Synthesis and Comparative Studies of Antimicrobial behavior of Ni(Ⅱ) and Cu(Ⅱ) novel chelates with novel mixed ligands (azo anils and 8-hydroxyquinoline)

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Abstract— The work presented in this thesis concerns the synthesis of chelate metal complexes by the chelation of mixed ligand agents (Azo Anil derivative and 8-Hydroxyquinoline) with the transitions metals Ni (II) and Cu (II).The antimicrobial evaluation of the chelate complexes is also included in this work. Azo anils were prepared and along with 8-hydroxyquinoline were coordinated with Ni (II) and Cu (II).The different aspects of the newly synthesized mixed ligands and their mixed ligand metal chelate complexes were characterized by using the different spectroscopic techniques. They were characterized by UV, FTIR and Atomic Absorption Spectroscopies. The comparative study of the antimicrobial activities of chelating agents and metal chelates is also concluded.

Index Terms—Azo anils,antimicrobial activities,chelate,chelating agents,mixed ligands,transition metals, .

1 Introduction

Azo complexes are very important compounds and have captivated much awareness in research field related to chemistry.[1] While Anils are the Schiff base compounds derived from the aromatic amines. Azo Anils which we are going to prepare in this project are the compounds containing azo group (N=N) as well as imine group (C=N) in them. [2] The presence of these two groups in the same compound interacting with the transition metal as a ligand can result in the formation of the chelate. Azo anils ligands can be easily synthesized by condensing an azo dye of salicyladehyde and aromatic amine [3,5]. The introduction of the azo group in these ligands can be carried out through the process of diazotization of the primary amines. [6,7]

In this project we have used different aromatic amine to prepare novel azo anils ligands to interact with Ni and Cu transition metals along with carboxylic acid derived ligand i-e 8-Hydrixyquinoline. The coordination complexes of the transition metals which had be prepared in this project are stable because of the chelate effect of the mixed ligands. Then the biological activities of these coordination complexes of Nickel and Copper were observed at the end of this project for further characterization of the the newly synthesis azo anil ligand and its mixed ligand complexes.

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2 Procedure

2.1 Synthesis Of Azo Anil Chelating agents

In this thesis also Schiff base ligands (Anils) had been prepared by the condensation reaction of primary amine and salicylaldehyde by applying certain conditions. A general way in which a Schiff base is synthesized is a condensation reaction between the amino group of the amine and the carbonyl group of the aldehyde or ketone, which result in the formation of an imne group. The Schiff bases in this project have been synthesized by the condensation of azo group containing derivative of salicylaldehyde with a primary amine (aniline). This condensation reaction has lead us to the preparation of Anils i-e the class of Schiff bases containing both functional groups, azo (N=N) as well as imine (C=N) group in it.

2.2 Experimental

2.2.1 Chemicals and Reagents:

All the chemical (Salicylaldehyde, Aniline, 8 Hydroxy-quinoline,o-amino phenol, Sodium nitrite, Sodium Hydroxide, Nickel Chloride, Copper nitrate) that had been used in this project were purchased from BDH. And the absolute solvents (Methanol and ethanol) used were purchased from Aldrich.

2.2.2 Equipment used:

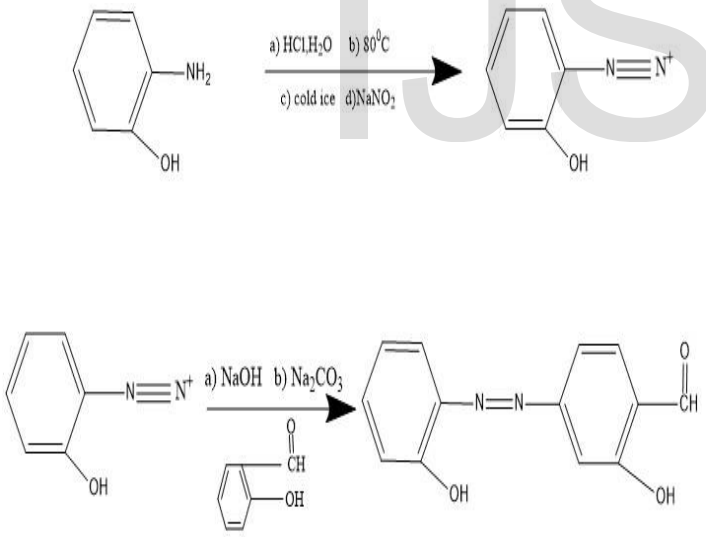
Following is the equipment used in the experimental project of the present project;

