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A NEW METHOD FOR DETERMINATION OF COMPLEX STABILITY CONSTANT BY ISOTACHOPHORESIS

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A method for the isotachophoretic determination of stability constant was examined by a computor analysis of the observed isotachopherograms. The obtained values for acetate complexes with Ba, Ca, Zn, Cd, Cu and Ca-tartrate complex were compared with those obtained by the other methods and discussed. The present method is applicable even for mixed samples.

Recently developed capillary type isotachophoresis has been widely used as an analytical method like gas- and liquid-chromatography, and yet scarcely as a method for the basic research of physical chemistry. In previous papers$^{1)}$, we have reported that the absolute mobilities of some organic acids and their acidity constants can be determined by a computor analysis of the observed isotachopherograms.

An object of the present paper is to determine the stability constants of acetate complexes with divalent metal ions and Ca-tartrate complex by the computation using a FORTRAN program.

Estimation of R_E values (ratio of potential gradients): Under an isotachophoretic equilibrium, the ratio of potential gradient of sample zone (E_V) to that of leading zone (E_L) can be transformed by the effective mobility of sample ($\bar{m}_V$) and leading ion ($\bar{m}_L$) as follows;

$$R_E = E_V/E_L = \bar{m}_L/\bar{m}_V = h_V/h_L \qquad (1)$$

where h is the stepheights of isotachopherogram which can be directly measured.

Effective mobilities of metal ions in the presence of acetate ion: In a given metallic sample zone, the following equilibrium may be continuously maintained during electrophoretic migration of the zone.

$$M^{2+} + H_2O \rightleftharpoons MOH^+ + H^+, \qquad K_{MOH} = [MOH^+][H^+]/[M^{2+}]$$

$$MOH^+ + H_2O \rightleftharpoons M(OH)_2 + H^+, \qquad K_{M(OH)_2} = [M(OH)_2][H^+]/[MOH^+]$$

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+, \qquad K_{Ac} = [CH_3COO^-][H^+]/[CH_3COOH]$$

$$CH_3COO^- + M^{2+} \rightleftharpoons CH_3COOM^+, \qquad K_{AcM} = [CH_3COOM^+]/[CH_3COO^-][M^{2+}]$$

where M^{2+} is the divalent metal ion, K is the acidity constant of subscript chemical species, and K_{AcM} is the stability constant of acetate complex (AcM^+). Effective mobility of metal ions ($\bar{m}_M$) can be expressed as follows;

$$\bar{m}_M = \frac{m_M[M^{2+}] + m_{MOH}[MOH^+] + m_{AcM}[AcM^+]}{[M^{2+}] + [MOH^+] + [M(OH)_2] + [AcM^+]}$$

$$= \frac{m_M + m_{MOH}K_{MOH}/C_H + m_{AcM}K_{AcM}C^t_{Ac}K_{Ac}/(K_{Ac} + C_H)}{1 + K_{MOH}/C_H + K_{MOH}K_{M(OH)_2}/C_H^2 + K_{AcM}C^t_{Ac}K_{Ac}/(K_{Ac} + C_H)} \qquad (2)$$

where m is the absolute mobility of subscript chemical species and C^t_{Ac} is the total concentration of acetic acid in given sample zones. In practice, the C^t_{Ac} value can be calculated with total concentration of leading ion $K^+(C^t_K)$, since C^t_{Ac} of zone is controlled automatically by C^t_K according to law of mass balance. Figure 1 shows R_E *vs.* pH curves of Cu (C^t_{Ac} = 0 ~0.015 mol/l). The curves were calculated substituting the known values of absolute mobility m, acidity constant K and stability constant K_{AcM} found in literatures[2,3] into Eq. (2). In Fig. 2, the difference of R_E in the pH range of 4 to 7 results in the different stability of acetate complexes and the extreme increment of R_E at pH > 7 arises from formation of MOH^+ and $M(OH)_2$. Therefore, in order to determine the stability constant of AcM^+ complex, one must measure R_E in the pH range of 5 to 6, without any obstruction of the hydroxy-complex formation. The pH range seems to be suitable for the separation of metallic ions as shown in Fig. 2.

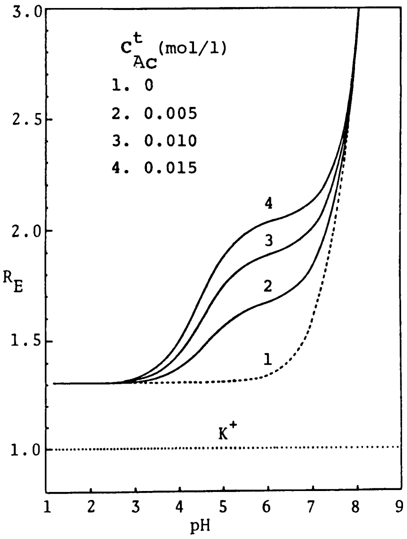

Fig. 1. R_E *vs.* pH of Cu-acetate.

