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Synthesis, Characterization of some Antidiabetic Copper Complexes with Ethylenediamine

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Abstract

*Biological trace metals such as iron, zinc, copper, and manganese are essential for human health. Development of inorganic pharmaceutical agents is very interesting creation in field of medicinal inorganic chemistry and these agents are being used to treat various chronic diseases with the help of essential elements and other biological trace metals. Metallotherapy is an expanding of interest in the research field of treatment of diabetes mellitus. Especially, orally active anti-diabetic and anti-metabolic syndrome copper complexes have been developed and progressed since last decades, where several highly potent anti-diabetic copper complexes with different coordination structures have quite recently been disclosed, using experimental diabetic animals and enzyme inhibitarory action (α-glucosidase inhibition) also . In the present study we have synthesized, characterized and evaluate % of α- glucosidase of Cu (II) metal complexes and concluded that [Cu (en)$_3$] 2NO$_3$ (complex **3**) shows lowest IC$_{50}$ value 0.475 mg/ml while [Cu (en)$_3$] 2Cl (Complex **2**) shows moderate activity.*

Keywords: Chronic diseases, metallotherapy, anti-diabetic, anti-metabolic syndrome and α-Glucosidase Inhibition.

Introduction

Diabetes mellitus (DM), a metabolic disorder related with multiple etiologies, is one of the five leading causes of death in the world. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 if successful strategies are not implemented for its prevention and control[1].

Diabetes mellitus is a clinically and genetically heterogeneous group of disorders, characterized by hyperglycemia. Several pathogenic processes are involved in the development of diabetes. These are destruction of the β-cells of the pancreas from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Deficient supply of insulin causes abnormalities in carbohydrate, fat, and protein metabolism and these results in disability and premature death [3, 2].

Metallotherapy is a new therapeutic strategy to treat diabetes with metal complexes. It is first studied by Coulson and Dandona in 1980 that $ZnCl_2$ stimulate lipogenesis in rat adipocytes similarly to the action of Insulin. In three decades there are many researchers reported insulin-mimetic activity, *α*-glucosidase and *α*-amylase inhibition with different coordination of different ligand with transition metals[4].

The development of modern chemotherapy is based on metals and metal complexes which play a key role in modifying the pharmacological properties of known drugs after coordinating to a metal. The resulting precursors allow these drugs to be released in a controlled fashion and at specific location. This approach may lead to the rescue of drugs that have failed because of poor pharmacology or high toxicity. For example, complexation of nonsteroidal anti-inflammatory drugs to copper overcomes some of the gastric side effects of these drugs[5].

In the present work we are synthesizing the copper complexes to evaluate their α-glucosidase activity.

Material and Methods

Chemicals: Water, DMSO, $CuSO_4.5H_2O$, $CuCl_2$, $Cu(NO_3)_2.H_2O$, Potassium Chloride, Potassium Bromide, p-nitrophenyl-α-D-glucopyranoside were purchased from SRL, India and ethylenediamine from alfa acear, Great Britain. Acarbose, α–glucosidase Rat intestinal Acetone powder was procured from Sigma Aldrich, USA. All solvent were HPLC grade, chemicals were A.R grade and used further any purification.

Synthesis of Complex: The $[Cu (L)_3]^{2+}$ complex was prepared from three different salts of copper and ethylenediamine. 2 mM aqueous solution of metal salts was stirred in a beaker and 6 mM of ethelenediamine were added drop by drop. With

continuous string, 2-3 ml of ethyl alcohol was added for precipitation. A deep blue colored solution were obtained, which were transferred in a petri dish to remove solvent in incubator at 45˚C. After few days a deep blue colored complex $[Cu (en)_3] SO_4$, $[Cu (en)_3] 2Cl$ and $[Cu (en)_3] 2NO_3$ (1, 2 and 3 respective) solid obtained[6].

Infra Red 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 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 means of the program OPUS 7.0.

Cyclic Volta metric: The cyclic voltammetric 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 was used as a working electrode, while a 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 volatammogram, peak position and area were calculated using NOVA 1.9 software.

α–Glucosidase Inhibition : Method for determination of α-Glucosidase was adopted from Misra S. et al.[7]. Rat intestinal acetone powder (Sigma chemicals,USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm × 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 50 µl of enzyme in a 96-well microplate for 5 mins. 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 spectrophotometerically (Multimode^{SynergyH4} 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 ($Y = 26.63X + 46.26$, $R^2 = 0.958$) results were expressed as milligram of acarbose equivalent per ml of extract. Percent α-Glucosidase inhibition was calculated using following equation.

