

Studying of transition metal complexes containing oxalate ion with antibacterial activity

Atheer. A. Mahmood*, Abdulqader M. Abdulqader *, Saffa A. Raheem Mahmood**, Mohammed Mosa Jafaar**, Anaam Mahmood Abid**

*Chemistry Department, College of Education, Iraqia University, Baghdad-IRAQ

** Industrial Microbiology Department; Food and Biotechnology Centre; Agricultural research directorate; Ministry of Science and Technology

Abstract

The research includes synthesis two types of complexes containing oxalate ion with metals Fe(III) and Cu(II). They had been characterized by molar conductance, melting point, atomic absorption measurements(A.A), magnetic moment measurements infrared (FTIR) and electronic (UV.VIS) spectra .

Also includes the studying of biological effect for these complexes on different pathogenic species of bacteria: Staphylococcus aureus gram positive (+ve), the others are gram negative (-ve) which are included Escherichia coli (E.coli) and Pseudomonas aeruginosa , by using Muller Inhibitors Concentration (MIC) method In order to evaluate the effect of metal ions upon chelation and show the different activity of inhibition on the growth of bacteria.

Key words: oxalate complexes, antibacterial activity, transition metal complexes

1- Introduction

The oxalate ion ($C_2O_4^{2-}$) abbreviated as OX^{2-} is an example of a bidentate dibasic ligand; having two bonding points for the metal ion occurs through its both negatively charged O-atoms⁽¹⁾ as figure (1):



Figure (1) : structure of oxalate ion

The ferrate(III) oxalate was reported for the first time by Blair and Jonesin⁽²⁾. This material was extensively investigated To its wide applications in photochemical studies ,actinometry ,sensors and magnetic materials ⁽³⁻⁵⁾.

The complexation of metals with ligands can drastically change the physico–chemical and biological properties of the metal species⁽⁶⁾. Metal ions play an important role in bioinorganic chemistry and metals such as Fe and Cu may exist in trace amounts in biological systems. Structural studies of the complexes of theses metals with biological compounds are extremely important⁽⁷⁾.

Scientific researchers working in the field of bioinorganic chemistry, the copper represents one of the most interesting biometals for the preparation of new metal-based drugs with strong potential for therapeutic applications ⁽⁸⁾. Copper is an essential micronutrient that, its ability to undergo Cu(I) to Cu(II) redox cycling, plays a role in cellular redox reactions⁽⁹⁾ . copper complexes are an essential as anti-inflammatory drugs are often more active than the parent ligands themselves⁽¹⁰⁾ . Several works have appeared concerning

models of copper enzymes⁽¹¹⁻¹³⁾. Copper deficiency has been reported to cause hematologic disorders, hypo pigmentation, defective connective tissue cross-linking and ataxia⁽¹⁴⁻¹⁵⁾. The antibacterial activity of the complexes was investigated against representative microorganisms using the method previously reported⁽¹⁶⁾.

2- Experimental

2.1 Materials

All chemicals used were of reagent grade (supplied by either Merck or Fluka) and used as supplied. All the metal ions Cu(II) and Fe(III) were of Analar grade (BDH). They were used without further purification.

2.2 Instruments

Electronic spectra of the prepared complexes were measured in the region (200- 1100) nm quartz cell Shimadzu, UV-160A Ultra Violet Visible- Spectrophotometer. FTIR spectra were recorded as KBr pellets using Fourier transform Infrared Spectrophotometer Shimadzu 24 FTI.R 8400s. While metal contents of the complexes were determined by Atomic Absorption(A.A) Technique using Japan A.A-67G Shimadzu. Electrical conductivity measurements of the complexes were recorded at room temperature for 10⁻³ M solutions of the samples in DMF using pw9527 Digital conductivity meter (Philips). Melting points were recorded to ligand and complexes by using Stuart melting point . Magnetic susceptibility measurements were measured using Bruker magnet BM6 instrument at 298°K following the Faraday's method. The proposed molecular structure of the complexes were determinate by using ChemBio office v.13.0 (2012) used for drawing the 2D and 3D - structure of compounds⁽¹⁷⁾.

2.3 preparation of complex potassium tris(oxalato) ferrate (III) trihydrate=

Ferric chloride hexahydrate (0.016 mole) is dissolved in distilled water, then is added to the solution Potassium oxalate hydrate (0.048 mole), the product is cooled in bath ice and filtered to obtain green crystals.

