*Synthesis, spectral studies and antimicrobial evaluation of 6,6⁻-(1,2-benzenediazo) bis (3-amino benzoic acid) complexes with some metal ions

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Abstract

The Ligand 6,6⁻-(1,2-benzenediazo) bis (3-aminobenzoicacid) derived from o-phenylenediamine and 3-aminobenzoicacid was synthesized. The prepared ligand was identified by Microelemental Analysis, ¹HNMR, FT-IR and UV-Vis spectroscopic techniques. Treatment of the ligand with the following metal ions (Co^{II}, Ni^{II}, Cu^{II} and Zn^{II}) in aqueous ethanol with a 1:1 M:L ratio and at optimum pH. Characterization of these compounds has been done on the basis of elemental analysis, electronic data, FT-IR and UV-Vis, as well as magnetic susceptibility and conductivity measurements. The nature of the complexes formed were studied following the mole ratio and continuous variation methods, Beer's law obeyed over a concentration range (1×10⁻⁴ - 3×10⁻⁴ M). High molar absorbtivity of the complex solutions were observed. On the basis of physicochemical data tetrahedral geometries were assigned for the complexes. The ligand and metal complexes were screened for their antimicrobial activity.

Keywords: - spectral studies, bisazo dye, antimicrobial activity

Chemistry classification : QD 146-197

Introduction

Azo dyes are a class of compounds containing a (N=N) double bond and due to their ability to absorb visible light, and ease of synthesis have been extensively used in the textile, fiber, leather, paint and printing industries for more than a century^(1,2). It is a subject of interest to develop a new structure of azo dyes which has commercial applications, in polyester, polyacrylic, polyamide as well as other kinds of fiber blends⁽³⁾. Azo containing polymers are macromolecules having the azo groups in the

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main ore side chain position. The azo benzene moiety has reasonable stability and high glass transition temperature (Tg). The thermal stability of the dye can be improved to a great extent by introducing azo benzene linkages in the polymeric main chain, this will also the application of the dye as an engineering plastic^(4,5). In recent years there is a great interest in coordination chemistry of the azo ligand⁽⁶⁻⁸⁾. Metal complexes of azo compounds containing hetroaryl ring systems find many application, such as indicator in complexometric, dyes and pigments in textile industry⁽⁹⁾. In this work, azo dye derived from o-phenylenediamine as diazo component and 3-aminobenzoicacid as coupling agent. The complex of this ligand with some metal ions has been prepared and characterized physciochemically.

Experimental

Instrumentation

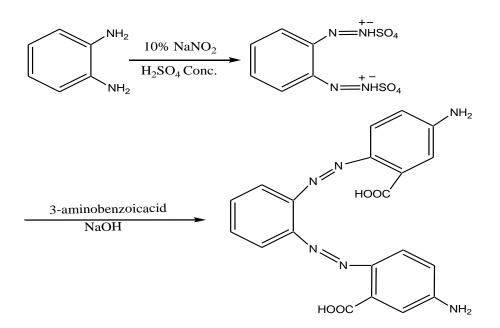
UV- Vis spectra were recorded on a Shimadzu UV- 160A Ultra Violet-Visible Spectrophotometer. IR- spectra were taken on a Shimadzu, FT-IR- 8400S Fourier Transform Infrared Spectrophotometer in the 4000- 400 cm⁻¹ spectral regions with samples prepared as KBr discs. Atomic absorption was obtained using a Shimadzu A.A-160A Atomic Absorption/Flame Emission Spectrophotometer. The ¹H-NMR spectra were recorded on a Brucker-300 MHz Ultra Shield spectrometer at the University of Al- al- Bayt using DMSO-d₆ as the solvent and TMS as the reference. Microelemental analysis (C, H, N) were performed at the Al- al- Bayt University, Jordan, using Euro vector EA 3000A Elemental Analyser. Conductivities were measured for 10^{-3} M solutions of complexes in ethanol at 25°C using Philips PW-Digital Conductimeter. Magnetic susceptibilities were performed by using Brucker Magnet B.M.6 instrument at 25C°. In addition, melting points were obtained using Stuart Melting Point Apparatus.

