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Rate and Equilibrium in Aqueous 1:2 Chelation of Copper(II) by 2-Pyridinecarboxylic Acid Hydrazide. Antituberculosis Activity

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Chelation reactions of 2-pyridinecarboxylic acid hydrazide, widely used drug in antituberculous regimens, with copper(II) have been investigated in aqueous solution at a temperature of 25°C. The ionization equilibria of the ligand and the stability of the metal-ligand complexes have also been characterized. Kinetic results show that the rate constant for monochelated complex formation is several times larger than the rate constant for dichelated complex formation. A detailed mechanism is proposed to account for the kinetic data.

Key words: copper(II), 2-pyridinecarboxylic acid hydrazide, kinetics, chelation, antituberculosis activity

Continuing with our studies on chelation of 1,3-diketones [1–4] and 1,3-ketoesters [5] with transition metal ions and looking for a chelating agent with biological capacity and multiple functionality, chelation of 2-pyridinecarboxylic acid hydrazide towards transition metal ions has been attempted. This ligand, and structurally similar hydrazides, have great potential owing to their antituberculous action, which increases in the presence of some ions such as Co(II), a biological capacity which has been extensively pointed out [6–8]. This is a very important property because tuberculosis still remains the leading cause of death in the world from a single infections disease. This ligand has high activity against *Mycobacterium tuberculosis* and is still one of most widely used drug in antituberculous regimens.

Although the activity of these compounds is only efficient against bacterium sensitive strains, more potent derivates may be active against the resistant strains as well [9]. It is important to understand the hydrazides mode of action before searching for a new related drug. Unfortunately, the exact mechanism of action is not known. Several hypotheses have been proposed to account for its mode of action [10,11]: (a) inhibition of mycolic acid synthesis; (b) inhibition of a catalase-peroxydase system causing biotransformation of the hydrazide; (c) depletion of intracellular nicotinamide ade-

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nine dinucleotide; and (d) reaction of hydrazide with tyrosine residues in mycobacterial proteins. It is assumed that these modes of action may not exclude each other and that the antimycobacterial activity of these compounds may be due to their cumulative effect. However, the first two hypotheses are favoured by more recent studies [12,13].

Furthermore, the metal ion involved presents also a special feature. Cupric ion with the d^9 configuration has ligand field stabilization in its complexes, and usually the Jahn-Teller effect is observed. This causes the hydrated ion $Cu(H_2O)_6^{2+}$ to have a distorted octahedral structure with weak absorption in the visible. Two of the water molecules in trans positions are further removed from Cu^{2+} ion than the other four, which are coplanar. Such structures usually lead to reduced stability for the fifth and the sixth bound ligands.

The present studies were undertaken to study the reaction kinetics of copper(II) with 2-pyridinecarboxylic acid hydrazide in aqueous solution to form the dichelated complex. The hydrogen ion concentration influence on the ligand and metal systems have been studied. In this sense, the ionization equilibria of the ligand and the stability of the metal–ligand complexes have also been characterized.

