Original article

Synthesis, Characterization, and Biological Activity of L-Leucine and Mixed Amino Acids Complexes of Nickel (II) ion

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Abstract

Amino acid complexes of Ni^{2+} with L-Leucine and mixed amino acid of Cysteine + Leucine have been synthesized and characterized in the present study. Characterization was carried out using powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), UV/Vis-spectroscopy, Conductivity measurement, Thermo gravimetric analysis (TGA) and Energy dispersive X-ray (EDX). The ligands with Ni^{+2}ion have been found to act as bidentate chelating agents coordinating through carboxyl group (COO^-) and amino group (NH_2). The antibacterial activity of amino acid complexes was evaluated against four bacteria strains, two gram positive (*Staphylococcus aureus* and *Enterococcus feacalis),* and two gram negative *(Escherichia coli* and *Klebsiella pneumonia*). The Ni^{+2}complexes were found to have a good degree of inhibitory effect against bacteria.

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Introduction

The microbial resistance represents a problem and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, measures must be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient (Kabbani *et al.,* 2007).For centuries, people have used cobalt and other ions to inhibit the growth of harmful microbes (Egorova *et al.,* 2017).

Much attention is currently focused on the rational design and controlled synthesis of metal-organic complexes with novel topological structure because of various potential applications of complexes as function materials, catalysts and medicaments (Chauhan and Carpenter 2015; Chauhan *et al.*, 2014; Chauhan *et al*., 2015). Nowadays many of antimony (III) complexes have been clinically used because of their biological activities and drug efficacies (Lizarazo-Jaimes *et al.,* 2014; Chauhan *et al.,* 2014; Piló *et al.,* 2015; Tunç *et al.*, 2015), such as the treatment of a variety of microbial infections including *Leishmaniasis*, parasitic diseases, diarrhea, peptic ulcers, *Helicobacter pylori* ...etc. Herbal treatment was the most common in the provinces of Sudan. In recent years more attention was put on plants' based traditional medicine and antibacterial study (Saif *et al.,* 2017 and Abdalrahman *et al.,* 2016).This study was proposed to create a new antibiotic from amino acids and nickel ion and to

form more stable complexes that have a substantial biological activity, and to minimize the side effects associated with traditional antibiotics.

Material and methods

Chemicals and reagents

The chemicals used in the synthesis complexes are, Ni^{2+} chloride hexahydrate, sodium hydroxide, Leucine and Cysteine, distilled water and deionized water, absolute ethanol, formic acid.

Instruments used: IR spectrometer (FTIR), UV/Vis-Spectrometer, Conduct meter, Thermo gravimetric analysis (TGA), X-Ray Diffraction (XRD), Energy dispersive X-ray (EDX).

Method

Nick amino acids synthesis

There were synthesized two amino acid complexes with Ni^{2+}ions using as ligands Leucine, and Cysteine + Leucine (Ni.Leu, and Ni. (Cys+Leu), respectively. The solid complexes, $ML_2.nH_2O$ and $MLL'.nH_2O$types, were prepared following the procedure described in the literature, (Stanila *et al.,* 2007). 1:2 molar ratio (aqueous solution) of metal chlorides and ligands (leucine, and cysteine), the pH of solution was adjusted to about 8-10 using sodium hydroxide, the mixture of solution were mixed under stirring about one hour in room temperature until the precipitate was formed, filtered off, dried and kept in a desiccator over anhydrous $CaCl_2$.

Physical Measurements

The conductance was measured in formic acid (10^{-3} M) on ELICO digital conductivity meter at room temperature, EDX analysis were recorded by using LEOS430 scanning, electron microscope coupled with energy dispersive X-ray analyzer model Oxford LINK ISIS, TGA was carried out on a Perkin–Elmer model TGS-2 instrument in temperature range (0-500ºC), IR spectra were recorded on Fourier-Transform (FT.IR) Spectrophotometer, Tensor 27 Co. Brucker 2003 at a range (400-4000 cm^{-1}) using KBr discs, Electronic spectra were recorded on a UV-Vis. Spectrophotometer (Shimadzu, UV-1650PCb Spectrophotometer) using formic acid as a solvent at room temperature, and X-ray diffraction data were recorded on Philips PW 3710 diffractometer attached to digitized computer along with graphical assembly in which radiation source was connected with the tube Cu-K α ,25 Kv/20 mA. The scan range was between 3 and 80º 2θ.The X-ray pattern have been indexed using computer software (PowdMoult 2.3 version)),and the lattice constants a, b and c, Inter axialangle α, β, and γ for unit cell, molecular formula, molecular weight, and density values, of the two complexes Ni.Leu, and Ni.Asn.Leu (Chohan *et al.,* 1998). The results were recorded in table (5).

