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Advances in the Research of Novel N/S Containing Metal Complexes as Potent Antioxidants

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Abstract: *In the thematic issue we synthesized transition metal complexes with N / S donor ligands. The structures of the obtained complexes were characterized by FT-IR, NMR, elemental analysis, ESR spectral studies, conductometric and magnetic moment measurements. The magnetic moments and electronic spectral studies suggests that the complex has distorted octahedral geometry with unpaired electron lying in* $d_{x^2-y^2}$ *orbital giving* $^2B_{1g}$ *as the ground state. The synthesized metal complexes were successfully investigated for biological activities namely antibacterial, antifungal, DNA binding and cleavage activity etc. In an effort towards the development of metallodrugs as chemotherapeutic agents with interesting antioxidant activity playing an important role in protecting the body against damages caused by reactive oxygen species, we report herein the synthesis and characterization of metal complexes and the antioxidant activity of the novel synthesized complexes.*

Keywords: *Antioxidants, Azoles, Copper, Flavonoid Content, Reducing Power.*

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I. Introduction

Antioxidants are the compounds that terminate the oxidation of other chemicals. The main characteristic of an antioxidant is its ability suppresses the oxidative damage by trapping the free radicals, thereby protecting the cell components and preventing oxidative stress. Oxidative stress is a state when appropriate balance of endogenous antioxidants and reactive oxygen species is diminished. Oxidative stress results in the damage of biopolymers including nucleic acids, lipids, proteins, fatty acids and carbohydrates. So, indirectly antioxidants can be called as vital substances possessing the ability to protect the body from damage caused by free radical. Also, they play important role in body defense system against reactive oxygen species (ROS) by nullifying their toxic effects. ROS are class of highly reactive molecule (includes superoxide radical, hydrogen peroxide radical, singlet oxygen and hydroxyl radical) found to oxidize DNA, proteins and essential cell structures resulting in sever oxidative stress damage. These damages initiate pathological disorders and diseases like arthritis, diabetes, cancer, Alzheimer s disease, Parkinson s disease etc. The body sustains a type of enzyme mechanism for protection against them. Supplementing antioxidants in diet, boosts the source of ammunition against such harmful detoriations. Metal-based antioxidants have gained attention recently for their capacity of protection from damage induced by oxidative stress or free radicals. These metal complex derivatives which show significant antioxidant activity may represent an interesting approach for designing new chemotherapeutic drugs. Benzothiazoles are well established as an important class of nitrogen donor ligands and found to be highly interesting when related to transition metal ions in this regards. The azole moiety represents an important structural component associated with a variety of bio – molecules and having biological activities such as antiviral, antitumor, antimicrobial activities, antituberculosis, antioxidants and antifungal activities [1-9]. During the last few decades there has been a growing interest in the pharmacological properties of these heterocycles and their transition metal complexes due to their ability to function as bio-potent ligands displaying manifold applications in medicine, industry and agriculture. The activity is found to be increased by complexation therefore to understand the properties of both ligands and metal can lead to the synthesis of highly active complexes. The influence of certain metals on the biological activity of these compounds and their intrinsic chemical interest as multi-dentate ligands has prompted a considerable increase in the study of their coordination behavior. In the current issue we deeply investigate the antioxidant power of aforesaid complexes. Our ongoing research work on transition metal complexes with nitrogen and sulphur donor heterocyclic complexes involving such systems led us to describe the synthesis, characterization and antioxidant activity of some transition metal complexes [10-17]. The preparation and study of inorganic compounds containing biologically important ligands is made easier because metal ions used are active in many biological processes. The fact that transition metals are essential metallic elements and exhibit great biological activity when associated with certain metal electronic transfer reactions or the storage of ion has created attention in the study of system.

