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Study of Microscopic Properties and Antioxidant Effect of Azo Ligand

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Abstract:

In coordination chemistry, azo ligands are organic chemicals capable of selectively absorbing and reflecting light at wavelengths within the visible range of the electromagnetic spectrum. A pigment is a colored substance that has an affinity for the colored substance. The dye needs a liquid medium often to be able to transfer to the colored material, and it may need a color anchor to improve the color fastness in the dyed fibers. Color results from the pigment, or pigment, as a result of their absorption of certain wavelengths of light. Pigments , unlike dyes in general, dissolve in water and have no affinity for dyed materials. Azo ligands have weak electron bonding in the conjugated system so that incident light can stimulate these electrons and give them the energy needed to jump from one energy level to another, which means absorbing some wavelengths of incident light. What is important is that a mixture of the remaining wavelengths is reflected, giving color.

Keywords: *inorganic ligand, TEM , coordination, azo.*

Introduction

Azo ligands are dyes that are synthesized from organic molecules in dye factories. These dyes are subjected to different adjustments, so that in the end they give an identical product each time for dyers [1-4]and printers. The process of obtaining an identical color from one batch to another requires a lot of skill, because the dye production process involves many variables that affect the dye absorption in the fibres [5-9] . Schiff bases, are more versatile compounds in coordination chemistry [10-14], bio –field , pharmaceutical field , as they have many significant biological applications including antifungal, antibacterial, antiviral, anticancer and antioxidant activities [15-18]. In addition, role of Schiff bases and their complexes in catalytic reactions such as oxidation, reduction [19-22], hydrolysis reactions, inhibition of corrosion and memory storage devices in electronics are also reported [9–12]. Over the years, mixed-ligand complexes have been attraction in worldwide due to their several biological, enzymatic and analytical applications [23-27]. Azo - derivative Imidazole ligands have attracted the attention of many researches working in the field of coordination chemistry [28-32] This type of ligands that contain the azo imine group (-N=N-C=N-), that give feature high stability to metallic complexes [33-38] due to the back bonding.

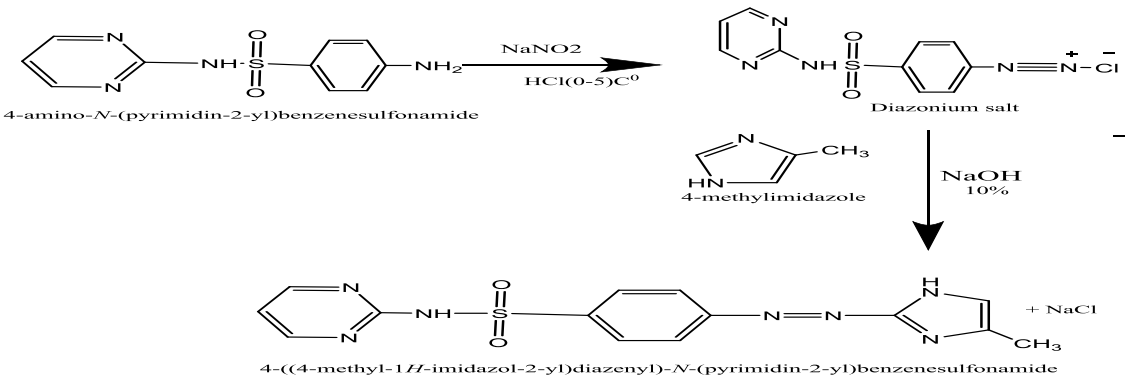
2. Experimental Part

All chemicals were supplied by BHD and Sigma Aldrich, and A.K.Scie and used without further purification .,The electro-thermal melting point model 9300 was used to measure the melting point of the ligand and its complexes. Elemental analyses were carried out by means of micro analytical unit of 1180 C.H.N elemental analyzer. Electronic spectra were recorded on Shimadzu spectrophotometer double beam model 1700 ultraviolet-visible (UV-Vis) spectrophotometer. Fourier-transform infrared (FTIR) spectra were recorded in KBr disc on FTIR Shimadzu spectrophotometer model 8400 in wave number 4000- 400/cm. Proton nuclear magnetic resonance (1H-NMR) and carbon nuclear magnetic resonance (13C-NMR) spectra in ppm unit were operating in dimethyl sulfoxide-d6 (DMSO-d6) as solvent using (Bruker) Ultra Shield 3000 MHz, Switzerland). And mass spectra were recorded on AB Sciex 3200 QTRAP LC/ MS/MS (mass range m/z 5- 2000 quad mode and 50-

