Research Article

Synthesis, Infrared (I.R), Thermal Gravimetric Analysis (TGA) Characterization and Antibacterial Activity of some Amino Acids Complexes

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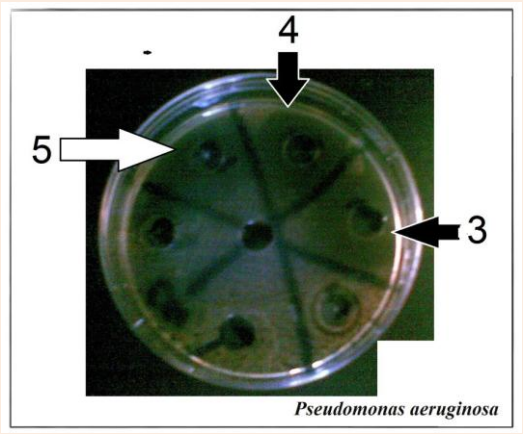
Abstract

Twelve amino acids-metals complexes were prepared. The amino acids including (Cysteine, Cystine and Methionine) . The metal which used including (lead, manganese, Cadmium and Iron).The identification of the complexes structure were measured by using IR,TGA and physical properties (conductivity, Melting point),also the concentrations of the heavy metal were determined. Antibacterial applications of the prepared complexes were used on some types of bacteria in clouding (Bacillus, E.coli ATCC, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus ATCC, Sarcina lutea, Mycobacterium phlei, Candida albicans). The results showed that there is strong effect for some complexes on the bacteria types which selected in this study and different form concentration to contraction. Also some complexes give good induction to use it in cosmetic manufication.

Keywords: I.R ,TGA and antibacterial activity amino acids complexes preparation



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Introduction

Amino acids are building blocks of proteins. More than100 amino acids have been isolated and identified but only 25 are obtained upon hydrolysis of typical proteins. All 25 except 2 are α-amino acids; the two exceptions are proline and hydroxy proline, which are amino acids. Only 20 amino acids are of general occurrence because they are usually found in all proteins. Ten of the amino acids are essential amino acids i.e. a deficiency in any one prevent growth in young animals, and may even cause death. They must be furnished in the diet as they are not synthesized in the body[(1)]. In addition to providing the monomer units from which the long polypeptide chains of proteins are synthesized, the L-α-amino acids and their derivatives participate in cellular functions as diverse as nerve transmission and the biosynthesis of prophyrins , Parines, pyrimidines, and urea. Short polymers of amino acids called peptides perform prominent roles in the neuroedocrine system as hormones, hormone-releasing factors, neuromdulators, or neurotransmitters. While proteins contain only L-α-amino acids[(2)].

Microorganisms elaborate peptides that contain both D and L-α-amino acids several of these peptides are of therapeutic value including the antibiotics backtracking and gramicidin A and antitumor agent bleomycin. Certain other microbial peptides are toxic. The cyan bacterial peptides microcystin and nodular in are lethal in large doses. While small guantities prom ore the formation of hepatic rumors. Neither humans norang other higher animals can synthesize 10 of 20 common L-α-amino acids in amounts adequate to support infant growth or to maintain health in adults consequently. The human diet must contain adequate quantities of these nutritionally essential amino acids[(3)].

Experimental

Materials:
All chemicals used in this investigation were laboratory grade. They include $MnCl_2.4H_2O$, $FeCl_2.4H_2O$, $CdCl_2.4H_2O$, $PbCl_2.4H_2O$, and cysteine, cystine ,methionine, ammonia, sodium hydroxide (NaOH),), hydrochloric acid (HCl), acetic acid (CH_3COOH), sodium chloride (NaCl), boric acid (H_3BO_3), Phosphoric acid (H_3PO_4), (KCl), ethanol, Methanol, DMSO, DMF, chloroform, distilled water was used distillation process was carried out using both of condensation process and ion exchange.

Ligands:

Cysteine:
10^{-4} M stock solution was prepared by dissolving 0.00302 g of cysteine in distilled water by gentle heating till complete dissolution .the solution was cooled and diluted to 250 ml. More diluted solutions were prepared by diluting the stock.

Cystine:
10^{-4} M stock solution was prepared by dissolving 0.0060 g of cystine in distilled water by gentle heating till complete dissolution. The solution was cooled and diluted to 250 ml. More diluted solutions were prepared by diluting the stock.

Methionine:
10^{-3}M stock solution was prepared by dissolving 0.0373 g of methionine in 250 ml distilled water, more diluted solutions were prepared by diluting the stock.

Synthesis of the metal complexes:-

synthesis of metal –L-cysteine –(H_2L) complexes :
0.8 mole of metal chloride in 50 ml ammonia was added with stirring to 0.6 mole of cysteine ligand in 50 ml distilled water .the reaction mixture was refluxed and then left overnight .the precipitated solid complex was separated out by filtration then washed with water and dried over P_2O_5.

***synthesis of metal –L-cystine –(H_2L) complexes*:**
0.6 mole of metal chloride in 50 ml distilled water was added with stirring to 0.8 mole sodium-L-cystinate (prepared by dissolving L-cystine in NaOH). The reaction mixture was refluxed and then left overnight. the precipitated solid complex was separated out by filtration then washed with water and dried over P_2O_5.

synthesis of metal –L-Methionine –(HL) complexes :
0.8 mole of metal chloride in 50 ml ammonia was added with stirring to 0.6 mole of cysteine ligand in 50 ml distilled water. The reaction mixture was refluxed and then left overnight. The precipitated solid complex was separated out by filtration then washed with water and dried over P_2O_5.

