Water soluble analytical reagent like 1-(2'-chloro-5'-sulpho phenyl)-3-methyl-4-azo-(2",5"dichloro-4''-sulphonic acid)-5-pyrazolone as a spectrophotometric reagent for Ni (II)

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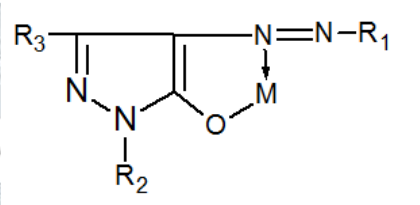
***Abstract* :** 2,5-dichlorosulphanilic acid is coupling with 1-(2'-Chloro-5'-Sulphophenyl)-3-Methyl-5-Pyrazolone in alkaline medium to give 1-(2'-Chloro-5'-Sulphophenyl)-3-Methyl-4-Azo–(2",5"dichloro-4''-sulphonic acid)-5-Pyrazolone. It was characterized by elemental analysis and spectral studies. The metal chelates of the same were prepared with Niickel (II), Copper (II), Cobalt (II) and Iron (III). The composition of complexes have been established by spectral (UV–vis, IR) studies and physico chemical studies. The antimicrobial activity of ligand and its metal chelates were screened against various gram-positive (+) and gram-negative (-) organism. The results show that all these samples are more or less active agents against various organisms.

***Keywords**:* 1-(2'-chloro-5'-sulphophenyl)-3-methyl-4-azo-(2",5"dichloro-4''-sulphonic acid)-5-pyrazolone, Metal chelates, Spectral studies, Physico chemical studies, Antibacterial activity.

I. INTRODUCTION

Pyrazolone and Azopyrazolone compounds are widely used as an analytical reagents, they are capable of forming chelates with a number of metal cations [1,2] Many metal complexes of these azo dyes have been studied. In pyrazolone ring 4-position is reactive, aryl diazonium chlorides is coupling with pyrazolone ring at 4-position to form 4-arylazo derivatives, which constitute an important class of azo dyes. These heterocyclic azo dyes are also good chelating agents. [3-9]



Where,

R_1 = aryl group
R_2 = alkyl or aryl group or hydrogen
R_3 = alkyl or aryl group or hydrogen

The azo-derivatives of 5-pyrazolones amd their metal complexes have wide applications in dye industry as well as analytical reagents for determination of trace metals and it is predicted to have some biological applications[10-11].The present paper comprise the synthesize and characterize the solid complexes of the newly ligand containing the pyrazolone moieties, 1-(2'-Chloro-5'-Sulphophenyl)-3-Methyl-4-Azo–(2",5"dichloro-4''-sulphonic acid)-5-Pyrazolone with Ni (II) investigate their spectral (UV–vis, IR) studies, physico chemical studies and antimicrobial effects.

II. MATERIALS AND METHODS

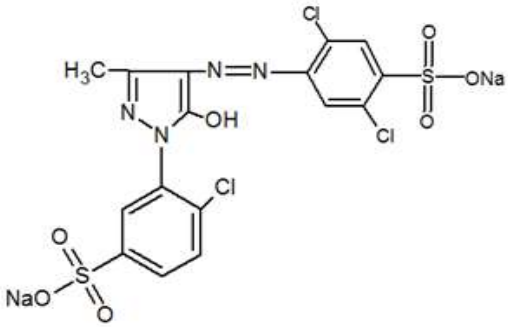
2.1 Materials

All the chemicals used were of pure grade (Merck and B.D.H). The melting points of all complexes were determined by open capillary method and were uncorrected.

2.2 Synthesis of Ligand

Diazotization of 2,5- dichlorosulphanilic acid (38gms, 66%) and then in alkaline medium coupling with 1-(2'-Chloro-5'-Sulphophenyl)-3-Methyl-5-Pyrazolone (2.8 gms, 0.01 M) 1-(2'-Chloro-5'-Sulphophenyl)-3-Methyl-4-Azo–(2", 5"dichloro-4''-sulphonic acid)-5-Pyrazolone (CSMADCSP) was prepared. The completion of reaction, NaCl solution (10 % w/v)

was added with stirring to precipitate out the solid material. It was then filtered off, dried and recrystallized from mixture of dimethyl formamide (DMF) and acetone. Reagent CSMADCSP is orange yellow crystals, soluble in water and DMF, insoluble in ethanol, methanol, acetone and chloroform. (M.P. above 310°C)



1-(2'-chloro-5'-sulpho phenyl)-3-methyl-4-azo- (2",5" dichloro-4''- sulphonic acid)-5-pyrazolone (CSMADCSP)

2.3 Synthesis of metal complex

The complex was synthesized by 0.01 M corresponding ligands were dissolved in water and 0.005 M metal salts also dissolved in minimum quantity of water. The pH was adjusted to 9.0 using suitable buffer solution. The resulting solutions refluxed on water bath for 4 hrs. The solid mass was filtered. The percentage yield of chelates was in the range of 80-90 %. All the chelates were powdered well and dried at 110°C over a period of 24 hr. The synthesized complexes were recrystallized from DMF.