Materials and Reagents

The following chemicals were used as received from suppliers; cobalt chloride hexahydrate 98.8%, nickel chloride hexahydrate 99.9%, copper chloride dihydrate 98% and zinc chloride 98.8%,(Merck), o-phenylenediamine and 3-aminoenzoicacid (B. D. H).

Preparation of the Ligand ⁽¹⁰⁾

A solution of o-phenylenediamine (0.27g,1mmole) (10ml) of EtOH solution contain (2ml) conc.H₂SO₄ was diluted with 10 ml H₂O, and diazotized at 5°C with NaNO₂ solution. The diazo solution was added drop wise with stirring to a cooled ethanolic solution of (0.685g, 2mmole) of 3-amino benzoic acid. 25 ml of 1M sodium hydroxide solution was added to the dark colored mixture. The precipitate was filtered, washed several times with (1:1) ethanol: water, mixture then left to dry. The reaction is shown in scheme 1, while Table 1 describes the physical properties and elemental analysis.



Scheme 1. Synthesis of the azo dye Ligand (L).

Buffer Solution

Buffer solution, covering the pH range of 4-9, were prepared as 0.01 M solutions of ammonium acetate in distilled water. The required pH was obtained by the addition of either ammonia solution or glacial acetic acid.

Metal Salt Solutions

Solutions of $(1.10^{-5}-1.10^{-3} \text{ M})$ of the metal salt were prepared by dissolving appropriate weight of the metal salt in the buffer solutions.

Ligand Solution

Ligand solutions of $(1.10^{-5} - 1.10^{-3} \text{ M})$ were prepared by dissolving a required weight of the ligand in ethanol.

Study of Biological Activity

Three selected types of bacteria were used in this study Escherichia Coli (E.Coli) as Gram Negative Bacteria, Staphylococcus Aureus (Staph. Aurous) as Gram Positive Bacteria and Pseudomonas Aeruginosa (Ps. Aeruginosa) in Neutrient Agar medium, using (DMSO) as a solvent and as a control, the concentration of the compounds in this solvent was 10^{-3} M, using disc sensitivity test. This method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plate. The plates were incubated for 24hrs at 37C°.

Preparation of Metal Complexes (general procedure)

An ethanolic solution of the ligand (0.404g,1mmole) was added gradually with stirring to the 0.118g, 0.118g, 0.085g and 0.068g (1mmole) of CoCl₂.6H₂O, NiCl₂.6H₂O, CuCl₂.2H₂O and ZnCl₂ respectively dissolved in the buffer solution of the required pH. The mixture was cooled until dark color precipitate was formed, filtered and washed several times with (1:1) water: ethanol then with acetone.

Results and Discussion

The ligand (L) was prepared by coupling 3-aminobenzoic acid with the o-phenylenediamine in alkaline solution. The ligand (L) was sparingly soluble in water but soluble in organic solvents such as DMSO and DMF, stable toward air and moisture.

Synthesized ligand (L) was characterized by ¹H-NMR, FT-IR, Elem.Anal. (C,H,N) and UV-Vis spectroscopic technique. The ¹HNMR spectrum of the ligand in DMSO (Fig 1) shows multiplet signal at δ = 6.866-8.317 ppm, which refers to aromatic protons⁽¹¹⁾. A signal at δ =13.246 ppm appears due to hydrogen atom of the carboxylic group⁽¹²⁾. The signal at δ = 4.570 ppm is assigned to δ (NH₂) and the signal at δ = 2.50 ppm referred to DMSO-d₆⁽¹³⁾.

Interaction of the metal ions Co(II),Ni(II),Cu(II) and Zn(II) with the prepared ligand (L) has been studied in solution; an aqueous- ethanolic solution was always performed over wide molar concentration and acidity range. Colours of these mixed solutions were varied from brown or green.

