Preparation, Characterization and Prevention Biological pollution of 4 (4-Benzophenylazo) Pyrogallol and their Metal Complexes

ZAINAB A. ABID ALRADAA AND ZEINA M. KADAM*

*Department of chemistry, college of science, University of Al-Qadisiyah, Diwaniyah, Iraq

Email: dr.zeinakadam@gmail.com

Abstract

New azo reagent was synthesized by reaction dizonium salt of Para aminobenzoic acid with Pyrogallol, also prepared three Chelate complexes of this reagent with the metal ions of Co(II),Ni(II), and Ag(I) .This reagent was characterized by FTIR(Fourier Transform Infrared) , UV.Visible spectrophotometer,FESEM and HNMR proton. The antibacterial activities of the compounds were studied and evaluated using gram positive and negative gram bacteria. The purity of the dye was checked by thin layer chromatography (TLC) using solvent system (Toluene-Methanol) (2:3).The melting point of the purified dye was measured in an open capillary tube. The results showed that some of these compounds have high levels of antibacterial activity.

Introduction

Azo compounds are a class of chemical compounds that are always getting awareness in logical study. They are typically powerfully colored compounds which are able to extremely yellow, red, orange, blue or even green, depending on the correct arrangement of the molecule. As a result of their colour, The compounds of azo structures are wonderful position as dyes and also as pigments for a while. In statement, almost high ratio of the dyes in industrial use today are azo dyes compounds, which are usually prepared from diazonium salts. The structural types in organic compounds, that naturally yield colour are > C = C <, –N = O, –N=N–, aromatic rings, > C = O and –NO₂. Most significantly, the compounds that always award colour are the azo (–N=N–)and nitroso (–N=O) while the other groups essentially do so under certain conditions. In addition, azo dyes have been studied mostly because of their exceptional thermal and optical characteristics in different applications such as optical recording medium, toner, inkjet printing, and oil-soluble light firm dyes. lately, azo structures as organic dyes have also paying care due to their attractive electronic features and application for molecular memory storage, nonlinear optical elements and organic photoconductors. Dyes make our world attractive, however they carry pollution. Wastewaters originating from dyes manufacture and application industries pose a major risk to surrounding ecosystems, because of their toxicity and potentially carcinogenic life. Azo compounds act as important analytical tools by providing a mightily chromophoric label, the concentration of which is simply determined by spectrofluorimetric, or colorimetric, spectrophotometric methods. Also, azo compounds are...
important analytical assist compounds serving as pH indicators, complex metric indicators and to a smaller extent, pre-concentration reagents. The pharmacological use of azo compounds originates from the detection of the antibacterial action of Prontosil on streptococcal infections by Dogmagk. moreover, azo compounds were reported to explain a variety of biological activities including antibacterial, antifungal, pesticide, antiviral and anti-inflammatory activities \(^\text{(13-14)}\).

**Chemicals and Practical Part:-**

Reagents and Chemicals were all of analytical grade and used further without purification.

**2-Synthesis of reagent 4(4-benzophenol Azo) pyrogallol**

2.1. Synthesis of first part of the reagent:

The above reagent was prepared based on the traditional azo method, so 2.7482 g (0.02 mol) of 4-aminobenzoc acid was dissolved in a mixture of 10 ml of concentrated hydrochloric acid (37%) and 80 ml of ionic water and the mixture was cooled to a temperature of (0-5) C. Then, ice cold solution 0.02 mol of sodium nitrite dissolved in 20 mL of deionized water was added to it and the mixture was mixed for half an hour.

2.2. Synthesis of second part of the reagent

The second part of the coupling reaction was done by dissolving a solution of 2.52g (0.02 mol) of pyrogallol in 30 ml ethanol plus 100 ml of sodium hydroxide prepared by dissolving (4 grams in 100 ml of ion-free water) drop by drop with constant stirring. -5) m. Then add 100 ml of deionized water and leave the mixture for the next day. Then the sediment was filtered and washed several times with ion-free water and recrystallized with absolute ethanol, then the sediment was dried using an electric furnace for several hours at 60 °C. The result product was purified with absolute ethanol and monitored by TLC on silica gel plates using 2:3 methanol: toluene as solvent. YIELD of the product was 43% and m.p of the reagent was (290-300).\(^\text{(15)}\)

