

SYNTHESIS AND CHARACTERIZATION OF A NEW AZO QUINOLINE LIGAND AND ITS METAL COMPLEXES WITH SPECTROPHOTOMETRIC DETERMINATION AND BIOLOGICAL EFFICACY STUDY OF ITS Hg(II) COMPLEX

Esraa Rasool Radhi^{1*}, Fatema Jabbar Ali¹ and Khdeeja Jabbar Ali²

¹Ministry of Education, Kufa, Iraq

²Chemistry Department, Education for Girls Faculty, Kufa University, Kufa, Iraq

(Received April 27, 2023; Revised June 21, 2023; Accepted June 29, 2023)

ABSTRACT. An azo quinoline ligand, (E)-2-((4-iodophenyl)diazanyl)-8-hydroxyquinoline (IPDHQ), was synthesized by the coupling reaction of 4-iodoaniline diazonium salt with 8-hydroxyquinoline. The synthesized ligand and its Cd(II), Pd(II), Cu(II) and Hg(II) complexes were spectrally characterized by UV-Vis spectrophotometry, FT-IR, mass spectral analysis, ¹H-NMR, magnetic sensitivity and molar conductivity. The general formula of the Cd(II), Cu(II) and Hg(II) complexes is [M(L)₂].Cl₂.H₂O with octahedral geometry, while the general formula of the Pd(II) complex is [M(L).Cl₂].H₂O with square planer geometry. A simple and rapid spectrophotometric procedure was suggested for the determination of Hg(II) using the synthesized ligand as a spectrophotometric reagent. By measuring the absorbance for the resulting orange complex at 430 nm, the linearity range was 0.5-15 µg mL⁻¹, and the interfering effect was also studied. The synthesized azo ligand and its Hg(II) complex were tested for their biological activity against four bacterial strains.

KEY WORDS: Azo quinoline ligand, 8-Hydroxy quinoline, 4-Iodoaniline, Azo metal complexes, Spectrophotometric determination, Biological efficacy study

INTRODUCTION

Azo compounds are a significant group of organic chemical compounds, and these compounds have one or more azo chromophores as (-N=N-), azo chromophore gives characteristic colors to these compounds [1]. Azo compounds can be employed as analytical reagents for the microquantity determination of different elements in multiple specimens [2-4]. Azo dyes are considered to be in general nonbiodegradable molecules under aerobic conditions and can be reduced to hazardous intermediate compounds under anaerobic conditions [5]. Azo-metal complexes are considered new and low-cost compounds that have a wide range of antimicrobial activities [6], anti-inflammatory, and anticancer [7]. This type of complexes has high significance in drug development, especially metal-based anticancer drugs [8, 9]. 8-Hydroxy quinoline is an important organic compound that has the capability to coordinate with different ions as a bidentate ligand [10-12]. The formation of the five-membered ring with the central metal ion helps to increase the stability of the formed complexes, in addition to their important biological activity [13]. 8-Hydroxy quinoline metal complexes can affect the human body by preventing the body from forming neurotoxic reactions specific to Alzheimer's disease [14].

Mercury is a common poisonous element in ecosystems, and even in low quantities, it can affect human health [15, 16]. Its poisoning effect occurs because of its accumulation in the tissues of the body from getting food [17, 18]. Mercury can be present in many forms and all of these forms are very poisonous [19]. Many analytical methods have been employed for Hg(II) determination, such as inductively coupled plasma-atomic emission spectrometry and inductively coupled plasma-mass spectrometry [20, 21], and cold-vapour atomic absorption spectroscopy [22]. Currently, as inexpensive and rapid techniques for Hg(II) determination, methods such as

*Corresponding author. E-mail: esraarasul@gmail.com

This work is licensed under the Creative Commons Attribution 4.0 International License

colorimetry [23], fluorimetry [24], and cloud point extraction methods (AALLME-SFO and AALLME-DES) [25] are employed. In the present work, an azo dye derived from the diazonium salt of 4-iodoaniline and 8-hydroxy quinoline and its Pd(II), Hg(II), Cu(II) and Cd(II) complexes were synthesized and spectrally characterized. The synthesized azo dye was used as an analytical reagent for the spectrophotometric determination of Hg(II). The synthesized azo ligand and its Hg(II)-complex were also checked for their biological activity.

