Research Paper

Volume : 4 | Issue : 7 | July 2015 • ISSN No 2277 - 8179

In Vitro Anticancer and Biological Activity Studies for New Synthesized Transition Metal Ternary Complexes

Chemistry

KEYWORDS : Leucine, Valine, Glutamic, ternary complexes, biological activity, cytotoxicity .

Eman H. Ismail — Chemistry Department, Faculty of Science, Taibah University, 344,Elmadinah , KSA. Chemistry Department, Faculty of Science, Ain Shams University, 11566, Abbassia,,Cairo, Egypt.

Amal I. Abdel-Mageed — Chemistry Department, Faculty of Science, Taibah University, 344,Elmadinah , KSA. Textile Technology Departement, Faculty of Industrial Education ,Beni-Suef University,Egypt.

Heba Mohamed — Chemistry Department, Faculty of Science, Ain Shams University, 11566, Abbassia,,Cairo, Egypt.

Mostafa M. H. Khalil — Chemistry Department, Faculty of Science, Ain Shams University, 11566, Abbassia,,Cairo, Egypt.

ABSTRACT

Ternary complexes of Co (II), Ni (II), Cu (II) and Zn (II) with glutamic acid (glu) as a primary ligand and leucine (leu) or valine (val) as secondary ligand were prepared in slightly acidic medium. The structures of the complexes were elucidated using elemental, IR, mass spectra, magnetic moment, UV–Vis spectrophotometer and thermal analyses. The ternary complexes were isolated in 1:1:1 molar ratio and the molecular structures were found to be M(glutamic)(leucine)$(H_2O)_2$] H_2O and [M(glutamic)(valine)(H2O)2].H2O, where M = Co(II), Ni(II). Thermogravimetric analysis confirmed that two water molecules coordinated to the central metal atom and one crystalline water molecule. UV–Vis spectra showed that the complexes have octahedral symmetry. On the other side, ternary copper complexes with molecular formula [Cu(glu)(leu)] and [Cu(glu)(val)] where the two amino acids coordinated using oxygen and nitrogen forming distorted square planar structure as further supported by electron spin resonance ESR spectra. The ternary zinc complexes showed that the glutamic acid acted as tridentate ligand while leucine or valine acted as monodentate ligands. Moreover, the ligands and their metal complexes were screened against bacteria and fungi using the inhibitory zone diameter. Furthermore, the complexes were tested their cytotoxicity effects against three types of human cancer cell lines hepatocellular carcinoma (HePG2), human colonic carcinoma (HCT) and breast cancer cell lines (MCF-7).

1. Introduction

Transition metal ions play an important role in biochemical processes taking place in living cells (Sigel, A.1973) [1]. The chelation between amino acids and metal ions are considered as models of the process occurring at the molecular level in metal protein system (Hay, *1984)* [2]. Ternary complexes which contain on amino acids as a secondary ligands are significance potential models for enzyme metal ion substrate complexes . (Sanap & Patil, 2013) [3]. In studies of, the model ternary complexes structure provide informations about how biological systems achieve their specificity and stability (Sigel, Operschall, and Massoud , 2006) (Czakis-Sulikowska, Czylkowska, and Radwanska, (2007) [4,5]. Ternary complexes are established to be biologically active against pathogenic microorganisms Crowe, Dobeli, and Gentz (1994) , Nieba-Axmann, Persson, and Hamalainen (1997) [6,7]. On the other hand, metal amino acids complexes exhibit significant biological and enzymatic activities (Perrin, & Agarwal, 1973 p. 167). [8]. Ternary complexes proved to be useful as antibacterial agents against *Staphylococcus aureus, Escherichia coli, Candida albicans* and are used as antitumor drugs against sarcoma and leukaemia (Sakyan, Logoglu, Arslan, Sari and, Akiyan 2004)

(Chohan, Arif, and Akhtar (2006) [9,10]. In addition, ternary complexes have been used in cartographic protein surfaces (Bocarsly & Barton 1992) [11] for biological redox centers (Thakkar & Thakkar 2000) [12] and in protein pick up for purification (Shivankar & Thakkar 2003) [13]. Glutamic acid is classified as-essential amino acid and very common in plants and animals. It is vital in the transmission of nerve impulses especially in brain. Glutamic acid belongs to the group of amino acids with a COOH side chain that ionized at pH above 4.25. Glutamic acid has three binding sites for metal ions coordination, the amino N atom and two COOH groups, their availability depends on the pH of solution. Leucine is an essential branched-chain amino acid used as source for synthesis of blood sugar in liver during starvation, stress and infection to aid in healing (Macotela, Emanuelli, Bang, Espinoza, Boucher, Beebe, Gall, and Khan (2011) [14]. Valine can be used as an energy source in the muscles and in doing so preserves the use of glucose. Valine helps stimulate the central nervous system and is needed for proper mental functioning (Chetty , Makings, Reavis, and Metz, (1991) [15]. It also may help treat liver and gall bladder diseases (Kawaguchi, Torimura, Takata, Satomi, and Sata , (2012) [16]. Many studies showed that the stability constants of the ternary complexes showed to be more stable than its binary ones(Ercag, Sismanglu, and Pura (2005) , (Paschevskaya, Bolotin, Sklyar, Trudnikova, Bukov, and Panyushkin (2006), (Şişmanoğlua, Pura, and Baştuğb (2006) ,(Bindu & Rao 2012) ,(Lahsasni1, Ammar, Amin,and Shoukry, (2012) [17-21]. The inorganic compounds of glutamine and glutamic acid with metal ions have antitumor activity (Dutta, Ray, and Nagarajan, (2013) [22] and these compounds are considered an ideal form for magnesium supplementation (Wagner & Baran 2003) [23]. The complexes of amino acid (L-lysine and L-ornithine) with copper and uses these complexes as models for metallo-proteins (Patil & Rasayan 2013) [24] act anti-inflammatory, antiulcer, anticonvulsant, anticancer (Conato, Gavioli, Guerrini, Kozlowski, Pasti, Pulidori, and Remelli (2001) [25]. Study of copper ternary complexes containing certain hydroxyl acids (Souaya, Khalil, Ismail, Bendas, and Neaz, (2014)[26] and / or amino acids is of interest for brain biochemistry (Chohan, Arif, and Sarfraz (2007) [27] and biological activity (Ramakrishna, Ashis, and Patra (2008) [28]. The interaction of Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) metal ions using aspartic acid (ASP) and glutamic acid (glu) as primary ligands, ibuprofen (IBP) and paracetamol (PC) as secondary ligands have been studied (Patil and Rasayan (2013) [24]. Chemical speciation of ternary complexes of Co(II), Ni(II), Cu(II), and Zn(II) ions with L-glutamic acid and L- methionine have been studied formed ternary complexes have been examined and discussed in relation to that of the corresponding ternary complexes as well as the nature of metal ion in which have been found

