

## 氨三乙酸、缬氨酸、亮氨酸的三元过渡金属配合物:合成和生物学应用

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**摘要:** 以氨三乙酸 HNTA<sup>2-</sup>为主要配体,缬氨酸(valine)或亮氨酸(leucine)为次要配体在微酸性介质中合成了 Ni(II), Cu(II), and Zn(II)的三元配合物。用元素分析、热分析、FTIR, UV-Vis 分光光度法,磁性测量和质谱法表征了合成的三元配合物。结果表明,三元配合物可在金属(M):氨三乙酸(HNTA):缬氨酸(valine)或亮氨酸(leucine)=1:1:1 时制得,其分子结构为 [M(HNTA)(valine)(H<sub>2</sub>O)<sub>2</sub>]·1.5H<sub>2</sub>O and [M(HNTA)(leucine)(H<sub>2</sub>O)<sub>2</sub>]·1.5H<sub>2</sub>O(其中 M=Ni(II) or Cu(II)和 H<sub>2</sub>[Zn(NTA)(valine)(H<sub>2</sub>O)]H<sub>2</sub>O。标题三元过渡金属配合物为八面体对称构型。同时研究了该三元配合物对大肠杆菌,金黄色葡萄球菌,白色念珠菌,黄曲霉菌(菌株从开罗大学理学院微分析中心获得)的抗菌活性。根据推荐的知名方法用标准的抗菌和抗真菌剂进行体外测试(in vitro)以评估我们的新制备的配合物对细菌和真菌物种的生长抑制活性。

**关键词:** 缬氨酸; 亮氨酸; 三元配合物; 生物学应用

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## Ternary Transition Metal Complexes of Nitrilotriacetic Acid and Valine or Leucine: Synthesis and Biological Applications

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**Abstract:** Ternary complexes of Ni(II), Cu(II), and Zn(II) with nitrilotriacetic acid HNTA<sup>2-</sup> as a primary ligand and valine or leucine as a secondary ligand were synthesized in slightly acidic medium. The new complexes were characterized by elemental analysis, FTIR, UV-Vis spectroscopy, thermal analysis, magnetic measurements and mass spectroscopy. The results indicate that the ternary complexes are isolated in M:HNTA:valine (or leucine) ratio of 1:1:1, and the molecular structures are suggested to be [M(HNTA)(valine)(H<sub>2</sub>O)<sub>2</sub>]·1.5H<sub>2</sub>O and [M(HNTA)(leucine)(H<sub>2</sub>O)<sub>2</sub>]·1.5H<sub>2</sub>O, where M=Ni(II) or Cu(II) and H<sub>2</sub>[Zn(NTA)(valine)(H<sub>2</sub>O)]H<sub>2</sub>O. The suggested geometry of our ternary metal complexes is an octahedral symmetry. The antimicrobial activities of prepared complexes against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus flavus* (isolates from Microanalytical Center, Faculty of Science, Cairo University) were also investigated. The complexes were tested in vitro to assess growth inhibitory activity against the bacterial and fungal species with standard antibacterial and antifungal agents according to the recommended known method.

**Key words:** valine; leucine; ternary complexes; biological applications

## 0 Introduction

Ternary complexes of metal (II) containing nitrogen and oxygen-donor ligands have received considerable attention newly because they could achieve exceptionally high stability<sup>[1-3]</sup>. In the other hand, ternary metal complexes have been recently studied due to their power as metal systems for metal-protein complexes such as metalloenzymes. They have been received particular attention and have been used in cartographic protein surfaces<sup>[4]</sup> for biological redox centers<sup>[5]</sup> and in protein pick up for both purification<sup>[6]</sup> and studying<sup>[7-8]</sup>.

As a strong example for ternary metal complexes, the high binding affinity of  $\text{Ni}^{2+}$  to multiple histidine (His) sites is employed to label His-tagged PsbH protein (protein belongs to a group of small protein subunits of photosystem II (PSII) complex)<sup>[9]</sup>.

On the other hand, nickel plays a key role in methane formation where nitrilotriacetic acid (NTA) improves the methane production because NTA favors the dissolution of nickel from their carbonates and sulphides. Thus a direct uptake of the complex NTA-Ni might have occurred and methane production is significantly improved<sup>[10]</sup>.

Also Ternary complexes of cobalt (II) with nitrilotriacetic acid as a primary ligand and some selected mono- and dicarboxylic acids as secondary ligands had been synthesized. These complexes were formed in a stepwise mechanism<sup>[11]</sup>.