Different range of molar concentrations $(10^{-5}-10^{-3} \text{ M})$ of the mixed solutions, only concentrations in the range of 10^{-4} M obey the Lambert- Beer's law, and only these solutions showed intense colour. A calibration curve was fitted to data points in the range $1.10^{-4} - 3.10^{-4}$ M, which shows absorbance against molar concentration (Figure 2). Best fit straight lines were obtained with correlation factor of R> 0.998.

The optimal concentration was chosen for complex solutions, and it was observed that the absorption maximum (λ_{max}) remained the same at different pH values. The influence of pH was also studied in the pH range of 4-9, and the absorbance- pH curves for each metal ion measured at λ_{max} are plotted in Figure 3. Figure 3 shows selective pH-absorbance curves. The plateaus of the curves represent the completion of the reaction and consequently represent the optimum pH value.

The composition of complexes formed in solutions has been established by mole ratio and job methods. In both cases the results reveal a 1:1 metal to ligand (L) ratio. A selected plot is presented in Figure 4.

Table 2 summarizes the results obtained, as well as conditions for the preparation of the complexes.

The solid complexes have been prepared by direct reaction of alcoholic solution of the ligand (L) with the aqueous solution of the metal ions at the optimum pH and in a M:L ratio of 1:1. The result of C.H.N analysis and the metal content of these complexes were in good agreements with the calculated values.

The molar conductance of 10⁻³ M in ethanolic solutions of these complexes indicated their non- electrolytic nature;⁽¹⁴⁾ data are presented in Table 2. The UV-Vis

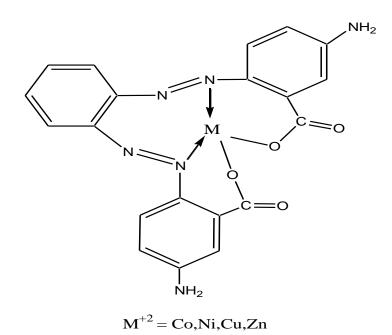
spectra of the prepared complexes dissolved in ethanol (10^{-3} M) have been measured and the data obtained are included in Table 2.

The effective magnetic moments (Table 2) of the complexes lie in the range (1.73-4.67) B.M. This value refers to a paramagnetic (high spin) which has been reported for most octahedral geometry. In case of Zn^{II} complex because of filled-d orbital, therefore the magnetic moment (μ =0) is diamagnetic ⁽¹⁵⁾.

The UV- Vis spectrum of an ethanolic solution of the ligand 10⁻³ M displayed mainly three peaks, the first and second peaks were observed at 263 nm and 340 nm were assigned to the moderate energy π - π^* transition of the aromatic rings. The third peak (λ_{max}) was observed at the 401 nm was referred to the π - π^* transition of intermolecular charge- transfer taken place from benzene through the azo group (-N=N)⁽¹⁶⁾. The spectrum of Co(II) complex showed peak at 319 nm due to charge transfer. Other three peaks at 397 nm, 644 nm and 822 nm were found to be caused by (d-d) electronic transition type ${}^{4}A_{2(F)} \rightarrow {}^{4}T_{1(P)}$, ${}^{4}A_{2(F)} \rightarrow {}^{4}T_{1(F)}$ and ${}^{4}A_{2(F)} \rightarrow {}^{4}T_{2(F)}$ respectively⁽¹⁷⁾. The spectrum of Ni(II) complex appeared absorption peak at 320 nm was related to charge transfer, then other three peaks at 437 nm, 630 nm and 761 nm were assigned to electronic transition type ${}^{3}T_{1(F)} \rightarrow {}^{3}T_{1(F)} \rightarrow {}^{3}A_{2(F)}$ and ${}^{3}T_{1(F)} \rightarrow {}^{3}T_{2(F)}$ respectively⁽¹⁸⁾. The spectrum of Cu(II) complex gave absorption peak at 322 nm due to charge transfer. The peak at 646 nm was caused by electronic transition⁽¹⁹⁾ ${}^{2}T_{2} \rightarrow {}^{2}E$. The spectrum of Zn(II) complex showed absorption peak at 314 nm due to charge transfer. The absence of absorption peaks in the visible region indicated no (d-d) electronic transition happened, this is a good result for tetrahedral complex.⁽²⁰⁾

