Synthesis, Determination and Antibacterial Activity Studies of Trace Amount of Cu(II) and Ni(II) with New Reagent 2-[2`-Iodophenyl azo)]-4,5-diphenyl imidazole by Spectrophotometric methods

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Abstract

New reagent and two complexes of general formula $[ML_2CL_2].H_2O$, where M = Cu(II), Ni(II),L = 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole have been prepared in ethanolic solution.

Solid compounds were isolated and characterized by elemental analysis and vibrational spectra. The above newly synthesized compounds were investigated for their antibacterial, antifungal activities as well as, a sensitive and selective method has been developed for the determination of micro amounts of Ni(II), Cu(II). The method is based on the chelation of metal ions with 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole (2-IPAI) to form an intense color soluble products, that are stable and have a maximum absorptions at 566 nm and at 534 nm, and ε_{max} of $1.26*10^4$ and $1.66*10^4$ $L.mol^{-1}.cm^{-1}$ for Ni(II), Cu(II), respectively. The stability constants and relative errors and a relative standard deviations for Ni(II) and Cu(II) were: $3.134*10^{10}$, $5.022*10^{10}$ $L^2\ mol^{-2}$ and (-1, -1.33%), (-0.6, 0.3%) and (0.31, 0.699%), (0.29, 0.63%) respectively.

Keywords:- 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole spectrophotometry, nickel and copper determination.

Introduction

Azo compounds play a vital role in analytical chemistry due to highly sensitive color reaction, stability and selectivity towards various metal ions[1-8].

Pyridylazo and thiazolylazo compounds have been synthesized and proposed as highly sensitive chromogenic reagents for the determination of several metal ions[9-11]. The azo compounds are known to form chelates with many metal ions which have molar absorptivities that exceed 10^5 $L.mole^{-1}.Cm^{-1}$. Although the analytical application of azo compounds has been extensive, investigations of imidazo thialylazo compounds are few. Imidazole has two nitrogen atoms,

respectively of the pyridine and pyrrole type; high reactivity with metals is expected due to the strong basicity of the pyridine – type nitrogen in comparison with pyridine itself[12,13]. Among various instrumental methods that have been developed or modified for determination of nickel and copper, flame AAS combined with preconcentration by chelating agents or modified resins[14-16] and electrothermal AAS[17,18] have been widely used. These methods suffer from some limitations in simplicity, analytical time, economics and environmental safety.

Spectrophotometric methods often suffer from limitations in sensitivity and selectivity but are widely used due to both the resulting experimental rapidity and simplicity. Therefore the objective of the investigation reported in this paper was to evaluate a spectro-photometric determination of nickel and copper based on the reaction of Ni(II) and Cu(II) with 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole as chromogenic reagent.

Experimental:

Apparatus:

- All spectral and absorbance measurement were carried out on a Shimadzu UV-visible 1700 double beam spectrometer using 1 cm glass cells.
- Vibration spectra were recorded on testscan FTIR Shimadzu 8000 series.
- Micro analytical data (C.H.N) were obtained using micro analytical unit, 1108 CHN.O Elemental analyzer.
- A digital pH meter was used.

Reagents:

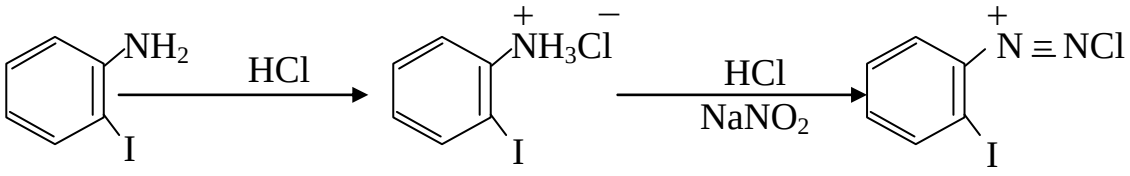
All the chemicals used were of analytical reagent (AR) grade. Distilled water was used throughout the present study.

- ***2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole (1×10^{-3} M)***. (0.1125 gm) of reagent (2-IPAI) was dissolved in 250 ml of ethanol. Working (2-IPAI) solution was prepared by simple dilution of the appropriate volume of the (2-IPAI) solution (1×10^{-3} M) with methanol.