2.4 preparation of complex sodium bis(oxalato) cuprate (II) dihydrate

Copper sulfate pentahydrate (0.008 mole) is dissolved in distilled hot water, then is added to the solution sodium oxalate (0.014 mole) with stirring and heating to 90 °C, the product is cooled in bath ice and filtered , washed and dried to obtain blue complex .

2.5 Antibacterial Activity Test

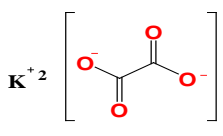
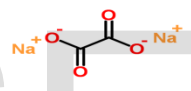
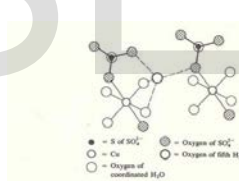
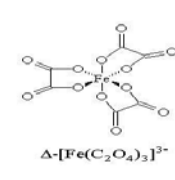
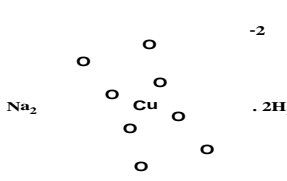
Antibacterial activities of the complexes were tested in different concentrations against three types of pathogenic species of standard bacteria which are isolated in general central lab in ministry of health; Staphylococcus aureus gram positive (+ve), the others are gram negative (-ve) which are included Escherichia coli (E.coli) and Pseudomonas aeruginosa by using Muller Inhibitors Concentration (MIC) method⁽¹⁸⁾.

The sterilized (autoclave at 121 °C for 15 min) medium (40-50 °C) was poured into petridishes to give a depth of 3-4 m.m and allowed to solidify. The suspension of the standard microorganism then streaked on plates which pre-incubated for 1 hr at room temperature and incubated at 37°C for 24 hr . The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (I.Z) around the hole in millimeter m.m⁽¹⁹⁾ .

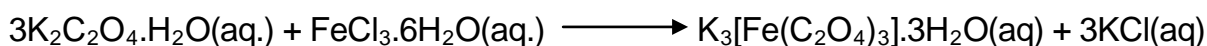
Results and Discussion

The physical properties of ligands and the complexes were shown in table (1) as :

Table (1): physical properties of ligands and the complexes

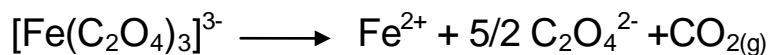
No.	Chemical formula and Nomenclature	Structure formula	M.Wt	M.P °C	Colour
1	$K_2C_2O_4 \cdot H_2O$ Potassium Oxalate monohydrate		184.23		White
2	$Na_2C_2O_4$ Sodium Oxalate		134		White
3	$CuSO_4 \cdot 5H_2O$ Cupric Sulfate Pentahydrate		249.6		Blue
4	$K_3[Fe(C_2O_4)_3] \cdot 3H_2O$ Potassium trisoxalatoferrate(III) trihydrate		491	230	Green
5	$Na_2[Cu(C_2O_4)_2] \cdot 2H_2O$ Sodium bisoxalato cuprate(II) dihydrate		321.5		Blue

Potassium trisoxalatoferrate(III) trihydrate, $K_3[Fe(C_2O_4)_3] \cdot H_2O$ is a green crystalline salt, soluble in hot water but rather insoluble when cold. It can be prepared by the reaction of $K_2C_2O_4 \cdot H_2O$ with $FeCl_3 \cdot 6H_2O$.



The synthesis of potassium tris(oxalato)ferrate(III)trihydrate was in 1939⁽²⁰⁾. This material was extensively investigated owing to its wide applications in photochemical studies, actinometry, sensors, magnetic materials, etc.⁽²¹⁻²³⁾.

This means that upon exposure to light of an appropriate wavelength (<450 nm in this case) the $[\text{Fe}(\text{C}_2\text{O}_4)_3]^{3-}$ undergoes an intermolecular redox reaction in which the Fe(III) anion is reduced to Fe(II) while one of the oxalate groups is oxidized to CO_2 .



As mentioned above, light causes an internal electron-transfer reaction to occur in $[\text{Fe}(\text{C}_2\text{O}_4)_3]^{3-}$ ion, producing CO_2 and Fe^{2+} ions.

