Potentiometric Study of Complex Formation Between Some Transition Metal Ions and 2 - Aminopyridine. Part 1. A Model for Therapeutic Agent for Wilson's Disease

***Joshaghani, Mohammad**[*+]*

Department of Chemistry, Faculty of Science, Razi University, P.O. Box 67148, Kermanshah, I.R. IRAN

Sotodehnejad, Masoud

Department of Chemical Research, ACECR, Kermanshah, I.R. IRAN

ABSTRACT: *The complexation reactions of some transition metal ions and 2-aminopyridine (2-ampy) were studied potentiometrically in aqueous solution at $\mu=0.1$ M and 25 °C. The overall stability constants log β's of all species are obtained by computer refinement of pH-volume data with using the BEST computer program. Several models were tested and the best one accepted according to the least sum of squared deviations. The main species are MHL, ML, ML$_2$, ML$_3$, and ML$_2$(OH)$_2$. For ML complexes the order of stability confirms the Irwing-Williams series.*

KEY WORDS: *Potentiometry, Complex formation, Stability constant, 2-Amino pyridine, Wilson's disease*

INTRODUCTION

The formation of complexes, in the aqueous solutions is a matter of great importance not only in inorganic but also in analytical, biochemistry and other scientific and industrial fields [1-2]. Metal ions can induce toxicity in humans. Classic examples being heavy metal poisons such as mercury and lead. Even essential metal ions can be toxic when present in excess. Iron is a common household poison in united states as a result of accidental ingestion of dietary supplement of ferrous sulfate.

Wilson's disease leads to an excessive accumulation of copper in the liver, kidney and brain which leads to various failure and neurological abnormalities.

One way for treatment of metal toxicity involves chelation therapy, in which metal-specific chealting agents are administered as drugs to complex and facilitate excertion of unwanted excess element. The use of desferridoxamin to treat iron poisoning or dimercaprol (BAL) and K$_2$Ca (EDTA) for treatment of Wilson's

disease are examples of this approach[3]. The stability constants of metal-chelates are valuable measure for designing and selecting of chelators[4]. Unfortunately, most of this chelates have similar affinities for metals. For example, Zink deficiency is an invetable result in Wilson's disease.

Potentiometry is one of the most convenient and successful techniques employed in metal complex equilibrium measurements. Such applications are based on studing the influence of the pH on three-component equilibrium system containing metal ion, ligand and proton. The titration curves are obtained at the same initial total hydrogen ion and total ligand concentration, in the presence and in the absence of metal ion. This principles was utilized as long ago as 1954 by Rossoti and Irving [5]. The difference between the titration curves is proportional to quantitative complex formation in the equilibrium process:

$$M + H L \rightleftharpoons M L + H \qquad (1)$$

where the number of proton released corresponds to the composition of the complexes formed in the whole range of titration curve.

Computerized method have become very important procedures in the calculation of complex formation equilibriums. Among the programs available for the calculation of equilibrium constants, the BEST program has evolved into a very useful and friendly interactive program [6]. The use of the algorithm for computing equilibrium constants in the BEST program involves the following sequences: (1) start with a set of known and estimated overall stability constants (β's) and compute $[H^+]$ at all equilibrium points; (2) compute the weighted sum of the squares of the deviations in p[H] as:

$$U = \Sigma\, W\, (p[H]_{obs} - p[H]_{cal})^2 \qquad (2)$$

Where w is a weighting factor which serves to lessen the influence of the less accurate p[H] values in the steeply sloped regions of the p[H] profile; and (3) adjust the unknown stability constants and repeat the calculation until no further minimization of U can be achieved, thus providing the final calculated β values. Minimization should lead to a minimum value for the standard deviation in p[H] units which is represented by σ_{fit} value:

$$\sigma_{fit} = \{U/N\}^{0.5} \qquad (3)$$

where $N = \Sigma\, W.$ $\qquad (4)$

Derivatives of pyridine and amine compounds are very important due to their applications in medicine. Schiff base complexes of nickel(II), copper(II) and zinc(II) derived from the 2-ampy have been studied as a model for carbonic anhydrase[7]. The effects of 2-ampy on inhibition and voltage dependence of K^+ current in smooth muscle cells from cerebral arterites have been studied [9].

Complex formation reaction of 2-ampy with Be^{2+} has been studied potentiometrically by Relan et al [9]. In 1972, polarographic determination of the formation constants of copper was studied by Coutuier et al [10]. In 1974 Foundov and co-workers reported stability constants of Ag^+ complexes with metal complex electrode via potentiometry [11]. In 1976, characterization of rhodium (I) complexes of 2-Ampy by Brodzki and co-workers show coordination occurred through both nitrogen atoms of amino group and heterocycle [12].

