

## Spectrophotometric Complexation Study of 8-Hydroxyquinoline Based AZO Dye with Pb(II) Metal ION and their Antimicrobial Activities

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### ABSTRACT

Heavy metals in particular are believed to be one of the main contributors of environmental contamination since trace elements have a significant effect on ecological quality. Here, we investigated the complexation of the hazardous heavy metal Pb(II) with the 8-HDQ azo dye and recorded the results using XRD, IR, and UV-visible analysis. The stoichiometry between M: L and the impact of pH on complexation. In addition, the synthetic complex tests its ability to suppress infections.

**Key words:** 8-Hydroxyquinoline, Antimicrobial activity study, Pb(II) metal ion, UV-Visible.

### 1. INTRODUCTION

Monitoring heavy metals in the environment, such as Pb(II), is critical due to their hazardous effects on living species. Although lead poisoning can have many negative impacts on human health, it is especially harmful to children's neurological development. Since heavy metals are not biodegradable, they often build up in the soft tissues of living things, which can lead to a variety of illnesses. Lead poisoning in humans can be lethal even at low concentrations and can cause problems with the kidney, liver, heart, immune system, and brain system [1]. Therefore, it is important to trace out the heavy metal from different resources. In routine laboratory work, spectrophotometric tests are crucial. In any laboratory that studies the environment, biochemistry, biotechnology, food safety, or quality, high throughput assays have become essential [2-6]. A specific class of synthetic organic dye known as an azodye has a number of chemical characteristics that increase the molecule's sensitivity to its surroundings. A chelating substance called 8-hydroxyquinoline (HQ) is utilized in the quantitative analysis of metal ions. In an aqueous solution, HQ has a pKa of 9.9 [7]. Outstanding work centered on the development and synthesis of HQ metal complexes [8-11].

8-HDQ is heavily utilized for metal chelation as well as analytical and separation applications due to its strong coordinating ability and good metal identification qualities [12]. Numerous biological, clinical, herbicidal, anti-inflammatory, antifungal, antibacterial, antituberculosis, antineoplastic, and antidiabetic uses can be made of the majority of azo compounds and their complexes [13-15]. Scientific study is focusing on azo compounds, a significant class of chemical molecules. They have a rich color and have long been used as pigments and dyes [16-18]. Since they have superior thermal and optical qualities for uses like optical recording media, they have been extensively researched [19-22]. The present work describes the synthesis and characterization of HQ azo dye. The structure of ligand and its Pb(II) complex was characterized by UV-visible, IR, and XRD study. The synthesized complex also checks with its inhibition property against pathogens viz. *Escherichia Coli*, *Salmonella typhi* and *Staphylococcus aureus*.

### 2. EXPERIMENTAL

All chemical compounds used in this study were supplied by S.D. fine chemicals. Electronic spectra were measured with a single beam UV-VIS Bio-Era spectrophotometer with quartz cell (10 mm). IR spectra were recorded with a PerkinElmer 1430 IR spectrophotometer within the range 400–4000  $\text{cm}^{-1}$  as KBr discs. Solvents of the AR grade were employed. The stock solution of 8-HDQ dye was made of  $2 \times 10^{-4}$  M and metal solutions were of  $1 \times 10^{-2}$  M.

### 3. PROCEDURE

#### 3.1. Preparation of 8-hydroxyquinoline azo dye (Scheme 1):

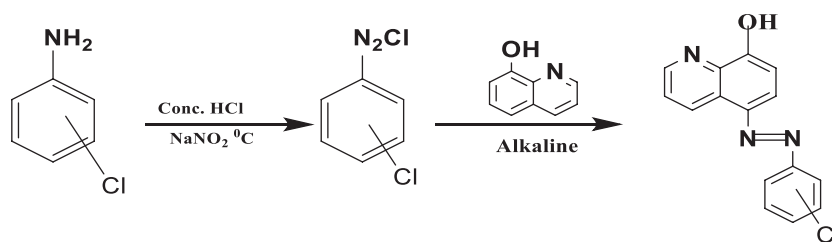
In a 250 mL beaker, 2.54 g (0.02 mol) of 2-chloroaniline was dissolved in 40 mL of ethanol. In a subsequent step, 9 mL of concentrated HCl and 9 mL of distilled water were added and cooled in an ice bath to 0°C. Then, while stirring, 20 mL of a cold solution of NaNO<sub>2</sub> (0.03 mol, 2.07 g) in water was added. The resulting mixture was then left for 1 h at 0°C. The mixture was then stirred while a 30 mL cold solution of HQ (0.03 mol, 4.35 g) containing 10% NaOH was added dropwise. The mixture was then kept at 0°C for a further hour. The resulting crude product was filtered, washed with cold water, and then dried and recrystallized with ethanol.

