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Determination of Stability Constants of Triazole Ligand Carrying Naphthol Group with Heavy Metal Ions in Aqueous Solutions

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Abstract
Interaction of 4-(2-hydroxy-1-naphthylmethylamino)-3-methyl-5-(4-tolyl)-4H-1,2,4-triazole with heavy metal cations such as Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} was investigated by using UV-visible spectrophotometric technique. The complex stability constants (Log β) were determined in aqueous as well as in methanol: water (1:1) system at 25 ± 0.1°C by Buschmann's method and Valeur's methods, respectively. The ligand showed good sensitivity for Co^{2+} with a linear range of 2×10^{-6}M to 3×10^{-5}M.

Keywords**:** Triazole; Complex; Stability constant .

Introduction

Azole compounds are clinically important antifungal drugs; fluconazole, tercanazole, voriconazole and posaconazole may be examples for these compounds. They can effectively inhibit the biosynthesis of ergosterol, which is the main sterol composition in the fungal cell membrane, which needs sterols lacking C-4 methyl groups. The nitrogen of N-4 in triazole compounds binds with the iron of HEM of cytochrome p450 at the molecular level. Thus, inhibition of cytochrome p450 is caused to stop the demethylation reaction [1-3]. This mechanism provides antifungal activity of triazole derivatives.

Heterocycles including amino group can be considered as useful intermediates in organic synthesis. The amino group is ready-made nucleophilic center for the synthesis of condensed heterocyclic ring [4-5]. Heterocyclic ligands can be obtained by the condensation reaction of small compounds with suitable reagents [6]. Therefore, ester ethoxycarbonylhydrazones are used as starting materials. Many azole derivatives starting from corresponding hydrazones were prepared [7-10].

It is important to investigate metal complexation of triazole compounds in solution because; such type of complexation is present in biological systems. Current efforts are directed toward the investigation of triazole systems, which are capable of acting as functional drug mimics and could be used in particular reactions in highly specific manner. Copper (II) aminomethyltriazole shows antiproliferative activity. Similarly, aminomethylthioxotriazoline complexes are discussed in literature [11]. Different antiproliferative species are more effective and less toxic anticancer drugs [12-13]. Several 1,2,4-triazole compounds having biological activities such as antielastase, antiurease, antioxidant, antibacterial, antifungal, and antitumor agents have

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*Corresponding Author Email: ngumrukcuoglu@ktu.edu.tr also been synthesized in our laboratories [7-9, 14]. The triazole compounds have antimicrobial activity for bacteria and yeast-like fungi [10].

This paper describes the complexation properties of 1,2,4-triazole compounds carrying naphthol group with some heavy metal cations by using UV-visible spectrometry. 4-Amino-3-methyl-5-(4-tolyl)-4H-1,2,4-triazole was prepared from ethyl N-acetyl-4-methylbenzenecarbohydrazonoate and hydrazine by using the earlier method [10]. The synthesis of certain 4-(2-hydroxy-1-naphthylmethylenamino)-3-methyl-5-(4-tolyl)-4H-1,2,4-triazole was carried out by treating the amino compound with 2-hydroxy-1-naphthaldehyde. In early studies, the selective reduction of only the imino group of compound 4-alkylidenamino-4H-1,2,4-triazole without affecting the rest part of the ring has been reported [9, 14-16]. Thus, the synthesis of 4-(2-hydroxy-1-naphthylmethylamino)-3-methyl-5-(4-tolyl)-4H-1,2,4-triazole was established in good yields by the use of $NaBH_4$ as a selective reducing agent.

Experimental

Chemicals and apparatus

Merck and Fluka were commercial source for all the chemicals that are used in this research. The anhydrous salts $Ni(NO_3)_2.6H_2O$, $Pb(NO_3)_2.2H_2O$, $Cu(NO_3)_2.3H_2O$, $Co(NO_3)_2.6H_2O$, $Cd(NO_3)_2.4H_2O$ had the highest purity available and they were used without further purification in doubly distilled water was used as solvent.

Thermo Scientific Evo 60 spectrophotometer was used to record the absorption spectra of the compound.

