

Synthesis and evaluation of the antibacterial activity of Cu(II) and Ni(II) complexes with mixed ligands based on glycine and dicarboxylic acids

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Abstract

A large number of metal complexes have the ability to inhibit bacterial growth. Cu(II) and Ni(II) complexes based on glycine (Gly), itaconic acid (Ita), and oxalic acid (Ox) were synthesized by conventional methods and evaluated for their antibacterial activity. The metal complexes were characterized by TGA, FTIR, UV-vis spectroscopy, and XRD. The metal: ligands (M:L₁:L₂) stoichiometry of these complexes is 1:2:2, and coordination around Cu(II) and Ni(II) seems to be octahedral, with the ligands bound through the N atom of the amino group and O atoms of the bridging carboxylate group. These compounds are crystalline and stable at temperatures between 250 to 300°C. The metal complexes were screened for their antibacterial activity against the bacterial species *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli*. These compounds were shown to have antibacterial activity mainly against gram-positive strains, with a minimum inhibitory concentration of 20 ppm.

Keywords: complexes; amino acids; glycine; itaconic acid; oxalic acid.

Síntesis y evaluación de la actividad antibacteriana de complejos de Cu(II) y Ni(II) con ligandos mixtos basados en glicina y ácidos dicarboxílicos

Resumen

Un gran número de complejos metálicos tienen la capacidad de inhibir el crecimiento bacteriano. Se sintetizaron complejos de Cu(II) y Ni(II) basados en glicina (Gly), ácido itacónico (Ita) y ácido oxálico (Ox) mediante métodos convencionales y se evaluó su actividad antibacteriana. Los complejos metálicos se caracterizaron por TGA, FTIR, espectroscopia UV-vis y XRD. La estequiometría metal:ligandos (M:L₁:L₂) de estos complejos es 1:2:2, y la coordinación alrededor de Cu(II) y Ni(II) parece ser octaédrica, con los ligandos unidos a través del átomo de N del grupo amino y los átomos de O del grupo carboxilato puente. Estos compuestos son cristalinos y estables a temperaturas entre 250 y 300°C. Los complejos metálicos se probaron para su actividad antibacteriana contra las especies bacterianas *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* y *Escherichia coli*. Estos compuestos demostraron tener actividad antibacteriana principalmente contra cepas gram-positivas, con una concentración mínima inhibitoria de 20 ppm.

Palabras clave: complejos; aminoácidos; glicina; ácido itacónico; ácido oxálico.

1 Introduction

Metal complexes with biological ligands are of great interest since they allow us to find new alternatives to combat

and/or control diseases [1]. Aminoacids (AA) are molecules of great importance for human health; they are necessary for the formation of proteins that allow the organism to carry out fundamental activities such as tissue repair, metabolic

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processes, growth, and energy production, among others. These biomolecules used as ligands have become an attractive alternative to synthesize metal complexes, as they provide high biological activity and are biocompatible and safe for the organism [2,3]. Complexes derived from different AA are used as antibacterial, antifungal and antioxidant agents [4,5]. The use of AA theoretically allows the use of any of them since they have two functional groups that can interact with the metal. Glycine (Gly) is the simplest amino acid that has facilitated the study of metal-ligand interactions and the determination of the parameters that affect the formation of stable compounds. Likewise, the amino acids methionine and cysteine have been used as ligands to enhance the antioxidant capacity and the use of histidine has allowed the polymerization of the chain in 2D and 3D [6,7]. The AA in complexes improved antibacterial activity against various strains of health concern, mainly those associated with foodborne diseases and water pollution [8]. However, the use of AA as the only ligand implies in most cases, that the complexes have low thermal stability and form 1D or 2D networks [9,10], very few complexes using only AA have the ability to form 3D networks and these mostly achieved with aromatic AA that allow the formation of hydrogen bonds to form supramolecular networks [11]. The use of ancillary ligands in metal complexes with AA is a necessary route to improve the properties exhibited by the compounds with AA, which degrade at temperatures between 150 to 250°C [12]. Dicarboxylic acids are used as ancillary ligands that act as a bridge between metal cations, which in theory allows improving the structural properties of the complex. Additionally, the functional groups that these acids contain can confer other chemical properties to the complex [13] Cao et al. (2020) used Ag(I) and 1,4-benzenedicarboxylic acid (BDC or terephthalic acid) to synthesize complexes with activity against oral bacterial strains [14]. The sustained release of ions is related to the chemical stability possessed by the complex due to the two -COOH groups that bind to the metal and extend the network. The use of pyridin-3,5-dicarboxylic acid with Ag (I) was studied by Alisir et al. (2015), the thermal stability of the synthesized complex was 190°C showing antibacterial activity against a wide variety of gram-positive and gram-negative strains, the complex showed reduced activity against fungi such as *Candida albicans* [15]. Meanwhile, Lucena et al. (2018) succeeded in synthesizing a porous complex for the transport and subsequent release of diclofenac sodium from Zn(II), biphenyl-4,4'-dicarboxylic acid and adenine linkers. It has been shown that drug delivery systems integrating biological components can be used to reduce adverse effects due to biological compatibility [16]. Undoubtedly, dicarboxylic acids are ligands that allow obtain complexes with a wide variety of properties, also improve thermal stability and dimensionality, while presenting good antibacterial activity against a wide variety of species of clinical and industrial interest [8,17,18]. The use of biological molecules helps to generate greater stability and compatibility with living systems. Because of these properties, the use of dicarboxylic acids and AA in metal complexes can improve the antibacterial activity against a wide variety of pathogenic species. Cu and Ni complexes