that L-glutamic acid can be coordinated by the amino and the two deprotonated α & 8-carboxylate groups to form seven member ring (Latha, Rao, Rao, and Rao (2007), (Latha & Rao 2008), (Pinto, Puppin, Behring, Alves, Rey, and Felcman, (2012)[29-31].

In previous study, ternary complexes of Ni(II),Cu(II),and Zn(II) with nitrilotriacetic acid (NTA) as a primary ligand and valine or leucine as secondary ligand in slightly acidic medium were prepared and their molecular formula were found to be [M(HNTA)(Leu or Val)(H_2O)$_2$]1.5H_2O (Souaya, Khalil, Ismail, and Rabie (2013) [33]. In addition, eight ternary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with NTA as a primary ligand and alanine (alaH) or phenylalanine (phealaH) as secondary ligand in slightly acidic medium were also prepared and their molecular formula were found to be [M(HNTA)(alaH or phealaH)(H_2O)$_2$] (Khalil, Hamed, Souaya, and Abdel Azim (2010) [34]. In this study, preparation and characterization eight of ternary complexes of Co (II), Ni (II), Cu (II) and Zn (II) with glutamic acid as primary ligand and leucine or valine as secondary ligand are studied with their cytotoxicity and biological activity investigations.

2. Experimental

2.1 Materials and reagents

All chemicals used in this study are reagent grade. glutamic acid, leucine and valine were Sigma-Aldrich products. $CuCO_3.Cu(OH)_2.H_2O$, $NiCO_3.2Ni(OH)_2.4H_2O$, $CoCO_3.3Co(OH)_2$, $ZnCO_3.2Zn(OH)_2.H_2O$ from B.D.H. products and used as received.

2.2 Synthesis of metal complexes The calculated amounts of the assigned metal carbonate ($CuCO_3.Cu(OH)_2.H_2O$ (pH=8.44), $NiCO_3.2Ni(OH)_2.4H_2O$ (pH=8.4), $CoCO_3.3Co(OH)_2$ (pH=7.4), $ZnCO_3.2Zn(OH)_2.H_2O$ (pH=7.56)) was mixed with glutamic acid (pH=3.65), and leucine (pH=6.62) or valine (pH=6.27) in 50 mL ~~ml~~ of distilled water to give the 1:1:1 complex, and the mixture was heated nearly to boiling. After complete reaction, 96% ethanol was added until dense precipitate was obtained. After filtration of the precipitate; it was washed with alcohol and dried in an oven at 80ºC then placed in the desiccators over night.

2.3 Instrumentation

All measurements were carried out at the microanalytical laboratories of Cairo University, Ain Shams University and The National Research Center, Cairo. C, H, and N were determined by Vario El Elemental. Co, Ni, Cu, and Zn percentages were determined by atomic absorption spectrometry (AAS), using Perkin-Elmer AAS 3100. IR spectra of the solid complexes were recorded on a JascoFTIR-300 E Fourier Transform Infrared spectrometer using KBr disks in the range 400–4,000 cm^{-1} and CsI Technique in the range 200–630 cm^{-1}. Thermogravimetric analysis was carried out using a Perkin-Elmer 7 series Thermal analyzer. The measurements were carried out under nitrogen atmosphere at a heating rate 10 C min^{-1}.Magnetic susceptibilities of the paramagnetic metal complexes were measured by using a magnetic susceptibility balance Johnson Mtthy, Alfa products; model No MKI atroom temperature. The electronic UV–Vis spectra were measured at room temperature on a Jasco model V-550 UV/Vis spectrophotometer. Mass spectra were recorded at350 C and 70 eV on a GC/MS finnigan mat SSQ 7000 apparatus.

2.4 biological Activity

Antimicrobial activity of the tested samples were determined using a modified Kirby-Bauer disc diffution method (Bauer, Kirby, Sherris, and Truck (1966) [35] 100 mL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 (cells mL^{-1}) for bacteria or 10^5 (cells mL^{-1}) for fungi. A 100 mL–of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism should be selected from primary agar plates and tested for susceptibility by disc diffusion method. Plates incubated with fungi at 25 ºC for 48 hours; bacteria at 35-37 ºC for 48 hours and ,then the diameters of the inhibition zones were measured in millimeters. Agar-based methods such as E tested and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods (Fresco, Coles, Heimberg, Liebowitz, Hami, Stein, and Goetz (2001),

Farnworth, 2003, p. 177) [36, 37].

2.5 Cytotoxicity

Potential cytotoxicity of the compound (s) was tested using the method of Skehan et al (Skehan, Storeng, Scudiero, Monks, McMahon, Vistica, Warren, Bokesch, Kenny, and Boyd (1990) [38]. Cells were plated in 96-multiwell plate (10^4 cells $well^{-1}$) for 24 h before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Different concentration of the compound under test (0,1,2.5,5 and 10) (mg mL^{-1}) were added to the cell monolayer triplicate wells were prepared for each individual dose, then incubated for 48 h at 37ºC and in atmosphere of 5% CO. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was recovered with Tris- EDTA buffer. Color intensity was measured in an ELISA read (Wang, Lin, Zhu, Liu, and Chen, (2002) [39].

