Journal of Molecular Structure 1039 (2013) 51–60

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Preparation and characterization of some gold nanometric compounds with simple organic materials as precursor: Spectroscopic, biological and anti-cancer assessments

Aly H. Atta [a,b,*], Ahmed I. El-Shenawy [a,c], Moamen S. Refat [d,e], Khaled M. Elsabawy [d,f]

[a] Department of Chemistry, Faculty of Education in Dammam, Dammam University, Dammam, Saudi Arabia
[b] Department of Chemistry, Faculty of Science, Suez University, Suez, Egypt
[c] Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt
[d] Department of Chemistry, Faculty of Science, Taif University, Al-Hawiah, P.O. Box 888, Taif 21974, Saudi Arabia
[e] Department of Chemistry, Faculty of Science, Port Said University, Port Said, Egypt
[f] Materials Science Unit, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt

HIGHLIGHTS

- This study reports the synthesis of the gold nano-particles assembly mediated by some amino acids.
- The general formula is $[Au_2(AA)_2(Cl)_2]^{2+}Cl_2$ with 1:1 (metal:ligand) stoichiometry.
- The biological and anti-cancer activities of the gold nano-sized complexes were done.

ARTICLE INFO

Article history:
Received 4 December 2012
Received in revised form 10 January 2013
Accepted 12 January 2013
Available online 5 February 2013

Keywords:
Gold nano-particles
Microbiological investigation
Spectroscopy
XRD
SEM
TEM

ABSTRACT

This study reports the synthesis of the gold nano-particles assembly mediated by some amino acids, such as ornithine (Orn), isoleucine (i-Leu), serine (Ser) and histidine (His). The structural characterization of $[Au_2(AA)_2(Cl)_2]^{2+}Cl_2$ (where AA = Orn, i-Leu, Ser and His) compounds were done by using elemental analysis, molar conductance, FT-IR, Raman, UV–vis, X-ray powder diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) spectroscopy. It has been found from the elemental analysis, spectroscopic and the thermal studies that the amino acids ligand behaves as bidentate ligand forming chelates with 1:1 (metal:ligand) stoichiometry. The molar conductance measurements of the complexes in DMSO indicate that the complexes are electrolyte. The thermal decomposition mechanisms were discussed for compounds Orn, i-Leu, Ser and His and occurred in two consecutive steps. The TG curves of the gold(III) compounds suggest the loss of the ligands (Orn, i-Leu, Ser and His) within two steps, with probable formation of a gold free metal. The biological and anti-cancer activities (Breast carcinoma cells (MCF-7-cell line) of the nano-sized complexes have also been studied.

1. Introduction

Biologically active molecules such as amino acids are usually attached to nano-particles to improve their bio-specificity and to expand application potentialities of these types of systems in biological and medical sciences. Amino acids are a promising class of organic compounds to be used in biofunctionalization of gold nano-particles, as protective layers and for their assembly. Functional groups such as –SH and $–NH_2$ present a high affinity for gold, and since amino acids contain some of these groups, they are expected to stabilize gold nanoparticles. Their capacity of generating structural diversity was recognized [1], and gold surfaces capped with amino acids are considered to represent the simplest mimics for protein surfaces [2]. However, there are relatively few reports in the literature on surface modifications of gold nano-particles with amino acid molecules [3–8], but no systematic study of amino acid interactions with gold nano-particles is available. Amino acids can be used in the assembly formation of inorganic nanoparticles. If such molecules are adsorbed and bonded to the nanoparticle surface, amino acids belonging to two different nanoparticles can be connected through a condensation reaction with peptide bonding formation, thus leading to a peptide-linked assembly of nanoparticles. The properties of such assemblies could be designed rationally

* Corresponding author at: Department of Chemistry, Faculty of Science, Dammam University, Dammam, Saudi Arabia.
E-mail address: aly_atta@yahoo.com (A.H. Atta).

0022-2860/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molstruc.2013.01.029

by choosing the initial amino acid. Alternatively, amino acids can be adsorbed on the metallic particle surface already during the formation of nanoparticles, using the amino acid itself as reduction agent [8–12], or latter, by ligand exchange reactions or binding on the former adsorbed stabilizing molecules. In this way protecting homogeneous or heterogeneous, mixed monolayers of metallic nanoparticles can be obtained. One of the problems which have attracted the attention of both science and industry is the integration of nanoparticles and organic molecules, in order to develop new materials for electronics and optics and new applications in biomedical and bioanalytical areas, such as controlled drug delivery, medical diagnosis devices and biosensors [13–18]. Gold compounds are increasingly attracting researchers' attention as a source of novel cytotoxic substances, of potential use in cancer treatment. In particular, several gold(III) compounds, with profoundly different molecular structures, have been designed, synthesized and tested as antiproliferative agents during the last decade [19–23]. Very relevant cytotoxic properties were disclosed for many gold(III) compounds and initial structure-function relationships outlined [24–26]. The complexes between different metal ions and biological molecules like amino acids play an important role in human life. Amino acids and their compounds with different metal ions play an important role in biology, pharmacy and industry [27].

The aim of this investigation is to gain insights into the assembly formation of gold compounds nanoparticles and interparticle interactions in the presence of ornithine (Orn), isoleucine (i-Leu), serine (Ser) and histidine (His), which could have potential application for the anti-microbial and anti-cancer activities. The gold(III) solid complexes were prepared by copreciptation method. The coordinate of Orn, i-Leu, Ser and His with Au^{3+} metal ions ($NaAuCl_4{\cdot}xH_2O$) was investigated in solid state by spectroscopic and thermal studies and were proved the molar ratio was 1:1. In view of the literature, the coordination chemistry of Orn, i-Leu, Ser and His with Au^{3+} metal ions is obscure. In the paper herein, we report the formation of four new gold(III) amino acid nano-particles complexes obtained from the reactions of ornithine (Orn), isoleucine (i-Leu), serine (Ser) and histidine (His) with the $NaAuCl_4{\cdot}xH_2O$ in aqueous media. The aim of this study is to make an assessment of the coordination behavior of the resulting new complexes formed. The results provide important information on the affinity of gold nano-particles to Orn, i-Leu, Ser and His molecules and medical applications.

Table 1
Analytical and physical data for gold(III)/Orn, i-Leu, Ser and His complexes.

Compounds	Mp (°C)	Colors	Λ_M (Ω^{-1} cm^2 mol^{-1})
$[Au_2(Orn)_2(Cl)_2]{\cdot}2Cl$	>230	Yellow–orange	102
$[Au_2(i\text{-}Leu)_2(Cl)_2]{\cdot}2Cl$	>230	Yellow–orange	94
$[Au_2(Ser)_2(Cl)_2]{\cdot}2Cl$	>230	Yellow–orange	110
$[Au_2(His)_2(Cl)_2]{\cdot}2Cl$	>230	Yellow–orange	97

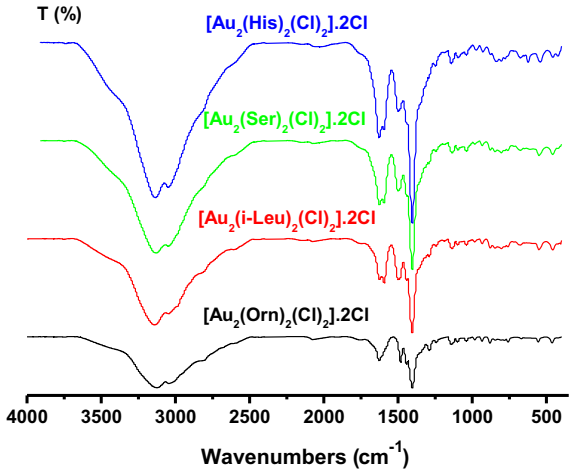


Fig. 1. Infrared spectra of Orn, i-Leu, Ser and His gold(III) complexes.