Trivalent iron has the electronic configuration of $3d^5$ which corresponds to a half-filled d-sub-shell and is particularly most stable. In crystalline fields, the usual high spin configuration is $t_{2g}^3 e_g^2$ with one unpaired electron in each of the orbitals and the low spin state has the t_{2g}^5 configuration with two pairs of paired electrons and one unpaired electron. The energy level in the crystal field is characterized by the following features.

The ground state of d^5 ion, 6S transforms into $^6A_{1g}$ - a singlet state. It is not split by the effect of crystal field and hence all the transitions are spin forbidden and are of less intensity. In excited state, d^5 ion gives rise to quartets (4G , 4F , 4D , 4P) and doublets (2I , 2H , 2G , 2F , 2D , 2P , 2S). The transitions from the ground to doublet state are forbidden because the spin multiplicity changes by two and hence they are too weak. Thus sextet-quartet forbidden transitions observed are: $^6A_{1g} \rightarrow ^4T_{1g}$ and $^6A_{1g} \rightarrow ^4T_{2g}$. The transitions which are independent of Dq and which result in sharp bands are $^6A_{1g} \rightarrow ^4E(4D)$, $^6A_{1g} \rightarrow ^4E_g + ^4E_{1g}$ etc.⁽²⁴⁾.

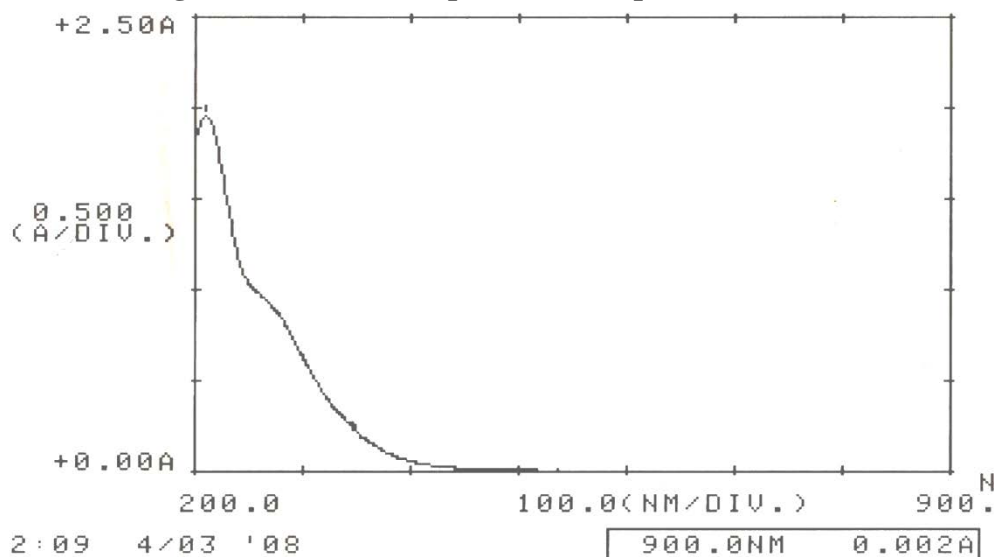
The electronic spectra of the Fe(III) complex exhibited λ_{max} at 210 nm (absorbance was 1.95 A) $n \rightarrow \pi^*$ which means ultraviolet region and charge transfer from L to M, an electron moves versus ligand-to-metal charge transfer (LMCT); take M with high ionization energy, i.e. low lying empty d orbitals, and L with low electron affinity, filled orbitals at high energies. Fe^{3+} , there are three transitions: $^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$ (ν_1), $^6A_{1g}(S) \rightarrow ^4T_{2g}(G)$ (ν_2). ν_1 occurs between 10525 cm^{-1} and ν_2 occurs between 15380 to 18180 cm^{-1} usually as a shoulder. The bands corresponding to $^6A_{1g}(S) \rightarrow ^4A_{1g}(G)$, $^4E_g(G)$ (ν_3) appear around 22000 cm^{-1} . The last transition is field independent. The ligand field spectrum of ferric iron appears as if the first (ν_1) and the third (ν_3) bands of octahedral symmetry are only present. The analysis of general features of the spectrum of Fe^{3+} containing plumbojarosite is discussed here. The first feature observed in the range 12000 to 15500 cm^{-1} is attributed to $^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$, the third band at 22730 cm^{-1} is sharp and is assigned to $^6A_{1g}(S) \rightarrow ^4A_{1g}(G)$, $^4E_g(G)$ transitions respectively. A broad and diffused band at 19045 cm^{-1} is assigned to the $^6A_{1g}(S) \rightarrow ^4T_{2g}(G)$ band. The other bands are also assigned to the transitions with the help of Tanabe-Sugano diagram.⁽²⁵⁻²⁷⁾