Microbial strains

Standard strains of microorganism used in this study and were obtained from The National Health Laboratory and Management of laboratories, Khartoum. The bacteria species used were *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus feacalis.* Bacteria were grown in Mueller Hinton Agar.

Antibacterial assay

Antibacterial activity of Ni^{2+} amino acid complexes was evaluated by disc diffusion method (Kil *et al.,* 2009). Ni^{2+} amino acid complexes solution (100 mg/ml) stock solution were prepared by diluting with 5% formic acid. The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10, 20, 30, 40 µl of Ni^{2+} amino acid complexes, separately, followed by drying off. Formic acid was used as a negative control, while gentamicin (10µg/disc) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Diameters of clear zone of inhibition produced around the discs were measured and recorded

Results and Discussion

Physical analysis

Molar conductivity of the dithiocarbamate complexes were measured in dimetylformamide (10^{-3}M) solvent indicates that theses complexes are 1:1 electrolyte, (Geary, 2014). The

corresponding nickel complexes, are soluble in formic acid but insoluble in THF, cyanomethane, benzene, dichloromethane, chloroform, DMSO, DMF+DMSO mixture and ethanol. Accordingly, the conductivity of the two complexes, were measured in 20% formic acid and the results obtain, indicate that all ions involved in complex formation were coordinated with Ni^{2+} ion, in the other word, two groups coordinated by negative charge and the other groups coordinated by lone pair electrons.

Energy dispersive x-ray spectra (EDX) study

The EDX profile of Ni.leu, and Ni. (Cys+Leu) complexes confirmed the presence of O, Ni, C, N. the prominent nitrogen and oxygen clearly suggests to the functional group of amino acid ligand and Ni peak presence indicated to formation of complexes, and the percentage of each atom give the number of ions in complex. These results had been summarized in table (1), and (2) for Ni.leu. The number of atoms was calculated according to their percentages shown by the apparatus whereNi.leu complex contains 12, 6, 1,and 2 ions of C, O, Ni, and N, respectively. In addition, Ni. Cys. Leu complex contains 9, 6, 1, 1, and 2 ions of C, O, Ni, S, and N, respectively.

Table (1) EDX (Energy dispersive X-ray) readings of Ni.Leu complex

δ3	Result (%)	Element
0.291	43.29	C
0.196	28.87	O
0.218	17.67	Ni
0.109	8.40	N
0.242	1.824	Trace element

Table (2). EDX readings of Ni.Cys.Leu complex

δ3	Result	Element
0.288	32.01	C
0.234	24.01	O
0.213	19.568	Ni
0.218	10.66	S
0.134	9.347	N
0.045	4.416	Trace element

Thermal analysis

Thermal Gravimetric Analysis (TGA) for Ni.Leu, and Ni.(Cys+Leu), complexes (Table (3), revealed identical characteristic bands and little differences in temperature and weight loss. TGA curve for the two complexes displays two stages of mass loss within the temperature range of 100–500 °C. The first stage is at 120–180°C, corresponding to the dehydration of two moles of crystal water and the second stage occurs approximately at 200–500 °C. According to (Rosu *et al.,* 2009), such phenomenon was caused in the Ni.leu and Ni.cys.leu TGA complexes.11% weight loss was reached for Ni.leu complex in temperature range 156-188ºC in the first stage, which corresponds to dehydration of the two moles of crystal water. The second stage involved weight loss of32% in temperature range 268-290 ºC, which corresponds to evaporation of CO_2, and NH_3 gases from the amino acid. The drop in weight associated with temperature range 472^0C was due to melting of Ni.Leu complex.

For Ni.cys.leu complex the weight loss of 11% was reached in the 1st stage at temperature range 119-160ºC corresponding to two moles of crystal water and 27% at 220-248 ºC in the 2nd stage. Another peak was evident (13%) at 280-300^0C also, which corresponds to evaporation carbon dioxide CO_2, and ammonium NH_3 gases from the amino acid. These results indicated the presence of two peaks in the second stage resulting from mixed amino acid.