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**Scheme 1:** Preparation of 8-hydroxyquinoline azo dye.

### 3.2. Preparation of Metal Complexes (General Procedure)

(The ligand (0.56 g, 2 mmole) was dissolved in ethanol and gradually added, while stirring, to the 0.331 g, 1 mmole of  $\text{Pb}(\text{NO}_3)_2$  that had been dissolved in the appropriate pH solution. The mixture was allowed to cool until a rich color precipitate was achieved, after which it was filtered and repeated washings with a 1:1 water: ethanol mixture and acetone.

### 3.3. Antimicrobial Activity Study

Utilizing three Gram-positive bacteria (*S. aureus*) and two Gram-negative bacteria (*E. coli* and *salmonella typhi*), the ligand and its complex were examined for their in vitro antibacterial properties. Concentration solutions, namely 0.01 mg/L, were made by dissolving the complex with 8-HQ in ethanol. For every agar well, 100  $\mu\text{L}$  of material was allocated. Ciprofloxacin (conc. 1 mg/mL) was used as a control.

## 4. RESULTS AND DISCUSSION

### 4.1. UV-Visible Study

UV-VIS spectroscopy provides important insights into the interactions of Pb(II) complexes with o-chloroaniline 8-HDQ and p-chloroaniline-8-HDQ, which is our main study goal. The ortho and para-chloroaniline-8-HDQ ligand and its complexes' UV/Vis absorption spectra were recorded in ethanol solvent at room temperature. Figure 1 clearly indicates that both ligands have strong absorption band at 420–450 nm is due to the  $n-\pi^*$  and  $\pi-\pi^*$  transition. The coordination of the azo ligand through metal (II) in metal complexes causes these transitions to shift to red or blue frequencies. Ligand exhibits a decrease in absorbance intensity following complex formation with Pb(II) metal ion, confirming the complex formation. This potential heavy metal effect could be the cause of this decline in absorption intensity. This result suggested that there is strong affinity for Pb(II) metal ions toward the 8-HDQ molecules.

### 4.2. Effect of pH on Complexation Study

To investigate the complicated dynamics of complexation between o-8-HDQ and its Pb(II) complex, as well as p-8-HDQ and its Pb(II) complex, a pH range of 4–9 was selected as the experimental range. A straight ratio between pH rise and absorption intensity is revealed by the analysis in Figure 2. At lower pH-4, the lower absorbance is due to form quinolinium ion result from the reaction hydrogen ions and the ion pair of electron which found in the nitrogen atoms for quinoline ring. A significant finding is that the o-8-HDQ-Pb(II) complex exhibits its maximum absorption intensity at pH-6, which is clearly detected at 420–450 nm. The p-8-HDQ-Pb(II) complex simultaneously showed a bathochromic shift and a peak absorption intensity at pH-8. The significant impact of pH on complexation not only highlights its importance but also designates it as the ideal pH level for more research.

### 4.3. Metal to Ligand Stoichiometry

To find the metal: ligand [M:L] ratio of the chelated complexes at a constant concentration and optimal pH at maximum wavelength

absorption ( $\lambda_{\text{max}}$ ), the molar ratio method was used. The plot of absorbance versus mole fractions is shown in Figure 3. Both the HDQ-Pb(II) complex exhibited a 1:2 stoichiometry in response to the greatest absorption seen at a mole ratio of 0.4 for complexes.