3. Result and discussion

3.1. Physical properties and elemental analysis of the ternary complexes

Reaction of glutamic acid (pH 3.65) and valine (pH 6.27) or leucine (pH 6.62) with Cu carbonate (pH 8.44), Ni carbonate (pH 8.4), Co carbonate pH (7.4), Zn carbonate (pH 7.56) gave complexes of Cu-glu-val (pH 4.24), Ni-glu-val (pH 4.36), Co-glu-val (pH 4.3), Zn-glu-val (5.8) at the end of the reaction and similar pH values with leucine which are suggested formation of the metal complexes with different pH values . Elemental analyses of the eight complexes are given in Table 1. All the prepared complexes have some common features such as thermal decomposition before melting. The molecular masses of complexes suggest the presence of two water molecules in case of Co (II), Ni(II) and Zinc(II), Table 1.

Table 1. Elemental analysis, mass spectrometry data, and physical properties of the complexes

Complexes	C%	H%	N%	Metal%	Colour	Molecular weight	Magnetic susceptibility	M.p
	Found Cal.	Found Cal.	Found Cal.	Found Cal.				
[Co(glu)(leu)(H$_2$O)$_2$]H$_2$O	37.69/38.10	7.80/8.30	6.10/6.32	17.32/17.40	Pink	389.11	4.8	336
[Ni(glu)(leu)(H$_2$O)$_2$]H$_2$O	35.80/36.16	7.89/8.10	5.97/6.32	17.29/18.40	Green	389.02	3.6	337
[Cu(glu)(leu)]	38.00/38.60	7.58/8.28	4.20/5.85	18.98/18.57	Blue	339.83	1.8	341
H[Zn(glu)(leu)(H$_2$O)$_2$]H$_2$O	38.15/38.40	7.90/8.14	7.13/7.40	19.10/18.11	Colourless	394.62	diamagnetic	393
[Co(glu)(val)(H$_2$O)$_2$]H$_2$O	36.25/37.10	8.15/8.67	5.80/6.19	18.10/18.23	Pink	376.99	5.1	323
[Ni(glu)(val)(H$_2$O)$_2$]H$_2$O	36.88/37.15	8.53/8.67	5.98/6.19	17.96/18.20	Green	376.79	3.36	325
[Cu(glu)(val)]	38.00/38.66	7.98/8.24	3.91/6.10	19.31/19.40	Blue	327.79	2.2	330
H[Zn(glu)(val)(H$_2$O)$_2$]H$_2$O	35.68/36.40	8.10/8.49	6.20/6.67	19.63/19.80	Colourless	383.59	diamagnetic	382

3.2 IR spectra

The IR frequencies for glutamic acid, leucine and valine are in good agreement with those reported in literature (Kumar , 2011), (Wagner & Argentina , *2003)* [40,41]. Table 2. The spectra of glutamic acid contain a strong band at 1685 cm^{-1} due to stretching vibrations of the un-ionized carboxy group appeared in all the complexes (except zinc complexes) as band (shoulder) at 1711-1695 cm^{-1}. This fact indicates that one of the carboxy

groups is not involved in the coordination. In the i.r. spectra of the amino acids the anti-symmetric stretching vibration frequency ν (N-H) appeared at 3108-3148 cm^{-1} assigned to NH_3^+ group, Table 2. This band has disappeared and new band appeared at 3245-3281 cm^{-1} assigned to N-H stretching modes in the cobalt, nickel and copper complexes suggested that the amino -NH_2 group is coordinated to the metal ion while in zinc ternary complex this band still exist (Batiu, Jelic, Leopold, Cozar, and David (2005)[42].

Table 2. Selected IR bands of the ligands and ternary complexes

νOH	—	-----	-----	3324	3369	-	3429	3424	3345	3336	3424
νNH_2	-----	----	-----	3271	3281	3245	----		3281 3166	3269 3158	3250 3113
ν_{as}NH3+	3148	3108	3148	–	–	–	3062	–	–	–	3140
νCOOH	1685 1638	-----	----	1711	1710	1705	----	----	1710	1695	---
νCOO	1552	1417 1587	1421 1586	1601 1582	1605 1586	1605 1513	1608 1583	1586	1616 1585	1619	1612 1583
δNH3+	1534	1513	1510	---	----	----	1513	----	----	---	1511
νCOO	1456	1424	1422	1403	1404	1416	1406	1398	1401	1401	1407
νM-N	-----	----	----	534	562	539	535	542	542	580	538
νM-O	-----	----	----	399	364	396	365	367	377	385	367

Note:

1=[Co(glu)(leu)(H2O)2]1H2O. 2=[Ni(glu)(leucine)$(H_2O)_2]_1H_2O$. $_3$= [Cu(glu(leucine)]

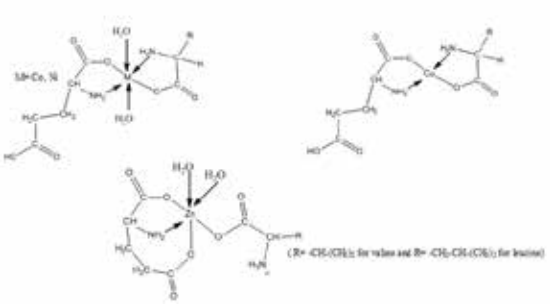
4=[Zn(glu)(leucine)$(H_2O)]_1H_2O$. 5=[Co(glu)(Valine)($H_2O)_2]_1H_2O$.

