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Synthesis, Characterization and α- Glucosidase Inhibition of Some Copper, Cobalt, Nickel and Zinc Complexes with N – Methylethylenediamine

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of current study to examine percentage inhibition of α-glucosidase by synthesized metal complexes.
Study Design: The α-glucosidase inhibitory activity assay was performed by Tripathi et al. method with minor modification
Place and Duration of Study: Faculty of Science and Environment and Department of Physical Sciences, between June 2009 and July 2010.
Methodology: In the present work we have synthesized metal [Cu(II), Co(II), Ni(II), Zn(II)] complexes with N - methylethylenediamine. We synthesized total twelve metal complexes by various salts i.e. nitrate, sulphate and chloride of copper, cobalt, nickel and zinc. Synthesized metal complexes characterized via IR spectroscopy and cyclovoltametry. We examined percentage inhibition of α-glucosidase by synthesized metal complexes. IC_{50} value of metal complexes was also calculated.
Results: All twelve complexes possess α-glucosidase inhibition activity, among them [Co $(men)_3]2NO_3$ have the highest α-glucosidase inhibition, having IC_{50} value 900 µg/ml and [Ni

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$(men)_3]2Cl$ have the lowest α-glucosidase inhibition, having IC_{50} value 2533.21 µg/ml.
Conclusion: Diabetes is a proven and inherited type of disorder transition metal ions are essential nutrients to all forms of life. Iron, copper, zinc, manganese, cobalt and nickel all have unique chemical and physical properties that make them useful metals for biological systems so this type of approach may be useful. We have done *in-vitro* study; however, need further work to validate reliability.

Keywords: Diabetes; α-glucosidase; α-glucosidase inhibition.

1. INTRODUCTION

Diabetes is a clinical and genetic type of disorder. It is very common disease having various complications i.e. atherosclerosis, cardiac dysfunction, retinopathy, neuropathy [1]. Diabetes is related with Dyslipidaemia, it is a main cardiovascular risk factor which accelerates the atherosclerotic process and its morbid consequences. Nutraceuticals can improve dyslipidaemia. Nutraceuticals and functional food ingredients that are beneficial to vascular health may represent useful compounds that are able to reduce the overall cardiovascular risk induced by dyslipidaemia [2]. Metal ions such as iron, zinc, copper, cobalt, nickel and manganese are essential to life and play an important role in biological systems [3]. These metals are useful in various diseases in the form of metal complexes, metal salt reacts with specific ligand and produces metal-ligand complex, which plays an important role in metallotherapy. For the treatment of diabetes mellitus, a new therapeutic approach was made through metallotherapy means treatment of diabetes and its complications by implementation of metal and its complexes of different ligands [4]. α- glucosidase is a digestive enzyme found in plants, animals, bacteria, fungi, yeast, and humans. α-glucosidase inhibitors are oral anti diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates (such as starch and table sugar). Carbohydrates are normally converted into simple sugars (monosaccharide), which can be absorbed through the intestine. Hence, alpha-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar [5].

In the present work we are synthesizing different types of metal complexes with N-methylethylenediamine to evaluate their α-glucosidase inhibition activity.

2. MATERIALS AND METHODS

2.1 Chemicals

Water, DMSO, $CuCl_2$, $Cu(NO_3)2.H2O$, $CuSO_4$, potassium chloride, potassium bromide, p-Nitrophenyl-β-D-Glucopyranoside were purchased from SRL, India, and N - methylethylenediamine from alfa aesar, Great Britain. Acarbose, α–glucosidase rat intestinal acetone powder was purchased from Sigma - Aldrich, USA. All solvents were HPLC grade, chemicals were analytical reagent grade and used without any purification.

2.2 Synthesis of Complex

Complexes were prepared from different salts (chloride, sulphate and nitrate) of metal ions (Cu(II), Co(II), Ni(II), Zn(II)) and n - methylethylenediamine. We have used three salts (given above) of each metal ion. 2 mM aqueous solution of metal salt was taken in a beaker and 6 mM solution of ligand was added drop wise with continuous stirring. Colored solution obtained now stirred it for three hours. 2-3 ml of ethyl alcohol was added for precipitation, now solution transferred in a Petri dish to remove solvent in incubator at 45℃. After 4-5 days colored complex obtained in the form of crystalline solid which was collected and placed in dessicator [6].

2.3 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 Global 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 [7].

