Indium(III) Complexes of DL-Penicillamine in Aqueous Solution. Evidence for the Formation of Protonated and Hydrolyzed Complexes

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The penicillamine–In(III) system has been investigated with potentiometric titration over a wide range of metal concentration at 21°C and $I=0.1$ (KNO_3). In dilute concentration of metal (0.00025—0.0005 M) and ligand (free base), single species (InL and InL_2) are predominantly formed. In higher concentration of metal (0.0025—0.005 M) and ligand (with an equimolar HCl), the protonated and hydrolyzed complexes (InLH, InL(LH), and InL(OH)) are also formed together with the simple species. The formation constants calculated with non-linear least-squares program were found to be: $\log \beta_{110}=15.330$, $\log \beta_{120}=29.789$, $\log \beta_{11-1}=18.858$, $\log \beta_{12-1}=33.391$, and $\log \beta_{111}=11.25$ for InL, InL_2, InLH, InL(LH), and InL(OH) species, respectively. The formation constants and ultraviolet spectra indicate that the proton in the protonated complex is attached to the amino-group of penicillamine.

^{113m}In[1] and ^{111}In[2] as well as ^{99m}Tc are a few of the radioisotopes recommended for the scanning of various organs or tissues among those introduced in the field of nuclear medicine. Recently, the use of some complexing agents with these radioisotopes has made a wide variety of scanning possible. However, knowledge of the complex formation of these metals is still insufficient for the development of new effective scanning agents. Some thiols, of which penicillamine is the most popular, have been used as the complexing agents with ^{99m}Tc. The complex formation together with hydrolysis reaction have been studied in connection with the preparation of the effective scanning agents which localize to some organs or tissues.[3] We planned a series of studies on the complex formation of In(III) with some complexing agents in an aqueous solution and also the reaction of In(III) with proteins for the better understanding on the mechanism of the localization of In(III) complexes *in vivo*. As the first step of this study, we adopted penicillamine as a complexing agent.

Although Habeeb and Tuck[4] have extensively investigated coordination compounds of In(III), most of which are prepared in organic solvent, studies on complex formation of In(III) in an aqueous solution are relatively few. Sarin and Munshi[5-7] reported that the order of the stability constants of β-substituted propionic acid–In(III) complexes decreases in the order $SH>OH>NH_2$ in various substituted groups. Their estimates for the stability constants are made on the assumption that three simple species, InL, InL_2 and InL_3 are present. On the other hand, Perrin and Sayce[8] detected the protonated penicillamine complexes with Zn(II) which has the isoelectronic configuration for d^{10}-In(III).

We have investigated the complex formation of In(III) with penicillamine with the use of potentiometric titration and absorption spectra. The presence of the protonated and hydrolyzed species is discussed.

Experimental

Materials. Indium metal (99.99%, Wako Pure Chemical Industries, Ltd.) was used. An accurately weighed amount of metal was dissolved in a small amount of hydrochloric acid by heating and diluted to 1 liter with deionized water after cooling. Solution of In(III) was freshly prepared and standardized with 8-quinolinol method.[9] Hydrochloric acid added to prevent hydrolysis of In(III) was standardized potentiometrically.

DL-Penicillamine (Sigma Chemical Company) was dried under vacuum over phosphoric oxide at room temperature. Stock solutions were stored under nitrogen, and checked by pH titration.

Carbonate-free potassium hydroxide solutions (1M and 0.1M) were prepared by an ion-exchange method,[10] and standardized by a conventional method. All other reagents used of commercial reagent grade.

Potentiometric Titrations. The potentiometric titrations were performed with a Radiometer titrator, Type TTT-2 equipped with a Radiometer G202B glass electrode and a K401 saturated calomel electrode. The pH readings with standard buffer solutions (0.05M potassium hydrogen phtalate, pH 4.00, and 0.05M sodium tetraborate, pH 9.22) were constant within 0.01 pH unit for periods exceeding 1 day. Titration was carried out with standard carbonate-free potassium hydroxide solutions with a specially designed, water-jacketed titration vessel at 21.00 ± 0.05°C in an air-conditioned room (21 ± 1°C), the solution being stirred continuously with a stirrer under pure nitrogen gas supply. In(III) (0—0.005 M), penicillamine (0.001—0.01 M) and potassium nitrate (0.1M) were placed in the vessel, and the total volume was adjusted to 20 ml by adding deionized water. All potentiometric measurements were repeated at least twice in order to obtain satisfactory reproducibility required for the computer-based analysis.

