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RESEARCH ARTICLE

STRUCTURAL, SPECTRAL, DFT, ION-FLOTATION AND BIOLOGICAL STUDIES ON TRANSITION METAL COMPLEXES OF 2-AMINOTHIAZOLE DERIVATIVES.

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Abstract

A series of Ni(II), Zn(II), Cu(II) and Fe(III) complexes of the 3-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-3-oxo-N-(thiazol-2-yl)propanamide (H₂L) were synthesized. The produced compounds were illustrated by different spectroscopic techniques. The geometry of the produced compounds was predicated by computational studying via DFT method. Furthermore, the flotation method was used to separate Zn(II) and Cu(II) ions. In addition, the antimicrobial, anti-oxidant and cytotoxic of the prepared compounds were tested.

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Introduction:-

Hydrazone complexes derived from heterocyclic compounds containing oxygen, nitrogen and/or sulfur as donor atoms are attention models for biological systems [1-3]. Heterocycles, especially thiazoles, occupy an important place owing to their versatile bioactivities due to the presence of multifunctional groups [4, 7]. Transition metal complexes have an energetic role in a numerous of biological systems. The chemical compounds that containing 2-aminothiazole moiety have significant role in pharmaceutical chemistry because it used in the improvement of drugs as anti-HIV agent, anti-inflammatory, anti-hypertensive, cytotoxic, anti-oxidant, cardiotoxic and anti-allergies. Still, these compounds were interested in organic as well as inorganic chemistry due to their wide applications in medicine.

Also, hydrazone derivatives were used as selective metal extracting agents in analytical chemistry in addition to the determination of certain transition metals in the spectroscopy [6-10]. A lot of preconcentration/separation methods (ion-flotation, column extraction, ion-selective electrode, cloud point extraction, liquid-liquid extraction, solid phase extraction and co-precipitation) were used to determine trace metal. In the current article we will apply ion-flotation techniques since it was a fast, simple and economical technique for metal analysis to overcome the problems attended with the use of highly classy apparatuses [11-14].

In addition of our work on hydrazone of 2-amino thiazoles derivatives [15-19], the present work aimed to the production and characterization of Ni(II), Fe(III), Zn(II) and Cu(II) complexes. The mode of chelation was elucidated by means of numerous spectroscopic methods. Also, the cytotoxic, anti-oxidant and antimicrobial of the prepared compounds were tested.

Experimental:-**Materials and reagents:-**

The chemicals used were pure (Merck, Aldrich or Sigma). They included:

- Organic substance as; oleic acid (HOL), 4-dimethylamino-benzaldehyde and 3-hydrazinyl-oxo-N-(thiazole-2-)propanamide
- Metal salts as; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, ZnCl_2 and FeCl_3
- Solvent as; absolute ethanol, diethyl ether and dimethyl formamide.

Solutions:-

A stock solution of HOL ($6.36 \times 10^{-2} \text{ mol.L}^{-1}$) was prepared by adding 20 mL in one liter of kerosene. Similarly, stock solutions of ZnCl_2 and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ($1 \times 10^{-2} \text{ mol.L}^{-1}$) were prepared in bidistilled water. Also, stock solution of $1 \times 10^{-2} \text{ mol.L}^{-1}$ of H_2L was prepared in absolute ethanol.

Instrumentation:-

- ❖ The infrared spectra were detected by using FTIR spectrophotometer Mattson 5000, Madison, USA in the range $4,000\text{--}400 \text{ cm}^{-1}$.
- ❖ The ^1H NMR spectra were detected by using EM-390 (200 MHz) on a Varian Mercury-300 instrument (Switzerland).
- ❖ The MS spectra were determined by using Mattson 5000 FTIR spectrophotometer.
- ❖ The values of magnetic moment were estimated at room temperature ($25 \pm 1^\circ\text{C}$) by using magnetic susceptibility balance "Johnson Matthey Wayne, Pennsylvania, USA".
- ❖ The electronic spectra was recorded in DMSO by using "Shimadzu UV 240 (P/N 204-58000) spectrophotometer (USA) in the range $200\text{--}900 \text{ nm}$ ".
- ❖ The GBC, Sensaa Series Atomic Absorption Spectrometry (computerized AAS) with air-acetylene flame was used to determine the analyte concentration.
- ❖ Two types of cells were used in the flotation and separation experiments. Which are cylindrical tube of (29, 45) cm length and (1.2, 6) cm inner diameter with a stopper at the top was used to training the various factors affecting the separation of the tested ions from aqueous solutions.
- ❖ The pH values were determined by using Hanna instrument 8519 digital pH meter.
- ❖ The percentage of C, H and N were determined by using Perkin-Elmer 2400 Series II Analyzer (Table 1).
- ❖ The standard methods used to determine the metal contents in the complexes [20].

Table 1: Analytical and Physical Data of H_2L and Its Metal Complexes.

N o.	Compound	Empirical formula (molecular mass)	Color	M.P ($^\circ\text{C}$)	Yield %	%Found (calculated)				
						C	H	N	M	Cl
1	H_2L	$\text{C}_{15}\text{H}_{17}\text{O}_2\text{N}_5\text{S}$ (331.353)	pale yellow	192	80	54.17 (54.37)	4.99 (5.17)	21.17 (21.14)	-	-
2	$[\text{Ni}(\text{H}_2\text{L})(\text{H}_2\text{O})\text{Cl}_2] \cdot 2\text{H}_2\text{O}$	$\text{Ni C}_{15}\text{H}_{23}\text{O}_5\text{N}_5\text{S Cl}_2$ (515.080)	brown	> 250	60	34.86 (34.98)	4.49 (4.50)	13.58 (13.60)	11.36 (11.39)	13.72 (13.78)
3	$[\text{Fe}(\text{H}_2\text{L})\text{Cl}_3] \cdot 2\text{H}_2\text{O}$	$\text{Fe C}_{15}\text{H}_{21}\text{O}_4\text{N}_5\text{S Cl}_3$ (529.733)	yellowish white	> 250	50	34.15 (34.01)	3.86 (3.99)	13.01 (13.22)	10.50 (10.54)	20.14 (20.19)
4	$[\text{Zn}(\text{HL})_2]$	$\text{Zn C}_{30}\text{H}_{32}\text{O}_4\text{N}_{10}\text{S}_2$ (690.04)	yellowish white	> 250	70	52.34 (52.22)	4.72 (4.67)	20.19 (20.30)	9.50 (9.47)	-
5	$[\text{CuL}(\text{H}_2\text{O})] \cdot 2\text{H}_2\text{O}$	$\text{Cu C}_{15}\text{H}_{21}\text{O}_5\text{N}_5\text{S}$ (446.930)	brown	> 250	60	40.02 (46.31)	4.31 (4.74)	15.58 (15.67)	14.18 (14.22)	-

Synthesis of H_2L :-

The ligand was synthesized by adding equimolar quantities of 4-Dimethylamino-benzaldehyde (0.01 mol; 1.5 g) and 3-hydrazinyl-oxo-N-(thiazole-2-)propanamide (0.01 mol; 2 g) in 50 ml absolute ethyl alcohol in presences of 1 ml glacial acetic acid. The H_2L was produced through reflux (3 hrs) and then separated by filtration followed by recrystallization from ethanol.

