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Using Python multi-paradigm programming language in evaluating the antibacterial activity of a Ni(II) complex compound compared to that of its free organic ligand

Research article

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Abstract
As antibiotics are indispensable in treating infections caused by bacteria, there has been much research done within this field, in order to identify new solutions against this kind of pathogen agents. The paper aims to provide information about the antibacterial activity exhibited by a Ni(II) complex compound in comparison with that shown by its free organic bidentate ligand (namely, the bidentate (N, S) heterocyclic ligand is 2-mercapto-3-niacinamido-1,4-naphthalenedione). As it refers to substances that have been described previously from other points of view, the current paper is intended to be the final part of their presentation. More precisely, to complete the description table by evaluating the antibacterial activity, Kirby-Bauer disk diffusion method has been used. The microbiological tests have been conducted against eight kinds of microorganisms: four gram-positive and four gram-negative bacteria. These tests were followed by a thorough statistical analysis of their results, performed within Python multi-paradigm programming language.

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Keywords: Python programming language, Kirby-Bauer disk diffusion method, antibacterial activity, square-planar Ni(II) complex compounds, naphthalenedionic ligands

1. INTRODUCTION

At the beginning of the third millennium, because of the changes in different human habits and also due to the climate changes, bacterial infections have become a main cause of disease and even death. As to overcome this grave medical issues, studying drugs able to treat them is a very significant and challenging matter. Though, drug overuse and/or misuse by people have been resulted in increased bacteria resistance, which is a major public health threat [1].

Consequently, during the current years, much research is concentrated on identifying paths to obtain new drugs, which may be active despides bacteria structural changes, solving the problem of increasing bacterial resistance [1-7].

Coordination chemistry represents a field of major interest, which can play a crucial role in developing new compounds with significant antibacterial activities and, therefore, with potential pharmaceutical applications [1-6].

The present paper reports the results of such a study, performed on a heterocyclic bidentate ligand and its Ni(II) complex compound, which have been tested as potential drugs against eight kinds of microorganisms: four gram-positive and four gram-negative bacteria.

2. MATERIALS AND METHODS

2.1. Materials for obtaining the free ligand and its Ni(II) complex compound

As previously presented [8], so as to synthesize the organic ligand, we have used the following Sigma-Aldrich reagents: 2,3-dichloro-1,4-naphthalenedione, niacinamide, thiourea, NaOH pellets, CH_3COOH and ethyl alcohol and then, with the aim of synthesizing the complex compound, we have also used Sigma-Aldrich reagents, namely:

tetrabutylammonium hydroxide solution, 40 wt. % in H_2O, nickel (II) chloride hexahydrate, diethyl ether and once again ethyl alcohol.

To obtain the solutions for the experimental investigation, we have used Sigma-Aldrich DMF as a solvent.

Furthermore, for the experimental study we have also used Sigma-Aldrich tetrabutylammonium perchlorate, as well as acetone and potassium bromide.

The synthesis paths, for both the free ligand and its Ni(II) complex compound, were already described elsewhere [8].

The ligand appears as a microcrystalline yellowish-orange air-stable powder, whereas its complex with Ni(II) appears as a micro-crystalline orange-red air-stable powder [8, 9].

2.2. Kirby-Bauer disk diffusion method

The Kirby-Bauer diffusion method has several variants, and the standardized technique of antibiotic-impregnated discs in Petri plates is currently used [10].

A number of factors, such as: the strain studied, the culture medium (such as: pH, density and thickness of the medium), the technique used and even the interpretation of the results can influence an antibiogram.

Therefore, all antibiograms must be performed in reproducible conditions, according to relevant standards.

The principle of the method is as follows: on the surface of an agar medium seeded with a standardized inoculum obtained from the strain under test, discs impregnated with antibiotic solutions of a certain concentration are placed at quite equal distances.

If the strain is sensitive to a particular antibiotic, growth will be inhibited on a certain surface around the antibiotic-containing disc, this area being called „the growth inhibition zone".