### 4.4. Infrared Analysis

FTIR spectra of ligands and synthesized complexes are shown in Figure 4. Table 1 shows the spectral data obtained by FTIR spectroscopy. A broad band of medium intensity was seen on the ligands. FTIR spectra in the area of  $3340\text{ cm}^{-1}$  and  $3354\text{ cm}^{-1}$  which would correspond to the O-H bond of both 8-HDQ ligands which was shifted to  $3381\text{ cm}^{-1}$  and  $3367\text{ cm}^{-1}$  on complex formation with Pb(II) metal ion. The band is due to the C-O bond was observed at  $1109\text{ cm}^{-1}$  and  $1104\text{ cm}^{-1}$  in ortho and para derivatives of 8-HDQ, respectively, both these bands notably shifted to  $1120\text{ cm}^{-1}$  and  $1097\text{ cm}^{-1}$  in complexes. The band C=N was recorded at  $1548\text{ cm}^{-1}$  ortho 8-HDQ, while in Pb(II) complexes, this band was shifted to  $1554\text{ cm}^{-1}$ ; however, in the para derivative, no shift was observed. The ligand-metal ion bonding is confirmed by the vibrational frequencies of the M-O and M-N bonds, which are around  $517\text{ cm}^{-1}$  and  $516\text{ cm}^{-1}$  and  $469\text{ cm}^{-1}$  and  $468\text{ cm}^{-1}$ , respectively, in both complexes [23].

### 4.5. XRD Analysis

The 8-HDQ ligand and its Pb(II) metal complexes' powder XRD patterns were captured within the  $2\theta = 10-90^\circ$  range. The powder XRD patterns of the complexes differ significantly from those of the initial  $\text{H}_2\text{L}$  ligand, confirming the production of coordination molecules. Table 2 summarizes the findings, which are also displayed in Figure 5. The nature of the patterns suggests that the compounds are not crystalline. Using the Scherrer formula, the average particle size of the ligand and metal complexes was determined.

$$D = \frac{K\lambda}{FWHM \times \cos\theta} \quad (1)$$

Where, K = Scherrer constant, FWHM = whole breadth at half the maximum of the diffraction pattern's reflection peak, which has the same maximum intensity,  $\lambda$  = wavelength of X-rays ( $1.54059\text{ \AA}$ ), and  $\theta$  = diffraction angle of X-rays. The Scherrer constant (K) in the formula accounts for particle shape and is commonly assumed to be 0.9. The ligand 2-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II) complex have average particle sizes of 33.26–29.53 nm and for 4-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II) complex the average particle size lies between 37.02 and 41.59 nm.

### 4.6. SEM Analysis

The substantial alteration in the complexes surface shape brought about by the metals coordination with the ligand was examined using SEM analysis. The morphologies of p-chloro-8-HDQ and o-chloro-8-HDQ and their Pb(II) complexes were examined in the SEM investigation. The SEM micrograph of the ligand shows an unevenly shaped and non-uniformly sized crystalline structure, as shown in Figure 6a

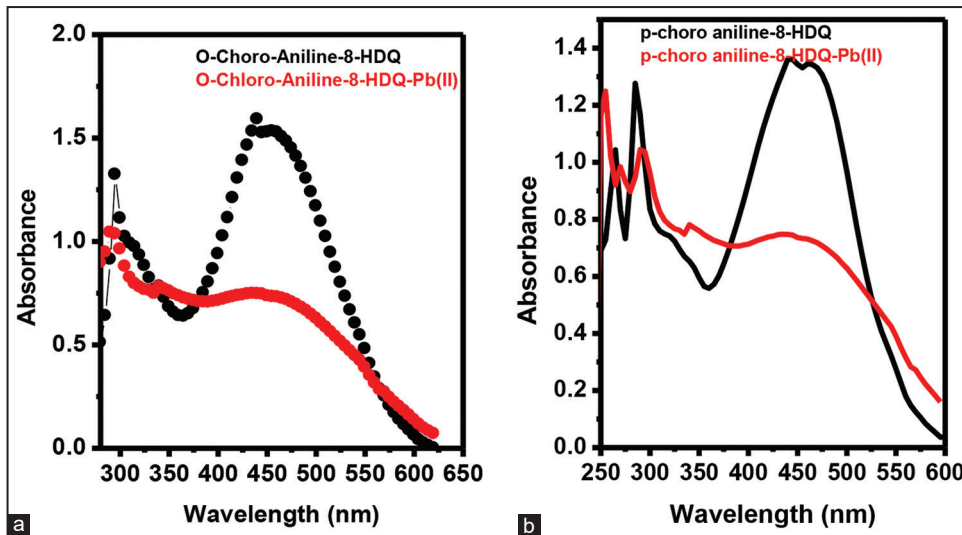


Figure 1: UV-Visible spectra of (a) o-chloro-8-HDQ and its Pb(II) complex and (b) p-chloro-8-HDQ and its Pb(II) complex.