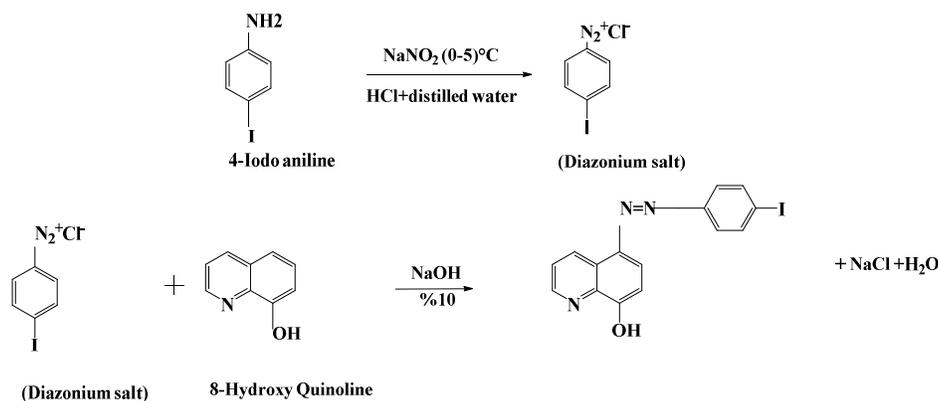
EXPERIMENTAL

Chemicals and instrumentation

All the chemicals used were of the highest analytical grade. The melting points of the prepared compounds were recorded by a Stuart-SMP10 melting point apparatus. UV-Vis spectra were obtained using a Shimadzu (UV-1700) spectrophotometer. FT-IR spectra were obtained using a Shimadzu FT-IR 8400 spectrophotometer by using KBr disks, and the mass spectrum was obtained by a Shimadzu Agilent HP (5973). ¹H-NMR spectra were obtained using Bruker Biospin Gmph (500 MHz) equipment with DMSO-d₆ as a solvent and TMS as an internal standard. Magnetic susceptibility was measured using a Sherwood Scientific balance. Molar conductivities were measured in DMSO as a solvent at room temperature by digital conductivity series ion. lab. 720.

Preparation of (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline (IPDHQ)

This azo ligand was synthesized by dissolving (2.19 g, 0.01 mol) 4-iodoaniline in a diluted solution of HCl (3 mL concentrated HCl and 20 mL distilled water). The solution was cooled to (0–5) °C, and then mixed with an aqueous solution of (0.70 g, 0.01 mol) sodium nitrite, noting that the temperature does not increase above (0–5) °C to form the diazonium salt of iodoaniline. The formed diazonium salt solution was added slowly with stirring to a basic alcoholic solution of 8-hydroxy quinoline (1.451 g, 0.01 mol) at the same temperature to form the azo dye (Scheme 1). The mixture was left until the next day, and then the acidic function was adjusted to be neutral (pH = 7) by adding a few drops of 0.1 N HCl to the solution to precipitate the red crystals. After one hour, the formed crystals were filtered and washed with distilled water. After drying in air, then it was re-crystallized by ethanol solvent, and dried, and its melting point was measured.



Scheme 1. The preparation steps of the IPDHQ ligand.

Preparation of (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline metal complexes

Metal complexes were prepared at optimal pH by mixing (0.375 g, 0.001 mol) IPDHQ dissolved in 15 mL ethanol and (0.001 mol) Cu(II), Cd(II), and Hg(II) chlorides dissolved in 5 mL ammonium acetate buffer solution with a 1:2 [M:L] molar ratio and (0.436 g, 0.001 mol) Pd(II) chloride dissolved in acetonitrile with a 1:1 [M:L] molar ratio. The mixtures were left overnight to complete the precipitation, and then the formed precipitate was filtered, dried, and recrystallized from hot absolute ethanol. The physical properties and elemental analysis for the prepared compounds are collected in Table 1.

Table 1. Physical properties and elemental analysis for the prepared compounds.

Compound	Color	m.p. °C	Yield %	M.f. (M.wt)	Found % (Calc.) %			
					C	H	N	M
Ligand = L (C ₁₅ H ₁₀ N ₃ OI)	Red	111	80	375.9	47.88 (47.85)	2.66 (2.61)	11.17 (11.12)	----
[Cu(L) ₂].Cl ₂ .H ₂ O	Brown	237	82	904.34	39.80 (39.75)	2.21 (2.19)	9.28 (9.27)	7.02 (7.00)
[Cd(L) ₂].Cl ₂ .H ₂ O	Brown	224	78	953.2	37.76 (37.73)	2.098 (2.095)	8.81 (8.77)	11.79 (11.78)
[Hg(L) ₂].Cl ₂ .H ₂ O	Orange	202	68	1041.39	34.50 (34.48)	1.920 (1.91)	8.066 (8.063)	11.79 (11.77)
[Pd(L)Cl ₂].H ₂ O	Violet	241	90	571.35	31.50 (31.49)	1.75 (1.73)	7.35 (7.33)	18.63 (18.60)

Found % = experimental values, (Calc.) % = calculated values.

RESULTS AND DISCUSSION*Characterization of (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline ligand and its complexes*

The ligand and its metal complexes are stable in air at room temperature. They are insoluble in water and freely soluble in ethanol, methanol, chloroform, acetone, dimethylformamide, dimethylsulfoxide, strongly acidic solutions and alkaline aqueous solutions. The ligand is reddish-orange, while the prepared metal complexes have different colors depending on the metal ion. The prepared ligand and its complexes were spectrally characterized by FT-IR, UV-Vis spectrophotometry, ¹H-NMR, mass spectral analysis, elemental analysis and several techniques.

¹H-NMR spectra of (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline

The ¹H-NMR spectrum of IPDHQ show a signal at $\delta = 11.03$ ppm due to the hydroxyl group of the 8-hydroxyquinoline ring and multiplet signals at $\delta = 6.89$ -7.64 ppm assigned to aromatic protons, whereas the signal at $\delta = 2.50$ ppm is assigned to DMSO-d₆ (Figure 1).

