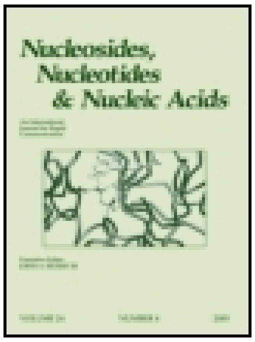

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Interactions of Some Divalent Metal Ions with Thymine and Uracil Thiosemicarbazide Derivatives

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ABSTRACT
The study of interactions between metal ions and nucleobases, nucleosides, nucleotides, or nucleic acids has become an active research area in chemical, biological, and therapeutic fields. In this respect, the coordination behavior of nucleobase derivatives to transition metals was studied in order to get a better understanding about DNA-metal interactions in in vitro and in vivo systems. Two nucleobase derivatives, 3-benzoyl-1-[3-(thymine-1-yl)propamido]thiourea and 3-benzoyl-1-[3-(uracil-1-yl)propamido]thiourea, were synthesized and their dissociation constants were determined at different temperatures and 0.3 ionic strength. Potentiometric studies were carried out on the interaction of the derivatives towards some divalent metals in 50% v/v ethanol-water containing 0.3 mol.dm^{-3} KCl, at five different temperatures. The formation constants of the metal complexes for both ligands follow the order: $Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Pb^{2+} > Cd^{2+} > Mn^{2+}$. The thermodynamic parameters were estimated; the complexation process has been found to be spontaneous, exothermic, and entropically favorable.

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Nucleic bases; thiourea derivatives; metal complexes; potentiometry; dissociation constants; stability constants; thermodynamic parameters

Introduction

DNA consists of two coupled polynucleotide strands that coil together to form the double helix; presenting the sugar-phosphate backbones on the outer side and the nucleobases projecting into the interior. DNA encodes the genetic information of life according to the sequence of nucleobases extending along its molecule. [1] Nucleic acids are polyanionic in nature, and thus their conformation and biological function depend strongly on association with metal ions. The presence of metal ions is essential in many natural processes where nucleic acids play the dominant role. [2] On the other hand, the binding of metals to the nucleobases usually disrupts base pair hydrogen bonding and weakens the double helix. [3] Thus, certain metal ions

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can cause structural alteration of nucleic acids, and induce chemical modification and cleavage. Such metal ions (Cr^{3+}, Ni^{2+}, Cd^{2+}, and Hg^{2+} ions) are even carcinogenic and mutagenic due to a different consequence of metal-DNA interaction. [4] Moreover, the binding of metal ions to nucleic acids may lead to rearrangement of electrons, the variation of p*K*a values, and the favoring of transition state geometries or the stabilization of rare tautomers. [5]

Current research to understand how nucleic acid functions at the most fundamental level requires deep knowledge of nucleic acid-metal ion interactions. This increased interest in metal-nucleic acid interactions originates from the mechanistic antitumor activity of platinum metal complexes, studies involving RNA catalysis, and the tremendous utilization of coordination compounds as structural probes of nucleic acids and as biotechnology tools. [6] Such studies can provide valuable information about the structure, function and stability of nucleic acids.

Literature survey reveals that Halli et al. reported a potentiometric study of some divalent metal complexes of 4-hydrazinobenzofuro[3,2-d]pyrimidine Schiff base with 5-methylsalicylaldehyde in alcohol-water (70:30, v/v) at 30°C and 0.1 M $NaClO_4$. [7] Sharma et al. had potentiometrically investigated the formation constants and thermodynamic parameters of 1,3-diethylphenyl-4,5,6-pyrimidinetrione-2-thioxo-5-oxime in 75% (v/v) aqueous-dioxane at different temperatures. [8] In another work, Abdalla et al. had potentiometrically studied the formation equilibria of binary and ternary complexes of Cu^{2+}, Co^{2+}, Ni^{2+} with glycine, DL-alanine and glutamic acid as primary ligand and 5-(arylazo)-2,6-diamino-4-pyrimidinol as secondary ligands in 40% (v/v) EtOH-H_2O at 25°C and 0.15 M $NaClO_4$. [9] Ashok et al. also used potentiometric techniques in order to determine the formation constants and complexation equilibria at 35°C and 0.01 M KNO_3 in aqueous solution for binary and ternary metal complexes of some divalent metal ions with 4,6-dihydroxy-2-mercaptopyrimidine (as primary or secondary ligand). [10] The formation constants of binary (1:1) and ternary (1:1:1) complexes of some divalent metals with aspartic acid as primary ligand and cytosine as secondary ligand were potentiometrically determined in aqueous solution by Dharmveer et al. [11] Shukla et al. reported the potentiometric determination of the stability constants and complexation equilibria at 30°C and 0.1 M $NaNO_3$ for quaternary metal complexes of some divalent metal ions containing thymine. [12] Krishna et al. had potentiometrically determined the formation constants of the ternary (1:1:1) and quaternary (1:1:1:1) complexes of some divalent metals with glutamic acid/L-cysteine as primary ligands and thymine as secondary ligand in aqueous medium. [13]

Since in many instances, metal ions or complexes coordinate preferentially to one of the common bases in DNA or RNA; and in order to develop a model that can help in assisting the study of larger systems such as nucleotides and nucleic acids, we have conducted a potentiometric study of the complexation behavior of two nucleobase-thiosemicarbazide derivatives to some divalent metals in 50% v/v ethanol-water medium, containing 0.3 $mol.dm^{-3}$ KCl, at different temperatures.

This work will significantly contribute to the elucidation of the general coordination chemistry of nucleobase derivatives and could result in the development of new metal-based antitumor or antiviral drugs.