In order to study the bonding mode of the ligand (L) with the metal ions, the FT-IR spectra of the free ligand (I) and the prepared complexes have been compared, and the data was tabulated in Table 3. The IR spectrum of the ligand (L) exhibited broad band at 3448 cm⁻¹, which was assigned to the stretching vibration of v(OH)group. This band was absent in the spectra of all prepared complexes, which indicated deprotonation and involvement of the enol oxygen in chelation⁽²¹⁾. The bands at 3371 cm⁻¹ and 3236 cm⁻¹ appears due to the stretching mode of $v(NH_2)$. Since no change in these bands was noticed, the possibility that coordination occurs via the coordination of nitrogen atom of this group was excluded⁽²²⁾. A significant change in the intensity and in position to lower wavenumber was observed on complexation with metal ion for the strong band in the free ligand (L) spectrum at 1589 cm^{-1} , which appears due to $v_{as}(COO)$ asymmetric vibration. The band at 1558 cm⁻¹ in the spectrum of the ligand (I), which was assigned to the $v_s(COO)$ symmetric vibration, suffered a great change to higher wavenumber on complexation with metal ion⁽²³⁾. Band characteristic of the azo bridge vibration at 1462 cm⁻¹ shifted to lower frequency with change in shape in spectra of complexes, which is an indication of the engagement of this group in the coordination with the metal $ion^{(24)}$. The appearance of new bands in 470-578 cm⁻¹ are tentatively assigned to v(M-N) and v(M-O)the region of (Metal-Ligand) stretching bands^(25,26).

According to the results obtained, a tetrahedral structure has been tentatively suggested to these complexes (Scheme 1).



Scheme 1

Finally, the biological activities of the ligand (L) and their complexes have also been examid against selected type of bacteria; Table 4 shows the deactivation capacity against the bacteria specimen of the prepared compounds under study. The results show that the Co- complex has a relatively strong deactivating capacity.

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Compounds	Color	M.P°C	Yield%	Analysis Calc (Found)			nd)
				M%	C%	H%	N%
Ligand(L)	Yellow	178	76	-	59.40	3.96	20.79
					(58.98)	(3.77)	(19.89)
[Co L]	Yellowish	235	71	12.74	51.83	3.45	18.14
	Orange			(12.37)	(51.25)	(2.98)	(17.78)
[Ni L]	Yellowish	238	73	12.55	51.94	3.46	18.18
	green			(11.88)	(49.79)	(3.11)	(17.65)
[Cu L]	Pal	244	77	13.67	51.28	3.42	17.94
	green			(12.95)	(50.81)	(2.96)	(16.84)
[Zn L]	Pal	240	82	13.85	51.17	3.41	17.91
	yellow			(13.25)	(50.93)	(3.11)	(16.97)

Table 1. Physical Properties and Elemental Analysis of the
Complexes.

Table 2. Conditions for the Preparation of the Complexes and UV-Vis, Magnetic Susceptibility and Conductance Measurements Data.

