ORIGINAL RESEARCH PAPER

Chemistry

STRUCTURAL, FREE RADICAL SCAVENGING ACTIVITY AND α- GLUCOSIDASE INHIBITION OF NEWLY SYNTHESIZED COMPLEXES OF COPPER(II) WITH L-CYSTEIN, METHIONINE AND THREONINE

KEY WORDS: copper(II), amino acids UV, IR, CV, antioxidant activity, enzymatic inhibition.

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ABSTRACT

A new series of closely related yet distinct dinuclear Cu(II) complexes, obtained upon subsequent complexation with all these ligands, are investigated for their structural properties and for their ability to exhibited effective -glucosidase inhibition. These activity studies are mainly focused on evaluating antioxidant activity. The complex of L-theonine effective on the proton release in DPPH assay with an IC50 value of 92.17µM and also exhibited the highest activity in ABTS scavenging with an IC50 of about 61.07 µM for L-cysteine amongst the synthesized metal complexes. A perusal of the data reveals that these complexes of Cu(II) with L-methionine shows effective alpha-glucosidase activity exhibits.

Introduction

L-amino acids possess biodegradable properties, and are therefore useful in various fields, including food science, medicine, and cosmetics (Grecu et al 1986 and Asma et al 2001). Amino acids have special importance compared to other chemical compounds in the sense that they are regarded as the foundation stones of living organisms. Fostered by the crucial role of amino acids in our life, studying their structural, chemical and physical properties becomes very necessary to explain their behavior and potential applications. Methionine and cystein are the amino acids containing sulfur, it helps to prevent disorders of the hair, skin and nails, in lowering the cholesterol levels by increasing the liver's production of lecithin and reduces fat build-up in the liver and body (Usmani et al 1991). In particular, amino acids containing sulphur have shown superior inhibition efficiency attributed to the presence of sulphur atoms as a result of which they can be adsorbed as bidentate ligands with coordination taking place through amino or carboxyl group and –SH (Barouni et al 2008, Zang et al 2005 and Badawy et al 2006). In this communication we report a study of the antioxidant and enzymatic inhibition activity of copper-amino acid complexes to understand their mode of action.

Experimental

Materials and Methods- Chemicals: Acarbose, α-glucosidase Rat intestinal powder was procured from Sigma Aldrich, USA. All solvents were HPLC grade and used further purification. $CuSO_4.5H_2O$, $Cu(NO_3)_2.H_2O$, $CuCl_2$, $Cu(CH_3COO)_2$, CH_3COONa were purchased from alfa acear, Great Britain.

Synthesis of complexes: amino acid-metal complexes were prepared in the deionized water by reacting the corresponding amino acid and metal ion in a 1:2 molar ratio.The $[Cu(L)_2]^{+2}$ complexes were prepared from three different salts of copper and amino acids as ligand. **Infrared Spectroscopy:** Infrared (IR) spectra were obtained by the KBr method using a Bruker Alfa-T model Fourier transform (FTIR) spectrometer (Bruker Instrument Germany). The spectrometer was equipped with a Glober IR source, KBr beam spillter and detector. For each spectrum, 16 scans were obtained with the resolution of 4 cm^{-1}. The obtained IR spectra were proceeding by mean of the program OPUS 7.0.

UV-VIS spectroscopy: The UV-visible transmittance spectra of the complexes were recorded at 25°C on a Shimadzu UV-Vis 160 spectrophotometer.

Cyclic voltametry: The cyclicvoltametric measurements were carried out with a Metrohm Instrument (Germany) having an electrochemical cell with a three-electrode system. The reference electrode was an $Ag/AgCl_2$. Platinum wire used an as a working electrode, Platinum wire electrode used as an auxiliary electrode. The 3 mg of complex were dissolved in supporting electrolyte 25 ml of 0.01 M solution of KCL solution. The voltamograme, peak position and area were calculated using NOVA 1.9 software.

Biological Activities of Metal Complexes α-Glucosidase Inhibition: Method for determination of α-Glucosidase was adopted from Tripathi et al (2013). Rat intestinal acetone powder (Sigma chemicals,USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm×30 minutes the supernatant was treated as crude intestinal α-Glucosidase. 50 µl various dilutions in DMSO (0.1mg /ml solution) were mixed and incubated with 50 µl of enzyme in a 96-well micro plate for 5 minutes. Reaction mixture was further incubated for another 10 minutes with 50 µl substrate (5 mM, pnitrophenyl-α-D-glucopyranoside) prepared in 100 mM phosphate buffer (pH~6.8) and release of nitrophenol was read at, 405 nm spectroph otometerically(MultimodeSynergy H4 micro plate reader, BioTek instrument, inc.Winoosci, VT, USA). All the samples were run in triplicate and acarbose was taken as standard reference compound. Several dilutions of primary solution (5mg/ml DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50% activity (IC_{50}) of the enzyme. Quantification was performed with respect to the standard curve of acarabose (Y = 26.63X +46.26, R_c = 0.958) results were expressed as milligram of acarbose equivalent per ml of extract.