Experimental

Materials and methods

Potentiometric measurements were carried out using Eutech pH-meter in well-stirred solutions. The temperature of the systems was maintained constant during titration, with a water bath (VWR, model 12101-10) and a glass titration cell with double jacket. Double-distilled water was boiled for 2 hours to minimize atmospheric carbon dioxide contamination. Thereafter, it was cooled to room temperature in a closed vessel leaving no headspace.

The ligands stock solutions were prepared in different percentages of ethanol-water media, whereas metal ions solutions were prepared by dissolving an accurately weighed amount of each in water. NaOH solution was prepared by dissolving Analar pellets in CO_2-free double-distilled water, and the solution was standardized with a standard potassium hydrogen phthalate solution. Potassium chloride solution in water was prepared as a supporting electrolyte ($\mu = 0.30$) to maintain constant ionic strength.

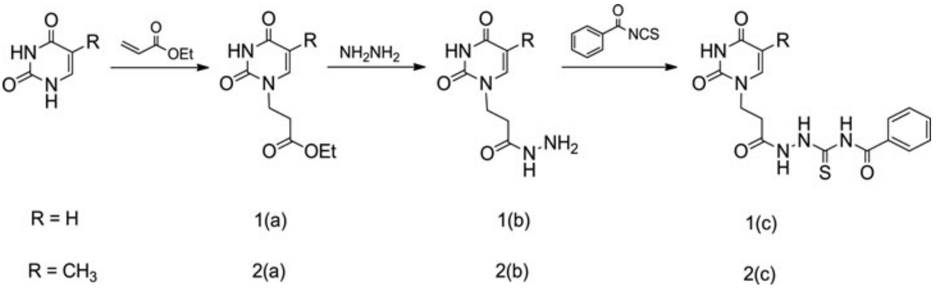
IR spectra were recorded in KBr pellet, on a Nicolet iS 10 FT/IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer.

Calibration of the glass electrode

The pH meter was calibrated using two standard aqueous buffer solutions. Correction of pH-meter readings (B) in ethanol–water media was done using the Van Uitert and Hass equation (1) for determination of the exact $[H^+]$, as follows:

$$-\log\,[\mathrm{H}^{+}] \;=\; \mathrm{B} \;+\; \log\,\mathrm{U_H} \tag{1}$$

where $\log U_H = \log U_H^o + \log \gamma_{\pm}$. $\gamma\pm$ is the activity coefficient of the hydrogen ions in the solvent mixture under consideration, at the same temperature and ionic strength; and U_H^o is a correction factor at zero ionic strength, which depends only on the solvent composition. The pH-meter readings (B) were recorded for different solvent mixtures containing 1.00×10^{-3} M hydrochloric acid at different temperatures and ionic strength = 0.3. The difference between the logarithm of known hydrogen ion concentrations and the corresponding values of B was used to calculate values of the correction term $\log U_H = \log(U_H^o \gamma_{\pm})$. [14–16]



Scheme 1. Synthesis of pyrimidine nucleobase derivatives.

Synthesis and characterization of the ligands

The two nucleobase derivatives were prepared as in scheme 1, according to our previously published procedure. [17]

Synthesis of the metal complexes

Some metal complexes were prepared as a preliminary study to confirm formation and stability of complexes in support of potentiometric complexation studies in solution:

Metal complexes were prepared by refluxing 15 mL methanolic solution of each ligand (0.2 g, 0.53 mmol) with sodium acetate (0.14 g, 3 mmol) for 15 mins, followed by drop-wise addition of 0.26 mmol hydrated metal salts (0.044 g copper chloride dihydrate; 0.062 g cobalt chloride hexahydrate; 0.061 g nickel chloride hexahydrate; 0.074 g zinc sulfate heptahydrate) with continuous stirring at room temperature for 12 hours. The resulting solid complexes were filtered, washed with ethanol and dried under reduced pressure, then characterized by FTIR.

Results and discussion

Thiosemicarbazide derivatives are becoming much more interesting due to their remarkable biological activities (antiviral, anticancer, etc.), possibly related to their ability to coordinate metal centers in enzymes. [18] Moreover, thiosemicarbazides are considered multifunctional N,O,S-donors in transition metal complexes. Such complexes have been proposed as analytical reagents that can be used in selective and sensitive determinations of metal ions. [19] Thus, the conductance of a potentiometric study on the complexation behavior of nucleobase-thiosemicarbazide derivatives is considered highly significant.

Acidity constant studies

The acid dissociation constant is a key physicochemical parameter of compounds. Along with pH, both parameters govern the extent of ionization of compounds, thus affecting different properties such as: water solubility, UV absorption, reactivity