In this paper we studied the interaction between 2-ampy and, Zn^{2+}, Cu^{+2}, Ni^{+2}, Co^{+2}, and Mn^{+2}. Since 2-ampy would be used as a model for investigation of the therapeutic effects of chelators in Wilson's disease, so the complexation reactions of these ligands with copper has a great importance.

EXPERIMENTAL
Reagents

2-aminopyridine was prepared [13]. Metal ions were prepared from analytical grade nitrate salts (Merck Company) and have dried over P_2O_5 in vacue for 72 hrs and the stock solutions were standardized complexometrically by EDTA titration [14]. Carbonate free solution of KOH was prepared from CO_2-free commercial concentrate and was standardized against primary standard oven-dried potassium hydrogen phthalate. The HCl solution was standardized with standard KOH. All solutions were prepared in triply distilled deionized water.

Apparatus

Potentiometric titrations were performed by means of a Metrohm 686 Titroprocessor equipped with a 665 Dosimat ý(with a 5.0 ml exchange unit) which was connected to a Pentium 200 MHz computer for data transfer and calculations. The Titroprocessor was

calibrated to read hydrogen ion concentration by titration of hydrochloric acid solution at $25\pm 0.5°C$ and $\mu = 0.5$ M KCl, with KOH solution according to Gran's method [15]. Sample solutions were titrated in a double-walled glass cell maintained at $25\pm0.5°C$ by circulating water and stirred magnetically under a continuous flow of purified nitrogen. Titrations were carried out over the pH range 2-10 using $50.0\pm0.01cm^3$ samplers. In some cases the titration was stopped in the beginning of precipitation.

Protonation Constants of 2-Ampy

The cell is charged with 10 mL of 0.0552 M of 2-ampy solution, 4.00 mL of 0.158 M of HCl, 5.00 mL of 1.00 M KCl and 31.00 mL of deionized doubly distilled water. It is sealed and remained under N_2 for 30 min., equilibrated, then slowly titrated with 8 mL of 0.094 M of KOH solution. The allowed changes of pH value, before the addition of subsequent increment, were less that %1 of that value. The ionic strength of the solution is nominally 0.100 M initially and remains nearly constant. This procedure was repeated three times and the mean values are selected. The species distribution curves are shown in Fig. 1. The obtained values of protonation constants are consistent with the Founder and coworker report [11].

Stability Constants of 2-Ampy Complexes

The conditions of measurements for the evaluation of the stability constants of 2-Ampy complexes were the same as for acidity constants, but in addition to the mentioned material a fraction of KCl was replaced by metal ion solution, so that the desired concentration ratio of ligand to metal 1:1 and 1:2 were obtained. The mean value of all results is selected. The species distribution curves for Zn^{2+} and Cu^{2+} are shown in Figs. 2 and 3, respectively.

Calculation Procedure

The protonation constants of the ligands and formation constants of the resulting complexes were computed from titration data using the FORTRAN programs PKAS and BEST that are loaded on a Pentium 200 MHz computer, respectively. The stoichiometries and formation constants of the resulting complexes were obtained by testing several possible composition models for the system under study. The model selected was that
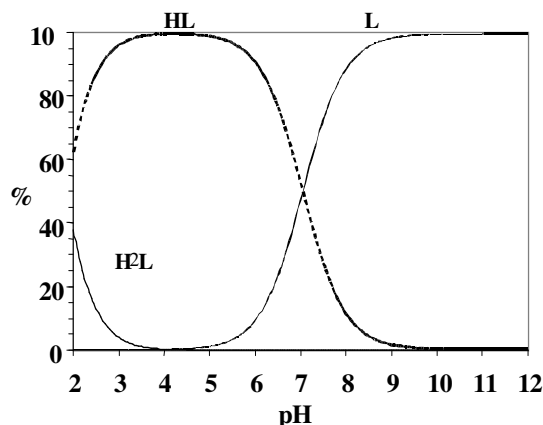

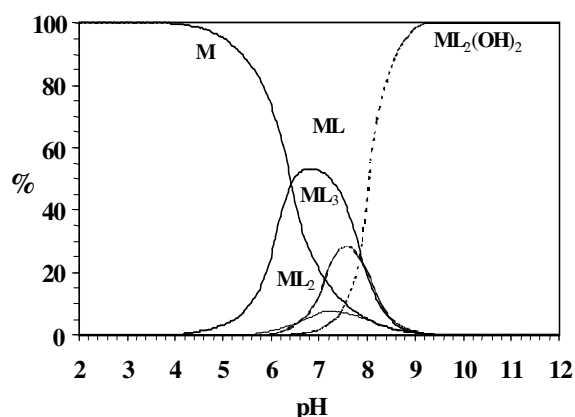**Fig. 1: Species distribution curve for 0.11 M of 2-Ampy at 0.10 M ionic strength (KCl) and 25 °C.**

