Synthesis and characterization and biological study of pyridylazo ligand and its compounds of Co, Ni and Cu divalent ions

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Abstract
In this work, we synthesized and characterized an azo dye of amino pyridine namely 6-[3-pyridylazo]-2,4-dichlorophenol (PADCP) furthermore its complexes of cobalt, nickel and copper divalent ions. The infrared spectra refereed that PADCP ligand coordinates by oxygen and nitrogen atoms to the metals in the complexes as bidentate ligand. The synthesized complexes have general formula [M(PADCP)₂] which confirmed by elemental analysis, magnetic moments, molar conductivity and electronic spectra. The synthesized complexes of PADCP have tetrahedral shape and non-ionic. The PADCP dye exhibit clear color changes under pH changes. The PADCP ligand and its complexes shown notable biological activity against E.coli, Staph.aureus and Aspergillus Niger

Keywords: biological activity, ligand, azo dye

Introduction
Azo dyes are organic compounds and the azo group considered the main component of azo dyes, as they represent the largest part of all types of dyes used at the present time [1]. This type of compounds discovered by Greiss and named in 1860. He indication to the double bound of (-N = N-) with hybridized (sp²) where both nitrogen atoms linked to aromatic system which is more stable than aliphatic system [2–4].

This type of dyes widely use as organic reagents due to its versatile properties like high stability and speed of interaction with metal ions with high sensitivity and selectivity under complexation [5–7]. The direct dye (Congo red dye) is a sulfonated azo pigment used on a large scale in the biological field, where it was the focus of many attention due to its effect on the growth of viruses and it showed interest effect as an anti-HIV and anti-Alzheimer agent [8–10].

The dye 4-(5- chloro- 2- hydroxyphenylazo)- N- thiazol- 2- ylbenzenesulfonamide was prepared from the reaction of sulfathiazole with para-chlorophenol. The copper-coordinating compound of this dye showed IC₅₀ 3.47 μg/ml with 5.53 μmolary against cancer liver cells, which means that it is a more effective drug against cancer than the standard cis-Platinum IC₅₀ = 3.67 μg/ ml with12.23 μmolary [11].

The azo-ruthenium (II) dye of bipyridyl was used as a sensitive dye in the photochemical treatment technique, it was characterized by high accumulation in the mitochondria of HeLa cells and it was
non-toxic in dark conditions. It also showed high absorption capacity for two-photon at 810 nm with high killing for cancer cell [12,13]. The azo dyes of pyridine exhibited large interesting like reagent for metal ions, indicator, and in biology[14–17], therefore we synthesis azo dye 6-[3-pyridylazo]-2,4-dichlorophenol and its complexes of Co, Ni and Cu.

Experimental

All chemicals and reagents used were of a high degree of purity, instruments used: UV-1650 pc UV – Visible Spectrophotometer (SHIMADZU), Digital Melting Point-SMP3, Shimadzu FT-IR 8400S Spectrophotometer, Cond.720(WT), Bruker 400 MHz Spectrophotometer, magnetic susceptibility, Autoclave Model HV-5 (Hirayama), memmert UE400.

We took 0.5g of 3-aminopridine and dissolve it in a mixture containing 3mL of concentrated HCl acid with 7mL of distilled water and place the mixture in an ice bath at 5 ° C. Then we prepared another solution by dissolving 0.37g of sodium nitrite in 5mL of distilled water and placing the solution in an ice bath at 5 ° C. The diazonium salt was prepared by mixing the solution of acidic amino aromatic with the solution of sodium nitrite slowly drop by drop while keeping the solution cool. 0.8g of sodium hydroxide dissolved in 6mL of distilled water then mixed with 0.83g of 2,4-dichlorophenol dissolving in 12 mL of alcohol, the new solution was put under cooling. The diazonium salt was added slowly with continuous stirring to the basic solution of 2,4-dichlorophenol while continuing with cooling. The coloration of the solution was observed in a yellowish brown color. When the addition process is completed, leaves the solution for the next day, filter the precipitate and wash with distilled water several times.