DPPH scavenging activity : free radical scavenging assay

The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. In brief, a 96-well microplate, 25 µl of various dilutions (10-100 µg/ml) of methanolic extract 125 µl of tris–HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004% w/v in methanol) were added. The reaction mixture was shaken well. The DPPH decolourization was recorded at 518 nm on a BioTek$^{Synergy\ H4}$hybrid multimode micro plate reader (BioTek instruments, IncWinoosci, VT, USA.), after 30 min incubation in dark. The percentage of DPPH scavenging by complex dilutions obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of Ascorbic acid (y = 0.731x+14.60; R2 = 0.947). Results were expressed as milligram of Ascorbic acid equivalentper ml of extract.

ABTS free radical Scavenging activity: For ABTS assay, the procedure followed the method of BibhabasuHazra et al. with modification suitable for micro well plates. The ability to test samples to scavenge $ABTS^+$ radical cation was compared to ascorbic acid standard the $ABTS^+$ radical cation was pre generated by mixing 7mM ABTS stock solution with 2.45mM potassium persulphate and incubating for 18 hrs in dark at room temperature until reaction was complete and absorbance of ABTS+ cation solution was 0.637 ($\pm$ 0.02) by diluting with water at room temperature then 20µl of test samples with different concentration were mixed with 180µl of ABTS solution and absorbance was measured at 734nm after 5min. Quantification was performed with respect to the standard curve of ascorbic acid (Y = 0.517X + 40.06, R^2 = 0.985). Experiments were done in triplicates. The concentration was calculated using the following equation: scavenging effect (%) = Ao – A1 / Ao X 100. Where Ao

was the absorbance of the control and A1 was the absorbance in the presence of the sample.

Results and Discussion:

Characterization of metal complexes

Elemental analysis: The elemental analysis confirms the stoichiometric of the compounds, the composition corresponded to a metal-ligand ratio of 1:2. The results of these investigations are presented as following

Table (1) : Elemental analysis data of copper complexes with amino acids.

S.no.	Complex	Empirical formula	Molecular weight	Color	Elemental analysis Calculated (Found)			
					M %	C %	H %	N%
1	$[Cu(cys)_2]$ $2SO_4$	$C_6H_{24}N_2O_4S_2Cu$	491.78	Shining blue	12.92	14.64 (14.45	4.88 (4.07)	5.69 (5.36)
	$[Cu(cys)_2]$ 2Cl	$C_6H_{14}N_2O_4S_2Cu$	378.8	Royal blue	16.86	19.11 (18.84	3.71 (3.89)	07.43 (7.69)
	$[Cu(cys)_2]$ $2NO_3$	$C_6H_{14}N_3O_4S_2Cu$	474.8	Shining blue	13.38	15.16 (15.02	4.00 (4.17)	08.85 (9.04)
	$[Cu(cys)_2]2CH_3COO$	$C_{10}H_{14}N_2O_4S_2Cu$	441.95	Deep blue	14.37	27.15 (26.48	4.53 (5.07)	06.33 (6.56)
2	$[Cu(thr)_2]$ $2SO_4$	$C_8H_{18}N_2O_6Cu$	487.7	Shining blue	13.02	19.68 (19.43)	5.74 (6.07)	5.74 (5.67)
	$[Cu(thr)_2]$ 2Cl	$C_8H_{18}N_2O_6Cu$	372.72	Royal blue	17.04	25.76 (25.45)	4.83 (4.35)	07.51 (7.67)
	$[Cu(thr)_2]$ $2NO_3$	$C_8H_{18}N_2O_6Cu$	370.72	Shining blue	17.13	25.89 (---)	6.20 (---)	11.33 (9.67)
	$[Cu(thr)_2]2CH_3COO$	$C_8H_{18}N_2O_6Cu$	337.87	Deep blue	14.45	32.88 (33.53)	5.48 (5.09)	06.39 (6.63)
3	$[Cu(met)_2]$ $2SO_4$	$C_{10}H_{32}N_2O_4S_2Cu$	547.9	Shining blue	12.41	21.90 (21.68)	05.84 (5.57)	5.11 (5.72)
	$[Cu(met)_2]$ 2Cl	$C_{10}H_{22}N_2O_4S_2Cu$	432.92	Royal blue	16.01	27.71 (---)	5.08 (5.15)	06.46 (6.23)
	$[Cu(met)_2]$ $2NO_3$	$C_{10}H_{22}N_2O_4S_2Cu$	530.98	Shining blue	12.84	22.59 (30.42)	5.08 (5.73)	07.90 (7.68)
	$[Cu(met)_2]2CH_3COO$	$C_{14}H_{22}N_2O_4S_2Cu$	498.07	Deep blue	13.75	33.73 (33.84)	5.62 (6.03)	05.62 (5.68)

Infra red spectroscopy:

The qualitative differences between the infrared spectrum of the free amino acid threonine, cystein and methionine complexes are discussed in order to ascertain ligand to metal ions bonding modes. The unchanged bands after complexation at 1578 and 743 cm^{-1} in the free ligand suggests non-involvement of the coordination which was assigned as ν(C=N) thiazole ring and ν(C–S–C), respectively. The band observed for all the complexes approximate at 3300 to 3380 cm^{-1} in the spectrum assigned to the (N-H), suggesting the possibility of the coordination of ligand through the nitrogen atom at the amine group. While another absorption band appeared at 1632 cm^{-1} range could be explained as υ(COO) where the υ(OCO)sym was noticed at 1420 cm^{-1} range for all the synthesised complexes (Lever et al 1968 and Ning et al 2000). In order to get further information about the metal ligand bonding approximate at 1580 cm^{-1}, this also indicates the involvement of this group in the metal-ligand bond formation. The characteristic infrared bonds which are assigned to most significant functional groups of the amino acids and its metal ion complexes are summarized in Tables (2) and its spectral graphs are represented as Fig-1(a), 1(b) and 1©.