6=[Ni(glu)(Valine)$(H_2O)_2]_1H_2O$. 7=[Cu(glu)(Valine)]
8=[Zn(glu)(Valine)$(H_2O)]_1H_2O$

The IR spectra of the cobalt, nickel, and zinc complexes show absorption band in the 3450-3350 cm^{-1}, characteristic of OH stretching vibration, suggesting the presence of water molecules. The elemental analysis of the ternary complexes suggests the presence of two coordinated water molecule in the complexes of cobalt and nickel and zinc complexes and its absence in the copper ternary complexes. Absorption bands assigned to M-N and M-O stretching modes are observed in all the complexes (table 2). Metal-nitrogen (M-N) and metal–oxygen) (M-O) bonds were further confirmed by the presence of the bands around (650-450) cm^{-1} and (570-400) cm^{-1} respectively ,(Bindu and Rao (2012) [20], (Sanap and Patil (2013) [3]

From the elemental analysis and spectroscopic data the structure of the obtained complexes can be as shown in Scheme 1, where Co(II), Ni(II) and Zn(II) form octahedral complexes and Cu(II) form square planar complexes. Also, the glutamic acid acts as tridentate in the Zn(II) complexes and as bidentate in the other complexes.

Scheme 1. The structure of the obtained complexes



3.3 UV-Vis spectra and magnetic moments

The UV–Visible spectra of our prepared metal complexes were carried out in deionized H_2O (10^{-3} mol L^{-1}) solution. For ternary Co(II) complexes, the effective magnetic moment values are 4.8 B.M. and 5.1 B.M. for [Co(glu)(leu)$(H_2O)_2$] and [Co(glu)(val)$(H_2O)_2$], respectively. These values are higher than spin only moment for three unpaired electrons 3.89 B.M. due to a considerable orbital contribution (Jianfang et al. (2011) [32]. The UV–Vis spectra of the two complexes show one band at 19,305 cm^{-1} and 20,408 cm^{-1} for [Co(glu)(val)$(H_2O)_2$] and [Co(glu)(leu)$(H_2O)_2$] which are assigned to $^4T_{1g}(F)^{a4}T_{1g}(P)$ transition, assuming an octahedral geometry for Co(II) complex. For ternary Ni(II) complexes, the magnetic moments μ_{eff} of [Ni(glu)(leu)$(H_2O)_2$] and [Ni(glu)(val)$(H_2O)_2$] have values of 3.2 B.M. and 3.16 B.M., respectively, which are very close to the value of a distorted octahedral environment (Jianfang et al. (2011) [32]. The electronic spectrum of Ni(II) complexes displays two bands in the range of 16,260 cm^{-1} and 26,315 cm^{-1} assigned as $^3A_{2g}$ ° $^3T_{1g}(F)$ and $^3A_{2g}$ °$^3T_{1g}(P)$, respectively for octahedral geometry, while the third spin-allowed transition showed maximum around 1000 cm^{-1} assigned as $^3A_{2g}$ ° $^3T_{2g}$. The mixed-ligand complexes of Cu(II) with Leu and Val showed magnetic moment values, μ_{eff}, in the range 1.8–2.2 B.M. These values correspond to one unpaired electron and thus offer evidence for mononuclear structures of the complexes. The UV–Vis spectrum of Cu(II) complexes consists of a broadband centered at 13,513 cm^{-1} that assigned to transition of distorted square planar complexes. Comparing the 10 Dq values of the complexes of this study with the literature values of Co, Ni, and Zn octahedral complexes, respectively, it was concluded that the octahedral structure is recommended for these ternary complexes of this study and the two water molecules are coordinated to the metal (Chaudhary, 2011) [43].

3.4 Mass spectra

The mass spectra of the eight complexes were recorded. All the spectra contain molecular ion peaks confirm the molecular mass of the complexes, with loss of three water molecules for the cobalt and nickel complexes as molecular ion peak (MIP) had very good agreement with molecular weight of these complexes (nickel and cobalt) without the three water molecules (MIP-3H_2O) two coordinated and one crystalline water. The data are presented in Table (1).

3.5 Thermal analysis

Thermo-gravimetric analysis results are shown in Table3, The first mass loss in the complexes is equivalent to the water molecules which supports the suggested structure for the complexes (Li, Murthy, Telser, Zakharov, Yap, Rheingold, Karlin, and Rokita (2006) [44] where the decomposing temperatures are very close to the coordinate water temperature decomposition. As the thermal decomposition is made under nitrogen atmosphere and that almost all products are gases, (CO, C_2H_2 and NH_3). The final thermal decomposition residue is the mixture of metal or metal oxides (Souaya, Ismail, Mohamed, and Milad, 2009) ,(Shengli, Mian, Sanping, Rongzu, and Qizhen (2001) [45,46].

Table 3. Temperature values for the decomposition along with the species lost in each step

Complex	Molar Wt.	TG range/°C	(mass Found) (Calc.) %	Total mass loss	Assignment
1	391.2	55.84~140.56	5.34 (4.6)	82.35	$1H_2O$
		141~242	220(20.96)		$2H_2O$+$2NH_3$
		342~363	16.82(17.99		H2C=CH2+C_4H_4
		364~623	39.86(36.8)		maleic acid+CO
		624~745	17.65(19.09		CoO
2	391.02	50.38~182.36	8.61(9.02)	71.16	$2H_2O$
		182.88~338.79	24.04(24.55)		$1H_2O$+2 NH_3+ CO_2
		339.30~405.41	19.75(18.02)		C_5H_9
		406.76~556.03	18.02(17.13)		C_4H_6
		557.02~780.8	28.83(27.28)		Mix. Ni and NiO

3	341.38	77.22~287.38	47.74(48.85)	74.83	CO_2+ $2NH_3$+C_2H_4+$C_2H_5O_2$
		288.39~50.6	27.42(27.20)		C_2H_2O
		503~690	25.17(24.25)		CuO
4	397.6	31.19~136.66	4.51(4.52)		$1H_2O$
		137.75~256.64	34.35(35.20)		$2H_2O$+ $2NH_3$+ CO+C_2H_4+CH_4
		257.15~518.88	37.68(38.73)		Maleic acid+C_2H_2
		518.95~840	22.87(22.45)		ZnO
5	376.99	40~141	5.37(4.77)	82.35	$1H_2O$
		142~262	18.79(1856)		$2H_2O$+2 NH_3
		263~391	20.66(18.03)		C_2H_2
		392~627	36.95(39.19)		Maleic acid+CO
		628~729	17.65(19.18)		CoO
6	391.02	50.38~182.36	8.61(9.02)	71.16	$2H_2O$
		182.88~338.79	24.04(24.55)		$1H_2O$+2 NH_3+ CO_2
		339.30~405.41	19.75(18.02)		C_2H_2
		406.76~556.03	18.02(17.13)		C_2H_2
		557.02~780.8	28.83(27.28)		Mix. Ni and NiO
7	381.79	170.93~434.27	67.09(66.5)	74.97	CO_2+ $2NH_3$+$C_2H_2O_2$
		434.78~657.27	8.24(8.54)		C3H3
		657~675	25.17(24.25)		CuO
8	383.95	60.15~148.57	3(4.69)	84.18	$1H_2O$
		149.52~425.78	62.52(63.08)		$2H_2O$+ $2NH_3$+2 CO+ Maleic acid
		425.79~762	18.82(17.72)		C_2H_2
		763~980	15.82(16.62)		Zn