The procedure is the following: in the first stage, the culture medium is inoculated by sowing in a cloth with a sterile cotton swab that is initially soaked in the inoculum or by flooding with a pipette, a sufficient volume of the inoculum being distributed for obtaining an uniform coverage of the entire surface of the plate.

In order to dry the surface of the medium, the plates are left either at room temperature for 15 minutes at 37 °C [10].

Then follows the individual arrangement of several discs impregnated with antibiotics, by using a sterile forceps on the surface of the culture medium, at a distance of approx. 3 cm from each other and at least 1.5 cm from the edge of the Petri plate. After the discs are placed, the plates are left to rest at room temperature for 20-30 minutes, to get an uniform diffusion of antibiotics in the medium before the multiplication of microorganisms begins. The plates are then incubated for 18-24 hours at 37 °C. The reading of the results should be done by measuring the diameters of the growth inhibition zones determined by different antibiotics, with the help of a ruler or by using callipers.

In our case, in order to minimize the errors, two plates of 15 cm diameter have been used for each determination, 10-12 impregnated discs of 6 mm being placed on each one (with either the ligand or the complex).

2.3. Software used to develop Python multi-paradigm programming language

In order to develop this statistical analysis in Python [10] (which is a multi-paradigm programming language used for mathematical applications), we have loaded and cleaned the data, after which we used Jupyter Notebook (a web application for creating and sharing documents that contain code, visualizations and text) for creating a reproducible analysis. Jupyter Notebook was relevant for our case study, since it can be used for data science, statistical modeling, machine learning and much more, being able to combine plots with mathematical details [11].

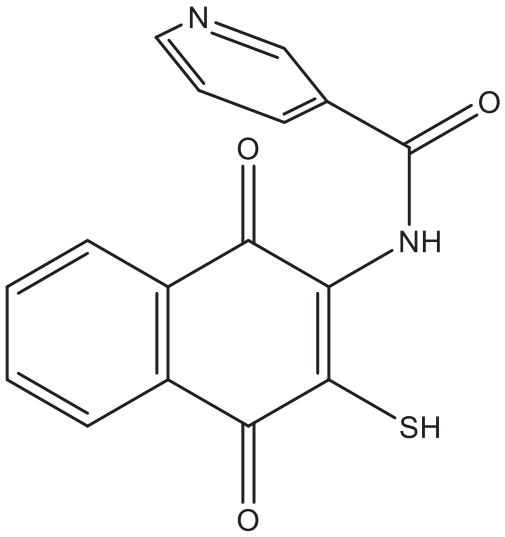
3. RESULTS AND DISCUSSION

3.1. Structure of the free ligand and its Ni(II) complex compound

The structural formulae have been deducted from our previous studies on this ligand and the complex compound formed by it with divalent nickel [8, 9].

The ligand, 2-mercapto-3-niacinamido-1,4-naphthalenedione, is a heterocyclic one, presenting interconvertible conformers due to the possible free turnings around two C–N bonds [8].

Its structure is presented below.