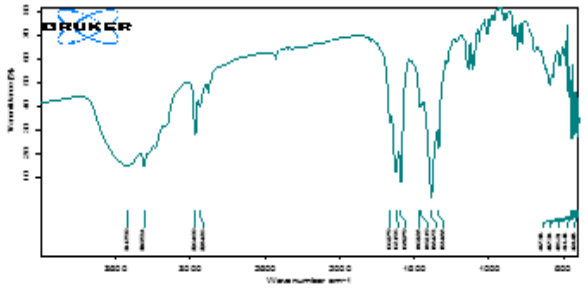

Fig-1(a) :IR Spectra of $[Cu(thr)_2]2NO_3$

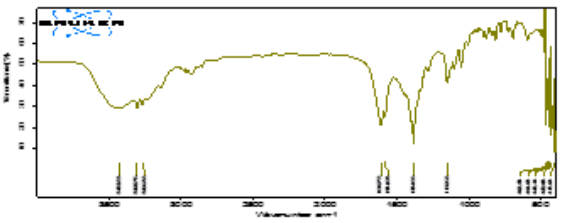

Fig-1(b) :IR Spectra of $[Cu(cys)_2]COOCH_3$

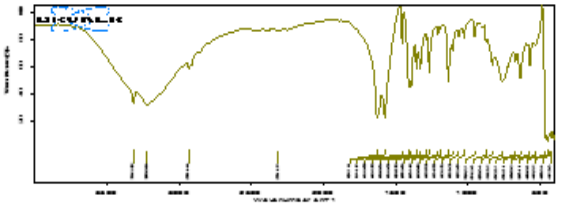

Fig-1C :IR Spectra of $[Cu(me)_2]2SO_4$

Table-2 :IR-frequencies (in cm^{-1}) for copper complexes with amino acids.

SN	Complex	Band cm^{-1}	Group
1	$[Cu(cys)_2]$ $2SO_4$	1583	N-H (bending) bounded with metal
		3309	N-H (stretching)
		1609	C=O bounded with metal
	$[Cu(cys)_2]$ 2Cl	1565	N-H (bending) bounded with metal
		3246-3318	N-H (stretching)
		---	C=O bounded with metal

	$[Cu(cys)_2]2NO_3$	1589	N-H (bending) bounded with metal
		2964-3309	N-H (stretching)
		1622	C=O bounded with metal
	$[Cu(cys)_2]2CH_3COO$	1589	N-H (bending) bounded with metal
		2964-3306	N-H (stretching)
		1621	C=O bounded with metal
2	$[Cu(thr)_2]2SO_4$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1610	C=O bounded with metal
	$[Cu(thr)_2]2Cl$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1609	C=O bounded with metal
	$[Cu(thr)_2]2NO_3$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1609	C=O bounded with metal
	$[Cu(thr)_2]2CH_3COO$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1609	C=O bounded with metal
3	$[Cu(met)_2]2SO_4$	1581	N-H (bending) bounded with metal
		3309	N-H (stretching)
		1621	C=O bounded with metal
	$[Cu(met)_2]2Cl$	1595	N-H (bending) bounded with metal
		3246	N-H (stretching)
		----	C=O bounded with metal
	$[Cu(met)_2]2NO_3$	1589	N-H (bending) bounded with metal
		3264-3309	N-H (stretching)
		1621	C=O bounded with metal
	$[Cu(met)_2]2CH_3COO$	1589	N-H (bending) bounded with metal
		3264-3309	N-H (stretching)
		1621	C=O bounded with metal

UV-visible spectroscopy The Cu(II) ion have the ground term arising from the $t^6 2g\ e^3 g$ configuration in an octahedral field is ^2E_g. The UV-VIS spectra of Cu(II) complexes with the amino acids show absorption bands assigned to intra ligands transitions and a large band around 620 nm (16000 cm^{-1}). There were two bands observed in the electronic spectrum of all the complex, at about 270 nm which can be assigned to $^2B_1g\ ^2B_2g$ and $^2B_1g\ ^2E_1g$ transitions(Tuncay et al 2010 and Benzite et al 2012). The electronic spectrum of the complexes with L-Methionine also exhibits a broad band at 696 nm attributable to d-d transitions. The absorption bands of the complexes corresponded to the n *, n π* and π* π* transitions of -NH_2 and -COO–, Shifts in these bands and the observed d-dtransitions of the compounds, as presented, indicated coordination. Characteristic π π* transitions are observed in all the spectrum of these complexes.The spectral graphs are represented as Fig-2(a), 2(b) and 2(c)absorptions and assignments related to the complexes are listed in Table-3.

