UDC 547-986:661.183.38

99. **Yoshikazu Matsushima**: Determination of Complex Stability Constants by Ion Exchange Method. (Extension of Schubert's Method to Lower pH Region).

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The quantitative measurements of complex stability constants using cation exchange resin and radioactive tracers was initiated by Schubert[1]) and studied furthermore by a number of researchers including the present author.[2,3]) Schubert studied the case in which only one kind of complex was formed and Fronaeus[4]) measured the consecutive stability constants on the inorganic ligand by the extension of this method.

In the present papar, the extension of Schubert's method to lower pH region and the correction for the formation of complexes with coexisting buffer substances are discussed.

Theoretical

(1) Corrections for the Hydrolyses of Ligands and Complexes

Schubert used ion exchange method at pH 7.2～7.3, where polybasic acids used as the ligands were expected to be perfectly dissociated. At lower pH, however, it would be necessary to consider the hydrogen complexes of the polybasic acid.

When the polybasic acid H_nA has the known acid dissociation constants K_1, $K_2, \cdots$, $K_i, \cdots$, K_n, where

$$K_i=\frac{(H^+)(H_jA^{-i})}{(H_{j+1}A^{1-i})} \qquad i=n-j \tag{1}$$

the ratio of species (H_jA^{-i}) to the total acid is readily calculated from the equation as follows:

$$F_i=\frac{(H_jA^{-i})}{A_t}=\frac{K_1K_2\cdots K_i(H^+)^j}{(H^+)^n+K_1(H^+)^{n-1}+\cdots+K_1K_2\cdots K_i(H^+)^j+\cdots+\prod\limits_{i=1} K_i} \tag{2}$$

where ( ) indicates molar concentration and A_t denotes the total concentration of the acid.

When divalent metal ion M^{+2} and H_jA^{-i} were present, following equilibrium may be involved.

$$M^{+2}+H_jA^{-i} \rightleftharpoons MH_jA^{+2-i} \tag{3}$$

Stability constants of complexes are expressed as

$$Ks_i=\frac{(MH_jA^{+2-i})}{(M^{+2})(H_jA^{-i})} \tag{4}$$

When aqueous solution containing tracer amounts of M and cation exchange resin are at equilibrium, distribution coefficient (Kd) is defined as

$$Kd=\frac{\text{Total concentration of } M \text{ in resin phase (mmole/mg.)}}{\text{Total concentration of } M \text{ in aqueous phase (mmole/ml.)}}$$

$$=\frac{(M)_R}{\sum(M)} \tag{5}$$

In the absence of complex forming substances, the distribution coefficient is expressed as Kd_0.

[*1] 250-Kurosuna-cho, Chiba-shi (松島美一).

1) J. Schubert: J. Phys. Chem., **56**, 113 (1952).

2) Z. Tamura, Y. Matsushima: Yakugaku Zasshi, **79**, 1526 (1959).

3) *Idem*: *Ibid.*, **82**, 1569 (1962).

4) S. Fronaeus: Proc. Symposium on Co-ordination Chem., 1953, p. 61, Danish Chem. Soc., Copenhagen.

Then

$$Kd_0=\frac{(M)_R}{(M^{+2})} \quad (6), \qquad Kd=\frac{(M)_R}{(M^{+2})+\sum(MH_jA^{+2-i})} \quad (7)$$

In the equation (7), the assumption that MH_jA^{+2-i} could not be appreciably absorbed by the cation exchange resin is involved.

Under the conditions that (M^{+2}) is sufficiently small relatively to the exchange capacity of the resin, that is the case the present paper discusses, Kd_0 is independent of (M^{+2}) over a wide range of concentration.