- Magnetic stirrer
- Gooch crucible
- Beakers
- Measuring cylinder
- Pipette

2.2.3 SYNTHESIS OF AZO DERIVATIVE: (2-hydroxy-5[(2-hydroxyphenyl) diazenyl] benzaldehyde) (HDPIMP)

To synthesize an azo derivative, into 3.68 g (30 mmol) of o-aminophenol, 30ml of hydrochloric acid and 20ml of distilled water was added. This was heated at 80 C until the complete dissolution. Then the other solution was made by adding 2.68g (30mmol) of sodium nitrite in 10 ml of distilled water and this was cooled below 5 C. The chilled solution of sodium nitrite and the room cooled solution of o-aminophenol were blended together. This diazonium salt solution was added into the salicylaldehyde solution. The salicylaldehyde solution was prepared by adding 3.88 g (30mmol) of salicylaldehyde in 57 ml of water, which also contain 11.1g of sodium carbonate , 1.2 g of sodium hydroxide and stirred over magnetic stirrer for 30 minutes in ice bath.
The azo derivative (2-hydroxy-5[(2-hydroxyphenyl) diazenyl] benzaldehyde) which was obtained following the above procedure was obtained using a gooch crucible. The azo derivative was firstly washed with 10% NaCl solution. Later washed extensively by distilled water and ethanol to remove the impurities.

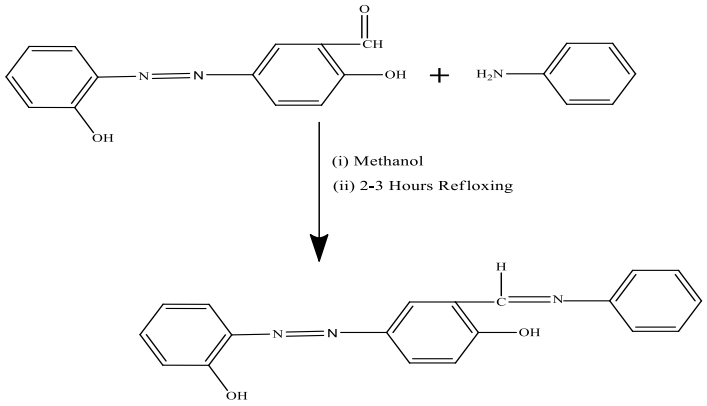
Chemical formula of Azo derivative: $C_{13}H_{10}0_3N_2$
Yield of reaction : 82 %
Colour of Azo Derivative : Reddish- Orange



Scheme 2.1 Synthesis of Azo derivative
(2-hydroxy-5[(2-hydroxyphenyl) diazenyl] benzaldehyde

2.2.3 SYNTHESIS OF AZO ANIL LIGAND:
2-{[2-hydroxyphenyl)imine]methyl}-3-{(2-hydroxyphenyl)diazenyl]phenol (HPIMHDP)

To prepare an azo Anil ligand (L1) into 50 mmol of methanol, 3 mmol of azo derivative prepared in above step was added and stirred for 30 minutes, afterwards 3 mmol of aniline was added into the solution along with the addition of few drops of glacial acetic acid as catalyst to the reaction. The reaction mixture was kept refluxed for almost 2-3 hours.
As the reaction mixture cooled , precipitates of azo Anil were obtained.
Chemical formula of Azo Anil (L1) : $C_{19}H_{15}N_3O_2$
Colour of Azo Anil (L1) : Reddish Brown
Yield : 77 %
The Chemical reaction of the synthesis of Azo Anil (L1) is mentioned as follow :



Scheme 2.2 Synthesis of Azo Anil (L1)

2.2.5 SYNTHSIS OF [(L1) Cu (8HQ)] :

The 1 mmol methanolic solution of Azo Anil ligand (L1) and 1 mmol of methanolic solution of 8 Hydroxy-quinoline (8HQ) were blended together. This mixture was added to the 1 mmol methanolic solution of the metal salt of copper ($CuNO_3.2H_20$). The color of the mixture changed in naked in just 2-3 minutes. Then the reaction mixture was refluxed for almost 3-3 hours. Afterward the precipitates formed were gained using a gooch crucible. The precipitates were washed with methanol , then later by extensive water to get rid of impurities.
Color : Yellowish Red
Yield : 50 %

