International Journal of Engineering& Scientific Research
Vol.7 Issue 11, November 2019,
ISSN: 2347-6532 Impact Factor: 6.660
Journal Homepage: http://esrjournal.com, Email: esrjeditor@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

EVALUATION OF ANTIOXIDENT – PONTENTIAL OF PHARMACOLOGICALLY SIGNIFICANT COMPLEXES DERIVED FROM COPPER – SURFACTANTS WITH SUBSTITUTED BENZO THIAZOLES.

Neha Mathur[1*]
Anil Kumar Sharma[2]

Abstract

In biological processes, inorganic compounds play critical roles and it has been established that many organic compounds used in medicine are activated or biotransformed by metal ions metabolism. Current research endeavor ascertains to propose antioxidant activity of macrocyclic complex compounds which will certainly have specified importance in the field of materials chemistry. Antioxidant activity of few compounds can be enhanced significantly with the complexation of metal ions with nitrogen and sulphur donor ligands. Thus in this backdrop the current research paper elucidates complexation of metallic Copper surfactants (derived from common fatty acids) with substituted 2-amino benzothiazoles. Thin layer chromatography evaluation was done in order to check purity[1-7]. Synthesized compounds were **Complex A** =$CS(BTA)_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole and **Complex B** = $CO(BTA)_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole. Antioxidant efficacy of synthesized compounds was assessed by DPPH assay. Which shows the metal complexes found to be good antioxidant, as comparable with ascorbic acid..

Keywords:

Copper Surfactant,
Metal Complexes,
Fatty acids, Antioxidant,
DPPH Assay,
Ascorbic acid.

Author correspondence:

[1]Department of Chemistry, PNKS Govt, P.G. College, Dausa-303303, Rajasthan
[2] Department of Chemistry, Career Point University, Kota, Rajasthan
Mail : nehavmathur@yahoo.co.in

1. Introduction

The living cells are disrupting by the chain reaction, that is, the attacked molecules lose its electrons and become a free radical which attacks the living cell. Natural and synthetic are the two basic types of antioxidants structures. Various alkyl substitutions containing phenolic group are in general natural antioxidants. Nitrogen compounds, phenolic compounds are categorized as synthetic antioxidants. The satirically hindered phenols and secondary aromatic amines form the primary antioxidants compounds. The hydrogen atom moves from the antioxidant molecule to radical intermediate is the first step of the radical termination.

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid oxidation. The *in* vitro antioxidant activity can be performed by three methods, DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide radical scavenging assay.

Furthermore certain thiazole derivatives are well known for their antioxidant. With the aim of finding a promising antioxidant agents, which may have improved efficacy and fewer side effects compared with existing antioxidants, we considered it of interest to synthesize some new complexes by incorporating Copper Surfactants with 2-amino-6-bromo benzothiazole analogues to investigate their antioxidant property.

MATERIALS AND METHODS:

LR/AR grade chemicals and reagents were used in standard operating procedures.

Synthesis of 2-amino 6-bromo benzothiazole[11-16]: In the current study, with the use of thiocyanogenation method, 2-amino 6-bromo benzothiazole was synthesized. During the experimentation (0.1mol) p- bromo aniline was treated with a mixture of 7.6 gm ammonium thiocyanate and 80 ml glacial acetic acid with (0.1mole) of cupric chloride in a 250 ml in 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. 100 ml concentrated HCl was added after cooling the reaction mixture, 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 with cold water, dried and recrystallised with ethanol.

Synthesis of Copper Surfactants[17-22]:

Second step undertaken was synthesis of Copper surfactants and was done in following discussed sequential manner

- Preapration of Copper Stearate and Copper Oleate by mixing one gm of Oleic acid and Stearic acid into 25 ml ethyl alcohol and shaking the mixture in hot water bath.
- Add one drop of phenolphthalein.
- A saturated solution of KOH in another beaker was prepared then it was added into Oleic acid / Stearic acid solution drop by drop until the light pink colour appears.
- Again in another beaker a saturated solution of $CuSO_4$ (about 2-3 g in 5 ml H_2O) was prepared and mixed into above solution with stirring till the blue coloured soap is formed.
- Filtered and washed with warm water and 10% ethyl alcohol then dried and recrystallised with hot benzene.

