Synthesis, characterization and bioactivity Study from azo – ligand derived frommethyl-2-amino benzoatewith some metal ions

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**Abstract:**  
A Ligand (ECA) methyl 2-((1-cyano-2-ethoxy-2-oxoethyl)diazenyl)benzoate with metals of (Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$) were prepared and characterization using H-NMR, atomic absorption spectroscopy, ultra violet (UV) visible, magnetic moments measurements, bioactivity, and Molar conductivity measurements in soluble ethanol. Complexes have been prepared using a general formula which was suggested as [M (ECA)$_{2}$] Cl$_{2}$, where M = (Cobalt(II), Nickel(II) and Copper(II), the geometry shape of the complexes is octahedral.

**Keywords:** Bioactivity, complexes, ethanol, Molar conductivity, Transition Metals.

**Introduction:**  
Azo compounds are very important class of chemical compounds that is attracting study attention, they are highly colorful and have long been used as dyes and pigments. Transition metal azo compounds were instrumental in the development of coordination chemistry are widely used in industrial and biological systems. Azo compounds are very important molecules, these compounds derivatives and their metal complexes are very important pigments for synthetic leather and vinyl polymers. Coordination compounds (complexes) play a significant role in our life. Coordination compounds characteristics are the most important way in determining the chemistry of the transitional elements. Their study contributes in quietly understanding the chemistry of organometallic and inorganic compounds, and chemical bonds as a whole. The figure of possible coordination compound is almost infinite. Inorganic compounds especially, transition metals, have played a significant part in the development of some cosmetic compounds and new metal-based drugs. Previously, complexes have been regarded as of interest only to the theoretical and to the inorganic chemists. However, these complexes are revealing a vital role in biochemistry, polymerization processes, and analytical chemistry through organic compounds preparation.

Transition metals, in particular, have played a key role in the creation of new metal-based pharmaceuticals and cosmetic compositions. Copper, for example, is one of many transition metals used in cosmetics (Cu). Nickel a transition metal is utilized as a skin sensitizer in cosmetics. Nickel, on the other hand, is one of the most poisonous transition metals. The aim of the work is preparation metal complexes from the Ligand (ECA) methyl 2-((1-cyano-2-ethoxy-2-oxoethyl)diazenyl)benzoate, Characterization of ligands and prepared complexes by different techniques and study of bioactivity of these complexes.

**Materials and Methods:**  
All the used chemical are supplied by either Fluka or Merck were of reagent grade and used as supplied. Ultra Shield 300 MHz (Bruker, Switzerland) was used to identify HNMR Spectra at University of Al-Bayt, Jordon. Shimadzu FT-IR infrared spectrophotometer was used to record absorption in the region of (4000-400cm$^{-1}$) by using a KBr disc. Shimadzu UV-Vis. (160A)
spectrophotometer was used to identify UV-Vis-
spectra in ethanol solution. Shimadzu AA680G
atomic absorption spectrophotometer was used to
identify metal content atomic absorption technique
of the complexes. A balanced magnetic
susceptibility model (MSBKT) in the Faraday
method was used to measure the magnetic moment
(µ_eff B.M). Melting points were measured by the
Stuart – melting point apparatus. Philips PW
Digital conductivity meter was used to measure
conductivity of the prepared compounds.

Preparation of the ligand (ECA) 5
Methyl-2-amino benzoate solution (1.3ml,
0.01mole) in HCl (3ml) was concentrated and
cooled to (0-5°C), then sodium nitrite (1.5g in 10ml
of water) cooled solution was added drop by drop
over 10 minutes. At the same temperature, the
reaction mixture was agitated for 30 minutes. Drop
by drop, over 15 minutes, the mixture was added
to an ice-cold combination of ethyl cyanoacetate
(0.01mole) and sodium acetate (4.1g, 0.05mole) in
ethanol (30ml). After that, the solution was stirred
for 30 minutes and then allowed to sit at room
temperature for (2 hours). The solid product was
recrystallized after it was produced and collected
from ethanol to give the orange crystals (ECA),
m.p (142-144˚C), and yield (75%). The reaction is
shown in scheme 1.

