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139. **Zenzo Tamura and Kozo Nagano**: Metal Complexes of Isonicotinoylhydrazine and Related Compounds. I. Consecutive Formation Constants of Isonicotinoylhydrazine-Cd(II) Complexes Determined from Polarographic Data.

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Isonicotinoylhydrazine (INH), one of the potential tuberculostatics, has an ability to form complexes with cupric and other metal ions in aqueous solutions. Erlenmeyer, *et al.* found that the antituberculous activity *in vitro* increased tenfold by adding cupric ion.[1] Cymerman-Craig, *et al.* further found that INH related compounds which could not form metal chelates, *e.g.* 1-isonicotinoyl-1-methylhydrazine (N-Me-INH), did not act against M. *tuberculosis.*[2] On the basis of these findings, Albert determined the formation constants of metal chelates of some hydrazides, ignoring the other complex species, and found no parallelism between their chelating ability and tuberculostatic activity.[3] Foye and Duvall isolated chelates having a 1:1 ratio of INH to metal from acidic media, and those having a 2:1 ratio on the alkaline side of neutrality (pH 7.5), and found that the 2:1 chelates were more lipophilic than the 1:1 chelates, but that there was no difference in antitubercular activities *in vivo* among these two types of chelates and INH itself.[4] On the other hand, Pope reported that the antituberculous action of INH was inhibited by pyridoxal, pyridoxamine, pyridoxine, α-ketoglutarate and pyruvate,[5] and Cymerman-Craig indicated a structural resemblance between the INH-metal complexes and those from pyridoxal, amino-acids and metal ions.[2,6,7] In spite of these findings, the mode of action of INH remains obscure. In order to make it clear, more precise informations will be necessary about the formation of INH complexes. Here, the authors intended to determine consecutive formation con stants of the complexes, and to elucidate their structures.

At first, the authors tried to determine consecutive formation constants of INH-Cd(II) complexes according to the polarographic method of De Ford and Hume.[8,9]

Experimental

Materials and Solutions——Analytical grade $Cd(NO_3)_2$ and INH (Sumifon powder: Sumitomo Chem. Co.) were used without further purification. No maximum suppressor was used as no maximum wave occurred. Nitrogen, after passing through an alkaline pyrogallol solution, was used to exclude air from the polarographic cell.

A series of solutions was prepared from standard stock solutions, each containing $0.001M$ $Cd(NO_3)_2$, $1.0M$ $NaNO_3$ and INH in concentrations varying from 0.05 to $1.0M$. pH Values of all solutions were adjusted to ca. 7.2 with an aqueous NaOH solution.

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1) E. Sorkin, W. Roth, H. Erlenmeyer: Helv. Chim. Acta, **35**, 1736 (1952).
2) J. Cymerman-Craig, D. Willis: J. Chem. Soc., **1955**, 4315; J. Cymerman-Craig, S. D. Rubbo, D. Willis, J. Edgar: Nature, **176**, 34 (1955).
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7) H. N. Christensen: *Ibid.*, **79**, 4073 (1957).
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Apparatus and Procedure——An apparatus used is shown schematically in Fig. 1. Current-voltage measurements were made with a Yanagimoto Recording Polarograph, Model PB-4. The capillary used had a value of 1.79 for $m^{2/3}t^{1/6}$ at the potential of the S.C.E. An modified "H" cell (Fig. 1) was employed with the solution to be examined in one arm and 1.0*M* $NaNO_3$ in the other arm. The two arms of the cell were separated by an agar bridge 1.0*M* with respect to $NaNO_3$. The connection to the saturated calomel electrode (hand-made; S.C.E. 1) was through a saturated KCl salt bridge dipping into the 1.0*M* $NaNO_3$ arm of the cell. The resistance of the cell system was found to be 1390 ohms.

A diffusion current, i_d, was measured from a polarographic wave, about which reversibility of the electrode reaction was confirmed from the graph of $\log(i_d-i)/i$ *vs.* $E_{d.e.}$ corrected for *i*R drop. After that, the rotating potentiometer was switched to manual, the pen was allowed to work at several potentials nearby $E_{1/2}$ and true values of the voltages between the saturated calomel reference electrode (Toa Denpa Co.; HC-205-2R; S.C.E. 2) and the dropping mercury electrode were measured with an outside potentiometer (Hokushin Denki Co.). From these and current values, the half-wave potential, $E_{1/2}$, was obtained by the use of interpolation. In this case, *i*R correction was unnecessary. The temperature of the water-bath was maintained at 25.0±0.1°. The water-bath was grounded.