Preparation of metal ion complexes with ligand (PADCP):

0.1g of (PADCP) dissolved in 10mL of alcohol then mixed with 3mL of aqueous solution of potassium hydroxide (0.02g). A hot solution of MCl$_2$ (0.044 g, 0.0314g and 0.044g, of CoCl$_2$.6H$_2$O, CuCl$_2$.2H$_2$O, and NiCl$_2$.6H$_2$O respectively dissolving in 4mL distilled water) was mixed with hot solution of PADCP, the mixture solution was heated under 60-80 ° C for 30 minutes. The mixture was left for the next day. The precipitate is filtered and washed with water.

We used Muller-Hinton agar to growth the bacteria and we use diffusion method with four halls for each Petri dish while in case of fungi we used potato dextrose agar to growth the fungi.

Results and discussion

The azo dye ligand 6-[3-pyridylazo]-2,4dichlorophenol (PADCP) formed by reacting diazonium salt of amino pyridine with 2,4-dichlorophenol in basic medium (scheme 1).

![Scheme 1 Preparation steps of PADCP dye](image)

The mass spectrum of PADCP dye (Figure 1) exhibited important fragments such as 268.5 as mother fragment and 233 as M-Cl with high abundance.
PADCP dye as indicator: The aqueous solutions of the PADCP dye exhibited exciting changes in the color under pH changes (Figure 2), the color of PADCP dye was red in basic aqueous, yellow in neutral aqueous and orange in acidic aqueous.

The PADCP dye showed high red shift in basic aqueous with hyperchromic effect comparing to the dye in neutral aqueous. The PADCP dye exhibited red shift in acidic aqueous with little bit hypochromic effect these changes are represented in (Figure 3)[18,19,7].
The Co, Ni and Cu complexes of PADCP dye were prepared by reacting hot ethanolic solution of deprotonation PADCP dye with hot aqueous solution of the metal ion with reflux under 79 °C for 30 minutes (scheme 2).

Scheme 2 Preparation steps of Co, Ni and Cu complexes of PADCP dye

The physical properties and elemental analysis of The PADCP ligand and its complexes are incorporated in Table 1. The PADCP ligand reacts with metals ions of Co, Ni and Cu under mole ratio equal to 2:1 as ligand : metal as shown in (Figure 4) for cobalt complex.
Table 1 The physical properties and elemental analysis of the PADCP ligand and its complexes

| Compound      | Empirical Formula | Elemental analysis Cal. (Found)% | color       | mp °C |
|---------------|-------------------|---------------------------------|-------------|-------|
| PADCP ligand  | C₁₁H₇N₃OCl₂       | C 49.28 (49.12) H 2.63 (2.42) N 15.67 (15.60) | Yellow      | 94.5  |
| Co complex    | CoC₂₂H₁₂N₆O₂Cl₄   | C 44.55 (44.35) H 2.04 (1.98) N 14.17 (14.01) | Dark red    | >350  |
| Ni complex    | NiC₂₂H₁₂N₆O₂Cl₄   | C 44.57 (44.43) H 2.04 (1.99) N 14.18 (13.97) | Red         | 294   |
| Cu complex    | CuC₂₂H₁₂N₆O₂Cl₄   | C 44.21 (44.02) H 2.02 (1.97) N 14.06 (13.97) | Dark brown  | 255   |

The infrared spectra of PADCP dye and its complexes (Figure 5-8) exhibited important characteristic peaks for example, CH aromatic, C=N stretching, C=C aromatic, N=N stretching, C-Cl and C-H bending [20] which are depicted in (Table 2). We noticed that frequencies of N=N in complexes exhibited in red shift and we did not see the stretching frequency of O-H that means that the azo group and oxygen atom of phenoxide ion coordinated to metals in complexes.
Table 2 The infrared frequencies in cm$^{-1}$ for important assignment groups of PADCP dye and its complexes

| Compounds       | OH  | CH aromatic | C=O | N=N | C=C | C-O | CH bending |
|-----------------|-----|-------------|-----|-----|-----|-----|-----------|
| PADCP dye       | 3360| 3063        | 1599| 1583| 1489| 1269| 696       |
| Co-complex      | -   | -           | 3068| 1589| 1540| 1475| 1215      |
| Ni-complex      | -   | -           | 3070| 1593| 1539| 1483| 1220      |
| Cu-complex      | 3379| large       | 3066| 1593| 1523| 1477| 1240      |
|                 |     | H$_2$O hydrate |     |     |     |     |           |
|                 | 3066| 1593        | 1523| 1477| 1240| 819 and 723 |

