Preparing and diagnosing the biological activity of some metallic complexes with ligand 4-Benzophenol Azopyrogallol(4)

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Abstract

Conducted a study of preparation, diagnosis and study of biological activity of Ligand 4(4-Benzophenol Azopyrogallol) The two-tooth containing the two groups of nitrogen with two similar rings, and the goal of the research is to prepare the ligand above, and after that to prepare the azo-ligand complexes above with the salts of the metal ions selected with a positive charge at fixed rates Sr (II), Pb (II), Cd (II), and these compounds were diagnosed with FTIR, UV and XRD, and the fungal activity of ligand and its complexes were diagnosed for staph, E-coli and fungus species. Aspergillus, and some complexes have shown to have antifungal and bacterial efficacy.

Key words: Ligand 4 (4-Benzophenol Azopyrogallol), Biological activity, Metal complexes

1. Introduction.

Azo compounds. Previous studies showed that\textsuperscript{1,2,3} azo compounds have a fast reaction rate and high stability with most elements of the periodic table, especially the transition ions, so this type of ligand attracted the interest of researchers\textsuperscript{4,5,6}. Because it has various advantages such as color trait and high molecular weights, and this has led to its spread in various fields of chemistry, as well as the formation of complexes is not limited to the transition elements, as it is, but also includes the elements represented\textsuperscript{7}, where metal ions from the represented elements can participate in the formation of complexes. As a result of the association of these ions with ions or organic or inorganic molecules called ligands, Among the organic compounds that have been widely used as reagents are compounds or azo dyes and their derivatives, because they are characterized by high sensitivity, maximum selectivity, and the speed of their interaction with metal ions to form complexes, as well as their high stability. The degree of stability is also affected by the types of groups associated with the two sides of the azo group, which may be aromatic or aliphatic\textsuperscript{8}. The following is the structural picture of the ligand 4(4-Benzophenol Azopyrogallol) containing the active group\((-\text{N = N-})\)
Azo dyes can be obtained easily and at a low cost by using diazonium and duplication or conjugation reactions, where the first two steps are prepared to form the diazonium salt of primary aromatic amines rapidly react with nitrous acid (HNO$_2$) prepared simultaneously from the reaction of sodium nitrite (NaNO$_2$) with concentrated hydrochloric acid (HCl) in degrees Low temperature ranging from (0-5) m due to the instability of the diazonium salt at high temperatures. The second step is the process of duplicating the diazonium salt resulting from the first step with the aromatic compound. The two steps can be illustrated by a simple chemical equation 

2. Biological activity

Fungi are widespread organisms that existed long ago in the same environment in which humans lived since ancient times. Fungi are distinguished by being eukaryotic organisms of different nutrition and have different shapes and sizes, including those that are unicellular, such as yeasts, some of which are filamentous, including fleshy, multicellular, and fungi live in all environments. And we find that there are residual fungi in the soil and other parasitic fungi that live on the bodies of living organisms and some of them live in the aquatic environment and are called aquatic fungi. There are many types of fungi, including the type Aspergillus, the opportunistic pathogen fungus pathogenic Opportunistic and causing Aspergillus, which is one of the most types of infections Common fungal.

Bacteria are single-celled micro-organisms that have a closed nucleus membrane or closed membrane organelles, such as mitochondrial green plastids, and have the ability to live in a variety of environments. Bacteria are usually found in soil and water, as well as on living organisms represented by humans, animals and plants.

The cell walls of all types of bacteria are mismatched, so the formation of the cell wall is one of the most important factors in the analysis and differentiation between bacterial species where there are two types of bacteria are positive bacteria and it consists of a thick layer of peptidoglycan connected by amino acid bridges. And peptidoglycan negative bacteria.

In this study, two types of bacteria were used: Escherichia Coli and Staphylococcus aureus, in addition to the fungus Aspergillus

3. practical part. Materials used. Distilled water, ethanol solvent (Haymankimian), p-amenobenzoic (COD), NaNO$_2$ sodium hydroxide, hydrochloric acid, pyrogallol, salts of some binary and tripartite elements

a. Preparation of ligand 4(4-Benzophenol Azopyrogallol) An amount of (p-amenobenzoic) 1.37 g is placed with a quantity of 5ml Hcl and 0.69g of NaNo2 with distilled water 40 m and mixed with each other and placed in Beaker with continuous stirring by stirring and the solution is placed in an ice bath on the hot plate device while maintaining that The temperature does not exceed 5 m for a period of 30 minutes, during this time we prepare a
cold NaOH sodium hydroxide solution (2.81 g) with 50 mL distilled water in a 50 mL volumetric bottle, and after the expiry of the prescribed time, the pyrogallol 1.26 g is placed by dissolving it with a solution of NaOH. The coolant is also applied to the remaining hydroxide solution, and by this, the aforementioned ligand has been prepared. In order to obtain the different absorbance's of the solutions (metal + liquid), the \((\lambda_{\text{max}})\) must be obtained by measuring in a UV .visible device, and this is done by mixing a weight of ligand at a concentration of \((1*10^{-4})\) and dissolving it with a solvent with ethanol in a volume of 10 ml with the weight of the metal at the same concentration. And dissolve it with distilled water in a volume of 10 ml, and for measurement we mix 2.5ml of liquor and also metal 2.5ml and continue to the mark 10ml in a tipe of 10 ml.