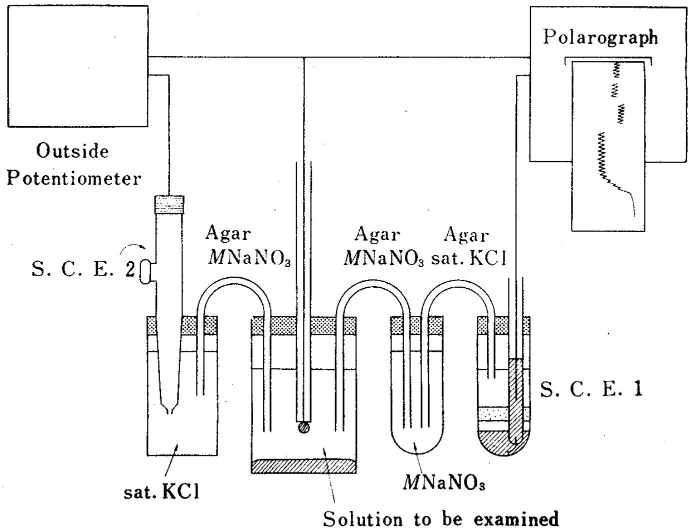

Fig. 1.

Treatment of Data——The method of De Ford and Hume[8] was improved to the more exact one by the authors.

The thermodynamical consecutive formation constants, K_j (j=1, 2, 3, ……), are defined as

$$K_j = f_{MX_j}C_{MX_j}/f_M C_M (f_X)^j (C_X)^j \qquad (1)$$

where f_M, f_X and f_{MX_j} are activity coefficients of cadmium ion (M), INH molecule (X), INH-Cd (II) (j:1) complex (MX_j) respectively. C_M, C_X and C_{MX_j} denote concentrations of those species respectively, expressed in term of molarity.

The first De Ford-Hume function, $F_0(X)$, is calculated from the following equation without ignoring f_M.

$$\begin{aligned} F_0(X) &= f_M \sum_{j=0} K_j (f_X)^j (C_X)^j / f_{MX_j} \\ &= \text{Antilog}[0.4343(nF/RT)\{(E_{1/2})_s-(E_{1/2})_c\}+\log(i_d)_s/(i_d)_c] \qquad (2) \end{aligned}$$

In equation (2), $(E_{1/2})_s$ and $(i_d)_s$ are the half-wave potential and the diffusion current obtained for the simple metal ion, and $(E_{1/2})_c$ and $(i_d)_c$ are those obtained for the solution containing both the metal ion and the complexing agent.

The second function, $F_1(X)$, was derived by De Ford and Hume as

$$F_1(X) = \{F_0(X)-1\}/C_X f_X$$

but f_X is unknown and should not be regarded as unity under the condition of constant ionic strength. Accordingly, $F_1(X)$ is unobtainable and only

$$F_1'(X) = \{F_0(X)-1\}/C_X = f_X F_1(X) \qquad (3)$$

can be calculated. Similarly, $F_2'(X)$, $F_3'(X)$, …… are derived as follows in stead of $F_2(X)$, $F_3(X)$, ……,

$$F_2'(X)=\{F_1'(X)-K_1'\}/C_X \qquad (4)$$
$$F_3'(X)=\{F_2'(X)-K_2'\}/C_X \qquad (5)$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$

where

$$K_j'=K_j f_M(f_X)^j/f_{MX_j}$$
$$=C_{MX_j}/C_M(C_X)^j \qquad (6)$$

When the function $F_j'(X)$ is plotted against C_X and is extrapolated to $C_X=0$, a concentration equilibrium constant K_j' is obtained.

Reliability of Data——The presence of liquid junction potentials of unknown magnitude was neglected. But, since the errors incurred by neglecting these potentials are approximately the same for each $E_{1/2}$ determination, the net error should approximate zero when the difference between the $(E_{1/2})_s$ and $(E_{1/2})_c$ values is taken. In any given experiment $E_{1/2}$ could be estimated to 1.0 mv. The errors of consecutive formation constants were calculated from the probable error of ±1 mv.

Results and Discussion

The experimental data are given in Table I, which gives also the values for the functions $F_0(X)$, $F_1'(X)$, $F_2'(X)$, and $F_3'(X)$. These values with ranges of error are plotted against C_X in Figs. 2 and 3. The graph of the $F_3'(X)$ function *vs.* concentration of INH

TABLE I. Analysis of $E_{1/2}$ of Cadmium in INH Medium

INH (*M*)	$E_{1/2}$ (v.)	i_d (μa.)	$F_0(X)$	$F_1'(X)$	$F_2'(X)$	$F_3'(X)$
0.00	0.579	6.07	1.00	....	....	....
0.05	0.591	5.29	2.92	38.4	68	960
0.10	0.599	4.98	5.78	47.8	128	1080
0.15	0.606	5.02	9.89	59.3	162	947
0.20	0.611	4.75	15.4	72.2	186	830
0.25	0.619	4.69	29.1	113	312	1168
0.30	0.623	4.28	43.6	142	357	1123
0.35	0.629	4.47	66.6	187	434	1183
0.40	0.634	4.42	99.4	246	528	1270
0.45	0.636	4.36	118	260	500	1067
0.50	0.640	4.28	164	326	582	1124
0.55	0.643	4.28	207	374	616	1084
0.60	0.645	4.19	247	410	625	1008
0.65	0.648	4.18	313	480	685	1023
0.70	0.651	4.16	397	565	757	1053
0.80	0.655	4.03	559	698	829	1011
0.90	0.659	3.84	801	889	949	1032
1.00	0.663	3.76	1118	1117	1082	1062
			$K_0=1.00$	$K_1'=35$	$K_2'=20$	$K_3'=1050$