Measurements

Buschmann's method

A spectrophotometric method to determine the complex stability constants of the ammonium and alkylammonium ions with many of dibenzo crown ethers in aqueous solution was developed by Buschmann [17]. These compounds are nearly insoluble ligands in aqueous media. Buschmann's method was used to calculate the stability constants of metal complexes with 1,2,4-triazole ligand **1** in water. Sufficiently high amounts of the solid ligands were thermostated at 25°C and shaken at intervals by adding then into salt solutions (1×10^{-3} - 1×10^{-2} mol L^{-1}) to ensure the formation of saturated solutions. Before recording their absorption spectra 3 days later, they were centrifuged and the resulting clear solutions were filtered through a membrane filter (polycarbonate, 0.4 µm) to remove any undissolved ligand. The maximum absorbance wavelength was used to evaluate complex formation. The stability constants for all metal ions, were measured at 302 nm.

Valeur's method

Modified Valeur's method was used to determine the complex stability constants in solution [18]. Stock solutions of the ligand and the metal salt were prepared in methanol and water, respectively. The absorbance of the solutions containing a fixed concentration of ligand (5×10^{-6} M) was measured at 206 nm with various salt concentrations using 1-cm long absorption cell. These concentrations were obtained by appropriate dilution of a 1×10^{-4} M stock solution.

Synthesis

Ligand **1** was prepared according to the earlier method [10].

Results and Discussion

Treatment of the experimental data for Buschmann's method

Equation (1) may represent the formation of a 1:1 complex between a ligand L and a cation M^{n+}.

$$L + M^{n+} \rightleftharpoons LM^{n+} \qquad (1)$$

The corresponding stability constant is defined as:

$$K = \frac{[LM^{n+}]}{[L][M^{n+}]} \qquad (2)$$

If only the ligand and the complex formed absorb at a given wavelength the experimentally measured absorptivity A' for an optical path length d is given by equation (3).

$$A = (A'/d) = \varepsilon_1 [L] + \varepsilon_2 [LM^{n+}] \quad (3)$$

Where, ε1 and ε_2 are the molar absorptivities of the ligand and complex, respectively. The first term in Equation (3) is constant because the salt solution is saturated with the ligand. Consequently:

$$A_o = \varepsilon_1 [L]_{sat} \quad (4)$$

Where, $[L]_{sat}$ denotes the solubility of the ligand in the pure solvent. The material balances (5) and (6) can be derivated by using Equation (2).

$$c_M = [M^{n+}] + [LM^{n+}] \quad (5)$$

$$c_L = [L]_{sat} + [LM^{n+}] \quad (6)$$

c_M and c_L are the analytical concentration of the ion and the ligand in solution, respectively. Equation (7) can be obtained from Equation (3).

$$(A/A_o)-1 = [(\varepsilon_2 / \varepsilon_1) Kc_{salt}] / (1+K [L]_{sat}) \quad (7)$$

Plotting (A/A_o)-1 as a function of the total salt concentration c_{salt} one gets a straight line with slope b. From this slope, the stability constant of the complex formed in solution can be calculated according to Equation (8).

$$K = b/ [(\varepsilon_2 / \varepsilon_1)-b [L]_{sat}] \quad (8)$$

The stability constant K is given by slope b, if molar absorptivities are nearly similar and the solubility of the ligand is low. The correctness of these assumptions has already been showed for some macrocyclic ligands such as benzo and aza crown ethers, cryptands, and cucurbit[n]urils [19-23].

Treatment of the experimental data for Valeur's method

In order to determine the complex stability constant, the ratio of $A_o/(A_o-A)$ was plotted against 1/[M], which gave a good straight line. A_o and A are the absorbance of the free ligand and the absorbance of the solution containing the cation, respectively. The stability constant was calculated from the ratio intercept/slope [18].

The complex stability constants of the ligand

Recently, Buschmann has shown that the determination of the total concentration of a ligand in a salt solution by using UV-Vis spectroscopy allows the determination of the stability constant of the complex formed in solution. It is not necessary to know definitely the relation between the measured signals and the ligand. The measured signal only has to be directly proportional [17]. The molar absorptivities of the ligand and complex, ε1 and ε_2, should be nearly the same to apply the method. In the earlier study, this approach was used to determine the complex stability constants of some 1,2,4-triazole ligands contaning substituted benzylidenamino group and their reduced forms for Cu^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Ag^{+} cations [24]. The measurements were carried out at an appropriate wavelength in which the enhancements of absorbance are regular when metal concentration increases. The regular evolution between (A/A_0)-1 and c_{salt} for the tested metal ions was observed. Therefore, the complex stability constants were calculated for the cations with the triazole ligands.