Scheme 1. Preparation route of Ligand (ECA)

Preparation of complexes
A solution of metal chloride containing
(0.11g, 0.11g and 0.076g) (1mmole) of
CoCl_2.6H_2O, NiCl_2.6H_2O, and CuCl_2.2H_2O
respectively was added to a solution of the ligand
(ECA) (0.25g, 2mmole) in ethanol (5ml). After
stirring for three hours, a colored precipitate was
formed at room temperature. The rustling solids
were filtered off from ethanol then dried at 50˚C in
oven.

Result and discussion
The isolated compounds which are
crystalline solids, were soluble ethanol, dimethyl
formamide (DMF), and dimethyl sulphoxide
(DMSO). The conductivity measurements in
ethanol pointed out the electrolytic behavior. The
molar ratio method was followed to measure the
ratio of metal ion to ligand in complexes (M:L).
Ethanol was used as a solvent. The (M: L) ratio was
(1:2).

Table. 1 includes the physical properties; the
magnetic measurements (µeff B.M) for the
complexes.

Spectra studies
The ¹HNMR In DMSO, the spectrum of the
ligand (ECA) was obtained, and Fig.1, shows
the following signals: (DMSO) singlet (s) at (2.50)
ppm, triplet (t) at (1.35) ppm for (3H, CH₃), singlet
(s) at (3.9) ppm for (3H, OCH₃), quartet (q) at
(4.34) ppm for (2H, OCH₂), and multiplet (m) at
(7.2-8.0) ppm for (4H, Ar-H). The IR spectra of the
ligand (ECA) and its complexes are shown in Table
2. In the free ligand, the prominent absorption bands
were at (1701, 1583) cm⁻¹ and (2222) cm⁻¹. The IR
spectrum of the ligand (ECA) resulting from the
(C=O) ester and (C=N) ⁴ in the complexes spectral
is shown in Fig. 2. The IR spectra of the cobalt complex is shown in Fig. 3.

The bands were in a range between (1637-1508) cm$^{-1}$ and deviated from lower frequencies by (64-193) cm$^{-1}$. This shows that the oxygen atom at the (C=O) group in methyl ester $^5$ is coordinated. A band of (C=N) was missing, indicating that the nitrogen atom of (C=N) was not coordinated. (N=N)$^6$ caused a band in the ligand spectra at (1454) cm$^{-1}$. Complex spectra show that this band was around (1436-1373) cm$^{-1}$ and that it deviated to lower frequencies by (81-18) cm$^{-1}$. This shows that the ligand (ECA) is coordinated through the azo nitrogen atom, and new bands formed in the spectra of metal complexes at (530-510) cm$^{-1}$ and (462-415) cm$^{-1}$, respectively, attributable to the (M-O) and (M-N).$^8$

The spectrum of the ligand

Table. 3 shows the assignments and absorptions that were related to the ligand and its complexes Fig. 4, displays an electronic spectra of ligand (ECA) with absorption bands at 253 nm (27548) cm$^{-1}$ and 361 nm (27710) cm$^{-1}$, which might be attributed to ($\pi \rightarrow \pi^*$) and ($\pi \rightarrow \pi^*$) transitions.$^9$

Spectra of Complexes

[Co(ECA)$_2$]Cl$_2$ complex

The bands at (250)nm (40000) cm$^{-1}$, (364)nm (27472) cm$^{-1}$, (665) nm (15037)cm$^{-1}$, and (841) nm (12285) cm$^{-1}$ in the spectra of deep green Co(II) complex were assigned to (C.T), $^4$T$_{1g}$P$_{1}$, $^4$T$_{1g}$P$_{2}$, $^4$A$_{2g}$, and $^4$T$_{3g}$ respectively.$^{10}$ The value of $\beta = B / B'$ is (0.388), indicating the presence of a covalent bond in the complex. The magnetic moment of the cobalt (II) complex is (4.34) B.M in the octahedral range.