Mass spectra of (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline

The mass spectrum of IPDHQ showed a molecular ion peak M⁺ at m/z⁺ = 375.1 attributed to the main molecular weight of the azo ligand (375.9 g mol⁻¹), and several peaks assigned to the molecular ions at m/z⁺ = 248 and 231 were due to the fragment ions [C₁₅H₁₀N₃O]⁺ and [C₁₅H₉N₃]⁺, respectively. These data are in good agreement with the corresponding molecular formula [26] (Figure 2).

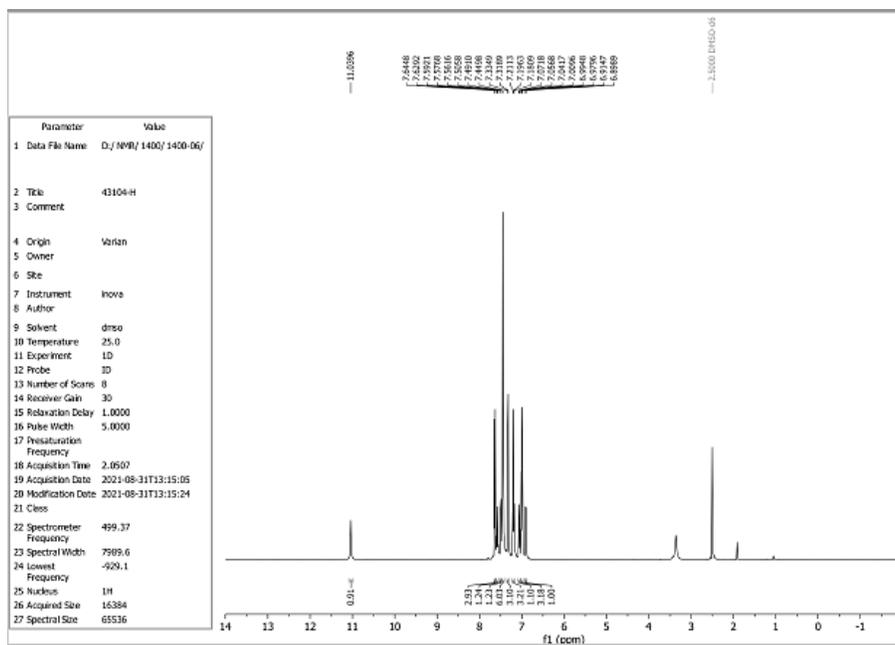
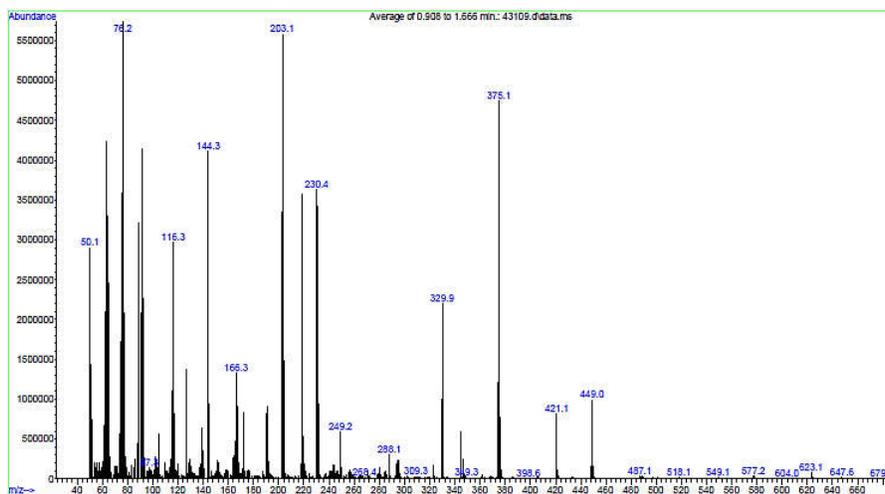
Figure 1. $^1\text{H-NMR}$ spectrum of IPDHQ.

Figure 2. Mass spectrum of IPDHQ.

Infrared spectra of (E)-2-((4-iodophenyl)diazanyl)-8-hydroxyquinoline and its metal complexes

The FT-IR spectrum of the free ligand exhibited a weak band at 1471 cm^{-1} due to the azo group. The band at $1687\text{-}1577\text{ cm}^{-1}$ appeared at the same position in the spectra of the ligand and the

complexes [27] (Figure 3). The spectra of the complexes showed a broad band at frequencies of 3425-3188 cm^{-1} , which are attributed to the symmetric and asymmetric vibrations of the N-H bond [28]. However, they were not observed in the spectrum of the ligand, which indicated that the ligand was coordinated with the metals through the azo group to form an M-N bond at 426-441 cm^{-1} in addition to the weak band at 532-580 cm^{-1} that was attributed to the M-O bond [29]. A broad band at 3410 cm^{-1} appears in the free ligand is attributed to the OH group of 8-hydroxy quinolone. (Table 2) lists the infrared data of IPDHQ and its complexes.

