Biological activity and latent fingerprints detection by azo quinoline dye and its complexes

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ABSTRACT

Azo dye of quinoline named 5-(3-pyridylazo)-8-hydroxyquinoline dye (PAHQ) was prepared via coupling reaction of diazonium salt of 3-aminopyridine and 8-hydroxyquinoline. The PAHQ dye was characterized by elemental analysis, mass spectroscopy and ¹H-NMR. The complexes of PAHQ dye were prepared under 2:1 (L:M) mole ratio. The complexes were characterized by elemental analysis, infrared spectroscopy, UV-Vis, molar conductance, magnetic susceptibility. The IR spectra data indicate that PAHQ ligand is bidentate through deprotonated phenolic oxygen and nitrogen atom of azomethine of quinoline. The IR spectra also indicate to presence of water in coordination state to metals. The PAHQ and its complexes were found to have high biological activity against different organisms of bacteria and fungi. The PAHQ ligand and zinc complex exhibited high toxicity against MCF-7 with high different values between IC₅₀ against infection cells and healthy cells.

Keywords: Azo dye, quinoline, photochromic, fingerprint

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1. Introduction

Coordination compounds are chemical compounds consisting of a central atom (a positively charged metal ion or a neutral metal atom) surrounded by a group of ions or neutral molecules (ligands) forming the coordination sphere, which may be found outside it negative or positive ions to equalize the charge and these compounds retain their identity in solutions [1-3]. Azo dyes and their complexes have a wide range of uses, including pigments, inks, leather, and food colourings, as well as biological action against bacteria, fungi, and cancer cells [4-6]. The most common type of organic dyes is azo dye, garnering the interest of academics due to their wide range of uses in a variety of fields [7-11]. The photo-switching of azo dye and sometimes their complexes [12, 13] serves large promising applications in many areas [14], like the design of optical recording systems of azo dyes which can be governed by converting stable E (trans) to Z (cis) isomeric and the convert process involves a change in dye geometry and polarity which can be managed by irradiation at different wavelengths [15-17]. Fingerprints are a crucial form of evidence for determining a person's identity. Fingerprints are one of the most important biometric features for personal identification in the forensic science field because of their uniqueness and long-term accuracy. In the crime scenes, latent fingerprints are normal [18, 19]. When a finger touches a surface, it leaves a stamping in the form of ridges (raised) and furrows (recessed). At the criterion, most fingerprints are unrealizable to the eye. In order to distinguish origins of fingerprints in habit forensic practice, they must be visualized in the right way [20-22]. Azo dyes are used to determine the fingerprint, such as Oil red O (lysochrome), which used in fingerprint disclosure. Sudan black is a popular lysochrome dye that is particularly helpful to disclosure the printing on waxy paper or wet surfaces [23]. Therefore, we are interested in preparation a ligand from quinoline and aminopyridine and some complexes for it and study their biological activity.
2. Experimental part

The salts, 8-hydroxyquinoline, sodium nitrite, 3-aminopyridine were got from Sigma-Aldrich. The infrared, UV-Vis and H-NMR spectroscopies were done by Shimadzu FT-IR 8400S Spectrophotometer by using KBr disk, UV-1650 Spectrophotometer and Bruker 400 MHz Spectrophotometer respectively. Microanalyses of C.H.N. of PAHQ dye and its complexes were done by elemental analyser. Hanna conductometer was used to measure the molar conductivity of prepared complexes of PAHQ dye. Melting point of PAHQ dye and its complexes were done Digital Melting Point-SMP3. Mass spectra were done by Shimadzu LCMS 2010. Magnetic susceptibility of the complexes of PAHQ ligand were done by magnetic susceptibility balance.

Preparation 5-(3-pyridylazo)-8-hydroxyquinoline dye (PAHQ): 0.5 g (0.0053 mole) of 3-aminopyridine dissolved in 20 mL of water then 4 mL of HCl was added to it. The solution was put under cooling, then mixed with 10 mL aqueous solution of NaNO2 (0.37 g, 0.0053 mole) to form diazonium salt of pyridine. The diazoinium salt mixed with 15 mL of ethanolic solution of (0.77 g, 0.0053 mole of 8-hydroxyquinoline with 0.8 g of sodium hydroxide) to form the azo dye. After one hour, the mixture solution of dye was filtrated and washed with water then it put in oven to dry at 50 °C, yield 90, mp= 221 °C.