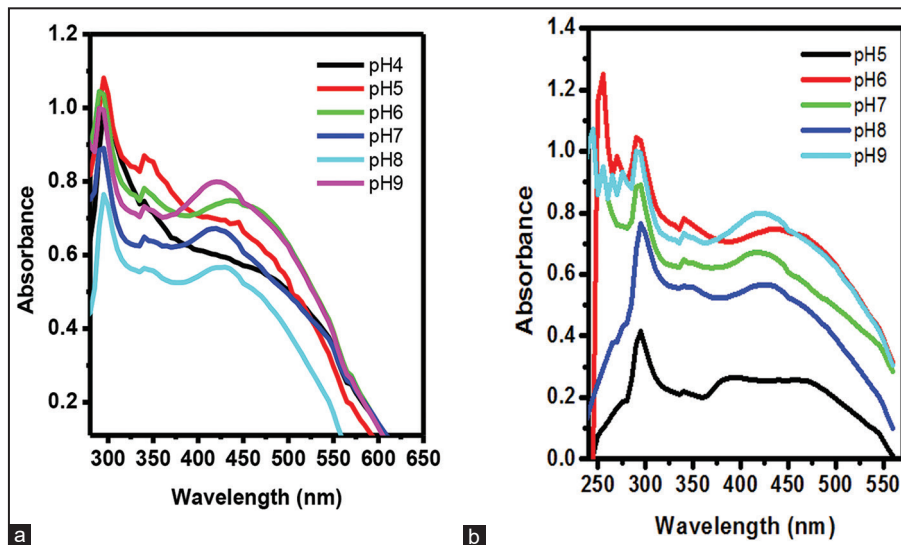


Figure 2: Effect of pH on complexation (a) o-chloro-aniline-8-HDQ and its Pb(II) complex and (b) p-chloro-aniline-8-HDQ and its Pb(II) complex.

