**Ni(II) and D-Penicillamine**

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

Yong-Kyu Kim and Sung-Nak Choi

Department of Chemistry, Pusan National University, Pusan 607, Korea

*Department (Rusan May 27, 1986)

**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 performed 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 therapeutic agent in Wilson's disease has focused attention on the metal bonding ability of this simple amino acid. Penicillamine has also been shown effective in certain metal toxicities in virtue of its exceptional ability to bind with metal ions.

Stability constants of some metal ions with DL-penicillamine has been reported. Lenz and Martell 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 \( \mu = 0 \), and kinetic investigation of the complex formation process was carried out by the use of pressure-jump method. 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 intermediate 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, the rate determining step is supposed to be the formation of the first bond between the metal ion and the ligand. However, in the second mechanism, 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 Na₂EDTA reagent with murexide as indicator in ammonical solution.

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 100ml jacket–titration cell which was fitted with a magnetic stirrer. Fifty milliters of a solution containing D-penicillamine and nickel(II) ion was titrated with 0.01N NaOH from a 5-ml 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 μ=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 BaSO₄. The solid BaSO₄ was removed by filtration. 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 on the cell and phosphorous-bronze diaphragm (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 NiSO₄ 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.
RESULTS AND DISCUSSION

Chelate-Stability Constants. The potentiometric titration was carried out by the use of Bjerrum's method. Dissociation constant of ligand and stability constants of complex were calculated by the method of Irving and Rossof [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 μ=0. The -logK<sub>H</sub> values were determined for D-penicillamine at various temperature and μ=0 and listed in Table 1. The measured dissociation constants are different slightly from the reported values. So, the effect of ionic strength on dissociation constant was investigated for this system. Table 2 gives the values of -logK<sub>H</sub> obtained for the D-penicillamine at various ionic strengths and at 25°C. The dissociation constants 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 μ=0.

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

<p>| Table 1. Dissociation constants of D-penicillamine |</p>
<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>-logK&lt;sub&gt;P&lt;/sub&gt;(-NH&lt;sub&gt;2&lt;/sub&gt;)</th>
<th>-logK&lt;sub&gt;P&lt;/sub&gt;(-SH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>7.88</td>
<td>10.17</td>
</tr>
<tr>
<td>20</td>
<td>7.80</td>
<td>10.05</td>
</tr>
<tr>
<td>25</td>
<td>7.70</td>
<td>9.87</td>
</tr>
<tr>
<td>30</td>
<td>7.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>35</td>
<td>7.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>μ=0.10(KNO<sub>3</sub>) for DL-penicillamine. <sup>b</sup>μ=0.15 (KNO<sub>3</sub>) for DL-penicillamine.

Fig. 1-a. Pressure-jump oscillogram. Temp. = 25°C, NiSO<sub>4</sub>=0.2M. The rise time of the apparatus 37 μsec.

Fig. 1-b. Experimental relaxation curve of nickel(II) - penicillamine at 30°C. C<sub>Ni</sub>=3.27×10<sup>-3</sup>M. C<sub>pen</sub>=2.27×10<sup>-3</sup>M. Relaxation time = 550 μsec.
Table 2. Ionic strength dependence of dissociation constants of D-Penicillamine at 25°C

<table>
<thead>
<tr>
<th>μ(NaClO₄)</th>
<th>logK²⁺(-NH₃)</th>
<th>logK⁻(-SH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>7.85</td>
<td>10.22</td>
</tr>
<tr>
<td>0.08</td>
<td>7.80</td>
<td>10.12</td>
</tr>
<tr>
<td>0.05</td>
<td>7.77</td>
<td>10.05</td>
</tr>
<tr>
<td>0.03</td>
<td>7.75</td>
<td>9.95</td>
</tr>
<tr>
<td>0.01</td>
<td>7.72</td>
<td>9.91</td>
</tr>
</tbody>
</table>

Table 3. Formation constants of nickel(II)-penicillamine complex at various temperature and μ = 0

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Formation Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log K₁</td>
</tr>
<tr>
<td>15</td>
<td>10.93</td>
</tr>
<tr>
<td>20</td>
<td>10.68</td>
</tr>
<tr>
<td>25</td>
<td>10.45</td>
</tr>
<tr>
<td>30</td>
<td>10.23</td>
</tr>
<tr>
<td>35</td>
<td>10.03</td>
</tr>
</tbody>
</table>

Fig. 2. Titration of D-penicillamine with NaOH in the absence and presence of Ni²⁺ ion at 25°C and μ = 0. (b) represents the number of equivalents of base added.

values of the formation constants are listed in Table 3, where K₃K₂ are the over-all formation constants.