**Solid complexes preparation:**

The preparation of Solid complexes were depending on the appropriate conditions of concentration, pH and molar ratio (metal: ligands)[1:1], and [1:2]. Cobalt complex was prepared by adding (00.002mol)(00.274gm) from the reagent and dissolved in ethanol gradually with continuous stirring to (00.001 mol)(00.237gm) of aqueous cobalt chloride (II) at pH 7, The mixture was heated to 60C° for (30 min) to complete the reaction, and a brown precipitate was observed. Then filtered and allowed to dry. While nickel complex was prepared by adding (0.137gm)(0.001mol) from the reagent and the reagent was dissolved in ethanol gradually with continuous mixing to (0.238gm) (0.001mol) of aqueous nickel chloride (II) at pH 7, The mixture was heated to 60C° for half an hour to finish the reaction, the result was a dark green precipitate, Then the reagent was filtered and left to dry. While the preparation of silver complex by adding (0.137gm,0.001mol) from silver nitrate (I) solution at pH 7,The mixture was heated to 60C° for half an hour to complete the reaction, and final reddish brown precipitate was formed. Then filtered and permitted to dry

**Antimicrobial activity:-**
The antimicrobial activity was evaluated by agar well plate method for determination of zone of inhibition which depends upon the inhibition of growth of microorganisms.

Evaluation of in vitro antibacterial of synthesized compounds was tested against Gram positive: Staphylococcus aurous and Klebsiella Pneumonia Gram-negative

Preparation of the agricultural medium:

Preparation of culture Media The agricultural medium was prepared according to the instructions of the company supplying it by adding 38 gm of nutritious agar powder (Mueller-Hinton agar) to 1000 ml of distilled water in a conical flask where the mixture was heated until melting of the clods, after which the medium was sterilized For a quarter of an hour at a temperature of 121 ° C and a pressure of 15 pounds / inch, pour into dishes called a Petri dish at a rate of (10-20) ml for the plate, and the dish was cooled until hardened and at room temperature.

Preparation of solutions

Preparation of solution (4-PABP) ligand solutions and its metallic complexes under study were prepared by dissolving 0.01 grams of each compound in (5) ml of pure DMSO for each of the ligand and its metallic complexes which were tested.

Biological activity.

Processing Method:

The bacteria were spread in the dishes and on the surface of the food medium (Mueller-Hinton agar) using (loopful). Also, three holes of 6 mm in diameter were made in these dishes by using the cork-borer sterilized with alcohol, taking into account leaving An appropriate distance between one hole and another to avoid overlapping areas of damping between them. The prepared solutions were added to these pits with a volume of 0.1 ml using a (Micropipette) and placed in an incubator for 24 hours at a temperature of 37 ° C. Then measure the inhibition zone of the compounds using a millimeter ruler.

The Results and discussion:-

The reagent and its metallic complexes Characterizations

Characterized of (4-PABA) and its metallic complexes

Spectra Analysis

The synthesized reagent compounds were characterized by their spectrum of Fourier-transformation --IR spectra proved successful preparation of the above reagent and its complexes as in Table (1) and Figure (1).

| Frequency (cm\(^{-1}\)) |
|-------------------------|
| \(\nu\) O-H            |
| \(\nu\) C-H            |
| \(\nu\) N=N            |
| \(\nu\) C=C            |
| \(\nu\) C-N            |
| \(\nu\) C-O            |
| \(\nu\) C=O            |
| 3433 cm\(^{-1}\)        |
| 3074 cm\(^{-1}\)       |
| 1425 cm\(^{-1}\)       |
| 1600 cm\(^{-1}\)       |
| 1280 cm\(^{-1}\)       |
| 1045 cm\(^{-1}\)       |
| 1687 cm\(^{-1}\)       |
Figure (1): Fourier Transform Infrared spectra for the reagent

The UV.Visible spectrophotometric analysis of the reagent

Figure (2) showed the UV spectra of the synthesized 4-PABA which bared three transitions at 446nm, 278nm and 235 nm , which absorbed at visible area because of their orange color but also gave multi transitions at far UV back to the shift towards (Blue shift) short wavelengths, because of the withdrawing groups.
Figure (2) : UV. Visible spectrophotometric analysis spectra for 4-PABA

The analysis of the prepared Ligand 1H-NMR 4-PBAP proton NMR spectrum

Proton nuclear magnetic Resonance for Ligand (4-PBAP)

H-NMR spectrophotometric as in Figure (3) of the prepared (4-PABP) ligand was performed using DMSO as a solvent, by studying the 1HNMR nuclear magnetic resonance (NMR) proton spectrum for ligand and compared to what was reported in the literature (16), we were able to interpret these apparent signals (17), as it resulted from This spectrum: a single beam at 13.49 ppm = belonging to the hydroxyl group, and a single beam at 12.79 ppm, 12.45 and 10.28 OH = belonging to the OH phenol group and OH groups compensated for the phenol ring, and the emergence of multiple beams belonging to the protons of the benzene ring at 7.48-8.75 ppm = also. The emergence of multiple beams at 6.57-7.53 ppm = belonging to the protons of the phenol ring. As for the beams at 2.50-2.54 ppm, they are for the DMSO solvent and at 1.05-1.22 ppm = belonging to the 12H proton of the phenol ring.
Figure (3): H-NMR1 proton spectrum of the prepared ligand 4-PABP using DMSO as solvent and TMS as the standard reference recipe.

