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Ni(II)와 *D*-Penicillamine 과의 착물형성반응에 대한 속도론적 및 평형에 관한 연구

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Kinetic and Equilibrium Studies on Complex Formation Between Ni(II) and *D*-Penicillamine in Aqueous Media

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요 약. Ni^{2+}와 *D*-penicillamine 사이의 착물형성반응에 대한 반응속도 및 평형에 관한 조사를 수용액중에서 실시하였다. 속도론적 실험은 압력-급변법을 사용하여 pH=8～9범위에서 실시하였다. *D*-Penicillamine은 질소와 유황원자를 주게로 하여 pH>9.2조건에서 Ni^{2+}이온에 배위하나 pH값 8.25～9.07범위에서 총괄 안정도 상수값이 급격히 감소하며 비해리된 mercapto기가 결합에 참여하지 않는 것으로 밝혀졌다. 또한 이 착물 형성반응에 있어 율속단계는 Ni^{2+}이온의 내부 배위권으로부터 물분자가 유리되는 과정임이 밝혀졌다.

ABSTRACT. Rates and equilibrium of complex formation between Ni^{2+} and *D*-penicillamine have been investigated in aqueous solutions. Kinetic study on the complex formation were perrmed in the pH range of 8～9 by the use of pressure-jump technique. *D*-Penicillamine coordinates to the nickel(II) ion utilizing sulfur and nitrogen as donor atoms in the high pH condition (pH 9.2). However, in the pH range of 8.25～9.07, the stepwise stability constant becomes drastically reduced and the undissociated mercapto group does not participate in bonding. The rate-determining step of the complexation reaction is found to be the release of a water molecule from the inner-coordination sphere of Ni^{2+} ion.

INTRODUCTION.

The chemistry of penicillamine as an oral therapautic agent in Wilson's disease[1] has focused attention on the metal bonding ability of this simple amino acid. Penicillamine has also been shown effective in certain metal toxicities[2] in verture of its exceptional ability to bind with metal ions.

Stability constants of some metal ions with DL-penicillamine has been reported.[3,4] Lenz and Martell[4] suggested that structure of chelate for nickel(II) ion with penicillamine is square planar where mercaptide and amino groups are coordinated to nickel(II) ion.

Because of the importance of *D*-penicillamine as a medicinal chelating agent, the hitherto-reported chelate-stability constants have been redetermined at various temperature and at ionic strength of $\mu \rightarrow 0$, and kinetic investigation of the complex formation process was carried out by the use of pressure-jump method.[5,6] When

the nickel(II) complex of the bidentate ligand is formed, two inner-coordinated waters should be replaced by one bidentate ligand such as *D*-penicillamine. Therefore, the mechanism of the bidentated chelate complex formation through the outer-sphere complex and the monodentated complex as an intermidiate has usually been proposed. If the complex is stable as a chelate, the rate-determining step might be postulated from one of the two possible mechanism.

According to the first mechanism[7~9], the rate dermining step is supposed to be the formation of the first bond between the metal ion and the ligand. However, in the second mechanism,[10,11] the chelate ring closure is assumed as the rate-determining step.

This study was undertaken primarily in an attempt to confirm if one of these two mechanism can be applied to the complex formation reaction between the nickel(II) ion and *D*-penicillamine.

EXPERIMENTAL

Chemicals. *D*-Penicillamine(3, 3-dimethyl-*D*-cysteine) was purchased from Fluka Chemical Co., and was used without further purification. Reagent grade nickel sulfate was obtained from Mallinckrodt Chemical Works, and was purified by recrystallization. A solution of nickel sulfate was standardized by titration with standard Na_2 EDTA reagent with murexide as indicator in ammonical solution[12]

Other chemicals used were reagent grade or first grade and were further purified by recrystallization. All aqueous solutions were prepared from deionized water.

Potentiometric Titrations. Titrations were carried out in a 100m*l* jacked-titration cell which was fitted with a magnetic stirrer. Fifty militers of a solution containing *D*-penicillamine and nickel(II) ion was titrated with 0.01N NaOH from a 5-m*l* microburet; pH measurements were made with a Metrohm 632 pH-meter equipped with an EA-120 combination electrode. Standard buffer solutions with pH's 4, 7 or 9 were used to standardize the instrument before titration and check the standardization after the titration. Nitrogen gas was bubbled through the solution during the entire titration procedure to avoid air oxidation of the mercapto group of the ligand. For the determination of formation constants of the chelate complex, the titration was employed at the condition of various molar ratio of metal to ligand. The titration was carried out in the temperature range of 15° to 35°C and ionic strength of $\mu \rightarrow 0$.