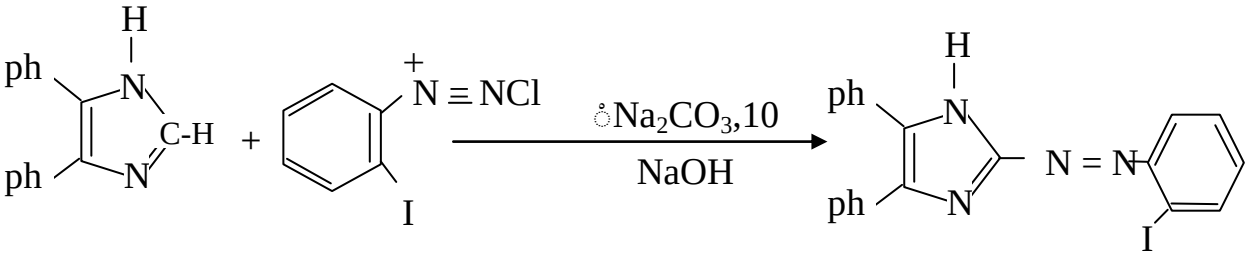
- ***Standard Ni(II) solution (1000 ppm)***. This solution was prepared by dissolving. (0.405) gm of $NiCl_2.6H_2O$ in 100 ml distilled water, working standard Ni(II) solutions were prepared by simple dilution of the appropriate volume of the standard Ni(II) solution (1000 ppm) with distilled water.
- ***Standard Cu(II) solution***. Standard Cu(II) solution was prepared by dissolving (0.268) gm of $CuCl_2.2H_2O$ in 100 ml of distilled water.
- ***Foreign ion: solution (1 mg.ml^{-1})***. These solutions were prepared by dissolving, an amount of the compound in distilled water completing the volume in a volumetric flask.

Preparation of reagent 2-(2-Iodophenyl azo)-4,5-diphenyl imidazole (2-IPAI):

The reagent was prepared by coupling 4,5 diphenylimidazole with the appropriate diazotate in alkaline alcoholic solution. A diazonium solution was prepared by taking 2 g. Iodo aniline in 2 ml of concentrated hydrochloric acid with 10 ml of distilled water, and adding sodium nitrite solution dropwise at 0 – 5°C. 4,5-diphenyl imidazole 2.0 g was dissolved in 150 ml of ethanol, and 50 ml of (0.1 M) sodium hydroxide and 50 ml of sodium bicarbonate were added at (–5°C). The mixture was allowed to stand overnight. The precipitate was filtered off. and recrystallized from ethanol. Schemes (1) [19,20].



2-Iodoaniline amine Salt Diazonium Salt



4,5-Diphenylimidazole (2-IPAI)

Scheme 1: preparation of the reagent (2-IPAI)

Preparation of Complexes:

$[Cu(2`\text{-}IPAI)_2].H_2O$ – the complex was prepared by mixing stoichiometric amounts of $CuCl_2.H_2O$. and ligand (2-IPAI) in a 1 : 2 ratio in aqueous and ethanolic solutions. The mixture was stirred at room temperature for 5 min. the pH of the solution was adjusted to 7 after buffer solution was added, then the solution left at room temperature for 24 h. the precipitate was filtrate by filter paper and washed with distilled water and dried at 70°C.

$[Ni(2\text{-}IPAI)_2].H_2O$ – the same procedure as described for $[Cu(2\text{-}IPAI)_2].H_2O$ was used for the preparation of this complex. The mole ratio of $NiCl_2$. : (2-IPAI) was can be formulated as $ML_2C\ L_2.H_2O$.

Procedure of Analytical Study:

In to a series of 5 ml calibrated flask, transfer increasing volumes of Ni(II) and Cu(II) working solution (100 μg/ml) to cover the range of the calibration curve, add (2 ml) of 1×10^{-4} M of (2-IPAI) solution and the pH was adjusted by 0.05 m hydroch loric acid and 0.05 M sodium hydroxide. The complexes formed were solubilised in water and diluted up to 5 ml with distilled water and allow the reaction mixture to stand for 15 min at room temperature.

Measure the absorbance at 566 nm for Ni(II) complex and at 534 nm for Cu(II) complex against a reagent blank prepared in the same way but containing no Ni(II) and Cu(II) respectively. The color of the formed complexes is stable for 24 h.

Results and Discussion:

The interaction of aqueous ions solution with aqueous ethanolic solution of (2-IPAI) to give fine crystalline precipitates in both cases which are insoluble in water.

Both reaction gave a quantitative of ratio 1:2 of metal ion to ligand. This result was confirmed by testing the need of supernatant solution for both metal ions and ligand. On the basis of analytical results, which are listed in table (1).