2.2.6 SYNTHSIS OF [(L1) Ni (8HQ)]:

The 1 mmol methanolic solution of Azo Anil ligand (L1) and 1 mmol of methanolic solution of 8 Hydroxy-quinoline (8HQ) were blended together. This mixture was added to the 1 mmol methanolic solution of the metal salt of nickel ($NiCl_2.6H_20$). The color of the mixture changed in naked in just 2-3 minutes. Then the reaction mixture was refluxed for almost 3-3 hours. Afterward the precipitates formed were gained using a gooch crucible. Then the washing of precipitates was done by methanol, later by extensive water to get rid of the impurities.
Color : Green
Yield : 70%

3.6.5 PHYSICAL PROPERTIES OF METAL CHELATES:

As the metal chelates were synthesized by using the above mentioned method, the following physical properties of the newly synthesized mixed ligand metal chelate were determined as well as noted:

a) Melting Point
b) Color of complex
c)Stability
d) Solubility

3.7 ANALYTICAL STUDIES OF METAL CHELATES

3.7.1 ATOMIC ABSORPTION SPECTROSCOPY :

The presence of the metal and its amount present in the solution was measured by using atomic absorption spectroscopy. The result of which is mentioned in the (Table 3.8). The parameters of the instrument used for atomic absorption spectroscopy of these metals Cu and Ni are given below. The schematic diagram of working of atomic absorption is as shown :

3.7.1.1 Preparation of metal solution by digestion:

The complex was treated with nitric acid to convert metal present in complex completely into its corresponding nitrate. The complex was digested completely for preparing metal solution. First of all, the metal complex was treated with conc. HNO_3 which converted the metal present in the complex into it's the corresponding nitrate. The procedure used is as follow:

0.05 g of metal complex was taken in the china dish and into it 10 ml of the conc.HNO3 was added. The mixture was heated in the sand bath until the acid was evaporated and only a film was left at the bottom of the china dish. To this film in china dish again 10 ml of HNO3 was added and the same procedure was repeated.

The whole of this procedure was repeated several times, until a thin brownish pasty film was left. To this thin paste distilled water was added to dissolve it completely. The it was poured into the 100 ml measuring flask and the volume was made up to the mark.

3.7.2 UV STUDIES OF METAL CHELATES:

The **λ max** of the metal chelates of Cu and Ni metals was determined with the help of UV-spectroscopy. The stability of the metal chelates was also determined by UV graphs. The UV studies were carried out to check the stability of the metal chelates both in solution as well in the air. The UV spectra of metals chelates were take on Carry 60 UV-visible (Agilent Technologies) present in Department of Chemistry, UET Lahore.

3.7.3 FTIR STUDIES OF METAL CHELATES:

The synthesized metal chelates of Ni and Cu were also characterized by using FTIR scan .To obtain Infra-red spectrum of synthesized **[(L1) Ni (8HQ)]** and **[(L1) Cu (8HQ)]** , Carry 630 FTIR spectrophotometer, present in Chemistry Department of UET, Lahore was used . They range available on this spectrophotometer was 650-3000 cm^{-1}.

3.7.3 ANTI-MICROBIAL ACTIVITY:

3.7.3.1 METHODS USED:

A) Test Organisms :

To determine the antimicrobial activity of the synthesized metal chelates the standard cultures of the test organisms were obtained from the microbiology department of UVAS, Lahore.

Fungi

Asprgillus oryzae, Aspergillus niger ,Aspergillus flavis Aspergillus fumigatus

BACTERIA

E.coli,Staphylococcus aureus, Lactobacilus

B) CULTURE MEDIA

Media used :

The following media were used both for the fungi as well bacteria in the present work to determine the antimicrobial activity of the synthesized metal chelate

a) Nutrient Agar (N.A)
b) Nutrient Broth (N.B)

3.Results and Disscussion

3.1 PHYSCIAL CHARCTERISTICS:

The physical state, color, melting points, solubility in polar solvents and solubility in non-polar solvents was checked for the azo derivative, azo anil derivative, mixed ligand chelate complex of Ni (II) and mixed ligand chelate complex of Cu (II).

3.1.1 Melting Point of Compounds:

The temperature at which the solid compound turn itself into liquid at normal temperature is known as Melting point. Generally, a pure substance at standard pressure conditions has a single value of Melting Point. In the present project the melting point of the compounds was taken using a digital melting point apparatus.