Kinetic Measurements. The solution of nickel(II)/penicillamine(molar ratio of 1 : 0.7 or 1 : 0.8) was titrated with a solution of $Ba(OH)_2$ until all the sulfate ion is precipitated as $BaSO_4$. The solid $BaSO_4$ was removed by filteration. The mixture solution was prepared immediately before kinetic runs. The pH values of the solution were in the range of 8.25~9.07, where most of the ligand exists as the monodissociated form. The concentration of the nickel-penicillamine was determined by Hitachi 124 spectrophotometer at 265nm. Pressure of approximately 30 atms was applied onthe cell and phosphorous-bronze diaphram(Poongsan Metal Co., thickness =0.2mm) was burst by stainless-steel plunger. The pressure of the two cells, one containing the sample solution and the other containing the reference KCl solution, decreases instantaneously from 30 atms to atmospheric pressure. The concentration change of the species was followed by means of the electric conductivity method. Time constant of the apparatus was calibrated with a 0.2M $NiSO_4$ solution which exhibits a relaxation effect faster than 37 μsec. From the obtained oscillogram(see *Fig.* 1-a), it was found that this pressure-jump apparatus is good for kinetic studies of reactions with relaxation time

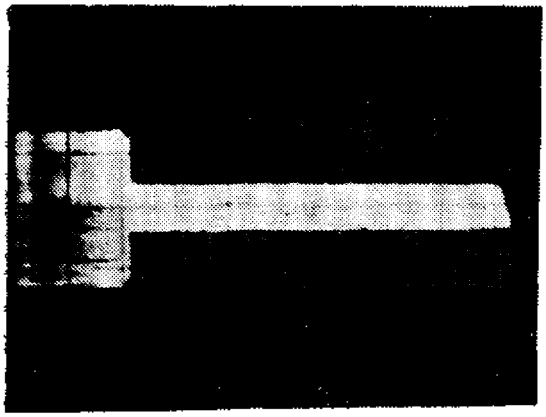


Fig. 1-a. Pressure-jump oscillogram. Temp. =25°C, $NiSO_4$=0.2M. The rise time of the apparatus 37 μsec.

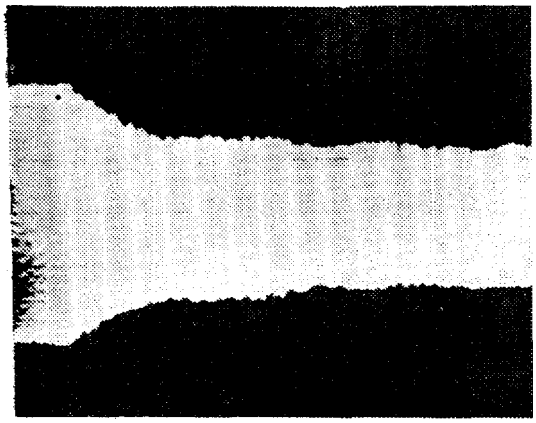


Fig. 1-b. Experimental relaxation curve of nicke(II)-penicillamine at 30°C. $C_{Ni^{2+}}=3.27\times10^{-3}$M. $C_{Pen}=2.27\times10^{-3}$M. Relaxation time=550$\mu$sec.

shorter than 100μsec. The temperature of the sample cell was controlled upto ±0.1°C by circulating water from the thermostat through a teflon tube around the cells. The temperature was checked by Shimadzu type TH-150 electro-thermometer just after the kinetic measurements.

In all nickel(II)-penicillamine solutions, the relaxation oscillograms were characterized by a single relaxation time. The relaxation time $\tau_{1/e}$ was evaluated directly from the oscillogram. The typical relaxation curve of the nickel(II)-penicillamine obtained is shown in *Fig.* 1-b.

RESULTS AND DISCUSSION

Chelate-Stability Constants. The potentiometric titration was carried out by the use of Bjerrum's method.[13] Dissociation constant of ligand and stability constants of complex were calculated by the method of Irving and Rossotti[14] using an Apple II personal computer.