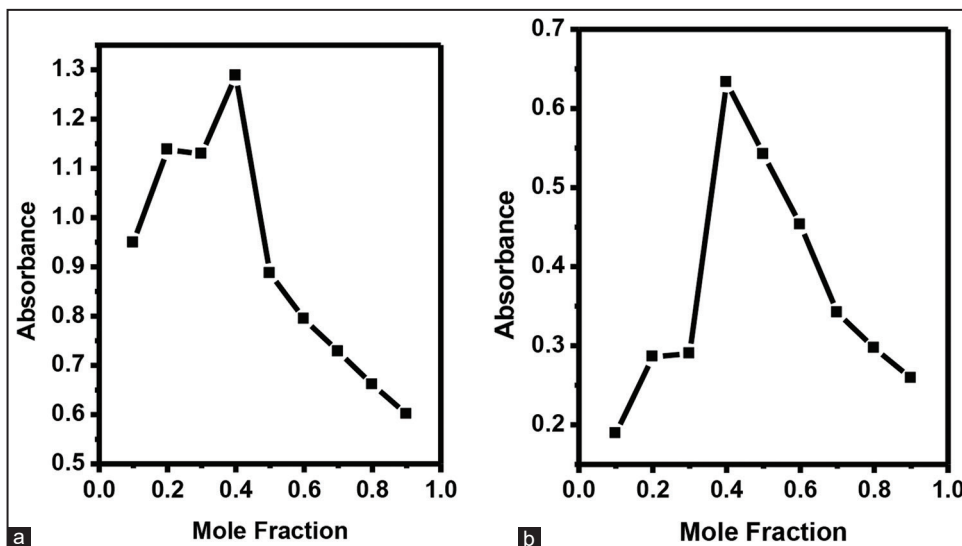
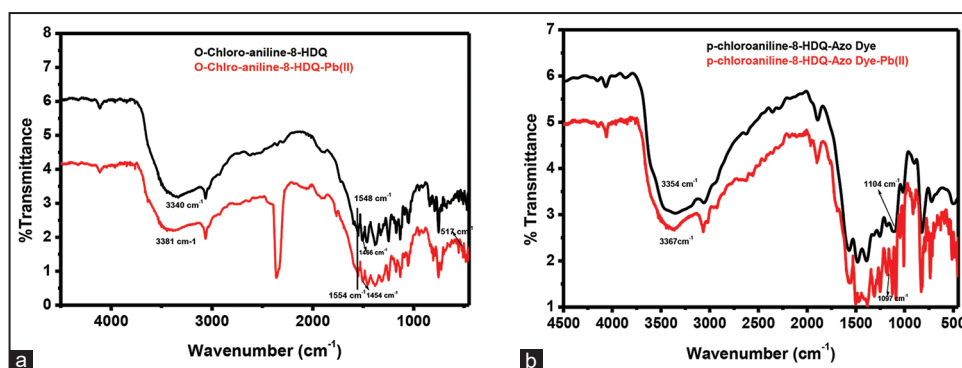
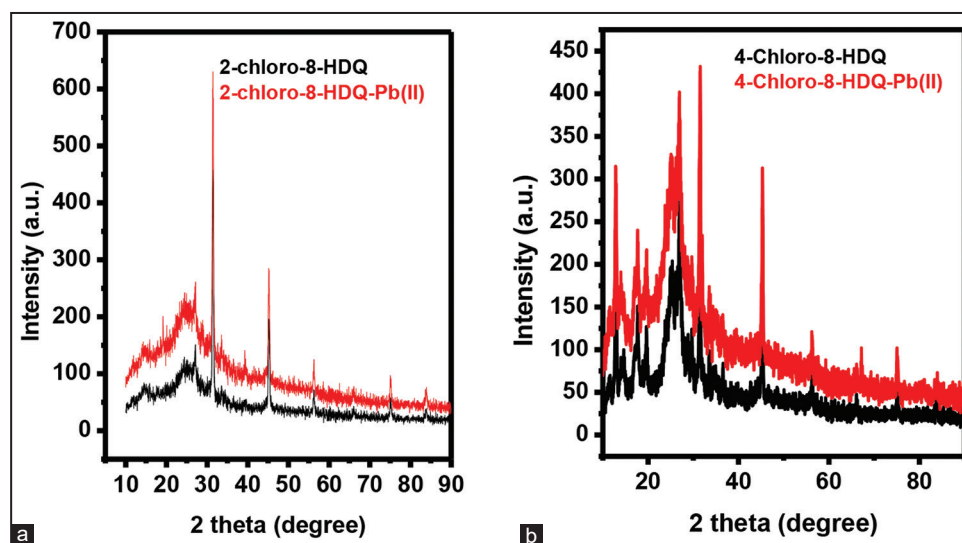


Figure 3: Stoichiometry of the complexes (a) o-chloro-aniline-8-HDQ and its Pb(II) complex and (b) p-chloro-aniline-8-HDQ and its Pb(II) complex.



**Figure 4:** FTIR spectra of (a) 2-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II) complex and (b) 4-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II) complex.



**Figure 5:** XRD patterns of (a) 2-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II) (b) 4-(8-hydroxyquinolin-5-azo)-chloroaniline and its Pb(II).

**Table 1:** Infrared spectral data for 8-HDQ and Pb (II) complexes.

Compound	OH	Infrared spectral bands $\nu$ $\text{cm}^{-1}$				
		C-O	C=N	N=N	M-O	M-N
o-chloro-8-HDQ	3340	1109	1548	1466	-	-
o-chloro-8-HDQ-Pb (II)	3381	1120	1554	1454	517	469
p-chloro-8-HDQ	3354	1104	1569	1483	-	-
p-chloro-8-HDQ-Pb (II)	3367	1097	1569	1487	516	468

and b. It is interesting to note that the complexes' surface shape was significantly changed by metal coordination to the ligand. In particular, the Pb(II) complex's SEM micrographs, which are shown in Figure 6c and d show a noticeably uneven texture that resembles fractured rocks. Further supporting the formation of the complexes, the EDX spectra, shown in Figure 6, prominently featured a peak corresponding to the Pb(II) ion, confirming successful complexation.