2.4 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/ AgCl2. Platinum wire was used as a working electrode, while another platinum wire electrode used as an auxiliary electrode. The 3

mg of complex were dissolved in 25 ml supporting electrolyte (0.1 M Sodium per chlorate) solution. The voltammogram, peak position and area were calculated using NOVA 1.9 software [8].

2.5 α - Glucosidase Inhibition

The α-glucosidase inhibitory activity assay was performed by following the method of Tripathi et al with minor modification. In brief, Rat-intestinal acetone powder was dissolved in 100 ml of saline water and sonicated properly at 4℃. After sonication, the suspension was centrifuged (3,000 rpm, 4℃, 30 minutes) and the resulting supernatant was used for the assay. A reaction mixture containing 50 µl of phosphate buffer (50 mM; pH 6.8), 20 µl of rat α-glucosidase and 25 µl sample of varying concentrations (100-1000 µg/ml) was pre-incubated for 5 min at 37℃, and then 25 µl of 3 mM PNPG was added to the mixture as a substrate. After incubation at 37℃ for 30 min, enzymatic activity was quantified by measuring the absorbance at 405 nm in a micro titer plate reader (Bio-TEK, USA). Acarbose was used as a positive control and water as negative control. Experiments were done in triplicates [9].

IC_{50} value was quantified using formula, Y= 0.026(x) - 46.26, R^2 = 0.958. The percentage of enzyme inhibition by the sample was calculated by the following formula:

$$\% \text{ Inhibition} = \{[(AC - AS)/AC] \times 100\}$$

Where, AC is the absorbance of the control and AS is the absorbance of the tested sample. The concentration of inhibitor required to inhibit fifty percent of enzyme activity under the mentioned assay conditions is defined as the IC_{50} value.

3. RESULTS AND DISCUSSION

3.1 Infra Red Spectroscopy

Infrared studies on coordination compounds of metal with ligand are a useful tool in structural studies [10]. Shifting of bands lower to the higher frequencies, suggesting the possibility of the coordination of ligand through the nitrogen atom at the amine group [11-13].

In the IR spectrum of complexes we have taken range 600-4000 cm^{-1} and found many bands are appearing in that region. In compound 1, the characteristic N-H bending vibration is observed as a strong bond at 1590 cm^{-1} which is indicating ligand is bounded with metal and chelation in N - methylethylenediamine complex. The N-H stretching vibrations are found in the range 3241-3445 cm^{-1} and the C-H stretching vibration 2915 cm^{-1}. In metal complexes water molecules are appearing via broad band at around 3400 cm^{-1}. In the IR spectrum of other complexes the spectra exhibited a marked difference between bands belonging to the stretching vibration of υ(N-H) of the amine group in the range between 3260-3204 cm-1, shifting of bands to the higher frequencies, suggesting the possibility of the coordination of ligand through the nitrogen atom at the amine group.

IR assignments and spectra of the complex 1 to 12 are given in Table 1 and IR spectrum of the complex 2, 4, 6 and 12 are shown in Figs. 1, 2, 3 and 4 respectively.

3.2 Electrochemical Studies of Complexes

Figs. 5, 6 and 7 are showing cyclic voltammogram of [Cu(men)3]2NO3, [Co(men)3]SO4, [Zn(men)3]SO4 respectively. CV of metal complexes scanned cathodically in the potential region between +0.00 and -1.000 V vs Ag/AgCl in 0.1 M sodium perchlorate solution $[M(Men)_3]^{2+}$ system. In scan range of $[Cu(men)_3]2NO_3$, the cyclic voltammetry showing two anionic peak at -424.0 mV, -300.0 mV at 2 mV/s. Peak position, peak hight, peak area and peak (1/2) of complex 3, 5 and 11 given in graphs showed in Figs. 5, 6, 7. Voltamogram clearly represents that reduced moiety of M(II) doesn't fully oxidized in further sweep.

3.3 α–Glucosidase Inhibition

After reviewing lots of literature found that metal ions are appearing as α- glucosidase inhibitor, lots of complexes of different metal salts with different ligands have been synthesized and evaluated their anti diabetic activity. α-glucosidase is a digestive enzyme which breaks polysaccharides into monosachharides. In the form of glucose monosachharide dissolves in blood. In a diabetic patient maintain the level of glucose we need α- glucosidase inhibitors. The previous researches have showed the potent alpha-glucosidase inhibitory effects of metal complexes [14-17]. So many cobalt (II) complexes have showed antidiabetic activity. Talba et al described antidiabetic effect of glucosaminic acid-cobalt (II) chelate [18].