[Co(ECA)$_2$]Cl$_2$ complex

The absorptions at (251) nm (39840) cm$^{-1}$, (360) nm (27777)cm$^{-1}$, (502) nm (19920) cm$^{-1}$, and (765) nm (13071) cm$^{-1}$ were ascribed to (C.T), $^3$A$_{2g}$ =$^3$T$_{1g}(E)$, $^3$A$_{2g}$, $^3$T$_{1g}(F)$, and $^3$A$_{2g}$ respectively.$^{11}$ in the spectra of red complex Fig. 5. The value of $\beta$ was determined to be (565.6) nm, and the value of $\beta' = B / B'$ is (0.543), indicating the presence of a covalent bond in the complex. The moment of induction is (3.29). The above-mentioned geometry was confirmed by B.M. From these results, the distorted Octahedral structure was proposed for this complex.

[Cu(ECA)$_2$]Cl$_2$ complex

The absorption bands observed at (254)nm(39370) cm$^{-1}$, (363)nm(27548) cm$^{-1}$ and (520)nm (19231) cm$^{-1}$, which were attributed to the (C.T), $^3$Eg =$^2$T$_{2g}$transition respectively.$^{12}$ The spectrum of deep red showed absorption bands at (39370) nm and (27548) nm, which were assigned to charge transfer transitions (C.T). The magnetic moment of the copper (II) complex is (1.75) B.M in the octahedral range.

| Table 1. The results of microanalysis and some physical properties of ligands and their complexes |
|-------|-------|-------|-------|-------|-------|-------|-------|
| Formula of compound | Molecular weight | Colour | M.p ^°C | Found (Calc.) | Metal % Found (Calc.) | Molar conductivity (S.cm$^{-1}$ mole$^{-1}$) in Ethanol (10$^{-3}$ M) | eff (β/M) Found (Calc.) | Suggested Structure |
| C$_3$H$_5$O$_3$N$_3$ | 275 | Orange | 142- | 56.7 | 4.7 | 15.2 | - | 2.4 | - |
| [ECA] | 679.93 | Deep green | 95(dec.) | 22.5 | 1.8 | 6.06 | 8.50 | 80 | 4.34 | octahedral |
| [Co(ECA)$_2$]Cl$_2$ | 679.69 | Red | 100(dec.) | 19.8 | 1.6 | 1.6 | 7.46 | 75 | 3.29 | octahedral |
| [Ni(ECA)$_2$]Cl$_2$ | 684.54 | Deep red | 98(dec.) | 24.7 | 2.06 | 6.6 | 10.07 | 81 | 1.75 | octahedral |

| Table 2. Infrared spectrum bands of the ligand (ECA) and its metal complexes |
|-------|-------|-------|-------|-------|-------|
| Comounds | $\nu$ (N=N) | $\nu$(C=O) Ester | $\nu$(C=N) | M-N | M-O |
| C13H13O4N3 [ECA] | 1454 | 1701, 1583 (s) | 2222(s) | - | - |
| [Co(ECA)$_2$]Cl$_2$ | 1435(m) | 1627(s) | 2220(s) | 420(w) | 526(w) |
| [Ni(ECA)$_2$]Cl$_2$ | 1436(m) | 1637(s) | 2215(s) | 424(w) | 530(w) |
| [Cu(ECA)$_2$]Cl$_2$ | 1404(w) | 1620(s) | 2210(s) | 418(m) | 520(w) |