Compounds	Optimum	Optimum	M:L	(λ	ABS	€ _{max}	$\Lambda_{\rm m}$	μ_{eff}
	pH	Molar Conc.	Ratio	nm)		(L.mol ⁻	(S.cm ² .mol ⁻¹)	(B.M)
	-	x 10 ⁻⁴		ŕ		¹ .cm ⁻¹)	In Absolute	
						· ·	ethanol	
Ligand(L)	-	-	-	263	0.836	836		-
-				340	1.532	1532		
				401	2.370	2370		
[Co L]	6.5	2.5	1:1	319	2.233	2233	13.86	4.68
				397	1.955	1955		
				644	0.235	235		
				822	0.121	121		
[Ni L]	6	2.5	1:1	320	0.235	235	10.73	2.96
				437	0.323	323		
				630	0.133	133		
				761	0.153	153		
[Cu L]	6	2	1:1	322	1.335	1335	20.32	1.73
				646	0.381	381		
[Zn L]	7	2.5	1:1	314	1.325	1325	18.31	Dia

Table 3. The Main Frequencies of the Ligand and Their Complexes (cm⁻¹).

Compounds	v (OH)	v(NH ₂)	v _{as} (COO)	v _s (COO)	v(-N=N-)	v (M-N)	v (M-O)
Ligand(L)	3448 br.	3371 br. 3325 br.	1589 s.	1489 sh.	1462 sh.	-	-
[Co L]	-	3369 s. 3323 s.	1578 s.	1496 sh.	1436 s.	532 w.	482 w.
[Ni L]	-	3370 sh. 3326 sh.	1550 sh.	1535 sho.	1425 sho.	547 w.	455 w.
[Cu L]	-	3371 s. 3324 s.	1566 s.	1496 sho.	1458 s.	524 w.	451 w.
[Zn L]	-	3368 s. 3324 s.	1558 sho.	1508 s.	1450 sho.	590 w.	470 w.

Table 4. Diameters(mm) of Deactivation of Bacteria for the Ligand and its Complexes.

Compounds	Staphylococcus	Escherichia	Pseudomonas	
	Aurous	Coli	Aeruginosa	
Ligand(L)	14	12	12	
[Co L]	38	40	28	
[Ni L]	13	13	0	
[Cu L]	12	0	0	
[Zn L]	13	15	10	

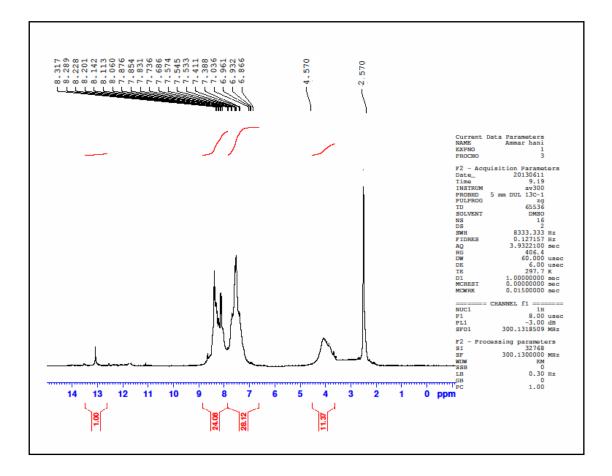


Figure 1. ¹H-NMR spectrum of the azo dye ligand (L).

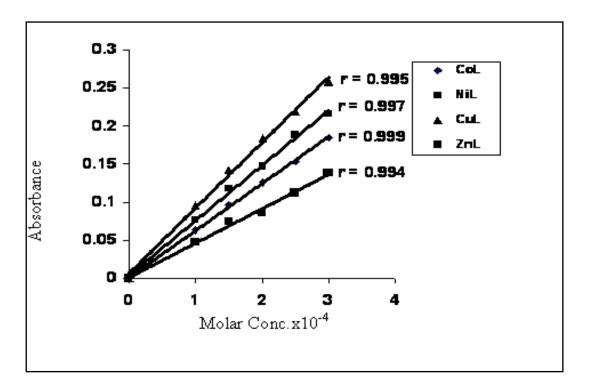


Figure 2. Linear correlation between molar concentration and absorbance.