In this study, the complex stability constants for Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} cations with a 1,2,4-triazole ligand carrying naphthole amino group (Fig. 1) have been reported. Firstly, Buschmann's method was used to determine the complex stability constants in aqueous solution. For Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} cations, straight lines were found in accordance with Eq. (7). Owing to the complex formation, the total concentration of the ligand in solution, monitored by spectrophotometric measurements, increased. The quantity (A/A_0)-1 was plotted against c_{salt}, and the stability constant was obtained from the slope. (Fig. 2a) shows the variation of the absorption spectra of ligand **1** with increasing Co(II) concentration. A linear response of (A/A_0)-1 versus c_{Co2+} for ligand **1** with Co^{2+} was given by equation y=13026×-1.43212 with a correlation coefficient of 0.9992 in (Fig. 2b).

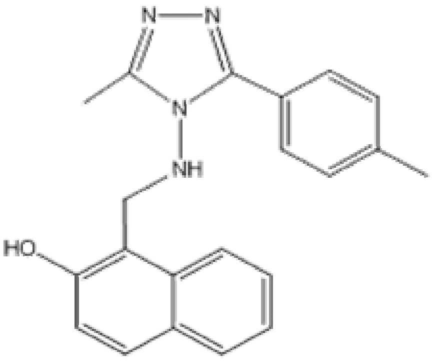

Figure 1. **The structure of ligand 1 used in the present study**

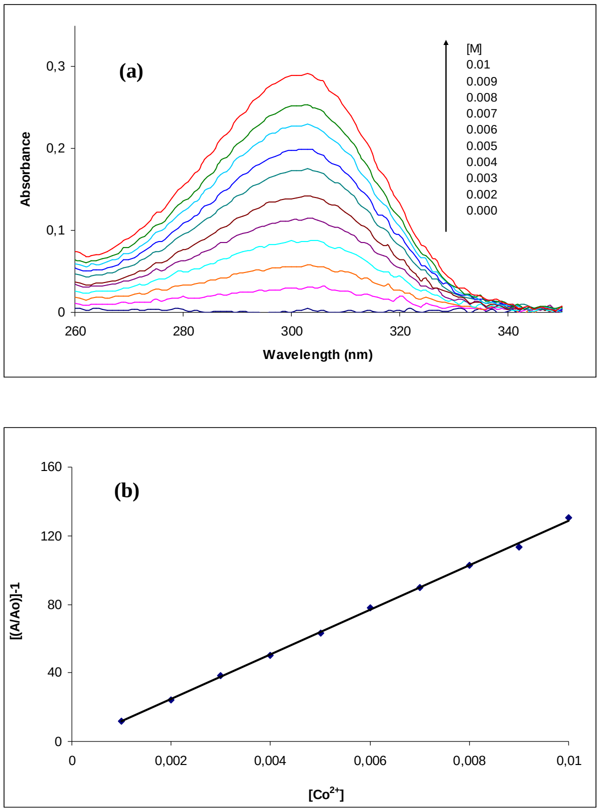

Figure 2. (**a) The variation of absorption spectra of ligand 1 (saturated) in water with increasing concentrations of Co^{2+}; (b) (A/A_o)-1 versus Co (II) concentration for the spectrophotometric titration of ligand 1 with Co (II) nitrate in water**

The stability constants for complexes of Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} with ligand **1** were determined by using Valeur's method in methanol:water (1:1). The various concentrations of metal nitrate were added to the methanolic solution of ligand **1**. The ligand concentration was constant at 5×10^{-6} M. The stability constants were determined from the change in absorbance as a function of cation concentration. Regular increase of absorbance with increasing metal ion concentration was observed in all spectrophotometric titrations.

Figure 3a shows the absorption spectra of ligand **1** in methanol:water (1:1) with increasing concentrations of Cu^{2+}. The complex stability constant (β) was calculated according to Valuer's method [18]. Accordingly, the quantity $A_0/(A_0\text{-}A)$ was plotted against $[Cu^{2+}]^{-1}$ with the stability constant given by the ratio of intercept/slope. (Fig. 3b), a plot of $A_0/(A_0\text{-}A)$ versus $[Cu^{2+}]^{-1}$ for ligand **1**, shows satisfactory linearity. Similar linearity for all metal cations with ligand **1** was obtained.