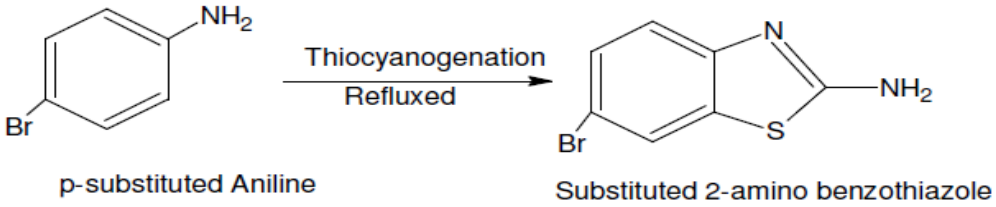
II. EXPERIMENTALS

The synthesis of complexes can be summarized in three steps as follows:

2.1 Synthesis of ligands

2-amino 6- bromo benzothiazole was synthesized using thiocyanogenation method. In this method (0.1 mole) p- bromo aniline was treated with a mixture of (0.1 mole) ammonium thiocyanate, (0.1 mole) cupric chloride and 80 ml glacial acetic acid in a 250 ml three necked round bottom flask, with stirrer, dropping funnel and reflux condenser at room temperature for one and half hour. The thiocyanogenation of aryl amine takes place in the presence of thiocynogen gas, which is generated insitu by the reaction of cupric chloride and ammonium thiocynate.

After cooling the reaction mixture, add 100 ml concentrated HCl, and heat again for half an hour, then cool it and then saturated solution of sodium carbonate (Na_2CO_3) is added to neutralize it, till the solid was formed. The solid separated out was filtered and washed cold water, dried and recrystallized with ethanol.



Scheme 1: Synthesis of 2-amino 6-bromo benzothiazole as ligand

2.2 Synthesis of Copper Surfactants

Copper Palmitate / Copper Caprylate were prepared by mixing one gm of Palmitic acid / Caprylic acid into 25 ml ethyl alcohol, shake the mixture in hot water bath and then add one drop of phenolphthalein. A saturated solution of KOH in another beaker was prepared then it was added into Palmitic acid / Caprylic acid solution drop by drop until the light pink color appears. Now again in another beaker prepare a saturated solution of $CuSO_4$ (about 2-3 gms in 5 ml H_2O) and mix it into above solution with stirring till the blue colored soap is formed. Filtered and washed with warm water and 10% ethyl alcohol then dried and recrystallised with hot benzene.

$$RCOOH + CuSO_4 + C_2H_5OH \longrightarrow (RCOO)_2Cu$$

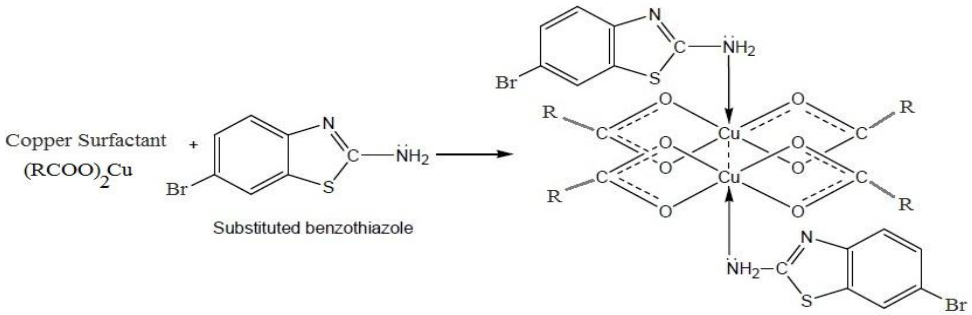
Fatty acids Copper Sulphate Ethanol Copper Surfactants

Here, R= $C_{15}H_{31}$ for Palmitic Acid and C_7H_{15} for Caprylic acid

Scheme 2: Synthesis of Copper Surfactants

2.3 Preparation of Copper complexes

The complexes of Copper Palmitate / Copper Caprylate and substituted benzothiazole were prepared by adding (0.001 mole) copper palmitate/ copper caprylate with (0.002 mole) substituted benzothiazoles in 25 – 30 ml ethyl alcohol and the mixtures were refluxed for about two hours with constant stirring. After cooling the precipitate were filtered, dried and recrystallized with hot benzene.



Scheme 3: Synthesis of Complex

III. RESULTS AND DISCUSSION

The IR spectral analysis was conducted at University of Rajasthan, Jaipur. The IR absorption spectra of the complexes were obtained as KBr discs in the range 400-4000 cm^{-1} on Shimadzu Spectrophotometer. ^1H NMR spectra were recorded at Therachem Laboratory, Jaipur using DMSO as solvent on Bruker Advance

Spectrometer. ESR studies were also obtained from SAIF, IIT Bombay with modulation frequency of 100 KHz at liquid nitrogen temperature (LNT). Magnetic susceptibility measurements were obtained at S.P. University, Vallabh Vidhyanagar, Gujarat.