Structural formula of the ligand,
2-mercapto-3-niacinamido-1,4-naphthalenedione

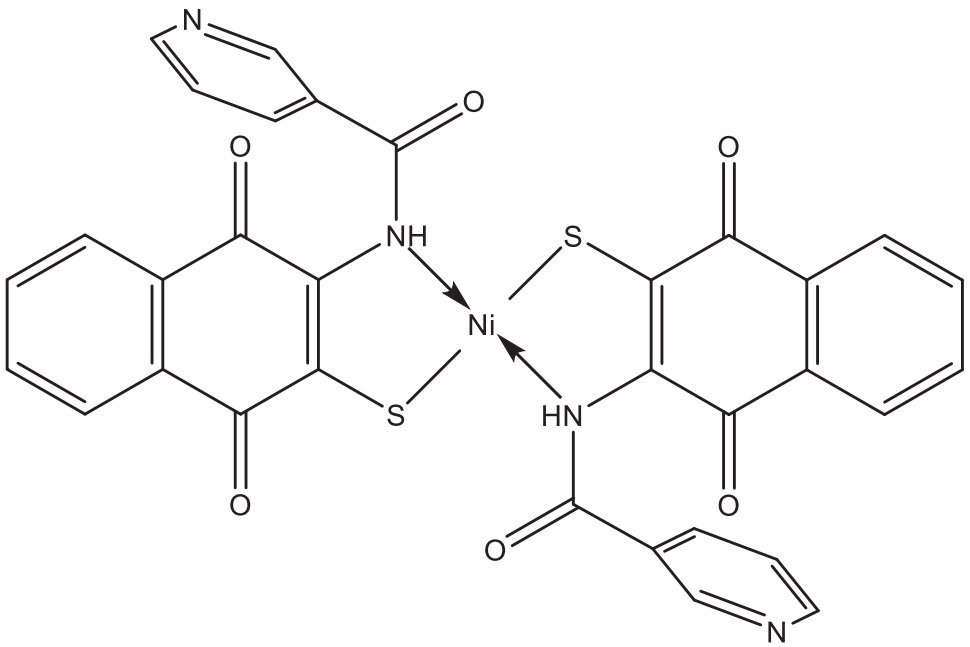
The ligand is a heterocyclic one, being able to change denticity and even to coordinate in several ways to the metal ion due its conformational isomerism, so we have already investigate the actual coordination manner.

Indeed, neglecting the free turnings of the mercapto group around the exocyclic C–S bond, of real interest are the free turnings of niacinamide heterocycle around the exocyclic C–N bond – on one side – and the free turnings of the same heterocycle around the C–N bond in its amidic part – on the other side [8].

The structure of the Ni(II) complex compound is also presented, showing that the ligand coordinates by means of the sulfur atom and the nitrogen atom in the amidic part of the molecule, so it turned out to be a tetracoordinate bis-chelated complex with two sulfur atoms and two nitrogen atoms involved into the coordination process, being of $[MN_2S_2]$-type – *trans* isomer [8].

The complex compound containing two identical bidentate ligands being denoted as [NiL_2] and taking into account that one hydrogen atom was lost by each ligand molecule as a consequence of its coordination to the central divalent transition metal ion, the ligand itself should be denoted as LH.

Its structure is presented below.



Structural formula of the complex compound formed by
2-mercapto-3-niacinamido-1,4-naphthalenedione with Ni(II)

3.2. Kirby-Bauer disk diffusion investigations results

The investigations have been carried out, for both LH and [NiL_2], against eight kinds of microorganisms: four gram-positive and four gram-negative bacteria, the selected microorganisms being the following:

- gram-positive bacteria:
  - (a) - *Streptococcus pneumoniae*;
  - (b) - *Staphylococcus aureus*;
  - (c) - *Enterococcus faecalis*;
  - (d) - *Bacillus anthracis*;
- gram-negative bacteria:
  - (e) - *Escherichia coli;*

- (f) - *Pseudomonas aeruginosa;*
- (g) - *Salmonella typhi;*
- (h) - *Helicobacter pylori.*

Reminding that two plates of 15 cm diameter have been used for each determination, with 10-12 discs of 6 mm diameter placed on each one (impregnated with either the ligand or the complex), it is obvious that we have obtained 20-24 results for the diameter of the growth inhibition zone in each of the 16 experiments.

On this basis, the susceptibility of the microorganisms is evaluated (a bacterium is called „susceptible/sensitive" to the action of an antibiotic if it exhibits a very broad zone of growth inhibition (the drug is more likely to eliminate the infection when administrated in a regular dose); „intermediary susceptible/sensitive" if it exhibits an appreciable, but not very broad zone of growth inhibition (the drug may be effective *in vivo* by topical administration in high concentrations in the organs or tissues where the infection is localized) and, finally, resistant if it exhibits a very small zone of growth inhibition or no inhibition zone at all (administration of the drug is most likely not to remove the infectious agent from the body). However, we must emphasize that the exact limits to define the classification for the susceptibility depends on the kind of bacteria and also on the drug concentration, being labeled in literature.