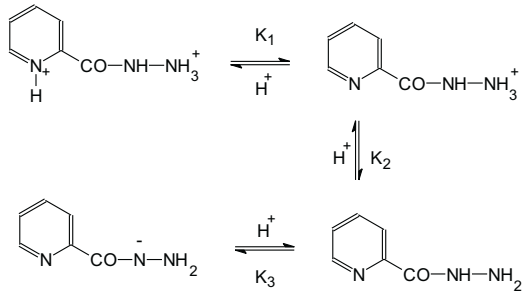
EXPERIMENTAL

Solutions of Cu(II) were prepared from reagent grade $CuSO_4 \cdot 5H_2O$ (Merck) and were standardized by reaction with an excess of potassium iodide. The liberated iodine was titrated with standard sodium thiosulphate. 2-Pyridinecarboxylic acid hydrazide (Hpcah) was prepared [14] from 2-pyridinecarboxylic acid (Merck), and characterized on the basis of elemental analysis, electronic and IR spectral measurements. Standard buffer solutions were obtained from HCl, potassium hydrogen phthalate, KH_2PO_4 and NaOH (Merck). Unless otherwise indicated, all materials were of analytical reagent grade, and they were used without further purification. A *Crison 2002* pH-meter, previously calibrated by titrating solutions of perchloric acid with standard sodium hydroxide solutions was used to read hydrogen ion concentration directly. Infrared spectra were recorded using *Perkin-Elmer 559* and *Beckman acculab 4* spectrophotometers. UV-visible spectra and kinetic runs were recorded using *Perkin-Elmer 200 UV-VIS* and *Spectronic 1201 UV-VIS* spectrophotometers, that included chart recorder and resident kinetic program. Kinetic experiments were performed by mixing a metal solution with a ligand solution, both of which have been previously adjusted to the same pH and ionic strength. The mixing time was less than 1 s.

RESULTS AND DISCUSSION

Equilibrium measurements: Prior to the aforementioned kinetic study, a spectrophotometric analysis of the ligand equilibria was carried out. From an analysis of UV spectra of 2-pyridinecarboxylic acid hydrazide in a sufficiently large pH-range, different equilibria have been identified (Scheme 1). The first equilibrium was characterized in the hydrogen ion concentration range 0.25 to 0.0125 mol dm^{-3}, the second between 6.3×10^{-3} and 1.0×10^{-5} and the third between 6.3×10^{-12} and 1.6×10^{-13}, and they presented isosbestic points at 205 nm, 235 nm and 270 nm, respectively. Ionization constants were determined spectrophotometrically [15] and the correspond-

Scheme 1



ing pK values were 1.11±0.02, 3.10±0.02 and 12.27±0.02, respectively. These values are in agreement with Nagano [1] conductimetrically obtained values. These spectra show two significant absorption maxim: the first around 225 nm is assigned to a transition ($\pi \longrightarrow \pi^*$) which is characteristic in amides of α-β-unsaturated acids; the second at 265 nm is attributed to a transition ($n \longrightarrow \pi^*$) characteristic of carbonyl groups. These assignations agree with values obtained from the literature, reference [16].

Complexation equilibrium: Once 2-pyridinecarboxylic acid hydrazide equilibria in aqueous solution have been established, copper(II) complexation reactions were studied. The complex, which Hpcah forms with copper(II) at low pH values, absorbs at 300 nm. However, a second complex is slowly formed at pH over 6, with a characteristic absorption maximum at 365 nm. These charge transfer transitions appear in complexes of transition metal ions with ligands which contain pyridinic structures [17–19].

The composition of the complexes that copper(II) forms with Hacph in aqueous solution has been investigated spectrophotometrically using, firstly, the method introduced by Job [20], in which the ligand molar fraction was increased by steps of 0.1 units keeping the total concentration ($[Cu^{2+}]+[Hacph]$) at the constant value of 1.0×10^{-3} mol dm^{-3}. Secondly, by using the "Yoe and Jones" method [21], a series of solutions of copper(II) (5.0×10^{-4} mol dm^{-3}) were prepared with varying ligand concentrations up to 2.5×10^{-3} mol dm^{-3}. On plotting absorption data against $[Hacph]/[Cu^{2+}]$, two straight lines were obtained whose crossing points show the stoichiometry. Finally, a COMIC plot was used. COMIC is a computer programme [22] for calculating equilibrium concentrations in a system of competing complexation.

Although the acidic complex presents a ML_1 composition, the second complex, obtained in neutral medium, shows a ML_2 stoichiometry which was confirmed by us even in solid state using IR spectroscopy. Infrared spectra of the pure compounds and their mixture in aqueous solution show that bonding between ligand and copper involves formation of five-membered chelate rings.

