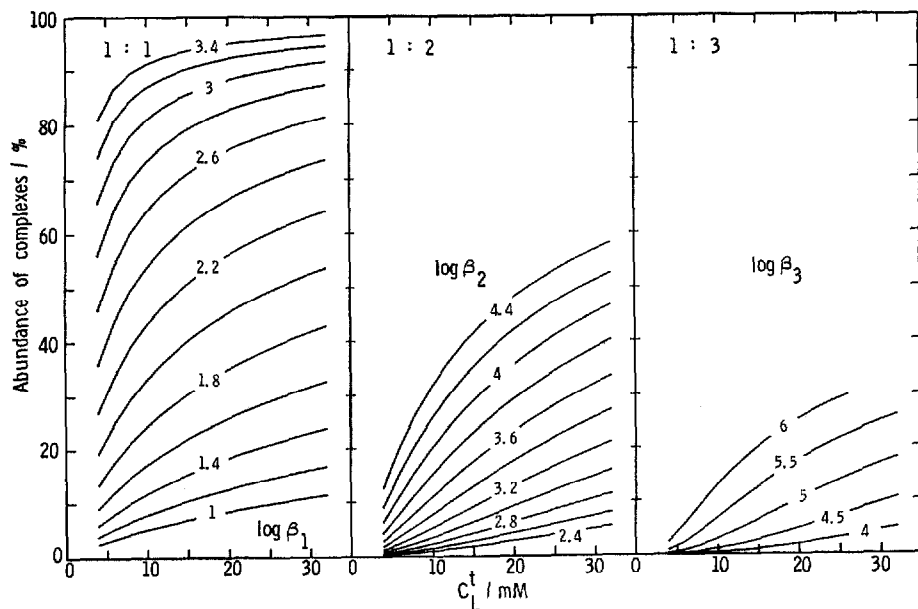


Fig. 4. Dependence on C_L^t of the abundance of free Zn^{2+} , and of one- (1:1), two- (1:2) and three-coordinated complexes (1:3) with α -hydroxyisobutyrate ions at the isotachophoretically steady state.

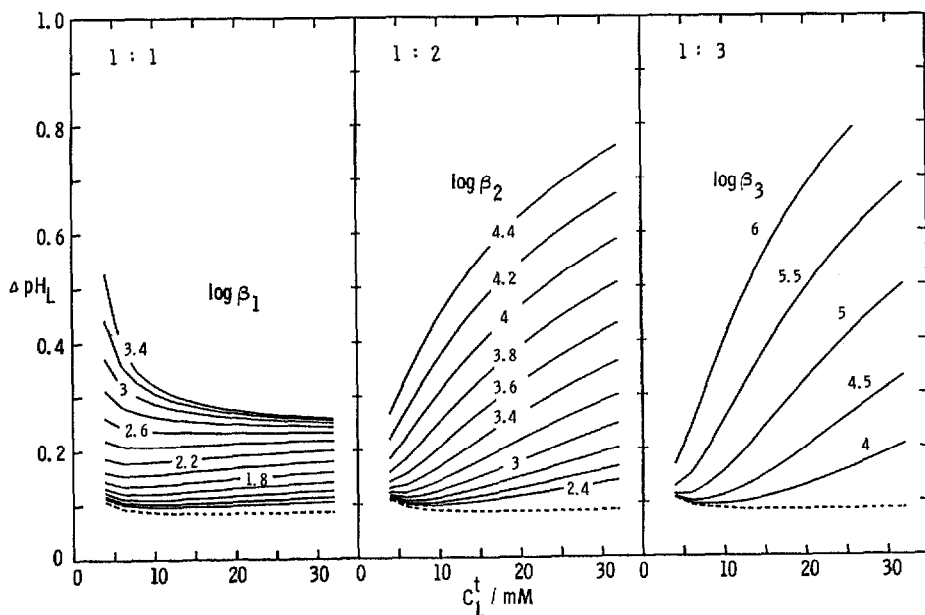


Fig. 5. Dependence on C_L^t of the pH shift, $pH_L - pH_s$, of the zinc zone containing one- (1:1), two- (1:2) and three-coordinated complexes (1:3) at the isotachophoretically steady state. The broken curves represent the pH shift of the zinc zone without any interaction.

