# The Study of Equilibrium and Formation Constants of Some Transition Metal Complexes with Vitamin $B_6$ in Solution by Potentiometry

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The protonation constants of vitamin $B_6$ (pyridoxol hydrochloride) and the formation constants of its complexes with some transition metal ions M(II) (M = Cd, Co, Ni, Cu, and Zn) were determined potentiometrically at $(25 \pm 0.1)\,°C$ and $I(NaCl) = 0.2$ mol $dm^{-3}$. Two computer programs MINIQUAD and MIQUV were used for calculation, and the results obtained with the two programs were in good agreement. There are two complexes species MLH and ML ascertained in the binary system, and the formation constant of complex ML for metal ions, which was found to be for Cd(II) < for Co(II) < for Ni(II) < for Cu(II) > for Zn(II), has an Irving—Williams order.

Vitamin $B_6$ is a significantly biological ligand, and the importance of metal ions in some enzymatic processes involving vitamin $B_6$ derivatives as cofactor is now well known. A recent report proposed that complexes containing vitamin $B_6$ could effectively inhibit the uptake of heavy metal on mammalian cell culture and reduce the cytotoxicity of heavy metal [1]. It is well known that metal complexes, especially the complexes of biological ligand, have the vital functions *in vivo*, whereas most studies were made *in vitro* as a model. Considering that the interaction of vitamin $B_6$ with metal ions can be responsible for a noticeable biological behaviour, the complexation equilibrium of vitamin $B_6$ with metal ions has not been extensively studied and only a few data by spectrophotometric technique have been reported up to now [2, 3].

In this investigation, the binary complexes of ligand vitamin $B_6$ (*i.e.* pyridoxol hydrochloride, see Fig. 1) with some transition metal ions M(II) (M = Cd, Co, Ni, Cu, and Zn) were considered. The formation constants as well as the stoichiometries of the complexes were determined at $(25 \pm 0.1)\,°C$ and $I(NaCl) = 0.2$ mol $dm^{-3}$ by potentiometric technique.

## EXPERIMENTAL

All reagents, obtained from Shanghai Chemical Reagents Supplier, were of anal. grade or high purity, and the water for preparing solutions was deionized by means of ion-exchanger equipment. The stock solutions of M(II) chloride (M = Cd, Co, Ni, Cu, and Zn) were standardized by EDTA complexometric titration. The stock solution of vitamin $B_6$, which dried under vacuum, was prepared by weighing and kept out of sunlight. Carbonate-freed titrant sodium hydroxide was prepared by diluting the saturated stock solution, and then standardized with potassium hydrogen phthalate (dried at $120\,°C$ for 4 h).

A model PXS-215 pH- or mV-meter (precision $\pm 0.2$ mV) assembled with model 231 glass electrode and model 232 calomel electrode (Shanghai Rex Instruments Factory, China) was employed for all potentiometric measurements. A model DAB-1B digital automatic burette (Jiangsu Electroanalytical Instrument Factory, China, precision $\pm 0.003$ $cm^3$) was used to deliver titrant base. Sample solutions were titrated in a double-walled glass cell maintained at $(25 \pm 0.1)\,°C$ by means of water circulation from a water thermostat (TB-85 Thermo Bath, Shimadzu) and were stirred magnetically under a continuous flow of pure nitrogen.