In this study we have done an attempt to prepare synthetic inhibitors for which we have

synthesized copper, cobalt, nickel and zinc complexes complexes with N - methylethylenediamine to examine their α-glucosidase inhibitory activity. We have examined the twelve complexes of N - methylethylenediamine with different metal salts for their percentage of α-glucosidase inhibition. Table 4 is describing the IC_{50} value of Acarbose and metal complexes. The lower IC_{50} value of complex indicated higher inhibitory activity while higher IC_{50} value of complex indicated lower inhibitory activity. Table 2 shows the absorbance of nitrophenol and Table 3 represents the percentage of α-glucosidase inhibition and Figs. 8, 9, 10 and 11 represents graph plotted according these values respectively.

Table 1. Representing the band assignment for complexes

S. no.	Complex	Group	Band (cm-1)
1.	[Cu $(men)_3$]2Cl	-NH (bending) bounded with metal	1590
		-NH (stretching)	3204
		-CH (stretching)	2930
		-OH	3564
		-CN	1075
2.	[Cu $(men)_3$]SO_4	-NH (bending) bounded with metal	1585
		-NH (stretching)	3206
		-CH (stretching)	2933
		-OH	3525
		-CN	1073
3.	[Cu $(men)_3$]$2NO_3$	-NH (bending) bounded with metal	1585
		-NH (stretching)	3207
		-CH (stretching)	2933
		-OH	3503
		-CN	1073
4.	[Co $(men)_3$]2Cl	-NH (bending) bounded with metal	1616
		-NH (stretching)	3210
		-CH (stretching)	3099
		-OH	3506
		-CN	1056
5.	[Co $(men)_3$]SO_4	-NH (bending) bounded with metal	1585
		-NH (stretching)	3324
		-CH (stretching)	2934
		-OH	3633
		-CN	-
6.	[Co $(men)_3$]$2NO_3$	-NH (bending) bounded with metal	-
		-NH (stretching)	-
		-CH (stretching)	-
		-OH	-
		-CN	-
7.	[Ni $(men)_3$]2Cl	-NH (bending) bounded with metal	1580
		-NH (stretching)	3258
		-CH (stretching)	2964
		-OH	-
		-CN	1061
8.	[Ni $(men)_3$]SO_4	-NH (bending) bounded with metal	1586
		-NH (stretching)	3227
		-CH (stretching)	2944
		-OH	3336
		-CN	1060

S. no.	Complex	Group	Band (cm-1)
9.	$[Ni (men)_3]2NO_3$	-NH (bending) bounded with metal	1603
		-NH (stretching)	-
		-CH (stretching)	2969
		-OH	3419
		-CN	1050
10.	$[Zn (men)_3]2Cl$	-NH (bending) bounded with metal	1597
		-NH (stretching)	3227
		-CH (stretching)	2952
		-OH	-
		-CN	1068
11.	$[Zn (men)_3]SO_4$	-NH (bending) bounded with metal	1585
		-NH (stretching)	-
		-CH (stretching)	2934
		-OH	3324
		-CN	-
12.	$[Zn (men)_3]2NO_3$	-NH (bending) bounded with metal	1593
		-NH (stretching)	3260
		-CH (stretching)	2971
		-OH	-
		-CN	1059