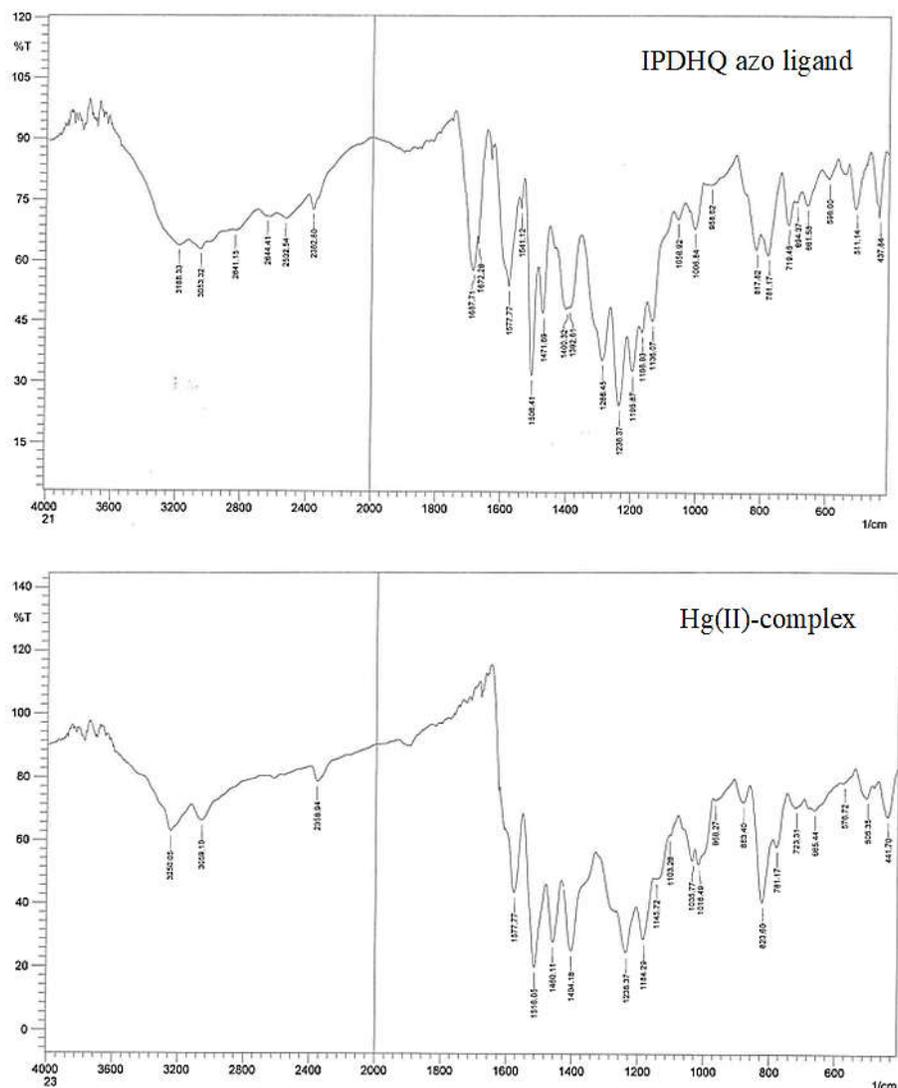


Figure 3. FT-IR spectra of IPDHQ azo ligand and its Hg(II)-complex.

Table 2. Selected infrared absorption bands (4000-400) cm^{-1} for IPDHQ and its metal complexes (KBr disc*).

Compound	ν (N-H) + ν O-H	ν (C-H) _{ar}	ν (C=N)	ν (N=N)	ν (M-O)	ν (M-N)
Ligand = L (C ₁₅ H ₁₀ N ₃ OI)	3410 br	3053 w	1687 m	1471 w	----	----
[Cu(L) ₂].Cl ₂ .H ₂ O	3375 br	3163 w	1581 m	1467 w	580 w	434 w
[Cd(L) ₂].Cl ₂ .H ₂ O	3441 br	3221 w	1577 m	1463 w	576 w	426 w
[Hg(L) ₂].Cl ₂ .H ₂ O	3250 br	3059 w	1577 m	1460 w	532 w	441 w
[Pd(L).Cl ₂].H ₂ O	3425 br	3066 w	1676 m	1469 w	----	432 w

*KBr disc: the examined powders are grinding with potassium bromide powder, and pressed into a disc
m = medium, w = weak, br = broad, ar = aromatic.