Effective mobility of tartrate ion in the presence of Ca^{2+} ion: The following equilibrium is expected in tartrate zone;

$$H_2Tar \rightleftharpoons HTar^- + H^+, \quad K_{HTar} = [HTar^-][H^+]/[H_2Tar]$$

$$HTar^- \rightleftharpoons Tar^{2-} + H^+, \quad K_{Tar} = [Tar^{2-}][H^+]/[Htar^-]$$

$$Tar^{2-} + Ca^{2+} \rightleftharpoons CaTar^0, \quad K_{CaTar} = [CaTar^0]/[Tar^{2-}][Ca^{2+}]$$

where Tar denotes the tartrate ion, K is the acidity constant of subscript chemical species, and K_{CaTar} is the stability constant. Effective mobility ($\bar{m}_{Tar}$) can be expressed as follows;

$$\bar{m}_{Tar} = \frac{m_{HTar}K_{HTar}/C_H + m_{Tar}K_{HTar}K_{Tar}/C_H^2}{1 + K_{HTar}/C_H + K_{HTar}K_{Tar}/C_H^2(1 + K_{CaTar}[Ca^{2+}])} \qquad (3)$$

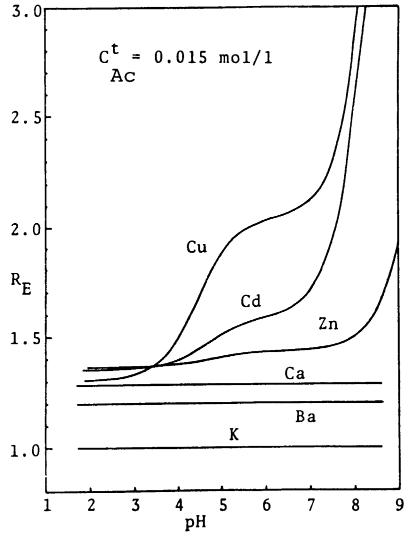

Fig. 2. R_E *vs.* pH of metal-acetate.

Figure 3 shows R_E *vs.* both pH and concentration of free Ca^{2+} ion (C_{Ca}). The values of m and K used for the calculation of Eq. (3) were taken from literatures[2,3]. Since K_{CaTar} is larger than those of metal-acetate complexes and the charge of the formed complex is null, the effect of the added Ca^{2+} ion on the increment of R_E is much significant.

Computor simulation of isotachophoretic equilibrium and refinement of stability constants: All calculations were carried out using HITAC 8700/8800 OS-7 of Hiroshima University. Everaerts' simple theory was extended to more general one, which is applicable to complex-forming equilibrium. With a FORTRAN program made by us, a correct pH of each sample zone was calculated iteratively to fulfill four isotachophoretic conditions[4). Then, effective mobilities of buffer, sample, and complex ions, their concentrations, and objective R_E values of samples were obtained. In the iterative procedure, Onsager's correction for the mobilities and activity correction for acidity constants were taken into account for the estimation of thermodynamic acidity constant$^{1)}$ or stability constant.

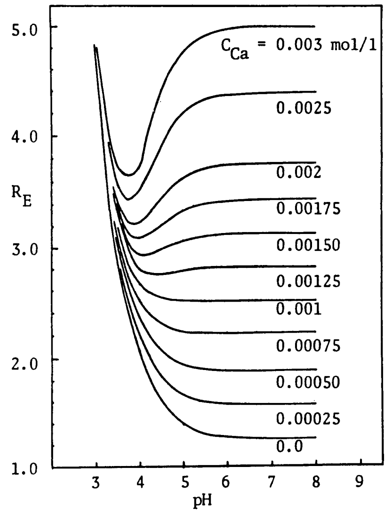

Fig. 3. R_E *vs.* pH and C_{Ca} of Ca-tartrate.

Experimental and Results: For the present experiments, a capillary type isotachophoretic analyzer was used (Shimadzu Seisakusho, Co., Ltd., Model IP-1B), which was equipped with a newly designed potential gradient detector$^{1)}$. All experiments were carried out in the 25 °C thermostatted box. For metal-acetate complexes, the leading electrolytes used were aq. KOH and the pH was adjusted to 5.10 by adding acetic acid. Terminating electrolyte was 0.005mol/l tris(hydroxymethyl)aminomethane and the pH was adjusted to *ca.* 5. For Ca-tartrate complex, the leading electrolyte was 0.01 mol/l HCl, containing Ca^{2+} ion. The pH was adjusted to 6.0 by adding histidine. Terminating electrolyte was 0.005 mol/l 2-(N-morpholino)ethanesulfonic acid. The observed R_E values shown in Figs. 4, 5, and 6 were the mean values of four to five runs. The capillary tube used was 20 cm in length and 0.5 mm in i.d.