At the EMF measurements, the glass electrode system was calibrated by means of titrating $1.01 \times 10^{-2}$ M-HCl solution with $9.91 \times 10^{-3}$ M-NaOH solution under the conditions $(25 \pm 0.1)\,°C$, NaCl ionic strength 0.2 mol $dm^{-3}$, and the initial volume of titrated solution was 50.0 $cm^3$. The electrode standard potential ($E°$) and the slope of glass electrode ($S_g$) were calcu-
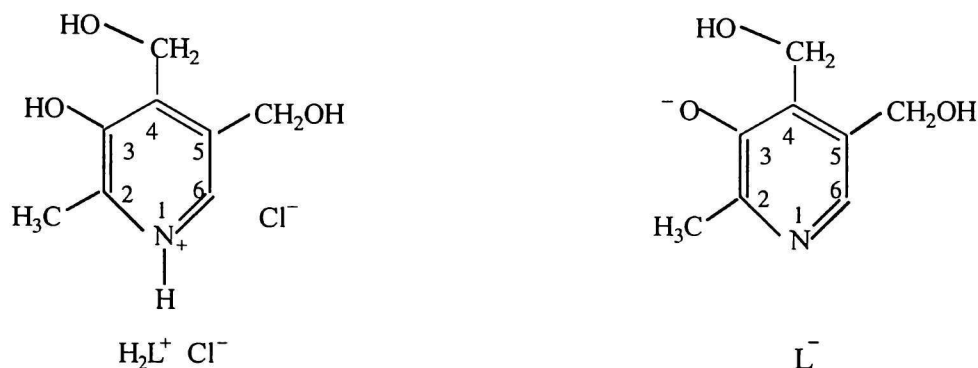
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Fig. 1. The structure of pyridoxol, protonated ($H_2L^+$ $Cl^-$) and deprotonated ($L^-$).

Table 1. The Result of the Calibration of Glass Electrode by Strong Acid-Base Titration at 25 °C and $I$(NaCl) = 0.2 mol dm$^{-3}$ [a]

| | Initial values | | Calculated values[b] |
|---|---|---|---|
| Sample volume/cm$^3$ | 50.0 | $E^\circ$/mV | 374.50(7) |
| $c$(HCl)/(mol dm$^{-3}$) | $1.01 \times 10^{-2}$ | $S_g$/mV | 59.18(2)[c] |
| $c$(NaOH)/(mol dm$^{-3}$) | $9.91 \times 10^{-3}$ | p$K_w$ | 13.66 |

a) The total experimental data number is 35 and 5 data points, which were found to be unbuffered by running program MAGEC, have been removed in the computation. b) Standard deviations are given in parentheses. c) The theoretical value is 59.16 mV.

lated by employing the computer program MAGEC [4] according to the Nernstian equation

$$E^{obs} = E^\circ + S_g \times \log\{[H^+]\}$$

where $E^{obs}$ and $[H^+]$ denote the measured EMF value, and the concentration of free hydrogen ion in working solution, respectively. As shown in Table 1, the experimentally estimated $S_g$ value was found to be in good agreement with the theoretical one, and this suggests that the experimental data obtained by this electrode system were accurate and reliable.

For the evaluation of the protonation constants, the sample solutions containing $(1.0 \leq \{c_L\} \leq 3.0) \times 10^{-3}$ mol dm$^{-3}$ of vitamin B$_6$ and acidified with a known excess of HCl were titrated with a standard NaOH solution, and the experiment was carried out in the pH range 2.4—10.3.

The determination of complexes formation constants was performed in a mole ratio of metal ions to ligand 1:1, 1:2, and 1:3, and all the solutions were acidified with a known excess of HCl before titration. Sample solutions that have a fixed concentration of ligand ($2.0 \times 10^{-3}$ mol dm$^{-3}$) and changed metal ions concentration $(6.0 \leq \{c_M\} \leq 20) \times 10^{-4}$ mol dm$^{-3}$ were titrated with a standardized NaOH·solution. Experimental data were collected within the pH range 2.4—6.2 and 2.4—7.1 for Cu(II) and Zn(II) ions, respectively, but 3.8—7.8 for Cd(II), Co(II), and Ni(II).