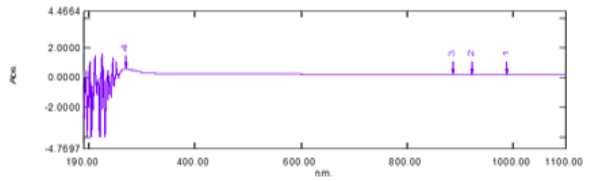

Fig. -2(a) :Uv-vis Spectra of $[Cu(th)_2]2Cl$

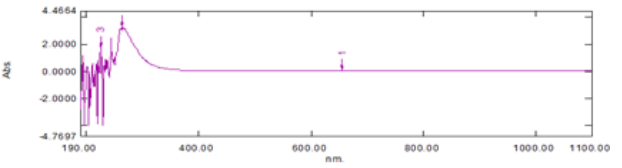

Fig. -2(b) :Uv-vis Spectra of $[Cu(cys)_2]2NO_3$

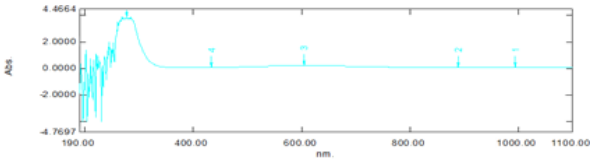

Fig. -2C :Uv-vis Spectra of $[Cu(met)_2]2SO_4$

Table-3: λmax(nm) values (in 100% DMSO solution) for copper complexes with amino acids.

SN	Complex	λ*max(nm)*
1	$[Cu\ (cys)_2]2SO_4$	244
	$[Cu\ (cys)_2]2Cl$	272
	$[Cu\ (cys)_2]2NO_3$	247
	$[Cu\ (cys)\ _2]2COOCH_3$	288
2	$[Cu\ (thr)_2]2SO_4$	267
	$[Cu\ (thr)_2]2Cl$	278
	$[Cu\ (thr)_2]2NO_3$	287
	$[Cu\ (thr)\ _2]2COOCH_3$	262
3	$[Cu\ (met)_2]2SO_4$	263
	$[Cu\ (met)_2]2Cl$	278
	$[Cu\ (met)_2]2NO_3$	267
	$[Cu\ (met)\ _2]2COOCH_3$	266

Cyclic voltammetry Cyclic voltamogram of complexes at Pt electrode shows a single step, one electron-transfer, reversible redox reaction. These observations are consistent with the occurrence of a two step mechanism due to the presence of stabilizing ligands in solution towards Cu(I) and Cu(II). In these cases, more than one complex for Copper may be present in the solution. Complex suggesting that a considerable interaction is occurred between the above amino acids with copper. All the voltamogram clearly represented that reduced moiety of Cu(II) doesn't fullyoxidized in further sweep (Przemyslaw et al 2004). Cyclic voltamogram of $[Cu(met)_2]^{2+}$ there are -456.54, $[Cu(thr)_2]^{2+}$ system at different pH (isoelectric point of amino acids)th e CVs show a single reduction peak at -457.33 mV and $[Cu(cys)_2]^{2+}$ the CVs show a single reduction peak at -456.78 mV. Electrochemical studies of complexes were performed at a Pt as a working electrode shows a single step, one electron-transfer, (Fig-3(a), 3(b) and 3(c)and CV results (in mV) for these complexes are given in Table-4