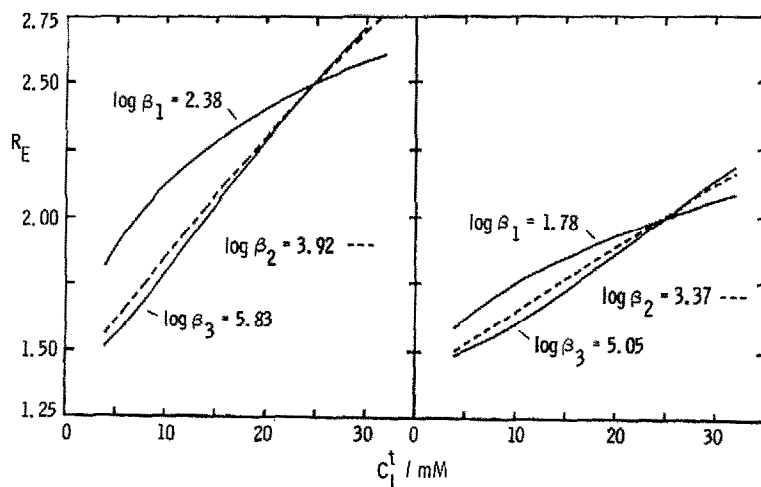


Fig. 6. Dependence on C_L^\dagger of the R_E values of Zn^{2+} co-existing with α -hydroxyisobutyrate ion forming one-, two- and three-coordinated complexes. The stability constants were selected so as to obtain $R_E = 2.5$ and 2.0 at $C_L^\dagger = 25$ mM. The broken curves represent the two-coordinated complexes.

respectively. For the one-coordination model (1:1) the R_E values are affected by the values of the stability constants even at low C_L^\dagger values and gradually approach a hypothetical R_E value for $ZnHIB^+$ with increasing stability constant. For the two-coordination model (1:2) the increase in R_E values is more significant and can be attributed to the formation of a non-ionic complex. The most significant increase was estimated for the three-coordination model (1:3), but it is suppressed due to the decrease in pH_S as shown later and therefore to the decrease in the ligand and complex concentrations. Fig. 4 shows the dependence on C_L^\dagger of the abundance (%) of the formed complexes with respect to the total concentration of zinc species in the zone. In contrast to the curves for the one-coordinated complex, several curves for the two- and three-coordinated complexes have points of inflection, *i.e.*, complex formation is suppressed at high C_L^\dagger values. Fig. 5 shows the dependence on C_L^\dagger of the pH shift of the sample zone, $\Delta pH_L = pH_L - pH_S$. Evidently the pH shift is smaller for the one-coordinate model forming a positive complex ion than for the other models forming non-ionic or negatively charged complexes. The pH shift is noticeable especially for the three-coordination model and in the simulation the establishment of the isotachophoretic equilibria was hindered by the increase of C_H when higher values of the stability constants were assumed.

Thus the observable R_E values increase with increasing C_L^\dagger due to complex formation and the differences among the systems are due to complexes with different coordination numbers. This suggests that when a unique kind of complex is formed the stability constant and the coordination number can be determined by analysing the R_E values obtained at different C_L^\dagger . Fig. 6 shows examples of the assessment of the coordination number. At $C_L^\dagger = 25$ mM two different R_E values of 2.50 and 2.00 were assumed. A best-fitting procedure based on the one-, two- and three-coordination models gave $\log \beta_1 = 2.38$, $\log \beta_2 = 3.92$ and $\log \beta_3 = 5.83$ for $R_E = 2.5$ and $\log \beta_1 = 1.78$, $\log \beta_2 = 3.37$ and $\log \beta_3 = 5.05$ for $R_E = 2.0$. Apparently, since

