Acid–base equilibria of the Zn(II) and Fe(III) complexes with condensation products of 2-acetylpyridine and the dihydrazide of oxalic and malonic acid

BRANKA DRAŽIĆ1, GORDANA POPOVIĆ1*, RATOMIR JELIĆ2, DUŠAN SLADIĆ3#, DRAGANA MITIĆ3#, KATARINA ANDELKOVIĆ3# and ŽIVOSLAV TEŠIĆ3#

1Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P.O. Box 146, 11000 Belgrade, 2Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac and 3Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, P.O. Box 158,11000 Belgrade, Serbia

(Received 22 May, revised 4 November 2008)

Abstract: Acid–base equilibria of Zn(II) and Fe(III) complexes with N',N2-bis-[(1E)-1-(2-pyridyl)ethylidene]ethanedihydrazide (ligand L1) and N',N2-bis[(1E)-1-(2-pyridyl)ethylidene]propanedihydrazide (ligand L2), i.e., [Fe(L1)Cl(H2O)]+, [Fe(L2)Cl(H2O)]2+, [Zn(L1)(H2O)3]+ and [Zn(L2)(H2O)2]2+, which expressed cytotoxic activity, were investigated in aqueous media. The equilibrium constants were determined potentiometrically at 25 °C at a constant ionic strength of 0.10 mol/dm3 (Na2SO4). The results showed that at pH < 8 both the Fe(III) complexes studied here have three, while [Zn(L1)(H2O)3]+ and [Zn(L2)(H2O)2]2+ have one and two titratable protons, respectively. Based on the obtained values for the equilibrium constants, protonation schemes of the examined complexes are proposed.

Keywords: metal complexes; d-metals; hydrazone; acid–base equilibria; potentiometry.

INTRODUCTION

Our studies have been directed for several years toward the synthesis, characterization and examination of the biological activity of d-metal complexes containing condensation products of 2-acetylpyridine and different hydrazides and hydrazines as ligands.1–5 Within the scope of these studies, Zn(II) and Fe(III) complexes with condensation derivatives of 2-acetylpyridine and the dihydrazide of either oxalic or malonic acid were synthesized.5 Among all the examined series of complexes, it was shown that the Zn(II) and Fe(III) complexes expressed the highest cytotoxic activity on HeLa and B16 cell cultures in vitro.6

* Corresponding author. E-mail: gpopovic@pharmacy.bg.ac.rs
# Serbian Chemical Society member.
doi: 10.2298/JSC0903269D

2009 Copyright (CC) SCS
Acid dissociation constants are particularly important in pharmaceutical research, especially for the discovery and evaluation of new compounds that could be pharmacologically active, i.e., that represent potential drugs. Knowledge on the distribution of the equilibrium forms of a new compound as a function of pH can be of value in the estimation of the absorption, distribution, metabolism and excretion of a drug, because different equilibrium forms will evolve on its contact with body fluids, disregarding the route of its application. This prompted us to study acid–base equilibria of Zn(II) and Fe(III) complexes with \( N',N'^2\)-bis-\([(1E)-1-(2-pyridyl)ethylidene]ethanedihydrazide and \( N',N'^2\)-bis-\([(1E)-1-(2-pyridyl)ethylidene]propanedihydrazide in aqueous media. The chemical structures of the investigated complexes are shown in Fig. 1.

![Fig. 1. Chemical structure of the examined Zn(II) and Fe(III) complexes.](image)

**EXPERIMENTAL**

**Apparatus and reagents**

UV–Vis spectra were recorded using a GBC UV–visible Cintra 6 spectrophotometer. For potentiometric titrations, a TTT60 titrator, with an automatic 2.5 cm\(^3\) ABU12 burette with an accuracy of 0.001 cm\(^3\) (Radiometer, Copenhagen, Denmark), was used. The titration mixtures were stirred with a mechanical M22 stirrer (Radiometer) at a constant temperature of...
25±0.1 °C, pH-Metric measurements were performed using a PHM240 pH-meter and a combined GK240B electrode (Radiometer). The measured pH values were transformed into p\text{c}H values \( (\text{pc}H = -\log[H_3O^+]) \) applying the relation:

\[
\text{pc}H = \text{pH} - A
\]

The correction factor, \( A = 0.55 ± 0.01 \), was determined based on the data of the potentiometric titration of HCl solution (0.007672–0.009424 mol/dm\(^3\)) with standard \( \text{NaOH} \) solution, at 25 °C and constant ionic strength of 0.10 mol/dm\(^3\) \( \text{Na}_2\text{SO}_4 \).