The copper ion has oxidation number (O.N) equal to (+2) and coordination

number (C.N) equal to (4) because the oxalate anion coordinates to a metal as bidentate Chelate. The copper ion has ground state 2D , while field configuration $e^4 t_2^5$. The electronic spectra of the Cu(II) complex exhibited λ_{max} at 251 nm (absorbance was 1.753 A) is attributed ($\pi \rightarrow \pi^*$) transition which means ultraviolet region and charge transfer from L to M, an electron moves versa ligand-to-metal charge transfer(LMCT); take M with high ionization energy. The other regions at 212 nm (absorbance was 2.176 A) and 235 nm (absorbance was 1.5416 A) which means ultraviolet region (near UV.) . The spectra of complexes Fe(III) and Cu(II) are shown in figures (2,3).

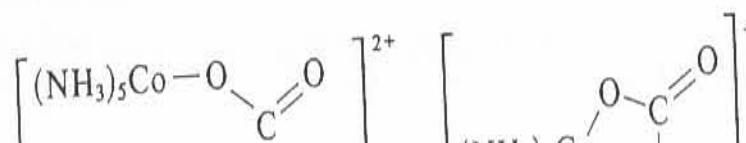
IJSER

Figure(2) : (UV-Vis) Spectra of complex Cu(II)