Table 1: Analytical data of prepared compounds

compound	Color	Found (Calc.) and data		
		C%	H%	N%
(2-IPAI)	yellow	56.00 (55.87)	3.33 (3.26)	12.44 (12.61)
$Cu(2\text{-}IPAI)_2.H_2O$	purple	48.10 (48.23)	3.05 (2.97)	10.68 (10.57)
$Ni(2\text{-}IPAI)_2.H_2O$	pruple	47.88	3.04	10.64

		(47.79)	(3.11)	(10.51)

Infrared Spectra of the Reagent and Complexes:

All the spectra were recorded in the solid state using (KBr) in the range (500 – 4000cm^{-1}) (2-IPAI) and their complexes displays *ν*(N–H) at 3100 – 3300 cm^{-1} and vibrations at 2625 – 2900 cm^{-1} of imidazole ring[21]. The reagent also displays distinct *ν*(–N=N–) at 1400 – 1520 cm^{-1} these bands reduced in intensity and slightly shifted to lower frequency upon complexation[22]. The coordination with the N–atom of imidazole ring it can be seen that bands at 600 – 800 cm^{-1} shifted to lower frequency in the complexes (by 20 – 50 cm^{-1})[23], and by appearance of (M–N) bands stretching[24]. Fig. (1, 2, 3).

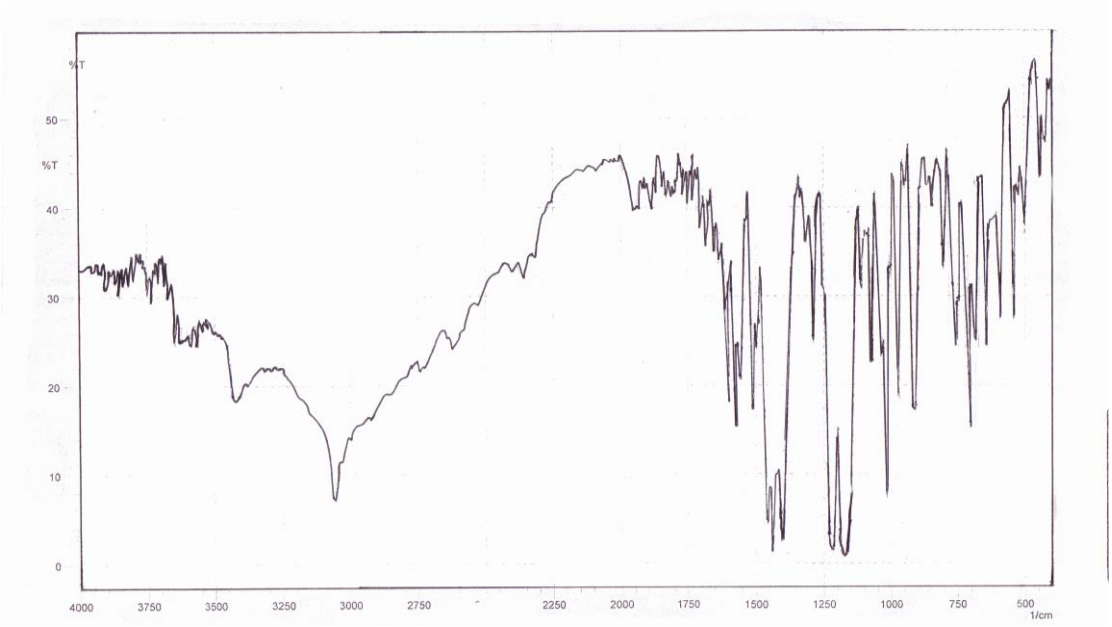


Fig. 1: FTIR spectrum of the reagent (2-IAPI)

