

Synthesis, Spectroscopic Studies and Biological Evaluation of Co(II), Ni(II), Cu(II) and Zr(IV) Complexes of Azo Dyes and Thiamine Hydrochloride as Antimicrobial Agents

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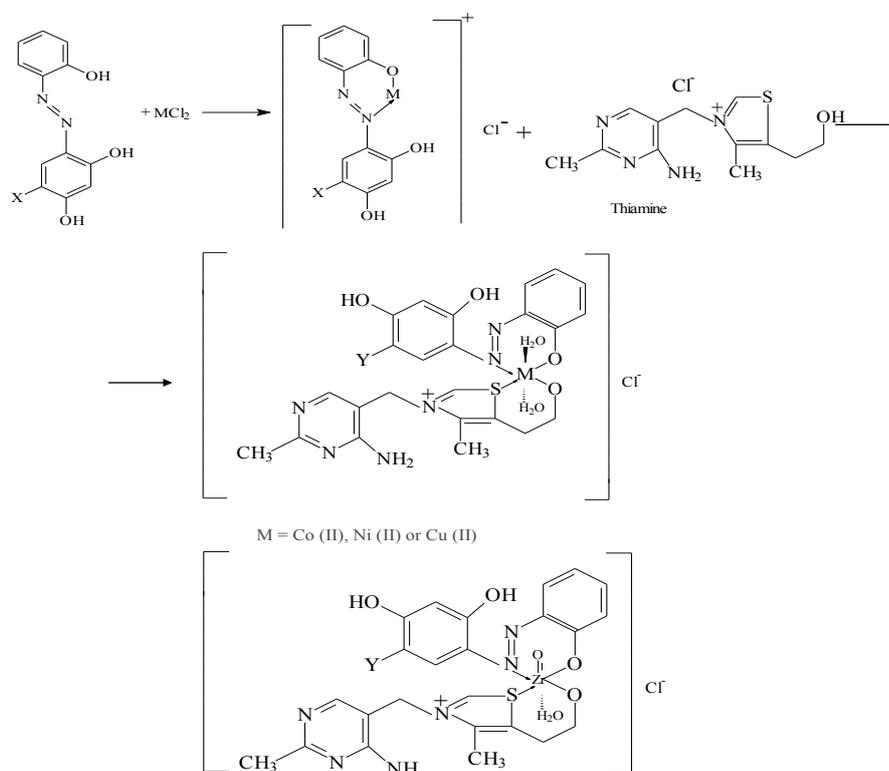
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Abstract

Four series of novel mixed ligand–drug complexes of thiamine hydrochloride as a primary ligand and four azo compounds, as secondary ligands, were prepared using Co (II), Ni (II), Cu (II) and Zr (IV) ions. The complexes were characterized by elemental analysis, thermogravimetric analysis, molar conductivity, FT-IR, magnetic susceptibility and UV-Visible spectroscopy. Based on the analytical and spectral data, the complexes were formulated as $[Azo - M - Thiamine (H_2O)_n]^+ Cl^-$ where $n=2$ for Co (II), Ni (II) and Cu (II) and $n=1$ for Zr (IV). All the complexes possessed octahedral geometry in which both the primary and secondary ligands act as monovalent monodentate coordinated via ON fashion in case of azo compounds and via OS fashion in case of thiamine hydrochloride. Biological activity of some complexes has been evaluated against a number of pathogenic bacteria and fungi. Obtained results indicated that the metal complexes exhibited better antimicrobial and antifungal activities.



Keywords: Thiamine hydrochloride; Azo dyes; Metal-drug complexes; Anti-microbial and antifungal activities

Introduction

It is well known that mixed ligand ternary complexes of some metal ion play an important role in the biological activity [1]. The early discovered Cis-platin was regarded as one of the most effective anticancer drugs, even if severe toxicities and drug resistance phenomena limit its clinical use. Therefore, in recent years there has been a rapid expansion in research and development of novel metal-based biologically active and anticancer complexes [2-4]. Numerous

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mixed ligands transition metal complexes have been investigated by various techniques and their biological activities were extensively studied [5-10]. Chelating ligands containing O, S and N donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions [11]. Thiamine, thiamin, or vitamin B₁ is a vitamin of the B complex. All living organisms use thiamine, but it is synthesized only in bacteria, fungi, and plants. Animals must obtain it from their diet, and thus, for humans, it is an essential nutrient. Azo dyes were found to be good ligating compounds with high biological activity and find many applications in industry. This paper reports the synthesis, characterization and spectral studies of mixed ligand Co(II), Ni(II), Cu(II) and Zr(VI) complexes prepared from thiamine hydrochloride as a primary ligand and four azo compounds as secondary ligands. The complexes were screened for their antibacterial and antifungal activities.