1700 linear ion trap mode). Magnetic susceptibility measurements were carried out on a balance magnetic MSB-MKI using faraday method. The diamagnetic corrections were made by Pascal's constants.

Formation of Azo Ligand and Schiff base ligand

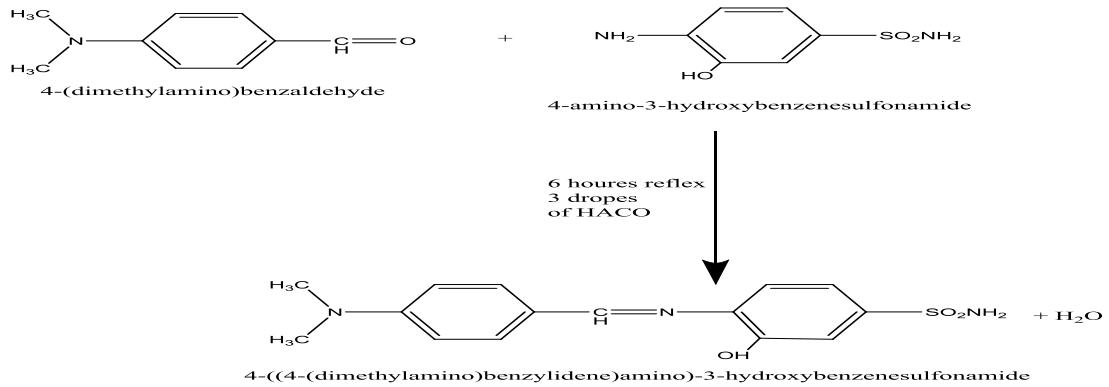
This heterocyclic azo ligand was prepared as described before [18] (Scheme.1). A solution of 4-amino N-(Pyrimidin-2-yl)benzenesulfonamide) (2.47 g,0.01mol) in (100 mL) water and (3 mL) concentrated HCl (37%) was whiskered until a clear solution was get. Where, this solution is cooled to (0–5 ^{O}C) and while maintaining the temperature below (5 ^{O}C) a solution of sodium nitrite (0.72 g, 0.01mol) in 10 mL water was then added drop by drop. The resulting mixture was stirred for (30 min) in an ice bath and the excess nitrite was removed with the addition of urea [19]. The solution of resulting diazonium chloride was mixed with coupling component 4-methyl-1H-imidazole (0.82 g, 0.01 mol) dissolved in (150 mL) from cooled alkaline ethanol under (5 ^{O}C). After the solution was left in the refrigerator for 24 hours, the mixture was acidified with hydrochloric acid diluted to (pH = 6). The red precipitate was washed and filtered several times by using distilled water, then dried in air and twice re-crystallized with hot ethanol, then it was dried using the oven at (50 ^{O}C). Some analytical and physical data for this azo dye was tabulated in Table (1) Scheme 1 shows the preparation steps of the azo ligand



Scheme 1: Preparation of azo Ligand (L_1)

Preparation of the Schiff base (HL_2):

(0.149.19 g ,0.01 mol) from (4-dimethylamino)benzaldehyde dissolved in ethanol (50 mL) and then mixed with (o.188 g ,0.01mol) of (4-amino3-hydroxobenzenesulfonamide) dissolved in ethanol. Three drops from glacial acetic acid ware added and the mixture was refluxed with stirring for 6 hrs. Schiff base ligand was isolated after the volume of the mixture was reduced to half by evaporation and precipitated product was collected by filtered off and dried over anhydrous $CaCl_2$. Yield:96%, mp:(198-200) ^{o}C. Scheme 2 show the preparation steps of the Schiff base ligand