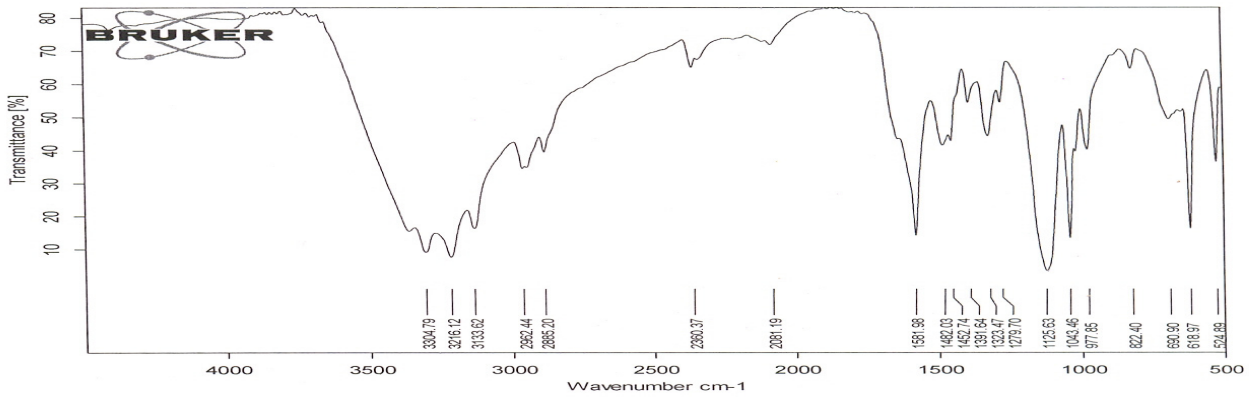
Results and Discussion

Infra Red Spectroscopy: In the IR spectrum of compound 1, the characteristic N-H bending vibration is observed as a strong bond at 1581 cm^{-1}, which is a commonly observed fact for chelated ethylenediamine complex. The N-H stretching vibrations are found in the range 3110-3304 cm^{-1} and the C-H stretching vibration 2962 and 2885 cm^{-1}. IR assignments and spectra of the complex 1, 2 and 3 are given in table 1 and figure 1, 2 and 3 respectively.

Electrochemical studies of Complexes: Figure 4 shows cyclic voltammogram (CV) scanned cathodically in the potential region between +0.00 and -1.00 V vs Ag/AgCl in 0.1 M KCl_2 solution $[Cu(II)en_3]^{2+}$ system. In this scan range, the CVs show a single reduction peak at -512.7 mV (B1) in the forward sweep and three overlapping oxidation waves A_1, A_2 and A_3 respectively at -285.6, -175.8 and - 65.9 mV at 2 mV/s (table 2 and figure 4). It must be mentioned that extension of negative potential limit to – 0.8 V did not result in any other reduction peak except B_1[8].

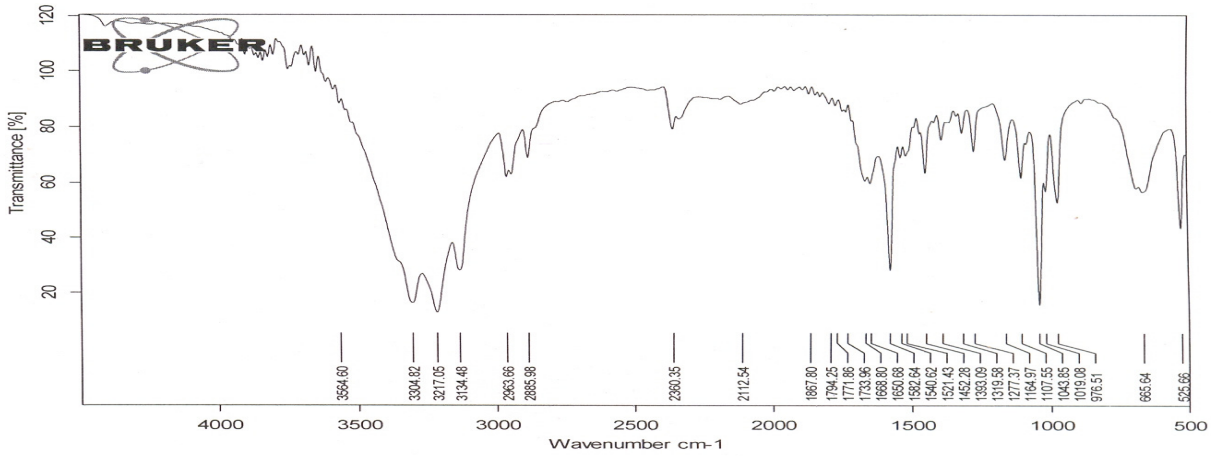
Table-1
Representing the band assignment for complex 2 and 3