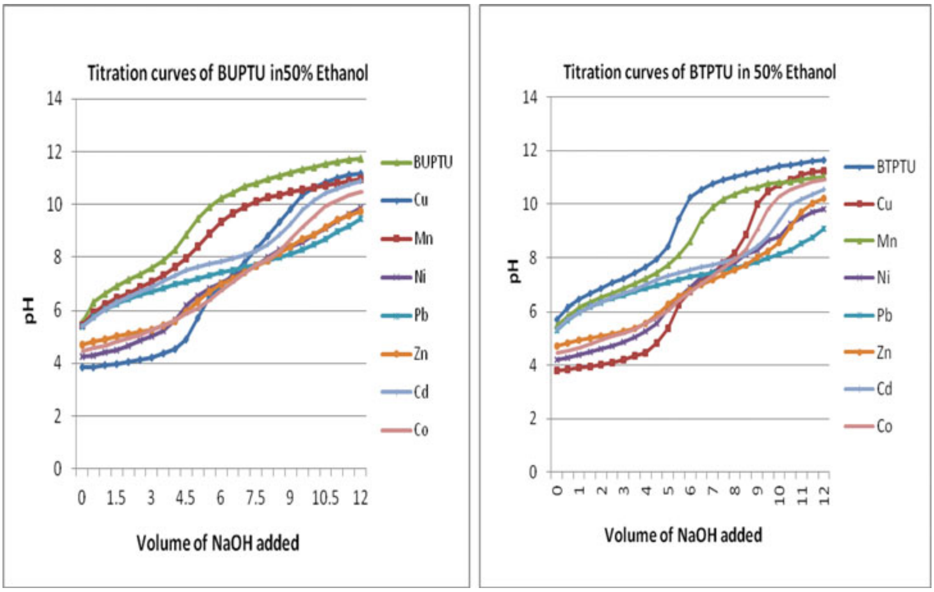


Figure 1. Potentiometric titration curves for 1×10^{-3} M **BUPTU** and **BTPTU** in 50% (v/v) ethanol–water medium at 20°C and at $\mu = 0.3$ M KCl, in the absence and presence of respective metal salts (1:2 ratio of metal to ligand).

and pharmacokinetics of compounds in biological systems. [20,21] Several methods were reported for the effective determination of dissociation constants, yet potentiometry remains one of the most useful techniques, due to its confirmed accuracy and reproducibility. [22]

Determination of the acid dissociation constants of the ligands: A 50 mL solutions of 10^{-3} M **BTPTU** (3-benzoyl-1-[3-(thymine-1-yl)propamido]thiourea) or **BUPTU** (3-benzoyl-1-[3-(uracil-1-yl)propamido]thiourea), in different percentages of ethanol-water media (40%, 50%, and 60%), were titrated potentiometrically against standard 5.00×10^{-3} M NaOH at ionic strength of 0.3, and the pH values were recorded, Fig. 1. At least three samples of each solution were titrated with NaOH to check data reproducibility.

The acid dissociation constants, K_{HL}^{H} for **BTPTU** and **BUPTU** as $H_1(L)$, were calculated by evaluation of $\bar{n}_A$ the average proton number associated with the ligand, at different pH, applying Irving and Rossotti equation (2):

$$\bar{n}_A = Y - (V_i N^o)/(V_o C_L^o), \tag{2}$$

where V_i denotes the volume of alkali required to reach a given pH on the titration curve, V_o is the initial volume of the ligand, N^o is the alkali concentration, C_L^o is the total concentration of the ligand, and Y is the number of displaceable hydrogen atoms in the ligand ($Y = 1$ for both ligands). The proton-ligand formation constant (Ka) was calculated from the formation curves obtained by plotting the corresponding $\bar{n}_A$ in different solvents and at different temperatures versus pH, where the pH values at $\bar{n}_A = 0.5$ give the pKa values. The pH titration curves of the free ligands

Table 1. The pKa values for **BUPTU** in different percentages of ethanol–water media at (20–40°C) and at $\mu = 0.3$.

	p*K*a				
%v/v Ethanol–Water	20°C	25°C	30°C	35°C	40°C
40	9.22 ± 0.04	9.21 ± 0.03	9.20 ± 0.03	9.19 ± 0.01	9.18 ± 0.01
50	9.38 ± 0.01	9.37 ± 0.02	9.36 ± 0.04	9.35 ± 0.01	9.34 ± 0.02
60	9.56 ± 0.02	9.55 ± 0.02	9.54 ± 0.02	9.53 ± 0.01	9.51 ± 0.02

against NaOH in presence of KCl ($\mu = 0.3$) showed only one inflection point, indicating that only one proton is titrated, as shown in Figure 1. The calculated $\bar{n}_A$ values for the proton-ligand systems in different solvents, different concentrations and at different temperatures were found to extend between 0 and 1. This means that the ligands have one ionizable proton.

A more accurate value of pKa was obtained from the plot of log $\bar{n}_A/(1 - \bar{n}_A)$ versus pH, equation (3):

$$\text{pKa} = \text{pH}+[\log \bar{n}_A/(1 - \bar{n}_A)]\ \bar{n}_A < 1 \tag{3}$$

The data are collected in Tables 1 and 2, which reveal that the pKa of **BUPTU** was higher than that of **BTPTU**.

According to Coetzee and Ritchie, the acidity constant in pure aqueous medium (Ka) can be related to that in water-organic solvent mixture (K′a) by equation (4), [23, 24]:

$$K_a = K'_a(\gamma_H^+ \gamma_A^-)/\gamma_{HA} \tag{4}$$

where γ is the activity coefficient of the respective species in a partly aqueous medium relative to that in pure water. The increase in pKa values of compounds with increasing mole fraction of organic solvents can be ascribed to less electrostatic effect and less hydrogen-bonding interaction (less stabilization) between conjugate base A^- and organic solvent compared to water. Accordingly, the pKa values increase as the mole fraction of the organic solvent is increased, which agree with equation (4). [25] The pKa values of the ligands decreased with increasing temperature and increased with increasing organic co-solvent content in the medium (Tables 1 and 2; Fig. 2 and 3).