Infrared spectra:
the infrared spectra of the ligands and their metal complexes were taken in potassium bromide discs using the I.R (Type thermo FT-IR 380Nicolet company) spectrophotometer covering the range from 200 to 4000 cm^{-1}.

Thermo gravimetric analysis:
The thermo gravimetric analysis of some amino acids complexes which contain water molecules was achieved by using thermal technique model TGA-H50 shimadzu (Japan).the weight lost of sample was measured from room temperature up to (1000^oC) in rate of 10 ^oC per min .

Antibacterial test:

Bacterial cultures:

Plate cultures of nutrient agar (OXID) medium were used for culture of bacteria . the medium was prepared by dissolving 28g of powder in 1liter of sterile distilled water. Then the medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes. The bacteria were cultured and incubated at 37 ^{o}C for 24h.

Antibacterial assay:

The antibacterial tests were assayed according to the diffusion method. The strains of bacteria used were Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and Sarcina *lutea*), Gram-negative bacteria (Escherichia *coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and representative fungal species yeast *(Candida albicans)*. All strains were isolated from patients in medicine academe .The identity of all the strains was confirmed. A bacterial suspension was prepared and added to the sterilized medium before solidification under aseptic condition. Different concentration of amino acid complexes were placed on the surface of the culture and incubated at 37 ^{o}C for 24h. After incubation the average inhibition zone recorded.

Result and Discussion

The physical properties of the synthesis complexes:

Table 1 The color, melting point and molar conductivity of the prepared complexes.

Metal Conce. ppm	***M.C Sm2 mol-1***	***M.P (C0)***	***Color***	**Complexes**
0.652	***1.21***	***300***	***Brown***	Fe(II)-MET
0.582	***2.08***	***450***	***White***	Cd(II)-MET
0.405	***1.69***	***305***	***Brown***	Mn(II)-MET
0.70	***4.45***	***401***	***White***	Pb(II)-MET
1.013	***2.04***	***298***	***Blake***	Fe(II)-Cysteine
0.278	***6.63***	***354***	***Yellow***	Cd(II)-Cysteine
1.177	***5.17***	***367***	***Black***	Mn(II)-Cysteine
1.417	***6.77***	***328***	***Black***	Pb(II)-Cysteine
0.673	***5.66***	***321***	***Orang***	Fe(II)-Cystine
0.693	***3.58***	***357***	***White***	Cd(II)- Cystine
1.565	***3.82***	***412***	***White***	Mn(II)-Cystine
0.70	***7.60***	***400***	***Black***	Pb(II)- Cystine

Infrared spectra studies:-

In the following, it is desired to know the mode of in the prepared complexes. This is simply done by comparing the infrared spectra of the complexes to that of the ligands.

A comparison of the IR spectra of the ligand and its metal complexes brings out the following:

1- The bands of cysteine located at 3420 cm^{-1}, 3000 cm^{-1} 1600 cm^{-1} are assigned to (N-H),) , N-H and δ N-H, respectively The first band of the free ligand is shifted to higher frequency in case of the copper complex and completely disappeared in case of the nickel complex and still existing in the same position in case of the cobalt complex. On the other hand, The δ N-H ligand band is subjected to changes in position in the copper and nickel

complexes but still exists in the same position in case of the CoL.4H_2O complex. From these results one can come to a conclusion that the amino group is of major importance for coordination in both the copper and the nickel complexes and is of minor importance in the cobalt complex.

2- The moderate band at 2540 cm $^{-1}$ of the free ligand could be argued to the presence of hydrogen bonds of O----- H—O type between the carboxyl group [4,5] .This band completely disappeared in all the metal complexes.

3- The cysteine ligand displays distinct γ(S - H) at 2100 cm^{-1} indicative that the ligand present in the thiol form rather than the thione structure. The γ(S-H) and δ(S-H) bands are completely absent in all metal complexes. This is evidence to coordination through the thiol sulphur on deprotonation [6].

4- The ligand gave two infrared spectral bands in the vicinity of 1600 cm^{-1} and 1400 cm^{-1} attributable to the asymmetric and symmetric vibrations of the carboxyl groups [7].The band at 1400 cm^{-1} is slightly shifted in case of copper and nickel complexes and shifted to lower frequency and becomes of broad nature in case of Co-L.4H_2O such finding suggests that the carboxyl group takes part in the cobalt complex through deprotonation [8].

It was reported that the metal-sulphur. Stretching frequencies lie within the range 480 - 210 cm^{-1} [9]). In most of the metal complexes possible coupling can occur. This can be attributed to γ (M-S) ring deformation [10].
In many instances two bands are observed: one of medium to strong intensity and a weaker band at frequency 10 - 40 cm $^{-1}$ lower than the stronger band. However, the frequency of γ (M-S) is not very sensitive to the atomic mass of M .

The monothio-β-diketone complexes of a wide range of bi- and tri valent metals [11, 12] display γ (M-S) within the range 399 - 376 cm • Further-more, There is no marked difference between thiolo complexes and thio ether complexes in regard to the frequency of γ (M-S) [13].