S. No.	Complex	Group	Band (cm^{-1})
1	$[Cu(en)_3] SO_4$	N-H (bending) bounded with metal	1581
		N-H (stretching)	3110-3304
		C-H (stretching)	2962 and 2885
2	$[Cu (en)_3] 2Cl$	N-H (bending) bounded with metal	1582
		N-H (stretching)	3110-3304
		C-H (stretching)	2963 and 2885
3	$[Cu (en)_3] 2NO_3$	N-H (bending) bounded with metal	1583
		N-H (stretching)	3110-3304
		C-H (stretching)	2962 and 2887



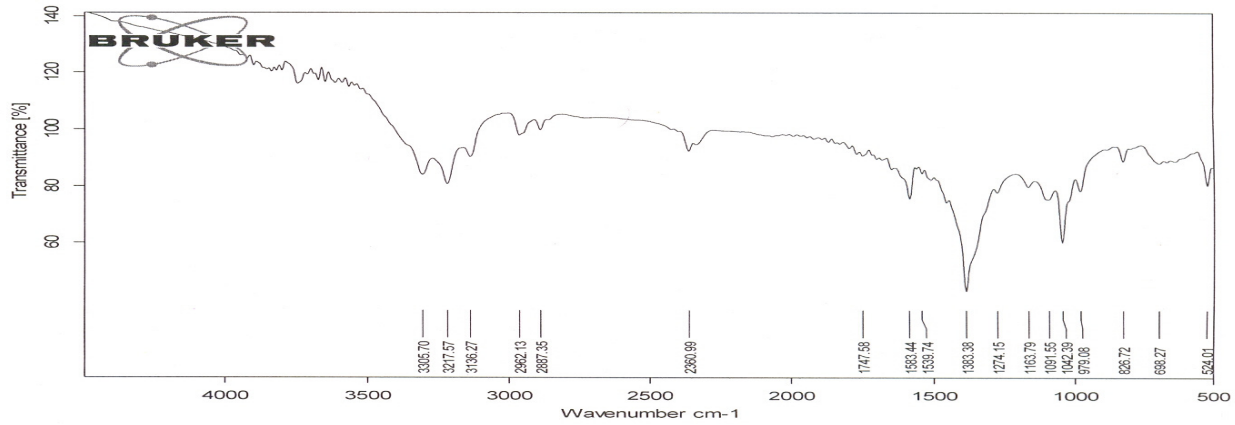
IR Spectra of $CuSO_4$ and Ethylenediamine Complex

Figure-1
IR Spectra of $[Cu(en)_3]SO_4$



IR Spectra of $CuCl_2$ and Ethylenediamine Complex

Figure-2
IR Spectra of $[Cu\ (en)_3]2Cl$



IR Spectra of $CuNO_3$ and Ethylenediamine Complex

Figure-3
IR Spectra of $[Cu(en)_3]2NO_3$

Table-2
Oxidation and reduction peaks

S.No.	Name of Peak	Peak (1/2)	Peak position V
1	B_1	-0.054234	-0.5127
2	A_1	0.040929	-0.28564
3	A_2	0.027677	-0.17578
4	A_3	0.025151	-0.065918

