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DOCTORAL SCHOOL OF CHEMISTRY**

ABSTRACT OF THE PhD THESIS

**COMPLEXES WITH AZAMACROCYCLIC LIGANDS
FUNCTIONALIZED WITH HETEROCYCLIC AMIDES –
SYNTHESIS, CHARACTERISATION AND BIOLOGICAL
ACTIVITY**

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The summary is from the thesis
The references are selective
The figures are renumbered

The formaldehyde condensation with a polyamine and a nucleophile species containing at least a reactive hydrogen atom like a carbonyl, amine, amide or an active methylene group generates usually an azamacrocyclic compound [1, 2]. The interest for this kind of compounds arises from both biological and coordinative point of view. Thus a broad spectrum of biological activities was evidenced for these species, including antibacterial [3-5], antifungal [5], antiviral [6] and antitumor [7] respectively.

Besides their biological properties, the polyazamacrocyclic derivatives are very interesting ligands and their complexes exhibit a high kinetic and thermodynamic stability that recommend them for biomedical applications [8, 9]. An improved antibacterial activity in comparison with free ligands was evidenced for such complexes [10, 11].

While the complexes with tetra- and hexaazamacrocyclic ligands have been intensively studied concerning synthesis, properties, crystallographic features as well as biological activity the complexes with pentaazamacrocyclic ligands were less studied. In the last years, the attention in the field was focused on the complexes with azamacrocyclic ligands bearing C- or N-pendant arms that add a new dimensionality to macrocycle unit. The substituents from this kind of species bring an additional group that can assist in coordination, afford an interesting packing in solid state and respectively confer the ability to interact with biological targets through a variety of covalent or non-covalent bonds such as hydrogen bonds, C-H \cdots π and π - π interactions [12].

The one-pot reactions have been widely used for synthesis of such complexes with a transition metal ion as template agent and by using formaldehyde and a variety of aliphatic polyamines and other organic derivatives such as aliphatic [13], aromatic [13] or heterocyclic [14-16] amine, carboxamide [13, 17] or sulfonamide [13, 18] as “capping” fragments.

On the other hand, nicotinamide is a component of nicotinamide adenine dinucleotide, a coenzyme found in all living cells that play an important role in human life [19]. Nicotinamide exhibit anti-inflammatory properties [20] and some complexes with nicotinic unit behave as antitumor [21] or antimicrobial agents [22-24] some of them turning into excellent models for the design of effective antibiotic drug substances.

Objectives

- Synthesis of new Ni(II), Cu(II) and Zn(II) complexes with penta- and hexaazamacrocyclic ligands functionalized with nicotinamide or 2-thioxo-4-thiazolidinone using “one pot” condensation;
- Characterization of complexes by suitable physicochemical methods;

- Antimicrobial and cytotoxic activity establishment.

Thesis content

The thesis entitled "**Complexes with azamacrocyclic ligands functionalized with heterocyclic amides - synthesis, characterization and biological activity**" contains data concerning new saturated azamacrocyclic ligands and their complexes obtained by "one-pot" reaction. The thesis is structured in three chapters.

The first chapter presents a survey of literature data concerning synthesis and characterization of some complexes with saturated monoaza- and poliazamacrocyclic ligands obtained either by template condensation or by direct reaction. Moreover the antiviral, antimicrobial, antitumor or SOD mimetic activity for such complexes are presented in detail.

The second chapter includes the original contributions on the synthesis and characterization of the 32 new complexes of Ni(II), Cu(II) and Zn(II) with penta- and hexaazamacrocyclic ligands functionalized with ketopyridine moiety provided by nicotinamide and of three compound with an acyclic ligand derived from 2-thioxo-4-thiazolidinone. The new azamacrocyclic ligands have been also isolated by the metal ion removal from a complex with sodium sulfide.

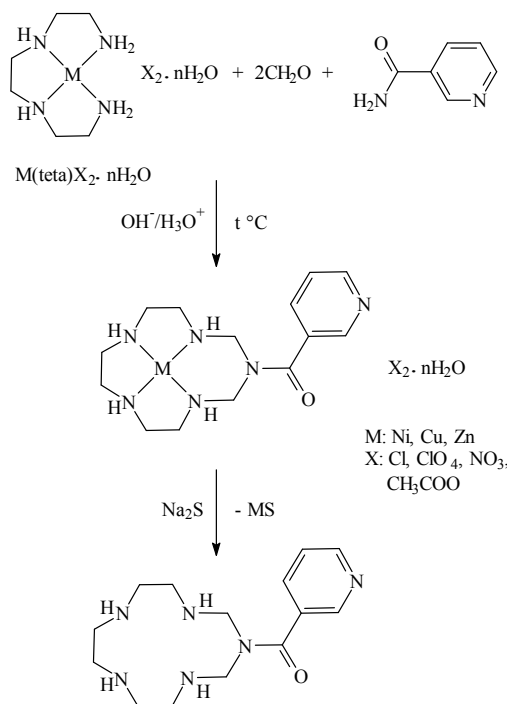
The new complexes and ligands were formulated on the basis of data provided by elemental chemical analysis, ESI-MS, electronic, IR, EPR, ^1H NMR, ^{13}C NMR spectroscopy, thermal analysis, magnetic measurements as well as cyclic voltammetry. The interest concerning this type of compounds comes from the fact that they may have biological activity comparable or improved in comparison with the free ligand. Therefore, the thesis presents the antimicrobial, anti-inflammatory and cytotoxic activity of complexes in comparison with that of ligands.

Chapter three contains data concerning the synthesis, analytical methods and equipments used in the characterization of complexes and corresponding ligands.

II.1. Synthesis of Ni(II), Cu(II) and Zn(II) complexes with pentaazamacrocyclic ligand derived from triethylenetetramine and nicotinamide

The first chapter of the original part of the thesis describes the synthesis of 10 new complexes by template condensation of nicotinamide, triethylenetetramine and formaldehyde assisted by chloride, perchlorate, nitrate or acetate of Ni(II), Cu(II) or Zn(II) (scheme 1). The complexes

and the pentaazamacrocyclic ligand were physico-chemical and biological characterized as presented below.



Scheme 1. The condensation reaction between nicotinamide, triethylenetetramine and formaldehyde in presence of metal salts

The formulation of complexes $[\text{NiL}^1\text{Cl}_2]$ (**1**), $[\text{CuL}^1\text{Cl}_2] \cdot \text{H}_2\text{O}$ (**2**), $[\text{ZnL}^1\text{Cl}_2]$ (**3**), $[\text{NiL}^1(\text{CH}_3\text{COO})_2] \cdot \text{H}_2\text{O}$ (**9**), $[\text{CuL}^1(\text{CH}_3\text{COO})_2] \cdot \text{H}_2\text{O}$ (**10**), was sustained by thermal analysis data. This method indicates that complexes (**1**) and (**3**) are anhydrous while the other species are monohydrated. The thermal behavior of complexes (**1**) and (**9**) is presented in Figure 1 (a and b) [25].

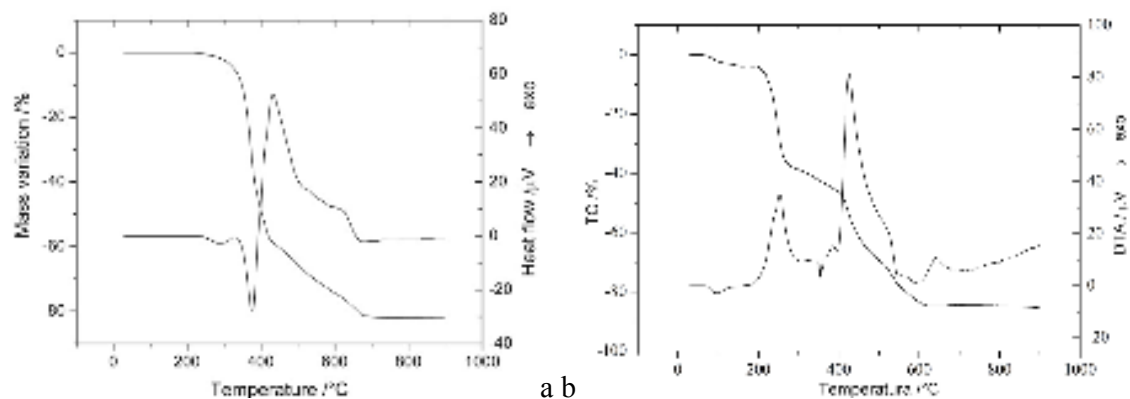


Fig. 1. The derivatograms of the complexes (**1**)–a and (**9**)–b

In the ESI-MS spectra of ligand and complexes $[\text{NiL}^1\text{Cl}_2]$ (**1**), $[\text{CuL}^1\text{Cl}_2]\cdot\text{H}_2\text{O}$ (**2**), $[\text{ZnL}^1\text{Cl}_2]$ (**3**) were identified peaks that correspond to molecular ions as can be seen in Figure 2 for complex (**1**).