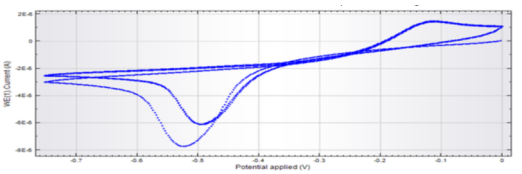

Fig – 3(a): Cyclic volatammogram of $[Cu(cys)_2]2NO_3$

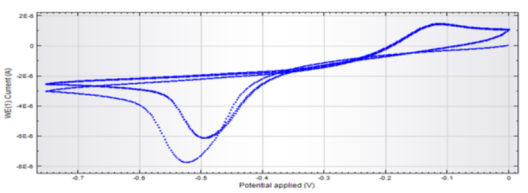

Fig –3(b): Cyclic volatammogram of $[Cu(thr)_2]2SO_4$

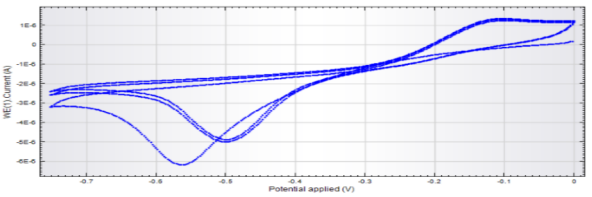

Fig- 3(c): Cyclic volatammogram of [Cu(met)$_2$]2Cl

Table-4: CV results (in mV) forcopper complexes with amino acids

SN	Complex	Reduction Peak(B1)	Oxidation Peak(A1)	Peak (1/2)	Peak width (1/2) V
1	[Cu (cys)$_2$]2SO$_4$	0.12253	-0.45678	0.064512	0.14003
	[Cu (cys)$_2$]2Cl	-0.12312	-0.43543	-0.063087	0.12233
	[Cu (cys)$_2$]2NO$_3$	-0.12321	-0.45622	0.0644667	0.12548
	[Cu (cys)$_2$]2COOCH$_3$	-0.12387	-0.45768	0.059981	0.13795
2	[Cu (thr)$_2$]2SO$_4$	0.12267	-0.45733	0.063789	0.12011
	[Cu (thr)$_2$]2Cl	-0.12309	-0.44556	-0.063156	0.12232
	[Cu (thr)$_2$]2NO$_3$	-0.12282	-0.45348	0.065985	0.12165
	[Cu (thr)$_2$]2COOCH$_3$	-0.12315	-0.45326	0.064771	0.12005
3	[Cu (met)$_2$]2SO$_4$	0.12312	-0.45654	0.063723	0.12231
	[Cu (met)$_2$]2Cl	-0.12306	-0.44759	-0.063365	0.12386
	[Cu (met)$_2$]2NO$_3$	-0.12276	-0.45105	0.064532	0.12237
	[Cu (met)$_2$]2COOCH$_3$	-0.12325	-0.45032	0.064718	0.12154

Biological screening. Biological route for synthesis of copper complexes of amino acids with therapeutic potential is a major challenge. In this research, Cu complexes were synthesized with L-amino acid as ligands which were further evaluated for free radical scavenging and antidiabetic activity. (Aaseth et al 2007, Araya et al 2000 and Decker et al 2000) Research has also shown significant progress in utilization of transition metal complexes as anti-inflammatory agents and free radical quenchers, anti-diabetic agents, anticancer agents, anti-infective agents, anti-proliferative effects (Goodman et al 2004 and Srivastava et al 2005). In view of these facts, there were studied the alpha-glucosidase and antioxidant activities of Cu(II) complexes with N, O, O donor bioactive ligands L-Amino acids.

α-Glucosidase Inhibition: Hereby an excellent inhibitor would limit the absorption of dietary carbohydrates and prevent the postprandial hyperglycemia. Inhibition of α-glucosidase and scavenging of free radicals is a important preventive measures of diabetes (Patil et al 2015, Parihar et al 2015, Markad 2014 and Siriwardena et al 2014).Table-6 demonstrates the IC_{50} of acarbose and metal complexes. Method for determination of α-Glucosidase was adopted from Tripathi et al (2013). Comparison with the predicted data has demonstrated a reasonable correspondence between the experimental and predicted values of inhibition of glucosidase. [Cu(met)$_2$] >[Cu(cys)$_2$]>[Cu(thr)$_2$]show were identified as the most potent antidiabetic agents among the synthesized compounds due to their lower IC_{50} values. Table-5 shows the % inhibition which were given by these complexes and figure-4 were plotted according these values.

DPPH free radical scavenging activity: The effect of antioxidants on DPPH radical scavenging is due to the hydrogen donating ability or radical scavenging activity of the samples (Arulpriya et al 2010). The scavenging reaction between (DPPH) and an antioxidant (H-D) can be written as:

DPPH) + (H-D) DPPH-H + (D)
(Purple) (Yellow)

The antioxidant properties were expressed as 50% inhibitory concentration (IC_{50}) values are illustrated. The antioxidant activity of these complexes can be attributed to the electron withdrawing effect of the Cu(II) ions which facilitates the release of hydrogen to reduce the DPPH radical. This proton release were very effective in [Cu(cys)$_2$], with an IC50 value of 97.43. The % free radical scavenging of DPPH for these complexes are given in Table-7and Table-8 shows their IC_{50} values. Figures-5represents graphical arrangement of % inhibition.

ABTS free radical scavenging activity: The ABTS radical scavenging ability of the tested compounds were given with thier IC_{50} values in Table -8. The scavenging of the $ABTS^+$ radical by the L-amino acid complexes was found to possess moderate to high activities relative to those of the standard.[Cu(cys)$_2$]2SO$_4$exhibited the highest activity with an IC_{50} of about 73.56 g/ml amongst the synthesized metal complexes. Figures-6 represents graphical arrangement of % inhibition and the % free radical scavenging of ABTS for these complexesare given in Table-7.

Table-5: % inhibition of α-Glucosidase for complexes

S.No.	Acarbose	Conc. in µg/ml	Cysteine								Threonine							
			Com-1	Error± SD	Com-2	Error± SD	Com-3	Error± SD	Com-4	Error± SD	Com-5	Error± SD	Com-6	Error± SD	Com-7	Error± SD	Com-8	Error± SD
1	17.87	200	6.47	0.02	5.83	0.01	6.26	0.02	4.55	0.01	4.94	0.02	4.66	0.03	5.67	0.02	4.43	0.02
2	26.04	400	13.63	0.03	12.39	0.03	11.53	0.04	11.43	0.03	15.33	0.01	13.34	0.01	14.49	0.01	12.55	0.04
3	48.39	600	21.81	0.01	18.75	0.04	20.69	0.01	17.38	0.02	20.61	0.04	17.72	0.02	22.51	0.04	17.35	0.01
4	59.15	800	29.64	0.04	28.14	0.06	30.44	0.03	27.93	0.04	28.62	0.03	22.85	0.05	25.13	0.03	27.51	0.06
5	68.23	1000	37.65	0.03	35.82	0.02	38.27	0.02	33.65	0.03	40.20	0.02	34.67	0.03	38.19	0.04	36.61	0.03
6	79.87	1200	47.36	0.06	45.22	0.01	46.57	0.06	41.64	0.02	54.17	0.01	47.95	0.02	43.87	0.06	48.46	0.01