Fig. 2 shows titration curves obtained for *D*-penicillamine in the absence and presence of nickel(II) ion at 25°C and $\mu\rightarrow0$. The $-\log K^H$ values were determined for *D*-penicillamine at various temperature and $\mu\rightarrow0$ and listed in *Table* 1. The measured dissociation constants are different slightly from the reported values.[4,5] So, the effect of ionic strength on dissociation constant was investigated for this system. *Table* 2 gives the values of $-\log K^H$ obtained for the *D*-penicillamine at various ionic strengths and at 25°C. The dissociation constans of *D*-penicillamine determined in this work were used throughout the calculations of the stability constants of metal complexes as these values were determined at $\mu\rightarrow0$.

In the titration curve(see *Fig.* 2), two inflections at b=1 and b=2 are observed. This clearly indicates that two moles of proton are displaced per mole of *D*-penicillamine. Thus a 1 : 2 metal to ligand chelate is formed. The

Table 1. Dissociation constants of *D*-penicillamine

Temperature (°C)	$-\log K_2^H(-NH_3^+)$	$-\log K_3^H(-SH)$
15	7.88	10.17
20	7.80	10.05
25	7.70	9.87
	7.88[a]	10.43[a]
	7.97[b]	10.46[b]
30	7.65	9.75
35	7.48	9.67

[a]μ=0.10(KNO_3) for *DL*-penicillamine. [b]μ=0.15 (KNO_3) for *DL*-penicillamine.

Table 2. Ionic strength dependence of dissociation constants of *D*-Penicillamine at 25°C

$\mu(NaClO_4)$	$-\log K_2^H(-NH_3^+)$	$-\log K_3^H(-SH)$
0.10	7.85	10.22
0.08	7.80	10.12
0.05	7.77	10.05
0.03	7.75	9.95
0.01	7.72	9.91

Table 3. Formation constants of nickel(II)-penicillamine complex at various temperature and $\mu \rightarrow 0$

Temperature (°C)	Formation Constant		
	log K_1	log K_2	log K_1K_2
15	10.93	7.03	17.96
20	10.68	6.97	17.64
25	10.45	6.92	17.37
30	10.23	6.87	17.10
35	10.03	6.82	16.85

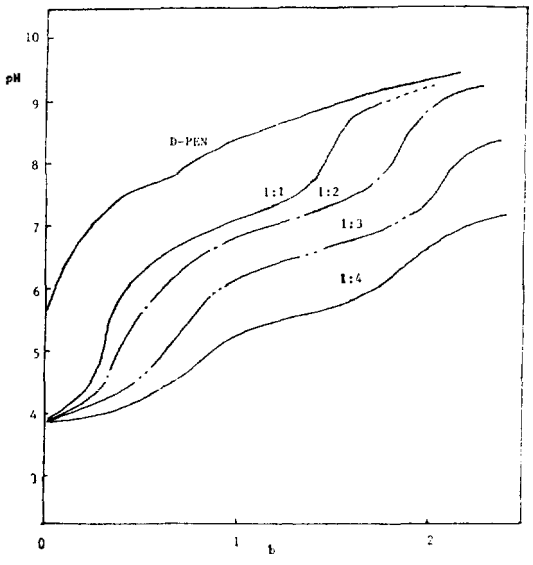
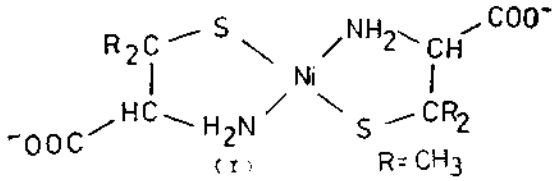


Fig. 2. Titration of *D*-penicillamine with NaOH in the absence and presence of Ni^{2+} ion at 25°C and $\mu \rightarrow 0$. (b represents the number of equivalets of base added.

values of the formation constants are listed in *Table* 3, where K_1K_2 are the over-all formation constants.