The experimental findings will be presented here in a form suitable for the purpose of this work, *i.e.*, performing the statistical analysis. Keeping the letters to designate each kind of bacteria, the arrays formed by the read results for the inhibition zone diameter are reported:

```
LH_a = np.array([8.4, 8.3, 7.8, 8.1, 8.2, 7.9, 7.0, 7.2, 7.7,
7.8, 7.9, 7.9, 8.4, 7.2, 8.3, 8.2, 8.2, 8.1, 8.0, 7.9, 7.4])
[NiL2]_a = np.array([14.1, 14.5, 13.9, 14.6, 14.5, 13.6, 13.8,
14.8, 14.0, 14.6, 14.9, 14.3, 14.4, 14.3, 14.0, 14.3, 14.5,
13.8, 13.8, 13.9, 14.4, 14.2])
LH_b = np. array([6.9, 7.1, 7.5, 7.6, 7.5, 6.6, 6.8, 7.8, 7.1,
7.2, 7.3, 7.5, 7.6, 7.9, 7.3, 6.8, 6.9, 7.4, 7.4, 7.3, 6.8, 7.2])
[NiL2]_b = np. array([11.5, 11.0, 11.1, 12.1, 12.3, 12.0, 11.6,
11.5, 11.6, 11.8, 12.3, 12.6, 12.0, 11.5, 11.8, 11.9, 12.2,
12.2, 12.5, 12.4, 11.4, 11.5, 11.6])
LH_c = np. array([7.1, 7.3, 6.9, 7.5, 6.8, 7.1, 7.6, 7.5, 7.6, 6.8,
7.3, 7.6, 6.9, 6.5, 6.8, 7.9, 7.2, 7.2, 7.5, 7.4, 7.4, 6.5, 6.6])
[NiL2]_c = np. array([17.1, 17.5, 16.6, 17.5, 17.6, 16.8, 16.8,
17.1, 17.6, 16.3, 17.8, 17.2, 17.9, 17.4, 17.3, 17.5, 16.4,
17.0, 16.8, 17.0])
```

```
LH_d = np. array([6.9, 7.1, 7.5, 6.6, 7.5, 7.6, 6.8, 6.8, 7.1,
7.6, 6.9, 6.3, 7.8, 7.2, 7.9, 7.4, 7.3, 7.5, 6.4, 7.3, 6.8, 7.2])
[NiL₂]_d = np. array([15.3, 15.0, 14.5, 15.6, 15.5, 15.6, 15.8,
14.8, 15.1, 15.0, 15.2, 15.5, 15.6, 14.9, 15.3, 14.9, 15.5,
15.0, 15.9, 15.4, 15.4])
LH_e = np. array([7.9, 8.6, 8.7, 8.7, 8.8, 8.2, 8.4, 8.4, 8.9,
8.6, 8.6, 7.8, 8.7, 8.1, 8.9, 8.6, 8.9, 8.3, 8.8, 8.8])
[NiL₂]_e = np. array([12.3, 12.9, 12.1, 12.4, 11.8, 12.8, 11.7,
12.1, 12.7, 12.6, 12.8, 12.9, 12.8, 12.6, 12.8, 12.7, 12.1,
12.2, 12.9, 12.7, 12.3, 12.6])
LH_f = np. array([8.8, 8.5, 8.0, 8.1, 8.7, 8.6, 8.8, 9.3, 9.1,
8.6, 8.9, 8.5, 8.0, 8.2, 8.2, 8.9, 8.6, 8.7, 8.5, 8.9, 9.4, 9.0,
8.3, 8.3])
[NiL₂]_f = np. array([11.9, 12.0, 11.5, 11.6, 12.3, 11.8, 11.9,
12.3, 11.5, 11.6, 11.5, 11.6, 11.8, 11.8, 12.1, 12.2, 11.9,
12.0, 11.4, 11.3])
LH_g = np. array([9.9, 9.3, 9.4, 9.4, 9.7, 10.2, 10.4, 9.9, 9.2,
9.2, 10.2, 9.2, 9.3, 9.4, 9.7, 9.9, 9.4, 10.0, 9.7, 9.9])
[NiL₂]_g = np. array([13.8, 14.5, 13.8, 14.1, 13.7, 13.6, 13.8,
14.3, 14.1, 13.6, 13.9, 13.5, 13.8, 14.2, 14.0, 13.9, 13.6,
13.7, 13.5, 13.9, 13.4, 13.4, 13.0, 13.3])
LH_h = np. array([7.0, 6.3, 6.3, 7.7, 6.7, 6.6, 7.0, 6.2, 6.8,
7.4, 6.5, 6.8, 7.1, 6.7, 7.2, 7.7, 7.6, 7.2, 6.8, 7.3, 7.5, 6.7])
[NiL₂]_h = np. array([10.8, 10.1, 10.9, 11.0, 10.5, 10.6, 10.8,
10.8, 10.9, 11.2, 10.3, 10.6, 10.9, 10.5, 11.2, 11.5, 11.0,
11.4, 10.6, 11.1])
```