2. Experimental

2.1. Chemicals

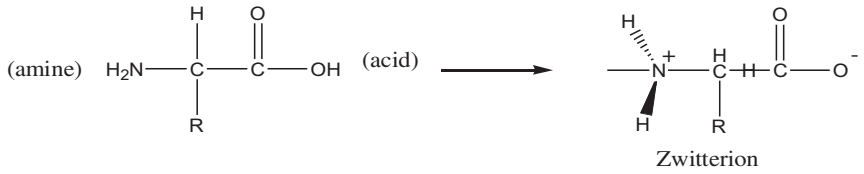
Ornithine, isoleucine, serine, histidine, $NaAuCl_4{\cdot}xH_2O$ and $LiOH{\cdot}H_2O$ (BDH) were purchased and used without further purification.

2.2. Synthesis of gold(III) complexes

First experiments were performed in order to find, if possible, a general method for the preparation of gold(III) complexes. Many

Table 2
Infrared characteristic bands frequencies (cm^{-1}) of gold(III)/Orn, i-Leu, Ser and His complexes.

Compounds	$[Au_2(Orn)_2(Cl)_2]{\cdot}2Cl$	$[Au_2(i\text{-}Leu)_2(Cl)_2]{\cdot}2Cl$	$[Au_2(Ser)_2(Cl)_2]{\cdot}2Cl$	$[Au_2(His)_2(Cl)_2]{\cdot}2Cl$
OH str.	–	–	3380	
NH str.				3418
NH_2 asym. str.	3127	3139	3139	3139
NH_2 sym. str.	3042	3042	3036	3042
CH aromatic str.	2815	2963	2801	2821
CH aliphatic str.		2873		
COO^- asym.	1626	1587	1633	1633
δNH_2	1477	1503	1503	
COO^- sym.	1445	1406	1406	1399
δCH	1405	1315		
CH in plane def.	1289	1256	–	1249
NH_2 wagging	1146	1185	1133	1146
CH out of plane def.	1094	1133	1087	1081
	1042	1074	1036	971
	983	1042	964	925
	931	996	912	834
	879	925	847	775
	840	853	801	
	763	801	769	
NH_2 rocking	672	684	678	665
M–N str.	555	542	522	536
M–O str.	464	451	438	419



Scheme 1. Zwitterion structure of amino acid.

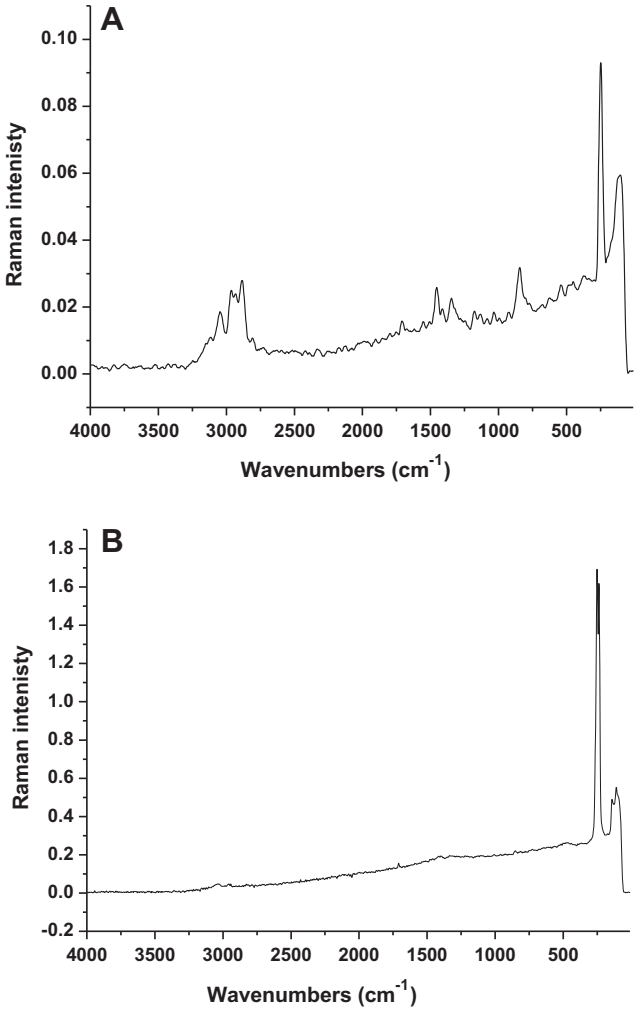


Fig. 2. Raman spectra of (A): isoleucine gold(III) complex and (B): serine gold(III) complex.

amino acids will react with metal ions in aqueous solutions, but reaction appeared to occur between solutions of L-tyrosine and metal salts in water, at room temperature or at 70 °C. It was found that lithium hydroxide powder was preferable to sodium hydroxide or potassium hydroxide pellets, as more accurate weight is possible with the powder. A second approach was via the reaction of ornithine, isoleucine, serine, histidine with lithium hydroxide in water at room temperature; subsequent addition of aqueous $NaAuCl_4{\cdot}xH_2O$ salt solutions did not appear to lead to complex formation. However, it was found that by heating the Amino acid/H-$LiOH{\cdot}H_2O$ (Eq. (1)) solution to 70 °C for 30 min, followed by addition of the $NaAuCl_4{\cdot}xH_2O$ salts, complexes formed readily (Eq. (2)).

Ornithine (0.133 g, 1.0 mmol) and $LiOH{\cdot}H_2O$ (0.043 g, 1.0 mmol) were dissolved in water (20 ml) and the solution heated to 70 °C for 30 min. The $NaAuCl_4{\cdot}xH_2O$ salt (0.362 g, 1.0 mmol) was dissolved in a minimum quantity of water and the solutions mixed with vigorous stirring (for a 1:1 metal:ligand ratio), the gold(III) salt was added to the OrnH–$LiOH{\cdot}H_2O$ solution. Precipitation was almost instantaneous, but stirring with heating were continued for 20 min. The Au(III) complex was filtered, washed with hot water (25 ml), and dried in *vacuo* over anhydrous $CaCl_2$. A similar procedure as that described for Au^{III}–Orn complex was carried out, but the weights of isoleucine, serine, and histidine are 0.132, 0.106 and 0.156 g with 1.0 mmol, respectively.

$$AA{-}H + LiOH \cdot H_2O \rightarrow AA{-}Li + 2H_2O \ (\text{where AA} = \text{Orn, i-Leu, Ser and His}) \quad (1)$$

$$2AA{-}Li + 2NaAuCl_4 \cdot xH_2O \rightarrow [Au_2(AA)_2(Cl)2] \cdot Cl_2 \downarrow + 2LiCl + 2NaCl \quad (2)$$

The yellow–orange precipitated compounds that separated were filtered, washed with water and ethanol, and dried over $CaCl_2$ in vacuum. Anal.: Calcd. For $[Au_2(Orn)_2(Cl)_2]{\cdot}Cl_2$: C, 15.05; H, 2.78; N, 7.02. Found: C, 14.97; H, 2.66; N, 6.98. Anal.: Calcd. For $[Au_2(i\text{-}Leu)_2(Cl)_2]{\cdot}Cl_2$: C, 18.10; H, 3.04; N, 3.52. Found: C, 18.02; H, 2.97; N, 3.46. Anal.: Calcd. For $[Au_2(Ser)_2(Cl)_2]{\cdot}Cl_2$: C, 9.69; H, 1.63; N, 3.77. Found: C, 9.54; H, 1.59; N, 3.74. Anal.: Calcd. For $[Au_2(His)_2(Cl)_2]{\cdot}Cl_2$: C, 17.08; H, 1.91; N, 9.96. Found: C, 16.98; H, 1.90; N, 9.94.