Schwarzenbach's stability constants for complexes, $K(ML_n) = [ML_n]/[M][L]^n$, were determined from absorption values at the stoichiometric point. These constants were $K(ML_1) = 3.85\pm0.1\times10^5$ and $K(ML_2) = 2.94\pm0.1\times10^9$. Since pH influence is so

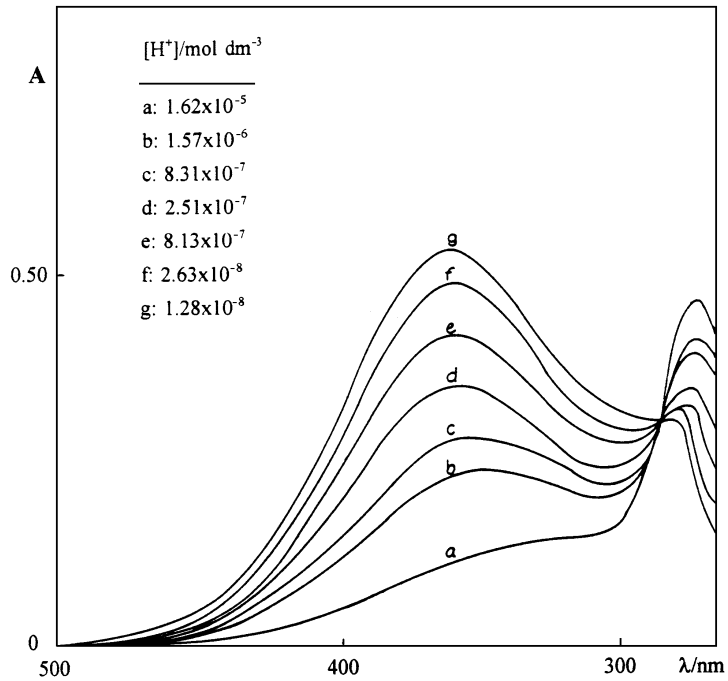


Figure 1. UV-visible spectra of complexes formed between copper(II) and 2-pyridinecarboxylic acid hydrazide in aqueous solutions.

important to the stability of these complexes, several experiments have been carried out in order to study the whole equilibrium. An isosbestic point was found located around 290 nm. The copper and ligand concentrations were 1.5×10^{-4} mol dm^{-3} and 2.5×10^{-4} mol dm^{-3}, respectively, and the proton concentrations ranged from 1.28×10^{-8} to 1.65×10^{-5} mol dm^{-3} (Fig. 1). Based on these experiments the following equilibrium between monochelated and bichelated complexes may be established, equilibrium which fits well with experimental results (It will be also demonstrated in Mechanism of Complex Formation section).

$$2Cu(Hpcah)^{2+} \rightleftarrows Cu(pcah)_2 + Cu^{2+} + 2\,H^{+} \quad (1)$$

The corresponding equilibrium constant was determined by a spectrophotometric method [23]. The wavelengths used were 260 nm and 320 nm and the equilibrium constant was 0.34±0.1. This value was the average of three determinations.

Kinetic study: The reaction of complex formation was carried out with the ligand concentration in excess of that of the metal ion in order to ensure pseudo-first-order conditions. The acidic conditions for kinetic measurements were limited by those hydrogen ion concentrations below which hydrolysis of copper(II) was appreciable. On the other hand, the pH was kept high enough to ensure dichelate formation.

The stability of $CuOH^+$ has been reported by several researchers. Pedersen and Perrin's [24] measurements in dilute $Cu(NO_3)_2$ give K_{11}~10^{-8} as the hydrolysis constant of Cu^{2+} to form $Cu(OH)^+$ and, therefore, we consider this value the most reliable in our ionic strength conditions. The second hydrolysis product of Cu^{2+}, which could be considered, is $Cu_2(OH)_2^{2+}$ (pK_{22} = 10.99). However, it also presents a negligible concentration during kinetic experiments. Pseudo-first-order constants, k_{exp}, were obtained by fitting absorbance data to the general first-order kinetic equation

$$\ln(A_\infty - A) = k_{exp}t + C \qquad (2)$$

where A and A_∞ are the absorbances of the reaction system at t seconds after the start of the reaction and at equilibrium, respectively. Each observed rate constant, k_{exp}, subsequently used for further calculation, is the average of at least three determinations. Several series of experiments were carried out to study the influence of the hydrogen ion and ligand concentrations on the observed rate constants, while the copper concentration, ionic strength, and temperature remained constant.