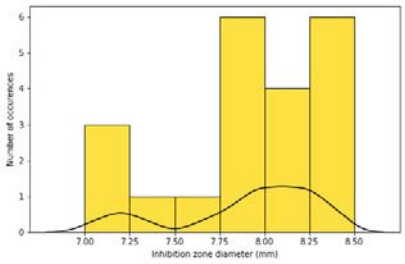
3.3. Python statistical analysis results

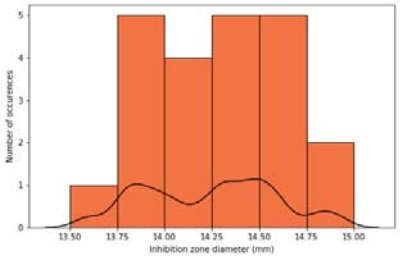
For each array reported above, by using Python multi-paradigm programming language we have determined the following parameters, which are representative in order to minimize the reading errors:

- the mean of the array;
- the median of the array (its second quartile);
- the standard deviation of the array;
- the empirical standard deviation rule interval of the array;
- the variance of the array;
- the confidence interval of the array.

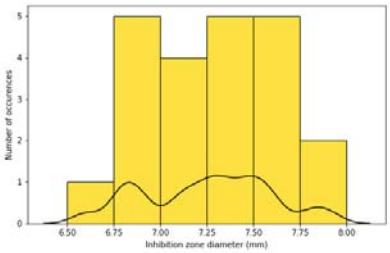
The most significant is considered to be the confidence interval, defined as „an interval computed from the statistics of the sample such that, if the sampling procedure generating the data was repeated, and the confidence interval was re-computed for each random realization, the fraction of such intervals which contains the true population parameter would tend towards 95%”.

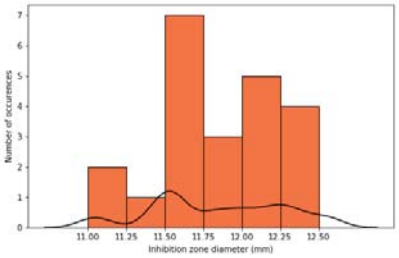
The two figures below present the sixteen situations investigated.



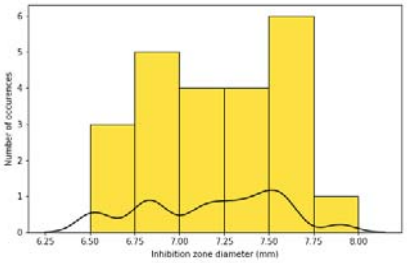


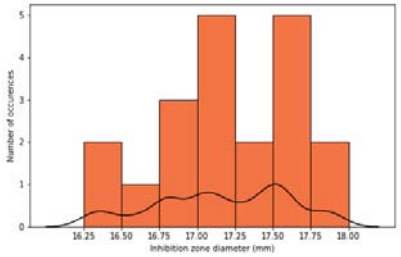
LH_a and [NiL$_2$]_a (a = *Streptococcus pneumoniae*)