The syntheses and characterization of the Zn(II) and Fe(III) complexes with \( \text{N}'\text{N}'_2\text{bis-}[\text{(1E)}-1\text{-}(2\text{-pyridyl})\text{ethylidene}]\text{ethanedihydrazide} \) (ligand \( \text{L}1 \)) and \( \text{N}'\text{N}'_2\text{bis-}[\text{(1E)}-1\text{-}(2\text{-pyridyl})\text{ethylidene}]\text{propanedihydrazide} \) (ligand \( \text{L}2 \)) were described previously. The complexes, prepared by direct and template synthesis and characterized by elemental analysis, \(^1\text{H}-\text{NMR}\) and \(^13\text{C}-\text{NMR}\) spectroscopy, and X-ray analysis, were kept in a cold, dry and dark place. During the experiments described herein, only freshly prepared solutions of the above complexes in deionized water were used. All other reagents were of analytical grade purity (Merck, Darmstadt, Germany). Hydrochloric acid and sodium hydroxide solutions were potentiometrically standardized.

**Reversibility of acid–base processes**

Three 5.0\times10\(^{-5}\) mol/dm\(^3\) solutions of each of the examined metal complex were prepared in 0.10 mol/dm\(^3\) \( \text{Na}_2\text{SO}_4 \); solution A, pH 3.3–3.5 (precisely measured), adjusted by adding \( \text{H}_2\text{SO}_4 \); solution B, pH 7.0–8.0, adjusted with \( \text{NaOH} \), and solution C, prepared by acidifying solution B with \( \text{H}_2\text{SO}_4 \) to the pH values of solution A.

The spectra of the above solutions were recorded within the wavelength range from 200–500 nm, using a 0.10 mol/dm\(^3\) \( \text{Na}_2\text{SO}_4 \) solution as the blank.

**Determination of the equilibrium constants**

Equilibrium constants of the examined complexes were determined potentiometrically in aqueous solutions at 25 °C and constant ionic strength of 0.10 mol/dm\(^3\) \( \text{Na}_2\text{SO}_4 \). Solutions of the complexes (25 cm\(^3\); \( c = 7.3\times10^{-4} \) mol/dm\(^3\)) were titrated with 0.020 cm\(^3\) aliquots of a standard carbonate-free \( \text{NaOH} \) solution (\( c = 0.0992–0.1052 \) mol/dm\(^3\)). The titrations were performed immediately after the solutions were prepared and after 1, 3, 5 and 7 h.

The equilibrium constants of the complexes were evaluated using the data obtained in three independent titrations. The experimental data were analyzed using the HYPERQUAD computer program.

**RESULTS AND DISCUSSION**

The acid–base equilibria of the metal complexes 1–4 were examined in aqueous medium within the pH range 3.0 to 8.0. The reversibility of these processes was confirmed spectrophotometrically for each complex on the basis of the absorption spectra of their solutions in acidic media (pH 3.52, 3.44, 3.34 and 3.52 for the complexes 1, 2, 3 and 4, respectively), then at pH 7.0–8.0 (pH 7.03, 7.00, 7.71 and 7.10 for the complexes 1, 2, 3 and 4, respectively) and the spectra of the solutions obtained by acidifying the neutral solutions to the desired pH value within acidic pH range. Reversible changes of the spectra during the change of acidity of all four examined complexes were registered, i.e., an overlapping of the spectrum of a complex prepared in an acidic medium with its spectrum after
the change of the pH of its solution from neutral to acidic apparently results from the acid–base equilibrium. The absorption spectra of complex 2 are shown in Fig. 2 as a representative example.

![Absorption spectra of complex 2 in solutions of different pH.](image)

Fig. 2. Absorption spectra of complex 2 in solutions of different pH. 1) pH 3.44; 2) pH 7.00; 3) pH 3.44 (solutions were prepared by acidifying the solution of pH 7.00).  

$c (\text{complex}) = 5.0 \times 10^{-5} \text{ mol/dm}^3$.  

In Fig. 2, curve 1 represents the spectrum of the solution prepared by dissolving complex 2 in 0.10 mol/dm$^3$ Na$_2$SO$_4$, the pH of the resulting solution being 3.44. Changing the pH of this solution to 7.00 (curve 2) led to a change in the absorption spectrum and a peak at 274 nm appeared. Curve 3 represents the spectrum of complex 2 after changing the pH from 7.00 back to the starting value of 3.44. As seen, curves 1 and 3 overlap each other.

The number of titratable protons in aqueous solutions of the examined complexes was determined on the basis of the moles of NaOH consumed per mole of the complex during potentiometric titration.  

From the corresponding titration curves presented in Fig. 3, it can be concluded that within the pH range from 3.0–8.0, the examined complexes contain a single (complex 3), two (complex 4) or three (complexes 1 and 2) acidic groups. The equilibrium constants ($\log \beta_{pqr} = c(M_pL_qH_r)/c(M)^pc(L)^qc(H)^r$) were calculated based on the data of the potentiometric titrations, assisted by the HYPERQUAD computer program (Table I).

The results of the pH-metric titrations up to pH 8.0 were used for the calculation of the constants. Acid–base processes of the complexes in alkaline media were not examined because of their decreased solubility and precipitation.