NTA has numerous commercial applications as a metal ion chelator, including principally its use in cleaning products, industrial water treatment, textile preparation, metal finishing and in agricultural herbicide formulations and micronutrient solutions. It has also been used in the pulp and paper industry. In addition, some metal NTA complexes have been used in the rubber processing, photographic products, the electrochemical industry, the tanning of leather, and in cosmetics<sup>[12-13]</sup>. Moreover, it has been evaluated as a soil additive in the phytoremediation of heavy metal contaminated soil<sup>[14]</sup>. The chelation of the metals with nitrilotriacetic acid is to mo metals for more rapid uptake by plants. Nitrilotriacetic acid has been

suggested for use as a therapeutic chelating agent for the treatment of manganese poisoning<sup>[15]</sup> and iron overloading<sup>[16]</sup>.

For the great biological applications, much of the studies on the coordination of nitrilotriacetic acid with transition metal to form binary or ternary complexes has been done in neutral or slightly basic media, where such ligands are in the ionized form ( $\text{NTA}^{3-}$ )<sup>[17-20]</sup>.

The amino ternary metal complexes have attracted much attention because they are useful as antibacterial agents against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Also they are used as antitumoral drugs against sarcoma and leukaemia; nutritive supplies for human and animals<sup>[21-22]</sup>. Leucine is an essential branched-chain amino acid used as a source for the synthesis of blood sugar in the liver during starvation, stress and infection to aid in healing<sup>[23]</sup>.

Synthesis, structure, DNA binding and oxidative cleavage activity of ternary (*L*-leucine/isoleucine) copper(II) complexes of heterocyclic bases have been studied<sup>[24]</sup>. Also synthesis of a new copper(II) complex with *L*-valine Schiff base and 1,10-phenanthroline [ $\text{Cu}(\text{sal-}L\text{-val})\text{phen}$ ] (*sal-}L\text{-val}*=a Schiff base derived from salicylaldehyde and *L*-valine, *phen*=1,10 phenanthroline) was studied<sup>[25]</sup>. Two new complexes of cobalt(II) and copper (II) with valine-derived Schiff bases were prepared. Biological studies for all these complexes were carried out *in vitro* for antimicrobial activity against Gram-positive, Gram-negative bacteria and human pathogenic fungi to give great results against pathogenic microorganisms<sup>[25-26]</sup>.

In the previous study, ternary complex of Co(II), Ni (II), Cu (II), and Zn (II) with nitrilotriacetic acid as a primary ligand and alanine or phenylalanine as secondary ligands in slightly acidic medium were prepared and their molecular structures were found to be  $[\text{M}(\text{HNTA})(\text{alaH})(\text{H}_2\text{O})_2]$ <sup>[27]</sup>.

In the present paper, synthesis and characterization of new Ni (II), Cu (II) and Zn (II) complexes using NTA as a primary ligand in the presence of valine or leucine as secondary ligand in slightly acidic medium are investigated. The biological

studies of the complexes as antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria and antifungal activity toward *Aspergillus flavus* and *Candida albicans* fungi are done.

## 1 Experimental

### 1.1 Materials and reagents

All chemicals used in this study were reagent grade and used without further purification.  $\text{H}_3\text{NTA}$ , valine, and leucine were Sigma-Aldrich products  $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$ ,  $\text{NiCO}_3 \cdot 2\text{Ni}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{CoCO}_3 \cdot 3\text{Co}(\text{OH})_2$ ,  $\text{ZnCO}_3 \cdot 2\text{Zn}(\text{OH})_2 \cdot \text{H}_2\text{O}$  from BDH chemicals were used.

### 1.2 Synthesis of metal complexes

The calculated amounts of metal carbonate,  $\text{H}_3\text{NTA}$ , and valine or leucine to give the 1:1:1 molar ratio were mixed in 100 mL of distilled water, and the mixture was heated nearly to boiling. After the completion of the reaction absolute ethanol was added until dense precipitate was appeared. Then the precipitate was obtained by filtration, washing with alcohol and drying in an oven at 100 °C and then placing in a desiccator over night. This procedure was followed for the preparation of all the 1:1:1 complexes.

### 1.3 Biological activities

The antimicrobial activities were carried out by Kirby-Bauer technique. One *Aspergillus* species (*A. flavus*), one *Candida* species (*C. albicans*) and two Bacteria (*Escherichia coli*, *Staphylococcus aureus*) were used for this study. All isolates were from Microanalytical Center, Faculty of Science, Cairo University.