Electronic spectra of (E)-2-((4-iodophenyl)diazanyl)-8-hydroxyquinoline and its metal complexes

UV-VIS spectrum of the synthesized ligand IPDHQ showed two main peaks at 284 nm due to electronic transition ($\pi \rightarrow \pi^*$) and at 371 nm due to the electronic transition ($n \rightarrow \pi^*$) of the azo group (N=N) [30], which shifted to higher wavelengths in the complexes due to coordination with the transition ions. The spectrum of the Cu(II) complex exhibits a peak at 454 nm, which is assigned to the (${}^4T_{1g} \rightarrow {}^4A_{2g}$) transition, and the magnetic moment exists at 1.76 B.M., which indicated that the complex geometry is octahedral [31], while the spectrum of the Cd(II) complex displayed one peak at 564 nm which was assigned to the (M \rightarrow L,CT) transition [32], which may be taken as evidence for the octahedral geometry. In addition, the spectrum of the Pd(II) complex illustrated a broadband at 592 nm, which refers to the (${}^1A_{1g} \rightarrow {}^1B_{1g}$) transition [33]. This complex has a diamagnetic moment, suggesting a square planar geometry. The analytical data of IPDHQ and its complexes are listed in (Table 3). Scheme 2 explains the suggested structures of the four synthesized complexes.

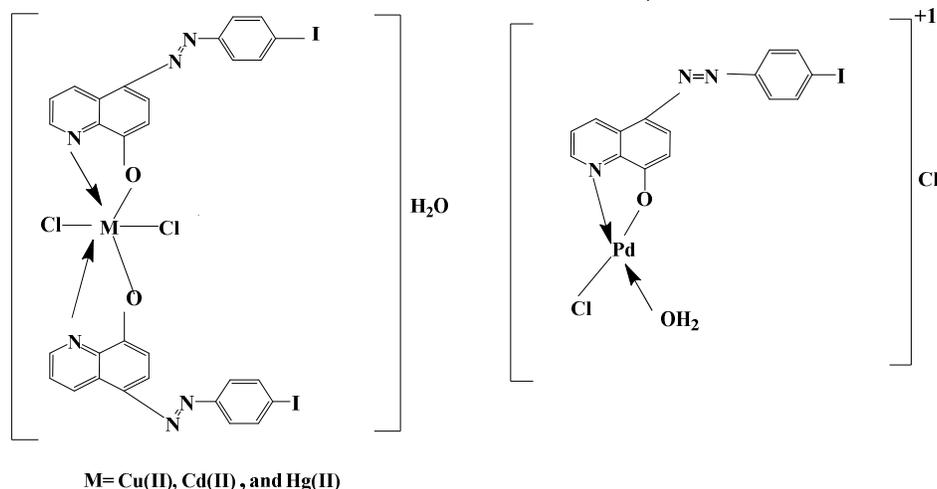
Table 3. Electronic spectra (nm and cm^{-1}), electronic transition, magnetic moments, proposed geometry and hybridization of IPDHQ and its complexes.

Compound	λ_{max} (nm)	Absorption bands (cm^{-1})	Transitions	μ_{eff} (B.M.)	Geometry	Hybridization
Ligand = L (C ₁₅ H ₁₀ N ₃ OI)	284 371	35211 27100	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	----	----	----
[Cu(L) ₂].Cl ₂ .H ₂ O	454	22026	${}^4T_{1g} \rightarrow {}^4A_{2g}$	1.76	Octahedral (regular)	d^2sp^3 (Low spin)
[Cd(L) ₂].Cl ₂ .H ₂ O	564	17730	M \rightarrow L,CT	Dia.	Octahedral (regular)	sp^3d^2 (High spin)
[Hg(L) ₂].Cl ₂ .H ₂ O	430	23094	M \rightarrow L,CT	Dia.	Octahedral (distorted)	sp^3d^2
[Pd(L).Cl ₂].H ₂ O	592	16891	${}^1A_{1g} \rightarrow {}^1B_{1g}$	Dia.	Square planer	dsp^2

Spectrophotometric determination of Hg(II)

Preparation of the standard and the work solutions

The stock solution of IPDHQ (1000 $\mu\text{g mL}^{-1}$) was prepared by dissolving 0.1 g of IPDHQ in 96% ethanol in a 100 mL volumetric flask, and then diluting using the same solvent to the flask mark. The working solutions were prepared by further dilutions with the same solvent. To obtain the stock solution of mercury ions at 100 $\mu\text{g mL}^{-1}$, dissolve 0.01 g of HgCl₂ salt in distilled water in a 100 mL volumetric flask, and then complete the volume using distilled water to the flask mark. To prepare the working solutions, further dilutions with distilled water were made.



Scheme 2. The suggested structures of IPDHQ complexes.

General determination procedure

Into a series of volumetric flasks (10 mL), transfer suitable volumes of Hg ion solution containing (0.5-15 μg), followed by adding 1 mL of 50 $\mu\text{g mL}^{-1}$ from IPDHQ solution to each flask, then complete the volume using distilled water to the flask mark. The absorbance value at 430 nm was calculated against the blank absorbance, and then the calibration curve of the concentration values and the corresponding absorbance values was drawn.

Absorption spectra of the IPDHQ and Hg(II) complex

The UV-Vis spectrum of IPDHQ in the neutral medium has a maximum absorption peak at 371 nm, which shifts to a higher wavelength (430 nm) when forming Hg(II) complex.

