

Spectroscopic Elucidation and Biological Assay of Hybrid Salicylaldehyde-Thiosemicarbazone Nickel & Copper Complexes

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Abstract

Thiosemicarbazide Salicylaldehyde legend and their nickel (II) and copper (II) complexes were prepared directly under optimum conditions for 30 minutes. Complexes have been characterized by physicochemical techniques (FT-IR and U.V.). The ligands and their nickel (II) and copper (II) complexes exhibited moderate water solubility. However, they are recorded as appropriate stability in an aqueous solution. I.R. spectrum showed the main functional groups C=N, C=S, N-N, C=C, and legend metal function. Maximum absorption of compounds was measured from 245 to 334 nm. Complexes predict approximately trigonal bipyramidal symmetry. Both the ligand and their complexes established biological evaluation against bacteria and fungi. These results pave the way for using nickel (II) and copper (II) complexes proactive as anti-bacteria and anti-fungi.

Keywords: Novel Complexes, Thiosemicarbazone, Electronic Absorption Spectral Bands, and Biological Assay.

DOI: 10.47750/pnr.2022.13.S03.133

INTRODUCTION

Schiff bases are condensation products carrying amines. Otherwise, azomethines (C=N) functional group by condensing derivatives of primary amines with carbonyl compounds, which Hygo Schiff first reported in 1864 (1). These compounds have essential reproduction as the most commonly used organic compounds and have many applications in diverse fields of analytical, biological, and inorganic chemistry. Moreover, have extended significance in medical and pharmaceutical areas due to a varied spectrum of physical activities like anti-inflammatory, analgesic, antimicrobial, anticonvulsant, and anticancer (2).

Thiosemicarbazones have a general formula $R_1R_2C=N-N.H.-C.S.-NR_3R_4$ ordinarily reacts as chelating ligands with transition metal ions by bonding through the sulfur hydrazinic nitrogen atom. The pharmacological activity is refereed via the N—C=S group, which is of substantial chemotherapeutic interest (3). Thiosemicarbazones of or-(N)-heterocyclic aldehydes and ketones have various chemotherapeutic characteristics, including antimalarial, antibacterial, and antiviral features (4). Condensation of thiosemicarbazides with aldehydes or ketones generates thiosemicarbazones (TSCs) (5). Sulfur and one of the hydrazinic nitrogen atoms coordinate in thiosemicarbazides and thiosemicarbazones, well-known ligands. A five-membered chelate ring is formed through hydrazinic nitrogen atom coordination.(6). In the solid state and in

solution, thiosemicarbazones exist in the thione form (IV), and they are known to tautomerize into a mixture of thione (IV) and thiol (V) forms. (7). When interacting with transition metal ions, the stereochemistry adopted by thiosemicarbazone ligands is mainly determined by an extra coordination center in the ligand moiety and the charge on the ligand, which is structured by the thione-thiol equilibrium (8). By using the disc diffusion method, tetradentate Schiff base ligands derived from Knoevenagel condensation of -ketoanilides and furfural with o-phenylenediamine and diethylmalonate, as well as their Cu (II) complexes, showed antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and Pse Complexes have been displayed to have more potent antibacterial activity than free ligands. (9). Cu (II) bidentate complexes with Schiff bases derived from 2, 6-diacetylpyridine (L1), 2-pyridine carboxaldehyde, 3-amino-5-methyl isoxazole with 5-methyl furan-2-carboxyaldehyde, 5-methyl thiophene-2-carboxyaldehyde, and pyridine-2-carboxyaldehyde coordinate through the azomethine nitrogen. The results exposed that the (L1) ligand is more effective against *E. coli* than the other bacteria, but does not affect *S. aureus*. Practically, all bacteria are more susceptible to metal compounds. (3). The (MICs) method was used to test the Schiff base of aroyl-hydrazone and its copper complexes for antibacterial activity against two Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and two Gram- negative