The agar used was Mueller-Hinton agar rigorously tested for composition and pH value. Nutrient agar was melted at 45 °C and inoculated by the microbial suspension (1 mL/100 mL). 100  $\mu\text{L}$  of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Plates were inoculated with filamentous fungi as *Aspergillus flavus* at 25 °C for 48 h; Gram (+) bacteria as *Staphylococcus aureus*, and Gram (–) bacteria as *Escherichia coli* were incubated at 35~37 °C for 48 h and yeast as *Candida albicans* was incubated at 30 °C

for 48 h.

The standard discs of Tetracycline (antibacterial agent), Amphotericin B (antifungal agent) were served as positive controls for antimicrobial activity but filter discs impregnated with 10  $\mu\text{L}$  of solvent (distilled water, chloroform, DMSO) were used as a negative control.

The filter paper disc impregnated with a tested chemical was placed on the agar. The chemicals would diffuse from the disc into the agar. This diffusion will place the chemicals in the agar only around the disc. The zone of inhibition diameters was measured.

### 1.4 Instrumentation

All measurements were carried out at the microanalytical laboratories of Cairo University, Ain Shams University and the National Research Center, Cairo. C, H and N were determined by Vario El Elemental. Ni, Cu and Zn percentages were determined by atomic absorption spectrometry (AAS), using a Perkin-Elmer AAS 3100. IR spectra of the solid complexes were recorded on a Jasco FTIR-300 E Fourier Transform Infrared Spectrometer, using KBr disks in the range of 400~4 000  $\text{cm}^{-1}$  and CsI in the range of 200~630  $\text{cm}^{-1}$ . Thermogravimetric analysis was carried out using a Perkin-Elmer 7 series thermal analyzer. The measurements were carried out under nitrogen atmosphere at a heating rate of 10 °C  $\cdot$  min<sup>-1</sup>. Magnetic susceptibilities of the paramagnetic metal complexes were measured by using a magnetic susceptibility balance Johnson Mithy, Alfa products; model No MKI at room temperature. The electronic UV-Vis spectra were recorded at room temperature on a Jasco model V-550 UV/Vis spectrophotometer. Mass spectra were recorded at 350 °C and 70 eV on a GC/MS Finnigan Mat SSQ 7000 apparatus

## 2 Results and discussion

### 2.1 Elemental analysis and physical properties

Elemental analysis, physical and chemical properties of the six complexes are given in Table 1. All the prepared complexes have some common features such as thermal decomposition before melting and effervescence as evolution of carbon dioxide on the

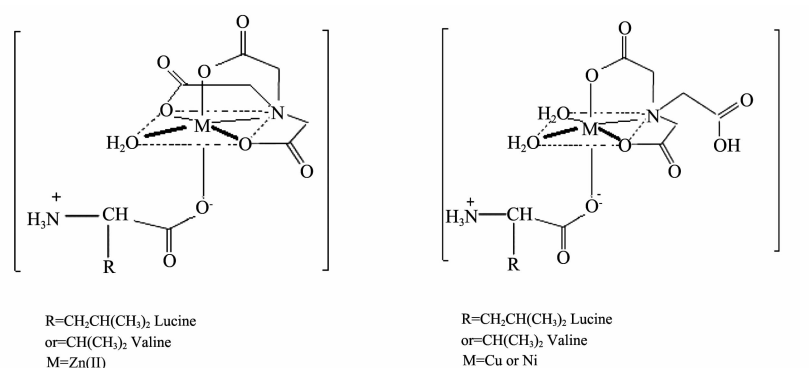
**Table 1** Elemental analysis, mass spectrometry data, and physical properties of the complexes

Complex	Color	Molar mass	$m/e$	$\mu_{\text{eff}}/\mu_B$	Elemental Analysis, Found(Calcd.) / %			
					C	H	N	Metal
1	Blue	432.54	415	2.61(val)	30.40(30.52)	6.50(6.47)	6.40(6.47)	16.23(14.70)
2	Green	427.71	451	4.75	30.65(30.86)	5.92(6.55)	6.80(6.55)	14.38(13.73)
3	White	407.38	450	Diam.	32.40(32.40)	6.60(5.40)	7.12(6.87)	15.23(16.05)
4	Green	446.54	439	4.13	33.20(32.24)	4.10(6.05)	6.49(6.27)	13.99(14.23)
5	Blue	441.71	435	2.36	32.20(32.60)	5.34(6.11)	6.14(6.34)	12.70(13.29)
6	White	421.38	447	Diam.	34.03(34.17)	3.44(5.70)	6.41(6.64)	16.00(15.52)