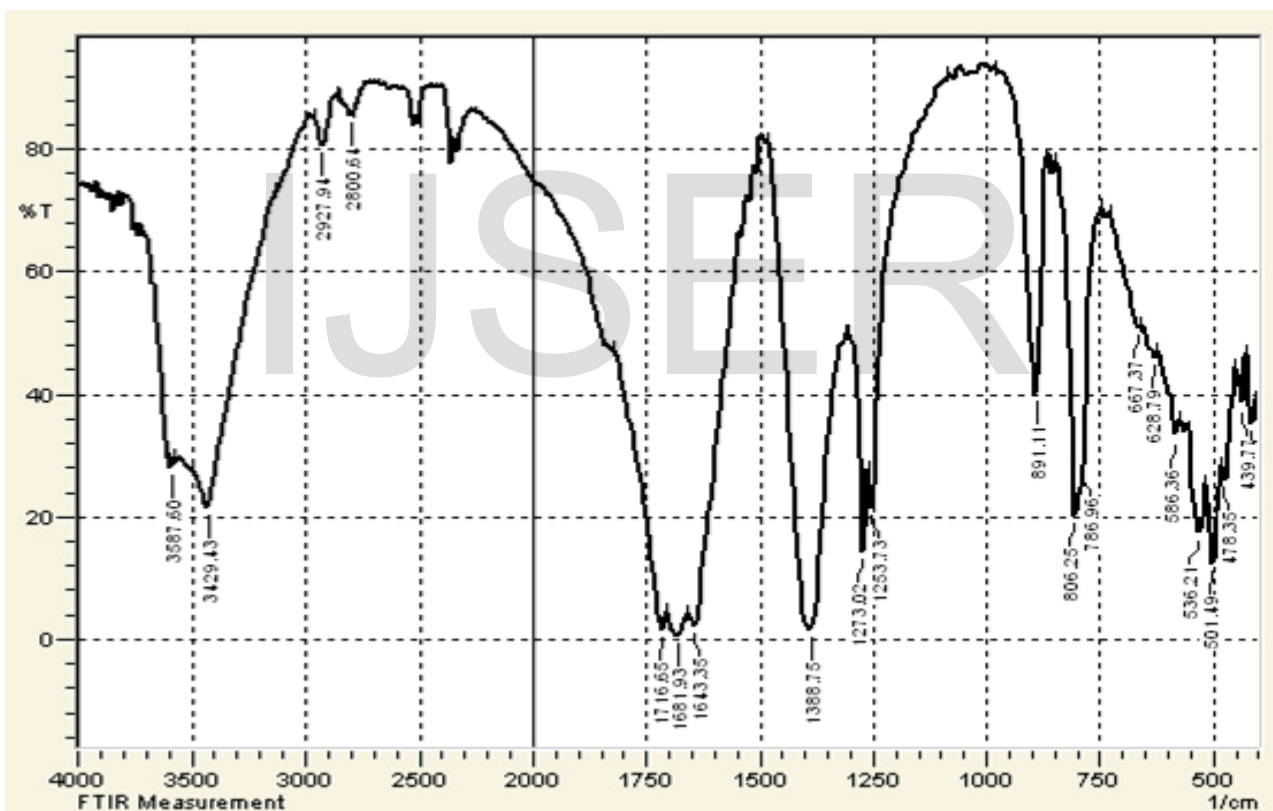


Figure(3) : (UV-Vis) Spectra of complex Fe(III)

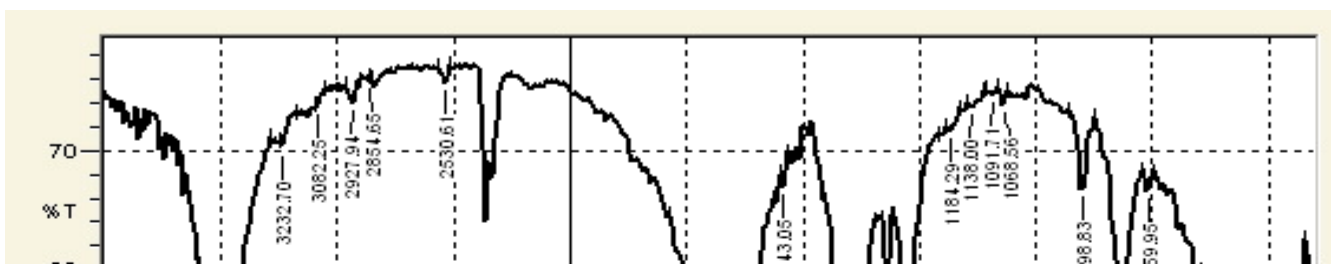
The oxalate anion (ox^{-2}) coordinates to a metal as an unidentate (I) or bidentate (II) ligand:



The bidentate chelate structure (II) is most common ⁽²⁸⁾. Carried out normal coordinate analyses on the 1:1 metal-ligand model of the $[M(OX)_2]^{2-}$ and $[M(OX)_3]^{3-}$ series, and obtained the band assignment listed in table (1). In the divalent metal series as Cu(II), $\nu(C=O)$ becomes higher, and $\nu(C-O)$ becomes lower. In the trivalent metal series Fe(III) found $\nu(MO_{stretching})$ follows the same trend as the crystal field stabilization energies (CFSE) of these metals ⁽²⁹⁻³⁰⁾. The spectra FT-IR of complexes are shown in figure (4,5).



figure(4) : FT-IR spectrum of complex Fe(II)



Figure(5) : FT-IR spectrum of complex Cu(II)

Antibacterial Activity

Metal complexes were screened for their antibacterial activities according to the respective literature protocol⁽³¹⁾ and the results are obtained in table (2).

Antibacterial activities of the complexes were tested in different concentrations against three types of pathogenic species of standard bacteria which are isolated in general central lab in ministry of health; Staphylococcus aureus gram positive (+ve), the others are gram negative (-ve) which are included Escherichia coli (E.coli) and Pseudomonas aeruginosa, and the minimum inhibitory concentration (MIC), then compared with the theoretical calculations with energies of docking .

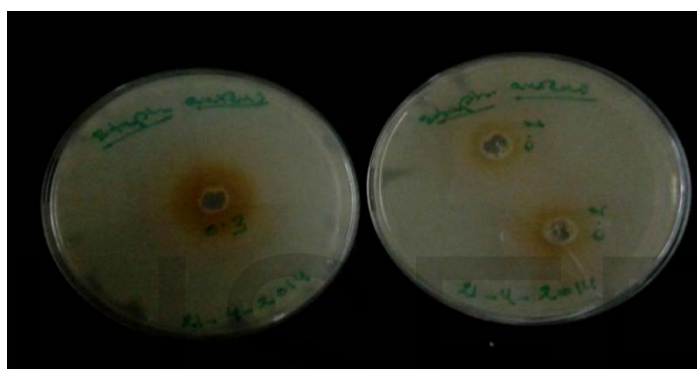
The results show antibacterial activity to complex $K_3[Fe(C_2O_4)_3].3H_2O$ best results which mean the diameter of inhibition in m.m, and the results were best on E.coli, Pseudomonas aeruginosa while Staphylococcus aureus was least .