with amino acids as single ligands have been shown to have antibacterial activity, greater stability and faster and more efficient syntheses. In contrast, other metals such as Zn, Ag and Co, although they have good antibacterial activity, have synthesis methods that are less efficient, more polluting and yield lower amounts [25].

In the present study, four complexes with Cu(II) and Ni(II) metal cations were synthesized using glycine (Gly) as amino acid and oxalic (Ox) and itaconic (Ita) acids as ligands, in order to determine their antibacterial properties. For this purpose, the antibacterial activity and the minimum inhibitory concentration (MIC) of the complexes against several gram-positive and gram-negative species were evaluated.

2 Experimental

2.1 Materials and reagents

All chemicals used were of the analytical reagent grade without further purification. $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ >98%, $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ >98%, NaOH 0.1 M 98%, methanol were acquired from Merck; itaconic acid 99% (Sigma-Aldrich); glycine 99.5%, oxalic acid 99% and pure absolute ethanol 99.5% were obtained of PanReac.

2.2 Synthesis of the metal complexes

A schematic of the synthesis procedure can be seen in Fig. 1, which was carried out following a molar ratio $\text{M:L}_1\text{:L}_2$ 1:2:2, where ligand 1 (L_1) is the amino acid and ligand 2 (L_2) is the dicarboxylic acid. The procedure is as follows: a mixture of copper acetate monohydrate or nickel acetate tetrahydrate in deionized water was heated at 70°C in a water bath for about 15 min with constant stirring. A small amount of ethanol was added to help with the homogenization of the solution and then the AA was incorporated.

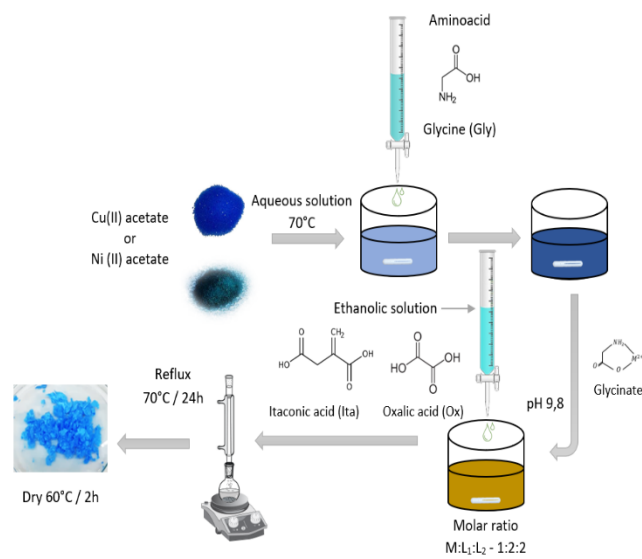


Figure 1. Scheme of the synthesis of metal complexes $\text{M:L}_1\text{:L}_2$
Source: Self-made image

The reaction mixture was left in agitation under controlled temperature (not exceeding 70°C). After approximately five minutes the formation of blue crystals could be appreciated. The pH was adjusted to 9.8 with the addition of 0.1M NaOH to obtain the glycinate species. Subsequently, an ethanolic solution with dicarboxylic acid (Ita or Ox) was added, preferably at 70°C to avoid thermal shock with the solution containing the AA, and stirred for 10 minutes. The solution was transferred to a two-necked round-bottom balloon and the system was refluxed at 70°C for 24 hours. Finally, after the reaction time, the system was filtered, dried at 60°C for 2 hours and finally stored for further analysis.