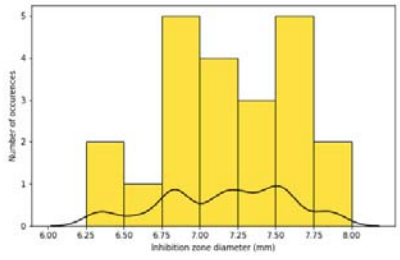


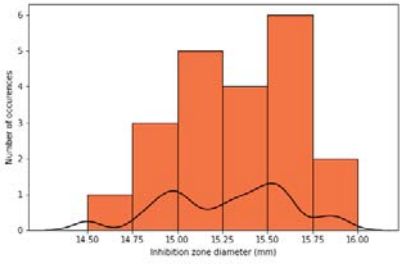
LH_b and [NiL$_2$]_b (b = *Staphylococcus aureus*)





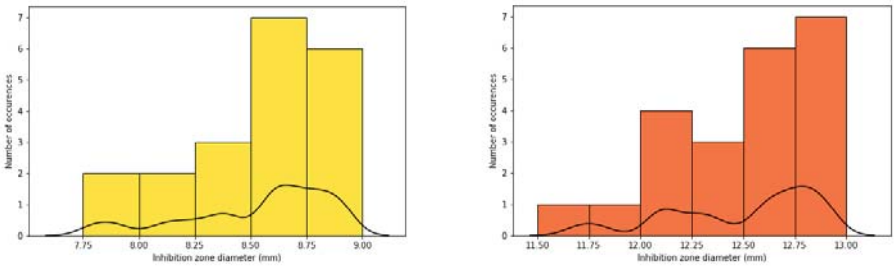
LH_c and [NiL$_2$]_c (c = *Enterococcus faecalis*)



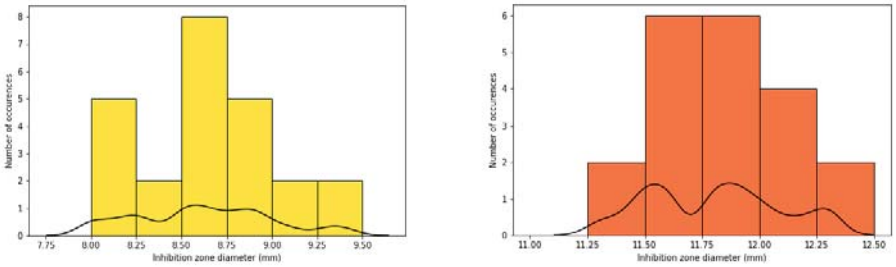


LH_d and [NiL$_2$]_d (d = *Bacillus anthracis*)

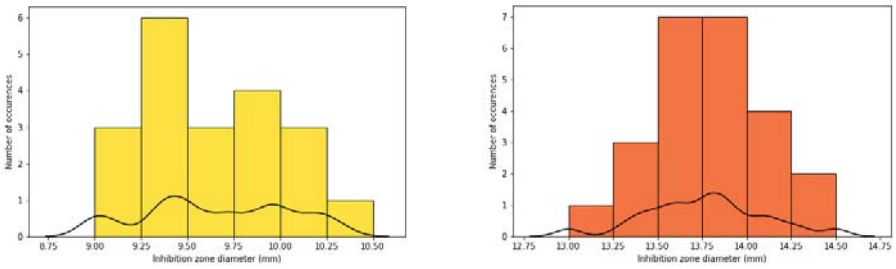
Figure 1. Results of the statistical analysis performed on the arrays containing the read results for the inhibition zone diameter within the experiments in which the ligand, LH (on the left) and its complex compound, [NiL$_2$] (on the right) were tested against the four chosen gram-positive bacteria