Preparation of PAHQ complexes: 0.021 g (0.00039 mole) of KOH was dissolved in 3 mL water then it was added to 0.1 g (0.00039 mole) of PAHQ ligand dissolving in 10 mL of ethanol. The hot solution of PAHQ ligand was added to 5 mL aqueous solution of metal salt 0.00019 mole (CoCl2.6H2O (0.045 g), CuCl2.2H2O (0.032 g), ZnCl2 (0.0258 g), CdCl2 (0.034 g)). The mixture solution was refluxed for 30 minute then the solution was left overnight. The solution was filtrated then the precipitate was washed with little water then it was put in oven at 50 °C to dry. The anticancer activity of PAHQ ligand and its complex of zinc against MCF-7 cell line of breast cancer was done by MTT assay. The biological activity was done by diffusion method.

3. Collection and identification of fingerprints

Fingerprints were left on slide glass. Following that, fingerprint powders were applied to these substrates with caution. Excessive powders were rubbed away with a brush with care. We took a small amount of dye and its complexes with grind well and dry it. Then we placed it on the surface to show the fingerprinted, spread the material with a special brush in one direction. Then the surface was cleaned from the excess amount of the material with another special brush. The results are recorded by using FX10AC and CRIMESCOPE –UV (CS-16-500) (USA) then photographed with Samsung galaxy s21[24].

4. Results and discussion

The azo dye of 8-hydroxy quinoline was formed by reacting daizonium salt of 3-aminopyridine with 8-hydroxy quinoline in basic medium as shown in the (Scheme 1) which namely 5-(3-pyridylazo)-8-hydroxyquinoline (PAHQ dye) in super yield 90%. The mass spectrum of PAHQ dye exhibited a signal for the molecular weight at 250.1 with high abundance as shown in Figure 1. The H-NMR spectrum of PAHQ dye as shown in Figure 2, shows nine signals as the following in agreement with the number of hydrogen in the dye: 9.41 (1H), 9.20(1H), 9.00(1H), 8.74 (1H), 8.68 (d, 2H), 8.59 (d, 2H), 8.22 (d, 4H), 7.81 (d, 2H), 7.55 (d, 2H), 6.89 (d, 2H) and 2.0 (s, 4H). The broad signal of OH of quinoline does not appear in the spectrum of zinc complex that means that the proton of hydroxyl of quinoline was deprotonated. Another notably notice, there is sharp singlet at 2.0 ppm equal to 4 protons which refers to coordinate the water to zinc metal in zinc complex.

![Scheme 1 Preparation steps of PAHQ dye](image-url)
Figure 1. Mass spectrum of PAHQ dye

Figure 2. H-NMR spectrum of PAHQ dye in DMSO d6
The UV-VIS spectra of PAHQ dye (Figure 4) have undergone dramatic changes under pH from 2 to 13. In basic medium, the dye was red with large red shift in absorption. In neutral medium, the dye was orange while in acidic medium, it was yellow with blue shift contrast to absorption in neutral medium.
Under irradiation at 395 nm for five minutes, the PAHQ dye has undergone high increasing in absorption of n-$\pi^*$ as shown in Figure 5 accompanied with decreasing in absorption of $\pi$- $\pi^*$ in agreement with isomerization behaviour of azo dyes, with full back to initial spectrum when we removed the source of light which is in agreement with real photochromic behavior [25-29].

![Figure 5. UV-Vis spectra of PAHQ azo dye in DMSO under irradiation (red line) at 395 nm and before irradiation (black line)](image)

The cobalt (II), copper (II), zinc (II) and cadmium (II) complexes of PAHQ ligand were formed by reacting PAHQ ligand with these salts (Scheme 2) under mole ratio 2:1 (Ligand: Metal like in Figure 6 for cobalt complex at 485 nm. The physical properties and elemental analysis data of the complexes are summarized in the Table 1. The molar conductivity data indicate that the complexes are non-ionic at 10^{-3} molar at room temperature in DMF solvent.

![Scheme 2. Preparation steps of PAHQ complexes, M(II) = Co, Cu, Zn and Cd](image)

![Figure 6. Mole ratio of PAHQ ligand with cobalt salt at 485 nm](image)
Table 1. Elemental analysis and physical properties of PAHQ and its complexes

| Compounds      | C exp, (theo) | H exp, (theo) | N exp, (theo) | color       | M.p | yield | Molar conductance Scm$^2$.mol$^{-1}$ |
|----------------|---------------|---------------|---------------|-------------|-----|-------|-------------------------------------|
| PAHQ ligand    | 67.01 (67.19) | 3.99 (4.03)   | 22.27 (22.39) | yellowish brown | 221 | 90    | -                                   |
| Co complex     | 56.25 (56.57) | 3.65 (3.74)   | 18.73 (18.88) | red         | >350 | 70    | 5                                   |
| Cu complex     | 56.05 (56.23) | 3.68 (3.71)   | 18.65 (18.74) | maroon      | >350 | 60    | 15                                  |
| Zn Complex     | 56.01 (56.06) | 3.62 (3.70)   | 18.67 (18.68) | dark red    | 350 | 75    | 10                                  |
| Cd complex     | 51.86 (51.98) | 3.38 (3.43)   | 17.25 (17.32) | Dark brown  | 235.5 | 80 | 5                                   |