Table 2. The pKa values for **BTPTU** in different percentages of ethanol–water media at (20–40°C) and at $\mu = 0.3$.

	p*K*a				
%v/v Ethanol–water	20°C	25°C	30°C	35°C	40°C
40	8.30 ± 0.01	8.28 ± 0.02	8.26 ± 0.04	8.25 ± 0.03	8.24 ± 0.01
50	8.38 ± 001	8.37 ± 0.02	8.36 ± 0.04	8.35 ± 0.01	8.33 ± 0.01
60	8.46 ± 0.03	8.45 ± 0.03	8.44 ± 0.05	8.42 ± 0.02	8.41 ± 0.03

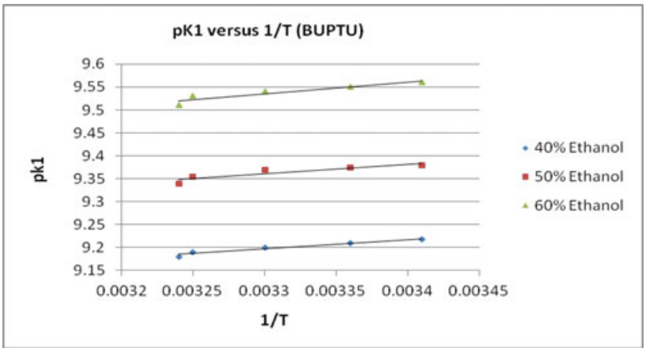


Figure 2. Van't Hoff Plot pKa of **BUPTU** against 1/T at different percentages of ethanol.

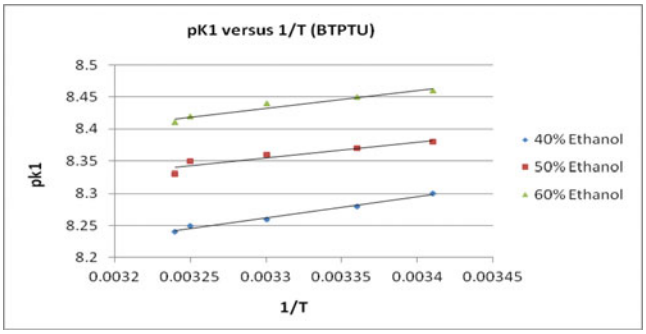


Figure 3. Van't Hoff Plot pKa of **BTPTU** against 1/T at different percentages of ethanol.

Thermodynamic parameters of ionization

The thermodynamic parameters for ionization of the compounds were evaluated in the temperature range 20–40°C, Tables 3 and 4. On plotting the pKa values versus 1/T, straight lines are obtained with a slope of $\Delta H°/2.3R$, from which the enthalpy values $\Delta H°$ (kcal/mol) are computed (Van't Hoff method), Figures 2 and 3. The free energy values $\Delta G°$ (kcal/mol) were calculated from the pKa values at different temperatures, whereas the entropy values $\Delta S°$ (e.u.) using Gibbs free energy equation.

The pKa values of both ligands decrease with increasing temperature revealing that there is a concomitant increase in their acidities. The values also increase with increasing the percent solvent composition at any temperature used (20–40°C).

Table 3. Thermodynamic parameters for the dissociation constants of **BUPTU** in different percentages of ethanol-water media at (20–40°C). $\Delta H°$, $\Delta G°$ in kcal mol^{-1} and $\Delta S°$ in cal mol^{-1} K^{-1} (e.u.).

% Ethanol		20°C	25°C	30°C	35°C	40°C
40	$\Delta H° \pm 0.05$	19.41				
	$\Delta G° \pm 0.02$	12.71	12.83	12.99	13.03	13.11
	$\Delta S° \pm 0.06$	22.85	22.07	21.17	20.86	20.36
50	$\Delta H° \pm 0.03$	27.90				
	$\Delta G° \pm 0.06$	12.65	12.82	13.21	14.04	15.29
	$\Delta S° \pm 0.05$	52.03	50.62	48.48	47.51	46.99
60	$\Delta H° \pm 0.05$	32.05				
	$\Delta G° \pm 0.03$	12.79	12.90	13.21	13.82	14.96
	$\Delta S° \pm 0.04$	65.74	64.27	62.17	60.82	59.35

Table 4. Thermodynamic parameters for the dissociation constants of **BTPTU** in different percentages of ethanol-water media at (20–40°C). $\Delta H°$, $\Delta G°$ in kcal mol^{-1} and $\Delta S°$ in cal mol^{-1} K^{-1} (e.u.).

% Ethanol		20°C	25°C	30°C	35°C	40°C
40	$\Delta H° \pm 0.05$	45.55				
	$\Delta G° \pm 0.04$	11.28	11.39	11.51	11.86	12.13
	$\Delta S° \pm 0.1$	116.97	114.64	112.35	110.77	109.96
50	$\Delta H° \pm 0.06$	31.86				
	$\Delta G° \pm 0.05$	11.32	11.40	11.59	11.84	12.06
	$\Delta S° \pm 0.2$	70.11	68.65	67.45	65.63	63.17
60	$\Delta H° \pm 0.03$	18.37				
	$\Delta G° \pm 0.03$	10.91	11.00	11.46	11.84	12.03
	$\Delta S° \pm 0.5$	25.48	24.74	22.81	21.23	20.90

Positive enthalpy, free energy and entropy values indicated that the acid dissociation is accompanied by an endothermic mechanism, non-spontaneous dissociation and the total number of solvent molecules bound with the dissociated ligand is greater than that originally accompanying the undissociated form.