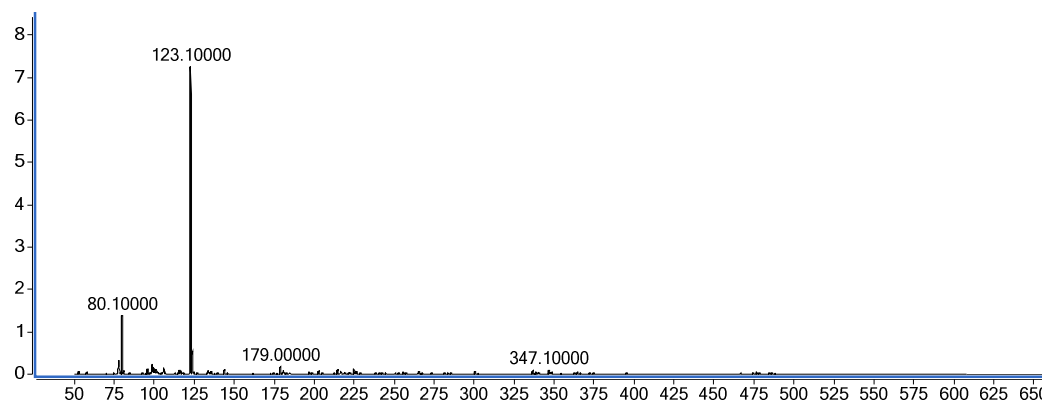


Fig. 2. ESI-MS spectrum of compound $[\text{NiL}^1\text{Cl}_2]$ (**1**)

In the ESI-MS spectra of the complexes the molecular ions were identified as $[\text{NiC}_{14}\text{H}_{24}\text{N}_6\text{O}]^+$ at 347.10; $[\text{CuC}_{14}\text{H}_{21}\text{N}_6\text{O}]^+$ at 352.10 and $[\text{ZnC}_{14}\text{H}_{24}\text{N}_6\text{O}+\text{CN}+3\text{H}]^+$ at 386.80. Another fragments such $[\text{C}_6\text{H}_7\text{N}_2\text{O}]^+$ (m/z: 123.10) and $[\text{C}_5\text{H}_6\text{N}]^+$ (m/z: 80.00) from the fragmentation of the ligand were identified in spectra of all compounds.

The condensation process was confirmed by the data obtained from IR spectra. This method provided also information concerning the anion role as unidentate ligand (e.g. $[\text{NiL}^1(\text{CH}_3\text{COO})_2]\cdot\text{H}_2\text{O}$ (**9**)) or free ion (e.g. $[\text{CuL}^1(\text{OH}_2)](\text{ClO}_4)_2\cdot 5\text{H}_2\text{O}$) [26-31].

The ^1H NMR spectrum of ligand reveals the characteristic pattern of protons from pyridyl group that generate signals in the range 7.85-8.93 ppm. The protons for ethylene and methylene groups were identified by the triplet at 3.11 ppm and the singlet at 3.87 ppm respectively. The secondary amine group was identified by signal at 1.90 ppm. The characteristic pattern for all groups was identified in the ^{13}C NMR spectrum as well.

These signals appear in the complex $[\text{ZnL}^1\text{Cl}_2]$ (**3**) spectrum with the mention that the peak assigned to secondary amine group is downfield shifted as result of coordination [27].

The electronic spectra of Ni(II) complexes display the three characteristic bands for Ni(II) in an octahedral surrounding [32] and the values of the magnetic moments and of the crystal field parameters are in good agreement with this geometry. The electronic spectra for Cu(II) complexes show a broad band characteristic to a square-pyramidal stereochemistry. The values in the range 1.85-2.04 BM for the magnetic moment of these complexes suggests no interactions between paramagnetic centers.

The powder EPR spectra at room temperature of the Cu(II) complexes confirmed the proposed geometry. The spectra of complexes (5) and (7) are according with an axial symmetry with the hyperfine structure well resolved (fig. 3 a and b).

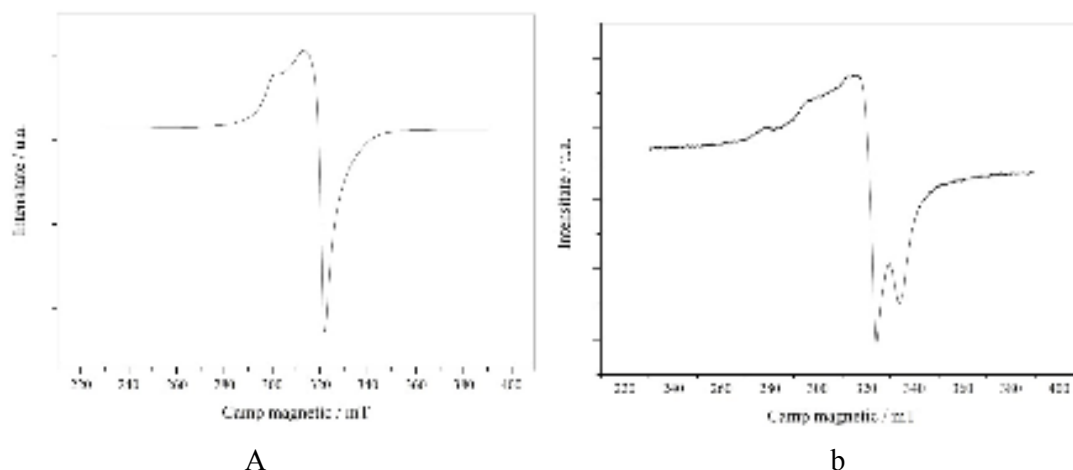
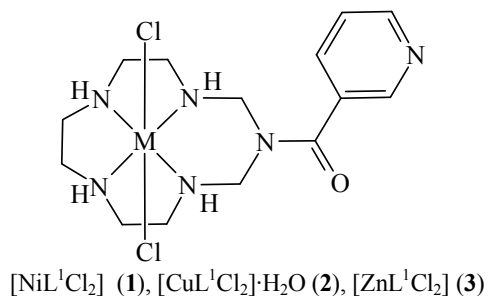


Fig. 3. Powder EPR spectra of complexes $[\text{CuL}^1(\text{OH}_2)](\text{ClO}_4)_2 \cdot 5\text{H}_2\text{O}$ (5) and $[\text{CuL}^1(\text{OH}_2)](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (7)

The redox behavior of the ligand and complexes $[\text{NiL}^1\text{Cl}_2]$ (1), $[\text{CuL}^1\text{Cl}_2] \cdot \text{H}_2\text{O}$ (2), $[\text{ZnL}^1\text{Cl}_2]$ (3) were studied by cyclic voltammetry and the informations provided indicate that the complexes with pentaazamacrocyclic ligand are reduced at more negative potential in comparison with DMSO solvated ions [33].

Taking into account the results provided by the chemical analysis, ESI-MS, electronic, IR, EPR, ^1H NMR, ^{13}C NMR spectra, thermal analysis, magnetic measurements and cyclic voltammetry the coordination for complexes (1) – (10) were proposed as presented in Figure 4.