Absorption Spectra. Optical spectra were determined in an aqueous solution with a Shimadzu recording spectrophotometer, Model Double 40-R. Sample solutions consisted of penicillamine (0.00016M), or the ligand and In(III) (0.00008 M), and a very small amount of 1M potassium hydroxide and 1M hydrochloric acid was added for the adjustment of pH. The visible and ultraviolet spectra of the penicillamine solutions containing Fe(III), Cu(II), Pb(II), Hg(II), Cd(II), or Zn(II) were also measured.

Calculations

The proton dissociation constant(Ka_1) of the carboxyl-group in penicillamine was obtained by the usual method

from the potentiometric titration data for the sample solution containing 0.05 M penicillamine and an equimolar HCl. The remaining two ionization constants Ka_2 and Ka_3 for the amino- and thiol-groups are defined by the following equation which can be deduced from the total ligand concentration($[L]_T$) and electroneutrality.

$$[H^+]([L]_T-\gamma)Ka_2 + (2[L]_T-\gamma)Ka_2Ka_3 = \gamma[H^+]^2 \quad (1)$$

In this equation, γ is defined as

$$\gamma = [H^+] + [K^+] - [OH^-]$$

where $[K^+]$ is the concentration of base added. The Ka_2 and Ka_3 values were defined simultaneously by the linear least-squares method for Eq. (1) under the condition of $[L]_T$=0.0015 M. These three constants were further refined by the non-linear Fortran IV computer program on a FACOM 230-75 Computer, the Kyoto University Computer Center. The values of pKw= 14.130[11] at 21°C and $[H^+]=10^{-pH}$ were used in the calculations.

The titration curve of 1:2 In(III) to penicillamine (free base) indicates the formation of the complex InL_2. Formation constants (K_1 and K_2) are defined as follows.

$$K_1 = [InL^+]/[In^{3+}][L^{2-}] \quad (2)$$

$$K_2 = [InL_2^-]/[InL^+][L^{2-}] \quad (3)$$

The constants were calculated by the least-squares method of the Irving and Rossotti equation.[12,13]

$$\bar{n}(\bar{n}-1)[L^{2-}] = (2-\bar{n})[L^{2-}]K_1K_2(\bar{n}-1) - K_1 \quad (4)$$

The theoretical $\bar{n}$ values are evaluated by the constants obtained. From the constants, theoretical titres (x ml) are also expressed as follows.

$$Ax^3 + Bx^2 + Cx + D = 0 \quad (5)$$

where

$$A = a(K_1K_2Qa^2+K_1PQa+P^2Q)$$

$$B = 3K_1K_2a^2b+K_1PQa(Va+2b)-0.5K_1[L]_TP^2Va + P^2Q(2Va+b)-[L]_TP^3V$$

$$C = 3K_1K_2a^2b+K_1PQb(2Va+b)-0.5K_1[L]_TP^2V(Va+b) + P^2QV(Va+2b)-2[L]_TP^3V^2$$

$$D = K_1K_2Qb^3+K_1PQVb^2-0.5K_1[L]_TP^2V^2b+P^2QV^2b - [L]_TP^3V^3$$

$$a = [OH^-] - [H^+] - [K^+]$$

$$b = V(2[L]_T+[Cl^-]+[OH^-]-[H^+])$$

$$P = 3(H^+)^3/Ka_1Ka_2Ka_3+2(H^+)^2/Ka_2Ka_3+(H^+)/Ka_3$$

$$Q = (H^+)^3/Ka_1Ka_2Ka_3+(H^+)^2/Ka_2Ka_3+(H^+)/Ka_3+1$$

V = initial volume

$[L]_T$ = total ligand concentration

$[Cl^-]$ = concentration of inorganic acid (if any)

Equation (5) was solved by the use of subroutine CALDND, Scientific Subroutine Library, the Kyoto University Computer Center.

No simple procedures exist for obtaining the stability constants of metal complexes from $[L^{2-}]$ and $\bar{n}$ values, if polynuclear, protonated or hydroxo complexes are formed. In such cases, various computer programs based on the non-linear least-squares method have been used.[14-17] At each point of the titration, equilibrium takes place among the various species expressed by the general formula $M_lL_mH_{-n}$ (M=metal, L=ligand), where n is positive for a hydrolyzed species and negative for a protonated species. The concentration of the species $M_lL_mH_{-n}$ at each point is given by

$$[M_lL_mH_{-n}] = \beta_{lmn}[M]^l[L]^m(H^+)^{-n} \quad (6)$$

where β_{lmn} is the formation constant of the species under consideration.