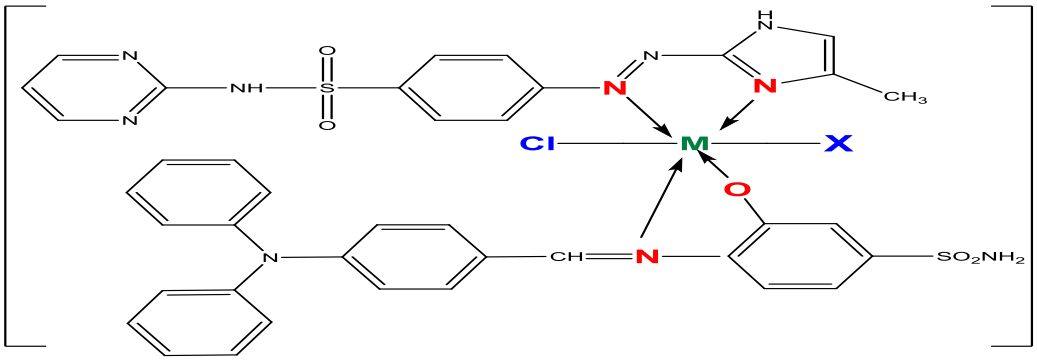
Scheme 2: preparation Ligand Schiff base (HL_2)

Preparation complex of Mixed ligand L_1 and HL_2:

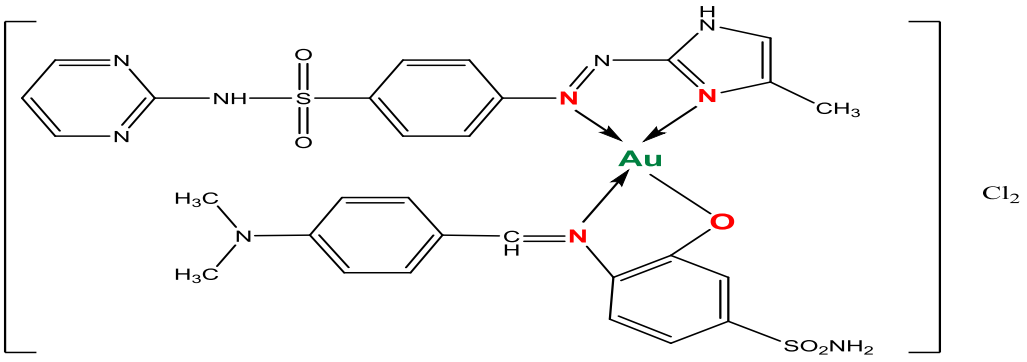
General procedure for preparation chelate complexes, the alcohol solution of respective salts [$NiCl_2.6H_2O$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$,$Cdcl_2$, $HgCl_2$ and $Na[AuCl_4].2H_2O$] was slowly mixed with hot mixture ethanolic solution of **(L_1)** and (HL_2) ligand, in (1:1:1) (L: M: L) molar ratio. After the addition was complete, the reaction mixture was refluxed for (2 houres) then cooled. The solids that precipitate were filtered off, washed with (5 mL) hot (50%) (ethanol:water) to take out any effects of the unreacted starting materials, air dried, re-crystallized from ethanol and heated in the oven at (60 ^{O}C). All data for these compounds tabulated in Table (1).