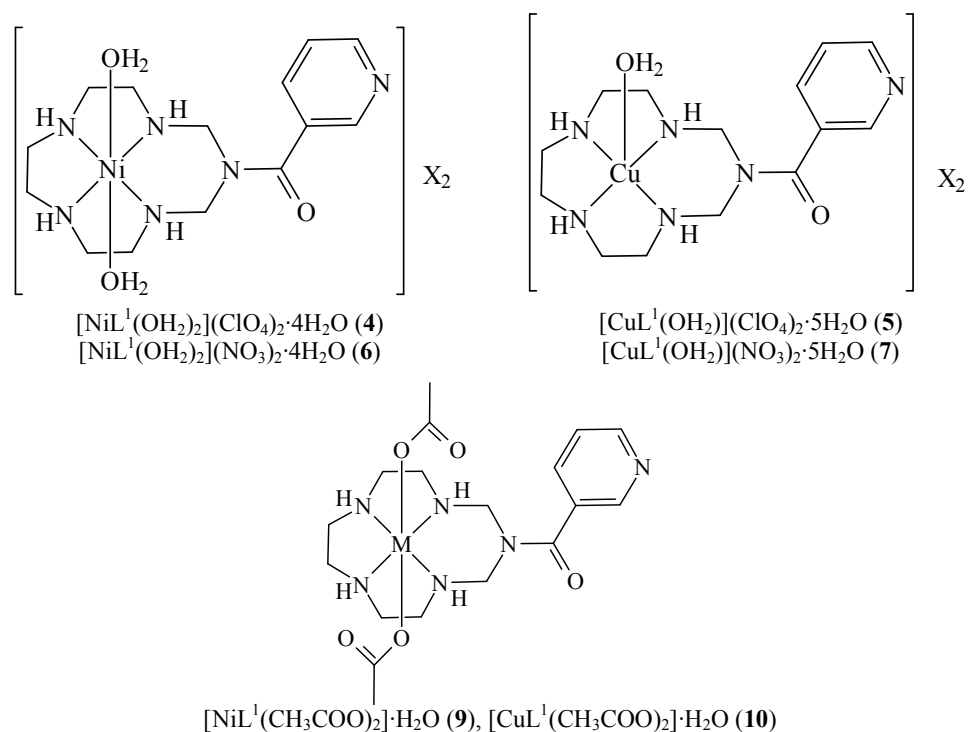
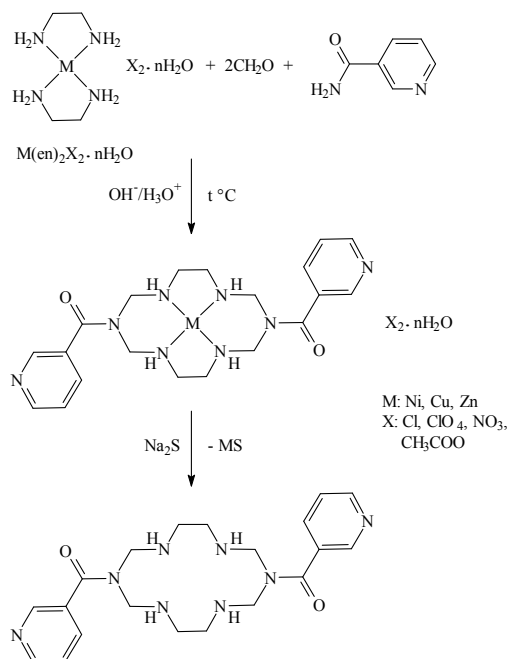


Fig. 4. The coordination proposed for the complexes (**1**) – (**10**)

The antimicrobial activity of the compounds was assayed against the reference and clinical microbial strains: *P. aeruginosa* 1397, *E. coli* ATCC 25922 and 714, *E. cloacae* 61R, *S. aureus* 0364, *B. subtilis* 12488, and *C. albicans* 249 and 10231. The complex (**2**) exhibits a good anti-fungal activity against the two *C. albicans* strains with a MIC value of 125 and 62.5 $\mu\text{g mL}^{-1}$ while the complex (**5**) against *B. subtilis* strain with a MIC value of 250 $\mu\text{g mL}^{-1}$. The cytotoxic assays were performed on Hep 2 (human cervical carcinoma derived from HeLa) and HT 29 (human colorectum adenocarcinoma) cancer cells. The evaluation of the cytotoxicity showed that Cu(II) complexes exhibit an effective cytotoxic activity by inducing the cellular cycle arrest in the G2/M phase.

II.2. Synthesis of Ni(II), Cu(II) and Zn(II) complexes with hexaazamacrocyclic ligand derived from ethylenediamine and nicotinamide

The second subchapter of the thesis describes the synthesis of 10 new complexes by template condensation of nicotinamide, ethylenediamine and formaldehyde in presence of metal salts (scheme 2). The complexes and the hexaazamacrocyclic ligand were studied from the perspective of their physico-chemical characterization and biological activity assessment.



Scheme 2. The condensation reaction between nicotinamide, ethylenediamine and formaldehyde in presence of metal salts

The complexes $[\text{NiL}^2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$ (**11**), $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**), $[\text{ZnL}^2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**13**), $[\text{Cu}_2\text{L}^2(\text{CH}_3\text{COO})](\text{CH}_3\text{COO})_3 \cdot 3\text{H}_2\text{O}$ (**19**), $[\text{ZnL}^2(\text{CH}_3\text{COO})_2]$ (**20**) were characterized by thermal analysis. By using this method, it was observed that complex (**20**) is anhydrous while the others compounds are hydrated species. This fact was confirmed by the mass loss in the first and second step of the complexes thermal decomposition (Fig. 5 a and b) [25].

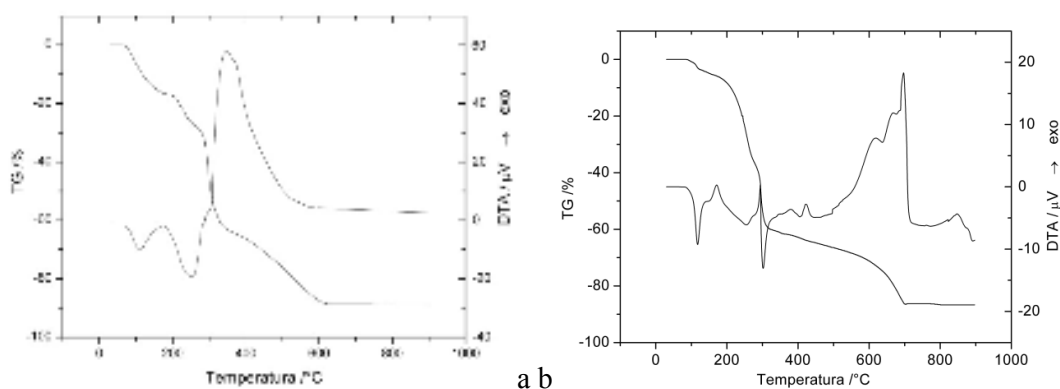


Fig. 5. The derivatograms of the complexes (**11**)–a and (**12**)–b

The ESI-MS spectra of the complexes $[\text{NiL}^2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$ (**11**), $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**), $[\text{ZnL}^2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**13**) show peaks that correspond to molecular ions such as $[\text{M} + 2\text{H}_2\text{O} + \text{H}]^+$ for

$[\text{NiL}^2\text{Cl}_2]\cdot 6\text{H}_2\text{O}$ (**11**), $[\text{M}+\text{H}_2\text{O}+3\text{H}]^+$ for $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2\cdot 0,5\text{H}_2\text{O}$ (**12**) and $[\text{M}-2\text{Cl}+\text{O}+\text{H}]^+$ for $[\text{ZnL}^2]\text{Cl}_2\cdot \text{H}_2\text{O}$ (**13**).

The IR spectra provide informations concerning both condensation process and ligands (macrocyclic, oxoanion or acetate) coordination.

The ^1H RMN spectrum of complex $[\text{ZnL}^2]\text{Cl}_2\cdot \text{H}_2\text{O}$ (**13**) shows signals that can be assigned to the protons of secondary amine (2.07 ppm), ethylene (2.67 ppm), methylene (3.06 ppm) and pyridyl groups (7.66-9.09 ppm) respectively.

The electronic spectra indicate that the metal ions adopt several types of stereochemistry such as octahedral for Ni(II) complexes and square–pyramidal for Cu(II) complexes [32]. These conclusions are confirmed by both the values of the magnetic moments and the values of the crystal field parameters for octahedral Ni(II) complexes.

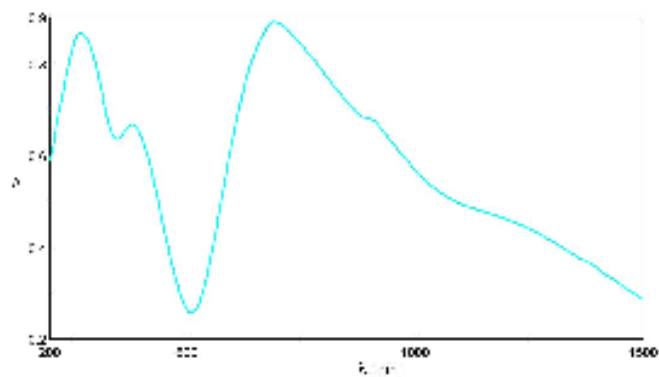


Fig. 6. Electronic spectrum of complex $[\text{Cu}_2\text{L}^2(\text{CH}_3\text{COO})](\text{CH}_3\text{COO})_3\cdot 1.5\text{H}_2\text{O}$ (**19**)

It is worth to mention that in the electronic spectrum of complex $[\text{Cu}_2\text{L}^2(\text{CH}_3\text{COO})](\text{CH}_3\text{COO})_3\cdot 1.5\text{H}_2\text{O}$ (**19**) (fig. 6), that is characteristic for a square-pyramidal stereochemistry, a new band that appear at 374 nm may be assigned to a simultaneous excitation of two copper ions from a dimer species. This data is sustained by the EPR spectrum also [34].