The highest absorbance of \(\text{PbCl}_2\) complex was = 0.054, while its wavelength was = 444 nm, and the highest absorbance was for \(\text{SrCl}_2\) complex = 0.123 and its wavelength was = 416 nm, and the highest absorbance of \(\text{CdCl}_2\) complex was = 0.121 and its wavelength = 427 nm .

Through \((\lambda_{\text{max}})\) that we obtained from the UV. visible device, we will obtain the absorbance's of all complexes by mixing 1ml of ligand with different proportions of metals starting from 0.25ml to 3ml) and then we complete the mark with 10ml ethanol solvent in a 10ml tipe. The following are the calibration curve (Figure 1) showing the different absorbance's of each complex against the concentrations.

![Figure 1: 1 shows absorbance versus concentration of lead complex](image1)

![Figure 1: 2 shows the absorbance versus concentration of the strontium complex](image2)
b. Preparation of solid ligand complexes. Each metal Sr (II), Pb (II), Cd (II), is weighed at \((1*10^{-2})\) and it is dissolved in the appropriate pH solution for each metal, while the liquor also takes the same concentration and is dissolved with ethanol solvent, and the two solutions are mixed with each other in a beaker and placed on a hot plate with a temperature ranging from (60-70) Celsius with continuous stirring by stirring And it continues until boiling, after which it is filtered and purified with distilled water, dried and preserved to be ready for the method of work of biological activity. 13,14,15,16,17.

c. Preparation of culture media. The culture medium (Muller Hinton Agar) was prepared according to the instructions of the Indian Laboratories Biomark by dissolving 38 g of the culture medium in 1000 milliliters of boiled distilled water in a glass flask and mixed well, to dissolve The culture medium was completely followed by placing the culture medium in the Autoclave device at a temperature of 121 and under pressure of 15 pounds per inch for 15 minutes, then pouring the medium into sterile glass dishes (dish Petri) at a rate of (15-20) milliliters per dish and left until the solidification is complete, then the dishes were placed in the incubator for 24 hours at a temperature of (37) degrees Celsius to make sure that there was no contamination in them.

d. Preparation of solutions. Ligand solutions and their complexes were prepared under study by dissolving 0.1 gram of each metal in \(C_2H_5OH\) ethanol solvent in a volume of 5mL. At a concentration of \((1*10^{-3})\) for both metal and ligand

4. Processing Method. The bacteria were spread once and the fungus again in the dishes and on the surface of the food medium (Mueller Hinton agar) using (Loopful), as well as making three holes with a diameter of \((6 \text{ mm})\) in these dishes by means of the cork-borer sterilized with alcohol, taking into account leaving an appropriate distance between one hole and another to avoid the inhibition areas overlapping between them.

The prepared solutions were added to these pits \((0.1 \text{ ml})\) using (Micropipette) and placed in the incubator for 24 hours at a temperature of 37 °C for bacteria, and for a whole week for the fungus, after which the inhibition amount was measured. (Inhibition Zone) for vehicles using a mm ruler

5. Results and discussion. X-Ray Diffraction (XRD). The crystalline structures of ligand in its solid state were studied using X-ray diffraction and the spectra were shown in Fig. (2) to
know some of its structural properties such as the crystal structure, crystal size and its purity can be estimated.

There are Ligand diffraction peaks that are exhibited for several things such as micro-strains, lack of lattice deformation, faulting due to deformations of the crystal, domain size of the crystal and field size distribution.

Figure (2) shows the measurement of X-Ray Diffraction (XRD) of ligand 4 (4-Benzophenol Azopyrogallol), the intensity of the (y) axis versus the degree (X) axis. The first peak was at $2\Theta = 26.5855$, the second peak was at $2\Theta = 21.4588 = 2\Theta$, the third peak was at $2\Theta = 29.4440$ and the forth peak was at $2\Theta = 19.6638$, and it was found that the second peak is the highest compared to the rest of the peaks, which indicates the presence of crystal levels and composition Crystalline with high homogeneity, while low peaks in X-ray spectroscopy indicate less homogeneous crystalline structures.