Table 3.1 Showing the melting points of the compounds

Sr.no	Name of Compound	Melting point (°C)
1	Azo Derivative	188
2	Azo Anil Derivative	287-290
3	Ni complex	300
3	Cu-complex	330

An increase in the melting point of the compound was observed after the chelation of the chelating agent with the metal. This increase in the melting point of the compound is also the proof of chelation found in the compound.

3.1.2 Physical State of Compounds:

Generally the properties of the compounds which are

observable are termed as Physical properties. The physical state refers to a state of compound in which it obtained or exist at Standard room conditions. The ligand and its complexes were obtained in crystalline form. The color of the ligand before chelation was different but after chelation with metal, the color of whole mixed ligand chelated complex was different.

Table 3.2 Showing the Physical state of and color the ligand and its chelate complexes

	Compound	Physical State	Color
	Azo Derivative	Crystalline	Reddish orange
	Azo Anil Derivative	Crystalline	Reddish brown
	Ni complex	Crystalline	Green
	Cu-complex	Crystalline	Yellowish Red

3.1.3 Solubility of Compounds:

The Solubility of the azo derivative, azo anil derivative, mixed ligand chelate complex of Ni and Cu, was checked both in the polar and non-polar solvents. The results are mentioned in the below tables.

Solubility in Polar Solvents

Table 3.3 Showing solubility of Azo derivative and Azo anil derivative in Polar solvents

Solvent	Azo derivative	AzoAnil derivative
Phenol	Soluble	Partially Soluble
Methanol	Soluble	Soluble
Water	Soluble	Soluble

Table 3.3 Showing solubility of Mixed Ligand Metal Chelate complexes in Polar solvents

Solvent	[(L1) Cu(8HQ)]	[(L1) Ni (8HQ)]
Phenol	Partially Soluble	Partially Soluble
Ethanol	Soluble	Soluble
Water	Insoluble	Insoluble

Solubility in Non-polar Solvents

Table 3.5 Showing solubility of chelating agents in Non-polar solvents

Solvent	Azo derivative	Azo Anil derivative
Acetone	Soluble	Partially Soluble
n-hexane	Insoluble	Insoluble
Ether	Partially Soluble	Partially Soluble

Table 3.6 Showing solubility of mixed ligand metal Chelate complexes in Non-polar solvents

Solvent	[(L1) Cu (8HQ)]	[(L1) Ni (8HQ)]
Acetone	Soluble	Soluble
CCl_3	Insoluble	Insoluble
Ether	Soluble	Soluble

3.2 FTIR DISCUSSIONS:

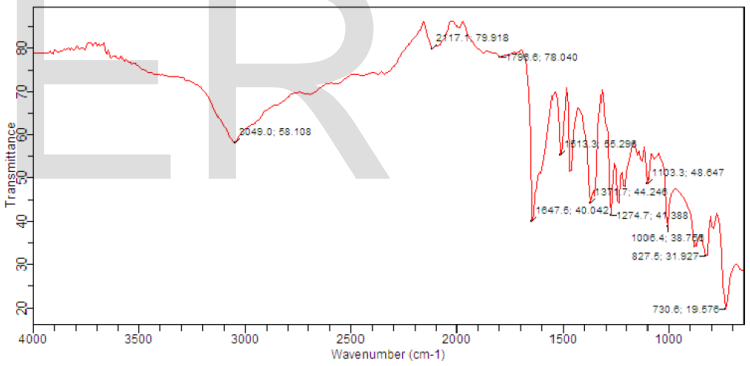
3.2.1 Discussion of Chelating Agents:

All the Schiff base derived chelating agents were characterized by IR spectroscopy. The satisfactory bands were obtained in their respective regions of the characteristic bonds present in the chelating agents (Azo derivative and Azo Anil Derivative). In comparison to the previous work that is present in the literature, the was no significant change or shift found in the expected value for each band observed in the IR spectrum.