Synthesis of metal complexes:-

The complexes were produced by reflux equimolar quantities of H₂L (3.31; 10.0 mmol) and 10.0 mmol of copper (II), nickel (II), zinc (II), and ferric (III) for 1–3 h. The formed precipitate was filtered off, washed with hot ethanol and distilled water (Scheme 1).

Scheme 1:- The outline of the synthesis of H₂L and its metal complexes

Molecular modeling:-

The cluster calculations was evaluate by using DMOL3 program in materials studio package [21, 22]. The geometry optimization of the prepared compounds was predicated by using the DFT (density functional theory) through the GAUSSIAN 09 program package. The DNP basis sets are of analogous class to 6-31G Gaussian basis sets [23]. The Gaussian basis sets are less accurate than DNP basis sets of the same size [24]. The PBEPBE functional [25] was considered the best brilliant exchange-correlation functional based on the GGA (generalized gradient approximation) [26].

Biological activity:-**Antibacterial and antifungal activities:-**

- ❖ By applying agar streak dilution technique the MIC of the produced compounds was detected [27].
- ❖ The strains included Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia*) and pathogenic fungi (*Candida albicans* and *Aspergillus flavus*).
- ❖ The Fluconazole (100 µg/ml) was used as standard for anti-fungal while Ciprofloxacin (100 µg/ml) used as standard for anti-bacterial.
- ❖ A 100 µg/ml stock solution of the tested compounds in DMSO was synthesized and then incorporated in definite amount of molten sterile.
- ❖ A definite quantity of the medium including investigated compound was poured into a Petri dish until reach 3-4 mm of depth at 40-50 °C, and then allowable to solidify.
- ❖ The micro-organism suspension was established to take about 10⁵cfu/ml and muddy to plates with diluted compounds in DMSO to be established and then, incubated at 37°C for 24-48 h.

- ❖ The MIC was determined till the lowermost concentration of the investigated substance viewing no visible growth of fungi or bacteria on the plate.

Anti-oxidant activity screening assay:-

Anti-oxidant activity screening assay - ABTS method:-

- ❖ In ABTS method; 3 ml MnO₂ (25 mg/ml) solution and 2 ml of ABTS (60 mM) solution was added to tested compounds in presence of 5 ml aqueous phosphate buffer solution (pH 7, 0.1 M).
- ❖ The prepared mix was shaken, centrifuged, filtered. Then the absorbance was recorded for the ABTS radical solution (resultant green-blue solution) at λ 734 nm.
- ❖ After that, 50 ml of methanol/phosphate buffer (1:1) was added to the tested compounds (2 mM) in the spectroscopic grade.
- ❖ The absorbance was measured by using L-ascorbic acid (positive control) as antioxidant standard. While, the ABTS and methanol/phosphate buffer (1:1) used as a negative control [28, 29].
- ❖ The intensity of color reduction was determined by using the following equation:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}) / (A_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the reaction control,

A_{sample} is the absorbance in the existence of the samples or standards.

Anti-oxidant screening assay for erythrocyte hemolysis:-

- ❖ Blood was obtained by cardiac puncture from rats and then collected in heparinized tubes.
- ❖ A regularly packed cell of Erythrocytes were prepared from the buffy coat and plasma. Where, it was washed by 0.15 M NaCl (three times), and centrifuged for 10 min at 2500 rpm.
- ❖ In this test, the erythrocyte hemolysis was mediated by peroxy radicals [30].
- ❖ In order to test the samples at different concentrations, 10% of erythrocytes suspension at pH 7.4 (PBS) was added to the similar volume of AAPH solution (200 mM) in PBS. Then the reaction mixture was shaken and being incubated at 37 °C for 1 h.
- ❖ Next, the mixture was separated, diluted with 8 volumes of PBS and centrifuged for ten min at 2500 rpm. The absorbance (A) of the supernatant was recorded at 540 nm.
- ❖ Similarly, the mixture was conserved with 8 volumes of distilled water to reach complete hemolysis. Then, after centrifugation the absorbance (B) of the supernatant was determined at 540 nm. The hemolysis percentage was measured by using this equation:

$$\% \text{ hemolysis} = (1 - A/B) \times 100\%$$

Cell proliferation assay:-

- ❖ The MTT colorimetric assay used to determine the inhibitory effects of the tested compounds on cell growth [31, 32].
- ❖ The Penicillin (100 units/ml) and Streptomycin (100 μ g/ml) are antibiotics that used under 5% CO₂ for 48 h incubator at 37 °C and seeds in a 96-well plate with density 1.0×10^4 cells/well [33, 34].
- ❖ After that, the incubated cells were preserved with various concentration of tested compounds for 24 h. following, 24 h of drug treatment, 20 μ l of MTT solution at 5 mg/ml was added and incubated for 4 h.
- ❖ Then 100 μ l of DMSO was added to each well to dissolve the purple formazan.
- ❖ The colorimetric test was measured by using a plate reader (EXL 800, USA) at 570 nm.
- ❖ The relative cell viability percentage was determined by applying this equation:

$$A_{570} \text{ of treated samples} / A_{570} \text{ of untreated sample} \times 100.$$

Flotation-separation procedure:-

A known quantity of copper (II) or zinc (II) solutions was added to a solution of H₂L. The pH of the prepared mixture was adjusted with sodium hydroxide and/or nitric acid to the wanted value. After that, the solution was completed with bidistilled water to 10 mL in the flotation cell. The cell was shaken well for 2 min and then 2 mL of known concentration of HOL were added to confirm complete complexation. After that, the cell was reversed upside down 20 times and left 5 min standing for complete flotation. Lastly, the concentration of copper (II) or zinc (II) ions stayed in the mother liquor was analyzed via AAS. The floatability (F %) of copper (II) or zinc (II) ions was measured by applying this equation:

$$F\% = (C_i - C_f) / C_i \times 100$$

Where: C_i and C_f are the initial and the final concentrations of copper (II) or zinc (II) ions in the mother liquor, respectively.

Results and Discussion:-

IR, ^1H NMR and mass spectra:-

The important IR bands H_2L and its metal complexes were considered to determine the effect of a metal bonding on the ligand vibration in the produced complexes. The IR spectrum of H_2L was exhibited a medium-intensity broad bands related to $\nu(\text{CH}_2)$, $\nu(\text{NH})_1$ and $\nu(\text{NH})_2$ at 3089, 3200 and 3174 cm^{-1} [35], respectively. Furthermore, there are three sharp bands related to $\nu(\text{C}=\text{O})_1$, $\nu(\text{C}=\text{O})_2$ [36] and $\nu(\text{C}=\text{N})$ [37] at 1688, 1667 and 1605 cm^{-1} , respectively (Table 2).