Table (3) TGA of Ni.Leu, and Ni.(Cys+Leu)complexes

Ni.(Cys+Leu)		Ni.Leu	
Weight loss (mg)	Temp (°C)	Weight loss (mg)	Temp(°C)
4.80	119160	0.55	156-188
4.41	220-248	1.60	268-290
4.10	248-300	Melted	>472
Steady decrease	>300		

Infrared spectra

Table (4) describes the infrared spectra of the Ni^{2+} complexes. Ni.leu complex spectra exhibit the observed frequencies ν_{asy}(COO-) was at 1668.31 cm-1 and the ν_{sy}(COO^-) at 1359.72 cm^{-1} are fairly in good agreement with the literature (Islam, *et al.,* 2018). The absence of the uncoordinated *ν*COOH (1730-1775 cm^{-1}) in the IR spectra indicates a clue for the coordination of the ligands to metal ions through the carboxylate anions (Islam *et al.,* 2018). The bands assigned due to the *ν*Ni-N at 447.45 cm^{-1}also fairly resemble to the literature (Temitayo *et al.,* 2012, Islam, *et al.,* 2018). The participation of the lone pairs of electrons on the N of the amino group in the ligand to the metals is supported by this band frequency (Osunlaja *et al.,* 2009, Islam, *et al.,* 2018) observed the chelation of the Ni^{2+} by the amino and carboxylate groups, The N-H stretching frequency at around 3444.8 cm-1 reduced on coordination, attributable to the reduction in bond order on coordination (Nakamoto 2009 and Islam *et al.,* 2018). *ν*OH stretching bands at around 3637.50 cm-1 which eventually indicate the presence of lattice water molecule. and*ν*N-H bending bands at aroundν_{asy}1595.09 cm-1 and ν_{sy} at around 1382.87. (Islam, *et al.,* 2018).For Ni.cys.leu complex spectra exhibit the observed frequencies ν_{asy}(COO^-) was at 1668.31cm^{-1} and the ν_{sy}(COO^-) at 1400.22cm^{-1} are fairly in good agreement with the literature (Islam, *et al.,* 2018). The absences of the uncoordinated νCOOH (1730 - 1775 cm^{-1}) in the IR spectra indicate a clue for the coordination of the ligands to metal ions through the carboxylate anions (Islam *et al.,* 2018). The bands assigned due to the *ν*Ni-N at 443.60cm^{-1} are also fairly resembling to the literature (Aiyelabola *et al.,* 2012) and (Islam *et al.,* 2018). The participation of the lone pairs of electrons on the N of the amino group in the ligand to the metals is supported by this band frequency (Osunlaja *et al.,* 2009 and Islam *et al.,* 2018) observed the chelation of the Ni^{2+} by the amino and carboxylate groups, The –NH stretching frequency at around 3427.27cm^{-1} reduced on coordination, attributable to the reduction in bond order on coordination (Nakamoto 2009 and Islam, *et al.,* 2018). *ν*OH stretching bands at around 3639.43cm^{-1} which eventually indicate the presence of lattice water molecule, and *ν*N-H bending bands at around ν_{asy}1596.95 cm^{-1} and ν_{sy} at around 1504.37Cm^{-1} (Islam *et al.,* 2018).

UV/Vis spectra study

The electronic distribution of Ni^{2+} ion (d^8) is $t_2g_6eg^2$which gives rise to 3F, 3P, 1D and 1S terms of which 3F is the ground state. In a cubic crystal, these terms transform as follows:

$$^3F \rightarrow {}^3T_1g(F) + {}^3T_2g(F) + {}^3A_2g\ (F)$$

$$^3F \rightarrow {}^3T_1g(P)$$

$$^1D \rightarrow {}^3T_2g(D) + {}^1Eg(D)$$

$$^1G \rightarrow {}^1T_1g(G) + {}^1T_2g(G) + {}^1Eg(G) + {}^1A_1g(G)$$

$$^1S \rightarrow {}^1A_1g(S)$$

Of these crystal field terms, $^3A_2g(F)$ is the ground state. Hence three spin allowed transitions are possible and the others are spin forbidden. The three spin allowed transitions are:

$^3A_2g(F) \rightarrow {}^3T_1g(P)$, $^3A_2g(F) \rightarrow {}^3T_1g(F)$ and $^3A_2g(F) \rightarrow {}^3T_2g(F)$.