The nitrogen atom tends to lower the solubility of the complexes in non- solvents [14]. So the complexes of sulphur-nitrogen ligands are ingenetal, either sparingly soluble or insoluble in non-polar solvents [15]. From the sparse data available, sulphur-nitrogen ligands appear to give rise to a smaller reduction, in -the interelectronic repulsion energy than sulphur-sulphur Ligands. This presumably is due to that the nitrogen atom having a low position compared to sulphur atom in the nephelauxetic series [16]. Also, the metal-nitrogen stretching frequencies can occur over a wide range, viz. from 600 to below 200 cm^{-1}. In nickel complexes of an NSN tridentate, γ(M- N) and γ (M-S) were reported to occur at 415 - 412 and 328 - 326 cm^{-1} ,respectively.

5- The band Located at 945 cm^{-1} in the free ligand could be assigned to dimeric structure [17]. Such band is absent in the prepared complexes except for Cul.2H_2O complex. In general, The data of the such ligand illustrate its bidentate and tridentate natures in the cobalt and both the copper and nickel complexes, respectively.

Based on the I.R data of the fundamental groups(S-H, NH_2 and COOH) and the data obtained from the electronic measurements gathered with the elemental analysis,

The structure of the prepared complexes could be suggested to be as follows:

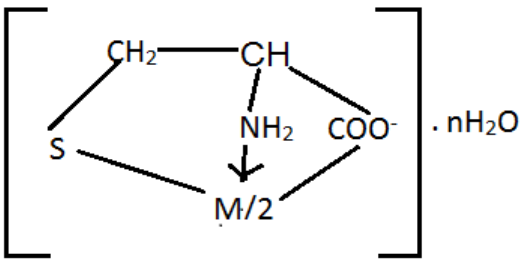

Table 2 Fundamental infrared band (cm^{-1}) for cysteine complexes

NH2	M–N	M–O	S–M	C=O	OH(H2O)	Complex
3027	460	600	500	1755	3405	[Pb(L)2].2H2O
3117	614	777	541	1793	3881	[Fe(L)2].2H2O
3052	467	800	555	1890	3887	[Cd(L)2].4H2O
3075	511	726	575	1734	3356	[Mn(L)2].2H2O

Similarly, by comparing the infrared spectra of the cystine ligand to that of the prepared complexes, the following points are obtained:

1- The bands located in the frequency range 3100 - 3160 cm^{-1} for the free ligand assigned to the stretching frequency of NH2 group are shifted to higher frequency in all the metal comp lexes, the NH_2 group , also, shifted to higher wave numbers on complexation , Table (3) .

2-The band at 1620cm^{-1} in the free ligand assigned as (N-H) of the amino group [5] is red in all metal complexes suggesting metal-nitrogen coordination.

3-The carboxyl group in the ligand is identified by the presence of two vibrational bands at 1580 and 1340 cm^{-1} assigned as asymmetric and symmetric stretching vibrations [6] , respectively. The first band is completely disappeared in all metal complexes while the second one is shifted to higher frequency. The data indicate that the carboxylate group acts as a centre complexation.

4 - The ωNH and ρNH_2 of the free ligands are affected on complexation (Table 3).This confirms again that the amino group coordinates to the metal.

5- The new bands which appeared in the spectra of yhe complexes at 320 , 330 and 340 cm^{-1} [18 ,19,20 and 21].

6- No sign for M-S bands is apparent.

From the spectral data the structure of the prepared complexes are suggested as follows:

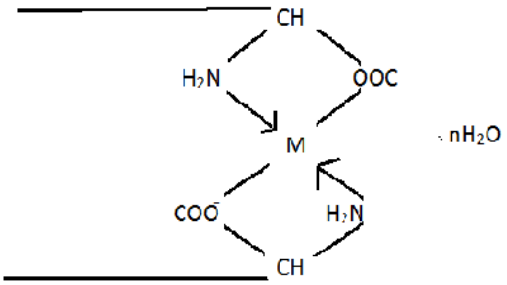

Table 3 Fundamental infrared band (cm-1) for cystine complexes

NH2	M–N	M–O	S–M	C=O	OH(H2O)	Complex
3500	530	776	-	1765	3025	$[Pb(L)].2H_2O$
3023	540	675	-	1800	2900	$[Fe(L)].2H_2O$
3015	540	673	-	1807	3013	$[Cd(L)].4H_2O$
3370	541	676	-	1729	3000	$[Mn(L)].2H_2O$

For the methionine systems (**Table 4**) the following conclusions are achieved:

1- The broad band at 3490 and 3400 cm^{-1} in the spectra of CoL3 and NiL_2-$2H_2O$, respectively, and the medium band at 3320 cm^{-1} in the CuL_2 complex are assigned to asymmetric stretching vibration of the NH_2 group .
The appearance of a band at 3300 and 3260 cm^{-1} in case of nickel and copper complexes, respectively, can be assigned to the symmetric stretching vibration of NH_2 group. However, the NH_2 group is affected on coordination with the transition metal ions.

2-The moderate band at 1590 cm^{-1} in th e free ligand assigned[5] as δ(NH) is strongly shifted to higher frequency in all metal complexes. Again, such data are in favor to suggest that the amino group is strongly affected on coordination with the transition metal ions.

3- The two strong bands at 1510 and 1415 cm^{-1} in the free ligand are attributed to asymmetric and symmetric vibrations of the carboxyl groups. Free first band is completely absent in the cobalt and nickel complexes and strongly blue shifted as in case of the cuL2 complex. The second band is slightly shifted in both the nickel and the copper complexes and almost absent in case of the complex. The data, in general, suggest the coordination of the carboxyl group to the central metal ion through deprotonation. So, the methionine compound acts as a bidentate ligand through the nitrogen and the carboxy oxygen atoms.