Results and Discussion

Physical properties

Based on elemental analysis and other techniques that support the chemical structure of the prepared complexes, it is suggested that metal chlorides react with the mixed ligands according to the following proposed equation:



Where Azo represents the secondary ligand azo compound, Thiam⁺HCl the primary ligand drug compound, n=2 for Co (II), Ni (II) and Cu (II) and n=1 for Zr (IV). All the synthesized complexes are non-hygroscopic solids with varying colors. They are freely soluble in DMSO and DMF but sparingly soluble in other common organic solvents. Results of elemental analysis (Table 1) showed the stoichiometric ratio (1:1:1) (Thiamine: M: Azo). The molar conductivity of complexes in DMF solution (10⁻³ M) at room temperature are in the range 18.8–32.4 ohm⁻¹cm²mol⁻¹ indicating the ionic nature of the complexes with number of ions equal two. The addition of AgNO₃ solution to the solubilized chelates in DMF led to the formation of white precipitate of AgCl confirming the presence of the Cl⁻ ion outside the coordination sphere.

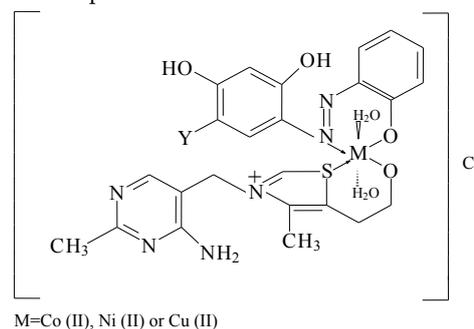
Thermal analysis

The thermogravical behavior of some selected solid complexes was studied in a flowing nitrogen atmosphere at constant heating rate of 10°C per minute. Representative thermograms are shown in Figures 1 and 2 and the thermal degradation data are summarized in Table 2. Inspection of these data shows that the complexes degrade through three main steps. The first is due to removal of coordinated water molecules and other gases resulting from the pre-decomposition of the complex such as HCl and /or CO₂. The second step is due to the decomposition of the azo compound representing the secondary ligand. The third step at higher temperature is due to the thermal decomposition of the thiamine moiety of the complex representing the primary ligand.

Infrared spectra

Fundamental IR spectral bands of the free azo compounds (secondary ligands) and thiamine hydrochloride were extensively investigated and compared to those of the mixed ligand complexes. The IR spectra of the free azo compounds are characterized mainly by strong bands at 3500-3420 cm⁻¹, 1675-1655 cm⁻¹, 1435-1405 cm⁻¹ and 1155-1145 cm⁻¹ attributed to stretching vibrations of V_{OH}, V_{C=O}, V_{N=N}

and V_{COOH}, respectively. On the other hand, the IR spectrum of thiamine hydrochloride is characterized by bands at 3310 cm⁻¹, 1635 cm⁻¹ and 1155 cm⁻¹ due to the stretching vibrations of V_{OH}, V_{C=N} (aromatic) and V_{C-S}, respectively. On comparing the spectra of the free ligands with those of the complexes showed that bands due to V_{OH} and V_{N=N} of the azo and V_{OH} and V_{C-S} of thiamine hydrochloride shifted to lower wavenumber indicating the contribution of these groups in complex formation. This is consistent with the appearance of new bands at 570-554 cm⁻¹, 532-505 cm⁻¹ and 463-452 cm⁻¹ due to the stretching vibrations of V_{M-S}, V_{M-O} and V_{M-N}, respectively [12]. Therefore, according to the IR spectra and the data of elemental analysis, it is concluded that both the primary and secondary ligands act as monovalent monodentate coordinated via ON fashion in case of azo compounds and via OS fashion in case of thiamine. This is represented as:



Electronic spectra and magnetic measurements

The electronic absorption spectra of the free ligands and their metal complexes were scanned in DMF and in solid state using Nujol mull technique, representative spectra are shown in Figures 3-6. The spectra in solid state show dramatic change from those in solution (DMF) due to the disturbance in crystal structure taking place when transferring from solid to solution states. Table 3 represents the average values of electronic transition bands (V_{max}; cm⁻¹) in Nujol mull and the corresponding assignments. The spectra of the free ligands, in the UV-Visible region show bands due to π-π*, n-π* and charge transfer (CT) transitions while those of the complexes show the characteristic d-d transition bands at far visible region. Co(II) spectra exhibit three d-d transition bands having average values at 26316, 24510 and 13699 cm⁻¹ due to ⁴T_{1g(F)} → ⁴T_{1g(F)} (V₃), ⁴T_{1g(F)} → ⁴A_{2g(F)} (V₁) and ⁴T_{1g(F)} → ⁴T_{2g(F)} (V₂) transitions in Oh field, respectively [13]. The spin-allowed transitions in Ni(II) complexes appear at 16949, 13423, 12821 cm⁻¹ which are assigned to electronic transitions ³A_{2g(F)} → ³T_{1g(F)}, ³A_{2g(F)} → ³T_{1g(F)} and ³A_{2g(F)} → ³T_{2g(F)}, respectively [14,15]. The electronic spectra of Cu(II) complexes show two bands whose average values centered at 15385, 13158 cm⁻¹ assigned to ²B_{1g} → ²A_{1g} (V₁) and ²B_{1g} → ²E_g (V₃) transitions, respectively suggesting tetragonally distorted octahedral geometry around Cu(II) ions [16,17]. The d-d transitions in Zr(IV) complexes are absent due to d⁰ electronic configuration. The spectral bands around 25974 and 23529 cm⁻¹ are due to n → π* or CT interaction (Zr → L). Magnetic susceptibilities were measured at room temperature. The μ_{eff} values, term symbols and ground state symbols are listed in Table 3. The average value of μ_{eff} of Co (II) complexes is 4.13 BM which lies between the spin-only, μ_{SO} = [4S(S+1)]^{1/2} = 3.88 BM and μ_{SL} = [4S(S+1) + L(L+1)]^{1/2} = 5.2 BM values, indicating an octahedral geometry around Cobalt(II) with orbital contribution to the spin – only magnetic moment values [18,19]. The Ni (II) complexes show magnetic moment values of 2.89 BM indicating an octahedral environment around Ni (II) ion [20,21]. The observed magnetic moments for the Cu (II) complexes lie within the range 1.72 BM suggesting a distorted octahedral geometry for Cu (II) complex

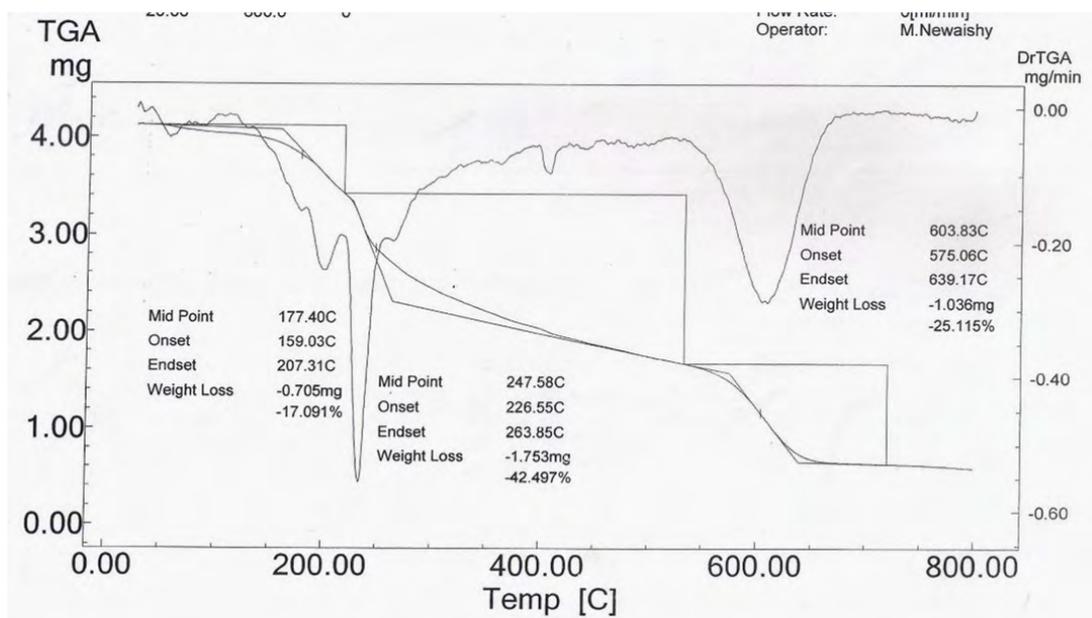


Figure 1: TG-DTA curves of [Thiam.-Ni- A1 (H₂O)₂]⁺Cl complex.