2.3 Antibacterial activity

The standardized diffusion method in semi-solid agar wells was followed to evaluate the antimicrobial activity of the synthesized compounds against gram-positive ATCC bacteria: *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* and gram-negative species: *Salmonella* and *Escherichia Coli*. The species were cultured at 37 °C for 24 h in nutrient broth. The cultures were adjusted with the McFarland standard and the petri dishes were prepared with 20 mL of the semisolid culture Müller Hinton (MH) and 50 µL of pathogen. In the petri dishes, 10 mm diameter wells were made to deposit the coordination compounds and the positive (oxytetracycline) and negative (peptone water) controls. The concentrations of the compounds were 5 mg/L, 10 mg/L and 15 mg/L. The cultures were incubated at 37 °C for 48 h, subsequently the diameter of the inhibition zone was measured to compare it with that of the antibiotic disks. The minimum inhibitory concentration (MIC) test was performed in a 96-well plate with the compounds that showed antibacterial activity against any of the strains studied. The technique used was half dilutions analyzing concentrations of 20 to 5 mg/L in duplicate. The bacteria were placed in liquid MH medium and shaken at 37 °C for 25 h. The growth of the pathogen was taken as a target. To adjust the McFarland standard to 106 CFU/mL (CFU= colony forming units) and the contamination controls of the medium and the compounds were carried out in duplicate [19]. The MIC values were determined in a UV-Vis spectrometer by the optical density method at 620 nm (OD_{620}), the bacterial growth curves were determined by scheduling readings every hour. All OD_{620} data were the average values of duplicate tests [20].

2.4 Characterization

The thermogravimetric analysis (TGA) was carried out on the TA Instruments model SDT 650 with a nitrogen atmosphere (20 mL/min), a heating ramp of 10 °C/min and a temperature range of 25 °C to 600 °C. The samples did not require additional preparation to the drying performed in the synthesis method. The infrared spectra of the coordination compounds were obtained on the Perkin Elmer Spectrum two series 93881 IR spectrometer equipped with an ATR diamond crystal performing 16 scans in a range between 4000 to 400 cm^{-1} . The UV-Vis studies of the coordination

compounds were carried out in the Boeco S-220 spectrophotometer in a range between 200 to 900 nm. For measurement in the visible range, solutions of the compounds were prepared at a concentration of 0.002 M in methanol. While, for the measurement in the ultraviolet range, the concentrations were 0.001 M. The diffractograms were obtained on a Malvern-PANalytical device model Empyrean 2012 with Pixel 3D detector and Cu source ($\lambda=1.541874 \text{ \AA}$) at 40 kV and 40 Ma, Goniometer: Omega/2 θ and platform configuration: Reflection transmission spinner rotating at 4 rpm. The step was 0.05° and a time per step was 50 s. Scanning electron microscopy images were taken on the JEOL JSM-5910LV equipment.

3 Results and discussion

3.1 Characterization of metal complexes

Four metal complexes were synthesized, Cu-Gly-Ita, Cu-Gly-Ox, Ni-Gly-Ita and Ni-Gly-Ox. The synthesis method was described in Fig. 1 following a 1:2:2 molar ratio $M:L_1:L_2$. The SEM images (Fig. 2) of some of these compounds show acicular crystals for the compounds with copper and laminar crystals for the compounds with nickel. It is also observed that the copper compounds are larger compared to the nickel compounds and have smooth and uniform surfaces.

Fig. 3 shows the thermal analysis of the complexes synthesized. The complex with itaconic acid Cu-Gly-Ita loses coordination waters around 150 °C, this generates a mass loss that does not exceed 10%. Subsequently, the complex remains stable up to 250 °C, at this temperature the degradation of the amino acid begins, generating a mass loss of approximately 40%, finally the respective metal oxide is obtained. On the other hand, the complex with oxalic acid Ni-Gly-Ox reaches temperatures close to 200°C before releasing coordination water. In this case, the released water generates a mass loss of 20%, slightly more than twice the mass lost by the Cu-Gly-Ita complex. The complex with oxalic acid remains stable up to 335°C, temperature at which the degradation of the amino acid begins. According to the studies reported by Sevgi F. et al (2018), the decomposition