Antioxidant activity

The free radical scavenging activity of the synthesized compounds was studied in vitro by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method.[20] Stock solution of the drug was diluted to different concentrations in the range of 50-200 mg/ mL in methanol. Methanolic solution of the synthesized compounds (2 mL) was added to 0.003% (w/v) methanol solution of DPPH (1 mL). The mixture was shaken vigorously and allowed to stand for 30 min. Absorbance at 517 nm was determined and the percentage of scavenging activity was calculated. Ascorbic acid was used as the standard drug. The inhibition ratio (I %) of the tested compounds was calculated according to the following equation: I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and As is the absorbance of the sample.



when M= Co(II) ,Ni(II) , Cu(II) ,Zn(II) ,Cd(II) ,Hg(II) , x=H2O
M=Fe , x=Cl



Scheme (4): the proposed structural formula of the complexes

Results and Discussion

Antioxidant Screening (DPPH radical scavenging activity)

The scavenging activity results of some of synthetic compounds showed in table (1)., From the results in table (1) and figures, the conc. (200) µg/mL is the most scavenging activity compared with other concentrations of

synthesized compounds. The DPPH is used in the laboratory and is widely used to evaluate the effectiveness of antioxidants. DPPH has absorption at 517 nm and disappears when DPPH is reduced to an antioxidant or becomes radical. The diamagnetic molecule is stable. As a result, the color changes from purple to yellow. This change in color is taken as an indicator of the ability y of hydrogen to donate to tested compounds.

Antioxidants can interact with DPPH and produce (1.1 - diphenyl - 2 - picryl – hydrazine). The limiting capabilities of the compounds examined were determined by their interaction with stable free-standing 1.1-di-vinyl-2-picryl-hydrazine (DPPH) in five different concentrations for 30 minutes [39-41] . The highest scavenger activity observed in compound (HL_2), this is probably due to the presence of hydroxyl group. Mostly electron withdrawing substituent's deactivate aromatic ring and have no capability to bind the free radicals. The results of evaluating the activity of antioxidants showed that all the prepared compounds have antioxidant properties when compared with standard antioxidants such as ascorbic acid as a reference in search of their antioxidant activity by the stable free radical method [42-44]

Table 1: Scavenging activity of some synthetic compound

DPPH scavenging activity%						
Conce.	Ascorbic acid	L_1	HL_2	$[NiL_1L_2H2OCl]$	$[CuL_1L_2H_2OCl]$	$[AuL_1L_2]Cl_2$
50	65	69.3	83.9	65.5	62.7	68.9
100	73	69.7	92.7	69.5	66	75.9
150	85.3	69.8	93.4	70.1	67.7	78
200	98.2	71.7	94.9	77.1	68.6	78.7

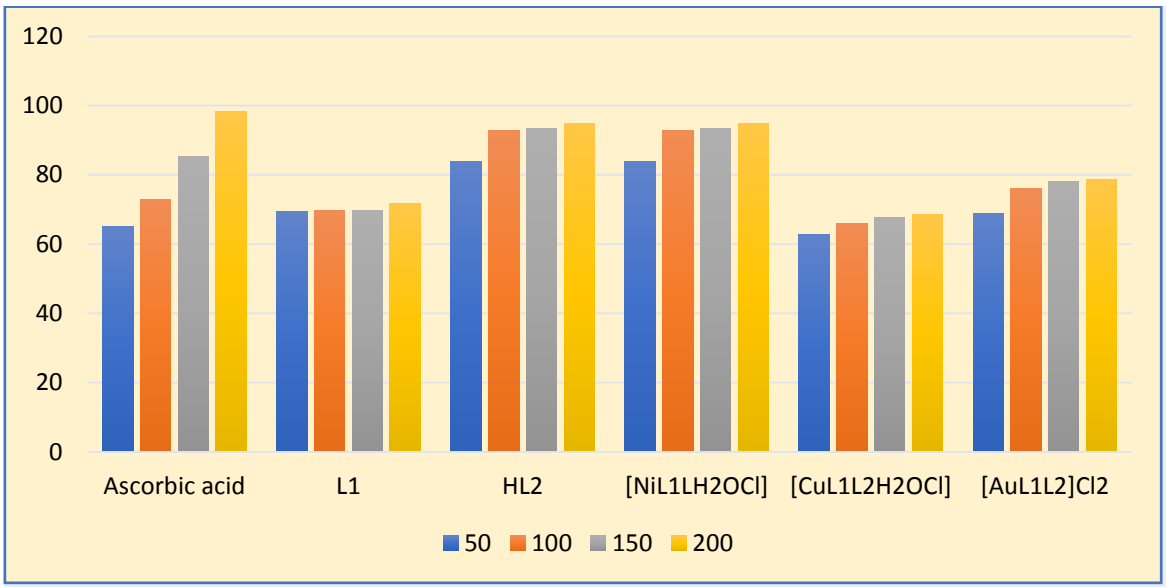


Figure (1): Scavenging activity of the compound using DPPH.