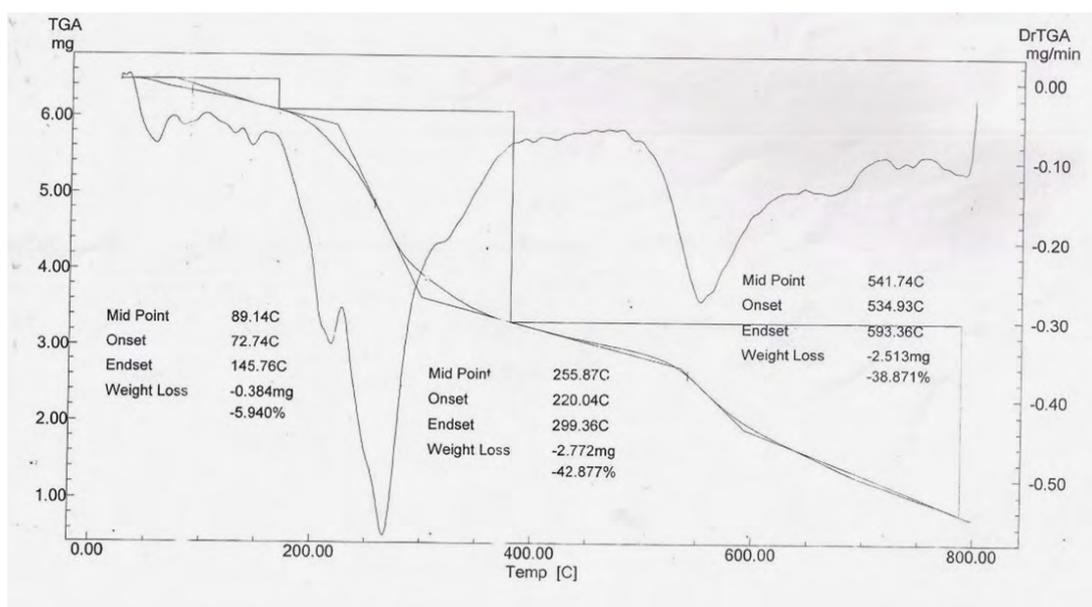


Figure 2: TG-DTA curves of [Thiam-Cu- A₂(H₂O)₂]⁺Cl complex.

[22]. The complexes of ZrO²⁺ are diamagnetic as expected from its d⁰ electronic configuration.

Biological activity

The mixed ligand complexes were tested against gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). Also, the antifungal activity against *Candida albicans*, *Aspergillus nigar* and *Aspergillus clavatus* was studied. The complexes were tested in concentration of 100, 200 and 300 mmol and in DMF as a negative control. On the other hand, media with ciprofloxacin (standard antibiotic for gram positive),

gentamicin (standard antibiotic for gram negative) and Griseofulvin (standard antifungi) were used as positive control. The growth inhibition percentage was calculated using the equation [23]

$$\text{Inhibition} = (C - T) / C \times 100$$

Where C is the diameter of the bacterial colony (mm) in the negative control plates after three days and T is the diameter of the colony (mm) of the treated plates after the same period.

The antibacterial and antifungal data are given in Tables 4 and 5. Inspection of the data given shows that: The percent inhibition increases

Complex	Tentative formula	M.Wt.	Elemental analysis*				Λ**
			%C	%H	%N	%M	
Thi-A ₁ -Co	[C ₂₅ H ₂₇ N ₆ O ₅ S Co (H ₂ O) ₂] Cl	652.99	45.98 (46.33)	4.17 (4.33)	12.87 (12.53)	9.02 (8.72)	25.5
Thi-A ₁ -Ni	[C ₂₅ H ₂₇ N ₆ O ₅ S Ni (H ₂ O) ₂] Cl	652.75	46.00 (45.72)	4.17 (4.64)	12.88 (12.08)	8.99 (9.23)	29.8
Thi-A ₁ -Cu	[C ₂₅ H ₂₇ N ₆ O ₅ S Cu (H ₂ O) ₂] Cl	657.61	45.66 (45.96)	4.14 (4.87)	12.78 (13.14)	9.66 (9.27)	20.6
Thi-A ₁ -Zr	[C ₂₅ H ₂₇ N ₆ O ₅ S Zr(H ₂ O)]Cl	683.28	43.95 (44.63)	3.98 (4.32)	12.30 (12.82)		30.3
Thi-A ₂ -Co	[C ₂₆ H ₂₇ N ₆ O ₆ S Co (H ₂ O) ₂] Cl	681.00	45.86 (46.22)	3.40 (4.16)	12.34 (12.90)	8.66 (9.08)	24.9
Thi-A ₂ -Ni	[C ₂₆ H ₂₇ N ₆ O ₆ S Ni (H ₂ O) ₂]Cl	680.76	45.87 (46.15)	4.00 (4.09)	12.34 (13.14)	8.62 (8.47)	22.4
Thi-A ₂ -Cu	[C ₂₆ H ₂₇ N ₆ O ₆ S Cu (H ₂ O) ₂]Cl	685.62	45.55 (45.78)	3.97 (3.88)	12.26 (12.98)	9.27 (9.86)	30.1
Thi-A ₂ -Zr	[C ₂₆ H ₂₇ N ₆ O ₆ S Zr (H ₂ O)] Cl	711.29	43.90 (44.23)	3.83 (4.09)	11.82 (12.64)		22.8
Thi-A ₃ -Co	[C ₂₅ H ₂₇ N ₆ O ₆ S Co (H ₂ O) ₂]Cl	665.99	45.09 (44.76)	4.09 (3.86)	12.62 (12.04)	8.85 (9.11)	18.8
Thi-A ₃ -Ni	[C ₂₅ H ₂₇ N ₆ O ₆ S Ni (H ₂ O) ₂]Cl	665.75	45.10 (45.16)	4.09 (4.41)	12.62 (12.85)	8.82 (8.41)	20.5
Thi-A ₃ -Cu	[C ₂₅ H ₂₇ N ₆ O ₆ S Cu (H ₂ O) ₂]Cl	670.61	44.78 (45.35)	4.06 (4.60)	12.53 (12.03)	9.48 (9.62)	32.4
Thi-A ₄ -Co	[C ₂₆ H ₂₇ N ₆ O ₇ S Co (H ₂ O) ₂]Cl	697.00	44.80 (44.34)	3.90 (4.33)	12.06 (12.66)	8.45 (8.93)	25.3
Thi-A ₄ -Ni	[C ₂₆ H ₂₇ N ₆ O ₇ S Ni (H ₂ O) ₂]Cl	696.76	44.80 (45.20)	3.91 (4.41)	12.06 (12.74)	8.42 (8.14)	19.6
Thi-A ₄ -Zr	[C ₂₆ H ₂₇ N ₆ O ₇ S Zr(H ₂ O)]Cl	727.29	42.94 (43.63)	3.74 (3.95)	11.56 (12.33)		26.7