Table 2:- Most important IR spectra bands of H_2L and its metal complexes.

Compound	$\nu(\text{NH})_1$	$\nu(\text{NH})_2$	$\nu(\text{CH}_2)$	$\nu(\text{C}=\text{O})_1$	$\nu(\text{C}=\text{O})_2$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{N})^*_1$	$\nu(\text{C}=\text{N})^*_2$	$\nu(\text{C}=\text{O})_{1(\text{enolic})}$	$\nu(\text{C}=\text{O})_{2(\text{enolic})}$
1	3200	3174	3089	1688	1667	1605	-	-	-	-
2	3227	3187	3093	1671	1656	1583	-	-	-	-
3	3385	3211	3080	1668	1640	1600	-	-	-	-
4	3244	-	3084	1665	-	1590	-	1603	-	1123
5	-	-	3082	-	-	1581	1636	1610	1170	1128

In ^1H NMR spectrum of H_2L there are two signals at 11.27 and 12.23 ppm ascribed to the protons of $(\text{NH})_1$ and $(\text{NH})_2$, respectively. Also, in 6.60-8.04 ppm region there are multiple signals related to the aromatic protons and $-\text{N}=\text{CH}-$. Moreover, there are two sharp singlet at 3.46 and 3.79 ppm associated to the protons of $(-\text{CH}_2)$ and $\text{ph}-\text{N}-(\text{CH}_3)_2$, respectively. (Figure1)

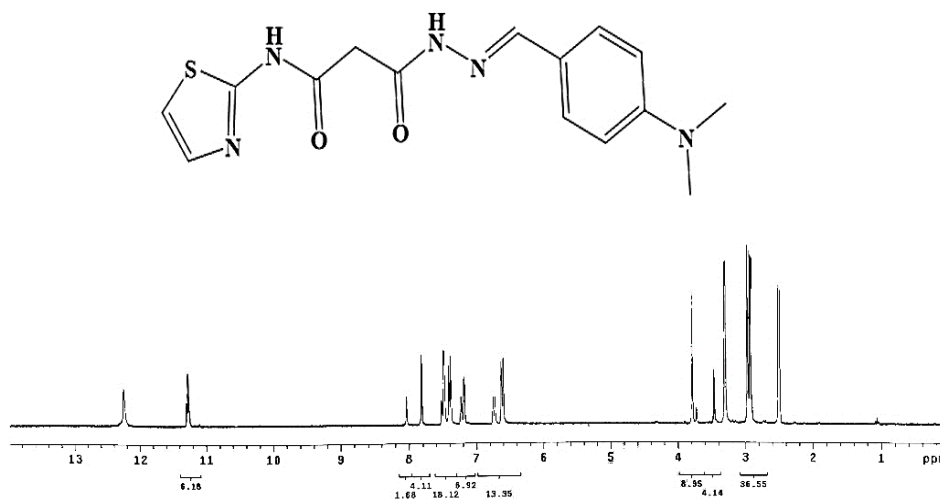


Fig.1:- ^1H NMR spectrum of H_2L in DMSO

The MS of H_2L displayed the molecular ion peak $[\text{M}]^+$ of H_2L at $m/z = 331$ (17.98%) which is equal to its molecular weight and relating to the moiety of the ligand $[(\text{C}_{15}\text{H}_{17}\text{O}_2\text{N}_3\text{S})$ atomic mass 331.353].

In IR spectra of $[\text{Ni}(\text{H}_2\text{L})(\text{H}_2\text{O})\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Fe}(\text{H}_2\text{L})\text{Cl}_3] \cdot 2\text{H}_2\text{O}$ complexes displayed that the H_2L acted as a neutral tridentate ligand coordinating via $(\text{C}=\text{O})_1$, $(\text{C}=\text{O})_2$ and $(\text{C}=\text{N})$. This manner of complexation was suggested by the negative shift of $\nu(\text{C}=\text{N})$, $\nu(\text{C}=\text{O})_1$ and $\nu(\text{C}=\text{O})_2$. The presence of new bands at (515; 519) and (467; 472) cm^{-1} which attributable to $(\text{M}-\text{O})$ and $(\text{M}-\text{N})$ [38], respectively (Figure 2).

Also, the IR spectrum of $[\text{Zn}(\text{HL})_2]$ complex showed that the H_2L acted as a mononegative tridentate ligand chelating through $(\text{C}=\text{N})$, $(\text{C}=\text{O})_1$ and $(-\text{C}=\text{O})_2$. This mode of chelation was confirmed by: (i) the absence of $\nu(\text{C}=\text{O})_2$ and $\nu(\text{NH})_2$ with instantaneous appearance of new bands at 1603 and 1123 cm^{-1} which assignable to $\nu(\text{C}=\text{N})^*_2$ and $\nu(\text{C}=\text{O})_{2(\text{enolic})}$, respectively [39], (ii) the negative shift of $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{O})_1$ and (iii) the existence of novel bands at 521 and 487 cm^{-1} which attributed to $\nu(\text{Zn}-\text{O})$ and $\nu(\text{Zn}-\text{N})$, respectively [38]. Also, the ^1H NMR

spectrum of the Zn(II) complex displayed the signal attributed to the (NH)₁ proton indicating that this group taken no part in coordination. However, the disappearance of signal related to (NH)₂ proton ensure the deprotonation of the enolized carbonyl oxygen.

Moreover, the IR spectrum of [CuL(H₂O)].2H₂O complex displayed that H₂L acted as a binegative tridentate ligand coordinating via (=C-O)₁, (=C-O)₂ and (C=N). This kind was suggested by (i) the negative shift of (C=N) to lower wavenumber, (ii) the absences of $\nu(\text{NH})_1$, $\nu(\text{NH})_2$, $\nu(\text{C}=\text{O})_1$ and $\nu(\text{C}=\text{O})_2$ with immediate presence of new band at 1636, 1610, 1170 and 1128 cm⁻¹ which related to $\nu(\text{=C-O})_{1(\text{enolic})}$, $\nu(\text{=C-O})_{2(\text{enolic})}$, $\nu(\text{C=N})^*_1$ and $\nu(\text{C=N})^*_2$, respectively, and (iii) the appearance of new bands at 513 and 410 cm⁻¹ which attributed to $\nu(\text{Cu-O})$ and $\nu(\text{Cu-N})$ respectively [39].