bacterium strains (*E. coli* and *P. fluorescence*). Schiff base was active against two Gram-negative bacterial strains with MIC, but not against two Gram-positive bacterial strains. Cu (II) mixed ligand complexes with the Schiff bases N-(2-hydroxy-1-naphthylidene)-4-chloroaniline (L1) and N-(2-hydroxybenzylidene)-2,3-dimethylaniline (L2) have been shown to have some antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis*, and *S. typhi*., but their complexes have a comparatively greater amount of activity. (9). N-(2-hydroxy-1-naphthylidene) phenylglycine and its copper complexes have been studied for antibacterial activity. The antifungal screening results revealed that after complexation, the ligand's activity improved (3). Cu (II) complexes have exhibited superior antifungal activity compared to the ligand and the corresponding metal salts. With using the excellent diffusion method, antifungal activity was demonstrated against *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataticola*, and *Candida albicans*. The antifungal activity of Clomiphene citrate Cu compounds has been tested using the Batemann poisoned food approach against two fungi, *A. flavus* and *A. niger* (10). Salicylaldehyde with formaldehyde and piperazine moiety, in addition to its metal polychelates, are fungicidal against *Candida albicans* and *Aspergillus niger*. Cu (II) polychelate had a high level of activity against *Candida albicans*, while the other had a low level of activity. Because enzymes that necessitate a free hydroxyl group for their activity tend to be especially sensitive to deactivation by the metal ion of polychelates, the presence of N and O donor groups in the ligand and its metal polychelates hindered enzyme synthesis. Metal polychelates in general are more poisonous than ligands. Antifungal action was seen in neutral Cu (II) complexes with Schiff bases generated from 3-nitrobenzylidene-4-aminoantipyrine and aniline, p-nitroaniline, or p-methoxyaniline. (11). For that reason, in continuation of the occurring study on Salicylaldehyde-

Thiosemicarbazide and their nickel (II) and copper (II) complexes, we report herein the spectroscopic elucidation and biological potency against fungi, Gram-positive and Gram-negative bacterial strains have been examined.

METHODS & MATERIALS

Absolute methanol CH₃OH

Thiosemicarbazide NH₂.CS.NH.NH₂

Salicylaldehyde for synthesis C₇H₆O₂

Nickel (II) Chloride (hexahydrate) NiCl₂. 6H₂O

Copper (II) Sulphate (pentahydrate) CuCl₂. 5H₂O

Preparation of Ligand

The ligand Salicylaldehyde-thiosemicarbazide was prepared due to method described by Pahontu et al (2013). mixing equimolecular amounts of 1.5 g thiosemicarbazide and 0.01 mol (1.52 g) salicylaldehyde in 100 ml absolute methanol and reflux on a water bath for 30 minutes. These Schiff bases were used to compose complexes of further metal ions of Cu²⁺, Ni²⁺, and their salts CuSO₄.5H₂O and NiCl₂.6H₂O. The condensation products were filtered, crystallized from methanol and dried. The percentage yield of the product was calculated using the following equation : (12)

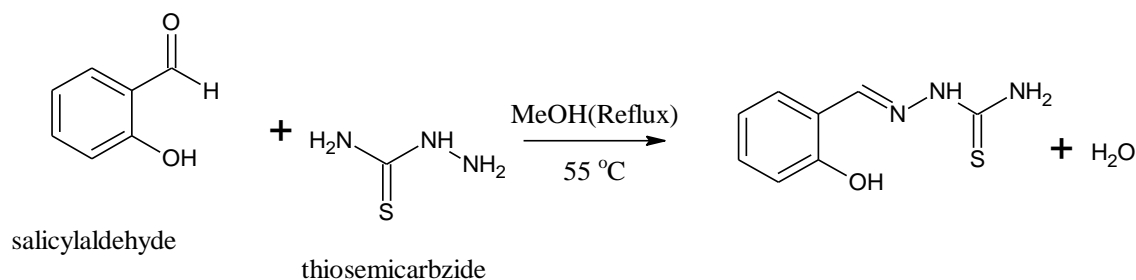
$$\text{Yield \%} = \frac{W_{\text{Prac}}}{W_{\text{Theo}}} (1)$$

Where:

W Prac. = Practical Weight.

W Theo. = Theoretical Weight.