Figure (2) shows the XRD measurement of Ligand a. Infrared Spectra. The beam locations were diagnosed in the spectra of the metal complexes based on what was available in the literature about the four peaks of the ligand and the complexes, which are the bridging azo group (-N = N-), the position of the OH link, the double pinion (C = C) and the linkage (C = H) The aromatic ring, and it is noticed in Figures (3-2,3-3,3-4) that the beams in the spectra of the metallic complexes have suffered from changes in intensity and position when compared with bands of ligand Free 4(4-Benzophenol Azopyrogallol) and shown in Figure (3-1). In addition, small or large displacements occur for most of these packages. These changes are evidence of the occurrence of symmetry and the formation of coordination complexes.

In the spectrum region confined between ((1600 - 3074 cm$^{-1}$)), the infrared spectrum of the azo compound ($L_1$), shown in Figure (3-1), showed a medium-intensity beam at the frequency (1440 cm$^{-1}$) of the(-N = N-) link, while the A bundle of (-C = C-) double bond at the frequency 1600 cm$^{-1}$ and a beam attributed to the OH bond)) of the hydroxyl group in the ligand at the frequency (3074 cm$^{-1}$), while this beam disappears in the spectra of the ligand metal complexes, indicating the occurrence of interaction by a group Phenolic (OH) after losing its proton, while two weak absorption bundles of the aromatic CH bond appear at the frequency ((3050 cm$^{-1}$) and another beam at the frequency (2885 cm$^{-1}$)), and these beams are stable in both sites of the free ligand and the prepared metal complexes.
While the spectrum region confined between (600-1681 cm$^{-1}$) is very important in the infrared spectrum when interpreting metallic complexes, as it includes most of the absorption beams belonging to the active groups in each of the ligand spectra and its complexes, including the groups (-N = N-), ( -C = C-) In addition to the vibrations belonging to the (metal-nitrogen) link, the spectra of the metal complexes showed new frequencies that differ from the free-ligand spectrum and the frequencies are (616-694 cm$^{-1}$), so the infrared spectrum indicates that the ligand behaves The triple-tooth ligand by means of the nitrogen atom of the ring and the nitrogen atom of the bridge azo group in addition to the oxygen atom of the phenolic hydroxyl group to give two rings that contribute to increasing the stability of the coordination complexes.

![Figure (3-1) the infrared spectrum of free ligand 4 (4-Benzopheno Azopyrogallol)](image-url)
Figure (3-2) complex infrared spectrum $[\text{Sr(L}_1\text{))]\text{Cl}_2$

Figure (3-3) a complex infrared spectrum $[\text{Pb(L}_1\text{))]\text{Cl}_2$

Figure (3-4) a complex infrared spectrum $[\text{Cd(L}_1\text{))]\text{Cl}_2$

b. Measurements of biological activity. The ligand solutions and complexes were prepared at a concentration of $1\times10^{-4}$ using an ethanol solvent, and their effect on two types of bacteria were positive and negative for the cream stain and one type of fungus, Aspergillus, and the amount of inhibition of ligand and complexes was measured with the ruler and table (1). It shows the amount of inhibition of ligand and complexes.

Table (1). shows the amount of inhibition of ligand and complexes

| Ligand with complex | Staph aureus Bacteria | E. Coli bacteria | Aspergillus fungus |
|---------------------|-----------------------|------------------|--------------------|
| $\text{L}_1$        | ++                    | +++              | _                  |
| SrCl$_2$L$_1$       | ++                    | ++               | _                  |
| PbCl$_2$L$_1$       | ++                    | +++              | _                  |
| CdCl$_2$L$_1$       | ++                    | ++               | _                  |

Notes (-)=no inhibition , (+)=inhibition
We note through the previous table containing the results obtained from the effect of the solutions on the two types of bacteria and fungi, it was found that all the solutions that gave (+) had a weak effect on the two types of bacteria, while the ones that gave (++) showed higher resistance on both types. Bacteria and this is due to the activity groups (-N = N-), (-C = C-) and (OH) that the ligand has with the complexes, which give stronger inhibition activity, and the inhibition ranged between mm (3.5 - 4.5) and this is also due to factors Hereditary and synthetic bacteria. On the contrary, with the fungus used, it showed resistance to solutions of complexes and ligand due to its potency and its possession of special genetic factors.

6. Conclusions. The results were for the ligand complexes of Staph and E. coli, with a diameter of 3.5 cm for each complex by measuring the plate containing the solution, and they all showed that the complexes resisted the two types of negative and positive bacteria.

a. The results showed in the measurements of the fungus its resistance to some complexes and the resistance of some of them to the fungus.

b. These rules can be used in the life sciences and pharmaceutical chemistry.
*Pictures of figures (7,8,9,10) show the results of the type of mushroom used with Ligand and its complexes

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