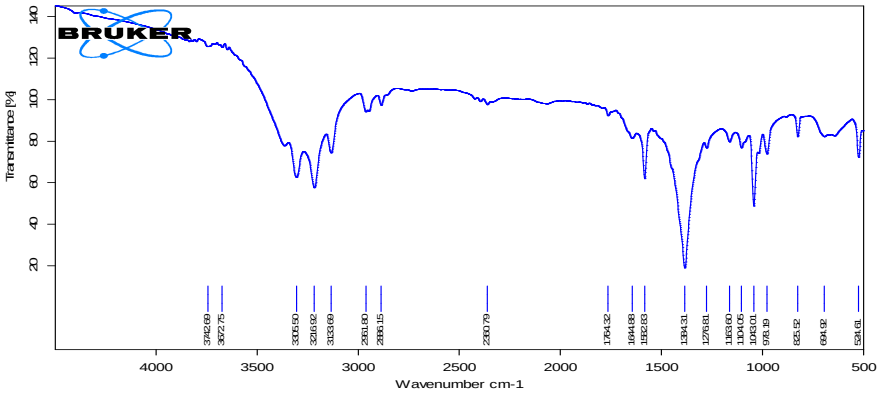


Fig. 1. IR spectrum of $[Cu(men)_3SO_4$

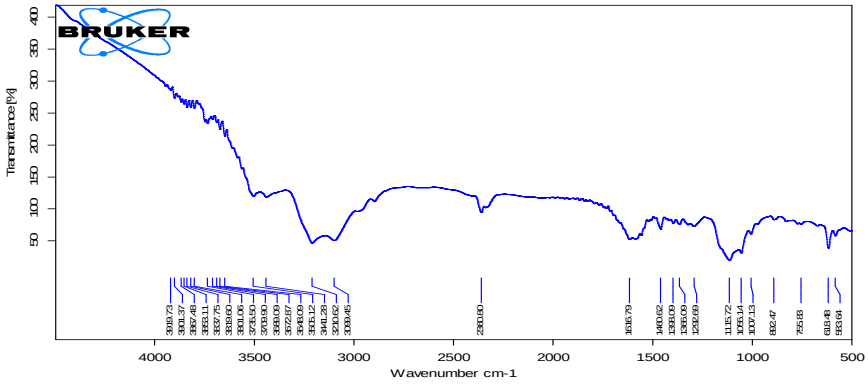


Fig. 2. IR spectrum of $[Co(men)_3]2Cl$

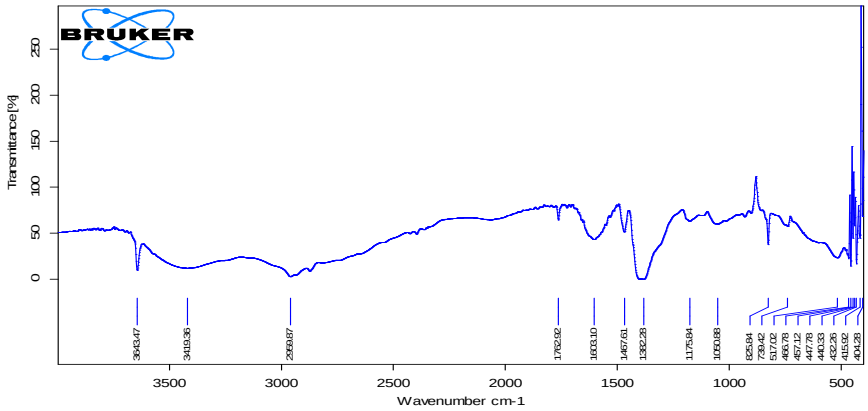


Fig. 3. IR spectrum of $[Ni(men)_3]NO_3$

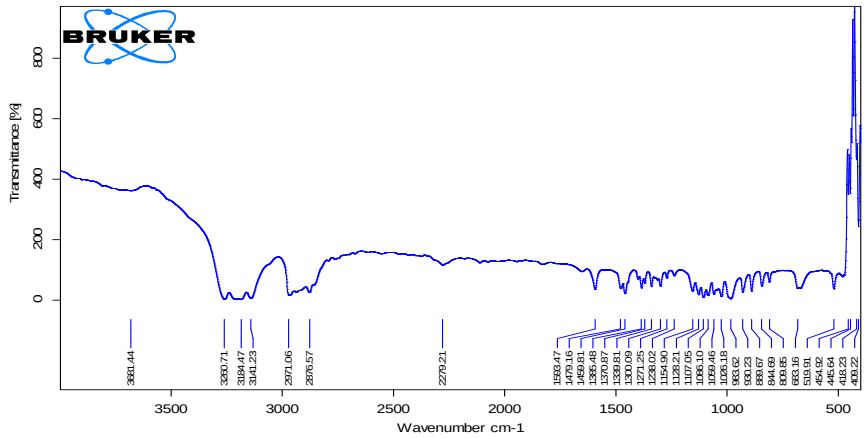
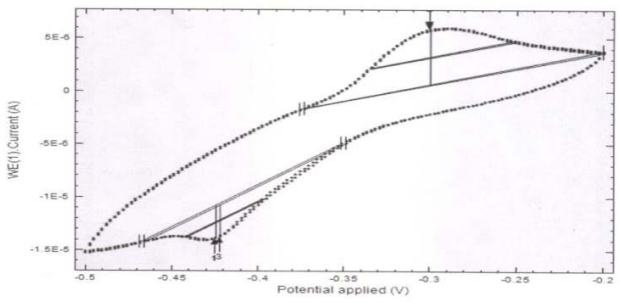