For each point, the followoing mass-balance equations should hold.

$$[M]_T = [M] + \sum l\beta_{lmn}[M]^l[L]^m(H^+)^{-n} \quad (7)$$

$$[L]_T = [L] + \sum m\beta_{lmn}[M]^l[L]^m(H^+)^{-n} \quad (8)$$

$$[H]_T = [H^+] + \sum(-n)\beta_{lmn}[M]^l[L]^m(H^+)^{-n} \quad (9)$$

where $[M]_T$, $[L]_T$ and $[H]_T$, are the analytical concentrations of the metal, ligand, and acid, respectively. The unknown parameters in these equations are [M] and [L] for each point and the stability constants β_{lmn}. The values of (H^+) are obtained from the potentiometric measurement. In the present case, [M] and [L] are obtained by considering a set of plausible values for the stability constants and by calculating the starting values of [M] and [L] from Eqs. (7) and (8) for each point, using the Newton-Raphson iteration. Computed [M] and [L] for the first point are used as initial estimates for the second point, and so on. Using the obtained [M] and [L], the concentration of $M_lL_mH_{-n}$ is calculated from Eq. (6), and $[H]_T$ from (9) for each data point. Theoretical titre of base is obtained from $[H]_T$. If the agreement between the experimental and calculated titres is not satisfactory, the values of the constants β_{lmn} are varied in turn so as to minimize the error-square sum in titre. The standard deviations of the parameters are the square-roots of the diagonal elements of the variance-covariance matrix of the parameters.

This program was also used for the calculation of pKa and hydrolysis constants of the metal ion.

Results and Discussion

Practical Association Constants. Table 1 shows the result of the calculation for "practical' association constants defined by the following equations.

$$\beta_{01-1} = [LH^-]/(H^+)[L^{2-}]$$

$$\beta_{01-2} = [LH_2]/(H^+)^2[L^{2-}]$$

$$\beta_{01-3} = [LH_3^+]/(H^+)^3[L^{2-}]$$

The constants were refined with non-linear program. The calculated $\log\beta_{01-1}$ and $\log\beta_{01-2}$ are in good agreement with that ($\log\beta_{01-1}$=10.679 and $\log\beta_{01-2}$=18.714) reported by Perrin and Sayce.[8] The value (pKa_1=

Table 1. Ionization constants of DL-penicillamine at 21°C and I=0.1 (KNO_3)[a]

LH	Log β_{01-1}=10.669±0.004
LH_2	Log β_{01-2}=18.774±0.006
LH_3	Log β_{01-3}=20.839±0.005[b]

a) Uncertainty intervals associated with all the values are the standard deviations. b) Obtained from 0.05 M solutions, others from 0.0015 M solutions.

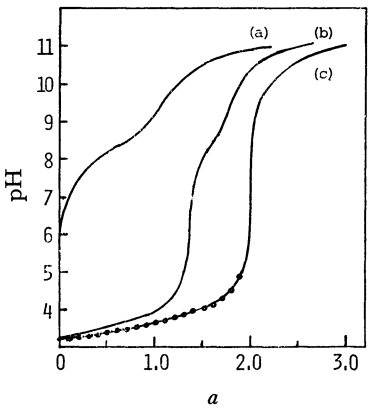

Fig. 1. Titration curves of the penicillamine (free base)–In(III) complexes: (a) ligand alone; (b) L: In=3: 1; (c) L: In=2: 1. Total ligand concentrations are 0.0015, 0.0015, and 0.001 M, respectively. Each point on the curve (c) was calculated by the use of the formation constants. a=moles of KOH added per mole of protonated ligand with regard to the thiol- and amino-groups.

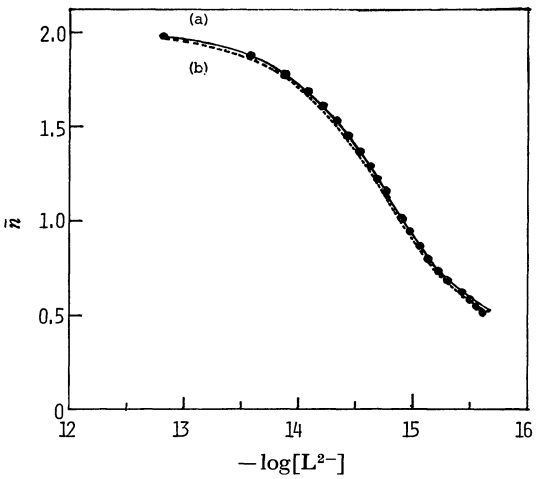

Fig. 2. Formation curves of the penicillamine (free base)–In(III) complexes: (a) L: In=2: 1, (b) L: In=3: 1. Total ligand concentrations are 0.001 and 0.0015 M respectively. Each point represents the calculated value as for curve (a).