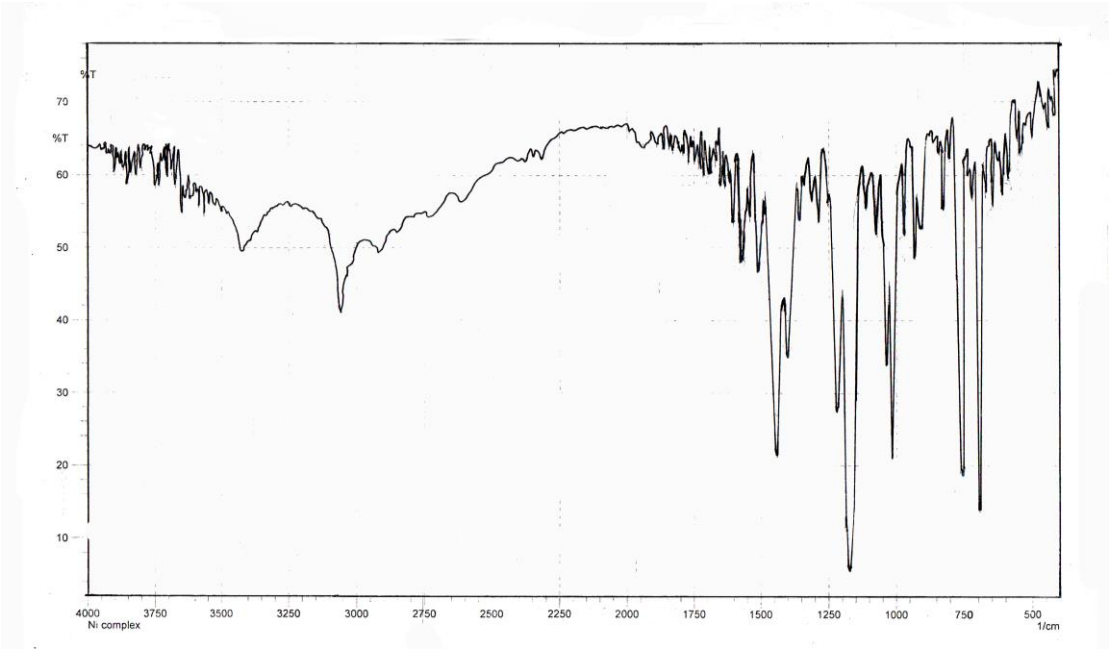


Fig. 2: FTIR spectrum of the Nil(II) complex

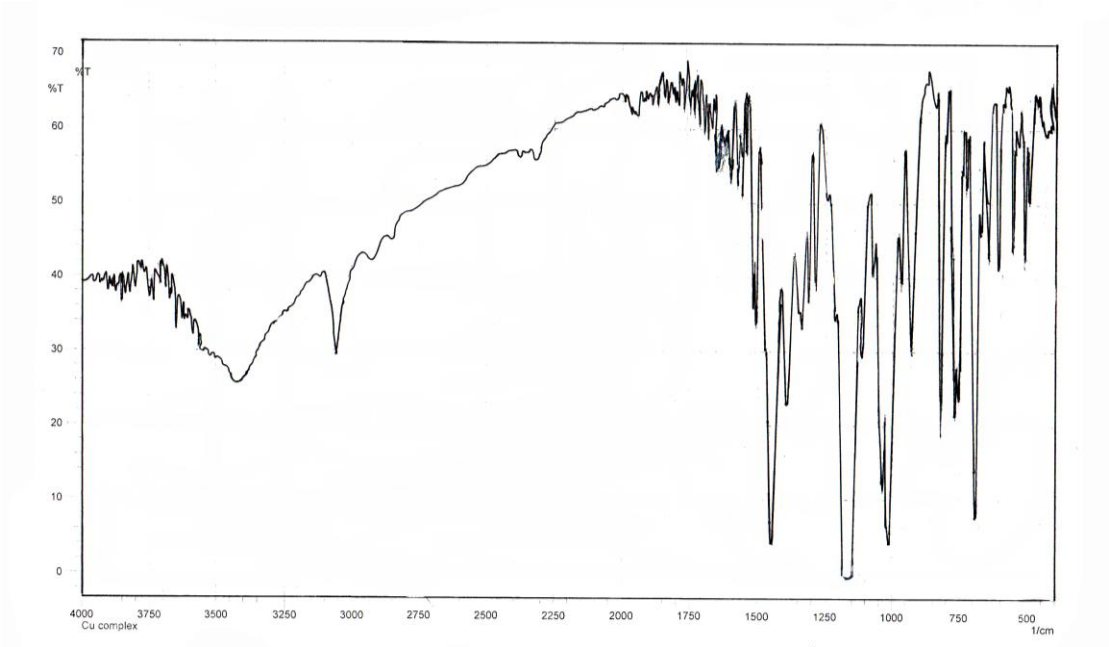


Fig. 3 :FTIR spectrum of the Cu(II) complex

The results of this investigation indicated that the reactions of Ni(II) and Cu(II) with 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole yields. Highly soluble colored complexes which can be utilized as a suitable assay procedures for determination of Ni(II) and Cu(II). These colored

complexes have a maximum absorptions at 566 nm for nickel complex and at 534 nm for copper complex, the blanks at these waves lengths shows zero absorbance (Fig. 4, 5).