Table 1: Elemental analysis and molar conductivity of some selected mixed thiamine-(A₁-A₄) complexes with Co²⁺, Ni²⁺, Cu²⁺ and ZrO²⁺ complexes.

Complex	Temp. (°C)	Weight loss %	Assignment
Thiam-Co A ₁	41-77	2.38	Removal of physically adsorbed water molecule.
	182-343	46.28	Decomposition of primary ligand (Azo 1)
	575-690	33.07	Decomposition of secondary ligand (thiamine)
Thiam-Co-A ₃	139-227	8.27	Removal of coordinated water molecule.
	264-424	33.42	Decomposition of primary ligand (Azo 3)
	553-684	43.44	Decomposition of secondary ligand (thiamine)
Thiam.Ni-A ₁	159-207	17.1	Removal of coordinated water molecule and HCl.
	227-264	42.5	Decomposition of primary ligand (Azo 1)
	575-639	25.12	Decomposition of secondary ligand (thiamine)
Thiam.Ni-A ₂	172-284	64.73	Removal of coordinated water molecules and decomposition of primary ligand (Azo 2)
	573-637	23.60	Decomposition of secondary ligand (thiamine)
Thiam.Cu-A ₂	73-146	5.94	Removal of coordinated water molecule.
	220-299	42.88	Decomposition of primary ligand (Azo 2)
	535-594	38.87	Decomposition of secondary ligand (thiamine)
Thiamzr-A ₁	49-107	2.69	Removal of physically adsorbed water molecule.
	199-261	44.09	Decomposition of primary ligand (Azo 1)
	473-616	34.14	Decomposition of secondary ligand (thiamine)
Thiam.Zr-A ₂	84-144	4.40	Removal of physically adsorbed water molecule.
	205-263	44.18	Removal of coordinated water molecule.
	485-554	19.44	Decomposition of primary ligand (Azo 2)
	578-643	14.22	Decomposition of secondary ligand (thiamine)

Table 2: Thermo gravimetric data for some selected complexes.

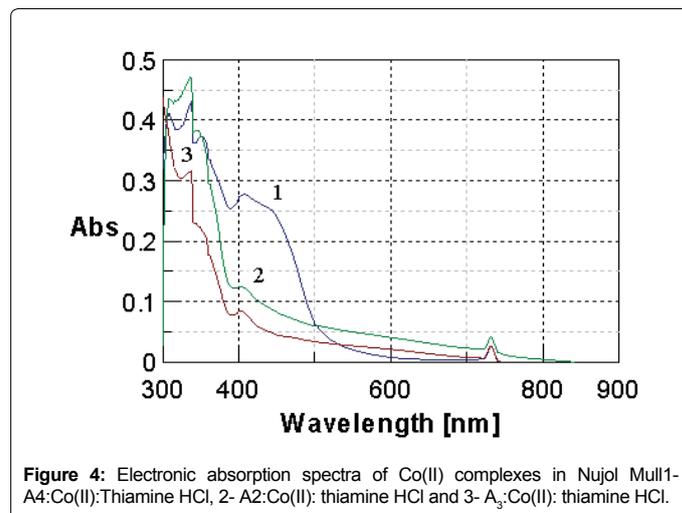
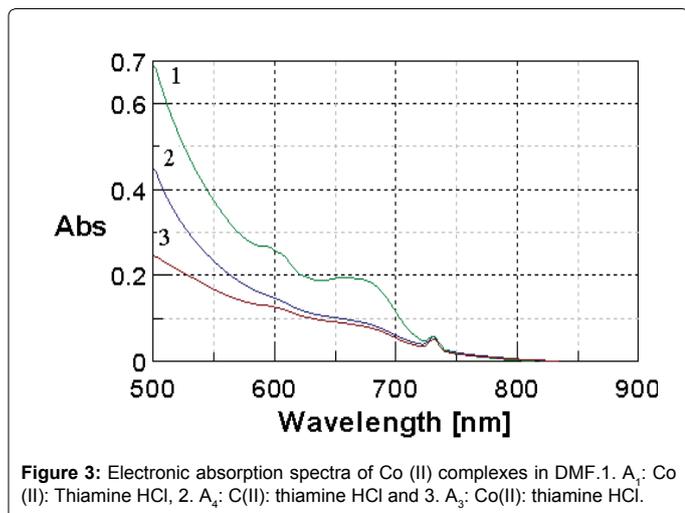
with increasing the concentration of the complexes from 100 to 300 mmol. Cu(II) complexes have the higher antibacterial and antifungal activities than the other metal complexes while ZrO complexes have the lower activity. In general, antifungal activity of metal-ligand complexes better than their antibacterial activities. The tested complexes are more active against gram negative than gram positive bacteria. It was reported that the antimicrobial activity is related to the cell wall structure of the bacteria because the latter is essential to the survival of bacteria. Gram positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acid, but in contrast, gram negative bacteria have a relatively thin cell wall consisting of few layers of peptidoglycan [24]. This difference in cell wall structure can produce