Albert and Li et. al. 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 remains 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).

\[
\text{Ni}^{2+} + 
\]
Ni(II) and D-Penicillamine react to form a complex, and the rate constants and equilibrium constants were studied

**Table 4.** Relaxation times and experimental conditions for the complex formation of nickel(II) ion and D-penicillamine (at 30°C)

<table>
<thead>
<tr>
<th>C^2 (10^-3)</th>
<th>C^4 (10^-3)</th>
<th>C_{MHL} (10^-3)</th>
<th>C_M (10^-4)</th>
<th>C_L (10^-4)</th>
<th>μ (10^-3)</th>
<th>1/τ</th>
<th>1/ττ (10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1.39</td>
<td>1.26</td>
<td>7.43</td>
<td>1.31</td>
<td>1.55</td>
<td>0.841</td>
<td>1.47</td>
</tr>
<tr>
<td>2.21</td>
<td>1.53</td>
<td>1.44</td>
<td>7.79</td>
<td>1.49</td>
<td>0.63</td>
<td>0.837</td>
<td>0.52</td>
</tr>
<tr>
<td>2.66</td>
<td>2.07</td>
<td>0.87</td>
<td>7.85</td>
<td>1.94</td>
<td>1.67</td>
<td>0.837</td>
<td>0.59</td>
</tr>
<tr>
<td>2.81</td>
<td>1.81</td>
<td>1.64</td>
<td>9.68</td>
<td>1.70</td>
<td>2.02</td>
<td>0.822</td>
<td>1.64</td>
</tr>
<tr>
<td>3.32</td>
<td>2.53</td>
<td>2.34</td>
<td>9.81</td>
<td>2.43</td>
<td>2.06</td>
<td>0.820</td>
<td>1.67</td>
</tr>
<tr>
<td>3.27</td>
<td>2.27</td>
<td>2.05</td>
<td>12.13</td>
<td>2.13</td>
<td>2.53</td>
<td>0.804</td>
<td>1.82</td>
</tr>
<tr>
<td>4.00</td>
<td>2.86</td>
<td>2.59</td>
<td>14.68</td>
<td>2.69</td>
<td>2.95</td>
<td>0.791</td>
<td>1.92</td>
</tr>
</tbody>
</table>

**Table 5.** The formation and dissociation constants of nickel(II)-penicillamine complex (μ=0)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>k_f (M^-1 s^-1)</th>
<th>k_r (s^-1)</th>
<th>K (M^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>5.26×10^5</td>
<td>4.29×10^5</td>
<td>1.23×10^5</td>
</tr>
<tr>
<td>20</td>
<td>6.38×10^5</td>
<td>5.50×10^5</td>
<td>1.16×10^5</td>
</tr>
<tr>
<td>25</td>
<td>8.21×10^5</td>
<td>7.22×10^5</td>
<td>1.14×10^5</td>
</tr>
<tr>
<td>30</td>
<td>9.88×10^5</td>
<td>8.86×10^5</td>
<td>1.12×10^5</td>
</tr>
<tr>
<td>35</td>
<td>11.44×10^5</td>
<td>10.33×10^5</td>
<td>1.11×10^5</td>
</tr>
</tbody>
</table>

Penicillamine and NiHL is the 1:1 nickel-D-penicillamine complex. The rate constants are related to the relaxation time by the equation.

$$1/\tau = kf f_2 (C_M + C_L) + kr$$  \hspace{1cm} \text{(2)}$$

where $k_f$ and $k_r$ are the rate constants of the complex formation and the dissociation at $\mu=0$, respectively and $C_M$ and $C_L$ are the concentration of Ni^{2+} and HL, and $f_2$ is the mean activity coefficient of the free ions. The $1/\tau$ values were plotted against $f_2 (C_M + C_L)$ 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 concentration 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.\textsuperscript{17} The calculations were repeated until a constant $K$ value was obtained. The final results are...
The rate constants, $k_f$ and $k_r$, and the equilibrium constants at each temperature were obtained from Fig. 3. The rate and equilibrium constants at various temperatures are summarized in Table 5. Kinetic parameters $\Delta H^\circ$ and $\Delta S^\circ$ were determined from the temperature dependencies of rate constants and are listed in Table 6.