Complex formation studies

It is documented that the nucleic acid monomers, guanine (G), adenine (A), thymine (T), and cytosine (C) have different metal ion affinities. The order of stability of 3D transition metal ion—nucleobase complexes are: G > A; C > T. The study of metal complexes formations and their stabilities is very important during drug design because it indicates the affinity of interaction between metal ions for a particular ligand. [26]

The stability constant of a complex in solution is usually determined by measuring the equilibrium constant at a given temperature; the higher the determined formation constant value, the greater the stability of the metal-ligand complex. [27]

The formation constant involved in the metal complex formation may be expressed as follows:

$$M^{n+} + L^{-} \rightleftharpoons ML^{+(n-1)} \tag{5}$$

$$K_{f1} = K_{ML^{+(n-1)}} = \frac{[ML^{+(n-1)}]}{[M^{n+}][L^{-}]} \tag{6}$$

$$ML^{+(n-1)} + L^{-} \rightleftharpoons ML_2^{+(n-2)} \tag{7}$$

$$K_{f2} = K_{ML_2^{+(n-2)}} = \frac{[ML_2^{+(n-2)}]}{[ML^{+(n-1)}][L^{-}]} \tag{8}$$

The free ligands acid-base properties facilitate the investigation of their coordinating behavior towards metals. The potentiometric determination of metal complex stability constant comprises the measurement of the change in hydrogen ion concentration upon displacement of one or more protons during complexation. This change can be observed in Fig. 1, which represents the titration curve for the ligand alone and ligand-metal mixtures against a standard alkali solution at constant ionic strength and temperature. Comparison of pH titration curves of free ligands with those of the complex solutions shows a drop in pH, attributed to the formation of strong metal ion complexes with H^+ liberation during complex formation. The stability constants of complexes were determined from titration curves according to Irving and Rossotti method.

A typical procedure for determination of stability constants involves the addition of 5 ml (5×10^{-3} M) metal salt solution to a 50 mL 50% v/v ethanol/water solution of 10^{-3} M **BUPTU** or **BTPTU** containing 0.3 M KCl (M:L = 1:2), and the mixture was titrated in triplicate against standard 5×10^{-3} M NaOH. The temperature was maintained constant at 20, 25, 30, 35, and 40 ± 0.1°C using a water bath and a glass titration cell with a double jacket.

The potentiometric titration data of the complexes of some selected metal ions Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, and Cd^{2+} with **BUPTU** and **BTPTU** are listed in Tables 5 and 6.

Modified Bjerrum's method was adopted and pH measurements of titration with NaOH solution of ligand in presence and absence of metal ions were used to calculate the free ligand exponent (pL) and the degree of formation of the system $\bar{n}$ (equations (9) and (10)).

$$pL = \log[L^-] = \log\left[\frac{(2-a)C_L - [H^+] + [OH^-]}{\frac{2[H^+]}{K_1}}\right] \tag{9}$$

The metal-ligand formation, $\bar{n}$, is defined as the average number of moles of ligand bound per mole of metal ion:

$$\begin{aligned}\bar{n} &= \frac{\text{total concentration of the ligand bound to metal}}{\text{total concentration of the metal}}\\ &= \frac{C_L - (concentration\ of\ the\ ligand\ not\ bound\ to\ the\ metal)}{C_M}\\ \bar{n} &= \frac{1}{C_M}\left(C_L - \left[\frac{[H^+]}{K_1}\right][L^-]\right)\end{aligned} \tag{10}$$

C_L is the total concentration of ligand, $[L^-]$ is the concentration of free ligand anion, a is the number of moles of base added per mole of ligand present and K_1 is the acid dissociaton constant of ligand.

The formation curves for the metal complexes were obtained by plotting the average number of ligands attached per metal ions ($\bar{n}$) versus the free ligand exponent (pL), according to Irving and Rossotti. The successive stability constants $\log K_{f1}$, and $\log K_{f2}$ values are computed at the pL values equivalent to 0.5 and 1.5, respectively [28–30].

It was clearly evident from the data collected in Tables 5 and 6, that $\log K_{f1}$ values for all formed complexes were greater than those of $\log K_{f2}$, ascribed to a weaker

Table 5. Metal-ligand stability constants and thermodynamic parameters of **BTPTU** with transition metal ions in 50% ethanol at 20–40°C and at $\mu = 0.3$. $\Delta H°$ and $\Delta G°$ (25°C) in kcal mol^{-1} and $\Delta S°$ (25°C) in cal mol^{-1}K^{-1} (e.u.).

	log $K_{f1} \pm 0.03$ (log $K_{f2} \pm 0.03$)							
System	20°C	25°C	30°C	35°C	40°C	$-\Delta G° \pm 0.2$	$-\Delta H° \pm 0.3$	$\Delta S° \pm 0.2$
Co^{+2}	4.239 (3.434)	4.178 (3.428)	4.152 (3.386)	4.061 (3.358)	4.051 (3.348)	23.839 (19.56)	20.62 (10.168)	149.19 (99.758)
Cu^{+2}	5.011 (3.945)	5.001 (3.923)	4.949 (3.896)	4.869 (3.861)	4.801 (3.813)	28.535 (22.384)	21.985 (12.945)	169.53 (118.55)
Ni^{+2}	4.609 (3.792)	4.572 (3.777)	4.522 (3.744)	4.469 (3.712)	4.417 (3.686)	26.087 (21.551)	19.785 (11.311)	153.93 (110.28)
Mn^{+2}	3.199 (1.492)	3.184 (1.426)	3.145 (1.388)	3.115 (1.344)	3.011 (1.337)	18.167 (8.136)	16.97 (16.731)	117.91 (−83.447)
Cd^{+2}	3.501 (2.442)	3.441 (2.418)	3.352 (2.376)	3.309 (2.345)	3.389 (2.326)	19.634 (13.797)	17.138 (12.725)	123.39 (88.998)
Pb^{+2}	3.855 (3.176)	3.825 (3.144)	3.799 (3.112)	3.755 (3.082)	3.711 (3.069)	21.825 (17.939)	14.36 (11.597)	121.42 (99.114)
Zn^{+2}	4.021 (3.282)	3.99 (3.268)	3.93 (3.254)	3.872 (3.153)	3.811 (3.117)	22.76 (18.647)	21.952 (17.769)	150.06 (122.2)

Table 6. Metal-ligand stability constants and thermodynamic parameters of **BUPTU** with transition metal ions in 50% ethanol at 20–40°C and at $\mu = 0.3$. $\Delta H°$ and $\Delta G°$ (25°C) in kcal mol^{-1} and $\Delta S°$ (25°C) in cal $mol^{-1}K^{-1}$ (e.u.).