The EPR spectrum of complex $[(\text{CuL}^2)_2(\text{CH}_3\text{COO})](\text{CH}_3\text{COO})_3\cdot 1.5\text{H}_2\text{O}$ (**19**) displays the pattern for a rhombic distorted stereochemistry with $g_{\text{iso}} = 2.233$ and a signal with low intensity at half field characteristic for dimer species (fig. 7).

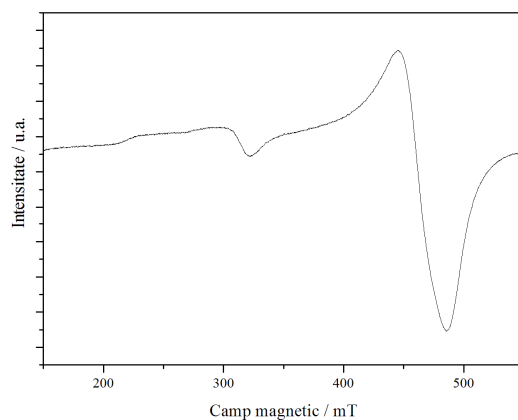
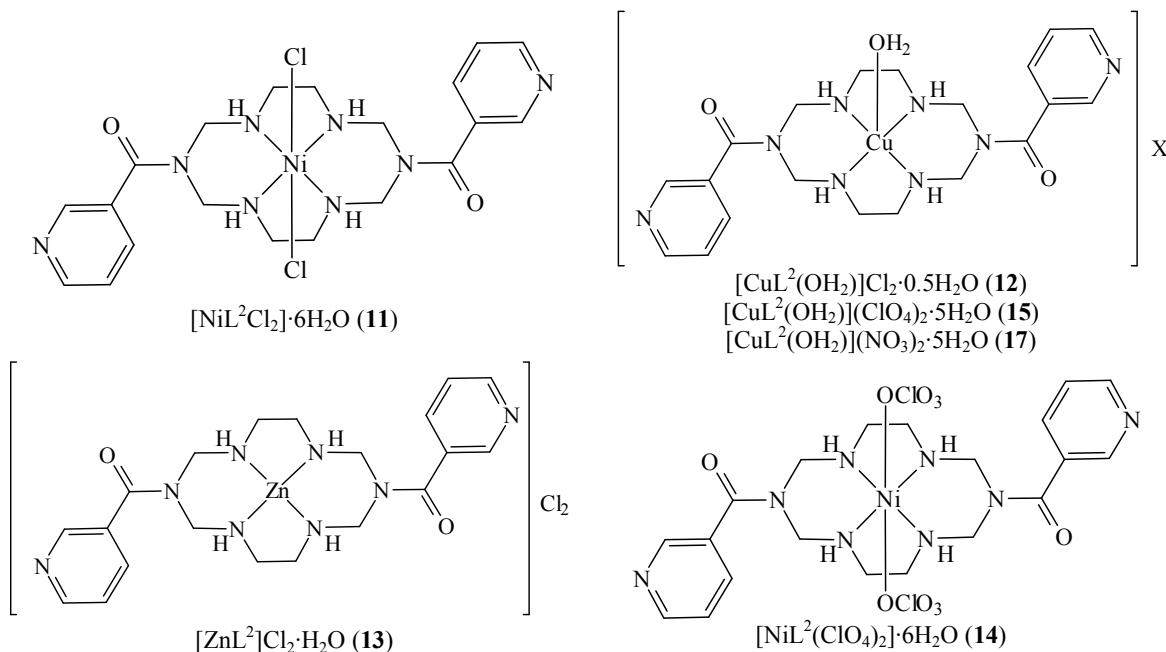


Fig. 7. Powder EPR spectrum of complex $[(\text{CuL}^2)_2(\text{CH}_3\text{COO})](\text{CH}_3\text{COO})_3 \cdot 1,5\text{H}_2\text{O}$ (**19**)

The electrochemical behavior of the ligand and complexes $[\text{NiL}^2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$ (**11**), $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**) and $[\text{ZnL}^2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**13**) studied by cyclic voltammetry provided information concerning reduction and oxidation processes and indicates the fact that the complexes with hexaazamacrocyclic ligand have a different behavior [33].

Taking into account the results provided by the analytical and physico-chemical methods it were proposed the coordination for complexes (**11**)–(**20**), presented in figure 8.



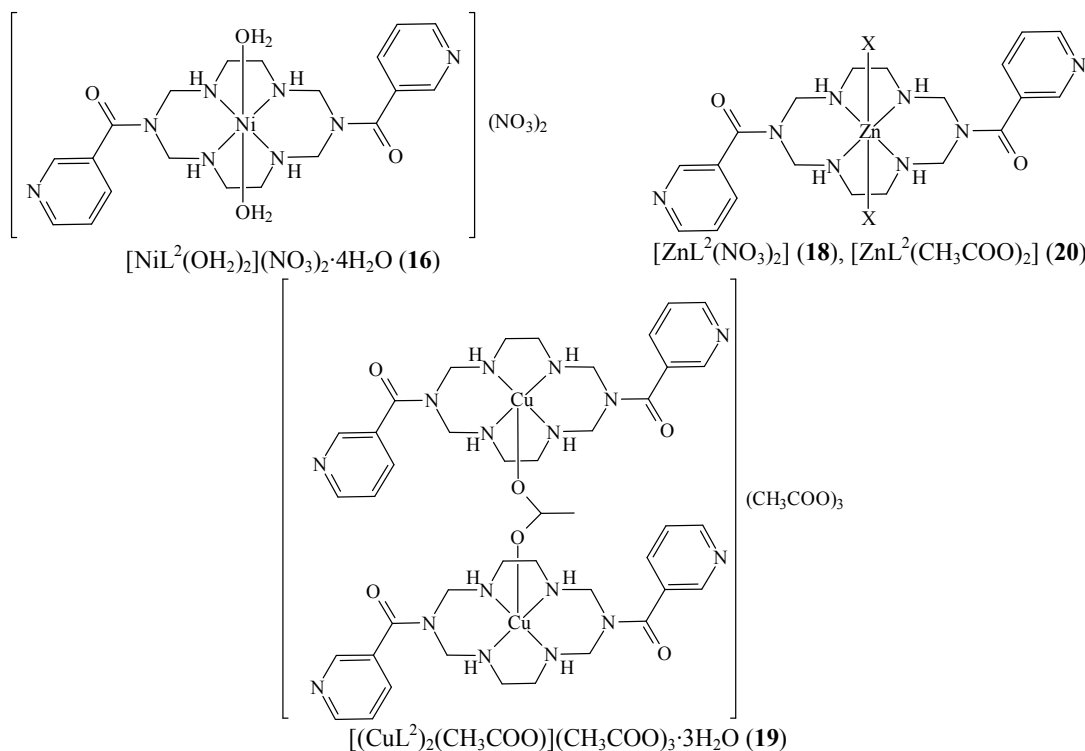


Fig. 8. The coordination proposed for the complexes (**11**) – (**20**)

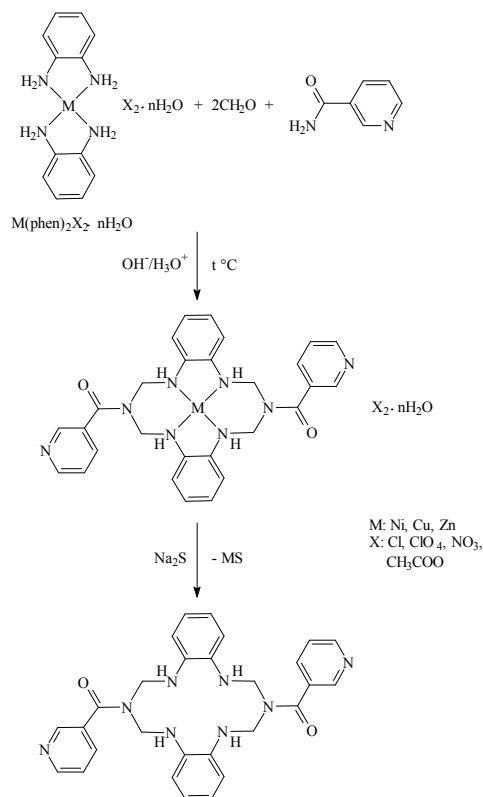
The complexes were also tested to determine the antimicrobial activity on pathological strains Gram negative (*Pseudomonas aeruginosa* 1397, *Escherichia coli* ATCC 25922 and 714, *Klebsiella pneumoniae* 2968, *Enterobacter cloacae* 61R), Gram positive (*Staphylococcus aureus* 0364, *Bacillus subtilis* 12488, *Enterococcus faecalis* ATCC 29212) and fungal strains *Candida albicans* 249 și 10231. In the case of bacterial strains, complexes exhibit a good activity against *B. subtilis*, with a MIC value of $250 \mu\text{g mL}^{-1}$ for $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**), $[\text{ZnL}^2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**13**), $[\text{NiL}^2(\text{ClO}_4)_2] \cdot 6\text{H}_2\text{O}$ (**14**), $[\text{ZnL}^2(\text{NO}_3)_2]$ (**18**) și $[\text{ZnL}^2(\text{CH}_3\text{COO})_2]$ (**20**) and $125 \mu\text{g mL}^{-1}$ for compound $[\text{NiL}^2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$ (**11**). For compound $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**) was also observed a good activity against fungal strains *C. albicans* 10231 with a MIC value of $62,5 \mu\text{g mL}^{-1}$ and *C. albicans* 249.