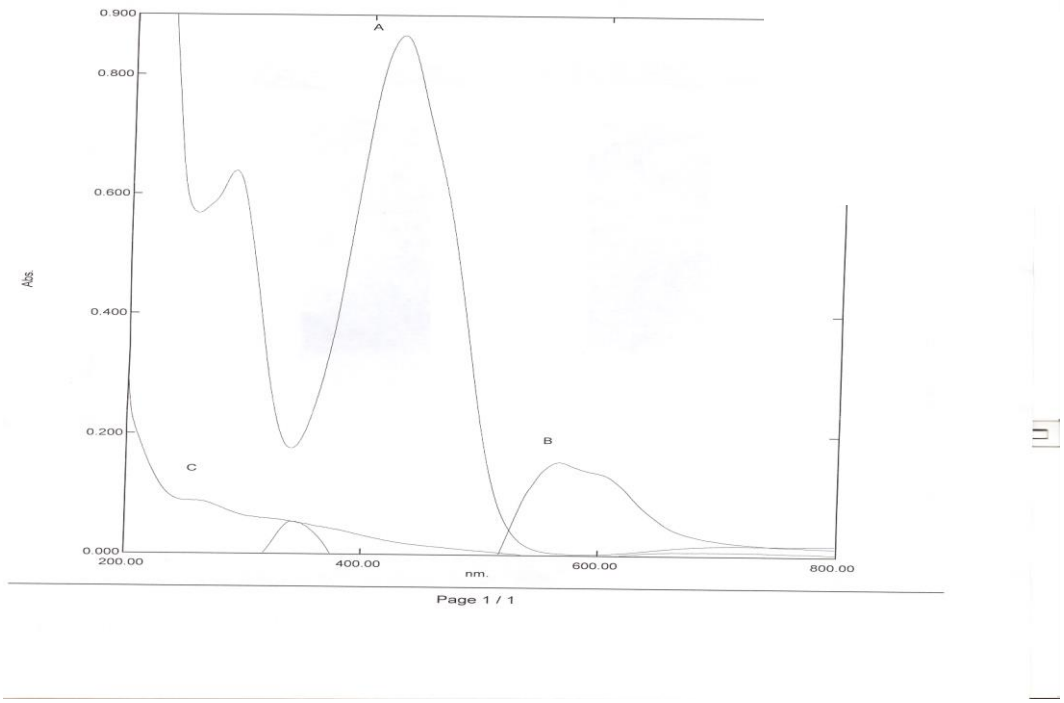


Fig. 4: Absorption Spectra of B(10 µg.ml^{-1}) of nickel(II) ion treated as described under procedure and measured against a regent blank and A the reagent blank measured against distilled water and C the metal ion measured against distilled water.