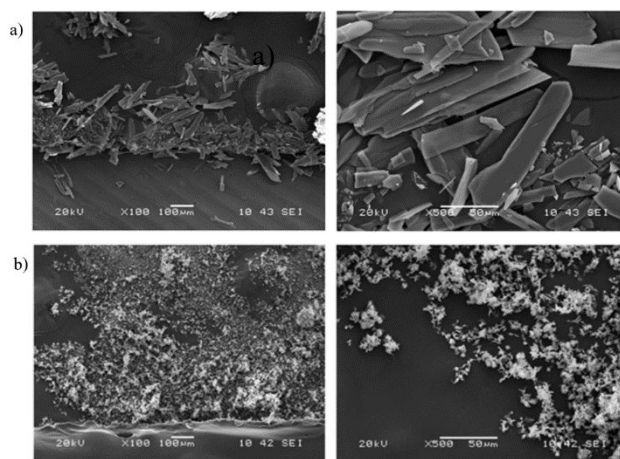


Figure 2. SEM images for a) Cu-Gly-Ita and b) Ni-Gly-Ita
Source: Self-made image

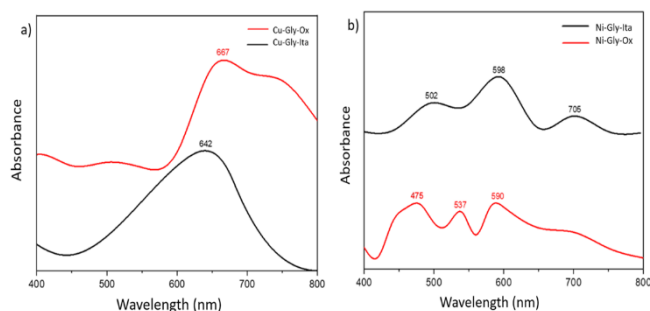


Figure 6. UV-Vis spectra of metal complexes. a) Cu-Gly-Ita and Cu-Gly-Ox. b) Ni-Gly-Ita and Ni-Gly-Ox

Source: Self-made image

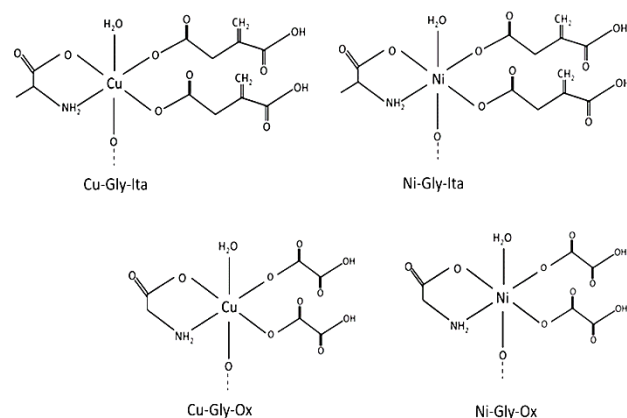


Figure 7. Proposed structures of metal complexes

Source: Self-made image

between 22123 and 13333 cm^{-1} (452 and 750 nm), according to Köse D. et al (2016) this is due to that Cu(II) complexes present octahedra distorted by the Jahn-Teller effect and the structure of the complex is pseudo-octahedral [25]. In these cases, the upper peak is designated as the absorption band and the d-d transition is set ($^2E_g \rightarrow ^2T_{2g}$) 14992 cm^{-1} (667 nm). On the other hand, for the Ni-Gly-Ita and Ni-Gly-Ox complexes, three absorption bands of d-d transitions were observed (Fig. 6b). The first transition ($^3A_{2g} \rightarrow ^3T_{2g}$)(F) 19920 and 21052 cm^{-1} (502 and 475 nm), the second transition ($^3A_{2g} \rightarrow ^3T_{1g}$)(F) 16722 and 18621 cm^{-1} (598 and 537 nm) and the last transition ($^3A_{2g} \rightarrow ^3T_{1g}$)(P) 16949 and 14184 cm^{-1} (590 and 705 nm) respectively. All transitions are low energy (from 400 nm onwards) which indicates interactions between d orbitals of transition metals or between orbitals of metals and ligands, as is the case.

