Synthesis, spectroscopic, and thermal studies of azo compounds from luminol and procaine with acetylacetone and their complexes

Noor Al AlBaheley*, T A Fahad and Asaad A Ali
Department of Chemistry, Basrah University's College of education for Pure Sciences, Iraq

Abstract. This study entails the synthesis of two newly synthesized azo dyes luminol and procaine, containing acetylacetone (N1 and N2, correspondingly). Elemental analysis, 1HNMR, TGA, and FTIR have all been used to characterize dyes. These new dyes were reacted with copper and nickel ions at various molar ratios to form complexes of metals (II) with a general stoichiometry: CuL2, and NiL2 in complexes. FTIR, as well as the corresponding metal (II) complex, were used to characterize them. The dye acts as a bidentate ligand, according to elemental analysis and spectral results. The thermal properties of these compounds were investigated using thermogravimetric analysis (TGA). Thermal decomposition of these compounds is a process that occurs in stages.

1. Introduction
Azo compounds are used in a wide range of practical applications, including fiber coloring [1, 2, 3], and have special importance in pharmaceutical industry because they play an active role in biological systems [4]. Non-linear and photonic creative methods [5], intelligent storage [6], nitrogen fixation, and carcinogenic [7-14]. Whereas certain azo compounds are moderately toxic and produce detectable adverse effects over time [15]. Azo-ketones exhibit a broad range of biological activity, acting as a major component of bimolecular compounds such as hemoglobin, vitamin B12 [15]. Because the –N=N–group and diketone moiety are combined in a single compound, its ability to form complexes increases and their stability increases [16, 17]. Due to their interesting electronic and geometrical properties, as well as their possible applications in molecular memory storage, azodye complexes are used in nonlinear optical elements, and printing systems [18-19]. Azo-metal complexes have a number of significant advantages. During synthesis, azo compounds have the potential to improve the ability to interact with the substrate to which they are applied, as well as increase the color intensity.

Recent research indicates that forming a complex between an azo dye and a metal such as Co(II) reduces cytotoxicity even at low complex concentrations [20]. Many azo dyes and their complexes have been studied and published [21]. The purpose of this work was to synthesis and characterization of two azo dyes (ligands N1 and N2) derived from luminol and procaine with acetylacetone. Using a variety of analytical instruments, we report on the coordination activity of these azo compounds with Cu and Ni ions.

2. Experimental
All chemicals are of a superior quality. On an ashimadzu 8400 FTIR Spectrometer, Complexes and ligands were analyzed using KBr pellets in the infrared (400-4000) cm⁻¹. Euro EA elemental analyzers were used to conduct the elemental analysis (C, H, N). On a BRUKER-500 MHz spectrophotometer, the 1HNMR spectrum of the ligand was determined in CDCl3 (internal standard TMS). Using an Agilent Technologies 5975C mass spectrometer, the ligand's mass spectrum was estimated at 70e. The electrical conductivity of the chelate complexes was measured (10⁻² M) in DMF solvent. The metal content of the complexes was determined using an atomic absorption meter Jenway Pcm3Phoenix-986. The melting points of the synthesis compounds were measured using an Electro thermal melting...
point 9300. A TGA Q500-V6.7 was used to conduct thermal analysis (TG) in a complex under nitrogen gas at 10°C per minute heating rate

2.1. prepare azo ligands
N1 (5-amino-2,3-dihydro-1,4-phthalazinedione azo-2,4-pentadione) N2, 3-[(2-(Diethylamino)ethyl 4-aminobenzoate azo-2,4-pentadione) [3,21]

Azo ligands were synthesized by dissolving 0.004 moles of each (luminol and procaine) in 1.4 ml concentrated HCl and 20 ml distilled water and 20 ml distilled water, then drop by drop, at -5°C, a stirring solution of (0.304 g sodium nitrite in 20 ml distilled water) was added to form diazonium salt. The above diazonium salt was added to an alkaline solution of 0.004 mole of (Acetyl acetone) in (1.2 percent w/v. NaOH). Then, by adding dilute HCl (to maintain a pH value of 6.5 – 7.5), convert the prepared dye from sodium to hydrogen. To remove any remaining unreacted substances. The product was purified by washing with distilled water and recrystallized by using ethanol as solvent and dried in a vacuum at 50°C for several hours scheme 1