Anticancer activity of complexes

In vitro cytotoxic activity against breast cancer (MCF 7) cell line at different concentrations was evaluated and compared with the healthy cells The anticancer activities of the gold complexes were performed with different concentrations such as 6.25 µ g / ml ,12.25 µ g / ml , 25 µ g / ml , 50 µ g / ml , 100 µ g / ml. The anticancer activity of gold complexes against breast cancer (MCF 7) cell line increased while in the concentration of gold

complexes (Fig. 6). Gold complexes exhibit good results when compare with the healthy cells [46-50]. Previously the cytotoxic effect of gold nanoparticles is the result of active physicochemical interaction of gold atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA .

It was observed that half of the inhibitory concentration of cancer cells, IC50, was (145.27µg / ml), which is low compared to healthy cells [45], where it was (192.21µg / ml), and this is a good result. That is, the gold nanocomplex kills breast cancer cells with high efficiency and has no effect on healthy cells. This is a very important result in the use of a complex of gold as a highly selective treatment for the treatment of breast cancer.

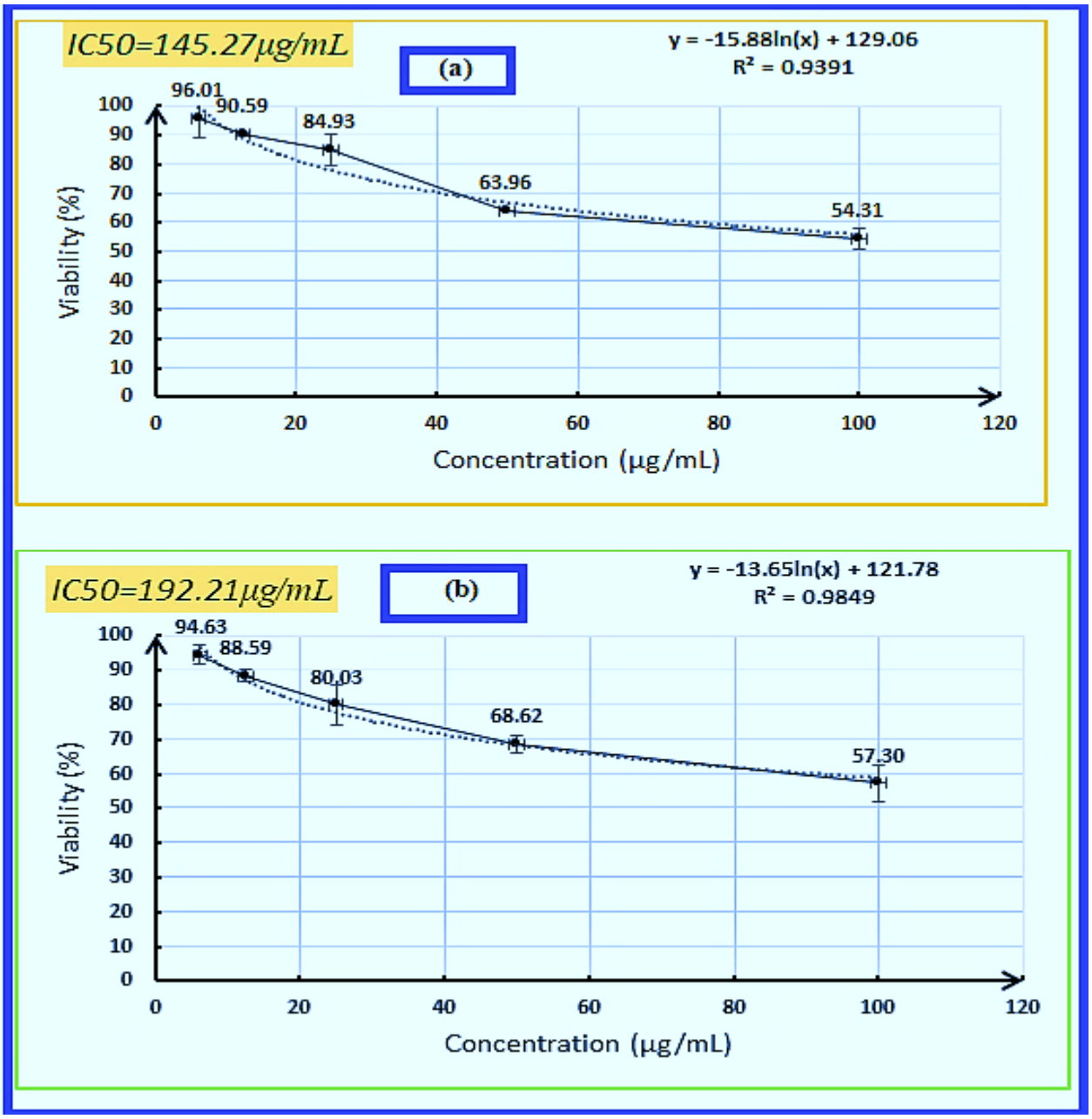


Figure (2): Percentage of inhibition in cells of (a) MCF-7 breast cancer line cell against the concentration of complex [Au L_1L_2]Cl_2 (b) normal line cell against the concentration of complex [Au L_1L_2]Cl_2