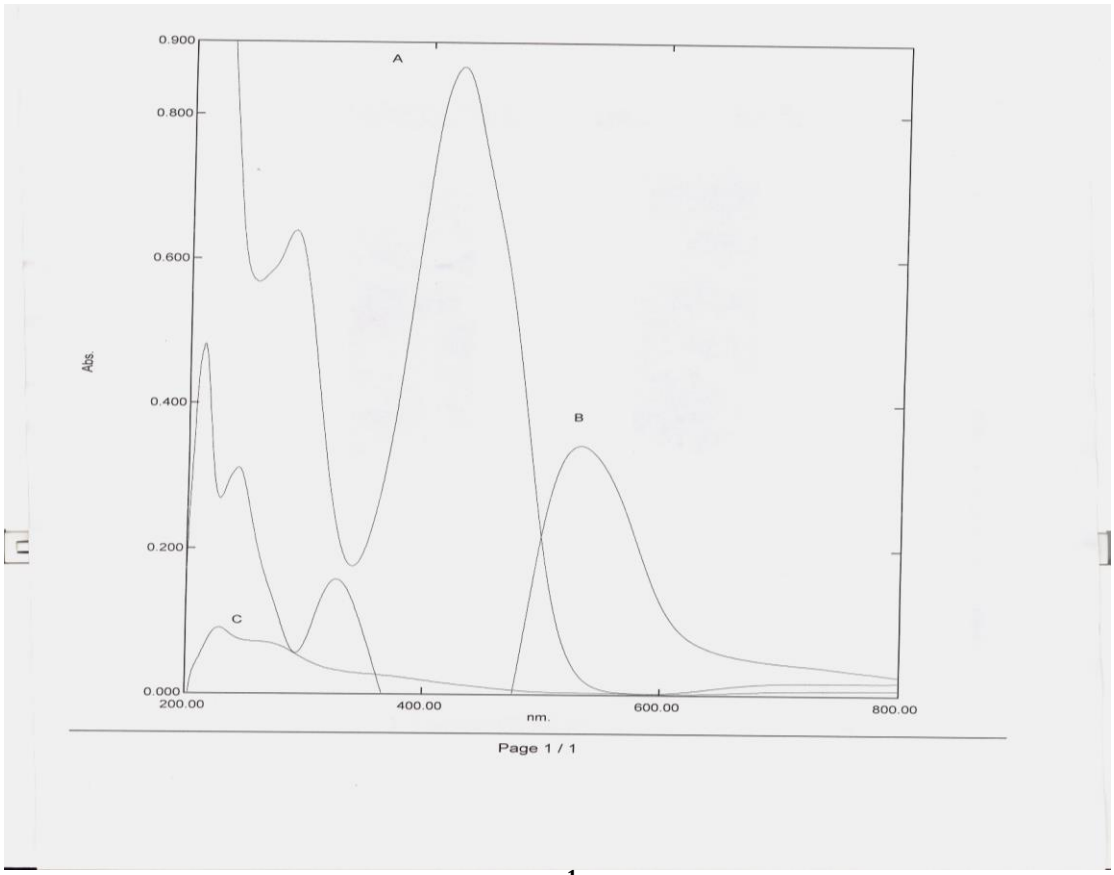


Fig. 5: Absorption Spectra of B(10 µg.ml^{-1}) of Cu(II) ion treated as described under procedure and measured against a regent blank and A the reagent blank measured against distilled water and C the metal ion measured against distilled water.

The effects of various parameter on the absorption intensity of the formed products were studied and the reaction condition were optimized.

- ***Effect of pH:***

The pH of metal complex solutions was adjusted using dilute solutions (0.05 M) HCl and (0.05 M) NaOH, and the effect on absorbance was studied. The absorbance of the complexes was maximum and constant in the pH range given in table (2).

- ***Effect of reagent concentration:***

When various concentration of 2-(2-Iodophenyl azo)-4,5-diphenyl imidazole solution were added to a fixed amount of Ni(II) and Cu(II), 2 ml of (1×10^{-3} M) reagent was found enough to

develop the color to its full intensity and give a minimum blank value and was considered to be optimum for the concentration range (0.05 – 5 μg/ml) of Ni(II) and Cu(II).

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- ***Calibration Curves:***

The calibration curves were constructed at their respective absorption maxima and these were linear over concentration range as given in table (2) for each metal ion. The molar absorptivity and Sandell's sensitivity are given in table (2).

Table 2: Analytical Characteristics of metal 2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole complexes

Characteristic	Ni(II)	Cu(II)
Beer's law range (μg/ml)	(0.05 – 4.5)	(0.05 – 4.5)
Absorption maxima	566	534
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.26×10^4	1.66×10^4
Sandell's sensitivity	0.0046	0.0038
pH range	6 – 8.5	5.5 – 8.5

- ***Development Time and Stability Period:***

The color intensity reached maximum after metal ions had been reacted with (2-IPAI). The colors obtained were stable for at least 24 h and this period was sufficient to allow several measurements to be performed sequentially.

- ***Composition of the Complexes:***

The composition of the complexes was studied[25,26] by Job's method and mole ratio method. A break at a 1:2 (M:L) suggested the formation of M ($C_{21}H_{15}N_4I$) where M = Ni(II), Cu(II). Fig. (6, 7).