2.3. Measurements

Elemental analyses (C, H, and N) were performed using a Perkin–Elmer CHN 2400 elemental analyzer. The percentages of the metal ions of the amino acid complexes were determined gravimetrically by converting the compounds into their gold metal. The molar conductivities of freshly prepared 1.0×10^{-3} mol/cm^3 dimethylsulfoxide (DMSO) solutions were measured for the dissolved Au(III) Orn, i-Leu, Ser and His complexes using Jenway 4010 conductivity meter. The electronic absorption spectra of gold(III) complexes were recorded in DMSO solvent within 900–200 nm range using an UV2 Unicam UV/vis Spectrophotometer fitted with a quartz cell of 1.0 cm path length. The infrared spectra with KBr disks were recorded on a Bruker FT-IR Spectrophotometer (4000–400 cm^{-1}), while Raman laser spectra of samples were measured on the Bruker FT-Raman with laser 50 mW. The thermal studies TG/DTG-50H were carried out on a Shimadzu thermogravimetric analyzer under static air till 800 °C. Scanning electron microscopy (SEM) images were taken in Quanta FEG 250 equipment and Joel JSM-6390 equipment; with an accelerating voltage of 20 and 25 kV also transmission electron microscopy (TEM) images were taken in Joel JSM-6390 equipment. The X-ray diffraction patterns for the Orn, i-Leu, Ser and His gold(III) complexes were recorded on X'Pert PRO PANanalytical X-ray powder diffraction, target copper with secondary monochromate.

2.4. Antibacterial and antifungal evaluation

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disk diffusion method [28]. Briefly, 100 μl of the test bacteria/fungi were grown in 10 mL of fresh

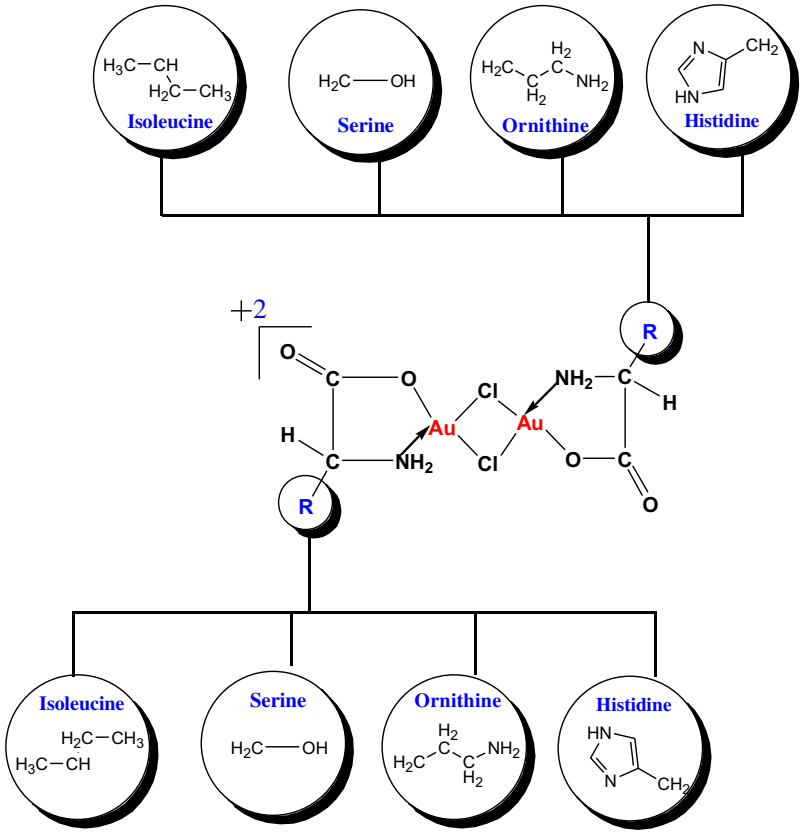


Fig. 3. General proposed structures of $[Au_2(AA)_2(Cl)_2]\cdot 2Cl$ complexes where AA = Ornithine, isoleucine, serine and histidine.

media until they reached a count of approximately108 cells/mL for bacteria or 105 cells/mL for fungi [29]. 100 μl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disk diffusion method [30,31]. Of the many media available, National Committee for Clinical Laboratory Standards (NCCLSs) recommends Mueller-Hinton agar due to: it results in good batch-to-batch reproducibility. Disk diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the NCCLS [32] for evaluating the susceptibility of filamentous fungi to antifungal agents. Disk diffusion method for

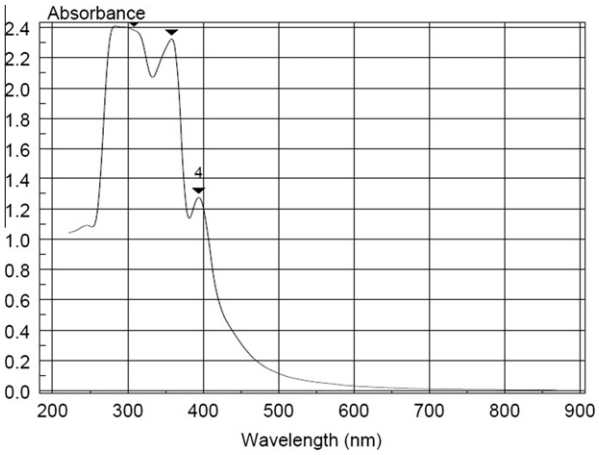


Fig. 4a. Raman spectra of histidine gold(III) complex.

