**ABSTRACT**

Complexes of some metal ions with amino acids can be used as models to study the pharmaco-dynamic effects of drugs or for increasing the biocompatibility and minimize toxic effects of some metal ions. Interactions between transitional metal ions and amino acids are very interesting in the biological applications. A series of complexes of Cu(II) and amino acid (L), i.e. glycine, valine, asparginine and arginine with formula [Cu(L)2]^{+2} have been synthesized and characterized as mononuclear species on the basis of elemental chemical analysis, infrared spectra, UV-Visible and cyclicvoltametry measurements. The IR spectra indicated the presence of amino acid coordinated through nitrogen and the oxygen from the carboxylic group. The experimental data suggest that the ligands act as bidentate and adopt an octahedral stereochemistry. In this study, we have also focused on the inhibitory activity of these metal complexes on α-glucosidase.

**KEYWORDS**

aminoacids, copper(II), and α-glucosidase inhibition

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**Introduction**

Cu(II) is a bio-essential element occurring in multitude of metalloproteins (Bhattacharjee et al 2010). Cu(II) complexes are interesting due to their biological applications and considerable amount of interest in the studies is due to their coordination modes when bound to metals, high pharmacological potentiality and good chelating property. Many metal ions are known to play very important roles in biological processes in the human body (Kaim et al 1996 and Xiao-Ming et al 1996). Cu(II) ions are the second and third most abundant transition metals in humans. They are found either at the active sites or as structural components of a good number of enzymes (Cotton et al 1988 and Greenwood et al 1984). Amino acid coordination to metals forms structural lability (Rombach et al 2002) and amino acid complexes are also of relevance in enzyme inhibition (Kahn et al 1999 and Farkas et al 2002). The interesting property of these compound ions may coordinate to the metal ions in the usual bidentate way through the amine and carboxylic group of the amino acids side chain. Pharmacological and toxicological properties of amino acid complexes are another area that has drawn lot of current attention (Chang et al 2005, Tanase et al 1985, Roberts et al 1983, Dondoni et al 2004 and Janiaj et al 2003). Complexes of Cu(II) with amino acids can be used as models to study the pharmaco-dynamic effects of drugs or for increasing the biocompatibility and minimize toxic effects of some metal ions (Grecu et al 1986 and AsmaI et al 2001). Metallotherapy is a very unique therapeutic method to treat many diseases like as diabetes. The goal of diabetes treatment is to control the blood glucose levels, body weight, blood pressure, and cholesterol and triglyceride levels, and prevent the development of complications (Pinhas et al 2007). Several metal ions and their complexes exhibit anti-diabetic effects (Schwarz et al 1959, Rubenstein et al 1962, Coulston et al 1980, Heyliger et al 1985, Sakurai et al 1990 and Yoshikawa et al 2000). In addition, some metal ions, such as tungsten (Dominguez et al 1985), vanadium (Heyliger et al 1985) and selenium (Esaki et al 1990) lower high blood glucose levels in the diabetic state. It appears attractive to many researchers to study the relationship between diabetes mellitus and metal ions. Therefore, this attraction we were synthesized the new Cu(II) complexes containing glycine, valine, asparginine and arginine as ligands. The synthesized complexes were characterized by elemental analysis, CV, IR, UV-visible and evaluated their enzymatic inhibition activity.

**Experimental**

**Materials and Methods**

**Chemicals:**

Triple distilled water, CuSO_{4}.5H_{2}O, Cu(NO_{3})_{2}.H_{2}O, CuCl_{2}, sodium acetate were purchased from alfa acear, Great Britain. Acarbose, α-glucosidase Rat intestinal powder was procured from Sigma Aldrich, USA. All solvent were HPLC grade and used further purification.

**Synthesis of complexes:**

The [Cu(L)]^{+2} complexes were prepared from three different salts of copper and amino acids (L-glycine, valine, asparginine and arginine) as ligand. 2 mM of amino acid was added in 20 ml of aqueous solution which containing 2 mM of sodium acetate and allow it to a clear solution with continuous string. Then 1 mM of metal salt in 2 ml of triple distilled water was added drop by drop into this aqueous solution with continuous stirring for 3 hours. A deep blue colored solution obtained which were transferred into petri dish for crystallization. After few days deep blue colored crystals obtained.