\*Note: A [Cu(HNTA)(val)(H<sub>2</sub>O)<sub>2</sub>][1.5(H<sub>2</sub>O)]; B [Ni(HNTA)(val)(H<sub>2</sub>O)<sub>2</sub>][1.5(H<sub>2</sub>O)]; C [Zn(NTA)(val)(H<sub>2</sub>O)](H<sub>2</sub>O)<sub>2</sub>; D [Cu(HNTA)(leu)(H<sub>2</sub>O)<sub>2</sub>][1.5(H<sub>2</sub>O)]; E [Ni(HNTA)(leu)(H<sub>2</sub>O)<sub>2</sub>][1.5(H<sub>2</sub>O)]; F [Zn(NTA)(leu)(H<sub>2</sub>O)](H<sub>2</sub>O)

reaction with sodium bicarbonate. The molecular masses of the six complexes suggest the presence of three water molecules (Table 1). Comparing the 10 Dq values of the complexes of this study with the literature values of Ni, and Cu octahedral complexes, respectively, for the ternary complexes of this study and two water molecules are coordinated to the metal. The pH values of the solution at which all the complexes under study are crystallized are ranged from 2.8 to 4.0. pK<sub>a</sub> value of leucine is 2.36 (carboxyl), 9.60 (amino)<sup>[28]</sup> and that of valine is 2.32 (carboxyl), 9.62 (amino)<sup>[28]</sup>. In this pH value range, HNTA<sup>2-</sup> and valine or leucine in their zwitter form predominate. At this pH value, HNTA<sup>2-</sup> has three coordination sites (N and two COO<sup>-</sup>) while valine or leucine coordinate in slightly acidic medium via its

carboxylic oxygen after being converted into the zwitter ion form (H<sub>3</sub>N<sup>+</sup> CH<sub>2</sub>COO<sup>-</sup>). This means that under the reaction condition used in this study, the second amino acids coordinated as monodentate ligand. Accordingly, the metal coordinates to three sites from HNTA<sup>2-</sup>, one from the second amino acids and two water molecules could be coordinated to form an octahedral structure. Fig.1 shows the suggested structure of the 1:1:1 complex. This suggested structure supposes that there are two ionisable protons; one due to uncoordinated COOH group and the other one from the protonated uncoordinated NH<sub>2</sub> group of amino acids used. In the following sections IR spectra, thermal analysis and mass spectra will be discussed to support the above conclusion about octahedral structure of our complexes.

**Fig.1** Proposed structure of our ternary complexes

## 2.2 IR spectra

The IR spectra of the H<sub>3</sub>NTA, valine, leucine, and their metal complexes were carried out in the range of 4 000~400 cm<sup>-1</sup> and the important bands are listed in

Table 2. IR spectra of nickel complexes with H<sub>3</sub>NTA and valine or leucine are shown in Fig.A in supplementary materials. The IR spectrum of nitrilotriacetic acid (H<sub>3</sub>NTA) shows bands at 3 041 and

Table 2 IR data of our ternary complexes( $\text{cm}^{-1}$ )

*Complex	$\nu\text{OH}$	$\nu\text{CO}$	$\nu\text{COO}$	$\nu\text{M-O}$	$\nu\text{M-N}$
<b>1</b>	3 406 (m)	1727 (s)	1 586 (vs), 1 464 (m), 1 413 (s)	377	543
<b>2</b>	3 588 (m), 3436(m)	1729(s)	1 603(vs), 1 407 (s)	359	462
<b>3</b>	3 520(m)	—	1 625, 1 586	336	544
<b>4</b>	3 486(s, br)	1729(s)	1 586(vs), 146 (vs), 1 412(s)	350	538
<b>5</b>	3 589(s)	1728(s)	1 604 (vs), 1 411(s)	364	467
<b>6</b>	3 520(m)	1 625(s), 1 585(vs)	324	585	585

\*Note: **1**  $[\text{Ni}(\text{HNTA})(\text{val})(\text{H}_2\text{O})_2]1.5(\text{H}_2\text{O})$ , **2**  $[\text{Cu}(\text{HNTA})(\text{val})(\text{H}_2\text{O})_2]1.5(\text{H}_2\text{O})$ , **3**  $[\text{Zn}(\text{HNTA})(\text{val})(\text{H}_2\text{O})](\text{H}_2\text{O})$ , **4**  $[\text{Ni}(\text{HNTA})(\text{leu})(\text{H}_2\text{O})_2]1.5(\text{H}_2\text{O})$ , **5**  $[\text{Cu}(\text{HNTA})(\text{leu})(\text{H}_2\text{O})_2]1.5(\text{H}_2\text{O})$ , **6**  $[\text{Zn}(\text{HNTA})(\text{leu})(\text{H}_2\text{O})](\text{H}_2\text{O})$