![Scheme 1. Synthesis of azo dyes (N1&N2)](image)

2.2 Preparation of azo complexes of nickel and copper [21]
Nickel and Cupper acetate (1 mmol) in ethanol (10 mL) was applied to (2 m mol) the ligand solution in 20 mL of an ethanol-chloroform mixture in a flask with a round bottom and a magnetic stirrer. We noticed a definite change in the color of the garment as well. While the reflux was happening, the mixture was stirred for 3 hours. The purple mass was removed and washed several times with ethanol; then it was vacuum-dried...

3. Results and Discussion
The new azole and its respective complexes have demonstrated good stability in DMF, methanol, ethanol, and aceton and do not degrade in air at room temperature. Table 1 summarizes the results of the CHN of complexes are strong accordance with the suggested molecular formula that the ligand behaves bidentate in prepared complexes

Table(1): Elements analysis and physical properties for novel azo- ligands) and their metal complexes

| Compound | Molecular formula (Mol.Wt) | Yield (%) | Color | m.p °C | Elemental Analysis Found (Calc.) | Cond. S cm2 mol⁻¹ |
|----------|-----------------------------|-----------|-------|--------|---------------------------------|------------------|
| Ni      | C₁₃H₁₂N₄O₄                  | 83        | brown | 235    | 54.16 (53.03) | 4.19 (4.34) | 19.34 (19.11) | - | - |
| NiCu    | C₂₆H₂₈N₈O₁₀Cu              | 77        | Brown green | >30 0 | 46.19 (45.82) | 4.17 (4.09) | 16.57 (16.38) | 9.39 (9.25) | 15.34 |
| NiNi    | C₂₆H₃₀N₈O₁₁Ni              | 73        | Brown yellow | >30 0 | 45.31 (44.22) | 4.38 (4.24) | 16.25 (16.13) | 8.51 (8.42) | 18.25 |
| N₂     | C₁₄H₂₂N₅O₄                 | 82        | black | 224    | 62.22 (61.01) | 7.25 (7.18) | 12.09 (12.01) | - | - |
| N₂Cu   | C₃₀H₃₄N₆O₁₀Cu              | 79        | Dark brown | >30 0 | 54.43 (53.24) | 6.85 (6.72) | 10.57 (10.39) | 7.99 (7.82) | 20.58 |
| N₂Ni   | C₃₆H₅₆N₆O₁₁Ni              | 75        | Light brown | 235   | 53.54 (52.42) | 6.89 (6.79) | 10.40 (10.31) | 7.62 (7.19) | 15.45 |

3.1. The infrared spectra
Table 2 shows the details on azoligands and their metal chelat complexes. The infrared spectrum of ligand displays a medium band about 3300-3400 cm⁻¹, which might be related to stretching vibrations (N-H) and (O-H). As a result, the (N-H) group's remaining hydrogen means that the ligand is not involved in metal ion coordination [22]. Due to the fact that the novel azo ligands contain (C-N), there is a strong strength band in the range of 1300-1270 cm⁻¹. In metal complexes, this band changed, suggesting imine nitrogen atom covalent bonding to metal ions [23]. At, one can observe the azo group. 1482 cm⁻¹, and At this time, this category is shifting to a lower frequency. (1450-1435) cm⁻¹, indicating that it is involved in metal ion bonding [20]. The (M-O) vibration is responsible for the appearance of new bands at (543-572) cm⁻¹ which aren't found in the free ligand. The presence of weakly absorbing bands in IR spectra of complexes (420-480) cm⁻¹ region, that can be traced back to (M-N) stretching, also indicated metal oxygen binding to form chelat complexes. The azo acts as a bidentate chelating agent according to IR spectra data.
A new band corresponding to C-O vibration appeared around 1260-1240 in the spectra of the complexes, and strong bands At low frequencies, the symbol v N=N appeared.
We can deduce from IR spectral data that azo act as an N,O-bidentate ligands [23].
Figure 1. IR spectra of N1