And some absorption bands are observed which are attributed to charge transfer from the non-bonding orbitals of the oxygen atoms in the ligand to the Ni^{2+}d orbitals. The last absorption bands are assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the ligand (Nassirinia *et al.,* 2008 and Rodríguez *et al.,* 2009). Figures (3), and (4) illustrates the assignment of the characteristic wave lengths of the electronic transition of Ni.Leu and Ni.(Cys.Leu),

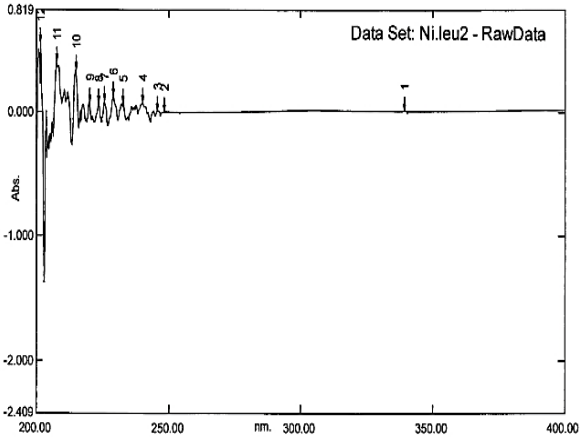


Figure (3) UV spectrum of Ni.Leu complex

Table (4) IR-Spectra of Ni.Leu, and Ni.(Cys+Leu) complexes

Compound	COO^-		NH_3^+			M-O	M-N	O-H
	V_{asy}	V_{sy}	$Str_{(broad)}$	$Bend_{asy}$	$Bend_{sy}$			
Ni.Leu	1668.31	1359.72	3444.83	1595.09	1382.87	682.75	447.45	3637.50
Ni.Cys.Leu	1668.31	1400.22	3427.27	1596.95	1504.37	516.89	443.60	3639.43

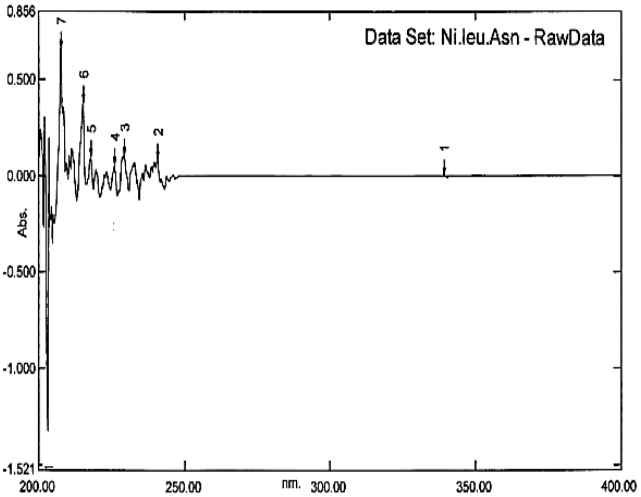


Figure (4) UV spectrum of Ni.Asn.Leu complex

X-ray Diffraction (XRD) study

The X-ray diffraction was performed to obtain further evidence about the structure of the metal complexes. The diffractogram of the coordination Ni (II) complex records 10 reflections between 10 to 80º (2 θ) with maxima reflection at 2 θ (10.14º) which corresponds to d(8.728Aº).These results were used to confirm the structure of Ni.Leu and Ni.Cys.Leu complexes.

		Ni.Leu	Ni.Cys.Leu
Molecular formula		$C_{12}H_{28}$ Ni N_2 O_6	C_9H_{22} Ni N_2 O_6 S
Crystal system		monoclinic	Monoclinic
Lattice constant	a	9.4390 Å	9.2340 Å
	b	9.998 Å	9.563 Å
	c	9.0076 Å	8.5676 Å
Inter axial angle	α	78.250º	79.75º
	β	104 º	91 º
	γ	119º	92.54º
Molecular weight		354.69	286.69
Density per g/cm3		1.039	0.840