1,733  $\text{cm}^{-1}$  which are attributed to  $\nu\text{OH}$  with intermolecular hydrogen bonding and undissociated carboxylic groups, respectively. The IR spectra of the ternary complexes with valine or leucine as secondary ligand exhibit bands at 1 727  $\text{cm}^{-1}$  and 729  $\text{cm}^{-1}$  (Table 2) suggesting the presence of non-coordinated free carboxylic group of  $\text{H}_3\text{NTA}$  ligand. On the other hand, the ternary complexes with valine exhibit new strong absorption at 1 727  $\text{cm}^{-1}$  and 1 729  $\text{cm}^{-1}$  for Ni and Cu complexes, respectively. These shifts can be assigned to the stretching vibration  $\nu(\text{CO})$  of the coordinated carboxylate group,  $\text{COOM}^{[29]}$ . Similar data for leucine ternary complexes are observed with appropriate shift of  $\text{COOM}$  due to complex formation (Table 2). The IR spectra of the free ligands show sharp bands at 1 586 and 1 510  $\text{cm}^{-1}$  for valine and at 1 587 and 1 514  $\text{cm}^{-1}$  for leucine, which are assigned for asymmetric and symmetric stretching vibrations of the carboxylate moiety, respectively. These two bands of the free ligands are either shifted to lower or higher frequencies, indicating that these ligands coordinate to the metal ions via deprotonated carboxylate group<sup>[30]</sup>. On the other hand, the IR spectra of valine and leucine show medium broad bands at 3 148 and 3 108  $\text{cm}^{-1}$ , respectively, which can attribute to  $\text{NH}_3^+$  group of the amino acid. The IR spectra then suggest that in ternary complexes  $\text{HNTA}^{2-}$  is a tridentate ligand (two  $-\text{COO}-$  and one nitrogen), and that the secondary ligands are monodentate (one  $-\text{COO}-$ ). All the prepared complexes exhibit bands in the range of 3 558 ~ 3 406  $\text{cm}^{-1}$  signifying that  $\text{H}_2\text{O}$  molecules exist in these complexes. The mass spectra of the complexes support the proposed

complexes with three water molecules coordinated to the central atom to form an octahedral structure.

Further elucidation of the participation of nitrogen and carboxylic groups in  $\text{HNTA}^{2-}$  and the used amino acids was confirmed by recording the IR spectra of the complexes in the range 50 ~ 650  $\text{cm}^{-1}$ . The  $\nu(\text{MO})$  frequencies of  $\text{NTA}^{3-}$  as ligand are at 362, 350 and 355  $\text{cm}^{-1}$  for Cu, Ni and Zn complexes, respectively<sup>[24]</sup>. Thus one may assign the bands in the complexes in the range of 350 ~ 377  $\text{cm}^{-1}$  to the M-O vibrational frequencies (Table 2). The M-N band frequencies are found in the range of 462 ~ 544  $\text{cm}^{-1}$  for Cu Ni, and Zn complexes, respectively (Table 2).

### 2.3 Magnetic moments and electronic spectra

The UV-Vis spectra of the metal complexes were recorded in distilled  $\text{H}_2\text{O}$  solution ( $10^{-2} \text{ mol} \cdot \text{L}^{-1}$ ). For ternary Ni(II) complexes, the magnetic moments  $\mu_{\text{eff}}$  of  $[\text{Ni}(\text{HNTA})(\text{valine})(\text{H}_2\text{O})_2] \cdot 1.5\text{H}_2\text{O}$  and  $[\text{Ni}(\text{HNTA})(\text{leucine})(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$  have values of 4.75  $\mu_{\text{B}}$  (B.M.) and 4.13  $\mu_{\text{B}}$ , respectively, which suggest an octahedral geometry<sup>[25]</sup>. The electronic spectrum of Ni(II) complexes displays two bands in the range of 626 nm (15 974  $\text{cm}^{-1}$ ) and 394 nm (25 380  $\text{cm}^{-1}$ ) assigned as  ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$  and  ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ , respectively. The mixed-ligand complexes of Cu(II), with valine and leucine show magnetic moment values,  $\mu_{\text{eff}}$ , of 2.61 and 2.36  $\mu_{\text{B}}$ . These values correspond to one unpaired electron and thus offer evidence for mononuclear structures of the complexes. The UV-Vis spectrum of Cu(II) complexes consists of a broad band centered at 812 nm (12 315  $\text{cm}^{-1}$ ) that is assigned to  ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$  transition with expected splitting of these states as a result of tetragonal distortion of the

octahedral Cu(II) ion,  $d^{9[27]}$ .

## 2.4 Mass spectra

The mass spectra of the six complexes were recorded. All the spectra contain molecular ion peaks and confirm the molecular mass of the complexes. The data are presented in (Table 1).