Fig. 2: Species distribution curve for 0.11 M of 2-Ampy and 8×10^{-4} M of Zn(II) at 0.10 M ionic strength (KCl) and 25 °C. Oriented axis is the percent of species with respect to $[Zn(II)]_t = 100\%$.
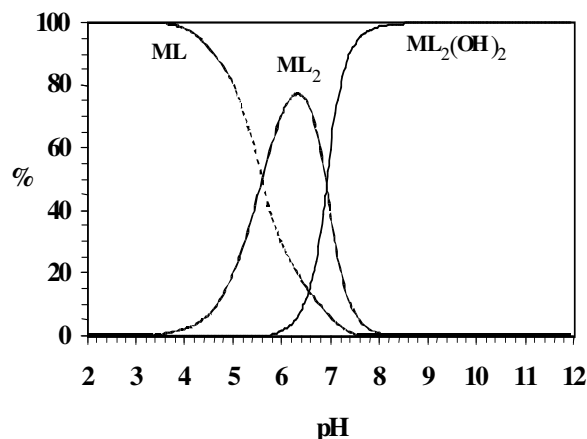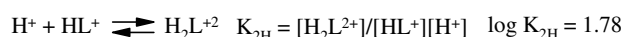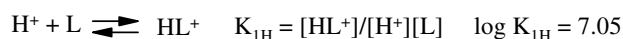

Fig. 3: Species distribution curve for 0.11 M of 2-Ampy and 4.2×10^{-3} M of Cu(II) at 0.10 M ionic strength (KCl) and 25 °C. Oriented axis is the percent of species with respect to $[Cu(II)]_t = 100\%$.

which gave the best statistical fit and which was chemically consistent with the titration data. The estimated error for each constant was less than 0.1 log units. The concentration distribution diagrams were obtained with the program SPEPLOT.

RESULTS AND DISCUSSION
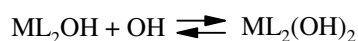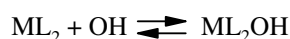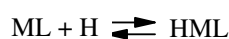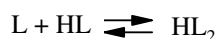### Protonation Constants

The protonation constants of the ligand were obtained from the titration data as follows:

$$H^+ + L \rightleftharpoons HL^+ \quad K_{1H} = [HL^+]/[H^+][L] \quad \log K_{1H} = 7.05$$

$$H^+ + HL^+ \rightleftharpoons H_2L^{+2} \quad K_{2H} = [H_2L^{2+}]/[HL^+][H^+] \quad \log K_{2H} = 1.78$$

The log k_{1H} is attribute to the pyridine N-atom of 2-ampy that is higher than that of the corresponding pyridine nitrogen (logK_{1H} = 5.18) and the logK_{2H} assigned to protonation of the amino group which is significantly lower than those of aniline. This is due to the fact that the pyridine ring is more electron-with-drawing than benzene.

Complex Formation Equilibria

The formation of any probable species was investigated using the BEST programs:

$$H + L \rightleftharpoons HL$$

$$L + HL \rightleftharpoons HL_2$$

$$M + L \rightleftharpoons ML$$

$$ML + L \rightleftharpoons ML_2$$

$$ML_2 + L \rightleftharpoons ML_3$$

$$ML + H \rightleftharpoons HML$$

$$ML_2 + OH \rightleftharpoons ML_2OH$$

$$ML_2OH + OH \rightleftharpoons ML_2(OH)_2$$
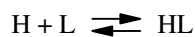
The equilibrium constants of various species are given in Table 1. In Co^{+2}, Ni^{+2} and Zn^{+2} cases, the formation constants were not high enough to prevent the precipitation of metal hydroxide [16]. These metal ions form complexes in the acidic pH range but dissociate to form metal hydroxide precipitates at above pH 9.5.

The stability constants obtained in both 1:1 and 1:2 metal to ligand concentration ratio were very similar for Cu (II) and Zn (II) cases and slightly different in Co (II)

Table 1: Logarithms of the stepwise formation constants for 2-Ampy and its complexes with some divalent metal ions at 0.1 M ionic strength (KCl or KNO$_3$) and 25 °C.

Metal ion (M)	Log K					
	HL	H$_2$L	HML	ML	ML$_2$	ML$_3$
H$^+$	7.05	1.77				
Co^{+2}			6.62 (5.85) [1.60]	1.78 (1.59) [2.58]	1.96 (2.11) [2.90]	2.29 (2.41) [3.10]
Ni^{+2}			3.30 (2.94) [1.95]	2.05 (1.95) [2.12]	1.59 (1.75) [1.65]	2.39 (2.52) [2.46]
Cu^{+2}				8.61 (8.54) [66]	3.41 (3.48) [3.45]	
Zn^{+2}			1.10 (1.05) [1.05]	2.60 (2.55) [2.67]	1.54 (1.60) [1.60]	2.61 (2.67) [2.65]

[a] *Values in parentheses are from 1:2 metal to ligand concentration ratio and the values in square brackets are in KNO$_3$ as supporting electrolyte.*

and Ni (II) cases. So it is concluded that 2-Ampy coordinate to studied metal via by both nitrogen atoms as a chelator.