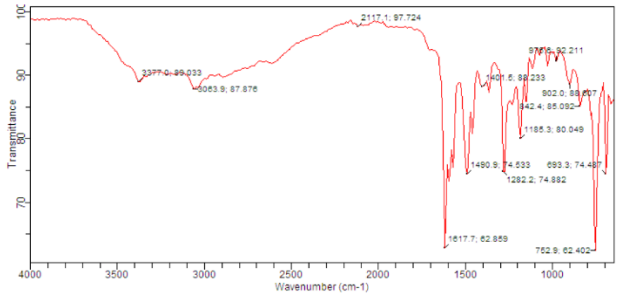
Table 3.7 I.R Data of Azo Derivative and Azo Anil Derivative

Ligand	C=N	N-C	O-H	C=C	C-O
AzoDerivative (HDPIMP)	-----	1371	3300	1637	1273
Azo Anil (L1) HPIMHDP	1520	1301	3377	1617	1282

FTIR SCAN FOR AZO DERIVATIVE



FTIR SCAN FOR AZO ANIL Derivative



3.2.2 Discussion of Mixed Ligand Metal Chelates:

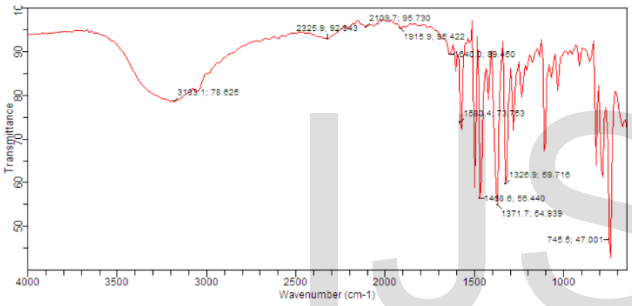
Table 3.8 Showing IR Data of the Mixed Ligand Metal

Compound	C=N	N-C	O-H	C=C	C-O
[(L1) Ni(8HQ)	1580	1368	3183	1630	1326
[(L1)Cu(8HQ)]	1580	1308	3337	1637	1107

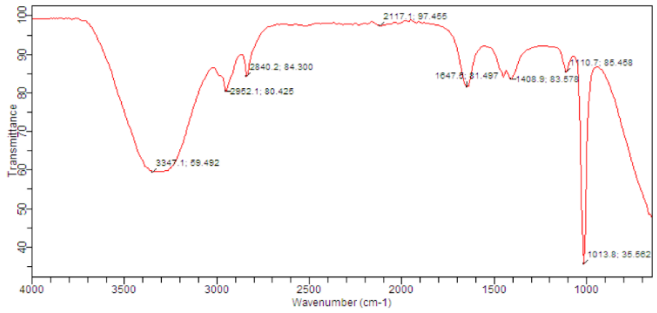
Chelates

All the Mixed ligand metal chelates of nickel and copper were characterized by IR spectroscopy. The satisfactory bands were obtained in their respective regions of the characteristic bonds present in the mixed ligand metal chelates of nickel and copper. In comparison to the previous work that is present in the literature, the was no significant change or shift found in the expected value for each band observed in the IR spectrum

FTIR SCAN FOR [(L1) Ni (8HQ)]



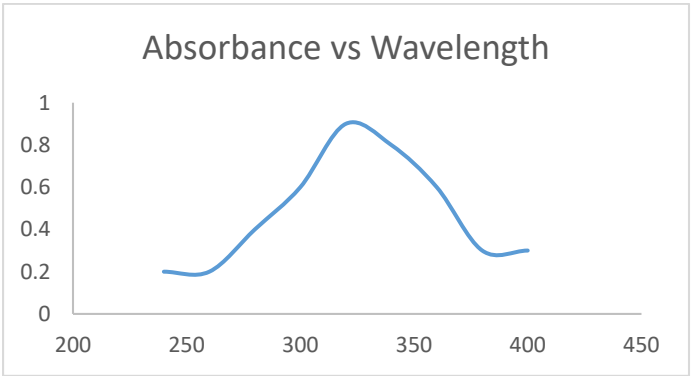
FTIR SCAN FOR [(L1) Cu (8HQ)]