Table (5). XRD study of Ni.Leu and Ni.Cys.Leu complexes

Biological Studies

The antibacterial activity of amino acids complexes was investigated against isolated gram positive strain (*Staphylococcus aureus* and *Enterococcus feacalis*), and gram negative (*Escherichia coli, Klebsiella pneumonia*). And take some commercial antibiotics sensitivity as standard drug, which were amoxyclav, gentamicin, cefotaxime, vancomycin, ciprofloxacin, co-trimoxazole, ceftriaxone, and ampicillin. The results presented in Table (2) and figure (1), showed various degrees in antibiotic resistance. All bacterial species showed resistance to amoxyclav, cefotaxime, vancomycin (except *S. aureus*), and ampicillin. Among the tested bacterial isolates, the strongest antibacterial activities of antibiotics were obtained by vancomycin and gentamicin against *S. aureus*, with inhibition zones of 22 and 19 mm, respectively, (Saif *et al.,* 2017). In Sudan, plant-based traditional medicine represents primary health care. The extracts of *Capparis decidua* L. twigs were found to have an antibacterial effects with different degrees of inhibition profiles against tested bacteria (Abdalrahman *et al.,* 2016). The ethyl acetate extract showed the highest activity against *S. aureus* (21 mm), *B. subtilis* (20 mm) and *P. pneumonia* (18 mm) while the n-butanol extract displayed best inhibition against *P. pneumoniae* (18 mm) and *E. coli* (16 mm). All extracts showed high antifungal activity against *A. niger* and *C. albicans* with inhibition zone ranged from 17 to 22 mm. The Ni^{2+}complexes of amino acid have inhibitory effect against

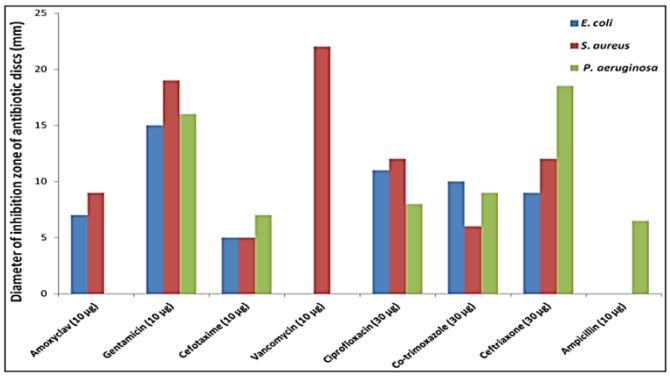
gram positive and gram negative. The sensitivity of these complexes was determined by measuring the diameter of inhibition zone that occurred and presented as mean value (X′) of four different concentrations of complexes. The sensitivity was then evaluated according to the following: (>12mm ≡ R), (12-15mm ≡ I), (16-22mm ≡ S) and (<22mm ≡ HS).

Two bacteria (*Escherichia coli, and Staphylococcus aure*us showed resistance to Ni^{2+} complexes, while the Ni^{2+} complexes have the strongest antibacterial activity toward *Pseudomonas aeruginosa*, and *Klebsiella pneumonia,* from the standard drug as used. This was illustrated in table (6), and figure (6), and (7). Each of the compounds was tested four times and the average data were recorded .

Table (6). Antibacterial sensitivity pattern of the test bacteria against 8 antibiotics using disc diffusion technique

Antibiotic	Diameter of inhibition zone of antibiotic discs (mm)		
	E. coli	*S. aureus*	*P. aeruginosa*
Amoxyclav (10 µg)	7 (R)	9 (R)	0 (R)
Gentamicin (10 µg)	15 (I)	19 (S)	16 (S)
Cefotaxime (10 µg)	5 (R)	5 (R)	7 (R)
Vancomycin (10 µg)	0 (R)	22 (S)	0 (R)
Ciprofloxacin (30 µg)	11 (R)	12 (R)	8 (R)
Co-trimoxazole (30 µg)	10 (R)	6 (R)	9 (R)
Ceftriaxone (30 µg)	9 (R)	12 (R)	18.5 (I)
Ampicillin (10 µg)	(0) R	(0) R	(6.5) R

S, sensitive; R, resistant; I, intermediate



Figure(5). Antibacterial sensitivity pattern of the test bacteria against 8 antibiotics using disc diffusion technique