#### 4.7. Stability Constant as well Free Gibbs Energy Determination

Using spectrophotometry, the stability constant of metal complexes is ascertained by measuring the absorbance of a mixture of Pb(II) metal ions and 8-HDQ azo ligand at a specified wavelength ( $\lambda_{\text{max}}$ ),

concentration, and optimal pH, complex's apparent stability constant (K) was derived using the equation below.

$$K = 1 - \alpha / 4\alpha^3 c^2 \quad (1)$$

$$\alpha = A_m - A_s / A_m \quad (2)$$

$$\text{Or } \alpha = A_s - A_m / A_s$$

Where c is the complex solution's concentration in mol/L, A is the degree of dissociation, and  $A_s$  is the solution's absorption when ligand and metal ions are present in stoichiometric amounts.  $A_m$  is the absorption of a solution with excess ligand and the same amount of metal. High values with (K) signify that the productive complexes are highly stable [24].

The thermodynamic parameters of Gibbs free energy (G) were also calculated using the equation [25].

$$\Delta G = -RT \ln K \quad (3)$$

Here, T is the absolute Kelvin temperature, and R is the gas constant, which is equivalent to  $8.314 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}$ . Table 3 shows that the negative values from (among the azo dyes and metal ions understudy) are spontaneous.

#### 4.8. Magnetic Measurements and Molar Conductance

The formula for calculating effective magnetic moments was  $\mu_{\text{eff}} = 2.828 (X \text{ M.T})^{1/2}$  B. M.



**Table 2:** (a) XRD data for 8-HDQ and its 8-HDQ-Pb (II) complexes.

2θ°	d (Å)	FWHM	Intensity	D (nm)	2θ°	d (Å)	FWHM	Intensity	D (nm)
Ligand					Complex				
27.11	3.286	0.17	14.7		19.19	4.620	0.14	5.4	
31.41	2.845	0.274	101.4		24.9	3.58	6.0	110	
45.16	2.006	0.321	51.4	33.26	31.38	2.849	0.335	26.8	29.53
56.27	1.633	0.34	12.0		45.13	2.007	0.33	12.5	
75.0	1.265	0.29	10.9		75.17	1.262	0.31	2.1	
83.82	1.153	0.43	9.0		83.80	1.1534	0.39	4.6	

**(b) XRD data for p-chloro 8-HDQ and its 8-HDQ-Pb (II) complexes**

2θ°	d (Å)	FWHM	Intensity	D (nm)	2θ°	d (Å)	FWHM	Intensity	D (nm)
Ligand					Complex				
26.903	3.3114	0.52	53		26.83	3.320	0.64	11	
31.561	2.8325	0.319	70		31.972	2.7970	0.090	9.1	
33.56	2.669	0.65	19		52.571	1.7394	0.05	0.9	
36.587	2.4541	0.12	6.8	37.02	53.063	1.7245	0.08	0.8	41.59
45.295	2.0005	0.189	52.8		54.04	1.6956	0.05	0.5	54.04
56.27	1.6335	0.31	12.2		67.189	1.3922	0.118	5.6	
66.13	1.4119	0.20	5.6		75.068	1.2643	0.152	10.8	
83.78	1.1537	0.27	4.7		73.023	1.2946	0.03	0.3	
					74.198	1.2770	0.03	0.3	
					81.029	1.1857	0.13	2.8	
					84.771	1.1426	0.11	0.9	
					87.411	1.1148	0.07	0.7	