All the calculations about the protonation constants and complexes formation constants were carried out with the aid of the least-squares computer program MINIQUAD and MIQUV [5]. Pro-

gram MINIQUAD was developed so that the minimization is based on the sum of the squared residuals between observed and calculated analytical concentrations whereas program MIQUV is based on the measured and calculated electromotive potentials (EMF value). The notation $\beta_{pqr}$ (concentration constants) was defined as follows (charges are omitted for simplicity).

$$p\text{M} + q\text{L} + r\text{H} \rightleftharpoons [\text{M}_p\text{L}_q\text{H}_r]$$

$$\beta_{pqr} = [\text{M}_p\text{L}_q\text{H}_r]/[\text{M}]^p[\text{L}]^q[\text{H}]^r$$

where $p$, $q$, and $r$ denote the numbers of moles of M(II) ions, deprotonated vitamin B$_6$, and proton (the negative value of $r$ denotes the hydroxyl-containing complexes), respectively. The complexes species are simply referred to as the combination $pqr$ and the formation constant is expressed as $\beta_{pqr}$.

## RESULTS AND DISCUSSION

The protonation constants for the ligand (protonated form $H_2L^+$) studied are given in Table 2. In addition, the recorded number of data points, the number of titrations, and the standard deviation (values in parentheses) are also listed. Potentiometric titration curves demonstrate clearly that there are two protonation steps, which correspond to the protonated nitrogen donor and the phenolic hydroxyl oxygen donor of the ligand, respectively. As shown in Table 2, the estimated protonation constants of vitamin B$_6$ by two

**Table 2.** Results[a] of the Determinations of Protonation Constants of Vitamin $B_6$ ($H_2L^+$) at 25 °C and $I$(NaCl) = 0.2 mol dm$^{-3}$

|  | MIQUV | MINIQUAD | Ref.[b] |
|---|---|---|---|
| $\log \beta_{011}$ ($pK_2$) | 8.782(2) | 8.785(3) | 8.84 |
| $\log \beta_{012}$ | 13.644(3) | 13.646(6) |  |
| $pK_1$ | 4.862 | 4.861 | 4.88 |
| pH range |  | 2.4—10.3 |  |
| Np/$\tau$[c] |  | 178/3 |  |

a) Standard deviations are given in parentheses. b) Values at 25 °C and $I$(NaCl) = 0.15 mol dm$^{-3}$. c) Np – number of experimental data, $\tau$ – number of titration.

programs with the identical experimental data are in a good agreement, and the values coincide with the literature [6] as well.

The complexes species of binary system that best fit the experimental data and their formation constants $\log \beta_{pqr}$ values are given in Table 3. For each and every binary system M(II)—vitamin $B_6$, two complexes species ML (110) and MLH (111) were ascertained in solutions. Moreover, in the Cu(II)—vitamin $B_6$ system, the third complex species CuL(OH) (11-1) was formed by the loss of a proton from the complex CuL.

Complex MLH can be thought as a monoacid, and its protonation constant $pK^H_{MLH}$ is shown in Table 3. The $pK^H_{MLH}$ value of complex MLH should be close to the $pK_2$ value of ligand vitamin $B_6$, and the difference between $pK_2$ and $pK^H_{MLH}$ reflects the acting strength of M(II) ions to the ligand. In this work the magnitude of ($pK_2 - pK^H_{MLH}$) for M(II) ions raised in the order for Cd(II) $\leq$ for Co(II) < for Ni(II) < for Zn(II) < for Cu(II); this suggests that the complex CuLH leaves the proton out more easily and the complex CuL can

be formed more easily or the complex CuL will be the most stable one to present experiments. As the prediction, the formation constants of the complex ML coincide with the order mentioned above.