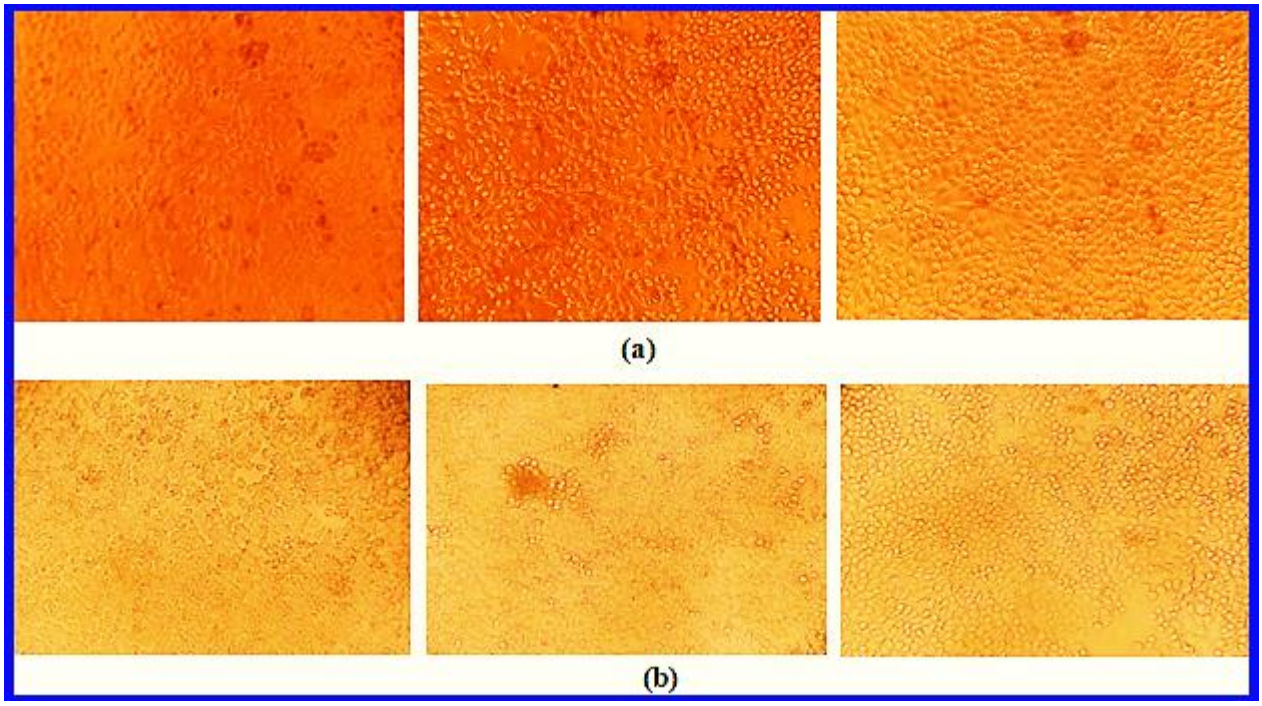


Figure (3):(a) Cancer cells treated with compound $[AuL_1L_2]Cl_2$ at different concentration after adding MTT (b)Normal cells treated with compound $[AuL_1L_2]Cl_2$ at different concentration after adding MTT

Transmission -Electron microscopy

The basis of the work of the electron microscope includes the use of electrons to take a picture of the samples. The source of the emission of electrons is the tungsten thread, which shoots electrons at high speed into a very thin layer of the sample, where the flow of electrons focuses on a small spot of the sample and photographs it, then moves to another spot until it photographs all the spots formed for the sample. Radiation passes through it. The transmission electron microscope is used to study the crystal structure in terms of the shape and size of the particles and the distribution of nanoparticle crystals, as the transmission electron microscope was used to take a picture of the shape of the crystals of the complexes of the triple gold ion ligand Cl2 [AuL1L2], as the pictures showed (3,2,1) in Figures (4, 5) The shape of the complex particles is spherical and the average particle size is about 20 nanometers, where the period of complexation by the reverse sublimation method was (123) two hours and under a temperature of (80) C, while the pictures are as in Figure (4) of the aforementioned complex prepared over a period of 18 hours under the same conditions of temperature and concentration showed a clear change in the shape of the complex particles [46-48] from spherical in shape to sheets of nano sizes, which indicates that increasing the time period for preparing the complex under temperature (80) helps to integrate the complex nanoparticles [49-51] And transform it into smaller nano sheets of irregular shape [52-54].