Where:  
$s$ = (strong)  
$m$ = (medium)  
$w$ = (weak)
Table 3. In ethanol solvent, electronic spectral data of ligand (ECA) and its complexes

| Compound | λmax  nm | Wave number cm⁻¹ | εmax Molar cm⁻¹ | Assignment |
|----------|----------|------------------|----------------|------------|
| Ligand [ECA] | 253 | 39525 | 567 | π→π* |
| | 361 | 27710 | 1207 | n→π* |
| [Co(ECA)₂]Cl₂ | 250 | 40000 | 428 | C.T |
| | 364 | 27472 | 870 | ⁴T₁g(F)→⁴T₁g(P) |
| | 665 | 15037 | 10 | ⁴T₁g(P) |
| | 814 | 12285 | 8 | ⁴T₁g→¹A₂g |
| | | | | ⁴T₁g→¹T₂g |
| [Ni(ECA)₂]Cl₂ | 251 | 39840 | 365 | C.T |
| | 360 | 27777 | 869 | 3A₂g→3T₁g(P) |
| | 502 | 19920 | 12 | 3A₂g→3T₁g(f) |
| | 765 | 13071 | 8 | 3A₂g→3T₂g |
| [Cu(ECA)₂]Cl₂ | 254 | 39370 | 1178 | C.T |
| | 363 | 27548 | 1497 | C.T |
| | 520 | 19231 | 143 | 2E₂g→2T₂g |

Figure 1. Ligand(ECA)HNMR Spectrum

Figure 2. Ligand (ECA)Infrared Spectrum
General Proposed Stereo Chemistry Structure of Complexes:

According to the results obtained from the elemental analysis, spectral studies, magnetic and conductivity measurements, the general structure of the prepared complexes can be illustrated as follows in Fig. 6.
Figure 6. The proposed structural formula of the complexes

Biological activity of the ligand and its complexes

The ligand (ECA) combined with Co, Ni, or Cu complexes showed biological activity which was studied by using the inhibition method for four types of pathogenic bacteria and Candida albicans fungi. Staphylococcus aureus and Bacillus subtilis are two forms of gram-positive bacteria. Escherichiacoli and Pseudomonas aeruginosa were the second and third gram-negative bacteria, respectively. To all species of bacteria, the chemicals show no inhibitory diameter. Under similar experimental settings, the results suggest that the complexes are more active than the ligand because of partial sharing of its positive charge with the donor groups and possible electron delocalization over the whole chelate ring such. The results for the compounds, the results as modified to the literature are shown in table 4 and Figure 7.

Table 4. The results of compounds

| Bactra comp. | Staphlococcus aureu (a) | Psedomonas aeruginosa(b) | Bacillus subtilis(c) | Escherichia coli(d) | Candida albicans fungi(e) |
|--------------|------------------------|--------------------------|---------------------|-------------------|---------------------------|
| Ligand [ECA] | -                      | -                        | -                   | -                 |                           |
| [Co(ECA)₂]Cl₂ | -                      | -                        | -                   | -                 |                           |
| [Ni(ECA)₂]Cl₂ | -                      | -                        | -                   | -                 |                           |
| [Cu(ECA)₂]Cl₂ | -                      | -                        | -                   | -                 |                           |

Where: (+) No significant Zone

Figure 7. (a-e) below shows bioactivity of the ligand and their compounds (L,Co,Ni and Cu) for types bacteria

Conclusions:

In this study new novel complexes are synthesized and recognized by a variety of spectral and physicochemical analyses. The findings show that the synthesized ligand behave as tridented coordinating with metal ions through three donor atoms (O in C = O, N in N=N and N in C=N), the complexes prepared have geometry octahedral, therefore the study of bioactivity for all compounds prepared show No significant Zone for all types bacteria Staphlococcus aureu, Psedomonas aeruginosa, Bacillus subtilis, Escherichia coliand Candida albicans fungi.

Authors' declaration:
- Conflicts of Interest: None.

Authors' contributions statement:

- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' contributions statement:

- The role of first authors I. H. I., Prepared higands and complexes did IR set and data analysis, The role of second authors N. S. M. (UV visible test, measure molarity conductivity and study biological activity) for the complexes, the role of the third and fourth authors writing the manuscript, drafting and designing the Ms.
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