All the species that can inhibit the growth of microorganisms also have the ability to inhibit their adherence to inert substratum at the inhibitory concentration.

All the copper compounds were tested for cytotoxicity and the results showed that the cytotoxicity on Hep 2 and HT 29 cells was enhanced for complexes $[\text{CuL}^2(\text{OH}_2)]\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ (**12**) and $[\text{CuL}^2(\text{OH}_2)](\text{NO}_3) \cdot 5\text{H}_2\text{O}$ (**17**), which showed an increase of cells blocked in the G2 / M phase, and the appearance of a subpeak associated with apoptosis.

II.3. Synthesis of Ni(II), Cu(II) and Zn(II) complexes with pentaazamacrocyclic ligand derived from 1,2-phenylenediamine and nicotinamide

This part of the thesis describes the synthesis of 12 new complexes by template condensation of nicotinamide, 1,2-phenylenediamine and formaldehyde as presented in Scheme 3.



Scheme 3. The condensation reaction between nicotinamide, 1,2-phenylenediamine and formaldehyde in presence of metal salts

The formulation proposed for complexes $[\text{NiL}^3]\text{Cl}_2 \cdot 4.5\text{H}_2\text{O}$ (**21**), $[\text{CuL}^3]\text{Cl}_2$ (**22**), $[\text{ZnL}^3]\text{Cl}_2$ (**23**), was confirmed by the data provided by thermal analysis. Using this method, it was observed that complexes (**22**) and (**23**) are anhydrous, while the other compound contain water molecules (Fig. 13 a and b). After water molecule elimination the oxidative degradation of the macrocyclic ligand occurs in one or two steps with generation the metallic oxide as residue [25].

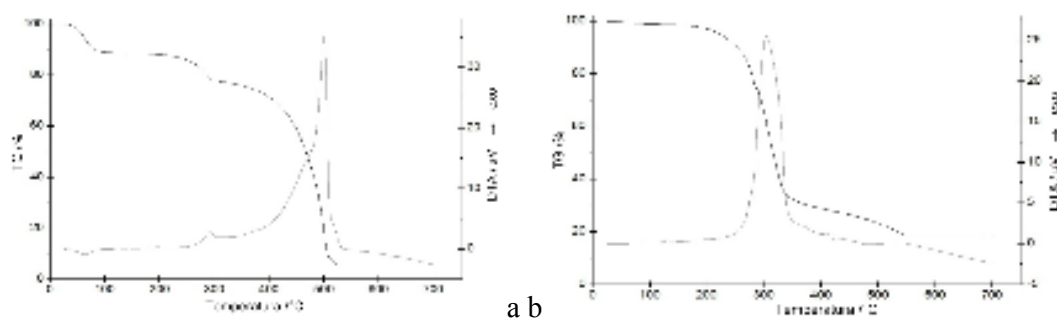


Fig. 9. The derivatograms of the complexes (21)–a and (22)–b

In the ESI-MS spectra for ligand and complexes $[\text{NiL}^3]\text{Cl}_2 \cdot 4.5\text{H}_2\text{O}$ (**21**), $[\text{CuL}^3]\text{Cl}_2$ (**22**), $[\text{ZnL}^3]\text{Cl}_2$ (**23**) were identified peaks that correspond to molecular ions $[\text{NiC}_{28}\text{H}_{21}\text{N}_8\text{O}_2 + 2\text{CN}]^{2+}$, $[\text{CuC}_{28}\text{H}_{22}\text{N}_8\text{O}_2\text{Cl} + \text{CN}]^+$ and $[\text{ZnC}_{28}\text{H}_{19}\text{N}_8\text{O}_2]^{2+}$. The ligand fragmentation generates $[\text{C}_9\text{H}_{19}\text{N}_6]^+$ (m/z : 211.10) and $[\text{C}_7\text{H}_5\text{N}_2\text{O}]^+$ (m/z : 133.10) that appear in the spectra of all complexes (fig. 10).

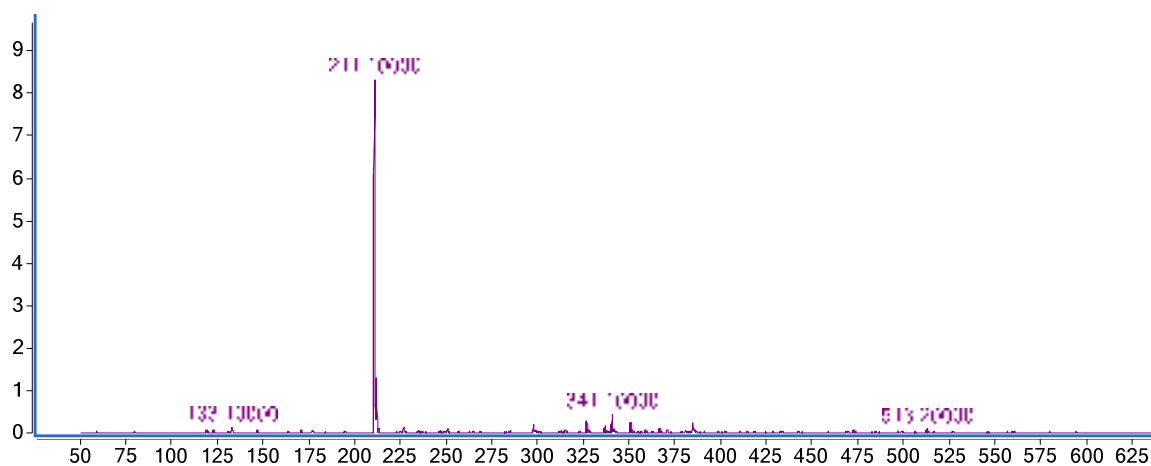


Fig. 10. ESI-MS spectrum of compound $[\text{NiL}^3]\text{Cl}_2 \cdot 4.5\text{H}_2\text{O}$ (**21**)

The condensation process is confirmed by the data obtained from IR spectra. This method offers additional information concerning the presence of anions as ligands (e.g. $[\text{CuL}^3(\text{CH}_3\text{COO})_2] \cdot \text{H}_2\text{O}$ (**31**)) or as free ions (e.g. $[\text{NiL}^3(\text{OH}_2)_2](\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (**27**)) [26-31].

In the ligand ^1H RMN spectrum were observed corresponding signals for the protons of ethylene, methylene, pyridyl and phenyl groups respectively. The signal assigned to protons from the secondary amine is downfield shifted, indicating the coordination of this group to metal ion [27]. The ^{13}C NMR spectra show the characteristic signals for all carbon atoms from the backbone of the macrocyclic ligand.

The electronic spectra of the complexes indicate that the metal ions adopt different geometries: octahedral for complexes $[\text{CuL}^3(\text{ClO}_4)_2] \cdot 2\text{H}_2\text{O}$ (**25**), $[\text{NiL}^3(\text{OH}_2)_2](\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (**27**) and $[\text{CuL}^3(\text{CH}_3\text{COO})_2] \cdot \text{H}_2\text{O}$ (**31**) and square-planar for the rest of the compounds [32].

The square-planar stereochemistry proposed for Ni(II) complexes is confirmed by the diamagnetic behaviour of these complexes.