Albert[15] and Li *et. al.*[16] have studied the chelates formed between various bivalent metal ions and cysteine. It has been suggested that cysteine binds to the metal ion by utilizing both sulfur and amino nitrogen atoms. The formation constants obtained for *D*-penicillamine complex with Ni(II) ion are comparable in its magnitude to those obtained for cysteine complex with nickel(II) ion. This might suggest a similar coordination pattern form cysteine and penicillamine. Furthermore, the formation constants obtained for alanine and valine are considerably lower than that of penicillamine. Therefore, it seems plausible to suggest that penicillamine entail a combination of the metal between the sulfur and amino nitrogen atoms, while the corboxylic group remaines free. Lenz and Matell also suggested that this chelate structure in the for mation of square planar complex involving the mercaptide and amino group as illustrated by (I).



(I)

Thermodynamic parameters of the nickel (II) -penicillamine complex formation were also determined from the temperature dependency of stability constants. The following thermodynamic parameters were obtained at $\mu \rightarrow 0$; $\Delta H^\circ = -93.8$KJ/mole, $\Delta S^\circ = 5.3$KJ/mol·K and $-\Delta G^\circ = 99.1$KJ/mole.

Kinetics. The experimental conditions and the relaxation times measured are summarized in *Table* 4. In the present study, the complex formation reaction may be expressed as follow:

$$Ni^{2+} + HL^- = NiHL^+ \qquad (1)$$

where HL^- is the mono-dissociated form of *D*-

Table 4. Relaxation times and experimental conditions for the complex formation of nickel(II) ion and *D*-penicillamine (at30°C)

$C_o^M(10^{-3})$	$C_o^M(10^{-3})$	$C_{MHL}(10^{-3})$	$C_M(10^{-4})$	$C_L(10^{-4})$	$\mu(10^{-3})$	$f_\pm$	$1/\tau(10^3s^{-1})$
2.00	1.39	1.26	7.43	1.31	1.55	0.841	1.47
2.21	1.58	1.44	7.79	1.49	0.63	0.837	0.52
2.66	2.07	0.87	7.85	1.94	1.67	0.837	0.59
2.61	1.81	1.64	9.68	1.70	2.02	0.822	1.64
3.32	2.58	2.34	9.81	2.43	2.08	0.820	1.67
3.27	2.27	2.05	12.13	2.13	2.53	0.804	1.82
4.00	2.86	2.59	14.08	2.69	2.95	0.791	1.92

Table 5. The formation and dissociation constants of nickl(II)-penicillamine complex ($\mu\rightarrow0$)

Temperature(°C)	$k_f(M^{-1}s^{-1})$	$k_r(s^{-1})$	$K(M^{-1})$
15	5.26×10^5	4.29×10^2	1.23×10^3
20	6.38×10^5	5.50×10^2	1.16×10^3
25	8.21×10^5	7.22×10^2	1.14×10^3
30	9.88×10^5	8.86×10^2	1.12×10^3
35	11.44×10^5	10.33×10^2	1.11×10^3

penicillamine and $NiHL^+$ is the 1 : 1 nickel–*D*-penicillamine complex. The rate constants are related to the relaxation time by the equation.

$$1/\tau=k_f f_\pm^2(C_M+C_L)+k_r \qquad (2)$$

where k_f and k_r are the rate constants of the complex formation and the disociation at $\mu=0$, respectively and C_M and C_L are the concentration of Ni^{2+} and HL^-, and $f_\pm$ is the mean activity coefficienty of the free ions. The $1/\tau$ values were plotted against $f_\pm^2(C_M+C_L)$ to give k_f and k_r from the slope and the intercept of the line. Knowledges of the stability constant, K, are required in order to calculate the concentraction of the free ions. However, an appropriate literature value was not available, and so the values of k_f, k_r and K were calculated from the kinetic data.

As an approximation, a value of K, which was roughly estimated from potentiometric titration data, was used to calculate the concentration of ionic species. The C_M and C_L were determined spectrophotometrically and were also used in calculation. The activity coefficients of the ions were calculated by the use of Davis equation.[17]