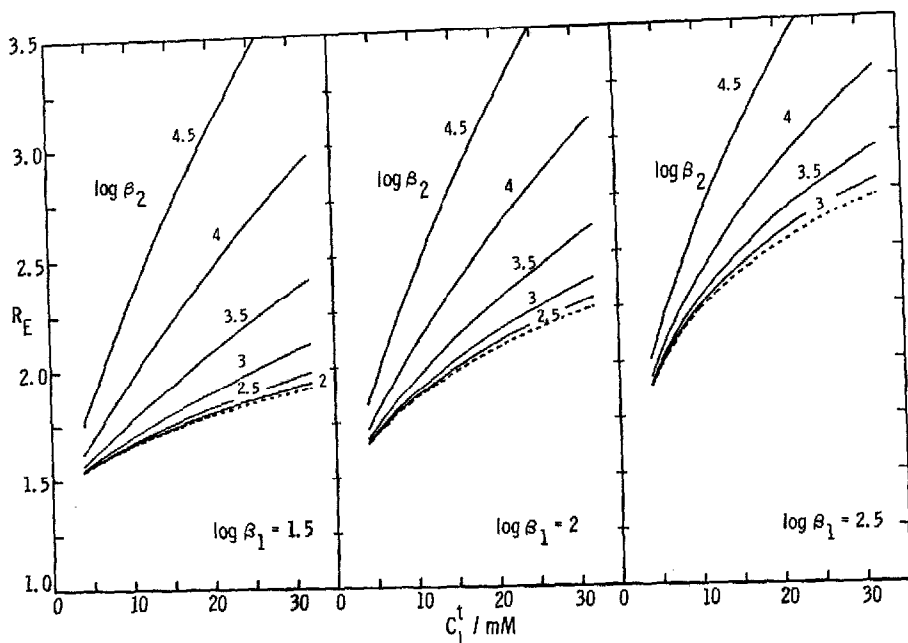


Fig. 7. Dependence on C_L^t of the R_E values of Zn^{2+} co-existing with α -hydroxyisobutyrate ion forming one- and two-coordinated complexes simultaneously. The broken curves represent the R_E values of Zn^{2+} forming a one-coordinated complex only.

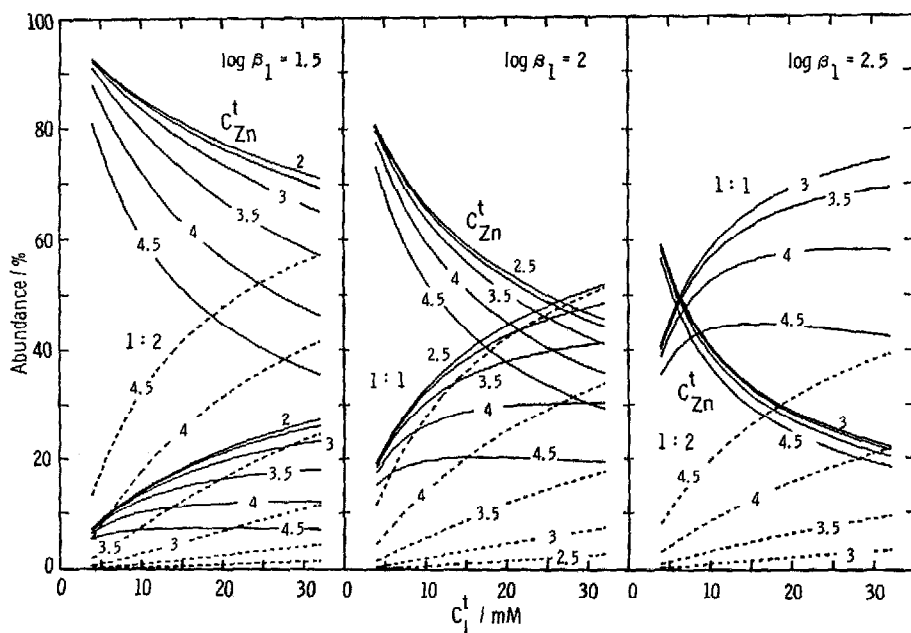


Fig. 8. Dependence on C_L^t of the abundance of free Zn^{2+} , and of one- (1:1) and two-coordinated complexes (1:2) with α -hydroxyisobutyrate ion at the isotachophoretically steady state. The broken curves represent the two-coordinated complexes.

the simulated R_E values at low C_L^t are different among the models they can be used for the determination of coordination number. In the present system it should be noted that the difference between the simulated R_E values for the two- and three-coordination models is small, suggesting that precise R_E measurement would be necessary to determine the actual coordination number. The maximum difference in the R_E values between the two models is 0.06, which may be distinguished with difficulty, since the accuracy of the observed R_E value is *ca.* ± 0.02 or less using a potential gradient detector and an internal standard to correct the asymmetric potential.