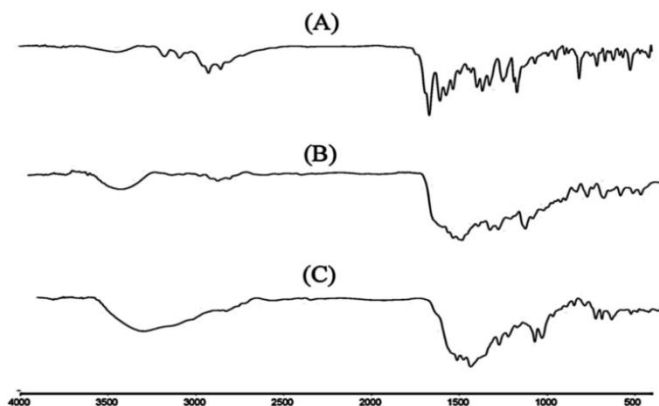


Fig.2:- Infrared spectra of (A) H₂L, (B) [CuL(H₂O)].2H₂O and (C) [Fe(H₂L)Cl₃].2H₂O

Electronic spectra:-

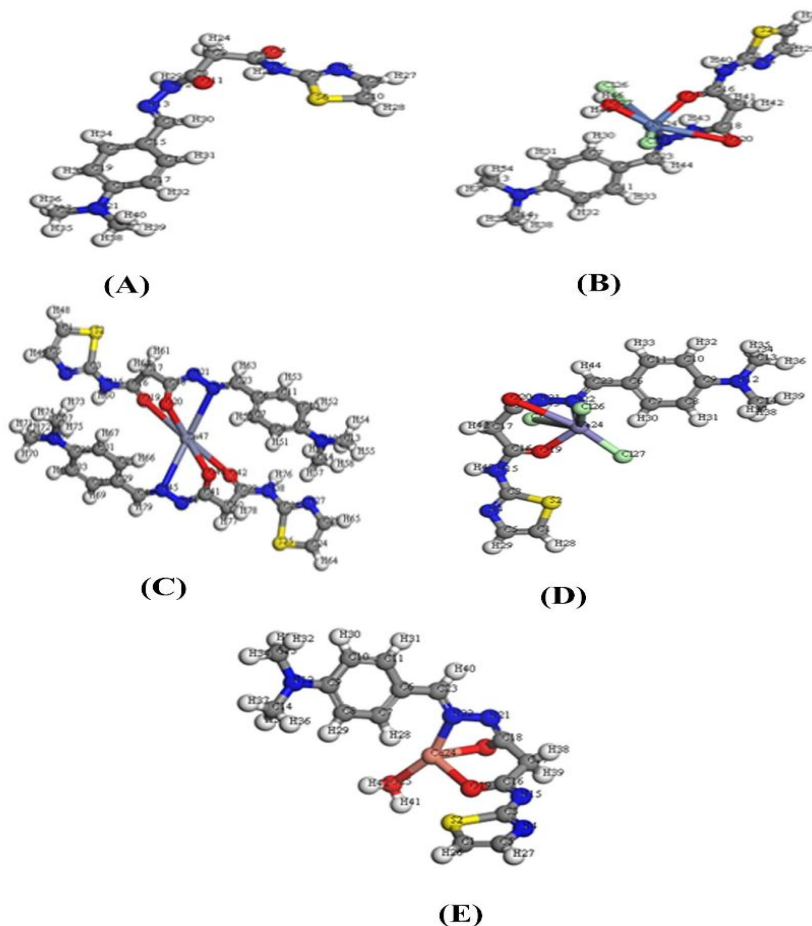
The electronic spectrum of [Ni(H₂L)(H₂O)Cl₂].2H₂O complex exhibited two bands at 31446 and 21739 cm⁻¹, assigned to ³A_{2g} → ³T_{1g} (F) and to ³A_{2g} → ³T_{1g} (P) transitions, respectively, in an octahedral geometry (Table 3). Also, the electronic spectrum of [CuL(H₂O)].2H₂O complex exhibited a broad band with a maximum at 14286 cm⁻¹ related to ²B_{1g} → ²A_{1g} transition as stated for square planar Cu(II) complex. The electronic spectrum of [Fe(H₂L)Cl₃].2H₂O complex displayed three bands at 12820, 20408 and 25000 cm⁻¹ ascribed to ⁶A_{1g} → ⁴T_{1g}(G), ⁶A_{1g} (F) → ⁴T_{2g}(G) and ⁶A_{1g} (F) → ⁴E_g(G) transitions, respectively, in octahedral configuration [40].

Table 3:- Magnetic moments, electronic spectra and ligand field parameters of metal complexes.

Compound	$\mu_{\text{eff.}}$ (BM)	Band position (cm ⁻¹)	Dq (cm ⁻¹)	B (cm ⁻¹)	β	ν_2/ν_1
2	3.2	31446; 21739	1378	689	0.709	1.468
3	5.2	25000; 20408; 12820	-	-	-	-
5	2.1	14286	-	-	-	-

Geometry optimization with DFT method:-

DFT calculations are performed to predict the molecular structure and numbering of atoms of H₂L and its metal complexes (Structures 1).



Structure 1:- Molecular modeling of (A) H₂L, (B) [Ni(H₂L)(H₂O)Cl₂].2H₂O, (C) [Zn(HL)₂], (D) [Fe(H₂L)Cl₃].2H₂O, (E) [CuL(H₂O)].2H₂O

Molecular parameters:-

The Quantum chemical parameters such as E_{HOMO}, E_{LUMO}, total energy, binding energy and dipole moment of investigated compounds were calculated (Table 4). From the outcome data we can propose that:

- ❖ The negative values of E_{HOMO} and E_{LUMO} indicated to the stability of complexes (Figure 3).
- ❖ The higher binding energy value of complexes indicated the great stability of complexes.
- ❖ The higher dipole moment value of H₂L indicated to the free ligand was more potent biological activities than the isolated complexes.

Table 4:- The molecular parameters of the ligand and their complexes.

Compound	Total Energy (Ha)	Binding Energy (Ha)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)
1	-1404.622293	-6.9256921	8.5025	-4.719	-2.096
2	-2595.687509	-8.2129894	5.4453	-5.435	-2.964
3	-2929.231760	-7.9761070	6.4118	-4.264	-3.036
4	-3063.2504092	-14.3447502	2.8675	-4.222	-2.482
5	-1702.907849	-7.5911838	8.4480	-4.733	-2.631