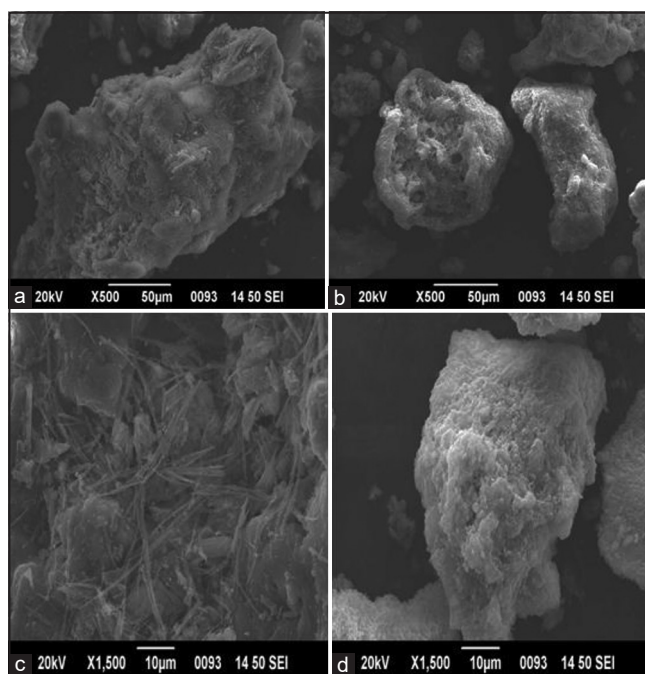
**Table 3:** The stability constant of the prepared complex.

Complexes	AS	Am	α	K	ln k	ΔG kJ.mol <sup>-1</sup>
o-8-HDQ-Pb (II)	0.650	1.528	0.574	1.4×10 <sup>6</sup>	16.45	-41.180
p-8-HDQ-Pb (II)	0.744	1.655	0.553	1.6×10 <sup>6</sup>	16.61	-41.589

**Table 4:** Inhibition zones for 8-HDQ azo dye and Pb (II) complexes.

Bacteria	Inhibition zone [mm]			
	8-HDQ Azo Dye		Pb (II)-8-HDQ Azo Dye	
	Ortho	Para	Ortho	Para
<i>Escherichia coli</i>	5	7	NZ	NZ
<i>Staphylococcus aureus</i>	12	11	9	6
<i>Salmonella typhi</i>	10	NZ	14	20

Where T is the absolute temperature and X M is the molar susceptibility corrected using Pascal's constants for the diamagnetism of all the atoms in the chelates. Lead(II) complexes of the 8-HDQ are found to be diamagnetic based on the experimental values of magnetic measurements. This suggests that Pb(II) in these complexes has the d<sup>10</sup> electronic configuration. The molar conductance of the metal complexes was measured for 10<sup>-3</sup> M solutions in ethanol at room temperature. The molar conductance values of both ortho and para metal complexes are approximately 6.3 and 5.2 Ω<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>, respectively. The complexes under



**Figure 6:** SEM images (a) 4-(8-hydroxyquinolin-5-azo)-chloroaniline (b) 4-(8-hydroxyquinolin-5-azo)-chloroaniline-Pb(II) complex and (c) 2-(8-hydroxyquinolin-5-azo)-chloroaniline (d) 2-(8-hydroxyquinolin-5-azo)-chloroaniline-Pb(II) complex.

investigation are non-electrolytic, as indicated by the low molar conductance values [26].

#### 4.9. Antimicrobial Studies

The outcomes of the 8-HDQ and Pb(II) complexes' antibacterial activities display in Table 4. It shows that 8-HDQ and its Pb(II) complex have significant antibacterial activity against tested strain, with zones of inhibition ranging from 5 to 20 mm. The antibacterial activity of 8-HDQ complexes is currently being studied, but the compound has long been recognized as an antimicrobial agent. Pathogenic bacteria such as *E. coli*, *Salmonella typhi*, and *S. aureus* were used to investigate the antimicrobial activity of the ligand and its complex at concentrations of 0.01 mg/mL.

#### 5. CONCLUSION

We have successfully synthesized a complex of HQ azo dye with Pb(II). The data obtained indicate that Pb(II) metal ions forms a complex of square-planar shape with 8-HDQ azo dye. After investigation, the stoichiometry of metal ions and ligands was found to be 1:2. The complex that forms between Pb (II) and the azo dye of 8-hydroxy quinoline has a higher stability constant, indicating that it is stable. The magnetic susceptibility demonstrates the synthesized complexes are diamagnetic in nature. There is significant antimicrobial activity against pathogenic bacteria in this complex.

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