2.065) obtained from $\log\beta_{01-3}$ and $\log\beta_{01-2}$ differs slightly from that (pKa_1=2.44) reported by Kuchinskas and Rosen.[18] The difference is due to the low concentration (0.0033 M) of the latter case.

Simple InL and InL_2 System. Figure 1 shows the titration curves of 2: 1 and 3: 1 penicillamine (free base) to In(III) (0.0005 M). The sharp inflections of pH were observed at a=1.33 and 2 in the 3: 1 and 2: 1 systems, respectively. The two formation curves could be superimposed (Fig. 2). It was impossible even under the condition of 3: 1 (ligand to metal) to obtain $\bar{n}$ values greater than 2. The findings indicate that in our experimental conditions the mononuclear species, InL and InL_2, are present predominantly. In the titration curve of the 3: 1 system, therefore, the inflections at a=1.33 and 1.67 are associated with the formation of the complex InL_2 and the dissociation of the thiol-group of the excess ligand, respectively.

The constants ($\log K_1$=15.44 and $\log K_1K_2$=29.85) were obtained from Eq. (4) using $\bar{n}$ and $[L^{2-}]$ values at each point. With use of these constants, theoretical $\bar{n}$ values were also calculated by Eq. (4). Validity of these constants was confirmed by the coincidence with the experimental and calculated formation curves (Fig. 2, (a)). From the K_1 and K_1K_2 values, the titration curve (Fig. 1, (c)) was recalculated by the use of Eq. (5) and compared with that obtained experimentally.

These constants determined by the Irving Rossotti method were further refined by the non-linear computer program. The final values ($\log\beta_{110}$=15.425±0.010 and $\log\beta_{210}$=29.844±0.006) fitted the data to within a standard deviation in titre of 0.0015 ml, as compared with the value (0.0021 ml) obtained in fitting the simple pKa titration data. From these constants, a distribution diagram of various complexes was calculated for various pH (Fig. 3).

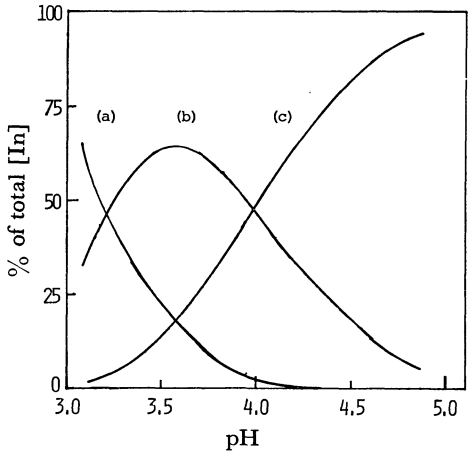

Fig. 3. Distribution diagram for the penicillamine (free base) (0.001M)–In(III)(0.0005M) system: (a) In^{3+}; (b) InL; (c) InL_2.

Penicillamine as a terdentate ligand has been predicted to form metal complexes with various coordination types. In dilute penicillamine(free base)–In(III) systems, however, the simple species InL and InL_2 only were formed. The constant ($\log\beta_{110}$=15.425) for the species InL is much larger than those for the corresponding penicillamine 1: 1 complex of Zn(II) (9.589), Ni(II) (10.749),[8] Cd(II) (10.92) and Pb(II) (12.88),[19] respectively. It is of interest that the formation constant is comparable to that (16.4) of the Hg(II) complex.[19]

InL, InL_2, InLH, InL(LH), and InL(OH) Systems. Titration was extensively carried out in the systems of various metal concentrations and metal-ligand ratios. An equimolar HCl was added to the penicillamine solution in order to protonate the carboxyl-group in the ligand. The total metal concentrations were 0.00025, 0.0005, 0.0025, and 0.005 M, and the total ligand concentrations were 0.001, 0.001, 0.01, and 0.01 M, respectively. Plots of $\bar{n}$ against $\log[L^{2-}]$ are shown in Fig. 4. Failure of superposition in the formation curve indicates that besides InL and InL_2, other complex species are formed with an increase in metal concentra-