Dividing equation (6) by (7)

$$\frac{Kd_0}{Kd}=1+\frac{\sum(MH_jA^{+2-i})}{(M^{+2})} \quad (8)$$

And from (2) and (4),

$$\frac{Kd_0}{Kd}-1=(\sum Ks_iF_i)A_t \quad (9)$$

In the equation (9), Kd_0, Kd and A_t are measurable and $\sum Ks_iF_i$ is constant so far as pH of the experimental systems is kept constant. Then a plot of $\left(\frac{Kd_0}{Kd}-1\right)$ versus A_t should give a straight line with slope $\sum Ks_iF_i$, provided that 1:1 complexes are the only predominantly formed species and their absorption by the resin would be negligible. Conversely, when the straight lines were experimentally observed, this could prove that above stated assumptions are right. From the acid dissociation constants K_1 and the values of the slopes obtained from more than n experiments at various pH, it is possible to calculate the values of the stability constants Ks_i.

To maintain the experimental systems at constant pH, the buffer substances are usually used. In the above description, it is postulated that the buffer substances do not appreciably form any complexes with metal ions, the comfirmation of that can be made by examining the constancy of Kd under various concentration of the buffer substances and definite concentration of the subject ligand.

(2) Corrections for the Complex Formation with Buffer Substances

When the complex formation of buffer substances can not be neglected, the corrections should be necessary.

In the system which does not contain the ligand but contains buffer substance at various concentration at its bufferring pH, the equation (9) is also utilized, transforming as,

$$\frac{Kd_0}{Kd_B}-1=K_B\cdot B_t \quad (10)$$

where

B_t=total concentration of the buffer substance

Kd_B=distribution coefficient under the existence of the buffer substance as the only complex forming substance.

K_B=constant under constant pH

In this case, Kd_0 is not directly measurable, because in the absence of buffer substance, it is impossible to maintain pH at desired levels. Then the equation (10) is further transformed as

$$\frac{1}{Kd_B}=\frac{1}{Kd_0}+\frac{K_B}{Kd_0}B_t \quad (11)$$

Provided that 1:1 complexes are the only appreciably formed complexes and their adsorption by cation exchange resin can be neglected, a plot of $1/Kd_B$ versus B_t should give a straight line, which can be extrapolated to $B_t=0$ to give the value of $1/Kd_0$. On the other hand, if the dissociation constants of buffer substance are known, the stability constants of complexes of the buffer substance can be calculated.

In the system which contains a fixed concentration of the buffer substance and various concentration of the complexing polybasic acid H_nA, following equations are obtained

$$Kd=\frac{(M)_R}{(M^{+2})+\sum(MB)+\sum(MH_jA^{+2-i})} \quad (12)$$

$$\frac{Kd_0}{Kd}=1+\frac{\sum(MB)}{(M^{+2})}+\frac{\sum(MH_jA^{+2-i})}{(M^{+2})} \tag{13}$$

where $\sum(MB)$ represents the total concentration of complexes formed between M and the buffer substance, and

$$\frac{Kd_0}{Kd}-1-K_B\cdot B_t=(\sum Ks_iF_i)A_t \tag{14}$$

In the equation (14), Kd_0, K_B can be determined from the system containing the buffer substance as an only complexing agent, and Kd, B_t and A_t are known or measurable from the system containing both the buffer substance and the acid. From the equation (14), as from (9), it is possible to obtain Ks_i corrected for the complex formation of buffer substance.

On the basis of the above described theories, treatments of the experimental data on the complexes of citrate with calcium, strontium, barium and zinc were carried out.

Experimental

All the experiments were carried out at 25°, ionic strength $\mu=0.16$ (NaCl). Reagents used were all analytical grade in purity.

(1) **Potentiometric Titration of Citric Acid**——Citric acid $C_6H_8O_7\cdot H_2O$ (E. Merck acc. to Sörensen), ca. 100 mg., dissolved in 0.16*M* NaCl solution was titrated with 0.16*N* NaOH. Titration and pH measurements were made with Metrohm Potentiograph E-336 (Metrohm LTD, Switzerland).

(2) **Ion Exchange Method**——The general methods used were almost the same as previously described,[1-3] the principal procedures of which were as follows.