Table 1 contains kinetic data for the reaction of copper(II) with Hacph in aqueous solution at 25°C and ionic strength 0.5 mol dm^{-3} $NaClO_4$.

Table 1. Kinetic data for reaction of copper(II) with Hpcah in aqueous solution at 25°C and ionic strength 0.5 mol dm^{-3}. $[Cu^{2+}] = 1.02\times10^{-4}$ mol dm^{-3}.

$[Hpcah]10^3$/mol dm^{-3}	10^7 $[H^+]$/mol dm^{-3}	10^3 k_{exp}/s^{-1}	10^3 k_{calc}/s^{-1}
1.0	2.5	0.99	0.94
1.0	5.1	0.48	0.46
1.0	7.6	0.33	0.31
1.0	10.1	0.24	0.23
1.5	2.5	1.36	1.41
1.5	5.1	0.66	0.69
1.5	7.6	0.45	0.47
1.5	10.1	0.33	0.35
2.0	2.5	1.85	1.88
2.0	5.1	0.88	0.92
2.0	7.6	0.64	0.62
2.0	10.1	0.47	0.47
2.5	2.5	2.42	2.35
2.5	5.1	1.18	1.15
2.5	7.6	0.81	0.77

Table 1 (continuation)

2.5	10.1	0.56	0.58
3.0	2.5	2.68	2.82
3.0	5.1	1.35	1.38
3.0	7.6	0.92	0.93
3.0	10.1	0.69	0.70
3.5	2.5	3.36	3.30
3.5	5.1	1.58	1.61
3.5	7.6	1.07	1.08
3.5	10.1	0.79	0.82

Mechanism of Complex Formation: Several mechanisms have been examined in order to explain the reaction under study. The dependence of k_{exp} on ligand and hydrogen ion concentration (Figure 2) shows that k_{exp} increases linearly with rising ligand concentrations. This is consistent with a mechanism that may be represented by the following scheme (solvent molecules are omitted for the sake of simplicity):

Scheme 2

$$Cu^{2+} + Hpcah \xrightarrow{k_1} Cu(Hpcah)^{2+} \quad (3)$$

$$Cu(Hpcah)^{2+} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Cu(pcah)^{*} + H^{+} \quad (4)$$

$$Cu(pcah)^{*} + Hpcah \xrightarrow{k_3} Cu(pcah)_2 + H^{+} \quad (5)$$

where $Cu(pcah)^{*}$ is an intermediate complex. Since a preliminary study of equilibrium and kinetics of complexation shows that $Cu(Hpcah)^{2+}$ formation is much faster than $Cu(pcah)_2$ formation, the rate of formation of $Cu(pcah)_2$ may be expressed as:

$$v = k_3[Cu(pcah)^{*}][Hpcah] \quad (6)$$

Assuming that $[Cu(pcah)^{*}]$ is present in a steady state, under pseudo-first-order conditions, Scheme 2 predicts that k_{exp} has the form of (7).