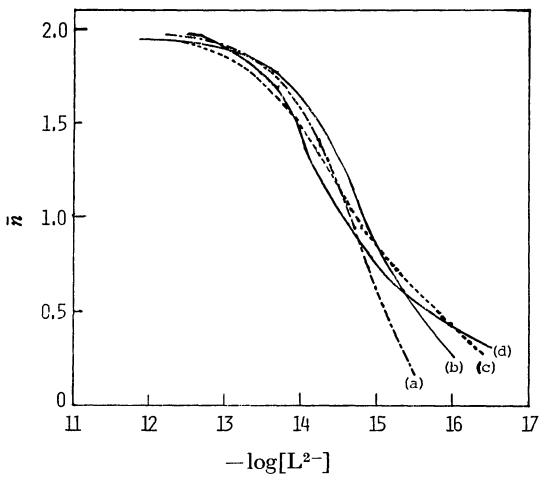

Fig. 4. Formation curves of the penicillamine (HCl)–In(III) complexes: (a) L: In=4: 1; (b) L: In=2: 1; (c) L: In=4: 1; (d) L: In=2: 1. Total ligand concentrations are 0.001, 0.001, 0.01, and 0.01M, respectively.

tion. However, the curves do not converge towards Biedermann and Sillén's "mononuclear wall."[20] Accordingly, the systems could contain no "core plus links" series of complexes.[21] Formation of mononuclear protonated and/or hydroxo species was assumed.

In order to characterize the hydrolyzed In(III) species which give the hydroxo complexes to associate with ligand, a brief inspection on the hydrolysis equilibrium of In(III) ion was carried out. Potentiometric titration of 0.0005 M In(III) solution containing 0.00026 M HCl at 21°C and I=0.1(KNO_3), afforded $\log \beta_{101} = -3.634 \pm 0.037$ after refinement by the non-linear program.

$$In^{3+} \rightleftarrows In(OH)^{2+} + H^+$$

$$[In(OH)^{2+}] = [InH^{2+}_{-1}] = \beta_{101}[In^{3+}](H^+)^{-1}$$

(β_{101} is not precisely the formation constant for the complex In(OH), but is related to it through $Kw = (H^+)(OH^-)$ since $\beta'[In^{3+}](OH^-) = \beta'[In^{3+}](H^+)^{-1}Kw$, *i.e.*, $\beta_{101} = \beta' \times Kw$, where β' is the true formation constant for the complex In(OH).) Besides a large amount of In(OH), a small amount of $In(OH)_2$ was also formed. Biedermann[22] reported $\log \beta_{202} = -5.21$ and $\log \beta_{304} = -9.90$ for 0.05 M In(III) perchlorate at 25° in 3 M $NaClO_4$. However, no such complexes were detected in our dilute system which is close to the "metal ion wall." The species In(OH) and $In(OH)_2$ were used as the hydrolyzed In(III) species which participate in the hydroxo complex formation with penicillamine.

On the other hand, the protonated mononuclear species to be considered are InL, InL_2, InLH, InL(LH), and $In(LH)_2$. The best fit to the experimental results was sought by the use of a computer program, with automatic refinement of estimated stability constants. The initial value of unknown constant for the complex InLH was estimated as below. Each equilibrium constant for the next three reaction were defined as follows.

$$In^{3+} + L^{2-} + H^+ \rightleftarrows InLH^{2+}; \quad \beta_{11-1}$$

$$In^{3+} + LH^- \rightleftarrows InLH^{2+}; \quad K^{In}_{InLH}$$

$$L^{2-} + H^+ \rightleftarrows LH^-; \quad K^{H}_{LH}$$

where β_{11-1} was given by

$$\beta_{11-1} = [InLH^{2+}]/[In^{3+}][L^{2-}](H^+)$$
$$= [InLH^{2+}]K^{H}_{LH}/[In^{3+}][LH^-]$$
$$= K^{In}_{InLH}K^{H}_{LH}$$

According to Sarin and Munshi,[5] the constant ($\log K_1$) for the In(III)–β-aminopropionic acid complex ($\widehat{NO}$ coordination type) was 2.72, which is much smaller than that of the In(III)–penicillamine complex. This suggests that the thiol-group coordinates to the metal in the protonated complex(InLH). The assignment of pKa value (10.67) to the dissociation of the protonated amino-group is supported by absorption spectral measurements. The constants ($\log K_1$) for the In(III) complexes of *N*-acetyl-*L*-cysteine ($\widehat{SO}$ coordination type) and 2-aminoethanethiol ($\widehat{SN}$ type) are 10.24 and 13.96, respectively. If the proton in InLH attaches to the carboxyl- or amino-group, $\log \beta_{11-1}$ values are obtained as follows.