3.3 UV DISCUSSION:

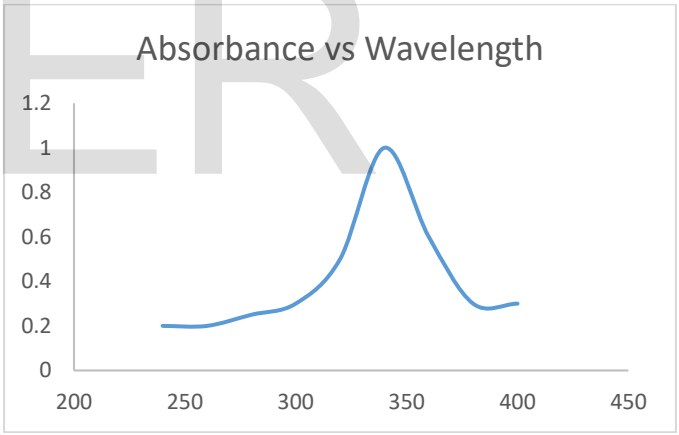
To determine the λ max of the of the chelating agents as well as the λ max of the mixed ligand metal chelate of nickel and copper, an appropriate solvent was selected to obtain the UV graphs, which were taken in relationship to the absorbance vs wavelength, and also absorbance vs days. The UV spectra were taken on Carry 60-UV-visible (Agilent Technologies) present in department of Chemistry, UET Lahore. According to the literature the λ max of the synthesized imine was expected to lie in the range of 320-370 nm. The λ max of the imine that is azo Anil derivative (L1) which has been designed in this present project, according to the literature lies in range of **320-370 nm.** The maximum wavelength for the chelating agents and its metal complexes with nickel and copper was observed near to the absorbance value of the chelating agent with a very small difference. The result for complexation with these two metals considered correct.

UV GRAPH FOR AZO DEIVATIVE



Graph 3.1

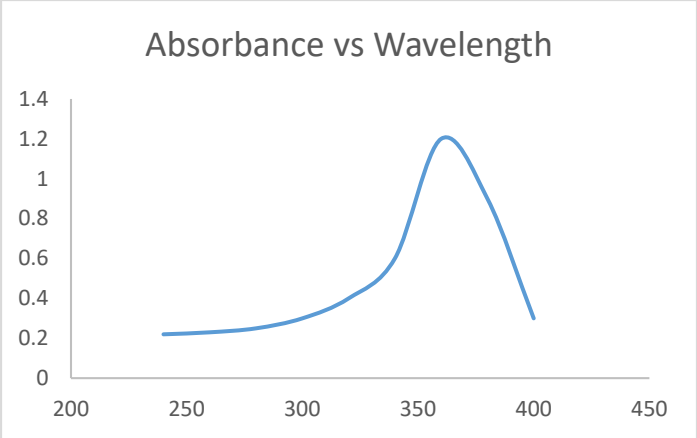
UV GRAPH FOR AZO ANIL DEIVATIVE



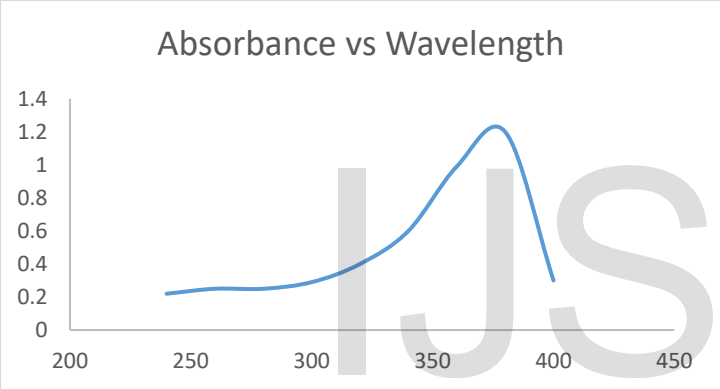
Graph 3.2

Table 3.9 Showing the λ max of the metal chelate

Sr.no	Compound	λ max(nm)
1	**AZO DERIVATIVE**	328
2	**AZOANIL DEIVATIVE**	330



Graph 3.3 Showing λ max of the metal chelate [(L1) Cu (8HQ)]



Graph 3.3 Showing λ max of the metal chelate [(L1) Ni (8HQ)]

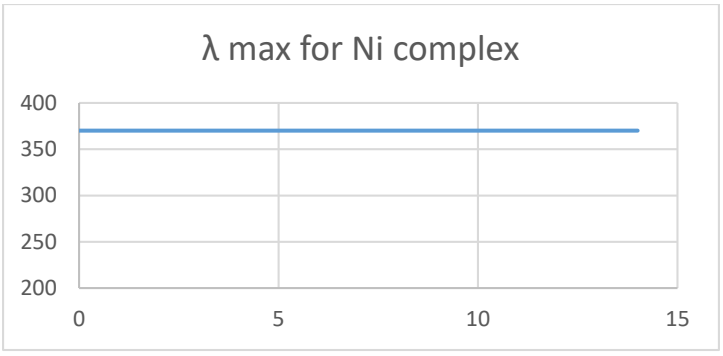
Table 3.10 Showing the λ max of the metal chelate