| Compound | v(O-H) | v(N-H) | vC-H  | vN=N | vC=O | νC=O (M-O) | vC=O (M-O) | vC=O (M-O) | vC=O (M-O) |
|----------|--------|--------|-------|------|-------|------------|------------|------------|------------|
| N1       | 3400 m | 3159.4 w | 1494 s | 1591.2 m | 1300.0 m | 1653.0 S | - | - | - |
| [N1Cu]   | 3250 w | 3008.9 m | 1492 s | 1591.2 m | 1367.5 s | 1651.0 S | 1245 s | 543 s | 450 w |
| [N1Ni]   | 3250 w | 290.23 m | 1481 s | 1571.9 m | 1357.8 s | 1643.3 S | 1255 m | 550w | 420w |
| N2       | 3300   | 3300w   | 1508 s | 1604.7 s | 1271.0 m | 1689.6 S | - | - | - |
| [N2Cu]   | 3300   | 2918.3 m | 1398 s | 1560.4 m | 1276.8 m | 1606.7 m | 1255.8 m | 561.2 m | 476w |
| [N2Ni]   | 3310   | 2918.3 m | 1386 s | 1597.0 m | 1315.4 m | 1666.5 m | 1254m | 572 w | 480w |

m = medium, s = strong, w = weak

3.2 The NMR spectra

The 1H-NMR spectra (N1 and N2) of the ligands synthesized were carried out in DMSO solutions, with TMS serving as an internal assessment. (Figure 2 and 3).

The DMSO solvent produced a peak at 2.4 ppm in H-NMR spectra, and dissolved water in DMSO produced a sharp peak at 3.4 ppm in some spectra. [23]

| Comp | Chemical shift |
|------|----------------|
| N1   | 2.4(S,6H-DMSO), 3.4(S,H,HOD), 2.3 (S,6H,CH3), 6.5-8.5(m,3H,Ar-H-H) -11.5(s,2H,NH) |
| N2   | 2.4(S,6H-DMSO), 3.4(S,H,HOD) -1.23(s,6H,CH3), 7.6-8.2(m,4H,Ar-H) -2.59(s,6H,CH3)14, (s, 1H, OH/NH), |
3.3-Mass spectra of N1 and N2

The structure of transition metal(II) complexes can be elucidated using mass spectra of synthesized compounds. The stoichiometry of complexes was compared using mass spectra obtained at room temperature (21) (Table 4). (figure 4and 5). Each synthesized complex matched the molecular ion fragment and supported the complexes’ proposed structure. [24]
Figure 4. Mass spectrum of Cupper complex of N1

Figure 5. Mass spectrum of Nickel complex of N1

(Table 4) - Mass spectra of synthesized complexes

| Compounds                   | Calculated mass | Obtain mass m/z | Peak assigned |
|-----------------------------|-----------------|-----------------|---------------|
| [Cu (N1)2]2H20             | 676             | 676             | M+            |
| [Ni ((N1)2(H2O)2]H2O       | 689.22          | 688             | [M-H]+        |
| Cu(N2)2H2O                 | 794             | 693             | [M-H]+        |
| [Ni ((N2)(H2O)2]H2O        | 809             | 793             | [M-H2O]+      |
Electrolytic studies of metal complex solutions shed light on their nature and composition. These studies provide insight into the number of ions present in a solution that are responsible for current conduction, and thus allow for the extraction of quite significant structural information.[25] The chelate complexes prepared in this study had molar conductance values ranging from (15-20) S.mole⁻¹.cm² in DMSO (10⁻³M) at room temperature, indicating that the Ni(II),Cu(II),complexes are not electrolytes and that no conductive species exist., indicating their non-electrolyte nature tale ( 1)

\[26\]

3.4. Interpretation of the structural
The structure of all ligand-containing complexes can be interpreted as having a common distribution of similar coordinating sites, as seen in fig(6), which is covalently linked to copper(II) and nickel (II) \[27\]