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Infra Red Spectroscopy:
Infrared (IR) spectra were obtained by the KBr method using a Bruker Alpha-T model Fourier transform (FTIR) spectrometer (Bruker Instrument Germany). The spectrometer was equipped with a Globar IR source, KBr beam splitter and detector. For each spectrum, 16 scans were obtained with the resolution of 4 cm⁻¹. The obtained IR spectra were processed 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, in quartz cells at the desired wavelength region. 3 mM solution of complexes in DMSO was used in all UV-visible measurements.

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/AgCl₂, Platinum wire used as an 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 voltamogram, peak position and area were calculated using NOVA 1.9 software.

α-Glucosidase Inhibition:
The determination of α-Glucosidase was adopted from the method (I.P. Tripathi et al 2014). Rat intestinal acetone powder (Sigma chemicals,USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm x 30 mins the supernatant was treated as crude intestinal α-Glucosidase. 50 µl various dilutions in DMSO (0.1mg/ml solution) were mixed and incubated with 50µl of enzyme in a 96-well microplate for 5mins. Reaction mixture was further incubated for another 10 mins with 50 µl substrate (5 mM, p-nitrophenyl-α-D-glucopyranoside) prepared in 100 mM phosphate buffer (pH- 6.8) and release of nitrophenol was read at, 405 nm spectrophotometrically (MultimodeSynergyHT micro plate reader, BioTek instrument, inc. Winoscoi, 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 (IC50) of the enzyme. Quantification was performed with respect to the standard curve of acarbose (Y = 26.63X + 46.26, R² = 0.958) results were expressed as milligram of acarbose equivalent per ml of extract.

Results and Discussion:
Characterization of metal complexes
All the complexes are colored, non-hygrosopic and thermally stable solids. Table-1, indicating a strong metal-ligand bond. The complexes are insoluble in common organic solvents such as ethyl alcohol, acetone, etc., but are fairly soluble in H₂O and DMSO.

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

| SN | Complex Formula | Molecular Weight | Color | Elemental Analysis Found (Calcd.) |
|----|----------------|------------------|-------|---------------------------------|
|    |                |                  |       | M % | C % | H % | N% |
| 1. | Cu(gly)₂SO₄     | C₃H₇N₂O₄Cu       | 399.83| Shining blue | 15.88  | 12.00  | 2.50  | 7.00 |
| 2. | Cu(val)₂Cl      | C₁₀H₁₂N₃O₄Cu     | 368.75| Dark blue     | 17.22  | 32.54  | 5.96  | 7.59 |
| 3. | Cu(asp)₂NO₃    | C₈H₁₈N₄O₆Cu      | 496.83| Royal blue    | 12.78  | 19.32  | 3.22  | 11.27|
| 4. | Cu(arg)₂CH₂COO  | C₁₁H₁₈N₄O₄Cu     | 548.05| Dark blue     | 11.58  | 26.27  | 5.10  | 20.43|

UV-VIS spectroscopy:
The electronic spectra data of the complexes were recorded in 100% DMSO and their assignments were given in Table-3 and one representative ligand field spectra of complex(2) [Cu(val)₂] is shown in Fig.-2 and band position are presented in Table-3. The UV-VIS spectra of Cu(II) complexes with the four ligands show absorption bands assigned to a large π-π* transitions of the compounds, as presented in Table-3, indicated coordination. Characteristic π-→π* transitions are observed in the spectrum at 236, 234, 249, 257 nm respectively (Eskander et al 2000 and Reddy et al 2000). The infrared spectra of the complex(1) was shifted to higher frequencies with the complexes, suggesting that the coordination of the metal ions with the ligand was via the nitrogen atom (Fessenden 1990, Nakamoto et al 2009 and Elzahany et al 2008). The infrared spectra of the complex(1) is given in Fig.-1. The absorption band at 1624 cm⁻¹ was attributed to the ν(C=O) stretching vibration in the spectrum. The consecutive bands at 1600 and 1572 cm⁻¹, in the spectrum of the ligand were assigned to the symmetric and asymmetric bending vibrations of N-H bond. The complexes 1, 2, 3 and 4 spectra, which involves the carboxylic group in the covalent bonding to the metal ion (David et al 2003). In the spectrum of the complexes are shifted to 1578 cm⁻¹ and 1584 cm⁻¹, which also indicates the involvement of this group in the metal-ligand bond formation. The important absorption at assignment of the complex 1, 2, 3 and 4 are listed in Table-2.