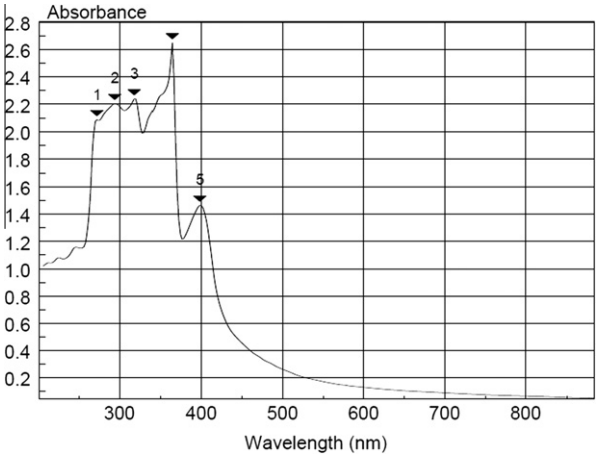


Fig. 4b. Raman spectra of serine gold(III) complex.

yeast developed standard method (M44-P) by the NCCLS [33]. Plates inoculated with filamentous fungi as *A. flavus* at 25 °C for 48 h; Gram (+) bacteria as *Staphylococcus aureus*, *Bacillus subtilis*; Gram (−) bacteria as *Escherichia coli*, *Pseudomonas aeruginosa* they were incubated at 35–37 °C for 24–48 h and yeast as *Candida albicans* incubated at 30 °C for 24–48 h and, then the diameters of the inhabitation zones were measured in millimeters [28]. Standard disks of tetracycline (antibacterial agent), Amphotericin B (antifungal agent) served as positive controls for antimicrobial activity but filter disk impregnated with 10 μl of solvent (distilled water and DMSO) were used as a negative control. The agar used is

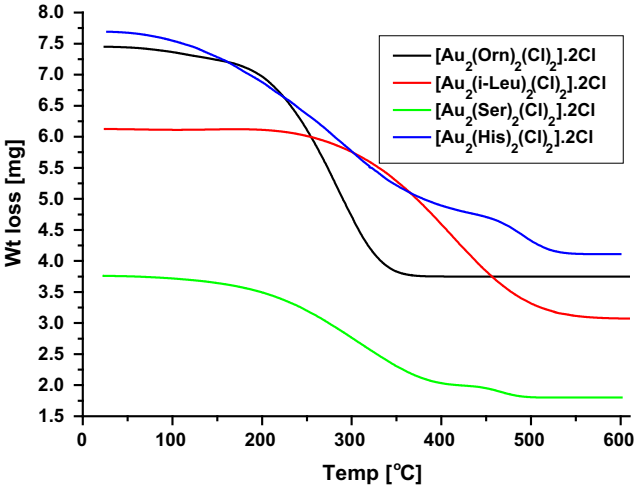


Fig. 5. TG curves of Orn, i-Leu, Ser and His gold(III) complexes.

Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disk diffusion method. This method is well documented and standard zones of inhabitation have been determined for susceptible values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 μl of tested concentration of the stock solutions. When a filter paper disk impregnated with a tested chemical is placed on agar the chemical will diffuse from the disk into the agar. This diffusion will place the chemical in the agar only around the disk. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disk. If an organism is placed on the agar it will not grow in the area around the disk if it is susceptible to the chemical. This area of no growth around the disk is known as a "Zone of inhibition" or "Clear zone". For the disk diffusion, the zone diameters were measured with slipping calipers of the National for Clinical Laboratory Standers [30]. Agar-based methods such as disk diffusion test can be good alternatives because they are simpler and faster than broth methods [34,35].

2.5. Anti-cancer evaluation

Cytotoxic and antitumor activity of Orn, i-Leu, Ser and His gold(III) synthesized compounds were tested against MCF-7 cell line according to the method of Mosmann [36] and Vijayan et al. [37]. Inhibitory activity against Breast carcinoma cells (MCF-7 cell line) was detected by using different concentration of the tested compounds (50, 25, 12.5, 6.25, 3.125 and 1.56 μg) and viability cells (%) were determined by colorimetric method.

3. Results and discussion

Four gold(III) complexes of ornithine, isoleucine, serine and histidine chelates have been synthesized according to the 1:1 molar ratio between $NaAuCl_4{\cdot}xH_2O$ and each of Orn, i-Leu, Ser and His in aqueous medium neutralized using monohydrated lithium hydroxide at 70 °C. The gold(III) complexes were investigated in this study, are stable at room temperature in the solid state. These complexes are insoluble in common organic solvents in cold or hot conditions except dimethylsulfoxide. No suitable crystals of the complexes were obtained in order to perform an X-ray structure determination. The molecular formula, molecular weights and molar conductance of the gold(III) complexes are given in Table 1. The analytical data are in a good agreement with the proposed stoichiometry of the complexes. Conductivity measurements in non-aqueous solutions have frequently been used in structural studies of metal chelates within the limits of their solubility. They provide a method of testing the degree of ionization of the complexes, the molar ions that a complex liberates in solution, the higher will be its molar conductivity and vice versa. The non-ionized complexes have negligible value of molar conductance. The molar conductivities of the solid gold(III) chelates were measured for 1.0×10^{-3} mol solution of 1:1 ratio in DMSO. The conductivity data reported for these complexes are given in Table 1. The product of the cell constant and the measured conductance of a solution give the specific conductivity K. The molar conductance ($\Omega^{-1}\,cm^{-1}\,mol^{-1}$) is given by the relation: $\Lambda_m = \frac{K}{C} \times 1000$, where C (mol/L) is the concentration of the solution. It is clear from the conductivity data that the complexes present behave as electrolytes [38] behavior. The molar conductivity values for all the complexes in organic solvent (DMSO) with 10^{-3} mol were in rang of (94–110) Λ_M ($\Omega^{-1}\,cm^2\,mol^{-1}$) (Table 1).

3.1. Infrared and Raman spectra

The infrared spectra within the (4000–400 cm^{-1}) region (Fig. 1) provide information regarding the coordination mode in the ornithine, isoleucine, serine and histidine gold(III) complexes and were analyzed by comparison with data for the free amino acids ligands. The most relevant bands and proposed assignments for all the complexes are mentioned in Table 2. In the FT-IR spectra, extensive coupling occurs for several vibrations, making qualitative deductions about the environment around gold metal ions difficult. However, the IR spectral data (Table 2) shown changes in the position and profiles of some bands, as compared to those of free ornithine, isoleucine, serine and histidine amino acids, suggesting participation of the groups that produce these bounds in the coordination with gold(III) metal ions. Major changes are related to the carboxylate and amino bands. Amino acid physical properties indicate a "salt-like" behavior. Amino acids are crystalline solids with relatively high melting points, and almost are quite soluble in water and insoluble in non-polar solvents. In solution, the amino acid molecule appears to have a change which changes with pH. As intermolecular neutralization reaction leads to a salt-like ion called a Zwitterion. The accepted practice is to show the amino acids in the Zwitterion form (Scheme 1). The amino group can lose a hydrogen ion to become negative charged; also the amino group can accept a hydrogen ion to become positive charged.

Assignment of observed frequencies; In free amino acids ligands spectra, the characteristic band of NH_2 group vibration appears at ~3300 and ~3400 cm^{-1} corresponding to $\nu_s(NH_2)$ and $\nu_{as}(NH_2)$, respectively. In the spectra of gold(III) complexes, this bands were shifted to lower wavenumbers at 3130 and 3040 cm^{-1} due to the involvement of $-NH_2$ group in the chelation bond. The band due to the NH_3^+ group $\nu(NH_3^+)$ at ~2500 cm^{-1} [39], which is very intense in the free ligand disappear in the spectra of the gold(III) compounds. The sharp bands at 3380 and 3418 cm^{-1} are assigned to the –OH and –NH stretching vibrations of Serine and histidine gold complexes, respectively. The presence of these peaks confirm the un-sharing of them in the complexation. The CH stretching vibrations of an aliphatic or aromatic group are observed in a lower frequency region than those of a benzene ring. Gold(III)-chelates; in all of the complexes it is apparent that the Orn, i-Leu, Ser and His moieties complexes as the ornithinate, isoleucinate, serinate and histidinate anion [40–43]. Amino acids can lose a proton from the carboxylic acid group. It is to be expected that carboxylic acid group will deprotonated preferentially, especially the α-amino acid moiety acts as a chelate. In the region 1700–500 cm^{-1} the assignments of the observed bands is accomplished by a comparison of the spectrum of each chelate with that of the ornithine, isoleucine,

Table 3
Antimicrobial activity data of gold(III)/Orn, i-Leu, Ser and His complexes.