## 2.5 Thermal analysis

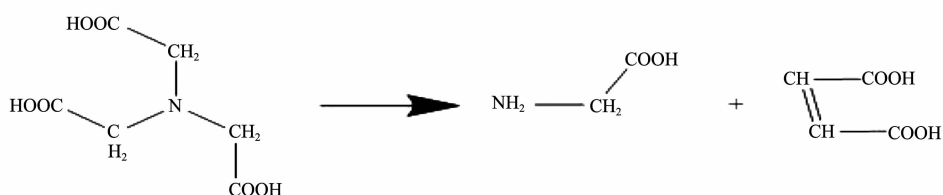
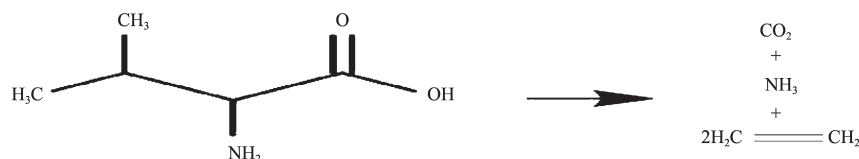
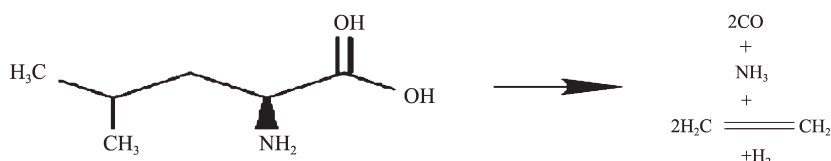
Thermogravimetric analysis results are shown in Table 3. And Thermogravimetric analysis curves are given in Fig.B in supplementary materials. Interpretation of the thermal mass losses shows that the complexes thermally decompose in the same patterns exhibited by the parent ligands. Scheme.1 is shown that  $H_3NTA$  thermally decompose in two overlapping steps<sup>[31]</sup>,

into glycine and maleic acid. Also, scheme 2 and scheme 3 are shown the supposed thermal decomposition for valine and lucine, respectively.

Both thermal products, on further heating, decompose to give different organic residues overlapping steps. The first mass loss in the complexes is equivalent to the water molecules which supports the suggested structure for the complexes<sup>[32]</sup> where the decomposing temperatures are very close to the coordinate water temperature decomposition. As the thermal decomposition is made under nitrogen atmosphere and that almost all products are gases, (CO,  $C_2H_2$  and  $HC \equiv CH$ ) which interprets the liberation of the metal or its carbide form as shown in Table 3. The final

**Table 3** Temperature values for the decomposition along with the species lost in each step

*Complex	Molar Wt.	TG range / °C	DTG / °C	Mass loss Found(Cald.) / %	Total mass loss / %	Assignment
<b>1</b>	427.71	48~40	113	5.56( 6.31)	78.78	1.5H <sub>2</sub> O
		141~276	225	29.73(29.93)		2H <sub>2</sub> O+NH <sub>3</sub> +glycine
		277~470	376	41.46(40.21)		maleic acid+2CO
		470~650	Above 470	23.25(23.05)		Mix. CO and NiC
<b>2</b>	432.54	34~76	54	7.2(6.24)	74.21	1.5H <sub>2</sub> O
		78~141	90	Overlap		2H <sub>2</sub> O+NH <sub>3</sub> +glycine+CO+CO <sub>2</sub> +H <sub>2</sub> C=CH <sub>2</sub>
		142~332	226	53.92(52.72)		
		334~451	—	—		
		451~620	522	14.05(13.87)		H <sub>2</sub> C=CH <sub>2</sub> +CH <sub>3</sub> OH
		620~816	Above 522	24.81(23.95)		Mix CO and CuC <sub>3</sub>
<b>3</b>	407.38	75~161	118	3.71(4.42)	69.30	H <sub>2</sub> O
		162~302	230	33.69(33.87)		H <sub>2</sub> O+CO+NH <sub>3</sub> +glycine
		303~397	363	24.19(24.56)		H <sub>2</sub> C=CH <sub>2</sub> +CO+CO <sub>2</sub>
		397~516	434	7.89(6.88)		H <sub>2</sub> C=CH <sub>2</sub>
		516~667	Above 434	30.52(29.80)		Mix. 2CO+Zn
<b>4</b>	441.71	42~131	101	11.41(12.26)	80.40	3H <sub>2</sub> O
		132~280	230	32.02(31.70)		0.5H <sub>2</sub> O+glycine+H <sub>2</sub> C=CH <sub>2</sub> +CO
		280~475	399	37.29(36.45)		Maleic acid+NH <sub>3</sub> +H <sub>2</sub> C=CH <sub>2</sub>
		47~600	Above 399	19.29(19.61)		Mix. CO and Ni
<b>5</b>	446.54	36~99	62	5.97(6.05)	80.30	1.5H <sub>2</sub> O
		100~321	221	64.63(63.38)		2H <sub>2</sub> O+glycine+maleic+acid+CO+H <sub>2</sub> C=CH <sub>2</sub>
		322~535	485	9.86(10.07)		NH <sub>3</sub> +H <sub>2</sub> C=CH <sub>2</sub>
		535~611	Above 485	19.54(20.47)		Mix CO and Cu
<b>6</b>	421.38	38~144	125	4.18(4.27)	73.10	H <sub>2</sub> O
		145~270	237	28.17(28.72)		H <sub>2</sub> O+CO+glycine
		270~404	377	25.71(24.92)		H <sub>2</sub> C=CH <sub>2</sub> +CO <sub>2</sub> +CH <sub>3</sub> OH
		404~514	457	9.42(10.68)		NH <sub>3</sub> +H <sub>2</sub> C=CH <sub>2</sub>
		514~604	Above 457	32.52(31.66)		2CO+ZnC