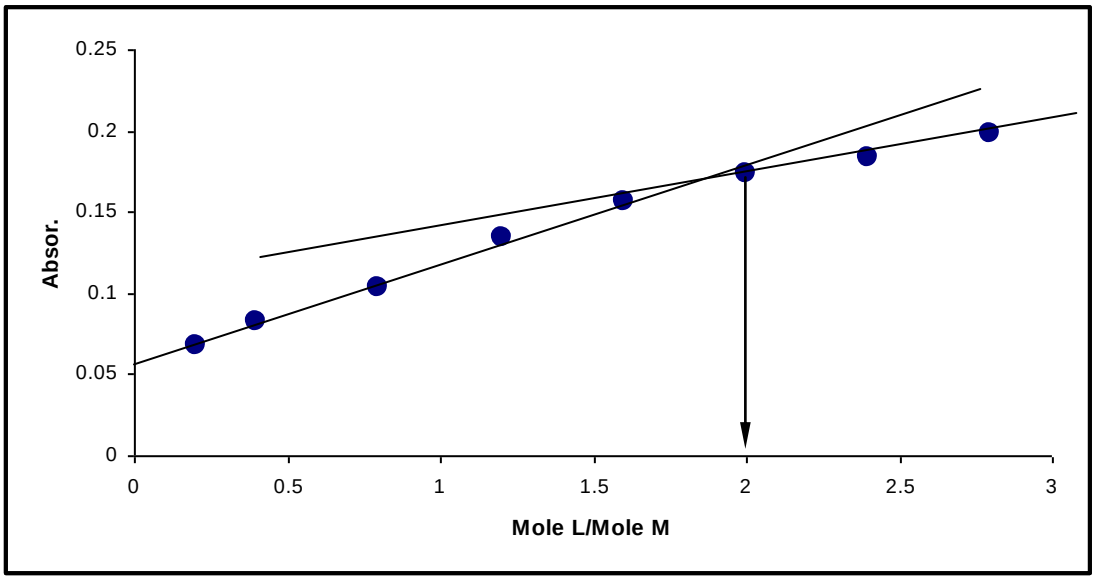


Fig. 6: Mole ratio of reagent to sample for the nickel complex formed

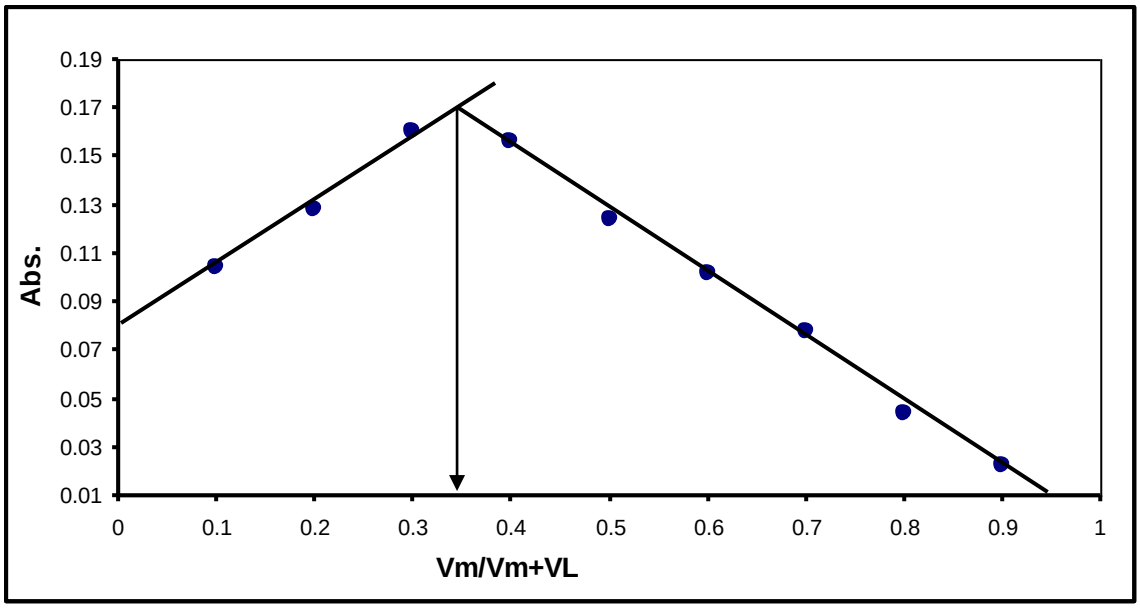
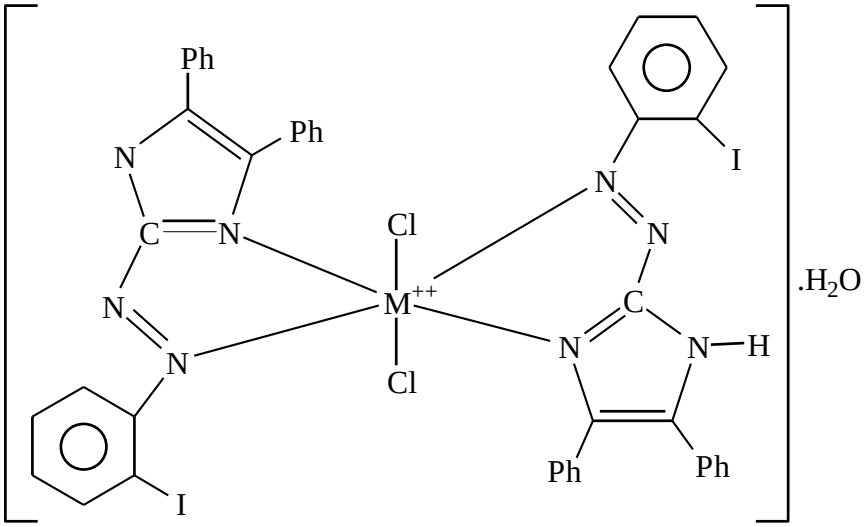


Fig**. 7: Study of the mole ratio of the reaction between Cu(II) and the reagent**

On the basis of the (C.H.N), IR, and analytical data the structure of both complexes can be suggested as follows:



where: M = Cu or Ni

- ***Sensitivity:***

To determine the accuracy and precision of the method, Nickel an Copper ions were determined at three different concentration. The results shown in (Table 3), indicate a satisfactory precision and could be obtained with proposed method.