Electrochemical studies of Complexes:
Fig-4 shows cyclic voltammogram (CV) scanned cathodically in the potential region between +0.00 and -0.750 V vs Ag/AgCl as 0.1M sodium perchlorate solution (Cu(III)₃+) system at different pH (isoelectric point of amino acids). In this scan range, the CVs show a single reduction peak at -498.05 mV (B1) in the forward sweep only one oxidation waves A1 at 124.51 mV/s at the scan rate of 0.01 V/s. Voltamogram clearly represents that reduced moiety of Cu(II) doesn’t fully oxidized in further sweep.

α-Glucosidase Inhibition:
The chemically inert and configurationally stable complexes revealed an astonishing range of interesting biological activi-
ties, such as the inhibition of the enzyme. In this study, we have also focused on the inhibitory activity of these complexes on α-glucosidase to analyze alternative action mechanisms of these metal ions. We have evaluated the α-glucosidase inhibitory activity of these metal ions. The previous researches have showed the potent alpha-glucosidase inhibitory effects (Bulut et al 2007, Yoshikawa et al 2009, Warra et al 2011, Hiromu et al 2010, Yutaka et al 2010 and Tripathi et al 2013). But in our case the synthesized copper complexes with amino acid doesn’t show any activity.

Table-2 : λmax (nm) values (in 100% DMSO solution) for Cu(II) complexes.

| SN | Complex     | λmax(nm) |
|----|-------------|----------|
| 1  | [Cu (gly)]^{2+} | 234      |
| 2  | [Cu (val)]^{2+}  | 237      |
| 3  | [Cu (asp)]^{2+}  | 249      |
| 4  | [Cu (arg)]^{2+}  | 257      |

Fig.-1: Uv-vis Spectra of [Cu (val)]^{2+}

Table -3: IR-frequencies (in cm^{-1}) of Cu(II) complexes

| SN | Complex     | Group                              | Band cm^{-1} |
|----|-------------|------------------------------------|--------------|
| 1  | [Cu (gly)]^{2+} | N-H (bending) bounded with metal | 1584         |
|    |              | N-H (stretching)                  | 3248-3383    |
|    |              | C=O bounded with metal             | 1624         |
| 2  | [Cu (val)]^{2+} | N-H (bending) bounded with metal | 1586         |
|    |              | N-H (stretching)                  | 3298-3385    |
|    |              | C=O bounded with metal             | 1631         |
| 3  | [Cu (asp)]^{2+} | N-H (bending) bounded with metal | 1583         |
|    |              | N-H (stretching)                  | 3277-3379    |
|    |              | C=O bounded with metal             | 1623         |
| 4  | [Cu (arg)]^{2+} | N-H (bending) bounded with metal | 1581         |
|    |              | N-H (stretching)                  | 3278-3378    |
|    |              | C=O bounded with metal             | 1683         |

Fig.-2 IR Spectra of [Cu (val)]^{2+}

Table-4 : CV results (in mV) for Cu(II) complex

| SN | Complex     | Reduction Peak(B1) | Oxidation Peak(A1) | Peak (1/2) | Peak width (1/2) V |
|----|-------------|--------------------|--------------------|------------|-------------------|
| 1  | [Cu (gly)]^{2+} | 0.12451            | -0.49805           | 0.061204   | 0.13785           |
| 2  | [Cu (val)]^{2+}  | -0.12939           | -0.49561           | -0.07415   | 0.14359           |
| 3  | [Cu (asp)]^{2+}  | -0.13184           | -0.50293           | 0.065609   | 0.13791           |
| 4  | [Cu (arg)]^{2+}  | -0.13184           | -0.46631           | 0.082223   | 0.16987           |

Fig-3 : Cyclic volatammogram of [Cu (gly)]^{2+}.

Conclusion:
The amino acids complexes of Cu(II) have global interest in scientific community due to their potential pharmacological activities. In this work, we have successfully synthesized the complex of copper (II) using amino acids as ligand. The coordination of the copper (II) with amino acids arises from the shift of the ν(C=O), ν(C-N) 1623 and 1584 cm^{-1} respectively. The assignment of copper (II) complexes with amino acids have corroborated by infrared, electro chemical and electronic spectral measurements. The cyclic voltamogram represents only reduction peak at -498.05mV. The broad band is observed at 16,638 cm^{-1} in the electronic spectrum of the Cu(II) complex assigned to 2E_g - 2T_{2g} transition which is conform the octahedral geometry of all the complexes (Dunn et al 1960)
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