When the R_E value of a sample is measured precisely and it is larger by at least *ca.* 0.04 than the R_E value of the sample in the absence of complex formation, the evaluated stability constant might be meaningful. Thus for the one-coordinated complexes the minimum evaluable stability constants may be $\log \beta_1 = \text{ca. } 0.8$ and for the two- and three-coordinated complexes the minimum values may be $\log \beta_2 = \text{ca. } 2.5$ and $\log \beta_3 = \text{ca. } 4$ respectively. These limiting values however can be improved statistically when a number of precise R_E values are treated by the least-squares method.

In practice, the complexes discussed above exist simultaneously and are equilibrated in a zone. Then the R_E values of multi-coordinated complexes of zinc were simulated assuming two models. One is the co-existence of one- and two-coordinated complexes and the other is that of one-, two- and three-coordinated complexes. For the former model, the stability constants of the one-coordinated complex were varied in the range 1.5–2.5 and those of the two-coordinated complex were varied in the

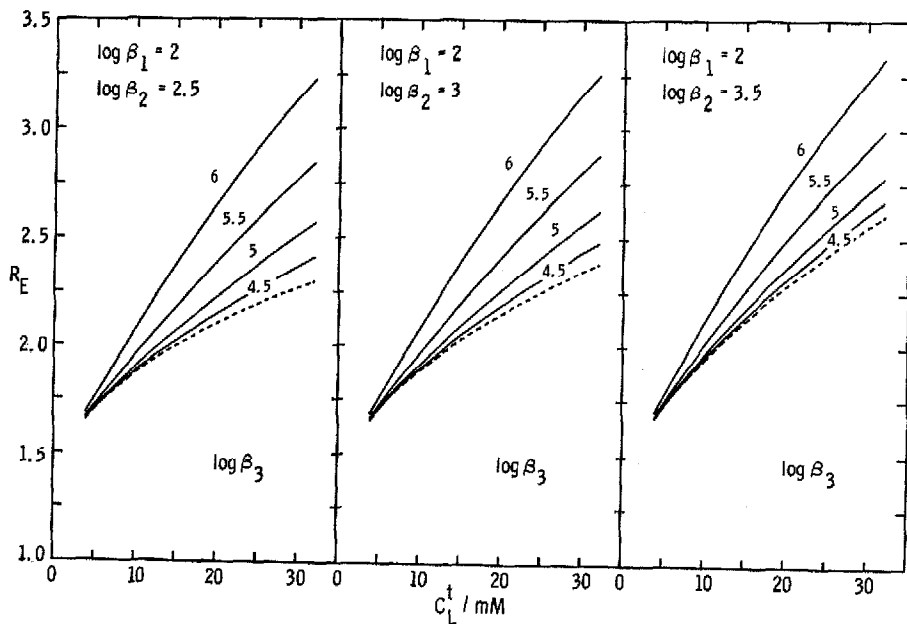


Fig. 9. Dependence on C_L^t of the R_E values of Zn^{2+} co-existing with α -hydroxyisobutyrate ion forming one-, two- and three-coordinated complexes simultaneously. The broken curves represent the R_E values of Zn^{2+} forming one- and two-coordinated complexes.