	$\log K_{f1} \pm 0.03$ ($\log K_{f2} \pm 0.03$)							
System	20°C	25°C	30°C	35°C	40°C	$-\Delta G° \pm 0.3$	$-\Delta H° \pm 0.5$	$\Delta S° \pm 0.2$
Co^{+2}	4.689 (2.399)	4.682 (2.372)	4.675 (2.361)	4.564 (2.269)	4.505 (2.242)	26.715 (13.534)	18.943 (16.841)	153.21 (−101.93)
Cu^{+2}	4.899 (4.345)	4.865 (4.339)	4.845 (4.293)	4.744 (4.263)	4.71 (4.219)	27.759 (24.758)	20.204 (13.168)	160.950 (127.27)
Ni^{+2}	4.798 (2.883)	4.761 (2.808)	4.725 (2.778)	4.688 (2.765)	4.601 (2.714)	27.166 (16.022)	18.271 (15.574)	152.470 (106.03)
Mn^{+2}	3.392 (1.352)	3.368 (1.269)	3.331 (1.257)	3.256 (1.179)	3.211 (1.164)	19.217 (7.241)	19.127 (17.063)	128.678 (−90.561)
Cd^{+2}	3.689 (1.882)	3.626 (1.845)	3.608 (1.821)	3.553 (1.745)	3.503 (1.714)	20.689 (10.527)	17.989 (17.766)	129.728 (−94.945)
Pb^{+2}	4.098 (1.986)	4.061 (1.977)	3.997 (1.955)	3.951 (1.910)	3.895 (1.816)	23.171 (11.280)	21.048 (15.34)	148.39 (−89.33)
Zn^{+2}	4.479 (2.289)	4.466 (2.286)	4.443 (2.261)	4.356 (2.180)	4.309 (2.173)	25.482 (13.044)	17.886 (13.892)	145.53 (−90.388)

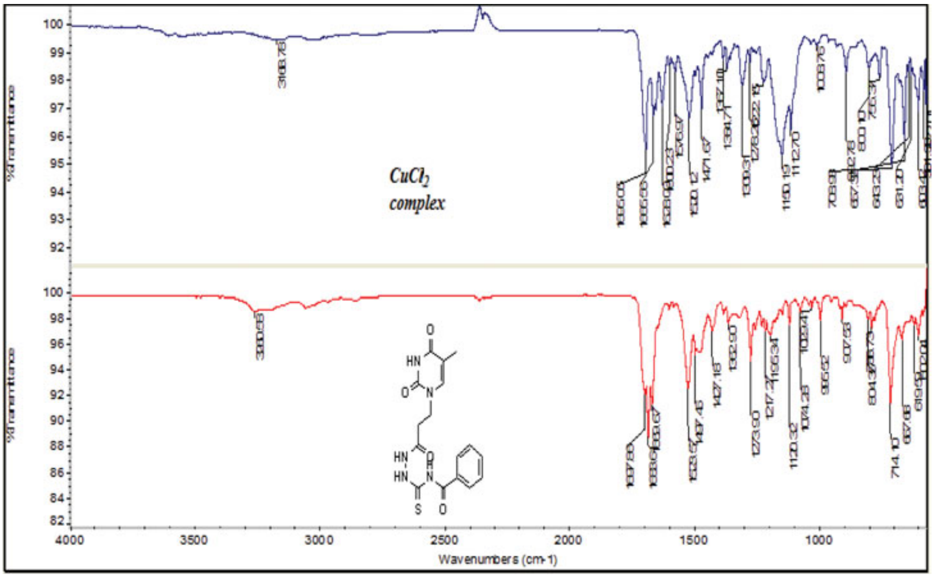


Figure 4. Stacked FT-IR Spectra of **BTPTU** and its Metal Complex with $CuCl_2$.

interaction of a second bulky ligand molecule compared to the first coordinated ligand and to the increase in the Lewis acidity of the free metal ion as compared to the 1:1 chelated ion; thus, ML_2 species do not form until complete formation of ML species had occurred. This was additionally supported by the values of enthalpy for **BTPTU** and **BUPTU**-complexes, where $\Delta H°_1 > \Delta H°_2$, indicating a strong steric hindrance for the second coordination.

The order of stability constants of the metal complexes for both ligands was in accordance with Irving-Williams series [31]: $Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Pb^{2+} > Cd^{2+} > Mn^{2+}$, and is governed by the ionic radii and polarizability of metal ions and the chelate crystal field strength.

In all cases, the possibility of hydroxyl products and polynuclear complexes formation was excluded due to the use of very low (4.5×10^{-5} M) metal ion concentrations.

FT-IR characterization of metal complexes:

Then, FT-IR spectroscopy was utilized in order to assign the interaction sites of the ligands with transition metals. The ligands FT-IR spectra show a moderate band at about 3,260 cm^{-1}, which can be attributed to the amide NH group. This band was shifted to lower wave number in all complexes, which indicates the involvement of this group in coordination. The ($\nu_{N\text{-}N}$) band observed at 1,029 cm^{-1} in free ligand was shifted to lower values in the complexes. Such displacement may be explained by the coordination of one of the two nitrogens from each N–N group to the metal ion, this cause the reduction of the lone pair-lone pair repulsive forces in the adjacent nitrogen atom. In addition, the pyrimidine carbonyl (C=O) and thiocarbonyl (C=S) bands observed at approximately 1,683 and 1,120 cm^{-1}, respectively, showed shift in the metal complexes as compared with free ligands, Fig. 4. This signifies the involvement of both groups in metal coordination.