Sample		Inhibition zone diameter (mm/mg sample)					
		Bacillus subtilis (G^+)	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0
Standard	Tetracycline antibacterial agent	34	32	34	30	–	–
	Amphotericin B antifungal agent	–	–	–	–	18	19
	$[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$	37	33	32	30	22	16
	$[Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$	32	28	37	42	17	12
	$[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$	41	38	29	34	19	26
	$[Au_2(His)_2(Cl)_2]^{2+}Cl_2$	30	24	32	31	25	10

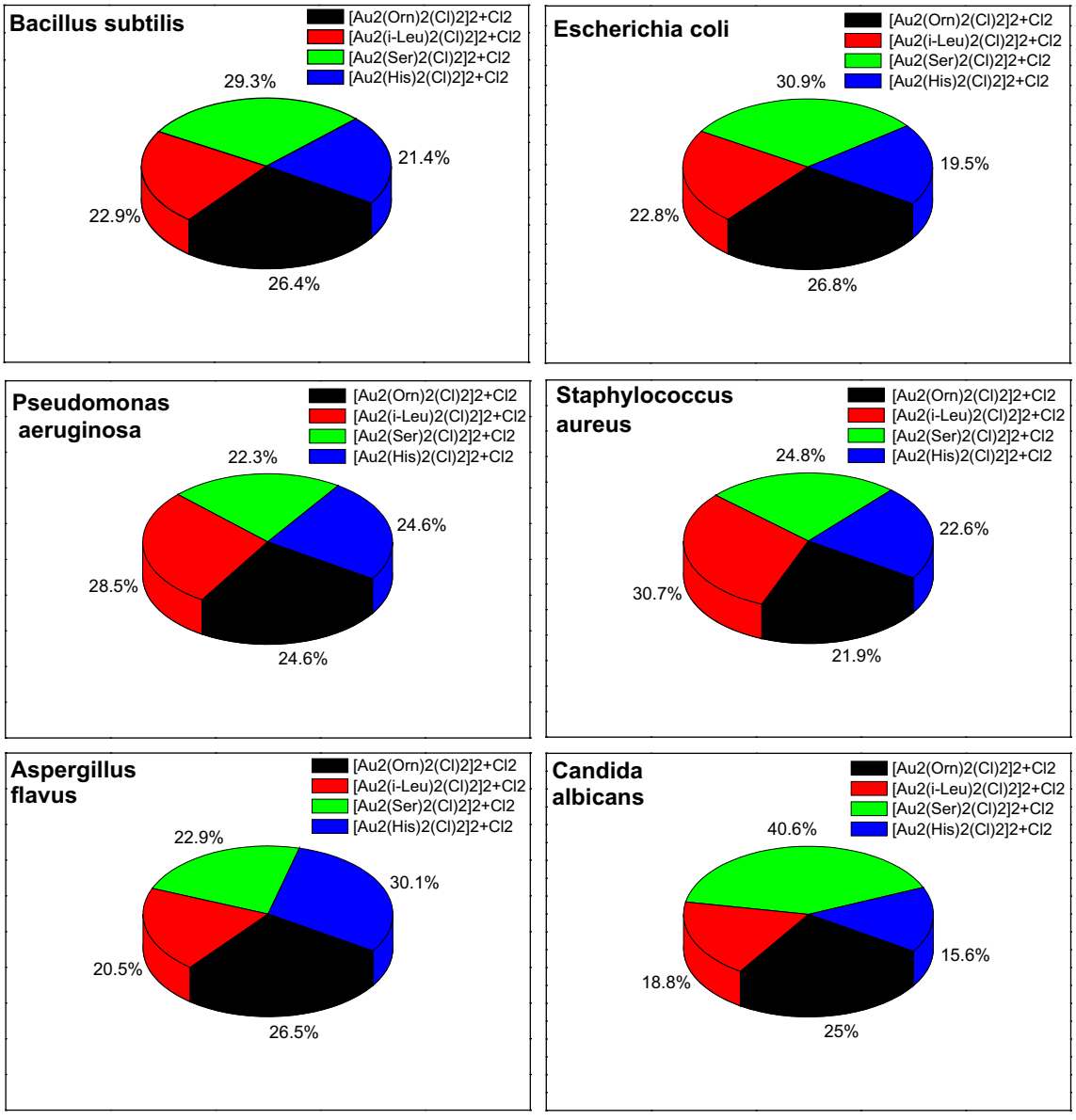


Fig. 6. Biological assessments of Orn, i-Leu, Ser and His gold(III) complexes.

serine and histidine free ligands. For the amino acids adducts, the observed $\nu_{as}(COO^-)$ band was exhibits at 1633–1587 cm^{-1} as well as the $\nu_s(COO^-)$ band which appears at 1445–1399 cm^{-1}, are in agreement with coordination involving an oxygen atom of the carboxylate group [44,45]. The $\Delta\nu$ difference (asymmetric–symmetric stretching) is ranged from 181 to 234 cm^{-1}, this value proved that, gold(III) ions coordinated to carboxylate group of ornithine, isoleucine, serine and histidine ligands as a monodentate chelates [39], taking account that the respective amino acids acts as a bidentate ligand through NH_2 and COO^- groups. The characteristic bands due

Table 4
Concentration (μg) and viability (%) of gold(III)/Orn, i-Leu, Ser and His complexes.

Sample conc. (μg)	Viability %			
	$[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$	$[Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$	$[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$	$[Au_2(His)_2(Cl)_2]^{2+}Cl_2$
50	10.2	16.23	19.45	22.34
25	19.32	23.45	26.34	31.54
12.5	31.22	31.98	33.54	43.45
6.25	35.11	43.21	46.34	53.31
3.125	36.09	49.19	52.19	61.84
1.56	60.32	66.03	66.45	80.43
0	100.00	100.00	100.00	100.00

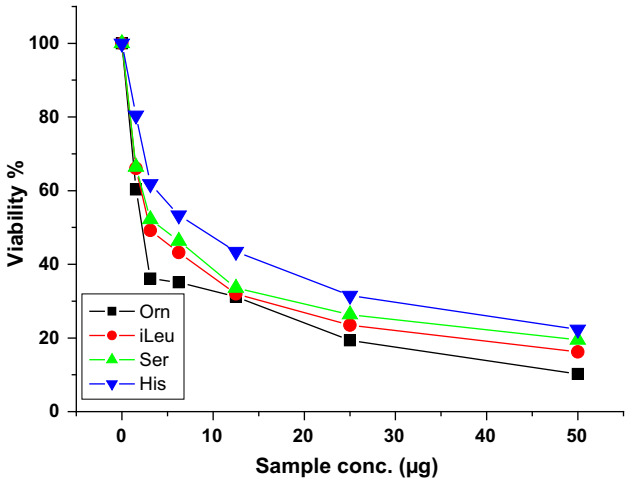


Fig. 7. Concentration (μg) and viability (%) of gold(III)/Orn, i-Leu, Ser and His complexes.

to Au–N and Au–O appears at 555–522 cm^{-1} and 464–419 cm^{-1}, respectively. Raman laser spectra is a good spectral tools within the region down 400 cm^{-1} (Fig. 2) to discussed the assignment of Au–Cl, that existed these bands at 248 and 113 cm^{-1}. A number of Raman studies on CNTs have taken advantage of the surface enhancement by local optical fields of silver or gold nanostructures [46].