3.1 Spectral Studies for Complex of copper palmitate with 2- amino 6- bromo benzothiazole [CP(BTA)$_{Br}$]

^{1}H NMR (400 MHz, DMSO-*d*6, *δ)* 0.84 (CH_3-, 12H), 1.22 (-CH_2-, 112H), 3.80 (-NH_2, 4H), 7.82 (Ar -H, 3H). IR (KBr); 1600 υ*(*N−H*)*, 490 υ*(*Cu−O*)*, 560 υ*(*Cu-N*)*, 1509 υ (COO-), 1180 υ (C=S), 832 υ (C-H ―oop$_l$) cm^{-1}; EIMS *m*/*z* (%): 722.5 (70), 678.5 (90), 668.3 (70), 634.5 (100), 590.5 (87). $C_{78}H_{134}O_8N_4S_2Br_2Cu_2$: Anal. Calcd. C 58.32, H 8.35, O 7.98, N 5.61, S 3.49, Br 9.95, Cu 7.91. Found C 58.30, H 8.33, O 7.76, N 5.59, S 3.43, Br 9.87, Cu 7.88.

3.2 Spectral studies for Complex of copper caprylate with 2- amino 6- bromo benzothiazole [CC(BTA)$_{Br}$]

^{1}H NMR (400 Mz, DMSO-*d*6, *δ)* 0.96 (CH_3- 12), 1.21(-CH_2- 48H), 3.99 (-NH_2, 4H), 7.82 (Ar-H), IR (KBr); 1580 υ*(*N−H*)*, 440 υ*(*Cu−O*)*, 500 υ*(*Cu-N*)*, 1558.4 υ (COO-), 1182 υ (C=S), 840 υ (C-H ―oop$_l$) cm^{-1}; EIMS *m*/*z* (%): 683.3 (60), 639.3 (75), 595.3 (95), 551.2 (100), 507.3 (90) Anal Calcd for C46H70O8N4S2Br2Cu2: C 47.71, H 6.05, O 11.06, N 4.48, S 5.53, Br 13.81, Cu 10.97. Found C 47.69, H 6.01, O 11.02, N 4.41, S 5.50, Br 13.77, Cu 10.95.

3.3 ESR spectrum

The ESR spectra of the complexes were recorded at X-Band at modulation frequency of 100KHZ at liquid nitrogen temperature. TCNE was used as the field marker. The ESR spectrum of the complex recorded at liquid nitrogen temperature. The values of g_{ll}, $g\perp$ and g_{av} calculated are 2.38 - 2.23, 2.04 - 2.07, and 2.166 - 2.198 resp. In the octahedral complexes the unpaired electron may lie in $d_x^2 - _y^2$ or d_z^2 orbital. The values of go was found to be 2.0027. Hence, the g tensor values are in the order $g_{ll} > g\perp > g_o$. These data when referred to literature suggests that the complex has distorted octahedral geometry with unpaired electron lying in $d_x^2 - _y^2$ orbital giving $^2B_{1g}$ as the ground state. The geometric parameter G is the measure of Cu-Cu interaction. According to Hathway, if the G value is greater than 4 the exchange coupling interaction between two copper centers is negligible. If the value of G is lesser than 4 a considerable interaction is reported. In the copper complexes the G values were found to be greater than 4, which clarifies that the local tetragonal axes aligned parallel or slightly misaligned and consistent with a $d_x^2 - _y^2$ ground state. Also, this is confirmation of negligible exchange coupling between the two copper centers in the complex.

3.4 Magnetic Moment Studies:

The studies that were reported earlier suggest that majority of Cu (II) complexes display magnetic moment values in range of 1.75-2.20 B.M. The lower magnetic moment values in propanol - benzene as to that of in solid state suggests [$Cu_2(RCOO)_4L_2$] nature of complex where L represents solvent molecule in solution but replaced by ligand on complexation.

3.5 Conductivity Studies:

The nature of synthesized complexes were found to be non electrolytic in nature as the molar conductance value in benzene were found to be 3.9- 6.14 mhos cm^2 mol^{-1}.