The powder EPR spectra at room temperature of the Cu(II) complexes confirmed the proposed geometry. The EPR spectra of complex $[\text{CuL}^3](\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (**28**) reveals an isotropic without hyperfine structure (fig. 11)

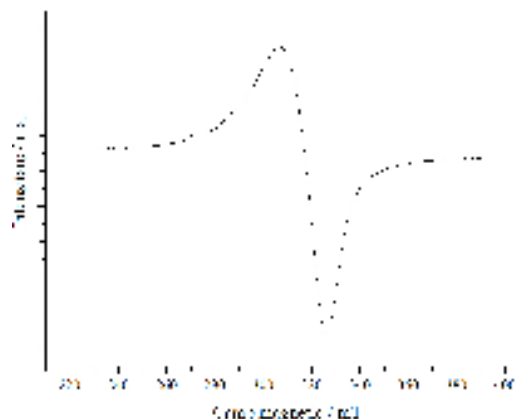
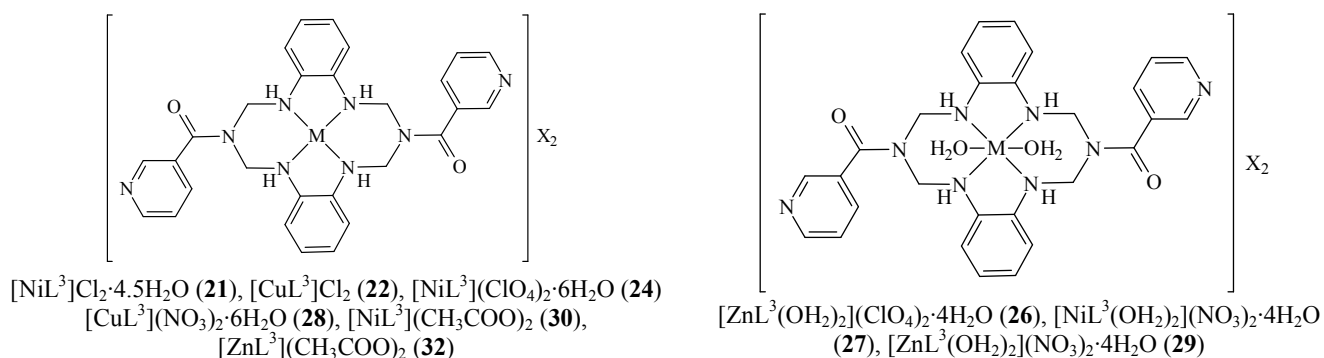
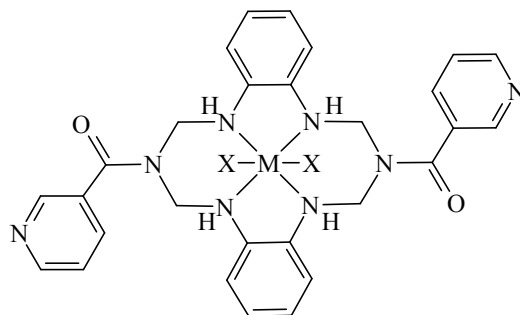


Fig. 11. Powder EPR spectrum of complex $[\text{CuL}^3](\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (**28**)

The redox properties of the ligand and complexes $[\text{NiL}^3]\text{Cl}_2 \cdot 4.5\text{H}_2\text{O}$ (**21**), $[\text{CuL}^3]\text{Cl}_2$ (**22**), $[\text{ZnL}^3]\text{Cl}_2$ (**23**) were studied by cyclic voltammetry and the data obtained from this analysis indicates that the hexaazamacrocyclic ligand stabilizes better the oxidation state (II) of metal ions [33].

Based on the results provided by the all analytical and physico-chemical data were proposed the coordination for complexes (**21**) – (**32**) as presented in Figure 12.





[ZnL³Cl₂] (**23**), [CuL³(ClO₄)₂] \cdot 2H₂O (**25**),
[CuL³(CH₃COO)₂] \cdot H₂O (**31**)

Fig. 12. Proposed coordination for complexes (**21**)-(32)

The ligand and the complexes were tested against pathological strains in order to assess the antimicrobial activity. The results show that the complexes from this set have a better activity than the free ligand against a large number of bacterial strains. It was observed a good antifungal activity for compound [ZnL³(OH₂)₂](ClO₄)₂ \cdot 4H₂O (**26**) with a MIC value 62.5 μ g mL⁻¹ in case of *C. albicans* 10231.

All the species that can inhibit the growth of micro-organisms also have the ability to inhibit adherence of microorganisms to inert substratum at the inhibitory concentration and also in case of *E. faecalis* ATCC 29212 where the complex [ZnL³(OH₂)₂](ClO₄)₂ \cdot 4H₂O (**26**) inhibit the adherence to inert substratum at 62.5 μ g mL⁻¹.

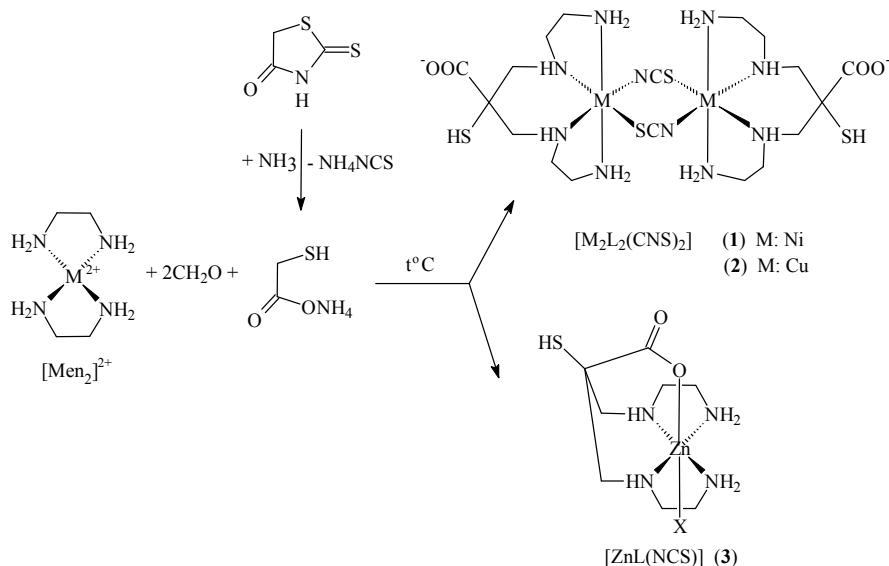
The complexes [CuL³]Cl₂ (**22**), [ZnL³Cl₂] (**23**), [ZnL³(OH₂)₂](ClO₄)₂ \cdot 4H₂O (**26**) and [ZnL³](CH₃COO)₂ (**32**) were investigated with the aim to determine the anti-inflammatory activity. These studies in rat acute inflammation induced by dextran, and kaolin showed anti-inflammatory effect for the [CuL³]Cl₂ (**22**) comparable with that of diclofenac.

All the copper compounds were tested for cytotoxicity assay and the results showed that the cytotoxicity activity on Hep 2 and HT 29 cells was higher for complexes [CuL³]Cl₂ (**22**) and [CuL³](NO₃)₂ \cdot 6H₂O (**28**), which presented an increase of cells blocked the G2 / M, and the appearance of a subpeak associated with apoptosis.

II.4. Synthesis of Ni(II), Cu(II) and Zn(II) complexes with acyclic ligand derived from ethylenediamine and 2-thioxo-4-thiazolidinone

This section presents the synthesis of three new complexes by template condensation of ethylenediamine, 2-thioxo-4-thiazolidinone and formaldehyde in presence of Ni(II), Cu(II) and Zn(II). It was observed that during the condensation the 2-thioxo-4-thiazolidinone

hydrolyze and the thioglycolic acid represent the nucleophile involved in condensation (scheme 4).



Scheme 4. Synthesis of complexes (33)-(35) their proposed formulation

The formulation proposed for $[NiL^4(NCS)]_2 \cdot H_2O$ (33), $[CuL^4SCN]_2 \cdot 9H_2O$ (34) and $[ZnL^4NCS] \cdot 0.5H_2O$ (35) based on chemical analysis was confirmed by the data obtained from the thermal analysis. The TG and DTA curves evidenced the crystallization nature of water molecules, the steps required for degradation of the organic ligand and thiocyanate respectively (Fig. 13 a and b) [35, 36].