The protolytic equilibria of the examined complexes, presented in Figs. 4–7, are proposed. The three constants obtained for complex 2 (Fig. 5) point to the substitution of the chloro ligand with a water molecule, as well as to the titration
TABLE I. Stability constants (log $\beta_{pqr}$) and the dissociation constants (pK) of the Fe(III) and Zn(II) complexes at $t = 25$ °C and $I = 0.10$ mol/dm$^3$ Na$_2$SO$_4$. Values in parentheses refer to the estimated standard deviations

<table>
<thead>
<tr>
<th>Complex</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>log $\beta_{111}$</td>
<td>$-2.54(12)$</td>
<td>$-3.01(4)$</td>
<td>$-4.44(1)$</td>
<td>$-3.88(2)$</td>
</tr>
<tr>
<td>log $\beta_{112}$</td>
<td>$-5.56(8)$</td>
<td>$-6.87(2)$</td>
<td>$-9.76(4)$</td>
<td>$-5.88(2)$</td>
</tr>
<tr>
<td>log $\beta_{113}$</td>
<td>$-11.09(9)$</td>
<td>$-12.72(3)$</td>
<td>$-9.76(4)$</td>
<td>$-5.88(2)$</td>
</tr>
<tr>
<td>pK$_1$</td>
<td>2.54</td>
<td>3.01</td>
<td>4.44</td>
<td>3.88</td>
</tr>
<tr>
<td>pK$_2$</td>
<td>3.02</td>
<td>3.86</td>
<td>$-$</td>
<td>5.88</td>
</tr>
<tr>
<td>pK$_3$</td>
<td>5.53</td>
<td>5.85</td>
<td>$-$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

Fig. 4. Protonation scheme of complex 1.
of protons from both coordinated water molecules and the third proton from ligand $L_2$ (–N(OH)–N=). The constant $pK_1 = 3.01$ corresponds to one coordinated water molecule and the other two constants $pK_2 = 3.86$ and $pK_3 = 5.85$ could be ascribed to the hydroxylimino group of ligand $L_2$ and the second coordinated water molecule, respectively. This conclusion was drawn based of a comparison of the constants for complex 2 with those of the Zn(II) complex containing the same ligand (complex 4) (Fig. 7). Namely, taking into consideration the higher charge of Fe(III) in relation to Zn(II), lower $pK$ values of the analogous groups in complex 2 would be expected. The constants $pK_1 = 3.88$ and $pK_2 = 5.88$ for complex 4 would belong to one coordinated water molecule and the hydroxylimino group of ligand $L_2$, respectively. The three constants of the Fe(III) complex 1 (Fig. 4) demonstrate the substitution of two chloro ligands with water molecules, as well as the involvement of all three water molecules in the acid–base equilibria (ligand $L_1$ contains no acidic groups). However, since ligand $L_1$ also contains one pyridine nitrogen atom that represents a non-coordinated basic center, it could be hypothesized that it also participates in these equilibria. Based on the pyridine $pK$ of 5.2, it could be supposed that a proton from one coordinated water molecule is transferred to the pyridine nitrogen atom and that the obtained three acidity constants belong to coordinated water molecules ($pK_1 = 2.54$ and $pK_3 = 5.53$) and the protonated pyridine nitrogen atom ($pK_2 = 3.02$). The increased acidity of the protonated pyridine of ligand $L_1$ in the complex in relation to pyridine itself was to be expected due to a decreased electron density at nitrogen atom resulting from electron-attractive effect of the ortho substituent, as well as from the influence of Fe(III) through the system of conjugated bonds. Since only one constant

![Protonation scheme of complex 2.](image-url)
was determined for complex 3 ($pK_1 = 4.44$) (Fig. 6), it was assumed that proton transfer from one molecule of coordinated water to the pyridine nitrogen atom in ligand $L_1$ occurs and due to the lower electron-attractive effect of Zn(II) as compared to that of Fe(III), the obtained value was higher in relation to the analogous Fe(III) complex (complex 1) (Fig. 4).

Fig. 6. Protonation scheme of complex 3.

Based on the determined constants, it was possible to calculate equilibrium concentrations of each particle present in solution at a defined pH value. Representative distribution of the equilibrium species of the complex 1 as a function of pH is depicted in Fig. 8.

Fig. 7. Protonation scheme of complex 4.

Fig. 8. Distribution of the equilibrium species of complex 1 as a function of pH.
From Fig. 8, it can be seen that at pH < 4.00, three forms of complex 1 are in equilibrium: the dication \([\text{FeL}^2+]\), the monocation \([\text{FeLH}^–1]^+\) and the neutral form \([\text{FeLH}^–2]^0\). Within the pH range 4.00 to 7.00, the neutral form and monoanion \([\text{FeLH}^–3]^–\) were dominant, while at pH > 7, the solution contained only the monoanion.

The uncharged forms of complexes 2–4 were predominant at pH 7.40 (the physiological pH of human body fluids), while complex 1 had a single negative charge. This result is significant for studies on the transport of the examined complexes through cell membranes, which are usually permeable for uncharged moieties, i.e., liposoluble species.

Acknowledgement. This work was supported by the Ministry for Science and Technological Development of Serbia, Contract No. 142062.

REFERENCES