1[Co(glu)(leu)($H_2O)_2$]$1H_2O$.2[Ni(glu)(leucine)

($H_2O)_2$]$1H_2O$.3[Cu(glu)(leucine)].

4[Zn(glu)(leucine)(H_2O)]H_2O.5[Co(glu)(Valine)($H_2O)_2$]$1H_2O$.

6[Ni(glu)(Valine)($H_2O)_2$]$1H_2O$.7[Cu(glu)(Valine)].8 [Zn(glu)(Valine)(H_2O)]H_2O

3.6 ESR spectra

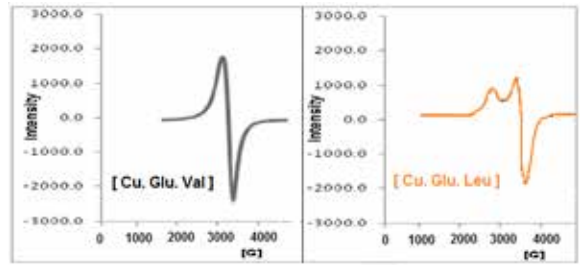
The ESR spectra of the two Cu complexes were similar and showed axial symmetry (g // = 2.11305, g ^ = 2.01538, G= 8.48), (g // = 2.06296, g ^ = 2.01201, G=6.25) for [Cu(glu)(leucine)] and [Cu(glu)(Valine)] as shown in figure.1 , where G is the exchange interaction value, the nearest surrounding of the metal ion; the spin Hamiltonian parameters which is associated with square planar coordination. In square planar complexes, the unpaired electron lies in the dx^2-y^2 sublevel orbital giving (g // > g ^ > 2) while the unpaired electron lies in the dz^2 sublevel orbital giving g^ > g// >2. This provides evidence that the unpaired electron is localized in the dx^2-y^2 sublevel orbital and the ground state is $2B_1g$ (Kadam, Sastry, Bhide, Chavan, Yakhmi, and Khan (1997), (Base, Ohta, Babu, and Sastry (2000), (Ray & Kaufiman 1990), (Jeyasubramanian, Samath, Thambidurai, Murugesan, and Ramalingam (1996) [47-50].

The value of the exchange interaction term G, estimated from the expression:

G = (g // – 2.0023) / (g ^ – 2.0023)

If G> 4, the local tetragonal axes were aligned parallel or only slightly misaligned. If G< 4, significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the Cu complexes (G> 4) suggests that the local tetragonal axes are aligned parallel or slightly misaligned, and the unpaired electron is present in the dx^2–y^2 sublevel orbital. This result also indicates that the exchange coupling effects are not operative in the present complex (Benial, Ramakrishnan, and Murugesan (2000) [51].

Figure 1. ESR of [Cu(glu)(leucine)] and [Cu(glu)(Valine)] complexes



3.7 Biological study

The antimicrobial activities of the metal complexes in vitro were tested against four microbes by the modified disc diffusion method ((Bauer et al. (1966) [35]. The zone of inhibition against the growth of bacteria or fungus for the complexes is given in table 4. It can be seen that, the ligands valine and leucine are inactive to the bacteria and fungus used in this study while glutamic acid shows activity against Escherichia coli. For the negative bacteria, *Escherichia coli*, all complexes exhibit antimicrobial activity in the range of glutamic acid value indicating no effect of leucine, valine or the metal ion. On the other hand, all the complexes gave higher activity against gram positive bacteria *Staphylococcus aureus***.** Only copper ternary complexes exhibit activity against *Aspergillus Flavus***.** Similar results were obtained for the ternary complexes with nitrilotriacetic acid as primary and leucine or valine as secondary ligand (Khalil, Souaya, Hamed, and Rabie (2013). [52]. The Biological activity against *Candida albicans* shows that [Co(glu)(leu)($H_2O)_2$] and [Ni(glu)(leu)($H_2O)_2$] causes inhibition zone of 18 and 21 mm/mg sample, respectively, which are comparable to the amphotericin B antifungal agent which causes 21 mm/mg sample inhibition zone which make these two complexes of more interest. For the ternary complexes with valine as secondary ligand only cobalt and copper and zinc complexes that shows antifungal activity against Candida albicans.

The results show that, as compared to the activity of metal salts and free ligand, the metal complexes show higher activity. The activity of metal complexes is enhanced due to chelation. The chelation reduces considerably the polarity of the metal ions in the complexes, which in turn increases the hydrophobic character of the chelate and thus enables its permeation through the lipid layer of microorganisms (Tweedy, 1964), (Stanila, Marcu, Rusu, Rusu, and David (2007), (Marcu, Stanila, Cozar, and David (2008), (Cornelia, Stanila , Rotar, Petran, and Socaciu, (2006), (Braicu, Stanila, and Socaciu , 2006, p. 69-170.) [53-56].