IPDHQ concentration effect study

The IPDHQ concentration effect on Hg(II)-complex formation with (2 mL, 10 $\mu\text{g mL}^{-1}$) Hg(II) aqueous solution was tested in the concentration range of (10-90) $\mu\text{g mL}^{-1}$ and a volume of 1 mL. The complex absorbance increased as the IPDHQ concentration increased up to 50 $\mu\text{g mL}^{-1}$ and then decreased after that.

Time and temperature effect study

The complexation time and temperature effect on the formation of the Hg(II)-complex was checked in the range of (0.5-30) minutes and (25-60) $^{\circ}\text{C}$, respectively. The complex absorbance increased with the complex formation time up to 1 min. and then remained stable with the same absorbance value for 24 hours in the temperature range of 25-30 $^{\circ}\text{C}$.

IPDHQ solution volume effect study

The IPDHQ volume effect on Hg(II)-complex formation with (2 mL, 10 $\mu\text{g mL}^{-1}$) Hg(II) aqueous solution was investigated in the volume range of (0.25-2) mL and a concentration of 50 $\mu\text{g mL}^{-1}$ IPDHQ. The complex maximum absorbance was reached when using 1 mL of IPDHQ, and then

the decrease was noticed when using greater volumes. Adding greater volumes can affect the coordination bonding in the formed complex, in addition to their effect on the ionic strength of the solution, causing a decrease in the concentration of the formed complex and its absorptivity.

Calibration curve

The determination calibration curve was established according to the obtained experimental optimum conditions. By preparing a series of increasing concentrations of Hg(II) solutions, and recording the absorbance values against the ligand blank, the complex absorbance values were plotted against the Hg(II) concentration. The linearity was in the range of (0.5-15) $\mu\text{g mL}^{-1}$. The calibration curve data and other analytical parameters are listed in Table 4.

Table 4. Analytical data of the proposed method for Hg(II) determination .

Analytical parameter	Hg(II)-complex
λ_{max} nm	430
Regression equation	$y = 0.0199x + 0.0016$
Specific absorption coefficient $\text{L gm}^{-1} \text{cm}^{-1}$	19.9
Molar absorption coefficient $\text{L mol}^{-1} \text{cm}^{-1}$	3.991×10^3
Sandells sensitivity $\mu\text{g cm}^{-2}$	0.050
Correlation coefficient r^2	0.9996
Detection limit $\mu\text{g mL}^{-1}$	0.174
Quantitation limit $\mu\text{g mL}^{-1}$	0.580
Linear range $\mu\text{g mL}^{-1}$	0.5 - 15
Standard deviation	0.001
Relative standard deviation %	0.180

Interferences effect study

At the optimum conditions determined in the above carried-out studies, the interference effect of different types of cations (Fe^{3+} , Pb^{2+} , Ni^{2+} , Mn^{2+} , Al^{3+} , Zn^{2+} , Co^{2+}) and anions (Cl^- , F^- , CH_3COO^- , NO_3^- , CrO_4^{2-} , SO_4^{2-} , CO_3^{2-}) on the complex formation was checked to estimate the method's utility. By following the general procedure and adding a known quantity of studied interfering ions at concentrations of 10 and 50 $\mu\text{g mL}^{-1}$ to Hg(II) solution (10 $\mu\text{g mL}^{-1}$). The study showed that the examined cations and anions have no interfering effect on Hg(II) complex formation at the two specified concentrations.

Biological efficacy study

The agar well diffusion method was applied to determine the biological efficacy of the synthesized azo ligand IPDHQ and its complex with Hg(II) using two types of gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and two types of gram-negative bacteria (*Klebsiella pneumonia* and *Escherichia coli*). Each type of chosen bacteria was diffused up a prepared agar surface. Five punctures with a 6 mm diameter were then perforated in the agar plates. A total of 0.1 mL of the azo ligand and its Hg(II) complex were individually added to five wells of each plate at five increasing concentrations (25, 50, 100, 200 and 400 $\mu\text{g mL}^{-1}$). All plates were incubated at 37 °C for one day; the biological efficacy was then recorded by calculating the diameter (in mm) of the clear inhibition zone around each puncture.

As shown in Table 5, the results indicated that the IPDHQ ligand and its Hg(II) complex at 25 $\mu\text{g mL}^{-1}$ have no inhibition activity against all the bacteria types, This also applies to the concentration of 50 $\mu\text{g mL}^{-1}$, except for the ligand which has a medium activity for inhibition equal to 12 mm diameter against *K. pneumonia* bacteria, while at concentrations in the range of

100-400 $\mu\text{g mL}^{-1}$, the two compounds will have high biological activity against all the bacteria types with inhibition diameter in the range of 13-18 mm with a slight difference in the inhibition diameter between the two compounds.

Table 5. The biological efficacy of IPDHQ and its Hg(II) complex.