The formation constants of complexes ML are generally higher than those given in the literature [2, 3] which were obtained by means of spectrophotometric method (see Table 3). The heterocyclic nitrogen of ligand is the coordination atom in the complex of ML proposed in the literature. According to the reports, vitamin $B_6$ exists under a Zwitterionic form around neutrality and the deprotonation occurs mainly on the phenolic group of C-3 site (Fig. 1) and partially on the pyridinium group in the pH range from 2 to 7 [7, 8]. Moreover, the relative position of the hydroxyl group existing in ligand may have a critical influence not only on the structure but also on the stability of the complex species formed [9, 10]. In the ligand vitamin $B_6$, besides phenolic hydroxyl of C-3 site there is an adjacent alcoholic hydroxyl occupying C-4 site, which should be beneficial to form more stable chelate. The formation constant of the complex of the related compound of pyridine with M(II) ions is by 1—2 logarithm units larger than that in the case of pyridine, and the observed higher values of the formation constants of ML can be rationalized in terms of chelation. This suggests that in the complex ML the coordination atom is phenolic oxygen in the C-3 site and alcoholic hydroxyl in the C-4 position of ligand vitamin $B_6$ then forms a chelated compound.

For the Cu(II)—vitamin $B_6$ system we estimated another acceptable complex species CuL(OH) (11-1). We assume that the hydroxyl group coordinated to Cu(II) ion is the alcoholic hydroxyl in the adjacent C-4 position of ligand vitamin $B_6$. That is to say that complex CuL may act as a monoacid, with the increase of basic capacity of the tested solutions the alcoholic

**Table 3.** Formation Constants of Vitamin $B_6$ with Metal Ions at 25 °C and $I$(NaCl) = 0.15 mol dm$^{-3}$

|  | Cd(II) | Co(II) | Ni(II) | Cu(II) | Zn(II) |
|---|---|---|---|---|---|
|  |  |  | Results of MIQUV[a] |  |  |
| $\log \beta_{110}$ ($\log K_1$) | 2.42(2) | 2.82(2) | 3.26(1) | 4.965(9) | 3.16(2) |
| $\log \beta_{111}$ | 10.52(4) | 10.85(2) | 10.47(6) | 10.73(2) | 9.88(7) |
| $\log \beta_{11-1}$ | – | – | – | −2.19(2) | – |
| $pK^H_{MLH}$ | 8.10 | 8.03 | 7.21 | 5.78 | 6.52 |
| $\log K^{LH}_{MLH}$ | 1.74 | 2.07 | 1.69 | 1.97 | 0.90 |
|  |  |  | Results of MINIQUAD |  |  |
| $\log \beta_{110}$ ($\log K_1$) | 2.39(5) | 2.81(3) | 3.23(3) | 4.97(2) | 3.17(4) |
| $\log \beta_{111}$ | 10.46(14) | 10.82(7) | 10.47(11) | 10.72(5) | 9.91(17) |
| $\log \beta_{11-1}$ | – | – | – | −2.22(6) | – |
| $pK^H_{MLH}$ | 8.07 | 8.01 | 7.24 | 5.76 | 6.74 |
| $\log K^{LH}_{MLH}$ | 1.68 | 2.04 | 1.69 | 1.94 | 1.13 |
| pH range | 3.8—8.8 | 3.8—8.4 | 3.8—8.4 | 2.4—6.2 | 2.4—7.4 |
| Np/$\tau$[b] | 162/3 | 104/3 | 89/3 | 140/3 | 80/3 |
| $\log K_1$ | – | 1.95[c] | 2.02[d] | 2.80[c] | – |