4- Similarly, the NH2, NH and NH modes of vibrations of the free ligand are affected on coordination (Table 4). This, again, gives an extra confirmation to that the amino group coordinates to the metal ions.

5- No sign for M-S vibrations is present.
From such discussion gathered the possible structure of the prepared complexes could be suggested to be as follows:

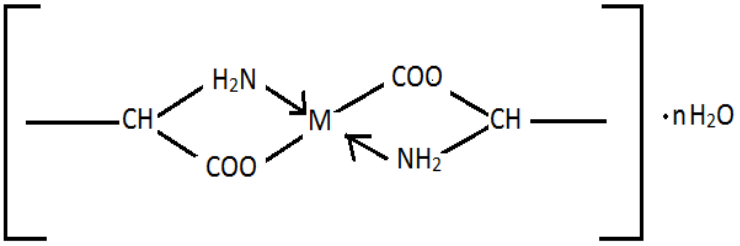

Table 4 Fundamental infrared band (cm-1) for Methionine complexes

NH_2	M–N	M–O	S–M	C=O	$OH(H_2O)$	Complexes
2985	543	667	-	1798	2622	[Fe(L)2].2H2O
3347	612	806	-	1780	2916	[Cd(L)2].4H2O
3390	516	797	-	1720	2955	[Mn(L)2].2H2O
3515	559	800	-	1736	2598	[Pb(L)2].2H2O

Thermogravimeteric analysis of some complexes:

The thermogravimeteric analysis curves allowed us to establish the temperature below which the characterized compound has a constant weight and which begins to decompose and how far the decomposition reaction can proceed The observed and stoichiometric decreases in weight of the substance make it possible to estimate an intermediate product which is formed during decomposition and at what temperature occasionally the temperature rang where this intermediate has constant weight [(23)].

Thermogravimeteric analysis for cystine complexes:

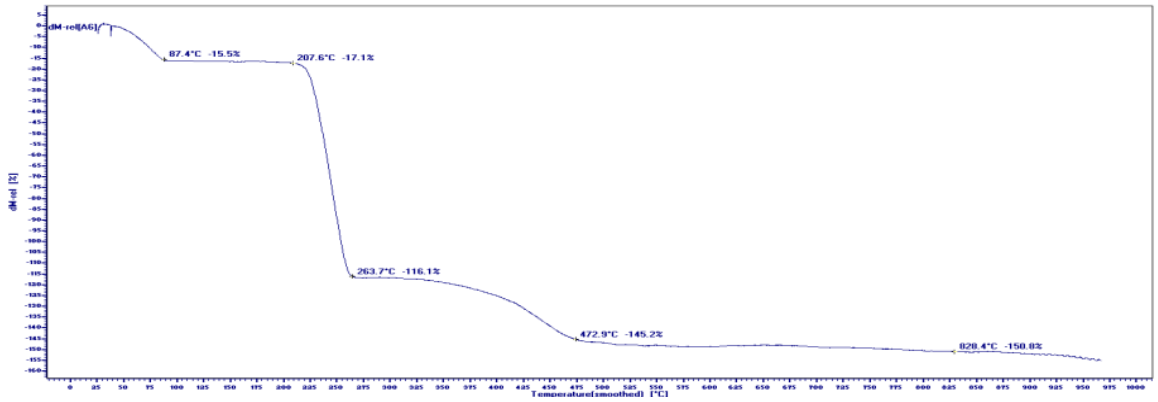

Figure 5 The thermogravimetric analysis for Fe(II)-cystine

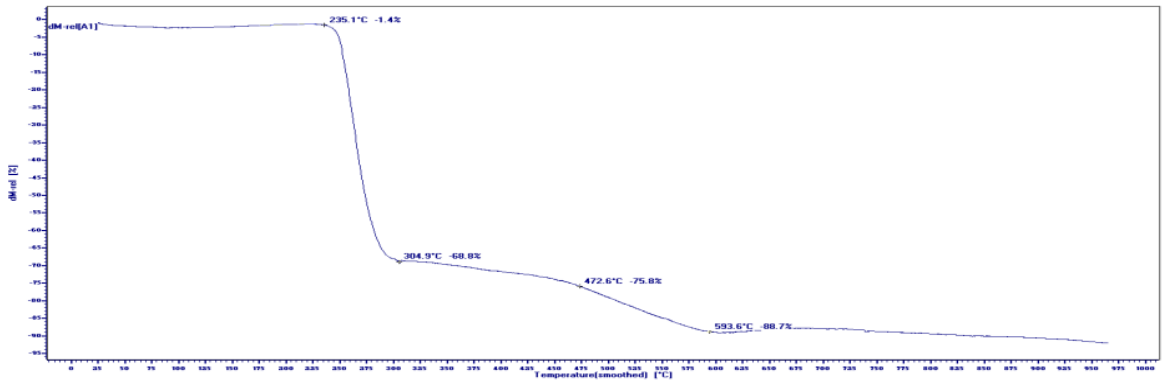

Figure 6 The thermogravimetric analysis for Mn(II)-Cystine

The thermogravimetric analysis data for cystine complexes are given in Table (5) Figures (5 -8).The weigh-loss of Fe(cys) , Mn(cys) , Cd(cys) , Pb(cys) corresponding to loss of water molecules at the temperature rang of (100 to 350 C^{O}). And the loss of CO_2 molecules occurred at the temperature range (400 to 500 ^{0}C). While the residual of metals oxides (MO_2) of (700 ^{o}C) appended at temperature for Fe(cys) , Mn(cys) , Cd(cys) , Pb(cys) respectively.