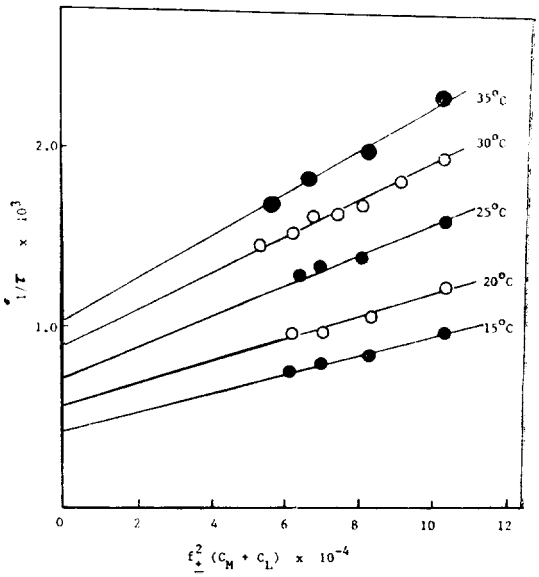


Fig. 3. The plots of $1/\tau$ against $f_\pm^2(C_M+C_L)$.

The calculations were repeated until a constant K value was obtained. The final results are

Table 6. Kinetic data for the complex formation of nickel (II)-ion and *D*-penicillamine at 25°C ($\mu \rightarrow 0$)

$k_f(M^{-1}sec^{-1})$	8.21×10^5
$k_r(sec^{-1})$	7.22×10^2
E_f^*(kcal mole^{-1})	1.78
ΔH_f^*(kcal mole^{-1})	1.18
ΔS_f^*(cal degK^{-1}mole^{-1})	1.29
ΔG_f^*(kcal mole^{-1})	1.56
$K_0(M^{-1})$	5.5
$k_1(sec^{-1})$	1.5×10^{-5}

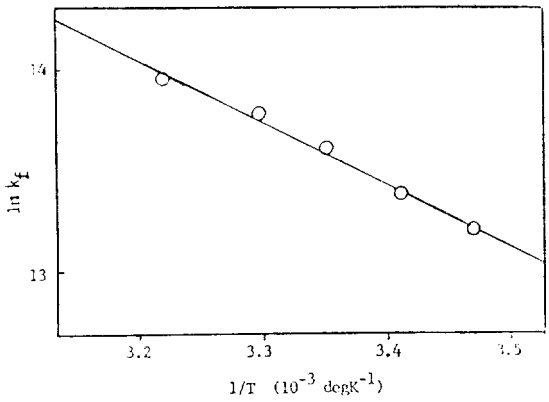


Fig. 4. Temperature dependence of K_f.

shown in *Fig.* 3. The rate constants, k_f, and k_r, and the the equlibrium constants at each temperature were obained from *Fig.* 3 The rate and equilibrium constants at various temperature are summarized in *Table* 5. Kinetic parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were determined from the temperature dependencies of rate constants and are listed in *Table* 6.

Previous investigations of complexation reaction of first row transiton metal ion suggested that the substitution requires at least two steps. The mechanism for complex formation reactions between metal ion and chelate ligand were represented by following equations;

$$M_{(aq)}+A-B_{(aq)} \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} w_2Mw_1A-B, \quad (\text{II})$$

$$K_0=\frac{k_0}{k_{-0}} \qquad (3)$$

$$w_2Mw_1A-B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} w_2M-A-B+H_2O, \quad (\text{III})$$

$$K_1=\frac{k_1}{k_{-1}} \qquad (4)$$

$$w_2M-A-B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} M\begin{matrix}A\\B\end{matrix}+H_2O, \quad (\text{IV})$$

$$K_2=\frac{k_2}{k_{-2}} \qquad (5)$$

where w_1 and w_2 represent the two water molecules in the inner-coordination sphere which are eventually replaced by the bidentate ligand. The two binding sites of the ligand are represented as A and B. Reaction (3) is the formation of the outer-sphere complex or ion-pair. The (III) and (IV) are the monodentated complex and bidentated chelate complex, respectively.

If we resrict our attention to the first substitution process, we may relate the experimentally determined rate constants k_f and k_r to the above mechanism.

The two assumptions made are: (a) that reaction (3) is very rapid with respect to reaction (4) and (5), and (b) that $d[w_2M-A-B]/dt=0$. The results are

$$k_f=K_0k_1\left(\frac{k_2}{k_{-1}+k_2}\right) \qquad (6)$$

$$k_r=k_{-1}\left(\frac{k_{-2}}{k_{-1}+k_2}\right) \qquad (7)$$

This mechanism shows two limiting types of behavior, depending upon the relative magnitudes of the two rate constants k_{-1} and k_2. In first case, if the rate determing step is explusion of a water molecule from the inner-coordination sphere, then, in terms of equation in the extreme where $k_2 > k_{-1}$, it follows that $k_f=k_1K_0$, $k_r=k_{-1}k_{-2}/k_2$ (limiting case A). In second case, if the complex is stable as a chelate, and reaction (5) is the rate-determining, then $k_{-1} > k_2$, it follows that $k_f=K_0K_1k_2$, $k_r=k_{-2}$ (limiting caae B).