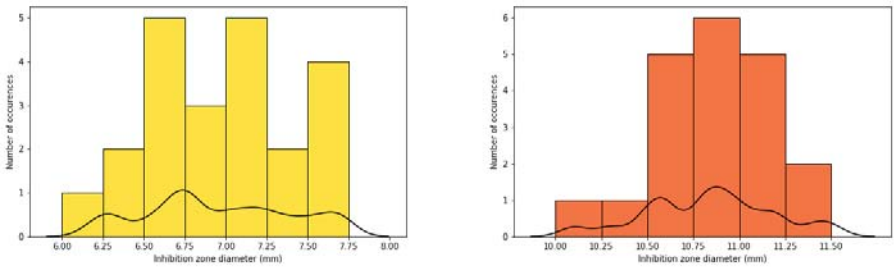
LH_e and [NiL$_2$]_e (e = *Escherichia coli*)



LH_f and [NiL$_2$]_f (f = *Pseudomonas aeruginosa*)



LH_g and [NiL$_2$]_g (g = *Salmonella typhi*)



LH_h and [NiL$_2$]_h (h = *Helicobacter pylori*)

Figure 2. Results of the statistical analysis performed on the arrays containing the read results for the inhibition zone diameter within the experiments in which the ligand, LH (on the left) and its complex compound, [NiL$_2$] (on the right) were tested against the four chosen gram-negative bacteria

Within all plots, the provided curve represents the kernel density estimate (usually denoted as „kde"), which is a metric showing the true distribution of the data across the total value range of the sample points, being independent on the number of bins chosen for plotting.

The results obtained are the following:

LH_a

- **mean: 7.90**
- **median (the second quartile): 8.0**
- **standard deviation: 0.4**
- **empirical standard deviation rule interval: [7.1, 8.7]**
- **variance: 0.16**
- **confidence interval: [7.74, 8.06]**

[NiL_2]_a

- **mean: 14.24**
- **median (the second quartile): 14.3**
- **standard deviation: 0.35**
- **empirical standard deviation rule interval: [13.54, 14.94]**
- **variance: 0.13**
- **confidence interval: [14.11, 14.37]**

LH_b

- **mean: 7.25**
- **median (the second quartile): 7.3**
- **standard deviation: 0.35**
- **empirical standard deviation rule interval: [6.55, 7.95]**
- **variance: 0.12**
- **confidence interval: [7.13, 7.37]**

[NiL_2]_b

- **mean: 11.84**
- **median (the second quartile): 11.8**
- **standard deviation: 0.44**
- **empirical standard deviation rule interval: [10.96, 12.72]**
- **variance: 0.12**
- **confidence interval: [11.72, 11.96]**

LH_c

- **mean: 7.17**
- **median (the second quartile): 7.2**
- **standard deviation: 0.39**
- **empirical standard deviation rule interval: [6.39, 7.95]**
- **variance: 0.15**
- **confidence interval: [7.02, 7.32]**

$[NiL_2]$_c

- **mean: 17.16**
- **median (the second quartile): 17.15**
- **standard deviation: 0.45**
- **empirical standard deviation rule interval: [16.26, 18.06]**
- **variance: 0.2**
- **confidence interval: [16.96, 17.36]**

LH_d

- **mean: 7.16**
- **median (the second quartile): 7.2**
- **standard deviation: 0.43**
- **empirical standard deviation rule interval: [6.3, 8.02]**
- **variance: 0.19**
- **confidence interval: [6.97, 7.35]**

$[NiL_2]$_d

- **mean: 15.28**
- **median (the second quartile): 15.3**
- **standard deviation: 0.36**
- **empirical standard deviation rule interval: [14.56, 16]**
- **variance: 0.13**
- **confidence interval: [15.15, 15.41]**

LH_e

- **mean: 8.54**
- **median (the second quartile): 8.6**
- **standard deviation: 0.33**
- **empirical standard deviation rule interval: [7.88, 9.2]**
- **variance: 0.1**
- **confidence interval: [8.44, 8.64]**

$[NiL_2]$_e

- **mean: 12.49**
- **median (the second quartile): 12.6**
- **standard deviation: 0.36**
- **empirical standard deviation rule interval: [11.77, 13.21]**
- **variance: 0.13**
- **confidence interval: [12.36, 12.62]**