range of 2.5–4.5. Fig. 7 shows the dependence on C_L^t of the simulated R_E values. The broken curves are for the simple one-coordination model without the two-coordinated complex. Comparison of Fig. 7 with Fig. 4 reveals that when a considerable amount of two-coordinated complexes coexist there is a remarkable change in profile of the dependence of R_E on C_L^t . At $C_L^t = 32$ mM the R_E value of the one-coordination model ($\log \beta_1 = 2.0$) is 2.25 and that of the co-existence model ($\log \beta_1 = 2.0$ and $\log \beta_2 = 2.5$) is 2.30; the difference of 0.05 may be critical to distinguish between observed R_E values. This suggests that when the difference between $\log \beta_1$ and $\log \beta_2$ is larger than *ca.* 0.5, one- and two-coordinated complexes are co-existent. The estimable value of $\log \beta_2$ decreases as the value of $\log \beta_1$ increases. Fig. 8 shows the dependence on C_L^t of the abundance (%) of free Zn^{2+} , and of one- and two-coordinated complexes. Apparently the lower limit of the detectable abundance of the two-coordinated complex is a few percent under the present conditions.

Similarly, the R_E values were simulated, for the mixture of one-, two- and three-coordinated complexes. The stability constant $\log \beta_1$ was fixed at 2; $\log \beta_2$ was varied in the range of 2.5–3.5 and $\log \beta_3$ was varied in the range 4–6. Fig. 9 shows the simulated R_E values; the broken curves represent the co-existence model of one- and two-coordinated complexes. The difference in R_E values between the solid and broken curves is due to the formation of three-coordinated complexes. Apparently, the minimum evaluable stability constant of the co-existing three-coordinated complexes is above *ca.* 4.5 by the present method. Fig. 10 shows the dependence on C_L^t of the abundance of free Zn^{2+} , and of one-, two- and three-coordinated complexes.

Thus we can conclude that the coordination numbers and the stability con-

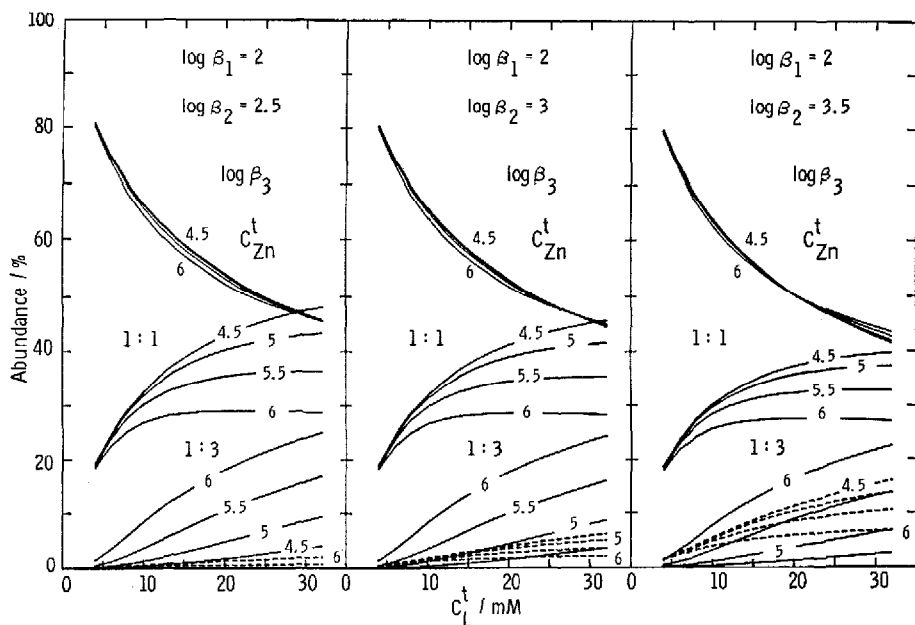


Fig. 10. Dependence on C_L^t of the abundance of free Zn^{2+} , and of one- (1:1), two- (1:2) and three-coordinated complexes (1:3) with α -hydroxyisobutyrate ion at the isotachophoretically steady state. The broken curves represent the two-coordinated complexes.

stants of complexes between metal ions and counter ions can be estimated by analysing the observed R_E values at different total concentrations of the counter ion in the leading electrolyte. Applications of the present method to several metal ions forming multi-coordinated complexes including Zn-HIB complexes will be reported in the following paper.

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