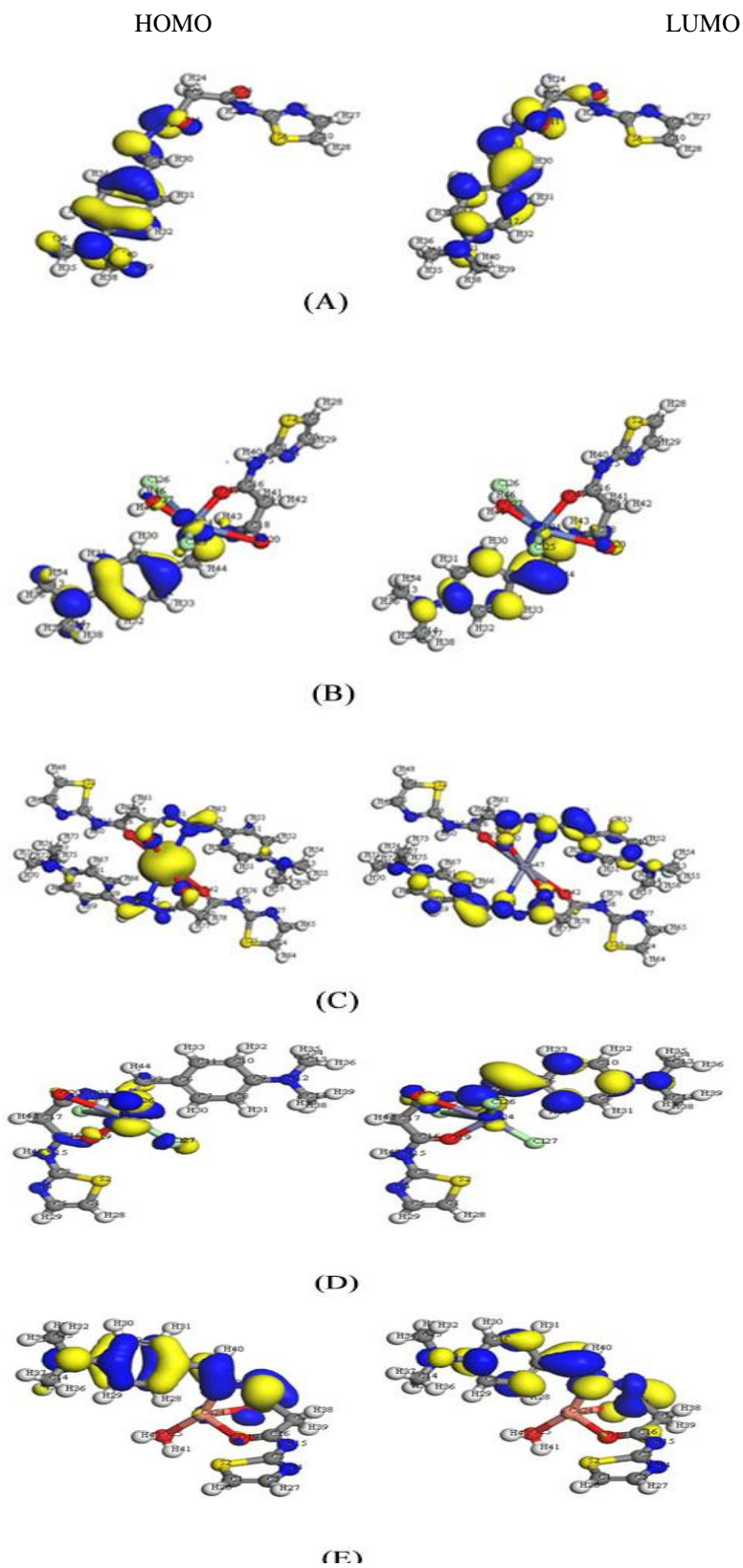


Fig.3:- The HOMO and LUMO of (A) H_2L , (B) $[Ni(H_2L)(H_2O)Cl_2] \cdot 2H_2O$, (C) $[Zn(HL)_2]$, (D) $[Fe(H_2L)Cl_3] \cdot 2H_2O$, and (E) $[CuL(H_2O)] \cdot 2H_2O$

Molecular electrostatic potential of H₂L and its metal complexes:-

The MEP was considered a good descriptor for significant positions for nucleophilic and electrophilic attack [41] where 3D plots of MEP were drawn for the ligand and its metal complexes (Figure 4). According to the MEP, the area with red color indicated the electron-rich sites which preferred the electrophilic attack. Conversely, the area with blue color indicated the electron-poor sites which preferred the nucleophilic attack [42]. While, the area with green color indicated the neutral electrostatic potential sites.

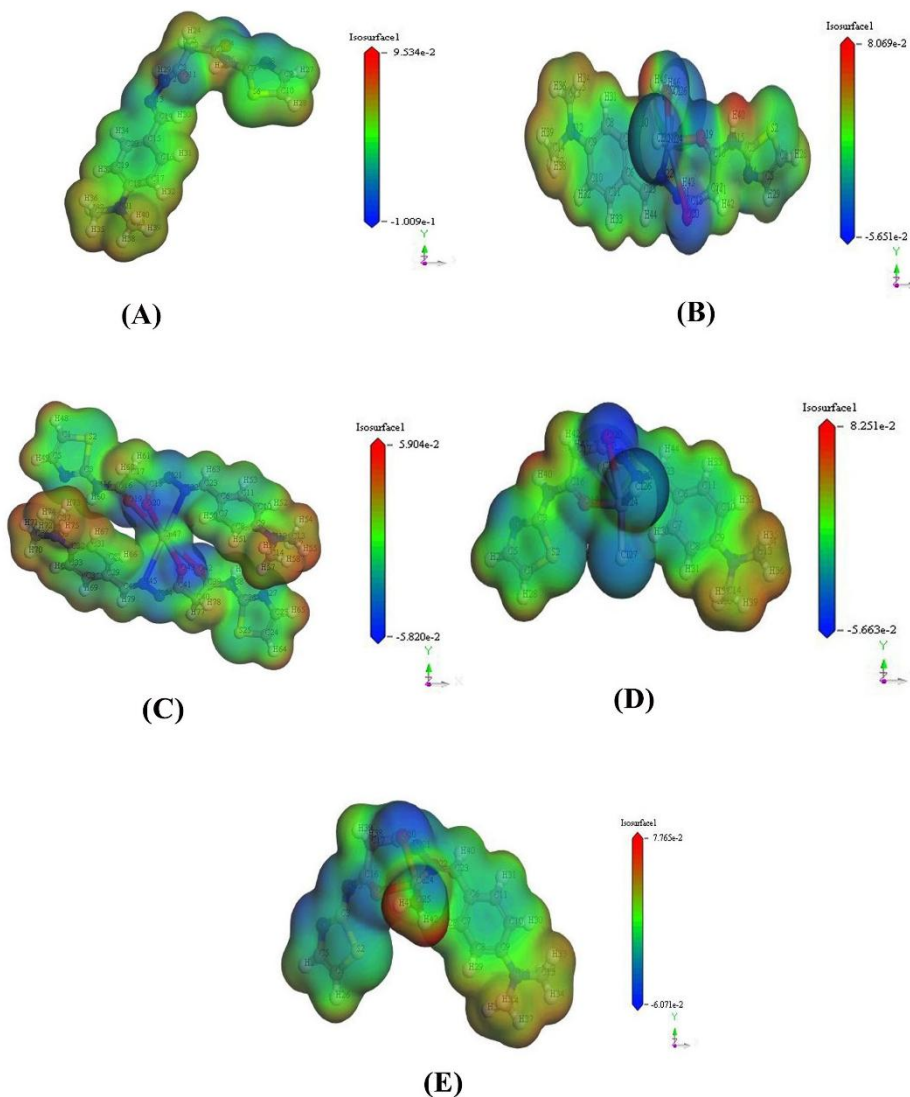


Fig.4:- Molecular electrostatic potential map for (A) H₂L, (B) [Ni(H₂L)(H₂O)Cl₂].2H₂O, (C) [Zn(HL)₂], (D) [Fe(H₂L)Cl₃].2H₂O, (E) [CuL(H₂O)].2H₂O

Biological activity:-

The biological activity of hydrazones was encouraged us to study their complexation effect and test their activity versus economically bacterial and fungal [43, 44].