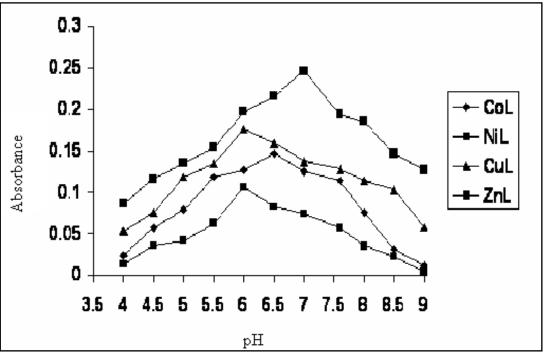


Figure 3. Effect of pH on absorbance (λ_{max}) for complexes.

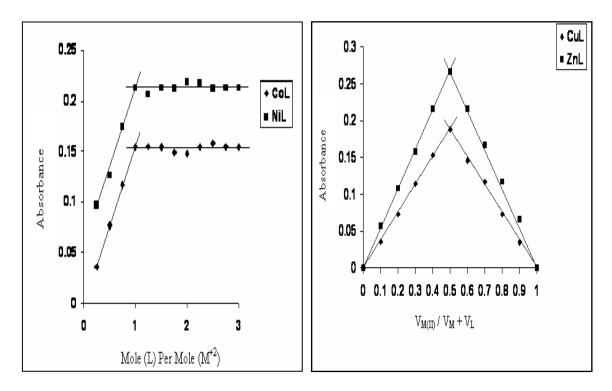


Figure 4. Mole ratio and Job methods for complexes, solutions.

تحضير، دراسة طيفية وتقييم الفعالية البايولوجية لمعقدات 6،6-(2،1- بنزين آزو) ثنائي (3- امينو حامض البنزويك) مع بعض الأيونات الفلزية.

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الخلاصة

حضرت الليكاند 6،6-(1،2- بنزين آزو) ثنائي (3- امينو حامض البنزويك) من تفاعل ازدواج 2،1-فنيلين ثنائي الأمين مع 3- امينو حامض البنزويك . شخص الليكاند المحضر بوساطة أطياف الرنين النووي المغناطيسي والأشعة تحت الحمراء وفوق البنفسجية- المرئية والتحليل الدقيق للعناصر (C.H.N) . تمت مفاعلة الليكاند مع بعض ألايونات ألفلزية المنتخبه (Co^{II}, Ni^{II}, Cu^{II} and Zn^{III}) في وسط ايثانول - ماء وبنسبة فلز: ليكاند (1:1) وفي الدالة الحامضية المثلى، وخضعت محاليل هذه المعقدات لقانون لامبرت –بير ضمن مدى التراكيز (M ⁴⁻¹0 × 2 - ^{4-10×11}). شخصت هذه المعقدات باستخدام تقنية الامتصاص الذري اللهبي، أطياف التراكيز (الا ⁴⁻¹0 × 2 - ^{4-10×11}). شخصت هذه المعقدات باستخدام تقنية الامتصاص الذري اللهبي، أطياف التراكيز والمنعة وولاشعة فوق البنفسجية – المرئية والتحليل الدقيق للعناصر (C.H.N)، فضلا عن قياسات التراكيز المنعر المراء والأشعة فوق البنفسجية – المرئية والتحليل الدقيق للعناصر (C.H.N)، فضلا عن قياسات التراكيز المتعراء والأشعة فوق البنفسجية – المرئية والتحليل الدقيق للعناصر (C.H.N)، فضلا عن قياسات والمتغيرات المستمرة ، ومن النتائج المحصول عليها تم اقتراح الشكل رباعي السطوح للمعقدات المولية والمتغيرات المستمرة ، ومن النتائج المحصول عليها تم اقتراح الشكل رباعي الماطوح المعقدات المحضرة. كما مت دراسة الفاعلية البايولوجية ووجد أن لهذه المعقدات قابلية متباينة على قتل الأنواع المنتخبة من البكتريا.

Chemistry classification : QD 146-197

*البحث مستل من اطروحة دكتوراه للباحث الثاني.