While the results of complex $Na_2[Cu(C_2O_4)_2].2H_2O$ on that bacteria appear effect the raising of concentration on the diameter of inhibition to E.coli, Pseudomonas aeruginosa bacteria, while the used concentration of complexes do not affect on Staphylococcus aureus bacteria.



**Inhibition Zones against E .coli
(gram -ve)**

**Inhibition Zones against Pseudomonas aeruginosa
(gram -ve)**



**Inhibition Zones against Staphylococcus aureus
(gram +ve)**

**The Antibacterial activity of complex $K_3[Fe(C_2O_4)_3].3H_2O$
appears the inhibition zones against three types of bacteria**



**Inhibition Zones against *Pseudomonas aeruginosa*
(gram -ve)**

**Inhibition Zones against *E. coli*
(gram -ve)**



Inhibition Zones against *Staphylococcus aureus* (gram +ve)

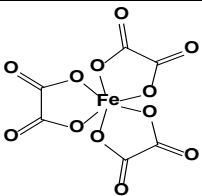
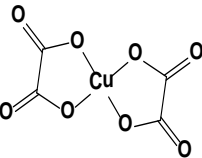
**The Antibacterial activity of complex
 $\text{Na}_2[\text{Cu}(\text{C}_2\text{O}_4)_2] \cdot 2\text{H}_2\text{O}$ appears the inhibition zones
against three types of bacteria**

Table(2): effect a concentration of complexes on diameter of inhibition in millimeter for bacteria

Compounds	Concentration (M)	E .Coli	Pseudomonas aeruginosa	Staphylococcus aureus
K₃[Fe(C₂O₄)₃].3H₂O	0.1	30	12	0
	0.2	30	14	0
	0.3	40	20	20
Na₂[Cu(C₂O₄)₂].2H₂O	0.1	18	0	0
	0.2	20	0	0
	0.3	22	40	0

The Docking (interaction) Energies in unit (k cal/mol) to the complexes against three types of bacteria appear the negative values which refer to theoretical calculations were performed by using a Personal Computer with a Core i7 processor (CPU 2.20GHz) and (RAM 8GB), with a Windows7 operating system, and these computational works used five software, and figures are sketched by ChemBio office v.13.0 (2012)⁽³²⁾. While molecular operating environment MOE v.(2009) software developed was by Chemical Computing Group, Montreal, Canada ⁽³³⁾ used for graphical illustrations and molecular interaction potentials, in addition that, used for docking calculations. whenever a value was greatest its best. The Docking (interaction) Energies were shown in table (2), and the possible binding mode of the complexes with bacteria was showed in figures (6,7).

Table (3): interaction Energies of complexes with bacteria

No.	Compound	Structure	E-Doc of A	E-Doc of B	E-Doc of C
1	K₃[Fe(C₂O₄)₃].3H₂O		-9.4	-7.4	-7.5
4	Na₂[Cu(C₂O₄)₂].2H₂O		-6.66	-6.4	-6.9
E-Doc. is the interaction energy in kcal/mol of: A- staphylococcus aureus (PDB:2z6f), B- pseudomonas aeruginosa (PDB:1lry), C-Escherichia coli (PDB:1llb)					