differences in antibacterial susceptibility and some antibiotics can kill only gram positive (or gram negative) bacteria and is ineffective against the other type.

Materials and Methods

Materials

All reagents used in the present study were of the highest quality (Merck, Aldrich and Fluka Research Laboratories) and were used without further purification. Thiamine hydrochloride was obtained from Egyptian Company for Chemicals and Pharmaceuticals. Freshly bi distilled water was used whenever water is necessary.

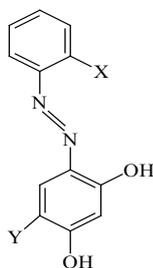


Complex	Wave No. (cm ⁻¹)	Assignment	Magnetic data		
			Term Symbol	Ground State	μ _{eff} (BM)
Co(II)	26316	⁴ T _{1g(F)}} → ⁴ A _{2g(F)}} (V ₂)	⁴ F	⁴ T _{1g}	4.13
	24510	→ ⁴ T _{1g(F)}} (V ₃)			
	13699	→ ⁴ T _{2g(F)}} (V ₁)			
Ni(II)	16949	³ A _{2g(F)}} → ³ T _{1g(P)}} (V ₃)	³ F	³ A _{2g}	2.89
	13423	→ ³ T _{1g(F)}} (V ₂)			
	12821	→ ³ T _{2g(F)}} (V ₁)			
Cu(II)	15385	² a _{1g(D)}} → ² b _{1g(D)}}	² D	² E _g	1.72
	13158	² e _{g(D)}} → ² b _{1g(D)}}			
Zr(IV)	25974 23529	M → L charge transere			Dia

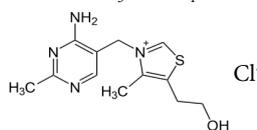
Table 3: Spectral and magnetic data for the mixed ligand complexes.

Preparation of the mixed ligands solid complexes

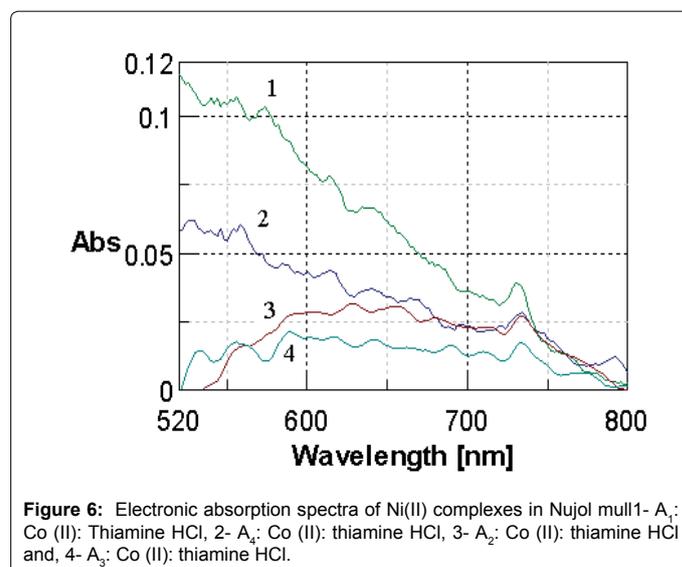
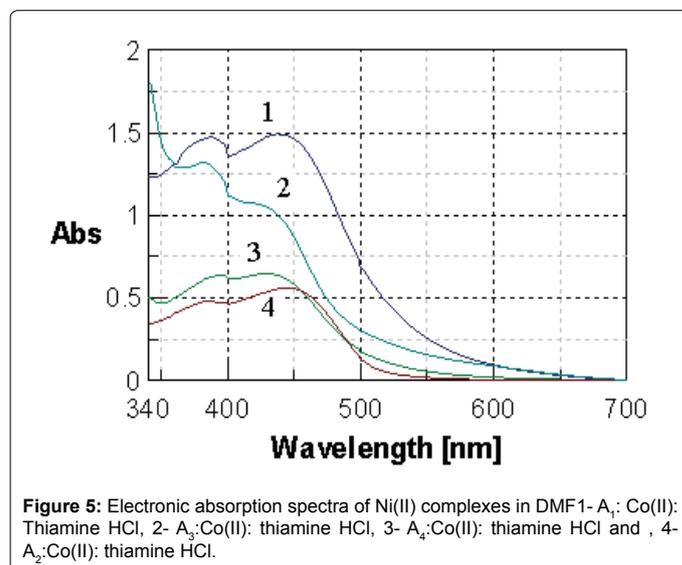
Mixed ligand Co(II), Ni(II), Cu(II) and Zr(IV) complexes were prepared from hydrated metal chloride (zirconyloxochloride, ZrOCl₂ for Zr complexes), thiamine hydrochloride as a primary ligand, and four azo compounds as secondary ligands. The azo compounds were prepared and characterized as described in our previous work [25]. The azo compounds and thiamine hydrochloride have the following structural formula:



X=OH (A₁ and A₃) and COOH (A₂ and A₄), Y=CHO (A₁ and A₂) and=COOH (A₃ and A₄)



Thiamine hydrochloride



The mixed ligand complexes were prepared by adding aqueous solution of 1.00 mmol of $MCl_2 \cdot xH_2O$ or $ZrOCl_2$ to an ethanolic solution of 1.00 mmol of each of the secondary ligand (A_1 - A_4) with constant stirring. The mixture was refluxed for \approx 3 hours then an ethanolic solution of thiamine hydrochloride (1.00 mmol) was added dropwise. The mixture (1:1:1 molar proportion) was again refluxed in a water bath for another 3 hours. The complexes were precipitated by adding few drops of ammonia buffer solution (pH 10). The mixture was cooled and the formed solid products obtained were filtered and washed with water followed by ethanol and dried under vacuum.

Physical measurements

Elemental analysis; (C, H and N) were carried out in the microanalytical centre, Cairo University, Giza, Egypt. Metal ion content (Co, Ni and Cu) was determined by EDTA titration under the appropriate conditions [26]. Infrared spectra were recorded as KBr disc technique using FT-IR spectrometer Model Nicolet is 10-thermo-scientific within the wavenumber range 4000-400 cm^{-1} . Thermo gravimetric analysis was carried out using Shimadzu TGA-50H thermal analyzer within the range 25°C-800°C under nitrogen atmosphere at heating rate of 10°C per minute using platinum crucible. The electronic absorption spectra of the complexes in solution (dimethylformamide; DMF as a solvent) and in solid state (Nujol mull technique) were recorded on Jasco V-530 (UV-Vis) double beam spectrophotometer (Japan) with scanning speed 400 nm/min and band width 2.0 nm using 10 mm matched quartz cell at room temperature in the range 800-200 nm. The spectral measurements were carried out at Faculty of Science, Benha University. Magnetic susceptibility measurements were carried out by Gouy method using $Hg[Co(CN)_4]$ as calibrant and magnetic susceptibility Johnson Matthey balance. The effective magnetic moments were calculated using the relation $\mu_{eff} = 2.828 (X_m T)^{1/2}$ BM where X_m is the molar susceptibility corrected for diamagnetism and T is the absolute temperature. Molar conductivities of the complexes (10^{-3} M) in DMF were measured using a conductivity bridge YSI model 32 applying the equation $A_m = \frac{1000 \times K \times M}{R \times m}$ where: A_m ; molar conductivity in $ohm^{-1}cm^2mol^{-1}$ M molecular weight of complex, m: mass of sample (g) in 10 mL of solvent, R; resistance of solution in ohm and K cell constant (Tables 4 and 5).

Antimicrobial screening

The complexes were incorporated in Czapeck's medium against Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and antifungal activity (*Candida albicans*, *Aspergillus niger* and

Aspergillus clavatus). The experiments were performed as mentioned in previous work [27]. The tested complexes were added directly to the cultivation media and their influence on the growth of microorganisms was considered. The complexes were tested in concentration of 100, 200 and 300 mmol. The medium with DMF as solvent was used as a negative control whereas media with ciprofloxacin (standard antibiotic for gram positive), gentamicin (standard antibiotic for gram negative) and Griseofulvin (standard antifungi) were used as positive control. The growth inhibition percentage was calculated using the equation

$$\text{Inhibition} = (C-T)/C \times 100$$

Where C is the diameter of the bacterial colony (mm) in the negative control plates after three days and T is the diameter of the colony (mm) of the treated plates after the same period.