Figure 5 Infrared spectrum of PADCP dye (KBr disk)
Figure 6 Infrared spectrum of cobalt complex of PADCP dye (KBr disk)

Figure 7 Infrared spectrum of nickel complex of PADCP dye (KBr disk)
Electronic spectra (Figure 9) refer that PADCP ligand exhibited a band at 319 for π-π* and large band at 390 nm for π-π* overlap with n-π*. The cobalt complex exhibited a band at 314 nm and 390 nm for electronic transition of PADCP ligand in the complex and a band at 570 nm due to $^4A_{2g} \rightarrow ^4T_1(F)$ with magnetic susceptibility 4.56 B.M. in agreement with tetrahedral complexes of cobalt. Nickel complex exhibited three bands, at 319 for π-π* of PADCP ligand in the complex, at 405 nm for $^3T_1(F) \rightarrow ^3A_2$ electronic transition, and at 560 nm for $^3T_1(F) \rightarrow ^3T_2 (F)$ electronic transition of d-d and magnetic susceptibility equal to 3.54 B.M. which is in agreement with tetrahedral complexes of nickel. Copper complex exhibited band at 319 nm for π-π* of PADCP dye and band at 392 nm due to charge transfer from copper to ligand with magnetic susceptibility 1.92 B.M. in agreement with tetrahedral complexes of copper [21]. On the other hand, the molar conductivity of synthesized complexes of Co, Ni and Cu in DMSO solvent at room temperature indicated that the complexes are not ionic [22].
Biological activity:

The PADCP dye and its complexes shown interesting biological activity against Aspergillus Niger as shown in Table 3. The PADCP dye exhibited excellent biological activity against Aspergillus Niger, it was 6.5 cm as shown in Figure 10. The complexes of Ni, Co and Cu exhibited good biological activity against Aspergillus Niger, it was 2- 3.3 cm respectively. The compounds exhibited the better role against fungi at 100 mg/mL.

Table 3 Inhibition zone of PADCP dye and its complexes in cm unit against Aspergillus Niger

| Concentration mg/ml | The diameter inhibition zone (cm) |
|---------------------|----------------------------------|
|                     | PADCP Ligand | Co | Ni | Cu |
| 100                 | 6.5          | 3  | 2  | 3.3|
| 75                  | 6            | 1  | -  | 2.5|
We tested our prepared materials against *E. coli* bacteria. The materials showed high biological efficacy (Table 4), ranging from 3.5 to 4 cm for the PADCP dye and cobalt complex respectively. Whereas, the nickel and copper complexes did not show biological efficacy against *E. coli* bacteria, noting that substances did not show biological efficacy at high concentration. The PADCP dye and its complex of Ni exhibited inhibition against *Staph. aureus* (Figure 11) from 2.5-1.5 cm respectively while other complexes did not show biological activity against *Staph. aureus*.

Table 4 Inhibition zone of PADCP dye and its complexes in cm unit against types of bacteria

| Bacteria           | Concentration mg/ml | The diameter inhibition zone (cm) |
|--------------------|---------------------|----------------------------------|
|                    |                     | PADCP   | Co | Ni | Cu |
| *E. coli* Gram-negtive | 100                 | -       | -  | -  | -  |
|                    | 75                  | 3.5     | 4  | -  | -  |
| *Staph. aureus* Gram-positive | 100               | 2.5     | -  | -  | -  |
|                    | 75                  | 2       | -  | 1.5| -  |

Figure 10 Biological activity of PADCP dye against *Aspergillus Niger* fungi

Figure 11 Biological activity of PADCP dye against Gram-positive *Staph. aureus*
Conclusion

The spectra of PADCP dye exhibited perfect agreement between the changes of color and the shift of spectra in different values of pH. The PADCP dye in its electronic spectra showed red shift in acid and basic aqueous. Infrared spectra confirmed that the PADCP dye is bidentate ligand. The behaviour of PADCP ligand is bidentate. The synthesized complexes of PADCP dye have tetrahedral environment. The PADCP dye and its synthesized compounds are consider a candidate as antibiotic against E.coli, Staph.aureus and Aspergillus Niger.