4.1. Infrared spectra

The infrared data of PAHQ dye and its complexes are depicted in Table 2. The spectrum of PAHQ dye (Figure 7) indicated to important functions such as sharp peak for O-H, C=N of quinoline and pyridine ring, azo group. The spectra of complexes exhibited large and strong peak at 3356-3395 cm$^{-1}$ with new peaks at 835-840 and 732-750 cm$^{-1}$ which are attributed to stretching, rocking and wagging vibrations of OH of water in coordination state to the metals [30-32]. The C=N group in quinoline ring of dye showed in the spectra of complexes under low frequency which indicate to coordinate it to the metal.

Table 2. Important frequencies in cm$^{-1}$ of PAHQ ligand and its complexes

| Compounds      | OH    | CH    | C=N  | C=N  | C=C  | N=N  | (OH) of H$_2$O |
|----------------|-------|-------|------|------|------|------|----------------|
| PAHQ ligand    | 3377  | 3033  | 1649 | 1570 | 1548 | 1506 | -              |
| Co-complex     | 3369  | 3033  | 1637 | 1579 | 1498 | 1464 | 750,835        |
| Cu-complex     | 3356  | 3065  | 1597 | 1579 | 1558 | 1498 | 744,839        |
| Zn-complex     | 3373  | 3072  | 1603 | 1577 | 1553 | 1502 | 736,840        |
| Cd-complex     | 3381  | 3061  | 1597 | 1566 | 1552 | 1502 | 734, 837       |
4.2. Electronic spectra of PAHQ dye and its complexes and magnetic properties data

The PAHQ ligand exhibited a large band at 520 nm due to intraligand transition\cite{33}. The PAHQ complexes of zinc and cadmium exhibited strong bands at 480 nm and 490 nm respectively as shown in (Figure 8) due to charge transfer transition (type metal to ligand) which is an allowed transition. The copper complex exhibited a broad band at 640 nm with low intensity due to d-d transition presenting $^2\text{E}_g \rightarrow ^2\text{T}_{2g}$, in agreement with octahedral environment of copper complexes \cite{31} with magnetic susceptibility equal to 1.92 B.M. The cobalt complex of PAHQ exhibited a band at 485 nm due to intraligand transition which is in the visible region. Therefore, the d to d transitions in this complex are obscured by this band \cite{34} with magnetic susceptibility equal to 3.87 B.M.
PAHQ and its complexes showed high effectiveness in showing the invisible fingerprint on smooth surfaces such as glass and paper, as it showed positive results through the appearance of fingerprints clearly and with high stability. In cooperation with the Forensic Evidence Directorate in Babylon, and the assistance of experts in the criminal edition therein, by examining the powder used to show the print of the fingers hidden in the crime scene and providing a standard model for a fingerprint that was shown by the black powder. Model for smooth surfaces. Crimescope (uv) (cs-16-500) was used to read the print and its clarity using different spectral frequencies in the device, as it gave results in the appearance of the print at wavelengths between (490-515 nm). Displayed by dye and some of their complexes and compared with standard editions shown by the black powder used by crime scene experts for every material separately, where the results were found to be well positive.

![Image](image1)

**Figure 9.** Detection of fingerprints by PAHQ ligand and its metal complexes of cobalt and zinc on smooth surface of glass

![Image](image2)

**Figure 10.** Comparison of fingerprints shown by PAHQ ligand and its metal complexes of zinc and cobalt with fingerprints shown by standard material (black ink)

### 4.3. Biological activity

Azo dyes, especially those containing heterocycle rings and their complexes are effective against types of fungi, bacteria and cancer. They also use in treatment the cancer by photodynamic therapy because they absorption in the visible to the red area. Quinoline derivatives, aminopyridine, pyridines and their complexes are effective against many diseases. Therefore, we linked these materials by azo dye, then we tested biological efficacy of this dye (PAHQ) and its complexes against types of human pathogen, *Staph. Aureus* and *E. coli*, and *Aspergillus Niger*. The cobalt, copper, cadmium complexes of the PAHQ dye exhibited inhibition zone (3.5 - 4 cm) against
E. Coli. The PAHQ dye and zinc complex did not show biological activity against E. Coli bacteria (Table 3). The PAHQ dye and cobalt complex exhibited inhibition zone (1.2-2 cm) against Staph. Aureus bacteria. The PAHQ dye and its complexes did not show activity at high concentration (100 mg/mL).