If rate-determining step is the step (4) (*i.e.* if the reaction follows to the limiting case A), then by using a calculated value for the ion-pair formation constant (K_0), one obtains a value of k_1, the rate constant for the elimination of a water from the first coordination sphere. The value of k_f obtained here for nicke(II) with *D*-penicillamine is $8.2\times10^5 M^{-1}sec^{-1}$ at 25°C, which is very close in magnitude to the case of other ligands.[7,10,18]

K_0 cannot be determined experimentally. However, this constant can be obtained by using Bjerrum's ion-pair constant with activity coefficient[19] or its equivalent calculated from diffusion theory.[20] Assuming a value for the distance of closest approach of 5Å (approximately the internuclear distance of a coordinate bond plus the effective thickness of a water molecule), Ko can be calculated for the various complexes and k_1 can be calculated from the experimentally determined rate constants. The calculated K_0 value was $5.5M^{-1}$ at 25°C and k_1 value was $1.5\times10^5\ sec^{-1}$. This value is about 5 times greater than the value obtained from NMR measureent; Swift and Connick[21] have determined the water exchangerate constant (k_1) by the use of NMR line-broading measurement. Their value is reported to be $2.7\times10^4 sec^{-1}$ for nickel(II) ion.

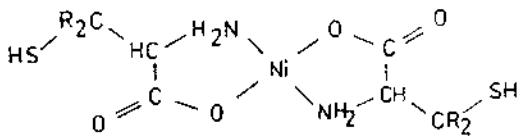
Therefore, this complexation reaction must follow the limiting case A; that is, the rate-determinig step is the release of a water molecule from the inner-coordination spehere in metal ion rather than chelate-ring closure for 1 : 1 metal to ligand complex formation.

The equilibrium constant determined experimentally was $1.14\times10^3M^{-1}$ at 25°C. The equilibrium study was carried out to determine whether -SH group will partake in binding, or if the -SH group is involved in the formation of this complex. The formation constants of nickel(II) complex obtained at 25°C in this work and literature values previously reported are summarized in *Table* 7. The log K_1 values of the nickel(II) ion complexes of mercaptoacetic acid, histidine, and cysteine are higher than that of the complex of *D*-penicillamine is close to the value of alanine and methionine for nickel(II) ion.

Table 7. The formation constants of nickel(II) ion complexes at 25°C

	log K_1	log K_2	Reference
D-Penicillamine	3.06	—	This work
Methionine	5.19	4.65	22
Alanine	5.48	4.53	22
Mercaptoacetic acid	6.2±0.6	6.81±0.02	23
Histidine	8.69	7.15	24
Cysteine	9.64	9.40	5

In the binding site of penicillamine with nickel(II) ion, there are three possible pairs of actual binding sites: (1) $-S^-$ and $-NH_2$, (2) $-S^-$ and $-COO^-$, and (3) $-NH_2$ and $-COO^-$. The possibility of the first pair as the binding sites is ruled out because the fromation constants of the nickel(II)-penicillamine complex are much lower than those of nickel (II)-histidine and nickel(II)-cysteine complexes. The possibility of the second pair as the binding sites is also ruled out because the formation constant of nickel (II)-penicillamine complxes from *D*-penicillamine in this pH condition entail a combination of the nickel(II) with the amino nitrogen atom and carboxylic group while mercapto group remains free (see V).



(V) R= CH_3

D-Penicillamine coordinates to the nickel(II)

ion utilizing sulfur and nitrogen as donor atoms in the high pH condition(pH>9.2). However, in the pH range of 8.25–9.07, the stepwise stability constant become drastically reduced(log K_1=3.06 for 1 : 1 complex). Eventhough this result was obtained from kinetic data, it is plausible to conclude that this result is ascribed to the fact that the undissociated mercapto group does not participate in binding. The difference of structure seems to be attributed to the difference in pH conditions.

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