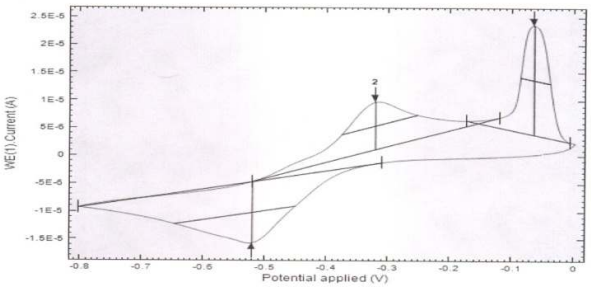
Fig. 4. IR spectrum of $[Zn(men)_3]NO_3$



CV of $[Cu(men)_3](NO_3)_2$ Complex

Index	Peak position	Peak height	Peak area	Base start	Base end	Peak width half	Peak (1/2)	Peak sum of derivatives
1	-0.42465	-3.1015E-06	1.5867E-07	-0.4686	-0.35141	0.035502	-0.027038	0.00019011
2	-0.30014	5.1676E-06	4.5311E-07	-0.37582	-0.20004	0.073984	0.03538	0.0001948

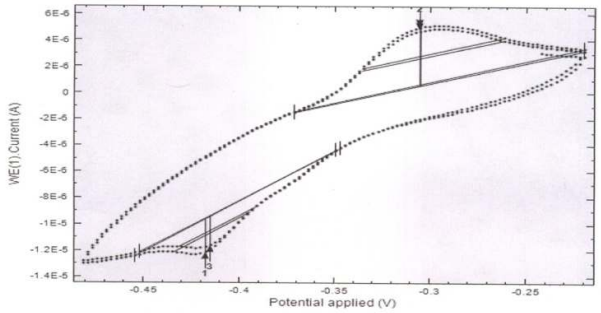
Fig. 5. CV of $[Cu(men)_3]2NO_3$ complex



CV of [Co(men)3] SO_4 Complex

Index	Peak position	Peak height	Peak area	Base start	Base end	Peak width half	Peak (1/2)	Peak sum of derivatives
1	-0.52124	-1.0883E-05	2.271E-06	-0.802	-0.30884	0.19628	-0.070088	0.00015239
2	-0.32104	8.5662E-06	1.258E-06	-0.52124	-0.11841	0.12343	0.054775	0.0001864
3	-0.064697	1.9495E-05	1.0559E-05	-0.17212	-0.0061035	0.04773	0.021677	0.0017887

Fig. 6. CV of $[Co(men)_3]SO_4$ Complex



CV of [Zn(men)3] SO_4 Complex

Index	Peak position	Peak height	Peak area	Base start	Base end	Peak width half	Peak (1/2)	Peak sum of derivatives
1	-0.41779	-2.5012E-06	1.1622E-07	-0.45441	-0.34943	0.039556	-0.023189	0.0001746
2	-0.30548	4.137E-06	3.2004E-01	-0.3714	-0.22003	0.074323	0.03073	0.00018270