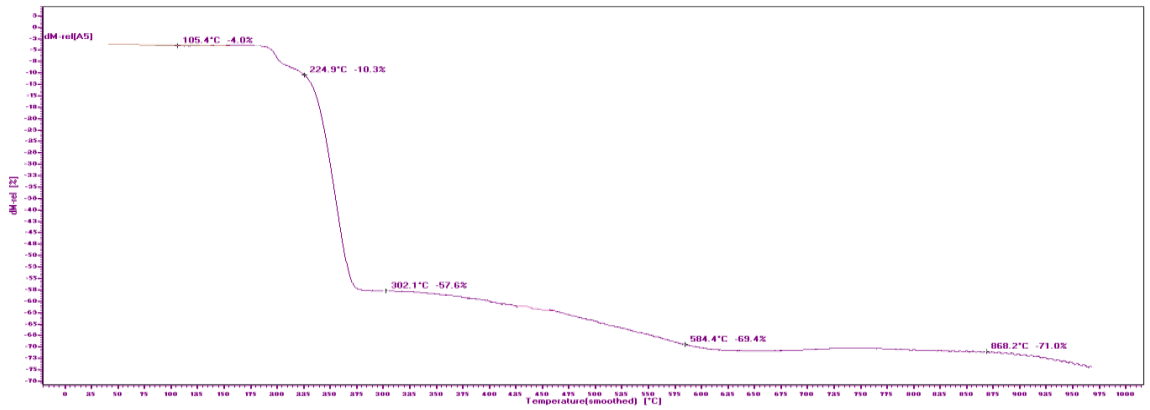

Figure 7 The thermogravimetric analysis for Cd(II)-Cystine

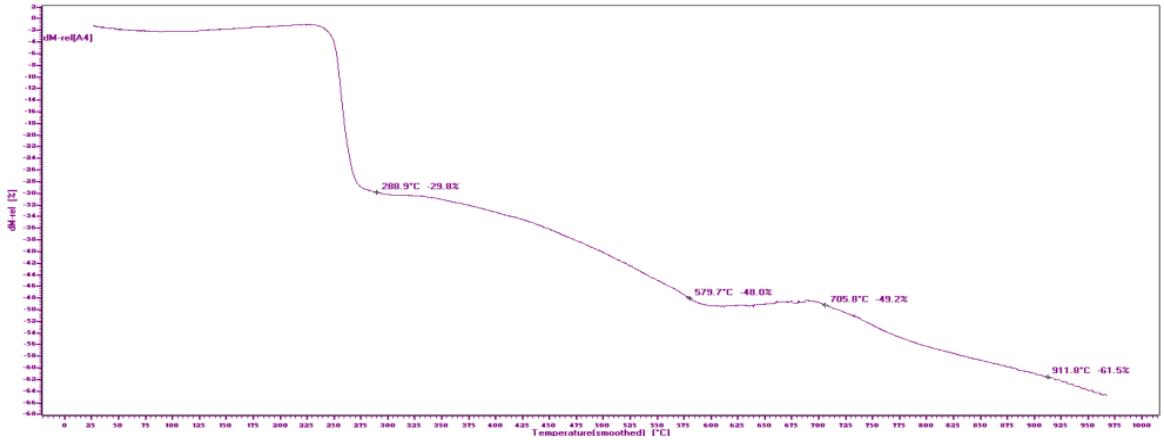

Figure 8 The thermogravimetric analysis for Pb(II)-cystine

Table 5 The thermogravimetric analysis data for cystine complexes

MO_2 Temp.rang C^{o}	***CO_2 Temp.rang C^{o}***	***H_2O Co***	***H_2O Temp .rang C^{o}***	**Decomposition *Compexes***
828.4	472.9	263.7	207	Fe(II)-Cystine
593.6	472.6	304.9	235.1	Mn(II)-Cystine
868.2	584.4	302.1	224.9	Cd(II)-Cystine
705.8	579.7	288.9	245	Pb(II)-Cystine

The thermogravimetric analysis for the another amino acids (cysteine and methionine) are show in tables (6) and(7) , where the first decomposition were appeared for the water molecules and lost decomposition are due to the residual of metal oxides(23).