The PAHQ dye and its complexes exhibited attractive results against Aspergillus Niger (1.2-9.5 cm) (Table 4). The PAHQ dye and zinc complex exhibited the highest inhibition zone (9.5 cm). The PAHQ dye and the complexes showed biological activity against Aspergillus Niger in both concentrations but the results were high at high concentration (100mg/mL).

| Bacteria         | Concentration mg/ml | The diameter inhibition zone (cm) |
|------------------|---------------------|-----------------------------------|
|                  | PAHQ                | Co      | Cu      | Zn      | Cd      |
| E.coli Gram-negative | 100                 | -       | -       | -       | -       |
| Staph. Aureus Gram-positive | 100                 | 4       | 3.5     | -       | 4       |
|                   | 75                  | 2       | 1.2     | -       | -       |

| Fungi            | Concentration mg/ml | The diameter inhibition zone (cm) |
|------------------|---------------------|-----------------------------------|
|                  | PAHQ                | Co      | Cu      | Zn      | Cd      |
| Aspergillus Niger | 100                 | 9.5     | 3.2     | 4       | 9.5     | 3.2     |
|                  | 75                  | 6       | 1.5     | 2       | 3.2     | 2.5     |

Figure 11. Biological activity of cobalt complex against Aspergillus Niger

4.4. The cytotoxicity and vitality assay (MTT) of the PAHQ ligand and its zinc complex

The female breast cancer cell line MCF-7 and normal, uninfected female breast cells (WRL-68) were exposed to concentrations ranging from 12.5-400 µg/mL to PAHQ ligand and its zinc complex for 24 hours at a temperature of 37 °C. Also, the toxic effect of PAHQ ligand and its zinc complex was evaluated based on the percentages of the inhibition rate of growth compared to the infected untreated normal-growth tumor cells. The results of cytotoxicity were included in Table 5 for the ligand and Table 6 for the zinc complex. The least inhibition of the growth of MCF-7 cancer cells when exposed to PAHQ ligand and zinc complex was at a concentration of 12.5 µg/ml, and the highest inhibition of PAHQ ligand and zinc complex for MCF-7 cancer cells was at concentration of 400 µg/ml. Also from Figures (12 and 13) we notice that the half inhibitory concentration of the PAHQ ligand IC50 with normal breast cells is 133.6, which means we need a high concentration of PAHQ ligand to kill half of the healthy cells and a much lower concentration of the same ligand
to kill half of the diseased cells. IC\textsubscript{50} of the zinc complex shows that the required concentration is 132.4 with the cell line MCF-7. We also note that the half inhibitory concentration of zinc complex IC\textsubscript{50} with normal breast cells is 387.2, which means we need a very high concentration of zinc complex to kill half of the healthy cells and a very much lower concentration of the same complex to kill half of the diseased cells.

Table 5. The effect of the PAHQ ligand on the cells of the breast cancer cell line MCF-7 using the MTT test

| Concentration | Mean | SD  | Mean | SD  |
|---------------|------|-----|------|-----|
| 400.00        | 46.10| 4.22| 64.97| 5.29|
| 200.00        | 56.64| 4.71| 73.30| 1.24|
| 100.00        | 66.98| 3.89| 83.10| 2.47|
| 50.00         | 79.09| 9.15| 94.79| 1.01|
| 25.00         | 91.82| 2.99| 94.29| 0.44|
| 12.50         | 94.75| 2.23| 95.64| 3.84|

Table 6. The effect of the zinc complex of PAHQ ligand on the MCF-7 using the MTT test

| Concentration | Mean | SD  | Mean | SD  |
|---------------|------|-----|------|-----|
| 400.00        | 50.19| 3.19| 73.28| 5.76|
| 200.00        | 65.32| 1.14| 82.91| 0.87|
| 100.00        | 70.87| 1.19| 90.28| 1.61|
| 50.00         | 88.39| 2.30| 93.79| 1.00|
| 25.00         | 95.22| 0.41| 94.25| 0.48|
| 12.50         | 95.33| 0.79| 96.64| 0.70|

Figure 12. The relationship between the biological activity of MCF-7 cells and normal WRL cells with the concentration of the PAHQ ligand
Figure 13. The relationship between the biological activity of MCF-7 cells and normal WRL cells with the concentration of the zinc complex of PHAQ ligand.

5. Conclusion

Azo dye of quinoline (PAHQ) and its complexes of Co(II), Cu(II), Zn(II) and Cd(II) were prepared characterized. The PAHQ ligand exhibited important photochromic properties. The PAHQ ligand is bidentate ligand depending on the IR data. The complexes have general formula \([M(PAHQ)_2(H_2O)_2]\) and nonionic with octahedral geometry. The PAHQ dye and the complexes showed high biological activity against types of bacteria and fungi. The PAHQ dye and zinc complex exhibited important cytotoxicity against breast cancer but the zinc complex shown better results.

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