Previous investigations of complexation reaction of first row transition metal ions 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:

$$M_{(aq)} + A - B_{(aq)} \rightarrow \frac{k_0}{k_{-1}} w_1 M_{(aq)} A - B$$  \hspace{1cm} (I)

$K_0 = \frac{k_0}{k_{-1}}$  \hspace{1cm} (3)

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

Previous investigations of complexation reaction of first row transition 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:

$$M_{(aq)} + A - B_{(aq)} \rightarrow \frac{k_0}{k_{-1}} w_1 M_{(aq)} A - B$$  \hspace{1cm} (I)

$K_0 = \frac{k_0}{k_{-1}}$  \hspace{1cm} (3)

$\frac{w_1 M w_2 A - B}{k_1} \frac{k_1}{k_2} \frac{w_2 M - A + H_2 O}{(III)}$

$k_1 = \frac{k_1}{k_2}$  \hspace{1cm} (4)

$\frac{w_2 M - A - B}{k_1} \frac{k_1}{k_2} \frac{M_{(aq)} A + H_2 O}{(IV)}$

$K_2 = \frac{k_2}{k_1}$  \hspace{1cm} (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 restrict 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 $d[w_1 M - A - B]/dt = 0$. The results are

$k_f = K_0 k_1 \frac{k_2}{k_{-1} + k_2}$  \hspace{1cm} (6)

$k_r = k_{-1} \frac{k_{-2}}{k_{-1} + k_2}$  \hspace{1cm} (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 determining step is expulsion of a water molecule from the inner-coordination sphere, then, in terms of equation in the extreme where $k_0 > k_{-1}$, it follows that $k_f = k_0 K_0, k_r = k_{-1} k_2/k_2$ (limiting case A). In second case, if the complex is stable as a chelate, and reaction (5) is the rate-determining, then $k_{-1} > k_0$, it follows that $k_f = K_0 K_1 k_2, k_r = k_{-2} (limiting case B)$. 

Journal of the Korean Chemical Society
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 $k_f$, the rate constant for the elimination of a water from the first coordination sphere. The value of $k_f$ obtained here for nickel(II) with $D$-penicillamine is $8.2 \times 10^{9} M^{-1} sec^{-1}$ 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), $K_0$ 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 $5.5 M^{-1}$ at 25°C and $k_1$ value was $1.6 \times 10^7 sec^{-1}$. This value is about 5 times greater than the value obtained from NMR measurement: Swift and Connick$^{21}$ have determined the water exchangerate constant ($k_1$) by the use of NMR line-broadening measurement. Their value is reported to be $2.7 \times 10^6 sec^{-1}$ for nickel(II) ion.

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

The equilibrium constant determined experimentally was $1.14 \times 10^{9} M^{-1}$ 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

<table>
<thead>
<tr>
<th>Complex</th>
<th>log $K_1$</th>
<th>log $K_2$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$-Penicillamine</td>
<td>3.06</td>
<td>0</td>
<td>This work</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.19</td>
<td>4.65</td>
<td>22</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.48</td>
<td>4.53</td>
<td>22</td>
</tr>
<tr>
<td>Mercaptoacetic acid</td>
<td>6.2±0.6</td>
<td>6.8±0.02</td>
<td>23</td>
</tr>
<tr>
<td>Histidine</td>
<td>8.69</td>
<td>7.15</td>
<td>24</td>
</tr>
<tr>
<td>Cysteine</td>
<td>9.64</td>
<td>9.40</td>
<td>5</td>
</tr>
</tbody>
</table>

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.

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 formation 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 complexes 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).
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 $K_i$ = 3.06 for 1:1 complex). Even through 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.

ACKNOWLEDGEMENT

Financial support from the basic science Research program, Ministry of Education is gratefully acknowledged.

REFERENCES