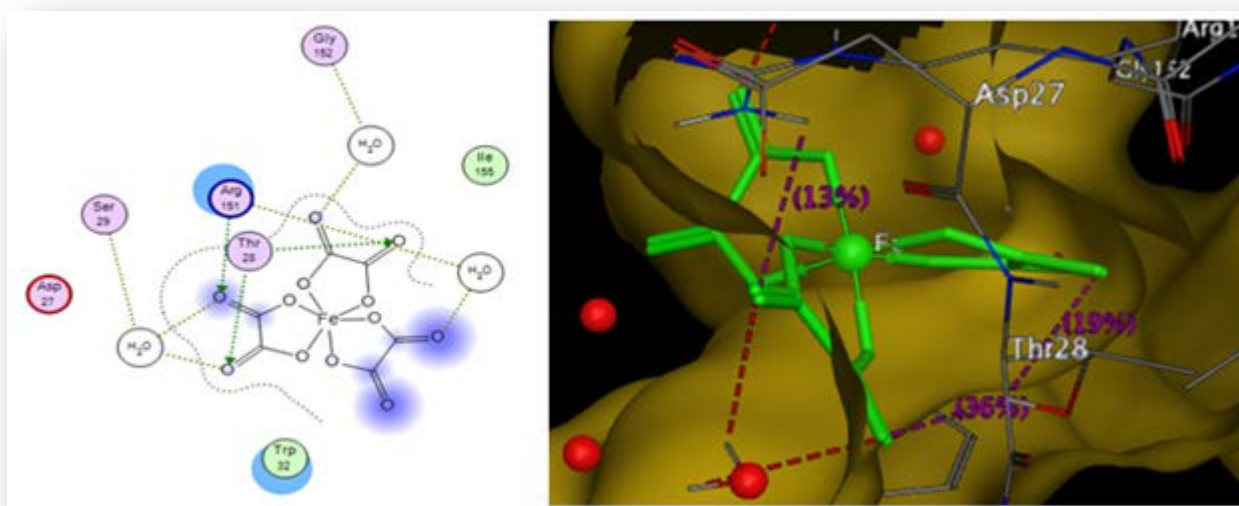


Figure (6): Binding mode of top ranked of complex $K_3[Fe(C_2O_4)_3].3H_2O$ in the binding site of Escherichia coli (**1llb**) from PDB.

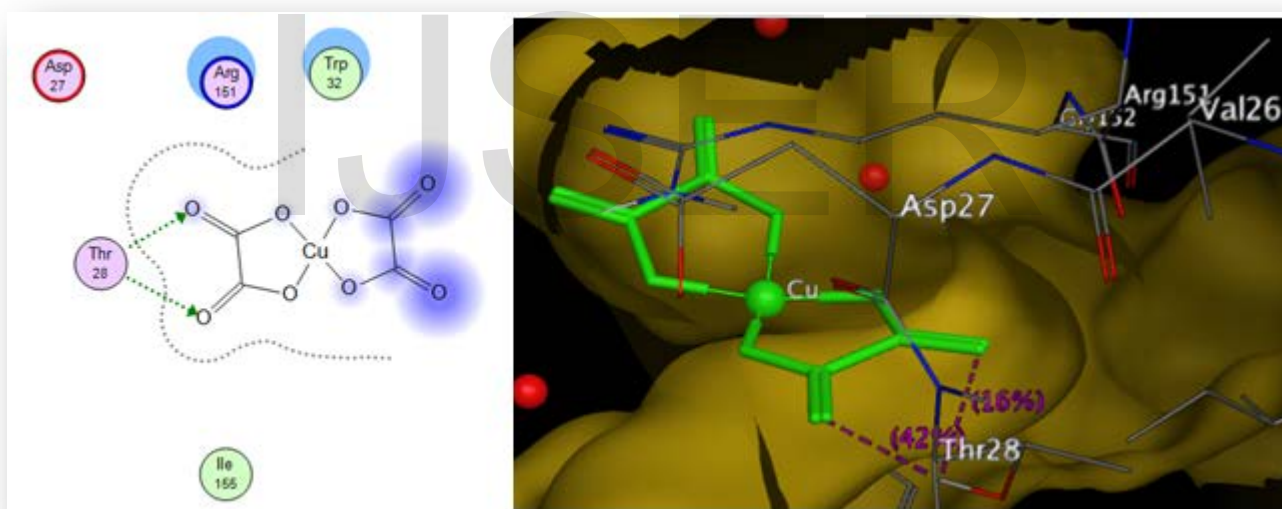


Figure (7): Binding mode of top ranked of complex $Na_2[Cu(C_2O_4)_2].2H_2O$ in the binding site of Escherichia coli (**1llb**) from PDB.