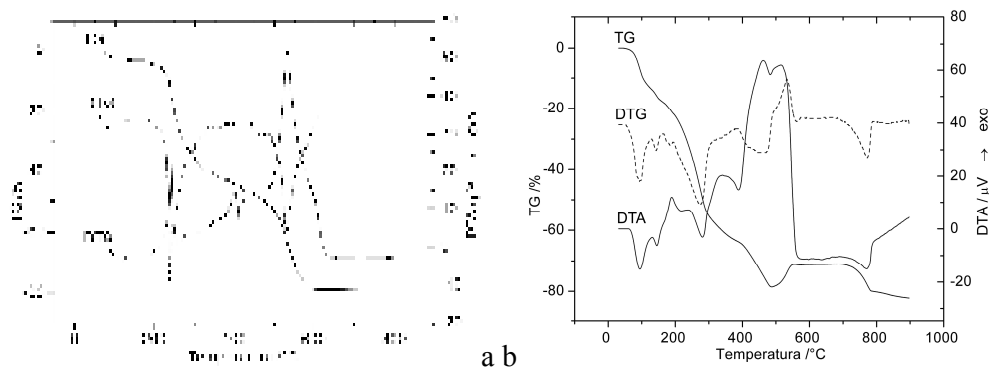


Fig. 13. The derivatograms of the complexes (32)-a and (34)-b

The complexes formulation was confirmed also by the ESI-MS data by identification the molecular ions as $[M+CH_3CN]^+$ for compound $[NiL^4NCS]_2 \cdot H_2O$ (33), $[M+H]^+$ for $[CuL^4SCN]_2 \cdot 9H_2O$ (34), and $[M+H]^+$ for $[ZnL^4NCS] \cdot 0.5H_2O$ (35).

The IR spectra provide information concerning the condensation process and the coordination mode of thiocyanate as unidentate (e.g. $[\text{ZnL}^4\text{NCS}]\cdot 0.5\text{H}_2\text{O}$ (**35**)) or as bridge (e.g. $[\text{NiL}^4\text{NCS}]_2\cdot \text{H}_2\text{O}$ (**33**)) [26-31].

In the ^1H RMN spectrum of complex $[\text{ZnL}^4\text{NCS}]\cdot 0.5\text{H}_2\text{O}$ (**35**) were observed corresponding signals for the protons from the primary and secondary amine at 5.76 and 1.90 ppm, ethylene and methylene at 2.69 ppm and 3.25 ppm and SH group at 1.25 ppm [27].

The electronic spectra of the complexes suggest the octahedral environment of metallic ions [32], sustained by the value of the magnetic moments for Ni(II) complex and by EPR spectrum for Cu(II) complex. The value 1.42 BM for the magnetic moment of the Cu(II) complex suggest an interactions between paramagnetic centers at room temperature.

EPR spectra of Cu(II) complexes reveal information concerning both local surrounding of metal ion and monomeric or dimeric structure. The X-band EPR spectrum of powder complex (**2**), shown in Fig. 14, exhibits at room temperature an asymmetric, large signal with $g_{\text{av}} = 2.14$, characteristic to an orthorhombic distorted local symmetry for copper ion. On lowering the temperature to 100 K the EPR spectrum corresponds to an axial symmetry ($g_{\parallel} = 2.33$, $g_{\perp} = 2.09$). The double integration of EPR signal is proportional to sample magnetic susceptibility. Thus, its temperature dependence evidences the magnetic behaviour. Inset Fig. 14 is presented The inverse of double integrated intensity of EPR spectra versus temperature. The linear fit correspondence to a weak antiferromagnetic coupling between copper(II) ions.

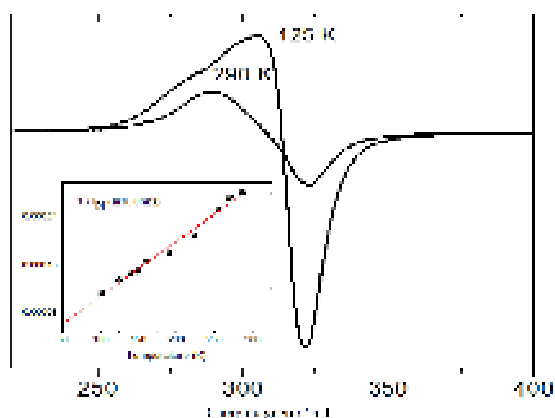


Fig. 14. X-band EPR spectrum of complex $[\text{CuL}^4\text{SCN}]_2\cdot 9\text{H}_2\text{O}$ (**34**); inset of figure is given the temperature dependence of inverse double integrated intensity

The antimicrobial activity of the compounds was tested against pathological strains isolated from clinical and reference strains, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*,

C. albicans and *B. subtilis* and the results showed a very good antimicrobial activity for complexes $[\text{CuL}^4\text{SCN}]_2 \cdot 9\text{H}_2\text{O}$ (**34**), with a MIC value of $1.95 \mu\text{g mL}^{-1}$ in the case of *P. aeruginosa* 1246.

Conclusions

- The chapter that presents the original scientific research of the thesis describes the synthesis and characterization of 32 new complexes of Ni(II), Cu(II) and Zn(II) with penta- and hexaazamacrocyclic ligands functionalized with nicotinamide and three new complexes with an acyclic ligand functionalized with 2-thioxo-4-thiazolidinone. All of them were screened for biological activity on various bacterial and fungal strains and tumor cell cytotoxicity on Hep 2 and HT 29.

- Complexes were synthesized by condensation reaction, from systems that contain metallic ions, triethylenetetramine / ethylenediamine/ o-phenylenediamine, formaldehyde and nicotinamide / 2-thioxo-4-thiazolidinone. Three macrocyclic ligands have been isolated from one of these complexes by treatment with sodium sulfide.

- The characterization of the complexes and ligands were based on data provided by elemental chemical analysis, thermal analysis, ESI-MS, electronic, IR, EPR, ^1H NMR, ^{13}C NMR spectroscopy, magnetic susceptibility and cyclic voltammetry at room temperature.

ESI -MS Mass spectroscopy confirmed the proposed formulation for the ligands and complexes by comparing the m/z values observed in the mass spectra with the molecular weight of fragments resulted.

- In the case of chloride and acetate type complexes the thermal analysis confirmed the proposed formula, the nature of water molecules, the thermal stability of the compounds and provided information concerning the steps corresponding to the thermal degradation as well as the thermal effects that accompanies them. IR spectrum and the powder X-ray diffraction confirmed the composition of the residue that consisted from metal oxide.

- The information supplied by IR spectra indicated that the azamacrocyclic ligands (coordinated through nitrogen atoms of secondary amine groups to metal ions) were formed through condensation reaction.

- ^1H NMR and ^{13}C NMR spectra have provided useful information about the ligands and diamagnetic complexes of Zn(II), confirming the nature of the protons and carbon atoms from functional groups of ligands.

- Electronic spectra correlated with the values of magnetic moment at room temperature have offered information about the oxidation state and the stereochemistry of Ni(II) and Cu(II) ions in corresponding complexes.

- EPR spectra confirmed the proposed stereochemistry for Cu(II) complexes.

- Voltammetric studies revealed the quasireversible reduction of the ligands due to carbonyl groups. In the case of complexes have been identified waves associated with redox couples Ni(II)/Ni(III), Ni(I)/Ni(II) and L_{red}/L for nickel compounds, Cu(II)/Cu(I) and Cu(I)/Cu(0) for copper compounds and Zn(II)/Zn(0) for zinc complexes). All the data from the cyclic voltammograms concluded that the azamacrocyclic ligands generated stable compounds compared with the solvated species.

- Antimicrobial tests were performed in order to determine minimum inhibitory concentration (MIC) and the influence of the tested compounds on the formation of microbial biofilms on inert substratum. It was observed that complexes were more active than the ligand. The species with hexaazamacrocyclic ligands derived from 1,2-phenylenediamine were more active against a large number of strains compared to complexes with ligands derived from ethylenediamine and triethylenetetramine. This behaviour may be correlated with their increased lipophilicity associated with the hydrophobicity of the benzene ring, which allows a simple transport through lipid membrane of pathogenic microorganisms.

- Complexes $[CuL^3]Cl_2$ (**22**), $[ZnL^3Cl_2]$ (**23**), $[ZnL^3(OH_2)_2](ClO_4)_2 \cdot 4H_2O$ (**26**) and $[ZnL^3](CH_3COO)_2$ (**32**) were tested in order to assess the inflammatory activity and the results indicate that the complex $[CuL^3]Cl_2$ (**22**) possess a good anti-inflammatory activity.

- Cytotoxicity tests showed that the integrity of the cells membrane of Hep 2 and HT 29 were destroyed by the Cu(II) compounds manifested by percent of cells blocked in the mitotic phase and in some cases the appearance of apoptotic cells was observed.