$$\log \beta_{11-1} = 13.96 + 2.07 = 16.03$$

$$\log \beta_{11-1} = 10.24 + 10.67 = 20.91$$

The two values were refined by the program in the system of 0.005 M In(III) and 0.01 M penicillamine (HCl), together with the constants for the complexes

Table 2. Values (logarithmic units) of the formation constants in the successive trials for the In(III) (0.005 M)–penicillamine(HCl) (0.01 M) system[a]

Trial	Complex species						Std. dev. in titre (ml)
	InL	InL_2	InLH	InL(LH)	$In(LH)_2$	InL(OH)	
1	15.803 (0.016)	29.610 (0.038)					0.0168
2	15.778 (0.008)	29.860 (0.011)	18.662 (0.014)				0.0022
3	15.728 (0.008)	29.761 (0.012)	18.718 (0.012)			11.752 (0.045)	0.0015
4	15.688 (0.016)	29.865 (0.007)	18.754 (0.015)	33.000 (0.065)			0.0015
5		29.885 (0.010)	18.796 (0.020)	33.604 (0.017)	36.61 (1.03)		0.0018
6	15.696 (0.014)	29.804 (0.017)	18.747 (0.014)	32.79 (0.14)		11.47 (0.13)	0.0014

a) Standard deviations in parentheses.

InL and InL_2. In each case, the final value of log β_{11-1} converged to 18.662, *viz.*, the intermediate between two initial estimates (Table 2, trial 2). Introduction of this complex (InLH) gave a remarkable improvement as compared with trial 1. For trial 1 inclusion of the hydroxo complex (InL(OH)) caused no improvement, but for trial 2 it gave a better fit to the experimental data (trial 3). However, the inclusion of $InL(OH)_2$ did not significantly improve the fit. Trials 4—6 gave acceptable fits of a series of a trial and error approach. Trial 5 indicates that the complex $In(LH)_2$ is negligible. Trials 3 and 4 gave the equal standard deviations in titre. In trial 6, the standard deviation in titre is slightly improved, neverthless the standard deviations of the constants of InL(LH) and InL(OH) are larger. This result may be due to the fact that a small amount of the two species are formed in similar pH regions. It is difficult to make a selection from trials 3, 4, and 6 by pH titration. Figure 5 shows the distribution diagram of the complexes obtained from trial 6.

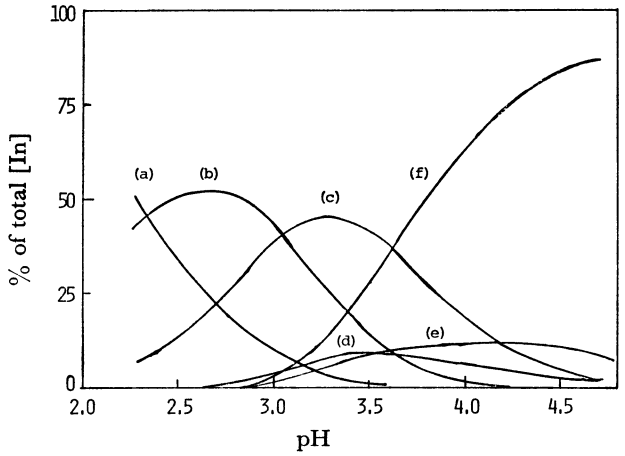

Fig. 5. Distribution diagram for the penicillamine(HCl) (0.01M)–In(III)(0.005M) system, as for Table 2, trial 6: (a) In^{3+}; (b) InLH; (c) InL; (d) InL(LH); (e) InL(OH); (f) InL_2.

The sulfur-bridged polymers are formed in aqueous solutions containing ligands of the types RS^- and the first transition metals.[8,23,24] However, introduction of various polynuclear or olated species caused no improvement in the results. Probably, the methyl groups in penicillamine prevent the formation of sulfur-bridging species.

The same investigation on the other three formation curves suggests that the presence of InL(LH) and InL(OH) becomes negligible with the decrease in concentration of the metal. A comparison with Figs. 3 and 5 also suggests that the species (InLH) becomes negligible, unless the system is acidified by addition of HCl in the lower metal concentration.