Scheme 1 Thermal decomposition of nitrilotriacetic acid<sup>[30]</sup>Scheme 2 Thermal decomposition of valine acid<sup>[30]</sup>Scheme 3 Thermal decomposition of *L*-leucine acid<sup>[30]</sup>

thermal decomposition residue is the mixture of metal or metal carbide with carbon monoxide gas where thermal decomposition is made under nitrogen atmosphere<sup>[31,33]</sup>.

## 2.6 Biological study

In testing the antimicrobial activity of these compounds, we used more than one test organism to increase the chance of detecting antibiotic principles in the tested materials. The sensitivity of a microorganism to antibiotics and other antimicrobial agents was determined by the assay plates which were incubated at 37 °C for 48 h for bacteria, at 25 °C for 48 h for *Aspergillus flavus* and yeast as *Candida albicans* incubated at 30 °C for 48 h. All the Ni and Cu tested compounds show a remarkable biological activity against different types of Gram-positive (G+) bacteria and Gram-negative (G-) bacteria. Only Ni·NTA·valine complex shows a biological activity against *Candida albicans* that can cause Candidiasis. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. The data are listed in Table 4. The data show that metal complexes under

investigation have the capacity of inhibiting the metabolic growth of the investigated bacteria and fungi to different extents. The size of the inhibition zone depends upon the culture medium, incubation conditions, rate of diffusion, and the concentration of the antibacterial agent. The activities of all the tested complexes may be explained on the basis of chelation theory. Chelation considerably reduces the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and possible *p*-electron delocalization over the chelater. Such chelation increases the lipophilic character of the central metal ion, which subsequently favors the permeation through the lipid layer of cell membrane. It is likely that the increased lipo-solubility of the ligand up on metal chelation may contribute to its facile transport into the bacterial cell which blocks the metal binding sides in the enzymes of microorganisms<sup>[34]</sup> and<sup>[35]</sup>.

On comparing the biological activity for our metal complexes, the following results are obtained.

Biological activity against Gram-positive bacteria follows the order: Cu·NTA·leucine=Cu·NTA·valine>Ni·NTA·leucine>Ni·NTA·valine. The biological

**Table 4** Biological activity of NTA , valine and leucine ligands and their metal complexes

Sample	Inhibition zone diameter / mm%	
	<i>Escherichia coli</i> (G <sup>-</sup> )	<i>Staphylococcus aureus</i> (G <sup>+</sup> )
	Mean±SD	Mean±SD
Control: DMSO	0.0	0.0
Standard:Tetracycline (Antibacterial agent)	30	29
H <sub>3</sub> NTA	14.000±0.214	16.333±0.215
Valine	0.000±0.000	0.000±0.000
Leucine	0.000±0.000	0.000±0.000
[Ni(HNTA)(valine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	12.667±0.164	10.667 ± 0.236
[Cu(HNTA)(valine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	13.167±0.096	14.000±0.286
[Zn(HNTA)(valine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	0.000±0.000	0.000±0.000
[Ni(HNTA)(leucine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	10.333±0.296	10.667±0.236
[Cu(HNTA)(leucine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	13.333±0.413	14.333±0.315
[Zn(HNTA)(leucine)(H <sub>2</sub> O) <sub>2</sub> ]·(H <sub>2</sub> O)	0.000±0.000	0.000±0.000