References:

- 1- Dr. R.D. MADAN, " Modern Inorganic Chemistry," RAM NAGAR, NEW DELHI, 2000.
- 2- J.C. Blair, E.M. Jones, in: H.S. Booth (Ed.), Inorganic Synthesis, vol. 1, McGraw-Hill Book Co., New York, 1939, p.35
- 3- H.E. Spencer, M.W. Schmidt, J. Phys. Chem. 75 (1971) 2986.
- 4- D. Armenatano, G. De Munno, F. Lioret, M. Julve, Cryst. Eng. Commun. 7 (2005) 57.
- 5- Tiexiang Fu, Jun Tao, Sens. Actuat. B 129 (2008) 339.
- 6- K. Gao, J. Pearce, J. Jones, C. Taylor, *Environ. Geochem. Health* 21 (1999) 13
- 7- (Canpolat et al, 2004)
- 8- Duncan, White, 2012
- 9- Jomová, Valko, 2011.
- 10- J. R. J. Sorenson, Prog. Med. Chem. 26, 437 (1989) and refs. Therein
- 11- K. D. Karlin, Z. Tyek'ar, Bioinorganic Chemistry of Copper, Chapman and Hall, New York (1993).
- 12- T. N. Sorel, Tetrahedron 45, 3 (1989).
- 13- P. Chaudhuri, Multi-copper Oxidases, World Scientific, Singapore (1997).
- 14- Koca, E.; Buyukasik, Y.; Cetiner, D.; Yilmaz, R.; Sayinalp, N.; Yasavul, U.; Uner, A. Copper deficiency with increased hematogones mimicking refractory anemia with excess blasts. *Leuk. Res.* 2008, 32, 495-499.
- 15- Twomey, P.J.; Reynolds, T.M.; Wierzbicki, A.S.; Viljoen, A. The relationship between serum copper and ceruloplasmin in routine clinical practice. *Int. J. Clin. Pract.* 2008, 62, 485-487.
- 16- Prachayasittikul, S.; Suksrichavalit, T.; Isarankura-Na-Ayudhya, C.; Ruchirawat, S.; Prachayasittikul, V. Antimicrobial and antioxidative activities of 1-adamantylthio derivatives of 3-substituted pyridines. *Excli J.* 2008, 7, 63-70.
- 17- ChemBio 2D, 3D Ultra, version 13.0, ChemBio Office (2012), Cambridge Soft Corporation, Cambridge, MA, USA, 2012
- 18- Anhalt JP, Washington JA. In Laboratory procedure in clinical Microbiology, 2nd ed., Washington JA(ed.). Springer: New York, 1985; 281-313 .
- 19- Bull. Chem. Soc. Ethiop. 2010, 24(3).
- 20- J.C. Blair, E.M. Jones, in: H.S. Booth (Ed.), Inorganic Synthesis, vol. 1, McGraw-Hill Book Co., New York, 1939, p. 35, in:
- 21- H.E. Spencer, M.W. Schmidt, J. Phys. Chem. 75 (1971) 2986.
- 22- D. Armenatano, G. De Munno, F. Lioret, M. Julve, Cryst. Eng. Commun. 7 (2005) 57.

- 23-** Tiexiang Fu, Jun Tao, *Sens. Actuat. B* 129 (2008) 339.
- 24-** C.J.Ballhausen, "Introduction to Ligand Field Theory", Mc Graw-Hill Book Co., New York (1962).
- 25-** Lever .A. B.P. "*Inorganic spectroscopy*" Elsevier publishing company , London , New York. (1984).
- 26-** Nishida. Y., Niinuma. A., Abe. K., *Inorg. Chem. Commun.* 12 198–200(2009).
- 27-** Shriver & Atkins "*Inorganic Chemistry*", 3rd Ed., , Freeman, (1999).
- 28-** J. Fujita A. E. Martell, and K. Nakamoto, *J. Chem. Phys.*, 36, 324, 331 (1962) .
- 29-** R. D. Hancock and D. A. Thornton, *J. Mol. Struct.*, 6, 441 (1970).
- 30-** Nakamoto K. "Infrared and Raman Spectra of Inorganic and Coordination Compounds Applications in Coordination Organometallic and Bioinorganic Chemistry, 4 th Ed., 244 (1997)
- 31-** A. M. Rahman, M. I. Choudhary and W. J. Thomsen, "Bio-assay Techniques for Drug Development," Harwood Academic, Netherlands, 2001 .
- 32-** ChemBio 2D, 3D Ultra, version 13.0, ChemBio Office (2012), Cambridge Soft Corporation, Cambridge, MA, USA, 2012.
- 33-** Vilar S., Cozza G., Moro S., (2008) "Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery", *Curr. Top. Med. Chem.*, 8 (18):1555-1572.