A final refinement of all these constants over the whole range of the experimental data (4 experiments, 166 data points) yielded the constants listed in Table 3. The calculated and experimental titres coincided within a standard deviation of 0.0087 ml. Although this value seems to be somewhat large, it is acceptable when the appreciable change in concentration of the complex

Table 3. Stability constants of In(III) complexes with DL-penicillamine at 21°C and I=0.1 (KNO_3)[a]

InL	log β_{110} =15.330±0.058
InL_2	log β_{120} =29.789±0.038
InLH	log β_{11-1}=18.858±0.043
InL(LH)	log β_{12-1}=33.391±0.079
InL(OH)	log β_{111} =11.25 ±0.11

a) Uncertainty intervals associated with all the values are the standard deviations.

species present in the various systems is taken into account.

Calculation of the constant for the protonated complex InLH yielded the intermediate value between the two initial estimates for the species of the $\widehat{SO}$ or $\widehat{SN}$ type. Figure 5 shows, however, that the two protonated species InLH and InL(LH) exhibit maxima at pH 2.7 and 3.3, respectively, where the carboxyl-group in penicillamine is fully ionized. Therefore, these protons are assumed to attach on the amino-group in the ligand. When the value of pKa_3=10.669 is assigned to the ionization constant of the amino-group, the constant K^{In}_{InLH} in the reaction ($In^{3+}+LH^- \rightleftarrows InLH^{2+}$) is calculated by the following equations using the refined constant log β_{11-1}=18.858.

$$K^{In}_{InLH} = \beta_{11-1}/Ka = 10^{18.858}/10^{10.669}$$

$$\log K^{In}_{InLH} = 8.189$$

The value is smaller than that (log β_{110}=10.242) of *N*-acetyl-L-cysteine complex with In(III). Presumably, the difference is attributed to the electrostatic effect, that is, the reaction species with In^{3+} is LH^- in penicillamine but L^{2-} in *N*-acetyl-L-cysteine. In the solutions containing 0.0015 M ligands, the 1: 1 (ligand to metal) complexes reach their maxima at pH 3.5 and 5.0 in *N*-acetyl-L-cysteine and 2-aminoethanethiol, respectively. This indicates that the coordination of the amino-group occurs at a relatively high pH.

Ultraviolet Absorption Spectra. The absorption of penicillamine in the 228—236 nm region is based on the form of RS^- ion. The absorbances increased rapidly in the pH range 7.5—8.5, and slightly in 10.5—11.5. Hence, we can assign the ionization constant pKa_2=

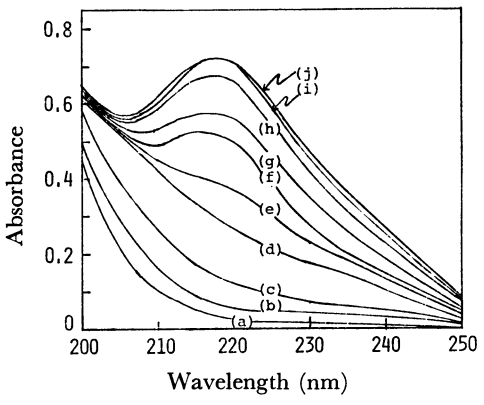

Fig. 6. Absorption spectra of the penicillamine (0.00016 M)–In(III)(0.00008M) system at various pH's: (a) pH 2.04; (b) pH 2.54; (c) pH 3.06; (d) pH 3.78; (e) pH 3.97; (f) pH 4.19; (g) pH 4.40; (h) pH 4.90; (i) pH 6.52; (j) pH 8.64.

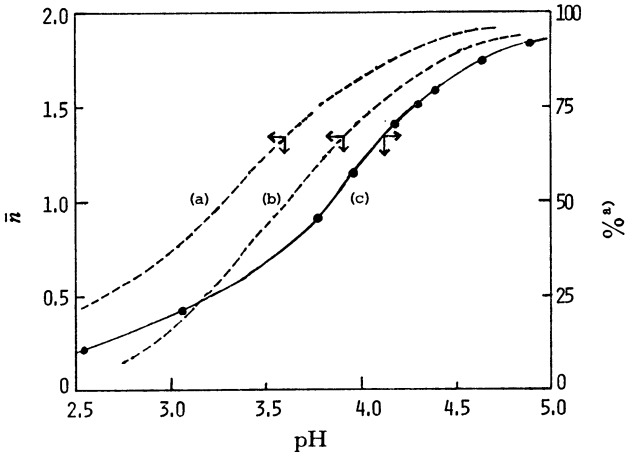

Fig. 7. Absorbance (solid line) and $\bar{n}$ values (dashed lines) of the 2:1 (penicillamine to In(III)) solutions: Total metal concentrations are 0.005M (a), 0.0005M (b), and 0.00008M (c), respectively.
a) Absorbance at maximum is taken to be 100%.