Fig 1 shows the preparation of salicylaldehyde-thiosemicarbazide



preparation

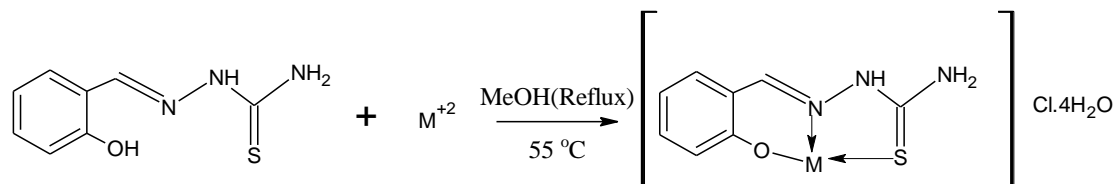
Preparation of Complexes

The metal complexes were prepared by mixing 3:1 molar ratios of Ni (II) and Cu (II) salts and the ligand, respectively, in absolute methanol. Depending on the metal cation used, the mixtures were refluxed in a water bath for a time. The formed complexes were filtered, washed several times with pure dry methanol, and dried, the yield of

Equation 1: Legend

complexes calculated as the equation above. Chemical characterization was applied to the complexes, FTIR (KBr) spectroscopy was measured from 200 to 4000cm⁻¹ on JASCO, FT/IR-4100 LE Serial No. B18736106, protect, Japan. While U.V./ visible- spectra were recorded at SHIMADZU, UV-VIS Spectrophotometer, Model: UV-2700 240V EN, Serial No.A11675100378 LP, Japan.

Spectrophotometer from 200 to 800 nm. With 1 cm path length. Water was used as a solvent.



M= Cu²⁺ Ni²⁺

Equation 2: preparation of complex

In addition, copper and nickel concentrations were calculated on Atomic Absorption Spectroscopy (AAS): Savanta AA, Serial No. AA7638 GBC.

The conditional stability constants (β') of the prepared complex were investigated by using the general formula;

$$\beta' = \frac{[\text{complex}]}{[M][\text{ligand}]} \quad \text{Equation 3 conditional stability}$$

The reaction was obtained at pH 5.75 by substituting with ethylenediaminetetraacetic acid (EDTA). During the experiment, the samples contained; 0.4 M Cu²⁺, and 5 m Mole TSC ligand while, EDTA concentration was performed up to 0.5 M. The computer program PSEQUAD aided in calculating proton dissociation hydrolysis constant, conditional stability continuous individual spectra of metal complexes and their species. The general stability of the copper complex complexes (β) was calculated from the dependent stability constants: $\beta = \beta' \times \alpha_H$, where

$$\alpha_H = 1 + ([H^+] \times 10^{pK_1}) \text{ and } [H^+] = 10^{-4.7} \quad (13).$$

Biological Assay

Copper and nickel complexes were used to test the antimicrobial activity using the disc diffusion method: Fungus - *Candida albicans* MTTC 227, Gram-positive bacteria - *Staphylococcus aureus* MTCC 96, Gram negative bacteria - *Escherichia Coli* MTCC. Inoculums of each bacterial strain were inoculated in 3ml of Mueller Hinton Broth and incubated at 37°C for 24h. After the incubation period, the culture was diluted.

Bacteria inoculums were prepared by growing cells in Mueller Hinton Broth (Himedia) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 10⁴ CFU/ml. Yeast was grown on Sabouraud Dextrose Broth (SDB) at 28°C for 48 h. Antimicrobial activity was carried out using disc diffusion method. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures (100 μ l of suspension containing 10⁸ CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at 1000 μ g/disc concentration of the compounds. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using the respective solvent. Gentamicin (10 mg /disc) was used as a positive control.

The plates were incubated for 24 h at 37°C. The zone of inhibition was recorded in millimeters, and the experiment was repeated twice.

RESULTS AND DISCUSSION

It is known from salicylaldehyde thiosemicarbazone, nickel, and copper, as central atoms, the complex compound is obtained by simply reacting metal salts and the ligands, all dissolved in methanol. Adding a base (soft or strong) leads, in general, to the anionic form of the complex. The strong base may be affected by forming a metal hydroxide precipitate instead of complexation. The deprotonated ligand is attached to metal ion yielding [M II (L)]⁺ entities. Contrary to this expectation, the complex with the twice deprotonated ligand was impossible to obtain with salicylaldehyde thiosemicarbazone.