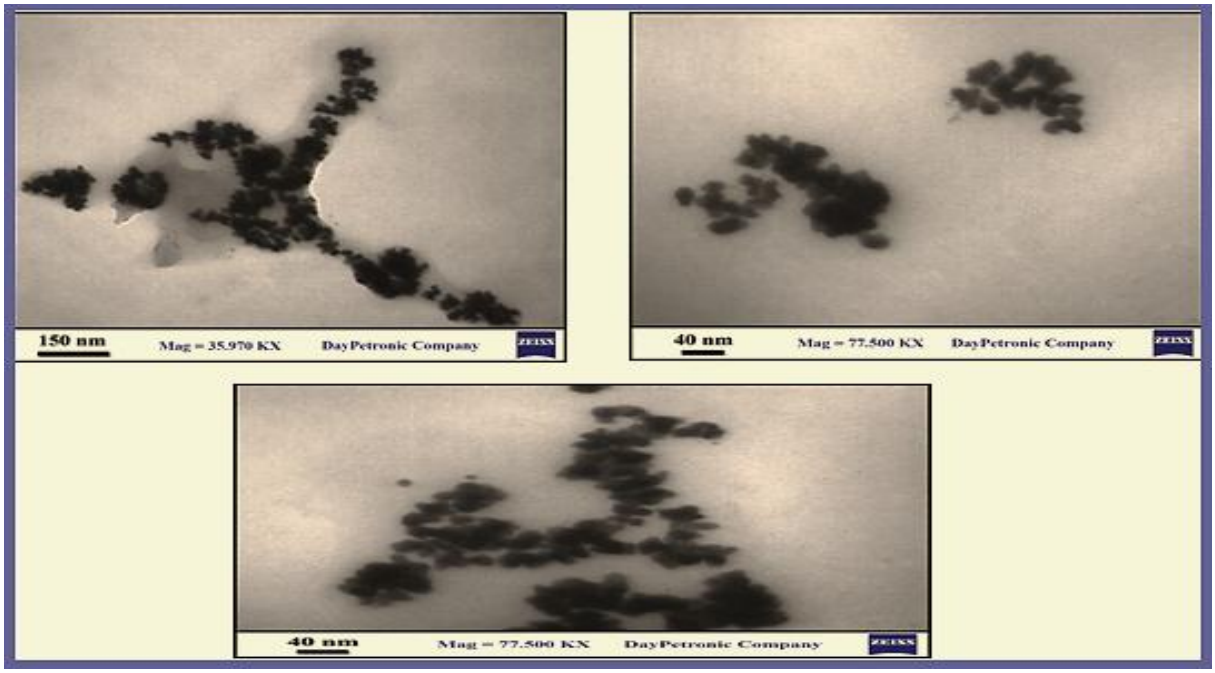


Fig.(4): TEM of [AuL1L2]Cl2 at refluxing 2 hrs

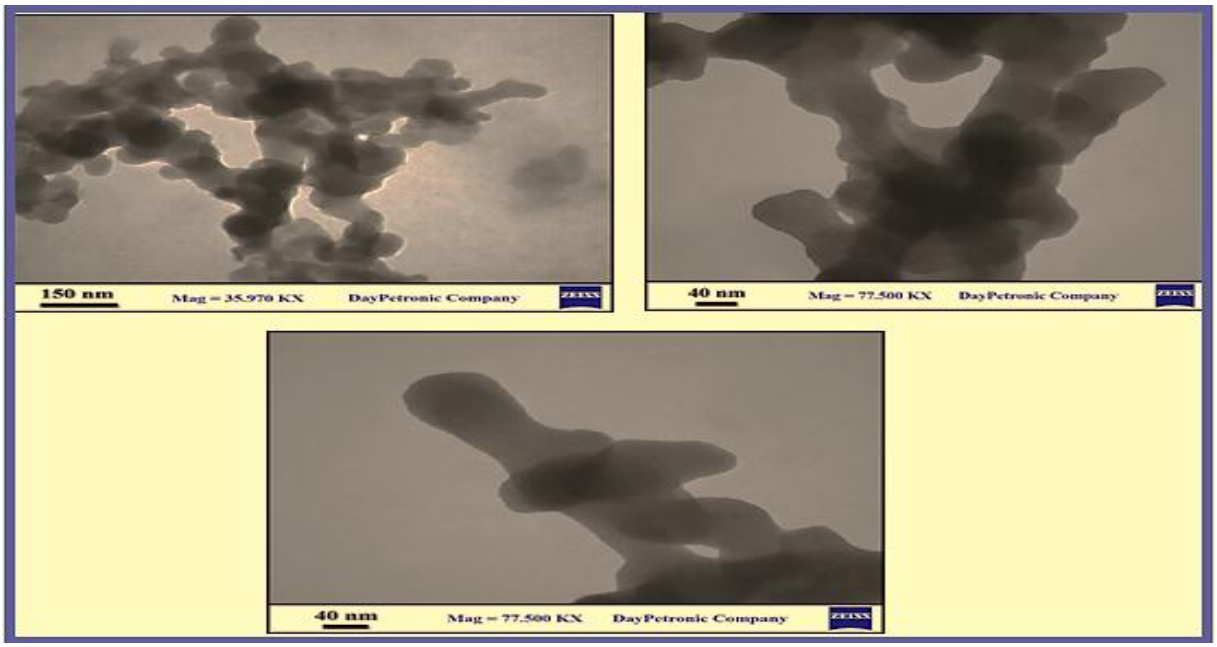


Fig.(5): TEM of [AuL1L2]Cl2 at refluxing 18 hrs

Conclusions :

All the compounds showed DPPH radical scavenging activity. In general, the results indicated that the complexes have potential and promising anti-oxidant activities. Previously the cytotoxic effect of gold nanoparticles is the result of active physicochemical interaction of gold atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA .

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