Antifungal activity:-

The outcomes data revealed that the H_2L and its metal complexes have a potent activity versus *Aspergillus flavus* and *Candida albicans* (Table 5). The H_2L and $[Ni(H_2L)(H_2O)Cl_2].2H_2O$ complex have the maximum antifungal activity against *Aspergillus flavus* than *Candida albicans* in comparison with the standard drug Fluconazole [45].

Table 5:- Antifungal activities of H_2L and its metal complexes in terms of MIC (mg/ml)

Compound	C. Albicans	A. flavus
Fluconazole	1.56	0.78
1	4.68	3.12
2	1.56	0.78
3	9.37	6.25
4	18.75	12.5
5	>100	>100

Antibacterial activity:-

Also, the outcomes data revealed that the H_2L and its metal complexes have a potent antibacterial activity versus different types of bacteria in comparison with the standard drug Ciprofloxacin [46-48]. The values of MIC displayed that the H_2L , $[Ni(H_2L)(H_2O)Cl_2].2H_2O$ and $[Fe(H_2L)Cl_3].2H_2O$ complexes have the highest antibacterial activity (Table 6).

Table 6:- Antibacterial activities in terms of (MIC) (mg/ml)

Compound	Gram -Ve		Gram +Ve	
	E. coli	P. aeruginosa	S. aureus	B. subtilis
Ciprofloxacin	1.56	0.78	1.56	0.39
1	9.37	6.25	4.68	2.34
2	1.56	0.78	1.56	1.17
3	4.68	2.34	6.25	3.12
4	12.5	9.37	12.5	9.37
5	>100	>100	>100	>100

The antioxidant activity of ligands and their metal complexes:-

The ABTS assay was applied to measure the antioxidant effect of the investigated compounds [49]. Amongst all tested compounds $[Ni(H_2L)(H_2O)Cl_2].2H_2O$ considered the good antioxidant in comparison with standard ascorbic acid (Table 7).

Table 7:- Anti-oxidant assays by ABTS method

Method	ABTS	
	Abs(control)-Abs(test)/Abs(control)X100	
Compounds	Absorbance of samples	% inhibition
Control of ABTS	0.51	0%
Ascorbic-acid	0.055	89.20%
1	0.156	69.40%
2	0.075	85.30%
3	0.22	56.90%
4	0.24	52.90%
5	0.287	43.70%

In addition, the anti-oxidant activity was tested for erythrocyte hemolysis of isolated compounds. The results revealed that $[\text{CuL}(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ demonstrated strong anti-oxidative activity in the hemolysis assay (Table 8).

Table 8:- Anti-oxidant assays by erythrocyte hemolysis.

Compounds	Erythrocyte hemolysis	
	A/B x 100	
	Absorbance of samples (A)	% hemolysis
Absorbance of H_2O (B)	0.896	---
Ascorbic-acid	0.042	4.70%
1	0.538	60.00%
2	0.531	59.30%
3	0.531	59.30%
4	0.179	20.00%
5	0.161	18.00%

The cytotoxicity of H_2L and its metal complexes on HCT-116 cell line:-

The cytotoxicity assays of H_2L and its metal complexes versus HCT-116 (human colorectal carcinoma cells lines) were demonstrated in (Table 9). The results showed that $[\text{Ni}(\text{H}_2\text{L})(\text{H}_2\text{O})\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ ($\text{IC}_{50} = 7.7 \pm 0.68 \mu\text{M}$) has greater inhibitory influence than the other investigated compounds. But, $[\text{CuL}(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ complex has lower inhibitory influence ($\text{IC}_{50} = 72.8 \pm 4.76 \mu\text{g/ml}$) nearly no activity [50].

Table 9: Cytotoxicity (IC_{50}) of tested compounds on HC

Compounds	In vitro Cytotoxicity IC_{50} ($\mu\text{g/ml}$)•	
	HCT-116	
5-FU	5.2 ± 0.31	
1	13.1 ± 1.47	
2	7.7 ± 0.68	
3	10.7 ± 1.24	
4	16.0 ± 1.73	
5	72.8 ± 4.76	

Ion-flotation separation:-

Influence of initial pH:-

Many experiments were carried out to study the relation between the floatability of metal ions ($2 \times 10^{-4} \text{ mol L}^{-1}$) and H_2L ($2 \times 10^{-4} \text{ mol L}^{-1}$) and HOL ($1 \times 10^{-3} \text{ mol L}^{-1}$). The outcomes data presented that the greater floatability in (6-10) pH range for Cu(II) and (5-10) for Zn(II) ions (Figure 5). This enables us to use the prepared ligand to separate the metal ions from various media.

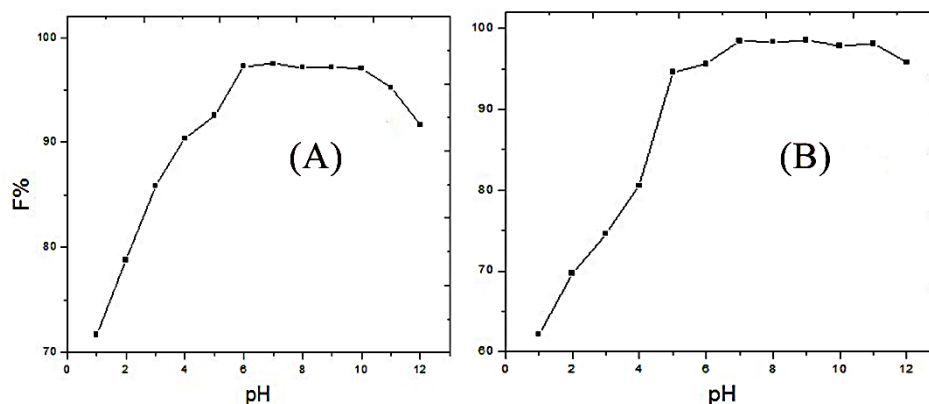


Fig.5:- Influence of pH on the floatability of $2 \times 10^{-4} \text{ mol.L}^{-1}$ (A) Cu(II) and (B) Zn(II) ions using $2 \times 10^{-4} \text{ mol.L}^{-1}$ of ligand and $1 \times 10^{-3} \text{ mol.L}^{-1}$ HOL

Influence of initial metal concentration:-

Many trials were applied to float various concentrations of Cu (II) and Zn (II) ions in presence of ($2 \times 10^{-4} \text{ mol L}^{-1}$) H_2L and ($1 \times 10^{-3} \text{ mol L}^{-1}$) HOL at pH~7. The maximum floatation efficiency of Cu (II) and Zn (II) ions was detected for H_2L when the ratio of M:L is (1:1) (Figure 6). The chelating agent provided quantitative separation of Cu (II) and Zn (II) ions (~100%) that attributed to the existence of enough quantities of H_2L to bind all Cu (II) and Zn (II) ions. So, the ratio of M:L of (1:1) was used throughout.