8.105 to the thiol-group approximately, although the acid strength of the amino- and thiol-groups cannot be easily defined. According to Benesch and Benesch[25] the red shift with increasing pH is related to the change in the ratio of the forms of S^-RNH_2 and $S^-RNH_3^+$. Penicillamine gives a larger shift than cysteine, their thiol- and amino-groups microscopic constants pK_S and pK_N being 8.45 and 8.58, respectively.[26]

The absorption spectrum of the In(III) complex with penicillamine exhibits maximum at 217.5 nm ($\varepsilon = 4\times10^3$), which differs a great deal from that of penicillamine only. On the other hand, In(III) ion(d^{10}) shows no absorption. The absorption spectra of the solution containing 0.00008 M In(III) and 0.00016 M penicillamine are shown in Fig. 6. The absorption curvature at 215 nm appears at pH≈4, reaches its maximum at pH≈6 and is accompanied by a red shift of 2.5 nm, when pH increases. Figure 7 shows plots of the absorbance against pH. In this case, the absorbance at maximum is regarded as 100%. In the lower pH range (having no curvature), the absorbances at 215 nm are plotted. Figure 7 also contains plots of $\bar{n}$ against pH obtained from the pH titration data in the 0.01 M and 0.001 M penicillamine solutions containing 0.005 M and 0.0005 M In(III), respectively. Curves are parallel with a spacing, $\Delta\log[In]_T$, proportional to ΔpH. However, the relation does not hold below pH 3.5, the absorption and formation curves intersecting each other.

The In(III) complex with $\widehat{SO}$ type ligand has λ_{max} at ≈200 nm, and exhibits the tail in the range of ≈215 nm. The absorption spectra in the pH range 2—3.5 are similar to those of the corresponding $\widehat{SO}$ type complex (Fig. 6). These findings also support the formation of the protonated complex InLH ($\widehat{SO}$ type), which has λ_{max} at <200 nm and ε differing from that of the $\widehat{SNO}$ type complex InL. The absorption of InLH appears as a tail at 215 nm, and is considered to be superimposed upon that of InL. Crossing between the absorption and formation curves occurs below pH 3.5, where the species InL is absent.

The shift from 215 to 217.5 nm seems to be caused by the reaction $InL(LH)\rightarrow InL_2$, which leads to the change in the ratio of the forms of $S^-RNH_3^+$ and S^-RNH_2. Under the condition of $[In]_T=0.00008$ M, however, no formation of the species InL(LH) was confirmed from the pH titration data. The shift is, therefore, due to the reaction $InL\rightarrow InL_2$.

Precipitation of the meta hydroxide appears at pH 3.1 in 0.05 M metal ion solution, because of high hydrolyzability of In(III). The In(III) complexes with the usual amino-acids are unstable, whereas the In(III) complexes containing the thiol-group are very stable. The high stability might be attributed to the $d\pi$ back-donation of d^{10} electrons to the empty d orbitals of sulfur. This π-bonding character is reflected in the ultraviolet absorption. The charge transfer bands (λ_{max}) of the various metal-sulfide complexes[27] are correlated to the absorption maxima of the correspond-

Table 4. Charge transfer bands (λ_{max}) of sulfides- and penicillamine–metals complexes

Metal	Sulfide[a] (nm)	Penicillamine (nm)
Fe(III)	>500	570
Cu(II)	>375	520
Pb(II)		268
Hg(II)	300	260
Cd(II)	~250	217
Zn(II)	<200	217
In(III)		217.5

a) Ref. 27.

ing penicillamine–metal complexes (Table 4). The absorption at 217.5 nm of the penicillamine–In(III) complex is assigned to the charge transfer band. The band of Zn(II) complex, having an isoelectronic configuration for In(III), is at <200 nm for the sulfides, and at 217 nm for penicillamine. This is related closely to the fact that the corresponding band of In(III) is at <200 nm for β-mercaptopropionic acid, at ≈217 nm for penicillamine and 2-aminoethanethlol. Thus it seems that the absorptions at ≈217 nm in the penicillamine–In(III) and –Zn(II) result from M–S and M–N coordinations.

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