References

[1] Zhao R, Tan C, Xie Y, Gao C, Liu H and Jiang Y 2011 One step synthesis of azo compounds from nitroaromatics and anilines Tetrahedron Lett. 52 3805–9
[2] Mohammed H S and Tripathi V D 2020 Medicinal Applications of Coordination Complexes J. Phys. Conf. Ser. 1664 012070
[3] Mohammed H S 2020 Synthesis, characterization, structure determination from powder X-ray diffraction data, and biological activity of azo dye of 3-aminopyridine and its complexes of Ni (II) and Cu (II) Bull. Chem. Soc. Ethiop. 34 523–32
[4] Mohammed H S 2011 Preparation and characterization of 4-[6-(purinyl)azo]-1-naphthol and some transition metal complexes Iraqi Natl. J. Chem. 347–60
[5] Mohammed H S, Tripathi V D and Darghouth A A 2019 Synthesis, Characterization, DFT calculation and Antimicrobial Activity of Co (II) and Cu (II) complexes with azo dye Journal of Physics: Conference Series vol 1294 (IOP Publishing) p 052051
[6] Deepak Tripathi V, Mohammed H and Shukla A Regioselective Three Component Domino Synthesis of Polyhydrospiro[indoline-3,3'-pyrrolizine]-2-one via [3+2] Cycloaddition Reaction
[7] Salam Hussein Ewaid et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012075
[8] Dur S, Husein I, Kadhim M I, Mohammed H S, Elveny M, Syah R and Hilda L 2020 An Optimally Solving Dentistry Internal Purity In Heat Polymerized Acrylic Resin With Different Polymerization Methods Syst. Rev. Pharm. 11 974–80
[9] Wilkinson G, Gillard R D and McCleverty J A 1987 Comprehensive coordination chemistry. The synthesis, reactions, properties and applications of coordination compounds. V. 3. Main group and early transition elements
[10] Geary W J 1971 The use of conductivity measurements in organic solvents for the characterisation of coordination compounds Coord. Chem. Rev. 7 81–122
[11] Setyawati H, Darmokoesoemo H, Murwani I K, Permata A J and Rochman F 2020 Functionalization of Congo red dye as a light harvester on solar cell Open Chem. 18 287–94
[12] Saad F A, El-Ghamry H A and Kassem M A 2019 Synthesis, structural characterization and DNA binding affinity of new bioactive nano-sized transition metal complexes with sulfathiazole azo dye for therapeutic applications Appl. Organomet. Chem. 33 e4965
[13] Zeng L, Kuang S, Li G, Jin C, Ji L and Chao H 2017 A GSH-activatable ruthenium(ii)-azo photosensitizer for two-photon photodynamic therapy Chem. Commun. Camb. Engl. 53 1977–80
[14] Mohammed H S 2021 Role of Coordination compounds as photosensitizes of photodynamic therapy: on overview MJPS 8
[15] Dutta P, Mondal S, Roy S, Lopez-Torres E and Sinha C 2015 The structural characterization and DNA binding of pyridyl-azo-acetylacetonato complexes of zinc (II), cadmium (II) and mercury (II) Polyhedron 89 203–12
[16] Panja A and Ghosh K 2018 Pyridylazo Derivatives with Dicyanovinyl Appendage in Selective Sensing of CN− in Sol-Gel Medium ChemistrySelect 3 1809–14
[17] Panja A and Ghosh K 2018 Azo and imine functionalized 2-naphthols: promising supramolecular gelators for selective detection of Fe3+ and Cu2+, reactive oxygen species and halides Mater. Chem. Front. 2 1866–75
[18] Raza R, Dey N, Panja A and Ghosh K 2019 Pyridyl Azo-Based Progelator in Selective Sensing of Hg2+ and Ag+ Ions via Sol to Gel Conversion ChemistrySelect 4 11564–71
[19] Rabbani M A D, Khalili B and Saeidian H 2020 Novel edaravone-based azo dyes: efficient synthesis, characterization, antibacterial activity, DFT calculations and comprehensive investigation of the solvent effect on the absorption spectra RSC Adv. 10 35729–39