The structures of the gold(II) complexes accordingly the above interpretation using elemental analysis, molar conductance, infrared and Raman spectra can be suggested as shown in Fig. 3.

3.2. Electronic spectra

The bands in the range 200–450 nm can be assigned to n–π* and/or π–π* intraligand transition associated to amino acid (Orn, i-Leu, Ser and His). Free ligand and complexes exhibit similar spectra in UV region in relation to the number of the absorption peaks. The electronic spectra of respective amino acids and its gold(III) complexes (Figs. 4a and 4b) recorded in DMSO. The electronic absorption spectrum of the free ligands shows three absorption peaks appearing at λ_{max} = 230, 240 and 280 nm. The first two bands are attributed to the intraligand π–π* transition [43], and the third can be attributed to the intraligand n–π* charge transfers [47,48]. The electronic absorption spectra of the complexes are different from the spectrum of the free ligand, There are four bands appearing in the spectrum of histidine gold(III) complex. The first band appeared at 282 nm, which represents the intraligand π–π* transition. The second and third bands appeared at 308 and 358 nm, which can be attributed to the intraligand n–π* transition. The fourth band appeared at range 394 nm, can be assigned to His–Au(III) charge transfers. In the spectrum of serine Au(III) complex there are also has a five bands, the first two bands at 272 and 294 nm are represent to the intraligand π–π* transition, and the third and fourth bands at 318 and 364 nm can be attributed to the intraligand n–π* transition. The fifth band at 398 nm can be assigned to Ser–Au(III) charge transfers.

3.3. Thermal analysis

Thermal techniques such as thermogravimetric analysis (TGA and DTG), has been successfully employed for the study of the energetic of interactions of metal cations with biological species, such as amino acids [49]. The weight loss profiles are analyzed the amount or percent of weight loss at any given temperature, and the temperature ranges of the degradation process were determined. The simultaneous TG–DTG curves of ornithine, isoleucine, serine and histidine complexes at the heating rate 10 °C/min in the static nitrogen atmosphere are given in Fig. 5. The overall loss of mass from the TG curves is 50.33%, 50.20%, 47.78% and 53.36% for $[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$, $[Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$, $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$ and $[Au_2(His)_2(Cl)_2]^{2+}Cl_2$ compounds, respectively. All the complexes have two endothermic maxima peaks. The theoretical mass loss 50.64%, 50.52%, 47.05% and 53.33% are in a good agreement with experimental data. The analysis of thermal curves of the gold(III) complexes clearly indicates that the weight loss in the first and second steps assigned to decomposition of two amino acids moieties. The end decomposition products gave gold metal pure with found percentage 49.67%, 49.80%, 52.22% and 46.64% matched with theoretical data 49.36%, 49.48%, 52.95% and 46.67%.

3.4. Antimicrobial assessments

The results of antibacterial actives in vitro of the gold(III) complexes are shown in Table 3 and Fig. 6. From the results we can see that all the test compounds have a higher antibacterial activity on *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. The $[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$ and $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$ compounds have a higher inhibitory on *B. subtilis* and *E. coli* than tetracycline antibacterial agent. The $Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$ compound has higher inhibitory on *P. aeruginosa* than tetracycline antibacterial agent. The $[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$ compound has the same inhibitory effect like tetracycline antibacterial agent on the *S. aureus*, but the $[Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$, $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$ and $[Au_2(His)_2(Cl)_2]^{2+}Cl_2$ compounds have a higher inhibitory on *S. aureus* than tetracycline antibacterial agent. The gold(III) complexes have been evaluated for their antifungal activity. The minimal inhibitory concentration values listed in Table 3 shows that all the test compounds have the order of antifungal activity as: for the *A. flavus* $[Au_2(His)_2(Cl)_2]^{2+}Cl_2 > [Au_2(Orn)_2(Cl)_2]^{2+}Cl_2 > [Au_2(Ser)_2(Cl)_2]^{2+}Cl_2 > [Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$, for the *C. albicans* the ordered as $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2 > [Au_2(Orn)_2(Cl)_2]^{2+}Cl_2 > [Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2 > [Au_2(His)_2(Cl)_2]^{2+}Cl_2$.

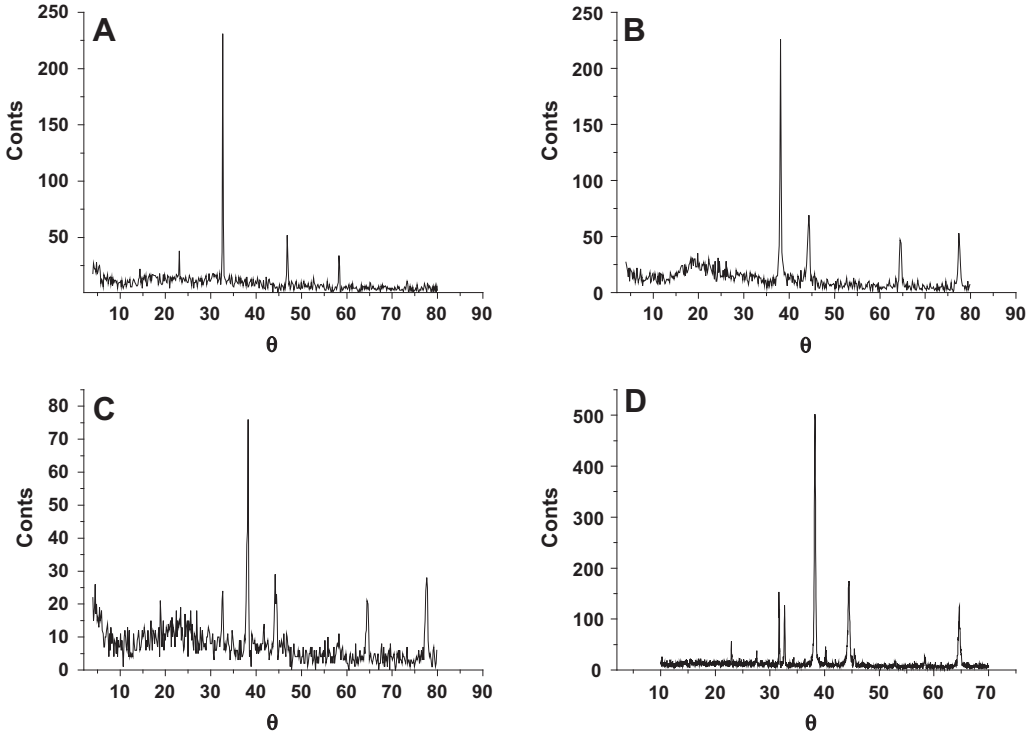


Fig. 8. XRD diagrams of: (A) Orn, (B) i-Leu, (C) Ser and (D) His gold(III) complexes.