$$k_{exp} = k_2k_3[Hpcah]/(k_{-2}[H^{+}] + k_3[Hpcah]) \quad (7)$$

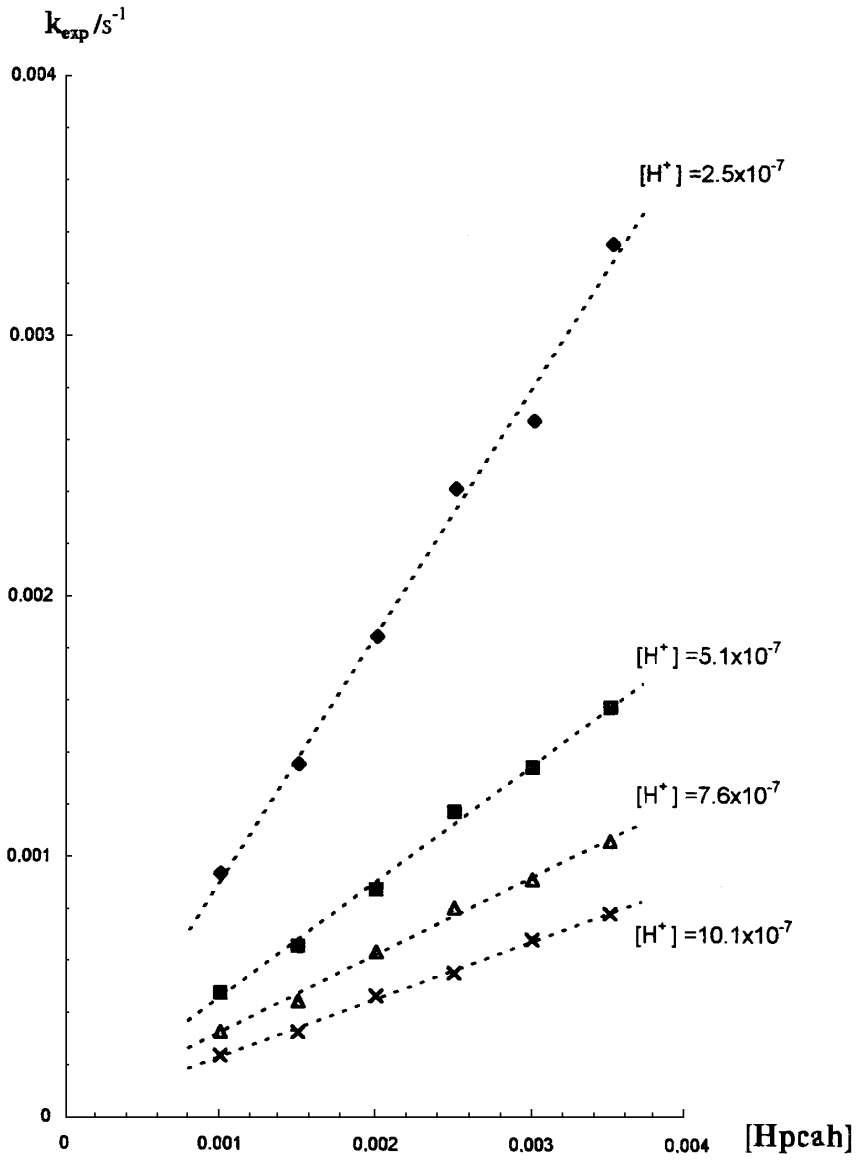


Figure 2. k_{exp} as a function of the total concentration of Hpcah at 25°C and ionic strength 0.5 mol dm^{-3}. [Cu^{2+}] = 1.02×10^{-4} mol dm^{-3}.

It is apparent that depending on the relative magnitude of k_{-2} and k_3 rate constants, a variety of kinetic behaviour can be exhibited. The kinetic data shown in Table 1 ([Hpcah], [Cu^{2+}] and k_{exp}) were fitted by a curve-fitting routine (NAG Fortran Library) to eqn. 7. The rate constant obtained for the reaction determining step was k_3 = 0.075±0.005 mol^{-1} dm^3 s^{-1}. The agreement between k_{exp} and k_{cal} values is quite satisfactory over the range of ligand and hydrogen ion concentrations studied as can be seen in Table 1. When Cu^{2+} water exchange rate [25] (k_s = 4.4×10^9 s^{-1}) is taken into

account, it is apparent that the rate constant for the reaction of Cu^{2+} with Hacph is considerably less than those predicted on the basis of the Eigen-Wilkins mechanism [26,27]. Assuming an ion pair association constant of 0.3 mol^{-1} dm^3 [28], the value of the rate constant for reaction of Cu^{2+} with 2-pyridinecarboxylic acid hydrazide should be approximately 9.9×10^9 mol^{-1} dm^3 s^{-1}.

In conclusion, it would appear that a reasonable interpretation of the kinetic results would be that conversion of Cu^{2+} to $Cu(pcah)_2$ proceeds at rates that are greatly retarded. This slow rate of dichelate formation can be rationalized by postulating that the closing of five membered chelate rings is sterically hindered.

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