Table 1 physical properties

Items	Yield %	Melting point	Color	stability constants
Cu-TSC	100	217	Pale blue	10 ^{-4,7}
Ni-TSC	89	212	Green	-

I.R. spectra

The characteristic bands of Ni-TSC and Cu-TSC complexes are summarized in Table 3. The ligand salicylaldehyde thiosemicarbazone (STSC) shows a sharp absorption band at 1603 cm⁻¹ corresponding to C=S frequency, and the band's presence in the region 1600-1614 cm⁻¹ supports the existence of C=S stretching in the metal complexes which used as reference measurement. A sharp band at 1536 cm⁻¹ corresponds to C=N stretching frequency for STSC. On the coordination of the azomethine nitrogen, the I.R. stretching frequency of C=N shows a shift and is observed in the region 1442- 1565 cm⁻¹ in STSC metal complexes (3). In the case of metal complexes, the appearance of bands in 426-474 cm⁻¹ and 445-551 cm⁻¹ correspond to the M-N and M-O vibrational frequencies, respectively. The band's appearance around 1110-1202 cm⁻¹ corresponds to N-N stretching frequency in ligands and metal complexes. Aromatic ν (C=C) value appears around 1439-1489 cm⁻¹. The strong

bands at 3318 cm^{-1} in the spectrum of STSC have been assigned to -O.H. cm^{-1} . The bands around 3114 to 3396 cm^{-1} in ligands are due to -O.H. vibration . (14).

Table 2 Characteristic bands of the ligands and their complexes

Compound	OH cm^{-1}	C=N cm^{-1}	C=S cm^{-1}	N-N cm^{-1}	C=C cm^{-1}	M-S/ M-O cm^{-1}	M-N cm^{-1}
STSC	3318	1536	1603	1110	1489	-	-
Ni (II)	3323	1565	1607	1159	1452	556	472
Cu (II)	3114	1442	1606	1172	1442	455	419

Electronic absorption spectral bands

U.V – Vis spectra were determined in an aqueous solution of the TSC and their complexes. All maximum

absorption values of the TSC as mentioned above and their legends showed a strong band because of $n \rightarrow \pi^*$ transition at 245, 295, and 334 nm for TSC-Ni, TSC-Cu, and TSC, respectively. Copper Complex assigned a d-d band at around 600 nm in its spectrum described by Hricovini and his co-work (15). The addition of Cu^{2+} ion to the solution Fig 3. The percentage of 1:1 of Cu (II)-to-ligand complexes in aqueous solutions has the highest absorption determined at (λ_{max} 295). On the other hand, the TSC value increased (λ_{max} 334nm) from the more extended conjugated p-electron system within the deprotonated form of the ligands. Thus, it likely belongs to the hydroxyl group. pKa values were calculated on the idea of deconvolution of recorded UV-Vis spectra UV-Vis spectra table 3. (16). The structure formula of the complexes as assigned in literature approximately trigonal bipyramidal symmetry (17).

Table 3. U.V. absorption bands of λ_{max}

Compound	STSC	Ni (II)	Cu (II)
λ_{max} nm	334	245	295

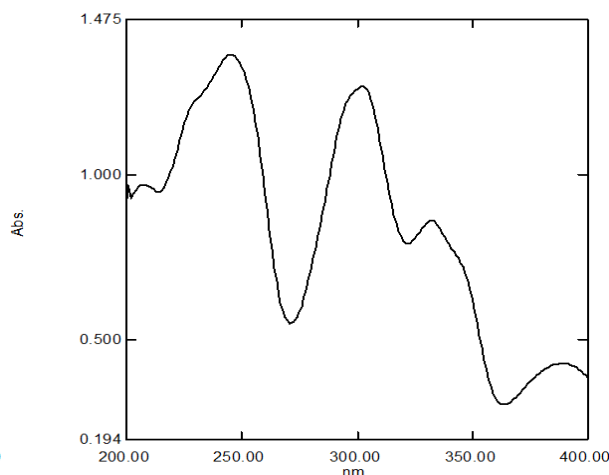
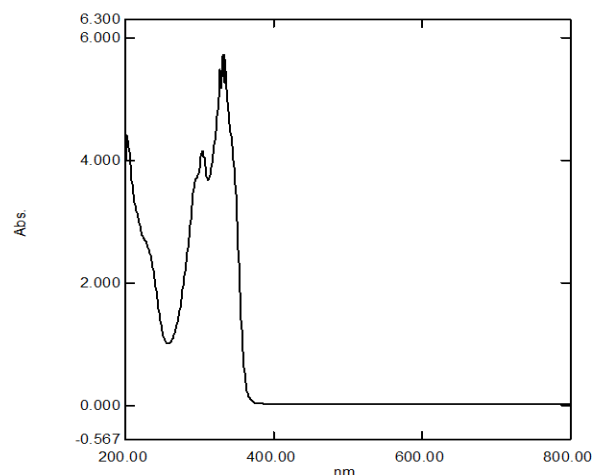