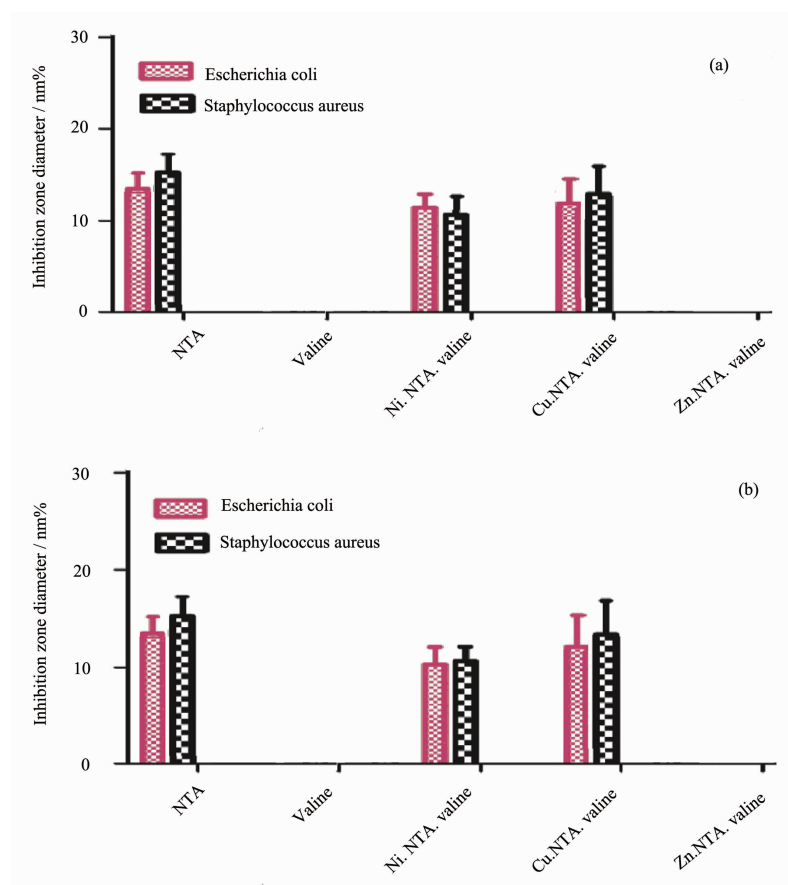


Fig.2 a) Percent of inhibition zone diameter ( $\text{mm} \cdot \text{mg}^{-1}$  sample) relative to standard vs. NTA ,leucine and its metal complexes;  
 b) Percent of inhibition zone diameter ( $\text{mm} \cdot \text{mg}^{-1}$  sample) relative to standard vs. NTA ,valine and its metal complexes

activity of metal complexes is lower than tetracycline antibacterial agent.

Biological activity against Gram-negative bacteria follows the order:  $\text{Cu} \cdot \text{NTA} \cdot \text{Leucine} > \text{Cu} \cdot \text{NTA} \cdot \text{valine} >$

$\text{Ni} \cdot \text{NTA} \cdot \text{valine} > \text{Ni} \cdot \text{NTA} \cdot \text{leucine}$ . The biological activity of metal complexes is lower than tetracycline antibacterial agent.

The Biological activity against *Candida albicans*

shows that Ni·NTA·valine causes inhibition zone of 13, 12 and 13 mm·mg<sup>-1</sup> sample. The biological activity of Ni·NTA·valine is lower than amphotericin B antifungal agent which causes 21, 20 and 21 mm·mg<sup>-1</sup> sample inhibition zone. Ni·NTA·valine is the only complex that shows antifungal activity against *Candida albicans* which makes this complex of interest.

The importance of this lies in the fact that these complexes could be applied fairly in the treatment of some common diseases caused by *Escherichia coli*, e.g., septicemia, gastroenteritis, urinary tract infections, and hospital-acquired infections<sup>[36-37]</sup>. It also can be used in treatment of *Candida albicans* that can cause Candidiasis. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases.

### 3 Conclusions

The Ni, Cu, and Zn, nitrilotriacetic acid and valine or leucine ternary complexes prepared in slightly acidic medium have an octahedral structure of the general form [M(HNTA)(valine)(H<sub>2</sub>O)<sub>2</sub>]·H<sub>2</sub>O and [M(HNTA)(leucine)(H<sub>2</sub>O)<sub>2</sub>]·H<sub>2</sub>O, in which nitrilotriacetic acid acts as a tridentate ligand and valine or leucine acts as a monodentate ligand. Two coordinated water molecules and one crystalline water molecule are required to complete octahedral coordination. These complexes behave as dibasic acids and also give enhanced effect in the inhibition zone of Gram-negative and positive bacteria for valine and leucine.

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