The experimental systems consisted of more than 12 flasks each containing 50 ml. of aqueous phase and the known quantity of cation exchange resin. The aqueous phase was prepared by mixing the definite volume of 0.1*N* HCl solution of radioactive tracer (^{45}Ca, ^{90}Sr, ^{133}Br or ^{65}Zn), the solution of sodium salt of buffer substance and the variable volume of citrate solution and finally adjusted to 50 ml. in total volume and $(Na^+)=0.16$ by adding NaCl solution. The flasks containing the mixture were agitated in a constant-temperature agitating device and, after equilibrium had been attained, a measured volume of the supernatant liquid was removed for radiochemical analysis. Sodium diethylbarbiturate and sodium acetate were used as buffer substances. Sodium form of Amberlite IR-120 was used as cation exchange resin.

^{45}Ca, ^{133}Ba and ^{65}Zn used were imported by the Japan Radioisotope Association from Oak Ridge National Laboratory U.S.A. and ^{90}Sr from the Radiochemical Center, Amersham, England. Radiochemical samples of ^{45}Ca and ^{90}Sr were assayed by means of G.M. tube and of ^{133}Ba and ^{65}Zn were counted by scintillation spectrophotometer.

Result

Dissociation Constants of Citric Acid——From the potentiometric titration curve, dissociation constants of citric acid at 25°, $\mu=0.16$ were calculated to be $pk_1=2.94$, $pk_2=4.31$, $pk_3=5.61$.*[2]

Stability Constants of Alkaline Earth Citrate Complexes——In the case of alkaline earth, the complex formation with buffer substances could be neglected under the experimental conditions. Relationships between $\left(\frac{Kd_0}{Kd}-1\right)$ and the total citrate concentration are shown with measured pH in Figs. 1~3. At pH7.10~7.15, A^{-3} is a predominating anion and the term Ks_2F_2 and Ks_1F_1 can be neglected compared with Ks_3F_3. From the slopes at pH 7.10~7.15, Ks_3 were calculated. From the value of Ks_3 and the

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*[2] Reported values under similar conditions are

Temp. (°C)	Ionic strength.	pK$_1$	pK$_2$	pK$_3$	References
30	0.1*M* NaNO$_3$	2.94	4.14	5.82	R.C. Warner, *et al.*: J. Am. Chem. Soc., **75**, 5086 (1953).
25	0.11 〃	2.95	4.38	5.80	N.C. Li, *et al.*: J. Phys. Chem., **65**, 1074 (1961).
25	0.15 〃	2.94	4.35	5.62	N.C. Li, *et al.*: J. Inorg. Nucl. Chem., **12**, 122 (1959).

slopes at pH 4.5~6.2, Ks_2 were also calculated. Ks_1 were of lower values compared with Ks_3 and Ks_2, and could not be obtained reliable values because of larger experimental errors from the data obtained in these pH region. At pH below 3.5 reproducible experimental results were not obtained, but the increased citrate concentration resulted in increased absorption of radioactivity to the cation exchange resin suggested that the absorption of cation complex to the resin took place.

Stability Constants of Zinc Citrate Complexes——In the zinc citrate system, correction for the formation of complexes of zinc with buffer anions was necessitated. Plots of $1/Kd_B$ versus B_t gave straight lines, showing 1:1 was the only complex formed and absorption of ZnB^+ to cation exchange resin was negligible under the experimental

TABLE I. Stability Constants of Citrate Complexes.