Figure 6. Metal complexes: proposed structural formulae

3.5. Thermal properties
At room temperature, the Cu(II) and Ni(II) complexes are stable and can be stored indefinitely. The obtained compounds were subjected to thermogravimetric (TG) analysis in N₂ atmospheres at temperatures ranging from ambient to 800 degrees. The TG curves are depicted in Figure 7,8)
The data from TGA clearly show that the complexes are decomposed in three or four phases. Between 25 and 160 °C, crystal water molecules were lost, and between 160 and 250 °C, coordinated water was lost[28]. The loss of organic moiety was the second step, and the loss of metal oxide and some organic species was the third and fourth steps.
3.6. **Antibacterial study**

The ligands and their complexes were found to have antibacterial activity was determined and tabulated against both gram positive and gram negative bacteria table(5).

| Isolates chemicals | Conc.mg/ml | Inhibition zone(mm) |
|---------------------|------------|---------------------|
|                     |            | S. aureus | E. coli |
| L1                  | 100        | 23        | -       |
| L2Cu                | 100        | 14        | -       |
| L2                  | 100        | 25        | -       |
| L2Cu                | 100        | 11        | -       |

The orbitals of each metal ion is made to overlap with the ligand's atoms. The delocalization of – electrons in the chelate ring increases the lipophilicity of complexes as activity rises. Increased lipophilicity can result In some instances, the cell's permeability barrier is compromised..[29]

4. References

[1] Hajipour, A.R., Zahmatkesh, S., Roosta, P. et al. Synthesis and characterization of new optically active poly(azo-ester-imide)s via interfacial polycondensation. *Amino Acids*, **36**, 511–518 (2009). https://doi.org/10.1007/s00726-008-0113-y

[2] Benkhaya, S. M'rabet, and A. El Harfi, (2020) Classifications, properties, recent synthesis and applications of azo dyes, *Heliyon*, **6**(1): e03271

[3] Ali A.A., Fahad, T.A and Al-muhsin, A.A. (2020) Preparation and pectroanalytical Studies of Two New Azo Dyes Based on Luminol IOP Conf. Series: Materials Science and Engineering **928**:52007, IOP Publishing, doi:10.1088/1757-899X/928/5/052007

[4]. Aljamali,N.M.,(2015) Review in Azo Compoundsand itsBiological Activity,*Biochem Ana.l Biochem.*, **4**:2 .http://dx.doi.org/10.4172/2161-1009.100016

[5].Mavila,S.S., Schitram,C., H.Jsekar,,(2017) Spectroscopic, DFT and Z-scan supported investigation of dicyanoisophorone based push-pull NLOphoric styryl dyes,*Optical Materials*, **64**: 453-460

[6]. Bae,J.S., Freemandan,H.S., El-Shafei,A. (2003). Metallization of non-genotoxic direct dyes, Dyes and Pigments, **57**(2):121-129

[7]. Badea, M., Olar, R., Cristurean, E., Marinescu, D., Emandi, A., Budrugace, P., Segal, E., 2004. Thermal stability study of some azo-derivatives and their complexes, Part 2. New azo-derivative pigmentsand their Cu(II) complexes. *J. Therm. Anal. Calor.* **77**(3): 815–824

[8]. Broekaert, J.A.C (1981) Separation of yttrium and rare earth elements from geological materials, *Anal. Chim. Acta*, **124**: 421-425.

[9]. Ggarg,H., and Prakash,C. (1972) Imidazo[1,2-a]pyridine anthelmintic and antifungal agents,*J Med. Chem.*, **15**(4): 435-436
[10]. VIDYA, V.G., and SADASIVAN, V. (2018) Synthesis, Characterization and Applications of Some Azo Dyes Derived from Various Aromatic Amines, *Asian Journal of Chemistry*, 30: 2049-2053

[11]. Chung, K.T. (2000) Mutagenicity and carcinogenicity of aromatic amines metabolically produced from Azo Dyes, *J. Environ. Sci. Health, Part C: Environ. Carcin. Ecotoxicol. Rev.*, 18: 51-74

[12]. Zhang, D., Zhang, M., Liu, Z., Huang, C. (2006) Highly selective colorimetric sensor for cysteine and homocysteine based on azo derivatives, *Tetrahedron Lett.*, 47: 7093

[13]. Gupa, R., Gizeiroglub, E., Kırkan, B. (2007) Synthesis and spectroscopic properties of new azo-dyes and azo-metalcomplexes derived from barbituric acid and aminoquinoline, *Dyes and Pigments*, 73: 40-46