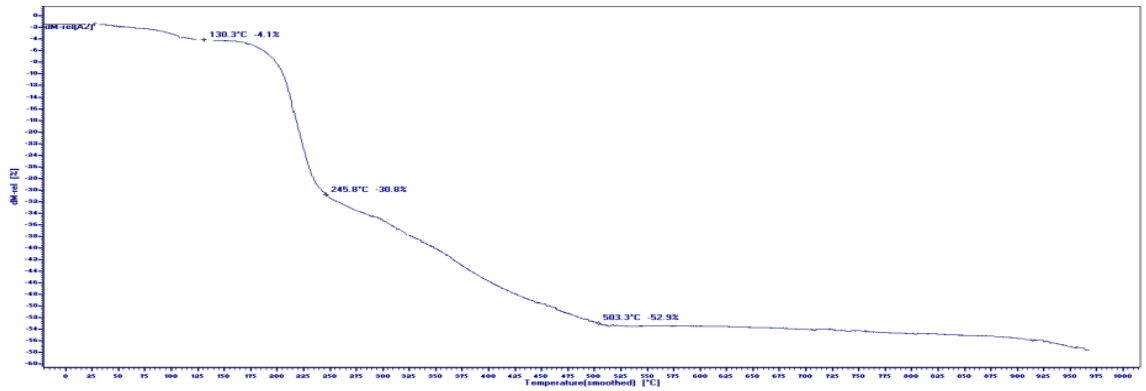

Figure 9 The thermogravimetric analysis for Fe(II)-Cysteine

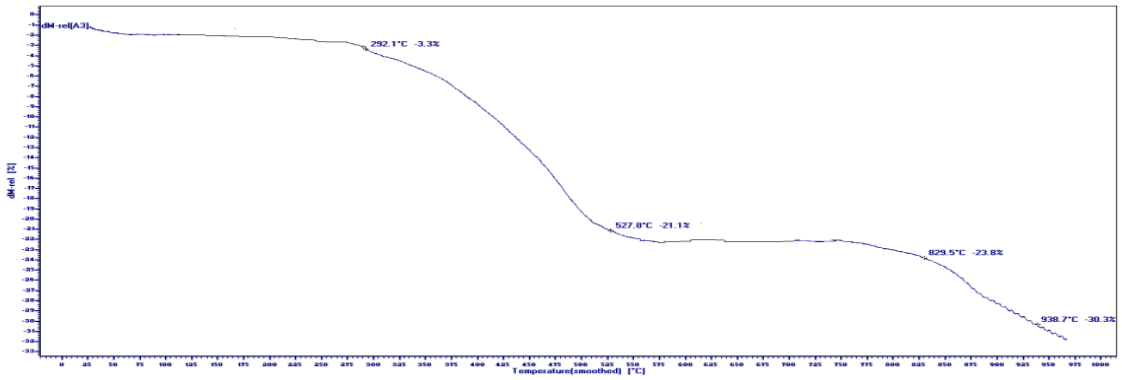

Figure 10 The thermogravimetric analysis for Mn(II)-Cysteine

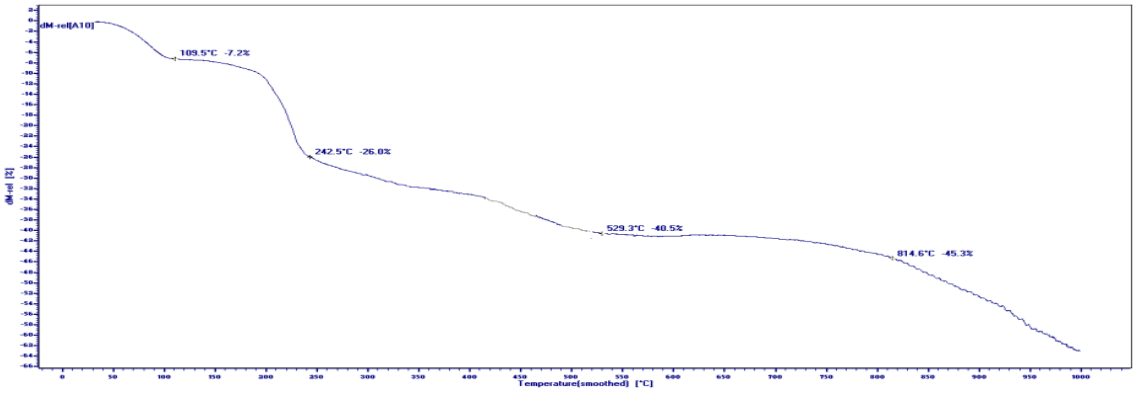

Figure 11 The thermogravimetric analysis for Cd(II)-Cysteine

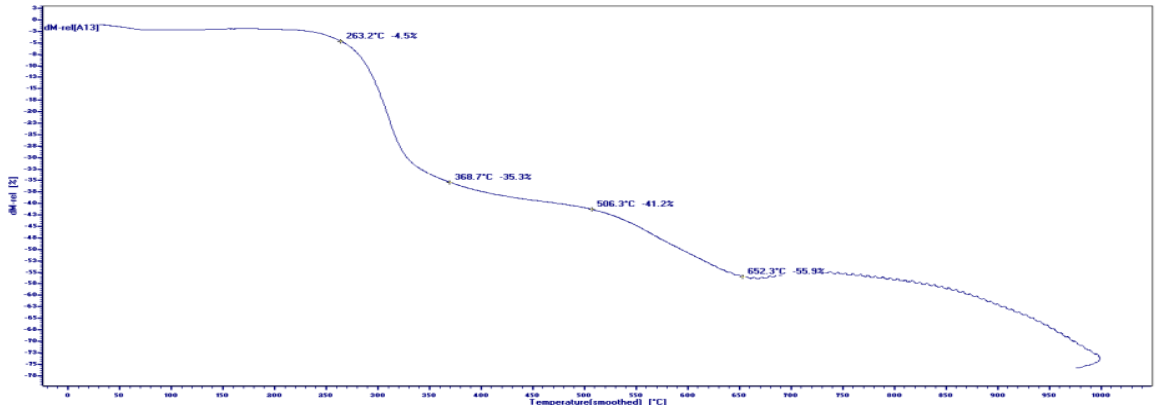

Figure 12 The thermogravimetric analysis for Pb(II)-Cysteine

Table 6 The thermogravimetric analysis data for cysteine complexes

MO_2 Temp.rang^0 C	**CO_2** Temp.rang^0 C	**H_2O Inter**	**H_2O** Temp.rang^{o}C	**Complexes**
503.3	245.8	130.3	101.4	Fe(II)-Cysteine
829.5	527.8	292.1	204.6	Mn(II)-Cysteine
814.6	529.3	242.5	109	Cd(II)-Cysteine
652.3	506.3	368.7	263.2	Pb(II)-Cysteine