Table (7). Amino acid complexes against the tested bacteria

complex / Type of bacteria	Ni.Leu		Ni.Cys.Leu	
	X′ ± SD	**Sensitivity**	X′ ± SD	**Sensitivity**
Escherichia coli	8.5 ± 0.00	R	-	R
Staphylococcus aure	7.5± 0.45	R	-	R
Pseudomonas aeruginosa	21.5± 0.34	HS	24.5 ± 0.59	HS
Klebsiella pneumonia	25.00± 0.26	HS	27.5± 0.59	HS

R, resistant; HS, high sensitivity

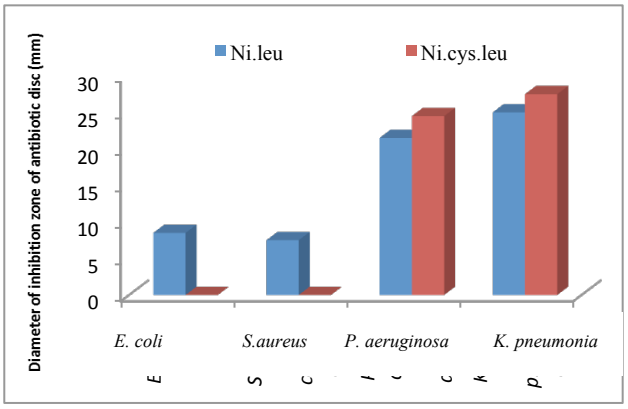


Figure (6). Antibacterial sensitivity pattern of the test bacteria against Ni^{2+} complexesusing disc diffusion technique

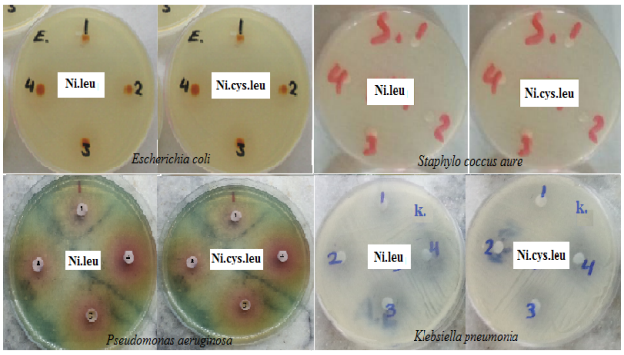


Figure (7). Antibacterial activity of Ni.leu and Ni.cys.leu complexes against thetested bacteria

Conclusions

*The Electrical conductivity of organometallic compounds

can provide information about the number of ions involvement in the complexes in solution, the electrical conductivity measurements were recorded for 2 mg/ml of complex solutions.

*The Ni.Leu, and Ni.Cys.Leu complexes are non-electrolyte solution, (neutral complex) this indicated that coordination to Ni through the two carboxyl groups COO^- and two amino groups NH_2, this proposal was supported by the IR-spectra of the complexes.

*The thermal Gravimetric Analysis (TGA) study forNi.Leu, and Ni.(Cys+Leu), complexes showed weight loss by 11% in temperature range 110-190ºC, this indicates that the complexes contain two molecules of water crystal. This proposal was supported by the IR-spectra, EDX, and XRD analysis of the complexes.

The antibacterial activity of amino acids complexes was investigated against isolated gram positive strain (*Staphylococcus aureas, Enterococcus feacalis*), and gram negative (*Escherichia coli, Klebsiella pneumonia*), two complexes showed resistanceto *Escherichia coli,* and *Staphylococcus aureas,* and high and more sensitivity than standard drug were used to*Pseudomonas aeruginosa,* and *Klebsiella pneumonia.*

As a result as the complexes form are $[Ni(leu)_2.(H_2O)_2]$ (a), and $[Ni(cys)(leu).(H_2O)_2]$ (b), respectively. This illustrated in Fig. (8).

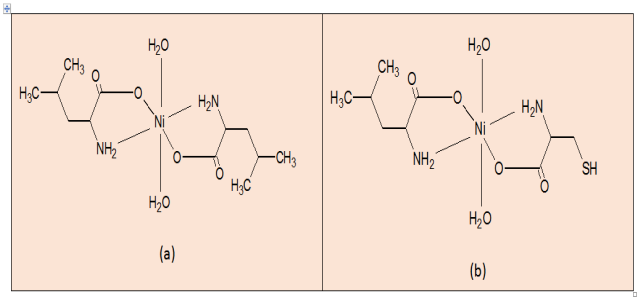


Figure (8) Structure of Ni.Leu (a), and Ni.Cys.Leu (b) complexes

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