Based on the characterization studies, the possible structures (Fig. 7) of the synthesized complexes can be proposed. The FTIR spectra showed the characteristic vibrations between the metal and the nitrogen or oxygen atoms of the amino acids and dicarboxylic acids; it was also possible to establish the coordination mode of the carboxyl group. The UV-Vis spectra allowed us to determine that the coordination environments are distorted octahedral for the complexes with copper and octahedral for the complexes with nickel due to the low energy signals obtained in the range of 400 to 900 nm that indicate interactions between d orbitals or between metal and ligand, as is the case.

3.2 Antibacterial activity of metal complexes

The synthesized metal complexes were tested against five bacterial species including gram-positive genera: *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* and gram-negative genera: *Salmonella* and *Escherichia Coli*. The activity was determined by measuring the zone of inhibition. The Ni-Gly-Ox complex did not present inhibition against the genera under study and the Cu-Gly-Ox complex only presented activity against *Listeria*. As mentioned before, all synthesized complexes are dense phases; the antibacterial activity of the complexes occurs by diffusion of ions of the respective metal [29,30]. If the complex is very dense, it interferes with the release of the ions, which directly affects the antibacterial capacity. In contrast, complexes with itaconic acid presented good activity against 4 of the 5 genera under study (Table 2). The Ni-Gly-Ita complex did not show activity against *Staphylococcus aureus* and its maximum activity was against *Listeria*. On the other hand, the complexes synthesized with copper presented better activity against the bacterial genera under study. Notably the compound Cu-Gly-Ita showed significant activity against *Listeria monocytogenes*. This bacterium causes serious diseases due to the consumption of foods contaminated with this pathogen. Listeriosis can cause septicemia, meningitis, encephalitis, pneumonia and intrauterine or cervical infection in pregnant women that can lead to spontaneous abortion [31].

In general, the best activity was against gram-positive species. This is because bacteria of this type do not have an outer membrane, which facilitates the penetration of metal ions through the peptidoglycan, causing cell lysis and subsequent bacterial death. In contrast, gram-negative bacteria have an outer membrane that provides an extra

Table 2.

Antibacterial activity of metal complexes

Compounds	Antibacterial activity (Zones of inhibition in cm)					Control +	Control -
	<i>Bacillus</i>	<i>Staphylococcus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>		
Cu-Gly-Ita	0.60±1.00	0.50±1.00	1.00±1.00	0.60±1.00	2.00±1.00	3.00±1.00	--
Cu-Gly-Ox	--	--	--	--	0.50±1.00	3.00±1.00	--
Ni-Gly-Ita	0.50±1.00	--	0.70±1.00	0.40±1.00	0.80±1.00	3.00±1.00	--
Ni-Gly-Ox	--	--	--	--	--	3.00±1.00	--

Control + : Oxitetracycline, Control - : Peptone water

Source: Self-made image

protective barrier, making the antibacterial action of some coordination complexes more difficult [32]. Fan et al. (2023) state that the mortality rate is between 20% and 30% in high-risk communities, such as the elderly, pregnant women, young children, and immunosuppressed people [33]. These same compounds have good activity against *Escherichia Coli*, another pathogen that causes food poisoning that can be very serious, mainly in children and vulnerable populations. The most common infections caused by *Escherichia Coli* are traveler's diarrhea, neonatal meningitis, cholangitis, urinary tract infection, cholecystitis, and pneumonia [34].

3.1 Minimum inhibitory concentration

The MIC test was performed for the complexes that showed significant activity against sensitive pathogens, these compounds were Cu-Gly-Ita and Ni-Gly-Ita against *Listeria*, *E-coli* and *Bacillus* bacteria. The MIC was determined using the optical density method measured at 620 nm. The MIC values for the copper compounds were around 20 mg/L and the nickel compounds between 5 to 10 mg/L. These results agree with those reported by authors such as Liu et al. (2012) whose coordination compounds with nickel and 5-phenyl-1H-pyrazole-3-carboxylic acid report MIC values between 15 to 20 ppm [35]. Jo et al. (2019) describes the minimum bactericidal concentration of compounds synthesized with copper, bipyridyl ligands and glutaric acid at values around 20 ppm [36]. Against *Listeria* (Fig. 8), the Cu-Gly-Ita complex presents minimum inhibitory activity at 20 ppm, during the first 750 minutes the inhibition activity is fast, however, after 1000 minutes an increase in the curve is observed. This indicates that the activity of the complex against the pathogen begins to decrease and the population of viable cells increases. On the other hand, the Ni-Gly-Ita complex presents inhibition at the concentration of 10 ppm during the first 1250 minutes; the antibacterial activity is sustained during these minutes for 20 and 10 ppm but the viable cells increase after 1250 minutes for the concentration of 10 ppm.