Conclusion

In this work

Synthesis, characterization, antibacterial and antifungal studies of some drug-azo compounds mixed metal complexes are presented. The structures of the newly prepared complexes are in good agreement with those of the proposed structure as gathered from analytical and physicochemical data.

In brief, the results obtained can be summarized as follows as The stoichiometric ratio of the complexes is (1:1:1) (thiamine: M: Azo) and the complexes are non-hygroscopic in air and have electrolytic nature with the number of ions equal two. - Both primary and secondary ligands act as monobasic monodentate ligands coordinated to metal ion through OS fashion in thiamine and through ON fashion in azo compounds. The complexes are of octahedral structure around metal ions. The spin allowed d-d spectral transitions were assigned by the aid of Tanabe Sugano diagrams. The complexes show high antibacterial and antifungal activities. The antifungal activity is, more or less, higher than that of antibacterial. Among the studied metal complexes, Cu (II) complexes are the most effective while ZrO complexes are the least.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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Authors Contributions

Dr. Islam Moustafa performed the preparation of the materials after collecting them from companies, he collected the material science from the sources, and he

Complex	<i>S. aureus</i>			<i>S. pyogenes</i>			<i>E. coli</i>			<i>P. aeruginosa</i>		
	100	200	400	100	200	400	100	200	400	100	200	400
Thi- A_1 -Co	40.2	45.3	55.0	41.3	48.5	55.2	50.2	58.3	61.3	43.7	50.3	59.4
Thi- A_1 -Ni	44.4	48.2	53.8	46.4	52.3	60.3	52.3	58.4	69.3	50.2	57.2	69.4
Thi- A_1 -Cu	50.2	55.6	62.2	51.0	55.2	63.4	55.4	63.6	78.4	54.3	64.3	77.4
Thi- A_1 -Zr	43.6	49.1	54.8	38.4	41.4	42.2	46.2	48.1	50.3	42.1	44.2	45.5
Thi- A_2 -Cu	46.3	48.4	50.6	48.3	52.1	58.4	63.3	71.1	79.4	56.7	66.4	78.4
Thi- A_2 -Zr	38.3	40.2	40.6	33.9	38.7	40.7	48.5	53.7	65.2	50.2	59.3	68.7
Thi- A_3 -Co	39.6	42.2	47.6	40.4	44.3	50.1	44.6	60.6	72.4	48.4	59.3	72.8
Thi- A_3 -Cu	39.7	44.2	49.9	41.1	44.4	50.3	44.2	51.3	60.6	44.9	52.4	66.9
Thi- A_3 -Co	40.4	44.2	57.3	39.5	46.3	52.8	48.4	58.2	67.3	49.5	58.3	66.8
Thi- A_3 -Ni	44.9	50.1	56.3	43.8	53.6	60.6	47.2	56.4	63.7	45.2	57.6	64.8
Ciprofloxacin: 95%												
Gentamicin 92%												

Table 4: Percentage inhibition of the investigated bacteria after three days using 100, 200 and 400 mmol of the mixed ligand complexes.

Complex	<i>Candida albicans</i>			<i>Aspergillus niger</i>			<i>Aspergillus clavatus</i>		
	100	200	400	100	200	400	100	200	400
Thi-A ₁ -Co	44.6	55.3	68.4	46.3	58.4	69.2	44.1	54.3	64.4
Thi-A ₁ -Ni	43.3	52.6	66.6	47.3	58.5	70.1	46.3	57.6	68.9
Thi-A ₁ -Cu	55.8	67.3	78.1	53.3	66.4	77.4	53.4	68.4	74.3
Thi-A ₁ -Zr	38.2	43.4	50.3	33.4	37.4	45.3	37.2	45.8	49.3
Thi-A ₂ -Cu	53.8	66.8	72.1	50.2	60.2	72.4	49.6	57.8	68.4
Thi-A ₂ -Zr	41.7	46.1	54.6	40.6	45.4	61.1	45.1	51.4	58.4
Thi-A ₃ -Co	44.7	54.7	66.4	49.9	57.8	69.3	49.0	58.2	68.2
Thi-A ₃ -Cu	54.3	63.7	77.1	51.1	68.4	71.1	48.4	59.3	68.4
Thi-A ₃ -Co	45.7	54.8	60.1	47.7	56.3	66.8	46.6	57.3	67.9
Thi-A ₃ -Ni	49.9	56.4	68.2	50.1	61.0	71.3	44.6	57.4	69.3
Griseofulvin	92 %			90%			93%		

Table 5: Percentage inhibition of the investigated fungi after three days using 100, 200 and 400 mmol of the mixed ligand complex.

also made the characterization of the compounds prepared by different physical and chemical tools.

Dr. Magda Abd-Ellattif wrote and reviewed the paper from the data collected from Dr. Islam, she also made the biological activities studies and she contacted the journal for publication, she is also responsible for the publications fees.

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