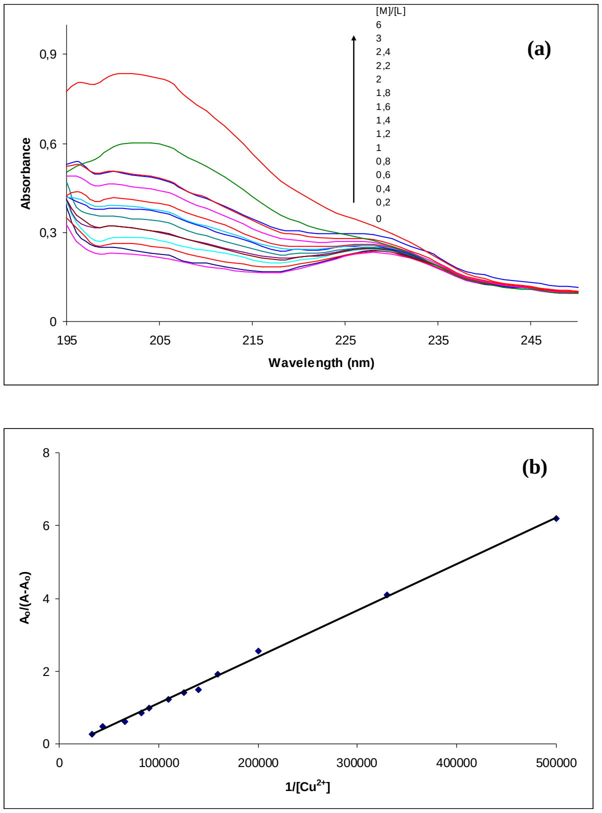

Figure 3. **(a) The variation of absorption spectra of ligand 1 (5×10^{-6} M) in methanol:water (1:1) with increasing concentrations of Cu^{2+}; (b) $A_o/(A_o\text{-}A)$ versus $1/[Cu^{2+}]$ for the spectrophotometric titration of ligand 1 with Cu^{2+} in methanol:water (1:1)**

A regular absorbance increase was detected for Co^{2+} in the spectrophotometric titration with ligand **1** at 206 nm. A linear response of the absorbance as a function of Co^{2+} concentration at this wavelength was observed from 2×10^{-6} to 3×10^{-5}M, where R^2 was 0.9925 (Fig. 4). Also, Job method was used to determine the complex composition. Figure 5 shows Job plot for Co^{2+}-ligand complex. As seen from (Fig. 5), Co^{2+} forms 1:1 complex with the ligand. The isosbestic point in (Fig. 5) verifies the equilibrium between Co^{2+}-ligand.

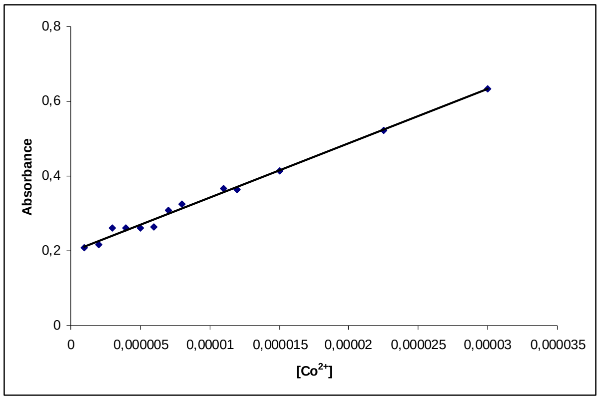

Figure 4. **The variation of absorbance of ligand 1 with increasing concentrations of Co^{2+} during the spectrophotometric titration. Wavelength:206 nm, Ligand concentration=5×10^{-6}M**

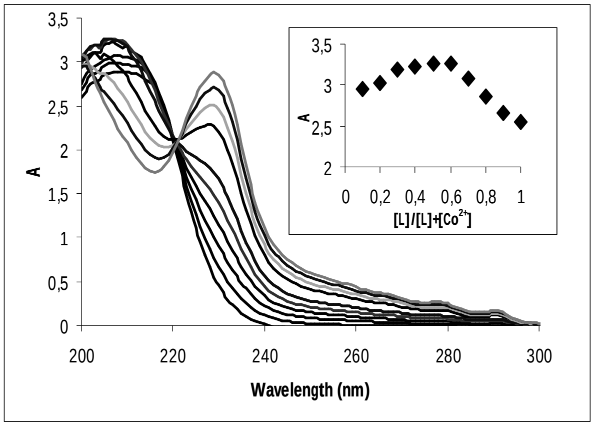

Figure 5. **Job plot for the Co^{2+}-ligand complex**

To propose a mode of metal-ligand coordination, ^{1}H NMR spectroscopic method was applied. DMSO-d_6 and metal nitrate was used in this experiment. As an example, Cd^{2+}-ligand interaction was investigated with this technique. While NH proton was observed at 6.70 ppm in ^{1}H NMR spectra of free ligand, it appeared in 6.89 ppm in case of equivalent Cd^{2+}-ligand mixture. Therefore, it is decided Cd^{2+} interacted with the NH nitrogen in the triazole ring. A reasonable change in the chemical shift of the OH proton after cadmium nitrate was added to the free ligand solution was not observed. Therefore, one can decide that hydroxyl group was not effective in the complexation. Also, the pH values of the solutions were measurement during the spectrophotometric titrations and no reasonable change in pH values indicating that there is no proton release during the complexation. This result shows that metal ion is covalently coordinated to nitrogen atoms in the triazole ring. (Fig. 6) shows the proposed mode of metal-ligand coordination for Co^{2+} ion.