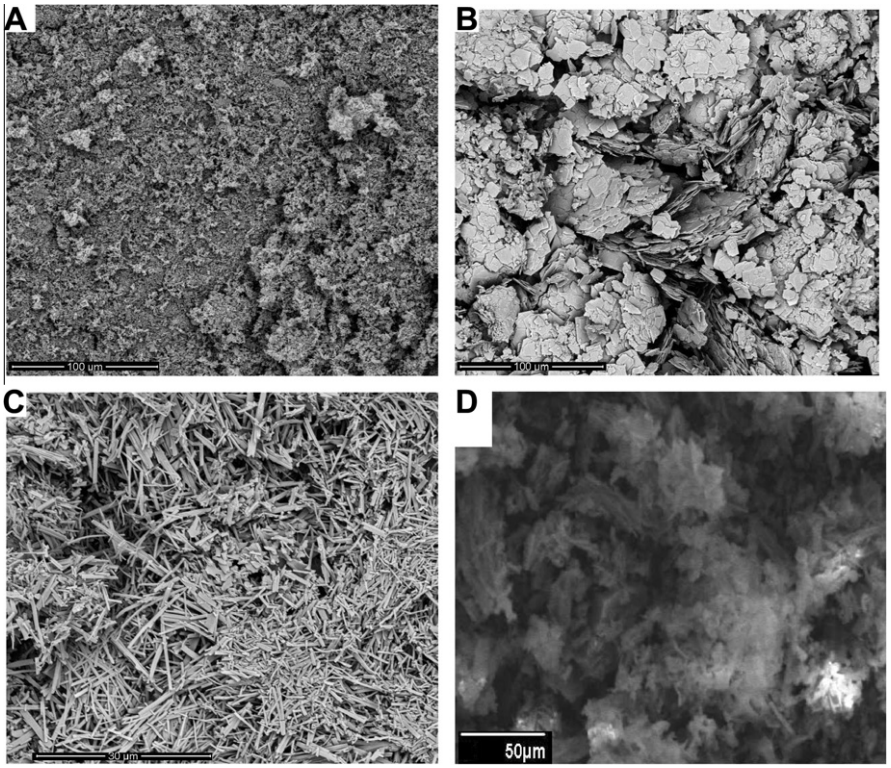


Fig. 9. SEM pictures of: (A) Orn, (B) i-Leu, (C) Ser and (D) His gold(III) complexes.

3.5. Anti-cancer evaluation

The inhibitory concentration fifty (IC_{50}) was calculated from Table 4 and Fig. 7. Results revealed that, all tested gold(III) compounds have cytotoxic and antitumor activity against Breast carcinoma cell line with superiority of compound $[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$ and compound $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$. Inhibitory concentration fifty (IC_{50}) was found to be ordered as

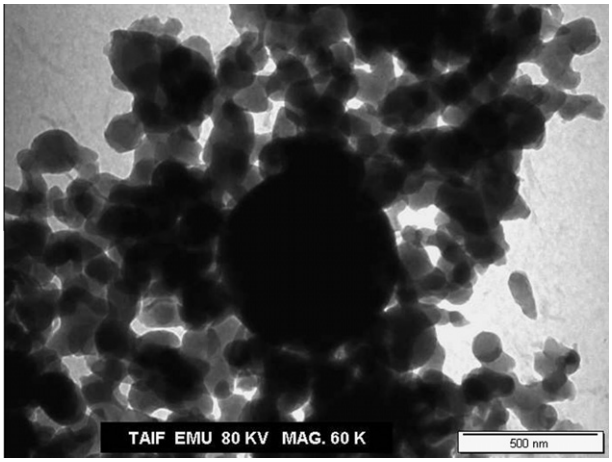


Fig. 10. TEM pictures of serine gold(III) complex.

$[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$ > $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$ > $[Au_2(i\text{-}Leu)_2(Cl)_2]^{2+}Cl_2$ > $[Au_2(His)_2(Cl)_2]^{2+}Cl_2$. Compound $[Au_2(Orn)_2(Cl)_2]^{2+}Cl_2$ contained terminal amino group. Compound $[Au_2(Ser)_2(Cl)_2]^{2+}Cl_2$ contained terminal hydroxyl group. The absence of a substituent on the terminal amino group of enhanced the cytotoxic activity [50].

3.6. XRD, SEM and TEM studies

Fig. 8 demonstrates the XRD patterns of the synthesized ornithine, isoleucine, serine and histidine nanoparticles. The X-ray diffraction data were recorded by using Cu Kα radiation (1.5406 Å). The intensity data were collected over a 2θ range of 0–80°. The average grain size of the samples was estimated with the help of the Scherrer equation, using the diffraction intensity peak. X-ray diffraction studies confirmed that the synthesized materials were containing gold(III) nanoparticles and all the diffraction peaks agreed with the reported standard data; no characteristic peaks were observed other than gold(III) amino acid (Orn, iso-Leu, Ser and His) complexes. The mean grain size (D) of the particles was determined from the XRD line broadening measurement using the Scherrer equation: $D = 0.89\lambda/(\beta \cos\theta)$. Where λ is the wavelength (Cu Kα), β is the full width at the half-maximum (FWHM) of the Orn, iso-Leu, Ser and His gold(III) complexes line and θ is the diffraction angle. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in the nanometer range. The lattice parameters calculated were also in agreement with the reported values. The complexation reaction greatly influences the particle morphology of as-prepared gold(III) complexes nanoparticles powders.

Scanning and transmission electron microscopy techniques give a general perception about microstructure, surface morphology, particle size and chemical composition of respective gold(III) complexes. Figs. 9 and 10 show the designed the SEM and TEM photographs of the gold(III) complexes. The uniformity and similarity between the particles forms of synthesized gold(III) complexes indicate that the existence of morphological phases of Orn, iso-Leu, Ser and His complexes have a homogeneous matrix. A homogeneous phase formation of gold(III) complexes having ice, crack morphologies in the form of a dispersed with particle size 30–100 μm is exhibited in Fig. 9. The serine gold(III) complex has a single-phase formation of straight bundle of sticks with particle size 0.5 μm, Fig. 9C. The serine complex nanoparticle was characterized with TEM technique. Transmission electron microscopy studies of nanoparticles were carried out to understand the shape and size of particles. The transmission electron photography for the nanoparticles of serine complex obtained in a neutral aqueous media with about 100 nm in diameter with spherical shape is shown in Fig. 10.

4. Conclusion

An important biological nucleus such as amino acids become more interesting when attached to nanoparticles that enhancement their antimicrobial and anti-cancer activities. The paper discussed chelation between gold salt and some of amino acids like ornithine, isoleucine, serine and histidine controlling with neutral pH using lithium hydroxide in water at room temperature. The formation of functionalization compound between sodium gold salt and some amino acids depends on the pH of amino acid solution and associated zwitterions form. These compounds were characterized using elemental analysis, molar conductance, FT-IR, Raman, UV–vis, XRD, SEM, TEM spectroscopy. The Breast carcinoma cancer cells were tested against the studied nano-sized gold compounds. This paper is considered the preliminary study in order to obtain a small scale gold nano-particle compounds.