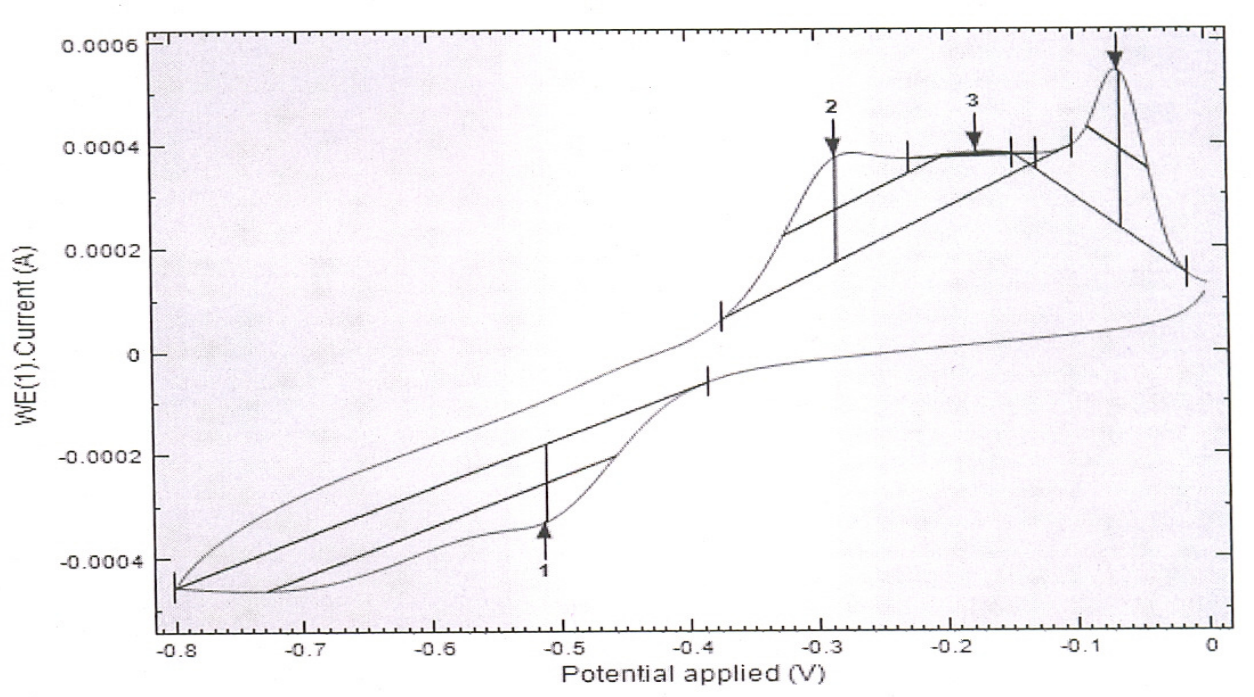


Figure-4
Cyclic volatammogram of [Cu $(en)_3$] species

α–Glucosidase Inhibition: A lot of literatures were reviewed and found that there are several complexes have been prepared to evaluate their anti diabetic activity. The development of a clinically useful metallopharmaceutics, the research of copper complexes on the long-term toxicity including side effects, clear-cut evidence of target molecule for the *in vivo* as well as *in vitro* pharmacological action and good pharmacokinetic property are essential[9-11].

Many copper complexes have been prepared to examine for their α-glucosidase inhibitory activity. We have examined the three complexes of ethylenediamine with copper (II) for their % α-glucosidase inhibition Table 3 demonstrate the IC_{50} value of Acarbose and metal complexes. Table 4 shows the absorbance of nitrophenol and Table 5 represents the % α-glucosidase inhibition and fogire-5 and figure-6 were plotted according these values respectively.

Table-3
IC_{50} value of Complexes

S.No.	Name of Complex	IC_{50} value in mg/ml
1	Acarbose	0.1403
2	[Cu $(en)_3$] SO_4 (Complex **1**)	0.6085
3	[Cu $(en)_3$] 2Cl (Complex **2**)	0.801
4	[Cu $(en)_3$] $2NO_3$ (Complex **3**)	0.4755

Table-4
The Absorbance of Nitrophenol

S.No.	Conc. in mg/ml	Absorbance of Nitrophenol							
		Acarbose	Error±SD	Complex1	Error±SD	Complex2	Error±SD	Complex3	Error±SD
1	0.1	0.155	00	0.249	0.001	0.269	0.001	0.257	0.0006
2	0.2	0.132	0.01	0.224	0.003	0.244	0.001	0.213	0.002
3	0.4	0.118	0.001	0.208	0.004	0.187	0.002	0.162	0.003
4	0.6	0.108	0.002	0.166	0.004	0.183	0.001	0.138	0.001
5	0.8	0.094	0.004	0.145	0.005	0.177	0.002	0.126	0.005
6	1.0	0.079	0.004	0.129	0.004	0.168	0.002	0.114	0.004

Table-5
% of α- glucosidase inhibition

S.No.	Conc. in mg/ml	% of α- glucosidase inhibition							
		Acarbose	Error±SD	Complex1	Error±SD	Complex2	Error±SD	Complex3	Error±SD
1	0.1	45.80	00	28.65	0.001	22.92	0.001	26.36	0.0006
2	0.2	53.85	0.01	35.82	0.003	30.08	0.001	38.96	0.002
3	0.4	58.74	0.001	40.40	0.004	46.42	0.002	53.58	0.003
4	0.6	62.24	0.002	52.43	0.004	47.56	0.001	60.46	0.001
5	0.8	67.13	0.004	58.45	0.005	49.28	0.002	63.90	0.005
6	1.0	72.38	0.004	63.04	0.004	51.86	0.002	67.33	0.004