Table (4). biological activity of the ternary complexes

Sample	Inhibition Zone Diameter (mm / mg sample)			
	Escherichia coli (G)	Staphylococcus aureus (G+)	Aspergillus Flavus (Fungus)	Candida albicans (Fungus)
Control : DMSO	0	0	0	0
Glutamic	11	0	0	0
Leucine	0	0	0	0
Valine	0	0	0	0
Co(glu)(leu)$(H_2O)_2$.H_2O	11	14	0	21R
Ni(glu)(leu)$(H_2O)_2$.H_2O	13	21	0	18R
Cu(glu)(leu)	13	14	10	0
Zn(glu)(leu)$(H_2O)_2$.H_2O	12	15	0	0
Co(glu)(val)$(H_2O)_2$.H_2O	11	10	0	10
Ni(glu)(val)$(H_2O)_2$.H_2O	16	22	0	0
Cu(glu)(val)	11	11	11	12
Zn(glu)(val)$(H_2O)_2$.H_2O	10	9	0	10
Streptomycin	25.6	25.1	------	------
amphotericin B	------	------	22.5	21

3.9 Cytotoxicity

Volume : 4 | Issue : 7 | July 2015 • ISSN No 2277 - 8179

In vitro anticancer activity evaluation of the newly synthesized ternary complexes as well as the amino acid ligands was carried out against three human cancer cell lines hepatocellular carcinoma (HePG2), human colonic carcinoma (HCT) and breast cancer cell lines (MCF-7) using Skehan et al. method (Skehan et al 1990) [38] . Cytotoxicity of the amino acid ligands and the ternary complexes were screened against the three cell lines using 200 mg mL^{-1} of each amino acid or ternary complexes. The highest inhibition percentages are found to be against HePG2 liver cancer followed by HCT then MCF-7 breast cancer cell lines, table 5. It could be seen that all the data for the pure amino acids and their ternary complexes are comparable, then the in vitro cytotoxcicity of the amino acids at different concentrations was carried out to determine the inhibition concentration required for 50% inhibition of cell viability (IC_{50}) of the amino acids and compare it with [Cu(glu)(val)] and [Cu(glu)(leu)] against HePG2 cell line. It was found that IC_{50} for amino acids > $50m^{g\ mL-1}$ while for [Cu(glu)(val)] and [Cu(glu)(leu)] it was 10.1 and 12.2 mg mL^{-1}, respectively. This result indicate that the ternary complexes are better in treatment of HePG2 liver tumers (57- (a ,b) C. Braicu, A. Stanila, C. Socaciu,) Spectroscopic studies of some copper (II) complexes with amino acids.. [57].

Table 5. Cytotoxicity effect of the amino acids ligands and ternary complexes using 200 mg mL^{-1} concentration of each.

Sample	HEPG2 Inhibition%	HCT Inhibition%	MCF-7 Inhibition%
Glutamic acid	85.77	42.83	44.34783
Leucine	87.21	54.84	50
Valine	90.71	53.28	53.47
[Co(Glu)(Leucine)($H_2O)_4$] H_2O	82.21	54.40	50.00
[Ni(Glu)(leucine)($H_2O)_4$] H_2O	79.34	54.06	49.13
[Cu(Glu)(leucine)]	80.27	35.95	33.91
[Co(Glu)(Valine)($H_2O)_4$]1H_2O	82.85	36.31	13.04
[Ni(Glu)(Valine)($H_2O)_4$]1H_2O	80.27	34.79	36.09
[Cu(Glu)(Valine)]	88.13	38.60	49.13

4. Conclusion

The eight ternary complexes of Co(II), Ni(II), Cu(II), Zn(II) glutamic acid and valine or lucine prepared in slightly acidic medium with different octahedral structures with Co(II) same with Ni(II) but different with Zn(II) . For Cu(II) complexes are square planar structure. The formation of the metal complexes with different pH values rather than in pure ligands or metals carbonate. The glutamic acid acts as tridentate ligand in the Zn(II) complexes and as bidentate in the other metal complexes. The lucine or valine acts as monodentate ligand in the Zn(II) complexes and as bidentate ligand in the other metal complexes. Copper complexes have no coordinate water molecules but the other prepared metal complexes have two coordinate water molecules. Co(II) and Ni(II) complexes behave as dibasic acid but for Zn(II) complex behave as monobasic acid.