Table 3: accuracy and precision of the method

Amount of Ni^{+2}, Cu^{+2} taken ppm	Error % for Ni(II)	Error % for Cu(II)	R.S.D % for Ni(II)	R.S.D % for Cu(II)
0.3	–1	–0.66	0.699	0.636
1	-1	-3	0.43	29
1.5	-1.33	-2	0.31	0.45

Results for determinations seven

- ***Interferences:***

The effects of diverse ions on the determination of these metal ions were studied in detail. To test of diverse ions were determined by the general procedure, in the presence of their respective foreign ions. Each of these metal ions can be determined without any interference in the presence of a 50 fold excess of cations (Table 4).

Table 4: Effect of foreign ions

Foreign ions	Amount added μg	Interferences with Ni(II)	Interferences with Cu(II)
Co^{++}	0.5	+4.1	+0.8
Hg^{++}	0.5	+5.0	+11.3
Pd^{++}	0.5	–4.4	–5.5
Bi^{+++}	0.5	0.0	0.0
Ba^{++}	0.5	0.0	–0.01
Cd^{++}	0.5	+6.6	–4.7
Zn^{++}	0.5	+11.2	–3.0
Ag^{++}	0.5	–10.5	–7.7
Mg^{++}	0.5	–0.01	+0.01
Cl^{-}	0.5	–0.08	–0.01
Br^{-}	0.5	0.0	–0.02
So_4^{--}	0.5	–0.01	0.0

Biological Effect:

Agar diffusion method[27], were used for the determination of antibacterial activity of the prepared compared compounds 0.1 ml of an overnight broth bacterial culture was spread on anutrient agar. Sterilized discs (6mm in diameter). Evolution of the above mentioned compounds for their antimicrobial activities showed that these compounds exhibited both antibacterial and antifungal actives. The results are presented in Table (5)., Fig. (8). The tested compounds showed activity against all types of bacteria.

However compound (2-IPAI) showed no activity against p.auroginosa. It should be mentioned that the antimicrobial results were obtained concentration 1×10^{-4} M for all tested compounds.

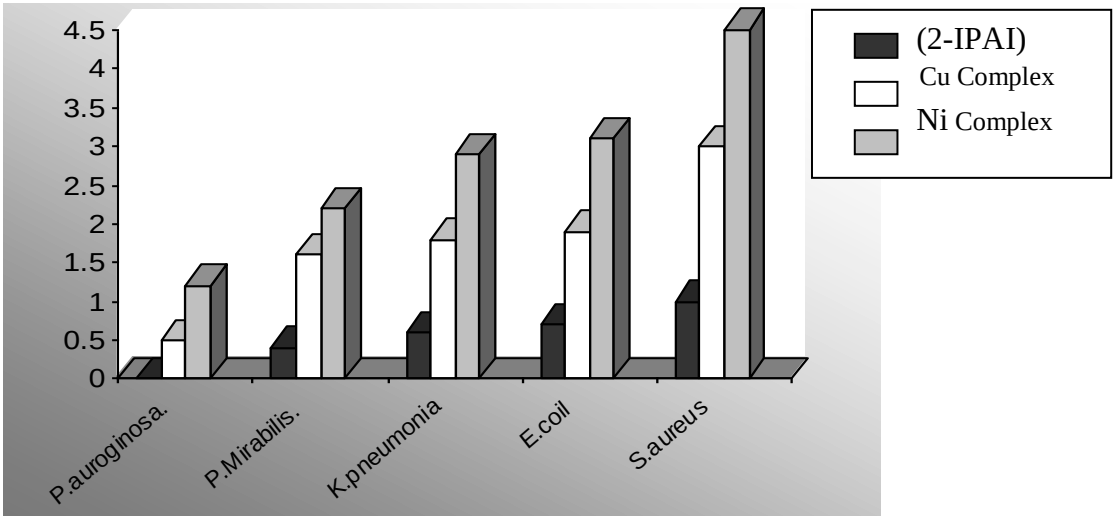


Fig. 8: Inhibition zones of the reagent and complexes of nickel(II) and copper (II) with the reagent.

Table 5: Antibacterial activities of the tested synthesized compound

Reagent and it's Complexes	Inhibition area (mm)				
	P.auroginosa.	P.mirabilis	K.Pneumonia.	E.Coli.	S.aureus.
(2-IPAI) (1B)	-	±	±	±	±
Ni(2-IPAI)(2B)	±	±	±	±	±
Cu(2-IPAI)(3B)	±	±	±	±	±

Note : mm = - (4-6) mm = + (0.2-4) mm = ±

Conclusions:

2-[(2`-Iodophenyl azo)]-4,5-diphenyl imidazole react with Cu(II) Ni(II), which form water insoluble complexes which can be easily dissolved in ethanol. The present method has been found to be simple, rapid and applicable for the determination of metals in the presence of each other, which makes it an alternative to the existing methods for the determination of these metals.

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