LH_f

- **mean: 8.62**
- **median (the second quartile): 8.6**
- **standard deviation: 0.38**
- **empirical standard deviation rule interval: [7.86, 9.38]**
- **variance: 0.18**
- **confidence interval: [8.44, 8.8]**

$[NiL_2]$_f

- **mean: 11.8**
- **median (the second quartile): 11.8**
- **standard deviation: 0.3**
- **empirical standard deviation rule interval: [11.2, 12.4]**
- **variance: 0.09**
- **confidence interval: [11.71, 11.89]**

LH_g

- **mean: 9.67**
- **median (the second quartile): 9.7**
- **standard deviation: 0.37**
- **empirical standard deviation rule interval: [8.93, 10.41]**
- **variance: 0.14**
- **confidence interval: [9.53, 9.81]**

$[NiL_2]$_g

- **mean: 13.77**
- **median (the second quartile): 13.8**
- **standard deviation: 0.34**
- **empirical standard deviation rule interval: [13.09, 14.45]**
- **variance: 0.12**
- **confidence interval: [13.65, 13.89]**

1. LH_h

- **mean: 6.96**
- **median (the second quartile): 6.9**
- **standard deviation: 0.45**
- **empirical standard deviation rule interval: [6.06, 7.86]**
- **variance: 0.21**
- **confidence interval: [6.75, 7.17]**

2. $[NiL_2]$_h

- **mean: 10.84**
- **median (the second quartile): 10.85**
- **standard deviation: 0.35**
- **empirical standard deviation rule interval: [10.14, 11.54]**
- **variance: 0.13**
- **confidence interval: [10.71, 10.97]**

3.4. Discussions

One may see that the antibacterial activity exhibited by 2-mercapto-3-niacinamido-1,4-naphthalenedione against the selected eight kind of microorganisms has not been proved to be as significant as expected.

More precisely, the gram-positive bacteria could be qualified as „resistant" to the action of this organic compound; however, a slight activity can be observed against the gram-negative ones (this is referring especially to *Salmonella typhi,* but *Pseudomonas aeruginosa* and *Escherichia coli* have also shown an observable growth inhibition zone).

On the other side, nevertheless, the biological activity of the complex compound formed by 2-mercapto-3-niacinamido-1,4-naphthalenedione with divalent nickel tends to be quite appreciable, which seems to show a good absorption of Ni(II) on the inoculated culture media.

Although none of the pathogen agents taken into study proved itself to be sensitive to the presence of the complex compound in the respective inoculated culture medium, they all were moderately (*i.e.*, intermediary) susceptible to its pharmaceutical action.

By looking at the data obtained and processed by the statistical analysis, one may note that, surprisingly, thought all the results could be looked at as satisfactory, the best three good behaviours have been recorded against gram-positive bacteria, namely *Enterococcus faecalis*, *Bacillus anthracis* and *Streptococcus pneumoniae* - in this particular order.

This observation may suggest the idea that the complexation process of 2-mercapto-3-niacinamido-1,4-naphthalenedione to divalent nickel plays a much more significant role in increasing the biological activity as far as gram-positive bacteria are concerned (comparing to the gram-negative ones).

However, taking into account the restricted number of pathogen agents that have been involved in this particular study, as well as the fact that one only coordinative compound has been submitted to it, it seems to be quite hazardous to draw a conclusion in this regard.

4. CONCLUSION

As a conclusion of this analysis, it might be observed that, generally, even though the ligand exhibited a quite reduced antibacterial activity, the antibacterial activity of the nickel complex is stronger than that of the free ligand, indicating a good absorption of the nickel divalent ion (moreover, there have even been some situations in which the investigated

coordinative compound behaved similarly to at least one of the antibiotics considered as standard in the specialized literature).

This means that the complex compound is indubitably suitable for this purpose, having a very consistent performance in terms of eliminating bacteria.

However, until such a substance can be expected to be assimilated to an approved medicine against these pathogen agents, it has to be carefully tested furthermore, in order to establish if it really represents a solution, because of its potential negative effects on the human health.

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