a) Standard deviations are given in parentheses. b) Np – number of experimental data, $\tau$ – number of titration. c) Ref. [3]. d) Ref. [2].
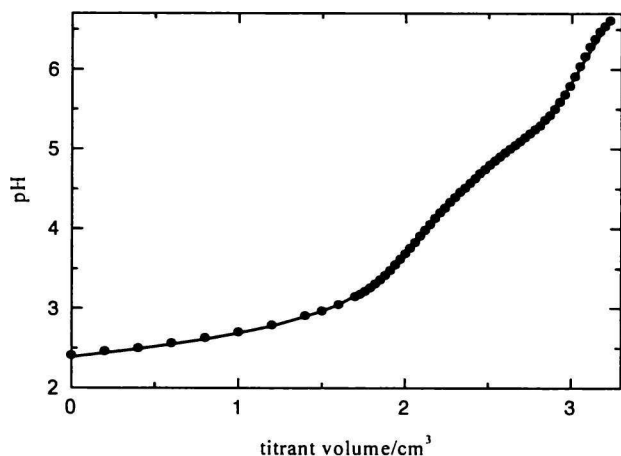
**Fig. 2.** Comparison of experimental (●) and calculated (——) curves of the Cu(II)—vitamin B$_6$ system. $c_{vit}$ = 1.98 × 10$^{-3}$ mol dm$^{-3}$; $c_{Cu}$ = 6.36 × 10$^{-4}$ mol dm$^{-3}$; $c_H$ = 6.03 × 10$^{-3}$ mol dm$^{-3}$; 25 °C and $I$(NaCl) = 0.2 mol dm$^{-3}$.
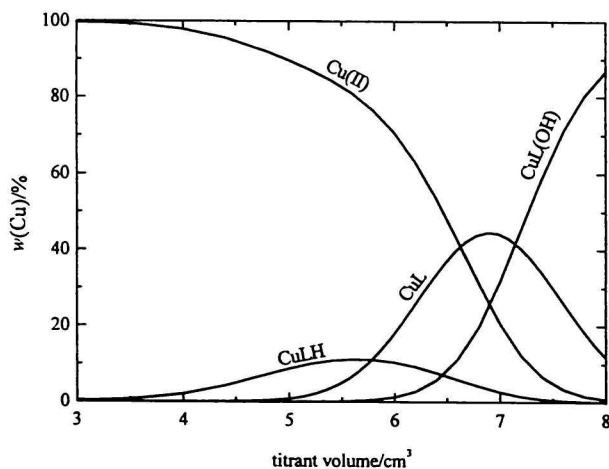


**Fig. 3.** Species distribution curves for Cu(II)—vitamin B$_6$ system at a mole ratio of 1:3 ($n$(metal):$n$(ligand)). $c_{vit}$ = 1.98 × 10$^{-3}$ mol dm$^{-3}$; $c_{Cu}$ = 6.36 × 10$^{-4}$ mol dm$^{-3}$.

hydroxyl group deprotonated, and the estimated protonation constant p$K_a$ (p$K_a$ = log $\beta_{110}$ − log $\beta_{11-1}$) value is 7.15. The alcoholic hydroxyl group generally has a p$K_a$ value about 12, and the low p$K_a$ value of the hydroxyl group in the complex CuL is due to the influence of its coordinative bond with Cu(II).

For Cu(II)-containing vitamin B$_6$ system, the experimental pH value and that calculated according to the ascertained complexes species are very close (Fig. 2), and this confirms the veracity of the se-

lected complexes species and the reliability of reported log $\beta_{pqr}$ values.

The species distribution curve of Cu(II)-containing vitamin B$_6$ system is shown in Fig. 3. The acidic complex CuLH formed preferentially (pH > 4) with a maximum content 11.2 %, then complex CuL formed (pH > 5) (maximum content: 45 %) with the increase in basic content of the sample solution, and the complex CuL(OH) formed (pH > 6) lastly with the rapidly enhanced content. When pH > 8, the complex species existing in solution is nearly the complex CuL(OH).

## CONCLUSION

In the binary M(II)—vitamin B$_6$ system, both complexes ML and MLH exist, and the formation constants of complex ML have an Irving—Williams order of stability [11]. Complex ML$_2$ has not been found in the present work, here we think that the stereo hindrance is the main factor. By comparing the formation constants of ML to analogous compound, such as pyridine, we proposed that the coordination atom of vitamin B$_6$ to M(II) ions in complexes ML is the oxygen of phenolic hydroxyl group but not the heterocyclic nitrogen.

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