Synthesis of Complexes

Last but not the least stem was **Synthesis of Complexes and was carried with following procedure:**

- Preparation of complexes adding (0.001 mole) Copper Stearate / Copper Oleate with (0.002) mole 2- amino-6-bromo benzothiazole in 25 – 30 ml ethyl alcohol
- Mixtures were refluxed for about two hours with constant stirring.
- After cooling the precipitate were filtered, dried.
- Recrystallized with hot benzene.

Identification of Complexes:

Structural elucidation and identification of synthesized complexes were confirmed by using various spectroscopic techniques viz IR, NMR, ESR and magnetic moment were studies as well as

elemental analysis was carried. Thin layer chromatography was undertaken in order to check the purity of the synthesized compound.

ABBREVATIOS USED FOR SYNTHESISIED COMPLEXES:

The synthesized complexes were abbreviated as follows:

Complex A =$CS(BTA)_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole.
Complex B = $CO(BTA)_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole.

ANTIOXIDANT ACTIVITY (DPPH ASSAY)[23-27]

The DPPH assay was used to study radical scavenging activity of test samples according to Scherer and Godoy. The measurement is taken at 517 nm. After the addition of an antioxidant the decrease in the absorption of the DPPH solution was taken. Standard taken is Ascorbic acid.

Principle of Technique

1, 1-Diphenyl- 2-picryl-hydrazyl (DPPH) is a stable free radical and a trap ("scavenger") for other radicals, widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H. Because of a strong absorption band at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, which is measured spectro-photometrically at 517 nm in NANODROP.

Required Reagents, Glassware and Equipments –

0.1mM solution of DPPH in methanol, DMSO (Dimethyl sulfoxide), amber colored reagent bottle, test tubes, ascorbic acid, three decimal place digital balance, and NANODROP.

Procedure

Preparation of Reagents and dilutions

1. **0.1mM DPPH-**

   Stock Solution = 1mM (.02 gm of DPPH in 50 ml of methanol)

   Working Concentration = .1mM (1 ml of 1mM DPPH in 9 ml of Methanol)

2. **Positive Control = Ascorbic Acid**

Stock Solution = 1mg/ml (10 mg in 10 ml DMSO)

Working Concentration = different working Concentration ranging 10 µg/ml to 1000 µg/ml were prepared from stock solution

Table: 1 Dilution of Ascorbic Acid

Stock Concentration (mg/ml)	Required Concentration (µg/ml)	Ascorbic Acid	DMSO	Final Volume
1	10	10	990	1.0 ml
1	100	100	900	1.0 ml
1	250	250	750	1.0 ml
1	500	500	500	1.0 ml
1	750	750	250	1.0 ml
1	1000	1000	0	1.0 ml

To Determine the Antioxidant of the Control

- Dilution of Ascorbic acid was prepared in DMSO solution.
- 500 µl of dilution and 500 µl of 0.1mM solution of DPPH in methanol were added into the eppendroff in dark condition.

- The mixture was shaken and incubated for 30 min at room temperature in the dark condition.
- After the incubation period absorbance was measured at 517 nm in NANODROP.
- Ascorbic Acid used as a positive control for this assay.
- Record the absorbance of ascorbic acid as a positive control as same absorbance as test compound i.e. 517 nm.
- Methanol serves as a blank prepared by adding 500 µl of 1mg/1ml of Ascorbic acid and 500 µl methanol.
- Control was prepared by adding 500 µl of 0.1mM DPPH and 500 µl of DMSO.

- The DPPH scavenging activity is calculated as follows –

$$\textbf{DPPH Scavenging Activity (\% Inhibition)} = \frac{\textbf{(OD of Control – OD of Sample)} \times 100}{\textbf{OD of Control}}$$

- The DPPH antioxidant activity for the test compound was expressed in the form of IC50 value. IC50 (half maximum inhibitory concentration) value was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Table 2 – % inhibition and IC50 value of positive control

Concentration (ug/ml)	OD Value (517 nm)	%Inhibition
10	1.011	49.851
100	0.638	68.321
250	0.463	77.010
500	0.298	85.203
750	0.137	93.197
1000	0.023	98.857
Control	2.014	-

To Determine the Antioxidant of the Given Complex.