[20] Darwish E S, Mosselhi M A, Altabawy F M and Saad H A 2011 Synthesis, Acidity Constants and Tautomeristic Structure of the Diazonium Coupling Products of 2-(Benzylsulfonyl)-7H-purin-6-one in Its Ground and Excited States Molecules 16 8788–802
[21] Hiremath C S, Yenagi J and Tonannavar J 2007 FT-Raman and infrared spectra and vibrational assignments for 3-chloro-4-methoxybenzaldehyde, as supported by ab initio, hybrid density functional theory and normal coordinate calculations Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 68 710–7
[22] Ahmed A and Lal R A 2017 Synthesis, characterization and electrochemical studies of copper(II) complexes derived from succinyl- and adipoyldihydrazone Arab. J. Chem. 10 S901–8
[23] Sahar, Y. J., & Mohammed, H. S. (2019). Synthesis and characterization some complexes of azo dye of pyrimidinyl and evaluating their biological activity. Al-Qadisiyah Journal of Pure Science, 24(3).
[24] Kareem Abass Al-Hassani, M. (2019). SEROLOGICAL DETECTION OF COXIELLA BURNETII CHRONIC INFECTION- PHASE 1 IN SERUM OF HUMAN AND SHEEP AT AL-QADISIYAH PROVINCE, IRAQ. Al-Qadisiyah Journal Of Pure Science, 24(1), 13 - 20.
[25] Jalil Abed, M. (2019). Hybrid density functional theory and normal coordinate calculations of some new pyrroline compounds. Al-Qadisiyah Journal Of Pure Science, 24(1).
[26] Abdul-Hamza, H. kadhum, & Mohammed, G. J. (2019). The inhibitory effect of some nanoparticles on biofilm formation of Streptococcus agalactiae. Al-Qadisiyah Journal Of Pure Science, 24(2).
[27] Hussein Al-abedi, K. J., & Abd Al-Mayahi, F. (2019). Molecular detection of metallo-β-lactamase genes in carbapenem-resistant isolates of Pseudomonas aeruginosa recovered from patients in Al-Diwaniyah province, Iraq. Al-Qadisiyah Journal Of Pure Science, 24(2).
[28] Alramahy, S. khoman, & karim, N. abdul. (2019). Evaluation of serum levels of IL-10and IL-6 in patients with HCV at Diwaniyah Teaching Hospitals, Iraq. Al-Qadisiyah Journal Of Pure Science, 24(2).
[29] Kareem, F., & Thamer, W. (2019). Study of some immunological effects of methotrexate in people with rheumatoid arthritis. Al-Qadisiyah Journal Of Pure Science, 24(2).
[30] Jwad Sahar, Y., & Shamran Mohammed, H. (2019). Synthesis and characterization some complexes of azo dye of pyrimidinyl and evaluating their biological activity. Al-Qadisiyah Journal Of Pure Science, 24(3).
[31] A. Ghazay, A., Mayar Hezam, A., M. Alkhuzaie, M., & Obayes, I. S. (2020). Study the effect of different temperatures on the biofilm production in Proteus mirabilis isolated from urinary tract infection patients. Al-Qadisiyah Journal Of Pure.
[32] Salah, A. (2020). The New Combination of Semi-Analytical Iterative Method and Elzaki Transform for Solving Some Korteweg-de Vries Equations. Al-Qadisiyah Journal Of Pure Science, 25(1), Math. 23 - 26.
[33] Ali, W., & R.Annon, M. (2020). Biological Effective of organic solvent extracts of Mirabilis jalapa Leaves in the Non-cumulative for mortality of Immature stages Culex quinquefasciatus Say ( Diptera : Culicidae ). Al-Qadisiyah Journal Of Pure Science, 25(1), Biol 1-6.
[34] Sami Abd ali, mohammed, Shaker Hussein, A., & mohammed hadi, H. (2020). Study The Current Density-Voltage (J-V) Characteristics of α-Fe2O3 Thin Film Prepared by Spray Pyrolysis Technique. Al-Qadisiyah Journal Of Pure Science, 25 (1), Phys 1-7.