2.4 Measurements

The metal contents were estimated using standard methods [12]. The IR spectra were recorded on "Perkin Elmer" FTIR spectrophotometer (RX-1) in KBr pallet. A reflectance spectrum of ligand was recorded on a Beckman –DK-2A spectrophotometer using MgO as reference. The antimicrobial properties of the above azo dye & their complexes are studied by using Agar diffusion plate technique described by Bryant [13].

2.4.1 Determination of the Molar Ratio of the Metal ions to the Ligands Spectrophotometrically

The mole ratio of the metal ions to the ligands was studied spectrophotometrically using molar ratio and continuous variation methods. The spectrophotometric method was used to confirm the data obtained by conductimetric and pH-metric methods. UV absorption can be used to determine stoichiometry of the complexes and this method appears to be valuable for studying complexes with low stabilities.

2.4.1.1 Molar Ratio Method

In this investigation, the concentration of the metal ions were maintained constant and the ligands concentrations were varied [14], so a series of metal-ligand aqueous ethanolic solutions were prepared with different [L]/[M] ratios. The absorptions of these solutions were measured using UV spectrophotometer at λmax of the expected complex ML2. Absorbance versus [L]/[M] curves were drawn for all complexes.

2.4.1.2 Continuous Variation Method

This method was used to confirm the data obtained using molar ratio and conductivity methods. In this method the mole fraction was varied by changing the concentrations of the two components, maintaining the total number of moles constant [13]. A series of metal-ligand aqueous ethanolic solutions were prepared with different metal mole fractions. The mole fraction of the metal was plotted against the absorbance of the expected complex at λmax of the complex. The measured absorbance increase as the molar ratio [M]/([L] + [M]) increase until the actual molar ratio of the complex is reached, after this point the absorbance becomes lower because the metal has no absorption at λmax of the complex.

III. RESULTS AND DISCUSSION

3.1 Infrared spectra

The IR spectra of the ligand and the complexes were recorded in the form of their KBr pellets. The important absorption bands observed were as follows:

Compound	-OH Stretching cm^{-1}	N = N cm^{-1}	C-O-H banding of –OH in Pyrazolone cm^{-1}	S = O Stretching in $–SO_3H$ group cm^{-1}
CSMADCSP Ligand	3515	1568	1376	1199,1042
Ni (II)-CSMADCSP	-	1570	1398	1195, 1038

The band of (OH) phenol group was absent in the spectra of all prepared complexes, which indicated deprotonation and involvement of the enol oxygen in chelation.

3.2 ^{1}H-NMR spectra

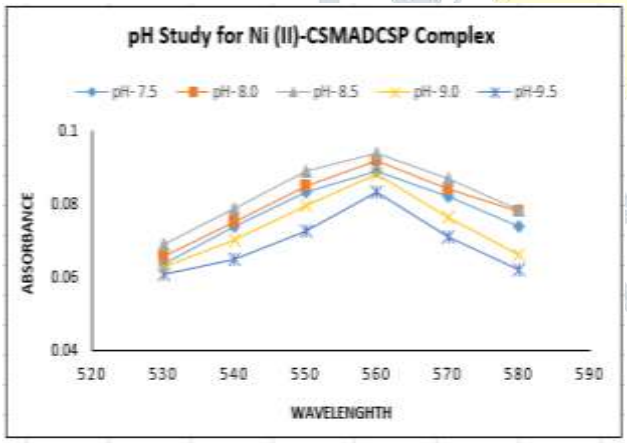
The NMR spectra of the ligand gave the multiplate between 7.6-7.9 δ ppm for aromatic protons, singlet at 4.6 to 4.6990 δ ppm for -OH and singlet at 2.0 to 2.2 δppm due to the aliphatic $–CH_3$ group protons. Thus the structure of ligand is confirmed.