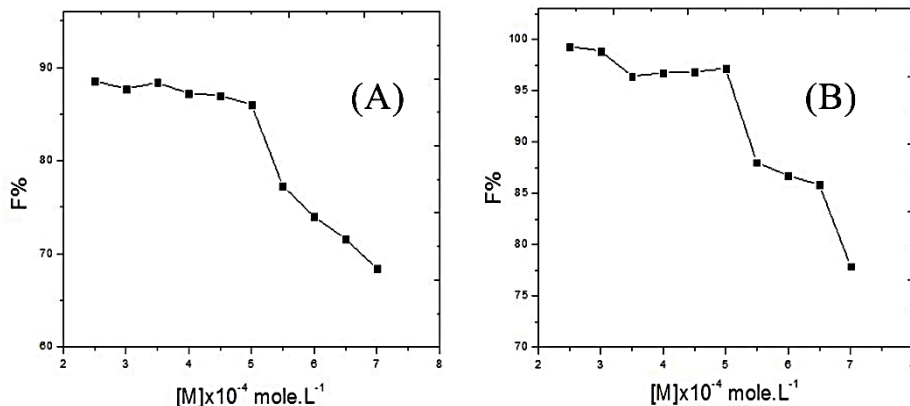


Fig. 6:- Floatability of different concentrations of (A) Cu (II) and (B) Zn (II) ions using $2 \times 10^{-4} \text{ mol.L}^{-1}$ of prepared ligand and $1 \times 10^{-3} \text{ mol.L}^{-1}$ HOL at pH ~7

Influence of ligand concentration:-

The effect of various concentrations of H_2L towards Cu(II) and Zn(II) ions was tested to measure the collecting ability of the ligand on the floatability of the analytes at pH~7 in presence of $1 \times 10^{-3} \text{ mol L}^{-1}$ of HOL. The outcomes data shown that, the floatability of Cu (II) and Zn (II) ions increases sharply until reaching its maximum value at M:L ratio of (1:1) (Figure 7). Extra ligand has no opposing effect on the flotation process, therefore $2 \times 10^{-4} \text{ mol L}^{-1}$ of H_2L was used throughout.

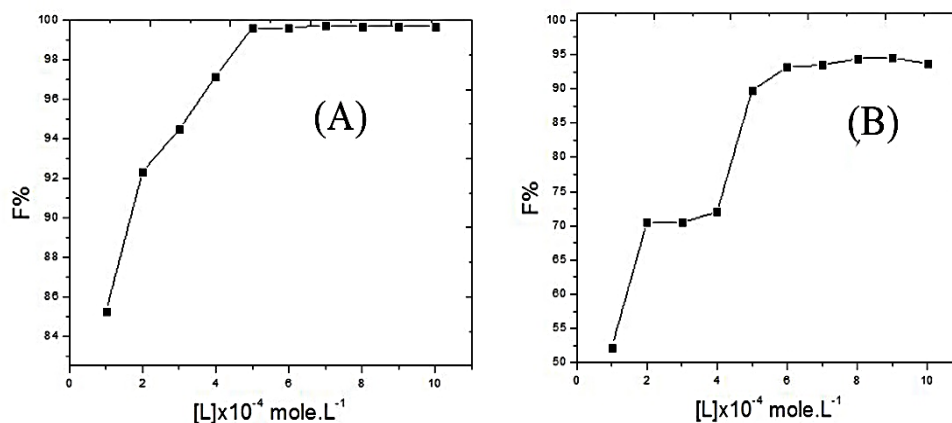


Fig. 7:- Floatability of $2 \times 10^{-4} \text{ mol.L}^{-1}$ (A) Cu(II) and (B) Zn(II) ions using different concentrations of prepared ligand and $1 \times 10^{-3} \text{ mol.L}^{-1}$ HOL at pH ~7

Influence of surfactant concentration:-

Many trials were achieved to float Cu (II) and Zn (II) ions with HOL only, but the recovery did not go above 43 %. Therefore, additional runs of experiments were occurred at pH~7 to float $2 \times 10^{-4} \text{ mol L}^{-1}$ Cu(II) and Zn(II) ions in the existence of $2 \times 10^{-4} \text{ mol L}^{-1}$ of H_2L and various concentrations of HOL ($1 \times 10^{-3} - 5 \times 10^{-2} \text{ mol L}^{-1}$). The outcomes data showed the highest floatation % of Cu (II) and Zn (II) ions in ($1 \times 10^{-3} - 9 \times 10^{-3} \text{ mol L}^{-1}$) concentration range of HOL (Figure 8).

The incomplete separation of Cu(II) and Zn(II) ions at higher concentration of surfactant was due to the surfactant changes the state of the Cu(II)-ligand and Zn(II)-ligand particles. An increase of HOL quantity was added to re-disperse the coagulated precipitate via coagulation flotation. Moreover, at high concentration of surfactant the weak flotation was occurred by the formation of a stable, hydrated envelope of surfactant or by forming a hydrate micelle coating on the solid surface on the air bubble surface [51, 52]. Therefore, the hydrophobicity of the surface was not appropriate for flotation. So, a 1×10^{-3} mol L⁻¹ concentration of HOL was fixed throughout.

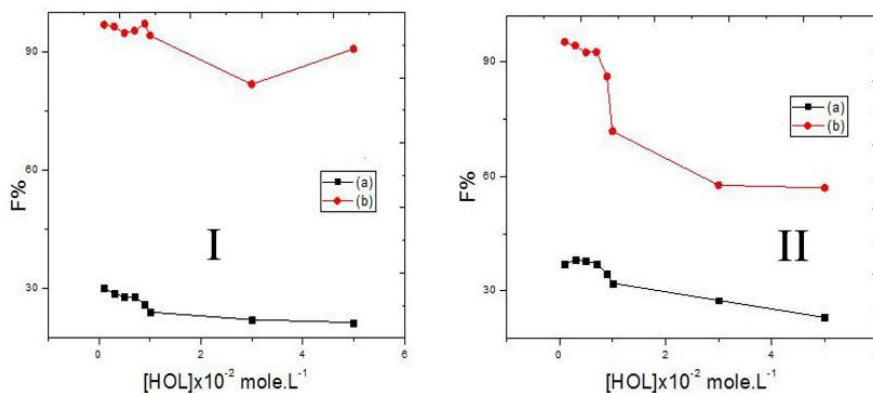


Fig. 8:- Floatability of 2×10^{-4} mol L⁻¹ (I) Cu²⁺ and (II) Zn²⁺ ions (a) in absence of HOL (b) in presence of different concentrations of HOL and 2×10^{-4} mol L⁻¹ of ligand at pH ~7.