Table 7. p*K*a and logK_f values of the ligands (**BTPTU, BUPTU)** and their complexes with cobalt and copper at different ionic strength in 50% ethanol at 25°C.

			Co(II)		Cu(II)	
μ	$\sqrt{\mu}$	p*K*a	logK_{f1}	logK_{f2}	logK_{f1}	logK_{f2}
3-Benzoyl-1-[3-(thymine-1-yl) propamido]thiourea						
0.05	0.2236	9.46	4.536	4.025	5.686	4.718
0.15	0.3873	8.92	4.315	3.715	5.335	4.315
0.3	0.5477	8.37	4.178	3.428	5.001	3.923
0.6	0.7746	7.89	3.912	3.109	4.797	3.625
3-Benzoyl-1-[3-(uracil-1-yl) propamido]thiourea						
0.05	0.2236	10.13	5.356	2.865	5.217	4.871
0.15	0.3873	9.8	5.062	2.553	5.015	4.651
0.3	0.5477	9.37	4.682	2.372	4.865	4.339
0.6	0.7746	8.94	4.532	2.249	4.749	4.019

From the complexes infrared spectra, it is apparent that, the chelation of the divalent metal ions to the ligands occurred from the nucleobase derivatives through the oxygen atom of the pyrimidine moiety, the sulfur atom and the NH group in the side chain of the ligands.

Effect of ionic strength on acidity and stability constants

We have studied the dependence of proton-ligand stability constants (pKa) and metal-ligand stability constants (logK_f) on ionic strength of medium, by using fixed concentration of metal salts and ligand solution during potentiometric titration. The system was evaluated at four different ionic strength (0.05, 0.15, 0.3, and 0.6), by varying the concentration of KCl at constant temperature (25°C). It was found that the values of proton-ligand stability constant decrease with increasing ionic strength of medium, Table 7. The metal-ligand stability constants of the metal ions (Cu^{2+}, Co^{2+}) complexes with the investigated ligands also decrease with increasing ionic strength, Table 7, which is in agreement with the Debye–Hückel equation (12), [32]:

$$pK_a = pK^\circ - [A\sqrt{\mu}/(1+\alpha)\sqrt{\mu}] + C_\mu \tag{11}$$

where, pKa is the ionization constant at a given ionic strength. pK° is the thermodynamic ionization constant at zero ionic strength; A and α are constants; μ is the ionic strength of the medium.

Determination of the metal complexes stability constants at physiological pH

The assessment of metal complex stability in solutions at physiological pH is highly significant, in view of possible use in medical practice. If complexes of appropriate stability can be formed, a range of in vitro and in vivo applications may exist. In this respect, a series of solutions were prepared with a constant concentration of metal ion $[M]_o$ and variable ligand concentration $[L]_o$ in 50% ethanol–water mixture. The

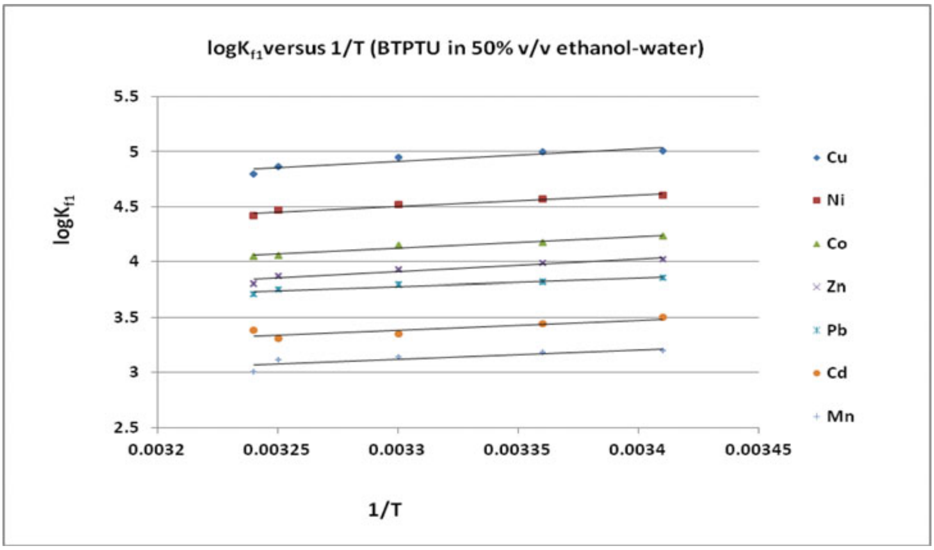


Figure 5. Log K_{f1} versus 1/T for metal complexes with **BTPTU** in 50% ethanol.

pH was adjusted to 7.4, at 37°C, and ionic strength was maintained constant using 0.3 M KCl. The mixture was left to stand for 15 min and the absorbance spectra were recorded. The stability constant β, was determined using Benesi-Hildebrand equation. [33] The metal complexes stability constant values were slightly decreased at the specified conditions. The determined logβ values for **BTPTU** were: 3.56 (Co^{2+}), 4.33 (Cu^{2+}), 4.05 (Ni^{2+}), 2.71 (Mn^{2+}), 2.94 (Cd^{2+}), 3.26 (Pb^{2+}), and 3.43 (Zn^{2+}).

Thermodynamic parameters of complexation

Determining the thermodynamic parameters for metal complexes provides information about the physicochemical behavior in solutions. The higher entropy (disorder of solution) of a reaction indicates greater product stability; whereas negative enthalpy indicates that a reaction needs higher reverse activation energy, so the product is considered to be more stable. The enthalpy change was calculated from the slopes of the plot logK_f versus $1/T$ using graphical representation of Van't hoff equation (Figs. 5–8). The change in free energy was calculated from the formation constants logK_f at various temperatures using Gibbs energy equation.