Sr.no	**Metal Chelate**	**λ max(nm)**
1	**[(L1) Cu (8HQ)]**	356
2	**[(L1) Ni (8HQ)]**	370

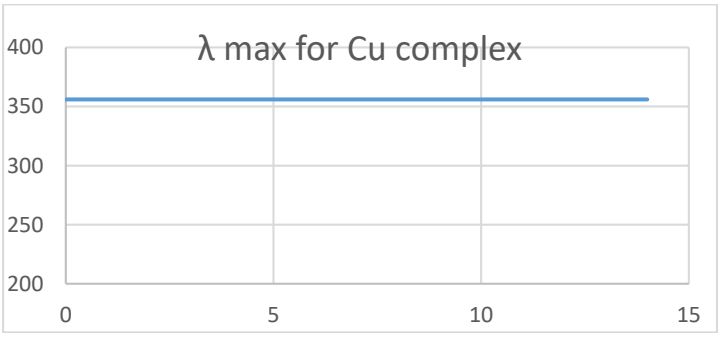
3.3.1 Stability Measurement:

The UV analysis was used to check the stability of the newly synthesized Azo Anil derivative (L1) and also the stability of the new synthesized mixed ligand complexes of nickel and copper. The UV analysis was made at the time when the compound was synthesized and after some time duration (two weeks). The similar analysis was also made for the mixed ligand metal complexes of Nickel and Copper.

To determine the variation in the value of the λ max, every time the analysis was made during the time duration of two weeks, solution made of the day of synthesis was run in comparison to the fresh solution.



Graph 3.5 Showing the Stability of [(L1) Ni (8HQ)]



Graph 3.6 Showing the Stability of [(L1) Cu(8HQ)]

The **λ max** of the fresh solution was to check the stability of newly synthesized compound in the solid crystalline form in air, while the first day solution **λ max** was obtained to determine the stability of the newly synthesized compound in the solution. Therefore, for checking the stability of the azo anil derivative and mixed ligand metal complexes, the sample is supposed to run once a day and constantly for the duration of two weeks. Since there was not much variation found in the value of λ max, therefore it was confirmed that the newly synthesized compounds are stable.

3.3 METAL ESTIMATION DISCUSSIONS:

The amount of the metal present in a certain mixed ligand metal chelate has been estimated by making use of Atomic Absorption Spectroscopy.

Table 3.8 Showing the metal estimation in Mixed Ligand Metal Chelate by AAS

Complex	**Metal to be estimated**	**Metal estimation by AAS (g/L)**	**Calculated amount (g/L)**
[(L1)Ni(8HQ)]	Ni	0.070	0.068
[(L1)Cu8HQ)]	Cu	0.058	0.053

The analysis of the metal estimation by Atomic absorption spectroscopy showed a very little difference between the estimated amount of the metal and the observed value.

3.5 ANTIMICROBIAL ACTIVITY DISCUSSIONS:

The analysis of antimicrobial activity of the chelating agents and metal chelates showed the large inhibition against all the Aspergillus species. When the comparison between antimicrobial activity the azo derivative, azo anil derivative and mixed ligand metal chelates was done, it was affirmed that the antimicrobial activity increase with the chelation of the chelating agents. The mixed ligand metals chelate of Nickel and Copper were found more active against the fungal species mentioned in comparison to their activity against bacterial species.

4 CONCULSION

In the present project an attempt was made to synthesize an azo anil derivative ligand as a primary chelating agent in metal chelate. the secondary ligand which was selected is 8-hydroxyquinoline. the mixed ligand metal chelate of nickel and copper were synthesized, the synthetic procedure of which is already mentioned in the thesis. the products were isolated in crystalline form.the ir studies conducted showed that there was not much significant change observes in the ir peaks of chelating agents after the chelation with metal. the colored crystalline product obtained confirmed the presence of the chromophoric group in the compound.the analysis of the uv studies also affirmed the stability of the mixed ligand metals chelates both in air as well as in solution, because the same because the same max was obtained during the duration of two weeks. The estimation of Ni and Cu metal in the mixed ligand metal chelates was done by using atomic absorption spectroscopy. The study on the antimicrobial activity of the chelating agents and the mixed ligand metal chelates revealed that the antimicrobial activity of the metal chelate was enhanced due to chelation.

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