3.6 Reducing Power [18]

- The reducing power can be determined by the method of Athukorala et al [19].
- 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min.
- Then, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm.
- The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance is measured at 700 nm.
- Ascorbic Acid (5-25mg/ml), was used as positive control.

The reducing power of a compound is referred to its electron transfer capacity in a redox reaction. Therefore, the reducing capacity of a complex may serve as a significant indicator of its potential antioxidant activity. Overall it leads to the conversion of free radicals in less reactive or inert products. Dietary antioxidant such as ascorbic acid was used for comparison. Transition metal complexes are called antioxidants because of their ability to scavenge free radicals and reducing effect [20-21].

Table No.1 Standard curve for Reducing Power using Ascorbic Acid

Concentrations (mg/ml)	O.D. at 700 nm
0	1.660
5	1.331
10	1.185
15	1.047
20	0.994
25	0.859

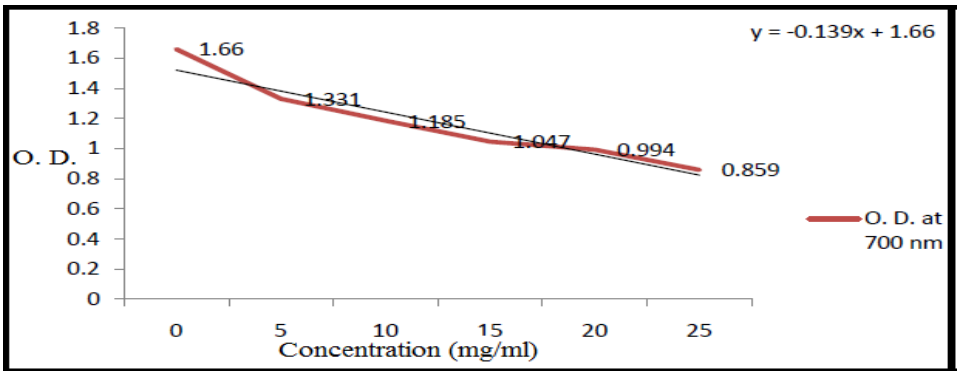


Fig 1. Standard Curve depicting Reducing Power using Ascorbic Acid

3.7 Nitric Oxide Radical Scavenging (NO) Assay [17]

- Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent (Green et al) [22].
- 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer is added to 2.0 ml of extract and reference compound in different concentrations (20 - 100 mg/ml).
- The resulting solutions are then incubated at 25°C for 60 min. A similar procedure is repeated with methanol as blank, which serves as control.
- To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylenediaminedihydrochloride in 2% H_3PO_3) is added and absorbance of the chromophore formed is measured at 540 nm.
- Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations.
- The nitric oxide radicals scavenging activity can be calculated according to the following equation:

$$\% \text{ Inhibition} = (A_0\text{-}A_1) / A_0 \times 100$$

Where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample.
Nitric oxide radical generated from the sodium nitroprusside is measured by the Greiss reduction. Sodium nitroprusside spontaneously generates nitric oxide, which thereby interacts with oxygen to produce nitrate ions that can be estimated using Greiss reagents. Thus, the scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide.