Com-1 [Cu (cys)$_2$]2SO$_4$ Com-2 [Cu (cys)$_2$]2Cl Com-3 [Cu (cys)$_2$]2NO$_3$ Com-4 [Cu (cys)$_2$]2COOCH$_3$
Com-5 [Cu (thr)$_2$]2SO$_4$ Com-6 [Cu (thr)$_2$]2Cl Com-7 [Cu (thr)$_2$]2NO$_3$ Com-8 [Cu (thr)$_2$]2COOCH$_3$

S.No.	Acarbose	Conc. in µg/ml	Methionine							
			Com-9	Error± SD	Com-10	Error± SD	Com-11	Error± SD	Com-12	Error± SD
7	17.87	200	3.41	0.01	3.44	0.02	4.06	0.01	2.95	0.03
8	26.04	400	12.27	0.03	13.62	0.03	11.57	0.03	10.34	0.01
9	48.39	600	21.29	0.02	19.38	0.04	18.61	0.05	15.77	0.02
10	59.15	800	29.98	0.04	26.26	0.03	28.03	0.02	25.84	0.05

11	68.23	1000	39.69	0.02	36.25	0.02	32.44	0.04	34.16	0.02
12	79.87	1200	50.09	0.01	42.56	0.01	46.06	0.01	39.8	0.04

Com-9 $[Cu(met)_2]2SO_4$ Com-10 $[Cu(met)_2]2Cl$ Com-11 $[Cu(met)_2]2NO_3$ Com-12 $[Cu(met)_2]2COOCH_3$

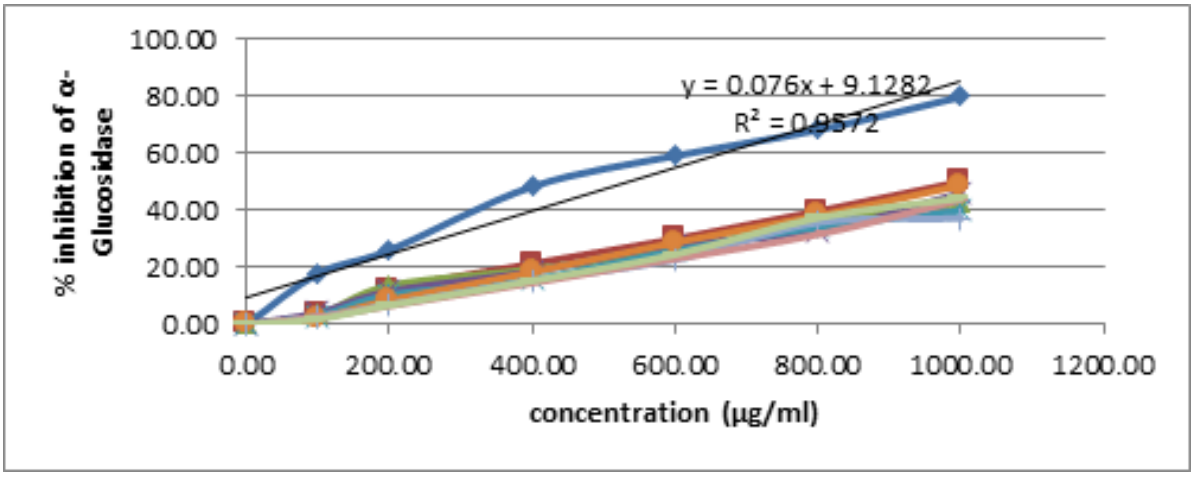

Fig- 4 Graphical representation of % inhibition of α-Glucosidase for Cu(II)-Methionine

Table-6:IC_{50} values of Cu(II)-Amino acid complexes for % inhibition of α-Glucosidase

Complex Name		Ic_{50} value in µg/ml
Acarbose		674
Cu(II)-Methionine	$[Cu(met)_2]2SO_4$	1056
	$[Cu(met)_2]2Cl$	1055
	$[Cu(met)_2]2NO_3$	1059
	$[Cu(met)_2]2COOCH_3$	1061
Cu(II)-Cysteine	$[Cu(cys)_2]2SO_4$	1257
	$[Cu(cys)_2]2Cl$	1255
	$[Cu(cys)_2]2NO_3$	1251
	$[Cu(cys)_2]2COOCH_3$	1254
Cu(II)-Threonine	$Cu(thr)_2]2SO_4$	1267
	$[Cu(thr)_2]2Cl$	1259
	$[Cu(thr)_2]2NO_3$	1258
	$[Cu(thr)_2]2COOCH_3$	1260