Compound	Concentration $\mu\text{g mL}^{-1}$	Inhibition zone diameter mm			
		Gram-positive bacteria		Gram-negative bacteria	
		<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Free ligand IPDHQ	25	0	0	0	0
	50	0	0	0	12
	100	15	13	14	15
	200	16	14	15	16
	400	18	15	16	17
Hg(II)- complex	25	0	0	0	0
	50	0	0	0	0
	100	14	13	15	12
	200	15	15	16	15
	400	16	16	18	17

CONCLUSION

The presented work describes the synthesis and spectral characterization of the azo quinoline ligand and its four transition metal complexes. The obtained results revealed that the proposed geometrical shape for Hg(II), Cu(II), and Cd(II) complexes is octahedral geometry with a 1:2 [M:L] molar ratio, while the geometrical shape for Pd(II)-complex is square-planar geometry with a 1:1 [M:L] molar ratio. The synthesized azo ligand was feasibly used as an analytical reagent for easy and efficient determination of the mercury ion at 430 nm in the linearity range 0.5-15 $\mu\text{g mL}^{-1}$, the interfering effect of different types of cations and anions was also studied, the study results showed there is no interfering effect from these ions on Hg(II) complex formation at the specified concentrations. The biological efficacy study was tested for the free ligand and its Hg(II)-complex against four strains of bacteria, the study indicates that the two compounds exhibited high inhibition activity at concentrations of 100-400 $\mu\text{g mL}^{-1}$.

REFERENCES

1. Mohammed, H.S. 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.* **2020**, 34, 523-532.
2. Jawad, S.K.; Husien, N.S.M. Solvent extraction method for separation and determination of Zn(II) by using of imidazole derivative. *Int. J. Eng. Technol.* **2018**, 7, 553-556.
3. Ammar, J.W.; Khan, Z.A.; Ghazi, M.N.; Naser, N.A. Synthesis of a new organic probe 4-(4-acetamidophenylazo) pyrogallol for spectrophotometric determination of Bi(III) and Al(III) in pharmaceutical samples. *Rev. Anal. Chem.* **2021**, 40, 108-126.
4. Kirem, H.; Naser, N.A. A Green product using selective compound for susceptible assessment of copper in blood serum. *Curr. Appl. Sci. Technol.* **2023**, 23, 10-55003.
5. Park, H.; Choi, W. Visible light and Fe(III) mediated degradation of acid orange 7 in the absence of H_2O_2 . *J. Photochem. Photobiol. A: Chem.* **2003**, 159, 241-247.
6. Reed, J.E.; Arnal, A.A.; Neidle, S.; Vilar, R. Stabilization of G-quadruplex DNA and inhibition of telomerase activity by square-planar nickel(II) complexes. *J. Am. Chem. Soc.* **2006**, 128, 5992-5993.

7. Al-Resayes, S.I.; Jarad, A.J.; Al-Zinke, J.M.; Al-Noor, T.H.; El-ajaily, M.M.; Abdalla, M.; Min, K.; Azam, M.; Mohapatra, R.K. Synthesis, characterization, antimicrobial studies, and molecular docking studies of transition metal complexes formed from a benzothiazole-based azo ligand. *Bull. Chem. Soc. Ethiop.* **2023**, *37*, 931-944.
8. Selvakumar, B.; Rajendiran, V.; Maheswari, P.U.; Stoeckli-Evans, H.; Palaniandavar, M. Structures, spectra, and DNA-binding properties of mixed ligand copper(II) complexes of iminodiacetic acid: The novel role of diimine co-ligands on DNA conformation and hydrolytic and oxidative double strand DNA cleavage. *J. Inorg. Biochem.* **2006**, *100*, 316-330.
9. Patra, A.K.; Nethaji, M.; Chakravarty, A.R. Synthesis, crystal structure, DNA binding and photo-induced DNA cleavage activity of (S-methyl-L-cysteine) copper(II) complexes of heterocyclic bases. *J. Inorg. Biochem.* **2007**, *101*, 233-244.
10. Vashi, R.T.; Patel, S.B.; Kadiya, H.K. Synthesis, Characterization and antimicrobial activity of metal chelates of 2-[(8-hydroxyquinolinyl)-5-aminomethyl]-3-(4-Bromophenyl)-3(H)-quinazolin-4-one. *Pharma Chem.* **2012**, *4*, 1506-1511.
11. Dixit, R.B.; Patel, J.A. Spectrophotometric determination of paracetamol drug using 8-hydroxyquinoline. *Int. J. Pharm. Sci. Res.* **2014**, *5*, 2393.
12. El-Wakiel, N.A.; Rizk, H.F.; Ibrahim, S.A. Synthesis and characterization of metal complexes of azo dye based on 5-nitro-8-hydroxyquinoline and their applications in dyeing polyester fabrics. *Appl. Organomet. Chem.* **2017**, *31*, e3723.
13. Abdelgawad, M.A.; Mohamed, A.M.; Musa, A.; Mostafa, E.M.; Awad, H.M. Synthesis, chromatographic separation and antimicrobial evolution of new azoquinoline-8-ol. *J. Pharm. Sci. Res.* **2018**, *10*, 1314-1318.
14. Suwanjang, W.; Prachayasittikul, S.; Prachayasittikul, V. Effect of 8-hydroxyquinoline and derivatives on human neuroblastoma SH-SY5Y cells under high glucose. *Peer J.* **2016**, *4*, e2389.
15. Yang, L.; Zhang, Y.; Wang, F.; Luo, Z.; Guo, S.; Strähle, U. Toxicity of mercury: Molecular evidence. *Chemosphere* **2020**, *245*, 125586.
16. Jawad, S.K.; Kadhium, M.U.; Azooz, E.A. Separation and spectrophotometric determination of iron(III) and mercury(II) via cloud point extraction with new azo-derivative. *Eurasian J. Anal. Chem.* **2018**, *13*, 1-11em48.
17. Rana, M.N.; Tangpong, J.; Rahman, M.M. Toxicodynamics of lead, cadmium, mercury and arsenic-induced kidney toxicity and treatment strategy: a mini review. *Toxicol. Rep.* **2018**, *5*, 704-713.
18. Orr, S. *Potential Mechanisms of Inorganic Mercury Intoxication in Rat Kidney Cells*, PhD Thesis, Mercer University, Macon, Georgia, **2018**.
19. Keshavayya, J. Synthesis, structural investigations and in vitro biological evaluation of N,N-dimethyl aniline derivatives based azo dyes as potential pharmacological agents. *J. Mol. Struct.* **2019**, *1186*, 404-412.
20. Huang, D.; Li, Z.; Zeng, G.; Zhou, C.; Xue, W.; Gong, X.; Yan, X.; Chen, S.; Wang, W.; Cheng, M. Megamerger in photocatalytic field: 2D g-C₃N₄ nanosheets serve as support of 0D nanomaterials for improving photocatalytic performance. *Appl. Catal. B* **2019**, *240*, 153-173.
21. Leopold, K.; Foulkes, M.; Worsfold, P. Methods for the determination and speciation of mercury in natural waters—a review. *Anal. Chim. Acta* **2010**, *663*, 127-138.
22. Lu, X.; Zhao, J.; Liang, X.; Zhang, L.; Liu, Y.; Yin, X.; Li, X.; Gu, B. The application and potential artifacts of Zeeman cold vapor atomic absorption spectrometry in mercury stable isotope analysis. *Environ. Sci. Technol. Lett.* **2019**, *6*, 165-170.
23. Radhi, E.R. Spectrophotometric determination of Hg(II) Using (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl) benzoic acid as analytical reagent. *J. Pharm. Negat. Results* **2022**, *13*, 530-536.
24. Peng, G.; Chen, Y.; Deng, R.; He, Q.; Liu, D.; Lu, Y.; Lin, J.M. Highly sensitive and selective determination of Hg(II) based on microfluidic chip with on-line fluorescent derivatization.