Table 7 The Thermogravimetric analysis data for Methionine complexes

MO2 Temp.rang	**CO2** Temp.rang	**H2O**	**H2O** Temp.rang	**Complexes**
817.5	529.6	412.4	175.7	Fe(II)-MET
583.6	468.0	336.9	98.2	Mn(II)- MET
888.1	527.1	284.6	199.4	Cd(II)- MET
799.	641.9	260.1	176.6	Pb(II)- MET

In general the steps of the investigated complexes may be occurred as:
[ML.nH2O].nH2O → [ML.nH2O]
[ML.nH2O] → [ML] → Intermediate
Intermediate → Metal oxide MO2
Intermediate (decomposition of organic ligand).

Antimicrobial Activity (AMA):

The antimicrobial activity of 12 chemical products were tested against representatives of acid fast bacilli (M*ycobacterium phlei*), Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*), Gram-negative bacteria (*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*) and representative fungal species yeast *(Candida albicans).* Apply the agar diffusion method by cup plate technique (1tap) using trypticase soy agar for bacteria. The products were dissolved in sterile dist. water at concentration of 10 mg/ml then 100 µl were aseptically

transferred to preformed cups (100 µg/cup) in the dried inoculated Tryticase soy agar plates. The plates were incubated inverted at 37 ºC for 24 hr in case of bacteria and at 25 ºC for 48 hr in case of fungi (yeasts). After incubation, the inhibition zones were recorded in mm. Diameter less than 10 mm indicates no effect. Discs of oflocxacine (OFX) and Amphotricine B,(AMP B), 5 µg/disc each were used as a positive control) [24]. The Minimum inhibitory concentration (MIC) was determined for bacterial species tested against serial dilution of the active compounds (no. 3, 4, 5) 520, 256, 128, 64, 32, 16, 8 and 4 (µg/ml) by agar diffusion cup method. Then 100µl of each dilution was transferred in cups preformed in nutrient agar inoculated with suspension of 105/ml microbial cells on the surface of agar plates and incubated at 37ºC for 24-48 hours. After incubation the lowest concentration producing inhibition was recorded as the minimum effective concentration [25]. Only 3(Cd(II)Methionine, Cd(II)cystine, Cd(II)cysteine) of the tested products revealed significant antimicrobial effect against both of Gram-positive & Gram negative bacteria, antimycobactrial activity as well as fungi table(8) and Figures (13 -17). With respect to MIC, the 3 tested compounds showed comparable antimicrobial activity. In general, the antimicrobial activity for the tested products was higher on Gram-positive than Gram negative bacteria. For Gram-negative bacteria, *E. coli* showed higher sensitivity to the tested products (MIC ranged between 1.3 – 4 µg /ml) than *Ps. aeruginosa* (MIC ranged between 4.5 -6 µg /ml). With respect to Gram-positive bacteria, Staph. auerus and Sarcina lutea the MIC level between 1 - 2 µg /ml). In addition, the antimycobaceterial actitivity was ranged between 3.8 -4.7 µg /ml. The observed results reveled that, the tested compounds were very active as antimicrobial compared with the reference drugs (Ofloxacine and Amphotericin B), (Table 9).

Table 8 Antimicrobial activity of the tested products (inhibition zone in mm)

OFX	12	**11**	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	***MICROORGANISMS***
28	-	-	-	-	-	-	-	22	24	28	-	-	***E.coli ATCC 10536***
25		-	-	-	-	-	-	15	13	15	-	-	***Pseudomonas aeruginosa***
25		-	-	-	-	-	-	16	15	17	-	-	***PROTEUS VULGARIS***
31	-	-	-	-	-	-	-	23	26	23	-	-	***Staphylococcus aureus ATCC***
28	-	-	-	-	-	-	-	31	30	32	-	-	***Sarcina lutea****
29	-	-	-	-	-	-	-	22	23	25	-	-	***BACILLUS SUBTILIS NCTC 6633***
27	-	-	-	-	-	-	-	20	22	25	-	-	***MYCOBACTERIUM PHLEI****
-	-	-	-	-	-	-	-	17	18	24	-	-	***CANDIDA ALBICANS****

*: OFX : ofloxacine (5 µg/disc) and AMP. B: amphotricine B (5 µg/disc) positive control.
- no inhibition zone; + diameter of inhibition zone 0 mm.