Table-7: % free radical scavenging of DPPH for complexes

S.No.	Ascorbic acid	Conc. in µg/ml	Cysteine								Threonine							
			Com-1	Error± SD	Com-2	Error± SD	Com-3	Error± SD	Com-4	Error± SD	Com-5	Error± SD	Com-6	Error± SD	Com-7	Error± SD	Com-8	Error± SD
1	29.17	200	42.54	0.03	37.12	0.01	23.1	0.01	62.18	0.04	54.28	0.01	58.5	0.02	33.49	0.03	37.78	0.02
2	41.47	400	45.6	0.01	37.47	0.03	25.32	0.01	62.44	0.03	67.32	0.03	53.27	0.01	38.82	0.01	42.73	0.01
3	59.38	600	45.38	0.02	41.8	0.06	26.31	0.02	64.7	0.06	69.2	0.02	58.78	0.01	37.5	0.02	47.9	0.06
4	67.15	800	42.56	0.02	47.57	0.01	38.53	0.04	62.23	0.01	63.42	0.01	62.46	0.02	40.04	0.03	48.74	0.02
5	76.09	1000	42.34	0.03	46.88	0.02	46.34	0.02	67.35	0.04	66.58	0.04	65.85	0.03	42.05	0.01	44.62	0.01

Com-1 $[Cu(cys)_2]2SO_4$ Com-2 $[Cu(cys)_2]2Cl$ Com-3 $[Cu(cys)_2]2NO_3$ Com-4 $[Cu(cys)_2]2COOCH_3$
Com-5 $[Cu(thr)_2]2SO_4$ Com-6 $[Cu(thr)_2]2Cl$ Com-7 $[Cu(thr)_2]2NO_3$ Com-8 $[Cu(thr)_2]2COOCH_3$

S.No.	Ascorbic acid	Conc. in µg/ml	Methionine							
			Com-9	Error± SD	Com-10	Error± SD	Com-11	Error± SD	Com-12	Error± SD
1	29.17	200	56.73	0.02	45.67	0.02	56.63	0.03	48.34	0.02
2	41.47	400	54.36	0.01	49.7	0.03	57.32	0.01	43.54	0.03
3	59.38	600	58.24	0.02	52.73	0.04	59.67	0.04	46.78	0.06
4	67.15	800	52.48	0.01	52.43	0.02	55.62	0.01	53.62	0.04
5	76.09	1000	52.17	0.03	42.05	0.01	53.61	0.03	57.31	0.01

Com-9 $[Cu(met)_2]2SO_4$ Com-10 $[Cu(met)_2]2Cl$ Com-11 $[Cu(met)_2]2NO_3$ Com-12 $[Cu(met)_2]2COOCH_3$

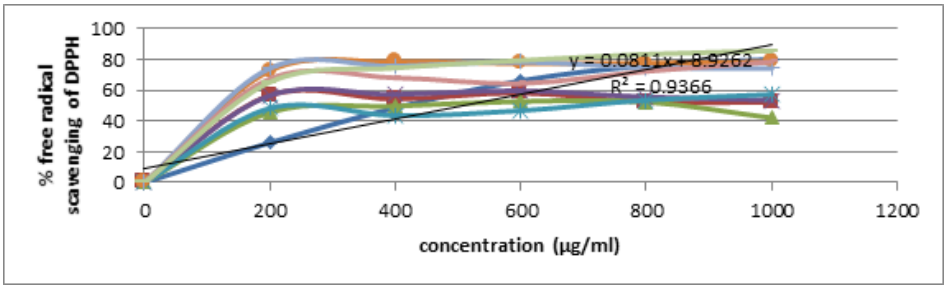

Fig- 5: Graphical representation of % free radical scavenging of DPPH for complexes Cu(II)-Cysteine

Table-7: IC_{50} values of Cu(II)-Amino acid complexes for % free radical scavenging of DPPH

Complex Name		Ic_{50} value in µg/ml
Ascorbic acid		47.58
Cu(II)-Cysteine	$[Cu(cys)_2]2SO_4$	97.43
	$[Cu(cys)_2]2Cl$	97.88
	$[Cu(cys)_2]2NO_3$	97.54
	$[Cu(cys)_2]2COOCH_3$	98.05
Cu(II)-Methionine	$[Cu(met)_2]2SO_4$	121.3
	$[Cu(met)_2]2Cl$	121.76
	$[Cu(met)_2]2NO_3$	121.61
	$[Cu(met)_2]2COOCH_3$	121.88
Cu(II)-Threonine	$[Cu(thr)_2]2SO_4$	133.65
	$[Cu(thr)_2]2Cl$	------
	$[Cu(thr)_2]2NO_3$	133.49
	$[Cu(thr)_2]2COOCH_3$	133.87

Table-8: % free radical scavenging of ABTS for complexes

S.No.	Ascorbic acid	Conc. in µg/ml	Cysteine								Threonine							
			Com-1	Error± SD	Com-2	Error± SD	Com-3	Error± SD	Com-4	Error± SD	Com-5	Error± SD	Com-6	Error± SD	Com-7	Error± SD	Com-8	Error± SD
1	21.57	200	19.67	0.02	16.22	0.02	17.38	0.04	13.62	0.03	12.43	0.02			11.83	0.01	7.75	0.02
2	32.73	400	21.39	0.01	18.92	0.02	17.72	0.01	13.87	0.02	15.39	0.03			12.74	0.02	11.32	0.03
3	43.65	600	22.71	0.01	21.02	0.01	22.05	0.01	21.38	0.01	17.82	0.03			15.48	0.03	12.07	0.01
4	59.18	800	25.58	0.04	22.59	0.02	23.63	0.03	23.42	0.01	19.03	0.01			17.27	0.02	12.65	0.01
5	72.26	1000	28.37	0.02	27.63	0.02	26.74	0.02	25.85	0.01	22.37	0.01			17.88	0.02	13.28	0.01
6	77.84	1200	25.23	0.03	27.88	0.01	28.62	0.01	26.55	0.03	25.44	0.02			19.56	0.01	13.78	0.04