All thermodynamic parameters of the dissociation process of metal complex are recorded in Tables 5 and 6. It was clearly evident that logK_f values decrease with increasing temperature revealing that the formation equilibrium is exothermic in nature, which is supported by negative enthalpy. The electron density on the metal ion generally increases upon the formation of ligand-metal coordinate bond and as a result, its affinity for a subsequent ligand decreases, leading to an increase in $\Delta G°$ and $\Delta H°$ of complexation.

The steric hindrance associated with second ligand chelation explains the obtained data $|\Delta G°_1| > |\Delta G°_2|$ and $|\Delta H°_1| > |\Delta H°_2|$.

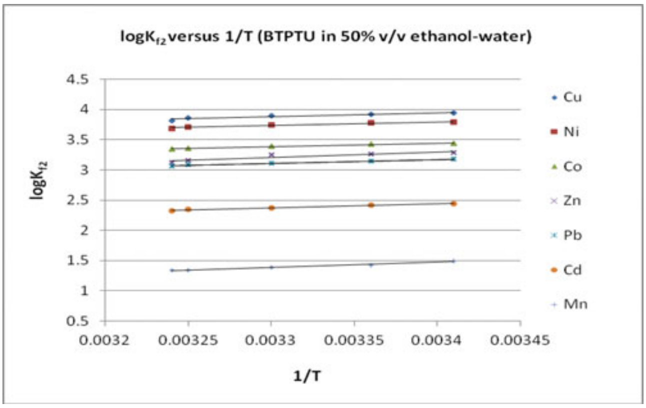


Figure 6. Log K_{f2} versus 1/T for metal complexes with **BTPTU** in 50% ethanol.

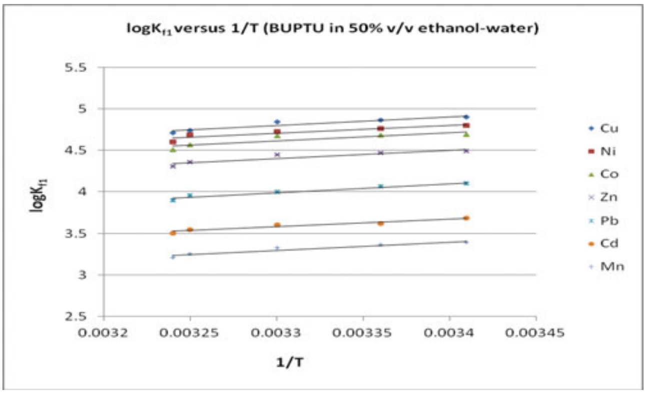


Figure 7. Log K_{f1} versus 1/T for metal complexes with **BUPTU** in 50% ethanol.

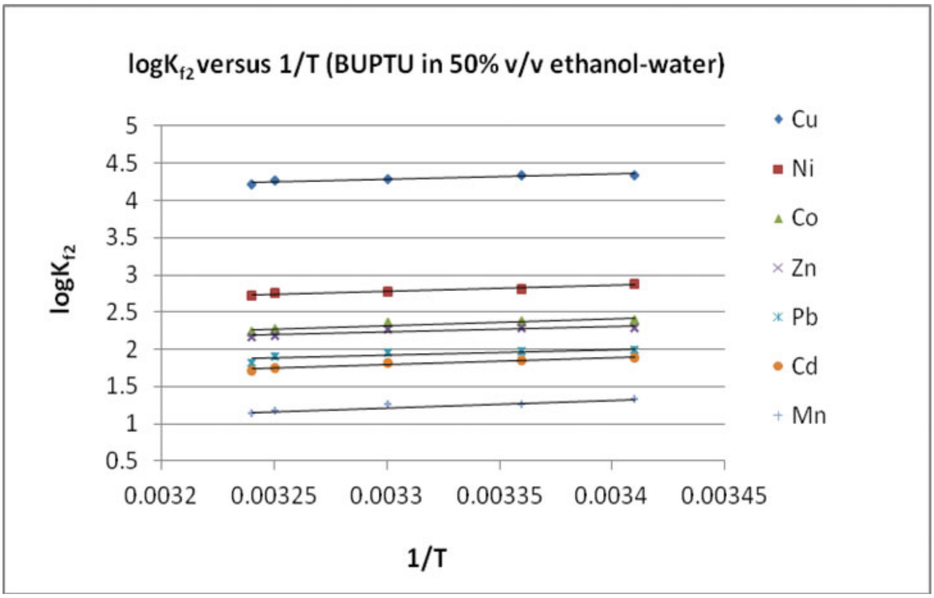


Figure 8. Log K_{f2} versus 1/T for metal complexes with **BUPTU** in 50% ethanol.

Negative values of free energy and positive values of entropy suggest the spontaneous nature of the complexation process and confirm that the ionization of the ligands is entropically favorable with complexation mechanism based upon hydrogen ion liberation and release of water.

For **BUPTU**, some entropy values are negative indicating that the complex keeps the primary solvation sphere of the metal ion upon complexation.

Conclusion

The binding of metal ions to nucleic acids has been an interesting subject for many years, where more research is still needed to unveil unknown mechanisms of this process. In this paper, we have utilized potentiometric technique for the interaction study of two nucleobase–thiosemicarbazide derivatives with some divalent transition metal ions in 50% (v/v) ethanol–water mixture at different temperatures. The data confirmed the formation of strong and stable metal ion complexes, in accordance with Irving–Williams series. Thermodynamic assessment indicated the spontaneous, exothermic, and entropic favoring of the complexation process.

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