Fig. 7. CV of $[Zn(men)_3]SO_4$

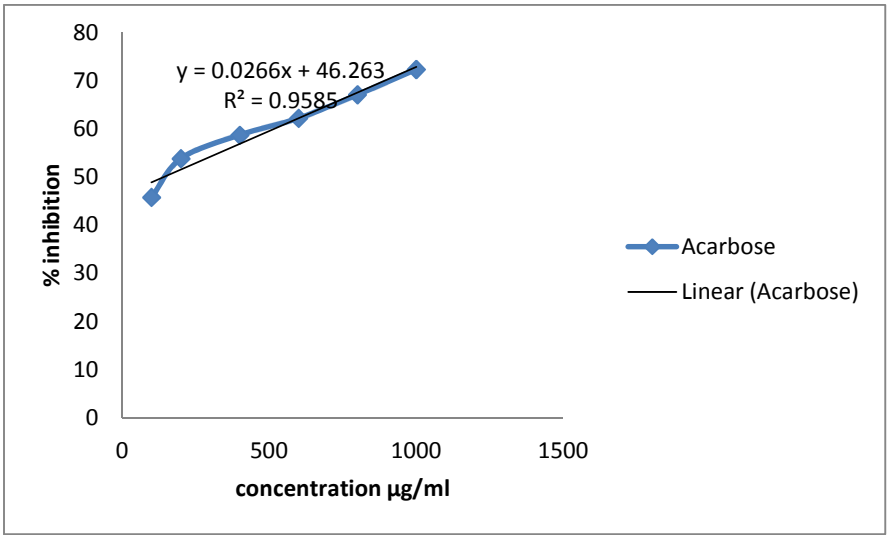


Fig. 8. Percentage Inhibition of α-glucosidase by acarbose

Table 2. The absorbance of nitrophenol

S N	Conc. In µg/ml	Acar-bose	Complexes											
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	100	0.155	0.328	0.320	0.326	0.366	0.367	0.348	0.398	0.392	0.386	0.34	0.342	0.334
2	200	0.132	0.304	0.285	0.296	0.34	0.347	0.301	0.384	0.381	0.366	0.324	0.335	0.306
3	400	0.118	0.284	0.265	0.274	0.304	0.301	0.276	0.364	0.355	0.354	0.304	0.327	0.274
4	600	0.108	0.253	0.254	0.257	0.273	0.287	0.234	0.353	0.337	0.347	0.293	0.3	0.235
5	800	0.094	0.234	0.202	0.201	0.254	0.24	0.201	0.334	0.312	0.31	0.284	0.287	0.21
6	1.0	0.079	0.210	0.197	0.187	0.227	0.211	0.187	0.324	0.287	0.289	0.261	0.264	0.194

Table 3. % inhibition of α- glucosidase

S N	Conc. in µg/ml	Acar-bose	Complexes											
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	100	45.8	6.02	8.31	6.59	5.67	5.41	10.30	2.68	4.15	5.62	3.68	3.11	5.38
2	200	53.85	12.89	18.33	15.18	12.37	10.56	22.42	6.11	6.84	10.51	8.21	5.09	13.31
3	400	58.74	18.62	24.06	21.48	21.64	22.42	28.86	11.00	13.20	13.44	13.88	7.36	22.37
4	600	62.24	27.50	27.22	26.36	29.63	26.03	39.69	13.69	17.60	15.15	16.99	15.01	33.42
5	800	67.13	32.95	42.12	42.40	34.53	38.14	48.19	18.33	23.71	24.20	19.54	18.69	40.50
6	1000	72.38	39.82	43.55	46.41	41.49	45.61	51.80	20.78	29.82	29.33	26.06	25.21	45.01