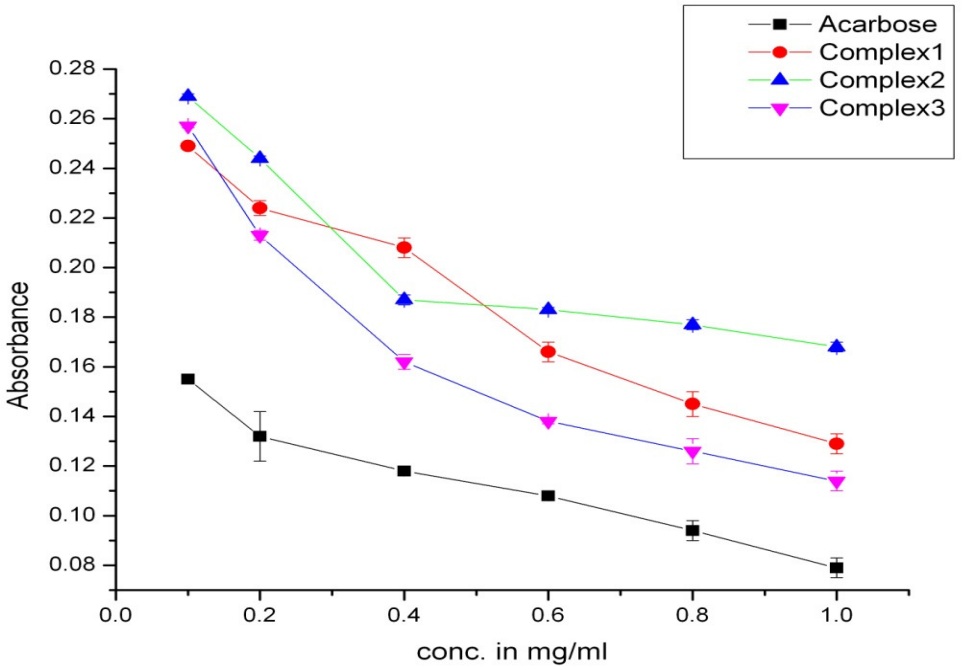


Figure-5
Representing graph between concentration (mg/ml) and absorbance

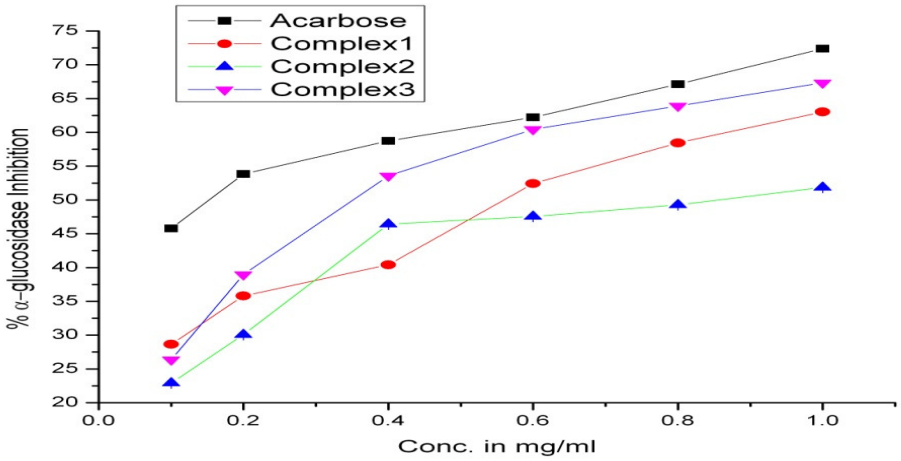


Figure-6
% inhibition curve of α-Glucosidase v/s Concentration of sample

Conclusion

Sharp and intense peak at 1581 cm^{-1} in IR spectrum shows the formation of [Cu $(en)_3$] complexes. The electrochemical behavior of Cu (II)-$(en)_3$ system in 0.01 M KCl solution complex as it comprises electroxidation and electroreduction of copper (0), copper (I) and copper (II) species. All three complexes possess α-glucosidase inhibition activity, among them [Cu $(en)_3$] $2NO_3$ have the highest α-glucosidase inhibition, having IC_{50} value 0.4755 mg/ml. In this complex the % inhibition of α-glucosidase may be due to the presence of NO_3^{2-} species.

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