In Fig. 4, solid lines were the results of simulations using the stability constant and m_M taken from literatures and dotted lines were obtained by the present experiments. The R_E values observed were significantly different from those calculated using K_{AcM} in literature$^{3)}$, except for Ca and Ba. Then, stability constants were refined by a least squres method$^{1)}$ to best-fit with the observed R_E values. For metal-acetate complexes, at first, a refinement of the absolute mobilities (m_{AcM}) and log K_{AcM} were made simultaneously. The results are shown in column I of Table 1. Converged m_{AcM} and log K_{AcM} could not be obtained for Ba-acetate, suggesting that the stability constant is very small, though the literature-cited value is 0.41 (ionic strength → 0). Also for Ca-acetate complex, we obtained smaller values of -0.26 with large dispersion. Next, m_{AcM} were estimated by the following relationship (Eq. 4) that effective mobility of given sample is proportional to the inverse of square-root of formula weight (FW), since the dispersion of m_{AcCu} was significantly large.

$$m_{AcM} = (244.2/\sqrt{FW} + 4.32) \times 10^{-5}\ cm^2/Vs \qquad (4)$$

where the coefficients are those obtained for a series of carboxylic acids($C_nH_{2n+1}COO^-$, n=3~10) by isotachophoresis$^{1)}$. With the values of m_{AcM} calculated from Eq. (4), log K_{AcM} was refined as shown in column II of Table 1 and the corrected R_E are shown in Fig. 5 together with the observed ones. The refined log K_{AcM} agreed well between columns I and II , if the dispersions are taken into consideration. In addition, Eq. (4) was adopted in the calculation for Figs. 1, 2, 4, and 5.

Fig. 6 shows the observed R_E for tartrate ion in the presence of Ca^{2+} as

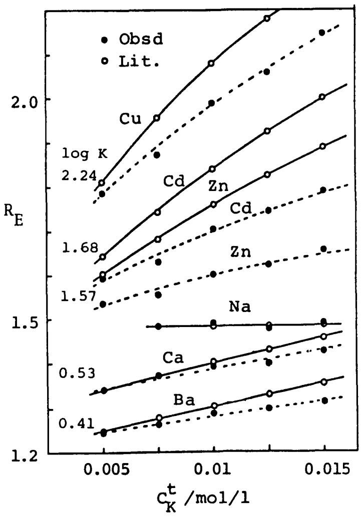

Fig. 4. Observed and calculated R_E of metal-acetate (log K_{AcM}: Ref.3).

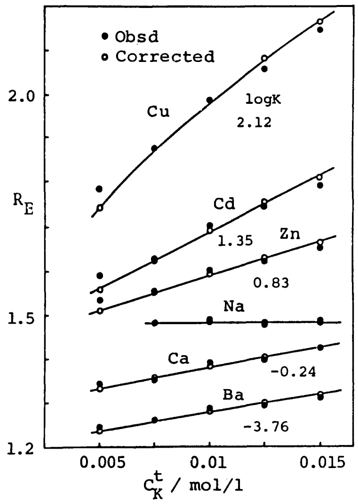

Fig. 5. Observed and corrected R_E of metal-acetate.

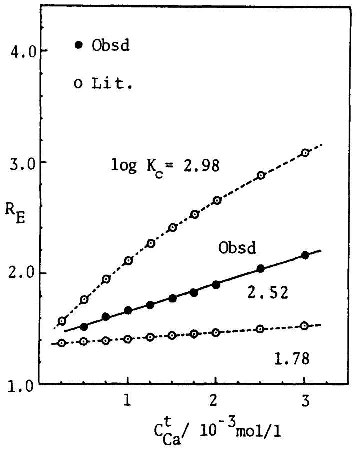

Fig. 6. Observed and calculated R_E of Ca-tartrate (log K_{CaTar} : Ref. 3).

counter ion and calculated R_E using log K_{CaTar} found in literature. The values of m_{HTar} and m_{Tar} used were obtained by isotachophoresis; 36.0 and $66.1\times10^{-5}cm^2/Vs$. The difference between the observed and calculated R_E is significant. Then, the value of log K_{CaTar} was refined. The obtained value was 2.52 and the dispersion was 0.006. The corrected values of R_E were not shown, since they agreed well with the observed ones. In this case, since m_{CaTar} is null, the obtained value seems to be more accurate than those of metal-acetate complexes. Refinement of stability constants of the other complexes between divalent metallic ions and di- and tri-carboxylic acids is now in progress.

Table 1. Absolute mobilities and stability constants of metal-acetate (25°C)

Samples	I $m^{1)}$	 $\sigma^{2)}$	 log $K^{3)}$	 σ	II m	 log K	 σ	log K (Ref. 3)
Ac-Ba		dispersed			21.7	-3.76	0.06	0.39, 0.41
Ac-Ca	27.5	0.34	-0.26	0.61	28.9	-0.24	0.53	0.0, 0.53, 0.77
Ac-Zn	22.9	0.53	0.77	0.10	26.2	0.83	0.08	0.66, 1.0, 1.46
Ac-Cd	13.0	0.86	1.18	0.05	23.0	1.35	0.04	1.33, 1.57, 1.68
Ac-Cu	20.9	7.21	1.95	0.28	26.4	2.12	0.02	1.62, 1.80, 2.24

1) Unit: $10^{-5}cm^2/Vs$. 2) Dispersion. 3) Ionic strength → 0.

References

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