The 1:1 complex of Cu (II):L is the most stable among other metal ion with the stability order of Cu(II)>> Zn(II)> Ni(II)> Co(II). This order has a good agreement with Irving-Williams series [17].

At pH below 6 CuL (Log β_1=8.61) species is formed with high amount. But CoL (log β_1=1.78), NiL (log β_1=2.05) and ZnL (log β_1=2.60) species are formed with very less amount. Therefore 2-ampy reacts with Cu (II) selectively at the acidic and neutral ranges.

At low pH for Co (II) system, pyridine N atom of ligand coordinated with hydrogen ion and formed complex of the type MHL. The corresponding equilibrium constants decrease with increasing stability of the chelates. Thus amount of MHL for Ni^{+2} and Zn^{+2} is very low and for Cu^{+2}, such a species is not formed at all. This result is in conformity with Jahn-Teller distortion necessary in a d^9 Cu (II) ion [18].

In order to investigate of the role of supporting electrolyte, the same reactions were repeated with KNO$_3$ instead of KCl. In the case of Cu (II) and Zn (II)

complexes, there are negligible differences in stability constants. But the differences become significant especially for Co (II). So it is suggested that chloride ion may act as a ligand in the later case and the formation of CoHL complex may be due to this situation.

By notice to high difference of stability constants of copper system respect to other metal ion specially Zinc, 2-ampy has some important cases such as a model for therapeutic chelating agent in excess copper disease [19], biological molecule models and copper ion separation of divalent cations mixture at acidic pH.

Because of the relatively low (L_D) of 2-ampy, we suggest that its safe substituents can be investigated as therapeutic agent for Wilson's disease. Also this ligand is a good choice for separation of Cu (II) from other metal ion studied at low pH.

By the same method as described in experimental section the complexation of 2-aminopyrimidine and 2-aminopyrazine are studied and it is found that the complexation is very poor. The result shows that the inductive effect of two nitrogen atoms in these rings and amine group are greater than that of 2-aminopyridine.

REFERENCES

[1] Martell, A. E., Motekaitis, R. J., in "The Determination and Use of Stability Constants", 2nd Ed., VCH Publishers, NY (1992).

[2] Bell, C. F., in "Principles and Applications of Metal Chelation", Oxford University Press. (1977).

[3] Rossoti, F. C., Rossoti, H., in "The Determination of stability constant"*,* Mc-Graw Hill, NY (1961).

[4] Motekaitis, R. J., Martell, A. E., *Can. J. Chem.* **60** 2403 (1982); Kim, W. D., Hrncir, D. C., Kiefer, G. E., Sherry, A. D., *Inorg Chem.* **34**, 2225 (1995).

[5] Hay, R. W., in "Bioinorganic Chemistry", 4th. Ed. Ellis Horwood Ltd., England (1991).

[6] Perrin, D. D., in "Medicinal Chemistry", Topics in Current Chemistry 64: Inorganic Biochemistry, chap. 3, Singapor Verlag, Berlin (1976).

[7] Chohan, Z. H., Kausar, S., *Chem. Pharm. Bull.* **40**, 2555 (1992).

[8] Robertson, B. E., Nelson, MT., *Am. J. Physiol. Cell Physiol.* **267**, 1589 (1994).

[9] Relan, P. S., Bhattacharya, P. K., *J. Indian Chem. Soc.* **44**(6), 536 (1967).

[10] Coutuier, Y., Petitfaun, C. R., *Acd. Sci. Ser.* **275,** 953 (1972).

[11] M. Foundou, E., Berthon, G., *Analusis,* **2,** 658, (1974).

[12] Brodzki, Pannetier, G., *J. Organomet. Chem.* **104,** 241 (1976).

[13] Vogel, A. I., in "Practical Organic Chemistry", 3rd. Ed. P. 1007 Longmans, London (1959).

[14] Vogel,A. I., in "A Textbook of quantitative inorganic analysis", Longmans, Green &Co. London (1961).

[15] Gran, G., *Analyst* **77**, 661 (1952).

[16] Li, Y., Martell, A. E., *Inorg. Chim. Acta* **214**, 103 (1993).

[17] Irving, H., Williams, R. J. P., *J. Chem. Soc.* 3192 (1953).

[18] Huheey, J. E., in "Inorganic Chemistry", 3rd. Ed., Ch. 9, p. 396, Harper and Row, NY (1983).

[19] Dabardly, A., *New Scientist,* **34** (1992).