1-Fe(II)Met; 2-Mn(II)Met; 3-Cd(II)Met; 4-Cd(II)cystine;

5-Cd(II)cysteine; 6-Pb(II)cystine; 7-Pb(II)Met; 8-Mn(II)cystine;

9-Fe(II)cysteine, 10-Fe(II)cystine, 11-Mn(II)cysteine , 12-Pb(II)cysteine

Table 9 Antimicrobial activity of the tested products (MIC μg/ml)

AMPB	**OFX**	**3**	**2**	**1**	**MICROORGANISMS**
-	1.5	2	1.3	4	*E.coli* ATCC 10536
-	2	6	4.5	5	*Pseudomonas aeruginosa* CNCM A21
-	2	1.5	1	1	*Staphylococcus aureus* ATCC 4175
-	2	2	1	1.5	*Sarcina lutea*
-	0.5	3.5	4	4	Bacillus subtilis NCTC 6633
-	2	4.7	3.8	4	MYCOBACTERIUM PHLEI*

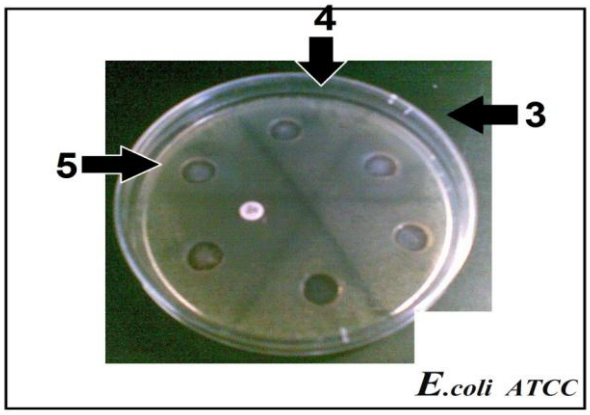

Figure 13 Effect 3-Cd(II)Met,4-Cd(II)cystine,5-Cd(II) cysteine on E. coli

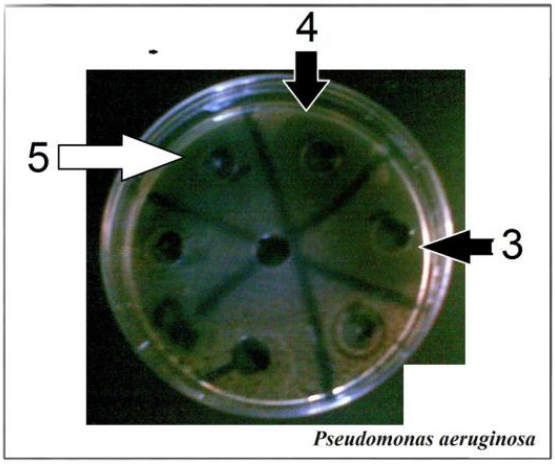

Figure 14 Effect 3-Cd(II)Met,4-Cd(II)cystine,5-Cd(II)cysteine on Pseudomonas

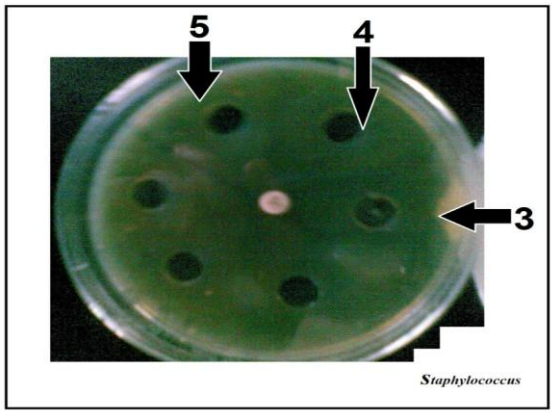

Figure 15 Effect 3-Cd(II)Met,4-Cd(II)cystine,5-Cd(II)cysteine on Staphylococcus

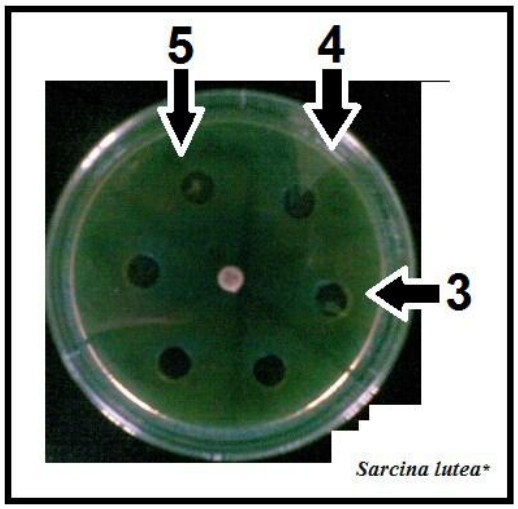

Figure 16 Effect 3-Cd(II)Met,4-Cd(II)cystine,5-Cd(II)cysteine on Sarcina lutea

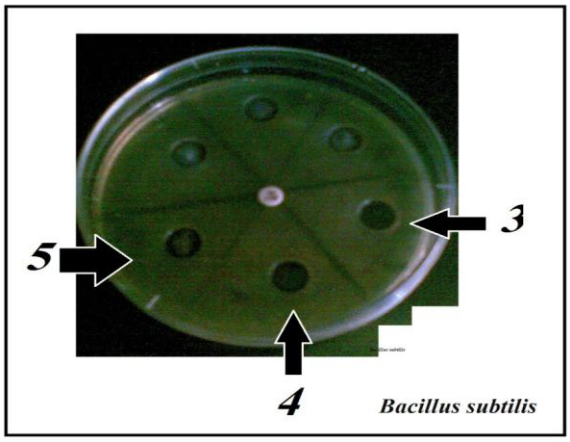

Figure 17 Effect 3-Cd(II)Met,4-Cd(II)cystine,5-Cd(II)cysteine on Bacillus subtilis

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