S.No.	Ascorbic acid	Conc. in µg/ml	Methionine							
			Com-9	Error± SD	Com-10	Error± SD	Com-11	Error± SD	Com-12	Error± SD
1	21.57	200	19.61	0.03	17.46	0.01	15.74	0.05	13.86	0.03
2	32.73	400	21.34	0.02	15.61	0.03	16.02	0.03	13.25	0.02
3	43.65	600	22.78	0.01	19.33	0.01	16.83	0.03	16.29	0.01
4	59.18	800	22.69	0.01	22.53	0.03	17.93	0.01	19.55	0.01
5	72.26	1000	25.08	0.04	23.36	0.05	22.18	0.02	19.79	0.03
6	77.84	1200	25.56	0.03	22.78	0.01	22.65	0.02	26.79	0.03

Com-1 $[Cu(cys)_2]2SO_4$ Com-2 $[Cu(cys)_2]2Cl$ Com-3 $[Cu(cys)_2]2NO_3$ Com-4 $[Cu(cys)_2]2COOCH_3$
Com-5 $[Cu(thr)_2]2SO_4$ Com-6 $[Cu(thr)_2]2Cl$ Com-7 $[Cu(thr)_2]2NO_3$ Com-8 $[Cu(thr)_2]2COOCH_3$
Com-9 $[Cu(met)_2]2SO_4$ Com-10 $[Cu(met)_2]2Cl$ Com-11 $[Cu(met)_2]2NO_3$ Com-12 $[Cu(met)_2]2COOCH_3$

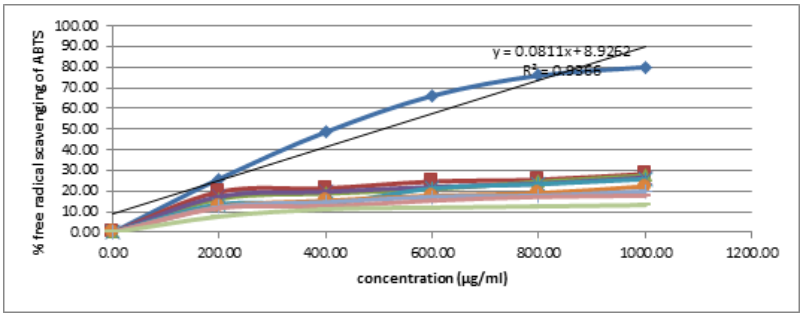

Fig- 6 Graphical representation of % free radical scavenging of ABTS for complexes Cu(II)-Cysteine

Table-17: IC_{50} values of Cu(II)-Amino acid complexes for % free radical scavenging of ABTS

Complex Name	IC_{50} value in µg/ml	
Ascorbic acid	19.57	
Cu(II)-Cysteine	$[Cu(cys)_2]2SO_4$	73.56
	$[Cu(cys)_2]2Cl$	------
	$[Cu(cys)_2]2NO_3$	73.64
	$[Cu(cys)_2]2COOCH_3$	73.75
Cu(II)-Methionine	$[Cu(met)_2]2SO_4$	86.39
	$[Cu(met)_2]2Cl$	86.73
	$[Cu(met)_2]2NO_3$	86.03
	$[Cu(met)_2]2COOCH_3$	88.54
Cu(II)-Threonine	$[Cu(thr)_2]2SO_4$	95.61
	$[Cu(thr)_2]2Cl$	-----
	$[Cu(thr)_2]2NO_3$	95.02
	$[Cu(thr)_2]2COOCH_3$	96.82

Conclusion: All tested Cu(II)-L-amino acids complexes reveal effective biological activities against enzyme inhibition and free radical scavenging activity significantly. These newly synthesised complexes of Cu(II) with L-amino acid as ligands have gained considerable attention due to their spectroscopic properties and biochemical applications through antioxidant and alpha glucosidase inhibition screening activity.It was observed that Cu(II) complexes had at the lowest concentration (100 µg/mL) the antioxidant activity of the these complexes found to be approximate 20-60%. The reason would be the presence of electron releasing hydroxyl and electron donating Nitrogen groups in ligand moiety endowed notable improvement in radical scavenging activity. After the complexation with metal ions reveals that the antioxidant activity increase due to the presence of positively charged metal ions as well as different electron withdrawing and electron donating groups present in the moiety.

The spectroscopic characterization showed that copper coordinated with ligands, forming dimeric species in the solid state. Among the synthesized metal complexes Cu(II)-L-Methionine complexes exhibited effective - glucosidase inhibition with IC_{50} of 1055 µg/ml and also effective on the proton release in DPPH assay with an IC_{50} value of 97.43 µg/ml. All the metal complexes showed moderate antioxidant activity. Cu(II)-L-Cystein exhibited the highest activity in ABTS scavenging with an IC50 of about 73.56 µg/ml amongst the synthesized metal complexes. Thus it can be claimed that metal complex formation is a useful strategy for enhancing the activity of the transition metal compounds.

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