REFERENCE

1- Base, M., Ohta, K., Babu, Y., & Sastry, M. D., (2000)," Orienting effect of magnetic field on some copper metallomesogen powders: single crystal-like alignment "Chemical Physics Letters,324,330-336 | 2- Batiu, C., Jelic, C., Leopold, N., Cozar, O., & David, L., (2005) ," Spectroscopic investigations of new Cu(II), Co(II), Ni(II) complexes with 8-L-glutamyl amide as ligand" Journal of Molecular Structure ,744 ,325-330. | 3- Bauer, A.W., Kirby, W. M. , Sherris, C., & Truck, M., (1966), "antibiotic susceptibility testing by a standardized single disk method", American journal of clinical pathology. 45 , 493-496. | 4- Benial , A.M. F., Ramakrishnan, V., & Murugesan, R. (2000), "Single crystal EPR of $Cu(C_2H_2NO)_6(BF_4)_2$: an example of admixed ground state "Spectrochim. Acta. A. 56 , 2775-2781 | | 5- Bindu G., & Rao, G. N., (2012), "Mixed ligand complexes of essential metal ions with L-glutamine and succinic acid in sodium dodecyl sulfate–water mixtures." Journal of the Serbian Chemical Society 77, 453-463. | 6- Bocarsly, J. R., & Barton, J. K. (1992)," Protein Surface Recognition and Covalent Binding by Chromium Nitrilotriacetate complexes: Elucidation Using NMR and CD Spectroscopies", Inorganic Chemistry. 31 , 2827-2832 | 7- (a) Braicu C, Stanila A, Rotar M-A, Petran M, Socaciu C (2006)." Antifungal activity of some amino acids complexes against Candida albicans". Bulletin USAMV Agriculture 62:207-212. | (b) Braicu, C., Stanila, A., & Socaciu, C. (2006) , " Studies for evaluation the effect of some aminoacids complex using Sacharomyces cerevisiae as a in vitro model for cytotoxicity" Proc 41st Croatian Internat Symp on Agricult, Opatija. 69-170. | 8- Chaudhary, R., (2011),"Synthesis, Spectral and Pharmacological Study of Cu(II), Ni(II) and Co(II)Coordination Complexes.", Research Journal Chemistry Environmental Science1 , 1-5. | 9- Chetty N., Makings E. V., Reavis, S. C., & Metz, J., (1991) "Methylma¬lon¬ic acid metabolism and nervous system fatty acids in cobalamin deficient fruit bats receiving supplements of methionine, valine and isoleucine." Journal of Biochemistry. 275 585-590 | 10- Chohan ,H. Z., Arif, M. Akhtar, M. A. Supuran, C.T., (2006) " Metal-Based Antibacterial and Antifungal Agents: Synthesis, Characterization, and In Vitro Biological Evaluation of Co(II), Cu(II), Ni(II), and Zn(II) Complexes With Amino Acid-Derived Compounds" bioinorganic chemistry application, 2006. 1-13 | 11- Chohan, H. Z. , Arif, M., Sarfraz, M., (2007) "Formation equilibria of nickel coplexes with glycyl-histidyl-lysine and two synthetic analogues", Applied Organometallic Chemistry,21 , 294-302. | 12- Conato, C., Gavioli, R., Guerrini, R., Kozlowski, H., Pasti, C., Pulidori, F., & Remelli , M., (2001) "Copper complexes of glycyl –histidyl –lysine and two of its synthetic analogues :chemical behavior and biological activity" Biochimica et Biophysica Acta, 1526 , 199-210. | 13- Cornelia, B., Stanila , A., Rotar, M. A., Petran, M., & Socaciu, C. ,(2006)," ANTIFUNGAL ACTIVITY OF SOME AMINO ACID", Buletin. USAMV-CN. 62 , 207-212. | 14- Crowe, J., Dobeli, H., & Gentz, R. ,(1994)" 6xHis-Ni-NTA chromatography as a superior technique in recombinant protein expression/purification", Methods in Molecular Biology 31, 371-387. | 15- Czakis-Sulikowska, D ., Czylkowska A.,& Radwanska, D., (2007), " Synthesis and characterization of new metal(II) complexes with formates and some nitrogen donor ligands" Journal of Thermal Analysis and Calorimetry, 90 , 557-564 | 16- Dutta, S., Ray, S., & Nagarajan, K. ,(2013)" Glutamic acid as anticancer agent: An overview", Saudi Pharm Journal 21 , 337-343. | 17- Ercag, A., Sismanglu, T., & Pura, S., (2005) ,"Thermodynamics of Binary and Ternary ... of 3-amino-1,2 ,4-triazole and Amino Acids with Ni(II) and Co(II) Metal Ions", Journal of the Serbian Chemical Society ,70 , 1057-1066. | 18- Farnworth, E. R., Handbook of Fermented Functional Foods. 2nd ed.; CRC Press, Taylor& Francis group: Boca Raton, London ,New York, 2003, 177-201. | 19- Fresco, D. M. , Coles, M. E. , Heimberg, R. G. , Liebowitz, M. R., Hami, S. , Stein, M. B., & Goetz, D., (2001)," The Liebowitz Social Anxiety Scale: a comparison of | the psychometric properties of self-report and clinician-administered formats" Psychological Medicine, 31 , 1025-1035. | | 20- Hay, R. W. , Bio-Inorganic Chemistry. Second ed., Ellis, Horwood, Chichester UK , 1984. | 21- Jeyasubramanian, K., Samath, S. A., Thambidurai, S. , Murugesan, R., & Ramalingam, S. K., (1996)," Cyclic voltammetric and ESR studies of a tetraaza 14-membered macrocyclic copper(II) complexderived from 3-salicylideneacetylacetone and ophenylenediamine: Stabilization and activation of unusual oxidation states" Transition metal chemistry", 20 , 76. | | 22- Jianfang, D. , Lianzhi, L., Guihua, L., Tao, X., & Daqi, W. ,(2011)," Synthesis, crystal structure and DNA- binding properties of a new copper(II) complex with L-valine Schiff base and 1,10- phenanthroline," Journal of Molecular Structure , 986 , 57-63. | 23- Kadam, R. M. , Sastry, M. D. , Bhide, M. K. , Chavan, S. A. , Yakhmi, J. V. , & Khan, O., (1997), "An EPR study of spin correlations and existence of ordered and disordered phases in (NBu4)2Mn2[Cu(opba)]3•6DMSO•1H2O",Chemical Physics Letters ,281 ,292-296. | 24- Kawaguchi, T. ,Torimura, T., Takata, A., Satomi, S., & Sata , M ., (2012), "Valine, a Branched-Chain Amino Acid, Reduced HCV Viral Load and Led to Eradication of HCV by Interferon Therapy in a Decompensated Cirrhotic Patient." Case Rep Gastroenterol , 6 , 660-667. | 25- Khalil, M. M. H., Hamed, E., Souaya, E. R. , & Abdel Azim, S. ,(2010)," Synthesis, characterization, and thermal