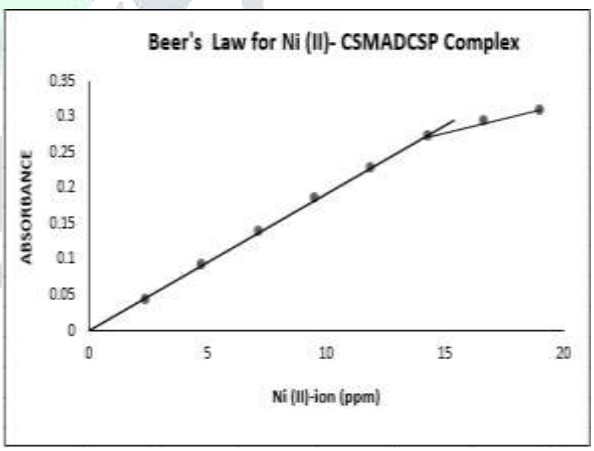
3.3 pH study

The Orange yellow coloured reagent CSMADCSP is soluble in water and shows maximum absorbance at 470 nm. The reagent CSMADCSP forms green coloured complex with Ni (II) in pH range 8.0 to 10.0. To study the effect of pH, 2 ml 0.002 M Ni (II) solution, was mixed with 2 ml 0.02 M solution of ligand in water was mixed. pH of solution was adjusted to 8.0, 8.5, 9.0 , 9.5 and 10.0 with suitable buffer solution. All solution was diluted to 25.0 ml in volumetric flask and absorbance was measured between 400 nm and 600 nm against reagent blank. Absorbance spectra shown in fig. (1) Shows that maximum absorbance is found at 570 nm in all cases. Maximum complex formation occurs at pH 8.5. Thus in all further studies pH 9.0 was used. The absorbance spectra of the reagent Shows λ max at 470 nm. At 570 nm where complex shows maximum absorbance [fig. (1)] the reagent does not absorb much, and hence 570 nm wavelength was used in further studies.

3.4 Verification of beer's law:

In 100 ml beakers, varying amount of 0.002 M Ni (II) solutions 0.5 ml, 1.0 ml …5.0 ml. were taken and 3.0 ml 0.01 M reagent solutions were added to each beaker. pH of each solution was adjusted at 9.0 using buffer. All solution was diluted to 25.0 ml with water in volumetric flask, and absorbance was measured at 570 nm against reagent blank. The Beer's law plot is shown in fig. (2).



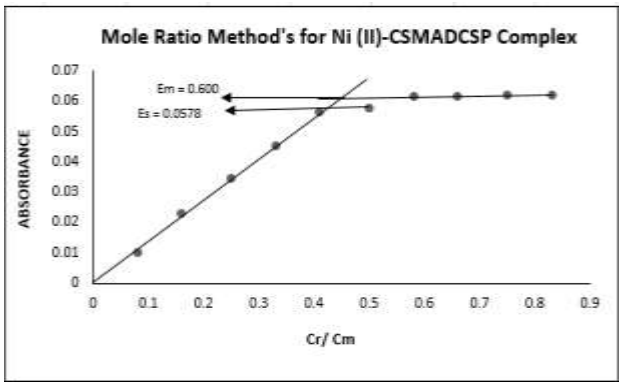


3.5 Molar Ratio Method

It was observed that the absorption increases linearly as the ligand concentration increase, because of the formation of the complex until the solution reaches the actual molar ratio of the investigated complex. At this point all of the added materials were completely reacted and the absorption observed is the absorption of the investigated complex alone. After this point, the excess amount of the added ligand causes an inflection in the straight line that is because the ligand has an absorption value differ from that of the complex at λmax of the complex [13]. [L]/[M] ratio corresponding to the inflection point in (Abs-[L]/[M] curve) indicates to the actual [L]/[M] ratio of the investigated complex. It was found that all of the complexes in this investigation are able to be stable in the form ML2.

In this method equimolar of metal ions as well as the reagent (0.001 M) were used. By keeping the Concentration of reagent solution (6.0 ml, 0.001 M) constant, while varying the amount of metal ion solution from 0.5 ml to 5.0 ml, 0.005 M with increment of 0.5 ml, a series of solutions were prepared.

By using buffer solution the pH of the solutions were adjusted to 9 and the contents were diluted to 25 ml in volumetric flask by using deionised water. At 570 nm wavelength Absorbances of these solutions were recorded and plotted against the ratio of metal ion concentration to reagent concentration. The results are show in the graph.