Table No. 2 Standard curve for Nitric Oxide using Ascorbic Acid

Concentrations (mg/ml)	O.D. at 540 nm
0	0
5	1.399
10	2.147
15	2.5

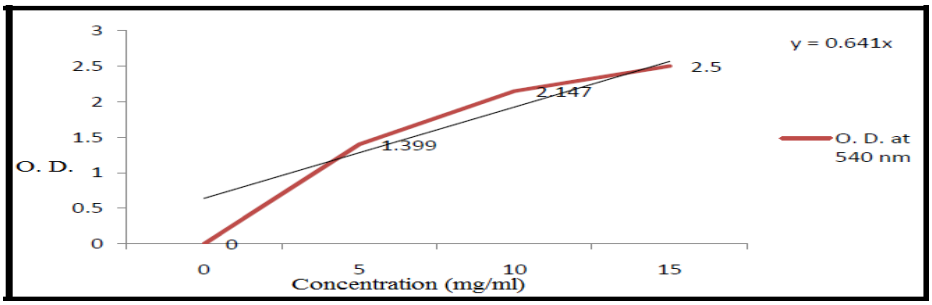


Fig 2. Standard Curve depicting the Nitric Oxide activity using Ascorbic Acid

3.8 Total Flavonoid (TF) [17]

- The amount of total flavonoid content can be determined by Aluminum chloride method (Chang et al) [23].
- The reaction mixture (3.0 ml) comprised of 1.0 ml of extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) is incubated at room temperature for 30 min and absorbance measured at 415 nm.
- Quercetin(5-25mg/ml) was used as a positive control.
- The flavonoid content is expressed in terms of standard equivalent (mg-1 of extracted compound).
- Ascorbic acid (5-25mg/ml**)** was used as a positive control.

Flavonoids are good metal chelators and can chelate many metal ions to form complexes. Previous findings propose that metal chelation reduces electron transfer from Quercetin after complex formation since Quercetin decreases the redox potential of metal-Quercetin complex[24-26].

Table No. 3 Standard curve for Total Flavonoids using Quercetine

Concentrations (mg/ml)	O.D. at 415 nm
0	0
5	0.56
10	0.937
15	1.722
20	2.234
25	2.445

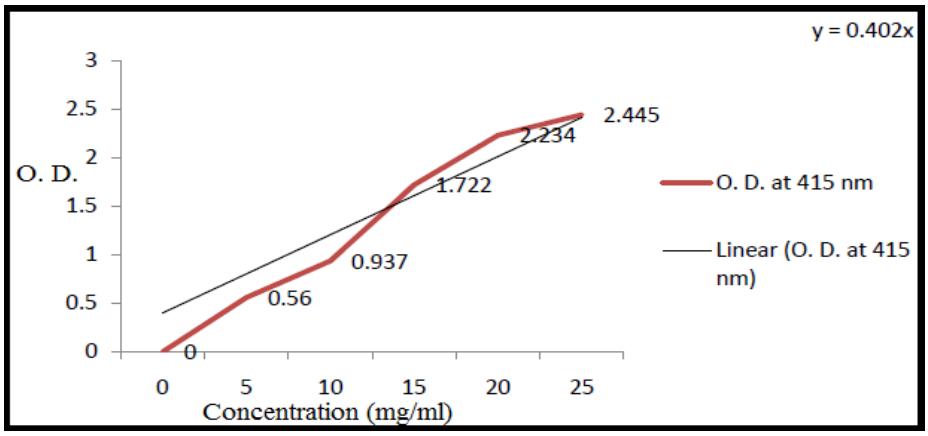


Fig 3. Standard Curve depicting the Total flavonoid activity using Quercitine

Table No.4 A summary of different parameters of synthesized complexes relating to antioxidant activity

S.No.	Analysis	Sample	Optical Density	Quantity	Optical Density	Quantity	Mean Quantity (mg/ml of Extract)
O. D. at 700 nm							
1.	Reducing Power	$CP(BTA)_{Br}$	0.254	2.023	2.059	2.015	2.0194
		$CC(BTA)_{Br}$	0.231	2.056	0.34	1.899	1.9776
O. D. at 540 nm							
2.	Nitric Oxide Radical Scavenging (NO) Assay	$CP(BTA)_{Br}$	1.001	0.312	1.266	0.395	0.3536
		$CC(BTA)_{Br}$	1.372	0.428	1.895	0.591	0.5096
O. D. at 415 nm							
3.	Total Flavonoid	$CP(BTA)_{Br}$	0.403	0.201	0.431	0.214	0.2074
		$CC(BTA)_{Br}$	0.444	0.218	0.512	0.254	0.2368

IV. Conclusion

In the present work, we report the synthesis and characterization of copper complexes with N/S donor ligands. The structure of the ligand and its complexes was determined on the basis of elemental analyses, molar conductivities, IR spectra, ^{1}H-NMR, ESR and conductometric analyses. All these studies give good evidence for the proposed structure. The results of this study clearly indicate that ligand and its metal (II) complexes have effective and potent antioxidant properties. In addition to that antioxidant property of ligand and its complexes was examined. The results shown that all the complexes were excellent scavengers as compared to the ligands. Biological activity studies revealed that the antioxidant activity of the ligand was found to be enhanced on complexation with metal ion. Hence, this study has widened the scope of developing this type of metal based drugs as promising antioxidant agents.

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