Field Emission Scanning Microscopy (FESM)

Scanning electron microscope technology was used for the purpose of identifying the crystal structure, surface shape, shape and size of minutes, and distribution of prepared reagent crystals (4-BPAP). (FESEM) was used because it gives high-quality images that are clearer and less distorted in relation to SEM. \(^{(18,19)}\) Pictures were taken of the surface of the reagent crystals as in Figure (4), and through the captured images showed a clear difference in the crystal structures and homogeneity of the surface, and by relying on the scanning electron microscope technology, images of the prepared reagent were taken at different distances in the cross section are (1) µm, 2µm and 5(µm), 500 (µm), µm (200) and µm (100), and the magnification force is within limits of Mag = 30.00kx, Mag = 10.0kx, Mag = 5.00kx, Mag = 50.0kx, 25.0kx, and the morphology of surface properties were studied. The prepared reagent (4-BPAP in terms of particle size and shape and aggregation) between them has been shown through the analysis of (FESEM) the appearance of the prepared reagent in the form of clusters with an average crystal size of (33.43-51.95nm), and that the presence of some aggregates leads to a process. Agglomeration that has the merit of the elementary particle pool, and the average crystal size was calculated by

( Image J) software to calculate the average particle size and Other features.\(^{(20,21)}\)
Figure (4): (FESEM images of the prepared reagent(4-BPAP).
Bioactivity dissection

Ligand azo-4-BPAP and its prepared complexes showed anti-bacterial efficacy, as its effectiveness was tested against two types of Staph.Aureus, which are Gram positive, and Klebsiella.pneumoniae, which is Gram negative bacteria, as in Figure (3).

The results of the bioactivity of 4-BPAP and its complexes against the bacterial species used in the study are included in Table (2).

Ligand 4-BPAP showed high activity against Klebsiella.pneum, where as in Figure (4) it was 2.4 cm at a concentration of 0.01 M, and the complexes also showed good biological activity against the same bacteria (1,1,0,9,1,2).

4-BPAP showed modest inhibition against Staph.Aureus as in Figure (5) growth as it was 1 cm at a concentration of 0.01 M. Cobalt (II), silver (I), and nickel (II) complexes showed biological activity, gradated in descending order (1,6,0,9,0,9) cm at a concentration of 0.01 molar against Staph.Aureus.

Table (2) Effect of Ligand 4-BPAP and its metallic complexes dissolved in ethanol on Staph.Aureus and Klebsiella.pneum.

| Bacterial               | 4-BPAP | Ag(I) | Co(II) | Ni(II) |
|-------------------------|--------|-------|--------|--------|
| Klebsiella.pneum        | 2.4    | 1     | 1.1    | 1.2    |
| (Gram-negative)         |        |       |        |        |
| Staph.Aureus            | 1      | 0.9   | 1.6    | 0.9    |
| (Gram-positive)         |        |       |        |        |

Bacterial activity of ligand and its complexes against bacteria

Klebsiella.pneum

Staph.aureus
Figure (5) Diagram showing the biological activity values for positive and negative bacteria

A2  A1

A4  A3

A3= [Ag(4-PABP)(H2O)] NO3
A1=[CO(4-PABP)_2]Cl2
A4=[Ni(4-PABP)]Cl2
A2=(4-PABP)

Figure (6) shows the biological activity of 4-BPAP and its metal complexes against K.PNEUM bacteria.

B1  B2
B1= (4-PABP)  \[\text{Ni}(4\text{-PABP})\text{Cl}_2\]

B3= \[\text{CO}(4\text{-PABP})_2\text{Cl}_2\]  B4= \[\text{Ag}(4\text{-PABP})(\text{H}_2\text{O})\] \text{NO}_3

Figure (7) shows the biological activity of ligand-4-BPAP and its metal complexes against Staph. Aureus

The Conclusion

The studies confirm the reagent 4-PABA was prepared of reacting diazonium salts of Para amino benzoic acid with pyrogallol, then their complexes were prepared. The products; 4-PABA were characterized by UV–Vis spectrophotometry, Fourier-transformation --IR spectra proved successful preparation confirmed by scanning electron microscopy and transmission electron microscopy. The analysis of the prepared Ligand by $^1$H-NMR 4-PBAP proton NMR spectrum confirmed the preparation. The compounds of Azo and their complexes were calculated in ranges of solutions of different pH and calculated the molar ratio for complexes and measurement UV-VIS spectra to know effect the solvent on ligand. The complexes were also prepared according to the conditions favorable to them of molar ratio, concentration, volume and temperature. Melting degrees were also measured for them. The biological activity of the prepared dye was also tested. Its complexes are directed towards two types of Gram positive and negative bacteria and showed good to moderate efficacy towards the bacteria.

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