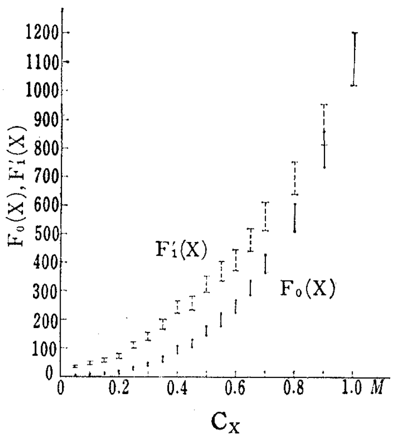

Fig. 2.

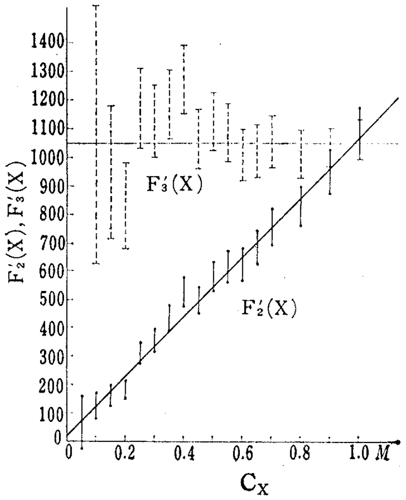

Fig. 3.

is a straight line with slope zero, indicating that INH, within the range of concentrations up to 1.0*M*, does not form any more highly coordinated complexes with cadmium ion than tris-species. The consecutive formation constants obtained are as follows :

$$K_1' = 35 \pm 5$$
$$K_2' = 20 \pm 50$$
$$K_3' = 1050 \pm 90$$

After the polarographic measurement, an addition of more cadmium ion to the solution containing 1.0*M* INH caused a precipitation of yellowish prisms. The composition of the crystal was proved to be $Cd(INH)_2(NO_3)_2 \cdot 2H_2O$ from the data of elemental analyses and chemical analyses of cadmium (sulfate), INH (bromine), nitrate ion (nitron) and water (Karl Fischer, drying). This result was inconsistent with the polarographic data, from which INH-Cd(II) (3:1) complex was suggested much more stable than (2:1) complex. On the other hand, values of the consecutive formation constants for INH-Cd(II) complexes were determined by means of pH titration as follows,[*2] and were compared with the corresponding values from the polarographic data in parentheses.

$$K_{M_1} = C_{MX}/C_M C_X = 12.2\ (35)$$
$$K_{M_2} = C_{MX_2}/C_{MX} C_X = 12.6\ (0.57)$$
$$K_{M_3} = C_{MX_3}/C_{MX_2} C_X = 3.4\ (52.5)$$

The data from the pH titration method indicated the considerable stability of the (2:1) complex, and well coincided with the composition of the crystal. Furthermore, the sequence of magnitude of K_{M_j} from the polarographic data was unreasonable according to the concept of statistical effect.10) Causes of such a doubtful result have not been completely explained, but it should not be disregarded that, when a concentration of a neutral molecule increase extremely, activity coefficients, f_M, f_X, f_{MX_j}, etc. might change considerably even if ionic strength of the solution is maintained constant. After that, the authors intended to make use of the pH titration method in determining consecutive formation constants of complexes. The works will be described in the following papers.[*2,*3]

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Summary

Consecutive formation constants of Cd(II) complexes of isonicotinoylhydrazine (INH) were determined by the polarographic method. A series of solutions containing 0.001*M* Cd^{2+}, 1.0*M* $NaNO_3$ and INH in concentration varying from 0.05 to 1.0*M* were used for the measurement of i_d and $E_{1/2}$. The method of De Ford and Hume was modified for calculation of concentration equilidrium constants K_j'.

The results were

$$K_1' = 35 \pm 5 \qquad K'_2 = 20 \pm 50 \qquad K'_3 = 1050 \pm 90$$

These results, especially K_3', however, did not agree with the constants obtained by pH titration method which will be shown in the later report.

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[*2] Details will be described in Part III : To be published in this Bulletin in near future (1963).

[*3] Part II : *Ibid.*, **11**, 797 (1963).

10) J. Bjerrum : "Metal Ammine Formation in Aqueous Solution," 40 (1957) Haase & Son, Copenhagen.