- First we diluted test complexes viz complex CS $(BTA)_{Br}$ and CO $(BTA)_{Br}$ in DMSO Solution.
- 500 µl of dilution and 500 µl of 0.1mM solution of DPPH in methanol were added into the eppendroff in dark condition.
- The mixture was shaken and incubated for 30 min at room temperature in the dark condition.
- After the incubation period absorbance was measured at 517 nm in NANODROP.
- Ascorbic Acid used as a positive control for this assay.
- Record the absorbance of ascorbic acid as a positive control as same absorbance as test compound i.e. 517 nm.
- Methanol serves as a blank prepared by adding 500 µl of 1mg/1ml of Ascorbic acid and 500 µl methanol.
- Control was prepared by adding 500 µl of 0.1mM DPPH and 500 µl of DMSO.
- The DPPH scavenging activity is calculated as follows –

$$\textbf{DPPH Scavenging Activity (\% Inhibition)} = \frac{\textbf{(OD of Control – OD of Sample)} \times 100}{\textbf{OD of Control}}$$

- The DPPH antioxidant activity for both the complexes was expressed in the form of IC50 value. IC50 (half maximum inhibitory concentration) value was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Table: 3 Amount of DMSO required to obtain certain Concentrations

Complex	Stock Concentration
Complex A $CS(BTA)_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole.	1mg/1ml
Complex B $CO\ (BTA)_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole	1mg/1ml

Table : 4 Dilution of concentrations with DMSO

Complex	Working concentration 1	Working concentration 2
Complex A $CS(BTA)_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole.	16.5ug/ml	8.0 ug/ml
Complex B $CO\ (BTA)_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole	16.5ug/ml	8.0 ug/ml

Table 5:- % inhibition value of Complex-'A'
$CS(BTA)_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo Benzothiazole.

Concentration (ug/ml)	OD Value (517 nm)	%Inhibition
Control	2.346	
16.5	0.268	88.576
8.0	0.488	79.198

Table 6:- % inhibition value of Complex-'B'
$CO\ (BTA)_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo Benzothiazole

Concentration (ug/ml)	OD Value (517 nm)	%Inhibition
Control	2.346	
16.5	0.350	85.08
8.0	0.552	76.38

RESULTS AND DISCUSSION

Synthesis and characterization of synthesized Complexes derived from Substituted benzothiozoles with Copper stearate and Copper Oleate were done in the present work. The synthesized complexes were tested for anti-oxidant activities by DPPH assay. Both the complexes showed good activity both in DPPH Scavenging radical assay.

Here complex 'A' shows very good activities as compare to Complex 'B' by DPPH assay, due to presence of stearic acid in the Copper Surfactant which is $C_{18}H_{36}O_2$ saturated acid, while Oleic acid having same formula but it is unsaturated acid.

Some of the benzothiazole derivatives exhibited excellent antioxidant property as responded by Bhat et al., Our result can be correlated with the benzothiazole azo ester derivatives as described by Bhat and Belagali 2014, in which compound show the activity at higher concentration .

CONCLUSION[28-31]:

- The given **complex A CS(BTA)$_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole** has the % Inhibition of 88.576 and 79.198 %.
- The given **compound B** The given **compound B CO(BTA)$_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole** has the % Inhibition of 85.05 and 76.38 %

% Inhibition = CS (BTA)$_{Br}$ > CO (BTA)$_{Br}$

Synthesis and characterization of Copper metal surfactants of compounds derived from 2-amino 6-bromo benzothiazole were done in the present work. The synthesized compounds were tested for anti-oxidant activities by DPPH assay. Synthesized compounds A **CS(BTA)$_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole** showed best antioxidant activity both in DPPH Scavenging radical assay, where as compound B **CO(BTA)$_{Br}$: Complex of Copper Oleate with 2-amino 6-bromo benzothiazole** showed comparatively less activity. So, their IC50 values were calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals. The synthesized compound A **CS(BTA)$_{Br}$: Complex of Copper Stearate with 2-amino 6-bromo benzothiazole** have 18 Carbon atom back bone with a carboxylic group and saturation, which show very good radical scavenging activities. Synthesised Copper surfactants compounds are electron releasing which are important in radical scavenging activity.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, Head of the Department of Chemistry, PNKS Govt. P.G. College Dausa, Rajasthan. Special thanks to Dr. B. Lal Clinic of Biotechnology for providing necessary laboratory facilities for the study.

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