Acknowledgement

This work was funded by Deanship of Scientific Research at Dammam University, Saudi Arabia under project Grants No. 2012044.

References

[1] G.M. Coppola, H.F. Schuster, Asymmetric Synthesis. Construction of Chiral Molecules Using Amino Acids, Wiley, New York, 1987.
[2] C.C. You, M. De, G. Han, V.M. Rotello, J. Am. Chem. Soc. 127 (2005) 12873.
[3] R. Levy, R.C. Doty, Stabilization and functionalization of metallic nanoparticles: the peptide route, in: C.S.S.R. Kumar (Ed.), Biofunctionalization of Nanomaterials, Wiley-VCH Verlag GmbH & Co KGaA, Weinheim, 2005, pp. 235–269.
[4] J. Zsang, Q. Chi, J.U. Nielsen, E.P. Friis, J.E.T. Andersen, J. Ulstrup, Langmuir 16 (2000) 7229.
[5] S. Mandal, S. Phadtare, M. Sastry, Curr. Appl. Phys. 4 (2005) 118.
[6] P.R. Selvakannan, S. Mandal, S. Phadtare, R. Pasricha, M. Sastry, Langmuir 19 (2003) 3545.
[7] H. Joshi, P.S. Shirude, V. Bansal, K.N. Ganesh, M. Sastry, J. Phys. Chem. B 108 (2004) 11535.
[8] Y. Shao, Y. Jin, S. Dong, Chem. Commun. 1104 (2004).
[9] S Mandal, P. Selvakannan, S. Phadtare, R. Pasricha, M. Sastry, Proc. Indian Acad. Sci. (Chem. Sci.) 114 (2002) 513.
[10] P. Selvakannan, S. Mandal, S. Phadtare, A. Gole, R. Pasricha, S.D. Adyanthaya, M. Sastry, J. Colloid Interface Sci. 269 (2004) 97.
[11] S. Aryal, B.K. Remant, B. Narayan, C.K. Kim, H.Y. Kim, J. Colloid Interface Sci. 299 (2006) 191.
[12] S.K. Bhargava, J.M. Booth, S. Agrawal, P. Coloe, G. Kar, Langmuir 32 (2005) 5949.
[13] M. Zheng, X. Huang, Biofunctionalization of gold nanoparticles, in: C.S.S.R. Kumar (Ed.), Biofunctionalization of Nanomaterials, Wiley-VCH Verlag GmbH & Co KGaA, Weinheim, 2005, pp. 99–124.
[14] D. Fitzmaurice, S. Connolly, Adv. Mater. 11 (1999) 1202.
[15] S. Mann, W. Shenton, M. Li, S. Connolly, D. Fitzmaurice, Adv. Mater. 12 (2000) 147.
[16] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293.
[17] N.L. Rosi, C.A. Mirkin, Chem. Rev. 105 (2005) 1547.
[18] J.C. Love, L.A. Estroff, J.K. Knebel, R.G. Nuzzo, G.M. Whitesides, Chem. Rev. 105 (2005) 1103.
[19] A. Casini, C. Hartinger, C. Gabbiani, et al., J. Inorg. Biochem. 102 (2008) 564.
[20] L. Messori, G. Marcon, Met. Ions Biol. Syst. 42 (2004) 385.
[21] I. Ott, Coord. Chem. Rev. 253 (2009) 1670.
[22] E.R.T. Tiekink, Crit. Rev. Oncol./Hematol 42 (2002) 225.
[23] V. Milacic, D. Chen, L. Ronconi, K.P. Landis-Piwowar, D. Fregona, Q.P. Dou, Cancer Res. 66 (2006) 10478.
[24] D. Saggioro, M.P. Rigobello, L. Paloschi, et al., Chem. Biol. 14 (2007) 1128.
[25] Y. Wang, Q.Y. He, R.W. Sun, C.M. Che, J.F. Chiu, Cancer Res. 65 (2005) 11553.
[26] L. Messori, F. Abbate, G. Marcon, et al., J. Med. Chem. 43 (2000) 3541.
[27] Norbert Sewald, Hans-Dieter Jakube. Peptides: Chemistry and Biology, Wiley-VCH verlag Gmb H&Co. KGaA, 2002.
[28] A.W. Bauer, W.M. Kirby, C. Sherris, M. Turck, Am. J. Clin. Pathol. 45 (1966) 493.
[29] M.A. Pfaller, L. Burmeister, M.A. Bartlett, M.G. Rinaldi, J. Clin. Microbiol. 26 (1988) 1437.
[30] National Committee for Clinical Laboratory Standards, Performance Vol. antimicrobial susceptibility of Flavobacteria, 1997.

[31] National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved standard M7-A3, National Committee for Clinical Laboratory Standards, Villanova, Pa, 1993.
[32] National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi: Proposed Standard M38-A, NCCLS, Wayne, PA, USA, 2002.
[33] National Committee for Clinical Laboratory Standards, Methods for Antifungal Disk Diffusion Susceptibility Testing of Yeast: Proposed Guideline M44-P, NCCLS, Wayne, PA, USA, 2003.
[34] L.D. Liebowitz, H.R. Ashbee, E.G.V. Evans, Y. Chong, N. Mallatova, M. Zaidi, D. Gibbs, Global Antifungal Surveillance Group, Diagn. Microbiol. Infect. Dis. 4 (2001) 27.
[35] M.J. Matar, L. Ostrosky-Zeichner, V.L. Paetznick, J.R. Rodriguez, E. Chen, J.H. Rex, Antimicrob. Agents Chemother. 47 (2003) 1647.
[36] T. Mosmann, J. Immunol. Methods 65 (1983) 55.
[37] P. Vijayan, C. Ragha, G. Ashok, S.A. Doanraj, B. Suresh, Indian J. Med. Res. 120 (2004) 24.
[38] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
[39] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination compounds, 4th ed., Wiley, New York, 1986.
[40] Xu. Hao, Liang. Chen, Spectrochim. Acta, A 59 (2003) 657.
[41] C.A. McAuliffe, S.G. Murray, Inorg. Chim. Acta 7 (1973) 171.
[42] Y. Inomata, T. Inomata, T. Moriwaki, Bull. Chem., Soc. Jpn. 47 (4) (1974) 818.
[43] J.F. Vilia, W.E. Hatfield, Chem. Phys. Lett. 9 (6) (1971) 568.
[44] R.F. de Farieas, J. Scatena Jr., C. Airoldi, J. Inorg. Biochem. 73 (1999) 253.
[45] R.F. de Farieas, C. Airoldi, J. Inorg. Biochem. 76 (1999) 273.
[46] K. Kneipp, H. Kneipp, M.S. Dresselhaus, S. Lefrant, Philos. Trans. R. Soc. Lond., Ser. A 362 (2004) 2361.
[47] R.H. Holm, F.A. Cotton, J. Am. Chem. Soc. 80 (1958) 5658.
[48] F.A. Cotton, C.W. Wilkinson, Advanced Inorganic Chemistry, 3rd ed., Interscience Publisher, New York, 1972.
[49] K. Burger, Coordination Chemistry: Experimental Methods, Akademiai Kiado, Budapest, 1973. p. 23.
[50] C. Marzano1, M. Pellei, F. Tisato, C. Santini, Anti-Cancer Agents Med. Chem. 9 (2009) 185.