- Spectrochim. Acta Part A* **2018**, 204, 1-6.
25. Azooz, E.A.; Al-Wani, H.S.A.; Gburi, M.S.; Al-Muhanna, E.H.B. Recent modified air-assisted liquid–liquid microextraction applications for medicines and organic compounds in various samples: A review. *Open Chem.* **2022**, 20, 525-540.
 26. Khan, B.A.; Zafar, S.; Mughal, E.U.; Ahmed, M.N.; Hamdani, S.S.; Akhtar, T.; Sadiq, A.; Khan, K.M. Design and synthesis of novel 1,3,4-oxadiazole derivatives bearing azo moiety as biologically significant scaffolds. *Lett. Drug Des. Discov.* **2018**, 15, 1346-1355.
 27. Keshavayya, J.; Pandurangappa, M.; Ravi, B.N. Synthesis, characterization and electrochemical investigations of azo dyes derived from 2-amino-6-ethoxybenzothiazole. *Chem. Data Collect.* **2018**, 17, 13-29.
 28. Methaq, S. Some transition metal complexes with new Schiff base ligand hexadentate. *Acta Chim. Pharm. Indica* **2013**, 3, 140-148.
 29. Jarad, A.J.; Kadhim, M.A.; Jabrallah, S.A. Synthesis and spectral studies of Zn II, Cd II and Hg II complexes with 5-(2-benzoic acid azo)-8-hydroxy quinoline ligand. *Baghdad Sci. J.* **2012**, 9, 178-186.
 30. Ali, F.J.; Radhi, E.R.; Ali, K.J. Synthesis and characterization of a new azo ligand derivative from 4,5-bis (4-methoxyphenyl) imidazol and its metal complexes and biological activity study of its Pd(II) complex. *Pak. J. Med. Health Sci.* **2022**, 16, 431-431.
 31. Atiyah, E.M.; Alwan, W.M.; Alkam, H.H. New mixed ligand complexes of new Schiff base 4,4'-((naphthalen-1-ylimino)methylene)dibenzene-1,3-diol and 8-hydroxy quinoline: Synthesis, spectral characterization, thermal studies and biological activities. *Syst. Rev. Pharm.* **2020**, 11, 725-735.
 32. Geary, W.J. The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. *Coord. Chem. Rev.* **1971**, 7, 81-122.
 33. Shaygan, S.; Pasdar, H.; Foroughifar, N.; Davallo, M.; Motiee, F. Cobalt(II) complexes with Schiff base ligands derived from terephthalaldehyde and *ortho*-substituted anilines: synthesis, characterization and antibacterial activity. *Appl. Sci.* **2018**, 8, 385.