Influence of temperature:-

In a wide range of temperature (10-80 °C) a series of experimentations was carried out to float Cu(II) and Zn(II) ions under the optional conditions. For this purpose, solutions of Cu(II), Zn(II) ions, H₂L and HOL were either heated or cooled to the similar temperature in a water bath. Where in this step the HOL solution was decanted into Cu(II) and Zn(II) ions solution. The mix was presented into the flotation cell jacketed with 1 cm thick fiberglass insulation. The maximum flotation of Cu (II) and Zn (II) ions in 15-80 °C range was presented in (Figure 9). So, the temperature of 25 °C was used throughout. The decrease in separation by increasing temperature further than 80 °C may be due to the increase in solubility of the precipitate and the instability of the foam giving rise to partial dissolution of the precipitate and deficient foam constancy to delay the precipitate [53].

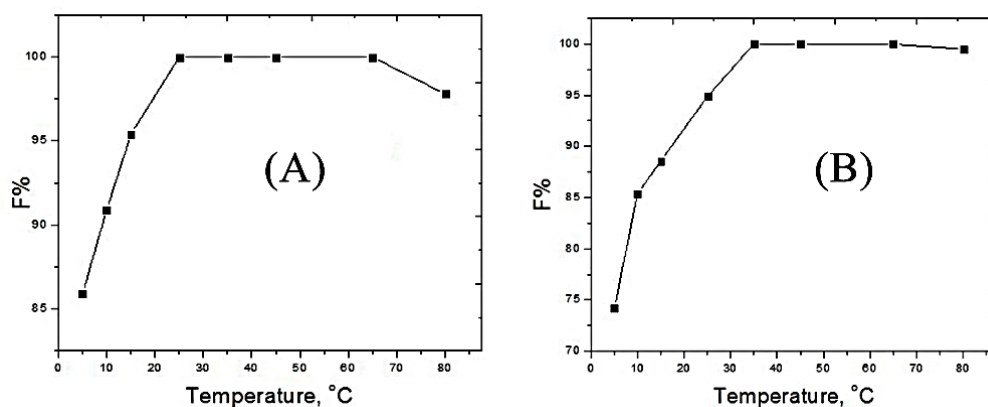


Fig. 9:- Floatability of 2×10^{-4} mol.L⁻¹ (A) Cu (II) and (B) Zn (II) ions at different temperatures using 2×10^{-4} mol.L⁻¹ of prepared ligand and 1×10^{-3} mol.L⁻¹ HOL at pH ~7

Interference of the presence of foreign ions:-

The removal percentage of 10 mg L⁻¹ Cu(II) or Zn(II) ions from a solution of 30 mg L⁻¹ of H₂L was studied in the presence of high concentrations of different anions and cations at pH 7. The used quantities of each ion, giving an error of $\pm 4\%$ in the removal efficiency of Cu (II) or Zn(II) ions were recorded in (Table 10). Examination of the data shown that, all the foreign ions with relatively high concentrations in comparison with that of Cu (II) and Zn (II) ions have no effect on the flotation of copper (II) or zinc (II). So, the suggested method may find its applications on water samples.

Table 10:- Effects of the foreign ions on the removal percentage of the examined metal ions

Foreign ions	*I/A ratio(mg.L ⁻¹)	Re % of (Cu ²⁺)	Re % of (Zn ²⁺)
Na ⁺	25	99.3	100
K ⁺	45	98.4	98.8
Mg ²⁺	35	100	97.9
Ca ²⁺	30	97.5	98.9
Cl ⁻	30	98.4	99.1
SO ₄ ²⁻	20	94.9	98.8
HCO ₃ ⁻	25	99.5	98.9
CH ₃ COO ⁻	40	99.2	97.5

*I/A = interference/ analyte ratio

Application:-

To investigate the applicability of the suggested method, a number of trials were performed to recover 15 mg L⁻¹ of Cu (II) and Zn (II) ions poured to 1L of aqueous and some water samples. The experiments of flotation were performed by using 50 ml clear, filtered, uncontaminated sample solutions at pH 7. The outcomes data displayed that the recovery was acceptable and quantitative under the suggested conditions of the applied flotation technique (Table 11).

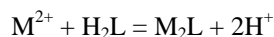
Table 11: Recovery of 15 mg L⁻¹ of studied metal ions from some water samples.

Water samples (location)	Added metal (mg.L ⁻¹)	Re % of Cu ²⁺	Re % of Zn ²⁺
Sharm El-Shiekh	15	98.57143	85.97368
Alexandria	15	97.71429	80.23684
New Valley	15	99.11905	77.89474
Mansoura	15	98.78571	83.65789

Suggested flotation mechanism:-

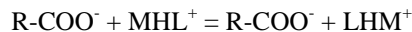
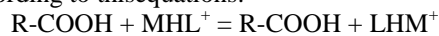
The mechanism of the flotation of metal-ligand precipitates was proposed depending on the following facts:

- ❖ Cu(II) and Zn(II) reacted with H₂L in a M:L ratio of (1:1) to provide the complex M₂L according to this equation:



The H₂L has several donating sites, as carbonyl oxygen (C=O), azomethine nitrogen (C=N) (Scheme 1).

- ❖ HOL started to dissociate at pH > 5.2 [54] and the percentage of various forms of oleic acid were detected by infra-red analysis (Table 12). The infra-red spectra of oleic acid with changing pH showed that at 1300-1800 cm⁻¹, there are bands characteristic of the COOH and CO²⁻ groups associated with Na⁺ [55]. These information approve the reported data, [56] that the (C=O) stretching band of oleic acid at 1705 cm⁻¹ was shifted to bands in the range 1520-1540 cm⁻¹ for sodium oleate on ionization. So, oleic acid can interact with extra systems, via hydrogen bonding, either in its undissociated (R-COOH) or dissociated (R-COO⁻) forms depending on the pH of the medium and according to these equations:



The combination of oleic acid surfactant with the copper-ligand or zinc-ligand chelate provided hydrophobic aggregates that float with the assistance of air bubbles to the solution surface by slight shaking [57].

Table 12:- Different forms of oleic acid determined by spectrophotometric

pH	HOL	Ol ⁻	NaOL	Total
5.2	100.0	0.0	0.0	100.0
8.0	6.5	34.2	0.0	100.0
8.2	38.5	57.7	3.8	100.0
9.0	13.6	68.2	18.2	100.0
11.5	0.0	80.0	20.0	100.0
12.0	0.0	52.2	47.8	100.0

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