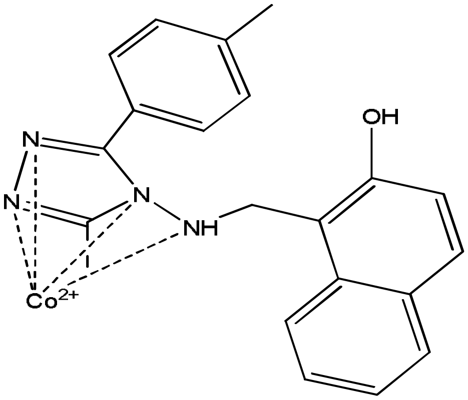

Figure 6. **The proposed mode of metal-ligand coordination**

Table 1 shows the stability constants with Log β determined by means of both methods for Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} ions with ligand **1**. For these cations, the value of the stability constants decreased in the order $Co^{2+} > Ni^{2+} > Cu^{2+} > Cd^{2+} > Pb^{2+}$ in aqueous solution. In methanol:water (1:1), nearly the same order is valid for these cations, except for Ni^{2+} and Cu^{2+}, where the Cu(II) complex (log β: 4.25) is more stable than the Ni(II) complex (log β: 3.99) (see Table 1). However, in water, the log β value is 3.50 for Cu(II) while it is 3.72 for Ni(II). The most stable complex is the Co (II) complex in water. The value of log β is 4.11 for this complex. The reason of this may be preference of Co (II) more water molecules binding to the ligand with respect to the other metal cations. This manner provides the most complex formation. The lowest log β value of 3.29 belongs to Pb(II) complex. Interestingly, methanol increases complex stability for all metal cations. The Co (II) complex is the most stable complex (4.77) in methanol: water (1:1) with an increase of 16% in log β value. However, the highest increase, i.e. 21% in log β value was observed for Cu(II) ion when solvent changed from water to methanol:water (1:1). This result shows that a change in solvent polarity is very important for Cu(II) complexation with ligand **1**.

Table 1. **Stability constants (Log β) for ligand 1 with metal salts in solution at 25°C[a].**

Cation	Log β	
	Solvent	
	Water	Methanol: Water (1:1)
Cd^{2+}	3.38±0.09	3.67±0.09
Co^{2+}	4.11±0.10	4.77±0.05
Cu^{2+}	3.50±0.04	4.25±0.08
Ni^{2+}	3.72±0.05	3.99±0.05
Pb^{2+}	3.29±0.04	3.60±0.06

[a] The values and standard deviations were calculated from three independent experiments. The confidence level is 95%.

In the present study, it was clearly demonstrated that the feasibility to quantitatively study the complexation reaction between a 1,2,4-triazole ligand carrying naphthole group and metal cations even if the ligand is nearly insoluble in water as in such earlier study with some substituted 1,2,4- triazole compounds [24]. Because of their nearly insoluble nature in water under the abovementioned conditions, very small quantities of 1,2,4-triazole ligand are sufficient to perform the spectrophotometric titrations according to Buschmann's method, which is more appropriate for the determination of complex stability constants in aqueous solutions in comparison to fast and simple Valeur's method, for which the ligand should be soluble in a proper solvent. Another advantage of Buschmann's method is the recovery of the ligand after the procedure. Because of the biological activity of 1,2,4-triazole compounds, demonstration of their complexation properties with metal ions in aqueous phase is noteworthy to utilize them in physiological studies.

Conclusions

The spectrophotometric titrations of triazole ligand **1** carrying naphthol group with Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} cations point out the complexation properties of the ligand in aqueous solutions and methanol:water media (1:1) by using Buschmann and Valeur's methods, respectively. The most stable complex was the cobalt complex according to both methods. The log β values were 4.11 and 4.77 in water and methanol:water (1:1), respectively. The work presents the complex stability constants for these metal cations, calculated from two different methods. Job method shows 1:1 complex composition for the cobalt complex. NMR spectroscopic data reveals a probable complexation model over nitrogen donor atoms.

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