Metal ion	log Ks_3	log Ks_2	log Ks_3 as reported in literature for similar ionic strength.
Ca^{+2}	3.16	2.67	3.15,5) 3.17,6) 3.227)
Sr^{+2}	2.76	2.20	2.82,5) 2.90,6) 2.707)
Ba^{+2}	2.56	1.28	2.98,6) 2.548)
Zn^{+2}	4.69	3.17*3	4.71,9) 4.68,9) 4.8010)

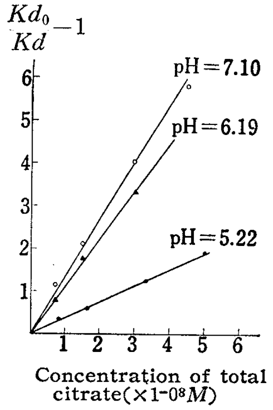

Fig. 1. Ion Exchange Results for Calcium Citrate

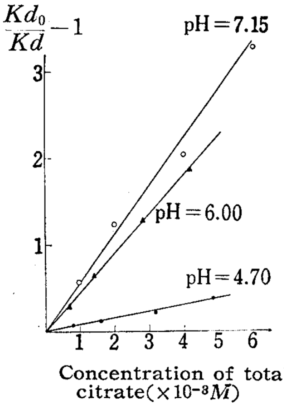

Fig. 2. Ion Exchange Results for Strontium Citrate

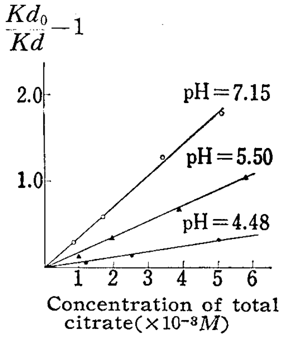

Fig. 3. Ion Exchange Results for Barium Citrate

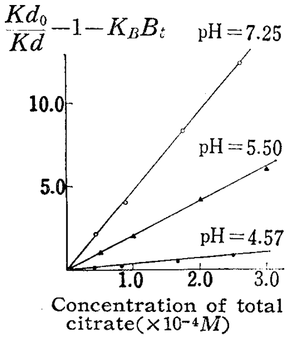

Fig. 4. Ion Exchange Results for Zinc Citrate

[*3] N.C. Li, *et al.*10) reported to be 2.96.

5) J. Schubert, A. Lindenbaum : J. Am. Chem. Soc., **74**, 3529 (1952).

6) N.R. Joseph : J. Biol. Chem., **164**, 529 (1952).

7) A.B. Hastings, *et al.* : *Ibid.*, **107**, 351 (1934).

8) J. Schubert : J. Am. Chem. Soc., **76**, 3442 (1954).

9) *Idem* : *Ibid.*, **80**, 4799 (1958).

10) N.C. Li, *et al.* : J. Inorg. Nucl. Chem., **12**, 122 (1959).

conditions. From the data obtained from the zinc acetate system and using the dissociation constant of acetic acid to be $10^{-4.64}$, stability constant for zinc acetate was calculated to be $\log K_{ZnC_2H_3O_2^+} = 1.01$.

Relationships between $\left(\frac{Kd_0}{Kd} - 1 - K_B \cdot B_t\right)$ and total citrate concentration in zinc citrate systems are shown in Fig. 4, where diethylbarbiturate and acetate were used as buffer substances. The value of Ks_3, Ks_2 calculated are listed in Table I.

Discussions

The above described method is applicable for the most organic acid that form exclusively 1:1 complexes with metal ions. In the cases 2:1 or higher complexes are formed, more complicated treatments will be necessary. With this method, Ks_n and Ks_{n-1} are usually measurable with fair accuracy, especially the differences of their values are not very large, while the reliable values of more hydrogenated complexes are not generally obtainable. That seems to be caused by the absorption of complexes by the resin, their low values and experimental errors.

The author expresses his gratitude to Prof. emeritus M. Ishidate and Prof. Z. Tamura of University of Tokyo for their unfailing guidance and encouragement throughout the course of this work. He is also indebted to Miss K. Tanaami for her technical assistance.

Summary

Theoretical considerations on the extension of ion exchange method for measuring complex stability constants were made. They are (1) the extension towards lower pH region, making possible to calculate the stability constants of hydrogenated complexes and (2) corrections for the formation of complexes with coexisting substances such as buffer substances. Applying these for the treatments of experimental data on the systems of calcium, strontium, barium and zinc with citrate, stability constants were calculated as listed in Table I.

(Received August 27, 1962)