Fig 1. TSC-SLD UV spectrum Fig 2. U.V. spectrum of Ni-TSC complex

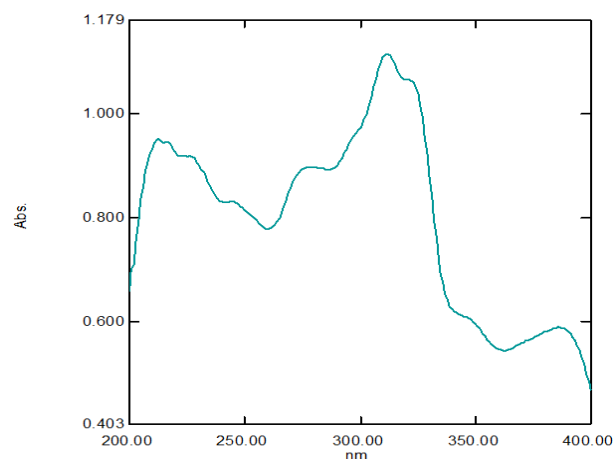


Fig 3. Spectrum of Cu-TSC - complex

Table 3: Absorption bands

S. No.	Sample	Absorption bands cm ⁻¹	
		n → π*	π → π*
1.	STSC	299.4	327.8
2.	Ni-TSC	331.1	408.1
4.	Cu-TSC	320.5	469.4

Biological Assay

The biological assay is presented in table 4. The ligand Salicylaldehyde Thiosemicarbazone displays absent activity against fungi (*Candida albicans*), gram +ve bacteria (*Staphylococcus aureus*), and gram -ve bacteria (*E. Coli*). The Cu (II) complex show high activity against gram +ve bacteria (*Staphylococcus aureus*) and moderate activity against gram -ve bacteria (*E.Coli*). The Ni (II) complex has moderate activity against gram -ve bacteria (*E.Coli*) and have no activity against fungi (*Candida albicans*) and gram +ve bacteria (*Staphylococcus aureus*).

Tell now; hydrogen peroxide is widely considered a normal oxygen metabolite in the aerobic metabolism of cells and tissues. And its interaction with copper and nickel ions can

generate highly reactive and damaging intermediates. Simplified redox behavior of the complex ion in the reaction with hydrogen peroxide, the oxidation number of copper +1, +2, and +3, as summarized by (15), (18), and (19).

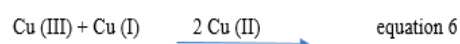
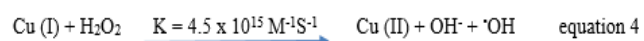
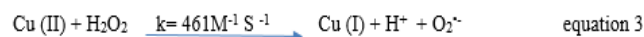


Table 4. Antimicrobial activity

Compound	Gram +ve	Gram -ve	Fungi
	<i>Staphylococcus</i>	<i>E. Coli</i>	<i>Candida albicans</i>
1. Ligand	7	8	9
2. Cu (II)	20	10	10
3. Ni (II)	9	8	9



CONCLUSION

Preparation of nickel and copper complexes from analogous thiosemicarbazide Salicylaldehyde ligand (Ni-TSC and Cu-TSC) 1:1 percentage complexes were formed due to trichelate. I.R. spectrometer results showed the main functional groups. The backbone of the complexes is assigned approximately to trigonal bipyramidal in the geometrical space. ¹H, and ¹³C NMR is required to calculate the singlet of detected functional groups. Besides, the highest absorption value was recorded to the pro legend TSC due to the high pKa value and the formation of the hydroxide group. Furthermore, other techniques are recommended, such as electron paramagnetic resonance (EPR) as a spin trapping technique it presents pro legend photoexcitation. The ligands salicylaldehyde-TSC and their complexes were more active against bacteria and fungi as well.

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