Selective References

1. G.A. Lawrance, M. Maeder, E.N. Wilkes. Rev. Inorg. Chem. 1993, 13, 199-232.
2. K.P. Wainwright. Coord. Chem. Rev. 1997, 166, 35-90.
3. L.F. Lindoy. Adv. Inorg. Chem. 1998, 45, 75-125.
4. T.J. Hubin. Coord. Chem. Rev. 2003, 241, 27-46.
5. L.F. Lindoy, G.V. Meehan, I.M. Vasilescu, H.J. Kim, J.-E. Lee, S.S. Lee. Coord. Chem. Rev. 2010, 254, 1713-1725.
6. J.C. Timmons, T.J. Hubin. Coord. Chem. Rev. 2010, 254, 1661-1685.

7. M. Licchelli, M. Milani, S. Pizzo, A. Poggi, D. Sacchi, M. Boiocchi. *Inorg. Chim. Acta.* 2012, 384, 210-218.
8. R.E. Mewis, S.J. Archibald. *Coord. Chem. Rev.* 2010, 254, 1686-1712.
9. T.J. Hubin, J.M. McCormick, S.R. Collinson, C.M. Perkins, N.W. Alcock, P.K. Kahol, A. Raghunathan, D.H. Busch. *J. Am. Chem. Soc.* 2000, 122, 2512-2522.
10. C. Bolm, N. Meyer, G. Raabe, T. Weyhermüller, E. Bothe. *Chem. Commun.* 2000, 2435-2436.
11. A. Grenz, S. Ceccarelli, C. Bolm. *Chem. Commun.* 2001, 1726-1727.
12. K. Mochizuki, S. Manaka, I. Takeda, T. Kondo. *Inorg. Chem.* 1996, 35, 5132-5136.
13. K. Wieghardt. *Angew. Chem. Int. Ed.* 1989, 28, 1153-1172.
14. W.B. Tolman. *Acc. Chem. Res.* 1997, 30, 227-237.
15. M.A. Halcrow, G. Christou. *Chem. Rev.* 1994, 94, 2421-2481.
16. P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo, P. Scrimin. *J. Am. Chem. Soc.* 1999, 121, 6948-6949.
17. C. Sissi, P. Rossi, F. Felluga, F. Formaggio, M. Palumbo, P. Tecilla, C. Toniolo, P. Scrimin. *J. Am. Chem. Soc.* 2001, 123, 3169-3170.
18. M.J. Young, J. Chin. *J. Am. Chem. Soc.* 1995, 117, 10577-10578.
19. P. Caravan, J.J. Ellison, T.J. McMurry, R.B. Lauffer. *Chem. Rev.* 1999, 99, 2293-2352.
20. D.E. Reichert, J.S. Lewis, C.J. Anderson. *Coord. Chem. Rev.* 1999, 184, 3-66. L. Thunus, R. Lejeune. *Coord. Chem. Rev.* 1999, 184, 125-155.
21. W.A. Volkert, T.J. Hoffmann. *Chem. Rev.* 1999, 99, 2269-2292.
22. L. Ronconi, P.J. Sadler. *Coord. Chem. Rev.* 2007, 251, 1633-1648.
23. C.G. Nirmala, A.K. Rahiman, S. Sreedaran, R. Jegadeesh, N. Raaman, V. Narayanan. *Polyhedron.* 2011, 30, 106-113.
24. D. Kong, Y. Xie. *Inorg. Chim. Acta.* 2002, 338, 142-148.
25. L. Findoráková, K. Györyová, D. Hudecová, D. Mudroňová, J. Kovářová, K. Homzová, F.A. Nour El-Dien. *J. Therm. Anal. Calorim.* 2013, 111, 1771-1781, D.A. Köse, H. Necefoğlu. *J. Therm. Anal. Calorim.* 2008, 93, 509-514.
26. D.A. Köse, H. Necefoğlu. *J. Therm. Anal. Calorim.* 2008, 93, 509-514.
27. A.T. Balaban, M. Banciu, I.I. Pogany. *Aplicații ale metodelor fizice în chimia organică.* Ed. Tehnica, București, 1983.
28. B.J. Hathaway. Oxyanions. In: Silkinson G, Gillard RD, McCleverty JA, editors. *Comprehensive Coordination Chemistry.* Oxford: Pergamon Press; 1986.
29. G.B. Deacon, J.R. Philips. *Coord. Chem. Rev.* 1980, 33, 227-250.
30. V. Zeleňák, Z. Vargová, K. Györyová, E. Večerníková, V. Balek, J. Therm. Anal. Calorim. 2005, 82, 747-754.
31. K. Nakamoto. *Infrared and Raman spectra of inorganic and coordination compounds.* Wiley, New York, 1986.
32. A.B.P. Lever. *Inorganic electronic spectroscopy.* Elsevier, Amsterdam, London, New York, 1986.
33. P. Zanello. *Inorganic Electrochemistry: Theory, Practice and Application.* Cambridge, U.K, Royal Society of Chemistry, 2003.

34. M. Brezeanu, L. Patron, M. Andruh. Combinații complexe polinucleare și aplicațiile lor. Ed. Academiei RSR, București, 1986.
35. H.F. Abd El-Halim, F.A. Nour El-Dien, G.G. Mohamed, N.A. Mohamed. J. Therm. Anal. Calorim. 2012, 109, 883-892.
36. M. Badea, E. Iosub, C.M. Chifiriuc, L. Marutescu, E.E. Iorgulescu, V. Lazar, D. Marinescu, C. Bleotu, R. Olar. J. Therm. Anal. Calorim. 2013, 111, 1753-1761

List of published articles:

1. "Thermal, spectral, magnetic and antimicrobial behaviour of new Ni(II), Cu(II) and Zn(II) complexes with a hexaazamacrocyclic ligand". **Florentina Pătrașcu**, Mihaela Badea, Maria Nicoleta Grecu, Nicolae Stănică, Luminița Măruțescu, Dana Marinescu, Cezar Spînu, Cristian Tigae, Rodica Olar. J. Therm. Anal. Calorim. 113, 2013, 1421–1429. (F.I.= 1.982)
2. "Synthesis and characterization of Ni(II), Cu(II) and Zn(II) complexes with an acyclic Mannich base functionalized with thioglycolate moiety". Anca Dumbrava, Rodica Olar, Mihaela Badea, **Florentina Pătrașcu**, Luminița Măruțescu, Nicolae Stănică. J. Therm. Anal. Calorim. 115, 2014, 2447-2455. (F.I.= 1.982)

Communications (poster presentations):

1. "Synthesis, spectral, biological and thermal characterization of some new complexes with hexaazamacrocyclic ligands". Florentina Pătrașcu, Rodica Olar, Mihaela Badea, Ioana Dorina Vlaicu, Anca Dumitra, Monica Raită, Dana Marinescu. 1st Central and Eastern Europe Conference – Thermal Analysis and Calorimetry, Craiova, 7-10 septembrie 2011.
2. "Complexes of Ni(II), Cu(II) and Zn(II) with Azamacrocyclic Ligands functionalized with nicotinamide as Antimicrobials Agents". Florentina Pătrașcu, Rodica Olar, Mihaela Badea, Ioana-Dorina Vlaicu, Dana Marinescu, Luminița Măruțescu, Carmen Balotescu, Veronica Lazăr/Workshop/poster/
3. "Synthese et Caracterisation spectrale et biologique de nouvelles combinaisons complexes avec une Base Schiff couronnee"/ Rodica Olar, Irina Zarafu, Mihaela Badea, Dana Marinescu, Petre Ioniță, Florentina Pătrașcu, Anca Păun, Lucia Ivan Coralia Bleotu/ Septième Colloque Franco-Roumain de Chimie Appliquée (CoFrRoCA2012), 27-29 iunie 2012, Bacău, România.
4. "Thermal, spectral, magnetic and antimicrobial behavior of new Ni(II), Cu(II) and Zn(II) complexes with a hexaazamacrocyclic ligand bearing nicotinamide moieties"/ Rodica Olar, Mihaela Badea, Florentina Pătrașcu, Romana Cerc Korošec, Dana Marinescu, Monica Raită, Mariana Carmen Chifiriuc, Luminița Măruțescu/2nd Central and Eastern Europe Conference – Thermal Analysis and Calorimetry, Vilnius 27-30 august 2013, Lithuania/ Poster/353

5. "Thermal, spectral and antimicrobial characterization of new Ni(II), Cu(II) and Zn(II) complexes with a pentaazamacrocyclic ligand with nicotinamide pendant". Mihaela Badea, Florentina Pătraşcu, Dana Marinescu, Mariana Carmen Chifiriuc, Luminița Măruțescu, Rodica Olar/ 2nd Central and Eastern Europe Conference – Thermal Analysis and Calorimetry (CEEC-TAC2), Vilnius 27-30 august 2013, Lithuania/ poster/351