[14]. Patil, C.J., and Rajput, S.V. (2019) Coupling reactions involving aryldiazonium salt: part-ix. review on synthesis of azo-phenolic derivatives, their applications and biological activities, *International Journal of Recent Scientific Research*, 10: 32144-32156

[15]. Aljamali, N.M., Jawd, S.M., JawadZ.M., Alfatlawi, I.O. (2017) Inhibition Activity of (Azo – Acetyl acetone) on Bacteria of Mouth. *Research J. Pharm. and Tech.*, 10(6): 1683-1686. doi: 10.5958/0974-360X.2017.00297.9

[16]. Abdallah, S.M. (2012) Metal complexes of azo compounds derived from 4-acetamidophenol and substituted aniline, *Arabian Journal of Chemistry*, 5: 251–256.

[17]. Gouda, M.A., Eldien, H.F., Girges, M.M., Berghot, M.A. (2016) Synthesis and antitumor evaluation of thiophene based azo dyes incorporating pyrazolone moiety. *J. Saudi Chem. Soc.* 20 (2) : 151–157

[18]. Mutlu, H., Geiselhart, C.M., Barner-Kowollik, C. (2018) Untapped potential for debonding on demand: The wonderful world of azocompounds. *Mater. Horizons*, 5:162-183,

[19]. Wang, S. Shen, S., Xu, H., (2000) Synthesis, spectroscopic and thermal properties of a series of azo metal chelate dyes, *Dyes Pigments*, 44:195-198

[20]. Farghaly, T.A. ; Abdalla, M.M. (2009) Synthesis, tautomerism, and antimicrobial, anti-HCV, anti-SSPE, antioxidant, and antitumor activities of aryazobenzosuberones. *Bioorg. Med. Chem.*, 17(23): 8012-8019

[21]. Dhumad, A.M., Hassan, Q.M., C.A., Fahad, T.A., Emshary, Raheem, N.A., Sultan, H.A., (2021). Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 251- 119487

[22]. Saritha, T.J., Metilda, P., (2021) Synthesis, spectroscopic characterization and biological applications of some novel Schiff base transition metal (II) complexes derived from curcumin moiety. *Journal of Saudi Chemical Society*, 25: 101245

[23]. Pesyan, N. N., Gholsanamloo, V, Par, M., Rashidnejad, H, Gharib, A., Nejati, K (2019) Synthesis, characterization and spectroscopic properties of new azo dyes derived from aniline
derivatives based on acetylacetone and azo-metal (II) complexes and singular value decomposition (SVD) investigation. *Iranian Chemical Communication*, 7:1-19

[24]. Dhumad, A.M., Hassan, Q.M., C.A., Fahad, T.A., Emshary, Raheem, N.A., Sultan, H.A.,(2021) Synthesis, structural characterization and optical nonlinear properties of two azo-β-diketones, *Journal of Molecular Structure* 1235:130196

[25]. Ali, I., W AWani, and Saleem, K., (2013), Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, 43:1162–1170,

[26]. El Wakiel, N.A., RizkHF, S.A., Ibrahim, A., (2017) Synthesis and characterization of metal complexes of azo dye based on 5-nitro-8-hydroxyquinoline and their applications in dyeing polyester fabrics. *ApplOrganometal Chem.*, e3723. https://doi.org/10.1002/aoc.3723

[27]. Chandra, S.; Gupta, L.K., (2004) Spectroscopic characterization of tetradeutate macrocyclic ligand: its transition metal complexes, *Spectrochim. Acta A*, 60:2767–2774.

[28]. Devi, J.M., Tharmaraj, P., Ramakrishnan, S. K., Ramachandran, (2008) On the thermal properties of metal (II) complexes of chalcone, *Materials Letters*, 62:852–856.

[29]. Vinodkumar, Keshavayya, J. , Pushpavathi, I., Keerthikumar C. T., Maliyappa M. R., Ravi B. N.,(2020) Synthesis, characterization, computational and biological studies of nitrothiazole incorporated heterocyclic azo dyes. *Struct Chem*, 31:1317–1329