For the Cu-Gly-Ita complex against *E-Coli* (Fig. 9), the antibacterial activity occurs at 20 ppm. At the concentrations of 10 and 5 ppm, the complex reduces bacterial growth, but does not maintain this trend. However, the Ni-Gly-Ita complex at 10 ppm shows inhibition during the first 500 minutes, after that time an increase in the optical density is observed, indicating, an increase in viable cells.

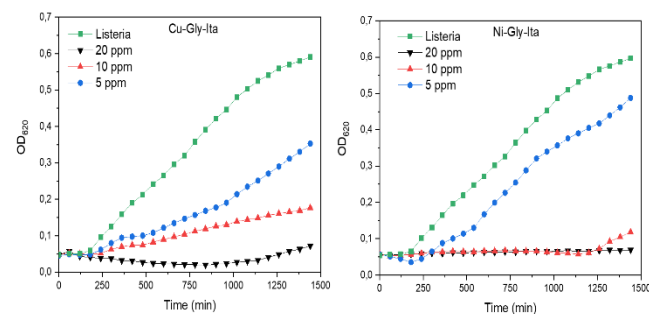


Figure 8. Growth curves of *Listeria* with 5, 10 and 20 ppm.
Source: Self-made image

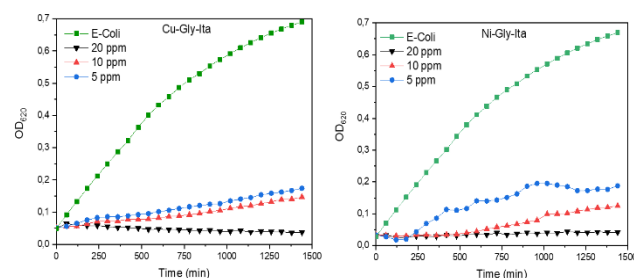


Figure 9. Growth curves of *E-Coli* with 5, 10 and 20 ppm.
Source: Self-made image

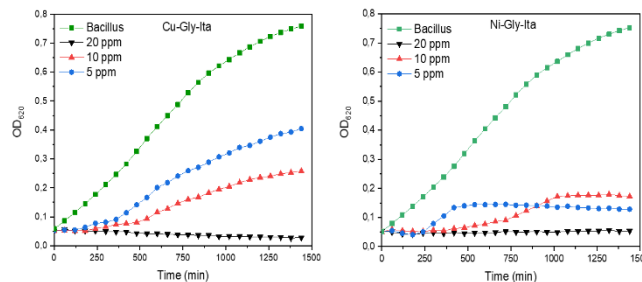


Figure 10. Growth curves of *Bacillus* with 5, 10 and 20 ppm.
Source: Self-made image

Against *Bacillus* (Fig. 10), the Cu-Gly-Ita complex presents inhibition at a concentration of 20 ppm, this activity is sustained over time showing the same pattern for the copper complexes described in this study. The behavior of the Ni-Gly-Ita complex during the first 250 minutes is inhibitory at the 3 concentrations (5, 10 and 20 ppm) however, bacterial growth is activated for the lower concentrations and is only maintained for 20 ppm.

4 Conclusions

The metal complexes synthesized with glycine present a monodentate coordination mode of the carboxyl group. This allows the ligand to have a free group to interact with neighboring molecules and act as a bridge. The improvement in thermal capacity occurs in the complexes with oxalic acid due to the formation of dense phases, making them more stable at temperatures ranging between 250 and 350°C, depending on the metal and the amino acid. However, the complexes synthesized with this acid do not exhibit significant antibacterial activity against the species studied. The biological activity of the complexes occurs mainly against gram-positive genera. The diffusion of metal ions through the peptidoglycan of the bacteria is favored, as they do not have an outer layer for additional protection. On the other hand, while the complexes with nickel show smaller zones of inhibition compared to those with copper, they have a lower MIC, around 10 ppm compared to the MIC of copper complexes, which is 20 ppm. This implies that less of the nickel complex is needed to achieve optimal and low-polluting results. However, the literature reports MICs around 40 ppm. The Cu-Gly-Ita complex exhibits greater antibacterial activity, particularly against the gram-positive species *Listeria monocytogenes*.

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