It is evident from the graph that, the absorbance gradually increases up to the molar composition of metal to the reagent and after that it becomes constant indicating 1 : 2 (M : L) Stoichiometry of the complex.

3.6 Continuous Variation Method

This method was used to confirm the data obtained using molar ratio and conductivity methods. In this method, The measured absorbance increases as the molar ratio [M]/([L] + [M]) increase until the actual molar ratio of the complex is reached, after this point the absorbance becomes lower because the metal has no absorption at λmax of the complex. It was found that all of the curves have inflection points at mole fraction around (0.33); this means that all of the complexes in this investigation have the form ML2 and this form is the most stable form.

3.7 Determination of Stability Constant and Gibbs Free Energy:

Stoichiometry was found (1:2) M: L using Job's method of continuous variation and Yoe and Jone's mole ratio method. Stability constant was determined from the graphs using following formula.

$$K_s = \frac{1-\alpha}{4\alpha^3 C^2}$$

$$\alpha = \frac{Em-E_s}{E_m}$$

E_m = maximum absorbance obtained from the horizontal portion of the curve.

E_s = absorbance at the stoichiometric molar ratio of the metal to reagent in the complex.

K_s = stability constant.

By using above relationships the stability constant was calculated using the data obtained from both methods. E_m and E_s were obtained from graph. The high values of (K) refers to high stability of prepared complexes.

Average stability constant found from two methods was 1.726×10^8.

The thermodynamic parameters of Gibbs free energy (DG) were also studied. The DG data have been calculated from the equation.

$$\Delta G^o = -RT \ln K_s$$

$$\Delta G^o = -15.3842 \text{ K}\cdot \text{Cal /mole}$$

The negative value of (DG) indicates that the reaction between Ligand and metal ions understudy are spontaneous.

3.8 DETERMINATION OF NICKEL IN GERMAN SILVER :

The standard sample (preanalysed) of German silver (0.2200 gm) was dissolved in nitric acid (1 : 2) by heating on a water-bath for 30 to 40 minutes. The solution was evaporated on a water-bath to a volume of 5 ml but not to dryness and excess of nitric acid was removed. The resulting solution was made upto 250 ml in a volumetric flask by using deionised water.10 ml of this solution was diluted to 100 ml in a volumetric flask.

In a 25 ml beaker an aliquot of the above diluted solution (4.0 ml) was taken. By using buffer solution pH of the solution was then adjusted to 9.0. Then 1.0 ml of 0.01 M CSMADCSP reagent solution was added. The solution was diluted to 25

ml with deionised water in a volumetric flask. The absorbance was measured at 570 nm against the reagent blank. The results are tabulated below; graph is shown in fig.4.2 (V).
German solution: 0.2200 gm German silver in 250 ml stock solution.
:. 5.0 ml stock solution contains 4.4 mg german silver; 5.0 ml aliquot contained 0.2200 mg german silver.

Reagent solution : 1.0 ml (0.01M)
pH : 9.0
Final volume : 25 ml
Wavelength : 570 nm

From the above experiment,
Absorbance was found to be 0.216, which corresponds to 0.1809 mg Nickel in 4.0 ml finally diluted solution of brass.

Nickel found in german silver sample = 23.96 %
(Reported nickel in german silver sample = 23.00 %
% Relative error = 0.96 %

3.9 Antimicrobial activity
After incubation, the zone of inhibition was measured the data for selected cultures are given table No. 1.

TABLE NO:-1
Antibacterial activity

Name of Azo dye Compound	**Zone of Inhibition in mm.**		
	Staphylococcus Aureus	***Bacillus Megaterium***	***Escherichia coli***
Ni (II) – Dye-I	➢ 60	100	75

The result indicates that the Ni (II) exhibits higher activity towards most of the plant pathogenic organisms studied than the ligands. The other metal complexes did not show any significant increase in activity as compared to the ligands. The results suggest that variation in structure on coordination affects the growth of microorganisms and may result in to inhibitory or reduction in toxicology of metal ions towards some organisms.

IV. CONCLUSIONS

- The dye forms intense coloured complexes with Ni (II) and few other metal ions which are completely soluble in water and hence, there is no necessity of extracting the complexes, This saves costly and toxic solvents. However this reduces its selectivity.
- The dye solution and the complexes are stable over a long period.
- It is well suited as metallochromic indicator. Compared to many other metallochromic indicators which are soluble in ethanol, it is soluble in water.
- It can be used to analyse alloys and medicinal sample with fairly high accuracy.

V. ACKNOWLEDGEMENT

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