analysis of ternary complexes of nitrilotriacetic acid and alanine or phenylalanine with some transition metals" Journal of Thermal Analysis and Calorimetry,101 , 129-135. | 26- Khalil, M. M. H., Souaya, E. R. , Hamed, E., & Rabie, E. ,(2013)," Synthesis and characterization of a novel ternary complexes of nitrilotriacetic acid and valine or leucine with some transition metals and their biological applications ", Chinese Journal of Inorganic Chemistry, 29 , 1-12. | 27- Kumar , S., (2011),"Spectroscopic studies of valine and leucine molecules a comparative study" Elixir, Vibrational Spectroscopy, 39 , 4996-4999. | 28- Lahsasni1, S. A., Ammar, R. A. , Amin, M. F. ,& Shoukry, E. M. , (2012), "Mixed-Ligand Complex Formation of Cu(II) with 1,2-Diphenyle-thylenediamine as Primary Ligand and Amino Acids as Secondary Ligands." Int. J. Electrochem. Sci. 7 , 7699-7711. | 29- Latha, M. P. , Rao, V. M., Rao, T. S. , & Rao, G. N. , (2007), Acta. Chim. Slov. 54 ,160-165. | 30- Latha, M. P. ,& Rao, V. M. , (2008), Acta. Chim. Slov. 55 , 248-256. | 31- Li, L., Murthy, N. N. ,Telser, J., Zakharov, L.N., Yap, G. P., Rheingold, A. L. , Karlin, K. D. ,& Rokita, S. E., (2006), Inorg. Chem. 45 , 7144-7159. | 33- Marcu, A. , Stanila,A., Cozar, O., & David, L., (2008), "Structural investigations of some metallic complexes with threonine as ligand" Journal of Optoelectronics and Advanced Materials,10 , 830-833. | 34- Nieba-Axmann, S. E., Persson, A., & Hamalainen, M., (1997), Anal. Bio-chem. 252, 217-222. | | 35- Paschevskaya,N. V., Bolotin, S. N., Sklyar, A. A., Trudnikova, N. M., Bukov, N. N., & Panyushkin,V. T., (2006), "Binary and ternary complexes of copper(II) with N -phosphonomethylglycine and valine ." J. Molec. Liqu. 126 , 89-94. | 36- Patil, A. , (2013), "Stability Constants of Binary and ternary complexes of ibuprofen and paracetamol" Rasayan. J. Chem. 6 , 168-171. | 37- Perrin, D. D., Agarwal , R. P., in: H. C. Sigel, (Eds), Metal Ions in Biological Systems. Marcel Dekker, New York, 1973, vol 2, 167-190. | 38- Pinto, L.D., Puppin, P. A. L., Behring , V. M., Alves , O. C., Rey, N. A., & Felcman, J., (2012), Solution and solid state study of copper(II) ternary complexes containing amino acids of interest for brain biochemistry – 2: Homocysteine with aspartate, glutamate or methionine", Inorganica Chimica Acta 386 , 60-67. | 39- Ramakrishna, R., Ashis , K., & Patra, P. R.,(2008), Polyhedron. 27 , 1343-1352. | 40- Ray, R. K.,& Kaufiman, G. B., (1990), Inorganica Chimica Acta ,173 , 207. | 41- Sakyan , I., Logoglu, E., Arslan, S., Sari, N., & Akiyan, N., (2004), "Antibacterial activities of some Amino acid Schiff bases", Bio metals, 17 , 115-120 | 42- Sanap, S .V.,& Patil ,S .V., (2013), "Synthesis, Characterisation and Biological Activity of Chiral Mixed Ligand Ni(II) Complexes." Research Journal Pharmaceutical Science, 2 , 1-10. | 43- Shengli, J., Mian, J., Sanping, C., Rongzu, H., & Qizhen , S., (2001), " The Thermokinetics of the Formation Reaction of Cobalt Histidine Complex ",Journal of Thermal Analysis and Calorimetry , 66, 423-429. | 44- Shivankar, V. S., & Thakkar ,N. V., (2003), "Synthesis, characterization and antimicrobial activity of some mixed ligandCo(II) and Ni(II) complexes" ,Acta Poloniae Pharmaceutica - Drug Research 60 , 45-50. | 45- Skehan, P., Storeng , R., Scudiero , D., Monks, A., McMahon , J., Vistica, D., Warren , J. T., Bokesch ,H., Kenny, S., & Boyd, M. R., (1990),: New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening",Journal of the National Cancer Institute,82 , 1107-1112. | | 46- Sigel , H., Operschall, B. P., & Massoud, S.S., (2006), "Evidence for Intramolecular Aromatic-Ring Stacking in the Physiological pH range of the Monodeprotonated Xanthine Residue in Mixed-Ligand Complexes Containing Xanthosine 5`-monophosphate" Journal Chemical Society Dalton Trans. 46 , 5521-5529. | | 47- Sigel, A., in: Sigel , H. C., (Eds.), Metal Ions in Biological Systems. Marcal Dekker, New York, 1973, vol 18 , 1-23. | 48- Şişmanoğlua, T., Pura, S., & Baştuğb, , A. S., (2006), " Binary and ternary metal complexes of Congo red with amino acids." Dyes and Pigments, 70 , 136-142. | 49- Souaya , E. R., Ismail, E. H., Mohamed , A. A., & Milad, N. E., (2009), "Preparation ,Characterization and Thermal Studies of Some Transition Metal Ternary complexes" Journal of Thermal Analysis and Calorimetry, 95 , 553-558. | 50- Souaya , E. R., Khalil, M. M. H., Ismail, E. H., & Rabie , E., (2013), "Ternary Transition Metal Complexes of Nitrilotriacetic Acid and Valine or Leucine: Synthesis and Biological Applications" Chin. J. Inorganic. Chem. 29 , 1969-1978. | 51- Souaya , E. R., Khalil , M. H., Ismail, E. H., Bendas , E. R., & Neaz , O. S., (2014), RJPBCS. 5 , 18-30. | 52- Stanila, A., Marcu, A., Rusu, D., Rusu, M., & David, L., (2007), " Spectroscopic studies of some copper (II) complexes with amino acids". Journal of Molecular Structure. 834 , 364-368. | 53- Thakkar, J. R., & Thakkar , N. V., (2000), " Synthesis and characterization of chiral mixed ligand Co(II) complexes of isonitrosopropiophenone and amino acids" Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 30 , 1871-1887. | 54- Tweedy B. G., (1964), "Plant extracts with metal ions as potential antimicrobial agents," Phytopathology, 55 ,910. | 55- Wagner , C. C., & Baran , E. J.,(2003), " Vibratioal and Magnetic properties of a Cu/Mg Glutamate complex. "Acta. Farm. Bonaerense. 22 , 137-142. | 56 -Wang, Z. M., Lin , H. K., Zhu, S. R., Liu , T. F.,& Chen, Y. T., (2002), " Spectroscopy, cytotoxicity and DNA-binding of the lanthanum (III) complex of an L-valine derivative of 1,10-phenanthroline, ",J. Inorg. Biochem. 89 , 97- 106.