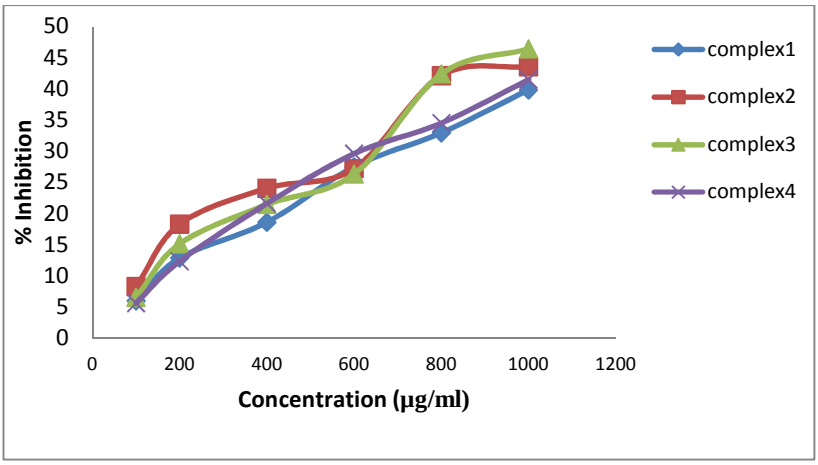


Fig. 9. Percentage inhibition of α - glucosidase by complexes

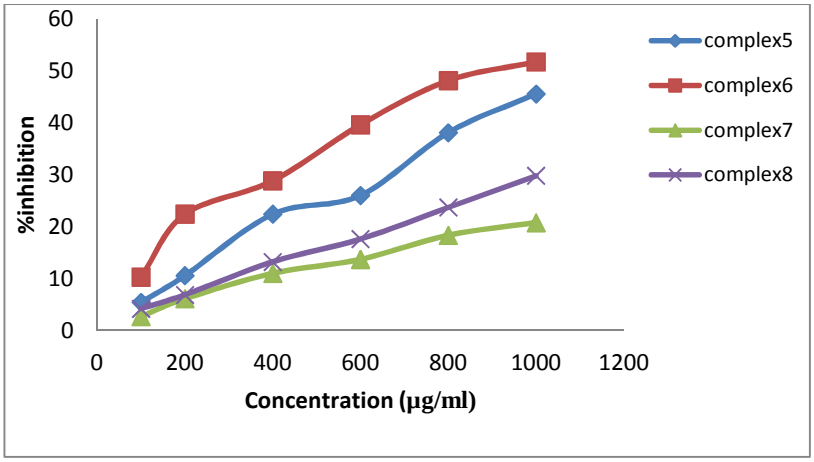


Fig. 10. Percentage inhibition of α - glucosidase by complexes

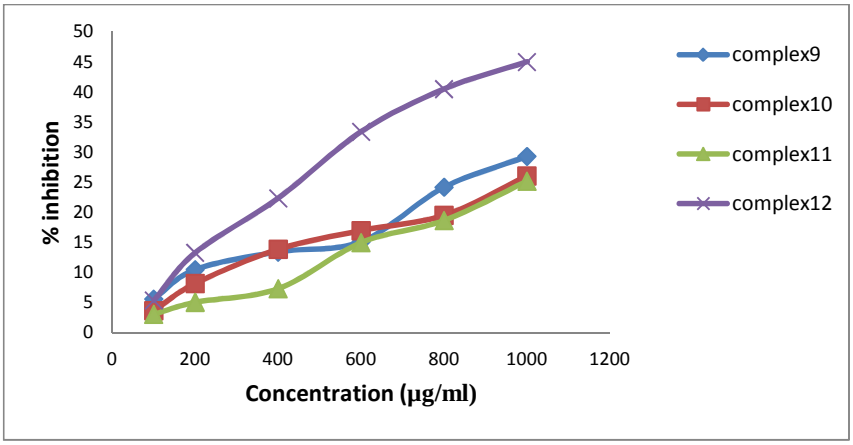


Fig. 11. Percentage inhibition of α - glucosidase by complexes

Table 4. IC_{50} value of complexes

S. no.	Name of complex	IC_{50} value in µg/ml
1.	Acarbose	143.84
2.	[Cu(men) $_3$]2Cl	1272.58
3.	[Cu $(men)_3]SO_4$	1116.31
4.	[Cu $(men)_3]2NO_3$	1073.81
5.	[Co $(men)_3]2Cl$	1203.65
6.	[Co $(men)_3]SO_4$	1092.57
7.	[Co $(men)_3]2NO_3$	900.00
8.	[Ni $(men)_3]2Cl$	2533.21
9.	[Ni $(men)_3]SO_4$	1738.91
10.	[Ni $(men)_3]2NO_3$	1933.91
11.	[Zn $(men)_3]2Cl$	2135.90
12.	[Zn $(men)_3]SO_4$	2070.91
13.	[Zn $(men)_3]2NO_3$	1050.52

4. CONCLUSION

Metal ions [copper(II), cobalt(II), nickel(II) and zinc(II)] are essential for life. These metals also play an important role in medicine and pharmacology. Sharp and intense peaks between 1580-1616 cm^{-1} in IR spectrum represents metal-ligand bonding which shows that $[M(men)_3]$ complexes have synthesized. The electrochemical behavior of M (II)-$(men)_3$ system in 0.01 M KCl solution complex as it comprises electroxidation and electroreduction of M (0), M (I) and M (II) species. All twelve complexes possess α-glucosidase inhibition activity, among them [Co $(men)_3]2NO_3$ have the highest α-glucosidase inhibition, having IC_{50} value 900 µg/ml and [Ni $(men)_3$]2Cl have the lowest α-glucosidase inhibition, having IC_{50} value 2533.21 µg/ml.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

This manuscript was presented in the conference "The Indian Science Congress Association 2016 available link is "http://isc103.in/materials_science%285thjan2016%29.html"
Date: 5-JAN-2016, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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